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BIOLOGICAL AND CHEMICAL SCIENCES

Perspectives to North East India

Editors
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Pyrimidines: A Universal Topic of Research in Organic and Medicinal Chemistry

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1. Introduction:

Research in bioactive molecules has long been targeted to discover new potential bioactive molecules. This is a never ending area of research. For this, synthetic chemists, medicinal chemists, theoretical chemists and biologists have come forward to put their hands and idea together. In this regard, the bioactive molecule, pyrimidines as chemical entity occupies a cardinal position. This is mainly due to the inherent bioactivity associated with such molecules and subsequent possible synthetic manipulation through various tactics.

Since the discovery, as an important constituent of nucleic acids, pyrimidineshave been occupying a distinct and unique place inhuman's life and consequently in organic and medicinal chemistry research. The core molecule pyrimidine is a member of diazine family (isomeric to pyridazine and pyrazine, **Fig.1)**. The pyrimidine and its various derivatives have significant biological and medicinal significances. Whilst cytosine, thymine and uracil (**Fig.2**) are the pyrimidine bases found in DNA and RNA, lots of other pyrimidine derivatives possess pharmacological activities[1] like tyrosine kinase activity, antibacterial activity, calcium channel antagonist activity, tuberculostatic activity, diuretic and potassium sparing activity, antiagressive activity etc.Simple examples include 5-Iodo-2'-deoxyuridine (IDU) (1), which has been used as anti-herpes virus drug and Zidovudine (2), the first U.S. government-approved drug for HIV, prescribed under the names Retrovir and Retrovis. In fact, Zidovudine is included in the World Health Organization's Model List of EssentialMedicines, which suggests the minimum medicinal needs for a basic health care system.VIAGRA® (3), the much talked molecule in present day's world, which is widely used in oral therapy of erectile dysfunction is the citrate saltof sildenafil, a selective inhibitor of cyclicguanosine monophosphate (cGMP)-specific phospodiesterase type 5 (PDE5).

Fig. 1: Three isomeric diazines

Fig. 2: Three pyrimidine bases in DNA &RNA

Numerous other lifesaving drugs are now available in the market; a partial list of which has been published very recently [2]. Feeling the ever-increasing vitality, demand and especially considering the bioactivity, pyrimidine chemistry now becomes an interdisciplinary subject of immense practical and theoretical importance. In this chapter, we will try to give a humble effort to manifest the credibility of pyrimidines as a universal topic of research in organic and medicinal chemistry taking an account from the first isolation of uric acid (7) (a purine derivative; purines are bicyclic compounds with one pyrimidine and one imidazole ring) to their current use in chemotherapy of cancer in brief.

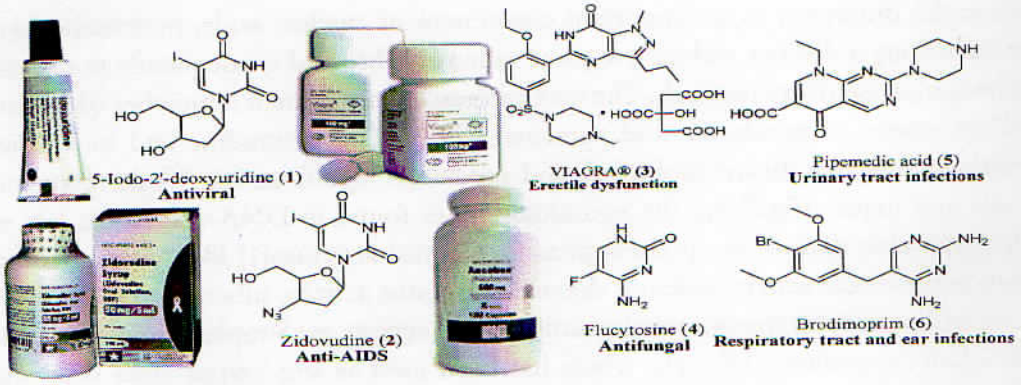


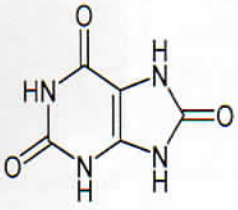
Fig.3: Some pyrimidine based marketed drugs

2.Early history:

As early as 1776, C. W. Scheele gave birth the researchonpyrimidine derivatives by isolating uric acid in pure form from urinary calculie(stones) [3], although another 42 years has been taken to isolate the first pyrimidine derivative alloxan (8) by Brugnatelli in 1818 [4]. Alloxan is known for its diabatogenic action in a number of animals. However, laboratory synthesis of a pyrimidine was not carried until 1879, when Grimaux

reported the first preparation of barbituric acid from urea and malonic acid in the presence of phosphorus oxychloride [5]. Actually, the systematic study of pyrimidines started with the work of Pinner, who synthesized pyrimidine derivatives by condensing ethyl acetoacetate with amidines [6]. He was the first to coin the term 'pyrimidine' to the unsubstituted parent bodyby combining the words pyridine and amidine. The parent compound was first synthesized by Gabriel & Colman in 1900, by conversion of *barbituric* acid to 2,4,6-*trichloropyrimidine* followed byreduction using zinc dust in hot water [7].

C.W. Scheele



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L. Brugnatelli

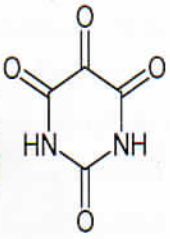

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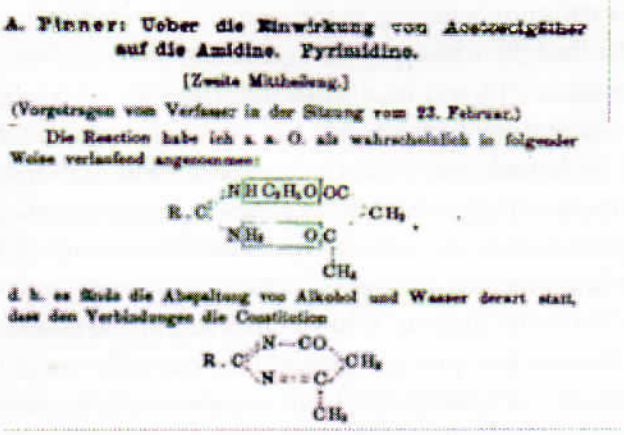
Fig. 4: Pioneers of *purine* and *pyrimidine* isolation



A. Pinner: Ueber die Einwirkung von Acetessigäther auf die Amidine. Pyrimidine.

[Zweite Mittheilung.]

(Vorgetragen vom Verfasser in der Sitzung vom 22. Februar.)

Die Reaction habe ich a. a. O. als wahrscheinlich in folgender Weise verlaufend angenommen:

d. h. es findet die Abspaltung von Alkohol und Wasser derart statt, dass den Verbindungen die Constitution

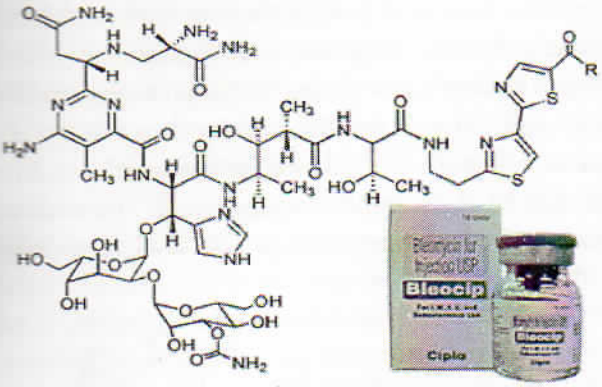
Fig. 5: Adolf Pinner started the systematic study of *pyrimidine*

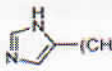
A.Kossel received the Nobel Prize for Medicine in 1910 as a recognition of his contribution to the knowledge of cell chemistry made through his work on proteins, including the nucleic substances. He was associated with the isolation and synthesis of all the three pyrimidine nucleobases thymine,cytosine and uracil. Thymine was isolated from hydrolyzates of bovine thymus in 1893 [8], while it took another eight years for laboratory synthesis. Cytosine was first isolated in 1894 from hydrolysis of calf thymus [9] and by 1903, it structure was confirmed by synthesis [10]. Isolation of uracil was first performed by the hydrolysis of herring sperm in 1900 [11] and its structure was established in 1901 by synthesis.

Fig. 6: A. Kossel was associated with isolation and synthesis of pyrimidine nucleobases

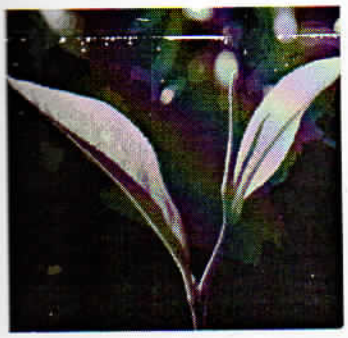
3.Natural Occurence:

Apart from the three pyrimidine and two purine (where one of the ring is a pyrimidine ring) bases found in DNA and RNA, pyrimidines have wide natural occurance. The most common example is caffeine (7), the stimulating agent present in tea and coffee. Willardiine (8), a non-proteinogenic *L□-amino acid* was isolated from the seeds of Acacia willardiana [12] and later from other species of Acacia [13]. Thiamine (9) or Vitamine B1, the anti-beriberi vitamin is a pyrimidine containing vitamin, first isolated in 1926 [14] by Jansen and Donath from rice bran and is known only in the form of salt. Bacimethrin (10), a naturally occuring thiamine antimetabolite, first isolated from Bacillus megatherium is the simplest pyrimidine antibiotic [15]. Bleomycin (11) is one of the very few anticancer drugs not attacking bone marrow and often used clinically to treat solid forms of tumour. It has a rapid action and is able to break single-stranded DNA in the tumour and prevent repair. This naturally occuring glycopeptide is isolated from Streptomyces verticillus[16]. For more naturally occurring pyrimidines, interested readers may go through the review by I. M. Lagoja [18].

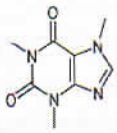


R= $MeS(O)(CH_2)_3NH$ A_1
R= $Me_2\overset{\oplus}{S}(CH_2)_3NH$ A_2
R= $MeS(CH_2)_3NH$ Demethyl-A2
R= $H_2N(CH_2)_4NH$ A_2'-a
R= $H_2N(CH_2)_3NH$ A_2'-b
R=  A_2'-c
R= $H_2N(CH_2)_4NH(CH_2)_2NH$ A_5
R= $H_2N(CH_2)_3NH(CH_2)_4NH(CH_2)_3NH$ A_6
R=NH_2 B_2'
R=  B_2

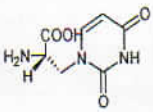
Some bleomycin (11) derivatives found in nature



Tea leaves

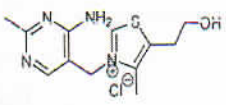


Caffeine (7)

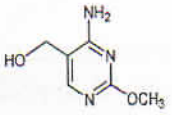


Willardiine (8)

Acacia willardiana



Thiamine (9)



Bacimethrin (10)

4.Medicinal significance

In the introduction and natural occurrence part of the chapter, we have already mentioned some pyrimidine drugs extracted from natural resources or synthesized in laboratory that deal various health issues. A large number of other pyrimidine drugs are in clinical use and trial whereas numerous others have been proposed and under research to treat various threats to human life. Here, in this section we will make a simple effort to discuss the chemistry and logic behind their current use of a few pyrimidine derivatives as drug and their potentiality to be drug.

4.1 Gout and its treatment:

Gout is the clinical term describing the physiological consequences accompanying excessive uric acid (7) accumulation in body fluids [19]. The most common symptom of gout is arthritic pain in the joints as a result of urate deposition in cartilaginous tissue.

During the process of degradation of nucleotide Adenosine monophosphate (AMP), hypoxanthine (12) is produced, which is further oxidized to xanthine (13) and then to

uric acid (7) by the enzyme xanthine oxidase. Uric acid loses a proton at physiological pH to form urate, which is the final product of purine degradation in human beings and is excreted through urine [20]. A high serum level of urate (hyperuricemia) is responsible for the painful joint disease gout. The small joint at the base of the big toe is very susceptible to sodium urate accumulation, although the salt builds up at other joints also. Painful inflammation results when cells of the immune system engulf the sodium urate crystals. Urate crystals may also appear as kidney stones and lead to painful obstruction of the urinary tract.

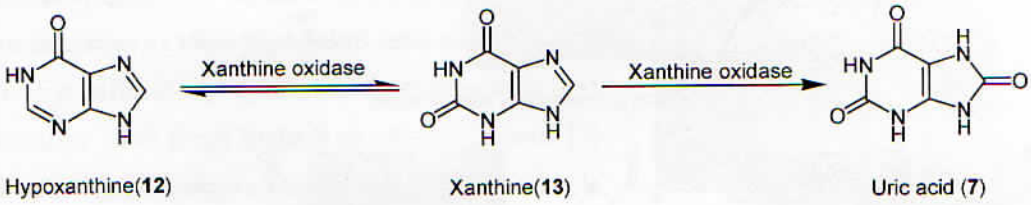


Fig. 7: Mechanism of *uric acid* production in mammals

The administration of allopurinol (14), an analogue of hypoxanthine, is an effective treatment for gout. The mechanism of action of allopurinol is quite interesting and is an example of suicide inhibition: it acts first as a substrate and then as an inhibitor of xanthine oxidase. The enzyme xanthine oxidase hydroxylates allopurinol to alloxanthine (15), which then remains tightly bound to the active site of the enzyme, an example of suicide inhibition. The synthesis of urate from hypoxanthine and xanthine decreases soon after the administration of allopurino. The serum concentrations of hypoxanthine andxanthine rise, and that of urate drops. Hypoxanthine and xanthine do not accumulate to harmful concentrations because they are more soluble and thus more easily excreted.

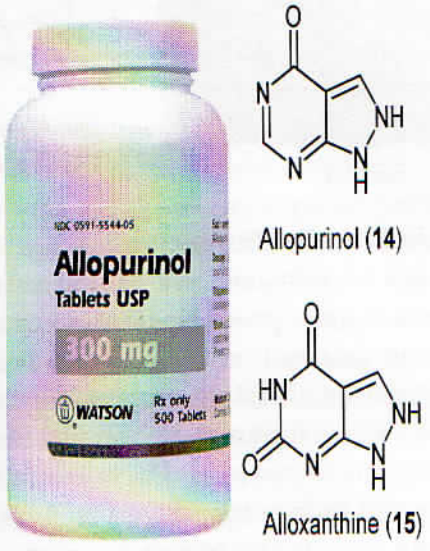


Fig. 8: Structure of allopurinol andalloxanthine

4.2 Anti-Parkinson drugs

MAO-B (monamine oxidase B) is an important target for the development of new anti-Parkinson's drugs (Parkinson's disease, a degenerative disorder of the central nervous system is mainly caused by the loss of dopaminergic neurons of the substantianigria located at the midbrain) and MAO-B inhibitors are promising drugs in the treatment of Parkinson's disease [21]. The enzyme MAO-B, located in the mitochondrial outer membrane plays an important role in the metabolism of dopamine and neuroactive and vasoactive amines in the central nervous system. Inhibition of this enzyme enhances the elevation of dopamine levels in adjunctive treatment with levodopa, and delays the onset of relapse following levodopa monotherapy.(E)-8-(3-Chlorostyryl) caffeine (CSC, 16) and KW-6002 (17) have been reported as inhibitors of MAO-B with a Ki value (enzyme inhibitor dissociation constant) of 128 nM and 11 μM, respectively. Numerous analogues of CSC and KW-6002with purine ring system have been synthesized from pyrimidine derivatives [22] later by different research groups among which J. P. Petzer et al. contributes the lion's part [23]. Jacobus P. Petzer, initially from USA and presently from South Africa has been contributing a lot to this area by his work of design, synthesis and biological evaluation of various pyrimidine derivatives and many of his synthesized compounds are proved as potential MAO-B inhibitor.

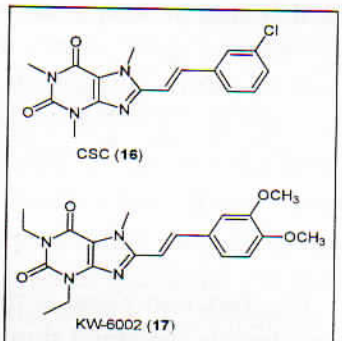


Fig. 9: The structure of MAO-B inhibitor

4.3Antibacterial drugs

The worldwide emergence over the past three decades of bacterial strains resistant to most current antibiotics raises a serious threat to global public health [24]. After several decades of continuously successful antibiotic therapy against bacterial infections, we are now really in trouble due to the accelerated evolution of antibiotic resistance to important human pathogens and the scarcity of new anti-infective drug families under development. Efflux is a general mechanism responsible for this bacterial resistance to antibiotics [25]. So, inhibition of bacterial efflux mechanisms now becomes a promising target in order to (i) increase the intracellular concentration of antibiotics that are expelled by efflux pumps, (ii) restore the drug susceptibility of resistant clinical strains and (iii) reduce the capability for acquired additional resistance. A flurry of structurally unrelated classes of efflux pump inhibitors (EPIs) have been described and tested in the last two decades, including some analogues of antibiotic substrates and new chemical molecules. The need for novel antibiotic classes to combat bacterial drug resistance has led to considerable efforts to identify and exploit new antibacterial targets and novel antimicrobial agents.

A large number of pyrimidine derivatives possessing antibacterial activity have been reported every year and some of them are in clinical trial. Recently, in Nature Reviews Drug Discovery [26], M. H. Flight highlighted the work of Stover and colleagues [27] screening of Pfizer compound library of 1.6 million compounds for antibacterial activity resulting the discovery of three pyrido[2,3-d]pyrimidine (18,19,20) derivatives as potent synthetic antibacterial that targeted the bacterial biotin carboxylase selectively. They made a particular note of their exquisite potency against clinical isolates of fastidious Gram negative pathogens such as Haemophilus influenza and Moraxella catarrhalis; causative agents of many respiratory tract infections. This series of pyridopyrimidines target the ATP-binding site of biotin carboxylate (BC) in the bacteria which catalyzes the first step of fatty acids.

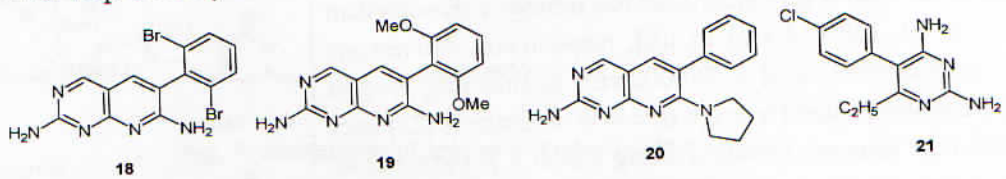


Fig. 10: Structure of **18, 19, 20&21**

The fact that these compounds can selectively target the BC in spite of having considerable structural similarity to eukaryoticprotein kinases including human kinases is established. In a very recent report [24], A. Zega et al. demonstrated 6-Aryl-pyrido[2,3-d]pyrimidines as novel ATP-competitiveinhibitors of bacterial D-alanine: D-alanine ligase and established a mechanism for their antibacterial activity. The bacterial enzyme D-alanine: D-alanine ligase(Ddl) catalyzes the ATP- dependent formation of a dipeptide D-Ala-D-Alathat subsequently takes part in the biosynthesis of disaccharide-pentapeptide peptidoglycan structure, which is a specific and essential component of the bacterial cell wall. 6-arylpyrido[2,3-d]pyrimidines target this bacterial enzyme and bind tightly to it by competing with ATP and thereby inhibits the formation of D-Ala-D-Ala. Another series of pyrimidine derivatives, 2,4-diaminopyrimidines are proved to be effective inhibitors of the enzyme dihydrofolatereductase (DHFR) [28]. Notable among them is pyrimithamine (21), a selective inhibitor of the DHFR of malarial plasmodia.

4.4 Anticancer drugs

For a cell to reproduce, it must first faithfully replicate the entire DNA in its genome. During DNA synthesis, purine and pyrimidine molecules must be made available to allow for the synthesis of the nucleotide building blocks and ultimately the new DNA molecules. A reduction in the availability of the raw materials needed to build DNA, such as is caused by the pyrimidine antagonists lead to stoppage of DNA synthesis and

Fig. 11: Some pyrimidine based anticancer drugs

5.Research in pyrimidine chemistry- National and International scenario:

As mentioned earlier, study on pyrimidines is an interdisciplinary one and the research field is common for both chemist and biologist and so their works are closely associated. While chemists, specially the organic chemists design and synthesize the compounds with possible biological activity, biologists work on them to establish the fact. Every year, hundreds of papers have been getting published by the collaborative work of organic and medicinal chemists. In addition, numerous other reports have been found describing solely either the novel synthesis of pyrimidine derivatives or the synthesis of novel pyrimidine derivatives. Some other reports emphasize on the structural aspects of various pyrimidine derivatives [31]. In India, Prof. K. C. Majumdar, now UGC Emeritus Fellow in Kalyani University is a leading scientist in this area and has contributed a lot to the field. In north-east India, NEIST, Jorhat has also been contributing significantly for the growth and knowledge of pyrimidine chemistry.

6.Conclusion and future scope:

Since the day of the first systematic study on pyrimidines, research in this area has been continuing with the same strength and spirit till date. Although the field has been explored quite well by the scientists across the globe for more than a century, still lots of reports have been publishing every year implying the universality of the field. Newer pyrimidine based drugs are still coming into the market. The very fast evolution of computational chemistry brings extra colour to the field providing the way for structure activity relationship (SAR). Green chemistry is one another aspect that is also incorporated during the designing of synthetic route to the targeted molecule.

Considering all these, i.e. medicinal significance of pyrimidine derivatives, increasing number of pyrimidine drugs in clinical use, greener way to synthesize them, their natural occurrence and structure activity relationship to understand the mode of action as drug, this challenging field of research will continually enjoy its good name as a frontline research area in organic and medicinal chemistry in future also.

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