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ADVANCES IN CHEMICAL SCIENCES

Volume - 3

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Dr. Anurika Mehta

Co-Editor
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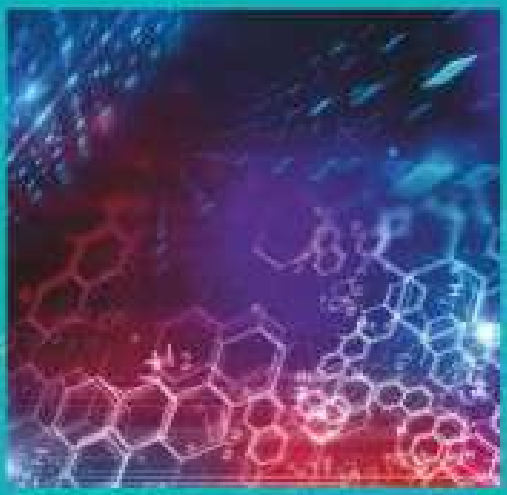

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Contents

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Chapter - 3
Ethnopharmacological Significance of Nitrogen Containing Heterocyclic Compounds

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Chapter - 3

Ethnopharmacological Significance of Nitrogen Containing Heterocyclic Compounds

Dr. Ramadevi Kyatham, Dr. Raju Bathula, Dr. Sadanandam Palle, Dr. Jyothi Vantikommu, Dr. S. Hemalatha and Mohammad Arif Pasha

Abstract

Heterocyclic compounds have a role in most fields of sciences such as medicinal chemistry, biochemistry also another area of sciences. More than 90% of new drugs contain heterocycles and the interface between chemistry and biology, at which so much new scientific insight, discovery and application is taking place is crossed by heterocyclic compounds. Compounds derived from heterocyclic rings in pharmacy, medicine, agriculture, plastic, polymer and other fields. Most active heterocycles that have shown considerable biological actions as anti-fungal, anti-inflammatory, anti-bacterial, anti-convulsant, anti-protozoal, anti-allergic, herbicidal, anti-cancer activity. Heterocyclic compounds are a highly valuable and unique class of compounds. Different heterocyclic analogues have been evaluated for their diverse biological activities. Heterocyclic compounds are present in abundance in our surroundings. They owe their importance in the biological system due to uniqueness in their structural skeleton parts. They are naturally found in nucleic acid, vitamins, anti-biotics, hormones etc. Nitrogen containing heterocyclic compounds are an important class of heterocyclic compounds that has paid significant contribution towards medicinal chemistry. This review highlights the trends in the use of nitrogen-based moieties in drug design and the development of different potent and competent candidates against various diseases. This review shall give researchers access and detailed understanding on various application of a novel heterocyclic subsidiary into diverse areas for new process or application. In this study, we will understand the biologically active heterocyclic compounds, biological importance of nitrogen-containing heterocyclic compounds and pyridine containing medicinal agents. The number of novel N-heterocyclic moieties with significant physiological properties and promising applications in medicinal chemistry is ever-growing. Pyrimidine is one of the most important

heterocycles compounds, most potent pyrimidines used to treat of various diseases such as cancer, leukemia. Pyrimidine also represents the backbone of RNA and DNA.

Keywords: Heterocyclic compounds, nitrogen, heteroatom, nitrogen-based heterocycles, biological activities, pyrimidine, nucleosides

Introduction

In organic chemistry, largest families of organic compounds belong to heterocyclic compounds. In place of a carbon atom incorporation of an oxygen, a nitrogen, a sulfur, or an atom of a related element gives rise to a heterocyclic compound. Heterocyclic compounds are of very essential for our day-to-day life. It has a broad range of applications in medicinal chemistry as well as in agrochemical products. Heterocyclic chemistry is the branch of chemistry dealing with the synthesis, properties and applications of heterocycles.

Medicinal chemistry had its beginning when chemists, pharmacist and physicians isolated and purified active principles of plants and animal tissues and taken from micro-organism and their fermentation products. These compounds have been associated with therapeutic properties. Medicinal chemistry which has leaned on the classical fields of chemistry, especially organic chemistry and biology. Various natural and synthetic compounds are serving directly as therapeutic agents and some other uses in agriculture. Most of the drugs belong to the class of heterocyclic compounds. These heterocyclic compounds played a vital role in the metabolism of all living cells; large numbers of them are five and six membered heterocyclic compounds having one, two and three hetero atoms in their nucleus. The compounds may be pyrimidine and purine basis of genetic material DNA, and these heterocyclic compounds may be isolated or fused heterocyclic systems. Some of the common heterocyclic compounds used in the medicines are as amino acids like proline, histidine and tryptophan, the vitamins and coenzymes precursors such as thiamine, riboflavin, pyridoxine, folic acid, biotin, vitamin B_{12} and vitamin E. There are various pharmacologically active heterocyclic compounds, many of which are in regular clinical use.

Heterocyclic compounds are frequently abundant in plants and animal products and they are one of the important constituents of almost one half of the natural organic compounds known. Alkaloids, natural dyes, drugs, proteins, enzymes etc. are some important classes of natural heterocyclic compounds. Heterocyclic compounds can be easily classified based on their electronic structure. Heterocyclic compounds are primarily classified as

saturated and unsaturated. The saturated heterocyclic compounds behave like the acyclic derivatives with modified steric properties. Piperidine and tetrahydrofuran are the conventional amines and ethers of this category.

However, unsaturated heterocyclic compounds of 5-and 6-member rings have been studied extensively because of their unstrained nature. The unstrained unsaturated heterocyclic compounds include pyridine, thiophene, pyrrole, furan and their benzo fused derivatives. Quinoline, isoquinoline, indole, benzothiophene and benzofuran are some important examples of benzo fused heterocycles. Heterocyclic compounds have a wide application in pharmaceuticals, agrochemicals and veterinary products. Many heterocyclic compounds are very useful and essential for human life. Various compounds such as hormones, alkaloids antibiotic, essential amino acids, hemoglobin, vitamins, dyestuffs and pigments have heterocyclic structure.

A large number of heterocyclic compounds, both synthetic and natural, are pharmacologically active and are in clinical use. Several heterocyclic compounds have applications in agriculture as insecticides, fungicides, herbicides, pesticides etc. They also find applications as sensitizers, developers, anti-oxidants, copolymers etc. They are used as vehicles in the synthesis of other organic compounds. Chlorophyll-photosynthesizing and haemoglobin-oxygen transporting pigments are also heterocyclic compounds. Heterocyclic is the largest and most varied family of organic compounds, heterocyclic system can be 3, 4, 5, 6, 7 membered rings.

Definition of heterocyclic compounds: Heterocyclic compounds are cyclic compounds which contains one/more atoms of other elements along with carbon atoms. *Definition of Heteroatom:* Hetero atoms are those which contains an atom other than carbon such as nitrogen, sulphur, oxygen, phosphorus etc.

Heterocycles are the main classical organic chemistry divisions and are of considerable biological and industrial significance. The bulk of pharmaceuticals and agrochemicals that are biologically active is heterocyclic, while the numerous additives and modifiers utilized by industry are often heterocyclic, such as cosmetics, reprography, information storage and plastics. One striking structural function inherent in heterocycles which the drug industry exploits greatly is their ability to display substituents in given three-dimensional images by a core scaffold. Heterocycles have been one of the major fields of study in organic chemistry for more than a century. Heterocycles have led to the biological and economic advancement of civilization as well as to the awareness of the mechanisms of life aimed at

improving the quality of life. More than two-thirds of the around 20 million chemical compounds found by the end of the second millennium are entirely or partly aromatic and about half are heterocyclic.

Synthetic heterocycles have widely used therapeutic uses such as anti-bacterial, anti-fungal, anti-mycobacterial, trypanocidal, anti-HIV, anti-leishmanial, genotoxic, anti-tumoral, anti-inflammatory, muscular relaxants, anti-convulsant, anti-cancer, and lipid prooxidants, hypnotic agents, non-depressants and anti-transplants. There are more synthetic heterocyclic compounds with other essential uses, such as fungicides, herbicides, anti-bodies, photo stabilizers, agrochemicals, dyestuffs, copolymers, development photographers, fluorescent whiteners, sensitizers, boosters, rubber anti-oxidants and flavorers. The compounds of pyrimidine (cytoses, uracils, and thymines) and purine (adenine and guanines) are monocyclic and bicyclic heterocycles, each of which consists of two and four nitrogen atoms. The deoxyribonucleic acid (DNA) molecules are essential components that engage directly in the decoding of genetic material.

Classification of heterocyclic compounds

Based on the structural and electronic arrangement the heterocyclic compounds may be classified into two categories.

i) Aliphatic heterocyclic compounds.

ii) Aromatic heterocyclic compounds.

The aliphatic heterocyclic compounds are the cyclic amines, cyclic amides, cyclic ethers and cyclic thioethers. Aliphatic heterocycles those do not contain double bonds are called saturated heterocycles. The properties of aliphatic heterocycles are mainly affected by the ring strain. Examples of aliphatic heterocyclic compounds are shown in Figure 1.

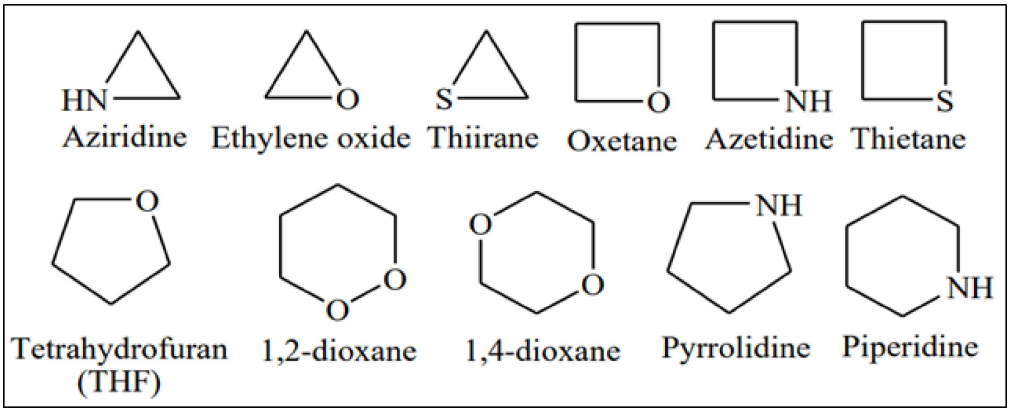


Fig 1: Aliphatic heterocyclic compounds

However, aromatic heterocyclic compounds are analogous of benzene. The aromatic heterocyclic compounds also follow the Huckel's rule. According to Huckel's rule an aromatic compounds must be cyclic in nature with planar geometry due to conjugate double bonds and must have $(4n+2)\pi$ electrons. Examples of aromatic heterocyclic compounds are shown in Figure 2.

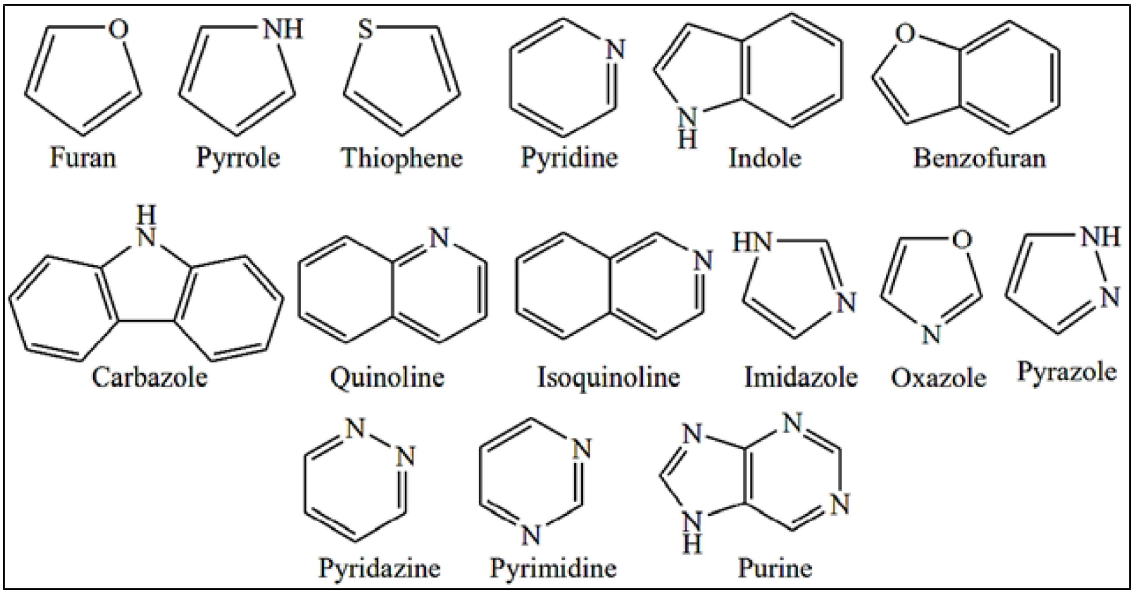


Fig 2: Examples of aromatic heterocyclic compounds

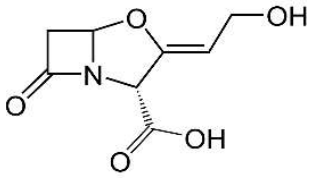
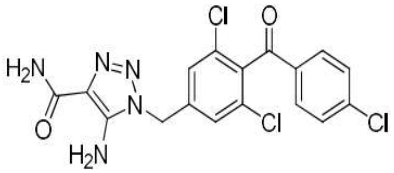
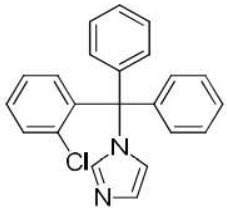
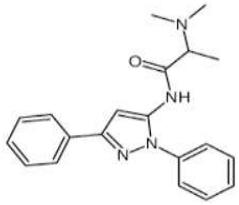
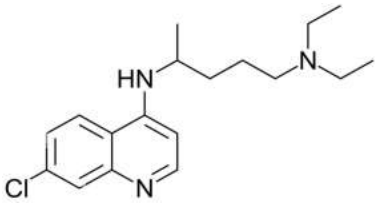
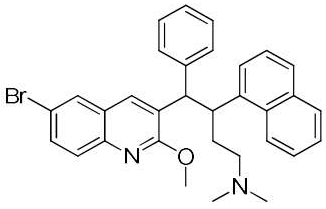
A heterocyclic ring may comprise of three or more than three atoms, which may be saturated or unsaturated. Also, heterocyclic ring may contain more than one heteroatom which may be either similar or different.

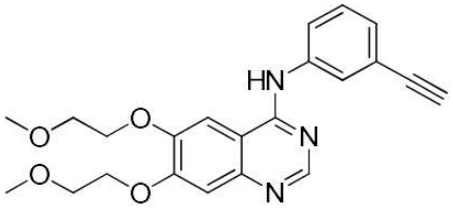
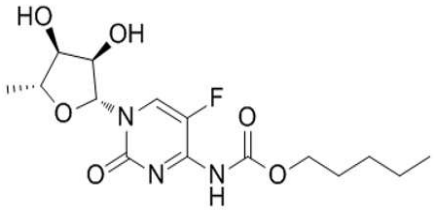
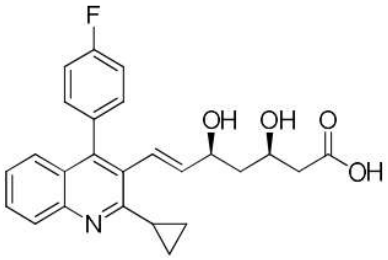
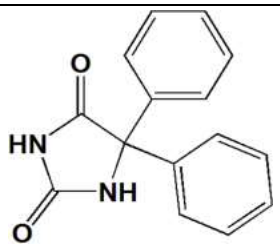
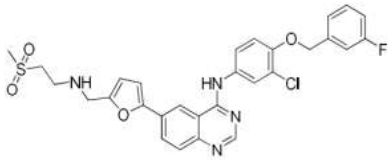
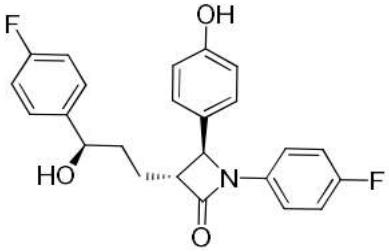
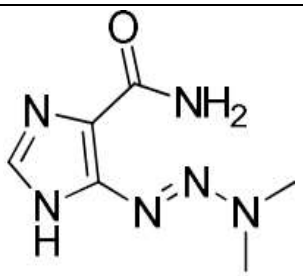
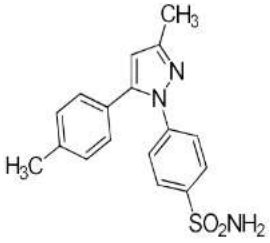
Nitrogen-based heterocyclic chemistry is an important and unique class among the applied branches of organic chemistry, with a significant amount of research dedicated to the development of novel molecules and composites. These molecules have received increasing attention over the past two decades. They contributed to the development of numerous organic synthesis protocols and found abundant applications in the chemical sciences. Many N-heterocyclic compounds that are broadly distributed in Nature, possess physiological and pharmacological properties and are constituents of many biologically important molecules, including many vitamins, nucleic acids, pharmaceuticals, antibiotics, dyes and agrochemicals, amongst many others. Moreover, they form an integral part of many pharmacologically active molecules. The base pairs of DNA and RNA (guanine, cytosine, adenine, and thymine) are also made up of N-heterocyclic compounds, namely purines, pyrimidines, etc.

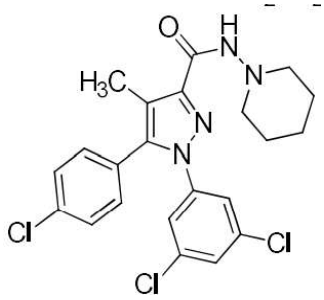
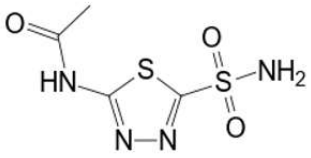
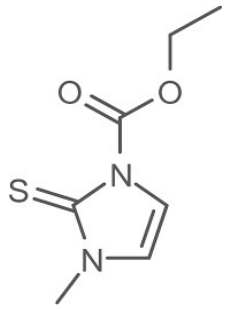
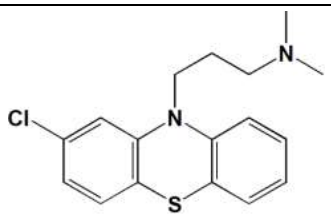
These nitrogen-containing heterocyclic molecules with distinct characteristics and applications have gained prominence in the rapidly

expanding fields of organic and medicinal chemistry and the pharmaceutical industry. A glance at the FDA databases reveals the structural significance of nitrogen-based heterocycles in drug design and engineering of pharmaceuticals. Nearly 75% unique small-molecule drugs contain a nitrogen heterocycle. The N-heterocyclic skeletons feature significantly various classes of therapeutic applications and are used as the building blocks of a number of new drug candidates, due to the ability of the nitrogen atom to easily form hydrogen bonding with biological targets.

Table 1: Examples of nitrogen containing pharmaceutical drugs

S. No.	Drug Name	Pharmacological activity	Structure
1.	Clavulanic acid	β-Lactamase inhibitor	
2.	Carboxyamidotriazole	Calcium channel blocker and anti-cancer	
3.	Clotrimazole	Anti-fungal	
4.	Difenamizole	Analgesic	
5.	Chloroquine	Anti-malarial	
6.	Bedaquiline	Anti-TB drug	

7.	Erlotinib	Treating metastatic non-small cell lung cancer	
8.	Capecitabine	Anti-breast cancer	
9.	Pitavastatin	Cholesterol lowering agent	
10.	Phenytoin	Anti-convulsant	
11.	Lapatinib	Anti-breast cancer	
12.	Ezetimibe	Cholesterol absorption inhibitor	
13.	Dacarbazine	Treatment of metastatic melanoma	
14.	Celecoxib	Anti-inflammatory	

15.	Rimonabant	Anti-obesity	
16.	Acetazolamide	Diuretic	
17.	Carbimazole	Anti-thyroid drug	
18.	Chlorpromazine	Anti-psychotic	

Pyrimidine-General introduction

Pyrimidines are the heterocyclic aromatic compounds similar to benzene and pyridine containing two nitrogen atoms at positions 1 and 3 of the six membered rings. Heterocycles containing pyrimidine moiety are of great interest because they constitute an important class of natural and non-natural products, many of which exhibit useful biological activities and clinical applications. Substituted purines and pyrimidines occur very widely in living organisms and were some of the first compounds studied by the organic chemists (Figure 3). Pyrimidines are biologically very important heterocycles and represent by far the most ubiquitous members of the diazine family with uracil and thymine being constituents of ribonucleic acid (RNA) and deoxyribonucleic acid (DNA) and with cytosine both being present in Figure 3. In addition to this, pyrimidines skeleton is also present in many natural products such as vitamin B_1 (thiamine-Figure 4) and many synthetic compounds, such as barbituric acid and veranal which are used as hypnotics.

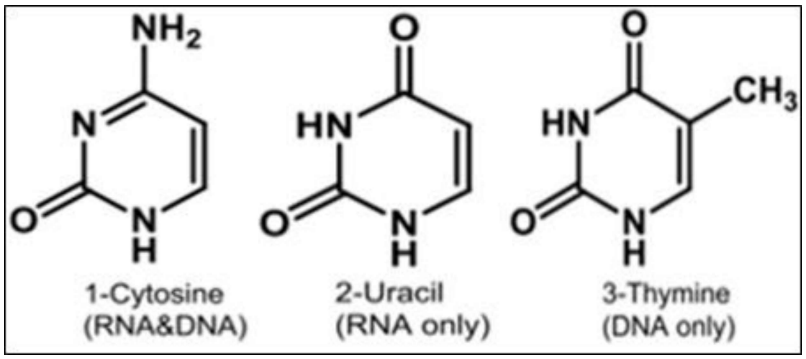


Fig 3: Pyrimidine nucleobases

Medicinal applications of pyrimidines

The presence of pyrimidine base in thymine, cytosine, and uracil, which are the essential building blocks of nucleic acids DNA and RNA, is one possible reason for their widespread therapeutic applications. The pyrimidines represent one of the most active classes of compounds possessing wide spectrum of biological activities like significant *in vitro* activity against unrelated DNA and RNA, viruses including polio herpes viruses, diuretic, anti-tumour, anti-HIV, and cardiovascular. The literature survey indicated that a wide range of pharmacological activities are exhibited by the compounds encompassing pyrimidines nucleus. In addition to this, various analogues of pyrimidines have been found to possess anti-bacterial, anti-fungal, anti-leishmanial, anti-inflammatory, analgesic, anti-hypertensive, anti-pyretic, anti-viral, anti-diabetic, anti-allergic, anti-convulsant, anti-oxidant, anti-histaminic, herbicidal, and anti-cancer activities and many of pyrimidine derivatives are reported to possess potential central nervous system (CNS) depressant properties and also act as calcium channel blockers.

Clinical and pharmacological applications of pyrimidine in the world: marketed drugs

The top selling active pharmaceutical ingredients comprise of pyrimidine nucleus are, flucytosine (5-FC), floxuridine, lopinavir, lamivudine, zidovudine, pyrimethamine and minoxidil. Recently, the US-FDA approved some pyrimidine and pyrimidinone derivatives (ibrutinib, capecitabine, folinic acid and monastrol) as anti-cancer agents (Figure 5). These pyrimidines and their scaffolds exhibit a broad spectrum of bioactivity; hence they occupy privileged positions in drug discovery studies. Lathyrine (tingitanine) containing a pyrimidine ring, which can be isolated from the seeds of *Lathyrus tingotanus*. Variolin B is an example of pyrimidine-based alkaloid that shows inhibiting cell growth and anti-viral activity (Figure 6).

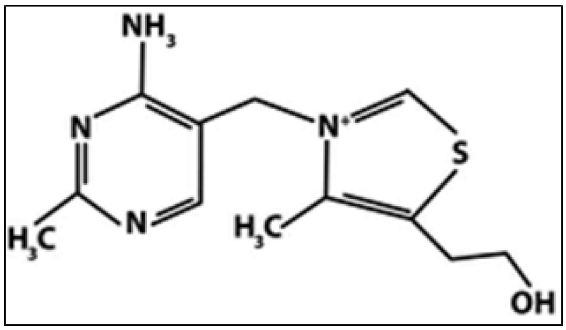


Fig 4: Chemical structure of thiamine

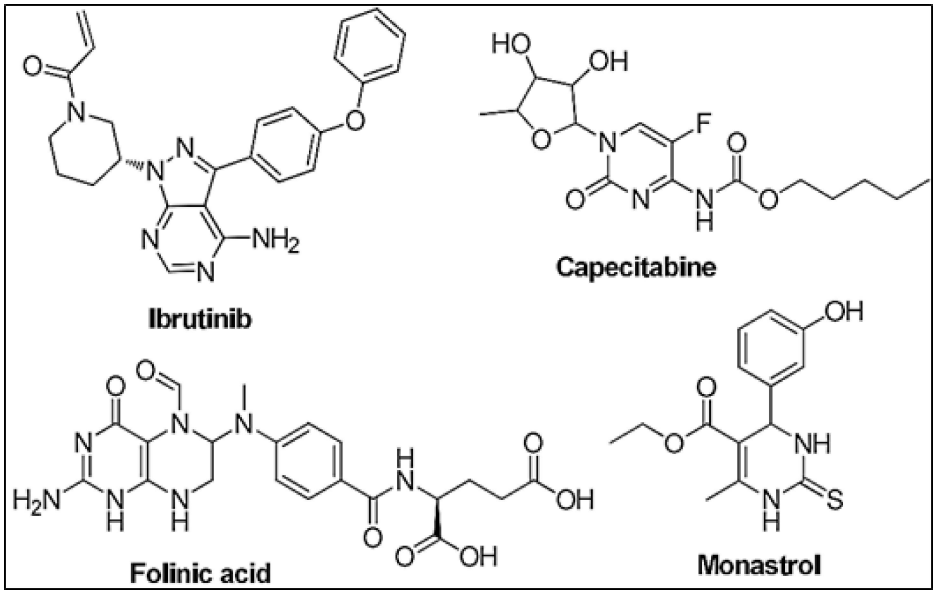


Fig 5: Pyrimidine clinical drugs

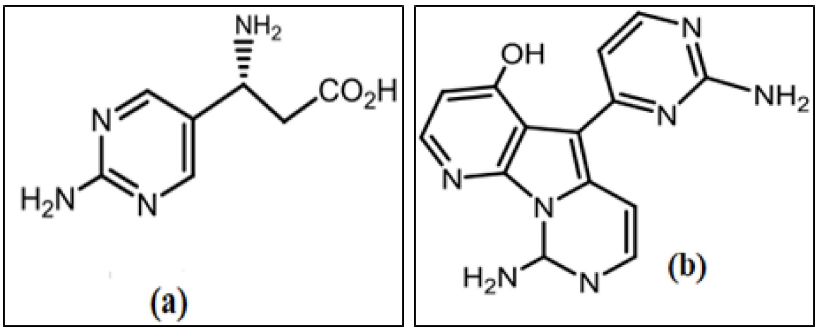


Fig 6: Naturally occurring pyrimidine compounds, structures of Lathyrine (a) and Variolin B (b)

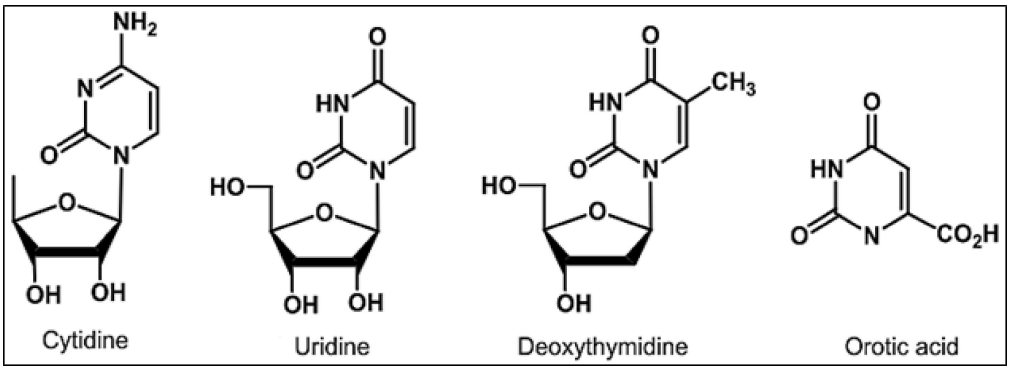


Fig 7: Pyrimidine based nucleosides

Combining thienopyridine and pyrimidine cores in the same molecular architecture, forming a pyridothienopyrimidine nucleus, serves as an attractive strategy for designing a novel scaffold with more favourable pharmacological effects. Recently, various pyridothienopyrimidine derivatives were reported to produce significant anti-microbial and anti-cancer activities, as well as to suppress protein kinases such as serine/threonine kinase and vascular endothelial growth factor receptor (VEGFR-2).

Brodiprim (1) is found to be an effective anti-bacterial compound. Iclaprim (2) which is a new selective dihydrofolate inhibitor was synthesized based on rational drug design and this drug is found to be active against methicillin-, TMP-, and vancomycin-resistant strains. Trimethoprim (3) is an anti-bacterial drug which selectively inhibits bacterial dihydrofolate reductase (DHFR). Pyrimethamine (4) is a selective inhibitor of the dihydrofolate reductase (DHFR) of malarial plasmodia (Figure 8).

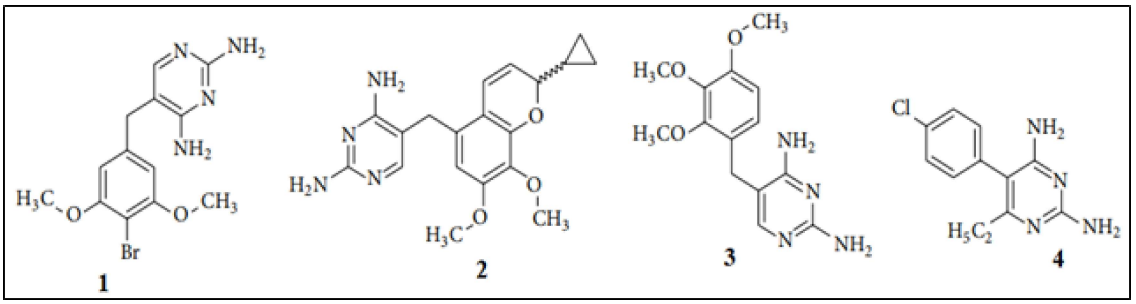


Fig 8: Anti-bacterial drugs containing substituted pyrimidines

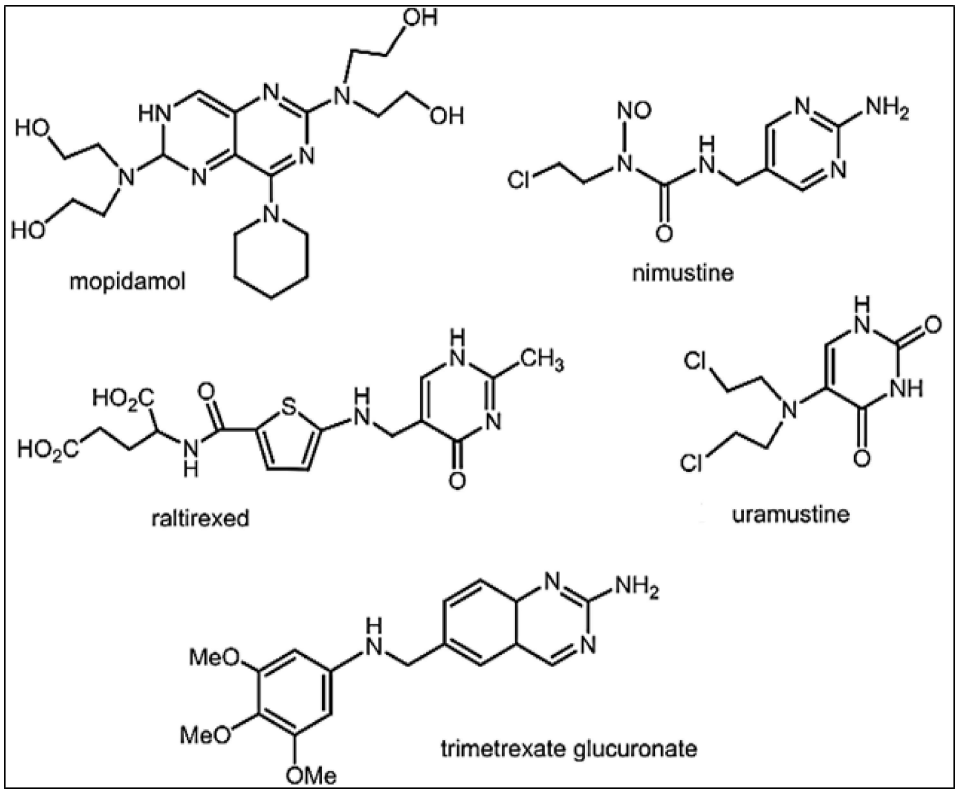


Fig 9: Anti-cancer drugs

Capreomycin produced by *Strepromyces capreolus* is a second-line bacteriostatic anti-tuberculin drug containing pyrimidine backbone (Figure 10).

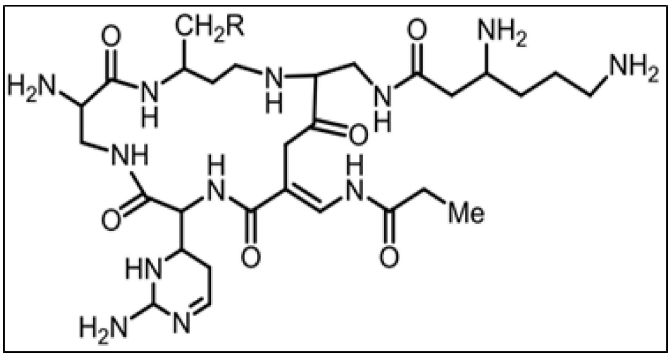


Fig 10: Molecular structure of capreomycin

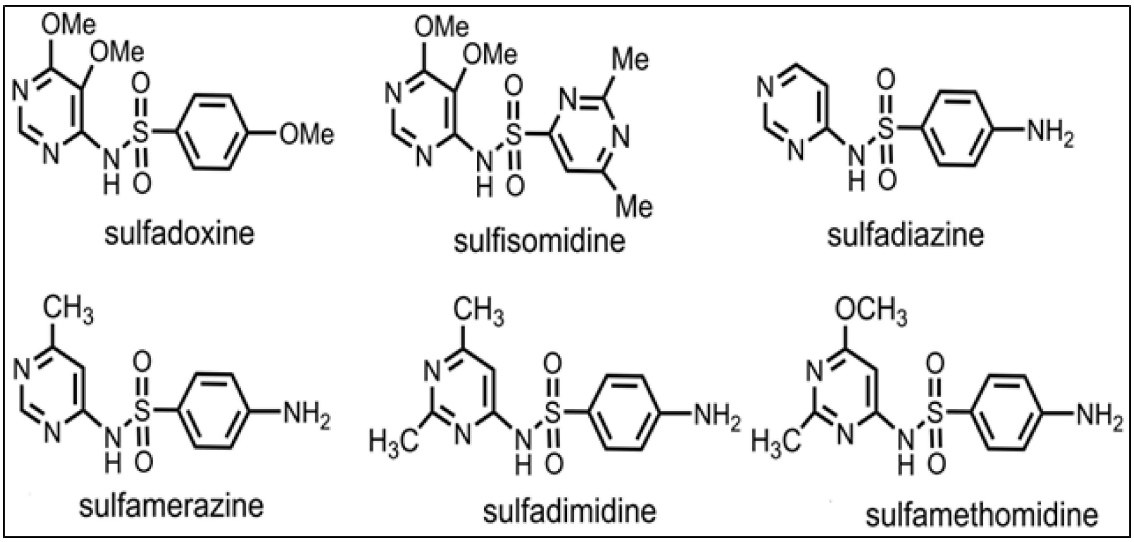
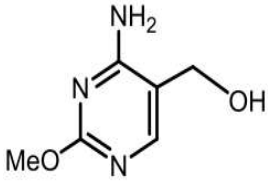
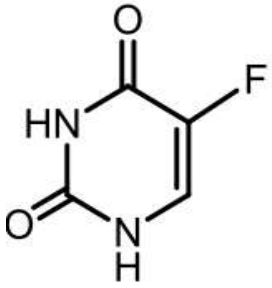
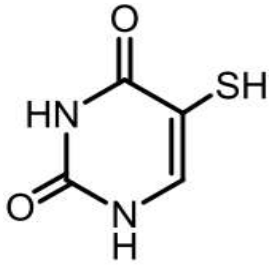
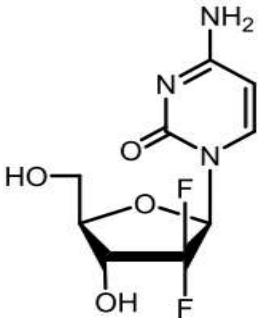
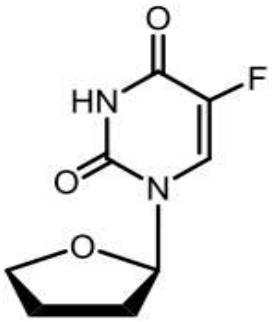
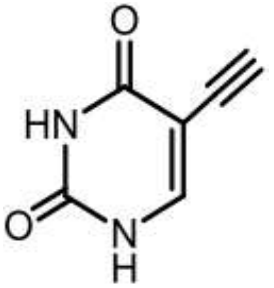
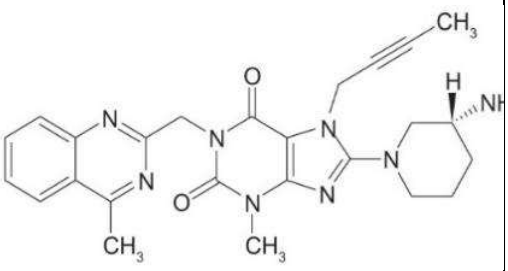
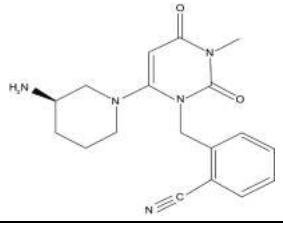
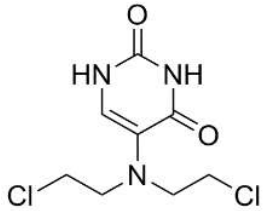
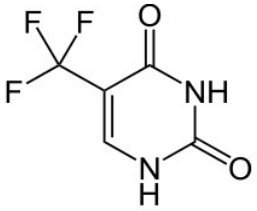
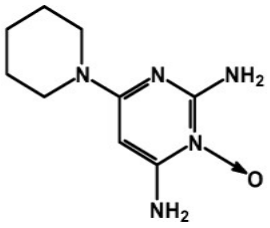
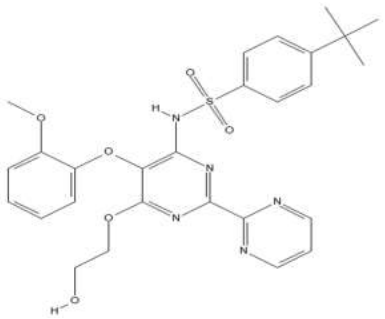


Fig 11: Sulfadrugs carrying pyrimidine skeleton

Table 2: List of pyrimidine containing medicinally active drugs

S. No.	Structure	Drug Name	Activity
1.		Bacimethrine	Anti-biotic
2		5- fluorouracil	Anti-cancer

3		5-Thiouracil	Anti-cancer
4		Gemcitabine	Anti-tumour
5		Tegafur	Anti-tumour
6		Eniluracil	Anti-tumour
7		Linagliptin	Anti-diabetic
8		Aglogliptin	Anti-diabetic

9		Uramustine	Anti-cancer
10		Triflurdine	Anti-viral
11		Minoxidil	Anti-hypertensive
12		Bosentan	Endothelin receptor antagonist used in the treatment of pulmonary artery hypertension

An interesting new agent for the treatment of chronic leukemia is the tyrosine kinase inhibitor imatinib mesylate (Gleevec), which contains a 4-pyridyl-substituted pyrimidine-2-amine structure as the aromatic heterocyclic element (Figure 12).

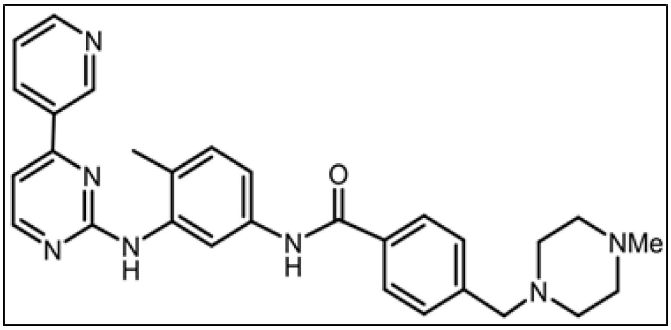


Fig 12: Chemical structure of gleevec

2-Thiouracil compound and its alkyl derivatives, methylthiouracil, propylthiouracil are effective drugs against hyperthyroidism (Figure 13).

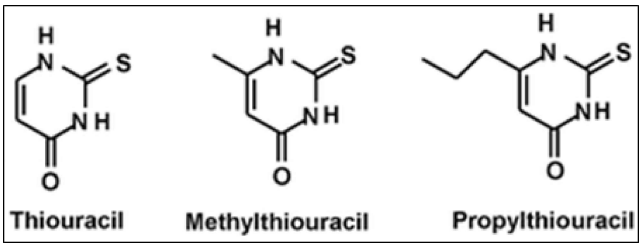


Fig 13: Anti-thyroid drugs

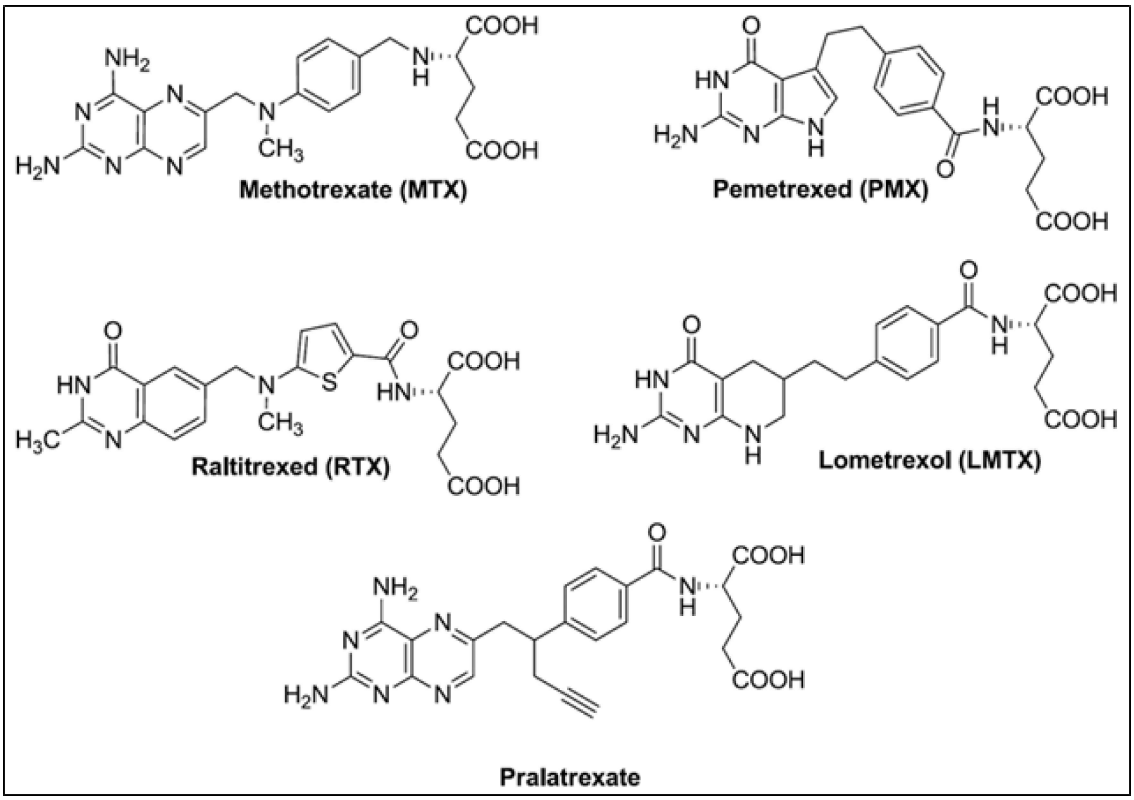


Fig 14: Anti-cancer agents

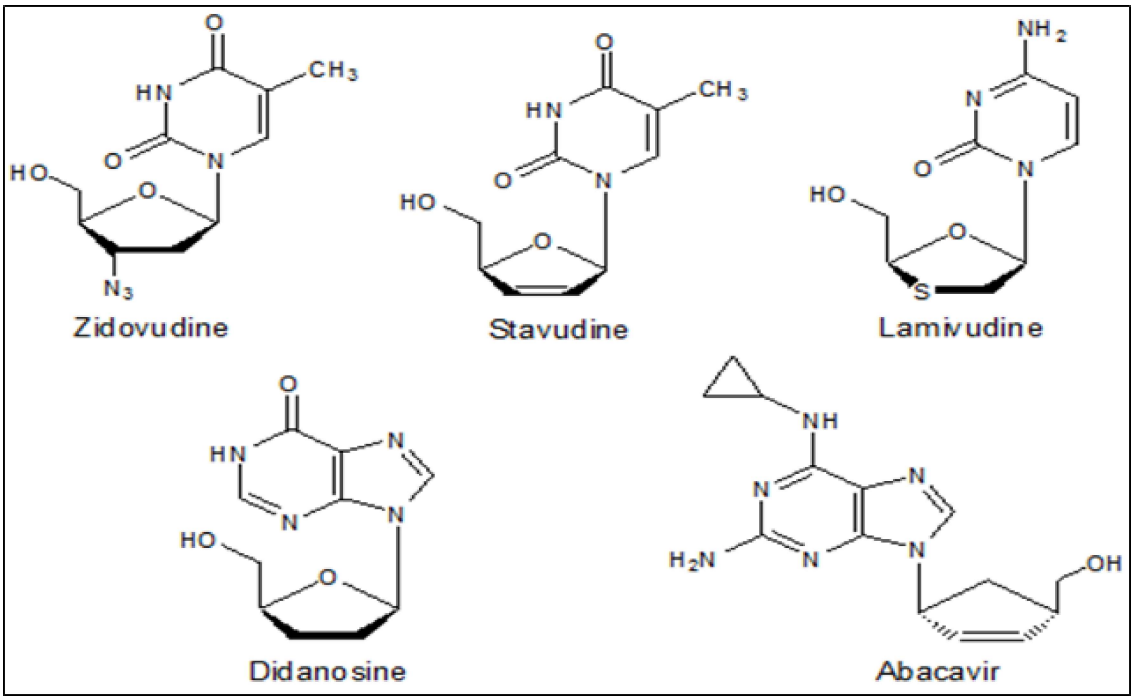


Fig 15: Anti-viral agents

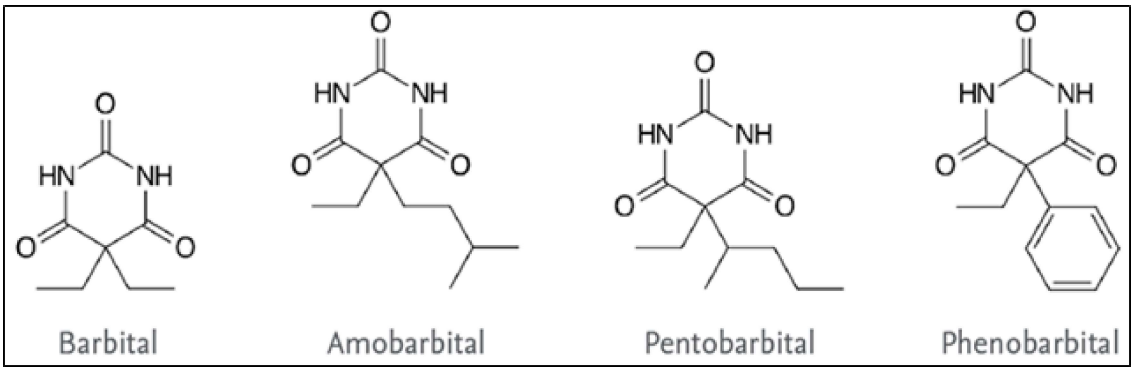


Fig 16: Hypnotic and sedatives and anti-convulsants

Importance to Life and Industry

Many heterocyclic compounds are biosynthesized by plants and animals and are biologically active. Over millions of years these organisms have been under intense evolutionary pressure, and their metabolites may be used to advantage; for example, as toxins to ward off predators, or as colouring agents to attract mates or pollinating insects. Some heterocycles are fundamental to life, such as haem derivatives in blood and the chlorophylls 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.

Nitrogen heterocycles are important structural subunits that occur widely in bioactive natural products, pharmaceuticals, agrochemicals, dyes, cosmetics, and functional materials. Considering the importance of these useful compounds in modern science, the synthesis of N-heterocycles and their derivatives has always been a hot topic in organic synthesis.

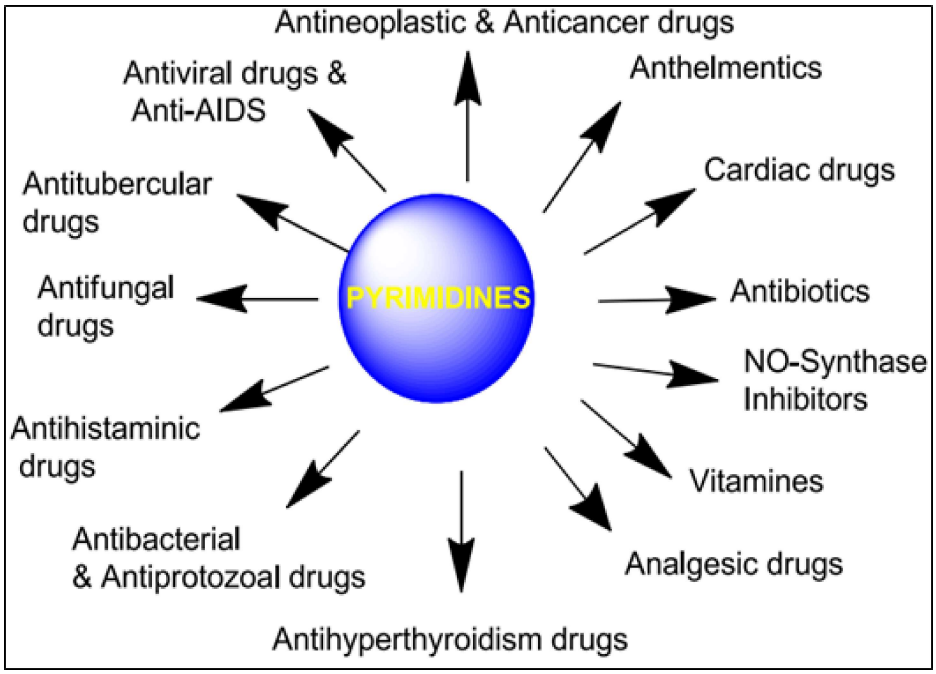


Fig 17: Medicinal significance of pyrimidines

Conclusion

The scope of nitrogen-based compounds in medicine is growing daily and their diverse analogues provide a viable and important path for the discover of drugs with various biological applications. The N-heterocyclic frameworks offer a high degree of structural diversity that has proven useful for the search of new therapeutic agents in improving the pharmacokinetics and other physicochemical features. Numerous drugs that are currently in clinical practice have fatal side-effects and have developed multidrug resistance, and have been extensively used in practice to treat various types of diseases with high therapeutic potency. Research and development of nitrogen-based compounds in medicinal chemistry has become a rapidly developing and increasingly active topic. A large amount of work has been made towards N-heterocyclic skeleton medicinal chemistry. The overwhelming advantages of nitrogen-containing drugs in the medicinal field, including easy preparation, low toxicity, less adverse effects, high bioavailability, lower drug resistance, good biocompatibility, etc., encourage efforts towards further research and development. Hence, the properties of these scaffolds are vital to the synthetic strategy in the current drug discovery and design system. These significant points confirm the enormous potential of various N-heterocyclic cores in pharmaceutical applications suggesting a massive scope for these promising moieties because of their diverse molecular targets. We believe that this review will be valuable for encouraging the structural design and development of sustainable and effective nitrogen-based drugs against various diseases, with minimal side-effects.

Nitrogen containing six membered heterocyclic compounds have numerous applications in the pharmaceutical field which are pharmacologically and physiologically active and it is used in the treatment of various diseases. On the basis of various literature surveys these derivatives show various activities like anti-microbial, anti-inflammatory, analgesic, anti-cancer, anti-depressant, anti-viral, anti-tubercular and anti-fungal. This chapter reviewed some of the biological activities of these compounds. The possible improvements in the activity can be further achieved by slight modifications in the substituents on the basic nucleus of these compounds. Thus, has been long focused for research interest in the field of medicine, due to excellent activities exhibited by its derivatives.

Heterocyclic chemistry is an extensive subject of feverish action and can be seen in several research papers in known journals, as well as in the abundance of monographs and studies of different topics including photochemistry, pharmacology, and the industry, to name a few. This increase in awareness and implementation presents pedagogical difficulties; only an organic chemist is challenging to understand the advancement of many of its essential fields. But the heterocycle region gives an organic chemist the ability to synthesize new molecules, whose structural activity relationships will include applications in the medical sciences and so on.

This review is endeavouring to find potential future directions in the development of more potent and specific analogues of nitrogen containing compounds for the biological target. The information illustrated in this review also encourage organic chemist for the design of novel molecules to identify many more biologically active heterocycles for the benefit of humanity.

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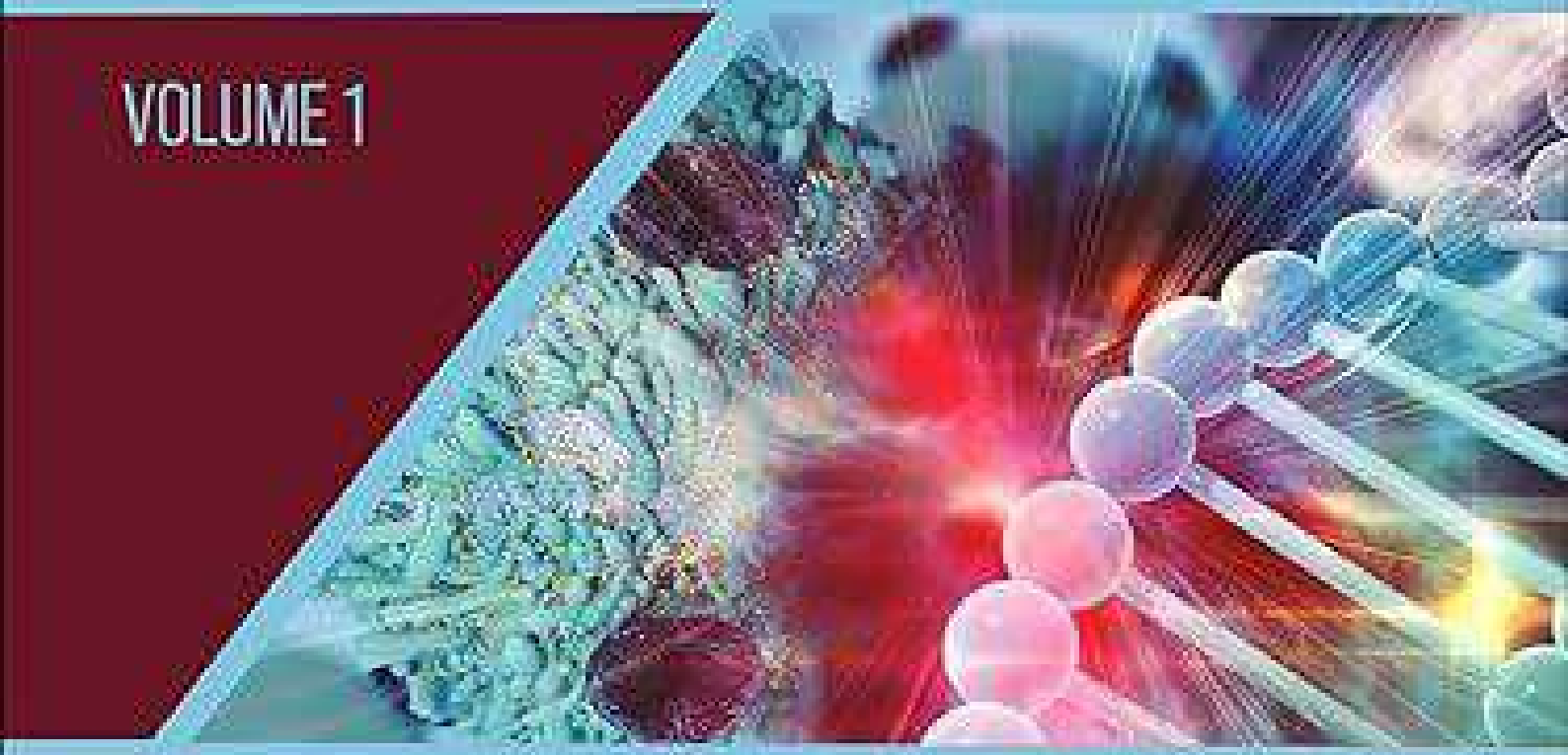

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CHAPTER

13

Telomerase in hepatocellular carcinoma

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Abstract

Hepatocellular carcinoma (HCC) is a very widely prevalent and deadliest cancer worldwide. HCC in pathogenesis, histopathology, and biological behavior is renowned for its great diversity, wherein dysregulated signaling pathways and different genetic mutations are commonly encountered. In the course of hepatocarcinogenesis, reactivation of telomerase is necessary for uncontrolled cell multiplication, leading to malignancy and HCC. During the development and progression of HCC, telomerase reactivation plays an important role and could serve as a biomarker to diagnose HCC, for prognostication and also a promising biological target. The essential catalytic component of telomerase complex, telomerase reverse transcriptase (TERT), and various telomerase reactivation mechanisms are reported in HCC which are in accordance with the previous research findings on other types of cancer. C228T and C250T are the two prime mutation hotspots observed in the region of TERT promoter. In addition, in hepatitis B virus (HBV)-related HCC cases involving integration of HBV-DNA there is a constant interruption of TERT promoter. TERT promoter mutations are believed to occur preliminarily during the carcinogenesis of HCC and remarkably contribute to HCC progression. In the present chapter, we have summarized the evidence about the somatic mutations of TERT promoter region alongside discussing the critical role of such mutations in the development of HCC and their robust utility as biological markers and targets in clinic.

Keywords: Hepatocellular carcinoma; telomerase; mutations; diagnosis; reverse transcriptase

Abbreviations

ACV-TP-T	acycloguanosyl-5′-thymidyltriphosphate
AFP	alpha-fetoprotein
CCNE1	cyclin E1
CNS	central nervous system
ctDNA	circulatory tumor DNA

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CTNNB1	catenin beta-1
DDR	DNA damage response
HBV	hepatitis B virus
HCA	hepatocellular adenoma
HCC	hepatocellular carcinoma
HCV	hepatitis C virus
iCCA	cholangiocarcinoma
ISTRAP	in situ TRAP
LGDN	low-grade dysplastic nodules
MLL4	mixed-lineage leukemia 4
mRNA	messenger RNA
NAFLD	nonalcoholic fatty liver disease
NIDDM	noninsulin-dependent diabetes mellitus
TERC	telomerase RNA component
TERT	telomerase reverse transcriptase
TPM	TERT promoter mutations
TPR	TERT promoter region
TRAP	telomeric repeat amplification protocol
ACV-TP-T	acycloguanosyl-5′-thymidyl triphosphate

Introduction

Liver cancer (LC) is well-known one diagnosed globally with a high mortality rate. It displays huge diversity in its etiology and diagnosis. Hence, the accurate prognosis of LC patients is very difficult. LC is classified into two types: hepatocellular carcinoma (HCC) and cholangiocarcinoma (iCCA) [1]. Among all the cases of LC, HCC accounts for 75% and iCCA accounts for nearly 20%. The occurrence rate of LC is more in East Asian men (above 75 years) than in females. LCs are often diagnosed in advanced stages due to several human and technical problems, thus reducing the survival rate. The therapeutic options for LC are very limited despite multiple studies. Drugs inhibiting multiple kinases like sorafenib and lenvatinib are used for first-line treatment, while regorafenib and cabozantinib constitute second-line treatment choice for patient's adversity by LC [2–4]. Recently, US FDA has approved the usage of a combination containing atezolizumab and bevacizumab for unresectable patients or metastatic HCC who have not undergone systemic therapy [5].

LC has several risk factors, major being chronic hepatitis B and hepatitis C viral infections, abuse of alcohol and tobacco, noninsulin-dependent diabetes mellitus (NIDDM), prolonged aflatoxin B1 exposure, obesity, and gender [6]. Most LCs arise due to preexisting chronic liver diseases like cirrhosis, fibrosis, hepatitis, and nonalcoholic steatohepatitis. In the United States and other Western countries, HCC is believed to be mainly because of hepatitis C virus (HCV) [7,8], unlike in Asia and other developing countries where hepatitis B virus (HBV) infections are quite common and held responsible for the majority of the HCC cases. On the contrary, HCC is sometimes observed in normal healthy liver devoid of any disease or liver with mild fibrosis [9]. However, in modern states of East Asia like Japan, HCC is related to HCV infection which is most prevalent [10]. There is an exponential rise in HCC cases in low-risk areas due to higher prevalence of obesity, NIDDM, and nonalcoholic fatty liver disease (NAFLD), thus increasing the global burden of HCC [11]. In view of the higher heterogeneity reported in liver tumors due to diverse risk factors, for

developing individualized therapies, molecular targets have to be identified. Based on some such studies, the various subtypes of HCC and iCCA have been previously reported [12,13]. Specific treatment options for different tumor subtypes can be derived from personalized studies, which enables significant improvement of the overall survival rate of LC patients [14–16]. Exploring and understanding the dysregulated molecular pathways in precancerous tissue help to detect HCC, quite early [17]. To achieve targeted therapy, greater insights on various molecular targets involved in different types of LC are highly beneficial.

Telomeres are the DNA sequences present at the chromosomal ends. They are composed of the repetitive TTAGGG nucleotide sequences and offer protection to the chromosomes from deterioration or damage. The telomeric sequences are shortened constantly during each cell division. Upon consecutive cell divisions, telomeres attain a critical size wherein cells stop division and become senescent [18]. In the somatic cells of humans, telomeres serve as a clock for mitosis; the decrease in their length, in turn, reduces cell division and, thus controls their division cycles [19,20]. Telomeric loss is slower in normal cells as compared to neoplastic cells where it occurs rapidly leading to instability of genes making such genes imperishable. Dysfunctioning of telomeres causes disturbances in mitotic cell division which further results in the rearrangement of chromosomes via a sequence of events like formation of anaphase bridges, multipolar mitosis, and genomic imbalance [21].

Telomerase enzyme with reverse transcriptase activity culpable for the synthesis of telomeric TTAGGG sequences from RNA template [18]. It is highly active during embryonic development and during the regeneration of organs like liver but cannot be observed in adult cells except for the gut and bone marrow stem cells where it helps in their renewal periodically [22]. Telomerase is a 670-kDa ribonucleoprotein complex consisting of a catalytic unit TERT (telomerase reverse transcriptase), a template TERC (telomerase RNA component), and dyskerin protein which serve to stabilize the telomerase complex. Among them, TERT plays a crucial part in telomerase activity [23–25]. DNA sequences are shortened when the activity of telomerase is impaired.

In general, telomeric length is stabilized depending on the rate at which nucleotides are lost at each cycle and the rate at which elongation of telomeres occurs due to enhanced telomerase activity. It is believed that cancer probability can be reduced in normal cells by suppressing telomerase. For instance, in majority of the human somatic cells, TERC is highly expressed, while TERT is suppressed and, thus limits the activity of telomerase. If TERT is overexpressed, most of the human cells get immortalized [23,26]. Nevertheless, maintaining a balance between the telomeric length and telomerase activity is of immense importance for tumor development in view of the fact that the telomeric length in many of the cancer cells is short and stable due to telomerase reactivation [19]. Hitherto there are very few reports on how telomerase is regulated in the pathophysiology of gut, pancreas, or liver. Hence, in this chapter an attempt is made to summarize the existing data on the role of the activity of telomerase in HCC and its relationship with cancer progression.

Telomerase and hepatocellular carcinoma

Downregulation of telomerase activity is observed in liver at early stages of embryogenesis, while in adult liver its activity is absent. Most of the hepatocytes are in inactive stage

(G_0 phase) in the healthy liver in which proliferation is weak, wherein only a minute fraction of cells are involved in cell cycle [27]. Hence, it can be assumed that in a healthy liver, the function of hepatocytes is independent of telomerase activity. But in injurious and chronic diseased conditions of liver, telomerase activity is insufficient in hepatocytes causing the shortening of telomeres [19]. It is very important to note that in adult hepatocytes and cholangiocytes, lowered or no telomerase activity is observed during regenerative process which clearly indicates that telomerase is potentially activated under physiological conditions [28,29]. Telomerase activity, telomere maintenance, and other factors associated with LC are summarized in the Fig. 13.1, [30].

Till date, two different underlying mechanisms were suggested to explain the shortening of telomere as a result of elevated signals of proliferation (due to the activation of oncogene or oncogenic protein expression in virus). They are (1) inadequate reactivation

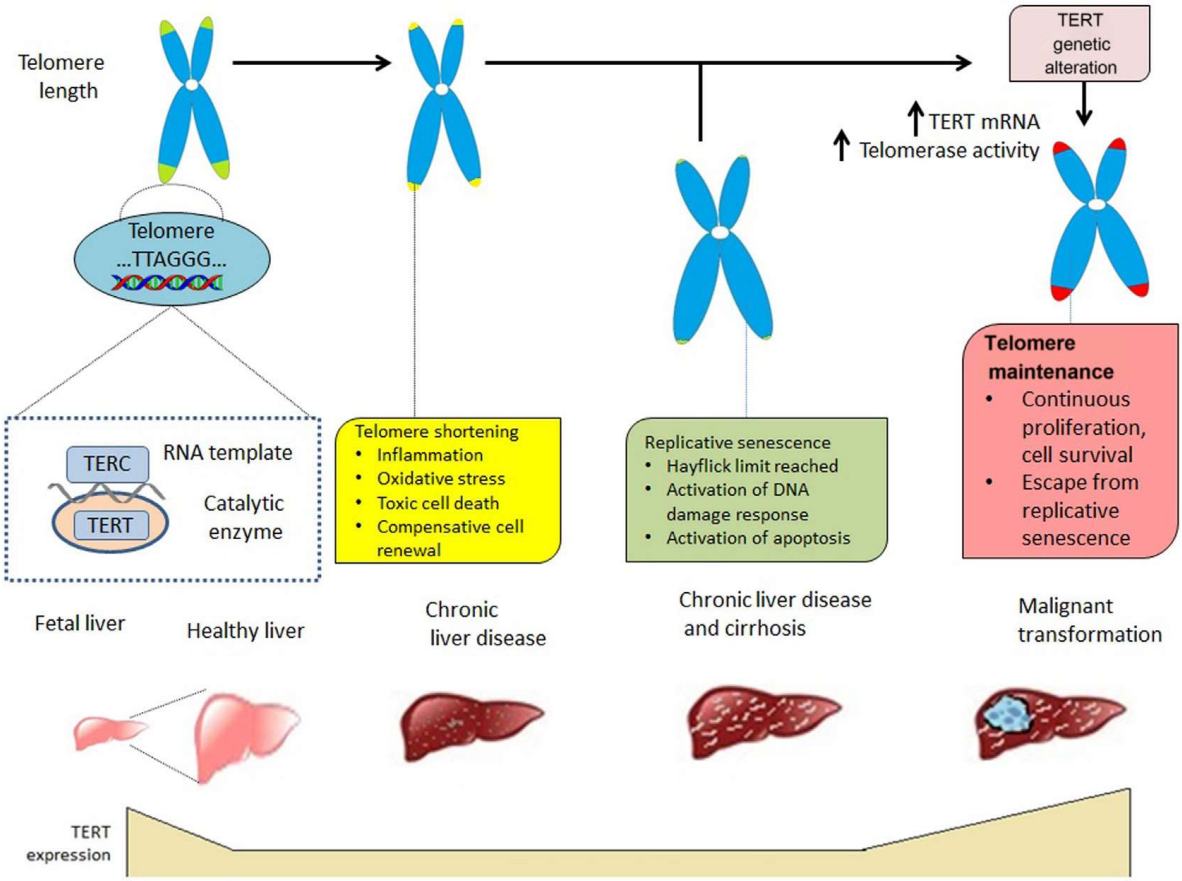

FIGURE 13.1 Expression of telomerase and maintenance of the telomere in the liver. Liver carcinogenesis is enabled in the context of cirrhosis by the reappearance of telomerase activity triggered through genetic modifications influencing TERT. HCC is characterized by evacuation from replicative senescence, with telomere conservation supporting tumor growth. *HCC*, Hepatocellular carcinoma; *TERT*, telomerase reverse transcriptase. Source: *Adapted from Nault J.C., et al. The role of telomeres and telomerase in cirrhosis and liver cancer. Nat Rev Gastroenterol Hepatol 2019;16(9):544–58.*

of telomerase, majorly [31,32] and (2) impaired telomerase activity in proliferating liver cells due to mutations, minorly [33,34]. Further, it is said that the activation of telomerase is a rate-determining step for the formation of LC. This is evident from the fact that in majority of HCCs, telomerase is reactivated [19,35,36]. Telomerase reactivation occurs when the two basic components of telomerase (TERT, TERC) are upregulated. Earlier studies revealed the occurrence of TERT reexpression and telomerase activation during the initial stages of premalignancy in cirrhotic nodules [30,37,38]. Notably, an equal rise of greater than 80% was observed in telomerase activity in the two major types of LC (HCC, iCCA) [35,39]. Hence, it is quite important to highlight that both these LCs show a similar telomerase response indicating the significance of telomerase activity for the functioning of telomeres and progression of tumors. Inadequate telomerase activity can accelerate shortening of telomere in proliferating hepatocytes, which causes instability of genome by breakage–fusion–bridge mechanism [40]. Senescence or apoptosis results from telomere shortening in cells containing intact DDR (DNA damage response) checkpoints, thus providing a mechanism for tumor suppression. Contrarily in cells devoid of functional DDR, shortening of telomeres can promote instability of genome and formation of tumor.

Several malignancies have telomerase reverse transcriptase promoter mutations

TERT promoter mutations (TPM) are often noticed mutations of somatic cells in many malignancies. There has been widespread speculation that TPM recur in two main hotspots C228T and C250T on chromosome 5. They are located at basepairs −124 and −146, respectively, upstream from ATG codon in TERT gene [41–44]. In a systematic investigation, including 1581 malignant cases of various kinds, TPM were observed in 27.0% [42]. Killela et al. [45] investigated 1230 tumor samples from 60 various kinds and found 231 TPM (18.8% of the total), with C228T and C250T mutations accounting for 98.3% of the total. Likewise, 327 (21.6%) of 1515 central nervous system (CNS) tumors tested positive for TPM, with all but two containing either C228T or C250T [46]. C228T mutations are more common than C250T mutations, according to statistics (Table 13.1) in multiple cancers, covering various subtypes of CNS, urogenital, melanoma, and thyroid cancers [42–54]. Glioma, melanoma, bladder cancer, and HCC are among the most frequent cancers impacted by TPM, according to most experts [42,45,55].

HCC TERT promoter point mutations

TPM were detected in approximately 30%–60% of case studied, according to a few significant HCC publications. The two most frequent alterations in HCC were C228T and C250T, which were similar to results in many other cancer types, with the former being more common than the latter (Table 13.2) [56–59,61–67]. There have been no instances with the C228T and C250T mutations, as reported in Table 13.2, suggesting that such two hot spot genetic changes are completely incompatible. Moreover, a thorough evaluation of TPM distribution in 1939 HCC cases from four continents revealed that TPM have been almost ubiquitously prevalent, with marginally greater mutation rates of 56.6% in Europe, 53.3% in Africa, 40% in America, and 42.5% in Asia, and that the C228T mutation was

TABLE 13.1 Telomerase reverse transcriptase promoter mutations in various cancer types: a statistical summary.

S. no	Type of cancer	Cancer cases	Mutations of TERT (%)	Various kinds of TERT promoter mutations (%) C228T, C250T, C228T,or C250T			Methods	Reference
1	Glioma, medulloblastoma, hepatocellular, etc.	1230	18.8	77.5	20.8	98.3	PCR/Sanger sequencing	[45]
2	Bladder, liver, glioma, etc.	1581	26.9	–	–	–	Whole-genome/lowpass whole-genome sequencing	[42]
3	CNS	1515	21.6	78.6	20.8	99.4	PCR/bidirectional sequencing	[46]
4	CNS, bladder, thyroid, etc.	741	19.2	69.6	30.3	98.6	PCR/Sanger sequencing	[47]
5	Urogenital	302	43.0	76.9	18.5	96.4	PCR/Sanger sequencing	[48]
6	Medulloblastoma	466	21.0	–	–	–	PCR/Sanger sequencing	[49]
7	Melanoma	287	38.0	46.8	36.7	91 (83.5)	PCR/Sanger sequencing	[50]
8	Bladder cancer	262	83.2	75.7	14.7	90.4	SNaPshot assay and Sanger sequencing	[51]
9	Melanoma	77	31.2	29.2	20.8	50.0	High-throughput sequencing/Sanger sequencing	[52]
	Cancer cell line							
1	Melanoma	168	74.4	36.8	51.2	88	High-throughput sequencing/Sanger sequencing	[52]
2	Melanoma, liver, bladder cancers, etc.	150	36.0	–	–	100	Whole-genome sequencing, Sanger sequencing	[43]
3	Urothelial bladder	23	87.0	80.0	10.0	90.0	PCR/Sanger sequencing	[53]
4	Urothelial bladder	32	87.5	89.3	10.7	100	PCR/Sanger sequencing	[54]

CNS, Central nervous system; *PCR*, polymerase chain reaction; *SNaPshot*, single-base primer extension assay.

TABLE 13.2 Telomerase reverse transcriptase promoter mutations in hepatocellular carcinoma.

S. no	No. of HCC cases	No. of TERT mutations (%)	No. of different types of TERT promoter mutations (%) C228T, C250T, C228T, or C250T			Methods	Reference
1	469	254 (54.2)	236 (92.9)	11 (4.3)	247 (97.2)	PCR/Bidirectional sequencing	[56]
2	316	103 (32.6)	96 (93.2)	5 (4.9)	101 (98.1)	PCR/Sanger sequencing	[57]
3	305	179 (58.7)	166 (92.7)	11 (6.1)	177 (98.9)	PCR/Sanger sequencing	[58]
4	276	85 (30.8)	84 (98.8)	1 (1.2)	85 (100)	PCR/Sanger sequencing	[59]
5	196	87 (44.4)	–	–	–	Whole-genome sequencing	[60]
6	195	57 (29.5)	54 (94.7)	3 (5.3)	57 (100)	PCR/Sanger sequencing	[61]
7	160	46 (28.8)	32 (69.6)	14 (30.4)	46 (100)	PCR/Sanger sequencing	[62]
8	44	15 (34.1)	10 (66)	5 (34)	15 (100)	PCR/Sanger Sequencing	[63]
9	190	57 (30.0)	50 (87.7)	7 (12.3)	57 (100)	PCR/Bidirectional sequencing	[64]
10	127	64 (50.4)	62 (96.9)	2 (3.2)	64 (100)	PCR/Sanger sequencing	[65]
11	123	45 (36.6)	43 (95.6)	2 (4.4)	45 (100)	PCR/Sanger sequencing	[66]
12	125	85 (68.0)	–	–	–	PCR/Sanger sequencing	[67]

PCR, Polymerase chain reaction.

universal [68]. Apart from the greater incidence of TPM in HCC, additional point of important information suggested by multiple lines of research is that TPM is influenced by a number of variables, such as the patient's viral status, gender, age, and size of tumor. TPM are highly witnessed in patients suffering from HCC and HCV coinfection [56,58,60,62,65,66,69,70] compared with HBV-infected patients. TPM rates were shown to be greater in men [58,62,69], in older patients [69,70], in patients who smoked [71], and in patients having small tumors in many investigations, in patients with lowered alpha-fetoprotein (AFP) serum levels [58], and in patients harboring catenin beta-1 (CTNNB1) mutations [56,58,65]. TPM are initial somatic genetic polymorphisms in hepatocarcinogenesis, and they play a vital part in the malignant development of preneoplastic cirrhotic lesions [58,72]. TPM frequency rose when premalignant lesions converted into HCC, according to Nault et al. [73], from dysplastic nodules of various grades (25%) to early

HCC (61%) and small and advanced HCC (42%); mutations in 10 other recurring genes only appeared in small and advanced HCC. Calderaro et al. [74] discovered that TPM were present in 64.6% of patients (208/322); HCC symptoms were closely linked to gene alterations, particularly TPM, and transcriptome categorization. There have been research examining TPM-linked HCC due to a rise in NAFLD-related HCC patients because of escalating incidence of metabolic syndrome, notably in Western nations [75–77]. TPM C228T was identified in 9/11 (82%) of malignant tumors from 10 NAFLD-HCC patients, according to one study [77]. On the other hand, in another research, individuals with NAFLD had a very low TPM C228T and C250T incidence (3.2%) [78].

Telomerase reverse transcriptase promoter insertional mutations

HBV infection has been linked to the development of HCC, particularly in Asians, where chronic hepatitis B infection is common. In HBV-related HCC, HBV-DNA insertion in the genome of human HCC cells is seen [56,60,63,79–85]. Incorporation of specific genomic locations such as those near or within TERT genes [56,60,80–86], mixed-lineage leukemia 4 (MLL4) [60,80,82–84,86], and cyclin E1 (CCNE1) [60,82–84,86] are more often recognized in HCC [60]. Till now, 262 different HBV-DNA integrations into TERT gene have been detected from 13 separate studies [69,79,86–96]. MLL4 (79 integrations), CCNE1 (22 integrations), and cyclin A2 are the genes most vulnerable to HBV incorporation (19 integrations) [97]. As per our pooled study of the literature cited [69,79,86–96], 73.28% (192/262) of the 262 HBV insertion in TERT happen in the TERT promoter region (TPR), with 26% occurring in the core functional segment (−223 bp to −14 bp before ATG codon). Because TERT expression is mainly controlled by the TPR, the incorporation of HBV with the TERT promoter might play an important part in HCC progression. HBV was shown to integrate at frequent chromosomal fragile spots, where DNA replication was slowed and DNA sequences were more vulnerable to breaking, according to a few studies [84,85]. The repeated occurrence of HBV-DNA incorporations into the TPR of host in HCC might be because of the advantage of extensive growth augmented TERT expression which leads to the expansion of clones and hepatocarcinogenesis [81,83]. HCCs with HBV-DNA incorporation into the TERT promoter have the greatest amount of TERT RNA expression in the TCGA database, implying that an HBV *cis*-activating event occurred [60]. HBV insertion enhances development and progression by causing global genomic instability, increasing expression of nearby genes, viral-host fusion transcripts, secondary mutations of host or viral genes, and also DNA copy number variations and carcinogenic proteins (X and preS gene products) [79,82,85,86]. Li et al. [90] suggested a new mechanism in which sex hormones, together with GA-binding protein alpha chain, play major part in regulating TERT expression, based on the observation of a higher frequency of HBV paired with TERT promoter somatic mutations in male patients with HCC. They analyzed 101 different HBV-linked HCC cases using hybrid-capture sequencing and came to the conclusion that the assimilation of HBV-DNA-containing hormone responsive elements (androgenic- and estrogenic-) into the TERT promoter allows the androgen receptor to upregulate and the estrogen receptor to downregulate TERT transcription via hepatocyte nuclear factor [83].

Telomerase in malignant transformation

Hepatocarcinogenesis associated with cirrhosis is a condition with multiple stages resulting as an outcome of the successive emergence of low-grade dysplastic nodules (LGDN) resembling regenerative nodule, high-grade dysplastic nodules (HGDN) resembling HCC, HCC (early and progressed), and portal vein tumor thrombosis to metastasis [98,99] (Fig. 13.2). Among the 96 nodules shown by 58 cirrhotic patients, the TERT promoter somatic mutations were observed in 6%, 19%, 61%, and 41% of LGDN, HGDN, early HCC, and small, established HCCs, respectively [9]. The previous observations indicate the involvement of TPM in initiation of tumor and its progress toward malignancy from cirrhosis, its role in tumor progression due to the occurrence of tumor protein 53, RP6SKA3, AT-rich interactive domain-containing protein 1A, and CTNNB1 gene mutations beyond small, established HCCs (Fig. 13.2). When dysplastic nodules exhibit TPM, TERT expression is increased as compared to dysplastic nodules devoid of TPM [9]. In

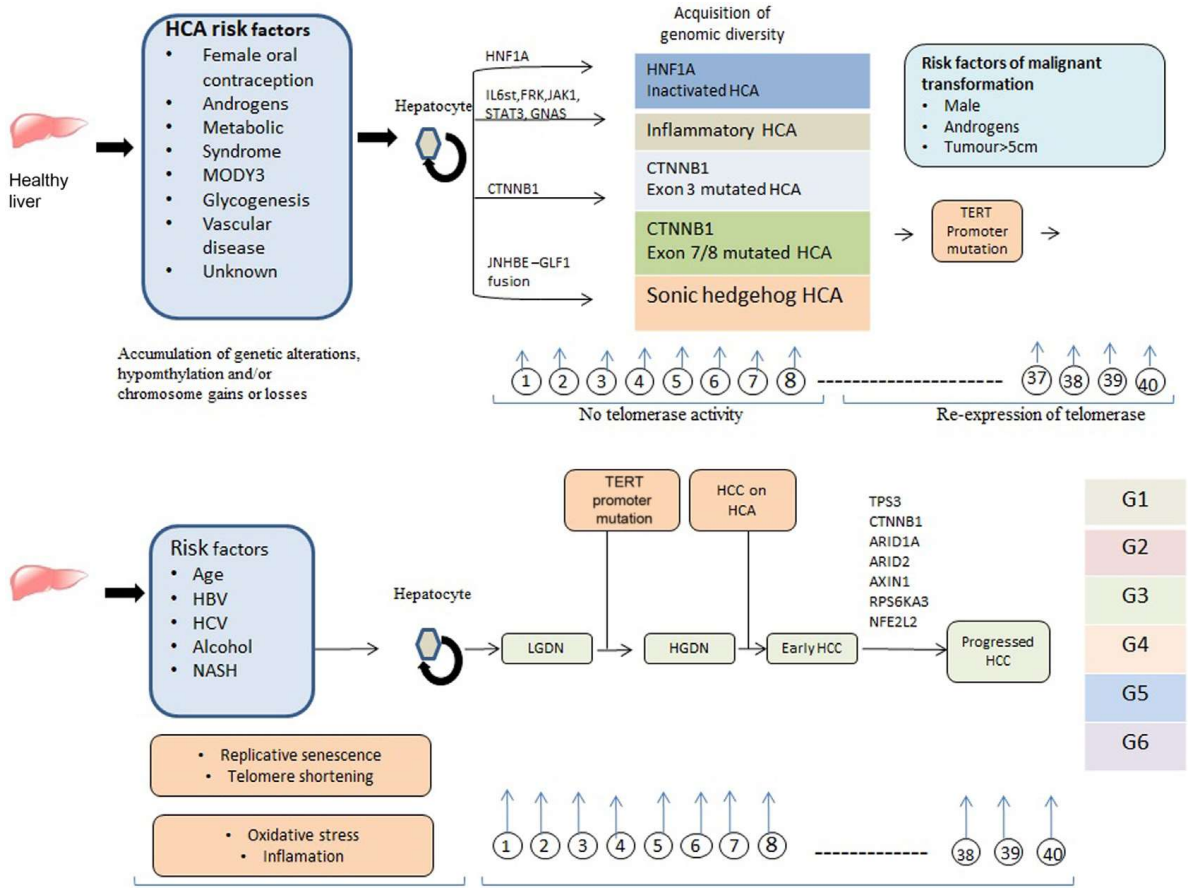

FIGURE 13.2 Factors leading to the reactivation of telomerase and its crucial role in carcinogenesis of the liver (A) and (B). The multistep HCA malignant transformation and carcinogenesis on a cirrhotic liver background are shown, respectively. *HCA*, Hepatocellular adenoma. Source: *Adapted from Nault J.C., et al. The role of telomeres and telomerase in cirrhosis and liver cancer. Nat Rev Gastroenterol Hepatol 2019;16(9):544–58.*

addition, if TPM are introduced into HCC cell lines of humans, TERT promoter activity is enhanced when compared with those without TPM [100]. It is, therefore inferred from this data that in human hepatocarcinogenesis, TPM increases the transcription of TERT. Certain HCCs develop in healthy liver when hepatocellular adenoma (HCA) undergoes malignant transformation [101,102]. HCAs occur seldom as benign liver tumors mostly in younger females using contraceptive pills. Sometimes, HCCs are formed by the degeneration of HCAs. HCAs have been categorized into several subtypes based on their molecular, morphological and clinical features, histopathology and immunohistochemistry, radiologic imaging modalities, etc. [101,103–109] (Fig. 13.2). Pathological conditions in which HCAs are associated with activating mutations in exon 3 of CTNNB1 possess the maximal risk of transforming into malignant liver tumors [101]. In classic HCAs, TPM are not identified. However, nearly half of the HCA/HCC borderline lesions and HCAs clearly indicating malignant transformation harbor TPM [105]. Further, different studies revealed the occurrence of TPM in tumor carcinoma region rather than in adenoma region unlike CTNNB1 mutations that occur in both the abovesaid regions. Hence, it can be said that HCAs are developed from CTNNB1 mutations that occur preliminarily, while TPM is most required for HCAs transformation into HCCs, of late (Fig. 13.2). In addition, it is exciting to note that TPM are also associated with the meningiomas [110] and thyroid adenomas during their malignant progression. In contrast, TPM are absent in benign lesions and can only be noticed in follicular cell-derived thyroid cancers like papillary thyroid cancers (5%–26%) and poorly differentiated thyroid cancers (21%–52%) [111].

Although TPM is vital for the malignant transformation of cirrhosis or HCA to HCC, a prominent difference is noticed in its acquisition time. In a cirrhotic patient, TPM occur as early carcinogenic event, whereas in HCA to HCC patients TPM occur toward the end of carcinogenesis following the initial CTNNB1 mutation [9,105]. Thus it explains the telomeric shortening and senescence phenomenon detected in the liver cells of patients suffering from cirrhosis, wherein the telomerase reactivation should occur rapidly in the early stages to avoid the senescence and to proliferate uncontrollably [23].

Diagnosis

TERT is a recognized primer for HCC advancement and its suppression has been shown to limit HCC progression, both in vivo and in vitro [112,113]. It has multiple pathways for activation: cmyc expression through its c-Myc/Max/Mad network binding [114] and TERT promoter binding [115]. Previous reports indicate that phosphatase and tensin homolog gene absence strongly corresponds to the better staining of HCC TERT, without their coexpression [116]. In addition, in HCC patients, genes, including RNA-binding protein fox-1 homolog 3 [117] or bromodomain PHD finger transcription factor, were associated with bad prognostic signs. Silencing of brain-derived neurotrophic factor gene is correlated to the rise of tumor apoptosis, thus exploring a possible biological target [118]. The telomeric repeat amplification protocol (TRAP) is highly sensitive to telomerase activity detection in tissue extracts. However, hybridization of the two vital telomerase components TERT mRNA and hTRin situ, has been indirectly attempted to better assess telomerase expression among the various types of cells and the concerned population, but

none of these are purely associated with the activity of telomerase: TERT mRNA is extensively expressed, while hTR undergoes alternative splicing and modifies posttranslationally presenting greater variability, despite being closely related. Unlike the conventional TRAP assay, the in situ TRAP assay (ISTRAP) enables to visualize the telomerase activity in tissue sections directly. Precisely 85% of the HCC checked were ISTRAP positive, many of which showed zonal distribution. There was a significant correlation between TRAP and ISTRAP techniques in direct comparison [38]. It has been established that the serum of breast cancer patient contains hTR as well as hTERT mRNA, indicating a potential role in its diagnosis. Few workers have attempted the usage of hTERT mRNA as a marker for HCC, as telomerase is reactivated in HCC [119]. With disease progression, there is a gradual increase in TERT positivity rate, HCC contributing its maximum with 63.6% specificity and 89.7% sensitivity. TERT expression was significantly and independently connected with AFP, size of the tumor and differentiated degree of HCC [120]. In early HCC diagnosis the advent of a new polymerase chain reaction assay enabled to detect TERT mRNA with high sensitivity and specificity. Particularly, it was found to be far superior to AFP. In addition, with HCC differentiation, mRNA expression was associated. The mRNA in sera was most significantly correlated with that in the HCC tissue [121]. Serum TERT mRNA has been screened for early diagnostic purposes in HCC patients. A correlation has been identified between tumor size, AFP, and TERT. Also when the tumor load is greater than 2 cm, a similar correlation is found between AFP and tumor [122]. The data observed, together with scientific advancement, enabled the implementation of TERT evaluation for clinical diagnosis and prognosis in HCC-affected patients. In such patients, TERT mutations can be identified by estimating the circulating tumor DNA (ctDNA) levels in plasma thereby rendering promising therapy and prognosis [123,124]. In particular, although the quantitative level of TERT-based ctDNA as analyzed appears to be independent of the chances relapsing HCC posttreatment, a remarkable association was observed connecting the existence of tumor-related mutations and a greater likelihood of recurrence of HCC postsurgery upon assessment of TERT-mutant ctDNA levels [125]. On the grounds of these data, it can be stated that by acquiring greater insights on the role of TERT and TERT-ctDNA in patients affected with HCC, better diagnostic tools can be developed using them to effectively assess the follow-up of hepatic cirrhotic patients and to assess the treatment efficacy of HCC.

Treatment perspectives

The therapeutic potential of a synthetic androgen Danazol (800 mg/day over a period of 2 years) in reducing the telomere attrition rate in 92% of male patients suffering from known telomere disease was noticed by Townsley et al. [126]. They also reported that in 79% of patients, a hematological response was achieved after danazol treatment. Acyclovir, an extremely choosy nucleotide analog for virus infected cells, was mostly coupled with thymidine-kinase herpes virus-carrying adenovirus, which further inhibits the replication of DNA. The prodrug of acyclovir, acycloguanosyl-5′-thymidyltriphosphate (ACV-TP-T), was identified to be a telomerase substrate which impedes cell proliferation. Curiously, TERT modulation affects the sensitivity of tumor cells to this prodrug. When

dominant-negative TERT gene was transfected, it enhanced the prodrug's half-maximal inhibitory concentration, suggesting a decreased usage of the compound. ACV-TP-T arrests the aimed cell-division cycle, stimulates apoptosis, and selectively causes relapse in HCC transgenic models. To counteract the proliferation of the same tumor cell, telomerase that is a striking characteristic of different HCCs can be utilized as a medical potential. The connection to sorafenib is shown, even though statistically significant findings are not conceivable [127]. In addition, it is vital to note that certain medications, like azidothymidine [128] and phosphoinositide 3-kinase delta inhibitors [129], have already shown impressive outcomes in murine models despite not showing up to have a direct impact on TERT, also displaying a decrease in TERT activity itself. Thus utmost importance has to be given in gaining ample knowledge on the role of TERT in the oncogenesis of HCC which ultimately can offer high therapeutic potential.

Conclusion

The activity of telomerase and its subunits is controlled by multiple factors. At a high frequency in HCC, telomerase reactivation is detectable. In HCCs, TERT promoter mutations have been observed more frequently. From these observations, it can be said that certain tumors are devoid of distinct mutations in TPR and additional telomerase reactivation mechanisms. Identifying the regulatory mechanisms that contribute to the reactivation of telomerase in HCC may highlight the divergent pathways of tumor commencement, development, and offer novel, specific treatment alternatives. The incidence of TERT promoter mutations during the initial stages of HCC reveals the importance of telomerase activity for tumor cell viability. Intriguingly, this phenomenon could serve as biomarker to study the development of malignant cancers from benign lesions. The disruption of transcriptional activation of TERT, on the other hand, is an interesting target to treat HCC especially in solid neoplasms.

Conflict of interest

No.

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