

The rRNAs are the major components of ribosomes and contribute upto 65% of their weight. There are three kinds of rRNA in prokaryotic ribosomes which are distinguished by their molecular weights.

In eukaryotic cells there exist two additional kinds of RNAs, such as heterogeneous nuclear RNA (hnRNA), a nuclear precursor of mRNA and small nuclear RNA (snRNA) which participates in RNA processing (c.f. Lehninger, 1982).

It has been reported, (c.f. Rottman, 1978) that 5'-ends of viral nuclear mRNA are frequently 'capped' with a structure in which 7-methyl-guanosine is joined by (5'-5') triphosphate link to a 2'-O-methylated ribonucleoside at its 5'-hydroxyl through the 3'-phosphate. The mRNA molecules frequently contain internal 6-methyladenylates and other 2'-O-ribose methylated nucleotides. The presence of 'cap' structure is needed for binding and translation of mRNA.

## 2: NUCLEOSIDE ANALOGUES

The significant role of nucleic acids and their components in living systems and the remarkable understanding of their genetic archives and cellular replication have contributed a great deal of research during the last 2 - 3 decades. Some of

the major ones have been inter alia in the area of syntheses of modified nucleosides and nucleotides, homo- and heteropoly-nucleotides, Jones (1979), and polynucleotides of defined sequences i.e. gene synthesis, Khorana (1979) Matteucci and Carithers (1981) and Sung and Narang (1982).

The analogues of nucleic acid bases and nucleosides have been synthesized by various workers in which extensive variations in purine, pyrimidine and pentose moiety have been made, Michelson (1963); Brown (1962, 1970); Elion and Hitchings (1965); Prusoff et al. (1974) and Clercq and Torrence (1978).

These analogues have been useful in therapy of cancer, Welsch (1961), metabolic disorders, therapy against various viral infectious agents e.g. herpes viruses, reterovirus, Varicella - zoster virus etc. The citation for the design and syntheses of such nucleic acid analogues may be found in the reviews of Elion and Hitchings (1965); Clercq and Torrence (1978); Smith (1980); Chabner (1981) and Robins and Revanker (1988). Prusoff et al. (1984a) and Clercq (1985) reviewed the possible target for viral chemotherapy. Prusoff et al. (1984b) have discussed the molecular basis for treatment of herpes virus infections with antiviral drugs based on nucleoside analogues. Prusoff et al. (1985) reviewed the basic biochemical and pharmacological aspects of antiviral agents.

Thus, with such wide uses and broad applicability as cited above, the entity of these analogues with the passage of time has now been confirmed as "Antiviral Drugs".

It would therefore be of interest to describe very briefly some important base and nucleoside analogues which are biologically active.

## **2.1: Clinically Important Synthetic Base Analogues**

6-Thioguanine, an analogue of guanine, has been used clinically in the treatment of acute myelocytic and chronic granulocytic leukemias. It is an antimetabolite of guanine which is converted into 6-thioguanine - ribose phosphate. It incorporates with DNA and RNA hence interferes with guanine synthesis (c.f. Remington, 1985).

The thio-analogue of hypoxanthine i.e. 6-mercaptopurine when used with methotrexate provides a combination of first choice in the maintenance of chemotherapy of acute lymphocytic leukemia. It is an antimetabolite precursor which is converted into 6-thioinosinic acid which acts as antimetabolite to inhibit the synthesis of adenine and guanine and also to prevent conversion of purine bases into nucleotides (c.f. Remington, 1985).

In 1957 Heidelberger synthesized 5-fluorouracil as an analogue of thymine on the basis of the observations that rat tumour cells utilized the base uracil more efficiently than the intestinal mucosa. In combination with other drugs it provides chemotherapy of first choice in the treatment of breast cancer, islet cells tumour, colorectal cancer and gastric carcinoma. It is regarded as a congener of uracil that acts both as a surrogate and as a metabolite. Its metabolite 5-fluorouridine 5'-monophosphate blocks the synthesis of thymidylic acid and hence of DNA (c.f. Remington, 1985). Heidelberger et al. (1957) also synthesized 5-fluorouridine (FUR) having antitumour activity, but it showed leukopenia and thrombopenia with gastro-intestinal toxicity as side effects, hence it was abandoned. Recently Ozaki et al. (1989) have prepared 5'-O-acyl derivatives of 5-fluorouridine by introducing twelve kinds of aliphatic acyl groups with carbon numbers 1-27 at the 5'-O-position of FUR. They have shown intraperitoneal antitumour activity of these compounds against leukemia 1210 in mice.

The purine analogue 4-hydroxypyrazole pyrimidine commonly known as "Allopurinol" has also been used as an inhibitor of de novo purine biosynthesis and xanthine oxidase. It is used for the treatment of hyperurecemia and gout (c.f. Martin et al. 198..).

Azothiopurine which is catabolized to 6-mercaptopurine is useful in organ transplantation as a support of events involved in immunologic reactions (c.f. Martin et al. 1981).

## 2.2: Nucleosides with Modification in Heterocyclic Base Moiety

A number of 5-substituted pyrimidine nucleosides and various other nucleoside analogues have been synthesized over the years. Their biological activities have been determined and most of them have been found active against one or several viruses. These nucleoside analogues have been divided into the categories such as shown below:

## 2.3: 5-Substituted Pyrimidine Deoxyribosides

The substitution of halogen groups such as -F, -Cl, -Br, and -I in deoxyuridine has been reported by Clercq et al. (1974). These are reported to inhibit the multiplication of DNA viruses and reteroviruses.

The 5-iodo-2'-deoxyuridine (Idoxuridine, IDU) has been reported to be the first antiviral nucleoside synthesized from 2'-deoxyuridine by Prusoff (1959). It is a prototype of pyrimidine nucleoside antiviral agent, active against various DNA viruses in vitro. It is used against herpes infection in man

to prevent blindness. IDU is the first chemotherapeutic agent to be licensed for human use by the FDA in United States, Prusoff et al. (1984a and 1985) and Prusoff and Lin (1988).

Another compound known as E-5(2-Bromovinyl)-2'-deoxyuridine was synthesized by Jones et al. (1979). Clercq et al. (1979) showed it to be a potent and selective inhibitory agent for HSV-1. This drug has also been recognized by the FDA in United States for treatment against viral infections, Prusoff et al. (1984a and 1985) and Prusoff and Lin (1988).

The 5-substituted trifluoromethyl-2'-deoxyuridine also known as Trifluridine ( $CF_3dUrd$ ) was originally synthesized to be an anticancer agent, but it was found to be more effective as antiviral drug, Heidelberger (1957). Its use is restricted for topical therapy of herpetic keratitis. It has also been recognized by the FDA in United States for clinical use against viral infections, Prusoff et al. (1984a and 1985) and Prusoff and Lin (1988).

In addition to the above, a variety of 5-substituted 2'-deoxyuridine have been cited in the literature, for example, hydroxymethyl ( $-CH_2OH$ ), Clercq and Torrence (1978); thiocyno ( $-S-C\equiv N$ ), Clercq and Shugar (1975); hydroxy ( $-OH$ ) and ethynyl ( $-C\equiv CH$ ), Clercq et al. (1977); and vinyl ( $-CH=CH_2$ ), Clercq

et al. (1977); Rahim et al. (1982) and Walker et al. (1982), carboxamidomethoxy and allyloxy, Torrence et al. (1978). These analogues have been reported to be effective against HSV and vaccinia virus, Clercq and Torrence (1978).

The 5-substituted cyano (-C≡N), Torrence et al. (1977) and nitro (-NO<sub>2</sub>) analogues of 2'-deoxyuridine, Clercq and Torrence (1978) are specific antivaccinial agents.

The substitution such as propyl (-CH<sub>2</sub>-CH<sub>2</sub>-CH<sub>3</sub>) has been reported by Cheng et al. (1976), Ruth and Burgstrom (1978) and Clercq and Torrence (1978), methylamino (-CH<sub>2</sub>NH<sub>2</sub>) by Shen et al. (1966), Names and Hilleman (1965) and Prusoff and Ward (1976); ethylamino (-CH<sub>2</sub>-CH<sub>2</sub>-NH<sub>2</sub>) by Shen et al. (1966); methoxymethyl by Meldrum et al. (1974) and propynyloxy by Torrence et al. (1978). These analogues are specific antiherpetic agents, Clercq and Torrence (1978).

The substitution such as allyl (-CH<sub>2</sub>=CH-CH<sub>2</sub>) has been reported by Cheng et al. (1976); mercaptomethyl (-S-CH<sub>2</sub>) by Gupta et al. (1975) and methylmercapto (-CH<sub>2</sub>-S) by Hardi et al. (1975). These analogues inhibit herpes simplex virus replication. The 2-halovinyl is effective against HSV-1 in vitro, Luke (1983). The methyl (-CH<sub>3</sub>) substituted analogue inhibits HSV- and vaccinia virus replication.

The substitution such as formyl (-CHO), oxime of formyl (-CH=NOH), azidomethyl (-CH<sub>2</sub>N<sub>3</sub>), 2-bromovinyl (-CH=CHBr[E]), ethyl (-CH<sub>2</sub>-CH<sub>3</sub>), 1,3-dithiolan-2-yl (-O-CH[SCH<sub>2</sub>]<sub>2</sub>), methyl-thio-methyl (-CH<sub>2</sub>SCH<sub>3</sub>); methylsulfonylmethyl (-CH<sub>2</sub>-SO<sub>2</sub>-CH<sub>3</sub>) have been cited from Clercq (1985). He reported that C-5 substituents like fluoro, trifluoromethyl, nitro and formyl derivatives of 2'-deoxyuridines were the most potent cytostatic agents. They also inhibited the proliferation of tumour cells.

The substitution of halogen groups such as -F, -Cl, -Br, and -I, nitro (NO<sub>2</sub>); ethynyl (-C≡CH) and ethyl (-CH<sub>2</sub>-CH<sub>3</sub>) in deoxycytidine and the biological properties of these analogues have been reported by Clercq and Torrence (1978). Their antiviral activities are quite similar to the 5-substituted deoxyuridine derivatives. The substitutions such as allyl (-CH<sub>2</sub>=CH-CH<sub>2</sub>), propyl (-CH<sub>2</sub>-CH<sub>2</sub>-CH<sub>3</sub>) and propenyl were reported by Ruth and Burgstrom (1978). The substitution such as vinyl (-CH=CH<sub>2</sub>) has been reported by Rahim et al. (1982) and ethynyl by Sharma et al. (1984).

Wyatt and Cohn (1953) found that DNA of E. coli T even bacteriophages e.g. T2, T4, and T6 contained, apart from normal deoxycytidine, a fair proportion of 5-hydroxymethyl cytidine. Khan and Qudrat-e-Khuda (1982) prepared 5-hydroxymethylcytidine by direct hydroxymethylation using formaldehyde.

## 2.4: Cytosine Arabinosides

Cytarabine (Ara-C) was synthesized in 1959 and has since been recognized as the most active antimetabolite for remission induction in adult non-lymphatic leukemia, Ellison (1968). Kremer (1975) reported its use for non-lymphatic leukemia which provided 60-70% remission in unselected cases. Band et al. (1973) found its use in the treatment of meningeal leukemia or lymphoma.

Another cytosine derivative known as 2'-fluoro-5-iodo-1-D-arabinofuranosyl cytosine (FIAC) was synthesized by Watnabe et al. (1979). It has been reported to protect mice against a lethal infection of HSV-1. In cancer patients it has been shown to abort cutaneous Varicella-zoster. The FIAC was found to be superior to Ara-A in this regard in a double blind study (c.f. Robins and Revanker, 1988).

The 1- $\beta$ -D-arabinofuranosylthymine: (Ara-T) was isolated from certain caribbean sponges hence also named as spongiothymidine. It was synthesized by Fox et al. (1957) and Underwood et al. (1964) who showed it to have activity against HSV-1 in the rabbit cornea.

From amongst purine nucleosides the adenine arabinoside, Vidarabine (Ara-A) was first synthesized by Lee et al. (1960). Miller et al. (1968) found it to be a nucleoside antibiotic. Sidwell et al. (1968) reported its antiviral activity in vitro against HSV-1 infections in mice and HSV-keratitis in hamsters. Ara-A represents a major advance in the chemotherapy of herpes virus infections. This drug has also received the approval of FDA in United States for treatment against herpes virus infection, Prusoff et al. (1984a and 1985) and Prusoff and Lin (1983).

Cytidine arabinosides have also been substituted by halogens such as -F, -Cl, -Br and -I by Buthala (1964); hydro and methyl by Chien et al. (1973) and various others. These derivatives were shown to inhibit viruses such as S.V.40, adeno, herpes simplex, Varicella-zoster, cytomegalo etc., Chien et al. (1973).

Uridine arabinosides have been substituted on 5-position by -hydro, -iodo, methyl, ethyl etc. which were found active against herpes virus. The 5-methyl substituted arabinothymidine has been reported to inhibit the growth of HSV and Varicella-zoster virus, Underwood et al. (1964). Schabel Jr. (1968) reported that the purine arabinoside such as arabinoadenine inhibited all the DNA and some RNA viruses.

Recently the synthesis of boron containing antimetabolites of nucleosides such as 2'-deoxyguanosine-N-7-cynoborane, 2'-deoxyinosine-N-7-cynoborane, 2'-deoxyadenosine-N-1-cynoborane and 2'-deoxycytidine-N-3-cynoborane have been reported by Sood et al. (1989). But only 2'-deoxyadenosine-N-1-cynoborane and 2'-deoxycytidine-N-3-cynoborane showed potent activity in T-molt-3 and human colorectal adenocarcinoma.

## **2.5: Clinically Important Nucleoside Analogues**

The 5-azacytidine, a new analogue, has been shown to possess promising activity against adult non-lymphocytic leukemia. It can either be synthesized chemically or produced microbiologically. It mainly effects the synthesis and function of RNA, Li et al. (1970).

The 3-deazauridine was synthesized by Robin and Curie in 1968. Bloch et al. (1973) found it to have anticancer activity against Ehrlich ascites and L-1210 tumour cells. It is an analogue of uridine. The primary action of 3-deazauridine, as the active triphosphate, is the inhibition of CTP synthetase. It does not incorporate into RNA and DNA. It also inhibits cytidine deaminase, deoxycytidylate deaminase and ribonucleotide reductase.

Amongst the azido-substituted nucleosides the 3'-azido-3'-deoxythymidine (AZT) has gained much importance. It was first prepared by Horwitz et al. (1964) and later with slight modification by Lin and Prusoff (1978). Ostertag et al. (1974) reported that AZT was active against reterovirus and Friend leukemia virus. Mitsuya et al. (1985) found it to inhibit various strains of human T-lymphotropic virus (HTLV-III); lymphadenopathy-associated virus (LAV); AIDS-associated virus (ARV) and human immunodeficiency virus (HIV).

The 2',3'-dideoxyadenosine was first prepared by Robins and Robins (1964). It was found to inhibit reterovirus 334C and murine leukemia virus in NIH Swiss 3T3 cells in culture, Wagner et al. (1984). Recently Mitsuya and Border (1986) have shown that this compound completely protected ATH 8 cells exposed to HIV. Similar inhibition is also shown by 2',3'-dideoxyguanosine.

The 2',3'-dideoxycytidine was first prepared by Hortwitz et al. (1967). It has been reported to inhibit HIV and its clinical trials have also been carried out at NIH in AIDS patients, Mitsuya and Border (1986).

The syntheses of azido nucleosides, AZT have also been

reported by various workers such as Horwitz et al. (1964); Torrence et al. (1973); Bobek et al. (1980); Lin and Mancini (1983); Mitsuya et al. (1985); Lin et al. (1988); Mansuri et al. (1989); Murata and Achiwa (1990) are worth mentioning. These compounds have been reported as active against human immunodeficiency virus (HIV) which is responsible for the Acquired Immunodeficiency Syndrome (AIDS).

Birnbaum et al. (1987, 1988) studied X-ray crystallography of such compounds and have correlated the biological activity of AZT with the conformation of their chemical structure and glycosidic torsion angle.

## **2.6: Miscellaneous Biologically Active Nucleosides**

A number of different types of nucleosides have been synthesized from time to time which are reported to be biologically active, for example, ribavirin or virazole is an important such nucleoside. It was synthesized by Witkowski et al. (1972) and consists of a triazole ring in place of natural heterocyclic base. It has been reported as one of the most powerful synthetic antiviral agents active against DNA viruses. Virazole has been approved by the FDA in United States for the treatment of viral infections, Prusoff et al. (1984a, 1985) and Prusoff and Lin (1988).

The pyrazofurin or pyrazomycin is a C-glycoside and has an activity comparable to ribavirin. It suppresses the replication of herpes virus, murine leukemia virus etc. Descamps and Clercq (1977).

The acyclovir (ACV) was first synthesized by Schaeffer et al. (1971). It is a structural analogue of guanosine, effective against herpes encephalitis, topical therapy of herpes keratitis, etc. (c.f. Prusoff et al. 1985).

## **2.7: Nucleosides with Modification in Sugar and Heterocyclic Base Moiety**

Numerous modifications have been carried out in nucleotide structure involving both sugar and heterocyclic base. Cheng et al. (1975) and Prusoff et al. (1977) have reported 5-iodo-5'-amino-2',5'-dideoxyuridine. Its activity is restricted only to herpes and reteroviruses. Lee and Chang (1978) reported the synthesis of N-methylated and P-methylated and N- and P- dimethylated CMP and UMP with dimethylsulphate. Holy and Pischal (1977) prepared 5'-O-carboxymethyluridine. They then prepared 5-substituted halogen derivatives of the 5'-O-carboxymethyluridine. Keppler and Kiefer (1983) patented bis-3'-O-(5-alkyl)-2'-deoxyuridine sulphoxides which showed antiviral activity. Lin et al. (1987) prepared analogues of pyrimidine deoxyribonucleo-

sides by substituting various functional groups in base and sugar moieties. These were tested against Maloney murine leukemia virus (M-MULV). Some of them were also tested in vitro against HTLV - II/LAV/AAV (AIDS virus). Kunio et al. (1989) patented the synthesis of 5'-aminoacyl-5-fluouridine derivative as useful antitumour, antiviral agent and immunosuppressant. Yasuko et al. (1989) patented the synthesis of 2',3'-dideoxycytidine by debromination and hydrogenation of 2'-bromo-2',3'-didehydro-2',3'-dideoxycytidine for the treatment of Acquired Immunodeficiency Syndrome (AIDS).

### 3: NUCLEOTIDES

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The nucleotides, as already indicated, are nucleosides phosphorylated on 5'-hydroxyl group of the sugar, D-ribose or deoxyribose. They are found in a variety of cells and tissues where they perform important functions such as structural components, biocatalysts, receptors, repositories of genetic informations etc. They are particularly well-known as building blocks of RNA and DNA in which they function as coding elements.

Purine ribonucleotides serve in biological systems as the ubiquitous high-energy source such as adenosine triphosphate (ATP) and as regulatory signals such as cyclic-AMP and cyclic-GMP in a wide variety of tissues and organisms. Flavin adenine