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Progress in development of HIV-1 integrase inhibitor

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Abstract

AIDS (Acquired Immuno Deficiency Syndrome) has found to be most fatal syndrome. The main causative agent of AIDS is HIV-1 integrase. Incorporation of viral DNA into the host cell genome could be translated as the basis of life-long infection. Therefore, this biochemical event, catalyzed by the enzyme integrase, is a pivotal step in viral life cycle and thus worthy of being exploited to develop anti-HIV chemotherapy. The review presented on different categories of compounds that have been studied for the inhibition of the HIV-1 integrase to develop anti-HIV agents. The categories are Oligonucleotides, Curcumin Analogues, Polyhydroxylated Aromatic Compounds, Diketo Acid,) Caffeoyl-based Inhibitors, Hydrazides and Amides, Tetracycline, Depsides and Depsidones.

Keywords : AIDS (Acquired Immuno Deficiency Syndrome), Oligonucleotides, Curcumin Analogues, Diketo Acid, Caffeoyl-based Inhibitors, Depsides and Depsidones.

INTRODUCTION

In the present era AIDS (Acquired Immuno Deficiency Syndrome) has found to be the most fatal syndrome. The main causative agent of AIDS is HIV-1 integrase (Human Immuno Deficiency Virus).The high-resolution electron microscopy has illustrated that HIV-1, the causative agent of AIDS is an enveloped virus of about 100 nm diameters [1]. It contains an outer lipid bilayer, derived from the host cell during maturation and consists of two major viral glycoproteins, the external gp120 and the transmembrane gp41 (gp stands for glycoprotein and the number refers to the mass of protein in thousands of Dalton). Immediately beneath the outer envelope is a membrane– sociated protein p18, which provides a matrix for the viral structure and is vital for the integrity of the virion. The matrix surrounds a characteristics dense, cylindrical nucleoid, containing capsid protein p24. Inside this nucleoid are two identical RNA strands with which the viral RNA- dependent DNA polymerase (pol) p66/p55, called reverse transcriptase, is in

association with nucleoprotein p9, integrase protein p12, and protease p15 components. The HIV life cycle begins with high-affinity binding of gp120 envelope protein to its receptor CD4 on the host cell surface [2]. The CD4 receptor is a protein molecule found predominantly on a subset of T-lymphocytes responsible for the helper or inducer function in the immature response. Following binding, the fusion of virus with host cell membrane occurs via the gp41 molecules and the HIV genomic RNA is uncoated and internalized. The enzyme reverses transcription of genomic RNA into double–stranded DNA. The DNA migrates to the nucleus to be integrated into the host cell chromosome through the action of virally encoded enzyme, integrase. The incorporation of this "provirus" into the cell genome is permanent. The provirus may remain transcriptionally inactive (latent) or manifest a high level of gene expression with active production of virus. The activation of provirus (the gene expression) from the latent state by selective and constitutive host transcription factors, notably the NF-Kβ family of DNA enhancer binding proteins, leads to the sequential production of various viral m-RNAs. These m-RNAs are translated into regulatory proteins – Tat, Rev, and Nef. The viral core is formed by the assembly of these proteins, enzymes, and genomic RNA at the plasma membrane of the cells. Budding of the progeny virion occurs through the host cell membrane, where the core acquires its external envelope. During the final budding process, the cleavage of gag-pol polyprotein precursor by HIV protease occurs, leading to morphological maturation of virions. Thus, the replicative cycle of HIV-1 presents several viable targets that could be exploited for the development of anti-HIV chemotherapy. Medicinal chemists have focused their attention predominantly on the following stages:(1) viral binding to target cells, (2) virus cell fusion, (3) virus uncoating, (4) reverse transcription of genomic RNA, (5) viral integration, (6) gene expression, (7) cleavage event, and (8) virion maturation. By hitting any of these stages, the viral replication can be terminated. For details readers may refer to De Clercq *et al.* [3].In the recent past, however, the chemists have focused their attention mainly on the inhibition of reverse transcription of genomic RNA and the cleavage event, for which variety of reverse transcriptase (RT) inhibitors [4] and HIV-1 protease inhibitors [5, 6], respectively, have been developed, and in abundance are available structure-activity relationship (SAR) studies on them [7, 8]. The third most important event that recently drew the attention of the chemists is now viral integration for which people have started developing integrase inhibitors.

Life cycle of HIV-1

The HIV life cycle begin with high affinity binding glycoprotein gp120 envelope protein to its receptors CD4+ on the host cell surface (Figure I) [9]. The CD4+ receptor is a protein molecule found predominantly on a subset of T-lymphocytes responsible for helper or inducer function in the immune response. Following binding, the fusion of virus with host cell membrane occurs via the glycoprotein gp41 molecules and the HIV genomic RNA is uncoated and internalized. The enzyme reverses transcription of genomic RNA into double stranded DNA. The DNA migrates to the nucleus to be integrated into the host cell chromosome through the action of this "provirus" into the cell genome is permanent. The provirus may remain transcriptionally inactive (latent) or manifest a high level of gene expression with action production of virus. The activation of provirus (the gene expression) from the latent state by selective and constructive host transcription factors, notably the NF-K β family of DNA enhancer binding protein, leads to the sequential production of various viral m-RNAs. These m-RNAs are translated into regulatory proteins, enzymes, and genomic RNA at the plasma membrane, where the core acquires its

external envelope. During the final budding process, the cleavage of *gag-pol*. Polyprotein precursor by HIV protease occurs, leading to morphological maturation of virion [10].

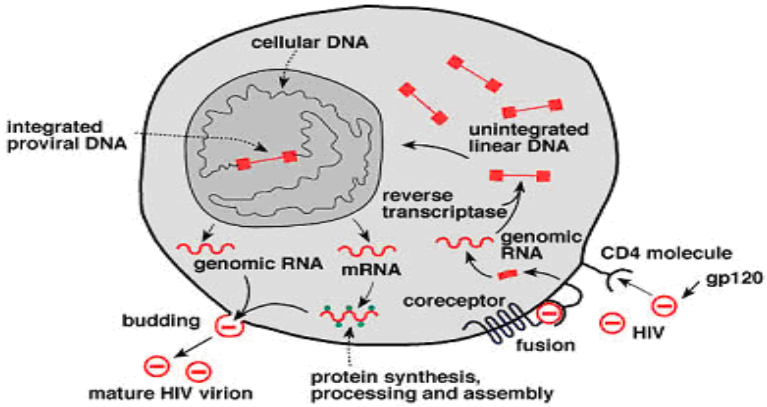


Figure 1: Life Cycle of HIV-1[10]

HIV-1 integrase

Incorporation of viral DNA into the host cell genome could be translated as the basis of life-long infection. Therefore, this biochemical event, catalyzed by the enzyme integrase, is a pivotal step in viral life cycle and thus worthy of being exploited to develop anti-HIV chemotherapy. HIV-1 integrase is comprised of three domains (Figure II) based on the susceptibility of the linker regions to proteolysis [11], functional studies [11-13], and the structures of the domains, which have been individually determined by X-ray crystallography or NMR. The catalytic core domain contains the invariant triad of acidic residues, the D, D-35-E motif [11, 14-16], comprising residues Asp64, Asp116, and Glu152. These catalytic residues of HIV-1 integrase are in close proximity, coordinate with divalent metal ion, and define the active site. However, the residues comprising the active site region exhibit considerable flexibility, suggesting that binding of DNA substrate is required to impose the precise configuration of residues that is required for catalysis.

ACTG ---------- CAGT (+) 3'-end
(-) 3'-end TGAC ---------- GTCA

(Figure II). The 3'-end processing of HIV double helical DNA prior to integration into host cell. Although the core domain of integrase is clearly responsible for catalysis, the functional roles of other two domains are less clear. The C-terminal domain binds DNA nonspecifically. The N-terminal domain of HIV-1 contains a conserved pair of His and Cys residues, a motif similar to the zinc coordinating residues of zinc fingers. Although this domain does indeed bind zinc [17, 18], its structure is totally different from that of zinc fingers. It consists of a bundle of three α-helices [19, 20] and has an SH3 fold, although there is no known functional relationship with SH3 domains of other proteins.

HIV transmission

HIV is transmitted in three ways [21, 22].

(i) Through unprotected vaginal or anal intercourse with an infected man or woman [23, 24].

(ii) The infected blood entering the blood stream through sharing injection needle, transfusion of infected blood or blood products, intravenous drug users (those who take injections regularly for pleasure), needle injury etc.[25].

(iii) From a woman with HIV to her baby either during pregnancy or during delivery [26, 27].
There is absolutely no evidence that HIV is transmitted by causal contact or that the virus can be spread by insects such as a mosquito bite.

Pathogenesis

HIV infects cells in the immune system and the central nervous system. The main cell HIV infection is called a T helper lymphocyte. The T helper cell is a crucial part of the immune system, and co-ordinates the actions of other immune system cells. A large reduction in the number of T helper cells seriously weakens the immune system. HIV infects the T Helper cell because it has the protein CD4 on its surface, which HIV uses to attach itself to the cell before gaining entry. This is why the T helper cell is sometimes referred to as a CD4+ lymphocyte. Once it has found its way into a cell, HIV produces new copies of itself, which can then go on to infect other cells. The infected cells are often destroyed or stop working properly. However, battling against HIV the immune system is rapidly killing virus particles and HIV-infected cells, and replacing the T helper cells that have been lost. HIV infection can generally be broken down into four distinct stages: primary infection, clinically asymptomatic stage, symptomatic HIV infection, and progression from HIV to AIDS. Events occurring in the days and weeks following infection are critical in determining the ultimate course of HIV disease. These events include:

(i) HIV spread to tissues and events that ultimately may represent hard to eradicate viral reservoirs.
(ii) Extensive damage to lymph node cellular architecture.
(iii) Stimulation of an immune response against HIV.
(iv) Loss of HIV specific CD4+.
(v) Rapid HIV replication and mutation creating a more genetically diverse population of HIV genome, some perhaps more virulent.

The extent, quality, and consequences of these events vary greatly among individuals, according in parts for differences in subsequent rate of HIV disease progression [28-32].

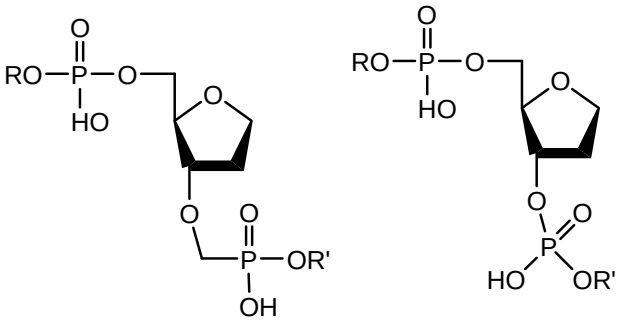
Recent Development

Gupta, S.P. and Nagappa, A.N. [33] presented on different categories of compounds that have been studied for the inhibition of the HIV-1 integrase to develop anti-HIV agents. Several DNA binding agents were found to inhibit HIV-1 integrase, probably due to a nonspecific interaction with the DNA binding domain of the enzyme [34]. Many catechol derivatives have also been found to act as integrase inhibitors but they have been postulated to elicit their effects by interfering with the coordination of metal ions that are required for the phosphoryl transfer [35]. However, catechol derivatives do not exhibit much antiviral specificity in the cell culture and hence are no longer considered to be worth pursuing [36]. However, varieties of other groups of integrase inhibitors have been recently developed that are discussed below:

(1) Oligonucleotides: Some oligonucleotides of natural origin are capable of interfering with the integration process by competing with viral DNA for binding to HIV integrase [37]. Based upon the inhibitory mechanism, reported anti-viral oligonucleotides generally belong to three categories [38]. (a) Antisense and double–stranded oligonucleotides, (b) Triplex inhibitors, and (c) G-quartet inhibitors.

(a) Antisense and Double-Stranded Oligonucleotides:

All modified 21-mer **(1, 2)** oligonucleotides were found to competitively inhibit 3'- processing (3'-P) as well as strand transfer (ST) reactions with nanomolar IC_{50} values. Studies with 19-mer **(1, 2)** oligonucleotides showed that the modifications of the 3'-OH significantly reduced the strand transfer reaction and that the proper orientation of this hydroxyl group and the presence of the furanose ring of adenosine significantly influence this reaction [39].



(1, 2)

(b) Triplex Inhibitors

DNA triplexes are formed from a Watson-Crick duplex and a homopyrimidine or purine rich third strand that binds in the major groove to form base triplets. The triplex inhibitor is the oligonucleotide designed to bind duplex DNA (or RNA) by a third nucleic acid strand to form a triple helix. A family of triplex oligonucleotide was studied by Mouscadet *et al.* [40] to target the integrase-binding site in U3 LTR (LTR: long-terminal repeat), which contains a purine motif 5'-GGAAGGG-3', by forming either homopyrimidine or homopurine short triple-helix conjugates. A single oligonucleotide, 5'-GGTTTTTGTGT- 3', can associate with the purine motif to form a DNA triplex. The IC_{50} of HIV-1 integrase inhibitor *in vitro* for triplex formation was observed to be in submicro molar concentration [41].

(c) G-Quartet Inhibitors

A 16-mer olignucleotide, 5'-(GGGT)4, referred to as T30695 (Figure III) represents actually the model of this oligonucleotide), was found to be a potent integrase inhibitor with IC_{50} equal to 43 nM for 3'-processing and 24 nM for strand transfer [42]. A 17-mer G-rich oligonucleotide, 5' GTGGGT(GGGT)3, referred to as T30177 or AR177, was also investigated for integrase inhibitor activity with IC_{50} values in nanomolar range for both 3'- processing and strand transfer reactions [43], but it was recently reported that this oligonucleotide targets the enveloped protein gp120 of HIV-1, instead of HIV-1integrase [44,45].

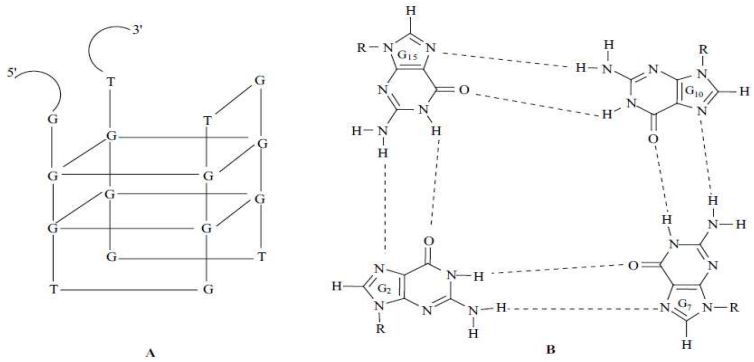
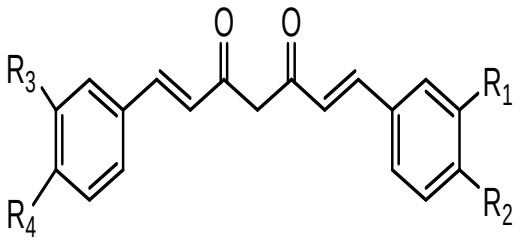


Figure 3: An intramolecular G-quartet model (A), in which four G-bases associate through hydrogen bondings to form a cyclic structure (B)

(2) Curcumin Analogues

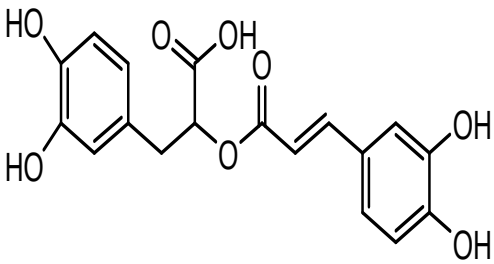
Curcumin belongs to the family of curcuminoids (phenolic diarylheptanoids), which are characteristic, yellow coloring constituents of turmeric, the roots/rhizomes of *Curcuma aromatica* SALISB., *C. longa L.*, *C. xanthorrhiza* ROXB, and *C.Zedoaria* (CHRISTM.) ROSC cultivated in tropical areas and used in traditional medicine and as spices in South and South East Asia [46].

Two closely related natural congeners of curcumin **(3) (a and b)** were found to have slightly better potency than cumurin, suggesting that methoxy groups are not preferred. Its two synthetic analogues with no methoxy groups, discaffeolymethane **(3) (c)** and rosmarinic acid (**4**) were found to be very potent [47].



a: R_1=R_3=H, R_2=R_4=OH
b: R_1=H, R_3=OCH_3, R_2=R_4=OH
c: R_1=R_2=R_3=R_4=OH

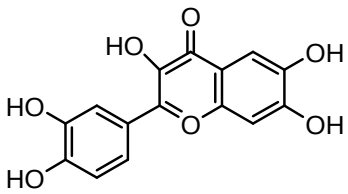
(3)



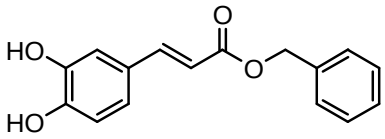
(4)

(3) Polyhydroxylated Aromatic Compounds

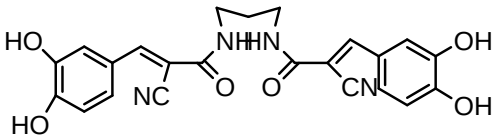
Multiaromatic rings and orthohydroxylation were found to lead to potent integrase inhibitors. Examples include flavones such as quercetin (**5**) [48], caffeic acid phenethyl ester (CAPE) (**6**) [48], and certain "tyrphostins" as **(7)** [49]. Based on these models, a series of bis-arylamides were reported [50] in which **(8)** was found to possess the highest potency.



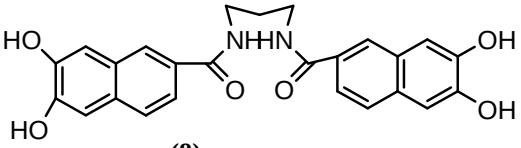
(5), quercetin



(6), CAPE



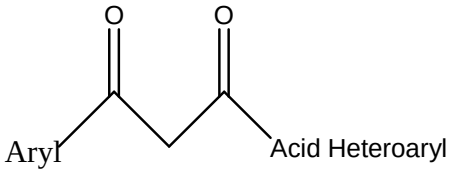
(7), tyrphostin



(8)

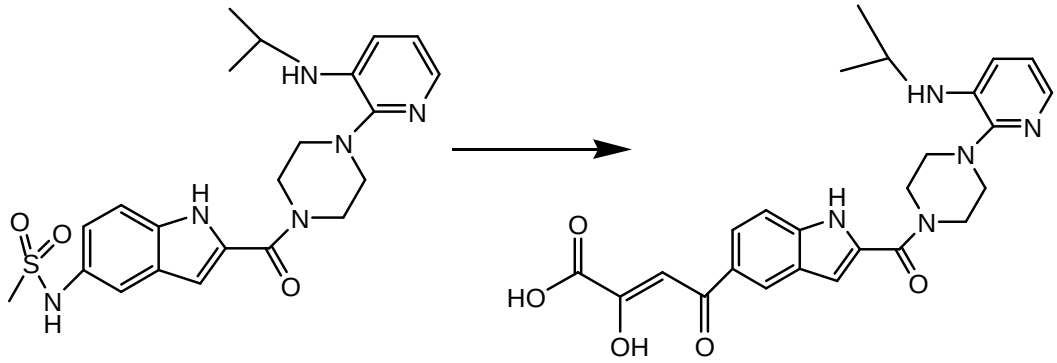
(4) Diketo Acid

Another emerging class of integrase inhibitors is related to aryl β-diketo (ADK) based agents with general structure **(9)**.



(9)

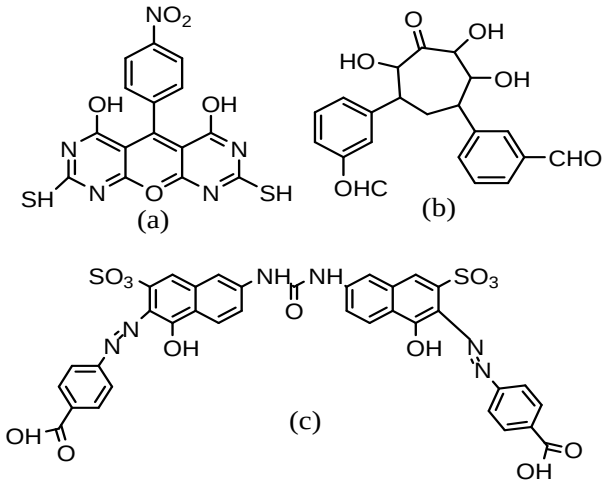
Wang, Z and Vince, R. [51] designed and synthesized dual inhibitors of HIV reverse transcriptase (RT) and integrase (IN) by introducing diketoacid (DKA) functionality into the tolerant C-5 site of RT inhibitor delavirdine **(10)**. The resulting compounds all demonstrate good activity against both RT and IN in enzymatic assays and HIV in cell-based assay.



Delavirdine, RT inhibitor RT/IN dual inhibitor

(10)

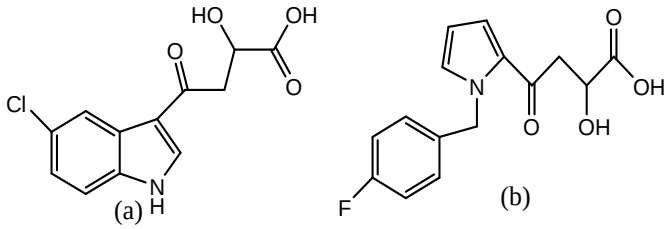
Wang et al. [52] designed and synthesized bifunctional inhibitors based on 1-[(2 hydroxyethoxy) methyl]-6-(phenylthio) thymine (HEPT) *a*1 nonnucleoside reverse transcriptase (RT) inhibitors and diketoacid (DKA) integrase (IN) inhibitors **(11)**. Biochemical studies revealed activity against RT and IN at low nanomolar and low micromolar concentrations, respectively. Exceptionally low IC_{50} values from a cell-based assay were achieved along with remarkably high therapeutic indices.



Structures of reported compounds active against RT and IN

(11)

Li, X. and Vince, R. [53] designed and synthesized a series of carbazolone-containing α & ɣ diketo acids **(12)** by applying conformational restraint onto the open-chain form of the diketo acid. These compounds showed anti-integrase activity in the low micromolar range, and integrase assay results indicated that the geometry of the diketo acid moiety is crucial to potency.

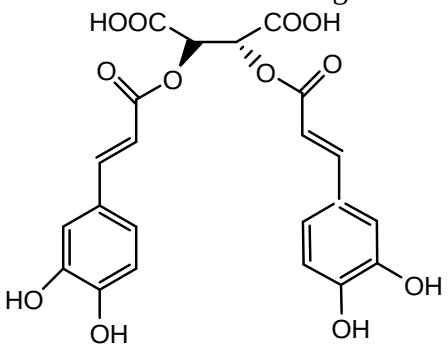


(12)

(5) Caffeoyl-based Inhibitors

Caffeoyl–based inhibitors are caffeic acid esters separated by aliphatic, alicyclic, or aromatic linkers [54, 55]. L-chicoric acid (**13**) containing two units of caffeoyl (**14**) is an example of this class of inhibitors.

Maurin et al. [56] synthesized a new series of catechol–DKA (diketoacid) hybrids. These compounds presented micromolar anti-integrase activities with moderate antiviral properties.



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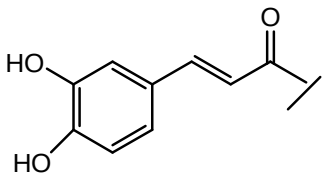
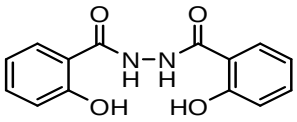


(14)

(6) Hydrazides and Amides

Hong *et al.* [57] investigated some hydrazides, in which four compounds (**15-18**) were found to possess good integrase inhibition activity. These four molecules, being nonionic and of low molecular weight and simple structure, were supposed to be promising lead compounds in the search of integrase inhibitors.



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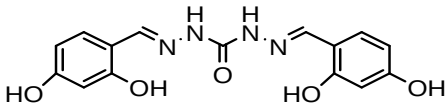

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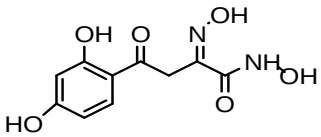

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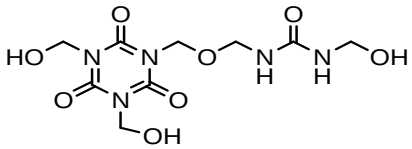
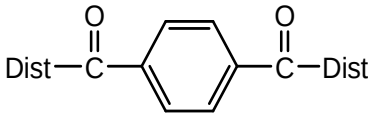


(18)

In the polyamide series, some bisdistamycin and lexitropsin derivatives were also studied for integrase inhibition activity, where the importance of dimeric structure was also pointed out along with the role of linker chains. In the series of bisdistamycin analogues, Neamati *et al.* [58] reported that 1,4-disubstituted para derivative **(19)** had markedly higher potency than 1,2- ortho or 1,3–meta disubstituted derivative and so was the case with a derivative containing pyridinyl nucleus **(20)**.



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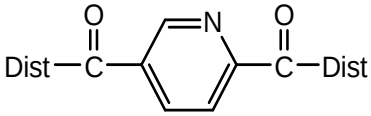
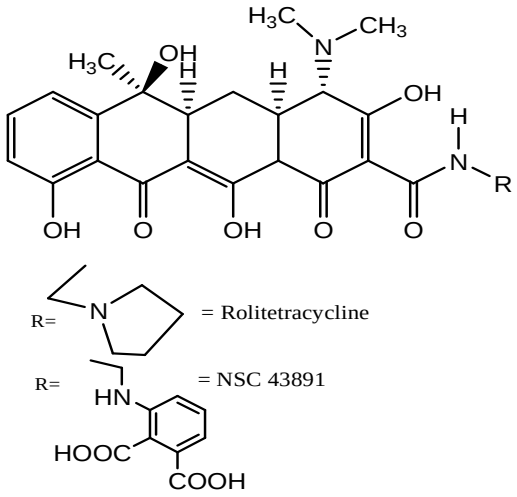


(20)

(7) Tetracycline

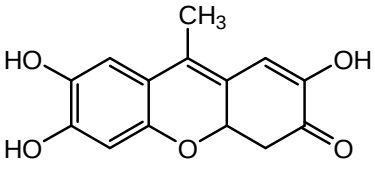
Neamati *et al.* [59] found that among the most potent inhibitors, a new class of inhibitors can be tetracyclines. Although the parent tetracycline exhibited marginal potency against purified integrase, all substituted analogues **(21)** tested showed 5-100-fold increased potency. The disparity in the potency of tetracycline and that of its analogues is attributed to the difference in their chelating properties.



(21)

(8) Depsides and Depsidones

Neamati *et al*. [60] also studied a series of depsides and depsidones as inhibitors of HIV-1 integrase. Certain compounds, e.g., **(22, 23)** were found to possess very high potency having IC_{50} values around 1 μM.



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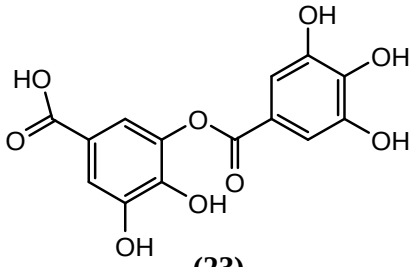
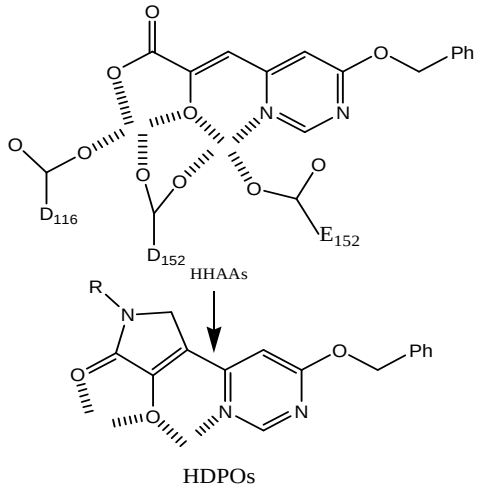


(23)

(9) Miscellaneous

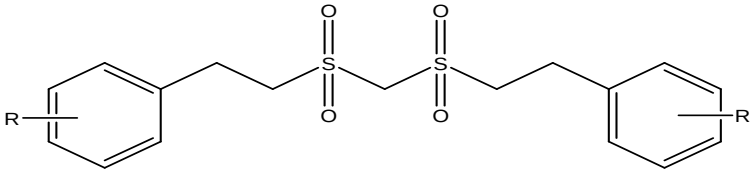
Kawasuji et al. [61] modified 2-hydroxy-3-heteroaryl acrylic acid inhibitors (HHAAs) **(24)** and presented a novel series of HIV IN inhibitors having a 3 hydroxy-1, 5-dihydro-pyrrol-2-one moiety (HDPO) **(25)** as an advanced analog of HHAAs. This cyclic modification of the chelating region of HHAA produces a favorable configuration to coordinate two-metal ions in HIV IN, which consequently gave improvements in not only enzymatic assay but also antiviral cell based assay in many cases.



Process of drug design from HHAAs to HDPOs. Two- metal binding model of HHAAs. D64, D116 and E152 indicate catalytic triad of HIV IN.

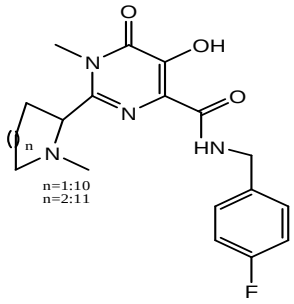
(24, 25)

Meadows et al. [62] designed and synthesized a series of vinyl geminal disulfone-containing compounds **(26)** possessing a range of ring substituents to probe the impact of structure on inhibitory mechanisms. Four active compounds were identified using HIV drug susceptibility assays. Three of the inhibitors possessing either no substituents or electron withdrawing substituents on the aromatic rings led to high levels of cytotoxicity and antiviral activity. The studies reported suggest that compounds lacking electron donating substituents on the aromatic ring are promiscuous acceptors of biological nucleophiles, whereas compounds possessing electron-donating substituents seem to resist addition or at least be more selective and significantly less toxic.



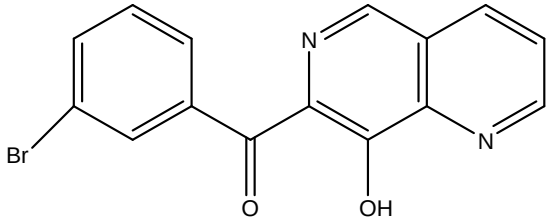
(26)

Gardelli et al. [63] discovered and synthesized N-Methyl Pyrimidones (HIV Integrase Inhibitors) **(27)** which was found to be Potent and Orally Bioavailable. The compounds have favorable pharmacokinetic properties in three preclinical species and show no liabilities in several counter screening assays. It represents a promising antiviral agent against HIV-1.



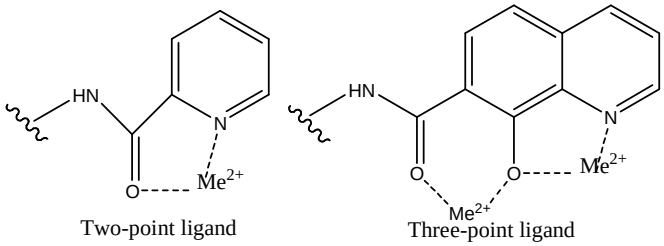
(27)

Zhuang et al. [64] designed and synthesized 8-Hydroxy 1, 6 Naphthyridines **(28)** as novel inhibitors of HIV-1 Integrase in Vitro and in infected cells. It does not exhibit cytotoxicity in cell culture at ≤12.5 µM and shows a good pharmacokinetic profile when dosed orally to rats. The antiviral activity of and its effect on integration was confirmed using viruses with specific integrase mutations.



(28)

Li, X. and Vince, R. [65] synthesized a series of amide substituted purine derivatives via palladium-catalyzed amidation reactions, and their biological activities against HIV integrase were evaluated. These purine derivatives showed anti-integrase activity at low micromolar range. The biological results indicated that the type of $Me2^{+}$ ligands, two-point ligand picolinamide or three-point ligand 8-hydroxy-quinoline-7-carboxamide, **(29)** affected inhibitory potency depending on the substitution position of the para fluorobenzyl group.



(29)

CONCLUSION

So far so many development has been done on integrase enzyme. The enzyme plays a critical role in causing life long infection in patient. Currently medicinal chemist are focussing on the integrase enzyme and lots of synthetic, Quantitative structure activity relationship work is under progress. The aim is to design a novel rationale design of molecule with more potency and less toxicity.

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