

Advances in antiviral drug discovery and development: Part I: Advancements in antiviral drug discovery



“...our knowledge about viruses has increased tremendously owing to rapid developments in science and technology.”

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The history of infectious diseases is as old as the human civilization, and need of protection against these infections always remains one of the prime concerns. Infectious diseases are a leading cause of death, and alone accounted for one-fourth to one-third of total deaths worldwide [101]. Among all the disease-causing agents in humans, viruses are the most notorious, active and important. Viruses are obligate parasites having DNA or RNA as their genetic material with markedly different mutation rates that can be quantified. Some viruses show remarkable genetic stability and others show variable rate of mutation. In the last 30 years, several new viruses have been reported from all over the world (FIGURE 1A) and have caused major outbreaks [102]. Meanwhile, we are struggling against viruses and lack foolproof antiviral therapies against them. It has helped to understand the genetic, molecular, structural as well as functional diversity of viruses, the intricacy of viral multiplication, immune escape, association of host cell machinery for replication and host immune response. Owing to such development, researchers were able to find out appropriate targets against viruses, and as a result many antiviral drugs have been developed (FIGURE 1B).

Antiviral therapeutics: addressing the entangled

Antiviral therapeutics deals specifically with the treatment of viral infections and refers to the use of drugs and the methods of their execution in the treatment of life-threatening viral diseases. Antiviral therapeutics may be differentiated into three groups: virucides, immunomodulators and antivirals. Virucides are agents that are capable

of neutralizing or destroying a virus; for example, phenol, sodium hypochlorite, ethanol, detergents and ultraviolet rays [1]. Immunomodulators consist of molecules that augment the host response to infections by promoting the secretion of antibodies, interferons or by intensifying cell-mediated immunity. They may be of immunoglobulin/antibody, cytokine, interferon, soluble receptor, receptor antagonist, hormone, cell, cell extract, low-molecular-weight compound, antigen, DNA or therapeutic vaccine origin [2]. Antivirals are the molecules that inhibit viral multiplication by targeting a particular stage such as viral adsorption, fusion, uncoating, reverse transcription, integration, nucleic acid synthesis and maturation during viral replication (FIGURE 2) without causing unacceptable side effects. To classify any molecule as antiviral, it should reach the infected organ, inhibit virus function without affecting the host, be readily absorbed and not be toxic, carcinogenic, allergenic or mutagenic [3].

Classification of antivirals: ramifications of mode of action

The first successful administration of antiviral drugs (thiosemicarbazone) to patients began in the 1960s against smallpox [4]. Since then, over 100 antivirals have been developed [5]. On the basis of targets, antivirals may be classified into seven groups: fusion inhibitors, DNA polymerase inhibitors (DPis), reverse transcriptase inhibitors (RTIs), integrase inhibitors, protease inhibitors, protease inhibitors and signaling inhibitors. Examples of various groups of antivirals and their representative drugs, with their target and mode of action have been elucidated in TABLE 1.

“Infectious diseases are a leading cause of death, and alone accounted for one-fourth to one-third of total deaths worldwide...”



Figure 1. Emergence of viruses (A) and discovery of antivirals (B) during the last three decades. The boxes represent the year of emergence/discovery and the virus/antiviral is mentioned against the respective years.
 CHIKV: Chikungunya virus; HHV-8: Human herpesvirus-8; HTLV: Human T lymphotropic virus; SARS: Severe acute respiratory syndrome.

“Fusion inhibitors inhibit binding/fusion of the virus to the host cell, and thus prevent noninfected cells from infection.”

Fusion inhibitors inhibit binding/fusion of the virus to the host cell, and thus prevent noninfected cells from infection. They target either viral surface proteins (enfuvirtide) or cell receptors (maraviroc). DPIs/RTIs target viral DNA polymerase/reverse transcriptase, either by binding directly to the active site of an enzyme, or terminate a growing chain of DNA by preventing further formation of phosphodiester bonds, thus preventing viral replication. DPIs may be nucleoside analogs (idoxuridine, vidarabine and acyclovir), nucleotide analogs (cidofovir) or pyrophosphate analogs (foscarnet and phosphonoacetic acid). RTIs may be divided into three classes [6]:

- Nucleoside analog RTIs (zidovudine, didanosine, zalcitabine, stavudine and lamivudine);
- Nucleotide analog RTIs (tenofovir and adefovir)
- Non-nucleoside RTIs (efavirenz, nevirapine, delavirdine and etravirine).

Integrase inhibitors are molecules that block the action of integrase and thus prevent integration of viral DNA into the host genome; for example, raltegravir [7]. Portmanteau inhibitors are a novel approach where researchers are attempting to design a drug that functions as a RTI as well

as an integrase inhibitor [8]. Protease inhibitors prevent virus replication by inhibiting the activity of viral proteases. They inhibit the cleavage of nascent viral proteins needed for the assembly and formation of new virions; for example, saquinavir, indinavir, amprenavir, nelfinavir and ritonavir [6]. Signaling inhibitors block signaling by interacting with key components of signaling pathways that are involved in viral replication; for example, ribavirin and viramidine are used as inosine monophosphate (IMP) dehydrogenase inhibitors [9]. Diethylthiocarbamate-mediated inhibition of Japanese encephalitis virus is believed to affect pathways responsible for enhanced production of inducible nitric oxide synthase and TNF- α [10]. Various chemotherapeutic strategies have been formulated to curb influenza virus infection: amantadine and rimantadine (as M2-channel inhibitors), T705 (as a viral RNA polymerase inhibitor), flutimide (as a viral endonuclease inhibitor) and oseltamivir, zanamivir and peramivir (as neuraminidase inhibitors). Helicase primase inhibitors such as BAY 57-1293 also appear very promising against Herpes simplex virus (HSV) and Varicella-zoster virus, and are currently in Phase II clinical trials [11,12].

Problems & limitations:
niggling would help

Toxicity & immunosuppression:
incurring immunostifle

Toxicity is a major problem with all antivirals as they lack absolute specificity against viruses, resulting in interference with normal cellular functions. Many antivirals used against HIV are highly toxic to white blood cells and cause megaloblastic anemia, late drug toxicity with drug-induced immunosuppression, mitochondrial toxicity and nerve injury [13–15]. Therefore, the immunosuppressive properties of antiviral agents should be identified so as to minimize the unwanted side effects. Hence, it is important to develop antivirals with fewer toxic effects and less immunosuppression.

Resistance: the refusal to comply

Almost all viruses have been found to develop resistance against their respective antiviral drugs. HIV has been found to develop resistance against tenofovir, emtricitabine, abacavir and lamivudine [16], and acyclovir-associated resistance has been observed during herpes virus infection [17]. Variable rates of mutation in viruses result in the generation of viral mutant pools inside the cells, which may lead to alteration in viral enzymes or structural components. The selective pressure caused by antiviral agents under these circumstances results in the replacement of wild-type viruses with mutant ones. The next important issue to be addressed is cross-resistance (CR) among antiviral drugs. Resistance to one drug is accompanied by the reduced susceptibility to another drug of the

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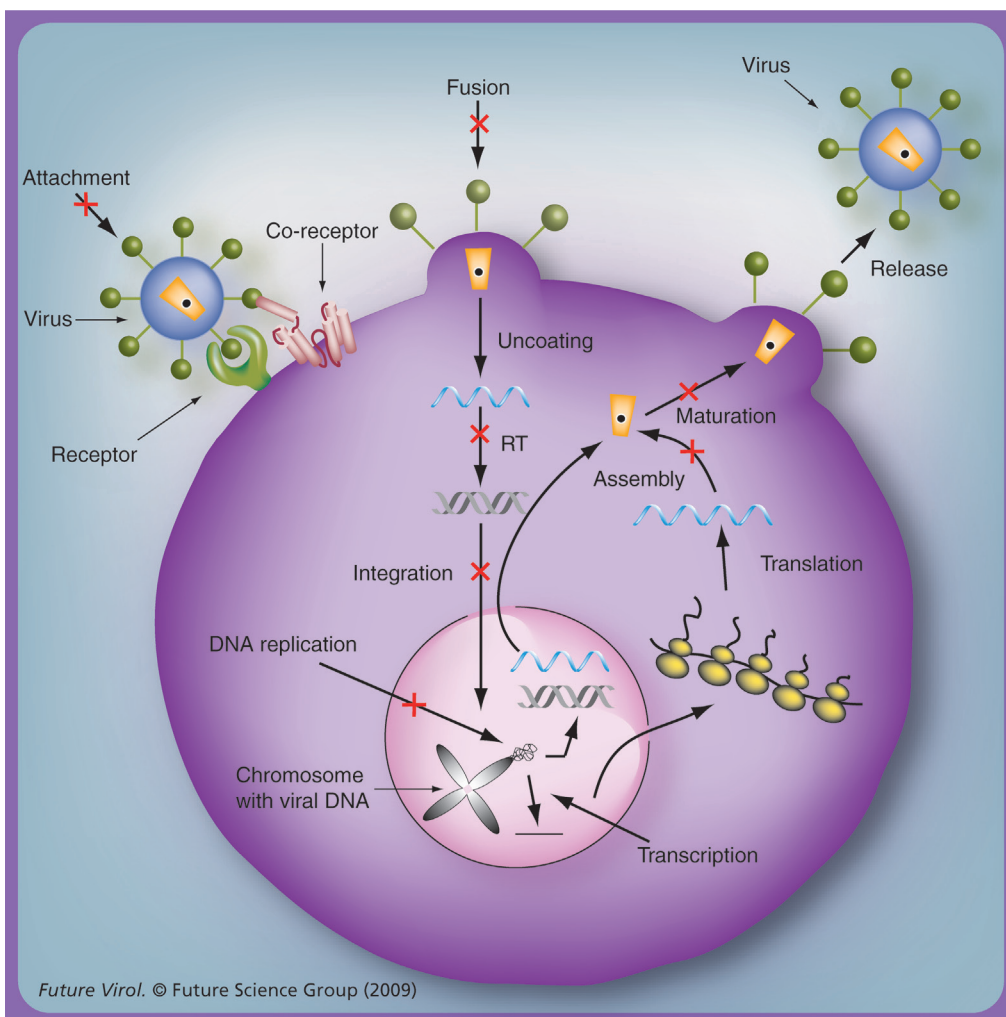


Figure 2. Restriction points in virus replication. Various restriction points (red x) in the virus life cycle are targeted against viruses to prevent viral replication. As mentioned in this figure, these restriction points (red x) may be utilized in the attachment and fusion of the virus to the host cell, uncoating and release of nucleic acids, reverse transcription, integration, DNA polymerization or assembly and maturation of the virus. RT: Reverse transcription.

Table 1. Mechanisms of action of the antiviral drugs.

Antiviral drugs	Target	Mode of action	Example
Fusion inhibitors	Viral surface proteins or host cell receptors	Inhibit binding, fusion and entry of virus to the host cell and thus prevent noninfected cells from infection	<ul style="list-style-type: none"> • Cyclosporine • Enfuvirtide • Maraviroc • Docosanol • Amantadine • Palivizumab
DNA polymerase inhibitors	Viral DNA polymerase	Binds directly to active site of DNA polymerase or terminate growing chain of DNA, as lack 3'OH group thus prevents the further formation of phosphodiester bonds	Nucleoside analog: <ul style="list-style-type: none"> • Idoxuridine • Vidarabine • Acyclovir Nucleotide analog: <ul style="list-style-type: none"> • Cidofovir Pyrophosphate analog: <ul style="list-style-type: none"> • Foscarnet • Phosphonoacetic acid
RT inhibitors	Viral replication	Inhibits action of RT by either binding directly to the enzyme or terminating the growing DNA chain	NRTIs: <ul style="list-style-type: none"> • Zidovudine • Didanosine • Zalcitabine • Stavudine • Lamivudine ntRTIs: <ul style="list-style-type: none"> • Tenofovir • Adefovir NNRTIs: <ul style="list-style-type: none"> • Efavirenz • Nevirapine • Delavirdine • Etravirine
Integrase inhibitors	Integration of viral DNA to host genome	Blocks the action of integrase	<ul style="list-style-type: none"> • Raltegravir
Portmanteau inhibitors	RT as well as integrase inhibitors		Researchers are currently attempting to design a suitable drug
Protease inhibitors	Activity of viral proteases	Inhibits the cleavage of nascent proteins of virus needed for assembly and formation of new virions	<ul style="list-style-type: none"> • Saquinavir • Indinavir • Amprenavir • Nelfinavir • Ritonavir
Signaling inhibitors	Signaling pathways	Prevents signaling by blocking any key component of the signaling pathways, which are essential for viral replication	<ul style="list-style-type: none"> • Resveratrol • Ribavirin • Diethylthiocarbamate

NNRTI: Non-nucleoside reverse transcriptase inhibitor; NRTI: Nucleoside analog reverse transcriptase inhibitor; ntRTI: Nucleotide analog reverse transcriptase inhibitor; RT: Reverse transcriptase.

same class. Nelfinavir/saquinavir causes CR with indinavir/ritonavir [18], and delaviridin shows CR against nevirapine [19]. Application of antivirals during co-infections and opportunistic infections is an alarming concern, as treatment with one antiviral may lead to antiviral resistance to various viruses. In order to minimize resistance, several measures have been suggested, which include the use of combination therapy and avoiding prolonged/discontinuing use of antiviral drugs.

Latency: dormant potential

The prolonged use of antivirals may result in viral latency, since antivirals are ‘virustatic’ not ‘virucidal’, and as a result, as soon as the antiviral treatment is stopped, the latent virus starts proliferating once more, which is evident from the fact that treatment with acyclovir does not prevent future recurrences of HSV [17]. The study of latency-associated factors in the virus may provide a solution to the problem.

Tedious & risky: the irksome

Antiviral discovery and development is a long, tedious, costly and risky process. The pharmaceutical industry is currently facing serious challenges, which include rising research and development costs, declining productivity in the approval of new drugs and the short life-span of antivirals. Nearly 10–15 years, and almost US\$1 billion is required to bring a new drug to market, and the expense is expected to double by 2010. Most of the expense is attributed to costly late-stage failure [103].

Alternative approaches: multiple guns against a single target

To overcome the aforementioned disadvantages, scientists are now looking towards host factors and pathways involved in virus replication and pathogenesis. By regulating the host system, viral infection may be tackled as the virus uses host cellular machinery for its replication, pathogenesis and maintenance in the host. Therefore, some alternative approaches such as antisense oligonucleotides, ribozymes, aptamers and drug delivery systems are in development.

Use of antisense oligonucleotides: biological ballistic missile

The antisense nucleotide approach involves the targeting of RNA, by RNA interference (RNAi). RNAi is a mechanism that inhibits gene expression either at the transcriptional or translational level by targeting viral RNA, and also plays a role in regulating development and genome maintenance. The RNAi approach has been used widely to control viral infection [20]. Small interfering RNAs (siRNAs) and microRNAs (miRNAs) are key to the RNAi process. siRNA and miRNA are 18–23-nt long ssRNA molecules acting as critical regulators in complex gene regulatory networks in eukaryotes by regulating the translation of mRNA by binding to it, effecting target cleavage or blocking translation. Many reports have shown that siRNA inhibits infection caused by HIV-1 [21], HCV [22] and poliovirus [23]. Application of miRNA mir-32 restricts the accumulation of the primate retrovirus foamy virus type 1 in human cells [24]. miRNAs are supposed to be able to tackle the high rate of mutations in viruses better than siRNAs [25]. siRNAs and miRNAs are promising and potent candidates for developing novel biotherapeutics against various viruses. RNAi is still in its developmental stage and many questions such as short-lived nature, efficiency,

efficacy and specificity during its application need to be addressed. Transient expression and low and variable transfection efficiency is also of great concern.

Aptamers: the knight in action

Aptamers are oligonucleic acid or peptide molecules that bind a specific target molecule and interfere with interacting proteins inside cells. They consist of a variable peptide loop attached at both ends to a protein scaffold [26]. Aptamers targeting NS-3 protease and helicase have been shown to inhibit HCV [27]. Peptide aptamers are capable of inhibiting HBV by blocking capsid formation, replication and virion formation [28]. The major limitation of this approach is the problem in delivery that is still unanswered. Pleiomorphism, high molecular mass, stability and restricted range of target sites are the problems associated with the usage of aptamers.

Ribozymes: a favorite warrior

Ribozymes are RNAs working as enzymes and catalyzing hydrolysis of one of their own phosphodiester bonds or of any other RNAs. Hairpin ribozymes effectively reduce HBV replication [29] and provide protection against HIV and HCV [30,31]. The use of ribozymes as therapeutic tools is important only at low-level viral gene expression, such as during later stages of treatment and latency. It is very difficult to treat using ribozyme when viral expression is fully activated [32]. This approach may be extended to develop genetically modified cells capable of producing ribozymes, which, when injected to the patient, may work even when viral expression is high. In order to implement it as an effective antiviral, specificity, instability and mutations in target RNA are the potential restrictions.

Drug delivery systems: launchpad targeting a bulls eye

Targeted delivery of antivirals are still of great concern. This helps to decrease toxicity and dosage, and may enhance the stability, specificity and efficiency of antivirals. Carriers such as liposomes [4] and nanoparticles [33] are used for this purpose, but owing to the instability of carriers, rapid release or the bioavailability of antivirals remains a major problem. Recently, some novel methods such as the use of heterodimer-loaded erythrocytes [34] with azidothymidine and self-microemulsifying systems with acyclovir [35] have shown encouraging results.

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Future perspective: peeping through the window

In spite of increasing knowledge about the virus and host, treatment regimes and antiviral developments, many questions still remain unanswered and we need to work on them to develop better therapeutics and antivirals in the future. The most important one is to address antiviral resistance, otherwise, like antibiotics, antivirals may also be rendered useless in future. Thus, there is a need to characterize newer molecules as antivirals for more specificity, less toxicity, proper bioavailability and multiple targets to overcome antiviral resistance-related concerns. For this, the secondary properties of antivirals need to be studied and proper assays based on computational screening, cell culture and animals models need to be developed. Antiviral resistance is most common in immunocompromized individuals. Thus, before the start of therapy, the immunological status of patients should be available to clinicians. Since there are very few available antivirals, there is a need to speed up the development of efficient antivirals so that available drugs may be supplemented with others. Combination therapy finds a good opportunity here. However, even in combination therapy, prolonged usage of a single antiviral needs to be avoided. Treatment regimes need to be followed strictly without discontinuous or inadequate usage. Techniques to check laboratory and clinical resistance should be worked out. Computational as well as laboratory methods also need to be developed to find out the crucial mutations important for resistance.

Biomedicines, in other words, medicines based on host factors, may be the best alternative, which will restrict viral replication and latency with little or no toxicity, and help us to fight antiviral resistance. We need to focus on the treatment of curable viruses such as HCV, as until such a date, there is no specific treatment regime available and, as a result, viruses are getting more resistant to antivirals used against HIV infection. Stable and targeted delivery with high transfection efficiency and bioavailability is also important to reduce cost, toxicity and to optimize dosage. Computational and *in vivo* techniques play a major role making screening easy, and to track

the movement and effects of the drug in living systems but, unfortunately, it is still in early stages. Developments in this field may help us to characterize the secondary characteristics of molecules and will reduce failure at the clinical level, which in turn would bring down the cost of antivirals.

Conclusion

The continuous emergence and re-emergence of viruses during the last few decades is a serious public health concern. This may be due to viral latency, improper diagnosis, viral resistance, toxicity and immunosuppression caused by antivirals. To date, we are struggling against viruses and lack foolproof antiviral therapies against them. The failure of drugs in human clinical trials is adding salt to the wound. Many alternative approaches, along with past drug discovery efforts and recent advances in biology and chemistry, have shown some promise to the therapeutic management of life-threatening viral infections. The recent development in gene inhibition by RNAi, regulation of gene expression by aptamers, ribozymes, various novel drug delivery systems, *in silico* resources and *in vivo* imaging may be used to validate a large number of potential antivirals in a site-specific manner in a short timespan. Therefore, the use of a combinatorial approach may result in the rapid development of better antivirals in the near future. The battle between humans and viruses is gaining greater magnitude, and both are rapidly improving strategies to succeed. The growing knowledge about viruses and the rapidly evolving tools and techniques are definitely promising, but there is still a long way to develop effective measures for combating the viral diseases. The journey is still continuing...

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