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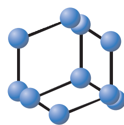

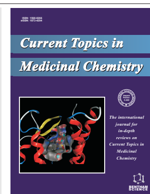

Discovery and Development of Anti-HIV Therapeutic Agents: Progress Towards Improved HIV Medication

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Abstract: The history of the human immunodeficiency virus (HIV)/AIDS therapy, which spans over 30 years, is one of the most dramatic stories of science and medicine leading to the treatment of a disease. Since the advent of the first AIDS drug, AZT or zidovudine, a number of agents acting on different drug targets, such as HIV enzymes (*e.g.* reverse transcriptase, protease, and integrase) and host cell factors critical for HIV infection (*e.g.* CD4 and CCR5), have been added to our armamentarium to combat HIV/AIDS. In this review article, we first discuss the history of the development of anti-HIV drugs, during which several problems such as drug-induced side effects and the emergence of drug-resistant viruses became apparent and had to be overcome. Nowadays, the success of Combination Antiretroviral Therapy (cART), combined with recently-developed powerful but nonetheless less toxic drugs has transformed HIV/AIDS from an inevitably fatal disease into a manageable chronic infection. However, even with such potent cART, it is impossible to eradicate HIV because none of the currently available HIV drugs are effective in eliminating occult "dormant" HIV cell reservoirs. A number of novel unique treatment approaches that should drastically improve the quality of life (QOL) of patients or might actually be able to eliminate HIV altogether have also been discussed later in the review.

Keywords: HIV, AIDS, Combination antiretroviral therapy, Reverse transcriptase inhibitors, Protease inhibitors, Integrase inhibitors.

1. INTRODUCTION

Since the identification of the first acquired immunodeficiency syndrome (AIDS) patient in the US in 1981, the progress of antiretroviral therapy for HIV-1 infection and AIDS has been very rapid, making it unique in the history of medicine [1, 2]. The first AIDS drug, zidovudine (or AZT) was developed and deployed within 10 years of the identification of the virus [3, 4]. First generation anti-HIV-1 drugs, such as AZT, are all HIV-1 reverse transcriptase inhibitors (RTIs) [3]. Subsequently, efforts by scientists enabled the identification of several additional targets for antiviral therapy, such as HIV-1 protease (PR) and Integrase (IN) [5, 6]. In the mid-1990s, treatment of HIV-1 infection/AIDS was revolutionized by the development of HIV-1 protease inhibitors and the introduction of combination antiretroviral therapy (cART). This new therapy strongly suppressed viral replication, reduced plasma HIV-1 viral load resulting in a significant reconstitution of the immune system [7-9]. The life expectancy of HIV-1-infected patients treated with cART improved significantly after 1996, and mortality rates for HIV-1-infected persons have now become close to general mortality rates [10-12]. Thus, successful development of cART has changed HIV-1 infection and AIDS from an inevitably fatal disease into a manageable chronic infection. However, a number of obstacles were encountered when conducting cART in the early days, such as drug-related toxicities and the emergence of drug resistance against all the then-existing antiretroviral regimens.

To overcome these problems, attempts have been made to develop more potent and safer anti-HIV-1 drugs effective against any existing drug-resistant HIV-1 strains and as a result, some new generation RTIs (*e.g.* tenofovir disoproxil fumarate) and protease inhibitors (PIs) (*e.g.* darunavir) were developed [13, 14]. In addition, new regimens focusing on other targets, such as integrase inhibitors (INSTI, *e.g.* dolutegravir) and entry inhibitors, have also been developed [15-18]. Thus, we can now say that most patients, even those with prehistory of treatment failure, can be successfully treated if they receive a new recommended Combination

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Antiretroviral Therapy (cART) regimen (consisting of 2 "backbone" NRTIs and a "key-drug" INSTI or PI). However, while nearly 22 million people with HIV-1/AIDS worldwide are receiving ART at present, that number accounts for slightly less than two-thirds of infected individuals worldwide, due to limited diagnosis and ineffective treatment in developing countries [19-21]. The UN has committed to the goal of ending the AIDS pandemic by 2030. In order to achieve this, UN has aimed for diagnosis of 90% of HIV-1 cases and treatment with cART to have sustained viral suppression by 2020 [22]. Hence, a continuous effort is still needed to establish a way to provide effective antiretroviral drugs around the world, including low-income countries in a cost-effective way.

This review will first describe the development of anti-HIV-1 drugs, especially the dramatic progress in increasing activity and reducing the toxicity of recently developed small molecule agents. Later some novel unique approaches toward developing safer and more effective treatment options have been discussed.

2. REVERSE TRANSCRIPTASE INHIBITORS (RTIS): DISCOVERY OF THE FIRST HIV-1/AIDS DRUGS

2.1. Factors Involved in the HIV-1 Life Cycle as Targets for Anti-HIV-1 Agents

The HIV-1 life cycle (Fig. **1**) consists of several steps, starting with the attachment of an HIV-1 particle to the host cell membrane, where interactions between HIV-1-gp120 (HIV-1 envelope) and the cell surface CD4 molecule are followed by binding to the chemokine receptors CXCR4 or CCR5 [23-32]. These specific interactions induce the activation of the HIV-1 fusion protein (gp41) and consequently fusion between the cell membrane and the viral membrane [33-36]. Thereafter, the contents of the virion are released into the cell's cytoplasm, where viral RNA is transcribed to double-stranded DNA by RNA-dependent DNA polymerase or HIV-1 reverse transcriptase (HIV-1-RT). Subsequently, viral DNA is integrated into the host chromosome. After transcription and translation into viral proteins using the cell's own machinery, Gag and Gag-Pol polyproteins thus produced the move to the cell membrane, where the assembly, budding, and maturation of virions occurs to finally release the functional HIV-1 particles.

In principle, anti-HIV-1 drugs should target either viral proteins or cellular proteins that are related to the HIV-1 life cycle (Fig. **1**). In addition, the interaction of such small molecules with target proteins should ideally yield HIV-1-specific inhibitory effects with low toxicity [37-39]. In fact, the first anti-HIV-1 small molecule agent, an HIV-1-RT inhibitor, had already been developed in the mid-1980s, based on this strategy [3, 38].

2.2. Development of Nucleoside/Nucleotide Reverse Transcriptase Inhibitors (NRTIs) and Non-nucleoside Reverse Transcriptase Inhibitors (NNRTIs)

The reverse transcriptase (RT) of HIV-1, which is a DNA polymerase capable of using RNA as a template, converts a single-stranded viral RNA into a double-stranded proviral DNA, which is subsequently integrated into the host cell genome. HIV-1-RT is a heterodimer consisting of a 66-kD polypeptide (p66) and a 51-kD polypeptide (p51) subunits. The structure of RT can be compared to that of the human hand. The palm domain contains the catalytic site for DNA polymerization. The flexible fingers and thumb domains fold over the nucleic acid form a cavity, where the template-primer is positioned for DNA synthesis. A connecting domain in the p66 subunit links the active site of RT to the RNase H domain, which contains the catalytic site for ribonuclease H [40, 41].

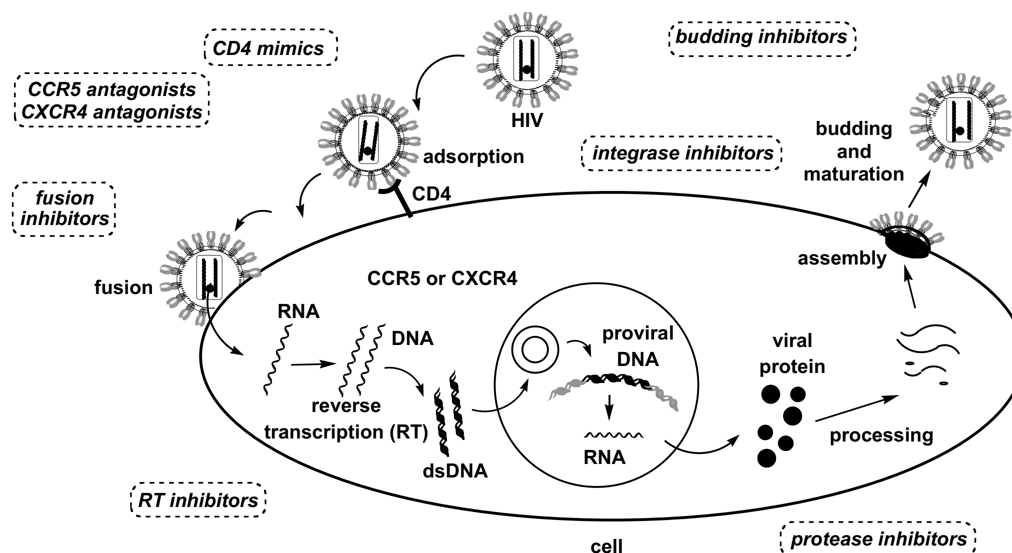

Fig. (1). HIV-1 replication cycle and anti-HIV-1 agents that target its several steps. Molecular mechanisms of replication cycle (life cycle) are well understood from entry of HIV to generation of new matured viral particles; (i) adsorption and membrane fusion, (ii) reverse transcription, (iii) integration, (iv) processing, (v) assembly, (vi) budding, (vii) maturation, *etc.* Several anti-HIV drugs have been reported in the last three decades: reverse transcriptase (RT) inhibitors including nucleoside reverse transcriptase inhibitors (NRTIs) and non-nucleoside reverse transcriptase inhibitors (NNRTIs), protease inhibitors (PIs), integrase inhibitors (INIs), entry/fusion inhibitors, *etc.*

The first-ever approved anti-HIV-1 drug, 3′-azido-2′,3′-dideoxythymidine (zidovudine or AZT) was a nucleoside analog (Fig. **2** and Table **1**) [3]. Subsequently, several other nucleoside analogues, such as 2,3 -dideoxynucleosides, have been approved for treating HIV-1/AIDS (Fig. **2**) [38, 42]. Such nucleoside analogs lack 3′-OH in their ribose moiety, which enables HIV-1-RT to differentiate them from physiologic dNTP substrates. Thus, the mechanism of action of NRTIs includes both competitive inhibition and termination of polymerization once the drug is incorporated. Chain termination occurs either during RNA-dependent DNA or DNA-dependent DNA synthesis, inhibiting the production of a strand of the proviral DNA [39, 43-45]. Nucleoside RT inhibitors enter cells and are then phosphorylated to achieve their active triphosphate form. On the other hand, Tenofovir disoproxil fumarate (TDF), a nucleotide RT inhibitor, exists in a monophosphate form and needs only two additional phosphorylation steps inside cells to become active. Currently, there are nine Food and Drug Administration (FDA)-approved NRTIs (excluding drug combinations), namely, zidovudine (AZT), didanosine (ddI), zalcitabine (ddC), stavudine (d4T), lamivudine (3TC), Abacavir (ABC), Tenofovir Disoprovil Fumarate (TDF), a nucleotide analog, emtricitabine (FTC), and Tenofovir Alafenamide (TAF) (Table **1** and Fig. **2**).

Small molecule inhibitors in clinical use that act by blocking HIV-1-RT are either nucleoside reverse transcriptase inhibitors (NRTIs) (Fig. **2**) or non-nucleoside reverse transcriptase inhibitors (NNRTIs) (Fig. **3**). The latter consist of chemically diverse compounds that bind to a hydrophobic pocket [the non-nucleoside inhibitor binding pocket (NNIBP)] located approximately 10 angstroms from the RT polymerase active site [46]. NNRTIs do not directly prevent template-primer or dNTP binding to RT, but cause misalignment of the template-primer to the catalytic site, thereby preventing incoming dNTP from being incorporated [47, 48]. Unlike NRTIs, NNRTIs do not inhibit RTs of other lentiviruses such as HIV-1-2 and simian immunodeficiency virus (SIV) [40, 49]. The first-generation NNRTIs were discovered by screening compounds that inhibited RT activity. Currently, there are 6 approved NNRTIs, namely etravirine (ETR), delavirdine (DLV), nevirapine (NVP), efavirenz (EFV), rilpivirine (RPV) and doravirine (DOR) (Fig. **3**).

2.3. Emergence of NRTI Drug Resistance and the Development of New NRTIs Active Against Drug-resistant HIV-1 Strains

The emergence of NRTI and NNRTI drug resistance during long-term therapy has been one of the biggest problems since AZT resistance was first recognized [50] (Table **2**). Resistance to NRTIs is caused by two mechanisms: 1) increased discrimination between the native deoxyribonucleotide substrate (dNTP) and the NRTI-triphosphate (NRTI-TP), resulting in decreased NRTI incorporation, and 2) ATP-mediated excision of incorporated NRTIs, resulting in the reversal of chain termination.

Among mutations associated with the first mechanism, Q151M was previously reported in patients during combination therapy with NRTIs. It resulted in high-level NRTI cross-resistance [51]. Multidrug-resistant HIV-1 strains with Q151M mutations were also found to contain common mutations at amino acid positions A62, V75, F77, and F116 of RT (referred to as the Q151M complex). The loss of the Q151 amide group causes a disruption in the hydrogen bond network necessary for positioning the 3′-OH of the incoming dNTP substrate. However, NRTIs that lack the 3′-OH are more sensitive to changes in the hydrogen bond network caused by Q151M substitution, resulting in the selective incorporation of dNTP over most NRTIs [52]. M184V and M184I are mutations that confer high-level resistance to 3TC and FTC [53-55]. M184 is located in the highly conserved YMDD motif found in all retroviruses and contributes two of the three catalytic aspartic acid residues of the RT polymerase domain. The side chains of either valine or isoleucine in the M184V or -I mutants lead to increased contact with the dNTP or NRTI-TP sugar ring. The bulky L-oxathiolane rings of NRTIs such as 3TC and FTC make these compounds particularly vulnerable to steric hindrance resulting from these mutations. Thus, M184V/I results in high-level resistance to lamivudine or emtricitabine [56-58]. K65 is located in the fingers domain and conformational changes initiated by nucleotide binding bring it into the proximity of the dNTP binding site, where it serves to orient the triphosphate moiety of the nucleotide [58, 59]. K65R is an important mutation found in clinical isolates that engenders resistance to NRTIs such as ddI, ddC, d4T, 3TC, ABC and Tenofovir (TDF) [60-63].

ATP-mediated excision of incorporated NRTIs represents another mechanism of NRTI resistance. Combined mutations, referred to as thymidine analog mutations (TAMs) or nucleoside analog mutations (NAMs), consist of changes at the following RT amino acid positions: M41L, D67N, K70R, T215Y/F, and K219Q/E/N [50]. These confer high levels of resistance to AZT and d4T and also cross-resistance (albeit lower-level) to other NRTIs [64-66] (Table **2**).

TDF (tenofovir disoproxil fumarate) (Fig. **2**), a prodrug of tenofovir, is the only NtRTI (nucleotide reverse transcriptase inhibitor) and one of the most commonly used anti-HIV-1/AIDS drugs. TDF has mostly been used as a once-a-day fixed-dose tablet (Truvada®) that is combined with emtricitabine (FTC) to treat HIV-1-infected individuals including those who carry HIV-1 strains that are resistant to other existing NRTIs, as well as to treat patients with HBV infection. As described above, K65R is a mutation related to resistance to TDF. K65R has not often emerged in patients receiving any AZT-containing regimen, as this mutation is thought to be phenotypically antagonistic to TAMs. In addition, the M184V mutation restores TDF susceptibility in the presence of K65R; thus, drug-resistant variants with the K65R mutation remain treatable in patients who failed regimens with 3TC or with FTC in the presence of an M184 mutation [67-69] (Table **2**).

All currently approved NRTIs lack a 3′-OH moiety, which was thought to be essential for NRTI mediation of chain terminator activity. However, a number of 4′-ethynyl-2′-deoxynucleoside analogues (EdNs) that maintain the 3′-OH in their sugar moiety and still show activity against HIV-1 have also been reported [70-72]. Through the optimization of such EdNs, 4′-ethynyl-2-fluoro-2′- deoxyadenosine (EFdA or MK8591) (Fig. **2**) has been recently developed

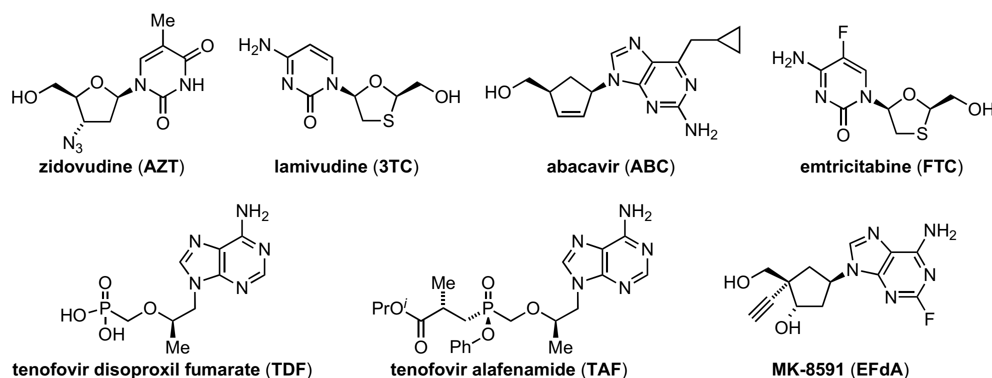

Fig. (2). Chemical structures of HIV-1 nucleoside/nucleotide reverse transcriptase inhibitors (NRTIs).

Table 1. Currently available FDA-approved anti-HIV drugs.

Class	Generic Name	Brand Name	Approved Year
Nucleoside reverse transcriptase inhibitors (NRTIs) [Figure 2]			
	zidovudine (AZT, ZDV)	Retrovir	1987
	lamivudine (3TC)	Epivir	1995
	abacavir (ABC)	Ziagen	1998
	tenoforvir disoproxil fumarate (TDF)	Viread	2001
	emtricitabine (FTC)	Emtriva	2003
	tenofovir alafenamide (TAF)	(combined drug)	2016
Non-nucleoside reverse transcriptase inhibitors (NNRTIs) [Figure 3]			
	nevirapine (NVP)	Viramune	1996
	efavirenz (EFV)	Sustiva	1998
	etravirine (ETR)	Intelence	2008
	rilpivirine (RPV)	Edurant	2011
	doravirine (DOR)	Pifeltro	2018
Protease inhibitors (PIs) [Figure 5]			
	saquinavir (SQV)	Invirase	1995
	ritonavir (RTV)	Norvir	1996
	lopinavir (LPV)	(combination drug)	2000
	atazanavir (ATV)	Reyataz	2003
	fosmaprenavir (FOS-APV)	Lexiva	2003
	tipranavir (TPV)	Aptivus	2005
	darunavir (DRV)	Prezista	2006
Integrase inhibitors (INSTIs) [Figure 7]			
	raltegravir (RAL)	Isentress	2007
	elvitegravir (EVG)	(combination drug)	2012
	dolutegravir (DTG)	Tivicay	2013
	bictegravir (BIC)	(combination drug)	2018

(Table 1) contd….

Class	Generic Name	Brand Name	Approved Year
Fusion inhibitor			
	enfuvirtide (T-20)	Fuzeon	2003
CCR5 inhibitor [Figure 13]			
	maraviroc (MVC)	Selzentry	2007
Attachment inhibitor			
	ibalizumab-uiyk (IBA)	Trogarzo	2018
Combination drugs			
	3TC/AZT	Combivir	1997
	LPV/RTV (LPVr)	Kaletra	2000
	ABC/3TC/AZT	Trizivir	2000
	FTC/TDF	Truvada	2004
	ABC/3TC	Epzicom	2004
	EFV/FTC/TDF	Atripla	2006
	FTC/RPV/TDF	Complera	2011
	EVG/cobisistat (COBI)*/FTC/TDF	Stribild	2012
	ABC/DTG/3TC	Trimeq	2014
	ATV/COBI	Evotaz	2015
	DRV/COBI	Prezcobix	2015
	EVG/COBI/FTC/TAF	Genvoya	2015
	FTC/RPV/TAF	Odefsey	2016
	FTC/TAF	Descovy	2016
	DTG/RPV	Juluca	2017
	EFV/3TC/TDF	Symfi	2018
	BIC/FTC/TAF	Biktarvy	2018
	3TC/TDF	Cimduo	2018
	DRV/COBI/FTC/TAF	Symtuza	2018
	DOR/3TC/TDF	Delstrigo	2018

Cobisistat (COBI): Inhibitor of CYP3A, used with other anti-HIV drug(s) to improve their PK and anti-HIV efficacy.

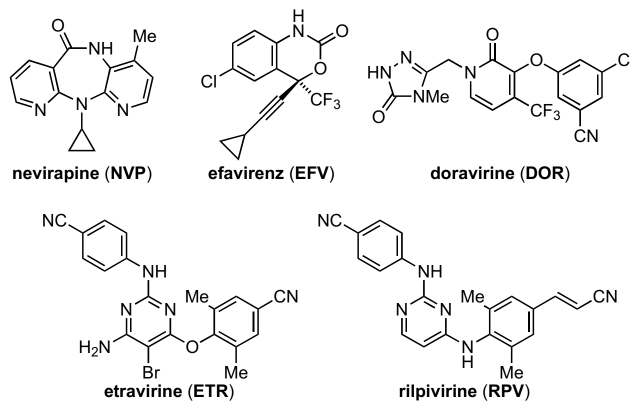

Fig. (3). Chemical structures of HIV-1 non-nucleoside reverse transcriptase inhibitors (NNRTIs).

Table 2. Drug resistance mutations.

NRTI Resistance Mutations													
Amino acid (Consensus)	**41 (M)**	**65 (K)**	**67 (D)**	**69 (T)**	**70 (K)**	**74 (L)**	**115 (Y)**	**151 (Q)**	**184 (M)**	**210 (L)**	**215 (T)**	**219 (K)**	
3TC	-	R	-	Ins*	-		-	M	VI	-	-	-	-
FTC	-	R	-	Ins	-		-	M	VI	-	-	-	-
ABC	L	R	-	Ins	E	VI	F	M	VI	W	FY	-	-
ddI	L	R	-	Ins	E	VI	-	M	VI	W	FY	-	-
TDF	L	R	-	Ins	ER	-	F	M	-	W	FY	-	-
d4T	L	R	N	Ins	ER	-	-	M	-	W	FY	QE	-
AZT	L	-	N	Ins	R	-	-	M	-	W	FY	QE	-
NNRTI Resistance Mutations													
Amino acid (Consensus)	**100 (L)**	**101 (K)**	**103 (K)**	**106 (V)**	**138 (E)**	**181 (Y)**	**188 (Y)**	**190 (G)**	**230 (M)**	**-**	**-**	**-**	**-**
DOR	I	EP	-	AMI	-	CIV	LHC	SE	L	-	-	-	-
EFV	I	EP	NS	AM	-	CIV	LCH	ASE	L	-	-	-	-
ETR	I	EP	-	-	AGKQ	CIV	L	ASE	L	-	-	-	-
NVP	I	EP	NS	AM	-	CIV	LCH	ASE	L	-	-	-	-
RPV	I	EP	-	-	AGKQ	CIV	L	ASE	L	-	-	-	-
PI Resistance Mutations													
Amino acid (Consensus)	**30 (D)**	**32 (V)**	**33 (L)**	**46 (M)**	**47 (I)**	**48 (G)**	**50 (I)**	**54 (I)**	**76 (L)**	**82 (V)**	**84 (I)**	**88 (N)**	**90 (L)**
ATV	-	I	F	IL	V	VM	L	VTALM	-	ATFS	V	S	M
DRV	-	I	F	-	VA	-	V	LM	V	F	V	-	-
FPV	-	I	F	IL	VA	-	V	VTALM	V	ATFS	V	-	M
IDV	-	I	-	IL	V	-	-	VTALM	V	ATFS	V	S	M
LPV	-	I	F	IL	VA	VM	V	VTALM	V	ATFS	V	-	M
NFV	N	-	F	IL	V	VM	-	VTALM	-	ATFS	V	DS	M
SQV	-	-	-	-	-	VM	-	VTALM	-	AT	V	S	M
TPV	-	I	F	IL	VA	-	-	VAM	-	TL	V	-	-
INSTI Resistance Mutations													
Amino acid (Consensus)	**66 (T)**	**92 (E)**	**118 (G)**	**138 (E)**	**140 (G)**	**143 (Y)**	**147 (S)**	**148 (Q)**	**155 (N)**	**263 (R)**	**-**	**-**	**-**
BIC	K	Q	R	KAT	SAC	-	-	HRK	H	K	-	-	-
DTG	K	Q	R	KAT	SAC	-	-	HRK	H	K	-	-	-
EVG	AIK	Q	R	KAT	SAC	-	G	HRK	H	K	-	-	-
RAL	AIK	Q	R	KAT	SAC	RCH	-	HRK	H	K	-	-	-

* Insertion mutation.

[71-74]. EFdA/MK8591 exerts highly potent activity against HIV-1, including multi-drug-resistant variants *in vitro* and *in vivo* [71, 72, 75, 76]. Previous reports showed that three amino acid substitutions (I142V, T165R, and M184V) in the RT were associated with HIV-1 developing a moderate resistance [72]. Another report demonstrated that the emergence of EFdA-resistant HIV-1 was significantly delayed when the selection was performed *in vitro*, and nine amino acid substi-

tutions, namely, M41L, D67delta, T69G, K70R, L74I, V75T, M184V, T215F, and K219Q, were associated with EFdA resistance [77]. Regarding the mechanism of the strong activity of EFdA against drug-resistant HIV-1s, a recent paper reported that EFdA and 4'-ethynyl-NRTIs (but not other 4'-modified NRTIs) formed strong Van der Waals interactions with critical amino acid residues of reverse transcriptase. Such interactions were maintained even in the presence of a broad resistance-endowing M184V substitution, thus potently inhibiting drug-resistant HIV-1 strains (Fig. **4**) [78]. MK8591 (EFdA) has now been forwarded to clinical development by Merck & Co. (See Section 6.3).

2.4. Emergence of NNRTI Drug Resistance and the Development of New NNRTIs Active Against Drug-resistant HIV-1 Strains

NNRTIs are allosteric inhibitors that induce conformational changes in the binding pocket. The allosteric binding site is located within a short distance (~15 A) of the catalytic site [79, 80], and consists of hydrophobic residues such as Y181 and Y188, and hydrophilic residues such as K101, K103, and D192 of the p66 subunit and E138 of the p51 subunit [81]. Of the NNRTI-associated drug-resistant mutations (Table **2**), Y181C and K103N often lead to resistance to many different NNRTIs because of their cross-resistance profile [82]. Second generation NNRTIs were developed to address this drug resistance problem. A pyrimidine added to form the diarylpyrimidine (DAPY) compound etravirine (ETR, TMC125), has significant activity against HIV-1 with K103N substitution. As described above, K103N causes cross-resistance to NNRTIs by interacting with Y188, preventing access of NNRTIs to the NNIBP [83]. The central pyrimidine linker of ETR may directly interact with the asparagine of K103N. This may prevent the K103N interaction with Y188 that results in NNRTI resistance. Another DAPY compound, rilpivirine (RPV or R278474), has been synthesized in an effort to improve on its dapivirine (TMC120) predecessor [84]. RPV contains a cyanovinyl group in one of its wing moieties that strengthens interactions with a conserved tryptophan at position W229, one of the conserved residues in RT [85]. Such strengthened interactions with W229 and the inherent flexibility of DAPY compounds are likely explanations as to why rilpivirine is more potent against NNRTI-resistant mutants, relative to 1st generation NNRTIs. RPV has a longer half-life and reduced side-effect profile compared with older NNRTIs and was approved for use in the United States in 2011. As with other currently employed drugs, RPV is used as a fixed-dose agent combining rilpivirine with FTC and tenofovir disoproxil (TDF), approved in 2011 (Complera®). A newer combination of RPV with FTC and tenofovir alafenamide (TAF) was approved in 2016 (Descovt®).

3. PROTEASE INHIBITORS (PIS): ADVENT OF COMBINATION ANTIRETROVIRAL THERAPY (CART)

3.1. Development of HIV-1 protease Inhibitors (PIs) and Initiation of cART

As of this writing, there are 41 approved anti-HIV-1 drugs (counting only those in current use) that belong to 7 different classes including combinations (Table **1**). The first anti-HIV-1 drugs were given as monotherapies or a cocktail of NRTI(s) in the early 1990s. Then, thanks to the introduction of the first protease inhibitor (PI), saquinavir, in 1995 (Fig. **5**), combination antiretroviral therapy (cART), also known as highly active antiretroviral therapy (HAART), was initiated for the treatment of HIV-1 infection. This resulted in reduced morbidity and mortality associated with HIV-1/AIDS [10-12].

The HIV-1 protease is an aspartyl protease that cleaves the HIV-1 Gag and Gag-Pol polypeptides to generate structural proteins along with enzymes of the virus. This processing occurs late in the HIV-1 life cycle-during assembly and release from infected cells, which is an essential step for the formation of mature viral particles. The HIV-1 protease consists of two identical 99 amino acid subunits and has an active site that lies at the dimer interface with each monomer contributing a single catalytic aspartic acid residue (D25 and D25′). Two flexible β-sheets with a conserved glycine residue form a flap region over the top of the active site. The flaps are flexible enough to allow entry and exit of the polypeptide substrates, thus inducing a conformational shift to enclose the active site when the enzyme is bound to a substrate [86, 87]. Polypeptide substrates bind to the enzyme in an extended conformation with a minimum of seven amino acid residues interacting with the enzyme, denoted P4 to P1 and P1′ to P4′ in the standard nomenclature (Fig. **6A**) [87].

There are currently seven PIs approved for the treatment of HIV-1 infection (Table **1** and Fig. **5**). All are competitive inhibitors that bind to the protease active site. Since saquinavir, many other first-generation inhibitors have been developed such as ritonavir, indinavir, nelfinavir, and amprenavir between 1996 and 1999. It is noteworthy that ritonavir was found to be a potent inhibitor of cytochrome P450 3A, a major metabolic enzyme for protease inhibitors [88]. Because of this finding, ritonavir is used more frequently as a pharmacokinetic booster.

3.2. Emergence of PI Drug Resistance and Development of New Generation PIs Active Against Drug-resistant HIV-1 Strains

Most PIs, especially those of the 1st generation, share relatively similar chemical structures (Fig. **5**) and cross-resistance is commonly observed (Table **2**). Primary drug-resistant mutations are mostly found near the active site of the enzyme, at positions located at the substrate/inhibitor binding site (*e.g.*, D30N, G48V, I50V, V82A, and I84V) (Fig. **6A**). Because of their location near the substrate binding cleft, these amino acid changes usually have a deleterious effect on replicative fitness. On the other hand, mutations outside the binding cavity that do not affect inhibitor binding directly, serve to compensate for deleterious effects on enzymatic activity caused by primary mutations. These compensatory mutations are referred to as secondary mutations [89-91]. Subsequently, additional mutations associated with PI-resistance have been found outside the protease enzyme coding region, near the cleavage sites of Gag substrates. These mutations in the gag region also appear to be secondary mutations that compensate for the reduced catalytic efficiency caused by primary protease mutations

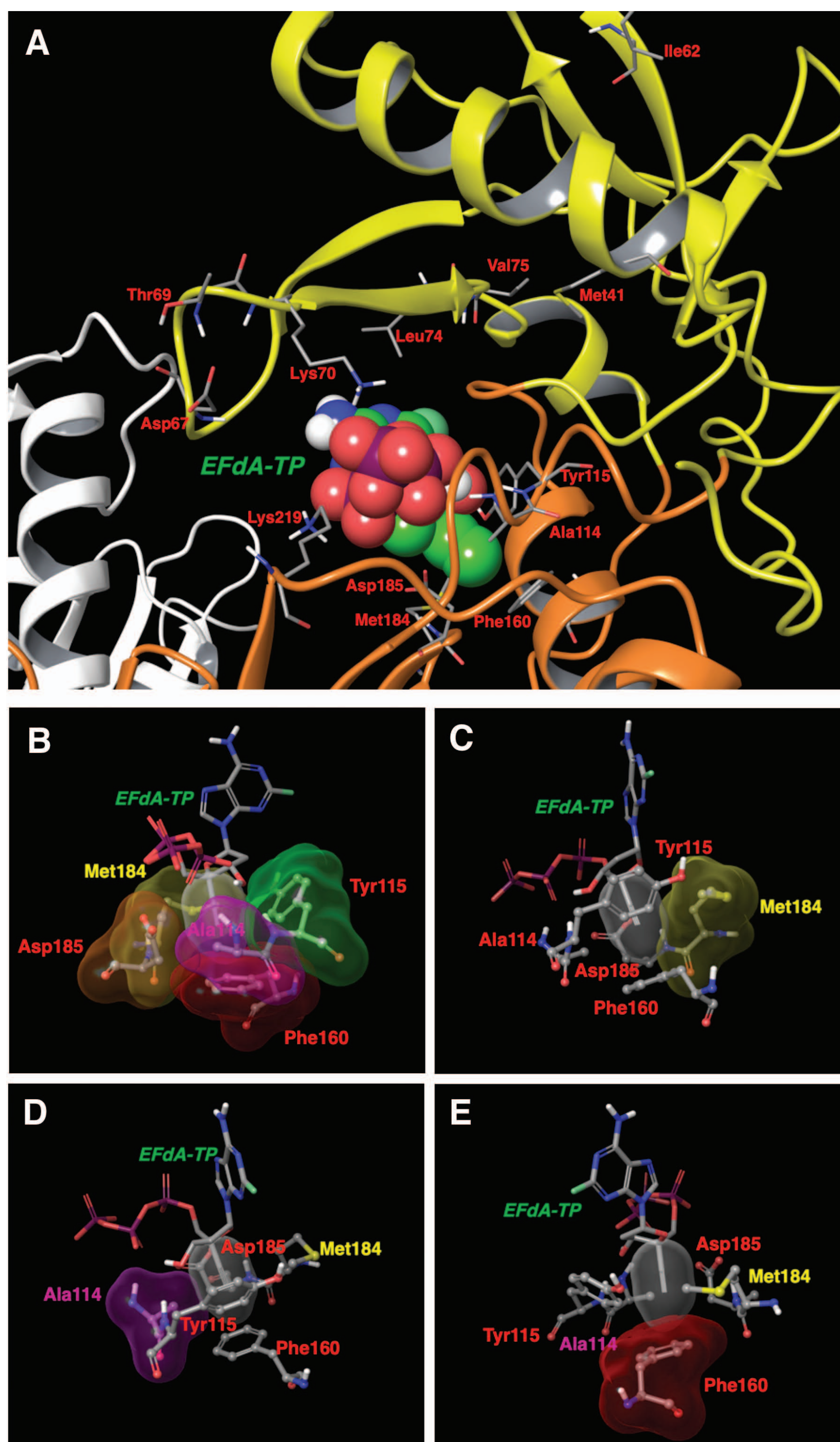

Fig. (4). Structure of wild-type HIV-1 RT with EFdA-TP. **A**: EFdA shown in CPK mode, selected RT residues are shown as thick sticks. Some of these RT residues are implicated in drug resistance, and some (Ala114, Phe160, and Asp185) are in the active site responsible for tight interaction with EFdA [77, 78]. The finger region of the RT is shown as a yellow ribbon, and the palm region is shown in orange. Parts of the other RT domains are shown as white ribbons. **B**: EFdA-TP in the active site cavity of HIV-1 RT (PDB: 5J2M) [243]. The hydrophobic pocket of the wild-type HIV-1 RT active site and EFdA-TP are shown. The 4'-ethynyl group of EFdA showed good vdW interactions with several residues, such as A114, Y115, F160, M184, and D185, in the active site cavity of RT. EFdA-TP strongly interacts with these residues and shifts positions inside the active site, thus terminating DNA polymerization [78, 244]. **C-E**: Detailed vdW interactions between the 4'-ethynyl group of EFdA and residues in the active site cavity of HIV-1 RT, namely, Met184 (**C**), Ala114 (**D**), and Phe160 (**E**). These amino acids show good interaction with the 4'-ethynyl group of EFdA. Surface colors: 4'-ethynyl, white; A114, magenta; Y115, green; F160, red; M184, yellow; D185, orange. RT residues are shown as ball and stick, EFdA shown as thick sticks.

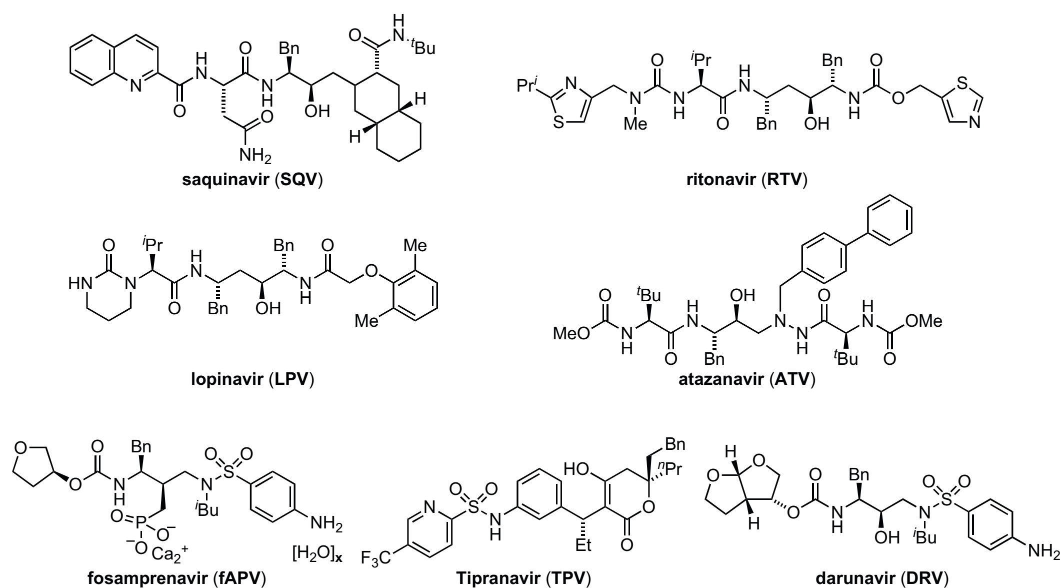

Fig. (5). Chemical structures of HIV-1 protease inhibitors (PIs).

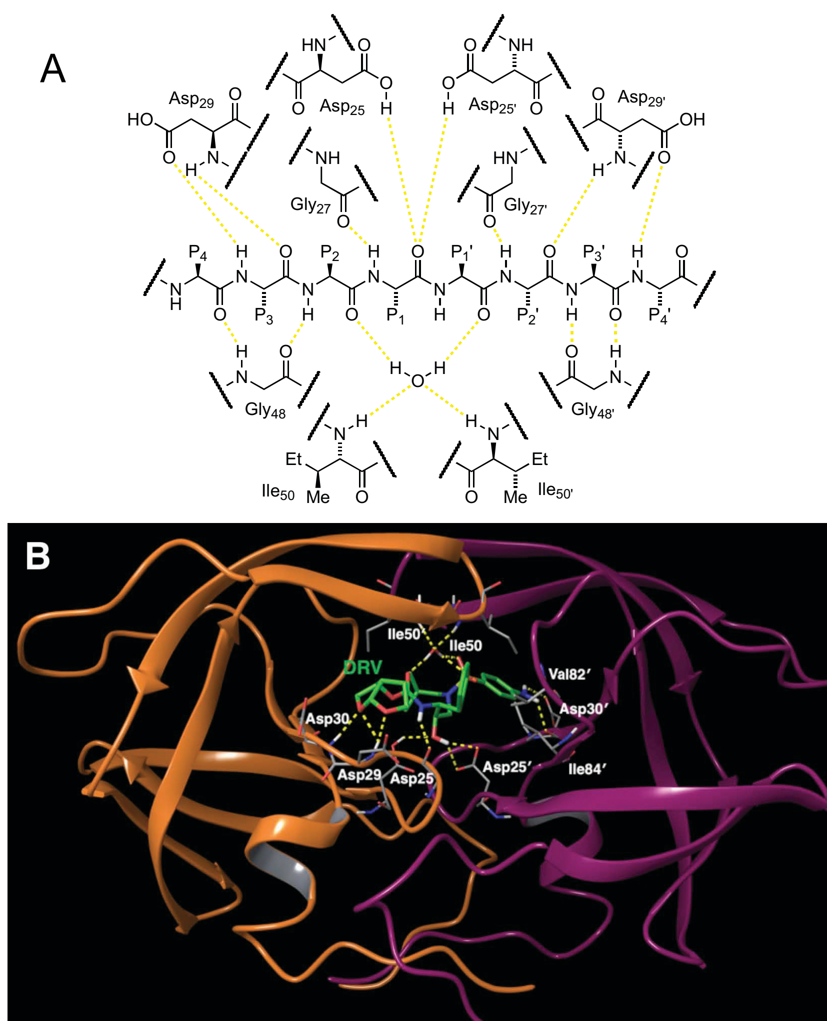

Fig. (6). A: Binding of polypeptides in the HIV-1 protease active site. Hydrogen bonds shown as yellow dotted lines. Polypeptide substrates bind to the enzyme in an extended conformation with a minimum of seven amino acid residues interacting with the enzyme, denoted P4 to P1 and P1′ to P4′ by standard nomenclature [14]. **B**: Structure of HIV-1 protease and an inhibitor (DRV) bound to the active site. Protease (PDB code 4HLA) ribbons in orange and maroon, DRV in green carbons, selected protease residues in gray carbons. Polar interactions are shown by yellow dashed lines.

[92-95]. Thus, high-level drug resistance to PIs requires the stepwise accumulation of multiple primary and secondary mutations to generate a protease capable of discriminating inhibitor from natural substrate, yet able to maintain adequate catalytic efficiency needed for virus replication [89, 96].

As described above, the therapeutic efficacy of first-generation PIs was limited due to the emergence of drug-resistant HIV-1 strains. In addition, because of their peptidic nature, they had a relatively short half-life and poor oral bioavailability, requiring frequent dosing. After 2000, some PIs such as lopinavir, atazanavir and amprenavir (Fig. **5**) came to the market. They had improved profiles in terms of their oral dosing (once daily) characteristics or a higher genetic barrier to drug resistance, requiring many mutations in the protease region.

The most recently approved PI, darunavir (DRV or TMC114), was approved in 2006 for treatment-experienced adult patients and then for treatment-naive patients in 2008 [13, 14, 97-99]. DRV, a highly active nonpeptidic PI, maintained potency against multidrug-resistant HIV-1 strains with a high genetic barrier for the development of resistance in preclinical studies [13, 100]. However, multidrug-resistant HIV-1 variants have nonetheless emerged in DRV-experienced patients. In clinical trials (POWER trial 1 and 2), it was found that 11 protease mutations (V11I, V32I, L33F, I47V, I50V, I54L/M, G73S, L76V, I84V, and L89V) were associated with diminished DRV virological responses. However, it is noteworthy that DRV demonstrated significantly greater efficacy than other control PIs in trials of treatment-experienced patients, regardless of baseline viral genotype or phenotype, while exhibiting a high genetic barrier to the development of resistance [97, 101-103].

In vitro structural analyses revealed that the close contact of DRV with the main chains of the protease active-site amino acids (D29 and D30) is important for its potency and wide spectrum of activity against multi-PI-resistant HIV-1 variants (Fig. **6B**) [13]. In addition, it is known that DRV strongly inhibits dimerization of HIV-1 protease [104]. Because dimerization of protease monomers is essential for the catalytic function of HIV-1 protease, inhibition of protease dimerization represents a novel approach to inhibiting HIV-1 progression with a high genetic barrier to resistance [14, 104].

4. DEVELOPMENT OF INTEGRASE STRAND TRANSFER INHIBITORS (INSTIS): KEY DRUGS OF CURRENT CART REGIMENS

4.1. Role of HIV-1 Integrase and the Development of Integrase Strand Transfer Inhibitors (INSTIs)

HIV-1 integrase is an enzyme that catalyzes the insertion of proviral cDNA synthesized from viral RNA genome into the genome of infected cells. The integration of viral DNA consists of two catalytic steps: 3'-processing and DNA strand transfer. In the first step, proviral DNA is primed for integration by integrase-mediated trimming of the 3′-ends. Integrase remains bound to the proviral DNA as a multimeric complex, designated the Preintegration Complex (PIC), consisting of reverse transcriptase, matrix, nucleocapsid and an accessory protein, Vpr, which enables PICs to enter the nucleus from the cytoplasm. Inside the nucleus, integrase then catalyzes the insertion of proviral DNA into the chromosomes of the host cells. This process, called “strand transfer” consists of the ligation of the proviral DNA’s 3′-OH ends (generated through the 3′- processing) to the 5′-DNA phosphate of the host cell chromosome. Subsequently, host enzymes complete the integration process by repairing the single-strand gaps abutting the unjoined viral DNA 5' ends, resulting in the establishment of a stable provirus. All integrase inhibitors block the strand transfer reaction [and are thus referred to as integrase strand transfer inhibitors (INSTIs)]. Additionally, INSTIs recognize and bind to the specific complex between integrase and the viral DNA during strand transfer (Fig. **7**) [105-112].

The first experimental integrase inhibitors were reported in the early 1990s, but the advent of the first-in-class, clinically approved integrase inhibitor took more than a decade [113, 114]. One of the diketo acid (DKA)-like compounds, 5CITEP [1-(5-chloroindol-3-yl)-3-hydroxy-3-(2*H*-tetrazol-5-yl)propenone] (Fig. **8**), discovered by scientists at Shionogi, possesses a tetrazole group in place of the common DKA carboxylic acid moiety. 5CITEP inhibited both 3′-processing and strand transfer. 5CITEP was subsequently reported in a complex with the CCD (catalytic core domain), providing the first crystal structure for HIV-1 integrase [115]. Later, scientists at Merck discovered a group of potent inhibitors, the most active of which proved to contain a DKA moiety that was capable of coordinating metal ions within the integrase active site [15].

Raltegravir (RAL or MK-0518) is the first in the class of INIs approved by the FDA for the treatment of HIV-1/AIDS (Table **1** and Fig. **7**). RAL is a selective strand-transfer inhibitor and also possesses a DKA scaffold. Clinical trials (BENCHMRK-1 and -2) were conducted to evaluate the safety and efficacy of RAL in combination with optimized background therapy of HIV-1-infected patients with limited treatment options. Patients were randomly assigned to RAL or placebo, and RAL plus optimized background therapy provided better viral suppression than optimized background therapy alone at week 48 [116]. Thus, RAL was initially approved for use in individuals resistant to other drugs in 2007, and thereafter it was also approved for use in drug-naive patients in 2009.

Elvitegravir (EVG, GS-9137 or JTK-303) (Table **1** and Fig. **7**) represents another DKA-like integrase inhibitor developed initially by Japan Tobacco and then by Gilead Sciences. Thanks to the favorable pharmacokinetic profile of EVG combined with ritonavir (RTV), once-daily dosing of EVG with RTV became possible. Thus, EVG (approved in 2012) brought about a major advantage for the treatment of HIV-1/AIDS with a once-daily single-tablet regimen (EVG/COBI/FTC/TDF). This significantly improved patients´ compliance [117, 118].

4.2. Drug-resistance to 1st Generation INSTIs and the Development of New Generation INSTI(s)

Once RAL came to the market as the first INSTI in 2007, it rapidly became a blockbuster anti-HIV-1 drug. However, it was also shown that HIV-1 drug-resistant mutations in the

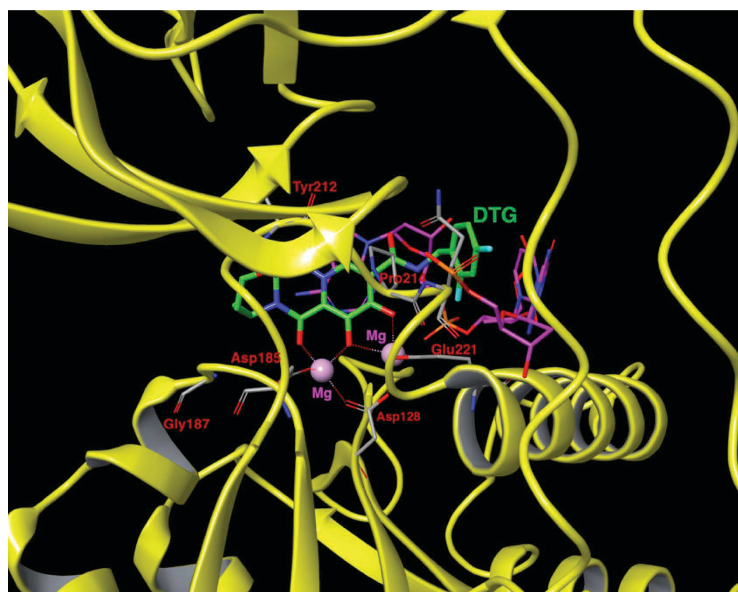

Fig. (7). Structure of HIV-1 integrase and an inhibitor (DTG) bound to the active site. Figure made from PDB ID: 3S3M. The figure has purple carbons for DNA, gray carbons for protein (yellow ribbons), and green carbons for DTG. Fluorine, phosphorous, oxygen and nitrogen atoms are shown in cyan, orange, red and blue respectively. Magnesium is shown as pink spheres. DTG is represented as thick sticks, the protein and DNA atoms are represented as thin sticks.

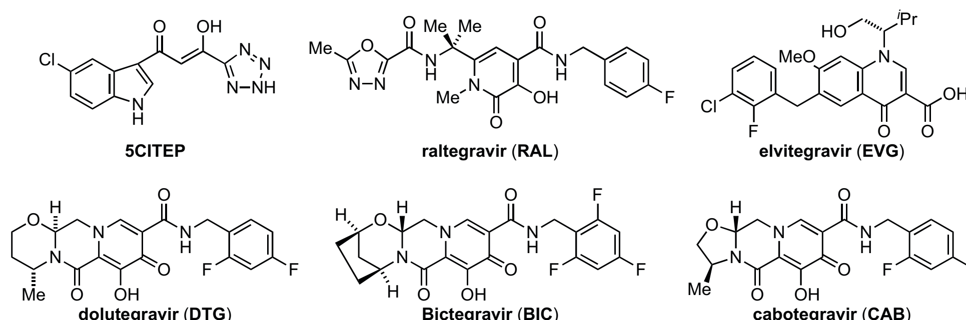

Fig. (8). Chemical structures of HIV-1 integrase inhibitors (INSTIs, strand-transfer inhibitors).

CCD of integrase appeared in patients receiving RAL-containing regimens (Table **2**). Mutations associated with RAL treatment include Q148 (H/R/K), E138 (A/K), G140 (A/S), T66A, Y143 (C/R), and N155H. Among these, mutations at Y143, N155, or Q148 are defined as signature mutations; for example, Q148K together with E138K and G140A mutations reduced susceptibility to RAL >100-fold [106, 119-121]. While EVG confers a moderate genetic barrier to integrase resistance, it was shown to elicit mutations including T66I, E92Q, Q146P. In addition, other mutations have also emerged which are associated with RAL-based treatment failure and result in cross-resistance between RAL and EVG [122, 123].

To overcome such RAL/EVG resistance, a new generation INSTI was developed. Dolutegravir (DTG or S/GSK1349572) is a potent INSTI, developed by Shionogi & Co. Ltd. and ViiV Healthcare, which came to the market in 2013. DTG displayed highly favorable antiviral activity against RAL-resistant clinical HIV-1 strains isolated from patients experiencing virologic failure while receiving RAL. In addition, the pharmacokinetic features of DTG supported once-daily dosing without boosting with RTV [16, 124]. Structurally, the carbonyl of the C-5 carboxamide on DTG (Fig. **7**) renders DTG more flexible, allowing it to be more embedded into the hydrophobic pocket of the integrase active site. In addition, it was reported that DTG readjusts its position and conformation in response to structural changes in the mutated active site that was responsible for resistance to RAL. Thus, DTG can maintain high potency against RAL/EVG-resistant HIV-1 strains [111, 112, 125, 126] (Table **2**).

5. HIV-1 ENTRY INHIBITORS (EIS): A PEPTIDE FUSION INHIBITOR (T-20) AND CCR5 INHIBITORS

5.1. HIV-1 Entry as a Target for Anti-HIV-1 Therapeutics

During HIV-1 replication, a dynamic supramolecular mechanism associated with HIV-1 entry steps has been elucidated in detail (Fig. **1**). The HIV-1 envelope protein gp120 interacts with a cell surface protein CD4, resulting in a conformational change of the former which subsequently binds to a second receptor, which can be a chemokine receptor such as CCR5 [27-31] or CXCR4 [32]. The conformational change of gp120 causes the exposure of another envelope protein gp41 and then penetration of its *N*-terminus through

the cell membrane. This phenomenon causes the formation of the gp41 trimer-of-hairpins structure and subsequent fusion of the HIV-1 membrane and the cell membrane [36]. Elucidation of the dynamic molecular machinery of this infection process has encouraged medicinal chemists to develop agents which block HIV-1-entry steps by targeting the cellular receptors CD4, CCR5 and CXCR4 and the viral proteins gp120 and gp41. In this regard, in 2003 the FDA approved the first "fusion inhibitor", designated enfuvirtide (fuzeon/T-20, Roche/Trimeris), for use in combination with other anti-HIV-1 drugs to treat advanced HIV-1 infections [127]. Subsequently, in 2007, the FDA approved a CCR5 co-receptor antagonist, maraviroc (MVC, Pfizer), in combination with other anti-HIV-1 drugs for the treatment of patients infected with the R5 HIV-1 virus [128]. In the case of the co-receptor CXCR4, several antagonists have been synthesized to date. However, no drug has been approved as an anti-HIV-1 agent, although some antagonists such as AMD3100 [129] and T140 derivatives [130, 131] have proven useful for different indications including hematopoietic stem cell mobilization and cancer treatment. In addition, CD4-related small compounds, CD4 mimics, which bind to gp120, will be described in section 7.2 below. Thus, this paper focuses on the success of a fusion inhibitor, enfuvirtide, and a CCR5 antagonist, MVC, including the development of other agents in these categories.

5.2. T-20 (Peptide Fusion Inhibitor)

The binding of gp120 to CD4 and CCR5 or CXCR4 causes the formation of the trimer-of-hairpins structure of gp41 and the subsequent fusion of the HIV-1/cell membrane as described in Section 5.1 above. The trimer-of-hairpins structure, which is referred to as a six-helical bundle structure, is formed by a central parallel trimer of the *N*-terminal helical region (HR1 region) surrounded by the *C*-terminal helical region (HR2 region) that is oriented in an antiparallel, hairpin fashion (Fig. **9**). To date, several HR2 region-related peptides, which inhibit bundle formation of six α-helices by the binding to the inner three-stranded coiled coils of the HR1 region, have been reported (Fig. **10**) [132]. An HR2 region peptide, C34, a 34-mer from the native sequence of gp41 of the HIV-1_{NL4-3} strain, has high inhibitory activity against membrane fusion [35]. In addition, a 36-residue peptide, T-20, with the native sequence of gp41 sharing 24 residues with C34, shows potent anti-HIV-1 activity. This has been used in the clinic as the first HIV-1 entry/fusion inhibitor [127] and was designated enfuvirtide (fuzeon, Roche/Trimeris). Combinational therapy of enfuvirtide with other antiretroviral agents was approved for the treatment of advanced HIV-1 disease in patients 6 years of age or older with evidence of HIV-1 replication notwithstanding current therapy and of resistance to current anti-HIV-1 drugs. Because of the clinical use of enfuvirtide, the dynamic supramolecular mechanism involving membrane fusion has become a valid and rational target for inhibitors. The success of enfuvirtide has encouraged us to develop entry/fusion inhibitors as a new class of anti-HIV-1 drugs. While inhibitors such as RTIs, PIs and INSTIs work internally in cells to inhibit functions of viral enzymes, entry/fusion inhibitors work extracellularly, preventing HIV-1 from invading into cells. The prevention of entry/fusion by inhibitors is a great advantage of these drugs. An HR2 region peptide, C34, has an exact interface that is capable of binding to a central parallel trimer of the HR1 region, whereas enfuvirtide has a poorly delineated interface. However, a disadvantage of C34 is its poor aqueous solubility. Therefore, highly soluble versions of C34 derivatives, SC peptides, have been developed through the artificial remodeling of C34 by Otaka *et al*. (Fig. **10**) [133]. The design of SC peptides maintained the amino acid residues at the a, d, and e positions of the helical wheel diagram of the C34 peptide. These positions are indispensable for binding to the inner three-stranded coiled coils of the HR1 region and cannot be substituted, whereas non-conserved residues at the b, c, f, and *g* positions, located in the exterior and solvent-contact region, were replaced by Glu-Lys pairs (Fig. **10B**). Salt bridges formed by several Glu-Lys side-chain pairs located between i and i+4 positions enhance solubility and α-helicity of the C34 derivatives (Fig. **10C**). In practice, the aqueous solubility of the SC peptides, SC34 and SC34EK, is more than three orders of magnitude higher than that of C34. The anti-HIV-1 activities of SC34 and SC34EK were superior or similar to that of C34, and an order of magnitude greater than enfuvirtide [134]. In addition, SC34 and SC34EK were even effective against an enfuvirtide-resistant strain. C34, SC34 and SC34EK lack the *C*-terminal lipid-binding domain of enfuvirtide, suggesting that these agents have a mechanism of action distinct from that of enfuvirtide [135]. Thus, these SC peptides have been leads for further refinement and clinical development. C34, T-649 [136], enfuvirtide and SC peptides are 34- to 36-mers derived from the HR2 region of gp41. T-1249 (Roche/Trimeris), a successor to enfuvirtide [136], has a hydrophobic *C*-terminal sequence, which might interact with lipid bilayers in a manner similar to the interaction mediated by enfuvirtide. However, the clinical trial of this agent was already discontinued in 2005 because of formulation problems. Development of small non-peptide inhibitors that block gp41 activation has also been attempted [137-139].

Regarding the development of an AIDS vaccines, the HR1 region is useful because the six-helical bundle structure is formed by the interaction of the HR2 regions to the central trimer of the HR1 regions. The development of artificial antigens that efficiently induce broadly neutralizing antibodies is desirable. An inspired strategy is to design molecules that mimic the natural inner three-stranded coiled coils of the HR1 region of gp41 on the virion surface. Thus, a novel three-helical bundle mimetic of the trimeric form of N36 completely equivalent to three N36 peptides was synthesized based on an original template with C_3-symmetric linkers (Fig. **11A**) [139]. The antiserum produced by immunization of mice with the N36 trimeric antigen showed an approximately 30-fold higher affinity for the N36 trimer than for the N36 monomer and thereby mediated a more potent neutralizing activity compared to antibodies generated by immunization with the N36 monomer. The exposure of the target epitopes is limited to the time of HIV-1 entry, and carbohydrates, which might disturb access of the antibodies to their epitopes, are not included in the amino acid sequences of the native HR1 region [140]. These two points are advantageous for vaccine design based on the HR1 region of gp41. In terms of HIV-1 inhibition, both the N36

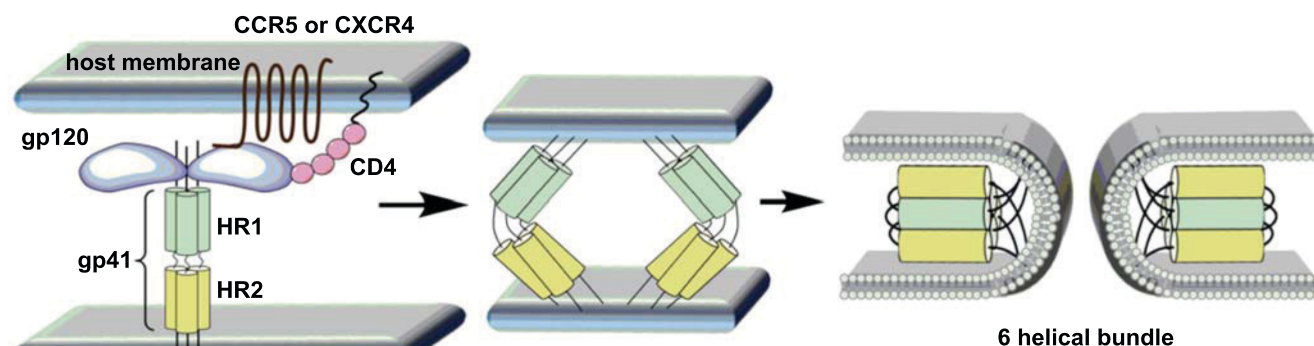

Fig. (9). Brief mechanisms of HIV-1 entry and fusion. The HIV-1 envelope protein gp120 interacts with a cell surface protein CD4, resulting in a conformational change of gp120, which subsequently binds to a second receptor, CCR5 or CXCR4. The conformational change of gp120 causes the exposure of another envelope protein gp41 and penetration of its *N*-terminus through cell membranes, and then the formation of the gp41 trimer-of-hairpins structure, which is referred as a six-helical bundle structure. This bundle structure is formed by a central parallel trimer of the *N*-terminal helical region (HR1 region) surrounded by the *C*-terminal helical region (HR2 region) that is oriented in an antiparallel and hairpin fashion. This bundle formation causes fusion of HIV/cell-membranes and results in completion of the infection.

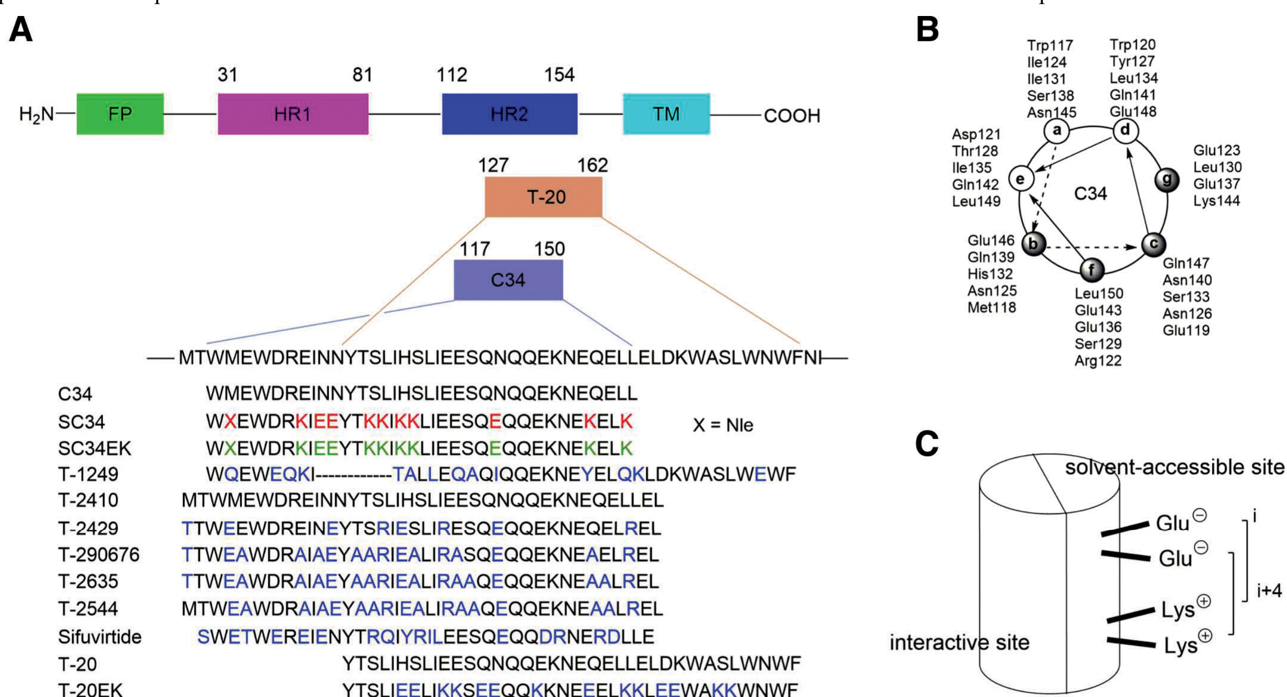

Fig. (10). A: Schematic representation of HIV-1 gp41 and amino acid sequences of HR2 region peptides (HIV-1_{NL4-3} strain). **B**: Helical wheel representation of the C34 peptide. Numbering of amino acid residues is based on gp41 of the HIV-1_{NL4-3} strain. **C**: The design concept of introduction of the Glu-Lys pairs to the solvent-accessible site.

monomer and trimer have modest but not strong inhibitory activity [141].

A novel three-helical bundle structure of the trimeric form of C34 completely equivalent to three C34 peptides has also been designed and synthesized. In this case, the novel C_3-symmetric template depicted in Fig. (**11B**) was prepared [142]. The inhibitory effect of the C34 trimer is 100-fold stronger than that of the C34 monomer. This suggests that a trimeric form is critical as the potent structure of the inhibitor. However, in terms of efficacy as an HIV-1 vaccine, neither the C34 trimer or the monomer is superior to the N36 trimer [143]. A C34-derived dimer with PEG linkers was prepared to elucidate the inhibitory mechanism against the fusion of the C34 trimer [144, 145]. The inhibitory activity of the C34 dimer was found to be approximately equal to that of the C34 trimer, suggesting that the C34 units in the dimer can bind to the inner three-stranded coiled coils of the HR1 region of gp41 in a cooperative manner [144]. A dimeric form of C34 is evidently a potent C34-related fusion inhibitor [145].

5.3. CCR5 Inhibitors

CCR5 and CXCR4 are members of the family of G protein-coupled, seven-transmembrane segment receptors (GPCRs), which comprise the largest superfamily of proteins in the body. CCR5 serves as one of the two essential co-receptors for HIV-1 entry into human CD4+ cells [24, 25, 27, 28, 31, 32], thereby representing an attractive target for possible intervention to prevent HIV-1 infection. Fig. (**12**) illustrates the crystal structure of CCR5 along with a small

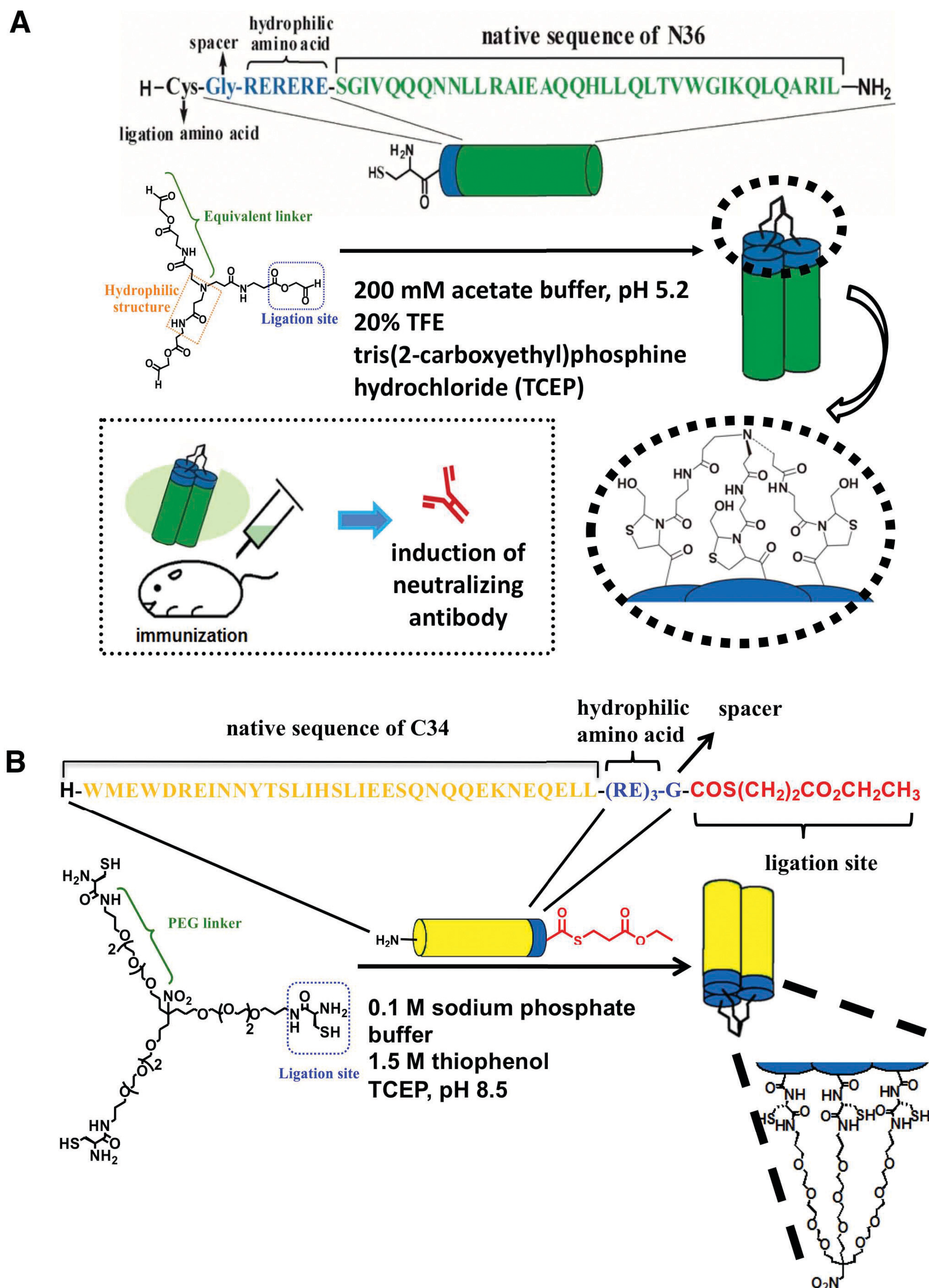

Fig. (11). A: Artificial remodeling of dynamic structures of HR1 regions leads to synthetic antigen molecules inducing neutralizing antibodies. **B:** Three equivalent HR2 region peptides lead to synthetic fusion inhibitors.

molecule CCR5 inhibitor, maraviroc (MVC, Selzentry®, UK-427, 857) (Fig. **13**). CCR5 has a seven trans-membrane helical structure [146, 147], and MVC binds to the largest hydrophobic cavity between extracellular loops and upper transmembrane domains (Fig. **12**). It had been predicted that the binding of a small molecule CCR5 inhibitor to the binding cavity under the extra-cellular domains would cause allosteric changes of CCR5, thus resulting in loss of binding of the HIV-1 envelope (gp120) to CCR5. However, recent studies using cryo-electron microscopy demonstrated that the V3 loop of gp120 directly interacts with amino acid residues in the CCR5 inhibitor binding cavity that locates inside the transmembrane regions of CCR5. Thus, the mechanism of the gp120 binding inhibition by a small molecule CCR5 inhibitor is competitive inhibition [23].

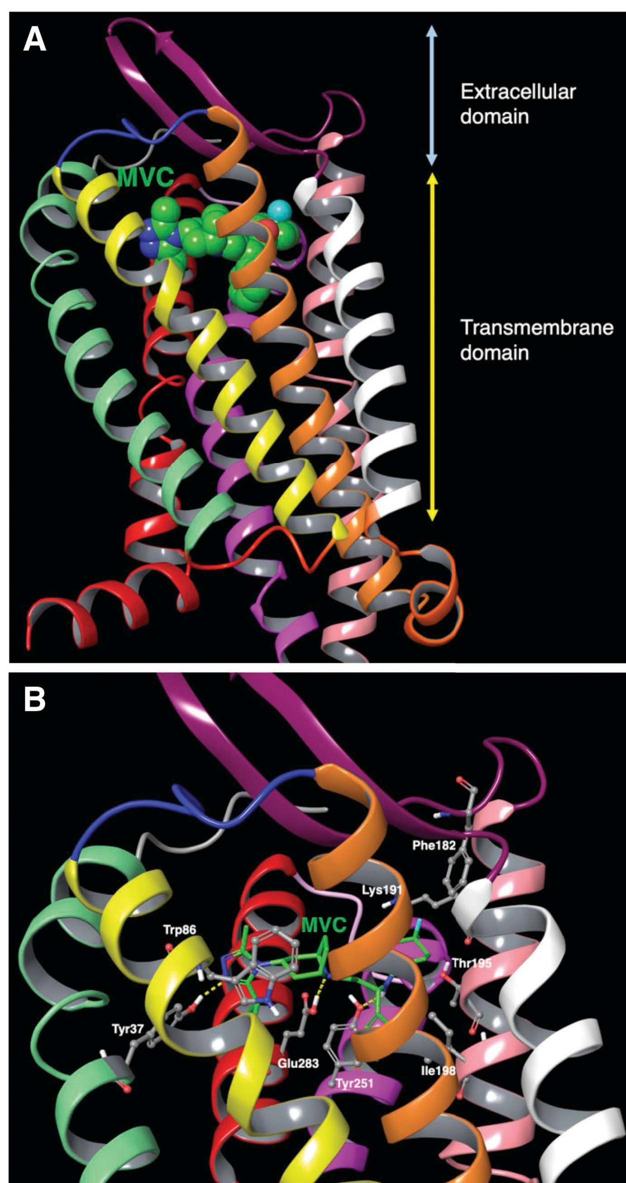

Fig. (12). Structure of CCR5 and a CCR5 inhibitor (MVC) bound in the hydrophobic cavity (PDB ID 4MBS). **A**: Bird's eye view of MVC (CPK mode) bound within CCR5. **B**: The interaction of MVC with selected active site residues of CCR5 as determined from a crystal structure. The hydrogen bond interactions are shown by yellow dotted lines. MVC is shown in thick sticks with green carbons. CCR5 residues are shown in ball and stick representation with gray carbons. Nitrogens, oxygens, polar hydrogens and fluorines are in blue, red, white and cyan respectively. The different CCR5 domains in ribbon representation are colored as follows: TM1 (transmembrane domain 1), aquamarine; TM2, Yellow; TM3, Orange; TM4, White; TM5, Pink; TM6, Purple; TM7, Red; N-term, Gray; ECL1 (extracellular domain 1), Blue; ECL2, Maroon; ECL3, Plum. Figure generated using Maestro version 10.7.015.

The first experimental small molecule CCR5 inhibitor, TAK779 (Fig. **13**), was reported in 1999 [17]. Subsequently, several agents were developed as anti-HIV-1 drugs [18, 148-150]. SCH-351125 (SCH-C) and vicriviroc (VVC or SCH-D) (Fig. **13**) are orally bioavailable CCR5 inhibitors with potent antiviral activity [148, 149]. VVC, which has greater *in vitro* potency than SCH-C, was forwarded to clinical trials. In a Phase IIb clinical study of treatment-experienced patients, VVC in combination with an optimized background regimen resulted in a > 1.5 log10 decrease of plasma viral load. However, in Phase III studies (VICTOR-E3 and VICTOR-E4) enrolling treatment-experienced individuals, no statistically significant difference was observed between the vicriviroc arm and the placebo arm after 48 weeks. For this reason, the development of vicriviroc for the treatment of HIV-1 infection was discontinued by Merck in 2010. Aplaviroc (APL or GSK873140) (Fig. **13**), a spirodiketopiperazine derivative, was developed and reported in 2004 [150]. APL has a high affinity for CCR5 (KD values of ~3 nM), blocked HIV-1-gp120/CCR5 binding, and exerted potent activity against a wide spectrum of R5-HIV-1 isolates including multi-drug-resistant HIV-1 strains *in vitro* (IC_{50} values of 0.1 - 0.6 nM) [150]. In Phase IIb clinical trials, patients receiving 600 mg of APL twice daily had a mean decrease in viral load of ~ 1.6 log10 from baseline. Phase III clinical trials of APL involving ~ 2,000 drug-experienced patients with AIDS were implemented by GSK in the United States in the summer of 2005; however, Grade 4 idiosyncratic hepatotoxicity occurred in a few patients and all trials were terminated in 2005 [151-153].

Among CCR5 inhibitors, in 2007 it was maraviroc (MVC) (Fig. **13**) that became the first-in-class drug approved by the FDA. The safety and efficacy of MVC in antiretroviral therapy-experienced patients were examined in clinical trials (MOTIVATE 1 and 2), and superior virological responses were recorded with lower baseline viral loads and higher $CD4^+$ T-cell counts than seen in the placebo arms [154, 155]. In these studies, before starting drug treatment, patients were confirmed to have R5 virus at the screening by the Trofile assay (Monogram Biosciences, South San Francisco, California, USA). Regarding the acquisition of resistance against CCR5 inhibitors, a clinical trial (the MERIT study) of MVC showed that the level of MVC resistance was low and that the virologic failure observed was mainly caused by the emergence of pre-existing X4-HIV-1 that was not detected by the tropism assay [156].

Recent studies have shown a possible use of CCR5 inhibitors for indications other than use as a conventional anti-HIV-1 drug. Another CCR5 inhibitor, cenicriviroc (CVC or TBR-652) (Fig. **13**) currently under clinical development, was reported to interact with CCR2 as well, which is associated with inflammation-related diseases and is expected to be a potential anti-inflammatory mediator [157]. In fact, Krenkel *et al.* reported therapeutic effects of inhibiting monocyte infiltration in Nonalcoholic Steatohepatitis (NASH) models by using CVC to inhibit the recruitment of Kupffer cells and monocyte-derived cells [158]. Joy *et al.* reported that CCR5 is expressed in cortical neurons after stroke and the administration of a CCR5 inhibitor led to early recovery after stroke and traumatic brain injury [159]. Additionally, the administration of MVC also reportedly results in an increase of $CD4^+$ T-cell counts in the blood, which was considered a possible advantage potentially contributing to increased immunological function in HIV-

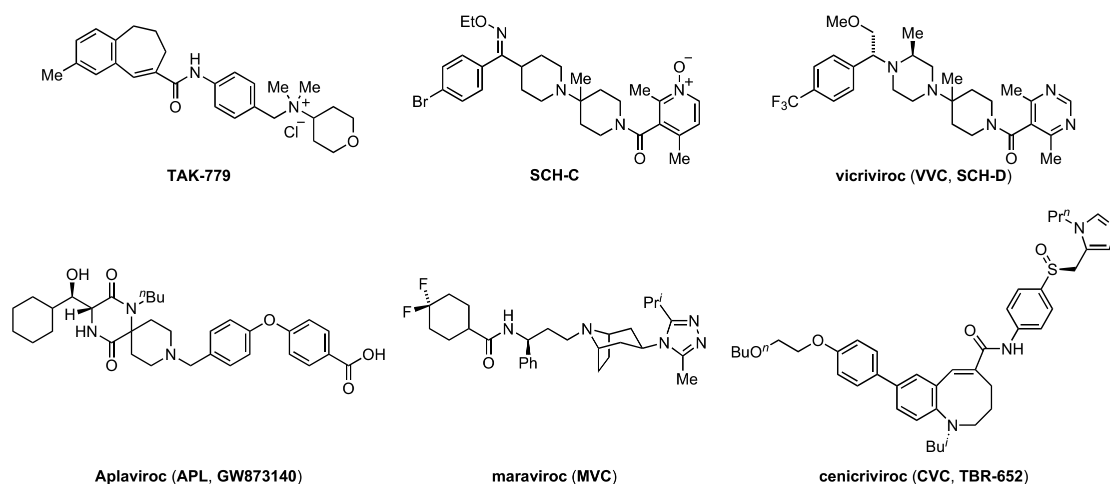

Fig. (13). Chemical structures of CCR5 inhibitors.

1/AIDS patients under treatment [160]. Moreover, another group recently reported that MVC reversed HIV-1 latency *in vitro* alone or in combination with a PKC agonist [161], suggesting the possibility of using MVC as a latency-reversing agent.

Currently, MVC and enfuvirtide are the only drugs that have been approved for clinical use as entry inhibitors. Thus, the development of other entry inhibitors, such as CXCR4 inhibitors, will also improve the utility of CCR5 inhibitors both for regular usage as anti-HIV-1/AIDS drugs, as well as for prophylactic use topically.

6. CURRENT AND FUTURE CART: TOWARDS MORE POTENT AND SAFER TREATMENTS WITH FEWER TABLETS

6.1. From "Many Times a day, Many-tablets" cART to a "Once Daily, Single-tablet" Regimen

As discussed above, the development of a number of new anti-HIV-1 drugs has greatly improved the efficacy of cART for HIV-1/AIDS patients. This improvement is not only seen in therapeutic potency, but also in the less frequent emergence of drug-resistant viruses resulting in reduced occurrence of severe side effects. One example of success in improving the toxicity profile is the development of tenofovir alafenamide fumarate (TAF) (Fig. **2**), approved in 2016. TAF is a prodrug of tenofovir, and is structurally related to tenofovir disoproxil fumarate (TDF) (Fig. **2**), which was developed by Gilead Sciences and approved in 2001 for the treatment of chronic hepatitis B as well as AIDS. TDF used to be one of the most widely used anti-HIV-1 drugs and had been considered generally safe and well-tolerated, but it sometimes causes decreases in bone mineral density and kidney injury in susceptible individuals [162, 163]. TAF, a new formulation pro-drug, can deliver the active form (tenofovir diphosphate) more efficiently to cells. In addition, TAF distributes better into lymphoid tissues than TDF, and is thus effective at lower concentrations in blood plasma with less drug exposure for the bones and kidneys [164, 165].

Another important issue in recent anti-HIV-1 drug development efforts is how to improve drug adherence and quality of life (QOL) of HIV-1/AIDS patients. Because cART consists of multiple drugs, patients receiving cART had been taking multiple tablets, many times a day. Thus, the effort to formulate a single tablet that contains 2-3 anti-HIV-1 drugs had been ongoing since early in the cART (or HAART) era. In addition, recent progress in developing new drugs with better pharmacokinetics profiles enabled the formulation of drugs that can be administered once a day (such as TDF, EVG, and DTG). RPV/TDF/FTC (Complera®) and EVG/COBI/FTC/TDF (Stribild®) are fixed-dose combination drugs approved by the FDA in 2011 and 2012, respectively, the first "once daily (QD) single-tablet" drugs for the treatment of HIV-1/AIDS. Subsequently, a number of combinations of drugs have been approved and made available as a once-daily single-tablet drug, for example, DTG/ABC/3TC (Triumeq®, 2014), EVG/COBI/TAF/FTC (Genvoya®, 2015), RPV/TAF/FTC (Odefsey®, 2016), and DTG/RPV (Juluca®, 2017) (Table **1**).

6.2. Recently-approved HIV-1/AIDS Drugs: Once-daily Single-tablet Regimen (and More)

In 2018, three agents (in addition to combinations) were newly approved as anti-HIV-1 drugs, namely, Doravirine (DOR) (Fig. **3**), Bictegravir (BIC) (Fig. **7**), and Ibalizumab-uiyk. DOR is an NNRTI developed by Merck & Co. and is also available in combination with 3TC/TAF (Delstrigo®). In a clinical trial, DOR (100 mg once daily) was non-inferior to the DRV/r arm. DOR also showed activity against many NNRTI-resistant HIV-1s [166]. BIC is a once-daily INSTI with a high genetic barrier to resistance developed by scientists at Gilead Sciences. In a Phase 3 trial, the efficacy of BIC was examined as a single tablet regimen in combination with TAF and FTC; the combination drug (BIC/FTC/TAF: Biktarvy®) was then approved in the United States in 2018. Ibalizumab-uiyk (TNX-355, Trogarzo®) is an inhibitor of HIV-1 attachment, which blocks the binding of the HIV-1 envelope molecule, gp120 to CD4 on the cell surface. It was recently approved for the treatment of HIV-1/AIDS patients in heavily treatment-experienced adults with multidrug-resistant HIV-1 infection failing their current antiretroviral regimen. Ibalizumab-uiyk is administered intravenously as a single loading dose of 2,000 mg followed by a maintenance dose of 800 mg every 2 weeks.

6.3. New HIV-1/AIDS Drugs Under Development: Introduction of Long-acting Therapies

Cabotegravir (CAB) (Fig. **7**) is an INSTI under development by ViiV Healthcare. In clinical trials for CAB, two different forms are being examined: a tablet form taken by mouth (oral CAB) and a long-acting (LA) injectable form (CAB LA). Results of the LATTE-2 trial (Phase IIb) demonstrated that patients treated with CAB LA showed noninferiority relative to those receiving CAB orally [167]. CAB is currently in phase III development and the long-acting form of CAB is considered for use in combination with a long-acting form of RPV (RPV LA) as an injectable maintenance therapy.

MK-8591 (EFdA) (Fig. **2**) is a potent investigational NRTI under development by Merck & Co. as part of a regimen for HIV-1 treatment with potential utility as a single agent for preexposure prophylaxis (PrEP). Recent clinical studies reported the pharmacokinetic (PK) profiles and antiviral efficacy of EFdA. Grobler *et al.* demonstrated that a single 10 mg dose in humans was able to exceed the projected efficacious concentration for at least 7 days [168]. In another study, treatment of cART-naive HIV-1-infected patients with a single 10 mg dose of EFdA rapidly reduced the viral load, which continued to decrease until day 10 after the treatment, with a mean reduction of 1.78 $\log_{10}$ and no evidence of recrudescence [169]. In addition, a report by scientists at Merck & Co. demonstrated that newly-designed drug-eluting implant devices successfully provided prolonged MK-8591 release *in vivo* [170]. The data were evaluated together with those generated in phase 1 clinical studies with once-weekly oral administration of MK-8591. After a single administration in animals, MK-8591 implants achieved clinically relevant drug exposures and sustained drug release with plasma levels corresponding to efficacious MK-8591-TP levels maintained >6 months, resulting in a 1.6-log reduction in viral load [170]. These findings suggest that EFdA can be administered orally at least once weekly (QW), or at much longer intervals with long-acting parenteral formulations.

7. SMALL MOLECULE ANTI-HIV-1 AGENTS UNDER DEVELOPMENT WITH NOVEL MECHANISM OF ACTION

7.1. Capsid Inhibitors

Currently, combinatorial antiretroviral therapy (cART) can reduce viral loads to undetectable levels in HIV-1-infected patients. However, the establishment of persistent infection is still a major barrier to complete viral eradication, which has remained elusive because latently infected reservoirs can evade host antiviral immune responses as well as cART. There, therefore, remains an unmet need to develop new anti-HIV-1 compounds which will be effective over the long term and to establish improved strategies to prevent HIV-1 infection at the early stages prior to provirus formation. It has been shown that the HIV-1 capsid (CA) is a multifunctional protein in HIV-1 replication [171]. Shortly after HIV-1 entry into target cells, the HIV-1 CA, a main component of the HIV-1 core, initiates dissociation of CA from the HIV-1 core. Proper disassembly of the HIV-1 core, a process called uncoating, is known to be a key step for efficient reverse transcription during the early stage of HIV-1 infection [172-174]. Many reports have also shown that the HIV-1 core dissociation process is triggered and regulated by host factors [175-178]. Despite many attempts to understand HIV-1 uncoating, the precise mechanisms involved in HIV-1 core disassembly, including the timing or triggering of uncoating, remain largely unclear. A previous report showed that CA mutations E128A/R132A increased the stability of the viral core and impaired viral cDNA synthesis, while CA mutations Q63A/Q67A accelerated CA disassembly and viral cDNA synthesis, but severely impaired viral infectivity [173]. Overall, these observations indicate that the HIV-1 CA is a highly attractive druggable target and this is expected to lead to the development of therapies to block or enhance these virus-host interactions in order to cause premature or delayed uncoating and potentially add to the cART armamentarium for HIV-1 patients.

Several compounds that bind to CA have been identified [179-189]. One of the best-studied CA inhibitors is PF-3450074, abbreviated as PF74 (Fig. **14**). Recent studies showed that PF74 binds at an intermolecular interface between the N-terminal and the C-terminal of CA within the CA hexamer of the HIV-1 core [190-192]. The PF74-binding site in CA is also shared by the host proteins CPSF6 and NUP153 [191]. Therefore, PF74 is expected to prevent the interaction between CA and CPSF6 or NUP153 [191, 193]. PF74 inhibits HIV-1 infection with EC50 ranging from 80 to 640 nM in PBMCs and T cells [183]. Intriguingly, high concentrations of PF74 (~5 to 10 μM) accelerate the uncoating process and reduce the efficiency of viral cDNA synthesis [192, 194, 195], whereas lower concentrations of PF74 (~2 μM) do not impede HIV-1 infection with little impact on reverse transcription [191, 196, 197]. In addition to the above-mentioned chemical inhibitors, peptide inhibitors such as NYAD-1 reduce the efficiency of both immature- and mature-virion formation [198] and inhibit HIV-1 replication [179, 189, 199].

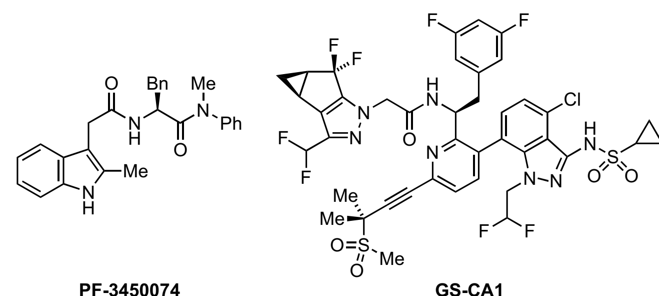

Fig. (14). Structures of Capsid inhibitors.

Recent studies showed that the CA inhibitors GS-CA1 and GS-CA2 (a drug similar to GS-CA1) have great potential compared with the currently-approved anti-HIV-1 drugs (Fig. **14**) [200, 201]. GS-CA1 binds at the same general site as PF74, CPSF6 and NUP153 [191, 193, 200] and inhibits HIV-1 replication in peripheral blood mononuclear cells (PBMCs) (EC50 = 140 pM) at very low concentrations [200]. Furthermore, GS-CA2 has an anti-HIV-1 effect in T cells (EC50 = 100 pM) and PBMCs (EC50 = 50 pM) against 23 HIV-1 primary isolates representing major subtypes [201]. More interestingly, studies in rats and dogs show that both GA-CA1 and GA-CA2 also have potential as long-

acting drugs [200-203]. These observations suggest that GS-CA compounds have a better potency than PF74.

7.2. HIV-1 Attachment Inhibitors Under Development

The HIV-1 envelope protein gp120 binds to the cell surface protein CD4, which causes a conformational change in gp120 and subsequent binding to the co-receptor CCR5 or CXCR4, as described in Section 5.1. Thus, CD4-derived molecules such as soluble CD4 (sCD4) could act as entry inhibitors, although attempts to develop effective sCD4 agents have been unsuccessful to date. Recently, several low molecular weight CD4 mimic molecules have been developed by ourselves and others, including NBD-556 [204, 205], YYA-021 [206-208], JRC-II-191 [209] and BMS806 [210] (Fig. **15**). NBD-556, YYA-021, and JRC-II-191 cause a conformational change of gp120 and inhibit attachment of the HIV-1 virion to CCR5 or CXCR4, while BMS806 binds to gp120 and blocks HR1 exposure following CD4 induction, with no significant effect on CD4 binding. YIR-821, which is a current lead compound derived from YYA-021, has high anti-HIV-1 activity and the CD4-like ability of inducing a conformational change of gp120 through interaction with the Phe43 cavity [211]. YIR-821 also shows a highly synergistic effect in combination with the neutralizing anti-V3 monoclonal antibody KD-247 [212], which may help to reduce the necessary dose of (possibly expensive) KD-247. It could also be that the interaction of CD4 mimics with the Phe43 cavity causes conformational changes of gp120, thereby exposing the neutralizing antibody-binding site of gp120 and enabling efficient access of KD-247 to gp120. CD4 mimics are attractive tools directed toward blocking HIV-1 entry and are important compounds, potentially useful for maintenance therapies together with neutralizing antibodies following initial treatment with conventional cART.

7.3. Novel Approaches Toward Treating HIV-1 Reservoirs: Development of Latency-reversing Agents (LRAs)

As described in the previous sections, cART strongly suppresses HIV-1 replication. However, even life-long cART cannot eradicate HIV-1 due to its persistence in a latent form in cell reservoirs [213-216]. In this regard, Latency-reversing Agents (LRAs) are considered to be vital tools to cure HIV-1. The approach to eliminate HIV-1 reservoirs using LRA has been referred to as "shock and kill" [217-219]. In this approach, HIV is reactivated by LRA in cells which are subsequently eliminated by immune effectors such as cytotoxic T lymphocytes (CTL), or undergo virally-induced cytopathic damage or apoptosis [220, 221]. However, there are still many obstacles to using LRA to reverse HIV-1 latency and eliminate reservoir cells *in vivo*. Several LRAs have been tested in clinical trials. However, most studies demonstrated that the size of the latent reservoir remained unchanged, indicating that these drugs failed to purge the virus *in vivo* [222-226]. These results imply that *in vitro* (*ex vivo*) LRA potency does not necessarily translate into clinical LRA potency, and this is presumably because multiple mechanisms are involved in the maintenance of HIV-1 latency *in vivo* [227]. For instance, a recent study showed that T-cell stimulation does not result in the activation of all functional latent proviruses and that a significant proportion of these non-induced proviruses is replication-competent [228]. Moreover, hypothetically, if LRAs are used alone in treatment-naïve HIV-1 patients, infectious viruses will be produced from reactivated reservoir cells and subsequent infection of uninfected cells could occur. Thus, for effective LRA therapy against HIV-1 reservoirs, it is essential to combine this with potent cART during treatment.

Many small molecule agents that are currently being developed as LRAs include protein kinase C (PKC) activators [*e.g.,* PEP005 (ingenol-3-angelate), prostratin, and bryostatin-1], HDAC inhibitors (*e.g.,* SAHA/vorinostat), or BRD4 inhibitors (*e.g.,* JQ1) (Fig. **16**) [229-235]. The first clinically evaluated LRA candidates were HDAC inhibitors such as valproic acid and vorinostat (SAHA) (Fig. **16**), some of which were originally developed as anti-cancer agents. In some clinical studies of HDAC inhibitors, transient increases in cell-associated and plasma HIV-1 RNA due to HIV-1 latency reversal were observed, but no obvious reduction of HIV-1 reservoir cells was reported [222, 223, 225, 226, 236]. Another concern with the use of HDAC inhibitors is that they (*e.g.* vorinostat) reportedly enhance the susceptibility of $CD4^+$ T cells to HIV-1, which possibly increases the susceptibility of uninfected $CD4^+$ T cells to HIV-1 [237].

One of the other classes of LRA comprise drugs that activate PKC, which plays a critical role in the regulation of cell growth, differentiation, and apoptosis [238, 239]. PKC activators induce transcription factors such as NF-κB, which binds to HIV-1-LTR and thus activates HIV-1 mRNA tran-

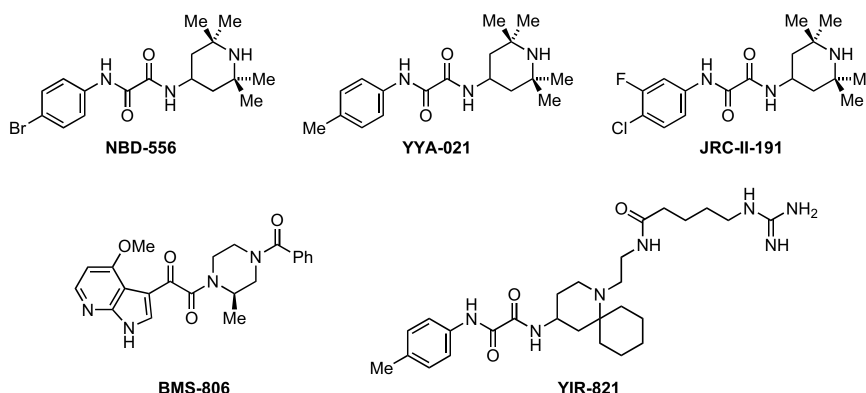

Fig. (15). Structures of representative small CD4 mimic molecules.

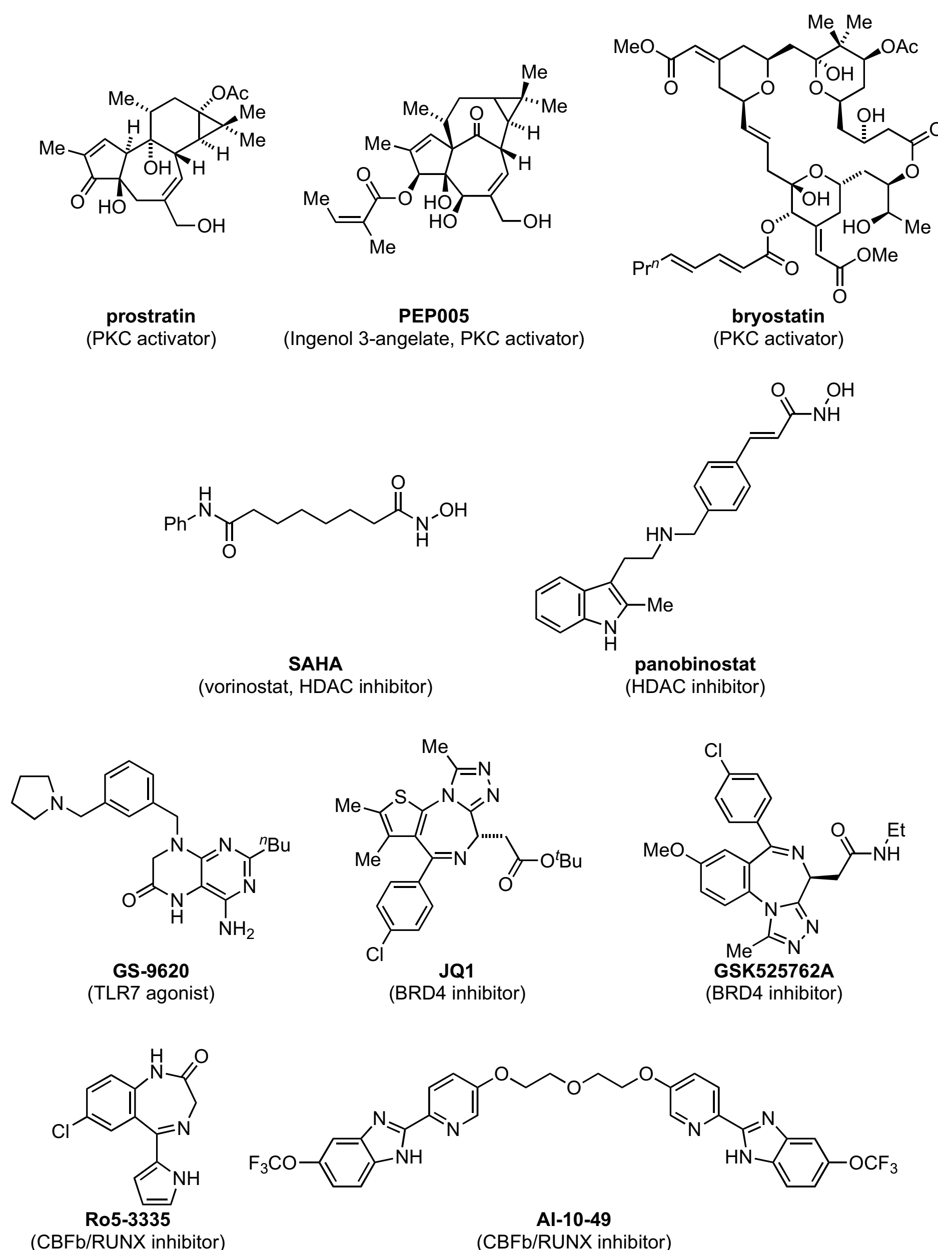

Fig. (16). Chemical structures of HIV-1 latency-reversing agents (LRAs).

scription [240]. Recent studies demonstrate that some PKC activators, such as PEP005 (Fig. **16**), show potent latency-reversing activity in many HIV-1 latent cell lines and primary cells derived from HIV-1 patients *in vitro* [229, 232, 234, 241]. In addition, it is also known that the potency of PKC activators to act as LRA is strongly enhanced by their combination with an LRA in a different class. Several groups have reported that combined treatment is important for LRAs to obtain maximum reactivation [232, 241], and among such combinations, JQ1 (Fig. **16**) plus a PKC activator is considered to be one of the most effective [232, 234, 241]. A recent clinical trial using the PKC agonist bryostatin-1 demonstrated that this agent was safe at the single doses administered. However, the drug did not show any effect on PKC activity or on the transcription of latent HIV-1, probably due to low plasma concentrations in that study [224].

Thus, it is now considered that such broad-acting LRAs alone (*e.g.* HDAC inhibitors or PKC activators) may not be sufficient to eradicate HIV-1 reservoirs *in vivo,* and addition of other strategies/techniques with a different mechanism of action will be necessary. In fact, Borducchi *et al.* recently reported that administration of an HIV-1 env-specific broadly neutralizing antibody (PGT121) together with an LRA (Toll-like receptor 7 agonist: GS-9620) (Fig. **16**) during ART delayed viral rebound following discontinuation of ART in simian human immunodeficiency virus (SHIV)-infected rhesus monkeys [242].

CONCLUSION AND FUTURE PERSPECTIVES

As discussed in this review, fortunately, many different anti-HIV-1/AIDS drugs targeting different stages of the HIV-1 life cycle are now available and more such drugs are

being tested in preclinical studies. The development of such increasingly effective therapeutics will undoubtedly improve our ability to manage HIV-1/AIDS. In addition, the introduction of cART with a single tablet regimen (STR) and future long-acting therapy using injectable drugs, long-acting nanomedicines or drug-eluting implants will drastically change the QOL of HIV-1/AIDS patients under treatment and expand the utility of preexposure prophylaxis (PrEP).

One of the most important key-factors in future anti-HIV-1/AIDS drug design will be to generate novel agents, rather than "me-too" drugs, that will be active against HIV-1 via new antiretroviral mechanisms, have convenient dosing regimens and be of low costs. In addition, a continuous effort is needed to establish a way to reduce/eliminate viral reservoirs to consolidate latency condition enough to stop treatment. This is what is meant by a "Cure of HIV-1/AIDS", and it is one of the most important themes for current HIV-1 research [243, 244].

CONSENT FOR PUBLICATION

Not applicable.

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CONFLICT OF INTEREST

The authors declare no conflict of interest, financial or otherwise.

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REFERENCES

[1] Barré-Sinoussi, F.; Chermann, J.C.; Rey, F.; Nugeyre, M.T.; Chamaret, S.; Gruest, J.; Dauguet, C.; Axler-Blin, C.; Vézinet-Brun, F.; Rouzioux, C.; Rozenbaum, W.; Montagnier, L. Isolation of a T-lymphotropic retrovirus from a patient at risk for acquired immune deficiency syndrome (AIDS). *Science,* **1983**, *220*(4599), 868-871. [http://dx.doi.org/10.1126/science.6189183] [PMID: 6189183]

[2] Gallo, R.C.; Sarin, P.S.; Gelmann, E.P.; Robert-Guroff, M.; Richardson, E.; Kalyanaraman, V.S.; Mann, D.; Sidhu, G.D.; Stahl, R.E.; Zolla-Pazner, S.; Leibowitch, J.; Popovic, M. Isolation of human T-cell leukemia virus in acquired immune deficiency syndrome (AIDS). *Science,* **1983**, *220*(4599), 865-867. [http://dx.doi.org/10.1126/science.6601823] [PMID: 6601823]

[3] Mitsuya, H.; Weinhold, K.J.; Furman, P.A.; St Clair, M.H.; Lehrman, S.N.; Gallo, R.C.; Bolognesi, D.; Barry, D.W.; Broder, S. 3′-Azido-3′-deoxythymidine (BW A509U): An antiviral agent that inhibits the infectivity and cytopathic effect of human T-lymphotropic virus type III/lymphadenopathy-associated virus *in vitro*. *Proc. Natl. Acad. Sci. USA,* **1985**, *82*(20), 7096-7100. [http://dx.doi.org/10.1073/pnas.82.20.7096] [PMID: 2413459]

[4] Yarchoan, R.; Klecker, R.W.; Weinhold, K.J.; Markham, P.D.; Lyerly, H.K.; Durack, D.T.; Gelmann, E.; Lehrman, S.N.; Blum, R.M.; Barry, D.W. Administration of 3′-azido-3′-deoxythymidine, an inhibitor of HTLV-III/LAV replication, to patients with AIDS or AIDS-related complex. *Lancet,* **1986**, *1*(8481), 575-580. [http://dx.doi.org/10.1016/S0140-6736(86)92808-4] [PMID: 2869302]

[5] Graves, M.C.; Lim, J.J.; Heimer, E.P.; Kramer, R.A. An 11-kDa form of human immunodeficiency virus protease expressed in *Escherichia coli* is sufficient for enzymatic activity. *Proc. Natl. Acad. Sci. USA,* **1988**, *85*(8), 2449-2453. [http://dx.doi.org/10.1073/pnas.85.8.2449] [PMID: 3282230]

[6] Farmerie, W.G.; Loeb, D.D.; Casavant, N.C.; Hutchison, C.A., III; Edgell, M.H.; Swanstrom, R. Expression and processing of the AIDS virus reverse transcriptase in *Escherichia coli*. *Science,* **1987**, *236*(4799), 305-308. [http://dx.doi.org/10.1126/science.2436298] [PMID: 2436298]

[7] Autran, B.; Carcelain, G.; Li, T.S.; Blanc, C.; Mathez, D.; Tubiana, R.; Katlama, C.; Debré, P.; Leibowitch, J. Positive effects of combined antiretroviral therapy on CD4+ T cell homeostasis and function in advanced HIV disease. *Science,* **1997**, *277*(5322), 112-116. [http://dx.doi.org/10.1126/science.277.5322.112] [PMID: 9204894]

[8] Komanduri, K.V.; Viswanathan, M.N.; Wieder, E.D.; Schmidt, D.K.; Bredt, B.M.; Jacobson, M.A.; McCune, J.M. Restoration of cytomegalovirus-specific CD4+ T-lymphocyte responses after ganciclovir and highly active antiretroviral therapy in individuals infected with HIV-1. *Nat. Med.,* **1998**, *4*(8), 953-956. [http://dx.doi.org/10.1038/nm0898-953] [PMID: 9701250]

[9] Lederman, M.M.; Connick, E.; Landay, A.; Kuritzkes, D.R.; Spritzler, J.; St Clair, M.; Kotzin, B.L.; Fox, L.; Chiozzi, M.H.; Leonard, J.M.; Rousseau, F.; Wade, M.; Roe, J.D.; Martinez, A.; Kessler, H. Immunologic responses associated with 12 weeks of combination antiretroviral therapy consisting of zidovudine, lamivudine, and ritonavir: results of AIDS clinical trials group protocol 315. *J. Infect. Dis.,* **1998**, *178*(1), 70-79. [http://dx.doi.org/10.1086/515591] [PMID: 9652425]

[10] Bhaskaran, K.; Hamouda, O.; Sannes, M.; Boufassa, F.; Johnson, A.M.; Lambert, P.C.; Porter, K.; Collaboration, C. Changes in the risk of death after HIV seroconversion compared with mortality in the general population. *JAMA,* **2008**, *300*(1), 51-59. [http://dx.doi.org/10.1001/jama.300.1.51] [PMID: 18594040]

[11] Walensky, R.P.; Paltiel, A.D.; Losina, E.; Mercincavage, L.M.; Schackman, B.R.; Sax, P.E.; Weinstein, M.C.; Freedberg, K.A. The survival benefits of AIDS treatment in the United States. *J. Infect. Dis.,* **2006**, *194*(1), 11-19. [http://dx.doi.org/10.1086/505147] [PMID: 16741877]

[12] Gupta, R.; Hill, A.; Sawyer, A.W.; Pillay, D. Emergence of drug resistance in HIV type 1-infected patients after receipt of first-line highly active antiretroviral therapy: A systematic review of clinical trials. *Clin. Infect. Dis.,* **2008**, *47*(5), 712-722. [http://dx.doi.org/10.1086/590943] [PMID: 18662137]

[13] Koh, Y.; Nakata, H.; Maeda, K.; Ogata, H.; Bilcer, G.; Devasamudram, T.; Kincaid, J.F.; Boross, P.; Wang, Y.F.; Tie, Y.; Volarath, P.; Gaddis, L.; Harrison, R.W.; Weber, I.T.; Ghosh, A.K.; Mitsuya, H. Novel bis-tetrahydrofuranylurethane-containing nonpeptidic protease inhibitor (PI) UIC-94017 (TMC114) with potent activity against multi-PI-resistant human immunodeficiency virus *in vitro*. *Antimicrob. Agents Chemother.,* **2003**, *47*(10), 3123-3129. [http://dx.doi.org/10.1128/AAC.47.10.3123-3129.2003] [PMID: 14506019]

[14] Ghosh, A.K.; Osswald, H.L.; Prato, G. Recent progress in the development of HIV-1 Protease inhibitors for the treatment of HIV/AIDS. *J. Med. Chem.,* **2016**, *59*(11), 5172-5208. [http://dx.doi.org/10.1021/acs.jmedchem.5b01697] [PMID: 26799988]

[15] Hazuda, D.J.; Felock, P.; Witmer, M.; Wolfe, A.; Stillmock, K.; Grobler, J.A.; Espeseth, A.; Gabryelski, L.; Schleif, W.; Blau, C.; Miller, M.D. Inhibitors of strand transfer that prevent integration and inhibit HIV-1 replication in cells. *Science,* **2000**, *287*(5453), 646-650. [http://dx.doi.org/10.1126/science.287.5453.646] [PMID: 10649997]

[16] Min, S.; Song, I.; Borland, J.; Chen, S.; Lou, Y.; Fujiwara, T.; Piscitelli, S.C. Pharmacokinetics and safety of S/GSK1349572, a next-generation HIV integrase inhibitor, in healthy volunteers. *Antimicrob. Agents Chemother.,* **2010**, *54*(1), 254-258. [http://dx.doi.org/10.1128/AAC.00842-09] [PMID: 19884365]

[17] Baba, M.; Nishimura, O.; Kanzaki, N.; Okamoto, M.; Sawada, H.; Iizawa, Y.; Shiraishi, M.; Aramaki, Y.; Okonogi, K.; Ogawa, Y.; Meguro, K.; Fujino, M. A small-molecule, nonpeptide CCR5 antagonist with highly potent and selective anti-HIV-1 activity. *Proc. Natl. Acad. Sci. USA,* **1999**, *96*(10), 5698-5703. [http://dx.doi.org/10.1073/pnas.96.10.5698] [PMID: 10318947]

[18] Dorr, P.; Westby, M.; Dobbs, S.; Griffin, P.; Irvine, B.; Macartney, M.; Mori, J.; Rickett, G.; Smith-Burchnell, C.; Napier, C.; Webster, R.; Armour, D.; Price, D.; Stammen, B.; Wood, A.; Perros, M. Maraviroc (UK-427,857), a potent, orally bioavailable, and selective small-molecule inhibitor of chemokine receptor CCR5 with broad-spectrum anti-human immunodeficiency virus type 1 activity. *Antimicrob. Agents Chemother.,* **2005**, *49*(11), 4721-4732. [http://dx.doi.org/10.1128/AAC.49.11.4721-4732.2005] [PMID: 16251317]

[19] World Health Organization. (Accessed at http://www.who.int/gho/hiv/en/).

[20] Piot, P.; Abdool Karim, S.S.; Hecht, R.; Legido-Quigley, H.; Buse, K.; Stover, J.; Resch, S.; Ryckman, T.; Møgedal, S.; Dybul, M.; Goosby, E.; Watts, C.; Kilonzo, N.; McManus, J.; Sidibé, M. Defeating AIDS-advancing global health. *Lancet,* **2015**, *386*(9989), 171-218. [http://dx.doi.org/10.1016/S0140-6736(15)60658-4] [PMID: 26117719]

[21] UNAIDS. (Accessed at: http://www.unaids.org/en).

[22] UNAIDS. 90-90-90: An ambitious treatment target to help end the aids epidemic. (Accessed at: http://www.unaids.org/en/resources/909090).

[23] Shaik, M.M.; Peng, H.; Lu, J.; Rits-Volloch, S.; Xu, C.; Liao, M.; Chen, B. Structural basis of coreceptor recognition by HIV-1 envelope spike. *Nature,* **2019**, *565*(7739), 318-323. [http://dx.doi.org/10.1038/s41586-018-0804-9] [PMID: 30542158]

[24] Wu, L.; Gerard, N.P.; Wyatt, R.; Choe, H.; Parolin, C.; Ruffing, N.; Borsetti, A.; Cardoso, A.A.; Desjardin, E.; Newman, W.; Gerard, C.; Sodroski, J. CD4-induced interaction of primary HIV-1 gp120 glycoproteins with the chemokine receptor CCR-5. *Nature,* **1996**, *384*(6605), 179-183. [http://dx.doi.org/10.1038/384179a0] [PMID: 8906795]

[25] Trkola, A.; Dragic, T.; Arthos, J.; Binley, J.M.; Olson, W.C.; Allaway, G.P.; Cheng-Mayer, C.; Robinson, J.; Maddon, P.J.; Moore, J.P. CD4-dependent, antibody-sensitive interactions between HIV-1 and its co-receptor CCR-5. *Nature,* **1996**, *384*(6605), 184-187. [http://dx.doi.org/10.1038/384184a0] [PMID: 8906796]

[26] Bleul, C.C.; Farzan, M.; Choe, H.; Parolin, C.; Clark-Lewis, I.; Sodroski, J.; Springer, T.A. The lymphocyte chemoattractant SDF-1 is a ligand for LESTR/fusin and blocks HIV-1 entry. *Nature,* **1996**, *382*(6594), 829-833. [http://dx.doi.org/10.1038/382829a0] [PMID: 8752280]

[27] Deng, H.; Liu, R.; Ellmeier, W.; Choe, S.; Unutmaz, D.; Burkhart, M.; Di Marzio, P.; Marmon, S.; Sutton, R.E.; Hill, C.M.; Davis, C.B.; Peiper, S.C.; Schall, T.J.; Littman, D.R.; Landau, N.R. Identification of a major co-receptor for primary isolates of HIV-1. *Nature,* **1996**, *381*(6584), 661-666. [http://dx.doi.org/10.1038/381661a0] [PMID: 8649511]

[28] Doranz, B.J.; Rucker, J.; Yi, Y.; Smyth, R.J.; Samson, M.; Peiper, S.C.; Parmentier, M.; Collman, R.G.; Doms, R.W. A dual-tropic primary HIV-1 isolate that uses fusin and the beta-chemokine receptors CKR-5, CKR-3, and CKR-2b as fusion cofactors. *Cell,* **1996**, *85*(7), 1149-1158. [http://dx.doi.org/10.1016/S0092-8674(00)81314-8] [PMID: 8674120]

[29] Choe, H.; Farzan, M.; Sun, Y.; Sullivan, N.; Rollins, B.; Ponath, P.D.; Wu, L.; Mackay, C.R.; LaRosa, G.; Newman, W.; Gerard, N.; Gerard, C.; Sodroski, J. The beta-chemokine receptors CCR3 and CCR5 facilitate infection by primary HIV-1 isolates. *Cell,* **1996**, *85*(7), 1135-1148. [http://dx.doi.org/10.1016/S0092-8674(00)81313-6] [PMID: 8674119]

[30] Dragic, T.; Litwin, V.; Allaway, G.P.; Martin, S.R.; Huang, Y.; Nagashima, K.A.; Cayanan, C.; Maddon, P.J.; Koup, R.A.; Moore, J.P.; Paxton, W.A. HIV-1 entry into CD4+ cells is mediated by the chemokine receptor CC-CKR-5. *Nature,* **1996**, *381*(6584), 667-673. [http://dx.doi.org/10.1038/381667a0] [PMID: 8649512]

[31] Alkhatib, G.; Combadiere, C.; Broder, C.C.; Feng, Y.; Kennedy, P.E.; Murphy, P.M.; Berger, E.A. CC CKR5: a RANTES, MIP-1alpha, MIP-1beta receptor as a fusion cofactor for macrophage-tropic HIV-1. *Science,* **1996**, *272*(5270), 1955-1958. [http://dx.doi.org/10.1126/science.272.5270.1955] [PMID: 8658171]

[32] Feng, Y.; Broder, C.C.; Kennedy, P.E.; Berger, E.A. HIV-1 entry cofactor: functional cDNA cloning of a seven-transmembrane, G protein-coupled receptor. *Science,* **1996**, *272*(5263), 872-877. [http://dx.doi.org/10.1126/science.272.5263.872] [PMID: 8629022]

[33] Kilby, J.M.; Eron, J.J. Novel therapies based on mechanisms of HIV-1 cell entry. *N. Engl. J. Med.,* **2003**, *348*(22), 2228-2238. [http://dx.doi.org/10.1056/NEJMra022812] [PMID: 12773651]

[34] Jahn, R.; Lang, T.; Südhof, T.C. Membrane fusion. *Cell,* **2003**, *112*(4), 519-533. [http://dx.doi.org/10.1016/S0092-8674(03)00112-0] [PMID: 12600315]

[35] Chan, D.C.; Fass, D.; Berger, J.M.; Kim, P.S. Core structure of gp41 from the HIV envelope glycoprotein. *Cell,* **1997**, *89*(2), 263-273. [http://dx.doi.org/10.1016/S0092-8674(00)80205-6] [PMID: 9108481]

[36] Chan, D.C.; Kim, P.S. HIV entry and its inhibition. *Cell,* **1998**, *93*(5), 681-684. [http://dx.doi.org/10.1016/S0092-8674(00)81430-0] [PMID: 9630213]

[37] De Clercq, E. Strategies in the design of antiviral drugs. *Nat. Rev. Drug Discov.,* **2002**, *1*(1), 13-25. [http://dx.doi.org/10.1038/nrd703] [PMID: 12119605]

[38] Mitsuya, H.; Broder, S. Strategies for antiviral therapy in AIDS. *Nature,* **1987**, *325*(6107), 773-778. [http://dx.doi.org/10.1038/325773a0] [PMID: 2434858]

[39] Mehellou, Y.; De Clercq, E. Twenty-six years of anti-HIV drug discovery: where do we stand and where do we go? *J. Med. Chem.,* **2010**, *53*(2), 521-538. [http://dx.doi.org/10.1021/jm900492g] [PMID: 19785437]

[40] Kohlstaedt, L.A.; Wang, J.; Friedman, J.M.; Rice, P.A.; Steitz, T.A. Crystal structure at 3.5 A resolution of HIV-1 reverse transcriptase complexed with an inhibitor. *Science,* **1992**, *256*(5065), 1783-1790. [http://dx.doi.org/10.1126/science.1377403] [PMID: 1377403]

[41] Jacobo-Molina, A.; Ding, J.; Nanni, R.G.; Clark, A.D., Jr; Lu, X.; Tantillo, C.; Williams, R.L.; Kamer, G.; Ferris, A.L.; Clark, P. Crystal structure of human immunodeficiency virus type 1 reverse transcriptase complexed with double-stranded DNA at 3.0 A resolution shows bent DNA. *Proc. Natl. Acad. Sci. USA,* **1993**, *90*(13), 6320-6324. [http://dx.doi.org/10.1073/pnas.90.13.6320] [PMID: 7687065]

[42] Mitsuya, H.; Broder, S. Inhibition of the *in vitro* infectivity and cytopathic effect of human T-lymphotrophic virus type III/lymphadenopathy-associated virus (HTLV-III/LAV) by 2′,3′-dideoxynucleosides. *Proc. Natl. Acad. Sci. USA,* **1986**, *83*(6), 1911-1915. [http://dx.doi.org/10.1073/pnas.83.6.1911] [PMID: 3006077]

[43] Cheng, Y.C.; Dutschman, G.E.; Bastow, K.F.; Sarngadharan, M.G.; Ting, R.Y. Human immunodeficiency virus reverse transcriptase. General properties and its interactions with nucleoside triphosphate analogs. *J. Biol. Chem.,* **1987**, *262*(5), 2187-2189. [PMID: 2434477]

[44] Balzarini, J.; Herdewijn, P.; De Clercq, E. Differential patterns of intracellular metabolism of 2′,3′-didehydro-2′,3′-dideoxythymidine and 3′-azido-2′,3′-dideoxythymidine, two potent anti-human immunodeficiency virus compounds. *J. Biol. Chem.,* **1989**, *264*(11), 6127-6133. [PMID: 2539371]

[45] Richman, D.D. HIV chemotherapy. *Nature,* **2001**, *410*(6831), 995-1001. [http://dx.doi.org/10.1038/35073673] [PMID: 11309630]

[46] De Clercq, E. The role of non-nucleoside reverse transcriptase inhibitors (NNRTIs) in the therapy of HIV-1 infection. *Antiviral Res.,* **1998**, *38*(3), 153-179. [http://dx.doi.org/10.1016/S0166-3542(98)00025-4] [PMID: 9754886]

[47] Esnouf, R.; Ren, J.; Ross, C.; Jones, Y.; Stammers, D.; Stuart, D. Mechanism of inhibition of HIV-1 reverse transcriptase by non-nucleoside inhibitors. *Nat. Struct. Biol.,* **1995**, *2*(4), 303-308. [http://dx.doi.org/10.1038/nsb0495-303] [PMID: 7540935]

[48] Spence, R.A.; Kati, W.M.; Anderson, K.S.; Johnson, K.A. Mechanism of inhibition of HIV-1 reverse transcriptase by nonnucleoside inhibitors. *Science,* **1995**, *267*(5200), 988-993. [http://dx.doi.org/10.1126/science.7532321] [PMID: 7532321]

[49] Witvrouw, M.; Pannecouque, C.; Van Laethem, K.; Desmyter, J.; De Clercq, E.; Vandamme, A.M. Activity of non-nucleoside reverse transcriptase inhibitors against HIV-2 and SIV. *AIDS,* **1999**, *13*(12), 1477-1483. [http://dx.doi.org/10.1097/00002030-199908200-00006] [PMID: 10465070]

[50] Larder, B.A.; Darby, G.; Richman, D.D. HIV with reduced sensitivity to zidovudine (AZT) isolated during prolonged therapy. *Science,* **1989**, *243*(4899), 1731-1734. [http://dx.doi.org/10.1126/science.2467383] [PMID: 2467383]

[51] Shafer, R.W.; Kozal, M.J.; Winters, M.A.; Iversen, A.K.; Katzenstein, D.A.; Ragni, M.V.; Meyer, W.A., III; Gupta, P.; Rasheed, S.; Coombs, R. Combination therapy with zidovudine and didanosine selects for drug-resistant human immunodeficiency virus type 1 strains with unique patterns of pol gene mutations. *J. Infect. Dis.,* **1994**, *169*(4), 722-729. [http://dx.doi.org/10.1093/infdis/169.4.722] [PMID: 8133086]

[52] Shirasaka, T.; Kavlick, M.F.; Ueno, T.; Gao, W.Y.; Kojima, E.; Alcaide, M.L.; Chokekijchai, S.; Roy, B.M.; Arnold, E.; Yarchoan, R. Emergence of human immunodeficiency virus type 1 variants with resistance to multiple dideoxynucleosides in patients receiving therapy with dideoxynucleosides. *Proc. Natl. Acad. Sci. USA,* **1995**, *92*(6), 2398-2402. [http://dx.doi.org/10.1073/pnas.92.6.2398] [PMID: 7534421]

[53] Schinazi, R.F.; Lloyd, R.M., Jr; Nguyen, M.H.; Cannon, D.L.; McMillan, A.; Ilksoy, N.; Chu, C.K.; Liotta, D.C.; Bazmi, H.Z.; Mellors, J.W. Characterization of human immunodeficiency viruses resistant to oxathiolane-cytosine nucleosides. *Antimicrob. Agents Chemother.,* **1993**, *37*(4), 875-881. [http://dx.doi.org/10.1128/AAC.37.4.875] [PMID: 7684216]

[54] Quan, Y.; Gu, Z.; Li, X.; Li, Z.; Morrow, C.D.; Wainberg, M.A. Endogenous reverse transcription assays reveal high-level resistance to the triphosphate of (-)2′-dideoxy-3′-thiacytidine by mutated M184V human immunodeficiency virus type 1. *J. Virol.,* **1996**, *70*(8), 5642-5645. [PMID: 8764080]

[55] Schuurman, R.; Nijhuis, M.; van Leeuwen, R.; Schipper, P.; de Jong, D.; Collis, P.; Danner, S.A.; Mulder, J.; Loveday, C.; Christopherson, C. Rapid changes in human immunodeficiency virus type 1 RNA load and appearance of drug-resistant virus populations in persons treated with lamivudine (3TC). *J. Infect. Dis.,* **1995**, *171*(6), 1411-1419. [http://dx.doi.org/10.1093/infdis/171.6.1411] [PMID: 7539472]

[56] Ferrer, E.; Podzamczer, D.; Arnedo, M.; Fumero, E.; McKenna, P.; Rinehart, A.; Pérez, J.L.; Barberá, M.J.; Pumarola, T.; Gatell, J.M.; Gudiol, F. Genotype and phenotype at baseline and at failure in human immunodeficiency virus-infected antiretroviral-naive patients in a randomized trial comparing zidovudine and lamivudine plus nelfinavir or nevirapine. *J. Infect. Dis.,* **2003**, *187*(4), 687-690. [http://dx.doi.org/10.1086/367987] [PMID: 12599088]

[57] Johnson, M.S.; McClure, M.A.; Feng, D.F.; Gray, J.; Doolittle, R.F. Computer analysis of retroviral pol genes: Assignment of enzymatic functions to specific sequences and homologies with non-viral enzymes. *Proc. Natl. Acad. Sci. USA,* **1986**, *83*(20), 7648-7652. [http://dx.doi.org/10.1073/pnas.83.20.7648] [PMID: 2429313]

[58] Huang, H.; Chopra, R.; Verdine, G.L.; Harrison, S.C. Structure of a covalently trapped catalytic complex of HIV-1 reverse transcriptase: implications for drug resistance. *Science,* **1998**, *282*(5394), 1669-1675. [http://dx.doi.org/10.1126/science.282.5394.1669] [PMID: 9831551]

[59] Tuske, S.; Sarafianos, S.G.; Clark, A.D., Jr; Ding, J.; Naeger, L.K.; White, K.L.; Miller, M.D.; Gibbs, C.S.; Boyer, P.L.; Clark, P.; Wang, G.; Gaffney, B.L.; Jones, R.A.; Jerina, D.M.; Hughes, S.H.; Arnold, E. Structures of HIV-1 RT-DNA complexes before and after incorporation of the anti-AIDS drug tenofovir. *Nat. Struct. Mol. Biol.,* **2004**, *11*(5), 469-474. [http://dx.doi.org/10.1038/nsmb760] [PMID: 15107837]

[60] Harrigan, P.R.; Stone, C.; Griffin, P.; Nájera, I.; Bloor, S.; Kemp, S.; Tisdale, M.; Larder, B. Resistance profile of the human immunodeficiency virus type 1 reverse transcriptase inhibitor abacavir (1592U89) after monotherapy and combination therapy. CNA2001 Investigative Group. *J. Infect. Dis.,* **2000**, *181*(3), 912-920. [http://dx.doi.org/10.1086/315317] [PMID: 10720512]

[61] Wainberg, M.A.; Miller, M.D.; Quan, Y.; Salomon, H.; Mulato, A.S.; Lamy, P.D.; Margot, N.A.; Anton, K.E.; Cherrington, J.M. *In vitro* selection and characterization of HIV-1 with reduced susceptibility to PMPA. *Antivir. Ther. (Lond.),* **1999**, *4*(2), 87-94. [PMID: 10682153]

[62] Margot, N.A.; Isaacson, E.; McGowan, I.; Cheng, A.K.; Schooley, R.T.; Miller, M.D. Genotypic and phenotypic analyses of HIV-1 in antiretroviral-experienced patients treated with tenofovir DF. *AIDS,* **2002**, *16*(9), 1227-1235. [http://dx.doi.org/10.1097/00002030-200206140-00004] [PMID: 12045487]

[63] García-Lerma, J.G.; MacInnes, H.; Bennett, D.; Reid, P.; Nidtha, S.; Weinstock, H.; Kaplan, J.E.; Heneine, W. A novel genetic pathway of human immunodeficiency virus type 1 resistance to stavudine mediated by the K65R mutation. *J. Virol.,* **2003**, *77*(10), 5685-5693. [http://dx.doi.org/10.1128/JVI.77.10.5685-5693.2003] [PMID: 12719561]

[64] Arion, D.; Kaushik, N.; McCormick, S.; Borkow, G.; Parniak, M.A. Phenotypic mechanism of HIV-1 resistance to 3′-azido-3′-deoxythymidine (AZT): increased polymerization processivity and enhanced sensitivity to pyrophosphate of the mutant viral reverse transcriptase. *Biochemistry,* **1998**, *37*(45), 15908-15917. [http://dx.doi.org/10.1021/bi981200e] [PMID: 9843396]

[65] Meyer, P.R.; Matsuura, S.E.; So, A.G.; Scott, W.A. Unblocking of chain-terminated primer by HIV-1 reverse transcriptase through a nucleotide-dependent mechanism. *Proc. Natl. Acad. Sci. USA,* **1998**, *95*(23), 13471-13476. [http://dx.doi.org/10.1073/pnas.95.23.13471] [PMID: 9811824]

[66] Boyer, P.L.; Sarafianos, S.G.; Arnold, E.; Hughes, S.H. Selective excision of AZTMP by drug-resistant human immunodeficiency virus reverse transcriptase. *J. Virol.,* **2001**, *75*(10), 4832-4842. [http://dx.doi.org/10.1128/JVI.75.10.4832-4842.2001] [PMID: 11312355]

[67] Parikh, U.M.; Bacheler, L.; Koontz, D.; Mellors, J.W. The K65R mutation in human immunodeficiency virus type 1 reverse transcriptase exhibits bidirectional phenotypic antagonism with thymidine analog mutations. *J. Virol.,* **2006**, *80*(10), 4971-4977. [http://dx.doi.org/10.1128/JVI.80.10.4971-4977.2006] [PMID: 16641288]

[68] White, K.L.; Chen, J.M.; Feng, J.Y.; Margot, N.A.; Ly, J.K.; Ray, A.S.; Macarthur, H.L.; McDermott, M.J.; Swaminathan, S.; Miller, M.D. The K65R reverse transcriptase mutation in HIV-1 reverses the excision phenotype of zidovudine resistance mutations. *Antivir. Ther. (Lond.),* **2006**, *11*(2), 155-163. [PMID: 16640096]

[69] Deval, J.; White, K.L.; Miller, M.D.; Parkin, N.T.; Courcambeck, J.; Halfon, P.; Selmi, B.; Boretto, J.; Canard, B. Mechanistic basis for reduced viral and enzymatic fitness of HIV-1 reverse transcriptase containing both K65R and M184V mutations. *J. Biol. Chem.,* **2004**, *279*(1), 509-516. [http://dx.doi.org/10.1074/jbc.M308806200] [PMID: 14551187]

[70] Kodama, E.I.; Kohgo, S.; Kitano, K.; Machida, H.; Gatanaga, H.; Shigeta, S.; Matsuoka, M.; Ohrui, H.; Mitsuya, H. 4′-Ethynyl nucleoside analogs: potent inhibitors of multidrug-resistant human immunodeficiency virus variants *in vitro*. *Antimicrob. Agents Chemother.,* **2001**, *45*(5), 1539-1546. [http://dx.doi.org/10.1128/AAC.45.5.1539-1546.2001] [PMID: 11302824]

[71] Nakata, H.; Amano, M.; Koh, Y.; Kodama, E.; Yang, G.; Bailey, C.M.; Kohgo, S.; Hayakawa, H.; Matsuoka, M.; Anderson, K.S.; Cheng, Y.C.; Mitsuya, H. Activity against human immunodeficiency virus type 1, intracellular metabolism, and effects on human DNA polymerases of 4′-ethynyl-2-fluoro-2′-deoxyadenosine. *Antimicrob. Agents Chemother.,* **2007**, *51*(8), 2701-2708. [http://dx.doi.org/10.1128/AAC.00277-07] [PMID: 17548498]

[72] Kawamoto, A.; Kodama, E.; Sarafianos, S.G.; Sakagami, Y.; Kohgo, S.; Kitano, K.; Ashida, N.; Iwai, Y.; Hayakawa, H.; Nakata, H.; Mitsuya, H.; Arnold, E.; Matsuoka, M. 2′-deoxy-4′-C-ethynyl-

2-halo-adenosines active against drug-resistant human immunodeficiency virus type 1 variants. *Int. J. Biochem. Cell Biol.,* **2008**, *40*(11), 2410-2420.
[http://dx.doi.org/10.1016/j.biocel.2008.04.007] [PMID: 18487070]

[73] Markowitz, M.; Sarafianos, S.G. 4′-Ethynyl-2-fluoro-2′-deoxyadenosine, MK-8591: a novel HIV-1 reverse transcriptase translocation inhibitor. *Curr. Opin. HIV AIDS,* **2018**, *13*(4), 294-299.
[http://dx.doi.org/10.1097/COH.0000000000000467] [PMID: 29697468]

[74] Stoddart, C.A.; Galkina, S.A.; Joshi, P.; Kosikova, G.; Moreno, M.E.; Rivera, J.M.; Sloan, B.; Reeve, A.B.; Sarafianos, S.G.; Murphey-Corb, M.; Parniak, M.A. Oral administration of the nucleoside EFdA (4′-ethynyl-2-fluoro-2′-deoxyadenosine) provides rapid suppression of HIV viremia in humanized mice and favorable pharmacokinetic properties in mice and the rhesus macaque. *Antimicrob. Agents Chemother.,* **2015**, *59*(7), 4190-4198.
[http://dx.doi.org/10.1128/AAC.05036-14] [PMID: 25941222]

[75] Murphey-Corb, M.; Rajakumar, P.; Michael, H.; Nyaundi, J.; Didier, P.J.; Reeve, A.B.; Mitsuya, H.; Sarafianos, S.G.; Parniak, M.A. Response of simian immunodeficiency virus to the novel nucleoside reverse transcriptase inhibitor 4′-ethynyl-2-fluoro-2′-deoxyadenosine *in vitro* and *in vivo*. *Antimicrob. Agents Chemother.,* **2012**, *56*(9), 4707-4712.
[http://dx.doi.org/10.1128/AAC.00723-12] [PMID: 22713337]

[76] Hattori, S.; Ide, K.; Nakata, H.; Harada, H.; Suzu, S.; Ashida, N.; Kohgo, S.; Hayakawa, H.; Mitsuya, H.; Okada, S. Potent activity of a nucleoside reverse transcriptase inhibitor, 4′-ethynyl-2-fluoro-2′-deoxyadenosine, against human immunodeficiency virus type 1 infection in a model using human peripheral blood mononuclear cell-transplanted NOD/SCID Janus kinase 3 knockout mice. *Antimicrob. Agents Chemother.,* **2009**, *53*(9), 3887-3893.
[http://dx.doi.org/10.1128/AAC.00270-09] [PMID: 19546363]

[77] Maeda, K.; Desai, D.V.; Aoki, M.; Nakata, H.; Kodama, E.N.; Mitsuya, H. Delayed emergence of HIV-1 variants resistant to 4′-ethynyl-2-fluoro-2′-deoxyadenosine: comparative sequential passage study with lamivudine, tenofovir, emtricitabine and BMS-986001. *Antivir. Ther. (Lond.),* **2014**, *19*(2), 179-189.
[http://dx.doi.org/10.3851/IMP2697] [PMID: 24162098]

[78] Takamatsu, Y.; Das, D.; Kohgo, S.; Hayashi, H.; Delino, N. S.; Sarafianos, S. G.; Mitsuya, H.; Maeda, K. The high genetic barrier of EFdA/MK-8591 stems from strong interactions with the active site of drug-resistant HIV-1 reverse transcriptase. *Cell Chem Biol ,* **2018**, *25*(10), 1268-1278.

[79] De Clercq, E. Non-nucleoside reverse transcriptase inhibitors (NNRTIs): past, present, and future. *Chem. Biodivers.,* **2004**, *1*(1), 44-64.
[http://dx.doi.org/10.1002/cbdv.200490012] [PMID: 17191775]

[80] Pauwels, R. New non-nucleoside reverse transcriptase inhibitors (NNRTIs) in development for the treatment of HIV infections. *Curr. Opin. Pharmacol.,* **2004**, *4*(5), 437-446.
[http://dx.doi.org/10.1016/j.coph.2004.07.005] [PMID: 15351347]

[81] Sluis-Cremer, N.; Temiz, N.A.; Bahar, I. Conformational changes in HIV-1 reverse transcriptase induced by nonnucleoside reverse transcriptase inhibitor binding. *Curr. HIV Res.,* **2004**, *2*(4), 323-332.
[http://dx.doi.org/10.2174/1570162043351093] [PMID: 15544453]

[82] Domaoal, R.A.; Demeter, L.M. Structural and biochemical effects of human immunodeficiency virus mutants resistant to non-nucleoside reverse transcriptase inhibitors. *Int. J. Biochem. Cell Biol.,* **2004**, *36*(9), 1735-1751.
[http://dx.doi.org/10.1016/j.biocel.2004.02.026] [PMID: 15183341]

[83] Das, K.; Clark, A.D., Jr; Lewi, P.J.; Heeres, J.; De Jonge, M.R.; Koymans, L.M.; Vinkers, H.M.; Daeyaert, F.; Ludovici, D.W.; Kukla, M.J.; De Corte, B.; Kavash, R.W.; Ho, C.Y.; Ye, H.; Lichtenstein, M.A.; Andries, K.; Pauwels, R.; De Béthune, M.P.; Boyer, P.L.; Clark, P.; Hughes, S.H.; Janssen, P.A.; Arnold, E. Roles of conformational and positional adaptability in structure-based design of TMC125-R165335 (etravirine) and related non-nucleoside reverse transcriptase inhibitors that are highly potent and effective against wild-type and drug-resistant HIV-1 variants. *J. Med. Chem.,* **2004**, *47*(10), 2550-2560.
[http://dx.doi.org/10.1021/jm030558s] [PMID: 15115397]

[84] Janssen, P.A.; Lewi, P.J.; Arnold, E.; Daeyaert, F.; de Jonge, M.; Heeres, J.; Koymans, L.; Vinkers, M.; Guillemont, J.; Pasquier, E.; Kukla, M.; Ludovici, D.; Andries, K.; de Béthune, M.P.; Pauwels, R.; Das, K.; Clark, A.D., Jr; Frenkel, Y.V.; Hughes, S.H.; Medaer, B.; De Knaep, F.; Bohets, H.; De Clerck, F.; Lampo, A.; Williams, P.; Stoffels, P. In search of a novel anti-HIV drug: multidisciplinary coordination in the discovery of 4-[[4-[[4-[(1E)-2-cyanoethenyl]-2,6-dimethylphenyl]amino]-2-pyrimidinyl]amino]benzonitrile (R278474, rilpivirine). *J. Med. Chem.,* **2005**, *48*(6), 1901-1909.
[http://dx.doi.org/10.1021/jm040840e] [PMID: 15771434]

[85] Pelemans, H.; Esnouf, R.; De Clercq, E.; Balzarini, J. Mutational analysis of trp-229 of human immunodeficiency virus type 1 reverse transcriptase (RT) identifies this amino acid residue as a prime target for the rational design of new non-nucleoside RT inhibitors. *Mol. Pharmacol.,* **2000**, *57*(5), 954-960.
[PMID: 10779379]

[86] Brik, A.; Wong, C.H. HIV-1 protease: mechanism and drug discovery. *Org. Biomol. Chem.,* **2003**, *1*(1), 5-14.
[http://dx.doi.org/10.1039/b208248a] [PMID: 12929379]

[87] Wlodawer, A.; Miller, M.; Jaskólski, M.; Sathyanarayana, B.K.; Baldwin, E.; Weber, I.T.; Selk, L.M.; Clawson, L.; Schneider, J.; Kent, S.B. Conserved folding in retroviral proteases: Crystal structure of a synthetic HIV-1 protease. *Science,* **1989**, *245*(4918), 616-621.
[http://dx.doi.org/10.1126/science.2548279] [PMID: 2548279]

[88] Kumar, G.N.; Rodrigues, A.D.; Buko, A.M.; Denissen, J.F. Cytochrome P450-mediated metabolism of the HIV-1 protease inhibitor ritonavir (ABT-538) in human liver microsomes. *J. Pharmacol. Exp. Ther.,* **1996**, *277*(1), 423-431.
[PMID: 8613951]

[89] Nijhuis, M.; Deeks, S.; Boucher, C. Implications of antiretroviral resistance on viral fitness. *Curr. Opin. Infect. Dis.,* **2001**, *14*(1), 23-28.
[http://dx.doi.org/10.1097/00001432-200102000-00005] [PMID: 11979111]

[90] Quiñones-Mateu, M.E.; Moore-Dudley, D.M.; Jegede, O.; Weber, J.; J Arts, E. Viral drug resistance and fitness. *Adv. Pharmacol.,* **2008**, *56*, 257-296.
[http://dx.doi.org/10.1016/S1054-3589(07)56009-6] [PMID: 18086415]

[91] Shafer, R.W.D.K.; Winters, M.A.; Eshleman, S.H. A guide to HIV-1 reverse transcriptase and protease sequencing for drug resistance studies. In: *HIV sequence compendium* **2000**; Cl, K., Ed.; Los Alamos National Laboratory: Los Alamos, NM, **2000**, pp. 1-51.
[PMID: 22324021]

[92] Doyon, L.; Payant, C.; Brakier-Gingras, L.; Lamarre, D. Novel Gag-Pol frameshift site in human immunodeficiency virus type 1 variants resistant to protease inhibitors. *J. Virol.,* **1998**, *72*(7), 6146-6150.
[PMID: 9621079]

[93] Gatanaga, H.; Suzuki, Y.; Tsang, H.; Yoshimura, K.; Kavlick, M.F.; Nagashima, K.; Gorelick, R.J.; Mardy, S.; Tang, C.; Summers, M.F.; Mitsuya, H. Amino acid substitutions in Gag protein at non-cleavage sites are indispensable for the development of a high multitude of HIV-1 resistance against protease inhibitors. *J. Biol. Chem.,* **2002**, *277*(8), 5952-5961.
[http://dx.doi.org/10.1074/jbc.M108005200] [PMID: 11741936]

[94] Zhang, Y.M.; Imamichi, H.; Imamichi, T.; Lane, H.C.; Falloon, J.; Vasudevachari, M.B.; Salzman, N.P. Drug resistance during indinavir therapy is caused by mutations in the protease gene and in its Gag substrate cleavage sites. *J. Virol.,* **1997**, *71*(9), 6662-6670.
[PMID: 9261388]

[95] Tamiya, S.; Mardy, S.; Kavlick, M.F.; Yoshimura, K.; Mistuya, H. Amino acid insertions near Gag cleavage sites restore the otherwise compromised replication of human immunodeficiency virus type 1 variants resistant to protease inhibitors. *J. Virol.,* **2004**, *78*(21), 12030-12040.
[http://dx.doi.org/10.1128/JVI.78.21.12030-12040.2004] [PMID: 15479842]

[96] Clavel, F.; Race, E.; Mammano, F. HIV drug resistance and viral fitness. *Adv. Pharmacol.,* **2000**, *49*, 41-66.
[http://dx.doi.org/10.1016/S1054-3589(00)49023-X] [PMID: 11013760]

[97] De Meyer, S.; Azijn, H.; Surleraux, D.; Jochmans, D.; Tahri, A.; Pauwels, R.; Wigerinck, P.; de Béthune, M.P. TMC114, a novel human immunodeficiency virus type 1 protease inhibitor active against protease inhibitor-resistant viruses, including a broad range

of clinical isolates. *Antimicrob. Agents Chemother.,* **2005**, *49*(6), 2314-2321. [http://dx.doi.org/10.1128/AAC.49.6.2314-2321.2005] [PMID: 15917527]

[98] Ghosh, A.K.; Anderson, D.D.; Weber, I.T.; Mitsuya, H. Enhancing protein backbone binding--a fruitful concept for combating drug-resistant HIV. *Angew. Chem. Int. Ed. Engl.,* **2012**, *51*(8), 1778-1802. [http://dx.doi.org/10.1002/anie.201102762] [PMID: 22290878]

[99] Deeks, E.D. Darunavir: a review of its use in the management of HIV-1 infection. *Drugs,* **2014**, *74*(1), 99-125. [http://dx.doi.org/10.1007/s40265-013-0159-3] [PMID: 24338166]

[100] Koh, Y.; Amano, M.; Towata, T.; Danish, M.; Leshchenko-Yashchuk, S.; Das, D.; Nakayama, M.; Tojo, Y.; Ghosh, A.K.; Mitsuya, H. *In vitro* selection of highly darunavir-resistant and replication-competent HIV-1 variants by using a mixture of clinical HIV-1 isolates resistant to multiple conventional protease inhibitors. *J. Virol.,* **2010**, *84*(22), 11961-11969. [http://dx.doi.org/10.1128/JVI.00967-10] [PMID: 20810732]

[101] de Meyer, S.; Vangeneugden, T.; van Baelen, B.; de Paepe, E.; van Marck, H.; Picchio, G.; Lefebvre, E.; de Béthune, M.P. Resistance profile of darunavir: combined 24-week results from the POWER trials. *AIDS Res. Hum. Retroviruses,* **2008**, *24*(3), 379-388. [http://dx.doi.org/10.1089/aid.2007.0173] [PMID: 18327986]

[102] Mitsuya, Y.; Liu, T.F.; Rhee, S.Y.; Fessel, W.J.; Shafer, R.W. Prevalence of darunavir resistance-associated mutations: patterns of occurrence and association with past treatment. *J. Infect. Dis.,* **2007**, *196*(8), 1177-1179. [http://dx.doi.org/10.1086/521624] [PMID: 17955436]

[103] Tie, Y.; Boross, P.I.; Wang, Y.F.; Gaddis, L.; Hussain, A.K.; Leshchenko, S.; Ghosh, A.K.; Louis, J.M.; Harrison, R.W.; Weber, I.T. High resolution crystal structures of HIV-1 protease with a potent non-peptide inhibitor (UIC-94017) active against multi-drug-resistant clinical strains. *J. Mol. Biol.,* **2004**, *338*(2), 341-352. [http://dx.doi.org/10.1016/j.jmb.2004.02.052] [PMID: 15066436]

[104] Koh, Y.; Matsumi, S.; Das, D.; Amano, M.; Davis, D.A.; Li, J.; Leschenko, S.; Baldridge, A.; Shioda, T.; Yarchoan, R.; Ghosh, A.K.; Mitsuya, H. Potent inhibition of HIV-1 replication by novel non-peptidyl small molecule inhibitors of protease dimerization. *J. Biol. Chem.,* **2007**, *282*(39), 28709-28720. [http://dx.doi.org/10.1074/jbc.M703938200] [PMID: 17635930]

[105] Espeseth, A.S.; Felock, P.; Wolfe, A.; Witmer, M.; Grobler, J.; Anthony, N.; Egbertson, M.; Melamed, J.Y.; Young, S.; Hamill, T.; Cole, J.L.; Hazuda, D.J. HIV-1 integrase inhibitors that compete with the target DNA substrate define a unique strand transfer conformation for integrase. *Proc. Natl. Acad. Sci. USA,* **2000**, *97*(21), 11244-11249. [http://dx.doi.org/10.1073/pnas.200139397] [PMID: 11016953]

[106] Hazuda, D.J.; Anthony, N.J.; Gomez, R.P.; Jolly, S.M.; Wai, J.S.; Zhuang, L.; Fisher, T.E.; Embrey, M.; Guare, J.P., Jr; Egbertson, M.S.; Vacca, J.P.; Huff, J.R.; Felock, P.J.; Witmer, M.V.; Stillmock, K.A.; Danovich, R.; Grobler, J.; Miller, M.D.; Espeseth, A.S.; Jin, L.; Chen, I.W.; Lin, J.H.; Kassahun, K.; Ellis, J.D.; Wong, B.K.; Xu, W.; Pearson, P.G.; Schleif, W.A.; Cortese, R.; Emini, E.; Summa, V.; Holloway, M.K.; Young, S.D. A naphthyridine carboxamide provides evidence for discordant resistance between mechanistically identical inhibitors of HIV-1 integrase. *Proc. Natl. Acad. Sci. USA,* **2004**, *101*(31), 11233-11238. [http://dx.doi.org/10.1073/pnas.0402357101] [PMID: 15277684]

[107] McColl, D.J.; Chen, X. Strand transfer inhibitors of HIV-1 integrase: bringing IN a new era of antiretroviral therapy. *Antiviral Res.,* **2010**, *85*(1), 101-118. [http://dx.doi.org/10.1016/j.antiviral.2009.11.004] [PMID: 19925830]

[108] Pommier, Y.; Johnson, A.A.; Marchand, C. Integrase inhibitors to treat HIV/AIDS. *Nat. Rev. Drug Discov.,* **2005**, *4*(3), 236-248. [http://dx.doi.org/10.1038/nrd1660] [PMID: 15729361]

[109] Chiu, T.K.; Davies, D.R. Structure and function of HIV-1 integrase. *Curr. Top. Med. Chem.,* **2004**, *4*(9), 965-977. [http://dx.doi.org/10.2174/1568026043388547] [PMID: 15134551]

[110] Engelman, A.; Cherepanov, P. The structural biology of HIV-1: mechanistic and therapeutic insights. *Nat. Rev. Microbiol.,* **2012**, *10*(4), 279-290. [http://dx.doi.org/10.1038/nrmicro2747] [PMID: 22421880]

[111] Grobler, J.A.; Stillmock, K.; Hu, B.; Witmer, M.; Felock, P.; Espeseth, A.S.; Wolfe, A.; Egbertson, M.; Bourgeois, M.; Melamed, J.; Wai, J.S.; Young, S.; Vacca, J.; Hazuda, D.J. Diketo acid inhibitor mechanism and HIV-1 integrase: implications for metal binding in the active site of phosphotransferase enzymes. *Proc. Natl. Acad. Sci. USA,* **2002**, *99*(10), 6661-6666. [http://dx.doi.org/10.1073/pnas.092056199] [PMID: 11997448]

[112] Hare, S.; Smith, S.J.; Métifiot, M.; Jaxa-Chamiec, A.; Pommier, Y.; Hughes, S.H.; Cherepanov, P. Structural and functional analyses of the second-generation integrase strand transfer inhibitor dolutegravir (S/GSK1349572). *Mol. Pharmacol.,* **2011**, *80*(4), 565-572. [http://dx.doi.org/10.1124/mol.111.073189] [PMID: 21719464]

[113] Cushman, M.; Sherman, P. Inhibition of HIV-1 integration protein by aurintricarboxylic acid monomers, monomer analogs, and polymer fractions. *Biochem. Biophys. Res. Commun.,* **1992**, *185*(1), 85-90. [http://dx.doi.org/10.1016/S0006-291X(05)80958-1] [PMID: 1599491]

[114] Fesen, M.R.; Kohn, K.W.; Leteurtre, F.; Pommier, Y. Inhibitors of human immunodeficiency virus integrase. *Proc. Natl. Acad. Sci. USA,* **1993**, *90*(6), 2399-2403. [http://dx.doi.org/10.1073/pnas.90.6.2399] [PMID: 8460151]

[115] Goldgur, Y.; Craigie, R.; Cohen, G.H.; Fujiwara, T.; Yoshinaga, T.; Fujishita, T.; Sugimoto, H.; Endo, T.; Murai, H.; Davies, D.R. Structure of the HIV-1 integrase catalytic domain complexed with an inhibitor: A platform for antiviral drug design. *Proc. Natl. Acad. Sci. USA,* **1999**, *96*(23), 13040-13043. [http://dx.doi.org/10.1073/pnas.96.23.13040] [PMID: 10557269]

[116] Steigbigel, R.T.; Cooper, D.A.; Kumar, P.N.; Eron, J.E.; Schechter, M.; Markowitz, M.; Loutfy, M.R.; Lennox, J.L.; Gatell, J.M.; Rockstroh, J.K.; Katlama, C.; Yeni, P.; Lazzarin, A.; Clotet, B.; Zhao, J.; Chen, J.; Ryan, D.M.; Rhodes, R.R.; Killar, J.A.; Gilde, L.R.; Strohmaier, K.M.; Meibohm, A.R.; Miller, M.D.; Hazuda, D.J.; Nessly, M.L.; DiNubile, M.J.; Isaacs, R.D.; Nguyen, B.Y.; Teppler, H.; Teams, B.S. Raltegravir with optimized background therapy for resistant HIV-1 infection. *N. Engl. J. Med.,* **2008**, *359*(4), 339-354. [http://dx.doi.org/10.1056/NEJMoa0708975] [PMID: 18650512]

[117] DeJesus, E.; Berger, D.; Markowitz, M.; Cohen, C.; Hawkins, T.; Ruane, P.; Elion, R.; Farthing, C.; Zhong, L.; Cheng, A.K.; McColl, D.; Kearney, B.P.; Study, T. Antiviral activity, pharmacokinetics, and dose response of the HIV-1 integrase inhibitor GS-9137 (JTK-303) in treatment-naive and treatment-experienced patients. *J. Acquir. Immune Defic. Syndr.,* **2006**, *43*(1), 1-5. [http://dx.doi.org/10.1097/01.qai.0000233308.82860.2f] [PMID: 16936557]

[118] Unger, N.R.; Worley, M.V.; Kisgen, J.J.; Sherman, E.M.; Childs-Kean, L.M. Elvitegravir for the treatment of HIV. *Expert Opin. Pharmacother.,* **2016**, *17*(17), 2359-2370. [http://dx.doi.org/10.1080/14656566.2016.1250885] [PMID: 27767362]

[119] Arts, E.J.; Hazuda, D.J. HIV-1 antiretroviral drug therapy. *Cold Spring Harb. Perspect. Med.,* **2012**, *2*(4)a007161. [http://dx.doi.org/10.1101/cshperspect.a007161] [PMID: 22474613]

[120] Stanford University. INSTI resistance notes. (Available at; https://hivdb.stanford.edu/dr-summary/resistance-notes/INSTI/).

[121] Fransen, S.; Gupta, S.; Danovich, R.; Hazuda, D.; Miller, M.; Witmer, M.; Petropoulos, C.J.; Huang, W. Loss of raltegravir susceptibility by human immunodeficiency virus type 1 is conferred via multiple nonoverlapping genetic pathways. *J. Virol.,* **2009**, *83*(22), 11440-11446. [http://dx.doi.org/10.1128/JVI.01168-09] [PMID: 19759152]

[122] Abram, M.E.; Hluhanich, R.M.; Goodman, D.D.; Andreatta, K.N.; Margot, N.A.; Ye, L.; Niedziela-Majka, A.; Barnes, T.L.; Novikov, N.; Chen, X.; Svarovskaia, E.S.; McColl, D.J.; White, K.L.; Miller, M.D. Impact of primary elvitegravir resistance-associated mutations in HIV-1 integrase on drug susceptibility and viral replication fitness. *Antimicrob. Agents Chemother.,* **2013**, *57*(6), 2654-2663. [http://dx.doi.org/10.1128/AAC.02568-12] [PMID: 23529738]

[123] Shimura, K.; Kodama, E.; Sakagami, Y.; Matsuzaki, Y.; Watanabe, W.; Yamataka, K.; Watanabe, Y.; Ohata, Y.; Doi, S.; Sato, M.; Kano, M.; Ikeda, S.; Matsuoka, M. Broad antiretroviral activity and resistance profile of the novel human immunodeficiency virus integrase inhibitor elvitegravir (JTK-303/GS-9137). *J. Virol.,* **2008**, *82*(2), 764-774. [http://dx.doi.org/10.1128/JVI.01534-07] [PMID: 17977962]

[124] Vandekerckhove, L. GSK-1349572, a novel integrase inhibitor for the treatment of HIV infection. *Curr. Opin. Investig. Drugs,* **2010**, *11*(2), 203-212.
[PMID: 20112170]

[125] Dow, D.E.; Bartlett, J.A. Dolutegravir, the second-generation of integrase strand transfer inhibitors (INSTIs) for the treatment of HIV. *Infect. Dis. Ther.,* **2014**, *3*(2), 83-102.
[http://dx.doi.org/10.1007/s40121-014-0029-7] [PMID: 25134686]

[126] Choi, E.; Mallareddy, J.R.; Lu, D.; Kolluru, S. Recent advances in the discovery of small-molecule inhibitors of HIV-1 integrase. *Future Sci. OA,* **2018**, *4*(9), FSO338.
[http://dx.doi.org/10.4155/fsoa-2018-0060] [PMID: 30416746]

[127] Wild, C.; Greenwell, T.; Matthews, T. A synthetic peptide from HIV-1 gp41 is a potent inhibitor of virus-mediated cell-cell fusion. *AIDS Res. Hum. Retroviruses,* **1993**, *9*(11), 1051-1053.
[http://dx.doi.org/10.1089/aid.1993.9.1051] [PMID: 8312047]

[128] Walker, D.K.; Abel, S.; Comby, P.; Muirhead, G.J.; Nedderman, A.N.; Smith, D.A. Species differences in the disposition of the CCR5 antagonist, UK-427,857, a new potential treatment for HIV. *Drug Metab. Dispos.,* **2005**, *33*(4), 587-595.
[http://dx.doi.org/10.1124/dmd.104.002626] [PMID: 15650075]

[129] De Clercq, E. Mozobil® (Plerixafor, AMD3100), 10 years after its approval by the US food and drug administration. *Antivir. Chem. Chemother.,* **2019**, *27,* 2040206619829382.
[http://dx.doi.org/10.1177/2040206619829382] [PMID: 30776910]

[130] Tamamura, H.; Hori, A.; Kanzaki, N.; Hiramatsu, K.; Mizumoto, M.; Nakashima, H.; Yamamoto, N.; Otaka, A.; Fujii, N. T140 analogs as CXCR4 antagonists identified as anti-metastatic agents in the treatment of breast cancer. *FEBS Lett.,* **2003**, *550*(1-3), 79-83.
[http://dx.doi.org/10.1016/S0014-5793(03)00824-X] [PMID: 12935890]

[131] BioLineRx. (Available at: http://www.biolinerx.com/ default.asp?pageid=98).

[132] Shimura, K.; Nameki, D.; Kajiwara, K.; Watanabe, K.; Sakagami, Y.; Oishi, S.; Fujii, N.; Matsuoka, M.; Sarafianos, S.G.; Kodama, E.N. Resistance profiles of novel electrostatically constrained HIV-1 fusion inhibitors. *J. Biol. Chem.,* **2010**, *285*(50), 39471-39480.
[http://dx.doi.org/10.1074/jbc.M110.145789] [PMID: 20937812]

[133] Otaka, A.; Nakamura, M.; Nameki, D.; Kodama, E.; Uchiyama, S.; Nakamura, S.; Nakano, H.; Tamamura, H.; Kobayashi, Y.; Matsuoka, M.; Fujii, N. Remodeling of gp41-C34 peptide leads to highly effective inhibitors of the fusion of HIV-1 with target cells. *Angew. Chem. Int. Ed. Engl.,* **2002**, *41*(16), 2937-2940.
[http://dx.doi.org/10.1002/1521-3773(20020816)41:16<2937::AID-ANIE2937>3.0.CO;2-J] [PMID: 12203417]

[134] Nishikawa, H.; Nakamura, S.; Kodama, E.; Ito, S.; Kajiwara, K.; Izumi, K.; Sakagami, Y.; Oishi, S.; Ohkubo, T.; Kobayashi, Y.; Otaka, A.; Fujii, N.; Matsuoka, M. Electrostatically constrained alpha-helical peptide inhibits replication of HIV-1 resistant to enfuvirtide. *Int. J. Biochem. Cell Biol.,* **2009**, *41*(4), 891-899.
[http://dx.doi.org/10.1016/j.biocel.2008.08.039] [PMID: 18834950]

[135] Liu, S.; Lu, H.; Niu, J.; Xu, Y.; Wu, S.; Jiang, S. Different from the HIV fusion inhibitor C34, the anti-HIV drug Fuzeon (T-20) inhibits HIV-1 entry by targeting multiple sites in gp41 and gp120. *J. Biol. Chem.,* **2005**, *280*(12), 11259-11273.
[http://dx.doi.org/10.1074/jbc.M411141200] [PMID: 15640162]

[136] Derdeyn, C.A.; Decker, J.M.; Sfakianos, J.N.; Zhang, Z.; O'Brien, W.A.; Ratner, L.; Shaw, G.M.; Hunter, E. Sensitivity of human immunodeficiency virus type 1 to fusion inhibitors targeted to the gp41 first heptad repeat involves distinct regions of gp41 and is consistently modulated by gp120 interactions with the coreceptor. *J. Virol.,* **2001**, *75*(18), 8605-8614.
[http://dx.doi.org/10.1128/JVI.75.18.8605-8614.2001] [PMID: 11507206]

[137] Pierson, T.C.; Doms, R.W.; Pöhlmann, S. Prospects of HIV-1 entry inhibitors as novel therapeutics. *Rev. Med. Virol.,* **2004**, *14*(4), 255-270.
[http://dx.doi.org/10.1002/rmv.435] [PMID: 15248253]

[138] Liu, S.; Jiang, S. High throughput screening and characterization of HIV-1 entry inhibitors targeting gp41: theories and techniques. *Curr. Pharm. Des.,* **2004**, *10*(15), 1827-1843.
[http://dx.doi.org/10.2174/1381612043384466] [PMID: 15180543]

[139] Si, Z.; Madani, N.; Cox, J.M.; Chruma, J.J.; Klein, J.C.; Schön, A.; Phan, N.; Wang, L.; Biorn, A.C.; Cocklin, S.; Chaiken, I.; Freire, E.; Smith, A.B., III; Sodroski, J.G. Small-molecule inhibitors of HIV-1 entry block receptor-induced conformational changes in the viral envelope glycoproteins. *Proc. Natl. Acad. Sci. USA,* **2004**, *101*(14), 5036-5041.
[http://dx.doi.org/10.1073/pnas.0307953101] [PMID: 15051887]

[140] Zwick, M.B.; Saphire, E.O.; Burton, D.R. gp41: HIV's shy protein. *Nat. Med.,* **2004**, *10*(2), 133-134.
[http://dx.doi.org/10.1038/nm0204-133] [PMID: 14760422]

[141] Nakahara, T.; Nomura, W.; Ohba, K.; Ohya, A.; Tanaka, T.; Hashimoto, C.; Narumi, T.; Murakami, T.; Yamamoto, N.; Tamamura, H. Remodeling of dynamic structures of HIV-1 envelope proteins leads to synthetic antigen molecules inducing neutralizing antibodies. *Bioconjug. Chem.,* **2010**, *21*(4), 709-714.
[http://dx.doi.org/10.1021/bc900502z] [PMID: 20359196]

[142] Nomura, W.; Hashimoto, C.; Ohya, A.; Miyauchi, K.; Urano, E.; Tanaka, T.; Narumi, T.; Nakahara, T.; Komano, J.A.; Yamamoto, N.; Tamamura, H. A synthetic C34 trimer of HIV-1 gp41 shows significant increase in inhibition potency. *ChemMedChem,* **2012**, *7*(2), 205-208.
[http://dx.doi.org/10.1002/cmdc.201100542] [PMID: 22247043]

[143] Hashimoto, C.; Nomura, W.; Ohya, A.; Urano, E.; Miyauchi, K.; Narumi, T.; Aikawa, H.; Komano, J.A.; Yamamoto, N.; Tamamura, H. Evaluation of a synthetic C34 trimer of HIV-1 gp41 as AIDS vaccines. *Bioorg. Med. Chem.,* **2012**, *20*(10), 3287-3291.
[http://dx.doi.org/10.1016/j.bmc.2012.03.050] [PMID: 22507207]

[144] Nomura, W.; Hashimoto, C.; Suzuki, T.; Ohashi, N.; Fujino, M.; Murakami, T.; Yamamoto, N.; Tamamura, H. Multimerized CHR-derived peptides as HIV-1 fusion inhibitors. *Bioorg. Med. Chem.,* **2013**, *21*(15), 4452-4458.
[http://dx.doi.org/10.1016/j.bmc.2013.05.060] [PMID: 23800723]

[145] Nomura, W.; Mizuguchi, T.; Tamamura, H. Multimerized HIV-gp41-derived peptides as fusion inhibitors and vaccines. *Biopolymers,* **2016**, *106*(4), 622-628.
[http://dx.doi.org/10.1002/bip.22782] [PMID: 26583370]

[146] Maeda, K.; Das, D.; Ogata-Aoki, H.; Nakata, H.; Miyakawa, T.; Tojo, Y.; Norman, R.; Takaoka, Y.; Ding, J.; Arnold, G.F.; Arnold, E.; Mitsuya, H. Structural and molecular interactions of CCR5 inhibitors with CCR5. *J. Biol. Chem.,* **2006**, *281*(18), 12688-12698.
[http://dx.doi.org/10.1074/jbc.M512688200] [PMID: 16476734]

[147] Tan, Q.; Zhu, Y.; Li, J.; Chen, Z.; Han, G.W.; Kufareva, I.; Li, T.; Ma, L.; Fenalti, G.; Li, J.; Zhang, W.; Xie, X.; Yang, H.; Jiang, H.; Cherezov, V.; Liu, H.; Stevens, R.C.; Zhao, Q.; Wu, B. Structure of the CCR5 chemokine receptor-HIV entry inhibitor maraviroc complex. *Science,* **2013**, *341*(6152), 1387-1390.
[http://dx.doi.org/10.1126/science.1241475] [PMID: 24030490]

[148] Strizki, J.M.; Xu, S.; Wagner, N.E.; Wojcik, L.; Liu, J.; Hou, Y.; Endres, M.; Palani, A.; Shapiro, S.; Clader, J.W.; Greenlee, W.J.; Tagat, J.R.; McCombie, S.; Cox, K.; Fawzi, A.B.; Chou, C.C.; Pugliese-Sivo, C.; Davies, L.; Moreno, M.E.; Ho, D.D.; Trkola, A.; Stoddart, C.A.; Moore, J.P.; Reyes, G.R.; Baroudy, B.M. SCH-C (SCH 351125), an orally bioavailable, small molecule antagonist of the chemokine receptor CCR5, is a potent inhibitor of HIV-1 infection *in vitro* and *in vivo*. *Proc. Natl. Acad. Sci. USA,* **2001**, *98*(22), 12718-12723.
[http://dx.doi.org/10.1073/pnas.221375398] [PMID: 11606733]

[149] Tagat, J.R.; McCombie, S.W.; Nazareno, D.; Labroli, M.A.; Xiao, Y.; Steensma, R.W.; Strizki, J.M.; Baroudy, B.M.; Cox, K.; Lachowicz, J.; Varty, G.; Watkins, R. Piperazine-based CCR5 antagonists as HIV-1 inhibitors. IV. Discovery of 1-[(4,6-dimethyl-5-pyrimidinyl)carbonyl]- 4-[4-[2-methoxy-1(R)-4-(trifluoromethyl)phenyl]ethyl-3(S)-methyl-1-piperazinyl]- 4-methylpiperidine (Sch-417690/Sch-D), a potent, highly selective, and orally bioavailable CCR5 antagonist. *J. Med. Chem.,* **2004**, *47*(10), 2405-2408.
[http://dx.doi.org/10.1021/jm0304515] [PMID: 15115380]

[150] Maeda, K.; Nakata, H.; Koh, Y.; Miyakawa, T.; Ogata, H.; Takaoka, Y.; Shibayama, S.; Sagawa, K.; Fukushima, D.; Moravek, J.; Koyanagi, Y.; Mitsuya, H. Spirodiketopiperazine-based CCR5 inhibitor which preserves CC-chemokine/CCR5 interactions and exerts potent activity against R5 human immunodeficiency virus type 1 *in vitro*. *J. Virol.,* **2004**, *78*(16), 8654-8662.
[http://dx.doi.org/10.1128/JVI.78.16.8654-8662.2004] [PMID: 15280474]

[151] Lalezari, J.; Thompson, M.; Kumar, P.; Piliero, P.; Davey, R.; Patterson, K.; Shachoy-Clark, A.; Adkison, K.; Demarest, J.; Lou, Y.; Berrey, M.; Piscitelli, S. Antiviral activity and safety of 873140, a novel CCR5 antagonist, during short-term monotherapy in HIV-infected adults. *AIDS,* **2005**, *19*(14), 1443-1448.

[http://dx.doi.org/10.1097/01.aids.0000183633.06580.8a] [PMID: 16135896]

[152] Nichols, W.G.; Steel, H.M.; Bonny, T.; Adkison, K.; Curtis, L.; Millard, J.; Kabeya, K.; Clumeck, N. Hepatotoxicity observed in clinical trials of aplaviroc (GW873140). *Antimicrob. Agents Chemother.,* **2008**, *52*(3), 858-865. [http://dx.doi.org/10.1128/AAC.00821-07] [PMID: 18070967]

[153] Demarest, J.F.; Amrine-Madsen, H.; Irlbeck, D.M.; Kitrinos, K.M.; Team, C.C.R.C.S. Virologic failure in first-line human immunodeficiency virus therapy with a CCR5 entry inhibitor, aplaviroc, plus a fixed-dose combination of lamivudine-zidovudine: nucleoside reverse transcriptase inhibitor resistance regardless of envelope tropism. *Antimicrob. Agents Chemother.,* **2009**, *53*(3), 1116-1123. [http://dx.doi.org/10.1128/AAC.01055-08] [PMID: 19075055]

[154] Gulick, R.M.; Lalezari, J.; Goodrich, J.; Clumeck, N.; DeJesus, E.; Horban, A.; Nadler, J.; Clotet, B.; Karlsson, A.; Wohlfeiler, M.; Montana, J.B.; McHale, M.; Sullivan, J.; Ridgway, C.; Felstead, S.; Dunne, M.W.; van der Ryst, E.; Mayer, H.; Teams, M.S. Maraviroc for previously treated patients with R5 HIV-1 infection. *N. Engl. J. Med.,* **2008**, *359*(14), 1429-1441. [http://dx.doi.org/10.1056/NEJMoa0803152] [PMID: 18832244]

[155] Fätkenheuer, G.; Nelson, M.; Lazzarin, A.; Konourina, I.; Hoepelman, A.I.; Lampiris, H.; Hirschel, B.; Tebas, P.; Raffi, F.; Trottier, B.; Bellos, N.; Saag, M.; Cooper, D.A.; Westby, M.; Tawadrous, M.; Sullivan, J.F.; Ridgway, C.; Dunne, M.W.; Felstead, S.; Mayer, H.; van der Ryst, E. Subgroup analyses of maraviroc in previously treated R5 HIV-1 infection. *N. Engl. J. Med.,* **2008**, *359*(14), 1442-1455. [http://dx.doi.org/10.1056/NEJMoa0803154] [PMID: 18832245]

[156] Cooper, D.A.; Heera, J.; Goodrich, J.; Tawadrous, M.; Saag, M.; Dejesus, E.; Clumeck, N.; Walmsley, S.; Ting, N.; Coakley, E.; Reeves, J.D.; Reyes-Teran, G.; Westby, M.; Van Der Ryst, E.; Ive, P.; Mohapi, L.; Mingrone, H.; Horban, A.; Hackman, F.; Sullivan, J.; Mayer, H. Maraviroc versus efavirenz, both in combination with zidovudine-lamivudine, for the treatment of antiretroviral-naive subjects with CCR5-tropic HIV-1 infection. *J. Infect. Dis.,* **2010**, *201*(6), 803-813. [http://dx.doi.org/10.1086/650697] [PMID: 20151839]

[157] Seto, M.; Aikawa, K.; Miyamoto, N.; Aramaki, Y.; Kanzaki, N.; Takashima, K.; Kuze, Y.; Iizawa, Y.; Baba, M.; Shiraishi, M. Highly potent and orally active CCR5 antagonists as anti-HIV-1 agents: synthesis and biological activities of 1-benzazocine derivatives containing a sulfoxide moiety. *J. Med. Chem.,* **2006**, *49*(6), 2037-2048. [http://dx.doi.org/10.1021/jm0509703] [PMID: 16539392]

[158] Krenkel, O.; Puengel, T.; Govaere, O.; Abdallah, A.T.; Mossanen, J.C.; Kohlhepp, M.; Liepelt, A.; Lefebvre, E.; Luedde, T.; Hellerbrand, C.; Weiskirchen, R.; Longerich, T.; Costa, I.G.; Anstee, Q.M.; Trautwein, C.; Tacke, F. Therapeutic inhibition of inflammatory monocyte recruitment reduces steatohepatitis and liver fibrosis. *Hepatology,* **2018**, *67*(4), 1270-1283. [http://dx.doi.org/10.1002/hep.29544] [PMID: 28940700]

[159] Joy, M. T.; Ben Assayag, E.; Shabashov-Stone, D.; Liraz-Zaltsman, S.; Mazzitelli, J.; Arenas, M.; Abduljawad, N.; Kliper, E.; Korczyn, A. D.; Thareja, N. S.; Kesner, E. L.; Zhou, M.; Huang, S.; Silva, T. K.; Katz, N.; Bornstein, N. M.; Silva, A. J.; Shohami, E.; Carmichael, S. T. CCR5 is a therapeutic target for recovery after stroke and traumatic. *Brain Injury,* **2019**, *176*(5), 1143-1157. [http://dx.doi.org/10.1016/j.cell.2019.01.044]

[160] Asmuth, D.M.; Goodrich, J.; Cooper, D.A.; Haubrich, R.; Rajicic, N.; Hirschel, B.; Mayer, H.; Valdez, H. CD4+ T-cell restoration after 48 weeks in the maraviroc treatment-experienced trials MOTIVATE 1 and 2. *J. Acquir. Immune Defic. Syndr.,* **2010**, *54*(4), 394-397. [http://dx.doi.org/10.1097/QAI.0b013e3181c5c83b] [PMID: 20009949]

[161] López-Huertas, M.R.; Jiménez-Tormo, L.; Madrid-Elena, N.; Gutiérrez, C.; Rodríguez-Mora, S.; Coiras, M.; Alcamí, J.; Moreno, S. The CCR5-antagonist Maraviroc reverses HIV-1 latency *in vitro* alone or in combination with the PKC-agonist Bryostatin-1. *Sci. Rep.,* **2017**, *7*(1), 2385. [http://dx.doi.org/10.1038/s41598-017-02634-y] [PMID: 28539614]

[162] Fernandez-Fernandez, B.; Montoya-Ferrer, A.; Sanz, A.B.; Sanchez-Niño, M.D.; Izquierdo, M.C.; Poveda, J.; Sainz-Prestel, V.; Ortiz-Martin, N.; Parra-Rodriguez, A.; Selgas, R.; Ruiz-Ortega, M.; Egido, J.; Ortiz, A. Tenofovir nephrotoxicity: 2011 update. *Aids Res. Treat.,* **2011**, **2011**354908. [http://dx.doi.org/10.1155/2011/354908] [PMID: 21716719]

[163] Malik, A.; Abraham, P.; Malik, N. Acute renal failure and Fanconi syndrome in an AIDS patient on tenofovir treatment--case report and review of literature. *J. Infect.,* **2005**, *51*(2), E61-E65. [http://dx.doi.org/10.1016/j.jinf.2004.08.031] [PMID: 16038754]

[164] Eisenberg, E.J.; He, G.X.; Lee, W.A. Metabolism of GS-7340, a novel phenyl monophosphoramidate intracellular prodrug of PMPA, in blood. *Nucleosides Nucleotides Nucleic Acids,* **2001**, *20*(4-7), 1091-1098. [http://dx.doi.org/10.1081/NCN-100002496] [PMID: 11562963]

[165] De Clercq, E. Tenofovir alafenamide (TAF) as the successor of tenofovir disoproxil fumarate (TDF). *Biochem. Pharmacol.,* **2016**, *119*, 1-7. [http://dx.doi.org/10.1016/j.bcp.2016.04.015] [PMID: 27133890]

[166] Ankrom, W. Effect of severe renal impairment on doravirine pharmacokinetics. The annual conference on retroviruses and opportunistic infections (CROI), Seattle, **2016**.

[167] Margolis, D.A.; Gonzalez-Garcia, J.; Stellbrink, H.J.; Eron, J.J.; Yazdanpanah, Y.; Podzamczer, D.; Lutz, T.; Angel, J.B.; Richmond, G.J.; Clotet, B.; Gutierrez, F.; Sloan, L.; Clair, M.S.; Murray, M.; Ford, S.L.; Mrus, J.; Patel, P.; Crauwels, H.; Griffith, S.K.; Sutton, K.C.; Dorey, D.; Smith, K.Y.; Williams, P.E.; Spreen, W.R. Long-acting intramuscular cabotegravir and rilpivirine in adults with HIV-1 infection (LATTE-2): 96-week results of a randomised, open-label, phase 2b, non-inferiority trial. *Lancet,* **2017**, *390*(10101), 1499-1510. [http://dx.doi.org/10.1016/S0140-6736(17)31917-7] [PMID: 28750935]

[168] Grobler, J. Long-acting oral and parenteral dosing of MK-8591 for HIV treatment or prophylaxis. The annual conference on retroviruses and opportunistic infections (CROI), Boston, **2016**.

[169] Friedman, E. A Single monotherapy dose of MK-8591, a Novel NRTI, Suppresses HIV for 10 days. *The Annual Conference on Retroviruses and Opportunistic Infections (CROI),* Boston, **2016**.

[170] Barrett, S.E.; Teller, R.S.; Forster, S.P.; Li, L.; Mackey, M.A.; Skomski, D.; Yang, Z.; Fillgrove, K.L.; Doto, G.J.; Wood, S.L.; Lebron, J.; Grobler, J.A.; Sanchez, R.I.; Liu, Z.; Lu, B.; Niu, T.; Sun, L.; Gindy, M.E. Extended-duration MK-8591-eluting implant as a candidate for HIV treatment and prevention. *Antimicrob. Agents Chemother.,* **2018**, *62*(10)e01058-18 [http://dx.doi.org/10.1128/AAC.01058-18] [PMID: 30012772]

[171] Campbell, E.M.; Hope, T.J. HIV-1 capsid: the multifaceted key player in HIV-1 infection. *Nat. Rev. Microbiol.,* **2015**, *13*(8), 471-483. [http://dx.doi.org/10.1038/nrmicro3503] [PMID: 26179359]

[172] von Schwedler, U.K.; Stray, K.M.; Garrus, J.E.; Sundquist, W.I. Functional surfaces of the human immunodeficiency virus type 1 capsid protein. *J. Virol.,* **2003**, *77*(9), 5439-5450. [http://dx.doi.org/10.1128/JVI.77.9.5439-5450.2003] [PMID: 12692245]

[173] Forshey, B.M.; von Schwedler, U.; Sundquist, W.I.; Aiken, C. Formation of a human immunodeficiency virus type 1 core of optimal stability is crucial for viral replication. *J. Virol.,* **2002**, *76*(11), 5667-5677. [http://dx.doi.org/10.1128/JVI.76.11.5667-5677.2002] [PMID: 11991995]

[174] Rihn, S.J.; Wilson, S.J.; Loman, N.J.; Alim, M.; Bakker, S.E.; Bhella, D.; Gifford, R.J.; Rixon, F.J.; Bieniasz, P.D. Extreme genetic fragility of the HIV-1 capsid. *PLoS Pathog.,* **2013**, *9*(6)e1003461 [http://dx.doi.org/10.1371/journal.ppat.1003461] [PMID: 23818857]

[175] Rawle, D.J.; Harrich, D. Toward the “unravelling” of HIV: Host cell factors involved in HIV-1 core uncoating. *PLoS Pathog.,* **2018**, *14*(10)e1007270 [http://dx.doi.org/10.1371/journal.ppat.1007270] [PMID: 30286189]

[176] Hilditch, L.; Towers, G.J. A model for cofactor use during HIV-1 reverse transcription and nuclear entry. *Curr. Opin. Virol.,* **2014**, *4*, 32-36. [http://dx.doi.org/10.1016/j.coviro.2013.11.003] [PMID: 24525292]

[177] Fassati, A. Multiple roles of the capsid protein in the early steps of HIV-1 infection. *Virus Res.,* **2012**, *170*(1-2), 15-24.

[http://dx.doi.org/10.1016/j.virusres.2012.09.012] [PMID: 23041358]

[178] Ambrose, Z.; Aiken, C. HIV-1 uncoating: connection to nuclear entry and regulation by host proteins. *Virology,* **2014**, *454-455*, 371-379.
[http://dx.doi.org/10.1016/j.virol.2014.02.004] [PMID: 24559861]

[179] Tang, C.; Loeliger, E.; Kinde, I.; Kyere, S.; Mayo, K.; Barklis, E.; Sun, Y.; Huang, M.; Summers, M.F. Antiviral inhibition of the HIV-1 capsid protein. *J. Mol. Biol.,* **2003**, *327*(5), 1013-1020.
[http://dx.doi.org/10.1016/S0022-2836(03)00289-4] [PMID: 12662926]

[180] Sticht, J.; Humbert, M.; Findlow, S.; Bodem, J.; Müller, B.; Dietrich, U.; Werner, J.; Kräusslich, H.G. A peptide inhibitor of HIV-1 assembly *in vitro*. *Nat. Struct. Mol. Biol.,* **2005**, *12*(8), 671-677.
[http://dx.doi.org/10.1038/nsmb964] [PMID: 16041387]

[181] Ternois, F.; Sticht, J.; Duquerroy, S.; Kräusslich, H.G.; Rey, F.A. The HIV-1 capsid protein C-terminal domain in complex with a virus assembly inhibitor. *Nat. Struct. Mol. Biol.,* **2005**, *12*(8), 678-682.
[http://dx.doi.org/10.1038/nsmb967] [PMID: 16041386]

[182] Kelly, B.N.; Kyere, S.; Kinde, I.; Tang, C.; Howard, B.R.; Robinson, H.; Sundquist, W.I.; Summers, M.F.; Hill, C.P. Structure of the antiviral assembly inhibitor CAP-1 complex with the HIV-1 CA protein. *J. Mol. Biol.,* **2007**, *373*(2), 355-366.
[http://dx.doi.org/10.1016/j.jmb.2007.07.070] [PMID: 17826792]

[183] Blair, W.S.; Pickford, C.; Irving, S.L.; Brown, D.G.; Anderson, M.; Bazin, R.; Cao, J.; Ciaramella, G.; Isaacson, J.; Jackson, L.; Hunt, R.; Kjerrstrom, A.; Nieman, J.A.; Patick, A.K.; Perros, M.; Scott, A.D.; Whitby, K.; Wu, H.; Butler, S.L. HIV capsid is a tractable target for small molecule therapeutic intervention. *PLoS Pathog.,* **2010**, *6*(12), e1001220.
[http://dx.doi.org/10.1371/journal.ppat.1001220] [PMID: 21170360]

[184] Bocanegra, R.; Rodríguez-Huete, A.; Fuertes, M.A.; Del Álamo, M.; Mateu, M.G. Molecular recognition in the human immunodeficiency virus capsid and antiviral design. *Virus Res.,* **2012**, *169*(2), 388-410.
[http://dx.doi.org/10.1016/j.virusres.2012.06.016] [PMID: 22728445]

[185] Curreli, F.; Zhang, H.; Zhang, X.; Pyatkin, I.; Victor, Z.; Altieri, A.; Debnath, A.K. Virtual screening based identification of novel small-molecule inhibitors targeted to the HIV-1 capsid. *Bioorg. Med. Chem.,* **2011**, *19*(1), 77-90.
[http://dx.doi.org/10.1016/j.bmc.2010.11.045] [PMID: 21168336]

[186] Fader, L.D.; Bethell, R.; Bonneau, P.; Bös, M.; Bousquet, Y.; Cordingley, M.G.; Coulombe, R.; Deroy, P.; Faucher, A.M.; Gagnon, A.; Goudreau, N.; Grand-Maître, C.; Guse, I.; Hucke, O.; Kawai, S.H.; Lacoste, J.E.; Landry, S.; Lemke, C.T.; Malenfant, E.; Mason, S.; Morin, S.; O'Meara, J.; Simoneau, B.; Titolo, S.; Yoakim, C. Discovery of a 1,5-dihydrobenzo[b][1,4]diazepine-2,4-dione series of inhibitors of HIV-1 capsid assembly. *Bioorg. Med. Chem. Lett.,* **2011**, *21*(1), 398-404.
[http://dx.doi.org/10.1016/j.bmcl.2010.10.131] [PMID: 21087861]

[187] Lemke, C.T.; Titolo, S.; von Schwedler, U.; Goudreau, N.; Mercier, J.F.; Wardrop, E.; Faucher, A.M.; Coulombe, R.; Banik, S.S.; Fader, L.; Gagnon, A.; Kawai, S.H.; Rancourt, J.; Tremblay, M.; Yoakim, C.; Simoneau, B.; Archambault, J.; Sundquist, W.I.; Mason, S.W. Distinct effects of two HIV-1 capsid assembly inhibitor families that bind the same site within the N-terminal domain of the viral CA protein. *J. Virol.,* **2012**, *86*(12), 6643-6655.
[http://dx.doi.org/10.1128/JVI.00493-12] [PMID: 22496222]

[188] Tremblay, M.; Bonneau, P.; Bousquet, Y.; DeRoy, P.; Duan, J.; Duplessis, M.; Gagnon, A.; Garneau, M.; Goudreau, N.; Guse, I.; Hucke, O.; Kawai, S.H.; Lemke, C.T.; Mason, S.W.; Simoneau, B.; Surprenant, S.; Titolo, S.; Yoakim, C. Inhibition of HIV-1 capsid assembly: optimization of the antiviral potency by site selective modifications at N1, C2 and C16 of a 5-(5-furan-2-yl-pyrazol-1-yl)-1H-benzimidazole scaffold. *Bioorg. Med. Chem. Lett.,* **2012**, *22*(24), 7512-7517.
[http://dx.doi.org/10.1016/j.bmcl.2012.10.034] [PMID: 23122820]

[189] Lamorte, L.; Titolo, S.; Lemke, C.T.; Goudreau, N.; Mercier, J.F.; Wardrop, E.; Shah, V.B.; von Schwedler, U.K.; Langelier, C.; Banik, S.S.; Aiken, C.; Sundquist, W.I.; Mason, S.W. Discovery of novel small-molecule HIV-1 replication inhibitors that stabilize capsid complexes. *Antimicrob. Agents Chemother.,* **2013**, *57*(10), 4622-4631.
[http://dx.doi.org/10.1128/AAC.00985-13] [PMID: 23817385]

[190] Gres, A.T.; Kirby, K.A.; KewalRamani, V.N.; Tanner, J.J.; Pornillos, O.; Sarafianos, S.G. Structural virology. X-ray crystal structures of native HIV-1 capsid protein reveal conformational variability. *Science,* **2015**, *349*(6243), 99-103.
[http://dx.doi.org/10.1126/science.aaa5936] [PMID: 26044298]

[191] Price, A.J.; Jacques, D.A.; McEwan, W.A.; Fletcher, A.J.; Essig, S.; Chin, J.W.; Halambage, U.D.; Aiken, C.; James, L.C. Host cofactors and pharmacologic ligands share an essential interface in HIV-1 capsid that is lost upon disassembly. *PLoS Pathog.,* **2014**, *10*(10), e1004459.
[http://dx.doi.org/10.1371/journal.ppat.1004459] [PMID: 25356722]

[192] Bhattacharya, A.; Alam, S.L.; Fricke, T.; Zadrozny, K.; Sedzicki, J.; Taylor, A.B.; Demeler, B.; Pornillos, O.; Ganser-Pornillos, B.K.; Diaz-Griffero, F.; Ivanov, D.N.; Yeager, M. Structural basis of HIV-1 capsid recognition by PF74 and CPSF6. *Proc. Natl. Acad. Sci. USA,* **2014**, *111*(52), 18625-18630.
[http://dx.doi.org/10.1073/pnas.1419945112] [PMID: 25518861]

[193] Price, A.J.; Fletcher, A.J.; Schaller, T.; Elliott, T.; Lee, K.; KewalRamani, V.N.; Chin, J.W.; Towers, G.J.; James, L.C. CPSF6 defines a conserved capsid interface that modulates HIV-1 replication. *PLoS Pathog.,* **2012**, *8*(8), e1002896.
[http://dx.doi.org/10.1371/journal.ppat.1002896] [PMID: 22956906]

[194] Shi, J.; Zhou, J.; Shah, V.B.; Aiken, C.; Whitby, K. Small-molecule inhibition of human immunodeficiency virus type 1 infection by virus capsid destabilization. *J. Virol.,* **2011**, *85*(1), 542-549.
[http://dx.doi.org/10.1128/JVI.01406-10] [PMID: 20962083]

[195] Xu, H.; Franks, T.; Gibson, G.; Huber, K.; Rahm, N.; Strambio De Castillia, C.; Luban, J.; Aiken, C.; Watkins, S.; Sluis-Cremer, N.; Ambrose, Z. Evidence for biphasic uncoating during HIV-1 infection from a novel imaging assay. *Retrovirology,* **2013**, *10*, 70.
[http://dx.doi.org/10.1186/1742-4690-10-70] [PMID: 23835323]

[196] Peng, K.; Muranyi, W.; Glass, B.; Laketa, V.; Yant, S.R.; Tsai, L.; Cihlar, T.; Müller, B.; Kräusslich, H.G. Quantitative microscopy of functional HIV post-entry complexes reveals association of replication with the viral capsid. *eLife,* **2014**, *3*e04114.
[http://dx.doi.org/10.7554/eLife.04114] [PMID: 25517934]

[197] Hulme, A.E.; Kelley, Z.; Foley, D.; Hope, T.J. Complementary assays reveal a low level of CA associated with viral complexes in the nuclei of HIV-1-infected cells. *J. Virol.,* **2015**, *89*(10), 5350-5361.
[http://dx.doi.org/10.1128/JVI.00476-15] [PMID: 25741002]

[198] Zhang, H.; Curreli, F.; Waheed, A.A.; Mercredi, P.Y.; Mehta, M.; Bhargava, P.; Scacalossi, D.; Tong, X.; Lee, S.; Cooper, A.; Summers, M.F.; Freed, E.O.; Debnath, A.K. Dual-acting stapled peptides target both HIV-1 entry and assembly. *Retrovirology,* **2013**, *10*, 136.
[http://dx.doi.org/10.1186/1742-4690-10-136] [PMID: 24237936]

[199] Kortagere, S.; Madani, N.; Mankowski, M.K.; Schön, A.; Zentner, I.; Swaminathan, G.; Princiotto, A.; Anthony, K.; Oza, A.; Sierra, L.J.; Passic, S.R.; Wang, X.; Jones, D.M.; Stavale, E.; Krebs, F.C.; Martín-García, J.; Freire, E.; Ptak, R.G.; Sodroski, J.; Cocklin, S.; Smith, A.B., III Inhibiting early-stage events in HIV-1 replication by small-molecule targeting of the HIV-1 capsid. *J. Virol.,* **2012**, *86*(16), 8472-8481.
[http://dx.doi.org/10.1128/JVI.05006-11] [PMID: 22647699]

[200] Tse, W.C.; Link, J.O.; Mulato, A.; Niedziela-Majka, A.; Rowe, W.; Somoza, J.R.; Villasenor, A.G.; Yant, S.R.; Zhang, J.R.; Zheng, J. In: Discovery of novel potent HIV capsid inhibitors with long-acting potential. *Conference on Retroviruses and Opportunistic Infections,* Seattle, Washington, USA, **2017**.

[201] Zheng, J.; Yant, S.R.; Ahmadyar, S.; Chan, T.Y.; Chiu, A.; Cihlar, T.; Link, J.O.; Lu, B.; Mwangi, J.; Rowe, W.; Schroeder, S.D.; Stepan, G.J.; Wang, K.W.; Subramanian, R.; Tse, W.C. 539. GS-CA2: A novel, potent, and selective first-in-class inhibitor of hiv-1 capsid function displays nonclinical pharmacokinetics supporting long-acting potential in humans. *Open Forum Infect Dis.*, **2018**, *5*(Suppl 1), S199-S200.
[DOI: 10.1093/ofid/ofy210.548]

[202] Jarvis, L.M. Conquering, H. IV's capsid. *Chem. Eng. News,* **2017**, *95(31),* 23-25.

[203] Carnes, S.K.; Sheehan, J.H.; Aiken, C. Inhibitors of the HIV-1 capsid, a target of opportunity. *Curr. Opin. HIV AIDS,* **2018**, *13*(4), 359-365. [http://dx.doi.org/10.1097/COH.0000000000000472] [PMID: 29782334]

[204] Zhao, Q.; Ma, L.; Jiang, S.; Lu, H.; Liu, S.; He, Y.; Strick, N.; Neamati, N.; Debnath, A.K. Identification of N-phenyl-N'-(2,2,6,6-tetramethyl-piperidin-4-yl)-oxalamides as a new class of HIV-1 entry inhibitors that prevent gp120 binding to CD4. *Virology,* **2005**, *339*(2), 213-225. [http://dx.doi.org/10.1016/j.virol.2005.06.008] [PMID: 15996703]

[205] Schön, A.; Madani, N.; Klein, J.C.; Hubicki, A.; Ng, D.; Yang, X.; Smith, A.B., III; Sodroski, J.; Freire, E. Thermodynamics of binding of a low-molecular-weight CD4 mimetic to HIV-1 gp120. *Biochemistry,* **2006**, *45*(36), 10973-10980. [http://dx.doi.org/10.1021/bi061193r] [PMID: 16953583]

[206] Yamada, Y.; Ochiai, C.; Yoshimura, K.; Tanaka, T.; Ohashi, N.; Narumi, T.; Nomura, W.; Harada, S.; Matsushita, S.; Tamamura, H. CD4 mimics targeting the mechanism of HIV entry. *Bioorg. Med. Chem. Lett.,* **2010**, *20*(1), 354-358. [http://dx.doi.org/10.1016/j.bmcl.2009.10.098] [PMID: 19926478]

[207] Narumi, T.; Ochiai, C.; Yoshimura, K.; Harada, S.; Tanaka, T.; Nomura, W.; Arai, H.; Ozaki, T.; Ohashi, N.; Matsushita, S.; Tamamura, H. CD4 mimics targeting the HIV entry mechanism and their hybrid molecules with a CXCR4 antagonist. *Bioorg. Med. Chem. Lett.,* **2010**, *20*(19), 5853-5858. [http://dx.doi.org/10.1016/j.bmcl.2010.07.106] [PMID: 20728351]

[208] Yoshimura, K.; Harada, S.; Shibata, J.; Hatada, M.; Yamada, Y.; Ochiai, C.; Tamamura, H.; Matsushita, S. Enhanced exposure of human immunodeficiency virus type 1 primary isolate neutralization epitopes through binding of CD4 mimetic compounds. *J. Virol.,* **2010**, *84*(15), 7558-7568. [http://dx.doi.org/10.1128/JVI.00227-10] [PMID: 20504942]

[209] Lalonde, J.M.; Elban, M.A.; Courter, J.R.; Sugawara, A.; Soeta, T.; Madani, N.; Princiotto, A.M.; Kwon, Y.D.; Kwong, P.D.; Schön, A.; Freire, E.; Sodroski, J.; Smith, A.B., III Design, synthesis and biological evaluation of small molecule inhibitors of CD4-gp120 binding based on virtual screening. *Bioorg. Med. Chem.,* **2011**, *19*(1), 91-101. [http://dx.doi.org/10.1016/j.bmc.2010.11.049] [PMID: 21169023]

[210] Lu, R.J.; Tucker, J.A.; Zinevitch, T.; Kirichenko, O.; Konoplev, V.; Kuznetsova, S.; Sviridov, S.; Pickens, J.; Tandel, S.; Brahmachary, E.; Yang, Y.; Wang, J.; Freel, S.; Fisher, S.; Sullivan, A.; Zhou, J.; Stanfield-Oakley, S.; Greenberg, M.; Bolognesi, D.; Bray, B.; Koszalka, B.; Jeffs, P.; Khasanov, A.; Ma, Y.A.; Jeffries, C.; Liu, C.; Proskurina, T.; Zhu, T.; Chucholowski, A.; Li, R.; Sexton, C. Design and synthesis of human immunodeficiency virus entry inhibitors: sulfonamide as an isostere for the alpha-ketoamide group. *J. Med. Chem.,* **2007**, *50*(26), 6535-6544. [http://dx.doi.org/10.1021/jm070650e] [PMID: 18052117]

[211] Ohashi, N.; Harada, S.; Mizuguchi, T.; Irahara, Y.; Yamada, Y.; Kotani, M.; Nomura, W.; Matsushita, S.; Yoshimura, K.; Tamamura, H. Small-molecule CD4 mimics containing mono-cyclohexyl moieties as HIV entry inhibitors. *ChemMedChem,* **2016**, *11*(8), 940-946. [http://dx.doi.org/10.1002/cmdc.201500590] [PMID: 26891461]

[212] Eda, Y.; Takizawa, M.; Murakami, T.; Maeda, H.; Kimachi, K.; Yonemura, H.; Koyanagi, S.; Shiosaki, K.; Higuchi, H.; Makizumi, K.; Nakashima, T.; Osatomi, K.; Tokiyoshi, S.; Matsushita, S.; Yamamoto, N.; Honda, M. Sequential immunization with V3 peptides from primary human immunodeficiency virus type 1 produces cross-neutralizing antibodies against primary isolates with a matching narrow-neutralization sequence motif. *J. Virol.,* **2006**, *80*(11), 5552-5562. [http://dx.doi.org/10.1128/JVI.02094-05] [PMID: 16699036]

[213] Chun, T.W.; Davey, R.T., Jr; Engel, D.; Lane, H.C.; Fauci, A.S. Re-emergence of HIV after stopping therapy. *Nature,* **1999**, *401*(6756), 874-875. [http://dx.doi.org/10.1038/44755] [PMID: 10553903]

[214] Finzi, D.; Hermankova, M.; Pierson, T.; Carruth, L.M.; Buck, C.; Chaisson, R.E.; Quinn, T.C.; Chadwick, K.; Margolick, J.; Brookmeyer, R.; Gallant, J.; Markowitz, M.; Ho, D.D.; Richman, D.D.; Siliciano, R.F. Identification of a reservoir for HIV-1 in patients on highly active antiretroviral therapy. *Science,* **1997**, *278*(5341), 1295-1300. [http://dx.doi.org/10.1126/science.278.5341.1295] [PMID: 9360927]

[215] Siliciano, J.D.; Kajdas, J.; Finzi, D.; Quinn, T.C.; Chadwick, K.; Margolick, J.B.; Kovacs, C.; Gange, S.J.; Siliciano, R.F. Long-term follow-up studies confirm the stability of the latent reservoir for HIV-1 in resting CD4+ T cells. *Nat. Med.,* **2003**, *9*(6), 727-728. [http://dx.doi.org/10.1038/nm880] [PMID: 12754504]

[216] Geeraert, L.; Kraus, G.; Pomerantz, R.J. Hide-and-seek: the challenge of viral persistence in HIV-1 infection. *Annu. Rev. Med.,* **2008**, *59*, 487-501. [http://dx.doi.org/10.1146/annurev.med.59.062806.123001] [PMID: 17845138]

[217] Hamer, D.H. Can HIV be Cured? Mechanisms of HIV persistence and strategies to combat it. *Curr. HIV Res.,* **2004**, *2*(2), 99-111. [http://dx.doi.org/10.2174/1570162043484915] [PMID: 15078175]

[218] Katlama, C.; Deeks, S.G.; Autran, B.; Martinez-Picado, J.; van Lunzen, J.; Rouzioux, C.; Miller, M.; Vella, S.; Schmitz, J.E.; Ahlers, J.; Richman, D.D.; Sekaly, R.P. Barriers to a cure for HIV: new ways to target and eradicate HIV-1 reservoirs. *Lancet,* **2013**, *381*(9883), 2109-2117. [http://dx.doi.org/10.1016/S0140-6736(13)60104-X] [PMID: 23541541]

[219] Richman, D.D.; Margolis, D.M.; Delaney, M.; Greene, W.C.; Hazuda, D.; Pomerantz, R.J. The challenge of finding a cure for HIV infection. *Science,* **2009**, *323*(5919), 1304-1307. [http://dx.doi.org/10.1126/science.1165706] [PMID: 19265012]

[220] Kim, Y.; Anderson, J.L.; Lewin, S.R. Getting the "kill" into "shock and kill": Strategies to eliminate latent HIV. *Cell Host Microbe,* **2018**, *23*(1), 14-26. [http://dx.doi.org/10.1016/j.chom.2017.12.004] [PMID: 29324227]

[221] Hattori, S.I.; Matsuda, K.; Tsuchiya, K.; Gatanaga, H.; Oka, S.; Yoshimura, K.; Mitsuya, H.; Maeda, K. Combination of a latency-reversing agent with a smac mimetic minimizes secondary HIV-1 infection *in vitro. Front. Microbiol.,* **2018**, *9*, 2022. [http://dx.doi.org/10.3389/fmicb.2018.02022] [PMID: 30283406]

[222] Archin, N.M.; Liberty, A.L.; Kashuba, A.D.; Choudhary, S.K.; Kuruc, J.D.; Crooks, A.M.; Parker, D.C.; Anderson, E.M.; Kearney, M.F.; Strain, M.C.; Richman, D.D.; Hudgens, M.G.; Bosch, R.J.; Coffin, J.M.; Eron, J.J.; Hazuda, D.J.; Margolis, D.M. Administration of vorinostat disrupts HIV-1 latency in patients on antiretroviral therapy. *Nature,* **2012**, *487*(7408), 482-485. [http://dx.doi.org/10.1038/nature11286] [PMID: 22837004]

[223] Rasmussen, T.A.; Tolstrup, M.; Brinkmann, C.R.; Olesen, R.; Erikstrup, C.; Solomon, A.; Winckelmann, A.; Palmer, S.; Dinarello, C.; Buzon, M.; Lichterfeld, M.; Lewin, S.R.; Østergaard, L.; Søgaard, O.S. Panobinostat, a histone deacetylase inhibitor, for latent-virus reactivation in HIV-infected patients on suppressive antiretroviral therapy: a phase 1/2, single group, clinical trial. *Lancet HIV,* **2014**, *1*(1), e13-e21. [http://dx.doi.org/10.1016/S2352-3018(14)70014-1] [PMID: 26423811]

[224] Gutiérrez, C.; Serrano-Villar, S.; Madrid-Elena, N.; Pérez-Elías, M.J.; Martín, M.E.; Barbas, C.; Ruipérez, J.; Muñoz, E.; Muñoz-Fernández, M.A.; Castor, T.; Moreno, S. Bryostatin-1 for latent virus reactivation in HIV-infected patients on antiretroviral therapy. *AIDS,* **2016**, *30*(9), 1385-1392. [http://dx.doi.org/10.1097/QAD.0000000000001064] [PMID: 26891037]

[225] Elliott, J.H.; McMahon, J.H.; Chang, C.C.; Lee, S.A.; Hartogensis, W.; Bumpus, N.; Savic, R.; Roney, J.; Hoh, R.; Solomon, A.; Piatak, M.; Gorelick, R.J.; Lifson, J.; Bacchetti, P.; Deeks, S.G.; Lewin, S.R. Short-term administration of disulfiram for reversal of latent HIV infection: a phase 2 dose-escalation study. *Lancet HIV,* **2015**, *2*(12), e520-e529. [http://dx.doi.org/10.1016/S2352-3018(15)00226-X] [PMID: 26614966]

[226] Søgaard, O.S.; Graversen, M.E.; Leth, S.; Olesen, R.; Brinkmann, C.R.; Nissen, S.K.; Kjaer, A.S.; Schleimann, M.H.; Denton, P.W.; Hey-Cunningham, W.J.; Koelsch, K.K.; Pantaleo, G.; Krogsgaard, K.; Sommerfelt, M.; Fromentin, R.; Chomont, N.; Rasmussen, T.A.; Østergaard, L.; Tolstrup, M. The depsipeptide romidepsin reverses HIV-1 latency *in vivo*. *PLoS Pathog.,* **2015**, *11*(9), e1005142. [http://dx.doi.org/10.1371/journal.ppat.1005142] [PMID: 26379282]

[227] Mbonye, U.; Karn, J. Transcriptional control of HIV latency: Cellular signaling pathways, epigenetics, happenstance and the hope for a cure. *Virology,* **2014**, *454-455*, 328-339. [http://dx.doi.org/10.1016/j.virol.2014.02.008] [PMID: 24565118]

[228] Ho, Y.C.; Shan, L.; Hosmane, N.N.; Wang, J.; Laskey, S.B.; Rosenbloom, D.I.; Lai, J.; Blankson, J.N.; Siliciano, J.D.; Siliciano, R.F. Replication-competent noninduced proviruses in the latent reservoir increase barrier to HIV-1 cure. *Cell,* **2013**, *155*(3), 540-551. [http://dx.doi.org/10.1016/j.cell.2013.09.020] [PMID: 24243014]

[229] Bullen, C.K.; Laird, G.M.; Durand, C.M.; Siliciano, J.D.; Siliciano, R.F. New *ex vivo* approaches distinguish effective and ineffective single agents for reversing HIV-1 latency *in vivo*. *Nat. Med.,* **2014**, *20*(4), 425-429. [http://dx.doi.org/10.1038/nm.3489] [PMID: 24658076]

[230] Boehm, D.; Calvanese, V.; Dar, R.D.; Xing, S.; Schroeder, S.; Martins, L.; Aull, K.; Li, P.C.; Planelles, V.; Bradner, J.E.; Zhou, M.M.; Siliciano, R.F.; Weinberger, L.; Verdin, E.; Ott, M. BET bromodomain-targeting compounds reactivate HIV from latency via a Tat-independent mechanism. *Cell Cycle,* **2013**, *12*(3), 452-462. [http://dx.doi.org/10.4161/cc.23309] [PMID: 23255218]

[231] Williams, S.A.; Chen, L.F.; Kwon, H.; Fenard, D.; Bisgrove, D.; Verdin, E.; Greene, W.C. Prostratin antagonizes HIV latency by activating NF-kappaB. *J. Biol. Chem.,* **2004**, *279*(40), 42008-42017. [http://dx.doi.org/10.1074/jbc.M402124200] [PMID: 15284245]

[232] Laird, G.M.; Bullen, C.K.; Rosenbloom, D.I.; Martin, A.R.; Hill, A.L.; Durand, C.M.; Siliciano, J.D.; Siliciano, R.F. *Ex vivo* analysis identifies effective HIV-1 latency-reversing drug combinations. *J. Clin. Invest.,* **2015**, *125*(5), 1901-1912. [http://dx.doi.org/10.1172/JCI80142] [PMID: 25822022]

[233] Gohda, J.; Suzuki, K.; Liu, K.; Xie, X.; Takeuchi, H.; Inoue, J.I.; Kawaguchi, Y.; Ishida, T. BI-2536 and BI-6727, dual Polo-like kinase/bromodomain inhibitors, effectively reactivate latent HIV-1. *Sci. Rep.,* **2018**, *8*(1), 3521. [http://dx.doi.org/10.1038/s41598-018-21942-5] [PMID: 29476067]

[234] Matsuda, K.; Kobayakawa, T.; Tsuchiya, K.; Hattori, S.I.; Nomura, W.; Gatanaga, H.; Yoshimura, K.; Oka, S.; Endo, Y.; Tamamura, H.; Mitsuya, H.; Maeda, K. Benzolactam-related compounds promote apoptosis of HIV-infected human cells *via* protein kinase C-induced HIV latency reversal. *J. Biol. Chem.,* **2019**, *294*(1), 116-129. [http://dx.doi.org/10.1074/jbc.RA118.005798] [PMID: 30413535]

[235] Sengupta, S.; Siliciano, R.F. Targeting the latent reservoir for HIV-1. *Immunity,* **2018**, *48*(5), 872-895. [http://dx.doi.org/10.1016/j.immuni.2018.04.030] [PMID: 29768175]

[236] Cillo, A.R.; Sobolewski, M.D.; Bosch, R.J.; Fyne, E.; Piatak, M., Jr; Coffin, J.M.; Mellors, J.W. Quantification of HIV-1 latency reversal in resting CD4+ T cells from patients on suppressive antiretroviral therapy. *Proc. Natl. Acad. Sci. USA,* **2014**, *111*(19), 7078-7083. [http://dx.doi.org/10.1073/pnas.1402873111] [PMID: 24706775]

[237] Lucera, M.B.; Tilton, C.A.; Mao, H.; Dobrowolski, C.; Tabler, C.O.; Haqqani, A.A.; Karn, J.; Tilton, J.C. The histone deacetylase inhibitor vorinostat (SAHA) increases the susceptibility of uninfected CD4+ T cells to HIV by increasing the kinetics and efficiency of postentry viral events. *J. Virol.,* **2014**, *88*(18), 10803-10812. [http://dx.doi.org/10.1128/JVI.00320-14] [PMID: 25008921]

[238] Tamamura, H.; Bienfait, B.; Nacro, K.; Lewin, N.E.; Blumberg, P.M.; Marquez, V.E. Conformationally constrained analogues of diacylglycerol (DAG). 17. Contrast between sn-1 and sn-2 DAG lactones in binding to protein kinase C. *J. Med. Chem.,* **2000**, *43*(17), 3209-3217. [http://dx.doi.org/10.1021/jm990613q] [PMID: 10966739]

[239] Newton, A.C. Protein kinase C: Structure, function, and regulation. *J. Biol. Chem.,* **1995**, *270*(48), 28495-28498. [http://dx.doi.org/10.1074/jbc.270.48.28495] [PMID: 7499357]

[240] Margolis, D.M. Confronting proviral HIV infection. *Curr. HIV/AIDS Rep.,* **2007**, *4*(2), 60-64. [http://dx.doi.org/10.1007/s11904-007-0009-6] [PMID: 17547826]

[241] Jiang, G.; Mendes, E.A.; Kaiser, P.; Wong, D.P.; Tang, Y.; Cai, I.; Fenton, A.; Melcher, G.P.; Hildreth, J.E.; Thompson, G.R.; Wong, J.K.; Dandekar, S. Synergistic reactivation of latent HIV expression by ingenol-3-angelate, PEP005, Targeted NF-kB signaling in combination with JQ1 induced p-TEFb activation. *PLoS Pathog.,* **2015**, *11*(7), e1005066. [http://dx.doi.org/10.1371/journal.ppat.1005066] [PMID: 26225771]

[242] Borducchi, E.N.; Liu, J.; Nkolola, J.P.; Cadena, A.M.; Yu, W.H.; Fischinger, S.; Broge, T.; Abbink, P.; Mercado, N.B.; Chandrashekar, A.; Jetton, D.; Peter, L.; McMahan, K.; Moseley, E.T.; Bekerman, E.; Hesselgesser, J.; Li, W.; Lewis, M.G.; Alter, G.; Geleziunas, R.; Barouch, D.H. Antibody and TLR7 agonist delay viral rebound in SHIV-infected monkeys. *Nature,* **2018**, *563*(7731), 360-364. [http://dx.doi.org/10.1038/s41586-018-0600-6] [PMID: 30283138]

[243] Salie, Z.L.; Kirby, K.A.; Michailidis, E.; Marchand, B.; Singh, K.; Rohan, L.C.; Kodama, E.N.; Mitsuya, H.; Parniak, M.A.; Sarafianos, S.G. Structural basis of HIV inhibition by translocation-defective RT inhibitor 4′-ethynyl-2-fluoro-2′-deoxyadenosine (EFdA). *Proc. Natl. Acad. Sci. USA,* **2016**, *113*(33), 9274-9279. [http://dx.doi.org/10.1073/pnas.1605223113] [PMID: 27489345]

[244] Michailidis, E.; Marchand, B.; Kodama, E.N.; Singh, K.; Matsuoka, M.; Kirby, K.A.; Ryan, E.M.; Sawani, A.M.; Nagy, E.; Ashida, N.; Mitsuya, H.; Parniak, M.A.; Sarafianos, S.G. Mechanism of inhibition of HIV-1 reverse transcriptase by 4′-Ethynyl-2-fluoro-2′-deoxyadenosine triphosphate, a translocation-defective reverse transcriptase inhibitor. *J. Biol. Chem.,* **2009**, *284*(51), 35681-35691. [http://dx.doi.org/10.1074/jbc.M109.036616] [PMID: 19837673]