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ORIGINAL ARTICLE

New Approach for Inhibition of HIV Entry: Modifying CD4 Binding Sites by Thiolated Pyrimidine Derivatives

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Abstract Thiolated pyrimidine derivatives have been synthetized and their antiretroviral effect against human immunodeficiency virus type 1 (HIV-1_{IIIB}) and HIV-1 chimeric pseudovirions have been quantitatively determined in cell-based viral infectivity assays including syncytium inhibition assay as well as a single-cycle viral infection assay on HeLaCD4-LTR/ß-gal cells. Pseudotype virions prepared bearing HIV-1 envelope preference for CCR5 coreceptor, CXCR4 coreceptor or for both, respectively, with a HIV-1 core containing luciferase reporter gene were able to infect susceptible cells but are replication defective so unable to replicate in the cells . Data indicate that thiolated pyrimidine derivatives inhibited effectively virally induced cell fusion in vitro as well as infectivity of primary HIV-1IIIB strain and HIV-1 pseudovirions using chemokine receptors CCR5 or CXCR4 or both for virus entry a dose dependent manner. Inhibition was selective, depended on the pseudovirus coreceptor preference. Our results suggest that some of these sulfur containing pyrimidines interact with redoxactive -SH groups required for successful HIV entry, including a redox active disulfide in the CD4 molecule as well as -SH groups in HIV viral envelope gp120. This mode of action is unique representing a new class of potential HIV entry inhibitors.

Keywords HIV entry · Thiolated pyrimidine derivatives · HIV-1 pseudotypes · Redox processes · CD4

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Introduction

Human immunodeficiency virus type 1 (HIV-1) infection and AIDS are still a serious public health issue. The introduction of highly active antiretroviral therapy (HAART) for AIDS has dramatically delayed disease progression and increased the lifespan of infected individuals [1], while decreased HIV transmission rates [2], but its effects are limited by the emergence of drug resistance HIV strains [3]. The drugs used so far are inhibitors of virally coded enzymes and are acting only while the virus has already infected the cells. HIV-associated morbidities [4–6] persist underlining the need for new strategies to control HIV infection.

Fully elucidating the early steps of HIV replication is crucial not only for identifying new antiretroviral drugs, but also for improving the design of retroviral vectors for gene therapy [7]. In addition to conformational changes in cell surface receptors, coreceptors of HIV [8, 9] and viral envelope proteins, redox changes in these proteins are also required for successful HIV-1 entry and infection. HIV Env has an unusually high amount of disulfide-bonded molecule with 9 out of a total of 10 disulfide bonds occurring within the outer viral membrane domain of gp120 [10]. Viral and cell membrane fusion are enabled by the reduction of disulfide bonds of Env by a lymphocyte surface-associated reductase activity [11]. The involvement of redox changes of Env as part of the HIV-lymphocyte interaction is supported by the observation that an unusually dense cluster of disulfide bonds occurs close to the receptor binding surfaces [12]. They concentrate on lipid rafts of the cell membrane, as we and others reported earlier [13, 14].

Redox processes active in viral entry could be potential targets for treatment of HIV infection [10, 14, 15, 17].

In this paper we report the inhibitory effects on primary HIV-1 infection of various pyrimidine nucleotide derivatives

synthesized in our laboratory. Based on our earlier results, these compounds likely to be inhibitors of HIV entry by interfering the redox processes (−SH group of CD4 molecule as well as –SH groups in HIV viral envelope gp120) required for successful HIV entry. We studied the action of the modified pyrimidines in specific quantitative in vitro HIV infectivity assays using unique recombinant HIV-1 viral pseudotypes utilizing CCR5 or CXCR4 or both of the cellular coreceptors for entry. One of our compounds (DS53) showed a remarkable antiviral activity and low toxicity on primary HIV-1 infected cells verifying our earlier suggestion [12, 18] that 4-thio pyrimidine derivatives may represent a new class of HIV entry inhibitors with unique mode of action.

Materials and Methods

Cells

H9 human T-cells, MT-2 human T-cells, HeLaCD4-LTR/ß-gal cells, HEK293T human embryonic kidney cells were obtained from the NIH AIDS Research and Reference Reagent Program (Bethesda, MD). 3T3.T4.CCR5 and 3T3.T4.CXCR4 mouse fibroblast cells expressing the respective human kemokine receptors, as well as P4-CCR5 transfected HeLa indicator cells [19] were obtained from Institute of Virology, University of Leipzig, Germany (C. Jassoy). 3T3.T4.CCR5, 3T3.T4.CXCR4 and P4-CCR5 cells (transfected HeLa cells that express CD4, CCR5 and CXCR4) were used for the determination of pseudovirion titer and tropism.

HIV-1_{IIIB} Virus Stocks

Human immunodeficiency virus type 1 (HIV-1_{IIIB}) was obtained from R. Weiss (London University, UK). HIV-1_{IIIB} strains were propagated in H9 human permanent T lymphocyte cells, HIV-1_{IIIB} stocks were prepared and stored as described earlier [20]. Infectious titer was determined by infectivity assays on MT-2 human T cells (see below).

Preparation of Plasmids

Preparation of plasmids containing genes encoding various HIV Env glycoproteins determining coreceptor preference of pseudovirions was described elsewhere [19]. Three different plasmids were constructed and used: *pEnv M-ad*, coding envelope glycoproteins for CCR5 coreceptor; *pEnv HXB2*, for CXCR4 and *pEnv M-ad/HXB2* coding genes for dual tropic envelope glycoproteins. (The plasmids were obtained from C. Jassoy, University of Leipzig, Germany). Lentiviral expression plasmid *pGJ3-luci* containing a reporter gene (firefly luciferase) and HIV *gag-pol* genes between LTR sequences [19] was used . To amplify plasmids competent DH5α *E. coli* was transformed, and isolated with Quiagen Plasmid Midi kit (Quiagen) according to the protocol and stored at −20 ° C.

Preparation of Pseudovirions

To produce HIV-1 pseudotype virus [16] stocks for infectivity and determination of virus entry, pEnv plasmids were transfected by the calcium phosphate method into HEK293T cells, using 2.5 μg DNA together with pGJ3-luci (2.5 μg DNA). 48 h after transfection cell culture supernatant that contains the chimeric constructs (pseudovirions) was harvested and stored at −80 °C until use. To examine transfection efficiency at the time of pseudovirion harvest, expression of the luciferase was determined in cell lysates by measuring the luciferase activity with a luminometer (Victor 3 Multilabel plate reader, Perkin Elmer). By this method infectious but replication defective HIV-1 pseudovirions were obtained: *M-ad* utilizing CCR5 chemokine receptor; *HXB2* utilizing CXCR4 and *HXB2/M-ad* utilizing both receptors as dual tropic pseudovirions.

Preparation of 4-Thiolated Pyrimidine Derivatives

The introduction of 4-thiono group to the pyrimidine ring was performed by H_2S treatment of the appropriate cytidine or cytidylate derivatives as we published earlier [12, 14, 18]. The 4-thiono group has a propensity toward tautomeric conversion to form a reactive –SH (thiol), which may affect redox processes mediated by protein –SH groups. Seven derivatives of 4-thio-uridylate were prepared and used (see Table 1) Compounds were dissolved in aqueous stock solution or in DMSO as 50 mM stock solutions and kept at −20 °C until use, but not longer than 30 days.

HIV-1 Infectivity and Inhibition of Infection Assays

Syncytium Induction and Inhibition of Syncytia Formation

The infectious units of biologically active HIV-1_{IIIB} were determined by their ability to induce syncytia (multinucleated giant cells) as we described earlier [15, 21, 22] on MT-2 human T-cell line, and in H9 cells. Inhibitory activity on syncytia formation of the thiolated pyrimidine compounds was determined by adding 100 μl of compounds in various concentrations (up to 100 μM) to the cells 30 min before, at the time of and 30 min after virus infection. Reduction in the number of syncytia compared to the ones without treatment of thiolated compounds was calculated.

Table 1 Effect of thiolated pyrimidines on the viabilty of HIV-1_{IIIB} infected and non-infected H9 T-lymphocyte cells and monolayer HeLaCD4-LTR-βgal cells

Thiolated compounds	*H9 cells*		*HeLaCD4-LTR-βgal cells*	
	uninfected	HIV-1_{IIIB} infected	uninfected	HIV-1_{IIIB} infected
	(per cent cell viability)			
UD29	100	53	100	55
UD30	100	43	90	54
UD31	100	45	88	77
UD29-new	88	36	80	50
MOD-94	100	45	85	95
MOD-2012	88	35	85	53
DS53	100	92	88	82

Viability values were calculated and adjusted to IC_{50} μM concentration of the respected compound
- HIV-1_{IIIB} (moi 0,2) infection 30 min after adding compounds
- cytotoxicity was determined 24 h post infection quantitatively by XTT Assay (see Material and methods)

Multinuclear Activation of Galactosidase Inhibition (MAGI) Assay

A single-round infectivity assay (MAGI assay) was used to determine infectious titer of HIV-1_{IIIB}, as we previously described [20]. HeLaCD4-LTR/β -gal cells and P4 cells were seeded in 96-well plates and infected with a multiplicity of infection (m.o.i) of 0.02, 0.2, and 0.4 of HIV-1 stock virus in the presence or in the absence of various concentrations (up to 100 μM) of thiolated compounds added to the cells 30 min before, at the time of and 30 min after virus infection. Forty hours after infection, infected cells were counted *in situ* with light microscope by virtue of their blue color after incubation with 5-bromo-4-chloro-3-indolyl-ß-D galactopyranoside (X-gal) [20]. The percentage of inhibition was calculated as mean numbers of blue cells of inhibitor-treated versus non-treated cultures using Hill analysis.

Determination of HIV-1 Pseudotype Infectivity and Inhibition by Thiolated Pyrimidine Compounds

Infectious but replication defective CCR5, CXCR4 or dual tropic HIV-1 chimeric pseudotype virions have been prepared by transfection of HEK293T cells (see above). 3T3.T4.CCR5 and 3T3.T4.CXCR4 permissive mouse fibroblast cells, as well as P4 cells in 96-well OptiPlate (Perkin Elmer) ($3–5 \times 10^4$cells/well) were infected by 5, 10 and 20 μl respectively of HIV-1 pseudotype virions prepared. Thiolated oligonucleotide compounds in concentrations of up to 100 μM were added respectively to cells 30 min before, at time of or 30 min after infection with pseudovirions. 48 h later pseudotype infectivity and/or inhibition of infection by thiolated pyrimidine compounds were quantitatively determined in cell lysates by measuring the luciferase activity in RLU (relative luminescence unit) with a luminometer (Victor 3 Multilabel plate reader, Perkin Elmer).

XTT Assay Measuring Cytotoxicity

Potential toxic effect of the compounds on HeLaCD4-LTR/ß-gal cells, as well in H9 cells has been determined quantitatively in vitro by XTT based Toxicology Assay Kit (Sigma-Aldrich) performed according to the protocol.

Statistical Methods

For the determination of inhibitory concentrations IC_{50} and IC_{90} values of thiolated compounds Hill analysis with 95 % confidence limit was used. For other bio statistical analysis two-tailed Student's test was used.

Results

Cytotoxic Effect of Thiolated Pyrimidine Compounds on H9 and HeLaCD4-LTR/ß-gal Cells

Potential cytotoxicity on cell viability of thiolated compounds was determined on uninfected H9 human T lymphoid cells and on HeLaCD4-LTR/ß-gal cells, and on HIV-1_{IIIB} infected cells (Table 1).

Cytotoxicity was determined 24 h post infection using the XTT assay. Viability values were calculated and adjusted to IC_{50} μM concentration of the respective compound. On both uninfected lymphoid and monolayer cells there was only minor fluctuation on viability using the various compounds. HIV-1 infection however significantly reduced viability of the cells treated with the compounds. Cytotoxicity was somehow more pronounced in H9 as compared to HeLaCD4-LTR/ß-gal

cells. Treatment with DS53 however did not show pronounced cytotoxicity either on uninfected or virus infected cells (Table 1).

Inhibition of Primary HIV-1_{IIIB} Infection of H9 and HeLaCD4-LTR/ß-gal Cells by Thiolated Pyrimidine Compounds

The antiviral potency of seven thiolated pyrimidine compounds (nucleotides and nucleosides) on HIV-1_{IIIB} infection was quantitatively determined by cell based infectivity assays on H9 human lymphocyte cultures. The various thiolated pyrimidine compounds inhibited HIV-1_{IIIB} infection in vitro by IC_{50} values ranging 10 μM (UD29-New) to 1.85 μM (DS53) (see Table 2). Quantitative determination of the compounds' cytotoxicity were also performed. The various compounds displayed different cytotoxic effect on H9 cells ranging in cytotoxic values of TC_{50} from 7.8 μM (MOD-2012) to >200 μM (UD29). Antiviral activity did not correlate with cytotoxicity.

In comparison to H9 lymphoid cells the inhibition of HIV-1_{IIIB} infection by the thiolated pyrimidine compounds was also analyzed in monolayer HeLaCD4-LTR/ß-gal cells. This construct allows only a single cycle of virus infection and therefore is suitable to test compounds with a potential of inhibition of viral entry. In these cells antiviral inhibitory concentration (IC_{50} value) of the compounds was slightly higher, ranging 4.5 μM (DS53) to 15 μM (UD29-new) with slightly less cytotoxicity (Table 2).

The theoretical in vitro therapeutic index (toxic versus effective dose; TC_{50} /IC_{50}) was also calculated. On both H9 cells and HeLaCD4-LTR/ß-gal cells UD29 is the less toxic and has the highest TI. DS53 proved to be the most effective against HIV-1_{IIIB} infection with the second highest TI value (Table 2). In all the cases antiviral activity of the compounds was dose dependent (data not shown).

Inhibition of Cell Fusion on MT-2 Cells

The thiolated pyrimidine derivate DS53 proved to be the most effective with favorable cytotoxicity and TI value. When the compounds were added to MT-2 human T-cells 30 min prior to infection with HIV-1_{IIIB} strain (m.o.i.: 0.2), DS53 prevented syncytia formation induced by HIV-1_{IIIB} infection with IC_{50}: 0.75 μM (Table 3).

DS53 Inhibits Infection of HIV-1 Pseudotypes with Different Coreceptor Preference Selectively *in P4 Cells*

P4 cells are suitable to analyze entry of HIV-1 virions with different coreceptor preference. P4 cells were infected with M-ad (CCR5), HXB2 (CXCR4) and the dual tropic HXB2/M-ad (X4/R5) pseudoviruses respectively at the indicated doses. 30 min before pseudovirus infection cells were treated with DS53 at concentrations indicated. Pseudovirus infectivity was quantitatively determined in cell lysates by measuring the luciferase activity indicating the quantity of viruses that entered the cells. DS53 inhibited pseudovirion infection in a dose dependent manner. This effect was somehow selective as it inhibited most effectively infection of M-ad (pseudovirus with CCR5 preference) with an IC_{50} value 0.75 μM and a slightly less effectively pseudoviruses

Table 2 Inhibitory effect of thiolated pyrimidines on primary HIV-1_{IIIB} virus infection of H9 and HeLaCD4-LTR-βgal cells

Thiolated compounds	H9 cells			HeLaCD4- LTR-βgal cells		
	IC_{50} (μM)	TC_{50} (μM)	TI	IC_{50} (μM)	TC_{50} (μM)	TI
UD29	7.5	>200	>27	12.2	>200	>27
UD30	8.6	>100	>12	14.8	>100	>12
UD31	7.8	>100	>13	14.2	>100	>13
UD29-new	10.0	100	10	15.0	150	10
MOD-94	7.4	20	2.7	12.7	45	3.5
MOD-2012	4.12	7.8	1.9	10.1	40	3.9
DS53	1.85	>40	>22	4.5	80	17

- HIV-1_{IIIB} (moi:0,2) infection 30 min after adding compounds
- cytotoxicity was determined 24 h post infection quantitatively by XTT Assay (see Material and methods)
- IC_{50} - 50 % inhibition of infection (μM)
- TC_{50} - 50 % reduction of cell viability (μM)
- TI - calculated in vitro therapeutic index (TC_{50} /IC_{50})

Table 3 Antiviral activity of UD29 and DS53 against HIV-1_{IIIB} and HIV-1 pseudotype viruses on inhibition of cell fusion and virus entry

Compounds inhibition assays	Viruses							
	HIV-1_{IIIB}		M-ad		HXB2		HXB2/M-ad	
	IC_{50}[a] (μM)	TC_{50}[b] (μM)	IC_{50} (μM)	TC_{50} (μM)	IC_{50} (μM)	TC_{50} (μM)	IC_{50} (μM)	TC_{50} (μM)
UD29								
Cell fusion[c]	11.7	>200	NT[e]		NT		NT	
Viral entry[d]	4.7	>200	NT		NT		NT	
DS53								
Cell fusion[c]	0.75	70	0.75	>40	1.2	>40	1.2	>40
Viral entry[f]	0.6	>40	0.75	>40	1.0	>40	1.0	>40

[a] 50% inhibition of virus entry

[b] 50% reduction of cell viability

[c] on MT-2 cells

[d] on HeLaCD4-LTR-βgal cells (MAGI assay)

[e] NT – not tested

[f] on P4 cells (Luciferase assay)

HXB2/M-ad (dual tropic) and HXB2 (CCR4 tropic) pseudovirions both with an IC_{50} value 1.2 μM (Table 3).

Antiviral Activity of UD29 and DS53 Against HIV-1_{IIIB} and HIV-1 Pseudoviruses on Inhibition Cell Fusion and Virus Entry

The antiviral activity of UD29, the most studied compound of this class [12, 14], and DS53 was compared quantitatively in cell fusion and viral entry assays using HIV-1_{IIIB} and HIV-1 pseudoviruses as is shown in Table 3. DS53 inhibited cell fusion (formation of syncytia of HIV-1_{IIIB} infected cells) 14 times more efficiently than UD29. The IC_{50} value of the viral entry inhibition of DS53 was 0.6 μM, 7.8 time less than that of UD29. DS53 inhibited infection with HIV-1 pseudoviruses with different coreceptor preference selectively. M-ad (CCR5) pseudovirus infection was inhibited more efficiently with IC_{50} 0.75 μM. Infection with HXB2/M-ad dual tropic pseudovirus and HXB2 pseudovirus utilizing CXCR4 coreceptors were inhibited with less efficiency (with IC_{50} 1.2 μM, respectively) (Table 3).

Discussion

In this paper we present experimental data showing that thiolated pyrimidine derivatives inhibit in vitro infectivity of primary HIV-1_{IIIB} strain and HIV-1 pseudovirions using chemokine receptors CCR5, CXCR4 or both for virus entry, as well as virally induced cell fusion. Our results suggest that these compounds may interfere with the function of the essential - SH groups of CD4 molecule as well as –SH groups in HIV viral envelope gp120. These compounds, especially DS53 may function as an effective new generation entry inhibitor for HIV.

HIV entry inhibitors are desirable therapeutic approaches that block virus from entering target cells and thereby prevent *de novo* infections and also limit both viral integration and subsequent viral spread in the already infected host. HIV-1 entry inhibitor licensed for clinical use is *maraviroc* which alter conformation of the CCR5, CXCR4 antagonist *plerixafo*r (influencing bone marrow cell homing as side effect) and the only FDA-approved HIV fusion inhibitor *enfuvirtide (T20)*, preventing the formation of the gp41 six-helix bundle required for membrane fusion [23]. However they are not orally bioavailable and viral variants resistant to all entry inhibitors have already been identified.

The gp120 subunit of HIV Env is composed of five relatively conserved (C1-C5) and five variable (V1-V5) domains containing intrachain disulfide bounds. D1, D2 and D4 domains of CD4 also contain intramolecular disulfide bounds. The second domain disulfide bond of CD4 is redox-active, and the redox process is controlled by cell surface thioredoxin secreted by T cells [17].

Modified envelope conformation induced by reforming the disulfide-bonding is required for the membrane fusion, which is the essential step for the virus entry [10].

In previous reports [12, 14, 15, 18], we identified that reactive – SH group of the enol form of a thiolated pyrimidine nucleotide UD29 may interfere with the function of some cell surface proteins including essential receptors. Thiol/disulfide exchange processes are required for successful HIV entry, both on the surface of host cells and in the gp120 Env proteins of HIV [10]. UD29 interferes with the thiol function, as it has an essential cystein in its active center, which was proven

by its inhibitory activity on glyceraldehyde-3-phosphate dehydrogenase (GAPDH) enzyme [10, 15].

UD29 and six more of its thiolated pyrimidine derivatives have been synthesized in order to analyze further their antiviral activity as entry inhibitors. Compounds have been administered in various concentrations to uninfected and HIV-1 infected H9 T lymphocyte cells as well as uninfected and HIV-1 infected HeLaCD4-LTR/β-gal cells. These are monolayer HeLa cells expressing human CD4 receptors on the cell surface and have been transfected with HIV-1 LTR as well as β-gal genes rendering them as useful model to analyze early steps of HIV-1 entry during a single cycle of infection [20]. Only in high dose (>200 μM) did compounds used influence cell viability of uninfected H9 cells. The cytotoxicity observed was not the consequence of direct cytopathogenic effect (CPE) of HIV-1 as experimental arrangements provided only a single cycle of infection.

In cell fusion experiments on MT-2 human T cells one of the compounds, DS53 had the capacity to prevent formation of syncytia (i.e., cell fusion) induced by HIV-1_{IIIB} with an IC_{50} value of 0.6 μM. This compound was active also in inhibition of primary HIV-1_{IIIB} infection in a cell fusion assay on HeLaCD4-LTR/β-gal cells.

In order to in vitro analyze in a more detailed way the molecular events of the mechanism of primary HIV infection, host cell infectivity (tropism) and virus entry, pseudotype virions were prepared. These pseudovirions are carrying HIV-1 envelope with the preference for CCR5, CXCR4 coreceptors or for both, with a core containing luciferase reporter gene. As the *env* gene is not inserted between LTR regions, moreover some of HIV regulator and accessor genes had been omitted, HIV pseudovirions are able to infect susceptible cells but are replication defective, are unable to replicate in the cells. HIV pseudovirions are containing reporter genes, so quantitative determination of pseudovirions infecting (entering) the host cells can be performed upon detecting these reporter genes [19]. Host cell infectivity, chemokine coreceptor preference and inhibition of virus infection of pseudovirions were studied in P4 cells. DS53 selectively inhibited pseudovirion infection in a dose dependent manner. Inhibition was depended on the pseudovirus coreceptor preference. This could be explained that both X4 and R5 tropic gp120 subunits on the surface on pseudovirion Env bind CD4 with the same strength, but they bind their respective coreceptors with different strengths. CD receptors are not randomly distributed at the surface of the plasma membrane of cells but rather confined into domains and partially located into lipid rafts [24]. For productive entry into cells HIV uses lipid rafts co-localized CD4. Our previous results [14] indicated that thiolated pyrimidine derivatives may interfere with –SH (thiol) groups concentrated in lipid rafts of cell membrane. It is possible that viruses with different tropisms require different numbers of Env spikes to mediate entry or that disabling subunits within a trimer has different effects in X4 vs. R5 tropic viruses [25].

Our results presented here further support the hypothesis that based on its interactive role in redox processes active in viral entry, thiolated pyrimidine compounds could have a potential for the treatment of HIV infection as HIV entry inhibitors.

Better understanding of how protein interactions, such as gp120 binding to CD4, affect the sensitivity of intra-molecular disulfide bounds to reduction is potentially very important for further insight into disulfide rearrangements that occur during HIV-mediated fusion.

Research on the mechanisms of HIV entry has led to the discovery and development of new antiviral agents. A critical future challenge is translating our molecular understanding of HIV entry into therapeutically useful information.

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Compliance with ethical standards

Conflict of Interest The authors declare there is no conflict of interest in this work.

References

1. Kaplan JE, Hanson D, Dworkin MS, Frederick T, Bertolli J, Lindegren ML, Holmberg S, Jones JL (2000) Epidemiology of human immunodeficiency virus-associated opportunistic infectiopns in the United States in the era of highly active antiretroviral therapy. Clin Infect Dis 30:S5–S14
2. Baeten JM, Donnell D, Ndase P, Mugo NR, Campbell JD, Wangisi J, Tappero JW, Bukusi EA, Cohen CR, Katabira E et al (2012) Antiretroviral prophylaxis for HIV prevention in heterosexual men and women. N Engl J Med 367:399–410
3. Kanizsai S, Ghidán Á, Ujhelyi E, Bánhegyi D, Nagy K (2001) Monitoring of drug resistance in therapy-naive HIV infected patients and detection of African HIV subtypes in Hungary. Acta Microbiol Immunol Hung 57(1):55–68
4. Triant VA, Lee H, Hadigan C, Grinspoon SK (2007) Increased acute myocardial infarction rates and cardiovascular risk factors among patients with human immundiodeficiency virus disease. J Clin Endocrinol Metab 92:2506–25012
5. Monforte AD, Abrams D, Pradier C, Weber R, Reiss P, Bonnet F, Kirk O, Law M, de Witt S, Friis-Moller N et al (2008) HIV-induced immunodeficiency and mortality from AIDS-defining and non-AIDS-defining malihgnancies. AIDS 22:2143–2153
6. Neuhaus J, Jacobs DR, Baker JV, Calmy A, Duprez D, la Rosa A, Kuller LH, Pett SL, Ristola M, Ross MJ (2010) J Infect Dis 201: 1788–1795
7. Didigu C, Doms RW (2014) Gene therapy targeting HIV entry. Viruses 6:1395–1409
8. Deng H, Liu R, Ellmeier W, Choe S, Unutmaz D, Burkhart M, di Marzio P, Marmon S, Sutton RE, Hill CM et al (1996) Identification of major co-receptor for primary isolates of HIV-1. Nature 381:661–666

9. Kemény B, Nagy K, Horváth A (2000) CCR5 and SDF1 gene polymorphism in HIV-infected and healthy individuals in Hungary. Hung Venerol Arch IV(2–3):89–92
10. Ryser HJ, Levy E, Mandel R, DiSciullo GJ (1994) Inhibition of human immunodeficiency virus infection by agents that interfere with thiol-disulfide interchange upon virus-receptor interaction. Proc Natl Acad Sci U S A 91:4559–4563
11. Barbouche R, Miquelist R, Jones IM, Fenouillet E (2003) Protein-disulphide isomerase-mediated reduction of two disulfide binds of HIV envelope glycoprotein 120 occurs poet-CXCR4 binding and is required for fusion. J Biol Chem 278(5):3131–3136
12. Beck Z, Kis A, Berenyi E, Kovacs P, Fesüs L, Aradi J (2009) 4-Thio-uridylate (UD29) interferes with the function of protein –SH and inhibits HIV replication in vitro. Pharmacol Rep 61:343–347
13. Campbell S, Crowe SM, Mak J (2001) Lipid refats and HIV-1: from viral entry to assembly of progeny virions. J Clin Virol 22:217–227
14. Kanizsai S, Ongrádi J, Aradi J, Nagy K (2014) Thiolated pyrimidine derivatives may interfere thiol groups concentrated at lipid rafts of HIV-infected cells. Acta Microbiol Immunol Hung 61(4): 447–458
15. Kanizsai S, Ghidán A, Ongrádi J, Nagy K (2012) Antiretroviral effect of 4-thio-uridylate against human immunodeficiency virus type. Acta Microbiol Immunol Hung 59(4):499–510
16. Nagy K (2007) HIV pseudovirions assay for coreceptor selection and entry fitness. Proc. HIV correlates (Ed:Fust G) p12., Budapest
17. Matthias LJ, Yam PT, Jiang XM, Vandegraaff N, Li P, Poumbourios P, Donoghue N, Hogg PJ (2002) Disulfide exchange in domain 2 of CD4 is required for entry of HIV-1. Nat Immunol 3:727–733
18. Horváth A, Tőkés S, Hartman T, Watson K, Turpin JA, Buckheit RW Jr, Sebestyén Z, Szöllősi J, Benkő I, Bardos TJ, Dunn J, Fésűs L, Tóth FD, Aradi J (2005) Potent inhibition of HIV-1 entry by Suligovir (s4dU)3. Virology 334: 214–223
19. Jármy G, Henekelein M, Weissbrich B, Jassoy C, Rethwilm A (2001) Phenotypic analysis of the sensitivity of HIV-1 to inhibitors of the reverse transcriptase, protease and integrase using a self-inactivating virus vector system. J Med Virol 64(3):223–231
20. Nagy K, Young M, Baboonian C, Merson J, Whittle P, Oroszlan S (1994) Antiviral activity of human immunodeficiency virus type 1 protease inhibitors in a single cycle of infection: Evidence for a role of protease in the early phase. J Virol 68:757–765
21. Nagy K, Clapham P, Cheingsong-Popov R, Weiss RA (1983) Human-T-cell leukemia virus type I: induction of syncytia and inhibition by patients' sera. Int J Cancer 32:321–328
22. Clapham P, Nagy K, Cheingsong-Popov R, Exley M, Weiss RA (1983) Productive infection and cell-free transmission of human T-cell leukemia virus in a non-lymphoid cell line. Science 222:1125–1127
23. Didigu C, Doms RW (2012) Novel approaches to inhibit HIV entry. Viruses 4:309–424
24. Dumas F, Preira P, Salome L (2014) Membrane organization of virus and target cell plays a role in HIV entry. Biochimie 107:22–27
25. Zarr M, Siliciano R (2015) Stoichiometric parameters of HIV-1 entry. Virology 474:1–9