Materials and methods: Reviewing of outpatient clinic records, case histories, and AIDS Center reports in the Northwest of Russia in association with studies of the clinical conditions of HIV patients encompassed 120 000 HIV cases. The data were treated statistically using MS Excel and Statistica 10 and software tools.

Results: The onset of HIV epidemic dates back to late 1990s when new found HIV cases amounted to hundreds and, further on, to thousands because of rapid spread of injection drug use with HIV-contaminated needles and syringes. At the time of diagnosis, most patients had normal CD4 cell counts suggesting a recent HIV infection, which had reached Stage II or III.

In 2003–2004, the epidemic subsided, having involved mostly men aged 20 to 25 years, who constituted the main group of HIV risk and at difference from the earlier time were infected with subtype A HIV-1 (more than 90%). The decline of the first wave of HIV incidence may be explained by the exhaustion of the main risk group and by the active preventive interventions. In the Northwest of Russia, the most vulnerable to HIV were territories featuring higher population density and more intense migrant streams (Saint-Petersburg and Leningrad, Kaliningrad, and Murmansk Oblasts).

In patients who according to the presence of opportunistic and concomitant diseases could be referred to HIV infection Stages 4 or 5, blood CD4 cell counts were below 200 $m1^{-1}$, and HIV loads were $10^{4,5}$ to $10^{6,0}$ RNA copies per 1 ml. The duration of infection from the time of HIV contracting, mainly by the injection route, to HIV detection was from 4 to 12 years, probably because a part of HIV cases detected during the first peak of the epidemic were not diagnosed timely. Other patients were infected in a relatively more recent time, during the second wave of HIV. The least numerous group comprised patients at advanced stages associated with immunosuppression who needed HAART at the time of diagnosis. The high numbers of HIV cases at Stage 4 was conducive to tuberculosis and associated with high death rate over the first year after the onset of therapy.

Lethal cases were registered in patients under HAART mainly because of pronounced immunosuppression, the development of severe concomitant infections including tuberculosis, patients' poor adherence to therapeutic regiments, and the discontinuation of therapy.

Conclusion: The current period of HIV epidemic may be characterized as associated with the advances stages of HIV infection associated with numerous concomitant diseases and high death rates upon HAART. All this warrants special attention to early HIV detection and subsequent management of HIV cases and to developing of a complex of preventive, organizational and socio-medical interventions adapted to megalopolises as well as to sparsely populated territories.

Novel dual inhibitors targeting HIV-1 integrase and reverse transcriptase-associated ribonuclease H activity

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Reverse transcriptase (RT) and integrase (IN) facilitate the early stages of HIV-1 replication. HIV-1 integrase (IN) and reverse transcriptase (RT)-associated ribonuclease H (RNase H) domains belong to the polynucleotidyl transferase superfamily and share common structural features. The catalytic sites of both enzymes contain a central core composed of three highly conserved amino acid residues forming the DDE motif or RNase H-like fold (Asp, Asp and Glu). Among the novel approaches aimed to identify new HIV-1 inhibitors, the development of dual-action inhibitors, single drugs that may act on two different enzymes, such as IN and RNase H. A number of attempts has been made to identify compounds that may act on both enzymes, but still there is no dual inhibitor passed the clinical trials [Corona A., et al. *Antomicrob. Agents Chemother.*, 2014, 58 (10), 6101–6110; Cuzzucolli Crucitti G., et al. *J. Med. Chem.*, 2015, 58 (4), 1915–1928; Esposito F., et al. *Antiviral Chem. Chemother.*, 2014, 23, 129–144].

In our previous works, we have studied in detail the integration inhibition by dimeric bis-benzimidazoles (DBI) [Zhuse A.L., et al. *J.Biomol. Struct. Dyn.*, 2007, 24(6), 666–668; Korolev S.P., et al. *Russian J. of Mol. Biol.*, 44 (4), 633–641.]. The inhibitory effect of these compounds are explainable by the fact that they interfere with a correct binding of the DNA substrate in the IN active center. To enable their potential use however requires to overcome their low solubility in water and to increase their bioavailability. Recently the new series of water soluble DBI has been obtained. The inhibitory activity of these compounds was determined for both recombinant enzymes IN and RNase H. It was shown that the inhibition ability for the set of compounds depends on the length of the spacer connecting the pairs of benzimidazole fragments. The IC_{50} values of the most active one were 50 nM for IN and 5 μM for RNase H. Moreover these compounds demonstrated extremely low cytotoxity (CC_{50}>100 μM). According to preliminary trials the most active DBI showed its inhibitor activity on replication-incompetent VSV-G-pseudotyped vector HIV-1 based. Overall, DBI may be considered as a potent dual-acting inhibitor for treatment of HIV-1 infections.

Inorganic pyrophosphate analogs as multitarget HIV replication inhibitors

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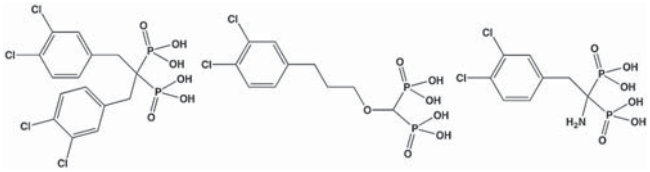
Despite the undoubted success in the development of human immunodeficiency virus type 1 (HIV-1) therapy, this

infection still remains a significant health threat. The key steps of the replication cycle of HIV-1 are the viral DNA synthesis and its integration into the cell genome. One of the current problems associated with HIV therapy is the emergence of drug-resistant virus variants. The resistance emergence is often associated with phosphorolytic excision of the 3'-drug-terminator. To date lots of inhibitors of different viral enzymatic activities are developed, however, a promising approach for development of novel and effective anti-HIV drugs is the design of multitarget compounds suppressing simultaneously several of these activities.

Herein, we synthesized 40 methylenebisphosphonates (BPs), five of which simultaneously inhibited phosphorolytic activity of native and drug-resistant forms of HIV-1 reverse transcriptase (RT), RT catalyzed elongation and RNAse H activity and, moreover, two integrase activities. We assessed structural elements required for simultaneous inhibition of these reactions. BPs should be constructed of three pharmacophores: the methylenebisphosphonate backbone, the aromatic halogenated pharmacophore linked to the backbone through the inert aliphatic linker, and the



Mg^{2+}-coordinating group. The activity of BPs was also affected by the nature of the second substituent at the bridging carbon. The most active BPs inhibiting activities of the main HIV-1 enzymes are demonstrated below.

Interaction between HIV-1 integrase and the host protein Ku70: a promising approach to antiretroviral therapy

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The Ku protein is a heterodimer composed of two subunits: Ku70 and Ku80. The main function of this heterodimer is the binding of DNA termini produced by double-strand DNA brakes during the first steps of the non-homologous end joining repair process, that makes Ku an essential complex for cellular survival during genotoxic stress. Besides its role in NHEJ, Ku can also take part in transcription regulation, telomere maintenance, protein turn-over, cytoplasmic DNA-sensing and some other processes. Recently, it has been reported that Ku participates in the HIV-1 replication. Ku favors different stages of the HIV-1 life cycle, such as the formation of 2-LTR circles, integration and transcription of the integrated viral DNA. Viral replication is diminished in cells depleted of either component of Ku and this effect is more pronounced during the early stages of viral replication. However, an exact mechanism by which Ku affects the replication of HIV-1 remains to be evaluated. It has been proposed, that the binding of Ku70 subunit to HIV-1 integrase (IN) protects the later from proteasomal degradation. Considering this, the inhibition of the complex formation between IN and Ku70 might affect viral replication. The drug design would be greatly facilitated if a detailed structure of the IN/Ku70 complex were present. Unfortunately, the exact structure of HIV-1 IN is not yet known, and only single domains of IN can be effectively crystallized.

We have shown that a stable complex can be formed between recombinant Ku70 and IN with a Kd ~ 70 nM. A series of deletion mutants were created both for Ku70 and IN that helped us localize the binding sites within both proteins. N-His6-tagged HIV-1 IN separate domains (N-terminal (1–50 aa), catalytic (51–220 aa) and C-terminal (220–270 aa)) were expressed in *E. coli* as well as several truncated IN variants containing amino acids 1–160, 1–220, 51–160 and 51–280. A full-size Ku70 with a GST-tag on its N-termini together with a number of truncated variants (Ku70(1–250), Ku70(250–609)) were purified also from *E. coli*. Using the GST-pull down technique we have gained data suggesting that the binding of Ku70 with HIV-1 IN relies at least on two sites in the proteins structure. Specifically, the Ku70(1–205) domain makes contacts with an α-helix located in the (160–230) IN region. This observation is further supported by inserting point mutations in various positions in this α-helix. Ku70(250–609) can also bind to IN but the site of this interaction localizes closer to the N-terminus of the protein around the region of IN(130–160). The data obtained from experiments on recombinant purified proteins were corroborated by expressing C-terminal HA-tagged full-length IN and its various deletion mutants in HEK 293T cells with or without a WT Ku70–3FLAG and truncated variants. The coexpression with Ku70 stabilized IN in the cells, while the IN expression in cell that were knocked down of Ku70 was greatly reduced. Based on the data collected in our experiments we intend to construct an optimized computer model of the complex between HIV-1 IN and Ku70 that will be further used for molecular docking of potential inhibitors of their interactions.

Diversity of HIV-1 recombinant forms in Russia and the former USSR countries

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Background: The massive HIV-1 epidemic in Russia and the former USSR started in the mid-1990 years. In the