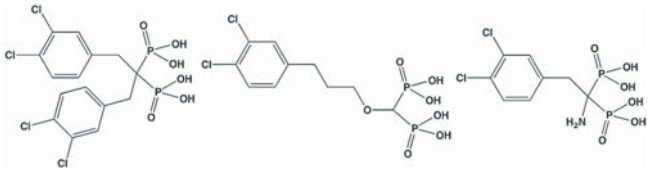
infection still remains a significant health threat. The key steps of the replication cycle of HIV-1 are the viral DNA synthesis and its integration into the cell genome. One of the current problems associated with HIV therapy is the emergence of drug-resistant virus variants. The resistance emergence 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