Abstract

Human Immuno Deficiency Virus type 1 (HIV-1) is the causative agent of Acquired Immuno Deficiency Syndrome (AIDS). HIV-1 mainly infects body T cells and macrophages. The loss of T cells during the viral infection leads to the weakened immune system. Feeble immune response of the immune system provides opportunity to establish different microbial infections in infected patients. The decrease in number of T cells, heavy viral load and damaging effect of opportunistic infections in patients leads to the death of infected persons. After identification of AIDS virus in 1980s, several approaches were developed to target both viral proteins and cellular proteins involved in HIV-1 replication. Most of the strategies target viral proteins like reverse transcriptase, protease, integrase, Tat, Rev, Nef, gp120 and gp41 etc. and also cellular proteins that are involved in viral entry, integration, transcription and maturation of viral life cycle. Although the currently available drugs reduces the viral load remarkably but fails to treat infected patients completely because of drug toxicities, generation of drug resistant mutant variants, existence of latent viral reservoirs and defective immune responses during the therapy. New inhibitors of viral replication are strongly required with novel mechanism of action and potent inhibitory effect.

Natural products from marine organisms are very useful source to develop novel therapeutics to human diseases. A large number of novel molecules and their derivatives have been isolated from marine organisms and are currently being clinically tested for treating human diseases. Several novel molecules were isolated from the extracts of marine organisms with potential antiviral activities. The extracts of a marine bivalve available in Russian coastal region is being used as a source of antiviral preparation and is being used to treat Influenza, Herpes Simplex Virus, and Hepatitis including HIV-1 infection.

In this study, nine species of Indian marine bivalves were selected and crude extracts of each bivalve was used in antiviral screening experiments to check their inhibitory effect on HIV-1 replication if any. CEM-GFP, a HIV-1 reporter
A T cell line was selected as a model system to screen antiviral activity of samples and green fluorescence and production of virus into the culture supernatants were analyzed to observe the inhibitory effect of samples. Black clam crude extracts showed potent inhibitory effect on HIV-1 replication as compared to other species in HIV-1NL4-3 and HIV-1IIIB infected CEM-GFP and Jurkat T cells. The active component of Black clam crude extract was purified by bioactivity guided fractionation. The structure of the active molecule in highly pure fraction was identified as Zinc diprolinate using NMR and Mass spectroscopic analysis and was synthesized by chemical synthesis. IC$_{50}$ concentration of Zinc diprolinate was calculated as 45 µM. Zn(pr)$_2$ inhibited HIV-1 replication of both subtype B and C viral isolates in T and monocytic cells. L-Proline was used at similar concentration as a negative control and had no effect on viral replication. Zinc diprolinate inhibited replication of laboratory as well as subtype C primary isolates in human PBMCs, indicating that it is a potent inhibitor of HIV-1 replication. The current antiretroviral drugs that are used to treat HIV-1 infected patients frequently fail to decrease the viral infection because of the generation of drug resistant variants. Novel molecule with inhibitory effect on known drug resistant mutants is useful for developing novel therapeutics. Zn(pr)$_2$ inhibited the replication of HIV-1JF4A, a ddC drug resistant RT mutant and HIV-1L10R/M461I63P/V82T, a protease inhibitor resistant virus. Time of addition experiments showed that Zn(pr)$_2$ inhibits at a early step of viral infection after the entry of HIV-1 into the target cells. With this evidence, Zn(pr)$_2$ was tested for its effect on reverse transcriptase enzymatic activity. Zn(pr)$_2$ inhibited the RNA dependent DNA polymerase activity (RDDP) of recombinant as well as RTs of HIV-1NL4-3, HIV-1IIIB (subtype B), HIV-1Indie-C1 (subtype C) and HIV-1JF4A (ddC resistant) virus isolates. It also inhibited RDDP activity of AMV and MMLV RTs, which indicated that Zn(pr)$_2$ is a retroviral reverse transcriptase inhibitor. HIV-1 reporter cell lines are useful tools in screening of anti-HIV compounds, which target different stages of viral life cycle. Subtype C viruses are prevalent in most of the highly affected countries in the world, like South Africa and
India. Although subtype C LTR-Luc reporter gene construct has been previously reported, however, no GFP based subtype C LTR reporter vector is currently available. We have constructed a novel dual reporter vector pLTRC-Luc-EGFP expressing both EGFP and Luciferase under the regulation of HIV-1 subtype C LTR along with two single reporter vectors, pLTRC-EGFP and pLTRC-Luc expressing EGFP and luciferase respectively. pLTRC-Luc, pLTRC-EGFP and pLTRC-Luc-EGFP showed basal transcriptional activities and also showed Tat mediated transactivation confirming the functional significance of the vectors. The treatment with viral transcription activator sodium butyrate and inhibitor DRB in dual reporter and Tat vector co-transfected cells enhanced and suppressed both GFP and luciferase reporter gene activities from dual reporter confirming the modulatory effect of the molecules on viral transcription. The constructed novel subtype C LTR transcriptional reporters are useful for easy visualization and sensitive quantitation of LTR mediated gene expression and also for screening of viral transcription modulators and inhibitors of viral replication. Finally, in this study, we have identified Zinc diprolinate as a novel inhibitor of HIV-1 replication and reverse transcription step as its inhibitory target in viral life cycle. Thus, Zinc diprolinate can be a potential lead molecule for future therapeutic use or can be used to generate novel analogues with enhanced anti-HIV activity.