ABSTRACT

Human Immune 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 lifecycle. Although the currently available drugs reduce the viral load remarkably but fail 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.

Plants are rich source of bioactive compounds with different modes of action. Some of these are known to possess anti-HIV activity. These natural products are being evaluated as microbicides candidates to prevent HIV infection and transmission. Donglie Yu et al in 2007 elaborated anti-HIV compounds from plants. These compounds mostly belong to tannins, terpenoids, alkaloids etc. Each of these molecules exhibit unique mechanism of action. Structure-Activity-Relationship studies will help in transforming these leads into useful drugs. Use of natural products along with existing anti-HIV drugs may help in treating drug resistant viruses.

Several anti-HIV compounds from plant sources are presently in different stages of development studies such as Buchapines, Harmines, Betulinic acid derivatives, Calliptin etc (Inder Pal Singh et al 2005). Some phloroglucnol derivatives like mallotojapnin
and mallotochromene have shown 70% RT inhibition while mallotophenone and mallotolerin exhibited weak RT inhibitory activity (Hideo N et al 1991).

In this study, we have selected these 7 analogues of dimeric phloroglucinol mallotojaponin and 10 of the analogues of quinoline 2,4-diol buchapine to explore their efficacy as anti-HIV-agents and potential to be used as HIV-1 microbicide candidates.

Since these compounds are novel, it may be possible that they may or may not exhibit mechanism of action different than the parent compound buchapine. We intended to evaluate the efficacy of these compounds as anti-HIV-agents and further explore mechanism of action of potent molecules. Further HIV-1 microbicidal potential of the lead molecules was assessed. To begin with, we assessed the cytotoxicity of these compounds in TZM-bl reporter cell line and used nontoxic concentration to evaluate anti-HIV-1 activity of the compounds. Two phloroglucinol analogues M1 (1-[3-Acetyl-5-[(3,5-diacetyl-2,4,6-trihydroxyphenyl)-cyclohexyl-methyl]-2,4,6-trihydroxyphenylethanone) and M7 (1-[3-Isopentanoyl-5-[(3,5-diisopentanoyl-2,4,6-trihydroxyphenyl)-4-benzyloxyphenyl-methyl]-2,4,6-trihydroxyphenyl]-3-ethylbutan-1-one) and two buchapine analogues B4 (4-(Isopytelyoxy) quinolin-2-ol) and B1 (3,3-Bis(3-methylbut-2-etyl)quinoline-2,4(1H,3H)-dione) were found to possess better safety index and anti-HIV-1 activity.

These four compounds were further tested for broad spectrum anti-HIV-1 activity against CXCR4 and CCR5 tropic viruses and dual tropic viruses in primary culture of human PBMCs. All four compounds showed potent activity irrespective of tropism of HIV-1. The compounds were highly active against HIV-1 subtype C clinical isolates from India invitro.

Further we assessed mechanism of action of these analogues by cell based and cell free assays.

We looked for the ability of compounds to inhibit strand transfer reaction of HIV-1 integrase. Except M1, none of the compounds were able to inhibit i vitro reaction catalyzed by HIV-1 integrase. M1 showed 60% inhibition of integrase enzyme.
Since buchapine is known to inhibit Reverse Transcriptase, we assessed the RT inhibitory activity of buchapine and its analogues using Reverse Transcriptase Assay colorimetric. Buchapine (B7) and B exhibited potent inhibition of RNA-dependent DNA polymerization activity of HIV-1 Reverse Transcriptase.

B showed moderate inhibition of Reverse Transcriptase activity indicating possibly a different mode of action. B1 inhibited infection of TZM-bl cells when added during the infection. Hence we looked for its role in entry/attachment of virus with the CD4+ cells. We evaluated effect of B1 on virus attachment to cell. For this purpose, cell-cell fusion assay was developed and optimized. In this assay, ability of B1 to inhibit gp120 binding to CD4 receptor was assessed. B1 showed 70% inhibition of virus attachment with CD4+ cells. B4 and B7 could not inhibit virus attachment with the cells.

Further we evaluated B4 and B7 as potential HIV-1 microbicide candidates. We found that B1, the prenyl substituted quinoline 2,4 diol has exhibited potent HIV-1NL4-3 microbicidal activity in dual chamber trans-well system.

The potent activity of B1 could be because of its dual mechanism of action i.e. it can block gp120 binding as well as inhibit Reverse Transcriptase enzyme at early phase of infection.

B4, the O-substituted quinoline 2,4-diol showed potent inhibition of virus transfer from the epithelium to T-cells when used at its highest nontoxic concentration but failed to inhibit at similar concentration of B1. Buchapine and its analogues did not cause inflammation of epithelium upon 24 h of exposure.

Finally, in this study, we identified M1 as novel HIV-1 Integrase Inhibitor. We have identified B1a buchapine analogue with dual mechanism of action, first it acts at the entry step of virus and second it interferes with reverse transcription of HIV-1. We have shown that B has potential to be used as HIV-1 microbicide candidate.