Anti-HIV Activity of Naphthalenesulfonic Acid Derivatives

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(received November 21)

Three species of naphthalenesulfonic acid analogs have been evaluated for anti-HIV activity in assays using MOLT-4 cells. Two species of 1-amino-8-hydroxy compounds 1 and 3 and 6-amino-1-hydroxy compound 2 showed inhibition against HIV as EC_{50} values 7.4 μg/mL, 9.7. μg/mL, and 17. μg/mL, respectively.

These naphthalenesulfonic acid analogs represents a new class of compounds which may be effective in the treatment of HIV infected patients. The structure activity relationships and a probable mode action of three compounds is discussed.

Acquired immunodeficiency syndrome (AIDS) is caused by a retrovirus now known as the human immune deficiency virus (HIV). AZT, the first FDA approved drug for therapeutic use against AIDS, has shown increased survival in AIDS patients. Since then, many research groups have provided numerous nucleoside and nucleoside analogs with potential anti-AIDS activities. These studies have led to the consideration of 2',3'-dideoxycytidine (ddC), 2',3'-dideoxyadenosine (ddA), 2',3'-dideoxyinosine (ddI), and 2',3'-didehydro-2',3'-dideoxythimidine (d4T) for clinical trials. The dideoxynucleosides, like AZT, exhibit their anti-HIV activity by two general modes of action. As the 5'-triphosphate they inhibit the retroviral reverse transcriptase enzyme. Due to the lack of a 3'-OH functionality, these compounds also block viral DNA chain elongation. More recently, patients receiving AZT have displayed toxicity, shown resumption of viral replication, the occurrence of cell to cell virus transmission during treatment, and the emergence of AZT resistant HIV-1 strains during prolonged therapy. Therefore, there still remains an urgent need to search for new inhibitors of HIV that could be useful in the treatment of patients with AIDS.

Research in the non-nucleoside anti-AIDS area has provided interesting compounds displaying novel modes of action. These include the natural products and/or their analogs, avarol and avarone(1), castanospermine(2), N-butyldeoxynojirimycin(3), colchicine(4), hypericin(5), amphotericin B methyl ester(6), chalcones and flavones(7), pepstain A(8), and benanomicine A and B(9). Other candidates that have demonstrated anti-HIV activity are the oligonucleotides(10), cholic acid derivatives(11), dextran sulfate(12), soluble CD4(13), D-penicillamine(14), and phosphonoformate(15). These include various natural products and/or their analogs and other diverse chemical entities. As part of an ongoing search for new anti-HIV leads, a previous study of the in vitro anti-HIV-1 activity of certain naphthalenedisulfonic acid derivatives provided the impetus to further design and biologically evaluate other analogs of this class of compounds for potential anti-HIV activity. In addition, since Congo Red and related anti-HIV dyes are known to be metabolized to carcinogenic aromatic amines in vivo, author wanted to explore the potential of using other dyes. Indeed, in this report author show that this try has been fruitful.

Chemicals

1-Amino-8-hydroxy-3,6-naphhalenedisulfonic acid derivatives, Direct Red 79 and Direct Blue 15, were purchased from DINIPON KAYAKU CO. LTD. and MITUI TOATU DYES LTD., respectively. 6-Amino-1-hydroxy-3-naphthalenesulfonic acid derivative, Direct Red 83, was purchased from DINIPON KAYAKU CO. LTD. All compounds were further purified by chromatography and/or repeated fractional recrystallizations and characterized by NMR, IR, and elemental analyses.

Assays for HIV-1 Inhibition

Activity of the compounds against the replication of HIV-1 was based on the inhibition of virus-induced cytopathogenicity in MT-4 cells were infected with HIV at a multiplicity of infection of 0.02 and incubated in the presence of various concentration of test compounds. After a 4-days incubation, the number of viable cells was determined by the MTT method, as previously described. The giant cell formation assays was carried out according to established protocol. MOLT-4 cells were cultured with an equal number of HUT-78/HTLV-VIIIB for 24 h, and the number of giant cells was determined microscopically. The HIV RT assays was carried out as follows. HIV-1 RT was obtained from disrupted virions which had been partially purified and concentrated. The assays was performed at 37 C for 30 min with 50 μL of a reaction mixture containing 50 mM Tris-HCl (pH 8.4), 2 mM of dithiothreitol, 100 mM KCl, 10 mM $MgCl_2$. 0.1% Triton X-100, 1 μCi of [methyl-^{3}H]dTTP (30 Ci/mmol), 0.01 unit of poly(rA)-oligo(dT), test compound, and enzyme. The reaction was terminated with 200 μL of trichloroacetic acid (5%, v/v). The precipitated materials were collected on glass-fiber filters and analyzed for their radioactivity in a liquid-scintillation counter. Variation in the experiments was 10% at maximum.

Results and Discussion

Inhibition of HIV-1 cytopathogenicity was studied in MT-4 cells. These results illustrate that compounds 1 and 2 effect 100% inhibition of HIV-1 at doses that are nontoxic to the cells. Compound 1 has EC_{50} (50% antiviral dose) values 7.4 μg/mL against HIV-1, demonstrating greater potency in the HIV-1 cytopathogenicity assay. Compound 1 has a CC_{50} values of 100 μg/mL and demonstrates the best in vitro therapeutic index (ratio of CC_{50} to EC_{50}). Compound 2 has EC_{50} values 17 μg/mL against HIV-1. Two compounds 1 and 2 are nontoxic at a dose of 100 μg/mL. However, compound 3 has toxic at a dose of 52 μg/mL and EC_{50} values 9.7 μg/mL.

Polar compounds have provided novel potential anti-HIV agents. Castanospermine, a polar natural product, probably provided the impetus to develop N-butylnojirimycin, a glycosidase inhibitor that has entered clinical trials. Many sulfated polysaccharides have shown anti-HIV activity below the anticoagulant threshold with a favorable therapeutic index. Dextran sulfate and heparin exhibit their antiviral activity by inhibiting viral adsorption to the target cell.

It is worthy to note that the sulfated polysaccharides do not need to enter cells to exhibit antiviral activity. In a clinical study, it has been shown that oral dextran sulfate is poorly absorbed. The activity of these agents seems to be related to the sulfate content and molecular weight of the molecule. Indeed, certain workers have used the tactics of sulfation to entrance of induce anti-HIV activity. Another polar compound, aurintricarboxylic acid (ATA), a triphenylmethane dye, had been shown to have anti-HIV activity and specific interaction with the CD4 receptor. A new structure for ATA has been assigned.

Few sulfonic acid compounds have displayed anti-HIV activity. The first such compounds was the hexasulfonic acid antitrypanosomial drug suramine, also the first agent to be used in patients with AIDS and found to be clinically ineffective. Other sulfonic acid dyes have also demonstrated anti-HIV activity. However, the dye properties of these molecules and their known ability to metabolized to mutagenic and/-or carcinogenic phenyl or biphenyl amine fragments will preclude their use as antiviral agents.

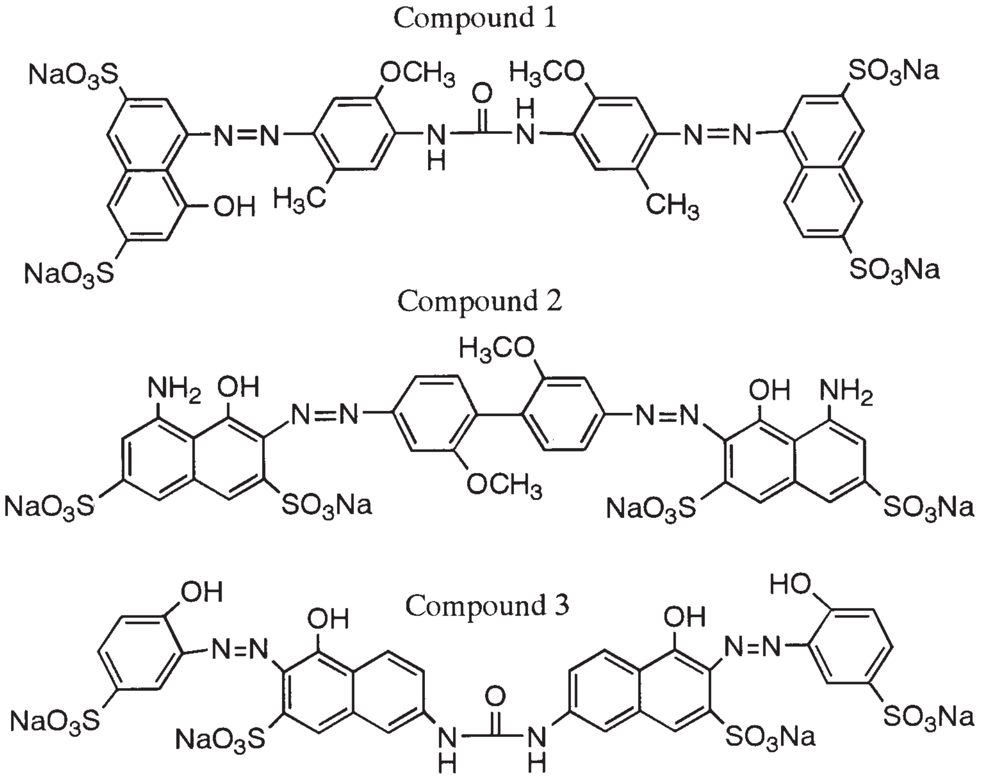
Among the sulfonic acid containing natural products, lignin and a group of sulfolipids have shown anti-HIV activity. Synthetic analogs of suramine have also demonstrated in vitro activity better than that of suramine. The general inactivity of 1,2-diamino-4-naphthalenesulfonic acid and the activity of Congo Red suggest the need for the bis(naphthalenenesulfonic acid) moiety for anti-HIV activity. The detailed mechanism of action of these agents remains to be determined.

However, the mode of action against RT may be due to the chelating potential of these compounds. The anti-HIV dyes, Congo Red and Evans Blue, are documented to form metal complexes. Congo Red is known to complex with rare earth salts and Evans Blue is used for the estimation of magnesium. Examination of the structures of the described bis(naphthalenesulfonic acid) derivatives reveals that they also have the potential to form metal complexes. It is known that HIV-1 RT is a zinc metalloenzyme requiring magnesium for optimum activity. Complexation may be expected to interfere with the activity of RT. The antiviral activity of chelating agents is well-known. It remains to be determined whether the activity of these compounds is due to the complexes they form with these crucial metal ions or whether they form in vivo complexes with other metals and it is these metal complexes that are responsible for the anti-HIV activity.

It may be envisioned that the activity of these compounds is due to their in vivo hydrolysis products. Many naphthalenesulfonic acids have demonstrated antiviral activity in a variety of screens other than for HIV. The potential in vivo hydrolysis products did not show any anti-HIV activity in MT-4 cells. The varied sensitivity of anti-HIV agents to different screens is well-documented. These studies indicate that the activity of these compounds is not due to any in vivo hydrolysis products.

Conclusions

These studies have demonstrated that the naphthalenesulfonic acids have potential for



Compound 1

Compound 2

Compound 3

Scheme. Antiviral active compounds against HIV future development in the area of potential anti-AIDS therapy. These observations suggest that the bis(naphthalenedisulfonic acid) unit may be requirement for increased activity of hydroxy-3,6-naphthlenedisulfonic acid derivatives. Studies to prepare products and analogues of these agents targeted for optimizing anti-HIV activity are in progress.

Acknowledgements

Author is indebted to the staffs of the Fukushima Medical College for the antiviral assays against HIV of the screening test of compounds.

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