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Development of Anti-viral Agents from Natural Sources

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ABSTRACT

Human immunodeficiency virus (HIV), the causative agent of AIDS, still continues to spread rapidly in the world population, especially in Africa and Southeast Asia. At present, two kinds of therapeutic approaches are used for treatment of AIDS. One is to target HIV reverse transcriptase, which is responsible for the viral genome transcription.

The other is to inhibit HIV protease PR, which is essential for the processing of viral proteins. Drug combinations based on these approaches can reduce the blood virus to an undetectable level. However, a small amount of virus may lurk inside the immune cells in a dormant state. Another major obstacle of long-term treatment of the disease is remarkable mutation in HIV. Most of the clinical chemotherapeutic agents have one or more of these problems. High cost and harmful side-effects further reduced the desirability of these drugs.

In the course our studies on development of anti-HIV agents from natural products, we investigated various crude drugs for their inhibitory activity against HIV-induced cytopathic effects (CPE) in culture cells, HIV-protease (PR), HIV-reverse transcriptase (RT) including ribonuclease H (RNase H), and HIV integrase (INT). In the present paper, some inhibitory substances relating to the development of anti-HIV agents are reported.

1. INHIBITORY SUBSTANCES AGAINST HIV-1-INDUCED CYTOPATHIC EFFECTS (CPE)

1.1 Croton tiglium [1,2]

From the MeOH extract of the seeds of *Croton tiglium*, five new phorbol diesters, together with the three known diesters, were isolated, and their structures were determined by spectroscopic methods and selective hydrolysis of acyl groups. These compounds were tested for their abilities to inhibit HIV-1-induced CPE on MT-4 cells and to activate protein kinase C (PKC) associated with tumor- promoting action. 12-*O*-Acetylphorbol 13-decanoate and 12-*O*-decanoylphorbol 13-(2-methylbutyrate) effect-ively inhibited the HIV-1-induced CPE [complete inhibitory concentrations (IC_{100}) of 7.6 ng/ml and 7.81 μg/ml, and minimum cytotoxic concentrations (CC_0) of 62.5 and 31.3 μg/ml, respectively]. Although 12-*O*-tetradecanoylphorbol-13-acetate was the most potent inhibitor of the CPE (IC_{100} value of 0.48 ng/ml), 12-*O*-acetylphorbol 13-decano-ate showed no activation of PKC at concentrations of 10 and 100 ng/ml, suggesting this compound may be one of the most promising anti-HIV agents without tumor-promoting activity.

1.2 Ganoderma lucidum [3]

From methanol extracts of the fruiting bodies and spores of Ganoderma lucidum, a variety of highly

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oxygenated lanostane-type triterpenes including new compounds (ganoderic acids α, β, γ, δ, ε, ξ, η, and θ, and lucidumols A and B) were isolated and their inhibitory activities against HIV-1-induced CPE. Of these, ganoderiol F and ganodermanontriol were appreciably inhibited the CPE at 7.8 μg/ml for both.

2. INHIBITORY SUBSTANCES AGAINST HIV-1-PROTEASE (PR) ACTVITY

2.1. Ganoderma lucidum [3, 4]

Of the compounds isolated from *Ganoderma lucidum*, ganoderic acid B, ganoderiol B, ganoderic acid C1, 3β,5α-dihydroxy-6β-methoxyergosta-7,22-diene, ganoderic acid α, ganoderic acid H and ganoderiol A were moderately active inhibitors against HIV-1 PR with IC_{50} values of 0.17-0.23 mM.

2.2 Cynomorium songaricum [5, 6]

From the MeOH extracts of the stems of Cynomorium songaricum Rupr. (Cynomoriaceae), ursolic acid and its hydrogen malonate were isolated as inhibitory substances against HIV-1 PR, their IC_{50} values being 8.0 and 6.0 μM, respectively. Of various dicarboxylic acid hemiesters of related triterpenes synthesized, the inhibitory activities tended to increase in the order of oxalic, malonyl, succinyl and glutaryl hemiesters of triterpenes such as ursolic acid, oleanolic acid and betulinic acid. The most potent inhibition was observed for the glutaryl hemiesters with an IC_{50} of 4.0 μM. Of the water extract of the stems of *C. songaricum*, flavan-3-ol polymers consisting of epicatechin as their extender flavan units were found to be the potent inhibitory principles against HIV-1 PR. Oleanolic acid derivatives with different lengths of 3-*O*-acidic acyl chains were synthesized and evaluated for their inhibitory activity against HIV-1 PR. The lengths of the acidic chains were optimized to 6 and 8 carbons. Changing a 3-ester bond to an amide bond or dimerization of the triterpenes retained their inhibitory activity against HIV-1 PR. Introduction of an additional acidic chain to C-28 of oleanolic acid increased the inhibitory activity appreciably, though a derivative with only one acidic chain linked at C-28 also showed potent activity against HIV-1 PR. The inhibitory mechanism was proved directly by size exclusion chromatography to be inhibition of dimerization of the enzyme polypeptides.

2.3 Xanthoceras sorbifolia [7]

From the methanol extract of the wood of Xanthoceras sorbifolia, two new compounds, xanthocerasic acid and epigallocatechin-(4β→8, 2β→O-7)-epicatechin, were isolated together with eleven known compounds. Of the isolated compounds, 3-oxotirucalla-7,24-dien-21-oic acid, oleanolic acid, and epigallocatechin-(4β→8, 2β→O-7)-epicatechin were found to be inhibitory substances against HIV-1 PR, with their IC_{50} values being 20, 10, and 70 μg/ml, respectively. Condensed tannins of high molecular weights with epicatechin and epiafzelechin as the main extender units were found to be the most active principles of this plant (IC_{50} values *ca.* 6.0 μg/ml).

2.4. Artemisia caruifolia [8]

From the methanol extract of *Artemisia caruifolia*, which showed a moderate inhibitory activity on HIV-1 PR in a preliminary screening, N^1, N^5, N^{10}-tri-*p*-coumaroylspermidine and three dicaffeoylquinic acids were isolated. The former compound was found to inhibit appreciably HIV-1 PR. Of related amides which were chemically synthesized, N^1, N^5, N^{19}, N^{14}-tetra-p-coumaroylspermine and N^1, N^4, N^7, N^{10}, N^{13}-penta-p-coumaroyltetra-ethylenepentamine inhibited HIV-1 PR more potently than N^1, N^5, N^{10}-tri-p-coumaroyl-spermidine.

3. INHIBITORY SUBSTANCES AGAINST

REVERSE TRANSCRIPTASE (RT) AND RIBONUCLEASE H (RNASE H) ACTIVITIES

3.1. Juglans mandshurica[9]

From the stem-bark of Juglans mandshurica, two new naphthalenyl glucopyranosides, 1,4,8-trihydroxynaphthalene 1-*O*-[α-L-arabinofuranosyl-(1→6)-β -D-glucopyranoside] and 1,4,8-trihydroxynaphthalene 1-*O*-β-D-[6' -*O*-(3" ,5" -dihydroxy-4" -methoxybenzo-yl)]glucopyranoside, and two new α-tetralonyl glucopyranosides, 4α,5,8-trihydroxy-α-tetralone 5-*O*-β-D-[6' -*O*-(3" ,5" -dihydroxy-4" -methoxybenzoyl)]glucopyranoside and 4α,5,8-trihydroxy-α-tetralone 5-*O*-β-D-[6' -*O*-(3" ,4" ,5" -trihydroxybenzoyl)]glucopyra-noside, were isolated together with three known naphthalenyl glucopyranosides, one α-tetralonyl glucopyranoside, four flavonoids, and two galloyl glucopyranosides.

Of the isolated compounds, 1,2,6-trigalloylglucopyranose and 1,2,3,6-terta-galloylglucopyranose exhibited the most potent inhibition of RT activity with IC_{50} values of 0.067 and 0.040 μM, respectively, while the latter compound also inhibited RNase H activity with an IC_{50} of 39 μM, comparable in potency to illimaquinone used as a positive control. 1,4,8-Trihydroxy-naphthalene 1-*O*-β-D-glucopyranoside, 1,4,8-trihydroxynaphthalene 1-*O*-β-D-[6' -O-(4" -hydroxy-3" ,5" -dimethoxybenzoyl)]gluco-pyranoside and 4α,5,8-trihydroxy-α-tetralone 5-O-β-D-[6' -*O*-(3" ,5" -dihydroxy-4" -methoxybenzoyl)]glucopyranoside showed moderate inhibition against both enzyme activities, and inhibitory potency of 1,4,8-trihydroxynaphthalene 1-*O*-β-D-gluco-pyranoside against RNase H activity (IC_{50}=156 μM) was slightly greater than that against the RT activity (IC_{50}=290 μM). The inhibitory potencies among 4α,5,8-trihydroxy-?-tetralone 5-glucopyranosides against RT activity increased accompanied by an increase in the number of free hydroxyls on the galloyl residues attached to C-6' , as represented by the IC_{50} values of >500, 330 and 5.8 μM, respectively.

4. INHIBITORY SUBSTANCES AGAINST HIV-INTEGRASE (INT)

4.1. Coleus parvifolius

Of 50 Thai plants, the EtOH extract of Benth. (aerial parts) showed potent activity against HIV-1 INT with an IC_{50} value of 9.2 μg/ml. From this extract, 11 compounds were isolated and identified as luteolin 5-*O*-β-glucopyranoside, luteolin, luteolin 7-methyl ether, luteolin 5-*O*-β -glucuronide, 5-*O*-β -D-glucopyranosylluteolin 7-methyl ether, rosmarinic acid, rosmarinic acid methyl ester, daucosterol, α- and β-amyrins and phytol. Of these compounds, rosmarinic acid methyl ester, rosmarinic acid, luteolin and luteolin 7-methyl ether exhibited high inhibitory activity against HIV-1 INT with IC_{50} values of 3.1, 5.0, 11.0 and 11.0 μ M, respectively. Among rosmarinic acid derivatives, the HIV-1 INT inhibitory activity increased in order of dimmer (IC_{50}=5.0μM), trimer (IC_{50}=1.4μM), and tetramer (IC_{50}=1.0μM).

Since HIV has a long latent period after infection in humans and clinically effective vaccine is not yet available at present, many medications in association with chemotherapy have been tried for treatment for AIDS. Traditional medicines, whose effectiveness has been established for various diseases since more than a thousand years, are seem to be promising sources of new anti-HIV agents.

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