Discovery of potent Vpr inhibitors as anti-HIV drugs from Myanmar medicinal plants and formulations

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The viral protein R (Vpr) is a small basic protein (14 kDa) and is well conserved in HIV-1, HIV-2, and simian immunodeficiency virus (SIV). Many reports have suggested that Vpr is a promising drug target for a comprehensive AIDS therapy. To date, only four natural products small molecule Vpr inhibitors, namely fumagillin, damnacanthal, vipirinin and quercetin have been reported. The structures of reported Vpr inhibitors are quite different in each other at the level of their scaffolds, and thus further presence of structurally quite different candidates as Vpr inhibitors are significantly expected in the natural medicines.

In the present study, we developed a screening system to investigate Vpr inhibitors. We established TREx-HeLa-Vpr cell line, in which Vpr expression was tightly regulated by tetracycline. In this assay system, addition of tetracycline led to the expression of Vpr which caused the death of TREx-HeLa cells whereas Vpr-induced cell death was not occurred in the presence of Vpr inhibitor. We screened the crude extracts of Myanmar medicinal plants against the established cell line, TREx-HeLa-Vpr, and found that $CHCl_3$ soluble fraction of rhizomes of *Kaempferia pulchra* exhibited anti-Vpr activity with 25 μg/mL. *Kaempferia pulchra* Ridl., a perennial herb of the Zingiberaceae family is cultivated in some tropical countries including Myanmar, Indonesia, Malaysia, and Thailand. It is commonly known as "Shan-pan-oot" in Myanmar, and has been extensively used for cough, blood stimulation, carminative, quenching heat, deodorant, urinary tract infection, diuretic, and mellitus diabetes. It is also one of the ingredients of Myanmar traditional medicinal formulations such as TMF-20, TMF-21, TMF-23, TMF-32, TMF-39, and TMF-43. It has been reported to possess anti-inflammatory and anti-tumor activities. The rhizomes have been used locally for the self-medication by cancer and AIDS patients in Myanmar. The isolation of the active $CHCl_3$ soluble extract of *K. pulchra* afforded ten new isopimarane diterpenoids, kaempulchraols A–J, along with 5 known analogues [9α-hydroxyisopimara-8(14),15-dien-7-one, 7β, 9α-dihydroxypimara-8(14),15-diene, 1α, 11α-dihydroxypimara-8(14),15-diene, 1α, 2α-dihydroxypimara-8(14),15-diene, and (2*R*)-*ent*-2hydroxyisopimara-8(14),15-diene]. The structures of new compounds were elucidated using extensive spectroscopic techniques including XRD and CD measurements. All the isolates were tested for anti-Vpr activity against TREx-HeLa-Vpr cells. Among the tested compounds, kaempulchraols B and G exhibited potent anti-Vpr activity with 1.56 μM. The investigation on the detailed inhibitory mechanisms and structure-activity-relationship (SAR) of isolated kaempulchraols against Vpr are currently inprogress.