Executive summary

Anti-HIV-1 integrase activity of compounds from *Eclipta prostrata* and anti-inflammatory activity of compounds from *Kaempferia parviflora*"

Introduction

AIDS has been a major problem in Thailand since the late 1980s (58,000 dealths/year, 21,000 new HIV positives reported/year and 570,000 total HIV-positive patients todate). Specific drug treatment is expensive, and only a very small number of AIDS patients have access to the cocktail of modern antiviral agents. Therefore the majority of AIDS patients resort to using Thai traditional doctors, who prescribe a range of plant-based products. However, scientific studies supporting this use (efficacy, specificity, toxicity) have in most cases not yet been carried out. A preliminary screening of Thai medicinal plants that are used as self-medication by AIDS patients revealed that the extract of *E. prostrata* exhibited high inhibitory activity against HIV-1 IN with an IC_{50} of 21.1 μg/mL (Tewtrakul *et al.*, 2006). In this study, we therefore report the isolation of active principles from *E. prostrata* and their HIV-1 IN inhibitory activities.

K. parviflora Wall. ex Baker, is one of the plants in the Zingiberaceae family, locally known in Thai as kra-chai-dam. The rhizome of this plant has been used for treatment of gout, apthous ulcer, abscesses, allergy and gastrointestinal disorders, as well as an aphrodisiac (Pengcharoen, 2002). *K. parviflora* has recently been reported to possess anti-allergic (Tewtrakul et al., 2008), antimycobacterial, antiplasmodial (Yenjai et al., 2004), anti-peptic ulcer (Rujjanawate et al., 2005) and anti-viral protease effects (Sookkongwaree et al., 2006). Moreover, it has been reported that the ethanolic extract of this plant promoted NO production in human umbilical vein endothelial cells (Wattanapitayakul et al., 2007). Since *K. parviflora* rhizomes have long been used for treatment of inflammation and possessed marked anti-NO activity, we thus investigated the inhibitory activity of compounds isolated from this plant against NO, PGE_2 and TNF-α releases using RAW264.7 macrophage cells.

Material and Methods

Assay for HIV-1 IN inhibitory activity

The integration reaction was evaluated according to the method previously described (Tewtrakul *et al.*, 2001).

Assay for NO, PGE_2, TNF-a inhibitory effects from RAW264.7 cells

Inhibitory effect on NO production by murine macrophage-like RAW264.7 cells was evaluated using a modified method from that previously reported (Banskota et al., 2003).

Results and discussions

Anti-HIV-1 integrase activity of compounds from *Eclipta prostrata*

Four compounds belonging to terthiophene derivatives were isolated from the CH_2Cl_2 extract of the whole plants of *Eclipta prostrata*. They were found to be 5-hydroxymethyl-(2, 2′:5′, 2′′)-terthienyl tiglate (**1**), 5-hydroxymethyl-(2, 2′: 5′, 2′′)-terthienyl agelate (**2**), 5-hydroxymethyl-(2, 2′: 5′, 2′′)-terthienyl acetate (**3**), ecliptal (**4**); whereas those of methanol fraction were orobol (**5**) and wedelolactone (**6**) (Figure 1). Of these compounds, wedelolactone (**6**) possessed the highest activity against HIV-1 IN with the IC_{50} value of 4.0 μM, followed by orobol (**5**) (IC_{50} = 8.1 μM), while all isolated terthiophene compounds were apparently inactive (IC_{50} > 100 μM). Wedelolactone exhibited potent activity, comparable to that of the positive control, suramin (IC_{50} = 2.4 μM). This finding also supports the use of *E. prostrata* in AIDS treatment which agrees with its traditional use for treatment of blood-related diseases.

Anti-inflammatory activity of compounds from *Kaempferia parviflora*

EtOH and water extracts from the rhizomes of five selected Zingiberaceous plants used for treatment of inflammation in Thai traditional medicine, including *C. mangga*, *K. galanga*, *K. parviflora*, *Z. officinale* and *Z. zerumbet* were investigated for their anti-inflammatory activities using RAW264.7 cell line. Among these, the EtOH extract of *K. parviflora* exhibited the most appreciable anti-inflammatory effect against LPS-induced NO release in RAW264.7 cells, with an IC_{50} value of 7.8 μg/ml.

From bioassay-guided fractionation of *K. parviflora*, the hexane fraction showed high activity against NO release with an IC_{50} value of 3.6 μg/ml, followed by $CHCl_3$ fraction (IC_{50} = 8.8 μg/ml), EtOAc- and water fractions (IC_{50} > 100 μg/ml), respectively. The hexane fraction was then chromatograpged further to obtain seven methoxyflavones and were tested for their NO inhibitory effects. The result indicated that compound **5** (5-hydroxy-3,7,3′,4′-tetramethoxyflavone) exhibited the highest activity against NO release with an IC_{50} value of 16.1 μM, followed by **4** (IC_{50} = 24.5 μM) and **3** (IC_{50} = 30.6 μM). These three compounds exhibited higher effect than L-NA, a positive control (NO synthase inhibitor, IC_{50} = 61.8 μM). Moreover, compounds **5** (IC_{50} = 16.1 μM) and **2** (IC_{50} = 24.5 μM) conferred higher effect than that of indomethacin (IC_{50} = 25.0 μM), a

clinical used drug. Compound **5** was also tested on LPS-induced PGE_2 and TNF-α releases from RAW264.7 cells. It was revealed that **5** again exhibited appreciable inhibitory effect on PGE_2 release (IC_{50} = 16.3 μM), but inactive on the release of TNF-α (IC_{50} > 100 μM).

The structure-activity trends of *K. parviflora* upon NO inhibition could be summarized as follow: 1) 3,7,3′,4′-tetramethoxyflavone was essential for NO inhibitory activity; and 2) 4′-methoxyl group on B-ring increased the activity as shown in **4** (IC_{50} = 24.5 μM) versus **2** (IC_{50} = 64.3 μM). The result of the present study are concurrent with the previous report that the methoxylation at position 3 or 4′ enhanced the activity. It has also been reported that some active flavonoids suppressed the iNOS induction in a dose-dependent manner (Matsuda et al., 2003). Nobiletin (5,6,7,8,3′,4′-hexamethoxy flavone), which was purified from the fruit peel of *Citrus sunki* at concentration 6-50 μM significantly suppressed NF-κB transcriptional activation, NO and PGE_2 production in LPS-activated RAW 264.7 cells. This result revealed that the methoxyl flavones may exert anti-inflammatory effect (Choi et al., 2007).

In conclusion, the present study may support the use in Thai traditional medicine of *K. parviflora* for treatment of inflammatory-related diseases through the inhibition of NO and PGE_2 releases, but partly due to that of TNF-α. It is suggested that the flavones isolated from this plant might involve in the suppression of iNOS and COX-2 genes. The anti-inflammatory mechanism in transcriptional level of active flavones from *K. parviflora* will be further investigated.

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