

**ภาคผนวก 11 : ผลงานวิจัยส่งเพื่อการพิจารณาตีพิมพ์ เรื่อง HIV-1 protease- and HIV-1 integrase inhibitory substances from *Eclipta prostrate*, *Planta Med.*, 2006 (submitted)**

# HIV-1 protease- and HIV-1 integrase inhibitory substances

from *Eclipta prostrata*

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**Abstract :** The bioassay-guided fractionation led to the isolation of six compounds from the whole plants of *Eclipta prostrata* extract. They were indentified as 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**), orobol (**5**) and wedelolactone (**6**). Among these, compound **6** showed the highest activity against HIV-1 integrase (IN) with IC<sub>50</sub> value of 4.0 ± 0.2 µM, followed by compound **5** (IC<sub>50</sub> = 8.1 ± 0.5 µM); whereas four terthiophene compounds were inactive (IC<sub>50</sub> > 100 µM). Regarding HIV-1 protease (PR) inhibitory activity, compound **1** exhibited appreciable activity against HIV-1 PR with IC<sub>50</sub> of 58.3 ± 0.8 µM, followed by compound **4** (IC<sub>50</sub> = 83.3 ± 1.6 µM) and compound **3** (IC<sub>50</sub> = 93.7 ± 0.8 µM), while compounds **2**, **5** and **6** were inactive against HIV-1 PR.

**Keywords :** HIV-1 protease, HIV-1 integrase, Inhibitory activities, *Eclipta prostrata*

## Introduction

*Eclipta prostrata* Linn. (syn : *E. alba* Hassk., *E. erecta* Hassk.) is one of the plants in Compositae family. It is a perennial herb that grows widely throughout the tropical areas especially in Asia. In Thai traditional medicine, the leaf of this plant has been used as hair dyeing and treatment of skin diseases. The root are used as antituberculosis, antiamoebiasis, antianaemia and antiasthma; whereas the stem are used as astringent, anticancer, hepatoprotectant and tonic (1). It was reported that *Eclipta prostrata* exhibited anti-inflammatory (2), immunomodulatory effect on T-lymphocytes (3), antimicrobial (4) and hepatoprotective activities (5).

From the screening of Thai medicinal plants used as self medication by AIDS patients, the methanol (MeOH) extract of *E. prostrata* exhibited high inhibitory activity against HIV-1 IN with an IC<sub>50</sub> of 21.1 µg/ml. Therefore, this plant was extracted into dichloromethane (CH<sub>2</sub>Cl<sub>2</sub>) and MeOH soluble fractions, respectively. In this paper, we report the isolation of active principles and their HIV-1 PR and HIV-1 IN inhibitory activities.

## Materials and methods

### *Plant materials and extraction of the extract*

Whole plants of *E. prostrata* were collected at Prince of Songkla University, Songkhla, Thailand and was identified by Assoc. Prof. Dr. Sanan Subhadhirasakul. The voucher specimen (No. SN 4412025) is deposited at Southern center of Thai medicinal plants at Faculty of Pharmaceutical Sciences, Prince of Songkla University.

### *Apparatus*

Melting points were determined on the Fisher-John melting point apparatus. UV spectra were measured with a SPECORD S 100 (Analytikjena). The IR spectra were measured with a

Perkin-Elmer FTS FT-IR spectrophotometer. The  $^1\text{H}$  NMR spectra was recorded using 300 MHz Bruker FTNMR Ultra Shield<sup>TM</sup> spectrometers. Chemical shifts are recorded in part per million ( $\delta$ ) in  $\text{CDCl}_3$  with tetramethylsilane (TMS) as an internal reference. The EIMS were obtained from a MAT 95 XL mass spectrometer. Quick column chromatography (QCC) and column chromatography (CC) were carried out on silica gel 60 F<sub>254</sub> (Merck) and silica gel 100 (Merck), respectively. Precoated plates of silica gel 60 F<sub>254</sub> was used for analytical purposes.

### *Extraction and isolation*

Dried-aerial parts (250.0 g) of *E. prostrata* were extracted with  $\text{CH}_2\text{Cl}_2$  and MeOH (2 l x 2, 5 days each) at room temperature. The mixtures were filtered and concentrated under reduced pressure to afford  $\text{CH}_2\text{Cl}_2$  and MeOH extracts, successively.

The  $\text{CH}_2\text{Cl}_2$  extract (1.95 g) was subjected to quick column chromatography (QCC) over silica gel (150 g) and elution with a gradient of hexane:EtOAc (17 : 3, 1 : 1, 500 ml each), EtOAc (200 ml) and EtOAc:MeOH (19 : 1, 200 ml) to give four fractions (D1-D4). Fraction D2 (441.4 mg) was further purified by CC over silica gel (30 g) with hexane : EtOAc (19 : 1, 500 ml) to yield **1** (10.0 mg), **2** (22.3 mg) and **3** (2.6 mg). Fraction D3 (50 mg) was separated by CC over silica gel (3 g) with hexane: $\text{CH}_2\text{Cl}_2$  (7 : 3, 250 ml) to afford **4** (4.6 mg).

The MeOH extract (1.0 g) was fractioned by CC over silica gel (60 g) with hexane and increasing polarity with  $\text{CH}_2\text{Cl}_2$  and MeOH (7 : 3 : 0, 1 : 1 : 0, 0 : 100 : 0, 0 : 19 : 1, 0 : 17 : 3, 250 ml each), respectively, to afford five fractions (M1-M5). Fraction M3 (12.5 mg) was further purified by preparative thin layer chromatography (prep. TLC) with  $\text{CH}_2\text{Cl}_2$  : MeOH (19:1) to give **5** (5.4 mg). Fraction M4 (10.0 mg) was subjected by prep. TLC with  $\text{CH}_2\text{Cl}_2$  : MeOH (19 : 1) to afford **6** (6.2 mg). These compounds were identified by comparison their

spectroscopic data with those reported in the literatures (6), (7), (8), (9). Copies of original spectra are available from the author of correspondence.

5-Hydroxymethyl-(2, 2' : 5', 2'')-terthienyl tiglate (**1**) : viscous oil; IR (neat) : 2914, 1704  $\text{cm}^{-1}$ ; UV (MeOH)  $\lambda_{\text{max}}$  ( $\log \epsilon$ ) : 352 (3.40), 248 (3.20) nm;  $^1\text{H}$  NMR (300 MHz,  $\text{CDCl}_3$ ) :  $\delta$  7.22 (1H, dd,  $J = 5.1, 1.2$  Hz, H-5''), 7.17 (1H, dd,  $J = 3.6, 0.9$  Hz, H-3''), 7.08-6.98 (5H, m, H-3, H-4, H-3', H-4', H-4''), 6.92 (1H, qq,  $J = 7.2, 1.5$  Hz, H-5'''), 5.29 (2H, s, H-1'''), 1.87 (3H, d,  $J = 1.5$  Hz, H-7'''), 1.80 (3H, d,  $J = 7.2$  Hz, H-6'''); EIMS  $m/z$  (% rel.) : 360 ( $\text{M}^+$ , 9), 359 ( $\text{M}^+ - 1, 32$ ), 260 (100), 130 (10), 83 (10).

5-Hydroxymethyl-(2, 2' : 5', 2'')-terthienyl agelate (**2**) : viscous oil; IR (neat): 2914, 1714  $\text{cm}^{-1}$ ; UV (MeOH)  $\lambda_{\text{max}}$  ( $\log \epsilon$ ) : 356 (2.93), 248 (2.74) nm;  $^1\text{H}$  NMR (300 MHz,  $\text{CDCl}_3$ ) :  $\delta$  7.22 (1H, dd,  $J = 5.1, 1.2$  Hz, H-5''), 7.17 (1H, dd,  $J = 3.6, 0.9$  Hz, H-3''), 7.08-7.00 (5H, m, H-3, H-4, H-3', H-4', H-4''), 6.11 (1H, qq,  $J = 7.2, 1.5$  Hz, H-5'''), 5.30 (2H, s, H-1'''), 2.05 (3H, d,  $J = 7.2$  Hz, H-6'''), 1.95 (3H, d,  $J = 1.5$  Hz, H-7'''); EIMS  $m/z$  (% rel.) : 360 ( $\text{M}^+$ , 7), 359 ( $\text{M}^+ - 1, 22$ ), 260 (100), 129 (42), 83 (84), 69 (95).

5-Hydroxymethyl-(2, 2' : 5', 2'')-terthienyl acetate (**3**): viscous oil; IR (neat): 2914, 1731  $\text{cm}^{-1}$ ; UV (MeOH)  $\lambda_{\text{max}}$  ( $\log \epsilon$ ) : 358 (3.80), 249 (3.47) nm;  $^1\text{H}$  NMR (300 MHz,  $\text{CDCl}_3$ ) :  $\delta$  7.23 (1H, brd,  $J = 5.1$  Hz, H-5''), 7.17 (1H, brd,  $J = 3.6$  Hz, H-3''), 7.08-6.99 (5H, m, H-3, H-4, H-3', H-4', H-4''), 5.20 (2H, s, H-1'''), 2.10 (3H, s,  $\text{OCOCH}_3$ ); EIMS  $m/z$  (% rel.) : 320 ( $\text{M}^+$ , 9), 319 ( $\text{M}^+ - 1, 46$ ), 260 (100), 148 (14), 83 (15), 69 (25).

Ecliptal (**4**): viscous oil; UV (MeOH)  $\lambda_{\max}$  (log  $\epsilon$ ): 352 (2.39), 265 (2.56) nm;  $^1\text{H}$  NMR (300 MHz,  $\text{CDCl}_3$ ):  $\delta$  9.87 (1H, s, CHO), 7.67 (1H, d,  $J = 3.6$  Hz, H-3), 7.28-7.04 (6H, m, H-4, H-3', H-4', H-3'', H-4'', H-5'').

Orobol (**5**): viscous oil; UV (MeOH)  $\lambda_{\max}$  (log  $\epsilon$ ): 337 (3.07), 288 (3.59), 261 (3.88), 217 (3.82) nm;  $^1\text{H}$  NMR (300 MHz,  $\text{CDCl}_3$ ):  $\delta$  7.80 (1H, s, H-2), 6.96 (1H, d,  $J = 1.8$  Hz, H-2'), 6.86 (1H, d,  $J = 8.1$  Hz, H-5'), 6.80 (1H, dd,  $J = 8.1, 1.8$  Hz, H-6'), 6.32, 6.18 (each 1H, each brs, H-6 or H-8); EIMS  $m/z$  (% rel.): 286 ( $\text{M}^+$ , 46), 285 ( $\text{M}^+-1$ , 100), 152 (54), 133 (45), 69 (32).

Wedelolactone (**6**): mp 323-324 °C, IR (KBr): 3476, 3416, 1620  $\text{cm}^{-1}$ ; UV (MeOH)  $\lambda_{\max}$  (log  $\epsilon$ ): 353 (3.38), 303 (2.94), 248 (3.30) nm;  $^1\text{H}$  NMR (300 MHz,  $\text{CDCl}_3$ ):  $\delta$  7.39, 7.21 (each 1H, each s, H-10 or H-13), 6.56, 6.45 (each 1H, each d,  $J = 2.4$  Hz, H-6 or H-8), 3.85 (3H, s,  $\text{OCH}_3$ ); EIMS  $m/z$  (% rel.): 314 ( $\text{M}^+$ , 24), 313 ( $\text{M}^+-1$ , 100), 298 (27), 242 (16), 157 (9).

### *Enzymes and chemicals*

Recombinant HIV-1 PR, substrate peptides and acetyl pepstatin, were purchased from Sigma Chemical Co., St. Louis, USA.

Recombinant HIV-1 IN was expressed in *Escherichia coli*, purified according to the method described in the previous paper (10), except that after removal of the His-tag with thrombin, chromatography on a Sephadex 200 column was the final purification step. The integrase enzyme was stored at -80 °C until use.

### *Assay of HIV-1 protease inhibitory activity*

This assay was modified from the previously reported method (11). In brief, the recombinant HIV-1 PR solution was diluted with a buffer composed of a solution containing 50 mM of sodium acetate (pH 5.0), 1 mM ethylenediamine disodium (EDTA.2Na) and 2 mM 2-mercaptoethanol (2-ME) and mixed with glycerol in the ratio of 3 : 1. The substrate peptides, Arg-Val-Nle-(*p*NO<sub>2</sub>-Phe)-Glu-Ala-Nle-NH<sub>2</sub>, was diluted with a buffer solution of 50 mM sodium acetate (pH 5.0). Two microliters of plant extract and 4 µl of HIV-1 PR solution (0.025 mg/ml) were added to a solution containing 2 µl of 50 mM buffer solution (pH 5.0) and 2 µl of substrate solution (2 mg/ml), and the reaction mixture 10 µl was incubated at 37°C for 1 hr. A control reaction was performed under the same condition but without the plant extract. The reaction was stopped by heating the reaction mixture at 90 °C for 1 min. Subsequently, 20 µl of sterile water was added and an aliquot of 10 µl was analyzed by HPLC using RP-18 column (4.6 x 150 mm I.D., Supelco 516 C-18-DB 5 µm, USA). Ten microlitres of the reaction mixture was injected to the column and gradiently eluted with acetonitrile (15-40%) and 0.2% trifluoroacetic acid (TFA) in water, at a flow rate of 1.0 ml/min. The elution profile was monitored at 280 nm. The retention times of the substrate and *p*-NO<sub>2</sub>-Phe-bearing hydrolysate were 11.336 and 9.490 min, respectively. The inhibitory activity on HIV-1 PR was calculated as follows : % inhibition =  $(A_{\text{control}} - A_{\text{sample}}) \times 100 / A_{\text{control}}$ ; whereas A is a relative peak area of the product hydrolysate. Acetyl pepstatin was used as a positive control.

#### *Assay for HIV-1 IN inhibitory activity*

##### *Oligonucleotide substrates*

Oligonucleotides of long terminal repeat donor DNA (LTR-D) and target substrate (TS) DNA were purchased from QIAGEN Operon, USA and stored at -25°C before use. The sequence of biotinylated LTR donor DNA and its unlabelled complement were 5'-biotin-

ACCCTTTTAGTCAGTGTGGAAAATCTCTAGCAGT-3' (LTR-D1) and 3'-GAAAATCAGTCACACCTTTTAGAGATCGTCA-5' (LTR-D2), respectively; while those of the target substrate DNA (digoxigenin-labelled target DNA, TS-1) and its 3'-labelled complement were 5'-TGACCAAGGGCTAATTCAGT-digoxigenin and digoxigenin-ACTGGTCCCGATTAAGTGA-5' (TS-2), respectively.

### *Multiplate integration assay (MIA)*

#### *Annealing of the Substrate DNA*

Firstly, LTR-D1 and LTR-D2, TS-1 and TS-2 were mixed separately and then the former solution was diluted to a concentration of 2 pmol/ml, while the later one was made to 5 pmol/ml using a buffer solution containing 10 mM Tris-HCl (pH 8.0), 1mM EDTA and 100 mM KCl. Both solutions were then heated at 85 °C for 15 min in an incubator. After heating, each solution is gradually cooled to room temperature and were stored at -20 °C until use.

#### *Pretreatment of the multiplate*

A 96-well plate was coated with 50 µl of a streptavidin solution containing 40 µg/ml streptavidin, 90 mM Na<sub>2</sub>CO<sub>3</sub> and 10 mM KCl. After discarding streptavidin coating solution, the coated plate was washed with 300 µl of sterile water twice and phosphate buffer saline (PBS, 300 µl) twice. The blocking buffer (300 µl) containing 1% skim milk in PBS was added into each well and the plate was kept at room temperature for 30 min. After discarding the blocking buffer, each well was washed with PBS solution (300 µl) four times and then the PBS solution was completely removed. A biotinylated LTR donor DNA (50 µl) solution containing 10 mM Tris-HCl (pH 8.0), 1mM NaCl and 40 fmol/ml of LTR donor DNA was added into each well and the plate was shaken well, centrifuged and kept gently at room temperature for 60 min. After discarding the LTR donor solution, the microplate was washed



with PBS solution four times and then each well was filled with 300  $\mu$ l of PBS solution. Just before the integration reaction, the PBS solution of each well was discarded and rinsed with 300  $\mu$ l of distilled water four times, and then the distilled water was removed completely.

### *Integration reaction*

The integration reaction was evaluated according to the method previously described (12). Briefly, a mixture (45  $\mu$ l) composed of 12  $\mu$ l of IN buffer [containing 150 mM 3-(N-morpholino)propane sulfonic acid, pH 7.2 (MOPS), 75 mM  $MnCl_2$ , 5 mM dithiothritol (DTT), 25% glycerol and 500  $\mu$ g/ml bovine serum albumin], 1  $\mu$ l of 5 pmol/ml digoxigenin-labelled target DNA and 32  $\mu$ l of sterilized water were added into each well of a 96-well plate. Subsequently, 6  $\mu$ l of sample solution and 9  $\mu$ l of 1/5 dilution of integrase enzyme was added to the plate and incubated at 37 °C for 80 min. After wells were washed with PBS 4 times, 100  $\mu$ l of 500 mU/ml alkaline phosphatase (AP) labelled anti-digoxigenin antibody were added and incubate at 37 °C for 1 hr. The plate was washed again with washing buffer containing 0.05% Tween 20 in PBS 4 times and with PBS 4 times. Then, AP buffer (150  $\mu$ l) containing 100 mM Tris-HCl (pH 9.5), 100 mM NaCl, 5 mM  $MgCl_2$  and 10 mM p-nitrophenyl phosphate was added to each well and incubated at 37°C for 1 hr. Finally, the plate was measured with a microplate reader at a wavelength of 405 nm. A control composed of a reaction mixture, 50% DMSO and an integrase enzyme, while a blank is buffer-E containing 20 mM MOPS (pH 7.2), 400 mM potassium glutamate, 1 mM ethylenediaminetetraacetate disodium salt (EDTA. 2Na), 0.1% Nonidet-P 40 (NP-40), 20% glycerol, 1 mM DTT and 4 M urea without the integrase enzyme. Suramin, a polyanionic HIV-1 IN inhibitor was used as a positive control.

$$\% \text{ Inhibition against HIV-1 IN} = \left[ \frac{(\text{OD control} - \text{OD sample})}{\text{OD control}} \right] \times 100$$

\* OD = absorbance detected from each well

### Statistics

For statistical analysis, the results of anti-HIV-1 PR activity were expressed as mean  $\pm$  S.D of three determinations, while anti-HIV-1 IN were as mean  $\pm$  S.D of four determinations. The  $IC_{50}$  values were calculated using the Microsoft Excel program.

### Results and discussion

Four compounds belonging to terthiophene derivatives were isolated from the  $CH_2Cl_2$  fraction 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). Among these compounds, wedelolactone (**6**) possessed the highest activity against HIV-1 IN with the  $IC_{50}$  value of  $4.0 \pm 0.2 \mu M$ , followed by orobol (**5**) ( $IC_{50} = 8.1 \pm 0.5 \mu M$ ); while all isolated terthiophene compounds were apparently inactive ( $IC_{50} > 100 \mu M$ ). Wedelolactone also exhibited potent activity comparable to that of a positive control, suramin ( $IC_{50} = 2.4 \pm 0.1 \mu M$ ). In the case of HIV-1 PR inhibitory activity, **1** exhibited appreciable activity with the  $IC_{50}$  of  $58.3 \pm 0.8 \mu M$ , followed by **4** ( $IC_{50} = 83.3 \pm 1.6 \mu M$ ) and **3** ( $IC_{50} = 93.7 \pm 0.8 \mu M$ ), while compounds **2**, **5** and **6** were inactive. This result implies that wedelolactone (**6**) and orobol (**5**) are selective inhibitors of HIV-1 IN, but not of HIV-1 PR; whereas thiophene derivatives are the contrary (Table 1, Figure 2 and Figure 3).

Several classes of photosensitizers including sulphur-containing compounds and terthiophenes have been reported for their anti-HIV-1 activities. Among these photodynamic

agents,  $\alpha$ -terthienyl was found to be the most potent activity against HIV and basic terthiophene structure was essential for good anti-HIV activity (13). From the present study, compound **1** (*trans*-form) which is a terthiophene ester, displayed significant activity against HIV-1 PR; whereas its isomer (**2**, *cis* form) was inactive. This indicated that the different stereoisomers might affect anti-HIV-1 PR activity of these two compounds.

Coumarins such as calanolides and inophyllums have been established as non-nucleotide specific inhibitors of HIV-1 reverse transcriptase (RT). Calanolides A and B isolated from *Calophyllum lanigerum* were previously reported to be the potent anti-HIV-1 agents (Cardellina et al., 1995). Two coumarins isolated from *Glycyrrhiza glabra* namely glycocoumarin and licopyranocoumarin were revealed to inhibit giant cell formation in HIV infected cell cultures (15). However, wedelolactone (**6**) which is also a coumarin derivative, still has not been reported for its anti-HIV activity. Flavonoids were also revealed to possess anti-HIV-1 activity as both HIV-RT (16) and HIV-1 IN inhibitors (12). It was found that the potent inhibition of HIV-1 IN was observed with flavonoids having at least one pair of vicinal hydroxyl groups and the activity was highly dependent on the number of vicinal hydroxyl groups (12). Orobol (**5**), whose structure bearing one vicinal hydroxyl groups with other two hydroxy substituents also showed high inhibitory activity against HIV-1 IN ( $IC_{50} = 8.1 \mu M$ ). From the present study, it may indicate that the inhibitory activity of *E. prostrata* and its isolated compounds against HIV-1 PR and HIV-1 IN are the scientific support for using this plant in AIDS treatment.

In conclusion, among the isolated compounds from *E. prostrata*, wedelolactone (**6**) and orobol (**5**) are responsible for anti-HIV-1 IN activity; whereas 5-hydroxymethyl-(2, 2': 5',

2'')-terthienyl tiglate (**1**), ecliptal (**4**) and 5-hydroxymethyl-(2, 2': 5', 2'')-terthienyl acetate (**3**) act as HIV-1 PR inhibitors.

**Table 1** Anti-HIV-1 PR and HIV-1 IN activities of compounds isolated from the whole plant of *Eclipta prostrata*

**Fig. 1** Chemical structures of compounds isolated from the whole plants of *Eclipta prostrata*.

**Fig. 2** Dose-dependent curve of anti-HIV-1 PR activity of compounds **1**, **3**, **4** and acetyl pepstatin. Each value represents the mean  $\pm$  S.D of three determinations.

**Fig. 3** Dose-dependent curve of anti-HIV-1 IN activity of compounds **5**, **6** and suramin. Each value represents the mean  $\pm$  S.D of four determinations.

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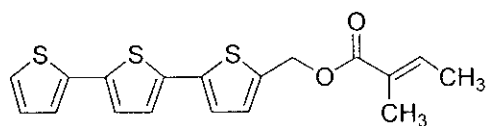
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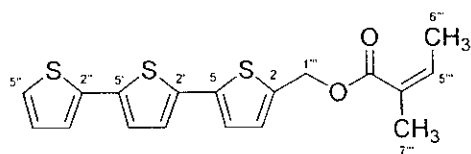
**Table 1** Anti-HIV-1 PR and HIV-1 IN activities ( $IC_{50}$ ,  $\mu M$ ) of compounds isolated from the whole plants of *Eclipta prostrata*

Compounds	$IC_{50}$ ( $\mu M$ ) $\pm$ S.D.	
	HIV-1 PR	HIV-1 IN
5-Hydroxymethyl-(2, 2':5', 2'')-terthienyl tiglate (1)	<b>58.3 <math>\pm</math> 0.8</b>	>100
5-Hydroxymethyl-(2, 2': 5', 2'')-terthienyl agelate (2)	>100	>100
5-Hydroxymethyl-(2, 2': 5', 2'')-terthienyl acetate (3)	<b>93.7 <math>\pm</math> 0.8</b>	>100
Ecliptal (4)	<b>83.3 <math>\pm</math> 1.6</b>	>100
Orobol (5)	>100	<b>8.1 <math>\pm</math> 0.5</b>
Wedelolactone (6)	>100	<b>4.0 <math>\pm</math> 0.2</b>
Acetyl pepstatin (Positive control for HIV-1 PR)	<b>3.4 <math>\pm</math> 0.2</b>	-
Suramin (Positive control for HIV-1 IN)	-	<b>2.4 <math>\pm</math> 0.1</b>

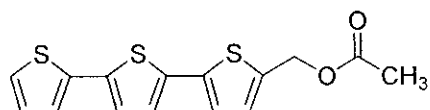
**Fig. 1** Chemical structures of compounds isolated from the whole plants of *Eclipta prostrata*



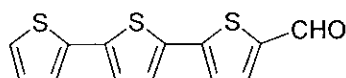
5-Hydroxymethyl-(2,2':5',2'')-terthienyl tiglate (**1**)



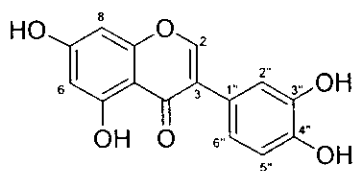
5-Hydroxymethyl-(2,2':5',2'')-terthienyl agelate (**2**)



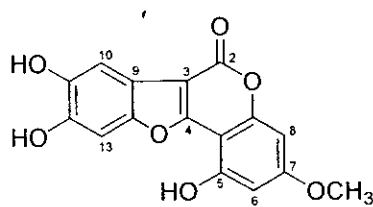
5-Hydroxymethyl-(2,2':5',2'')-terthienyl acetate (**3**)



Ecliptal (**4**)



Orobol (**5**)

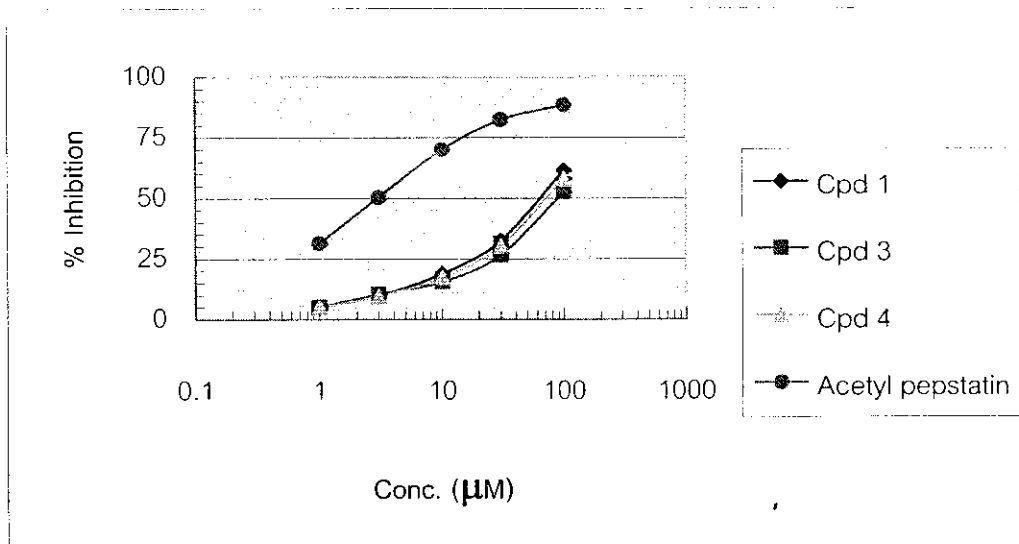


Wedelolactone (**6**)



**Fig. 2** Dose-dependence of anti-HIV-1 PR activity of compounds **1**, **3**, **4** and acetyl pepstatin.

Each value represents the mean  $\pm$  S.D of three determinations.



**Fig. 3** Dose-dependence of anti-HIV-1 IN activity of compounds **5**, **6** and suramin. Each value represents the mean  $\pm$  S.D of four determinations.

