

Antimycobacterial Activity of Cinnamate-Based Esters of the Triterpenes Betulinic, Oleanolic and Ursolic Acids

Tanud TANACHATCHAIRATANA,^a John Barnard BREMNER,^b Ratchanaporn CHOKCHAISIRI,^a and Apichart SUKSAMRARN*^a

^a Department of Chemistry, Faculty of Science, Ramkhamhaeng University; Bangkok, Bangkok 10240, Thailand; and

^b School of Chemistry, University of Wollongong; Wollongong, NSW 2522, Australia.

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Betulinic acid, oleanolic acid and ursolic acid have been modified at the C-3 position to cinnamate-based esters and *in vitro* antimycobacterial activity against *Mycobacterium tuberculosis* H₃₇Ra has been determined. The results indicated that modification of the parent structures of betulinic acid, oleanolic acid and ursolic acid to the *p*-coumarate and, in the case of the latter two triterpenes, the ferulate ester analogues resulted in high antimycobacterial activity. Structure–activity relationships within the lupane, oleanane and ursane analogues and between these triterpenes are discussed.

Key words triterpene ester; antimycobacterial activity; structure–activity relationship; betulinic acid; oleanolic acid; ursolic acid

Tuberculosis is the leading cause of mortality among all infectious diseases worldwide and is responsible for two million deaths annually.¹⁾ The situation has recently been complicated by the human immunodeficiency virus (HIV) pandemic and the increased prevalence of multi-drug resistant strains of *Mycobacterium tuberculosis*.^{2,3)} The recent increase in the number of multi-drug resistant clinical isolates of *M. tuberculosis* has created an urgent need for the evolution of new antituberculosis therapeutics.^{4,5)} The structural modification of natural products is one potential strategy for the development of new antitubercular drugs which are different from the drugs currently used. In this context, triterpenes and derivatives are of particular interest.

Triterpenes exist abundantly in the plant kingdom. This class of natural products and their derivatives have been reported to have interesting biological activities, such as anti-HIV,^{6,7)} antiplasmodial^{8,9)} and cytotoxic activities.^{10,11)} Previous investigations have shown that triterpenes also exhibit moderate to high *in vitro* antimycobacterial activity against *M. tuberculosis*.^{12–14)} It was reported¹²⁾ that betulinic acid (**1**) was more active than its C-3 epimer, *epi*-betulinic acid. Oleanolic acid (**2**) was 4-fold less active than its C-3 epimer, 3-*epi*-oleanolic acid^{12,15)} whereas the acetate derivative of **2** was as active as its parent compound **2**.¹⁶⁾ The 3-keto analogue, oleanonic acid, exhibited an equivalent MIC to that of 3-*epi*-oleanolic acid.¹⁵⁾ The presence of an ester function at C-22 of **2** resulted in a 2-fold decrease in activity.¹⁷⁾ Addition of the hydroxylic functionality at the C-19, and C-2 and C-19 positions of ursolic acid (**3**) to give pomolic acid and tormentic acid, resulted in an equivalent or a 2-fold decrease in antimycobacterial activity, respectively.^{12,15)} The C-3 epimer of the triterpene **3**, 3-*epi*-ursolic acid, exhibited relatively high activity.¹⁸⁾ Modifications of triterpene rings also affected their antimycobacterial activity.^{19,20)} The results observed among the various groups of triterpenes are rather conflicting, thus making it difficult to predict structural requirements for antimycobacterial activity. We have discovered, however, that the presence of a *p*-coumarate moiety at the C-2 hydroxyl group of alphitolic acid (2 α -hydroxybetulinic acid) resulted in increased antimycobacterial activity.²¹⁾ It was thus

of interest to investigate whether such an ester moiety would also increase antimycobacterial activity with respect to the triterpenes **1–3**. In order to study the relationships between the structure of the ester moiety and antimycobacterial activity in triterpenes, other substituted cinnamate ester analogues were also prepared for biological evaluation. This present paper describes the *in vitro* antimycobacterial activity of the triterpenes **1–3** and their C-3 substituted cinnamate ester analogues.

The chemical modifications described in this paper were focused on the introduction of the cinnamoyl and substituted cinnamoyl groups at the C-3 hydroxyl group of betulinic acid (**1**), oleanolic acid (**2**) and ursolic acid (**3**). The ester moieties selected for this study were the unsubstituted cinnamate, *p*-coumarate, the acetate and methyl ether derivatives of the *p*-coumarate esters, the ferulate (4-hydroxy-3-methoxycinnamate) esters and their acetate derivatives, the caffeate (3,4-dihydroxycinnamate) esters and their acetate derivatives, and the *p*-chlorocinnamate esters. The last group of esters were selected as representatives of non-oxygenated substituted cinnamate esters. The esters were obtained by treatment of the triterpenes **1–3** with the appropriate acid chloride in the presence of 4-*N,N*-dimethylaminopyridine (DMAP). Treatment of **1** with cinnamoyl, *p*-acetoxycinnamoyl, *p*-methoxycinnamoyl, 4-*O*-acetylferuloyl, 3,4-di-*O*-acetylcaffeoyl, and *p*-chlorocinnamoyl chlorides (the latter five acid chlorides being prepared by reaction of the corresponding carboxylic acids with oxalyl chloride) in benzene in the presence of DMAP furnished the cinnamate esters **4–9** in 59–97% yields (Chart 1). Treatment of **2** with the foregoing acid chlorides gave the corresponding cinnamate esters **10–15** in 62–81% yields (Chart 2). The cinnamate esters **16–21** were similarly prepared from **3** in 60–87% yields (Chart 2). Deacetylation of the *p*-acetoxycinnamate esters **5**, **11** and **17**, the 4-*O*-acetylferulate esters **7**, **13** and **19**, and the 3,4-di-*O*-acetylcaffeate esters **8**, **14** and **20** with 10% aqueous K₂CO₃ afforded the corresponding *p*-coumarate esters **5a**, **11a** and **17a**, the ferulate esters **7a**, **13a** and **19a**, and the caffeate esters **8a**, **14a** and **20a** in 67–96% yields. All synthetic compounds were purified by column chromatography and their

* To whom correspondence should be addressed. e-mail: apichart@ram1.ru.ac.th

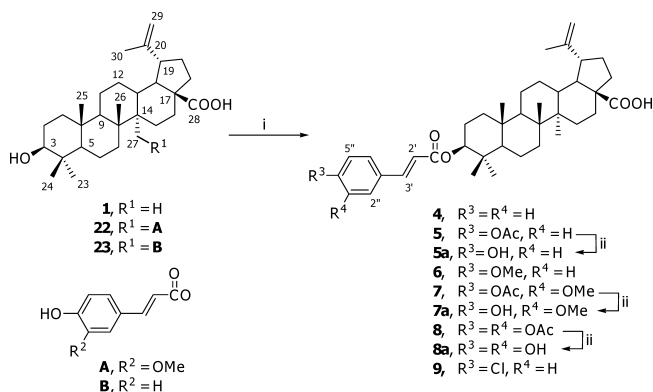


Chart 1. Synthesis of Cinnamate-Based Esters of Betulinic Acid (1)

Reagents and conditions: (i) RCOCI/DMAP/benzene, 60 °C, 0.5–1 h; (ii) 10% K₂CO₃/MeOH, 30 min.

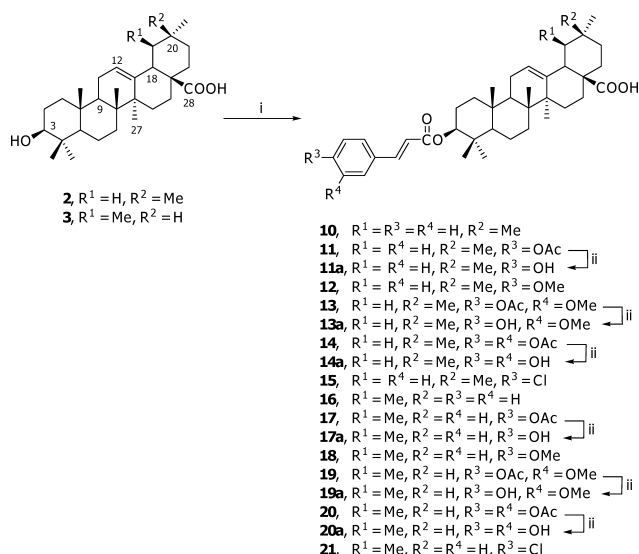


Chart 2. Synthesis of Cinnamate-Based Esters of Oleanolic Acid (2) and Ursolic Acid (3)

Reagents and conditions: (i) RCOCI/DMAP/benzene, 60 °C, 0.5–1 h; (ii) 10% K₂CO₃/MeOH, 30 min.

structures were established by spectroscopic (IR, ¹H-NMR and MS) analysis (see Experimental).

All cinnamate esters of betulinic acid (1), oleanolic acid (2) and ursolic acid (3) were screened for their *in vitro* antimycobacterial activity against *M. tuberculosis* H₃₇Ra by the Microplate Alamar Blue Assay (MABA)²² for the determination of MIC, which is defined as the minimum concentration of compound required to exhibit 90% inhibition of bacterial growth; MICs of the compounds are reported in Table 1. The known active antimycobacterial compounds kanamycin sulfate, isoniazid and rifampicin were included in the assays as controls and reference points. Their MIC values are 2.5, 0.06 and 0.004 μg/ml (4.29, 0.44, 0.005 μM), respectively.

Betulinic acid (1) exhibited low antimycobacterial activity with an MIC of 50 μg/ml. The cinnamoyl, *p*-methoxycinnamoyl and *p*-chlorocinnamoyl analogues 4, 6 and 9 were inactive in this assay. The *p*-coumaroyl analogue 5a, on the other hand, showed very good activity with an MIC of 6.25 μg/ml. The corresponding acetate derivative 5 was 2-fold less active than its parent compound 5a. The ferulate

Table 1. Antimycobacterial Activity (*in Vitro*) of the Triterpenes 1–3 and Their Cinnamate-Based Ester Analogues

Triterpene group	Compound	MIC ^{a,b} (μg/ml)
Betulinic acid group	1	50
	4	>200
	5	12.5
	5a	6.25
	6	>200
	7	200
	7a	200
	8	200
	8a	200
	9	>200
Oleanolic acid group	2	50
	10	50
	11	12.5
	11a	6.25
	12	200
	13	50
	13a	12.5
	14	12.5
	14a	200
	15	>200
Ursolic acid group	3	12.5
	16	>200
	17	25
	17a	6.25
	18	100
	19	3.13
	19a	3.13
	20	100
	20a	200
	21	>200

a) MIC at >200 μg/ml is regarded as inactive. b) The standard drugs, kanamycin sulfate, isoniazid and rifampicin, showed MIC values of 2.5, 0.06 and 0.004 μg/ml, respectively.

ester analogue 7a and its acetate derivative 7 as well as the caffeate ester analogue 8a and its acetate derivative 8 were only weakly active (MICs 200 μg/ml).

In the oleanolic acid (2) group, almost all compounds exhibited antimycobacterial activity, including the cinnamate ester 10 which exhibited equal activity (MIC 50 μg/ml) to that of the parent triterpene 2. The *p*-coumarate ester 11a showed the highest activity of the group, with an MIC of 6.25 μg/ml. The corresponding acetate 11 was 2-fold less active than the parent *p*-hydroxy analogue 11a. Methylation of the parent compound 11a to the corresponding methyl ether analogue 12 resulted in sharp decrease in antimycobacterial activity. The *p*-chlorocinnamoyl analogue 15 was the only inactive analogue of the group. The ferulate ester analogue 13a was 2-fold less active than the *p*-coumarate ester 11a, the most active analogue of the oleanane group. The 4-*O*-acetyl analogue 13 was 4-fold less active than the ferulate ester 13a. In contrast to the ferulate ester 13a and its acetate derivative 13, the acetate derivative of the caffeate ester (compound 14) was as active as the ferulate ester 13a, while the parent compound 14a was only weakly active (MIC 200 μg/ml).

In the ursolic acid (3) group, the parent compound 3 exhibited antimycobacterial activity (MIC 12.5 μg/ml), whereas its cinnamoyl and *p*-chlorocinnamoyl analogues 16 and 21 were inactive. The *p*-coumarate ester 17a was highly active, with an MIC value of 6.25 μg/ml. The corresponding acetate and methyl ether derivatives, 17 and 18, showed lower activ-

ity with MICs of 25 and 100 $\mu\text{g/ml}$ respectively. Both the ferulate ester **19a** and its acetate **19** were highly active with equal MICs of 3.13 $\mu\text{g/ml}$. However, the corresponding caffeate ester **20a** and its acetate **20** were much less active, with respective MICs of 200 and 100 $\mu\text{g/ml}$.

The results indicated that the presence of unsubstituted cinnamate ester functionality in the lupane and ursane triterpenes (**1**, **3** respectively) lead to inactive compounds, while no improvement in the antimycobacterial activity was seen with respect to the oleanane triterpene parent (**2**). Acetylation of the hydroxyl group of the *p*-coumarate moiety resulted in a 2- to 4-fold decrease in activity whereas methylation of the *p*-coumarate hydroxyl group led to a sharp decrease in activity. The presence of the *p*-chloro group resulted in inactivity or did not improve antimycobacterial activity of the triterpene esters. The presence of an extra methoxyl group at the 3-position of the *p*-coumarate moiety to yield the ferulate ester gave rise to varying effects: almost complete loss of activity was observed in the betulinic acid group, whereas a 2-fold decrease and increase in activity were noted for the oleanolic acid and ursolic acid group, respectively. The ferulate analogue **19a** and its acetate **19** showed very high antimycobacterial activity, both with MICs of 3.13 $\mu\text{g/ml}$ (4.64, 4.95 μM respectively). Their MIC values were comparable to the reference drug kanamycin sulfate (MIC 4.29 μM), though isoniazid and rifampicin (MIC 0.44, 0.005 μM , respectively) are considerably more active.

Among the three triterpene groups, ursolic acid (**3**) was the most active compound; it was 4-fold more active than both compounds **1** and **2**. The *p*-coumarate esters of each of these parents (compounds **5a**, **11a**, **17a**) exhibited the same antimycobacterial activity (MICs 6.25 $\mu\text{g/ml}$). The ferulate ester **19a** of ursolic acid (**3**) was 4-fold more active than the ferulate ester **13a** of oleanolic acid (**2**). Rather surprisingly, the ferulate ester **7a** of betulinic acid (**1**) was only weakly active (MIC 200 $\mu\text{g/ml}$). The weak activity of the ferulate ester of betulinic acid was, in fact, not unusual, since it has also been observed in other biological evaluations. For example, it has been reported⁶ that winchic acid (**22**), the C-27 ferulate ester analogue of betulinic acid (**1**), was inactive in an antiplasmodial evaluation, while messagenic acid B (**23**), the C-27 *p*-coumarate ester analogue of betulinic acid (**1**), was moderately active (IC₅₀ 3.8 $\mu\text{g/ml}$) in this assay. It is also worth noting that, while having a 3,4-dioxygenated cinnamoyl moiety as in the ferulate ester analogues **19a** and **19**, the caffeate ester analogues **20a** and **20** were much less active. This finding implied that the methyl ether function at the 3-position of the ferulate moiety contributed to high antimycobacterial activity in the ursolic acid group.

The results indicated that introduction of the *p*-coumarate moiety at the C-3 position of betulinic acid (**1**), oleanolic acid (**2**) and ursolic acid (**3**) resulted in an 8-fold increase in antimycobacterial activity of the parent triterpenes **1** and **2**, and a 2-fold increase in this activity of the triterpene **3**. Introduction of a methoxyl group at the 3-position of the *p*-coumarate moiety to give the ferulate moiety caused further 2-fold increase in activity in the ursane group. Thus the presence of the ferulate ester group at the 3-hydroxyl position of ursolic acid (**3**) to afford **19a** resulted in a 4-fold increase in antimycobacterial activity. The compound **19a** constitutes an interesting potential lead for the development of a new

antimycobacterial drug, although an esterase-resistant ester bioisosteric replacement would probably need to be introduced. The presence of the ferulate moiety resulted in a 4-fold increase in activity of compound **2**. In contrast to this, the introduction of the ferulate ester in the triterpene **1** caused a 4-fold decrease in antimycobacterial activity. In all three triterpene groups, the *p*-chloro group decreased the activity of the cinnamate esters. The unsubstituted cinnamate and its *p*-methoxyl and *p*-chloro analogues decreased the activity. Within the cinnamate esters tested, the *p*-hydroxyl group contributed to high antimycobacterial activity, while methylation and acetylation of the phenolic hydroxyl group, with the exception of the caffeate esters, decreased activity.

Experimental

General Procedures Melting points were determined using a Galenkamp melting point apparatus and were uncorrected. IR spectra were recorded in KBr on a Perkin-Elmer FT-IR Spectrum BX spectrophotometer. ¹H-NMR spectra were recorded on a Bruker AVANCE 400 spectrometer. Mass spectra were obtained using a Finnigan LC-Q mass spectrometer. High resolution mass spectra were obtained using a Finnigan MAT 90, a Bruker micrOTOF and a Micromass Q-ToF 2 mass spectrometer. Column chromatography and TLC were carried out using Merck silica gel 60 (<0.063 mm) and precoated silica gel 60 F₂₅₄ plates, respectively. Betulinic acid (**1**) was isolated from *Ziziphus cambodiana*²¹ whereas oleanolic acid (**2**) and ursolic acid (**3**) were purchased from Sigma-Aldrich, Inc. Unless indicated otherwise, TLC of the triterpenes and analogues was run with CH₂Cl₂-MeOH (80:1) as developing solvent and compounds were detected under UV light (254 nm) and by spraying with anisaldehyde-H₂SO₄ reagent followed by heating. Solvent ratios were all v/v. All purified synthetic analogues were homogeneous on TLC, and ¹H-NMR spectra indicated >95% purity. The *R_f* values for compounds **1**, **2** and **3** are 0.28, 0.20 and 0.24, respectively. References to the literature are given for known synthesized compounds.

Procedure for the Preparation of Cinnamate Ester Analogues of

Triterpenes To a stirred triterpene (0.03 mmol) solution in dry benzene (2 ml) was added 4–8 eq of the cinnamic acid chloride and 4–5 eq of DMAP and the solution was stirred at 60 °C for 0.5–1 h. Except for the commercially available cinnamoyl chloride, all the other acid chlorides were prepared by reacting the corresponding carboxylic acids with oxalyl chloride at reflux for 2 h; the remaining oxalyl chloride was distilled off and the residue was dissolved in dry benzene (1 ml). After the completion of the esterification, water was added and the solution mixture was extracted three times with EtOAc. The combined organic layer was washed with water, and then dried over anhydrous Na₂SO₄. The solvent was then evaporated and the residue chromatographed on a column by elution with CH₂Cl₂-MeOH (80:2).

3-*O*-(*E*)-Cinnamoylbetulinic Acid (**4**): 97% yield from compound **1**; amorphous solid from MeOH, mp >300 °C (lit.²³ 320 °C); *R_f*=0.54; IR (KBr) cm⁻¹: 3247, 2941, 2869, 1725, 1696, 1450, 1296, 1191, 1005, 982; ¹H-NMR data (CDCl₃) were consistent with the reported values²³; ES-MS *m/z*: 609 [M+Na]⁺.

3-*O*-(*E*)-Cinnamoyloleanolic Acid (**10**): 72% yield from compound **2**; amorphous solid from MeOH, mp 251–253 °C; *R_f*=0.42; IR (KBr) cm⁻¹: 3614, 2945, 2870, 1713, 1693, 1462, 1450, 1365, 1305, 1280, 1203, 1173, 1144; ¹H-NMR (400 MHz, CDCl₃+4 drops CD₃OD) δ: 0.76, 0.90, 0.90, 0.91, 0.92, 0.96, 1.13 (each 3H, each s, CH₃), 2.80 (1H, br d, *J*=10.2 Hz, H-18), 4.63 (1H, dd, *J*=8.5, 7.4 Hz, H-3), 5.27 (1H, br s, H-12), 6.43 (1H, d, *J*=16.0 Hz, H-2'), 7.36 (3H, br s, H-3''–H-5''), 7.51 (2H, br s, H-2' and H-6'), 7.65 (1H, d, *J*=16.0 Hz, H-3'); ES-MS *m/z*: 609 [M+Na]⁺; HR-FAB-MS (–ve) *m/z*: 585.3942 [M–H][–] (Calcd for C₃₉H₅₄O₄–H: 585.3944).

3-*O*-(*E*)-Cinnamoylursolic Acid (**16**): 78% yield from compound **3**; amorphous solid from MeOH, mp 255–257 °C (lit.²³ 258 °C); *R_f*=0.50; IR (KBr) cm⁻¹: 3500, 2971, 2926, 1709, 1693, 1638, 1450, 1303, 1280, 1204, 1172, 1002, 767; ¹H-NMR data (CDCl₃) were consistent with the reported values²³; ES-MS *m/z*: 609 [M+Na]⁺.

3-*O*-(*E*)-*p*-Acetoxycinnamoylbetulinic Acid (**5**): 61% yield from compound **1**; amorphous solid from MeOH, mp 141–143 °C; *R_f*=0.48; IR (KBr) cm⁻¹: 3301, 2946, 1734, 1700, 1636, 1508, 1458, 1374, 1246, 1201, 1166, 1014, 980; ¹H-NMR (400 MHz, CDCl₃) δ: 0.81, 0.82, 0.86, 0.91, 0.95 (each 3H, each s, CH₃), 1.67 (3H, s, CH₃-30), 2.29 (3H, s, OAc), 2.98 (1H,

m, H-19), 4.45 (1H, m, H-3), 4.59 (1H, brs, H-29a), 4.72 (1H, brs, H-29b), 6.37 (1H, d, $J=16.0$ Hz, H-2'), 7.10 (2H, d, $J=8.0$ Hz, H-3" and H-5"), 7.52 (2H, d, $J=8.0$ Hz, H-2" and H-6"), 7.61 (1H, d, $J=16.0$ Hz, H-3'); ES-MS m/z : 667 [M+Na]⁺; HR-ES-TOF-MS (-ve): m/z 643.3987 [M-H]⁻ (Calcd for C₄₁H₅₆O₆-H: 643.4004).

3-*O*-(*E*)-*p*-Acetoxycinnamoyloleanolic Acid (**11**): 62% yield from compound **2**; needles from MeOH, mp 269–271 °C; $R_f=0.36$; IR (KBr) cm⁻¹: 3289, 2945, 2855, 1769, 1700, 1634, 1507, 1459, 1368, 1307, 1280, 1203, 1166, 1016, 912, 836; ¹H-NMR data (CDCl₃) were consistent with the reported values²⁴; ES-MS m/z : 667 [M+Na]⁺.

3-*O*-(*E*)-*p*-Acetoxycinnamoylursolic Acid (**17**): 87% yield from compound **3**; amorphous solid from MeOH, mp >300 °C; $R_f=0.42$; IR (KBr) cm⁻¹: 3420, 2926, 1769, 1691, 1678, 1508, 1462, 1371, 1313, 1277, 1248, 1205, 1167, 1017, 833; ¹H-NMR data (CDCl₃) were consistent with the reported values²⁵; ES-MS m/z : 667 [M+Na]⁺.

3-*O*-(*E*)-*p*-Methoxycinnamoylbetulinic Acid (**6**): 96% yield from compound **1**; amorphous solid from CH₂Cl₂-MeOH, mp >300 °C (lit.²³ 320 °C); $R_f=0.55$; IR (KBr) cm⁻¹: 3217, 2942, 1725, 1686, 1665, 1638, 1604, 1513, 1459, 1304, 1252, 1172, 1035, 977, 826; ¹H-NMR data (CDCl₃) were consistent with the reported values^{23,20}; ES-MS m/z : 615 [M-H]⁻.

3-*O*-(*E*)-*p*-Methoxycinnamoyloleanolic Acid (**12**): 81% yield from compound **2**; amorphous solid from CH₂Cl₂-MeOH, mp 292–293 °C; $R_f=0.45$; IR (KBr) cm⁻¹: 3356, 2930, 2854, 1707, 1636, 1604, 1513, 1451, 1253, 1170, 1020; ¹H-NMR (400 MHz, CDCl₃+4 drops CD₃OD) δ: 0.80, 0.89, 0.90, 0.92, 0.92, 0.94, 1.14 (each s, each 3H, CH₃), 2.81 (1H, d, $J=10.2$ Hz, H-18), 3.82 (3H, s, OCH₃), 4.61 (1H, t, $J=8.0$ Hz, H-3), 5.30 (1H, brs, H-12), 6.29 (1H, d, $J=15.9$ Hz, H-2'), 6.88 (2H, d, $J=8.7$ Hz, H-3" and H-5"), 7.46 (2H, d, $J=8.7$ Hz, H-2" and H-6"), 7.60 (1H, d, $J=15.9$ Hz, H-3'); ES-MS m/z : 615 [M-H]⁻; HR-ES-TOF-MS (-ve): m/z : 615.4059 [M-H]⁻ (Calcd for C₄₀H₅₆O₅-H: 615.4055).

3-*O*-(*E*)-*p*-Methoxycinnamoylursolic Acid (**18**): 86% yield from compound **3**; amorphous solid from CH₂Cl₂-MeOH, mp 283–284 °C (lit.²³ 267 °C); $R_f=0.51$; IR (KBr) cm⁻¹: 3622, 2929, 2855, 1707, 1636, 1604, 1513, 1253, 1170, 1024; ¹H-NMR data (CDCl₃) were consistent with the reported values²³; ES-MS m/z : 615 [M-H]⁻.

3-*O*-(*E*)-(4-*O*-Acetylferuloyl)betulinic Acid (**7**): 59% yield from compound **1**; amorphous solid from MeOH, mp 187–188 °C; $R_f=0.45$; IR (KBr) cm⁻¹: 3394, 2946, 1768, 1705, 1687, 1638, 1600, 1513, 1453, 1375, 1259, 1192, 1173, 1155, 1033, 979, 883; ¹H-NMR (400 MHz, CDCl₃) δ: 0.80 (1H, d, $J=4.9$ Hz, H-5), 0.84, 0.85, 0.88, 0.91, 0.94 (each 3H, each s, CH₃), 1.66 (3H, s, CH₃-30), 2.29 (3H, s, OAc), 2.97 (1H, m, H-19), 3.83 (3H, s, OCH₃), 4.57 (1H, brs, H-3), 4.58 (1H, brs, H-29a), 4.70 (1H, brs, H-29b), 6.34 (1H, d, $J=16.0$ Hz, H-2'), 7.01 (1H, d, $J=8.0$ Hz, H-5"), 7.07 (1H, brs, H-2"), 7.08 (1H, brd, $J=8.0$ Hz, H-6"), 7.57 (1H, d, $J=16.0$ Hz, H-3'); ES-MS m/z : 697 [M+Na]⁺; HR-ES-TOF-MS (-ve) m/z : 673.4121 [M-H]⁻ (Calcd for C₄₂H₅₆O₆-H: 673.4110).

3-*O*-(*E*)-(4-*O*-Acetylferuloyl)oleanolic Acid (**13**): 68% yield from compound **2**; amorphous solid from MeOH, mp 176–177 °C; $R_f=0.32$; IR (KBr) cm⁻¹: 3310, 2946, 2870, 1771, 1700, 1637, 1601, 1509, 1466, 1368, 1259, 1198, 1157, 1123, 1011, 902; ¹H-NMR (400 MHz, CDCl₃) δ: 0.75, 0.890, 0.894, 0.91, 0.92, 0.95, 1.13 (each 3H, each s, CH₃), 2.30 (3H, s, OAc), 2.81 (1H, dd, $J=10.1$, 3.4 Hz, H-18), 3.84 (3H, s, OCH₃), 4.62 (1H, t, $J=8.0$ Hz, H-3), 5.27 (1H, brs, H-12), 6.36 (1H, d, $J=16.0$ Hz, H-2'), 7.02 (1H, d, $J=7.9$ Hz, H-5"), 7.08 (1H, brs, H-2"), 7.09 (1H, brd, $J=8.4$ Hz, H-6"), 7.59 (1H, d, $J=16.0$ Hz, H-3'); HR-FAB-MS (-ve) m/z : 673.4108 [M-H]⁻ (Calcd for C₄₂H₅₆O₇-H: 673.4104).

3-*O*-(*E*)-(4-*O*-Acetylferuloyl)ursolic Acid (**19**): 63% yield from compound **3**; amorphous solid from MeOH, mp 202–204 °C; $R_f=0.39$; IR (KBr) cm⁻¹: 3427, 2926, 1772, 1686, 1654, 1636, 1560, 1508, 1458, 1260, 1199, 1034; ¹H-NMR (400 MHz, CDCl₃) δ: 0.80, 0.91, 0.93, 0.98, 1.09 (each 3H, each s, CH₃), 0.87 (3H, d, $J=6.1$ Hz, H-29), 0.94 (3H, d, partially obscured signal, H-30), 2.19 (1H, dd, $J=10.1$, 3.4 Hz, H-18), 2.32 (3H, s, OAc), 3.86 (3H, s, OCH₃), 4.64 (1H, t, $J=8.1$ Hz, H-3), 5.26 (1H, brs, H-12), 6.37 (1H, d, $J=15.9$ Hz, H-2'), 7.04 (1H, d, $J=7.9$ Hz, H-5"), 7.10 (1H, brs, H-2"), 7.11 (1H, brd, $J=7.9$ Hz, H-6"), 7.61 (1H, d, $J=15.9$ Hz, H-3'); HR-FAB-MS (-ve) m/z : 673.4105 [M-H]⁻ (Calcd for C₄₂H₅₈O₇-H: 673.4104).

3-*O*-(*E*)-(3,4-Di-*O*-acetylcaffeoyl)betulinic Acid (**8**): 66% yield from compound **1**; powder from MeOH, mp 218–220 °C; $R_f=0.44$; IR (KBr) cm⁻¹: 3443, 2929, 1776, 1688, 1640, 1504, 1454, 1379, 1205, 1176, 1111, 1016, 902; ¹H-NMR (400 MHz, CDCl₃) δ: 0.80 (1H, obscured signal, H-5), 0.86, 0.86, 0.88, 0.93, 0.97 (each 3H, each s, CH₃), 1.68 (3H, s, CH₃-30), 2.28 (3H, s, OAc), 2.29 (3H, s, OAc), 2.98 (1H, m, H-19), 4.58 (1H, m, H-3), 4.60 (1H, brs, H-29a), 4.72 (1H, brs, H-29b), 6.35 (1H, d, $J=16.0$ Hz,

H-2'), 7.18 (1H, d, $J=8.4$ Hz, H-5"), 7.34 (1H, d, $J=1.8$ Hz, H-2"), 7.38 (1H, dd, $J=8.4$, 1.8 Hz, H-6"), 7.56 (1H, d, $J=16.0$ Hz, H-3'); HR-ES-TOF-MS (+ve) m/z : 725.4029 [M+Na]⁺ (Calcd for C₄₃H₅₈O₈+Na: 725.4023).

3-*O*-(*E*)-(3,4-Di-*O*-acetylcaffeoyl)oleanolic Acid (**14**): 62% yield from compound **2**; powder from MeOH, mp 211–213 °C; $R_f=0.31$; IR (KBr) cm⁻¹: 3429, 2944, 1780, 1695, 1640, 1505, 1462, 1367, 1204, 1179, 1110, 1028, 997; ¹H-NMR (400 MHz, CDCl₃) δ: 0.77, 0.89, 0.89, 0.91, 0.92, 0.95, 1.13 (each 3H, each s, CH₃), 2.27 (3H, s, OAc), 2.28 (3H, s, OAc), 2.81 (1H, brd, $J=10.1$ Hz, H-18), 4.61 (1H, dd, $J=8.6$, 7.4 Hz, H-3), 5.28 (1H, brs, H-12), 6.36 (1H, d, $J=16.0$ Hz, H-2'), 7.19 (1H, d, $J=8.4$ Hz, H-5"), 7.34 (1H, d, $J=1.8$ Hz, H-2"), 7.38 (1H, dd, $J=8.4$, 1.8 Hz, H-6"), 7.57 (1H, d, $J=16.0$ Hz, H-3'); HR-ES-TOF-MS (+ve) m/z : 725.4030 [M+Na]⁺ (Calcd for C₄₃H₅₈O₈+Na: 725.4023).

3-*O*-(*E*)-(3,4-Di-*O*-acetylcaffeoyl)ursolic Acid (**20**): 60% yield from compound **3**; amorphous solid; $R_f=0.40$; IR (KBr) cm⁻¹: 3452, 2930, 1777, 1693, 1636, 1503, 1457, 1372, 1205, 1111, 1045, 1017, 902; ¹H-NMR (400 MHz, CDCl₃) δ: 0.80, 0.88, 0.90, 0.96, 1.07 (each 3H, each s, CH₃), 0.87 (3H, d, $J=6.1$ Hz, H-29), 0.95 (3H, d, partially obscured signal, H-30), 2.14 (1H, brd, $J=11.6$ Hz, H-18), 2.28 (2×3H, s, 2×OAc), 4.61 (1H, dd, $J=8.9$, 6.9 Hz, H-3), 5.32 (1H, m, H-12), 6.36 (1H, d, $J=16.0$ Hz, H-2'), 7.20 (1H, d, $J=8.4$ Hz, H-5"), 7.36 (1H, brs, H-2"), 7.38 (1H, dd, $J=8.4$, 1.9 Hz, H-6"), 7.57 (1H, d, $J=16.0$ Hz, H-3'); HR-ES-TOF-MS (+ve) m/z : 725.4029 [M+Na]⁺ (Calcd for C₄₃H₅₈O₈+Na: 725.4023).

3-*O*-(*E*)-*p*-Chlorocinnamoylbetulinic Acid (**9**): 67% yield from compound **1**; amorphous solid from MeOH, mp >300 °C; $R_f=0.54$; IR (KBr) cm⁻¹: 3232, 2939, 1725, 1642, 1492, 1462, 1328, 1305, 1196, 1014, 981; ¹H-NMR (400 MHz, CDCl₃+4 drops CD₃OD) δ: 0.84, 0.85, 0.87, 0.92, 0.95 (each 3H, each s, CH₃), 1.66 (3H, s, CH₃-30), 2.96 (1H, m, H-19), 4.57 (1H, m, H-3), 4.58 (1H, brs, H-29a), 4.70 (1H, brs, H-29b), 6.38 (1H, d, $J=16.0$ Hz, H-2'), 7.32 (2H, d, $J=8.4$ Hz, H-3" and H-5"), 7.43 (2H, d, $J=8.4$ Hz, H-2" and H-6"), 7.56 (1H, d, $J=16.0$ Hz, H-3'); ES-MS m/z : 643 [M+Na]⁺; HR-ES-TOF-MS (-ve) m/z : 655.3319 [M+Cl]⁻ (Calcd for C₃₉H₅₃ClO₄+Cl: 655.3326).

3-*O*-(*E*)-*p*-Chlorocinnamoyloleanolic Acid (**15**): 65% yield from compound **2**; amorphous solid from CH₂Cl₂-MeOH, mp >300 °C; $R_f=0.44$; IR (KBr) cm⁻¹: 3461, 2942, 1716, 1689, 1639, 1491, 1465, 1303, 1274, 1202, 1176, 1093, 1010; ¹H-NMR (400 MHz, CDCl₃+4 drops CD₃OD) δ: 0.73, 0.84, 0.84, 0.86, 0.86, 0.89, 1.08 (each 3H, each s, CH₃), 4.56 (1H, t, $J=7.8$ Hz, H-3), 5.22 (1H, brs, H-12), 6.35 (1H, d, $J=16.0$ Hz, H-2'), 7.29 (2H, d, $J=8.3$ Hz, H-3" and H-5"), 7.40 (2H, d, $J=8.3$ Hz, H-2" and H-6"), 7.54 (1H, d, $J=16.0$ Hz, H-3'); ES-MS m/z : 643 [M+Na]⁺; HR-ES-TOF-MS (-ve) m/z : 655.3328 [M+Cl]⁻ (Calcd for C₃₉H₅₃ClO₄+Cl: 655.3326).

3-*O*-(*E*)-*p*-Chlorocinnamoylursolic Acid (**21**): 66% yield from compound **3**; amorphous solid from CH₂Cl₂-MeOH, mp >300 °C; $R_f=0.51$; IR (KBr) cm⁻¹: 3404, 2927, 1716, 1696, 1637, 1458, 1274, 1172, 1093, 1014, 826; ¹H-NMR (400 MHz, CDCl₃) δ: 0.78, 0.89, 0.91, 0.97, 1.07 (each 3H, each s, CH₃), 0.85 (3H, d, $J=6.2$ Hz, CH₃-29), 0.93 (3H, d, partially obscured signal, CH₃-30), 2.18 (1H, d, $J=11.0$ Hz, H-18), 4.61 (1H, t, $J=7.8$ Hz, H-3), 5.24 (1H, m, H-12), 6.39 (1H, d, $J=16.0$ Hz, H-2'), 7.33 (2H, d, $J=8.5$ Hz, H-3" and H-5"), 7.44 (2H, d, $J=8.5$ Hz, H-2" and H-6"), 7.59 (1H, d, $J=16.0$ Hz, H-3'); ES-MS m/z : 643 [M+Na]⁺; HR-ES-TOF-MS (-ve) m/z : 655.3308 [M+Cl]⁻ (Calcd for C₃₉H₅₃ClO₄+Cl: 655.3326).

Procedure for the Deacetylation of Acetoxycinnamic Acid Esters of Triterpenes To a stirred solution of the acetoxycinnamic acid ester (0.02 mmol) in MeOH (1 ml) was added excess 10% aqueous K₂CO₃ (0.5 ml) and the solution was stirred at ambient temperature for 30 min. Water (20 ml) was added and the solution mixture was acidified with 5% aqueous HCl and extracted three times with EtOAc (3×20 ml) and the combined organic phase was then washed with water (1×20 ml). The organic layer was dried over anhydrous Na₂SO₄; the solvent was then evaporated and the residue chromatographed by elution with CH₂Cl₂-MeOH (80 : 2).

3-*O*-(*E*)-*p*-Coumaroylbetulinic Acid (**5a**): 92% yield from compound **5**; amorphous solid from MeOH, mp 188–189 °C; $R_f=0.25$; IR (KBr) cm⁻¹: 3422, 2945, 1696, 1606, 1515, 1458, 1169, 1020, 668; ¹H-NMR data (CDCl₃+4 drops CD₃OD) were consistent with the reported values²⁷; ES-MS m/z : 625 [M+Na]⁺.

3-*O*-(*E*)-*p*-Coumaroyloleanolic Acid (**11a**): 89% yield from compound **11**; amorphous solid from MeOH, mp >300 °C; $R_f=0.18$; IR (KBr) cm⁻¹: 3348, 2949, 1706, 1606, 1516, 1458, 1270, 1181, 1010; ¹H-NMR data (CDCl₃+4 drops CD₃OD) were consistent with the reported values²⁴; ES-MS m/z : 625 [M+Na]⁺.

p-Coumaroylursolic Acid (**17a**): 96% yield from compound **17**; amorphous solid from MeOH, mp >300 °C; $R_f=0.23$; IR (KBr) cm⁻¹: 3348, 2925, 1707, 1690, 1606, 1516, 1458, 1371, 1277, 1168, 1100, 1017, 826;

¹H-NMR data (CDCl₃+4 drops CD₃OD) were consistent with the reported values^{25,27,28}; ES-MS *m/z*: 625 [M+Na]⁺.

3-*O*-(*E*)-Feruloylbutelnic Acid (Lawsonic Acid) (**7a**): 96% yield from compound **7**; amorphous solid from MeOH, mp 289–291 °C (lit.²⁹) 299–300 °C); *R*_f=0.32; IR (KBr) cm⁻¹: 3532, 2940, 1702, 1638, 1606, 1515, 1464, 1429, 1376, 1319, 1267, 1207, 1169, 1157, 1036, 974, 887, 817; ¹H-NMR data (CDCl₃) were consistent with the reported values³⁰; ES-MS *m/z*: 655 [M+Na]⁺.

3-*O*-(*E*)-Feruloyloleanolic Acid (Scaphopetalumate) (**13a**): 80% yield from compound **13**; amorphous solid from MeOH, mp 160–162 °C (lit.³¹) amorphous powder); *R*_f=0.20; IR (KBr) cm⁻¹: 3420, 2928, 1702, 1516, 1458, 1267, 1169, 1035; ¹H-NMR data (CDCl₃) were consistent with the reported values³¹; ES-MS *m/z*: 655 [M+Na]⁺.

3-*O*-(*E*)-Feruloylursolic Acid (**19a**): 71% yield from compound **19**; amorphous solid from MeOH, mp 176–178 °C; *R*_f=0.31; IR (KBr) cm⁻¹: 3420, 2926, 1701, 1636, 1595, 1515, 1458, 1379, 1268, 1170, 1034; ¹H-NMR (400 MHz, CDCl₃) δ: 0.78, 0.90, 0.92, 0.97, 1.07 (each 3H, each s, CH₃), 0.85 and 0.93 (each 3H, d, *J*=6.2 Hz and d, *J*=ca. 7.0 Hz, CH₃-29 and CH₃-30), 2.17 (1H, br d, *J*=11.3 Hz, H-18), 3.91 (3H, s, OCH₃), 4.62 (1H, dd, *J*=8.7, 7.2 Hz, H-3), 5.24 (1H, br s, H-12), 6.27 (1H, d, *J*=15.9 Hz, H-2'), 6.89 (1H, d, *J*=8.2 Hz, H-5''), 7.02 (1H, br s, H-2''), 7.05 (1H, br d, *J*=8.2 Hz, H-6''), 7.57 (1H, d, *J*=15.9 Hz, H-3'); ES-MS *m/z*: 655 [M+Na]⁺; HR-FAB-MS (-ve) *m/z*: 631.3998 [M-H]⁻ (Calcd for C₄₀H₅₆O₆-H: 631.3999).

3-*O*-(*E*)-Caffeoylbutelnic Acid (**8a**): 79% yield from compound **8**; amorphous solid from EtOAc, mp >300 °C (lit.³²) >300 °C); *R*_f=0.04; *R*_f=0.40 (CH₂Cl₂-MeOH, 25:1); IR (KBr) cm⁻¹: 3436, 2943, 1687, 1645, 1620, 1531, 1450, 1352, 1281, 1218, 1175, 1121, 1043, 975, 850, 817; ¹H-NMR data (CDCl₃+3 drops CD₃OD) were consistent with the reported values³²; HR-ES-TOF-MS (+ve) *m/z*: 641.3818 [M+Na]⁺ (Calcd for C₃₉H₅₄O₆+Na: 641.3812).

3-*O*-(*E*)-Caffeoyloleanolic Acid (**14a**): 74% yield from compound **14**; powder from EtOAc, mp >300 °C (lit.³³) >300 °C); *R*_f=0.02; *R*_f=0.29 (CH₂Cl₂-MeOH, 25:1); IR (KBr) cm⁻¹: 3434, 2945, 1687, 1645, 1619, 1600, 1531, 1450, 1352, 1326, 1280, 1217, 1175, 1120, 1031, 975, 850, 817; ¹H-NMR data (CDCl₃+3 drops CD₃OD) were consistent with the reported values³³; HR-ES-TOF-MS (+ve) *m/z*: 641.3818 [M+Na]⁺ (Calcd for C₃₉H₅₄O₆+Na: 641.3812).

3-*O*-(*E*)-Caffeoylursolic Acid (**20a**): 67% yield from compound **20**; amorphous solid, mp >300 °C (lit.^{34,35}) 324–326 °C); *R*_f=0.03; *R*_f=0.33 (CH₂Cl₂-MeOH, 25:1); IR (KBr) cm⁻¹: 3435, 2928, 1687, 1645, 1619, 1532, 1450, 1376, 1280, 1218, 1175, 1121, 975, 817; ¹H-NMR (400 MHz, CDCl₃) δ: 0.78, 0.85, 0.88, 0.95, 1.05 (each 3H, each s, CH₃), 0.82 and 0.89 (each 3H, d, *J*=6.4 Hz and d, *J*=ca. 7.0 Hz, CH₃-29 and CH₃-30), 4.57 (1H, dd, *J*=8.6, 7.2 Hz, H-3), 5.21 (1H, br s, H-12), 6.18 (1H, d, *J*=16.0 Hz, H-2'), 6.78 (1H, d, *J*=8.1 Hz, H-5''), 6.91 (1H, br d, *J*=8.1 Hz, H-6''), 7.00 (1H, br s, H-2''), 7.48 (1H, d, *J*=16.0 Hz, H-3'); HR-ES-TOF-MS (+ve) *m/z*: 641.3818 [M+Na]⁺ (Calcd for C₃₉H₅₄O₆+Na: 641.3812).

Antimycobacterial Assay Antimycobacterial activity was assessed against *Mycobacterium tuberculosis* H₃₇Ra using the Microplate Alamar Blue Assay.²² This testing was undertaken by the National Center for Genetic Engineering and Biotechnology, Thailand. In our system, the standard drugs, kanamycin sulfate, isoniazid and rifampicin showed MIC values of 2.5, 0.06 and 0.004 μg/ml, respectively. The assay results are presented in Table 1.

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