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

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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* H_{37}Ra 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 reported[12)] 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-epioleanolic 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 (2α-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 K_2CO_3 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

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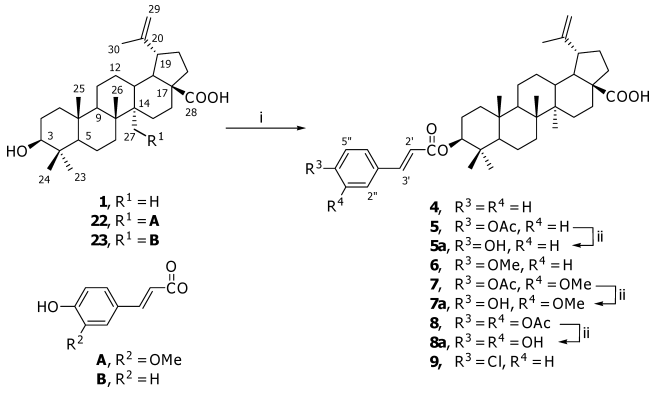

Chart 1. Synthesis of Cinnamate-Based Esters of Betulinic Acid (**1**)

Reagents and conditions: (i) RCOCl/DMAP/benzene, 60 °C, 0.5—1 h; (ii) 10% K_2CO_3/MeOH, 30 min.

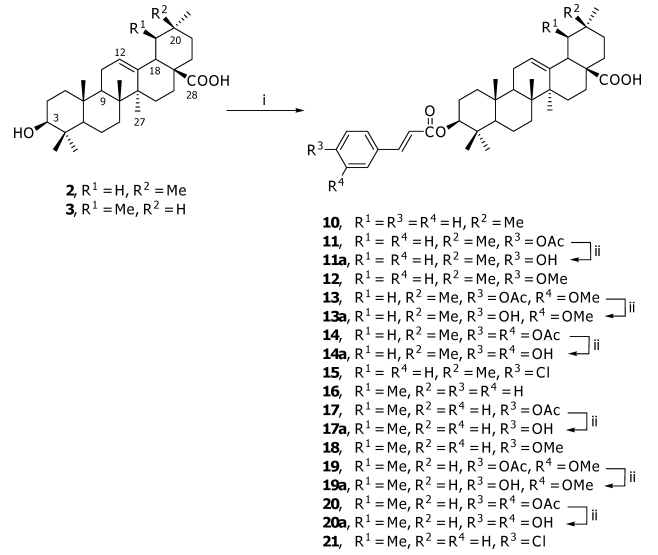

Chart 2. Synthesis of Cinnamate-Based Esters of Oleanolic Acid (**2**) and Ursolic Acid (**3**)

Reagents and conditions: (i) RCOCl/DMAP/benzene, 60 °C, 0.5—1 h; (ii) 10% K_2CO_3/MeOH, 30 min.

structures were established by spectroscopic (IR, ^{1}H-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* $H_{37}Ra$ 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 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).

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.

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 μg/ml respectively. Both the ferulate ester **19a** and its acetate **19** were highly active with equal MICs of 3.13 μg/ml. However, the corresponding caffeate ester **20a** and its acetate **20** were much less active, with respective MICs of 200 and 100 μg/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 μg/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 μg/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 μg/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 reported[6] 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 (IC_{50} 3.8 μg/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 Gallenkamp melting point apparatus and were uncorrected. IR spectra were recorded in KBr on a Perkin-Elmer FT-IR Spectrum BX spectrophotometer. ^{1}H-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 F_{254} 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 CH_2Cl_2–MeOH (80 : 1) as developing solvent and compounds were detected under UV light (254 nm) and by spraying with anisaldehyde–H_2SO_4 reagent followed by heating. Solvent ratios were all v/v. All purified synthetic analogues were homogeneous on TLC, and ^{1}H-NMR spectra indicated >95% purity. The *Rf* 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 Na_2SO_4. The solvent was then evaporated and the residue chromatographed on a column by elution with CH_2Cl_2–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); *Rf*=0.54; IR (KBr) cm^{-1}: 3247, 2941, 2869, 1725, 1696, 1450, 1296, 1191, 1005, 982; ^{1}H-NMR data ($CDCl_3$) were consistent with the reported values[23]; 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; *Rf*=0.42; IR (KBr) cm^{-1}: 3614, 2945, 2870, 1713, 1693, 1462, 1450, 1365, 1305, 1280, 1203, 1173, 1144; ^{1}H-NMR (400 MHz, $CDCl_3$+4 drops CD_3OD) δ: 0.76, 0.90, 0.90, 0.91, 0.92, 0.96, 1.13 (each 3H, each s, CH_3), 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 $C_{39}H_{54}O_4$−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); *Rf*=0.50; IR (KBr) cm^{-1}: 3500, 2971, 2926, 1709, 1693, 1638, 1450, 1303, 1280, 1204, 1172, 1002, 767; ^{1}H-NMR data ($CDCl_3$) were consistent with the reported values[23]; 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; *Rf*=0.48; IR (KBr) cm^{-1}: 3301, 2946, 1734, 1700, 1636, 1508, 1458, 1374, 1246, 1201, 1166, 1014, 980; ^{1}H-NMR (400 MHz, $CDCl_3$) δ: 0.81, 0.82, 0.86, 0.91, 0.95 (each 3H, each s, CH_3), 1.67 (3H, s, CH_3-30), 2.29 (3H, s, OAc), 2.98 (1H,

m, H-19), 4.45 (1H, m, H-3), 4.59 (1H, br s, H-29a), 4.72 (1H, br s, 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 $C_{41}H_{56}O_6$-H: 643.4004).

3-*O*-(*E*)-*p*-Acetoxycinnamoyloleanolic Acid (**11**): 62% yield from compound **2**; needles from MeOH, mp 269—271 °C; Rf=0.36; IR (KBr) cm^{-1}: 3289, 2945, 2855, 1769, 1700, 1634, 1507, 1459, 1368, 1307, 1280, 1203, 1166, 1016, 912, 836; ^{1}H-NMR data ($CDCl_3$) were consistent with the reported values[24]; 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; Rf=0.42; IR (KBr) cm^{-1}: 3420, 2926, 1769, 1691, 1678, 1508, 1462, 1371, 1313, 1277, 1248, 1205, 1167, 1017, 833; ^{1}H-NMR data ($CDCl_3$) were consistent with the reported values[25]; ES-MS m/z: 667 $[M+Na]^+$.

3-*O*-(*E*)-*p*-Methoxycinnamoylbetulinic Acid (**6**): 96% yield from compound **1**; amorphous solid from CH_2Cl_2–MeOH, mp >300 °C (lit.[23] 320 °C); Rf=0.55; IR (KBr) cm^{-1}: 3217, 2942, 1725, 1686, 1665, 1638, 1604, 1513, 1459, 1304, 1252, 1172, 1035, 977, 826; ^{1}H-NMR data ($CDCl_3$) were consistent with the reported values[23,26]; ES-MS m/z: 615 $[M-H]^-$.

3-*O*-(*E*)-*p*-Methoxycinnamoyloleanolic Acid (**12**): 81% yield from compound **2**; amorphous solid from CH_2Cl_2–MeOH, mp 292—293 °C; Rf=0.45; IR (KBr) cm^{-1}: 3356, 2930, 2854, 1707, 1636, 1604, 1513, 1451, 1253, 1170, 1020; ^{1}H-NMR (400 MHz, $CDCl_3$+4 drops CD_3OD) δ: 0.80, 0.89, 0.90, 0.92, 0.92, 0.94, 1.14 (each s, each 3H, CH_3), 2.81 (1H, d, J=10.2 Hz, H-18), 3.82 (3H, s, OCH_3), 4.61 (1H, t, J=8.0 Hz, H-3), 5.30 (1H, br s, 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 $C_{40}H_{56}O_5$−H: 615.4055).

3-*O*-(*E*)-*p*-Methoxycinnamoylursolic Acid (**18**): 86% yield from compound **3**; amorphous solid from CH_2Cl_2–MeOH, mp 283—284 °C (lit.[23] 267 °C); Rf=0.51; IR (KBr) cm^{-1}: 3622, 2929, 2855, 1707, 1636, 1604, 1513, 1253, 1170, 1024; ^{1}H-NMR data ($CDCl_3$) were consistent with the reported values[23]; 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; Rf=0.45; IR (KBr) cm^{-1}: 3394, 2946, 1768, 1705, 1687, 1638, 1600, 1513, 1453, 1375, 1259, 1192, 1173, 1155, 1033, 979, 883; ^{1}H-NMR (400 MHz, $CDCl_3$) 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, CH_3), 1.66 (3H, s, CH_3-30), 2.29 (3H, s, OAc), 2.97 (1H, m, H-19), 3.83 (3H, s, OCH_3), 4.57 (1H, br s, H-3), 4.58 (1H, br s, H-29a), 4.70 (1H, br s, 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, br s, H-2″), 7.08 (1H, br d, 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 $C_{42}H_{56}O_6$−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; Rf=0.32; IR (KBr) cm^{-1}: 3310, 2946, 2870, 1771, 1700, 1637, 1601, 1509, 1466, 1368, 1259, 1198, 1157, 1123, 1011, 902; ^{1}H-NMR (400 MHz, $CDCl_3$) δ: 0.75, 0.890, 0.894, 0.91, 0.92, 0.95, 1.13 (each 3H, each s, CH_3), 2.30 (3H, s, OAc), 2.81 (1H, dd, J=10.1, 3.4 Hz, H-18), 3.84 (3H, s, OCH_3), 4.62 (1H, t, J=8.0 Hz, H-3), 5.27 (1H, br s, 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, br s, H-2″), 7.09 (1H, br d, 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 $C_{42}H_{58}O_7$−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; Rf=0.39; IR (KBr) cm^{-1}: 3427, 2926, 1772, 1686, 1654, 1636, 1560, 1508, 1458, 1260, 1199, 1034; ^{1}H-NMR (400 MHz, $CDCl_3$) δ: 0.80, 0.91, 0.93, 0.98, 1.09 (each 3H, each s, CH_3), 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, OCH_3), 4.64 (1H, t, J=8.1 Hz, H-3), 5.26 (1H, br s, 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, br s, H-2″), 7.11 (1H, br d, 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 $C_{42}H_{58}O_7$−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; Rf=0.44; IR (KBr) cm^{-1}: 3443, 2929, 1776, 1688, 1640, 1504, 1454, 1379, 1205, 1176, 1111, 1016, 902; ^{1}H-NMR (400 MHz, $CDCl_3$) δ: 0.80 (1H, obscured signal, H-5), 0.86, 0.86, 0.88, 0.93, 0.97 (each 3H, each s, CH_3), 1.68 (3H, s, CH_3-30), 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, br s, H-29a), 4.72 (1H, br s, 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 $C_{43}H_{58}O_8$+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; Rf=0.31; IR (KBr) cm^{-1}: 3429, 2944, 1780, 1695, 1640, 1505, 1462, 1367, 1204, 1179, 1110, 1028, 997; ^{1}H-NMR (400 MHz, $CDCl_3$) δ: 0.77, 0.89, 0.89, 0.91, 0.92, 0.95, 1.13 (each 3H, each s, CH_3), 2.27 (3H, s, OAc), 2.28 (3H, s, OAc), 2.81 (1H, br d, J=10.1 Hz, H-18), 4.61 (1H, dd, J=8.6, 7.4 Hz, H-3), 5.28 (1H, br s, 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 $C_{43}H_{58}O_8$+Na: 725.4023).

3-*O*-(*E*)-(3,4-Di-*O*-acetylcaffeoyl)ursolic Acid (**20**): 60% yield from compound **3**; amorphous solid; Rf=0.40; IR (KBr) cm^{-1}: 3452, 2930, 1777, 1693, 1636, 1503, 1457, 1372, 1205, 1111, 1045, 1017, 902; ^{1}H-NMR (400 MHz, $CDCl_3$) δ: 0.80, 0.88, 0.90, 0.96, 1.07 (each 3H, each s, CH_3), 0.87 (3H, d, J=6.1 Hz, H-29), 0.95 (3H, d, partially obscured signal, H-30), 2.14 (1H, br d, 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, br s, 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 $C_{43}H_{58}O_8$+Na: 725.4023).

3-*O*-(*E*)-*p*-Chlorocinnamoylbetulinic Acid (**9**): 67% yield from compound **1**; amorphous solid from MeOH, mp >300 °C; Rf=0.54; IR (KBr) cm^{-1}: 3232, 2939, 1725, 1642, 1492, 1462, 1328, 1305, 1196, 1014, 981; ^{1}H-NMR (400 MHz, $CDCl_3$+4 drops CD_3OD) δ: 0.84, 0.85, 0.87, 0.92, 0.95 (each 3H, each s, CH_3), 1.66 (3H, s, CH_3-30), 2.96 (1H, m, H-19), 4.57 (1H, m, H-3), 4.58 (1H, br s, H-29a), 4.70 (1H, br s, 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 $C_{39}H_{53}ClO_4$+Cl: 655.3326).

3-*O*-(*E*)-*p*-Chlorocinnamoyloleanolic Acid (**15**): 65% yield from compound **2**; amorphous solid from CH_2Cl_2–MeOH, mp >300 °C; Rf=0.44; IR (KBr) cm^{-1}: 3461, 2942, 1716, 1689, 1639, 1491, 1465, 1303, 1274, 1202, 1176, 1093, 1010; ^{1}H-NMR (400 MHz, $CDCl_3$+4 drops CD_3OD) δ: 0.73, 0.84, 0.84, 0.86, 0.86, 0.89, 1.08 (each 3H, each s, CH_3), 4.56 (1H, t, J=7.8 Hz, H-3), 5.22 (1H, br s, 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 $C_{39}H_{53}ClO_4$+Cl: 655.3326).

3-*O*-(*E*)-*p*-Chlorocinnamoylursolic Acid (**21**): 66% yield from compound **3**; amorphous solid from CH_2Cl_2–MeOH, mp >300 °C; Rf=0.51; IR (KBr) cm^{-1}: 3404, 2927, 1716, 1696, 1637, 1458, 1274, 1172, 1093, 1014, 826; ^{1}H-NMR (400 MHz, $CDCl_3$) δ: 0.78, 0.89, 0.91, 0.97, 1.07 (each 3H, each s, CH_3), 0.85 (3H, d, J=6.2 Hz, CH_3-29), 0.93 (3H, d, partially obscured signal, CH_3-30), 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 $C_{39}H_{53}ClO_4$+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 K_2CO_3 (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 Na_2SO_4; the solvent was then evaporated and the residue chromatographed by elution with CH_2Cl_2–MeOH (80 : 2).

3-*O*-(*E*)-*p*-Coumaroylbetulinic Acid (**5a**): 92% yield from compound **5**; amorphous solid from MeOH, mp 188—189 °C; Rf=0.25; IR (KBr) cm^{-1}: 3422, 2945, 1696, 1606, 1515, 1458, 1169, 1020, 668; ^{1}H-NMR data ($CDCl_3$+4 drops CD_3OD) were consistent with the reported values[27]; 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; Rf=0.18; IR (KBr) cm^{-1}: 3348, 2949, 1706, 1606, 1516, 1458, 1270, 1181, 1010; ^{1}H-NMR data ($CDCl_3$+4 drops CD_3OD) were consistent with the reported values[24]; ES-MS m/z: 625 $[M+Na]^+$.

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

^{1}H-NMR data ($CDCl_3$+4 drops CD_3OD) were consistent with the reported values[25,27,28]; ES-MS *m/z*: 625 $[M+Na]^+$.

3-*O*-(*E*)-Feruloylbetulinic Acid (Lawsonic Acid) (**7a**): 96% yield from compound **7**; amorphous solid from MeOH, mp 289—291 °C (lit.[29] 299—300 °C); *Rf*=0.32; IR (KBr) cm^{-1}: 3532, 2940, 1702, 1638, 1606, 1515, 1464, 1429, 1376, 1319, 1267, 1207, 1169, 1157, 1036, 974, 887, 817; ^{1}H-NMR data ($CDCl_3$) were consistent with the reported values[30]; 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); *Rf*=0.20; IR (KBr) cm^{-1}: 3420, 2928, 1702, 1516, 1458, 1267, 1169, 1035; ^{1}H-NMR data ($CDCl_3$) were consistent with the reported values[31]; 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; *Rf*=0.31; IR (KBr) cm^{-1}: 3420, 2926, 1701, 1636, 1595, 1515, 1458, 1379, 1268, 1170, 1034; ^{1}H-NMR (400 MHz, $CDCl_3$) δ: 0.78, 0.90, 0.92, 0.97, 1.07 (each 3H, each s, CH_3), 0.85 and 0.93 (each 3H, d, *J*=6.2 Hz and d, *J*=*ca.* 7.0 Hz, CH_3-29 and CH_3-30), 2.17 (1H, br d, *J*=11.3 Hz, H-18), 3.91 (3H, s, OCH_3), 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 $C_{40}H_{56}O_6$−H: 631.3999).

3-*O*-(*E*)-Caffeoylbetulinic Acid (**8a**): 79% yield from compound **8**; amorphous solid from EtOAc, mp >300 °C (lit.[32] >300 °C); *Rf*=0.04; *Rf*=0.40 (CH_2Cl_2–MeOH, 25 : 1); IR (KBr) cm^{-1}: 3436, 2943, 1687, 1645, 1620, 1531, 1450, 1352, 1281, 1218, 1175, 1121, 1043, 975, 850, 817; ^{1}H-NMR data ($CDCl_3$+3 drops CD_3OD) were consistent with the reported values[32]; HR-ES-TOF-MS (+ve) *m/z*: 641.3818 $[M+Na]^+$ (Calcd for $C_{39}H_{54}O_6$+Na: 641.3812).

3-*O*-(*E*)-Caffeoyloleanolic Acid (**14a**): 74% yield from compound **14**; powder from EtOAc, mp >300 °C (lit.[33] >300 °C); *Rf*=0.02; *Rf*=0.29 (CH_2Cl_2–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; ^{1}H-NMR data ($CDCl_3$+3 drops CD_3OD) were consistent with the reported values[33]; HR-ES-TOF-MS (+ve) *m/z*: 641.3818 $[M+Na]^+$ (Calcd for $C_{39}H_{54}O_6$+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); *Rf*=0.03; *Rf*=0.33 (CH_2Cl_2–MeOH, 25 : 1); IR (KBr) cm^{-1}: 3435, 2928, 1687, 1645, 1619, 1532, 1450, 1376, 1280, 1218, 1175, 1121, 975, 817; ^{1}H-NMR (400 MHz, $CDCl_3$) δ: 0.78, 0.85, 0.88, 0.95, 1.05 (each 3H, each s, CH_3), 0.82 and 0.89 (each 3H, d, *J*=6.4 Hz and d, *J*=*ca.* 7.0 Hz, CH_3-29 and CH_3-30), 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 $C_{39}H_{54}O_6$+Na: 641.3812).

Antimycobacterial Assay Antimycobacterial activity was assessed against *Mycobacterium tuberculosis* H_{37}Ra 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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