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CONJUGATES OF SEVERAL LUPANE, OLEANANE, AND URSANE TRITERPENOIDS WITH THE ANTITUBERCULOSIS DRUG ISONIAZID AND PYRIDINECARBOXALDEHYDES

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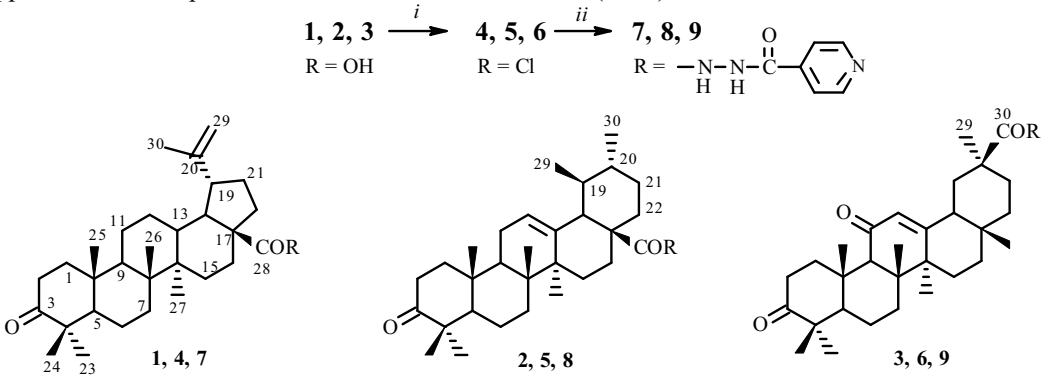
Conjugates of betulinic, oleanolic, ursolic, and glycyrrhetic acids and several of their derivatives with the antituberculosis drug isoniazid and 3- and 4-pyridinecarboxaldehydes were synthesized.

Keywords: triterpenoids, betulinic, oleanolic, ursolic, glycyrrhetic acids, 28-oxoallobetulone, conjugates, antituberculosis activity, isoniazid.

Betulinic, oleanolic, ursolic, and glycyrrhetic acids have been successfully modified in the last decades to produce new biologically active compounds [1–6] of various structures including hydrazides and hydrazones [4] with antiviral and antitumor activity. It was shown recently that functionalization of the diterpenoid oxo-acid isosteviol by N-containing groups increased its antituberculosis activity, reducing the minimal inhibitory concentrations (MIC) from 50 to 1.5 μg/mL [7]. Because antituberculosis activity is also typical of triterpenoids, namely betulin [8] and betulinic [8–11], oleanolic [10–13], and ursolic [10, 14] acids, it seemed interesting to perform an analogous investigation of the isoprenoid series by studying the effect of their functionalization by the same *N*-containing groups that were used for isosteviol [7].

Herein we report the synthesis of conjugates of betulinic, oleanolic, ursolic, and glycyrrhetic acids and 28-oxoallobetulone with the antituberculosis drug isoniazid (4-pyridinecarboxylic acid hydrazide) and 3- and 4-pyridinecarboxaldehydes.

Mixed hydrazides **7**–**9** (Scheme 1) were synthesized via reaction of acid chlorides **4**–**6**, which were prepared from triterpene acids **1**–**3**, with isoniazid by refluxing in $CHCl_3$ in analogy with the previous work [15]. The target products were isolated by column chromatography in 71–77% yields. The PMR spectra turned out to be sufficiently characteristic to establish their structures. They showed resonances for amide protons at weak field (δ 6.35 ppm, 8.15, 9.00–9.25, 10.15–10.90). Doubled resonances for the amide protons were noted in **7**. This indicated that conformational equilibrium around the C(O)–N bond was occurring in solution. Protons of the pyridine rings of **7**–**9** appeared as doublets at δ 7.56–7.68 (H-3′, H-5′) and 8.50–8.67 (H-2′, H-6′) ppm. ^{13}C NMR spectra exhibited resonances for C-28 (C-30) and C-7′ at δ 174.3–176.3 and 161.4–163.7 ppm.



i. $(COCl)_2/CHCl_3$, 25°C; *ii*. 4-pyridinecarboxylic acid hydrazide, $CHCl_3$, reflux

Scheme 1

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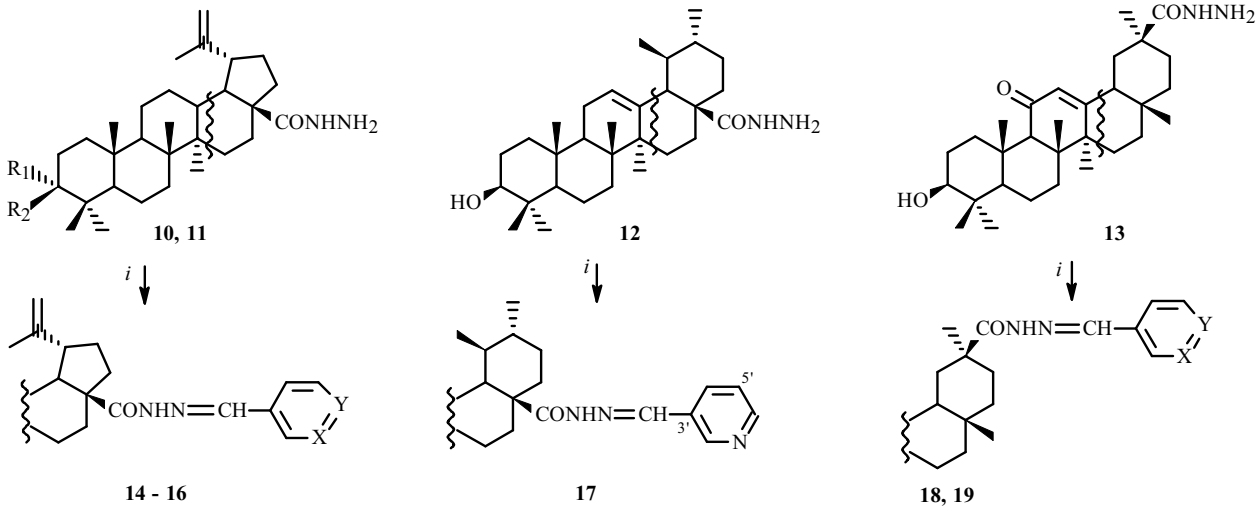
Pyridinylmethylidenehydrazides **14**–**19** (Scheme 2) were synthesized via reaction of the hydrazides of triterpene acids **10**–**13** with 3- or 4-pyridinecarboxyaldehydes by refluxing in EtOH in analogy with the previous work [16]. The products were isolated by column chromatography in 51–59% yields. Resonances of protons of C(28 or 30)ONH- and =C(7′)H-groups were observed at δ 8.20–10.40 and 8.00–8.70 ppm, respectively, in PMR spectra of **14**–**19**. The pyridine protons of **14**, **15**, **17**, and **18** were observed at weak field at 7.31–7.35 ppm (H-5′, multiplet), 8.14–8.22 (H-4′, doublet), 8.45–8.55 (H-6′, doublet), and 8.65–9.35 (H-2′, singlet). Protons H-3′, H-5′ and H-2′, H-6′ resonated in spectra of **16** and **19** as doublets at δ 7.39–7.50 and 8.63–8.70 ppm, respectively. ^{13}C NMR spectra of **18**–**27** contained resonances of C-28 (C-30) and C-7′ at δ 172.9–173.4 and 142.7–150.6 ppm, respectively.



10, 14: R_1 = H, R_2 = OH; **11, 15, 16:** $R_1 + R_2$ = NOH
14, 15: X = N, Y = CH; **16:** X = CH, Y = N

18: X = N, Y = CH
19: X = CH, Y = N

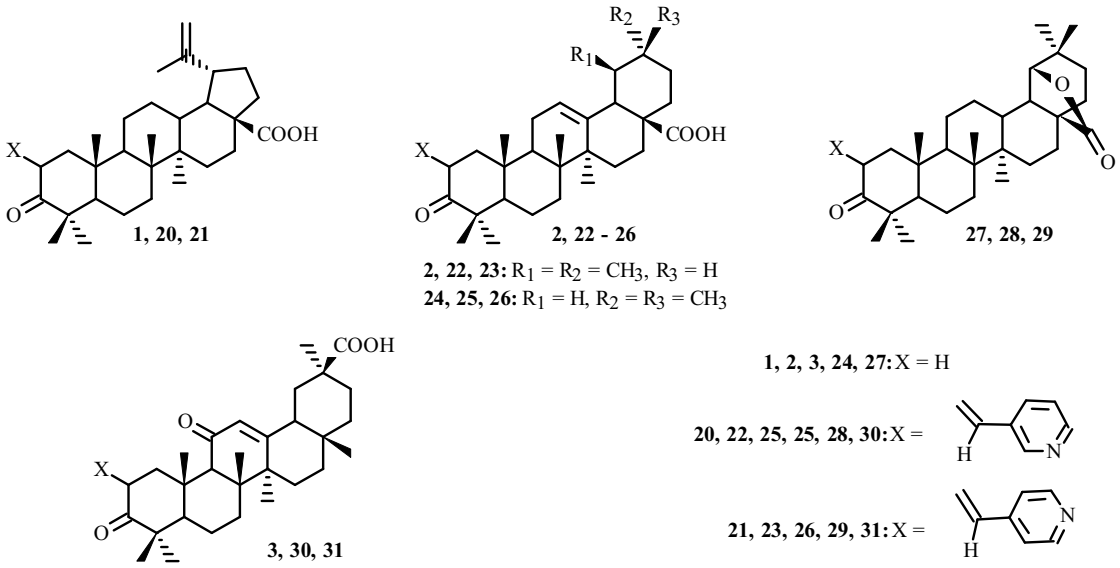
i. 3- or 4-pyridinecarboxaldehyde, EtOH, reflux

Scheme 2

Compounds **20**–**23**, **25**, **26**, and **28**–**31** (Scheme 3) with a pyridinylmethylidene substituent in the C-2 position were prepared via reaction of 3-oxotriterpenoids **1**–**3**, **24**, and **27** with 3- or 4-pyridinecarboxaldehydes in basic EtOH in analogy with the previous work [17] in 88–95% yields (after recrystallization). PMR spectra of **20**–**23**, **25**, **26**, and **28**–**31** had the resonance for the methylidene proton at δ 6.85–8.50 ppm. The pyridine protons in spectra of **20**, **22**, **25**, **28**, and **30** resonated as multiplets at δ 7.30–7.41 ppm (H-5′), doublets at 7.70–8.55 (H-4′), doublets at 7.72–8.62 (H-6′), and singlets at 8.65–8.70 ppm (H-2′). Spectra of **21**, **23**, **26**, **29**, and **31** showed doublets at δ 7.06–7.41 and 8.45–8.65 ppm corresponding to protons H-3′, H-5′ and H-2′, H-6′. ^{13}C NMR spectra of **20–23**, **25**, **26**, and **28–31** showed resonances of C-7′ at δ 136.2–144.1 ppm; of the pyridine C atoms, at weak field in the range from δ 121.8 to 181.9 ppm.

The antituberculosis activity of the synthesized compounds was evaluated preliminarily at the National Institute of Allergy and Infectious Diseases (USA, www.niaid-aacf.org). It was found that conjugates **7**–**9**, **15**–**18**, **20**, **22**, **23**, **25**, **26**, **30**, and **31** did not inhibit growth of *Mycobacterium tuberculosis* (strain H_{37}Rv *in vitro*) at MIC values <10 μg/mL. Unfortunately, the conditions of the preliminary screening did not provide for a study of the antituberculosis activity at MIC values >10 μg/mL. We were unable in this stage to evaluate the effect of covalent binding of isoniazid and pyridinecarboxaldehydes with triterpenoid betulinic, oleanolic, ursolic, and glycyrrhetic acids on the known inhibiting capability of the last (MIC = 12.5–100 μg/mL [8–14]). Therefore, syntheses of conjugates of triterpenoid metabolites with antituberculosis activity with synthetic mycostatics and studies of their antituberculosis activity will be continued.

Thus, we synthesized conjugates of several triterpenoid acids and their derivatives with the antituberculosis drug isoniazid and pyridinecarboxaldehydes in addition to 2-pyridinylmethylidene derivatives of 28-oxoallobetulone.



i. 3-pyridinecarboxaldehyde, 40% KOH/EtOH, 22°C; *ii*. 4-pyridinecarboxaldehyde, 40% KOH/EtOH, 22°C

Scheme 3

EXPERIMENTAL

PMR and ^{13}C NMR spectra were recorded in $CDCl_3$ with TMS internal standard on a Bruker AM-300 spectrometer (300 and 75.5 MHz, respectively). Melting points were determined on a Boetius microstage. Optical density was measured in a 1-dm tube on a Perkin–Elmer 241 MC polarimeter. TLC analysis used Sorbil plates (ZAO Sorbpolimer, Russia) and solvent system $CHCl_3$:EtOAc (40:1). Compounds were detected by H_2SO_4 solution (10%) with subsequent heating at 100–120°C for 2–3 min. We used oleanolic and ursolic acids and 3- and 4-pyridinecarboxaldehydes (Aldrich Chemical Co.). Betulonic acid (**1**) was synthesized from betulin by the literature method [18]. Ursolic and oleanolic acids were oxidized by Jones reagent in acetone to the 3-oxo derivatives **2** and **24** in analogy with previous work [18]. 3-Oxoglycyrrhetic acid (**3**) and 28-oxoallobetulone (**27**) were prepared as before [19, 20]. Constants of the starting compounds agreed with those published. Acid chlorides **4**–**6** were prepared by the standard method [15] via reaction with oxalylchloride in $CHCl_3$. Triterpene acid hydrazides **10**–**13** were synthesized via reaction of their acid chlorides with hydrazine hydrate in $CHCl_3$ as before [16]. Hydrazides of 3- and 4-pyridinecarboxylic acids were prepared by the literature method [21].

Synthesis of 7–9. A solution of freshly prepared acid chloride **4**–**6** (1 mmol) in anhydrous $CHCl_3$ (20 mL) was treated with the hydrazide of 4-pyridinecarboxylic acid (1.5 mmol), refluxed until the reaction was complete (TLC monitoring), and diluted with HCl solution (30 mL, 5%). The precipitate was filtered off, washed with H_2O, and dried. The product was chromatographed over a column with elution successively by C_6H_6 and $CHCl_3$.

17β-(4-Pyridinoylhydrazinocarbonyl)-3-oxolup-20(29)-ene (7). Yield 0.42 g (73%), mp 192–194°C, $[\alpha]_D^{20}$ +8° (*c* 0.1, $CHCl_3$), $C_{36}H_{51}O_3N_3$ (MW 573.81).

PMR spectrum ($CDCl_3$, δ, ppm, J/Hz): 0.80, 0.89, 0.90, 0.95, 0.95, 1.59 (18H, 6s, $6CH_3$), 1.10–2.50 (24H, m, CH_2, CH), 2.90 (1H, m, H-19), 4.50 (1H, s, H-29a), 4.60 (1H, s, H-29b), 7.60 (2H, d, J = 5.3, H-3′, H-5′), 8.50 (2H, d, J = 2.4, H-2′, H-6′), 8.15 (2H, br. signal, $C^{28}ONH$, $C^{7'}ONH$).

^{13}C NMR spectrum (δ, ppm): 14.5, 15.9, 19.3, 19.6, 21.0, 21.4, 25.6, 26.6, 29.4, 30.8, 33.1, 33.5, 34.1, 36.9, 37.7, 38.4, 39.0, 39.6, 40.7, 42.5, 46.7, 47.3, 49.9, 50.3, 55.0, 55.4, 109.7 (C-29), 121.2 (C-3′), 121.2 (C-5′), 138.9 (C-4′), 149.0 (C-20), 150.3 (C-2′), 150.3 (C-6′), 163.6 (C-7′), 176.3 ($\underline{C}^{28}ONH$), 218.1 (C-3).

17β-(4-Pyridinoylhydrazinocarbonyl)-3-oxours-12-ene (8). Yield 0.44 g (77%), mp 168–170°C, $[\alpha]_D^{20}$ +27° (*c* 0.1, $CHCl_3$), $C_{36}H_{51}O_3N_3$ (MW 573.81).

PMR spectrum ($CDCl_3$, δ, ppm, J/Hz): 0.77, 0.86, 0.88, 0.93, 1.01, 1.05, 1.09 (21H, 7s, $7CH_3$), 1.15–2.58 (23H, m, CH_2, CH), 5.50 (1H, s, H-12), 7.68 (2H, d, J = 3.3, H-3′, H-5′), 8.67 (2H, d, J = 3.3, H-2′, H-6′), 9.25 (1H, br. signal, $C^{28}ON\underline{H}$), 10.90 (1H, br. signal, $C^{7'}ON\underline{H}$).

^{13}C NMR spectrum (δ, ppm): 15.3, 16.4, 17.1, 19.6, 21.1, 21.5, 23.3, 23.6, 24.9, 26.5, 27.8, 30.7, 32.2, 34.1, 36.6, 36.9, 38.9, 39.3, 39.5, 39.5, 42.4, 46.8, 47.4, 47.7, 52.9, 55.2, 121.2 (C-12), 127.1 (C-3′), 127.1 (C-5′), 138.6 (C-4′), 138.9 (C-13), 150.3 (C-2′), 150.3 (C-6′), 161.4 (C-7′), 174.3 (C-28), 217.4 (C-3).

20β-(4-Pyridinoylhydrazinocarbonyl)-3,11-dioxoolean-12-ene (9). Yield 0.41 g (71%), mp 197°C, $[\alpha]_D^{20}$ +129° (*c* 0.1, $CHCl_3$), $C_{36}H_{49}O_4N_3$ (MW 587.79).

PMR spectrum ($CDCl_3$, δ, ppm, J/Hz): 0.62, 0.95, 0.99, 1.01, 1.13, 1.15, 1.25 (21H, 7s, $7CH_3$), 1.20–2.60 (20H, m, CH_2, CH), 2.80 (1H, m, H-18), 5.65 (1H, s, H-12), 7.56 (2H, d, J = 5.7, H-3′, H-5′), 8.57 (2H, d, J = 5.1, H-2′, H-6′), 9.00 (1H, br. signal, $C^{7'}ONH$), 10.15 (1H, br. signal, $C^{30}ONH$).

^{13}C NMR spectrum (δ, ppm): 15.6, 18.4, 18.7, 19.5, 21.3, 23.2, 26.3, 26.5, 28.4, 28.9, 31.2, 31.7, 32.0, 34.1, 36.6, 37.1, 39.7, 41.5, 43.2, 43.3, 45.2, 47.7, 47.9, 55.3, 61.0, 121.1 (C-3′), 121.1 (C-5′), 128.1 (C-12), 138.6 (C-4′), 150.4 (C-2′), 150.4 (C-6′), 163.7 (C-7′), 170.2 (C-13), 175.4 ($\underline{C}^{30}ONH$), 199.8 (C-11), 217.1 (C-3).

Synthesis of 14–19. A solution of the acid hydrazides **10**–**13** (1 mmol) in EtOH (30 mL) was treated with 3- or 4-pyridinecarboxaldehyde (1.3 mmol), refluxed until the reaction was complete (TLC monitoring), and poured into HCl solution (100 mL, 5%). The precipitate was filtered off, washed with H_2O until neutral, and dried. The product was chromatographed over a column with elution successively by $CHCl_3$ and $CHCl_3$:MeOH (50:1).

17β-(Pyridin-3-ylmethylidenehydrazinocarbonyl)-3β-hydroxylup-20(29)-ene (14). Yield 0.28 g (51%), mp 143–145°C, $[\alpha]_D^{20}$ –1° (*c* 0.1, $CHCl_3$), $C_{36}H_{53}O_2N_3$ (MW 559.82).

PMR spectrum ($CDCl_3$, δ, ppm, J/Hz): 0.69, 0.78, 0.90, 0.92, 0.96 (15H, 5s, $5CH_3$), 1.10–2.30 (25H, m, CH_2, CH), 1.65 (3H, s, $C^{30}\underline{H}_3$), 2.70 (1H, m, H-3), 4.30 (1H, br. signal, –O<u>H</u>), 4.56 (1H, s, H-29a), 4.69 (1H, s, H-29b), 7.35 (1H, dd, J = 7.85, 7.90, H-5′), 8.15 (1H, s, H-7′), 8.22 (1H, d, J = 7.90, H-4′), 8.45 (1H, d, J = 7.85, H-6′), 8.65 (1H, s, H-2′), 10.10 (1H, br. signal, $C^{28}ONH$).

^{13}C NMR spectrum (δ, ppm): 14.3, 15.0, 15.9, 17.5, 18.0, 19.0, 20.8, 25.4, 26.7, 27.6, 29.3, 30.4, 32.2, 33.7, 34.2, 36.9, 37.1, 38.6, 40.5, 42.1, 46.1, 50.3, 50.5, 54.9, 55.3, 57.2, 78.4 (C-3), 109.1 (C-29), 123.9 (C-5′), 130.9 (C-3′), 134.0 (C-4′), 142.7 (C-7′), 148.4 (C-20), 149.6 (C-2′), 150.5 (C-6′), 174.0 (<u>C</u>ONH).

17β-(Pyridin-3-ylmethylidenehydrazinocarbonyl)-3-hydroxyiminolup-20(29)-ene (15). Yield 0.31 g (55%), mp 195–197°C, $[\alpha]_D^{20}$ +5° (*c* 0.1, $CHCl_3$), $C_{36}H_{52}O_2N_4$ (MW 572.82).

PMR spectrum ($CDCl_3$, δ, ppm): 0.85, 0.95, 1.10, 1.35, 1.45, 1.70 (18H, 6s, $6CH_3$), 1.80–3.20 (25H, m, CH_2, CH), 4.60 (1H, s, H-29a), 4.70 (1H, s, H-29b), 7.30-9.00 (5H, m, H-2′, H-4′, H-5′, H-6′, H-7′), 9.92 (2H, br. signal, CON<u>H</u>, NO<u>H</u>).

^{13}C NMR spectrum (δ, ppm): 11.6, 14.6, 17.0, 19.4, 21.1, 23.0, 26.6, 27.1, 29.7, 29.8, 30.8, 34.2, 36.9, 37.2, 38.8, 40.3, 40.8, 42.5, 42.6, 43.7, 46.3, 47.4, 50.1, 50.4, 55.5, 55.9, 109.6 (C-29), 123.9 (C-5′), 130.5 (C-3′), 134.4 (C-4′), 143.6 (C-7′), 146.1 (C-20), 150.6 (C-6′), 159.8 (C-2′), 166.9 (C-3), 173.4 (<u>C</u>ONH).

17β-(Pyridin-4-ylmethylidenehydrazinocarbonyl)-3-hydroxyiminolup-20(29)-ene (16). Yield 0.33 g (58%), mp 185–190°C, $[\alpha]_D^{20}$ +8° (*c* 0.1, $CHCl_3$), $C_{36}H_{52}O_2N_4$ (MW 572.82).

PMR spectrum ($CDCl_3$, δ, ppm): 0.92, 0.98, 1.08, 1.31, 1.41, 1.62 (18H, 6s, $6CH_3$), 1.20–3.15 (25H, m, CH_2, CH), 4.60 (1H, s, H-29a), 4.70 (1H, s, H-29b), 7.50-8.71 (5H, m, H-2′, H-3′, H-5′, H-6′, H-7′), 10.00 (2H, br. signal, CON<u>H</u>, NO<u>H</u>).

^{13}C NMR spectrum (δ, ppm): 14.6, 15.9, 16.2, 17.4, 19.6, 21.0, 22.0, 22.9, 25.6, 26.6, 29.6, 29.8, 30.4, 30.7, 33.7, 34.1, 36.9, 39.6, 40.7, 42.3, 44.2, 46.2, 47.3, 50.1, 55.0, 58.0, 109.4 (C-29), 121.1 (C-3′), 121.1 (C-5′), 143.6 (C-7′), 144.3 (C-4′), 150.1 (C-2′), 150.1 (C-6′), 150.5 (C-20), 166.9 (C-3), 173.4 (<u>C</u>ONH).

17β-(Pyridin-3-ylmethylidenehydrazinocarbonyl)-3β-hydroxyurs-12-ene (17). Yield 0.33 g (59%), mp 186–188°C, $[\alpha]_D^{20}$ –2° (*c* 0.1, $CHCl_3$), $C_{36}H_{53}O_2N_3$ (MW 559.82).

PMR spectrum ($CDCl_3$, δ, ppm, J/Hz): 0.70, 0.72, 0.84, 0.86, 0.88, 0.95, 1.10 (21H, 7s, $7CH_3$), 1.20–2.10 (22H, m, CH_2, CH), 2.70 (1H, d, J = 11.5, H-18), 3.20 (1H, dd, J = 11.0, 4.1, H-3), 5.48 (1H, s, H-12), 7.31 (2H, m, H-5′, OH), 8.14 (1H, d, J = 7.8, H-4′), 8.20 (1H, s, CON<u>H</u>), 8.55 (1H, d, J = 4.1, H-6′), 8.70 (1H, s, H-7′), 9.35 (1H, s, H-2′).

^{13}C NMR spectrum (δ, ppm): 15.5, 15.6, 16.9, 17.2, 18.2, 21.2 23.4, 23.6, 25.1, 27.2, 27.9, 28.2, 30.8, 32.6, 36.9, 36.9, 38.7, 38.8, 39.0, 39.6, 39.7, 42.5, 47.5, 48.1, 53.4, 55.1, 78.8 (C-3), 123.7 (C-5′), 128.3 (C-12), 130.2 (C-3′), 133.8 (C-4′), 140.2 (C-13), 144.3 (C-7′), 149.4 (C-2′), 150.9 (C-6′), 174.9 (<u>C</u>ONH).

20β-(Pyridin-3-ylmethylidenehydrazinocarbonyl)-11-oxo-3β-hydroxyolean-12-ene (18). Yield 0.32 g (56%), mp 166–168°C, $[\alpha]_D^{20}$ +103° (*c* 0.1, $CHCl_3$), $C_{36}H_{51}O_3N_3$ (MW 573.81).

PMR spectrum ($CDCl_3$, δ, ppm, J/Hz): 0.80, 0.80, 1.00, 1.10, 1.10, 1.20, 1.40 (21H, 7s, $7CH_3$), 1.20–2.40 (20H, m, CH_2, CH), 2.70 (1H, d, J = 11.5, H-18), 3.20 (1H, br. signal, H-3), 5.78 (1H, s, H-12), 8.00–8.60 (5H, m, H-2′, H-4′, H-5′, H-6′, H-7′), 10.4 (2H, br. signal, –O<u>H</u>, CON<u>H</u>).

^{13}C NMR spectrum (δ, ppm): 15.5, 16.2, 17.4, 18.3, 18.6, 23.3, 26.3, 27.1, 28.1, 28.3, 29.0, 31.2, 31.8, 32.7, 37.0, 37.4, 39.1, 39.2, 41.1, 43.2, 45.4, 48.0, 54.9, 58.0, 61.8, 78.6 (C-3), 123.7 (C-5′), 128.3 (C-12), 130.1 (C-3′), 133.7 (C-4′), 144.5 (C-7′), 149.0 (C-2′), 150.5 (C-6′), 169.6 (C-13), 172.9 (CONH), 200.3 (C-11).

20β-(Pyridin-4-ylmethylidenehydrazinocarbonyl)-11-oxo-3β-hydroxyolean-12-ene (19). Yield 0.34 g (59%), mp 201°C, $[\alpha]_D^{20}$ +162° (*c* 0.1, $CHCl_3$), $C_{36}H_{51}O_3N_3$ (MW 573.81).

PMR spectrum ($CDCl_3$, δ, ppm): 0.78, 1.00, 1.05, 1.10, 1.21, 1.29, 1.34 (21H, 7s, $7CH_3$), 1.40–2.85 (21H, m, CH_2, CH), 5.78 (1H, s, H-12), 7.51 (2H, br.s, H-3′, H-5′), 8.21 (1H, s, H-7′), 8.52 (2H, br. signal, H-2′, H-6′), 10.26 (1H, br. signal, CONH).

^{13}C NMR spectrum (δ, ppm): 15.5, 18.4, 18.6, 21.3, 23.2, 26.3, 28.4, 28.5, 28.9, 31.2, 31.7, 32.0, 34.1, 36.6, 37.3, 39.7, 40.9, 43.1, 43.3, 43.5, 45.1, 47.7, 47.9, 55.3, 61.0, 121.4 (C-3′), 121.4 (C-5′), 128.2 (C-12), 141.6 (C-4′), 145.0 (C-7′), 149.9 (C-2′), 149.9 (C-6′), 170.3 (C-13), 173.0 (CONH), 199.9 (C-11), 217.2 (C-3).

Synthesis of 20–23, 25, 26, 28–31. A solution of **1**–**3**, **24**, or **27** (1 mmol) in EtOH (20 mL) was stirred, treated with 3- or 4-pyridinecarboxaldheyde (1.5 mmol) and KOH solution (2.5 mL, 40%) in EtOH, left for 1 d, and poured into HCl solution (100 mL, 5%). The precipitate was filtered off, washed with H_2O until neutral, dried, and crystallized from EtOH (MeOH).

3-Oxo-2-(pyridin-3-ylmethylideno)-lup-20(29)-en-28-oic Acid (20). Yield 0.48 g (89%), mp 183°C, $[\alpha]_D^{20}$ +17° (*c* 0.1, $CHCl_3$), $C_{36}H_{49}O_3N$ (MW 543.78).

PMR spectrum ($CDCl_3$, δ, ppm, J/Hz): 0.97, 1.02, 1.12, 1.14, 1.46, 1.72 (18H, 6s, $6CH_3$), 1.20–2.40 (21H, m, CH_2, CH), 3.00 (2H, m, H-19, H-1a), 4.61 (1H, s, H-29a), 4.71 (1H, s, H-29b), 7.41 (2H, m, H-5′, H-7′), 7.72 (1H, d, J = 7.88, H-4′), 8.54 (1H, d, J = 4.8, H-6′), 8.69 (1H, s, H-2′), 10.80 (1H, br. signal, COOH).

^{13}C NMR spectrum (δ, ppm); 14.5, 15.5, 15.8, 19.4, 20.3, 21.6, 22.3, 25.5, 29.4, 29.6, 30.5, 32.1, 33.0, 36.5, 37.0, 38.4, 40.5, 42.5, 44.4, 45.2, 46.8, 48.4, 49.1, 52.8, 56.3, 109.7 (C-29), 123.5 (C-5′), 132.0 (C-3′), 133.1 (C-4′), 136.6 (C-7′), 137.2 (C-2), 148.4 (C-2′), 150.5 (C-6′), 150.6 (C-20), 181.3 (COOH), 207.7 (C-3).

3-Oxo-2-(pyridin-4-ylmethylideno)-lup-20(29)-en-28-oic Acid (21). Yield 0.49 g (90%), mp 183°C, $[\alpha]_D^{20}$ +29° (*c* 0.1, $CHCl_3$), $C_{36}H_{49}O_3N$ (MW 543.78).

PMR spectrum ($CDCl_3$, δ, ppm, J/Hz): 0.96, 0.99, 1.08, 1.11, 1.43, 1.69 (18H, 6s, $6CH_3$), 1.10–2.40 (20H, m, CH_2, CH), 2.20 (1H, d, J_{gem} = 16.9, H-1b), 3.00 (2H, dd, J_{gem} = 16.9, 8.7, H-1a, H-19), 4.62 (1H, s, H-29a), 4.72 (1H, s, H-29b), 7.25 (1H, s, H-7′), 7.30 (2H, d, J = 5.3, H-3′, H-5′), 8.63 (2H, d, J = 5.3, H-2′, H-6′), 10.80 (1H, br. signal, COOH).

^{13}C NMR spectrum (δ, ppm): 14.7, 15.6, 15.9, 19.6, 20.4, 21.8, 22.4, 25.7, 29.3, 29.8, 30.8, 32.3, 33.1, 36.7, 37.2, 38.5, 40.6, 42.6, 44.4, 45.5, 47.0, 48.5, 49.3, 53.0, 56.4, 109.7 (C-29), 124.4 (C-3′), 124.4 (C-5′), 133.6 (C-2), 139.0 (C-4′), 144.4 (C-7′), 149.1 (C-2′), 149.1 (C-6′), 150.7 (C-20), 180.8 (COOH), 207.9 (C-3).

3-Oxo-2-(pyridin-3-ylmethylideno)-urs-12-en-28-oic Acid (22). Yield 0.49 g (91%), mp 139–145°C, $[\alpha]_D^{20}$ –18° (*c* 0.1, $CHCl_3$), $C_{36}H_{49}O_3N$ (MW 543.78).

PMR spectrum ($CDCl_3$, δ, ppm, J/Hz): 0.81, 0.85, 0.89, 0.91, 0.96, 1.13, 1.13 (21H, 7s, $7CH_3$), 1.15–2.35 (20H, m, CH_2, CH), 2.95 (1H, d, J_{gem} = 16.3, H-1a), 5.25 (1H, s, H-12), 7.35 (1H, dd, J = 4.8, 8.0, H-5′), 7.45 (1H, s, H-7′), 7.72 (1H, d, J = 8.0, H-6′), 8.55 (1H, d, J = 3.5, H-4′), 8.70 (1H, s, H-2′), 11.0 (1H, br. signal, COOH).

^{13}C NMR spectrum (δ, ppm): 15.5, 16.7, 17.1, 20.3, 21.2, 22.7, 23.4, 23.5, 24.1, 28.0, 29.6, 30.7, 32.1, 36.3, 36.7, 38.9, 39.2, 39.4, 42.2, 44.1, 45.2, 45.3, 48.0, 52.8, 53.1, 123.6 (C-5′), 125.2 (C-12), 132.0 (C-3′), 133.4 (C-4′), 136.2 (C-7′), 137.5 (C-2), 138.3 (C-13), 148.5 (C-2′), 150.4 (C-6′), 183.0 (COOH), 207.3 (C-3).

3-Oxo-2-(pyridin-4-ylmethylideno)-urs-12-en-28-oic Acid (23). Yield 0.50 g (92%), mp 207°C, $[\alpha]_D^{20}$ –5° (*c* 0.1, $CHCl_3$), $C_{36}H_{49}O_3N$ (MW 543.78).

PMR spectrum ($CDCl_3$, δ, ppm, J/Hz): 0.76, 0.79, 0.83, 0.85, 0.90, 1.60, 1.60 (21H, 7s, $7CH_3$), 1.10–2.30 (20H, m, CH_2, CH), 2.90 (1H, d, J_{gem} = 16.5, H-1a), 5.20 (1H, s, H-12), 7.20 (2H, d, J = 4.8, H-3′, H-5′), 7.40 (1H, s, H-7′), 8.60 (2H, d, J = 4.8, H-2′, H-6′), 11.0 (1H, br. signal, COOH).

^{13}C NMR spectrum (δ, ppm): 15.5, 16.7, 17.1, 20.2, 21.2, 22.7, 23.5, 23.5, 24.2, 28.0, 29.4, 30.7, 32.1, 36.4, 36.7, 38.8, 39.2, 39.4, 42.2, 43.9, 45.2, 45.4, 48.0, 52.8, 53.2, 124.2 (C-3′), 124.2 (C-5′), 125.1 (C-12), 133.9 (C-4′), 138.2 (C-13), 138.2 (C-2), 144.1 (C-7′), 149.3 (C-2′), 149.3 (C-6′), 182.7 (COOH), 207.3 (C-3).

3-Oxo-2-(pyridin-3-ylmethylideno)-olean-12-en-28-oic Acid (25). Yield 0.52 g (95%), mp 192–194°C, $[\alpha]_D^{20}$ +44° (*c* 0.1, $CHCl_3$), $C_{36}H_{49}O_3N$ (MW 543.78).

PMR spectrum ($CDCl_3$, δ, ppm, J/Hz): 0.89, 0.91, 0.95, 1.05, 1.10, 1.12, 1.38 (21H, 7s, $7CH_3$), 1.20–2.50 (18H, m, CH_2, CH), 2.24 (1H, d, J_{gem} = 16.8, H-1b), 3.00 (1H, d, J_{gem} = 16.8, H-1a), 4.20 (1H, m, H-18), 5.69 (1H, s, H-12), 7.35 (1H, d, J = 7.7, H-5′), 7.66 (1H, s, H-7′), 7.89 (1H, d, J = 7.7, H-4′), 8.50 (1H, s, H-2′), 8.62 (1H, s, H-6′), 11.0 (1H, br. signal, COOH).

^{13}C NMR spectrum (δ, ppm): 15.3, 18.4, 19.4, 22.5, 22.8, 23.4, 23.4, 27.9, 29.5, 30.7, 31.6, 32.8, 33.7, 36.4, 41.6, 41.9, 43.0, 43.7, 44.6, 44.8, 45.5, 45.9, 46.0, 53.4, 59.3, 123.7 (C-12), 127.8 (C-5′), 132.5 (C-3′), 137.0 (C-4′), 137.2 (C-7′), 147.8 (C-2), 150.7 (C-13), 170.0 (C-2′), 181.9 (C-6′), 199.5 (COOH), 207.1 (C-3).

3-Oxo-2-(pyridin-4-ylmethylideno)-olean-12-en-28-oic Acid (26). Yield 0.48 g (88%), mp 191–193°C, $[\alpha]_D^{20}$ +5° (*c* 0.1, $CHCl_3$), $C_{36}H_{49}O_3N$ (MW 543.78).

PMR spectrum ($CDCl_3$, δ, ppm, J/Hz): 0.80, 0.83, 0.90, 0.92, 1.12, 1.14, 1.18 (21H, 7s, $7CH_3$), 1.00–2.10 (18H, m, CH_2, CH), 2.27 (1H, d, J_{gem} = 16.8, H-1b), 2.88 (1H, m, H-18), 2.94 (1H, d, J_{gem} = 16.8, H-1a), 5.30 (1H, s, H-12), 7.25 (2H, d, J = 5.8, H-3′, H-5′), 7.35 (1H, s, H-7′), 8.61 (2H, d, J = 5.8, H-2′, H-6′), 11.0 (1H, br. signal, COOH).

^{13}C NMR spectrum (δ, ppm): 15.2, 16.5, 20.2, 20.5, 22.6, 23.0, 23.5, 25.6, 27.7, 29.5, 30.7, 31.8, 32.4, 33.0, 33.8, 36.3, 39.1, 41.2, 41.9, 44.0, 45.3, 45.9, 46.5, 49.2, 53.0, 121.8 (C-3′), 121.8 (C-52), 124.3 (C-12), 130.6 (C-2), 133.7 (C-4′), 138.3 (C-13), 144.1 (C-7′), 149.1 (C-2′), 149.1 (C-6′), 182.8 (COOH), 207.3 (C-3).

3,28-Dioxo-2-(pyridin-3-ylmethylideno)-19*β*,28-epoxyoleanane (28). Yield 0.49 g (90%), mp 154–156°C, $[\alpha]_D^{20}$ +22° (*c* 0.1, $CHCl_3$), $C_{36}H_{49}O_3N$ (MW 541.76).

PMR spectrum ($CDCl_3$, δ, ppm, J/Hz): 0.78, 0.88, 0.91, 0.93, 0.99, 1.99, 1.13 (21H, 7s, $7CH_3$), 1.10–2.15 (20H, m, CH_2, CH), 2.21 (1H, d, J_{gem} = 16.1, H-1b), 3.00 (1H, d, J_{gem} = 16.2, H-1a), 3.91 (1H, m, H-19), 7.30 (1H, m, H-5′), 7.40 (1H, s, H-7′), 7.70 (1H, d, J = 8.0, H-4′), 8.51 (1H, d, J = 4.1, H-6′), 8.66 (1H, s, H-2′).

^{13}C NMR spectrum (δ, ppm): 13.6, 15.1, 16.2, 20.3, 21.7, 22.4, 24.0, 25.6, 26.6, 27.9, 28.8, 29.5, 32.0, 32.4, 32.5, 33.6, 36.2, 36.7, 40.1, 40.4, 44.8, 45.3, 46.1, 46.7, 49.1, 53.0, 86.0 (C-19), 123.4 (C-5′), 131.9 (C-3′), 133.6 (C-4′), 136.4 (C-7′), 137.1 (C-2), 149.1 (C-2′), 150.9 (C-6′), 179.7 (C-28), 207.6 (C-3).

3,28-Dioxo-2-(pyridin-4-ylmethylideno)-19*β*,28-epoxyoleanane (29). Yield 0.49 g (92%), mp 247–250°C, $[\alpha]_D^{20}$ +109° (*c* 0.1, $CHCl_3$), $C_{36}H_{49}O_3N$ (MW 541.76).

PMR spectrum ($CDCl_3$, δ, ppm, J/Hz): 0.88, 0.94, 0.96, 0.97, 1.01, 1.03, 1.05 (21H, 7s, $7CH_3$), 1.10–1.90 (20H, m, CH_2, CH), 3.35 (1H, d, J = 14.9, H-1a), 3.58 (1H, d, J = 14.7, H-1b), 3.92 (1H, s, H-19), 6.85 (1H, s, H-7′), 7.06 (2H, d, J = 5.8, H-3′, H-5′), 8.45 (2H, d, J = 5.8, H-2′, H-6′).

^{13}C NMR spectrum (δ, ppm): 13.6, 16.3, 19.1, 20.1, 21.4, 21.5, 24.0, 25.6, 26.4, 27.8, 28.3, 28.8, 32.0, 32.4, 33.1, 33.6, 36.2, 36.3, 39.2, 41.5, 44.7, 45.5, 46.1, 46.7, 49.3, 53.4, 85.9 (C-19), 124.1 (C-3′), 124.1 (C-5′), 133.7 (C-4′), 149.3 (C-7′), 149.7 (C-2′), 149.7 (C-6′), 156.4 (C-2), 179.6 (C-28), 204.4 (C-3).

3,11-Dioxo-2-(pyridine-3-ylmethylideno)-olean-12-en-30-oic Acid (30). Yield 0.51 g (91%), mp 140–143°C, $[\alpha]_D^{20}$ +94° (*c* 0.1, $CHCl_3$), $C_{36}H_{47}O_4N$ (MW 557.76).

PMR spectrum ($CDCl_3$, δ, ppm, J/Hz): 0.81, 1.10, 1.15, 1.18, 1.21, 1.39, 1.50 (21H, 7s, $7CH_3$), 1.35–2.50 (18H, m, CH_2, CH), 4.20 (1H, d, J_{gem} = 16.5, H-1a), 5.79 (1H, s, H-12), 7.39 (1H, dd, J = 4.8, 8.0, H-5′), 7.42 (1H, s, H-7′), 7.92 (1H, d, J = 8.0, H-6′), 8.54 (1H, d, J = 3.5, H-4′), 8.70 (1H, s, H-2′), 11.0 (1H, br. signal, COOH).

^{13}C NMR spectrum (δ, ppm): 15.3, 17.9, 19.4, 22.4, 23.1, 26.3, 26.4, 28.5, 29.4, 29.5, 30.9, 31.3, 31.8, 36.2, 37.6, 41.1, 43.3, 43.6, 44.2, 44.9, 45.4, 48.2, 53.3, 59.1, 123.9 (C-5′), 128.3 (C-12), 131.9 (C-3′), 132.5 (C-4′), 137.3 (C-7′), 138.0 (C-2), 146.9 (C-2′), 149.6 (C-6′), 170.7 (C-13), 180.4 (COOH), 199.2 (C-11), 206.9 (C-3).

3,11-Dioxo-2-(pyridine-4-ylmethylideno)-olean-12-en-30-oic Acid (31). Yield 0.49 g (88%), mp 147–149°C, $[\alpha]_D^{20}$ +75° (*c* 0.1, $CHCl_3$), $C_{36}H_{47}O_4N$ (MW 557.76).

PMR spectrum ($CDCl_3$, δ, ppm, J/Hz): 0.85, 1.00, 1.10, 1.21, 1.23, 1.35, 1.42 (21H, 7s, $7CH_3$), 1.00–2.70 (19H, m, CH_2, CH), 5.78 (1H, s, H-12), 7.41 (2H, d, J = 5.3, H-3′, H-5′), 8.50 (1H, s, H-7′), 8.65 (2H, d, J = 5.3, H-2′, H-6′), 11.0 (1H, br. signal, COOH).

^{13}C NMR spectrum (δ, ppm): 15.4, 16.2, 18.1, 18.4, 19.5, 20.6, 22.8, 26.4, 28.5, 31.1, 32.0, 36.5, 37.8, 41.5, 43.3, 43.8, 45.0, 46.0, 48.7, 49.4, 53.8, 54.4, 55.9, 59.2, 124.6 (C-3′), 124.6 (C-5′), 128.3 (C-12), 133.3 (C-2), 138.9 (C-4′), 144.6 (C-7′), 148.8 (C-2′), 148.8 (C-6′), 170.5 (C-13), 179.9 (COOH), 199.1 (C-11), 206.8 (C-3).

The methods for studying the antituberculosis activity of **7**–**9**, **15**–**18**, **20**, **22**, **23**, **25**, **26**, **30**, and **31** are given at the website www.niaid-aacf.org.

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