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SYNTHESIS OF BETULIN DERIVATIVES CONTAINING TRIAZOLE FRAGMENTS

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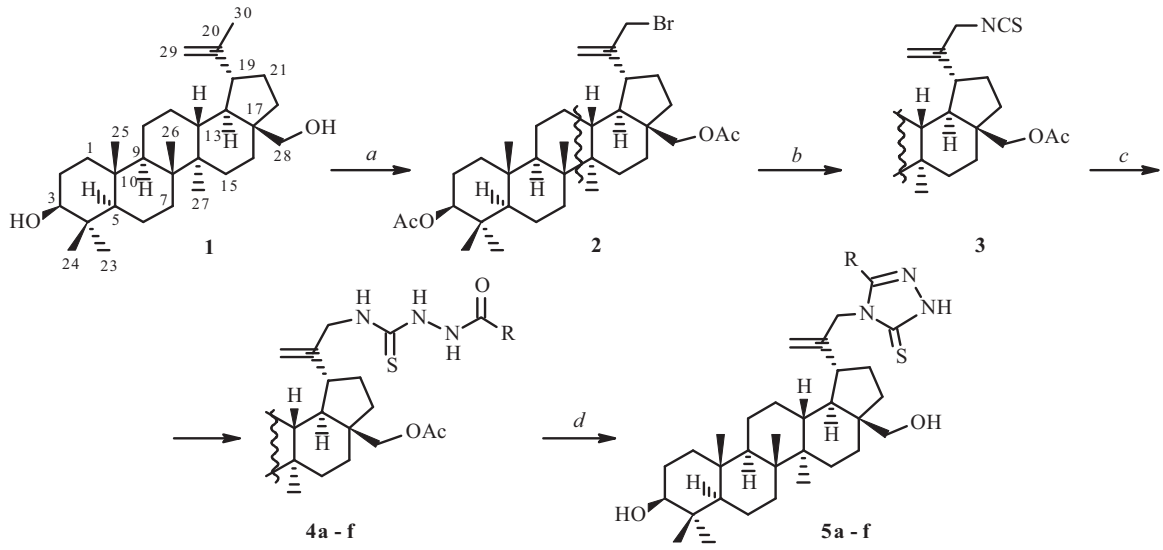
30-Triazole derivatives of betulin were synthesized through isothiocyanation, hydrazidation, and cyclization steps using betulin as a starting material.

Keywords: betulin, bromination, isothiocyanation, deprotection.

Betulin is a member of the pentacyclic triterpene family; its derivatives have interesting bioactivities such as anti-HIV activity [1–3], antitumor activity [4–6], and anti-inflammatory activity [7, 8]. Furthermore, 1,2,4-triazole-3-thiones were identified as potent anti-inflammatory compounds [9]. Proceeding with our search for biologically active compounds, we have synthesized six new betulin derivatives at the C-30 position with 1,2,4-triazole-3-thione. The synthesis of the target molecules is shown in Scheme 1.

The 3,28-di-*O*-acetyl-30-isothiocyanatobetulin (**3**) was prepared by isothiocyanation of 3,28-di-*O*-acetyl-30-bromobetulin (**2**) with potassium isothiocyanate in ethanol–chloroform mixture as solvent. The yield after silica gel chromatography was 58.3%. In the PMR, H-30 of **2** exhibited an unusual quartet (the two outer peak intensities are very low because $\Delta\nu/J = 0.77$ in the AB system) at δ 4.10.

Compounds **4a**–**f** were obtained using the hydrazidation reaction of **3** with hydrazides at 90–100°C. The yields of **4a**–**f** were 41.6–61.8% after chromatographic purification. In the PMR, signals for the three amine protons are clearly visible in the range δ 6.99–9.63 ppm. Furthermore, we verified the resonance for the aldehyde proton of **4a** at δ 8.14 ppm.



Scheme 1

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Intramolecular cyclization of **4** in the presence of aqueous 2 N NaOH directly afforded di-deactylation compounds **5a**–**f**. The IR spectra of compounds **5a**–**f** indicate that there was no absorption of the SH group at 2600–2500 cm^{-1} and also show the presence of two absorption bands at 1186–1335 cm^{-1}, which confirms that compounds **5a**–**f** exist predominantly in the thione form. The PMR spectra of compounds **5a**–**f** do not indicate the disappearance of the three amidic NH-proton characteristic signals for the thiosemicarbazides **4a**–**f**, and the peak at δ 11.5 due to S-H-proton was absent, but a pair of doublets downfield resonating for H-30 at δ 4.49–4.55 and δ 4.67–4.70 ppm, respectively, was evident. In addition, the upfield shifts of the H-3 (δ 3.20 ppm) and H-28 (δ ~3.30 and δ ~3.39 ppm) signals were observed, which can be attributed to the cyclization step occurring with simultaneous deprotection.

EXPERIMENTAL

IR spectra were recorded on a Perkin–Elmer FT-IR 1730 infrared spectra photometer. PMR spectra were recorded on a Bruker AV-300 spectrometer (300 MHz) in $CDCl_3$ with $SiMe_4$ as internal standard. Melting points were determined on an X-5 microscope melting point apparatus. MALDI-TOF-MS data were obtained using a Shimadzu AXIMA-CFR plus mass spectrometry employing a 1,8,9-anthracenetriol (DITH) matrix. Elemental analyses corresponded with those calculated.

3,28-Di-*O*-acetyl-30-bromobetulin was prepared from betulin (**1**) by the literature method [10].

3,28-Di-*O*-acetyl-30-isocyanatobetulin (3). A solution of **2** (1.0 mmol, 0.61g) in chloroform (6 mL) was added dropwise to a solution of potassium isothiocyanate (1.2 mmol, 0.12 g) in anhydrous ethanol (3 mL) at 95–100°C. After the addition was completed, the reaction mixture was cooled to room temperature and the organic solvent removed under reduced pressure. Then 20 mL of dichloromethane and 10 mL of water were added, and the organic phase was separated, dried, and evaporated. The residue was purified by silica-gel column chromatography.

Yield 0.34 g (58.3%), $C_{35}H_{53}NO_4S$. PMR spectrum (300 MHz, $CDCl_3$, δ, ppm, J/Hz): 0.84, 0.83, 0.97, 1.03 (15H, 4s, $5CH_3$), 2.04 and 2.07 (6H, 2s, $2OCOC\underline{H}_3$), 2.26–2.39 (1H, m, H-19), 3.81 and 4.23 (2H, both d, J = 11.1, H-28), 4.03–4.20 (2H, m, H-30), 4.46 (1H, dd, J = 5.4, 9.6, H-3), 5.02 and 5.04 (2H, both s, H-29).

General Synthesis of 1-Acyl-4-(3,28-diacetylbetulin-30-yl)-aminothiourea (4a–f). A solution of **3** (0.80 mmol, 0.45 g) in chloroform (10 mL) was added dropwise to a solution of hydrazide (0.88 mmol) in anhydrous ethanol (5 mL) at 95–100°C. The solution was continuously stirred at the same temperature for 10–12 h and cooled, and the organic solvent was removed under reduced pressure. The residue was purified by silica-gel column chromatography.

1-Formyl-4-(3,28-diacetylbetulin-30-yl)-aminothiourea (4a). Yield 0.35 g (54.2%), $C_{36}H_{57}N_3O_5S$. PMR spectrum (300 MHz, $CDCl_3$, δ, ppm, J/Hz): 0.84, 0.83, 0.98, 1.02 (15H, 4s, $5CH_3$), 2.04 and 2.07 (6H, 2s, $2OCOC\underline{H}_3$), 2.28–2.45 (1H, m, H-19), 3.83 (1H, d, J = 10.8, H-28), 4.15–4.33 (3H, m, H-30, H-28), 4.39–4.51 (1H, m, H-3), 4.78 and 4.90 (2H, both s, H-29), 6.97, 9.02, 9.16 (3H, each br.s, 3NH), 8.14 (1H, br.s, CHO).

1-Acetyl-4-(3,28-diacetylbetulin-30-yl)-aminothiourea (4b). Yield 0.389 g (58.7%), $C_{37}H_{59}N_3O_5S$. PMR spectrum (300 MHz, $CDCl_3$, δ, ppm, J/Hz): 0.84, 0.83, 0.98, 1.02 (15H, 4s, $5CH_3$), 2.04 and 2.07 (6H, both s, $2OCOC\underline{H}_3$), 2.09 (3H, s, $NHCOC\underline{H}_3$), 2.28–2.46 (1H, m, H-19), 3.82 (1H, d, J = 11.1, H-28), 4.13–4.31 (3H, m, H-30, H-28), 4.38–4.52 (1H, m, H-3), 4.89 and 4.77 (2H, both s, H-29), 6.99, 8.71, 8.99 (3H, each br.s, 3NH).

1-Propionyl-4-(3,28-diacetylbetulin-30-yl)-aminothiourea (4c). Yield 0.410 g (60.7%), $C_{38}H_{61}N_3O_5S$. PMR spectrum (300 MHz, $CDCl_3$, δ, ppm, J/Hz): 0.82, 0.96, 1.04 (15H, 3s, $5CH_3$), 2.01 and 2.04 (6H, both s, $2OCOC\underline{H}_3$), 2.24–2.43 (3H, m, H-19, $COC\underline{H}_2$), 3.79 (1H, d, J = 11.1, H-28), 4.10–4.30 (3H, m, H-30, H-28), 4.34–4.52 (1H, m, H-3), 4.73 and 4.84 (2H, both s, H-29), 7.20, 9.06, 9.36 (3H, each br.s, 3NH).

1-Butanoyl-4-(3,28-diacetylbetulin-30-yl)-aminothiourea (4d). Yield 0.291 g (41.6%), $C_{39}H_{63}N_3O_5S$. PMR spectrum (300 MHz, $CDCl_3$, δ, ppm, J/Hz): 0.82, 0.83, 0.97, 1.02 (15H, 4s, $5CH_3$), 2.03 and 2.06, (6H, both s, $2OCOC\underline{H}_3$), 2.22–2.44 (3H, m, H-19, $COC\underline{H}_2$), 3.81 (1H, d, J = 10.8, H-28), 4.12–4.31 (3H, m, H-30, H-28), 4.49–4.50 (1H, m, H-3), 4.86 and 4.76 (2H, both s, H-29), 7.26, 9.09, 9.66 (3H, each br.s, 3NH).

1-Pentanoyl-4-(3,28-diacetylbetulin-30-yl)-aminothiourea (4e). Yield 0.349 g (50.4%), $C_{40}H_{65}N_3O_5S$. PMR spectrum (300 MHz, $CDCl_3$, δ, ppm, J/Hz): 0.83, 0.84, 0.98, 1.03 (15H, 4s, $5CH_3$), 2.04 and 2.07, (6H, both s, $2OCOC\underline{H}_3$), 2.24–2.47 (3H, m, H-19, $COC\underline{H}_2$), 3.81 (1H, d, J = 11.1, H-28), 4.23–4.27 (3H, m, H-30, H-28), 4.59 (1H, dd, J = 5.1, 9.3, H-3), 4.78 and 4.88 (2H, both s, H-29), 7.26, 8.98, 9.61 (3H, each br.s, 3NH).

1-Hexanoyl-4-(3,28-diacetylbetulin-30-yl)-aminothiourea (4f). Yield 0.437 g (61.8%), $C_{41}H_{67}N_3O_5S$. PMR spectrum (300 MHz, $CDCl_3$, δ, ppm, J/Hz): 0.83, 0.84, 0.98, 1.03 (15H, 4s, $5CH_3$), 2.04 and 2.07 (6H, both s, $2OCOC\underline{H}_3$), 2.23–2.44 (3H, m, H-19, $COC\underline{H}_2$), 3.81 (1H, d, J = 11.1, H-28), 4.11–4.33 (3H, m, H-30, H-28), 4.59 (1H, dd, J = 5.1, 9.6, H-3), 4.78 and 4.88 (2H, both s, H-29), 7.21, 8.95, 9.60 (3H, each br.s, 3NH).

5-Alkyl-4-(betulin-30-yl)-2,4-dihydro-3*H*-1,2,4-triazole-3-thione (5a–f). A solution of **3** (0.35 mmol) in a mixture of ethanol (4.8 mL), THF (6.7 mL) and 4N NaOH (2.6 mL) was refluxed for 5–6 h. After cooling to room temperture, the mixture was acidified with 10% AcOH until pH 6–7 and concentrated under reduced pressure. Then 20 mL of dichloromethane and 10 mL of water were added, and the organic phase was separated, washed with water, dried, and evaporated. The residue was purified by silica-gel column chromatography.

4-(Betulin-30-yl)-2,4-dihydro-3*H*-1,2,4-triazole-3-thione (5a). Yield 0.101 g (54.6%), mp 186.2–186.7°C, $C_{32}H_{51}N_3O_2S$. IR spectrum (KBr, ν, cm^{-1}): 3414 (OH, NH), 1649, 1556 (C=C, C=N), 1269, 1211 (C=S), 1028 (C-O). PMR spectrum (300 MHz, $CDCl_3$, δ, ppm, J/Hz): 0.76, 0.83, 0.97, 1.00, 1.03 (each 3H, s, CH_3), 2.30–2.45 (1H, m, H-19), 3.20 (1H, dd, J = 4.8, 10.8, H-3), 3.29 and 3.78 (2H, both d, J = 10.8, H-28), 4.55 and 4.68 (2H, both d, J_{gem} = 16.2, H-30), 4.64 and 5.06 (1H, both s, 2H-29), 7.76 (1H, s, triazole). MS (Maldi-TOF, DITH as matrix): *m/z* (100%): 510.28 $[M - CH_2OH]^+$, 527.11 $[M + H - CH_3]^+$, 580.32 $[M + K]^+$.

5-Methyl-4-(betulin-30-yl)-2,4-dihydro-3*H*-1, 2, 4-triazole-3-thione (5b). Yield 0.120 g (59.4%), mp 198.1–198.6°C, $C_{33}H_{53}N_3O_2S$. IR spectrum (KBr, ν, cm^{-1}): 3374 (OH, NH), 1649, 1585 (C=C, C=N), 1251, 1192 (C=S), 1038 (C-O). PMR spectrum (300 MHz, $CDCl_3$, δ, ppm, J/Hz): 0.76, 0.83, 0.97, 1.00, 1.03 (each 3H, s, CH_3), 2.31 (3H, s, CH_3), 2.33–2.51 (1H, m, H-19), 3.20 (1H, dd, J = 5.1, 10.8, H-3), 3.32 and 3.79 (2H, both d, J = 10.8, H-28), 4.34 and 4.96 (2H, both s, H-29), 4.49 and 4.70 (2H, both d, J_{gem} = 16.8, H-30). MS (Maldi-TOF, DITH as matrix): *m/z* (100%): 524.38 $[M - CH_2OH]^+$, 541.16 $[M + H - CH_3]^+$, 594.37 $[M + K]^+$.

5-Ethyl-4-(betulin-30-yl)-2,4-dihydro-3*H*-1,2,4-triazole-3-thione (5c). Yield 0.132 g (61.9%), mp 214.2–214.7°C, $C_{34}H_{55}N_3O_2S$. IR spectrum (KBr, ν, cm^{-1}): 3389 (OH, NH), 1647, 1576 (C=C, C=N), 1261, 1186 (C=S), 1028 (C-O). PMR spectrum (300 MHz, $CDCl_3$, δ, ppm, J/Hz): 0.76, 0.82, 0.97, 1.00, 1.02 (each 3H, s, CH_3), 1.23 (3H, t, J = 7.2, $CH_2C\underline{H}_3$), 2.28–2.52 (1H, m, H-19), 2.53 (2H, q, J = 7.2, $C\underline{H}_2CH_3$), 3.20 (1H, dd, J = 4.8, 10.8, H-3), 3.32 and 3.79 (2H, both d, J = 10.8, H-28), 4.30 and 4.94 (2H, both s, H-29), 4.50 and 4.69 (2H, both d, J_{gem} = 16.5, H-30). MS (Maldi-TOF, DITH as matrix) *m/z* (100%): 538.44 $[M - CH_2OH]^+$, 555.27 $[M + H - CH_3]^+$, 608.48 $[M + K]^+$.

5-Propyl-4-(betulin-30-yl)-2,4-dihydro-3*H*-1,2,4-triazole-3-thione (5d). Yield 0.068 g (33.6%), mp 190.5–190.9°C, $C_{35}H_{57}N_3O_2S$. IR spectrum (KBr, ν, cm^{-1}): 3352 (OH, NH), 1647, 1577 (C=C, C=N), 1335, 1275, (C=S), 1049 (C-O). PMR spectrum (300 MHz, $CDCl_3$, δ, ppm, J/Hz): 0.76, 0.83, 0.97, 1.01, 1.03 (each 3H, s, CH_3), 2.35–2.49 (1H, m, H-19), 2.53 (2H, t, J = 7.5, $C\underline{H}_2CH_2CH_3$), 3.20 (1H, dd, J = 4.8, J = 10.8, H-3), 3.33 and 3.79 (2H, both d, J = 10.8, H-28), 4.26 and 4.94 (2H, both s, H-29), 4.51 and 4.67 (2H, both d, J_{gem} = 16.8, H-30). MS (Maldi-TOF, DITH as matrix) *m/z* (100%): 552.46 $[M - CH_2OH]^+$, 569.30 $[M + H - CH_3]^+$, 622.51 $[M + K]^+$.

5-Butyl-4-(betulin-30-yl)-2,4-dihydro-3*H*-1,2,4-triazole-3-thione (5e). Yield 0.112 g (54.2%), mp 188.4–188.9°C, $C_{36}H_{59}N_3O_2S$. IR spectrum (KBr, ν, cm^{-1}): 3379 (OH, NH), 1647, 1574 (C=C, C=N), 1277, 1184 (C=S), 1031 (C-O). PMR spectrum (300 MHz, $CDCl_3$, δ, ppm, J/Hz): 0.76, 0.83, 0.97, 1.00, 1.03 (each 3H, s, CH_3), 2.35–2.50 (1H, m, H-19), 2.55 (2H, t, J = 7.2, $C\underline{H}_2(CH_2)_2CH_3$), 3.20 (1H, dd, J = 4.8, 10.8, H-3), 3.33 and 3.79 (2H, both d, J = 10.8, H-28), 4.24 and 4.94 (2H, both s, H-29), 4.53 and 4.66 (2H, both d, J_{gem} = 16.8, H-30). MS (Maldi-TOF, DITH as matrix) *m/z* (100%): 566.49 $[M - CH_2OH]^+$, 583.32 $[M + H - CH_3]^+$, 636.53 $[M + K]^+$.

5-Pentyl-4-(betulin-30-yl)-2,4-dihydro-3*H*-1,2,4-triazole-3-thione (5f). Yield 0.137 g (63.1%), mp 234.3–234.6°C, $C_{37}H_{61}N_3O_2S$. IR spectrum (KBr, ν, cm^{-1}): 3356 (OH, NH), 1649, 1577 (C=C, C=N), 1292, 1253 (C=S), 1026 (C-O). PMR spectrum (300 MHz, $CDCl_3$, δ, ppm, J/Hz): 0.76, 0.83, 0.85, 0.97, 1.03 (each 3H, s, CH_3), 2.35–2.68 (3H, m, H-19, $C\underline{H}_2(CH_2)_3CH_3$), 3.14–3.28 (1H, m, H-3), 3.33 and 3.79 (1H, both d, J = 10.5, H-28), 4.25 and 4.94 (1H, both s, H-29), 4.45–4.75 (2H, m, H-30). MS (Maldi-TOF, DITH as matrix) *m/z* (100%): 580.52 $[M - CH_2OH]^+$, 597.37 $[M + H - CH_3]^+$, 650.56 $[M + K]^+$.

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