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The First Naturally Occurring Tie2 Kinase Inhibitor

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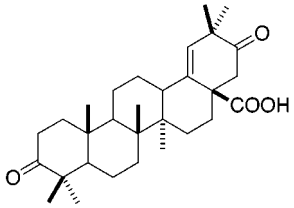
ABSTRACT



Bioassay-guided fractionation of the plant *Acacia aulacocarpa*, guided by a bioassay for Tie2 tyrosine kinase activity, yielded the novel triterpene 3,21-dioxo-olean-18-en-oic acid (1) as the first naturally occurring non-protein inhibitor of Tie2 kinase. The structure of 1 was assigned by analysis of spectral data. In addition to its activity as an inhibitor of Tie2 kinase, compound 1 also shows modest activity against a variety of cultured mammalian cells.

The uncontrolled growth of tumor cells depends on angiogenesis, or the generation of new blood vessels, and the inhibition of angiogenesis is a promising approach to cancer chemotherapy. The control of angiogenesis is complex, and several factors are believed to be involved. Vascular endothelial growth factor (VEGF) is believed to be necessary for tumor angiogenesis, since blocking the VEGF pathway inhibits the growth of various murine and human xenograft tumors.[1] The tyrosine kinase Tie2 kinase has also been implicated in the process of angiogenesis, since the natural Tie2 antagonist angiopoietin-2 disrupts angiogenesis in vivo.[2] Blocking Tie2 activation with the recombinant Tie2 receptor AdExTek significantly inhibited the growth rate of two different murine tumors.[3] Inhibitors of Tie2 kinase are thus attractive candidates for evaluation as cancer chemotherapeutic agents.

In continuation of our search for natural products with potential anti-cancer activity,[4] we screened our extract bank for inhibitors of Tie2 kinase, and an extract of *Acacia aulacocarpa* (Fabaceae) designated UM 4195-P[5] showed inhibitory bioactivity against Tie2 kinase with $IC_{50} = 6.7$ μg/mL. Although there is no previously reported work on *Acacia aulacocarpa* other than a report on the phytotoxicity of its leaf extracts,[6] work on other *Acacia* species includes

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(5) Leaves of *Acacia aulacocarpa* A. Cunn. ex. Benth (Fabaceae) were collected in Australia as PR-8300 in July 1961; a voucher specimen is deposited in the Herbarium of the National Arboretum, Agricultural Research Service, USDA, Washington, DC. The dried leaves were ground and extracted with MeOH/CH_2Cl_2 to give NCI B632381 (UM 4195-P).

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the isolation of alkaloids,[7] diterpenoids,[8] triterpenoids,[9] flavonoids,[10] anthraquinones,[11] and glycosides.[12] Using the Tie2 kinase bioassay as a guide, liquid−liquid partition and chromatography of the crude extract led to the isolation of the novel bioactive triterpene **1**.[13]

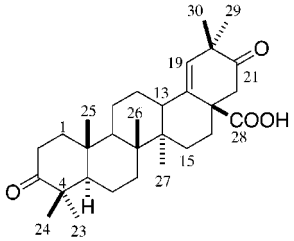

Figure 1. The structure of compound **1**.

Compound **1**[14] was obtained as a colorless gel-like substance; $[\alpha]^{23}_D$ +124.2° (*c* 0.0512, $CHCl_3$); UV (MeOH) λ_{max} (log ϵ) 209 (3.67), 278 (2.90). Its HRFABMS gave the molecular formula as $C_{30}H_{45}O_4$ (found *m*/*z* 469.3313 (M + H)$^+$; calc 469.3318). Its NMR data showed the presence of seven methyl groups [δ_H in ppm 0.87 (s), 0.97 (s), 1.04 (s), 1.05 (s), 1.08 (s), 1.15 (s), and 1.21 (s); and δ_C in ppm 15.2, 15.9, 16.5, 20.9, 24.6, 26.8, 28.9], one carboxylic acid group [δ_C 178.2], one trisubstituted double bond [δ_H 5.26 (d, J = 2.0 Hz) and δ_C 132.4 (d) and 137.0 (s)] and two carbonyl groups [δ_C 212.7 and 218.2] in addition to nine methylenes, three methines, six quarternary carbons. No oxygenated carbons (other than the carbonyl carbons) were observed in its ^{13}C NMR spectrum. Its formula, coupled with the observation of seven singlet methyl signals in its ^{1}H NMR spectrum, indicated that compound **1** belonged to the oleanoic acid class of triterpenoids.

The two carbonyl signals observed at low field suggested their placement at the sterically hindered C-3 and C-21 positions. The long-range correlations of H-23/C-3 and H-24/C-3 in the HMBC spectrum confirmed the location of one carbonyl group at C-3, the long-range correlation of H-22/C-28 identified the H-22 protons, and the correlations H-22/C21 and H-19/C21 placed the second carbonyl group at C-21. Correlations of H-19/C-29, H-19/C-30, H-19/C-21, H-22/C-18, and H-22/C-28 located the double bond at $\Delta^{18,19}$. The above data confirmed the related positions of the functional groups in ring E as shown in Figure 2.

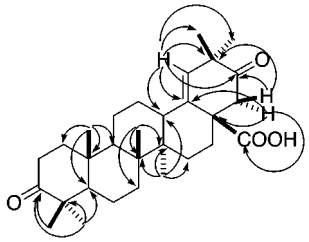

Figure 2. Selected HMBC correlations of **1**.

The structure of compound **1** was thus assigned as the new triterpenoid 3,21-di-oxo-olean-18-en-28-oic acid. The ^{13}C NMR chemical shifts of the A, B, and C ring carbons and their attached methyl groups in compound **1** were very close to the published shifts of the corresponding carbons in 3-oxo-olean-18-en-28-oic acid.[15]

Compound **1** was active in the Tie2 kinase bioassay with an IC_{50} = 4.2 μM (2.0 μg/mL). This is the first example of a non-protein natural product inhibitor of Tie2 kinase; the only previously reported inhibitors are the natural protein angiopoietin-2[2] and some 4-naphthyl-5-pyridyl imidazoles reported in the patent literature.[16] Because the potency of **1** is not in the nM range and it is unlikely to be a good cell penetrant, it was not evaluated in direct angiogenesis models.

Since some triterpenoid acids such as quinovic acid have been reported to be cytotoxic,[17] compound **1** was also tested for cytotoxicity against several cultured mammalian cell

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(13) A portion of the crude extract (UM 4195-P, 522.8 mg) was taken and partitioned between *n*-hexane and aqueous MeOH, followed by dilution of the MeOH extract with H_2O and extraction with CH_2Cl_2. Both the *n*-hexane and the CH_2Cl_2 fractions were active. Repeated column chromatography on Si gel with the solvents CH_2Cl_2/MeOH, 100:2, with the fractions monitored by bioassay, yielded compound **1** (6.43 mg) from the *n*-hexane fraction and a further amount (5.72 mg) from the CH_2Cl_2 fraction. The total yield of **1** was 12.15 mg (2.3% based on crude UM 4195-P extract).

(14) The NMR data of compound **1** were assigned by ^{1}H and ^{13}C NMR, DEPT, DQCOSY, HMQC, and HMBC spectra: δ_H (ppm in $CDCl_3$) 1.98 (1H, m, H-1a), 1.43 (1H, m, H-1b), 2.4−2.6 (2H, m, H-2a and H-2b), 1.35 (1H, m, H-5), 1.60 (1H, m, H-6a), 1.43 (1H, m H-6b), 1.52 (1H, m, H-7a), 1.42 (1H, m, H-7b), 1.39 (1H, m, H-9), 1.58 (1H, m H-11a), 1.39 (1H, m, H-11b), 1.74 (1H, br dd, J = 12.0, 2.7 Hz, H-12a), 1.27 (1H, m, H-12b), 2.23 (1H, m, H-13), 1.85 (1H, ddd, J = 13.50, 13.50, and 4.05 Hz, H-15a), 1.30 (1H, m, H-15b), 2.24 (1H, m, H-16a), 1.43 (1H, m, H-16b), 5.26 (1H, d, J = 1.9 Hz, H-19), 2.60 (2H, s, H-22), 1.08, (3H, s, H-23), 1.04 (3H, s, H-24), 0.97 (3H, s, H-25), 1.05 (3H, s, H-26), 0.87 (3H, s, H-27), 1.15 (3H, s, H-29), and 1.21 (3H, s, H-30). δ_C (ppm in $CDCl_3$) 39.8 (C-1), 34.0 (C-2), 218.2 (C-3), 47.3 (C-4), 54.9 (C-5), 19.6 (C-6), 33.8 (C-7), 40.1 (C-8), 50.3 (C-9), 36.9 (C-10), 21.3 (C-11), 26.2 (C-12), 41.1 (C-13), 42.2 (C-14), 28.8 (C-15), 32.8 (C-16), 51.5 (C-17), 137.0 (C-18), 132.4 (C-19), 44.8 (C-20), 212.7 (C-21), 47.6 (C-22), 26.8 (C-23), 20.9 (C-24), 15.9 (C-25), 16.5 (C-26), 15.2 (C-27), 178.2 (C-28), 24.6 (C-29), and 28.9 (C-30).

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lines. Its IC_{50} values for cell growth during 72 h were determined by the standard XTT assay[18] and were 2 μM for human diploid fibroblasts (MRC-5) or cervical carcinoma (HeLaS3) cells and 10 μM for mouse endothelial (MS-1) cells.

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