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A new antitumor compound from the plant *Oryctanthus* sp. as a VEGF receptor binding inhibitor

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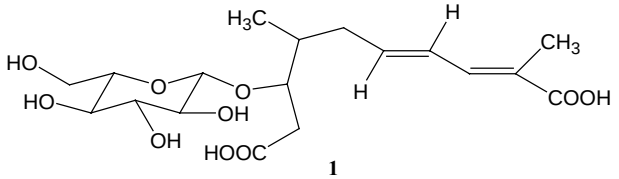
Abstract—The 70% aqueous methanolic extract of the Peruvian plant *Oryctanthus* sp. was found to contain a novel saccharide of a diene α,ω-diacid. Compound **1** was identified as an inhibitor of the VEGF receptor. The structure of this compound was established based on NMR studies. Compound **1** inhibited ligand binding to the VEGF receptor with an IC_{50} of 5.0 μM.

Tumor angiogenesis is a critical step for the growth and metastasis of solid tumors. Vascular endothelial growth factor (VEGF) is the most important angiogenic molecule associated with tumor-induced neovascularization. Vascular endothelial growth factor receptor 2 (VEGFR2—also referred to as KDR for kinase insert domain-containing receptor) is a receptor tyrosine kinase belonging to the platelet derived growth factor receptor (PDGFR) family. The kinase domain of VEGFR2 contains a 68-amino acid insert referred to as the kinase insert domain (KID),[1] which contains two tyrosine residues that have been shown to be autophosphorylation sites and required for maximal kinase activity.[2]

VEGF promotes angiogenesis[3] when binding to receptors on vascular endothelial cells. Angiogenesis is beneficial when attributed to embryogenesis, organ development, and other growth circumstances. However, VEGF has been associated with tumor growth, arthritis, and macular degeneration, and its receptor has been found in tumors of the breast,[4] colon,[5,6] and kidney.[7] The ligand for VEGFR (VEGF) has been shown to be overexpressed by human tumor cell lines in culture.[8] Specific inhibitors of VEGFR signal transduction have been reported in the literature[9] and have been shown to block mitogenesis of human endothelial cells without affecting the growth of human tumor cell lines. A high-throughput screen was developed to find inhibitors that are specific for the VEGF receptor.

As part of our continuing investigation of natural products as leads for inhibiting the VEGF receptor, we screened semi-purified fractions of aqueous methanolic extracts of many plants. One of these fractions, which was derived from a plant identified as *Oryctanthus* sp. from the lorantaceae family, was active in the VEGF inhibition assay. This parasitic plant grows about a meter in height in South America around Peru. A specimen of this plant is deposited at PeruBotanica, Iquitos, Peru, under Voucher # 8848. Bioassay-guided fractionation of this extract led to the isolation of the monosaccharide **1**, of a diene α,ω-diacid.



1

The detanninized aqueous methanolic extract (2.0 g) was loaded on a reverse-phase diaion CHP-20 P (Mitsubishi Chemicals, 1.0 in. × 12 in.) column equilibrated with water and chromatographed using a water and methanol gradient system. These fractions were screened for their activity in the VEGF receptor assay. The active

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fractions were combined and dried to yield 36.0 mg of enriched complex. Separation of the active compound was achieved by reverse-phase preparative HPLC on a Phenomenex Luna C-18 silica column (21.2 × 250 mm), eluting with a mixture of acetonitrile and water (25:75 v/v). Acetonitrile was removed from the active peak eluate and the aqueous solution was freeze-dried to yield 1.6 mg of **1**.

Compound **1** showed a molecular ion at *m/z* 422 $(M+NH_4)^+$ in the FAB mass spectrum, suggesting a molecular weight of 404 Da. The molecular formula of **1** was established as $C_{18}H_{28}O_{10}$ by high resolution mass spectrum (HRMS),[10] suggesting five unsaturations in the molecule. The UV spectrum (MeOH) showed absorption maxima at 266 nm and the IR spectrum in KBr showed peaks at 3393, 1696, 1462, 1418, 1204, and 1020 cm^{-1}, suggesting the presence of acid functionality. ^1H and ^{13}C NMR chemical shifts of **1** are listed in Table 1. The ^1H NMR indicated the presence of three sp^2 protons and the presence of one sugar. The ^{13}C NMR also showed 18 carbon signals in agreement with the number of carbons revealed by HRMS. APT ^{13}C NMR identified them as two >C=O, four olefinic (three =CH–, one =C<), one anomeric methine (O–CH–O), five >CH–O, one O–CH_2–, one >CH–, two >CH_2–, and two –CH_3 carbons. The data indicated that **1** contains two double bonds and one sugar. When we include the two carbonyl functionalities, we can account for all the degrees of unsaturations. Using contemporary 2D NMR techniques (COSY, HMQC, and HMQC-TOCSY) the structure was established as shown in **1** (2,7-dimethyl-2,4-diene-deca-α,ω-diacid) and confirmed by HMBC.

Compound **1** was active in the VEGF receptor assay[11] with an IC_{50} value of 5.0 μM.

Table 1. ^1H and ^{13}C NMR chemical shifts for **1**

C#	^1H	^{13}C
1		172.3
2		126.5
3	7.18 (d, *J* = Hz)	140.3
4	6.47 (dd, *J* = Hz)	129.1
5	6.12 (dt, *J* = Hz)	142.8
6	2.45 (dt, *J* = Hz)	37.1
	2.15 (dt, *J* = Hz)	
7	1.96 (m)	38.7
8	4.02 (m)	81.9
9	2.64 (dd, *J* = Hz)	39.2
	2.56 (dd, *J* = Hz)	
10		176.4
11	0.97 (d, *J* = Hz)	12.8
12	1.89 (s)	15.7
1^1	4.33 (d, *J* = Hz)	104.9
2^1	3.16 (dd, *J* = Hz)	75.4
3^1	3.32 (dd, *J* = Hz)	78.3
4^1	3.29 (dd, *J* = Hz)	71.6
5^1	3.22 (m)	77.8
6^1	3.82 (dd, *J* = Hz)	62.9
	3.65 (dd, *J* = Hz)	

Compound **1** is a novel compound. The aglycone C12-acid (diene α,ω-diacid) has been isolated previously from the seeds of *Phaseolus multiflorus*.[13]

Several natural plant products, such as polyphenolic pentameric procyanidins,[14] 1,2,3,4,6-penta-*O*-galloyl-β-D-glucose,[15] and other phenolic compounds, *cis*-hinokiresinol, brazilin,[16] salvianolic acid B,[17] and capsaicin,[18] are reported to reduce VEGF activity and angiogenic activity. Aplidine, a depsipeptide derived from the mediterranean tunicate,[19,20] and nakijiquinone C[21,22] another natural product derived from a marine sponge inhibit VEGF secretion and block the VEGF-VEGFR-1 (flt-1) autocrine loop in human leukemia cells MOLT-4. Compound **1** represents a novel natural product inhibitor of the VEGF receptor.

Acknowledgment

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10. Spectral data for **1**: FABMS *m/z* 422$(M+NH_4)^+$; HR-FABMS for $C_{18}H_{28}O_{10}$ (obsd 422.2042 $(M+NH_4)^+$, calcd for $C_{18}H_{32}NO_{10}$ 422.2016); IR (Neat): 3393, 1696, 1462, 1418, 1204, and 1020 cm^{-1}.
11. *Methods*: VEGFR tyrosine kinase assay: An antibody-competition-based fluorescence polarization assay was developed utilizing recombinant, glutathione *S*-transferase (GST)-tagged human vascular endothelial growth factor receptor 2 which was truncated to express the cytoplasmic domain of the receptor only (VEGFR2CD). GST-tagged VEGFR2CD was expressed Baculovirus and purified to ~90% homogeneity in a single purification step by GST-agarose affinity chromatography. Reactions were

carried out in 20 mM Tris–HCl buffer, pH 7.5, containing 5 mM $MgCl_2$, 2 mM $MnCl_2$, 1 mM DTT, 5 μM ATP, and 5 μg/ml polyglutamate tyrosine (Sigma P-0275) as the phosphate acceptor substrate and 1.5 ng VEGFR2CD. Inhibitor samples were dissolved in DMSO and were tested at a final DMSO (dimethylsulfoxide) concentration of 2%. A fluorescent phosphopeptide/antiphosphotyrosine antibody detection system (PanVera P2836) was used to measure reaction velocity.[12] Fluorescence polarization of reactions was measured kinetically over 30–40 min on an Analyst AD (Molecular Devices).

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