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Vegfrecine, an Inhibitor of VEGF Receptor Tyrosine Kinases Isolated from the Culture Broth of *Streptomyces* sp.

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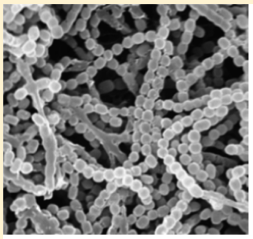
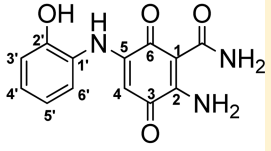
Supporting Information

ABSTRACT: A new inhibitor of VEGF receptor tyrosine kinases, vegfrecine (**1**), was isolated from the culture broth of *Streptomyces* sp. MK931-CF8. The molecular structure of **1** was determined by NMR and MS analysis combined with synthesis. Compound **1** showed potent inhibitory activity against vascular endothelial growth factor receptor (VEGFR) tyrosine kinases in *in vitro* enzyme assays, but platelet-derived growth factor receptors (PDGFRs), fibroblast growth factor receptor (FGFR), and epidermal growth factor receptor (EGFR) responded only weakly. Compound **1** is a promising new selective VEGFR inhibitor for investigating new treatments of cancer and inflammatory diseases.



Streptomyces sp. MK931-CF8



Vegfrecine

Vascular endothelial growth factors (VEGFs) are important regulators of angiogenesis, both in normal physiological processes such as embryonic development[1,2] and in abnormal disease states. Abnormal angiogenesis is associated with various pathological processes such as inflammation, rheumatoid arthritis, ocular neovascularization, psoriasis, and tumor development. It has been shown that VEGFs are important for tumor growth and metastasis formation.[3−7] VEGF expression is markedly upregulated in tumors,[8] and inhibition of VEGF-induced angiogenesis significantly inhibits tumor growth *in vivo*.[9−13] Avastin, the monoclonal antibody against VEGF-A, has been used as a therapeutic drug for cancer.[14]

VEGF-A interacts with two potent receptor tyrosine kinases, Flt-1 (VEGFR-1) and KDR (VEGFR-2). Both Flt-1 and KDR exist almost exclusively in endothelial cells, and VEGF-A stimulates vascular endothelial cell proliferation, migration, and protease production. Several studies showed that KDR is strongly autophosphorylated in endothelial cells upon VEGF stimulation and mediates a mitogenic response.[15] On the other hand, tyrosine kinase activity of Flt-1 is weak, leading to no significant mitogenic response in endothelial cells. Flt-1 signaling does not appear to be involved in physiological angiogenesis, but in a recent study, activation of Flt-1 was found to be important for angiogenesis in pathological conditions. Furthermore, Flt-1 is functionally expressed in monocytes and macrophages, which play a crucial role in promoting inflammation.[16−21] Indeed, blocking the Flt-1 signal is sufficient to inhibit strongly pathological angiogenesis such as cancer, atherosclerosis, arthritis, ocular neovascular disease, and metastasis formation.[22−24] In the course of our screening of microbial metabolites to identify novel inhibitors of Flt-1 tyrosine kinase, compound **1** was isolated from the culture broth of *Streptomyces* sp. Compound **1** exhibited potent inhibitory activity against VEGFR tyrosine kinases in *in vitro* enzyme assays, but only weak inhibitory activity against PDGFRs, FGFR, and EGFR.

In the screening program for microbial metabolites with inhibitory activity against Flt-1 tyrosine kinase, we used the kinase domain of recombinant human Flt-1 expressed in a baculovirus/insect cell system and ^{32}P-ATP as a tracer to measure phosphorylation. Strong Flt-1 tyrosine kinase inhibitory activity was observed in the ethanol extract of the broth of *Streptomyces* sp. MK931-CF8 cultured in solid pressed barley medium. Ethanol extracts of the solid cultures were loaded onto Diaion HP-20 and eluted with MeOH. The active fractions were combined and evaporated to give a brown solid. The solid was subjected to silica gel column chromatography followed by Sephadex LH-20 column chromatography to give **1** as a magenta solid. The molecular formula of **1** was determined to be $C_{13}H_{11}N_3O_4$ by HRESIMS. UV spectra of **1** showed characteristic absorption maxima at 248, 268, 334, and 524 nm and bathochromic shifts (297, 340, 592 nm) in alkaline solution, suggesting the presence of a phenol. In addition,

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absorption at 1633 cm^{-1} in the IR spectrum of **1** revealed the presence of quinone carbonyl groups. The structure of **1** was established by ^{1}H, ^{13}C, HMQC, HMBC, and COSY spectra. The ^{13}C and DEPT135 spectra revealed the presence of eight sp^2 quaternary carbons including three carbonyls and five sp^2 methines, respectively. Uncorrelated proton signals (δ 7.42, 8.66, 8.95, 9.19, 10.09, and 11.00) in the HMQC spectrum were assigned as exchangeable protons. The COSY spectrum of **1** revealed the connectivities between H-3′ (δ 6.96) and H-6′ (δ 7.26), δ 7.42 and 8.66. The HMBC spectrum of **1** showed connectivities from H-3′ and H-5′ (δ 6.86) to C-1′ (δ 124.4), H-4′ (δ 7.12) and H-6′ to C-2′ (δ 151.0), and from δ 7.42 to 167.9, indicating the presence of ortho-disubstituted benzene and carboxamide moieties. However the structure of **1** could not be thoroughly clarified because full HMBC correlations were missing. Therefore, compound **1** was converted into the MOM derivative **2** (Figure 1). The $^{1}H-^{13}C$ HMBC spectrum

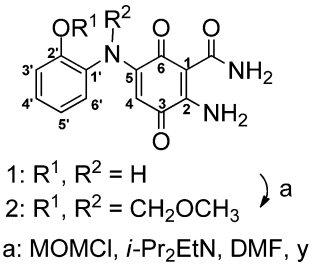

Figure 1. Structures of vegfrecine (**1**) and its MOM derivative (**2**).

of **2** revealed the following connections: from the MOM1 methylene at δ 5.13 to C-2′ (δ 150.6); from the MOM2 methylene at δ 5.01 to C-1′ (δ 135.8) of ortho-disubstituted benzene and C-5 (δ 154.7); from H-4 (δ 5.89) to C-2 (δ 154.5) and C-6 (δ 178.7); from the carboxamide protons (δ 7.17, 8.18) to C-1 (δ 97.2); and from NH_2-2 (δ 8.56, 10.50) to C-1 and C-3 (δ 178.2). These data suggested a partial structure of the 2-amino-3,6-dioxocyclohexa-1,4-dienecarboxamide moiety. Furthermore, $^{1}H-^{15}N$ HMBC experiments with **2** demonstrated the couplings of H-4, H-6′, and MOM2 methylene to a tertiary amine, resulting in the connectivity between C-1′ of ortho-disubstituted benzene and C-5 of the quinone ring with an imino linkage. The protective groups of MOM1 and MOM2 were attached to oxygen and nitrogen, respectively. All of the carbon connections in structure **2** were established except that between C-1 and C-6 (Figure 2). Therefore we planned to synthesize **1** based on the reasonable assumption that a quinone is present. Scheme 1 illustrates the synthetic plan.

Oxidative amination of 2,5-dihydroxybenzamide **5** with the TBS derivative **4**[25] afforded the amino benzoquinone **6**. Ammonolysis of **6** gave compound **7**, with an amino group at

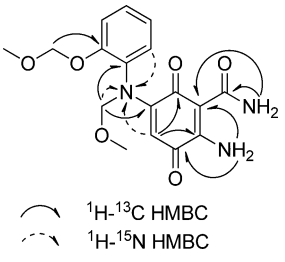

Figure 2. HMBC correlations of **2**.

the 2-position. Deprotection of the TBS group of **7** produced the desired compound **1** (see Supporting Information). Thus, the chemical structure of the active compound **1** was established as 2-amino-5-((2′-hydroxyphenyl)amino)-3,6-dioxocyclohexa-1,4-dienecarboxamide and named vegfrecine. Compound **1** has a unique structure with a *p*-benzoquinone ring possessing the same array of amino and carboxamide groups that are found in the antibiotic G-7063-2[26] and sarubicin A.[27] The quinone skeleton of sarubicin A is derived from 6-hydroxyanthranilic acid via oxidation of the hydroquinone, 3,6-dihydroxyanthranylamide. Such oxidation of the hydroquinone was mimicked in the synthesis of **6** from **5**.

We investigated the inhibitory activities of **1** against several different tyrosine kinases. VEGFRs, PDGFR-α and -β, FGF, and EGF tyrosine kinases were selected to cover a broad spectrum of tyrosine kinases. The VEGFRs are predominantly expressed on endothelial cells. Flt-1 (VEGFR-1) and KDR (VEGFR-2) are responsible for endothelial cell proliferation and blood vessel permeability, while Flt-4 (VEGFR-3) seems to be critical for lymphatic vessel development. These receptor tyrosine kinases are essential for tumor angiogenesis and lymphangiogenesis, respectively.[28−30] Compound **1** showed selective inhibition against VEGFRs *in vitro* and weakly inhibited PDGFR-α and -β, FGF, and EGF tyrosine kinase. The inhibitory potency of **1** surpassed that of the positive control compound, SU5416.[31]

Compound **1** showed a weak *in situ* inhibitory activity of over 10 μM against the ligand-induced phosphorylation of Flt-1 in NIH3T3-Flt-1 cells at two hours after treatment of **1**, at which time cytotoxicity was not observed. The inhibitory activity of **1** *in situ* is 100-fold less active than that *in vitro* (Figure 3). The weak inhibitory activity *in situ* could originate from poor cell membrane permeability and nonspecific interaction of **1** with cell components.

On the other hand, the cytotoxicity of **1** against NIH3T3-Flt-1 and NIH3T3-KDR cells at 48 h after treatment of **1** was 3 and 12 μM, respectively. The structure−activity relationship study is ongoing to improve the profile of activity *in situ*.

EXPERIMENTAL SECTION

General Experimental Procedures. UV spectra were obtained on a U-2800 spectrophotometer (Hitachi High-Tech, Tokyo, Japan). The IR spectrum was obtained on a FT-210 Fourier transform infrared spectrometer (Horiba, Kyoto, Japan). NMR spectra were recorded using a JNM-ECA600 (JEOL RESONANCE Inc., Tokyo, Japan) and an AVANCE 700 spectrometer (Bruker BioSpin, Rheinstetten, Germany) with TMS as an internal standard. HRESIMS spectra were measured using an LTQ Orbitrap mass spectrometer (Thermo Fisher Scientific, San Jose, CA, USA).

Taxonomy. Strain MK931-CF8 was isolated from a soil sample collected at Sendai-shi, Miyagi Prefecture, Japan, in April 1997. Strain MK931-CF8 formed well-branched vegetative mycelia and aerial hyphae that bore straight spore chains. The aerial hyphae of the strain were light brownish-gray. The vegetative mycelia were pale yellow to pale yellowish-brown. These characteristics were observed on yeast extract-starch agar. The diaminopimelic acid isomers in whole-cell hydrolysates of strain MK931-CF8 were determined to be the LL-form by the method of Staneck and Roberts.[33] The 16S rRNA gene sequence (1478 bp, positions 28−1524, *Escherichia coli* numbering system[34] of the strain) was determined and deposited in Genbank under the accession number AB688982. The sequence of the strain showed high identity with those of the genus *Streptomyces* such as *Streptomyces tanashiensis* (NBRC 12919^{T}, T: Type strain, Genbank/EMBL/DDBJ database accession no. AB184245, 1467/1475 bp, 99%), *S. nashvillensis* (NBRC 13064^{T}, AB184286, 1467/1477 bp, 99%), *and*

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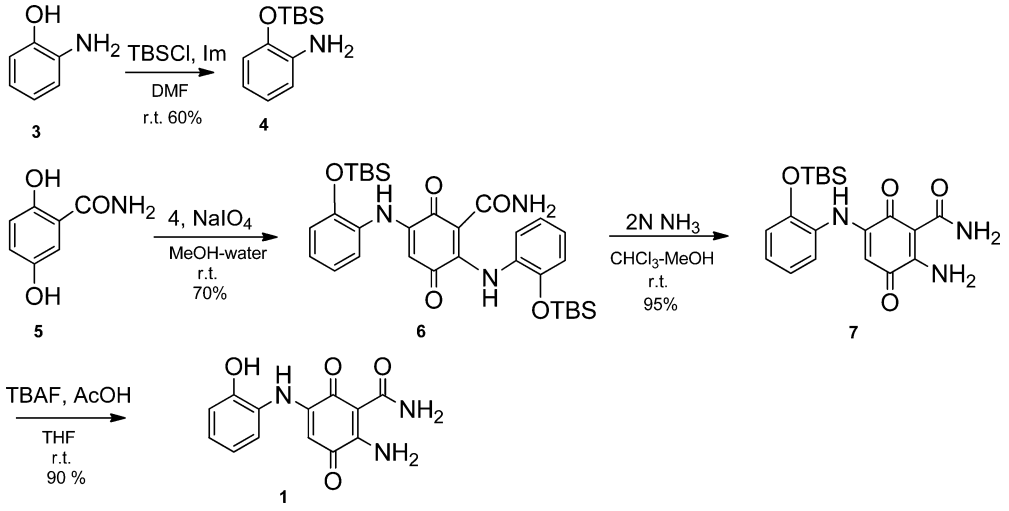
Scheme 1. Synthesis of 1



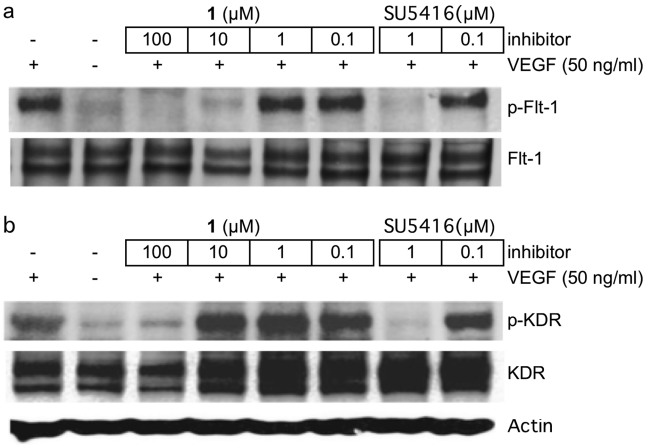


Figure 3. Effect of **1** on the autophosphorylation of Flt-1 (VEGFR-1) (a) and KDR (VEGFR-2) (b) in NIH3T3 cells.

S. polychromogenes (NBRC 13072^T, AB184292, 1454/1470 bp, 98%). These morphological characteristics together with genetic analysis of strain MK931-CF8 suggested that the strain belongs to the genus *Streptomyces*. Therefore, the strain was designated as *Streptomyces* sp. MK931-CF8

Fermentation and Isolation of 1. A slant culture of *Streptomyces* sp. MK931-CF8 was inoculated into 110 mL of medium in 500 mL baffled flasks and incubated for 2 days at 27 °C on a rotary shaker at 180 rpm. Each flask contained 110 mL of seed medium consisting of 2% galactose, 2% dextrin, 1% soypeptone, 0.5% corn steep liquor, 0.2% $(NH_4)_2SO_4$, and 0.2% $CaCO_3$ in deionized water and was adjusted to pH 7.2 before sterilization. Aliquots (7 mL) of the seed culture were transferred to fifty 500 mL Erlenmeyer flasks containing sterilized solid production medium composed of barley (15 g) and deionized water (25 mL), and the medium was cultivated statically at 30 °C for 17 days. Ethanol was added to the medium, and the combined ethanol extracts (2000 mL) from the solid cultures were evaporated to give a brown residue, which was dissolved in 8000 mL of water. The aqueous solution of the residue was loaded onto HP-20 (600 mL) (Mitsubishi Chemical Co., Tokyo, Japan), washed with 60% MeOH, and then eluted with MeOH. The MeOH fractions were combined and evaporated to give a brown solid (2.27 g). The solid was subjected to silica gel column chromatography (62 g, Cica silica gel 60N, Kanto Chemical Co. Inc., Tokyo, Japan) and eluted with 200 mL of $CHCl_3$/MeOH (50:1) followed by 400 mL of $CHCl_3$/MeOH (40:1). The active fractions (140 mL) were combined and evaporated to give solids, which were further subjected to column chromatography over Sephadex LH-20 (GE Healthcare Bio-Sciences AB, Uppsala, Sweden) and eluted with MeOH to yield **1** (43 mg).

Vegfrecine (1): magenta, amorphous solid; UV (MeOH) λ_{max} (log ε) 248 (4.02), 268 (4.06), 334 (4.04), 524 (3.30) nm, (MeOH-HCl) λ_{max} (log ε) 248 (4.02), 268 (4.06), 334 (4.04), 523 (3.29) nm, (MeOH/NaOH) λ_{max} (log ε) 297 (3.94), 340 (3.95), 592 (3.72) nm; IR (KBr) ν_{max} 3417, 3311, 1633, 1581, 1519, 1459, 1375, 1349 cm^{-1}; ^{1}H and ^{13}C NMR data are listed in Table 1; HRESIMS (positive) m/z 274.0822 (M + H)$^+$ (calcd for $C_{13}H_{12}N_3O_4$, 274.0822).

Synthesis of 2. To a solution of **1** (4 mg, 0.0146 mmol) in dimethyl formamide (0.2 mL) were added methoxymethyl chloride (11 μL, 0.146 mmol) and *N*,*N*-diisopropylethylamine (51 μL, 0.292 mmol), and the reaction mixture was stirred overnight. The reaction mixture was diluted with ethyl acetate, washed with water, dried over $MgSO_4$, and filtered. The filtrate was evaporated to give a crude solid, which was subjected to preparative TLC on silica gel developed with a mixture of chloroform and methanol (50:1) to give **2** (4.2 mg, 80% yield). **2**: ^{1}H and ^{13}C NMR data are listed in Table 1; HRESIMS (positive) m/z 384.1158 (M + Na)$^+$ (calcd for $C_{17}H_{19}N_3O_6Na$, 384.1166).

Synthesis of 1. To a solution of **5** (3.13 g, 20.4 mmol) in a mixture of MeOH and water (1:2, 664 mL) were added compound **4**[25] (10.5 g, 47 mmol) in MeOH (66 mL) and $NaIO_4$ (15.27 g, 71.4 mmol) in water (132 mL), and the reaction mixture was stirred overnight at room temperature. The reaction mixture was diluted with ethyl acetate, washed with water, dried over $MgSO_4$, and filtered. The filtrate was evaporated to give a crude solid, which was subjected to column chromatography on silica gel. Elution with a mixture of toluene and acetone (20:1) gave **6** (8.4 g, 70% yield).

6: ^{1}H NMR (600 MHz, $CDCl_3$) δ 0.16 (6H, s), 0.23 (6H, s), 0.91 (9H, s), 1.02 (9H, s), 5.54 (1H, brd, *J* 3.3 Hz), 6.05 (1H, s), 6.85 (1H, d, *J* 8.5 Hz), 6.90 (1H, dd, *J* 1.4 and 7.8 Hz), 6.91−7.0 (2H, m), 7.03 (1H, dd, *J* 0.6 and 3.1 Hz), 7.08−7.16 (2H, m), 7.32 (1H, dd, *J* 0.6 and 3.2 Hz), 8.53 (1H, s), 9.36 (1H, brs); ^{13}C NMR (150 MHz, $CDCl_3$) δ −4.35, 18.1, 18.2, 25.6, 25.7, 98.6, 98.8, 119.4, 119.9, 121.1, 121.5, 121.6, 125.4, 125.6, 127.6, 129.0, 131.7, 144.9, 147.6, 148.8, 157.1, 171.0, 177.5, 178.6; HRESIMS (positive) m/z 616.2620 (M + Na)$^+$ (calcd for $C_{31}H_{43}N_3O_5NaSi_2$, 616.2633).

To a solution of **6** (10.8 g, 18.1 mmol) was added a 7 M ammonia in methanol solution (Aldrich) (114 mL), and the reaction mixture was stirred at room temperature for 2 h. Evaporation of the solvent gave a crude solid, which was subjected to column chromatography on silica gel. Elution with a mixture of $CHCl_3$ and MeOH (20:1) gave **7** (6.65 g, 95%).

7: ^{1}H NMR (600 MHz, $CDCl_3$) δ 0.04 (6H, s), 0.81 (9H, s), 5.37 (1H, brs), 5.97 (1H, s), 6.72 (1H, dd, *J* 1.2 and 8.4 Hz), 6.81 (1H, dt, *J* 1.2 and 7.8 Hz), 6.90 (1H, dt, *J* 1.2 and 7.8 Hz), 7.17 (1H, dd, *J* 1.8 and 7.8 Hz), 7.45 (1H, brs), 8.54 (1H, brs), 8.79 (1H, brs), 10.65 (1H,

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Table 1. ^{1}H and ^{13}C NMR Data of 1 and 2[a]

	1		2	
position	δ_C, type	δ_H (J in Hz)	δ_C, type	δ_H (J in Hz)
1	96.5 =C		97.2 =C	
2	157.3 =C		154.5 =C	
3	175.7 C=O		178.2 C=O	
4	95.9 =CH	5.55, s	104.8 =CH	5.89, s
5	148.2 =C		154.7 =C	
6	176.6 C=O		178.7 C=O	
1′	124.4 =C		135.8 =C	
2′	151.0 =C		150.6 =C	
3′	116.2 =CH	6.96, dd (1.0, 7.7)	115.3 =CH	7.10, dd (1.0, 7.6)
4′	127.4 =CH	7.12, dt (1.5, 7.7)	126.7 =CH	7.21, dt (1.0, 7.6)
5′	119.2 =CH	6.86, dt (1.0, 7.7)	122.1 =CH	7.01, dt (1.0, 7.6)
6′	124.2 =CH	7.26, dd (1.5, 7.7)	127.4 =CH	7.19, dd (1.0, 7.6)
1-CONH$_2$	167.9		169.4	
1-CO*NH*$_2$		7.42, d (3.2)		7.17, d (3.5)
		8.66, d (3.2)		8.18, d (3.5)
2-NH$_2$		8.95, br		8.56, br
		11.00, br		10.50, br
5-NH		9.19, s		
2′-OH		10.09, s		
MOM1			94.3 CH$_2$	5.13, s
			55.7 CH$_3$	3.30, s
MOM2			85.0 CH$_2$	5.01, s
			55.9 CH$_3$	3.24, s

[a]NMR spectra were measured at 600 MHz for **1** in DMSO-d_6 and 700 MHz for **2** in DMSO-d_6. Chemical shifts are expressed in ppm with TMS as an internal standard.

Table 2. Inhibitory Activities of 1 and SU5416

	IC_{50} (μM)	
RTK	1	SU5416
Flt-1 (VEGFR-1)	0.018	3
KDR (VEGFR-2)	0.011	1
Flt-4 (VEGFR-3)	0.0018	0.5
PDGF-α	3.2	0.2
PDGF-β	0.48	0.4
EGF	2.6	>100
FGF	0.73	5

brs); ^{13}C NMR (150 MHz, $CDCl_3$) δ −4.30, 18.1, 25.5, 25.6, 96.7, 97.2 (br), 119.5, 121.7, 122.1, 126.6, 127.8, 128.2, 146.8, 148.2, 149.6, 156.6, 170,0, 176.4, 177.1; HRESIMS (positive) m/z 388.1681 $(M + H)^+$ (calcd for $C_{19}H_{26}N_3O_4Si$, 388.1687).

To a solution of **7** (1.04 g, 2.71 mmol) in tetrahydrofuran (303 mL) were added acetic acid (5.89 mL, 102.9 mmol) and a 1 M tetrabutylammonium fluoride solution (2.7 mL) in tetrahydrofuran, and the reaction mixture was stirred at room temperature for 5 min. The reaction mixture was diluted with ethyl acetate, washed with water, dried over $MgSO_4$, and filtered. The filtrate was evaporated to give a solid, which was washed with MeOH to afford synthetic compound **1** (660 mg, 90% yield). The ^{1}H and ^{13}C NMR spectra of synthetic compound **1** were identical to those of vegfrecine (see Supporting Information).

***In Vitro* Tyrosine Kinase Assay.** The kinase domain of Flt-1 was expressed as a GST-fusion protein by infection of Sf9 cells with engineered baculoviruses. GST-Flt-1 was purified from infected Sf9 cell lysates on glutathione sepharose. The enzymes KDR, Flt-4 (VEGFR-3), PDGFR-α and -β, EGFR, and FGFR were purchased from Invitrogen Co Ltd. The assays were performed in 96-well filter plates. The assay buffers were 20 mM Tris-HCl, 3 mM $MnCl_2$, 3 mM $MgCl_2$, 50 μg/mL poly GluTyr, 100 mM NaCl, 1 μM Na_3VO_4, 1 μM DTT, and 0.5% BSA at pH 7.4 for Flt-1, Flt-4, and PDGFR-α and -β, and 20 mM Tris-HCl, 1 mM $MnCl_2$, 10 mM $MgCl_2$, 50 μg/mL poly GluTyr, 100 mM NaCl, 1 μM Na_3VO_4, 1 μM DTT, and 0.5% BSA at pH 7.4 for KDR, EGFR, and FGFR. Each kinase (55 ng/20 μL buffer) and the compound dissolved in 2% DMSO buffer solution (10 μL) were added to a well, and the reaction was started by addition of 4 μM ATP/10 μCi/mL ^{32}P-ATP buffer solution (10 μL). After incubation for 20 min at room temperature the reaction was stopped by the addition of 30% TCA. Filter plates were washed three times with 15% TCA, and radioactivity was measured on a scintillation counter (Tri-Carb 2800TR, PerkinElmer, Waltham, MA, USA).

Cell Culture. Flt-1 overexpressing NIH3T3-Flt-1 cells and KDR overexpressing NIH3T3-KDR cells were grown in Dulbecco's modified Eagle's medium supplemented with 10% fetal calf serum, 2 mmol of glutamine, and 200 μg/mL G418. Human umbilical vein endothelial cells were obtained from LONZA (Walkersville, MD, USA) and cultured in endothelial growth medium (LONZA).

Cytotoxicity Assay. Cell viability was measured by the MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) colorimetric method.[35] The cells were seeded in 96-well microplates at densities of 3×10^3cells in 100 μL of growth medium per well in the absence or presence of test samples. After 2 days' incubation, MTT (10 μL of 5 mg/mL) was added to each well, and the plate was further incubated for 2 h. Resultant MTT formazan was solubilized with 10% sodium dodecyl sulfate overnight, and the absorbance at 570 nm was measured using an ARBO-SX 1420 multilabeled counter (PerkinElmer).

Immunoblotting. Subconfluent cells were starved for 16 h and then incubated for 2 h with samples. Cells were subsequently stimulated for 5 min with 50 ng/mL recombinant human VEGF-A (R&D Systems Inc., Minneapolis, MN, USA). Cells were then lysed in lysis buffer (50 mM HEPES [pH 7.2], 1% Triton X-100, 150 mM NaCl, 10% glycerol, 1 mM EGTA, 1 mM Na_3VO_4, 1 mM PMSF, 500 U/mL aprotinin), and the insoluble material was removed by centrifugation. Equal amounts of protein were separated electrophoretically on 7.5% polyacrylamide-SDS gels and transferred to polyvinylidene difluoride membranes. The membranes were blocked in blocking agent (5% nonfat milk in Tris-buffered saline containing

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0.1% Tween 20) and incubated with anti-p-tyr antibody (Cell Signaling Technology), anti-Flt-1 goat polyclonal antibody (R&D systems Inc.), anti-KDR rabbit polyclonal antibody (SantaCruz Biotechnology Inc., Santa Cruz, CA, USA), and anti-actin mouse monoclonal antibody (Abcam PLC., Cambridge, UK).

To analyze the phosphorylation of Flt-1, cell lysates were immunoprecipitated with anti-Flt-1 rabbit polyclonal antibody (Santa Cruz Biotechnology Inc.), and immune complexes were then separated by SDS-PAGE gel and blotted with the antibodies described above.

■ ASSOCIATED CONTENT

Ⓢ Supporting Information

^{1}H and ^{13}C NMR, $^{1}H-^{1}H$ COSY, $^{1}H-^{13}C$ HMQC, and $^{1}H-^{13}C$ HMBC spectra of **1**. ^{1}H and ^{13}C NMR, $^{1}H-^{13}C$ HMQC, $^{1}H-^{13}C$ HMBC, and $^{1}H-^{15}N$ HMBC spectra of **2**. ^{1}H and ^{13}C NMR spectra of synthetic vegfrecine (**1**). This material is available free of charge via the Internet at http://pubs.acs.org.

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Author Contributions

The manuscript was written through contributions of H. Adachi, C. Nosaka, R. Sawa, and N. Kinoshita. All authors have given approval to the final version of the manuscript.

Notes

The authors declare no competing financial interest.

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