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RP-1776, a Novel Cyclic Peptide Produced by *Streptomyces* sp., Inhibits the Binding of PDGF to the Extracellular Domain of Its Receptor

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RP-1776, a novel cyclic peptide, was isolated from the culture broth of *Streptomyces* sp. KY11784. RP-1776 selectively inhibited the binding of PDGF BB to the extracellular domain of the PDGF β-receptor with an IC_{50} value of $11\pm6\,\mu M$. Detailed binding experiments suggested that RP-1776 directly interacts with PDGF BB. RP-1776 inhibited the phosphorylation of the PDGF β-receptor induced by PDGF BB. These results suggested that RP-1776 antagonizes the signaling of PDGF BB probably through the inhibition of PDGF BB binding to the PDGF β-receptor.

Platelet-derived growth factor (PDGF) is a potent mitogen and chemotactic molecule for various cells, released from activated platelets.[1,2] PDGF is also expressed in embryonal tissues, and has neurotrophic activity for neuronal cells in rat brain, suggesting involvement of PDGF in development or maintenance of cells. And the fact that PDGF can stimulate growth of connective tissue and the production of matrix constituents indicates a role of PDGF in wound healing.

Evidence indicates that PDGF may be involved in the pathogenesis of various proliferative diseases including tumorigenesis, fibrosis, atherosclerosis, and restenosis after angioplacity.[3~5] Recent finding showed that anti-PDGF antibody reverted the transformed phenotype of smooth muscle cells into normal or prevented vascular intimal hyperplasia.[6] Therefore, compounds which block the activation of the PDGF receptor would be potential antitumor drugs or could be effective for the treatment of atherosclerosis.

There are two subtypes of PDGF, PDGF A and PDGF B, which form homo- or hetero-dimer, resulting three isoforms, AA, AB, and BB. These isoforms can interact with the extracellular domains of their specific receptor, designated as α-receptor and β-receptor with different affinities.[7~9] Both receptors have tyrosine kinase domains in their intracellular regions, and the receptor dimerization induced by PDGFs followed by autophosphorylation of the receptor is essential for the signal transduction of PDGF into the cells.[10,11]

In the course of our screening work to obtain inhibitors of the binding of PDGF to its receptor, we found a novel cyclic peptide, RP-1776, produced by an actinomycete, *Streptomyces* sp. KY11784. Here we report the fermentation, purification, and biochemical properties of RP-1776.

Materials and Methods

Materials

The extracellular domain of the recombinant human PDGF α-receptor, the extracellular domain of the

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recombinant human PDGF β-receptor, recombinant human PDGF AA with KT3 epitope (TPPPEPET), and anti-KT3 antibody were prepared as described by FRETTO *et al.*[12] PDGF BB was purchased from R & D Research (Minneapolis, MN, USA). Anti-PDGF BB antibody was from Collaborative Biomedical Products (Bedford, MA, USA). Anti-PDGF β-receptor antibody (1B5B11) was raised as described previously.[13] Anti-phosphotyrosine antibody was purchased from Transduction Laboratories (Lexington, KY, USA). Peroxidase-coupled anti-goat IgG, peroxidase-coupled anti-rabbit IgG, and peroxidase-coupled anti-mouse IgG were from Boeringer Mannheim (Indianapolis, IN, USA). All chemicals were of analytical grade.

Microorganism

The RP-1776 producing strain KY11784 was isolated from a soil sample collected in Sakai-shi, Osaka, Japan. This strain has been deposited at the National Institute of Bioscience and Human-Technology, Agency of Industrial Science and Technology, Tsukuba-shi, Ibaraki, Japan, as *Streptomyces* sp. KY11784 with the accession number FERM BP-5396.

Taxonomical Characterization

Cultural and physiological characteristics of strain KY11784 were determined by the methods of the International *Streptomyces* Project[14] (ISP). Color codes were assignated to the substrate and aerial mass pigments according to the Color Harmony Manual.[15] Morphology and spore surface ornamentation of the strain were determined by light and scanning electron microscopy (HITACHI S-570). The temperature ranges for growth of the strain was determined after submerged cultivation with ISP No. 5 medium for 14 days. The diaminopimelic acid isomer was determined by the method of HASEGAWA *et al.*[16] on the hydrolysate of aerial mycelia grown of the solid medium ISP No. 4. Menaquinones were extracted as described by ATHALYE *et al.*[17] and analyzed by reversed-phase HPLC.

Culture and Medium Conditions

Mycelia of the microorganism grown on an agar slant were inoculated into 10 ml of seed medium composed of D-glucose 1%, soluble starch 1%, Bactotryptone 0.5%, yeast extract 0.5%, meat extract 0.3%, and $Mg_3(PO_4)_2 \cdot 8H_2O$ 0.05% (pH 7.2 before sterilization) in a 50-ml culture tube. The inoculated tube was incubated for 5 days at 28°C on a reciprocating shaker. A 5-ml aliquot of the seed culture was transferred into a 300-ml Erlenmeyer flask containing 50-ml seed medium and the inoculated flask was incubated for 2 days at 28°C on a rotary shaker. A 50-ml aliquot of the cultured broth of the second culture was transferred into a 2-liter Erlenmeyer flask containing 500-ml seed medium and the inoculated flask was incubated for 3 days at 28°C on a rotary shaker. A 1500-ml aliquot of the cultured broth of the third culture was transferred into a 30-liter jar fermenter containing 15-liter fermentation medium consisting of soluble starch 4%, soybean meal 1%, corn steep liquor 0.5%, dried yeast 0.5%, KH_2PO_4 0.05%, $Mg_3(PO_4)_2 \cdot 8H_2O$ 0.05%, $ZnSO_4 \cdot 7H_2O$ 10 μg/ml, $CoCl_2 \cdot 6H_2O$ 1 μg/ml, and Diaion HP-20 10% (v/v) (pH 7.0 before sterilization). The jar fermenter was operated for 4 days at 28°C with agitation at 200 rpm and aeration of 1 v/v/m.

Determination of RP-1776

To detect RP-1776, HPLC analysis was adopted. The condition of HPLC was described as follows. The cultured broth was centrifuged to obtain mycelia. The mycelia were extracted with methanol, and the methanol extract was injected onto an HPLC column (YMC ODS AQ-312, 6 mm i.d.×150 mm) developed at 40°C with 78% methanol containing 0.1% trifluoroacetic acid at a flow rate of 1 ml/minute monitoring absorbance at 220 nm.

Ligand Binding Assays

The standard PDGF binding assay was performed for routine measurements by a solid-phase method using the specific antibody for PDGF according to the method of FRETTO *et al.*[12] In brief, the extracellular domain of the PDGF α- or β-receptor protein was immobilized in 96-well plates, and incubated with 13 ng/ml PDGF AA having the KT3 epitope or 5 ng/ml PDGF BB, and a sample dissolved in methanol or dimethyl sulfoxide for 2 hours at room temperature in the reaction mixture composed of 25 mM HEPES (pH 7.8), 100 mM NaCl, 0.01% Tween 20, and 0.3% gelatin. Each well was washed with the wash buffer consisting of 25 mM HEPES, 100 mM NaCl, and 0.01% Tween 20 (pH 7.6). The amount of PDGF bound to the receptors was detected with the anti-KT3 antibody or the anti-PDGF BB antibody. After incubation of the wells with 5 μg/ml of the anti-KT3 antibody or 2.5 μg/ml of the anti-PDGF BB in the reaction mixture for an hour at 37°C, the wells were washed again with the wash buffer. These wells were incubated with the secondary antibody (peroxidase-conjugated antibody, ×1,000 dilution) for an hour at 37°C. After washing the plates, the substrates of peroxidase, azinobis-3-ethylbenzthiazoline-6-sulfonic acid (ABTS) and H_2O_2, were added. The product of the enzyme reaction was

Table 1. Cultural characteristics of the strain KY11784.

Medium	Amount of growth	Color of: Aerial mycelium	Color of: Substrate mycelium	Soluble pigment
Yeast extract-malt extract agar (ISP No.2)	abundant	white (a)	mustardbrown (2pi)	brown
Oatmeal agar (ISP No.3)	abundant	oatmeal (2ec)	Lt mustard tan (2ie)	brown
Inorganic salt-starch agar (ISP No. 4)	abundant	oatmeal (2ec)	bamboo (2ge)	brown
Glycerol-asparagine agar (ISP No. 5)	abundant	white (a)	oatmeal (2ec)	none
Tyrosine agar (ISP No. 7)	abundant	oatmeal (2ec)	bamboo (2ge)	brown
Sucrose-nitrate agar	moderate	parchment (1cb)	oatmeal (2ec)	none
Glucose-asparagine agar	abundant	white (a)	beige (3ge)	none
Nutrient agar	abundant	white (a)	Lt gold (2ic)	brown

detected by monitoring the absorbance at 650 nm.

Another PDGF binding assay was also carried out using the immobilized PDGF ligand in 96-well plates. The PDGF BB-immobilized plates were incubated for 2 hours at room temperature with 5 μg/ml PDGF β-receptor extracellular domain and a sample dissolved in methanol or dimethyl sulfoxide in the reaction mixture described above. Each well was washed with the wash buffer described above. The amount of PDGF β-receptor bound to the ligand were detected with the anti-PDGF β-receptor antibody. After incubation of the wells with 0.1 μg/ml of the anti-PDGF β-receptor antibody in the reaction mixture for an hour at 37°C, the wells were washed again with the wash buffer. These wells were incubated with the secondary antibody (peroxidase-conjugated antibody, $\times$1,000 dilution) for an hour at 37°C. After washing the plates, the substrates of peroxidase were added. The product of the enzyme reaction was detected by monitoring the absorbance at 650 nm.

The inhibitory activity was determined from the following equation; inhibition (%)=100−[(OD_{650} in the presence of a sample)−(background)]/[(OD_{650} in the absence of a sample)−(background)]$\times$100, where background was measured in the absence of both samples and the ligands.

PDGF β-Receptor Phosphorylation in HR5 Cells

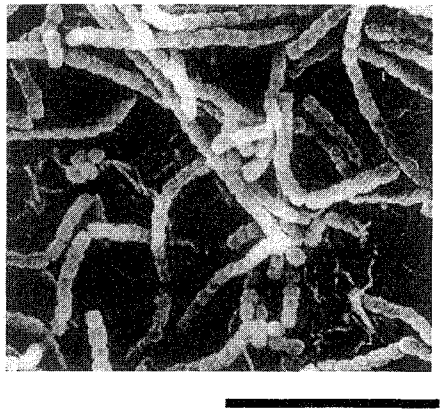
The detection of phosphorylation of the PDGF β-receptors was carried out according to the method by LOKKER *et al.*[13] In brief, a Chinese hamster ovary cell line, HR5, which stably overexpresses the human PDGF β-receptors was plated on 96-well plates, and cultured in RPMI1960 medium containing 10% fetal calf serum for 3 to 7 days. After reaching cofluency, cells were starved by withdrawal of serum and cultured for additionally 24 hours. The cells were preincubated with RP-1776 dissolved in methanol for 15 minutes at room temperature, and stimulated with 300 ng/ml PDGF BB for 1 hours on ice. The medium was discarded, and the cells were lysed with the lysis buffer containing 20 mM Tris-HCl (pH 7.5), 1% Triton X-100, 1 mM sodium vanadate, 10 mM sodium orthopyrophosphate, 50 mM sodium fluoride, 10 μg/ml leupeptin, 10 μg/ml aprotinin, and 1 mM phenylmethane-sulphonyl fluoride. The cell lysate was transferred to a 96-well plate coated with the anti-PDGF β-receptor antibody (1B5B11), and incubated for 2 hours to capture the PDGF β-receptors in the cell lysates. The wells were washed, and the phosphorylation of the PDGF receptor was detected with anti-phosphotyrosine antibody and peroxidase-conjugated anti-rabbit IgG. The product of the peroxidase reaction using ABTS as substrates were detected by monitoring absorbance at 650 nm, or that using tetra-methylbenzidine were detected by monitoring at 450 nm.

Results

Cultural and Morphological Characteristics of Strain KY11784

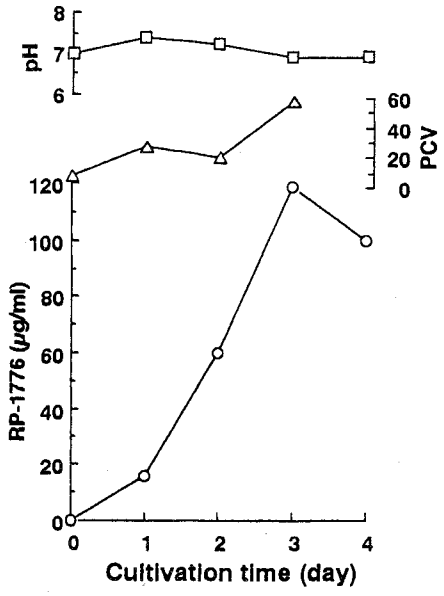
Strain KY11784 grew well on various agar media but grew only moderately on the sucrose-nitrate agar medium

Fig. 1. Scanning electron micrograph showing the smooth surfaces of spores of the strain KY11784.



Bar represents 10 μm.

Fig. 2. Time course of cultivation of *Streptomyces* sp. KY11784.



The small amount of cultured broth sampled every day was centrifuged to obtain mycelia. The productivity of RP-1776 was determined as described in Materials and Methods. The production of RP-1776 in the mycelia (○), packed cell volume (%) (△), and pH (□) are indicated.

as shown in Table 1. The color of the aerial mycelium of strain KY11784 was white or oatmeal on any agar media listed in Table 1 after incubation at 28°C for 14 days. Strain

Table 2. Physiological properties of the strain KY11784.

Characteristics	KY11784
Temperature for growth	6℃～35℃
Optimum temperature	28℃～30℃
Liquefaction of gelatin	positive
Hydrolysis of starch	positive
Coagulation of milk	positive
Peptonization of milk	positive
Melanine pigment production	
on peptone-yeast extract-iron agar	negative
on tyrosine agar	positive
Utilization	
D-glucose	positive
L-arabinose	positive
D-xylose	positive
D-fructose	positive
L-rhamnose	positive
sucrose	negative
raffinose	negative
D-mannitol	positive
myo-innositol	negative

KY11784 produced a brown soluble pigment during cultivation on ISP No. 2, No. 3, No. 4, No. 7 and the nutrient agar medium (Table 1). Strain KY11784 produced the aerial mycelium which consisted of straight chains of 20 or more short rod-shaped smooth-surfaced spores (0.6～0.8 by 0.7～0.9 μm) as shown in Figure 1. No fragmentation of substrate mycelia was observed, and sclerotia, flagellated spores were not formed in the cultures. The physiological characteristics of strain KY11784 are shown in Table 2. This strain utilized glucose, arabinose, xylose, fructose, rhamnose, and mannitol for growth, but not sucrose, raffinose, and *myo*-inositol. Analysis of cell hydrolysates of the strain revealed that the cell walls contained LL-diaminopimelic acid. The predominant menaquinone was MK-9 (H_6), and there was a significant amount of MK-9 (H_8). These taxonomic observations indicate that strain KY11784 belongs to the genus *Streptomyces*.

Production of RP-1776 by Fermentation

The time course of RP-1776 production in a 30-liter jar fermenter is shown in Figure 2. The production of RP-1776 in the culture broth initiated on day 1 and the amount of RP-1776 reached maximum on day 3.

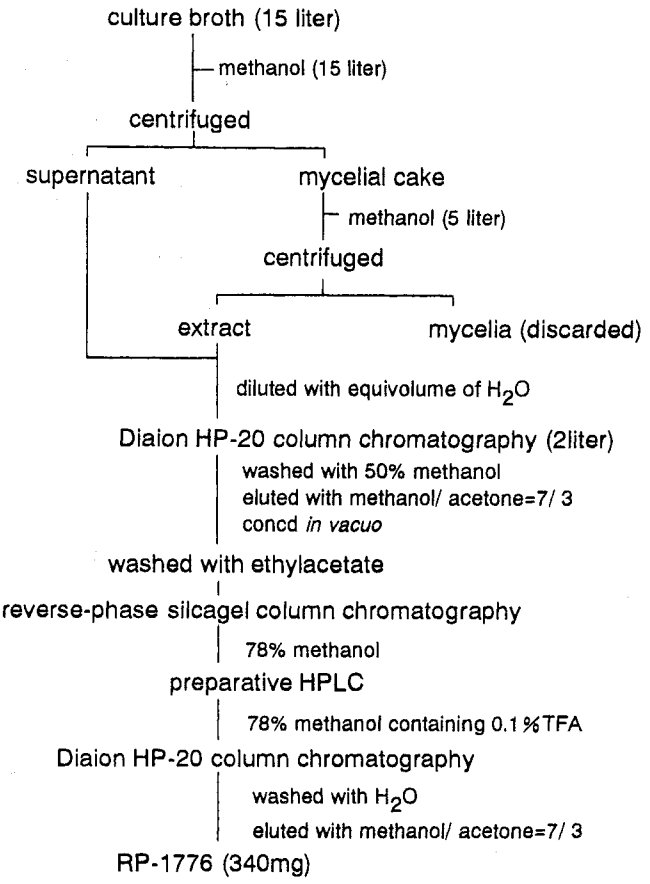
Isolation and Purification

The isolation procedures for RP-1776 are outlined in Figure 3. Fifteen liter of methanol was added to the whole fermentation broth (15 liter) and centrifuged. The obtained mycelial cake was extracted again with methanol. The formerly obtained supernatant and the latter methanol extract were mixed together and diluted with the equal volume of water, and applied to a Diaion HP-20 column (2 liter). The column was washed with 50% methanol, and adsorbed material was eluted with methanol - acetone (7 : 3). Fractions containing RP-1776 were pooled and concentrated *in vacuo*. The crude materials were suspended in water and washed with ethyl acetate. The aqueous layer was collected and concentrated *in vacuo* to dryness to yield brownish powder. The crude powder was dissolved in a small amount of 78% methanol and applied to a reverse-phase silica gel column (YMC ODS AQ-S50, 200 ml) and developed with 78% methanol monitoring absorbance at 220 nm. The fractions containing RP-1776 were pooled and evaporated *in vacuo* to dryness. The crude RP-1776 was dissolved in a small amount of 78% methanol containing 0.1% trifluoroacetic acid, and injected to a preparative HPLC column (YMC D-ODS-5AQ, 2 cm i.d.×25 cm). The HPLC was performed with 78% methanol containing 0.1% trifluoroacetic acid as elution solvent monitoring absorbance at 220 nm. The fractions containing RP-1776 were combined, and diluted with equivolume of water. The diluted fraction was loaded on a Diaion HP-20 column. The column was washed with water, then eluted with methanol - actetone (7 : 3). The evaporation of the elution solvent gave 340 mg of RP-1776.

Physico-chemical Properties of RP-1776

Physico-chemical properties and ^{13}C and ^{1}H NMR data of RP-1776 are summarized in Table 3 and Table 4, respectively. RP-1776 is readily soluble in methanol, and ethanol, and virtually insoluble in chloroform and water. The structures of RP-1776 was determined as a novel cyclic peptide shown in Figure 4. RP-1776 consists of eleven amino acids including five unusual amino acids.

Fig. 3. The purification procedure of RP-1776.



Biochemical Properties of RP-1776

RP-1776 inhibited the binding of PDGF BB to the extracellular domain of PDGF β-receptor in a dose-dependent manner (Figure 5). An IC_{50} value for the binding was calculated to be $11 \pm 6\ \mu M$ (mean±SD, n=4).

PDGF BB can bind to both PDGF α-receptor and PDGF β-receptor, while PDGF AA can bind to only the α-receptor. RP-1776 inhibited PDGF BB binding to the PDGF α-receptor extracellular domain and the PDGF β-receptor extracellular domain in the same concentration range (Figure 5). Nevertheless, RP-1776 inhibited the binding of PDGF AA to the PDGF α-receptor extracellular domain much less potently (approximately 20% of the total binding) even at a concentration of 132 μM. These results suggest that the effect of RP-1776 depends on the ligand isoform but not the subtype of the PDGF receptor, and indicate the possibility of the interaction of RP-1776 with PDGF BB.

In order to confirm the interaction of RP-1776 with PDGF BB, the effect of RP-1776 on PDGF BB immobilized on a 96-well plate was examined. RP-1776

Table 3. Physico-chemical properties of RP-1776.

appearance		white powder
melting point		228°C-230°C
$[\alpha]_D^{25}$		+27.9 ° (*c* 0.5, MeOH)
high resolution FAB-MS		
found	*m/z*	1505.6598$[M+Na]^+$
calc.	*m/z*	1505.6605$[M+Na]^+$
		($C_{75}H_{94}N_{12}O_{20}Na$)
elemental analysis		
found		C 58.59; H 6.62; N 10.50 %
calc.		C 58.58; H 6.55; N 10.93 %
fomula		$C_{75}H_{94}N_{12}O_{20}$
UV	λ_{max} (MeOH)(ε)	222 (81,600), 282 (21,500), 290 (18,800) nm
IR	ν_{max} (KBr)	3317, 1734, 1657, 1516, 1456, 1250, 1063 cm^{-1}
TLC (Rf value)		0.22[a]
		0.79[b]
		0.74[c]
colorization reaction		
positive		I_2 vapor, H_2SO_4, anisaldehyde, Ridon-Smith
negative		ninhydrin
solubility		
soluble		methanol, ethanol
insoluble		chloroform, H_2O

[a] Silicagel $60F_{254}$; chloroform/ methanol=9/ 1
[b] Silicalgel $60F_{254}$; chloroform/ methanol/ ethanol/ H_2O=10/ 4/ 4/ 2
[c] RP-$18F_{254}$s; methanol

was incubated for 1 hour with the immobilized PDGF BB prior to the addition of the PDGF β-receptor extracellular domain, and removed by washing the plates followed by the addition of the receptor extracellular domain. After incubation with the receptor, the amount of the PDGF β-receptor extracellular domain bound to the RP-1776-treated PDGF BB was measured. The PDGF β-receptor extracellular domain did not bind to the RP-1776-treated PDGF BB (Figure 6A). On the other hand, RP-1776 prevented the binding of the PDGF β-receptor extracellular domain to the immobilized PDGF BB without washing out (Figure 6A). These data suggest that RP-1776 binds to PDGF BB tightly and inhibits the binding of the PDGF β-receptor extracellular domain to the immobilized PDGF BB. To the contrary, PDGF BB bound to the immobilized RP-1776-treated, and washed PDGF β-receptor extracellular domain (Figure 6B). This suggests that RP-1776 interacts weakly, if any, with the immobilized receptor.

Inhibition of Phosphorylation of PDGF β-Receptor by RP-1776

PDGF BB stimulates phosphorylation of the PDGF β-receptors expressed on the cell surface of HR5 cells. The phosphorylation was inhibited by RP-1776 in a dose-dependent manner with an IC_{50} value of 30±9 μM (mean±SD, n=6) (Figure 7). Approximately 90% of HR5 cells were viable in the presence of 133 μM of RP-1776.

Antimicrobial Activity of RP-1776

RP-1776 exhibited weak antimicrobial activity against *Bacillus subtilis* No. 10707 and *Staphylococcus aureus* ATCC 6538P at concentrations of 105 μM and 140 μM, respectively, and no activity at 555 μM against *Candida albicans* ATCC 10231, *Enterococcus hirae* ATCC 10541, *Pseudomonas aeruginosa* BMH No. 1, *Escherichia coli* ATCC 26, *Proteus vulgaris* ATCC 6897, *Shigella sonnei* ATCC 9290, and *Klebsiella pneumoniae* ATCC 10031.

Discussion

We have isolated a novel compound, RP-1776, from the culture broth of *Streptomyces* sp. KY11784, which inhibited the PDGF BB binding to the PDGF β-receptor extracellular domain with an IC_{50} value of 11±6 μM.

Table 4. ^{13}C and ^{1}H NMR data for RP1776 in DMSO-d_6 (30°C).

	^{13}C NMR δ_C		^{1}H NMR δ_H		J (Hz)
Acyl					
1	168.68	s			
2	121.36	d	7.32	d	15.9
3	137.62	d	7.73	d	15.9
4	132.14	s			
5	125.52	d	7.67	d	7.8
6	126.95[1)]	d	7.24	m	
7	129.74	d	7.40	t	7.6
8	130.05	d	7.24	m	
9	137.17	s			
10	126.95	d	6.45	dd	11.5, 1.7
11	129.11	d	5.99	dq	11.5, 7.1
12	14.00	q	1.61	dd	7.1, 1.7
Thr					
NH			8.99	d	4.2
α	59.71	d	5.26	d	4.2
β	68.67	d	5.30	q	6.8
γ	15.61	q	1.05	d	6.8
C=O	173.21	s			
Ala					
NH			7.92	d	2.9
α	51.02	d	3.85	m	
β	16.37	q	1.28	d	7.3
C=O	172.01	s			
β-Me-Asp					
NH			7.54	d	10.0
α	53.01	d	4.86	dd	10.0, 8.1
β	40.20	d	3.09	m	
γ	174.92	s	12.20	br s	(COOH)
β-Me	13.16	q	1.08	d	7.1
C=O	170.26	s			
Gly					
NH			7.44	m	
α	42.23	t	4.00	dd	17.8, 8.6
			3.40	m	
C=O	169.27	s			
β-OH-Phe					
NH			7.25	m	
α	57.86	d	4.15	m	
β	70.57	d	4.19	m	
1	142.24	s			
2,6	125.64[1)]	d	7.31	m	
3,5	127.09[1)]	d	7.25	m	
4	127.51[1)]	d	7.25	m	
β-OH			5.44	d	5.4
C=O	172.12[2)]	s			
Pro					
α	60.12	d	4.32	m	
β	29.04	t	1.95	m	
			1.86	m	
γ	23.54	t	1.86	m	
			1.77	m	
δ	46.64	t	3.77	m	
			3.66	m	
C=O	169.88	s			
β-OH-*O*-Me-Tyr					
NH			6.83	d	10.3
α	56.69	d	4.95	dd	10.3, 5.1
β	72.39	d	4.41	d	5.1
1	131.48	s			
2,6	127.28	d	6.11	d	8.3
3,5	112.38	d	6.22	d	8.3
4	158.23	s			
β-OH					
4-OMe	54.60	q	3.57	s	
C=O	169.34	s			
Trp					
NH			8.84	d	8.1
α	53.04	d	4.60	m	
β	28.10	t	3.10	m	
			2.98	m	
1			10.79	d	2.0
2	124.83	d	7.34	m	
3	109.71	s			
4	127.02	s			
5	119.37	d	7.89	d	7.8
6	118.09	d	6.97	t	7.8
7	120.80	d	7.05	t	7.8
8	111.19	d	7.27	d	7.8
9	136.19	s			
C=O	172.04	s			
α-OH-Gly					
NH			9.28	d	8.8
α	71.32	d	5.81	d	8.8
α-OH			7.02	br s	
C=O	170.10[2)]	s			
Leu					
NH			8.99	m	
α	54.90	d	4.37	m	
β	38.55	t	1.91	m	
			1.53	m	
γ	24.49	d	1.75	m	
δ	22.62	q	0.96	d	6.3
	20.96	q	0.93	d	6.6
C=O	175.33	s			
β-OH-Leu					
NH			7.84	d	8.1
α	55.25	d	4.26	d	8.1
β	74.42	d	3.74	m	
γ	30.55	d	1.72	m	
δ	19.28	q	0.97	d	6.1
	18.21	q	0.74	d	6.8
β-OH			5.65	br s	
C=O	169.41	s			

[1), 2)] Assignments may be interchanged.

Fig. 4. The structure of RP-1776.

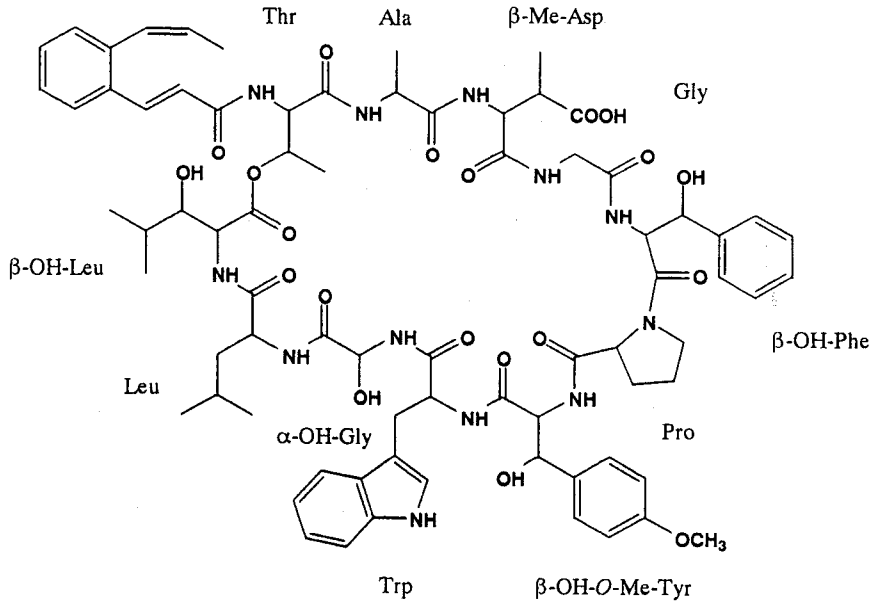
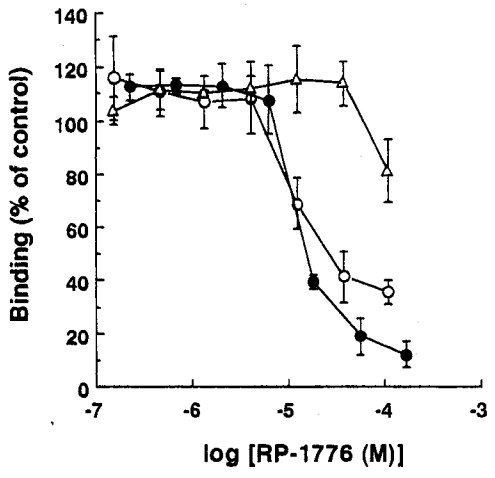


Fig. 5. The displacement curves of RP-1776 for the binding of various PDGF isoforms.



The effect of RP-1776 on the binding of PDGF BB to the α-receptor extracellular domain (○), the binding of PDGF BB to the β-receptor extracellular domain (●), and the binding of PDGF AA to the α-receptor extracellular domain (△) was indicated. Each error bar indicates SD. The experiment was performed at least in triplicate.

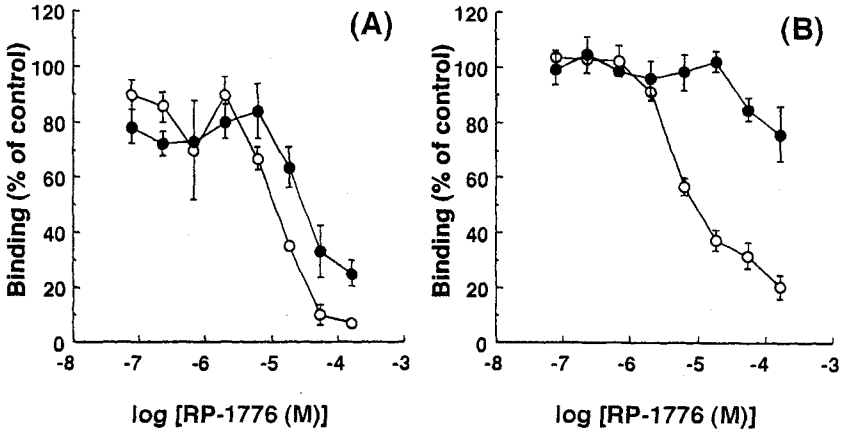
Several PDGF receptor antagonists have been reported so far. A 13-amino acid peptide corresponding to amino acid 116~121 and 157~163 in PDGF B chain was shown to inhibit the binding to the PDGF receptors selectively with IC_{50} values between 20 and 40 μM.[18] Neomycin blocked the PDGF binding with an IC_{50} value of 1 mM.[19] A synthetic compound, SCH 13929, selectively inhibited the PDGF binding to its receptor with an IC_{50} value of 0.12 μM.[20] The inhibitory potency of RP-1776 is almost equal to that of the 13-amino acid peptide.

RP-1776 did not block the binding of the PDGF AA to the PDGF α-receptor extracellular domain at concentrations up to 66 μM, and partially inhibited the binding at 132 μM, the maximal concentration of RP-1776 under the assay conditions because of the solubility limitation. These data indicate that RP-1776 selectively inhibits PDGF BB binding at these concentration ranges.

RP-1776 is a cyclic peptide possessing 2-[1-(*z*)-propenyl]-cinnamoyl moiety in its *N* terminal. There is a report of isolation of a cyclic peptide with the same moiety, WA9326A, of microbial origin as an antagonist of tachykinin.[21] Unlike WA9326A, which has seven natural amino acids, RP-1776 has eleven amino acids containing five unusual amino acids.

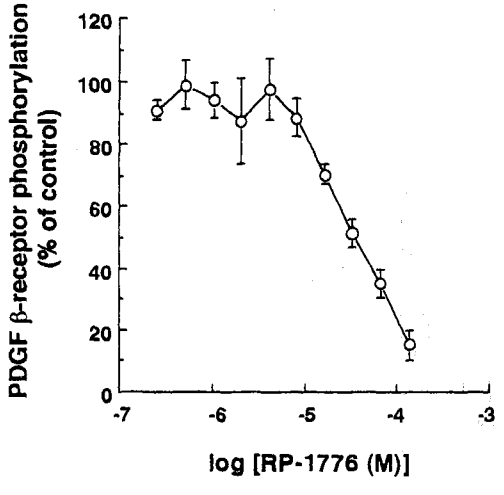
In conclusion, RP-1776 selectively inhibits the binding of PDGF BB and blocks phosphorylation of the PDGF β-receptor induced by PDGF BB probably through the

Fig. 6. The effect of preincubation of the immobilized PDGF BB or the immobilized β-receptor extracellular domain with RP-1776 on subsequent PDGF BB/the β-receptor binding.



The binding of the β-receptor extracellular domain to the immobilized PDGF BB (A) or the ligand to the immobilized receptor (B) was measured without preincubation with RP-1776 as described in Materials and Methods. Open circles; the ligand/the receptor binding was measured in the presence of various concentrations of RP-1776. Closed circles; RP-1776 at various concentrations was preincubated with the immobilized PDGF (A) or immobilized β-receptor extracellular domain (B) for 1 hour. After removal of RP-1776, subsequent the ligand/the receptor binding was measured. Each error bar indicates SD. The experiments were performed at least in triplicate.

Fig. 7. The effect of RP-1776 on PDGF BB-induced phosphorylation of the PDGF β-receptor.



The phosphorylation of the PDGF β-receptor expressed on HR5 cells was determined as described in Materials and Methods. Each error bar indicates SD. The experiments were performed at least in triplicate.

interaction with PDGF BB. RP-1776 could be a new tool to investigate the interaction of PDGF BB and the PDGF receptors and provide information on the design of new types of PDGF antagonists.

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