VOL. 52, NO. 3, MAR. 1999 THE JOURNAL OF ANTIBIOTICS pp. 235 — 244

RP-1551s, a Family of Azaphilones Produced by *Penicillium* sp., Inhibit the Binding of PDGF to the Extracellular Domain of Its Receptor

SHINICHIRO TOKI*, TAKEO TANAKA, YOUICHI UOSAKI, MAYUMI YOSHIDA, YASUHIRO SUZUKI†, KATSUNORI KITA†, AKIRA MIHARA†, KATSUHIKO ANDO, NATALIE A. LOKKER††, NEILL A. GIESE†† and YUZURU MATSUDA

Tokyo Research Laboratories, Kyowa Hakko Kogyo Co., Ltd., 3-6-6 Asahimachi, Machida-shi, Tokyo 194-8533, Japan
†Pharmaceutical Research Laboratories, Kyowa Hakko Kogyo Co., Ltd., 1188 Shimotogari, Nagaizumicho, Sunto-Gun, Shizuoka 411-8731, Japan
††COR Therapeutics, Inc., 256 E. Grand Avenue, South San Francisco, CA 94080, U.S.A.

(Received for publication December 4, 1998)

Nine azaphilones designated RP-1551-1, -2, -3, -4, -5, -6, -7, -M1, and -M2 were isolated from the culture broth of *Penicillium* sp. SPC-21609 as inhibitors of PDGF binding to its receptor. RP-1551s inhibit the binding of PDGF AA to the extracellular domain of PDGF α-receptor with IC_{50} values ranging from 0.1 to 2 μM without affecting PDGF BB binding to the extracellular domain of PDGF β-receptor. PDGF binding was not restored after the PDGF α-receptor extracellular domain was washed in an attempt to remove the RP-1551-1 bound to the receptor. This result suggests that RP-1551-1 may irreversibly interact with the PDGF α-receptor. Since many azaphilone compounds possess high reactivity with an amino group, RP-1551-1 may prevent PDGF AA binding by reacting with amino groups on the α-receptor extracellular domain.

Platelet-derived growth factor (PDGF) plays a role as a potent mitogen and chemotactic molecule for various cells such as fibroblasts, vascular smooth muscle cells, and glial cells.[1,2] PDGF and its receptor are believed to participate in various physiological events such as development or healing.

There are two subtypes of PDGF; PDGF A and PDGF B. These molecules form three types of homo or heterodimers (PDGF AA, PDGF BB, and PDGF AB), and can interact with the extracellular domains of their specific receptors, designated α-receptor and β-receptor, with different affinities.[3~5] Both the receptors have tyrosine kinase domains in their intracellular regions, and the dimerization of the receptor following autophosphorylation induced by the ligands is thought to be essential for signal transduction in cells.[6,7]

Evidence indicates that PDGF may be involved in the pathogenesis of various proliferative diseases including tumorigenesis,[8] fibrosis, atherosclerosis, and restenosis.[9,10] Recent findings showed that anti-PDGF antibody can revert the transformed phenotype or can prevent vascular intimal hyperplasia.[11] Therefore, drugs which block the activation of the PDGF receptor would have potential as antitumor substances or for treatment of atherosclerosis.

In the course of our screening work to obtain inhibitors of the binding of the PDGFs to their receptors, we found that azaphilones, designated RP-1551s, produced by a fungus, *Penicillium* sp. SPC-21609 have inhibitory activity. Here we report the fermentation, purification, and biochemical properties of RP-1551s.

Materials and Methods

Materials

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

combinant human PDGF β-receptor, recombinant human PDGF AA with the 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 obtained from Collaborative Biomedical Products (Bedford, MA, USA). Peroxidase-coupled anti-goat IgG and peroxidase-coupled anti-mouse IgG were from Boehringer Mannheim (Indianapolis, IN, USA). (+)-Sclerotiorin[13] was purified by conventional methods from an in-house fermentation of a fungus, *Penicillium* sp. RP-1719, which was recently isolated from soil. The structure of sclerotiorin was established by the analyses of its ^{1}H and ^{13}C NMR, UV, and mass spectra. All other chemicals were of analytical grade.

Microorganism

The producing microorganism, *Penicillium* sp. SPC-21609 (FERM BP-4768), was isolated from a soil sample collected in Kanagawa prefecture, Japan.

Culture and Medium Condition

Mycelia grown on an agar slant were inoculated into 10 ml of seed medium composed of mashed potato (Yukijirushi) 3%, D-glucose 10%, and yeast extract 0.5% (pH 6.5 before sterilization) in a 50-ml culture tube. The inoculated tube was incubated for 7 days at 25°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 5 days at 25°C on a rotary shaker. The entire culture broth of the second culture was transferred into a 2-liter Erlenmeyer flask containing 500 ml fermentation medium consisting of sucrose 3%, soluble starch 2%, dried yeast (Ebios) 0.5%, malt extract 1%, corn steep liquor 0.5%, V8 vegetable juice (Campbell) 20% (v/v), $CaCO_3$ 0.5% (pH 6.5 before sterilization) for 7 days at 25°C. For the purification of RP-1551-M1 and RP-1551-M2, the fermentation was carried out in a large scale with 2,000-liter tank fermenter. Mycelia, grown on an agar slant, were inoculated into 30 ml of seed medium described above in a 250-ml Erlenmeyer flask, and the inoculated tube was incubated for 3 days at 28°C. A 6-ml aliquot of the seed culture was transferred into a 2-liter Erlenmeyer flask containing 300 ml seed medium and the inoculated flask was incubated for 3 days at 28°C on a rotary shaker. Two liters of the second seed culture were transferred into a 200-liter tank fermenter containing 100 liters of the seed medium. The tank was operated for 3 days at 28°C with agitation at 200 rpm and aeration of 60 liters per minute. The entire culture broth of the tank fermenter was transferred into a 2,000-liter tank fermenter containing 1,000 liters of the fermentation medium composed of sucrose 3%, soluble starch 2%, dried yeast (Ebios) 0.5%, malt extract 1%, corn steep liquor 0.5%, V8 vegetable juice (Campbell) 5% (v/v), $CaCO_3$ 1.5% (pH 6.5 before sterilization). The fermentation was conducted for 6 days at 25°C with agitation at 120 rpm and aeration of 400 liters per minute.

Determination of RP-1551-1

To detect the RP-1551-1, HPLC analysis was adopted. The conditions of HPLC analysis are described as follows. The culture broth was centrifuged to obtain mycelia. The mycelia were extracted with the same amount of methanol. An appropriate amount of the methanol extract was injected into HPLC column (YMC ODS AQ-312, 6 mm i.d. × 150 mm) developed at 40°C with 85% methanol at flow rate of 1 ml/minute with monitoring of absorbance at 254 nm.

Ligand Binding Assays

The PDGF binding assays were performed by a solid-phase method using a specific antibody for the PDGF according to the method of FRETTO, *et al.*[12] For detection of PDGF AA binding to the α-receptor, the extracellular domain of the PDGF α-receptor protein was immobilized on a 96-well plate, and incubated with 13 ng/ml PDGF AA (containing the KT3 epitope) and a test sample dissolved in methanol or dimethylsulfoxide for 2 hours at room temperature in incubation buffer composed of 25 mM HEPES (pH 7.8), 100 mM NaCl, 0.01% Tween 20, and 0.3% gelatin. After incubation, each well was washed with the incubation buffer. The amount of PDGF bound to the receptors were detected with anti-KT3 antibody. The wells were incubated with 5 μg/ml of the anti-KT3 antibody in the incubation buffer for an hour at 37°C, and the wells were washed again with the incubation buffer. These wells were incubated with secondary antibody (peroxidase-conjugated antibody, 1 : 10,000 diluted with the incubation buffer) for an hour at 37°C. After washing the plate, the peroxidase substrates, 2,2′-azinobis[3-ethylbenzothiazoline-6-sulfonic acid]-diammonium salt (ABTS) and H_2O_2, were added. The product of the enzyme reaction was detected by monitoring the absorbance at 650 nm.

The assay for PDGF BB binding were carried out in the same way as those of PDGF AA binding. In brief, the incubation buffer containing 5 ng/ml of PDGF BB

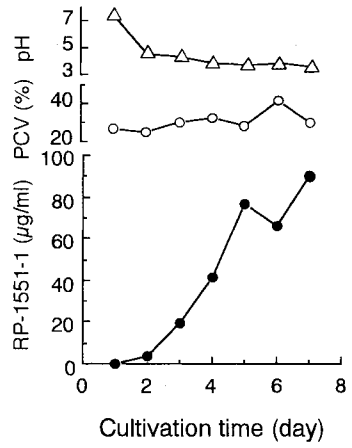
and a test sample was incubated with the extracellular domain of the PDGF β-receptor protein immobilized on a plate for 2 hours at room temperature. After washing the plate, the plate was sequentially incubated with 2.5 μg/ml of anti-PDGF BB and secondary antibody (peroxidase-conjugated antibody, 1:10,000 diluted with the incubation buffer) for an hour at 37°C. The reaction of peroxidase was performed as described above.

Results

Production of RP-1551s by Fermentation

The time course of RP-1551-1 production in 2-liter flask is shown in Figure 1. The production of RP-1551-1 in the culture broth was initiated on day 3 and the amount of RP-1551-1 increased up to day 7.

Fig. 1. Time course of cultivation of *Penicillium* sp. SPC21609.



A small amount of culture broth sampled every day was centrifuged to obtain mycelia. The productivity of RP-1551-1 was determined as described in Materials and Methods.

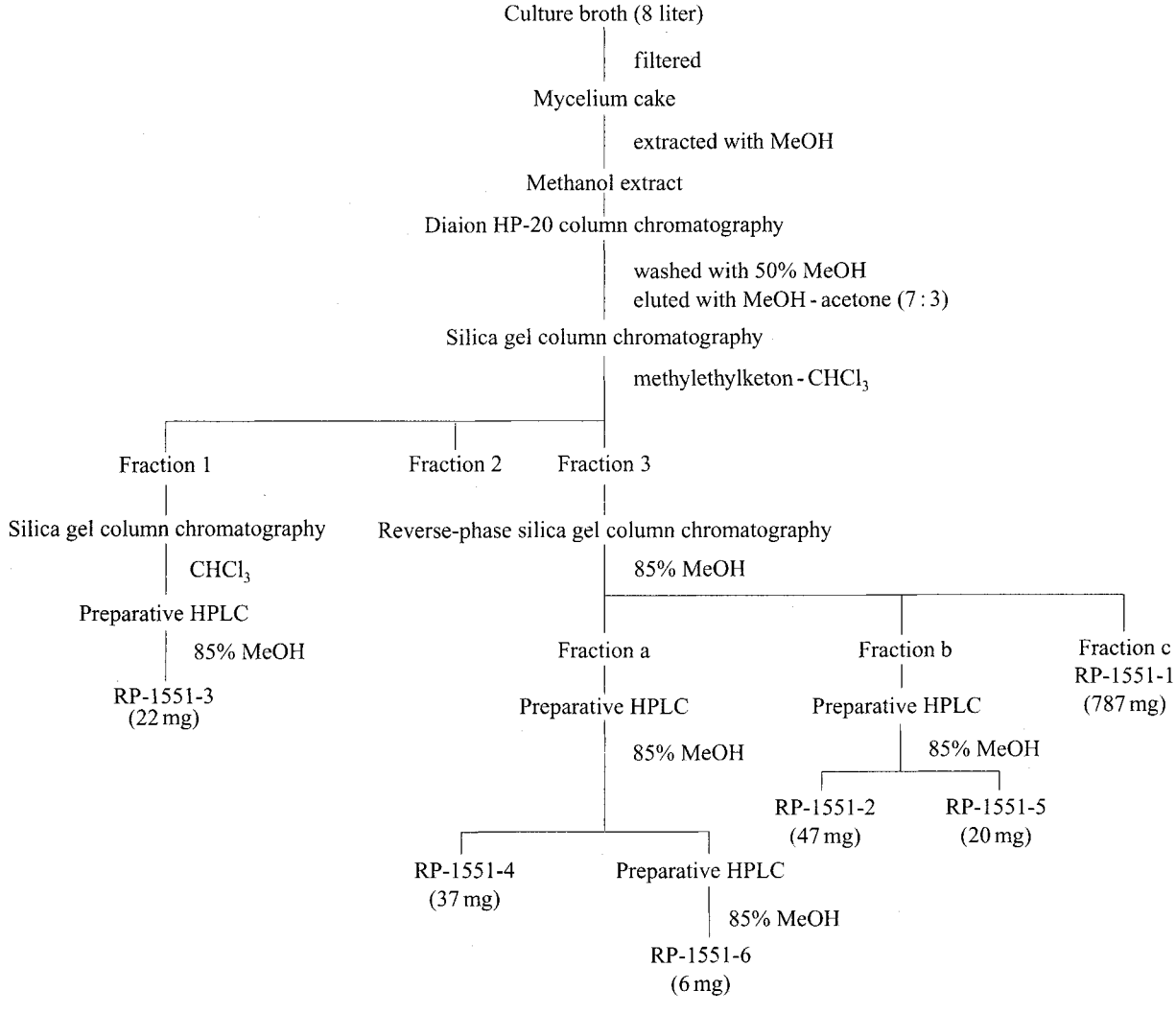
The productivity of RP-1551-1 in the mycelia (●), packed cell volume (%) (○), and pH (△) are indicated.

Isolation and Purification

The isolation procedures for RP-1551s are outlined in Figure 2 and Figure 3. The RP-1551s (other than RP-1551-M1 and -M2) were purified from the fermentation broth cultured in 2-liter flasks. The fermentation broth was filtered to obtain mycelia. The mycelial cake was extracted with methanol. The extract was diluted with an equal volume of water, and applied to a Diaion HP-20 column (3 liter). The column was washed with 50% methanol, and the absorbed material was eluted with methanol-acetone (7:3). Fractions containing RP-1551s were pooled and concentrated *in vacuo*. The crude material was applied to a silica gel column (Merck Art. 7734, 1 liter) prepacked with chloroform. The column was sequentially eluted with 3 liters of chloroform, 3 liters of methylethylketone-chloroform (1:9), and 9 liters of methylethylketone-chloroform (2:8); RP-1551-3 was eluted at elution volumes between 3 liters to 4 liters (Fraction 1), RP-1551-7 was at elution volumes between 4 liters to 5 liters (Fraction 2), and RP-1551-1, RP-1551-2, RP-1551-4, RP-155-5, and RP-1551-6 were at volumes between 5 liters to 15 liters (Fraction 3). Each fraction was concentrated *in vacuo* to dryness, to yield yellow oily material. The material obtained from the Fraction 3 was dissolved in 85% methanol, and loaded on a reverse-phase silica gel column (YMC ODS AQ-S50, 2 cm i.d. × 50 cm). The chromatography was developed with 85% methanol, and three fractions (Fraction a; RP-1551-4- and RP-1551-6-rich fraction, Fraction b; RP-1551-2- and RP-1551-5-rich fraction, and Fraction c; RP-1551-1-rich fraction) were collected. Fraction c was concentrated *in vacuo* to dryness, to yield 787 mg of RP-1551-1 as a yellow powder. Fraction a was dissolved in a small amount of 85% methanol, and injected onto a preparative HPLC column (YMC D-ODS-5AQ, 2 cm i.d. × 25 cm). The HPLC was developed with 85% methanol as elution solvent. The fractions containing RP-1551-4 were combined, and concentrated *in vacuo* to dryness, to yield RP-1551-4 (37 mg). The final purification of RP-1551-6 was achieved by preparative HPLC (YMC D-ODS-5AQ, 2 cm i.d. × 25 cm) with 85% methanol as elution solvent, and the resulting yellow powder of RP-1551-6 (6 mg) was obtained. RP-1551-2 and RP-1551-5 in the Fraction b were separated with preparative HPLC according to the purification procedure of RP-1551-6. The fractions containing RP-1551-2 or RP-1551-5 were pooled separately; the evaporation of the elution solvent gave 47 mg of RP-1551-2 and 20 mg of RP-1551-5 as yellow powders.

The Fraction 1 was concentrated *in vacuo* and subjected to silica gel column chromatography (Merck, Lichroprep Si60 Größe B) with chloroform as the solvent to yield a crude RP-1551-3. The crude RP-1551-3 was further purified using HPLC by means described in the

Fig. 2. The purification procedure of RP-1551-1, -2, -3, -4, -5, and -6.

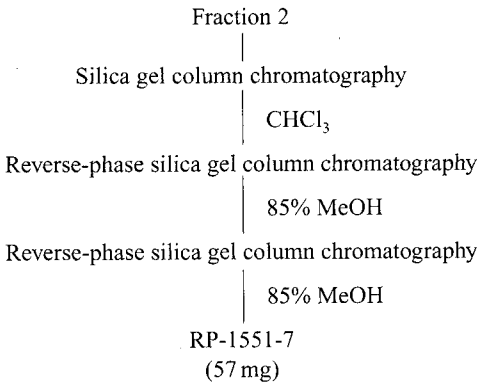


experimental procedures, and a yellow powder of RP-1551-3 (22 mg) was obtained.

RP-1551-7 was purified by silica gel column chromatography and HPLC from the Fraction 2 as was done for the other RP-1551 compounds (Fig. 3). Consequently, RP-1551-7 (57 mg) was obtained as a yellow powder.

RP-1551-M1 and RP-1551-M2 were isolated from the 1,000-liter culture broth. The purification procedures of these compounds are described in Figure 4. The culture broth was filtered, and the resulting mycelial cake was extracted with methanol (1,000 liter). The methanol extract was diluted with an equal amount of water, and passed through a Diaion HP-20 column (50 liter). The column was washed with 50% methanol, eluted with methanol - acetone (7 : 3), and the eluate was concentrated *in vacuo* to yield a brown oily material. One-fifth of the material was loaded on a silica gel column (Wakogel C-200, 15 liter), followed by elution with methylethylketone - chloroform (3 : 17). The fraction containing RP-1551-M1 and RP-1551-M2 was collected, and evaporated *in vacuo*. The resulting material was subjected to reverse-phase silica gel column chromatography (YMC ODS-AQ S50, 10 liter) developed with 85% methanol. RP-1551-M2 and RP-1551-M1 were eluted at elution volumes between 20 liters to 26 liters, and between 34 liters to 38 liters, respectively. The crude RP-1551-M1 was applied to a reverse-phase silica gel column (YMC ODS AQ-S50, 5 liter). The chromatography was performed with 85% methanol as the solvent.

Fig. 3. The purification procedure of RP-1551-7.



RP-1551-M1 (47 mg) was purified by silica gel column chromatography (Develosil Lop60) developed with methylethylketone - chloroform (1 : 9) in combination with preparative HPLC (Develosil ODS-K-10) developed with 75% methanol. The crude RP-1551-M2 was loaded onto a reverse-phase silica gel column (YMC ODS AQ-S50, 5 cm i.d. × 30 cm), and the column was sequentially developed with 80% and 85% methanol. RP-1551-M2 (73 mg) was purified from the fractions by silica gel column chromatography and preparative HPLC as described in the purification procedure for RP-1551-M1.

Physico-chemical Properties of RP-1551s

Physico-chemical properties of RP-1551s are summarized in Table 1. All RP-1551s were obtained as yellow

Fig. 4. The purification procedure of RP-1551-M1 and -M2.

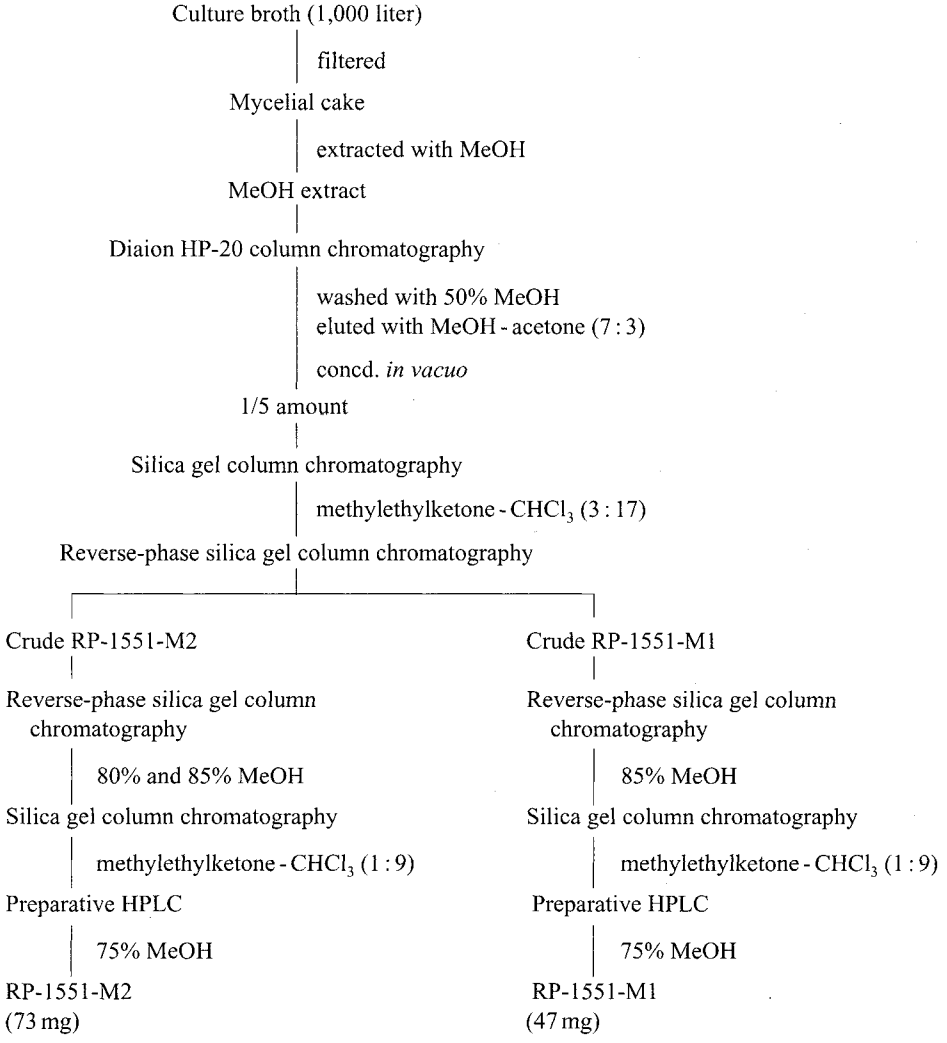


Table 1. Physico-chemcal properties of RP-1551s.

Compound	RP-1551-1	RP-1551-2	RP-1551-3	RP-1551-4	RP-1551-5
Appearance	Yellow powder	Yellow powder	Yellow powder	Yellow powder	Yellow powder
Molecular formula	$C_{25}H_{29}O_6Cl$	$C_{24}H_{31}O_5Cl$	$C_{25}H_{27}O_5Cl$	$C_{25}H_{29}O_6Cl$	$C_{25}H_{33}O_5Cl$
FAB-MS (*m/z*)	461 $(M+H)^+$	435 $(M+H)^+$	443 $(M+H)^+$	461 $(M+H)^+$	449 $(M+H)^+$
MP (°C)	124~128	54~55	91~93	82~84	71~73.5
TLC (Rf)					
$CHCl_3$: methylethylketone (1 : 1)[a]	0.64	0.57	0.93	0.82	0.74
$CHCl_3$: MeOH (9 : 1)[a]	0.69	0.55	0.78	0.57	0.68
70% MeOH[b]	0.08	0.07	0.07	0.12	0.07
Color reaction					
Positive	I_2, H_2SO_4	I_2, H_2SO_4	I_2, H_2SO_4	I_2, H_2SO_4	I_2, H_2SO_4
Negative	Ninhydrin	Ninhydrin	Ninhydrin	Ninhydrin	Ninhydrin
Solubility					
Soluble	$CHCl_3$, MeOH, EtOH, acetone	MeOH	MeOH	MeOH	MeOH
Insoluble	*n*-Hexane				

Compounds	RP-1551-6	RP-1551-7	RP-1551-M1	RP-1551-M2
Appearance	Yellow powder	Yellow powder	Yellow powder	Yellow powder
Molecular formula	$C_{25}H_{29}O_6Cl$	$C_{19}H_{23}O_4Cl$	$C_{26}H_{31}O_6Cl$	$C_{19}H_{25}O_4Cl$
FAB-MS	461 $(M+H)^+$	351 $(M+H)^+$	475 $(M+H)^+$	353 $(M+H)^+$
MP (°C)	126~127	64~64.7	98~99	78~79
TLC (Rf)				
$CHCl_3$: methylethylketone (1 : 1)[a]	0.61	0.67	NT	NT
$CHCl_3$: MeOH (9 : 1)[a]	NT	0.51	0.78	0.52
70% MeOH[b]	NT	0.08	0.04	0.09
Color reaction				
Positive	I_2, H_2SO_4	I_2, H_2SO_4	I_2, H_2SO_4	I_2, H_2SO_4
Negative	Ninhydrin	Ninhydrin	Ninhydrin	Ninhydrin
Solubility				
Soluble	MeOH	MeOH	MeOH	MeOH
Insoluble				

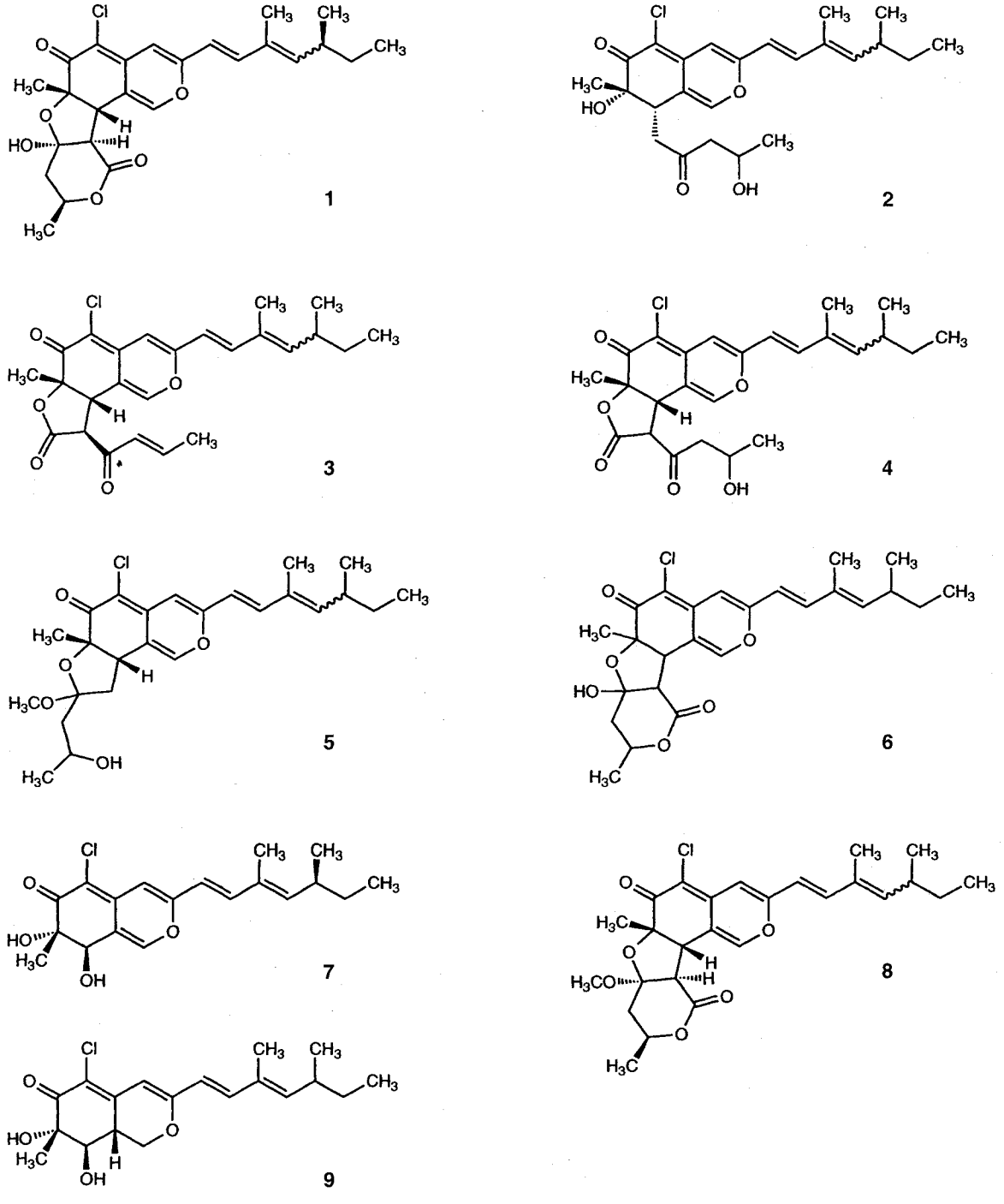
[a] Merck Kieselgel 60F254 Art. 5628.
[b] Merck Kieselgel RP-18WF254 Art. 13124.
NT; Not tested.

powder. RP-1551-1 is readily soluble in chloroform, methanol, ethanol, acetone, and virtually insoluble in *n*-hexane and water. The structures of RP-1551-1, -2, -3, -4, -5, -6, -7, -M1, and -M2 were determined as **1** to **9**, respectively (Figure 5). RP-1551-7 is identical to the known azaphilone, luteusin A. RP-1551-1 and RP-1551-6 are stereoisomers each other, and these are distinct stereoisomers from the known azaphilones, luteusin C and luteusin D.[14,15] The structural elucidation will be described in a separate paper.

Biochemical Properties

RP-1551-1 inhibited the binding of PDGF AA to the extracellular domain of the PDGF α-receptor in a dose-dependent manner (Figure 6). An IC_{50} value was calculated to be 1.7 μM. RP-1551-1 did not affect the binding of PDGF BB to the PDGF β-receptor at concentrations up to 50 μM. The inhibitory potency of the RP-1551s are summarized in Table 2. All RP-1551s selectively blocked the binding of PDGF AA to the α-receptor extracellular domain. On the other hand, sclerotiorin, a well-characterized azaphilone produced by

Fig. 5. The structures of RP-1551s.

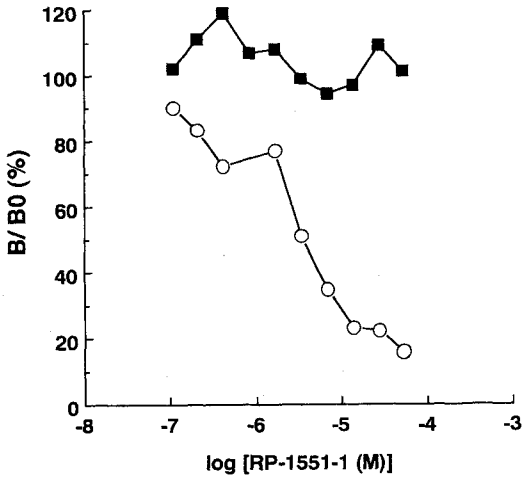


1 RP-1551-1, **2** RP-1551-2, **3** RP-1551-3, **4** RP-1551-4, **5** RP-1551-5, **6** RP-1551-6, a diastereomer of RP-1551-1, **7** RP-1551-7, identical to luteusin A, **8** RP-1551-M1, **9** RP-1551-M2.

fungi, inhibited both the binding of PDGF AA to the PDGF α-receptor extracellular domain and the binding of PDGF BB to the PDGF β-receptor extracellular domain with IC_{50} values of 3.1 μM and 10.7 μM, respectively.

In order to further characterize the inhibitory property of RP-1551-1 on the PDGF α-receptor, the following experiments were carried out. In the binding experi-

Fig. 6. The displacement curve of RP-1551-1 for various ligand binding.



Specific binding of PDGF AA to the α-receptor extracellular domain (○) or PDGF BB to the β-receptor extracellular domain (■) was measured in the presence of various concentrations of RP-1551-1. All experiments were performed at least in duplicate.

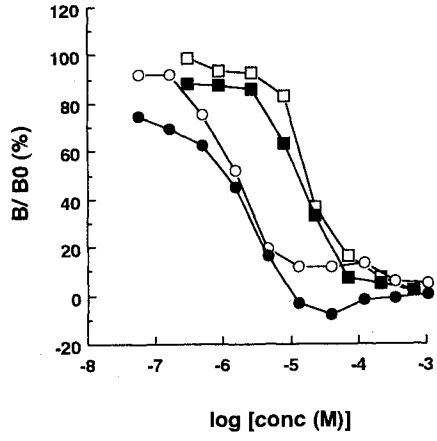
Table 2. The inhibitory activities of various RP-1551s for the PDGF binding.

Compounds	IC_{50} values (μM)	
	PDGF AA/ α-receptor	PDGF BB/ β-receptor
RP-1551-1	1.7	*ca.* 100
RP-1551-2	0.32	>100
RP-1551-3	0.11	NT
RP-1551-4	1.4	*ca.* 100
RP-1551-5	0.76	>100
RP-1551-6	2.2	>100
RP-1551-7	0.25	>100
RP-1551-M1	0.42	>100
RP-1551-M2	26	>100
(+)-Sclerotiorin	3.1	11

NT: Not tested.

ment, prior to the addition of the ligand, RP-1551-1 or sclerotiorin was removed after the compound was incubated for 1 hour with the immobilized receptor on the plate. PDGF AA failed to bind to the immobilized

Fig. 7. Irreversible inhibition by RP-1551-1 or sclerotiorin against the PDGF AA binding to the PDGF α-receptor extracellular domain.



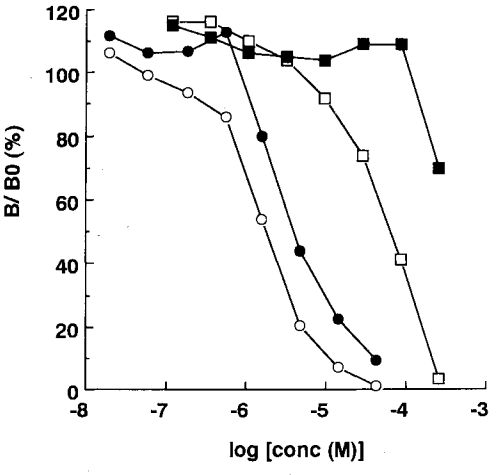
Open symbol; The inhibitory activity of RP-1551-1 or sclerotiorin was measured as described in Materials and Methods. Closed symbol; RP-1551-1 or sclerotiorin was incubated with immobilized PDGF α-receptor extracellular domain on 96-well plate for 1 hour. After that, the wells were washed twice to remove RP-1551-1 or sclerotiorin. The remaining binding ability of the immobilized receptor was determined as described in Materials and Methods. The circles and the squares indicate the inhibitory curves by RP-1551-1 and sclerotiorin, respectively. The experiments were performed at least in duplicate.

PDGF α-receptor treated with RP-1551-1 or sclerotiorin in this manner. The inhibitory potency was almost equal to that observed without preincubation with the compound and the receptor (Figure 7).

The displacement curve by RP-1551-1 was shifted only slightly to the right when RP-1551-1 was incubated with 1 mg/ml bovine serum albumin (BSA) prior to the binding assay, whereas the inhibition by the sclerotiorin was dramatically restored by preincubation with BSA (Figure 8).

The antimicrobial activity of RP-1551-1 is described as follows. RP-1551-1 has weak activity against *Bacillus subtilis*, *Enterococcus faecium*, and *Staphylococcus aureus*.

Fig. 8. The effect of BSA on the inhibition by RP-1551-1 or sclerotiorin.



Open symbols; the inhibition by RP-1551-1 or sclerotiorin was measured in the absence of BSA. Closed symbols; the binding buffer containing various concentrations of RP-1551-1 or sclerotiorin was incubated for 1 hour in the presence of 1 mg/ml BSA. The mixture was transferred into the 96-well plate coated with the extracellular domain of the PDGF α-receptor, and the binding assay was achieved. The circles and the squares indicate the inhibitory curve by RP-1551-1 and sclerotiorin, respectively. The experiments were carried out in triplicate.

Discussion

In this paper, we report that azaphilones, the RP-1551s, were isolated from *Penicillium* sp. as inhibitors of the PDGF AA binding to the PDGF α-receptor extracellular domain. While RP-1551-7 is identical to a known azaphilone luteusin A, which has been reported as an inhibitor of monoamine oxdase,[14,16] the other RP-1551s are novel compounds.

Recently, novel azaphilones isochromophilones have been isolated from the culture broth of *Penicillium* sp. as inhibitors of the interaction of gp120, an envelope protein of HIV, with CD4.[17] PAIRET *et al.* reported selective endothelin receptor binding activity of the azaphilones produced by *Penicillium sclerotiorum*.[18] Moreover, azaphilones have been reported as inhibitors of acylcholesterol acyl-transferase,[19] monoamine oxidase,[20] or inhibitors of tumor promotion induced by phorbol ester.[21] Although differential biological and physiological activities of various azaphilones produced by microorganisms have been demonstrated, there has been no report describing the interaction of azaphilones with the PDGF receptors. This article is the first report showing the inhibition of PDGF binding by azaphilones.

The RP-1551s inhibited the binding of PDGF AA to the α-receptor extracellular domain with IC_{50} values ranging from 0.1 to 2 μM, whereas these compounds had no effect on the binding of PDGF BB to the β-receptor at the same concentration ranges. These results suggest that the RP-1551s may be selective blockers of the PDGF α-receptor extracellular domain.

We also found the inhibitory effect of sclerotiorin, one of azaphilone compounds which has been demonstrated to possess high reactivity with amino groups,[22] on PDGF binding to their receptor's extracellular domains. Moreover, sclerotiorin incubated with BSA showed less potent inhibition than non-treated sclerotiorin, suggesting that BSA added to the reaction mixture may chemically react with the sclerotiorin and neutralize the inhibitory effect of the sclerotiorin. Therefore, it is plausible that sclerotiorin may inhibit PDGF binding by chemical reaction with the receptor protein. The reactivities of the RP-1551s with amino groups have not been examined precisely, however, preliminary data show that RP-1551-1 decomposed in the presence of monomethylamine at room temperature (data not shown). The result implies that RP-1551-1 may be modified by amino groups. Thus, RP-1551-1 may prevent PDGF AA binding to the α-receptor extracellular domain due to its reactivity with the amino groups, such as ε-amino groups of lysine residues, in the receptor molecule. The fact that both sclerotiorin and RP-1551-1 irreversibly inhibited the PDGF AA binding support this hypothesis.

The mode of action of the RP-1551s may not be simple, because the RP-1551s did not affect PDGF BB binding to the β-receptor but did affect PDGF AA binding to the α-receptor, while sclerotiorin inhibited both PDGF AA and PDGF BB binding. Unlike sclerotiorin, the effect of RP-1551-1 on PDGF AA binding was shifted slightly to the right in the presence of BSA. These results suggest that RP-1551-1 appears to react more preferentially with the PDGF α-receptor extracellular domain than with BSA. Therefore, it is likely that this effect is due to the reactivity of RP-1551-1 on protein molecules, and the selectivity of RP-1551-1 to the PDGF α-receptor extracellular domain may be due to the differential reactivity of RP-1551-1 to proteins.

Acknowledgments

We acknowledge with thanks the expert assistance of Ms. MAYUKO NISHIIE-OKUDA.

References

1) ROSS, R.; E. W. RAINES & D. F. BOWEN-POPE: The biology of platelet-derived growth factor. Cell 46: 155～169, 1986
2) HELDIN, C.-H.: Structural and functional studies on platelet-derived growth factor. EMBO J. 11: 4251～4259, 1992
3) HART, C. E.; J. W. FORSTROM, J. D. KELLY, R. A. SEIFERT, R. A. SMITH, R. ROSS, M. J. MURRAY & D. F. BOWEN-POPE: Two classes of PDGF receptor recognize different isoforms of PDGF. Science 240: 1529～1531, 1988
4) ESCOBEDO, J. A.; S. NAVANKASATUSSAS, L. S. COUSENS, S. R. COUGHLIN, G. I. BELL & L. T. WILLIAMS: A common PDGF receptor is activated by homodimeric A and B forms of PDGF. Science 240: 1532～1534, 1988
5) HELDIN, C.-H.; G. BACKSTRÖM, A. ÖSTMAN, A. HAMMACHER, L. RÖNNSTRAND, K. RUBIN, M. NISTÉR & B. WESTERMARK: Binding of different dimeric forms of PDGF to human fibroblasts: evidence for two separate receptor types. EMBO J. 7: 1387～1393, 1988
6) ESCOVEDO, J. A.; P. J. BARR & L. T. WILLIAMS: Role of tyrosine kinase and membrane-spanning domains in signal transduction by the platelet-derived growth factor receptor. Mol. Cell. Biol. 8: 5126～5131, 1988
7) CLAESSON-WELSH, L.: Platelet-derived growth factor receptor signals. J. Biol. Chem. 269: 32023～32026, 1994
8) PECH, M.; A. GAZIT, P. ARNSTEIN & S. A. AARONSON: Generation of fibrosarcomas *in vivo* by a retrovirus that expresses the normal B chain of platelet-derived growth factor and mimics the alternative splice pattern of the v-sis oncogene. Proc. Natl. Acad. Sci. USA 86: 2693～2697, 1989
9) DEFEUDIS, F. V.: Coronary atherosclerosis: current therapeutic approaches and future trends. Life Sci. 49: 689～705, 1991
10) SANDERS, M.: Molecular and cellular concepts in atherosclerosis. Pharmacol. Ther. 61: 109～153, 1994
11) FERNS, G. A.; E. W. RAINES, K. H. SPRUGEL, A. S. MOTANE, M. A. REIDY & R. ROSS: Inhibition of neointimal smooth muscle accumulation after angioplasty by an antibody to PDGF. Science 253: 1129～1132, 1991
12) FRETTO, L. J.; A. J. SNAPE, J. E. TOMLINSON, J. J. SEROOGY, D. L. WOLF, W. J. LAROCHELLE & N. A. GIESE: Mechanism of platelet-derived growth factor (PDGF) AA, AB, and BB binding to α and β PDGF receptor. J. Biol. Chem. 268: 3625～3631, 1993
13) CURTIN, T. P. & J. REILLY: Sclerotiorin, $C_{20}H_{20}O_5Cl$, a chlorine-containing metabolic product of *Penicillium sclerotiorum* Van Beyma. Biochem. J. 34: 1419～1421, 1940
14) YOSHIDA, E.; H. FUJIMOTO & M. YAMAZAKI: Isolation of three new azaphilones, luteusins C, D, and E, from an ascomycete, *Talaromyces luteus*. Chem. Pharm. Bull. 44: 284～287, 1996
15) YOSHIDA, E.; H. FUJIMOTO & M. YAMAZAKI: Revised stereostructures of luteusins C and D. Chem. Pharm. Bull. 44: 1775, 1996
16) SATOH, Y. & M. YAMAZAKI: Studies on the monoamine oxidase (MAO) inhibitory potency of TL-1, isolated from a fungus, *Talaromyces luteus*. Chem. Pharm. Bull. 37: 206～207, 1989
17) ŌMURA, S.; H. TANAKA, K. MATSUZAKI, H. IKEDA & R. MASUMA: Isochromophilones I and II, novel inhibitors against gp120-CD4 binding from *Penicillium* sp. J. Antibiotics 46: 1908～1911, 1993
18) PAIRET, L.; S. K. WRIGLEY, I. CHETLAND, E. E. REYNOLDS, M. A. HAYES, J. HOLLOWAY, A. M. AINSWORTH, W. KATZER, X.-M. CHENG, D. J. HUPE, P. CHARLTON & A. M. DOHERTY: Azaphilones with endothelin receptor binding activity produced by *Penicillium sclerotiorum*: Taxonomy, fermentation, isolation, structure elucidation and biological activity. J. Antibiotics 48: 913～923, 1995
19) YANG, D.-J.; H. TOMODA, N. TABATA, R. MASUMA & S. ŌMURA: New isochromophilones VII and VIII produced by *Penicillium* sp. FO-4164. J. Antibiotics 49: 223～229, 1996
20) TAKAHASHI, M.; K. KOYAMA & S. NATORI: Four new azaphilones from *Chaetomium globosum* var. *flavoviridae*. Chem. Pharm. Bull. 38: 625～628, 1990
21) YASUKAWA, K.; M. TAKAHASHI, S. NATORI, K. KAWAI, M. YAMAZAKI, M. TAKEUCHI & M. TAKIDO: Azaphilones inhibit tumor promotion by 12-*O*-tetradecanoylphorbol-13-acetate in two-stage carcinogenesis in mice. Oncology 51: 108～112, 1994
22) NATSUME, M.; Y. TAKAHASHI & S. MARUMO: Chlamydospore-like cell-induceing substances of fungi: close correlation between chemical reactivity with methylamine and biological activity. Agric. Biol. Chem. 52: 307～312, 1988