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ORIGINAL ARTICLE

A new series of the SMTP plasminogen modulators with a phenylamine-based side chain

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SMTPs are a family of small-molecule plasminogen modulators that enhance plasminogen activation. SMTP-7, one of the most potent congeners, is effective in treating thrombotic cerebral infarction. The SMTP molecule consists of a tricyclic γ-lactam moiety, a geranylmethyl group, and an *N*-linked side chain. The presence of both an aromatic group and a negatively ionizable group in the *N*-linked side chain is crucial for activity. Investigations of the congeners with a phenylglycine-based side chain suggest that a phenolic hydroxy group affects potency. In this study, we isolate and characterize a series of novel SMTP congeners with a phenylamine-based *N*-linked side chain. Of the 11 congeners isolated, SMTP-19 (with a 4-phenylcarboxylic acid moiety), SMTP-22 (with a 3-hydroxyphenyl-4-carboxylic acid moiety) and SMTP-25 (with a 2-hydroxyphenyl-3-carboxylic acid moiety) are as potent as SMTP-7 in plasminogen-modulating activity. Their isomers with a carboxylic acid group and/or a phenolic hydroxy group at different positions have <40% of the activity of these congeners. Both SMTP-22 and SMTP-25 have >1.7 times more oxygen radical absorbance capacity as compared with SMTP-7.
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Keywords: fibrinolysis; plasminogen modulator; proteolysis; radical scavenger; structure-activity relationships

INTRODUCTION

The plasminogen/plasmin system has a crucial role in blood clot lysis and other pathophysiological events involving localized extracellular proteolysis.[1,2] Plasminogen is a zymogen that is proteolytically activated, via cleavage at Arg[561]-Val[562], to plasmin by tissue-type and urokinase-type plasminogen activators (t-PA and u-PA, respectively).[1] Plasminogen adopts tight conformation because of intramolecular binding of Lys[50] and/or Lys[62] to the lysine-binding site in the fifth kringle domain,[3,4] rendering the plasminogen molecule less sensitive to activation by plasminogen activators. Upon binding to fibrin and cellular receptors, plasminogen adopts relaxed conformation and is efficiently activated on these substrata, leading to extracellular proteolysis.[5]

SMTPs, a family of triprenyl phenol metabolites produced by *Stachybotrys microspora*, enhance plasminogen activation by modulating plasminogen conformation.[5–7] SMTP-7, one of the most potent congeners, is effective in treating thrombotic stroke in animal models,[8–11] possibly involving a neuroprotective mechanism.[10–12] The SMTP molecule consists of a tricyclic γ-lactam moiety, a geranylmethyl group, and an *N*-linked side chain. Previous studies have identified 31 SMTP congeners, most of which differ in the *N*-linked side chain.[13–20] The *N*-linked side chain structure of SMTP affects the plasminogen-modulating activity of the congeners. It has been suggested that a negatively ionizable group in the side chain is crucial for activity.[19] Among congeners with a negatively ionizable side chain, one with an aromatic group is more active than that with an aliphatic group.[19] Investigations of the congeners with a phenylglycine-based side chain suggest that a phenolic hydroxy group in the side chain affects potency of a congener.[20] In this study, we isolated 11 new SMTP congeners with a phenylamine-based side chain and investigated the roles for a side chain-phenolic hydroxy group and a carboxylic acid group in the plasminogen-modulating activity. This paper deals with the isolation and characterization of these congeners. Part of the results has been disclosed as a patent.[21] Biological activities of some of the new congeners have also been described in the patent literatures.[22–24]

RESULTS AND DISCUSSION

Production, isolation, and physico-chemical properties

The 11 new SMTP congeners were produced by *S. microspora* fed with various phenylamines (Table 1). In this precursor amine-fed culture method, the fed amines were incorporated as the *N*-linked side chain of the SMTP molecule.[18,25] The products were isolated by reversed-phase HPLC. The yields of the 11 congeners varied from 95 to 993 $mg\,l^{-1}$ (Table 1).

Physico-chemical properties of the new congeners are summarized in Table 2. NMR signals (Supplementary Figures S1–S11) are assigned as shown in Table 3, according to the results from ^1H-^1H-correlation, hetero-nuclear multiple quantum coherence and hetero-nuclear multiple-bond connectivity spectroscopies. Based on these results obtained, we propose the structures of the new congeners as shown in Figure 1a. The conclusion is consistent with the idea that the fed

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Table 1 Organic amines used for the production, HPLC analysis, and yield of new SMTP congeners

Compound	*Amine added*	*Analytical HPLC* *Solvent*[a]	t_R *(min)*	*Yield* *(mg l*$^{-1}$*)*
SMTP-18	*p*-Aminophenol	A in 80% MeOH	16.8	993
SMTP-19	*p*-Aminobenzoic acid	A in 70% MeOH	19.1	524
SMTP-20	*m*-Aminobenzoic acid	A in 75% MeOH	11.9	404
SMTP-21	*o*-Aminobenzoic acid	A in 75% MeOH	13.5	146
SMTP-22	4-Aminosalicylic acid	A in 75% MeOH	13.5	185
SMTP-23	4-Amino-3-hydroxybenzoic acid	A in 75% MeOH	9.6	95
SMTP-24	3-Hydroxyanthranilic acid	A in 75% MeOH	9.2	311
SMTP-25	3-Aminosalicylic acid	A in 75% MeOH	11.8	233
SMTP-26	5-Aminosalicylic acid	A in 70% MeOH	21.0	663
SMTP-27	3-Amino-4-hydroxybenzoic acid	A in 65% MeOH	22.7	789
SMTP-28	5-Hydroxyanthranilic acid	B in 80% MeOH	13.8	434

[a]Solvent A, 50 mM ammonium acetate; solvent B, 0.1% (vol/vol) formic acid.

amine is introduced as the *N*-linked side chain of an SMTP molecule.[17,18,25]

Plasminogen-modulating activity

Plasminogen-modulating activities of the 11 new SMTP congeners (Figure 1b) were assessed as the activity to enhance plasminogen activation catalyzed by u-PA. SMTP-18, which had a 4-phenol moiety as the *N*-linked side chain, was weak in activity, giving 10-fold enhancement (EC_{10}) at 182 μM and maximum enhancement (E_{max}) of 18-fold. The ratio E_{max}/EC_{10} was 0.10-fold μM^{-1}, which was ∼1/16 of that of SMTP-7 (1.57-fold μM^{-1}), one of the most potent congener to be identified (Figure 1c). SMTP-19, an analog with 29-COOH, gave EC_{10} at 86 μM and E_{max} of 126-fold, resulting in E_{max}/EC_{10} of 1.47-fold μM^{-1}, which was comparable to that of SMTP-7. The isomers SMTP-20 (with 28-COOH) and SMTP-21 (with 27-COOH) were much less active than SMTP-19 (E_{max}/EC_{10} = 0.26- and 0.37-fold μM^{-1}, respectively). SMTP-22, an analog of SMTP-19 with 28-OH as well as 29-COOH, was as potent as SMTP-19 (E_{max}/EC_{10} = 1.57-fold μM^{-1}), whereas SMTP-23, the isomer with 27-OH and 29-COOH, was significantly less active than SMTP-22 (E_{max}/EC_{10} = 0.50-fold μM^{-1}). The isomers SMTP-24 (with 27-COOH and 31-OH) and SMTP-28 (with 27-COOH and 29-OH) were less active than SMTP-22 (E_{max}/EC_{10} = 0.22- and 0.50-fold μM^{-1}, respectively). The isomer SMTP-25, with 27-OH and 28-COOH, was comparable to SMTP-22 in activity (EC10 = μM; E_{max} = 91-fold; E_{max}/EC_{10} = 1.30-fold μM^{-1}). Two other 28-COOH isomers, SMTP-26 (with 28-COOH and 29-OH) and SMTP-27 (with 28-COOH and 31-OH), were much less active than SMTP-25.

Thus, among the series of SMTP congeners with a phenylamine-based side chain, SMTP-19 (with 29-COOH), SMTP-22 (with 28-OH and 29-COOH) and SMTP-25 (with 27-OH and 28-COOH) are potent in enhancing plasminogen activation catalyzed by u-PA. The activity of each of these compounds is comparable to that of SMTP-7, which has two-triprenyl-phenol units with a MW of 868. The new potent congeners have a single triprenyl phenol unit, and their MWs are 506.25–522.25. A phenolic hydroxy group reduces the activity of a congener with a phenylglycine-based side chain,[20] but has differential effects in activity in combination with a carboxylic acid group. For example, with respect to the congeners with 28-COOH, the presence of 27-OH (SMTP-25) greatly increases activity compared with the congener with 28-COOH but without a hydroxy group (SMTP-20). The isomers with 29-OH (SMTP-26) or 31-OH (SMTP-27) are much less active than SMTP-25.

Antioxidant activity

The core structure of SMTP resembles tocopherols, which have significant antioxidant activities. Both *in vivo* and *in vitro* studies suggest that part of the SMTP-7 activity in the amelioration of ischemic stroke can be attributable to its antioxidant property.[10–12] Hence, we evaluated antioxidant activities of the new SMTP congeners utilizing the oxygen radical absorbance capacity (ORAC) method, which measured peroxy radical scavenging activity (Figure 1c). SMTP-7 had approximately two times as high ORAC value as the water-soluble tocopherol analog trolox (2.08 TE (trolox equivalent)). SMTP-19, -20 and -21 had ORAC values of 1.36–3.57 TE. Except for SMTP-26, the congeners with a phenolic hydroxy group in addition to a carboxylic acid group at any positions had higher ORAC values (3.63–6.89 TE) compared with the congeners with a carboxylic acid group alone. Thus, the *N*-linked side chain structure of the SMTP molecule affects antioxidant activity, and the structural requirement for antioxidant activity seems unrelated to that for plasminogen modulator activity. Both SMTP-22 and SMTP-25 have higher plasminogen-modulating and antioxidant activities.

EXPERIMENTAL PROCEDURE

Materials

Human native plasminogen (Glu[1]-plasminogen) was isolated on lysine-Sepharose affinity chromatography. H-Val-Leu-Lys-*p*-nitroanilide (VLK-*p*NA), a chromogenic substrate for plasmin, was obtained from Bachem (Bubendorf, Switzerland). Two-chain u-PA (tcu-PA) was purchased from JCR Pharmaceuticals (Kobe, Japan). SMTP-7 was prepared as described previously.[16] SMTP congeners were converted to sodium salt before assay of plasminogen activation and antioxidant activity.

Production and isolation of new SMTP congeners

S. microspora IFO 30018 was incubated at 25 °C for 4 days in a 500-ml Erlenmeyer flask containing 100 ml of a seed medium consisting of glucose (4%), soybean meal (0.5%), peptone (0.3%), yeast extract (0.3%) and the antifoam CB442 (Nippon Oil & Fat Co, Tokyo, Japan.) (0.01%), pH 5.8. Aliquot of the seed culture (5 ml) was transferred to a 500-ml Erlenmeyer flask containing 100 ml of a production medium consisting of sucrose (5%), yeast extract (0.1%), KNO_3 (0.7%), K_2HPO_4 (1.5%), $MgSO_4{\bullet}7H_2O$ (0.05%), KCl (0.05%), $CoCl_2{\bullet}6H_2O$ (0.00025%), $FeSO_4{\bullet}7H_2O$ (0.0015%), $CaCl_2{\bullet}2H_2O$ (0.00065%) and CB442 (0.01%), pH 5.8. Flasks were incubated at 25 °C on a rotary shaker at 180 r.p.m. After 96 h, 100 mg of organic amine (*see* Table 1) was added, and the flask was incubated further for 40 h.

The culture was mixed with 200 ml of MeOH, and the mixture was filtered and concentrated to remove MeOH. After adjusting pH to 2 with phosphoric acid, the concentrate was settled overnight at 4 °C. Precipitates formed were collected by centrifugation and dissolved in acetone. After evaporation, resulting oily residue was dissolved in MeOH, treated with Lichrolut RP-18 (Merck KGaA, Darmstadt, Germany), and subjected to preparative HPLC on an Inertsil PREP-ODS (30 × 250 mm; GL Science, Tokyo, Japan). The column was developed at a rate of 25 ml min^{-1} at 40 °C with a solvent mixture shown in Table 1. Fractions containing desired compound were evaporated to remove MeOH. Purified materials were obtained after ethyl acetate extraction (with ammonium acetate-containing solvent) or direct evaporation (with MeOH solvent). The yield of each congener is shown in Table 1.

Assay for plasminogen activation

The activation of plasminogen was assayed by measuring initial velocity for tcu-PA-catalyzed plasmin generation using the chromogenic substrate

Table 2 Physico-chemical properties of new SMTP congeners

	SMTP-18	*SMTP-19*	*SMTP-20*	*SMTP-21*	*SMTP-22*	*SMTP-23*
Appearance	Dark brown oil	Pale yellow solid	Pale yellow oil	Colorless oil	Pale yellow oil	Pale yellow oil
Molecular formula	$C_{29}H_{35}NO_5$	$C_{30}H_{35}NO_6$	$C_{30}H_{35}NO_6$	$C_{30}H_{35}NO_6$	$C_{30}H_{35}NO_7$	$C_{30}H_{35}NO_7$
MALDI-TOF-MS						
Found $(M+H)^+$:	478.2591	506.2549	506.2549	506.2565	522.2534	522.2533
Calculated:	478.2593 for $C_{29}H_{36}NO_5$	506.2543 for $C_{30}H_{36}NO_6$	506.2543 for $C_{30}H_{36}NO_6$	506.2543 for $C_{30}H_{36}NO_6$	522.2492 for $C_{30}H_{36}NO_7$	522.2492 for $C_{30}H_{36}NO_7$
UV λ_{max} nm (ε) MeOH	214 (sh) (83 952)	298 (39 690)	217 (46 786)	215 (sh) (48 908)	212 (sh) (47 641)	212 (sh) (63 635)
	260 (sh) (21 465)		281 (17 583)	260 (13 440)	288 (18 973)	263 (16 471)
	291 (30 051)			301 (sh) (5255)	306 (18 243)	297 (13 865)
IR ν_{max} (neat) cm^{-1}	3309, 2971, 2919, 2863, 1664, 1618, 1513, 1461, 1373, 1247, 1168, 1074	3392, 2969, 2915, 2858, 1691, 1610, 1513, 1463, 1429, 1365, 1303, 1267, 1187, 1076	3352, 2970, 2920, 2858, 2634, 2540, 1693, 1616, 1593, 1462, 1367, 1290, 1244, 1155, 1072	3398, 2970, 2920, 2860, 2630, 2488, 1707, 1612, 1466, 1369, 1240, 1159, 1078, 1036	3396, 2968, 2922, 2860, 2553, 1689, 1622, 1462, 1362, 1253, 1218, 1157, 1074	3388, 2968, 2922, 2858, 2578, 1697, 1614, 1466, 1371, 1215, 1076
Specific rotation $[\alpha]_D^{27}$	−99.8° (*c* 0.23, MeOH)	−59.3° (*c* 1.0, MeOH)	−45.8° (*c* 0.44, MeOH)	−29.2° (*c* 0.32, MeOH)	−46.7° (*c* 1.0, MeOH)	−37.4° (*c* 1.0, MeOH)

	SMTP-24	*SMTP-25*	*SMTP-26*	*SMTP-27*	*SMTP-28*
Appearance	Pale orange oil	Light brown oil	Pale yellow oil	Light brown oil	Yellow ocher oil
Molecular formula	$C_{29}H_{35}NO_7$	$C_{30}H_{35}NO_7$	$C_{30}H_{35}NO_7$	$C_{30}H_{35}NO_7$	$C_{30}H_{35}NO_7$
MALDI-TOF-MS					
Found $(M+H)^+$:	522.2533	522.2537	522.2505	522.2529	522.2516
Calculated:	522.2492 for $C_{30}H_{36}NO_7$	522.2492 for $C_{30}H_{36}NO_7$	522.2492 for $C_{30}H_{36}NO_7$	522.2492 for $C_{30}H_{36}NO_7$	522.2492 for $C_{30}H_{36}NO_7$
UV λ_{max} nm (ε) MeOH	212 (61 055)	214 (57 962)	214 (50 665)	215 (52 124)	214 (66 614)
	257 (10 300)	256 (10 633)	259 (sh) (9591)	252 (22 205)	260 (16 471)
	300 (8272)	304 (11 676)	295 (14 490)	308 (sh) (6255)	297 (sh) (9695)
IR ν_{max} (neat) cm^{-1}	3356, 2968, 2920, 2858, 1697, 1616, 1470, 1294, 1159, 1076, 762	3221, 2850, 2918, 2976, 1678, 1616, 1464, 1240, 1157, 1074	3394, 2970, 2920, 2858, 1676, 1618, 1489, 1464, 1370, 1203, 1161, 1076	3803, 3429, 3068, 2970, 2924, 2860, 2549, 2517, 1691, 1601, 1464, 1302, 1076, 1036	3373, 3329, 2970, 2920, 2860, 1705, 1660, 1610, 1504, 1464, 1338, 1296, 1219, 1074, 1032
Specific rotation $[\alpha]_D^{27}$	−19.3° (*c* 1.0, MeOH)	−27.2° (*c* 0.16, MeOH)	−49.9° (*c* 0.44, MeOH)	−23.4° (*c* 1.0, MeOH)	−25.3° (*c* 1.0, MeOH)

Table 3 NMR spectral data for new SMTP congeners

	SMTP-18			SMTP-19		
No.	δ_C	δ_H		δ_C	δ_H	
2	166.36			167.16		
3	131.83			131.00		
4	99.63	6.71	(1H, s)	99.66	6.76	(1H, s)
5	156.29			156.35		
6	112.01			112.97		
7	26.67	2.86	(1H, dd, *J*=5.4, 17.4)	26.56	2.87	(1H, dd, *J*=5.9, 17.6)
		2.52	(1H, dd, *J*=7.2, 17.4)		2.55	(1H, dd, *J*=7.3, 17.6)
8	65.96	3.76	(1H, m)	65.81	3.79	(1H, m)
9	78.80			78.89		
11	148.28			148.30		
12	118.53			118.74		
13	48.16	4.63	(1H, d, *J*=16.2)	47.49	4.73	(1H, d, *J*=16.1)
		4.61	(1H, d, *J*=16.2)		4.78	(1H, d, *J*=16.1)
14	37.12	1.63	(2H, m)	37.00	1.66	(2H, m)
15	21.00	2.14	(2H, m)	20.91	2.16	(2H, m)
16	124.18	5.13	(1H, m)	124.06	5.16	(1H, m)
17	134.23			134.12		
18	39.08	1.91	(2H, m)	38.93	1.93	(2H, m)
19	26.10	1.99	(2H, m)	25.99	2.00	(2H, m)
20	123.98	5.03	(1H, m)	123.86	5.04	(1H, m)
21	130.52			130.33		
22	25.27	1.60	(3H, s)	25.05	1.60	(3H, s)
23	17.35	1.51	(3H, s)	17.17	1.52	(3H, s)
24	15.55	1.55	(3H, s)	15.43	1.57	(3H, s)
25	18.05	1.19	(3H, s)	17.99	1.22	(3H, s)
26	131.34			143.32		
27, 31	121.45	7.59	(2H, m)	117.84	8.01	(2H, d, *J*=8.8)
28, 30	115.17	6.77	(2H, m)	130.01	7.97	(2H, d, *J*=8.8)
29	154.00			125.34		
32				166.55		

	SMTP-20			SMTP-21			SMTP-22		
No.	δ_C	δ_H		δ_C	δ_H		δ_C	δ_H	
2	168.26			169.04			168.67		
3	133.14			132.94			132.78		
4	100.86	6.84	(1H, s)	101.19	6.83	(1H, m)	100.77	6.85	(1H, s)
5	157.45			157.22			157.49		
6	113.76			113.15			114.18		
7	27.75	3.05	(1H, dd, *J*=5.4, 17.4)	27.75	3.05	(1H, dd, *J*=5.4, 17.4)	27.70	3.04	(1H, dd, *J*=5.5, 17.6)
		2.69	(1H, dd, *J*=7.2, 17.4)		2.69	(1H, dd, *J*=7.8, 17.4)		2.69	(1H, dd, *J*=7.7, 17.6)
8	67.67	3.98	(1H, dd, *J*=5.4, 7.2)	67.89	3.96	(1H, m)	67.56	3.97	(1H, dd, *J*=5.5, 7.7)
9	80.14			80.04			80.18		
11	149.87			149.78			149.85		
12	120.52			121.78			120.61		
13	48.61	4.81	(2H, s)	51.01	4.70	(2H, s)	48.62	4.78	(2H, s)
14	38.44	1.76	(2H, m)	38.66	1.76	(2H, m)	38.42	1.77	(2H, m)
15	22.23	2.25	(2H, m)	22.28	2.24	(2H, m)	22.21	2.26	(2H, m)
16	125.31	5.19	(1H, t, *J*=6.6)	125.36	5.19	(1H, t, *J*=6.6)	125.31	5.20	(1H, m)
17	135.65			135.72			135.65		
18	40.44	1.96	(2H, m)	40.48	1.97	(2H, m)	40.45	1.97	(2H, m)
19	27.39	~2.03	(2H, m)	27.48	~2.06	(2H, m)	27.39	~2.06	(2H, m)
20	125.11	5.07	(1H, t, *J*=6.6)	125.19	5.08	(1H, t, *J*=6.6)	125.10	5.08	(1H, m)
21	131.66			131.69			131.67		
22	25.79	1.61	(3H, s)	25.80	1.63	(3H, s)	25.78	1.62	(3H, m)
23	17.68	1.54	(3H, s)	17.72	1.56	(3H, s)	17.68	1.55	(3H, s)
24	16.01	1.61	(3H, s)	16.05	1.60	(3H, s)	16.00	1.62	(3H, s)
25	18.62	1.32	(3H, s)	18.51	1.30	(3H, s)	18.57	1.32	(3H, s)
26	141.55			139.54			147.49		
27	120.58	8.60	(1H, s)	130.85			106.43	7.69	(1H, d, *J*=2.2)
28	132.34			131.66	7.95	(1H, d, *J*=7.2)	163.73		
29	125.43	7.79	(1H, d, *J*=7.2)	127.80	7.44	(1H, t, *J*=7.2)	108.46		
30	129.85	7.52	(1H, t, *J*=7.8)	133.27	7.65	(1H, t, *J*=7.8)	132.00	7.89	(1H, d, *J*=8.8)
31	123.76	8.26	(1H, d, *J*=7.8)	128.88	7.53	(1H, d, *J*=7.8)	110.15	7.54	(1H, dd, *J*=2.2, 8.8)
32	167.62			167.62			172.24		

	SMTP-23		SMTP-24		SMTP-25		SMTP-26		SMTP-27		SMTP-28	
No.	δ_C	δ_H	δ_C	δ_H	δ_C	δ_H	δ_C	δ_H	δ_C	δ_H	δ_C	δ_H
2	170.02		170.06		168.80		167.84		169.92		169.32	
3	132.48		132.63		132.48		133.34		131.78		133.00	
4	100.86	6.87 (1H, s)	101.04	6.83 (1H, d, *J*=4.8)	100.96	6.86 (1H, s)	100.78	6.84 (1H, s)	100.82	6.87 (1H, s)	100.97	6.82 (1H, d, *J*=2.9)
5	157.57		156.97		157.22		157.31		157.43		157.08	
6	114.25		112.87		113.12		113.23		113.91		112.83	
7	27.71	3.06 (1H, dd, *J*=5.5, 17.6)	27.62	3.05 (1H, dd, *J*=5.4, 17.4)	27.66	3.03 (1H, dd, *J*=5.5, 17.6)	27.68	3.04 (1H, dd, *J*=5.5, 17.2)	27.69	3.05 (1H, dd, *J*=5.4, 17.4)	27.65	3.04 (1H, dd, *J*=5.5, 17.6)
		2.71 (1H, dd, *J*=7.7, 17.6)		2.69 (1H, dd, *J*=7.8, 17.4)		2.67 (1H, dd, *J*=7.7, 17.6)		2.68 (1H, dd, *J*=7.7, 17.2)		2.70 (1H, dd, *J*=7.8, 17.4)		2.68 (1H, dd, *J*=7.7, 17.6)
8	67.55	3.98 (1H, dd, *J*=5.5, 7.7)	67.78	3.95 (1H, dd, *J*=5.4, 7.8)	67.67	3.97 (1H, dd, *J*=5.5, 7.7)	67.61	3.96 (1H, dd, *J*=5.5, 7.7)	67.54	3.98 (1H, dd, *J*=5.4, 7.8)	67.75	3.94 (1H, dd, *J*=5.5, 7.7)
9	80.23		79.81		79.90		80.02		80.15		79.89	
11	149.82		149.63		149.69		149.76		149.83		149.64	
12	122.48		122.29		121.74		120.38		122.39		121.70	
13	50.64	4.93 (2H, d, *J*=4.0)	49.76	4.60 (2H, brs)	49.67	4.69 (2H, s)	48.98	4.73 (2H, s)	50.58	4.88 (2H, d, *J*=1.8)	51.48	4.61 (2H, s)
14	38.40	1.76 (2H, m)	38.57	1.75 (2H, m)	38.45	1.74 (2H, m)	38.46	1.76 (2H, m)	38.45	1.76 (2H, m)	38.56	1.74 (2H, m)
15	22.18	2.24 (2H, m)	22.15	2.22 (2H, m)	22.15	2.21 (2H, m)	22.21	2.24 (2H, m)	22.18	2.24 (2H, m)	22.17	2.22 (2H, m)
16	125.21	5.18 (1H, m)	125.21	5.18 (1H, t, *J*=6.6)	125.25	5.17 (1H, t, *J*=7.0)	125.31	5.19 (1H, m)	125.26	5.18 (1H, m)	125.25	5.18 (1H, m)
17	135.67		135.62		135.55		135.62		135.63		135.63	
18	40.41	1.96 (2H, m)	40.38	1.95 (2H, t, *J*=7.8)	40.36	1.94 (2H, m)	40.43	1.96 (2H, m)	40.40	1.95 (2H, m)	40.42	1.95 (2H, m)
19	27.38	~2.05 (2H, m)	27.35	~2.07 (2H, m)	27.34	2.06 (2H, m)	27.38	~2.06 (2H, m)	27.36	~2.06 (2H, m)	27.39	~2.06 (2H, m)
20	125.07	5.07 (1H, m)	125.07	5.07 (1H, t, *J*=6.6)	125.06	5.05 (1H, m)	125.11	5.07 (1H, m)	125.09	5.06 (1H, m)	125.10	5.07 (1H, m)
21	131.53		131.63		131.63		131.64		131.63		131.65	
22	25.77	1.62 (3H, s)	25.76	1.62 (3H, s)	25.76	1.61 (3H, s)	25.78	1.61 (3H, m)	25.78	1.61 (3H, s)	25.79	1.62 (3H, m)
23	17.67	1.55 (3H, s)	17.65	1.55 (3H, s)	17.64	1.53 (3H, s)	17.68	1.54 (3H, s)	17.68	1.54 (3H, s)	17.68	1.55 (3H, s)
24	15.98	1.60 (3H, s)	15.97	1.59 (3H, s)	15.94	1.58 (3H, s)	16.00	1.60 (3H, s)	15.98	1.60 (3H, s)	15.97	1.59 (3H, s)
25	18.57	1.31 (3H, s)	18.37	1.29 (3H, s)	18.39	1.28 (3H, s)	18.59	1.31 (3H, s)	18.54	1.31 (3H, s)	18.42	1.28 (3H, s)
26	131.66		126.16		127.82		132.64		127.96		131.22	
27	151.74		132.97		158.51		122.02	8.36 (1H, d, *J*=2.6)	156.64		131.58	
28	121.30	7.65 (1H, d, *J*=1.8)	120.91	7.49 (1H, d, *J*=7.2)	115.53		114.88		119.43	7.08 (1H, d, *J*=8.4)	118.08	7.44 (1H, d, *J*=2.9)
29	130.51		129.32	7.33 (1H, t, *J*=7.8)	130.02	7.88 (1H, dd, *J*=1.5, 7.7)	159.31		130.36	7.87 (1H, dd, *J*=2.4, 8.4)	157.16	
30	122.53	7.63 (1H, dd, J=1.8, 8.4)	122.60	7.23 (1H, d, *J*=7.8)	119.13	6.98 (1H, t, *J*=7.7)	117.86	6.94 (1H, d, *J*=9.2)	123.67		120.07	7.09 (1H, dd, *J*=2.9, 8.4)
31	125.13	7.59 (1H, d, *J*=8.4)	155.83		135.41	7.65 (1H, dd, *J*=1.5, 7.7)	127.82	8.07 (1H, dd, *J*=2.6, 9.2)	128.11	8.09 (1H, d, *J*=2.4)	130.95	7.34 (1H, d, *J*=8.4)
32	167.05		167.31		173.03		173.07		167.05		167.09	

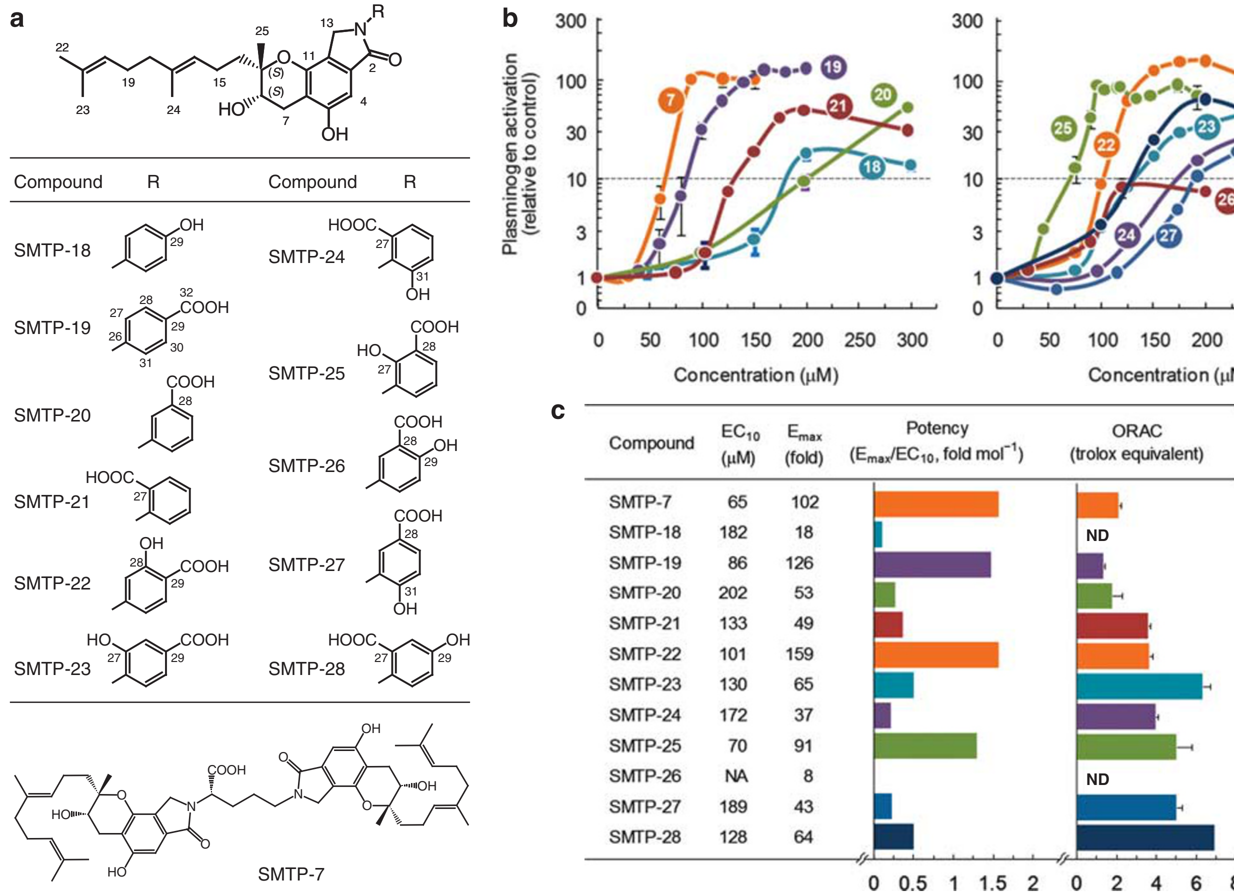

Figure 1 Structure and activity of new SMTP congeners. (**a**) Structures of the new SMTP congeners and SMTP-7. (**b**) The activation of plasminogen was assayed in the presence of the indicated concentrations of each SMTP congener. Numbers in circle represent the SMTP number. Each value represents the mean ± s.d. from triplicate determinations. Percent of control values are shown. (**c**) Summary of the results in panel **b**. EC_{10}, concentration (μM) of SMTP that causes 10-fold enhancement of plasminogen activation; E_{max}, maximum level of enhancement (fold increase in plasminogen activation compared with control). E_{max} and the reciprocal of EC_{10} are independent indexes that represent the potency of the compound. The ratio E_{max}/EC_{10} represents comprehensive potency. NA, not available (owing to that enhancement did not reach 10-fold at concentrations tested). Along with the plasminogen-modulating activity, ORAC values for SMTP congeners are shown in panel **c**. ORAC was assessed in the presence of the SMTP congeners. Each value represents the mean ± s.d. from triplicate determinations. ND, not determined due to experimental limitations.

VLK-*p*NA. A reaction mixture consisting of 50 nM plasminogen, 50 U ml^{-1} tcu-PA, and 0.1 mM VLK-*p*NA in 50 μl of buffer (50 mM Tris-HCl, 100 mM NaCl and 0.01% Tween 80, pH 7.4) was incubated in the presence or absence of SMTP congeners at 37 °C. The hydrolysis of VLK-*p*NA (absorbance at 405 nm) was kinetically monitored for up to 60 min. From the slope of the plots of A_{405} versus t^2, the initial velocity of plasmin generation was calculated.

Assay for ORAC

The ORAC was assayed according to the method of Wu *et al.*[26] using trolox as the standard. For assay, various concentrations of sodium salts of SMTP congeners (50 μl) were mixed with fluorescein (140 nM, 100 μl) for 10 min at 37 °C. Subsequently, 2,2′-azobis(2-amidinopropane) dihydrochloride (48 mM, 100 μl) was added to the mixture. The decay of fluorescence (emission at 535 nm; excitation at 485 nm) was monitored every 2 min to calculate area under the curve. The data obtained were compared with those of trolox. Results were expressed as TE on a molar basis.

General procedures

UV spectrum was measured in MeOH on a model 320 spectrometer (Hitachi, Tokyo, Japan) and IR spectrum on a JIR-WINSPEC (JEOL, Tokyo, Japan) with NaCl. MALDI-TOF-MS spectrum was taken on a Voyager DE STR (Applied Biosystem, Foster City, CA, USA) using α-cyano-4-hydroxycinnamic acid as a matrix. NMR spectra were measured in DMSO-d_6 or acetone-d_6 on a JNM-Alpha-600 (JEOL). Optical rotation was measured in MeOH on a model DIP-360 (JASCO, Tokyo Japan).

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