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NOTE

A new series of the SMTP plasminogen modulator with a phenylglycine-based side chain

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The plasminogen/plasmin system has a central role in blood clot lysis.[1] The system is also important in other pathophysiological events, where localized proteolysis is involved.[2] Plasminogen is proteolytically activated to plasmin by plasminogen activators through specific cleavage at Arg^{561}-Val^{562}.[1] Plasminogen adopts tight conformation due to intramolecular binding of Lys^{50} and/or Lys^{62} to the lysine-binding site in the fifth kringle domain.[3,4] The tight conformation renders plasminogen less sensitive to activation by plasminogen activators. Plasminogen binding to fibrin or cellular receptors allows relaxation of plasminogen conformation, enabling efficient activation. This mechanism facilitates localized activation of plasminogen and following extracellular proteolysis.[5]

Stachybotrys microspora triprenyl phenols (SMTPs) are triprenyl phenol metabolites from the fungus *Stachybotrys microspora*.[5] SMTP enhances both activation and fibrin binding of plasminogen by modulating plasminogen conformation.[5–7] SMTP-7, one of the most potent congeners, is effective in treating thrombotic stroke.[8–11] The SMTP molecule consists of a tricyclic γ-lactam moiety, a geranylmethyl group, and an *N*-linked side chain. Our previous studies identified 26 SMTP congeners, most of which differ in the side chain.[12–18] Plasminogen modulator activities of the congeners differ depending on the *N*-linked side-chain structure. It has been suggested that a negatively ionizable group in the *N*-linked side chain is crucial for activity.[18] Among congeners with a negatively chargeable side chain, one with an aromatic group as the side chain is more active than that with an aliphatic group.[18] In this study, we isolated five new SMTP congeners with a phenylglycine-based side chain to investigate structure–activity relationships further. This paper deals with the isolation and characterization of these congeners. Part of the results has been disclosed as a patent.[19] Biological activities of some of the new congeners have also been described in patent literatures.[20,21]

Our previous studies established fermentation conditions that enable efficient selective production of an SMTP congener through feeding of a precursor amine to *S. microspora* cultures.[17,22] Based on this method, we produced five new SMTP congeners with a phenylglycine-based *N*-linked side chain (Supplementary Materials and Methods). Precursor amines fed for the production is shown in Supplementary Table S1. A culture fed with an optically active precursor afforded single major product (SMTP-43, -43D, -44, or -44D), whereas a culture fed with racemic 3-hydroxy-D,L-phenylglycine gave two major products, which were separable each other on reversed-phase HPLC (retention times at 11.5 and 13.3 min under conditions described in Supplementary Table S1). The slow-eluting isomer was successfully purified on preparative HPLC, whereas the fast-eluting isomer could not be obtained as a homogenous state. The yield varied among the five congeners, ranging from 94 to 1351 mg l^{-1} (Supplementary Table S1).

Physicochemical properties of the new congeners are summarized in Table 1 (see Supplementary Materials and Methods for detailed conditions for the analyses). NMR signals (Supplementary Figures S1S5) are assigned as shown in Supplementary Table S2 according to the results from ^1H–^1H-correlation, heteronuclear multiple quantum coherence and heteronuclear multiple-bond connectivity spectroscopies. Based on these results, we propose the structures of the new congeners as shown in Figure 1a. The conclusion is consistent with the idea that the fed amine is introduced as the *N*-linked side chain of an SMTP molecule.[16,17,22] The stereochemistry of SMTP-45D, the slow-eluting isomer produced in a culture fed with the racemic precursor, was proposed based on the following facts: (i) the 26*R* epimers, SMTP-43D and -44D, were eluted slower on reversed-phase HPLC compared with respective 26*S* epimers, SMTP-43 and -44 (Supplementary Table S1); (ii) specific optical rotations of SMTP-43 and -44 were in the plus sign, while those of SMTP-43D and -44D were in the minus sign (Table 1); (iii) retention time of SMTP-45D was slower than the its epimer (13.3 min compared with 11.5 min), and specific rotation of SMTP-45D was in the minus sign (Table 1); (iv) *S. microspora* culture fed with 3-hydroxy-L-phenylglycine ((*S*)-3-hydroxyphenylglycine) afforded the fast-eluting epimer but not the slow-eluting one (Supplementary Figure S6). Therefore, *R* configuration was assigned to position 26 in SMTP-45D.

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Table 1. Physicochemical properties of new SMTP congeners

	SMTP-43	*SMTP-43D*	*SMTP-44*	*SMTP-44D*	*SMTP-45D*
Appearance	Pale yellow oil	Pale yellow oil	Reddish brown oil	Reddish brown oil	Yellowish oil
Molecular formula	$C_{31}H_{37}NO_6$	$C_{31}H_{37}NO_6$	$C_{31}H_{37}NO_7$	$C_{31}H_{37}NO_7$	$C_{31}H_{37}NO_7$
MALDI-TOF-MS					
Found $(M + H)^+$:	520.2662	520.2662	536.2656	536.2671	536.2723
Calculated:	520.2699 for $C_{31}H_{38}NO_6$	520.2699 for $C_{31}H_{38}NO_6$	536.2648 for $C_{31}H_{38}NO_7$	536.2648 for $C_{31}H_{38}NO_7$	536.2648 for $C_{31}H_{38}NO_7$
UV λ_{max} nm (ε) MeOH	214 (46 214)	215 (44 864)	216 (42 714)	216 (46 490)	215 (57 915)
	259 (11 112)	259 (11 008)	262 (11 026)	261 (11 730)	261 (13 703)
	300 (3 012)	300 (3 012)	300 (2 783)	300 (2 946)	300 (3 747)
IR ν_{max} (neat) cm^{-1}	3423, 2968, 2920, 2864, 1726, 1660, 1620, 1464, 1350, 1205, 1169, 1074	3354, 2968, 2922, 2862, 1714, 1664, 1620, 1466, 1356, 1207, 1167, 1074	3348, 2974, 2922, 2856, 1718, 1660, 1612, 1514, 1464, 1365, 1211, 1173, 1072, 922, 827, 768	3325, 2970, 2922, 2858, 1711, 1662, 1612, 1512, 1464, 1365, 1217, 1173, 1074, 922, 831, 771	3309, 2974, 2924, 2864, 1707, 1662, 1603, 1464, 1365, 1224, 1163, 1076, 847, 771, 712, 656, 536
Specific rotation $[\alpha]_D^{27}$	47.3° (*c* 0.45, MeOH)	−110.6° (*c* 0.45, MeOH)	49.0° (*c* 0.45, MeOH)	−89.1° (*c* 0.45, MeOH)	−110.2° (*c* 0.45, MeOH)

Abbreviation: SMTP, Stachybotrys microspora triprenyl phenol.

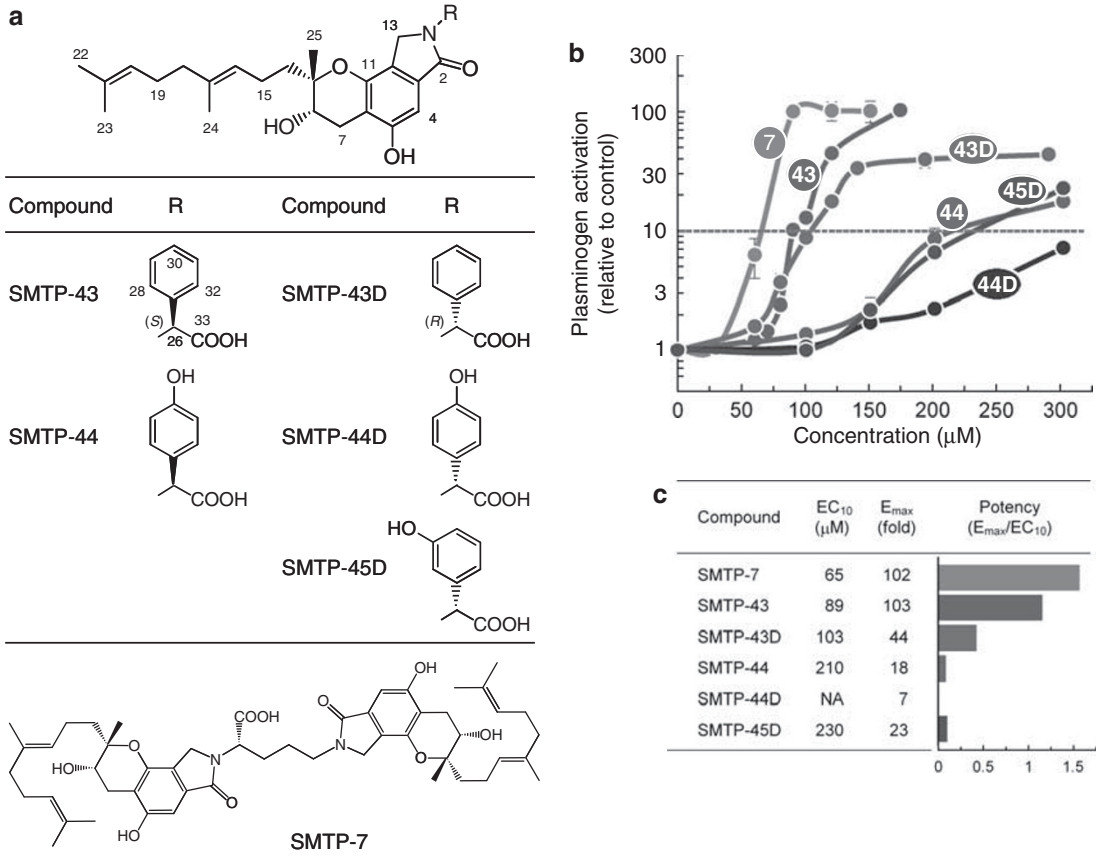

Figure 1 Structure and activity of new SMTP congeners. (**a**) Structures of new SMTP congeners and SMTP-7. (**b**) The activation of plasminogen was assayed in the presence of the indicated concentrations of each SMTP congener. Numbers in circle represent the SMTP number. Each value represents the mean ± 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 (due to that enhancement did not reach 10-fold at concentrations tested). A full color version of this figure is available at *The Journal of Antibiotics* journal online.

Plasminogen modulator activities of the five new SMTP congeners (Figures 1b and c) were assessed as their activity to enhance plasminogen activation (see Supplementary Materials and Methods) in comparison with SMTP-7, one of the most potent congeners identified so far. SMTP-43, which had an L-2-phenylglycine moiety as the *N*-linked side chain, was potent in enhancing plasminogen activation. The concentration that caused 10-fold enhancement (EC_{10}) was 89 μM, and the maximum level of the enhancement (E_{max})

exceeded 100-fold (Figures 1b and c). These parameters were comparable to those of SMTP-7 (EC_{10}=65 μM; E_{max}=102-fold). The 26*R* epimer SMTP-43D (E_{max}/EC_{10}=0.43-fold μM^{-1}), which had a D-2-phenylglycine moiety, was significantly weaker than SMTP-43 (E_{max}/EC_{10}=1.16-fold μM^{-1}). SMTP-44, an analog of SMTP-43 with a hydroxyl group at position 30, was ~13 times less active than SMTP-43 in terms of the E_{max}/EC_{10} value (Figures 1b and c). SMTP-44D was much less active than its epimer, SMTP-44, giving sevenfold enhancement even at 300 μM. The potency (E_{max}/EC_{10}) of SMTP-45D was ~1/4 of that of SMTP-43D, while SMTP-45D was significantly more active than the regioisomer SMTP-44D.

Thus, among the series of SMTP congeners with a phenylglycine-based side chain, SMTP-43, which has no hydroxyl group in the side chain, is most potent. Its activity is comparable to the two-unit congener SMTP-7, which has two triprenyl phenol units. The introduction of a phenolic hydroxyl group into the *N*-linked side chain of SMTP-43 results in a decrease in potency. The position of the hydroxyl group affects the potency of a congener. A congener with an *S* configuration at the phenylglycine moiety is more active than that with an *R* configuration.

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Supplementary Information accompanies the paper on The Journal of Antibiotics website (http://www.nature.com/ja)