NOTE

Pre-SMTP, a key precursor for the biosynthesis of the SMTP plasminogen modulators

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SMTPs are triprenyl phenol metabolites obtained from the fungus *Stachybotrys microspora*.¹ SMTP enhances the activation of plasminogen by modulating its conformation.^{1–4} SMTP-7, one of the most potent congeners, is effective in enhancing blood clot clearance and treating thrombotic stroke.^{4–8} The SMTP molecule consists of a tricyclic γ -lactam moiety, a geranylmethyl group and an *N*-linked side chain. Our previous studies identified 30 SMTP congeners, which differ in the *N*-linked side chain.^{9–16} Plasminogen modulator activities of the congeners differ depending on the *N*-linked side-chain structure.^{15,16} These congeners can be produced by a precursor amine-fed culture.¹⁷ In this method, some metabolites

existing in the culture are consumed following the amine feeding, with a concomitant formation of an SMTP congener, which has the fed amine as the *N*-linked side chain. Thus, the presence of a key precursor for the biosynthesis of SMTPs is suggested. In this study, we isolated a compound, designated pre-SMTP (**1**), which directly affords SMTP congeners by reacting nonenzymatically with amines.

In the precursor amine-fed culture, *S. microspora* is grown in medium containing limited amounts of amine compounds before amine feeding. During this “starvation phase”, significant amounts of possible precursors are accumulated. We purified two of the candidates, compounds **1** and **2** (Figure 1a), by solvent extraction

Figure 1 Structures of SMTP-0, pre-SMTP (**1**), LL-Z1272 β (**2**), SMTP-54 (**3**) and SMTP-55 (**4**). (a) Structures of SMTP-0, pre-SMTP (**1**) and LL-Z1272 β (**2**). (b) Key ^1H - ^{13}C correlations observed in the HMBC spectrum of **1**. (c) Structures of SMTP-54 (**3**) and SMTP-55 (**4**), which are derived from **1** through nonenzymatic reaction with L-glutamine and L-glutamic acid, respectively.

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and reversed-phase HPLC (see Supplementary Methods and Results). The physicochemical properties (Supplementary Table S1) and the NMR spectra of **1** (Supplementary Table S2 and Supplementary Figure S1) suggested that **1** is a new compound. The physicochemical properties of **2** (Supplementary Methods and Results) were consistent with those of LL-Z1272 β , which was isolated from *Fusarium* sp. as an antiprotozoan.¹⁸ The presence of a geranylmethyl group and a chroman skeleton in the structure of **1**, which were identical to the partial structure of the SMTP family compounds, was confirmed by the NMR spectral data, including hetero-nuclear multiple-quantum coherence and HMBC spectroscopies. The NMR data suggested that **1** did not have the lactam structure, as the carbonyl signal at position 2 (δ_c 170.24) and the methylene signals at position 13 (δ_c 41.95; δ_H 4.09 and 4.05) present in SMTP-0 were missing. Instead, the low-field signals at δ_c 193.06 and 190.06, as well as δ_H 10.46 (1H, s) and 10.37 (1H, s), suggested the presence of two aldehyde groups. An *o*-dialdehyde structure and the relative position of the two low-field oxygen-bearing olefinic carbons (δ_c 161.96 and 159.17) and the olefinic methine (δ_c 106.66; δ_H 6.85) were confirmed by the HMBC spectrum (Figure 1b). On the basis of these observations, the structure of **1** was elucidated as shown in Figure 1. The stereochemistries at positions 8 and 9 shown in Figure 1 were based on the fact that **1** afforded SMTP-0, SMTP-4 and SMTP-6 on reaction with ammonium chloride, L-phenylalanine and L-tryptophan, respectively (see below). Thus, we designate compound **1** pre-SMTP.

The structural features of **1** and **2**, and the fact that these compounds are rapidly consumed following the amine feeding, suggest that these compounds are precursors of SMTPs. Compound **2** has an uncyclized farnesyl side chain, and hence is assumed to be a precursor of pre-SMTP, which has a geranylmethyl side chain attached to a chroman structure. In addition, **2** lacks 8-OH and has a methyl group instead of an aldehyde group in **1**. Thus, there may be at least 3 steps, a cyclization, a hydroxylation (position 8) and an oxidation (position 2), in the conversion of **2** to **1**.

Although SMTP-7 enhanced the plasminogen activation catalyzed by urokinase-type plasminogen activator activities of compounds **1** and **2** in promoting plasminogen activation were minimal (Supplementary Figure S2).

Pre-SMTP (**1**) has an *o*-phthalaldehyde partial structure, which can react with an amine compound to afford a phthalimidine structure such as that present in SMTPs. The formation of phthalimidines via a 1:1 condensation between *o*-phthalaldehyde and several kinds of amines has been reported to proceed under mild conditions.^{19,20} Therefore, we tested **1** for reaction with some amines. Although **1** was stable in the absence of amine in buffer (20 mM potassium phosphate, pH 7.4) or 50% aqueous acetone in the presence or absence of acetic acid (1%, v/v) (Figure 2a), the addition of ammonium acetate resulted in a decrease in pre-SMTP and a concomitant formation of SMTP-0 (Figure 2b) under these three conditions. Similarly, the reaction between **1** and L-phenylalanine and L-tryptophan afforded SMTP-4 and SMTP-6, respectively (Figures 2c and d). These results suggest that **1** is a direct precursor of SMTPs.

The formation of SMTPs through nonenzymatic reaction between **1** and an amine enables the synthesis of a variety of congeners with different *N*-linked side-chain structures. The microbial production of SMTP congeners through the precursor amine-feeding method cannot be adapted for certain amines, possibly because of the problems with cellular uptake. For example, congeners with L-glutamine and L-glutamic acid as the *N*-linked side chain have not been produced through the precursor amine-feeding method. Nevertheless, the nonenzymatic reaction between **1** and these amines

Figure 2 Conversion of pre-SMTP (**1**) to SMTPs. Pre-SMTP (100 $\mu\text{g ml}^{-1}$ in acetone) was incubated with 1 volume of water (a), ammonium acetate (b), L-phenylalanine (c) or L-tryptophan (d) in the presence of 1.5% (v/v) acetic acid, and the products were analyzed by HPLC. The arrowhead indicates the peak at which UV spectrum (shown in the inset) and mass spectrum were taken. The elution positions, UV spectra and mass spectra of the indicated peaks in panels (a–d) were consistent with those of authentic pre-SMTP, SMTP-0, SMTP-4 and SMTP-6, respectively. Essentially the same results were obtained with the incubations of pre-SMTP and amines in the absence of acetic acid or the incubation in buffer, where acetone concentration was 1% (v/v).

afforded congeners with an expected side chain, SMTP-54 and SMTP 55, respectively (Figure 1c; Supplementary Methods and Results; Supplementary Table S3; Supplementary Figures S3 and S4).

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