Telomestatin, a Novel Telomerase Inhibitor from *Streptomyces anulatus*

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Telomeres are the guanine-rich, simple repeat sequences of TTAGGG that constitute the physical termini of eukaryotic chromosomes. Maintenance of telomeres, in which the specialized ribonucleoprotein complex known as telomerase mediates, is significant for immortalization in cancer cells.[1] Since the correlation between telomerase activity and tumors has led to the hypothesis that tumor growth requires reactivation of telomerase and that telomerase inhibitors represent a class of chemotherapeutic agents, we attempted to screen telomerase inhibitors from the metabolites of the microorganism. A wide range of screening resulted in the isolation of a potent specific telomerase inhibitor designated as telomestatin (**1**) from *Streptomyces anulatus* 3533-SV4. Although telomerase consists of several components with DNA polymerase or reverse transcriptase activity in addition to its intrinsic telomerase component, **1** specifically inhibited telomerase without affecting DNA polymerases and reverse transcriptases (RT) such as Taq polymerase and HIV-RT. Here, we describe the structure and biological properties of **1**.The telomestatin-producing organism, *Streptomyces anulatus* 3533-SV4, was cultivated in a production medium consisting of 2% glycerol, 1.0% molasses, 0.5% casein, 0.1% polypepton, and 0.4% $CaCO_3$ for 3 days in a jar fermenter. The whole culture broth was centrifuged and the collected mycelium was extracted with the same volume of acetone as that of the culture broth. After concentration in vacuo, the residual aqueous layer was partitioned between EtOAc and H_2O. The concentrated organic layer was applied to a silica gel column and eluted with $CHCl_3$−MeOH (20:1 to 10:1). The active eluate was chromatographed on a silica gel column with $CHCl_3$−MeOH−NH_4OH (700:100:1) as the solvent system. Finally, a pure sample of **1** was obtained as a white yellowish powder by HPLC using a PEGASIL ODS column developed with 70% CH_3CN containing 0.1% trifluoroacetic acid.

A high-resolution FAB-MS of **1** (*m*-nitrobenzyl alcohol) $[\alpha]_D$ −9.4° (*c* 0.13, MeOH) [mp 134−143 °C dec] established the molecular formula of **1** as $C_{26}H_{14}N_8O_7S$ [$(M + H)^+$, *m*/*z* 583.0790 (calcd 583.0784)]. The ^1H and ^{13}C NMR spectral data together with direct $^{13}C-^1H$ correlation established by an HMQC experiment for **1** are summarized.[2]

The ^1H NMR spectrum of **1** showed five isolated aromatic proton signals 12-H (δ_H 8.12), 15-H (δ_H 8.24), 18-H (δ_H 8.13), 21-H (δ_H 8.34), and 24-H (δ_H 8.12), which were connected to carbon signals appearing at δ_C 137.5−141.2 by HMQC correlations. Long-range couplings from these aromatic protons to quaternary aromatic carbons at δ_C 130.4−136.7 and at δ_C 156.2−157.3 proved the presence of five oxazole moieties (rings D to H) as shown in Figure 2 (**2** and **3**), though their connectivities remained unclear at this point due to negligible long-range couplings through the bonds connecting oxazole rings.[3,4] The presence of the oxazole rings in **1** was supported by comparison of the ^{13}C-chemical shifts with a similar unit in kabiramide C[4] shown in Figure 2 (**4**) and related compounds.[3,5]

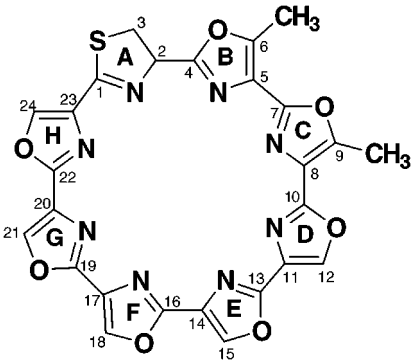

Figure 1. Structure of telomestatin (**1**).

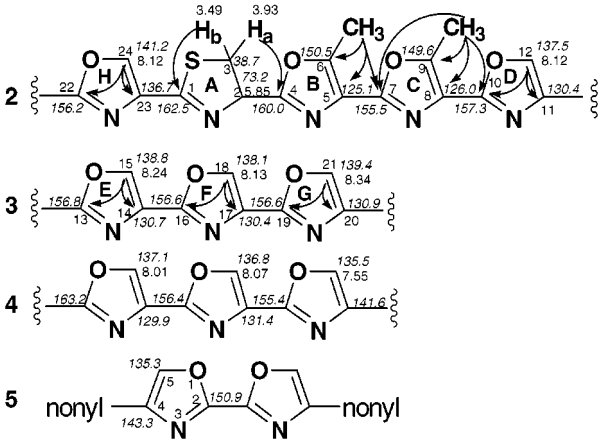

Figure 2. Partial structures of **1**. Arrows show $^1H-^{13}C$ long-range correlations observed in HMBC. **4** and **5** show the model compounds.[4,10]

Long-range couplings from two isolated arylic methyl protons 6-CH_3 (δ_H 2.55) and 9-CH_3 (δ_H 2.65) to carbon signals at δ_C 150.5 and 125.1 and δ_C 149.6 and 126.0, respectively, proved two methyloxazole moieties in Figure 2 (**2**). These chemical shift values are in good agreement with those of tantazole F.[6] In addition, key long-range couplings from 9-CH_3 to 10-C of the

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(2) ^{13}C NMR (125 MHz, $CDCl_3-CD_3OD$ (1:2)) (ppm): thiazoline ring A, 162.5 (s, C-1*), 73.2 (d, C-2), 38.7 (t, C-3); methyloxazole ring B, 160.0 (s, C-4*), 125.1 (s, C-5), 150.5 (s, C-6), 11.5 (q, 6-CH_3); methyloxazole ring C, 155.5 (s, C-7), 126.0 (s, C-8), 149.6 (s, C-9), 11.5 (q, 9-CH_3); oxazole ring D, 157.3 (s, C-10), 130.4 (s, C-11), 137.5 (d, C-12); oxazole ring E, 156.8 (s, C-13), 130.7 (s, C-14), 138.8 (d, C-15); oxazole ring F, 156.6 (s, C-16), 130.4 (s, C-17), 138.1 (d, C-18); oxazole ring G, 156.6 (s, C-19), 130.9 (s, C-20), 139.4 (d, C-21); oxazole ring H, 156.2 (s, C-22), 136.7 (s, C-23), 141.2 (d, C-24). ^1H NMR (500 MHz, $CDCl_3-CD_3OD$ (1:2)) (ppm): thiazoline ring A, 5.95 (bs, 2-H), 3.93 (m, 3-H_a), 3.49 (t, 12 Hz, 3-H_b); methyloxazole ring B, 2.55 (3H, s, 6-CH_3); methyloxazole ring C, 2.65 (3H, s, 9-CH_3); oxazole ring D, 8.17 (s, 12-H); oxazole ring E, 8.24 (s, 15-H); oxazole ring F, 8.13 (s, 18-H); oxazole ring G, 8.34 (s, 21-H); oxazole ring H, 8.12 (s, 24-H). ^{13}C chemical shifts identified with an asterisk are exchangeable. Assignments for oxazole rings E, F, and G are exchangeable.

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oxazole ring D and to 7-C of the ring C established the linkage between ring D and ring C which was further connected to the methyloxazole ring B by long-range coupling between 6-CH_3 to 7-C of ring C. Assignment of 4-C of the ring B remained unclear due to the lack of long-range coupling with 6-CH_3.

The remaining units, CH_2, CH, −C═, S, and N, were connected to give a thiazoline ring A by observation of a spin coupling between a methine proton 2-H (δ_H 5.95, δ_C 73.2) and methylene protons 3-H_a and 3-H_b (δ_H 3.93 and 3.49, respectively, δ_C 38.7) in the phase-sensitive DQF spectrum of **1**, the methylene proton 3-H_b being long-range coupled to a quaternary aromatic carbon C-1 (δ_C 162.5 or 160.0). Line broadening of 2-H of the ring A prevented the determination of any long-range coupling information with this proton. The ^{1}H and ^{13}C chemical shift values of the thiazoline ring in **1** well coincided with those of didehydrotantazole A and tantazole F,[6] dolastatin E,[7] cyclothiazomycin,[8] and thiangazole.[9]

Based on its chemical shift value, the only remaining carbon signal appearing at δ_C 160.0 (or 162.5) was assigned to C-4 which was long-range coupled to the methylene proton 3-H_a in the thiazoline unit to afford the connectivity between thiazoline ring A and methyloxazole ring B. Thus, the sequence from thiazoline ring A to oxazole ring D through methyloxazole rings B and C was elucidated as shown in Figure 2 (**2**).

The ^{13}C chemical shift values of the second oxazole ring (ring F) in the trisoxazole moiety were typical and similar to those reported for kabiramide C (Figure 2, **4**).[4] The ^{13}C chemical shift values of oxazole rings except those of oxazole ring H in **1** well coincided with those of conjugated oxazole units, revealing the existence of a sequential oxazole moiety.[3,4,9] Among the two possible connecting patterns of oxazole rings, the linkage between C_2 and C_4 is considered to be preferable over the linkage C_2-C_2 based on their ^{13}C chemical shifts. The ^{13}C chemical shift of the C_2 carbon bound to the C_4 carbon[4] (Figure 2, **3**) was observed at a lower field than that bound to the C_2 carbon (Figure 2, **5**).[10] In addition to these ^{13}C chemical shifts, biosynthetic considerations of the oxazole moieties also supported the sequence of this trisoxazole moiety. The ^{13}C chemical shifts of C_4 and C_5 carbons of the remaining oxazole ring H were observed at a relatively low field reflecting the environmental difference of ring H from rings D to G. Thus, the trisoxazole moiety was finally inserted between oxazole rings D and H to construct the cyclic structure as shown in Figure 1. To the best of our knowledge, **1** is the first example with a macrocyclic system containing a sequential pentaoxazole moiety. Some related compounds with linear trisoxazole or sequential thiazoline units have been reported.[3,4,6,8]

Inhibitory effects against telomerase, which was semipurified from the cell lysates of human B lymphoma Namalwa cells, were estimated by using a modified TRAP assay with the addition of an internal standard.[1,11] **1** specifically inhibited telomerase activity with an IC_{50} value of 0.005 μM, whereas it did not show activities against DNA polymerases such as Taq polymerase. Since telomerase is a multisubunit ribonucleoprotein complex that includes an RNA component and a reverse transcriptase (RT) catalytic subunit,[12,13] the inhibitory effects of **1** against RT were investigated. It showed weak activities against RTs such as HIV- and MMLV (Moloney Murine Leukemia Virus), RTs with IC_{50} values of 19.4 and 13.4 μM, respectively. Thus, **1** was proved to be a specific telomerase inhibitor; the ratios of IC_{50} values of **1** against telomerase to those of **1** against HIV-RT and MMLV-RT were 3880 and 2680, respectively. It is to be noted that the inhibitory activities of representative synthetic telomerase inhibitors TMPyP4[14] and BSU1051[15] against telomerase were not remarkable with IC_{50} values of 0.63 and 80.0 μM, respectively, and their ratios to IC_{50} values against HIV- and MMLV-RT were also less selective with values of 6.98, 9.68, 0.65, and 0.35, respectively. These results reveal that **1** is the strongest and most specific telomerase inhibitor ever reported.

Although telomerase plays a significant role in cellular senescence and tumorigenic conversion, its role in proliferation and immortality is yet to be clarified, since a few rare tumors and some experimentally immortalized cells, do not exhibit detectable telomerase activity. Therefore, **1** would be a useful tool for studying the characteristics of telomerase. Detailed studies on biological activities such as mechanistic, kinetics, and cytotoxicity are now under way. The planar structure of **1** has recently been confirmed by chemical synthesis. Details will be published elsewhere.

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