COMMUNICATIONS TO THE EDITOR

NA22598A$_1$, a Novel Antitumor Substance Produced by *Streptomyces* sp. NA22598

Sir:

In the course of our screening program for new antitumor compounds, we isolated a new type of peptide, NA22598A$_1$ (**1**) (Fig. 1) from a culture broth of *Streptomyces* sp. NA22598 (FERM P-14686). This compound proved to inhibit the anchorage-independent growth of a human colon-cancer cell line, DLD-1, on poly 2-hydroxyethylmethacrylate (HEMA) coated plates[1]. In this paper we report the production, isolation, physico-chemical properties and biological properties of **1**.

The strain was cultured at 27°C for 5 days in 500-ml Erlenmeyer flasks containing 100 ml of a medium composed of galactose 2.0%, dextrin 2.0%, corn steep liquor 0.5%, Bacto-soytone (Difco) 1.0%, $(NH_4)_2SO_4$ 0.2% and $CaCO_3$ 0.2%, adjusted to pH 7.4 before sterilization.

Isolation was followed by measuring the antitumor activity against the anchorage-independent growth of DLD-1 on poly HEMA coated plates. The culture broth (20 liters) pH was adjusted to 4.0 with 6 N HCl and was allowed to stand at r.t. for an hour, and was centrifuged at 15,000 rpm. The supernatant was applied to a Dowex 50W × 2 (H^+) column. The active fractions were eluted with 1.5 N NH_4OH after washing with water and the eluate was concentrated *in vacuo* for removing ammonia. The concentrated solution was applied to an Amberlite CG-50 (H^+) column. The activity was eluted with 1.5 N NH_4OH and then the eluate was evaporated *in vacuo* to dryness. This residue was dissolved in water and applied to a TSK gel SP-Toyopearl 650C (NH_4^+) column. After washing the column with water, the elution was carried out by a linear gradient from H_2O to 1.0 M NH_4Cl. The active fractions were collected and then absorbed on a Diaion SP-207 column for desalination. After washing the column with water, the activity was eluted with 50% aqueous acetone. The eluate was concentrated and was applied to a TSK gel SP-Toyopearl 650C (NH_4^+) column which was pre-equilibrated with 0.05 M NH_4OAc buffer (pH 3.7) before use. The elution was carried out by a linear gradient from 0.05 to 1.00 M NH_4OAc buffer (pH 3.7). The active fractions were eluted approximately at 0.6 M NH_4OAc buffer, collected and desalinated as described above using Diaion SP-207. The active compounds were purified by a preparative HPLC (ODS, Capcell pak UG-120) using the mobile phase of 5% CH_3CN in 50 mM phosphate buffer (pH 9.2). The active fractions were collected and desalinated by a Dowex 50W × 8 (H^+) column with 1.5 N NH_4OH elution. The eluate was finally concentrated and lyophilized to afford 20 mg of **1**.

NA22598A$_1$ (**1**) was a colorless powder with a melting point of 190~195°C. The molecular formula was determined to be $C_{20}H_{38}N_8O_7$ by HRFAB-MS (Found: *m/z* 503.2943 $(M+H)^+$, calcd. for $C_{20}H_{39}N_8O_7$ 503.2942). It is easily soluble in DMSO and water, slightly soluble in methanol and insoluble in chloroform. It showed positive reactions to ninhydrin, Rydon-Smith and phosphomolybdic acid tests, but was negative to the Sakaguchi test. Amino acid analysis indicated the presence of alanine and valine in it. Both amino acids were determined to be *L* by HPLC analysis of *o*-phthalaldehyde (OPA) derivatives[2]. The UV spectrum showed end absorption. The IR spectrum (KBr) showed

Fig. 1. Structure of NA22598A$_1$ (**1**).

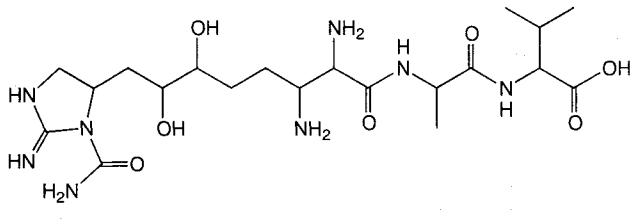
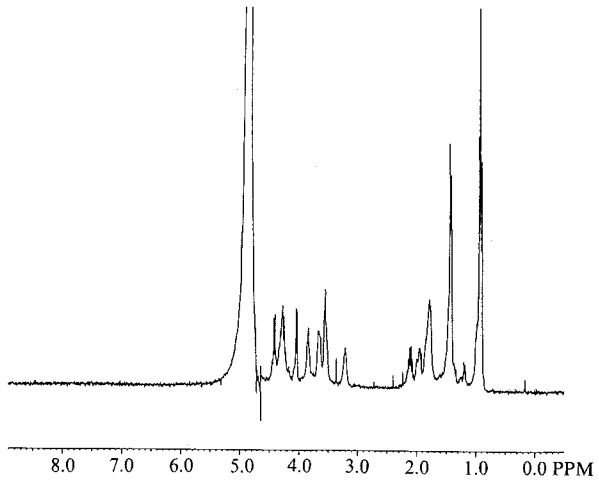


Fig. 2. ^1H NMR spectrum of NA22598A$_1$ in D_2O (300 MHz).



absorption bands at 3370, 2963, 1731, 1654, 1589 and 1399 cm^{-1}. The ^1H NMR spectrum of **1** is shown in Fig. 2. The ^{13}C NMR spectrum displayed signals at δ 179.6, 175.6, 175.4, 157.6, 157.1, 75.5, 72.8, 62.2, 58.9, 55.5, 52.7, 51.7, 51.0, 37.3, 31.8, 29.5, 28.4, 20.1, 18.6, and 17.8 ppm, which accounts for the presence of 20 carbon signals. All ^1H and ^{13}C NMR signals of NA22598A_1 were assigned by analyses of 2D NMR spectra. Based on the NMR data, the presence of 8-(2-iminoimidazolin-4-yl)-2,3-diamino-6,7-dihydroxyoctanoic acid, alanine and valine units were elucidated. Analysis of HMBC spectra revealed the connectivities among units described above. The structure of NA22598A_1 is a unique peptide containing the 1-carbamoyl-2-iminoimidazolin moiety[3]. Details of the structural determination of **1** and its congeners will be reported in a separate paper. NA22598A_1 was inactive at 200 μg/ml against Gram-positive and Gram-negative bacteria, yeast and fungi.

NA22598A_1 inhibited the anchorage-independent growth of DLD-1 cells on poly (HEMA)-coated plates at the concentration of 0.32 μM (IC_{50}), but did not inhibit the growth on uncoated plates at the same concentration. This result suggests that NA22598A_1 may be an inhibitor of oncogenic signal-transduction pathways[4]. Details of biological activity and the mechanism of action studies of NA22598A_1 will be reported in a separated paper.

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