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ACTINOMYCIN D, C_2 AND VII, INHIBITORS OF GRB2-SHC INTERACTION PRODUCED BY *STREPTOMYCES*

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Abstract: Actinomycin D, C_2 and VII, cyclic peptides, inhibited Grb2 SH2 domain association (IC_{50} 5-7 μM) with a phosphotyrosine containing peptide derived from the Shc protein (pTyr317). Actinomycins are the first examples of nonphosphorylated natural ligands of SH2 domain.

Growth factor receptor-bound protein 2 (Grb2) and Src homologous and collagen (Shc) protein are adaptor proteins containing a Src homology 2 (SH2) domain that mediate the interaction with growth factor receptors or adaptor proteins by binding to specific phosphotyrosine motifs. Grb2 associates with phosphotyrosine sites of the activated receptors or Shc via their SH2 domain to link receptor tyrosine kinases to Ras signaling.[1] This process of activation can lead to run away cell proliferation, differentiation and apoptosis in many kinds of cell types, resulting in diseases including cancer.[2] Thus, blocking of the Grb2-Shc complexes may be lead to intervene the oncogenic signal transduction pathways and to develop a new antitumor drug.

In the course of screening for Grb2-Shc interaction inhibitors of microbial origin, we have isolated actinomycin C_2 and VII from *Streptomyces* sp. A1525.[3] Actinomycins are a group of peptide antibiotics produced by various species of *Streptomyces*. The increasing interest in these natural compounds has been due to the biological significance of their action: they bind noncovalently to DNA and strongly inhibit the transcription of DNA to RNA.[4] Therefore, actinomycins have become a powerful tool in biochemistry, molecular and cell biology. The whole broth (8 liters) of *Streptomyces* sp. A1525 was extracted with BuOH (8 liters x 2) to afford a crude extract (2.4g). The extract was applied on chromatography (C18, SiO_2, and HPLC) to give actinomycin D (1.5 mg/L), C_2 (10 mg/L), and VII. (8 mg/L). Active compounds were isolated by the monitoring of binding affinity against GST-Grb2 SH2 domain. Their UV spectrum data show the presence of chromophore, actinocin (λ_{max} in CH_3OH at 220 and 420nm). The molecular formulas of the inhibitors were determined as $C_{62}H_{86}N_{12}O_{16}$, $C_{63}H_{88}N_{12}O_{16}$ and $C_{64}H_{90}N_{12}O_{16}$, respectively by

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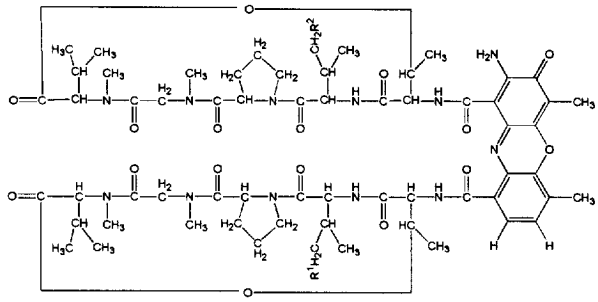
HRFAB-MS. The actinomycin analogues were identified as the known actinomycin D, C_2, and VII according to the spectroscopic data.[3]



Actinomycin D:
$R^1 = H$, $R^2 = H$
Actinomycin C_2:
$R^1 = CH_3$, $R^2 = H$
Actinomycin VII:
$R^1 = CH_3$, $R^2 = CH_3$

The binding affinity of actinomycins to the Grb2 SH2 domain was measured in the presence of various concentrations (from 0.62 μM to 39.8 μM) of actinomycins. Actinomycin D, C_2, and VII inhibited the binding between GST-Grb2 SH2 domain and [H^3]-labeled-phosphopeptide (Ac-SpYVNK-NH-C(O)-$CH_2CH_2H^3$, derived from Shc pY317) with IC_{50} values of 5.0 μM, 5.9 μM, and 7.6 μM, respectively. And the displacement assay of Grb2 SH2 binding to labeled phosphopeptide were also performed with cold phosphopeptide (Ac-SpYVNVK-NH_2) to give IC_{50} of 0.5 μM.[5,6]

Even though, a couple of peptide or peptidomimetic inhibitors were reported,[6] actinomycins are the first inhibitors of the Grb2-Shc complex from natural sources. They will serve as useful compounds in understanding *ras* signal transduction pathways.

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