Inhibition of DNA Topoisomerases by Nidulalin A Derivatives

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Three cytotoxic dihydroxanthone derivatives, nidulalin A(1), F390B(2), and F390C(3) were evaluated for inhibitory activity against DNA topoisomerases. Compounds 1 and 2 inhibited DNA topoisomerase II with IC₅₀ values of 2.2 μ M and 16 μ M, and 3 inhibited DNA topoisomerase I with an IC₅₀ value of 5.9 μ M.

Key words topoisomerase inhibitor; nidulalin A; F390B; F390C; dihydroxanthone

DNA topoisomerases are nuclear enzymes which regulate the state of DNA topology. The enzymes are important for DNA replication, recombination, and transcription.¹⁾ In the previous report, we reported the isolation of nidulalin A (1), which was originally isolated from *Emericella nidulans*,²⁾ and its derivatives, F390B (2) and F390C (3) (Fig. 1), from *Penicillium* sp. as cytotoxic agents.³⁾

The mechanism of cytotoxic activity of these natural products was not clear. To elucidate the mode of action, inhibitory activity against DNA topoisomerases was studied. We found that F390C was a selective inhibitor of DNA topoisomerase I (Topo I), and nidulalin A and F390B were inhibitors of DNA topoisomerase II (Topo II). We report herein the inhibitory activity of nidulalin A and its derivatives against DNA topoisomerases.

Nidulalin A (1), F390B (2), and F390C (3) were evaluated for inhibitory activity against Topo I⁴⁾ and Topo II.⁵⁾ Nidulalin A, the major metabolite of the producing strain, and F390B were inhibitory to Topo II with IC₅₀ values of 2.2 μ M and 16 μ M, respectively. In contrast, F390C inhibited Topo I with an IC₅₀ value of 5.9 μ M. Nidulalin A and F390C did not stabilize the cleavable complex (data not shown). This result indicates that these dihydroxanthone derivatives inhibit the enzymes in a different manner from that of camptothecin or VP-16. The analogs, diketone (4) and 4*S* isomer of nidulalin A (5), both derived from 1,³⁾ retained weak inhibitory activity against Topo II with IC₅₀ values of 32 μ M and 49 μ M, respectively. In addition, weak inhibition by 5 against Topo I was observed. Reduction of 1 by NaBH₄ in MeOH gave a compound 6⁶⁾ in 88% yield, which exhibited decreased effect on Topo II and marginal activity against Topo I at a higher dose (Table 1).

Among the compounds with a methyl group at the C-6 position nidulalin A inhibited Topo II to a greater extent than

diketone (4) or alcohol (5). These results indicate that the conjugated dienone moiety is necessary for inhibitory activity against Topo II, and the hydroxyl group at the C-4 position with *R* stereochemistry is required as well. The dienone moiety makes the cyclohexadiene-ring conformation rigid for favorable interaction with the target enzyme. This moiety is reactive as a Michael acceptor possibly to form a covalent bond with nucleophile in the active site of the enzyme, and its reactivity may be related to inhibitory activity. Interestingly, substitution of a hydroxyl group for the hydrogen of the methyl group at the C-6 altered inhibition selectivity. Further evaluation of details of the inhibition mechanism, and preparation of related compounds are now in progress.

MATERIALS AND METHODS

General Melting point (uncorrected) was determined on a Yanako MP-S3. Optical rotation value was measured on a JASCO DIP-370 polarimeter. ¹H-NMR spectrum was recorded on a Varian XL-300 spectrometer. Mass spectra were recorded on a JEOL JMS-HX110 spectrometer. Preparative TLC was carried out on a silica gel plate (Merck Kieselgel 60 art. 13794).

Preparation of Compounds 4 and 5 Preparation of 4 and 5 was shown in ref 3.

Table 1. Inhibitory Activity of Nidulalin A Derivatives against Topo I and Topo II

	IC ₅₀ (μ M)	
	Topo I	Topo II
1	172	2.2
2	175	16
3	5.9	154
4	>300	32
5	78	49
6	220	196
Camptothecin	<5	nd. ^{a)}
ICRF193	nd.	3.1

^{a)} not determined

Fig. 1

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Reduction of Nidulalin A To a solution of **1** (9 mg) in MeOH (2 ml) was added NaBH₄ (1.5 mg) at 5 °C, and the mixture was stirred for 5 min at the same temperature. After addition of 5% HCl (15 ml), the mixture was extracted with EtOAc (10 ml). The organic layer was concentrated *in vacuo*. Further purification was achieved by preparative TLC developed with *n*-hexane/EtOAc (3 : 2, v/v) to give **6** (8 mg, 88%).

mp 156–159 °C. $[\alpha]_D^{25} -334^\circ$ ($c=0.07$, CHCl₃). ¹H-NMR (CDCl₃) δ : 2.28 (3H, s), 2.60–2.77 (3H, m), 3.63 (3H, s), 4.67 (1H, d, $J=5.7$ Hz), 5.88 (1H, ddt, $J=2.4, 5.7, 9.9$ Hz), 6.06 (1H, ddd, $J=3.0, 4.2, 9.9$ Hz), 6.30 (1H, br s), 6.38 (1H, br s), 11.52 (1H, s). HR-FAB-MS m/z : 304.0955 (Calcd for C₁₆H₁₆O₆: 304.0947). FAB-MS m/z 304 (M⁺).

Preparation of Topo I and Topo II Human Topo I cDNA was inserted into the EcoRI site of the Baculovirus transfer vector pVL1392 (Pharmingen), and cotransfected with BaculoGold DNA (Pharmingen) in H5 cells. The recombinant human Topo I was purified from the nuclear extract of the transfected H5 cells by affinity chromatography using Heparin Sepharose CL-6B (Pharmacia Fine Chemicals). The recombinant human Topo II was prepared in a similar manner as described above. Details of preparation of these enzymes will be described elsewhere.

The Topo I Relaxation Assay Topo I activity was measured by detecting the conversion of supercoiled pT2GN DNA to its relaxed form. The reaction mixture containing 50 mM Tris-HCl (pH 8.0), 50 mM NaCl, 0.5 mM EDTA, 0.5 mM dithiothreitol (DTT), 10% glycerol, 30 μ g/ml bovine serum albumin (BSA), 1 μ l sample solution, 0.2 μ g pT2GN DNA and 1 unit Topo I, in a final volume of 20 μ l, was incubated at 37 °C for 15 min. The reaction was terminated by the addition of 4 μ l of a solution containing 4.5% SDS, 45% glycerol

and 0.1% bromophenol blue. The sample was electrophoresed in 0.8% agarose gel at 50 V for 1.5 h. The gel was stained with ethidium bromide and washed with water. The DNA band was visualized under UV light.

The Topo II Decatenation Assay Topo II activity was measured by detecting the conversion of catenated kinetoplast DNA (kDNA) to monomer minicircles. The reaction mixture containing 50 mM Tris-HCl (pH 8.0), 50 mM NaCl, 0.5 mM EDTA, 0.5 mM DTT, 10 mM MgCl₂, 1 mM ATP, 10% glycerol, 30 μ g/ml BSA, 1 μ l sample solution, 0.2 μ g kDNA and 1 unit Topo II, in a final volume of 20 μ l, was incubated at 37 °C for 15 min. After termination of the reaction, the sample was analyzed by agarose gel electrophoresis as described above.

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