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SYNTHESES OF NOVEL ANTITUMOR DIHYDROXANTHONE DERIVATIVES WITH INHIBITORY ACTIVITY AGAINST DNA TOPOISOMERASE II

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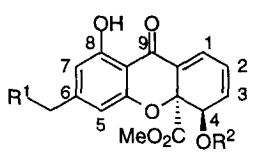
Abstract: A series of methoxycarbonyl group modified nidulalin A analogs were synthesized to improve stability against esterases. The amide derivatives showed cytotoxic activity along with inhibitory activity against DNA topoisomerase II. Among the analogs, amide **9a** exhibited antitumor activity in Colon 26 murine tumor model.

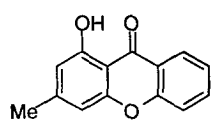
Introduction

DNA topoisomerases are the enzymes which regulate the state of DNA topology. The enzymes are, therefore, important for DNA metabolism, such as replication, recombination and transcription.[1] Nidulalin A(**1**)[2] and its derivatives, F390B(**2**) and C(**3**), were isolated from *Penicillium* sp. as potent cytotoxic agents (Figure 1).[3] Nidulalin A, a major metabolite of the producing strain, showed potent inhibitory activity against DNA topoisomerase II(Topo II) *in vitro*.[4] The antitumor activity of nidulalin A against Colon 26 murine adenocarcinoma was evaluated *in vivo*, however, no antitumor effect was observed. Further investigation revealed that rapid hydrolysis and decarboxylation of the methyl ester moiety of **1** by esterases in murine plasma gave an inactive xanthone derivative **4**, which was identical to the compound derived from **1** by alkaline hydrolysis. This result prompted us to synthesize stable analogs against hydrolysis. In this communication, we report synthesis and antitumor activity of derivatives of nidulalin A with amide moieties in place of the methoxycarbonyl group.

Figure 1



Nidulalin A(**1**) R^1=R^2=H
F390B(**2**) R^1=H, R^2=Ac
F390C(**3**) R^1=OH, R^2=H



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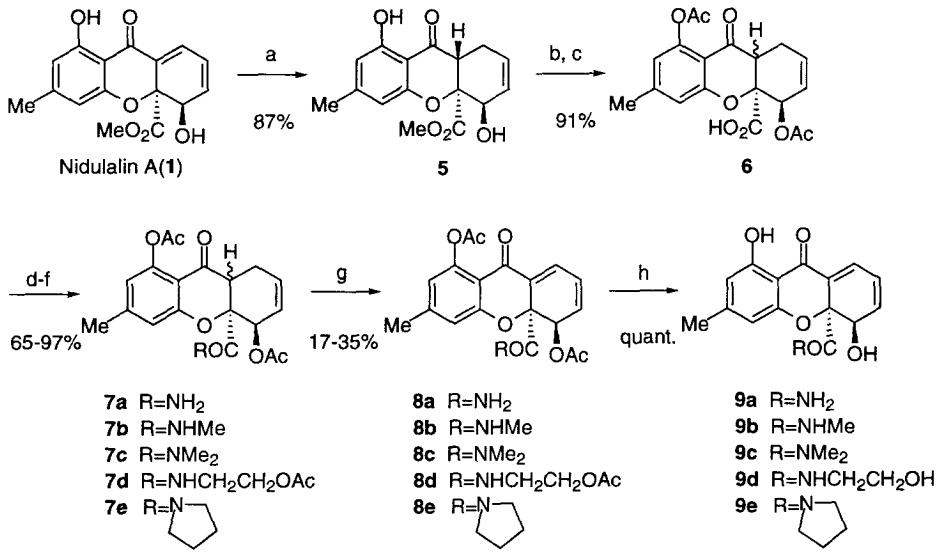
Synthesis

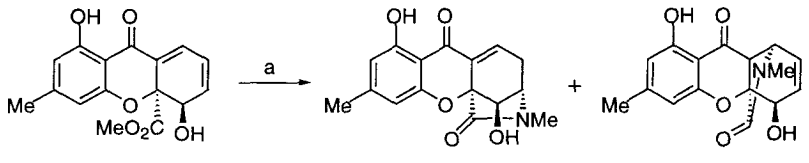
The syntheses of amide analogs of nidulalin A are outlined in Scheme 1. Direct amidation by methylamine in MeOH afforded approximately 4:3 mixture of lactams **10a** and **10b** *via* Michael addition followed by intramolecular aminolysis(Scheme 2). To avoid lactam formation, nidulalin A was reduced by $NaBH_4$ in MeOH to give a tetrahydroxanthone derivative **5** in 87% yield,[5] which was treated with 1N NaOH followed by acetylation with acetic anhydride in pyridine to give carboxylic acid **6**. Condensation of **6** with various amines, including ammonia, methylamine, dimethylamine, 2-aminoethanol, and pyrrolidine, *via* acid chloride followed by acetylation of a hydroxyl group, which was generated during amine treatment, gave corresponding amides **7a-e**, respectively. Oxidation of **7a** with SeO_2 in dioxane at 65°C gave a dihydroxanthone derivative **8a** in 18% yield. In the same manner, **7b-e** were converted to **8b-e**, respectively. The acetyl groups of **8a-e** were removed by alkaline hydrolysis to give amide analogs **9a-e**, respectively.[6]

Scheme 1



a) $NaBH_4$, MeOH, 5°C b) 1N NaOH, 5°C c) Ac_2O, pyridine, rt d) $SOCl_2$, cat. DMF, CH_2Cl_2, rt
e) aq. amines, 5°C f) Ac_2O, pyridine, rt g) SeO_2, dioxane, 65°C h) 1N NaOH, MeOH, 5°C

Scheme 2



a) $MeNH_2$, MeOH, rt

Result and Discussion

To test the stability, the amide derivatives were treated with murine plasma. The inactive compound **4** was not detected by HPLC analyses, and it was revealed that the amide analogs were stable against esterases.[7] The biological activities of the semisynthetic compounds of nidulalin A are shown in Table 1. The amide derivatives **9a-e** retained cytotoxic activity against both human and murine cancer cell lines *in vitro*. **9a** inhibited Topo II in dose dependent manner with IC_{50} value of 29μM. Furthermore, **9a** possessed weak inhibitory activity against topoisomerase I(Topo I) with IC_{50} value of 63μM. In spite of decreased activity against Topo II, cytotoxicity of **9a** was comparable to that of **1**. Lactam **10a** inhibited neither Topo I nor Topo II since a conjugated dienone moiety was required for inhibitory activity against Topo II.[4] The reason for the discrepancy between cytotoxicity and inhibitory activity against Topo I and Topo II of these compounds is not clear. There might be other mechanisms for cytotoxicity of these analogs or instability of **1** in cell culture might decrease its cytotoxicity.

Table 1. Biological Activities of Nidulalin A Derivatives *in vitro*

	Cytotoxicity[a] IC_{50}(μM)			Inhibitory Activity IC_{50}(μM)	
Compound	HCT-116	K562	P388	Topo I[b]	Topo II[c]
9a	0.33	0.59	0.052	63	29
9b	1.13	2.26	0.24	>300	132
9c	0.79	0.98	0.15	nd[d]	nd
9d	7.88	10.61	2.67	102	23
9e	1.64	1.52	0.56	nd	nd
10a	19.6	6.31	nd	>300	>300
1(Nidulalin A)	0.14	0.32	0.024	172	2.2

a) Cells were treated with each compound for 72 hours. Cell viability was evaluated by MTT assay. b) Relaxation assay of Topo I was performed as shown in ref. 8. c) Decatenation assay of Topo II was performed as shown in ref. 9. d) not determined

Table 2. Antitumor Activity of 9a *in vivo*

Compound	Route	IR(%)[a](dose)
9a	iv	42[b](10mg/Kg)
1	iv	24(20mg/Kg)

a) Tumor growth suppression was determined as shown in note 10. b) P<0.01 by T-test

Antitumor activity of **9a** was evaluated against Colon 26 murine tumor model *in vivo*(Table 2). **9a**, at a dose of 10mg/Kg, showed moderate antitumor activity in this model, while **1** showed only marginal effect even at a higher dose (20mg/Kg). From above results, it is demonstrated that the methoxycarbonyl group modified nidulalin A analogs are stable against esterases and effective in murine tumor model *in vivo*. Further evaluation of antitumor activity and synthesis of other stable analogs are underway.

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References and Notes

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2. Nidulalin A was originally isolated from *Emericella nidulans*. Kawahara, N.; Sekita, S.; Satake, M.; Udagawa, S.; Kawai, K. *Chem. Pharm. Bull.*, **1994**, *42*, 1720.
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5. Fujimoto, H.; Sato, Y.; Yamaguchi, K.;Yamazaki, M. *Chem. Pharm. Bull.* **1998**, *46*, 1506.
6. **9a**: $[\alpha]_D^{25}$-767°(*c* 0.06, MeOH); FAB MS *m/z* 288(M+H)$^+$; HR MS *m/z* 288.0866(M+H)$^+$ calcd. for $C_{15}H_{14}O_5N$(Δ-0.6mmu); ^{1}H NMR(300MHz, CD_3CN) δ 12.20(1H, brs), 7.43(1H, dd, *J*=1.5, 5.4Hz), 6.60(1H, brs), 6.47(1H, brs), 6.42(1H, brs), 6.34-6.40(2H, m), 6.00(1H, brs), 4.46(1H, d, *J*=4.5Hz), 2.33(3H, s).
   9b: $[\alpha]_D^{25}$-443°(*c* 0.18, $CHCl_3$); FAB MS *m/z* 302(M+H)$^+$; HR MS *m/z* 302.1019(M+H)$^+$ calcd. for $C_{16}H_{16}O_5N$(Δ-0.9mmu); ^{1}H NMR(300MHz, CD_3CN) δ 12.20(1H, s), 7.44(1H, dd, *J*=1.5, 5.1Hz), 6.80(1H, brs), 6.34-6.45(4H, m), 4.43(1H, d, *J*=4.8Hz), 2.59(3H, d, *J*=4.8Hz), 2.32(3H, s).
   9c: $[\alpha]_D^{25}$-278°(*c* 0.05, $CHCl_3$); FAB MS *m/z* 316(M+H)$^+$; HR MS *m/z* 316.1168(M+H)$^+$ calcd. for $C_{17}H_{18}O_5N$(Δ-1.7mmu); ^{1}H NMR(300MHz, $CDCl_3$) δ 12.15(1H, s), 7.48(1H, dd, *J*=1.2, 5.7Hz), 6.42-6.47(2H, m), 6.34(1H, brs), 6.32(1H, ddd, *J*=1.2, 5.4, 9.3Hz), 4.66(1H, dd, *J*=4.8, 5.4Hz), 3.40(3H, br), 2.92(3H, br), 2.57(1H, d, *J*=4.8Hz), 2.31(3H, s).
   9d: $[\alpha]_D^{27}$-287°(*c* 0.06, MeOH); ESI MS *m/z* 332(M+H)$^+$; HR MS *m/z* 332.1142(M+H)$^+$ calcd. for $C_{17}H_{18}O_6N$(Δ+0.8mmu); ^{1}H NMR(300MHz, pyridine-d_5) δ 8.80(1H, br), 7.69(1H, dd, *J*=1.2, 5.7Hz), 6.64(1H, ddd, *J*=1.2, 5.7, 9.6Hz), 6.44(1H, dd, *J*=5.7, 9.6Hz), 6.40(2H, s), 5.22(1H, d, *J*=5.7Hz), 3.85(2H, t, *J*=6.0Hz), 3.64(2H, q, *J*=6.0Hz), 2.01(3H, s).
   9e: $[\alpha]_D^{23}$-283°(*c* 0.04, $CHCl_3$); ESI MS *m/z* 342(M+H)$^+$; HR MS *m/z* 342.1327(M+H)$^+$ calcd. for $C_{19}H_{20}O_5N$(Δ-1.4mmu); ^{1}H NMR(400MHz, $CDCl_3$) δ 12.22(1H, s), 7.40(1H, d, *J*=5.6Hz), 6.43-6.46(2H, m), 6.29-6.32(2H, m), 4.57(1H, d, *J*=5.6Hz), 3.83(2H, m), 3.47(2H, m), 2.31(3H, s), 1.60-1.99(4H, m).
7. The test compounds, including **1**, **9a**, **9b**, and **9c**, were incubated in plasma/DMSO(9/1, v/v) at 37°C for 2 hours. Then resulting solutions were analyzed by HPLC. HPLC was carried out on a column (Inertsil ODS-3, 0.6 i.d. x 15cm) with a linear gradient from 30% to 80% aqueous MeCN at a flow rate of 1 ml/min. The compound **4** showed no cytotoxic activity against HCT-116 human cancer cell line *in vitro* at a dose of 40μM.
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10. Fragments of Colon 26 tumor(10mg) were inoculated subcutaneously(sc) into CDF1 mice. Test compounds were given iv on day 7, 11, and 15. On day 17, the tumor weights were measured. The inhibition ratio was evaluated as (1-T/C)x100(%)(T; the mean tumor weight of the treated group, C; the mean tumor weight of the control group). Each group consisted of 5 mice.