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Synthesis and Biological Activities of NB-506 Analogues: Effects of the Positions of two Hydroxyl Groups at the Indole Rings

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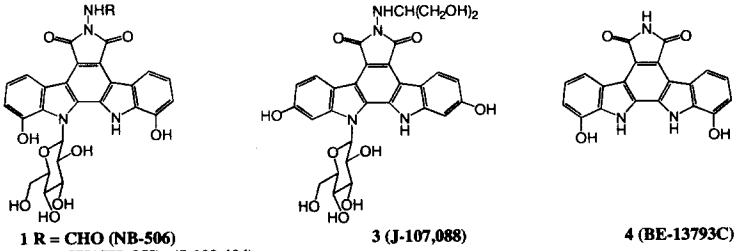
Abstract: In the course of a study of 6-*N*-amino-substituted analogues of NB-506 (**1**), a more potent anticancer drug, J-109,404 (**2**), in which the formyl group of NB-506 was replaced with a 1,3-dihydroxypropane group, was reported. A study of further modification in the positions of two hydroxyl groups at the indole rings of **2** resulted in the discovery of a 2,10-dihydroxy analogue, J-107,088 (**3**), which is a promising anticancer agent with a broader therapeutic window than J-109,404.

Keywords: Anticancer agents; Topoisomerase; Indolocarbazole; Substituent effects

DNA topoisomerase I has been reported to be an attractive target for the development of anticancer agents.[1] Recently, NB-506 (**1**)[2], a DNA topoisomerase I inhibitor derived from a natural compound, BE-13793C (**4**)[3], was reported to be a potent anticancer drug. Previous studies of the 6-*N*-amino analogues of NB-506 to improve the potency as well as aqueous solubility yielded a more potent anticancer drug, J-109,404 (**2**), which has a 1,3-dihydroxypropane group at the 6-*N*-amino position.[4] This paper reports on the synthesis and biological activities of a new series of analogues of J-109,404 focused on the hydroxyl groups at the indole rings on an indolocarbazole skeleton. The *in vivo* anticancer effects of several potent compounds in mice are also discussed.

Fig. 1



1 R = CHO (NB-506)
2 R = $CH(CH_2OH)_2$ (J-109,404)

3 (J-107,088)

4 (BE-13793C)

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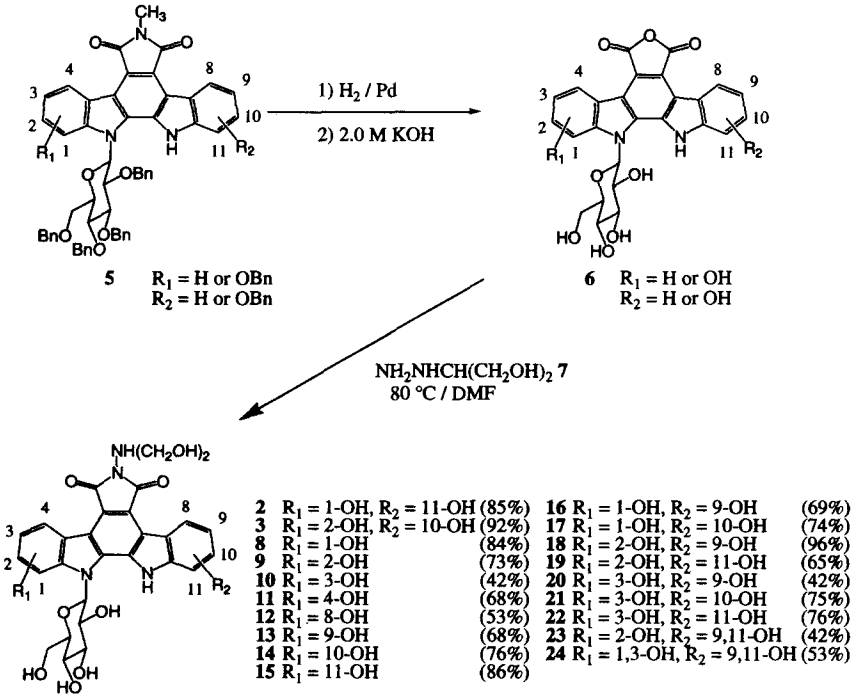
A new series of J-109,404 analogues focused on the position of the hydroxyl groups on the indole rings was synthesized from 6-*N*-methyl compounds **5** by the same method as for J-109,404, shown in **Scheme 1**, and the chemical yield was also summarized. The benzyl groups of the 6-*N*-methyl compounds **5** were removed by hydrogenolysis with palladium hydroxide followed by treatment with 2.0 M aqueous potassium hydroxide to yield anhydride compound **6** . Final compounds, **2**, **3**, **8-24** were obtained by the coupling reaction of **6** with hydrazine **7** in dimethylformamide (DMF) at 80 °C in 42-96% yields.

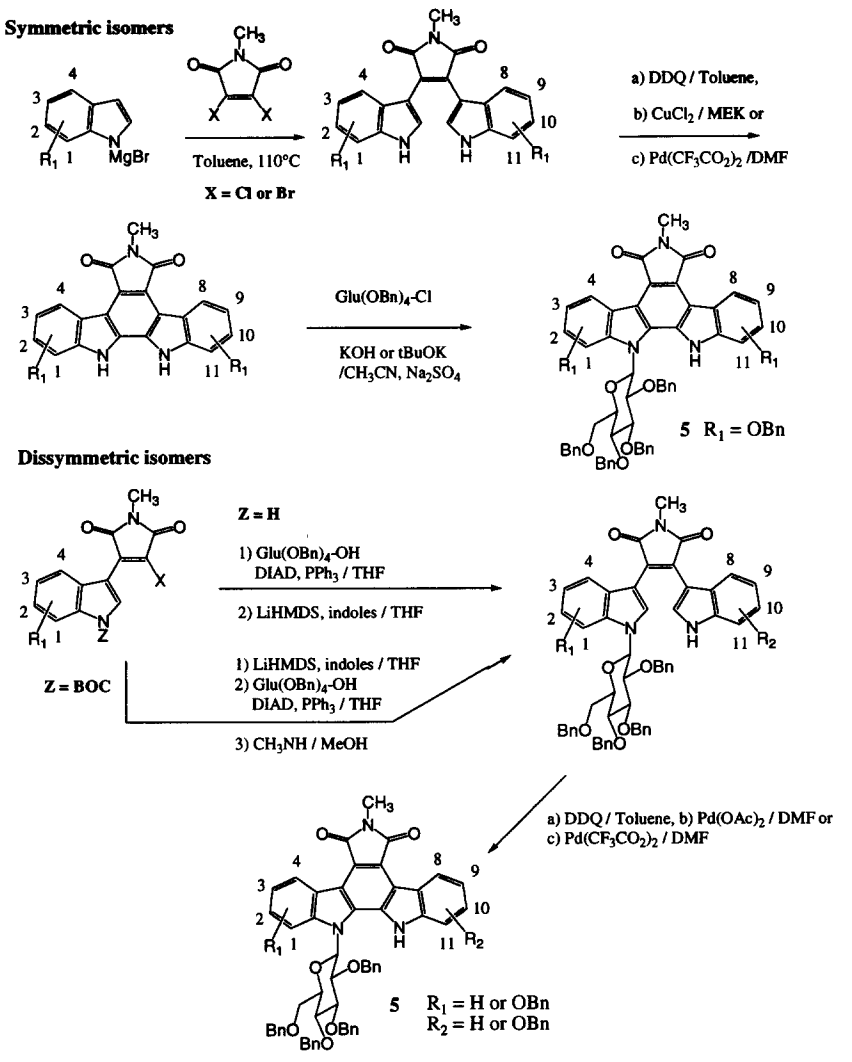
Scheme 1



2 R_1 = 1-OH, R_2 = 11-OH (85%) **16** R_1 = 1-OH, R_2 = 9-OH (69%)
3 R_1 = 2-OH, R_2 = 10-OH (92%) **17** R_1 = 1-OH, R_2 = 10-OH (74%)
8 R_1 = 1-OH (84%) **18** R_1 = 2-OH, R_2 = 9-OH (96%)
9 R_1 = 2-OH (73%) **19** R_1 = 2-OH, R_2 = 11-OH (65%)
10 R_1 = 3-OH (42%) **20** R_1 = 3-OH, R_2 = 9-OH (42%)
11 R_1 = 4-OH (68%) **21** R_1 = 3-OH, R_2 = 10-OH (75%)
12 R_1 = 8-OH (53%) **22** R_1 = 3-OH, R_2 = 11-OH (76%)
13 R_1 = 9-OH (68%) **23** R_1 = 2-OH, R_2 = 9,11-OH (42%)
14 R_1 = 10-OH (76%) **24** R_1 = 1,3-OH, R_2 = 9,11-OH (53%)
15 R_1 = 11-OH (86%)

The most important issues for the effective synthesis of β-glycosides, 6-*N*-methyl compounds **5**, were regioselectivity and stereoselectivity in a glycosylation step. The novel synthetic pathways of an important intermediate, 6-*N*-methyl compounds **5**, are summarized in **Scheme 2**. In the case of symmetric analogues, 6-*N*-methyl compounds **5** could be readily prepared using the same glycosylation reactions as previously reported in the synthesis of NB-506.[5] In fact, the glycosylation reactions of 2,10- and 3,9-dibenzyloxy-indolocarbazoles with 1-chloro-2,3,4,6-tetra-*O*-benzylglucose using potassium hydroxide or potassium *tert*-butoxide as a base were each carried out with more than 95% β-selectivity. On the other hand, non-symmetric β-glycosides were effectively obtained by the same method as previously reported,[6] in which the Mitsunobu reaction was used for the glycosylation reaction of mono-indole compounds or mono-*N*-tert-butoxycarbonyl (BOC) bisindole compounds with 2,3,4,6-tetra-*O*-benzylglucose. The stereoselectivity of the Mitsunobu reaction was greater than 90 %.

Scheme 2



Results and Discussion

Several biological activities of J-109,404 analogues are summarized in Table 1. As for topoisomerase-mediated DNA cleavage activity, the high selectivity of these analogues for topoisomerase II and protein kinase C (PKC) was completely maintained, and the hydroxyl group at the C-2 position obviously improved the topoisomerase I-mediated DNA cleavage activity, and in the case of di-hydroxyl analogues, the hydroxyl group at the C-10 position was also effective (**3**, **17** and **21**). The inhibitory activity against topoisomerase I tested by an enzyme assay (Topo-I cleavage, EC_{50}) did not always correlate to that determined by a cellular assay (K^+/SDS, EC_{200}), probably because of their differences in penetration into the cells. However, from the results of the K^+/SDS assay, two hydroxyl groups seemed to enhance penetration, because neither the mono-hydroxyl analogues nor the tri- or tetra-hydroxyl analogues showed potent EC_{200} values, except for 2-OH compound **9**. As for cytotoxicity (CTX) toward P388 (murine leukemia), MKN-45 (human stomach cancer) and DLD-1

(human colon cancer) cells, the structure-activity relationships (SAR) were nearly the same as that in the K^+/SDS assay. These results suggested that the number of hydroxyl groups influenced penetration into the cells while the positions of the hydroxyl groups affected the inhibitory activity against topoisomerase I; in particular, hydroxyl group at C-2 position seemed to be most important. Among the compounds, a 2,10-dihydroxy analogue, J-107,088 (**3**), showed not only the greatest activity in stabilizing a DNA-topoisomerase I cleavable complex (EC_{200} = 0.10 μM), but also more potent cytotoxicity against human cancer cells than J-109,404.

Table 1 *In vitro* activities of J-109,404 analogues

No.	R_1	R_2	Topo-I [a] Cleavage EC_{50} (μM)	Topo-II [a] Cleavage EC_{50} (μM)	K^+/SDS [b] (P388/S) EC_{200} (μM)	PKC [c] IC_{50}(μM)	CTX [d] P388/S IC_{50} (nM)	CTX [e] MKN-45 IC_{50} (nM)	CTX [e] DLD-1 IC_{50} (nM)
8	1-OH	H	0.65	>50	2.6	>200	22	640	2600
9	2-OH	H	0.23	>50	0.40	>200	3.1	25	1000
10	3-OH	H	1.9	>50	4.50	>200	47	4200	>30000
11	4-OH	H	0.49	NT [f]	>10	>200	910	7100	>30000
12	H	8-OH	0.57	>50	>10	>200	700	2100	>30000
13	H	9-OH	1.1	>50	2.0	>200	130	720	5500
14	H	10-OH	0.65	>50	1.2	>200	13	110	2000
15	H	11-OH	0.21	>50	>10	>200	140	1600	10000
16	1-OH	9-OH	0.51	>50	0.55	>200	32	250	220
17	1-OH	10-OH	0.16	>50	0.40	>200	5.4	87	89
2 (J-109,404)	1-OH	11-OH	0.58	>50	0.45	>200	17	130	520
18	2-OH	9-OH	0.037	>50	0.35	>200	5.2	56	79
3 (J-107,088)	2-OH	10-OH	0.051	>50	0.10	100	1.5	4.8	120
19	2-OH	11-OH	0.055	>50	0.80	>200	10	65	200
20	3-OH	9-OH	0.21	>50	0.60	>200	6.8	250	11000
21	3-OH	10-OH	0.055	>50	0.65	90	3.5	120	340
22	3-OH	11-OH	0.13	>50	3.00	>200	31	300	5100
23	2-OH	9,11-OH	0.090	>50	>10	40	15000	>30000	>30000
24	1,3-OH	9,11-OH	0.35	>50	5.5	>200	>30000	>30000	>30000

a) Topoisomerase-mediated DNA cleavage assay was carried out using supercoiled pBR322 plasmid DNA.[2b] *b*) Effects on the formation of protein-DNA complex in P388 cells were investigated by the K^+/SDS method.[2b] *c*) The histone II-As was used as a substrate for protein kinase C.[2b] *d*) Cytotoxicity (CTX) against murine leukemia cells (P388) was measured by the colorimeric tetrazolium-formazan method.[2b] *e*) Cytotoxicity (CTX) against human stomach cancer cells (MKN-45) and colon cancer cells (DLD-1) was measured by the colorimeric tetrazolium-formazan method and the sulforhodamine B dye-staining method.[2b] *f*) NT: not tested.

Several analogues were tested for anticancer effects in mice. As shown in Table 2, a good correlation between cytotoxicity and anticancer activity against human stomach cancer cells, MKN-45, was observed for the tested compounds, while toxicity (LD_{10}) did not correlate with cytotoxicity. In conclusion, a 2, 10-dihydroxy analogue, J-107,088 (**3**), was found to have potent anticancer activity and a very wide safety margin.
J-107,088 (**3**) is now being tested clinically.[7]

Table 2 Anticancer activity

	R_1	R_2	CTX MKN-45 IC_{50} (μM)	GID_{75} [c] MKN-45 (mg / m^2)	LD_{10} [d] (mg / m^2)	Safety Margin [e] LD_{10} / GID_{75}
9 [a]	2-OH	H	0.025	800	1000	1.3
14 [a]	H	10-OH	0.11	820	2700	3.3
16 [a]	1-OH	9-OH	0.25	2500	1900	0.8
17 [a]	1-OH	10-OH	0.087	220	370	1.7
2 (J-108,404) [a]	1-OH	11-OH	0.13	78	390	5.0
18 [b]	2-OH	9-OH	0.056	290	1000	3.4
3 (J-107,088) [b]	2-OH	10-OH	0.0048	45	>1600	>36
19 [a]	2-OH	11-OH	0.064	320	350	1.1
20 [a]	3-OH	9-OH	0.25	300	380	1.3
21 [b]	3-OH	10-OH	0.12	710	1000	1.4
22 [a]	3-OH	11-OH	0.3	2200	1800	0.8

a) Compounds were injected intravenously five times / week for 2 weeks, and treatment was initiated when tumors grew to 0.2 cm^3 or larger. *b)* Compounds were injected intravenously two times / week for 2 weeks, and treatment was initiated when tumors grew to 0.2 cm^3 or larger. *c*) Anticancer effect on MKN-45 human stomach cancer cells implanted subcutaneously. into flanks of nude mice. GID_{75}; approximate 75% Growth Inhibition Dose. *d*) LD_{10}; approximate 10% Lethal Dose at the treatment schedule. *e*) Safety margin: the ratio of LD_{10} / GID_{75}.

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8. Physical data for a representative compound, J-107,088 (**3**): mp >250 °C; $[\alpha]^{20}_{D}$ +163 °; ^{1}H-NMR (300 MHz, DMSO-d6), δ_{H} (ppm) : 3.2-3.3 (1H, m), 3.4-3.6 (6H, m), 3.78 (1H, m), 3.85-3.95 (2H, m), 4.02 (1H, m), 4.53 (2H, t, J = 5.4 Hz), 4.91 (1H, m), 5.11 (1H, d, J = 5.3 Hz), 5.32 (1H, d, J = 4.6 Hz), 5.55 (1H, d, J = 2.6 Hz), 5.86 (1H, t, J = 3.8 Hz), 5.97 (1H, d, J = 8.3 Hz), 6.80 (1H, dd, J = 2.0, 8.6 Hz), 6.82 (1H, dd, J = 2.0, 8.6 Hz), 6.98 (1H, d, J = 2.0 Hz), 7.18 (1H, d, J = 1.7 Hz), 8.79 (1H, d, J = 8.6 Hz), 8.87 (1H, d, J = 8.6 Hz), 9.75 (1H, s), 9.78 (1H, s), 11.20 (1H, s); HRMS (FAB) calcd for $C_{29}H_{29}N_4O_{11}$ 609.1833, found 609.1816.