SYNTHETIC DUAL INHIBITORS OF DNA TOPOISOMERASE I AND II

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A new type of synthetic dual inhibitor of DNA topoisomerase I and II was explored. This series of compounds shows high antineoplastic activities against cancer cells, but *in vivo* activity against P388 was not as high as *in vitro* activities.

KEY WORDS dual inhibitor; DNA topoisomerase I; DNA topoisomerase II; pyrazolo[1,5-*a*]indole derivatives; antineoplastic activity

Among large numbers of antitumor agents with various activities, agents targeting topoisomerase I and II have attracted particular interest[1] and some potential anticancer agents have been explored.[2] These agents are selective, but there have been few studies on dual inhibitors of these enzymes due to poor chemical sources, which are mostly restricted to natural products.[3] We have been researching the chemistry of pyrazolo[1,5-*a*]indoles with the hope of discovering biologically active compounds. In this context, we have prepared a series of compounds and found that some derivatives have fairly strong dual inhibitory activity against DNA topoisomerase I and II. Active compounds **4-9** are summarized in Chart 1. These compounds were prepared by coupling pyrazolium salts **1**[4] and **2** with benzladehyde derivatives **3** in acetic acid.[5] The structures were characterized by ^{1}H- and ^{13}C-NMR spectra. Characteristic signals of 3-H and 10-H were observed in **4-9** at δ 7.2-7.6 and 8.2-8.6 ppm as distinct singlet signals, respectively. Also the signals for 3-C were detected at δ 102-104 and those for 10-C at 135-140 ppm, both in isolated areas except **7** which shows a 3-C signal at δ 111.9 and 10-C at 140 ppm. The stereochemistry of the phenylmethylene substituent at C-4 was determined by positive NOE observations between 3-H and *o*-protons of the phenyl group. When the formation of **5** was incomplete, contamination of the geometrical isomer was detected in the ^{1}H-NMR spectrum by the signals of 3-H and 10-H at δ 7.49 and 8.28 ppm, respectively. A longer reaction period, however, allowed transformation of this isomer to **5** and **5** was obtained as the sole product.

Inhibition assay of **4-9** against human DNA topoisomerase I and II (TopoGen, U.S.A.) was carried out by measuring the relaxation of superhelical DNA.[6] The activity was evaluated as IC_{50} (μg/mL) and summarized in Table 1. Selective inhibitors SN-38 and VP-16 were employed as references. The listed compounds except for **10** showed quite strong activities. Among them, **5** was the strongest and was unique in its accessibility. Thus these compounds constitute novel synthetic dual inhibitors of topoisomerase I and II among the compounds with the ammonium nitrogen atom.[7] Information available from this table is as follows: a) the tricyclic system is important for high activity

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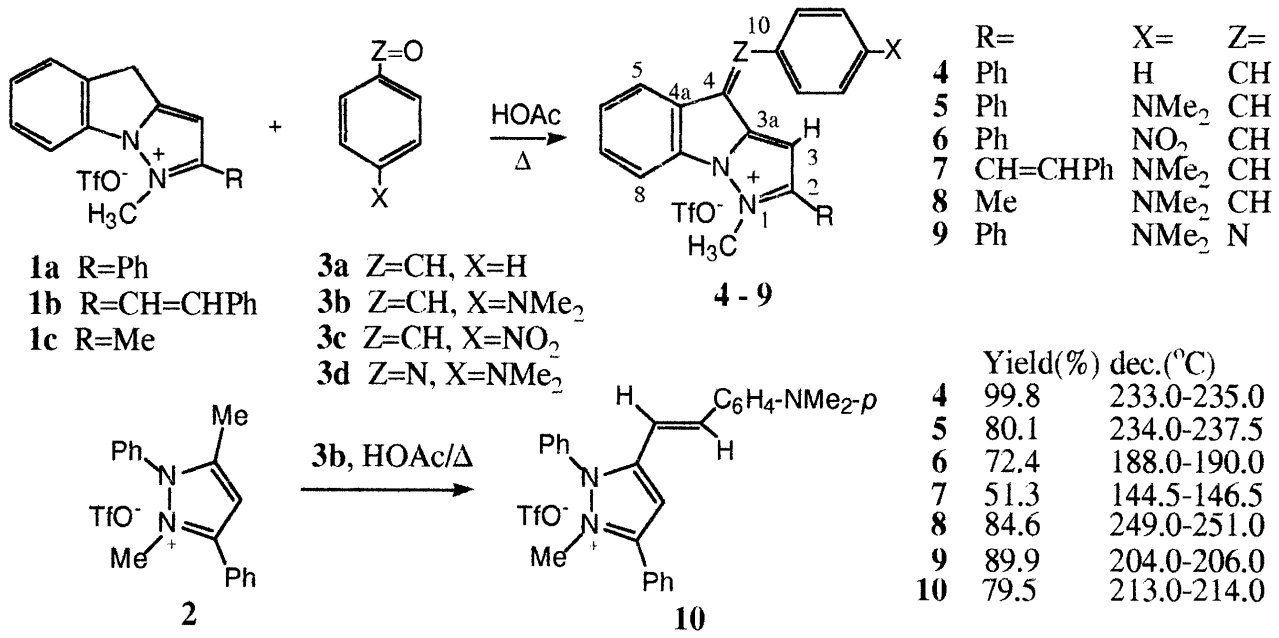

	R=	X=	Z=
4	Ph	H	CH
5	Ph	NMe_2	CH
6	Ph	NO_2	CH
7	CH=CHPh	NMe_2	CH
8	Me	NMe_2	CH
9	Ph	NMe_2	N

	Yield(%)	dec.(°C)
4	99.8	233.0-235.0
5	80.1	234.0-237.5
6	72.4	188.0-190.0
7	51.3	144.5-146.5
8	84.6	249.0-251.0
9	89.9	204.0-206.0
10	79.5	213.0-214.0

Chart 1

Table 1. Inhibition of Human DNA Topoisomerase I and II (IC_{50}; μg/mL)[a] and Cytotoxicity toward Cultured Cancer Cells (GI_{50} : ng/mL)[b] of **4-10**

Comp.	Top I	Top II	P388	PC-6	NUGC-3	SW-620	MCF-7
5-FU	-	-	48.8	240	1290	1250	732
CDDP	-	-	13	105	76.5	403	1510
SN-38	8.9	-	1.79	0.43	0.8	1.11	4.27
VP-16	-	6.99	8.12	99	343	292	414
4	>10	3.98	197	211	962	967	506
5	0.54	0.38	8.6	37.6	153	141	62.9
6	4.76	3.5	668	828	1224	2060	720
7	5.52	0.54	11.5	21	69.5	95.5	29.3
8	1.46	4.74	10	35.2	216	234	77.8
9	0.5	0.39	293	164	577	1280	329
10	>10	9.04	0.85	28.7	292	399	98.5

a) SN-38, 11-ethyl-9-hydroxycamptothecin; VP-16, etoposide.
b) Activities against five cell lines (P388, murine leukemia; PC-6, human lung; NUGC-3, gastric; SW-620, colon; MCF-7, breast) obtained[8] were measured by MTT assay after 3 days of incubation. Four human cell lines were maintained in RPMI 1640 (Nissui Pharm. Co., Ltd., Tokyo) supplemented with 10% fetal bovine serum and 2 mM L-glutamine. A mouse cell line, P388, was maintained in the RPMI 1640 medium supplemented with 20 μM 2-mercaptoethanol.

as exemplified in **10**; b) substitution at position C-10 with nitrogen (*cf.* **9**) does not affect the activity; c) selectivity ranges from 10 to 0.3 in the ratios of topoisomerase I over topoisomerase II.

Antineoplastic activities of these compounds were tested by measuring the proliferation of cancer cells by the microculture tetrazolium method[8]. The results are shown in Table 1. As references, 5-

FU (5-fluorouracil) and CDDP (cisplatin) were tested. Topoisomerase inhibition is principally reflected in antineoplastic activities. The potencies of **5** and **7** were noticeable compared with the reference compounds. The exception was **9**, in which imine hydrolysis in biological conditions may yield inactive products. The high potency of the open compound **10** in this test may suggest that a different reacting mechanism is operative in the antineoplastic action of **10**, which is also possible in **4**-**9**. However, the trend observed between the topoisomerase inhibition and antineoplastic activities of these compounds supports the hypothesis that topoisomerase inhibition may play a major role in these *in vitro* activities. When the antitumor activity of these compounds against P388 was investigated (*ip* inoculation into CDF1 mice on days 1 and 5), considerable ILS (increase of life span) values (26% *vs* 70% of 5-FU) were observed in **5** and **7** at the dose of 2 mg/kg x 2. However, a dose increase reduced the ILS value because of high toxicity. Although their *in vivo* activity is discouraging, the above compounds are unique as novel synthetic dual inhibitors of DNA topoisomerase I and II. Detailed analyses of the mechanism of action and SAR (structure-activity relationship) analyses may provide an opportunity to discover a new type of anticancer agent. We are now attempting to solve these problems.

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