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Antitumor agents. 256. Conjugation of paclitaxel with other antitumor agents: Evaluation of novel conjugates as cytotoxic agents

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Abstract—Sixteen different taxoid conjugates were prepared by linking various anticancer compounds, including camptothecin (CPT), epipodophyllotoxin (EP), colchicine (COL), and glycyrrhetinic acid (GA), at the 2'- or 7-position on paclitaxel (TXL, 1) through an ester, imine, amine, or amide bond. Newly synthesized conjugates were evaluated for cytotoxic activity against replication of several human tumor cell lines. Among them, TXL–CPT conjugates, **8–10**, were more potent than TXL itself against the human prostate carcinoma cell line PC-3 (ED₅₀ = 14.8, 3.1, 19.4 nM compared with 55.5 nM), and conjugate **10** was also 8-fold more active than TXL against the LN-CAP prostate cancer cell line. These compounds also possessed anti-angiogenesis ability as well as lower inhibitory effects against a normal cell line (MRC-5). Thus, conjugates **8–10** are possible antitumor drug candidates, particularly for prostate cancer.

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Paclitaxel (TXL, 1, Fig. 1), a plant derived antimitotic agent,^{1,2} is currently in clinical use against ovarian and breast cancer. It promotes the irreversible assembly of tubulin into microtubules by binding to and stabilizing microtubules. This mechanism of action is unique among the established antitumor drugs, including the vinca alkaloids, vincristine and vinblastine, which prevent microtubule assembly by microtubule binding.³ Other natural products, including camptothecin (CPT),⁴ which is approved for clinical use in the United State, epipodophyllotoxin (EP),⁵ and colchicine (COL),⁶ also possess potent antitumor activities with different mechanisms of action³ from TXL. Some EP derivatives, such as etoposide (2d), topotecan, and irinotecan, are approved by the FDA for cancer treatment, although their therapeutic use is often limited by undesired side effects, such as myelosuppression, multi-drug resistance, and poor water solubility. In addition, glycyrrhetinic

acid (GA),⁷ which is a well-known natural compound isolated from plants, shows antitumor-promoter activity, as well as anti-allergic, anti-inflammatory, and anti-ulcer effects.

Conjugation of two antitumor agents with different mechanisms of action can possibly augment the potency of both compounds or reduce the side effects and drug-resistance development. We have already reported the unique antitumor activities of such conjugates, including TXL–CPT,⁸ TXL–EP,⁹ and CPT–EP.¹⁰ In our continuous effort to develop new classes of antitumor agents based on TXL, we investigated the syntheses and evaluation of TXL conjugates with the above-mentioned antitumor or antitumor-promoter compounds through various linkages at the TXL C-2' and C-7 positions as reported herein.

Newly synthesized conjugates are shown in Figure 2. In conjugates 6–15, EP, CPT, COL, and GA are connected to the TXL C-2' position through various linkages, while conjugates 16–21 contain EP, COL, and GA connected to the C-7 position of TXL. As shown in

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Figure 1. Structures of paclitaxel (1) and other antitumor agents used for conjugation.

Scheme 1, a linking group was introduced directly at the TXL C-2' position using the appropriate anhydride or carboxylic acid to provide 22-25.11 For the C-7 linked conjugates, the TXL C-2' hydroxyl group was first protected with a Cbz or TBS group, the linking carboxylic acid was added at C-7, and the protecting group was then removed to afford 26-28. Compounds 22-24, 26, and 27 were conjugated with EP derivative 2c,¹² COL derivatives 4b-d, ¹³ and GA (5), respectively, by using EDCI in the presence of DMAP to provide 6, 7, 11-13, 15-17, and 21. Heating intermediates 22 and 23 with 7-formyl-CPT $(3b)^{14}$ in benzene produced the corresponding imines 8 and 10, respectively. In the same way, imines 14, 18, and 20 were prepared from 25 with 4d and 28 with 2b or 4d, respectively. Hydrogenation of 8 and 18 produced the corresponding amines 9 and 19.

All synthesized compounds were evaluated for cytotoxic activity against replication of several human tumor cell lines, human ovarian carcinoma (1A9), human lung carcinoma (A549), breast cancer (MCF-7), human prostate carcinoma (LN-CAP, PC-3, DU-145), human epidermoid of the nasopharynx (KB), and multi-drug resistant

expressing P-glycoprotein (KB-VIN) and against the normal cell line, human embryonic fibroblast (MRC-5). The results are shown in Table 1 with the values for TXL (1), EP derivatives 2b-c, etoposide (2d), CPT (3a), 7-formyl-CPT (3b), 2-demethyl-COL (4b), 2-demethylthio-COL (4c), and GA (5) for comparison. All conjugated compounds showed better activity than the partner compounds, although most of them were less potent than TXL itself. However, TXL-CPT conjugates 8–10 displayed different patterns of inhibition against the LN-CAP and PC-3 prostate cancer cell lines, with less effect on the normal cell line, MRC-5. Imine 10 had an ED_{50} value of 0.34 nM and was 7.7-fold more potent than TXL against LN-CAP cells, while compound 9 had an ED₅₀ value of 3.1 nM and was 18-fold more potent than TXL against PC-3 cells.

Cytotoxic activity was somewhat dependent on the conjugated position on TXL as well as the type of linkage. From comparison of the TXL-EP conjugates 6, 7, 16, and 17, the conjugates linked at the C-2' position (6, 7) showed better activity than conjugates linked at the C-7 position (16, 17) against all cell lines. Moreover,

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Figure 2. Structures of conjugates.

the linkage with two methylenes (6, 16) gave better results than the one with three methylenes (7, 17). However, TXL-COL conjugates 14 (2'-linkage) and 20(7linkage) showed similar potency against most cell lines. A phenyl imino linkage was better than the linear amido linkage, as TXL-COL conjugate 14 was 4- to 9-fold more potent than 13 against 1A9, A549, and KB cells.

The results of selected compounds in an anti-angiogenesis assay are shown in Table 2. Compared with the other conjugates, imines 8 and 10 possessed significant activity with ED_{50} values of 0.73 and 0.98 µg/mL, respectively.

In conclusion, we have synthesized 16 different conjugates between paclitaxel (TXL) and other

1 ────► BzHN´	Ph 2 2 R	Aco O HO BZÔACÔ		a) EDC b) PhH c) H ₂ , I	I, DMAP	Conjugates
Condition		R	R'	Cor	ndition	
1) EDCI, DMAP, HO ₂ C(CH ₂) _n NHCbz 2) H ₂ , Pd/C	22	-C(O)-(CH ₂) ₂ NH ₂	Н	2c 3b	(a) (b)	6 89
	23	-C(O)-(CH ₂) ₃ NH ₂	Н	2c 3b	(a) (b)	7 10
Succinic anhydride, py	24	-C(O)CH ₂ CH ₂ CO ₂ H	н	5 4b 4c 4d	(a) (a) (a) (a)	15 11 12 13
4-Carboxybenzaldehyde, EDCI, DMAP	25	-С(О)-СНО	н	4d	(b)	14
1) Benzylchloroformate, py 2) EDCI, DMAP, HO ₂ C(CH ₂) _n NHCbz 3) H ₂ , Pd/C	26	H -C(C))-(CH ₂) ₂ NH ₂	2c 5	(a) (a)	16 21
	27	H -C(C))-(CH ₂) ₃ NH ₂	2c	(a)	17
1) TBSCI, imid. 2) 4-Carboxybenzaldehyde, EDCI, DMAP 3) HF-Py, Py	28	H -C(O)	- Сно) 2b 4d	(b) (b)	18 <mark>──→</mark> 19 20

Scheme 1. Synthesis of conjugates.

Table 1. Cytotoxic activity data of taxol conjugates

Compound	ED_{50}^{a} (nM)							ED ₅₀ (µM)	
	1A9	A549	MCF-7	LN-CAP	PC-3	DU-145	KB	KB-VIN	MRC-5
1	1.0	2.3	1.1	2.6	55.5	1.3	1.8	311	NT ^a
2b	739	1074	686	680	1234	3107	111.2	2050	NT
2d	284.9	1609	11659	530.1	9452	1600	1365	14447	>30
3a	3.2	6.9	3.2	2.0	16.7	10.9	20.9	15	NT
3b	4.3	12.8	4.3	4.3	18.4	7.7	80.6	25	NT
4b	183.7	950.6	361.0	324.7	475.3	611.9	146.1	28977	NT
4c	43.6	99.8	59.9	59.9	112.2	113.5	47.9	7426	NT
5	>20 ^b	>20 ^b	>20 ^b	>20 ^b	>20 ^b	>20 ^b	>20 ^b	>20 ^b	NT
6	2.9	10.1	6.4	4.2	12.1	8.6	8.4	9964	>10 ^b
7	8.1	23.7	22.1	10.2	42.8	32.5	13.9	6231	6174
8	1.6	2.6	26.7	3.0	14.8	3.7	1.3	62.4	177.7
9	1.5	2.6	2.7	1.2	3.1	2.3	1.9	2050	6133
10	1.0	1.9	34.1	0.34	19.4	1.9	1.5	56.3	243.6
11	10.6	27.3	26.5	12.9	50.7	31.9	19.0	>20 ^b	NT
12	10.3	23.2	24.7	19.5	71.8	29.8	17.0	>20 ^b	NT
13	14.0	28.5	29.2	23.7	70.4	34.4	21.57	>20 ^b	NT
14	2.3	3.8	NT	NT	NT	NT	5.4	308	NT
15	45.3	77.4	38.7	46.2	103.6	68.3	53.6	>20 ^b	NT
16	20.7	45.6	43.9	22.1	86.3	83.4	31.6	>20 ^b	3279
17	104	1319	125.0	NT	NT	NT	347.2	NA	NT
18	60.1	111.0	48.6	90.1	145.3	139.1	53.8	6220	NT
19	75.7	123.5	68.2	128.5	191.5	167.5	87.4	5200	NT
20	2.3	3.8	NT	NT	NT	NT	2.3	308	NT
21	154.3	224.8	129.8	192.7	652.1	311.0	147.3	>20 ^b	NT

Human ovarian carcinoma (1A9), human lung carcinoma (A549), breast cancer (MCF-7), human prostate carcinoma (LN-CAP, PC-3, DU-145), human umbilical vein endothelial cell (HUVEC), human epidermoid carcinoma of the nasopharynx (KB), multi-drug resistant expressing P-glycoprotein (KB-VIN), and human embryonic fibroblast (MRC-5).

^a Cytotoxicity as ED₅₀ values for each cell line, the concentration of compound that caused 50% reduction in absorbance at 562 nm relative to untreated cells using the sulforhodamine B assay.

 $^{\rm b}$ Test compound (20 µg/mL or 10 µg/mL) did not reach 50% inhibition.

Table 2. Anti-angiogenesis assay data of selected compounds inHUVECs

Compound	ED ₅₀ (nM)
6	4.93
8	0.73
9	2.09
10	0.98
11	13.84
12	9.33
13	15.8
14	43.0
18	230
20	293
21	150

antitumor agents, including camptothecin (CPT), epipodophyllotoxin (EP), colchicine (COL), and glycyrrhetinic acid (GA). The two components were joined by an ester, imine, amine or amide linkage at the 2'- or 7-position of TXL. Among them, TXL-CPT conjugates, 8–10, showed different in vitro cytotoxic activity profiles against human prostate carcinoma, LN-CAP and PC-3, with less effect against normal cells. These compounds also possessed anti-angiogenesis ability; therefore, conjugates 8–10 are possible antitumor drug candidates, particularly for prostate cancer.

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