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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

Fifteen 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 (ED_{50} = 14.8, 3.1, 19.4 nM compared with 55.5 nM), and conjugate **10** was also eightfold 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.

Keywords

Paclitaxel; Conjugation; Cytotoxity; Prostate cancer

Paclitaxel (TXL, **1**, Figure 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 action[3] 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, multidrug resistance and poor water solubility. In addition, glycyrrhetinic

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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 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-demthylthio-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 ED_{50} value of 3.1 nM and was 18-fold more potent than TXL against PC-3 cells.

Cytotoxic activity was somewhat dependant 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, 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** (7-linkage) showed similar potency against most cell lines. A phenyl imino linkage was better than the linear amido linkage, as TXL-COL conjugate **14** was four- to nine-fold more potent than **13 a**gainst 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 fifteen different conjugates between paclitaxel (TXL) and other 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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References and notes

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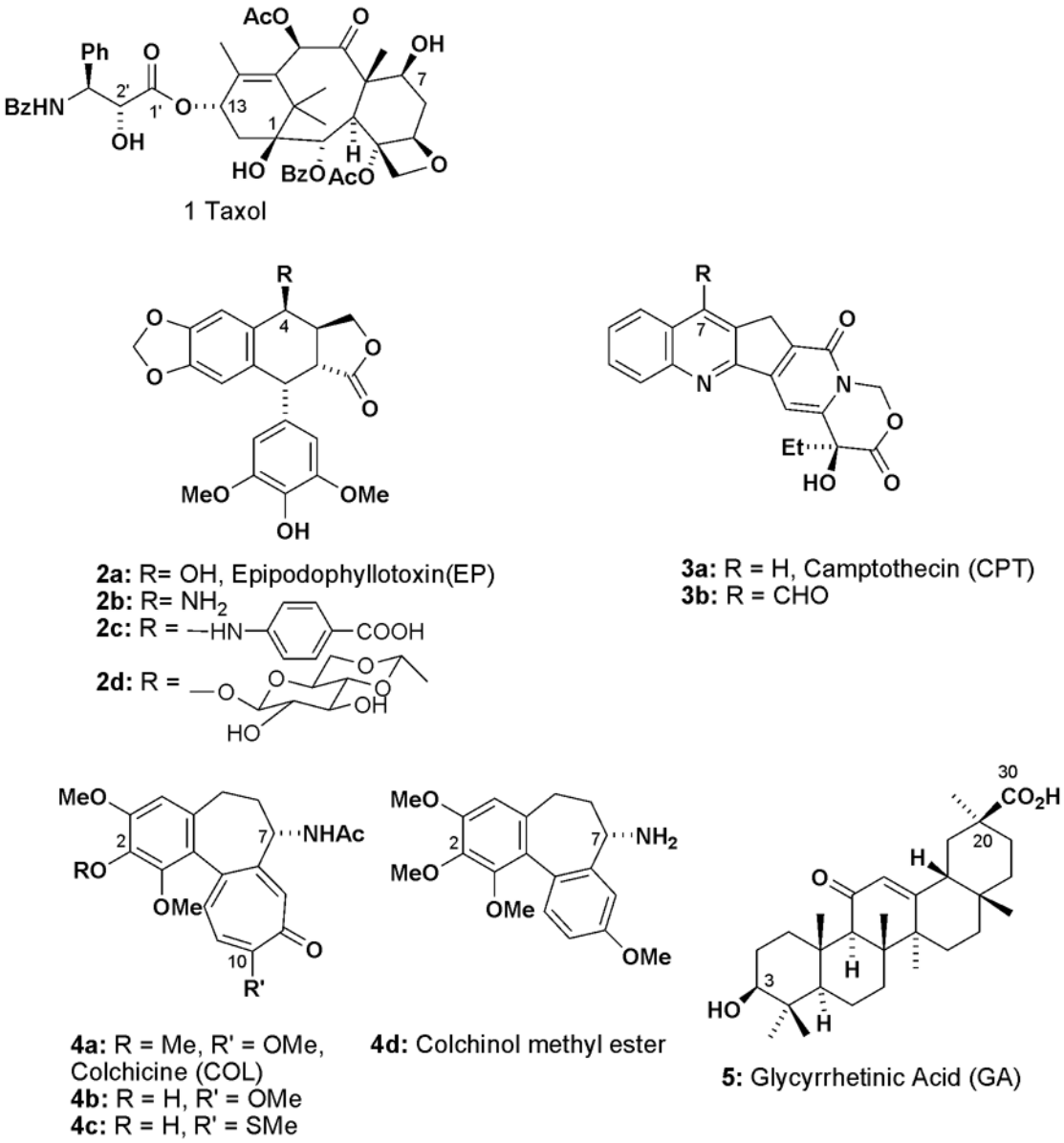


Figure 1.
Structures of paclitaxel (**1**) and other antitumor agents used for conjugation.

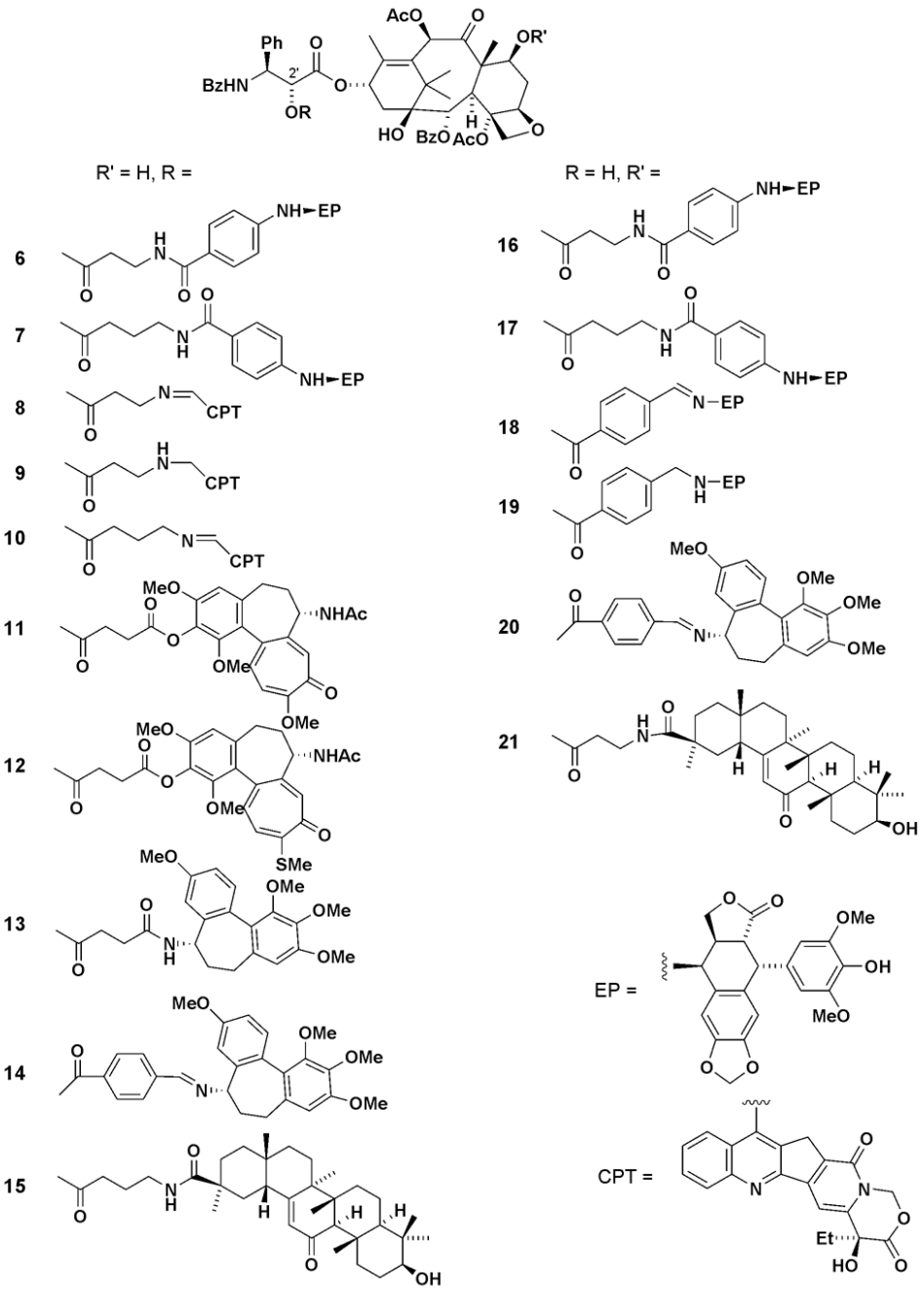
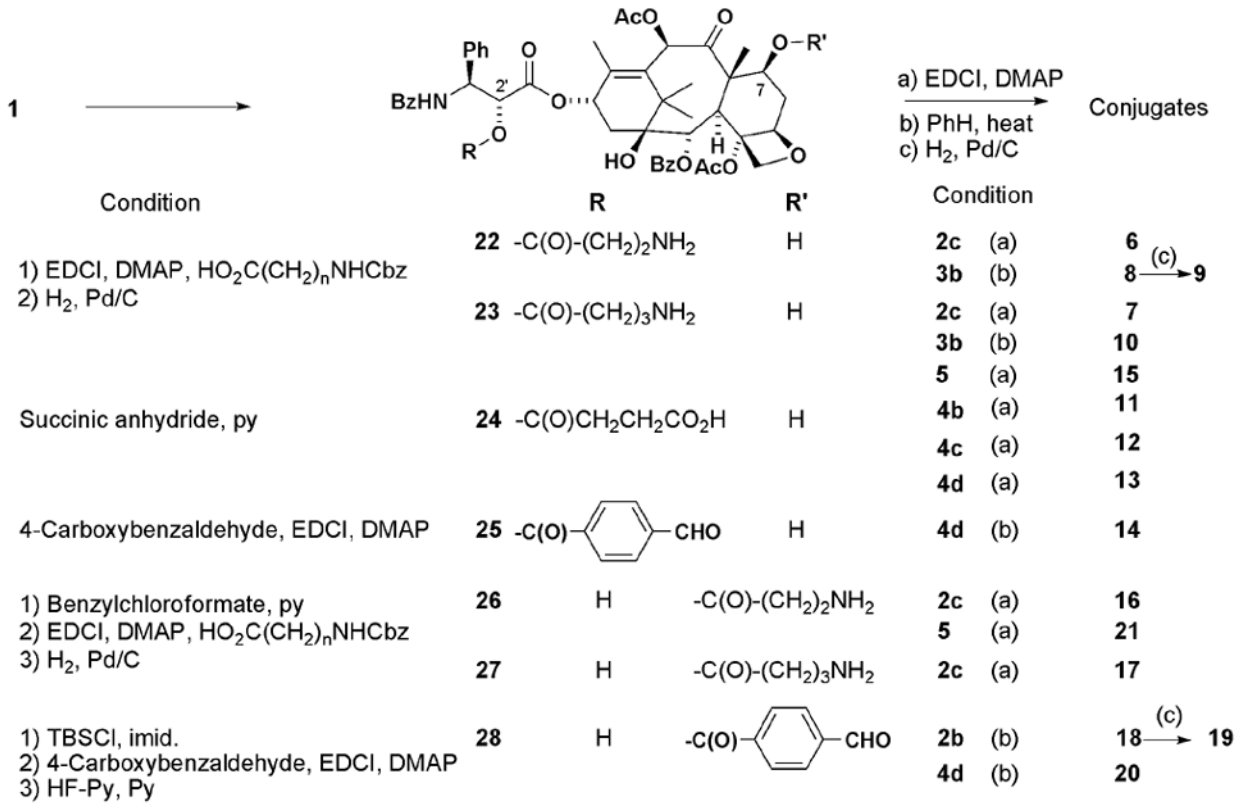


Figure 2.
Structures of conjugates.



Scheme 1.
Synthesis of conjugates.

Table 1

Cytotoxic activity data of taxol conjugates

Cmpd	ED_{50} (nM)[a]							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 [c]
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 ED_{50} 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.

[b] Test compound (20 μg/mL) did not reach 50% inhibition.

Table 2

Anti-angiogenesis assay data of selected compounds in HUVEC cells

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