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Reductively Activated Disulfide Prodrugs of Paclitaxel

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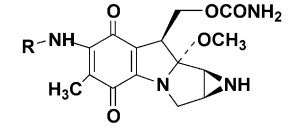
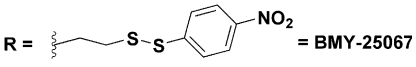
Abstract—A series of unsymmetrical polar disulfide prodrugs **2**–**5** of paclitaxel were designed and synthesized as reductively activated prodrugs. These compounds behaved as prodrugs in vitro on L2987 lung carcinoma cells. In vivo evaluation in mice demonstrated that the mutual prodrug **5** with captopril exhibited significant regressions and cures.

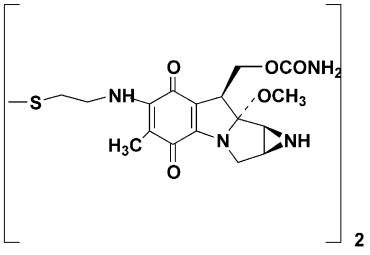
The microenvironment in which solid tumors thrive is characterized by hypoxia due to abnormal vasculature. Tumor hypoxia leads to diminished supplies of oxygen and nutrients. The hypoxic environment of tumors has been delineated in cervical cancer, squamous cell carcinoma of the head and neck, melanoma, and mammary tumors. Hypoxia has also been implicated in resistance to chemotherapy and radiation and is an excellent target for development of cancer therapeutics.[1] The hypoxic environment is also congenial for reductive transformations. It is known that the anticancer drug mitomycin C[2a,2b] and agents such as tirapazamine elicit their cytotoxicity through reductive activation.[3] Nitroreductase mediated reductive activation of a prodrug of the minor groove alkylating agent amino-*seco*-CBI-TMI,[4a] and a number of other cytotoxic agents has recently been reported.[4b] The use of aromatic nitro and azido groups as bioreductive triggers to release paclitaxel (**1**) from its 2′-ester prodrugs via 1,6- or 1,8-elimination reactions has also been described.[5] Cleavage of a disulfide bond is a facile biochemical transformation in a reductive environment. In an attempt to make more potent mitosane derivatives, several disulfide containing mitosanes were prepared and tested. Compounds such as BMY-25067 and RR-150 are examples of such agents.[6a,6b]



R = H = Mitomycin C



= BMY-25067



RR-150

Paclitaxel (**1**) is one of the most widely used chemotherapeutics and has activities in many types of cancer.[7] Due to its aqueous insolubility, paclitaxel is administered in a vehicle containing cremophor EL®, which can produce hypersensitivity reactions in patients.[7] Water soluble prodrugs of paclitaxel, which would be activated in the reductive hypoxic environment of tumors may comprise useful new drugs in cancer chemotherapy.

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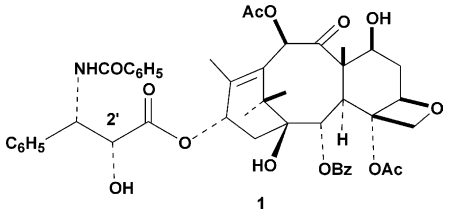

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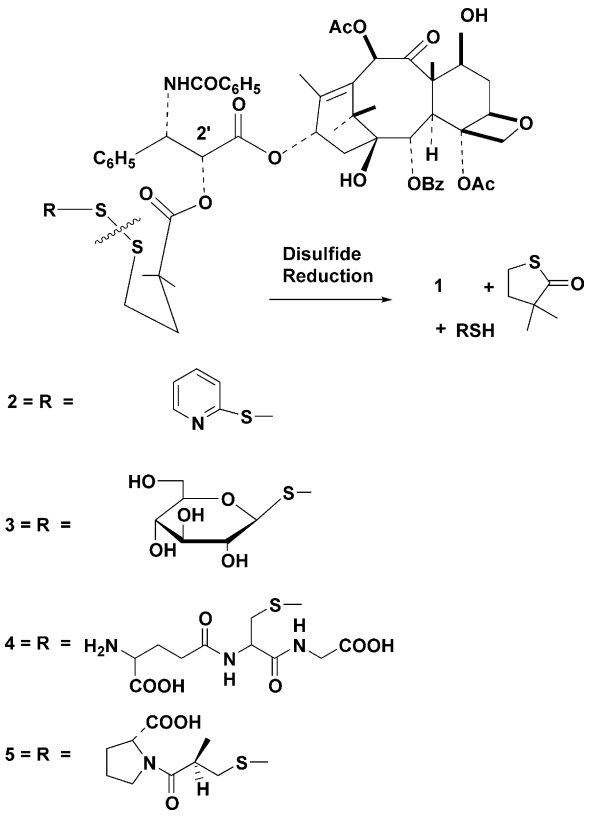
Esterification of the 2′-OH group of paclitaxel generally results in paclitaxel esters of diminished cytotoxicity. Paclitaxel esters derived from 2′-OH that release paclitaxel either by endogenous esterase activity[8] or by a cyclization reaction resulting in expulsion of paclitaxel have been prepared previously.[9] In this report we describe the synthesis of unsymmetrical disulfide prodrugs of **1** derived from 2,2-dimethyl-4-mercaptobutyric acid, which contain polar moieties as part of the disulfide residue for increased hydrophilicity. Geminal dimethylation of carbon α to the carbonyl group in the linker may offer stability toward serum esterases in addition to facilitating cyclization of the intermediate thiol due to the Thorpe–Ingold effect.[10]



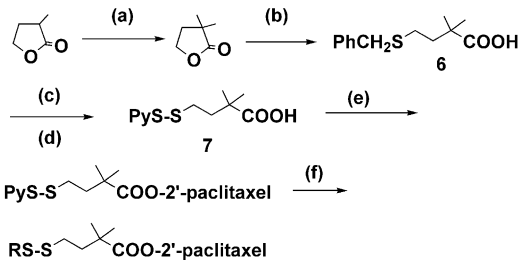
It was expected that the enhanced hydrophilicity of compounds **2–5** would impair their abilities to traverse cell membranes and would therefore diminish their cytotoxic activities. The key intermediate for the syntheses of these disulfides is the activated 2-thiopyridyldisulfide (**2**). To enhance hydrophilicity, compounds in which the variable component of the unsymmetrical disulfide contained highly polar moieties such as thioglucose (**3**) and glutathione (**4**) were prepared. The ACE inhibitor captopril was recently reported to have antiangiogenic effects.[11] Compound **5** utilizing captopril as one of the disulfide components is thus a mutual prodrug.[12]

The synthesis of pyridyldisulfide carboxylic acid (**7**) necessary for coupling to paclitaxel was carried out as shown in Scheme 1. α,α-Dimethylbutyrolactone[13] was subjected to nucleophilic ring opening with benzylmercaptan. The resulting *S*-benzyl derivative (**6**) was debenzylated with sodium in liquid ammonia to give the disodium salt which upon treatment in situ with 2,2′-dipyridyl disulfide in methanol gave (**7**) in 62% yield. DCC mediated esterification of paclitaxel with the hindered carboxylic acid was slow and resulted in the formation of **2** in a 43% isolated yield. In order to prepare disulfides **3–5**, the activated disulfide **2** was treated with excess thiol under basic conditons and the excess thiolate was quenched with *N*-ethylmaleimide. In this manner disulfides **3–5** were prepared in 45–53% yields.[14]

Reversed-phase analytical HPLC studies were carried out to investigate the release of **1** from **2–5** under reducing conditions. When treated with dithiothreitol (DTT), disulfides **2–5** underwent reduction and released paclitaxel. Since the steric and electronic environment around the disulfide bond is different in each of these disulfides, they were expected to undergo reductive cleavage at differing rates. The half-lives for the release of paclitaxel after cyclization (Table 1) ranged from 4 min for the activated pyridyl disulfide (**2**) to >60 min for the glutathione derived disulfide (**4**).

Human serum stability studies were done at 37 °C with compounds **2** and **3** using reversed-phase HPLC methods. Both these compounds released <10% paclitaxel after 125 min.

Cytotoxicity assays using L2987 lung carcinoma cells were carried out using incorporation of [^{3}H]-thymidine as a measure of cellular replication. Cells were treated with various concentrations of disulfides or a combination of disulfides and DTT for reductive activation. The percentage of control of [^{3}H]-thymidine incorporation against concentration was plotted. Prior treatment of disulfide derivatives with DTT (10 mM) for an hour was used to measure reductive activation in this assay. IC_{50} values shown in Table 2 indicated that compounds **2–5** were all prodrugs. The diminishment in cytotoxicity in comparison with **1** ranged from 30-fold for the activated pyridyl disulfide **2** to 650-fold with captopril prodrug **5**. Compounds **2–5** underwent reductive activation in presence of DTT. The cytotoxicity of **2** and **3** in reductive



Scheme 1. (a) MeI, NaH, dioxane; (b) NaH, $PhCH_2SH$, toluene, reflux; (c) Na, $liqNH_3$; (d) PyS-SPy, MeOH; (e) DCC, **1**, CH_2Cl_2; (f) RSH, MeOH, Et_3N.

Table 1. Half lives values for release of **1** from **2–5**

Compd	Half-life for release of paclitaxel (min)
2	4
3	12
4	>60
5	25

Table 2. IC_{50} values[a] from cytotoxicity assay on L2987 lung carcinoma cells

Compd	IC_{50}, μM of compd	IC_{50}, μM of compd + DTT
1	0.2	
2	6.2	0.2
3	5.7	0.2
4	10.1	0.4
5	130.0	2.3

[a]Values are averages from experiments run in triplicate.

environment matched that of **1** while **4** and **5** in the presence of DTT were slightly less toxic.

Prodrugs **2–5** were evaluated for antitumor activity in vivo in athymic nude mice that had subcutaneous L2987 human lung adenocarcinoma xenografts. Treatment was initiated on day 20-post implant and was repeated every two days for a total of three treatments. Paclitaxel (**1**) and the prodrugs were administered in a 1:1 solution of cremaphor ethanol intravenously. Control animals received only the vehicle diluted in saline. The data were plotted until any of the animals within the group had a tumor that reached 1000 mm^3.

While **2–4** at doses of 125 mg/kg for **2**, 85 mg/kg for **3** and 150 mg/kg for **4**, proved to be no more effective than those with paclitaxel (data not shown), the results obtained with the mutual (i.e., captopril containing) prodrug **5** were very pronounced (Fig. 1). Prodrug **5** was well tolerated with a 10–20% loss of body weight even at a dose of 125 mg/kg. In both groups no toxicities were observed. In animals treated with **5** at 125 mg/kg dose, there was a 60% tumor cure rate and the remaining animals experienced marked regressions. Prodrug **5** was clearly superior to paclitaxel in this model. Paclitaxel did not regress tumors at the maximum tolerated dose of 30 mg/kg (Fig. 1). It is possible that some of the superiority of **5** can be attributed to the possible anti-angiogenic effects of captopril combined with the cytotoxic effects of paclitaxel. Further studies are required to establish if this is the case.

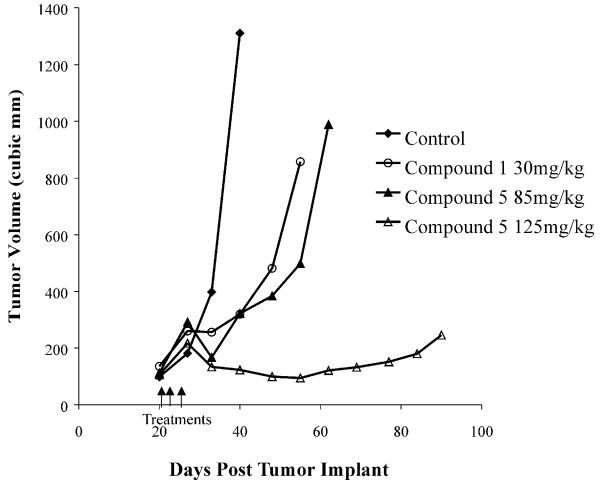


Figure 1. In vivo evaluation of **5** in athymic nude mice implanted with L2987 lung carcinoma.

In conclusion, we have demonstrated that reductively activated polar disulfide derivatives of **1**, incorporating a range of thiol-containing agents, can be prepared. The disulfide derivatives have reduced cytotoxic activities, and are reductively activated leading to the formation of paclitaxel. The mutual prodrug **5** served as a prodrug of both paclitaxel and captopril, and led to regressions and cures of established tumor xenografts. The activities were superior to the clinically approved anticancer drug paclitaxel.

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14. *Spectral characteristics of 2*: Letter T is used to denote paclitaxel. MS: $[M+H]=1093$, $[M+NH_4+MeCN]=1151$. IR (KBr) 532.8 cm^{-1} (–S–S– stretching). ^1H NMR ($CDCl_3$) δ 8.37 (m, Py-H), 8.16–7.31 (5×m, 16H, ArH, PyH,), 7.06–7.03 (m, 2H, NHCO, PyH), 6.28 (s, 1H, T-10), 6.23 (t, 1H, T-13, $J_{12,13}=9.0$ Hz), 5.98 (dd, 1H, T-3′, $J_{NH,3}=9.2$ Hz, $J_{2,3}=3.2$ Hz), 5.67 (d, 1H, T-2, $J_{2,3}=7.1$ Hz), 5.40 (d, 1H, T-2′), 4.97 (m, 1H, T-5) 4.97 (m, 1H, T-5), 4.44 (dd, H-7, T-7, $J=6.7$ Hz,

and 10.9 Hz), 4.32 (d, 1H, T-20A, $J_{AB}=8.4$ Hz), 4.20 (d, 1H, T-20B), 3.81 (d, 1H, T-3), 2.79 (dt, 1H, S–$\underline{CH_{2A}}$-, $J_{AB}=4.1$, $J_{vic}=12.3$ Hz), 2.58–2.34 (m and s, 7H, T-6A,–S–$\underline{CH_{2B}}$–, T-14, CO$\underline{CH_3}$), 2.22–1.60 (m and 3×s, 13H,–S–C–$\underline{CH_2}$–, T-6B, CO$\underline{CH_3}$, OH, T-18 and angular $\underline{CH_3}$), 1.20 and 1.04 (2×s, 6H, T-*gem* di Me), 1.22 (s, 6H, linker *gem* di Me). Representative procedure for the synthesis of disulfides. Preparation of **3**: A solution of **2** (0.116 g, 0.106 mmol) and β-D-thioglucose sodium salt (76% free SH content by estimation with Ellman's reagent, 36 mg) in argon bubbled MeOH (10 mL) at 0 °C was stirred for 30 min. At the end *N*-ethylmaleimide (14 mg, 0.106 mmol) in MeOH (1 mL) was added. After 10 min purified by C18 Column chromatography with a step gradient of MeCN/H_2O (2:3) to MeCN/H_2O (3:2). Fractions containing required compound were analyzed on 5 μ Brownlee reversed-phase column with MeCN: Sod.phosphate buffer (50 mM, pH 3.5, 2:3) at λ 230 nm. Fractions containing pure compound were combined and lyophilized to give **3** (63 mg, 53% yield).

Spectral characteristics of ***3***: MS = [M – H] = 1176, [M + NH4] = 1195, IR (KBr) 536.2 cm^{-1} (–S–S– stretching), ^{1}H NMR ($CDCl_3$) δ 8.14–7.29 (5×m, 15H, ArH,), 7.00 (m, 1H, $\underline{NH}$CO), 6.31 (s, 1H, T-10), 6.20 (t, 1H, T-13, $J_{12,13}=9.0$ Hz), 5.94 (dd, 1H, T-3, $J_{NH,\,3'}=9.0$ Hz, $J_{2,3}=3.2$ Hz), 5.67 (d, 1H, T-2, $J_{2,3}=7.2$ Hz), 5.46 (d, 1H, T-2′), 4.96 (m, 1H, T-5), 4.42 (m, 1H, T-7), 4.30 (d, 1H, T-20A, $J_{AB}=8.4$ Hz), 4.20–4.16 (m, 2H, T-20B and anomeric H), 3.85–3.0 (4×m, 10H, T-3, 4×thioglucose-$\underline{CH}$, thioglucose–$\underline{CH_2}$–, and 3×OH), 3.10 (br.s, 1H, OH), 2.71–2.52 (m, 4H, S-$\underline{CH_2}$–, T-6A, 2.45– 1.80 (2×m, and 3×s, 13H, T-14, 3×Me,–$\underline{CH_2}$–C(Me)$_2$–CO–, 1.67 (s, 3H, T-18), 1.24–1.12 (4×s, 12H, 2 sets of *gem* di Me).

Spectral characteristics for ***4***: MS: [M + H] = 1289, [M + MeCN + H] = 1330, IR (KBr) 536.8 cm^{-1} (–S–S– stretching), ^{1}H NMR (DMSO + D_2O) δ 7.96–7.17 (6×m, ArH), 6.27 (s, 1H, T-10), 5.79 (t, 1H, T-13, $J_{12,13}=9.0$ Hz), 5.55 (d, 1H, T-3′, $J_{2,3}=9.6$ Hz), 5.39 (d, 1H, T-2, $J_{2,3}=7.2$ Hz), 5.30 (d, 1H, T-2′), 4.90 (m, 1H, T-5), 4.48 (dd, 1H, T-7, $J_{6,7}=4.3$ Hz and 9.9 Hz), 4.51–3.95 (m, 3H, Cys–$\underline{CH}$- and T-20), 3.28 (t, 1H, Glu–CH–, $J_{vic}=6.6$ Hz), 3.2–2.5 (3×m, Cys–$\underline{CH_2}$–, Linker–S–CH_2–, T-6A), 2.45–1.47 (2×m and 4×s, 16H, 16H, T-14,–$\underline{CH_2}$C(Me)$_2$CO–, T-18, 2×$COCH_3$ and angular Me), 1.13–0.98 (4×s, 2×*gem* di Me).

Spectral characteristics for ***5***: MS: [M + H] = 1199, [M + NH_4 + MeCN] = 1257, [M–H] = 1198, IR (KBr) 535.1 cm^{-1} (–S–S– stretching), ^{1}H NMR ($CDCl_3$ + D_2O) δ 8.17–7.31 (5×m, 15H, ArH), 6.28 (s, 1H, T-10), 6.26 (t, 1H, $J_{12,13}=9.0$ Hz), 6.02 (d, 1H, T-3′, $J_{2,3}=3.1$ Hz), 5.68 (d, 1H, T-2, $J_{2,3}=7.2$ Hz), 5.43 (d, 1H, T-2′, 4.97 (m, 1H, T-5), 4.98–4.41 (m, 2H, T-5, prolyl–$\underline{CH}$–), 4.32 (d, 1H, T-20$_A$, $J_{AB}=8.4$ Hz), 4.20 (d, 1H, T-20$_B$), 3.81 (d, 1H, T-3), 3.60–3.40 (m, 2H, Prolyl–CH_2–NCO), 3.00–2.80 (m, 2H, Prolyl–$\underline{CH}$—S–, Prolyl–CH_{2A}), 2.8–1.6 (5×m and 4×s, 24H, T-6, T-14,–S–S–$\underline{CH_2}$–$\underline{CH_2}$–,Prolyl–CH_{2B}, Prolyl–C–$\underline{CH_2}$–C, 2×$COCH_3$, T-18 and angular CH_3), 1.77, 1.66, 1.21 (3×s, 12H, 2×gem diMe), 1.08 (d, 3H, Cys–CH_3).

Spectral characteristics for ***6***: MS: [M–H] = 237. ^{1}H NMR (DMSO-d_6) δ 12.20 (s, 1H, COO$\underline{H}$), 7.38–7.20 (m, 5H, Ar$\underline{H}$), 3.71 (s, 2H. Ph$\underline{CH_2}$), 2.33–2.26 (m, 2H, S$\underline{CH_2}$), 1.74–1.67(m, 2H, $\underline{CH_2}$COOH), 1.05 (s, 6H, C$\underline{Me_2}$); ^{13}C NMR (DMSO–d_6) δ 178.22, 138.64, 128.82, 128.29, 126.70, 41.32, 34.90, 26.15, 24.78.

Spectral characteristics for ***7***: ^{1}H NMR ($CDCl_3$) δ 8.47–8.41 (m, 1H, Ar$\underline{H}$), 7.74–7.56 (m, 2H, Ar$\underline{H}$), 7.10–7.02 (m, 1H, Ar$\underline{H}$), 2.84–2.70 (m, 2H, S$\underline{CH_2}$), 2.05–1.86 (m, 2H, $\underline{CH_2}$COOH), 1.17 (s, 6H, C$\underline{Me_2}$).