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Nitroarylmethylcarbamate prodrugs of doxorubicin for use with nitroreductase gene-directed enzyme prodrug therapy

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Abstract—A series of nitrobenzyl- and nitroimidazolylmethyl carbamate prodrugs of doxorubicin were prepared and evaluated for their potential use in nitroreductase (NTR) mediated gene-directed enzyme prodrug therapy (GDEPT). The carbamate prodrugs and doxorubicin were tested in a cell line panel comprising parental and NTR transfected human (SKOV3/SKOV3–NTR^{neo}, WiDr/WiDr–NTR^{neo}), Chinese hamster (V79/V79–NTR^{puro}) and murine (EMT6/EMT6–NTR^{puro}) cell line pairs, and were compared with the established NTR substrates CB 1954 (an aziridinyl dinitrobenzamide) and the analogous dibromomustard SN 29427. The low solubility of the prodrugs (from 3 to 39 μM) precluded the determination of IC_{50} values against the parent cell lines in some instances. All of the prodrugs were unstable in culture medium with 5% added fetal calf serum over a 24 h period, although release of doxorubicin was not observed. The prodrugs were 20- to >336-fold less toxic than doxorubicin in the human cells lines SKOV3 and WiDr, with overall less deactivation seen in the V79 cell line (11- to >286-fold) and EMT6 cell line (1.8- to >178-fold). Prodrugs with the nitrobenzyl unit directly conjugated to doxorubicin showed modest selectivity for NTR across the cell line panel (1- to 5.9-fold) but this was increased to between >10- and >370-fold with the interpolation of an 4-aminobenzyl spacer unit between the bioreductive unit and doxorubicin. A 2-nitroimidazolylmethyl carbamate provided deactivation of doxorubicin (8- to 124-fold) but showed only modest selectivity for NTR (2- to 14-fold) across the panel. The interpolation of a 4-aminobenzyl spacer gave slightly lower deactivation (3- to 64-fold) and similar selectivity for NTR (>1.2- to >12-fold) for 2- and 5-nitroimidazolylmethyl prodrugs. The activity of two nitrobenzyl prodrugs containing an aminobenzyl spacer, providing excellent selectivity for NTR+ve cells in culture, was evaluated against EMT6 tumours comprising ca. 10% NTR+ve cells, but neither showed statistically significant levels of killing even of NTR+ve cells. This lack of activity in tumours, despite potent and selective activity in culture, indicates that pharmacokinetic optimization is needed to achieve in vivo efficacy against solid tumours with this new class of NTR prodrugs.

1. Introduction

The development of gene-directed enzyme prodrug therapy (GDEPT)[1–5] using an oxygen-insensitive nitroreductase from *Escherichia coli* B (the *nfsB* gene product NTR) has been the subject of considerable study[6] since the first description of NTR.[7–10] Two main classes of nitroaromatic substrate have been identified as prodrugs activated by NTR: 2,4-dinitrobenzamides and 4-nitrobenzylcarbamates. The first-identified NTR prodrug, the 2,4-dinitrobenzamide, CB 1954 (**1**)[7,11–13] has advanced to clinical trial[14–16] and structure–activity relationships (SAR) have been determined for related aziridines[17] and nitrogen mustards.[18–21] The bromomustard **2** (SN 29427) shows increased in vivo activity and a larger bystander effect compared to CB 1954 in preclinical models.[22] A second class of prodrugs under investigation is based on the 4-nitrobenzylcarbamate moiety (**3**), which undergoes reduction to the hydroxylamine and subsequent fragmentation to release a cytotoxic amine.[23–25] Masking the amine function as a carbamate provides significant deactivation of the cytotoxin by either electronic effects, where electronic release from the amine is required for activation of the cytotoxin [$\sigma_{p(NH_2)} = -0.66$, $\sigma_{p(OCONH)} = -0.17$], or by steric effects limiting binding to the site of action. A variety of cytotoxic agents bearing a critical amine group have been examined as their 4-nitrobenzylcarbamate prodrugs for NTR including aniline mustard,[26] mitomycin,[26] enediynes,[27,28] *seco*-cyclopropylindoline derivatives,[29] pyrrolobenzodiazepines,[30] anthracyclines[26] and tallimustine analogues.[31] Related nitrobenzyl phosphorodiamidate[32]

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and nitrobenzyl phosphoroamide[33] prodrugs of alkylating agents have also been reported.

Recently we extended the 4-nitrobenzylcarbamate class of prodrugs to include 2-alkoxy-4-nitrobenzylcarbamates (e.g., **4**), where the addition of the 2-alkoxy substituents improved solubility and provided faster fragmentation kinetics.[24] Several prodrugs of 5-aminobenz[*e*]indoline alkylating agents were good substrates for NTR in human cell lines in vitro and were more selectively cytotoxic to NTR+ve cells than the unsubstituted 4-nitrobenzyl carbamate.[34] Additionally, we identified a range of nitroheterocyclic methyl carbamates of a 5-aminobenz[*e*]indoline alkylating agent (e.g., **5**), which were good substrates for NTR in human cell lines in vitro and were as selectively cytotoxic to NTR+ve cells as the 2-alkoxy-4-nitrobenzylcarbamate prodrugs.[35,36]

In an effort to consolidate the initial promise shown by **4** and **5** as prodrugs for NTR we sought to conjugate the bioreductive elements identified in our previous studies[24,34–36] with doxorubicin (**6**) via its 3′-amino group. Doxorubicin (**6**) has been a popular choice[37–42] as a cytotoxic effector in numerous prodrug strategies because of the requirement of a free 3′-amino group for activity, high potency, broad-spectrum activity and established clinical efficacy. The 4-nitrobenzylcarbamate linked to the 3′-amine of doxorubicin (**7**) has been prepared previously and although **7** was a substrate for NTR, the authors concluded doxorubicin was not released upon reduction.[26] There is evidence[43–48] that the rate of activation of some anthracycline prodrugs is enhanced by inclusion of a 4-aminobenzyloxycarbonyl 'spacer', which undergoes spontaneous fragmentation via a 1,6-elimination process.[23]

We report here the synthesis of, and in vitro and in vivo studies on directly linked 4-nitroarylcarbamates **7**–**11** and 4-nitroarylcarbamates containing a 'self-immolative' linker **12**–**14** as prodrugs of doxorubicin (**6**) for an NTR-mediated GDEPT approach.

2. Results and discussion

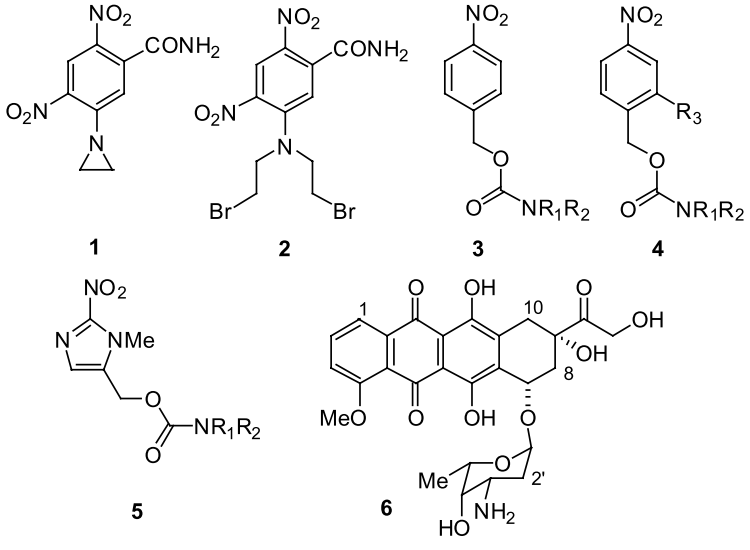
2.1. Chemistry

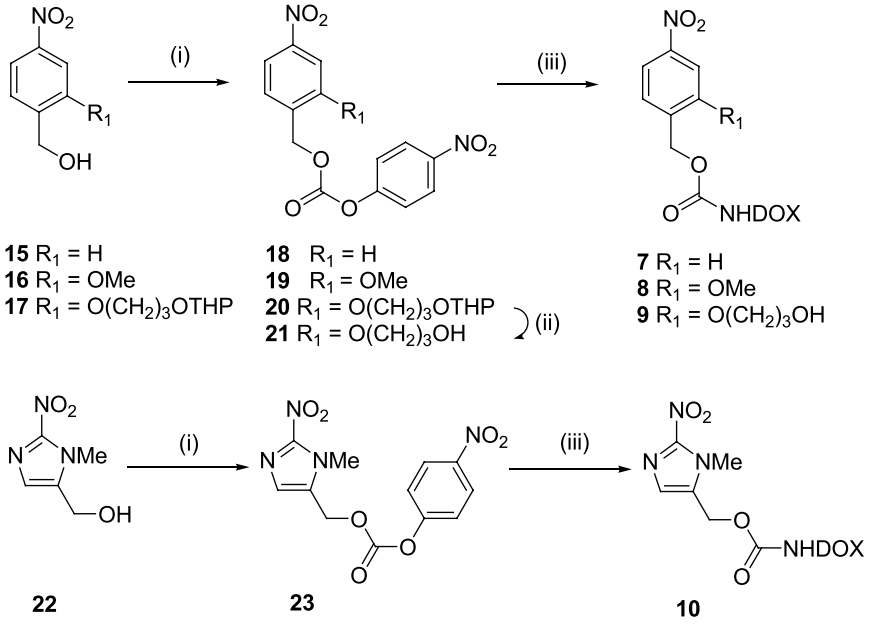
4-Nitrobenzyl alcohol **15** and 2-methoxy-4-nitrobenzyl alcohol **16**[24] were activated as the 4-nitrophenyl carbonates **18**[26] and **19** (Scheme 1). Displacement of the carbonates **18** and **19** with **6** gave **7** and **8**, respectively, in excellent yield. Similarly, reaction of the THP-protected propyloxy ether **17**, prepared by alkylation of methyl 4-nitrosalicylate with the protected iodopropanol and subsequent DIBAL-H reduction of the ester, with 4-nitrophenylchloroformate gave carbonate **20**. Deprotection under acidic conditions gave **21**, which reacted with **6** to give carbamate **9**. Similarly, 2-nitroimidazole-5-methanol (**22**) was activated as the carbonate **23**[49] and reacted with **6** to give a directly linked 2-nitroimidazole carbamate **10**.

An iterative process was used to obtain carbamates with a 4-aminobenzyl spacer between the nitroaryl trigger and **6**. Reaction of 4-nitrobenzylchloroformate **24** with 4-aminobenzyl TBDMS ether **25** in the presence of HOBT[50] gave carbamate **26**, which was deprotected to give **27** (Scheme 2). Activation of alcohol **27** with 4-nitrophenylchloroformate gave carbonate **30**, which reacted with **6** to give carbamate **11**. Similarly, reaction of carbonate **19** with **25** gave carbamate **28**, which was deprotected to give **29**. Activation of alcohol **29** as the 4-nitrophenyl carbonate **30** facilitated reaction with **6** to give carbamate **12**. Similar reaction sequences with 2-nitroimidazolyl-5-methyl carbonate (**23**) and 5-nitroimidazolyl-2-methyl carbonate (**35**)[49] produced the corresponding nitroimidazole-spacer-doxorubicin prodrugs **13** (Scheme 3) and **14** (Scheme 4), respectively.

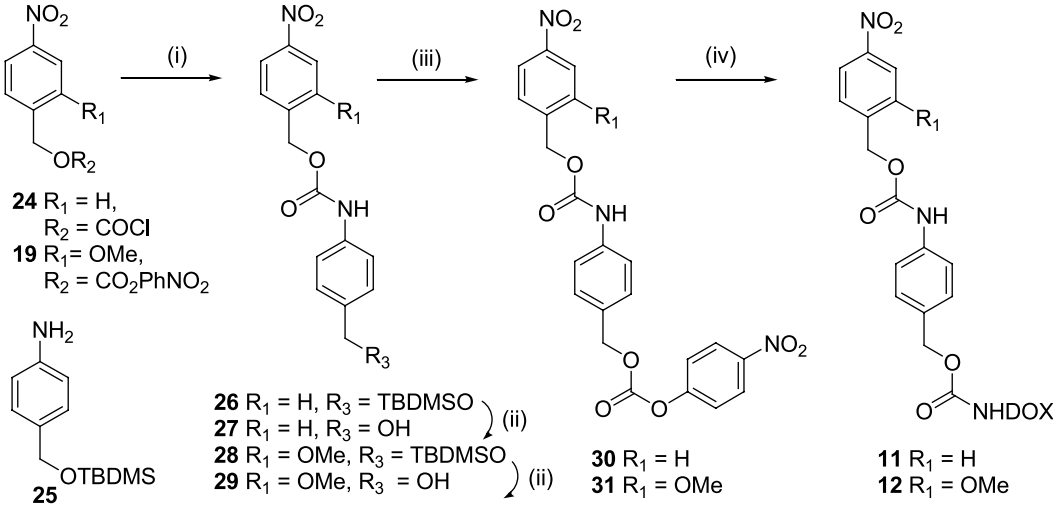
2.2. Solubility and stability

The purity of the compounds **7**–**14** was shown by HPLC analysis to be 98% or greater. The amount of effector **6** detected was generally less than 0.1%, although 0.3% was detected in samples of **11** and **14** and 0.5% in **10**.

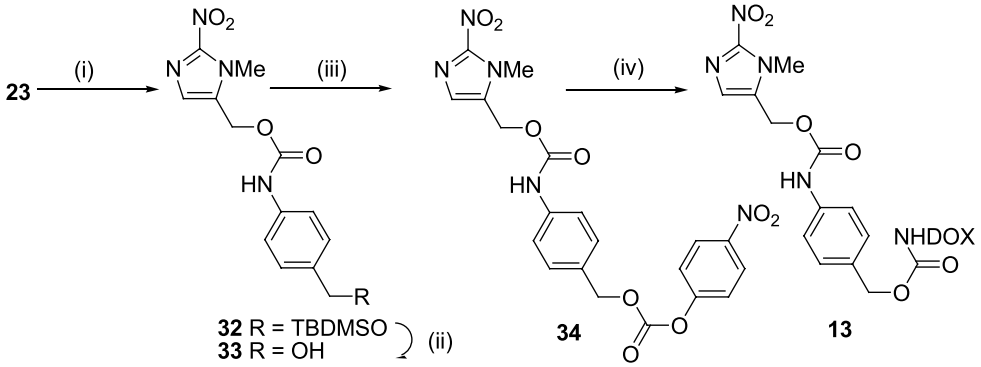




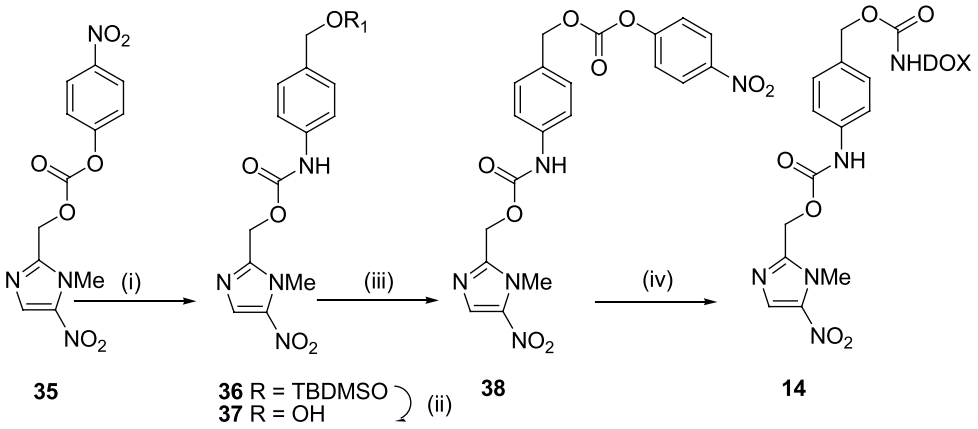
Scheme 1. Reagents: (i) $NO_2PhOCOCl$, DIEA, THF; (ii) HCl, MeOH; (iii) DOX, Et_3N, DMF.



Scheme 2. Reagents: (i) (**25**), Et_3N, HOBT, 4 Å sieves, THF; (ii) HCl, MeOH; (iii) 4-$NO_2PhOCOCl$, DIEA, THF; (iv) DOX, Et_3N, DMF.



Scheme 3. Reagents: (i) (**25**), Et_3N, HOBT, 4 Å sieves, THF; (ii) HCl, MeOH; (iii) 4-$NO_2PhOCOCl$, DIEA, THF; (iv) DOX, Et_3N, DMF.



Scheme 4. Reagents: (i) (**25**), Et_3N, HOBT, 4 Å sieves, THF; (ii) HCl, MeOH; (iii) 4-NO_2PhOCOCl, DIEA, THF; (iv) DOX, Et_3N, DMF.

The solubility of compounds (**1**, **2**, **7**–**14**) in α-MEM culture medium with 5% added fetal calf serum (FCS) was determined by HPLC analysis of the supernatant of a saturated solution (Table 1). The prodrugs **7**–**14** had solubilities ranging from 3 to 39 μM: considerably less soluble than CB 1954 (**1**) and similar to the bromomustard **2**. The addition of a 2-OMe group provided an appreciable increase in solubility over the unsubstituted nitrobenzyl analogue **7** in the directly linked prodrug **8**, but this effect was not seen when a 4-aminobenzyl linker unit was present, that is, **11** and **12**. The prodrugs **7**–**14** were unstable in α-MEM + 5% FCS, with 20–87% prodrug remaining after 24 h whereas both **1** and **2** were stable under these conditions.

2.3. In vitro cytotoxicity

The effectors and prodrugs were evaluated for cytotoxicity in four pairs of cell lines, each comprising a transfectant stably expressing NTR and its non-NTR-expressing counterpart. The parental lines were SKOV3 (human ovarian carcinoma), WiDr (human colon carcinoma), $V79^{puro}$ (Chinese hamster fibroblast) and EMT6 (mouse mammary carcinoma). Cytotoxicity was measured as IC_{50} values following an 18 h drug exposure in the NTR−ve lines, and these values are reported in Table 1, together with the ratios of the IC_{50} values between the NTR−ve and NTR+ve lines which are a measure of selectivity for NTR-expressing cells. The compounds were compared with the established NTR prodrugs CB 1954 (**1**) and the related dibromomustard **2** in order to evaluate their potential as GDEPT candidates.[22] Doxorubicin (**6**) is a potent cytotoxin with IC_{50} values ranging from 30 to 56 nM against the cell line panel (Table 1) and showed no selectivity for NTR+ve cells. The directly-linked prodrugs **7**–**10** were ca. 20- to 124-fold less toxic than **6** to the human NTR-ve cell lines (SKOV and WiDr) and similarly ca. 11- to 88-fold less toxic in the hamster NTR−ve line (V79), but were only 1.4- to 8-fold less toxic against the mouse cell line (EMT6). The nitrobenzyl prodrugs containing a spacer unit, **11** and **12**, were considerably less toxic overall, to the extent that cytotoxicity in NTR−ve cell lines could not be quantified at the solubility limit. Compounds **11** and **12** were >286- to >336-fold less toxic than **6** in the human and hamster cell lines (SKOV, WiDr and V79) and 57- to >178-fold less toxic than **6** in the murine cell line (EMT6). The 2- and 5-nitroimidazole spacer-linked prodrugs **13** and **14** were more toxic than their nitrobenzyl counterparts **11** and **12**; with **13** and **14** being 34- to 64-fold less toxic than **6** in SKOV, WiDr and V79 cells, and 3- to 4-fold less toxic than **6** in EMT6 cells. Directly linked prodrugs **7**–**10** displayed modest selectivity for NTR in human cell lines (1.8- to 14-fold) and lower selectivity in the rodent lines with only the 2-nitroimidazole **10** being selective across the panel (2.6- to 14-fold). Similarly, the nitroimidazole prodrugs **13** and **14** containing a spacer unit displayed only modest selectivity. However, the nitrobenzyl prodrugs containing a spacer unit, **11** and **12**, showed good to excellent selectivity (>16- to >370-fold) although solubility prevented determination of an upper limit. The selectivity for NTR observed for **11** and **12** was comparable to **1** and **2** in the human cell line pairs and only ca. 2.5- to 6-fold lower in the EMT6 cell line pair.

2.4. In vivo evaluation

Prodrugs **11** and **12** were selected for in vivo evaluation because of their selectivity for NTR across the cell line panel. The maximum tolerated dose of **11** given by intraperitoneal (i.p.) injection in DMSO to C3H mice was >1000 μmol kg^{-1}, whereas the MTD of **12** given i.p. in DMSO to Swiss nude mice was >750 μmol kg^{-1}. The MTD of **1** was 240 μmol kg^{-1} given i.p. in DMSO to C3H mice whereas **2** was considerably less toxic (>1330 μmol kg^{-1}).[34] The activity of **11** and **12** were evaluated against NTR-expressing EMT6 tumours comprising mixtures of NTR−ve and NTR+ve cells. In this model nude mice are inoculated with 2:1 mixtures of $EMT6–NTR^{puro}$ and EMT6 (NTR−ve) cells, respectively, providing ca. 10% NTR+ve cells at the time of tumour treatment.[34,51] This tumour model represents the likely situation in a GDEPT protocol where low rates of transfection of tumour tissue are expected. Compound **11** provided no killing of either NTR+ve or −ve tumour cells and although a small decrease in tumour cells was observed after treatment with **12** these

Table 1. Solubility, stability, IC_{50} data and cell line ratios (NTR−ve/NTR+ve) for **6–14**

No	Spacer	Sol[a] (μM)	Stab[b] (%)	SKOV (μM)[c]	SKOV/SKOV–NTR^{neo}[c,d]	WiDr (μM)[c]	WiDr/WiDr–NTR^{neo}[c,d]	V79 (μM)[c]	V79/V79–NTR^{puro}[c,d]	EMT6 (μM)[c]	EMT6/EMT6–NTR^{puro}[c,d]
1		1600	100	174 ± 10	317 ± 21	54 ± 3	51 ± 2	374 ± 14	2090 ± 210	71 ± 7	930 ± 140
2		50	100	58 ± 10	211 ± 16	40 ± 3	174 ± 31	43 ± 5	302 ± 93	54	1380
6	–		ND	0.0298 ± 0.0003	1.2 ± 0.3	0.043 ± 0.005	1.2 ± 0.2	0.035 ± 0.003	1.2 ± 0.2	0.056 ± 0.009	0.9 ± 0.2
7	–	14	36	0.59 ± 0.9	5.4 ± 0.1	1.0 ± 0.2	4.3 ± 1.8	0.39 ± 0.06	0.97 ± 0.04	0.10 ± 0.02	2.9 ± 1.0
8	–	39	20	2.48 ± 0.05	5.9 ± 1.4	2.77 ± 0.05	3.1 ± 0.3	1.53 ± 0.05	1.35 ± 0.02	0.27 ± 0.03	1.8 ± 1.0
9	–	11	30	1.49 ± 0.06	2.5	2.5 ± 0.2	1.76 ± 0.1	2.1 ± 0.3	1.17 ± 0.05	0.08 ± 0.02	1.4 ± 0.4
10	–	33	48	3.7 ± 0.5	14.4 ± 2.6	3.6 ± 0.9	10.4 ± 2.4	3.1 ± 0.8	2.6 ± 0.6	0.46 ± 0.08	6.4 ± 1.0
11	+	15	30	>10	>213	>10	>18.4	>10	ND[e]	>10	>370
12	+	8	87	>10	>57	>10	>16	>10	ND	3.2 ± 0.5	231 ± 15
13	+	1.5	50	>1.5	>12.3	>1.5	>5.6	>1.5	>1.2	0.24 ± 0.03	5.0
14	+	3	66	1.9 ± 0.5	7.0 ± 0.4	1.9 ± 0.4	2.4 ± 0.4	2.1 ± 0.4	1.97 ± 0.06	0.19 ± 0.04	3.1 ± 0.3

[a] Solubility in α-MEM culture medium, determined by HPLC.
[b] Stability after 24 h in α-MEM culture medium, determined by HPLC.
[c] Values are mean ± sem for up to four independent experiments.
[d] Intra-experiment ratios.
[e] Not determined.

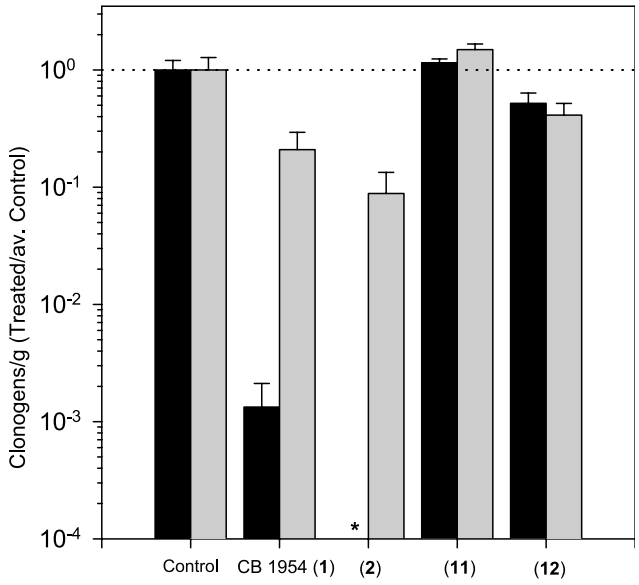


Figure 1. Comparison of in vivo activity and bystander effects for CB 1954 (**1**) bromomustard **2**, doxorubicin prodrugs **11** and **12**. Prodrugs given at 200, 1330, 1000 and 750 μmol kg^{-1} i.p. For experiments with **1**, **2** and **12**, control tumours at excision comprised 10.3% EMT6–NTR^{puro}, and for experiments with **11** control tumours at excision comprised 9.5% EMT6–NTR^{puro}, as assessed by the proportion of puromycin-resistant cells. Filled bars, EMT6–NTR^{puro}; shaded bars, EMT6. *EMT6–NTR^{puro} < 10^{-4} clonogens/g.

effects were not significant (Fig. 1). In contrast, we have previously demonstrated[34] that dinitrobenzamides **1** and **2** given at 200 and 1330 μmol kg^{-1}, respectively, show significant killing of activator (EMT6–NTR^{puro}) cells and target (EMT6) cells, indicating the operation of a bystander effect.

2.5. Discussion

Directly linked compounds showed modest deactivation of doxorubicin and relatively low selectivity for NTR. In the case of 2-nitroimidazole **13** the addition of a spacer did not provide any significant increase in either the deactivation of the cytotoxin or in selectivity for NTR compared to **10**. However, in the two nitrobenzyl analogues, **11** and **12**, the addition of a linker unit provided a substantial deactivation of **6** and also an increase in selectivity for NTR+ve cells, in accord with the previously noted requirement for a spacer for optimal prodrugs of doxorubicin.[43–48] The instability of the prodrugs in culture medium is a potential problem for this class of compounds even though release of effector **6** was not detected.

The high MTD values for **11** and **12**, >1000 and >750 μM kg^{-1}, respectively, suggest the prodrug is effectively deactivating doxorubicin, which has an MTD of 23.7 μmol kg^{-1} also in C3H mice.[52] However, it has not been established that the compounds distribute from the intraperitoneal injection site. The lack of in vivo activity against both activator and target cells suggests either poor plasma pharmacokinetics leading to low amounts of prodrug reaching the tumour or poor

tumour penetration resulting in little activation of the prodrug in the activator (NTR+ve) cell population. The high molecular weight of **12** (ca. 900 Da), the presence of seven hydrogen bond donors and 20 hydrogen bond acceptors, and a calculated log $D_{7.4}$ of 5.6 (ACD log *D* Calculator v7.0: Advanced Chemistry Development Inc, Toronto, Canada) altogether present an unfavorable profile for drug distribution[53] and studies of the penetration of anthracyclines through a three-dimensional cell culture models have shown that doxorubicin itself displays limited drug penetration.[54,55]

3. Conclusions

These studies of nitrobenzyl- and nitroimidazolylmethyl carbamates of doxorubicin identified two nitrobenzyl carbamates, **11** and **12**, linked to doxorubicin via an aminobenzyl carbamate spacer, as potential prodrugs for NTR. These compounds were considerably less potent than doxorubicin across the cell line panel and showed marked selective cytotoxicity to NTR+ve cells in vitro, but did not show any significant in vivo activity against tumours comprising NTR+ve and −ve cells in mice. As a clinical agent, doxorubicin is an appealing drug candidate for a GDEPT approach from a regulatory point of view. However, the lack of in vivo activity of the prodrugs may reflect the physicochemical properties of these compounds that are probably outside the acceptable range for drug-like properties. This points to the importance of early 'drugability' screening in drug discovery.[53] This work brings to a conclusion a series of exploratory studies[24,25,27,28,34–36] searching for prodrugs, based on the 4-nitrobenzylcarbamate motif, for NTR-mediated GDEPT. In the course of these studies we have identified 2-alkoxy-4-nitrobenzyl, 2-nitro-5-methylimidazolyl and 2-methyl-5-nitroimidazolyl carbamates as potential prodrugs. These carbamates provided considerable deactivation of cytotoxins and displayed good in vitro selectivity for activation by NTR in vitro, but were not active against NTR-expressing cells in tumours. Further development of these triggers is contingent on the optimization of pharmacokinetic and pharmacodynamic parameters of such prodrugs to ensure in vivo activity.

4. Experimental

4.1. General

Analyses were carried out in the Microchemical Laboratory, University of Otago, Dunedin, NZ. Melting points were determined on an Electrothermal 2300 Melting Point Apparatus. NMR spectra were obtained on a Bruker Avance 400 spectrometer at 400 MHz for ^{1}H and 100 MHz for ^{13}C spectra. Spectra were obtained in $CDCl_3$ unless otherwise specified and are referenced to Me_4Si. Chemical shifts and coupling constants were recorded in units of ppm and hertz, respectively. Assignments were determined using APT, COSY, HSQC and HMBC two-dimensional experiments. Mass spectra were determined on a VG-70SE mass spectrometer using an ionizing potential of 70 eV at a nominal resolution of 1000. High-resolution spectra were obtained at nominal resolutions of 3000, 5000 or 10,000 as appropriate. All spectra were obtained as electron impact (EI) using PFK as the reference unless otherwise stated. Solutions in organic solvents were dried with anhydrous Na_2SO_4. Solvents were evaporated under reduced pressure on a rotary evaporator. Thin-layer chromatography was carried out on aluminium-backed silica gel plates (Merck $60F_{254}$) with visualization of components by UV light (254 nm) or exposure to I_2. Column chromatography was carried out on silica gel, (Merck 230–400 mesh). All compounds designated for biological evaluation were analyzed by reverse phase HPLC using an Agilent 1100 liquid chromatograph, an Altima C18 (5 μM) stainless steel column (150 mm × 3.2 mm i.d.) and an Agilent 1100 diode array detector. Chromatograms were run using various gradients of aqueous (0.045 M ammonium formate and formic acid at pH 3.5) and organic (80% MeCN/MilliQ water) phases. DCM refers to dichloromethane; DIEA refers to diisopropylethylamine; DMF refers to dry dimethylformamide; EtOAc refers to ethyl acetate; HOBT refers to 1-hydroxybenzotriazole; MeOH refers to methanol; pet. ether refers to petroleum ether, boiling range 40–60 °C; THF refers to tetrahydrofuran dried over sodium benzophenone ketyl. All solvents were freshly distilled. Doxorubicin hydrochloride (**6**) in a lactate buffer was dissolved in water, neutralized with dilute aqueous NH_3 and extracted with $CHCl_3$, dried and the solvent evaporated immediately prior to use.

4.1.1. Preparation of methanols. {4-Nitro-2-[3-(tetrahydro-2*H*-pyran-2-yloxy)propoxy]phenyl}methanol (17). A mixture of methyl 4-nitrosalicylate (2.3 g, 11.7 mmol) and K_2CO_3 (2.42 g, 17.5 mmol) in DMF (25 mL) was stirred at 20 °C for 20 min. A solution of 3-iodopropyl tetrahydro-2*H*-pyran-2-yl ether (4.7 g, 17.5 mmol) in DMF (5 mL) was added and the mixture stirred at 100 °C for 2 h. The mixture was poured into water, extracted with EtOAc (3 × 100 mL), the combined organic extracts washed with water (2 × 50 mL) and brine (50 mL), dried and the solvent evaporated. The residue was purified by chromatography, eluting with 20% EtOAc/pet. ether to give methyl 4-nitro-2-[3-(tetrahydro-2*H*-pyran-2-yloxy)propoxy]benzoate (3.66 g, 92%) as a colourless oil, ^{1}H NMR δ 7.89 (d, J = 8.5 Hz, 1H, H-6), 7.80–7.84 (m, 2H, H-3, H-5), 4.60–4.62 (m, 1H, OCHO), 4.27 (t, J = 6.2 Hz, 2H, CH_2O), 3.95–4.00 (m, 1H, CH_2O), 3.94 (s, 3H, OCH_3), 3.79–3.86 (m, 1H, CH_2O), 3.59–3.66 (m, 1H, CH_2O), 3.47–3.52 (m, 1H, CH_2O), 2.13–2.17 (m, 2H, CH_2), 1.78–1.84 (m, 1H, CH_2), 1.68–1.75 (m, 1H, CH_2), 1.47–1.62 (m, 4H, 2 × CH_2); ^{13}C NMR δ 164.5 (CO_2), 158.6 (C-2), 150.7 (C-4), 132.0 (C-6), 126.2 (C-1), 114.8 (C-5), 107.9 (C-3), 99.0 (OCO), 66.5 (CH_2O), 63.4 (CH_2O), 62.4 (CH_2O), 52.5 (OCH_3), 30.6 (CH_2), 29.3 (CH_2), 25.4 (CH_2), 19.6 (CH_2); MS *m*/*z* 339 (M^+, 2), 322 (12), 239 (20), 222 (40), 85 (100); HRMS calcd for $C_{16}H_{21}NO_7$ (M^+) *m*/*z* 339.1318. Found 339.1317.

DIBALH (1 M in DCM, 34 mL, 34 mmol) was added dropwise to a solution of benzoate (3.46 g, 10.2 mmol)

in THF (100 mL) at 5 °C and the solution stirred at 5 °C for 1 h. The solution was poured into a solution of sodium potassium tartrate (1 M, 100 mL, 0.1 mol) and stirred for 30 min. The mixture was extracted with EtOAc (3 × 100 mL), the combined organic fraction washed with water (100 mL) and brine (50 mL), dried and the solvent evaporated. The residue was purified by chromatography, eluting with 50% EtOAc/pet. ether, to give **17** (3.11 g, 98%) as a pale yellow solid, mp (EtOAc/pet. ether) 64–65.5 °C; ^{1}H NMR δ 7.84 (dd, J = 8.2, 2.1 Hz, 1H, H-5), 7.72 (d, J = 2.1 Hz, 1H, H-3), 7.50 (d, J = 8.2 Hz, 1H, H-6), 4.74 (dd, J = 14.8, 4.2 Hz, 2H, CH_2O), 4.58–4.61 (m, 1H, OCHO), 4.24 (t, J = 6.1 Hz, 2H, CH_2O), 3.96 (dt, J = 10.0, 5.8 Hz, 1H, CH_2O), 3.80–3.86 (m, 1H, CH_2O), 3.62 (dt, 10.0, 5.8 Hz, 1H, CH_2O), 3.46–3.51 (m, 1H, CH_2), 2.30 (br s, 1H, OH), 2.08–2.11 (m, 2H, CH_2), 1.79–1.85 (m, 1H, CH_2), 1.69–1.77 (m, 1H, CH_2), 1.48–1.62 (m, 4H, 2 × CH_2); ^{13}C NMR δ 156.5 (C-2), 148.2 (C-4), 136.8 (C-1), 128.1 (C-6), 115.9 (C-5), 105.8 (C-3), 99.3 (OCO), 65.9 (CH_2O), 63.9 (CH_2O), 62.8 (CH_2O), 60.8 (CH_2O), 30.6 (CH_2), 29.3 (CH_2), 25.3 (CH_2), 19.7 (CH_2); MS (CI) *m/z* 312 (MH^+, 0.5), 294 (1), 245 (15), 227 (30), 85 (100); HRMS (CI) calcd for $C_{15}H_{22}NO_6$ (MH^+) *m/z* 312.1447. Found 312.1438. Anal. Calcd for $C_{15}H_{21}NO_6$: C, 57.9; H, 6.8; N, 4.5. Found: C, 58.1; H, 6.7; N, 4.5.

4.1.2. 4-Nitrobenzyl 4-({[*tert*-butyl(dimethyl)silyl]oxy}-methyl)phenylcarbamate (26). A solution of 4-nitrobenzyl chloroformate (**24**) (1.0 g, 4.6 mmol) in THF (10 mL) was added dropwise to a stirred solution of 4-({[*tert*-butyl(dimethyl)silyl]oxy}methyl)aniline (**25**) (0.91 g, 4.2 mmol) and Et_3N (0.76 mL, 5.5 mmol) in THF (20 mL) at 0 °C and the solution was stirred at 20 °C for 16 h. The solvent was evaporated and the residue dissolved in EtOAc (200 mL), washed consecutively with 1 M HCl (100 mL), water (2 × 100 mL), aqueous Na_2CO_3 (100 mL), water (100 mL) and brine (50 mL), dried and the solvent evaporated. The residue was purified by chromatography, eluting with 10% EtOAc/pet. ether to give **26** (1.10 g, 63%) as a white solid, mp (EtOAc/pet. ether) 107–109 °C; ^{1}H NMR δ 8.22 (ddd, J = 8.7, 2.3, 1.8 Hz, 2H, H-3′, H-5′), 7.55 (d, J = 8.7 Hz, 2H, H-2′, H-6′), 7.34 (br d, J = 8.1 Hz, 2H, H-2, H-6), 7.26 (d, J = 8.6 Hz, 2H, H-3, H-5), 6.73 (br s, 1H, OCONH), 5.29 (s, 2H, CH_2O), 4.70 (s, 2H, CH_2OSi), 0.93 [s, 9H, $SiC(CH_3)_3$], 0.09 [s, 6H, $Si(CH_3)_2$]; ^{13}C NMR δ 152.9 (OCONH), 147.7 (C-4′), 143.5 (C-1′), 137.1 (C-1), 136.1 (C-4), 128.3 (C-2′, C-6′), 127.1 (C-3, C-5), 123.8 (C-3′, C-5′), 118.7 (C-2, C-6), 65.4 (CH_2O), 64.5 (CH_2O), 25.9 [$SiC(CH_3)_3$], 18.4 [$SiC(CH_3)_3$], −5.2 [$Si(CH_3)_2$]; MS *m/z* 416 (M^+, 0.2), 401 (1), 359 (50), 241 (40), 206 (55), 162 (55), 132 (100); HRMS calcd for $C_{21}H_{28}N_2O_5Si$ (M^+) *m/z* 416.1768. Found 416.1760. Anal. Calcd for $C_{21}H_{28}N_2O_5Si$: C, 60.6; H, 6.8; N, 6.7. Found: C, 60.7; H, 6.7; N, 6.8.

4.1.3. 4-Nitrobenzyl 4-(hydroxymethyl)phenylcarbamate (27). HCl (1 M, 3.9 mL, 3.9 mmol) was added to a stirred solution of silyl ether **26** (0.81 g, 1.9 mmol) and the solution stirred at 20 °C for 15 min. The solution was poured into brine (50 mL), extracted with EtOAc (2 × 100 mL), the combined organic fraction washed with water (50 mL), dried and the solvent evaporated. The residue was purified by chromatography, eluting with a gradient (20–50%) of EtOAc/pet. ether to give **27** (442 mg, 75%) as a colourless solid, mp (EtOAc/pet. ether) 137–140 °C; ^{1}H NMR [$(CD_3)_2SO$]: δ 9.83 (s, 1H, OCONH), 8.26 (ddd, J = 8.7, 2.4, 1.9 Hz, 2H, H-3′, H-5′), 7.69 (d, J = 8.7 Hz, 2H, H-2′, H-6′), 7.42 (d, J = 8.5 Hz, 2H, H-2, H-6), 7.22 (d, J = 8.5 Hz, 2H, H-3, H-5), 5.30 (s, 2H, CH_2O), 5.07 (t, J = 5.7 Hz, 1H, OH), 4.42 (d, J = 5.7 Hz, 2H, CH_2O); ^{13}C NMR [$(CD_3)_2SO$]: δ 153.0 (OCONH), 147.0 (C-4′), 144.5 (C-1′), 137.4 (C-1), 136.7 (C-4), 129.3 (C-3, C-5), 127.0 (C-2′, C-6′), 123.5 (C-3′, C-5′), 117.9 (C-2, C-6), 64.3 (CH_2O), 62.5 (CH_2O). Anal. Calcd for $C_{15}H_{14}N_2O_5$: C, 59.6; H, 4.7; N, 9.3. Found: C, 59.7; H, 4.8; N, 9.3.

4.1.4. 2-Methoxy-4-nitrobenzyl 4-({[*tert*-butyl(dimethyl)-silyl]oxy}methyl)phenylcarbamate (28). Et_3N (0.40 mL, 2.8 mmol) was added to a stirred suspension of carbonate **19** (0.90 g, 2.6 mmol), 4-({[*tert*-butyl(dimethyl)silyl]oxy}methyl)aniline (**25**) (0.64 g, 2.7 mmol), HOBT (0.35 g, 2.6 mmol) and 4 Å molecular sieves (900 mg) in THF (80 mL) and the mixture stirred at 20 °C for 16 h. The solvent was evaporated and the residue partitioned between EtOAc (100 mL) and water (100 mL). The organic fraction was washed with 1 M HCl (2 × 40 mL), water (100 mL) and brine (50 mL), dried and the solvent evaporated. The residue was purified by chromatography, eluting with 20% EtOAc/pet. ether to give **28** (0.89 g, 77%) as a white solid, mp (EtOAc/pet. ether) 120–122 °C; ^{1}H NMR δ 7.84 (dd, J = 8.3, 2.1 Hz, 1H, H-5′), 7.72 (d, J = 2.1 Hz, 1H, H-3′), 7.51 (d, J = 8.3 Hz, 1H, H-6′), 7.35 (d, J = 8.3 Hz, 2H, H-2, H-6), 7.26 (d, J = 8.3 Hz, 2H, H-3, H-5), 6.76 (br s, 1H, OCONH), 5.30 (s, 2H, CH_2O), 4.69 (s, 2H, CH_2O-Si), 3.93 (s, 3H, OCH_3), 0.92 [s, 9H, $SiC(CH_3)_3$], 0.09 [s, 6H, $Si(CH_3)_2$]; ^{13}C NMR δ 157.3 (C-2′), 153.0 (OCONH), 148.7 (C-4′), 137.0 (C-4), 136.4 (C-1), 132.1 (C-1′), 128.7 (C-6′), 126.9 (C-3, C-5), 118.6 (C-2, C-6), 115.7 (C-5′), 105.2 (C-3′), 64.6 (CH_2O), 61.4 (CH_2O), 56.0 (OCH_3), 26.9 [$SiC(CH_3)_3$], 18.4 [$SiC(CH_3)_3$], −5.2 [$Si(CH_3)_2$]. Anal. Calcd for $C_{22}H_{30}N_2O_6Si$: C, 59.2; H, 6.8; N, 6.4. Found: C, 59.0; H, 6.9; N, 6.3.

4.1.5. 2-Methoxy-4-nitrobenzyl 4-(hydroxymethyl)phenylcarbamate (29). HCl (1 M, 4 mL, 4 mmol) was added to a stirred solution of silylether **28** (0.89 g, 0.2 mmol) in MeOH (10 mL) and stirred at 20 °C for 1 h. The solution was poured into brine (50 mL) and extracted with EtOAc (3 × 50 mL). The combined organic fraction was washed with water (50 mL), dried and the solvent evaporated. The residue was purified by chromatography, eluting with a gradient (20–50%) of EtOAc/pet. ether to give **29** (628 mg, 95%) as a white solid, mp (EtOAc/pet. ether) 164–165 °C; ^{1}H NMR [$(CD_3)_2SO$]: δ 9.83 (br s, 1H, OCONH), 7.90 (dd, J = 8.3, 2.1 Hz, 1H, H-5′), 7.80 (d, J = 2.1 Hz, 1H, H-3′), 7.63 (d, J = 8.3 Hz, 1H, H-6′), 7.41 (d, J = 8.4 Hz, 2H, H-2, H-6), 7.22 (d, J = 8.4 Hz, 2H, H-3, H-5), 5.21 (s, 2H, CH_2O), 5.07 (t, J = 5.6 Hz, 1H, OH), 4.41 (t, J = 5.6 Hz, 2H, CH_2O), 3.97 (s, 3H, OCH_3); ^{13}C NMR

[$(CD_3)_2SO$]: δ 157.0 (C-2′), 153.0 (OCONH), 148.2 (C-4′), 137.4 (C-4), 136.7 (C-1), 132.3 (C-1′), 128.8 (C-6′), 127.0 (C-3, C-5), 117.9 (C-2, C-6), 115.5 (C-5′), 105.4 (C-3′), 62.5 (CH_2O), 60.4 (CH_2O), 56.0 (OCH_3). Anal. Calcd for $C_{16}H_{16}N_2O_6$: C, 57.8; H, 4.9; N, 8.4. Found: C, 58.0; H, 4.7; N, 8.5.

4.1.6. (1-Methyl-2-nitro-1*H*-imidazol-5-yl)methyl 4-({[*tert*-butyl(dimethyl)silyl]oxy}methyl)phenylcarbamate (32). Et_3N (0.26 mL, 1.9 mmol) was added to a stirred suspension of (1-methyl-2-nitro-1*H*-imidazol-5-yl)methyl 4-nitrophenyl carbonate (**23**)[49] (0.50 g, 1.6 mmol), 4-({[*tert*-butyl(dimethyl)silyl]oxy}methyl)aniline (**25**) (0.40 g, 1.7 mmol), HOBT (0.21 g, 1.6 mmol) and 4 Å molecular sieves (500 mg) in THF (80 mL) and the mixture stirred at 20 °C for 16 h. The solvent was evaporated and the residue partitioned between EtOAc (100 mL) and water (100 mL). The organic fraction was washed with 1 M HCl (2 × 40 mL), water (100 mL) and brine (50 mL), dried and the solvent evaporated. The residue was purified by chromatography, eluting with 40% EtOAc/pet. ether to give **32** (0.43 mg, 66%) as a white solid, mp (EtOAc/pet. ether) 131–132 °C; ^{1}H NMR δ 7.33 (br d, J = 8.8 Hz, 2H, H-3, H-5), 7.27 (d, J = 8.8 Hz, 2H, H-2, H-6), 7.23 (s, 1H, H-4′), 6.83 (br s, 1H, OCONH), 5.22 (s, 2H, CH_2O), 4.69 (s, 2H, CH_2O), 4.05 (s, 3H, NCH_3), 0.93 [s, 9H, $SiC(CH_3)_3$], 0.09 [s, 6H, $Si(CH_3)_2$]; ^{13}C NMR δ 152.3 (OCONH), 146.1 (C-2′), 137.4 (C-1), 135.8 (C-4), 132.5 (C-5′), 129.6 (C-4′), 126.9 (C-2, C-6), 118.8 (C-3, C-5), 64.5 (CH_2O), 55.4 (CH_2O), 34.3 (NCH_3), 25.9 [$SiC(CH_3)_3$], 18.4 [$SiC(CH_3)_3$], −5.3 [$Si(CH_3)_2$]. Anal. Calcd for $C_{19}H_{28}N_4O_5Si$: C, 54.3; H, 6.7; N, 13.3. Found: C, 54.5; H, 6.6; N, 13.4.

4.1.7. (1-Methyl-2-nitro-1*H*-imidazol-5-yl)methyl 4-(hydroxymethyl)phenylcarbamate (33). HCl (1 M, 2 mL, 2 mmol) was added to a stirred solution of silylether **32** (0.39 g, 0.9 mmol) in MeOH (10 mL) and stirred at 20 °C for 1 h. The solution was poured into brine (50 mL) and extracted with EtOAc (3 × 50 mL). The combined organic fraction was washed with water (50 mL), dried and the solvent evaporated. The residue was purified by chromatography, eluting with a gradient (50–100%) of EtOAc/pet. ether to give **33** (247 mg, 87%) as a pale yellow solid, mp (EtOAc) 180–181 °C; ^{1}H NMR [$(CD_3)_2SO$]: δ 9.77 (br s, 1H, OCONH), 7.40 (d, J = 8.5 Hz, 2H, H-3, H-5), 7.31 (s, 1H, H-4′), 7.22 (d, J = 8.5 Hz, 2H, H-2, H-6), 5.27 (s, 2H, CH_2O), 5.08 (t, J = 5.6 Hz, 1H, OH), 4.42 (d, J = 5.6 Hz, 2H, CH_2O), 3.97 (s, 3H, NCH_3); ^{13}C NMR [$(CD_3)_2SO$]: δ 152.6 (OCONH), 146.0 (C-2′), 137.2 (C-1), 136.8 (C-4), 133.3 (C-5′), 128.7 (C-4′), 127.0 (C-2, C-6), 118.0 (C-3, C-5), 62.4 (CH_2O), 55.0 (CH_2O), 34.2 (NCH_3). Anal. Calcd for $C_{13}H_{14}N_4O_5$: C, 51.0; H, 4.6; N, 18.3. Found: C, 51.0; H, 4.5; N, 18.2.

4.1.8. 1-Methyl-5-nitro-1*H*-imidazol-2-yl 4-({[*tert*-butyl(dimethyl)silyl]oxy}methyl)phenylcarbamate (36). Et_3N (1.10 mL, 7.9 mmol) was added to a stirred suspension of (1-methyl-5-nitro-1*H*-imidazol-2-yl)methyl 4-nitrophenyl carbonate (**35**)[49] (2.31 g, 7.2 mmol), 4-({[*tert*-butyl(dimethyl)silyl]oxy}methyl)aniline (**25**) (1.79 g, 7.9 mmol), HOBT (0.97 g, 7.2 mmol) and 4 Å molecular sieves (2.5 g) in THF (100 mL) and the mixture stirred at 20 °C for 48 h. The solvent was evaporated and the residue partitioned between EtOAc (100 mL) and water (100 mL). The organic fraction was washed with 1 M HCl (2 × 40 mL), water (100 mL) and brine (50 mL), dried and the solvent evaporated. The residue was purified by chromatography, eluting with 50% EtOAc/pet. ether to give **36** (2.57 g, 85%) as a white solid, mp (EtOAc/pet. ether) 145–146 °C; ^{1}H NMR δ 7.99 (s, 1H, H-4′), 7.32 (br d, J = 8.1 Hz, 2H, H-3, H-5), 7.27 (d, J = 8.1 Hz, 2H, H-2, H-6), 6.96 (br s, 1H, OCONH), 5.30 (s, 2H, CH_2O), 4.69 (s, 2H, CH_2O), 4.05 (s, 3H, NCH_3), 0.93 [s, 9H, $SiC(CH_3)_3$], 0.09 [s, 6H, $Si(CH_3)_2$]; ^{13}C NMR δ 152.3 (OCONH), 147.0 (C-5′), 139.6 (C-2′), 137.4 (C-1), 135.8 (C-4′), 129.6 (C-4), 126.9 (C-2, C-6), 118.8 (C-3, C-5), 64.5 (CH_2O), 58.0 (CH_2O), 33.7 (NCH_3), 25.9 [$SiC(CH_3)_3$], 18.4 [$SiC(CH_3)_3$], −5.3 [$Si(CH_3)_2$]. Anal. Calcd for $C_{19}H_{28}N_4O_5Si$: C, 54.3; H, 6.7; N, 13.3. Found: C, 54.5; H, 7.0; N, 13.5.

4.1.9. (1-Methyl-5-nitro-1*H*-imidazol-2-yl)methyl 4-(hydroxymethyl)phenylcarbamate (37). HCl (1 M, 16 mL, 16 mmol) was added to a stirred solution of silylether **36** (1.36 g, 3.2 mmol) in MeOH (50 mL) and stirred at 20 °C for 1 h. The solution was poured into brine (50 mL) and extracted with EtOAc (3 × 50 mL). The combined organic fraction was washed with water (50 mL), dried and the solvent evaporated. The residue was recrystallized to give **37** (0.86 g, 47%) as a white solid, mp (EtOAc/pet. ether) 181–183 °C; ^{1}H NMR [$(CD_3)_2SO$]: δ 9.85 (br s, 1H, OCONH), 8.09 (s, 1H, H-4′), 7.40 (d, J = 8.5 Hz, 2H, H-3, H-5), 7.22 (d, J = 8.5 Hz, 2H, H-2, H-6), 5.29 (s, 2H, CH_2O), 4.42 (s, 2H, CH_2O), 3.96 (s, 3H, NCH_3), 3.79 (br s, 1H, OH); ^{13}C NMR [$(CD_3)_2SO$]: δ 152.5 (OCONH), 147.8 (C-5′), 139.3 (C-2′), 137.4 (C-1), 136.8 (C-4), 131.7 (C-2, C-6), 127.0 (C-3, C-5), 118.0 (C-4), 62.4 (CH_2O), 57.5 (CH_2O), 33.4 (NCH_3). Anal. Calcd for $C_{13}H_{14}N_4O_5$: C, 51.0; H, 4.6; N, 18.3. Found: C, 51.0; H, 4.6; N, 18.4.

4.1.10. Preparation of carbonates. 2-Methoxy-4-nitrobenzyl 4-nitrophenyl carbonate (19). A solution of 4-nitrophenyl chloroformate (1.00 g, 5.0 mmol) in THF (10 mL) was added dropwise to a stirred solution of 2-methoxy-4-nitrobenzyl alcohol (**16**)[24] (617 mg, 3.3 mmol) and DIEA (0.75 mL, 4.3 mmol) in THF (40 mL) at 20 °C and the solution stirred for 16 h. The solution was evaporated and the residue partitioned between EtOAc (100 mL) and water (100 mL). The organic fraction was washed with water (3 × 50 mL) and brine (50 mL), dried and the solvent evaporated. The residue purified by chromatography, eluting with a gradient (20–50%) EtOAc/pet. ether to give **19** (928 mg, 80%) as pale yellow solid, mp (EtOAc/pet. ether) 105–106 °C; ^{1}H NMR δ 8.28 (ddd, J = 9.2, 3.1, 2.1 Hz, 2H, H-3, H-5), 7.89 (dd, J = 8.3, 2.1 Hz, 1H, H-5′), 7.77 (d, J = 2.1 Hz, 1H, H-3′), 7.58 (d, J = 8.3 Hz, 1H, H-6′), 7.40 (ddd, J = 8.3, 3.1, 2.1 Hz, 2H, H-2, H-6), 5.41 (s, 2H, CH_2O), 4.00 (s, 3H, OCH_3); ^{13}C NMR δ 157.6 (C-2′), 155.4 (OCO_2), 152.3 (C-1), 149.2 (C-4′), 145.5 (C-4), 129.8 (C-1′), 129.3 (C-6′), 125.3 (C-2, C-6), 121.7 (C-3, C-5), 115.8 (C-5′), 105.5 (C-3′), 65.3

(CH_2O), 56.2 (OCH_3). Anal. Calcd for $C_{15}H_{12}N_2O_8$: C, 51.7; H, 3.5; N, 8.1. Found: C, 51.8; H, 3.3; N, 7.8.

4.1.11. 4-Nitrophenyl 4-nitro-2-[3-(tetrahydro-2*H*-pyran-2-yloxy)propoxy]benzyl carbonate (20). Similarly, **17** (0.44 g, 1.4 mmol) gave (i) starting material **17** (176 mg, 40%); and (ii) **20** (380 mg, 56%) as a pale yellow oil, ^{1}H NMR δ 8.30 (ddd, J = 9.2, 3.1, 2.1 Hz, 2H, H-3′, H-5′), 7.87 (dd, J = 8.4, 2.1 Hz, 1H, H-5), 7.79 (d, J = 2.1 Hz, 1H, H-3), 7.57 (d, J = 8.4 Hz, 1H, H-6), 7.41 (ddd, J = 9.2, 3.1, 2.1 Hz, 2H, H-2′, H-6′), 5.42 (s, 2H, CH_2O), 4.58–4.61 (m, 1H, OCHO), 4.28 (t, J = 6.3 Hz, 2H, CH_2O), 3.96 (dt, J = 10.0, 6.0 Hz, 1H, CH_2O), 3.78–3.83 (m, 1H, CH_2O), 3.59 (dt, J = 10.0, 6.0 Hz, 1H, CH_2O), 3.45–3.52 (m, 1H, CH_2O), 2.13–2.18 (m, 2H, CH_2O), 1.79–1.86 (m, 1H, CH_2), 1.67–1.76 (m, 1H, CH_2), 1.48–1.60 (m, 4H, $2\times CH_2$); ^{13}C NMR δ 157.0 (C-1′), 155.4 (C-2), 153.4 (OCONH), 149.2 (C-4), 145.5 (C-4′), 129.9 (C-1), 129.2 (C-6), 125.3 (C-3′, C-5′), 121.7 (C-2′, C-6′), 115.6 (C-5), 106.3 (C-6), 99.1 (OCO), 66.1 (CH_2O), 65.3 (CH_2O), 63.5 (CH_2O), 60.4 (CH_2O), 30.6 (CH_2), 29.4 (CH_2), 25.4 (CH_2), 19.7 (CH_2); MS m/z 476 (M^+, 2), 459 (5), 392 (2), 210(30), 85 (100); HRMS calcd for $C_{22}H_{24}N_2O_{10}$ (M^+) m/z 476.1431. Found 476.1425.

4.1.12. 2-(3-Hydroxypropoxy)-4-nitrobenzyl 4-nitrophenyl carbonate (21). A solution of ether **20** (207 mg, 0.5 mmol) in THF (20 mL) and 1 M HCl (5 mL) was stirred at 20 °C for 16 h. The solvent was evaporated and the residue partitioned between EtOAc (50 mL) and water (50 mL). The organic fraction was dried, the solvent evaporated and the residue purified by chromatography, eluting with 50% EtOAc/pet. ether, to give **21** (125 mg, 68%) as a white solid, mp (EtOAc/pet. ether) 116–117 °C; ^{1}H NMR δ 8.29 (ddd, J = 9.1, 3.2, 2.1 Hz, 2H, H-3, H-5), 7.88 (dd, J = 8.3, 2.1 Hz, 1H, H-5′), 7.80 (d, J = 2.1 Hz, 1H, H-3′), 7.58 (d, J = 8.3 Hz, 1H, H-6′), 7.40 (ddd, J = 9.1, 3.2, 2.1 Hz, 2H, H-2, H-6), 5.41 (s, 2H, CH_2O), 4.30 (t, J = 6.0 Hz, 2H, CH_2O), 3.90 (dt, J = 5.4, 4.6 Hz, 2H, CH_2O), 2.10–2.15 (m, 2H, CH_2), 1.65 (br s, 1H, OH); ^{13}C NMR δ 157.0 (C-2′), 155.3 (C-1), 152.3 (OCONH), 149.3 (C-4′), 145.5 (C-4), 129.8 (C-1′), 129.6 (C-6′), 125.4 (C-2, C-6), 121.7 (C-3, C-5), 115.8 (C-5′), 106.4 (C-3′), 66.2 (CH_2O), 65.3 (CH_2O), 59.5 (CH_2O), 31.7 (CH_2). Anal. Calcd for $C_{17}H_{16}N_2O_9$: C, 52.0; H, 4.1; N, 7.2. Found: C, 52.3; H, 4.1; N, 6.9.

4.1.13. 4-({[(4-Nitrobenzyl)oxy]carbonyl}amino)benzyl 4-nitrophenyl carbonate (30). Similarly, reaction of **27** (320 mg, 1.1 mmol) gave **30** (81 mg, 16%) as a white powder, mp (EtOAc/pet. ether) 165–166.5 °C; ^{1}H NMR [$(CD_3)_2SO$]: δ 10.42 (s, 1H, OCONH), 8.32 (ddd, J = 9.0, 3.2, 2.3 Hz, 2H, H-3, H-5), 8.28 (br d, J = 8.8 Hz, 2H, H-3″, H-5″), 7.70 (br d, J = 8.8 Hz, 2H, H-2″, H-6″), 7.58 (ddd, J = 9.0, 3.2, 2.3 Hz, 2H, H-2, H-6), 7.54 (br d, J = 8.5 Hz, 2H, H-3′, H-5′), 7.41 (br d, J = 8.5 Hz, 2H, H-2′, H-6′), 5.32 (s, 2H, CH_2O), 5.24 (s, 2H, CH_2O); ^{13}C NMR [$(CD_3)_2SO$]: δ 155.2 (OCO_2), 153.0 (OCONH), 151.9 (C-1), 147.0 (C-4″), 145.1 (C-4), 144.4 (C-1″), 139.3 (C-4′), 129.5 (C-2′, C-6′), 128.6 (C-1′), 128.4 (C-2, C-6), 125.3 (C-2″, C-6″), 123.5 (C-3″, C-5″), 122.5 (C-3, C-5), 118.1 (C-3′, C-5′), 70.2 (CH_2O), 64.5 (CH_2O). Anal. Calcd for $C_{22}H_{17}N_3O_9$: C, 56.5; H, 3.7; N, 9.0. Found: C, 56.7; H, 3.7; N, 8.8.

4.1.14. 4-({[(2-Methoxy-4-nitrobenzyl)oxy]carbonyl}amino)benzyl 4-nitrophenyl carbonate (31). Similarly, reaction of **29** (282 mg, 0.9 mmol) gave **31** (238 mg, 56%) as a white powder, mp (EtOAc/DCM) 144–146 °C; ^{1}H NMR [$(CD_3)_2SO$]: δ 10.01 (s, 1H, OCONH), 8.31 (ddd, J = 9.1, 3.4, 2.2 Hz, 2H, H-3, H-5), 7.91 (dd, J = 8.3, 2.2 Hz, 1H, H-5″), 7.81 (d, J = 2.2 Hz, 1H, H-3″), 7.64 (d, J = 8.3 Hz, 1H, H-6″), 7.56 (ddd, J = 9.1, 3.4, 2.2 Hz, 2H, H-2, H-6), 7.53 (br d, J = 8.6 Hz, 2H, H-3′, H-5′), 7.41 (br d, J = 8.6 Hz, 2H, H-2′, H-6′), 5.24 (s, 4H, $2\times CH_2O$), 3.98 (s, 3H, OCH_3); ^{13}C NMR [$(CD_3)_2SO$]: δ 157.0 (C-2″), 155.2 (OCO_2), 153.0 (OCONH), 151.9 (C-1), 148.2 (C-4″), 145.1 (C-4), 139.4 (C-1), 132.2 (C-1′), 129.6 (C-2′, C-6′), 128.9 (C-6″), 128.5 (C-4′), 125.3 (C-2, C-6), 122.6 (C-3, C-5), 118.0 (C-3′, C-5′), 115.5 (C-5″), 105.5 (C-3″), 70.2 (CH_2O), 60.5 (CH_2O), 56.2 (OCH_3); MS (FAB^+) m/z 498 (MH^+, 0.5); HRMS (FAB^+) calcd for $C_{23}H_{20}N_3O_{10}$ (MH^+) m/z 498.1149. Found 498.1151. Anal. Calcd for $C_{23}H_{19}N_3O_{10}$: C, 55.5; H, 3.9; N, 8.5. Found: C, 55.3; H, 3.7; N, 8.5.

4.1.15. 4-({[(1-Methyl-2-nitro-1*H*-imidazol-5-yl)methoxy]carbonyl}amino)benzyl 4-nitrophenyl carbonate (34). Similarly, reaction of **33** (219 mg, 0.7 mmol) gave **34** (62 mg, 18%) as a white solid, ^{1}H NMR [$(CD_3)_2SO$]: δ 9.96 (s, 1H, OCONH), 8.31 (ddd J = 9.2, 3.3, 2.2 Hz, 2H, H-3, H-5), 7.56 (ddd, J = 9.2, 3.3, 2.2 Hz, 2H, H-2, H-6), 7.51 (d, J = 8.6 Hz, 2H, H-3′, H-5′), 7.40 (d, J = 8.6 Hz, 2H, H-2′, H-6′), 7.31 (s, 1H, H-4″), 5.33 (s, 2H, CH_2O), 5.24 (s, 2H, CH_2O), 3.98 (s, 3H, NCH_3); ^{13}C NMR [$(CD_3)_2SO$]: δ 155.2 (OCO_2), 152.6 (OCONH), 151.9 (C-1), 145.1 (C-2″), 139.2 (C-1′), 133.2 (C-4′), 129.6 (C-3, C-5), 128.8 (C-4″), 128.7 (C-5″), 125.6 (C-2′, C-6′), 122.2 (C-2, C-6), 118.8 (C-2′, C-6′), 70.2 (CH_2O), 55.2 (CH_2O), 34.2 (NCH_3); MS (FAB^+) m/z 472 (MH^+, 1), 443 (0.5); HRMS (FAB^+) calcd for $C_{20}H_{18}N_5O_9$ (MH^+) m/z 472.1105. Found 472.1106.

4.1.16. 4-({[(1-Methyl-5-nitro-1*H*-imidazol-2-yl)methoxy]carbonyl}amino)benzyl 4-nitrophenyl carbonate (38). Similarly, reaction of **37** (0.73 g, 2.4 mmol) gave **38** (0.71 g, 63%) as a white solid, ^{1}H NMR [$(CD_3)_2SO$]: δ 10.02 (s, 1H, OCONH), 8.31 (ddd J = 9.1, 3.3, 2.1 Hz, 2H, H-3, H-5), 8.08 (s, 1H, H-4″), 7.57 (ddd, J = 9.1, 3.3, 2.1 Hz, 2H, H-2, H-6), 7.51 (d, J = 8.5 Hz, 2H, H-3′, H-5′), 7.40 (d, J = 8.5 Hz, 2H, H-2′, H-6′), 5.32 (s, 2H, CH_2O), 4.70 (s, 2H, CH_2O), 3.97 (s, 3H, NCH_3); MS (FAB^+) m/z 472 (MH^+, 1.5); HRMS (FAB^+) calcd for $C_{20}H_{18}N_5O_9$ (MH^+) m/z 472.1105. Found 472.1108.

4.1.17. Preparation of carbamates (7–14). 4-Nitrobenzyl doxorubicincarbamate (7). A solution of 4-nitrobenzyl 4-nitrophenyl carbonate (**18**) (32 mg, 103 μmol), prepared[26] from 4-nitrobenzyl alcohol (**15**) and 4-nitrophenyl chloroformate, in DMF (2 mL) was added to a solution of doxorubicin (**6**) (46 mg, 86 μmol) and Et_3N

(15 μL, 104 μmol) in DMF (5 mL) at 20 °C and the solution stirred for 16 h. The solvent was evaporated and the residue purified by chromatography, eluting with a gradient (0–5%) of MeOH/DCM, to give **7** (55 mg, 88%) as a red solid, mp (DCM) 153–158 °C; ^{1}H NMR δ 13.95 (s, 1H, 6-OH), 13.19 (s, 1H, 11-OH), 8.15 (d, J = 8.5 Hz, 2H, H-3″), 8.01 (dd, J = 7.7, 0.7 Hz, 1H, H-1), 7.78 (dd, J = 8.0, 7.7 Hz, 1H, H-2), 7.45 (d, J = 8.5 Hz, 2H, H-2″), 7.39 (dd, J = 8.0, 0.7 Hz, 1H, H-3), 5.50 (d, J = 3.5 Hz, 1H, H-1′), 5.30 (d, J = 8.7 Hz, 1H, OCONH), 5.28 (br s, 1H, H-7), 5.14 (d, J = 13.3 Hz, 1H, CH_2O), 5.09 (d, J = 13.3 Hz, 1H, CH_2O), 4.75 (s, 2H, H-14), 4.51 (s, 1H, 9-OH), 4.12–4.17 (m, 1H, H-5′), 4.08 (s, 3H, 4-OCH_3), 3.83–3.09 (m, 1H, H-3′), 3.67 (br s, 1H, H-4′), 3.24 (d, J = 18.7 Hz, 1H, H-10), 3.04 (br s, 1H, 14-OH), 2.95 (d, J = 18.7 Hz, 1H, H-10), 2.34 (d, J = 15.0 Hz, 1H, H-8), 2.17 (dd, J = 15.0, 4.0 Hz, 1H, H-8), 2.13 (br d, J = 7.2 Hz, 1H, 4′-OH), 1.89 (dd, J = 13.3, 4.9 Hz, 1H, H-2′), 1.79 (dt, J = 13.3, 3.8 Hz, 1H, H-2′), 1.29 (d, J = 6.6 Hz, 3H, 6′-CH_3); ^{13}C NMR δ 213.7 (C-13), 187.1 (C-5), 186.6 (C-12), 161.0 (C-4), 156.1 (C-6), 155.5 (C-11), 155.0 (OCONH), 147.5 (C-4″), 143.8 (C-1″), 135.8 (C-2), 135.4 (C-12a), 133.5 (C-6a), 13.4 (C-10a), 128.1 (C-2″, C-6″), 123.7 (C-3″, C-5″), 120.7 (C-4a), 120.0 (C-1), 118.5 (C-3), 111.6 (C-5a), 111.4 (C-11a), 100.7 (C-1′), 76.6 (C-9), 69.8 (C-7), 69.5 (C-4′), 67.2 (C-5′), 65.4 (C-14), 65.2 (CH_2O), 56.6 (4-OCH_3), 47.1 (C-3′), 35.6 (C-8), 33.9 (C-10), 30.2 (C-2′), 16.8 (6′-CH_3); MS (FAB$^+$) m/z 723 (MH^+, 0.2); HRMS (FAB$^+$) calcd for $C_{35}H_{35}N_2O_{15}$ (MH^+) m/z 723.2037. Found 723.2039. Anal. Calcd for $C_{35}H_{34}N_2O_{15}$: C, 58.2; H, 4.7; N, 3.9. Found: C, 57.8; H, 4.9; N, 3.8.

4.1.18. 2-Methoxy-4-nitrobenzyl doxorubicincarbamate (8). Similarly, reaction of **19** (23 mg, 66 μmol) and **6** (30 mg, 55 μmol) gave **8** (37 mg, 88%) as a red solid, mp (DCM) 159–161 °C; ^{1}H NMR δ 13.97 (s, 1H, 6-OH), 13.22 (s, 1H, 11-OH), 8.02 (dd, J = 8.0, 1.0 Hz, 1H, H-1), 7.77–7.81 (m, 2H, H-2, H-5″), 7.66 (br s, 1H, H-3″), 7.41 (d, J = 8.0 Hz, 1H, H-6″), 7.39 (dd, J = 8.0, 1.0 Hz, 1H, H-3), 5.52 (br d, J = 3.3 Hz, 1H, H-1′), 5.29 (br s, 1H, H-7), 5.25 (d, J = 8.7 Hz, 1H, OCONH), 5.13 (2 d, J = 14.0 Hz, 2H, CH_2O), 4.75 (s, 2H, H-14), 4.51 (s, 1H, 9-OH), 4.13–4.17 (m, 1H, H-5′), 4.08 (s, 3H, 4-OCH_3), 3.90 (s, 3H, 2″-OCH_3), 3.84–3.88 (m, 1H, H-3′), 3.69 (s, 1H, H-4′), 3.24 (dd, J = 18.9, 1.3 Hz, 1H, H-10), 3.03 (s, 1H, 14-OH), 3.01 (d, J = 18.9 Hz, 1H, H-10), 2.34 (br d, J = 14.7 Hz, 1H, H-8), 2.18 (dd, J = 14.7, 4.0 Hz, 1H, H-8), 2.02 (br s, 1H, 4′-OH), 1.90 (dd, J = 13.2, 4.7 Hz, 1H, H-2′), 1.79 (dd, J = 13.2, 3.3 Hz, 1H, H-2′), 1.30 (d, J = 6.5 Hz, 3H, H-6′); ^{13}C NMR δ 213.7 (C-13), 187.1 (C-5), 186.7 (C-12), 161.0 (C-4), 157.0 (C-2″), 156.1 (C-6), 155.6 (C-11), 155.2 (OCONH), 148.5 (C-4″), 135.8 (C-2), 135.5 (C-12a), 133.5 (C-6a), 133.4 (C-10a), 132.5 (C-1″), 128.4 (C-6″), 120.8 (C-4a), 119.9 (C-1), 118.5 (C-3), 115.7 (C-5″), 111.6 (C-5a), 111.4 (C-11a), 105.1 (C-3″), 100.7 (C-1′), 76.6 (C-9), 69.8 (C-7), 69.6 (C-4′), 67.2 (C-5′), 65.5 (C-14), 61.1 (CH_2O), 56.7 (4-OCH_3), 56.0 (2′-OCH_3), 47.1 (C-3′), 35.6 (C-8), 34.0 (C-10), 30.2 (C-2), 16.8 (C-6′); MS (FAB$^+$) m/z 753 (MH^+, 0.3); HRMS (FAB$^+$) calcd for $C_{36}H_{37}N_2O_{16}$ (MH^+) m/z 753.2143. Found 753.2100. Anal. Calcd for $C_{36}H_{36}N_2O_{16}$: C, 57.4; H, 4.8; N, 3.7. Found: C, 57.2; H, 5.1; N, 3.9.

4.1.19. 2-(3-Hydroxypropoxy)-4-nitrobenzyl doxorubicincarbamate (9). Similarly, reaction of **21** (41 mg, 104 μmol) and **6** (46 mg, 86 μmol) gave **9** (69 mg, 84%) as a red solid, mp (DCM) 154–160 °C; ^{1}H NMR [$(CD_3)_2SO$]: δ 14.00 (s, 1H, 6-OH), 13.24 (s, 1H, 11-OH), 7.85–7.89 (m, 2H, H-1, H-3), 7.80 (dd, J = 8.3, 1.8 Hz, 1H, H-5″), 7.71 (d, J = 1.8 Hz, 1H, H-3″), 7.62 (dd, J = 6.6, 2.8 Hz, 1H, H-2), 7.50 (d, J = 8.3 Hz, 1H, H-6″), 7.07 (d, J = 8.0 Hz, 1H, OCONH), 5.42 (s, 1H, 9-OH), 5.14 (br s, 1H, H-1′), 5.05 (d, J = 18.4 Hz, 1H, CH_2O), 4.99 (d, J = 18.4 Hz, 1H, CH_2O), 4.92 (br s, 1H, H-7), 4.85 (t, J = 6.0 Hz, 1H, 14-OH), 4.74 (d, J = 5.8 Hz, 1H, 4′-OH), 4.58 (d, J = 6.0 Hz, 2H, H-14), 4.55 (t, J = 5.3 Hz, 1H, H-5′), 4.14–4.20 (m, 2H, CH_2O), 3.97 (s, 3H, 4-OCH_3), 3.69–3.76 (m, 1H, H-3′), 3.54 (dt, J = 6.0, 5.7 Hz, 2H, CH_2O), 3.48 (br s, 1H, H-4′), 3.30 (br s, 1H, OH), 2.98 (d, J = 18.2 Hz, 1H, H-10), 2.90 (d, J = 18.2 Hz, 1H, H-10), 2.22 (br d, J = 14.4 Hz, 1H, H-8), 2.09 (dd, J = 14.4, 5.5 Hz, 1H, H-8), 1.88–1.92 (m, 1H, H-2′), 1.82–1.87 (m, 2H, CH_2), 1.50 (dd, J = 12.4, 3.7 Hz, 1H, H-2′), 1.13 (d, J = 6.4 Hz, 3H, H-6′); ^{13}C NMR [$(CD_3)_2SO$]: δ 213.7 (C-13), 186.4 (C-5), 186.3 (C-12), 160.7 (C-4), 156.0 (C-2″), 155.9 (C-6), 154.9 (C-11), 154.4 (OCONH), 147.8 (C-4″), 136.1 (C-2), 135.4 (C-12a), 134.5 (C-6a), 134.0 (C-10a), 133.2 (C-1″), 127.8 (C-6″), 119.9 (C-4a), 119.6 (C-1), 118.9 (C-3), 115.3 (C-5″), 110.6 (C-5a), 110.5 (C-11a), 105.8 (C-3″), 100.2 (C-1′), 74.8 (C-9), 69.8 (C-7), 67.9 (C-4′), 66.6 (C-5′), 65.6 (CH_2O), 63.6 (C-14), 59.8 (CH_2O), 57.0 (CH_2O), 56.5 (4-OCH_3), 47.2 (C-3′), 36.5 (C-8), 32.0 (C-10), 31.7 (CH_2), 29.7 (C-2′), 16.9 (C-6′); MS (FAB$^+$) m/z 797 (MH^+, 0.3); HRMS (FAB$^+$) calcd for $C_{38}H_{40}N_2O_{17}$ (MH^+) m/z 797.2405. Found 797.2953. Anal. Calcd for $C_{38}H_{40}N_2O_{17}\cdot\frac{1}{2}H_2O$: C, 56.6; H, 5.1; N, 3.5. Found: C, 56.7; H, 5.1; N, 3.5.

4.1.20. (1-Methyl-2-nitro-1*H*-imidazol-5-yl)methyl doxorubicincarbamate (10). Similarly, reaction of **23**[49] (33 mg, 104 μmol) and **6** (46 mg, 86 μmol) gave **10** (44 mg, 70%) as a red solid, mp (DCM) 162–166 °C; ^{1}H NMR [$(CD_3)_2SO$]: δ 13.96 (s, 1H, 6-OH), 13.21 (s, 1H, 11-OH), 7.82–7.87 (m, 2H, H-1, H-3), 7.58 (dd, J = 7.5, 2.1 Hz, 1H, H-2), 7.18 (s, 1H, H-4″), 7.02 (d, J = 7.9 Hz, 1H, OCONH), 5.42 (s, 1H, 9-OH), 5.21 (d, J = 2.6 Hz, 1H, H-1′), 5.07 (s, 2H, CH_2O), 4.86–4.91 (m, 2H, H-7, 14-OH), 4.73 (d, J = 5.9 Hz, 1H, 4-OH), 4.58 (d, J = 5.9 Hz, 2H, H-14), 4.13–4.17 (m, 1H, H-5′), 3.96 (s, 3H, 4-OCH_3), 3.88 (s, 3H, NCH_3), 3.66–3.74 (m, 1H, H-3′), 3.41–3.46 (m, 1H, H-4′), 2.97 (d, J = 18.3 Hz, 1H, H-10), 2.87 (d, J = 18.3 Hz, 1H, H-10), 2.21 (d, J = 14.0 Hz, 1H, H-8), 2.17 (dd, J = 14.0, 5.4 Hz, 1H, H-8), 1.84 (dt, J = 12.8, 3.5 Hz, 1H, H-2′), 1.57 (dd, J = 12.8, 3.8 Hz, 1H, H-2′), 1.12 (d, J = 6.4 Hz, 3H, H-6′); ^{13}C NMR [$(CD_3)_2SO$]: δ 213.7 (C-13), 186.3 (C-5), 186.2 (C-12), 160.7 (C-4), 156.0 (C-6), 154.6 (C-11), 154.4 (OCONH), 145.8 (C-2″), 136.1 (C-2), 135.4 (C-12a), 134.5 (C-6a), 134.0 (C-10a), 133.8 (C-5″), 128.3 (C-4″), 119.8 (C-4a), 119.6 (C-1),

118.9 (C-3), 110.6 (C-5a), 110.5 (C-11a), 100.3 (C-1′), 74.9 (C-9), 69.8 (C-7), 67.8 (C-4′), 66.6 (C-5′), 63.7 (C-14), 56.5 (4-OCH_3), 54.7 (CH_2O), 47.3 (C-3′), 38.4 (C-8), 34.1 (NCH_3), 32.0 (C-10), 29.7 (C-2′), 17.0 (C-6′); MS (FAB^+) *m/z* 727 (MH^+, 0.2); HRMS (FAB^+) calcd for $C_{33}H_{35}N_4O_{15}$ (MH^+) *m/z* 727.2099. Found 727.2075. Anal. Calcd for $C_{33}H_{34}N_4O_{15}\cdot\frac{1}{2}H_2O$: C, 53.9; H, 4.8; N, 7.6. Found: C, 53.7; H, 4.8; N, 7.3.

4.1.21. 4-({[(4-Nitrobenzyl)oxy]carbonyl}amino)benzyl doxorubicincarbamate (11). Similarly, reaction of **30** (48 mg, 104 μmol) and **6** (46 mg, 86 μmol) gave **11** (61 mg, 81%) as a red solid, mp (DCM) 160–166 °C; ^1H NMR [$(CD_3)_2SO$]: δ 13.98 (s, 1H, 6-OH), 13.22 (s, 1H, 11-OH), 9.88 (br s, 1H, OCONH), 8.24 (d, *J* = 8.7 Hz, 2H, H-3‴, H-5‴), 7.82–7.86 (m, 2H, H-1, H-3), 7.66 (d, *J* = 8.7 Hz, 2H, H-2‴, H-6‴), 7.58 (dd, *J* = 6.6, 2.9 Hz, 1H, H-2), 7.40 (d, *J* = 8.4 Hz, 2H, H-3″, H-5″), 7.22 (d, *J* = 8.4 Hz, 2H, H-2″, H-6″), 6.81 (d, *J* = 7.9 Hz, 1H, OCONH), 5.42 (s, 1H, 9-OH), 5.27 (s, 2H, CH_2O), 5.21 (d, *J* = 2.5 Hz, 1H, H-1′), 4.89–4.92 (m, 1H, H-7), 4.87 (s, 2H, CH_2O), 4.85 (br s, 1H, 14-OH), 4.70 (d, *J* = 5.7 Hz, 1H, 4′-OH), 4.59 (d, *J* = 6.0 Hz, 2H, H-14), 4.13–4.17 (m, 1H, H-5′), 3.95 (s, 3H, 4-OCH_3), 3.68–3.78 (m, 1H, H-3′), 3.45 (br s, 1H, H-4′), 2.98 (d, *J* = 18.2 Hz, 1H, H-10), 2.90 (d, *J* = 18.2 Hz, 1H, H-10), 2.21 (br d, *J* = 12.8 Hz, 1H, H-8), 2.08–2.12 (m, 1H, H-8), 1.84 (dt, *J* = 12.8, 3.5 Hz, 1H, H-2′), 1.47 (dd, *J* = 12.8, 4.5 Hz, 1H, H-2′), 1.14 (d, *J* = 6.4 Hz, 3H, 6′-H); ^{13}C NMR [$(CD_3)_2SO$]: δ 213.7 (C-13), 186.4 (C-5), 186.2 (C-12), 160.7 (C-4), 156.0 (C-6), 155.2 (C-11), 154.5 (OCONH), 153.0 (OCONH), 147.0 (C-4‴), 144.5 (C-1‴). 138.4 (C-4″), 136.1 (C-2), 135.5 (C-12a), 134.5 (C-6a), 131.1 (C-10a), 128.6 (C-2″, C-6″), 128.4 (C-2‴, C-6‴), 128.2 (C-1″), 123.5 (C-3‴, C-5‴), 119.8 (C-4a), 119.6 (C-1), 118.7 (C-3), 118.0 (C-3″, C-5″), 110.7 (C-5a), 110.5 (C-11a), 100.2 (C-1′), 74.9 (C-9), 69.8 (C-7), 67.9 (C-4′), 66.6 (C-5′), 64.9 (CH_2O), 64.4 (CH_2O), 63.6 (C-14), 56.5 (4-OCH_3), 47.0 (C-3′), 36.5 (C-8), 32.0 (C-10), 29.8 (C-2′), 16.9 (C-6′); MS (FAB^+) *m/z* 872 (MH^+, 0.25); HRMS (FAB^+) calcd for $C_{43}H_{42}N_3O_{17}$ (MH^+) *m/z* 872.2514. Found 872.2499. Anal. Calcd for $C_{43}H_{41}N_3O_{17}\cdot\frac{1}{2}H_2O$: C, 58.6; H, 4.8; N, 4.8. Found: C, 58.6; H, 5.1; N, 4.6.

4.1.22. 4-({[(2-Methoxy-4-nitrobenzyl)oxy]carbonyl}amino)benzyl doxorubicincarbamate (12). Similarly, reaction of **31** (52 mg, 103 μmol) and **6** (45 mg, 86 μmol) gave **12** (61 mg, 80%) as a red solid, mp (DCM) 128–131 °C; ^1H NMR [$(CD_3)_2SO$]: δ 14.01 (s, 1H, 6-OH), 13.25 (s, 1H, 11-OH), 9.88 (s, 1H, OCONH), 7.87–7.90 (m, 3H, H-1, H-2, H-5‴), 7.79 (d, *J* = 2.2 Hz, 1H, H-3‴), 7.59–7.63 (m, 2H, H-3, H-6‴), 7.41 (d, *J* = 8.3 Hz, 2H, H-3″, H-5″), 7.22 (d, *J* = 8.3 Hz, 2H, H-2″, H-6″), 6.81 (d, *J* = 8.0 Hz, 1H, OCONH), 5.44 (s, 1H, H-7), 5.21 (d, *J* = 3.0 Hz, 1H, H-1′), 5.19 (s, 2H, CH_2O), 4.91–4.94 (m, 1H, 9-OH), 4.87 (s, 2H, CH_2O), 4.83 (dd, *J* = 6.3, 5.9 Hz, 1H, 14-OH), 4.69 (d, *J* = 5.7 Hz, 1H, 4-OH), 4.58 (d, *J* = 6.0 Hz, 2H, H-14), 4.12–4.18 (m, 1H, H-5′), 3.97 (s, 3H, OCH_3), 3.95 (s, 3H, OCH_3), 3.68–3.75 (m, 1H, H-3′), 3.43–3.47 (m, 1H, H-4′), 2.99 (d, *J* = 18.4 Hz, 1H, H-10), 2.92 (d, *J* = 18.4 Hz, 1H, H-10), 2.20 (br d, *J* = 14.1 Hz, 1H, H-8), 2.12 (dd, *J* = 14.1 Hz, 1H, H-8), 1.85 (dt, *J* = 12.8, 3.7 Hz, 1H, H-2′), 1.47 (dd, *J* = 12.8, 4.1 Hz, 1H, H-2′), 1.13 (d, *J* = 6.5 Hz, 3H, H-6′); ^{13}C NMR [$(CD_3)_2SO$]: δ 213.7 (C-13), 186.4 (C-5), 186.3 (C-12), 160.7 (C-4), 157.0 (C-2‴), 156.0 (C-6), 155.2 (C-11), 154.4 (OCONH), 152.9 (OCONH), 148.2 (C-4‴), 138.4 (C-4″), 136.1 (C-2), 135.4 (C-12a), 134.6 (C-6a), 134.0 (C-10a), 132.2 (C-1″), 131.0 (C-1‴), 128.9 (C-2‴), 128.6 (C-2″, C-6″), 119.9 (C-4a), 119.6 (C-1), 118.9 (C-3), 117.9 (C-3″, C-5″), 115.4 (C-5‴), 110.7 (C-5a), 110.6 (C-11a), 105.4 (C-3‴), 100.2 (C-1′), 74.9 (C-9), 69.8 (C-7), 67.9 (C-4′), 66.6 (C-5′), 64.8 (C-14), 63.6 (CH_2O), 60.4 (CH_2O), 56.5 (OCH_3), 56.2 (OCH_3), 47.0 (C-3′), 36.5 (C-8), 32.0 (C-10), 29.7 (C-2′), 16.9 (C-6′); MS (FAB^+) *m/z* 902 (MH^+, 0.2). Anal. Calcd for $C_{44}H_{43}N_3O_{18}\cdot H_2O$: C, 57.5; H, 4.9; N, 4.6. Found: C, 57.4; H, 5.1; N, 5.6.

4.1.23. 4-({[(1-Methyl-2-nitro-1*H*-imidazol-5-yl)methoxy]carbonyl}amino)benzyl doxorubicincarbamate (13). Similarly, reaction of **34** (81 mg, 172 μmol) and **6** (45 mg, 86 μmol) gave **13** (57 mg, 75%) as a red solid, mp (DCM) 160–162 °C; ^1H NMR [$(CD_3)_2SO$]: δ 13.99 (s, 1H, 6-OH), 13.24 (s, 1H, 11-OH), 9.82 (s, 1H, OCONH), 7.84–7.89 (m, 2H, H-1, H-2), 7.60–7.63 (m, 1H, H-3), 7.40 (d, *J* = 8.3 Hz, 2H, H-3″, H-5″), 7.29 (s, 1H, H-4‴), 7.23 (d, *J* = 8.3 Hz, 2H, H-2″, H-6″), 6.81 (d, *J* = 8.0 Hz, 1H, OCONH), 5.43 (s, 1H, H-7), 5.25 (s, 2H, CH_2O), 5.21 (d, *J* = 2.9 Hz, 1H, H-1′), 4.89–4.91 (m, 1H, 9-OH), 4.87 (s, 2H, CH_2O), 4.84 (dd, *J* = 6.3, 5.8 Hz, 1H, 14-OH), 4.69 (d, *J* = 5.7 Hz, 1H, 4-OH), 4.58 (d, *J* = 6.0 Hz, 2H, H-14), 4.13–4.17 (m, 1H, H-5′), 3.97 (s, 3H, OCH_3), 3.95 (s, 3H, NCH_3), 3.68–3.75 (m, 1H, H-3′), 3.43–3.46 (m, 1H, H-4′), 2.99 (d, *J* = 18.3 Hz, 1H, H-10), 2.91 (d, *J* = 18.3 Hz, 1H, H-10), 2.21 (br d, *J* = 14.1 Hz, 1H, H-8), 2.10 (dd, *J* = 14.1 Hz, 1H, H-8), 1.84 (dt, *J* = 12.9, 3.6 Hz, 1H, H-2′), 1.47 (dd, *J* = 12.9, 3.8 Hz, 1H, H-2′), 1.13 (d, *J* = 6.5 Hz, 3H, H-6′); ^{13}C NMR [$(CD_3)_2SO$]: δ 213.7 (C-13), 186.4 (C-5), 186.3 (C-12), 160.7 (C-4), 156.0 (C-6), 155.2 (C-11), 154.4 (OCONH), 152.6 (OCONH), 146.0 (C-2‴), 138.2 (C-4″), 136.1 (C-2), 135.4 (C-12a), 134.6 (C-6a), 134.0 (C-10a), 13.2 (C-5‴), 131.2 (C-1″), 128.7 (C-4‴), 128.6 (C-2″, C-6″), 119.9 (C-4a), 119.6 (C-1), 118.9 (C-3), 118.0 (C-3″, C-5″), 110.7 (C-5a), 110.5 (C-11a), 100.2 (C-1′), 74.9 (C-9), 69.8 (C-7), 67.9 (C-4′), 66.6 (C-5′), 64.8 (C-14), 63.6 (CH_2O), 56.5 (OCH_3), 55.1 (CH_2O), 47.0 (C-3′), 36.5 (C-8), 34.1 (NCH_3), 32.0 (C-10), 29.8 (C-2′), 16.9 (C-6′); MS (FAB^+) *m/z* 876 (MH^+, 0.2). Anal. Calcd for $C_{41}H_{41}N_5O_{17}\cdot H_2O$: C, 55.1; H, 4.9; N, 7.8. Found: C, 54.7; H, 4.9; N, 7.5.

4.1.24. 4-({[(1-Methyl-5-nitro-1*H*-imidazol-2-yl)methoxy]carbonyl}amino)benzyl doxorubicincarbamate (14). Similarly, reaction of **38** (61 mg, 129 μmol) and **6** (45 mg, 86 μmol) gave **14** (50 mg, 66%) as a red solid, mp (DCM) 170–173 °C; ^1H NMR [$(CD_3)_2SO$]: δ 14.00 (s, 1H, 6-OH), 13.24 (s, 1H, 11-OH), 9.91 (s, 1H, OCONH), 8.07 (s, 1H, H-4‴), 7.86–7.90 (m, 2H, H-1, H-2), 7.60–7.63 (m, 1H, H-3), 7.40 (d, *J* = 8.4 Hz, 2H, H-3″, H-5″), 7.23 (d, *J* = 8.4 Hz, 2H, H-2″, H-6″), 6.81 (d, *J* = 8.0 Hz, 1H, OCONH), 5.43 (s, 1H, H-7), 5.27

(s, 2H, CH_2O), 5.21 (d, $J = 2.9$ Hz, 1H, H-1′), 4.92–4.95 (m, 1H, 9-OH), 4.87 (s, 2H, CH_2O), 4.84 (dd, $J = 6.3$, 5.9 Hz, 1H, 14-OH), 4.69 (d, $J = 5.7$ Hz, 1H, 4-OH), 4.58 (d, $J = 6.0$ Hz, 2H, H-14), 4.12–4.18 (m, 1H, H-5′), 3.97 (s, 3H, OCH_3), 3.95 (s, 3H, NCH_3), 3.68–3.75 (m, 1H, H-3′), 3.43–3.46 (m, 1H, H-4′), 2.98 (d, $J = 18.3$ Hz, 1H, H-10), 2.91 (d, $J = 18.3$ Hz, 1H, H-10), 2.20 (br d, $J = 14.1$ Hz, 1H, H-8), 2.11 (dd, $J = 14.1$ Hz, 1H, H-8), 1.84 (dt, $J = 12.9$, 3.7 Hz, 1H, H-2′), 1.47 (dd, $J = 12.9$, 4.0 Hz, 1H, H-2′), 1.12 (d, $J = 6.5$ Hz, 3H, H-6′); ^{13}C NMR [$(CD_3)_2SO$]: δ 213.7 (C-13), 186.4 (C-5), 186.3 (C-12), 160.7 (C-4), 156.0 (C-6), 155.2 (C-11), 154.4 (OCONH), 152.4 (OCONH), 147.8 (C-5‴), 139.3 (C-2‴), 138.2 (C-4″), 136.1 (C-2), 135.4 (C-12a), 134.6 (C-6a), 134.0 (C-10a), 131.7 (C-4‴), 131.2 (C-1″), 128.6 (C-2″, C-6″), 119.9 (C-4a), 119.6 (C-1), 118.9 (C-3), 118.0 (C-3″, C-5″), 110.7 (C-5a), 110.5 (C-11a), 100.2 (C-1′), 74.9 (C-9), 69.8 (C-7), 67.9 (C-4′), 66.6 (C-5′), 64.8 (C-14), 63.6 (CH_2O), 57.6 (CH_2O), 56.5 (OCH_3), 47.0 (C-3′), 36.5 (C-8), 33.4 (NCH_3), 32.0 (C-10), 29.7 (C-2′), 16.9 (C-6′); MS (FAB^+) *m/z* 876 (MH^+, 0.6%); HRMS (FAB^+) calcd for $C_{41}H_{42}N_5O_{17}$ (MH^+) *m/z* 876.2576. Found 876.2573. Anal. Calcd for $C_{41}H_{41}N_5O_{17}\cdot H_2O$: C, 55.1; H, 4.9; N, 7.8. Found: C, 55.2; H, 4.9; N, 7.9.

4.2. Biological testing

4.2.1. Cell lines. Four pairs of cell lines, each comprising a tumour cell line and corresponding transfectant stably expressing NTR, were grown as monolayers in αMEM containing 5% fetal bovine serum. V79–NTR^{puro}, also known as T79-A3, is a Chinese hamster fibroblast that expresses NTR from an CMV promoter; the corresponding NTR−ve line here referred to as V79^{puro} has been transfected with the empty shuttle vector and is also known as T78-1.[20] SKOV–NTR^{neo} and WiDr–NTR^{neo}, also known as SC3.2 and WC14, respectively, are human ovarian and colon carcinoma lines derived from SKOV3 and WiDr, and also express NTR from a CMV promoter.[56] EMT6–NTR^{puro}, also known as EN2A, is a murine breast carcinoma line derived from EMT6 and expresses NTR from a bicistronic cassette with an EF-1α promoter.[51] Selection for NTR expression was maintained during passage, but not during experiments, using 15 μM puromycin (V79–NTR^{puro}), 5 μM puromycin (EMT6–NTR^{puro}) or 300 μg/mL G418 (WiDr–NTR^{neo}; SKOV–NTR^{neo}).

4.2.2. Growth inhibition assays. Growth inhibitory potencies were determined under aerobic conditions using log-phase cultures in 96-well plates, as described previously.[57,58] Cultures were initiated 24 h before an 18 h drug exposure, with cell densities determined 4–5 days later by staining with sulforhodamine B. IC_{50} values were calculated as the drug concentration providing 50% inhibition of growth relative to controls on the same plate.

4.2.3. Mouse toxicity. Compounds were formulated in DMSO immediately before use. Groups of six male C3H mice (ca. 25 g) were treated i.p. with single doses of compounds at 1 μL/g body weight, using $10^{1/8}$-fold dose increments, and were observed daily for 60 days. Any animals losing >15% body weight or becoming moribund during the study were terminated.

4.2.4. In vivo excision assay. Activity against NTR-expressing and parental (NTR−ve) tumour cells was assessed by treating mice with tumours containing mixtures of EMT6–NTR^{puro} cells and EMT6 cells. CD-1 nude mice were inoculated subcutaneously with 3×10^6 cells using a 2:1 mixture of EMT6–NTR^{puro} and EMT6 cells. When the tumours reached a mean diameter (length × width) of 9 ± 1 mm the animals were randomized to treatment groups (5 animals/group). Mice were treated i.p. with single doses of prodrugs, at the MTD as determined in C_3H mice and tumours were removed 18 h later to determine cell killing by clonogenic assay as reported elsewhere.[51] Briefly, tumours were dissected, weighed and dissociated in a pronase/collagenase/DNAase cocktail. Cell numbers were determined with a particle counter (Coulter Electronics) and up to 10^5 cells were plated in medium containing 3 μM puromycin or nonselective medium to quantify survival of EMT6–NTR^{puro} and total tumour cells respectively. Plates were incubated for 8 days, and colonies of >50 cells counted. The plating efficiency of EMT6 cells was estimated from the difference between plating efficiency in puromycin and nonselective medium, and the number of clonogens of both types was calculated per gram of tumour tissue for control and treated tumours. Statistical significance of drug effects was determined by ANOVA using Dunnett's test to compare groups.

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References and notes

1. Huber, B. E.; Richards, C. A.; Austin, E. A. *Adv. Drug Delivery Rev.* **1995**, *17*, 279–292.
2. Martin, L.-A.; Lemoine, N. R. *Cancer Metastasis Rev.* **1996**, *15*, 301–316.
3. Niculaescu-Duvaz, I.; Spooner, R.; Marais, R.; Springer, C. J. *Bioconjugate Chem.* **1998**, *9*, 4–22.
4. Springer, C. J.; Niculescu-Duvaz, I. *J. Clin. Invest.* **2000**, *105*, 1161–1167.
5. Xu, G.; McLeod, H. L. *Clin. Cancer Res.* **2001**, *7*, 3314–3324.
6. Denny, W. A. *Curr. Pharm. Des.* **2002**, *15*, 1349–1361.
7. Anlezark, G. M.; Melton, R. G.; Sherwood, R. F.; Coles, B.; Friedlos, F.; Knox, R. J. *Biochem. Pharmacol.* **1992**, *44*, 2289–2296.

8. Knox, R. J.; Friedlos, F.; Sherwood, R. F.; Melton, R. G.; Anlezark, G. M. *Biochem. Pharmacol.* **1992**, *44*, 2297–2301.
9. Michael, N. P.; Brehm, J. K.; Anlezark, G. M.; Minton, N. P. *FEMS Microbiol. Lett.* **1994**, *124*, 195–202.
10. Skelly, J. V.; Collins, P. J.; Knox, R. J.; Anlezark, G. M.; Melton, R. G. *J. Mol. Biol.* **1994**, *238*, 852–853.
11. Grove, J. I.; Searle, P. F.; Weedon, S. J.; Green, N. K.; McNeish, I. A.; Kerr, D. J. *Anti-Cancer Drug Des.* **1999**, *14*, 461–472.
12. Djeha, A. H.; Hulme, A.; Dexter, M. T.; Mountain, A.; Young, L. S.; Searle, P. F.; Kerr, D. J.; Wrighton, C. J. *Cancer Gene Ther.* **2000**, *7*, 721–731.
13. Djeha, A. H.; Thomson, T. A.; Leung, H.; Searle, P. F.; Young, L. S.; Kerr, D. J.; Harris, P. A.; Mountain, A.; Wrighton, C. J. *Mol. Ther.* **2001**, *3*, 233–240.
14. Chung-Faye, G.; Palmer, C.; Anderson, D.; Clark, J.; Downes, M.; Baddeley, J.; Hussain, S.; Murray, P. I.; Searle, P.; Seymour, L.; Harris, P. A.; Ferry, D.; Kerr, D. *J. Clin. Cancer Res.* **2001**, *7*, 2662–2668.
15. Palmer, D. H.; Mautner, V.; Mirza, D.; Buckels, J.; Olliff, D.; Hull, D.; Mountain, A.; Harris, P.; Djeha, H.; Ellis, J.; Horne, M.; Hill, S.; Wrighton, C.; Searle, P. F.; Kerr, D. J.; Young, L. S.; James, N. D. *Br. J. Cancer* **2002**, *86*(Suppl 1), S30.
16. Palmer, D. H.; Mautner, V.; Mirza, D.; Oliff, S.; Gerritsen, W.; van der Sijp, Jr.; Hubscher, S.; Reynolds, G.; Bonney, S.; Rajaratnam, R.; Hull, D.; Horne, M.; Ellis, J.; Mountain, A.; Hill, S.; Harris, P. A.; Searle, P. F.; Young, L. S.; James, N. D.; Kerr, D. J. *J. Clin. Oncol.* **2004**, *22*, 1546–1552.
17. Helsby, N. A.; Atwell, G. J.; Yang, S.; Palmer, B. D.; Anderson, R. F.; Pullen, S. M.; Ferry, D. M.; Wilson, W. R.; Denny, W. A. *J. Med. Chem.* **2004**, *47*, 3295–3307.
18. Anlezark, G. M.; Melton, R. G.; Sherwood, R. F.; Wilson, W. R.; Denny, W. A.; Palmer, B. D.; Friedlos, F.; Williams, A. *Biochem. Pharmacol.* **1995**, *50*, 609–618.
19. Atwell, G. J.; Boyd, M.; Palmer, B. D.; Anderson, R. F.; Pullen, S. M.; Wilson, W. R.; Denny, W. A. *Anti-Cancer Drug Des.* **1996**, *11*, 553–567.
20. Friedlos, F.; Denny, W. A.; Palmer, B. P.; Springer, C. J. *J. Med. Chem.* **1997**, *40*, 1270–1275.
21. Helsby, N. A.; Wheeler, S. J.; Pruijn, F. B.; Palmer, B. D.; Yang, S.; Denny, W. A.; Wilson, W. R. *Chem. Res. Toxicol.* **2003**, *16*, 469–478.
22. Wilson, W. R.; Pullen, S. M.; Hogg, A.; Helsby, N. A.; Hicks, K. O.; Denny, W. A. *Cancer Res.* **2002**, *62*, 1425–1432.
23. Carl, P. L.; Chakravarty, P. K.; Katzenellenbogen, J. A. *J. Med. Chem.* **1981**, *24*, 479–480.
24. Hay, M. P.; Sykes, B. M.; O'Connor, C. J.; Denny, W. A. *J. Chem. Soc., Perkin Trans. 1* **1999**, 2759–2770.
25. Sykes, B. M.; Hay, M. P.; Bohinc-Herceg, D.; Denny, W. A.; O'Connor, C. J. *J. Chem. Soc., Perkin Trans. 1* **2000**, 1601–1608.
26. Mauger, A. B.; Burke, P. J.; Somani, H. H.; Friedlos, F.; Knox, R. J. *J. Med. Chem.* **1994**, *37*, 3452–3458.
27. Hay, M. P.; Wilson, W. R.; Denny, W. A. *Bioorg. Med. Chem. Lett.* **1995**, *5*, 2829–2834.
28. Hay, M. P.; Wilson, W. R.; Denny, W. A. *Bioorg. Med. Chem. Lett.* **1999**, *9*, 3417–3422.
29. Tercel, M.; Denny, W. A.; Wilson, W. R. *Bioorg. Med. Chem. Lett.* **1996**, *6*, 2741–2744.
30. Sagnou, M. J.; Howard, P. W.; Gregson, S. J.; Eno-Amooquaye, E.; Burke, P. J.; Thurston, D. E. *Bioorg. Med. Chem. Lett.* **2000**, *10*, 2083–2086.
31. Lee, M.; Simpson, J. E., Jr.; Woo, S.; Kaenzig, C.; Anlezark, G. M.; Eno-Amooquaye, E.; Burke, P. J. *Bioorg. Med. Chem. Lett.* **1997**, *7*, 1065–1070.
32. Mulcahy, R. T.; Gipp, J. J.; Schmidt, J. P.; Joswig, C.; Borch, R. F. *J. Med. Chem.* **1994**, *37*, 1610–1615.
33. Hu, L.; Yu, C.; Jiang, Y.; Han, J.; Li, Z.; Browne, P.; Race, P. R.; Knox, R. J.; Searle, P. F.; Hyde, E. I. *J. Med. Chem.* **2003**, *46*, 4818–4821.
34. Hay, M. P.; Atwell, G. J.; Wilson, W. R.; Pullen, S. M.; Denny, W. A. *J. Med. Chem.* **2003**, *46*, 2456–2466.
35. Hay, M. P.; Sykes, B. M.; Denny, W. A.; Wilson, W. R. *Bioorg. Med. Chem. Lett.* **1999**, *9*, 2237–2242.
36. Hay, M. P.; Anderson, R. F.; Ferry, D. M.; Wilson, W. R.; Denny, W. A. *J. Med. Chem.* **2003**, *46*, 5533–5545.
37. Jungheim, L. N.; Shepherd, T. A. *Chem. Rev.* **1994**, *94*, 1553–1566.
38. Hay, M. P.; Denny, W. A. *Drugs Future* **1996**, *21*, 917–931.
39. Springer, C. J.; Niculescu-Duvaz, I. *Expert Opin. Invest. Drugs* **1996**, *3*, 289–308.
40. Denny, W. A. *Curr. Pharm. Des.* **1996**, *2*, 281–294.
41. Papot, S.; Tranoy, I.; Tillequin, F.; Florent, J.-C.; Gesson, J.-P. *Curr. Med. Chem.—Anti-Cancer Agents* **2002**, *2*, 155–185.
42. Rooseboom, M.; Commandeur, J. N. M.; Vermeulen, N. P. E. *Pharmacol. Rev.* **2004**, *56*, 53–102.
43. Haisma, H. J.; van Muijen, M.; Pinedo, H. M.; Boven, E. *Cell Biophys.* **1994**, *24/25*, 185–192.
44. Azoulay, M.; Florent, J.-C.; Monneret, C.; Gesson, J. P.; Jacquesy, J.-C.; Tillequin, F.; Koch, M.; Bosslet, K.; Czech, J.; Hoffman, D. *Anti-Cancer Drug Des.* **1995**, *10*, 441–450.
45. Houba, P. H. J.; Leenders, R. G. G.; Boven, E.; Scheeren, J. W.; Pinedo, H. M.; Haisma, H. J. *Biochem. Pharmacol.* **1996**, *52*, 455–563.
46. Leenders, R. G. G.; Damen, E. W. P.; Bijsterveld, E. J. A.; Scheeren, H. W.; van der Meulen-Muileman, I. H.; Boven, E.; Haisma, H. J. *Bioorg. Med. Chem.* **1999**, *7*, 1597–1610.
47. Dubowchik, G. M.; Firestone, R. A. *Bioorg. Med. Chem. Lett.* **1998**, *8*, 3341–3346.
48. Dubowchik, G. M.; Mosure, K.; Knipe, J. O.; Firestone, R. A. *Bioorg. Med. Chem. Lett.* **1998**, *8*, 3341–3346.
49. Hay, M. P.; Wilson, W. R.; Denny, W. A. *Tetrahedron* **2000**, *56*, 645–657.
50. Dubrovchik, G. M.; King, H. D.; Pham-Kaplita, K. *Tetrahedron Lett.* **1997**, *38*, 5261–5264.
51. Wilson, W. R.; Pullen, S. M.; Hogg, A.; Hobbs, S. M.; Pruijn, F. B.; Hicks, K. O.. In *Suicide Gene Therapy: Methods and Protocols for Cancer*; Springer, C. J., Ed.; Humana: Totowa, NJ, 2003, pp 403–432.
52. Siim, B. G.; Lee, A. E.; Shalal-Zwain, S.; Pruijn, F. B.; McKeage, M. J.; Wilson, W. R. *Cancer Chemother. Pharmacol.* **2003**, *51*, 43–52.
53. Lipinski, C. A. *J. Pharmacol. Toxicol. Method* **2000**, *44*, 235–249.
54. Kyle, A. H.; Huxham, L. A.; Chiam, A. S. J.; Sim, D. H.; Minchinton, A. I. *Cancer Res.* **2004**, *64*, 6304–6309.
55. Durand, R. E. *Cancer Chemother. Pharmacol.* **1990**, *26*, 198–204.
56. Friedlos, F.; Court, S.; Ford, M.; Denny, W. A.; Springer, C. J. *Gene Ther.* **1998**, *5*, 105–112.
57. Finlay, G. J.; Baguley, B. C.; Wilson, W. R. *Anal. Biochem.* **1984**, *139*, 272–277.
58. Palmer, B. D.; Wilson, W. R.; Anderson, R. F.; Boyd, M.; Denny, W. A. *J. Med. Chem.* **1996**, *39*, 2518–2528.