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The effect of a bromide leaving group on the properties of nitro analogs of the duocarmycins as hypoxia-activated prodrugs and phosphate pre-prodrugs for antitumor therapy

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ABSTRACT

Nitro *seco* analogs (nitroCBIs) of the antitumor antibiotic duocarmycins are a new class of hypoxia activated prodrugs. These compounds undergo hypoxia-selective metabolism to form potent DNA alkylating agents. A series of four nitroCBI alcohol prodrugs containing a bromide rather than chloride or sulfonate leaving group was synthesized. In assays for in vitro hypoxia-selective cytotoxicity against human tumor cell lines the two bromides with DNA minor groove binding basic side chains displayed hypoxic cytotoxicity ratios (HCRs) of 52–286 in HT29 cells and 41–43 in SiHa cells. These values compare well with a related previously reported chloride analog. The corresponding more water soluble phosphate pre-prodrugs of the bromides were synthesized and evaluated for in vivo antitumor activity against SiHa human tumor xenografts. All four phosphates, with both neutral and basic side chains, demonstrated activity providing statistically significant hypoxic $\log_{10}$ cell kills of 0.87–2.80 at non-toxic doses, matching or proving superior to those of their chloride analogs.

1. Introduction

We have recently reported[1–6] a class of hypoxia-activated prodrugs (HAPs), the nitroCBIs (**1**, Fig. 1), that are selectively cytotoxic at low oxygen concentrations. Hypoxia often correlates with resistance to cancer treatment and negative prognosis;[7] thus the HAP concept may offer a useful strategy to tumor therapy.[8–10] The bioreduced 'effectors' (aminoCBIs, **2**, Fig. 1) of these compounds are analogs of a small family of antitumor antibiotics including the duocarmycins (e.g., (+)-duocarmycin SA,[11] **4**, Fig. 2). These natural products and a number of synthetic analogs monoalkylate in a sequence-selective manner at N3 of adenine in the minor groove of DNA and are extremely cytotoxic.[12] Myelotoxicity has so far precluded successful completion of clinical trials of such compounds.[13,14] A number of prodrug strategies for tumor-selective liberation of the cytotoxin have been attempted to address this issue. Tietze has used a glycosidic prodrug strategy (e.g., **5**, Fig. 2).[15,16] Boger[17] and Lee[18] have described quinone-containing prodrugs (e.g., **6**, Fig. 2). More recently Boger has synthesized *N*-acyl *O*-amino phenol derivatives of CBIs as prodrugs (e.g., **7**, Fig. 2).[19] Our laboratory has prepared Co(III) complexes of an aza-hydroxyCBI as prodrugs (e.g., **8**, Fig. 2).[20,21] Conjugation to tumor-specific antibodies has also been pursued.[22] A recent review[10] outlines the challenges, opportunities and strategies (molecular target inhibitors or bioreductive prodrugs) in targeting tumor hypoxia.

Some time ago we established that aminoCBIs are very potent DNA minor groove alkylating agents.[23,24] We also showed that nitroCBIs containing 5,6,7-trimethoxyindole (TMI) or 5-[(dimethylamino)ethoxy]indole (DEI) DNA minor groove binding side chains (for structures see Table 1) were up to 300-fold more cytotoxic under hypoxic than oxic conditions.[1] Unfortunately the effect was cell line-selective and hypoxic reduction rates were low. In an attempt to overcome this, we introduced electron-withdrawing groups (EWGs) into the A-ring (Fig. 1) of a series of nitroCBIs, thereby raising the one-electron reduction potentials.[2] We found that a primary 7-sulfonamide (e.g., **9**, Fig. 2) or 7-carboxamide (e.g., **10**, Fig. 2) A-ring substituent combined with the DEI side

Abbreviations: CBI, *seco*-1,2,9,9a-tetrahydrocyclopropa[*c*]benz[*e*]indol-4-one; DEI, 5-[(dimethylamino)ethoxy]indole; DIPEA, diisopropylethylamine; DMA, dimethylacetamide; EDCI·HBr, *N*-ethyl-*N*′-(3-dimethylaminopropyl)carbodiimide hydrobromide; EWG, electron-withdrawing group; HAP, hypoxia-activated prodrug; HCR, hypoxic cytotoxicity ratio; LCK, $\log_{10}$ cell kill; MEI, 5-[(morpholino)ethoxy]indole; MS, 4-methoxystyrene; MES, 4-[(morpholino)ethoxy]styrene; TMI, 5,6,7-trimethoxyindole.

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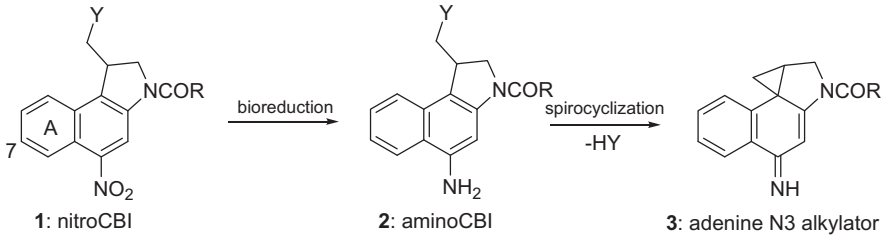


Figure 1. Suggested mechanism of action of nitroCBI hypoxia-activated prodrugs. R is a DNA minor groove binding side chain. Y is a leaving group (e.g., Cl, Br, sulfonate).

chain provided compounds with high hypoxic cytotoxicity ratios (HCRs) in SKOV3 (275 and 77 for the two compounds respectively) and in HT29 (330 and 60) human tumor cell lines. Furthermore, HCRs of 19–330 were observed for the 7-sulfonamide **9** across an 11-cell line panel. Significant activity against hypoxic cells in a SiHa human tumor xenograft was observed when **9** was combined with radiation in vivo.[2] In an in vitro comparison with known HAPs tirapazamine[25] and PR-104A,[26] the 7-sulfonamide **9** proved competitive, demonstrating similar hypoxic selectivity and much greater cytotoxicity (300- to 500-fold) under hypoxic conditions.[2] We also found that hypoxic selectivity is reduced by more than 25-fold when the side chain is changed from basic DEI to neutral TMI.[2]

In an attempt to improve aqueous solubility, we attached a tertiary amine to the 7-sulfonamide moiety (**11**, Fig. 2),[3] but this provided only a moderate increase in solubility.

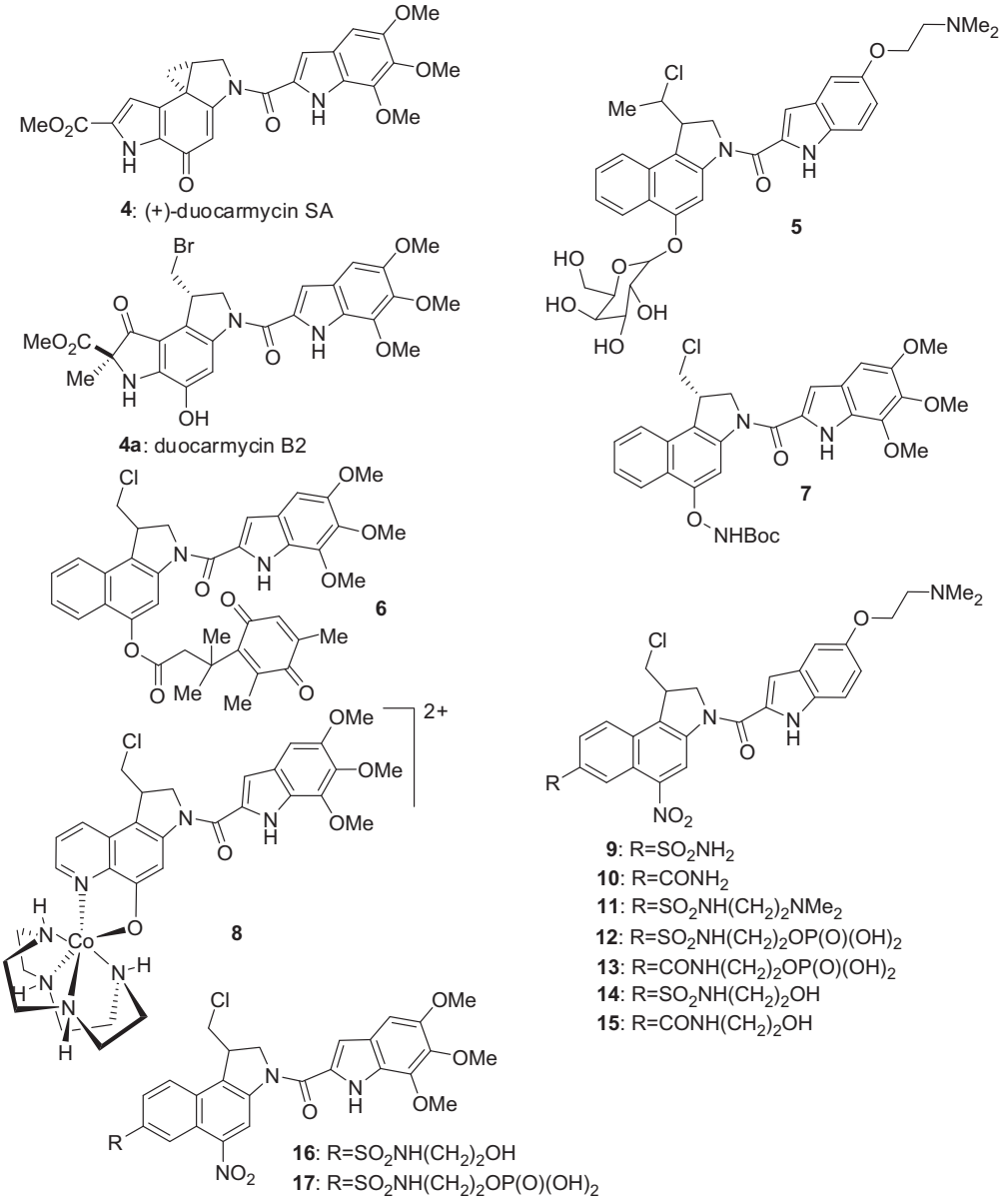
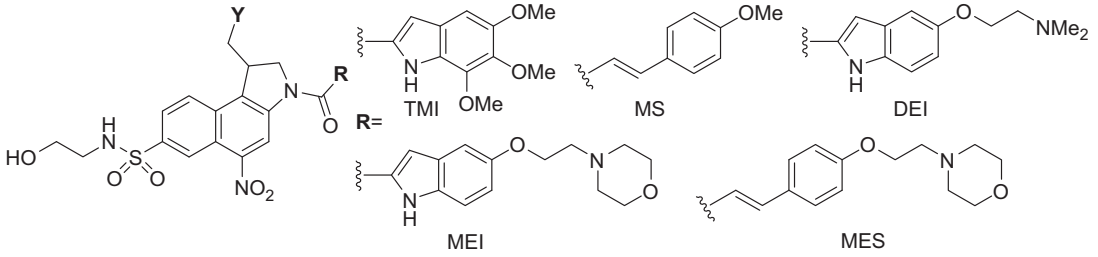


Figure 2. (+)-Duocarmycin SA, duocarmycin B2 and some previously reported prodrug and pre-prodrug analogs of the duocarmycins.

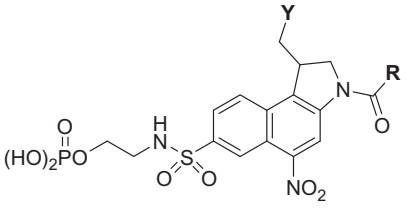
Table 1
Cytotoxicity data for nitroCBI alcohols **18**–**21** (bromides) compared with known **14** and **16** (chlorides)



Compd	R	Y	IC_{50}[a] (μM)					
			HT29			SiHa		
			Oxic	Hypoxic	HCR[b]	Oxic	Hypoxic	HCR[b]
18	TMI	Br	12.3 ± 2.9	1.35 ± 0.13	10 ± 2	2.61 ± 0.36	0.27 ± 0.05	10 ± 1.6
19	MS	Br	2.45 ± 0.1	<0.1[c]	>25	1.44 ± 0.11	0.12 ± 0.03	17 ± 4.8
20	MEI	Br	22.4 ± 2.8	0.44 ± 0.14	52 ± 16	11.7 ± 1.3	0.28 ± 0.03	43 ± 6
21	MES	Br	11.2 ± 1.2	0.08 ± 0.042	286 ± 124	2.72 ± 0.24	0.09 ± 0.014	41 ± 11
16[d]	TMI	Cl	28 ± 2	11 ± 2	2.1 ± 0.1	13 ± 1	3.5 ± 2.5	16 ± 4
14[d]	DEI	Cl	9.3 ± 1	0.09 ± 0.02	160 ± 40	2.0 ± 0.2	0.07 ± 0.01	46 ± 10

[a] Drug concentration to reduce cell density to 50% of that of controls, 5 days after 4 h exposure (mean ± SEM for 3–6 experiments).
[b] Hypoxic cytotoxicity ratio = IC_{50}(oxic)/IC_{50}(hypoxic). Values are means of intraexperiment ratios (± SEM for 2–6 experiments).
[c] Single determination.
[d] Synthesis and cytotoxicity data (2–14 experiments) of nitroCBIs with chloride leaving groups (Fig. 2) previously reported.[4]

Table 2
Antitumor activity for nitroCBI phosphates **22**–**25** (bromides) compared with known **12** and **17** (chlorides) in SiHa human tumor xenografts



Compd	R[a]	Y	Control[b]	NitroCBI[c]	p[d]	Radiation[e]	Radiation + nitroCBI[f]	Hypoxic LCK[g]	p[h]
22	TMI	Br	7.15 ± 0.18	6.51 ± 0.16	NS	5.46 ± 0.04	3.62 ± 0.26	1.84 ± 0.21	<0.001
23	MS	Br	7.15 ± 0.20	7.07 ± 0.03	NS	5.37 ± 0.04	4.50 ± 0.23	0.87 ± 0.18	0.005
24	MEI	Br	7.37 ± 0.01	6.05 ± 0.08	<0.001	5.40 ± 0.05	2.60 ± 0.21	2.80 ± 0.17[i]	<0.001
25	MES	Br	7.46 ± 0.10	7.01 ± 0.13	0.038	5.34 ± 0.12	3.78 ± 0.06	1.56 ± 0.10	<0.001
17[j]	TMI	Cl	7.62 ± 0.10	7.29 ± 0.31	NS	5.50 ± 0.15	4.96 ± 0.14	0.55 ± 0.17	NS
12[j]	DEI	Cl	7.52 ± 0.12	6.89 ± 0.26	NS	5.73 ± 0.05	3.90 ± 0.18	1.83 ± 0.15	<0.001

NS: not significant.
[a] See Table 1.
[b] Values are mean of $\log_{10}$ clonogens/g tumor ± SEM for a control group of 3 mice assayed by excision and clonogenic assay 18 h after iv dosing.
[c] Values are mean of $\log_{10}$ clonogens/g tumor ± SEM for groups of 3 mice treated with nitroCBI alone. Dose was 42 μmol/kg accept for **25** (30 μmol/kg).
[d] p-values for nitroCBI alone vs control. Calculated using intraexperiment controls.
[e] Values are mean of $\log_{10}$ clonogens/g tumor ± SEM for groups of 5 mice for γ irradiation (15 Gy).
[f] Values are mean of $\log_{10}$ clonogens/g tumor ± SEM for groups of 5 mice for γ irradiation (15 Gy) plus nitroCBI. Dose was 42 μmol/kg except for compound **25** (30 μmol/kg).
[g] Hypoxic $\log_{10}$ cell kill: radiation + nitroCBI (vs radiation alone).
[h] p-values for radiation + nitroCBI versus radiation alone. Calculated using intraexperiment controls
[i] No clonogens detected in 1 of 5 treated tumors.
[j] Synthesis and in vivo data for chlorides **12** (included highest LCK of four reported experiments: LCK 1.36–1.82) and **17** previously reported.[4]

A modified series of 7-sulfonamide and 7-carboxamide nitroCBIs were prepared by attaching a phosphate-containing moiety to the sulfonamide (e.g., **12**, Fig. 2) or carboxamide (e.g., **13**, Fig. 2).[4] It has been shown that phosphate **12** rapidly hydrolyzes to alcohol **14** in plasma,[4] and thus the corresponding alcohols (i.e., **14** and **15**, Fig. 2) were prepared. As expected, the phosphates of these had much greater aqueous solubility (>2 mM) than the alcohols.[4] Furthermore, the phosphate **12** was highly selective for and active against hypoxic cells in SiHa human tumor xenografts in vivo (Table 2).[4] The corresponding alcohol **14** exhibited HCRs of 9.4–250 in a panel of 14 human tumor cell lines (examples in Table 1).[4] Alcohol **16** containing the neutral TMI side chain provided low HCRs (2.1–16) in vitro and that was matched by phosphate **17** having less activity (LCK 0.55) than phosphate **12** (1.36–1.82) in vivo.[4]

We have recently reported the effect of sulfonate leaving groups on the hypoxia-selective toxicity of A-ring unsubstituted nitroCBIs and observed that unlike their chloride counterparts, compounds with neutral DNA minor groove binding side chains (e.g., TMI) provided the greatest hypoxic selectivity.[5] Duocarmycin B2 (**4a**, Fig. 2), a *seco* hydroxyCBI containing a bromide leaving group, is among the antitumor antibiotics isolated from the culture broth of *Steptomyces* sp.[27] Nagamura and co-workers have reported the syntheses and antitumor activity of duocarmycin derivatives and prodrugs

containing a bromide leaving group.[28–35] We now report the syntheses of nitroCBIs containing a bromide leaving group[36,37] and the effects of the bromide leaving group in combination with A-ring one-electron reduction potential-modifying substituents and DNA minor groove binding side chains on cytotoxicity and antitumor activity.

2. Results and discussion

2.1. Chemistry

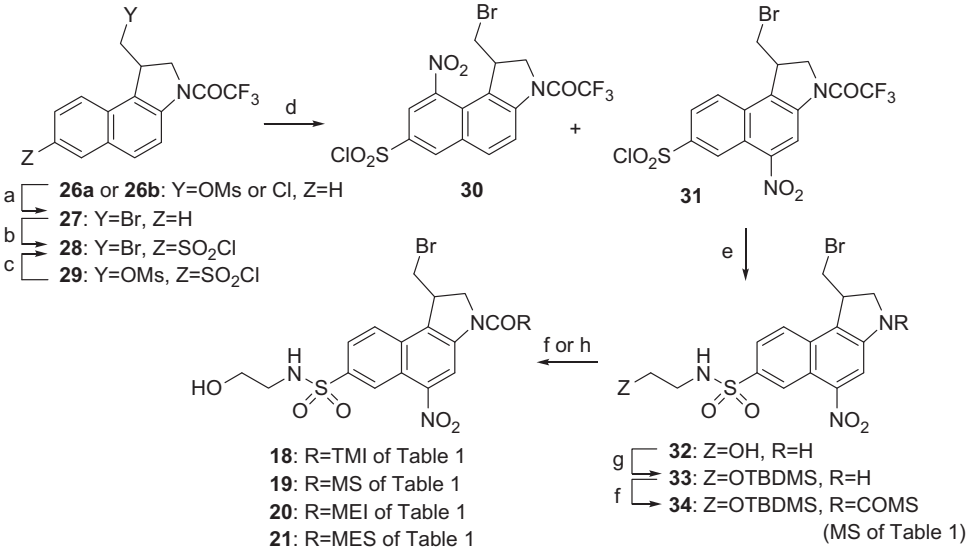
Syntheses of the nitroCBI bromides of Table 1 began by bromide displacement of the known chloride **26b**,[2] or the mesylates **26a**[5] or **29**[5] (Scheme 1). Starting with **26a** rather than **26b** proved a better option, as chloride starting material was difficult to remove from the product bromide. However, chlorosulfonylation of bromide **27** was more successful than converting the mesylate **29** into bromide **28**. During the formation of 7-sulfonyl chloride **28** from bromide **27**, a considerable amount of the 7-sulfonic acid formed as a precipitate which could be converted to **28** by dissolving the acid in DMF and treating the solution with oxalyl chloride, ultimately giving a 93% yield of **28** from **27**. Nitration of **28** (KNO_3/H_2SO_4) gave a mixture of the 9-nitro and 5-nitro regioisomers (**30** and **31**, respectively, determined by ^1H NMR). The desired major isomer **31** was separated by either flash chromatography (59%) or precipitation from $CH_2Cl_2/i\text{-}Pr_2O$ (75%). This intermediate and all of the following products had to be handled with extra care—rings A and B both contain EWGs that enhance elimination of bromide (producing HBr), particularly under basic conditions, and of halide ion scrambling from any chloride ions present. Thus whenever reactions were subjected to basic conditions or reagents, or whenever basic workup was required, the system was routinely kept cold to minimize these potential side reactions. The intermediate sulfonamide alcohol **32** was formed quantitatively by reaction of **31** with ethanolamine, followed by trifluoroacetamide cleavage with aqueous Cs_2CO_3. EDCI-mediated couplings of aniline **32** with carboxylic acids using EDCI·HCl produced a considerable amount (up to 40%) of chloride ion scrambling, which was circumvented by the use of EDCI·HBr (requiring CH_2Cl_2 as a co-solvent to dissolve it). In addition, where salts of basic side chains were used, these were converted from HCl to HBr forms by ion exchange chromatography prior to the coupling reaction. The known 5-[(morpholino)ethoxy]indole[38] (MEI) and 4-[(morpholino)ethoxy]styrene[37] (MES) side chains were introduced in this way. As previously reported[2] the EDCI-mediated reactions were most successful under acidic conditions (catalytic anhydrous TsOH). Thus, EDCI·HBr-mediated couplings of aniline **32** were carried out with four carboxylic acids to introduce two neutral DNA minor groove binding side chains, 5,6,7-trimethoxyindole (TMI) (**18**, 87%) and 4-methoxystyrene (MS) (**19**, 67%), and two weakly basic minor groove binding side chains, MEI (**20**, 57%) and MES (**21**, 57%) shown in Table 1. Although there was no advantage, **19** was also alternatively prepared from the TBDMS-protected alcohol **33** (Scheme 1).

Reduction of bromide-containing nitroCBIs to aminoCBIs was not attempted due to the presumed instability via facile ring-closure to an imino version (**3**, Fig. 1) of a spirocyclopropylcyclohexadienone. We have previously reported on the difficulty of obtaining aminoCBIs with sulfonate leaving groups.[5]

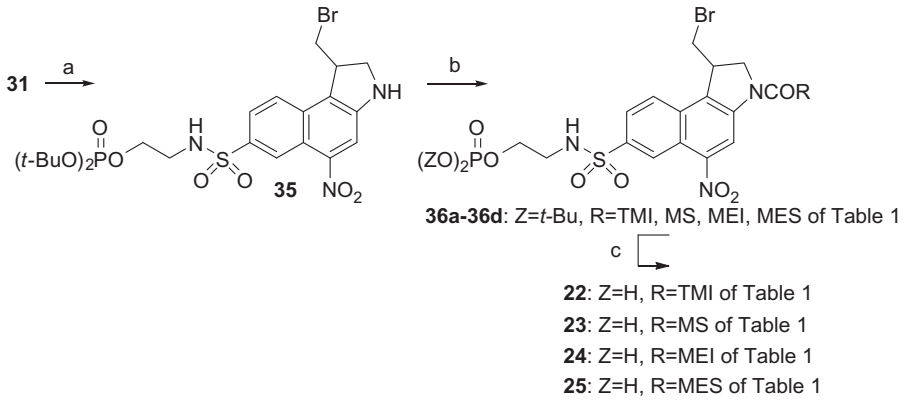
The corresponding phosphates (Scheme 2) were prepared via *tert*-butyl phosphate esters, as previously,[4] in a synthetic strategy designed to minimize handling of the very polar phosphates themselves. The intermediate sulfonamide phosphate ester **35** was formed (81%) by reaction of sulfonyl chloride **31** with 2-aminoethyldi-*tert*-butyl phosphate and deprotection of the trifluoroacetamide-protected aniline with aqueous Cs_2CO_3. EDCI·HBr-mediated couplings using **35** were carried out with the same four carboxylic acids as above, introducing the TMI (**36a**, 55%), MS (**36b**, 38%), MEI (**36c**, 38%) and MES (**37d**, 50%) minor groove binding side chains. The final step required phosphate ester cleavage with TFA, to produce compounds **22**–**25** (Scheme 2, 100%).

2.2. In vitro cytotoxicity

In accordance with our previous protocol,[4] hypoxia-selective cytotoxicity in vitro was assessed with the cell-permeable alcohols rather than the highly polar phosphates. Thus alcohols **18**–**21** were compared by measuring IC_{50} values under oxic and hypoxic conditions and deriving a hypoxic cytotoxicity ratio [HCR = IC_{50}(oxic)/IC_{50}(hypoxic)] (Table 1, Fig. 3). Data for previously reported[4] alcohols containing a chloride leaving group (**14** and **16**) are presented for comparison. Compound **14** is suitable for comparison as it provided the highest HCRs of all sulfonamides reported and was the



Scheme 1. Synthesis of nitroCBI alcohols **18**–**21** of Table 1. Reagents and conditions: (a) LiBr, THF or LiBr, 2-butanone, 85 °C; (b) (i) $ClSO_3H$, CH_2Cl_2, −78 °C to 5 °C, (ii) oxalyl chloride, DMF, 0 °C; (c) LiBr, THF; (d) (from **28**) KNO_3, 98% H_2SO_4, −12 °C; (e) (i) ethanolamine, CH_2Cl_2, 0 °C, (ii) Cs_2CO_3, MeOH, H_2O, 0 °C; (f) (from **32**, for **18**–**21**) RCO_2H, EDCI·HBr, TsOH, DMA, CH_2Cl_2; (g) TBDMSCl, DIPEA, DMF, 0 °C; (h) (from **34**, for **19**) TFA, CH_2Cl_2.



Scheme 2. Synthesis of nitroCBI phosphates **22**–**25** of Table 2. Reagents and conditions: (a) (i) $H_2N(CH_2)_2OP(O)(Ot\text{-}Bu)_2$, DIPEA, CH_2Cl_2, −50 °C, (ii) Cs_2CO_3, MeOH, H_2O, −10 °C; (b) RCO_2H, EDCI·HBr, TsOH, DMA, CH_2Cl_2; (c) TFA, CH_2Cl_2.

only compound in this series with a basic side chain. We have previously demonstrated that nitroCBIs (chlorides) containing a basic side chain (e.g., DEI in **14**) provide significantly greater HCRs than those with neutral side chains (e.g., TMI in **16**).[4] We now show that this is also true for nitroCBIs containing a bromide leaving group. Bromide **21** containing the MES basic side chain has an HCR of 286 in HT29 human colon carcinoma cells and an HCR of 41 in SiHa human cervical carcinoma. The ratios were less but still highly significant for bromide **20** containing the MEI basic side chain with an HCR of 52 in HT29 cells and an HCR of 43 in SiHa cells. This compares well with the HCRs of their chloride analog **14** containing the DEI basic side chain (160 in HT29 cells and 46 in SiHa cells). As with their chloride analogs, the bromides with neutral side chains provided lower HCRs. Bromide **18** containing the TMI side chain has an HCR of 10 in both HT29 cells and SiHa cells while bromide **19** containing the MS side chain has an HCR >25 in HT29 cells and 17 in SiHa cells. These lower ratios follow the trends of the chloride analog **16** containing the TMI side chain with HCR 2.1 in HT29 and HCR 16 in SiHa. Although we did not synthesize the corresponding bromide aminoCBIs, cytotoxicity values under hypoxia for bromide nitroCBIs are comparable to those of their chloride nitroCBI counterparts (Table 1). From these we infer that bromide aminoCBIs would have comparable cytotoxicity values to chloride aminoCBIs.

2.3. In vivo activity

We have previously shown that the phosphate moiety of nitroCBI **12** is rapidly hydrolyzed in plasma to the corresponding alcohol, and that a dose of 42 μmol/kg provides significant hypoxic tumor cell kill.[4] Higher doses (>100 μmol/kg) promoted acute toxicity in some animals and a maximum tolerated dose was difficult to define.[4] Thus, in the present study, an iv dose of 30 or 42 μmol/kg of phosphates was administered to SiHa tumor-bearing mice either alone or 5 min after a single 15 Gy dose of ionizing radiation.

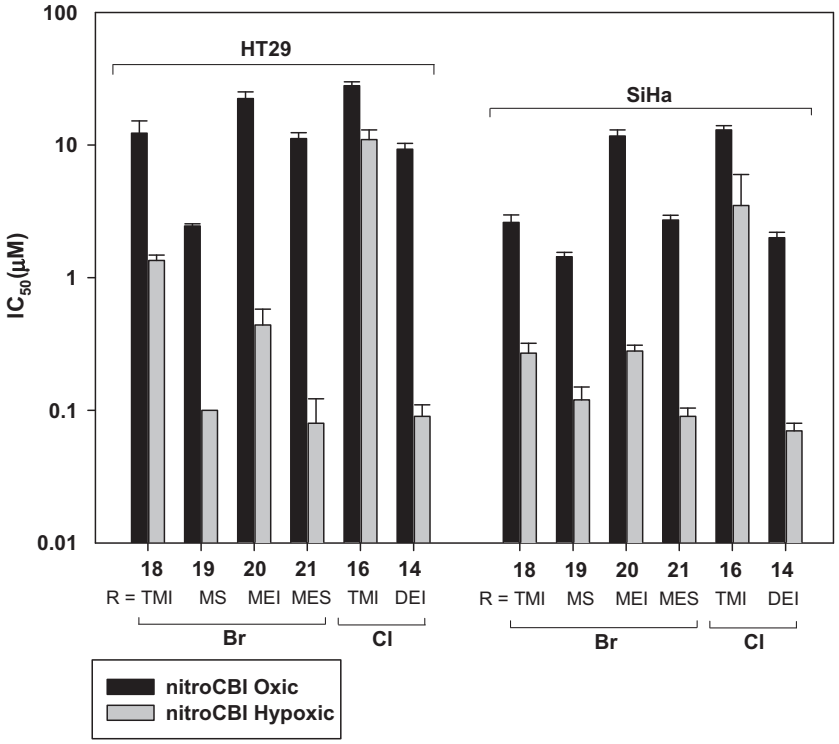


Figure 3. Comparative cytotoxicity of bromides **18**–**21** versus known[4] chlorides **14** and **16** under oxic and hypoxic conditions in HT29 and SiHa human tumor cell lines. R refers to the DNA minor groove binding side chains shown in Table 1.

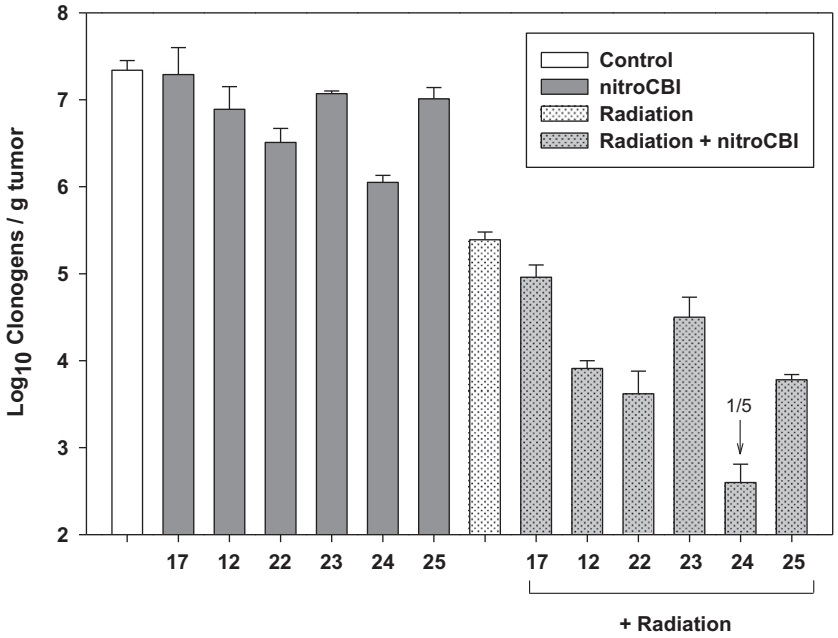


Figure 4. Antitumor activity of bromides **22**–**25** versus known[4] chlorides **12** and **17** in SiHa human tumor xenografts assayed by tumor excision and clonogenic assay 18 h after iv dosing with nitroCBI (30 or 42 μmol/kg) only or γ irradiation (15 Gy) plus nitroCBI. Values are mean ± SEM for groups of 3 mice (control or nitroCBI alone) or 5 mice (radiation alone or radiation + nitroCBI). Values for control and radiation alone are averages ± SEM from six independent experiments. Downward arrow: the number of tumors for which no surviving clonogens could be detected.

At these doses no acute toxicity was observed for any of the analogs. Eighteen hours later the tumors were excised and surviving clonogens assessed. An irradiation dose of 15 Gy is sufficient to sterilize aerobic tumor cells and provided 1.69–2.12 logs of cell kill compared to control in 6 independent experiments (Table 2). When bromides **22**–**25** were combined with radiation, a greater kill was achieved (2.65–4.77 log), indicating elimination of radioresistant hypoxic cells within the tumors by the nitroCBIs. This compares favorably with the two chloride comparators **17** (2.66 log) and **12** (3.62 log) (Table 2, Fig. 4). Phosphate **17** contains the TMI side chain while **12** contains the DEI basic side chain and was the most active sulfonamide previously reported.[4] The hypoxic $\log_{10}$ cell kill (LCK) of radiation plus drug (vs radiation alone) for **22**–**25** ranged from 0.87–2.80. The most active compound was **24** containing the MEI basic side chain, for which no clonogens could be detected in 1 of 5 treated tumors. All four bromides were much more active (p-values $\leqslant 0.005$) in combination with radiation (vs radiation alone). As single agents (nitroCBI alone) **24** and **25**, the two bromides with basic side chains, exhibited statistically significant activity with p-values of <0.001 and 0.038 versus control, respectively. Single agent activity may be due to hypoxic cell kill, oxic cell kill or aminoCBI diffusion from hypoxic regions (bystander effect). Bromides **22** and **23** with neutral side chains exhibited weak but not statistically significant single agent activity, as did the chlorides **12** and **17**.

3. Conclusions

The current study was performed to investigate structure–activity relationships influencing cytotoxicity and hypoxic selectivity for a small series of nitroCBI prodrugs and more water soluble phosphate pre-prodrugs containing a bromide leaving group, and to compare the results with those of previously reported chloride analogs. Undoubtedly the bromides are synthetically more challenging than their chloride counterparts. However, in both in vitro and in vivo the bromides matched or proved superior to their chloride analogs in the small series examined. Thus nitroCBIs with a bromide leaving group offer an alternative and promising approach in antitumor therapy, especially targeting radioresistant tumor cells. It would be useful to understand toxicity differentials between bromide prodrug and effector, made difficult by the intrinsic instability of the latter due to the powerful leaving group effect of the bromide. The superior activity of **24** in vivo could be due to bromide instead of chloride or the MEI side chain versus the DEI side chain. Future investigations will involve in vivo studies of dose-dependent tumor hypoxic cell kill, tumor growth delay assays and an in depth study incorporating morpholine side chains with different leaving groups. Bromide is slightly more lipophilic and bulky than chloride and the issues of plasma pharmacokinetics, tissue penetration and bystander effect also need to be investigated.

4. Experimental

4.1. Chemistry

Final products were analyzed by reverse-phase HPLC (Alltima C18 5 μm column, 150 × 3.2 mm; Alltech Associated, Inc., Deerfield, IL) using an Agilent HP1100 equipped with a diode-array detector. Mobile phases were gradients of 80% CH_3CN/20% H_2O (v/v) in 45 mM NH_4O_2CH at pH 3.5 and 0.5 mL/min. Purity was determined by monitoring at 330 ± 50 nm and was $\geqslant$95% for all final products. Final product purity was also assessed by combustion analysis carried out in the Campbell Microanalytical Laboratory, University of Otago, Dunedin, New Zealand. 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.

4.1.1. 1-(1-(Bromomethyl)-1*H*-benzo[*e*]indol-3(2*H*)-yl)-2,2,2-trifluoroethanone (27)

4.1.1.1. Method A. A solution of (3-(2,2,2-trifluoroacetyl)-2,3-dihydro-1*H*-benzo[*e*]indol-1-yl)methyl methanesulfonate[5] (**26a**) (8.9 g, 23.9 mmol) and LiBr (41.5 g, 477 mmol) in THF (100 mL)

was stirred in the dark for 36 h. EtOAc was added and the solution was washed with water, brine and dried (Na_2SO_4). The organic layer was evaporated and the crude product was recrystallized (MeOH/H_2O) to give **27** (8.1 g, 95%) as colorless crystals: mp 152–154 °C; ^{1}H NMR ($CDCl_3$) δ 8.43 (d, J = 9.0 Hz, 1H), 7.92 (d, J = 9.1 Hz, 1H), 7.88 (d, J = 9.3 Hz, 1H), 7.79 (d, J = 8.3 Hz, 1H), 7.58 (td, J = 8.1, 1.2 Hz, 1H), 7.49 (td, J = 8.1, 1.1 Hz, 1H), 4.62 (dt, J = 11.5, 1.4 Hz, 1H), 4.43 (dd, J = 11.4, 8.5 Hz, 1H), 4.25 (t, J = 9.1 Hz, 1H), 3.84 (ddd, J = 10.7, 3.2, 0.8 Hz, 1H), 3.39 (t, J = 10.2 Hz, 1H). Anal. Calcd for $C_{15}H_{11}BrF_3NO$: C, 50.30; H, 3.10; N, 3.91. Found: C, 50.58; H, 3.07; N, 3.92.

4.1.1.2. Method B. A solution of 1-(1-(chloromethyl)-1*H*-benzo[*e*]indol-3(2*H*)-yl)-2,2,2-trifluoroethanone[2] (**26b**) (500 mg, 1.60 mmol) and LiBr (2.19 g, 26.39 mmol) in 2-butanone (15 mL) was heated (85 °C) for 9 days. EtOAc and water were added and the organic portion was washed with water, brine and dried (Na_2SO_4). Flash chromatography (petroleum ether/EtOAc; 100:0 then 99.5:0.5) gave **27** (333 mg, 55%) contaminated with **26b** (37 mg, 10%), which could not be removed by recrystallization (EtOAc/petroleum ether).

4.1.2. 1-(Bromomethyl)-3-(2,2,2-trifluoroacetyl)-2,3-dihydro-1*H*-benzo[*e*]indole-7-sulfonyl chloride (28)

4.1.2.1. Method A. A solution of **27** (7.50 g, 21.0 mmol) in CH_2Cl_2 (40 mL) was added dropwise to a cooled (−78 °C) solution of $ClSO_3H$ (9.72 g, 83.8 mmol) in CH_2Cl_2 (40 mL). The stirred mixture was allowed to warm to 5 °C over 7 h producing a gray precipitate. The mixture was kept at 5 °C overnight and then cooled (0 °C). DMF was slowly added (the minimum amount required to dissolve the precipitate) and oxalyl chloride (4 mL) was added dropwise. After 1 h the mixture was poured into ice water. The system was extracted with cold EtOAc and the organic portion was washed with cold water, cold brine and dried (Na_2SO_4). After filtration through a plug of Celite/silica gel and removal of solvent, the crude product was dissolved in CH_2Cl_2/*i*-Pr_2O. Slow removal of CH_2Cl_2 under vacuum gave **28** (8.88 g, 93%) as a white powder: mp 194–196 °C; ^{1}H NMR ($CDCl_3$) δ 8.65 (d, J = 7.5 Hz, 1H), 8.63 (d, J = 2.0 Hz, 1H), 8.11 (d, J = 9.0 Hz, 1H), 8.09 (dd, J = 9.0, 2.0 Hz, 1H), 7.99 (d, J = 9.0 Hz, 1H), 4.67 (dt, J = 11.7, 1.6 Hz, 1H), 4.52 (dd, J = 11.4, 8.7 Hz, 1H), 4.32 (tt, J = 8.7, 2.6 Hz, 1H), 3.80 (dd, J = 10.8, 3.3 Hz, 1H), 3.47 (dd, J = 10.8, 9.1 Hz, 1H). Anal. Calcd for $C_{15}H_{10}BrClF_3NO_3S{\cdot}0.1i$-$Pr_2O$: C, 40.13; H, 2.46; N, 3.00. Found: C, 40.17; H, 2.27; N, 2.97.

4.1.2.2. Method B. A solution of **29**[5] (80 mg, 0.17 mmol) and LiBr (400 mg, 4.60 mmol) in THF (8 mL) was stirred for 4 days. EtOAc and water were added and the organic portion was washed with water, brine and dried (Na_2SO_4). Flash chromatography (petroleum ether/EtOAc; 100:0 to 4:1) gave **28** (36 mg, 47%).

4.1.3. 1-(Bromomethyl)-5-nitro-3-(2,2,2-trifluoroacetyl)-2,3-dihydro-1*H*-benzo[*e*]indole-7-sulfonyl chloride (31) and 1-(bromomethyl)-9-nitro-3-(2,2,2-trifluoroacetyl)-2,3-dihydro-1*H*-benzo[*e*]indole-7-sulfonyl chloride (30)

A cooled (0 °C) solution of KNO_3 (117 mg, 1.16 mmol) in 98% H_2SO_4 (1 mL) was added dropwise to a cooled (−12 °C) solution of **28** (440 mg, 0.97 mmol) in 98% H_2SO_4 (40 mL). After 15 min, the mixture was poured into ice water and extracted with cold EtOAc. The organic portion was washed with cold water, cold brine and dried (Na_2SO_4). Flash chromatography (petroleum ether/EtOAc; 100:0 to 7:3) gave **31** (287 mg, 59%) as a white powder and **30** (10 mg, 2%) as a white powder. A portion of **31** was recrystallized (CH_2Cl_2/*i*-Pr_2O) to give colorless crystals: mp 217–219 °C; ^{1}H NMR ($CDCl_3$) δ 9.35 (s, 1H), 9.29 (d, J = 1.7 Hz, 1H), 8.23 (dd, J = 9.0, 1.9 Hz, 1H), 8.11 (d, J = 9.1 Hz, 1H), 4.72 (br d, J = 11.5 Hz, 1H), 4.61 (dd, J = 11.5, 8.9 Hz, 1H), 4.43 (tt, J = 8.6, 3.0 Hz, 1H), 3.81 (dd, J = 11.0, 3.2 Hz, 1H), 3.58 (dd, J = 11.0, 7.8 Hz, 1H). Anal. Calcd for $C_{15}H_9BrClF_3N_2O_5S$: C, 35.91; H, 1.81; N, 5.58. Found: C, 36.00; H, 1.77; N, 5.27. A portion of **30** was recrystallized (CH_2Cl_2/*i*-Pr_2O) to give colorless crystals: mp 238–243 °C; ^{1}H NMR ($CDCl_3$) δ 8.91 (d, J = 9.1 Hz, 1H), 8.81 (d, J = 1.9 Hz, 1H), 8.36 (d, J = 2.0 Hz, 1H), 8.26 (d, J = 9.1 Hz, 1H), 4.58–4.45 (m, 2H), 4.18–4.11 (m, 1H), 3.47 (dd, J = 8.1, 3.2 Hz, 1H), 3.18 (dd, J = 11.0, 7.3 Hz, 1H). Anal. Calcd for $C_{15}H_9BrClF_3N_2O_5S{\cdot}0.2i$-$Pr_2O$: C, 37.27; H, 2.28; N, 5.37. Found: C, 37.10; H, 2.11; N, 5.18.

A repeat reaction with **28** (870 mg, 1.91 mmol) gave **31** (724 mg, 75%) after re-precipitation (CH_2Cl_2/*i*-Pr_2O) of the crude product. Compound **30** could not be detected in the precipitated material.

4.1.4. 1-(Bromomethyl)-*N*-(2-hydroxyethyl)-5-nitro-2,3-dihydro-1*H*-benzo[*e*]indole-7-sulfonamide (32)

A solution of ethanolamine (18 mg, 0.30 mmol) in CH_2Cl_2 (0.5 mL) was added dropwise to a cooled (0 °C) solution of **31** (50 mg, 0.10 mmol) in CH_2Cl_2 (10 mL). After 15 min Cs_2CO_3 (67 mg, 0.20 mmol), MeOH (3 mL) and water (2 drops) were added. After a further 15 min ice was added and the mixture was poured into cold water/cold EtOAc. The organic portion was washed with cold water, cold brine and dried (Na_2SO_4). Recrystallization (EtOAc/*i*-Pr_2O) gave **32** (43 mg, 100%) as red crystals: mp 144–147 °C; ^{1}H NMR [$(CD_3)_2SO$] δ 8.59 (d, J = 1.7 Hz, 1H), 8.02 (d, J = 8.9 Hz, 1H), 7.79 (dd, J = 8.9, 1.8 Hz, 1H), 7.69 (t, J = 5.9 Hz, 1H), 6.73 (s, 1H), 4.63 (t, J = 5.6 Hz, 1H), 4.33–4.25 (m, 1H), 3.89 (td, J = 10.4, 1.9 Hz, 1H), 3.82 (dd, J = 10.2, 3.4 Hz, 1H), 3.74–3.66 (m, 2H), 3.43–3.30 (m, 3H), 2.81 (q, J = 6.1 Hz, 2H). Anal. Calcd for $C_{15}H_{16}BrN_3O_5S{\cdot}0.1i$-$Pr_2O$: C, 42.54; H, 3.98; N, 9.54. Found: C, 42.73; H, 3.92; N, 9.30.

4.1.5. 1-(Bromomethyl)-*N*-(2-hydroxyethyl)-5-nitro-3-(5,6,7-trimethoxy-1*H*-indole-2-carbonyl)-2,3-dihydro-1*H*-benzo[*e*]indole-7-sulfonamide (18)

A solution of EDCI·HCl (9.0 g, 58.1 mmol) in CH_2Cl_2 (20 mL) was washed with a solution of 40% aq K_2CO_3. Addition of pyridinium·HBr (7.5 g, 46.5 mmol) to the organic portion followed by addition of Et_2O produced a precipitate. The precipitate was filtered off and the solid was washed with Et_2O to give EDCI·HBr (8.63 g, 79%) as a white powder.

A solution of EDCI·HBr (165 mg, 0.69 mmol), TsOH (4 mg, 0.02 mmol), 5,6,7-trimethoxyindole-2-carboxylic acid (58 mg, 0.23 mmol) and **32** (50 mg, 0.12 mmol) in CH_2Cl_2 (6 mL) and DMA (0.5 mL) was stirred for 1 h and the mixture was poured into cold EtOAc/cold water. The organic layer was washed with cold water, cold brine and then dried (Na_2SO_4) and evaporated. The residue was purified by re-precipitation (CH_2Cl_2/MeOH) followed by trituration (EtOAc) to give **18** (67 mg, 87%) as a yellow powder: mp 248–250 °C; ^{1}H NMR [$(CD_3)_2SO$] δ 11.61 (s, 1H), 9.24 (s, 1H), 8.86 (d, J = 1.4 Hz, 1H), 8.42 (d, J = 8.9 Hz, 1H), 8.02 (dd, J = 8.9, 1.6 Hz, 1H), 7.91 (t, J = 5.8 Hz, 1H), 7.20 (d, J = 2.1 Hz, 1H), 7.00 (s, 1H), 4.93 (t, J = 10.3 Hz, 1H), 4.67 (t, J = 5.5 Hz, 2H), 4.61 (dd, J = 10.9, 2.1 Hz, 1H), 4.07–3.97 (m, 2H), 3.94 (s, 3H), 3.83 (s, 3H), 3.81 (s, 3H), 3.38 (q, J = 5.8 Hz, 2H), 2.87 (q, J = 6.1 Hz, 2H). Anal. Calcd for $C_{27}H_{27}BrN_4O_9S{\cdot}$EtOAc: C, 49.54; H, 4.69; N, 7.45. Found: C, 49.71; H, 4.58; N, 7.46.

4.1.6. (*E*)-1-(Bromomethyl)-*N*-(2-hydroxyethyl)-3-(3-(4-methoxyphenyl)acryloyl)-5-nitro-2,3-dihydro-1*H*-benzo[*e*]indole-7-sulfonamide (19)

4.1.6.1. Method A. A solution of EDCI·HBr (430 mg, 1.81 mmol), TsOH (10 mg, 0.06 mmol), (*E*)-3-(4-methoxyphenyl)acrylic acid (65 mg, 0.36 mmol) and **32** (130 mg,

0.30 mmol) in CH_2Cl_2 (8 mL) was stirred for 1 h. Further portions of EDCI·HBr (100 mg, 0.42 mmol), TsOH (5 mg, 0.03 mmol) and (*E*)-3-(4-methoxyphenyl)acrylic acid (50 mg, 0.28 mmol) were added and the solution was stirred for 3 h and the mixture was poured into cold EtOAc/cold water. The organic layer was washed with cold water, cold brine and then dried (Na_2SO_4) and evaporated. The residue was purified by precipitation (CH_2Cl_2/MeOH) to give **19** (120 mg, 67%). Further purification by preparative HPLC (Synergi-Max RP column; flow rate 15 mL/min; pump 1: H_2O/TFA, pH 2.5, gradient 80%-5%-80%; pump 2: CH_3CN/H_2O, 9:1, gradient 20%–95%–20%) gave a yellow powder (106 mg, 99.4% purity): mp 246 °C (dec); ^{1}H NMR [$(CD_3)_2SO$] δ 9.35 (s, 1H), 8.83 (d, J = 1.4 Hz, 1H), 8.37 (d, J = 8.9 Hz, 1H), 8.01 (dd, J = 8.9, 1.6 Hz, 1H), 7.88 (t, J = 5.9 Hz, 1H), 7.81 (d, J = 8.8 Hz, 2H), 7.73 (d, J = 15.3 Hz, 1H), 7.10 (d, J = 15.3 Hz, 1H), 7.02 (d, J = 8.7 Hz, 2H), 4.72–4.63 (m, 3H), 4.56 (t, J = 7.7 Hz, 1H), 4.03–3.94 (m, 2H), 3.83 (s, 3H), 3.39 (q, J = 5.8 Hz, 2H), 2.87 (q, J = 6.1 Hz, 2H). Anal. Calcd for $C_{25}H_{24}BrN_3O_7S$: C, 50.86; H, 4.10; N, 7.12. Found: C, 50.97; H, 4.13; N, 6.95.

4.1.6.2. Method B. A solution of TBDMSCl (21 mg, 0.14 mmol) and DIPEA (30 mg, 0.23 mmol) in DMF (0.5 mL) was added dropwise to a cooled (0 °C) solution of **32** (50 mg, 0.12 mmol) in DMF (2 mL) and the mixture was allowed to warm to 20 °C over 1 h. Further portions of TBDMSCl (42 mg, 0.28 mmol) and DIPEA (30 mg, 0.23 mmol) were added and after 15 min the mixture was poured into cold water/cold EtOAc. The organic portion was washed with cold water, cold brine and dried (Na_2SO_4). Trituration with petroleum ether and Et_2O gave 1-(bromomethyl)-*N*-(2-((*tert*-butyldimethylsilyl)oxy)ethyl)-5-nitro-2,3-dihydro-1*H*-benzo[*e*]indole-7-sulfonamide (**33**) (60 mg, 95%) as a red powder: mp 69–72 °C (dec); ^{1}H NMR [$(CD_3)_2SO$] δ 8.58 (s, 1H), 8.02 (d, J = 9.0 Hz, 1H), 7.80 (dd, J = 8.9, 1.6 Hz, 1H), 7.78–7.72 (m, 2H), 6.74 (s, 1H), 4.34–4.24 (m, 1H), 3.88 (t, J = 10.4 Hz, 1H), 3.81 (dd, J = 10.3, 3.4 Hz, 1H), 3.74–3.65 (m, 2H), 3.52 (t, J = 6.3 Hz, 2H), 2.85 (q, J = 6.0 Hz, 2H), 0.79 (s, 9H), −0.05 (s, 6H). Anal. Calcd for $C_{21}H_{30}BrN_3O_5SSi{\cdot}0.3H_2O$: C, 45.87; H, 5.61; N, 7.64. Found: C, 45.55; H, 5.28; N, 8.04.

A solution of EDCI·HBr (143 mg, 0.61 mmol), TsOH (4 mg, 0.02 mmol), (*E*)-3-(4-methoxyphenyl)acrylic acid (27 mg, 0.15 mmol) and **33** (55 mg, 0.10 mmol) in CH_2Cl_2 (2 mL) was stirred for 3 h. Further portions of EDCI·HBr (143 mg, 0.61 mmol), TsOH (4 mg, 0.02 mmol) and (*E*)-3-(4-methoxyphenyl)acrylic acid (27 mg, 0.15 mmol) were added and the solution was stirred for 1 h and the mixture was poured into cold EtOAc/cold water. The organic layer was washed with cold water, cold brine and then dried (Na_2SO_4) and evaporated. The residue was purified by precipitation (CH_2Cl_2/MeOH) followed by trituration (petroleum ether) to give (*E*)-1-(bromomethyl)-*N*-(2-((*tert*-butyldimethylsilyl)oxy)ethyl)-3-(3-(4-methoxyphenyl)acryloyl)-5-nitro-2,3-dihydro-1*H*-benzo[*e*]indole-7-sulfonamide (**34**) (50 mg, 70%) as a yellow powder: mp 128–132 °C; ^{1}H NMR [$(CD_3)_2SO$] δ 9.39 (s, 1H), 8.89 (d, J = 1.3 Hz, 1H), 8.42 (d, J = 8.9 Hz, 1H), 8.05 (dd, J = 8.9, 1.6 Hz, 1H), 8.03 (t, J = 5.9 Hz, 1H), 7.86 (d, J = 8.7 Hz, 2H), 7.78 (d, J = 15.3 Hz, 1H), 7.15 (d, J = 15.3 Hz, 1H), 7.07 (d, J = 8.7 Hz, 2H), 4.77–4.67 (m, 2H), 4.65–4.58 (m, 1H), 4.08–3.97 (m, 2H), 3.88 (s, 3H), 3.59 (t, J = 6.1 Hz, 2H), 2.97 (q, J = 6.0 Hz, 2H), 0.83 (s, 9H), 0.00 (s, 6H). Anal. Calcd for $C_{31}H_{38}BrN_3O_7SSi{\cdot}0.4$petroleum ether: C, 54.27; H, 5.95; N, 5.69. Found: C, 54.54; H, 5.65; N, 6.03.

TFA (0.5 mL) was added dropwise to a solution of **34** (47 mg, 0.07 mmol) in CH_2Cl_2 (2 mL) and the mixture was stirred for 4 h. Solvents were removed and the residue was triturated (Et_2O) to give **19** (39 mg, 100%). Further purification by preparative HPLC (Synergi-Max RP column; flow rate 15 mL/min; pump 1: H_2O/TFA, pH 2.5, gradient 80%–5%–80%; pump 2: CH_3CN/H_2O, 9:1, gradient 20%–95%–20%) gave a yellow powder (29 mg, 99.4% purity).

4.1.7. 1-(Bromomethyl)-*N*-(2-hydroxyethyl)-3-(5-(2-morpholinoethoxy)-1*H*-indole-2-carbonyl)-5-nitro-2,3-dihydro-1*H*-benzo[*e*]indole-7-sulfonamide (20)

Biorad AG 1-X4 (Cl^- form) resin (45 g) was converted into the Br^- form by eluting with a solution of NaBr (45 g) in water (450 mL). After washing the resin with water (450 mL) and MeOH (450 mL), a solution of the HCl salt of 5-(2-morpholinoethoxy)-1*H*-indole-2-carboxylic acid[38] (1.5 g) in MeOH (20 mL) was passed through the resin to give 5-(2-morpholinoethoxy)-1*H*-indole-2-carboxylic acid hydrobromide (1.33 g, 78%) as a cream powder after removal of MeOH.

EDCI·HBr (230 mg, 0.98 mmol), TsOH (6 mg, 0.033 mmol) and 5-(2-morpholinoethoxy)-1*H*-indole-2-carboxylic acid hydrobromide (121 mg, 0.33 mmol) were added to a solution of **32** (70 mg, 0.16 mmol) in CH_2Cl_2 (15 mL) and DMA (2 mL). After 30 min further portions of EDCI·HBr (230 mg, 0.98 mmol) and TsOH (20 mg, 0.12 mmol) were added. After 1.5 h a further portion of 5-(2-morpholinoethoxy)-1*H*-indole-2-carboxylic acid hydrobromide (60 mg, 0.16 mmol) was added. After a further 5 h the mixture was poured into cold water/cold EtOAc. The organic portion was washed with cold water, cold brine and dried (Na_2SO_4). After removal of solvent the solid was dissolved in CH_2Cl_2/MeOH and slow removal of CH_2Cl_2 under vacuum gave **20** (65 mg, 57%) as a yellow powder: mp 210–216 °C (dec); ^{1}H NMR [$(CD_3)_2SO$] δ 11.74 (s, 1H), 9.29 (s, 1H), 8.85 (d, J = 1.5 Hz, 1H), 8.43 (d, J = 8.9 Hz, 1H), 8.02 (dd, J = 8.9, 1.6 Hz, 1H), 7.91 (t, J = 5.9 Hz, 1H), 7.42 (d, J = 8.9 Hz, 1H), 7.22–7.15 (m, 2H), 6.96 (dd, J = 8.9, 2.3 Hz, 1H), 4.97 (t, J = 10.5 Hz, 1H), 4.73–4.62 (m, 3H), 4.13 (t, J = 5.8 Hz, 2H), 4.08–3.98 (m, 2H), 3.63–3.56 (m, 4H), 3.39 (q, J = 6.1 Hz, 2H), 2.88 (q, J = 6.1 Hz, 2H), 2.73 (t, J = 5.7 Hz, 2H), 4 aliphatic protons not observed. Anal. Calcd for $C_{30}H_{32}BrN_5O_8S{\cdot}0.5H_2O$: C, 50.64; H, 4.67; N, 9.84. Found: C, 50.47; H, 4.60; N, 9.67.

4.1.8. (*E*)-1-(Bromomethyl)-*N*-(2-hydroxyethyl)-3-(3-(4-(2-morpholinoethoxy)phenyl)acryloyl)-5-nitro-2,3-dihydro-1*H*-benzo[*e*]indole-7-sulfonamide (21)

Biorad AG 1-X4 (Cl^- form) resin (45 g) was converted into Br^- form by eluting with a solution of NaBr (45 g) in water (450 mL). After washing the resin with water (450 mL) and MeOH (450 mL), a solution of the HCl salt of (*E*)-3-(4-(2-morpholinoethoxy)phenyl)acrylic acid[37] (1.0 g) in MeOH (20 mL) was passed through the resin to give (*E*)-3-(4-(2-morpholinoethoxy)phenyl)acrylic acid hydrobromide (1.0 g, 88%) as a cream powder after removal of MeOH.

EDCI·HBr (329 mg, 1.40 mmol), TsOH (8 mg, 0.047 mmol) and (*E*)-3-(4-(2-morpholinoethoxy)phenyl)acrylic acid hydrobromide (166 mg, 0.47 mmol) were added to a solution of **32** (100 mg, 0.23 mmol) in CH_2Cl_2 (8 mL) and DMA (1 mL). After 2 h further portions of (*E*)-3-(4-(2-morpholinoethoxy)phenyl)acrylic acid hydrobromide (166 mg, 0.47 mmol), EDCI·HBr (329 mg, 1.40 mmol) and TsOH (8 mg, 0.047 mmol) were added. After a further 2 h the mixture was poured into cold water/cold EtOAc. The organic portion was washed with cold water, cold brine and dried (Na_2SO_4). After removal of solvent the solid was dissolved in CH_2Cl_2/MeOH and slow removal of CH_2Cl_2 under vacuum gave **21** (91 mg, 57%) as a yellow powder: mp 185–190 °C (dec); ^{1}H NMR [$(CD_3)_2SO$] δ 9.34 (s, 1H), 8.83 (s, 1H), 8.35 (d, J = 8.8 Hz, 1H), 8.01 (d, J = 8.9 Hz, 1H), 7.90 (t, J = 5.8 Hz, 1H), 7.79 (d, J = 8.7 Hz, 2H), 7.73 (d, J = 15.3 Hz, 1H), 7.09 (d, J = 15.4 Hz, 1H), 7.03 (d, J = 8.7 Hz, 2H), 4.73–4.63 (m, 3H), 4.59–4.51 (m, 1H), 4.16 (t, J = 5.7 Hz, 3H), 4.05–3.92 (m, 3H), 3.63–3.55 (m, 4H), 3.54–3.47 (m, 3H), 2.86 (q, J = 6.0 Hz, 3H), 2.72 (t, J = 5.7 Hz, 2H). Anal. Calcd for $C_{30}H_{33}BrN_4O_8S{\cdot}$MeOH: C, 51.60; H, 5.17; N, 7.76. Found: C, 51.89; H, 5.07; N, 7.60.

4.1.9. 2-(1-(Bromomethyl)-5-nitro-3-(5,6,7-trimethoxy-1*H*-indole-2-carbonyl)-2,3-dihydro-1*H*-benzo[*e*]indole-7-sulfonamido)ethyl dihydrogen phosphate (22)

A solution of DIPEA (155 mg, 1.20 mmol) and $H_2N(CH_2)_2O$-P(O)(O*t*-Bu)$_2$[4] (300 mg, 1.20 mmol) in CH_2Cl_2 (1 mL) was added dropwise to a cooled (−50 °C) solution of **31** (500 mg, 1.00 mmol) in CH_2Cl_2 (10 mL). After 30 min Cs_2CO_3 (650 mg, 2.0 mmol), MeOH (5 mL) and water (2 mL) were added. The cooled (−10 °C) mixture was stirred for 15 min and poured into cold water/cold EtOAc. The organic portion was washed with cold water, cold brine, dried (Na_2SO_4) and filtered through a plug of silica gel (EtOAc/petroleum ether: 9:1) to give 2-(1-(bromomethyl)-5-nitro-2,3-dihydro-1*H*-benzo[*e*]indole-7-sulfonamido)ethyl di-*tert*-butyl phosphate (**35**) (500 mg, 81%). A small portion was recrystallized (CH_2Cl_2/*i*-Pr_2O) to give red crystals: mp 76 °C (dec); ^{1}H NMR [$(CD_3)_2SO$] δ 8.59 (d, J = 1.6 Hz, 1H), 8.04 (d, J = 8.9 Hz, 1H), 7.94 (t, J = 5.6 Hz, 1H), 7.80 (dd, J = 9.0, 1.8 Hz, 1H), 7.75 (s, 1H), 6.75 (s, 1H), 4.33–4.25 (m, 1H), 3.89 (td, J = 10.5, 1.9 Hz, 1H), 3.84–3.76 (m, 3H), 3.74–3.65 (m, 2H), 3.01 (q, J = 5.6 Hz, 2H), 1.35 (s, 18H). Anal. Calcd for $C_{23}H_{33}BrN_3O_8PS$: C, 44.38; H, 5.34; N, 6.75. Found: C, 44.48; H, 5.20; N, 6.65.

A solution of EDCI·HBr (273 mg, 1.16 mmol), TsOH (7 mg, 0.04 mmol), 5,6,7-trimethoxyindole-2-carboxylic acid (97 mg, 0.39 mmol) and **35** (120 mg, 0.19 mmol) in CH_2Cl_2 (5 mL) was stirred for 1 h and the mixture was poured into cold EtOAc/cold water. The organic layer was washed with cold water, cold brine and then dried (Na_2SO_4) and evaporated. The residue was purified by trituration with EtOAc followed by Et_2O to give 2-(1-(bromomethyl)-5-nitro-3-(5,6,7-trimethoxy-1*H*-indole-2-carbonyl)-2,3-dihydro-1*H*-benzo[*e*]indole-7-sulfonamido)ethyl di-*tert*-butyl phosphate (**36a**) (90 mg, 55%) as a yellow powder: mp 206–210 °C (dec); ^{1}H NMR [$(CD_3)_2SO$] δ 11.61 (d, J = 1.7 Hz, 1H), 9.24 (s, 1H), 8.87 (d, J = 1.6 Hz, 1H), 8.44 (d, J = 8.9 Hz, 1H), 8.17 (t, J = 5.9 Hz, 1H), 8.02 (dd, J = 8.9, 1.7 Hz, 1H), 7.19 (d, J = 2.1 Hz, 1H), 6.09 (s, 1H), 4.93 (t, J = 9.5 Hz, 1H), 4.70–4.63 (m, 1H), 4.61 (dd, J = 10.9, 2.3 Hz, 1H), 4.08–3.96 (m, 2H), 3.94 (s, 3H), 3.87–.81 (m, 2H), 3.84 (s, 3H), 3.81 (s, 3H), 3.06 (q, J = 5.9 Hz, 2H), 1.36 (s, 18H), 2 protons not observed. Anal. Calcd for $C_{35}H_{44}BrN_4O_{12}PS$: C, 49.13; H, 5.18; N, 6.55. Found: C, 49.37; H, 5.21; N, 6.43.

TFA (66 mg, 0.58 mmol) was added dropwise to a solution of **36a** (50 mg, 0.06 mmol) in CH_2Cl_2 (2 mL) and stirred for 15 h. Solvents were removed and the residue was triturated (MeOH) to give **22** (43 mg, 100%) as a yellow powder: mp 215–219 °C (dec); ^{1}H NMR [$(CD_3)_2SO$] δ 11.60 (s, 1H), 9.24 (s, 1H), 8.87 (d, J = 1.6 Hz, 1H), 8.42 (d, J = 8.9 Hz, 1H), 8.23 (br s, 1H), 8.03 (dd, J = 8.9, 1.6 Hz, 1H), 7.19 (d, J = 2.1 Hz, 1H), 6.99 (s, 1H), 4.92 (t, J = 10.4 Hz, 1H), 4.70–4.63 (m, 1H), 4.61 (dd, J = 10.9, 2.2 Hz, 1H), 4.07–3.98 (m, 2H), 3.94 (s, 3H), 3.84 (s, 3H), 3.82–3.75 (m, 2H), 3.81 (s, 3H), 3.03 (t, J = 5.7 Hz, 2H). Anal. Calcd for $C_{27}H_{28}BrN_4O_{12}PS$: C, 43.62; H, 3.80; N, 7.54. Found: C, 43.86; H, 3.88; N, 7.37.

4.1.10. (*E*)-2-(1-(Bromomethyl)-3-(3-(4-methoxyphenyl)acryloyl)-5-nitro-2,3-dihydro-1*H*-benzo[*e*]indole-7-sulfonamido)ethyl dihydrogen phosphate (23)

A solution of EDCI·HBr (340 mg, 1.45 mmol), TsOH (8 mg, 0.05 mmol), (*E*)-3-(4-methoxyphenyl)acrylic acid (56 mg, 0.31 mmol) and **35** (150 mg, 0.24 mmol) in CH_2Cl_2 (6 mL) was stirred for 2 h. Further portions of EDCI·HBr (114 mg, 0.48 mmol), TsOH (8 mg, 0.05 mmol) and (*E*)-3-(4-methoxyphenyl)acrylic acid (30 mg, 0.17 mmol) were added and the solution was stirred for 2 h and the mixture was poured into cold EtOAc/cold water. The organic layer was washed with cold water, cold brine and then dried (Na_2SO_4) and evaporated. The residue was purified by precipitation (CH_2Cl_2/MeOH) to give (*E*)-2-(1-(bromomethyl)-3-(3-(4-methoxyphenyl)acryloyl)-5-nitro-2,3-dihydro-1*H*-benzo[*e*]indole-7-sulfonamido)ethyl di-*tert*-butyl phosphate (**36b**) (72 mg, 38%) as a yellow powder: mp 186–189 °C; ^{1}H NMR [$(CD_3)_2SO$] δ 9.35 (s, 1H), 8.84 (d, J = 1.6 Hz, 1H), 8.38 (d, J = 8.9 Hz, 1H), 8.15 (t, J = 5.8 Hz, 1H), 7.99 (dd, J = 8.9, 1.6 Hz, 1H), 7.81 (d, J = 8.8 Hz, 2H), 7.74 (d, J = 15.3 Hz, 1H), 7.10 (d, J = 15.3 Hz, 1H), 7.02 (d, J = 8.8 Hz, 2H), 4.71–4.64 (m, 2H), 4.57 (t, J = 7.8 Hz, 1H), 4.02–3.94 (m, 2H), 3.87–3.80 (m, 2H), 3.83 (s, 3H), 3.05 (q, J = 5.9 Hz, 2H), 1.36 (s, 18H). Anal. Calcd for $C_{33}H_{41}BrN_3O_{10}PS·H_2O$: C, 49.51; H, 5.41; N, 5.25. Found: C, 49.13; H, 5.04; N, 5.56.

TFA (98 mg, 0.86 mmol) was added dropwise to a solution of **36b** (67 mg, 0.09 mmol) in CH_2Cl_2 (1.5 mL) and stirred for 15 h. Solvents were removed and the residue was triturated (Et_2O) to give **23** (57 mg, 100%) as a yellow powder: mp 191 °C (dec); ^{1}H NMR [$(CD_3)_2SO$] δ 9.35 (s, 1H), 8.85 (d, J = 1.6 Hz, 1H), 8.37 (d, J = 8.9 Hz, 1H), 8.12 (t, J = 5.8 Hz, 1H), 8.01 (dd, J = 8.9, 1.7 Hz, 1H), 7.81 (d, J = 8.8 Hz, 2H), 7.72 (d, J = 15.3 Hz, 1H), 7.10 (d, J = 15.3 Hz, 1H), 7.03 (d, J = 8.8 Hz, 2H), 4.72–4.62 (m, 2H), 4.56 (t, J = 7.8 Hz, 1H), 4.03–3.94 (m, 2H), 3.83 (s, 3H), 3.82–3.76 (m, 2H), 3.03 (q, J = 5.6 Hz, 2H), 2 protons not observed. Anal. Calcd for $C_{25}H_{25}BrN_3O_{10}PS·H_2O$: C, 43.61; H, 3.95; N, 6.10. Found: C, 43.83; H, 3.82; N, 5.77.

4.1.11. 2-(1-(Bromomethyl)-3-(5-(2-morpholinoethoxy)-1*H*-indole-2-carbonyl)-5-nitro-2,3-dihydro-1*H*-benzo[*e*]indole-7-sulfonamido)ethyl dihydrogen phosphate trifluoroacetate (24)

EDCI·HBr (3.74 g, 15.8 mmol), TsOH (109 mg, 0.63 mmol) and 5-(2-morpholinoethoxy)-1*H*-indole-2-carboxylic acid hydrobromide (1.30 g, 3.48 mmol) were added to a solution of **35** (1.97 g, 3.17 mmol) in CH_2Cl_2 (55 mL) and DMSO (5 mL). After 1 h further portions of EDCI·HBr (3.0 g, 12.7 mmol), TsOH (200 mg, 1.16 mmol) and 5-(2-morpholinoethoxy)-1*H*-indole-2-carboxylic acid hydrobromide (600 mg, 1.61 mmol) were added. After a further 3 h the mixture was poured into cold water/cold EtOAc. The organic portion was washed with cold water, cold brine and dried (Na_2SO_4). Precipitation (EtOAc) followed by trituration (acetone) gave 2-(1-(bromomethyl)-3-(5-(2-morpholinoethoxy)-1*H*-indole-2-carbonyl)-5-nitro-2,3-dihydro-1*H*-benzo[*e*]indole-7-sulfonamido)ethyl di-*tert*-butyl phosphate (**36c**) (1.08 g, 38%) as a yellow powder: mp 228–233 °C (dec); ^{1}H NMR [$(CD_3)_2SO$] δ 11.73 (s, 1H), 9.30 (s, 1H), 8.87 (d, J = 1.7 Hz, 1H), 8.45 (d, J = 8.9 Hz, 1H), 8.18 (t, J = 5.9 Hz, 1H), 8.02 (dd, J = 8.9, 1.7 Hz, 1H), 7.42 (d, J = 8.9 Hz, 1H), 7.23–7.17 (m, 2H), 6.96 (dd, J = 8.9, 2.4 Hz, 1H), 4.97 (t, J = 10.4 Hz, 1H), 4.73–4.63 (m, 2H), 4.13 (t, J = 5.7 Hz, 2H), 4.08–3.99 (m, 2H), 3.83 (q, J = 6.0 Hz, 2H), 3.60 (t, J = 4.5 Hz, 4H), 3.07 (q, J = 5.8 Hz, 2H), 2.74 (t, J = 5.4 Hz, 2H), 1.36 (s, 18H), 4 protons not observed. Anal. Calcd for $C_{38}H_{49}BrN_5O_{11}PS·0.5H_2O$: C, 50.50; H, 5.58; N, 7.75. Found: C, 50.68; H, 5.57; N, 7.82.

A solution of **36c** (140 mg, 0.16 mmol) and TFA (178 mg, 1.56 mmol) in CH_2Cl_2 (5 mL) was stirred for 20 h. Removal of solvents gave **24** (146 mg, 100%) as a yellow powder: mp 206–210 °C (dec); ^{1}H NMR [$(CD_3)_2SO$] δ 11.78 (s, 1H), 9.28 (s, 1H), 8.87 (d, J = 1.5 Hz, 1H), 8.42 (d, J = 8.9 Hz, 1H), 8.19 (br s, 1H), 8.01 (dd, J = 8.9, 1.5 Hz, 1H), 7.43 (d, J = 8.9 Hz, 1H), 7.24 (d, J = 2.0 Hz, 1H), 7.21 (d, J = 1.4 Hz, 1H), 6.99 (dd, J = 8.9, 2.3 Hz, 1H), 4.97 (t, J = 10.5 Hz, 1H), 4.72–4.63 (m, 2H), 4.27 (t, J = 5.9 Hz, 2H), 4.08–3.97 (m, 2H), 3.82 (q, J = 6.1 Hz, 2H), 3.87–3.70 (m, 2H), 3.03 (t, J = 5.7 Hz, 2H), 9 aliphatic protons not observed (obscured by large water peak). After D_2O addition the following peaks became visible: δ 3.56 (t, J = 5.6 Hz, 2H), 3.52–3.41 (m, 2H), 3.30–3.15 (br m, 2H). Anal. Calcd for $C_{32}H_{34}BrF_3N_5O_{13}PS$: C, 42.87; H, 3.82; N, 7.81. Found: C, 43.21; H, 4.11; N, 7.73.

4.1.12. (*E*)-2-(1-(Bromomethyl)-3-(3-(4-(2-morpholinoethoxy)phenyl)acryloyl)-5-nitro-2,3-dihydro-1*H*-benzo[*e*]indole-7-sulfonamido)ethyl dihydrogen phosphate trifluoroacetate (25)

EDCI·HBr (820 mg, 3.47 mmol), TsOH (20 mg, 0.12 mmol) and (*E*)-3-(4-(2-morpholinoethoxy)phenyl)acrylic acid hydrobromide (410 mg, 1.16 mmol) were added to a solution of **35** (360 mg, 0.58 mmol) in CH_2Cl_2 (15 mL) and DMA (1 mL). After 3 h the mixture was poured into cold water/cold EtOAc. The organic portion was washed with cold water, cold brine and dried (Na_2SO_4). Precipitation (CH_2Cl_2/MeOH) followed by trituration (MeOH) gave (*E*)-2-(1-(bromomethyl)-3-(3-(4-(2-morpholinoethoxy) phenyl)acryloyl)-5-nitro-2,3-dihydro-1*H*-benzo[*e*]indole-7-sulfonamido)ethyl di-*tert*-butyl phosphate (**36d**) (250 mg, 50%) as a yellow powder: mp 177–182 °C (dec); ^{1}H NMR [$(CD_3)_2SO$] δ 9.35 (s, 1H), 8.86 (d, J = 1.7 Hz, 1H), 8.39 (d, J = 8.9 Hz, 1H), 8.16 (t, J = 5.9 Hz, 1H), 8.00 (dd, J = 8.9, 1.7 Hz, 1H), 7.79 (d, J = 8.8 Hz, 2H), 7.73 (d, J = 15.3 Hz, 1H), 7.10 (d, J = 15.3 Hz, 1H), 7.03 (d, J = 8.8 Hz, 2H), 4.71–4.63 (m, 2H), 4.61–4.53 (m, 1H), 4.17 (t, J = 5.7 Hz, 2H), 4.04–3.93 (m, 2H), 3.82 (q, J = 6.0 Hz, 2H), 3.59 (t, J = 4.6 Hz, 4H), 3.06 (q, J = 5.8 Hz, 2H), 2.72 (t, J = 5.7 Hz, 2H), 1.35 (s, 18H), 4 protons not observed. Anal. Calcd for $C_{38}H_{50}BrN_4O_{11}PS$: C, 51.76; H, 5.72; N, 6.35. Found: C, 51.80; H, 5.91; N, 6.21.

A solution of **36d** (250 mg, 0.28 mmol) and TFA (320 mg, 2.84 mmol) in CH_2Cl_2 (12 mL) was stirred for 20 h. Removal of solvents gave **25** (250 mg, 100%) as a yellow powder: mp 178–182 °C (dec); ^{1}H NMR [$(CD_3)_2SO$] δ 9.35 (s, 1H), 8.85 (s, 1H), 8.38 (d, J = 8.8 Hz, 1H), 8.18–8.08 (m, 1H), 8.01 (d, J = 8.4 Hz, 1H), 7.86 (d, J = 8.4 Hz, 2H), 7.76 (d, J = 15.2 Hz, 1H), 7.14 (d, J = 15.2 Hz, 1H), 7.11 (d, J = 8.4 Hz, 2H), 4.73–4.63 (m, 2H), 4.60–4.52 (m, 1H), 4.46–4.36 (m, 2H), 4.07–3.94 (m, 2H), 3.79 (q, J = 5.9 Hz, 4H), 3.02 (t, J = 5.6 Hz, 2H), 11 protons not observed (large water peak). After D_2O addition the following peaks became visible: δ 3.54 (t, J = 5.6 Hz, 2H), 3.50–3.10 (br m, 6H). Anal. Calcd for $C_{32}H_{35}BrF_3N_4O_{13}PS{\cdot}H_2O$: C, 42.63; H, 4.14; N, 6.21. Found: C, 42.93; H, 4.25; N, 6.02.

4.2. In vitro cytotoxicity

Inhibition of proliferation of log-phase monolayers was assessed in 96-well plates as previously described.[3] The drug exposure time was 4 h under aerobic (20% O_2) or anoxic (<20 ppm O_2) conditions followed by sulforhodamine B staining 5 days later. The IC_{50} was determined by interpolation as the drug concentration required to inhibit cell density to 50% of that of the controls on the same plate.

4.3. In vivo activity

Antitumor activity by excision assay was performed as previously described.[2] SiHa tumors were grown in male mice by subcutaneous inoculation of 10^7 cells from tissue culture, and mice were randomized to treatment groups when tumors reached a mean diameter of 8–10 mm. In each experiment mice received vehicle alone (phosphate-buffered saline; n = 3), compound alone (dissolved in phosphate-buffered saline with 4 equiv of sodium bicarbonate; n = 3), radiation alone (15 Gy, whole body cobalt-60 γ irradiation, n = 5), or radiation followed 5 min later by compound (n = 5) administered via the tail vein. Significance of treatment effects was tested using ANOVA with Holm-Sidak post hoc test on log-transformed data with SigmaPlot v11.2 (Sysat Software, Inc.).

All animal studies were approved by the University of Auckland Animal Ethics Committee. Experiments were performed using CD1-Foxn1$^{nu/nu}$ (nude) mice.

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