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Nitro *seco* Analogues of the Duocarmycins Containing Sulfonate Leaving Groups as Hypoxia-Activated Prodrugs for Cancer Therapy

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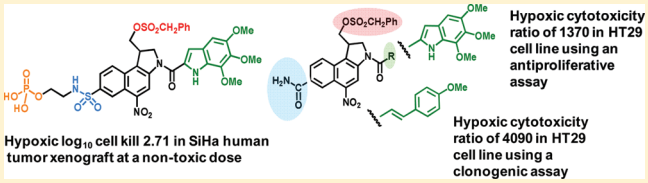
Supporting Information

ABSTRACT: The synthesis of 19 (5-nitro-2,3-dihydro-1*H*-benzo[*e*]indol-1-yl)methyl sulfonate prodrugs containing sulfonate leaving groups and 7-substituted electron-withdrawing groups is reported. These were designed to undergo hypoxia-selective metabolism to form potent DNA minor groove-alkylating agents. Analogues **17** and **24**, containing the benzyl sulfonate leaving group and a neutral DNA minor groove-binding side chain, displayed hypoxic cytotoxicity ratios (HCRs) of >1000 in HT29 human cancer cells in vitro in an antiproliferative assay. Four analogues maintained large HCRs across a panel of eight human cancer cell lines. In a clonogenic assay, **19** showed an HCR of 4090 in HT29 cells. Ten soluble phosphate preprodrugs were also prepared and evaluated in vivo, alone and in combination with radiation in SiHa human tumor xenografts at a nontoxic dose. Compounds **34** and **39** displayed hypoxic $\log_{10}$ cell kills (LCKs) of 1.78 and 2.71, respectively, equivalent or superior activity to previously reported chloride or bromide analogues, thus showing outstanding promise as hypoxia-activated prodrugs.



INTRODUCTION

The DNA minor groove (adenine N3) alkylating agents exemplified by the natural antitumor antibiotic duocarmycin SA[1] (**1**) and by a range of simpler synthetic precursor *seco*-form analogues [e.g., hydroxyCBI (*seco*-1,2,9,9a-tetrahydrocyclopropa[*c*]benz[*e*]indol-4-one) (**2**, Figure 1)][2] are extremely potent (sub-nM IC_{50}) cytotoxins. Their high potency is considered to be due to their ability to become much more reactive alkylators following initial noncovalent DNA binding[3] and to then form DNA monoadducts following a single alkylation event, with these lesions generating little DNA distortion and thus evading some routes of DNA repair. Some analogues of this class [e.g., adozelesin (**3**) and carzelesin (**4**), Figure 1][4,5] did undergo clinical trials for cancer treatment but were too myelotoxic.

This class has become a major focus for the development of selective prodrug forms, including those designed to release these potent "effectors" by exploiting the specific physiological property of hypoxia in solid tumors. A recent review outlines the challenges, opportunities, and strategies in targeting tumor hypoxia in this regard.[6] A CBI analogue (**5**, Figure 1) with a quinone "trigger" showed no oxic/hypoxic differential, which was suggested to be due to prodrug instability.[7] Other analogues (e.g., **6**, Figure 1) were tested against cells overexpressing or lacking the two-electron reductase DT-diaphorase, but selective hypoxic cytotoxicity was not evaluated.[8]

We have previously shown[9,10] that nitro analogues (e.g., **7**, Figure 1) of duocarmycins are hypoxia-selective, undergoing oxygen-reversible one-electron reduction that ultimately results in generation of the corresponding amino compounds (e.g., **7a**, Figure 1) under hypoxia. The latter amines are potent cytotoxins[11] with a similar mechanism to the phenolic *seco*-duocarmycins, alkylating at N3 of adenine in AT-rich regions of DNA,[9,12] presumably via an imino form (Figure S1 in the Supporting Information) of the spirocyclohexadienone. In contrast, the hypoxia-activated prodrug (HAP) nitro compounds are relatively nontoxic, consistent with their inability to undergo spirocyclization. The hypoxic cytotoxicity ratios [HCR = IC_{50}(oxic)/IC_{50}(hypoxic)] of compounds such as **7** were variable among cell lines, suggested due to their relatively low one-electron reduction potentials [$E(1)$ = −512 mV for **8**, Figure 1].[13] Analogues bearing electron-withdrawing groups (EWGs) on ring A (as defined in Figure 1) at the 7-position had the greatest effect [$E(1)$ values from −350 to −420 mV] in raising the reduction potential. The 7-sulfonamide **9** (Figure 1) had HCRs of 19−330 across an 11-cell line panel[13] and also presented the opportunity of further modification to provide increased aqueous solubility. The sulfonamide alcohol **10** (Figure 1) showed HCRs from 10 to 250 across a 14-cell line panel, and the corresponding phosphate preprodrug **11** (Figure 1, which rapidly hydrolyzed to **10** in plasma) showed significant hypoxic $\log_{10}$ cell kills [additional cell kill, on a log scale, for the combination of radiation and nitroCBI as compared to radiation alone; $\log_{10}$ cell kills (LCKs) of 0.41−1.82] in mice in combination with radiation (to sterilize the oxygenated cells) in excision assays with five different human tumor xenograft models.[14] In SiHa cervical carcinoma xenografts, the combination of **11** and radiation gave complete tumor sterilization in 3/

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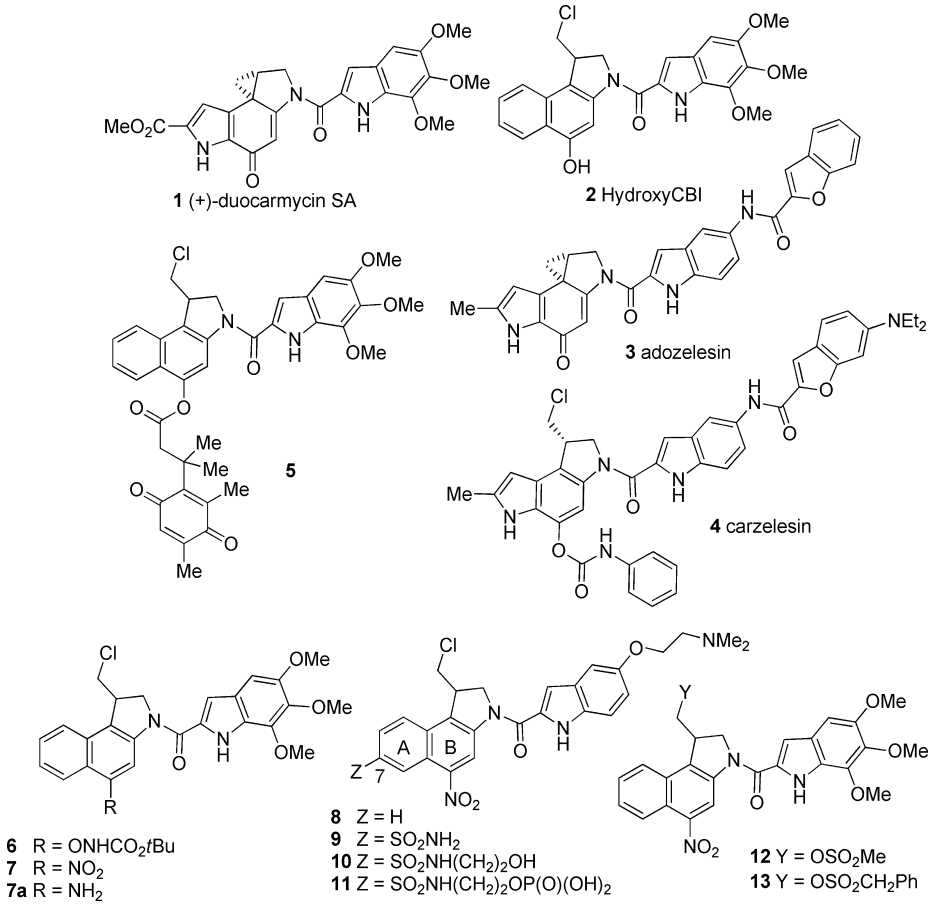

Figure 1. (+)-Duocarmycin SA, adozelesin, carzelesin, and some previously reported analogues of the duocarmycins.

5 mice, whereas the corresponding aminoCBI **10** was completely inactive. Similar trends were observed for 7-carboxamide analogues.[14] We recently showed that related nitroCBIs bearing a bromide leaving group also provide high HCRs (10−286) in HT29 colon carcinoma and SiHa cell lines, with their phosphate preprodrugs also showing high hypoxic LCKs (0.87−2.80) in SiHa human tumor xenografts.[15]

Finally,[16] we have also compared chloride versus sulfonate leaving groups (e.g., **12** and **13**, Figure 1), since the latter are better leaving groups than chloride, with significantly greater nucleofugalities.[17] In nitroCBIs lacking an A ring substituent, sulfonates with neutral DNA minor groove-binding side chains[18] had consistently higher HCRs (IC_{50} ratios) than the corresponding chloride compounds; a comparison of **7**, **12**, and **13** in SKOV3 ovarian carcinoma cells showed HCRs of 2.8, 39, and 246, respectively.[16] The general conclusion was that sulfonate leaving groups offer a way of increasing the hypoxic selectivity of nitroCBIs without introducing A ring substituents. In this manuscript, we now systematically explore combinations of sulfonate leaving groups, A ring $E(1)$-raising 7-EWGs, and various DNA minor groove-binding side chains and evaluate the products as HAPs in vitro and phosphate preprodrugs in vivo.

RESULTS AND DISCUSSION

Chemistry. The new nitroCBI prodrugs **14**−**32** described in this manuscript are listed in Table 1, and their syntheses are shown in Schemes 5−10. New nitroCBI phosphate preprodrugs **33**−**42** are listed in Table 3, and their syntheses are shown in Schemes 11−14. The syntheses of precursors and important intermediates are shown in Schemes 1−4.

We previously demonstrated that nitroCBIs containing a chloride leaving group and A ring EWGs raise $E(1)$ relative to unsubstituted parent compounds, thus providing HAPs with enhanced reductive metabolism and improved hypoxia-selective cytotoxicity.[9,13] While all A ring substituents (i.e., 6-, 7-, 8-, and 9-) raised $E(1)$, the effect was strongest when the substituent was in the 7-position [$\Delta E(1)$ = 83−159 mV]. Four examples of 7-substituents (NO_2, SO_2Me, CN, and SO_2NH_2) raised $E(1)$ values to above −400 mV. Although higher $E(1)$ alone was not sufficient to observe hypoxic selectivity and significant HCRs (>10) were found for several nitroCBIs (e.g., 7-$CONH_2$) with $E(1) < -400$ mV,[13] the combination of 7-substituents with H-bond donor capacity and higher $E(1)$ generally provided the most highly selective compounds. In the current work, we chose to limit our targets to 7-substituted nitroCBIs. This provides a direct comparison of the new nitroCBIs as HAPs with the 7-substituted prodrugs[13,14] and 7-substituted phosphate preprodrugs[14] previously reported. It was demonstrated in the chloride series that while the final products are structurally more complex than the corresponding unsubstituted analogues, their syntheses were generally easier.[13] This was a direct consequence of the A ring EWGs, which direct nitration of the benzindoline intermediates to the desired 5-regioisomer. Although this key step was not completely selective, the major isomer was readily purified by recrystallization or chromatography, and we postulated that this may also be the case in the sulfonate series. In the current study, we

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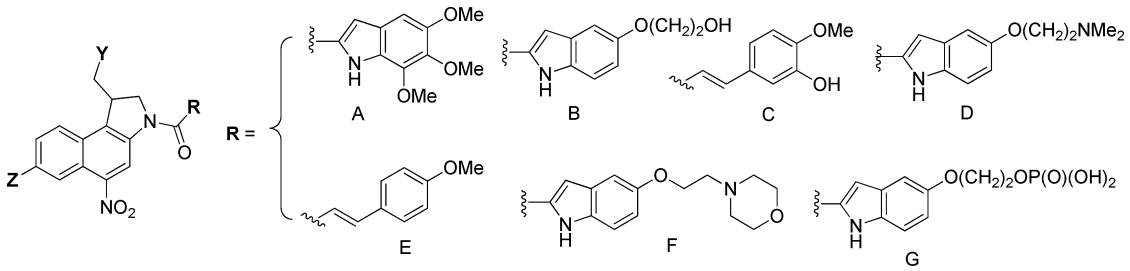
Table 1. Cytotoxicity Data for NitroCBIs 14−32 (Sulfonates) and Known[14] Chlorides 10 and 144−146[a]



				IC_{50}[b] (μM)					
				HT29			SiHa		
compd	Z	R	Y	oxic	hypoxic	HCR[c]	oxic	hypoxic	HCR[c]
14	CN	A	OSO_2CH_2Ph	2.6 ± 0.6	0.043 ± 0.01	94 ± 24	0.65 ± 0.14	0.026 ± 0.007	31 ± 11
15	CN	B	OSO_2CH_2Ph	2.5 ± 0.3	0.49 ± 0.06	5.2 ± 1.2	0.71 ± 0.12	0.035 ± 0.008	23 ± 7
16	CN	C	OSO_2CH_2Ph	3.4 ± 0.3	0.62 ± 0.03	5.4 ± 0.3	0.57 ± 0.07	0.019 ± 0.002	31 ± 2
17	$CONH_2$	A	OSO_2CH_2Ph	9.2 ± 2.4	0.016 ± 0.006	1370 ± 502	0.99 ± 0.29	0.18 ± 0.02	5.8 ± 1.9
18	$CONH_2$	D	OSO_2CH_2Ph	2.2 ± 0.1	0.038 ± 0.01	58 ± 4	0.53 ± 0.08	0.12 ± 0.03	4.9 ± 1.4
19	$CONH_2$	E	OSO_2CH_2Ph	7.0 ± 0.5	0.17 ± 0.02	40 ± 4	2.1 ± 0.5	0.046 ± 0.005	45 ± 8
20	$CONH_2$	B	OSO_2CH_2Ph	22 ± 7	0.22 ± 0.05	105[d]	18.5 ± 0.3	0.24 ± 0.04	64 ± 3
21	$CONH_2$	C	OSO_2CH_2Ph	4.6 ± 1.7	0.43 ± 0.22	21 ± 14	2.1 ± 1.3	0.096 ± 0.015	22 ± 14
22	$CONH(CH_2)_2OH$	A	OSO_2CH_2Ph	15 ± 3	0.61 ± 0.22	13 ± 2	0.90 ± 0.11	0.16 ± 0.05	10 ± 3
23	$CONH(CH_2)_2OH$	D	OSO_2CH_2Ph	4.3 ± 0.2	0.57 ± 0.19	12 ± 6	2.3 ± 0.23	0.21 ± 0.06	12 ± 3
144[e]	$CONH(CH_2)_2OH$	A	Cl	5.7 ± 1.8	3.2 ± 0.2	1.7 ± 0.5	ND	ND	ND
145[e]	$CONH(CH_2)_2OH$	D	Cl	12 ± 2	0.11 ± 0.07	485 ± 196	4.2 ± 1.1	0.24 ± 0.14	20 ± 4
24	SO_2NH_2	A	OSO_2CH_2Ph	35 ± 4	0.042 ± 0.007	1010 ± 188	13 ± 2	0.18 ± 0.01	70 ± 8
25	SO_2NH_2	D	OSO_2CH_2Ph	1.6 ± 0.3	0.090 ± 0.029	18 ± 3	0.37 ± 0.06	0.045 ± 0.012	10 ± 3
26	$SO_2NH(CH_2)_2OH$	A	OSO_2Me	14 ± 1	14 ± 7	8.6 ± 7.9	6.3 ± 0.7	2.1 ± 0.3	3.2 ± 0.3
27	$SO_2NH(CH_2)_2OH$	D	OSO_2Me	13 ± 1	2.2 ± 0.7	7.4 ± 1.7	4.1 ± 0.8	0.38 ± 0.17	17 ± 8
28	$SO_2NH(CH_2)_2OH$	E	OSO_2Me	26 ± 3	4.1 ± 2.3	31 ± 13	11 ± 1	0.32 ± 0.03	27 ± 4
29	$SO_2NH(CH_2)_2OH$	A	OSO_2CH_2Ph	8.4 ± 0.7	0.36 ± 0.03	23 ± 2	0.94 ± 0.11	0.11 ± 0.008	8.4 ± 1.0
30	$SO_2NH(CH_2)_2OH$	D	OSO_2CH_2Ph	38 ± 8	3.9 ± 0.4	9.7 ± 3.0[f]	4.5 ± 1.6	0.055 ± 0.012	66 ± 17
31	$SO_2NH(CH_2)_2OH$	E	OSO_2CH_2Ph	47[d]	0.95 ± 0.39	50[f]	7.3 ± 1.5	0.37 ± 0.03	19 ± 3
32	$SO_2NH(CH_2)_2OH$	F	OSO_2CH_2Ph	50 ± 2	>20[d]	<2.5[f]	23 ± 2	0.039 ± 0.003	600 ± 51
146[e]	$SO_2NH(CH_2)_2OH$	A	Cl	28 ± 2	11 ± 2	2.1 ± 0.1	13 ± 1	3.5 ± 2.5	16 ± 4
10[e]	$SO_2NH(CH_2)_2OH$	D	Cl	9.3 ± 1	0.09 ± 0.02	160 ± 40	2.0 ± 0.2	0.07 ± 0.01	46 ± 10

[a]ND, not determined. [b]Drug concentration to reduce cell density to 50% of that of controls, 5 days after 4 h of exposure (mean ± SEM for 3−9 experiments). [c]HCR = IC_{50}(oxic)/IC_{50}(hypoxic). Values are means of intraexperiment ratios (±SEM for 3−6 experiments). [d]Single determination. [e]Synthesis and cytotoxicity data of nitroCBIs with chloride leaving groups previously reported.[14] [f]Values are interexperimental.

Table 2. HCRs[b] of Compounds 17, 19, 20, and 24 in a Panel of Human Tumor Cell Lines[a]

compd[c]	SKOV3[d]	A549[d]	C33A[d]	H1299[d]	H460[d]	HCT116[d]	HCT8[d]	PC3[d]
17	85 ± 17	6.7 ± 0.5	76 ± 62	86 ± 45	32 ± 22	13 ± 5	55 ± 40	ND
19	ND	34 ± 1	184 ± 149	644 ± 153	104 ± 74	403 ± 280	283 ± 201	ND
20	ND	38 ± 3	93 ± 6	255[e]	>114[e]	127 ± 37	374[e]	ND
24	119 ± 17	20 ± 4	177[e]	226 ± 28	0.67[e]	193 ± 70	ND	278 ± 72

[a]ND, not determined. [b]HCR = IC_{50}(oxic)/IC_{50}(hypoxic). Values are means of intraexperiment ratios (±SEM for 2−5 experiments). [c]See Table 1. [d]SKOV3, ovarian carcinoma; A549, nonsmall-cell lung carcinoma; C33A, cervical carcinoma; H1299, lung carcinoma; H460, large-cell lung carcinoma; HCT116, colorectal carcinoma; HCT8, colon adenocarcinoma; and PC3, prostate carcinoma. [e]Single determination.

chose EWGs [Z in Table 1, i.e., CN,[13] $CONH_2$,[13] SO_2NH_2,[13] $CONH(CH_2)_2OH$,[14] and $SO_2NH(CH_2)_2OH$[14]] based on our established syntheses for chloride analogues and/or improved hypoxia-selective cytotoxicity for chloride analogues.[9,13,14] A series of DNA minor groove-binding side chains (R in Table 1) were selected based on our previous work with chloride,[13,14] bromide,[15] and sulfonate[16] analogues. We have recently noted that alternative leaving groups (i.e., bromide[15] or sulfonates[16]) are especially susceptible to halide ion scrambling and elimination in a basic environment. These potential side reactions are enhanced by having EWGs in rings A and B (as defined in Figure 1). Thus, once sulfonate leaving groups were incorporated into the nitroCBI scaffolds, extra care was required (e.g., use of dilute base and cold temperatures) to avoid elimination of RSO_2OH. Generally, we chose to introduce sulfonate leaving groups late in the synthesis.

The intermediate 7-cyano acetate **47** was obtained by several routes (Scheme 1), the best of which was halogen exchange of **43** to give iodide **44** followed by protecting group exchange to give **45** and quantitative conversion to **47**. This sequence provided the highest overall yield with no separation issues. Nitration of **47** was conducted using concentrated H_2SO_4 with

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Table 3. Antitumor Activity for NitroCBI Phosphates 33−42 (Sulfonates), Nonphosphate 19, and Known[14] 11, 147, and 148 (Chlorides) in SiHa Human Tumor Xenografts[a]

compd	Z[b]	R[b]	Y[b]	control[c]	nitroCBI[d]	p[e]	radiation[f]	radiation + nitroCBI[g]	p[h]	hypoxic LCK[i]
19	$CONH_2$	E	OSO_2CH_2Ph	7.15 ± 0.18	6.87 ± 0.20	NS	5.46 ± 0.04	4.30 ± 0.04	0.003	1.16 ± 0.04
33	$CONH_2$	G	OSO_2CH_2Ph	7.15 ± 0.18	6.91 ± 0.36	NS	5.46 ± 0.04	5.09 ± 0.06	NS	0.37 ± 0.06
34	$CONH(CH_2)_2OP(O)(OH)_2$	A	OSO_2CH_2Ph	7.37 ± 0.01	6.71 ± 0.08	<0.001	5.40 ± 0.05	3.62 ± 0.31	<0.001	1.78 ± 0.25
35	$CONH(CH_2)_2OP(O)(OH)_2$	D	OSO_2CH_2Ph	7.37 ± 0.01	6.92 ± 0.11	<0.001	5.40 ± 0.05	4.42 ± 0.10	0.001	0.98 ± 0.09
147[j]	$CONH(CH_2)_2OP(O)(OH)_2$	D	Cl	7.36 ± 0.12	6.56 ± 0.20	<0.001	5.32 ± 0.09	3.11 ± 0.22	<0.001	2.21 ± 0.19[k]
36	$SO_2NH(CH_2)_2OP(O)(OH)_2$	A	OSO_2Me	7.35 ± 0.12	7.15 ± 0.06	NS	4.98 ± 0.13	4.38 ± 0.24	0.073	0.60 ± 0.22
37	$SO_2NH(CH_2)_2OP(O)(OH)_2$	D	OSO_2Me	7.35 ± 0.12	7.18 ± 0.13	NS	4.98 ± 0.13	4.65 ± 0.21	NS	0.33 ± 0.19
38	$SO_2NH(CH_2)_2OP(O)(OH)_2$	E	OSO_2Me	7.15 ± 0.18	7.09 ± 0.11	NS	5.46 ± 0.04	4.75 ± 0.17	0.057	0.71 ± 0.14
39	$SO_2NH(CH_2)_2OP(O)(OH)_2$	A	OSO_2CH_2Ph	7.24 ± 0.07	6.53 ± 0.29	NS	5.10 ± 0.13	2.39 ± 0.03	<0.001	2.71 ± 0.11[l]
40	$SO_2NH(CH_2)_2OP(O)(OH)_2$	D	OSO_2CH_2Ph	7.24 ± 0.07	6.92 ± 0.21	NS	5.10 ± 0.13	4.92 ± 0.26	NS	0.18 ± 0.23
41	$SO_2NH(CH_2)_2OP(O)(OH)_2$	E	OSO_2CH_2Ph	7.24 ± 0.07	6.74 ± 0.23	NS	5.10 ± 0.13	4.32 ± 0.20	0.01	0.78 ± 0.18
42	$SO_2NH(CH_2)_2OP(O)(OH)_2$	F	OSO_2CH_2Ph	7.24 ± 0.07	7.08 ± 0.16	NS	5.10 ± 0.13	4.76 ± 0.09	NS	0.34 ± 0.12
148[j]	$SO_2NH(CH_2)_2OP(O)(OH)_2$	A	Cl	7.62 ± 0.10	7.29 ± 0.31	NS	5.50 ± 0.15	4.96 ± 0.14	NS	0.54 ± 0.17
11[j]	$SO_2NH(CH_2)_2OP(O)(OH)_2$	D	Cl	7.52 ± 0.12	6.89 ± 0.26	NS	5.73 ± 0.05	3.90 ± 0.18	<0.001	1.83 ± 0.15

[a]NS, not significant ($p > 0.05$). [b]See Table 1. [c]Values are mean of $\log_{10}$ clonogens/g tumor ± SEM for a control group of three mice assayed by excision 18 h after iv dosing, culturing, and determining the number of surviving colony-forming cells after 14 days. [d]Values are mean of $\log_{10}$ clonogens/g tumor ± SEM for groups of three mice for nitroCBI alone (42g or 13 μmol/kg for **19**). [e]Statistical significance for drug alone vs control. Calculated using intraexperiment controls. [f]Values are mean of $\log_{10}$ clonogens/g tumor ± SEM for groups of five mice for γ-irradiation (15 Gy). [g]Values are mean of $\log_{10}$ clonogens/g tumor ± SEM for groups of 4−5 mice for γ-irradiation (15 Gy) plus nitroCBI (42 or 13 μmol/kg for **19**). [h]Statistical significance for radiation + drug vs radiation alone. Calculated using intraexperiment controls. [i]Hypoxic LCK: additional cell kill, on a log scale, for the combination of radiation + nitroCBI (vs radiation alone). [j]Synthesis and in vivo data for chlorides **11** (value in the table is highest LCK of four separate experiments: LCK 1.36−1.83), **147** (value in the table is highest LCK of two separate experiments: LCK 2.04−2.21), and **148** previously reported.[14] [k]No clonogens detected in one of five treated tumors: within sample size and detection limits of assay (~1 in 100 000 surviving cells), no colony-forming cells could be detected. [l]No clonogens detected in four of four treated tumors.

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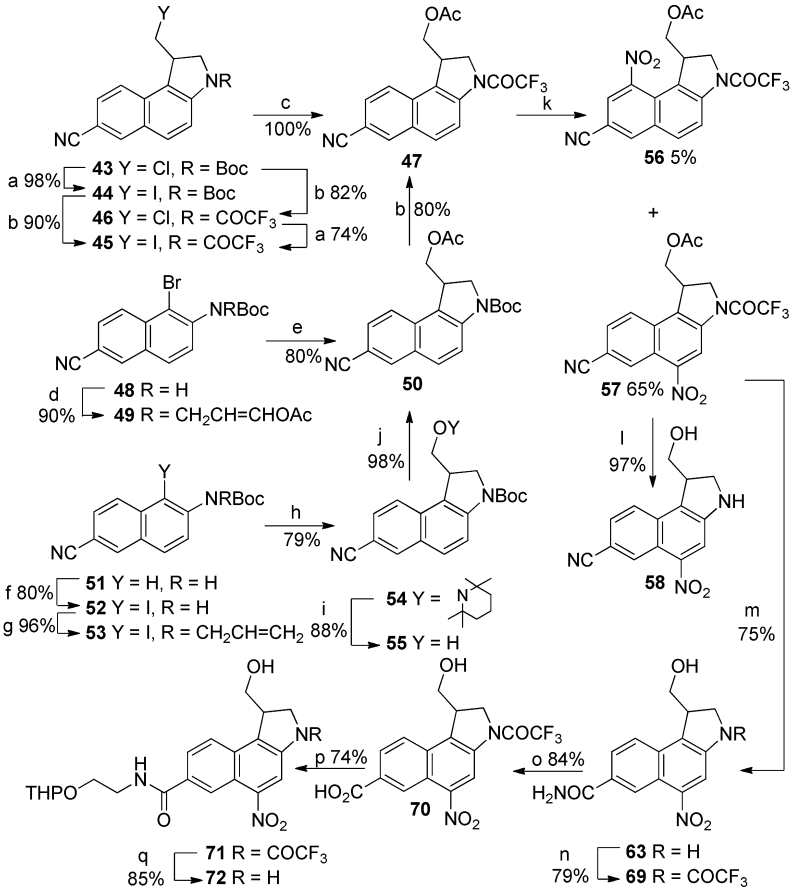
Scheme 1. Synthesis of Intermediates 58, 63, 70, and 72[a]



[a]Reagents and conditions: (a) NaI, 2-butanone, reflux. (b) (i) TFA, CH_2Cl_2; (ii) TFAA, pyridine, 0 °C. (c) (From **45**) AgOAc, HOAc, reflux. (d) $AcOCH_2CH{=}CHOAc$, DMF, NaH, 0 °C. (e) (From **49**) Bu_3SnH, AIBN, benzene, reflux. (f) NIS, TsOH, CH_3CN, 35 °C. (g) NaH, $CH_2{=}CHCH_2Br$, DMF, 0 °C. (h) (From **53**) TEMPO, Bu_3SnH, benzene, reflux. (i) Zn, HOAc, THF, H_2O, reflux. (j) (From **55**) Ac_2O, Et_3N, DMAP, THF. (k) KNO_3, 98% H_2SO_4, −10 °C. (l) Cs_2CO_3, KOH, THF, reflux. (m) H_2O_2, K_2CO_3, DMSO. (n) TFA, EDCI·HCl, TsOH, DMA. (o) (From **69**) $NaNO_2$, TFA, 0 °C. (p) $THPO(CH_2)_2NH_2$, PyBOP, DIPEA, THF, 0 °C. (q) Cs_2CO_3, MeOH, 0 °C.

a slight excess of KNO_3, giving the desired 5-NO_2 isomer **57** (structure confirmed by 2D NMR experiments) (65%) and 9-NO_2 isomer **56** (5%). Short reaction times and low temperatures in this and subsequent nitrations were required to preserve the acetate protecting group. Base-promoted cleavage of the trifluoroacetamide and acetate gave indoline **58**. The neutral minor groove-binding side chain 5,6,7-trimethoxyindole (TMI) was introduced using 5,6,7-trimethoxy-1*H*-indole-2-carboxylic acid and *N*-ethyl-*N*′-(3-dimethylaminopropyl)carbodiimide hydrochloride (EDCI·HCl) as a coupling reagent (Scheme 5). As previously reported, the EDCI-mediated reactions were most successful under acidic conditions (catalytic anhydrous TsOH).[13] The alcohol **59** thus obtained was converted to the benzyl sulfonate **14** using benzylsulfonyl chloride in pyridine. Two polar neutral side chains were introduced by first forming benzyl sulfonate **61** from Boc-protected indoline **60**, followed by Boc-deprotection to give **62**, and then coupling with the carboxylic acids in the final step giving benzyl sulfonates **15** and **16** (Scheme 5).

Five primary 7-carboxyamido benzyl sulfonates (Scheme 6) were synthesized using the 5-nitro-7-cyano acetate **57** (Scheme 1) as a starting material. Attempts to hydrolyze nitrile **57** under acidic conditions (90% H_2SO_4) led to loss of the acetate and further reaction of the primary alcohol. Instead, hydrolysis was achieved using H_2O_2 in DMSO under basic conditions[19] that also resulted in cleavage of the trifluoroacetamide and acetate to provide **63** in good overall yield (Scheme 1). Coupling of **63** with 5,6,7-trimethoxy-1*H*-indole-2-carboxylic acid, 5-(2-(dimethylamino)ethoxy)-1*H*-indole-2-carboxylic acid hydrochloride, introducing the 5-[(dimethylamino)ethoxy]indole (DEI) basic side chain, and (*E*)-3-(4-methoxyphenyl)acrylic acid, introducing the 4-methoxystyrene (MS) side chain, gave 7-carboxyamido alcohols **64**−**66** (Scheme 6). Sulfonylation of **64**−**66** gave the benzyl sulfonates **17**−**19**. Benzyl sulfonates **19**−**21** were synthesized by an alternative route via Boc-protected indoline alcohol **67**, which was first converted to benzyl sulfonate **68** before undergoing Boc deprotection and couplings.

Two secondary 7-carboxamido benzyl sulfonates (**22** and **23**, Scheme 7) were synthesized using **63** (Scheme 1) as a starting material. Coupling with trifluoroacetic acid (TFA) allowed selective protection of the indoline to give **69**, which was converted into carboxylic acid **70** by diazotization ($NaNO_2$/TFA). The secondary amide was formed by benzotriazol-1-yl-oxytripyrrolidinophosphonium hexafluorophosphate (pyBOP)-mediated coupling to give the tetrahydropyranyl (THP)-protected alcohol **71**. Coupling of the deprotected amine **72** with carboxylic acids introduced the TMI and DEI side chains giving **73** and **74** (Scheme 7). Sulfonylation of the alcohols **73** and **74** gave the benzyl sulfonates **75** and **76**. The final step

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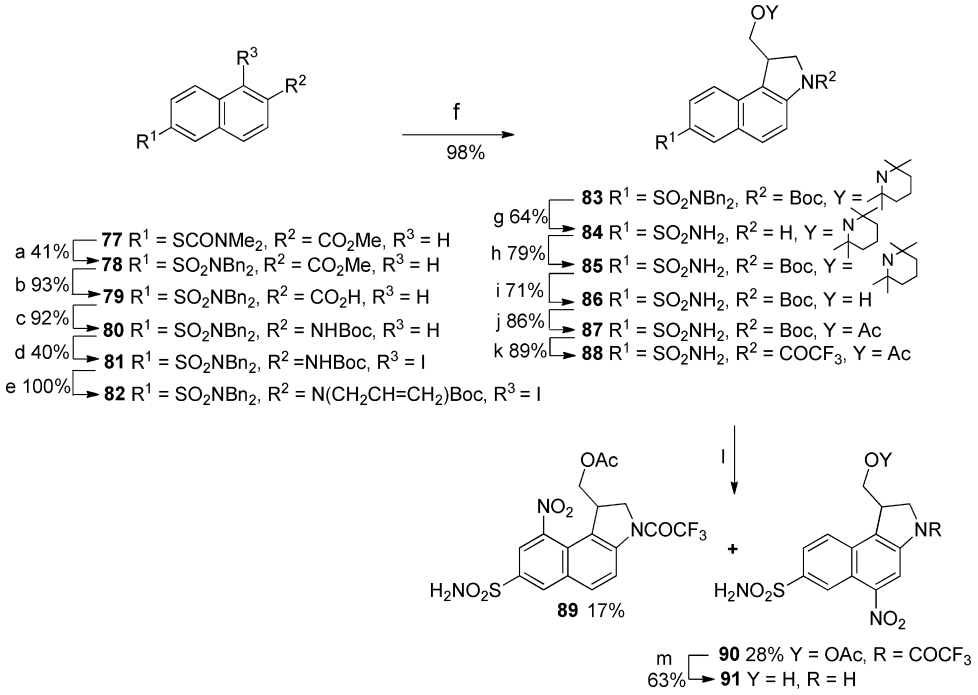
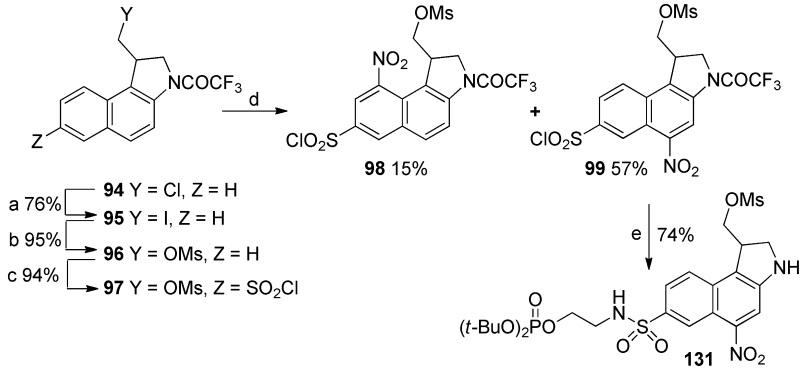
Scheme 2. Synthesis of Intermediate 91[a]



[a]Reagents and conditions: (a) (i) KNO_3, SO_2Cl_2, CH_3CN, 0 °C; (ii) Bn_2NH, Et_3N, THF. (b) KOH, MeOH, H_2O, reflux. (c) DPPA, *tert*-BuOH, Et_3N, reflux. (d) NIS, TsOH, CH_2Cl_2, CH_3CN. (e) NaH, allyl bromide, DMF, 0 °C to rt. (f) (From **82**) TEMPO, Bu_3SnH, C_6H_6. (g) H_2SO_4, 0 °C. (h) Boc_2O, THF, reflux. (i) Zn, HOAc, THF, H_2O, reflux. (j) Ac_2O, Et_3N, DMAP, THF. (k) (i) HCl, dioxane; (ii) TFAA, Et_3N, THF, 0 °C. (l) (From **88**) KNO_3, H_2SO_4, 0 °C. (m) Cs_2CO_3, MeOH, THF.

Scheme 3. Synthesis of Intermediates 99 and 131[a]



[a]Reagents and conditions: (a) NaI, 2-butanone, reflux. (b) AgOMs, CH_3CN. (c) (i) $ClSO_3H$, CH_2Cl_2, −78 to 15 °C; (ii) oxalyl chloride, DMF, 0 °C. (d) (From **97**) KNO_3, 98% H_2SO_4, −5 °C. (e) (i) $(t\text{-BuO})_2(O)PO(CH_2)_2NH_2$, DIPEA, CH_2Cl_2, 0 °C; (ii) Cs_2CO_3, MeOH, H_2O, 0 °C.

required the acid-mediated deprotection of the THP-protected alcohols **75** and **76** giving **22** and **23**, respectively.

Two primary 7-sulfonamido benzyl sulfonates (**24** and **25**, Scheme 8) were synthesized via a multistep route starting with the synthesis[20] of the protected dibenzylsulfonamide **78** from known[13] naphthoate **77** (Scheme 2). Ester hydrolysis, modified Curtius reaction, iodination, and allylation followed by radical ring cyclization[21,22] produced (2,2,6,6-tetramethylpiperidin-1-yl)oxyl (TEMPO) adduct[23] **83**. Acid-mediated deprotection removed both the Boc and the benzyl groups of **83** giving 7-sulfonamide **84**. Reductive cleavage of the TEMPO group at this stage gave an unstable product, while the corresponding trifluoroacetamide was also unstable to the cleavage conditions, so the Boc protecting group was reinstalled to give **85**. Zn/HOAc-mediated reduction of the N−O bond then gave alcohol **86**, followed by acetylation to **87** (using only a slight excess of Ac_2O to avoid acetylation of the sulfonamide) and a protecting group exchange to give the trifluoroacetamide **88**. Nitration gave the desired 5-NO_2 product **90** (28%) and a significant amount of the 9-NO_2 isomer **89** (17%). Base treatment of **90** removed both the trifluoroacetamide and the acetate groups to give alcohol **91**. Coupling of **91** with carboxylic acids introduced the TMI (**92**) and DEI (**93**) side chains (Scheme 8). The final step required benzylsulfonylation of **92** to give **24** and of **93** to give **25**.

Three secondary 7-sulfonamido methane sulfonates (**26**−**28**, Scheme 9) were synthesized using known chloride **94**[13] (Scheme 3) as a starting material. Halide exchange of **94** gave iodide **95**, and reaction of **95** with AgOMs gave methane sulfonate **96**. During the formation of 7-sulfonyl chloride **97**

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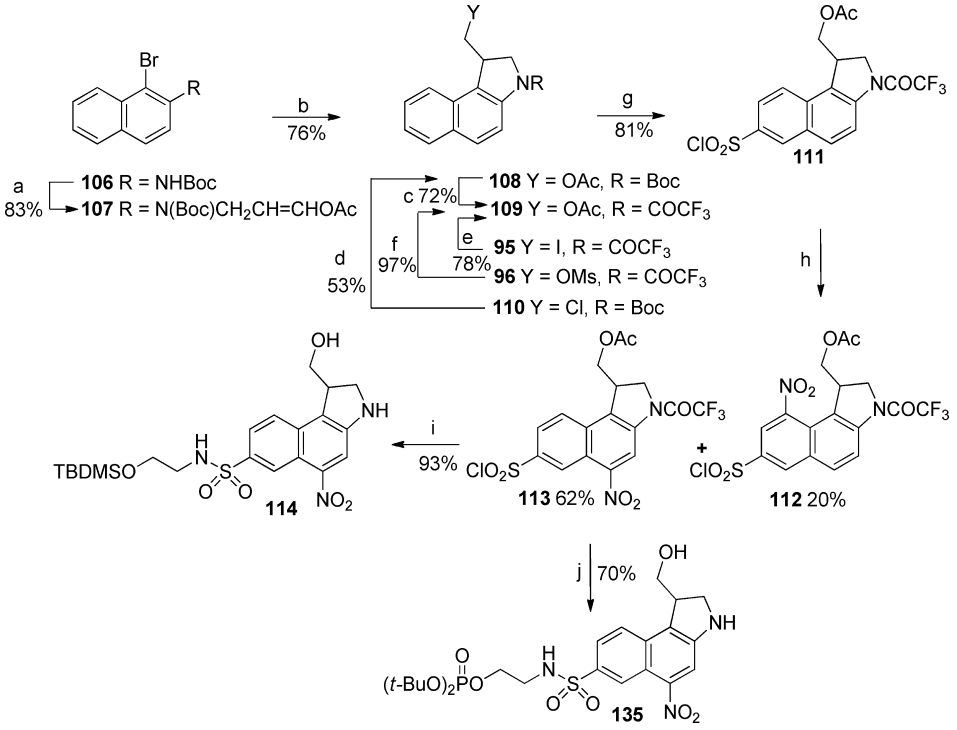
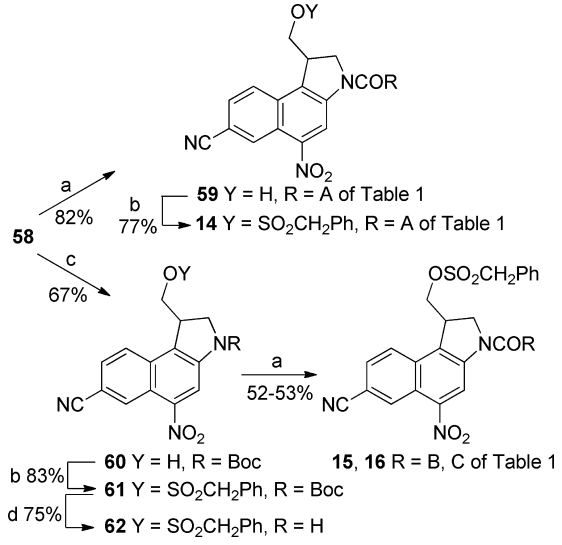
Scheme 4. Synthesis of Intermediates 114 and 135[a]



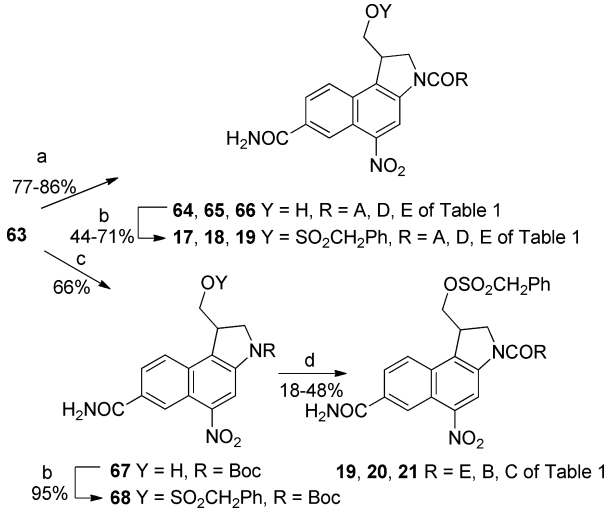
[a]Reagents and conditions: (a) NaH, AcOCH═CH(CH$_2$)OAc, DMF, 0 °C to rt. (b) Bu_3SnH, AIBN, benzene, reflux. (c) (i) $HCl_{(g)}$, dioxane; (ii) TFAA, py, 0 °C. (d) AgOAc, HOAc, 80 °C. (e) KOAc, 18-crown-6, HOAc, 100 °C. (f) AgOAc, HOAc. (g) (From **109**) (i) $ClSO_3H$, CH_2Cl_2, −80 to 5 °C; (ii) oxalyl chloride, DMF, −10 to 5 °C. (h) KNO_3, 98% H_2SO_4, −12 °C. (i) (i) $TBDMSO(CH_2)_2NH_2$, DIPEA, CH_2Cl_2, 0 °C; (ii) Cs_2CO_3, MeOH, H_2O, 0 °C to rt. (j) (i) $(t\text{-BuO})_2(O)PO(CH_2)_2NH_2$, DIPEA, CH_2Cl_2, 0 °C; (ii) Cs_2CO_3, MeOH, H_2O, 0 °C.

Scheme 5. Synthesis of Compounds 14−16 of Table 1[a]



[a]Reagents and conditions: (a) RCO_2H, EDCI·HCl, TsOH, DMA. (b) $PhCH_2SO_2Cl$, pyridine, CH_2Cl_2, 0 °C. (c) Boc_2O, THF, reflux. (d) TFA, CH_2Cl_2.

Scheme 6. Synthesis of Compounds 17−21 of Table 1[a]



[a]Reagents and conditions: (a) RCO_2H, EDCI·HCl, TsOH, DMA. (b) $PhCH_2SO_2Cl$, pyridine or Et_3N, 0 °C. (c) Boc_2O, dioxane, reflux. (d) (i) TFA, CH_2Cl_2; (ii) RCO_2H, EDCI·HCl, TsOH, DMA.

from methane sulfonate **96**, a considerable amount of the corresponding sulfonic acid formed as a precipitate. However, this could be reconverted to **97** by treating a solution of the acid in CH_2Cl_2/DMF with oxalyl chloride, ultimately giving a 94% yield of **97** from **96**. Nitration of **97** gave the desired 5-NO_2 isomer **99** (57%) and some 9-NO_2 isomer **98** (15%). The sulfonamide was formed by reacting **99** with ethanolamine or alcohol-protected (THP or TBDMS) ethanolamine, followed by cleavage of the trifluoroacetamide, to give compounds **100**−**102** (Scheme 9). Coupling of 7-sulfonamides **100**−**102** with carboxylic acids introducing TMI, DEI, and MS side chains, followed by TFA-promoted deprotection where required, gave methane sulfonates **26**−**28**. EDCI·HCl-mediated coupling of **100** with 4-methoxycinnamic acid resulted in a long reaction time and significant exchange of methane sulfonate to chloride,

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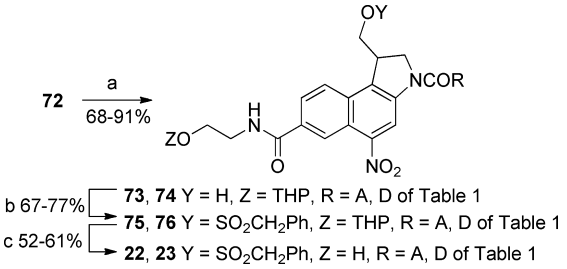
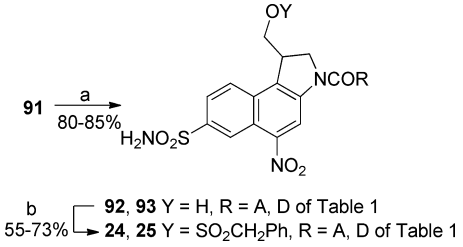
Scheme 7. Synthesis of Compounds 22 and 23 of Table 1[a]



[a]Reagents and conditions: (a) RCO_2H, EDCI·HCl, TsOH, DMA. (b) $PhCH_2SO_2Cl$, pyridine, 0 °C. (c) $MeSO_3H$, MeOH or TFA, CH_2Cl_2.

Scheme 8. Synthesis of Compounds 24 and 25 of Table 1[a]

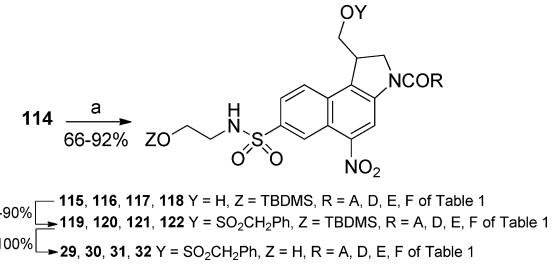


[a]Reagents and conditions: (a) RCO_2H, EDCI·HCl, TsOH, DMA. (b) $PhCH_2SO_2Cl$, pyridine, 0 °C.

prompting the use of EDCI·TsOH with the THP-protected **101**. In the event, the two-step route gave an improved overall yield of 63% (up from 43%) for **28**.

Four secondary 7-sulfonamido benzyl sulfonates (**29**–**32**, Scheme 10) were synthesized by several routes (all Scheme 4). The reaction of methane sulfonate **96** with AgOAc in acetic acid proved to be the most convenient method of forming acetate **109** (97%) (Scheme 4). Chlorosulfonylation followed by nitration gave the desired 5-NO_2 isomer **113** (62%) and 9-NO_2 isomer **112** (20%). The sulfonamide was introduced by reacting **113** with alcohol-protected (TBDMS) ethanolamine to give **114**. The presence of base [diisopropylethylamine (DIPEA) and subsequent addition of aqueous Cs_2CO_3] in this step also removed both trifluoroacetamide and acetate groups. Coupling of **114** with carboxylic acids introduced the TMI (**115**), DEI (**116**), MS (**117**), and 5-[(morpholino)ethoxy]-indole[24] (MEI) (**118**) side chains (Scheme 10). Benzylsulfonylation of alcohols **115**–**118** gave benzyl sulfonates **119**–**122**,

Scheme 10. Synthesis of Compounds 29–32 of Table 1[a]

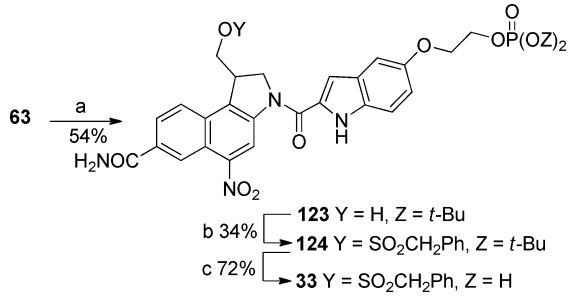


[a]Reagents and conditions: (a) RCO_2H, EDCI·HCl, TsOH, DMA. (b) $PhCH_2SO_2Cl$, pyridine, 0 °C. (c) TFA, CH_2Cl_2, MeOH.

and the final TFA-mediated cleavage of the TBDMS group gave **29**–**32**.

The phosphate preprodrug **33** (Scheme 11) was prepared by coupling of **63** (Scheme 1) with a phosphate ester-containing

Scheme 11. Synthesis of Compound 33 of Table 3[a]



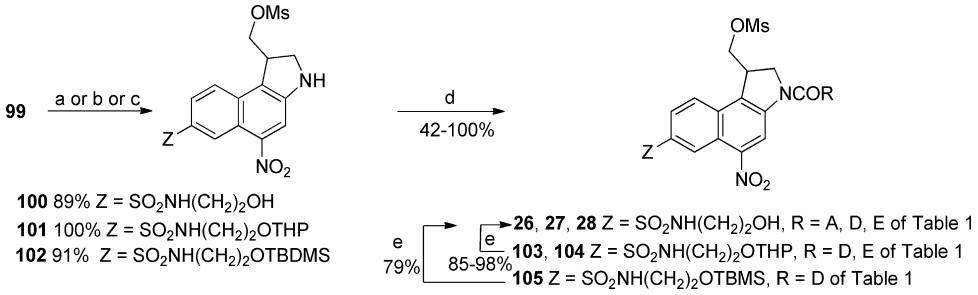
[a]Reagents and conditions: (a) 5-(2-((Di-*tert*-butoxyphosphoryl)oxy)-ethoxy)-1*H*-indole-2-carboxylic acid, EDCI·HCl, TsOH, DMA. (b) $PhCH_2SO_2Cl$, pyridine, CH_2Cl_2, 0 °C. (c) TFA, CH_2Cl_2.

carboxylic acid[14] to give **123**, followed by formation of benzyl sulfonate **124** and TFA-mediated deprotection of the phosphate ester.

Phosphates **34**–**42** (Table 3) are more water-soluble preprodrugs of alcohols **22**, **23**, and **26**–**32** of Table 1, and their syntheses (Schemes 12–14) are based on methodology previously reported.[14]

In Vitro Cytotoxicity. In accordance with our previous studies,[14] hypoxia-selective cytotoxicity in vitro was assessed with the cell-permeable alcohols and other 7-substituted compounds rather than the highly polar phosphates. Thus

Scheme 9. Synthesis of Compounds 26–28 of Table 1[a]



[a]Reagents and conditions: (a) (From **99** to **100**) (i) ethanolamine, CH_2Cl_2, 0 °C; (ii) Cs_2CO_3, MeOH, H_2O, 0 °C. (b) (From **99** to **101**) (i) $THPO(CH_2)_2NH_2$, CH_2Cl_2, 0 °C; (ii) Cs_2CO_3, MeOH, H_2O, 0 °C. (c) (From **99** to **102**) (i) $TBDMSO(CH_2)_2NH_2$, Et_3N, CH_2Cl_2, 0 °C; (ii) Cs_2CO_3, MeOH, H_2O, 0 °C. (d) RCO_2H, EDCI·HCl or EDCI·TsOH, TsOH, DMA or CH_2Cl_2. (e) TFA, CH_2Cl_2, MeOH.

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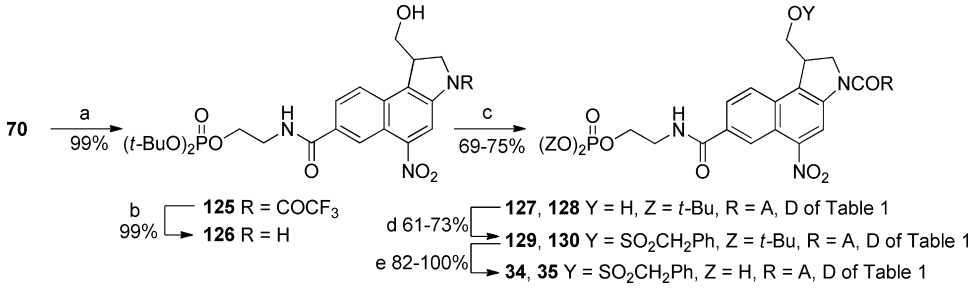
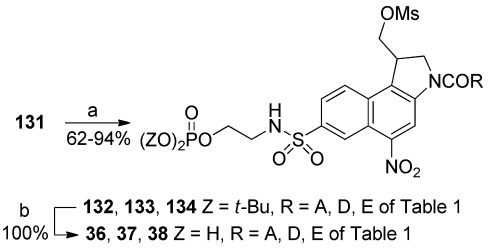
Scheme 12. Synthesis of Compounds 34 and 35 of Table 3[a]



[a]Reagents and conditions: (a) $(t\text{-BuO})_2(O)PO(CH_2)_2NH_2$, PyBOP, DIPEA, 0 °C. (b) Cs_2CO_3, MeOH, 0 °C. (c) RCO_2H, EDCI·HCl, TsOH, DMA. (d) $PhCH_2SO_2Cl$, pyridine, 0 °C. (e) TFA, CH_2Cl_2.

Scheme 13. Synthesis of Compounds 36–38 of Table 3[a]



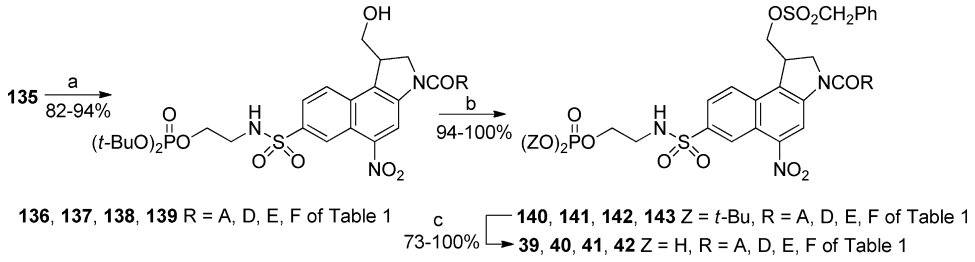
[a]Reagents and conditions: (a) RCO_2H, EDCI·HCl, TsOH, DMA. (b) TFA, CH_2Cl_2.

compounds **14–32** were evaluated by measuring IC_{50} values under oxic (20% O_2) and hypoxic (<20 ppm O_2) conditions and deriving a HCR [HCR = IC_{50}(oxic)/IC_{50}(hypoxic)] (Table 1 and Figures S2 and S3 in the Supporting Information). Data for previously reported[14] alcohols containing a chloride leaving group (**10**, **144–146**) are presented for comparison. These chlorides are suitable for comparison because of their closely matching structures, and **10** and **145** are of interest as they provided the highest HCRs of the eight sulfonamides or carboxamides in our previous report.[14] In our recent study on nitroCBIs containing sulfonate leaving groups but lacking A ring substituents, we reported that sulfonates with neutral side chains (e.g., TMI) showed consistently higher HCRs than the corresponding chloride analogues using IC_{50}-based assays.[16] We have also shown that nitroCBIs containing a bromide leaving group follow a similar trend to chlorides in regards to the side chain structure–activity relationship (SAR): those with basic side chains provide larger HCRs.[15]

Figure 2 illustrates data from Table 1 where IC_{50} values under oxic versus hypoxic conditions are plotted separately for HT29 and SiHa. In both cell lines, almost all compounds are displaced from the line indicating HCR = 1 (equivalent toxicity under both conditions). In HT29, the majority of the benzyl sulfonates are more toxic under both oxic and hypoxic conditions and also more hypoxia-selective than the methane sulfonates. The most selective benzyl sulfonates **17** and **24**, containing a neutral TMI side chain, have very large HCRs of 1370 and 1010, respectively. As previously suggested,[13] it is possible that H-bond donor capacity of sulfonamides or carboxamides enhances their reductase-substrate ability for hypoxic activation. This feature combined with higher $E(1)$ (7-EWG in A ring) both contribute to the high selectivity. In contrast, their previously reported chloride analogues (TMI side chain) showed low HCRs of 2.8 and 3.6, respectively, while the chlorides with a basic DEI side chain exhibited large HCRs of 60 and 330, respectively.[13] Other benzyl sulfonates exhibiting significant hypoxic selectivity are **14**, **18–21**, **22**, **25**, **29**, and **31** (HCR = 13–105), the majority of which have neutral side chains. The two highly selective previously reported[14] chlorides **10** (HCR = 160) and **145** (HCR = 485) containing a basic DEI side chain, compare well on the basis of hypoxia-selectivity with most of the benzyl sulfonates in HT29 cells.

The SAR in regards to leaving group and side chain in SiHa cells is more difficult to interpret than in HT29 cells (Figure 2). For instance, methane sulfonates **27** and **28** do show significant HCRs (17 and 27), faring better than several benzyl sulfonates. However, in general, the benzyl sulfonates are more selective. Notably, two of the most selective, **30** (HCR = 66) and **32** (HCR = 600), contain basic side chains. Other benzyl sulfonates exhibiting significant HCRs (19–70) are **14–16**, **19–21**, **24**, and **31**, none of which contain a basic side chain. In SiHa cells, the selective chlorides[14] **10** (HCR = 46) and **145** (HCR = 20) were comparable to the benzyl sulfonates. However, in both HT29 (**17** and **24**) and SiHa (**32**) cell lines, we here provide examples of nitroCBIs containing a benzyl sulfonate leaving group that appear markedly more hypoxia-

Scheme 14. Synthesis of Compounds 39–42 of Table 3[a]



[a]Reagents and conditions: (a) RCO_2H, EDCI·HCl, TsOH, DMA. (b) $PhCH_2SO_2Cl$, pyridine, 0 °C. (c) TFA, CH_2Cl_2.

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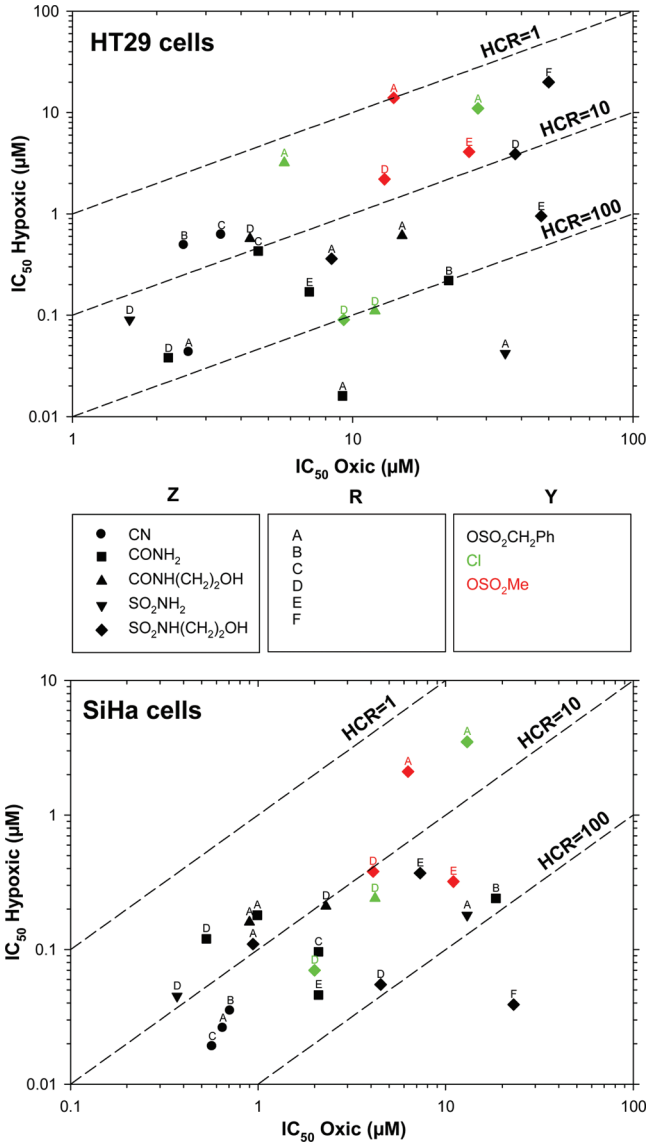

Figure 2. Cytotoxicity of sulfonates **14**–**32** (Table 1) and known[14] chlorides **10** and **144**–**146** under oxic and hypoxic conditions in HT29 and SiHa human tumor cell lines assessed using an antiproliferative assay. Z, R, and Y: see Table 1. Dashed lines: HCR = 1, 10, or 100.

selective than previously reported nitroCBIs containing a chloride leaving group. It is noteworthy that **17** is much less selective in SiHa than HT29 cells, and **32** is much less selective in HT29 than SiHa cells. Figure S4 (Supporting Information) illustrates cytotoxicity under oxic and hypoxic conditions in SiHa versus HT29 cells for sulfonates **14**–**32** and known[14] chlorides **10** and **144**–**146** of Table 1. For the oxic data, the intercept is significantly different (from zero) and indicates that HT29 IC_{50} values are, on average, 4.2-fold higher than in SiHa. None of the compounds are more toxic in HT29 than in SiHa under oxic conditions. There is no significant correlation under hypoxia, and this suggests different reductases at work in SiHa versus HT29. Furthermore, the spread of hypoxic IC_{50} data is much greater in HT29 (~1000-fold) than in SiHa (~20-fold). We recently reported[25] that for chloride **10** in SiHa, the oxic toxicity is a consequence of a low level of oxic reduction. If the same mechanism is true for the new sulfonates, then Figure S4 in the Supporting Information suggests that oxic reductases are shared between SiHa and HT29. It appears that in general SiHa is more sensitive than HT29. A combined summed ranking of HCRs for the 19 sulfonates evaluated in HT29 and SiHa indicates that compounds **24** and **20** receive the highest ranks (Figure S4 in the Supporting Information).

Benzyl sulfonates **17**, **19**, **20**, and **24** with high hypoxic selectivity in either HT29 or SiHa cells were examined in a panel of eight human tumor cell lines of various tissue origins using an antiproliferative assay (Tables 2 and S1 in the Supporting Information). All four compounds have neutral side chains, and compound **20** contains an alcohol that could be converted into a solubilizing phosphate group. Sizable HCRs in all cell lines (with the exception of **24** in H460 cells) were observed, indicating that the unidentified enzymes responsible for nitro reduction are widely expressed and that the bulky benzyl sulfonate does not hinder reduction to the point of compromising hypoxic selectivity. The TMI side chain presents no special advantages as compounds **19** and **20** exhibit HCRs of 34–644 and 38–374, respectively, that is, at least as large as for **17**. While **17**, **19**, and **20** contain a 7-$CONH_2$ substituent, **24** contains a 7-SO_2NH_2 group and, with the exception of H460, similarly showed substantial HCRs of 20–278.

The question arises as to whether there is an HCR advantage to combining both a bulky sulfonate leaving group and an A ring EWG. For a given A ring substituent, a switch from chloride to benzyl sulfonate almost always results in an improvement in HCR when the side chain is neutral and a reduction when the side chain is strongly basic, with the effect mostly being a product of different hypoxic IC_{50} values. If a bulky sulfonate leaving group is present, then a compound with an A ring EWG will generally show an improvement in HCR over a compound lacking such a substituent, but the effect depends on both the side chain and the cell line. The two effects (A ring substitution vs nature of the leaving group) have such different SARs that making a generalization is difficult.

We have previously reported cytotoxicities in both an antiproliferative assay (based on IC_{50} values) and a clonogenic assay (based on C_{10} values).[13,14] In the current study, the cytotoxicity of **19** was further evaluated in HT29 cells and **16**, **17**, **20**, **21**, and **23** in SiHa cells using a clonogenic assay.[27] All compounds exhibit much higher toxicity under hypoxic (<20 ppm O_2) than oxic (20% O_2) conditions [HCR = C_{10}(oxic)/C_{10}(hypoxic)], where cell killing (C_{10}) was quantified as the drug concentration required to lower the surviving fraction to 10% of that of controls. For instance, compound **19** provided a remarkably high HCR of 4090 in HT29 cells (cf. HCR = 40 in antiproliferative assay). The compounds evaluated in SiHa cells showed HCRs of 41–422 (cf. HCR = 5.8–64 in antiproliferative assay), and although the clonogenic assay ratios are higher (1.3–100-fold) than those of the antiproliferative assay, both assays demonstrate similar trends.

In Vivo Activity. We have previously shown that nitroCBIs incorporating a solubilizing phosphate group have excellent aqueous solubility (in the mM range), that the phosphate moiety of nitroCBI **11** is rapidly hydrolyzed in plasma to the corresponding alcohol, and that **11** at a nontoxic dose of 42 μmol/kg provides significant hypoxic tumor cell kill in a range of human tumor xenografts.[14] Higher doses (>100 μmol/kg) caused acute toxicity in some animals, and a maximum tolerated dose was difficult to define.[14] Thus, in the present study, the phosphates were administered at an intravenous (iv) dose of 42 μmol/kg to SiHa tumor-bearing immunocompromised mice either alone or 5 min after a single 15 Gy dose of ionizing

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radiation (sufficient to sterilize aerobic tumor cells). At this dose, no acute toxicity was observed for any of the analogues. Eighteen hours later, the tumors were excised, and surviving clonogens were assessed after 14 days to determine the number of colony-forming cells per gram of tumor tissue (Figure S5 in the Supporting Information).

The 7-carboxamide **33**, a phosphate analogue of alcohol **20**, contains a benzyl sulfonate leaving group and a phosphate moiety incorporated in the DNA-binding side chain. It exhibited weak activity as a single agent (nitroCBI alone), and the hypoxic LCK of radiation plus drug (vs radiation alone) was relatively low (0.37), with neither measure being statistically significant (Table 3 and Figure 3 where values for

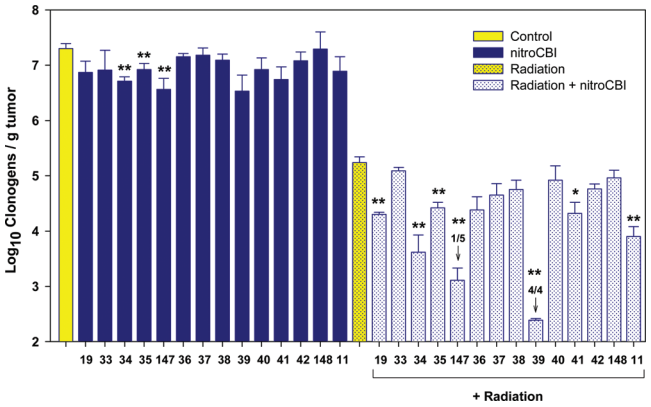

Figure 3. Antitumor activity of sulfonates **19** and **33**–**42** (Table 3) and known[14] chlorides **11**, **147**, and **148** in SiHa human tumor xenografts assayed by tumor excision 18 h after iv dosing with nitroCBI (42 or 13 μmol/kg for **19**) only or γ-irradiation (15 Gy) plus nitroCBI, followed by culturing for 14 days. Values are means ± SEMs for groups of three mice (control or nitroCBI alone) or five mice (radiation alone or radiation + nitroCBI). Values for control and radiation alone are averages ± SEMs from seven independent experiments. Downward arrow: the number of tumors for which no surviving clonogens could be detected within sample size and detection limits of the assay. *, $p < 0.05$; **, $p < 0.01$; calculated using intraexperiment controls.

control alone and radiation alone are averages of all independent experiments). Interestingly, 7-carboxamide **19** with the neutral MS side chain and the absence of a phosphate moiety provided a statistically significant hypoxic LCK of 1.16 at the lower dose (limited due to poor solubility) of 13 μmol/kg.

Secondary 7-carboxamides **34** and **35**, which incorporate a phosphate in the carboxamide side chain and contain a benzyl sulfonate leaving group, both provided statistically significant hypoxic LCKs of 1.78 and 0.98, respectively. They also exhibited weak but statistically significant single agent activity. Although **34** (having a neutral TMI side chain) is superior to **35** (having a basic DEI side chain), it was not as active as the previously reported[14] chloride analogue **147** (having a basic DEI side chain) with hypoxic LCK of 2.21.

Secondary 7-sulfonamides **36**–**38** contain a methane sulfonate leaving group and provided a hypoxic LCK range of 0.33–0.71 (Table 1); **36** (with a neutral TMI side chain) and **38** (with a neutral MS side chain) are superior to **37** (with a basic DEI side chain).

Secondary 7-sulfonamides **39**–**42** incorporate a phosphate in the sulfonamide side chain and contain a benzyl sulfonate leaving group. These compounds exhibited hypoxic LCKs of 0.18–2.71. An irradiation dose of 15 Gy provided 2.14 logs of cell kill as compared to control (Table 3 for **39**). When **39** was combined with radiation, a greater cell kill was achieved (4.85 log), indicating elimination of radioresistant hypoxic cells within the tumors by the nitroCBI. Compound **39** (with a neutral TMI side chain) was easily the most active of all compounds tested with no clonogens detected in four of four treated tumors (the number quoted is calculated assuming one colony detected for each tumor at the highest number of cells plated; i.e., the true hypoxic LCK will be greater than the reported value of 2.71). This compares favorably with the previously reported[14] two chloride comparators **148** (hypoxic LCK = 0.54) and **11** (hypoxic LCK = 1.83). In the sulfonate series, compounds **39** and **41** with neutral side chains provide greater hypoxic LCKs than **40** (having a DEI side chain) and **42** (having a MEI side chain) with basic side chains, whereas in the chloride series the activity is reversed with **13** (with a basic DEI side chain) exhibiting the greater hypoxic LCK. The moderate in vivo data for **40** and **42** is in contrast to their alcohol analogues **30** and **32**, which display high selectivity in SiHa cells in vitro (Table 1). In a recent report,[15] we have shown that nitroCBIs containing a bromide leaving group follow a similar trend to chlorides in regards to side chain with the bromide analogue of **42** (with a basic side chain) providing a hypoxic LCK of 2.80 in SiHa tumor-bearing mice and the bromide analogue of **39** (with a neutral side chain) providing a hypoxic LCK of 1.84.

Six phosphates were also assayed in H460 (human large-cell lung tumor) xenografts at 75 μmol/kg, which again proved to be a nontoxic dose (Table S2 in the Supporting Information). Although large HCRs were observed in vitro (Tables 2 and S1 in the Supporting Information) for H460, the relatively high IC_{50} values (Table S1 in the Supporting Information) indicate that it seems to be a relatively resistant cell line. In the event, hypoxic LCKs were mostly disappointing ranging from 0 to 0.60 with little evidence of any single agent activity. The most active compound was the methane sulfonate **36** (having a TMI side chain) with a hypoxic LCK of 0.60.

CONCLUSIONS

The current study was performed to investigate SARs influencing cytotoxicity and hypoxic selectivity for a series of nitroCBI prodrugs and their more water-soluble phosphate preprodrugs. The new compounds contain a sulfonate leaving group (benzyl sulfonate or methane sulfonate) and a 7-EWG, two structural modifications that were shown to independently increase the hypoxic selectivity of related nitroCBIs.[13−16] Although the target nitroCBIs are structurally more complex than the unsubstituted parents,[16] their syntheses proved generally easier, as previously reported for chlorides.[13,14] Sulfonate leaving groups[16] are more prone to elimination under basic conditions than the chloride leaving group; however, if the sulfonate group was introduced late in the synthesis and appropriate care was taken, this side reaction was greatly minimized. Moreover, we found during syntheses of nitroCBIs that the sulfonate leaving group was less susceptible to elimination than a bromide.[15] In general, the very good hypoxic selectivity and antitumor activity observed for a number of the sulfonates justified the synthetic effort.

The more bulky benzyl sulfonates proved more hypoxia-selective in vitro than methane sulfonates (Table 1 and Figure 2), as observed previously with A ring unsubstituted

sulfonates.[16] A subset of the 7-substituted nitroCBIs with neutral side chains (e.g., **17** and **24**) was significantly more hypoxia-selective than those with basic side chains, as also shown previously for unsubstituted parents.[16] Although a subset of three primary 7-carboxamides and one 7-sulfonamide with neutral side chains showed significant hypoxic selectivity across a cell line panel, there were some exceptions to this correlation. For instance, compound **32** (having a weakly basic MEI side chain) showed the largest HCR (IC_{50} ratio) in SiHa cells of all of the compounds tested. Unlike their primary parents (e.g., **17** and **24**), neutral side chains combined with secondary 7-carboxamides (e.g., **22**) or 7-sulfonamides (e.g., **26** and **29**) did not appear to provide a marked increase in hypoxic selectivity in vitro. This leaves open the possibility that the very high HCRs seen for some sulfonates bearing a TMI side chain are not a consequence of the fact that the side chain is neutral but of some other as yet unexplained property. It would be useful to understand toxicity differentials between sulfonate prodrug and effector, made difficult by the intrinsic instability of the latter due to the powerful leaving group effect of the sulfonate, as better leaving groups might be expected to increase cytotoxic potency. However, several sulfonates displayed nanomolar hypoxic cytotoxicities (Table 1).

We have described two ways of increasing the hypoxic selectivity of nitroCBIs: adding an appropriate EWG to the A ring[13,14] or incorporating a bulky leaving group.[16] Although both are capable of providing compounds with high HCRs, the former is possibly more powerful since the A ring substituent alone can give consistently high HCRs across a cell line panel,[13,14] whereas the effect of the sulfonate leaving group is more cell line-dependent,[16] as shown for **17** and **32**. The current study demonstrates that combining both effects can give highly hypoxia-selective compounds.

The antitumor activity for the phosphate analogues also demonstrated the previously observed correlation in regard to side chain, with **34** and **39** (both having a benzyl sulfonate leaving group and neutral TMI side chain) providing the greatest hypoxic LCKs of the new compounds tested. Overall, phosphates with neutral side chains provided greater hypoxic LCKs than those with basic side chains, which was opposite to that shown previously for chloride[14] or bromide[15] analogues. Although we do not have an explanation for this observation, it is potentially useful as basic side chains may compromise distribution (by sequestration in acidic organelles) or metabolism. The association between in vitro and in vivo data is not complete; for instance, the alcohol analogue (**29**) of phosphate **39** exhibited relatively moderate HCRs (IC_{50} ratios) in vitro in the two cell lines in which it was examined. Nevertheless, **39** was extremely active against hypoxic cells in xenografts of one of these two cell lines. This outstanding in vivo activity of **39** advocates it as a viable hypoxia-activated preprodrug for cancer therapy, effective against the most resistant tumor cell population.

EXPERIMENTAL SECTION

General Chemistry Methods. Final products were analyzed by reverse-phase HPLC (Alltima C18 5 μm column, 150 mm × 3.2 mm; Alltech Associated, Inc., Deerfield, IL) using an Agilent HP1100 equipped with a diode array detector. The mobile phase was 80% CH_3CN/20% H_2O (v/v) in 45 mM HCO_2NH_4 at pH 3.5 and 0.5 mL/min. The purity was determined by monitoring at 330 ± 50 nm and was ≥95% for final products unless otherwise stated. The 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 and 100 MHz for ^{13}C spectra. "Methods" indicate alternative routes to the same compound.

*Synthesis of Compounds in Table 1 and their Intermediates. tert-Butyl 7-Cyano-1-(iodomethyl)-1H- benzo[e]indole-3(2H)-carboxylate (**44**) (Scheme 1).* A solution of *tert*-butyl 1-(chloromethyl)-7-cyano-1*H*-benzo[*e*]indole-3(2*H*)-carboxylate[13] (**43**) (10.00 g, 29.2 mmol) in anhydrous 2-butanone (100 mL) was treated with NaI (26.30 g, 175.5 mmol) and heated under reflux for 5 days. The mixture was cooled to room temperature and then evaporated to dryness, and the residue was dissolved in $CHCl_3$, washed with 5% aqueous sodium disulfite and brine, dried, and concentrated under reduced pressure. The residue was purified by column chromatography on silica gel eluting with petroleum ether/EtOAc (5:1) and further recrystallized from CH_2Cl_2/petroleum ether to give **44** as a pale yellow solid (12.40 g, 98%): mp 141−143 °C. ^{1}H NMR [$(CD_3)_2SO$]: δ 8.52 (d, J = 1.2 Hz, 1H), 8.11 (br, s, 1H), 8.04 (dd, J = 14.3, 8.7 Hz, 1H), 8.01 (dd, J = 8.8, 3.7 Hz, 1H), 7.73 (dd, J = 8.7, 1.5 Hz, 1H), 4.22−4.10 (m, 2H), 3.89 (dd, J = 11.1, 2.4 Hz, 1H), 3.68 (dd, J = 10.2, 3.0 Hz, 1H), 3.64−3.60 (m, 1H), 1.55 (s, 9H). Anal. calcd for $C_{19}H_{19}IN_2O_2$: C, 52.55; H, 4.41; N, 6.45. Found: C, 52.83; H, 4.41; N, 6.50.

*1-(Iodomethyl)-3-(2,2,2-trifluoroacetyl)-2,3-dihydro-1H-benzo[e]-indole-7-carbonitrile (**45**) (Scheme 1). Method A.* A solution of **44** (12.30 g, 28.3 mmol) in CH_2Cl_2 (350 mL) was treated with TFA (65 mL, 849.0 mmol) at 0 °C. The mixture was stirred overnight at room temperature and concentrated under reduced pressure to give 1-(iodomethyl)-7-cyano-1,2-dihydro-3*H*-benzo[*e*]indole as a red foam. The residue was then dissolved in pyridine (200 mL), cooled to 0 °C, and treated with trifluoroacetic anhydride (TFAA) (10 mL, 71.8 mmol). The mixture was stirred at 0 °C for 2 h and then poured into a beaker of ice water, and the resulting precipitate was extracted into CH_2Cl_2 (3×). The combined organic layers were washed with HCl (1 M), water, and brine, dried, and concentrated under reduced pressure. The residue was purified by column chromatography on silica gel eluting with petroleum ether/EtOAc (7:3) and further recrystallized from CH_2Cl_2/petroleum ether to give **45** as a pale yellow solid (10.93 g, 90%): mp 187−189 °C. ^{1}H NMR [$(CD_3)_2SO$]: δ 8.66 (d, J = 1.5 Hz, 1H), 8.44 (d, J = 9.0 Hz, 1H), 8.20 (d, J = 8.7 Hz, 1H), 8.12 (d, J = 9.0, 1H), 7.86 (dd, J = 8.7, 1.5 Hz, 1H), 4.57 (dd, J = 10.8, 9.3 Hz, 1H), 4.37−4.33 (m, 1H), 4.28 (dt, J = 11.3, 1.8 Hz, 1H), 3.79 (dd, J = 10.4, 3.1 Hz, 1H), 3.72 (dd, J = 10.3, 5.9 Hz, 1H). HRMS (FAB) calcd for $C_{16}H_{11}F_3IN_2O$ (MH^+) m/z, 430.9868; found, 430.9867.

*(7-Cyano-3-(2,2,2-trifluoroacetyl)-2,3-dihydro-1H-benzo[e]indol-1-yl)methyl Acetate (**47**) (Scheme 1). Method A.* A solution of **45** (15.40 g, 35.8 mmol) in glacial acetic acid (300 mL) was treated with AgOAc (18.80 g, 112.6 mmol) and heated under reflux for 48 h. The mixture was cooled to room temperature and then evaporated to dryness, and the dark brown residue was dissolved in EtOAc, passed through a short pad of Celite, washed with water and brine, dried, and concentrated under reduced pressure. The residue was purified by chromatography eluting with petroleum ether/EtOAc (5:1, 4:1 and 3:1) to give **47** as a pale yellow solid (12.97 g, 100%): mp 158−160 °C. ^{1}H NMR [$(CD_3)_2SO$]: δ 8.64 (d, J = 1.3 Hz, 1H), 8.43 (d, J = 9.0 Hz, 1H), 8.21 (d, J = 8.7 Hz, 1H), 8.12 (d, J = 9.0 Hz, 1H), 7.87 (dd, J = 8.7, 1.6 Hz, 1H), 4.53 (dd, J = 10.8, 8.8 Hz, 1H), 4.46−4.36 (m, 2H), 4.35−4.29 (m, 1H), 4.28−4.22 (m, 1H), 1.89 (s, 3 H). ^{13}C NMR [$(CD_3)_2SO$]: δ 170.0, 153.6 (q, J_{CF} = 37 Hz), 142.1, 135.0, 130.6, 130.3, 130.1, 127.5, 127.0, 125.1, 118.9, 118.0, 116.4 (q, J_{CF} = 288 Hz), 107.7, 64.8, 52.2, 38.7, 20.3. Anal. calcd for $C_{18}H_{13}F_3N_2O_3$: C, 59.67; H, 3.62; N, 7.73. Found: C, 59.72; H, 3.72; N, 7.81.

*(7-Cyano-5-nitro-3-(2,2,2-trifluoroacetyl)-2,3-dihydro-1H-benzo-[e]indol-1-yl)methyl Acetate (**57**) and (7-Cyano-9-nitro-3-(2,2,2-trifluoroacetyl)-2,3-dihydro-1H-benzo[e]indol-1-yl)methyl Acetate (**56**) (Scheme 1).* Compound **47** (2.36 g, 6.51 mmol) was ground to a powder and dissolved in 98% H_2SO_4 (20 mL) at −10 °C and stirred for 10 min. Anhydrous KNO_3 (857 mg, 8.49 mmol) was added portion wise, and the mixture was stirred for a further 7 min, then

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poured into a beaker of ice water, and stirred for 10 min. The resulting precipitate was extracted with EtOAc (3×), and the combined organic layers were washed with water and brine, dried, and concentrated under reduced pressure. The residue was purified by column chromatography on silica gel eluting with petroleum ether/EtOAc (3:2) and further recrystallized from CH_2Cl_2/MeOH to give **57** as a yellow solid (1.72 g, 65%): mp 188−190 °C. ^{1}H NMR [$(CD_3)_2SO$]: δ 9.10 (s, 1H, H-4), 8.90 (d, J = 1.0 Hz, 1H, H-6), 8.44 (d, J = 8.7 Hz, 1H, H-9), 8.09 (dd, J = 8.8, 1.5 Hz, 1H, H-8), 4.59 (dd, J = 11.0, 9.1 Hz, 1H, H-2), 4.52−4.41 (m, 3H, H-1, H-2, CH_2OAc), 4.34 (dd, J = 11.0, 5.4 Hz, 1H, CH_2OAc), 1.88 (s, 3H, $COCH_3$). ^{13}C NMR [$(CD_3)_2SO$]: δ 170.0 ($COCH_3$), 154.1 (q, J_{CF} = 37 Hz, $COCF_3$), 146.4 (C-5), 140.7 (C-3a), 134.1 (C-9b), 130.7 (C-9a), 130.1 (C-6), 128.9 (C-8), 126.2 (C-9), 121.6 (C-5a), 119.7 (CN), 118.3 (C-4), 115.5 (q, J_{CF} = 288 Hz, CF_3), 111.0 (C-7), 64.7 (CH_2OAc), 52.5 (C-2), 39.2 (C-1), 20.2 ($COCH_3$) (full assignments and 5-nitro structure determined by 2D NMR experiments: COSY, NOESY, HSQC, HMBC). Anal. calcd for $C_{18}H_{12}F_3N_3O_5$: C, 53.08; H, 2.97; N, 10.32. Found: C, 53.03; H, 2.93; N, 10.28; and **56** as a yellow solid (140 mg, 5%): mp 176−178 °C (MeOH). ^{1}H NMR [$(CD_3)_2SO$]: δ 9.00 (d, J = 1.6 Hz, 1H), 8.67 (d, J = 9.1 Hz, 1H), 8.62 (d, J = 1.6 Hz, 1H), 8.36 (d, J = 9.1 Hz, 1H), 4.55 (dd, J = 11.1, 8.5 Hz, 1H), 4.32−4.26 (m, 1H), 4.05 (dd, J = 11.1, 5.3 Hz, 1H), 3.96 (dd, J = 11.1, 5.8 Hz, 1H), 3.89−3.82 (m, 1H), 1.76 (s, 3H). ^{13}C NMR [$(CD_3)_2SO$]: δ 169.6, 154.0 (q, J_{CF} = 37 Hz), 146.6, 145.5, 139.9, 132.3, 131.7, 125.3, 123.9, 122.0, 119.4, 117.0, 115.5 (q, J_{CF} = 288 Hz), 106.4, 65.3, 51.8, 39.6, 20.0. Anal. calcd for $C_{18}H_{12}F_3N_3O_5$: C, 53.08; H, 2.97; N, 10.32. Found: C, 53.15; H, 3.01; N, 10.27.

*1-(Hydroxymethyl)-5-nitro-2,3-dihydro-1H-benzo[e]indole-7-carbonitrile (**58**) (Scheme 1).* A solution of **57** (320 mg, 0.79 mmol) in THF (20 mL) and water (6 mL) was treated with Cs_2CO_3 (768 mg, 2.36 mmol). The mixture was heated under reflux for 2 h, cooled to room temperature, treated with KOH (142 mg, 2.53 mmol) in water (1 mL), and heated under reflux for a further 3 h. The solution was concentrated under reduced pressure, and the residue was dissolved in EtOAc, washed with water and brine, dried, and concentrated under reduced pressure. The crude product was purified by column chromatography on silica gel using petroleum ether/EtOAc (1:1 then neat EtOAc) to give **58** as a red solid (206 mg, 97%): mp (EtOAc/petroleum ether) 206−209 °C. ^{1}H NMR [$(CD_3)_2SO$]: δ 8.55 (d, J = 1.4 Hz, 1H, H-6), 7.93 (d, J = 8.8 Hz, 1H, H-9), 7.75 (s, 1H, H-4), 7.70 (dd, J = 8.8, 1.6 Hz, 1H, H-8), 6.71 (s, 1H, NH), 4.99 (t, J = 5.5 Hz, 1H, OH), 3.88−3.76 (m, 2H, H-1, H-2), 3.71 (dd, J = 9.6, 2.5 Hz, 1H, H-2), 3.65−3.58 (m, 1H, CH_2OH), 3.51−3.42 (m, 1H, CH_2OH). ^{13}C NMR [$(CD_3)_2SO$]: δ 151.8 (C-3a), 146.7 (C-5), 131.8 (C-9a), 129.8 (C-6), 127.3 (C-8), 127.0 (C-9b), 124.4 (C-9), 119.3 (CN), 116.7 (C-5a), 110.5 (C-4), 105.4 (C-7), 62.3 (CH_2OH), 50.2 (C-2), 43.5 (C-1) (full assignments and structure determined by 2D NMR experiments: COSY, NOESY, HSQC, HMBC). HRMS (EI) calcd for $C_{14}H_{11}N_3O_3$ (M+) m/z, 269.08004; found, 269.07969.

*1-(Hydroxymethyl)-5-nitro-3-(5,6,7-trimethoxy-1H-indole-2-carbonyl)-2,3-dihydro-1H-benzo[e]indole-7-carbonitrile (**59**) (Scheme 5).* A mixture of **58** (101 mg, 0.38 mmol), 5,6,7-trimethoxy-1*H*-indole-2-carboxylic acid (123 mg, 0.49 mmol), anhydrous TsOH (71 mg, 0.42 mmol), and EDCI·HCl (0.29 g, 1.52 mmol) in dry dimethylacetamide (DMA) (2 mL) was stirred at room temperature for 17 h. Dilute aqueous $NaHCO_3$ was added, and the mixture was stirred for several minutes. The precipitated solid was filtered off, washed with water, and dried in a vacuum desiccator. The solid was triturated with hot EtOAc to give **59** as a yellow solid (155 mg, 82%): mp 254−256 °C. ^{1}H NMR [$(CD_3)_2SO$]: δ 11.57 (d, J = 1.4 Hz, 1H), 9.26 (s, 1H), 8.87 (d, J = 1.1 Hz, 1H), 8.33 (d, J = 8.6 Hz, 1H), 7.98 (dd, J = 8.8, 1.5 Hz, 1H), 7.21 (d, J = 2.2 Hz, 1H), 6.98 (s, 1H), 5.06 (t, J = 5.6 Hz, 1H), 4.86−4.79 (m, 1H), 4.65 (dd, J = 10.5, 2.3 Hz, 1H), 4.22−4.16 (m, 1H), 3.94 (s, 3H), 3.83 (s, 3H), 3.81 (s, 3H), 3.78−3.71 (m, 1H), 3.70−3.63 (m, 1H). Anal. calcd for $C_{26}H_{22}N_4O_7\cdot H_2O$: C, 60.00; H, 4.65; N, 10.76. Found: C, 59.94; H, 4.39; N, 10.75.

*(7-Cyano-5-nitro-3-(5,6,7-trimethoxy-1H-indole-2-carbonyl)-2,3-dihydro-1H-benzo[e]indole-1-yl)methyl phenylmethanesulfonate (**14**) (Scheme 5).* α-Toluenesulfonyl chloride (39 mg, 0.20 mmol) was added to a solution of **59** (85 mg, 0.17 mmol) in pyridine (2 mL) at 0 °C, and the mixture was stirred at this temperature for 10 min. More α-toluenesulfonyl chloride (19 mg, 0.10 mmol) was added, and after a further 20 min, ice-cold water (5 mL) was added. The mixture was stirred at 0 °C for 10 min, and then, the precipitated solid was filtered off, washed with water, and dried in a vacuum desiccator. The crude product was triturated with EtOAc at room temperature to give **14** as a yellow solid (85 mg, 77%): mp 219−222 °C. ^{1}H NMR [$(CD_3)_2SO$]: δ 11.62 (d, J = 1.6 Hz, 1H), 9.24 (s, 1H), 8.87 (d, J = 1.1 Hz, 1H), 8.31 (d, J = 8.8 Hz, 1H), 8.03 (dd, J = 8.8, 1.5 Hz, 1H), 7.23−7.12 (m, 6H), 6.98 (s, 1H), 4.92−4.86 (m, 1H), 4.62−4.49 (m, 6H), 3.94 (s, 3H), 3.84 (s, 3H), 3.82 (s, 3H). Anal. calcd for $C_{33}H_{28}N_4O_9S\cdot H_2O$: C, 58.75; H, 4.48; N, 8.30. Found: C, 58.78; H, 4.28; N, 8.23.

*tert-Butyl 7-Cyano-1-(hydroxymethyl)-5-nitro-1H-benzo[e]-indole-3(2H)-carboxylate (**60**) (Scheme 5).* A solution of **58** (187 mg, 0.69 mmol) in anhydrous THF (30 mL) and under N_2 was treated with di-*tert*-butyl dicarbonate (606 mg, 2.78 mmol). The mixture was heated under reflux for 24 h, cooled to room temperature, and then evaporated to dryness, and the residue was purified by column chromatography eluting with petroleum ether/EtOAc (4:1, 3:2 and 1:1) to give **60** as a yellow solid (171 mg, 67%): mp (petroleum ether/EtOAc) 107−111 °C. ^{1}H NMR [$(CD_3)_2SO$]: δ 8.87 (br, s, 1H), 8.82 (d, J = 1.1 Hz, 1H), 8.23 (d, J = 8.8 Hz, 1H), 7.93 (dd, J = 8.8, 1.5 Hz, 1H), 5.02 (m, 1H), 4.21−4.12 (m, 2H), 4.07−4.00 (m, 1H), 3.72−3.69 (m, 1H), 3.63−3.59 (m, 1H), 1.56 (s, 9H). Anal. calcd for $C_{19}H_{19}N_3O_5\cdot 0.2$EtOAc: C, 61.45; H, 5.37; N, 10.86. Found: C, 61.92; H, 5.77; N, 10.51.

*tert-Butyl 1-(((Benzylsulfonyl)oxy)methyl)-7-cyano-5-nitro-1H-benzo[e]indole-3(2H)-carboxylate (**61**) (Scheme 5).* Compound **60** (170 mg, 0.46 mmol) was dissolved in CH_2Cl_2 (15 mL) and pyridine (149 μL, 1.84 mmol), cooled to 0 °C, and then treated with α-toluenesulfonyl chloride (263 mg, 1.38 mmol). The mixture was stirred at room temperature for a further 6 h, diluted with CH_2Cl_2, washed with water and brine, dried, and concentrated under reduced pressure. The residue was purified by column chromatography eluting with petroleum ether/EtOAc (4:1) to give **61** as a yellow solid (201 mg, 83%): mp (petroleum ether/EtOAc) 139−141 °C. ^{1}H NMR [$(CD_3)_2SO$]: δ 8.82 (d, J = 1.2 Hz, 1H), 8.82 (br, s, 1H), 8.19 (d, J = 8.8 Hz, 1H), 7.97 (dd, J = 8.8, 1.5 Hz, 1H), 7.34−7.22 (m, 5H), 4.65 (br, s, 2H), 4.47 (d, J = 2.0 Hz, 1H), 4.45 (br, s, 1H), 4.41−4.35 (m, 1H), 4.27−4.22 (m, 1H), 4.08 (dd, J = 11.5, 2.7 Hz, 1H), 1.56 (s, 9H). Anal. calcd for $C_{26}H_{25}N_3O_7S$: C, 59.65; H, 4.81; N, 8.03. Found: C, 59.68; H, 4.92; N, 8.15. Preparation of this intermediate using Et_3N as a base was also successful, but a lower yield (64%) was obtained.

*(7-Cyano-5-nitro-2,3-dihydro-1H-benzo[e]indol-1-yl)methyl Phenylmethanesulfonate (**62**) (Scheme 5).* A solution of **61** (200 mg, 0.38 mmol) in CH_2Cl_2 (10 mL) was treated with TFA (883 μL, 11.5 mmol) and stirred overnight at room temperature. The mixture was then poured into a beaker of ice water and extracted with CH_2Cl_2 (3×), and the combined organic layers were washed with water and brine, dried, and concentrated under reduced pressure to give a red solid. The residue was purified by column chromatography eluting with petroleum ether/EtOAc (1:1) and further recrystallized from CH_2Cl_2/*i*-Pr_2O to give **62** as a red solid (120 mg, 75%): mp 154−156 °C. ^{1}H NMR [$(CD_3)_2SO$]: δ 8.55 (d, J = 1.1 Hz, 1H), 7.86 (d, J = 8.7 Hz, 1H), 7.77 (s, 1H), 7.75 (dd, J = 8.8, 1.6 Hz, 1H), 7.34−7.30 (m, 5H), 6.87 (br s, 1H), 4.69 (s, 2H), 4.30−4.29 (m, 2H), 4.22−4.15 (m, 1H), 3.86−3.80 (m, 1H), 3.63 (dd, J = 10.5, 2.5 Hz, 1H). Anal. calcd for $C_{21}H_{17}N_3O_5S\cdot 0.2$EtOAc: C, 59.37; H, 4.25; N, 9.53. Found: C, 59.07; H, 4.18; N, 9.59.

*(7-Cyano-3-(5-(2-hydroxyethoxy)-1H-indole-2-carbonyl)-5-nitro-2,3-dihydro-1H-benzo[e]indol-1-yl)methyl Phenylmethanesulfonate (**15**) (Scheme 5).* A solution of **62** (39 mg, 0.09 mmol) in dry DMA (1 mL) was treated with anhydrous TsOH (17 mg, 0.10 mmol), 5-(2-hydroxyethoxy)-1*H*-indole-2-carboxylic acid (26 mg, 0.12 mmol), and EDCI·HCl (71 mg, 0.37 mmol). The mixture was stirred at room temperature for 2 h, then cooled to 0 °C, treated with 5% aqueous $NaHCO_3$, and stirred for further 10 min. The precipitate was collected

by filtration, washed with cold water and small portions of ice-cold EtOAc, and then dried in a vacuum desiccator. The resulting solid was recrystallized from CH_2Cl_2/diethyl ether in a cold room (+5 °C) to give **15** as a yellow solid (30 mg, 53%): mp 239−242 °C. ^{1}H NMR [$(CD_3)_2SO$]: δ 11.74 (d, J = 1.6 Hz, 1H), 9.29 (s, 1H), 8.86 (d, J = 1.2 Hz, 1H), 8.32 (d, J = 8.8 Hz, 1H), 8.03 (dd, J = 8.8, 1.5 Hz, 1H), 7.44 (d, J = 8.9 Hz, 1H), 7.32−7.11 (m, 7H), 6.98 (dd, J = 8.9, 2.4 Hz, 1H), 4.95−4.90 (m, 1H), 4.84 (t, J = 5.5 Hz, 1H), 4.67−4.51 (m, 6H), 4.02 (t, J = 5.1 Hz, 2H), 3.76 (q, J = 5.3 Hz, 2H). Anal. calcd for $C_{32}H_{26}N_4O_8S{\cdot}0.5CH_2Cl_2$: C, 58.34; H, 4.07; N, 8.37. Found: C, 58.10; H, 4.21; N, 8.53.

*1-(Hydroxymethyl)-5-nitro-2,3-dihydro-1H-benzo[e]indole-7-carboxamide (**63**) (Scheme 1).* A solution of **57** (1.71 g, 4.20 mmol) in DMSO (35 mL) was treated with H_2O_2 (10 mL, 35% aqueous solution, 103.4 mmol) and K_2CO_3 (2.10 g, 15.2 mmol). The mixture was stirred overnight at room temperature and then poured into a beaker of ice water and stirred for further 2 h. The solution was extracted with EtOAc (3×), and the combined organic layers were washed with water and brine and concentrated under reduced pressure. The residue was triturated with diethyl ether and dried in a vacuum oven (35 °C) for 48 h. Purification by column chromatography eluting with EtOAc/MeOH (20:1) followed by recrystallization from CH_2Cl_2/MeOH afforded **63** as dark red needles (910 mg, 75%): mp 219−221 °C. ^{1}H NMR [$(CD_3)_2SO$]: δ 8.63 (d, J = 1.3 Hz, 1H), 8.06 (br, s, 1H), 7.93 (dd, J = 8.9, 1.7 Hz, 1H), 7.83 (d, J = 8.8 Hz, 1H), 7.64 (s, 1H), 7.37 (br s, 1H), 6.38 (d, J = 1.4 Hz, 1H), 4.96 (t, J = 5.5 Hz, 1H), 3.87−3.80 (m, 1H), 3.78−3.62 (m, 3H), 3.47−3.39 (m, 1H). ^{13}C NMR [$(CD_3)_2SO$]: δ 167.6, 150.4, 147.5, 131.8, 129.2, 126.7, 125.5, 123.4, 122.8, 117.3, 109.4, 62.1, 50.1, 43.9. Anal. calcd for $C_{14}H_{13}N_3O_4$: C, 58.53; H, 4.56; N, 14.63. Found: C, 58.26; H, 4.69; N, 14.40.

TLC and product analysis showed that the order of reaction was as follows: hydrolysis of the trifluoroacetamide, then nitrile, then acetate, to give sequentially more polar products. If the reaction was worked up at shorter times, the product with the acetate still intact could be isolated by column chromatography eluting with EtOAc to give (7-carbamoyl-5-nitro-2,3-dihydro-1*H*-benzo[*e*]indol-1-yl)methyl acetate as a red-orange solid: mp 211−214 °C. ^{1}H NMR [$(CD_3)_2SO$]: δ 8.63 (s, 1H), 8.08 (br, s, 1H), 7.97 (d, J = 8.8 Hz, 1H), 7.86 (d, J = 8.8 Hz, 1H), 7.67 (s, 1H), 7.40 (br, s, 1H), 6.49 (br, s, 1H), 4.28 (dd, J = 10.0, 4.4 Hz, 1H), 4.13−4.00 (m, 2H), 3.82−3.74 (m, 1H), 3.65−3.57 (m, 1H), 2.00 (s, 3H). ^{13}C NMR [$(CD_3)_2SO$]: δ 170.3, 167.5, 150.5, 148.0, 131.8, 129.3, 125.9, 124.7, 123.4, 122.4, 117.3, 109.5, 63.9, 50.4, 39.9, 20.6. Anal. calcd for $C_{16}H_{15}N_3O_5$: C, 58.36; H, 4.59; N, 12.76. Found: C, 58.33; H, 4.66; N, 12.31.

*1-(Hydroxymethyl)-5-nitro-3-(5,6,7-trimethoxy-1H-indole-2-carbonyl)-2,3-dihydro-1H-benzo[e]indole-7-carboxamide (**64**) (Scheme 6).* A mixture of **63** (62 mg, 0.22 mmol), 5,6,7-trimethoxy-1*H*-indole-2-carboxylic acid (70 mg, 0.29 mmol), anhydrous TsOH (41 mg, 0.24 mmol), and EDCI·HCl (165 mg, 0.88 mmol) in dry DMA (1.5 mL) was stirred at room temperature for 16 h. Dilute aqueous $NaHCO_3$ was added, and the brown suspension was stirred at room temperature for 20 min. The solid was filtered off, washed with aqueous $NaHCO_3$ and then water, dried, and triturated with hot EtOAc to give **64** as a brown solid (87 mg, 77%): mp 242−246 °C (dec). ^{1}H NMR [$(CD_3)_2SO$]: δ 11.54 (s, 1H), 9.14 (s, 1H), 8.86 (d, J = 1.3 Hz, 1H), 8.26 (br, s, 1H), 8.22 (d, J = 8.8 Hz, 1H), 8.13 (dd, J = 8.8, 1.6 Hz, 1H), 7.58 (br s, 1H), 7.19 (s, 1H), 6.98 (s, 1H), 5.07 (t, J = 5.2 Hz, 1H), 4.84−4.76 (m, 1H), 4.65 (dd, J = 10.5, 2.2 Hz, 1H), 4.19−4.12 (m, 1H), 3.95 (s, 3H), 3.83 (s, 3H), 3.81 (s, 3H), 3.80−3.75 (m, 1H), 3.68−3.62 (m, 1H). Anal. calcd for $C_{26}H_{24}N_4O_8{\cdot}H_2O$: C, 57.99; H, 4.87; N, 10.40. Found: C, 58.31; H, 4.75; N, 10.36.

*(7-Carbamoyl-5-nitro-3-(5,6,7-trimethoxy-1H-indole-2-carbonyl)-2,3-dihydro-1H-benzo[e]indol-1-yl)methyl Phenylmethanesulfonate (**17**) (Scheme 6).* α-Toluenesulfonyl chloride (62 mg, 0.32 mmol) was added to a solution of **64** (85 mg, 0.16 mmol) in pyridine (5 mL) at 0 °C, and the mixture was stirred at this temperature for 20 min. More α-toluenesulfonyl chloride (14 mg, 0.08 mmol) was added, and after a further 15 min, ice water (10 mL) was added. The mixture was stirred at 0 °C for several minutes, and the precipitated solid was filtered off, washed with water, and dried. The crude product was triturated with EtOAc to give **17** as a yellow-orange solid (53 mg, 49%): mp 164−168 °C. ^{1}H NMR [$(CD_3)_2SO$]: δ 11.67 (s, 1H), 9.14 (s, 1H), 8.86 (d, J = 1.1 Hz, 1H), 8.33 (br, s, 1H), 8.21 (d, J = 8.8 Hz, 1H), 8.15 (dd, J = 8.8, 1.5 Hz, 1H), 7.66 (br, s, 1H), 7.22−7.11 (m, 6H), 6.98 (s, 1H), 4.92−4.85 (m, 1H), 4.66−4.49 (m, 6H), 3.94 (s, 3H), 3.83 (s, 3H), 3.81 (s, 3H). Anal. calcd for $C_{33}H_{30}N_4O_{10}S{\cdot}0.5H_2O$: C, 57.97; H, 4.57; N, 8.19. Found: C, 57.81; H, 4.51; N, 8.08.

*tert-Butyl 7-Carbamoyl-1-(hydroxymethyl)-5-nitro-1H-benzo[e]-indole-3(2H)-carboxylate (**67**) (Scheme 6).* A solution of **63** (110 mg, 0.38 mmol) in dioxane (30 mL) and under N_2 was treated with di-*tert*-butyl dicarbonate (498 mg, 2.28 mmol). The mixture was heated under reflux for 24 h and concentrated to dryness under reduced pressure. The residue was purified by column chromatography eluting with CH_2Cl_2/MeOH (19:1) followed by trituration with *i*-Pr_2O to give **67** as a yellow solid (97 mg, 66%): mp 199−201 °C. ^{1}H NMR [$(CD_3)_2SO$]: δ 8.82 (s, 1H), 8.72 (br, s, 1H), 8.21 (br, s, 1H), 8.11 (d, J = 8.8 Hz, 1H), 8.07 (dd, J = 8.8, 1.5 Hz, 1H), 7.54 (br, s, 1H), 5.01 (t, J = 5.5 Hz, 1H), 4.15 (br, s, 1H), 4.17 (br, s, 1H), 4.04−3.98 (m, 1H), 3.71−3.76 (m, 1H), 3.57−3.62 (m, 1H), 1.56 (s, 9H). Anal. calcd for $C_{19}H_{21}N_3O_6{\cdot}0.2MeOH{\cdot}0.1i\text{-}Pr_2O$: C, 58.89; H, 5.74; N, 10.41. Found: C, 58.86; H, 5.64; N, 10.03.

*tert-Butyl 1-(((Benzylsulfonyl)oxy)methyl)-7-carbamoyl-5-nitro-1H-benzo[e]indole-3(2H)-carboxylate (**68**) (Scheme 6).* Compound **67** (95 mg, 0.25 mmol) was dissolved in CH_2Cl_2 (10 mL) and Et_3N (101 μL, 0.65 mmol), cooled to 0 °C, treated with α-toluenesulfonyl chloride (124 mg, 0.65), and stirred for 20 min. The mixture was diluted with CH_2Cl_2, washed with water and brine, dried, and concentrated under reduced pressure. The residue was purified by column chromatography eluting with CH_2Cl_2/MeOH (16:1) to give **68** as a yellow solid (128 mg, 95%): mp 173−175 °C. ^{1}H NMR [$(CD_3)_2SO$]: δ 8.82 (s, 1H), 8.73 (br, s, 1H), 8.25 (br, s, 1H), 8.13−8.07 (m, 2H), 7.57 (br, s, 1H), 7.34−7.22 (m, 5H), 4.65 (br, s, 2H), 4.48 (br, s, 1H), 4.47 (br, s, 1H), 4.38−4.33 (m, 1H), 4.26−4.21 (m, 1H), 3.14 (dd, J = 11.4, 2.3 Hz, 1H), 1.56 (s, 9H). Anal. calcd for $C_{26}H_{27}N_3O_8S$: C, 57.66; H, 5.03; N, 7.76. Found: C, 57.79; H, 5.22; N, 7.46.

*(E)-(7-Carbamoyl-3-(3(4-methoxyphenyl)acryloyl)-5-nitro-2,3-dihydro-1H-benzo[e]indol-1-yl)methyl phenylsulfonate (**19**) (Scheme 6). Method A.* Compound **66** (140 mg, 0.31 mmol) was dissolved in CH_2Cl_2 (6 mL) and pyridine (6 mL) at 38 °C in a warm water bath. The solution was cooled to 0 °C, treated with α-toluenesulfonyl chloride (298 mg, 1.56 mmol), and stirred for 10 min, then poured into a beaker of ice water. The precipitate was collected by filtration, washed with water, and dried in a vacuum desiccator. The residue was purified by column chromatography eluting with CH_2Cl_2/MeOH (99:1 and 49:1) and further triturated with small portions of CH_2Cl_2 and then ice-cold MeOH to provide **19** as a yellow solid (133 mg, 71%): mp 207−209 °C. ^{1}H NMR [$(CD_3)_2SO$]: δ 9.22 (s, 1H), 8.83 (s, 1H), 8.27 (br, s, 1H), 8.17−8.12 (m, 2H), 7.81−7.78 (m, 2H), 7.74 (d, J = 15.3 Hz, 1H), 7.59 (br, s, 1H), 7.26−7.21 (m, 5H), 7.07−7.02 (m, 3H), 4.66−4.59 (m, 3H), 4.56−4.51 (m, 4H), 3.83 (s, 3H). HRMS (FAB) calcd for $C_{31}H_{28}N_3O_8S$ (MH^+) m/z, 602.1597; found, 602.1595. HPLC purity, 94.1%.

*1-(Hydroxymethyl)-5-nitro-3-(2,2,2-trifluoroacetyl)-2,3-dihydro-1H-benzo[e]indole-7-carboxamide (**69**) (Scheme 1).* A solution of **63** (586 mg, 2.04 mmol) in DMA (10 mL) was treated with anhydrous TsOH (386 mg, 2.24 mmol), TFA (616 μL, 8.16 mmol), and EDCI·HCl (1.56 g, 8.14 mmol). The mixture was stirred at room temperature overnight, then cooled to 0 °C, treated with 5% aqueous $NaHCO_3$, and stirred for further 10 min. The resulting solution was poured into a beaker of ice water and extracted with EtOAc, and the combined organic layers were washed with brine, dried with Na_2SO_4, and concentrated under reduced pressure to give an orange solid. The solid was triturated with *i*-Pr_2O and small portions of cold diethyl ether to provide **69** as an orange solid (615 mg, 79%): mp 249−251 °C. ^{1}H NMR [$(CD_3)_2SO$]: δ 9.01 (s, 1H), 8.87 (d, J = 1.3 Hz, 1H), 8.29 (br, s, 1H), 8.28 (d, J = 8.9 Hz, 1H), 8.17 (dd, J = 8.8, 1.6 Hz, 1H), 7.62 (br, s, 1H), 5.05 (t, J = 5.4 Hz, 1H), 4.61−4.49 (m, 2H),

4.24–4.20 (m, 1H), 3.83–3.78 (m, 1H), 3.75–3.69 (m, 1H). Anal. calcd for $C_{16}H_{12}F_3N_3O_5$: C, 50.14; H, 3.16; N, 10.96. Found: C, 50.39; H, 3.45; N, 10.76.

*1-(Hydroxymethyl)-5-nitro-3-(2,2,2-trifluoroacetyl)-2,3-dihydro-1H-benzo[e]indole-7-carboxylic acid (**70**) (Scheme 1).* Compound **69** (288 mg, 0.75 mmol) was dissolved in TFA (10 mL) at room temperature, cooled to 0 °C in an ice water bath, and stirred for 5 min. The mixture was treated with $NaNO_2$ (569 mg, 8.25 mmol), further stirred for 10 min, and then poured into a beaker of ice water with stirring. The precipitate was collected by filtration, dissolved in EtOAc, washed with water and brine, dried, and concentrated under reduced pressure. The residue was triturated with small portions of cold CH_2Cl_2 to provide **70** as a yellow solid (241 mg, 84%): mp 246–250 °C. ^{1}H NMR [$(CD_3)_2SO$]: δ 13.44 (br s, 1H), 9.06 (s, 1H), 9.02 (d, J = 1.1 Hz, 1H), 8.28 (d, J = 8.8 Hz, 1H), 8.19 (dd, J = 8.8, 1.5 Hz, 1H), 5.06 (br, s, 1H), 4.63–4.49 (m, 2H), 4.24–4.21 (m, 1H), 3.83–3.80 (m, 1H), 3.74–3.70 (m, 1H). Anal. calcd for $C_{16}H_{11}F_3N_2O_6.0.1CH_2Cl_2$: C, 49.24; H, 2.87; N, 7.13. Found: C, 49.32; H, 3.06; N, 7.39.

*1-(Hydroxymethyl)-5-nitro-N-(2-((tetrahydro-2H-pyran-2-yl)oxy)-ethyl)-3-(2,2,2-trifluoroacetyl)-2,3-dihydro-1H-benzo[e]indole-7-carboxamide (**71**) (Scheme 1).* A solution of **70** (110 mg, 0.29 mmol) in anhydrous THF (15 mL) was treated with DIPEA (150 μL, 0.86 mmol) at room temperature. The solution was cooled to 0 °C and treated with 2-(tetrahydro-2*H*-2-pyranyloxy)ethanamine[26] (46 mg, 0.32 mmol) and PyBOP (194 mg, 0.37 mmol), stirred at room temperature for 1 h, and then evaporated to dryness. The residue was dissolved in EtOAc, washed with ice water and brine, dried, and concentrated under reduced pressure, and the crude product was purified by column chromatography eluting with diethyl ether/MeOH (24:1) to provide **71** as a yellow solid (110 mg, 74%): mp 122–124 °C. ^{1}H NMR [$(CD_3)_2SO$]: δ 9.01 (s, 1H), 8.87 (t, J = 5.4 Hz, 1H), 8.83 (d, J = 1.3 Hz, 1H), 8.27 (d, J = 8.9 Hz, 1H), 8.13 (dd, J = 8.8, 1.6 Hz, 1H), 5.05 (t, J = 5.4 Hz, 1H), 4.63 (t, J = 3.6 Hz, 1H), 4.58–4.48 (m, 2H), 4.23–4.20 (m, 1H), 3.83–3.69 (m, 4H), 3.59–3.49 (m, 3H), 3.46–3.40 (m, 1H), 1.78–1.72 (m, 1H), 1.66–1.61 (m, 1H), 1.50–1.43 (m, 4H). HRMS (ESI) calcd for $C_{23}H_{24}F_3N_3NaO_7$ (MNa^+) m/z, 534.1459; found, 534.1454.

*1-(Hydroxymethyl)-5-nitro-N-(2-((tetrahydro-2H-pyran-2-yl)oxy)-ethyl)-2,3-dihydro-1H-benzo[e]indole-7-carboxamide (**72**) (Scheme 1).* A solution of **71** (160 mg, 0.31 mmol) in MeOH (16 mL) was cooled to 0 °C in an ice water bath and stirred for 10 min. The solution was then treated with Cs_2CO_3 (102 mg, 0.31 mmol), stirred for further 20 min, and then evaporated to dryness. The residue was purified by column chromatography eluting with CH_2Cl_2/MeOH (19:1) and followed by recrystallization from CH_2Cl_2 to provide **72** as a red solid (109 mg, 85%): mp (CH_2Cl_2) 119–121 °C. ^{1}H NMR [$(CD_3)_2SO$]: δ 8.61 (m, 1H), 8.59 (d, J = 1.1 Hz, 1H), 7.90 (dd, J = 8.9, 1.6 Hz, 1H), 7.85 (d, J = 8.9 Hz, 1H), 7.64 (s, 1H), 6.37 (d, J = 1.1 Hz, 1H), 4.95 (t, J = 5.5 Hz, 1H), 4.61 (t, J = 3.6 Hz, 1H), 3.86–3.62 (m, 6H), 3.57–3.52 (m, 1H), 3.49–3.40 (m, 4H), 1.80–1.71 (m, 1H), 1.66–1.60 (m, 1H), 1.50–1.42 (m, 4H). Anal. calcd for $C_{21}H_{25}N_3O_6$: C, 60.71; H, 6.07; N, 10.11. Found: C, 60.65; H, 6.13; N, 9.80.

*1-(Hydroxymethyl)-5-nitro-N-(2-((tetrahydro-2H-pyran-2-yl)oxy)-ethyl)-3-(5,6,7-trimethoxy-1H-indole-2-carbonyl)-2,3-dihydro-1H-benzo[e]indole-7-carboxamide (**73**) (Scheme 7).* A solution of **72** (147 mg, 0.35 mmol) in DMA (2 mL) was treated with anhydrous TsOH (67 mg, 0.39 mmol), 5,6,7-trimethoxy-1*H*-indole-2-carboxylic acid (116 mg, 0.46 mmol), and EDCI·HCl (270 mg, 1.41 mmol). The mixture was stirred at room temperature for 2 h, then cooled to 0 °C, treated with 5% aqueous $NaHCO_3$, and stirred for a further 10 min. The precipitate was collected by filtration, washed with cold water, diethyl ether, and a small portion of cold MeOH, and then dried in a vacuum desiccator. The residue was triturated with diethyl ether to give **73** as a yellow solid (154 mg, 68%): mp 243–247 °C. ^{1}H NMR [$(CD_3)_2SO$]: δ 11.53 (d, J = 1.6 Hz, 1H), 9.14 (br, s, 1H), 8.83–8.81 (m, 2H), 8.22 (d, J = 8.8 Hz, 1H), 8.09 (dd, J = 8.8, 1.5 Hz, 1H), 7.18 (d, J = 2.1 Hz, 1H), 6.98 (br, s, 1H), 5.06 (t, J = 5.6 Hz, 1H), 4.83–4.77 (m, 1H), 4.67–4.62 (m, 2H), 4.18–4.12 (m, 1H), 3.94 (s, 3H), 3.83 (s, 3H), 3.81 (s, 3H), 3.80–3.76 (m, 3H), 3.467–3.41 (m, 5H), 1.80–1.71 (m, 1H), 1.67–1.60 (m, 1H), 1.50–1.44 (m, 4H). HRMS (FAB) calcd for $C_{33}H_{36}N_4O_{10}$ (M^+) m/z, 648.2431; found, 648.2423.

*(5-Nitro-7-((2-((tetrahydro-2H-pyran-2-yl)oxy)ethyl)carbamoyl)-3-(5,6,7-trimethoxy-1H-indole-2-carbonyl)-2,3-dihydro-1H-benzo-[e]indol-1-yl)methyl Phenylmethanesulfonate (**75**) (Scheme 7).* A solution of **73** (137 mg, 0.21 mmol) in dry pyridine (10 mL) and was treated with α-toluenesulfonyl chloride (121 mg, 0.63 mmol) at 0 °C. The mixture was stirred for 10 min and then poured into a beaker of ice water and stirred for further 10 min. The solution was extracted with CH_2Cl_2 (3×), and the combined organic layers were washed with water and brine, dried, and concentrated under reduced pressure. The residue was purified by column chromatography eluting with CH_2Cl_2/MeOH (19:1 and 9:1) and further recrystallized from CH_2Cl_2/diethyl ether in cold room (+5 °C) to give **75** as a yellow solid (113 mg, 67%): mp (CH_2Cl_2/diethyl ether) 126–129 °C. ^{1}H NMR [$(CD_3)_2SO$]: δ 11.58 (d, J = 1.6 Hz, 1H), 9.13 (br, s, 1H), 8.85 (t, J = 5.4 Hz, 1H), 8.81 (d, J = 1.1 Hz, 1H), 8.21 (d, J = 8.8 Hz, 1H), 8.12 (dd, J = 8.8, 1.4 Hz, 1H), 7.21–7.12 (m, 6H), 6.98 (br, s, 1H), 4.90–4.85 (m, 1H), 4.64–4.53 (m, 7H), 3.94 (s, 3H), 3.84 (s, 3H), 3.82 (s, 3H), 3.80–3.75 (m, 2H), 3.60–3.42 (m, 4H), 1.80–1.71 (m, 1H), 1.67–1.61 (m, 1H), 1.50–1.45 (m, 4H). HRMS (FAB) calcd for $C_{40}H_{42}N_4O_{12}S$ (M^+) m/z, 802.2520; found, 802.2526.

*(7-((2-Hydroxyethyl)carbamoyl)-5-nitro-3-(5,6,7-trimethoxy-1H-indole-2-carbonyl)-2,3-dihydro-1H-benzo[e]indol-1-yl)methyl Phenylmethanesulfonate (**22**) (Scheme 7).* A solution of **75** (28 mg, 0.035 mmol) in MeOH (2 mL) was treated with methane sulfonic acid (4 drops), stirred at room temperature for 1 h, and concentrated under reduced pressure. The residue was dissolved in CH_2Cl_2, washed with water and brine, dried, and concentrated under reduced pressure. The crude product (88% HPLC purity) was further purified by preparative HPLC (Synergi-Max RP column; flow rate, 14 mL/min; pump 1: H_2O/TFA, pH 2.5, gradient 60%–1%–1%–60%; pump 2: CH_3CN/H_2O, 9:1, gradient 40%–99%–99%–40%) to provide **22** as a yellow solid (13 mg, 52%): mp 126–130 °C. ^{1}H NMR [$(CD_3)_2SO$]: δ 11.59 (d, J = 1.7 Hz, 1H), 9.12 (br, s, 1H), 8.82 (d, J = 1.2 Hz, 1H), 8.77 (t, J = 5.5 Hz, 1H), 8.21 (d, J = 8.8 Hz, 1H), 8.14 (dd, J = 8.8, 1.5 Hz, 1H), 7.23–7.12 (m, 6H), 6.98 (s, 1H), 4.90–4.85 (m, 1H), 4.75 (t, J = 5.6 Hz, 1H), 4.53 (m, 5H), 3.94 (s, 3H), 3.84 (s, 3H), 3.82 (s, 3H), 3.60–3.55 (m, 2H), 3.43–3.39 (m, 3H). HRMS (FAB) calcd for $C_{35}H_{35}N_4O_{11}S$ (MH^+) m/z, 719.2023; found, 719.2017. HPLC purity, 96.9%. Attempted deprotection of the THP group by TFA/CH_2Cl_2 (1:1) did not go to completion, and a byproduct was formed.

*Methyl 6-(N,N-dibenzylsulfamoyl)-2-naphthoate (**78**) (Scheme 2).* Solid KNO_3 (9.32 g, 92 mmol) and then SO_2Cl_2 (7.40 mL, 92 mmol) were added to a suspension of methyl 6-(dimethylcarbamoylthio)-2-naphthoate[13] (**77**) (11.11 g, 38.4 mmol) in CH_3CN (250 mL) at 0 °C, and the mixture was stirred at this temperature for 3 h. More SO_2Cl_2 (7.40 mL, 92 mmol) was added, and after a further 30 min at 0 °C, the mixture was cautiously diluted with ice-cold aqueous $NaHCO_3$. The mixture was extracted with EtOAc, and the extract was washed with aqueous $NaHCO_3$ and brine and then dried ($MgSO_4$) and evaporated. The crude sulfonyl chloride (9.9 g, ca. 35 mmol) was dissolved in THF (100 mL), and Bn_2NH (8.0 mL, 41.8 mmol) and Et_3N (5.8 mL, 41.8 mmol) were added. The mixture was stirred at room temperature for 4 days, then diluted with aqueous HCl (2 N), and extracted with EtOAc. The EtOAc extract was washed with brine and then dried ($MgSO_4$) and evaporated. The resulting solid was triturated with hot MeOH (350 mL) to give **78** as a cream solid (5.65 g, 33%). ^{1}H NMR [$(CD_3)_2SO$]: δ 8.77 (s, 1H), 8.61 (d, J = 1.6 Hz, 1H), 8.37 (d, J = 8.8 Hz, 1H), 8.28 (d, J = 8.8 Hz, 1H), 8.13 (dd, J = 8.6, 1.7 Hz, 1H), 7.97 (dd, J = 8.7, 1.9 Hz, 1H), 7.22–7.15 (m, 6H), 7.13–7.06 (m, 4H), 4.39 (s, 4H), 3.96 (s, 3H). ^{1}H NMR analysis also showed the presence of some impurities, one of which was later identified as the corresponding 5-chloro analogue [methyl 5-chloro-6-(*N*,*N*-dibenzylsulfamoyl)-2-naphthoate]. An analytically pure sample of **78** was obtained by recrystallization from MeOH, mp 150–152 °C. Anal. calcd for $C_{26}H_{23}NO_4S$: C, 70.09; H, 5.20; N, 3.14. Found: C, 70.14; H, 5.10; N, 3.09. A second crop of **78** was obtained from the MeOH triturate (1.29 g, 8%).

*6-(N,N-Dibenzylsulfamoyl)-2-naphthoic Acid (**79**) (Scheme 2).* A solution of KOH (4.52 g, 81 mmol) in water (100 mL) was added to a suspension of ester **78** (6.92 g, 15.5 mmol) in MeOH (200 mL), and the mixture was stirred at reflux for 30 min, by which time all of the suspended solid had dissolved. The mixture was cooled and acidified with aqueous HCl (2 N, 60 mL) and then diluted with water. After the mixture was stirred overnight, the precipitated solid was filtered off, washed with water, and dried to give **79** as a white solid (6.21 g, 93%), mp 260−261 °C. ^{1}H NMR [$(CD_3)_2SO$]: δ 13.31 (br s, 1H), 8.74 (s, 1H), 8.60 (d, J = 1.5 Hz, 1H), 8.35 (d, J = 8.9 Hz, 1H), 8.25 (d, J = 8.8 Hz, 1H), 8.12 (dd, J = 8.6, 1.6 Hz, 1H), 7.95 (dd, J = 8.7, 1.9 Hz, 1H), 7.22−7.16 (m, 6H), 7.13−7.08 (m, 4H), 4.39 (s, 4H). Anal. calcd for $C_{25}H_{21}NO_4S$: C, 69.59; H, 4.91; N, 3.25. Found: C, 69.66; H, 4.98; N, 3.26.

*tert-Butyl (6-(N,N-Dibenzylsulfamoyl)naphthalen-2-yl)-carbamate (**80**) (Scheme 2).* A mixture of acid **79** (6.21 g, 14.4 mmol), diphenylphosphoryl azide (DPPA; 3.41 mL, 15.8 mmol), and Et_3N (2.4 mL, 17.3 mmol) in dry *tert*-BuOH (100 mL) was stirred at reflux under a $CaCl_2$ drying tube for 17 h. The yellow solution was allowed to cool, and the *tert*-BuOH was evaporated. The residue was dissolved in CH_2Cl_2, and the solution was washed with dilute aqueous HCl (2×) and then brine. The CH_2Cl_2 layer was dried ($MgSO_4$), diluted with MeOH, and concentrated under reduced pressure until a solid began to precipitate. The mixture was allowed to stand at room temperature until precipitation was complete. The solid was filtered off and dried to give **80** as a cream solid (6.62 g, 92%). A sample was recrystallized from EtOAc/petroleum ether as a white solid, mp 191−193 °C. ^{1}H NMR [$(CD_3)_2SO$]: δ 9.80 (s, 1H), 8.39 (d, J = 1.6 Hz, 1H), 8.25 (d, J = 1.4 Hz, 1H), 8.04 (d, J = 9.0 Hz, 1H), 7.98 (d, J = 8.9 Hz, 1H), 7.78 (dd, J = 8.7, 1.9 Hz, 1H), 7.64 (dd, J = 8.9, 2.1 Hz, 1H), 7.22−7.16 (m, 6H), 7.12−7.07 (m, 4H), 4.34 (s, 4H), 1.53 (s, 9H). Anal. calcd for $C_{29}H_{30}N_2O_4S$: C, 69.30; H, 6.02; N, 5.57. Found: C, 69.05; H, 6.22; N, 5.61.

*tert-Butyl (6-(N,N-Dibenzylsulfamoyl)-1-iodonaphthalen-2-yl)-carbamate (**81**) (Scheme 2).* Carbamate **80** (6.37 g, 12.7 mmol) was dissolved in CH_2Cl_2 (150 mL), and the solution was diluted with CH_3CN (120 mL). *N*-Iodosuccinimide (3.14 g, 14.0 mmol) and $TsOH·H_2O$ (60 mg, 0.32 mmol) were added, and the resulting orange-brown solution was stirred at room temperature in the dark for 6 days. The solution was washed with aqueous $Na_2S_2O_5$ (10%), then dried ($MgSO_4$), and evaporated. The residue was dissolved in the minimum amount of CH_2Cl_2, and the solution was diluted with EtOAc and allowed to stand at room temperature. The precipitated solid was filtered off and dried to give **81** as a cream solid (3.16 g, 40%). A sample was recrystallized from EtOAc as a white solid, mp 194−195 °C. ^{1}H NMR [$(CD_3)_2SO$]: δ 8.94 (s, 1H), 8.52 (d, J = 1.9 Hz, 1H), 8.28 (d, J = 9.0 Hz, 1H), 8.14 (d, J = 8.7 Hz, 1H), 7.98 (dd, J = 9.0, 2.0 Hz, 1H), 7.76 (d, J = 8.8 Hz, 1H), 7.22−7.16 (m, 6H), 7.13−7.08 (m, 4H), 4.36 (s, 4H), 1.51 (s, 9H). Anal. calcd for $C_{29}H_{29}IN_2O_4S$: C, 55.42; H, 4.65; N, 4.46. Found: C, 55.42; H, 4.82; N, 4.55. The mother liquor was evaporated, and the residue was purified by column chromatography (eluting with EtOAc/petroleum ether/CH_2Cl_2, 10:75:15) and recrystallization (CH_2Cl_2/EtOAc) to give more **77** as a cream solid (3.09 g, 39%).

*tert-Butyl Allyl(6-(N,N-dibenzylsulfamoyl)-1-iodonaphthalen-2-yl)carbamate (**82**) (Scheme 2).* Sodium hydride (60% dispersion in oil, 0.53 g, 13.3 mmol) was added to a solution of iodide **81** (6.43 g, 10.2 mmol) and allyl bromide (1.77 mL, 20.4 mmol) in dry DMF (100 mL) at 0 °C. The mixture was stirred at this temperature for 15 min and then allowed to warm to room temperature. After 2.5 h, the mixture was cooled again in an ice bath, ice-cold water (3 mL) was added, and the mixture was allowed to warm to room temperature once more. The solvent was evaporated under reduced pressure, and the residue was partitioned between EtOAc and brine. The aqueous layer was extracted again with EtOAc (2×), and the combined organic layer was washed with brine (3×) and then dried ($MgSO_4$) and evaporated to give **82** as a yellow foam (6.86 g, 100%). ^{1}H NMR [$(CD_3)_2SO$]: δ 8.58−8.53 (m, 1H), 8.33 (d, J = 9.0 Hz, 1H), 8.22−8.15 (m, 1H), 8.00 (d, J = 8.6 Hz, 1H), 7.53 (d, J = 8.5 Hz, 1H), 7.23−7.04 (m, 10H), 6.05−5.90 (m, 1H), 5.19−5.02 (m, 2H), 4.58−4.31 (m, 5H), 4.06−3.88 (m, 1H), 1.51 and 1.29 (2 s, 9H). HRMS (FAB) calcd for $C_{32}H_{34}IN_2O_4S$ (MH+) m/z 669.12841; found, 669.12875.

*tert-Butyl 7-(N,N-Dibenzylsulfamoyl)-1-(((2,2,6,6-tetramethylpiperidin-1-yl)oxy)methyl)-1H-benzo[e]indole-3(2H)-carboxylate (**83**) (Scheme 2).* TEMPO (1.91 g, 12.2 mmol) was added to a solution of iodide **82** (6.86 g, 10.2 mmol) in dry benzene (350 mL) under nitrogen at room temperature. The mixture was stirred at reflux, and Bu_3SnH (97%, 3.29 mL, 12.2 mmol) was added dropwise over 5 min. After 10 min, the mixture was cooled to room temperature. The addition of TEMPO and Bu_3SnH was repeated under the same conditions a further two times until TLC analysis indicated complete consumption of the starting material. The mixture was evaporated, and the residue was purified by column chromatography (eluting with EtOAc/petroleum ether, 1:20 then 1:15 then 1:10). The product-containing fractions were evaporated to dryness and triturated with MeOH. A small quantity of white solid was filtered off, and the filtrate was evaporated to give **83** as an orange oil-foam (7.00 g, 98%). ^{1}H NMR ($CDCl_3$): δ 8.31 (d, J = 1.7 Hz, 1H), 7.88 (d, J = 8.9 Hz, 1H), 7.80 (d, J = 8.9 Hz, 1H), 7.76 (dd, J = 8.9, 1.9 Hz, 1H), 7.23−7.17 (m, 6H), 7.11−7.06 (m, 4H), 4.36 (s, 4H), 4.31−4.24 (m, 1H), 4.17−4.11 (m, 1H), 4.05−3.98 (m, 1H), 3.93−3.86 (m, 2H), 1.61 (s, 9H), 1.45−1.26 (m, 6H), 1.15−0.94 (m, 12H), 1 proton not observed. HRMS (FAB) calcd. for $C_{41}H_{52}N_3O_5S$ (MH+) m/z, 698.36277; found, 698.36256.

Analysis of the white solid by ^{1}H NMR and MS showed that it consisted largely of the 6-chloro analogue [*tert*-butyl 6-chloro-7-(*N*,*N*-dibenzylsulfamoyl)-1-((2,2,6,6-tetramethylpiperidin-1-yloxy)methyl)-1*H*-benzo[*e*]indole-3(2*H*)-carboxylate] formed as an impurity in the chlorosulfonylation step. Compound **83** isolated as above was free of this impurity.

*1-(((2,2,6,6-Tetramethylpiperidin-1-yl)oxy)methyl)-2,3-dihydro-1H-benzo[e]indole-7-sulfonamide (**84**) (Scheme 2).* Ice-cold concentrated H_2SO_4 (20 mL) was added to dibenzylsulfonamide **83** (4.58 g, 6.56 mmol) cooled in an ice bath, and the mixture was stirred at 0 °C until TLC analysis indicated complete debenzylation of the sulfonamide (a total of 14 h, interrupted overnight when the reaction mixture was allowed to stand in the freezer). The mixture was poured into ice-cold water, EtOAc was added, and then concentrated NH_3 was cautiously added with stirring until the aqueous phase was alkaline. The mixture was extracted with EtOAc (3×), and the combined extracts were washed with brine (2×), then dried ($MgSO_4$), and evaporated. The residue was triturated with EtOAc to give **84** as a white solid (0.68 g, 22%), mp 199−201 °C (MeOH). ^{1}H NMR [$(CD_3)_2SO$]: δ 8.18 (d, J = 1.7 Hz, 1H), 7.77 (d, J = 8.6 Hz, 1H), 7.72 (d, J = 8.9 Hz, 1H), 7.68 (dd, J = 8.9, 1.8 Hz, 1H), 7.22 (s, 2H), 7.04 (d, J = 8.6 Hz, 1H), 6.13 (d, J = 2.4 Hz, 1H), 3.90−3.82 (m, 2H), 3.77−3.62 (m, 2H), 3.62−3.57 (m, 1H), 1.57−1.22 (m, 6H), 1.09 (s, 6H), 1.06 (s, 3H), 0.99 (s, 3H). Anal. calcd for $C_{22}H_{31}N_3O_3S$: C, 63.28; H, 7.48; N, 10.06. Found: C, 63.50; H, 7.31; N, 10.07. The mother liquor was evaporated, and the residue was purified by column chromatography (eluting with EtOAc/petroleum ether, 1:2 then 1:1) to give more **84** (1.29 g, 42%).

*tert-Butyl 7-Sulfamoyl-1-(((2,2,6,6-tetramethylpiperidin-1-yl)-oxy)methyl)-1H-benzo[e]indole-3(2H)-carboxylate (**85**) (Scheme 2).* A mixture of indoline **84** (1.97 g, 4.72 mmol) and di-*tert*-butyldicarbonate (4.12 g, 18.9 mmol) in THF (70 mL) was stirred at reflux for 16 h and then cooled to room temperature. The THF was evaporated, and the residue was purified by column chromatography (eluting with EtOAc/petroleum ether, 1:2) to give **85** as a cream solid (1.93 g, 79%), mp 200−202 °C (MeOH).; ^{1}H NMR [$(CD_3)_2SO$]: δ 8.35 (d, J = 1.8 Hz, 1H), 8.13 (v br s, 1H), 8.03 (d, J = 8.7 Hz, 1H), 8.01 (d, J = 8.8 Hz, 1H), 7.83 (dd, J = 8.9, 1.9 Hz, 1H), 7.34 (s, 2H), 4.23 (dd, J = 11.1, 1.4 Hz, 1H), 4.13−4.06 (m, 1H), 4.00−3.86 (m, 3H), 1.54 (s, 9H), 1.46−1.18 (m, 6H), 1.12 (s, 3H), 0.98 (s, 3H), 0.76 (s, 3H), 0.64 (s, 3H). Anal. calcd for $C_{27}H_{39}N_3O_5S$: C, 62.64; H, 7.59; N, 8.12. Found: C, 62.67; H, 7.35; N, 8.14.

*tert-Butyl 1-(Hydroxymethyl)-7-sulfamoyl-1H-benzo[e]indole-3(2H)-carboxylate (**86**) (Scheme 2).* A mixture of TEMPO adduct **85** (1.93 g, 3.73 mmol) and Zn powder (2.44 g, 37 mmol) in THF (40 mL), HOAc (30 mL), and H_2O (10 mL) was stirred at reflux. More

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Zn powder (3 × 2.4 g) was added after 7, 25, and 29 h, and more HOAc was added after 29 h. After a total of 33 h, TLC analysis showed complete consumption of the starting material. The mixture was cooled to room temperature, diluted with EtOAc, and filtered through Celite washing with EtOAc. The solvents were evaporated, the residue was dissolved in EtOAc, and the solution was washed with aqueous $NaHCO_3$ and brine and then dried ($MgSO_4$) and evaporated. The residue was purified by column chromatography (eluting with EtOAc/petroleum ether, 1:2 then 1:1 then 2:1) to give **87** (152 mg, 10%, identical to the product from the following step) and **86** as a pale yellow solid (997 mg, 71%). A sample was triturated with CH_2Cl_2/petroleum ether to give a white solid, mp 198−200 °C. ^{1}H NMR [$(CD_3)_2SO$]: δ 8.35 (d, J = 1.8 Hz, 1H), 8.12 (v br s, 1H), 8.02 (d, J = 8.7 Hz, 2H), 7.84 (dd, J = 8.9, 1.9 Hz, 1H), 7.35 (s, 2H), 4.98 (t, J = 5.4 Hz, 1H), 4.17−4.04 (m, 2H), 3.91−3.83 (m, 1H), 3.78−3.70 (m, 1H), 3.51−3.40 (m, 1H), 1.55 (s, 9H). Anal. calcd for $C_{18}H_{22}N_2O_5S{\cdot}0.25H_2O$: C, 56.46; H, 5.92; N, 7.32. Found: C, 56.49; H, 5.66; N, 7.19.

*tert-Butyl 1-(Acetoxymethyl)-7-sulfamoyl-1H-benzo[e]indole-3(2H)-carboxylate (**87**) (Scheme 2).* Acetic anhydride (261 μL, 2.76 mmol) was added dropwise to a solution of alcohol **86** (997 mg, 2.63 mmol), Et_3N (0.73 mL, 5.3 mmol), and DMAP (3 mg, 0.03 mmol) in THF (60 mL), and the mixture was stirred at room temperature for 2 h. TLC analysis showed the presence of starting material, so more acetic anhydride (52 μL, 0.53 mmol) was added. The mixture was stirred for a further 2 h, and then, the THF was evaporated. The residue was dissolved in EtOAc, and the solution was washed with aqueous HCl (1 N), aqueous $NaHCO_3$, and brine, then dried ($MgSO_4$), and evaporated. The residue was purified by column chromatography (eluting with EtOAc/petroleum ether, 2:3 then 1:1) to give acetate **87** as a pale yellow solid (953 mg, 86%). A sample was recrystallized from CH_2Cl_2/petroleum ether as a white solid, mp 161−162 °C. ^{1}H NMR [$(CD_3)_2SO$]: δ 8.37 (d, J = 1.7 Hz, 1H), 8.17−8.01 (m, 3H), 7.87 (dd, J = 8.9, 1.9 Hz, 1H), 7.37 (s, 2H), 4.42−4.35 (m, 1H), 4.18−4.04 (m, 4H), 1.96 (s, 3H), 1.55 (s, 9H). Anal. calcd for $C_{20}H_{24}N_2O_6S$: C, 57.13; H, 5.75; N, 6.66. Found: C, 57.03; H, 5.79; N, 6.70.

Further elution (EtOAc/petroleum ether, 3:2) gave a sample that was shown by ^{1}H NMR and MS analysis to be a close-running mixture of recovered starting material (6%) and the corresponding diacetate [*tert*-butyl 1-(acetoxymethyl)-7-(*N*-acetylsulfamoyl)-1*H*-benzo[*e*]-indole-3(2*H*)-carboxylate, 5%]. Reaction with a larger excess of acetic anhydride gave a clean sample of this diacetate as a white foam. ^{1}H NMR [$(CD_3)_2SO$]: δ 12.08 (br s, 1H), 8.52 (d, J = 1.7 Hz, 1H), 8.17−8.13 (m, 2H), 8.08 (d, J = 8.9 Hz, 1H), 7.86 (dd, J = 8.9, 1.9 Hz, 1H), 4.44−4.36 (m, 1H), 4.18−4.03 (m, 4H), 1.96 (s, 3H), 1.91 (s, 3H), 1.55 (s, 9H). LRMS (APCI, -ve) calcd for $C_{22}H_{26}N_2O_7S$ (M − H) m/z, 461; found, 461.5 (100%).

*(7-Sulfamoyl-3-(2,2,2-trifluoroacetyl)-2,3-dihydro-1H-benzo[e]-indol-1-yl)methyl Acetate (**88**) (Scheme 2).* A solution of carbamate **87** (953 mg, 2.27 mmol) in HCl-dioxane (4 M, 20 mL) was stirred at room temperature for 2 h and then evaporated. The residue was partitioned between EtOAc and aqueous $NaHCO_3$, and the aqueous layer was again extracted with EtOAc (6×). The combined EtOAc layers were dried ($MgSO_4$) and evaporated to give crude (7-sulfamoyl-2,3-dihydro-1*H*-benzo[*e*]indol-1-yl)methyl acetate as a pale yellow solid (0.76 g). TLC analysis showed the presence of minor impurities that were not completely separated by attempted trituration (EtOAc) or column chromatography (eluting with EtOAc/petroleum ether, 2:3 then 3:2). The remaining apparently unstable material (614 mg, 85%) was used directly in the next step. ^{1}H NMR [$(CD_3)_2SO$]: δ 8.19 (d, J = 1.7 Hz, 1H), 7.82−7.77 (m, 2H), 7.73 (dd, J = 8.8, 1.9 Hz, 1H), 7.20 (s, 2H), 7.06 (d, J = 8.6 Hz, 1H), 6.18 (d, J = 2.4 Hz, 1H), 4.29−4.21 (m, 1H), 4.00−3.92 (m, 2H), 3.74−3.67 (m, 1H), 3.56−3.51 (m, 1H), 2.02 (s, 3H). LRMS (APCI, -ve) calcd for $C_{15}H_{16}N_2O_4S$ (M − H) m/z, 319; found, 319.3 (80%), 212.3 (100%).

The crude indoline (614 mg, 1.92 mmol) and Et_3N (0.53 mL, 3.8 mmol) were dissolved in THF (30 mL), and the solution was cooled to 0 °C. TFAA (284 μL, 2.02 mmol) was added dropwise, and the mixture was stirred at 0 °C. TLC analysis after 20 min indicated incomplete reaction, so further portions of TFAA (a total of 204 μL, 1.45 mmol) and Et_3N (0.27 mL, 1.9 mmol) were added over 3 h until the starting material was consumed. Ice-cold water and a slight excess of aqueous HCl (2 N) were added, and the THF was evaporated. The aqueous residue was warmed with EtOAc (100 mL) until all suspended solid dissolved, the EtOAc layer was separated, and the aqueous phase was extracted again with EtOAc (2×). The combined organic layers were washed with brine, dried ($MgSO_4$), and concentrated to a small volume. On standing at room temperature a solid separated which was filtered off and dried to give **88** as a light tan solid (543 mg, 68%), mp 200−202 °C. ^{1}H NMR [$(CD_3)_2SO$]: δ 8.47 (d, J = 1.7 Hz, 1H), 8.41 (d, J = 9.0 Hz, 1H), 8.24 (d, J = 8.9 Hz, 1H), 8.19 (d, J = 9.0 Hz, 1H), 7.96 (dd, J = 8.8, 1.9 Hz, 1H), 7.45 (s, 2H), 4.57−4.51 (m, 1H), 4.47−4.37 (m, 2H), 4.36−4.25 (m, 2H), 1.89 (s, 3H). Anal. calcd for $C_{17}H_{15}F_3N_2O_5S$: C, 49.04; H, 3.63; N, 6.73. Found: C, 49.31; H, 3.71; N, 6.63. The mother liquor was evaporated, and the residue was purified by column chromatography (eluting with EtOAc/petroleum ether, 2:3) to give more **88** (200 mg, 21%).

*(5-Nitro-7-sulfamoyl-3-(2,2,2-trifluoroacetyl)-2,3-dihydro-1H-benzo[e]indol-1-yl)methyl Acetate (**90**) and (9-Nitro-7-sulfamoyl-3-(2,2,2-trifluoroacetyl)-2,3-dihydro-1H-benzo[e]indol-1-yl)methyl Acetate (**89**) (Scheme 2).* Ice-cold concentrated H_2SO_4 (8 mL) was added to trifluoroacetamide **88** (730 mg, 1.75 mmol) cooled in an ice bath, and the mixture was stirred at this temperature for 20 min until all of the suspended solid had dissolved. Solid KNO_3 (213 mg, 2.1 mmol) was added, giving a color change from brown to green to yellow-brown over several minutes. After 12 min at 0 °C, ice-cold water was added, and the mixture was extracted with EtOAc (2×). The combined extracts were washed with brine (2×) and then dried ($MgSO_4$) and evaporated. The residue was dissolved in THF, and the solution was evaporated onto silica gel. Purification by column chromatography (eluting with EtOAc/petroleum ether, 1:2 then 1:1 then 3:2) gave **89** as a yellow solid (138 mg, 17%), mp 254−256 °C (MeOH). ^{1}H NMR [$(CD_3)_2SO$]: δ 8.81 (d, J = 1.8 Hz, 1H), 8.66 (d, J = 9.0 Hz, 1H), 8.51 (d, J = 9.1 Hz, 1H), 8.50 (d, J = 1.8 Hz, 1H), 7.69 (s, 2H), 4.56 (dd, J = 11.1, 8.5 Hz, 1H), 4.34−4.26 (m, 1H), 4.09−3.95 (m, 2H), 3.93−3.86 (m, 1H), 1.75 (s, 3H). Anal. calcd for $C_{17}H_{14}F_3N_3O_7S$: C, 44.26; H, 3.06; N, 9.11. Found: C, 44.55; H, 3.18; N, 8.89; and **90** as a yellow-orange solid (224 mg, 28%), mp 240−241 °C (MeOH). ^{1}H NMR [$(CD_3)_2SO$]: δ 9.10 (s, 1H), 8.89 (d, J = 1.5 Hz, 1H), 8.47 (d, J = 8.9 Hz, 1H), 8.12 (dd, J = 8.9, 1.7 Hz, 1H), 7.65 (s, 2H), 4.65−4.56 (m, 1H), 4.51−4.43 (m, 3H), 4.41−4.34 (m, 1H), 1.87 (s, 3H). Anal. calcd for $C_{17}H_{14}F_3N_3O_7S$: C, 44.26; H, 3.06; N, 9.11. Found: C, 44.31; H, 3.11; N, 8.99.

*1-(Hydroxymethyl)-5-nitro-2,3-dihydro-1H-benzo[e]indole-7-sulfonamide (**91**) (Scheme 2).* Cs_2CO_3 (0.47 g, 1.5 mmol) was added to a solution of acetate **90** (224 mg, 0.49 mmol) in THF (10 mL) and MeOH (6 mL), and the mixture was stirred at room temperature for 1 h. During this time, the color changed from yellow to red-brown to brown, and a brown solid separated. The mixture was diluted with more MeOH, and the solid was filtered off. The solid was redissolved in a mixture of THF and EtOAc, and the solution was diluted with water. The mixture was concentrated to a small volume and allowed to stand at room temperature. The precipitated solid was filtered off and dried to give **91** as a red-brown solid (99 mg, 63%), mp 229−231 °C. ^{1}H NMR [$(CD_3)_2SO$]: δ 8.62 (d, J = 1.6 Hz, 1H), 7.98 (d, J = 8.9 Hz, 1H), 7.83 (dd, J = 9.0, 1.8 Hz, 1H), 7.74 (s, 1H), 7.39 (s, 2H), 6.53 (d, J = 1.2 Hz, 1H), 4.98 (t, J = 5.5 Hz, 1H), 3.90−3.82 (m, 1H), 3.80−3.68 (m, 2H), 3.67−3.60 (m, 1H), 3.50−3.42 (m, 1H). Anal. calcd for $C_{13}H_{13}N_3O_5S{\cdot}0.5H_2O$: C, 46.98; H, 4.25; N, 12.64. Found: C, 46.77; H, 4.44; N, 12.23.

*1-(Hydroxymethyl)-5-nitro-3-(5,6,7-trimethoxy-1H-indole-2-carbonyl)-2,3-dihydro-1H-benzo[e]indole-7-sulfonamide (**92**) (Scheme 8).* A mixture of amine **91** (52 mg, 0.16 mmol), 5,6,7-trimethoxy-1*H*-indole-2-carboxylic acid (53 mg, 0.21 mmol), anhydrous TsOH (30 mg, 0.18 mmol), and EDCI·HCl (123 mg, 0.64 mmol) in dry DMA (2 mL) was stirred at room temperature for 19 h. Dilute aqueous $NaHCO_3$ was added, and the mixture was stirred at room temperature for 1 h. The precipitated solid was filtered off, washed with H_2O, dried, and triturated with EtOAc to give **92** as a yellow solid (72 mg, 80%),

mp 280−285 °C (dec). ^{1}H NMR [$(CD_3)_2SO$]: δ 11.56 (s, 1H), 9.26 (s, 1H), 8.88 (d, J = 1.6 Hz, 1H), 8.37 (d, J = 8.9 Hz, 1H), 8.05 (dd, J = 8.9, 1.7 Hz, 1H), 7.60 (s, 2H), 7.20 (s, 1H), 6.98 (s, 1H), 5.07 (t, J = 5.6 Hz, 1H), 4.87−4.79 (m, 1H), 4.66 (dd, J = 10.6, 2.2 Hz, 1H), 4.21−4.14 (m, 1H), 3.95 (s, 3H), 3.83 (s, 3H), 3.81 (s, 3H), 3.80−3.75 (m, 1H), 3.71−3.64 (m, 1H). Anal. calcd for $C_{25}H_{24}N_4O_9S$: C, 53.95; H, 4.35; N, 10.07. Found: C, 53.65; H, 4.56; N, 9.87.

*(5-Nitro-7-sulfamoyl-3-(5,6,7-trimethoxy-1H-indole-2-carbonyl)-2,3-dihydro-1H-benzo[e]indol-1-yl)methyl Phenylmethanesulfonate (**24**) (Scheme 8).* α-Toluenesulfonyl chloride (28 mg, 0.14 mmol) was added to a solution of alcohol **92** (62.5 mg, 0.11 mmol) in pyridine (10 mL) at 0 °C, and the mixture was stirred at this temperature for 20 min. More α-toluenesulfonyl chloride (28 mg, 0.14 mmol) was added, and after a further 20 min, ice-cold water was added. The mixture was stirred at 0 °C for several minutes, and the precipitated solid was filtered off and dried. ^{1}H NMR analysis indicated that the reaction had only proceeded to ca. 20% completion but that there was no competing elimination or reaction on the sulfonamide. The procedure was repeated using a large excess of α-toluenesulfonyl chloride until TLC analysis showed complete conversion of the starting material. The crude product obtained was dissolved in THF, and the solution was evaporated onto silica gel. Purification by column chromatography (eluting with EtOAc/petroleum ether, 1:1 then 3:1) followed by trituration with EtOAc gave **24** as a yellow-orange solid (44 mg, 55%), mp 243−247 °C (dec). ^{1}H NMR [$(CD_3)_2SO$]: δ 11.62 (d, J = 1.6 Hz, 1H), 9.24 (s, 1H), 8.86 (d, J = 1.7 Hz, 1H), 8.34 (d, J = 8.9 Hz, 1H), 8.07 (dd, J = 8.9, 1.7 Hz, 1H), 7.64 (s, 2H), 7.23−7.12 (m, 6H), 6.98 (s, 1H), 4.94−4.86 (m, 1H), 4.66−4.51 (m, 6H), 3.95 (s, 3H), 3.84 (s, 3H), 3.82 (s, 3H). Anal. calcd for $C_{32}H_{30}N_4O_{11}S_2$: C, 54.08; H, 4.26; N, 7.88. Found: C, 54.28; H, 4.39; N, 7.68.

*2,2,2-Trifluoro-1-(1-(iodomethyl)-1H-benzo[e]indol-3(2H)-yl)-ethanone (**95**) (Scheme 3).* A solution of NaI (7.19 g, 47.9 mmol) in 2-butanone (30 mL) was heated (85 °C) for 1 h. Compound **94**[13] (5.0 g, 16.0 mmol) was added, and the mixture was heated (85 °C) for 17 h after which EtOAc and water were added. The organic layer was washed with aqueous sodium disulfite (10%), water, and brine, dried (Na_2SO_4), and evaporated. The crude product was precipitated (EtOAc/petroleum ether) to give **95** (4.91 g, 76%) as a cream powder: mp 132−135 °C. ^{1}H NMR ($CDCl_3$): δ 8.42 (d, J = 9.0 Hz, 1H), 7.92 (d, J = 7.9 Hz, 1H), 7.89 (d, J = 8.9 Hz, 1H), 7.77 (d, J = 8.4 Hz, 1H), 7.58 (td, J = 8.1, 1.1 Hz, 1H), 7.48 (td, J = 8.1, 1.1 Hz, 1H), 4.49 (d, J = 11.5 Hz, 1H), 4.43 (dd, J = 11.5, 8.2 Hz, 1H), 4.21 (tt, J = 9.8, 2.3 Hz, 1H), 3.65 (dd, J = 10.5, 2.3 Hz, 1H), 3.23 (t, J = 10.2 Hz, 1H). Anal. calcd for $C_{15}H_{11}F_3INO$: C, 44.47; H, 2.74; N, 3.46. Found: C, 44.74; H, 3.04; N, 3.41.

*(3-(2,2,2-Trifluoroacetyl)-2,3-dihydro-1H-benzo[e]indol-1-yl)-methyl Methanesulfonate (**96**) (Scheme 3).* A solution of iodide **95** (3.6 g, 8.89 mmol) and AgOMs (10.8 g, 53.3 mmol) in CH_3CN (50 mL) was stirred in the dark for 24 h. CH_3CN was removed, EtOAc was added, and the mixture was filtered through Celite. After removal of EtOAc, the crude product was recrystallized (EtOAc/petroleum ether) to give **96** (3.15 g, 95%) as colorless crystals: mp 122−124 °C. ^{1}H NMR ($CDCl_3$) δ 8.43 (d, J = 9.0 Hz, 1H), 7.91 (d, J = 7.8 Hz, 1H), 7.88 (d, J = 8.8 Hz, 1H), 7.83 (dd, J = 8.3, 0.5 Hz, 1H), 7.61 (td, J = 8.2, 1.2 Hz, 1H), 7.51 (td, J = 8.1, 1.1 Hz, 1H), 4.65 (dd, J = 3.8, 0.6 Hz, 1H), 4.59 (dt, J = 11.5, 1.4 Hz, 1H), 4.42 (dd, J = 11.5, 8.2 Hz, 1H), 4.29 (td, J = 8.2, 3.4 Hz, 1H), 4.14 (dd, J = 10.6, 9.1 Hz, 1H), 2.95 (s, 3H). Anal. calcd for $C_{16}H_{14}F_3NO_4S$: C, 51.47; H, 3.78; N, 3.75. Found: C, 51.77; H, 3.82; N, 3.86.

*(7-(Chlorosulfonyl)-3-(2,2,2-trifluoroacetyl)-2,3-dihydro-1H-benzo[e]indol-1-yl)methyl Methanesulfonate (**97**) (Scheme 3).* A solution of mesylate **96** (1.40 g, 3.75 mmol) in CH_2Cl_2 (5 mL) was added dropwise to a cooled (−78 °C) solution of $ClSO_3H$ (1.74 g, 15.0 mmol) in CH_2Cl_2 (20 mL). The stirred mixture was allowed to warm to 15 °C over 12 h, producing a gray precipitate. The mixture was then cooled (0 °C). A minimum amount of DMF was slowly added to dissolve the precipitate, and oxalyl chloride (1.5 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 and cold brine and dried (Na_2SO_4). After filtration through a plug of Celite/silica gel and removal of EtOAc, the crude product was precipitated (CH_2Cl_2/i-Pr_2O) to give **97** (1.64 g, 94%) as a white powder: mp 169−172 °C. ^{1}H NMR [$(CD_3)_2SO$] δ 8.32 (d, J = 8.9 Hz, 1H), 8.21 (d, J = 1.4 Hz, 1H), 8.06 (d, J = 9.0 Hz, 1H), 8.00 (d, J = 8.7 Hz, 1H), 7.81 (dd, J = 8.7, 1.6 Hz, 1H), 4.58 (dd, J = 10.0, 3.2 Hz, 1H), 4.58−4.54 (m, 1H), 4.49−4.36 (m, 3H), 3.05 (s, 3H). HRMS calcd for $C_{16}H_{13}{}^{35}ClF_3NO_6S_2$ m/z, 470.9825; found, 470.9824; calcd for $C_{16}H_{13}{}^{37}ClF_3NO_6S_2$ m/z, 472.9795; found, 472.9801.

*(7-(Chlorosulfonyl)-5-nitro-3-(2,2,2-trifluoroacetyl)-2,3-dihydro-1H-benzo[e]indol-1-yl)methyl Methanesulfonate (**99**) and (7-(Chlorosulfonyl)-9-nitro-3-(2,2,2-trifluoroacetyl)-2,3-dihydro-1H-benzo[e]indol-1-yl)methyl Methanesulfonate (**98**) (Scheme 3).* A cooled (0 °C) solution of KNO_3 (450 mg, 4.44 mmol) in 98% H_2SO_4 (2 mL) was added dropwise to a cooled (−5 °C) solution of **97** (1.90 g, 4.03 mmol) in 98% H_2SO_4 (30 mL). After 20 min at −5 °C, the mixture was poured into ice water and extracted with cold EtOAc. The organic layer was washed with cold water and cold brine and then dried (Na_2SO_4) and evaporated. The residue was purified by flash chromatography (petroleum ether/EtOAc; gradient, 100:0 to 2:3) followed by recrystallization (CH_2Cl_2/i-Pr_2O) to give **99** (1.19 g, 57%) as a white powder: mp 180−182 °C. ^{1}H NMR ($CDCl_3$): δ 9.32 (s, 1H), 9.27 (d, J = 1.7 Hz, 1H), 8.27 (dd, J = 9.0, 1.8 Hz, 1H), 8.20 (d, J = 9.0 Hz, 1H), 4.71 (d, J = 11.6 Hz, 1H), 4.65−4.53 (m, 2H), 4.47−4.40 (m, 1H), 4.24 (dd, J = 11.0, 8.2 Hz, 1H), 3.05 (s, 3H). Anal. calcd for $C_{16}H_{12}ClF_3N_2O_8S_2$: C, 37.18; H, 2.34; N, 5.42. Found: C, 37.05; H, 2.43; N, 5.31; and **98** (313 mg, 15%) as a white powder: mp 206−210 °C. ^{1}H NMR ($CDCl_3$): δ 8.93 (d, J = 9.1 Hz, 1H), 8.82 (d, J = 2.0 Hz, 1H), 8.40 (d, J = 2.0 Hz, 1H), 8.29 (d, J = 9.0 Hz, 1H), 4.55−4.50 (m, 2H), 4.19−4.04 (m, 3H), 2.95 (s, 3H). Anal. calcd for $C_{16}H_{12}ClF_3N_2O_8S_2$: C, 37.18; H, 2.34; N, 5.42. Found: C, 37.22; H, 2.35; N, 5.47.

*(7-(N-(2-Hydroxyethyl)sulfamoyl)-5-nitro-2,3-dihydro-1H-benzo-[e]indol-1-yl)methyl Methanesulfonate (**100**) (Scheme 9).* A solution of ethanolamine (46 mg, 0.76 mmol) in CH_2Cl_2 (0.5 mL) was added dropwise to a cooled (0 °C) solution of **99** (130 mg, 0.25 mmol) in CH_2Cl_2 (10 mL). After 15 min at 0 °C, Cs_2CO_3 (164 mg, 0.50 mmol), MeOH (5 mL), and water (1 mL) were added. After a further 15 min, ice was added, and the mixture was poured into cold water/cold EtOAc. The organic layer was separated and then washed with cold water and cold brine, then dried (Na_2SO_4), and evaporated to give **100** (100 mg, 89%). A portion was recrystallized (EtOAc/CH_2Cl_2) to give red crystals: mp 142−146 °C. ^{1}H NMR [$(CD_3)_2SO$]: δ 8.59 (d, J = 1.6 Hz, 1H), 8.0 (d, J = 9.0 Hz, 1H), 7.82 (dd, J = 9.0, 1.7 Hz, 1H), 7.79 (s, 1H), 7.69 (br s, 1H), 6.74 (d, J = 1.4 Hz, 1H), 4.63 (t, J = 5.6 Hz, 1H), 4.40−4.34 (m, 1H), 4.31−4.19 (m, 2H), 3.86 (td, J = 9.6, 2.0 Hz, 1H), 3.70 (dd, J = 9.9, 2.3 Hz, 1H), 3.36 (q, J = 5.8 Hz, 2H), 3.14 (s, 3H), 2.81 (t, J = 6.3 Hz, 2H). Anal. calcd for $C_{16}H_{19}N_2O_8S_2$·0.1EtOAc: C, 43.36; H, 4.39; N, 9.25. Found: C, 43.09; H, 4.39; N, 8.91.

*(7-(N-(2-Hydroxyethyl)sulfamoyl)-5-nitro-3-(5,6,7-trimethoxy-1H-indole-2-carbonyl)-2,3-dihydro-1H-benzo[e]indol-1-yl)methyl Methanesulfonate (**26**) (Scheme 9).* A solution of EDCI·HCl (58 mg, 0.31 mmol), TsOH (3 mg, 0.02 mmol), TMI-2-carboxylic acid (25 mg, 0.10 mmol), and **100** (34 mg, 0.08 mmol) in DMA (1 mL) was stirred for 15 h. Cold 5% aqueous $NaHCO_3$ was added, and the precipitate was filtered and washed with cold water to give **26** (52 mg, 100%): mp 169−173 °C (dec.). ^{1}H NMR [$(CD_3)_2SO$]: δ 11.59 (s, 1H), 9.24 (s, 1H), 8.86 (s, 1H), 8.41 (d, J = 8.9 Hz, 1H), 8.03 (dd, J = 8.9, 1.5 Hz, 1H), 7.92 (t, J = 5.7 Hz, 1H), 4.96−4.85 (m, 1H), 4.71−4.61 (m, 2H), 4.61−4.49 (m, 3H), 3.94 (s, 3H), 3.83 (s, 3H), 3.81 (s, 3H), 3.05 (s, 3H), 2.88 (q, J = 6.0 Hz, 2H), 2 protons not observed. Anal. calcd for $C_{28}H_{30}N_4O_{12}S_2$·0.25H_2O: C, 49.23; H, 4.50; N, 8.20. Found: C, 49.16; H, 4.43; N, 8.03.

*(3-(2,2,2-Trifluoroacetyl)-2,3-dihydro-1H-benzo[e]indol-1-yl)-methyl Acetate (**109**) (Scheme 4). Method D.* A solution of **96** (2.0 g, 4.94 mmol) and AgOAc (2.47 g, 14.8 mmol) in HOAc (50 mL) was stirred in the dark for 48 h. EtOAc was added, and the mixture was filtered through Celite. The organic layer was washed with water and brine, dried (Na_2SO_4), and evaporated. The residue was purified by

flash chromatography (petroleum ether/EtOAc; gradient, 100:0 to 9:1) to give **109** (1.61 g, 97%) as a white powder.

*(7-(Chlorosulfonyl)-3-(2,2,2-trifluoroacetyl)-2,3-dihydro-1H-benzo[e]indol-1-yl)methyl Acetate (**111**) (Scheme 4).* A solution of **109** (5.50 g, 16.3 mmol) in CH_2Cl_2 (40 mL) was added dropwise to a cooled (−80 °C) solution of $ClSO_3H$ (7.57 g, 65.3 mmol) in CH_2Cl_2 (80 mL). The stirred mixture was allowed to warm to 5 °C over 5 h producing a gray precipitate. The mixture was kept at 5 °C overnight and then cooled (−10 °C). A minimum amount of DMF was slowly added to dissolve the precipitate, oxalyl chloride (3 mL) was added dropwise, and the mixture was allowed to warm to 5 °C over 3 h. The mixture was poured into ice water and extracted with cold EtOAc, and the organic layer was separated and washed with cold water and cold brine, dried (Na_2SO_4), and evaporated. After filtration through a plug of silica gel, the crude product was precipitated (CH_2Cl_2/*i*-Pr_2O) to give **111** (5.77 g, 81%). A portion was recrystallized (petroleum ether/EtOAc) to give colorless crystals: mp 153−157 °C. ^{1}H NMR ($CDCl_3$) δ 8.64 (d, J = 8.6 Hz, 1H), 8.62 (d, J = 1.9 Hz, 1H), 8.16 (d, J = 9.0 Hz, 1H), 8.08 (2dd, J = 9.0, 2.1 Hz, 2H), 4.58 (dd, J = 11.4, 4.0 Hz, 1H), 4.53 (d, J = 11.4 Hz, 1H), 4.44 (dd, J = 11.3, 8.4 Hz, 1H), 4.24−4.16 (m, 1H), 4.02 (dd, J = 11.4, 8.3 Hz, 1H), 2.08 (s, 3H). Anal. calcd for $C_{17}H_{13}ClF_3NO_5S$·0.3EtOAc: C, 47.29; H, 3.36; N, 3.03. Found: C, 47.50; H, 3.09; N, 3.20.

*(7-(Chlorosulfonyl)-5-nitro-3-(2,2,2-trifluoroacetyl)-2,3-dihydro-1H-benzo[e]indol-1-yl)methyl Acetate (**113**) and (7-(Chlorosulfonyl)-9-nitro-3-(2,2,2-trifluoroacetyl)-2,3-dihydro-1H-benzo[e]indol-1-yl)methyl Acetate (**112**) (Scheme 4).* A cooled (0 °C) solution of KNO_3 (56 mg, 0.55 mmol) in 98% H_2SO_4 (0.5 mL) was added dropwise to a cooled (−12 °C) solution of **111** (200 mg, 0.46 mmol) in 98% H_2SO_4 (20 mL). After 15 min at −12 °C, the mixture was poured into ice water and extracted with cold EtOAc. The organic layer was separated and washed with cold water and cold brine, dried (Na_2SO_4), and evaporated. The residue was purified by flash chromatography (petroleum ether/EtOAc; gradient, 100:0 to 3:2) followed by recrystallization (CH_2Cl_2/*i*-Pr_2O) to give **113** (137 mg, 62%) as colorless crystals: mp 162−164 °C. ^{1}H NMR ($CDCl_3$): δ 9.33 (s, 1H), 9.28 (d, J = 1.6 Hz, 1H), 8.30 (d, J = 9.0 Hz, 1H), 8.22 (dd, J = 9.0, 1.9 Hz, 1H), 4.64−4.57 (m, 2H), 4.52 (dd, J = 11.3, 8.5 Hz, 1H), 4.34−4.27 (m, 1H), 4.07 (dd, J = 11.4, 7.8 Hz, 1H), 2.08 (s, 3H). Anal. calcd for $C_{17}H_{12}ClF_3N_2O_7S$: C, 42.47; H, 2.52; N, 5.83. Found: C, 42.32; H, 2.49; N, 5.57; and **112** (45 mg, 20%) as a white powder: mp 152−154 °C. ^{1}H NMR ($CDCl_3$) δ 8.89 (d, J = 9.0 Hz, 1H), 8.79 (d, J = 2.0 Hz, 1H), 8.41 (d, J = 2.0 Hz, 1H), 8.24 (d, J = 9.1 Hz, 1H), 4.48 (dd, J = 11.1, 7.9 Hz, 1H), 4.39 (dt, J = 11.2, 1.4 Hz, 1H), 4.12−3.96 (m, 3H), 1.90 (s, 3H). Anal. calcd for $C_{17}H_{12}ClF_3N_2O_7S$: C, 42.47; H, 2.52; N, 5.83. Found: C, 42.60; H, 2.46; N, 5.65.

*N-(2-((tert-Butyldimethylsilyl)oxy)ethyl)-1-(hydroxymethyl)-5-nitro-2,3-dihydro-1H-benzo[e]indole-7-sulfonamide (**114**) (Scheme 4).* A solution of TBDMSO$(CH_2)_2NH_2$ (142 mg, 0.81 mmol)[27] and DIPEA (242 mg, 1.88 mmol) in CH_2Cl_2 (2 mL) was added dropwise to a cooled (0 °C) solution of **113** (300 mg, 0.63 mmol) in CH_2Cl_2 (15 mL). After 20 min at 0 °C, Cs_2CO_3 (400 mg, 1.23 mmol), MeOH (5 mL), and water (1 mL) were added. After a further 30 min, a further portion of Cs_2CO_3 (400 mg, 1.23 mmol) was added, and the mixture was allowed to warm to room temperature over 2 h. The mixture was poured into water/EtOAc, and the organic layer was separated and washed with cold water and cold brine and dried (Na_2SO_4). Filtration through a silica gel plug followed by removal of EtOAc and trituration (petroleum ether) gave **114** (280 mg, 93%) as a red oil. ^{1}H NMR [$(CD_3)_2SO$]: δ 8.60 (d, J = 1.6 Hz, 1H), 7.97 (d, J = 9.0 Hz, 1H), 7.77 (dd, J = 9.0, 1.7 Hz, 1H), 7.77−7.71 (m, 2H), 6.57 (s, 1H), 4.98 (t, J = 5.4 Hz, 1H), 3.88−3.82 (m, 1H), 3.79−3.68 (m, 2H), 3.66−3.61 (m, 1H), 3.52 (t, J = 6.3, 2H), 2.85 (q, J = 6.1 Hz, 2H), 0.78 (s, 9H), −0.05 (s, 6H). HRMS (FAB) calcd for $C_{21}H_{32}N_3O_6SSi$ m/z, 482.1781; found, 482.1786.

*N-(2-((tert-Butyldimethylsilyl)oxy)ethyl)-1-(hydroxymethyl)-5-nitro-3-(5,6,7-trimethoxy-1H-indole-2-carbonyl)-2,3-dihydro-1H-benzo[e]indole-7-sulfonamide (**115**) (Scheme 10).* A solution of EDCI·HCl (64 mg, 0.33 mmol), TsOH (3 mg, 0.02 mmol), TMI-2-carboxylic acid (25 mg, 0.10 mmol), and **114** (40 mg, 0.08 mmol) in DMA (2 mL) was stirred for 1.5 h. Further portions of EDCI·HCl (15 mg, 0.08 mmol), TsOH (2 mg, 0.01 mmol), and TMI-2-carboxylic acid (5 mg, 0.01 mmol) were added, and after 2 h, cold 5% aqueous $NaHCO_3$ was added, and the precipitate was filtered and washed with cold water. The residue was purified by trituration (Et_2O) followed by precipitation (CH_2Cl_2/MeOH) to give **115** (55 mg, 92%) as a yellow powder: mp 233−237 °C. ^{1}H NMR [$(CD_3)_2SO$]: δ 11.56 (s, 1H), 9.27 (s, 1H), 8.87 (d, J = 1.6 Hz, 1H), 8.36 (d, J = 8.9 Hz, 1H), 8.01 (dd, J = 8.9, 1.6 Hz, 1H), 7.97 (br s, 1H), 7.20 (d, J = 2.0 Hz, 1H), 6.98 (s, 1H), 5.07 (t, J = 5.5 Hz, 1H), 4.82 (t, J = 10.2 Hz, 1H), 4.67 (dd, J = 10.5, 2.0 Hz, 1H), 4.21−4.13 (m, 1H), 3.94 (s, 3H), 3.83 (s, 3H), 3.81 (s, 3H), 3.80−3.74 (m, 1H), 3.69−3.62 (m, 1H), 3.56 (t, J = 6.1 Hz, 2H), 2.96−2.85 (m, 2H), 0.80 (s, 9H), −0.03 (s, 6H). Anal. calcd for $C_{34}H_{42}N_4O_{10}SSi$·0.5H_2O: C, 55.50; H, 5.89; N, 7.61. Found: C, 55.44; H, 6.00; N, 7.95.

*(7-(N-(2-((tert-Butyldimethylsilyl)oxy)ethyl)sulfamoyl)-5-nitro-3-(5,6,7-trimethoxy-1H-indole-2-carbonyl)-2,3-dihydro-1H-benzo[e]-indol-1-yl)methyl Phenylmethanesulfonate (**119**) (Scheme 10).* α-Toluenesulfonyl chloride (42 mg, 0.22 mmol) was added to a cooled (0 °C) solution of **115** (40 mg, 0.06 mmol) in N_2-purged dry pyridine (4 mL). After 1 h, a further portion of α-toluenesulfonyl chloride (84 mg, 0.44 mmol) was added, and after 1 h at 0 °C, ice water was added, and the precipitate was filtered and washed with cold water and Et_2O to give **119** (37 mg, 76%) as a yellow powder: mp 114−117 °C. ^{1}H NMR [$(CD_3)_2SO$]: δ 11.62 (s, 1H), 9.25 (s, 1H), 8.85 (d, J = 1.6 Hz, 1H), 8.34 (d, J = 8.9 Hz, 1H), 8.02 (dd, J = 8.9, 1.7 Hz, 1H), 7.99 (t, J = 5.8 Hz, 1H), 7.25−7.09 (m, 6H), 6.98 (s, 1H), 4.89 (t, J = 9.3 Hz, 1H), 4.66−4.56 (m, 3H), 4.56−4.48 (m, 3H), 3.94 (s, 3H), 3.84 (s, 3H), 3.82 (s, 3H), 3.57 (t, J = 6.2 Hz, 2H), 2.91 (q, J = 6.0 Hz, 2H), 0.80 (s, 9H), −0.03 (s, 6H). Anal. calcd for $C_{41}H_{48}N_4O_{12}S_2Si$·0.5$H_2O$: C, 55.33; H, 5.55; N, 6.30. Found: C, 55.32; H, 5.56; N, 6.24.

*(7-(N-(2-Hydroxyethyl)sulfamoyl)-5-nitro-3-(5,6,7-trimethoxy-1H-indole-2-carbonyl)-2,3-dihydro-1H-benzo[e]indol-1-yl)methyl Phenylmethanesulfonate (**29**) (Scheme 10).* TFA (0.5 mL) was added dropwise to a solution of **119** (34 mg, 0.04 mmol) in CH_2Cl_2 (2 mL) and MeOH (0.5 mL). After 30 h, solvents were removed, and the residue was triturated (petroleum ether/Et_2O) to give **29** (29 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 (99.9% purity): mp 191−193 °C. ^{1}H NMR [$(CD_3)_2SO$]: δ 11.61 (s, 1H), 9.25 (s, 1H), 8.85 (s, 1H), 8.34 (d, J = 8.9 Hz, 1H), 8.03 (d, J = 8.5 Hz, 1H), 7.92 (br s, 1H), 7.24−7.10 (m, 6H), 6.98 (s, 1H), 4.94−4.84 (m, 1H), 4.68 (t, J = 5.5 Hz, 1H), 4.64−4.50 (m, 6H), 3.95 (s, 3H), 3.85 (s, 3H), 3.82 (s, 3H), 3.40 (q, J = 5.8 Hz, 2H), 2.87 (q, J = 5.7 Hz, 2H). HRMS (FAB) calcd for $C_{34}H_{35}N_4O_{12}S_2$ m/z, 755.1693; found, 755.1699.

*Synthesis of Compounds in Table 3 and Their Intermediates. Di-tert-butyl (2-((2-(7-Carbamoyl-1-(hydroxymethyl)-5-nitro-2,3-dihydro-1H-benzo[e]indole-3-carbonyl)-1H-indol-5-yl)oxy)ethyl) Phosphate (**123**) (Scheme 11).* A solution of **63** (110 mg, 0.38 mmol) in DMA (3 mL) was treated with anhydrous TsOH (72 mg, 0.42 mmol), 5-(2-((di-*tert*-butoxyphosphoryl)oxy)ethoxy)-1*H*-indole-2-carboxylic acid[14] (207 mg, 0.50 mmol), and EDCI·HCl (300 mg, 1.57 mmol). The mixture was stirred at room temperature for 4 h, then cooled to 0 °C, treated with 5% aqueous $NaHCO_3$ (10 mL), and stirred for further 10 min. The precipitate was collected by filtration, washed with cold water and small portions of ice-cold EtOAc, and then dried in a vacuum desiccator. The residue was triturated with diethyl ether to give **123** as a yellow solid (140 mg, 54%): mp 206−210 °C. ^{1}H NMR [$(CD_3)_2SO$]: δ 11.71 (d, J = 1.7 Hz, 1H), 9.19 (s, 1H), 8.85 (d, J = 1.3 Hz, 1H), 8.25 (br, s, 1H), 8.22 (d, J = 8.9 Hz, 1H), 8.13 (dd, J = 8.8, 1.5 Hz, 1H), 7.57 (br, s, 1H), 7.43 (d, J = 8.9 Hz, 1H), 7.20 (dd, J = 8.1, 1.7 Hz, 2H), 6.96 (dd, J = 8.9, 2.4 Hz, 1H), 5.06 (t, J = 5.6 Hz, 1H), 4.84 (t, J = 10.3 Hz, 1H), 4.72 (dd, J = 10.4, 2.1 Hz, 1H), 4.22−4.18 (m, 5H), 3.78 (m, 1H), 3.70−3.64 (m, 1H), 1.43 (s, 18H). HRMS (FAB) calcd for $C_{33}H_{39}N_4O_{10}P$ (M^+) m/z, 682.2404; found, 682.2405.

(7-Carbamoyl-3-(5-(2-((di-tert-butoxyphosphoryl)oxy)ethoxy)-1H-indole-2-carbonyl)-5-nitro-2,3-dihydro-1H-benzo[e]indol-1-yl)-

*methyl Phenylmethanesulfonate (**124**) (Scheme 11).* A solution of **123** (100 mg, 0.15 mmol) in dry pyridine (3 mL) was treated with α-toluenesulfonyl chloride (140 mg, 0.73 mmol) at 0 °C. The mixture was stirred for 1 h and then poured into a beaker of ice water and stirred for further 10 min. The solution was extracted with CH_2Cl_2 (3×), and the combined organic layers were washed with water and brine, dried, and concentrated under reduced pressure. The residue was triturated with diethyl ether to give **124** as a yellow solid (43 mg, 34%): mp 166−170 °C. ^{1}H NMR [$(CD_3)_2SO$]: δ 11.75 (d, *J* = 1.7 Hz, 1H), 9.18 (s, 1H), 8.85 (d, *J* = 1.2 Hz, 1H), 8.29 (br, s, 1H), 8.22 (d, *J* = 8.8 Hz, 1H), 8.16 (dd, *J* = 8.8, 1.4 Hz, 1H), 7.61 (br, s, 1H), 7.45 (d, *J* = 8.9 Hz, 1H), 7.20−7.11 (m, 7H), 6.97 (dd, *J* = 8.9, 2.4 Hz, 1H), 4.93−4.91 (m, 1H), 4.66−4.55 (m, 6H), 4.22−4.20 (m, 4H), 1.43 (s, 18H). HRMS (FAB) calcd for $C_{40}H_{45}N_4O_{12}$ PS (M^+) *m*/*z*, 836.2492; found, 836.2497.

*(7-Carbamoyl-5-nitro-3-(5-(2-(phosphonooxy)ethoxy)-1H-indole-2-carbonyl)-2,3-dihydro-1H-benzo[e]indol-1-yl)methyl phenylmethanesulfonate (**33**) (Scheme 11).* A solution of **124** (40 mg, 0.05 mmol) in CH_2Cl_2 (2 mL) was treated with TFA (41 μL, 0.53 mmol), stirred at room temperature for 24 h, and then concentrated under reduced pressure. The residue was repeatedly dissolved in CH_2Cl_2 and then concentrated to remove the excess TFA. The crude product was triturated with diethyl ether to give **33** as a yellow solid (26 mg, 72%): mp 191−194 °C. ^{1}H NMR [$(CD_3)_2SO$]: δ 11.74 (s, 1H), 11.20 (br, s, 1H), 9.18 (s, 1H), 8.85 (d, *J* = 1.0 Hz, 1H), 8.28 (br, s, 1H), 8.21 (d, *J* = 8.8 Hz, 1H), 8.16 (dd, *J* = 8.8, 1.3 Hz, 2H), 7.61 (br, s, 1H), 7.45 (d, *J* = 8.9 Hz, 1H), 7.21−7.11 (m, 6H), 6.99 (dd, *J* = 8.9, 2.3 Hz, 1H), 4.94−4.90 (m, 1H), 4.66−4.55 (m, 6H), 4.19−4.13 (m, 4H), 2 protons not observed. HRMS (ESI) calcd for $C_{32}H_{28}N_4O_{12}PS$ (MH^-) *m*/*z*, 723.1168; found, 723.1157. HPLC purity, 99.7%.

*Di-tert-butyl (2-(1-(Hydroxymethyl)-5-nitro-3-(2,2,2-trifluoroacetyl)-2,3-dihydro-1H-benzo[e]indole-7-carbonylamino)ethyl) Phosphate (**125**) (Scheme 12).* A solution of **70** (120 mg, 0.31 mmol) in anhydrous THF (15 mL) was treated with DIPEA (160 μL, 0.86 mmol) at room temperature. The mixture was cooled to 0 °C, treated with 2-aminoethyl di(*tert*-butyl) phosphate[14] (153 mg, 0.64 mmol) and PyBOP (211 mg, 0.41 mmol), and stirred at room temperature for 1 h. The mixture was evaporated to dryness, and the residue was dissolved in EtOAc, washed with ice-cold water and brine, dried, and concentrated under reduced pressure. The crude product was purified by column chromatography eluting with diethyl ether/MeOH (24:1) to provide **125** as a yellow solid (190 mg, 99%): mp 76−79 °C. ^{1}H NMR [$(CD_3)_2SO$]: δ 9.00 (s, 1H), 8.98 (t, *J* = 5.5 Hz, 1H), 8.84 (d, *J* = 1.3 Hz, 1H), 8.28 (d, *J* = 8.8 Hz, 1H), 8.15 (dd, *J* = 8.8, 1.6 Hz, 1H), 5.05 (t, *J* = 5.4 Hz, 1H), 4.58−4.49 (m, 2H), 4.23−4.21 (m, 1H), 4.03 (q, *J* = 5.9 Hz, 2H), 3.83−3.78 (m, 1H), 3.75−3.69 (m, 1H), 3.56 (q, *J* = 5.7 Hz, 2H), 1.39 (s, 18H). HRMS (ESI) calcd for $C_{26}H_{33}F_3N_3NaO_9P$ (MNa^+) *m*/*z*, 642.1799; found, 642.1792.

*Di-tert-butyl (2-(1-(Hydroxymethyl)-5-nitro-2,3-dihydro-1H-benzo[e]indole-7-carbonylamino)ethyl) Phosphate (**126**) (Scheme 12).* A solution of **125** (100 mg, 0.16 mmol) in MeOH (10 mL) was cooled to 0 °C in an ice water bath and stirred for 10 min. The mixture was then treated with Cs_2CO_3 (53 mg, 0.16 mmol), stirred for a further 30 min, and then evaporated to dryness. The residue was purified by column chromatography eluting with CH_2Cl_2/MeOH (49:1 and 48:1) to provide **126** as a red gum (83 mg, 99%). ^{1}H NMR [$(CD_3)_2SO$]: δ 8.73 (t, *J* = 5.5 Hz, 1H), 8.60 (d, *J* = 1.3 Hz, 1H), 7.91 (dd, *J* = 8.9, 1.6 Hz, 1H), 7.85 (d, *J* = 8.8 Hz, 1H), 7.63 (s, 1H), 7.39 (s, 1H), 4.95 (t, *J* = 5.4 Hz, 1H), 4.00 (q, *J* = 6.0 Hz, 2H), 3.86−3.82 (m, 1H), 3.76−3.63 (m, 3H), 3.52 (q, *J* = 5.6 Hz, 2H), 3.46−3.41 (m, 1H), 1.39 (s, 18H). HRMS (FAB) calcd for $C_{24}H_{34}N_3O_8P$ (M^+) *m*/*z*, 523.2084; found, 523.2083.

*Di-tert-butyl (2-(1-(Hydroxymethyl)-5-nitro-3-(5,6,7-trimethoxy-1H-indole-2-carbonyl)-2,3-dihydro-1H-benzo[e]indole-7-carbonylamino)ethyl) Phosphate (**127**) (Scheme 12).* A solution of **126** (142 mg, 0.27 mmol) in DMA (3 mL) was treated with anhydrous TsOH (51 mg, 0.30 mmol), 5,6,7-trimethoxy-1*H*-indole-2-carboxylic acid (88 mg, 0.35 mmol), and EDCI·HCl (206 mg, 1.08 mmol). The mixture was stirred at room temperature for 2 h, then cooled to 0 °C, treated with 5% aqueous $NaHCO_3$, and stirred for further 10 min. The precipitate was collected by filtration, washed with cold water and *i*-Pr_2O, and then dried in a vacuum desiccator to give **127** as a yellow solid (140 mg, 69%): mp 150−154 °C.; ^{1}H NMR [$(CD_3)_2SO$]: δ 11.53 (d, *J* = 1.8 Hz, 1H), 9.14 (br, s, 1H), 8.94 (t, *J* = 5.4 Hz, 1H), 8.83 (d, *J* = 1.3 Hz, 1H), 8.23 (d, *J* = 8.8 Hz, 1H), 8.11 (dd, *J* = 8.8, 1.5 Hz, 1H), 7.19 (d, *J* = 2.3 Hz, 1H), 6.98 (br, s, 1H), 5.06 (t, *J* = 5.5 Hz, 1H), 4.80 (t, *J* = 10.4 Hz, 1H), 4.66 (dd, *J* = 10.6, 2.1 Hz, 1H), 4.18−4.12 (m, 1H), 4.03 (m, 2H), 3.94 (s, 3H), 3.83 (s, 3H), 3.81 (s, 3H), 3.80−3.76 (m, 1H), 3.68−3.61 (m, 1H), 3.59−3.52 (m, 2H), 1.40 (s, 18H). HRMS (ESI) calcd for $C_{36}H_{45}N_4NaO_{12}P$ (MNa^+) *m*/*z*, 779.2664; found, 779.2684.

*(7-((2-((Di-tert-butoxyphosphoryl)oxy)ethyl)carbamoyl)-5-nitro-3-(5,6,7-trimethoxy-1H-indole-2-carbonyl)- 2,3-dihydro-1H-benzo[e]indol-1-yl)methyl Phenylmethanesulfonate (**129**) (Scheme 12).* A solution of **127** (140 mg, 0.19 mmol) in dry pyridine (5 mL) was treated with α-toluenesulfonyl chloride (210 mg, 1.11 mmol) at 0 °C. The mixture was stirred for 1 h, then poured into a beaker of ice water, stirred for further 10 min, and then extracted with EtOAc (3×). The combined organic layers were washed with water and brine, dried, and concentrated under reduced pressure. The residue was purified by column chromatography eluting with CH_2Cl_2/MeOH (19:1) to give **129** as a yellow solid (106 mg, 61%): mp 109 °C. ^{1}H NMR [$(CD_3)_2SO$]: δ 11.59 (d, *J* = 1.7 Hz, 1H), 9.12 (br, s, 1H), 8.97 (t, *J* = 5.5 Hz, 1H), 8.82 (d, *J* = 1.2 Hz, 1H), 8.22 (d, *J* = 8.8 Hz, 1H), 8.13 (dd, *J* = 8.8, 1.5 Hz, 1H), 7.22−7.12 (m, 6H), 6.98 (br, s, 1H), 4.90−4.85 (m, 1H), 4.60−4.53 (m, 6H), 4.04 (q, *J* = 5.7 Hz, 2H), 3.94 (s, 3H), 3.84 (s, 3H), 3.82 (s, 3H), 3.57 (q, *J* = 5.4 Hz, 2H), 1.39 (s, 18H). Anal. calcd for $C_{43}H_{51}N_4O_{14}PS$: C, 56.70; H, 5.64; N, 6.15. Found: C, 56.57; H, 5.86; N, 6.07.

*(5-Nitro-7-((2-(phosphonooxy)ethyl)carbamoyl)-3-(5,6,7-trimethoxy-1H-indole-2-carbonyl)-2,3-dihydro-1H-benzo[e]indol-1-yl)-methyl Phenylmethanesulfonate (**34**) (Scheme 12).* A solution of **129** (100 mg, 0.11 mmol) in CH_2Cl_2 (5 mL) was treated with TFA (93 μL, 1.21 mmol), stirred at room temperature for 24 h, and then evaporated to dryness. The residue was repeatedly dissolved in CH_2Cl_2 and then concentrated under reduced pressure to remove the excess TFA. The crude product was triturated with diethyl ether to give **34** as a yellow solid (88 mg, 100%): mp 163−167 °C. ^{1}H NMR [$(CD_3)_2SO$]: δ 11.59 (d, *J* = 1.7 Hz, 1H), 9.13 (br, s, 1H), 8.98 (t, *J* = 5.3 Hz, 1H), 8.84 (d, *J* = 1.1 Hz, 1H), 8.22 (d, *J* = 8.9 Hz, 1H), 8.15 (dd, *J* = 8.8, 1.4 Hz, 1H), 7.22−7.13 (m, 6H), 6.98 (br, s, 1H), 4.88−4.83 (m, 1H), 4.61−4.53 (m, 6H), 4.04−3.98 (m, 2H), 3.94 (s, 3H), 3.84 (s, 3H), 3.82 (s, 3H), 3.57 (q, *J* = 5.7 Hz, 2H), 2 protons not observed. HRMS (FAB) calcd for $C_{35}H_{36}N_4O_{14}PS$ (MH^+) *m*/*z*, 799.1686; found, 799.1678. HPLC purity, 96.5%.

*(7-(N-(2-((Di-tert-butoxyphosphoryl)oxy)ethyl)sulfamoyl)-5-nitro-2,3-dihydro-1H-benzo[e]indol-1-yl)methyl Methanesulfonate (**131**) (Scheme 3).* A solution of DIPEA (90 mg, 0.70 mmol) in CH_2Cl_2 (1 mL) followed by a solution of 2-aminoethyl di(*tert*-butyl) phosphate (90 mg, 0.35 mmol) in CH_2Cl_2 (4 mL) were added dropwise to a cooled (0 °C) solution of **99** (120 mg, 0.23 mmol) in CH_2Cl_2 (18 mL). After 15 min, Cs_2CO_3 (160 mg, 0.46 mmol), MeOH (7 mL), and water (1 mL) were added. After a further 15 min, ice was added, and the mixture was poured into cold water/cold EtOAc. The organic layer was separated and then washed with cold water and cold brine, then dried (Na_2SO_4), and evaporated to give **131** (110 mg, 74%). A portion was recrystallized (petroleum ether/EtOAc) to give red crystals: mp 146−151 °C. ^{1}H NMR [$(CD_3)_2SO$]: δ 8.60 (d, *J* = 1.6 Hz, 1H), 8.01 (d, *J* = 8.9 Hz, 1H), 7.95 (br s, 1 H), 7.81 (dd, *J* = 8.9, 1.7 Hz, 1H), 7.80 (s, 1 H), 4.41−4.33 (m, 1H), 4.29−4.19 (m, 2H), 3.90−3.77 (m, 3H), 3.71 (dd, *J* = 9.8, 2.0 Hz, 1H), 3.15 (s, 3H), 2.90 (t, *J* = 5.8 Hz, 2H), 1.35 (s, 18H). Anal. calcd for $C_{24}H_{36}N_3O_{11}PS_2$: C, 45.21; H, 5.69; N, 6.59. Found: C, 45.50; H, 5.82; N, 6.45.

*(7-(N-(2-((Di-tert-butoxyphosphoryl)oxy)ethyl)sulfamoyl)-5-nitro-3-(5,6,7-trimethoxy-1H-indole-2-carbonyl)-2,3-dihydro-1H-benzo[e]indol-1-yl)methyl Methanesulfonate (**132**) (Scheme 13).* A solution of EDCI·HCl (280 mg, 1.44 mmol), TsOH (12 mg, 0.07 mmol), TMI-2-carboxylic acid (118 mg, 0.47 mmol), and **131** (230 mg, 0.36 mmol) in DMA (2 mL) was stirred for 5 h. Cold 5% aqueous $NaHCO_3$ was added, and the precipitate was filtered and washed with cold water to give **132** (295 mg, 94%) as a yellow powder. ^{1}H NMR [$(CD_3)_2SO$]: δ 11.60 (s, 1H), 9.25 (s, 1H), 9.88 (d, *J* = 1.6 Hz, 1H),

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8.42 (d, J = 8.9 Hz, 1H), 8.15 (br s, 1H), 8.03 (dd, J = 8.9, 1.7 Hz, 1H), 7.20 (d, J = 2.0 Hz, 1H), 6.98 (s, 1H), 4.90 (t, J = 10.5 Hz, 1H), 4.66 (d, J = 11.1 Hz, 1H), 4.62−4.48 (m, 3H), 3.95 (s, 3H), 3.86−3.79 (m, 2H), 3.83 (s, 3H), 3.81 (s, 3H), 3.08−3.02 (m, 2H), 3.07 (s, 3H), 1.37 (s, 18H).

*(5-Nitro-7-(N-(2-(phosphonooxy)ethyl)sulfamoyl)-3-(5,6,7-trimethoxy-1H-indole-2-carbonyl)-2,3-dihydro-1H-benzo[e]indol-1-yl)methyl Methanesulfonate (**36**) (Scheme 13).* A solution of TFA (380 mg, 3.33 mmol) in CH_2Cl_2 (1 mL) was added dropwise to a solution of **132** (290 mg, 0.33 mmol) in CH_2Cl_2 (15 mL). After 15 h, solvents were removed, and the residue was triturated with Et_2O to give **36** (260 mg, 100%): mp 137−141 °C (dec.). ^{1}H NMR [$(CD_3)_2SO$]: δ 11.59 (s, 1H), 9.24 (s, 1H), 8.87 (s, 1H), 8.42 (d, J = 8.9 Hz, 1H), 8.26 (br s, 1H), 8.03 (d, J = 8.4 Hz, 1H), 7.18 (d, J = 1.8 Hz, 1H), 6.98 (s, 1H), 4.91 (t, J = 9.5 Hz, 1H), 4.66 (d, J = 10.7 Hz, 1H), 4.62−4.49 (m, 3H), 3.95 (s, 3H), 3.84 (s, 3H), 3.84−3.75 (m, 2H), 3.82 (s, 3H), 3.06 (s, 3H), 3.06−3.00 (m, 2H), 3 protons not observed. Anal. calcd for $C_{28}H_{31}N_4O_{15}PS_2{\cdot}H_2O$: C, 43.30; H, 4.28; N, 7.21. Found: C, 43.42; H, 4.53; N, 7.04.

*Di-tert-butyl (2-(1-(Hydroxymethyl)-5-nitro-2,3-dihydro-1H-benzo[e]indole-7-sulfonamido)ethyl) Phosphate (**135**) (Scheme 4).* A solution of DIPEA (350 mg, 2.69 mmol) and 2-aminoethyl di(*tert*-butyl) phosphate (272 mg, 1.08 mmol) in CH_2Cl_2 (2 mL) was added dropwise to a cooled (0 °C) solution of **113** (430 mg, 0.90 mmol) in CH_2Cl_2 (10 mL). After 30 min, Cs_2CO_3 (580 mg, 1.78 mmol), MeOH (5 mL), and water (1 mL) were added, and the mixture was allowed to warm to room temperature over 2 h. A further portion of Cs_2CO_3 (580 mg, 1.78 mmol) was added, and after 1 h, water/EtOAc were added. The organic layer was separated and washed with cold water and cold brine, dried (Na_2SO_4), and evaporated. The residue was purified by flash chromatography (CH_2Cl_2/MeOH; gradient, 100:0 to 95:5) followed by trituration (Et_2O) to give **135** (350 mg, 70%) as a red powder: mp 90 °C (dec.). ^{1}H NMR [$(CD_3)_2SO$]: δ 8.61 (d, J = 1.6 Hz, 1H), 7.99 (d, J = 9.0 Hz, 1H), 7.91 (br s, 1H), 7.77 (dd, J = 9.0, 1.7 Hz, 1H), 7.75 (s, 1H), 6.58 (s, 1H), 4.98 (t, J = 5.4 Hz, 1H), 3.88−3.75 (m, 4H), 3.71 (dd, J = 9.7, 2.8 Hz, 1H), 3.67−3.60 (m, 1H), 3.50−3.42 (m, 1H), 2.98 (t, J = 5.6 Hz, 2H), 1.34 (s, 18H). Anal. calcd for $C_{23}H_{34}N_3O_9PS{\cdot}0.5H_2O$: C, 48.59; H, 6.21; N, 7.39. Found: C, 48.81; H, 6.24; N, 7.39. HRMS (FAB) calcd for $C_{23}H_{35}N_3O_9PS$ m/z, 560.1832; found, 560.1843.

*Di-tert-butyl (2-(1-(Hydroxymethyl)-5-nitro-3-(5,6,7-trimethoxy-1H-indole-2-carbonyl)-2,3-dihydro-1H-benzo[e]indole-7-sulfonamido)ethyl) Phosphate (**136**) (Scheme 14).* A solution of EDCI·HCl (154 mg, 0.81 mmol), TsOH (6 mg, 0.03 mmol), TMI-2-carboxylic acid (49 mg, 0.19 mmol), and **135** (90 mg, 0.16 mmol) in DMA (5 mL) was stirred for 2 h. Further portions of EDCI·HCl (20 mg, 0.11 mmol), TsOH (2 mg, 0.01 mmol), and TMI-2-carboxylic acid (12 mg, 0.05 mmol) were added, and after 1 h, cold 5% aqueous $NaHCO_3$ was added, and the precipitate was filtered and washed with cold water. The residue was purified by precipitation (CH_2Cl_2/MeOH) to give **136** (112 mg, 88%) as a yellow powder: mp 216−220 °C (dec.). ^{1}H NMR [$(CD_3)_2SO$]: δ 11.56 (d, J = 1.4 Hz, 1H), 2.28 (s, 1H), 8.88 (d, J = 1.5 Hz, 1H), 8.38 (d, J = 8.9 Hz, 1H), 8.13 (t, J = 5.8 Hz, 1H), 8.00 (dd, J = 8.9, 1.6 Hz, 1H), 7.20 (d, J = 2.1 Hz, 1H), 6.98 (s, 1H), 5.07 (t, J = 5.6 Hz, 1H), 4.82 (t, J = 9.6 Hz, 1H), 4.66 (dd, J = 10.5, 2.0 Hz, 1H), 4.21−4.13 (m, 1H), 3.94 (s, 3H), 3.87−3.82 (m, 2H), 3.83 (s, 3H), 3.81 (s, 3H), 3.81−3.74 (m, 1H), 3.71−3.62 (m, 1H), 3.06 (q, J = 5.8 Hz, 2H), 1.36 (s, 18H). Anal. calcd for $C_{35}H_{45}N_4O_{13}PS$: C, 53.03; H, 5.72; N, 7.07. Found: C, 52.77; H, 5.81; N, 7.00.

*(7-(N-(2-((Di-tert-butoxyphosphoryl)oxy)ethyl)sulfamoyl)-5-nitro-3-(5,6,7-trimethoxy-1H-indole-2-carbonyl)-2,3-dihydro-1H-benzo-[e]indol-1-yl)methyl Phenylmethanesulfonate (**140**) (Scheme 14).* α-Toluenesulfonyl chloride (120 mg, 0.63 mmol) was added to a cooled (0 °C) solution of **136** (100 mg, 0.13 mmol) in N_2-purged dry pyridine (5 mL). After 15 min, a further portion of α-toluenesulfonyl chloride (120 mg, 0.63 mmol) was added, and after 1 h at 0 °C, ice water was added, and the precipitate was filtered and washed with cold water to give **140** (118 mg, 100%) as a yellow powder: mp 156−160 °C (dec.). ^{1}H NMR [$(CD_3)_2SO$]: δ 11.62 (d, J = 1.6 Hz, 1H), 9.26 (s, 1H), 8.86 (d, J = 1.6 Hz, 1H), 8.35 (d, J = 8.9 Hz, 1H), 8.17 (t, J = 5.6 Hz, 1H), 8.02 (dd, J = 8.9, 1.7 Hz, 1H), 7.23−7.11 (m, 6H), 6.98 (s, 1H), 4.88 (t, J = 9.3 Hz, 1H), 4.67−4.48 (m, 4H), 4.62 (d, J = 3.0 Hz, 2H), 3.95 (s, 3H), 3.88−3.80 (m, 2H), 3.84 (s, 3H), 3.82 (s, 3H), 3.06 (q, J = 5.6 Hz, 2H), 1.36 (s, 18H). Anal. calcd for $C_{42}H_{51}N_4O_{15}PS_2{\cdot}H_2O$: C, 52.28; H, 5.54; N, 5.81. Found: C, 52.36; H, 5.41; N, 5.76.

*(5-Nitro-7-(N-(2-(phosphonooxy)ethyl)sulfamoyl)-3-(5,6,7-trimethoxy-1H-indole-2-carbonyl)-2,3-dihydro-1H-benzo[e]indol-1-yl)methyl Phenylmethanesulfonate (**39**) (Scheme 14).* TFA (140 mg, 1.23 mmol) was added dropwise to a solution of **140** (116 mg, 0.12 mmol) in CH_2Cl_2 (2 mL). After 15 h, solvents were removed to give **39** (102 mg, 100%) as a yellow powder: mp 212−215 °C. ^{1}H NMR [$(CD_3)_2SO$]: δ 11.70 (s, 1H), 9.27 (s, 1H), 8.87 (d, J = 1.5 Hz, 1H), 8.36 (d, J = 8.9 Hz, 1H), 8.19 (t, J = 5.7 Hz, 1H), 8.02 (dd, J = 8.9, 1.6 Hz, 1H), 7.2−7.10 (m, 6H), 6.98 (s, 1H), 4.95−4.85 (m, 1H), 4.65−4.50 (m, 6H), 3.94 (s, 3H), 3.83 (s, 3H), 3.83−3.80 (m, 2H), 3.81 (s, 3H), 3.02 (q, J = 5.8 Hz, 2H), 2 protons not observed. Anal. calcd for $C_{34}H_{35}N_4O_{15}PS_2{\cdot}0.5H_2O$: C, 48.40; H, 4.30; N, 6.64. Found: C, 48.51; H, 4.19; N, 6.61.

In Vitro Cytotoxicity. Inhibition of proliferation of log-phase monolayers was assessed in 96-well plates as previously described.[13,28] 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.

Clonogenic Assay. Clonogenic assays were performed as previously described.[25] Drug exposures were performed in 96-well plates (Nunc) using either a 37 °C humidified incubator (95% air, 5% CO_2) or in the incubator compartment (37 °C) of an anaerobic chamber where palladium catalyst-scrubbed gas (90% N_2, 5% H_2, 5% CO_2) ensured severe anoxia (<0.001% O_2). Cell cultures were grown in αMEM (Gibco BRL, Grand Island, NY) containing 5% heat-inactivated fetal calf serum (FCS; Gibco BRL, Auckland, New Zealand) and maintained in exponential growth phase. For each experiment, 3 × 10^5 cells in 150 μL were seeded into wells in αMEM + 10% FCS + 10 mmol L^{-1} added D-glucose + 200 μmol L^{-1} 2′-deoxycytidine (2′-dCyd) containing penicillin (100 units mL^{-1}) and streptomycin (100 μg mL^{-1}) (P/S) and allowed to attach for at least 2 h. High glucose (final concentration, 17 mmol L^{-1}) and the presence of 2′-dCyd minimize hypoxia-induced cell-cycle arrest. Drug solutions were added (150 μL), and cells were exposed in duplicate for 4 h. At the end of the incubation, the extracellular medium was removed, and the cells were washed with 100 μL of PBS and trypsinized. Subsequently, cells were plated for clonogenic survival in plastic Petri dishes in αMEM + 10% FCS containing P/S. The dishes were stained with methylene blue 14 days later, and colonies containing more than 50 cells were counted.

In Vivo Activity. Antitumor activity by excision assay was performed as previously described.[13,14] SiHa and H460 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 posthoc test on log-transformed data with SigmaPlot v11.2 (Sysat Software, Inc.). Compound **19** was formulated in 10% DMSO, 40% PEG-400, and 50% water. All animal studies were approved by the University of Auckland Animal Ethics Committee. Experiments were performed using CD1-Foxn1$^{nu/nu}$ (nude) mice.

■ ASSOCIATED CONTENT

Ⓢ Supporting Information

Experimental details for the syntheses of intermediates **46**, **45**, **49**, **50**, **52**−**55**, **50** (method B), **47**, **65**, **66**, **74**, **76**, **93**, **101**, **103**, **102**, **105**, **104**, **107**, **108** (method A), **108** (method B),

109 (method A), **109** (method B), **116**, **120**, **117**, **121**, **118**, **122**, **128**, **130**, **133**, **134**, **137**, **141**, **138**, **142**, **139**, and **143**. Syntheses of compounds in Table 1: **16**, **18**, **19** (method B), **20**, **21**, **23**, **25**, **27** (method A), **27** (method B), **27** (method C), **28** (method A), **28** (method B), and **30**−**32**. Syntheses of compounds in Table 3: **35**, **37**, **38**, and **40**−**42**. Figures and tables. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

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ABBREVIATIONS USED

AIBN, azobisisobutyronitrile; CBI, *seco*-1,2,9,9a-tetrahydrocyclopropa[*c*]benz[*e*]indol-4-one; DIPEA, diisopropylethylamine; DEI, 5-[(dimethylamino)ethoxy]indole; DMA, dimethylacetamide; DPPA, diphenylphosphoryl azide; *E*(1), one-electron reduction potential; EDCI·HCl, *N*-ethyl-*N*′-(3-dimethylaminopropyl)carbodiimide hydrochloride; EWG, electron-withdrawing group; HAP, hypoxia-activated prodrug; HCR, hypoxic cytotoxicity ratio; iv, intravenous; LCK, $\log_{10}$ cell kill; MEI, 5-[(morpholino)ethoxy]indole; MS, 4-methoxystyrene; pyBOP, benzotriazol-1-yl-oxytripyrrolidinophosphonium hexafluorophosphate; SAR, structure−activity relationship; TEMPO, (2,2,6,6-tetramethylpiperidin-1-yl)oxyl; TFA, trifluoroacetic acid; TFAA, trifluoroacetic anhydride; THP, tetrahydropyranyl; TMI, 5,6,7-trimethoxyindole

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