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Influence of a Basic Side Chain on the Properties of Hypoxia-Selective Nitro Analogues of the Duocarmycins: Demonstration of Substantial Anticancer Activity in Combination with Irradiation or Chemotherapy

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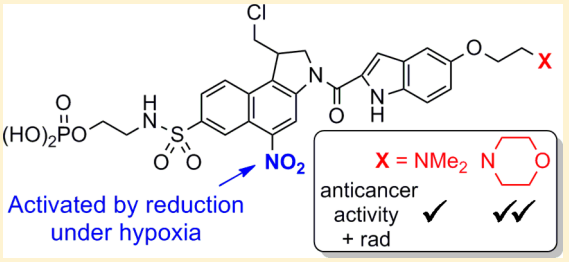
Supporting Information

ABSTRACT: A new series of nitro analogues of the duocarmycins was prepared and evaluated for hypoxia-selective anticancer activity. The compounds incorporate 13 different amine-containing side chains designed to bind in the minor groove of DNA while spanning a wide range of base strength from pK_a 9.64 to 5.24. The most favorable in vitro properties were associated with strongly basic side chains, but the greatest in vivo antitumor activity was found for compounds containing a weakly basic morpholine. This applies to single-agent activity and for activity in combination with irradiation or chemotherapy (gemcitabine or docetaxel). In combination with a single dose of γ irradiation **50** at 42 μmol/kg eliminated detectable clonogens in some SiHa cervical carcinoma xenografts, and in combination with gemcitabine using a well-tolerated multidose schedule, the same compound caused regression of all treated A2780 ovarian tumor xenografts. In the latter experiment, three of seven animals receiving the combination treatment were completely tumor free at day 100.



INTRODUCTION

The duocarmycins, exemplified by duocarmycin SA (**1**, Figure 1), comprise a small group of extremely cytotoxic natural products that alkylate adenine in the minor groove of DNA.[1,2] Several synthetic analogues showed very promising preclinical antitumor activity,[3,4] and four examples advanced to clinical trial.[5−8] However, all proved to be myelotoxic and lacking in efficacy at tolerated doses.

Subsequent efforts have sought to improve the therapeutic index by introducing a level of tumor-selective delivery or release, aided by an understanding of the duocarmycin mechanism of action. This is illustrated in Figure 1 for 1,2,9,9a-tetrahydrocyclopropa[*c*]benz[*e*]indol-4-one (CBI, **3**), a widely used synthetic variant of the alkylating subunit which, when linked to appropriate minor groove-binding side chains R, retains similar cytotoxic potency to that of the natural products.[9] In the presence of DNA, **3** undergoes selective attack by N3 of adenine to generate a thermally unstable adduct; this depurinates on heating to release **4**. The active alkylating agent **3** can be formed by ring closure of a *seco* precursor **2**. Modifications of the phenol in **2** which prevent ring closure also prevent DNA alkylation and can therefore form the basis of potentially tumor-selective prodrug strategies.[10] Examples of these strategies include phenol glycosides,[11] *N*-acyl *O*-amino phenol derivatives,[12] phenol carbamates as payloads for antibody-drug conjugates (ADCs)[13] or small molecule-drug conjugates,[14] and removal of the phenol functional group to give precursors designed for activation by cytochrome P450 oxidation.[15]

In an alternative structural modification the phenol of the *seco* precursor can be replaced with an amino functional group. This has been reported for a number of different alkylating subunits[16−18] but has been studied in most depth for aminoCBI **7**.[19] We have provided evidence that aminoCBIs share many of the properties of their phenol analogues; particular examples alkylate DNA sequence selectively in AT-rich regions[20] and form exclusively the N3 adenine adduct,[21] with the *S*-enantiomer showing superior DNA alkylation efficiency.[20] We have also shown that aminoCBIs react with nucleophiles in dilute aqueous solution exclusively via the cyclopropyl intermediate **8**, an imine analogue of **3**.[22] Intermediate **8** forms readily from **7** under physiological conditions but is extremely reactive because it is fully protonated. Nevertheless, a direct comparison of **7** and **2** (where R is the 5,6,7-trimethoxyindole (TMI) side chain found in duocarmycin SA) showed very similar cytotoxicity across a cell line panel, especially for the more potent *S* enantiomers.[22]

AminoCBIs have also been employed as the active species in several tumor-selective prodrug strategies. Various carbamates

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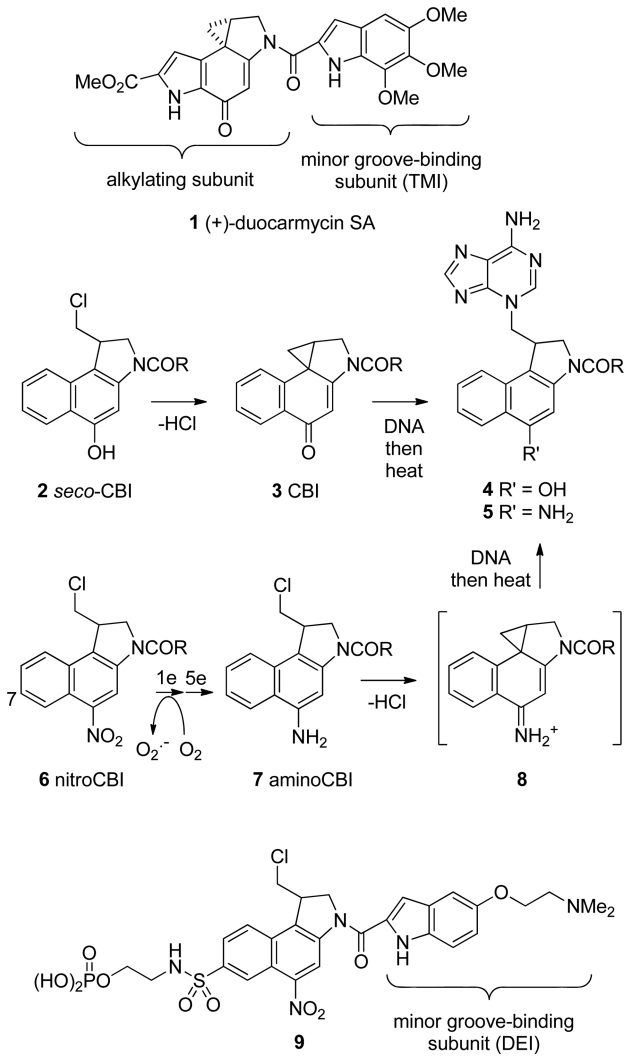

Figure 1. Structure of the natural product duocarmycin SA, DNA alkylation pathway illustrated with **3** (a synthetic variant of the alkylating subunit), and the hypoxia-selective mechanism of action of nitroCBI prodrugs. R is a side chain that can bind in the minor groove of DNA; examples include TMI (5,6,7-trimethoxyindole) and DEI [5-(2-(dimethylamino)ethoxy)indole]. NitroCBIs do not undergo spirocyclization but are reduced selectively in the absence of oxygen to the corresponding aminoCBIs which alkylate DNA by a mechanism analogous to that of the phenol congeners.

have been designed as ADC payloads[23,24] or for activation following reduction by exogenous enzymes[25] or in the presence of ferrous iron.[26] We have proposed an alternative strategy with nitroCBIs of general structure **6**. In this scheme, the strongly electron-withdrawing nitro substituent suppresses spirocyclization but is reduced by endogenous reductases via an initial 1-electron oxygen-sensitive step to ultimately yield the corresponding aminoCBI **7**. NitroCBIs are thus examples of hypoxia-activated prodrugs (HAPs), compounds designed to take advantage of the low oxygen concentrations commonly found in tumors but not in healthy tissues.[27,28] Hypoxic cells in tumors have been linked to multiple types of treatment resistance[29,30] (to radiotherapy, to some forms of chemotherapy, and more recently to immunotherapy[31]) in part because the selective pressures of a hypoxic microenvironment drive the development of a more aggressive and metastatic phenotype. The promise of HAPs is that they may be combined to advantage with a variety of existing therapies, an outlook supported in preclinical and in some clinical trials,[32,33] but to date a HAP has yet to achieve registration as an anticancer agent.

In preclinical experiments, we showed that **6** (R = TMI) is selectively toxic to some tumor cell lines in the absence of oxygen and that the corresponding adduct **5** can be isolated from cells incubated with **6** under hypoxic but not oxic conditions.[21] The introduction of electron-withdrawing substituents, particularly sulfonamides or carboxamides in the 7-position of the nitroCBI, provided analogues that were more efficiently reduced by endogenous reductases and exhibited substantial hypoxic cytotoxicity ratios (HCRs) in all tumor cell lines tested.[34] This substituent in turn provided a convenient site for the introduction of a phosphate "preprodrug" that conferred excellent water solubility while being rapidly cleaved after iv administration.[35] The particular example **9** is highly active against hypoxic cells in human tumor xenografts at well-tolerated doses in mice and provides a substantial increase in tumor growth delay when combined with γ irradiation. Although **9** is racemic and the enantiomers differ at least 20-fold in potency, there is no enantioselectivity in the hypoxia-selective activation step in vitro; both enantiomers show clean metabolism from nitro to amino in the presence of hypoxic tumor cells and selectively kill cells under hypoxic conditions.[36] Hypoxia-selective metabolism of **9** was subsequently demonstrated in a panel of 23 human tumor cell lines in a study which identified three flavoreductases capable of performing the one-electron reductive activation step.[37]

In the development of **9**, a set of five different minor groove-binding side chains was examined.[35] One of these, 5-(2-dimethylaminoethoxy)indole (DEI), the only example with a basic group in the side chain, outperformed the others in terms of both HCR in vitro and activity against radioresistant tumor cells in animal models. Superior hypoxic selectivity of DEI versus TMI side chains had previously been noted with a series of substituted nitroCBIs.[34] However, a basic side chain is not always preferred; when the leaving group of a nitroCBI is changed from chloride to sulfonate, strong hypoxia-selective activity is sometimes associated with neutral (TMI) rather than basic (DEI) side chains.[38,39] Because of these observations, and to build on the promising antitumor activity of **9**, we decided to explore the influence of a wide range of basic side chains on the in vitro and in vivo antitumor properties of nitroCBIs related to **9**.

RESULTS AND DISCUSSION

Chemistry. The structures of the new compounds prepared, together with relevant reference compounds, are collected in Table 1. The compounds fall into three groups: nitroCBI prodrugs, aminoCBI cytotoxins, and phosphate preprodrugs of the nitroCBIs. The first two are used to assess in vitro properties because their alcohol side chain (Y = H) is compatible with cell permeability, while the highly polar and water-soluble phosphates are used for in vivo evaluation. In addition to TMI and DEI 13 other side chains (A–M in Table 1) were explored, all of which carry a basic substituent. Eight of the new side chains are indoles (A–H), each functionalized at the 5-position. Incorporating a substituent at this site which projects toward the base of the DNA minor groove can have a substantial influence on cytotoxicity, and a variety of such substituents are known that generate potent CBI[40] and aminoCBI analogues.[41] In addition, three cinnamic acid-based

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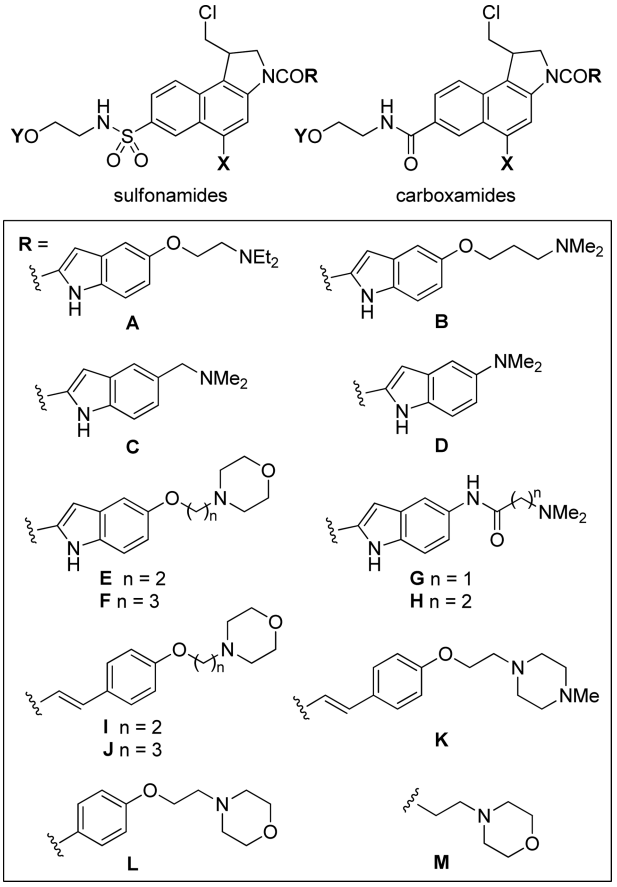
Table 1. Structures of New and Reference Compounds



R	nitroCBI X = NO_2, Y = H	pK_a[a]	aminoCBI X = NH_2, Y = H	phosphate X = NO_2, Y = P(O) $(OH)_2$
sulfonamides				
TMI[b]	**10**[c]	na	**29**[c]	**45**[c]
DEI[b]	**11**[c]	8.70	**30**[c]	**9**[c]
A	**12**	9.64	**31**	**46**
B	**13**	9.35	**32**	**47**
C	**14**	8.84	**33**	**48**
D	**15**	5.24	**34**	**49**
E	**16**	6.47	**35**	**50**
F	**17**	7.16	**36**	**51**
G	**18**	7.45	**37**	**52**
H	**19**	8.86	**38**	**53**
I	**20**	6.39	**39**	**54**
J	**21**	7.15	**40**	**55**
K	**22**	7.58		**56**
L	**23**	6.30	**41**	**57**
M	**24**	6.59	**42**	**58**
carboxamides				
TMI[b]	**25**[c]	na		
DEI[b]	**26**[c]		**43**[c]	**59**[c]
E	**27**	6.47	**44**	**60**
I	**28**	6.39		**61**

[a]Calculated using ACD/Laboratories software ACD/pK_a v12.01. [b]The structures of the TMI and DEI side chains are illustrated in Figure 1. [c]Reference compounds previously reported.[35]

side chains were investigated (I–K, again substituted in a position known to be compatible with cytotoxic potency[41]), along with a benzoic acid derivative L and a single example M, where the aromatic system was replaced with a methylene linker to a morpholine base.

Carboxylic acids corresponding to the selected side chains were commercially available in one case (M) or previously reported for three others (D,[40] E,[42] G[42]). For the remaining side chains, the required acids were prepared using standard methods as shown in Scheme 1. The basic substituent was generally introduced via Mitsunobu reaction or alkylation of a phenol (B, F, I–L) or by acylation of an aniline (H). In addition, side chain A was accessed using a Fischer indole synthesis and side chain C by displacing the bromide of a suitably protected (bromomethyl)indole intermediate using dimethylamine.

Incorporation of the new side chains into nitroCBIs and aminoCBIs bearing a 7-sulfonamide substituent is shown in Scheme 2, and the corresponding routes to 7-carboxamides are presented in Scheme 3. In general, these schemes make use of previously described intermediates and methods. For example, nitroCBIs **12**–**14** and **16**–**19** were prepared by 1-ethyl-3-(3-(dimethylamino)propyl)carbodiimide hydrochloride (EDCI)-mediated coupling of the alkylating subunit **87**[35] to the appropriate side chain acid under acidic conditions (in the presence of toluenesulfonic acid). This approach is generally high-yielding, with the only difficulty being the tendency of the product to eliminate HCl, giving an exomethylene byproduct[36] hard to separate from the desired material, particularly if basic conditions are used to remove the excess acid side chain (see Supporting Information). A slight modification of this method employs a TBDMS protecting group on the alkylating subunit, previously reported in the synthesis of the enantiomers of **11** to give more soluble and more easily purified intermediates.[36] For the current application, TBDMS-protected sulfonamide and carboxamide subunits **89** and **92** were prepared as shown and used to make 6 nitroCBI analogues (four sulfonamide and two carboxamide examples), with a subsequent HCl-mediated deprotection of the TBDMS group. An alternative approach for the amide coupling involved preparation of the acid chloride of the side chain using thionyl chloride, which gave good yields of the desired products in the two cases it was employed (sulfonamides **90i** and **90l**). AminoCBIs were prepared from the nitroCBIs by hydrogenation over PtO_2, followed by HCl treatment to remove the TBDMS protecting group if present. This method was not suitable for the cinnamate side chains because of competing saturation of the double bond. Instead, for two examples, conversion to the aminoCBI (**39** and **40**) was achieved in moderate yield using selective reduction with Zn and NH_4Cl.

Synthesis of water-soluble phosphates suitable for in vivo experiments also followed previous methods using the protected *t*-Bu phosphate esters **94**[35] and **96**[35] as starting materials (Scheme 4). The amide bond to the side chain was again formed using EDCI, with the single exception of side chain L, for which the acid chloride was employed, giving the desired amide in high yield. Final deprotection to produce the free phosphates was achieved using TFA (**46**–**58**), except for two examples where HCl was employed. This proceeded smoothly for **61**, but in the case of **60**, acid exposure also gave an impurity which was tentatively identified as the corresponding *N*-(2-chloroethyl) carboxamide.

Base Strength. The relative basicity of the side chains was assessed by calculating pK_a for the nitroCBIs **11**–**24** and **27** and **28** using ACD software.[43] Not surprisingly, the nitroCBI 7-substituent (sulfonamide versus carboxamide) had no influence

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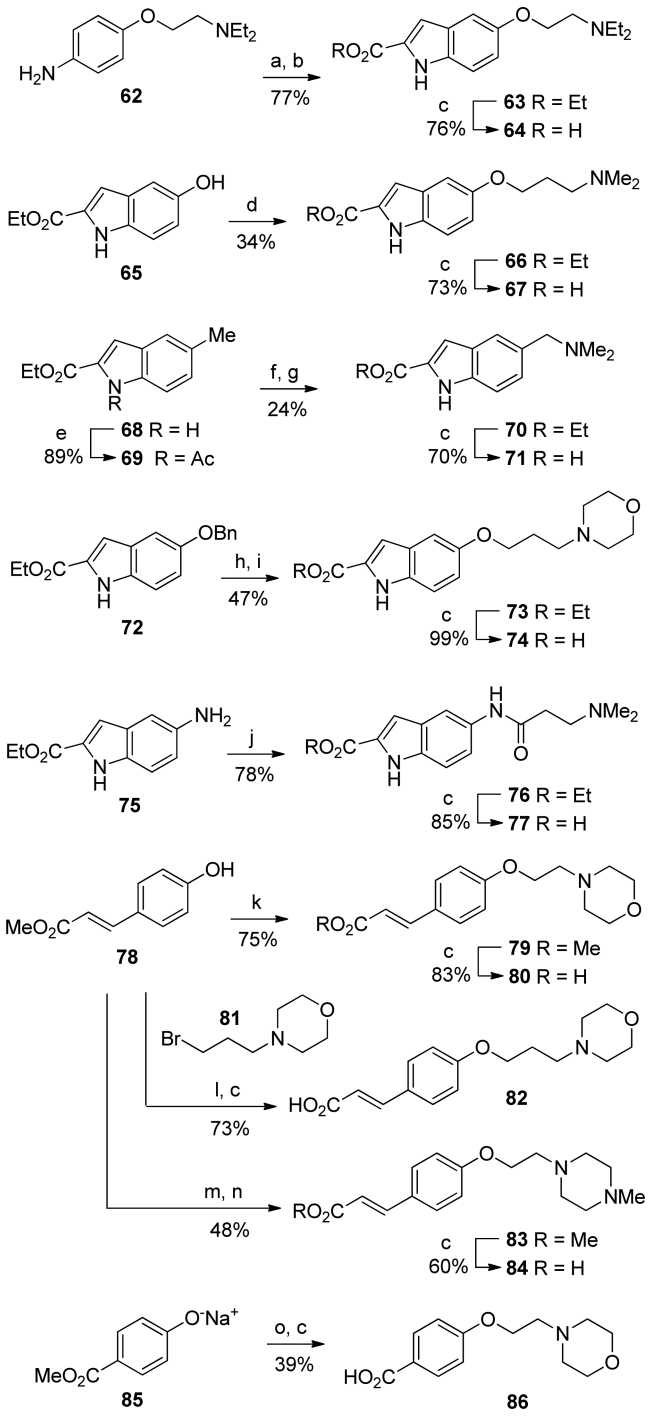
Scheme 1. Synthesis of Side Chain Acids[a]



[a]Conditions: (a) $NaNO_2$, HCl; (b) ethyl 2-methylacetoacetate, NaOAc, then HCl; (c) KOH, then HCl; (d) 3-(dimethylamino)-1-propanol, DEAD, PPh_3; (e) AcCl, NaH; (f) NBS, AIBN; (g) dimethylamine; (h) H_2, Pd/C; (i) 3-morpholino-1-propanol, DEAD, PPh_3; (j) 3-(dimethylamino)propanoic acid hydrochloride, EDCI; (k) 4-(2-chloroethyl)morpholine, NaH; (l) **81** (prepared by reaction of morpholine with 1,3-dibromopropane), NaH; (m) 1,2-dibromoethane, NaH; (n) 1-methylpiperazine then HCl; (o) 4-(2-chloroethyl)-morpholine.

on the calculated base strength. The observed trends in pK_a match expectations based on an increase of basicity with Et versus Me amine substitution and a decrease of basicity in the presence of σ-acceptor ether or electron-withdrawing carboxamide substituents in the γ- or especially β- or α-positions.[44] Overall the set provides a large range of pK_a, with examples more basic than DEI (those with side chains A–C, H) and less basic (D–G, I–M) to the point where limited protonation would be expected even in the acidic microenvironment often found in solid tumors.[45]

In Vitro Cytotoxicity. Cytotoxicity was determined using an antiproliferative assay in two human tumor cell lines, the cervical carcinoma SiHa and the colon carcinoma HT29, for which reference data were available for the TMI and DEI analogues (Figure 2 and Supporting Information, Tables S1 and S2). In general, most of the new aminoCBIs retained strong cytotoxic activity with IC_{50}s in SiHa in the low nM range, suggesting that even the bulkier basic substituents (containing diethylamine or cyclic amine groups) were compatible with binding in the DNA minor groove. While a protonated side chain might be expected to influence cytotoxicity by a number of mechanisms (e.g., affinity to negatively charged DNA or sequestration in acidic organelles), there was no obvious trend in aminoCBI toxicity with calculated pK_a. Instead, side chains L and M, which share the morpholine common to several other side chains, stand out as generating less cytotoxic aminoCBIs (about 100 and 250 times less toxic than DEI, respectively), perhaps because of a poorer fit with the minor groove (L) or decreased binding affinity in the absence of an aromatic group (M). In addition, side chains G and H also tend to generate slightly weaker aminoCBI cytotoxicity. A similar observation was noted for an aminoCBI with a side chain indole 5-NHCOMe substituent[41] even though an amide in this position mimics a structural motif found in some of the natural products. AminoCBI toxicity in the two cell lines was very strongly correlated (Supporting Information, Figure S2), indicating a common mechanism of action, although IC_{50}s were on average 4.2-fold higher in HT29, in keeping with this being the most resistant of 14 cell lines surveyed against reference aminoCBI **30**.[35] As expected, there was no dependence in either cell line on oxic versus hypoxic incubation conditions for aminoCBI cytotoxicity (i.e., HCRs close to 1, see Supporting Information, Table S2).

All nitroCBIs exhibited oxic IC_{50}s in the low μM range in both cell lines, with little dependence on the structure of the side chain, thus showing good deactivation of cytotoxicity in the prodrug form. In contrast, the cytotoxicity of nitroCBIs incubated under hypoxic conditions was much more variable. For some side chains, the shift from oxic to hypoxic conditions gave hardly any enhancement in cytotoxicity (e.g., I, J), while for others sizable HCRs were observed (e.g., A, B, C; HCRs of 27 to 68 for **12**–**14** in the two cell lines) but none superior to those for the two DEI reference compounds **11** and **26** in HT29 (Supporting Information, Table S1). In HT29, the greatest hypoxic selectivity was clearly associated with strongly basic side chains (all HCRs >10 were found for the five nitroCBIs with pK_a $\geq$ 8.7), and in each of these cases the nitroCBI became as potent, or nearly so, as the corresponding aminoCBI under hypoxic conditions (Figure 2). In SiHa, the nitroCBIs rarely achieved equipotency with aminoCBIs under hypoxia but HCRs >10 were observed for a larger selection of side chains, including some of much weaker base strength. Despite the discrepancy between absolute HCRs between the two cell lines, nitroCBI cytotoxicity under hypoxia was again highly correlated between them (Supporting Information, Figure S3), with the more basic side chains clustered at the

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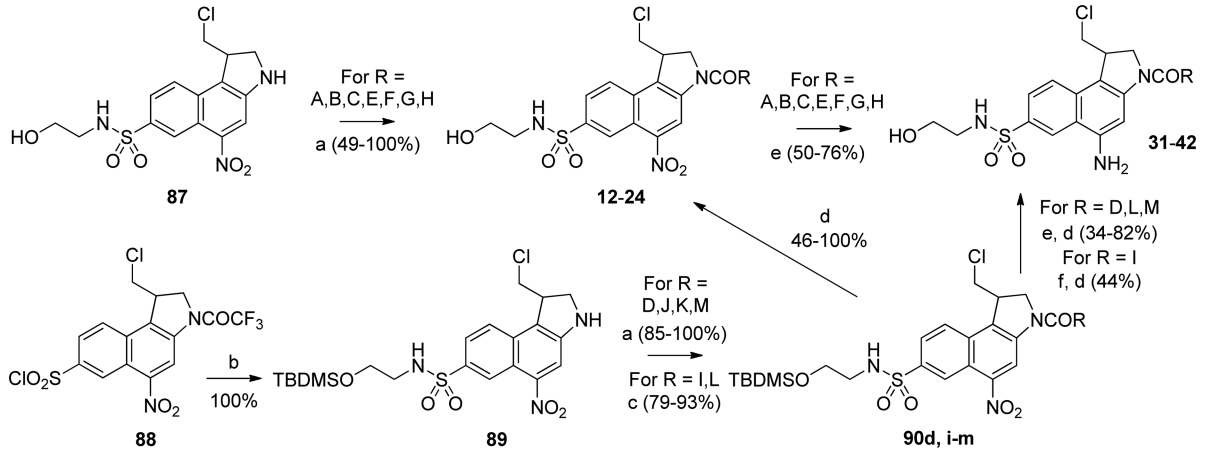
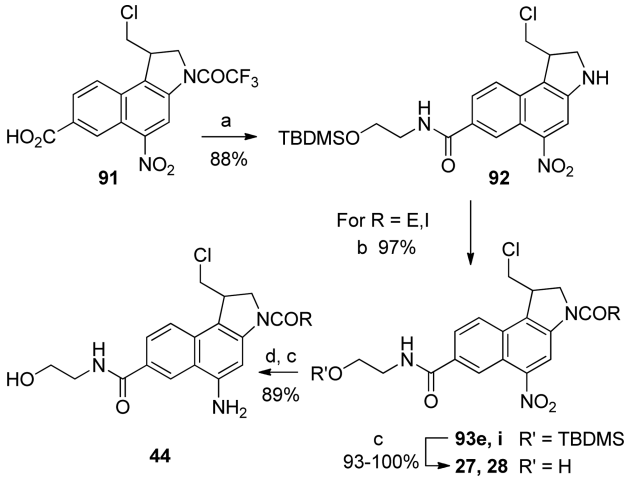
Scheme 2. Synthesis of Sulfonamide-Substituted Nitro- and AminoCBIs[a]



[a]Side chain R is defined in Table 1. Conditions: (a) EDCI, RCO_2H·HCl, toluenesulfonic acid, DMA; (b) (*tert*-butyldimethylsilyloxy)ethylamine, *i*-$PrNEt_2$, then Cs_2CO_3; (c) RCO_2H, $SOCl_2$, DMF, then **89**, *i*-$PrNEt_2$; (d) HCl; (e) H_2, PtO_2; (f) Zn, NH_4Cl. For aminoCBI **40** (R = J), the amide formation and Zn-mediated nitro group reduction were done sequentially in a one-pot reaction in 39% overall yield. For intermediates **90**, the lower case letter refers to the corresponding side chain R, e.g., **90d** has R = D.

Scheme 3. Synthesis of Carboxamide-Substituted Nitro- and AminoCBIs[a]



[a]Side chain R is defined in Table 1. Conditions: (a) (*tert*-butyldimethylsilyloxy)ethylamine, *i*-$PrNEt_2$, pyBOP, then Cs_2CO_3; (b) EDCI, RCO_2H·HCl, toluenesulfonic acid, DMA; (c) HCl; (d) H_2, PtO_2. For intermediates **93**, the lower case letter refers to the corresponding side chain R, e.g., **93e** has R = E.

more potent end of the spectrum, possibly indicating a link between basicity and the extent of reductive metabolism in vitro.

Antitumor Activity in Combination with Irradiation. In our previous evaluation of **9** in nude mice, a sporadic acute toxicity was noted in some animals treated at doses >100 μmol/kg.[35] Similar observations were made for some of the new nitroCBI phosphates, making it difficult to determine MTDs. The new compounds were therefore compared on an equimolar basis. Two xenografts were chosen, SiHa and the lung carcinoma H460, both characterized as containing a moderate hypoxic fraction when grown as sc xenografts.[46] These xenografts differed in their response to the hypoxia-selective activity of **9**,[35] and accordingly the new analogues were compared at a lower dose in the more sensitive SiHa (42 μmol/kg) and a higher dose in the more resistant H460 (75 μmol/kg). These doses were not achievable for one of the new nitroCBI phosphates: iv administration of **53** caused death within a few seconds at 13 μmol/kg, and this compound was therefore dropped from further study. Notably, **52**, which differs only by the removal of one methylene from the side chain (and a consequent reduction in pK_a from 8.86 to 7.45) was well-tolerated, with no toxicity noted at the highest dose tested (75 μmol/kg).

Formulation for iv administration was achieved using PBS containing 2−4 equiv of $NaHCO_3$, which gave solutions with pH in the range 6.8−8.0. Previously, this approach produced a more than 1000-fold increase in aqueous solubility for phosphate **9** compared to alcohol **11**. However, some of the new phosphates proved to be less water-soluble, and the target concentration of 7.5 mM (required to dose mice at 75 μmol/kg) was not achieved in four cases (for details, see Supporting Information, Tables S3 and S4).

Antitumor activity in combination with irradiation was assessed using a previously reported excision assay[35] as outlined in Figure 3A. A large single dose of γ irradiation was used to eliminate the well-oxygenated tumor cell population, with 15 Gy causing on average a 2.0 and 1.2 log reduction in the number of colony-forming cells that could be isolated from SiHa and H460 xenografts, respectively. The nitroCBI phosphates were administered 5 min after irradiation to avoid any potential for radiosensitization. Thus, any further reduction in the residual radioresistant population after the combination treatment indicates activity of the nitroCBI against hypoxic tumor cells in vivo. This is illustrated in Figure 3B for the new analogues in gray compared to **9** and **59** in black (and white for **45**, which was not tested in H460). Although there was some variation on repeat testing (see Supporting Information, Tables S3 and S4), there are clearly several analogues with superior HAP activity compared to the reference compounds. The most marked activity is seen for the sulfonamides **50**, **51**, and **54** (side chains E, F, and I, respectively), which outperform **9** on an equimolar basis in both tumor models. Notably, these compounds share a morpholine in the side chain and are not the compounds that might have been predicted to be highly active on the basis of their in vitro properties; the corresponding nitroCBI alcohols **16**, **17**, and **20** were not

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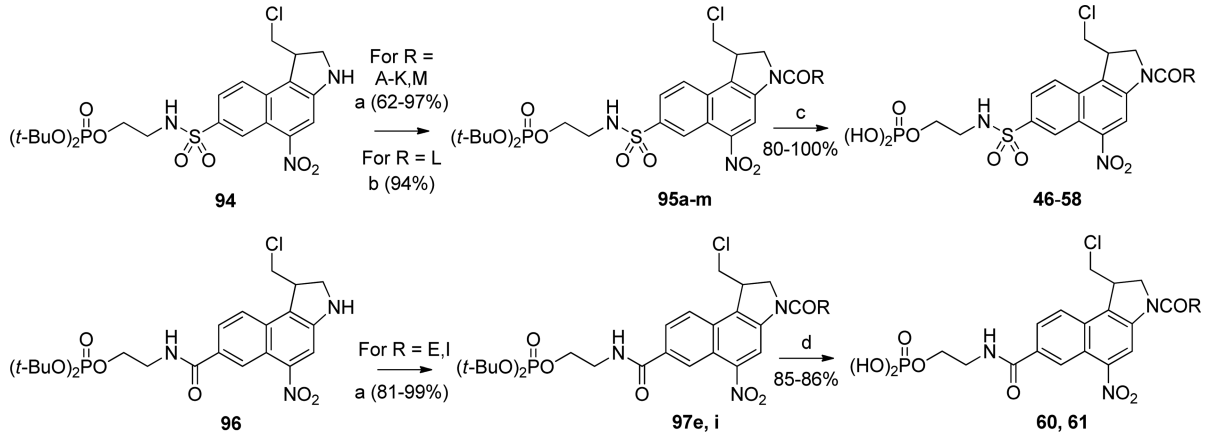
Scheme 4. Synthesis of NitroCBI Phosphates[a]



[a]Side chain R is defined in Table 1. Conditions: (a) EDCI, RCO_2H·HCl, toluenesulfonic acid, DMA; (b) **86**, $SOCl_2$, DMF, then **94**, *i*-$PrNEt_2$, (c) TFA; (d) HCl. For intermediates **95** and **97**, the lower case letter refers to the corresponding side chain R, e.g., **95a** has R = A.

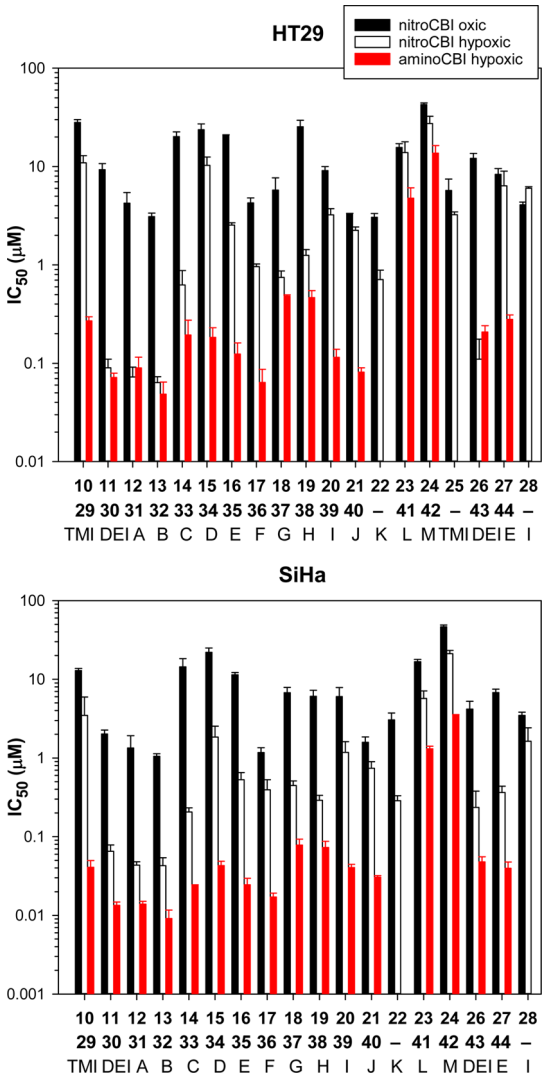


Figure 2. Cytotoxicity of nitroCBIs and aminoCBIs in two human carcinoma cell lines. Cell monolayers were exposed to the compounds for 4 h under oxic or hypoxic conditions, and proliferation was measured 5 days later in comparison to untreated control cells. Side chain structures are shown in Table 1.

remarkable in terms of either HCR or potency under hypoxic conditions (Figure 2). Ranking the best compounds is difficult in SiHa because their activity at the dose used is approaching the limit of the dynamic range of the assay (about 1 in 100000 surviving cells). This is indicated in Figure 3B, with the arrows identifying complete sterilization of the tumor sample (no colonies detected even at the highest plating concentration) for three of five animals receiving **50** or **54** in combination with irradiation. In H460, **54** appears particularly effective, especially considering that solubility limited the dose of this compound to 56 μmol/kg. In contrast, there are several compounds that are not significantly active against hypoxic cells in H460 and only weakly active in SiHa, a group that includes **57** and **58** (side chains L and M associated with weakly cytotoxic aminoCBIs) but also **46** and **48** with strongly basic side chains (A and C) that generated the most favorable in vitro properties. Remarkably, **55** also falls within this group even though it differs only by the insertion of one methylene (side chain J versus I) from the highly active **54**. A comparison of sulfonamides and carboxamides also provides unexpected results. Previously, with the DEI side chain, we observed somewhat stronger HAP activity in SiHa for carboxamide **59** compared to sulfonamide **9**,[35] but with the new examples, sulfonamides **50** and **54** clearly outperform the carboxamide analogues **60** and **61** in both SiHa and H460.

As well as in combination with irradiation, single-agent activity of the nitroCBI phosphates was assessed in comparison to control tumors. Highly significant activity in both tumor models was associated with side chains E, F, and I (Supporting Information, Tables S3 and S4), with **50**, **51**, **54**, and **60** all causing more than 1 log of cell kill in SiHa (Figure 3C). This represents a considerably greater proportion than the hypoxic fraction of this xenograft,[46] implying nitroCBI toxicity to well-oxygenated tumor cells. Possible mechanisms include a low level of reductive activation in these cells or a bystander effect in which aminoCBI produced in hypoxic zones diffuses to and kills cells in surrounding better-oxygenated regions of the tumor. Because we have observed evidence for each mechanism in in vitro experiments,[21,36] it is possible that a combination of both is in action in these tumors. Nevertheless, if single-agent activity against the entire tumor cell population is compared to hypoxia-selective activity against the radioresistant fraction (Figure 3C), there is clearly much greater activity against the

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J. Med. Chem. XXXX, XXX, XXX−XXX

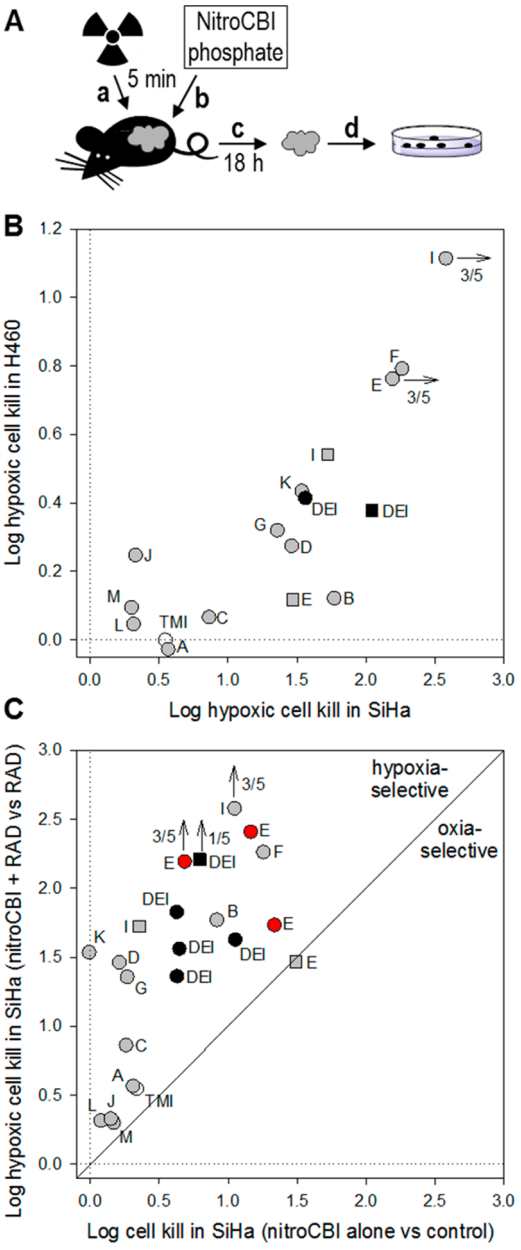

Figure 3. Antitumor activity in combination with irradiation. (A) Experimental design. Immunocompromised mice bearing SiHa or H460 xenografts were treated with or without (a) a large single dose of γ irradiation (15 Gy) and 5 min later with (b) an iv dose of nitroCBI phosphate **9**, **45**–**51**, or **53**–**61**. After 18 h (c) the tumor was excised, dissociated, and plated (d) to determine the number of surviving clonogens. Group sizes were $n = 3$ for control and nitroCBI alone and $n = 5$ for radiation alone and radiation plus nitroCBI. Hypoxic (radioresistant) cell kill is the *additional* cell kill for the combination treatment compared to radiation alone. (B) Efficacy of nitroCBIs against hypoxic cells in the two xenograft models. Circles represent sulfonamides and squares carboxamides with the side chain structures given in Table 1. The TMI compound **45** (white circle) was not tested in H460. Standard doses of 42 and 75 μmol/kg were used for SiHa and H460, respectively, with exceptions noted in the Supporting Information. Where repeat experiments were performed single representative examples are shown. Arrows indicate the number of tumors/group for which no surviving clonogens were detected after combination treatment. (C) Comparison of single agent activity (*x*-axis) versus activity against radioresistant cells (*y*-axis) for all experiments (including replicates) in SiHa. Symbols as for (B) apart from highlighting of **50** in red.

latter. The appearance of data points exclusively in the upper left portion of this graph is strong evidence supporting a hypoxia-selective mechanism of action for this class of compounds in vivo.

For a subset of compounds activity in combination with irradiation was also examined in other human tumor xenografts (Figure 4 and Supporting Information, Tables S5–S7). The

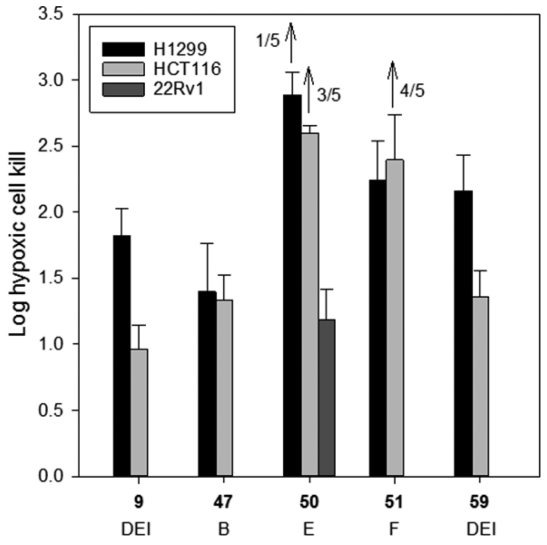

Figure 4. Activity of nitroCBI phosphates against hypoxic cells in lung, colon, and prostate cancer xenografts. Efficacy for a subset of nitroCBI phosphates was assessed using the excision assay illustrated in Figure 3. Doses used were 75, 56, and 30 μmol/kg for H1299, HCT116, and 22Rv1, respectively. Arrows indicate the number of tumors/group for which no surviving clonogens were detected after the combination treatment of irradiation (15 Gy) plus nitroCBI.

lung carcinoma cell line H1299 is relatively insensitive to aminoCBI **11**[35] but as a xenograft has an extremely high hypoxic fraction,[46] whereas colon carcinoma HCT116 and prostate carcinoma 22Rv1 are quite sensitive to **11**[35] in vitro and as xenografts display a moderate hypoxic fraction slightly larger than that of SiHa and H460.[46] Highly significant activity was observed in combination with irradiation for all compounds tested, but once again the morpholine-containing side chains of **50** and **51** conferred superior activity to that of the stronger bases of the DEI and B side chains (**9**, **47**, **59**). At the doses used, **50** and **51** with irradiation eliminated detectable clonogens in several H1299 and HCT116 tumors, and even at the low dose of 30 μmol/kg, **50** was significantly active against hypoxic cells in 22Rv1 tumors.

Antitumor Activity in Combination with Chemotherapy. We have previously shown that combination of **9** with irradiation can lead to substantial tumor growth delay[35] but have not to date reported combination of nitroCBIs with chemotherapy despite the recognition that hypoxic tumor cells are resistant to many types of cytotoxic treatment.[29] For example, limited drug penetration to slowly proliferating hypoxic cells is considered to contribute to resistance to both gemcitabine[47] and docetaxel,[48] and for this reason these agents have been investigated in combination with other HAPs in preclinical[49–51] and clinical studies.[33,52] Here we investigate the ability of either **9** or **50** to enhance the growth delay activity provided by gemcitabine or docetaxel in three different

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J. Med. Chem. XXXX, XXX, XXX–XXX

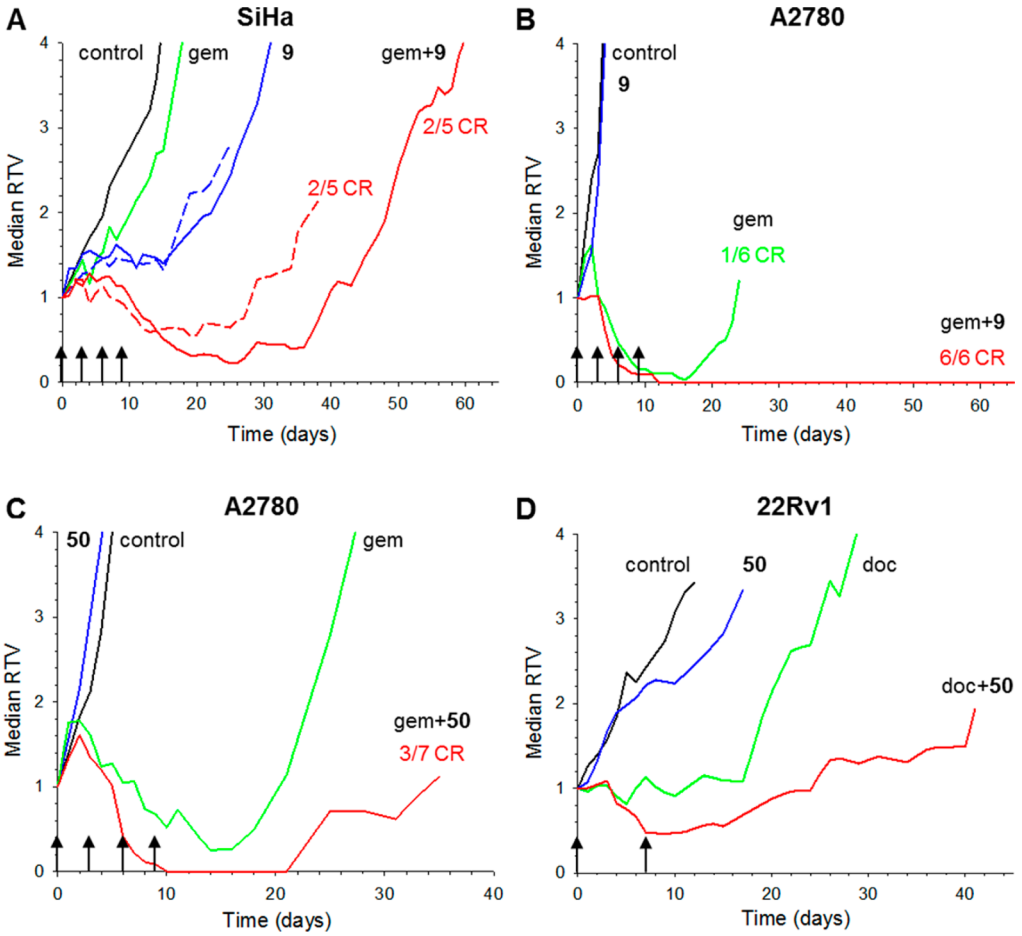

Figure 5. Antitumor activity in combination with chemotherapy. Growth delay curves are displayed as median relative tumor volume (RTV, calculated until the first animal in each group passes end point) for black (control), green (chemotherapy alone), blue (nitroCBI alone), or red (combination) treatment groups. Doses for: (A) gemcitabine 237 μmol/kg, **9** 42 and 56 μmol/kg (dashed and solid lines respectively), $n = 4–5$; (B) gemcitabine 133 μmol/kg, **9** 56 μmol/kg, $n = 6–7$; (C) gemcitabine 100 μmol/kg, **50** 15 μmol/kg, $n = 7$; (D) docetaxel 32 μmol/kg, **50** 14 μmol/kg, $n = 6–7$. Treatment schedule is indicated with arrows and complete responses/group (no tumor at end of observation period at 100–120 days) noted in colored text. Acute deaths occurred in A (four mice) and B (one mouse) (for details, see Experimental Section); these animals were excluded from the analysis.

xenograft models (Figure 5, Supporting Information, Figure S4 and Tables S8, S9).

The schedule for administration of gemcitabine (four ip doses every 3 days, q3d × 4) optimizes its activity against sc xenografts.[53] Mice bearing established SiHa tumors were treated with gemcitabine (237 μmol/kg) in combination with two different doses of **9** and tumor growth followed as relative tumor volume (RTV) over time (Figure 5A). In this experiment, gemcitabine alone had little activity while **9** alone gave a period of tumor stasis followed by regrowth some days after the treatment period was completed. The survival advantage was calculated on the basis of time to quadrupling of the initial tumor volume (RTV4, Kaplan–Meier analyses are presented in the Supporting Information), showing that **9** had significant single-agent activity at both doses used. The combination treatment was very effective, causing regressions in all treated tumors (more durable at the higher dose of **9**), a significant growth delay compared to gemcitabine alone (an extra 39 days at the higher dose of **9**, $p = 0.004$), and several complete responses in which the tumors became undetectable and remained so until the experiment was terminated at >100 days. Although the dose of **9** was limited to 42 and 56 μmol/kg, sporadic acute toxicity was still seen on four occasions (mostly on repeat dosing); these animals were excluded from analysis. Otherwise, the treatment was well-tolerated; although there was a trend to greater body weight loss after combination treatment, none of the groups had an average body weight nadir significantly different from that of the controls (Supporting Information, Table S9).

Gemcitabine was also combined with **9** in A2780 ovarian tumors (Figure 5B). This is a very rapidly growing xenograft with a high hypoxic fraction[46] and marked sensitivity to gemcitabine.[53] For this reason, the gemcitabine dose was reduced to 133 μmol/kg, which produced substantial regressions and one complete response with little associated toxicity (maximum body weight loss of 5%, although one mouse was culled with >15% weight loss). In contrast, **9** alone was inactive and in some cases the tumors passed the end point before the treatment schedule could be completed. However, the combination treatment was dramatically active. A single mouse in this group was excluded following acute toxicity, but the remaining six generated complete responses. In these cases, all tumors became undetectable 8–23 days after treatment commenced, and the animals remained in good health and completely tumor-free for the remainder of the 100 days.

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J. Med. Chem. XXXX, XXX, XXX–XXX

Two further growth delay experiments were conducted to investigate **50**, a representative of the new nitroCBIs with a morpholine-containing side chain. Given the excellent activity of **50** in combination with irradiation, and in an effort to avoid any acute toxicity, considerably lower doses of this compound were employed. The dose of gemcitabine was also further reduced to 100 μmol/kg for the treatment of A2780 (Figure 5C). At this level, gemcitabine provided a similar growth delay to that observed in Figure 5B but without any complete responses. Single-agent **50** at 15 μmol/kg (3.7 times lower dose than **9** in Figure 5B) had no effect on tumor growth, but once again the combination gave striking results, universal tumor regression, an additional 14 day median growth delay (p = 0.001), and in three of seven cases lasting complete responses. These results were obtained without any acute toxicity or any significant body weight losses. **50** was also combined with docetaxel in the aggressive prostate cancer model 22Rv1 (Figure 5D). Antitumor activity of docetaxel is relatively schedule-independent,[54] and the convenient and widely used[50] qw × 2 schedule was employed. At 32 μmol/kg, docetaxel produced short-term tumor stasis and a 13-day growth delay compared to controls but at a cost of significant body weight loss. There was little activity for **50** alone, but combination with docetaxel induced several regressions and a significantly increased growth delay compared to single-agent docetaxel (17 days, p = 0.007). Concomitant with the low dose of **50**, there was no acute toxicity and no measurable effect of nitroCBI on body weight.

CONCLUSIONS

This study was prompted by the observation that the strongest hypoxia-selective activity, both in vitro and in vivo, for a series of nitroCBIs was associated with the only DNA minor groove-binding side chain that contained a basic rather than neutral substituent.[35] In the present study, we describe 13 new side chains (A to M in Table 1) which contain a variety of basic groups spanning a pK_a range from a strongly basic 9.64 to an effectively neutral 5.24, as compared to the previously reported DEI side chain with pK_a 8.70. The necessary side chain acids were synthesized by straightforward routes (Scheme 1) and used to prepare a set of nitroCBI prodrugs and aminoCBI cytotoxins for in vitro analysis and water-soluble phosphate preprodrugs for administration to tumor-bearing mice. This work was done with racemic alkylating subunits rather than the more potent *S* enantiomers because our previous studies had shown no enantioselectivity in the crucial reductive activation step.[36]

Evaluation in vitro (Figure 2) showed that most of the new side chains were able to deliver strongly cytotoxic aminoCBIs and that cytotoxicity was more dependent on structural features other than that of the particular basic substituent. All aminoCBIs with side chains based on a 5-substituted indole or 4-substituted styrene generated submicromolar IC_{50}s after brief exposure to two different human carcinoma cell lines even though these structures covered the widest range of pK_a. In contrast, two side chains sharing a morpholine but attached to a phenyl ring (L) or directly to the alkylating subunit (M) generated considerably less cytotoxic aminoCBIs, presumably because of weaker binding to the minor groove. Some dependence on the nature of the basic substituent was however noted in the in vitro properties of the nitroCBIs. While all of these prodrugs demonstrated good deactivation (relatively weak cytotoxicity under oxic conditions), the greatest potency under hypoxia and the highest HCRs were associated with the most basic side chains, particularly A–C and the previously reported DEI. NitroCBIs with these side chains became as cytotoxic, or nearly so, as the corresponding aminoCBIs when incubated under hypoxic conditions. This suggests efficient metabolic activation for these compounds, even in HT29, which was one of the least proficient cell lines at converting reference nitroCBI **11** to aminoCBI **30**.[37]

The ability of the new nitroCBI phosphates to eliminate hypoxic tumor cells in vivo was investigated in combination with irradiation (Figure 3). Despite the clear preference for strongly basic side chains in vitro, the greatest activity in vivo was associated with weakly basic morpholine-containing side chains E, F, and I, particularly in combination with the sulfonamide-substituted alkylating subunit. Moderate single doses of **50** and **51** were sufficient to eliminate detectable clonogens in the radioresistant subpopulation of some SiHa tumors (and other xenografts, Figure 4), and to cause considerably more hypoxic cell killing than the reference compound **9** in the more resistant H460 tumor model. Discrepancies between in vitro and in vivo data have been noted previously in HAP development. For example, for nitroCBIs containing a benzylsulfonate leaving group, the standout activity in vivo was found for a compound with only a moderate HCR in vitro.[39] In a more comprehensive study of 281 analogues of the HAP tirapazamine, no correlation was found between HCR or hypoxic potency in vitro and the ability to kill hypoxic tumor cells in vivo.[55] Instead, the in vitro data was used as a screen to eliminate less interesting candidates. A model was then developed that specifically included extravascular transport and PK properties and was able to predict the extent of HAP activity in vivo. Interestingly, the two best tirapazamine analogues to emerge from this work both incorporated a morpholine-containing side chain.

Evaluation of the new nitroCBIs in vivo also provided evidence of single-agent activity that was once again particularly marked for side chains E, F, and I. Single-agent activity could be a consequence of a bystander effect or a low level of reductive activation in better-oxygenated cells. Whatever the mechanism, the greater sensitivity of the radioresistant rather than bulk population of tumor cells to all nitroCBIs tested (Figure 3C) is compelling evidence of hypoxia-selective activity for this class of compounds in vivo.

One attractive feature of a HAP approach is its potential to be combined to therapeutic advantage with many standard treatments that spare hypoxic cells. Radiotherapy clearly falls within this category, as do several chemotherapy agents such as gemcitabine and docetaxel. We present here the first evidence that nitroCBIs can potentiate the tumor growth delay activity of both these agents (Figure 5). In three different xenograft models, coadministration of **9** or **50** significantly increased the growth delay provided by chemotherapy alone, in several cases leading to complete elimination of the tumor. Significant additional growth delay is observed even for xenografts in which the nitroCBI has no activity as a single agent; this is consistent with a relatively small hypoxic tumor fraction that nevertheless harbors cells resistant to chemotherapy and capable of repopulating the tumor after challenge with chemotherapy alone.

A possible limitation for nitroCBIs is the sporadic acute toxicity that was previously observed following large single doses of **9** and seen in the present study for some animals treated with multiple lower doses of the same compound. For

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J. Med. Chem. XXXX, XXX, XXX–XXX

this reason, growth delay experiments with **50**, which at an equimolar level was more effective than **9** in combination with irradiation, were conducted at further reduced doses. This approach completely eliminated acute toxicity while preserving excellent antitumor activity. Until further information is obtained (PK characterization, and a basis for toxicity comparisons other than MTDs), it is difficult to identify the best candidate from the nitroCBIs so far prepared. Nevertheless, we note that **50** has very favorable HAP properties, and in addition to the information presented here, there is preliminary evidence suggesting utility against triple-negative breast cancer[56] and for the development of clickable analogues suitable as biomarkers for preclinical investigation of the mechanism of action.[57] Further studies of **50** and related compounds are in progress with a view to selection of a lead compound for clinical development.

EXPERIMENTAL SECTION

General Chemistry Methods. All 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. Mobile phases were gradients of 80% acetonitrile/20% H_2O (v/v) in 45 mM ammonium formate at pH 3.5 and 0.5 mL/min. Purity of final products was determined by monitoring at 330 ± 200 nM. The following final products had purity ≥95%: **12**, **15**−**17**, **19**−**21**, **24**, **27**, **28**, **35**, **36**, **38**, **40**−**42**, **44**, **47**, **49**−**57**, **60**. For those cases where purity was <95% (**13**, **14**, **18**, **22**, **23**, **31**−**34**, **37**, **39**, **46**, **48**, **58**, **61**), the chromatograms (at 330 ± 200 nm and at λ_{max} ± 16 nm), along with identified impurities, are presented in the Supporting Information.

Solvents were distilled prior to use by common laboratory methods. Petroleum ether refers to the fraction with a bp = 40−60 °C. Organic solutions were dried over anhydrous Na_2SO_4 or $MgSO_4$. Column chromatography was carried out on silica gel (Merck 230−400 mesh). 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. HRMS was performed with a Bruker micrOTOF-QII or an Agilent 6530B Accurate Mass Q-TOF mass spectrometer. Combustion analyses were carried out in the Campbell Microanalytical Laboratory, University of Otago, Dunedin, New Zealand.

General Method A, EDCI-Mediated Amide Formation. A mixture of the appropriate indoline (**87**,[35] **89**, **92**, **94**,[35] or **96**[35]), side chain acid hydrochloride salt (1.2 to 1.5 equiv), EDCI (4 equiv), and anhyd TsOH (0.15 to 0.4 equiv) in DMA was stirred at rt for 1−5 h. Reactions with the cinnamic acids **80**, **82**, and **84** were incomplete at this stage and required the addition of a second batch of acid, EDCI, and TsOH and stirring overnight. The mixtures were cooled in an ice bath and cold alkali (generally 5% $NaHCO_3$ or 2% Na_2CO_3) added. The precipitated solids were filtered off, washed with water, and dried.

*1-(Chloromethyl)-3-(5-(2-(diethylamino)ethoxy)-1H-indole-2-carbonyl)-N-(2-hydroxyethyl)-5-nitro-2,3-dihydro-1H-benzo[e]indole-7-sulfonamide (**12**).* Prepared by general method A from 1-(chloromethyl)-*N*-(2-hydroxyethyl)-5-nitro-2,3-dihydro-1*H*-benzo[*e*]-indole-7-sulfonamide (**87**) (100 mg, 0.26 mmol) and **64**·HCl (97 mg, 0.31 mmol). The crude product was obtained as an oil which was dissolved in MeOH (5 mL). This solution was stirred with HCl in MeOH (1.25M, 1.0 mL), and EtOAc was added to precipitate the product, which was collected, washed with EtOAc, and dried to give the hydrochloride salt of **12** as a yellow−orange solid (151 mg, 85%): mp 204−206 °C (dec). HPLC purity 97.0%. ^{1}H NMR (DMSO-d_6) δ 11.83 (s, 1H), 9.80 (s, 1H), 9.29 (s, 1H), 8.86 (d, J = 1.7 Hz, 1H), 8.44 (d, J = 8.9 Hz, 1H), 8.03 (dd, J = 8.9, 1.7 Hz, 1H), 7.93 (t, J = 5.9 Hz, 1H), 7.46 (d, J = 8.9 Hz, 1H), 7.30−7.20 (m, 2H), 7.02 (dd, J = 8.9, 2.3 Hz, 1H), 4.97 (t, J = 10.1, 1H), 4.77−4.60 (m, 3H), 4.43−4.29 (m, 2H), 4.20−4.07 (m, 2H), 3.60−3.48 (m, 2H), 3.38 (q, J = 5.9 Hz, 2H), 3.31−3.17 (m, partially obscured by water peak, 4H), 2.87 (q, J = 6.1 Hz, 2H), 1.27 (t, J = 7.1 Hz, 6H). Anal. $C_{30}H_{34}ClN_5O_7S$·HCl·H_2O requires C, 51.58; H, 5.34; N, 10.02. Found: C, 51.51; H, 5.30; N, 10.07.

*1-(Chloromethyl)-3-(5-(3-(dimethylamino)propoxy)-1H-indole-2-carbonyl)-N-(2-hydroxyethyl)-5-nitro-2,3-dihydro-1H-benzo[e]-indole-7-sulfonamide (**13**).* Prepared by general method A from **87** (91 mg, 0.24 mmol) and **67**·HCl (99 mg, 0.33 mmol). Filtration of the product was slow, resulting in elimination of HCl to give an exomethylene byproduct that was apparent in the ^{1}H NMR of the crude product (in DMSO-d_6 diagnostic signals centered at δ 6.3, 5.8, and 5.5 in a 1:1:2 integral ratio), comprising about 10% of the material. The crude product (115 mg, 77%) was dissolved in DCM (4 mL) and MeOH (2 mL), and HCl-saturated MeOH (2 mL) was added. The precipitated solid was filtered off and dried, then dissolved in DMF (3 mL) and precipitated by the addition of EtOAc. The solid was filtered off and dried to give the hydrochloride salt of **13** as a yellow powder (77 mg, 49%): mp 224−228 °C. HPLC purity 93.8%. ^{1}H NMR (DMSO-d_6) δ 11.78 (d, J = 1.7 Hz, 1H), 9.82 (br s, 1H), 9.29 (s, 1H), 8.85 (d, J = 1.7 Hz, 1H), 8.44 (d, J = 8.9 Hz, 1H), 8.03 (dd, J = 8.9, 1.7 Hz, 1H), 7.93 (t, J = 5.9 Hz, 1H), 7.44 (d, J = 8.9 Hz, 1H), 7.21 (d, J = 1.7 Hz, 1H), 7.19 (d, J = 2.3 Hz, 1H), 6.97 (dd, J = 8.9, 2.4 Hz, 1H), 5.00−4.93 (m, 1H), 4.76−4.61 (m, 3H), 4.19−4.12 (m, 2H), 4.08 (t, J = 6.0 Hz, 2H), 3.42−3.36 (m, 2H), 3.28−3.20 (m, 2H), 2.90−2.85 (m, 2H), 2.81 (s, 6H), 2.20−2.11 (m, 2H). HRMS (FAB) calcd for $C_{29}H_{33}{}^{35}ClN_5O_7S$ (MH^+) m/z 630.17892, found 630.17846. Calcd for $C_{29}H_{33}{}^{37}ClN_5O_7S$ (MH^+) m/z 632.17597, found 632.17615.

*1-(Chloromethyl)-3-(5-((dimethylamino)methyl)-1H-indole-2-carbonyl)-N-(2-hydroxyethyl)-5-nitro-2,3-dihydro-1H-benzo[e]-indole-7-sulfonamide (**14**).* Prepared by general method A from **87** (150 mg, 0.39 mmol) and **71**·HCl (120 mg, 0.47 mmol) to give **14** as a yellow−orange solid (225 mg, 98%): mp 242−245 °C (dec). HPLC purity 92.8%. ^{1}H NMR (DMSO-d_6) δ 11.83 (s, 1H), 9.30 (s, 1H), 8.85 (d, J = 1.6 Hz, 1H), 8.43 (d, J = 8.9 Hz, 1H), 8.02 (dd, J = 8.9, 1.7 Hz, 1H), 7.91 (t, J = 5.9 Hz, 1H), 7.59 (s, 1H), 7.46 (d, J = 8.5 Hz, 1H), 7.31−7.22 (m, 2H), 4.98 (dd, J = 10.8, 9.7 Hz, 1H), 4.73 (dd, J = 11.0, 2.4 Hz, 1H), 4.70−4.62 (m, 2H), 4.20−4.10 (m, 2H), 3.48 (s, 2H), 3.37 (q, J = 6.0 Hz, 2H), 2.87 (q, J = 6.0 Hz, 2H), 2.18 (s, 6H). Anal. $C_{27}H_{28}ClN_5O_6S$·$^1/_2H_2O$ requires C, 54.50; H, 4.91; N, 11.77. Found: C, 54.61; H, 4.93; N, 11.79.

General Method B, TBDMS Ether Cleavage. A solution of the TBDMS ether in DCM or MeOH or dioxane was treated with HCl (1.25 M in MeOH, 5−10 equiv), and the mixture was stirred at rt for 30 min to 2 h. The mixture was diluted with EtOAc to precipitate the product, which was filtered off either directly or after standing at 5 °C overnight. The product was washed with EtOAc and dried.

*1-(Chloromethyl)-3-(5-(dimethylamino)-1H-indole-2-carbonyl)-N-(2-hydroxyethyl)-5-nitro-1,2-dihydro-3H-benzo[e]indole-7-sulfonamide (**15**).* Prepared by general method B from **90d** (185 mg, 0.27 mmol) to give the hydrochloride salt of **15** as a yellow−orange solid (142 mg, 87%): mp >300 °C. HPLC purity 97.3%. ^{1}H NMR (DMSO-d_6) δ 12.61 (br s, 1H), 12.18 (br s, 1H), 9.29 (s, 1 H), 8.86 (br d, J = 1.6 Hz, 1H), 8.45 (d, J = 8.9 Hz, 1H), 8.10 (br s, 1H), 8.04 (dd, J = 8.9, 1.7 Hz, 1H), 7.75−7.55 (m, 2H), 7.42 (s, 1H), 4.99 (br t, J = 10.6 Hz, 1H), 4.77−4.62 (m, 2H), 4.21−4.09 (m, 2H), 3.39 (t, J = 6.2 Hz, 2H), 2.99 (s, 6H), 2.88 (br q, J = 6.1 Hz, 2H), 1H obscured by water peak. Anal. $C_{26}H_{26}ClN_5O_6S$·HCl·H_2O requires C, 49.85; H, 4.67; N, 11.18. Found: C, 50.07; H, 4.69; N, 10.88.

*1-(Chloromethyl)-N-(2-hydroxyethyl)-3-(5-(2-morpholinoethoxy)-1H-indole-2-carbonyl)-5-nitro-1,2-dihydro-3H-benzo[e]-indole-7-sulfonamide (**16**).* Prepared by general method A from **87** (88 mg, 0.23 mmol) and 5-(2-morpholinoethoxy)-1*H*-indole-2-carboxylic acid hydrochloride[42] (104 mg, 0.32 mmol) to give **16** as a yellow solid (150 mg, 100%): mp 131−135 °C. HPLC purity 96.0%. ^{1}H NMR (DMSO-d_6) δ 11.73 (d, J = 1.7 Hz, 1H), 9.29 (s, 1H), 8.85 (d, J = 1.7 Hz, 1H), 8.43 (d, J = 8.9 Hz, 1H), 8.03 (dd, J = 8.9, 1.7 Hz, 1H), 7.91 (t, J = 5.6 Hz, 1H), 7.41 (d, J = 8.9 Hz, 1H), 7.21 (d, J = 1.8 Hz, 1H), 7.19 (d, J = 2.3 Hz, 1H), 6.96 (dd, J = 8.9, 2.4 Hz, 1H), 5.01−4.94 (m, 1H), 4.73 (dd, J = 10.9, 2.4 Hz, 1H), 4.68−4.62 (m, 1H), 4.67 (t, J = 5.6 Hz, 1H), 4.20−4.10 (m, 4H), 3.64−3.58 (m, 4H), 3.39 (q, J = 6.0 Hz, 2H), 2.87 (q, J = 6.2 Hz, 2H), 2.73 (t, J = 5.7 Hz, 2H), ca. 2.52−2.46 (m, 4H, partially obscured by DMSO peak). Anal.

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J. Med. Chem. XXXX, XXX, XXX−XXX

$C_{30}H_{32}ClN_5O_8S\cdot{}^3/_4H_2O$ requires C, 53.65; H, 5.03; N, 10.43. Found: C, 53.79; H, 4.85; N, 10.37.

*1-(Chloromethyl)-N-(2-hydroxyethyl)-3-(5-(3-morpholinopropoxy)-1H-indole-2-carbonyl)-5-nitro-1,2-dihydro-3H-benzo[e]-indole-7-sulfonamide (**17**).* Prepared by general method A from **87** (87 mg, 0.23 mmol) and **74**·HCl (123 mg, 0.36 mmol) to give **17** as a yellow solid (153 mg, 100%): mp 130−134 °C. HPLC purity 95.0%. ^{1}H NMR (DMSO-d_6) δ 11.72 (d, J = 1.7 Hz, 1H), 9.29 (s, 1H), 8.85 (d, J = 1.6 Hz, 1H), 8.44 (d, J = 8.9 Hz, 1H), 8.04 (dd, J = 8.9, 1.7 Hz, 1H), 7.91 (t, J = 5.9 Hz, 1H), 7.42 (d, J = 8.9 Hz, 1H), 7.22 (d, J = 1.8 Hz, 1H), 7.16 (d, J = 2.3 Hz, 1H), 6.95 (dd, J = 8.9, 2.4 Hz, 1H), 5.01−4.94 (m, 1H), 4.72 (dd, J = 10.9, 2.4 Hz, 1H), 4.69−4.63 (m, 1H), 4.67 (t, J = 5.6 Hz, 1H), 4.21−4.10 (m, 2H), 4.04 (t, J = 6.4 Hz, 2H), 3.63−3.57 (m, 4H), 3.39 (q, J = 6.0 Hz, 2H), 2.88 (q, J = 6.1 Hz, 2H), 2.47 (t, J = 7.2 Hz, 2H), 2.43−2.36 (m, 4H), 1.96−1.88 (m, 2H). Anal. $C_{31}H_{34}ClN_5O_8S\cdot H_2O$ requires C, 53.95; H, 5.26; N, 10.15. Found: C, 53.97; H, 5.21; N, 10.29.

*N-(2-(1-(Chloromethyl)-7-(N-(2-hydroxyethyl)sulfamoyl)-5-nitro-2,3-dihydro-1H-benzo[e]indole-3-carbonyl)-1H-indol-5-yl)-2-(dimethylamino)acetamide (**18**).* Prepared by general method A from **87** (200 mg, 0.52 mmol) and 5-(2-(dimethylamino)acetamido)-1*H*-indole-2-carboxylic acid hydrochloride[42] (185 mg, 0.62 mmol) to give **18** as a yellow−orange solid (305 mg, 88%): mp 260 °C (dec). HPLC purity 93.3%. ^{1}H NMR (DMSO-d_6) δ 11.81 (br d, J = 1.4 Hz, 1H), 9.74 (s, 1H), 9.30 (s, 1H), 8.86 (d, J = 1.6 Hz, 1H), 8.44 (d, J = 8.9 Hz, 1H), 8.13 (s, 1H), 8.01 (dd, J = 8.9, 1.6 Hz, 1H), 7.92 (t, J = 6.0 Hz, 1H), 7.50−7.41 (m, 2H), 7.30 (br d, J = 2.0 Hz, 1H), 4.98 (br t, J = 10.8 Hz, 1H), 4.74 (br dd, J = 11.5, 2.4 Hz, 1H), 4.71−4.63 (m, 2H), 4.15 (br d, J = 4.3 Hz, 2H), 3.39 (q, J = 6.0 Hz, 2H), 3.23 (br s, 2H), 2.88 (q, J = 6.0 Hz, 2H), 2.39 (s, 6H). Anal. $C_{28}H_{29}ClN_6O_7S\cdot H_2O$ requires C, 51.97; H, 4.83; N, 12.99. Found: C, 52.30; H, 4.63; N, 12.71.

*N-(2-(1-(Chloromethyl)-7-(N-(2-hydroxyethyl)sulfamoyl)-5-nitro-2,3-dihydro-1H-benzo[e]indole-3-carbonyl)-1H-indol-5-yl)-3-(dimethylamino)propanamide (**19**).* Prepared by general method A from **87** (150 mg, 0.39 mmol) and **77**·HCl (146 mg, 0.47 mmol). After the addition of cold aq Na_2CO_3 (2%), the mixture was extracted with DCM (2 × 250 mL) and the extracts were dried and filtered. HCl (1.25 M in MeOH, 1.0 mL) was added, and the mixture was evaporated at 25 °C. The resulting oil was dissolved in MeOH (2 mL), and the solution was diluted with EtOAc. The precipitated solid was filtered off, washed with EtOAc, and dried to give the hydrochloride salt of **19** as a yellow−orange solid (185 mg, 70%): mp 225−227 °C (dec). HPLC purity 95.8%. ^{1}H NMR (DMSO-d_6) δ 11.84 (s, 1H), 10.18 (s, 1H), 9.65 (s, 1H), 9.29 (s, 1H), 8.85 (d, J = 1.6 Hz, 1H), 8.44 (d, J = 8.9 Hz, 1H), 8.07 (br s, 1H), 8.03 (dd, J = 8.9, 1.7 Hz, 1H), 7.93 (t, J = 5.9 Hz, 1H), 7.46 (d, J = 8.8 Hz, 1H), 7.41 (dd, J = 8.9, 1.9 Hz, 1H), 7.30 (d, J = 1.7 Hz, 1H), 4.97 (t, J = 10.2, 1H), 4.78−4.61 (m, 3H), 4.21−4.09 (m, 2H), 3.46−3.34 (m, 4H), 2.91−2.83 (m, 4H), 2.81 (s, 6H). Anal. $C_{29}H_{31}ClN_6O_7S\cdot HCl\cdot H_2O$ requires C, 49.93; H, 4.91; N, 12.05. Found: C, 50.31; H, 5.09; N, 11.95.

*(E)-1-(Chloromethyl)-N-(2-hydroxyethyl)-3-(3-(4-(2-morpholinoethoxy)phenyl)acryloyl)-5-nitro-1,2-dihydro-3H-benzo-[e]indole-7-sulfonamide (**20**).* Prepared by general method B from **90i** (142 mg, 0.187 mmol) to give the hydrochloride salt of **20** as a yellow−orange solid (127 mg, 99%): mp 280−282 °C (dec). HPLC purity 97.7%. ^{1}H NMR (DMSO-d_6) δ 10.75 (br s, 1H), 9.35 (s, 1H), 8.83 (d, J = 1.6 Hz, 1H), 8.38 (d, J = 8.9 Hz, 1H), 8.02 (dd, J = 8.9, 1.6 Hz, 1H), 7.92 (t, J = 6.0 Hz, 1H), 7.84 (d, J = 8.7 Hz, 2H), 7.75 (d, J = 15.3 Hz, 1H), 7.14 (d, J = 15.3 Hz, 1H), 7.09 (d, J = 8.7 Hz, 2H), 4.72−4.60 (m, 3H), 4.49 (br s, 2H), 4.10 (br d, J = 4.1 Hz, 2H), 3.99 (br d, J = 13.8, 2H), 3.80 (br t, J = 11.7 Hz, 2H), 3.63−3.49 (m, 4H), 3.39 (t, J = 6.2 Hz, 2H, partially obscured by water peak), 3.30−3.16 (m, 2H, partially obscured by water peak), 2.87 (q, J = 6.0 Hz, 2H). HRMS (FAB) calcd for $C_{30}H_{34}{}^{35}ClN_4O_8S$ (MH^+) m/z 645.1785, found 645.1787. Calcd for $C_{30}H_{34}{}^{37}ClN4O_8S$ (MH^+) m/z 647.1756, found 647.1770. Anal. $C_{30}H_{33}ClN_4O_8S\cdot HCl\cdot H_2O\cdot{}^1/_2EtOAc$ requires C, 51.68; H, 5.42; N, 7.53. Found: C, 51.41; H, 5.31; N, 7.50.

*(E)-1-(Chloromethyl)-N-(2-hydroxyethyl)-3-(3-(4-(3-morpholinopropoxy)phenyl)acryloyl)-5-nitro-1,2-dihydro-3H-benzo[e]indole-7-sulfonamide (**21**).* Prepared by general method B from **90j** (112 mg, 0.145 mmol) to give the hydrochloride salt of **21** as a yellow−orange solid (98 mg, 97%): mp 262−263 °C (dec). HPLC purity 97.9%. ^{1}H NMR (DMSO-d_6) δ 10.73 (br s, 1H), 9.35 (s, 1H), 8.83 (br d, J = 1.6 Hz, 1H), 8.38 (d, J = 8.9 Hz, 1H), 8.01 (dd, J = 8.9, 1.7 Hz, 1H), 7.92 (t, J = 5.8 Hz, 1H), 7.82 (d, J = 8.8 Hz, 2H), 7.74 (d, J = 15.3 Hz, 1H), 7.11 (d, J = 15.3 Hz, 1H), 7.05 (d, J = 8.8 Hz, 2H), 4.75−4.57 (m, 3H), 4.16 (t, J = 6.04 Hz, 2H), 4.09 (br d, J = 4.0 Hz, 2H), 4.04−3.71 (m, 4H), 3.54−3.00 (m, 8H), 2.86 (q, J = 6.1 Hz, 2H), 2.28−2.13 (m, 2H). Anal. $C_{31}H_{35}ClN_4O_8S\cdot HCl\cdot{}^1/_2H_2O$ requires C, 52.84; H, 5.29; N, 7.95. Found: C, 52.98; H, 5.33; N, 7.97.

*1-(Chloromethyl)-N-(2-hydroxyethyl)-3-(4-(2-(4-methylpiperazin-1-yl)ethoxy)benzoyl)-5-nitro-1,2-dihydro-3H-benzo[e]indole-7-sulfonamide (**22**).* Prepared by general method B from **90k** (98 mg, 0.13 mmol) to give the dihydrochloride salt of **22** as a yellow−orange solid (93 mg, 100%): mp 225 °C (dec). HPLC purity 94.8%. ^{1}H NMR (DMSO-d_6) δ 11.10 (v br s, 1H), 9.35 (br s, 1H), 8.83 (br d, J = 1.6 Hz, 1H), 8.39 (d, J = 8.9 Hz, 1H), 8.02 (dd, J = 8.9, 1.6 Hz, 1H), 7.92 (t, J = 5.9 Hz, 1H), 7.83 (d, J = 8.7 Hz, 2H), 7.74 (d, J = 15.3 Hz, 1H), 7.13 (d, J = 15.3 Hz, 1H), 7.08 (d, J = 8.7 Hz, 2H), 4.72−4.59 (m, 3H), 4.39 (br s, 2H), 4.08 (br d, J = 4.0 Hz, 2H), 3.38 (t, J = 6.2 Hz, 2H), 3.35−2.91 (m, 10H), 2.93−2.75 (m, 5H). Anal. $C_{31}H_{36}ClN_5O_7S\cdot 2HCl\cdot H_2O$ requires C, 49.70; H, 5.38; N, 9.35. Found: C, 50.14; H, 5.32; N, 9.03.

*1-(Chloromethyl)-N-(2-hydroxyethyl)-3-(4-(2-morpholinoethoxy)-benzoyl)-5-nitro-1,2-dihydro-3H-benzo[e]indole-7-sulfonamide (**23**).* Prepared by general method B from **90l** (65.5 mg, 0.089 mmol) to give the hydrochloride salt of **23** as an orange solid (45 mg, 82%): mp 203−206 °C (dec). HPLC purity 94.0%. ^{1}H NMR (DMSO-d_6) δ 10.75 (br s, 1H), 8.91 (br s, 1H), 8.84 (br d, J = 1.6 Hz, 1H), 8.40 (d, J = 8.9 Hz, 1H), 8.02 (dd, J = 8.9, 1.7 Hz, 1H), 7.93 (t, J = 6.0 Hz, 1H), 7.72 (d, J = 8.8 Hz, 2H), 7.17 (d, J = 8.8 Hz, 2H), 4.63 (br dd, J = 10.9, 9.4 Hz, 1H), 4.56−4.44 (m, 3H), 4.17 (br dd, J = 11.1, 1.8 Hz, 1H), 4.12−3.95 (m, 3H), 3.88−3.75 (m, 2H), 3.68−3.48 (m, 4H), 3.44−3.15 (m, 6H, partially obscured by water peak), 2.87 (q, J = 6.0, Hz, 2H). Anal. $C_{28}H_{32}Cl_2N_4O_8S\cdot{}^1/_2H_2O$ requires C, 50.61; H, 5.01; N, 8.43. Found: C, 50.60; H, 5.03; N, 8.15.

*1-(Chloromethyl)-N-(2-hydroxyethyl)-3-(3-morpholinopropanoyl)-5-nitro-1,2-dihydro-3H-benzo[e]indole-7-sulfonamide (**24**).* Prepared by general method B from **90m** (110 mg, 0.17 mmol) to give the hydrochloride salt of **24** as an orange solid (45 mg, 46%): mp 193 °C (dec). HPLC purity 96.1%. ^{1}H NMR (C_5D_5N) δ ca. 11.1 (v br s, 1H), 9.84 (t, J = 5.6 Hz, 1H), 9.66 (br s, 1H), 9.47 (d, J = 1.6 Hz, 1H), 8.36 (dd, J = 8.9, 1.6 Hz, 1H), 8.18 (d, J = 8.9 Hz, 1H), 4.61−4.39 (m, 3H), 4.13 (dd, J = 11.3, 3.3 Hz, 1H), 4.04 (t, J = 5.6 Hz, 2H), 3.98 (dd, J = 11.3, 8.0 Hz, 1H), 3.79 (t, J = 4.6 Hz, 4H), 3.61 (q, J = 5.6 Hz, 2H), 3.05−2.86 (m, 4H), 2.59 (t, J = 4.6 Hz, 4H), 1H obscured by water peak. HRMS (FAB) calcd for $C_{22}H_{28}{}^{35}ClN_4O_7S$ (MH^+) m/z 527.1367, found 527.1361. Calcd for $C_{22}H_{28}{}^{37}ClN_4O_7S$ (MH^+) m/z 529.1338, found 529.1357.

*1-(Chloromethyl)-N-(2-hydroxyethyl)-3-(5-(2-morpholinoethoxy)-1H-indole-2-carbonyl)-5-nitro-1,2-dihydro-3H-benzo[e]-indole-7-carboxamide (**27**).* Prepared by general method B from **93e** (83 mg, 0.113 mmol) to give the hydrochloride salt of **27** as a yellow−orange solid (69 mg, 93%): mp 214−217 °C. HPLC purity 99.1%. ^{1}H NMR (DMSO-d_6) δ 11.80 (s, 1H), 10.70 (s, 1H), 9.16 (s, 1H), 8.82 (br d, J = 1.3 Hz, 1H), 8.78 (t, J = 5.6 Hz, 1H), 8.30 (d, J = 8.8 Hz, 1H), 8.16 (dd, J = 8.8, 1.5 Hz, 1H), 7.46 (d, J = 8.9 Hz, 1H), 7.28 (br s, 1), 7.22 (br d, J = 1.6 Hz, 1H), 7.04 (br dd, J = 8.9, 2.0 Hz, 1H), 4.96 (br t, J = 10.1 Hz, 1H), 4.70 (br dd, J = 10.9, 2.3 Hz, 1H), 4.68−4.59 (m, 1H), 4.43 (s, 2H), 4.19−4.07 (m, 2H), 4.05−3.71 (m, 4H), 3.69−3.48 (m, 5H), 3.40 (q, J = 5.9 Hz, 2H), 3.36 (m, 4H). Anal. $C_{31}H_{32}ClN_5O_7\cdot HCl\cdot{}^1/_2H_2O$ requires C, 55.78; H, 5.13; N, 10.49. Found: C, 55.65; H, 5.12; N, 10.43.

*(E)-1-(Chloromethyl)-N-(2-hydroxyethyl)-3-(3-(4-(2-morpholinoethoxy)phenyl)acryloyl)-5-nitro-1,2-dihydro-3H-benzo-[e]indole-7-carboxamide (**28**).* Prepared by general method B from **93i** (97 mg, 0.134 mmol) to give the hydrochloride salt of **28** as a yellow−orange solid (87 mg, 100%): mp 239−241 °C (dec). HPLC purity 98.1%. ^{1}H NMR (DMSO-d_6) δ 10.81 (br s, 1H), 9.22 (s, 1H), 8.82−8.74 (m, 2H), 8.24 (d, J = 8.8 Hz, 1H), 8.13 (dd, J = 8.8, 1.5 Hz, 1H), 7.84 (d, J = 8.7 Hz, 2H), 7.74 (d, J = 15.3 Hz, 1H), 7.18−7.05

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J. Med. Chem. XXXX, XXX, XXX−XXX

(m, 3H), 4.72−4.58 (m, 3H), 4.48 (br s, 2H), 4.09 (br d, J = 4.2 Hz, 2H), 3.98 (br d, J = 12.0, 2H), 3.81 (br t, J = 11.7 Hz, 2H), 3.66−3.47 (m, 5H), 3.40 (q, J = 5.9 Hz, 2H), 3.26 (br s, 4H). Anal. $C_{31}H_{33}ClN_4O_7 \cdot HCl \cdot H_2O$ requires C, 56.11; H, 5.47; N, 8.44. found: C, 56.08; H, 5.42; N, 8.44.

General Method C, Hydrogenation of NitroCBIs. A solution of the nitroCBI in THF with PtO_2 was hydrogenated at 40−50 psi for 30 min to 4 h. The catalyst was filtered off through Celite, the filtrate was evaporated, and the residue was triturated with EtOAc. The solid was filtered off and dried.

*5-Amino-1-(chloromethyl)-3-(5-(2-(diethylamino)ethoxy)-1H-indole-2-carbonyl)-N-(2-hydroxyethyl)-2,3-dihydro-1H-benzo[e]-indole-7-sulfonamide (**31**).* Prepared by general method C from **12** (34 mg, 0.053 mmol) to give **31** as a yellow solid (16.4 mg, 50%): mp 283−286 °C (dec). HPLC purity 93.1%. ^{1}H NMR (DMSO-d_6) δ 11.56 (s, 1H), 8.54 (d, J = 1.7 Hz, 1H), 7.92 (d, J = 8.9 Hz, 1H), 7.80 (s, 1H), 7.72 (dd, J = 8.8, 1.7 Hz, 1H), 7.42 (t, J = 6.0 Hz, 1H), 7.38 (d, J = 8.9 Hz, 1H), 7.15 (d, J = 2.3 Hz, 1H), 7.08 (br d, J = 1.5 Hz, 1H), 6.91 (dd, J = 8.8, 2.3 Hz, 1H), 6.29 (s, 2H), 4.79−4.70 (m, 1H), 4.63 (t, J = 5.6 Hz, 1H), 4.52 (dd, J = 10.9, 1.8 Hz, 1H), 4.21−4.10 (m, 1H), 4.07−3.95 (m, 3H), 3.79 (dd, J = 11.0, 7.6 Hz, 1H), 3.38 (q, J = 6.1 Hz, 2H), 2.90−2.76 (m, 4H), 2.68−2.46 (m, partially obscured by DMSO peak, 4H) 1.00 (t, J = 7.0 Hz, 6H). HRMS (FAB) calcd for $C_{30}H_{37}ClN_5O_5S$ (MH^+) m/z 614.2203, found 614.2204.

*5-Amino-1-(chloromethyl)-3-(5-(3-(dimethylamino)propoxy)-1H-indole-2-carbonyl)-N-(2-hydroxyethyl)-2,3-dihydro-1H-benzo[e]-indole-7-sulfonamide (**32**).* A solution of $NaHCO_3$ (32 mg, 0.38 mmol) in water (3 mL) was added to a suspension of **13**·HCl (38.5 mg, 0.058 mmol) in THF (4 mL) at 0 °C, and the mixture was stirred at this temperature until all the solid dissolved. The mixture was diluted with water and extracted with EtOAc (×2), and the extracts were washed with brine and then dried and evaporated. The resulting free base was hydrogenated using general method C to give **32** as a pale-green solid (26 mg, 75%): mp 235−240 °C (dec). HPLC purity 92.1%. ^{1}H NMR (DMSO-d_6) δ 11.56 (d, J = 1.7 Hz, 1H), 8.54 (d, J = 1.7 Hz, 1H), 7.93 (d, J = 8.9 Hz, 1H), 7.81 (s, 1H), 7.74 (dd, J = 8.9, 1.7 Hz, 1H), 7.44 (t, J = 5.9 Hz, 1H), 7.40 (d, J = 8.9 Hz, 1H), 7.15 (d, J = 2.3 Hz, 1H), 7.09 (d, J = 1.7 Hz, 1H), 6.91 (dd, J = 8.9, 2.4 Hz, 1H), 6.29 (s, 2H), 4.75 (dd, J = 10.8, 9.0 Hz, 1H), 4.65 (t, J = 5.6 Hz, 1H), 4.53 (dd, J = 10.9, 1.8 Hz, 1H), 4.20−4.14 (m, 1H), 4.05−3.96 (m, 3H), 3.81 (dd, J = 11.0, 7.7 Hz, 1H), 3.39 (q, J = 6.1 Hz, 2H), 2.84 (q, J = 6.2 Hz, 2H), 2.39 (t, J = 7.1 Hz, 2H), 2.16 (s, 6H), 1.90−1.83 (m, 2H). HRMS (FAB) calcd for $C_{29}H_{35}{}^{35}ClN_5O_5S$ (MH^+) m/z 600.20474, found 600.20449. Calcd for $C_{29}H_{35}{}^{37}ClN_5O_5S$ (MH^+) m/z 602.20179, found 602.20117.

*5-Amino-1-(chloromethyl)-3-(5-((dimethylamino)methyl)-1H-indole-2-carbonyl)-N-(2-hydroxyethyl)-2,3-dihydro-1H-benzo[e]-indole-7-sulfonamide (**33**).* Prepared by general method C from **14** (50 mg, 0.085 mmol) to give **33** as a yellow solid (16.4 mg, 50%): mp 290 °C (dec). HPLC purity 87.7%. ^{1}H NMR (DMSO-d_6) δ 11.67 (s, 1H), 8.52 (br d, J = 1.3 Hz, 1H), 7.92 (d, J = 8.8 Hz, 1H), 7.81 (s, 1H), 7.74 (dd, J = 8.8, 1.6 Hz, 1H), 7.56 (s, 1H), 7.48−7.40 (m, 2H), 7.21 (dd, J = 8.5, 1.4 Hz, 1H), 7.16 (br d, J = 1.2 Hz, 1H), 6.29 (s, 2H), 4.75 (dd, J = 10.8, 9.0 Hz, 1H), 4.63 (t, J = 5.6 Hz, 1H), 4.53 (br dd, J = 11.0, 1.7 Hz, 1H), 4.21−4.11 (m, 1H), 4.03−3.96 (m, 1H), 3.84−3.76 (m, 1H), 3.46 (s, 2H), 3.38 (q, J = 6.1 Hz, 2H), 2.83 (q, J = 6.2 Hz, 2H), 2.16 (s, 6H). HRMS (FAB): calcd for $C_{27}H_{31}ClN_5O_4S$ (MH^+) m/z 556.1785, found 556.1786.

*5-Amino-1-(chloromethyl)-3-(5-(dimethylamino)-1H-indole-2-carbonyl)-N-(2-hydroxyethyl)-1,2-dihydro-3H-benzo[e]indole-7-sulfonamide (**34**).* Prepared by general method C followed by general method B from **90d** (60 mg, 0.088 mmol) to give the hydrochloride salt of **34** as a yellow solid (42 mg, 82%): mp >300 °C. HPLC purity 93.1%. ^{1}H NMR (DMSO-d_6) δ 12.70 (br s, 1H), 12.10 (s, 1H), 8.56 (br d, J = 1.6 Hz, 1H), 8.14 (br s, 1H), 7.97 (d, J = 8.8 Hz, 1H), 7.86 (br s, 1H), 7.76 (dd, J = 8.8, 1.6 Hz, 1H), 7.65 (br s, 2H), 7.50 (br t, J = 5.5 Hz, 1H), 7.32 (br d, J = 1.9 Hz, 1H), 4.79 (br dd, J = 10.7, 9.1 Hz, 1H), 4.52 (br dd, J = 10.9, 1.8 Hz, 1H), 4.26−4.18 (m, 1H), 4.01 (dd, J = 11.0, 3.1 Hz, 1H), 3.83 (dd, J = 10.1, 7.5 Hz, 1H), 3.39 (t, J = 6.3 Hz, 2H), 3.21 (s, 6H), 2.84 (br q, J = 5.9 Hz, 2H). HRMS (FAB) calcd for $C_{26}H_{29}{}^{35}ClN_5O_4S$ (MH^+) m/z 542.1628, found 542.1618. Calcd for $C_{26}H_{29}{}^{37}ClN_5O_4S$ (MH^+) m/z 544.1599, found 544.1607.

*5-Amino-1-(chloromethyl)-N-(2-hydroxyethyl)-3-(5-(2-morpholinoethoxy)-1H-indole-2-carbonyl)-1,2-dihydro-3H-benzo[e]indole-7-sulfonamide (**35**).* Prepared by general method C from **16** (49.6 mg, 0.075 mmol) to give **35** as a pale-green solid (35 mg, 74%): mp 220−225 °C (dec). HPLC purity 98.6%. ^{1}H NMR (DMSO-d_6) δ 11.57 (s, 1H), 8.54 (d, J = 1.6 Hz, 1H), 7.94 (d, J = 8.9 Hz, 1H), 7.82 (s, 1H), 7.74 (dd, J = 8.8, 1.7 Hz, 1H), 7.45 (br s, 1H), 7.40 (d, J = 8.9 Hz, 1H), 7.18 (d, J = 2.3 Hz, 1H), 7.09 (d, J = 1.2 Hz, 1H), 6.93 (dd, J = 8.9, 2.4 Hz, 1H), 6.29 (s, 2H), 4.76 (dd, J = 10.8, 9.0 Hz, 1H), 4.65 (t, J = 5.4 Hz, 1H), 4.53 (dd, J = 10.9, 1.8 Hz, 1H), 4.21−4.14 (m, 1H), 4.12 (t, J = 5.8 Hz, 2H), 4.01 (dd, J = 11.0, 3.1 Hz, 1H), 3.80 (dd, J = 11.0, 7.7 Hz, 1H), 3.63−3.58 (m, 4H), 3.40 (q, J = 6.0 Hz, 2H), 2.84 (br t, J = 6.2 Hz, 2H), 2.72 (t, J = 5.8 Hz, 2H), ca. 2.52−2.46 (m, 4H, partially obscured by DMSO peak). Anal. $C_{30}H_{34}ClN_5O_6S$ requires C, 57.37; H, 5.46; N, 11.15. Found: C, 57.12; H, 5.36; N, 11.00.

*5-Amino-1-(chloromethyl)-N-(2-hydroxyethyl)-3-(5-(3-morpholinopropoxy)-1H-indole-2-carbonyl)-1,2-dihydro-3H-benzo[e]indole-7-sulfonamide (**36**).* Prepared by general method C from **17** (52.6 mg, 0.078 mmol) to give **36** as a pale-green solid (38 mg, 76%): mp 220−225 °C (dec). HPLC purity 95.0%. ^{1}H NMR (DMSO-d_6) δ 11.56 (d, J = 1.5 Hz, 1H), 8.54 (d, J = 1.6 Hz, 1H), 7.94 (d, J = 8.9 Hz, 1H), 7.81 (s, 1H), 7.74 (dd, J = 8.9, 1.7 Hz, 1H), 7.49−7.44 (m, 1H), 7.40 (d, J = 8.9 Hz, 1H), 7.16 (d, J = 2.3 Hz, 1H), 7.09 (d, J = 1.5 Hz, 1H), 6.92 (dd, J = 8.9, 2.4 Hz, 1H), 6.30 (s, 2H), 4.76 (dd, J = 10.7, 9.0 Hz, 1H), 4.65 (t, J = 5.4 Hz, 1H), 4.53 (dd, J = 10.9, 1.7 Hz, 1H), 4.21−4.15 (m, 1H), 4.06−3.98 (m, 3H), 3.82 (dd, J = 11.0, 7.7 Hz, 1H), 3.63−3.56 (m, 4H), 3.39 (q, J = 6.0 Hz, 2H), 2.88−2.81 (m, 2H), 2.46 (t, J = 7.2 Hz, 2H), 2.42−2.36 (m, 4H), 1.95−1.87 (m, 2H). Anal. $C_{31}H_{36}ClN_5O_6S$ requires C, 57.98; H, 5.65; N, 10.91. Found: C, 57.70; H, 5.70; N, 11.10.

*5-Amino-1-(chloromethyl)-N-(2-hydroxyethyl)-3-(5-(2-(dimethylamino)acetamido)-1H-indole-2-carbonyl)-1,2-dihydro-3H-benzo[e]indole-7-sulfonamide (**37**).* Prepared by general method C from **18** (60 mg, 0.095 mmol) to give **37** as a yellow solid (38 mg, 68%): mp 287 °C (dec). HPLC purity 92.8%. ^{1}H NMR (DMSO-d_6) δ 11.65 (br d, J = 1.3 Hz, 1H), 9.63 (s, 1H), 8.54 (br d, J = 1.5 Hz, 1H), 8.08 (s, 1H), 7.94 (d, J = 8.8 Hz, 1 H), 7.81 (s, 1 H), 7.74 (dd, J = 8.8, 1.6 Hz, 1 H), 7.51−7.38 (m, 3 H), 7.16 (br d, J = 2.0 Hz, 1H), 6.29 (s, 1H), 4.76 (br dd, J = 10.8, 9.2 Hz, 1H), 4.65 (t, J = 5.6 Hz, 1H), 4.53 (br dd, J = 10.9, 1.7 Hz, 1H), 4.24−4.13 (m, 1H), 3.98 (dd, J = 10.9, 3.0 Hz, 1H), 3.83 (dd, J = 10.9, 7.5 Hz, 1H), 3.39 (q, J = 6.1 Hz, 2H), 3.13 (br s, 2H), 2.84 (q, J = 6.2 Hz, 2H), 2.34 (s, 6H). Anal. $C_{28}H_{31}ClN_6O_5S \cdot {}^1/_2H_2O$ requires C, 55.30; H, 5.30; N, 13.82. Found: C, 55.25; H, 5.16; N, 13.53.

*N-(2-(5-Amino-1-(chloromethyl)-7-(N-(2-hydroxyethyl)-sulfamoyl)-2,3-dihydro-1H-benzo[e]indole-3-carbonyl)-1H-indol-5-yl)-3-(dimethylamino)propanamide (**38**).* Prepared by general method C from **19** (42 mg, 0.065 mmol) to give **38** as a yellow solid (30 mg, 76%): mp 320 °C (dec). HPLC purity 95.5%. ^{1}H NMR (DMSO-d_6) δ 11.62 (s, 1H), 9.93 (s, 1H), 8.54 (d, J = 1.5 Hz, 1H), 8.05 (d, J = 1.5 Hz, 1H), 7.93 (d, J = 8.9 Hz, 1H), 7.81 (s, 1H), 7.73 (dd, J = 8.9, 1.6 Hz, 1H), 7.48−7.38 (m, 2H), 7.34 (dd, J = 8.9, 1.8 Hz, 1H), 7.15 (d, J = 1.5 Hz, 1H), 6.29 (s, 2H), 4.77 (dd, J = 10.7, 9.1 Hz, 1H), 4.63 (t, J = 5.6 Hz, 1H), 4.52 (dd, J = 10.8, 1.7 Hz, 1H), 4.22−4.12 (m, 1H), 3.98 (dd, J = 10.5, 3.0 Hz, 1H), 3.82 (dd, J = 11.0, 7.5 Hz, 1H), 3.38 (q, J = 6.1, Hz, 2H), 2.83 (q, J = 6.2 Hz, 2H), 2.60 (t, J = 7.0 Hz, 2H), 2.45 (t, J = 7.0 Hz, 2H), 2.21 (s, 6H). Anal. $C_{29}H_{33}ClN_6O_5S \cdot {}^3/_4H_2O$ requires C, 55.58; H, 5.55; N, 13.41. Found: C, 55.55; H, 5.55; N, 13.15.

*(E)-5-Amino-1-(chloromethyl)-N-(2-hydroxyethyl)-3-(3-(4-(2-morpholinoethoxy)phenyl)acryloyl)-1,2-dihydro-3H-benzo[e]-indole-7-sulfonamide (**39**).* Saturated aq NH_4Cl (4 mL) and then Zn powder (450 mg) were added to a stirred solution of **90i** (107 mg, 0.141 mmol) in acetone (8 mL) and water (4 mL). The mixture was stirred at rt for 1 h then filtered through Celite, and the Celite pad was rinsed with acetone. The combined filtrates were concentrated to remove acetone, and the aqueous residue was extracted with DCM (×2). The extracts were washed with water and then brine and then dried and evaporated. The residue was dissolved in dioxane (1.5 mL)

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J. Med. Chem. XXXX, XXX, XXX−XXX

and MeOH (0.75 mL), and HCl (4 M in MeOH, 1 mL) was added. The resulting mixture was stirred at rt for 30 min, concentrated, and diluted with DCM (2 mL). The mixture was cooled at 0 °C, and ice-cold aq $NaHCO_3$ (10%, 10 mL) was added, followed by water (10 mL). The mixture was stirred for 30 min and then extracted with DCM (×2). The extracts were washed with water and then brine and then dried and evaporated. The residue was dissolved in the minimum quantity of MeOH, cooled to 0 °C, and diluted with Et_2O. After stirring at 0 °C for 30 min, the resulting precipitate was filtered off and washed with water and then Et_2O to provide **39** as a yellow powder (38 mg, 44%): mp 225−230 °C (dec). HPLC purity 93.4%. ^{1}H NMR (DMSO-d_6) δ 8.51 (d, J = 1.5 Hz, 1H), 7.89−7.84 (m, 2H), 7.76−7.68 (m, 3H), 7.63 (d, J = 15.3 Hz, 1H), 7.41 (br s, 1H), 7.06 (d, J = 15.6 Hz, 1H), 7.01 (d, J = 8.8 Hz, 2H), 6.25 (s, 2H), 4.63 (t, J = 5.6 Hz, 1H), 4.51−4.38 (m, 2H), 4.16−4.02 (m, 3H), 3.95 (dd, J = 10.9, 2.9 Hz, 1H), 3.79 (dd, J = 11, 8 Hz, 1H), 3.61−3.56 (m, 4H), 3.38 (q, J = 12, 6.3 Hz, 2H), 2.85−2.81 (m, 2H), 2.71 (t, J = 5.8 Hz, 2H), 2.53−2.46 (4H obscured by DMSO peak). Anal. $C_{30}H_{35}ClN_4O_6S$ requires C, 58.58; H, 5.73; N, 9.11. Found: C, 58.31; H, 5.63; N, 8.74.

*(E)-5-Amino-1-(chloromethyl)-N-(2-hydroxyethyl)-3-(3-(4-(3-morpholinopropoxy)phenyl)acryloyl)-1,2-dihydro-3H-benzo[e]-indole-7-sulfonamide (**40**).* A mixture of **87** (60 mg, 0.155 mmol), **82**·HCl (101 mg, 0.31 mmol), EDCI (180 mg, 3.63 mmol), and toluenesulfonic acid (2.6 mg, 0.0155 mmol) in DMA (6 mL) was stirred at rt overnight. Acetone (15 mL) and water (5 mL) were added, followed by saturated aq NH_4Cl (2 mL) and Zn powder (145 mg). The suspension was stirred at rt for 2 h then filtered through Celite, and the Celite pad was rinsed with acetone. The combined filtrates were concentrated to remove most of acetone. The aqueous residue was cooled in an ice-bath, diluted with cool aq $NaHCO_3$ (10 mL) and water (10 mL), and then extracted with DCM. The organic layer was washed successively with water (×2) and brine and then dried and evaporated. The residue was dissolved in the minimum quantity of MeOH, and the solution was cooled to 0 °C and diluted with Et_2O. After stirring at 0 °C for 30 min, the precipitate was filtered off and washed with water and Et_2O to provide **40** as a yellow powder (38 mg, 39%): mp 225−230 °C (dec). HPLC purity 97.1%. ^{1}H NMR (DMSO-d_6) δ 8.51 (d, J = 1.5 Hz, 1H), 7.89−7.86 (m, 2H), 7.76−7.69 (m, 3H), 7.61 (d, J = 15.3 Hz, 1H), 7.40 (t, J = 5.4 Hz, 1H), 7.08−6.95 (m, 3H), 6.25 (s, 2H), 4.63 (t, J = 5.6 Hz, 1H), 4.49−4.38 (m, 2H), 4.19−4.04 (m, 2H), 3.95 (dd, J = 11.3 Hz, 1H), 3.82−3.44 (m, 1H), 3.01−3.55 (m, 4H), 2.87−2.79 (m, 2H), 2.38−2.31 (m, 7H), 1.91−1.82 (m, 2H), 2H not observed. Anal. $C_{31}H_{37}ClN_4O_6S\cdot{}^1/_2Et_2O$ requires C, 59.49; H, 6.35; N, 8.41. Found: C, 59.65; H, 6.09; N, 8.21.

*5-Amino-1-(chloromethyl)-N-(2-hydroxyethyl)-3-(4-(2-morpholinoethoxy)benzoyl)-1,2-dihydro-3H-benzo[e]indole-7-sulfonamide (**41**).* Prepared by general method C followed by general method B from **90l** (42 mg, 0.057 mmol) to give **41** as a gray−brown solid (31 mg, 78%). HPLC analysis showed that this material was 86% pure. Purification by column chromatography ($CHCl_3$ then $CHCl_3$:MeOH 9:1 then $CHCl_3$:MeOH:NH_3 10:50:0.25) gave a yellow oil which was dissolved in dioxane (1 mL) and treated with HCl (4 M in dioxane, 0.2 mL). The mixture was diluted with EtOAc (20 mL), stirred at 0 °C for 5 min, and then left to stand at 5 °C overnight. The precipitated solid was filtered off, washed with EtOAc, and dried to give **41** as a beige solid (12.9 mg, 34%): mp 265−268 °C. HPLC purity 97.1%. ^{1}H NMR (C_5D_5N) δ 11.10 (br s, 1H), 9.39 (d, J = 1.6 Hz, 1H), 9.23 (t, J = 5.8 Hz, 1H), 8.22 (dd, J = 8.8, 1.6 Hz, 1H), 7.95−7.76 (m, 4H), 7.08 (d, J = 8.8 Hz, 2H), 4.53−4.40 (m, 2H), 4.16 (t, J = 5.7 Hz, 2H), 4.03−3.95 (m, 4H), 3.82−3.71 (m, 5H), 3.50 (q, J = 5.8 Hz, 2H), 2.78 (t, J = 5.7 Hz, 2H), 2.61−2.53 (m, 4H). HRMS (FAB) calcd for $C_{28}H_{34}{}^{35}ClN_4O_6S$ (MH^+) m/z 589.1887, found 589.1886. Calcd for $C_{28}H_{34}{}^{37}ClN_4O_6S$ (MH^+) m/z 591.1858, found 591.1874.

*5-Amino-1-(chloromethyl)-N-(2-hydroxyethyl)-3-(3-morpholinopropanoyl)-1,2-dihydro-3H-benzo[e]indole-7-sulfonamide (**42**).* Prepared by general method C followed by general method B from **90m** (50 mg, 0.078 mmol) to give the hydrochloride salt of **42** as a gray−white solid (30 mg, 71%): mp 225 °C (dec). HPLC purity 95.0%. ^{1}H NMR (DMSO-d_6) δ 10.64 (br s, 1H), 8.53 (d, J = 1.6 Hz, 1H), 7.91 (d, J = 8.9 Hz, 1H), 7.86 (s, 1H), 7.73 (dd, J = 8.9, 1.6 Hz, 1H), 7.49 (br s, 1H), 4.33 (t, J = 10.2 Hz, 1H), 4.22−4.14 (m, 2H), 4.03−3.95 (m, 2H), 3.84−3.78 (m, 2H), 3.72−3.62 (m, 2H), 3.51−3.34 (m, 6H), 3.20−2.91 (m, 4H), 2.82 (t, J = 6.2 Hz, 2H), 3H obscured by water peak. HRMS (FAB) calcd for $C_{22}H_{30}{}^{35}ClN_4O_5S$ (MH^+) m/z 497.1625, found 497.1615. Calcd for $C_{22}H_{30}{}^{37}ClN_4O_5S$ (MH^+) m/z 499.1596, found 499.1588.

*5-Amino-1-(chloromethyl)-N-(2-hydroxyethyl)-3-(5-(2-morpholinoethoxy)-1H-indole-2-carbonyl)-1,2-dihydro-3H-benzo[e]indole-7-carboxamide (**44**).* Prepared by general method C followed by general method B from **93e** (39.4 mg, 0.054 mmol) to give the hydrochloride salt of **44** as a pale-green solid (30 mg, 89%): mp >300 °C. HPLC purity 95.3%. ^{1}H NMR (C_5D_5N) δ 12.98 (s, 1H), ca. 11.0 (v br s, 1H), 9.59 (br d, J = 1.3 Hz, 1H), 9.35 (t, J = 5.4 Hz, 1H), 8.55 (br s, 1H), 8.43 (br dd, J = 8.7, 1.5 Hz, 1H), 7.86 (d, J = 8.7 Hz, 1H), 7.68 (d, J = 8.8 Hz, 1H), 7.41 (d, J = 2.3 Hz, 1H), 7.34−7.24 (m, 2H), 4.93 (br dd, J = 10.8, 1.7 Hz, 1H), 4.73 (br t, J = 8.8 Hz, 1H), 4.36 (t, J = 5.7 Hz, 2H), 4.23−4.12 (m, 3H), 4.10−3.96 (m, 3H), 3.87−3.75 (m, 4H), 3.65 (dd, J = 10.9, 9.5 Hz, 1H), 2.93 (t, J = 5.7 Hz, 2H), 2.73−2.63 (m, 4H), 3H obscured by water peak. HRMS (FAB) calcd for $C_{31}H_{35}{}^{35}ClN_5O_5$ (MH^+) m/z 592.2327, found 592.2318. Calcd for $C_{31}H_{35}{}^{37}ClN_5O_5$ (MH^+) m/z 594.2297, found 594.2310.

General Method D, Phosphate *tert*-Butyl Ester Cleavage. TFA (10 equiv) was added to a suspension of the phosphate ester in DCM, and the mixture was stirred at rt for 16−25 h. The mixture was evaporated, the residue was resuspended in DCM and evaporated once more, and the residue was triturated with EtOAc. The solid was filtered off and dried.

*2-(1-(Chloromethyl)-3-(5-(2-(diethylamino)ethoxy)-1H-indole-2-carbonyl)-5-nitro-2,3-dihydro-1H-benzo[e]indole-7-sulfonamido)-ethyl Dihydrogen Phosphate (**46**).* Prepared by general method D from **95a** (482 mg, 0.576 mmol) to give the trifluoroacetate salt of **46** as a yellow solid (452 mg, 94%): mp 215 °C (dec). HPLC purity 93.6%. ^{1}H NMR (DMSO-d_6) δ 11.82 (s, 1H), 9.28 (s, 1H), 8.86 (d, J = 1.6 Hz, 1H), 8.42 (d, J = 8.9 Hz, 1H), 8.24 (br s, 1H), 8.03 (dd, J = 8.9, 1.6 Hz, 1H), 7.45 (d, J = 8.9 Hz, 1H), 7.26 (d, J = 2.2 Hz, 1H), 7.23 (d, J = 1.3 Hz, 1H), 7.01 (dd, J = 8.9, 2.3 Hz, 1H), 4.94 (t, J = 10.1 Hz, 1H), 4.77−4.59 (m, 2H), 4.32 (t, J = 4.9 Hz, 2H), 4.20−4.07 (m, 2H), 3.79 (q, J = 6.6 Hz, 2H), 3.57−3.49 (m, 2H), 3.25 (br q, J = 6.9 Hz, 4H), 3.02 (br t, J = 5.5 Hz, 2H), 1.25 (t, J = 7.2 Hz, 6H), 3H not observed. Anal. $C_{30}H_{35}ClN_5O_{10}PS\cdot{}^3/_4CF_3CO_2H$ requires C, 46.73; H, 4.45; N, 8.65. Found: C, 46.77; H, 4.48; N, 8.35.

*2-(1-(Chloromethyl)-3-(5-(3-(dimethylamino)propoxy)-1H-indole-2-carbonyl)-5-nitro-2,3-dihydro-1H-benzo[e]indole-7-sulfonamido)ethyl Dihydrogen Phosphate (**47**).* Prepared by general method D from **95b** (144 mg, 0.18 mmol) to give the trifluoroacetate salt of **47** as a pale-yellow solid (141 mg, 98%): mp 148−151 °C. HPLC purity 96.7%. ^{1}H NMR (DMSO-d_6) δ 11.79 (s, 1H), 9.7 (v br s, 1H), 9.27 (s, 1H), 8.86 (d, J = 1.6 Hz, 1H), 8.43 (d, J = 8.9 Hz, 1H), 8.30 (br s, 1H), 8.02 (dd, J = 8.9, 1.7 Hz, 1H), 7.37 (d, J = 8.8 Hz, 1H), 7.21 (d, J = 1.6 Hz, 1H), 7.15 (s, 1H), 6.89 (br d, J = 9.1 Hz, 1H), 5.00−4.91 (m, 1H), 4.74−4.60 (m, 2H), 4.18−4.02 (m, 4H), 3.81 (q, J = 6.6 Hz, 2H), 3.29−3.23 (m, 2H), 3.07−3.00 (m, 2H), 2.83 (s, 6H), 2.17−2.09 (m, 2H). Anal. $C_{29}H_{33}ClN_5O_{10}PS\cdot CF_3CO_2H$ requires C, 45.18; H, 4.16; N, 8.50. Found C, 44.96; H, 4.23; N, 8.79.

*2-(1-(Chloromethyl)-3-(5-((dimethylamino)methyl)-1H-indole-2-carbonyl)-5-nitro-2,3-dihydro-1H-benzo[e]indole-7-sulfonamido)-ethyl Dihydrogen Phosphate (**48**).* Prepared by general method D from **95c** (476 mg, 0.612 mmol) to give the trifluoroacetate salt of **48** as a yellow−orange solid (425 mg, 89%): mp 212−215 °C (dec). HPLC purity 92.8%. ^{1}H NMR (DMSO-d_6) δ 11.98 (s, 1H), 9.20 (s, 1H), 8.83 (s, 1H), 8.55 (br s, 1H), 8.33 (d, J = 8.8 Hz, 1H), 8.01 (dd, J = 8.8, 1.6 Hz, 1H), 7.75 (s, 1H), 7.41−7.11 (m, 3H), 4.94 (t, J = 10.0 Hz, 1H), 4.71−4.50 (m, 2H), 4.30−4.02 (m, 4H), 3.92−3.78 (m, 2H), 3.19−3.01 (m, 2H), 2.67 (s, 6H), 3H not observed. Anal. ($C_{27}H_{29}ClN_5O_9PS\cdot{}^3/_4CF_3CO_2H$) requires C, 45.55; H, 3.99; N, 9.32. Found: C, 45.66; H, 4.21; N, 9.35.

*2-(1-(Chloromethyl)-3-(5-(dimethylamino)-1H-indole-2-carbonyl)-5-nitro-1,2-dihydro-3H-benzo[e]indole-7-sulfonamido)ethyl Dihydrogen Phosphate (**49**).* Prepared by general method D from **95d** (350 mg, 0.458 mmol) to give the trifluoroacetate salt of **49** as a

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J. Med. Chem. XXXX, XXX, XXX−XXX

yellow−orange solid (305 mg, 87%): mp >300 °C. HPLC purity 96.9%. ^{1}H NMR (C_5D_5N) δ 12.93 (s, 1H), 9.67 (s, 1H), 9.46 (br d, J = 1.4 Hz, 1H), 8.40 (br dd, J = 8.8, 1.5 Hz, 1H), 8.18 (d, J = 8.8 Hz, 1H), 7.76 (v br s, 4H), 7.66 (d, J = 8.9 Hz, 1H), 7.37 (br d, J = 1.5 Hz, 1H), 7.27−7.17 (m, 2H), 5.08 (dd, J = 10.8, 2.5 Hz, 1H), 5.02 (t, J = 9.8 Hz, 1H), 4.63−4.46 (m, 3H), 4.19 (dd, J = 11.3, 3.4 Hz, 1H), 4.04 (dd, J = 11.3, 8.1 Hz, 1H), 3.72 (t, J = 5.2 Hz, 2H), 2.93 (s, 6H). Anal. ($C_{26}H_{27}ClN_5O_9PS\cdot{}^3/_4CF_3CO_2H$) requires C, 44.78; H, 3.79; N, 9.50. Found: C, 44.94; H, 4.19; N, 9.25.

*2-(1-(Chloromethyl)-3-(5-(2-morpholinoethoxy)-1H-indole-2-carbonyl)-5-nitro-1,2-dihydro-3H-benzo[e]indole-7-sulfonamido)ethyl Dihydrogen Phosphate (**50**).* Prepared by general method D from **95e** (164 mg, 0.19 mmol) to give the trifluoroacetate salt of **50** as a yellow solid (158 mg, 98%): mp 166−169 °C. HPLC purity 97.8%. ^{1}H NMR (DMSO-d_6) δ 11.84 (s, 1H), ca. 10.7 (v br s, 1H), 9.29 (s, 1H), 8.87 (d, J = 1.7 Hz, 1H), 8.45 (d, J = 8.9 Hz, 1H), 8.27−8.20 (m, 1H), 8.03 (dd, J = 8.9, 1.7 Hz, 1H), 7.44 (d, J = 8.9 Hz, 1H), 7.26 (d, J = 2.2 Hz, 1H), 7.24 (d, J = 1.8 Hz, 1H), 7.02 (dd, J = 8.9, 2.3 Hz, 1H), 5.01−4.94 (m, 1H), 4.71 (dd, J = 10.8, 2.1 Hz, 1H), 4.68−4.62 (m, 1H), 4.34−4.29 (m, 2H), 4.19−4.09 (m, 2H), 3.87−3.76 (m, 6H), 3.26−3.13 (m, 4H), 3.06−3.00 (m, 2H), 2H not observed. Anal. ($C_{30}H_{33}ClN_5O_{11}PS\cdot CF_3CO_2H$) requires C, 45.11; H, 4.02; N, 8.22. Found: C, 44.72; H, 4.36; N, 8.57.

*2-(1-(Chloromethyl)-3-(5-(3-morpholinopropoxy)-1H-indole-2-carbonyl)-5-nitro-1,2-dihydro-3H-benzo[e]indole-7-sulfonamido)-ethyl Dihydrogen Phosphate (**51**).* Prepared by general method D from **95f** (162 mg, 0.19 mmol) to give the trifluoroacetate salt of **51** as a pale-yellow powder (165 mg, 100%): mp 165−168 °C. HPLC purity 98.4%. ^{1}H NMR (DMSO-d_6) δ 11.82 (d, J = 1.6 Hz, 1H), ca. 9.7 (v br s, 1H), 9.30 (s, 1H), 8.87 (d, J = 1.6 Hz, 1H), 8.44 (d, J = 8.9 Hz, 1H), 8.20 (t, J = 5.9 Hz, 1H), 8.02 (dd, J = 8.9, 1.7 Hz, 1H), 7.44 (d, J = 8.9 Hz, 1H), 7.23 (d, J = 1.7 Hz, 1H), 7.20 (d, J = 2.2 Hz, 1H), 6.97 (dd, J = 8.9, 2.4 Hz, 1H), 5.02−4.93 (m, 1H), 4.71 (dd, J = 10.9, 2.2 Hz, 1H), 4.68−4.62 (m, 1H), 4.20−3.97 (m, 6H), 3.81 (q, J = 6.5 Hz, 2H), 3.34−3.28 (m, 2H), 3.20−3.08 (m, 2H), 3.02 (q, J = 5.9 Hz, 2H), 2.21−2.10 (m, 2H), 4H not observed. Anal. ($C_{31}H_{35}ClN_5O_{11}PS\cdot CF_3CO_2H\cdot H_2O$) requires C, 44.83; H, 4.33; N, 7.92. Found: C, 44.96; H, 4.41; N, 8.20.

*2-(1-(Chloromethyl)-3-(5-(2-(dimethylamino)acetamido)-1H-indole-2-carbonyl)-5-nitro-1,2-dihydro-3H-benzo[e]indole-7-sulfonamido)ethyl Dihydrogen Phosphate (**52**).* Prepared by general method D from **95g** (348 mg, 0.424 mmol) to give the trifluoroacetate salt of **52** as a yellow−orange solid (321 mg, 92%): mp 215−218 °C (dec). HPLC purity 98.2%. ^{1}H NMR (DMSO-d_6) δ 11.85 (s, 1H), 10.39 (s, 1H), 9.27 (s, 1H), 8.86 (d, J = 1.6 Hz, 1H), 8.37 (br s, 1H), 8.10 (br d, J = 1.5 Hz, 1H), 8.03 (dd, J = 8.9, 1.6 Hz, 1H), 7.40 (d, J = 8.8 Hz, 1H), 7.38−7.25 (m, 2H), 4.94 (t, J = 10.2 Hz, 1H), 4.69 (br dd, J = 10.8, 1.9 Hz, 1H), 4.67−4.57 (m, 1H), 4.20−4.07 (m, 2H), 3.87−3.77 (m, 2H), 3.11−3.02 (m, 2H), 2.78 (s, 6H), 3H not observed. Anal. ($C_{28}H_{30}ClN_6O_{10}PS\cdot{}^3/_4CF_3CO_2H$) requires C, 44.59; H, 3.90; N, 10.58. Found: C, 44.19; H, 4.11; N, 10.41.

*2-(1-(Chloromethyl)-3-(5-(3-(dimethylamino)propanamido)-1H-indole-2-carbonyl)-5-nitro-2,3-dihydro-1H-benzo[e]indole-7-sulfonamido)ethyl Dihydrogen Phosphate (**53**).* Prepared by general method D from **95h** (476 mg, 0.57 mmol) to give the trifluoroacetate salt of **53** as a yellow solid (438 mg, 92%): mp 215−218 °C (dec). HPLC purity 95.4%. ^{1}H NMR (DMSO-d_6) δ 11.70 (s, 1H), 10.25 (s, 1H), 9.14 (s, 1H), 8.83 (s, 1H), 8.52 (br s, 1H), 8.24 (br s, 1H), 8.06 (br s, 1H), 8.00 (dd, J = 8.8, 1.5 Hz, 1H), 7.27 (br s, 1H), 7.12 (br s, 1H), 6.89 (br s, 1H), 4.88−4.79 (m, 1H), 4.65−4.43 (m, 2H), 4.12−3.96 (m, 2H), 3.86−3.76 (m, 2H), 3.37 (t, J = 7.2 Hz, 2H), 3.12−3.04 (m, 2H), 2.85−2.73 (m, 8H), 3 H not observed. Anal. ($C_{29}H_{32}ClN_6O_{10}PS\cdot{}^3/_4CF_3CO_2H$) requires C, 45.30; H, 4.08; N, 10.39. Found: C, 45.65; H, 4.34; N, 10.43.

*(E)-2-(1-(Chloromethyl)-3-(3-(4-(2-morpholinoethoxy)phenyl)-acryloyl)-5-nitro-1,2-dihydro-3H-benzo[e]indole-7-sulfonamido)-ethyl Dihydrogen Phosphate (**54**).* Prepared by general method D from **95i** (366 mg, 0.437 mmol) to give the trifluoroacetate salt of **54** as a yellow solid (292 mg, 80%): mp 212 °C (dec). HPLC purity 98.3%. ^{1}H NMR (C_5D_5N) δ ca. 10.7 (v br s, 1H), 9.81 (br s, 1H), 9.46 (d, J = 1.6 Hz, 1H), 8.39 (dd, J = 8.9, 1.6 Hz, 1H), 8.22 (d, J = 15.2 Hz, 1H), 8.14 (d, J = 8.9 Hz, 1H), 7.74 (d, J = 8.8 Hz, 2H), 7.16 (d, J = 15.2 Hz, 2H), 7.10 (d, J = 8.8 Hz, 2H), 4.80 (br dd, J = 10.8, 2.6 Hz, 1H), 4.65 (br t, J = 10.0 Hz, 1H), 4.59−4.51 (m, 2H), 4.50−4.42 (m, 1H), 4.21−4.13 (m, 3H), 4.01 (br dd, J = 11.3, 8.3 Hz, 1H), 3.73 (br t, J = 4.6 Hz, 6H), 2.76 (t, J = 5.7 Hz, 2H), 2.53 (t, J = 4.6 Hz, 4H). Anal. ($C_{30}H_{34}ClN_4O_{11}PS\cdot{}^1/_2CF_3CO_2H$) requires C, 47.61; H, 4.45; N, 7.16. Found: C, 47.69; H, 4.65; N, 6.97.

*(E)-2-(1-(Chloromethyl)-3-(3-(4-(3-morpholinopropoxy)phenyl)-acryloyl)-5-nitro-1,2-dihydro-3H-benzo[e]indole-7-sulfonamido)-ethyl Dihydrogen Phosphate Trifluoroacetate (**55**).* Prepared by general method D from **95j** (218 mg, 0.256 mmol) to give the trifluoroacetate salt of **55** as an orange solid (177 mg, 81%): mp 215 °C (dec). HPLC purity 97.0%. ^{1}H NMR (DMSO-d_6) δ ca. 10.7 (v br s, 1H), 9.33 (br s, 1H), 8.84 (d, J = 1.6 Hz, 1H), 8.36 (d, J = 8.9 Hz, 1H), 8.23 (br s, 1H), 8.01 (dd, J = 8.9, 1.6 Hz, 1H), 7.76 (d, J = 8.7 Hz, 2H), 7.70 (d, J = 15.2 Hz, 1H), 7.08 (d, J = 15.2 Hz, 1H), 6.97 (d, J = 8.7 Hz, 2H), 4.70−4.55 (m, 3H), 4.17−4.02 (m, 4H), 4.85−3.66 (m, 6H), 3.10−2.82 (m, 8H), 2.12−2.00 (m, 2H). Anal. ($C_{31}H_{36}ClN_4O_{11}PS\cdot{}^1/_2CF_3CO_2H$) requires C, 48.28; H, 4.62; N, 7.04. Found: C, 47.94; H, 4.85; N, 6.80.

*2-(1-(Chloromethyl)-3-(4-(2-(4-methylpiperazin-1-yl)ethoxy)-benzoyl)-5-nitro-1,2-dihydro-3H-benzo[e]indole-7-sulfonamido)-ethyl Dihydrogen Phosphate (**56**).* Prepared by general method D from **95k** (1.57 g, 1.85 mmol) to give the trifluoroacetate salt of **56** as a yellow−orange solid (1.37 g, 87%): mp 230 °C (dec). HPLC purity 95.0%. ^{1}H NMR (DMSO-d_6) δ 9.35 (br s, 1H), 9.35 (br s, 1H), 8.85 (br d, J = 1.6 Hz, 1H), 8.38 (d, J = 8.9 Hz, 1H), 8.13 (t, J = 5.9 Hz, 1H), 8.02 (dd, J = 8.9, 1.6 Hz, 1H), 7.82 (d, J = 8.8 Hz, 2H), 7.74 (d, J = 15.2 Hz, 1H), 7.11 (d, J = 15.2 Hz, 1H), 7.04 (d, J = 8.8 Hz, 2H), 4.12−4.58 (m, 3H), 4.21 (t, J = 5.2 Hz, 2H), 4.09 (br d, J = 4.9 Hz, 2H), 3.82 (q, J = 6.2 Hz, 2H), 3.51−2.85 (m, 12H), 2.77 (s, 3H), 2H not observed. Anal. ($C_{31}H_{37}ClN_5O_{10}P\cdot 1{}^1/_2CF_3CO_2H\cdot$EtOAc) requires C, 45.77; H, 4.70; N, 7.02. Found: C, 45.72; H, 4.67; N, 7.09.

*2-(1-(Chloromethyl)-3-(4-(2-morpholinoethoxy)benzoyl)-5-nitro-1,2-dihydro-3H-benzo[e]indole-7-sulfonamido)ethyl Dihydrogen Phosphate (**57**).* Prepared by general method D from **95l** (458 mg, 0.565 mmol) to give the trifluoroacetate salt of **57** as a yellow solid (424 mg, 92%): mp 194 °C (dec). HPLC purity 96.7%. ^{1}H NMR (DMSO-d_6) δ ca. 10.6 (v br s, 1H), 8.90 (br s, 1H), 8.85 (br d, J = 1.6 Hz, 1H), 8.39 (d, J = 8.9 Hz, 1H), 8.16 (br s, 1H), 8.02 (d, J = 8.9, 1.7 Hz, 1H), 7.71 (d, J = 8.8 Hz, 2H), 7.16 (d, J = 8.8 Hz, 2H), 4.64 (dd, J = 10.9, 9.4 Hz, 1H), 4.52−4.43 (m, 1H), 4.37 (t, J = 5.0 Hz, 2H), 4.17 (dd, J = 11.1, 1.7 Hz, 1H), 4.11−3.99 (m, 2H), 3.86−3.72 (m, 6H), 3.32 (br s, 2H), 3.15−2.97 (m, 6H). Anal. ($C_{28}H_{32}ClN_4O_{11}PS\cdot{}^3/_4CF_3CO_2H$) requires C, 45.16; H, 4.21; N, 7.14. Found: C, 45.43; H, 4.49; N, 6.94.

*2-(1-(Chloromethyl)-3-(3-morpholinopropanoyl)-5-nitro-1,2-dihydro-3H-benzo[e]indole-7-sulfonamido)ethyl Dihydrogen Phosphate (**58**).* Prepared by general method D from **95m** (407 mg, 0.566 mmol) to give the trifluoroacetate salt of **58** as a yellow−orange solid (388 mg, 95%): mp 192 °C (dec). HPLC purity 94.6%. ^{1}H NMR (C_5D_5N) δ ca. 10.7 (v br s, 1H), 9.65 (br s, 1H), 9.44 (br d, J = 1.5 Hz, 1H), 8.38 (dd, J = 8.9, 1.6 Hz, 1H), 8.13 (d, J = 8.9 Hz, 1H), 4.61−4.38 (m, 5H), 4.15 (dd, J = 11.3, 3.2 Hz, 1H), 3.97 (dd, J = 11.3, 8.1 Hz, 1H), 3.76 (t, J = 4.5 Hz, 4H), 3.71 (t, J = 5.2 Hz, 2H), 3.04−2.81 (m, 4H), 2.56 (t, J = 4.5 Hz, 4H), 3H not observed. Anal. ($C_{22}H_{28}ClN_4O_{10}PS\cdot CF_3CO_2H$) requires C, 39.98; H, 4.05; N, 7.77. Found: C, 39.91; H, 4.08; N, 7.54.

*2-(1-(Chloromethyl)-3-(5-(2-morpholinoethoxy)-1H-indole-2-carbonyl)-5-nitro-1,2-dihydro-3H-benzo[e]indole-7-carboxamido)ethyl Dihydrogen Phosphate (**60**).* HCl (saturated solution in DCM, 1.0 mL) was added to a solution of **97e** (197 mg, 0.242 mmol) in DCM (10 mL), and the mixture was stirred at rt for 22 h and then evaporated. EtOAc (50 mL) was added, and the mixture was stirred at 0 °C for 2 h. The solid was filtered off, washed with EtOAc, and dried to give crude **60** as the hydrochloride salt (172 mg, 96%). LC-MS analysis showed the crude product contained 85% of the desired **60** and 13% *N*-(2-chloroethyl)-1-(chloromethyl)-3-(5-(2-morpholinoethoxy)-1*H*-indole-2-carbonyl)-5-nitro-1,2-dihydro-3*H*-benzo[*e*]indole-7-carboxamide. The crude product was dissolved in the minimum amount of DMSO (3 mL), and DCM (20 mL) was added slowly. The

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J. Med. Chem. XXXX, XXX, XXX−XXX

resulting mixture was stirred at rt for 1 h, and the precipitated solid was filtered off, washed with DCM, and dried to give the hydrochloride salt of **60** as a yellow−orange solid (153 mg, 85%), mp 225 °C (dec). HPLC purity 98.1%. ^{1}H NMR (DMSO-d_6) δ 11.80 (br d, J = 1.6 Hz, 1H), ca. 11.0 (v br s, 3H), 9.17 (s, 1H), 8.97 (t, J = 5.5 Hz, 1H), 8.84 (br d, J = 1.3 Hz, 1H), 8.32 (d, J = 8.8 Hz, 1H), 8.16 (dd, J = 8.8, 1.5 Hz, 1H), 7.46 (d, J = 8.9 Hz, 1H), 7.27 (br d, J = 2.3 Hz, 1H), 7.22 (br d, J = 1.6 Hz, 1H), 4.96 (br t, J = 10.2 Hz, 1H), 4.75−4.58 (m, 2H), 4.41 (t, J = 4.9 Hz, 2H), 4.20−3.77 (m, 8H), 3.64−3.12 (m, 8H). Anal. ($C_{31}H_{33}ClN_5O_{10}$·HCl·H_2O) requires C, 49.22; H, 4.80; N, 9.26. Found: C, 49.14; H, 4.83; N, 9.17.

*(E)-2-(1-(Chloromethyl)-3-(3-(4-(2-morpholinoethoxy)phenyl)-acryloyl)-5-nitro-1,2-dihydro-3H-benzo[e]indole-7-carboxamido)-ethyl Dihydrogen phosphate (**61**).* HCl (saturated solution in DCM, 10 mL) was added to a solution of phosphate ester **97i** (190 mg, 0.237 mmol) in DCM (10 mL), and the mixture was stirred at rt for 18 h and then evaporated. EtOAc was added, and the mixture was stirred at 0 °C for 2 h. The solid was filtered off, washed with EtOAc, and dried to give the hydrochloride salt of **61** as a yellow−orange solid (147 mg, 86%): mp 229 °C (dec). HPLC purity 93.8%. ^{1}H NMR (DMSO-d_6) δ ca. 10.6 (v br s, 1H), 9.23 (br s, 1H), 8.97 (br t, J = 4.7 Hz, 1H), 8.82 (br d, J = 0.9 Hz, 1H), 8.26 (d, J = 8.8 Hz, 1H), 8.14 (br d, J = 8.7 Hz, 1H), 7.83 (d, J = 8.5 Hz, 2H), 7.73 (d, J = 15.3 Hz, 1H), 7.12 (d, J = 15.3 Hz, 1H), 7.08 (d, J = 8.5 Hz, 2H), 4.72−4.56 (m, 3H), 4.39 (br s, 2H), 4.12−3.95 (m, 4H), 3.80 (br s, 4H), 3.55 (q, J = 5.6 Hz, 2H), 3.37 (br s, 2H), 3.15 (br s, 4H). Anal. ($C_{31}H_{34}ClN_4O_{10}P$·HCl·$^1/_4H_2O$) requires C, 51.07; H, 4.89; N, 7.68. Found: C, 51.00; H, 4.90; N, 7.68.

*Ethyl 5-(2-(Diethylamino)ethoxy)-1H-indole-2-carboxylate (**63**).* 4-(2-(Diethylamino)ethoxy)aniline (**62**)[58] was subjected to a Fischer indole synthesis, as described for the morpholine analogue[42] to give **63** as an orange oil (77%). ^{1}H NMR (DMSO-d_6) δ 11.68 (s, 1H), 7.33 (d, J = 8.9 Hz, 1H), 7.11 (d, J = 2.4 Hz, 1H), 7.04−7.01 (m, 1H), 6.90 (dd, J = 8.9, 2.4 Hz, 1H), 4.32 (q, J = 7.1 Hz, 2H), 4.00 (t, J = 6.2 Hz, 2H), 2.77 (t, J = 6.2 Hz, 2H), 2.55 (q, J = 7.1 Hz, 4H), 1.33 (t, J = 7.1 Hz, 3H), 0.80 (t, J = 7.1 Hz, 6H). HRMS (EI) calcd for $C_{17}H_{24}N_2O_3$ (M^+) m/z 304.1787, found 304.1790. **63** was treated with 4 M HCl in dioxane to give the corresponding hydrochloride salt as a beige solid: mp 95−97 °C. Anal. $C_{17}H_{24}N_2O_3$·HCl requires C, 59.90; H, 7.39; N, 8.22. Found: C, 59.97; H, 7.55; N, 7.83.

*5-(2-(Diethylamino)ethoxy)-1H-indole-2-carboxylic Acid (**64**).* KOH (9.8 g, 175 mmol) was added to a suspension of **63** (17.7 g, 58.2 mmol) in MeOH (120 mL) and water (60 mL), and the mixture was stirred at rt for 3 h. The MeOH was evaporated, and the aqueous residue was diluted with water (150 mL). The mixture was acidified with concd HCl at 0 °C to pH 6−7. The solid was collected, washed with cold water (3 × 25 mL), and dried to give **64** (16.1 g, 100%): mp 238−240 °C. ^{1}H NMR (DMSO-d_6) δ 11.52 (s, 1H), 7.31 (d, J = 8.9 Hz, 1H), 7.10 (d, J = 2.4 Hz, 1H), 6.97−6.92 (m, 1H), 6.88 (dd, J = 8.9, 2.4 Hz, 1H), 4.04 (t, J = 6.0 Hz, 2H), 2.89 (t, J = 6.0 Hz, 2H), 2.65 (q, J = 7.1 Hz, 4H), 1.02 (t, J = 7.1 Hz, 6H), 1H not observed. Anal. $C_{15}H_{20}N_2O_3$·$^1/_2H_2O$ requires C, 63.14; H, 7.42; N, 9.82. Found: C, 62.93; H, 7.32; N, 9.90. **64** (15.8 g, 57.4 mmol) was treated with 4 M HCl in dioxane to give the corresponding hydrochloride salt (13.6 g, 76%): mp 198−200 °C (dec). ^{1}H NMR (DMSO-d_6) δ 12.86 (s, 1H), 11.66 (s, 1H), 10.11 (s, 1H), 7.37 (d, J = 8.9 Hz, 1H), 7.19 (d, J = 2.4 Hz, 1H), 7.04−6.99 (m, 1H), 6.97 (dd, J = 8.9, 2.4 Hz, 1H), 4.34 (t, J = 4.9 Hz, 2H), 3.55−3.47 (m, 2H), 3.28−3.14 (m, 4H), 1.25 (t, J = 7.2 Hz, 6H). Anal. $C_{15}H_{21}ClN_2O_3$·H_2O requires C, 54.46; H, 7.01; N, 8.47. Found: C, 54.55; H, 7.04; N, 8.44.

*Ethyl 5-(3-(Dimethylamino)propoxy)-1H-indole-2-carboxylate (**66**).* DEAD (1.52 g, 8.7 mmol) was added dropwise to a solution of 3-(dimethylamino)-1-propanol (1.03 mL, 8.7 mmol), ethyl 5-hydroxy-1*H*-indole-2-carboxylate (**65**) (1.45 g, 5.8 mmol), and triphenylphosphine (2.29 g, 8.7 mmol) in dry THF (40 mL) while cooling in a water bath. The solution was stirred at rt for 4 h. The mixture was diluted with EtOAc, washed with water (×2), and then extracted with aq HCl (2N, ×2). The acidic extracts were cooled in an ice bath and basified with concd aq NH_3. The mixture was extracted with EtOAc (×2), and the extracts were dried and evaporated to give a light-brown oil. Purification by column chromatography (EtOAc then EtOAc:MeOH 9:1, 8:2, 7:3, 6:4) followed by recrystallization from aq EtOH gave **66** as cream crystals (0.57 g, 34%): mp 118−120 °C. ^{1}H NMR (DMSO-d_6) δ 11.67 (s, 1H), 7.33 (d, J = 8.9 Hz, 1H), 7.09 (d, J = 2.3 Hz, 1H), 7.03 (s, 1H), 6.90 (dd, J = 8.9, 2.5 Hz, 1H), 4.32 (q, J = 7.1 Hz, 2H), 3.97 (t, J = 6.5 Hz, 2H), 2.36 (t, J = 7.3 Hz, 2H), 2.14 (s, 6H), 1.85 (quin, J = 6.8 Hz, 2H), 1.33 (t, J = 7.1 Hz, 3H). Anal. $C_{16}H_{22}N_2O_3$ requires C, 66.19; H, 7.64; N, 9.65. Found: C, 65.95; H, 7.85; N, 9.79.

*5-(3-(Dimethylamino)propoxy)-1H-indole-2-carboxylic Acid (**67**).* A solution of KOH (430 mg, 7.7 mmol) in water (10 mL) was added to a solution of **66** (543 mg, 1.9 mmol) in EtOH (20 mL), and the mixture was stirred at reflux for 5 min and then cooled to rt. The EtOH was evaporated, and the aq portion was neutralized by the addition of aq HCl (2N, 3.8 mL). The mixture was allowed to stand at 4 °C overnight, and the white precipitate was filtered off and dried (475 mg, 97% as free base). This solid was stirred with HCl-saturated dioxane (30 mL) for 2 h. The solid was filtered off and dried to give the hydrochloride salt of **67** as a white solid (407 mg, 73%): mp 229−231 °C. ^{1}H NMR (DMSO-d_6) δ 12.8 (v br s, 1H), 11.61 (s, 1H), 10.2 (v br s, 1H), 7.34 (d, J = 8.9 Hz, 1H), 7.12 (d, J = 2.4 Hz, 1H), 7.00−6.97 (m, 1H), 6.91 (dd, J = 8.9, 2.4 Hz, 1H), 4.05 (t, J = 6.0 Hz, 2H), 3.25−3.19 (m, 2H), 2.79 (s, 6H), 2.18−2.10 (m, 2H). Anal. $C_{14}H_{18}N_2O_3$·HCl·$^1/_4H_2O$ requires C, 55.45; H, 6.48; N, 9.24. Found: C, 55.57; H, 6.34; N, 9.25.

*Ethyl 1-Acetyl-5-methyl-1H-indole-2-carboxylate (**69**).* NaH (60% in oil, 1.42 g, 59.0 mmol) was added in portions to a stirred solution of ethyl 5-methyl-1*H*-indole-2-carboxylate (**68**) (10.0 g, 49.2 mmol) in DMF (40 mL) at rt, and the mixture was stirred for 15 min. The reaction flask was cooled in an ice bath, and AcCl (4.89 mL, 68.9 mmol) was added dropwise over 5 min. The mixture was stirred at rt for 3 h and then poured into ice−water (400 mL) and extracted with EtOAc (500 mL). The extract was dried and evaporated to give an oil that was purified by column chromatography (EtOAc:petroleum ether 1:20) to give **69** (10.8 g, 89%) as a yellow oil. ^{1}H NMR ($CDCl_3$) δ 8.01 (d, J = 8.6 Hz, 1H), 7.41−7.37 (m, 1H), 7.29−7.22 (m, 2H), 4.40 (q, J = 7.1 Hz, 2H), 2.6 (s, 3H), 2.44 (s, 3H), 1.41 (s, 3H). HRMS (FAB) calcd for $C_{14}H_{16}NO_3$ (MH^+) m/z 246.1130, found 246.1130.

*Ethyl 5-((Dimethylamino)methyl)-1H-indole-2-carboxylate (**70**).* NBS (12.0 g, 67.6 mmol) and AIBN (1.51 g, 9.2 mmol) were added to a solution of **69** (10.8 g, 44.1 mmol) in CCl_4 (150 mL), and the mixture was stirred at reflux for 3 h. The mixture was cooled, diluted with DCM (300 mL), and washed with water (500 mL). The organic layer was separated, dried, and evaporated to give a yellow oil which was dissolved in DMF (70 mL) and cooled to °C. Gaseous dimethylamine was bubbled into this solution for 10 min, and then the mixture was stirred at rt for 18 h. The mixture was partitioned between EtOAc (400 mL) cold aq Na_2CO_3 (2%). The EtOAc layer was separated, dried, and evaporated to give an amber oil which was purified by column chromatography (DCM:MeOH 10:1) to give **70** as a pale-amber gum (2.61 g, 24%). ^{1}H NMR ($CDCl_3$) δ 8.86 (s, 1H), 7.58 (s, 1H), 7.37 (d, J = 8.5 Hz, 1H), 7.31 (dd, J = 8.5, 1.5 Hz, 1H), 7.21−7.15 (m, 1H), 4.40 (q, J = 7.1 Hz, 2H), 3.52 (s, 2H), 2.27 (s, 6H), 1.41 (t, J = 7.1 Hz, 3H). **70** was treated with 4 M HCl in dioxane and EtOAc to give the corresponding hydrochloride salt as a pale brown solid: mp 133−135 °C. Anal. $C_{14}H_{18}N_2O_2$·HCl·H_2O requires C, 55.90; H, 7.04; N, 9.31. Found: C, 55.53; H, 6.91; N, 9.11.

*5-((Dimethylamino)methyl)-1H-indole-2-carboxylic Acid (**71**).* KOH (1.57 g, 28.1 mmol) was added to a mixture of **70** (2.3 g, 9.35 mmol), MeOH (40 mL), and water (20 mL), and the mixture was stirred at rt for 5 h. The MeOH was evaporated, and the aqueous residue was stirred with petroleum ether for 15 min. The petroleum ether layer was separated and discarded. The aqueous layer was acidified with concd HCl to pH ca. 6−7 at 0 °C. The solid was filtered off, washed with cold water (3 × 5 mL), and dried to give **71** (1.43 g, 70%): mp 266−268 °C (dec). ^{1}H NMR (DMSO-d_6) δ 11.58 (s, 1H), 7.52 (s, 1H), 7.37 (d, J = 8.5 Hz, 1H), 7.19 (dd, J = 8.5, 1.5 Hz, 1H), 7.01−6.96 (m, 1H), 3.54 (s, 2H), 2.22 (s, 6H), 1H not observed. **71** was treated with 4 M HCl in dioxane to give the corresponding hydrochloride salt: mp 273−275 °C. Anal. $C_{12}H_{14}N_2O_2$·HCl requires C, 56.58; H, 5.94; N, 11.00. Found: C, 56.51; H, 5.91; N, 10.78.

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J. Med. Chem. XXXX, XXX, XXX−XXX

*Ethyl 5-(3-Morpholinopropoxy)-1H-indole-2-carboxylate (**73**).* Pd/C (5%) was added to a solution of ethyl 5-(benzyloxy)-1*H*-indole-2-carboxylate (**72**) (5.10 g, 17.3 mmol) in EtOAc (40 mL), THF (40 mL), and EtOH (30 mL). The mixture was hydrogenated at 50 psi for 17 h, then filtered through Celite. The filtrate was evaporated to give crude ethyl 5-hydroxy-1*H*-indole-2-carboxylate as a gray solid. A portion of this material (5.51 mmol) was dissolved in THF (40 mL) with triphenylphosphine (2.17 g, 8.3 mmol) and 4-(3-hydroxypropyl)morpholine (1.20 g, 8.3 mmol). DEAD (1.44 g, 8.3 mmol) was added dropwise, and the mixture was stirred at rt for 2.5 h. The THF was evaporated, and the residue was partitioned between EtOAc and water. The EtOAc layer was washed with brine (×3) and extracted with aq HCl (2N, ×2). The combined acid extracts were cooled to 0 °C, basified with concd aq NH_3, and extracted with EtOAc (×3). The combined organic extracts were dried and evaporated to give a tan solid. Recrystallization from EtOH gave **73** as pale-yellow crystals (0.86 g, 47%): mp 152−155 °C. ^{1}H NMR (DMSO-d_6) δ 11.68 (s, 1H), 7.33 (d, J = 8.9 Hz, 1H), 7.10 (d, J = 2.3 Hz, 1H), 7.03 (s, 1H), 6.91 (dd, J = 8.9, 2.4 Hz, 1H), 4.33 (q, J = 7.1 Hz, 2H), 3.99 (t, J = 6.4 Hz, 2H), 3.60−3.56 (m, 4H), 2.43 (t, J = 7.2 Hz, 2H), 2.39−2.35 (m, 4H), 1.93−1.84 (m, 2H), 1.33 (t, J = 7.1 Hz, 3H). Anal. $C_{18}H_{24}N_2O_4$ requires C, 65.04; H, 7.28; N, 8.43. Found: C, 64.70; H, 7.58; N, 8.56.

*5-(3-Morpholinopropoxy)-1H-indole-2-carboxylic Acid (**74**).* A solution of KOH (651 mg, 11.6 mmol) in water (10 mL) was added to a suspension of **73** (811 mg, 2.44 mmol) in EtOH (20 mL). The mixture was stirred at reflux for 10 min then cooled to rt. The EtOH was evaporated, and the aqueous residue was neutralized by the addition of aq HCl (2N, 5.80 mL, 11.6 mmol). An oil separated that solidified on standing. The solid was filtered off and dried, then suspended in dioxane (30 mL) and treated with HCl-saturated dioxane (10 mL). After stirring at room temperature for 3 h, the solid was filtered off and dried to give the hydrochloride salt of **74** as a white solid (824 mg, 99%): mp 246−249 °C. ^{1}H NMR (DMSO-d_6) δ 12.81 (br s, 1H), 11.61 (s, 1H), 10.69 (br s, 1H), 7.34 (d, J = 8.9 Hz, 1H), 7.13 (d, J = 2.3 Hz, 1H), 6.99 (dd, J = 2.0, 0.6 Hz, 1H), 6.91 (dd, J = 8.9, 2.4 Hz, 1H), 4.06 (t, J = 6.0 Hz, 2H), 4.01−3.92 (m, 2H), 3.85−3.74 (m, 2H), 3.53−3.43 (m, 2H), 3.16−3.03 (m, 2H), 2.24−2.14 (m, 2H), 2H obscured by water peak observed after D_2O exchange. Anal. $C_{16}H_{20}N_2O_4$·HCl·$^1/_2H_2O$ requires C, 54.94; H, 6.34; N, 8.01. Found: C, 55.00; H, 6.62; N, 7.64.

*Ethyl 5-(3-(Dimethylamino)propanamido)-1H-indole-2-carboxylate (**76**).* A mixture of ethyl 5-amino-1*H*-indole-2-carboxylate (**75**)[42] (2.74 g, 13.4 mmol), 3-(dimethylamino)propanoic acid hydrochloride (3.09 g, 20.1 mmol), and EDCI (7.71 g, 40.2 mmol) in DMA (50 mL) was stirred at rt for 18 h. The mixture was cooled in an ice bath, and EtOAc (500 mL) and water (500 mL) were added. The aq layer was basified with aq Na_2CO_3 (2%), and the EtOAc layer was separated. The aqueous layer was extracted with more EtOAc (2 × 200 mL). The combined EtOAc layers were dried and evaporated to give a brown solid, which was purified by column chromatography (DCM:EtOAc 1:1 then DCM:MeOH 10:1) to give **76** as a light-brown foam (3.17 g, 78%). ^{1}H NMR ($CDCl_3$) δ 10.78 (s, 1H), 8.82 (s, 1H), 7.98 (s, 1H), 7.37−7.30 (m, 2H), 7.17 (d, J = 1.5 Hz, 1H), 4.40 (q, J = 7.1 Hz, 2H), 2.68 (t, J = 7.3 Hz, 2H), 2.53 (t, J = 7.3 Hz, 2H), 2.40 (s, 6H), 1.41 (t, J = 7.1, 3H). HRMS (FAB) calcd for $C_{16}H_{22}N_3O_3$ (MH^+) m/z 304.1661, found 304.1659.

*5-(3-(Dimethylamino)propanamido)-1H-indole-2-carboxylic Acid (**77**).* KOH (1.73 g, 30.9 mmol) was added to a suspension of **76** (3.12 g, 10.3 mmol) in MeOH (40 mL) and water (20 mL), and the mixture was stirred at rt for 3 h. The MeOH was evaporated, and the aqueous residue was diluted with water (15 mL) and acidified with concd HCl at 0 °C to pH 6−7. The solvent was evaporated to give a brown residue, which was extracted with DCM:MeOH (5:1) several times. The extracts were evaporated to give a gray solid (3.29 g), which was stirred with water (20 mL) at 0 °C for 2 h. The precipitate was collected, washed with cold water (5 × 3 mL), and dried to give **77** as the free base (2.39 g, 85%): mp 215−217 °C. ^{1}H NMR (DMSO-d_6) δ 11.54 (s, 1H), 9.90 (s, 1H), 7.96 (d, J = 1.5 Hz, 1H), 7.33 (d, J = 8.8 Hz, 1H), 7.27 (dd, J = 8.8, 1.9 Hz, 1H), 6.97 (d, J = 1.4 Hz, 1H), 2.65 (t, J = 7.1 Hz, 2H), 2.47 (t, J = 7.1 Hz, 2H), 2.24 (s, 6H), 1H not observed. Anal. $C_{14}H_{17}N_3O_3$·$^3/_4H_2O$ requires C, 58.22; H, 6.46; N, 14.55; found: C, 57.88; H, 6.57; N, 14.47. **77** (2.38 g, 8.65 mmol) was treated with 4 M HCl in dioxane to give the corresponding hydrochloride salt (2.71 g, 100%): mp 221−223 °C (dec). ^{1}H NMR (DMSO-d_6) δ 12.85 (br s, 1H), 11.68 (s, 1H), 10.18 (s, 1H), 9.95 (br s, 1H), 7.99 (br s, 1H), 7.37 (d, J = 8.8 Hz, 1H), 7.34 (dd, J = 8.8, 1.8 Hz, 1H), 7.04 (d, J = 1.6 Hz, 1H), 3.37 (t, J = 7.1 Hz, 2H), 2.85 (t, J = 7.1 Hz, 2H), 2.79 (s, 6H).

*(E)-Methyl 3-(4-(2-Morpholinoethoxy)phenyl)acrylate (**79**).* A mixture of 4-(2-chloroethyl)morpholine hydrochloride (6.42 g, 34.5 mmol), NaOH (1.52 g, 38.1 mmol), water (24 mL), and toluene (26 mL) was stirred at 0 °C and saturated with solid NaCl. The toluene layer was separated, and the alkaline solution was extracted with toluene (4 × 15 mL). The combined toluene extracts were dried over KOH and used for the following reaction. NaH (60% in oil, 1.28 g, 32.0 mmol) was added in portions to a solution of (*E*)-methyl 3-(4-hydroxyphenyl)acrylate (**78**) (5.43 g, 30.5 mmol) in dry ether (100 mL) and stirred under nitrogen at rt. After the addition was complete, the mixture was stirred for a further 2 h. Most of the ether was evaporated, petroleum ether (100 mL) was added, and the mixture was stirred for 5 min. The petroleum ether was decanted, and the process was repeated once more. To the residue was added toluene (26 mL), followed by the toluene solution of 4-(2-chloroethyl)morpholine prepared above. The mixture was stirred at reflux for 92 h and then cooled and filtered through a short column of neutral Al_2O_3 eluting with hot toluene. The combined eluates were washed successively with cold aq $NaHCO_3$ and then with water, then dried and evaporated to give **79** as a colorless solid (6.66 g, 75%): mp 61−62 °C. ^{1}H NMR ($CDCl_3$) δ 7.64 (d, J = 16.0 Hz, 1H), 7.51−7.45 (m, 2H), 6.93−6.88 (m, 2H), 6.31 (d, J = 16.0 Hz, 1H), 4.14 (t, J = 5.7 Hz, 2H), 3.79 (s, 3H), 3.77−3.70 (m, 4H), 2.81 (t, J = 5.7 Hz, 2H), 2.61−2.55 (m, 4H). Anal. $C_{16}H_{21}NO_4$ requires C, 65.96; H, 7.27; N, 4.81. Found: C, 65.98; H, 7.33; N, 4.82.

*(E)-3-(4-(2-Morpholinoethoxy)phenyl)acrylic Acid (**80**).* A mixture of **79** (6.58 g, 22.6 mmol), MeOH (40 mL), water (20 mL), and KOH (3.16 g, 56.5 mmol) was stirred at 20 °C for 21 h. The mixture was evaporated at 35 °C to remove the MeOH. The resulting alkaline mixture was washed with CH_2Cl_2, and the aqueous layer was filtered. The alkaline filtrate was acidified with concd HCl at 0 °C. The resulting precipitate was filtered off, washed with 6 N HCl, and dried to give the hydrochloride salt of **80** as a colorless solid (5.90 g, 83%): mp 252−254 °C. ^{1}H NMR (DMSO-d_6) δ 12.22 (br s, 1H), 10.90 (br s, 1H), 7.69 (d, J = 8.8 Hz, 2H), 7.56 (d, J = 16.0 Hz, 1H), 7.05 (d, J = 8.8 Hz, 2H), 6.41 (d, J = 16.0 Hz, 1H), 4.46 (br s, 2H), 4.06−3.73 (m, 4H), 3.67−3.42 (m, 4H), 3.28−3.14 (m, 2H). Anal. $C_{15}H_{19}NO_4$·HCl·$^1/_3H_2O$ requires C, 56.34; H, 6.51; N, 4.38. Found: C, 56.49; H, 6.46; N, 4.42.

*4-(3-Bromopropyl)morpholine (**81**).* A solution of morpholine (8.71 g, 100 mmol) in EtOAc (500 mL) was added dropwise over 3 h to a solution of 1,3-dibromopropane (20.2 g, 100 mmol) in EtOAc (500 mL) at 0 °C. The mixture was stirred at 0 °C for 5 h then at rt for a further 110 h. The solid was filtered off and washed with EtOAc (3 × 100 mL). The combined filtrates were washed successively with cold aq Na_2CO_3 (2%) and water (×2) and then dried. The resulting solution was acidified with HCl (4 M in dioxane, ca. 10 mL) and then evaporated to dryness. The residue was dried to give the hydrochloride salt of **81** as a colorless solid (5.1 g, 21%): mp 134−136 °C. ^{1}H NMR ($CDCl_3$) δ 13.37 (br s, 1H), 4.30 (br s, 2H), 4.00 (br s, 2H), 3.52 (t, J = 6.0 Hz, 2H), 3.45 (br s, 2H), 3.15 (t, J = 8.0 Hz, 2H), 2.91 (s, 2H), 2.63−2.51 (m, 2H). Anal. $C_7H_{14}BrNO$·HCl requires C, 34.38; H, 6.18; N, 5.73. Found: C, 34.54; H, 6.20; N, 5.72.

*(E)-3-(4-(3-Morpholinopropoxy)phenyl)acrylic Acid (**82**).* A mixture of the hydrochloride salt of **81** (8.44 g, 34.5 mmol), NaOH (1.52 g, 38.1 mmol), water (24 mL), and toluene (26 mL) was stirred at 0 °C and saturated with solid NaCl. The toluene layer was separated, and the alkaline solution was extracted with toluene (4 × 15 mL). The combined toluene extracts were dried over KOH and used for the following reaction. A toluene solution of the sodium salt of **78** (5.39 g, 30.3 mmol) was prepared as described in the preparation of **79**. To

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J. Med. Chem. XXXX, XXX, XXX−XXX

this was added the toluene solution of 4-(3-bromopropyl)morpholine, and the mixture was stirred at reflux for 46 h, then cooled and filtered through a short column of neutral Al_2O_3, eluting with hot toluene. The combined eluates were washed successively with cold aq $NaHCO_3$ and water and then dried and evaporated to give a beige solid. The solid was treated with KOH (4.25 g, 75.8 mmol) in MeOH (60 mL) and water (30 mL) at rt for 20 h. The mixture was evaporated at 35 °C to remove the MeOH then washed with DCM and filtered. The alkaline filtrate was acidified with concd HCl at 0 °C to pH < 1. The precipitated solid was filtered off, washed with 6 N HCl, and dried to give the hydrochloride salt of **82** as a colorless solid (7.2 g, 73%): mp 225−227 °C. ^{1}H NMR (DMSO-d_6) δ 12.19 (br s, 1H), 11.22 (s, 1H), 7.64 (d, J = 8.7 Hz, 2H), 7.54 (d, J = 16.0 Hz, 1H), 6.98 (d, J = 8.7 Hz, 2H), 6.40 (d, J = 16.0 Hz, 1H), 4.12 (t, J = 6.1 Hz, 2H), 4.03−3.76 (m, 4H), 3.53−3.00 (m, 6H), 2.33−2.14 (m, 2H). Anal. $C_{16}H_{21}NO_4$·HCl requires C, 58.62; H, 6.77; N, 4.27. Found: C, 58.49; H, 6.94; N, 4.25.

*(E)-Methyl 3-(4-(2-(4-Methylpiperazin-1-yl)ethoxy)phenyl)-acrylate (**83**).* NaH (60% in oil, 0.77 g, 32.0 mmol) was added to a solution of **78** (5.4 g, 30.5 mmol) in dry ether (100 mL), and the mixture was stirred under nitrogen at rt for 6 h. The ether was evaporated, and DMA (20 mL) was added, followed by 1,2-dibromoethane (13.2 mL, 153 mmol). The mixture was stirred at 130−135 °C for 21 h and then cooled to rt. More NaH (60% in oil, 0.77 g, 32.0 mmol) and 1,2-dibromoethane (13.2 mL, 153 mmol) were added, and the mixture was stirred at 150−160 °C for 5 h, then cooled and diluted with toluene (100 mL). The mixture was filtered, and the filter pad was washed with hot toluene several times. The combined filtrates were washed successively with cold water, cold aq $NaHCO_3$, and again with water. The organic layer was dried and evaporated to give an oil which was dissolved in DMF (30 mL) and stirred with 1-methylpiperazine (14.7 mL, 133 mmol) at rt for 3 h. The mixture was diluted with EtOAc (200 mL) and then stirred with aq Na_2CO_3 (2%) at 0 °C for 5−10 min. The organic layer was separated, washed with water (×2), and then dried and evaporated. The resulting oil was dissolved in EtOAc, and the solution was treated with HCl (4 M in dioxane, 17 mL, 68 mmol). The mixture was stirred and then left to stand at 5 °C overnight. The solid was filtered off, washed with EtOAc several times, and dried to give the hydrochloride salt of **83** as a beige solid (5.47g, 48%): mp 235−237 °C (dec). ^{1}H NMR (DMSO-d_6) δ 11.78 (br s, 2H), 7.71 (d, J = 8.8 Hz, 2H), 7.64 (d, J = 16.0 Hz, 1H), 7.05 (d, J = 8.8 Hz, 2H), 6.52 (d, J = 16.0 Hz, 1H), 4.44 (poorly resolved t resolved after D_2O exchange, J = 4.9 Hz, 2H), 3.71 (s, 3H), 3.52−3.31 (m, partially obscured by water peak, revealed after D_2O exchange, 10H), 2.81 (s, 3H). Anal. $C_{17}H_{26}Cl_2N_2O_3\cdot{}^1/_2H_2O$ requires C, 52.86; H, 7.05; N, 7.25. Found: C, 52.79; H, 6.98; N, 6.94.

*(E)-3-(4-(2-(4-Methylpiperazin-1-yl)ethoxy)phenyl)acrylic Acid (**84**).* **83** (5.18 g, 13.7 mmol) was treated with KOH (3.5 g, 62 mmol) in a mixture of MeOH (30 mL) and water (15 mL) at rt for 29 h. The mixture was evaporated at 35 °C to remove the MeOH. The alkaline remainder was washed with DCM and filtered. The filtrate was acidified with concd HCl at 0 °C and left to stand at 5 °C overnight. The resulting precipitate was filtered off, washed with cold 6 N HCl (3 × 1 mL), and dried to give hydrochloride salt of **84** as a beige solid (2.97 g, 60%): mp 264−267 °C (dec). ^{1}H NMR (DMSO-d_6) δ 11.89 (br s, 3H), 7.67 (d, J = 8.8 Hz, 2H), 7.56 (d, J = 16.0 Hz, 1H), 7.05 (d, J = 8.8 Hz, 2H), 6.41 (d, J = 16.0 Hz, 1H), 4.46 (unresolved t resolved after D_2O exchange, J = 4.8 Hz, 2H), 3.71 (s, 3H), 3.57−3.30 (m, partially obscured by water peak, revealed after D_2O exchange, 10H), 2.82 (s, 3H). Anal. $C_{16}H_{24}Cl_2N_2O_3\cdot{}^1/_2H_2O$ requires C, 51.62; H, 6.77; N, 7.53. Found: C, 51.77; H, 6.63; N, 7.17.

*4-(2-Morpholinoethoxy)benzoic Acid (**86**).* A mixture of 4-(2-chloroethyl)morpholine hydrochloride (20.4 g, 110 mmol), NaOH (4.85 g, 121 mmol), water (75 mL), and toluene (83 mL) was stirred at 0 °C and saturated with solid NaCl. The toluene layer was separated and the aqueous layer extracted with toluene (4 × 33 mL). The combined toluene extracts were dried over solid KOH and then added to a mixture of sodium 4-(methoxycarbonyl)phenolate (**85**) (16.9 g, 97 mmol) and toluene (83 mL). The mixture was stirred at reflux for 4 h. The mixture was cooled and filtered, and the filter pad was washed with hot toluene several times. The combined filtrates were washed successively with cold aq $NaHCO_3$ and water and then dried and evaporated to give crude methyl 4-(2-morpholinoethoxy)benzoate (18.5 g) as an amber oil. This product was contaminated with excess 4-(2-chloroethyl)morpholine. The crude product was treated with KOH (9.8 g, 175 mmol) in a mixture of MeOH (100 mL) and water (50 mL) at rt for19 h. The mixture was concentrated at 35 °C to remove MeOH. The alkaline remainder was washed with DCM and filtered. The filtrate was acidified with concd HCl at 0 °C. The resulting precipitate was filtered off and dried to give the hydrochloride salt of **86** as a colorless solid (10.9 g, 39%): mp 266−268 °C. ^{1}H NMR (DMSO-d_6) δ 12.66 (br s, 1H), 11.06 (br s, 1H), 7.96−7.89 (m, 2H), 7.12−7.06 (m, 2H), 4.57−4.43 (m, 2H), 3.96 (br d, J = 12.4 Hz, 2H), 3.81 (br t, J = 12.0 Hz, 2H), 3.65−3.43 (m, 4H), 3.27−3.14 (m, 2H). Anal. $C_{14}H_{18}ClNO_4$ requires C, 54.26; H, 6.31; N, 4.87. Found: C, 54.00; H, 6.38; N, 4.90.

*N-(2-(tert-Butyldimethylsilyloxy)ethyl)-1-(chloromethyl)-5-nitro-1,2-dihydro-3H-benzo[e]indole-7-sulfonamide (**89**).* **88**[34] (1.84 g, 4.03 mmol) and i-Pr_2NEt (0.84 mL, 4.85 mmol) were added to a solution of 2-((*tert*-butyldimethylsilyl)oxy)ethylamine (850 mg, 4.85 mmol) in THF (100 mL), and the mixture was stirred at 0 °C for 40 min. Cs_2CO_3 (1.31 g, 4.03 mmol) and MeOH (50 mL) were added, and the mixture was stirred at rt for 45 min then allowed to stand at 5 °C for 14 h. The mixture was concentrated under reduced pressure at 25 °C to remove most of the THF. Ice−water was added, and the mixture was stirred at 0 °C for 1 h 30 min. The solid was filtered off, washed with cold water, and dried to give **89** as a red−orange solid (2.0 g, 100%): mp 127−129 °C. ^{1}H NMR ($CDCl_3$) δ 8.92 (d, J = 1.6 Hz, 1H), 7.90 (dd, J = 8.9, 1.6 Hz, 1H), 7.79 (d, J = 8.9 Hz, 1H), 7.73 (s, 1H), 4.88 (t, J = 5.8 Hz, 1H), 4.40 (br s, 1H), 4.17−4.08 (m, 1H), 4.05−3.95 (m, 2H), 3.83−3.74 (m, 1H), 3.68 (t, J = 5.2 Hz, 2H), 3.60 (dd, J = 11.1, 10.0 Hz, 1H), 3.18−3.11 (m, 2H), 0.84 (s, 9H), 0.00 (s, 6H). Anal. $C_{21}H_{30}ClN_3O_5SSi$ requires C, 50.44; H, 6.05; N, 8.40. Found: C, 50.71; H, 6.35; N, 8.32.

*N-(2-(tert-Butyldimethylsilyloxy)ethyl)-1-(chloromethyl)-3-(5-(dimethylamino)-1H-indole-2-carbonyl)-5-nitro-1,2-dihydro-3H-benzo[e]indole-7-sulfonamide (**90d**).* Prepared by general method A from **89** (195 mg, 0.39 mmol) and 5-(dimethylamino)-1*H*-indole-2-carboxylic acid hydrochloride (113 mg, 0.47 mmol) to give **90d** as a brown−orange solid (268 mg, 100%): mp 125 °C (dec). ^{1}H NMR (DMSO-d_6) δ 11.53 (br d, J = 1.6 Hz, 1H), 9.30 (s, 1H), 8.85 (d, J = 1.6 Hz, 1H), 8.43 (d, J = 8.9 Hz, 1H), 8.06−7.95 (m, 2H), 7.38 (d, J = 9.0 Hz, 1H), 7.15 (br d, J = 1.8 Hz, 1H), 7.04 (dd, J = 9.0, 2.3 Hz, 1H), 6.92 (br d, J = 2.2 Hz, 1H), 4.96 (br t, J = 10.2 Hz, 1H), 4.72 (br dd, J = 10.9, 2.4 Hz, 1H), 4.69−4.61 (m, 1H), 4.20−4.08 (m, 2H), 3.55 (t, J = 6.1 Hz, 2H), 2.91 (q, J = 6.1 Hz, 2H), 2.79 (s, 6H), 0.788 (s, 9H), −0.046 (s, 6H). HRMS (ESI) calcd for $C_{32}H_{41}ClN_5O_6SSi$ (MH^+) m/z 686.2230, found 686.2232.

*(E)-N-(2-(tert-Butyldimethylsilyloxy)ethyl)-1-(chloromethyl)-3-(3-(4-(2-morpholinoethoxy)phenyl)acryloyl)-5-nitro-1,2-dihydro-3H-benzo[e]indole-7-sulfonamide (**90i**).* A mixture of **80**·HCl (68.1 mg, 0.24 mmol), DMF (one drop), and $SOCl_2$ (2 mL) was stirred at reflux for 10 min. The mixture was cooled and evaporated to dryness. The reaction flask was immersed in an ice bath and **89** (108 mg, 0.215 mmol) was added, followed by a mixture of DMA (2 mL) and i-Pr_2NEt (0.041 mL, 0.237 mmol). The resulting mixture was stirred at 0 °C for 2 h. Dilute aq $NaHCO_3$ (5%, 7 mL) was added, followed by water (7 mL), and the mixture was stirred at 0 °C for 10 min. The precipitated solid was filtered off, washed with cold water, then dried and triturated with EtOAc:petroleum ether (1:10) to give **90i** as a yellow−orange solid (128 mg, 79%): mp 203−205 °C. ^{1}H NMR ($CDCl_3$) δ 9.42 (br s, 1H), 8.99 (d, J = 1.6 Hz, 1H), 8.03 (dd, J = 8.9, 1.6 Hz, 1H), 7.95 (d, J = 8.9 Hz, 1H), 7.90 (d, J = 15.2 Hz, 1H), 7.58 (d, J = 8.8 Hz, 2H), 6.96 (d, J = 8.8 Hz, 2H), 6.74 (d, J = 15.2 Hz, 1H), 4.96 (t, J = 5.9, 1H), 4.64 (dd, J = 10.7, 2.4 Hz, 1H), 4.56 (br t, J = 9.7 Hz, 1H), 4.35−4.26 (m, 1H), 4.18 (t, J = 5.7, 2H), 3.95 (br dd, J = 11.4, 3.3 Hz, 1H), 3.80−3.72 (m, 4H), 3.68 (t, J = 5.1 Hz, 2H), 3.63 (br dd, J = 11.4, 9.5 Hz, 1H), 3.22−3.13 (m, 2H), 2.84 (t, J = 5.7 Hz, 2H), 2.65−2.55 (m, 4H), 0.833 (s, 9H), −0.008 (s, 6H, obscured by TMS). Anal. $C_{36}H_{47}ClN_4O_8SSi$ requires C, 56.94; H, 6.24; N, 7.38. Found: C, 57.09; H, 6.14; N, 7.27.

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*(E)-N-(2-(tert-Butyldimethylsilyloxy)ethyl)-1-(chloromethyl)-3-(3-(4-(3-morpholinopropoxy)phenyl)acryloyl)-5-nitro-1,2-dihydro-3H-benzo[e]indole-7-sulfonamide (**90j**).* Prepared by general method A from **89** (90 mg, 0.18 mmol) and **82**·HCl (71 mg, 0.22 mmol) to give **90j** as a yellow−orange solid (136 mg, 98%): mp 130 °C. ^{1}H NMR ($CDCl_3$) δ 9.41 (br s, 1H), 8.98 (br d, J = 1.5 Hz, 1H), 8.03 (dd, J = 8.9, 1.7 Hz, 1H), 7.94 (d, J = 8.9 Hz, 1H), 7.90 (d, J = 15.2 Hz, 1H), 7.57 (d, J = 8.7 Hz, 2H), 6.94 (d, J = 8.7 Hz, 2H), 6.73 (d, J = 15.2 Hz, 1H), 4.97 (t, J = 5.8, 1H), 4.63 (dd, J = 10.7, 2.4 Hz, 1H), 4.56 (br t, J = 9.7 Hz, 1H), 4.44−4.24 (m, 1H), 4.09 (t, J = 6.3, 2H), 3.94 (br dd, J = 11.4, 3.3 Hz, 1H), 3.79−3.56 (m, 7H), 3.16 (q, J = 5.4 Hz, 2H), 2.58−2.43 (m, 6H), 2.05−1.95 (m, 2H), 0.832 (s, 9H), −0.013 (s, 6H, obscured by TMS). Anal. $C_{37}H_{49}ClN_4O_8SSi$ requires C, 57.46; H, 6.39; N, 7.24. Found: C, 57.80; H, 6.45; N, 7.30.

*N-(2-(tert-Butyldimethylsilyloxy)ethyl)-1-(chloromethyl)-3-(4-(2-(4-methylpiperazin-1-yl)ethoxy)benzoyl)-5-nitro-1,2-dihydro-3H-benzo[e]indole-7-sulfonamide (**90k**).* Prepared by general method A from **89** (90 mg, 0.18 mmol) and **84**·HCl (78 mg, 0.22 mmol) to give **90k** as a yellow−orange solid (118 mg, 85%): mp 202 °C (dec). ^{1}H NMR ($CDCl_3$) δ 9.42 (br s, 1H), 8.98 (br d, J = 1.6 Hz, 1H), 8.03 (dd, J = 8.8, 1.6 Hz, 1H), 7.95 (d, J = 8.8 Hz, 1H), 7.91 (d, J = 15.2 Hz, 1H), 7.57 (d, J = 8.7 Hz, 2H), 6.95 (d, J = 8.7 Hz, 2H), 6.73 (d, J = 15.2 Hz, 1H), 4.99 (t, J = 5.8 Hz, 1H), 4.63 (dd, J = 10.8, 2.5 Hz, 1H), 4.45 (br t, J = 9.7 Hz, 1H), 4.34−4.26 (m, 1H), 4.17 (t, J = 5.8 Hz, 2H), 3.94 (dd, J = 11.4, 3.3 Hz, 1H), 3.69 (t, J = 5.0 Hz, 2H), 3.62 (dd, J = 11.4, 9.5 Hz, 1H), 3.22−3.13 (m, 2H), 2.85 (t, J = 5.8 Hz, 2H), 2.73−2.42 (m, 8H), 2.30 (s, 3H), 0.84 (s, 9H), −0.008 (s, 6H, obscured by TMS). Anal. $C_{37}H_{50}ClN_5O_7SSi$ requires C, 57.53; H, 6.52; N, 9.07. Found: C, 57.51; H, 6.52; N, 8.86.

*N-(2-(tert-Butyldimethylsilyloxy)ethyl)-1-(chloromethyl)-3-(4-(2-morpholinoethoxy)benzoyl)-5-nitro-1,2-dihydro-3H-benzo[e]-indole-7-sulfonamide (**90l**).* A mixture of **86**·HCl (68.1 mg, 0.237 mmol), DMF (one drop), and $SOCl_2$ (2 mL) was stirred at reflux for 10 min. The mixture was cooled and evaporated to dryness. The flask was immersed in an ice bath and **89** (108 mg, 0.215 mmol) was added, followed by a mixture of DMA (2 mL) and *i*-Pr_2NEt (0.041 mL, 0.237 mmol). The mixture was stirred at 0 °C for 1 h. Aqueous $NaHCO_3$ (5%, 4 mL) was added, followed by water (4 mL), and the mixture was stirred at 0 °C for 10 min. The solid was filtered off, washed with cold water, and dried to give **90l** as an orange solid (147 mg, 93%): mp 86−89 °C. ^{1}H NMR ($CDCl_3$) δ 9.00 (br d, J = 1.5 Hz, 1H), 8.76 (br s, 1H), 8.83 (dd, J = 8.9, 1.7 Hz, 1H), 7.95 (d, J = 8.9 Hz, 1H), 7.64 (d, J = 8.8 Hz, 2H), 7.02 (d, J = 8.8 Hz, 2H), 4.95 (t, J = 5.9, 1H), 4.55−4.41 (m, 2H), 4.25−4.12 (m, 3H), 3.88 (dd, J = 11.4, 3.4 Hz, 1H), 3.80−3.62 (m, 7H), 3.20−3.13 (m, 2H), 2.86 (t, J = 5.7 Hz, 2H), 2.65−2.56 (m, 4H), 0.84 (s, 9H), 0.002 (s, 6H, obscured by TMS). Anal. $C_{34}H_{45}ClN_4O_8SSi$ requires C, 55.68; H, 6.18; N, 7.64. Found: C, 55.63; H, 6.30; N, 7.64.

*N-(2-(tert-Butyldimethylsilyloxy)ethyl)-1-(chloromethyl)-3-(3-morpholinopropanoyl)-5-nitro-1,2-dihydro-3H-benzo[e]indole-7-sulfonamide (**90m**).* Prepared by general method A from **89** (195 mg, 0.39 mmol) and 3-morpholinopropanoic acid hydrochloride (92 mg, 0.47 mmol) to give **90m** as a yellow-orange solid (229 mg, 92%): mp 135−137 °C. ^{1}H NMR (DMSO-d_6) δ 9.23 (br s, 1H), 8.82 (br d, J = 1.5 Hz, 1H), 8.37 (d, J = 9.0 Hz, 1H), 8.02−7.95 (m, 2H), 4.63−4.47 (m, 2H), 4.37 (br d, J = 8.5 Hz, 1H), 4.12−4.00 (m, 2H), 3.58 (t, J = 4.6 Hz, 4H), 3.54 (t, J = 6.1 Hz, 2H), 2.91 (t, J = 6.1 Hz, 2H), 2.87−2.65 (m, 4H), 2.44 (br t, J = 4.4 Hz, 4H), 0.78 (s, 9H), −0.06 (s, 6H). HRMS (ESI) calcd for $C_{28}H_{42}ClN_4O_7SSi$ (MH^+) m/z 641.2226, found 641.2225.

*N-(2-(tert-Butyldimethylsilyloxy)ethyl)-1-(chloromethyl)-5-nitro-1,2-dihydro-3H-benzo[e]indole-7-carboxamide (**92**).* (Benzotriazol-1-yloxy)tripyrrolidinophosphonium hexafluorophosphate (pyBOP, 352 mg, 0.675 mmol) was added to a solution of 1-(chloromethyl)-5-nitro-3-(2,2,2-trifluoroacetyl)-1,2-dihydro-3*H*-benzo[*e*]indole-7-carboxylic acid (**91**)[35] (209 mg, 0.519 mmol), 2-((*tert*-butyldimethylsilyl)oxy)ethylamine (109 mg, 0.62 mmol), and *i*-Pr_2NEt (0.27 mL, 1.56 mmol) in THF (20 mL) at 0 °C and the mixture was stirred at this temperature for 1 h. Cs_2CO_3 (339 mg, 1.04 mmol) and MeOH (10 mL) were added, and the mixture was stirred at rt for 2 h then allowed to stand at 5 °C overnight. The mixture was concentrated at 25 °C to remove most of the THF. Ice−water was added, and the mixture was stirred at 0 °C for 5 h 30 min. The resulting solid was filtered off, washed with cold water, and dried. Purification by column chromatography (DCM:EtOAc 5:1) gave **92** as a red solid (212 mg, 88%): mp 156−158 °C. ^{1}H NMR ($CDCl_3$) δ 8.75 (br d, J = 1.4 Hz, 1H), 8.02 (dd, J = 8.8, 1.7 Hz, 1H), 7.77 (d, J = 8.8 Hz, 1H), 7.71 (s, 1H), 6.67 (br s, 1H), 4.26 (s, 1H), 4.14−4.07 (m, 1H), 4.01−3.91 (m, 2H), 3.87−3.77 (m, 3H), 3.67−3.55 (m, 3H), 0.92 (s, 9H), 0.11 (s, 6H). Anal. $C_{22}H_{30}ClN_3O_4Si$ requires C, 56.94; H, 6.52; N, 9.06. Found: C, 57.07; H, 6.67; N, 9.20.

*N-(2-(tert-Butyldimethylsilyloxy)ethyl)-1-(chloromethyl)-3-(5-(2-morpholinoethoxy)-1H-indole-2-carbonyl)-5-nitro-1,2-dihydro-3H-benzo[e]indole-7-carboxamide (**93e**).* Prepared by general method A from **92** (126 mg, 0.27 mmol) and 5-(2-morpholinoethoxy)-1*H*-indole-2-carboxylic acid hydrochloride[42] (107 mg, 0.33 mmol) to give **93e** as a yellow solid (193 mg, 97%): mp 124 °C. ^{1}H NMR ($CDCl_3$) δ 9.38 (br s, 1H), 9.28 (s, 1H), 8.79 (br d, J = 1.3 Hz, 1H), 8.15 (dd, J = 8.8, 1.6 Hz, 1H), 7.94 (d, J = 8.8 Hz, 1H), 7.38 (d, J = 8.9 Hz, 1H), 7.14 (br d, J = 2.3 Hz, 1H), 7.09−7.04 (m, 2H), 6.79 (t, J = 5.2 Hz, 1H), 4.92 (dd, J = 11.3, 2.1 Hz, 1H), 4.81 (br t, J = 9.8 Hz, 1H), 4.39−4.29 (m, 1H), 4.18 (t, J = 5.7 Hz, 1H), 3.99 (dd, J = 11.4, 3.2 Hz, 1H), 3.86 (t, J = 5.2 Hz, 2H), 3.82−3.74 (m, 4H), 3.71−3.59 (m, 3H), 2.86 (t, J = 5.7 Hz, 2H), 2.67−2.58 (m, 4H), 0.94 (s, 9H), 0.12 (s, 6H). Anal. $C_{37}H_{46}ClN_5O_7Si\cdot{}^1/_3H_2O$ requires C, 59.86; H, 6.34; N, 9.43. Found: C, 59.76; H, 6.41; N, 9.58.

*(E)-N-(2-(tert-Butyldimethylsilyloxy)ethyl)-1-(chloromethyl)-3-(3-(4-(2-morpholinoethoxy)phenyl)acryloyl)-5-nitro-1,2-dihydro-3H-benzo[e]indole-7-carboxamide (**93i**).* Prepared by general method A from **92** (80 mg, 0.17 mmol) and **80**·HCl (65 mg, 0.21 mmol) to give **93i** as a yellow−orange solid (121 mg, 97%): mp 164−165 °C. ^{1}H NMR ($CDCl_3$) δ 9.35 (br s, 1H), 8.75 (br d, J = 1.3 Hz, 1H), 8.12 (dd, J = 8.8, 1.6 Hz, 1H), 7.93−7.86 (m, 2H), 7.58 (d, J = 8.8 Hz, 2H), 6.95 (d, J = 8.8 Hz, 2H), 6.78−6.69 (m, 2H), 4.63 (dd, J = 10.8, 2.4 Hz, 1H), 4.53 (t, J = 9.7 Hz, 1H), 4.33−4.25 (m, 1H), 4.17 (t, J = 5.7 Hz, 2H), 3.97 (dd, J = 10.4, 3.2 Hz, 1H), 3.85 (t, J = 5.2 Hz, 2H), 3.75 (br t, J = 4.7 Hz, 4H), 3.69−3.56 (m, 3H), 2.83 (t, J = 5.7 Hz, 2H), 2.59 (br t, J = 4.7 Hz, 4H), 0.93 (s, 9H), 0.11 (s, 6H). Anal. $C_{37}H_{47}ClN_4O_7Si$ requires C, 61.44; H, 6.55; N, 7.75. fFund: C, 61.31; H, 6.66; N, 7.99.

*Di-tert-Butyl (2-(1-(Chloromethyl)-3-(5-(2-(diethylamino)ethoxy)-1H-indole-2-carbonyl)-5-nitro-2,3-dihydro-1H-benzo[e]indole-7-sulfonamido)ethyl)phosphate (**95a**).* Prepared by general method A from di-*tert*-butyl 2-(1-(chloromethyl)-5-nitro-2,3-dihydro-1*H*-benzo-[*e*]indole-7-sulfonamido)ethyl phosphate (**94**)[35] (387 mg, 0.67 mmol) and **64**·HCl (272 mg, 0.87 mmol) to give **95a** as a yellow−orange solid (521 mg, 93%): mp 225−227 °C (dec). ^{1}H NMR ($CDCl_3$) δ 9.36 (s, 2H), 9.00 (d, J = 1.5 Hz, 1H), 8.07 (dd, J = 8.9, 1.6 Hz, 1H), 7.98 (d, J = 8.9 Hz, 1H), 7.37 (d, J = 8.9 Hz, 1H), 7.14 (d, J = 2.2 Hz, 1H), 7.09−7.01 (m, 2H), 6.00 (br s, 1H), 4.90 (dd, J = 10.7, 2.2 Hz, 1H), 4.82 (t, J = 10.7 Hz, 1H), 4.40−4.31 (m, 1H), 4.27−4.15 (m, 2H), 4.12−4.02 (m, 2H), 3.95 (dd, J = 11.5, 3.4 Hz, 1H), 3.65 (dd, J = 11.5, 9.0 Hz, 1H), 3.32 (t, J = 4.6 Hz, 2H), 3.06 (br s, 2H), 2.92−2.74 (m, 4H), 1.47 (s, 9H), 1.46 (s, 9H), 1.17 (t, J = 7.1 Hz, 6H). Anal. $C_{38}H_{51}ClN_5O_{10}PS\cdot{}^1/_2H_2O$ requires C, 53.99; H, 6.20; N, 8.29. Found: C, 53.77; H, 6.07; N, 8.00.

*Di-tert-butyl (2-(1-(Chloromethyl)-3-(5-(3-(dimethylamino)-propoxy)-1H-indole-2-carbonyl)-5-nitro-2,3-dihydro-1H-benzo[e]-indole-7-sulfonamido)ethyl)phosphate (**95b**).* Prepared by general method A from **94** (179 mg, 0.31 mmol) and **67**·HCl (120 mg, 0.40 mmol). The crude product was triturated with acetone to give **95b** as a pale-yellow solid (157 mg, 62%): mp 195−198 °C. ^{1}H NMR (DMSO-d_6) δ 11.76 (d, J = 1.6 Hz, 1H), 9.30 (s, 1H), 8.87 (d, J = 1.7 Hz, 1H), 8.46 (d, J = 8.9 Hz, 1H), 8.20 (t, J = 5.7 Hz, 1H), 8.01 (dd, J = 8.9, 1.7 Hz, 1H), 7.41 (d, J = 8.9 Hz, 1H), 7.22 (d, J = 1.8 Hz, 1H), 7.16 (d, J = 2.3 Hz, 1H), 6.95 (dd, J = 8.9, 2.4 Hz, 1H), 5.01−4.93 (m, 1H), 4.73 (dd, J = 10.9, 2.3 Hz, 1H), 4.69−4.62 (m, 1H), 4.20−4.10 (m, 2H), 4.02 (t, J = 6.4 Hz, 2H), 3.83 (q, J = 6.3 Hz, 2H), 3.09−3.04 (m, 2H), 2.39 (t, J = 7.1 Hz, 2H), 2.16 (s, 6H), 1.92−1.85 (m, 2H), 1.36 (s, 18H). Anal. $C_{37}H_{49}ClN_5O_{10}PS$ requires C, 54.04; H, 6.01; N, 8.52. Found: C, 53.94; H, 5.84; N, 8.44.

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*Di-tert-butyl (2-(1-(Chloromethyl)-3-(5-((dimethylamino)methyl)-1H-indole-2-carbonyl)-5-nitro-2,3-dihydro-1H-benzo[e]indole-7-sulfonamido)ethyl)phosphate (**95c**).* Prepared by general method A from **94** (387 mg, 0.67 mmol) and **71**·HCl (222 mg, 0.87 mmol) to give **95c** as a yellow−orange solid (494 mg, 95%): mp 242−245 °C (dec). ^{1}H NMR (DMSO-d_6) δ 11.70 (s, 1H), 9.30 (s, 1H), 8.87 (br d, J = 1.6 Hz, 1H), 8.45 (d, J = 8.9 Hz, 1H), 8.15 (t, J = 5.8 Hz, 1H), 8.02 (dd, J = 8.9, 1.7 Hz, 1H), 7.60 (s, 1H), 7.46 (d, J = 8.5 Hz, 1H), 7.32−7.21 (m, 2H), 5.03−4.91 (m, 1H), 4.75−4.60 (m, 2H), 4.18−4.05 (m, 2H), 3.83 (q, J = 7.0 Hz, 2H), 3.53 (br s, 2H), 3.05 (q, J = 5.8 Hz, 2H), 2.21 (s, 6H), 1.36 (s, 9H), 1.35 (s, 9H). Anal. $C_{35}H_{45}ClN_5O_9PS$ requires C, 54.02; H, 5.83; N, 9.00. Found: C, 53.61; H, 5.90; N, 9.10.

*Di-tert-butyl 2-(1-(Chloromethyl)-3-(5-(dimethylamino)-1H-indole-2-carbonyl)-5-nitro-1,2-dihydro-3H-benzo[e]indole-7-sulfonamido)ethyl)phosphate (**95d**).* Prepared by general method A from **94** (289 mg, 0.50 mmol) and 5-(dimethylamino)-1*H*-indole-2-carboxylic acid hydrochloride (151 mg, 0.63 mmol) to give **95d** as a yellow−brown solid (371 mg, 97%): mp >300 °C. ^{1}H NMR (DMSO-d_6) δ 11.53 (br d, J = 1.5 Hz, 1H), 9.30 (s, 1H), 8.86 (br d, J = 1.6 Hz, 1H), 8.44 (d, J = 8.9 Hz, 1H), 8.14 (t, J = 5.8 Hz, 1H), 8.02 (dd, J = 8.9, 1.7 Hz, 1H), 7.38 (d, J = 9.0 Hz, 1H), 7.15 (br d, J = 1.7 Hz, 1H), 7.03 (dd, J = 9.0, 2.3 Hz, 1H), 6.92 (br d, J = 2.2 Hz, 1H), 4.96 (t, J = 10.2 Hz, 1H), 4.72 (dd, J = 10.9, 2.4 Hz, 1H), 4.70−4.58 (m, 1H), 4.21−4.07 (m, 2H), 3.83 (m, 2H), 3.06 (q, J = 5.8 Hz, 2H), 2.88 (s, 6H), 1.358 (s, 9H), 1.355 (s, 9H). Anal. $C_{34}H_{43}ClN_5O_9PS$ requires C, 53.44; H, 5.67; N, 9.16. Found: C, 53.32; H, 5.84; N, 9.11.

*Di-tert-butyl 2-(1-(Chloromethyl)-3-(5-(2-morpholinoethoxy)-1H-indole-2-carbonyl)-5-nitro-1,2-dihydro-3H-benzo[e]indole-7-sulfonamido)ethyl)phosphate (**95e**).* Prepared by general method A from **94** (182 mg, 0.31 mmol) and 5-(2-morpholinoethoxy)-1*H*-indole-2-carboxylic acid hydrochloride[42] (134 mg, 0.40 mmol). The crude product was triturated with acetone to give **95e** as a yellow solid (176 mg, 66%): mp 200−204 °C (dec). ^{1}H NMR (DMSO-d_6) δ 11.77 (d, J = 1.6 Hz, 1 H), 9.30 (s, 1H), 8.86 (d, J = 1.7 Hz, 1H), 8.45 (d, J = 8.9 Hz, 1H), 8.20 (t, J = 5.9 Hz, 1H), 8.00 (dd, J = 8.9, 1.7 Hz, 1H), 7.39 (d, J = 8.9 Hz, 1H), 7.21 (d, J = 1.7 Hz, 1H), 7.08 (d, J = 2.3 Hz, 1H), 6.95 (dd, J = 8.9, 2.4 Hz, 1H), 5.01−4.94 (m, 1H), 4.71 (dd, J = 10.9, 2.2 Hz, 1H), 4.69−4.63 (m, 1H), 4.20−4.09 (m, 4H), 3.83 (q, J = 6.3 Hz, 2H), 3.63−3.58 (m, 4H), 3.06 (q, J = 5.8 Hz, 2H), 2.73 (t, J = 5.7 Hz, 2H), ca. 2.52−2.46 (m, 4H, partially obscured by DMSO peak), 1.35 (s, 18H). Anal. $C_{38}H_{49}ClN_5O_{11}PS$ requires C, 53.68; H, 5.81; N, 8.24. Found: C, 53.59; H, 5.61; N, 8.19.

*Di-tert-butyl 2-(1-(Chloromethyl)-3-(5-(3-morpholinopropoxy)-1H-indole-2-carbonyl)-5-nitro-1,2-dihydro-3H-benzo[e]indole-7-sulfonamido)ethyl)phosphate (**95f**).* Prepared by general method A from **94** (176 mg, 0.30 mmol) and **74**·HCl (156 mg, 0.45 mmol). The crude product was triturated with acetone to give **95f** as a pale-yellow solid (175 mg, 66%): mp 200−203 °C (dec). ^{1}H NMR (DMSO-d_6) δ 11.76 (d, J = 2.0 Hz, 1H), 9.30 (s, 1H), 8.87 (d, J = 1.7 Hz, 1H), 8.46 (d, J = 8.9 Hz, 1H), 8.20 (t, J = 5.9 Hz, 1H), 8.02 (dd, J = 8.9, 1.7 Hz, 1H), 7.42 (d, J = 8.9 Hz, 1H), 7.23 (d, J = 1.7 Hz, 1H), 7.16 (d, J = 2.3 Hz, 1H), 6.95 (dd, J = 8.9, 2.4 Hz, 1H), 5.02−4.95 (m, 1H), 4.74 (dd, J = 10.9, 2.3 Hz, 1H), 4.70−4.63 (m, 1H), 4.20−4.11 (m, 2H), 4.03 (t, J = 6.3 Hz, 2H), 3.84 (q, J = 6.3 Hz, 2H), 3.62−3.56 (m, 4H), 3.05 (q, J = 5.8 Hz, 2H), 2.46 (t, J = 7.2 Hz, 2H), 2.41−2.36 (m, 4H), 1.35 (s, 18H). Anal. $C_{39}H_{51}ClN_5O_{11}PS$ requires C, 54.20; H, 5.95; N, 8.10. Found: C, 54.13; H, 5.91; N, 7.82.

*Di-tert-butyl 2-(1-(Chloromethyl)-3-(5-(2-(dimethylamino)-acetamido)-1H-indole-2-carbonyl)-5-nitro-1,2-dihydro-3H-benzo-[e]indole-7-sulfonamido)ethyl)phosphate (**95g**).* Prepared by general method A from **94** (258 mg, 0.45 mmol) and 5-(2-(dimethylamino)-acetamido)-1*H*-indole-2-carboxylic acid hydrochloride[42] (167 mg, 0.56 mmol) to give **95g** as a yellow−orange solid (355 mg, 97%): mp 235−238 °C (dec). ^{1}H NMR (DMSO-d_6) δ 11.80 (br d, J = 1.5 Hz, 1H), 9.63 (s, 1H), 8.87 (br d, J = 1.6 Hz, 1H), 8.46 (d, J = 8.9 Hz, 1H), 8.21−8.12 (m, 2H), 8.02 (dd, J = 8.9, 1.7 Hz, 1H), 7.44 (br d, J = 1.1 Hz, 2H), 7.30 (br d, J = 2.1 Hz, 1H), 4.98 (t, J = 10.8 Hz, 1H), 4.74 (dd, J = 11.0, 2.4 Hz, 1H), 4.73−4.62 (m, 1H), 4.22−4.11 (m, 2H), 3.83 (q, J = 6.3 Hz, 2H), 3.12−3.03 (m, 4H), 2.32 (s, 6H), 1.358 (s, 9 H), 1.355 (s, 9H). Anal. $C_{36}H_{46}ClN_6O_{10}PS\cdot{}^1/_2H_2O$ requires C, 52.08; H, 5.71; N, 10.12. Found: C, 51.87; H, 5.58; N, 10.08.

*Di-tert-butyl (2-(1-(Chloromethyl)-3-(5-(3-(dimethylamino)-propanamido)-1H-indole-2-carbonyl)-5-nitro-2,3-dihydro-1H-benzo[e]indole-7-sulfonamido)ethyl)phosphate (**95h**).* Prepared by general method A from **94** (387 mg, 0.67 mmol) and **77**·HCl (271 mg, 0.87 mmol) to give **95h** as a yellow−orange solid (507 mg, 91%): mp 237−240 °C (dec). ^{1}H NMR (DMSO-d_6) δ 11.79 (s, 1H), 9.96 (s, 1H), 9.30 (s, 1H), 8.87 (d, J = 1.6 Hz, 1H), 8.45 (d, J = 8.9 Hz, 1H), 8.15 (t, J = 5.6 Hz, 1H), 8.09 (br s, 1H), 8.02 (dd, J = 8.9, 1.7 Hz, 1H), 7.42 (d, J = 8.8 Hz, 1H), 7.35 (dd, J = 8.8, 1.8 Hz, 1H), 7.29 (d, J = 1.5 Hz, 1H), 5.02−4.92 (m, 1H), 4.76−4.61 (m, 2H), 4.19−4.10 (m, 2H), 3.83 (q, J = 7.0 Hz, 2H), 3.06 (q, J = 5.6 Hz, 2H), 2,59 (t, J = 7.0 Hz, 2H), 2,45 (t, J = 7.0 Hz, 2H), 2.21 (s, 6H), 1.36 (s, 9H), 1.35 (s, 9H). Anal. $C_{37}H_{48}ClN_6O_{10}PS$ requires C, 53.20; H, 5.79; N, 10.06. Found: C, 53.07; H, 5.82; N, 10.01.

*(E)-Di-tert-butyl 2-(1-(Chloromethyl)-3-(3-(4-(2-morpholinoethoxy)phenyl)acryloyl)-5-nitro-1,2-dihydro-3H-benzo-[e]indole-7-sulfonamido)ethyl)phosphate (**95i**).* Prepared by general method A from **94** (290 mg, 0.50 mmol) and **80**·HCl (2 batches of 198 mg, 0.60 mmol) to give **95i** as a yellow−orange solid (395 mg, 94%): mp 245−248 °C (dec). ^{1}H NMR ($CDCl_3$) δ 9.41 (br s, 1H), 8.97 (d, J = 1.6 Hz, 1H), 8.50 (dd, J = 8.9, 1.6 Hz, 1H), 7.96 (d, J = 8.9 Hz, 1H), 7.91 (d, J = 15.2 Hz, 1H), 7.58 (d, J = 8.7 Hz, 2H), 6.96 (d, J = 8.7 Hz, 2H), 6.74 (d, J = 15.2 Hz, 1H), 5.95 (t, J = 5.7 Hz, 1H), 4.63 (br dd, J = 10.8, 2.5 Hz, 1H), 4.56 (br t, J = 9.8 Hz, 1H), 4.34−4.26 (m, 1H), 4.18 (t, J = 5.6, 2H), 4.12−4.04 (m, 2H), 3.96 (br dd, J = 11.5, 3.2 Hz, 1H), 3.75 (br t, J = 4.6 Hz, 4H), 3.62 (br dd, J = 11.4, 9.9 Hz, 1H), 3.33 (br q, J = 5.4 Hz, 2H), 2.85 (br t, J = 5.6 Hz, 2H), 2.65−2.57 (m, 4H), 1.46 (s, 9H), 1.45 (s, 18H). Anal. $C_{38}H_{50}ClN_4O_{11}PS\cdot{}^1/_2H_2O$ requires C, 53.93; H, 6.07; N, 6.62. Found: C, 53.83; H, 5.99; N, 6.53.

*(E)-Di-tert-butyl 2-(1-(Chloromethyl)-3-(3-(4-(2-morpholinopropoxy)phenyl)acryloyl)-5-nitro-1,2-dihydro-3H-benzo[e]indole-7-sulfonamido)ethyl)phosphate (**95j**).* Prepared by general method A from **94** (183 mg, 0.32 mmol) and **82**·HCl (2 batches of 125 mg, 0.38 mmol) to give **95j** as a yellow−orange solid (236 mg, 87%): mp 230 °C (dec). ^{1}H NMR ($CDCl_3$) δ 9.41 (br s, 1H), 8.97 (br d, J = 1.6 Hz, 1H), 8.04 (dd, J = 8.9, 1.7 Hz, 1H), 7.94 (d, J = 8.9 Hz, 1H), 7.90 (d, J = 15.2 Hz, 1H), 7.58 (d, J = 8.8 Hz, 2H), 6.95 (d, J = 8.8 Hz, 2H), 6.73 (d, J = 15.2 Hz, 1H), 5.92 (t, J = 5.7 Hz, 1H), 4.62 (dd, J = 10.8, 2.5 Hz, 1H), 4.55 (br t, J = 9.7 Hz, 1H), 4.34−4.23 (m, 1H), 4.14−4.02 (m, 4H), 3.94 (br dd, J = 11.4, 3.3 Hz, 1H), 3.73 (br t, J = 4.6 Hz, 4H), 3.62 (dd, J = 11.4, 9.4 Hz, 1H), 3.32 (br q, J = 5.4 Hz, 2H), 2.59−2.42 (m, 6H), 2.06−1.93 (m, 2H), 1.46 (s, 9H), 1.45 (s, 9H). Anal. $C_{39}H_{52}ClN_4O_{11}PS$ requires C, 55.02; H, 6.16; N, 6.58. Found: C, 54.77; H, 6.32; N, 6.63.

*Di-tert-butyl 2-(1-(Chloromethyl)-3-(4-(2-(4-methylpiperazin-1-yl)ethoxy)benzoyl)-5-nitro-1,2-dihydro-3H-benzo[e]indole-7-sulfonamido]ethyl)phosphate (**95k**).* Prepared by general method A from **94** (1.16 g, 2.00 mmol) and **84**·HCl (2 batches of 0.87 g, 2.40 mmol) to give **95k** as a yellow−orange solid (1.58 g, 93%): mp 118−120 °C (dec). ^{1}H NMR ($CDCl_3$) δ 9.41 (br s, 1H), 8.98 (br d, J = 1.3 Hz, 1H), 8.04 (dd, J = 8.8, 1.4 Hz, 1H), 7.95 (d, J = 8.8 Hz, 1H), 7.90 (d, J = 15.2 Hz, 1H), 7.57 (d, J = 8.6 Hz, 2H), 6.94 (d, J = 8.6 Hz, 2H), 6.73 (d, J = 15.2 Hz, 1H), 5.96 (br s, 1H), 4.63 (dd, J = 10.7, 2.3 Hz, 1H), 4.55 (br t, J = 9.7 Hz, 1H), 4.32−4.23 (m, 1H), 4.17 (t, J = 5.8 Hz, 2H), 4.12−4.02 (m, 2H), 3.95 (dd, J = 11.4, 3.2 Hz, 1H), 3.62 (dd, J = 11.3, 9.5 Hz, 1H), 3.32 (br s, 2H), 2.85 (t, J = 5.8 Hz, 2H), 2.66 (br s, 4H), 2.52 (br s, 4H), 2.32 (s, 3H), 1.46 (s, 9H), 1.45 (s, 9H). Anal. $C_{39}H_{53}ClN_5O_{10}S\cdot{}^1/_2H_2O$ requires C, 54.51; H, 6.33; N, 8.15. Found: C, 54.52; H, 6.34; N, 7.91.

*Di-tert-butyl 2-(1-(Chloromethyl)-3-(4-(2-morpholinoethoxy)-benzoyl)-5-nitro-1,2-dihydro-3H-benzo[e]indole-7-sulfonamido)-ethyl)phosphate (**95l**).* A mixture of **86**·HCl (136 mg, 0.474 mmol), DMF (one drop), and $SOCl_2$ (3 mL) was stirred at reflux for 10 min. The mixture was cooled and evaporated to dryness. The residue was cooled in an ice bath, and **94** (248 mg, 0.430 mmol) was added, followed by a mixture of DMA (3 mL) and *i*-Pr_2NEt (0.082 mL, 0.474 mmol). The reaction mixture was stirred at 0 °C for 1 h. Aqueous $NaHCO_3$ (5%, 6 mL) was added, followed by water (6 mL), and the mixture was stirred at 0 °C for 10 min. The precipitated solid was filtered off, washed with cold water, and dried to give **95l** as a yellow−

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J. Med. Chem. XXXX, XXX, XXX−XXX

orange solid (326 mg, 94%): mp 113−115 °C. ^{1}H NMR ($CDCl_3$) δ 8.99 (br d, J = 1.5 Hz, 1H), 8.75 (br s, 1H), 8.05 (dd, J = 8.9, 1.7 Hz, 1H), 7.95 (d, J = 8.9 Hz, 1H), 7.64 (d, J = 8.8 Hz, 2H), 7.02 (d, J = 8.8 Hz, 2H), 5.95 (t, J = 5.7 Hz, 1H), 4.54−4.41 (m, 2H), 4.23 (t, J = 5.4 Hz, 2H), 4.21−4.12 (m, 1H), 4.11−4.04 (m, 2H), 3.88 (dd, J = 11.5, 3.4 Hz, 1H), 3.83−3.72 (m, 4H), 3.66 (dd, J = 11.5, 8.3 Hz, 1H), 3.33 (br q, J = 5.6 Hz, 2H), 2.93−2.82 (m, 2H), 2.63 (br s, 4H), 1.47 (s, 9H), 1.46 (s, 9H). Anal. $C_{36}H_{48}ClN_4O_{11}PS\cdot{}^1/_3H_2O$ requires C, 52.91; H, 6.00; N, 6.86. Found: C, 52.89; H, 6.06; N, 6.83.

*Di-tert-butyl 2-(1-(Chloromethyl)-3-(3-morpholinopropanoyl)-5-nitro-1,2-dihydro-3H-benzo[e]indole-7-sulfonamido)ethyl)-phosphate (**95m**).* Prepared by general method A from **94** (194 mg, 0.34 mmol) and 3-morpholinopropanoic acid hydrochloride (85 mg, 0.44 mmol) to give **95m** as a yellow−orange solid (219 mg, 91%): mp 107−110 °C. ^{1}H NMR ($CDCl_3$) δ 9.31 (br s, 1H), 8.97 (br d, J = 1.5 Hz, 1H), 8.03 (dd, J = 8.9, 1.7 Hz, 1H), 7.93 (d, J = 8.9 Hz, 1H), 5.95 (br t, J = 5.3 Hz, 1H), 4.50−4.36 (m, 2H), 4.30−4.20 (m, 1H), 4.10−4.02 (m, 2H), 3.92 (dd, J = 11.5, 3.3 Hz, 1H), 3.74 (t, J = 4.6 Hz, 4H), 3.62 (dd, J = 11.4, 8.9 Hz, 1H), 3.32 (br q, J = 5.1 Hz, 2H), 2.94−2.66 (m, 4H), 2.57 (br t, J = 4.2 Hz, 4H), 1.46 (s, 9H), 1.45 (s, 9H). Anal. $C_{30}H_{44}ClN_4O_{10}PS\cdot{}^1/_2H_2O$ requires C, 49.48; H, 6.23; N, 7.69. Found: C, 49.22; H, 6.28; N, 7.50.

*Di-tert-butyl 2-(1-(Chloromethyl)-3-(5-(2-morpholinoethoxy)-1H-indole-2-carbonyl)-5-nitro-1,2-dihydro-3H-benzo[e]indole-7-carboxamido)ethyl)phosphate (**97e**).* Prepared by general method A from **96** (143 mg, 0.26 mmol) and 5-(2-morpholinoethoxy)-1*H*-indole-2-carboxylic acid hydrochloride[42] (104 mg, 0.32 mmol) to give **97e** as a yellow−orange solid (213 mg, 99%): mp 130−132 °C. ^{1}H NMR ($CDCl_3$) δ 9.34 (br s, 1H), 9.24 (s, 1H), 8.93 (br d, J = 1.3 Hz, 1H), 8.20 (dd, J = 8.8, 1.6 Hz, 1H), 8.06 (t, J = 4.9 Hz, 1H), 7.92 (d, J = 8.8 Hz, 1H), 7.38 (d, J = 8.9 Hz, 1H), 7.14 (br d, J = 2.3 Hz, 1H), 7.10−6.99 (m, 2H), 4.91 (dd, J = 10.8, 2.1 Hz, 1H), 4.80 (br t, J = 9.7 Hz, 1H), 4.38−4.13 (m, 5H), 3.98 (dd, J = 11.5, 3.3 Hz, 1H), 3.86−3.72 (m, 6H), 3.61 (dd, J = 11.4, 9.5 Hz, 1H), 2.87 (t, J = 5.6 Hz, 2H), 2.70−2.58 (m, 4H), 1.503 (s, 9H), 1.502 (s, 9H). Anal. $C_{39}H_{49}ClN_5O_{10}P\cdot{}^1/_3H_2O$ requires C, 57.11; H, 6.10; N, 8.54. Found: C, 57.02; H, 6.06; N, 8.44.

*(E)-Di-tert-butyl 2-(1-(Chloromethyl)-3-(3-(4-(2-morpholinoethoxy)phenyl)acryloyl)-5-nitro-1,2-dihydro-3H-benzo-[e]indole-7-carboxamido)ethyl)phosphate (**97i**).* Prepared by general method A from **96** (176 mg, 0.33 mmol) and **80**·HCl (2 batches of 123 mg, 0.39 mmol) to give **97i** as a yellow−orange solid (211 mg, 81%): mp 128−131 °C. ^{1}H NMR ($CDCl_3$) δ 9.30 (br s, 1H), 8.90 (br d, J = 1.4 Hz, 1H), 8.17 (dd, J = 8.7, 1.4 Hz, 1H), 8.01 (t, J = 4.6 Hz, 1H), 7.94−7.86 (m, 2H), 7.58 (d, J = 8.7 Hz, 2H), 6.96 (d, J = 8.7 Hz, 2H), 6.76 (d, J = 15.2 Hz, 1H), 4.62 (dd, J = 10.8, 2.1 Hz, 1H), 4.52 (br t, J = 9.7 Hz, 1H), 4.32−4.20 (m, 3H), 4.17 (t, J = 5.7 Hz, 2H), 3.96 (dd, J = 11.4, 3.1 Hz, 1H), 3.81 (q, J = 4.6 Hz, 2H), 3.74 (br t, J = 4.6 Hz, 4H), 3.59 (br t, J = 10.6 Hz, 1H), 2.84 (t, J = 5.7 Hz, 2H), 2.60 (br t, J = 4.5 Hz, 4H), 1.50 (s, 18H). Anal. $C_{39}H_{50}ClN_4O_{10}P\cdot{}^1/_2H_2O$ requires C, 57.81; H, 6.34; N, 6.91. Found: C, 57.51; H, 6.10; N, 6.95.

Calculation of pK_a. Approximated apparent pK_as were calculated for nitroCBIs using ACD/Laboratories software ACD/pK_a v12.01.[43]

In Vitro Cytotoxicity. The sources of the cell lines used in this study are as previously described.[37] All lines were confirmed to be *Mycoplasma*-free. Inhibition of proliferation of log-phase monolayers was assessed in 96-well plates as previously described.[59] The drug exposure time was 4 h under aerobic or hypoxic conditions (5% CO_2 in air or nitrogen, respectively), 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 untreated controls on the same plate.

Animal Experiments. All animal studies were approved by the University of Auckland Animal Ethics Committee (approval numbers R256 and CR590) and were conducted using CD1-Foxn1$^{nu/nu}$ (nude) mice.

Antitumor Activity by Excision Assay. Excision assays were performed as previously described.[34,35] Tumors were grown in the flank of male mice by sc inoculation of cells from tissue culture (10^7 for SiHa and H460, 5×10^6 for H1299, HCT116, and 22Rv1), and mice were randomized to treatment groups when tumors reached a mean diameter of 8−10 mm. In each experiment, mice received vehicle alone (PBS, n = 3), nitroCBI alone (dissolved in PBS containing 2−4 equiv of $NaHCO_3$ and administered iv through the lateral tail vein, n = 3), radiation alone (15 Gy, whole body cobalt-60 gamma irradiation, n = 5), or radiation followed 5 min later by nitroCBI (n = 5).

Antitumor Activity by Growth Delay Assay. Growth delay assays were performed as previously described.[49] Tumors were grown in the flank of female mice by sc inoculation of cells from tissue culture (as above), and mice were randomized to treatment groups when tumors reached treatment size (mean volume of 250, 160, and 200 mm^3 for SiHa, A2780, and 22Rv1 tumors, respectively). Gemcitabine was dissolved in saline (0.9% NaCl) solution and docetaxel in polysorbate 80 (40 mg/mL) then diluted 14-fold with 13% (w/w) ethanol in water, and each was administered ip. NitroCBIs **9** and **50** were dissolved in PBS containing 2−4 equiv of $NaHCO_3$ and administered iv through the lateral tail vein. Doses are reported in μmol/kg, conversion to mg/kg is as follows: gemcitabine 100, 133, 237 μmol/kg (26, 35, 62 mg/kg); docetaxel 32 μmol/kg (26 mg/kg); **9** 42, 56 μmol/kg (34, 45 mg/kg); **50** 14, 15 μmol/kg (12, 13 mg/kg). Tumor volume was calculated as $\pi(L \times w^2)/6$, where L is the major axis and w is the perpendicular minor axis. Animals were culled 100−120 days after start of treatment or when the mean tumor diameter exceeded 15 mm. Acute deaths were noted in experiments A and B (see Figure 5) as follows: (A) **9** at 42 μmol/kg (one mouse after third iv dose), **9** at 56 μmol/kg (one mouse after fourth iv dose), gemcitabine plus **9** at 56 μmol/kg (one mouse after first iv dose and 1 mouse after fourth iv dose); (B) gemcitabine plus **9** (one mouse after fourth iv dose). These animals were replaced to maintain group sizes and were excluded from the growth delay analyses. The biological end point used in the Kaplan−Meier analyses (Supporting Information, Figure S4) was the time required for the tumors to quadruple their volume relative to initial volume at the start of the treatment (i.e., RTV4). Statistically significant differences for pairwise comparisons were determined using Log-Rank test with SigmaPlot v13 (Systat Software, Inc.) (Supporting Information, Table S8).

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.jmedchem.7b00563.

Information on purity of final products, tabulated IC_{50} data for **10**−**28** and **29**−**44** in HT29 and SiHa, tabulated data for all excision assays shown in Figures 3 and 4, and Kaplan−Meier analyses and body weight data for all tumor growth delay experiments shown in Figure 5 (PDF)

Molecular formula strings (CSV)

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Notes

The authors declare no competing financial interest.

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J. Med. Chem. XXXX, XXX, XXX−XXX

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■ ABBREVIATIONS USED

ADC, antibody-drug conjugate; CBI, 1,2,9,9a-tetrahydrocyclopropa[*c*]benz[*e*]indol-4-one; DEI, 5-(2-dimethylaminoethoxy)indole; EDCI, 1-ethyl-3-(3-(dimethylamino)propyl)carbodiimide hydrochloride; HAP, hypoxia-activated prodrug; HCR, hypoxic cytotoxicity ratio; RTV, relative tumor volume; TMI, 5,6,7-trimethoxyindole

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J. Med. Chem. XXXX, XXX, XXX−XXX