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# Hypoxia-Activated Prodrugs: Substituent Effects on the Properties of Nitro *seco*-1,2,9,9a-Tetrahydrocyclopropa[*c*]benz[*e*]indol-4-one (nitroCBI) Prodrugs of DNA Minor Groove Alkylating Agents

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Nitrochloromethylbenzindolines (nitroCBIs) are a new class of hypoxia-activated prodrugs for antitumor therapy. The recently reported prototypes undergo hypoxia-selective metabolism to form potent DNA minor groove alkylating agents and are selectively toxic to some but not all hypoxic tumor cell lines. Here we report a series of 31 analogues that bear an extra electron-withdrawing substituent that serves to raise the one-electron reduction potential of the nitroCBI. We identify a subset of compounds, those with a basic side chain and sulfonamide or carboxamide substituent, that have consistently high hypoxic selectivity. The best of these, with a 7-sulfonamide substituent, displays hypoxic cytotoxicity ratios of 275 and 330 in Skov3 and HT29 human tumor cell lines, respectively. This compound (**28**) is efficiently and selectively metabolized to the corresponding aminoCBI, is selectively cytotoxic under hypoxia in all 11 cell lines examined, and demonstrates activity against hypoxic tumor cells in a human tumor xenograft in vivo.

## Introduction

The disorganized vasculature of solid tumors frequently gives rise to regions of chronic or acute hypoxia. Cells within these regions show resistance to radiotherapy and some forms of chemotherapy and experience selective pressures that promote a more malignant phenotype.[1,2] One possible approach to treating hypoxic cells involves the development of hypoxia-activated prodrugs (HAPs[a]). These are relatively nontoxic compounds that are activated by metabolic reduction in the absence of oxygen. Since severe hypoxia is rarely found in normal tissues, HAPs represent an avenue to target the most refractory cells of solid tumors in a selective fashion.[3]

Successful exploitation of the HAP concept requires careful prodrug design.[4,5] Important considerations include (i) the toxicity differential between prodrug and its reduction product(s), (ii) the selectivity for activation by one-electron reductases rather than two-electron (oxygen insensitive) reductases,[6] (iii) tissue penetration properties that allow the prodrug to reach hypoxic cells distant from the vasculature,[7] and (iv) whether the reduction product is capable of diffusing from the site of activation to kill surrounding cells. This last property (the bystander effect) may allow for effective killing of hypoxic cells without requiring metabolic activation in every one.[8] Examples of HAPs in recent or current clinical trial include the *N*-oxides tirapazamine[9,10] (**1**, Figure 1) and banoxantrone[11,12] and the nitro compounds **2** (PR-104[13]) and (1-methyl-2-nitro-1*H*-imidazol-5-yl)methyl *N*,*N*-bis(2-bromoethyl)phosphordiamidate (TH-302[14]), the latter of which are both prodrugs of nitrogen mustard DNA cross-linking agents.

We have investigated a class of DNA monoalkylating agents as possible "effectors" for various tumor selective prodrug strategies. These compounds are related to a family of antitumor antibiotics (including duocarmycin SA and yatakemycin) that alkylate in a sequence-selective manner at the N3 position of adenine in the minor groove of DNA and are exceptionally potent cytotoxins.[15,16] Four analogues of the natural products were evaluated clinically on the basis of excellent antitumor activity in preclinical models,[17,18] but all proved to be myelotoxic in patients and lacking in antitumor activity at the low doses that could be administered systemically.[19,20] Such observations have prompted efforts to target similar compounds to tumor tissue, for example, by the use of antibody−drug conjugates[21] or the formation of prodrugs such as glycosides[22] or metal complexes.[23]

We reasoned that amino analogues of the alkylating subunit of these compounds (see, for example, structure **5** in Figure 2) would undergo spirocyclization and DNA alkylation in a similar fashion to the natural products, which carry a phenol at this position, and further that such amino compounds would be amenable to the formation of a range of new prodrugs. We have reported the syntheses of three types of amino alkylating agents (CI,[24] CBI,[25] and DSA[26]) along with their DNA alkylation properties[27,28] and cytotoxicity data. The last shows that when the amino substituent is replaced by an electron withdrawing group (a carbamate[29] or nitro group[25,26,30] for instance), the cytotoxicity is dramatically reduced, presumably because the compounds can no longer undergo spirocyclization and DNA alkylation. We recently reported two particular

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[a] Abbreviations: CBI, chloromethylbenzindoline; DEI, 5-[(dimethylamino)ethoxy]indole; EDCI, *N*-ethyl-*N*′-(3-dimethylaminopropyl)carbodiimide hydrochloride; EWG, electron-withdrawing group; HAP, hypoxia-activated prodrug; HCR, hypoxic cytotoxicity ratio; TMI, 5,6,7-trimethoxyindole.

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examples of nitroCBI **4** that show promise as HAPs:[31] **6** (Table 1), bearing the neutral 5,6,7-trimethoxyindole (TMI) side chain as found in duocarmycin SA, and **19** (Table 2) with a basic 5-[(dimethylamino)ethoxy]indole (DEI) side chain. **6** was found to be up to 300-fold more cytotoxic to cultured tumor cells under hypoxic compared to aerobic conditions, while **19** was found to generate the corresponding aminoCBI (**45**, Table 3) selectively in hypoxic cell culture. **45** was demonstrated to be a diffusible metabolite capable of bystander killing of surrounding cells in multicellular layer cultures. In addition **19** proved to be considerably less toxic to mice than **45** by ip administration and provided selective killing of hypoxic cells in murine RIF-1 tumors in vivo.[31]

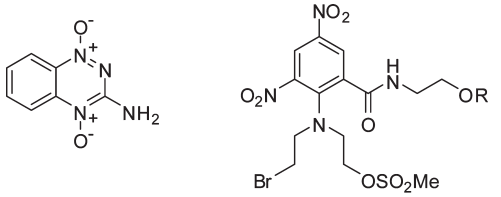



**Figure 1.** Structures of some known HAPs.

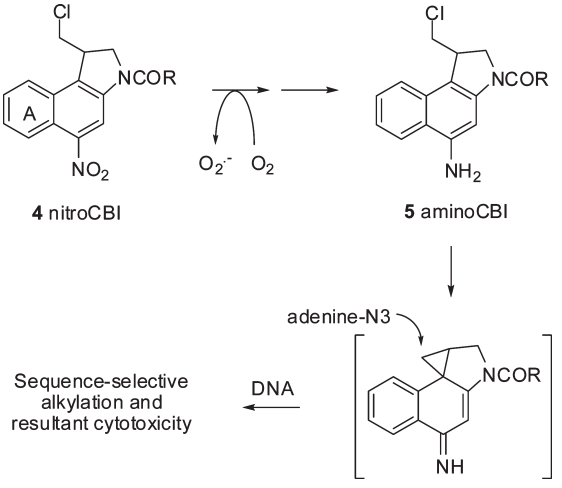
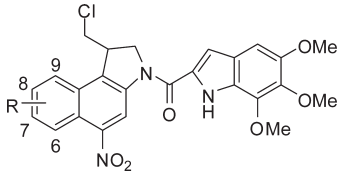



**Figure 2.** Proposed mechanism of action of nitroCBI hypoxia-activated prodrugs. If **4** is reduced to **5** via an initial one-electron step, this process can be reversed in the presence of oxygen. R is a side chain that can bind in the minor groove of DNA.

Some shortcomings in the properties of **6** and **19** as HAPs were also noted.[31] The hypoxic cytotoxicity ratio (HCR) was only high in some cell lines and was generally weaker when the TMI side chain of **6** was replaced by the somewhat more soluble DEI side chain of **19** (to the point where the HCR for **19** in HT29 cells was only 2.5 as measured by clonogenic assay). In addition, significant activity in vivo was only achieved at or above the maximally tolerated dose. One limitation may be that the reduction potential of **4** is too low for efficient bioreduction, which could contribute to the slow rates of cellular metabolism observed for **19** and explain why **6**, although clearly reduced under hypoxia, fails to become as cytotoxic as the corresponding aminoCBI (**39**, Table 3) under the cell culture conditions used. Here we describe the synthesis of a number of new analogues of **4** where the A-ring bears an extra electron-withdrawing group (EWG) at any one of the available positions and the effect of this substituent on the reduction potential and cytotoxicity of the compounds under oxic and hypoxic conditions. We identify a small group of A-ring substituents that confer consistently high hypoxic selectivity across a panel of human tumor cell lines and a particular compound with activity against hypoxic cells in SiHa human tumor xenografts.

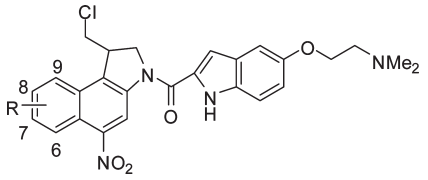
**Table 1.** Structure and in Vitro Cytotoxicity Data for NitroCBIs Bearing a TMI (5,6,7-Trimethoxyindole) Side Chain



| | | | Skov3 | | | HT29 | | |
|---|---|---|---|---|---|---|---|---|
| | | | $IC_{50}$ ($\mu$M)[a] | | | $IC_{50}$ ($\mu$M)[a] | | |
| compd | R | $\sigma_p$[b] | oxic | hypoxic | HCR[c] | oxic | hypoxic | HCR[c] |
| **6** | H | 0.00 | 4.2 ± 1.3 | 1.7 ± 0.2 | 2.8 ± 0.9 | 3.4 ± 0.4 | 1.6 ± 0.7 | 2.5 ± 0.7 |
| **7** | 6-$NO_2$ | | > 20[d] | > 20[d] | | > 20[d] | > 20[d] | |
| **8** | 6-COMe | | 0.50 ± 0.07 | 0.14 ± 0.05 | 3.4 ± 0.7 | 0.68 ± 0.11 | 0.33 ± 0.01 | 2.0 ± 0.6 |
| **9** | 7-$NO_2$ | 0.78 | 16 ± 1 | 0.24 ± 0.02 | 70 ± 8 | 14 ± 2 | 1.3 ± 0.1 | 12 ± 2 |
| **10** | 7-$SO_2$Me | 0.72 | 17 ± 1 | 9.3 ± 1.6 | 1.9 ± 0.4 | 14 ± 1 | 7.2 ± 1.7 | 2.0 ± 0.3 |
| **11** | 7-CN | 0.66 | 6.4 ± 2.5 | 1.0 ± 0.4 | 13 ± 9 | 5.3 ± 1.4 | 2.4 ± 0.5 | 2.5 ± 0.6 |
| **12** | 7-$SO_2NH_2$ | 0.57 | 37 ± 3 | 3.7 ± 0.4 | 10 ± 1 | 43 ± 5 | 12 ± 1 | 3.6 ± 0.2 |
| **13** | 7-COMe | 0.50 | 5.1 ± 1.9 | 0.44 ± 0.15 | 19 ± 8 | 4.1 ± 1.4 | 0.93 ± 0.21 | 5.2 ± 2.1 |
| **14** | 7-$CO_2$Me | 0.45 | 1.1 ± 0.3 | 0.51 ± 0.20 | 4.0 ± 2.8 | 1.9 ± 0.5 | 0.81 ± 0.27 | 3.2 ± 1.8 |
| **15** | 7-$CONH_2$ | 0.36 | 5.8 ± 1.0 | 1.6 ± 0.5 | 6.1 ± 0.4 | 7.9 ± 0.7 | 3.6 ± 0.7 | 2.8 ± 0.4 |
| **16** | 8-$SO_2$Me | | 11 ± 5 | 3.9 ± 1.1 | 4.2 ± 2.3 | 14 ± 5 | 7.2 ± 1.6 | 2.5 ± 1.4 |
| **17** | 8-$SO_2NH_2$ | | 12 ± 2 | 4.9 ± 0.6 | 2.6 ± 0.6 | 24 ± 2 | 14 ± 2 | 1.9 ± 0.1 |
| **18** | 9-$NO_2$ | | 55 ± 3 | > 50[d] | < 1.1 | 34 ± 7 | 33 ± 10 | 1.2 ± 0.1 |

[a] Drug concentration to reduce cell density to 50% of that of the controls following 4 h of exposure. Values are the mean ± SEM for two to nine experiments. [b] $\sigma_p$ from ref 37. [c] Hypoxic cytotoxicity ratio = $IC_{50}$(oxic)/$IC_{50}$(hypoxic). Values are the mean of intraexperiment ratios ± SEM for two to seven experiments. [d] $IC_{50}$ greater than solubility limit.

**Table 2.** Structure and in Vitro Cytotoxicity Data for NitroCBIs Bearing a DEI [(5-Dimethylaminoethoxy)indole] Side Chain




| | | | Skov3 | | | HT29 | | |
|---|---|---|---|---|---|---|---|---|
| | | | $IC_{50}$ ($\mu$M)[a] | | | $IC_{50}$ ($\mu$M)[a] | | |
| compd | R | $E(1)$ (mV)[b] | oxic | hypoxic | HCR[c] | oxic | hypoxic | HCR[c] |
| **19** | H | −512 ± 8 | 1.4 ± 0.5 | 0.87 ± 0.12 | 1.9 ± 0.6 | 0.47 ± 0.05 | 0.48 ± 0.11 | 1.1 ± 0.1 |
| **20** | 6-$NO_2$ | −467 ± 8 | 4.1 ± 1.1 | 1.4 ± 0.5 | 2.9 ± 0.1 | 2.8 ± 0.3 | 1.3 ± 0.1 | 2.2 ± 0.2 |
| **21** | 6-CN | −491 ± 8 | 0.17 ± 0.01 | 0.10 ± 0.01 | 1.7 ± 0.1 | 0.12 ± 0.02 | 0.10 ± 0.01 | 1.1 ± 0.3 |
| **22** | 6-$SO_2NH_2$ | −497 ± 7 | 3.7 ± 0.7 | 0.80 ± 0.04 | 4.6 ± 0.6 | 2.7 ± 0.1 | 1.2 ± 0.2 | 2.3 ± 0.4 |
| **23** | 6-COMe | −489[d] | 0.64 ± 0.11 | 0.052 ± 0.016 | 12 ± 2 | 0.20 ± 0.03 | 0.035 ± 0.004 | 5.2 ± 0.7 |
| **24** | 6-$CONH_2$ | −481 ± 8 | 50 ± 2 | 3.2 ± 0.9 | 18 ± 5 | 23 ± 3 | 1.5 ± 1.0 | 43 ± 5 |
| **25** | 7-$NO_2$ | −353[d] | 1.1 ± 0.1 | 0.12 ± 0.01 | 9.3 ± 0.6 | 0.89 ± 0.06 | 0.093 ± 0.035 | 9.6 ± 0.7 |
| **26** | 7-$SO_2$Me | −362 ± 8 | 1.3 ± 0.1 | 0.12 ± 0.03 | 11 ± 3 | 0.97 ± 0.13 | 0.27 ± 0.07 | 4.0 ± 1.1 |
| **27** | 7-CN | −385 ± 8 | 0.81 ± 0.18 | 0.19 ± 0.08 | 4.2 ± 1.3 | 0.70 ± 0.16 | 0.43 ± 0.08 | 1.7 ± 0.4 |
| **28** | 7-$SO_2NH_2$ | −390 ± 9 | 6.9 ± 1.5 | 0.028 ± 0.002 | 275 ± 57 | 4.6 ± 0.6 | 0.018 ± 0.004 | 330 ± 110 |
| **29** | 7-COMe | −409[d] | 0.64 ± 0.25 | 0.20 ± 0.05 | 4.0 ± 2.5 | 0.17 ± 0.04 | 0.12 ± 0.03 | 1.5 ± 0.5 |
| **30** | 7-$CO_2$Me | −429 ± 8 | 0.44 ± 0.18 | 0.22 ± 0.07 | 1.9 ± 0.7 | 0.32 ± 0.15 | 0.26 ± 0.04 | 1.2 ± 0.4 |
| **31** | 7-$CONH_2$ | −422 ± 10 | 3.9 ± 0.5 | 0.047 ± 0.007 | 77 ± 10 | 4.1 ± 0.5 | 0.14 ± 0.06 | 60 ± 25 |
| **32** | 8-$SO_2$Me | −420 ± 8 | 1.9 ± 0.5 | 0.68 ± 0.17 | 2.7 ± 0.1 | 0.98 ± 0.02 | 0.88 ± 0.20 | 1.2 ± 0.3 |
| **33** | 8-CN | −419 ± 8 | 1.2 ± 0.3 | 0.88 ± 0.34 | 1.5 ± 0.2 | 1.4 ± 0.3 | 1.2 ± 0.5 | 1.4 ± 0.3 |
| **34** | 8-$SO_2NH_2$ | −456 ± 8 | 4.9 ± 0.4 | 0.25 ± 0.03 | 21 ± 4 | 9.0 ± 1.7 | 0.36 ± 0.07 | 26 ± 6 |
| **35** | 8-COMe | −446[d] | 0.20 ± 0.04 | 0.15 ± 0.01 | 1.3 ± 0.3 | 0.15 ± 0.04 | 0.14 ± 0.06 | 1.1 ± 0.2 |
| **36** | 8-$CO_2$Me | −452[d] | 0.52 ± 0.05 | 0.21 ± 0.03 | 2.4 ± 0.1 | 0.55 ± 0.09 | 0.36 ± 0.10 | 1.6 ± 0.2 |
| **37** | 8-$CONH_2$ | −447 ± 9 | 3.4 ± 0.6 | 0.12 ± 0.03 | 34 ± 11 | 2.8 ± 0.2 | 0.20 ± 0.07 | 22 ± 10 |
| **38** | 9-$NO_2$ | −477 ± 8 | 1.2 ± 0.1 | 0.84 ± 0.03 | 1.5 ± 0.1 | 1.4 ± 0.3 | 1.1 ± 0.2 | 1.3 ± 0.3 |

[a] Drug concentration to reduce cell density to 50% of that of the controls following 4 h of exposure. Values are the mean ± SEM for two to ten experiments. [b] One-electron reduction potential measured by pulse radiolysis. [c] Hypoxic cytotoxicity ratio = $IC_{50}$(oxic)/$IC_{50}$(hypoxic). Values are the mean of intraexperiment ratios ± SEM for two to six experiments. [d] Calculated from linear regression analysis.

## Results

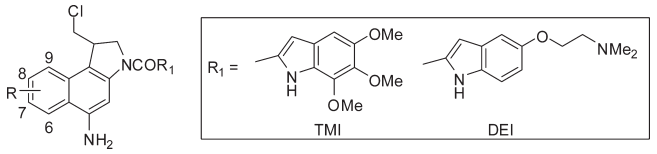
**Chemistry.** The new nitroCBIs prepared in this paper are listed in Tables 1 and 2, and the general route for their preparation is shown in Scheme 1. In order to obtain the correct substitution pattern, our previous synthesis of nitroCBIs (unsubstituted in the A-ring) introduced the nitro group early, followed by a multistep sequence involving malonate displacement and Curtius rearrangement to construct the indoline ring.[25] However, when the A-ring bears an EWG, as shown in Scheme 1, this directs nitration predominantly to the desired 5-position of intermediate **59** or **60**. Late introduction of the nitro group allows the use of previously described radical chemistry to construct the indoline ring.[32] The four-step sequence shown in Scheme 1 (from **53** to **57**), involving a modified Curtius rearrangement, bromination, chloroallylation, and radical ring closure, proved to be consistently high yielding and reliable and was used in the synthesis of all the nitroCBIs described here.

Suitable substituted 2-naphthoic acids employed as starting materials in Scheme 1 were known (6-cyano, 6-methoxycarbonyl)[33] or prepared as shown in Scheme 2 (6-methylsulfonyl, 7-methylsulfonyl, 7-cyano, 7-dibenzylaminosulfonyl, 7-acetyl, and 7-methoxycarbonyl).

An alternative route to nitration substrate **57** or **58** employed electrophilic aromatic substitution of the benzindoline **89** (Scheme 3), itself prepared by the same radical ring closure method from *tert*-butyl 2-naphthylcarbamate (**85**). The bromination procedure at the beginning of this sequence, which provides **86** in one step and 90% yield, is considerably superior to that previously reported (four steps and 49% overall yield).[34,35] Nitration of **89** yielded the 7-nitro and 9-nitro analogues **90** and **91** (note that with an unsubstituted A-ring only 3% of the 5-nitro isomer was produced in this reaction), while acetylation and chlorosulfonylation provided the separable 6- and 7-substituted products **92**−**95**. The isomer distribution in the acetylation reaction was quite sensitive to the conditions, with reaction in $CS_2$ at 70 °C giving the 6-acetyl isomer as the major product, while reaction in nitrobenzene at room temperature favored the 7-acetyl isomer. The position of the substituents was determined on the basis of 2D NMR experiments (details are given in the Supporting Information). The sulfonyl chloride **95** was further converted to the 6-cyano and 6-amide nitration substrates **97** and **98**.

Nitration reactions were either carried out on the trifluoroacetyl- (**58**) or Boc-protected indoline (**57**) (with the latter undergoing deprotection under the acidic nitration conditions) as shown in Scheme 1. The reactions were generally conducted using concentrated $H_2SO_4$ with a slight excess of $KNO_3$ or, for some of the less reactive 6-substituted examples, using fuming $HNO_3$. Where the substituent was 8-$SO_2NBn_2$, pretreatment with concentrated $H_2SO_4$ was used to deprotect the dibenzylsulfonamide to give the primary sulfonamide nitration substrate. In almost all cases the 5-$NO_2$ isomer was the major product, with yields generally

**Table 3.** Structure and in Vitro Cytotoxicity Data for AminoCBIs




| | | | Skov3 | | | HT29 | | |
|---|---|---|---|---|---|---|---|---|
| | | | $IC_{50}$ (nM)[a] | | | $IC_{50}$ (nM)[a] | | |
| compd | R | $R_1$ | oxic | hypoxic | HCR[b] | oxic | hypoxic | HCR[b] |
| **39** | H | TMI | 1.3 ± 0.1 | 1.7 ± 0.2 | 1.0 ± 0.1 | 0.86 ± 0.31 | 2.5 ± 0.3 | 0.76 ± 0.07 |
| **40** | 7-$SO_2Me$ | TMI | 32 ± 6 | 25 ± 1 | 1.2 ± 0.2 | 32 ± 3 | 36 ± 5 | 0.86 ± 0.14 |
| **41** | 7-CN | TMI | 10 ± 1 | 9.0 ± 1.9 | 1.3 ± 0.2 | 15 ± 2 | 11 ± 4 | 1.9 ± 0.7 |
| **42** | 7-$SO_2NH_2$ | TMI | 41 ± 5 | 33 ± 6 | 1.3 ± 0.1 | 86 ± 18 | 91 ± 26 | 1.1 ± 0.5 |
| **43** | 7-$CONH_2$ | TMI | 34 ± 1 | 27 ± 5 | 1.2 ± 0.2 | 59 ± 7 | 28 ± 11 | 2.3 ± 0.7 |
| **44** | 8-$SO_2Me$ | TMI | 19 ± 1 | 14 ± 2 | 1.4 ± 0.5 | 25 ± 2 | 28 ± 5 | 1.0 ± 0.2 |
| **45** | H | DEI | 6.8 ± 0.6 | 6.6 ± 0.6 | 0.97 ± 0.05 | 5.0 ± 0.6 | 3.5 ± 0.8 | 1.3 ± 0.1 |
| **46** | 7-$SO_2Me$ | DEI | 14 ± 4 | 8.2 ± 0.1 | 1.7 ± 0.5 | 11 ± 1 | 7.3 ± 2.0 | 1.6 ± 0.4 |
| **47** | 7-CN | DEI | 13 ± 4 | 12 ± 2 | 1.1 ± 0.2 | 17 ± 1 | 8.6 ± 2.0 | 2.0 ± 0.4 |
| **48** | 7-$SO_2NH_2$ | DEI | 23 ± 2 | 16 ± 1 | 1.4 ± 0.1 | 35 ± 6 | 23 ± 11 | 1.8 ± 0.6 |
| **49** | 7-COMe | DEI | 11 ± 2 | 8.2 ± 2.7 | 1.4 ± 0.2 | 7.6 ± 0.7 | 5.6 ± 1.7 | 1.5 ± 0.3 |
| **50** | 7-$CO_2Me$ | DEI | 7.7 ± 2.0 | 8.3 ± 0.3 | 0.91 ± 0.21 | 8.6 ± 0.8 | 5.7 ± 1.8 | 1.7 ± 0.7 |
| **51** | 7-$CONH_2$ | DEI | 13 ± 1 | 13 ± 1 | 1.0 ± 0.1 | 61 ± 13 | 48 ± 15 | 1.4 ± 0.3 |
| **52** | 8-$SO_2Me$ | DEI | 18 ± 4 | 12 ± 4 | 1.5 ± 0.2 | 29 ± 1 | 16 ± 1 | 1.9 ± 0.2 |

[a] Drug concentration to reduce cell density to 50% of that of the controls following 4 h of exposure. Values are the mean ± SEM for two to seven experiments. [b] Hypoxic cytotoxicity ratio = $IC_{50}$(oxic)/$IC_{50}$(hypoxic). Values are the mean of intraexperiment ratios ± SEM for two to four experiments.

higher for 7-substituents or for more electron withdrawing substituents. Some other nitro isomers were also isolated: the 7-$NO_2$ byproduct for 8-$SO_2Me$, 8-$SO_2NH_2$, and 9-$NO_2$ and the 9-$NO_2$ byproduct for 6-$CONH_2$ and 7-$CO_2Me$. The position of the nitro group was firmly established by X-ray crystallography (for **60** where EWG = 8-$SO_2Me$), 2D NMR experiments, and comparison of the NMR spectra of the remaining compounds to these reference examples (details in Supporting Information).[36]

Where the nitration was carried out on the trifluoroacetamide, this was subsequently cleaved in practically quantitative yield by mild base treatment (Scheme 1, **59** to **60**). For the two sulfonyl chlorides (**59**, 6- and 7-$SO_2Cl$) prior treatment with concentrated $NH_3$ served to generate the corresponding sulfonamides. Two nitrile examples (**99** and **100**) were further hydrolyzed to the amides (**101** and **102**) by exposure to aqueous $H_2SO_4$ (Scheme 4).

The only exception to this general route to intermediates **60** was for the 6-$NO_2$ example **105** (Scheme 5), which was prepared by nitration of **104**, itself formed by deprotection of the previously reported **103**. The structure of the dinitro product **105** was established by X-ray crystallography (see Supporting Information).

Two different minor groove binding side chains were employed, TMI and DEI as previously incorporated in the A-ring unsubstituted nitroCBIs **6** and **19**. The side chains were introduced using the indole-2-carboxylic acids and EDCI [*N*-ethyl-*N*′-(3-dimethylaminopropyl)carbodiimide hydrochloride] as a coupling reagent (or for some of the TMI compounds, using the corresponding acid chloride). The EDCI reactions were found to proceed best under acidic conditions, which were achieved by preforming the indoline hydrochloride salt or by adding anhydrous TsOH to the reaction mixture. Excess side chain acid could be removed following the reaction by washing with dilute aqueous $NaHCO_3$, but care is required in this step, since the products are susceptible to elimination of HCl.

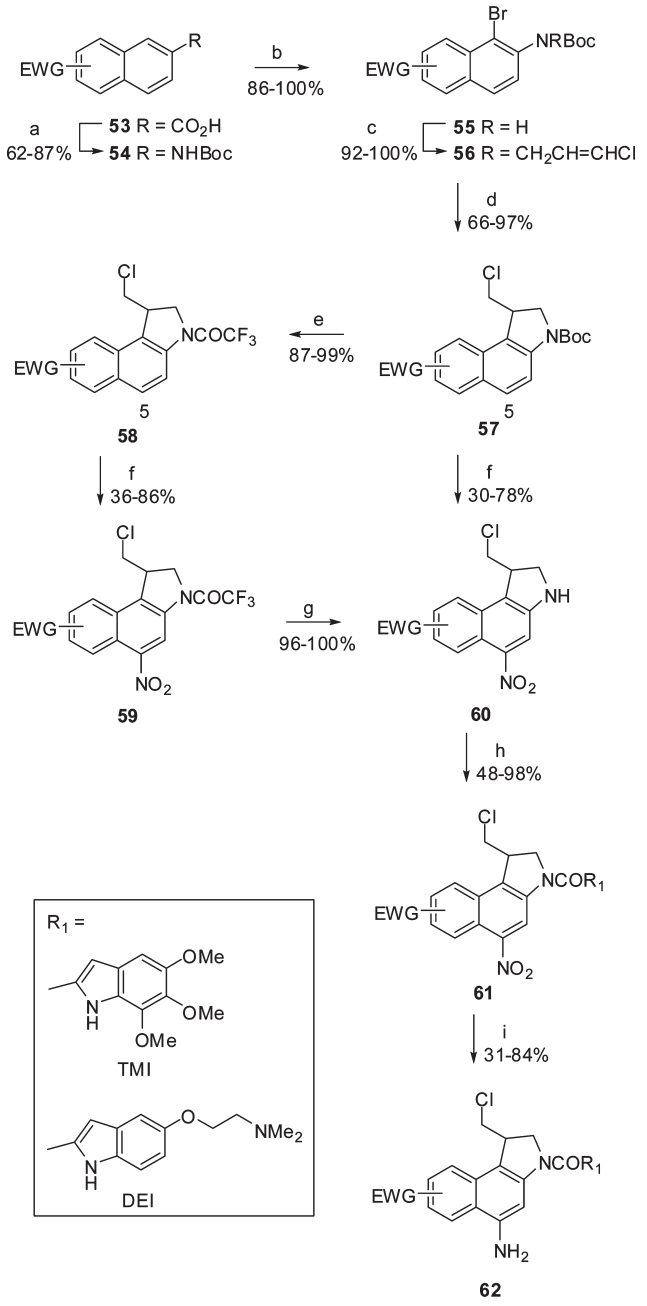
Several of the nitroCBIs were reduced to the corresponding aminoCBIs (Scheme 1) as listed in Table 3. A $PtO_2$ catalyst was employed to avoid hydrogenolysis of the chloride.

**Reduction Potentials.** One-electron reduction potentials, $E(1)$, were determined in aqueous solution by measuring the equilibrium constants, established within a few tens of microseconds, for electron transfer between the radical anions of the compounds and reference standards. Only the more water-soluble DEI analogues of Table 2 were investigated, and even some of these, including all acetyl substituted examples, were too insoluble to permit reduction potential measurement.

The experimentally determined values are plotted in Figure 3 as a function of $\sigma_p$ [37] of the A-ring substituent. While all substituents raised $E(1)$ above that of **19** (−512 mV), the effect was strongest when the substituent was in the 7-position [$\triangle E(1)$ = 15−93 mV for 6-, 8-, 9-substituents but 83−150 mV for 7-substituents]. The measured reduction potentials exhibited a reasonable correlation with $\sigma_p$ when the substituent was in either the 7- or 8-position ($r^2$ = 0.97 and 0.88, respectively), and the regression lines shown in Figure 3 were used to calculate $E(1)$ for the poorly soluble analogues.[38] The reduction potential was much less sensitive to the electron-withdrawing nature of a substituent in the 6-position (or for the single example of a 9-substituted analogue), with even the most electron-deficient nitro substituent only raising the reduction potential by 35−45 mV compared to **19**.

**In Vitro Cytotoxicity.** Our previous investigation of the cytotoxicity of **6** and **19** under oxic and hypoxic conditions used a clonogenic survival assay, but in order to compare a large number of analogues in the present study,

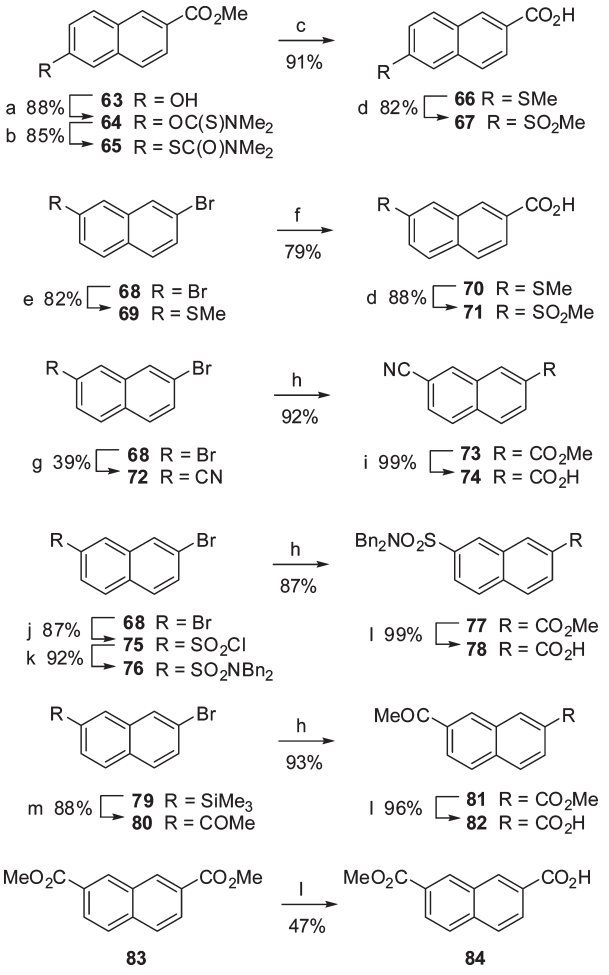
**Scheme 1**[a]




[a] Conditions: (a) DPPA, $Et_3N$, $^tBuOH$; (b) NBS; (c) NaH, 1,3-dichloropropene; (d) $Bu_3SnH$, AIBN; (e) HCl, then TFAA, py; (f) conc $H_2SO_4$, $KNO_3$, or fuming $HNO_3$ (for 6-CN, 6-COMe, 6-$CONH_2$); (g) $Cs_2CO_3$; (h) 5,6,7-trimethoxyindole-2-carbonyl chloride, py, DMAP, or 5,6,7-trimethoxyindole-2-carboxylic acid or 5-[2-(dimethylamino)-ethoxy]indole-2-carboxylic acid, EDCI, TsOH; (i) $H_2$, $PtO_2$.

an antiproliferative assay using a 96-well plate format was used instead. Oxic and hypoxic cytotoxicities were determined as $IC_{50}$ values after 4 h of drug exposure in two human tumor cell lines: the ovarian carcinoma Skov3 and the colon carcinoma HT29. **6** and **19** had previously demonstrated only modest hypoxia-selective cytotoxicity in HT29, and a similar result was noted using the $IC_{50}$-based assay (Tables 1 and 2, HCR = 2.5, 1.1). The Skov3 cell line, which had not previously been examined, also exhibited little hypoxia-selective sensitivity to these two compounds. In comparison, **1** and **3** (PR-104A, the alcohol derived by phosphate

**Scheme 2**[a]



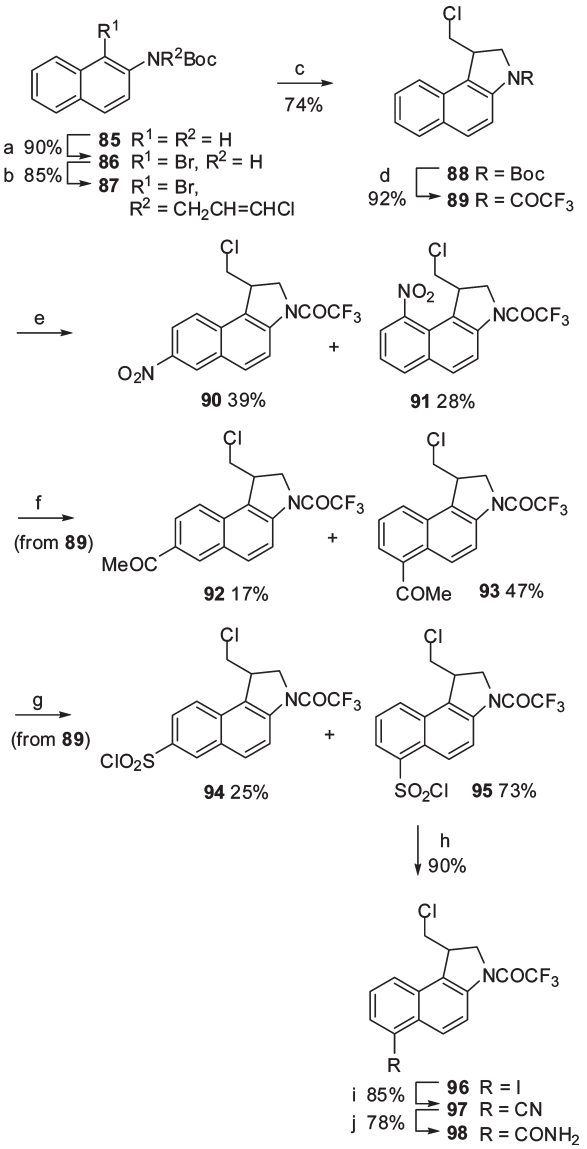

[a] Conditions: (a) $Me_2NC(S)Cl$, DABCO; (b) 225 °C; (c) KOH, then $Me_2SO_4$; (d) $NaBO_3$, AcOH; (e) BuLi, $(MeS)_2$, −78 °C; (f) BuLi, $CO_2$, −78 °C; (g) CuCN, py, NMP; (h) $Pd(OAc)_2$, DPPP, CO(g), $Et_3N$, MeOH; (i) NaOH; (j) BuLi, $SO_2$(g), −78 °C, then NCS; (k) $Bn_2NH$, $Et_3N$; (l) KOH; (m) $Ac_2O$, $AlCl_3$.

hydrolysis of the "preprodrug" **2**) exhibit HCRs of 75 and 5−10, respectively, in these two cell lines.[13]

For the new nitroCBI analogues both oxic and hypoxic cytotoxicity were found to be highly variable and sensitive to the nature of the A-ring substituent and side chain. The DEI side chain confers greater cytotoxicity than TMI under both oxic and hypoxic conditions (on the order of a 10-fold differential under oxic conditions). Further, oxic and hypoxic cytotoxicity both vary over about 2 orders of magnitude depending on the substituent, with some compounds being significantly more toxic (e.g., 6-COMe) and some significantly less toxic (e.g., 6-$CONH_2$) than the unsubstituted parent. Neither oxic nor hypoxic cytotoxicity correlates with reduction potential (illustrated in Supporting Information Figure S1 for the DEI compounds).

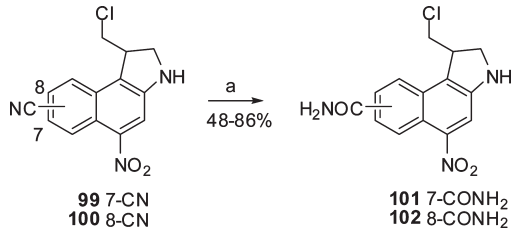
These trends can be seen from the data in Tables 1 and 2 and also in Figure 4 where $IC_{50}$ values under oxic versus hypoxic conditions are plotted for both cell lines and both side chains. Figure 4 also depicts HCR as displacement from the solid line which indicates equivalent toxicity under both conditions. For the TMI compounds, the HCR values are generally modest, with only the 7-$NO_2$ substituent generating an HCR greater than 10 (indicated by the dotted line) in

**Scheme 3**[a]




[a] Conditions: (a) NBS; (b) NaH, 1,3-dichloropropene; (c) $Bu_3SnH$, AIBN; (d) HCl, then TFAA, py; (e) fuming $HNO_3$, 20 °C, 5 min; (f) AcCl, $AlCl_3$, $CS_2$, 70 °C, 3 h (gives 47% **93**) or $PhNO_2$, 0 °C, 16 h (gives 20% **92** and recovered **89**); (g) $ClSO_3H$; (h) $ZnI_2$, LiCl, $PdCl_2(PhCN)_2$, $Ti(OiPr)_4$; (i) KCN, $Pd(PPh_3)_4$, CuI; (j) 90% $H_2SO_4$.

**Scheme 4**[a]




[a] Conditions: (a) 90% $H_2SO_4$, 65 °C, 1 h.

both cell lines. For the DEI compounds a different pattern emerges. While most of the substituents again display modest hypoxic selectivity, a subset containing the primary amides and sulfonamides gives large HCRs in both cell lines. The greatest effect is seen for the 7-$SO_2NH_2$ substituent with

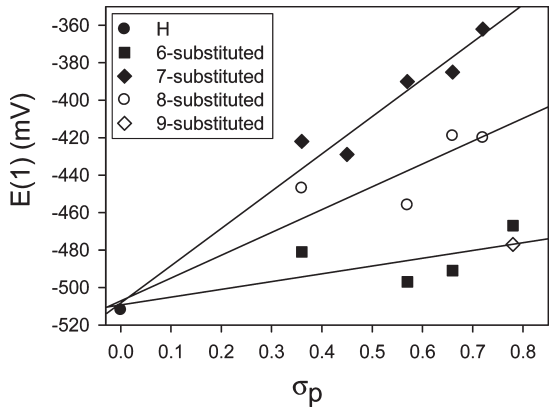
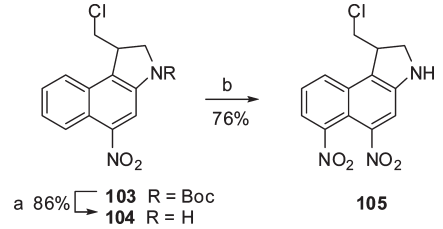



**Figure 3.** Measured one-electron reduction potentials $E(1)$ as a function of $\sigma_p$ for the compounds of Table 2. Equations for the regression lines for 6-, 7-, and 8-substituents are given in the Experimental Section.

**Scheme 5**[a]




[a] Conditions: (a) HCl; (b) conc $H_2SO_4$, $KNO_3$.

HCRs of 275 and 330 in Skov3 and HT29, respectively. For this subset the observed HCR appears to correlate with $E(1)$, as shown for the Skov3 data in Figure 5, $r^2 = 0.96$ (for HT29, $r^2 = 0.75$; see Supporting Information Figure S2), with the highest HCR being observed for the substituent with the highest one-electron reduction potential.

The cytotoxicity of several amino compounds was also determined, and the results are collected in Table 3. For all of these compounds the addition of an A-ring substituent decreases the cytotoxicity compared to the unsubstituted parent but leads to little variation in effect; all the A-ring substituted aminoCBIs investigated remain potent cytotoxins with $IC_{50}$ values in the 5−90 nM range. None of them display any hypoxic selectivity. The superior hypoxic selectivity of nitroCBIs bearing amide and sulfonamide substituents does not appear to be related to an intrinsically more toxic reduction product, since the two substituents that generate the highest HCRs as nitroCBIs (7-$SO_2NH_2$, 7-$CONH_2$) are associated with the weakest cytotoxicity as aminoCBIs. This can also be seen in Figure 6 where cytotoxicities of nitroCBI and aminoCBI compounds are compared in the HT29 cell line for all examples where the aminoCBI-DEI compounds were available. In this comparison the 7-$SO_2NH_2$ and 7-$CONH_2$ substituents stand out as being those where the nitroCBI under hypoxia becomes approximately as cytotoxic as the corresponding aminoCBI.

While there is a very large variation in nitroCBI cytotoxicity dependent on the A-ring substituent, the effect is consistent for the two cell lines examined, with correlation coefficients of 0.95 for $IC_{50}$ values obtained under both oxic and hypoxic conditions (Supporting Information Figure S3). This suggests that there is a common mechanism of toxicity for nitroCBIs in these two cell lines. A weaker correlation,

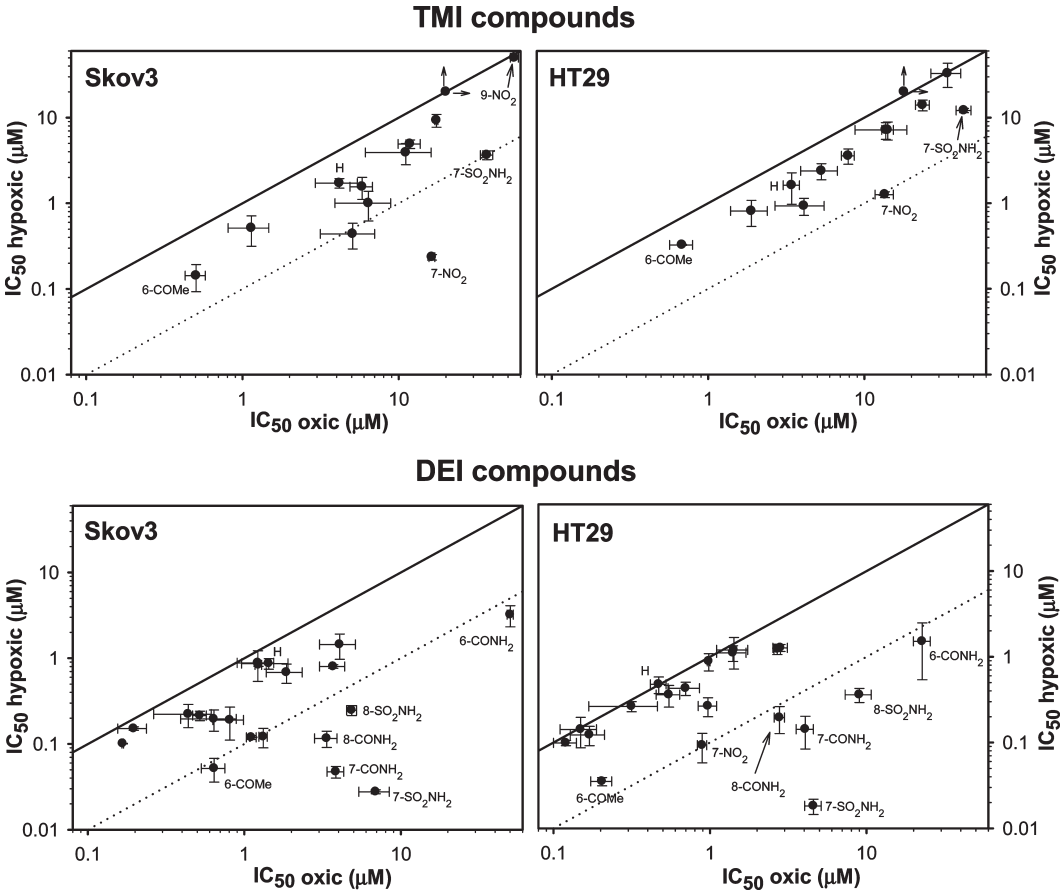



**Figure 4.** Comparison of nitroCBI cytotoxicity measured under oxic and hypoxic conditions. The TMI compounds are from Table 1 and DEI compounds from Table 2. The solid line represents equivalent toxicity under both conditions (HCR = 1) while the dotted line represents HCR = 10. Arrows indicate that the $IC_{50}$ is above the solubility limit.

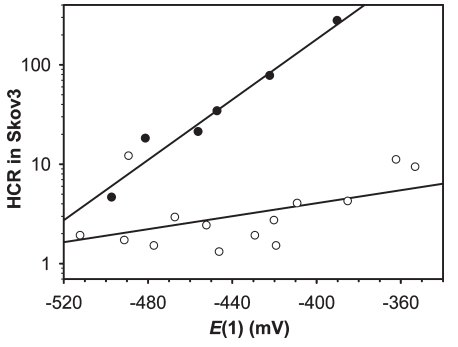



**Figure 5.** Relationship between hypoxic cytotoxicity ratio (HCR) in Skov3 and one-electron reduction potential [$E(1)$] for the compounds of Table 2. The A-ring substituents are divided into two groups: (●) 6-, 7-, and 8-$SO_2NH_2$ and 6-, 7-, and 8-$CONH_2$ (linear regression gives $r^2 = 0.96$); (○) all other substituents (linear regression gives $r^2 = 0.23$).

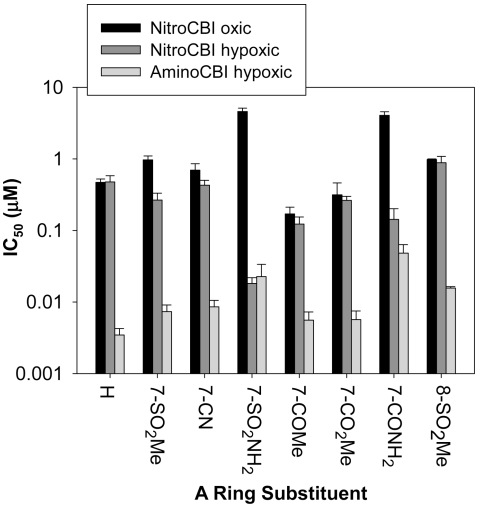



**Figure 6.** Comparative cytotoxicity of nitroCBI-DEI compounds (**19**, **26**−**30**, **32**) under oxic and hypoxic conditions and the matching aminoCBI-DEI compounds (**45**−**52**) under hypoxic conditions in HT29.

$r^2 = 0.75$, is observed for the smaller group of aminoCBIs investigated, suggesting that they also have a common mechanism of toxicity in the two cell lines.

Cytotoxicity in a panel of human tumor cell lines was examined for the most hypoxia-selective agent **28** as shown in Table 4. All cell lines exhibited an oxic $IC_{50}$ in the low micromolar range (0.5−7 $\mu$M) which was significantly enhanced under hypoxia (Figure 7), reaching low nanomolar levels in several cell lines. The panel includes a cell line pair that differ only in the level of expression of NADPH/cytochrome P450 oxidoreductase,[23] with the overexpressing cell line being more sensitive to **28** under hypoxic but not oxic conditions. All 11 cell lines examined display significant hypoxic selectivity (HCR 19−330) to this compound. When compared to **1** and **3** (Supporting Information Figure S4), it is clear that **28** is a significantly more potent cytotoxin under

**Table 4.** Cytotoxicity and Hypoxic Selectivity of NitroCBI **28** in a Panel of Human Tumor Cell Lines

| | | $IC_{50}$ ($\mu$M)[a] | | |
|---|---|---|---|---|
| tissue | cell line | oxic | hypoxic | HCR[b] |
| colon | HCT 116 | 1.7 ± 0.4 | 0.015 ± 0.001 | 110 ± 20 |
| cervix | C33A | 0.50 ± 0.02 | 0.0030 ± 0.0011 | 240 ± 110 |
| | SiHa | 1.7 ± 0.4 | 0.034 ± 0.008 | 71 ± 8 |
| NSCLC | A549 | 0.95 ± 0.14 | 0.035 ± 0.013 | 30 ± 7 |
| | A549 P450R$^{puro}$ [c] | 0.86 ± 0.22 | 0.0060 ± 0.0017 | 140 ± 10 |
| | H1299 | 6.3 ± 0.2 | 0.11 ± 0.08 | 180 ± 100 |
| LCLC | H460 | 3.8 ± 0.4 | 0.11 ± 0.01 | 36 ± 4 |
| prostate | PC3 | 2.9 ± 0.3 | 0.037 ± 0.026 | 180 ± 80 |
| melanoma | A375 | 2.9 ± 0.6 | 0.15 ± 0.01 | 19 ± 3 |

[a] Drug concentration to reduce cell density to 50% of that of the controls following 4 h of exposure. Values are the mean ± SEM for two to six experiments. [b] Hypoxic cytotoxicity ratio = $IC_{50}$(oxic)/$IC_{50}$(hypoxic). Values are the mean of intraexperiment ratios ± SEM for two to four experiments. [c] Stable transfectant of A549 overexpressing human NADPH/cytochrome P450 oxidoreductase (9-fold higher rate of cytochrome *c* reduction than parent cell line) (ref 23).

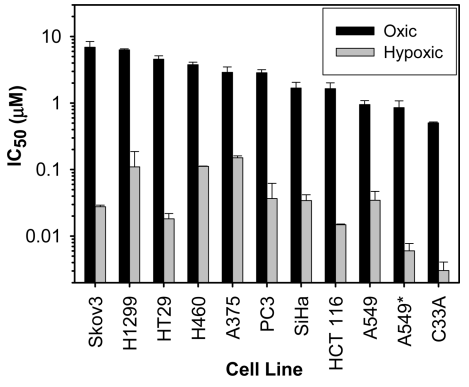



**Figure 7.** Cytotoxicity of nitroCBI **28** under oxic and hypoxic conditions in a panel of human tumor cell lines. A549* is the A549 P450R$^{puro}$ cell line of Table 4.

hypoxic conditions than either of these established HAPs (up to 300-fold more potent than **3** and 500-fold more potent than **1**). In addition, **28** exhibits hypoxic cell selectivity equal to or greater than **3** in each cell line examined.

**In Vitro Metabolism.** To investigate whether the superior HCR of **28** compared to **19** is due to enhanced reduction under hypoxic conditions, the metabolism of these two compounds was examined using subcellular fractions prepared from mouse livers and HT29 xenografts. Postmitochondrial S9 fractions were supplemented with NADPH and incubated with the nitroCBIs under either oxic or hypoxic conditions. LC/MS analysis showed that under hypoxic conditions the only significant metabolite was the corresponding aminoCBI (illustrated for liver S9 in Figure 8A, B), which was identified by mass spectrometry, UV−visible spectroscopy, and comparison with authentic samples (see Supporting Information Figure S5). Under otherwise identical incubation conditions there was clearly more extensive reduction of the sulfonamide-substituted nitroCBI **28** compared to the A-ring unsubstituted **19**. Qualitatively similar results were observed using S9 derived from HT29 tumors (data not shown), although higher protein concentrations and longer incubation times were required to achieve a similar level of metabolism. Control experiments showed that there was no reaction in the absence of cofactor or if the S9 was inactivated by prior heat treatment.

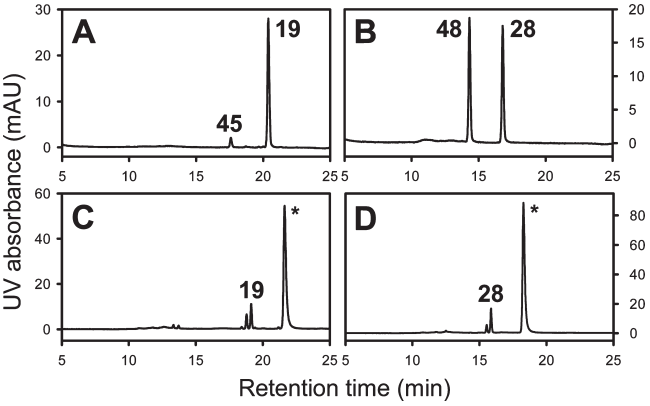



**Figure 8.** HPLC chromatograms following incubation of 10 $\mu$M nitroCBI **19** (A and C) and **28** (B and D) with mouse liver S9 (protein concentration of 8 mg/mL) at 37 °C for 30 min under either hypoxic (A and B) or oxic (C and D) conditions. HPLC conditions were slightly different between the oxic and hypoxic samples. The peaks marked with an asterisk are tentatively identified as the products of N-oxidation of the dimethylamino side chain (see text and Supporting Information).

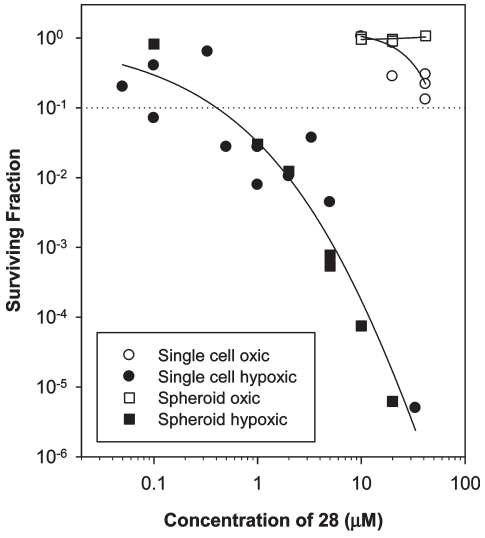



**Figure 9.** Clonogenic survival curves for HT29 single cell suspensions (circles) and intact spheroids (squares) incubated with **28** for 4 h under either oxic (open symbols) or hypoxic (closed symbols) conditions. Each point is a separate cell or spheroid suspension, and the data are pooled from four experiments.

Under oxic conditions no aminoCBI was produced, and the significant new metabolite (marked with an asterisk in Figure 8C,D), which shared the same UV−visible spectrum as the parent nitroCBI but with a mass spectrum 16 *m*/*z* units higher (Supporting Information), was tentatively identified as the corresponding *N*-oxide of the dimethylamino side chain. Similar levels of this metabolite were observed for each of the two nitroCBIs.

**Cytotoxicity of 28 in Dissociated and Intact Spheroids.** The cytotoxicity of **28** was further evaluated in HT29 cells using a clonogenic assay. In single cell suspensions (circles in Figure 9) there was a clear dependence of cytotoxicity on the nature of the gas phase; under oxic conditions the surviving fraction remained > 10% even at the solubility limit of 30 $\mu$M, while under hypoxic conditions a $C_{10}$ (concentration for 1 log of cell kill) of 0.40 $\mu$M was calculated from the fitted curve shown in Figure 9. Extrapolation of the oxic data suggests

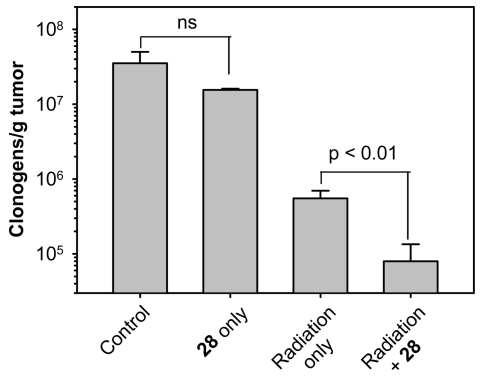



**Figure 10.** Antitumor activity of **28** in SiHa human tumor xenografts assayed by tumor excision and clonogenic assay 18 h after iv dosing with nitroCBI (42 $\mu$mol/kg) only or nitroCBI plus $\gamma$ irradiation (15 Gy). Values are the mean ± SEM for groups of three mice (control or **28** alone) or five mice (radiation alone or radiation + **28**).

that the HCR for **28** by clonogenic assay is approximately 125. The large improvement compared to **19** (HCR = 2.5 in this cell line[31]) appears to be due to the increased sensitivity of HT29 cells to **28** under hypoxic conditions, as also observed in the $IC_{50}$ assay (Table 2).

The single cell suspensions described above were generated by dissociation of HT29 spheroids. When the intact spheroids themselves were incubated with **28** under the same conditions, there was again a hypoxia-selective response (squares in Figure 9). Under oxic conditions no significant cell kill was achieved even up to the solubility limit, suggesting that the multicellular structure provides some resistance to nitroCBI-induced aerobic toxicity. In contrast, under hypoxic conditions, the survival curves for spheroids and single cell suspensions were superimposable. One possible explanation for these results is that a reduction product is formed under hypoxic conditions that is capable of diffusing from and killing cells distinct from those in which it is generated.

**In Vivo Activity.** To examine whether **28** has therapeutic activity in vivo, the compound was formulated in a DMSO−lactate buffer−PEG vehicle and administered as a single dose to mice. By ip administration, no toxicity was evident up to 100 $\mu$mol/kg, but at this dose there was clear precipitation in the peritoneum at the site of injection. As an alternative, an iv dose of 42 $\mu$mol/kg was administered to SiHa tumor-bearing mice either alone or 5 min after a single 15 Gy dose of ionizing radiation. Eighteen hours later the tumors were excised and surviving clonogens assessed (Figure 10). An irradiation dose of 15 Gy is sufficient to sterilize aerobic tumor cells and provided 1.79 logs of cell kill compared to control in this experiment. When **28** was combined with radiation, a greater cell kill was achieved (2.70 log), indicating elimination of radioresistant hypoxic cells within the tumor by the nitroCBI prodrug. As a single agent, **28** exhibited weak but not statistically significant activity.

## Discussion and Conclusions

This study was undertaken to test the idea that the addition of an EWG to the A-ring of **6** or **19** would raise the reduction potential and provide HAPs with enhanced reductive metabolism and improved hypoxia-selective cytotoxicity. While the target compounds are structurally more complex than the unsubstituted parents, their syntheses are generally easier. This is a direct consequence of the presence of the EWG, since it directs nitration to the desired 5-position of intermediates **57** and **58**, allowing preparation of the indoline ring by a well-precedented and reliable radical route. As an illustration, our previous synthesis of **6** proceeded in 14 steps and 4% overall yield,[25] whereas **14** can be obtained from 6-(methoxycarbonyl)-2-naphthoic acid in six steps and 12% overall yield. While the other appropriately substituted 2-naphthoic acids are not commercially available, they can be obtained in a few steps on a multigram scale (as previously described or as reported in Scheme 2), as can the alternative nitration substrates illustrated in Scheme 3. The key nitration step is not completely selective, but in all cases the desired 5-nitro isomer could be readily purified by recrystallization or column chromatography.

Measurement of one-electron reduction potentials for the more water-soluble DEI analogues established that the unsubstituted nitroCBI **19** has $E(1) = -512$ mV. This is outside the window of −400 to −200 mV predicted to be suitable for the observation of hypoxic selectivity with nitroaromatic compounds, in that it may be too negative to allow efficient enzymatic reduction.[39,40] As a comparison, the nitrogen mustard *N*,*N*-bis(2-chloroethyl)-4-nitroaniline, proposed as a potential HAP, was calculated to have $E(1) = -508$ mV, and it was not until analogues bearing extra EWGs were prepared that significant HCRs were achieved for these compounds in vitro.[41,42] The addition of any EWG to the A-ring of the nitroCBI raises the reduction potential (Table 2 and Figure 3), but the magnitude is sensitive to the substituent location. A variety of EWGs in the 6-position only raise $E(1)$ by a maximum of 45 mV, even for the strongly electron-deficient $NO_2$ substituent. It is possible that steric interaction with the 5-$NO_2$ group twists the 6-substituent out of plane with the aromatic ring, diminishing the effective conjugation. A similar effect may also explain the weak impact that a 9-$NO_2$ substituent has on $E(1)$. When the EWG is in the 8- or especially 7-position, a larger effect on reduction potential is observed and found to correlate with $\sigma_p$ of the substituents examined. Four examples of 7-substituents ($NO_2$, $SO_2Me$, CN, and $SO_2NH_2$) were found (or calculated) to provide nitroCBIs with one-electron reduction potentials above −400 mV.

The A-ring substituents also have a substantial effect on the cytotoxicity of the nitroCBIs under both oxic and hypoxic conditions. It seems reasonable to expect that such substituents will influence the binding of the compounds to the minor groove of DNA and that bulky substituents positioned at the base of the groove (for example, 9-$NO_2$ or 8-$SO_2Me$) may hinder binding, while smaller or planar substituents that can enhance van der Waals contacts could have the opposite effect. Even with these considerations it is not obvious why 6-substituents, directed toward the mouth of the minor groove, have such a large and disparate effect on $IC_{50}$; a 6-COMe substituent confers greater toxicity than the unsubstituted parent (compare **8** with **6** and compare **23** with **19**) and a 6-$CONH_2$ substituent the opposite (compare **24** with **19**), to the point where **23** is approximately 100 times more cytotoxic than **24** under oxic conditions. At this point the mechanism of oxic toxicity for the nitroCBI compounds is not known; possibilities include noncovalent DNA binding, direct DNA alkylation, redox cycling, and oxygen-insensitive reductive metabolism (or a combination of these). However, it is interesting to note that neither oxic nor hypoxic cytotoxicity correlate with reduction potential (Figure S1).

The nature of the minor groove binding side chain (TMI versus DEI) also has an effect on cytotoxicity. Although poor aqueous solubility precluded reduction potential measurement for the TMI compounds, it is considered highly unlikely that the side chain has any significant impact on $E(1)$. DEI nitroCBIs may be more toxic than the TMI compounds because of enhanced DNA binding by the positively charged basic side chain. The difference is smaller when comparing aminoCBI compounds (here a DEI side chain generates only marginally more toxicity than TMI), and it is possible that the basic side chain affects nitroCBI toxicity by influencing cell uptake or subcellular distribution relative to the enzyme(s) responsible for reductive activation.

Whatever the explanation for the cytotoxicity of individual compounds under particular conditions, it is clear that a subset of the nitroCBIs investigated have substantial hypoxic selectivity. These compounds (with the exception of **9**) are those with a DEI side chain and a primary sulfonamide or carboxamide in one of the 6-, 7-, or 8-positions on the A-ring. For this set hypoxic selectivity increases with increasing reduction potential (Figure 5), although significant HCRs ($>10$ in both cell lines) are observed for several compounds with $E(1)$ well below $-400$ mV (**24**, **31**, **34**, **37**). Higher reduction potential alone is not sufficient to observe hypoxic selectivity; the 7-substituted analogues **27** and **28** have the same $E(1)$ within experimental error, but the former (7-CN) gives HCRs of 1.7 and 4.2 in the two cell lines tested, while the latter (7-$SO_2NH_2$), the most selective of all the compounds surveyed, has HCRs of 275 and 330. It is possible that the H-bond donor capacity of the sulfonamide and carboxamide substituents makes them better substrates for the reductase(s) responsible for hypoxic activation, and when H-bond donor capacity is combined with higher reduction potential, then highly selective compounds are produced.

Further investigation of **28** shows that it has a number of attractive features as a HAP and that the improvements compared to the previously reported **19** are consistent with enhanced metabolism under hypoxic but not oxic conditions. Metabolism studies using S9 fractions from liver or tumor (Figure 8) show conversion to the corresponding aminoCBI as the sole product under hypoxic incubation, a reaction that is completely suppressed in the presence of oxygen. There is clearly more extensive reductive metabolism of **28** than **19**, so much so that after only 4 h of hypoxic incubation **28** becomes as cytotoxic to HT29 (Figure 6) or Skov3 cells (Tables 2 and 3) as directly administered aminoCBI **48**, which is clearly not the case for **19**. Although the enzyme(s) responsible for the reductive activation of **28** have not been identified, beyond the implication of NADPH/cytochrome P450 oxidoreductase, they appear to be widely expressed, since **28** displays substantial hypoxic selectivity in every cell line so far investigated (Figure 7). Hypoxic selectivity is also demonstrated using clonogenicity as the end point (Figure 9).

The major limitation remaining for the application of nitroCBI **28** as a HAP is its poor water solubility. Although **28** could be formulated for in vivo analysis, ip administration to mice led to precipitation in the peritoneum. When dosed iv, **28** produced a highly significant increase in tumor cell kill in combination with radiation. This encouraging result suggests that analogues of nitroCBI **28** that retain the hypoxia-selective activity in combination with improved water solubility would be very interesting candidates as hypoxia-activated prodrugs for antitumor therapy.

## Experimental Section

**General Chemistry Methods.** All final products were analyzed by reverse-phase HPLC (Alltima C18 5 $\mu$m column, 150 mm $\times$ 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 was determined by monitoring at $330 \pm 50$ nm and was $>95\%$ in all cases. Final product purity was also assessed by combustion analysis carried out in the Campbell Microanalytical Laboratory, University of Otago, Dunedin, New Zealand. Melting points were determined on an Electrothermal 2300 melting point apparatus. NMR spectra were obtained on a Bruker Avance 400 spectrometer at 400 MHz for $^1$H and 100 MHz for $^{13}$C spectra.

Illustrative examples are provided for the synthesis of all 6-substituted nitroCBI final products of Tables 1 and 2, and an example of the general method for the synthesis of an aminoCBI of Table 3 is provided. The preparation of all other compounds is fully documented in the Supporting Information.

**Preparation of 1-(Chloromethyl)-5,6-dinitro-3-(5,6,7-trimethoxyindol-2-carbonyl)-1,2-dihydro-3*H*-benzo[*e*]indole (7). 1-(Chloromethyl)-5-nitro-1,2-dihydro-3*H*-benzo[*e*]indole (104).** A solution of *tert*-butyl 1-(chloromethyl)-5-nitro-1,2-dihydro-3*H*-benzo[*e*]indole-3-carboxylate[25] (**103**) (600 mg, 1.65 mmol) in dioxane (15 mL) was saturated with dry HCl, stirred at 20 °C for 1 h, and then evaporated under reduced pressure below 30 °C. The residue was partitioned between $CH_2Cl_2$ and dilute aqueous $KHCO_3$ and the organic phase was washed with water, dried, and then filtered through a column of silica gel to give **104** (372 mg, 86%) as a red solid: mp ($CH_2Cl_2$/petroleum ether) 100−101 °C; $^1$H NMR [$(CD_3)_2SO$] $\delta$ 8.11 (d, $J$ = 8.7 Hz, 1 H), 7.87 (d, $J$ = 8.4 Hz, 1 H), 7.65 (s, 1 H), 7.55 (ddd, $J$ = 8.2, 7.0, 1.2 Hz, 1 H), 7.40 (ddd, $J$ = 8.7, 6.8, 1.0 Hz, 1 H), 6.27 (br s, 1 H), 4.23−4.15 (m, 1 H), 3.89 (dd, $J$ = 11.0, 3.7 Hz, 1 H), 3.81 (t, $J$ = 9.7 Hz, 1 H), 3.78−3.66 (m, 2 H). Anal. ($C_{13}H_{11}ClN_2O_2$) C, H, N.

**General Method of Nitration Using $H_2SO_4$ and $KNO_3$. Preparation of 1-(Chloromethyl)-5,6-dinitro-1,2-dihydro-3*H*-benzo[*e*]indole (105).** A stirred solution of **104** (500 mg, 1.90 mmol) in concentrated $H_2SO_4$ (5 mL) was cooled to $-5$ °C and treated with powdered $KNO_3$ (288 mg, 2.85 mmol). The mixture was stirred at 0 °C for a further 15 min, then poured into ice−water, and the solid was collected and dissolved in $CH_2Cl_2$. The solution was filtered through a column of silica gel and the product was recrystallized from EtOAc/$^i$Pr$_2$O to give **105** (446 mg, 76%) as a red solid: mp 206−207 °C; $^1$H NMR [$(CD_3)_2SO$] $\delta$ 8.23 (dd, $J$ = 8.7, 1.0 Hz, 1 H), 8.00 (dd, $J$ = 7.7, 0.9 Hz, 1 H), 7.76 (s, 1 H), 7.67 (dd, $J$ = 8.4, 7.6 Hz, 1 H), 6.72 (s, 1 H), 4.32−4.22 (m, 1 H), 3.94−3.83 (m, 2 H), 3.83−3.75 (m, 2 H). Anal. ($C_{13}H_{10}ClN_3O_4$) C, H, N.

**General Method of Amide Formation Using 5,6,7-Trimethoxyindole-2-carbonyl Chloride. Preparation of 7.** A suspension of 5,6,7-trimethoxyindole-2-carboxylic acid (122 mg, 0.49 mmol) in dry $CH_2Cl_2$ (10 mL) was treated with oxalyl chloride (0.13 mL, 1.49 mmol) followed by DMF (10 $\mu$L). The mixture was stirred at room temperature for 15 min, then evaporated under reduced pressure and azeotroped dry with benzene. The resulting acid chloride was cooled to $-5$ °C and treated with an ice-cold solution of amine **105** (100 mg, 0.33 mmol) in dry pyridine (2 mL) containing DMAP (5 mg). The stirred mixture was warmed to room temperature for 30 min, then poured into dilute aqueous $KHCO_3$. The solid was collected, purified by chromatography on silica gel, eluting with $CH_2Cl_2$/EtOAc (19:1), then crystallized from $CH_2Cl_2$/EtOAc to give **7** (84 mg, 48%) as a yellow solid: mp 278−279 °C; $^1$H NMR [$(CD_3)_2SO$] $\delta$ 11.67 (s, 1 H), 9.16 (s, 1 H), 8.61 (d, $J$ = 8.0 Hz, 1 H), 8.38 (d, $J$ = 7.4 Hz, 1 H), 7.92 (t, $J$ = 8.0 Hz, 1 H), 7.21 (d, $J$ = 1.9 Hz, 1 H), 6.99 (s, 1 H), 4.94 (t, $J$ = 10.6 Hz, 1 H), 4.73−4.60 (m, 2 H),

4.19−4.05 (m, 2 H), 3.94 (s, 3 H), 3.83 (s, 3 H), 3,81 (s, 3 H). HRMS (FAB) calcd for $C_{25}H_{21}{}^{35}ClN_4O_8$ ($M^+$) $m/z$ 540.1048, found 540.1051. Anal. ($C_{25}H_{21}ClN_4O_8$) C, H, N.

**Preparation of 6-Acetyl-1-(chloromethyl)-5-nitro-3-(5,6,7-trimethoxyindol-2-carbonyl)-1,2-dihydro-3*H*-benzo[*e*]indole (8). 7-Acetyl-1-(chloromethyl)-3-(trifluoroacetyl)-1,2-dihydro-3*H*-benzo[*e*]indole (92) and 6-Acetyl-1-(chloromethyl)-3-(trifluoroacetyl)-1,2-dihydro-3*H*-benzo[*e*]indole (93).** Solid **89** (4.7 g, 15 mmol) was added to a mixture of $AlCl_3$ (7.0 g, 52 mmol) and AcCl (2.5 mL, 35 mmol) in $CS_2$ (60 mL) at 0 °C, and the stirred mixture was heated at 70 °C for 3 h. Solvent was boiled off at 60 °C, and the black residue was cooled and treated with ice and concentrated HCl. The mixture was extracted with $CH_2Cl_2$ (3 × 100 mL). The extracts were dried and concentrated under reduced pressure, and the residue was chromatographed on silica gel. Elution with EtOAc/petroleum ether (1:4) gave a product (3.9 g, 73%) that was shown by NMR to be a mixture of 64% **93** and 23% **92**, with the remainder of the material being a mixture of other acetylated products. Pure **93** was obtained by crystallization from EtOAc/petroleum ether as a white solid: mp 121−123 °C; $^1$H NMR [$(CD_3)_2SO$] $\delta$ 8.72 (d, $J$ = 1.6 Hz, 1 H), 8.60 (d, $J$ = 9.4 Hz, 1 H), 8.38 (d, $J$ = 9.3 Hz, 1 H), 8.25 (d, $J$ = 8.4 Hz, 1 H), 8.00 (dd, $J$ = 7.1, 0.9 Hz, 1 H), 7.70 (dd, $J$ = 8.3, 7.3 Hz, 1 H), 4.60−4.40 (m, 3 H), 4.18−4.10 (m, 1 H), 4.07−3.99 (m, 1 H), 2.76 (s, 3 H); $^{13}$C NMR $\delta$ 201.8, 153.2 (q, $J_{C-F}$ = 36.9 Hz), 139.9, 136.1, 129.6, 128.1, 127.8, 127.5, 127.2, 126.8, 126.3, 124.9, 116.1 (q, $J_{C-F}$ = 288 Hz), 52.5, 47.6, 41.1, 30.0. Anal. ($C_{17}H_{13}ClF_3NO_2$) C, H, N.

**6-Acetyl-1-(chloromethyl)-5-nitro-3-(trifluoroacetyl)-1,2-dihydro-3*H*-benzo[*e*]indole (59, EWG = 6-COMe). 93** was nitrated using fuming $HNO_3$ as described in the general method below to give **59** (EWG = 6-COMe) (57%) as a brown solid: mp 182−184 °C (EtOAc/petroleum ether); $^1$H NMR ($CDCl_3$) $\delta$ 9.18 (s, 1 H), 9.06 (d, $J$ = 1.4 Hz, 1 H), 8.28 (dd, $J$ = 8.8, 1.6 Hz, 1 H), 7.95 (d, $J$ = 8.8 Hz, 1 H), 4.68−4.63 (m, 1 H), 4.57−4.49 (m, 1 H), 4.48−4.30 (m, 1 H), 3.93−3.87 (m, 1 H), 3.65−3.58 (m, 1 H), 2.70 (s, 3 H); $^{13}$C NMR $\delta$ 200.4, 154.7 (q, $J_{C-F}$ = 39.2 Hz), 148.7, 139.2, 138.2, 130.9, 130.4, 127.7, 127.5, 125.9, 119.6, 115.7, 115.6 (q, $J_{C-F}$ = 288 Hz), 52.7, 45.4, 42.7, 28.5. Anal. ($C_{17}H_{12}ClF_3N_2O_4$) C, H, N.

**Preparation of 8.** A solution of **59** (EWG = 6-COMe) (53 mg, 0.13 mmol) in $CH_2Cl_2$/MeOH (1:1, 20 mL) was treated with $Cs_2CO_3$ (100 mg, 0.31 mmol), and the mixture was stirred at room temperature for 15 min, then poured into water (100 mL) and extracted with $CH_2Cl_2$ (3 × 50 mL). The extracts were dried and concentrated under reduced pressure. The crude product was reacted with 5,6,7-trimethoxyindole-2-carbonyl chloride as described in the general method above [chromatography with EtOAc/petroleum ether (1:1), followed by crystallization from $CH_2Cl_2$/petroleum ether] to give **8** (40 mg, 57%) as a yellow solid: mp 180−183 °C; $^1$H NMR ($CDCl_3$) $\delta$ 9.41 (s, 1 H), 9.11 (s, 1 H), 7.89 (dd, $J$ = 8.4, 1.0 Hz, 1 H), 7.72 (dd, $J$ = 6.9, 0.9 Hz, 1 H), 7.61 (dd, $J$ = 8.3, 7.3 Hz, 1 H), 7.00 (d, $J$ = 2.4 Hz, 1 H), 6.86 (s, 1 H), 4.85−4.80 (m, 1 H), 4.74−4.67 (m, 1 H), 4.33−4.25 (m, 1 H), 4.08 (s, 3 H), 3.94 (s, 3 H), 3.91 (s, 3 H), 3.93−3.87 (m, 1 H), 3.59−3.51 (m, 1 H), 2.70 (s, 3 H); $^{13}$C NMR $\delta$ 200.5, 160.5, 150.5, 148.6, 141.4, 140.9, 138.9, 138.3, 130.6, 129.7, 128.7, 127.2, 126.6, 126.0, 125.7, 123.5, 118.8, 116.6, 107.1, 97.7, 61.5, 61.1, 56.3, 54.7, 45.6, 43.4, 28.5. Anal. ($C_{27}H_{24}ClN_3O_7 \cdot {}^1/_2H_2O$) C, H, N.

**Preparation of 1-(Chloromethyl)-3-{5-[2-(dimethylamino)-ethoxy]indol-2-carbonyl}-5,6-dinitro-1,2-dihydro-3*H*-benzo[*e*]-indole (20). General Method of Amide Formation Using Indole-2-carboxylic Acids and EDCI.** A mixture of amine **105** (100 mg, 0.33 mmol), 5-[2-(dimethylamino)ethoxy]indole-2-carboxylic acid hydrochloride (111 mg, 0.39 mmol), EDCI (249 mg, 1.30 mmol), and anhydrous TsOH (40 mg, 0.23 mmol) in dry DMA (4 mL) was stirred at room temperature under $N_2$ for 3 h, then poured into dilute aqueous $NH_3$. The solid was collected, dissolved in $CH_2Cl_2$ at room temperature, dried, and concentrated under reduced pressure below 30 °C. The residue was triturated with EtOAc to give crude **20**. Treatment of a solution of the free base in $CH_2Cl_2$ with $HCl_{(g)}$/EtOAc/hexane, followed by crystallization from MeOH/$Me_2CO$/EtOAc, gave **20**·HCl (129 mg, 69%) as a yellow solid: mp 225−226 °C; $^1$H NMR [$(CD_3)_2SO$] $\delta$ 11.88 (d, $J$ = 1.6 Hz, 1 H), 10.12 (br s, 1 H), 9.22 (s, 1 H), 8.63 (d, $J$ = 7.9 Hz, 1 H), 8.40 (dd, $J$ = 7.6, 0.6 Hz, 1 H), 7.93 (t, $J$ = 8.0 Hz, 1 H), 7.47 (d, $J$ = 8.9 Hz, 1 H), 7.27 (d, $J$ = 2.3 Hz, 1 H), 7.26 (d, $J$ = 1.6 Hz, 1 H), 7.04 (dd, $J$ = 8.9, 2.4 Hz, 1 H), 4.99 (t, $J$ = 10.2 Hz, 1 H), 4.79−4.66 (m, 2 H), 4.36 (t, $J$ = 4.4 Hz, 2 H), 4.20−4.07 (m, 2 H), 3.53 (t, $J$ = 5.0 Hz, 2 H), 2.87 (s, 6 H). Anal. ($C_{26}H_{24}ClN_5O_6 \cdot HCl \cdot 1{}^1/_2H_2O$) C, H, N.

**Preparation of 1-(Chloromethyl)-3-{5-[2-(dimethylamino)-ethoxy]indol-2-carbonyl}-5-nitro-1,2-dihydro-3*H*-benzo[*e*]indole-6-carbonitrile (21). *tert*-Butyl 1-Bromo-2-naphthylcarbamate (86).** A stirred solution of *tert*-butyl 2-naphthylcarbamate (**85**)[34,35] (20.3 g, 83 mmol) in MeCN (150 mL) was treated portionwise at 0 °C with NBS (17.82 g, 100 mmol), then stirred for a further 2 h at 0 °C. The mixture was concentrated under reduced pressure, and the residue was dissolved in $CH_2Cl_2$. The solution was filtered through a short column of silica gel, and the product was recrystallized from MeOH to give **86**[35] (24.09 g, 90%) as a white solid: mp 90−91 °C; $^1$H NMR [$(CD_3)_2SO$] $\delta$ 8.82 (s, 1 H), 8.15 (d, $J$ = 8.5 Hz, 1 H), 7.96 (d, $J$ = 9.6 Hz, 1 H), 7.93 (d, $J$ = 9.3 Hz, 1 H), 7.71 (d, $J$ = 8.8 Hz, 1 H), 7.66 (t, $J$ = 7.7 Hz, 1 H), 7.56 (t, $J$ = 7.4 Hz, 1 H), 1.49 (s, 9 H). Anal. ($C_{15}H_{16}BrNO_2$) C, H, N, Br.

***tert*-Butyl 1-Bromo-2-naphthyl-(3-chloro-2-propen-1-yl)carbamate (87).** A stirred solution of **86** (800 mg, 2.48 mmol) in DMF (6 mL) was treated portionwise at 0 °C with NaH (119 mg, 60% in oil, 2.98 mmol). The mixture was warmed to room temperature for 30 min, then cooled to 0 °C and treated with 1,3-dichloropropene (0.72 mL, 7.8 mmol, mixed isomers). The mixture was stirred at room temperature for a further 4 h, then diluted with 10% aqueous NaCl and extracted with EtOAc (×2). The combined organic extracts were washed with water (×3), dried, and concentrated under reduced pressure at 100 °C. The residue was chromatographed on silica gel, eluting with $CH_2Cl_2$/petroleum ether (7:3), to give **87** (958 mg, 97%) as an oil: $^1$H NMR [$(CD_3)_2SO$] (mixture of rotamers and *E* and *Z* forms) $\delta$ 8.23 (d, $J$ = 8.4 Hz, 1 H), 8.07−7.94 (m, 2 H), 7.71 (t, $J$ = 7.5 Hz, 1 H), 7.65 (t, $J$ = 7.4 Hz, 1 H), 7.51, 7.45 (2 d, $J$ = 8.6 Hz, 1 H), 6.44−6.26 (m, 1 H), 6.21−5.99 (m, 1 H), 4.58−4.46, 4.44−4.17, 4.14−3.96 (3 m, 2 H), 1.50, 1.26 (2 s, 9 H). HRMS (EI) calcd for $C_{18}H_{19}{}^{79}Br^{35}ClNO_2$ ($M^+$) $m/z$ 395.0288, found 395.0261.

***tert*-Butyl 1-(Chloromethyl)-1,2-dihydro-3*H*-benzo[*e*]indole-3-carboxylate (88).** A mixture of **87** (23.0 g, 58 mmol), $Bu_3SnH$ (16.4 mL, 61 mmol), and AIBN (1.2 g, 7.3 mmol) in dry benzene (200 mL) was stirred at reflux under $N_2$ for 2 h, then concentrated under reduced pressure. The residue was chromatographed on silica gel, eluting with $CH_2Cl_2$/petroleum ether, to provide an oil. This was dissolved in MeOH, and following prolonged refrigeration the precipitate was collected and recrystallized from petroleum ether to give **88**[35] (13.6 g, 74%) as a white solid: mp 107−108 °C; $^1$H NMR [$(CD_3)_2SO$] $\delta$ 8.07 (v br, 1 H), 7.94−7.80 (m, 3 H), 7.52 (t, $J$ = 7.4 Hz, 1 H), 7.39 (t, $J$ = 7.5 Hz, 1 H), 4.29−4.11 (m, 2 H), 4.08 (dd, $J$ = 11.1, 2.3 Hz, 1 H), 4.03 (dd, $J$ = 11.1, 2.9 Hz, 1 H), 3.88 (dd, $J$ = 11.0, 7.1 Hz, 1 H), 1.55 (s, 9 H). Anal. ($C_{18}H_{20}ClNO_2$) C, H, N.

**1-(Chloromethyl)-3-(trifluoroacetyl)-1,2-dihydro-3*H*-benz[*e*]-indole (89).** A solution of **88** (400 mg, 1.26 mmol) in dioxane (15 mL) was saturated with dry HCl, stirred at room temperature for 1 h, and then evaporated under reduced pressure below 30 °C. The residue was dissolved in pyridine (3 mL) and treated dropwise at 0 °C with trifluoroacetic anhydride (0.21 mL, 1.49 mmol). The mixture was warmed to room temperature for 5 min, then diluted with water, and the precipitated solid was collected, dissolved in $CH_2Cl_2$, and filtered through a column of

silica gel to give **89** (363 mg, 92%) as a white solid: mp ($CH_2Cl_2$/petroleum ether) 157 °C; $^1$H NMR [$(CD_3)_2SO$] $\delta$ 8.32 (d, $J$ = 9.0 Hz, 1 H), 8.07−7.96 (m, 3 H), 7.62 (ddd, $J$ = 8.2, 6.9, 1.2 Hz, 1 H), 7.53 (ddd, $J$ = 8.1, 6.9, 1.1 Hz, 1 H), 4.61−4.52 (m, 1 H), 4.51−4.39 (m, 2 H), 4.15 (dd, $J$ = 11.3, 3.0 Hz, 1 H), 4.04 (dd, $J$ = 11.3, 5.9 Hz, 1 H). Anal. ($C_{15}H_{11}ClF_3NO$) C, H, N.

**1-(Chloromethyl)-3-(trifluoroacetyl)-1,2-dihydro-3*H*-benzo[*e*]-indole-7-sulfonyl Chloride (94) and 1-(Chloromethyl)-3-(trifluoroacetyl)-1,2-dihydro-3*H*-benzo[*e*]indole-6-sulfonyl Chloride (95).** Solid **89** (1.6 g, 5.1 mmol) was gradually added to chlorosulfonic acid (6.0 mL, 90 mmol) with ice bath cooling. The mixture was then heated to 60 °C for 2 h, and the reaction was quenched by pouring the mixture slowly, with stirring, into ice−water. The precipitated solid was collected, washed with water, dried, and chromatographed on silica gel. Elution with EtOAc/petroleum ether (from 1:4 to 1:1) gave **94** (0.53 g, 25%) as a pale-yellow solid: mp (EtOAc/petroleum ether) 189−192 °C; $^1$H NMR ($CDCl_3$) $\delta$ 8.70−8.64 (m, 2 H), 8.13−8.08 (m, 2 H), 8.00 (d, $J$ = 9.0 Hz, 1 H), 4.72−4.68 (m, 1 H), 4.55−4.48 (m, 1 H), 4.33−4.25 (m, 1 H), 3.98−3.93 (m, 1 H), 3.68−3.61 (m, 1 H); $^{13}$C NMR $\delta$ 154.9 (q, $J_{C-F}$ = 38.4 Hz), 143.9, 140.7, 132.7, 131.8, 130.1, 130.0, 125.8, 124.8, 123.2, 119.6, 115.9 (q, $J_{C-F}$ = 288 Hz), 52.8, 45.4, 42.4. Anal. ($C_{15}H_{10}Cl_2F_3NO_3S$) C, H, N, Cl.

Later eluates gave **95** (1.54 g, 73%): mp (EtOAc/petroleum ether) 181−183 °C; $^1$H NMR ($CDCl_3$) $\delta$ 8.87 (d, $J$ = 9.5 Hz, 1 H), 8.73 (d, $J$ = 9.5 Hz, 1 H), 8.36 (dd, $J$ = 7.5, 1.0 Hz, 1 H), 8.19 (d, $J$ = 8.4 Hz, 1 H), 7.72 (dd, $J$ = 8.3, 7.5 Hz, 1 H), 4.72−4.66 (m, 1 H), 4.52−4.44 (m, 1 H), 4.31−4.23 (m, 1 H), 3.95−3.81 (m, 1 H), 3.63−3.56 (m, 1 H); $^{13}$C NMR $\delta$ 154.8 (q, $J_{C-F}$ = 38.3 Hz), 141.5, 140.9, 131.0, 130.6, 128.6, 126.7, 126.5, 125.8, 125.5, 120.3, 115.9 (q, $J_{C-F}$ = 288 Hz), 52.6, 45.4, 43.0. Anal. ($C_{15}H_{10}Cl_2F_3NO_3S$) C, H, N, Cl.

**1-(Chloromethyl)-6-iodo-3-(trifluoroacetyl)-1,2-dihydro-3*H*-benzo[*e*]indole (96).** Diglyme (10 mL) and Ti($O^iPr$)$_4$ (200 mg, 0.7 mmol) were added to a mixture of **95** (660 mg, 1.6 mmol), $ZnI_2$ (653 mg, 2.4 mmol), LiCl (63 mg, 1.45 mmol), and $PdCl_2(PhCN)_2$ (16 mg, 0.04 mmol) under $N_2$, and the mixture was stirred and heated at 155 °C for 30 min. The reaction mixture was poured into aqueous HCl (0.05M, 50 mL) and filtered through a wad of Celite. The filter cake was mixed with $CH_2Cl_2$ (50 mL, then 3 × 30 mL), and each time the mixture was filtered. The filtrate was dried and concentrated, and the residue was chromatographed on silica gel. Elution with EtOAc/petroleum ether (from 1:5 to 1:2) gave **96** as a pale-yellow solid (630 mg, 90%): mp (EtOAc/petroleum ether) 174−177 °C; $^1$H NMR ($CDCl_3$) $\delta$ 8.48 (d, $J$ = 9.3 Hz, 1 H), 8.14 (d, $J$ = 9.3 Hz, 1 H), 8.08 (d, $J$ = 7.3 Hz, 1 H), 7.76 (d, $J$ = 8.4 Hz, 1 H), 7.28−7.22 (m, 1 H), 4.70−4.62 (m, 1 H), 4.48−4.39 (m, 1 H), 4.21−4.13 (m, 1 H), 3.96−3.89 (m, 1 H), 3.56−3.48 (m, 1 H); $^{13}$C NMR $\delta$ 154.6 (q, $J_{C-F}$ = 38.3 Hz), 140.8, 137.3, 135.0, 132.8, 129.5, 128.4, 125.8, 123.4, 118.6, 116.0 (q, $J_{C-F}$ = 288 Hz), 100.6, 52.8, 45.4, 42.8. Anal. ($C_{15}H_{10}ClF_3INO$) C, H, N.

**1-(Chloromethyl)-3-(trifluoroacetyl)-1,2-dihydro-3*H*-benzo[*e*]-indole-6-carbonitrile (97).** A mixture of **96** (148 mg, 0.34 mmol), KCN (120 mg, 1.9 mmol), Pd($PPh_3$)$_4$ (10 mg, 0.01 mmol), and CuI (50 mg, 0.26 mmol) in dry THF (30 mL) was heated to reflux under $N_2$ with vigorous stirring for 30 min. The mixture was cooled to room temperature, diluted with EtOAc (50 mL), and then filtered through Celite. The filtrate was washed with water and brine, dried, and concentrated under reduced pressure. Chromatography of the residue on silica gel, eluting with EtOAc/petroleum ether (from 1:5 to 1:2), gave **97** (97 mg, 85%): mp (EtOAc/petroleum ether) 158−160 °C; $^1$H NMR ($CDCl_3$) $\delta$ 8.63 (d, $J$ = 9.1 Hz, 1 H), 8.26 (d, $J$ = 9.2 Hz, 1 H), 8.05 (d, $J$ = 8.5 Hz, 1 H), 7.90 (dd, $J$ = 7.2, 1.0 Hz, 1 H), 7.64 (dd, $J$ = 8.2, 7.2 Hz, 1 H), 4.70−4.63 (m, 1 H), 4.51−4.43 (m, 1 H), 4.28−4.20 (m, 1 H), 3.95−3.89 (m, 1 H), 3.64−3.55 (m, 1 H); $^{13}$C NMR $\delta$ 154.7 (q, $J_{C-F}$ = 38.3 Hz), 141.5, 132.0, 130.7, 129.0, 127.7, 127.5, 126.7, 119.7, 117.2, 115.9 (q, $J_{C-F}$ = 288 Hz), 111.7, 52.7, 45.4, 42.6. Anal. ($C_{16}H_{10}ClF_3N_2O \cdot {}^1/_3H_2O$) C, H, N.

**General Method of Nitration Using fHNO$_3$. Preparation of 1-(Chloromethyl)-5-nitro-3-(trifluoroacetyl)-1,2-dihydro-3*H*-benzo[*e*]indole-6-carbonitrile (59, EWG = 6-CN).** A solution of **97** (60 mg, 0.18 mmol) in $CH_2Cl_2$ (10 mL) was treated with fuming $HNO_3$ (1.5 mL) and stirred for 30 min at room temperature. The reaction was quenched with ice and extracted with $CH_2Cl_2$ (3 × 50 mL). The extracts were dried and concentrated under reduced pressure. The residue was chromatographed on silica gel, eluting with EtOAc/petroleum ether (from 1:4 to 1:1), to give **59** (EWG = 6-CN) (28 mg, 41%) as a brown solid: mp (EtOAc/petroleum ether) 201−205 °C (dec); $^1$H NMR [$(CD_3)_2SO$] $\delta$ 8.87 (s, 1 H), 8.63 (dd, $J$ = 8.5, 1.1 Hz, 1 H), 8.40 (dd, $J$ = 7.3, 1.0 Hz, 1 H), 7.93 (dd, $J$ = 8.5, 7.3 Hz, 1 H), 4.73−4.61 (m, 2 H), 4.55−4.49 (m, 1 H), 4.22−4.15 (m, 1H), 4.12−4.07 (m, 1 H); $^{13}$C NMR $\delta$ 153.8 (q, $J_{C-F}$ = 37.6 Hz), 146.8, 139.8, 137.7, 132.6, 130.3, 129.4, 128.3, 118.7, 115.4 (q, $J_{C-F}$ = 288 Hz), 115.1, 114.8, 105.4, 52.7, 47.4, 41.1. Anal. ($C_{16}H_9ClF_3N_3O_3$) C, H, N.

**Preparation of 21.** $Cs_2CO_3$ (0.5 g, 1.5 mmol) was added to a solution of **59** (EWG = 6-CN) (100 mg, 0.26 mmol) in $CH_2Cl_2$/MeOH (1:1, 20 mL). The mixture was stirred at room temperature for 15 min, then poured into water (100 mL) and extracted with $CH_2Cl_2$ (3 × 50 mL). The extracts were dried, and the solution was mixed with a solution of dry HCl in dioxane (10 mL). After 30 min the mixture was evaporated under reduced pressure. To the residue was added 5-[2-(dimethylamino)-ethoxy]indole-2-carboxylic acid hydrochloride (100 mg, 0.34 mmol), followed by EDCI (100 mg, 0.55 mmol), anhydrous TsOH (20 mg, 0.12 mmol), and DMA (3 mL), and the mixture was stirred at room temperature overnight. The mixture was poured into dilute ice-cold aqueous $NaHCO_3$ and extracted with EtOAc (3 × 50 mL). The combined organic phases were washed with water (3 × 30 mL) and then brine, dried, and evaporated to give **21** (88 mg, 66%): mp ($CH_2Cl_2$/MeOH) > 300 °C; $^1$H NMR ($CDCl_3$) $\delta$ 9.26 (br, 1 H), 9.10 (s, 1 H), 8.14 (dd, $J$ = 8.5, 1.0 Hz, 1 H), 8.02 (dd, $J$ = 8.2, 0.9 Hz, 1 H), 7.73 (dd, $J$ = 8.5, 7.3 Hz, 1 H), 7.38 (d, $J$ = 8.9 Hz, 1 H), 7.15−7.05 (m, 3 H), 4.95−4.90 (m, 1 H), 4.84−4.77 (m, 1 H), 4.37−4.29 (m, 1 H), 4.17−4.11 (m, 2 H), 3.96−3.90 (m, 1 H), 3.67−3.58 (m, 1 H), 2.81−2.76 (m, 2 H), 2.37 (s, 6 H); $^{13}$C NMR $\delta$ 160.7, 154.3, 148.3, 142.4, 135.9, 131.7, 130.2, 129.1, 128.7, 128.2, 127.9, 127.6, 119.2, 118.3, 116.3, 115.2, 112.8, 107.9, 106.8, 103.7, 66.8, 58.5, 54.8, 46.0, 45.5, 43.4. Anal. ($C_{27}H_{24}ClN_5O_4 \cdot {}^1/_4H_2O$) C, H, N.

**Preparation of 1-(Chloromethyl)-3-{5-[2-(dimethylamino)-ethoxy]indol-2-carbonyl}-5-nitro-1,2-dihydro-3*H*-benzo[*e*]indole-6-sulfonamide (22). 1-(Chloromethyl)-5-nitro-3-(trifluoroacetyl)-1,2-dihydro-3*H*-benzo[*e*]indole-6-sulfonyl Chloride (59, EWG = 6-SO$_2$Cl).** The 6-sulfonyl chloride **95** (750 mg, 1.9 mmol) was dissolved in concentrated $H_2SO_4$ (20 mL), the solution was cooled in an ice bath, and a solution of $KNO_3$ (195 mg, 1.95 mmol) in $H_2SO_4$ (5 mL) was added slowly. The mixture was stirred vigorously for 30 min, quenched with cold water, and extracted with EtOAc (3 × 50 mL). The extracts were dried and concentrated under reduced pressure, and the resulting solid was separated by column chromatography on silica gel. Elution with EtOAc/petroleum ether (from 1:4 to 1:1) gave **59** (EWG = 6-$SO_2$Cl) (202 mg, 59%, based on consumption of starting material): mp (EtOAc/petroleum ether) 169 °C (dec); $^1$H NMR [$(CD_3)_2SO$] $\delta$ 8.63 (s, 1 H), 8.22 (dd, $J$ = 7.6, 0.9 Hz, 1 H), 8.13 (dd, $J$ = 8.4 Hz, 1 H), 7.71 (dd, $J$ = 7.8, 7.8 Hz, 1 H), 4.66−4.56 (m, 2 H), 4.49−4.43 (m, 1 H), 4.17−4.02 (m, 2 H); $^{13}$C NMR $\delta$ 153.4 (q, $J_{C-F}$ 37.4 Hz), 149.2, 145.2, 137.7, 131.4, 130.5, 129.9, 127.7, 124.9, 118.6, 115.6 (q, $J_{C-F}$ = 288 Hz) 114.3, 52.6, 47.5, 41.4. Anal. ($C_{15}H_9Cl_2F_3N_2O_5S$) C, H, N. **95** (457 mg, 61%) was also recovered.

**Preparation of 22.** A solution of **59** (EWG = 6-$SO_2$Cl) (300 mg, 0.66 mmol) in $CH_2Cl_2$/THF (1:1, 50 mL) was treated with concentrated $NH_3$ (0.5 mL) at room temperature for 30 min, followed by $Cs_2CO_3$ (0.5 g, 1.5 mmol) and stirring for another

15 min. The mixture was poured into water (100 mL) and extracted with $CH_2Cl_2$ (3 × 50 mL), and the extracts were dried. To this solution was added a solution of dry methanolic HCl (10 mL). After 10 min the mixture was evaporated to dryness under reduced pressure. To the residue was added 5-[2-(dimethylamino)ethoxy]indole-2-carboxylic acid hydrochloride (160 mg, 0.55 mmol), EDCI (200 mg, 1.1 mmol), anhydrous TsOH (20 mg, 0.12 mmol), and DMA (5 mL). The mixture was stirred at room temperature overnight, then poured into a dilute solution of $NaHCO_3$ in ice−water and extracted with EtOAc (3 × 50 mL). The combined organic phases were washed with water (3 × 30 mL) and then brine, dried, and concentrated under reduced pressure, and the residue was crystallized from $CH_2Cl_2$/MeOH to give **22** (200 mg, 53%): mp > 320 °C; $^1$H NMR [$(CD_3)_2SO$] $\delta$ 11.73 (s, 1 H), 9.03 (s, 1 H), 8.44 (d, $J$ = 7.5 Hz, 1 H), 8.40 (d, $J$ = 8.3 Hz, 1 H), 7.92 (dd, $J$ = 8.0, 7.8 Hz, 1 H), 7.47 (s, 2 H), 7.41 (d, $J$ = 8.9 Hz, 1 H), 7.16−7.21 (m, 2 H), 6.95 (dd, $J$ = 8.7, 2.4 Hz, 1 H), 4.98−4.89 (m, 1 H), 4.73−4.61 (m, 2 H), 4.17−4.02 (m, 4 H), 2.68−2.63 (m, 2 H), 2.24 (s, 6 H); $^{13}$C NMR $\delta$ 160.4, 153.0, 147.3, 141.1, 140.8, 131.9, 131.5, 130.6, 130.3, 129.7, 127.8, 127.5, 127.4, 116.8, 116.7, 116.4, 113.2, 106.1, 103.1, 66.0, 57.7, 54.7, 47.8, 45.4, 41.6. Anal. ($C_{26}H_{26}ClN_5O_6S$) C, H, N, Cl.

**Preparation of 6-Acetyl-1-(chloromethyl)-3-{5-[2-(dimethylamino)ethoxy]indol-2-carbonyl}-5-nitro-1,2-dihydro-3*H*-benzo[*e*]indole (23).** The trifluoroacetamide **59** (EWG = 6-COMe) was deprotected and the amide formed using EDCI as described in the general method above to give **23** (75%): mp ($CH_2Cl_2$/MeOH) > 300 °C; $^1$H NMR [$(CD_3)_2SO$] $\delta$ 11.71 (s, 1 H), 9.01 (s, 1 H), 8.35 (dd, $J$ = 8.5, 0.9 Hz, 1 H), 8.13 (dd, $J$ = 7.2, 0.8 Hz, 1 H), 7.80 (dd, $J$ = 8.5, 7.2 Hz, 1H), 7.41 (d, $J$ = 8.9 Hz, 1 H), 7.20−7.15 (m, 2 H), 6.94 (dd, $J$ = 8.9, 2.4 Hz, 1 H), 4.98−4.89 (m, 1 H), 4.73−4.68 (m, 1 H), 4.67−4.60 (m, 1 H), 4.16−4.04 (m, 4 H), 2.69 (s, 3 H), 2.68−2.63 (m, 2 H), 2.24 (s, 6 H); $^{13}$C NMR $\delta$ 200.7, 200.6, 160.5, 153.0, 147.2, 141.3, 136.8, 131.9, 131.7, 130.3, 129.7, 127.6, 127.4, 127.0, 117.4, 116.3, 115.7, 113.2, 106.1, 103.1, 66.1, 57.7, 54.7, 47.7, 45.4, 41.5, 28.5. Anal. ($C_{28}H_{27}ClN_4O_5 \cdot {}^1/_2H_2O$) C, H, N, Cl.

**Preparation of 1-(Chloromethyl)-3-{5-[2-(dimethylamino)-ethoxy]indol-2-carbonyl}-5-nitro-1,2-dihydro-3*H*-benzo[*e*]indole-6-carboxamide (24). 1-(Chloromethyl)-3-(trifluoroacetyl)-1,2-dihydro-3*H*-benzo[*e*]indole-6-carboxamide (98).** Solid **97** (500 mg, 1.48 mmol) was added to 90% $H_2SO_4$ (5 mL) and heated to 70 °C for 1 h. After cooling to room temperature, the mixture was poured into ice−water and extracted with EtOAc (3 × 50 mL). The extracts were dried and concentrated under reduced pressure. Chromatography of the residue on silica gel, eluting with EtOAc/petroleum ether (from 1:1 to 1:0), gave **98** (410 mg, 78%) as a white solid: mp (EtOAc/petroleum ether) 190−193 °C; $^1$H NMR ($CDCl_3$) $\delta$ 8.54−8.45 (m, 2 H), 7.89 (d, $J$ = 5.8 Hz, 1 H), 7.69 (dd, $J$ = 7.1, 1.1 Hz, 1 H), 7.59 (dd, $J$ = 8.4, 7.1 Hz, 1 H), 5.91 (br, 2 H), 4.69−4.61 (m, 1 H), 4.48−4.40 (m, 1 H), 4.25−4.17 (m, 1 H), 3.97−3.89 (m, 1 H), 3.58−3.50 (m, 1 H); $^{13}$C NMR $\delta$ 170.7, 153.8 (q, $J_{C-F}$ = 38.0 Hz), 140.5, 134.6, 129.7, 128.6, 126.5, 125.6, 125.4, 124.8, 118.4, 115.4 (q, $J_{C-F}$ = 288 Hz), 53.4, 45.4, 43.0. Anal. ($C_{16}H_{12}ClF_3N_2O_2$) C, H, N, Cl.

**1-(Chloromethyl)-5-nitro-3-(trifluoroacetyl)-1,2-dihydro-3*H*-benzo[*e*]indole-6-carboxamide (59, EWG = 6-CONH$_2$). 98** was nitrated using fuming $HNO_3$ as described in the general method above [chromatography with EtOAc/petroleum ether/methanol (from 5:1:0 to 9:0:1)] to give **59** (EWG = 6-$CONH_2$) (150 mg, 45%) as yellow solid: mp 272−277 °C (EtOAc/petroleum ether); $^1$H NMR [$(CD_3)_2SO$] $\delta$ 8.78 (s, 1 H), 8.30 (dd, $J$ = 8.4, 1.1 Hz, 1 H), 8.24 (s, 1 H), 7.91 (dd, $J$=7.1, 1.0 Hz, 1 H), 7.80 (dd, $J$=8.4, 7.1 Hz, 1 H), 7.57 (s, 1 H), 4.69−4.61 (m, 2 H), 4.52−4.47 (m, 1 H), 4.21−4.15 (m, 1 H), 4.13−4.07 (m, 1 H); $^{13}$C NMR $\delta$ 169.3, 153.6 (q, $J_{C-F}$ = 37.8 Hz), 148.1, 138.6, 133.4, 132.4, 130.1, 128.7, 127.9, 126.1, 119.3, 115.6 (q, $J_{C-F}$ = 288 Hz), 114.4, 52.7, 47.5, 41.2. Anal. ($C_{16}H_{11}ClF_3N_3O_4$) C, H, N, Cl.

Further elution gave the 9-nitro isomer 1-(chloromethyl)-9-nitro-3-(trifluoroacetyl)-1,2-dihydro-3*H*-benzo[*e*]indole-6-carboxamide (100 mg, 30%), characterized only by NMR: $^1$H NMR ($CDCl_3$) $\delta$ 8.71 (d, $J$ = 9.2 Hz, 1 H), 8.49 (d, $J$ = 9.2 Hz, 1 H), 7.83 (d, $J$ = 7.6 Hz, 1 H), 7.67 (d, $J$ = 7.6 Hz, 1 H), 6.30 (br, 1 H), 6.13 (br, 1 H), 4.57−4.50 (m, 1 H), 4.47−4.39 (m, 1 H), 4.02 (s, 1 H), 3.59−3.57 (m, 1 H), 3.33−3.25 (m, 1 H).

**Preparation of 24.** The trifluoroacetamide **59** (EWG = 6-$CONH_2$) was deprotected and the amide formed using EDCI as described in the general method above to give **24** (48 mg, 72%): mp ($CH_2Cl_2$/MeOH) > 350 °C; $^1$H NMR [$(CD_3)_2SO$] $\delta$ 11.7 (s, 1 H), 8.95 (s, 1 H), 8.26 (dd, $J$ = 8.4, 1.0 Hz, 1 H), 8.20 (s, 1 H), 7.84 (dd, $J$ = 7.1, 1.0 Hz, 1 H), 7.76 (dd, $J$ = 8.3, 7.1 Hz, 1 H), 7.52 (s, 1 H), 7.40 (d, $J$ = 8.9 Hz, 1 H), 7.18 (d, $J$ = 2.1 Hz, 2 H), 6.95 (dd, $J$ = 8.9, 2.4 Hz, 1 H), 4.97−4.90 (m, 1 H), 4.72−4.58 (m, 2 H), 4.16−4.04 (m, 4 H), 2.68−2.63 (m, 2 H), 2.24 (s, 6 H); $^{13}$C NMR $\delta$ (one C not observed) 169.5, 160.4, 153.0, 147.9, 140.8, 133.4, 131.8, 131.0, 130.3, 129.9, 127.7, 127.4, 125.7, 118.3, 116.3, 115.2, 113.2, 106.0, 103.1, 66.2, 57.7, 54.7, 47.8, 45.5, 41.5. Anal. ($C_{27}H_{26}ClN_5O_5$) C, H, N, Cl.

**General Method for Hydrogenation of Nitro Compounds to Anilines. Preparation of 5-Amino-1-(chloromethyl)-7-(methylsulfonyl)-3-(5,6,7-trimethoxyindol-2-carbonyl)-1,2-dihydro-3*H*-benzo[*e*]indole (40).** A solution of **10** (63 mg, 0.11 mmol) in THF (15 mL) with $PtO_2$ (25 mg) was hydrogenated at 45 psi for 90 min. The mixture was filtered through Celite, the filtrate was evaporated under reduced pressure, and the residue was triturated with EtOAc to give **40** (25 mg, 42%) as a yellow solid: mp 266−268 °C; $^1$H NMR [$(CD_3)_2SO$] $\delta$ 11.42 (s, 1 H), 8.69 (d, $J$ = 1.7 Hz, 1 H), 7.96 (d, $J$ = 8.9 Hz, 1 H), 7.81 (dd, $J$ = 8.8, 1.8 Hz, 1 H), 7.76 (s, 1 H), 7.08 (d, $J$ = 2.0 Hz, 1 H), 6.97 (s, 1 H), 6.40 (s, 2 H), 4.71 (dd, $J$ = 10.9, 9.0 Hz, 1 H), 4.44 (dd, $J$ = 11.0, 1.8 Hz, 1 H), 4.18−4.11 (m, 1 H), 3.97 (dd, $J$ = 11.0, 3.2 Hz, 1 H), 3.94 (s, 3 H), 3.82 (s, 3 H), 3.80 (s, 3 H), 3.78 (dd, $J$ = 11.0, 7.6 Hz, 1 H), 3.25 (s, 3 H). Anal. ($C_{26}H_{26}ClN_3O_6S$) C, H, N.

**One-Electron Reduction Potentials.** Pulse radiolysis experiments were performed using The University of Auckland's linear accelerator equipped with a radical detection system, as previously described.[43] One-electron reduction potentials, $E(1)$, were determined at pH 7 in deaerated solutions containing 2-propanol (0.5 M) and phosphate buffer (2.5 mM) (compounds **19**, **24**, and **31**) or imidazole buffer (3 mM) (all other compounds). Deaeration by $N_2$ gas exchange without bubbling the solutions helped to prevent precipitation of the less soluble compounds. The following reference compounds and reduction potentials were used: triquat (7,8-dihydro-6*H*-dipyrido[1,2-*a*:2′,1′-*c*][1,4]diazepinium dibromide), −548 ± 7 mV (for **19**); methylviologen (1,1′-dimethyl-4,4′-bipyridinium dichloride), −447 ± 7 mV (for all other compounds). Regression analysis provided the following equations:

6-Substituted:

$$E(1)\ (\mathrm{mV}) = -509 + 42\sigma_p \qquad (n = 5,\quad r^2 = 0.57)$$

7-Substituted:

$$E(1)\ (\mathrm{mV}) = -508 + 199\sigma_p \qquad (n = 6,\quad r^2 = 0.97)$$

8-Substituted:

$$E(1)\ (\mathrm{mV}) = -507 + 122\sigma_p \qquad (n = 5,\quad r^2 = 0.88)$$

**In Vitro Cytotoxicity.** For the cell lines in Tables 1−4 inhibition of proliferation of log phase monolayers was assessed in 96-well plates as previously described.[44] The drug exposure time was 4 h under aerobic (20% $O_2$) or anoxic (< 20 ppm $O_2$) conditions followed by sulforhodamine B staining 5 days later.

The $IC_{50}$ was determined by interpolation as the drug concentration required to inhibit cell density to 50% of that of the controls on the same plate. Isolation of the A549 P450R$^{puro}$ cell line has been previously described.[23]

**In Vitro Metabolism.** Liver S9 was prepared by homogenizing livers from female homozygous nude (CD1-Foxn1$^{nu/nu}$) mice bred by The University of Auckland using breeding mice from Charles River Laboratories (Wilmington, MA) in 3 volumes of phosphate buffer (67 mM, pH 7.4, containing 1.15% KCl). The homogenate was centrifuged at 4 °C (10000*g* for 20 min) and stored at −80 °C. Tumor S9 was prepared by homogenizing HT29 tumors grown in the same strain of mice in 5 volumes of Tris buffer (100 mM, pH 7.4). The homogenate was centrifuged at 4 °C (10000*g* for 5 min followed by 9000*g* for 15 min). The protein concentrations of the liver and tumor S9 were 40.1 and 12.1 mg/mL respectively. Incubations were performed by adding nitroCBI (50 $\mu$M in formate buffer, diluted from a 5 mM stock solution in DMSO) to a mixture of S9, NADPH (10 mM in phosphate buffer), and sufficient phosphate buffer (67 mM, pH 7.4) to give a total volume of 250 $\mu$L and final concentrations of 8 mg/mL protein, 1 mM NADPH, and 10 $\mu$M nitroCBI. Vials were sealed and incubated at 37 °C; for incubations under hypoxia the experiment was set up in an anaerobic chamber using deoxygenated solvents. Reactions were quenched by the addition of an equal volume of ice-cold acetonitrile and centrifuged at 4 °C (12 000 rpm for 10 min). A 50 $\mu$L aliquot of the supernatant was diluted with 25 $\mu$L of ammonium formate buffer (45 mM, pH 4.5) and analyzed by HPLC using an Altima 150 mm × 2.1 mm column (5 $\mu$m C8 packing) and an aqueous ammonium formate (45 mM, pH 4.5)/acetonitrile gradient. Gradients from 80% to 10% aqueous phase over 21 min and from 80% to 20% to 10% aqueous phase over 16 min were used. Detection was by diode array absorbance, or for MS analysis by electrospray (positive mode and Auto MS(n); capillary voltage 4.5 kV) with an Agilent LC/MSD Trap-SL equipped with Agilent capillary HPLC using a drying gas flow rate of 4.4 L/min, nebulizer pressure of 12 psi, and a drying gas temperature of 325 °C.

**Clonogenic Assay Using Single Cell Suspensions or Spheroids.** Clonogenic assay with HT29 cells comparing single-cell suspensions with intact spheroids was performed as described previously.[45] The mean cell density during the intact and dissociated exposures (typically $1 \times 10^6$ cells/mL) and ambient conditions (5% $CO_2$ in $O_2$ or $N_2$, 37 °C) were identical, with drug exposure for 4 h. Colonies were grown for 14 days and stained with methylene blue, and those with >50 cells were counted to determine the plating efficiency (PE). Surviving fraction (SF) was calculated as PE(treated)/PE(controls). Cell killing was quantitated as the drug concentration required to lower the surviving fraction to 10% ($C_{10}$).

**Excision Assay.** SiHa tumors were grown in female homozygous nude (CD1-Foxn1$^{nu/nu}$) mice by subcutaneous inoculation of $10^7$ cells from tissue culture, and mice were randomized to treatment when tumors reached a mean diameter of 8−10 mm. **28** was formulated in 5% DMSO, 20% PEG-400, 75% lactate buffer, pH 4.0 (v/v/v), and administered via a tail vein. Tumors were collected 18 h after treatment and dissociated enzymatically to determine clonogenic survivors per gram of tumor as previously described.[46] Significance of treatment effects was tested using ANOVA with Holm−Sidak post hoc test on log-transformed data.

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**Supporting Information Available:** Synthesis of all 7-, 8-, and 9-substituted nitroCBIs of Tables 1 and 2 and aminoCBIs of Table 3 (compounds **9**−**18**, **25**−**38**, **41**−**52**); combustion analysis results for new compounds; 2D NMR spectra of **105**, **162**, **161**, **97**, **59** (EWG = 6-COMe), **92**, **119**, **144**; X-ray crystallographic data for **133** and **105**; relationship between one-electron reduction potential [$E(1)$] and cytotoxicity ($IC_{50}$) for the nitroCBIs of Table 2; relationship between hypoxic cytotoxicity ratio (HCR) in HT29 and one-electron reduction potential [$E(1)$] for the compounds of Table 2; correlation between cytotoxicity in HT29 and Skov3; and MS and UV−visible spectroscopy characterization of **28** and its major S9 metabolites under oxic and hypoxic incubation conditions. This material is available free of charge via the Internet at http://pubs.acs.org.

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