Bioorganic & Medicinal Chemistry 19 (2011) 4851–4860

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry

journal homepage: www.elsevier.com/locate/bmc

The effect of sulfonate leaving groups on the hypoxia-selective toxicity of nitro analogs of the duocarmycins

Amir Ashoorzadeh, Graham J. Atwell, Frederik B. Pruijn, William R. Wilson, Moana Tercel, William A. Denny *, Ralph J. Stevenson

Auckland Cancer Society Research Centre, Faculty of Medical and Health Sciences, The University of Auckland, Private Bag 92019, Auckland 1142, New Zealand

ARTICLE INFO

Article history:
Received 18 May 2011
Revised 22 June 2011
Accepted 26 June 2011
Available online 29 June 2011

Keywords:
Cyclopropylindoline
Hypoxic selectivity
Prodrug
Sulfonate leaving group
DNA minor groove alkylator
Antitumor
Neutral side chain

ABSTRACT

A series of 3-substituted (5-nitro-2,3-dihydro-1*H*-benzo[*e*]indol-1-yl)methyl sulfonate (nitroCBI) prodrugs containing sulfonate leaving groups undergo hypoxia-selective metabolism to form potent DNA minor groove alkylating agents. They were evaluated (along with chloride leaving group analogs for comparison) for their cytotoxicity against cultures of SKOV3 and HT29 human tumor cell lines under both aerobic and hypoxic conditions. Sulfonates with neutral side chains (e.g., 5,6,7-trimethoxyindole; TMI) show consistently higher hypoxic cytotoxicity ratios (HCRs) (34–246) than the corresponding chloro analogs (2.8–3.1) in SKOV3 cells, but these trends do not hold for compounds with cationic or polar neutral side chains.

1. Introduction

The class of compounds broadly known as the hydroxyCBIs (e.g., **1**)[1], evolved from natural product cyclopropylindolines such as duocarmycin SA[2] (**2**), have been widely explored as very potent cytotoxins targeted at the DNA minor groove, where they alkylate the N3 of adenine in a sequence-selective manner (Fig. 1).[1] It has been shown that the precursor *seco* analogs such as **1** are equally cytotoxic, due to their rapid conversion to the cyclopropylindoline form.[4] *N*-Acyl *O*-amino- (e.g., **3**) and quinone-containing analogs (e.g., **4**) of these have been explored[5,6] as bioreductively-activated prodrugs. We have taken a different approach, using 1-(chloromethyl)-5-nitro-2,3-dihydro-1*H*-benzo[*e*]indoles as hypoxia-activated prodrugs.[3,7–9]

We previously showed that replacement of the phenol in **1** with an amino group gave a compound (**5a**) of similar cytotoxic potency (IC_{50}s <1 nM in AA8 CHO cells following a 4 h exposure),[10] and with a similar mechanism of action; alkylation at adenine-N3 sites in AT-rich regions of DNA.[11] We also showed[12] that the corresponding nitro compound **5** was about 3000-fold less toxic than **5a** in AA8 CHO cells, presumably because it can no longer undergo spirocyclization and DNA alkylation, but could undergo hypoxia-selective reduction in cells to the much more potent **5a**.[8]

Nitro compounds such as **5** thus have the potential to be selectively activated in the hypoxic regions that are known to be present in a majority of human solid tumors.[13] Development of this class to date has sought to optimize the properties suggested to be needed for such prodrugs:[13,14] (i) high toxicity differential between prodrug and reduction product(s), (ii) rapid and selective activation by 1-electron reductases in an oxygen-sensitive process, (iii) high tissue penetration in order to reach the target hypoxic cells distant from the vasculature, and (iv) an ability of the active reduction product(s) to back-diffuse from the site of activation to kill surrounding cells (the bystander effect).

While nitroCBI **5** showed significantly lower cytotoxicity than its reduction product **5a**, its selective cytotoxicity for hypoxic over aerobic cell cultures was modest, with an HCR (hypoxic cytotoxicity ratio: IC_{50}aerobic/IC_{50}hypoxic) of only 2.5 in HT29 colon carcinoma cells after a 4 h exposure.[7] The reason for this was hypothesized to be the relatively low 1-electron reduction potential of the parent class; for example the more soluble analog **6,** which also has a low HCR, had a one electron reduction potential ($E(1)$) of −512 mV, and that of analog **5** is expected to be similar. This led to the study of a series of analogs of higher reduction

Abbreviations: CBI, *seco*-1,2,9,9a-tetrahydrocyclopropa[*c*]benz[*e*]indol-4-one; DIPEA, diisopropylethylamine; DMA, dimethylacetamide; EDCI·HCl, *N*-ethyl-*N*′-(3-dimethylaminopropyl)carbodiimide hydrochloride; HCR, hypoxic cytotoxicity ratio; TMI, 5,6,7-trimethoxyindole.

* Corresponding author. Tel.: +649 923 6144; fax: +64 9 373 7502.
E-mail address: b.denny@auckland.ac.nz (W.A. Denny).

0968-0896/$ - see front matter © 2011 Elsevier Ltd. All rights reserved.
doi:10.1016/j.bmc.2011.06.073

potentials, with electron-withdrawing groups on the A-ring. An example is the 7-SO_2NH_2 compound **7**, which had an *E*(1) of −390 mV and showed an HCR in the HT29 cell line of 330.[7] A later study on analogs of compound **7** showed that the related 7-$SO_2NH(CH_2)_2OH$ analog **8** had similarly high hypoxic selectivities across a range of cell lines.[9] The corresponding phosphate **9**, prepared as a much more water-soluble pre-prodrug that releases **8** in plasma, showed marked hypoxic cell killing and corresponding substantial tumor growth delays when used combination with radiation (to kill the well-oxygenated tumor cells) in mice bearing SiHa human cervical tumor xenografts.[9]

To date, while there have been substantial variations in the core alkylating subunit, the minor groove-binding side chain and the A-ring substituents of the nitroCBIs, little attention has been paid to variation of the leaving group which has essentially been restricted to chloro. A-ring substituents generally diminish the cytotoxicity of aminoCBIs but were found essential to observe significant HCRs for nitroCBIs in vitro when the leaving group was chloride. One sulfonate analog has been reported[15]; the aminoCBI methanesulfonate **10a**, which was equipotent with the corresponding chloro compound **5a** (IC_{50}s between 0.4 and 1.2 nM in EMT6 and SKOV3 human tumor cell lines).

Alternative leaving groups have been more extensively used in the aniline mustard class of DNA-alkylating drugs, where it is known that cytotoxicity correlates with the nucleofugality of the leaving group (e.g., bromo mustards are more reactive and more potent than chloro mustards).[16] Mesylate (OSO_2Me) and benzyl sulfonate (OSO_2CH_2Ph) groups have relative nucleofugalities comparable to that of iodide (ω 1.63, 1.34, and 1.33 eV, respectively) and greater than those of chloride or bromide (ω 0.77 and 0.90 eV, respectively).[17,18]

In this study we therefore explored structure-activity relationships for a number of nitroCBI prodrugs bearing sulfonate-based leaving groups and compared their cytotoxicities and HCRs with those of their chloride analogs and the known sulfonate **10**.[10,15] Questions to be considered included whether the steric bulk of the larger sulfonates might hinder binding at the DNA base in the minor groove and reduce prodrug toxicity, as has been suggested[19] with the hydroxyCBI compounds **11** and **12**, where the methyl compound **11** is considerably less potent than **12** (IC_{50}s 0.75 and 0.026 nM, respectively, in A549 human bronchial carcinoma cells, 24 h exposure).

2. Results and discussion

2.1. Chemistry

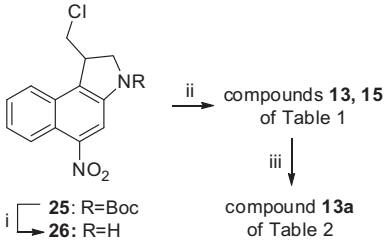
The new chloromethyl-5-nitroCBI reference analogs were prepared as shown in Scheme 1. Deprotection of the known Boc-protected intermediate **25**,[10] followed by the coupling reaction of the resulting crude amine **26** with 5-(2-hydroxyethoxy)indole-2-carboxylic acid[20] and (*E*)-3-(3-hydroxy-4-methoxyphenyl)acrylic acid using EDCI·HCl gave compounds **13** and **15,** respectively. Hydrogenation of **13** in THF over PtO_2 gave the corresponding aminoCBI **13a** in excellent yield. AminoCBIs **16a** and **18a** were prepared from nitroCBI precursors in the same way.

The cyclohexyl sulfonates **16** and **17** were made in the same way as the known[15] **10**, from hydroxymethyl-5-nitroCBI intermediate **27**[10] (Scheme 2) via formation of the cyclohexyl sulfonate **28**, followed by Boc deprotection and coupling of the resulting amine **29** with the appropriate side chain acids. Benzyl sulfonates **20** and **21** (Scheme 3), which contain free hydroxyl groups in the side chains, were prepared the same way, by initially sulfonating **27** to give **33**, followed by deprotection to amine **34**, to which the side chains were added as above. Analogs **18** and **19** were made by an alternative route, initially deprotecting **27** and coupling the resulting hydroxyl amine **30** with the appropriate side chains to give **31** and **32**, which were then sulfonated to give the end products (Scheme 3).

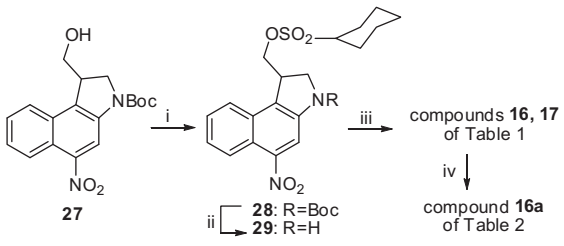
The synthesis of the tosylates **22**–**24** (Scheme 4) followed the former route (previous paragraph), with initial tosylation of **27** to give **35**, followed by Boc deprotection to give common intermediate **36**, to which the appropriate side chains were coupled. The latter reaction was complicated by the tosylate appearing to be a better leaving group than the other sulfonates. Thus coupling of **36** with 5-methoxy-1*H*-indole-2-carboxylic acid using EDCI·HCl mainly afforded the undesired chloromethyl product **14**, presumably due to chloride ion scrambling. Use of 1-ethyl-3-(3′-dimethylaminopropyl)carbodiimide methiodide (EDCI·MeI) gave the desired product, but the reaction was very slow and the yield was relatively low. Coupling of **36** with 5,6,7-trimethoxyindole-2-carboxylic acid using EDCI·HCl also gave the chloromethyl product **5** together with the desired product **22** in a ratio of 1:3, but these could be separated by column chromatography. Surprisingly, reaction of **36** with 5-(2-hydroxyethoxy)-1*H*-indole-2-carboxylic acid using EDCI·HCl provided the desired product **24** in quantitative yield. Special care, including cooling, had to be taken during basic workup of the sulfonates as these leaving groups were more prone to base-promoted elimination (of RSO_3H) than their chloride analogs.

It should be noted that while several aminoCBIs were able to be synthesized straightforwardly from their nitroCBI precursors via hydrogenation (H_2/PtO_2) and isolated, others (e.g., **18a**) appeared to undergo facile spirocyclization to the resultant imine.[7] Those with cinnamate side chains could not be prepared in this way due to reduction of the alkene.

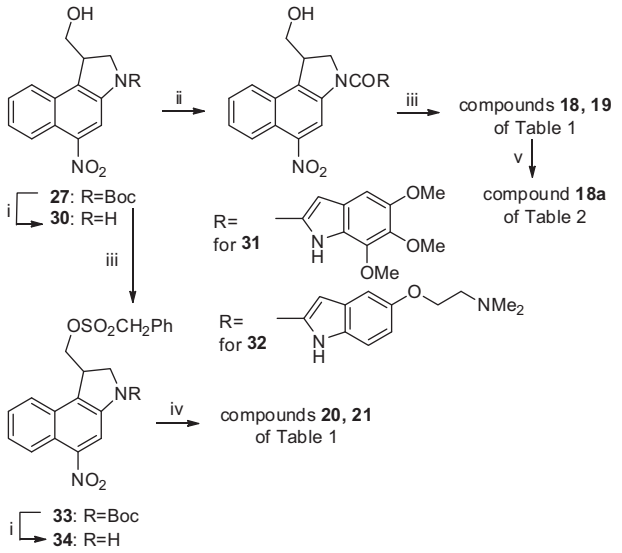
In all cases (with the sole exception of **24**) the nitroCBI sulfonates with neutral side chains showed higher solubility in culture medium than the corresponding chloro compounds, although this gain in aqueous solubility was not seen with nitroCBIs bearing a basic dimethylaminoethyl side chain (Table 1).



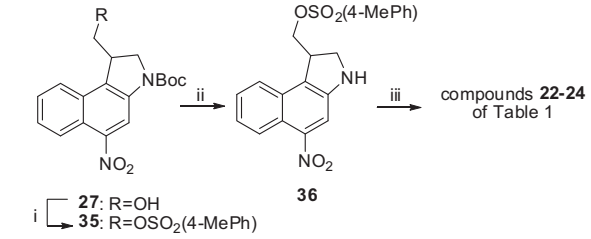
Scheme 1. Synthesis of compounds **13, 15,** and **13a**. Reagents and conditions: (i) HCl, dioxane, 10 °C; (ii) 5-(2-hydroxyethoxy)indole-2-carboxylic acid or (*E*)-3-(3-hydroxy-4-methoxyphenyl)acrylic acid, EDCI·HCl, DMA; and (iii) H_2, PtO_2, THF.



Scheme 2. Synthesis of compounds **16, 17,** and **16a**. Reagents and conditions: (i) cyclohexanesulfonyl chloride, DIPEA, CH_2Cl_2, 0 °C; (ii) HCl, dioxane, 10 °C; (iii) 5,6,7-trimethoxyindole-2-carboxylic acid or 5-(2-(dimethylamino)ethoxy)-1*H*-indole-2-carboxylic acid, EDCI·HCl, DMA; and (iv) H_2, PtO_2,THF.



Scheme 3. Synthesis of compounds **18**–**21** and **18a**. Reagents and conditions: (i) HCl, dioxane, 10 °C; (ii) 5,6,7-trimethoxyindole-2-carboxylic acid or 5-(2-(dimethylamino)ethoxy)-1*H*-indole-2-carboxylic acid hydrochloride, EDCI·HCl, DMA; (iii) α-toluenesulfonyl chloride, pyridine, 0 °C; (iv) 5-(2-hydroxyethoxy)indole-2-carboxylic acid or (*E*)-3-(3-hydroxy-4-methoxyphenyl)acrylic acid, EDCI·HCl, TsOH, DMA; (v) H_2, PtO_2,THF.



Scheme 4. Synthesis of compounds **22**–**24**. Reagents and conditions: (i) TsCl, pyridine, CH_2Cl_2, 0 °C or TsCl, pyridine, DMAP, CH_2Cl_2, 0 °C; (ii) TFA, CH_2Cl_2 or HCl, dioxane, 10 °C; and (iii) 5,6,7-trimethoxyindole-2-carboxylic acid or 5-methoxy-1*H*-indole-2-carboxylic acid or 5-(2-hydroxyethoxy)-1*H*-indole-2-carboxylic acid, TsOH, EDCI·HCl or EDCI·MeI, DMA.

2.2. Biology

The nitroCBIs bearing sulfonate leaving groups were evaluated for their cytotoxicity against cultures of SKOV3 (ovarian carcinoma) and HT29 (colon carcinoma), under both aerobic (20% O_2) and hypoxic (<20 ppm O_2) conditions as described previously,[21] and the results are given in Table 1, Figures. 2 and 3. Compounds **5, 6, and 13**–**15** are the comparators with the chloride leaving group, bearing a range of lipophilic (**5, 14**), polar neutral (**13, 15**) and cationic (**6**) side chains. Compounds **10, 16**–**24** are ordered according to the four different leaving groups studied; OSO_2Me (**10**), OSO_2cyclohexyl (**16, 17**), OSO_2CH_2Ph (**18**–**21**) and OSO_2(4-MePh) (**22**–**24**). Overall, the cytotoxicities of the compounds under both oxic and hypoxic conditions were broadly similar in SKOV3 and HT29 cell lines, although the HCR values for the more selective compounds were higher in SKOV3.

The sulfonates with A side chains (TMI) show consistently higher HCRs than the corresponding chloride compounds. Thus, compounds **5** (Cl) and **10** (OSO_2Me) have HCRs of 2.8 and 39, respectively, in SKOV3 cells. Similarly, the OSO_2cyclohexyl (**16**), OSO_2CH_2Ph (**16**) and OSO_2(4-MePh) (**22**) leaving groups all provided much higher HCRs (146, 246 and 72; Table 1) than **5**. The 5-OMe-indole pair showed a similar trend: **14/24** (OSO_2(4-MePh)) 3.1/34 in SKOV3. The higher HCR values for the sulfonates were mainly due to increased cytotoxicity under hypoxia, although decreased aerobic toxicity also contributed. The highest HCRs were shown by the most bulky sulfonates (**16, 18**, and **22**). This was, surprisingly, not true for the compounds with cationic or polar neutral side chains, where there was little difference in HCRs between chlorides and sulfonates in either cell line. Thus for compounds with cationic side chains the ratios for SKOV3 were: **6/17** (OSO_2cyclohexyl) 1.9/1.5; and **6/19** (OSO_2CH_2Ph) 1.9/1.8. Similarly, for polar neutral side chains the ratios were: **13/20** (OSO_2CH_2Ph) 3.8/7.1; **13/23** (OSO_2(4-MePh)) 3.8/2.8; and **15/21** (OSO_2CH_2Ph) 3.3/3.9.

Table 2 records the cytotoxicities and HCRs for two of the chloro nitroCBIs (**5, 13**) and their corresponding amine reduction products (**5a, 13a**); the active metabolites of these prodrugs.[7,8] For compounds with the TMI side chain only, there is comparison of nitro and amino pairs bearing two different sulfonate leaving groups; OSO_2Me (**10, 10a**) and OSO_2cyclohexyl (**16, 16a**). As previously shown[7,8] for the chloro compounds the nitro group provides a large degree of deactivation of cytotoxicity, with average nitro/amino oxic IC_{50} ratios of about 3000 for **5/5a** and **13/13a** in the two cell lines. The amine effectors of two sulfonates (**10a, 16a**) showed similar levels of potency (IC_{50}s 1–5 nM) and degrees of deactivation (nitro/amino ratios of 2000- to 3000-fold). As expected, all of the amino analogs showed HCRs close to unity.

As noted above, while about half of the nitroCBI sulfonates studied showed significant hypoxic cell selectivity (>30 in SKOV3 cells), the effect did vary with the nature of the side chain. The most hypoxia selective compound in SKOV3 and HT29 cultures was the OSO_2CH_2Ph/TMI analog **18**. Compound **18** was evaluated in a panel of cell lines, and the results are shown in Table 3. The HCR values showed large differences between the cell lines, varying from a low of 3.6 for H1299 cells to a high of 246 for SKOV3 cells, with a median value of 15.6-fold. To evaluate whether this reflects differences in reductase activity between cell lines, we compared a transfected A459 line which over-expresses NADPH: cytochrome P450 reductase (P450R). Forced P450R expression, to ninefold higher than the parental line[22] caused a fourfold reduction in the hypoxic IC_{50} but had no significant effect on the aerobic IC_{50} (Table 3). This establishes that **18** is a substrate for P450R, and that redox cycling as a result of its one-electron reduction in aerobic cells is not responsible for its aerobic cytotoxicity in A459 cells. We therefore evaluated whether differences in P450R activity in these cell lines (measured as NADPH-dependent cyanide-sensitive cytochrome c reductase activity)[23] might account for their differences in sensitivity under hypoxia, but there was no significant correlation between P450R activity and hypoxic IC_{50} of **18** by Pearson product moment correlation (correlation coefficient −0.159, $p = 0.682$).

3. Conclusions

The current study was performed to investigate structure–activity relationships influencing cytotoxicity and hypoxic selectivity for a series of nitroCBI prodrugs containing sulfonate leaving groups, with chloride leaving group analogs as comparators. The general conclusion is that sulfonate leaving groups offer a way of increasing hypoxic selectivity in nitroCBIs without introducing A-ring substituents. Furthermore, basic side chains do not appear to be necessary to produce high HCRs, in contrast to chloro analogs. This is potentially useful as basic side chains may compromise distribution (by sequestration in acidic organelles) or metabolism.

The sulfonates without solubilizing side chains showed modestly higher solubility in culture medium than the corresponding chloro compounds, although additional solubilizing strategies will

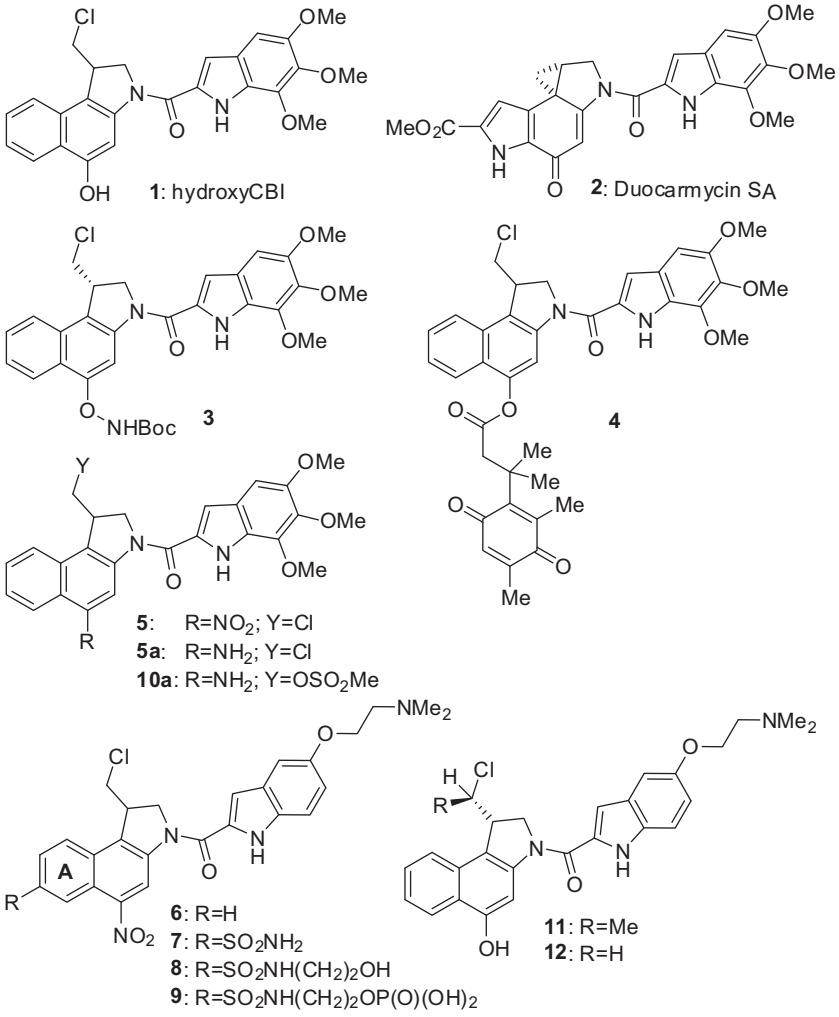


Figure 1. Structures of some previously reported CBIs.

be required to formulate these prodrugs for in vivo evaluation. The results demonstrate the utility of sulfonate leaving groups, due to the equivalent potencies of the amino compounds and the generally much larger HCRs of the nitro compounds compared with the chloro series. This study suggests that combining suitable A-ring substituents, which may be useful (even if not needed for high HCR) for appending functionality to allow preparation of phosphate pre-prodrugs as recently demonstrated in the chloro series,[9] and sulfonate leaving groups could provide analogs with higher hypoxic selectivity and improved water solubility. Regarding the criteria listed earlier for prodrugs, several of the sulfonates do show high toxicity differentials between prodrug and effector and effective oxygen-sensitive activation. The transport issues have not been addressed here.

4. Experimental

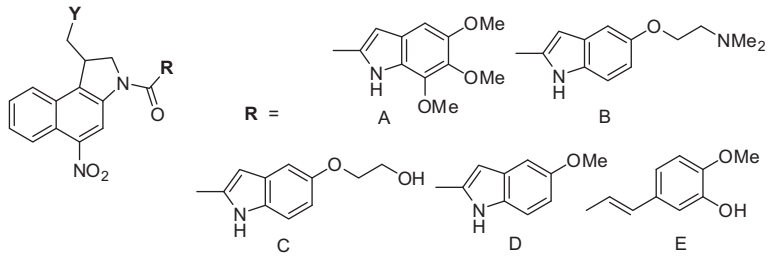
4.1. Chemistry

Final products were analyzed by reverse-phase HPLC (Alltima C18 5 μm column, 150 × 3.2 mm; Alltech Associated Inc., Deerfield, IL) using an Agilent HP1100 equipped with a diode-array detector. Mobile phases were gradients of 80% CH_3CN/20% H_2O (v/v) in 45 mM NH_4O_2CH at pH 3.5 and 0.5 mL/min. Purity was determined by monitoring at 330 ± 50 nm and was >95%. 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 ^1H and 100 MHz for ^{13}C spectra.

4.1.1. (1-(Chloromethyl)-5-nitro-1*H*-benzo[*e*]indol-3(2*H*)-yl)(5-(2-hydroxyethoxy)-1*H*-indol-2-yl)methanone (13) (Scheme 1)

A solution of *tert*-butyl 1-(chloromethyl)-5-nitro-1*H*-benzo[*e*]indole-3(2*H*)-carboxylate[10] (**25**) (600 mg, 1.65 mmol) in dioxane (15 mL) was saturated with HCl gas at 10 °C, stirred at room temperature for 1 h, and then concentrated under reduced pressure. The resulting crude amine **26** was dissolved in DMA (5 mL) and 5-(2-hydroxyethoxy)indole-2-carboxylic acid[20] (402 mg, 1.82 mmol) and EDCI·HCl (793 mg, 4.14 mmol) were added, and the mixture was stirred at room temperature for 4 h. Addition of aqueous $KHCO_3$ provided a solid that was further recrystallized from DMF/EtOAc/petroleum ether (2×) to give **13** (620 mg, 80%): mp 249–250 °C; ^1H NMR [$(CD_3)_2SO$] δ 11.71 (s, 1H), 9.16 (s, 1H), 8.35 (dd, *J* = 7.2, 2.5 Hz, 1H), 8.22 (dd, *J* = 6.9, 2.3 Hz, 1H), 7.79–7.70 (m, 2H), 7.42 (d, *J* = 8.9 Hz, 1H), 7.19 (d, *J* = 1.3 Hz, 1H), 7.17 (d, *J* = 2.2 Hz, 1H), 6.96 (dd, *J* = 8.9, 2.4 Hz, 1H), 4.93 (t, *J* = 10.2 Hz, 1H), 4.88 (t, *J* = 5.5 Hz, 1H), 4.70 (dd, *J* = 10.9, 2.3 Hz, 1H), 4.66–4.57 (m, 1H), 4.18–4.08 (m, 2H), 4.01 (t, *J* = 5.0 Hz, 2H), 3.76 (dd,

Table 1
Structure, solubility and in vitro cytotoxicity data for nitroCBIs with varying leaving groups and side chains



Compd	Y	R	Sol[a] (μM)	IC_{50}[b] average (μM)					
				SKOV3			HT29		
				Oxic	Hypoxic	HCR[c]	Oxic	Hypoxic	HCR[c]
5[d]	Cl	A	5	4.2±1.3	1.7±0.22	2.8±0.9	3.4±0.4	1.6±0.6	2.5±0.7
6[e]	Cl	B	193	1.4±0.5	0.87±0.12	1.9±0.6	0.47±0.05	0.48±0.11	1.1±0.13
13	Cl	C	13	9.3±2.0	3.3±0.06	3.8±0.2	6.6±0.9	5.7±2.0	1.1±0.2
14[e]	Cl	D	38	4.8±0.2	1.54±0.02	3.1±0.1	9.7±0.2	1.30±0.14	7.4±0.1
15	Cl	E	9	0.89±0.19	0.40±0.06	3.3±0.9	1.7±0.1	0.59±0 l.14	4.5±2.3
10[d]	OSO_2Me	A	69	2.2±1.0	0.14±0.04	39±29	1.5±0.6	0.31±0.04	4.6±1.4
16	OSO_2cyclohexyl	A	70	16±1.3	0.19±0.06	146±74	9.9±2.9	0.44±0.09	26±11
17	OSO_2cyclohexyl	B	22	0.47±0.04	0.30±0.03	1.5±0.28	0.41±0.13	0.20±0.03	2.3±0.3
18	OSO_2CH_2Ph	A	17	12±1.5	0.082±0.02	246±57	9.8±1.4	0.36±0.09	37±7
19	OSO_2CH_2Ph	B	91	0.27±0.02	0.15±0.01	1.8±0.2	0.24±0.04	0.08±0.02	4±2
20	OSO_2CH_2Ph	C	46	1.2±0.3	0.21±0.07	7.1±0.9	1.8±0.8	0.66±0.18	2.4±0.5
21	OSO_2CH_2 Ph	E	35	0.57±0.08	0.16±0.04	3.9±1.5	1.0±0.24	1.0±0.36	1.1±0.14
22	OSO_2(4-MePh)	A	89	8.8±1.1	0.22±0.06	72±19	12±0.8	0.47±0.07	28±2
23	OSO_2(4-MePh)	C	29	3.0±0.08	0.77±0.09	2.8±0.08	3.3±1.0	2.7±0.15	0.81±0.04
24	OSO_2(4-MePh)	D	12	11±2	0.38±0.09	34±15	6.5±3.5	0.7±0.6	7.9±2.2

[a] Solubility in culture medium (αMEM) containing 5% FCS.
[b] Drug concentration to reduce cell density to 50% of that of controls, 5 days after 4 h exposure (mean ± SEM for two to seven experiments).
[c] Hypoxic cytotoxicity ratio = IC_{50}(oxic)/IC_{50}(hypoxic). Values are means of intraexperiment ratios (±SEM for two to six experiments).
[d] Previously reported.[10,15]
[e] Previously reported.[15,20]

J = 5.1, 2.5 Hz, 2H). Anal. Calcd for $C_{24}H_{20}ClN_3O_5$: C, 61.87; H, 4.33; N, 9.02. Found: C, 61.61; H, 4.54; N, 9.31.

4.1.2. (*E*)-1-(1-(Chloromethyl)-5-nitro-1*H*-benzo[*e*]indol-3(2*H*)-yl)-3-(3-hydroxy-4-methoxyphenyl)prop-2-en-1-one (15)

Similar deprotection of **25** (352 mg, 0.97 mmol) with HCl/dioxane, followed by reaction with (*E*)-3-(3-hydroxy-4-methoxyphenyl)acrylic acid (207 mg, 1.07 mmol) and EDCI·HCl (558 mg, 2.91 mmol) in DMA (3 mL) gave **15** (308 mg, 72%): mp 258–259 °C; ^{1}H NMR [$(CD_3)_2SO$] δ 9.21 (br s, 1H), 9.16 (s, 1H), 8.32 (d, *J* = 8.7 Hz, 1H), 8.17 (d, *J* = 8.1 Hz, 1H), 7.77–7.66 (m, 2H), 7.62 (d, *J* = 15.3 Hz, 1H), 7.28 (d, *J* = 1.9 Hz, 1H), 7.22 (dd, *J* = 8.4, 2.0 Hz, 1H), 7.02–6.94 (m, 2H), 4.69–4.52 (m, 3H), 4.07 (d, *J* = 4.4 Hz, 2H), 3.84 (s, 3H). Anal. Calcd for $C_{23}H_{19}ClN_2O_5$: C, 62.94; H, 4.36; N, 6.38. Found: C, 62.71; H.4.33; N, 6.36.

4.1.3. (1-(Chloromethyl)-5-amino-1*H*-benzo[*e*]indol-3(2*H*)-yl)(5-(2-hydroxyethoxy)-1*H*-indol-2-yl)methanone (13a)

A solution of **13** (127 mg, 0.27 mmol) in THF (100 mL) was hydrogenated over PtO_2 (30 mg) at 45 psi for 2 h. The catalyst was removed by filtration and the solution was concentrated under reduced pressure to a small volume and diluted with *i*-Pr_2O to give **13a** (114 mg, 96%): mp >250 °C; ^{1}H NMR [$(CD_3)_2SO$] δ 11.55 (d, *J* = 1.6 Hz, 1H), 8.08 (d, *J* = 8.5 Hz, 1H), 7.76 (d, *J* = 8.3 Hz, 1H), 7.71 (s, 1H), 7.46 (t, *J* = 7.6 Hz, 1H), 7.40 (d, *J* = 8.9 Hz, 1H), 7.28 (t, *J* = 7.5 Hz, 1H), 7.15 (d, *J* = 2.3 Hz, 1H), 7.07 (d, *J* = 1.3 Hz, 1H), 6.92 (dd, *J* = 8.9, 2.4 Hz, 1H), 5.97 and 5.95 (2s, 2H), 4.85 (t, *J* = 5.6 Hz, 1H), 4.73 (dd, *J* = 10.7, 9.0 Hz, 1H), 4.51 (dd, *J* = 10.9, 1.6 Hz, 1H), 4.16–4.08 (m, 1H), 4.03–3.93 (m, 3H), 3.80–3.70 (m, 3H). Anal. Calcd for $C_{24}H_{22}ClN_3O_3$: C, 66.13; H, 5.09; N, 9.64; Cl, 8.13. Found: C, 66.15; H, 5.10; N, 9.50; Cl, 7.88.

4.1.4. (5-Nitro-3-(5,6,7-trimethoxy-1*H*-indole-2-carbonyl)-2,3-dihydro-1*H*-benzo[*e*]indol-1-yl)methyl cyclohexanesulfonate (16) (Scheme 2)

Cyclohexanesulfonyl chloride (350 mg, 1.92 mmol) was added dropwise to a stirred solution of *tert*-butyl 1-(hydroxymethyl)-5-nitro-1*H*-benzo[*e*]indole-3(2*H*)-carboxylate (**27**)[10] (330 mg, 0.96 mmol) and DIPEA (310 mg, 2.40 mmol) in CH_2Cl_2 (25 mL) at 0 °C. The mixture was stirred at 0 °C for 1 h and at room temperature for further 16 h, and then diluted with CH_2Cl_2, washed with aqueous $KHCO_3$ and water, dried and concentrated under reduced pressure. The residue was purified by column chromatography eluting with CH_2Cl_2 followed by trituration of the resulting oil with *i*-Pr_2O to give *tert*-butyl 1-(((cyclohexylsulfonyl)oxy)methyl)-5-nitro-1*H*-benzo[*e*]indole-3(2*H*)-carboxylate (**28**) as a yellow solid (349 mg, 74%): mp (CH_2Cl_2/*i*-Pr_2O) 201–204 °C; ^{1}H NMR [$(CD_3)_2SO$] δ 8.74 (br s, 1H), 8.31 (d, *J* = 8.6 Hz, 1H), 8.14 (d, *J* = 8.2 Hz, 1H), 7.78–7.60 (m, 2H), 4.56–4.44 (m, 2H), 4.42–4.32 (m, 1H), 4.26 (t, *J* = 10.4 Hz, 1H), 4.14 (dd, *J* = 11.4, 2.4 Hz, 1H) 3.08 (br, s, 1H), 1.88–1.80 (m, 1H), 1.73–1.43 (m, 13H), 1.24–0.90 (m, 5H). Anal. Calcd for $C_{24}H_{30}N_2O_7S$: C, 58.76; H, 6.16; N, 5.71. Found: C, 58.49; H, 6.33; N, 5.62.

A solution of **28** (112 mg, 0.23 mmol) in dioxane (2 mL) was saturated with HCl gas at 10 °C, stirred at room temperature for 1 h, and then concentrated under reduced pressure. The resulting crude amine **29** was dissolved in DMA (2 mL) and 5,6,7-trimethoxyindole-2-carboxylic acid (63 mg, 0.25 mmol) and EDCI·HCl (131 mg, 0.68 mmol) were added, and the mixture was stirred at room temperature for 2 h. Addition of aqueous $KHCO_3$ precipitated a solid that was purified by column chromatography eluting with CH_2Cl_2/EtOAc (9:1) followed by trituration of the resulting oil with *i*-Pr_2O to give **16** as a yellow solid (74 mg, 52%): mp (EtOAc) 191–

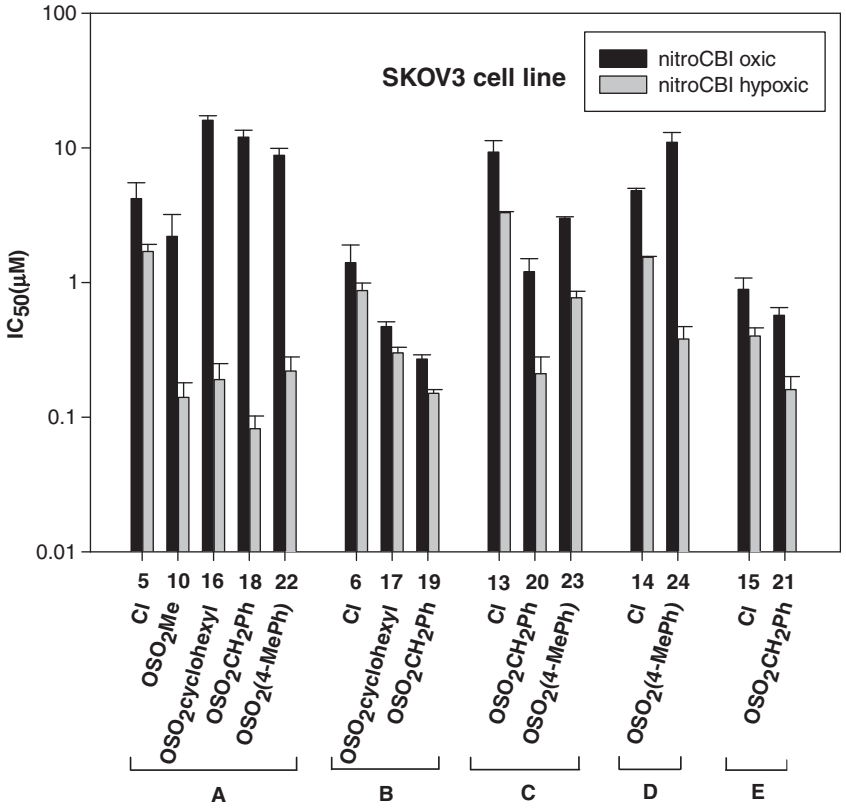


Figure 2. Cytotoxicity of nitroCBI compounds under oxic and hypoxic conditions in the SKOV3 cell line. The compounds are grouped according to side chains **A**–**E** of Table 1.

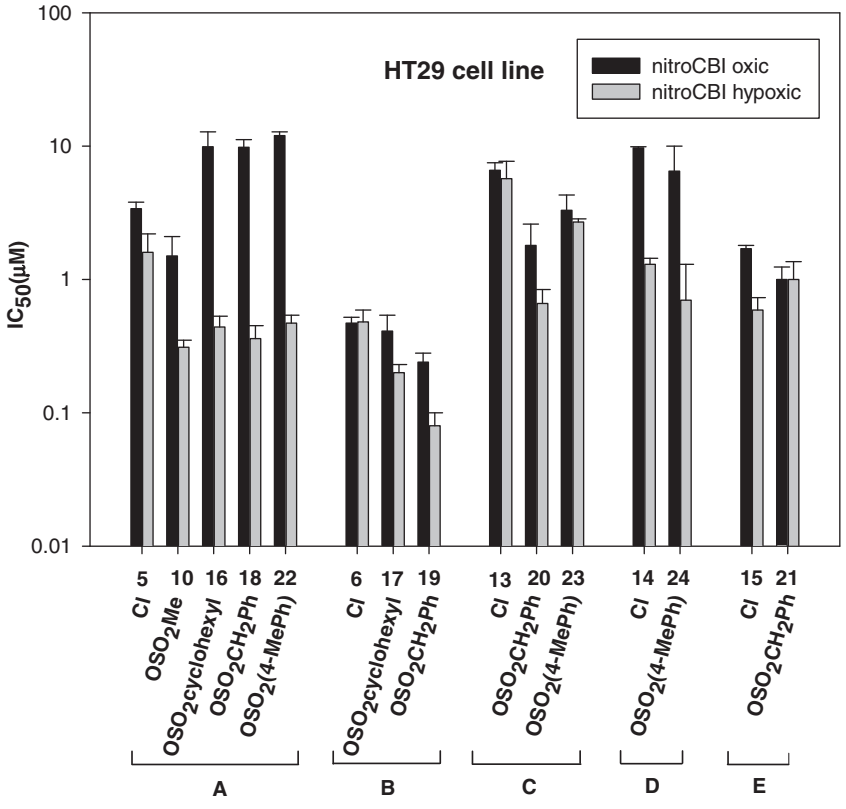
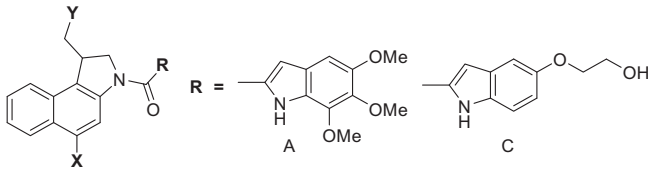


Figure 3. Cytotoxicity of nitroCBI compounds under oxic and hypoxic conditions in the HT29 cell line. The compounds are grouped according to side chains **A**–**E** of Table 1.

Table 2
Cytotoxicity of corresponding aminoCBI analogs



Compd	X	Y	R	Sol[a] (μM)	IC_{50}[b] (SKOV3) average (μM)			
					Oxic	Hypoxic	HCR[c]	Oxic NO_2/NH_2
5a[d]	NH_2	Cl	A	197	0.0013±0.00014	0.0017±0.00020	1.0±0.1	3230
13a	NH_2	Cl	C	22	0.0033±0.00018	0.0042±0.0001	0.8±0.04	2820
10a[d]	NH_2	OSO_2Me	A	15	0.0012±0.00012			1830
16a	NH_2	OSO_2cyclo hexyl	A	37	0.0049±0.0019	0.0065±0.0029	1.0±0.3	3265
1	OH	Cl	A		0.00061±0.00017			

[a] Solubility in culture medium (αMEM) containing 5% FCS.
[b] Drug concentration to reduce cell density to 50% of that of controls following 4 h exposure (mean ± SEM for two to seven experiments).
[c] Hypoxic cytotoxicity ratio = IC_{50}(oxic)/IC_{50}(hypoxic). Values are means of intraexperiment ratios (±SEM for two to six experiments).
[d] Previously reported.[10,15]

Table 3
Cytotoxicity of **18** in a panel of human tumor cell lines.

Cell line[a]	IC_{50}[b] (μM)		
	Oxic	Hypoxic	HCR[c]
A549	1.08±0.48	0.40±0.18	3.73±0.36
A549-P450R	0.93±0.39	0.095±0.008	16.5±3.2
H460	0.25±0.05	0.10±0.07	7.19±4.6
H1299	0.51±0.04	0.16±0.03	3.60±0.93
C33A	0.43±0.14	0.033±0.005	15.6±0.26
SiHa	3.00±2.15	0.45±0.42	30.1±16.8
HCT116	0.53±0.08	0.05±0.02	12.1±3.4
HT29	9.8±1.4	0.36±0.09	37.1±6.9
A375	1.23±0.07	0.41±0.22	4.12±2.08
PC3	1.9±0.47	0.21±0.04	10.2±3.9
SKOV3	12±1.5	0.082±0.02	246±57

[a] A549: nonsmall-cell lung carcinoma; A549-P450R: stable transfectant of A549 overexpressing human NADPH/cytochrome P450 oxidoreductase (ninefold higher rate of cytochrome *c* reduction than parent cell line; H460: large-cell lung carcinoma; H1299: lung carcinoma; C33A, SiHa: cervical carcinoma; HCT116: colorectal carcinoma; HT29: colon carcinoma; A375: melanoma; PC3: prostate carcinoma; SKOV3: ovarian carcinoma.
[b] Drug concentration to reduce cell density to 50% of that of controls following 4 h exposure (mean ± SEM for two to seven experiments).
[c] Hypoxic cytotoxicity ratio = IC_{50}(oxic)/IC_{50}(hypoxic). Values are means of intraexperiment ratios (±SEM for two to six experiments).

192 °C; ^{1}H NMR [$(CD_3)_2SO$] δ 11.59 (s, 1H), 9.13 (s, 1H), 8.35 (d, J = 8.7 Hz, 1H), 8.24 (d, J = 8.2 Hz, 1H), 7.81–7.70 (m, 2H), 7.16 (d, J = 2.1 Hz, 1H), 6.97 (s, 1H), 4.90 (t, J = 10.1 Hz, 1H), 4.65 (dd, J = 12.4, 1.7 Hz, 1H), 4.61–4.46 (m, 3H), 3.94 (s, 3H), 3.83 (s, 3H), 3.81 (s, 3H), 3.06–2.94 (m, 1H), 1.97 (d, J = 11.5 Hz, 1H), 1.61–1.34 (m, 4H), 1.20–0.79 (m, 5H). Anal. Calcd for $C_{31}H_{33}N_3O_9S$: C, 59.70; H, 5.33; N, 6.74. Found: C, 59.67; H, 5.48; N, 6.70.

4.1.5. (3-(5-(2-(Dimethylamino)ethoxy)-1*H*-indole-2-carbonyl)-5-nitro-2,3-dihydro-1*H*-benzo[*e*]indol-1-yl)methyl cyclohexanesulfonate (17)

Similar deprotection of **28** (200 mg, 0.41 mmol) with HCl/dioxane, followed by reaction with 5-(2-(dimethylamino)ethoxy)-1*H*-indole-2-carboxylic acid hydrochloride[21] (139 mg, 0.49 mmol) and EDCI·HCl (313 mg, 1.63 mmol) in DMA (6 mL) gave a solid that was recrystallized from CH_2Cl_2/*i*-Pr_2O/petroleum ether (2×) to give **17** (120 mg, 47%): ^{1}H NMR [$(CD_3)_2SO$] δ 11.68 (s, 1H), 9.16 (s, 1H), 8.35 (d, J = 8.7 Hz, 1H), 8.25 (d, J = 8.1 Hz, 1H), 7.82–7.70 (m, 2H), 7.42 (d, J = 8.9 Hz, 1H), 7.22–7.13 (m, 2H), 6.94 (dd, J = 8.9, 2.4 Hz, 1H), 4.94 (t, J = 10.0 Hz, 1H), 4.77–4.70 (m, 1H), 4.63–4.50 (m, 3H), 4.07 (t, J = 5.9 Hz, 2H), 3.04–2.95 (m, 1H), 2.65 (t, J = 5.8 Hz, 2H), 2.24 (s, 6H), 1.78–1.68 (m, 1H), 1.60–1.37 (m, 4H), 1.17–0.81 (m, 5H). Treatment of the free base with CH_2Cl_2/petroleum ether/HCl gave the hydrochloride salt, mp 150–154 °C. Anal. Calcd for $C_{32}H_{37}ClN_4O_7\,S{\cdot}\frac{1}{2}H_2O$: C, 57.69; H, 5.75; N, 8.41. Found: C, 57.67; H, 5.50; N, 8.47.

4.1.6. (5-Amino-3-(5,6,7-trimethoxy-1*H*-indole-2-carbonyl)-2,3-dihydro-1*H*-benzo[*e*]indol-1-yl)methyl cyclohexanesulfonate (16a)

A solution of **16** (95 mg, 0.15 mmol) in THF (20 mL) was hydrogenated over PtO_2 (25 mg) at 45 psi for 30 min. The catalyst was removed by filtration and the solution was concentrated under reduced pressure and diluted with *i*-Pr_2O to give **16a** as a yellow solid (79 mg, 87%): mp (CH_2Cl_2/*i*-Pr_2O) 214–217 °C; ^{1}H NMR [$(CD_3)_2SO$] δ 11.38 (s, 1H), 8.08 (d, J = 8.5 Hz, 1H), 7.77 (d, J = 8.1 Hz, 1H), 7.66 (br s, 1H), 7.46 (t, J = 7.7 Hz, 1H), 7.28 (t, J = 7.7 Hz, 1H), 7.03 (d, J = 2.1 Hz, 1H), 6.95 (s, 1H), 5.97 (s, 2H), 4.67 (dd, J = 10.8, 9.1 Hz, 1H), 4.47–4.36 (m, 2H), 4.25 (dd, J = 10.2, 7.1 Hz, 1H), 4.12–4.03 (m, 1H), 3.94 (s, 3H), 3.82 (s, 3H), 3.80 (s, 3H), 3.05 (dd, J = 11.8, 3.3 Hz, 1H) 1.88–1.79 (m, 1H), 1.71–1.40 (m, 4H), 1.28–0.90 (m, 5H). Anal. Calcd for $C_{31}H_{35}N_3O_7S{\cdot}\frac{1}{2}H_2O$: C, 61.77; H, 6.02; N, 6.97. Found: C, 61.75; H, 6.04, N, 6.83.

4.1.7. (5-Nitro-3-(5,6,7-trimethoxy-1*H*-indole-2-carbonyl)-2,3-dihydro-1*H*-benzo[*e*]indol-1-yl)methyl phenylmethanesulfonate (18) (Scheme 3)

A solution of **27** (1.00 g, 2.90 mmol) in dioxane (20 mL) was saturated with HCl gas at 10 °C, stirred at room temperature for 30 min, and then evaporated under reduced pressure below 30 °C. The resulting crude amine **30** was dissolved in DMA (10 mL) and 5,6,7-trimethoxyindole-2-carboxylic acid (0.77 g, 3.06 mmol) and EDCI·HCl (1.95 g, 10.2 mmol) were added, and the mixture was stirred at room temperature for 3 h. Addition of aqueous $KHCO_3$ precipitated a solid that was dissolved in EtOAc/CH_2Cl_2 and the solution was filtered through a silica gel plug, concentrated to a small volume and diluted with *i*-Pr_2O to give (1-(hydroxymethyl)-5-nitro-1*H*-benzo[*e*]indol-3(2*H*)-yl)(5,6,7-trimethoxy-1*H*-indol-2-yl)methanone (**31**) as a yellow solid (0.92 g,

66%): mp (EtOAc/CH_2Cl_2/*i*-Pr_2O) 211–212 °C; ^{1}H NMR [$(CD_3)_2SO$] δ 11.55 (s, 1H), 9.14 (s, 1H), 8.40–8.31 (m, 1H), 8.20–8.10 (m, 1H), 7.77–7.65 (m, 2H), 7.17 (s, 1H), 6.98 (s, 1H), 5.09 (t, J = 5.2 Hz, 1H), 4.78 (t, J = 9.8 Hz, 1H), 4.65 (dd, J = 10.6, 2.0 Hz, 1H), 4.17–4.09 (m, 1H), 3.94 (s, 3H), 3.86–3.74 (m, 7H), 3.65–3.56 (m, 1H). Anal. Calcd for $C_{25}H_{23}N_3O_7$: C, 62.88; H, 4.85; N, 8.80. Found: C, 62.64; H, 4.99; N, 8.62.

A solution of **31** (155 mg, 0.32 mmol) in pyridine (2 mL) was treated with α-toluenesulfonyl chloride (74 mg, 0.39 mmol) at 0 °C, and then stirred at this temperature for a further 15 min. The mixture was diluted with water and the resulting solid was purified by column chromatography eluting with CH_2Cl_2/EtOAc (9:1) to give **18** as a yellow solid (171 mg, 83%): mp (EtOAc/CH_2Cl_2) 185 °C; ^{1}H NMR [$(CD_3)_2SO$] δ 11.61 (s, 1H), 9.12 (s, 1H), 8.35 (dd, J = 7.9, 1.7 Hz, 1H), 8.15 (dd, J = 7.3, 1.5 Hz, 1H), 7.81–7.69 (m, 2H), 7.22–7.08 (m, 6H), 6.98 (s, 1H), 4.90–4.80 (m, 1H), 4.67–4.43 (m, 6H), 3.94 (s, 3H), 3.83 (s, 3H), 3.81 (s, 3H). Anal. Calcd for $C_{32}H_{29}N_3O_9S$: C, 60.84; H, 4.63; N, 6.65. Found: C, 60.85; H, 4.76; N, 6.64.

4.1.8. (3-(5-(2-(Dimethylamino)ethoxy)-1*H*-indole-2-carbonyl)-5-nitro-2,3-dihydro-1*H*-benzo[*e*]indol-1yl)methyl phenylmethanesulfonate (19)

Similar reaction of **30** (250 mg, 0.73 mmol) in dry DMA (5 mL) with 5-(2-(dimethylamino)ethoxy)-1*H*-indole-2-carboxylic acid hydrochloride (227 mg, 0.80 mmol) and EDCI·HCl (418 mg, 2.18 mmol), and crystallization of the product from EtOAc (2×) gave (5-(2-(dimethylamino)ethoxy)-1*H*-indol-2-yl)(1-(hydroxymethyl)-5-nitro-1*H*-benzo[*e*]indol-3(2*H*)-yl)methanone (**32**) as a yellow solid (202 mg, 59%): mp 135–137 °C; ^{1}H NMR [$(CD_3)_2SO$] δ 11.65 (s, 1H), 9.17 (s, 1H), 8.39–8.31 (m, 1H), 8.20–8.12 (m, 1H), 7.77–7.67 (m, 2H), 7.41 (d, J = 8.9 Hz, 1H), 7.18 (s, 2H), 6.94 (dd, J = 8.9, 2.4 Hz, 1H), 5.13–5.03 (m, 1H), 4.81 (t, J = 9.8 Hz, 1H), 4.72 (dd, J = 10.5, 2.3 Hz, 1H), 4.21–4.12 (m, 1H), 4.07 (t, J = 5.9 Hz, 2H), 3.85–3.76 (m, 1H), 3.69–3.58 (m, 1H), 2.65 (t, J = 5.8 Hz, 2H), 2.24 (s, 6H). Anal. Calcd for $C_{26}H_{26}N_4O_5$: C, 65.81; H, 5.52; N, 11.81. Found: C, 65.43; H, 5.82; N, 11.67.

A solution of **32** (96 mg, 0.20 mmol) in pyridine (2 mL) at 0 °C was treated portionwise with α-toluenesulfonyl chloride (115 mg, 0.60 mmol), and the mixture was stirred at 0 °C for 1 h and at room temperature for a further 6 h, then basified with aqueous NH_3. The resulting solid was recrystallized from EtOAc/*i*-Pr_2O to give **19** (64 mg, 50%): mp 136–139 °C; ^{1}H NMR [$(CD_3)_2SO$] δ 11.69 (s, 1H), 9.15 (s, 1H), 8.35 (dd, J = 7.6, 2.0 Hz, 1H), 8.15 (dd, J = 7.2, 1.9 Hz, 1H), 7.81–7.70 (m, 2H), 7.43 (d, J = 8.9 Hz, 1H), 7.22–7.09 (m, 7H), 6.95 (dd, J = 8.9, 2.4 Hz, 1H), 4.94–4.84 (m, 1H), 4.67–4.47 (m, 6H), 4.07 (t, J = 5.9 Hz, 2H), 2.66 (t, J = 5.8 Hz, 2H), 2.24 (s, 6H). Anal. Calcd for $C_{33}H_{32}N_4O_7S$: C, 63.04; H, 5.13; N, 8.91. Found: C, 62.67; H, 5.02; N, 8.71.

4.1.9. (5-Amino-3-(5,6,7-trimethoxy-1*H*-indole-2-carbonyl)-2,3-dihydro-1*H*-benzo[*e*]indol-1-yl)methyl phenylmethanesulfonate (18a)

A solution of **18** (55 mg, 0.09 mmol) in THF (10 mL) was hydrogenated over PtO_2 at 45 psi for 45 min. The mixture was filtered through a Celite pad, washed with CH_2Cl_2, and the filtrate was concentrated under reduced pressure and diluted with petroleum ether to give **18a** (42 mg, 80%): mp 215–220 °C; ^{1}H NMR [$(CD_3)_2SO$] δ 11.42 (s, 1H), 8.08 (d, J = 8.9 Hz, 1H), 7.73–7.61 (m, 2H), 7.48 (t, J = 7.4 Hz, 1H), 7.34–7.15 (m, 6H), 7.00 (d, J = 2.0 Hz, 1H), 6.96 (s, 1H), 4.70–4.58 (m, 3H), 4.44–4.31 (m, 2H), 4.17–3.99 (m, 2H), 3.94 (s, 3H), 3.83 (s, 3H), 3.81 (s, 3H). Anal. Calcd for $C_{32}H_{31}N_3O_7S{\cdot}H_2O$: C, 62.02; H, 5.37; N, 6.78. Found: C, 62.15; H, 5.64; N, 6.80.

4.1.10. (3-(5-(2-Hydroxyethoxy)-1*H*-indole-2-carbonyl)-5-nitro-2,3-dihydro-1*H*-benzo[*e*]indol-1-yl)methyl phenylmethanesulfonate (20)

Et_3N (91 μL, 0.65 mmol) and then α-toluenesulfonyl chloride (115 mg, 0.60 mmol) were added to a stirred solution of **30** (173 mg, 0.50 mmol) in CH_2Cl_2 (15 mL) at 0 °C. After 15 min further Et_3N (91 μL, 0.65 mmol) and then α-toluenesulfonyl chloride (115 mg, 0.60 mmol) were added and the mixture was stirred at 0 °C for a further 5 min. Water was added and the CH_2Cl_2 layer was separated. The aqueous phase was extracted with CH_2Cl_2 and the combined organic layers were dried with $MgSO_4$ and concentrated under reduced pressure. The residue was purified by column chromatography eluting with EtOAc/petroleum ether (1:4) to give *tert*-butyl 1-((benzylsulfonyloxy)methyl)-5-nitro-1*H*-benzo[*e*]indole-3(2*H*)-carboxylate (**33**) as a yellow foam (251 mg, 100%). A sample was crystallized from benzene/petroleum ether: mp 148–151 °C; ^{1}H NMR ($CDCl_3$) δ 8.79 (br s, 1H), 8.39 (d, J = 7.8 Hz, 1H), 7.68 (d, J = 7.8 Hz, 1H), 7.62–7.53 (m, 2H), 7.38–7.30 (m, 5H), 4.36 (s, 2H), 4.35–4.30 (m, 1H), 4.21–4.16 (m, 1H), 4.09–3.98 (m, 2H), 3.95–3.89 (m, 1H), 1.61 (s, 9H); ^{13}C NMR ($CDCl_3$) (one C not observed) δ 152.0, 148.1, 140.3, 130.5, 130.4, 129.3, 129.0, 128.5, 127.4, 127.2, 124.1, 122.8, 121.8, 113.3, 82.4, 70.2, 57.2, 51.6, 39.6, 28.4. Anal. Calcd for $C_{25}H_{26}N_2O_7S$: C, 60.23; H, 5.26; N, 5.62. Found: C, 60.33; H, 5.28; N, 5.57.

Trifluoroacetic acid (1.0 mL, 13 mmol) was added to a solution of **33** (122 mg, 0.24 mmol) and anisole (40 μL, 0.36 mmol) in CH_2Cl_2 (10 mL). The mixture was stirred at room temperature for 2.5 h and then evaporated to dryness. The residue was suspended in water and extracted with CH_2Cl_2, and the extract was dried with $MgSO_4$ and concentrated under reduced pressure. The residue was purified by column chromatography eluting with EtOAc/petroleum ether (1:4 then 2:3) to give (5-nitro-2,3-dihydro-1*H*-benzo[*e*]indol-1-yl)methyl phenylmethanesulfonate (**34**) as a red-orange oil (89 mg, 91%) that was used directly. ^{1}H NMR ($CDCl_3$) δ 8.35 (d, J = 8.7 Hz, 1H), 7.63 (s, 1H), 7.62–7.59 (m, 1H), 7.54–7.50 (m, 1H), 7.45–7.40 (m, 1H), 7.38–7.32 (m, 5H), 4.39–4.31 (m, 2H), 4.26–4.20 (m, 1H), 4.07–3.97 (m, 3H), 3.81–3.71 (m, 2H).

Amine **34** (89 mg, 0.22 mmol) was dissolved in dry DMA (3 mL) and 5-(2-hydroxyethoxy)-1*H*-indole-2-carboxylic acid[20] (64 mg, 0.29 mmol), anhydrous toluenesulfonic acid (38 mg, 0.22 mmol), and EDCI·HCl (0.17 g, 0.88 mmol) were added. The mixture was stirred at room temperature for 1 h, then cooled to 0 °C and diluted with cold dilute aqueous $NaHCO_3$. The mixture was extracted with EtOAc (2×) and the extracts were washed with cold dilute aqueous $NaHCO_3$ and then dried with $MgSO_4$ and concentrated under reduced pressure. The residue was recrystallized from CH_2Cl_2/MeOH to give **20** as a yellow solid (119 mg, 81%): mp 211–213 °C; ^{1}H NMR [$(CD_3)_2SO$] δ 11.70 (s, 1H), 9.17 (s, 1H), 8.37–8.33 (m, 1H), 8.17–8.14 (m, 1H), 7.81–7.72 (m, 2H), 7.44 (d, J = 8.9 Hz, 1H), 7.22–7.11 (m, 7H), 6.97 (dd, J = 8.9, 2.4 Hz, 1H), 4.94–4.84 (m, 2H), 4.67–4.50 (m, 6H), 4.02 (t, J = 5.1 Hz, 2H), 3.76 (q, J = 5.1 Hz, 2H). HRMS (FAB) calcd for $C_{31}H_{28}N_3O_8S$ (MH^+) m/z 602.15971, found: 602.15998. HPLC analysis showed this material to have a purity of 98.8%.

4.1.11. (*E*)-(3-(3-(3-Hydroxy-4-methoxyphenyl)acryloyl)-5-nitro-2,3-dihydro-1*H*-benzo[*e*]indol-1-yl)methyl phenylmethanesulfonate (21)

Crude amine **34** (76 mg, 0.20 mmol) was obtained as above from **31** (107 mg, 0.21 mmol), TFA (1.0 mL, 13 mmol), and anisole (35 μL, 0.31 mmol) in CH_2Cl_2 (8 mL). The amine was then dissolved in dry DMA (2 mL) and (*E*)-3-(3-hydroxy-4-methoxyphenyl)acrylic acid (49 mg, 0.25 mmol), anhydrous toluenesulfonic acid (37 mg, 0.22 mmol), and EDCI·HCl (150 mg, 0.78 mmol) were added. The mixture was stirred at room temperature for 2 h, then cooled to 0 °C and diluted with cold dilute aqueous $KHCO_3$. The precipitated solid was filtered off and washed with dilute aqueous $KHCO_3$ and

water. The solid was dissolved in THF and the solution was dried, concentrated to a small volume and then diluted with MeOH. The precipitated solid was filtered off and dried to give **21** (mono-THF solvate) as a yellow solid (79 mg, 57%): mp 190–192 °C; ^{1}H NMR [$(CD_3)_2SO$] δ 9.21 (s, 1H), 9.14 (s, 1H), 8.33 (d, J = 8.0 Hz, 1H), 8.11 (d, J = 7.7 Hz, 1H), 7.79–7.70 (m, 2H), 7.64 (d, J = 15.3 Hz, 1H), 7.29–7.20 (m, 7H), 7.01 (d, J = 8.4 Hz, 1H), 6.93 (d, J = 15.3 Hz, 1H), 4.66 (s, 2H), 4.62–4.56 (m, 1H), 4.54–4.46 (m, 4H), 3.84 (s, 3H), 3.63–3.58 (m, 4H, THF), 1.79–1.74 (m, 4H, THF). Anal. Calcd for $C_{30}H_{26}N_2O_8S$·THF: C, 63.15; H, 5.30; N, 4.33. Found: C, 62.90; H, 5.08; N, 4.40.

4.1.12. (5-Nitro-3-(5,6,7-trimethoxy-1*H*-indole-2-carbonyl)-2,3-dihydro-1*H*-benzo[*e*]indol-1-yl)methyl 4-methylbenzenesulfonate (22) (Scheme 4)

A solution of **28** (120 mg, 0.35 mmol) in CH_2Cl_2 (10 mL) was treated with pyridine (340 μL, 4.20 mmol) and tosyl chloride (667 mg, 3.50 mmol) at 0 °C. The mixture was stirred at room temperature for 48 h, diluted with CH_2Cl_2, washed with water and brine, dried with Na_2SO_4, and concentrated under reduced pressure. The residue was purified by column chromatography eluting with petroleum ether/EtOAc (4:1 then 3:2) followed by trituration of the product with petroleum ether/i-Pr_2O to give *tert*-butyl 1-(tosyloxymethyl)-5-nitro-1*H*-benzo[*e*]indole-3(2*H*)-carboxylate (**35**) as a yellow solid (174 mg, 100%): mp 131–132 °C; ^{1}H NMR [$(CD_3)_2SO$] δ 8.65 (br s, 1H), 8.24 (d, J = 9.3 Hz, 1H), 7.86 (d, J = 8.2 Hz, 1H), 7.54–7.63 (m, 2H), 7.32–7.34 (m, 2H), 7.10–7.12 (m, 2H), 4.33–4.41 (m, 2H), 4.14–4.23 (m, 2H), 4.00–4.06 (m, 1H), 2.30 (s, 3H), 1.55 (s, 9H). Anal. Calcd for $C_{25}H_{26}N_2O_7S$: C, 60.23; H, 5.26; N, 5.62. Found: C, 60.48; H, 5.27; N, 5.61.

A solution of **35** (340 mg, 0.68 mmol) in dioxane (3 mL) was saturated with HCl gas at 10 °C, stirred at room temperature for 30 min, and then concentrated under reduced pressure. The residue was purified by column chromatography eluting with petroleum ether/EtOAc (3:2) to give (5-nitro-2,3-dihydro-1*H*-benzo[*e*]indol-1-yl)methyl-4-methylbenzenesulfonate (**36**) as a red solid (241 mg, 89%): mp 121–123 °C; ^{1}H NMR [$(CD_3)_2SO$] δ 8.06 (d, J = 8.6 Hz, 1H), 7.63 (d, J = 8.3 Hz, 1H), 7.58 (s, 1H), 7.48–7.80 (m, 2H), 7.42–7.47 (m, 1H), 7.32–7.38 (m, 1H), 7.23–7.25 (m, 2H), 6.20 (d, J = 2.4 Hz, 1H), 4.07–4.19 (m, 3H), 3.72 (ddd, J = 13.0, 10.2, 2.9 Hz, 1H), 3.51 (dd, J = 10.2, 2.1 Hz, 1H), 2.34 (s, 3H). Anal. Calcd for $C_{20}H_{18}N_2O_5S$: C, 60.29; H, 4.55; N, 7.03. Found: C, 59.93; H, 4.65; N, 6.71.

A solution of **36** (241 mg, 0.60 mmol) in DMA (2 mL) was treated with 5,6,7-trimethoxyindole-2-carboxylic acid (188 mg, 0.75 mmol) and EDCI·HCl (522 mg, 2.72 mmol), and the mixture was stirred at room temperature for 2 h. Addition of dilute aqueous $KHCO_3$ gave a solid that was purified by column chromatography eluting with CH_2Cl_2/EtOAc (19:1) firstly to give compound **5** (80 mg, 27%) and then a product that was recrystallized from CH_2Cl_2/i-Pr_2O (2×) to provide **22** (268 mg, 71%): mp 212–214 °C; ^{1}H NMR [$(CD_3)_2SO$] δ 11.62 (s, 1H), 9.05 (s, 1H), 8.28 (d, J = 8.6 Hz, 1H), 7.98 (d, J = 8.3 Hz, 1H), 7.72–7.58 (m, 2H), 7.28 (d, J = 8.3 Hz, 2H), 7.06 (d, J = 1.6 Hz, 1H), 6.99–6.89 (m, 3H), 4.79 (t, J = 10.1 Hz, 1H), 4.52–4.48 (m, 4H), 3.97 (s, 3H), 3.84 (s, 3H), 3.83 (s, 3H). Anal. Calcd for $C_{32}H_{29}N_3O_9S$·1½H_2O: C, 58.25; H, 4.90; N, 6.38. Found: C, 58.34; H, 4.58; N, 6.35.

4.1.13. (3-(5-Methoxy-1*H*-indole-2-carbonyl)-2,3-dihydro-1*H*-benzo[*e*]indol-1-yl)methyl 4-methylbenzenesulfonate (23)

A solution of **36** (40 mg, 0.10 mmol) in DMA (1 mL) was treated with anhydrous toluenesulfonic acid (19 mg, 0.11 mmol), 5-methoxy-1*H*-indole-2-carboxylic acid (25 mg, 0.13 mmol) and EDCI·MeI (119 mg, 0.40 mmol). The mixture was stirred at room temperature overnight, then cooled to 0 °C, treated with 5% aqueous $NaHCO_3$ (3 mL) and stirred for a further 10 min. The precipitate was collected by filtration, washed with cold water and i-Pr_2O and then dried. The residue was recrystallized from $CHCl_3$/i-Pr_2O to give **22** as a yellow solid (16 mg, 28%): mp ($CHCl_3$/i-Pr_2O) 212–216 °C; ^{1}H NMR [$(CD_3)_2SO$] δ 11.71 (s, 1H), 9.07 (s, 1H), 8.28 (d, J = 8.5 Hz, 1H), 7.99 (d, J = 8.1 Hz, 1H), 7.71–7.61 (m, 2H), 7.45 (d, J = 8.9 Hz, 1H), 7.29–7.27 (m, 2H), 7.16 (d, J = 2.3 Hz, 1H), 7.09 (d, J = 1.7 Hz, 1H), 6.99–6.94 (m, 3H), 4.85–4.80 (m, 1H), 4.52–4.49 (m, 1H), 4.37–4.47 (m, 3H), 3.81 (s, 3H), 2.16 (s, 3H). HRMS (FAB) calcd for $C_{30}H_{26}N_3O_7S$ (MH^+) m/z 572.1492, found: 572.1489. HPLC analysis showed this material to have a purity of 100%.

4.1.14. (3-(5-(2-Hydroxyethoxy)-1*H*-indole-2-carbonyl)-5-nitro-2,3-dihydro-1*H*-benzo[*e*]indol-1-yl)methyl 4-methylbenzenesulfonate (24)

A solution of **36** (40 mg, 0.10 mmol) in DMA (1 mL) was treated with anhydrous toluenesulfonic acid (19 mg, 0.11 mmol), 5-(2-hydroxyethoxy)-1*H*-indole-2-carboxylic acid (29 mg, 0.13 mmol) and EDCI·HCl (77 mg, 0.40 mmol). The mixture was stirred at room temperature overnight, then cooled to 0 °C, treated with 5% aqueous $NaHCO_3$ (3 mL) and stirred for a further 10 min. The precipitate was collected by filtration, washed with cold water and dried to give **24** as a yellow solid (59 mg, 98%): mp ($CHCl_3$/i-Pr_2O) 127–129 °C; ^{1}H NMR [$(CD_3)_2SO$] δ 11.70 (d, J = 1.4 Hz, 1H), 9.07 (s, 1H), 8.28 (d, J = 8.3 Hz, 1H), 7.99 (d, J = 8.1 Hz, 1H), 7.60–7.71 (m, 2H), 7.45 (d, J = 8.9 Hz, 1H), 7.27–7.29 (m, 2H), 7.16 (d, J = 2.3 Hz, 1H), 7.08 (d, J = 1.7 Hz, 1H), 6.94–7.00 (m, 3H), 4.80–4.86 (m, 1H), 4.50–4.53 (m, 1H), 4.39–4.47 (m, 3H), 4.02 (t, J = 5.1 Hz, 2H), 3.76 (dd, J = 10.3, 5.3 Hz, 2H), 2.16 (s, 3H). Anal. Calcd for $C_{31}H_{27}N_3O_8S$: C, 61.89; H, 4.52; N, 6.98. Found: C, 61.75; H, 4.53; N, 7.05.

4.2. Solubility in culture medium

The solubility of the compounds in αMEM containing 5% FCS (equilibrated with 5% CO_2/air) at 22 °C was determined as previously described.[3]

4.3. In vitro cytotoxicity

Inhibition of proliferation of log-phase monolayers was assessed in 96-well plates as previously described.[24] 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.

Acknowledgments

The authors are grateful for the assistance of Sisira Kumara for HPLC and solubility determinations, Maruta Boyd for NMR spectrometry, and Jane Botting and Wouter van Leeuwen for cytotoxicity studies. This work was funded by the Foundation for Research, Science and Technology (NERF grant), the Auckland Division of the Cancer Society of New Zealand, and the Health Research Council of New Zealand.

References and notes

1. Boger, D. L.; Johnson, D. S. *Angew. Chem., Int. Ed.* **1996**, *35*, 1438.
2. Yasuzawa, T.; Saitoh, Y.; Ichimura, M.; Takahashi, I.; Sano, H. *J. Antibiot.* **1991**, *44*, 445.
3. Tercel, M.; Yang, S.; Atwell, G. J.; Smith, E.; Gu, Y.; Anderson, R. F.; Denny, W. A.; Wilson, W. R.; Pruijn, F. B. *Bioorg. Med. Chem.* **2010**, *18*, 4997.
4. Boger, D. L.; Ishizaki, T.; Kitos, P. A.; Suntornwat, O. *J. Org. Chem.* **1990**, *55*, 5823.
5. Wei, J.; Trzupek, J. D.; Rayl, T. J.; Broward, M. A.; Vielhauer, G. A.; Weir, S. J.; Hwang, I.; Boger, D. L. *J. Am. Chem. Soc.* **2007**, *129*, 15391.
6. Townes, H.; Summerville, K.; Purnell, B.; Hooker, M.; Madsen, E.; Hudson, S.; Lee, M. *Med. Chem. Res.* **2002**, *11*, 248.

7. Tercel, M.; Atwell, G. J.; Yang, S.; Stevenson, R. J.; Botting, K. J.; Boyd, M.; Smith, E.; Anderson, R. F.; Denny, W. A.; Wilson, W. R.; Pruijn, F. B. *J. Med. Chem.* **2009**, *52*, 7258.
8. Wilson, W. R.; Stribbling, S. M.; Pruijn, F. B.; Syddall, S. P.; Patterson, A. V.; Liyanage, H. D. S.; Smith, E.; Botting, K. J.; Tercel, M. *Mol. Cancer Ther.* **2009**, *8*, 2903.
9. Tercel, M.; Atwell, G. J.; Yang, S.; Ashoorzadeh, A.; Stevenson, R. J.; Botting, K. J.; Gu, Y.; Mehta, S. Y.; Denny, W. A.; Wilson, W. R.; Pruijn, F. B. *Angew. Chem., Int. Ed.* **2011**, *50*, 2606.
10. Atwell, G. J.; Tercel, M.; Boyd, M.; Wilson, W. R.; Denny, W. A. *J. Org. Chem.* **1998**, *63*, 9414.
11. Tercel, M.; Gieseg, M. A.; Milbank, J. B.; Boyd, M.; Fan, J. Y.; Tan, L. K.; Wilson, W. R.; Denny, W. A. *Chem. Res. Toxicol.* **1999**, *12*, 700.
12. Atwell, G. J.; Wilson, W. R.; Denny, W. A. *Bioorg. Med. Chem. Lett.* **1997**, *7*, 1493.
13. Wilson, W. R.; Hay, M. P. *Nat. Rev. Cancer* **2011**, *11*, 393.
14. Chen, Y.; Hu, L. *Med. Res. Rev.* **2009**, *29*, 29.
15. Atwell, G. J.; Milbank, J. B. J.; Wilson, W. R.; Hogg, A.; Denny, W. A. *J. Med. Chem.* **1999**, *42*, 3400.
16. Panthananickal, A.; Hansch, C.; Leo, A. *J. Med. Chem.* **1978**, *21*, 16.
17. Jaramillo, P.; Domingo, L. R.; Perez, P. *Chem. Phys. Lett.* **2006**, *420*, 95.
18. Pacheco, D. Y.; Stratton, N. K.; Gibson, N. W. *Cancer Res.* **1989**, *49*, 5108.
19. Tietze, L. F.; Birgit Krewer, B. *Anti-Cancer Agents Med. Chem.* **2009**, *9*, 304.
20. Denny, W. A.; Wilson, W. R.; Stevenson, R. J.; Tercel, M.; Atwell, G. J.; Yang, S.; Patterson, A. V.; Pruijn, F. B. PCT Int. Appl. WO 2006043839A1, **2006**.
21. Milbank, J. B. J.; Tercel, M.; Atwell, G. J.; Wilson, W. R.; Hogg, A.; Denny, W. A. *J. Med. Chem.* **1999**, *42*, 649.
22. Ahn, G.-O.; Botting, K. J.; Patterson, A. V.; Ware, D. C.; Tercel, M.; Wilson, W. R. *Biochem. Pharmacol.* **2006**, *71*, 1683.
23. Su, J.; Wang, J.; Wilson, W. A. Personal communication.
24. Hay, M. P.; Gamage, S. A.; Kovacs, M. S.; Pruijn, F. B.; Anderson, R. F.; Patterson, A. V.; Wilson, W. R.; Brown, J. M.; Denny, W. A. *J. Med. Chem.* **2003**, *46*, 169.