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Weight loss effects of quaternary salts of 5-amino-1-(chloromethyl)-1,2-dihydro-3*H*-benz[*e*]indoles; structure–activity relationships

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ABSTRACT

Quaternary salt analogues based on the DNA minor groove binder and adenine N3 alkylating agent 5-amino-1-(chloromethyl)-1,2-dihydro-3*H*-benz[*e*]indole (aminoCBI) show remarkable effects on the body weight of mice (a long-term failure to gain weight relative to matched controls with no loss of appetite or perceptible deterioration in health) following administration of a single (non-toxic) dose between about 0.5–5 μmol/kg. The nature of the quaternizing group was not important, but a related hydroxyCBI analogue was much less effective. Compounds where the chloro group was replaced by a hydrogen or hydroxy group (thus abrogating DNA alkylating capability) showed no weight control activity. It is speculated, based on other studies, that the marked long-term weight control effect is due to inhibition of bile flow into the intestine and reduced absorption of triglycerides, together with accelerated cell death in spleen and white adipose tissues due to drug accumulation there. This class of compound may serve as interesting tools for further study of these phenomena.

1. Introduction

Obesity is a major and rising health problem world-wide, as it is associated with many other disease states, including type II diabetes, coronary artery diseases, osteoarthritis, and some types of cancer.[1] Much is now known about the biochemical mechanisms that regulate energy metabolism and body weight,[2] and drugs are available that lower food intake by modulating the hypothalamic system,[1] block nutrient absorption[3] or increase energy output (thermogenesis).[4] Of these only orlistat (**1**) and sibutramine (**2**) (Fig. 1) have been approved by the FDA for long-term treatment (and orlistat was withdrawn in 2010).

Orlistat is an inhibitor of pancreatic lipase that reduces energy intake by preventing fat absorption,[3] and sibutramine is a selective inhibitor of serotonin and noradrenalin re-uptake, although much of the activity appears to reside in its desmethyl metabolites.[5] However, these compounds must be taken continuously, and both have significant side-effects, which have limited their use.[1] Sibutramine has sympathetic nervous system effects that can result in elevated blood pressure and heart rate,[6] while the side-effects of orlisat are primarily gastrointestinal (flatulence and frequent loose stools).[7]

The 5-amino-1-(chloromethyl)-1,2-dihydro-3*H*-benz[*e*]indole (aminoCBI) class of DNA minor groove alkylators are very potent cytotoxins,[8] and have been utilised as effector units in a number of classes of prodrugs designed for cancer therapy. These have included antibody conjugates,[9] prodrugs for gene therapy based on nitrobenzyl carbamates,[10] and the corresponding nitroCBIs as hypoxia-activated prodrugs.[11] In studies of the antiproliferative effects of the *N*,*N*-dimethyl-*N*-(pyrrolylmethyl) quaternary salt **3** (see Table 1) (designed as a putative hypoxia- and radiation-activated cytotoxin based on previous studies with related mustard

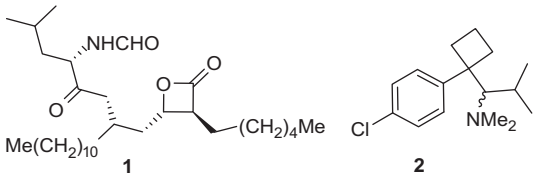

Figure 1. Structures of orlistat (**1**) and sibutramine (**2**).

Abbreviations: AIBN, 2,2′-azobis[isobutyronitrile]; CBI, *seco*-1,2,9,9a-tetrahydrocyclopropa[*c*]benz[*e*]indol-4-one; CSA, camphorsulfonic acid; DIPEA, diisopropylethylamine; DMA, dimethylacetamide; DMAP, 4-dimethylaminopyridine; EDCI·HCl, *N*-ethyl-*N*′-(3-dimethylaminopropyl)carbodiimide hydrochloride; ip, intra-peritoneal; NMP, *N*-methylpyrrolidone; pyBOP, benzotriazol-1-yl-oxytripyrrolidinophosphonium hexafluorophosphate.

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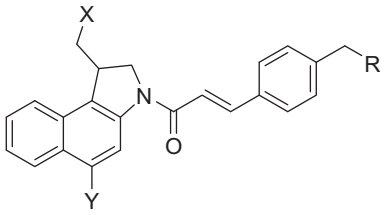
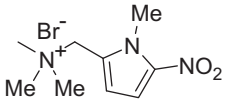
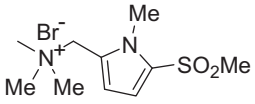
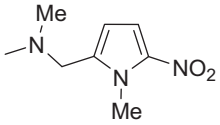
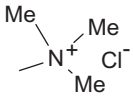
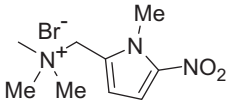
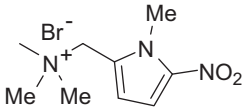
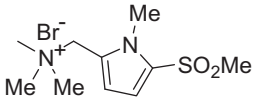
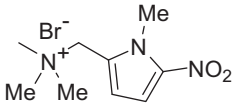
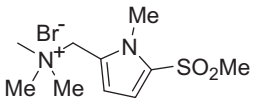
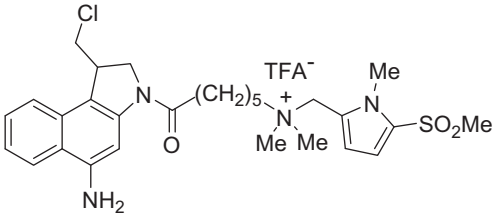
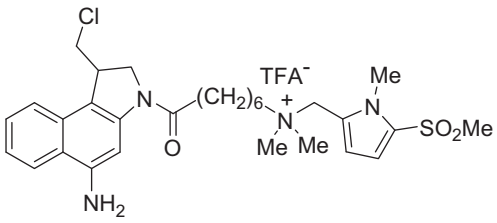
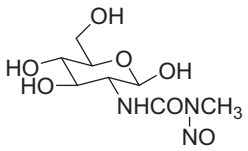
Table 1
Structural and biological data for benzindole quaternary salts



No.	X	Y	R	Dose[a] (μmol/kg)	Weight gain[b] (%)		Significance
					Treated	Control	
3	Cl	NH_2		0.47 1.4 4.2	38.3 ± 4.9 19.0 ± 1.8 −4.1 ± 2.8	27.7 ± 4.6 31.5 ± 8.2 30.1 ± 5.9	P <0.02[c] NS P <0.0037[c]
4	Cl	NH_2		0.14 0.42 1.4	44.1 ± 5.3 −7.6 ± 6.2 −22.8 ± 7.0	41.6 ± 8.7 23.6 ± 3.7 26.1 ± 7.9	NS P <0.0035[c] P <0.0007[c]
5	Cl	NH_2		4.2	21.0 ± 0.4[d]	26.1 ± 7.8	NS
6	Cl	NH_2		0.47 4.2	17.0 ± 2.6 Early deaths	27.7 ± 4.6	P <0.03[c]
7	Cl	OH		4.2	18.8 ± 6.8	27.7 ± 4.6	NS
8	OH	NH_2		12.6 37.8[e]	23.7 ± 3.7 18.8 ± 2.5	18.8 ± 1.8 31.5 ± 8.2	NS NS
9	OH	NH_2		37.8 50.2 117	36.8 ± 4.9 42.6 ± 17.0 Early deaths	23.9 ± 4.6 27.4 ± 9.4	P <0.0166[f] P <0.0287[c]
10	H	NH_2		4.2 37.8	26.0 ± 11.9 43.2 ± 14.6	27.7 ± 4.6 35.6 ± 3.1	NS NS
11	H	NH_2		37.8	30.4 ± 5.0	35.6 ± 3.1	NS
12				4.2 12.6	16.8 ± 5.2 Early deaths	33.3 ± 5.0	P <0.0178[c]

(*continued on next page*)

Table 1 (*continued*)

No.	X	Y	R	Dose[a] (μmol/kg)	Weight gain[b] (%)		Significance
					Treated	Control	
13				4.2 12.6	11.6 ± 5.9 2.9 ± 7.9	33.3 ± 5.0 41.6 ± 8.7	P <0.0124[c] P <0.0035[c]
14 (STZ)				377 1130[g]	23.7 ± 4.2 −22.1 ± 10.1	24.5 ± 3.5 12.8 ± 1.5	NS P <0.0003[h]

[a] Dose in 42% aqueous DMSO, given ip as a single treatment on day 1 to groups of 4 animals.
[b] Body weight was measured at day 60, averaged for each treatment group and for the control (vehicle-treated) group, and is reported as the mean % loss (or gain) in weight relative to the pre-treatment value.
[c] Paired Student's '*t*' test.
[d] 2/4 died by day 15.

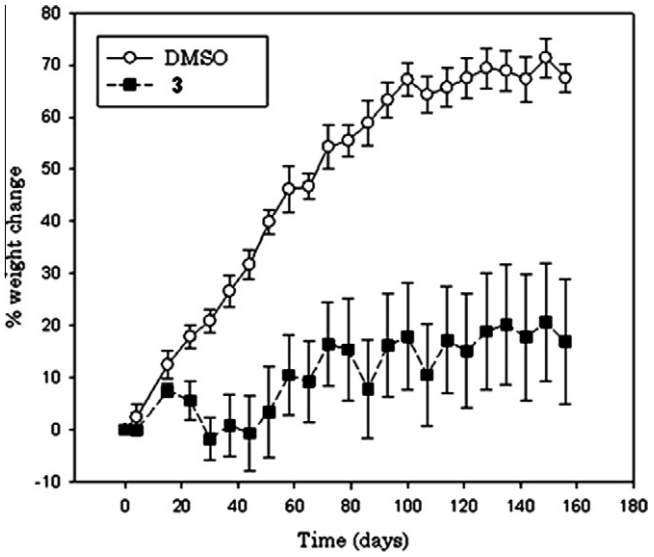


Figure 2. Effect of a single ip dose of **3** (4.2 μM/kg) on the body weight of female C3H/HeN mice over time. Values are mean and SEM for 6 mice/group.

quaternary salts[12,13]) we observed a remarkable body weight effect in female C3H mice. Following a single ip dose the animals showed a long-term failure to gain weight relative to matched controls with no perceptible deterioration in health and no loss of appetite or difficulty in eating or drinking[14] (see below, and Fig. 2). We undertook an initial exploration of this by seeking to define structure–activity relationships (SAR) for the phenomenon, and report the results in the present paper.

2. Results and discussion

2.1. Chemistry

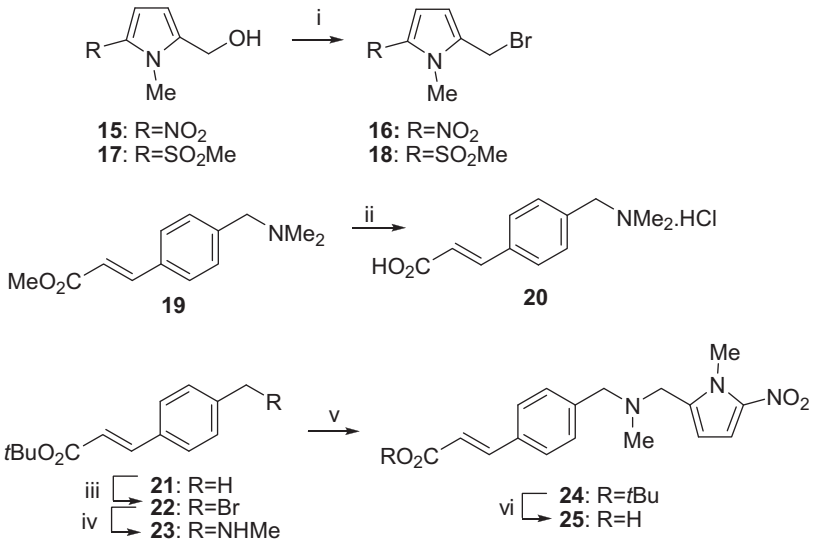
The (bromomethyl)heterocycles were prepared as in Scheme 1. The known[15] alcohol 2-(hydroxymethyl)-1-methyl-5-nitropyrrole (**15**) was treated with bromine/triphenylphosphine to give the somewhat unstable bromide **16**. Similar treatment of the known[16] methylsulfone alcohol **17** gave the bromide **18**.

To prepare the aminoCBI agents **3–6** of Table 1 (Scheme 2), the 5-amino substituent of the known[8] trifluoroacetamide **26** was protected as the bisBoc derivative **27** (Boc_2O/DMAP) to avoid alkylation during the quaternization step. This was then treated with aqueous Cs_2CO_3 to cleave the trifluoroacetamide and provide the free amine, which was reacted directly with the acid chloride of 4-(dimethylaminomethyl)cinnamic acid **20** (prepared as shown in Scheme 1 from ester **19**) to generate **29**. Quaternization of **29** was performed with **16**, **18**, or with methyl iodide, in THF or EtOAc, to give the intermediates **30a–c**. Cleavage of the Boc protecting groups from these with HBr or HCl in dioxane then gave the desired quaternary salts **3, 4** and **6**. For **6** the chloride salt was obtained by ion exchange chromatography. The compounds were shown by HPLC analysis to be contaminated with 0.3–1.0% of the unquaternized amine **31**, an authentic sample of which was prepared by Boc-deprotection of **29**. The tertiary amine **5** was prepared by triflate deprotection of **27** and coupling with the acid chloride of **25** (prepared as shown in Scheme 1) to give **28**, which was deprotected with HCl to generate **5**.

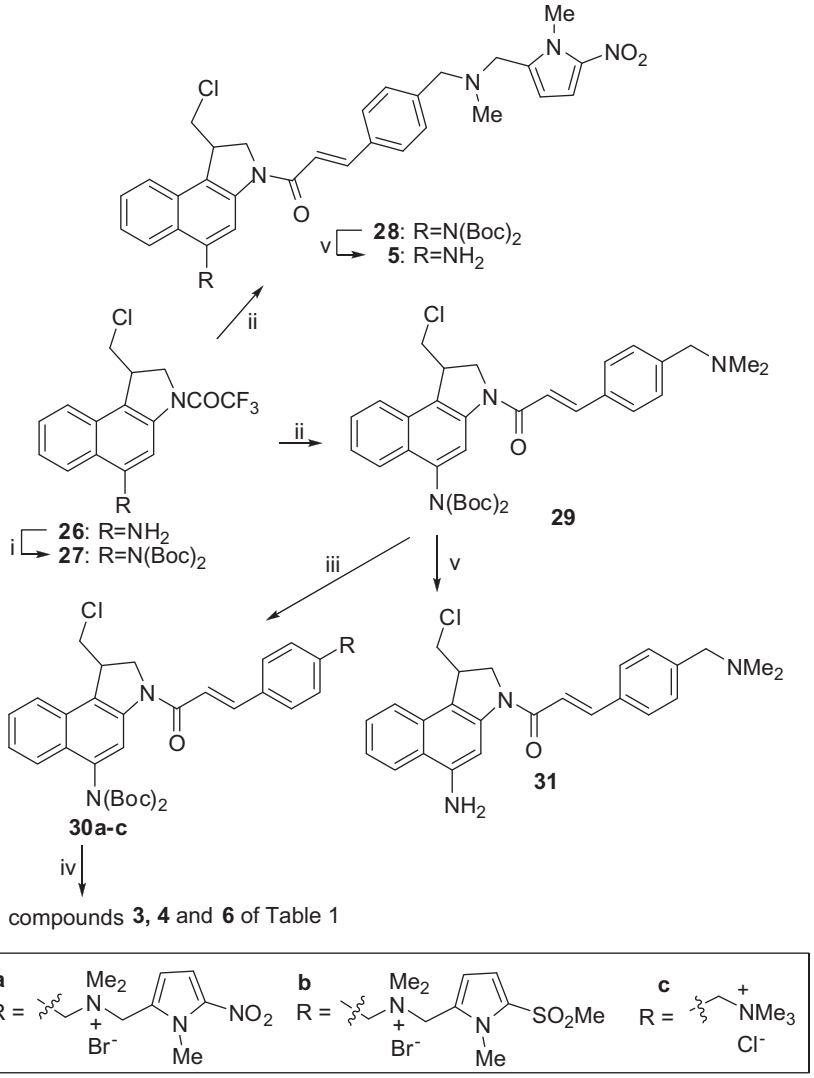
The phenol CBI **7** was prepared from the previously described[17] phenol **32**, which was Boc-deprotected and allowed to react with 4-(dimethylaminomethyl)cinnamic acid (**20**) in the presence of EDCI (Scheme 3). The crude amine could then be directly quaternized using **16** as above. The quaternary salt product still contained traces of **33** (0.15% by HPLC analysis).

The 1-(hydroxymethyl) derivatives **8** and **9** (Scheme 4) were prepared from the known[18] 1-(hydroxymethyl)-5-nitroCBI derivative **34**. The Boc protecting group was first changed to trifluoroacetamide, then **35** was hydrogenated over PtO_2 to give the somewhat unstable 5-amino compound that was protected as the trisBoc derivative **36**. Deprotection of the trifluoroacetamide and coupling with *E*-4-(dimethylaminomethyl)cinnamoyl chloride gave the key benzylamine intermediate **37**. Quaternization of this with bromides **16** or **18** gave **38a** and **38b**, respectively, which were deprotected with HBr in dioxane to give the hydroxy compounds **8** and **9**.

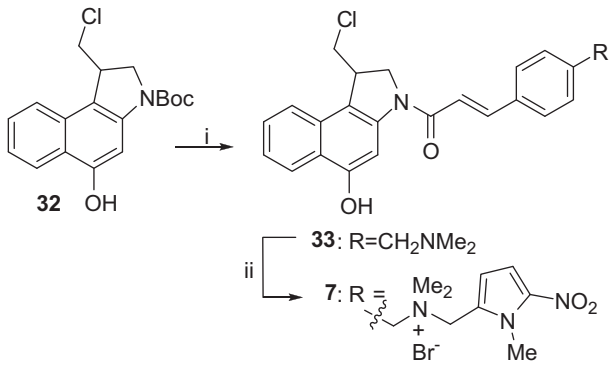
The 1-methyl compounds **10** and **11** were synthesized from the known[19] aminoCBI precursor **39**, which was cyclized with Bu_3SnH/AIBN to **40** (Scheme 5). This was deprotected with TFA, then



Scheme 1. Reagents and conditions: (i) Br_2, PPh_3, MeCN, 15–35 min, 20 °C; (ii) NaOH, MeOH/H_2O, 2 h, reflux, then HCl; (iii) AIBN, NBS, CCl_4, reflux, 15 h; (iv) aqueous $MeNH_2$, EtOH, DCM, 2 h, 20 °C; (v) DIPEA, THF, **16**, 15 h, 20 °C (N_2); (vi) TFA, 1 h, 20 °C.



Scheme 2. Reagents and conditions: (i) $(Boc)_2O$, DMAP, dioxane, 18 h, reflux (N_2); (ii) Cs_2CO_3, THF/H_2O, 8 h, 20 °C, then *E*-4-(dimethylaminomethyl)cinnamoyl chloride or the acid chloride of **25**, MeCN, DMF, 2–5.5 h, 20 °C; (iii) **16**, **18**, or MeI, THF or EtOAc, 16 h–3 days, 20 °C (dark); (iv) HBr or HCl, dioxane, 12–16 h, 20 °C (dark); (v) HCl(g), dioxane, 1 h, 20 °C.



Scheme 3. Reagents and conditions: (i) HCl(g), dioxane, 1 h, 20 °C, then **20**, EDCI·HCl, DMA, 16 h, 20 °C; (ii) **16**, THF, 18 days, 20 °C (dark).

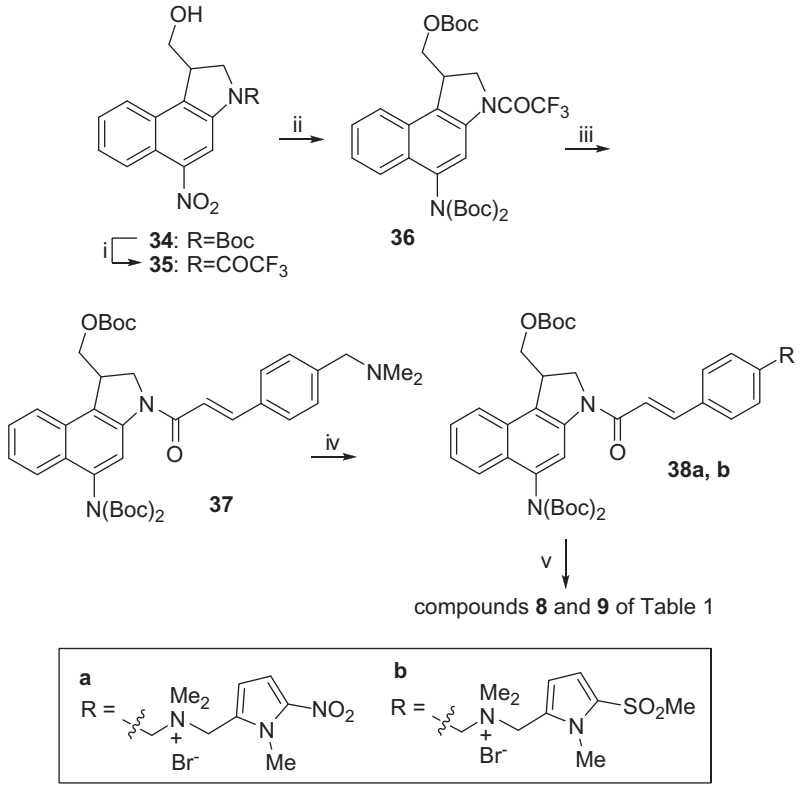
coupled with (*E*)-4-(dimethylaminomethyl)cinnamoyl chloride to give **41** selectively. The allyl group was removed using the method of Honda et al.[20] with $Pd(Ph_3P)_4/PhSO_2Na$/camphorsulfonic acid (CSA) to give **42** in excellent yield. Re-protection of **42** as the bisBoc derivative **43** was followed by quaternization with bromides **16** and **18** to give **44a** and **44b**, respectively. Boc-deprotection of these with HCl, followed by ion exchange chromatography, gave the required compounds **10** and **11**.

Finally, the longer-chain quaternary compounds **12** and **13** were prepared from the known[19] 5-allylaminoCBI derivative **45** (Scheme 6). The Boc groups were removed with TFA, and the resulting diamine was selectively coupled with 6-(dimethylamino)hexanoyl chloride[21] or 7-(dimethylamino)heptanoyl chloride[21] to give **46a** and **46b**. These were then de-allylated to give **47a** and **47b** and re-protected to give the 5-$N(Boc)_2$ derivatives **48a** and **48b**. These compounds were then quaternized with **18** to give the intermediates **49a** and **49b**, which were deprotected with HCl to give the desired compounds **12** and **13**.

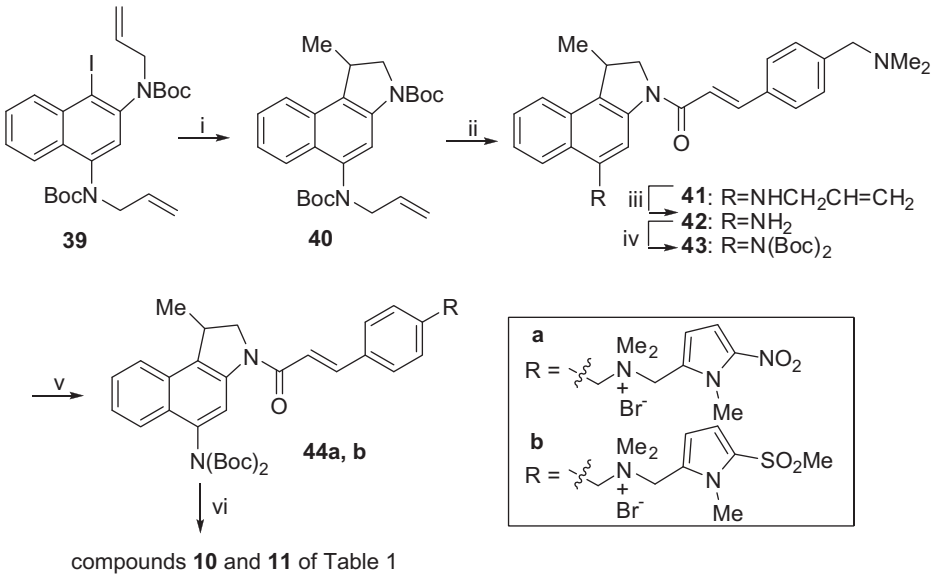
Following an analysis of the SAR studies, which suggested that **4** was a highly active compound, a radio-labelled sample of this was prepared, to enable drug disposition studies[22] (Scheme 7). The most accessible site for introducing a radiolabel is the methyl group of the quaternary nitrogen, allowing use of commercially available radiolabelled methylating agents. To this end, the monomethyl version (**55**) of compound **30b** (Scheme 2) was prepared starting from **18** and **23** and progressing through allylamine **53** (Scheme 7). This was the deallylated and re-protected as the bisBoc compound **55**, which was alkylated with tritium-enriched methyl iodide to give **56**. Deblocking then gave tritium-labelled **4** (^{3}**H-4**) (chemical purity 85%, radiochemical purity 71%, specific activity 107 GBq/mmol). Intensive attempts to improve the radiochemical purity by fractional crystallisation or HPLC purification were not successful.

2.2. Biology

The structures of the compounds studied are shown in Table 1. Compounds **3** (SN28127) and **4** (SN29220) were studied in greater detail elsewhere[22] to explore the scope and mechanism of their effects. In the present paper, we study a wider range of analogues in a single assay, seeking to define structure–activity relationships. Male C3H/HeN mice (~50 days old at time of drug treatment; groups of 4) were monitored for 7–10 days to establish normal eating and weight gain behaviour, then were given drug as a single ip injection in 42% aqueous DMSO. Body weight and food and water intake were measured for a further 60 days. Individual animals in



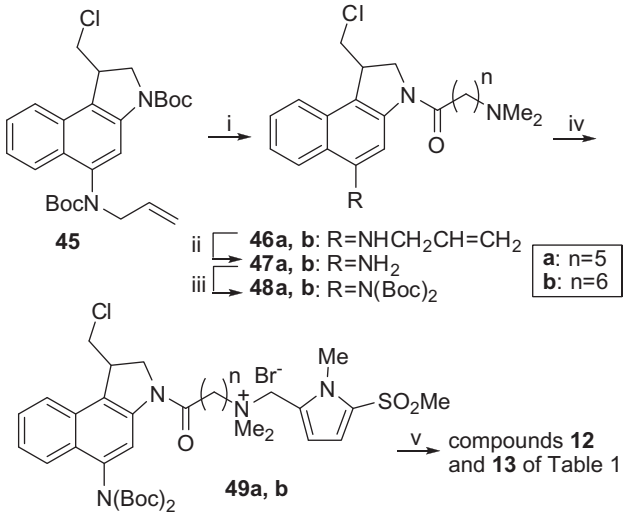
Scheme 4. Reagents and conditions: (i) HCl(g), dioxane, 15 h, 20 °C, then $(CF_3CO)_2O$, pyridine, 30 min, 0 °C; (ii) $PtO_2{\cdot}xH_2O$, THF, MeOH, H_2, 40 psi, 1 h, then $(Boc)_2O$, DMAP, dioxane, 20 h, reflux (N_2); (iii) Cs_2CO_3, DCM, MeOH 1 h, 20 °C, then *E*-4-(dimethylaminomethyl)cinnamoyl chloride, Et_3N, THF, 15 h, 20 °C; (iv) **16** or **18**, THF, 5–6 days, 20 °C (dark); (v) HBr(g), dioxane, 15 h, 20 °C (dark).



Scheme 5. Reagents and conditions: (i) AIBN, Bu_3SnH, benzene, 15 min, reflux; (ii) TFA, 2 h, 20 °C, then *E*-4-(dimethylaminomethyl)cinnamoyl chloride (from acid **20**), THF, 12 h, 20 °C; (iii) $Pd(Ph_3P)_4$, $PhSO_2Na$, CSA, DCM , 3 h, 20 °C (N_2); (iv) $(Boc)_2O$, DMAP, dioxane, 48 h, reflux; (v) **16** or **18**, THF, 48–85 h, 20 °C (N_2, dark); (vi) HCl(g), dioxane, 15 h, 20 °C (dark).

treatment and control groups (given vehicle only) were weighed regularly and the mean body weight change at the end of the experiment for the treatment and control (vehicle-only) groups are recorded in Table 1. Food and water intake for each group were also monitored over the course of the experiment. As explored in more detail elsewhere,[22] with most compounds there was a substantial dose-dependent but transient increase in both food and water intake from about days 15–25 after treatment (up to a maximal 50% increase in average food intake, and up to a maximal threefold increase in water consumption). In all cases, food and water intakes had returned to control levels before 60 days.[22]

The original nitroimidazole analogue **3** demonstrated a consistent dose–response effect for body weight; a dose of 0.47 μmol/kg was ineffective (both with regard to body weight and food and water intake), but the higher doses of 1.4 and 4.2 μmol/kg resulted in substantial and prolonged weight control with respect to untreated animals at 60 days post-dosing (Table 1 and Fig. 2).

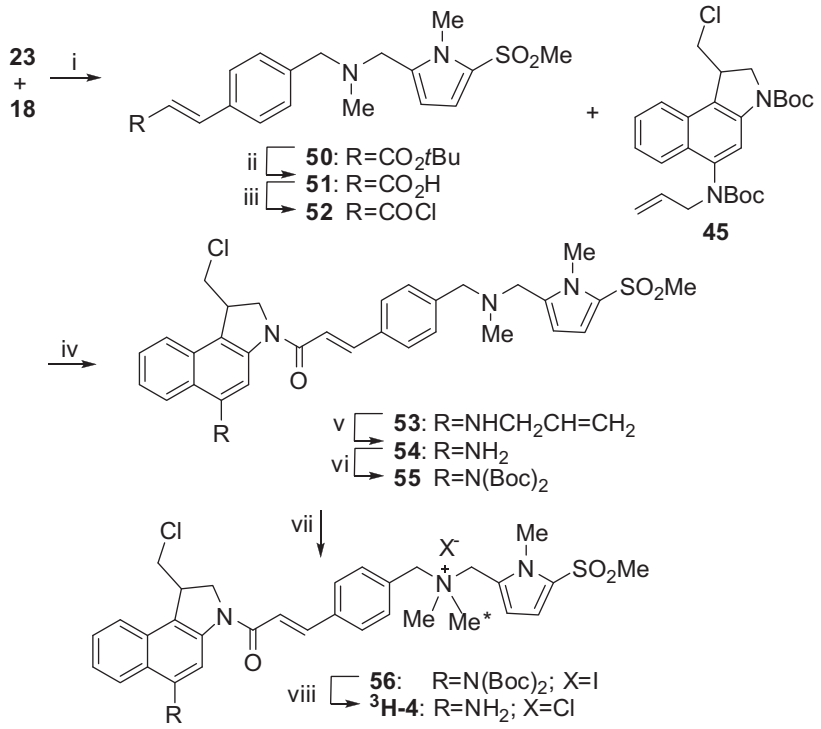


Scheme 6. Reagents and conditions: (i) TFA, 3 h, 20 °C, then 6-(dimethylamino)hexanoyl chloride hydrochloride or 7-(dimethylamino)heptanoyl chloride hydrochloride, DCM, 30 min, 20 °C; or HCl, dioxane, 3 h, 20 °C, then 7-(dimethylamino)heptanoyl chloride hydrochloride, DMF, DIPEA, 16 h, 20 °C; (ii) $Pd(Ph_3P)_4$, $PhSO_2Na$, CSA, DCM, 30 min, 20 °C (N_2); (iii) $(Boc)_2O$, DMAP, THF, dioxane, 12–15 h, reflux; (iv) **18**, K_2CO_3, THF, 2 h, 20 °C; (v) TFA, DCM, 6 h, 20 °C.

To explore the possible influence of metabolic reduction of the nitro group (which would be expected to result in fragmentation and release of the very cytotoxic DNA minor groove alkylator **32**),[12] the nitro group was replaced in compound **4** with a methylsulfonyl group, which has similar electronic properties, but is not a ready substrate for bioreduction. This compound proved more potent as a weight controller, showing substantial prolonged effects at doses of both 0.42 and 1.4 μmol/kg, but caused some deaths at 12.6 μmol/kg. To explore whether the quaternary salt function was necessary, a limited study was done with compound **5** (the des-methyl, non-quaternized analogue of **3**), but this was not effective at a dose (4.2 μmol/kg) where **3** showed substantial effects, and also caused some deaths at that dose. Finally, to evaluate the importance of the nature of the quaternary centre, we prepared the simple trimethylammonium compound **6**. This showed modest weight control (P <0.03) at a non-toxic dose (0.47 μmol/kg), and was toxic at 4.2 μmol/kg (Fig. 3). An experiment with repeat dosing was conducted with **6**, which was given at both day 0 and day 40. The results (Fig. 3) show that with this compound a second dose generated a similar but attenuated response.

We then turned to exploration of the benzindole chromophore. Compound **7** is a hydroxyCBI (the phenol analogue of **3**). Non-quaternary hydroxy- and aminoCBIs have broadly similar mechanisms of cytotoxicity, through alkylation of DNA at the N3 of adenines in poly-AT sequences.[23] However, in the weight loss assay **7** showed no significant effect on mouse body weight at a dose (4.2 μmol/kg) where **3** was significantly active (P <0.0037).

Of more potential interest were analogues where the chloro leaving group on the chromophore was replaced, to abrogate the DNA alkylating capability of the compounds. This was investigated firstly with the corresponding alcohols **8** and **9** (the direct analogues of chloro compounds **3** and **4**, respectively) since these were synthetically accessible from available intermediates. Even at much higher doses (38 for compound **8** and 50 μmol/kg for compound **9**), the former showed no and the latter only modest weight control activity. Compound **9** was also tested at 117 μmol/kg, but was acutely toxic at that dose. Because it was not possible to conclusively prove that **8** and **9** did not contain small amounts of potentially active bromide from the HBr deblocking step (different



Scheme 7. Reagents and conditions: (i) DIPEA, THF, 15 h, 20 °C; (ii) TFA, 1 h, 20 °C; (iii) $(COCl)_2$, DMF, DCM, 3 h, 20 °C; (iv) TFA, 3 h, 0 °C; then **52**, DIPEA, DCM, 3 h, 20 °C; (v) CSA, $PhSO_2Na$, $Pd(PPh_3)_4$, DCM, 3 h, 20 °C; (vi) $(Boc)_2O$, DMAP, THF, dioxane, reflux overnight; (vii) ^{3}H-MeI, NMP, sealed vial, 8 days, 40 °C; (viii) 4 N HCl, dioxane, 15 h, 20 °C.

batches of **8** gave considerably different biological results), we also prepared and evaluated the corresponding methyl compounds **10** and **11** as unequivocal non-alkylating analogues. These are the analogues of compounds **3** and **4**, respectively, but with the chloromethyl group replaced by methyl, and were completely ineffective in terms of weight control at doses of 38 μmol/kg, suggesting that DNA alkylating ability is required for the weight loss effects.

Having thus established the requirement for the 5-amino-1-(chloromethyl)-1,2-dihydro-3*H*-benz[*e*]indole (potentially DNA alkylating) chromophore and the quaternary centre for weight loss in this series, we finally looked at the effect of varying the link between these units, to see whether their relative positioning was important. Replacing the cinnamide unit with more flexible alkyl linkers gave compounds **12** and **13**, which showed effective long-term weight control at a dose of 4.2 μmol/kg (P <0.0178). Compound **13** was also effective (P <0.0035) at a dose of 12.6 μmol/kg, whereas **12**, different only by one methylene unit) was toxic at that dose.

While no true positive controls are available for this unique long-term weight control effect, the compounds were compared with the glucosylnitrosourea streptozotocin (STZ; **14**), since both compounds show selective toxicity to the pancreas.[22,24] However, **14** showed no weight loss effect at 377 μmol/kg, and was toxic at 1130 μmol/kg, with excessive and continuing weight loss resulting in termination of the experiment at day 18. The mechanism of action and spectrum of action may not be the same; **14** shows promiscuous methylation of DNA, including at adenine N-3 sites,[25] whereas the 5-aminoCBIs are exclusive adenine-N3 alkylators.[23] More importantly, **14** readily induces type 1 diabetes, whereas a detailed study[22] of compounds **3** and **4** shows that these do not.

3. Conclusions

Single non-toxic doses of *N*,*N*-dimethyl-*N*-(pyrrolylmethyl) and other quaternary salts of 5-amino-1-(chloromethyl)-1,2-dihydro-3*H*-benz[*e*]indole can cause substantial and permanent control of body weight without any apparent toxicity, compared to control-treated animals in a mouse model. The reduction is sustained for 60 days post injection (and for up to 160 days in other experiments[21]), while the mice continue to eat as much food as control mice. Drug-treated mice showed an increase in water intake between about 15–25 days post injection, but this then returned to normal. In all cases where dose–response studies were done, reduction in body weight and transient increase in thirst were both dose-dependent.[22] The body weight loss appeared to maximize at about 25 days post drug injection and then remained fairly stable. More detailed studies[22] indicated that the weight loss was largely due to substantial (50–75%) reductions in body fat. The SAR shows that a DNA alkylating capability is a necessary but not sufficient (compound **7**) condition for these compounds to show this sustained body weight control. Mechanism of action studies[22] with compounds **3** and **4** tentatively suggest that these cause inhibition of bile flow into the intestine and reduced absorption of triglycerides, together with accelerated cell death in spleen and white adipose tissues due to drug accumulation there. Tissue distribution studies[22] with ^{3}**H-4** showed that within 1–2 days most of the radioactivity accumulated in the pancreas, spleen, liver, bile duct, stomach, kidneys, visceral fat, and retroperitoneal fat, and was retained in the spleen, visceral fat and retroperitoneal fat for up to 30 days after treatment. While this class of compound may not be suitable as drugs due to their DNA-alkylating capability, the observation that they appear to reset the 'set-point' body weight in mice is intriguing.

4. Experimental

4.1. Chemistry

All reagents used were of analytical grade. Analyses were carried out in the Microchemical Laboratory, University of Otago, Dunedin, NZ. Melting points were determined on an Electrothermal 2300 Melting Point Apparatus. Final products were analysed

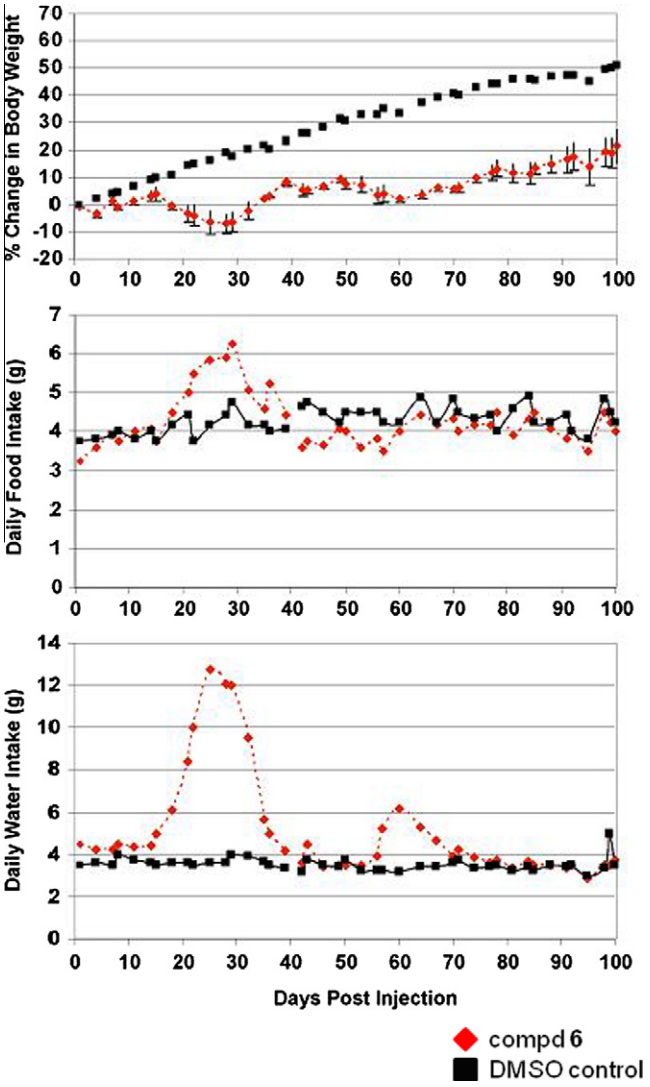


Figure 3. Variation in (from top) average weight change and food and water intake following treatment of male C3H/HeN mice with compound **6** at 0.42 μmol/kg at day 0 and again at day 40. Body weight changes are shown as a % of mouse body weight on day 0. Food and water intake are averaged per mouse. Data are shown as means ($n = 4$ mice).

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% MeCN/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%, unless otherwise stated. NMR spectra were obtained on a Bruker Avance 400 spectrometer at 400 MHz for ^1H spectra. Spectra were obtained in DMSO-d_6 or $CDCl_3$ unless otherwise specified, and are referenced to Me_4Si. Chemical shifts and coupling constants were recorded in units of ppm and Hz, respectively. Assignments were determined using COSY, HSQC, and HMBC two-dimensional experiments where appropriate. Mass spectra were determined on a VG-70SE mass spectrometer using an ionizing potential of 70 eV at a nominal resolution of 1000. High-resolution spectra were obtained at nominal resolutions of 3000, 5000, or 10000 as appropriate. FAB+ spectra used *m*-nitrobenzyl alcohol as the matrix and a xenon atom gun. Accurate mass calculations were referenced to polyethyleneglycol (PEG). Solutions in organic solvents were dried with anhydrous Na_2SO_4. Solvents were evaporated under reduced pressure on a rotary evaporator. Thin-layer chromatography was carried out on aluminum-backed silica gel plates (Merck 60 F_{254}), with visualization of components by UV light (254 nm) or exposure to I_2. Column chromatography was carried out on silica gel, (Merck 230–400 mesh). DCM refers to dichloromethane; DMSO refers to dimethylsulfoxide; EtOAc refers to ethyl acetate; MeOH refers to methanol; MeCN refers to acetonitrile; petroleum ether refers to petroleum ether, boiling range 40–60 °C. All solvents were freshly distilled.

4.1.1. 2-(Bromomethyl)-1-methyl-5-nitropyrrole (16) (Scheme 1)

A solution of Br_2 (0.45 mL, 8.7 mmol) in dry MeCN (5 mL) was added dropwise to a solution of PPh_3 (2.28 g, 8.7 mmol) in MeCN (30 mL) at 0 °C, and the pale yellow solution was allowed to warm to 20 °C. A solution of 2-(hydroxymethyl)-1-methyl-5-nitropyrrole[15] (**15**) (1.04 g, 6.7 mmol) in MeCN (15 mL) was added and the purple solution was stirred at 20 °C for 35 min. The mixture was evaporated and the residue purified by column chromatography (EtOAc/petroleum ether, 1:9) to give **16** as a pale yellow crystalline solid (1.35 g, 92%): mp 92–92 °C; ^1H NMR ($CDCl_3$) δ 7.16 (d, $J = 4.4$ Hz, 1H), 6.27 (d, $J = 4.4$ Hz, 1H), 4.48 (s, 2H), 3.99 (s, 3H). Anal. Calcd for $C_6H_7BrN_2O_2$: C, 32.90; H, 3.22; N, 12.79. Found: C, 33.31; H, 2.58; N, 12.71. Compound **16** was stable for several months when stored in the freezer, but underwent solvent displacement of the bromide on attempted recrystallization from MeOH.

4.1.2. 2-(Bromomethyl)-1-methyl-5-(methylsulfonyl)pyrrole (18)

A solution of Br_2 (373 mg, 2.33 mmol) in dry MeCN (5 mL) was added dropwise to a cooled solution of Ph_3P (527 mg, 2.33 mmol) in dry MeCN (9 mL) at 0 °C. After the mixture became colorless, a solution of 2-(hydroxymethyl)-1-methyl-5-(methylsulfonyl)pyrrole[16] (**17**) (400 mg, 2.12 mmol) in dry MeCN (3 mL) was added at 20 °C. After 15 min MeCN was removed and column chromatography (petroleum ether/EtOAc, 1:1) gave **18** (437 mg, 82%) as a white crystalline solid: mp 131–134 °C; ^1H NMR ($CDCl_3$) δ 6.84 (d, $J = 4.1$ Hz, 1H), 6.27 (d, $J = 4.1$ Hz, 1H), 4.49 (s, 2H), 3.90 (s, 3H), 3.10 (s, 3H). ^{13}C NMR δ 134.8, 130.7, 117.0, 110.4, 45.4, 31.9, 22.8. Anal. Calcd for $C_7H_{10}BrNO_2S$: C, 33.35; H, 4.00; N, 5.56. Found: C, 33.88; H, 4.08; N, 5.35. Compound **18** was stable for several months when stored in the freezer.

4.1.3. *E*-4-(Dimethylaminomethyl)cinnamic acid hydrochloride (20)

A solution of NaOH (974 mg, 24.4 mmol) in water (8 mL) was added to a solution of methyl *E*-4-(dimethylaminomethyl)cinnamate[26] (**19**) (2.67 g, 12.2 mmol) in MeOH (40 mL), and the mixture was stirred at reflux for 2 h, then cooled to 20 °C. Aqueous HCl (12.2 mL of 2.0 N solution, 24.4 mmol) was added and the solution was evaporated to dryness. The residue was dried in a vacuum desiccator and then extracted overnight with MeCN in a Soxhlet apparatus. The MeCN was reduced to a small volume (ca. 30 mL), allowed to cool to 20 °C, and the white solid was filtered off and dried to give acid **20** (1.10 g, 44%): mp 181–183 °C; ^1H NMR (DMSO-d_6) δ 7.63 (d, $J = 8.1$ Hz, 2H), 7.56 (d, $J = 16$ Hz, 1H), 7.33 (d, $J = 8.1$ Hz, 2H), 6.49 (d, $J = 16$ Hz, 1H), 3.42 (s, 2H), 2.15 (s, 6H); ^{13}C NMR δ 167.6, 143.4, 141.0, 133.0, 129.1, 128.0, 119.0, 62.8, 44.8. Anal. Calcd for $C_{12}H_{15}NO_2 \cdot 1/4H_2O$: C, 68.71; H, 7.45; N, 6.68. Found: C, 68.82; H, 7.70; N, 6.87.

4.1.4. *E*-[4-({Methyl[(1-methyl-5-nitro-2-pyrrolyl)methyl]-amino}methyl)cinnamic acid trifluoroacetate (25)

AIBN (80 mg, 0.459 mmol) and NBS (830 mg, 4.59 mmol) were added to a solution of *tert*-butyl *E*-4-methylcinnamate[27] (**21**) (1.00 g, 4.59 mmol) in CCl_4 (20 mL) at reflux. After 15 h the mixture was cooled and DCM was added. The solution was washed with water and brine and then dried (Na_2SO_4) and evaporated. The

residue was purified by column chromatography (petroleum ether/EtOAc, 49:1) to give *tert*-butyl *E*-4-(bromomethyl)cinnamate (**22**) (686 mg, 50%) as a colorless oil: ^{1}H NMR ($CDCl_3$) δ 7.57 (d, J = 16.1 Hz, 1H), 7.46 (d, J = 8.1 Hz, 2H), 7.38 (d, J = 8.2 Hz, 2H), 6.35 (d, J = 16.0 Hz, 1H), 4.80 (s, 2H), 1.53 (s, 9H); HRMS (^{79}Br) m/z 296.0401 (M^+), $C_{14}H_{17}BrO_2$ requires 296.0412.

A solution of **22** (380 mg, 1.28 mmol) in EtOH (10 mL) and DCM (3 mL) was added to a solution of methylamine (1.49 g, 19.2 mmol, 40% w/w) in water and the mixture was stirred for 2 h. DCM (30 mL) was added and the organic layer was separated. The organic layer was washed with 2 M NaOH, water and brine and then dried (Na_2SO_4) and evaporated. The residue was purified by column chromatography (DCM/MeOH, 99:1 then 49:1) to give *tert*-butyl *E*-4-(methylaminomethyl)cinnamate (**23**) (195 mg, 62%) as a yellow oil: ^{1}H NMR ($CDCl_3$) δ 7.57 (d, J = 16.0 Hz, 1H), 7.47 (d, J = 8.1 Hz, 2H), 7.32 (d, J = 8.1 Hz, 2H), 6.34 (d, J = 16.0 Hz, 1H), 3.77 (s, 2H), 2.44 (s, 3H), 2.04 (s, 1H), 1.52 (s, 9H); HRMS m/z 247.1572 (M^+), $C_{15}H_{21}NO_2$ requires 247.1572.

A solution of **23** (69 mg, 0.279 mmol), bromide **16** (51 mg, 0.233 mmol) and DIPEA (39 mg, 0.303 mmol) in THF (3 mL) was stirred under N_2 for 15 h. The solvent was removed under reduced pressure and the residue was purified by column chromatography (petroleum ether/EtOAc, 9:1) to give *tert*-butyl *E*-[4-({methyl[(1-methyl-5-nitro-2-pyrrolyl)methyl]amino}methyl)cinnamate (**24**) (102 mg, 94%) as a yellow oil: ^{1}H NMR ($CDCl_3$) δ 7.57 (d, J = 16.0 Hz, 1H), 7.46 (d, J = 8.1 Hz, 2H), 7.29 (d, J = 8.1 Hz, 2H), 7.14 (d, J = 4.3 Hz, 1H), 6.35 (d, J = 16.0 Hz, 1H), 6.10 (d, J = 4.3 Hz, 1H), 3.96 (s, 3H), 3.52 (s, 2H), 3.48 (s, 2H), 2.17 (s, 3H), 1.53 (s, 9H); HRMS m/z 385.2002 (M^+), $C_{21}H_{27}N_3O_4$ requires 385.2002.

TFA (1 mL) was added to cooled (0 °C) **24** (40 mg, 0.104 mmol) and the resulting solution was stirred for 1 h. The TFA was removed under reduced pressure and the residue was triturated with Et_2O to give **25** as a hygroscopic colorless solid that turned to an oil in air: ^{1}H NMR (DMSO-d_6) δ 7.74 (d, J = 8.0 Hz, 2H), 7.59 (d, J = 16.0 Hz, 1H), 7.48 (d, J = 7.9 Hz, 2H), 7.25 (d, J = 4.4 Hz, 1H), 6.57 (d, J = 16.0 Hz, 1H), 6.47 (d, J = 4.4 Hz, 1H), 4.23 (s, 2H), 4.14 (s, 2H), 3.88 (s, 3H), 4 protons not observed; HRMS (FAB) m/z 330.1454 (M+1−CF_3CO_2H) $C_{17}H_{20}N_3O_4$ requires 330.1454.

4.1.5. (*E*)-*N*-(4-(3-(5-Amino-1-(chloromethyl)-1*H*-benzo[*e*]indol-3(2*H*)-yl)-3-oxoprop-1-enyl)benzyl)-*N*,*N*-dimethyl-1-(1-methyl-5-nitro-1*H*-pyrrol-2-yl)methanaminium bromide hydrobromide (3) (Scheme 2)

A solution of 5-amino-1-(chloromethyl)-3-(trifluoroacetyl)-1,2-dihydro-3*H*-benz[*e*]indole[8] (**26**) (0.64 g, 1.95 mmol) in dioxane (50 mL) was treated with di-*tert*-butyl dicarbonate (1.5 g, 6.9 mmol) and DMAP (5 mg) and the solution was stirred at reflux under N_2 for 16 h. More di-*tert*-butyl dicarbonate (1.2 g, 5.5 mmol) was added and the solution stirred at reflux for a further 2 h. The solvent was evaporated and the residue purified by column chromatography (EtOAc/petroleum ether, 4:1) to give 5-[bis(*tert*-butoxycarbonyl)]amino-1-(chloromethyl)-3-(trifluoroacetyl)-1,2-dihydro-3*H*-benz[*e*]indole (**27**) as a cream solid (0.94 g, 91%). A sample was recrystallized from benzene/petroleum ether as white prisms: mp 181–182 °C; ^{1}H NMR ($CDCl_3$) δ 8.37 (s, 1H), 7.87 (d, J = 8.3 Hz, 1H), 7.80 (d, J = 8.1 Hz, 1H), 7.59 (dt, J = 7.6, 1.2 Hz, 1H), 7.53 (dt, J = 7.6, 1.3 Hz, 1H), 4.64 (d, J = 11.4 Hz, 1H), 4.45 (dt, J = 11.4, 8.5 Hz, 1H), 4.26–4.18 (m, 1H), 3.97 (dd, J = 11.5, 3.2 Hz, 1H), 3.57 (dd, J = 11.5, 9.4 Hz, 1H), 1.38 (s, 9H), 1.35 (s, 9H). Anal. Calcd for $C_{25}H_{28}ClF_3N_2O_5$: C, 56.77; H, 5.34; N, 5.30. Found: C, 56.63; H, 5.49; N, 5.23.

A solution of Cs_2CO_3 (1.4 g, 4.3 mmol) in water (30 mL) was added to a solution of **27** (440 mg, 0.83 mmol) in THF (40 mL), and the two-phase mixture was stirred vigorously at 20 °C for 8 h. The THF was evaporated and the residue was diluted with aqueous NaCl and extracted with EtOAc (×3). The extracts were washed with aqueous NaCl, dried (Na_2SO_4), and evaporated to provide crude amine as a yellow oil, which was immediately dissolved in THF (40 mL) and treated with *E*-4-(dimethylaminomethyl)cinnamoyl chloride [prepared from *E*-4-(dimethylaminomethyl)cinnamic acid (**20**) (256 mg, 1.24 mmol) and oxalyl chloride in dry MeCN 20 mL and DMF (one drop) stirred at 20 °C for 5.5 h, followed by evaporation], followed by Et_3N (0.52 mL, 3.73 mmol). The resulting light brown suspension was stirred at 20 °C for 45 min, then a solution of KOH (0.58 g, 10 mmol) in H_2O (6 mL) was added and the mixture was stirred at 20 °C for a further 12 h (to remove co-eluting by-product methyl *E*-4-(dimethylaminomethyl)cinnamate). Solvent was evaporated and the aqueous residue was extracted with EtOAc (×3) and purified by column chromatography (EtOAc then EtOAc/MeOH, 4:1) to give 5-[bis(*tert*-butoxycarbonyl)]amino-1-(chloromethyl)-3-[(*E*)-4-(dimethylaminomethyl)cinnamoyl]-1,2-dihydro-3*H*-benz[*e*]indole (**29**) as a yellow foam (480 mg, 77%): ^{1}H NMR ($CDCl_3$) δ 8.58 (br s, 1H), 7.87 (d, J = 15.3 Hz, 1H), 7.82 (d, J = 8.4 Hz, 1H), 7.76 (d, J = 8.2 Hz, 1H), 7.58 (d, J = 8.0 Hz, 2H) 7.53 (t, J = 7.5 Hz, 1H), 7.45 (t, J = 7.6 Hz, 1H), 7.37 (d, J = 8.0 Hz, 2H), 6.90 (d, J = 15.3 Hz, 1H), 4.59–4.53 (m, 1H), 4.46 (t, J = 9.5 Hz, 1H), 4.24–4.14 (m, 1H), 3.99 (dd, J = 11.3, 2.9 Hz, 1H), 3.53 (t, J = 10.8 Hz, 1H), 3.50 (s, 2H), 2.29 (s, 6H), 1.36 (s, 18H); ^{13}C NMR δ 164.4, 151.8, 151.7, 144.0, 141.1, 140.8, 137.3, 133.9, 129.7, 129.6, 128.4, 128.2, 127.3, 125.3, 123.9, 123.6, 122.7, 118.3, 118.0, 82.9, 63.8, 53.1, 46.1, 45.2, 42.7, 27.8; HRMS (FAB, ^{35}Cl) m/z 620.29015 (M+H), $C_{35}H_{43}ClN_3O_5$ requires 620.28912.

A solution of **29** (181 mg, 0.29 mmol) and bromide **16** (76.7 mg, 0.35 mmol) in THF (6 mL) was stirred in the dark at 20 °C for 3 days, giving a fine yellow precipitate. The precipitate was allowed to settle and the supernatant was decanted off. The remaining solid was triturated several times with MeCN, then filtered off and dried to give *N*-4-((*E*)-3-{5-[bis(*tert*-butoxycarbonyl)]amino-1-(chloromethyl)-1,2-dihydro-3*H*-benz[*e*]indol-3-yl}-3-oxo-1-propenyl)benzyl-*N*,*N*-dimethyl-*N*-[(1-methyl-5-nitro-2-pyrrolyl)methyl]ammonium bromide (**30a**) as a pale yellow solid (166 mg, 68%): mp 221 °C (dec.); ^{1}H NMR (DMSO-d_6) δ 8.40 (s, 1H), 8.03 (d, J = 8.3 Hz, 1H), 7.98 (d, J = 8.0 Hz, 2H), 7.76 (d, J = 15.4 Hz, 1H), 7.68 (d, J = 8.3 Hz, 1H), 7.64 (d, J = 8.3 Hz, 2H), 7.60 (t, J = 7.6 Hz, 1H), 7.53 (t, J = 7.5 Hz, 1H), 7.38 (d, J = 4.4 Hz, 1H), 7.35 (d, J = 16 Hz, 1H), 6.70 (d, J = 4.5 Hz, 1H), 4.83 (s, 2H), 4.66 (s, 2H), 4.65–4.47 (m, 3H), 4.09–3.99 (m, 2H), 4.02 (s, 3H), 2.95 (s, 6H), 1.37 (s, 9H), 1.36 (s, 9H). Anal. Calcd for $C_{41}H_{49}BrClN_5O_7$: C, 58.68; H, 5.88; N, 8.35; Br, 9.52. Found: C, 58.91; H, 5.77; N, 8.23; Br, 9.74.

Dioxane (3 mL) saturated with HBr gas was added to a suspension of **30a** (144 mg, 0.17 mmol) in dioxane (6 mL); the mixture was stirred in the dark at 20 °C for 12 h. Excess HBr was removed under reduced pressure, then the solid was filtered off and dried to give **3** as a yellow solid (120 mg, 97%): mp 170–180 °C (dec.); ^{1}H NMR (DMSO-d_6) δ 8.20 (br s, 1H), 8.04 (d, J = 8.5 Hz, 1H), 7.97 (d, J = 8.2 Hz, 2H), 7.86 (d, J = 8.4 Hz, 1H), 7.73 (d, J = 15.3 Hz, 1H), 7.64 (d, J = 8.2 Hz, 2H), 7.54 (t, J = 7.5 Hz, 1H), 7.41 (t, J = 7.6 Hz, 1H), 7.37 (d, J = 4.5 Hz, 1H), 7.35 (d, J = 16 Hz, 1H), 6.70 (d, J = 4.5 Hz, 1H), 4.84 (s, 2H), 4.67 (s, 2H), 4.55–4.46 (m, 2H), 4.31–4.26 (m, 1H), 4.03 (s, 3H), 3.99 (dd, J = 11.1, 2.7 Hz, 1H), 3.85 (dd, J = 10.4, 8.1 Hz, 1H), 2.96 (s, 6H); HRMS (FAB, ^{35}Cl) m/z 558.22205, $C_{31}H_{33}ClN_5O_3$ requires 558.22719.

HPLC analysis indicated the presence of 0.3% of **31**, an authentic sample of which was prepared from **30** as described below.

4.1.6. 5-Amino-1-(chloromethyl)-3-[(*E*)-4-(dimethylaminomethyl)cinnamoyl]-1,2-dihydro-(3*H*)benz[*e*]indole (31)

A solution of **29** (188 mg, 0.30 mmol) in dioxane (15 mL) was saturated with HCl gas and stirred at 20 °C for 1 h, during which time the HCl salt of the product precipitated. Aqueous $NaHCO_3$ was added and the mixture was extracted with EtOAc (×3). The

extracts were dried (Na_2SO_4) and evaporated, and the residue was purified by column chromatography (EtOAc then EtOAc/MeOH, 9:1 then 4:1), and recrystallized from benzene, giving **31** (as a yellow solid (91 mg, 71%): mp 230–240 °C (dec.): ^{1}H NMR ($CDCl_3$) δ 8.04 (br s, 1H), 7.86 (d, J = 15.3 Hz, 1H), 7.81 (d, J = 8.5 Hz, 1H), 7.68 (d, J = 8.3 Hz, 1H), 7.57 (d, J = 8.1 Hz, 2H), 7.50 (t, J = 7.3 Hz, 1H), 7.39–7.34 (m, 3H), 6.91 (d, J = 15.2 Ha, 1H), 4.51 (d, J = 10.6 Hz, 1H), 4.37 (t, J = 9.5 Hz, 1H), 4.32 (s, 2H), 4.09–4.02 (m, 1H), 3.95 (dd, J = 11.2, 2.8 Hz, 1H), 3.47 (s, 2H), 3.43 (t, J = 11.1 Hz, 1H), 2.27 (s, 6H); ^{13}C NMR δ 164.6, 144.2, 143.7, 142.1, 141.2, 133.9, 130.1, 129.6, 128.1, 127.2, 123.3, 122.9, 122.1, 121,1, 118,3, 114.6, 101.4, 64.0, 53.3, 46.3, 45.4, 42.6. Anal. Calcd for $C_{25}H_{26}ClN_3O$: C, 71.50; H, 6.24; N, 10.01. Found: C, 71.66; H, 6.18; N, 9.93.

4.1.7. (*E*)-*N*-(4-(3-(5-Amino-1-(chloromethyl)-1*H*-benzo[*e*]indol-3(2H)-yl)-3-oxoprop-1-enyl)benzyl)-*N*,*N*-dimethyl-1-(1-methyl-5-(methylsulfonyl)-1*H*-pyrrol-2-yl)methanaminium bromide hydrobromide (4)

A solution of **29** (159 mg, 0.256 mmol) and bromide **18** (97 mg, 0.385 mmol) in THF (4 mL) was stirred in the dark at 20 °C for 3 days. The precipitate was filtered off and triturated with Et_2O to give *N*-4-((*E*)-3-{5-[bis(*tert*-butoxycarbonyl)]amino-1-(chloromethyl)-1,2-dihydro-3*H*-benz[*e*]indol-3-yl}-3-oxo-1-propenyl)benzyl-*N*,*N*-dimethyl-*N*-[(1-methyl-5-methylsulfonyl-2-pyrrolyl)methyl]ammonium bromide (**30b**) (206 mg, 92%) as a pale yellow solid: mp 182–185 °C (dec.); ^{1}H NMR (DMSO-d_6) δ 8.40 (s, 1H), 8.03 (d, J = 8.3 Hz, 1H), 7.98 (d, J = 8.0 Hz, 2H), 7.77 (d, J = 15.4 Hz, 1H), 7.72–7.64 (m, 3H), 7.61 (td, J = 7.5, 1.1 Hz, 1H), 7.54 (td, J = 7.6, 1.0 Hz, 1H), 7.35 (d, J = 11.5 Hz, 1H), 6.92 (d, J = 4.1 Hz, 1H), 6.64 (d, J = 4.1 Hz, 1H), 4.77 (s, 2H), 4.65 (s, 2H), 4.62–4.54 (m, 2H), 4.53–4.46 (m, 1H), 4.07 (dd, J = 11.3, 3.3 Hz, 1H), 4.01 (dd, J = 11.2, 6.4 Hz, 1H), 3.97 (s, 3H), 3.32 (s, 3H), 2.92 (s, 6H), 1.37 (s, 9H), 1.35 (s, 9H). Anal. Calcd for $C_{42}H_{52}BrClN_4O_7S$: C, 57.83; H, 6.01; N, 6.42. Found: C, 57.55; H, 6.01; N, 6.34.

A solution of **30b** (40 mg, 0.046 mmol) in dioxane (1 mL) was treated with dioxane (2 mL) saturated with HBr gas and the mixture was stirred in the dark at 20 °C for 15 h. Excess HBr was removed under reduced pressure, then the solid was filtered off and triturated with Et_2O to give **4** as a yellow solid (28 mg, 80%): mp 178–182 °C (dec.); ^{1}H NMR (DMSO-d_6) δ 8.07 (d, J = 8.5 Hz, 1H), 8.01 (s, 1H), 7.96 (d, J = 8.2 Hz, 2H), 7.80 (d, J = 8.4 Hz, 1H), 7.71 (d, J = 15.4 Hz, 1H), 7.65 (d, J = 8.2 Hz, 2H), 7.50 (t, J = 7.5 Hz, 1H), 7.34 (t, J = 7.5 Hz, 1H), 7.33 (d, J = 15.4 Hz, 1H), 6.92 (d, J = 4.1 Hz, 1H), 6.65 (d, J = 4.1 Hz, 1H), 4.78 (s, 2H), 4.64 (s, 2H), 4.53–4.42 (m, 2H), 4.25–4.16 (m, 1H), 4.00–3.93 (m, 1H), 3.95 (s, 3H), 3.79 (dd, J = 11.1, 8.1 Hz, 1H), 3.28 (s, 3H), 2.91 (s, 6H); HRMS (FAB, ^{35}Cl) m/z 591.2213 (M+1), $C_{32}H_{36}ClN_4O_3S$ requires 591.2197. HPLC purity 98.4%.

Compound **4** was also obtained as a mixture of bromide and trifluoroacetate salts by Boc-deprotection of **30b** with TFA/DCM (1:1).

4.1.8. (*E*)-1-(4-(3-(5-Amino-1-(chloromethyl)-1*H*-benzo[e]indol-3(2*H*)-yl)-3-oxoprop-1-enyl)phenyl)-*N*,*N*,*N*-trimethylmethanaminium chloride hydrochloride (6)

A solution of **29** (180 mg, 0.29 mmol) and methyl iodide (22 μL, 0.35 mmol) in EtOAc (2 mL) was allowed to stand in the dark at 20 °C for 16 h. Et_2O was added dropwise, causing an oil to separate, and the supernatant was decanted off. The remaining oil was triturated with EtOAc, and the resulting solid was filtered off to give *N*-4-((*E*)-3-{5-[bis(*tert*-butoxycarbonyl)]amino-1-(chloromethyl)-1,2-dihydro-3*H*-benz[*e*]indol-3-yl}-3-oxo-1-propenyl)benzyl-*N*,*N*,*N*-trimethylammonium iodide (**30c**) (201 mg, 91%): mp >320 °C; ^{1}H NMR (DMSO-d_6) δ 8.40 (s, 1H), 8.03 (d, J = 8.3 Hz, 1H), 7.97 (d, J = 8.0 Hz, 2H), 7.75 (d, J = 15.4 Hz, 1H), 7.68 (d, J = 8.5 Hz, 1H), 7.63–7.58 (m, 3H), 7.53 (t, J = 7.6 Hz, 1H), 7.34 (d, J = 15.4 Hz, 1H), 4.67–4.47 (m, 5H), 4.08–3.98 (m, 2H), 3.05 (s, 9H), 1.37 (s, 9H), 1.36 (s, 9H). Anal. Calcd for $C_{36}H_{45}ClIN_3O_5\cdot H_2O$: C, 55.43; H, 6.07; N, 5.39. Found: C, 55.66; H, 5.99; N, 5.33.

A solution of **30c** (175 mg, 0.23 mmol) in dioxane (4 mL) was treated with dioxane (2 mL) saturated with HCl gas. A precipitate began to form within a few minutes. The mixture was stirred at 20 °C for 16 h, and the excess HCl was removed under reduced pressure. The solid was filtered off, washed with dioxane and dried to give a yellow-orange solid (122 mg): mp >310 °C; ^{1}H NMR (DMSO-d_6) δ 8.15 (br s, 1H), 8.07 (d, J = 8.5 Hz, 1H), 7.96 (d, J = 8.2 Hz, 2H), 7.85 (d, J = 8.3 Hz, 1H), 7.73 (d, J = 15.4 Hz, 1H), 7.61 (d, J = 8.2 Hz, 2H), 7.53 (d, J = 7.6 Hz, 1H), 7.39 (t, J = 7.6 Hz, 1H), 7.33 (d, J = 15.4 Hz, 1H), 4.57 (s, 2H), 4.54–4.45 (m, 2H), 4.30–4.23 (m, 1H), 3.99 (dd, J = 11.0, 3.0 Hz, 1H), 3.84 (dd, J = 11.0, 7.8 Hz, 1H), 3.07 (s, 9H). Microanalysis indicated a mixture of chloride and iodide counterions. The mixed salt (110 mg) was dissolved in MeOH (2 mL) and purified by ion exchange chromatography (Biorad AG-1X4 resin in Cl^- form), eluting with MeOH. The yellow product-containing fractions were concentrated to a small volume and diluted with EtOAc to give **6** as a yellow solid (83 mg, 79%). Anal. Calcd for $C_{26}H_{30}Cl_3N_3O\cdot H_2O\cdot 1/2EtOAc$: C, 59.11; H, 6.38; N, 7.39; Cl, 18.69. Found: C, 58.90; H, 6.41; N, 7.77; Cl, 18.24.

4.1.9. (*E*)-1-(5-Amino-1-(chloromethyl)-1*H*-benzo[*e*]indol-3(2*H*)-yl)-3-(4-((methyl((1-methyl-5-nitro-1*H*-pyrrol-2-yl)methyl)amino)methyl)phenyl)prop-2-en-1-one dihydrochloride (5)

A solution of **27** (60 mg, 0.114 mmol) and Cs_2CO_3 (153 mg, 0.456 mmol) in DCM (5 mL) and MeOH (5 mL) was stirred for 15 min. DCM (20 mL) and water were added and the organic layer was separated. The organic layer was washed with water, brine, and dried (Na_2SO_4), and then evaporated to provide crude amine (49 mg), which was immediately dissolved in THF (3 mL) with Et_3N (58 mg, 0.57 mmol). The solution was then treated with a solution of *E*-4-(dimethylaminomethyl)cinnamoyl chloride [prepared from acid **20** (76 mg, 0.171 mmol) and oxalyl chloride (187 mg, 1.54 mmol) in dry MeCN (3 mL) and DMF (one drop) stirred for 2 h and then evaporated under reduced pressure] in THF (2 mL)] and the mixture was stirred for 2 h at 20 °C. After 2 h the solvents were removed under reduced pressure and EtOAc and H_2O were added to the residue. The EtOAc extract was washed with brine and dried (Na_2SO_4). Column chromatography (petroleum ether/EtOAc, 7:3 then 3:2) followed by trituration with petroleum ether gave 5-[bis(*tert*-butoxycarbonyl)]amino-1-(chloromethyl)-3-{(*E*)-3-[4-({methyl[(1-methyl-5-nitro-2-pyrrolyl)methyl]amino}methyl)phenyl]-2-propenoyl}-1,2-dihydro-3*H*-benz[*e*]indole (**28**) (53 mg, 63%) as a yellow powder: mp 110–115 °C (dec.); ^{1}H NMR ($CDCl_3$) δ 8.57 (s, 1H), 7.88 (d, J = 15.3 Hz, 1H), 7.82 (d, J = 8.4 Hz, 1H), 7.75 (d, J = 8.3 Hz, 1H), 7.58 (d, J = 8.0 Hz, 2H), 7.53 (td, J = 7.1, 0.9 Hz, 1H), 7.44 (td, J = 7.2, 1.0 Hz, 1H), 7.34 (d, J = 7.9 Hz, 2H), 7.15 (d, J = 4.3 Hz, 1H), 6.91 (d, J = 15.2 Hz, 1H), 6.12 (d, J = 4.3 Hz, 1H), 4.58 (d, J = 10.8 Hz, 1H), 4.47 (t, J = 9.1 Hz, 1H), 4.27–4.16 (m, 1H), 4.03–3.96 (m, 1H), 9.98 (s, 3H), 3.58–3.49 (m, 1H), 3.57 (s, 2H), 3.52 (s, 2H), 2.20 (s, 3H), 1.36 (s, 18H). Anal. Calcd for $C_{40}H_{46}ClN_5O_7$: C, 64.55; H, 6.23; N, 9.41. Found: C, 64.20; H, 6.07; N, 9.05.

A solution of **28** (55 mg, 0.074 mmol) in dioxane (1 mL) was treated with dioxane (2 mL) saturated with HCl gas and the mixture was stirred in the dark at 20 °C for 15 h. Excess HCl was removed under reduced pressure, then the precipitate was filtered off and triturated with Et_2O to give **5** as a yellow solid (45 mg, 100%): mp 192–197 °C (dec.); ^{1}H NMR (DMSO-d_6) δ 7.95 (d, J = 8.4 Hz, 1H), 7.83 (s, 1H), 7.82 (d, J = 8.1 Hz, 2H), 7.64 (d, J = 15.4 Hz, 1H), 7.59–7.47 (m, 2H), 7.55 (d, J = 8.2 Hz, 2H), 7.39 (t, J = 7.8 Hz, 1H), 7.25 (d, J = 4.5 Hz, 1H), 7.19 (d, J = 15.4 Hz, 1H),

6.54 (d, J = 4.5 Hz, 1H), 4.49–4.33 (m, 2H), 4.45 (s, 2H), 4.37 (s, 2H), 4.30–4.22 (m, 1H), 3.77 (s, 3H), 2.66 (s, 3H), 2 protons not observed. Anal. Calcd for $C_{30}H_{32}Cl_3N_5O_3 \cdot H_2O \cdot 1/2$dioxane: C, 56.60; H, 5.64; N, 10.31. Found: C, 56.91; H, 5.48; N, 10.47.

4.1.10. (*E*)-*N*-(4-(3-(1-(Chloromethyl)-5-hydroxy-1*H*-benzo[*e*]indol-3(2*H*)-yl)-3-oxoprop-1-enyl)benzyl)-*N*,*N*-dimethyl-1-(1-methyl-5-nitro-1*H*-pyrrol-2-yl)methanaminium bromide hydrobromide (7) (Scheme 3)

A solution of 3-(*tert*-butoxycarbonyl)-1-(chloromethyl)-5-hydroxy-1,2-dihydro-3*H*-benz[*e*]indole[17] (**32**) (181 mg, 0.54 mmol) in dioxane (10 mL) was saturated with HCl gas and allowed to stand at 20 °C until TLC indicated complete deprotection of the Boc group (ca. 1 h). The dioxane was evaporated, and **20** (111 mg, 0.54 mmol), EDCI·HCl (207 mg, 1.08 mmol), and dry DMA (3 mL) were added to the residue. The resulting mixture was stirred at 20 °C for 16 h, then diluted with aqueous $NaHCO_3$ and extracted with EtOAc (×3). The extracts were dried (Na_2SO_4) and evaporated, and the residue was triturated with DCM to give 1-(chloromethyl)-3-[(*E*)-4-(dimethylaminomethyl)cinnamoyl]-5-hydroxy-1,2-dihydro-3*H*-benz[*e*]indole (**33**) as a yellow solid (72 mg, 32%): mp 225–233 °C (dec.). ^{1}H NMR (DMSO-d_6) δ 10.42 (s, 1H), 8.12–8.07 (m, 2H), 7.81 (d, J = 8.3 Hz, 1H), 7.76 (d, J = 8.1 Hz, 2H), 7.67 (d, J = 15.4 Hz, 1H), 7.51 (dt, J = 7.6, 0.8 Hz, 1H), 7.38–7.31 (m, 3H), 7.21 (d, J = 15.4 Hz, 1H), 4.57–4.45 (m, 2H), 4.27–4.18 (m, 1H), 3.99 (dd, J = 11.0, 2.9 Hz, 1H), 3.84 (dd, J = 11.0, 7.8 Hz, 1H), 3.42 (s, 2H), 2.16 (s, 6H); ^{13}C NMR δ 163.5, 154.2, 142.0, 140.8, 133.6, 129.8, 129.1, 128.4, 128.2, 127.2, 123.1, 122.8, 122.5, 121.8, 119.6, 114.6, 100.0, 62.9, 52.9, 47.7, 44.8, 40.6. Anal. Calcd for $C_{25}H_{25}ClN_2O_2$: C, 71.33; H, 5.99; N, 6.65. Found: C, 71.24; H, 6.07; N, 6.81.

The triturate was evaporated and the residue was purified by column chromatography (EtOAc/MeOH, 9:1 then 4:1). The fractions containing product were evaporated, and the residue was dissolved in THF and filtered. The filtrate was evaporated and the residue triturated with EtOAc to give further **33** (69 mg, 30%).

A solution of **33** (28 mg, 0.066 mmol) in THF (6 mL) was treated with bromide **16** (17.5 mg, 0.11 mmol) and the mixture was stirred in the dark at 20 °C for 18 days. The precipitate was filtered off to give **7** as a yellow powder (33 mg, 78%): ^{1}H NMR (DMSO-d_6) δ 10.46 (s, 1H), 8.12 (d, J = 8.5 Hz, 1H), 8.08 (s, 1H), 7.97 (d, J = 8.1 Hz, 2H), 7.82 (d, J = 8.3 Hz, 1H), 7.73 (d, J = 15.4 Hz, 1H), 7.65 (d, J = 8.1 Hz, 2H), 7.52 (t, J = 7.5 Hz, 1H), 7.39–7.32 (m, 3H), 6.70 (d, J = 4.5 Hz, 1H), 4.89 (s, 2H), 4.72 (s, 2H), 4.57–4.45 (m, 2H), 4.27–4.20 (m, 1H), 4.04 (s, 3H), 4.01 (dd, J = 11.0, 2.8 Hz, 1H), 3.84 (dd, J = 11.0, 8.1 Hz, 1H), 2.96 (s, 6H); ^{13}C NMR δ 163.2, 154.2, 141.9, 141.0, 139.7, 136.6, 133.5, 129.8, 129.2, 128.6, 127.7, 127.2, 123.1, 122.9, 122.6, 121.9, 121.8, 115.7, 114.7, 113.1, 99.9, 66.0, 58.1, 53.0, 48.2, 47.6, 40.6, 34.9. HPLC analysis indicated the presence of 0.15% of **33**.

A sample was prepared as the chloride salt: Anal. Calcd for $C_{31}H_{32}Cl_2N_4O_2 \cdot 2H_2O$: C, 58.96; H, 5.75; N, 8.87. Found: C, 59.12; H, 5.50; N, 8.83.

4.1.11. (*E*)-*N*-(4-(3-(5-Amino-1-(hydroxymethyl)-1*H*-benzo[*e*]indol-3(2*H*)-yl)-3-oxoprop-1-enyl)benzyl)-*N*,*N*-dimethyl-1-(1-methyl-5-nitro-1*H*-pyrrol-2-yl)methanaminium bromide hydrobromide (8) (Scheme 4)

A solution of 1-(hydroxymethyl)-5-nitro-3-(*tert*-butoxycarbonyl)-1,2-dihydro-3*H*-benz[*e*]indole[18] (**34**) (200 mg, 0.581 mmol) in dioxane (20 mL) saturated with HCl gas was stirred for 15 h. The dioxane was removed under reduced pressure and pyridine (5 mL) and $(CF_3CO)_2O$ (439 mg, 2.09 mmol) were added to the residue at 0 °C. After 30 min the mixture was poured into ice water and extracted with DCM. The DCM extract was washed with 1 M HCl and brine and then dried (Na_2SO_4) and evaporated. Column chromatography (petroleum ether/EtOAc, 4:1–1:1) gave 1-(hydroxymethyl)-5-nitro-3-(trifluoroacetyl)-1,2-dihydro-3*H*-benz[*e*]indole (**35**) (177 mg, 89%) as an orange powder: mp 150–151 °C; ^{1}H NMR ($CDCl_3$) δ 9.11 (s, 1H), 8.49–8.40 (m, 1H), 7.99–7.92 (m, 1H), 7.74–7.67 (m, 2H), 4.67 (dt, J = 11.2, 1.5 Hz, 1H), 4.46 (dd, J = 11.1, 8.7 Hz, 1H), 4.15–4.05 (m, 2H), 3.90–3.80 (m, 1H), 1.68 (t, J = 4.2 Hz, 1H). Anal. Calcd for $C_{15}H_{11}F_3N_2O_4$: C, 53.00; H, 3.26; N, 8.23. Found: C, 53.48; H, 3.34; N, 8.26.

A solution of **35** (200 mg, 0.588 mmol) in THF (15 mL) and MeOH (3 mL) was hydrogenated over $PtO_2 \times H_2O$ (Acros, Pt content 79–84%, 30 mg) at 40 psi for 1 h. The mixture was filtered through Celite and the filtrate was evaporated to give crude 5-amino-1-(hydroxymethyl)-3-(trifluoroacetyl)-1,2-dihydro-3*H*-benz[*e*]-indole as a cream solid (180 mg, 99%), which was used directly in the next step. The entire sample (180 mg, 0.581 mmol) was dissolved in dioxane (10 mL) with di-*tert*-butyl dicarbonate (1.01 g, 4.65 mmol) and DMAP (5 mg) and the solution was stirred at reflux under N_2 for 15 h. More di-*tert*-butyl dicarbonate (506 mg, 2.32 mmol) was added and the solution was stirred at reflux for a further 5 h. The solvent was evaporated and the residue purified by column chromatography (petroleum ether/EtOAc, 9:1). Trituration with petroleum ether gave 5-[bis(*tert*-butoxycarbonyl)]amino-1-{[(*tert*-butoxycarbonyl)oxy]methyl}-3-(trifluoroacetyl)-1,2-dihydro-3*H*-benz[*e*]indole (**36**) (263 mg, 74%) as a cream solid: mp 147–150 °C; ^{1}H NMR ($CDCl_3$) δ 8.37 (s, 1H), 7.94 (d, J = 8.3 Hz, 1H), 7.84 (d, J = 8.4 Hz, 1H), 7.59 (td, J = 7.6, 1.1 Hz, 1H), 7.52 (td, J = 7.7, 1.1 Hz, 1H), 4.61 (dd, J = 11.3, 3.4 Hz, 1H), 4.55 (d, J = 11.4 Hz, 1H), 4.38 (dd, J = 11.3, 8.5 Hz, 1H), 4.25–4.17 (m, 1H), 3.90 (dd, J = 11.1, 10.0 Hz, 1H), 1.52 (s, 9H), 1.37 (s, 9H), 1.36 (s, 9H). Anal. Calcd for $C_{30}H_{37}F_3N_2O_8$: C, 59.01; H, 6.11; N, 4.59. Found: C, 59.03; H, 6.14; N, 4.54.

A solution of **36** (150 mg, 0.246 mmol) and Cs_2CO_3 (400 mg, 1.23 mmol) in DCM (8 mL) and MeOH (8 mL) was stirred for 1 h. The solvents were removed under reduced pressure and the residue partitioned between EtOAc and water. The organic layer was washed with brine, then dried (Na_2SO_4) and evaporated. The crude product (126 mg) was dissolved in THF (4 mL) with Et_3N (124 mg, 1.23 mmol) and treated with a solution of *E*-4-(dimethylaminomethyl)cinnamoyl chloride (0.369 mmol) in THF (4 mL). The mixture was stirred for 15 h, then solvents were removed and the residue was partitioned between DCM and water. The DCM extract was washed with brine, and then dried (Na_2SO_4) and evaporated. Column chromatography (petroleum ether/EtOAc, 1:1 then DCM/MeOH, 95:5) followed by trituration with petroleum ether gave 5-[bis(*tert*-butoxycarbonyl)]amino-1-[(*tert*-butoxycarbonyl)oxy]-methyl-3-[(*E*)-4-(dimethylaminomethyl)cinnamoyl]-1,2-dihydro-3*H*-benz[*e*]indole (**37**) (160 mg, 93%) as a yellow powder: mp 127–131 °C; ^{1}H NMR ($CDCl_3$) δ 8.57 (s, 1H), 7.91 (d, J = 8.3 Hz, 1H), 7.87 (d, J = 15.3 Hz, 1H), 7.80 (d, J = 8.3 Hz, 1H), 7.58 (d, J = 8.1 Hz, 2H), 7.53 (t, J = 7.2 Hz, 1H), 7.43 (t, J = 7.1 Hz, 1H), 7.37 (d, J = 7.9 Hz, 2H), 4.68 (d, J = 9.6 Hz, 1H), 4.53 (d, J = 10.2 Hz, 1H), 4.42 (t, J = 8.7 Hz, 1H), 4.26–4.13 (m, 1H), 3.85 (t, J = 10.9 Hz, 1H), 3.48 (s, 2H), 2.28 (s, 6H), 1.52 (s, 9H), 1.35 (s, 18H). Anal. Calcd for $C_{40}H_{51}N_3O_8 \cdot$MeOH: C, 67.10; H, 7.55; N, 5.73. Found: C, 67.05; H, 7.20; N, 5.80.

A solution of **37** (46 mg, 0.066 mmol) and bromide **16** (17 mg, 0.079 mmol) in THF (2 mL) was stirred under N_2 in the dark for 6 days. The THF was removed and the residue was dissolved in EtOAc. Et_2O was added and the resulting precipitate was filtered off and triturated with Et_2O to give *N*-4-((*E*)-3-{5-[bis(*tert*-butoxy carbonyl)]amino-1-[(*tert*-butoxycarbonyl)oxy]methyl-1,2-dihydro-3*H*-benz[*e*]indol-3-yl}-3-oxo-1-propenyl)benzyl-*N*,*N*-dimethyl-*N*-[(1-methyl-5-nitro-2-pyrrolyl)methyl]ammonium bromide (**38a**) (50 mg, 83%) as a yellow powder: mp 166–170 °C (dec.); ^{1}H NMR (DMSO-d_6) δ 8.41 (s, 1H), 8.06 (d, J = 8.4 Hz, 1H), 7.98 (d, J = 8.1 Hz, 2H), 7.76 (d, J = 15.4 Hz, 1H), 7.69 (d, 8.4 Hz, 1H), 7.65–7.58 (m,

1H), 7.63 (d, J = 8.4 Hz, 2H), 7.53 (td, J = 7.7, 1.0 Hz, 1H), 7.36 (d, J = 4.5 Hz, 1H), 7.35 (d, J = 15.4 Hz, 1H), 6.70 (d, J = 4.5 Hz, 1H), 4.82 (s, 2H), 4.65 (s, 2H), 4.60–4.53 (m, 2H), 4.47 (dd, J = 10.8, 3.7 Hz, 1H), 4.36–4.28 (m, 1H), 4.10 (dd, J = 10.7, 8.1 Hz, 1H), 4.02 (s, 3H), 2.94 (s, 6H), 1.37 (s, 27H); HRMS m/z 840.4187 (M^+), $C_{46}H_{58}N_5O_{10}$ requires 840.4184.

Dioxane (2 mL) saturated with HBr gas was added to a solution of **38a** (50 mg, 0.054 mmol) in dioxane (1 mL) and the mixture was stirred in the dark at 20 °C for 15 h. Excess HBr was removed under reduced pressure and the precipitate was filtered off and triturated with Et_2O to give **8** as a yellow solid (38 mg, 100%): mp 180–185 °C (dec.); ^{1}H NMR (DMSO-d_6) δ 8.21 (s, 1H), 8.02 (d, J = 8.5 Hz, 1H), 7.95 (d, J = 8.3 Hz, 2H), 7.83 (d, J = 8.4 Hz, 1H), 7.72 (d, J = 15.4 Hz, 1H), 7.63 (d, J = 8.2 Hz, 2H), 7.52 (t, J = 7.3 Hz, 1H), 7.39 (t, J = 7.2 Hz, 1H), 7.35 (d, J = 4.5 Hz, 1H), 7.33 (d, J = 15.5 Hz, 1H), 6.69 (d, J = 4.5 Hz, 1H), 4.83 (s, 2H), 4.66 (s, 2H), 4.52 (d, J = 10.8 Hz, 1H), 4.45–4.36 (m, 1H), 4.02 (s, 3H), 3.92–3.82 (m, 1H), 3.78 (dd, J = 10.8, 3.9 Hz, 1H), 2.95 (s, 6H), 2 protons not observed; HRMS m/z 540.2614 (M^+), $C_{31}H_{34}N_5O_4$ requires 540.2611. HPLC purity 95.2%.

A sample was prepared as the hydrochloride salt: Anal. Calcd for $C_{31}H_{32}Cl_2N_4O_2\cdot 2H_2O$: C, 58.96; H, 5.75; N, 8.87. Found: C, 59.12; H, 5.50; N, 8.83.

4.1.12. (*E*)-*N*-(4-(3-(5-Amino-1-(hydroxymethyl)-1*H*-benzo[*e*]indol-3(2*H*)-yl)-3-oxoprop-1-enyl)benzyl)-*N*,*N*-dimethyl-1-(1-methyl-5-(methylsulfonyl)-1*H*-pyrrol-2-yl)methanaminium bromide hydrobromide (9)

A solution of **36** (40 mg, 0.057 mmol) and bromide **18** (17 mg, 0.068 mmol) in THF (1.5 mL) was stirred under N_2 in the dark for 5 days. The THF was removed, the residue was dissolved in EtOAc, and this solution was diluted with Et_2O. The resulting precipitate was filtered off and triturated with Et_2O to give *N*-4-((*E*)-3-{5-[bis(-*tert*-butoxycarbonyl)]amino-1-[(*tert*-butoxycarbonyl)oxy]methyl-1,2-dihydro-3*H*-benz[*e*]indol-3-yl}-3-oxo-1-propenyl)benzyl-*N*,*N*-dimethyl-*N*-[(1-methyl-5-methylsulfonyl-2-pyrrolyl)methyl] ammonium bromide (**38b**) (38 mg, 78%) as a yellow powder: mp 170 °C (dec.); ^{1}H NMR (DMSO-d_6) δ 8.41 (s, 1H), 8.06 (d, J = 8.4 Hz, 1H), 7.98 (d, J = 8.0 Hz, 2H), 7.75 (d, J = 15.3 Hz, 1H), 7.70–7.59 (m, 2H), 7.64 (d, J = 8.4 Hz, 2H), 7.52 (t, J = 8.0 Hz, 1H), 7.34 (d, J = 15.5 Hz, 1H), 6.92 (d, J = 4.1 Hz, 1H), 6.65 (d, J = 4.1 Hz, 1H), 4.77 (s, 2H), 4.65 (s, 2H), 4.59–4.52 (m, 2H), 4.47 (dd, J = 10.7, 3.9 Hz, 1H), 4.36–4.28 (m, 1H), 4.11 (dd, J = 10.6, 8.0 Hz, 1H), 3.95 (s, 3H), 3.31 (s, 3H), 2.92 (s, 6H), 1.37 (s, 27H); HRMS m/z 873.4105 (M^+), $C_{47}H_{61}N_4O_{10}S$ requires 873.4108.

Dioxane (3 mL) saturated with HBr gas was added to a solution of **38b** (98 mg, 0.103 mmol) in dioxane (2 mL) and the mixture was stirred in the dark at 20 °C for 15 h. Excess HBr was removed under reduced pressure, then the precipitate was filtered off and triturated with Et_2O to give **9** as a yellow solid (68 mg, 90%): mp 176–180 °C (dec.); ^{1}H NMR (DMSO-d_6) δ 8.10 (s, 1H), 8.02 (d, J = 8.5 Hz, 1H), 7.96 (d, J = 8.2 Hz, 2H), 7.80 (d, J = 8.6 Hz, 1H), 7.72 (d, J = 15.4 Hz, 1H), 7.66 (d, J = 8.2 Hz, 2H), 7.50 (t, J = 7.3 Hz, 1H), 7.36 (t, J = 7.3 Hz, 1H), 7.32 (d, J = 15.4 Hz, 1H), 6.92 (d, J = 4.1 Hz, 1H), 6.65 (d, J = 4.1 Hz, 1H), 4.78 (s, 2H), 4.64 (s, 2H), 4.51 (d, J = 11.6 Hz, 1H), 4.43–4.34 (m, 1H), 3.96 (s, 3H), 3.88–3.79 (m, 1H), 3.77 (dd, J = 11.0, 4.0 Hz, 1H), 2.91 (s, 6H), 5 protons not observed; HRMS m/z 573.2529 (M^+), $C_{32}H_{37}N_4O_4S$ requires 573.2536. HPLC purity 94.2%.

4.1.13. (*E*)-*N*-(4-(3-(5-Amino-1-methyl-1H-benzo[e]indol-3(2*H*)-yl)-3-oxoprop-1-enyl)benzyl)-*N*,*N*-dimethyl-1-(1-methyl-5-nitro-1*H*-pyrrol-2-yl)methanaminium bromide hydrobromide (10) (Scheme 5)

AIBN (140 mg, 0.851 mmol) and Bu_3SnH (1.49 g, 5.11 mmol) were added to a solution of *N*,*N*′-bis[allyl(*tert*-butoxycarbonyl)]-1-iodo-2,4-naphthalenediamine[19] (**39**) (2.40 g, 4.25 mmol) in dry benzene at reflux. After 15 min the mixture was cooled and the solvent was removed under reduced pressure. The residue was partitioned between EtOAc and water, then the EtOAc layer was washed with brine, dried (Na_2SO_4) and evaporated. The residue was purified by column chromatography (petroleum ether then petroleum ether/EtOAc, 99:1–93:7) gave 5-[allyl(*tert*-butoxycarbonyl)amino]-3-(*tert*-butoxycarbonyl)-1-methyl-1,2-dihydro-3*H*-benzo[*e*]indole (**40**) (1.44 g, 77%) as a brown oil: ^{1}H NMR ($CDCl_3$) δ 8.10 (br s, 1H), 7.80–7.70 (m, 2H), 7.45 (t, J = 8.3 Hz, 1H), 7.35 (t, J = 8.1 Hz, 1H), 6.08–5.88 (m, 1H), 5.21–4.98 (m, 2H), 4.65–4.30 (m, 1H), 4.25–4.09 (m, 2H), 3.88–3.70 (m, 2H), 1.58 (s, 9H), 1.41 (d, J = 6.9 Hz, 3H), 1.23 (s, 9H); HRMS m/z 438.2516 (M^+), $C_{26}H_{34}N_2O_4$ requires 438.2519.

TFA (15 mL) was added to **40** (1.40 g, 3.20 mmol) at 0 °C and the solution was stirred at 20 °C for 2 h. The TFA was removed under reduced pressure and the residue was partitioned between DCM and aqueous $NaHCO_3$. The DCM layer was washed with brine, dried (Na_2SO_4), and evaporated to give the crude amine (760 mg, 100%). This was dissolved in THF (10 mL) and treated with a solution of (*E*)-3-(4-((dimethylamino)methyl)cinnamoyl chloride [4.79 mmol, prepared from (*E*)-3-(4-((dimethylamino)methyl)cinnamic acid (**20**) with oxalyl chloride/catalytic DMF] in THF (10 mL). The mixture was stirred for 12 h, then diluted with H_2O and extracted with EtOAc. The EtOAc extract was washed with brine, dried (Na_2SO_4), and evaporated. The residue was purified by column chromatography (DCM then DCM/MeOH, 99:1–9:1) to give 5-allylamino-3-[(*E*)-4-(dimethylaminomethyl)cinnamoyl]-1-methyl-1,2-dihydro-3*H*-benz[*e*]indole (**41**) (836 mg, 62%) as a brown oil: ^{1}H NMR ($CDCl_3$) δ 7.98 (s, 1H), 7.86 (d, J = 15.7 Hz, 1H), 7.82 (d, J = 8.5 Hz, 1H), 7.72 (d, J = 8.3 Hz, 1H), 7.56 (d, J = 8.1 Hz, 2H), 7.47 (t, J = 7.2 Hz, 1H), 7.37–7.31 (m, 1H), 7.36 (d, J = 8.1 Hz, 2H), 6.89 (d, J = 15.3 Hz, 1H), 6.17–6.05 (m, 1H), 5.41 (d, J = 17.1 Hz, 1H), 5.25 (d, J = 10.0 Hz, 1H), 4.55–4.40 (m, 1H), 4.46 (br s, 1H), 4.06–3.97 (m, 2H), 3.87–3.79 (m , 1H), 3.51–3.43 (m, 1H), 3.47 (s, 2H), 2.28 (s, 6H), 1.42 (d, J = 6.8 Hz, 3H); HRMS m/z 425.2466 (M^+), $C_{28}H_{31}N_3O$ requires 425.2467.

Allyl amine **41** (70 mg, 0.165 mmol) was dissolved in dry DCM (5 mL) and the solution was purged with N_2. $Pd(Ph_3P)_4$ (13 mg, 0.012 mmol), $PhSO_2Na$ (68 mg, 0.412 mmol) and CSA (115 mg, 0.494 mmol) were added[20] and the solution was stirred under N_2 for 3 h, then diluted with aqueous $NaHCO_3$ and extracted with DCM. The DCM extract was washed with brine, dried (Na_2SO_4), and evaporated. The residue was purified by column chromatography (DCM then DCM/MeOH, 99:1–23:2) to give 5-amino-3-[(*E*)-4-(dimethylaminomethyl)cinnamoyl]-1-methyl-1,2-dihydro-3*H*-benz[*e*]indole (**42**) (80 mg, 91%) as a yellow foam: ^{1}H NMR ($CDCl_3$) δ 8.06 (s, 1H), 7.87–7.82 (m, 1H), 8.81 (d, J = 8.2 Hz, 1H), 7.74 (d, J = 8.6 Hz, 1H), 7.56 (d, J = 8.1 Hz, 2H), 7.46 (t, J = 7.4 Hz, 1H), 7.38–7.31 (m, 1H), 7.37 (d, J = 8.1 Hz, 2H), 6.87 (d, J = 15.1 Hz, 1H), 4.45 (t, J = 8.8 Hz, 1H), 4.22 (s, 2H), 4.02 (d, J = 9.5 Hz, 1H), 3.91–3.78 (m, 1H), 3.47 (s, 2H), 2.27 (s, 6H), 1.42 (d, J = 6.8 Hz, 3H); HRMS m/z 386.2235 (M^+), $C_{25}H_{27}N_3O$ requires 386.2232.

A solution of **42** (80 mg, 0.208 mmol), $(Boc)_2O$ (905 mg, 4.15 mmol), and DMAP (8 mg) in dioxane (2 mL) was heated at reflux for 48 h. The mixture was diluted with H_2O and extracted with EtOAc. The EtOAc layer was washed with H_2O, brine, and dried (Na_2SO_4) and evaporated. Column chromatography (DCM then DCM/MeOH, 99:1–97:3) gave 5-[bis(*tert*-butoxycarbonyl)]amino-3-[(*E*)-4-(dimethylaminomethyl)cinnamoyl]-1-methyl-1,2-dihydro-3*H*-benz[*e*]indole (**43**) (45 mg, 37%) as a yellow gum: ^{1}H NMR ($CDCl_3$) δ 8.57 (s, 1H), 7.87 (d, J = 15.4 Hz, 1H), 7.80 (d, J = 8.2 Hz, 1H), 7.79 (d, J = 8.2 Hz, 1H), 7.55 (d, J = 8.1 Hz, 2H), 7.48 (t, J = 6.9 Hz, 1H), 7.41 (t, J = 7.7 Hz, 1H), 7.34 (d, J = 8.0 Hz, 2H), 6.86 (d, J = 14.9 Hz, 1H), 4.50 (t, J = 9.4 Hz, 1H), 4.09 (d, J = 9.1 Hz, 1H), 3.99–3.89 (m, 1H), 3.46 (s, 2H), 2.26 (s, 6H), 1.47 (d, J = 6.9 Hz,

3H), 1.37 (s, 9H), 1.35 (s, 9H); HRMS *m/z* 586.3286 (M^+), $C_{35}H_{43}N_3O_5$ requires 586.3281.

A solution of **43** (45 mg, 0.077 mmol) and bromide **16** (20 mg, 0.092 mmol) in THF (2 mL) was stirred under N_2 in the dark for 48 h. The resulting precipitate was filtered off and washed with THF to give *N*-4-((*E*)-3-{5-[bis(*tert*-butoxycarbonyl)]-amino-1-methyl-1,2-dihydro-3*H*-benz[*e*]indol-3-yl}-3-oxo-1-propenyl) benzyl-*N*,*N*-dimethyl-*N*-[(1-methyl-5-nitro-2-pyrrolyl)methyl] ammonium bromide (**44a**) (51 mg, 82%) as a yellow powder: mp 168–172 °C (dec.); ^{1}H NMR (DMSO-d_6) δ 8.38 (s, 1H), 7.99–7.92 (m, 3H), 7.74 (d, *J* = 15.4 Hz, 1H), 7.69–7.62 (m, 3H), 7.58 (t, *J* = 7.5 Hz, 1H), 7.51 (t, *J* = 7.6 Hz, 1H), 7.37 (d, *J* = 4.5 Hz, 1H), 7.32 (d, *J* = 15.4 Hz, 1H), 6.70 (d, *J* = 4.5 Hz, 1H), 4.84 (s, 2H), 4.67 (s, 2H), 4.57 (t, *J* = 9.9 Hz, 1H), 4.31 (d, *J* = 9.4 Hz, 1H), 4.09–4.01 (m, 1H), 4.02 (s, 3H), 2.94 (s, 6H), 1.42–1.37 (m, 3H), 1.39 (s, 9H), 1.36 (s, 9H). Anal. Calcd for $C_{41}H_{50}BrN_5O_7 \cdot 11/2H_2O$: C, 59.20; H, 6.42; N, 8.42. Found: C, 59.13; H, 6.03; N, 8.33.

Dioxane (10 mL) saturated with HCl gas was added to a solution of **44a** (80 mg, 0.100 mmol) in dioxane (10 mL). The mixture was stirred in the dark at 20 °C for 15 h and the solvent was removed under reduced pressure. The residue was dissolved in MeOH (1 mL) and purified by ion exchange chromatography (Biorad AG-1X4 resin in Cl^- form), eluting with MeOH to give **10** as a yellow solid (38 mg, 64%): mp >300 °C (dec.); ^{1}H NMR (DMSO-d_6) δ 8.15 (s, 1H), 8.05 (d, *J* = 8.5 Hz, 1H), 7.95 (d, *J* = 8.2 Hz, 2H), 7.80 (d, *J* = 8.0 Hz, 1H), 7.72 (d, *J* = 15.4 Hz, 1H), 7.64 (d, *J* = 8.2 Hz, 2H), 7.51 (t, *J* = 7.9 Hz, 1H), 7.41–7.32 (m, 1H), 7.38 (d, *J* = 4.5 Hz, 1H), 7.32 (d, *J* = 15.4 Hz, 1H), 6.69 (d, *J* = 4.5 Hz, 1H), 4.85 (s, 2H), 4.67 (s, 2H), 4.54–4.43 (m, 1H), 4.18 (d, *J* = 9.8 Hz, 1H), 4.03 (s, 3H), 2.94 (s, 6H), 1.34 (d, *J* = 6.8 Hz, 3H); HRMS *m/z* 524.2670 (M^+), $C_{31}H_{34}N_5O_3$ requires 524.2662. HPLC purity 95.4%.

4.1.14. (*E*)-*N*-(4-(3-(5-Amino-1-methyl-1*H*-benzo[*e*]indol-3(2*H*)-yl)-3-oxoprop-1-enyl)benzyl)-*N*,*N*-dimethyl-1-(1-methyl-5-(methylsulfonyl)-1*H*-pyrrol-2-yl)methanaminium bromide hydrobromide (11)

A solution of **43** (100 mg, 0.171 mmol) and bromide **18** (52 mg, 0.205 mmol) in THF (10 mL) was stirred under N_2 in the dark for 85 h, then concentrated under reduced pressure to a small volume. EtOAc was added and the resulting precipitate was filtered off and washed with EtOAc to give *N*-4-((*E*)-3-{5-[bis(*tert*-butoxy carbonyl)]-amino-1-methyl-1,2-dihydro-3*H*-benz[*e*]indol-3-yl}-3-oxo-1-propenyl)benzyl]-*N*,*N*-dimethyl-*N*-[(1-methyl-5-methylsulfonyl-2-pyrrolyl)methyl]ammonium bromide (**44b**) (132 mg, 92%) as a yellow powder: mp 171–174 °C (dec.); ^{1}H NMR (DMSO-d_6) δ 8.38 (s, 1H), 8.01–7.92 (m, 3H), 7.74 (d, *J* = 15.4 Hz, 1H), 7.68–7.62 (m, 3H), 7.59 (t, *J* = 7.5 Hz, 1H), 7.52 (t, *J* = 7.6 Hz, 1H), 7.32 (d, *J* = 15.5 Hz, 1H), 6.93 (d, *J* = 4.1 Hz, 1H), 6.64 (d, *J* = 4.1 Hz, 1H), 4.77 (s, 2H), 4.63 (s, 2H), 4.57 (t, *J* = 9.9 Hz, 1H), 4.31 (d, *J* = 9.4 Hz, 1H), 4.10–4.02 (m, 1H), 3.95 (s, 3H), 3.31 (s, 3H), 2.92 (s, 6H), 1.43–1.38 (m, 3H), 1.39 (s, 9H), 1.36 (s, 9H). Anal. Calcd for $C_{42}H_{53}BrN_4O_7S \cdot H_2O$: C, 58.94; H, 6.48; N, 6.55. Found: C, 59.04; H, 6.20; N, 6.43.

Dioxane (5 mL) saturated with HCl gas was added to a solution of **44b** (50 mg, 0.060 mmol) in dioxane (5 mL). The mixture was stirred in the dark at 20 °C for 15 h and the solvent was removed under reduced pressure. The residue was dissolved in MeOH (1 mL) and purified by ion exchange chromatography (Biorad AG-1X4 resin in Cl^- form), eluting with MeOH to give **11** as a yellow solid (33 mg, 89%): mp >300 °C (dec.); ^{1}H NMR (DMSO-d_6) δ 8.21 (s, 1H), 8.04 (d, *J* = 8.5 Hz, 1H), 7.94 (d, *J* = 8.0 Hz, 2H), 7.86–7.78 (m, 1H), 7.72 (d, *J* = 15.3 Hz, 1H), 7.66 (d, *J* = 7.9 Hz, 2H), 7.57–7.48 (m, 1H), 7.44–7.35 (m, 1H), 7.31 (d, *J* = 15.4 Hz, 1H), 6.92 (d, *J* = 4.1 Hz, 1H), 6.65 (d, *J* = 4.1 Hz, 1H), 4.81 (s, 2H), 4.67 (s, 2H), 4.54–4.43 (m, 1H), 4.25–4.17 (m, 1H), 3.97 (s, 3H), 3.94–3.84 (m, 1H), 3.40 (s, 3H), 2.93 (s, 6H), 1.34 (d, *J* = 6.6 Hz, 3H); HRMS *m/z* 557.2576 (M^+), $C_{32}H_{37}N_4O_3S$ requires 557.2586. HPLC purity 95.8%.

4.1.15. (*E*)-5-(4-(3-(5-Amino-1-(chloromethyl)-1*H*-benzo[*e*]indol-3(2*H*)-yl)-3-oxoprop-1-enyl)phenyl)-*N*,*N*-dimethyl-*N*-((1-methyl-5-(methylsulfonyl)-1*H*-pyrrol-2-yl)methyl)pentan-1-aminium trifluoroacetate hydrotrifluoroacetate (12) (Scheme 6)

TFA (5 mL) was added to 5-[allyl(*tert*-butoxycarbonyl)amino]-3-(*tert*-butoxycarbonyl)-1-(chloromethyl)-1,2-dihydro-3*H*-benz[*e*]indole[19] (**45**) (300 mg, 0.636 mmol) at 0 °C and the solution was stirred at 20 °C for 3 h. The TFA was removed under reduced pressure and the residue was partitioned between DCM and aqueous $NaHCO_3$. The DCM layer was washed with brine, dried (Na_2SO_4), and evaporated to give the crude amine (173 mg, 100%) which was used directly. The entire sample was dissolved in DCM (5 mL) and treated slowly with a solution of 6-(dimethylamino)hexanoyl chloride hydrochloride[21] (203 mg, 0.953 mmol) in THF (5 mL). The mixture was stirred for 30 min, then diluted with H_2O and extracted with DCM. The DCM extract was washed with brine, dried (Na_2SO_4), and evaporated. The residue was purified by column chromatography (DCM/MeOH/NH_3, 9:1:12 drops/100 mL of solution) to give 5-allylamino-1-(chloromethyl)-3-[6-(dimethylamino)hexanoyl]-1,2-dihydro-3*H*-benz[*e*]indole (**46a**) (130 mg, 50%) as a brown powder: mp 185–190 °C (dec.); ^{1}H NMR (DMSO-d_6) δ 8.13 (d, *J* = 8.5 Hz, 1H), 7.71 (d, *J* = 8.2 Hz, 1H), 7.62 (s, 1H), 7.45 (t, *J* = 7.3 Hz, 1H), 7.28 (t, *J* = 7.4 Hz, 1H), 6.54 (s, 1H), 6.20–5.89 (m, 1H), 5.25 (dd, *J* = 17.2, 1.3 Hz, 1H), 5.13 (d, *J* = 9.4 Hz, 1H), 4.27 (t, *J* = 9.6 Hz, 1H), 4.14–4.02 (m, 2H), 3.94 (dd, *J* = 10.9, 2.9 Hz, 1H), 3.90–3.82 (m, 2H), 3.71 (dd, *J* = 10.5, 8.7 Hz, 1H), 2.93–2.83 (m, 2H), 2.63 (s, 6H), 2.57–2.48 (m, 2H), 1.70–1.57 (m, 4H), 1.43–1.34 (m, 2H). This was unstable, and was used directly in the next step.

Compound **46a** (200 mg, 0.484 mmol) was dissolved in dry DCM (10 mL) and the solution was purged with N_2. $Pd(Ph_3P)_4$ (39 mg, 0.034 mmol), $PhSO_2Na$ (200 mg, 1.21 mmol), and CSA (337 mg, 1.45 mmol) were added and the solution was stirred under N_2 for 30 min. The mixture was diluted with aqueous $NaHCO_3$ and extracted with DCM. The DCM extract was washed with brine, dried (Na_2SO_4), and evaporated to give crude 5-amino-1-(chloromethyl)-3-[6-(dimethylamino)hexanoyl]-1,2-dihydro-3*H*-benz[*e*]indole (**47a**) (87 mg, 48%), which was unstable and was used directly. A solution of **47a** (45 mg, 0.121 mmol), $(Boc)_2O$ (237 mg, 1.09 mmol), and DMAP (1 mg) in THF (6 mL) and dioxane (2 mL) was heated at reflux for 12 h. The mixture was diluted with H_2O and extracted with EtOAc. The EtOAc extract was washed with H_2O, aqueous Na_2CO_3, and brine, and then dried (Na_2SO_4) and evaporated. The residue was purified by column chromatography [DCM/MeOH, 9:1 containing c.NH_3 (12 drops per 100 mL of eluant)] to give 5-[bis(*tert*-butoxy-carbonyl)] amino-1-(chloromethyl)-3-[6-(dimethylamino)hexanoyl]-1,2-dihydro-3*H*-benz[*e*]indole (**48a**) (47 mg, 68%) as a yellow foam. ^{1}H NMR (DMSO-d_6) δ 8.28 (s, 1H), 7.98 (d, *J* = 8.3 Hz, 1H), 7.65 (d, *J* = 8.1 Hz, 1H), 7.56 (td, *J* = 7.5, 1.2 Hz, 1H), 7.48 (td, *J* = 7.6, 1.0 Hz, 1H), 4.49–4.34 (m, 2H), 4.23 (d, *J* = 8.7 Hz, 1H), 4.05 (dd, *J* = 11.3, 2.8 Hz, 1H), 3.50 (dd, *J* = 11.1, 6.2 Hz, 1H), 2.56–2.25 (m, 2H), 2.28–2.22 (m, 2H), 2.15 (s, 6H), 1.67–1.59 (m, 2H), 1.51–1.42 (m, 2H), 1.40–1.30 (m, 2H), 1.35 (s, 18H); HRMS (FAB, ^{35}Cl) *m/z* 574.3048 (M+1), $C_{32}H_{45}ClN_3O_5$ requires 574.3048.

A solution of **48a** (43 mg, 0.075 mmol), bromide **18** (21 mg, 0.083 mmol) and dry K_2CO_3 (25 mg) in THF (2 mL) was stirred under N_2 in the dark at room temperature for 2 h, and the inorganic solids were then filtered off. The filtrate was evaporated and the resulting residue was triturated with EtOAc/Et_2O and then with Et_2O to give *N*-6-{5-[bis(*tert*-butoxycarbonyl)amino]-1-(chloromethyl)-1,2-dihydro-3*H*-benz[*e*]indol-3-yl}-6-oxo-1-hexyl-*N*,*N*-dimethyl-*N* -{[1-methyl-5-(methylsulfonyl)-pyrrol-2-yl]methyl}ammonium

bromide (**49a**) (57 mg, 92%) as a cream powder: mp 150–153 °C (dec.).^{1}H NMR (DMSO-d_6) δ 8.29 (s, 1H), 7.99 (d, J = 8.3 Hz, 1H), 7.65 (d, J = 8.4 Hz, 1H), 7.57 (td, J = 7.5, 1.0 Hz, 1H), 7.49 (td, J = 7.6, 1.0 Hz, 1H), 6.90 (d, J = 4.1 Hz, 1H), 6.58 (d, J = 4.1 Hz, 1H), 4.66 (s, 2H), 4.48–4.40 (m, 2H), 4.23 (d, J = 8.1 Hz, 1H), 4.09–4.04 (m, 1H), 3.97 (dd, J = 11.1, 6.2 Hz, 1H), 3.91 (s, 3H), 3.41–3,28 (m, 2H), 3.28 (s, 3H), 2.98 (s, 6H), 2.71–2.54 (m, 2H), 1.87–1.78 (m, 2H), 1.74–1.67 (m, 2H), 1.46–1.38 (m, 2H), 1.35 (s, 9H), 1.34 (s, 9H). Anal. Calcd for $C_{38}H_{54}BrClN_4O_7S{\cdot}11/2H_2O$: C, 53.49; H, 6.73; N, 6.57. Found: C, 53.49; H, 6.37; N, 6.43.

TFA (1 mL) was added to a cooled (0 °C) solution of **49a** (54 mg, 0.065 mmol) in DCM (2 mL) and stirred for 6 h. Solvents were removed and the residue was triturated with EtOAc/Et_2O and then with Et_2O to give **12** (39 mg) as a pale yellow powder: mp 158–162 °C (dec.). ^{1}H NMR (DMSO-d_6) δ 8.02 (d, J = 8.4 Hz, 1H), 7.76 (s, 1H), 7.70 (d, J = 8.3 Hz, 1H), 7.43 (t, J = 7.3 Hz, 1H), 7.23 (t, J = 7.4 Hz, 1H), 6.90 (d, J = 4.1 Hz, 1H), 6.57 (d, J = 4.1 Hz, 1H), 5.80 (br s, 1H), 4.66 (s, 2H), 4.28 (t, J = 9.8 Hz, 1H), 4.14–4.02 (m, 2H), 3.94 (dd, J = 11.0, 2.8 Hz, 1H), 3.91 (s, 3H), 3.69 (dd, J = 10.3, 8.8 Hz, 1H), 3.48–3.34 (m, 2H), 3.28 (s, 3H), 2.97 (s, 6H), 2.60–2.40 (m, 2H), 1.88–1.76 (m, 2H), 1.75–1.63 (m, 2H), 1.48–1.34 (m, 2H); HRMS (FAB, ^{35}Cl) m/z 545.22353 (M^+), $C_{28}H_{38}ClN_4O_3S$ requires 545.2353. HPLC purity 92.3%.

4.1.16. (*E*)-6-(4-(3-(5-Amino-1-(chloromethyl)-1*H*-benzo[*e*]indol-3(2*H*)-yl)-3-oxoprop-1-enyl)phenyl)-*N*,*N*-dimethyl-N-((1-methyl-5-(methylsulfonyl)-1*H*-pyrrol-2-yl)methyl)hexan-1-aminium trifluoroacetate hydrotrifluoroacetate (13)

A solution of **45** (1.25 g , 2.64 mmol) in DCM (5 mL) was treated with HCl in dioxane (4 M, 30 mL) and the mixture was stirred at room temperature for 3 h, during which time a white precipitate formed. The solvent was evaporated and the off-white solid obtained (the amine hydrochloride) was dissolved in dry DMF (30 mL). To this solution was added 7-(dimethylamino)heptanoyl chloride hydrochloride[21] (611 mg, 2.92 mmol), DIPEA (2.13 mL, 13.25 mmol), and (benzotriazol-1-yloxy)tripyrrolidinophosphonium hexafluorophosphate, (1.793 g, 3.44 mmol), and the mixture was stirred at room temperature for 16 h. The solvent was evaporated under reduced pressure and the residue was dissolved in DCM. The solution was washed with dilute aqueous $NaHCO_3$, dried (Na_2SO_4), and evaporated. The crude product was purified by column chromatography [Et_3N/MeOH/DCM, 1:10:400 then 1:10:200 then 1:10:100 followed by a second column EtOAc then Et_3N/EtOAc, 1:40 then 1:20 then 1:10] to give *N*-allyl-1-(chloromethyl)-3-[7-(dimethylamino)heptanoyl]-2,3-dihydro-1*H*-benzo[*e*]indol-5-amine (**46b**) as white solid (867 mg, 77%): mp 168–170 °C (dec.); ^{1}H NMR (DMSO-d_6) δ 8.13 (d, J = 8.5 Hz, 1H), 7.71 (d, J = 8.1 Hz, 1H), 7.61 (s, 1H), 7.44 (t, J = 7.3 Hz, 1H), 7.27 (t, J = 7.7 Hz, 1H), 6.59 (s, 1H), 6.02–5.90 (m, 1H), 5.26 (dd, J = 17.3, 1.4 Hz, 1H), 5.12 (d, J = 10.3 Hz, 1H), 4.28 (t, J = 9.6 Hz, 1H), 4.15–4.00 (m, 2H), 3.93 (dd, J =10.9, 2.8 Hz, 1H), 3.86 (s, 2H), 3.70 (dd, J = 10.4, 8.6 Hz, 1H), 2.90–2.83 (m, 2H), 2.62 (s, 6H), 2.56–2.45 (m, 2H), 1.68–1.56 (m, 4H), 1.43–1.30 (m, 4H). This was unstable, and was used directly for the next step.

Compound **46b** (867 mg, 2.03 mmol), $PhSO_2Na$ (836 mg, 5.08 mmol), and CSA (1.415 g, 6.09 mmol) were dissolved in dry DCM (100 mL) and the solution was purged with N_2. $Pd(Ph_3P)_4$ (164 mg, 0.14 mmol) was added and the solution was stirred under N_2 for 30 min. The mixture was diluted with aqueous $NaHCO_3$ and extracted with DCM. The DCM extract was washed with brine, dried (Na_2SO_4), and evaporated. The crude product was purified by column chromatography [EtOAc then Et_3N/EtOAc, 1:40 then 1:20 then 1:10] to give to give 5-amino-1-(chloromethyl)-3-[7-(dimethylamino)heptanoyl]-1,2-dihydro-3*H*-benz[*e*]indole (**47b**) (650 mg, 83%) as a white solid: mp 195 °C (dec.); ^{1}H NMR ($CDCl_3$) δ 7.95 (s, 1H), 7.79 (d, J = 8.4 Hz, 1H), 7.66 (d, J = 8.4 Hz, 1H), 7.48 (td, J = 8.4, 1.0 Hz, 1H), 7.34 (td, J = 8.4, 1.0 Hz, 1H), 4.26 (br, 3H including NH_2), 4.19 (t, J = 9.5 Hz, 1H), 4.00 (br t, J = 9.2 Hz, 1H), 3.92 (dd, J = 11.3, 3.1 Hz, 1H), 3.39 (t, J = 10.8 Hz, 1H), 2.40–2.60 (m, 2H), 2.26 (t, J = 7.4 Hz, 2H), 2.22 (s, 6H), 1.74–1.82 (m, 2H), 1.35–1.54 (m, 6H); HRMS (FAB, ^{35}Cl) m/z 388.21532 (M+H), $C_{22}H_{31}{}^{35}ClN_3O$ requires 388.21557.

A solution of **47b** (644 mg, 1.71 mmol), $(Boc)_2O$ (3.73 g, 17.1 mmol), and DMAP (15 mg) in THF (60 mL) and dioxane (20 mL) was heated at reflux for 15 h. The solvents were evaporated under reduced pressure and the residue was dissolved in DCM. The DCM solution was washed with H_2O, aqueous $NaHCO_3$, and brine, and then dried (Na_2SO_4) and evaporated. The residue was purified by column chromatography [EtOAc then Et_3N/EtOAc, 1:40 then 1:20] to give 5-[bis(*tert*-butoxycarbonyl)]amino-1-(chloromethyl)-3-[7-(dimethylamino)heptanoyl-1,2-dihydro-3*H*-benz[*e*]indole (**48b**) (742 mg, 74%) as a white gum: ^{1}H NMR ($CDCl_3$): δ 8.48 (s, 1H), 7.80 (d, J = 8.4 Hz, 1H), 7.73 (d, J = 8.4 Hz, 1H), 7.51 (td, J = 7.6, 1.0 Hz, 1H), 7.42 (td, J = 7.6, 1.0 Hz, 1H), 4.33 (d, J = 10.1, 1H), 4.27 (t, J = 9.6 Hz, 1H), 4.13 (br t, J = 9.0 Hz, 1H), 3.97 (dd, J = 11.3, 3.1 Hz, 1H), 3.48 (t, J = 10.8 Hz, 1H), 2.42–2.61 (m, 2H), 2.26 (t, J = 7.5 Hz, 2H), 2.22 (s, 6H), 1.75–1.82 (m, 2H), 1.39–1.54 (m, 6H), 1.37 (s, 9H), 1.35 (s, 9H); HRMS (FAB, ^{35}Cl) m/z 588.3192 (M+1), $C_{32}H_{47}ClN_3O_5$ requires 588.3204.

A solution of **48b** (31 mg, 0.053 mmol), bromide **18** (15 mg, 0.058 mmol) and dry K_2CO_3 (25 mg) in THF (2 mL) was stirred under N_2 in the dark for 2 h. Inorganic solids were then filtered off and the filtrate was evaporated and the resulting residue was triturated with EtOAc/Et_2O and then with Et_2O gave 6-[5-[bis(*tert*-butoxycarbonyl)amino]-1-(chloromethyl)-1,2-dihydro-3*H*-benzo[*e*]indol-3-yl]-*N*,*N*-dimethyl-*N*-{[1-methyl-5-(methylsulfonyl)-1*H*-pyrrol-2-yl]methyl}-6-oxo-1-heptane ammonium bromide (**49b**) (40 mg, 91%) as a cream powder: mp 148–151 °C (dec.); ^{1}H NMR (DMSO-d_6) δ 8.29 (s, 1H), 7.99 (d, J = 8.3 Hz, 1H), 7.65 (d, J = 8.2 Hz, 1H), 7.57 (t, J = 7.9 Hz, 1H), 7.49 (t, J = 8.1 Hz, 1H), 6.90 (d, J = 4.1 Hz, 1H), 6.58 (d, J = 4.1 Hz, 1H), 4.66 (s, 2H), 4.47–4.37 (m, 2H), 4.23 (d, J = 8.3 Hz, 1H), 4.07 (dd, J = 9.9, 2.7 Hz, 1H), 3.97 (dd, J = 11.3, 6.2 Hz, 1H), 3.91 (s, 3H), 3.33–3,28 (m, 2H), 3.27 (s, 3H), 2.97 (s, 6H), 2.61–2.51 (m, 2H), 1.83–1.74 (m, 2H), 1.71–1.62 (m, 2H), 1.50–1.40 (m, 2H), 1.40–1.32 (m, 2H), 1.35 (s, 9H), 1.34 (s, 9H). Anal. Calcd for $C_{39}H_{56}BrClN_4O_7S{\cdot}H_2O$: C, 54.58; H, 6.81; N, 6.53. Found: C, 54.39; H, 6.72; N, 6.32.

TFA (1 mL) was added to a cooled (0 °C) solution of **49b** (37 mg, 0.044 mmol) in DCM (2 mL) and stirred for 6 h. Solvents were removed and the residue was triturated with EtOAc/Et_2O and then with Et_2O to give **13** (21 mg, 100%) as a pale yellow powder: mp 160–163 °C (dec.). ^{1}H NMR (DMSO-d_6) δ 8.02 (d, J = 8.4 Hz, 1H), 7.76 (s, 1H), 7.70 (d, J = 8.2 Hz, 1H), 7.43 (t, J = 7.7 Hz, 1H), 7.23 (t, J = 7.7 Hz, 1H), 6.90 (d, J = 4.1 Hz, 1H), 6.57 (d, J = 4.1 Hz, 1H), 5.80 (br s, 1H), 4.65 (s, 2H), 4.28 (t, J = 9.1 Hz, 1H), 4.15–4.02 (m, 2H), 3.95 (dd, J = 10.9, 2.8 Hz, 1H), 3.91 (s, 3H), 3.69 (dd, J = 10.3, 8.9 Hz, 1H), 3.46–3.31 (m, 2H), 3.28 (s, 3H), 2.95 (s, 6H), 2.60–2.40 (m, 2H), 1.85–1.72 (m, 2H), 1.71–1.61 (m, 2H), 1.50–1.31 (m, 4H); HRMS (FAB, ^{35}Cl) m/z 559.2506 (M^+), $C_{29}H_{40}ClN_4O_5S$ requires 559.2510. HPLC purity 92.4%.

4.1.17. Tritium-labeled (*E*)-*N*-(4-(3-(5-amino-1-(chloromethyl)-1*H*-benzo[*e*]indol-3(2*H*)-yl)-3-oxoprop-1-enyl)benzyl)-*N*,*N*-dimethyl-1-(1-methyl-5-(methylsulfonyl)-1*H*-pyrrol-2-yl) methanaminium bromide hydrobromide (^{3}H-4) (Scheme 7)

A solution of **18** (960 mg, 3.81 mmol) and **23** (1.035 g, 4.19 mmol) and DIPEA (1.062 mL, 6.10 mmol) in THF (50 mL) was stirred under N_2 for 15 h. The solvent was removed under reduced pressure and the residue was purified by column chromatography (petroleum ether/EtOAc, 1:1) to give (*E*)-*tert*-butyl 3-(4-((methyl((1-methyl-5-(methylsulfonyl)-1*H*-pyrrol-2-yl)methyl) amino)methyl)phenyl)acrylate (**50**) (1.53 g, 96%) as a white solid:

^{1}H NMR ($CDCl_3$) δ 7.56 (d, J = 16.0 Hz, 1H), 7.45 (d, J = 8.1 Hz, 2H), 7.29 (d, J = 8.1 Hz, 2H), 6.82 (d, J = 4.0 Hz, 1H), 6.35 (d, J = 16.0 Hz, 1H), 6.10 (d, J = 4.0 Hz, 1H), 3.85 (s, 3H), 3.50 (s, 2H), 3.47 (s, 2H), 3.06 (s, 3H), 2.16 (s, 3H), 1.53 (s, 9H); LRMS (ACPI+): *m/z* 419.5 (M+H), $C_{22}H_{31}N_2O_4S$ requires 419.2005.

TFA (1 mL) was added to cooled (0 °C) **50** (1.23 g, 2.95 mmol) and the resulting solution was stirred at 20 °C for 1 h. The TFA was removed under reduced pressure and the residue was triturated with DCM and Et_2O to give (*E*)-3-(4-((methyl((1-methyl-5-(methylsulfonyl)-1*H*-pyrrol-2-yl)methyl)amino)methyl)phenyl) acrylic acid (**51**) as a hygroscopic colorless solid (trifluorosulfonate salt) that turned to a gum in air: ^{1}H NMR (DMSO-d_6) δ 12.49 (br, 1H), 9.93 (br, 1H), 7.79 (d, J = 7.5 Hz, 2H), 7.62 (d, J = 16.0 Hz, 1H), 7.54 (d, J = 7.0 Hz, 2H), 6.82 (d, J = 3.6 Hz, 1H), 6.60 (d, J = 16.0 Hz, 1H), 6.51(br, 1H), 4.51 (br, 2H), 4.37 (br, 2H), 3.80 (s, 3H), 3.25 (s, 3H), 2.62 (br, 3H); HRMS (ESI): *m/z* 363.1381 (M−OTFA), $C_{18}H_{23}N_2O_4S$ requires 363.1373.

Oxalyl chloride (620 μL, 7.10 mmol) and then DMF (two drops) were added to a suspension of **51** (TFA salt) (678 mg, 1.42 mmol) in DCM (10 mL). The mixture was stirred for 3 h and then the solvents were removed under high vacuum to give the crude HCl salt of the acyl chloride **52**.

A solution of **45** (645 mg, 1.37 mmol) in TFA was held at 0 °C for 3 h, then solvent was pumped off and the dark brown gum obtained (amine HCl salt) was suspended in DCM (10 mL) and the solution was cooled in an ice bath. DIPEA (2.50 mL, 14.00 mmol) was added before a solution of **52** (0.54 g, 1.432 mmol) in DCM (10 mL) was added slowly to give a yellow solution. This solution was stirred in an ice bath for 1 h and at room temperature for 3 h, and then washed with aqueous $NaHCO_3$. The crude product purified by silica gel column chromatography twice both using EtOAc/petroleum ether (gradient: v/v = 1;2, 1:1, 2;1) as eluents to give (*E*)-1-(1-(chloromethyl)-5-(vinylamino)-1*H*-benzo[*e*]indol-3(2H)-yl)-3-(4-((methyl((1-methyl-5-(methylsulfonyl)-1*H*-pyrrol-2-yl)methyl)amino)methyl)phenyl)prop-2-en-1-one (**53**) as a yellow solid (475 mg, 56%). ^{1}H NMR ($CDCl_3$): 7.93 (br, 1H), 7.87 (d, J = 15.1 Hz, 1H), 7.81 (d, J = 8.5 Hz, 1H), 7.69 (d, J = 8.3 Hz, 1H), 7.57 (d, J = 8.1 Hz, 2H), 7.50 (d, J = 7.1 Hz, 1H), 7.38–7.33 (m, 3H), 6.93 (br d, J = 15.2 Hz, 1H), 6.84 (d, J = 4.0 Hz, 1H), 6.11 (d, J = 4.0 Hz, 1H), 6.11–6.05 (br, 1H), 5.40 (d, J = 17.1 Hz, 1H), 5.25 (d, J = 10.4 Hz, 1H), 4.62 (br, 1H), 4.52 (d, J = 10.7 Hz, 1H), 4.40 (t, J = 9.1 Hz, 1H), 4.06–4.02 (br s, 2H), 3.98–3.94 (dd, J = 11.3, 2.9 Hz, 1H), 3.88 (s, 3H), 3.54 (s, 2H), 3.50 (s, 2H), 3.43 (t, J = 11.0 Hz, 1H), 3.08 (s, 3H), 2.19 (s, 3H). LRMS (ACPI+): *m/z* 617.2 (M+H), $C_{34}H_{38}{}^{35}ClN_4O_3S$ requires 617.2353.

A solution of **53** (866 mg, 1.40 mmol), $PhSO_2Na$ (577 mg, 3.51 mmol) and CSA (978 mg, 4.21 mmol) in dry DCM (60 mL) was purged with N_2. $Pd(PPh_3)_4$ (114 mg, 0.10 mmol) was then added and the mixture was stirred for 3 h. After $NaHCO_3$ workup, the residue obtained was subjected to silica gel column chromatography twice using MeOH/DCM (v/v 5%) as eluent firstly and EtOAc/petroleum ether/DCM/TEA (v/v 3:1:1:0.02) secondly to give 688 mg (85%) of (*E*)-1-(5-amino-1-(chloromethyl)-1*H*-benzo[*e*]indol-3(2*H*)-yl)-3-(4-((methyl((1-methyl-5-(methylsulfonyl)-1*H*-pyrrol-2-yl)methyl)amino)methyl)phenyl)prop-2-en-1-one (**54**) as a bright yellow solid. ^{1}H NMR ($CDCl_3$): δ 8.03 (br s, 1H), 7.87–7.80 (m, 2H), 7.69 (d, J = 8.2 Hz, 1H), 7.57 (d, J = 8.1 Hz, 2H), 7.52 (t, J = 7.6 Hz, 1H), 7.39–7.33 (m, 3H), 6.92 (br d, J = 15.6 Hz, 1H), 6.83 (d, J = 4.0 Hz, 1H), 6.11 (d, J = 4.0 Hz, 1H), 4.51 (d, J = 9.6 Hz, 1H), 4.39 (t, J = 9.4 Hz, 1H), 4.31 (s, 2H), 4.09–4.05 (m, 1H), 3.98–3.94 (dd, J = 11.2, 2.8 Hz, 1H), 3.88 (s, 3H), 3.54 (s, 2H), 3.50 (s, 2H), 3.44 (t, J = 10.9 Hz, 1H), 3.08 (s, 3H), 2.19 (s, 3H). HRMS (FAB, ^{35}Cl) *m/z* 577.20413 (M+H), calcd for $C_{31}H_{34}{}^{35}ClN_4O_3S$: 577.20402.

A mixture of **54** (685 mg, 1.19 mmol), Boc_2O (2.60 g, 11.87 mmol) and DMAP (10 mg) in THF (60 mL) and dioxane (20 mL) was allowed to reflux overnight. All solvent was removed and the residue was dissolved in DCM. After a basic (aqueous $NaHCO_3$) workup, the crude product was purified through silica gel column chromatography. A mixture of EtOAc/DCM/petroleum ether (v/v/v = 2:1:1) was used to elute out (*E*)-di(*tert*-butyl) 1-(chloro methyl)-3-((2*E*)-3-(4-((methyl((1-methyl-5-(methylsulfonyl)-1*H*-pyrrol-2-yl)methyl)amino)methyl)phenyl)-2-propenoyl)-2,3-dihydro-1*H*-benzo[*e*]indol-5-ylimidodicarbonate (**55**) as bright yellow solid (797 mg, 86%). ^{1}H NMR (MeOH-*d*4): δ 8.45 (br s, 1H), 7.96 (d, J = 8.3 Hz, 1H), 7.78 (d, J = 5.9 Hz, 1H), 7.75 (s, 1H), 7.67 (d, J = 8.1 Hz, 2H), 7.61–7.57 (td, J = 7.6, 1.0 Hz, 1H), 7.53–7.49 (td, J = 7.6, 1.0 Hz, 1H), 7.39 (d, J = 8.1 Hz, 2H), 7.13 (d, J = 15.4 Hz, 1H), 6.77 (d, J = 4.0 Hz, 1H), 6.16 (d, J = 4.0 Hz, 1H), 4.62–4.58 (m, 2H), 4.12 (br s, 1H), 4.06–4.03 (dd, J = 11.3, 3.1 Hz, 1H), 3.90–3.86 (dd, J = 11.3, 7.0 Hz, 1H), 3.87 (s, 3H), 3.57 (s, 2H), 3.56 (s, 2H), 3.12 (s, 3H), 2.19 (s, 3H), 1.33 (s, 9H), 1.30 (s, 9H). HRMS (ESI): *m/z* 777.3071 (M+H), $C_{41}H_{50}{}^{35}ClN_4O_7S$ requires 777.3089.

A solution of **55** (40 mg, 0.051 mmol) in *N*-methyl-2-pyrrolidinone (2 mL) in a 5 mL vial with a screw cap was treated with methyl iodide (0.5 μL, 0.008 mmol). The vial was sealed and the mixture was stirred at 40 °C for 4 h, then cooled with ice and ^{3}H-methyl iodide (0.003 mmol, 250 mCi, in 0.25 mL of toluene, American Radiolabeled Chemicals, Inc.) was added. The mixture was then stirred at 40 °C for 4 days, cooled with ice, further MeI (2.6 μL, 0.042 mmol) was added and the mixture was stirred at 40 °C for 4 further days. The solution was transferred into a flask and the vial was washed by a little DCM. Et_2O (150 mL) was added and the resulting suspension was left at −20 °C overnight. The precipitate was collected by decantation and then recrystallized from DCM and Et_2O (×2) to give **56** as a white solid (40 mg, 85%); ^{1}H NMR (MeOH-d_4): δ 8.45 (br s, 1H), 7.98 (d, J = 8.3 Hz, 1H), 7.91 (d, J = 8.1 Hz, 1H), 7.83 (d, J = 15.4 Hz, 1H), 7.77 (d, J = 8.3 Hz, 1H), 7.68 (d, J = 8.2 Hz, 2H), 7.64–7.60 (td, J = 8.2, 1.0 Hz, 1H), 7.55–7.51 (m, 1H), 7.30 (d, J = 15.4 Hz, 2H), 7.02 (d, J = 4.2 Hz, 1H), 6.68 (d, J = 4.2 Hz, 1H), 4.78 (s, 2H), 4.67 (s, 2H), 4.64 (d, J = 5.3 Hz, 2H), 4.44 (br s, 1H), 4.08–4.05 (dd, J = 11.3, 3.1 Hz, 1H), 4.00 (s, 3H), 3.91–3.87 (dd, J = 11.3, 7.1 Hz, 1H), 3.24 (s, 3H), 3.02 (s, 6H), 1.33 (s, 9H), 1.31 (s, 9H). This was used directly in the next step.

A solution of **56** (40 mg, 0.043 mmol) in 4 M HCl in dioxane (10 mL) was kept at room temperature for 15 h, and the resulting precipitate was collected by filtration and recrystallized from MeOH/dioxane, then triturated with Et_2O ether to give **^{3}H-4** (27 mg, 79%) as a white solid. HPLC chemical purity 85%, radiochemical purity 71%, specific activity 107 GBq/mmol. ^{1}H NMR (MeOH-d_4) δ 8.83 (s, 1H), 8.09 (d, J = 8.3 Hz, 1H), 7.91 (d, J = 8.2 Hz, 1H), 7.93 (d, J = 8.2 Hz, 2H), 7.87 (d, J = 15.5 Hz, 1H), 7.77–7.66 (m, 4H), 7.32 (d, J = 15.5 Hz, 2H), 7.02 (d, J = 4.2 Hz, 1H), 6.69 (d, J = 4.2 Hz, 1H), 4.80 (s, 2H), 4.70 (s, 2H), 4.67 (d, J = 5.1 Hz, 2H), 4.48 (br s, 1H), 4.09–4.06 (dd, J = 11.3, 3.2 Hz, 1H), 4.02 (s, 3H), 3.96–3.92 (dd, J = 11.4, 6.7 Hz, 1H), 3.24 (s, 3H), 3.02 (s, H).

4.2. Biology

4.2.1. In vivo weight loss experiments

C3H/HeN mice (~50 days old at time of treatment) were obtained from the Vernon Jansen Animal Unit, University of Auckland, and housed in groups of four per cage with unrestricted access to Teklad Global 18% protein rodent diet was purchased from Harlan Teklad Global Diet (Oxford, UK) and tap water. Baseline body weight and food and water intake were measured for 7–10 days prior to drug injection to determine that the animals were eating and gaining weight normally (data not shown). A single ip injection of test compound in 42% aqueous DMSO, or 42% aqueous DMSO alone (vehicle control), was administered on day

1 to groups of 4 animals, and body weight and food and water intake were monitored 2–3 times per week for 60 days. The results are shown in Table 1. Tissue distribution studies with ^{3}**H-4** were conducted as described.[22]

The University of Auckland Animal Ethics Committee approved the animal protocols, which permitted continuing observation of animals with severe body weight loss provided that daily monitoring of clinical signs indicated the animals to be in good general health.

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmc.2011.12.007.

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