Organic & Biomolecular Chemistry

Cite this: *Org. Biomol. Chem.*, 2011, **9**, 6700

www.rsc.org/obc

PAPER

First synthesis of an aziridinyl fused pyrrolo[1,2-*a*]benzimidazole and toxicity evaluation towards normal and breast cancer cell lines†

Sarah Bonham,[a] Liz O'Donovan,[a] Michael P. Carty[b] and Fawaz Aldabbagh*[a]

Received 2nd May 2011, Accepted 22nd June 2011
DOI: 10.1039/c1ob05694h

Anionic aromatic *ipso*-substitution has allowed an aziridine ring to be fused onto pyrrolo[1,2-*a*]benzimidazole. This diazole analogue of aziridinomitosene, and *N*-[(aziridinyl)methyl]-1*H*-benzimidazole are shown to be significantly more cytotoxic towards the human breast cancer cell lines MCF-7 and HCC1937 than towards a human normal fibroblast cell line (GM00637). The aziridinyl fused pyrrolo[1,2-*a*]benzimidazole is less cytotoxic than the non-ring fused aziridinyl analogue towards all three cell lines. The *BRCA1*-deficient HCC1937 cells are more sensitive to mitomycin C (MMC) compared to GM00637 and MCF-7 cells. The evidence provided indicates that different pathways may mediate cellular response to benzimidazole-containing aziridine compounds compared to MMC.

Introduction

The mitomycin natural products are a family of pyrrolo[1,2-*a*]indolequinones containing a fused aziridine.[1] The most important is mitomycin C (MMC, Fig. 1) used to treat solid tumours (including breast cancer), despite its side-effects and sometimes poor clinical efficacy.[2] It is the archetypal bioreductive drug incorporating a quinone for reductive activation and subsequent electrophilic sites for alkylation of DNA.[3] MMC induces apoptosis, and is reported to be more cytotoxic than other drugs, such as docetaxel and cisplatin, towards human breast cancer cells (MCF-7).[4] Other breast cancer cell lines such as HCC1937, derived from a primary breast cancer carcinoma from a patient with germ-line mutation in the breast cancer susceptibility gene *BRCA1*,[5] have been reported to be hypersensitive to MMC and cisplatin.[6] The BRCA1 protein plays a key role in repair of DNA-strand breaks arising as a result of intrastrand and interstrand DNA-crosslinks induced by chemotherapeutic agents.

There have been many reported syntheses of the mitomycin skeleton or that of its bioactivated derivative aziridinomitosene.[7] Many related heterocyclic quinones have been prepared, and evaluated as anti-tumour agents,[8] including alicyclic ring fused benzimidazolequinones,[9–12] and imidazobenzimidazolequinones.[13,14] Some synthesized benzimidazolequinones contain an aziridine ring substituent.[9,12,15,16] For diazoles the aziridinyl substituent and not the quinone is required for hypersensitive killing of Fanconi anaemia (FA) cells deficient in FANCD2, with the effect on FA cell viability being similar to that observed with MMC.[12,16] The quinone functionality decreases specificity towards cancer cells by increased toxicity towards human normal fibroblast cells (GM00637) for aziridinyl substituted diazoles precluding its requirement in the present work.[12]

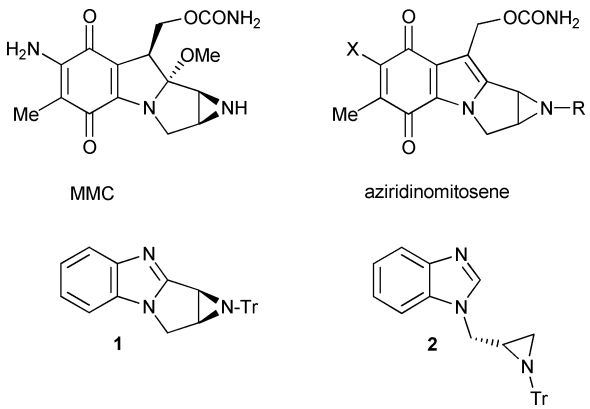


Fig. 1 Aziridinyl-fused natural products and synthetic targets.

In this article we report heterocyclic system **1**, a diazole analogue of aziridinomitosene—it represents the first time an aziridine is fused onto pyrrolo[1,2-*a*]benzimidazole. The synthesis uses a novel protocol for fusion of aziridine involving an intramolecular anionic aromatic *ipso*-substitution by the aziridinyl functionality onto the benzimidazol-2-yl position. The toxicity of compound **1** is evaluated towards human normal (GM00637) and two breast cancer cell lines; MCF-7 and HCC1937, and compared with *N*-[(1-tritylaziridin-(2*S*)-yl)methyl]-1*H*-benzimidazole **2** (Fig. 1) in order to assess the impact of ring fusion on toxicity.

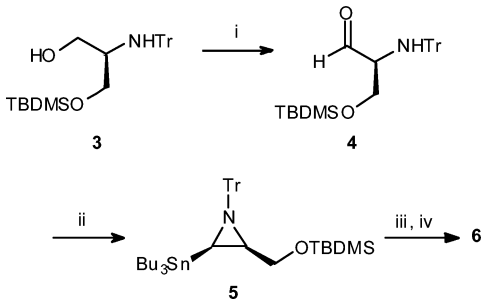
[a]*School of Chemistry, National University of Ireland Galway, University Road, Galway, Ireland. E-mail: fawaz.aldabbagh@nuigalway.ie*
[b]*Biochemistry, School of Natural Sciences, National University of Ireland Galway, University Road, Galway, Ireland*

† Electronic supplementary information (ESI) available: ^{1}H and ^{13}C NMR spectra and HPLC chromatograms. See DOI: 10.1039/c1ob05694h

Results and discussion

Synthesis

The required aziridine fragment was prepared from *N*-trityl-*O*-(*tert*-butyldimethylsilyl)-*R*-serinol **3**,[17] which was converted to aldehyde **4** (Scheme 1) using a 2,2,6,6-tetramethyl-1-piperidinyloxy radical (TEMPO)/sodium hypochlorite oxidation.[18]



Scheme 1 (i) TEMPO, NaClO, $NaHCO_3$, NaBr, Tol, EtOAc, H_2O, 0 °C, 92% (ii) *n*-Bu_3SnLi, THF, −78 °C, diisopropyl azodicarboxylate (DIAD), PPh_3, Tol, 0 °C, 56% (iii) *n*-$Bu_4N^+F^-$, THF, rt, 96%, (iv) 4-$NO_2C_6H_4SO_2Cl$, Et_3N, CH_2Cl_2, 0 °C, 75%.

The formation of the new aziridine **5** used Bu_3SnLi followed by a Mitsunobu-type ring closure according to a modification of a literature procedure.[19] Our approach to aziridine **6** *via* TBDMS-protected **5** circumvents the requirement for protection using (*tert*-butyldimethylsilyloxy)methyl chloride, which requires prior synthesis.[20] TBDMS-protected aziridine **5** was converted to the nosylate **6**,[19] and used rather than the analogous mesylate to give reasonable yields (41%) of 1-{[(2*S*,3*R*)-3-(tributylstannyl)-1-tritylaziridin-2-yl]methyl}-1*H*-benzimidazole **9** (Scheme 2). Steric hindrance between the bulky tributylstannyl (Bu_3Sn) and phenylsulfanyl (PhS) groups prevented the successful coupling of 2-phenylsulfanyl-1*H*-benzimidazole with **6** to directly give cyclization precursor **10**, as indicated by reacting mesylate **7** lacking the Bu_3Sn group with 2-phenylsulfanyl-1*H*-benzimidazole to give **8a** in good yield (72%). Target aziridine **2** was prepared in good yield (83%) by coupling benzimidazole with mesylate **7**. Vedejs and co-workers have reported a Michael addition approach to the aziridinomitosene skeleton, and lithiation of the α-position on pyrrole,[19] and indole.[21]

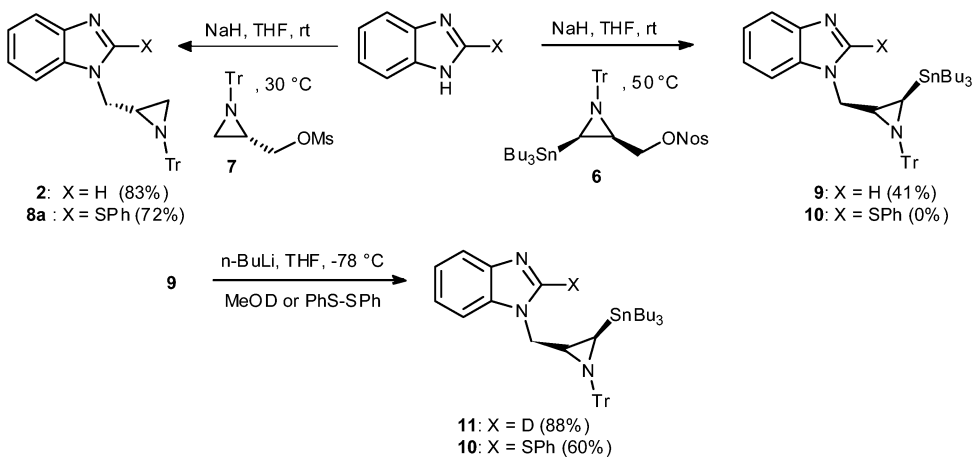
Selective lithiation at the benzimidazol-2-yl position was then investigated by treatment of **9** with *n*-BuLi in THF at −78 °C with the generated anion quenched with MeOD (Scheme 2). Selective deuterium incorporation at the benzimidazol-2-yl position yielded **11** in 88% yield and replacing MeOD with diphenyl disulfide gave **10** in 60% yield.

The less bulky organolithium, MeLi in THF at −78 °C was used to initiate the cyclization from **10** by generating the aziridin-3-yl anion (Scheme 3). This adds onto the electrophilic benzimidazol-2-yl position followed by elimination of SPh. The latter *ipso*-substitution gave tetracyclic target **1** in 75% yield with sulfide **8b** (enantiomer of **8a**) also isolated in 18% yield. Isolation of by-product **8b** confirmed the *in situ* generation of the aziridin-3-yl anion, which was quenched by adventitious hydration. Repeated attempts at this reaction did not improve the yield of target **1**. Organosulfur leaving groups have been previously displaced in radical *ipso*-substitutions,[22,23] however in this ionic case, SPh allows deactivation of the benzimidazol-2-yl position (otherwise acidic) during aziridine lithiation, as well as acting as an effective leaving group for the subsequent cyclization.

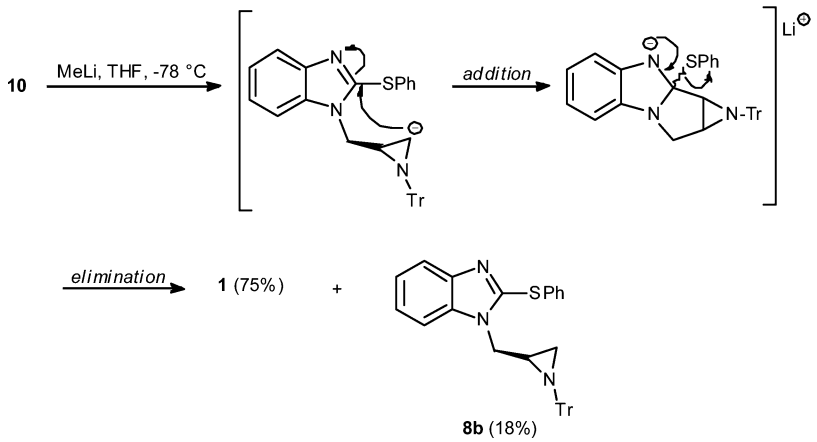
Cytotoxicity evaluation

The toxicity of compounds was evaluated towards human normal skin fibroblast cells (GM00637) and two human breast cancer cell lines (MCF-7) and (HCC1937). Cells were treated in parallel with MMC, which acts as a positive control in the MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) colorimetric assay.[24] Aziridinyl-fused pyrrolo[1,2-*a*]benzimidazole **1** was found to be largely non-toxic at concentrations of ≤1 μM with non-fused aziridinyl benzimidazole **2**, and MMC showing greater toxicity towards human normal fibroblast cells (GM00637, Fig. 2).

Aziridinyl fused compounds **1** and MMC show similar toxicity towards the breast cancer cell line (MCF-7), while cell response towards benzimidazole **2** is significantly greater (Fig. 3). In contrast, the *BRCA1*-deficient breast cancer cell line HCC1937 showed a significantly greater response towards MMC, with an effect on cell viability evident at doses as low as 10 nanomolar (0.01 μM, Fig. 4). Non-ring fused aziridine **2** was 4 times more



Scheme 2 Synthesis of cyclization precursor.



Scheme 3 Anionic aromatic *ipso*-substitution onto benzimidazole.

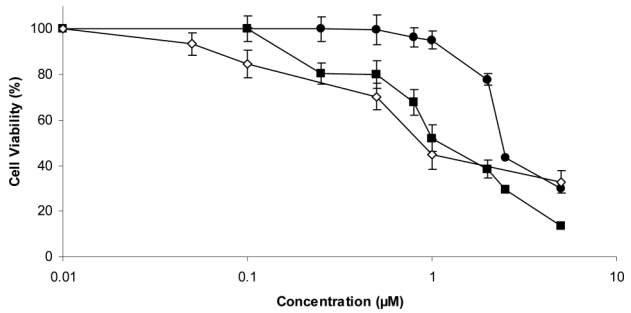


Fig. 2 Viability of human normal skin fibroblast cell line (GM00637) determined using the MTT assay following treatment with compounds **1** (●), **2** (■) and MMC (◇) under aerobic conditions for 24 h at 37 °C. Each data point is the mean of at least three independent experiments.

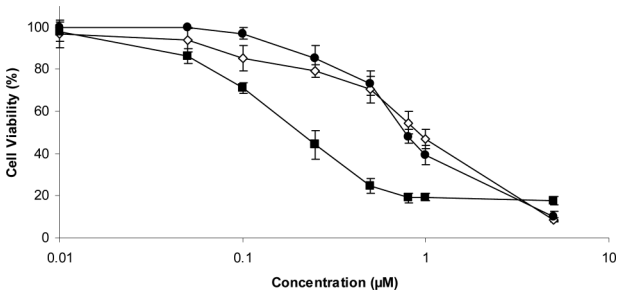


Fig. 3 Viability of human breast cancer cell line (MCF-7) determined using the MTT assay following treatment with compounds **1** (●), **2** (■) and MMC (◇) under aerobic conditions for 24 h at 37 °C. Each data point is the mean of at least three independent experiments.

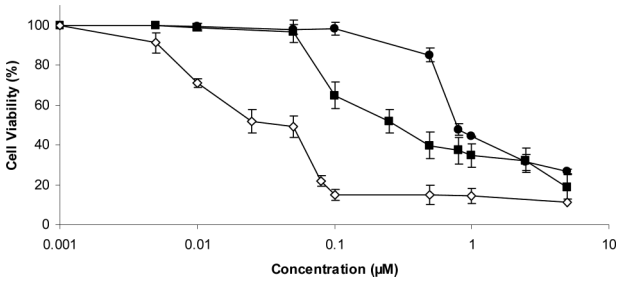


Fig. 4 Viability of human breast cancer cell line (HCC1937) determined using the MTT assay following treatment with compounds **1** (●), **2** (■) and MMC (◇) under aerobic conditions for 24 h at 37 °C. Each data point is the mean of at least three independent experiments.

toxic than fused system **1** towards HCC1937 and 2–4 times more toxic towards the other two cell lines evaluated (Table 1). The reduction in cytotoxicity for compound **1** compared to analogue **2** is similar to a previously observed general increase in cytotoxicity for 2-aromatic ring substituted benzimidazole-4,7-diones compared to analogous alicyclic ring fused systems, towards human normal and cancer cell lines.[11] Table 1 shows the aziridine-containing benzimidazoles **1** and **2** to be approximately 4–5 and 6–8 times, respectively, more toxic towards the breast cancer cell lines than towards the normal cell line, which may be of therapeutic advantage. In contrast the clinically used drug MMC shows similar cytotoxicity towards the normal and the breast cancer cell line, MCF-7.

Table 1 Effect of aziridines on the viability of human normal skin fibroblast cell line (GM00637) and human breast cancer cell lines (MCF-7) and (HCC1937)

Compound	IC_{50}[a] GM00637 [μM]	IC_{50}[a] MCF-7 [μM]	IC_{50}[a] HCC1937 [μM]
MMC (◇)	0.77 ± 0.18	0.93 ± 0.11	0.03 ± 0.01
1 (●)	3.11 ± 0.44	0.84 ± 0.14	0.67 ± 0.01
2 (■)	1.26 ± 0.13	0.22 ± 0.04	0.16 ± 0.03

[a] IC_{50} represents the compound concentration required for the reduction of the mean cell viability to 50% of the control after incubation for 24 h at 37 °C, as determined using the MTT assay.

For MMC it is reported that the formation of crosslinks upon DNA alkylation are primarily responsible for cell death.[3,25] The hypersensitivity of *BRCA1*-deficient cells (such as HCC1937) towards MMC implicates BRCA1 in cellular response to crosslinks.[6] The comparatively smaller cytotoxicity of benzimidazole containing aziridines **1** and **2** towards HCC1937 supports an alternative mechanism underlying cellular response to these agents. Furthermore the chemical structures of **1** and **2** do not allow for the formation of crosslinks, because there is only one position for DNA-alkylation (at the aziridine) and the absence of a quinone moiety indicates a lack of bioreductive activation. The latter is however in line with reports of DNA-alkylation and reactions of aziridinomitosene with nucleophiles in the absence of reduction.[26]

Future studies will examine detailed biochemical analysis of cell response towards compounds **1** and **2**, preparation of analogues, as well as extending their toxicity evaluation to further cancer cell lines.

Conclusions

A new protocol for fusion of aziridine using anionic aromatic *ipso*-substitution is described. This gave the first diazole analogue of aziridinomitosene. The latter along with non-fused, *N*-[(1-tritylaziridin-(2*S*)-yl)methyl]-1*H*-benzimidazole are shown to possess 4–8 times greater toxicity towards human breast cancer cell lines (MCF-7 and HCC1937) than towards a human normal fibroblast (GM00637) cell line. Aziridine-ring fusion onto diazole was found to reduce overall toxicity. The mitomycin mechanism for cytotoxicity involving bioreductive activation with subsequent formation of DNA-crosslinks is ruled out for the new benzimidazole compounds because of differences in their chemical structure. Bioreduction cannot take place since there is no aminoquinone motif as with MMC, and the formation of crosslinks is not possible since there is only one position for DNA-alkylation. The inability to form DNA crosslinks is supported by the comparatively smaller cell response of the *BRCA1*-mutated cell line, HCC1937 towards the new aziridine compounds.

Experimental

General

Materials. All materials were obtained from Sigma–Aldrich, except (*S*)-serine methyl ester hydrochloride, which was obtained from TCI Fine Chemicals. Solvents were purified and dried prior to use according to conventional methods. All reactions were carried out under a nitrogen atmosphere. NaH was obtained as 60% dispersion in oil and used without further purification. *n*-BuLi and MeLi solutions were obtained as 1.6 M in hexanes and 1.4 M in diethyl ether respectively, and titrated against diphenylacetic acid before use. Monitoring of reactions by thin layer chromatography (TLC) was carried out on aluminium-backed plates coated with silica gel (Merck Kieselgel 60 F_{254}). Column chromatography was carried out using Merck Kieselgel silica gel 60 (particle size 0.040–0.063 mm), and dry column vacuum chromatography was carried out using Merck Kieselgel silica gel 60 (particle size 0.015–0.040 mm).[14,27] The two steps of the synthesis of *N*-trityl-*O*-(*tert*-butyldimethylsiloxy)-*R*-serinol **3** from commercial (*S*)-serine methyl ester hydrochloride were carried out according to literature procedures.[17,28] [(2*S*)-1-Tritylaziridin-2-yl]methyl methanesulfonate **7** and 2-phenylsulfanyl-1*H*-benzimidazole were prepared according to our previously reported methods.[16,23] Cell culture reagents were obtained from Sigma–Aldrich and sterile plasticware was obtained from Sarstedt AG (Numbrecht, Germany).

Measurements. Melting points were determined on a Stuart Scientific melting point apparatus SMP3. IR spectra were obtained using a Perkin–Elmer Spectrum 1000 FT-IR spectrophotometer with ATR accessory. NMR spectra were recorded using a JEOL GXFT 400 MHz instrument equipped with a DEC AXP 300 computer workstation. Chemical shifts are reported relative to Me_4Si as internal standard and NMR assignments were supported by DEPT and ^1H–^{13}C NMR 2D spectra. Coupling constants (*J*) are expressed in Hertz (Hz). High resolution mass spectra (HRMS) for all compounds were carried out using electrospray ionization (ESI) on a Waters LCT Premier XE spectrometer by manual peak matching. The precision of all accurate mass measurements is better than 5 ppm. Optical rotations were recorded on a UniPol L1000 polarimeter. HPLC analysis was carried out using an Agilent Technologies 1200 series instrument with a UV detector at the specified wavelength. Absorbance was measured in the MTT assay using a Wallac Victor 2 1420 multi-label Counter.

Synthesis of *N*-[(1-tritylaziridin-(2*S*)-yl)methyl]-1*H*-benzimidazole (2). Mesylate **7** (0.450 g, 1.14 mmol) was added to benzimidazole (96 mg, 0.81 mmol) and NaH (20 mg, 0.83 mmol) in THF (5 mL), and heated at 30 °C for 24 h. The cooled mixture was evaporated to dryness, and purified by dry column vacuum chromatography with gradient elution of EtOAc and hexane. Evaporation of the fractions containing the second component gave the aziridine **2** (0.281 g 83%) as a clear oil; R_f 0.31 (1 : 9 ethyl acetate/hexane); $[\alpha]_D^{20}$ −58.9 (*c* 0.18 in $CHCl_3$); ν_{max} (neat, cm^{-1}) 2962, 1594, 1490, 1446, 1382, 1261, 1232, 1085, 1029; δ_H (400 MHz, $CDCl_3$) 1.18 (1H, d, *J* 6.0, aziridinyl–C*H*H), 1.67–1.75 (2H, m), 4.25 (1H, dd, J^2 14.8, J^3 5.6, NC*H*H), 4.59 (1H, dd, J^2 14.8, J^3 4.2, NCH*H*), 7.18–7.31 (12H, m), 7.44–7.46 (6H, m), 7.76–7.79 (1H, m, BnIm-4-H), 7.94 (1H, s, BnIm-2-H); δ_C (100 MHz, $CDCl_3$) 26.6 (CH_2), 31.9 (CH), 47.4 (NCH_2), 74.2 (*C*Ph_3), 109.8 (BnIm-7-CH), 120.5 (BnIm-4-CH) 122.4, 123.0 (BnIm-5,6-CH), 127.0, 127.7, 129.4 (Ph–CH), 134.2 (C), 143.1 (BnIm-2-CH), 143.8 (C), 144.1 (C); HRMS (ESI): found MH^+, 416.2109. $C_{29}H_{26}N_3$ requires 416.2127.

2-(Phenylsulfanyl)-1-{[(2*S*)-1-tritylaziridin-2-yl]methyl}-1*H*-benzimidazole (8a). was prepared using the same procedure as compound **2** (above), but replacing benzimidazole with 2-phenylsulfanyl-1*H*-benzimidazole. Spectroscopic data were found to be identical to enantiomer **8b** (see below) with $[\alpha]_D^{20}$ −8.7.

Synthesis of *N*-trityl-*O*-(*tert*-butyldimethylsiloxy)-*S*-serinal (4). An aqueous solution of NaClO (10.8 mL of 12% w/v, 17.48 mmol), $NaHCO_3$ (2.030 g, 24.17 mmol) was added dropwise to a mixture of alcohol **3** (1.370 g, 3.06 mmol), NaBr (0.33 g, 3.20 mmol), TEMPO (57 mg, 0.37 mmol), EtOAc (20 mL), toluene (20 mL) and water (4 mL) at 0 °C, and stirred at room temperature for 96 h. The aqueous layer was extracted with Et_2O (2 × 30 mL) and the combined organic layers washed with KI in 10% $KHSO_4$ (2 × 50 mL), 10% $Na_2S_2O_3$ (2 × 50 mL), brine (2 × 50 mL), and dried ($MgSO_4$). The solution was evaporated and the residue purified by column chromatography using silica gel as absorbent with gradient elution of EtOAc and hexane to give the aldehyde **4** (1.250 g, 92%) as a colourless oil; R_f 0.72 (1 : 9 ethyl acetate/hexane); $[\alpha]_D^{20}$ +32.1 (*c* 0.50 in $CHCl_3$); ν_{max} (neat, cm^{-1}) 2950, 2929, 2856, 1732 (C═O), 1573, 1522, 1491, 1447, 1462, 1377, 1330, 1253, 1205, 1176, 1109, 1031, 1005; δ_H (400 MHz, $CDCl_3$) 0.14 (3H, s, CH_3Si), 0.15 (3H, s, CH_3Si), 0.97 (9H, s, *t*Bu), 3.25 (1H, dd, J^2 9.6, J^3 5.6, C*H*H), 3.51–3.54 (1H, m, CH), 3.82 (1H, dd, J^2 9.6, J^3 4.0, CH*H*), 7.30–7.35 (9H, m), 7.59–7.61 (6H, m), 9.31 (1H, s, CHO); δ_C (100 MHz, $CDCl_3$) −5.5 ((CH_3)$_2$Si), 18.2 (*C*(CH_3)$_3$), 25.6 (C(*C*H_3)$_3$), 63.0 (CH), 64.2 (CH_2), 70.8 (*C*Ph_3), 126.9, 128.0, 128.8 (Ph–CH), 146.2 (Ph-*ipso*-C), 205.1 (CHO). Aldehyde **4** was found to be unstable

(could not be stored), and was used immediately in the next step.

Synthesis of (2*S*,3*R*)-2-({[*tert*-butyl(dimethyl)silyl]oxy}methyl)-3-(tributylstannyl)-1-tritylaziridine (5). *n*-BuLi (2.87 mL, 4.59 mmol) was added to *N*,*N*-diisopropylamine (0.43 mL, 3.05 mmol) in THF (3 mL) at −20 °C. After 20 min, *n*-Bu_3SnH (0.82 mL, 3.05 mmol) was added and the yellow solution stirred for 1 h. The solution was cooled to −78 °C and aldehyde **4** (0.680 g, 1.53 mmol) in THF (2 mL) added dropwise. The resultant blue–black solution was stirred at −78 °C for 1 h. Saturated NH_4Cl (30 mL) was added, and the solution extracted with Et_2O (3 × 40 mL), dried (Na_2SO_4), and evaporated to dryness to give the amino alcohol intermediate as a yellow oil; HRMS (ESI): found MH^+, 738.3705. $C_{40}H_{64}NO_2SiSn^{120}$ requires 738.3728. This was unstable to purification and used directly in the following Mitsunobu ring closure: DIAD (0.60 mL, 3.05 mmol) was added to the latter amino alcohol and Ph_3P (0.800 g, 3.05 mmol) in toluene (35 mL) at 0 °C. The solution was stirred for 48 h at room temperature, water (30 mL) added, extracted with CH_2Cl_2 (3 × 30 mL), and dried (Na_2SO_4). The solution was evaporated to dryness to give an orange residue, which was purified by column chromatography using silica gel as absorbent with gradient elution of CH_2Cl_2 and hexane to give the aziridine **5** (0.620 g, 56%) as a colourless oil; R_f 0.60 (1 : 4 dichloromethane/hexane); $[\alpha]_D^{20}$ −6.1 (*c* 0.30 in $CHCl_3$); ν_{max} (neat, cm^{-1}) 2954, 2926, 2854, 1489, 1462, 1254, 1074, 907; δ_H (400 MHz, $CDCl_3$) 0.04 (3H, s, CH_3Si), 0.06 (3H, s, CH_3Si), 0.88–0.91 (18H, m, CH_2CH_3, and *t*Bu), 0.93–1.03 (6H, m, CH_2), 1.26–1.40 (7H, m, CH_2 and aziridinyl–CHN), 1.43–1.51 (7H, m, CH_2 and aziridinyl–CHN), 3.65 (1H, dd, J^2 10.8, J^3 5.0, C*H*H), 3.82 (1H, dd, J^2 10.8, J^3 6.4, CH*H*), 7.19–7.23 (3H, m), 7.26–7.30 (6H, m), 7.53 (6H, d, *J* 7.2); δ_C (100 MHz, $CDCl_3$) −5.2 (CH_3Si), −5.1 (CH_3Si), 10.2 (CH_2), 13.8 (CH_3), 18.4 ($C(CH_3)_3$), 24.1 (CH), 26.0 ($C(CH_3)_3$), 27.5 (CH_2), 29.4 (CH_2), 38.2 (CH), 67.5 (OCH_2), 75.2 (CPh_3), 126.5, 127.4, 129.8 (Ph–CH), 144.6 (Ph-*ipso*-C); HRMS (ESI): found MH^+, 720.3625. $C_{40}H_{62}NOSiSn^{120}$ requires 720.3623; *m*/*z* 720 (M + H^+, 100%), 719 ($C_{40}H_{62}NOSiSn^{119}$, 60%), 718 ($C_{40}H_{62}NOSiSn^{118}$, 80%). Aziridine **5** was converted into [(2*S*,3*R*)-3-(tributylstannyl)-1-tritylaziridin-2-yl]methyl 4-nitrobenzenesulfonate **6** using the methods of Vedejs *et al.*[19]

Synthesis of 1-{[(2*S*,3*R*)-3-(tributylstannyl)-1-tritylaziridin-2-yl]methyl}-1*H*-benzimidazole (9). Nosylate **6** (0.400 g, 0.51 mmol) was added to benzimidazole (55 mg, 0.47 mmol) and NaH (13 mg, 0.54 mmol) in THF (5 mL), and heated at 50 °C for 24 h. The cooled mixture was evaporated to dryness, and purified by dry column vacuum chromatography with gradient elution of EtOAc and hexane. Evaporation of fractions containing the second component gave the aziridine **9** (0.134 g, 41%) as a yellow oil; R_f 0.28 (1 : 4 EtOAc/hexane); $[\alpha]_D^{20}$ −8.6 (*c* 0.50 in $CHCl_3$); ν_{max} (neat, cm^{-1}) 2927, 1597, 1492, 1447, 1216, 1032; δ_H (400 MHz, $CDCl_3$) 0.87 (9H, t, *J* 7.2, CH_3), 0.94–1.07 (6H, m, CH_2), 1.09 (1H, d, *J* 6.8, aziridinyl–H), 1.24–1.33 (6H, m, CH_2), 1.43–1.51 (6H, m, CH_2), 1.77 (1H, ddd, *J* 6.8, 6.8, 4.8, aziridinyl–H), 4.16 (1H, dd, J^2 14.0, J^3 4.8, NC*H*H), 4.27 (1H, dd, J^2 14.0, J^3 6.8, NCH*H*), 7.14–7.16 (9H, m), 7.23–7.29 (3H, m, BnIm-5,6,7-H), 7.35–7.38 (6H, m), 7.75–7.77 (1H, m, BnIm-4-H), 7.79 (1H, s, BnIm-2-H); δ_C (100 MHz, $CDCl_3$) 10.3 (CH_2), 13.8 (CH_3), 25.8 (CH), 27.5 (CH_2), 29.3 (CH_2), 35.5 (CH), 49.4 (NCH_2), 75.8 (CPh_3) 109.6 (BnIm-7-CH), 120.5 (BnIm-4-CH), 122.2, 122.9 (BnIm-5,6-CH), 126.8, 127.5, 129.3 (Ph–CH), 134.1 (C), 142.3 (BnIm-2-CH), 143.9 (C), 148.7 (C); HRMS (ESI): found MH^+, 706.3204. $C_{41}H_{52}N_3Sn^{120}$ requires 706.3183; *m*/*z* 706 (M + H^+, 100%), 705 ($C_{41}H_{52}N_3Sn^{119}$, 56%), 703 ($C_{41}H_{52}N_3Sn^{117}$, 39%), 702 ($C_{41}H_{52}N_3Sn^{116}$, 42%).

General procedure for the synthesis of 2-substituted benzimidazoles 10 and 11. *n*-BuLi (0.10 mL, 0.16 mmol) was added dropwise to benzimidazole **9** (0.100 g, 0.14 mmol) in THF (4 mL) at −78 °C. The solution turned deep red, and after 15 min MeOD or diphenyl disulfide (0.28 mmol) was added in THF (1 mL). The solution was stirred at room temperature for 30 min, evaporated to dryness, and the residue purified by column chromatography with gradient elution of EtOAc and hexane. Evaporation of fractions containing the second component gave the 2-substituted benzimidazoles.

2-Deutero-1-{[(2*S*,3*R*)-3-(tributylstannyl)-1-tritylaziridin-2-yl]methyl}-1*H*-benzimidazole (11). (87 mg, 88%) as a clear oil; R_f 0.21 (1 : 4 EtOAc/hexane); ν_{max} (neat, cm^{-1}) 2929, 1489, 1466, 1439, 1216, 1100; δ_H (400 MHz, $CDCl_3$) 0.86 (9H, t, *J* 7.2, CH_3), 0.95–1.08 (6H, m, CH_2), 1.09 (1H, d, *J* 6.8, aziridinyl–H), 1.23–1.33 (6H, m, CH_2), 1.42–1.50 (6H, m, CH_2), 1.76 (1H, ddd, *J* 6.8, 6.8, 4.8, aziridinyl–H), 4.15 (1H, dd, J^2 14.0, J^3 4.8, NC*H*H), 4.26 (1H, dd, J^2 14.0, J^3 6.8, NCH*H*), 7.14–7.16 (9H, m), 7.26–7.27 (3H, m, BnIm-5,6,7-H), 7.35–7.37 (6H, m), 7.74–7.77 (1H, m, BnIm-4-H); δ_C (100 MHz, $CDCl_3$) 10.3 (CH_2), 13.8 (CH_3), 25.8 (CH), 27.5 (CH_2), 29.3 (CH_2), 35.5 (CH), 49.4 (NCH_2), 75.7 (CPh_3) 109.6 (BnIm-7-CH), 120.5 (BnIm-4-CH), 122.1, 122.9 (BnIm-5,6-CH), 126.8, 127.8, 129.3 (Ph–CH), 134.1, 143.9 (C); HRMS (ESI): found MH^+, 707.3204. $C_{41}H_{51}DN_3Sn^{120}$ requires 707.3205.

2-(Phenylsulfanyl)-1-{[(2*S*,3*R*)-3-(tributylstannyl)-1-tritylaziridin-2-yl]methyl}-1*H*-benzimidazole (10). (68 mg, 60%) as a clear oil; R_f 0.56 (1 : 4 EtOAc/hexane); $[\alpha]_D^{20}$ −30.0 (*c* 0.10 in $CHCl_3$); ν_{max} (neat, cm^{-1}) 2929, 1596, 1490, 1447, 1328, 1251, 1080, 1033, 907; δ_H (400 MHz, $CDCl_3$) 0.87 (9H, t, *J* 7.4, CH_3), 0.97–1.13 (7H, m, CH_2 and aziridinyl–H), 1.25–1.35 (6H, m, CH_2), 1.45–1.53 (6H, m, CH_2), 2.05 (1H, ddd, *J* 8.4, 8.4, 2.8, aziridinyl–H), 4.15 (1H, dd, J^2 14.4, J^3 8.4, NC*H*H), 4.25 (1H, dd, J^2 14.4, J^3 2.8, NCH*H*), 7.00–7.04 (9H, m), 7.06–7.10 (5H, m), 7.21–7.30 (9H, m), 7.71–7.73 (1H, m, BnIm-4-H); δ_C (100 MHz, $CDCl_3$) 10.3 (CH_2), 13.8 (CH_3), 25.5 (CH), 27.5 (CH_2), 29.3 (CH_2), 35.5 (CH), 49.8 (NCH_2), 75.8 (CPh_3), 110.2 (BnIm-7-CH), 120.1 (BnIm-4-CH), 122.3, 123.1 (BnIm-5,6-CH), 126.6, 127.2, 127.5, 129.4, 129.5, 129.9 (Ph–CH), 132.3, 136.3, 143.6, 143.9, 147.0 (all C); HRMS (ESI): found MH^+, 814.3229. $C_{47}H_{56}N_3SSn^{120}$ requires 814.3217; *m*/*z* 814 (M + H^+, 100%), 813 ($C_{47}H_{56}N_3SSn^{119}$, 54%), 812 ($C_{47}H_{56}N_3SSn^{118}$, 66%).

Synthesis of (1a*S*,8a*S*)-1-trityl-1,1a,8,8a-tetrahydroazireno-[2′,3′:3,4]pyrrolo[1,2-*a*]benzimidazole (1). MeLi (0.19 mL, 0.26 mmol) was added dropwise to benzimidazole **10** (60 mg, 0.074 mmol) in THF (2 mL) at −78 °C. The solution turned deep red, and was brought to room temperature over 30 min. The mixture was evaporated to dryness, and the residue purified by column chromatography with gradient elution of EtOAc and hexane. The following compounds were isolated in order of elution: **2-(Phenylsulfanyl)-1-{[(2*R*)-1-tritylaziridin-2-yl]methyl}-1*H*-benzimidazole 8b)**; (7 mg, 18%) as a yellow oil; R_f 0.65 (1 : 3 EtOAc/hexane); $[\alpha]_D^{20}$ +8.7 (*c* 0.52 in $CHCl_3$); ν_{max} (neat,

cm^{-1}) 1581, 1480, 1441, 1442, 1353, 1326, 1281, 1246, 1202, 1154, 1083, 1024; δ_H (400 MHz, $CDCl_3$) 1.05 (1H, d, *J* 6.0, aziridinyl–C*H*H), 1.60–1.65 (1H, m, aziridinyl–CH), 1.81 (1H, d, *J* 2.8, aziridinyl–CH*H*), 4.23 (1H, dd, J^2 14.8, J^3 7.6, NC*H*H), 4.81 (1H, dd, J^2 14.8, J^3 4.0, NCH*H*), 7.20–7.26 (17H, m), 7.40–7.42 (6H, m), 7.74–7.76 (1H, m, BnIm-4-H); δ_C (100 MHz, $CDCl_3$) 28.1 (aziridinyl–CH_2), 31.6 (CHN), 47.7 (NCH_2), 74.2 (*C*Ph_3), 110.0 (BnIm-7-CH), 120.1 (BnIm-4-CH), 122.6, 123.5 (BnIm-5,6-CH), 126.9, 127.7, 129.4, 129.5, 130.3 (Ph–CH), 132.3, 136.1, 143.4, 144.1, 147.3 (all C); HRMS (ESI): found MH^+, 524.2162. $C_{35}H_{30}N_3S$ requires 524.2160, and the target compound (**1**) (23 mg, 75%) as a pale yellow oil; R_f 0.50 (1 : 3 EtOAc/hexane); $[\alpha]_D^{20}$ −9.3 (*c* 0.15 in $CHCl_3$); ν_{max} (neat, cm^{-1}) 2929, 1623, 1545, 1490, 1448, 1354, 1265, 1216, 1151, 1051, 909; δ_H (400 MHz, $CDCl_3$) 2.97 (1H, d, *J* 4.8, 1*a*-H), 3.08–3.10 (1H, m, 8*a*-H), 4.06 (1H, dd, J^2 11.2, J^3 4.0, 8-*H*H), 4.42 (1H, d, *J* 11.2, 8-H*H*), 7.23–7.32 (12H, m), 7.47–7.49 (6H, m, Ph-H), 7.76–7.79 (1H, m, 3-H); δ_C (100 MHz, $CDCl_3$) 33.3 (1*a*-CH), 40.2 (8*a*-CH), 45.2 (CH_2), 73.4 (*C*Ph_3), 108.6 (6-CH), 119.5 (3-CH), 120.8, 121.6 (4,5-CH), 126.2, 127.0, 128.3 (Ph–CH), 132.0, 142.9, 146.9 (C), 157.6 (1*b*-C); HRMS (ESI): found MH^+, 414.1971. $C_{29}H_{24}N_3$ requires 414.1970.

Cell culture. A SV40-transformed normal human skin fibroblast cell line (repository number GM00637) was obtained from the National Institute for General Medical Sciences (NIGMS) Human Genetic Cell Repository (Coriell Institute for Medical Research, New Jersey, USA). The MCF-7 cell line, a human breast cancer cell line was obtained from Dr Adrienne Gorman, Biochemistry, School of Natural Sciences, National University of Ireland Galway. The HCC1937 breast cancer cell line was obtained from Dr Paul Mullan, Centre for Cancer Research and Cell Biology, Queen's University Belfast. The SV40-transformed normal human skin fibroblast cell line (GM00637) was grown in minimum essential media Eagle–Earle BSS (MEM) supplemented with 15% non heat-inactivated fetal bovine serum (FBS), 1% penicillin-streptomycin, 2mM L-glutamine, 2X essential and non-essential amino acids and 2X vitamins. MCF-7 cells and HCC1937 cells were cultured in Dulbecco's modified Eagle's medium (DMEM) containing high glucose (4.5g mL^{-1}) and supplemented with 10% heat-inactivated FBS and 1% penicillin-streptomycin.

Cytotoxicity measurements. Growth inhibition (cell viability) was determined using the MTT colorimetric assay. Cells were plated into 96-well plates at a density of 10 000 cells per well for GM00637 and HCC1937 (200 µL per well) and 1,000 cells per well for MCF-7 (200 µL per well), and allowed to adhere over a period of 48 h. Compound **1** evaluation solutions were applied in ethanol, MMC and compound **2** were applied in DMSO (1% v/v final concentration in well). All cells were incubated at 37 °C under a humidified atmosphere containing 5% CO_2 for 24 h. Control cells were exposed to an equivalent concentration of ethanol and DMSO alone. MTT (20 µL, 5 mg ml^{-1} solution) was added and the cells were incubated for another 3 h. The supernatant was then removed carefully by pipetting. The resultant MTT formazan crystals were dissolved in 100 µL of DMSO and absorbance was determined on a plate reader at 550 nm with a reference at 690 nm. Cell viability is expressed as a percentage of the control solution value. Dose–response curves were analysed by nonlinear regression analysis and IC_{50} values were estimated by using GraphPad Prism software, v.5.02 (GraphPad Inc., San Diego, CA, USA).

Acknowledgements

The authors thank Science Foundation Ireland (07/RFP/CHEF227) for financial support and Drs. Adrienne Gorman and Paul Mullan (NUI Galway and QUB respectively) for the breast cancer cell lines. We are grateful to Prof. Ian D. Jenkins (Griffith University, Brisbane, Australia) on advice regarding the Mitsunobu reaction.

References

1 W. A. Remers, in *Anticancer Agents from Natural Products*, ed., G. M. Cragg, D. G. I. Kingston and D. J. Newman, Taylor & Francis, Boca Raton, FL, USA, 2005, p. 475.
2 W. T. Bradner, *Cancer Treat. Rev.*, 2001, **27**, 35.
3 H. A. Seow, P. G. Penketh, R. P. Baumann and A. C. Sartorelli, *Methods Enzymol.*, 2004, **382**, 221.
4 F. Pirnia, E. Schneider, D. C. Betticher and M. M. Borner, *Cell Death Differ.*, 2002, **9**, 905.
5 G. E. Tomlinson, T. T.-L. Chen, V. A. Stastny, A. K. Virmani, M. A. Spillman, V. Tonk, J. L. Blum, N. R. Schneider, I. I. Wistuba, J. W. Shay, J. D. Minna and A. F. Gazdar, *Cancer Res.*, 1998, **58**, 3237.
6 (*a*) P. Tassone, P. Tagliaferri, A. Perricelli, S. Blotta, B. Quaresima, M. L. Martelli, A. Goel, V. Barbieri, F. Costanzo, C. R. Boland and S. Venuta, *Br. J. Cancer*, 2003, **22**, 1285; (*b*) J. Yun, Q. Zhong, J.-Y. Kwak and W.-H. Lee, *Oncogene*, 2005, **24**, 4009; (*c*) M. Santarosa, L. D. Col, E. Tonin, A. Caragnano, A. Viel and R. Maestro, *Mol. Cancer Ther.*, 2009, **8**, 844.
7 J.-C. Andrez, *Beilstein J. Org. Chem.*, 2009, **5**No. 33.
8 (*a*) Y. Chen and L. Hu, *Med. Res. Rev.*, 2009, **29**, 29; (*b*) M. A. Colucci, C. J. Moody and G. D. Couch, *Org. Biomol. Chem.*, 2008, **6**, 637.
9 (*a*) E. B. Skibo and W. G. Schulz, *J. Med. Chem.*, 1993, **36**, 3050; (*b*) R. Zhou and E. B. Skibo, *J. Med. Chem.*, 1996, **39**, 4321; (*c*) E. B. Skibo and C. Xing, *J. Med. Chem.*, 2001, **44**, 3545; (*d*) E. B. Skibo, A. Jamil, B. Austin, D. Hansen and A. Ghodousi, *Org. Biomol. Chem.*, 2010, **8**, 1577.
10 (*a*) M. Lynch, S. Hehir, P. Kavanagh, D. Leech, J. O'Shaughnessy, M. P. Carty and F. Aldabbagh, *Chem.–Eur. J.*, 2007, **13**, 3218; (*b*) S. Hehir, L. O' Donovan, M. P. Carty and F. Aldabbagh, *Tetrahedron*, 2008, **64**, 4196.
11 E. Moriarty, M. Carr, S. Bonham, M. P. Carty and F. Aldabbagh, *Eur. J. Med. Chem.*, 2010, **45**, 3762.
12 K. Fahey, L. O'Donovan, M. Carr, M. P. Carty and F. Aldabbagh, *Eur. J. Med. Chem.*, 2010, **45**, 1873.
13 (*a*) W. G. Schulz and E. B. Skibo, *J. Med. Chem.*, 2000, **43**, 629; (*b*) A. Suleman and E. B. Skibo, *J. Med. Chem.*, 2002, **45**, 1211.
14 V. Fagan, S. Bonham, M. P. Carty and F. Aldabbagh, *Org. Biomol. Chem.*, 2010, **8**, 3149.
15 C. M. Ahn, S. K. Kim and J. L. Han, *Arch. Pharmacal Res.*, 1998, **21**, 599.
16 L. O-Donovan, M. P. Carty and F. Aldabbagh, *Chem. Commun.*, 2008, 5592.
17 E. Vedejs, A. Klapars, D. L. Warner and A. H. Weiss, *J. Org. Chem.*, 2001, **66**, 7542.
18 J. Jurczak, D. Gryko, E. Kobrzycka, H. Gruza and P. Prokopowicz, *Tetrahedron*, 1998, **54**, 6051.
19 E. Vedejs, J. D. Little and L. M. Seaney, *J. Org. Chem.*, 2004, **69**, 1788.
20 L.-L. Gundersen, T. Benneche and K. Undheim, *Acta Chem. Scand.*, 1989, **43**, 706.
21 (*a*) E. Vedejs and J. Little, *J. Am. Chem. Soc.*, 2002, **124**, 748; (*b*) E. Vedejs and J. D. Little, *J. Org. Chem.*, 2004, **69**, 1794; (*c*) M. Kim and E. Vedejs, *J. Org. Chem.*, 2004, **69**, 7262.
22 (*a*) W. B. Motherwell and A. M. K. Pennell, *J. Chem. Soc., Chem. Commun.*, 1991, 877; (*b*) S. Caddick, K. Aboutayab, K. Jenkins and R. I. West, *J. Chem. Soc., Perkin Trans. 1*, 1996, 675; (*c*) S. Caddick, C. L. Shering and S. N. Wadman, *Tetrahedron*, 2000, **56**, 465; (*d*) W. B. Motherwell and S. Vázquez, *Tetrahedron Lett.*, 2000, **41**, 9667; (*e*) S.

M. Allin, W. R. Bowman, R. Karim and S. S. Rahman, *Tetrahedron*, 2006, **62**, 4306.
23 F. Aldabbagh and W. R. Bowman, *Tetrahedron*, 1999, **55**, 4109.
24 T. Mosmann, *J. Immunol. Methods*, 1983, **65**, 55.
25 (*a*) V. N. Iyer and W. Szybalski, *Science*, 1964, **145**, 55; (*b*) M. Tomasz, R. Lipman, D. Chowdary, J. Pawlak, G. L. Verdine and K. Nakanishi, *Science*, 1987, **235**, 1204.
26 (*a*) V.-S. Li, D. Choi, M.-S. Tang and H. Kohn, *J. Am. Chem. Soc.*, 1996, **118**, 3765; (*b*) E. Vedejs, B. N. Naidu, A. Klapars, D. L. Warner, V.-S. Li, Y. Na and H. Kohn, *J. Am. Chem. Soc.*, 2003, **125**, 15796.
27 (*a*) L. M. Harwood, *Aldrichim. Acta*, 1985, **18**, 25; (*b*) D. S. Pedersen and C. Rosenbohm, *Synthesis*, 2001, 2431.
28 J.-M. Lee, H.-S. Lim and S.-K. Chung, *Tetrahedron: Asymmetry*, 2002, **13**, 343.

