First synthesis of *N*-[(aziridin-2-yl)methyl]benzimidazolequinone and analysis of toxicity towards normal and Fanconi anemia cells†

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A diazole is N-substituted with 1-trityl-2-methylaziridine and demethylated and oxidised with NBS under acidic conditions to give a benzimidazolequinone; this novel anti-tumour agent is marginally more cytotoxic than mitomycin C (MMC) towards the normal human fibroblast cell line GM00637, while the MMC-hypersensitive human Fanconi anaemia (FA) cell line, PD20i, lacking the FANCD2 protein, is also hypersensitive to the benzimidazolequinone, with expression of FANCD2 protein decreasing sensitivity to both MMC and the benzimidazolequinone.

Many bioreductive antitumour agents utilise a quinone for reductive activation and a strained aziridine ring as a DNA-alkylating center.[1,2] This includes mitomycin C (MMC, Fig. 1) the naturally occurring prototype bioreductive antitumour agent.[1–3] Cytotoxicity is known to be initiated by enzymatic reduction giving rise to electrophilic sites at C1 and C10 due to respective aziridine ring-opening and carbamate elimination. The C1 DNA-alkylation always precedes reaction at C10, resulting in inter- and intrastrand crosslinks that prevent DNA replication.[4]

Skibo and co-workers introduced pyrrolo[1,2-*a*]benzimidazoles (PBI) as bioreductive antitumour agents, and an alternative hydrolytic strand cleavage mechanism was reported *via* nucleophilic attack by the DNA phosphate to open the aziridine of PBI.[5] In contrast to MMC, human cancer cell lines were found to be more sensitive than mouse cancer cell lines to benzimidazolequinones with aziridine substituted directly onto the quinone.[6] Other cytotoxic benzimidazolequinones without aziridine exist,[7–11] including [1,2-*a*] alicyclic ring fused benzimidazolequinones containing an additional fused cyclopropane ring **1**.[9–11] It is speculated that, upon single electron reductive activation, ring-opening of the cyclopropane produces a highly reactive cyclopropyl radical capable of hydrogen abstraction from DNA leading to strand cleavage.[2,10,12] However, benzimidazoles and the corresponding benzimidazolequinones N-substituted with 1-trityl-2-methylaziridines (in order to impart DNA-alkylating ability) are an unknown class of compounds. We now report the synthesis of *N*-[(1-tritylaziridin-(2*S*)-yl)methyl]-1*H*-benzimidazole-4,7-dione **2** and compare its cytotoxicity with the clinically used drug MMC. Cells from Fanconi anemia (FA) patients are known to be hypersensitive to killing by DNA-crosslinking agents such as MMC.[13] We therefore compared the cytotoxic effects of MMC and **2** towards FA cell line, PD20i, lacking FANCD2 protein,[14] as well investigating the effect of FANCD2 protein expression on the cellular sensitivity towards MMC and **2**.

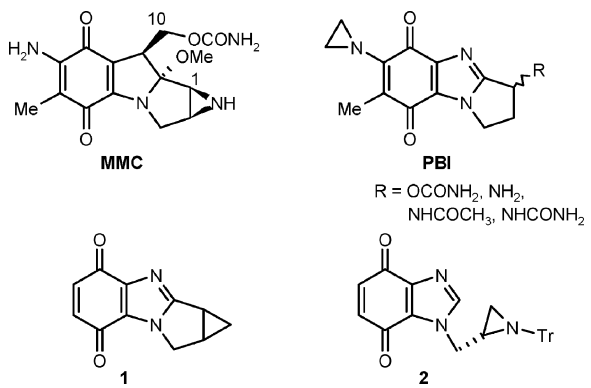
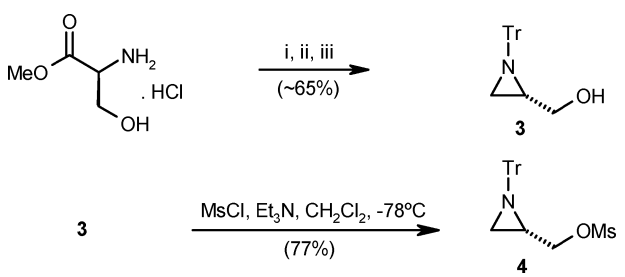


Fig. 1

The aziridine fragment originated from commercial (*S*)-serine methyl ester hydrochloride, which was used to prepare (2*S*)-1-tritylaziridine-2-methanol **3** using three literature synthetic steps[15–18] in an overall ~65% yield (Scheme 1). The reaction of the alcohol **3** with triethylamine and methanesulfonyl chloride gave the novel mesylate **4** in 77% yield. Treatment of 4,7-dimethoxybenzimidazole[19] with sodium hydride followed by **4** in DMF gave 4,7-dimethoxy-*N*-[(1-tritylaziridin-(2*S*)-yl)methyl]-1*H*-benzimidazole **5** in 80% yield (Scheme 2). We believe that this is the first time that *N*-diazole nucleophiles have been used to substitute at the methyl carbon of 2-methyl aziridines.

As perhaps expected, conversion of the 4,7-dimethoxy substituents of **5** into the target quinone **2**, while maintaining the integrity of the fragile aziridine moiety proved challenging



Scheme 1 (i) TrCl, Et_3N, CH_2Cl_2, 0 °C,[15] (ii) SO_2Cl_2, Et_3N, Tol, −50 °C,[16] (iii) $LiAlH_4$, THF, −20 °C,[17] or DIBAL, Tol, −78 °C.[18]

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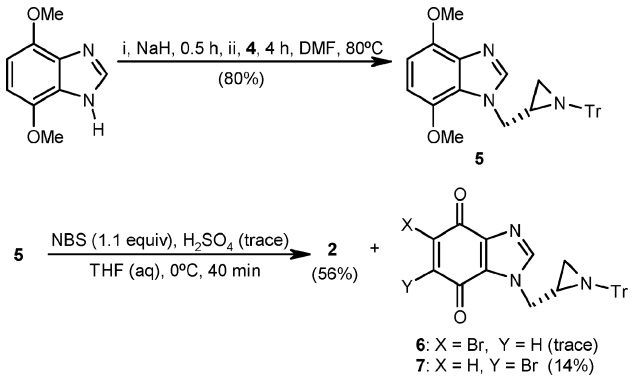
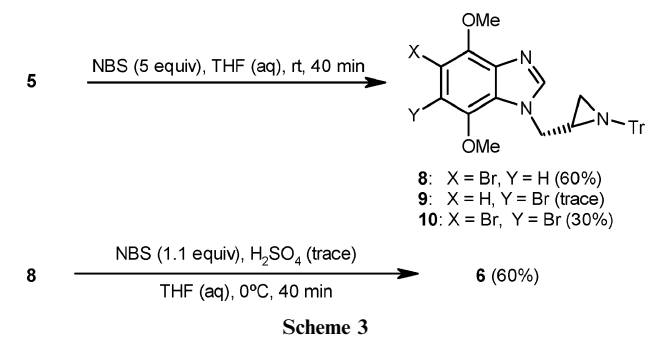
† Electronic supplementary information (ESI) available. Synthetic and biological procedures, and NMR spectra for compounds **2**, and **4**–**10**. See DOI: 10.1039/b814706j



Scheme 2

initially. Hydrobromic acid induced demethylation of **5** followed by room temperature oxidation with ferric chloride of the *in situ* formed hydroquinone, as successfully utilised in our [1,2-*a*] alicyclic ring fused benzimidazolequinone syntheses (including in the synthesis of **1**)[9,10] resulted in the opening of the aziridine. The aziridine of **5** equally did not survive oxidative-demethylation attempts using cerium(IV) ammonium nitrate (CAN)[7] or hypervalent iodine(III) reagents.[20] This led us to the procedure reported by Chi and co-workers[21] for the facile room temperature conversion of fused 1,4-dimethoxybenzenes and 5,8-dimethoxy-2-methylquinoline to the respective quinones. To our delight, this procedure of *N*-bromosuccinimide (NBS, 1.1 equiv.) and a catalytic amount of H_2SO_4 in aqueous THF resulted in the isolation of **2** in 56% yield with minor amounts of 5-bromo and 6-bromo-*N*-[(1-tritylaziridin-(2*S*)-yl)methyl]-1*H*-benzimidazole-4,7-diones (**6** and **7**, respectively)[22] separated after column chromatography (Scheme 2). Less than full equivalents of NBS per OMe of **5** are required for oxidative-demethylation,[21] as the electrophilic bromide supplied by NBS is required to substitute at only one OMe position in order to facilitate hydrolytic-demethylation.

In order to unambiguously confirm the location of the bromine substituent in benzimidazolequinones **6** and **7** (due to very similar spectroscopic data of these isomers[22]), we treated **5** with an excess of NBS (5 equiv.) in aqueous THF in the absence of H_2SO_4 in order to favour the bromination reaction.[21] This gave 5-bromo-4,7-dimethoxy-*N*-[(1-tritylaziridin-(2*S*)-yl)methyl]-1*H*-benzimidazole **8** in 60% yield, with trace amounts of the 6-bromo isomer **9** and 5,6-dibrominated benzimidazole **10** isolated in 30% yield (Scheme 3). Isomers **8** and **9** were principally distinguished by ^{1}H NMR spectroscopy using the upfield shift of the 6-H of **8** at 6.72 ppm relative to



Scheme 3

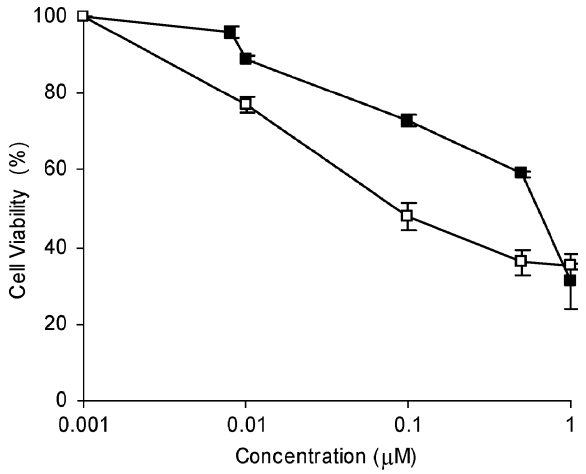


Fig. 2 Viability of normal human skin fibroblast cells (GM00637) determined using the MTT assay following treatment with MMC (■) and benzimidazolequinone **2** (□) for 24 h at 37°C (logarithmic scale). Each data point is the mean of at least three independent experiments. IC_{50}, the drug concentration required to reduce viability to 50%, was calculated by drawing lines of best fit on linear scale plots.

the 5-H of **9** at 7.10 ppm. The 5-H of **9** would be expected to be relatively downfield due to it being in closer proximity to the more electronegative pyridine-like N-3 of imidazole compared to the 6-H of **8**.[11,23]

Purified 5-bromo isomer **8** was then readily oxidised to benzimidazolequinone **6** using NBS under acidic conditions (Scheme 3). This indicated that quinone formation occurred prior to bromination in Scheme 2, seemingly due to the benzimidazolequinone **2** and 4,7-dimethoxy precursor **5** being predominantly brominated at different positions by NBS.

The cytotoxicity of synthetic target **2** was first measured using the MTT ((3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium

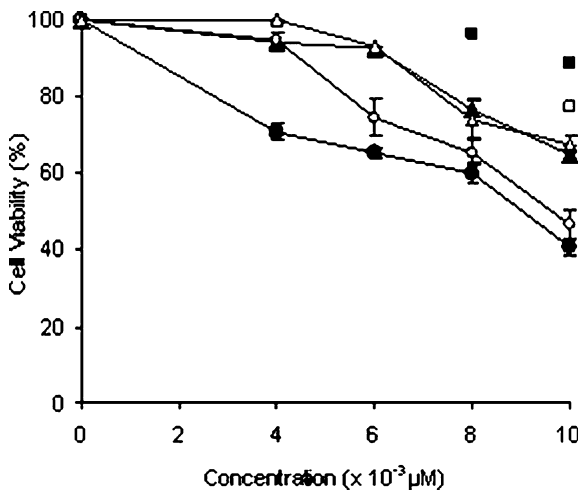


Fig. 3 Viability of PD20i (●, ○) and PD20:RV (▲, △) cells determined using the MTT assay to measure the effect of FANCD2 expression on cell viability following treatment with MMC (closed symbols) and benzimidazolequinone **2** (open symbols) for 24 h at 37°C. For comparison, the viability of human skin fibroblast (GM00637) (■, □ from Fig. 2) is also shown. Each data point is the mean of at least three independent experiments.

bromide) assay[10,11,24] following treatment of the normal human skin fibroblast cell line, GM00637, with **2** and MMC in parallel. MMC acts as a positive control for cytotoxicity in the MTT assay. Benzimidazolequinone **2** was found to be marginally more cytotoxic than MMC, with IC_{50} = 0.5 μM for **2** and 0.8 μM for MMC (Fig. 2).

FA is a rare human genetic disease, characterised by an increased incidence of cancer in early adulthood. FA cells are characterised by a hypersensitivity to agents that induce cross-links in DNA, in particular MMC.[13] FA cells are mutant in one of a number of genes encoding proteins in the FANC multiprotein complex, which plays a key role in processing MMC-induced DNA damage.[13] The human FA fibroblast cell line (PD20i) that lacks the FANCD2 protein and, as a control, an isogenic cell line (PD20:RV) expressing wild-type FANCD2 protein from an inserted transgene[14] were treated with benzimidazolequinone **2** and MMC in parallel (Fig. 3). MMC and **2** exhibited cytotoxicity in the nanomolar range (10^{-9} M) towards the FA (PD20i) cell line, which lacks the FANCD2 protein. PD20:RV cells, which express FANCD2, were found to be less sensitive to both MMC and **2** (Fig. 3).

The observations that MMC-sensitive FANCD2-deficient cells are also more sensitive to **2**, and that expression of wild-type FANCD2 protein partially corrects the cellular sensitivity to both MMC and **2**, provide evidence that DNA damage induction and the FANC pathway are important in the cytotoxicity of **2**. As there is only one position for DNA-alkylation (at the aziridine), the formation of crosslinks is not possible with **2**; this indicates that other forms of DNA damage may be involved in this response.

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22 Isomers may be tentatively assigned using NMR spectroscopy: 5-bromo-*N*-[(1-tritylaziridin-(2*S*)-yl)methyl]-1*H*-benzimidazole-4,7-dione **6**: ^{1}H NMR: δ 7.10, Ar-6-H; ^{13}C NMR: δ 137.1, Ar-6-CH; 6-bromo-*N*-[(1-tritylaziridin-(2*S*)-yl)methyl]-1*H*-benzimidazole-4,7-dione **7**: ^{1}H NMR: δ 7.16–7.26 (within), Ar-5-H; ^{13}C NMR: δ 137.7, Ar-5-CH.

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