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Synthesis by Radical Cyclization and Cytotoxicity of Highly Potent Bioreductive Alicyclic Ring Fused [1,2-*a*]Benzimidazolequinones

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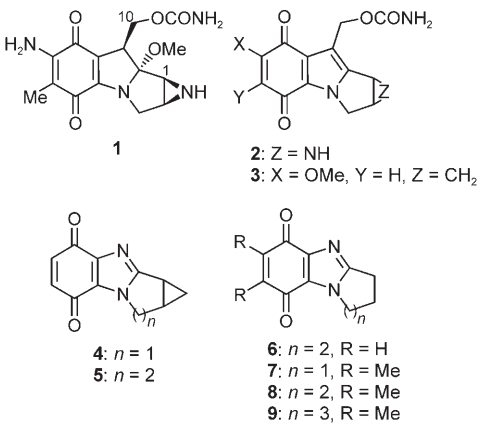
Abstract: The key step in the synthesis of new five, six and seven-membered alicyclic ring [1,2-*a*]-fused bioreductive benzimidazolequinones was radical cyclisation. Six and seven-membered tributyltin hydride-mediated homolytic aromatic substitutions of nucleophilic *N*-alkyl radicals onto the benzimidazole-2-position occurred in high yields (63–70%) when quaternising the pyridine-like 3-*N* of imidazole with camphorsulfonic acid and using large excesses of the azo-initiator, 1,1′-azobis-(cyclohexanecarbonitrile), to supplement the non-chain reaction. Elaboration of benzimidazoles to the benzimidazolequinones occurred in excellent yields. The IC_{50} values for the cytotoxicity of benzimidazolequinones towards the human skin fibroblast cell line GM00637 were in the nanomolar range, as determined by using the MTT assay. The benzimidazolequinones were much more cytotoxic than indolequinone analogues. 1,2,3,4-Tetrahydropyrido[1,2-*a*]benzimidazole-6,9-dione was the most potent compound prepared being more than 300 times more cytotoxic than the clinically used bioreductive drug, mitomycin C. The latter benzimidazolequinone was more potent under hypoxic conditions (associated with solid tumors), being 4.4 times more cytotoxic than under aerobic conditions, while mitomycin C was 1.8 times more selective towards hypoxia. The cyclopropane fused pyrido[1,2-*a*]benzimidazolequinone, 1a,2,3,9b-tetrahydro-1*H*-cyclopropa-[3,4]pyrido[1,2-*a*]benzimidazole-5,8-dione was less cytotoxic and selective than the five-membered ring analogue, 1,1a,8,8a-tetrahydrocyclopropa-[3,4]pyrrolo[1,2-*a*]benzimidazole-3,6-dione. Modifying the structure of the most potent pyrido[1,2-*a*]benzimidazolequinone by attaching methyl substituents onto the quinone moiety increased reductive potentials and decreased cytotoxicity and selectivity towards hypoxia.

Keywords: antitumor agents · benzimidazolequinones · radicals · synthetic methods

Introduction

O_2-deficient (anaerobic) hypoxic cells prevalent in solid tumors may be selectively targeted by using bioreductive compounds.[1] Quinone is the structural moiety commonly associated with reductive activation, and is present in many bioreductive compounds.[2] The naturally occurring indolequinone, mitomycin C (**1**) is the archetypical clinically used bioreductive antitumor agent. Mitomycin C owes its cytotoxicity to DNA-alkylation initiated by a single (SET) or



1

2: Z = NH
3: X = OMe, Y = H, Z = CH_2

4: *n* = 1
5: *n* = 2

6: *n* = 2, R = H
7: *n* = 1, R = Me
8: *n* = 2, R = Me
9: *n* = 3, R = Me

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two-electron transfer, to give the semiquinone radical anion or the hydroquinone, respectively. Although several enzymes can reduce **1**, evidence indicates that the one-electron reducing enzyme NADPH-cytochrome *c* reductase is involved under hypoxic conditions. The reversibility of the SET activation by oxygen has allowed researchers to use bioreductive compounds including prodrugs to selectively target hypoxia.[1–6] Alternatively, **1** can act as a substrate for the obligate and non-reversible two-electron reduction mediated by the enzyme DT-diaphorase.[2]

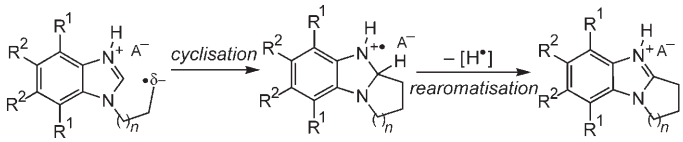
In organic solvents, the reduction of **1**, aziridinomitosene (**2**) and synthetic analogues, such as cyclopropamitosene **3**, is known to proceed by the reversible SET process.[1–3] In the case of **1**, under aqueous conditions, both one and two-electron reductions are thought to provide similar DNA alkylation pathways, and rapid disproportionation of the mitomycin semiquinone into the quinone and hydroquinone under anaerobic conditions indicated that the hydroquinone is the common intermediate.[7] Moreover many bioreductive compounds are known to be toxic under oxygenated conditions, due to either direct or indirect enzymatic reduction to the hydroquinone or the formation of reactive oxygen species, such as superoxide ($O_2^{\bullet-}$), the product of the reaction of the semiquinone radical anion with molecular oxygen.[1,2]

Moody and co-workers related selectivity of indolequinones to redox potentials obtained by using cyclic voltammetry.[3b] Indolequinone **3** was shown to have a lower reductive potential in DMF (−1.395 V versus Ferrocene, Fc) than **1** (−1.421 V versus Fc) and greater cytotoxicity and selectivity towards hypoxia. Cyclopropamitosene **3** was reported to be 34 times more cytotoxic under hypoxic than oxygenated conditions, while **1** was only twice more cytotoxic under hypoxic conditions.[3] This is despite **3** not being capable of alkylating DNA at C-1, as the aziridine is replaced by a cyclopropane ring. The formation of the activated semiquinone radical anion of **3** was speculated to induce radical ring opening of the cyclopropane ring to give a highly reactive radical capable of a cytotoxic effect through hydrogen abstraction from DNA leading to strand cleavage.[3,4] Chemical evidence of cyclopropane ring opening was presented under oxidative conditions for other cyclopropyl indolequinones,[4] and Naylor et al.[5] showed that the 2-cyclopropyl substituent in indolequinones was up to 2 orders of magnitude more effective towards hypoxia than the 2-isopropyl substituent.

More recently, we have reported the synthesis of new tetracyclic ring systems, pyrrolo and pyrido[1,2-*a*]benzimidazolequinones **4** and **5** containing a fused cyclopropane ring prepared by means of cycloaddition reactions induced by thermolysis of Eschenmoser hydrazones.[8] Cyclopropa-[3,4]pyrrolo[1,2-*a*]benzimidazole-3,6-dione (**4**) was considered to be a diazole analogue of **3**. Benzimidazolequinone **4** (−1.052 V versus Fc) and cyclopropa[3,4]pyrido[1,2-*a*]benzimidazole-5,8-dione (**5**) (−1.074 V, versus Fc) were found to have lower reductive potentials than **1** and **3** under analogous cyclic voltammetry conditions, which we anticipated may lead to greater cytotoxicity and selectivity towards hypoxia. Other benzimidazolequinones possessing potent anti-tumour activity exist,[9–11] but such compounds are excellent substrates for DT-diaphorase and are not necessarily selective towards hypoxia.[9,10]

We now report the synthesis of novel benzimidazolequinones **6–9** containing [1,2-*a*]-fused five-, six- and seven-membered alicyclic rings, but minus the cyclopropane ring. This allows us to assess the influence of the fused cyclopropane and different sized [1,2-*a*] alicyclic rings on cytotoxicity. The cytotoxicity of the dimethyl-substituted pyrrolo[1,2-*a*]benzimidazole-5,8-dione **7** and azepino[1,2-*a*]benzimidazole-1,4-dione **9** will be presented in a subsequent paper.[12] In the present paper, the effect of a five compared to a six-membered fused [1,2-*a*] alicyclic ring on cytotoxicity is assessed by using tetracycles **4** and **5**, while the activity of the cyclopropane ring is assessed by comparing the cytotoxicity of **5** with **6**. The influence of the quinone dimethyl substituents on reductive potentials and cytotoxicity is also evaluated by comparing the unsubstituted pyrido[1,2-*a*]benzimidazole-6,9-dione **6** with the 7,8-dimethyl-substituted analogue **8**. Cytotoxicity is measured by using the MTT assay on human skin fibroblast cells after treatment with benzimidazolequinones with selectivity deduced from assays under aerobic and hypoxic conditions.

The key step in the synthesis of **6–9** is tributyltin hydride (Bu_3SnH)-mediated radical cyclisations of nucleophilic *N*-alkyl radicals onto the benzimidazole 2-position activated by quaternising the pyridine-like 3-N of imidazole with camphorsulfonic acid (CSA) (Scheme 1). The addition of CSA



Scheme 1. Intramolecular homolytic aromatic substitution mechanism, where R^1=OMe, R^2=H, n = 2 for synthesis of **6** and R^1=H, R^2=Me, n = 1, 2, 3, respectively, for synthesis of **7–9** and AH=CSA.

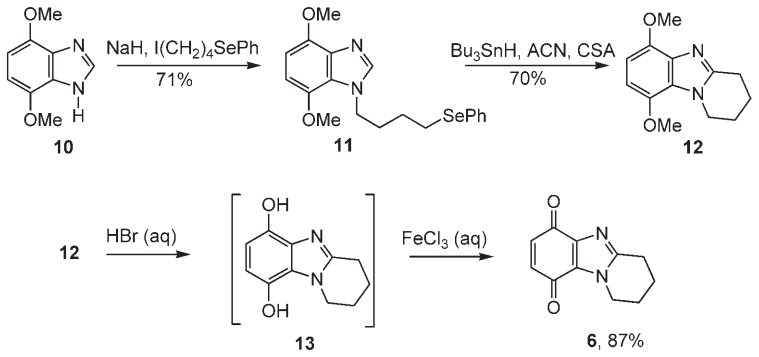
to protonate the basic imidazole 3-N to facilitate intramolecular 1,2-conjugate additions of alkyl radicals was first reported by Gagosz and Zard for xanthate precursors by using a peroxide initiator.[13] However, the radical cyclisations herein are non-chain "oxidative" homolytic aromatic substitutions carried out in the presence of the "reductant" Bu_3SnH.[14–16] The abstraction of the hydrogen atom or the oxidation to regenerate aromaticity is now understood to be carried out by the azo-initiator and/or derived radical.[14,15] Thus, such reactions should proceed in higher yields with excess azo-initiator added, as the latter will provide radicals for initiation as well as oxidative rearomatisation.

The following paper gives efficient preparations of a new series of benzimidazolequinones containing [1,2-*a*]-fused alicyclic rings (compounds **6–9**), prepared by means of homolytic aromatic substitutions onto the benzimidazole 2-position. Structure activity/relationships are presented for benzi-

midazolequinones **4**–**5**, **6** and **8**. This is part of our quest to find more selective and potent antitumor agents.

Results and Discussion

Synthesis benzimidazolequinones 6–9: The synthesis of **6** began with the preparation of 4,7-dimethoxy-1*H*-benzimidazole (**10**) from the condensation of 3,6-dimethoxybenzene-1,2-diamine with formic acid.[17] Benzimidazole **10** was alkylated by using less than one equivalent (0.75 equiv) of 1-iodo-4-(phenylselanyl)butane and sodium hydride to avoid dialkylation of the imidazole ring, giving radical precursor **11** in 71% yield (Scheme 2). Phenylselanides were used in place of alkyl bromides and iodides due to their efficient radical, but poor S_N2 leaving group ability enabling facile preparation of diazole radical precursors.[18,19]



Scheme 2. Preparation of target **6**.

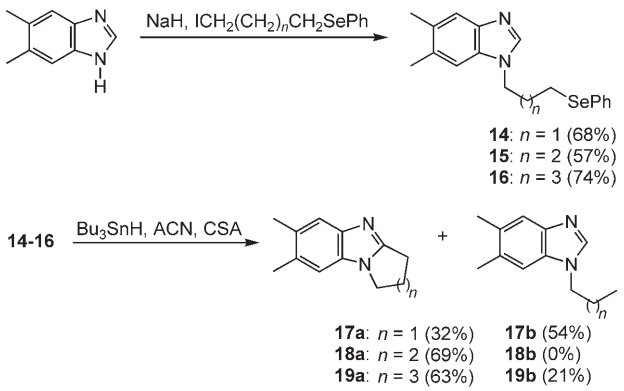
Radical cyclisation to give pyrido[1,2-*a*]benzimidazole **12** was carried out over 18 hours by using syringe-pump addition of Bu_3SnH (2.5 equiv) and 1,1′-azobis(cyclohexanecarbonitrile) (ACN, 4.0 equiv) to a solution of **11** (1 equiv) and CSA (1 equiv) heated under reflux in toluene. The addition of further portions of ACN (1.5 equiv) during the reaction was found to further improve yields of cyclisation products. Furthermore, ACN was found to give higher product yields than the azo-initiator AIBN (2,2′-azobis(isobutyronitrile)) used under the same reaction conditions, presumably because of its longer half-life allowing continual and slow generation of initiating radicals as required by the non-chain mechanism of the reaction (ACN, $t_{1/2} \approx 40$ min, AIBN, $t_{1/2} <$ 2 min in toluene at 110 °C[20]).

Despite the requirement for excess azo-initiator, the cyclisations described in this paper have several advantages over the synthetic protocol described by Aldabbagh and Bowman to access [1,2-*a*] alicyclic ring fused benzimidazoles by intramolecular *N*-(*ω*-alkyl) radical *ipso*-substitutions of the 2-phenylsulfanyl group.[18] Firstly, cyclisation yields are generally improved, secondly there is no requirement for the synthetic steps used to prepare the intermediate 2-(phenylsulfanyl)benzimidazole containing the necessary leaving group for *ipso*-substitution and thirdly the use of malodorous thiol compounds is avoided. Bowman's group have recently extended the *ipso*-substitution protocol to 2-(phenylsulfanyl)-benzimidazole radical precursors attached to solid-phase resins, and given an alternative synthesis of the precursors from 2-chlorobenzimidazole and benzenethiols.[19]

Purification by using KF/silica-gel column chromatography[21a] (later discussion) was found to be less successful for purification of **12**, and the Corey procedure of CsF/CsOH (an anhydrous fluoride source) on silica gel was not attempted.[21b] We found that tin residues were efficiently eliminated by repeated addition of KF to the acidic imidazolium salt solution with subsequent filtration of insoluble Bu_3SnF.[22,23] The basified free imidazole was purified by column chromatography to give **12** in 70% yield with all traces of alkyltin impurities eliminated, as indicated by analysis of ^1H and ^{13}C NMR spectra (Supporting Information), and supported by clean NMR spectra and elemental analysis (within 0.2% of the calculated C, H, N) for target quinone **6**. It is interesting that the dimethoxy substituents seem not to impart electronic conjugative donation effects onto the imidazole-2-position of **11** to decrease the efficiency of the cyclisation, as no alkyl radical reduction product was observed from this reaction.

The dimethoxybenzimidazole **12** was converted to quinone **6** in high yield (87%) by using hydrobromic acid-induced demethylation to give the hydroquinone **13** in situ, which underwent facile oxidation at room temperature with ferric chloride.[8] Quinones **6**–**9** were all found to be stable to repeated purification by chromatography, and could be stored in a freezer without any decomposition observed over periods of months.

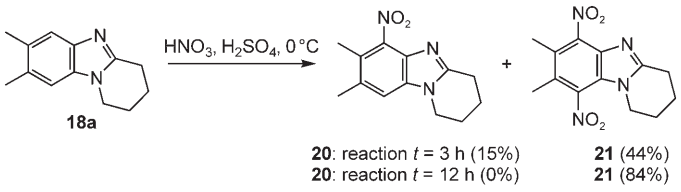
The synthesis of benzimidazolequinones **7**–**9** began with N-alkylation of commercially available 5,6-dimethylbenzimidazole with 1-iodo-*ω*-(phenylselanyl)alkanes to give radical precursors **14**–**16** in 57–74% yield (Scheme 3). Radical cycli-



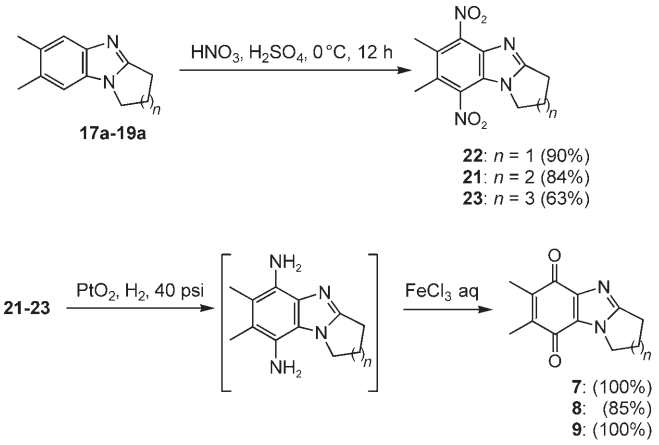
Scheme 3. Preparation of radical precursors and cyclisations towards the synthesis of targets **7**–**9**.

sations using Bu_3SnH and ACN were performed under identical conditions to the reaction that gave **12**. The six-membered cyclisation gave the highest yield of cyclised product, giving pyrido[1,2-*a*]benzimidazole **18a** in 69% yield without any radical reduction detected. The more difficult five- and seven-membered cyclisations both gave radical reduction products **17b** and **19b** in yields of 54 and 21%, as well as cyclisation products **17a** and **19a** in yields of 32 and 63%, respectively. Recovery yields of imidazole products were good, and separation of cyclised from reduced products and alkyltin residues was efficiently achieved. This was carried out by using the direct column chromatography procedure devised by Harrowven and Guy[21a] by purification of the concentrated reaction mixture using gradient elution on 10% w/w of finely ground KF and 90% silica. The latter procedure was an improvement on the cumbersome workup procedure of repeated washing of the acidic imidazolium salts with petrol to remove tin residues[18,19] and resulted in improved overall recovery yields of diazoles (≈70–85%). The yield of azepino[1,2-*a*]benzimidazole **19a** was particularly impressive, as the equivalent seven-membered cyclisations by the *ipso*-substitution protocol only occurred in yields of <17% for radical precursors without functionality on the fused benzene ring.[18,19] The low yield of the five-membered cyclisation product **17a** was consistent with similar literature radical reactions, and was presumably due to strain associated with forming pyrrolo[1,2-*a*]benzimidazole ring system.[16,18,19] Under non-reducing conditions, the equivalent five-membered cyclisation proceeded in 37–57% yield by using xanthate radical precursors.[13]

The incorporation of methyl substituents on the fused benzene part enabled conversion of cyclised compounds **17a**–**19a** to quinones **7**–**9** by use of a nitration, reduction and oxidation sequence.[24] Nitration was carried out by stirring **17a**–**19a** at 0°C in a 50:50 mixture of concentrated sulfuric and fuming nitric acid (Schemes 4 and 5). The first nitration carried out was on **18a**, which resulted in a mixture of the mono and dinitrated derivatives **20** and **21** in 15 and 44% yields, respectively (Scheme 4). This indicated that nitration occurred first at the 6-position on the fused benzene ring of **18a**, as indicated by isolation of **20**, and supported by the ^{13}C NMR spectra. Analogous selectivity in nitration occurred by using 6-bromo-7-methyl-2,3-dihydro-1*H*-pyrrolo[1,2-*a*]benzimidazole.[9] Although, **20** could probably be transformed to the quinone **8**, we decided to extend the reaction time from 3 to 12 h to enable a more efficient approach towards **8**. This gave 6,9-dinitro analogue **21** as the exclusive product in 84% yield. Thus, we decided to use the longer reaction time to nitrate **17a** and **19a** to exclusively give dinitro compounds **22** and **23** in 90 and 63% yield, respectively (Scheme 5). Hydrogenation in the presence of Adam's catalyst (PtO_2) in ethanol at 40 psi gave the in situ diamines that were readily oxidized by using a solution of ferric chloride at room temperature to give the target quinones **7**–**9** in high or quantitative yields.



Scheme 4. Attempted nitrations of **18a**.



Scheme 5. Preparation of targets **7**–**9**.

Cytotoxicity of benzimidazolequinones 4, 5, 6 and 8: By using the MTT assay we have found that the treatment of human skin fibroblast GM00637 cells with up to 0.005 μM of cyclopropane fused compounds **4** and **5** under aerobic conditions resulted in a similar effect (Figure 1). Under hypoxic conditions, both **4** and **5** showed greater potency resulting in hypoxic cytotoxicity ratios (HCR),[4] or efficiency towards hypoxia, of 2.9 and 1.2, respectively (Table 1). However, **4** and **5** (as well as **6** and **8**) were significantly more toxic regardless of the environment than **1**. The cytotoxicity of **1** was measured by using the same assay as for the benzimidazolequinones and the IC_{50} values for **1** (Table 1 and Supporting Information) are similar to those reported in the literature using Chinese hamster V79 cells, with an equivalent

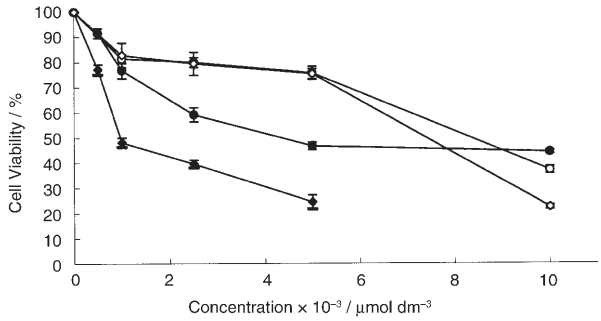


Figure 1. Assessing the effect of the [1,2-*a*] alicyclic ring on cytotoxicity: MTT assay of human skin fibroblast cells following treatment with cyclopropapyrrolo[1,2-*a*]benzimidazolequinone **4** (◇, ◆) and cyclopropapyrido[1,2-*a*]benzimidazolequinone **5** (○, ●) under aerobic (open symbols) and hypoxic (filled symbols) conditions for 24 h at 37°C, respectively.

Table 1. Reductive potentials and cytotoxicity.[a]

Compound	E_{redox} [V] versus Fc	IC_{50}(air) [μmol dm^{-3}]	$IC_{50}(N_2)$ [μmol dm^{-3}]	HCR
1	−1.421[3b]	0.9	0.5	1.8
4	−1.052[8]	0.0069	0.0024	2.9
5	−1.074[8]	0.0083	0.0070	1.2
6	−1.080	0.0070	0.0016	4.4
8	−1.168	0.0076	0.0026	2.9

[a] Reductive potentials were obtained by using cyclic voltammetry by dissolving compounds in DMF, containing 0.1 M tetrabutylammoniumperchlorate as electrolyte and 1 mM ferrocene (Fc) as reference. E_{redox} (± 0.010 V) calculated as $(E_{pc}+E_{pa})/2$ from 100 mV s^{-1} cyclic voltammograms. E_{pc} = cathodic peak potential, E_{pa} = anodic peak potential. Cytotoxicity was measured by using the MTT assay on human skin fibroblast cells (GM00637). IC_{50} is the concentration required to reduce viability by 50% and it was obtained by drawing lines of best fit. Hypoxic cytotoxicity ratios (HCR) were defined as the IC_{50} value for aerobic conditions divided by the IC_{50} value for hypoxic conditions.

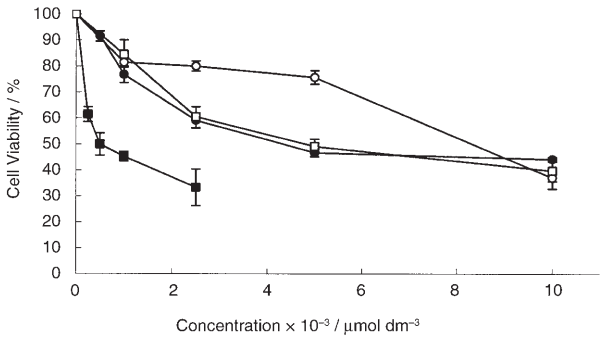


Figure 2. Assessing the effect of the fused cyclopropane ring on the cytotoxicity of **5**: MTT assay of human skin fibroblast cells following treatment with cyclopropapyrido[1,2-*a*]benzimidazolequinone **5** (○, ●) and pyrido[1,2-*a*]benzimidazolequinone **6** (□, ■) under aerobic (open symbols) and hypoxic (filled symbols) conditions for 24 h at 37 °C, respectively.

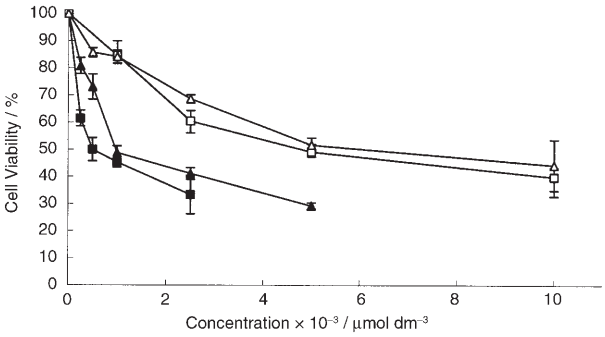


Figure 3. Assessing the effect of the 7,8-dimethyl substituents on cytotoxicity of **8**: MTT assay of human skin fibroblast cells following treatment with pyrido[1,2-*a*]benzimidazolequinones **6** (□, ■) and **8** (△, ▲) under aerobic (open symbols) and hypoxic (filled symbols) conditions for 24 h at 37 °C, respectively.

HCR of ≈2.[3] As cytotoxicity of mitomycin towards GM00637 cells is similar to that reported with V79 cells, this allows us to compare the cytotoxicity of the benzimidazolequinones used in the present study with published IC_{50} values for cyclopropamitosene **3** measured under the same conditions as **1**.[3] Indolequinone **3** has an impressive HCR of 34, and the structurally related benzimidazolequinone **4** is almost 700 and 90 times more potent under respective aerobic and hypoxic conditions.

The greater potency of **4** compared to **5** under hypoxic conditions may be related to the greater strain in the pyrrolo- compared to the pyrido[1,2-*a*]benzimidazolequinone ring system allowing more facile ring opening of the fused cyclopropane ring upon reductive activation. However, Moody and co-workers observed an opposite trend in cytotoxicity when comparing the hydroxymethyl-substituted pyrrolo[1,2-*a*]indolequinone with the pyrido[1,2-*a*]indolequinone containing a fused cyclopropane ring with the latter showing marginally greater cytotoxicity and HCR.[4] The hydroxymethyl-substituted cyclopropane fused indolequinones were also less effective than **3** indicating an involvement of the carbamate (−OCONH$_2$−) substituent in cytotoxicity.[4,6]

To evaluate the role of the cyclopropane ring in the cytotoxicity of the pyrido[1,2-*a*]benzimidazolequinones, the cytotoxicity of **5** was compared with **6** (Figure 2). The pyrido-[1,2-*a*]benzimidazolequinone lacking a cyclopropane ring **6** was found to have a lower IC_{50} value than **5** under aerobic, as well as hypoxic conditions (Table 1). Furthermore, **6** was the most potent and selective of all the benzimidazolequinones tested, being more than 120 and 310 times more cytotoxic under aerobic and hypoxic conditions, respectively, than **1**. Thus, it appears that the additional fused cyclopropane ring is reducing the effectiveness of [1,2-*a*] alicyclic ring-fused benzimidazolequinone **5** to act as a cytotoxin.

The role of the dimethyl substituents was assessed by comparing the cytotoxicity of unsubstituted **6** with 7,8-dimethyl-substituted quinone **8** (Figure 3). The inductive donation of the methyl substituents was expected to increase reductive potential (later discussion), and affect the rate of conversion of the prodrug quinone to the reductively activated biologically active forms. Compounds **6** and **8** exhibited very similar cytotoxicity under aerobic conditions, but **6** was ≈1.6 times more cytotoxic under hypoxic conditions than **8**. HCR values of 4.4 and 2.9 were obtained for **6** and **8**, respectively (Table 1). In the latter cases, increasing the reductive potential seems to slightly decrease cytotoxicity and selectivity toward hypoxic conditions, which is in agreement with general decreases in cytotoxicity with increasing reductive potential observed with large sets of indolequinones.[3b] As the most effective compound **6** lacks DNA damaging functionality (such as a strained three-membered ring) it is speculated that the cytotoxicity of the present benzimidazolequinones may be largely due to reduction–reoxidation cycles in the presence of O_2 generating toxic oxygen species, such as H_2O_2, $O_2^{\bullet-}$ and HO$^{\bullet}$.[7,25] This may explain the low HCR values for the benzimidazolequinones.

Cyclic voltammetry of benzimidazolequinones 6 and 8: This was carried out in DMF rather than in aqueous solution to avoid the complexities of protonation.[3b,7] As only the one and not the two-electron reduction can be reversed by

oxygen, we were only interested in the former as a guide to selectivity toward hypoxia. The one-electron reductive potentials are $E_{redox} = -1.080$ and -1.168 V versus Fc for the unsubstituted pyrido[1,2-*a*]benzimidazolequinone **6** and 7,8-dimethyl analogue **8**, respectively (Table 1). Thus, the inductive effect of the dimethyl substituents increased the E_{redox} of **8** by 88 mV (or 44 mV per methyl group). Compound **6** had a very similar reductive potential to pyrido[1,2-*a*]benzimidazolequinone **5** containing a fused cyclopropane ring. The values of $E_{pc} - E_p/2$ (≈ 0.056 V) obtained at scan rates of < 100 mVs^{-1} for benzimidazolequinones **4**, **5**, **6** and **8** indicated a reversible single-electron transfer with a stable semiquinone radical anion (Supporting Information). However, this does not rule out the disproportionation of the latter to the quinone and hydroquinone under aqueous conditions.[7] Benzimidazolequinones are easier to reduce than the indolequinones due to the additional electronegative pyridine-like nitrogen.

Conclusion

A new series of benzimidazolequinones containing [1,2-*a*]-fused five, six and seven-membered alicyclic rings has been prepared. The key synthetic step involves Bu_3SnH-mediated cyclisations of nucleophilic *N*-alkyl radicals onto the benzimidazole-2-position activated by quaternising the pyridine-like 3-*N* of imidazole with camphorsulfonic acid. The substituted aromatic product is formed upon loss of H$^{\bullet}$, and is an example of a formal oxidation in the presence of the reductant Bu_3SnH. Optimised cyclisation yields required the addition of a large excess azo-initiator, 1,1′-azobis(cyclohexanecarbonitrile) to maintain the non-chain reaction mechanism. The six-membered cyclisations were regioselective giving only the cyclised product in $\approx 70\%$ yield. The five and seven-membered cyclisations of 5,6-dimethyl *N*-[ω-(phenylselanyl)alkyl]benzimidazoles, respectively, gave 32 and 63% yields of cyclised product with 54 and 21% Bu_3SnH reduction products obtained. This new radical cyclisation protocol compares favourably with literature radical cyclisations onto the benzimidazole-2-position.

The IC_{50} values for benzimidazolequinone cytotoxicity towards the human skin fibroblast cell line GM00637 were in the nanomolar (10^{-9}) range, as determined by using the MTT assay. The most potent benzimidazolequinone tested was the six-membered alicyclic ring-fused pyrido[1,2-*a*]benzimidazole-6,9-dione **6**, which was more than 100 times more cytotoxic than the clinically used antitumour drug mitomycin C (**1**) under aerobic conditions, and 300 times more cytotoxic under hypoxic conditions. It was also more selective than **1**, being 4.4 times more cytotoxic under hypoxic than aerobic conditions (the cytotoxicity of **1** doubles under hypoxic conditions). The fused cyclopropane ring of pyrido-[1,2-*a*]benzimidazole-5,8-dione **5** and the 7,8-dimethyl substituents of pyrido[1,2-*a*]benzimidazole-6,9-dione **8** were found to reduce cytotoxicity and selectivity towards hypoxia compared to the pyrido[1,2-*a*]benzimidazole **6** lacking these functional groups. The higher cytotoxicity of the benzimidazolequinones compared to the indolequinones of comparable structure may be related to their lower reductive potentials. Future work will aim to establish precise enzymatic reduction and induction of DNA damage pathways, toxicity toward cancer cell lines and the synthesis of further analogues.

Experimental Section

Materials: All chemicals were purchased from Sigma–Aldrich, and all solvents were purified and dried prior to use according to conventional methods. Monitoring of reactions by TLC was performed by using aluminium-backed plates coated with silica gel (Merck Kieselgel 60 F_{254}). Column chromatography by using silica gel was carried out with Merck Kiesel 60 H silica. All reactions were conducted under a nitrogen atmosphere by using anhydrous solvents apart from those involving aqueous solutions.

Instrumentation: Melting points were measured on a Stuart Scientific melting point apparatus SMP3. Elemental analysis was carried out on a Perkin–Elmer 2400 Series II analyser. IR spectra were acquired by using a Perkin–Elmer Spec 1 with ATR attached.

All NMR spectra were recoded by using $CDCl_3$ as solvent, and tetramethylsilane as internal standard at room temperature. All ^{1}H and ^{13}C NMR spectra were recorded at 400 and 100 MHz, respectively, by using a Jeol GXFT 400 MHz instrument equipped with a DEC AXP 300 computer work station. NMR assignments were supported by HMQC ^{1}H–^{13}C NMR 2D spectra and distortionless enhancement polarization transfer (DEPT).

Low and high resolution electron impact (EI) and chemical ionisation (CI) mass spectra were obtained on a Micro Mass GTC spectrometer. The precision of this method for accurate mass measurement by manual peak matching was better than 5 ppm. EPSRC National Mass Spectrometry Service (University of Swansea) carried out low resolution EI on the Micromass Quattro II triple quadrupole instrument and high-resolution mass spectrometry on the Finnigan MAT 900 XLT by manual peak matching by using Electrospray (ESI) for compounds **6**, **19a**, **23**, **7** and **8**. The precision of this method was better than 3 ppm. Elemental analysis was carried out on a Perkin–Elmer 2400 Series II analyzer.

Absorbance was measured in the MTT assay by using a Wallac Victor2 1420 multilabel Counter (plate reader).

Syntheses: The preparation of 1,1a,8,8a-tetrahydrocyclopropa-[3,4]pyrrolo[1,2-*a*]benzimidazole-3,6-dione (**4**) and 1a,2,3,9b-tetrahydro-1*H*-cyclopropa[3,4]pyrido[1,2-*a*]benzimidazole-5,8-dione (**5**) was described in earlier papers.[8]

4,7-Dimethoxy-1-[4-(phenylseleno)butyl]-1*H*-benzimidazole (11): 4,7-Dimethoxy-1*H*-benzimidazole (**10**) (1.39 g, 7.80 mmol) and sodium hydride (0.25 g, 10.42 mmol) in THF (150 mL) were heated at reflux for 0.5 h. 1-Iodo-4-(phenylselanyl)butane (2.00 g, 5.9 mmol) was added and heating continued for an additional 2.5 h. The reaction was cooled, filtered through Celite and evaporated to dryness. The resultant yellow residue was purified by column chromatography using silica gel as absorbent with gradient elution of hexane and EtOAc to give **11** (1.63 g, 71%) as a yellow oil. $R_f = 0.40$ (EtOAc); ^{1}H NMR: $\delta = 7.63$ (s, 1H; Im-2-H), 7.43 (m, 2H; PhH), 7.22 (m, 3H; PhH), 6.54 (AB_q, $J = 8.5$ Hz, 2H; Ar-5-H, Ar-6-H), 4.31 (t, $J = 7.0$ Hz, 2H; NCH_2), 3.97 (s, 3H; Me), 3.86 (s, 3H; Me), 2.88 (t, $J = 7.1$ Hz, 2H; CH_2SePh), 1.96 (m, 2H; 2′-CH_2), 1.69 ppm (m, 2H; 3′-CH_2); ^{13}C NMR: $\delta = 146.0$ (C), 142.0 (Im-2-CH), 141.6 (C), 136.0 (C), 132.6 (PhCH), 129.6 (C), 129.0 (PhCH), 126.8 (PhCH), 124.6 (C), 103.0 (ArCH), 101.6 (ArCH), 55.9 (Me), 55.5 (Me), 46.3 (NCH_2), 31.5 (CH_2SePh), 27.1 (2′-CH_2), 26.8 ppm (3′-CH_2); IR (neat): $\tilde{\nu} = 963$, 1060, 1093, 1153, 1183, 1226, 1260, 1373, 1490, 1517, 1580, 2840, 2926 cm^{-1}; elemental analysis calcd (%) for $C_{19}H_{22}N_2O_2Se$: C 58.4, H 5.7, N 7.2; found: C 58.5, H 5.7, N 7.1.

6,9-Dimethoxy-1,2,3,4-tetrahydropyrido[1,2-*a*]benzimidazole (12): Bu_3SnH (1.15 g, 3.95 mmol) and ACN (1.54 g, 6.32 mmol) in PhMe (55 mL) were added over 18 h by a syringe pump to **11** (0.62 g, 1.58 mmol) and CSA (0.36 g, 1.58 mmol) in PhMe (95 mL) heated at reflux. Further ACN (0.58 g, 2.37 mmol) in 10 mL of PhMe was divided into three equal portions, and added after 1, 5 and 9 h to the reaction mixture. The reaction was stirred at reflux for an additional 1 h, and cooled. The diazole products were extracted with 2 M HCl (75 mL), and KF was added. The solution was gravity filtered, and further KF was added until no more precipitate of Bu_3SnF was formed. Na_2CO_3 was added to the filtrate until it was pH 8. The free diazole was extracted with $CHCl_3$, dried ($MgSO_4$) and evaporated to dryness and the residue purified by column chromatography using silica gel as absorbent with gradient elution of hexane and EtOAc to give **12** (0.26 g, 70%) as an orange oil. R_f=0.23 (EtOAc); ^{1}H NMR: δ=6.49 (s, 2H; ArH), 4.42 (t, J=5.9 Hz, 2H; 1-CH_2), 3.94 (s, 3H; Me), 3.86 (s, 3H; Me), 3.06 (t, J=6.4 Hz, 2H; 4-CH_2), 2.04, 1.96 ppm (m, 4H; 2-CH_2, 3-CH_2); ^{13}C NMR: δ=150.1 (4a-C), 145.4 (C), 141.8 (C), 134.5 (C), 125.3 (C), 102.2 (ArCH), 101.4 (ArCH), 55.7 (2×Me), 45.3 (1-CH_2), 25.4 (all CH_2), 23.0, 20.4 ppm; IR (neat): $\tilde{\nu}$=1091, 1157, 1193, 1260, 1311, 1402, 1424, 1449, 1523, 2837, 2932 cm^{-1}; HRMS (EI): m/z: calcd for $C_{13}H_{16}N_2O_2$: 232.1212; found: 232.1215 $[M]^+$; HRMS (EI): m/z (%): 232 (80) $[M]^+$, 231 (100), 217 (97), 203 (25).

1,2,3,4-Tetrahydropyrido[1,2-*a*]benzimidazole-6,9-dione (6): Compound **12** (0.57 g, 2.45 mmol) in 48% hydrobromic acid (20 mL) was heated at reflux for 3 h. The reaction was cooled and evaporated to dryness. $FeCl_3$ aq (0.7 M, 20 mL) was added to the residue and stirred at room temperature overnight. The solution was extracted with $CHCl_3$, the combined organic extracts dried ($MgSO_4$) and evaporated to dryness to give a red solid, which was purified by column chromatography using silica gel as absorbent with gradient elution of hexane and EtOAc to yield **6** (0.43 g, 87%) as a yellow solid. R_f=0.26 (EtOAc); m.p. 153–155 °C; ^{1}H NMR: δ=6.60 (AB_q, J=10.5 Hz, 2H; 7-H, 8-H), 4.33 (t, J=6.1 Hz, 2H; 1-CH_2), 3.01 (t, J=6.3 Hz, 2H; 4-CH_2), 2.05, 1.98 ppm (m, 4H; 3-CH_2, 2-CH_2); ^{13}C NMR: δ=181.3 (C=O), 178.3 (C=O), 151.9 (4a-C), 141.4 (C), 136.2 (ArCH), 136.0 (ArCH), 130.1 (C), 45.5 (1-CH_2), 25.0 (all CH_2), 22.3, 19.8 ppm; IR (neat): $\tilde{\nu}$=965, 1035, 1058, 1074, 1157, 1205, 1244, 1302, 1354, 1376, 1409, 1429, 1458, 1483, 2919, 1510, 1590, 1650 cm^{-1} (C=O); HRMS (ESI): m/z: calcd for $C_{11}H_{11}N_2O_2$: 203.0815; found: 203.0818 $[M+H]^+$; elemental analysis calcd (%) for $C_{11}H_{10}N_2O_2$: C 65.1, H 5.0, N 13.8; found: C 65.0, H 4.9, N 13.8.

5,6-Dimethyl-1-[3-(phenylseleno)propyl]-1*H*-benzimidazole (14): 5,6-Dimethyl-1*H*-benzimidazole (1.50 g, 10.26 mmol) and sodium hydride (0.40 g, 16.67 mmol) in DMF (120 mL) were heated at 80 °C for 0.5 h. 1-Iodo-3-phenylselanylpropane (2.69 g, 8.27 mmol) was added and heating continued for an additional 2.5 h. The reaction was cooled, filtered through Celite and evaporated to dryness. The resultant brown residue was purified by column chromatography using silica gel as absorbent with gradient elution of hexane and EtOAc to give **14** (1.93 g, 68%) as a yellow oil. R_f=0.23 (EtOAc); ^{1}H NMR: δ=7.71 (s, 1H; Im-2-H), 7.54 (s, 1H; Ar-4-H), 7.46 (m, 2H; PhH), 7.25 (m, 3H; PhH), 7.10 (s, 1H; Ar-7-H), 4.24 (t, J=6.7 Hz, 2H; NCH_2), 2.80 (t, J=6.8 Hz, 2H; CH_2SePh), 2.36 (s, 6H; Me), 2.20 ppm (m, 2H; 2′-CH_2); ^{13}C NMR: δ=142.7 (C), 142.4 (Im-2-CH), 133.2 (PhCH), 132.2 (C), 131.2 (C), 129.4 (PhCH), 129.1 (C), 127.5 (PhCH), 120.5 (Ar-4-CH), 109.9 (Ar-7-CH), 44.2 (NCH_2), 29.6 (2′-CH_2), 24.3 (CH_2SePh), 20.7 (Me), 20.4 ppm (Me); IR (neat): $\tilde{\nu}$=903, 1020, 1216, 1326, 1366, 1436, 1493, 1576, 1740, 2206, 2926 cm^{-1}; HRMS (EI): m/z: calcd for $C_{18}H_{20}N_2Se$: 344.0792; found: 344.0796 $[M]^+$; HRMS (EI): m/z (%): 344 $[M]^+$, 147 (34), 190 (100).

5,6-Dimethyl-1-[4-(phenylseleno)butyl]-1*H*-benzimidazole (15): The procedure given for the preparation of **14** was followed; 5,6-dimethyl-1*H*-benzimidazole (1.46 g, 10.00 mmol), sodium hydride (0.36 g, 15.00 mmol) and 4-iodo-1-(phenylselanyl)butane (2.56 g, 7.55 mmol) in DMF (180 mL) gave **15** (1.54 g, 57%) as a yellow solid after column chromotography. R_f=0.28 (EtOAc); m.p. 74–76 °C; ^{1}H NMR: δ=7.70 (s, 1H; Ar-2-H), 7.54 (s, 1H; Ar-4-H), 7.43 (m, 2H; PhH), 7.24 (m, 3H; PhH), 7.13 (s, 1H; Ar-7-H), 4.09 (t, J=7.1 Hz, 2H; NCH_2), 2.88 (t, J=7.1 Hz, 2H; CH_2SePh), 2.38 (d, J=2.9 Hz, 6H; Me), 1.99 (m, 2H; 2′-CH_2), 1.70 ppm (m, 2H; 3′-CH_2); ^{13}C NMR: δ=142.9 (C), 142.2 (Im-2-CH), 133.0 (PhCH), 132.1 (C), 131.0 (C), 130.0 (C), 129.2 (PhCH), 127.2 (PhCH), 120.5 (Ar-4-CH), 109.9 (Ar-7-CH), 42.5 (NCH_2), 29.7 (2′-CH_2), 27.3 (3′-CH_2, CH_2SePh), 20.7 (Me), 20.4 ppm (Me); IR (neat): $\tilde{\nu}$=1023, 1215, 1326, 1436, 1473, 1493, 1570, 2933 cm^{-1}; HRMS (CI): m/z: calcd for $C_{19}H_{23}N_2Se$: 359.1021; found: 359.1015 $[M+H]^+$; HRMS (EI): m/z (%): 358 (20) $[M]^+$, 201 (100), 200 (37), 159 (95), 91 (50), 78 (78).

5,6-Dimethyl-1-[5-(phenylseleno)pentyl]-1*H*-benzimidazole (16): The procedure given for the preparation of **14** was followed; 5,6-dimethyl-1*H*-benzimidazole (1.67 g, 11.42 mmol), sodium hydride (0.41 g, 17.11 mmol) and 5-iodo-1-(phenylselanyl)pentane (3.03 g, 8.58 mmol) in DMF (200 mL) gave **16** (2.37 g, 74%) as a yellow solid after column chromatography. R_f=0.27 (EtOAc); m.p. 67–69 °C; ^{1}H NMR: δ=7.72 (s, 1H; Im-2-H), 7.56 (s, 1H; Ar-4-H), 7.44 (m, 2H; PhH), 7.24 (m, 3H; PhH), 7.12 (s, 1H; Ar-7-H), 4.08 (t, J=7.1 Hz, 2H; NCH_2), 2.85 (t, J=7.3 Hz, 2H; CH_2SePh), 2.38 (d, J=2.9 Hz, 6H; Me), 1.84 (m, 2H; 2′-CH_2), 1.71, 1.43 ppm (m, 4H; 3′-CH_2, (4′)-CH_2); ^{13}C NMR: δ=142.7 (C), 142.3 (Im-2-CH), 132.7 (2×C), 132.1 (PhCH), 131.1 (C), 131.0 (C), 129.2 (PhCH), 127.0 (PhCH), 120.4 (Ar-4-CH), 109.9 (Ar-7-CH), 44.9 (NCH_2), 29.7 (4′-CH_2), 29.4 (2′-CH_2), 27.5 (CH_2SePh), 27.0 (3′-CH_2), 20.8 (Me), 20.4 ppm (Me); IR (neat): $\tilde{\nu}$=1020, 1223, 1326, 1446, 1493, 1580, 2860, 2926, 3086 cm^{-1}; HRMS (EI): m/z: calcd for $C_{20}H_{24}N_2Se$: 372.1105; found: 371.1108 $[M]^+$; HRMS (EI): m/z (%): 189 (94), 156 (39), 68 (40).

6,7-Dimethyl-2,3-dihydro-1*H*-pyrrolo[1,2-*a*]benzimidazole (17a): Bu_3SnH (1.36 g, 4.66 mmol) and ACN (1.82 g, 7.46 mmol) in PhMe (60 mL) were added over 18 h by a syringe pump to **14** (0.64 g, 1.86 mmol) and CSA (0.43 g, 1.86 mmol) in PhMe (60 mL) heated at reflux. Further ACN (0.68 g, 2.79 mmol) in 9 mL of PhMe was divided into three equal portions and added after 1, 5 and 9 h to the reaction mixture. The solution was stirred at reflux for an additional 1 h, cooled and evaporated to dryness to give a brown residue, which was purified by column chromatography with 10% w/w of finely ground KF and 90% w/w silica gel as absorbent and gradient elution with hexane and EtOAc to give in order of elution 6,7-dimethyl-1-propyl-1*H*-benzimidazole **17b** (0.19 g, 54%), as a yellow solid and **17a** (0.11 g, 32%) as a yellow powder.

*Product **17a***: R_f=0.10 (EtOAc); m.p. 159–161 °C (lit[26] m.p. 177–179 °C); ^{1}H NMR: δ=7.42 (s, 1H; Ar-5-H), 7.04 (s, 1H; Ar-8-H), 4.00 (m, 2H; 1-CH_2), 2.99 (m, 2H; 3-CH_2), 2.66 (m, 2H; 2-CH_2), 2.34 ppm (s, 6H; Me); ^{13}C NMR: δ=160.5 (Ar-3a-C), 147.6 (C), 131.0 (C), 130.7 (C), 130.4 (C), 119.8 (Ar-5-CH), 109.9 (Ar-8-CH), 42.8 (1-CH_2), 26.2 (2-CH_2), 23.6 (3-CH_2), 20.5 (Me), 20.4 ppm (Me); IR (neat): $\tilde{\nu}$=1000, 1130, 1293, 1300, 1446, 1523, 1410, 2940 cm^{-1}.

*Product **17b***: R_f=0.33 (EtOAc); m.p. 81–83 °C; ^{1}H NMR: δ=7.74 (s, 1H; Im-2-H), 7.55 (s, 1H; Ar-4-H), 7.14 (s, 1H; Ar-7-H), 4.06 (t, J=6.9 Hz, 2H; NCH_2), 2.37 (d, J=8.2 Hz, 6H; Me), 1.85 (m, 2H; 2′-CH_2), 0.91 ppm (t, J=7.3 Hz, 3H; CH_2CH_3); ^{13}C NMR: δ=142.4, 142.3, 132.1 (C), 132.0 (C), 130.9 (C), 120.3 (Ar-4-CH), 109.9 (Ar-7-CH), 46.8 (NCH_2), 23.2 (2′-CH_2), 20.8 (Me), 20.5 (Me), 11.5 ppm (CH_2CH_3); IR (neat): $\tilde{\nu}$=986, 1055, 1137, 1215, 1351, 1374, 1449, 1486, 1500, 1676, 2876, 2926 cm^{-1}; elemental analysis calcd (%) for $C_{12}H_{16}N_2$: C 76.5, H 8.6, N 14.9; found: C 76.2, H 8.5, N 14.7.

7,8-Dimethyl-1,2,3,4-tetrahydropyrido[1,2-*a*]benzimidazole (18a): The procedure given for the preparation of **17a** was followed; Bu_3SnH (1.26 g, 4.33 mmol), ACN (2.33 g, 9.51 mmol) CSA (0.40 g, 1.73 mmol) and **15** (0.62 g, 1.73 mmol) in PhMe (170 mL) gave **18a** (0.24 g, 69%) as an off-white powder after column chromatography. R_f=0.23 (EtOAc); m.p. 161–163 °C; ^{1}H NMR: δ=7.41 (s, 1H; Ar-6-H), 7.01 (s, 1H; Ar-9-H), 3.97 (t, J=6.0 Hz, 2H; 1-CH_2), 3.01 (t, J=6.3 Hz, 2H; 4-CH_2), 2.34 (d, J=4.6 Hz, 6H; Me), 2.05 (m, 2H; 2-CH_2), 1.96 ppm (m, 2H; 3-CH_2); ^{13}C NMR: δ=150.9 (Ar-4a-C), 141.4 (C), 133.2 (C), 130.7 (C), 130.6 (C), 119.0 (Ar-6-CH), 109.2 (Ar-9-CH), 42.4 (1-CH_2), 25.5 (4-CH_2), 22.8 (3-CH_2), 20.9, 20.5, 20.4 ppm; IR (neat): $\tilde{\nu}$=900, 1003, 1253, 1314, 1363, 1413, 1455, 1516, 1723, 2863, 2937 cm^{-1}; HRMS (EI): m/z: calcd for $C_{13}H_{16}N_2$: 200.1388; found: 200.1106 $[M]^+$; HRMS (EI): m/z (%): 200 $[M]^+$ (100), 185 (99), 157 (40), 171 (41), 121 (9).

2,3-Dimethyl-7,8,9,10-tetrahydro-6*H*-azepino[1,2-*a*]benzimidazole (19a): The procedure given for the preparation of **17a** was followed; Bu_3SnH (0.98 g, 3.38 mmol), ACN (1.81 g, 7.7 mmol), CSA (0.31 g, 1.35 mmol)

and **16** (0.50 g, 1.35 mmol) in PhMe (160 mL) gave in order of elution after column chromatography 5,6-dimethyl-1-pentyl-1*H*-benzimidazole (**19b**) (60 mg, 21%) as a yellow solid and **19a** (0.18 g, 63%) as a yellow crystals

Product ***19a***: R_f=0.14 (EtOAc); m.p. 129–131°C; ^{1}H NMR: δ=7.44 (s, 1H; Ar-4-H), 7.03 (s, 1H; Ar-1-H), 4.10 (t, J=5 Hz, 2H; 10-CH_2), 3.06 (t, J=5.6 Hz, 2H; 6-CH_2), 2.37 (d, J=2.5 Hz, 6H; Me), 1.90 (m, 2H; 9-CH_2), 1.79 ppm (m, 4H; 7-CH_2, 8-CH_2); ^{13}C NMR: δ=156.7 (Ar-5a-C), 140.9 (C), 134.3 (C), 130.9 (C), 130.3 (C), 119.4 (Ar-4-CH), 109.1 (Ar-1-CH), 44.5 (10-CH_2), 31.0 (9-CH_2), 30.2 (6-CH_2), 28.9, 25.7 (7-CH_2, 8-CH_2), 20.7 (Me), 20.3 ppm (Me); IR (neat): $\tilde{\nu}$=910, 1003, 1183, 1313, 1416, 1463, 1516, 1616, 2933 cm^{-1}; HRMS (CI): m/z: calcd for $C_{14}H_{19}N_2$: 215.1543; found: 215.1540 $[M+H]^+$; HRMS (ESI): m/z (%): 214 $[M]^+$ (100), 199 (35), 185 (30), 159 (20), 77 (35).

Product ***19b***: R_f=0.27 (EtOAc); m.p. 74–75°C; ^{1}H NMR: δ=7.76 (s, 1H; Im-2-H), 7.56 (s, 1H; Ar-4-H), 7.15 (s, 1H; Ar-7-H), 4.10 (t, J=7.1 Hz, 2H; NCH_2), 2.39 (d, J=9.2 Hz, 6H; Me), 1.86 (m, 2H; 2′-CH_2), 1.33 (m, 4H, 3′-CH_2, 4′-CH_2), 0.88 ppm (t, J=7.0 Hz, 3H; CH_2CH_3); ^{13}C NMR: δ=142.5 (Im-2-CH), 142.4 (C), 132.3 (C), 131.8 (C), 130.8 (C), 120.3 (Ar-4-CH), 109.8 (Ar-7-CH), 45.0 (NCH_2), 29.6, 28.9 (2′-CH_2, 3′-CH_2), 22.2 (4′-CH_2), 20.7 (Me), 20.3 (Me), 14.0 ppm (CH_2CH_3); IR (neat): $\tilde{\nu}$=910, 1003, 1183, 1313, 1416, 1463, 1516, 1616, 2933 cm^{-1}; elemental analysis calcd (%) for $C_{14}H_{20}N_2$: C 77.7, H 9.3, N 13.0; found: C 77.8, H 9.5, N 12.9.

7,8-Dimethy-6-nitro-1,2,3,4-tetrahydropyrido[1,2-*a*]benzimidazole (20) and 7,8-dimethyl-6,9-dinitro-1,2,3,4-tetrahydropyrido[1,2-*a*]benzimidazole (21): Compound **18a** (0.22 g, 1.10 mol) in a 50:50 mixture of concentrated H_2SO_4 and fuming concentrated HNO_3 (20 mL) was stirred at 0°C for 3 h. The solution was basified with NH_4OH to pH 12 and extracted with $CHCl_3$ (3×50 mL). The organic extracts were combined, dried (Na_2SO_4) and evaporated to dryness. The resultant yellow solid was purified by column chromatography using silica gel as absorbent with gradient elution of hexane and EtOAc to give in order of elution **21** (0.14 g, 44%) as a yellow crystals and **20** (40 mg, 15%) as a yellow solid.

Product ***20***: R_f=0.20 (EtOAc); m.p. 186–188°C; ^{1}H NMR: δ=7.22 (s, 1H; Ar-9-H), 4.05 (t, J=6.3 Hz, 2H; NCH_2), 3.09 (t, J=6.3 Hz, 2H; 4-CH_2), 2.41 (s, 3H; Me), 2.34 (s, 3H; Me), 2.12 (m, 2H; 2-CH_2), 2.01 ppm (m, 2H; 3-CH_2); ^{13}C NMR: δ=153.7 (Ar-4a-C), 141.2 (C), 134.4 (C), 134.1 (C), 131.6 (C), 123.2 (Ar-6-C), 112.5 (Ar-9-CH), 42.9 (1-CH_2), 25.7 (4-CH_2), 22.6 (2-CH_2), 21.1 (3-CH_2), 20.6 (8-Me), 14.9 ppm (7-Me); IR (neat): $\tilde{\nu}$=1027, 1165, 1276, 1309, 1370 (NO_2), 1418, 1449, 1516 (NO_2), 2922 cm^{-1}.

Product ***21***: R_f=0.70 (EtOAc); m.p. 175–176°C; ^{1}H NMR: δ=3.95 (t, J 6.0 Hz, 2H; 1-CH_2), 3.13 (t, J=6.3 Hz, 2H; 4-CH_2), 2.36 (s, 3H; Me), 2.32 (s, 3H; Me), 2.08 (m, 2H; 2-CH_2), 2.00 ppm (m, 2H; 3-CH_2); ^{13}C NMR: δ=155.9 (Ar-4a-C), 142.0 (C), 136.9 (C), 136.0 (C), 125.1 (C), 123.5 (C), 123.4 (C), 43.7 (1-CH_2), 26.0 (4-CH_2), 22.4 (2-CH_2), 19.7 (3-CH_2), 15.1 (Me), 14.9 ppm (Me); IR (neat): $\tilde{\nu}$=1036, 1250, 1306, 1373 (NO_2), 1410, 1506 (NO_2), 2913 cm^{-1}; HRMS (EI): m/z: calcd for $C_{13}H_{14}N_4O_4$: 290.1015; found: 290.1043 $[M]^+$; HRMS (EI): m/z (%): 290 (26) $[M]^+$, 284 (65), 226 (24), 199 (30), 189 (88), 121 (100).

6,7-Dimethyl-5,8-dinitro-2,3-dihydro-1*H*-pyrrolo[1,2-*a*]benzimidazole (22): Compound **17a** (0.18 g, 0.96 mmol) in a 50:50 mixture of concentrated H_2SO_4 and fuming concentrated HNO_3 (20 mL) was stirred at 0°C for 12 h. The reaction was basified by using NH_4OH to pH 12 and extracted with $CHCl_3$. The organic extracts were combined, dried (Na_2SO_4) and evaporated to dryness to give **22** (0.24 g, 90%) as an orange solid. R_f=0.67 (EtOAc); m.p. 163–165°C; ^{1}H NMR: δ=4.13 (t, J=7.1 Hz, 2H; 1-CH_2), 3.10 (t, J=7.1 Hz, 2H; 3-CH_2), 2.75 (m, 2H; 2-CH_2), 2.42 (s, 3H; Me), 2.37 ppm (s, 3H; Me); ^{13}C NMR: δ=165.2 (Ar-3a-C), 140.9 (C), 125.6 (C), 125.2 (C), 123.2 (overlapping C), 45.4 (1-CH_2), 26.1 (2-CH_2), 23.4 (3-CH_2), 15.7 (Me), 15.1 ppm (Me); IR (neat): $\tilde{\nu}$=810, 1000, 1300 (NO_2), 1523 (NO_2), 2933 cm^{-1}.

2,3-Dimethyl-1,4-dinitro-7,8,9,10-tetrahydro-6*H*-azepino[1,2-*a*]benzimidazole (23): The procedure given for the preparation of **22** was followed; **19a** (0.10 g, 0.47 mmol) in a 50:50 mixture of concentrated H_2SO_4 and fuming concentrated HNO_3 (20 mL) gave after workup **23** (90 mg, 63%) as a yellow crystals. R_f=0.77 (EtOAc); m.p. 129–131°C; ^{1}H NMR: δ=3.94 (m, 2H; 10-CH_2), 3.14 (m, 2H; 6-CH_2), 2.36 (d, J=14.1 Hz, 6H; Me), 1.91 (m, 2H; 9-CH_2), 1.81 ppm (m, 4H; 7-CH_2, 8-CH_2); ^{13}C NMR: δ=161.8 (Ar-5a-C), 142.5 (C), 136.8 (C), 135.4 (C), 125.4 (C), 123.6 (C), 122.9 (C), 45.4 (10-CH_2), 30.1 (9-CH_2), 29.4 (6-CH_2), 28.1, 25.0 (7-CH_2, 8-CH_2), 15.0 (Me), 14.9 ppm (Me); IR (neat): $\tilde{\nu}$=973, 1086, 1196, 1310, 1366 (NO_2), 1526 (NO_2), 1723, 2840, 2920 cm^{-1}; HRMS (ESI): m/z: calcd for $C_{14}H_{17}N_4O_4$: 305.1244; found: 305.1241 $[M+H]^+$.

6,7-Dimethyl-2,3-dihydro-1*H*-pyrrolo[1,2-*a*]benzimidazole-5,8-dione (7): Compound **22** (0.23 g, 0.83 mmol) and Adam's catalyst (PtO_2, 50 mg) in ethanol (20 mL) was shaken under a 40 psi hydrogen atmosphere by using a Parr apparatus for 18 h. The mixture was filtered through Celite and evaporated to dryness. $FeCl_3$ aq (0.7 M, 30 mL) was added and stirring continued for 12 h. Saturated NaOAc aq was added and the mixture extracted with $CHCl_3$. The combined organic extracts were dried ($MgSO_4$) and evaporated to dryness. The resultant orange solid was purified by column chromatography using silica gel as absorbent with a gradient elution of hexane and EtOAc to give **7** (0.18 g, 100%) as yellow powder. R_f=0.32 (EtOAc); m.p. 235–237°C; ^{1}H NMR: δ=4.20 (t, J=7.3 Hz, 2H; 1-CH_2), 2.93 (t, J=7.8 Hz, 2H; 3-CH_2), 2.71 (m, 2H; 2-CH_2), 2.03 ppm (d, J=20 Hz, 6H; Me); ^{13}C NMR: δ=181.1 (C=O), 178.1 (C=O), 160.4 (Im-3a-C), 145.9 (C), 141.0 (C), 138.7 (C), 129.3 (C), 45.0 (1-CH_2), 26.5 (3-CH_2), 22.8 (2-CH_2), 12.5 (Me), 11.9 ppm (Me); IR (neat): $\tilde{\nu}$=930, 1170, 1236, 1370, 1466, 1518, 1600, 1640 (C=O), 1663, 2920 cm^{-1}; HRMS (ESI): m/z: calcd for $C_{12}H_{13}N_2O_2$: 217.0972; found: 217.0972 $[M+H]^+$; HRMS (EI): m/z (%): 216 (100) $[M]^+$, 187 (40), 159 (78), 53 (60); elemental analysis calcd (%) for $C_{12}H_{12}N_2$: C 66.6, H 5.6, N 13.0; found: C 66.2, H 5.3, N 13.1.

7,8-Dimethyl-1,2,3,4-tetrahydropyrido[1,2-*a*]benzimidazole-6,9-dione (8): The procedure given for the preparation of **7** was followed; **21** (0.18 g, 0.64 mmol) and Adam's catalyst (PtO_2, 50 mg) in ethanol (20 mL) was shaken under 40 psi hydrogen for 18 h followed by $FeCl_3$ oxidation to give **8** (0.13 g, 85%) as yellow crystals after column chromatography. R_f=0.25 (ethyl acetate); m.p. 205–207°C; ^{1}H NMR: δ=4.32 (m, 2H; 1-CH_2), 2.99 (m, 2H; 4-CH_2), 2.06 ppm (m, 10H; 2-CH_2, 3-CH_2, Me); ^{13}C NMR: δ=181.5 (C=O), 178.6 (C=O), 151.5 (Im-4a-C), 141.6 (C), 141.1 (C), 140.4 (C), 129.9 (C), 45.5 (1-CH_2), 25.0 (4-CH_2), 22.3 (2-CH_2), 19.8 (3-CH_2), 12.5 (Me), 12.1 ppm (Me); IR (neat): $\tilde{\nu}$=915, 1037, 1199, 2925, 1229, 1265, 1307, 1416, 1579, 1644 cm^{-1} (C=O); HRMS (ESI): m/z: calcd for $C_{13}H_{14}N_2O_2$: 230.1055; found: 230.1066 $[M]^+$; elemental analysis calcd (%) for $C_{13}H_{14}N_2O_2$: C 67.9, H 6.1, N 12.2; found: C 67.7, H 6.1, N 12.1.

2,3-Dimethyl-7,8,9,10-tetrahydro-4*H*-azepino[1,2-*a*]benzimidazole-1,4-(6*H*)-dione (9): The procedure given for the preparation of **7** was followed; **23** (0.31 g, 1.02 mmol) and Adam's catalyst (PtO_2, 50 mg) in ethanol (20 mL) were shaken under 40 psi hydrogen for 18 h followed by $FeCl_3$ oxidation to give **9** (0.25 g, 100%) as yellow crystals, after column chromatography; R_f=0.37 (EtOAc); m.p. 129–131°C; ^{1}H NMR: δ=4.56 (m, 2H; 10-CH_2), 2.99 (t, J=5.7 Hz, 2H; 6-CH_2), 2.03 (d, J=16 Hz, 6H; Me), 1.88 (m, 2H; CH_2), 1.79 (m, 2H; CH_2), 1.70 ppm (m, 2H; 8-CH_2); ^{13}C NMR: δ=181.4 (C=O), 179.2 (C=O), 157.9 (Im-5a-C), 140.7 (C), 140.2 (C), 139.9 (C), 130.3 (C), 45.9 (10-CH_2), 30.9 (9-CH_2), 29.3 (6-CH_2), 28.2 (7-CH_2), 25.0 (8-CH_2), 12.5 (Me), 12.2 ppm (Me); IR (neat): $\tilde{\nu}$=1046, 1200, 1306, 1410, 1516, 1636, 2933 cm^{-1}; HRMS (EI): m/z: calcd for $C_{14}H_{16}N_2O_2$: 244.1212; found: 244.1206 $[M]^+$; elemental analysis calcd (%) for $C_{14}H_{16}N_2O_2$: C 68.8, H 6.6, N 11.5; found: C 68.8, H 6.6, N 11.5.

Biological studies

Cell culture: The SV40-transformed normal human skin fibroblast cell line (GM00637I) was grown in minimum essential media eagle-earle BSS (MEM) supplemented with 15% uninactivated fetal bovine serum (FBS), penicillin/streptomycin, 2×essential and non-essential amino acids and vitamins, with 2 mM L-glutamine. The cells were incubated at 37°C under a humidified atmosphere containing 5% CO_2.

Cytotoxicity measurement: MTT assay: Growth inhibition was determined by using the MTT colorimetric assay. Cells (GM00637I) were plated in 96-well plates at a density of 50000 cells/well and allowed to adhere over a period of 48 h. Benzimidazolequinone solutions (5 μL) were applied in DMSO (2.5% concentration in well), and the plates were incubated at 37°C under a humidified atmosphere containing 5%

CO_2 for 24 h (aerobic conditions). MTT (100 μg) 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 reference at 690 nm. Under hypoxic conditions 96-well plates were incubated in a hypoxic chamber (0.5% O_2, 5% CO_2, 2% H_2, 92.5% N_2) at 37°C under a humidified atmosphere for 3 h before addition of the benzimidazolequinone solutions. The plates were not removed from the chamber until after incubation with MTT was complete. Each data point given in figures represents the mean of at least three independent experiments ± one standard deviation. IC_{50}, the drug concentration required to reduce viability to 50%, was obtained from plots of cell viability (%) versus concentration, by drawing lines of best fit. Cell viability is expressed as a percentage of the DMSO-only treated control value.

Electrochemistry

Cyclic voltammagrams were recorded at scan rates 20, 50, 100 and 200 $mV s^{-1}$ by using an EcoChemie Autolab with PGSTAT12 potentiostat controlled by GPES software. Quinones **6** and **8** were dissolved in DMF, containing 0.1 M tetrabutylammoniumperchlorate as electrolyte and 1 mM ferrocene (Fc) as reference. Cyclic voltammetry was recorded at a platinum disk electrode in a single compartment electrochemical cell (2 mL volume) containing Ag/AgCl reference electrode and a platinum wire counter electrode. All measurements were carried out at RT. The platinum disc electrode was previously polished on a microcloth pad in 0.05 μm alumina slurry, and nitrogen was bubbled through solvents.

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