One-pot double intramolecular homolytic aromatic substitution routes to dialicyclic ring fused imidazobenzimidazolequinones and preliminary analysis of anticancer activity†

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Bu_3SnH/1,1′-azobis(cyclohexanecarbonitrile) (ACN)-mediated five, six, and seven-membered double alkyl radical cyclizations onto imidazo[5,4-*f*]benzimidazole and imidazo[4,5-*f*]benzimidazole are described. The quinone derivatives evaluated show selective toxicity towards human cervical (HeLa) and prostate (DU145) cancer cell lines (with negligible toxicity towards a normal human cell line, GM00637). Only the Fremy oxidation of the 6-aminoimidazo[5,4-*f*]benzimidazole gave iminoquinone, which showed high specificity towards the prostate cancer cell line (DU145).

Introduction

Bu_3SnH/azo-initiator mediated intramolecular homolytic aromatic substitution or radical cyclization onto (hetero)aromatics is now a valuable protocol in organic synthesis.[1–11] The protocol has attracted mechanistic interest, since the intermediate π-cyclohexadienyl radical (σ-complex) generated upon radical addition formally loses a hydrogen atom to give the aromatic substitution product, so is described as an oxidation in the presence of the "reductant" Bu_3SnH.[1,9] Most recent examples achieve optimized (often high) reaction yields in the presence of excess amounts of appropriate azo-initiator.[2–4,9–13] The latter is thought to fulfil a dual role in the non-chain reaction, providing continual initiation, as well as taking part in the oxidative rearomatization. We reported the use of the protocol in the synthesis of highly potent alicyclic [1,2-*a*] ring fused benzimidazolequinones *via* five to seven-membered radical cyclizations of nucleophilic *N*-alkyl radicals onto the benzimidazole-2-position activated by quaternizing the pyridine-like 3-*N* of imidazole with camphorsulfonic acid (CSA).[10] The pyrido[1,2-*a*]benzimidazolequinone **1** (Fig. 1) was the most potent compound prepared being more than 300 times more cytotoxic than the clinically used drug, mitomycin C (**MMC**) towards hypoxic (low pO_2) human skin fibroblast cells (GM00637).[10] Moreover, compound **1** was more cytotoxic than other benzimidazolequinones containing "DNA-damaging functionality", such as aziridine **2**[14] or cyclopropane **3**[10,15]

This has led us to the present synthesis of the novel anticancer agent dipyridoimidazo[5,4-*f*]benzimidazolequinone **4** and dipyridoimidazo[4,5-*f*]benzimidazolequinone **5**. Dialicyclic ring fused imidazo[4,5-*f*]benzimidazolequinones (including **5**) have been reported by Skibo and co-workers,[16,17] and shown

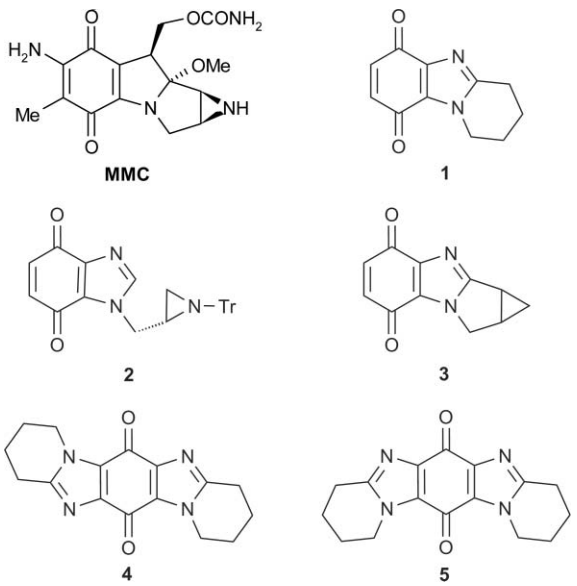

Fig. 1 Mitomycin C and synthetic benzimidazolequinone and imidazobenzimidazolequinone anticancer agents.

to be excellent substrates for the quinone reductase enzyme, NAD(P)H:quinone oxidoreductase (NQO1,[18] also known as DT-diaphorase). This article contains the first cytotoxicity evaluation of **4** and **5**, which we have carried out using normal cells (GM00637), and two human cancer cell lines known to express high levels of NQO1: cervical (HeLa, CCL-2)[19] and prostate (DU145)[20] cancer.

The required double annulations of imidazobenzimidazole have been achieved by the first one-pot double intramolecular radical substitution onto heteroarenes. We are aware of only one example of this protocol with arenes, which was reported by Harrowven and co-workers[7] involving the Bu_3SnH/azoinitiator mediated six-membered cyclization of aryl radicals to give [5 and 7]helicenes. As part of our study, we now report Bu_3SnH/1,1′-azobis(cyclohexanecarbonitrile) (ACN)-mediated five, six and seven-membered alkyl radical cyclizations at the

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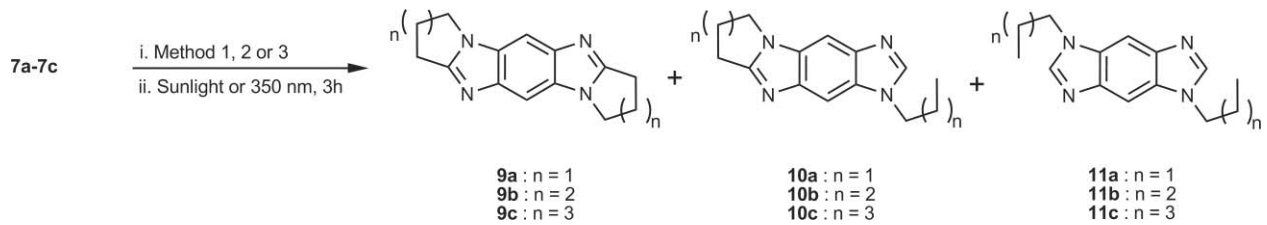
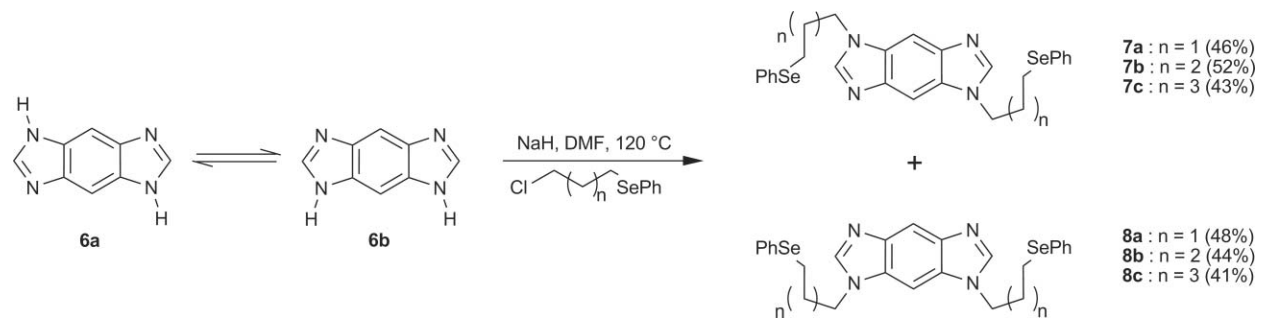
Table 1 One-pot double intramolecular homolytic aromatic substitutions onto imidazo[5,4-*f*]benzimidazole[a]



Entry	Precursors	Method	Products (%)[b]					
1	**7a**	1	**9a**	(32)	**10a**	(31)	**11a**	(10)
2	**7a**	2	—[c]		—[c]		—[c]	
3	**7a**	3	**9a**	(47)	**10a**	(30)	**11a**	(0)
4	**7b**	1	**9b**	(90)	**10b**	(0)	**11b**	(0)
5	**7c**	1	**9c**	(29)	**10c**	(32)	**11c**	(8)
6	**7c**	2	**9c**	(54)	**10c**	(13)	**11c**	(0)
7	**7c**	3	**9c**	(34)	**10c**	(31)	**11c**	(0)

[a] i. **Method 1:** Bu_3SnH (5 equiv, 0.06 M), ACN (5 equiv.), toluene, syringe pump addition (2.2 mL h^{-1}), reflux, air; **Method 2:** Method 1 & CSA (2.1 equiv.); **Method 3:** Method 1 & Ac_2O (2.1 equiv.); ii. Sunlight (Ireland), 15–20 °C or 350 nm, Rayonet reactor, rt. [b] Isolated yields; [c] Intractable mixture.



Scheme 1 Synthesis of radical precursors.

imidazobenzimidazole 2- and 6-positions. This represents the first protocol for annulation onto imidazobenzimidazole.

Results and discussion

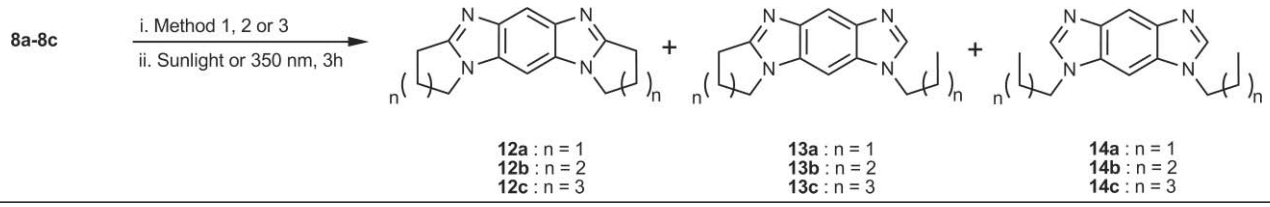
The two targeted isomeric heterocyclic systems were simultaneously accessed from imidazo[5,4-*f*(4,5-*f*)]benzimidazole **6a–6b** (benzo-bis(imidazole)).[21,22] Tautomers **6a–6b** exist in a 1 : 1 ratio observable in the ^{1}H NMR spectrum as an "apparent triplet" at 7.49–7.78 ppm. Reaction with 2.1 equivalents of NaH and *ω*-chloroalkyl phenyl selenides gave 1,5- and 1,7-dialkylphenylselenide radical precursors **7a–7c** and **8a–8c** in excellent combined yields (Scheme 1). The obtained approximate 1 : 1 mixture of dialkylated isomers **7a–7c** and **8a–8c** was readily separable by chromatography. Phenylselenides have previously been shown to be effective diazole radical precursors[8,10,11,23] because of their efficient radical, but poor S_N2 leaving group, ability. Unlike previous radical precursor preparations, alkylation of the basic heterocycle was achieved without prior conversion of the *ω*-chloroalkyl phenyl selenide to the corresponding iodide.

The conditions for the one-pot double homolytic aromatic substitution protocol were optimized using the six-membered cyclizations of dibutylphenylselenide **7b**. Since two cyclizing radicals are generated per molecule of substrate, at least two equivalents of Bu_3SnH are required. However optimized yields were obtained using five equivalents of Bu_3SnH and ACN (or 2.5 equivalents per phenylselenide moiety). This gave dipyrido ring fused imidazo[5,4-*f*]benzimidazole **9b** in excellent yield of 90% (Table 1, entry 4, method 1).

It is noteworthy, that for all cyclizations (in Tables 1 and 2), the homolytic aromatic substitutions were more efficient if carried out in the absence of an inert atmosphere. Attempts to exclude air or increase the number of ACN equivalents led to the formation of complex mixtures of non-aromatic trapped π-radical adducts.[4,13] The rearomatization of the inseparable mixture is aided by the presence of air (or O_2),[3,24] with additional prolonged heating of the reaction mixture or exposure to low-energy light (incl. sunlight) required for full conversion to aromatic products. The requirement for excess amounts of initiator indicates a non-chain mechanism, however some direct hydrogen atom abstraction from intermediate π-radicals by O_2 resulting in propagating cycles cannot be ruled out.[25]

The more difficult five and seven-membered radical cyclizations onto imidazo[5,4-*f*]benzimidazole gave low yields of diannulated products **9a** (32%) and **9c** (29%) with similar respective yields of single cyclization products **10a** (31%) and **10c** (32%) isolated (Table 1, entries 1 and 5). It was apparent that radical reduction was competing effectively with cyclization, with non-cyclized compounds **11a** (10%) and **11c** (8%) also obtained. Thus

Table 2 One-pot double intramolecular homolytic aromatic substitutions onto imidazo[4,5-*f*]benzimidazole



Entry	Precursors	Method[a]	Products (%)[b]					
1	**8a**	3	**12a**	(48)	**13a**	(27)	**14a**	(0)
2	**8b**	1	**12b**	(81)	**13b**	(0)	**14b**	(0)
3	**8c**	2	**12c**	(48)	**13c**	(16)	**14c**	(0)

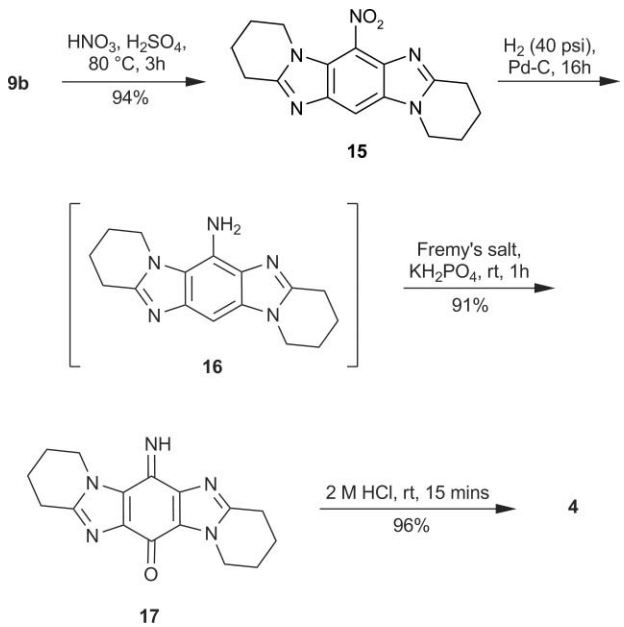
[a] Reaction conditions as in Table 1. [b] Isolated yields.

activation[10,26,27] of imidazo[5,4-*f*]benzimidazole by protonation of the 3,7-*N* basic sites (making the heterocycle more electrophilic) was deemed necessary, and CSA (2.1 equivalents) was added (method 2). Although the acid improved the double seven-membered nucleophilic alkyl radical cyclization to give **9c** in 54% yield (and **10c** (13%) entry 6), it led to an intractable mixture for the attempted five-membered analogue (entry 2). Replacing CSA with acetic anhydride (2.1 equivalents, method 3) facilitated the five membered cyclization to give improved, but modest yields of **9a** (47%) and **10a** (30%) (entry 3). Nevertheless, the present one-pot double annulation yields compare favorably with literature single alkyl radical cyclizations onto diazoles,[8,10,11,23] since a 54% and 47% yield for the double five and seven-membered cyclizations (to give **9a** and **9c**) correspond to a cumulative yield for two separate cyclizations each proceeding in about 70% yield.

The optimized reaction conditions in Table 1 were applied to the cyclizations onto imidazo[4,5-*f*]benzimidazole (Table 2). The double six-membered cyclization proceeded in excellent yield without activation giving **12b** in 81% yield (entry 2). Acetic anhydride and CSA quaternization of the 3,5-*N* basic sites of imidazo[4,5-*f*]benzimidazole allowed respective five and seven membered radical cyclizations to occur, with reduction minimized (entries 1 and 3).

The new heterocyclic quinone **4** was obtained in high overall yield by nitration, reduction and oxidation, as depicted in Scheme 2. Unexpectedly, iminoquinone **17** was obtained from the Fremy oxidation of the intermediate amine **16** carried out at pH 4 in the presence of KH_2PO_4, with **17** easily hydrolyzed to **4** with hydrochloric acid. The oxidation conditions were analogous to those used by Suleman and Skibo,[17] which we repeated to give imidazo[4,5-*f*]benzimidazolequinone **5** in high yield (69% from **12b**). In agreement with the latter report no iminoquinone was observed as part of the synthesis of quinone **5**.

The cytotoxicity of **4**, **5** and **17** was evaluated against two cancer cell lines known to contain high NQO1 activity: cervical (HeLa, CCL-2)[19] and prostate (DU145)[20] cancer. For comparison purposes cytotoxicity against a normal human fibroblast cell line (GM00637)[10,14] was also carried out. Each cell line was treated with solutions of up to 5 μM of **4**, **5**, **17** and **MMC** in parallel for 72 h. Cell viability was determined using the well-known MTT colorimetric assay.[28] Both **4** and **5** showed negligible toxicity towards the normal cell line in this dose range (Table 3). This may be of therapeutic advantage, as it represents



Scheme 2 Functionalization to give the target quinone.

Table 3 Cytotoxicity evaluation: IC_{50} values (μM)[a]

Compound	Cell lines		
	GM00637	HeLa CCL-2	DU145
MMC	0.46 ± 0.09	0.27 ± 0.16	0.17 ± 0.02
4	> 5	1.67 ± 0.05	1.99 ± 0.39
5	> 5	3.33 ± 0.47	1.73 ± 0.23
17	3.63 ± 0.52	1.55 ± 0.35	0.30 ± 0.01

[a] IC_{50} represents the compound concentration required for the reduction of the mean cell viability to 50% of the control value after incubation for 72 h at 37 °C, as determined using the MTT assay.

an improvement on earlier cytotoxicity evaluation of pyrido[1,2-*a*]benzimidazolequinone **1**,[10] that deemed the compound to be too toxic towards normal cells for further investigation in cancer cell lines. Moreover, quinone **4** is found to be selectively toxic (in comparison to normal cells) towards the two cancer cell lines, with **5** more toxic towards prostate than cervical cancer. Iminoquinone **17** showed a high specificity towards prostate cancer, being up to twelve times more toxic towards this cell line than to normal

cells. The elevated toxicity of **17** may indicate different cytotoxicity pathways in comparison to quinones **4** and **5**. In contrast, submicromolar concentrations of **MMC** were found to be toxic towards all three cell lines, with marginal selectivity towards the two cancer cell lines.

Conclusions

The first one-pot double intramolecular homolytic aromatic substitution onto heterocycles is reported, as represented by the annulation of imidazobenzimidazoles. Excellent yields are obtained for the Bu_3SnH/azo-initiator mediated double six-membered radical cyclizations onto imidazo[5,4-*f*]benzimidazole and imidazo[4,5-*f*]benzimidazole, while quaternization of the basic nitrogen atoms is required for five and seven-membered analogous alkyl radical cyclizations to proceed in reasonable yields. The derived annulated imidazobenzimidazolequinones show specificity towards cancer cell lines known to express elevated levels of NQO1, with the evaluated iminoquinone intermediate showing very high toxicity and specificity towards the prostate cancer cell line DU145. More detailed anticancer activity screening is currently being carried out, and the biochemical mechanisms for cytotoxicity investigated.

Experimental

General

Materials. All commercially available reagents were purchased from Sigma Aldrich and were used throughout. Dimethylformamide (DMF) and toluene were freshly distilled over CaH. Thin layer chromatography (TLC) was carried out on aluminium-backed plates coated with silica gel (Merck Kieselgel 60 F_{254}). Dry column chromatography[29] was carried out using Merck Kieselgel silica gel 60 (particle size 0.015–0.040 mm) using the specified eluents.

Measurements. Melting points were measured on a Stuart Scientific melting point apparatus SMP3. Infrared spectra were recorded using a Perkin-Elmer Spec 1 with ATR attached. 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. High resolution mass spectra for compounds **7b**, **7c**, **8b**, **8c**, **9b**, **9c** and **12b** were carried out at University College London, Mass Spectroscopy Facility using a Thermo Finnigan MAT900xp operating in chemical ionization (CI) mode with methane as carrier gas, and accurate masses were calculated against reference 'heptacosa'. High resolution mass spectra for all other 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.

Synthesis of imidazo[5,4(4,5)-*f*]benzimidazole (6a–6b). 1,2,4,5-Benzenetetraamine tetrahydrochloride (1.00 g, 3.5 mmol), concentrated formic acid (50 mL) and concentrated hydrochloric acid (10 mL) were heated at reflux for 16 h. The cooled solution was evaporated to dryness and sodium carbonate solution (5%, 50 mL) was added. The precipitated product was collected *via* vacuum filtration, washed with water and dried under vacuum ($CaCl_2$) to give an approximate 1 : 1 mixture of the two tautomers of the *title compound* **6a–6b** (0.542 g, 98%) isolated as a pale brown solid; mp >350 °C (lit.,[21] > 300 °C); δ_H (400 MHz; DMSO-d_6) 7.49 (1H, bs, 8-H, 6b), 7.65 (2H, bs, 4,8-H, 6a), 7.78 (1H, bs, 4-H, 6b), 8.14 (4H, s, 2,6-H), 12.17 (4H, bs, *N*-H).

General procedure for the synthesis of radical precursors

1,5(7)-Di(ω-(phenylselano)alkyl)imidazo[5,4-*f*(4,5-*f*)]benzimidazoles. Imidazo[5,4-*f*(4,5-*f*)]benzimidazole **6a–6b** (0.790 g, 5.0 mmol) and sodium hydride (0.252 g, 10.5 mmol) in DMF (50 mL) were heated at 120 °C for 10 min. ω-Chloroalkyl phenyl selenide (10.5 mmol) was added, and the mixture stirred for 1 h. The cooled mixture was evaporated, dichloromethane added, and the insoluble solid removed by filtration. The filtrate was evaporated, and the residue purified by dry column vacuum chromatography with gradient elution of ethyl acetate and methanol yielding the separated 1,5-di(ω-(phenylselano)alkyl)imidazo[5,4-*f*]benzimidazole **7a–7c** and 1,7-di(ω-(phenylselano)alkyl)imidazo[4,5-*f*]benzimidazole **8a–8c** isomers.

1,5-Di(3-(phenylselano)propyl)imidazo[5,4-*f*]benzimidazole (7a). (1.271 g, 46%); white solid; R_f 0.49 (EtOAc–MeOH 9 : 1); mp 144–146 °C; ν_{max} (neat/cm^{-1}) 3080, 2928, 1576, 1518, 1495, 1476, 1437, 1381, 1357, 1313, 1218, 1162, 1127, 1070, 1043, 1020; δ_H (400 MHz, $CDCl_3$) 2.17–2.24 (4H, m, CH_2), 2.76 (4H, t, *J* 6.7, CH_2SePh), 4.29 (4H, t, *J* 6.6, NCH_2), 7.18–7.21 (6H, m, Ph-H), 7.41–7.44 (4H, m, Ph-H), 7.68 (2H, s, Ar-4,8-H), 7.85 (2H, s, Ar-2,6-H); δ_C (100 MHz, $CDCl_3$) 24.0 (CH_2SePh), 28.9 (CH_2), 44.0 (NCH_2), 98.9 (Ar-4,8-CH), 127.1 (Ph-CH), 128.8 (C), 129.0 (Ph-CH), 131.1 (C), 132.8 (Ph-CH), 141.4 (Ar-3a,7a-C), 144.1 (Ar-2,6-CH); *m*/*z* (ESI) 555.0557 (M + H$^+$, $C_{26}H_{27}N_4{}^{80}Se^{80}Se$ requires 555.0566).

1,7-Di(3-(phenylselano)propyl)imidazo[4,5-*f*]benzimidazole (8a). (1.326 g, 48%); white solid; R_f 0.39 (EtOAc–MeOH 9 : 1); mp 89–91 °C; ν_{max} (neat, cm^{-1}) 3359, 3093, 2921, 2851, 1639, 1576, 1524, 1493, 1476, 1435, 1364, 1292, 1232, 1213, 1159, 1113, 1070, 1020, 1000; δ_H (400 MHz, $CDCl_3$) 2.14–2.22 (4H, m, CH_2), 2.77 (4H, t, *J* 6.9, CH_2SePh), 4.25 (4H, t, *J* 6.6, NCH_2), 7.16–7.21 (7H, m, Ph-H & Ar-8-H), 7.38–7.43 (4H, m, Ph-H), 7.81 (2H, s, Ar-2,6-H), 8.17 (1H, s, Ar-4-H); δ_C (100 MHz, $CDCl_3$) 24.3 (CH_2SePh), 29.3 (CH_2), 44.2 (NCH_2), 88.5 (Ar-8-CH), 110.5 (Ar-4-CH), 127.4 (Ph-CH), 129.1 (C), 129.4 (Ph-CH), 132.0 (C), 133.0 (Ph-CH), 141.2 (Ar-3a,4a-C), 143.8 (Ar-2,6-CH); *m*/*z* (ESI) 555.0576 (M + H$^+$, $C_{26}H_{27}N_4{}^{80}Se^{80}Se$ requires 555.0566).

1,5-Di(4-(phenylselano)butyl)imidazo[5,4-*f*]benzimidazole (7b). (1.509 g, 52%); white solid; R_f 0.50 (EtOAc–MeOH 9 : 1); mp 156–158 °C; ν_{max} (neat, cm^{-1}) 3074, 2930, 1797, 1579, 1521, 1477, 1446, 1356, 1236, 1212, 1124, 1072, 1022; δ_H (400 MHz, $CDCl_3$) 1.67–1.74 (4H, m, CH_2), 2.00-2.01 (4H, m, CH_2), 2.86 (4H, t, *J* 7.2, CH_2SePh), 4.18 (4H, t, *J* 7.1, NCH_2), 7.19–7.24 (6H, m, Ph-H), 7.39–7.43 (4H, m, Ph-H), 7.70 (2H, s, Ar-4,8-H), 7.86 (2H, s, Ar-2,6-H); δ_C (100 MHz, $CDCl_3$) 27.2 (CH_2SePh), 27.3 (CH_2), 29.4 (CH_2), 44.9 (NCH_2), 99.2 (Ar-4,8-CH), 127.1 (Ph-CH), 129.2 (Ph-CH), 129.8 (C), 131.5 (C), 133.0 (Ph-CH), 141.7 (Ar-3a,7a-C), 144.2 (Ar-2,6-CH); *m*/*z* (CI) 583.0853 (M + H$^+$, $C_{28}H_{31}N_4{}^{80}Se^{80}Se$ requires 583.0879).

1,7-Di(4-(phenylselano)butyl)imidazo[4,5-*f*]benzimidazole (8b). (1.277 g, 44%); white solid; R_f 0.40 (EtOAc–MeOH 9 : 1); mp 125-127 °C; ν_{max} (neat, cm^{-1}) 3345, 2922, 1579, 1524, 1500, 1364, 1283, 1212, 1111, 1071, 1022; δ_H (400 MHz, $CDCl_3$) 1.68–1.76 (4H, m, CH_2), 2.01–2.08 (4H, m, CH_2), 2.87 (4H, t, *J* 7.0, CH_2SePh), 4.15 (4H, t, *J* 7.1, NCH_2), 7.14–7.19 (7H, m, Ph-H & Ar-8-H), 7.36–7.39 (4H, m, Ph-H), 7.81 (2H, s, Ar-2,6-H), 8.18 (1H, s, Ar-4-H); δ_C (100 MHz, $CDCl_3$) 27.2 (CH_2SePh), 27.3 (CH_2), 29.2 (CH_2), 44.7 (NCH_2), 88.4 (Ar-8-CH), 110.6 (Ar-4-CH), 127.2 (Ph-CH), 129.2 (Ph-CH), 129.7 (C), 132.0 (C), 133.0 (Ph-CH), 141.3 (Ar-3a,4a-C), 143.6 (Ar-2,6-CH); *m/z* (CI) 583.0857 ($M + H^+$, $C_{28}H_{31}N_4{}^{80}Se^{80}Se$ requires 583.0879).

1,5-Di(5-(phenylselano)pentyl)imidazo[5,4-*f*]benzimidazole (7c). (1.308 g, 43%); white solid; R_f 0.50 (EtOAc–MeOH 9 : 1); mp 115–117 °C; ν_{max} (neat, cm^{-1}) 3033, 2934, 2853, 1702, 1574, 1517, 1492, 1475, 1446, 1356, 1331, 1269, 1224, 1203, 1165, 1125, 1072, 1021; δ_H (400 MHz, $CDCl_3$) 1.41–1.49 (4H, m, CH_2), 1.69–1.77 (4H, m, CH_2), 1.89–1.96 (4H, m, CH_2), 2.86 (4H, t, *J* 7.3, CH_2SePh), 4.20 (4H, t, *J* 6.9, NCH_2), 7.22-7.27 (6H, m, Ph-H), 7.44–7.47 (4H, m, Ph-H), 7.71 (2H, s, Ar-4,8-H), 7.91 (2H, s, Ar-2,6-H); δ_C (100 MHz, $CDCl_3$) 26.9 (CH_2), 27.4 (CH_2SePh), 28.9 (CH_2), 29.6 (CH_2), 45.1 (NCH_2), 99.1 (Ar-4,8-CH), 126.9 (Ph-CH), 129.0 (Ph-CH), 130.1 (C), 131.4 (C), 132.6 (Ph-CH), 141.6 (Ar-3a,7a-C), 144.1 (Ar-2,6-CH); *m/z* (CI) 611.1177 ($M+H^+$, $C_{30}H_{35}N_4{}^{80}Se^{80}Se$ requires 611.1192).

1,7-Di(5-(phenylselano)pentyl)imidazo[4,5-*f*]benzimidazole (8c). (1.248 g, 41%); white solid; R_f 0.40 (EtOAc–MeOH 9 : 1); mp 78–81 °C; ν_{max} (neat, cm^{-1}) 3089, 2936, 1577, 1522, 1477, 1446, 1361, 1288, 1261, 1211, 1142, 1107, 1070, 1021; δ_H (400 MHz, $CDCl_3$) 1.43–1.51 (4H, m, CH_2), 1.70–1.77 (4H, m, CH_2), 1.87–1.95 (4H, m, CH_2), 2.86 (4H, t, *J* 7.2, CH_2SePh), 4.17 (4H, t, *J* 7.1, NCH_2), 7.17–7.28 (7H, m, Ph-H & Ar-8-H), 7.42–7.46 (4H, m, Ph-H), 7.88 (2H, s, Ar-2,6-H), 8.20 (1H, s, Ar-4-H); δ_C (100 MHz, $CDCl_3$) 27.0 (CH_2), 27.5 (CH_2SePh), 29.0 (CH_2), 29.7 (CH_2), 45.1 (NCH_2), 88.5 (Ar-8-CH), 110.2 (Ar-4-CH), 126.9 (Ph-CH), 129.2 (Ph-CH), 130.2 (C), 132.0 (C), 132.6 (Ph-CH), 140.9 (Ar-3a,4a-C), 143.7 (Ar-2,6-CH); *m/z* (CI) 611.1185 ($M + H^+$, $C_{30}H_{35}N_4{}^{80}Se^{80}Se$ requires 611.1192).

One-pot double homolytic aromatic substitution methods

Method 1: radical cyclizations without activation. To a solution of 1,5(7)-di(*ω*-(phenylselano)alkyl)imidazo[5,4-*f*(4,5-*f*)]benzimidazole (0.6 mmol) in toluene (50 mL) at reflux, and open to the air, was added a solution of 1,1′-azobis(cyclohexanecarbonitrile) (ACN) (0.733 g, 3.0 mmol) and tributyltin hydride (0.873 g, 3.0 mmol) in toluene (50 mL) using a syringe pump at a rate of 2.2 mL h^{-1}.

Method 2: radical cyclizations with activation using camphorsulfonic acid. To a solution of 1,5(7)-di(*ω*-(phenylselano)alkyl)-imidazo[5,4-*f*(4,5-*f*)]benzimidazole (0.6 mmol) and camphorsulfonic acid (0.302 g, 1.3 mmol) in toluene (50 mL) at reflux, and open to the air, was added a solution of ACN (0.733 g, 3.0 mmol) and tributyltin hydride (0.873 g, 3.0 mmol) in toluene (50 mL) using a syringe pump at a rate of 2.2 mL h^{-1}.

Method 3: radical cyclizations with activation using acetic anhydride. To a solution of 1,5(7)-di(*ω*-(phenylselano)alkyl)imidazo-[5,4-*f*(4,5-*f*)]benzimidazole (0.6 mmol) and acetic anhydride (0.123 mL, 1.3 mmol) in toluene (50 mL) at reflux, and open to the air, was added a solution of ACN (0.733 g, 3.0 mmol) and tributyltin hydride (0.873 g, 3.0 mmol) in toluene (50 mL) using a syringe pump at a rate of 2.2 mL h^{-1}.

General work up and purification for methods 1–3

The cooled mixture was left in sunlight for 3 h and extracted with acetic acid (80%, 5 × 20 mL). The combined acid extracts were washed with petroleum ether (3 × 20 mL), and evaporated. Sodium carbonate solution (5%, 50 mL) was added to the acid residue, and extracted with dichloromethane (5 × 50 mL). The combined organic extracts were dried (Na_2SO_4), and evaporated to dryness. The residue was purified by dry column vacuum chromatography with a gradient elution of ethyl acetate and methanol.

One-pot double five-membered radical cyclizations onto imidazo[5,4-*f*]benzimidazole. Following method 1, using **7a** and after purification by chromatography; 2,3,8,9-Tetrahydro-1*H*,7*H*-pyrrolo[1,2-a]pyrrolo[1′,2′:1,2]imidazo[5,4-*f*]benzimidazole (**9a**). (46 mg, 32%), white solid; R_f 0.24 (CH_2Cl_2–Et_2O–MeOH 7 : 2 : 1); mp 295–305 °C decomp; ν_{max} (neat, cm^{-1}) 3009, 2942, 2895, 1546, 1489, 1434, 1411, 1347, 1324, 1267, 1161, 1100; δ_H (400 MHz, $CDCl_3$) 2.77–2.84 (4H, m, 2,8-CH_2), 3.19 (4H, t, *J* 7.6, 3,9-CH_2), 4.22 (4H, t, *J* 7.1, 1,7-CH_2), 7.59 (2H, s, 5,11-H); δ_C (100 MHz, $CDCl_3$) 23.9 (3,9-CH_2); 26.0 (2,8-CH_2), 43.0 (1,7-CH_2), 98.5 (5,11-CH), 129.6 (5a,11a-C), 145.5 (4a,10a-C), 161.7 (3a,9a-C); *m/z* (ESI) 239.1307 ($M + H^+$, $C_{14}H_{15}N_4$ requires 239.1297),

1-Propyl-7,8-dihydro-6*H*-imidazo[5,4-*f*]pyrrolo[1,2-*a*]benzimidazole (**10a**). (45 mg, 31%), white solid; R_f 0.32 (CH_2Cl_2–Et_2O–MeOH 7 : 2 : 1); mp 210–213 °C; ν_{max} (neat, cm^{-1}) 3019, 2935, 1538, 1493, 1444, 1418, 1357, 1291, 1222, 1189, 1163, 1110, 1056; δ_H (400 MHz, $CDCl_3$) 0.97 (3H, t, *J* 7.3, CH_3); 2.01–1.92 (2H, m, CH_2CH_3), 2.71–2.79 (2H, m, 7-CH_2), 3.11 (2H, t, *J* 7.6, 8-CH_2), 4.15–4.21 (4H, m, NCH_2), 7.63 (2H, s, Ar-4,10-H), 7.92 (1H, s, Ar-2-H); δ_C (100 MHz, $CDCl_3$) 11.4 (CH_3), 22.8 (CH_2), 23.8 (8-CH_2), 25.9 (CH_2), 42.9 (NCH_2), 46.9 (NCH_2), 98.4 (Ar-CH), 98.9 (Ar-CH), 130.0 (C), 131.0 (C), 140.6 (C), 143.5 (Ar-2-CH), 146.6 (C), 162.4 (Ar-8a-C); *m/z* (ESI) 241.1452 ($M + H^+$, $C_{14}H_{17}N_4$ requires 241.1453), and

1,5-Dipropylimidazo[5,4-*f*]benzimidazole (**11a**). (14 mg, 10%), white solid; R_f 0.39 (CH_2Cl_2-Et_2O–MeOH 7 : 2 : 1); mp 205–208 °C; ν_{max} (neat, cm^{-1}) 3091, 3025, 2960, 2930, 2874, 1741, 1518, 1491, 1450, 1382, 1361, 1312, 1227, 1208, 1167, 1113, 1084; δ_H (400 MHz, $CDCl_3$) 0.94 (6H, t, *J* 7.3, CH_3), 1.90–1.99 (4H, m, CH_2), 4.18 (4H, t, *J* 7.1, NCH_2), 7.72 (2H, s, Ar-4,8-H); 7.93 (2H, s, Ar-2,6-H); δ_C (100 MHz, $CDCl_3$) 11.3 (CH_3), 22.7 (CH_2), 46.9 (NCH_2), 99.0 (Ar-4,8-CH), 131.4 (Ar-4a,8a-C), 141.6 (Ar-3a,7a-C), 144.2 (Ar-2,6-CH); *m/z* (ESI) 243.1618 ($M + H^+$, $C_{14}H_{19}N_4$ calcd 243.1610).

Following method 2, using **7a** an intractable mixture was obtained. Following method 3, using **7a** and after purification by chromatography, **9a** (67 mg, 47%) and **10a** (43 mg, 30%) were isolated only.

One-pot double six-membered radical cyclizations onto imidazo[5,4-*f*]benzimidazole. Following method 1, using **7b** and after purification by chromatography; 1,2,3,4,8,9,10,

11-Octahydropyrido[1,2-*a*]pyrido[1′,2′:1,2]imidazo[5,4-*f*]benzimidazole (**9b**). (0.144 g, 90%) white solid; R_f 0.23 (CH_2Cl_2–Et_2O–MeOH 7 : 2 : 1); mp 285–295 °C decomp (lit.,[30] >300 °C); ν_{max} (neat, cm^{-1}) 2942, 1534, 1488, 1445, 1417, 1362, 1316, 1279, 1259, 1190, 1165, 1130, 1092; δ_H (400 MHz, $CDCl_3$) 2.02–2.08 (4H, m, CH_2), 2.15–2.21 (4H, m, CH_2), 3.16 (4H, t, *J* 6.4, 4,11-CH_2), 4.14 (4H, t, *J* 6.0, 1,8-CH_2), 7.55 (2H, s, 6,13-H); δ_C (100 MHz, $CDCl_3$) 20.5 (CH_2), 22.6 (CH_2), 25.4 (4,11-CH_2), 42.8 (1,8-CH_2), 96.9 (6,13-CH), 132.1 (6a,13a-C), 138.1 (5a,12a-C), 152.4 (4a,11a-C); *m*/*z* (CI) 267.1605 (M + H^+, $C_{16}H_{19}N_4$ requires 267.1610).

One-pot double seven-membered radical cyclizations onto imidazo[5,4-*f*]benzimidazole. Following method 1, using **7c** and after purification by chromatography; 2,3,4,5,10,11,12,13-Octahydro-1*H*,9*H*-azepino[1,2-*a*]azepino[1′,2′:1,2]imidazo[5,4-*f*]benzimidazole (**9c**). (51 mg, 29%) white solid; R_f 0.38 (CH_2Cl_2–Et_2O–MeOH 7 : 2 : 1); mp 275–285 °C decomp; ν_{max} (neat, cm^{-1}) 2931, 2850, 1534, 1478, 1440, 1414, 1365, 1311, 1256, 1178, 1157, 1116, 1085; δ_H (400 MHz, $CDCl_3$) 1.78–1.91 (8H, m, CH_2), 1.92–1.98 (4H, m, CH_2), 3.11 (4H, t, *J* 5.6, 5,13-CH_2), 4.19 (4H, t, *J* 5.0, 1,9-CH_2), 7.46 (2H, s, 7,15-H); δ_C (100 MHz, $CDCl_3$) 25.7 (CH_2), 28.8 (CH_2), 30.5 (5,13-CH_2), 31.0 (CH_2), 44.7 (1,9-CH_2), 97.1 (7,15-CH), 133.2 (7a,15a-C), 139.5 (6a,14a-C), 158.2 (5a,13a-C); *m*/*z* (CI) 295.1916 (M + H^+, $C_{18}H_{23}N_4$ requires 295.1923),

1-Pentyl-7,8,9,10-tetrahydro-6*H*-azepino[1,2-*a*]imidazo[5,4-*f*]-benzimidazole (**10c**). (57 mg, 32%) white solid; R_f 0.43 (CH_2Cl_2–Et_2O–MeOH 7 : 2 : 1); mp 198–200 °C; ν_{max} (neat, cm^{-1}) 2924, 2855, 1528, 1476, 1447, 1358, 1313, 1260, 1205, 1188, 1118; δ_H (400 MHz, $CDCl_3$) 0.87 (3H, t, *J* 6.8, CH_3), 1.27–1.38 (4H, m, CH_2), 1.80–2.00 (8H, m, CH_2), 3.13 (2H, t, *J* 5.7, 10-CH_2), 4.19–4.23 (4H, m, NCH_2), 7.61 (2H, s, Ar-4,12-H), 7.91 (1H, s, Ar-2-H); δ_C (100 MHz, $CDCl_3$) 14.0 (CH_3), 22.3, 25.7, 28.8, 29.0, 29.2 (all CH_2), 30.5 (10-CH_2), 31.0 (CH_2), 44.7 (NCH_2), 45.4 (NCH_2), 98.0 (Ar-CH), 98.2 (Ar-CH), 131.1, 133.7, 140.3, 141.0 (all C), 143.6 (Ar-2-CH), 158.9 (Ar-10a-C); *m*/*z* (ESI) 297.2091 (M + H^+, $C_{18}H_{25}N_4$ requires 297.2079), and

1,5-Dipentylimidazo[5,4-*f*]benzimidazole (**11c**). (15 mg, 8%) white solid, R_f 0.49 (CH_2Cl_2–Et_2O–MeOH 7 : 2 : 1); mp 161–162 °C; ν_{max} (neat, cm^{-1}) 2923, 2855, 1715, 1517, 1494, 1452, 1375, 1357, 1318, 1222, 1194, 1164, 1118, 1061, 1029; δ_H (400 MHz, $CDCl_3$) 0.77 (6H, t, *J* 6.9, CH_3), 1.17–1.28 (8H, m, CH_2), 1.77–1.85 (4H, m, CH_2), 4.10 (4H, t, *J* 7.1, NCH_2), 7.64 (2H, s, Ar-4,8-H), 7.85 (2H, s, Ar-2,6-H); δ_C (100 MHz, $CDCl_3$) 13.9 (CH_3), 22.2, 29.0, 29.2 (all CH_2), 45.3 (NCH_2), 99.1 (Ar-4,8-CH), 131.5 (Ar-4a,8a-C), 141.6 (Ar-3a,7a-C), 144.2 (Ar-2,6-CH); *m*/*z* (ESI) 299.2238 (M + H^+, $C_{18}H_{27}N_4$ requires 299.2236).

Following method 2, using **7c** and after purification by chromatography **9c** (95 mg, 54%) and **10c** (23 mg, 13%) were isolated only. Following method 3, using **7c** and after purification by chromatography, **9c** (60 mg, 34%) and **10c** (55 mg, 31%) were isolated only.

One-pot double five-membered radical cyclizations onto imidazo[4,5-*f*]benzimidazole. Following method 3, using **8a** and after purification by chromatography, 2,3,8,9-Tetrahydro-1*H*,7*H*-pyrrolo[1,2-*a*]pyrrolo[1′,2′:1,2]imidazo[4,5-*f*]benzimidazole (12a). (69 mg, 48%) white solid; R_f 0.22 (CH_2Cl_2–Et_2O–MeOH 7 : 2 : 1); mp 266–268 °C decomp (lit.[16] 240 °C decomp); ν_{max} (neat, cm^{-1}) 3229, 2956, 1644, 1543, 1490, 1431, 1352, 1300, 1228, 1117; δ_H (400 MHz, $CDCl_3$) 2.64–2.71 (4H, m, 2,8-CH_2), 3.02 (4H, t, *J* 7.7, 3,7-CH_2), 4.06 (4H, t, *J* 7.1, 1,9-CH_2), 7.04 (1H, s, 11-H), 7.95 (1H, s, 5-H); δ_C (100 MHz, $CDCl_3$) 23.8 (3,7-CH_2), 26.1 (2,8-CH_2), 42.8 (1,9-CH_2), 88.7 (11-CH), 108.9 (5-CH), 129.5 (10a,11a-C), 145.6 (4a,5a-C), 161.2 (3a,6a-C); *m*/*z* (ESI) 239.1300 (M + H^+, $C_{14}H_{15}N_4$ requires 239.1297), and

1-Propyl-7,8-dihydro-6*H*-imidazo[4,5-*f*]pyrrolo[1,2-*a*]benzimidazole (**13a**). (40 mg, 27%) white solid; R_f 0.30 (CH_2Cl_2–Et_2O–MeOH 7 : 2 : 1); mp 168–171 °C; ν_{max} (neat, cm^{-1}) 3351, 3079, 2927, 1641, 1544, 1513, 1492, 1453, 1423, 1356, 1322, 1298, 1253, 1215, 1115; δ_H (400 MHz, $CDCl_3$) 0.98 (3H, t, *J* 7.6, CH_3), 1.91-2.00 (2H, m, CH_2CH_3), 2.70–2.78 (2H, m, 7-CH_2), 3.09 (2H, t, *J* 7.8, 6-CH_2), 4.12–4.17 (4H, m, NCH_2), 7.16 (1H, s, Ar-10-H), 7.89 (1H, s, Ar-4-H), 8.08 (1H, s, Ar-2-H); δ_C (100 MHz, $CDCl_3$) 11.5 (CH_3), 22.9 (CH_2), 23.8 (6-CH_2), 26.1 (CH_2), 42.9 (NCH_2), 47.0 (NCH_2), 88.5 (Ar-10-CH), 109.7 (Ar-4-CH), 130.6, 131.1, 140.7 (all C), 143.2 (Ar-2-CH), 146.2 (C), 161.9 (Ar-5a-C); *m*/*z* (ESI) 241.1457 (M + H^+, $C_{14}H_{17}N_4$ requires 241.1453).

One-pot double six-membered radical cyclizations onto imidazo[4,5-*f*]benzimidazole. Following method 1, using **8b** and after purification by chromatography; 1,2,3,4,8,9,10,11-Octahydropyrido[1,2-*a*]pyrido[1′,2′:1,2]imidazo[4,5-*f*]benzimidazole (**12b**). (0.129 g, 81%) white solid; R_f 0.20 (CH_2Cl_2–Et_2O–MeOH 7 : 2 : 1); mp 266–270 °C decomp (lit.,[17] 104–108 °C); ν_{max} (neat, cm^{-1}) 3372, 2950, 2891, 1659, 1635, 1526, 1487, 1438, 1417, 1365, 1313, 1255, 1238, 1151, 1134; δ_H (400 MHz, $CDCl_3$) 1.99–2.05 (4H, m, CH_2), 2.12–2.18 (4H, m, CH_2), 3.09 (4H, t, *J* 6.3, 4,8-CH_2), 4.07 (4H, t, *J* 6.0, 1,11-CH_2), 7.02 (1H, s, 13-H), 7.95 (1H, s, 6-H); δ_C (100 MHz, $CDCl_3$) 20.9 (CH_2), 22.9 (CH_2), 25.8 (4,8-CH_2), 42.6 (1,11-CH_2), 87.1 (13-CH), 107.4 (6-CH), 132.0 (12a,13a-C), 139.9 (5a,6a-C), 151.8 (4a,7a-C); *m*/*z* (CI) 267.1595 (M + H^+, $C_{16}H_{19}N_4$ requires 267.1610).

One-pot double seven-membered radical cyclizations onto imidazo[4,5-*f*]benzimidazole. Following method 2, using **8c** and after purification by chromatography; 2,3,4,5,10,11,12,13-Octahydro-1*H*,9*H*-azepino[1,2-*a*]azepino[1′,2′:1,2]imidazo[4,5-*f*]benzimidazole (**12c**). (85 mg, 48%) white solid; R_f 0.32 (CH_2Cl_2–Et_2O–MeOH 7 : 2 : 1); mp 214–217 °C decomp; ν_{max} (neat, cm^{-1}) 3377, 2928, 2852, 1638, 1527, 1470, 1447, 1422, 1367, 1345, 1323, 1230, 1200, 1140; δ_H (400 MHz, $CDCl_3$) 1.79–1.97 (12H, m, CH_2), 3.11 (4H, t, *J* 5.6, 5,9-CH_2), 4.18 (4H, t, *J* 5.1, 1,13-CH_2), 7.00 (1H, s, 15-H), 7.95 (1H, s, 7-H); δ_C (100 MHz, $CDCl_3$) 25.7 (CH_2), 28.9 (CH_2), 30.4 (5,9-CH_2), 31.0 (CH_2), 44.7 (1,13-CH_2), 87.0 (15-CH), 108.0 (7-CH), 133.3 (14a,15a-C), 139.1 (6a,7a-C), 157.6 (5a,8a-C); *m*/*z* (ESI) 295.1911 (M + H^+, $C_{18}H_{23}N_4$ requires 295.1923).

1-Pentyl-7,8,9,10-tetrahydro-6*H*-azepino[1,2-*a*]imidazo[4,5-*f*]-benzimidazole (**13c**). (29 mg, 16%); colourless oil; R_f 0.41 (CH_2Cl_2–Et_2O–MeOH 7 : 2 : 1); ν_{max} (neat, cm^{-1}) 2926, 2855, 1638, 1535, 1510, 1489, 1448, 1365, 1286, 1254, 1220, 1193, 1126; δ_H (400 MHz, $CDCl_3$) 0.89 (3H, t, *J* 6.9, CH_3), 1.31–1.39 (4H, m, CH_2), 1.82-1.96 (8H, m, CH_2), 3.11 (2H, t, *J* 5.6, 6-CH_2), 4.16-4.21 (4H, m, NCH_2), 7.10 (1H, s, Ar-12-H), 7.88 (1H, s, Ar-4-H), 8.07 (1H, s, Ar-2-H); δ_C (100 MHz, $CDCl_3$) 14.0 (CH_3), 22.3, 25.7, 28.9, 29.1, 29.2 (all CH_2), 30.5 (6-CH_2), 31.0 (CH_2), 44.8 (NCH_2), 45.3 (NCH_2), 87.6 (Ar-12-CH), 109.2 (Ar-4-CH), 131.3, 134.1, 139.7, 140.7 (all C), 143.2 (Ar-2-CH), 158.2 (Ar-5a-C); *m*/*z* (ESI) 297.2089 (M + H^+, $C_{18}H_{25}N_4$ requires 297.2079).

Preparation of imidazobenzimidazolequinones

6-Nitro-1,2,3,4,8,9,10,11-octahydropyrido[1,2-*a*]pyrido[1′,2′:1,2]imidazo[5,4-*f*]benzimidazole (15). Concentrated HNO_3 (10 mL) was added to a solution of **9b** (0.240 g, 0.9 mmol) in concentrated H_2SO_4 (10 mL) at 0 °C and heated to 80 °C for 3 h. The cooled solution was neutralised with solid sodium carbonate and extracted with dichloromethane (6 × 50 mL). The combined organic extracts were dried ($MgSO_4$) and evaporated. The residue was purified by dry column vacuum chromatography with a gradient elution of ethyl acetate and methanol to yield the *title compound* **15** (0.263 g, 94%); yellow solid; R_f 0.48 (EtOAc–MeOH 9 : 1); mp 230–236 °C decomp; ν_{max} (neat, cm^{-1}) 2952, 1735, 1542, 1514 (NO_2), 1478, 1431, 1415, 1360 (NO_2), 1335, 1310, 1272, 1255, 1205, 1170, 1145, 1076, 1043; δ_H (400 MHz, $CDCl_3$) 2.01–2.29 (8H, m, CH_2), 3.13–3.22 (4H, m, 4,11-CH_2), 4.12–4.21 (4H, m, 1,8-CH_2), 7.69 (1H, s, 13-H); δ_C (100 MHz, $CDCl_3$) 20.1, 20.5, 22.5, 23.2 (all CH_2), 26.0, 26.4 (4,11-CH_2), 43.0, 45.7, (1,8-CH_2), 102.8 (13-CH), 124.5, 124.6, 132.5, 134.1, 141.0 (all C), 154.4, 155.4, (4a,11a-C); *m/z* (ESI) 312.1460 (M + H^+, $C_{16}H_{18}N_5O_2$ requires 312.1461).

6-Imino-1,2,3,4,8,9,10,11-octahydropyrido[1,2-*a*]pyrido[1′,2′:1,2]imidazo[5,4-*f*]benzimidazol-13-one (17). A mixture of **15** (0.275 g, 0.9 mmol) and Pd–C (10%, 25 mg) in methanol (100 mL) was stirred under 40 psi H_2 at room temperature for 16 h. The catalyst was removed by filtration and the filtrate evaporated to give 6-amino-1,2,3,4,8,9,10,11-octahydropyrido[1,2-a]pyrido[1′,2′:1,2]imidazo[5,4-f]benzimidazole (**16**) as a brown residue (not purified further); δ_H (400 MHz, $CDCl_3$) 1.97–2.08 (4H, m, CH_2), 2.10–2.20 (4H, m, CH_2), 3.10–3.06 (4H, m, 4,11-CH_2), 4.07 (2H, t, *J* 6.1, NCH_2), 4.36 (2H, bs, NH_2), 4.62 (2H, t, *J* 6.1, NCH_2), 6.97 (1H, s, 13-H). Monobasic potassium phosphate solution (50 mL, 0.2 M) was added to the residue of **16**, and a separate solution containing potassium nitrosodisulfonate (Fremy's salt) (0.725 g, 2.7 mmol) in monobasic potassium phosphate solution (50 mL, 0.2 M) was added. The solution was stirred for 1 h at room temperature and extracted with dichloromethane (6 × 50 mL). The combined organic extracts were dried ($MgSO_4$) and evaporated. The residue was purified by dry column vacuum chromatography with gradient elution of ethyl acetate and methanol to yield the *title compound* **17** (0.238 g, 91%) as a yellow solid; R_f 0.30 (EtOAc–MeOH 9 : 1); mp 230–240 °C decomp; ν_{max} (neat, cm^{-1}) 2948, 2867, 1656, (C=O), 1515, 1494, 1442, 1425, 1405, 1377, 1336, 1306, 1272, 1219, 1167, 1075, 1042; δ_H (400 MHz, $CDCl_3$) 1.93–2.08 (8H, m, CH_2), 2.92–3.00 (4H, m, 4,11-CH_2), 4.37–4.43 (4H, m, 1,8-CH_2), 10.55 (1H, s, NH); δ_C (100 MHz, $CDCl_3$) 19.9, 20.0, 22.3, 22.7 (all CH_2), 25.0, 25.3 (4,11-CH_2), 45.4, 46.5, (1,8-CH_2), 126.0 (C), 131.5 (C), 139.7 (2 × C), 150.2 (2 × C), 155.5 (C=N), 172.0 (C=O); *m/z* (ESI) 296.1502 (M + H^+, $C_{16}H_{18}N_5O$ requires 296.1511).

1,2,3,4,8,9,10,11-Octahydropyrido[1,2-*a*]pyrido[1′,2′:1,2]imidazo[5,4-*f*]benzimidazol-6,13-dione (4). 6-Iminoquinone **17** (0.238 g, 0.8 mmol) was dissolved in hydrochloric acid (50 mL, 2 M) and stirred for 15 min. The pH of the solution was adjusted to pH 6 using solid sodium carbonate and the product extracted using dichloromethane (6 × 50 mL). The combined organic extracts were dried ($MgSO_4$) and evaporated to dryness. The residue was purified by dry column vacuum chromatography to yield the *title compound* **4** (0.229 g, 96%) as a yellow solid; R_f 0.32 (EtOAc–MeOH 9 : 1); mp 265–275 °C decomp; ν_{max} (neat, cm^{-1}) 2948, 1655 (C=O), 1493, 1377, 1336, 1305, 1218, 1076, 1042; δ_H (400 MHz, $CDCl_3$) 1.98–2.10 (8H, m, CH_2), 2.99 (4H, t, *J* 6.2, 4,11-CH_2), 4.35 (4H, t, *J* 5.9, 1,8-CH_2); δ_C (100 MHz, $CDCl_3$) 19.8 (CH_2), 22.2 (CH_2), 25.1 (4,11-CH_2), 45.6 (1,8-CH_2), 130.2 (6a,13a-C), 141.8 (5a,12a-C), 151.4 (4a,11a-C), 171.6 (C=O); *m/z* (ESI) 297.1365 (M + H^+, $C_{16}H_{17}N_4O_2$ requires 297.1352).

1,2,3,4,8,9,10,11-Octahydropyrido[1,2-*a*]pyrido[1′,2′:1,2]imidazo[4,5-*f*]benzimidazol-6,13-dione (5). Following a literature procedure[17] pyrido[1,2-*a*]pyrido[1′,2′:1,2]imidazo[4,5-*f*]benzimidazole **12b** (0.158 g, 0.6 mmol) yielded the *title compound* **5** (0.121 g, 69%) as a yellow solid; R_f 0.30 (EtOAc–MeOH 9 : 1); mp 221–223 °C decomp (lit.,[17] >260 °C); ν_{max} (neat, cm^{-1}) 2941, 1675, 1652 (C=O), 1493, 1442, 1421, 1331, 1295, 1163, 1073, 1039; δ_H (400 MHz, $CDCl_3$) 1.94-2.09 (8H, m, CH_2), 2.98 (4H, t, *J* 6.4, 4,8-CH_2), 4.32 (4H, t, *J* 6.0, 1,11-CH_2); δ_C (100 MHz, $CDCl_3$) 19.8 (CH_2), 22.4 (CH_2), 25.0 (4,8-CH_2), 45.5 (1,11-CH_2), 130.3 (12a,13a-C), 142.5 (5a,6a-C), 151.4 (4a,7a-C), 169.8 (13-C=O), 175.5 (6-C=O); *m/z* (ESI) 297.1352 (M + H^+, $C_{16}H_{17}N_4O_2$ requires 297.1355).

Cell culture and cytotoxicity evaluation

Cell lines. An 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 HeLa cervical cancer cell line (repository number CCL-2) was obtained from the American Type Culture Collection (ATCC). The DU145 prostate cancer cell line (ATCC repository number HTB-81) was obtained from Prof. R.W.G Watson, School of Medicine & Medical Science, University College Dublin, Ireland.

Cell culture. The SV40-transformed normal human skin fibroblast cell line (GM00637) was grown in minimum essential media (MEM) Eagle-Earle BSS supplemented with 15% heat-inactivated fetal bovine serum (FBS), penicillin-streptomycin, 2 mM L-glutamine, 2× essential and non-essential amino acids, and vitamins. HeLa-CCL-2 cervical cancer cells were grown in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% heat-inactivated fetal bovine serum, penicillin-streptomycin, 2 mM L-glutamine, and MEM non-essential amino acids. DU145 prostate cancer cells were grown in RPMI-1640 medium supplemented with 10% heat-inactivated fetal bovine serum, penicillin-streptomycin and 2 mM L-glutamine.

Cytotoxicity measurement. Cell viability was determined using the MTT colorimetric assay.[28] Normal cells were plated into 96-well plates at a density of 10000 cells per well (200 μL per well) and allowed to adhere over a period of 24 h. HeLa cells were plated into 96-well plates at a density of 1000 cells per well (100 μL per well) and allowed to adhere over a period of 24 h. DU145 cells were plated into 96-well plates at a density of 2000 cells per well (200 μL per well) and allowed to adhere over a period of 24 h.

Compound solutions were applied in ethanol–H_2O (for **4**) or ethanol (for **5** and **17**) (1% final concentration in well), and the plates were incubated at 37 °C under a humidified atmosphere containing 5% CO_2 for 72 h. Control cells were

exposed to an equivalent concentration of ethanol or ethanol–H_2O alone. **MMC** (Sigma) solutions were applied in DMSO (1% final concentration in well) and the plates were incubated at 37 °C under a humidified atmosphere containing 5% CO_2 for 72 h. Control cells were exposed to an equivalent concentration of DMSO alone. MTT (20 μL, 5 mg ml^{-1} solution) was added, and the cells were incubated for another 3 h. The supernatant was 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 EtOH–H_2O, EtOH or DMSO-only treated value. Dose–response curves were analysed by nonlinear regression analysis and IC_{50} values were estimated using GraphPad Prism software, v. 5.02 (GraphPad Inc., San Diego, CA, USA).

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