European Journal of Medicinal Chemistry 45 (2010) 3762–3769

Contents lists available at ScienceDirect

European Journal of Medicinal Chemistry

journal homepage: http://www.elsevier.com/locate/ejmech

Original article

Synthesis and toxicity towards normal and cancer cell lines of benzimidazolequinones containing fused aromatic rings and 2-aromatic ring substituents

Eoin Moriarty [a], Miriam Carr [a], Sarah Bonham [a], Michael P. Carty [b], Fawaz Aldabbagh [a,*]

[a] School of Chemistry, National University of Ireland, Galway, Ireland
[b] Biochemistry, School of Natural Sciences, National University of Ireland, Galway, Ireland

ARTICLE INFO

Article history:
Received 15 April 2010
Received in revised form
7 May 2010
Accepted 10 May 2010
Available online 19 May 2010

Keywords:
Bioreductive
Heterocyclic compounds
NQO1
Quinones

ABSTRACT

A facile 6-*exo*-*trig* cyclization of σ-aromatic radicals has allowed the synthesis of various aromatic ring fused benzimidazoles and benzimidazolequinones. The most highly conjugated naphthyl fused benzimidazolequinone, (5-methyl-5,6-dihydrobenzimidazo[2,1-*a*]benzo[*f*]isoquinoline-8,11-dione) showed the highest specificity towards human cervical (HeLa) and prostate (DU145) cancer cell lines with little toxicity towards a human normal (GM00637) cell line at doses of <1 μM. In contrast, 2-aromatic ring substituted (benzimidazole-4,7-diones) analogues, benzimidazolequinone with a pyridine ring and mitomycin C were more toxic than the highly conjugated naphthyl fused benzimidazolequinone towards the normal cell line.

1. Introduction

Many antitumor agents require metabolic activation to exert their cytotoxic or cytostatic effects [1,2]. The clinically used natural product, mitomycin C (**MMC**, Fig. 1) is a prodrug that requires bioreductive activation in order to exert antitumor activity by damaging DNA through monofunctional and bifunctional alkylation [3]. One or two electron reductive activation occurs via the quinone moiety leading to reactive sites at C-1 (after aziridinyl ring-opening) and C-10 (after carbamate elimination) for DNA alkylation. Benzimidazolequinone alternatives containing aziridinyl [4–8] and other labile groups [9–11] have been developed; however there are a number of reported benzimidazolequinones that have anticancer activity despite lacking reactive sites that upon reductive activation can undergo DNA alkylation [12–16]. This includes the series of benzimidazole-4,7-diones containing pyridinyl [12,14] and thiazolyl [13] groups at the 2-position, reported by Garuti and co-workers. The nature of the quinone substituent, and the structure of the heterocyclic group attached at the benzimidazole-2-position were shown to influence cytotoxicity against selected human tumor cell lines. Others have prepared benzimidazole-4,7-dione with a 2-phenyl substituent reporting little toxicity towards human tumor cell lines (incl. DU145 [17]), and 6-arylamino-5-chloro-2-(2-pyridinyl)benzimidazole-4,7-diones exhibiting antifungal activity [18].

We now report the first biological evaluation of benzimidazolequinones containing fused aromatic rings (aryl, pyridinyl or naphthyl rings: compounds **1**–**3**, Fig. 1), and cytotoxicity of 2-aromatic ring substituted (benzimidazole-4,7-diones) analogues (compounds **4**–**6**). The premise is that the highly conjugated structure will lead to an increased tendency for bioreduction due to the subsequent stability of the reduced intermediates formed. This is expected to be manifested in selective toxicity towards cancer cell lines having elevated levels of reductase enzymes. There is evidence for several benzimidazolequinones [4,11,19] acting as substrates for the obligate two electron quinone reductase enzyme, NAD(P)H:quinone oxidoreductase (NQO1, also known as DT-diaphorase [20,21]). This article evaluates the cytotoxicity of compounds using two human cancer cell lines known to have high NQO1 activity: cervical (HeLa [22]) and prostate (DU145 [23]) cancer cell lines. Cytotoxicity towards these cancer cell lines is compared to a normal human skin fibroblast cell line (GM00637 [7,8,16]).

2. Results and discussion

2.1. Chemistry

2.1.1. Synthesis of benzimidazole-4,7-diones possessing 2-aromatic ring substituents

2-Phenyl and 2-pyridinyl benzimidazoles **7** and **8** (Scheme 1) were obtained using literature procedures [14,18] from the

* Corresponding author. Tel.: +353 91 493120; fax: +353 91 495700.
E-mail address: fawaz.aldabbagh@nuigalway.ie (F. Aldabbagh).

0223-5234/$ – see front matter © 2010 Elsevier Masson SAS. All rights reserved.
doi:10.1016/j.ejmech.2010.05.025

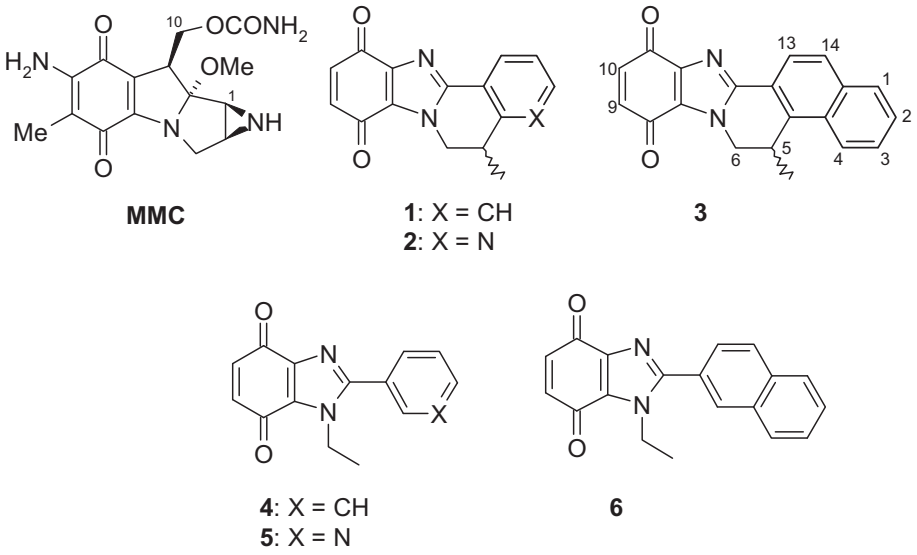


Fig. 1. Mitomycin C and aromatic ring fused and 2-substituted benzimidazolequinones.

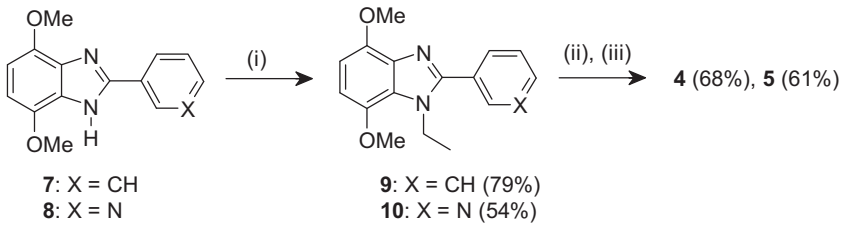
condensation of 3,6-dimethoxybenzene-1,2-diamine with the appropriate aromatic aldehyde. Analogous condensation using 2-naphthaldehyde gave novel 4,7-dimethoxy-2-(2-naphthyl)-1*H*-benzimidazole **11** in 79% yield (Scheme 2). Reaction with ethyl iodide in the presence of sodium hydride gave 1-ethyl-4,7-dimethoxy-2-aromatic ring substituted benzimidazoles in yields of 54–79%. Formation of target quinones **4**–**6** via hydrobromic acid induced demethylation of the 4,7-dimethoxy substituents of **9**, **10** and **12**, and oxidation of the reactive hydroquinones occurred in 61–77% yield. It should be noted that the latter intermediates are difficult to isolate and characterize prior to room temperature oxidation using ferric chloride, and thus are not evaluated in this article.

2.1.2. Synthesis of aromatic ring fused benzimidazolequinones

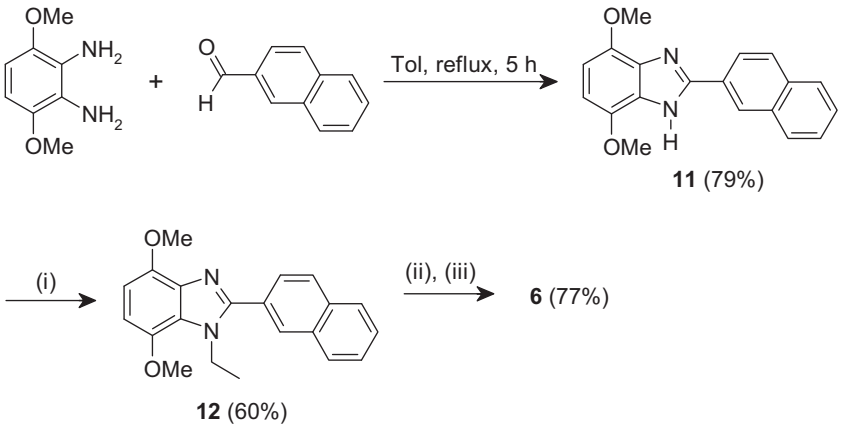
The formation of the new 5,6-dihydrobenzimidazo[2,1-*f*]-1,6-naphthyridine ring system was achieved via a 6-*exo*-*trig* radical cyclization of reactive σ-pyridinyl radical **15a** closing to form the more stable methylene radical **15b** under chain reaction conditions (Scheme 3). The efficient Bu_3SnH-mediated six-membered cyclization of σ-aryl and σ-naphthyl radicals was the subject of a recent communication [24]. We now report the synthesis of 5-methyl-5,6-dihydrobenzimidazo[2,1-*a*]benzo[*f*]isoquinoline-8,11-dione **3** in full (Scheme 4). The condensation of (3,6-dimethoxy)benzene-1,2-diamine with 1-bromo-2-naphthoic acid **17** in the presence of polyphosphoric acid (PPA) was effective in giving 2-(1-bromo-2-naphthyl)-1*H*-benzimidazoles **18a** and **18b**, but attempts to condense 2-bromonicotinic acid did not give high yields of 2-(2-bromopyridin-3-yl)-1*H*-benzimidazoles **13a** and **13b** [24,25]. The latter compounds could be efficiently prepared in 85–98% yield from an ammonium chloride-mediated condensation using nicotinaldehyde, according to a modification of a literature method for making 2-aryl substituted benzimidazoles [26]. Radical precursors **14a** and **14b** and **19a** and **19b** were obtained in yields of 86–95% by reaction with allyl bromide in the presence of sodium hydride. The radical cyclizations proceeded to give **16a** and **16b** and **20a** and **20b** in about 70% yield. The presence of 4,7-methoxy substituents in **14b** and **19b** was found to have no discernible effect on the cyclization. Target quinones **2** and **3** were obtained in over 75% yield from dimethoxy cyclized adducts **16b** and **20b** respectively using the well-established hydrobromic acid/ferric chloride procedure [16].

2.2. Cytotoxicity evaluation

The cytotoxicity of aromatic ring fused benzimidazolequinones **1**–**3** and non-cyclized analogues with an aromatic ring at the 2-position **4**–**6** was evaluated against cancer cell lines reported to contain high DT-diaphorase activity: cervical (HeLa [22]) and prostate (DU145 [23]) cancer. For comparison purposes cytotoxicity against a normal human skin fibroblast cell line (GM00637) was also determined. Each cell line was treated with solutions of 0.001–10 μM of compound, and with **MMC** in parallel. Aryl ring fused compounds **1** and **3** were about 10–60 times less toxic towards the three cell lines than 2-phenyl and 2-naphthyl substituted benzimidazolequinone analogues **4** and **6** (Table 1). Aryl ring fused compounds were more toxic towards the cancer cell lines than the normal GM00637 cell line, with the highest toxicity towards the prostate cancer cell line. The naphthyl fused compound **3** showed the highest specificity towards the cancer cell lines in this dose range (up to 5 μM, Fig. 2). The negligible toxicity of **3** towards



Scheme 1. Reagents and conditions; (i) NaH, Et-I, THF, reflux, 2 h; (ii) 48% HBr (aq), reflux, 3 h; (iii) $FeCl_3$ (aq), rt, 18 h.

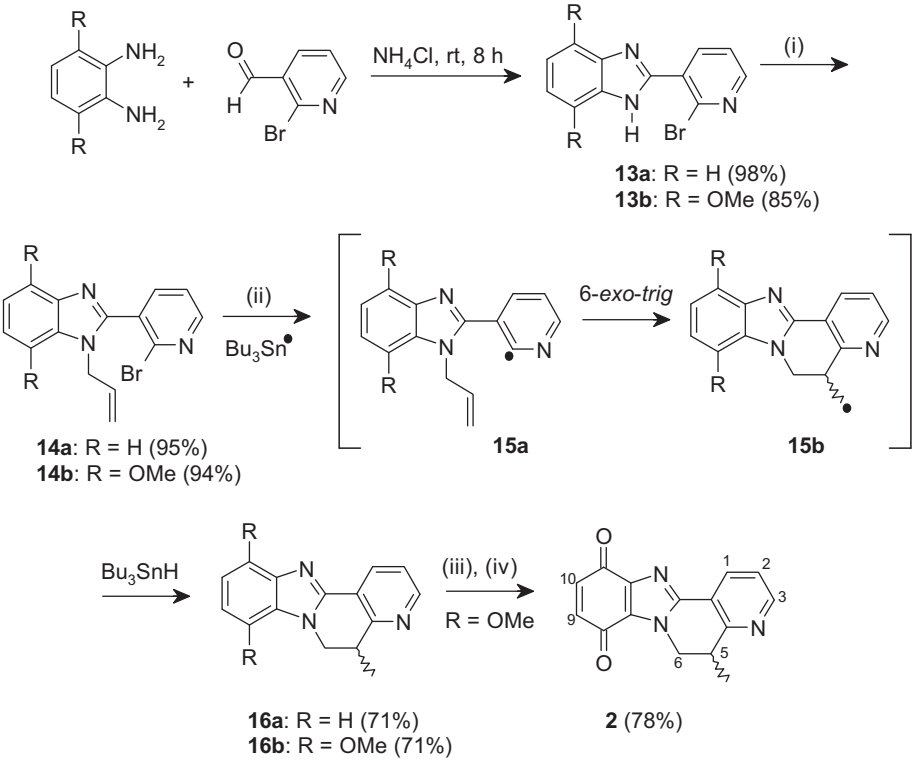


Scheme 2. Reagents and conditions; (i) NaH, Et-I, THF, reflux, 2 h; (ii) 48% HBr (aq), reflux, 3 h; (iii) $FeCl_3$ (aq), rt, 18 h.

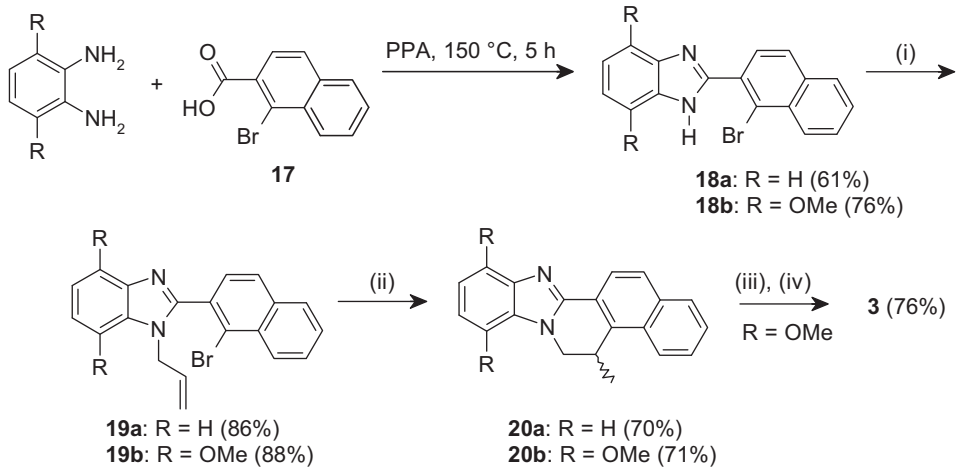
the normal cell line at sub-micromolar concentrations may be of therapeutic advantage. The higher specificity of compound **3** (compared to other benzimidazolequinones evaluated) towards cancer cell lines reported to have elevated reductase activity, may be related to the resonance stabilization of the chemically reduced intermediates formed, since this is the most conjugated quinone system prepared. In contrast benzimidazole-4,7-diones **4**–**6** without fused aromatic rings showed little specificity towards the two cancer cell lines, with 1-ethyl-2-(2-naphthyl)benzimidazole-4,7-dione **6** showing very high toxicity towards all three cell lines evaluated. Perhaps the flexibility of the 2-aromatic ring substituent in aryl substituted compounds **4** and **6** increases the overall toxicity, and reduces the specificity of compounds towards the cancer cell lines. Pyridinyl fused and substituted compounds **2** and **5** were more toxic towards the prostate than the cervical cancer cell line, however both compounds were significantly toxic towards the normal cell line. The requirement for the quinone moiety in cytotoxicity is confirmed by evaluating benzimidazole **20a**, the analogue of the most promising benzimidazolequinone **3**. This non-quinone containing compound was found to be essentially non-toxic towards the normal and cervical cancer cell lines evaluated. Similarly to 1-ethyl-2-phenyl-1*H*-benzimidazole-4,7-dione **4**, sub-micromolar concentrations of **MMC** were cytotoxic towards all three cell lines, although **MMC** showed marginal selectivity towards the two cancer cell lines.

3. Conclusion

The synthesis of new aromatic ring fused benzimidazoles and benzimidazolequinones has been achieved via a facile 6-*exo-trig*



Scheme 3. Reagents and conditions; (i) NaH, CH_2=$CHCH_2Br$, THF, reflux, 4 h; (ii) Bu_3SnH (1.4 equiv), ACN (0.2 equiv), 8 h, Tol, reflux; (iii) 48% HBr (aq), reflux, 3 h; (iv) $FeCl_3$ (aq), rt, 18 h.



Scheme 4. Reagents and conditions; (i) NaH, CH_2=$CHCH_2Br$, THF, reflux, 4 h; (ii) Bu_3SnH (1.4 equiv), ACN (0.2 equiv), 8 h, Tol, reflux; (iii) 48% HBr (aq), reflux, 3 h; (iv) $FeCl_3$ (aq), rt, 18 h.

cyclization of σ-aromatic radicals. The naphthyl fused benzimidazolequinone **3** showed the greatest specificity towards two cancer cell lines (HeLa and DU145). This supports our premise of increasing toxicity towards cancer cell lines that express higher levels of reductases, with increased quinone conjugation. Incorporating a pyridine ring was found to increase toxicity towards the normal (GM00637) cell line. 1-Ethyl-2-aromatic ring substituted benzimidazole-4,7-diones showed higher toxicity than aromatic ring fused analogues towards GM00637 cells, with little specificity towards cancer cell lines. 1-Ethyl-2-(2-naphthyl)benzimidazole-4,7-dione **6** (the non-fused analogue of **3**) was exceptionally toxic towards all three cell lines evaluated.

4. Experimental

4.1. Materials

Syntheses were carried out using commercially available starting materials from Sigma–Aldrich (apart from 2-bromonicotinaldehyde obtained from Frontier Scientific). 3,6-Dimethoxybenzene-1,2-diamine was obtained from the reduction of 1,4-dimethoxy-2,3-dinitrobenzene [27]. All compounds described below are reported for the first time, apart from the preparation of 5-methyl-5,6-dihydrobenzimidazo[2,1-*a*]isoquinoline-8,11-dione **1** and 5-methyl-5,6-dihydrobenzimidazo[2,1-*a*]benzo[*f*]isoquinoline **20a** described in the preliminary synthetic communication on this work [24]. 4,7-Dimethoxy-2-phenyl-1*H*-benzimidazole **7** and 4,7-dimethoxy-2-pyridin-3-yl-1*H*-benzimidazole **8** were prepared according to the literature from the condensation of 3,6-dimethoxybenzene-1,2-diamine with benzaldehyde and nicotinaldehyde respectively [14,18]. 1-Bromo-2-naphthoic acid **17** was prepared according to the literature oxidation of 1-bromo-2-methylnaphthalene [28].

Table 1
Effect of compounds on the viability of human normal skin fibroblast (GM00637), and cervical (HeLa) and prostate (DU145) cancer cell lines.

Compound	IC_{50}[a] (μmol/L)	IC_{50}[a] (μmol/L)	IC_{50}[a] (μmol/L)
	GM00637	HeLa	DU145
MMC	0.46 ± 0.09	0.27 ± 0.16	0.17 ± 0.02
1	1.65 ± 0.57	1.49 ± 0.48	1.09 ± 0.38
2	0.74 ± 0.14	1.51 ± 0.41	0.70 ± 0.11
3	2.44 ± 0.65	1.17 ± 0.31	0.78 ± 0.40
4	0.15 ± 0.04	0.18 ± 0.08	0.07 ± 0.02
5	0.48 ± 0.06	1.50 ± 0.08	1.27 ± 0.05
6	0.04 ± 0.01	0.07 ± 0.01	0.06 ± 0.02
20a	>10	>10	–

[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 MMT assay.

4.2. Instrumentation

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 Joel 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 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.

4.3. Preparation of 4,7-dimethoxy-2-(2-naphthyl)-1H-benzimidazole (*11*)

3,6-Dimethoxybenzene-1,2-diamine (1.800 g, 11 mmol) and 2-naphthaldehyde (1.400 g, 9 mmol) in toluene (30 mL) were

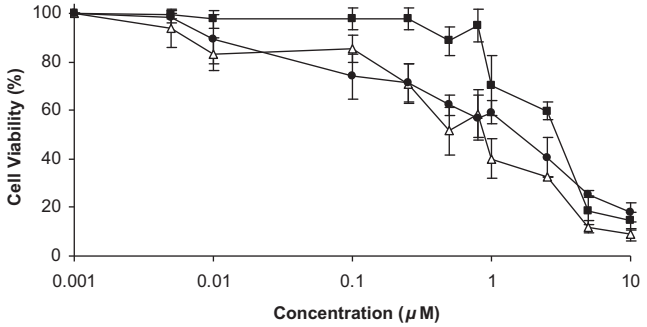


Fig. 2. Viability of a normal human skin fibroblast cell line (GM00637) (■) and human cervical (HeLa) (●) and prostate (DU145) (△) cancer cell lines determined using the MTT assay following treatment with 5-methyl-5,6-dihydrobenzimidazo[2,1-*a*]benzo[*f*]isoquinoline-8,11-dione **3** under aerobic conditions for 72 h at 37 °C. Each data point is the mean of at least three independent experiments.

refluxed for 5 h. The solution was cooled and evaporated to dryness to yield a brown residue, which was purified by column chromatography using silica gel as absorbent with chloroform as eluent to give **11** (2.181 g, 79%) as a brown solid; R_f 0.45 (50% petrol:50% EtOAc); mp 235–237 °C. IR (neat) 1071, 1094, 1167, 1204, 1264, 1338, 1401, 1418, 1444, 1499, 1519, 1541, 2933 cm^{-1}; ^1H NMR (400 MHz, DMSO-d_6): δ 8.83 (s, 1H, Naph-1-H), 8.35 (m, 1H, Naph-H), 8.01–7.95 (m, 3H, Naph-H), 7.58–7.52 (m, 2H, Naph-H), 6.64 (d (ABq), $J = 8.3$, 1H, BnIm-5(6)-H), 6.55 (d(ABq), $J = 8.3$, 1H, Ar-(5)6-H), 3.89 (s, 3H, OCH_3), 3.88 (s, 3H, OCH_3), NH not observed; ^{13}C NMR (100 MHz, DMSO-d_6): δ 150.4 (BnIm-2-C), 146.1, 141.0, 136.0, 133.8, 133.4 (all C), 129.0, 128.8, 128.3, 128.2, 127.5, 127.3, 127.1 (all CH), 126.5, 124.7 (C), 103.8, 103.0 (BnIm-5,6-CH), 56.3 ($2 \times OCH_3$); HRMS (ESI^-): m/z calcd for $C_{19}H_{15}N_2O_2$: 303.1134; found 303.1148 $[M - H]^-$.

4.4. General method for preparation of 1-ethyl-4,7-dimethoxy-2-aromatic ring substituted benzimidazoles

4.4.1. 1-Ethyl-4,7-dimethoxy-2-phenyl-1H-benzimidazole (**9**)

Benzimidazole **7** (0.740 g, 2.9 mmol) and NaH (86 mg, 3.6 mmol) in THF (10 mL) were refluxed for 30 min. Iodoethane (0.23 mL, 2.9 mmol) was added and refluxed for 1.5 h. The solution was evaporated to dryness and the residue purified by column chromatography using silica gel as absorbent with gradient elution of petrol and EtOAc to give **9** (0.647 g, 79%) as a yellow oil; R_f 0.43 (50% petrol:50% EtOAc). IR (neat) 1059, 1102, 1145, 1204, 1280, 1380, 1462, 1518, 2834 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): δ 7.70–7.66 (m, 2H), 7.45–7.42 (m, 3H), 6.56 (d(ABq), $J = 8.6$, 1H, BnIm-(6)5-H), 6.52 (d(ABq), $J = 8.6$, 1H, BnIm-(5)6-H), 4.37 (q, $J = 7.1$, 2H, CH_2), 3.94 (s, 3H, OCH_3), 3.88 (s, 3H, OCH_3), 1.35 (t, $J = 7.1$, 3H, CH_3); ^{13}C NMR (100 MHz, $CDCl_3$): δ 152.8 (BnIm-2-C), 146.1, 141.6, 135.2, 130.7 (all C), 129.7, 129.6, 128.5 (all CH), 126.2 (C), 103.2, 101.7 (BnIm-5,6-CH), 55.9 ($2 \times OCH_3$), 41.5 (CH_2), 17.3 (CH_3); HRMS (ESI^+): m/z calcd for $C_{17}H_{19}N_2O_2$: 283.1447; found 283.1445 $[M + H]^+$.

4.4.2. 1-Ethyl-4,7-dimethoxy-2-pyridin-3-yl-1H-benzimidazole (**10**)

Benzimidazole **8** (0.200 g, 0.8 mmol), NaH (24 mg, 1.0 mmol), and iodoethane (0.06 mL, 0.8 mmol), after purification by column chromatography gave **10** (0.120 g, 54%) as a yellow oil; R_f 0.33 (90% EtOAc:10% MeOH). IR (neat) 1059, 1102, 1145, 1204, 1280, 1380, 1462, 1518, 2834, 2934 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): δ 8.93 (d, $J = 1.8$, 1H, Pyr-2-H), 8.70 (dd, $J = 4.8$, $J = 1.8$, 1H, Pyr-6-H), 8.06 (d, $J = 7.6$, 1H, Pyr-4-H), 7.43–7.40 (dd, $J = 7.6$, $J = 4.8$, 1H, Pyr-5-H), 6.60 (d(ABq), $J = 8.5$, 1H, BnIm-(6)5-H), 6.55 (d(ABq), $J = 8.5$, 1H, BnIm-(5)6-H), 4.41 (q, $J = 7.1$, 2H, CH_2), 3.96 (s, 3H, OCH_3), 3.91 (s, 3H, OCH_3), 1.39 (t, $J = 7.1$, 3H, CH_3); ^{13}C NMR (100 MHz, $CDCl_3$): δ 150.5 (Pyr-6-CH), 149.9 (Pyr-2-CH), 149.6, 146.1, 141.6 (all C), 137.4 (Pyr-4-CH), 135.3, 127.0, 126.3 (all C), 123.5 (Pyr-5-CH), 103.6, 101.9 (BnIm-5,6-CH), 56.0 ($2 \times OCH_3$), 41.7 (CH_2), 17.4 (CH_3); HRMS (ESI^+): m/z calcd for $C_{16}H_{18}N_3O_2$: 284.1399; found 284.1413 $[M + H]^+$.

4.4.3. 1-Ethyl-4,7-dimethoxy-2-(2-naphthyl)-1H-benzimidazole (**12**)

Benzimidazole **11** (0.500 g, 1.6 mmol), NaH (48 mg, 2.0 mmol) and iodoethane (0.12 mL, 1.6 mmol) after purification by column chromatography gave **12** (0.319 g, 60%) as a brown oil; R_f 0.52 (50% petrol:50% EtOAc). IR (neat) 1079, 1102, 1145, 1222, 1260, 1354, 1389, 1462, 1520, 2836, 2934 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): δ 8.23 (s, 1H, Naph-1-H), 7.93–7.80 (m, 4H, Naph-H), 7.52–7.49 (m, 2H, Naph-H), 6.59 (d(ABq), $J = 8.6$, 1H, Ar-5(6)-H), 6.55 (d(ABq), $J = 8.6$, 1H, Ar-(5)6-H), 4.46 (q, $J = 7.1$, 2H, CH_2), 3.98 (s, 3H, OCH_3), 3.89 (s, 3H, OCH_3), 1.39 (t, $J = 7.1$, 3H, CH_3); ^{13}C NMR (100 MHz, $CDCl_3$): δ 152.8 (BnIm-2-C), 146.2, 141.7, 135.5, 133.6, 133.0 (all C), 129.7 (Naph-1-CH), 128.2 (CH), 128.1 (C), 127.9, 127.1, 127.0, 126.8, 126.7 (all CH), 126.3 (C), 103.3, 101.8 (BnIm-5,6-CH), 56.1, 56.0 (OCH_3), 41.6 (CH_2), 17.3 (CH_3); HRMS (ESI^+): m/z calcd for $C_{21}H_{21}N_2O_2$: 333.1603; found 333.1606 $[M + H]^+$.

4.5. General method for preparation of benzimidazolequinones

4.5.1. 1-Ethyl-2-phenyl-1H-benzimidazole-4,7-dione (**4**)

Benzimidazole **9** (0.530 g, 1.9 mmol) in 48% hydrobromic acid (13 mL) was refluxed for 3 h. The reaction was cooled and evaporated to dryness. $FeCl_3$ solution (14.4 mL, 0.7 M) was added, and stirred at room temperature for 18 h. The solution was extracted with $CHCl_3$, the combined organic extracts dried and evaporated to dryness to give an orange solid, which was purified by column chromatography using silica gel as absorbent with gradient elution of petrol, CH_2Cl_2 and EtOAc to give **4** (0.321 g, 68%) as an orange solid; R_f 0.51 (50% CH_2Cl_2:50% EtOAc); mp 129–132 °C. IR (neat) 1046, 1116, 1213, 1278, 1353, 1429, 1483, 1589, 1650 (C=O), 1675 (C=O) cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): δ 7.61–7.59 (m, 2H), 7.47–7.41 (m, 3H), 6.62 (d(ABq), $J = 10.4$, 1H, BnIm-(6)5-H), 6.58 (d (ABq), $J = 10.4$, 1H, BnIm-(5)6-H), 4.34 (q, $J = 7.1$, 2H, CH_2), 1.39 (t, $J = 7.1$, 3H, CH_3); ^{13}C NMR (100 MHz, $CDCl_3$): δ 181.2 (C=O), 178.2 (C=O), 153.7 (BnIm-2-C), 142.0 (C), 136.6, 136.1 (BnIm-5,6-CH), 130.8 (C), 130.6, 129.3, 129.2, 128.9 (all CH), 128.4 (C), 41.7 (CH_2), 16.1 (CH_3); HRMS (ESI^+): m/z calcd for $C_{15}H_{13}N_2O_2$: 253.0977; found 253.0970 $[M + H]^+$.

4.5.2. 1-Ethyl-2-pyridin-3-yl-1H-benzimidazole-4,7-dione (**5**)

Benzimidazole **10** (55 mg, 0.2 mmol) was treated with 48% hydrobromic acid (1.5 mL) and $FeCl_3$ solution (1.5 mL, 0.7 M), and after purification by column chromatography gave **5** (30 mg, 61%) as an orange solid; R_f 0.25 (EtOAc); mp 147–148 °C. IR (neat) 1024, 1052, 1114, 1215, 1288, 1379, 1412, 1528, 1595, 1657 (C=O), 1676 (C=O), 2853, 2927 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): δ 8.93 (d, $J = 1.4$, 1H, Pyr-2-H), 8.78 (dd, $J = 5.0$, $J = 1.4$, 1H, Pyr-6-H), 8.08 (d, $J = 8.3$, 1H, Pyr-4-H), 7.50 (dd, $J = 8.3$, $J = 5.0$, 1H, Pyr-5-H), 6.75 (d (ABq), $J = 10.1$, 1H, Ar-5(6)-H), 6.69 (d(ABq), $J = 10.1$, 1H, Ar-(5)6-H), 4.46 (q, $J = 7.1$, 2H, CH_2), 1.51 (t, $J = 7.1$, 3H, CH_3); ^{13}C NMR (100 MHz, $CDCl_3$): δ 181.0 (C=O), 178.2 (C=O), 151.5 (Pyr-6-CH), 150.8 (C), 149.4 (Pyr-2-CH), 142.2 (C), 137.1 (Pyr-4-CH), 136.6, 136.4 (BnIm-5,6-CH), 131.1 (C), 125.0 (C), 123.8 (Pyr-5-CH), 42.0 (CH_2), 16.3 (CH_3); HRMS (ESI^+): m/z calcd for $C_{14}H_{12}N_3O_2$: 254.0930; found 254.0924 $[M + H]^+$.

4.5.3. 1-Ethyl-2-(2-naphthyl)-1H-benzimidazole-4,7-dione (**6**)

Benzimidazole **12** (0.100 g, 0.3 mmol) was treated with 48% hydrobromic acid (2.5 mL) and $FeCl_3$ solution (2.5 mL, 0.7 M), and after purification by column chromatography gave **6** (70 mg, 77%) as an orange solid; R_f 0.57 (50% petrol:50% EtOAc); mp 135–137 °C. IR (neat) 1069, 1287, 1426, 1471, 1527, 1590, 1651 (C=O), 1676 (C=O) cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): δ 8.21 (s, 1H, Naph-1-H), 7.98 (d, $J = 8.5$, 1H), 7.93–7.91 (m, 2H), 7.76 (d, $J = 8.7$, 1H), 7.62–7.56 (m, 2H), 6.73 (d(ABq), $J = 10.4$, 1H, Ar-5(6)-H), 6.67 (d(ABq), $J = 10.4$, 1H, Ar-(5)6-H), 4.50 (q, $J = 7.1$, 2H, CH_2), 1.51 (t, $J = 7.1$, 3H, CH_3); ^{13}C NMR (100 MHz, $CDCl_3$): δ 181.3 (C=O), 178.2 (C=O), 153.8 (BnIm-2-C), 142.2 (C), 136.6, 136.2 (BnIm-5,6-CH), 133.0, 132.9, 130.9 (all C), 129.7 (Naph-1-CH), 128.8, 128.7, 127.9, 127.8, 127.1, 125.7 (all CH), 41.9 (CH_2), 16.2 (CH_3); HRMS (ESI^+): m/z calcd for $C_{19}H_{15}N_2O_2$: 303.1134; found 303.1143 $[M + H]^+$.

4.5.4. 5-Methyl-5,6-dihydrobenzimidazo[2,1-f]-1,6-naphthyridine-8,11-dione (**2**)

Benzimidazole **16b** (0.500 g, 1.7 mmol) was treated with 48% hydrobromic acid (13 mL) and $FeCl_3$ solution (0.7 M, 15 mL), and

after purification by column chromatography gave **2** (0.351 g, 78%) as an orange solid; R_f 0.55 (100% EtOAc); mp 199–200 °C. IR (neat) 1094, 1249, 1413, 1489, 1517, 1570, 1585, 1656 (C=O) cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): δ 8.63 (dd, $J = 4.9$, $J = 1.6$, 1H, 3-H), 8.47 (dd, $J = 7.9$, $J = 1.6$, 1H, 1-H), 7.34 (dd, $J = 7.9$, $J = 4.9$, 1H, 2-H), 6.72 (d (ABq), $J = 10.4$, 1H, (9)10-H), 6.66 (d(ABq), $J = 10.4$, 1H, 9(10)-H), 4.73 (dd, $J = 13.9$, $J = 5.7$, 1H, 6-H*H*), 4.51 (dd, $J = 13.9$, $J = 6.8$, 1H, 6-*H*H), 3.54–3.49 (m, 1H, 5-H), 1.44 (d, $J = 7.0$, 3H, CH_3); ^{13}C NMR (100 MHz, $CDCl_3$): δ 181.0 (C=O), 178.5 (C=O), 158.0 (C), 151.6 (3-CH), 148.2 (C), 142.7 (C), 136.7, 136.3 (9,10-CH), 133.2 (1-CH), 130.5 (C), 123.1 (2-CH), 120.4 (C), 48.2 (6-CH_2), 35.0 (5-CH), 17.3 (CH_3); HRMS (ESI^+): *m/z* calcd for $C_{15}H_{12}N_3O_2$: 266.0930; found 266.0923 $[M + H]^+$.

4.5.5. 5-Methyl-5,6-dihydrobenzimidazo[2,1-a]benzo[f] isoquinoline-8,11-dione (**3**)

Benzimidazole **20b** (0.100 g, 0.3 mmol) was treated with 48% hydrobromic acid (2.5 mL) and $FeCl_3$ solution (0.7 M, 5.0 mL), and after purification by column chromatography gave **3** (70 mg, 76%) as a red solid; R_f 0.48 (50% petrol:50% EtOAc); mp 145–147 °C. IR (neat) 1057, 1092, 1196, 1239, 1257, 1272, 1424, 1479, 1512, 1654 (C=O) cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): δ 8.35 (d, $J = 8.7$, 1H), 8.08 (d, $J = 8.2$, 1H), 7.92–7.87 (m, 2H), 7.64–7.56 (m, 2H), 6.72 (d(ABq), $J = 10.4$, 1H, (9)10-H), 6.66 (d(ABq), $J = 10.4$, 1H, 9(10)-H), 5.08 (d, $J = 13.7$, 1H, 6-H*H*), 4.34 (dd, $J = 13.7$, $J = 5.0$, 1H, 6-*H*H), 4.14–4.05 (m, 1H, 5-H), 1.34 (d, $J = 7.3$, 3H, CH_3); ^{13}C NMR (100 MHz, $CDCl_3$): δ 181.3 (C=O), 178.5 (C=O), 149.5 (C), 142.7 (C), 136.7, 136.3 (9,10-CH), 136.4, 135.1, 130.7, 129.8 (all C), 129.5, 128.5, 127.5, 127.4, 123.4, 122.7 (all CH), 121.2 (C), 48.3 (CH_2), 28.8 (5-CH), 19.9 (CH_3); HRMS (ESI^+): *m/z* calcd for $C_{20}H_{15}N_2O_2$: 315.1134; found 315.1131 $[M + H]^+$.

4.6. General method for preparation of 2-(2-bromopyridin-3-yl)-1H-benzimidazoles

4.6.1. 2-(2-Bromopyridin-3-yl)-1H-benzimidazole (**13a**)

2-Bromonicotinaldehyde (0.930 g, 5.0 mmol), benzene-1,2-diamine (0.540 g, 5.0 mmol) and NH_4Cl (1.100 g, 20.5 mmol) in CH_3OH (25 mL) were stirred at room temperature for 8 h. The solution was evaporated to dryness, $CHCl_3$ (25 mL) added, filtered, and recrystallized from toluene to give 13a (1.350 g, 98%) as a brown solid; mp 206–208 °C. IR (neat) 1052, 1097, 1261, 1399, 1531, 2922 cm^{-1}; ^1H NMR (400 MHz, DMSO-d_6): δ 8.52 (d, $J = 3.0$, 1H, Pyr-6-H), 8.16 (d, $J = 6.9$, 1H, Pyr-4-H), 7.69 (bs, 1H, BnIm-H), 7.63–7.56 (m, 2H, Pyr-5-H and BnIm-H), 7.23 (bs, 2H, BnIm-H), NH not observed; ^{13}C NMR (100 MHz, DMSO-d_6): δ 151.0 (Pyr-6-CH), 148.7 (C), 143.3 (C), 140.8 (Pyr-4-CH), 140.6 (C), 134.6 (C), 130.0 (C), 123.4 (Pyr-5-CH), 123.0, 121.9, 119.3 (all BnIm-CH), 111.8 (BnIm-7-CH); HRMS (ESI^+): *m/z* calcd for $C_{12}H_{10}{}^{79}BrN_3$: 273.9980; found 273.9991 $[M + H]^+$.

4.6.2. 2-(2-Bromopyridin-3-yl)-4,7-dimethoxy-1H-benzimidazole (**13b**)

2-Bromonicotinaldehyde (1.116 g, 6.0 mmol), 3,6-dimethoxybenzene-1,2-diamine (1.008 g, 6.0 mmol) and NH_4Cl (1.320 g, 24.6 mmol) in CH_3OH (30 mL), after recrystallization from toluene gave **13b** (1.710 g, 85%) as a brown solid; mp 160–161 °C. IR (neat) 1057, 1226, 1279, 1315, 1378, 1553, 1578 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): δ 8.81 (dd, $J = 7.8$, $J = 2.1$, 1H, Pyr-6-H), 8.40 (dd, $J = 4.6$, $J = 2.1$, 1H, Pyr-4-H), 7.40 (dd, $J = 7.8$, $J = 4.6$, 1H, Pyr-5-H), 6.60 (s, 2H, BnIm-5,6-H), 3.97 (s, 6H, CH_3), NH not observed; ^{13}C NMR (100 MHz, $CDCl_3$): δ 150.4 (Pyr-6-CH), 145.6 (C), 141.3 (Pyr-4-CH), 138.7 (C), 128.2 (C), 123.2 (Pyr-5-CH), 103.1 (BnIm-5,6-CH), 56.0 (2 × CH_3); HRMS (ESI^+): *m/z* calcd for $C_{14}H_{13}{}^{79}BrN_3O_2$: 334.0191; found 334.0198 $[M + H]^+$.

4.7. General method for preparation of 2-(1-bromo-2-naphthyl)-1H-benzimidazoles

4.7.1. 2-(1-Bromo-2-naphthyl)-1H-benzimidazole (**18a**)

A mixture of naphthoic acid **17** (3.000 g, 12 mmol), 1,2-phenylenediamine (1.296 g, 12 mmol) and polyphosphoric acid (17.000 g) was heated at 150 °C for 6 h. The mixture was poured onto crushed ice, and the precipitate filtered. Na_2CO_3 solution (0.5 M, 300 mL) was added and the mixture stirred for 30 min. The benzimidazole free base was filtered, and recrystallized from methanol to give **18a** (2.390 g, 61%) as a white solid; mp 267–268 °C. IR (neat) 1014, 1125, 1226, 1276, 1337, 1408, 1441, 1499, 1534 cm^{-1}; ^1H NMR (400 MHz, DMSO-d_6): δ 8.34 (d, $J = 8.2$, 1H, Naph-3(4)-H), 8.10–8.06 (m, 2H, Naph-H), 7.80 (d, $J = 8.2$, 1H, Naph-(3),4-H), 7.78–7.67 (m, 2H, Naph-H), 7.65–7.62 (m, 2H, BnIm-H), 7.25–7.22 (m, 2H, BnIm-H), NH not observed; ^{13}C NMR (100 MHz, DMSO-d_6): δ 151.7 (BnIm-2-C), 134.7, 132.1, 131.6 (all C), 129.2, 129.1, 129.0 (×2), 127.9, 127.9 (all Naph-CH), 122.8 (C-Br), 122.7, 122.6, 115.9, 115.8 (all BnIm-CH); HRMS (ESI^+): *m/z* calcd for $C_{17}H_{11}{}^{79}BrN_2$: 322.0100; found 322.0108 $[M]^+$.

4.7.2. 2-(1-Bromo-2-naphthyl)-4,7-dimethoxy-1H-benzimidazole (**18b**)

Naphthoic acid **17** (2.000 g, 8.0 mmol), 3,6-dimethoxybenzene-1,2-diamine (1.360 g, 8.1 mmol) and polyphosphoric acid (11.400 g) after recrystallization from methanol gave **18b** (2.329 g, 76%) as a light brown solid; mp 189–191 °C. IR (neat) 1105, 1263, 1452, 1508, 1538, 2547, 3302 cm^{-1}; ^1H NMR (400 MHz, DMSO-d_6): δ 8.43 (d, $J = 8.4$, 1H, Naph-H), 8.19–8.16 (m, 2H, Naph-H), 7.88–7.85 (m, 1H, Naph-H), 7.82–7.78 (m, 2H, Naph-H), 6.76 (s, 2H, BnIm-5,6-H), 4.02 (s, 6H, 2 × CH_3), NH not observed; ^{13}C NMR (100 MHz, DMSO-d_6): δ 150.4 (BnIm-2-C), 134.7, 132.0, 131.6 (all C), 129.1, 129.0, 128.9, 128.3, 128.1, 127.8 (all Naph-CH), 123.3 (C-Br), 103.4 (2 × BnIm-5,6-CH), 56.2 (2 × CH_3); HRMS (EI^+): *m/z* calcd for $C_{19}H_{15}{}^{79}BrN_2O_2$: 383.0395; found 383.0396 $[M + H]^+$.

4.8. General method for preparation of radical precursors

4.8.1. 1-Allyl-2-(2-bromopyridin-3-yl)-1H-benzimidazole (**14a**)

Bromide **13a** (1.000 g, 3.6 mmol) and NaH (81 mg, 3.4 mmol) in THF (40 mL) were refluxed for 1 h. Allyl bromide (0.50 mL, 5.8 mmol) in THF (20 mL) was added over 30 min, and refluxed for 8 h. The mixture was filtered, evaporated to dryness and purified by column chromatography using silica gel as absorbent with gradient elution of petrol and EtOAc to give **14a** (1.080 g, 95%) as an oil; R_f 0.38 (50% petrol:50% EtOAc). IR (neat) 1330, 1381, 1454, 1553 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): δ 8.45 (dd, $J = 4.8$, $J = 2.0$, 1H, Pyr-6-H), 7.85–7.82 (m, 1H, BnIm-4-H), 7.78 (dd, $J = 7.5$, $J = 2.0$, 1H, Pyr-4-H), 7.45–7.40 (m, 1H, BnIm-7-H), 7.36 (dd, $J = 7.5$, $J = 4.8$, 1H, Pyr-5-H), 7.33–7.28 (m, 2H, BnIm-5,6-H), 5.85–5.75 (m, 1H, 2′-H), 5.10 (d, $J = 10.3$, 1H, 3′-*cis*-H), 4.90 (d, $J = 17.2$, 1H, 3′-*trans*-H), 4.63 (d, $J = 5.0$, 2H, NCH_2); ^{13}C NMR (100 MHz, $CDCl_3$): δ 151.4 (Pyr-6-CH), 149.9, 142.9, 142.8 (all C), 140.7 (Pyr-4-CH), 134.7 (C), 131.8 (2′-CH), 129.7 (C), 123.6 (BnIm-CH), 122.8 (BnIm-CH), 122.7 (Pyr-5-CH), 120.2 (BnIm-4-CH), 118.0 (3′-CH_2), 110.8 (BnIm-7-CH), 47.1 (NCH_2); HRMS (ESI^+): *m/z* calcd for $C_{15}H_{13}{}^{79}BrN_3$: 314.0293; found 314.0295 $[M + H]^+$.

4.8.2. 1-Allyl-2-(2-bromopyridin-3-yl)-4,7-dimethoxy-1H-benzimidazole (**14b**)

Bromide **13b** (2.590 g, 7.8 mmol), NaH (0.188 g, 7.8 mmol), allyl bromide (0.680 mL, 7.8 mmol) in THF (80 mL) after purification by column chromatography gave **14b** (2.726 g, 94%) as a brown oil; R_f 0.42 (50% petrol:50% EtOAc); mp 119–120 °C. IR (neat) 1102, 1259, 1378, 1446, 1519 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): δ 8.34 (dd,

$J = 4.8$, $J = 1.8$, 1H, Pyr-6-H), 7.67 (dd, $J = 7.6$, $J = 1.8$, 1H, Pyr-4-H), 7.24 (dd, $J = 7.6$, $J = 4.8$, 1H, Pyr-5-H), 6.50 (d(ABq), $J = 8.4$, 1H, BnIm-5(6)-H), 6.44 (d(ABq), $J = 8.4$, 1H, BnIm-(5)6-H), 5.75–5.65 (m, 1H, 2′-H), 4.84 (d, $J = 10.3$, 1H, 3′-*cis*-H), 4.76 (bs, 2H, NCH_2), 4.49 (d, $J = 17.2$, 1H, 3′-*trans*-H), 3.83 (s, 3H, CH_3), 3.74 (s, 3H, CH_3); ^{13}C NMR (100 MHz, $CDCl_3$): δ 151.2 (Pyr-6-CH), 149.1, 146.0, 143.0, 141.6 (all C), 141.1 (Pyr-4-CH), 134.9 (C), 133.8 (2′-CH), 129.9 (C), 125.5 (C), 122.4 (Pyr-5-CH), 116.4 (3′-CH_2), 104.1, 102.1 (BnIm-5,6-CH), 56.0 (CH_3), 55.9 (CH_3), 48.5 (NCH_2); HRMS (ESI^+): *m*/*z* calcd for $C_{17}H_{17}{}^{79}BrN_3O_2$: 374.0504; found 374.0498 $[M + H]^+$.

4.8.3. 1-Allyl-2-(1-bromo-2-naphthyl)-1H-benzimidazole (**19a**)

Bromide **18a** (2.907 g, 9.0 mmol), NaH (0.216 g, 9.0 mmol) and allyl bromide (0.78 mL, 9.0 mmol) in THF (80 mL) after purification by column chromatography gave **19a** (2.823 g, 86%) as a yellow oil; R_f 0.48 (50% petrol:50% EtOAc). IR (neat) 1248, 1281, 1327, 1388, 1455, 1492 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): δ 8.34 (d, $J = 8.3$, 1H, Naph-5(8)-H), 7.95–7.92 (m, 1H, BnIm-4-H), 7.85 (d, $J = 8.5$, 1H, Naph-3(4)-H), 7.82 (d, $J = 8.0$, 1H, Naph-(5)8-H), 7.61–7.51 (m, 2H, Naph-6,7-H), 7.48 (d, $J = 8.5$, 1H, Naph-(3)4-H), 7.42–7.39 (m, 1H, BnIm-7-H), 7.35–7.30 (m, 2H, BnIm-5,6-H), 5.78–5.71 (m, 1H, 2′-H), 5.06 (d, $J = 10.3$, 1H, 3′-*cis*-H), 4.91 (d, $J = 17.2$, 1H, 3′-*trans*-H), 4.63–4.52 (bs, 2H, NCH_2); ^{13}C NMR (100 MHz, $CDCl_3$): δ 152.7 (BnIm-2-C), 143.0 (BnIm-3a-C), 134.6 (2 × C), 131.9 (2′-CH), 131.8 (C), 130.0 (C), 128.3, 128.2, 128.0, 127.9, 127.8, 127.7 (all Naph-CH), 124.9 (C-Br), 123.0, 122.4 (BnIm-5,6-CH), 120.2 (BnIm-4-CH), 117.7 (3′-CH_2), 110.6 (BnIm-7-CH), 46.9 (NCH_2); HRMS (ESI^+): *m*/*z* calcd for $C_{20}H_{16}{}^{79}BrN_2$: 363.0497; found 363.0498 $[M + H]^+$.

4.8.4. 1-Allyl-2-(1-bromo-2-naphthyl)-4,7-dimethoxy-1H-benzimidazole (**19b**)

Bromide **18b** (2.000 g, 5.2 mmol), NaH (0.144 g, 6.0 mmol) and allyl bromide (0.45 mL, 5.2 mmol) in THF (80 mL) gave after purification by column chromatography **19b** (1.937 g, 88%) as a yellow oil; R_f 0.46 (50% petrol:50% EtOAc). IR (neat) 1095, 1173, 1195, 1259, 1384, 1448, 1518, 2932 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): δ 8.36 (d, $J = 7.8$, 1H, Naph-5(8)-H), 7.88 (m, 2H, 2 × Naph-H), 7.66–7.57 (m, 2H, Naph-6,7-H), 7.51 (d, $J = 8.4$, 1H, Naph-3(4)-H), 6.63 (d(ABq), $J = 8.5$, 1H, BnIm-5(6)-H), 6.58 (d(ABq), $J = 8.5$, 1H, BnIm-(5)6-H), 5.84–5.76 (m, 1H, 2′-H), 5.20–5.10 (bs, 1H, NCH*H*), 4.95 (d, $J = 10.3$, 1H, 3′-*cis*-H), 4.65 (d, $J = 17.2$, 1H, 3′-*trans*-H), 4.58–4.47 (bs, 1H, NC*H*H), 3.98 (s, 3H, CH_3), 3.89 (s, 3H, CH_3); ^{13}C NMR (100 MHz, $CDCl_3$): δ 152.0 (BnIm-2-C), 146.3, 141.7, 135.1, 134.8 (all C), 134.1 (2′-CH), 132.0 (C), 130.4 (C), 128.3, 128.2, 127.9, 127.7, 127.6, 127.5 (all Naph-CH), 125.5 (C), 125.2 (C), 116.4 (3′-CH_2), 103.8, 101.9 (BnIm-5,6-CH), 56.1 (CH_3), 56.0 (CH_3), 48.5 (NCH_2); HRMS (ESI^+): *m*/*z* calcd for $C_{22}H_{20}N_2O_2$ ^{79}Br: 423.0708; found 423.0718 $[M + H]^+$.

4.9. General method for radical cyclization

4.9.1. 5-Methyl-5,6-dihydrobenzimidazo[2,1-f]-1,6-naphthyridine (**16a**)

Bu_3SnH (1.250 g, 4.3 mmol) and ACN (0.150 g, 0.6 mmol) in toluene (50 mL) were added over 8 h via a syringe pump to bromide **14a** (1.000 g, 3.2 mmol) in toluene (85 mL) at reflux. The cooled solution was evaporated to dryness and purified by column chromatography using silica as absorbent with gradient elution of petrol, CH_2Cl_2 and EtOAc to give **16a** (0.535 g, 71%) as a white solid; R_f 0.32 (43% petrol:43% EtOAc:14% methanol); mp 119–120 °C. IR (neat) 1327, 1413, 1448, 1484, 1570, 2926, 2967, 3397 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): δ 8.59 (dd, $J = 4.8$, $J = 1.6$, 1H, 3-H), 8.49 (dd, $J = 7.8$, $J = 1.6$, 1H, 1-H), 7.80–7.78 (m, 1H), 7.37–7.26 (m, 4H), 4.43 (dd, $J = 12.5$, $J = 5.6$, 1H, 6-H*H*), 4.13 (dd, $J = 12.5$, $J = 6.0$, 1H, 6-*H*H), 3.58–3.54 (m, 1H, 5-H), 1.44 (d, $J = 7.1$, 3H, CH_3); ^{13}C NMR (100 MHz, $CDCl_3$): δ 158.6 (C), 150.7 (3-CH), 147.6, 144.2, 134.9 (all C), 133.0 (1-CH), 123.4 (CH), 122.9 (2 × CH), 121.9 (C), 119.9 (CH), 109.4 (11-CH), 46.6 (CH_2), 35.4 (5-CH), 18.0 (CH_3); HRMS (ESI^+): *m*/*z* calcd for $C_{15}H_{14}N_3$: 236.1188; found 236.1185 $[M + H]^+$.

4.9.2. 8,11-Dimethoxy-5-methyl-5,6-dihydrobenzimidazo[2,1-f]-1,6-naphthyridine (**16b**)

Bromide **14b** (2.200 g, 5.9 mmol), Bu_3SnH (2.390 g, 8.2 mmol) and ACN (0.287 g, 1.2 mmol) gave after purification by column chromatography **16b** (1.237 g, 71%) as a white solid; R_f 0.28 (50% petrol:50% EtOAc); mp 137–139 °C. IR (neat) 1084, 1107, 1165, 1246, 1259, 1458, 1527 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): δ 8.57–8.52 (m, 2H, 1 and 3-H), 7.25 (dd, $J = 7.7$, $J = 5.0$, 1H, 2-H), 6.52 (d(ABq), $J = 8.5$, 1H, 10(9)-H), 6.47 (d(ABq), $J = 8.5$, 1H, (10)9-H), 4.71 (dd, $J = 13.1$, $J = 5.5$, 1H, 6-H*H*), 4.55 (dd, $J = 13.1$, $J = 6.5$, 1H, 6-*H*H), 3.94 (s, 3H, OCH_3), 3.86 (s, 3H, OCH_3), 3.45–3.43 (m, 1H, 5-H), 1.41 (d, $J = 6.9$, 3H, CH_3); ^{13}C NMR (100 MHz, $CDCl_3$): δ 158.5 (C), 150.3 (3-CH), 146.6, 146.1, 141.7, 136.0 (all C), 133.0 (1-CH), 125.8 (C), 122.7 (2-CH), 121.9 (C), 103.6, 101.7 (9,10-CH), 55.9 (2 × OCH_3), 48.8 (CH_2), 35.7 (5-CH), 17.6 (CH_3); HRMS (ESI^+): *m*/*z* calcd for $C_{17}H_{18}N_3O_2$: 296.1399; found 296.1408 $[M + H]^+$.

4.9.3. 8,11-Dimethoxy-5-methyl-5,6-dihydrobenzimidazo[2,1-a]benzo[f]isoquinoline (**20b**)

Bromide **19b** (1.000 g, 2.4 mmol), Bu_3SnH (1.000 g, 3.4 mmol) and ACN (0.117 g, 0.5 mmol) gave after purification by column chromatography **20b** (0.587 g, 71%) as a white solid; R_f 0.41 (50% petrol:50% EtOAc); ^1H NMR (400 MHz, $CDCl_3$): δ 8.54 (d, $J = 8.7$, 1H), 8.02 (d, $J = 8.2$, 1H), 7.82 (d, $J = 8.7$, 2H), 7.53–7.44 (m, 2H), 6.53 (d (ABq), $J = 8.5$, 1H, 10(9)-H), 6.50 (d(ABq), $J = 8.5$, 1H, 9(10)-H), 5.04 (d, $J = 13.0$, 1H, 6-H*H*), 4.36–4.32 (dd, $J = 13.0$, $J = 4.6$, 1H, 6-*H*H), 3.99 (s, 3H, OCH_3), 3.96–3.84 (m, 1H, 5-H), 3.87 (s, 3H, OCH_3), 1.32 (d, $J = 7.1$, 3H, CH_3); ^{13}C NMR (100 MHz, $CDCl_3$): δ 148.2, 146.2, 141.7, 136.2, 136.0, 134.6, 130.2 (all C), 129.2, 127.7, 126.9, 126.6 (all CH), 126.2 (C), 123.4 (CH), 123.3 (CH), 122.9 (C), 103.4, 101.5 (9,10-CH), 56.0 (OCH_3), 55.9 (OCH_3), 48.8 (CH_2), 29.3 (5-CH), 19.9 (CH_3); HRMS (ESI^+): *m*/*z* calcd for $C_{22}H_{21}N_2O_2$: 345.1603; found 345.1605 $[M + H]^+$.

4.10. Cell culture and cytotoxicity evaluation

4.10.1. 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 and Medical Science, University College Dublin, Ireland.

4.10.2. 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.

4.10.3. Cytotoxicity measurement

Cell viability was determined using the MTT colorimetric assay [29]. Normal cells were plated into 96-well plates at a density of 10,000 cells per well (200 μL per well). HeLa cells were plated into 96-well plates at a density of 1000 cells per well (100 μL per well). DU145 cells were plated into 96-well plates at a density of 2000 cells per well (200 μL per well). Cells were allowed to adhere for 24 h before exposure to benzimidazolequinone or **MMC**.

Benzimidazolequinone and **MMC** (Sigma) 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 DMSO-only treated value. Each data point used is the mean of at least three independent experiments. 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).

Acknowledgements

This publication has emanated from research conducted with financial support from Science Foundation Ireland (SFI) (07/RFP/CHEF227). The PhD of EM was supported by the Environmental Protection Agency (EPA) Doctoral Scheme (2006-PhD-ET-6). We thank the SFI and the EPA, Department of Environment, Heritage and Local Government funded under the National Development Plan 2000–2006 for this financial support. We thank Prof. William Watson of the Conway Institute, School of Medicine and Medical Science, University College Dublin, Ireland for the DU145 cell line.

References

[1] L. Garuti, M. Roberti, D. Pizzirani, Mini Rev. Med. Chem. 7 (2007) 481–489.
[2] Y. Chen, L. Hu, Med. Res. Rev. 29 (2009) 29–64.
[3] H.A. Seow, P.G. Penketh, R.P. Baumann, A.C. Sartorelli, in: H. Sies, L. Packer (Eds.), Quinones and Quinone Enzymes, Part B, Methods in Enzymology, vol. 382, Elsevier, 2004, pp. 221–233.
[4] E.B. Skibo, S. Gordon, L. Bess, R. Boruah, M.J. Heileman, J. Med. Chem. 40 (1997) 1327–1339.
[5] E.B. Skibo, I. Islam, W.G. Schulz, R. Zhou, L. Bess, R. Boruah, Synlett (1996) 297–309.
[6] C.M. Ahn, S.K. Kim, J.L. Han, Arch. Pharm. Res. 21 (1998) 599–609.
[7] L. O'Donovan, M.P. Carty, F. Aldabbagh, Chem. Commun. (2008) 5592–5594.
[8] K. Fahey, L. O'Donovan, M. Carr, M.P. Carty, F. Aldabbagh, Eur. J. Med. Chem. 45 (2010) 1873–1879.
[9] A.K. Singh, J.W. Lown, Anticancer Drug Des. 15 (2000) 265–275.
[10] A. Gellis, H. Kovacic, N. Boufatah, P. Vanelle, Eur. J. Med. Chem. 43 (2008) 1858–1864.
[11] C. Flader, J. Liu, R.F. Borch, J. Med. Chem. 43 (2000) 3157–3167.
[12] L. Garuti, M. Roberti, M. Malagoli, T. Rossi, M. Castelli, Bioorg. Med. Chem. Lett. 10 (2000) 2193–2195.
[13] L. Garuti, M. Roberti, A. Pession, E. Leoncini, S. Hrelia, Bioorg. Med. Chem. Lett. 11 (2001) 3147–3149.
[14] L. Garuti, M. Roberti, D. Pizzirani, A. Pession, E. Leoncini, V. Cenci, S. Hrelia, IL Farmaco 59 (2004) 663–668.
[15] K.-H. Chung, S.-Y. Hong, H.-J. You, R.-E. Park, C.-K. Ryu, Bioorg. Med. Chem. 14 (2006) 5795–5801.
[16] M. Lynch, S. Hehir, P. Kavanagh, D. Leech, J. O'Shaughnessy, M.P. Carty, F. Aldabbagh, Chem. Eur. J. 13 (2007) 3218–3226.
[17] O. Lavergne, A.-C. Fernandes, L. Bréhu, A. Sidhu, M.-C. Brézak, G. Prévost, B. Ducommun, M.-O. Contour-Galcera, Bioorg. Med. Chem. Lett. 16 (2006) 171–175.
[18] C.-K. Ryu, E.-H. Song, J.-Y. Shim, H.-J. You, K.U. Choi, I.H. Choi, E.Y. Lee, M. J. Chae, Bioorg. Med. Chem. Lett. 13 (2003) 17–20.
[19] J.J. Newsome, M.A. Colucci, M. Hassani, H.D. Beall, C.J. Moody, Org. Biomol. Chem. 5 (2007) 3665–3673.
[20] M.A. Colucci, C.J. Moody, G.D. Couch, Org. Biomol. Chem. 6 (2008) 637–656.
[21] D. Ross, D. Siegel, in: H. Sies, L. Packer (Eds.), Quinones and Quinone Enzymes, Part B, Methods in Enzymology, vol. 382, Elsevier, 2004, pp. 115–144.
[22] R.I. Bello, C. Gómez-Díaz, F. Navarro, F.J. Alcaín, J.M. Villalba, J. Biol. Chem. 276 (2001) 44379–44384.
[23] S.A. Fitzsimmons, P. Workman, M. Grever, K. Paull, R. Camalier, A.D. Lewis, J. Natl. Cancer Inst. 88 (1996) 259–269.
[24] E. Moriarty, F. Aldabbagh, Tetrahedron Lett. 50 (2009) 5251–5253.
[25] K.R. Reddy, G.G. Krishna, Tetrahedron Lett. 46 (2005) 661–663.
[26] B.C. Raju, N.D. Theja, J.A. Kumar, Synth. Commun. 39 (2009) 175–188.
[27] I.A. Shaikh, F. Johnson, A.P. Grollman, J. Med. Chem. 29 (1986) 1329–1340.
[28] D. Hellwinkel, S. Bohnet, Chem. Ber. 120 (1987) 1151–1173.
[29] T. Mosmann, J. Immunol. Methods 65 (1983) 55–63.