Benzimidazole- and benzothiazole-quinones: excellent substrates for NAD(P)H:quinone oxidoreductase 1†

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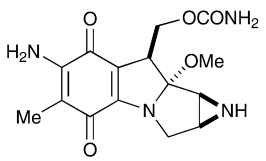
A series of benzimidazole- and benzothiazole-quinones has been synthesized. The ability of these heterocyclic quinones to act as substrates for recombinant human NAD(P)H:quinone oxidoreductase (NQO1), a two-electron reductase upregulated in tumour cells, was determined. Overall, the quinones were excellent substrates for NQO1.

Introduction

One group of compounds that exhibit wide-ranging properties are the quinones. Quinones, particularly the terpenoid benzoquinones, are widespread in nature,[1–3] and constitute a large group of natural pigments, although surprisingly their contribution to natural colouring is relatively small. Their major role is to participate in important biological redox processes. For example, the ubiquinones act as electron-transfer agents in the respiratory chain, and pyrroloquinolinequinone (coenzyme PQQ) is a redox co-factor. Other quinones also possess potent biological activity, doxorubicin (adriamycin) being a front-line cancer chemotherapy treatment. However, our own interest in quinones with anticancer properties stems from the natural product mitomycin C **1** and synthetic analogues (represented by the general indole-quinone structure **2**).



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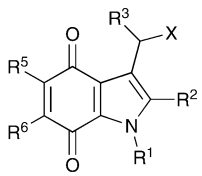

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Quinones are readily reduced *in vivo*, and the bioreduction of MMC, a key step in its activation into a DNA alkylating agent, has been widely studied.[4–7] Likewise, the bioreduction of a range of synthetic indole-quinones **2** has been investigated, in particular the generation of the cytotoxic electrophile formed upon elimination of a leaving group X from the (indol-3-yl)methyl position following two-electron reduction to the hydroquinone radical anion.[8–13] Such one- or two-electron reductions would be catalyzed in biological systems by, for example, NADPH:cytochrome *c* (P450) reductase[12] or by NAD(P)H:quinone oxidoreductase 1 (NQO1, DT-diaphorase) respectively.[14–18] To date we have focused on the two-electron reduction pathway involving NQO1,[15,19–23] and have investigated a range of indole-quinone-based substrates and inhibitors.[16,18,24–30] Although we have also studied the quinoline-5,8-dione system,[31,32] the range of heterocyclic quinones remains rather small. Therefore in an attempt to widen the group of NQO1 substrates/inhibitors, and to probe further the active site of the enzyme, we have explored a new series of heterocyclic quinones based on benzimidazole and benzothiazole.[33]

Results and discussion

Chemistry

Both benzimidazole-4,7-diones **3** and benzothiazole-4,7-diones **4** have been described previously, and reported to have a range of biological properties. The benzimidazole-quinones are better known, and pyrrolo[1,2-*a*]benzimidazoles such as **5** have been extensively investigated by Skibo and co-workers as analogues of MMC.[34,35] Benzimidazole-quinone phosphorodiamidates **3** (R^1 = Me, R^2 = $CH_2OP(NH_2)N(CH_2CH_2Cl)_2$, R^5 = R^6 = H) have also been studied as potential prodrugs for bioreductive activation,[36] whilst quinones **3** (R^1 = R^6 = H, R^2 = Et, R^5 = $NHCH_2CH_2NMe_2$) have been reported as inhibitors of the phosphatase CDC25C.[37] Related benzothiazole-quinones **4** (R^2 = Me, R^5 = $NHCH_2CH_2NMe_2$, R^6 = H) also inhibit the same phosphatase,[37] and 5- and 6-arylamino derivatives **4** (R^2 = Me, R^5 = NHAr, R^6 = H) and **4** (R^2 = Me, R^5 = H, R^6 = NHAr) are reported to inhibit cyclin-dependent kinase 4 and possess antifungal activity respectively.[38,39] However, the most widely studied benzothiazole-quinone is 5-undecyl-6-hydroxybenzothiazole-4,7-dione (UHDBT) **6**, an analogue of ubiquinone that inhibits electron transport by binding to cytochrome bc_1.[40,41]

In order to make meaningful comparisons with the more widely studied indole-quinones, we initially elected to investigate relatively simple 5-methoxybenzimidazole-quinones. Thus the known 4-methoxy-*N*-methyl-2-nitroaniline **8**[42] was reduced to the corresponding *o*-phenylenediamine derivative, which was immediately converted into benzimidazole **9** by reaction with glycolic acid. In order to effect the desired nitration reaction, prior acetylation of the primary alcohol proved necessary, and thereafter nitration in nitric/sulfuric acids gave a mixture of 4- and 6-nitro compounds (2 : 1), from which the desired 4-nitro isomer could be isolated in 36% yield. The acetyl group is lost during

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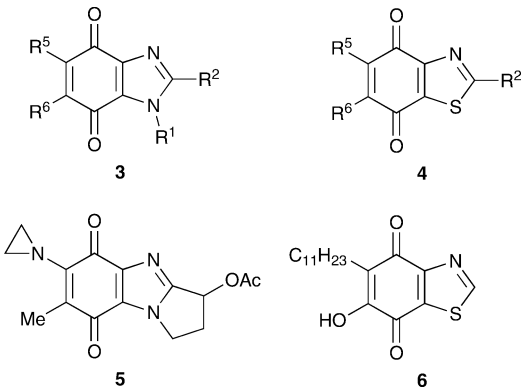
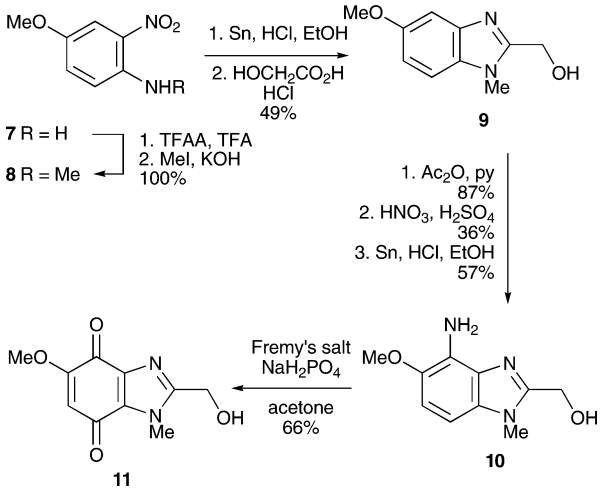


the reaction. Following reduction of the nitro group, the quinone **11** was obtained by oxidation of the aniline **10** with Fremy's salt (potassium nitrosodisulfonate) (Scheme 1).

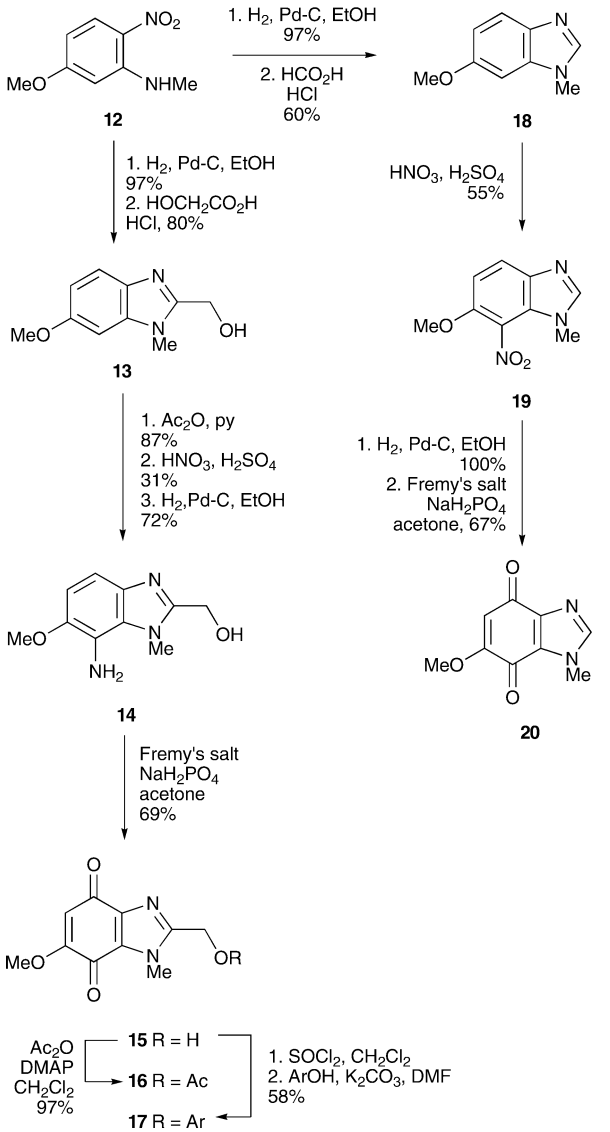


Scheme 1 Synthesis of 5-methoxybenzimidazolequinones.

The isomeric series of 6-methoxybenzimidazoles was also explored starting from the known 5-methoxy-*N*-methyl-2-nitroaniline **12**.[43] As before, reduction of the nitro group was followed by condensation with glycolic acid to give the 2-hydroxymethyl benzimidazole **13**. Again, acetylation of the primary alcohol prior to nitration proved necessary, and the desired 7-nitro compound was obtained in 31% yield after separation from the 5-nitro isomer. Reduction gave the 7-aminobenzimidazole **14**. Fremy's salt oxidation delivered the desired benzimidazole-quinone **15** in good yield, and in anticipation of such quinones bearing leaving groups being inhibitors of NQO1 (*q.v.*), this was subsequently converted into the acetate **16** and 4-nitrophenyl derivative **17** by standard methodology (Scheme 2).

For comparison purposes, the 2-unsubstituted benzimidazole-quinone **20** was also prepared. Heating the *o*-phenylenediamine derived by reduction of **12** with formic acid gave the known benzimidazole **18**.[44] Nitration gave a mixture of 7- and 5-nitro compounds (*ca.* 5 : 3) in good yield, with the desired 7-nitrobenzimidazole **19** being isolated in 55% yield. Finally, reduction of the nitro group and oxidation of the aniline gave the desired quinone **20** (Scheme 2).

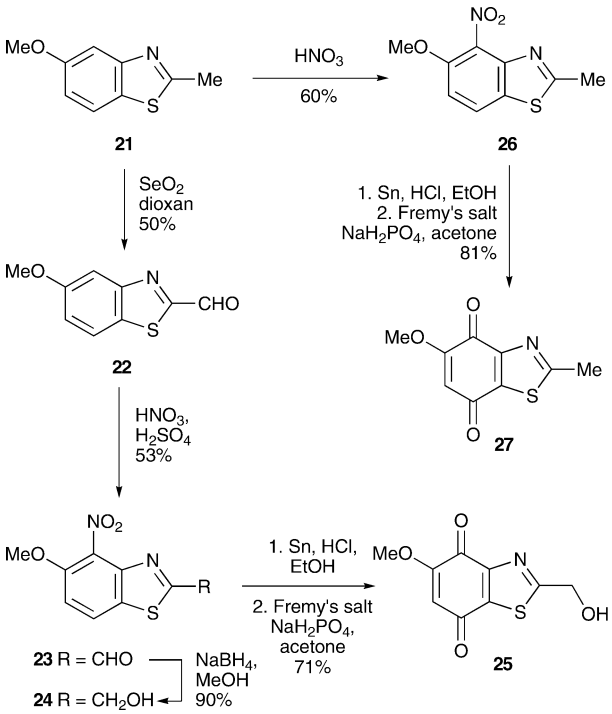
The synthesis of the benzothiazole-quinones started with commercially available 5-methoxy-2-methylbenzothiazole **21**. Oxida-



Scheme 2 Synthesis of 6-methoxybenzimidazolequinones.

tion of the methyl group with selenium dioxide in dioxan gave the known aldehyde **22**,[45] nitration of which gave a 6 : 1 mixture of 4- and 6-nitro compounds, with the desired 4-nitro compound **23** isolated in 53% yield after chromatography. Sequential reduction of the aldehyde with sodium borohydride, and of the nitro group with tin in hydrochloric acid, was followed by oxidation to the benzothiazole-quinone **25** with Fremy's salt (Scheme 3). The 2-methylbenzothiazole-quinone **27** was also prepared for comparison purposes: nitration of **21** gave the 4-nitro compound **26**, which was reduced and then oxidized to the quinone **27** (Scheme 3).

Electrochemical experiments were performed on benzimidazole- and benzothiazole-quinones **11** and **25** in DMF as solvent with tetra-*n*-butylammonium tetrafluoroborate as supporting electrolyte as previously described.[16] The E_{redox} values, with reference to ferrocene (Fc) are shown in Fig. 1; values for the closely related indole-quinones **28** and **29** are also shown. The data show that whilst the benzimidazole-quinone has a similar redox potential to the indole-quinones previously studied (E_{redox} *vs.* Fc



Scheme 3 Synthesis of benzothiazolequinones.

in the range −1.20 to −1.40 V), the benzothiazole-quinone is considerably easier to reduce.

Enzyme studies

We next examined the ability of the new quinones to act as substrates for NQO1. In our earlier studies on indole-quinone metabolism by recombinant human NQO1, we used an HPLC system that is capable of quantifying both NADH oxidation and quinone reduction.[16,18] To simplify comparisons with these earlier compounds, we have also used this method for some of the quinones in the present study. Quinone reduction is reversible due to redox cycling of the hydroquinone, so results (Table 1)

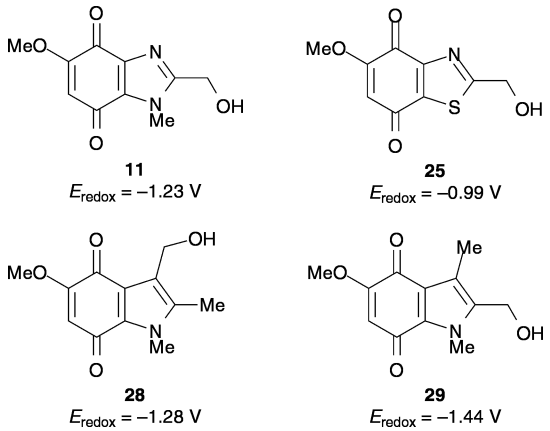
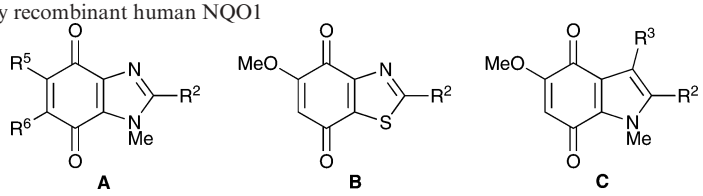


Fig. 1 E_{redox} values (*vs.* Fc) for benzimidazole- and benzothiazole-quinones **11** and **25** compared to related indole-quinones **28** and **29**.[16,24]

are reported as μmol NADH oxidized min^{-1} mg^{-1} NQO1. This HPLC method gives average rates of reduction over a 30–40 minute period. An alternative spectrophotometric method for determining quinone metabolism uses cytochrome *c* as the terminal electron acceptor and gives initial rates of reduction.[32] All the quinones in the present study were assayed by this method (Table 1). This assay generally gives higher reduction rates than the HPLC method, but the relative order of metabolism is essentially the same with the two methods.

The enzyme data show that the new quinones are excellent substrates for rhNQO1, with some of them approaching the reduction rate observed for menadione (1225 ± 15 μmol min^{-1} mg^{-1}), a simple naphthoquinone that has been used to measure activity of the enzyme. Reduction rates for the benzimidazole- and benzothiazole-quinones were similar (Table 1), but all of the new quinones were much better substrates for NQO1 than the indole-quinones from our previous work.[16,18,24] Indole-quinones **28** and **29**, two of the better indole-quinone substrates possessing hydroxymethyl groups, are included in Table 1 for comparison. Previously, the quinoline-quinones had given the highest reduction

Table 1 Metabolism of quinones by recombinant human NQO1



Ring	Cpd	R^2	R^3	R^5	R^6	NQO1 (av.)[a]/μmol min^{-1} mg^{-1}	NQO1 (init.)[a]/μmol min^{-1} mg^{-1}
A	**11**	CH_2OH	—	MeO	H	43.5 ± 6.3	679 ± 53
A	**15**	CH_2OH	—	H	MeO	nd[b]	784 ± 134
A	**16**	CH_2OAc	—	H	MeO	nd	712 ± 103
A	**17**	CH_2OAr[c]	—	H	MeO	nd	687 ± 104
A	**20**	H	—	H	MeO	nd	576 ± 39
B	**25**	CH_2OH	—	—	—	38.3 ± 8.0	702 ± 50
B	**27**	Me	—	—	—	49.7 ± 4.0	776 ± 114
C	**28**	Me	CH_2OH	—	—	1.25 ± 0.03[d]	nd
C	**29**	CH_2OH	Me	—	—	2.49 ± 1.27[e]	nd

[a] The abbreviations 'av.' and 'init.' refer to average and initial rates of metabolism as measured by the HPLC and spectrophotometric assays respectively. [b] nd = not determined. [c] Ar = 4-nitrophenyl. [d] Ref. 16. [e] Ref. 24.

rates by NQO1,[31,32] but the benzimidazole- and benzothiazole-quinones were generally better substrates. The new quinones also appeared to be much better substrates for NQO1 than the benzimidazole-quinones of Skibo[35] and Borch,[36] an observation based on comparisons of reduction rates for the experimental quinones to menadione.

Previous studies have demonstrated that indole-quinones bearing good leaving groups at the (indol-3-yl)methyl position are poor substrates for the two-electron reducing enzyme NQO1.[16,17] In fact the indole-quinone with a 4-nitrophenoxy group at the (indol-3-yl)methyl position, *i.e.* 5-methoxy-1,2-dimethyl-3-(4-nitrophenoxy)methylindole-4,7-dione (ES936), and its 6-methoxy analogue, are potent mechanism-based inhibitors of the enzyme.[27–30] Therefore it was of interest to examine benzimidazole-quinones containing potential leaving groups such as acetate or 4-nitrophenoxide. However, as can be seen from Table 1, the quinones **16** and **17** bearing such leaving groups are good substrates for the enzyme. In a separate experiment, it was established that **17** caused no inhibition of NQO1 up to 5 μM.[46] This is in contrast to indole-quinones such as ES936 that are potent mechanism-based inhibitors.

The results presented here complement our previous work on bioreductive activation of indole-quinone antitumour agents by NQO1. Novel heterocyclic quinones have been synthesized, characterized and studied biologically as substrates for recombinant human NQO1. These data add to our understanding of the structural requirements for efficient metabolism by the quinone reductase enzyme.

Experimental

Chemistry

For general details see the Electronic Supplementary Information†.

5-Methoxy-1-methylbenzimidazole-2-methanol 9

To a suspension of 4-methoxy-*N*-methyl-2-nitroaniline **8** (5.00 g, 27.5 mmol) in ethanol (460 ml) were added tin powder (14.80 g, 123.6 g-atom) and hydrochloric acid (3 M; 185 ml). The mixture was heated under reflux for 30 min. Upon cooling, the solution was decanted from the excess of tin and neutralized with saturated aqueous sodium hydrogen carbonate. The precipitate was extracted with dichloromethane (4 × 300 ml), filtered through a pad of Celite and $MgSO_4$, and evaporated under reduced pressure to yield the 1,2-diamine, which was used in the next step with no further purification. The 1,2-diamine was dissolved in hydrochloric acid (4 M; 43 ml), and glycolic acid (8.34 g, 109.7 mmol) was added to the reaction mixture, which was stirred under reflux for 4 h. After cooling, the mixture was basified with sodium hydrogen carbonate to pH = 6.5. The mixture was extracted with dichloromethane and the combined organic layers dried over $MgSO_4$, filtered and the filtrate evaporated under reduced pressure to yield the *title compound* (2.60 g, 49%) as a beige crystalline solid, recrystallized from ethyl acetate–pentane; mp 193–195 °C (lit.,[47] mp 191 °C); (Found: C, 62.2; H, 6.3; N, 14.5. $C_{10}H_{12}N_2O_2$ requires C, 62.5; H, 6.3; N, 14.6%); δ_H (300 MHz; $CDCl_3$) 7.15 (1 H, d, *J* 8.8, H-7), 7.14 (1 H, d, *J* 2.2, H-4), 6.90 (1 H, dd, *J* 8.8, 2.2, H-6), 4.84 (2 H, s, CH_2), 3.84 (3 H, s, OMe), 3.79 (3 H, bs, NMe); δ_C (100 MHz; $CDCl_3$) 156.2 (C), 153.9 (C), 142.2 (C), 130.4 (C), 113.0 (CH), 109.7 (CH), 101.4 (CH), 56.7 (CH_2), 55.8 (Me), 29.9 (Me).

2-Acetoxymethyl-5-methoxy-1-methylbenzimidazole

5-Methoxy-1-methylbenzimidazole-2-methanol **9** (1.31 g, 6.85 mmol), dry pyridine (818 μl, 10.28 mmol), DMAP (1 mg) and acetic anhydride (917 μl, 10.28 mmol) were dissolved in dry dichloromethane and stirred under reflux for 2 h. The reaction mixture was evaporated and the crude material obtained was purified by chromatography, eluting with methanol–ethyl acetate (1 : 19), to yield the *title compound* (1.40 g, 87%) as a white–beige crystalline solid, recrystallized from ethyl acetate–pentane; mp 121–122 °C; (Found: C, 61.4; H, 6.0; N, 11.9. $C_{12}H_{14}N_2O_3$ requires C, 61.5; H, 6.0; N, 12.0%); (Found: M^+, 234.1008. $C_{12}H_{14}N_2O_3$ requires 234.1004); ν_{max} (KBr)/cm^{-1} 2938, 2838, 1742, 1493, 1371, 1239, 1212, 1149, 1028; δ_H (300 MHz; $CDCl_3$) 7.25 (1 H, d, *J* 2.4, H-4), 7.23 (1 H, d, *J* 8.8, H-7), 6.98 (1 H, dd, *J* 8.8, 2.4, H-6), 5.35 (2 H, s, CH_2), 3.85 (3 H, s, OMe), 3.80 (3 H, s, NMe), 2.13 (3 H, s, Me); δ_C (75 MHz; $CDCl_3$) 170.5 (C), 156.6 (C), 148.7 (C), 143.4 (C), 131.0 (C), 113.9 (CH), 110.1 (CH), 102.2 (CH), 58.4 (Me), 56.0 (CH_2), 30.4 (Me), 20.9 (Me); *m/z* (EI) 234 (M^+, 30%), 191 (100), 175 (40), 161 (20), 147 (18).

5-Methoxy-1-methyl-4-nitrobenzimidazole-2-methanol

To a solution of nitric acid–sulfuric acid (9 : 1; 6 ml), cooled in a salt and ice bath, was added 2-acetoxymethyl-5-methoxy-1-methylbenzimidazole (616 mg, 2.63 mmol) portionwise. The mixture was stirred at room temperature overnight. The mixture was basified with saturated aqueous sodium hydrogen carbonate, extracted with dichloromethane, dried over $MgSO_4$, and evaporated. The crude product, which consisted of a 1 : 2 mixture of the 4-nitro and the 6-nitro products, was purified by chromatography, eluting with methanol–ethyl acetate (1 : 19), to yield the *title compound* (223 mg; 36%) as a light yellow crystalline solid, recrystallized from dichloromethane–pentane; mp 194–197 °C; (Found: C, 50.3; H, 4.5; N, 17.5. $C_{10}H_{11}N_3O_4$ requires C, 50.6; H, 4.7; N, 17.7%); (Found: M^+, 237.0790. $C_{10}H_{11}N_3O_4$ requires 237.0790); ν_{max} (KBr)/cm^{-1} 3199, 2951, 2927, 2848, 1625, 1584, 1522, 1488, 1341, 1278, 1219, 1093, 1049; δ_H (300 MHz; $CDCl_3$) 7.43 (1 H, d, *J* 9.1, ArH), 7.04 (1 H, d, *J* 9.1, ArH), 4.93 (2 H, s, CH_2), 3.97 (3 H, s, NMe), 3.87 (3 H, s, OMe); δ_C (75 MHz; $CDCl_3$) 157.4 (C), 146.4 (C), 134.7 (C), 131.9 (C), 113.8 (CH), 108.7 (CH), 57.5 (CH_2), 56.3 (Me), 30.4 (Me); one ArC unobserved; *m/z* (EI) 237 (M^+, 10%), 205 (50), 190 (100), 162 (15), 134 (15).

4-Amino-5-methoxy-1-methylbenzimidazole-2-methanol 10

To a mixture of 5-methoxy-1-methyl-4-nitrobenzimidazole-2-methanol (220 mg, 0.93 mmol) in ethanol (20 ml) was added tin powder (271 mg, 4.18 g-atom) and hydrochloric acid (3 M; 7.0 ml). The reaction mixture was stirred under reflux for 1 h. After cooling, the mixture was decanted from the excess of tin, neutralized to pH = 9 with a saturated sodium hydrogen carbonate, extracted with dichloromethane, dried over $MgSO_4$, filtered and evaporated under reduced pressure to yield the *title compound* (110 mg, 57%) as a colourless crystalline solid,

recrystallized from dichloromethane–pentane; mp 175–178 °C; (Found: C, 57.6; H, 6.4; N, 20.4. $C_{10}H_{13}N_3O_2$ requires C, 58.0; H, 6.3; N, 20.3%); (Found: M^+, 207.1014. $C_{10}H_{13}N_3O_2$ requires 207.1008); ν_{max} (KBr)/cm^{-1} 3458, 3350, 3108, 2931, 2835, 1617, 1510, 1483, 1341, 1272, 1195, 1176, 1068, 1033; δ_H (300 MHz; $CDCl_3$) 6.89 (1 H, d, *J* 8.6, ArH), 6.57 (1 H, d, *J* 8.6, ArH), 4.82 (2 H, s, CH_2), 4.50 (2 H, br, NH_2), 3.87 (3 H, s, NMe), 3.72 (3 H, s, OMe); δ_C (100 MHz; $CDCl_3$) 152.7 (C), 141.5 (C), 131.9 (C), 130.6 (C), 127.8 (C), 110.1 (CH), 96.8 (CH), 57.6 (Me), 56.5 (CH_2), 29.8 (Me); *m*/*z* (EI) 207 (M^+, 9%), 190 (9), 175 (40), 155 (10), 149 (20), 97 (28), 85 (50), 71 (70), 57 (100).

2-Hydroxymethyl-5-methoxy-1-methylbenzimidazole-4,7-dione 11

To a solution of 4-amino-5-methoxy-1-methylbenzimidazole-2-methanol **10** (60 mg, 0.29 mmol) in acetone (18 ml) was added a solution of potassium nitrosodisulfonate (317 mg, 1.16 mmol) in sodium dihydrogen phosphate buffer (0.3 M; 15 ml). The mixture was stirred at room temperature for 1 h, and then evaporated. The residue was extracted with dichloromethane and the combined organic layers dried over $MgSO_4$, filtered and evaporated under reduced pressure to yield the *title compound* (39 mg, 66%) as a yellow crystalline solid; mp 210–212 °C; (Found: MH^+, 223.0724. $C_{10}H_{10}N_2O_4$ + H requires 223.0719); λ_{max} (acetonitrile)/nm 272 (log ε 3.98), 290 (3.96), 401 (2.80); ν_{max} (KBr)/cm^{-1} 3319, 2924, 2852, 1695, 1654, 1588, 1526, 1316, 1244, 1116, 1091; δ_H (300 MHz; $CDCl_3$) 5.74 (1 H, s, 6-H), 4.82 (2 H, s, CH_2), 4.01 (3 H, s, NMe), 3.86 (3 H, s, OMe); δ_C (100 MHz; $CDCl_3$) 179.3 (C), 179.0 (C), 174.0 (C), 160.0 (C), 150.9 (C), 140.9 (C), 108.1 (CH), 62.6 (CH_2), 57.0 (2 × Me); *m*/*z* (CI) 223 (MH^+, 100%), 207 (35), 193 (10).

6-Methoxy-1-methylbenzimidazole-2-methanol 13

To a solution of 2-amino-5-methoxy-*N*-methylaniline (4.98 g, 32.76 mmol) in hydrochloric acid (4 M; 40 ml) was added glycolic acid (8.60 g, 113.82 mmol). The reaction mixture was heated under reflux for 4 h. After cooling, the reaction mixture was basified with a saturated aqueous solution of sodium hydrogen carbonate to pH 6.5. The reaction mixture was extracted into dichloromethane (3 × 50 ml), and the combined organic layers dried ($MgSO_4$), filtered and the solvent removed *in vacuo*. The crude product obtained was purified by chromatography, gradient elution with methanol–dichloromethane (1–5%), to give the *title compound* (5.01 g, 80%) as an orange crystalline solid; mp 159–162 °C (from chloroform–hexane); (Found: MH^+, 193.0973. $C_{10}H_{12}N_2O_2$ + H requires 193.0977); ν_{max} (KBr)/cm^{-1} 3370, 3145, 3001, 2935, 2848, 2059, 1885, 1634, 1593, 1491, 1475, 1460, 1429, 1342, 1209, 1040; δ_H (300 MHz; $CDCl_3$) 7.52 (1 H, d, *J* 8.8, 4-H), 6.85 (1 H, dd, *J* 8.8, 2.3, 5-H), 6.64 (1 H, d, *J* 2.3, 7-H), 4.83 (2 H, s, C*H*$_2$OH), 3.85 (3 H, s, OMe), 3.76 (3 H, s, NMe); δ_C (75 MHz; $CDCl_3$) 157.1 (C), 153.3 (C), 136.8 (C), 136.1 (C), 120.0 (CH), 112.0 (CH), 93.1 (CH), 57.4 (CH_2), 56.2 (Me), 30.3 (Me); *m*/*z* (CI) 193 (MH^+, 100%), 177 (38), 191 (22), 175 (16).

2-Acetoxymethyl-6-methoxy-1-methylbenzimidazole

6-Methoxy-1-methylbenzimidazole-2-methanol **13** (4.91 g, 25.57 mmol), dry pyridine (3.1 ml), a catalytic amount of DMAP (200 mg) and acetic anhydride (3.62 ml, 38.36 mmol) were dissolved in dry dichloromethane and the mixture heated under reflux for 4 h. The reaction mixture was concentrated *in vacuo*, washed with $CuSO_4$ solution, extracted into dichloromethane (2 × 100 ml), dried ($MgSO_4$), filtered and the solvent removed *in vacuo*. The crude product obtained was purified by chromatography, gradient elution with methanol–dichloromethane (1–5%), to give the *title compound* (5.24 g, 87%) as a beige crystalline solid; mp 113–115 °C (from ethyl acetate); (Found: MH^+, 235.1078. $C_{12}H_{14}N_2O_3$ + H requires 235.1082); ν_{max} (KBr)/cm^{-1} 3012, 2996, 2970, 2935, 1737, 1619, 1481, 1250, 1224, 1214, 1019; δ_H (300 MHz; $CDCl_3$) 7.65 (1 H, d, *J* 8.8, 4-H), 6.93 (1 H, dd, *J* 8.8, 2.2, 5-H), 6.79 (1 H, d, *J* 2.2, 7-H), 5.35 (2 H, s, C*H*$_2$OH), 3.89 (3 H, s, OMe), 3.78 (3 H, s, NMe), 2.14 (3 H, s, Me); δ_C (75 MHz; $CDCl_3$) 170.8 (C), 157.6 (C), 147.9 (C), 137.1 (C), 137.1 (C), 121.2 (CH), 112.4 (CH), 93.2 (CH), 58.7 (CH_2), 56.3 (Me), 30.5 (Me), 21.1 (Me); *m*/*z* (CI) 235 (MH^+, 100%), 175 (77).

6-Methoxy-1-methyl-7-nitrobenzimidazole-2-methanol

A mixture of nitric acid and sulfuric acids (9 : 1; 47 ml) was cooled in an ice bath and added slowly to 2-acetoxymethyl-6-methoxy-1-methylbenzimidazole (4.2 g, 17.95 mmol) cooled in an ice bath. This was stirred for 10 min at −5 °C and then at room temperature for 18 h. The reaction mixture was basified with a saturated aqueous solution of potassium carbonate to pH 9, stirred for 30 min, and extracted into dichloromethane (4 × 50 ml), dried ($MgSO_4$), filtered and the solvent removed *in vacuo*. The crude product, a 1 : 1.6 mixture of 5- and 7-nitro isomers, was purified by chromatography, eluting with ethyl acetate, to give the *title compound* (1.32 g, 31%) as a yellow crystalline solid; mp 207.5–209.5 °C (from ethyl acetate); (Found: C, 50.3; H, 4.4; N, 17.9. $C_{10}H_{11}N_3O_4$ requires C, 50.6; H, 4.7; N, 17.7%); (Found: M^+, 237.0740. $C_{10}H_{11}N_3O_4$ requires 237.0750); ν_{max} (KBr)/cm^{-1} 3132, 1636, 1583, 1521, 1472, 1393, 1334, 1315, 1228, 1244, 1166, 1121, 1076; δ_H (300 MHz; DMSO-*d*) 7.83 (1 H, d, *J* 8.9, ArH), 7.20 (1 H, d, *J* 8.9, ArH), 5.71 (1 H, t, *J* 5.8, CH_2OH), 4.70 (2 H, d, *J* 5.8, C*H*$_2$OH), 3.93 (3 H, s, OMe), 3.63 (3 H, s, NMe); δ_C (75 MHz; DMSO-*d*) 155.9 (C), 147.4 (C), 137.9 (C), 126.6 (C), 125.5 (C), 122.7 (CH), 107.5 (CH), 57.3 (Me), 56.1 (CH_2), 30.8 (Me); *m*/*z* (EI) 237 (M^+, 100%), 161 (61), 160 (20), 131 (45), 104 (20).

7-Amino-6-methoxy-1-methylbenzimidazole-2-methanol 14

To a solution of 6-methoxy-1-methyl-7-nitrobenzimidazole-2-methanol (1.50 g, 6.33 mmol) in ethanol (95 ml) was added palladium-on-carbon (10%; 270 mg). The mixture was stirred under a hydrogen atmosphere for 18 h. The reaction mixture was filtered through Celite and the solvent removed *in vacuo* to give the *title compound* (1.08 g, 72%) as a colourless crystalline solid; mp 208.5–210.5 °C (from methanol–pentane); (Found: C, 57.9; H, 6.5; N, 20.5. $C_{10}H_{13}N_3O_2$ requires C, 58.0; H, 6.3; N, 20.3%); (Found: M^+, 207.1009. $C_{10}H_{13}N_3O_2$ requires 207.1008); ν_{max} (KBr)/cm^{-1} 3402, 3309, 3221, 2930, 2839, 2719, 1623, 1508, 1487, 1476, 1451, 1400, 1231, 1217, 1195, 1029; δ_H (400 MHz; DMSO-*d*) 6.87–6.81 (2 H, m, ArH), 5.48 (1 H, t, *J* 5.7, CH_2OH), 4.61–4.58 (4 H, m, C*H*$_2$OH + NH_2), 4.03 (3 H, s, OMe), 3.78 (3 H, s, NMe); δ_C (100 MHz; DMSO-*d*) 153.3 (C), 142.4 (C), 138.3 (C), 126.0 (C), 123.0 (C), 108.6 (CH), 107.7 (CH), 57.2 (Me), 56.3 (CH_2), 31.9 (Me); *m*/*z* (EI) 207 (M^+, 40%), 192 (100), 162 (35).

2-Hydroxymethyl-6-methoxy-1-methylbenzimidazole-4,7-dione 15

To a solution of 7-amino-6-methoxy-1-methylbenzimidazole-2-methanol **14** (470 mg, 2.27 mmol) in acetone (140 ml) was added a solution of potassium nitrosodisulfonate (2.44 g, 9.08 mmol) in sodium dihydrogen phosphate buffer (0.3 M; 108 ml). The reaction mixture was stirred at room temperature for 2 h. The acetone was removed *in vacuo* and the resulting residue extracted into dichloromethane (2 × 100 ml), washed with water (2 × 50 ml), dried ($MgSO_4$), filtered and the solvent removed *in vacuo* to yield an imine intermediate as a yellow solid. This imine intermediate was then stirred in a mixture of acetone (10 ml) and hydrochloric acid (2 M; 10 ml)) for 1 h. The acetone was removed *in vacuo*, and the reaction mixture basified with a saturated aqueous solution of sodium hydrogen carbonate to pH 7, extracted into dichloromethane (3 × 50 ml), dried ($MgSO_4$) and filtered. The crude product was purified by chromatography, eluting with methanol–ethyl acetate (1 : 9), to give the *title compound* (0.35 g, 69%) as a yellow crystalline solid; mp 223–226 °C (decomp.) (from methanol); (Found: C, 53.7; H, 4.2; N, 12.3. $C_{10}H_{10}N_2O_4$ requires C, 54.1; H, 4.5; N, 12.6%); (Found: MH^+, 223.0710. $C_{10}H_{10}N_2O_4$ + H requires 223.0719); λ_{max} (acetonitrile)/nm 222 (log ε 4.26), 286 (4.20), 391 (2.49); ν_{max} (KBr)/cm^{-1} 3445, 3292, 3061, 2945, 2361, 1681, 1659, 1593, 1539, 1508, 1479, 1407, 1384, 1332, 1262, 1193, 1175; δ_H (300 MHz; DMSO-*d*) 6.91 (1 H, s, 5-H), 5.68 (1 H, t, *J* 5.8, CH_2OH), 4.61, (2 H, d, *J* 5.8, CH_2OH), 3.90 (3 H, s, OMe), 3.80 (3 H, s, NMe); δ_C (100 MHz; DMSO-*d*) 181.3 (C), 172.5 (C), 159.8 (C), 155.1 (C), 141.3 (C), 130.4 (C), 106.7 (CH), 57.3 (CH_2), 55.9 (Me), 32.7 (Me); *m*/*z* (CI) 223 (MH^+, 100%).

2-Acetoxymethyl-6-methoxy-1-methylbenzimidazole-4,7-dione 16

To a stirred solution of 2-hydroxymethyl-6-methoxy-1-methylbenzimidazole-4,7-dione **15** (50 mg, 0.225 mmol) in dichloromethane (5 ml) and acetone (1 ml) containing DMAP (2.75 mg, 0.025 mmol)) was added acetic anhydride (0.11 ml, 1.13 mmol). The solution was stirred at room temperature for 5 min. The solvent was removed *in vacuo* and the crude product was dissolved in ethyl acetate (50 ml), washed with a saturated aqueous solution of sodium hydrogen carbonate (20 ml), dried ($MgSO_4$), filtered and the filtrate evaporated. The residue was purified by chromatography, eluting with ethyl acetate–light petroleum (4 : 1), to give the *title compound* (58 mg, 97%) as a bright yellow crystalline solid; mp 172–173 °C (from ethyl acetate–hexane); (Found: M^+, 264.0735. $C_{12}H_{12}N_2O_5$ requires 264.0746); λ_{max} (acetonitrile)/nm 223 (log ε 4.31), 286 (4.23), 391 (2.52); ν_{max} (KBr)/cm^{-1} 1756, 1747, 1677, 1661, 1594, 1529, 1514, 1225, 1215, 1189, 1172, 1045, 1028; δ_H (300 MHz; $CDCl_3$) 5.85 (1 H, s, 5-H), 5.26 (2 H, s, CH_2OAc), 4.00 (3 H, s, OMe), 3.87 (3 H, s, NMe), 2.13 (3 H, s, Me); δ_C (100 MHz; $CDCl_3$) 180.5 (C), 172.6 (C), 170.1 (C), 159.4 (C), 149.8 (C), 142.2 (C), 129.8 (C), 106.6 (CH), 57.0 (CH_2), 56.9 (Me), 32.7 (Me), 20.6 (Me); *m*/*z* (EI) 264 (M^+, 100%).

6-Methoxy-1-methyl-2-(4-nitrophenoxymethyl)benzimidazole-4,7-dione 17

To a stirred solution of 2-hydroxymethyl-6-methoxy-1-methylbenzimidazole-4,7-dione **15** (50 mg, 0.23 mmol) in dichloromethane (5 ml) at 0 °C was added dropwise thionyl chloride (0.82 ml, 11.26 mmol). The reaction mixture was stirred at room temperature for 18 h. The solvent was removed *in vacuo* and the crude product was used directly in the next step without further purification.

The crude 3-chloromethyl-6-methoxy-1methylbenzimidazole-4,7-dione, 4-nitrophenol (125 mg, 0.90 mmol) and potassium carbonate (156 mg, 1.13 mmol) were stirred in DMF (5 ml) for 18 h. The solvent was removed *in vacuo* and the crude product was dissolved in dichloromethane (50 ml), washed with water (2 × 20 ml), dried ($MgSO_4$), filtered and the solvent removed *in vacuo*. The crude product was purified by chromatography, gradient elution with ethyl acetate–light petroleum (50–75%), to give the *title compound* (45 mg, 58%) as a bright yellow crystalline solid; mp 245.5–246.5 °C (from ethyl acetate–hexane); (Found: MH^+, 344.0869. $C_{16}H_{13}N_3O_6$ + H requires 344.0883); λ_{max} (acetonitrile)/nm 224 (log ε 4.38), 290 (4.33); ν_{max} (neat)/cm^{-1} 1780, 1664, 1594, 1508, 1496, 1348, 1337, 1251, 1113, 1017; δ_H (400 MHz; $CDCl_3$) 8.21 (2 H, d, *J* 9.2, ArH), 7.16 (2 H, d, *J* 9.2, ArH), 5.86 (1 H, s, 5-H), 5.40 (2 H, s, CH_2OAr), 4.09 (3 H, s, OMe), 3.86 (3 H, s, NMe); δ_C (100 MHz; $CDCl_3$) 180.4 (C), 172.5 (C), 162.0 (C), 159.4 (C), 149.1 (C), 142.5 (C), 141.8 (C), 130.2 (C), 125.6 (CH), 114.9 (CH), 106.7 (CH), 62.3 (CH_2), 56.8 (Me), 32.8 (Me); *m*/*z* (ES) 344 (MH^+, 100%), 366 (M + Na, 29).

6-Methoxy-1-methylbenzimidazole 18

To a solution of 2-amino-5-methoxy-*N*-methylaniline (2.38 g, 15.6 mmol) in hydrochloric acid (4 M; 22 ml) was added formic acid (2.36 ml, 62.6 mmol). The reaction mixture was heated under reflux for 4 h. After cooling, the reaction mixture was basified with a saturated aqueous solution of sodium hydrogen carbonate to pH 6.5. The reaction mixture was extracted into dichloromethane (3 × 50 ml) and the combined organic layer dried ($MgSO_4$), filtered and the solvent removed *in vacuo*. The crude product obtained was purified by chromatography, gradient elution with methanol–ethyl acetate (1–10%), to give the *title compound* (1.52 g, 60%) as an orange crystalline solid; mp 66–67 °C (lit.,[44] mp 67–68 °C); δ_H (300 MHz; $CDCl_3$) 7.71 (1 H, s, 2-H), 7.61 (1 H, d, *J* 8.8, 4-H), 6.86 (1 H, dd, *J* 8.8, 2.3, 5-H), 6.76 (1 H, d, *J* 2.3, 7-H), 3.82 (3 H, s, OMe), 3.74 (3H, s, NMe); δ_C (75 MHz; $CDCl_3$) 157.3 (C), 143.1 (C), 138.5 (C), 135.5 (C), 121.1 (CH), 111.9 (CH), 93.1 (CH), 56.3 (Me), 31.4 (Me).

6-Methoxy-1-methyl-7-nitrobenzimidazole 19

A mixture of nitric and sulfuric acids (9 : 1; 11.9 ml) was cooled in an ice bath and added slowly to 6-methoxy-1-methylbenzimidazole **18** (1.25 g, 7.72 mmol) cooled in an ice bath. The mixture was stirred for 10 min at −5 °C and then at room temperature for 16 h. The reaction mixture was basified with a saturated aqueous solution of potassium carbonate and extracted into dichloromethane (3 × 100 ml), dried ($MgSO_4$), filtered and the solvent removed *in vacuo*. The crude product, composed of a 1 : 1.3 mixture of the 5-and 7-nitro isomers, was purified by chromatography, gradient elution with methanol–ethyl acetate (0–5%), to give (i) the *title compound* (0.87 g, 55%) as a yellow crystalline solid; mp 123–125 °C (from ethyl acetate); (Found: MH^+, 208.0725. $C_9H_9N_3O_3$ + H requires 208.0722); ν_{max} (KBr)/cm^{-1} 3436, 3095, 2946, 1636, 1526, 1462, 1372, 1265, 1079, 1058; δ_H (300 MHz; $CDCl_3$) 7.87 (1 H, d, *J* 9.0, ArH), 7.81 (1 H, s,

2-H), 7.03 (1 H, d, *J* 9.0, ArH), 3.99 (3 H, s, OMe), 3.76 (3 H, s, NMe); δ_C (75 MHz; $CDCl_3$) 149.1 (C), 146.1 (CH), 140.6 (C), 126.8 (C), 126.7 (C), 124.2 (CH), 108.2 (CH), 57.9 (Me), 33.1 (Me); *m/z* (CI) 208 (MH^+, 100%), 178 (28), and (ii) 6-methoxy-1-methyl-5-nitrobenzimidazole (0.50 g, 31%) as a bright yellow solid; mp 157 159 °C (from ethyl acetate); δ_H (300 MHz; $CDCl_3$) 8.33 (1 H, s, ArH), 7.91 (1 H, s, 2-H), 6.93 (1 H, s, ArH), 4.02 (3 H, s, OMe), 3.87 (3 H, s, NMe).

7-Amino-6-methoxy-1-methylbenzimidazole

To a solution of 6-methoxy-1-methyl-7-nitrobenzimidazole **19** (460 mg, 3.22 mmol) in ethanol (25 ml) was added palladium-on-carbon (10%; 50 mg). The mixture was stirred under a hydrogen atmosphere for 25 h. The reaction mixture was filtered through Celite and the solvent removed *in vacuo* to yield the *title compound* (393 mg, 100%) as a brown crystalline solid; mp 162–164 °C (from ethanol); (Found: MH^+, 178.0980. $C_9H_{11}N_3O$ + H requires 178.0980); ν_{max} (KBr)/cm^{-1} 3414, 3297, 3197, 2953, 1625, 1508, 1466, 1421, 1328, 1264, 1234, 1200, 1062, 1042; δ_H (300 MHz; DMSO-*d*) 8.01 (1 H, s, 2-H), 7.06 (1 H, d, *J* 8.7, ArH), 7.02 (1 H, d, *J* 8.7, ArH), 4.77 (2 H, bs, NH_2), 4.20 (3 H, s, OMe), 3.95 (3 H, s, NMe); δ_C (75 MHz; $CDCl_3$) 144.5 (CH), 144.1 (C), 140.8 (C), 125.6 (C), 122.0 (C), 110.9 (CH), 109.2 (CH), 57.7 (Me), 33.8 (Me); *m/z* (CI) 178 (MH^+, 100%).

6-Methoxy-1-methylbenzimidazole-4,7-dione 20

To a solution of 7-amino-6-methoxy-1-methylbenzimidazole (328 mg, 1.77 mmol) in acetone (90 ml) was added a solution of potassium nitrosodisulfonate (1.99 g, 7.41 mmol) in sodium dihydrogen phosphate buffer (0.3 M; 72 ml). The reaction mixture was stirred at room temperature for 2 h. The excess acetone was removed *in vacuo* and the resulting residue extracted into dichloromethane (2 × 100 ml), washed with water (2 × 50 ml), dried ($MgSO_4$), filtered and the solvent removed *in vacuo* to yield an imine intermediate as a yellow solid. This imine intermediate was then stirred in a mixture of acetone (20 ml) and hydrochloric acid (2 M; 20 ml)) for 1 h. The acetone was removed *in vacuo* and the reaction mixture basified with a saturated aqueous solution of sodium hydrogen carbonate to pH 7, extracted into dichloromethane (6 × 50 ml), dried ($MgSO_4$) and filtered. The solvent was removed *in vacuo* to yield the *title compound* (240 mg, 67%) as a yellow crystalline solid; mp 225–228 °C (decomp.) (from methanol); (Found: C, 55.9; H, 3.9; N, 14.6. $C_9H_8N_2O_3$ requires C, 56.3; H, 4.2; N, 14.6%); (Found: M^+, 192.0533. $C_9H_8N_2O_3$ requires 192.0535); λ_{max} (acetonitrile)/nm 218 (log ε 3.98), 285 (3.97), 368 (2.19); ν_{max} (KBr)/cm^{-1} 3436, 3097, 3076, 2924, 1654, 1588, 1526, 1455, 1423, 1322, 1249, 1211, 1189, 1164, 1031; δ_H (400 MHz; $CDCl_3$) 7.70 (1 H, s, 5-H), 5.82 (1 H, s, 2-H), 3.99 (3 H, s, OMe), 3.85 (3 H, s, NMe); δ_C (100 MHz; $CDCl_3$) 180.6 (C), 172.7 (C), 159.3 (C), 144.3 (CH), 143.6 (C), 129.1 (C), 106.9 (CH), 56.8 (Me), 33.8 (Me); *m/z* (EI) 192 (M^+, 100%), 164 (54).

5-Methoxybenzothiazole-2-carboxaldehyde 22

5-Methoxy-2-methylbenzothiazole **21** (5.00 g, 27.93 mmol) was added at once to a solution of selenium dioxide (4.00 g, 36.04 mmol) in dioxan (10 ml) and water (1 ml) heated to 55–60 °C. The reaction mixture was stirred and heated under reflux for 2 h. The reaction mixture was filtered through Celite and evaporated. The crude product obtained was purified by chromatography, eluting with dichloromethane, to yield the *title compound* (2.72 g, 50%) as a yellow crystalline solid, recrystallized from dichloromethane–pentane; mp 114–115 °C (lit.,[45] mp 100–101 °C); (Found: C, 55.7; H, 3.5; N, 7.0. $C_9H_7NO_2S$ requires C, 55.9; H, 3.6; N, 7.2%); δ_H (300 MHz; $CDCl_3$) 10.24 (1 H, s, CHO), 7.86 (1 H, d, *J* 8.9, 7-H), 7.65 (1 H, d, *J* 2.5, 4-H), 7.24 (1 H, dd, *J* 8.9, 2.5, 6-H), 3.93 (3 H, s, OMe); δ_C (100 MHz; $CDCl_3$) 185.4 (CH), 166.2 (C), 159.8 (C), 155.0 (C), 128.7 (C), 122.9 (CH), 120.0 (CH), 106.4 (CH), 55.7 (Me).

5-Methoxy-4-nitrobenzothiazole-2-carboxaldehyde 23

5-Methoxybenzothiazole-2-carboxaldehyde **22** (2.20 g, 11.34 mmol) was added to a solution of nitric acid (22 ml) and sulfuric acid (3.3 ml) at −10 °C. The reaction mixture was stirred at room temperature overnight, followed by addition of ice and neutralization of the acid with a saturated solution of sodium hydrogen carbonate. The mixture was extracted with dichloromethane, dried over $MgSO_4$, filtered and evaporated under reduced pressure. NMR of the crude product showed a 6 : 1 mixture of the 4- and 6-nitro derivatives. The crude product was purified by flash chromatography, eluting with dichloromethane, to yield the *title compound* (1.45 g, 53%) as a light yellow solid; mp 188–190 °C; (Found: MH^+, 239.0132. $C_9H_6N_2O_4S$ + H requires 239.0127); ν_{max} (KBr)/cm^{-1} 3437, 2937, 2879, 1686, 1605, 1521, 1475, 1371, 1282, 1205, 1128, 1094; δ_H (400 MHz; $CDCl_3$) 10.14 (1 H, s, CHO), 8.08 (1 H, d, *J* 9.1, ArH), 7.42 (1 H, d, *J* 9.1, ArH), 4.06 (3 H, s, OMe); δ_C (100 MHz; $CDCl_3$) 184.9 (CH), 169.0 (C), 150.6 (C), 146.3 (C), 129.3 (C), 125.3 (CH), 115.0 (CH), 57.5 (Me); one ArC unobserved; *m/z* (CI) 239 (MH^+, 5%), 227 (5), 208 (5), 202 (5), 186 (5), 153 (5), 144 (5), 61 (100).

5-Methoxy-4-nitrobenzothiazole-2-methanol 24

Sodium borohydride (64 mg, 1.68 mmol) was added at once to a solution of 5-methoxy-4-nitrobenzothiazole-2-carboxaldehyde **23** (100 mg, 0.42 mmol) in dry methanol (10 ml) cooled to 0 °C. The reaction mixture was stirred at room temperature for 2 h. The reaction mixture was evaporated and the crude product obtained was purified by flash chromatography, eluting with ethyl acetate–dichloromethane (1 : 1), to yield the *title compound* (90 mg, 90%) as an orange solid; mp 150–152 °C; (Found: MH^+, 241.0280. $C_9H_8N_2O_4S$ + H requires 241.0283); ν_{max} (KBr)/cm^{-1} 3356, 2940, 2839, 1613, 1537, 1521, 1476, 1420, 1376, 1292, 1232, 1180, 1132, 1096, 1064, 1040; δ_H (300 MHz; $CDCl_3$) 7.93 (1 H, d, *J* 8.9, ArH), 7.18 (1 H, d, *J* 8.9, ArH), 5.09 (2 H, s, CH_2OH), 4.00 (3 H, s, OMe), 2.85 (1 H, bs, OH); δ_C (100 MHz; $CDCl_3$) 178.1 (C), 149.9 (C), 145.8 (C), 134.0 (C), 128.1 (C), 124.3 (CH), 110.9 (CH), 62.9 (CH_2), 57.3 (Me); *m/z* (CI) 241 (MH^+, 80%), 239 (MH^-, 100), 223 (35), 209 (50), 193 (10).

2-Hydroxymethyl-5-methoxybenzothiazole-4,7-dione 25

To a suspension of 5-methoxy-4-nitrobenzothiazole-2-methanol **24** (454 mg, 1.81 mmol) in ethanol (31 ml) was added tin powder (272 mg, 7.57 g-atom) and hydrochloric acid (3 M; 13 ml). The mixture was stirred and heated under reflux for 1 h. Upon cooling, the reaction mixture was decanted from the excess of

tin and neutralized with a saturated aqueous solution of sodium hydrogen carbonate. The suspension obtained was added to an equal volume of water. The precipitate and aqueous layer were filtered through Celite and extracted with dichloromethane. The combined organic layer was dried over $MgSO_4$ and evaporated under reduced pressure to yield the *amino derivative* as a yellow solid, which was used in the next step with no further purification.

To a solution of the above amino derivative in acetone (110 ml) was added a solution of potassium nitrosodisulfonate (1.97 g, 7.22 mmol) in sodium dihydrogen phosphate buffer (0.3 M; 88 ml). The reaction was stirred at room temperature for 1 h. The excess acetone was removed *in vacuo*. The resulting residue was extracted with dichloromethane and the combined organic layers evaporated off. The residue obtained was stirred at room temperature in a 1 : 1 mixture of 2 M hydrochloric acid and acetone (220 ml) for 1 h. The acetone was removed *in vacuo* and the residue extracted with dichloromethane. The organic layer was washed with water, dried over $MgSO_4$, filtered and evaporated under reduced pressure. The crude material was purified by chromatography, eluting with ethyl acetate–light petroleum (1 : 1), to yield the *title compound* (288 mg, 71%) as a yellow crystalline solid; mp 193–194 °C; (Found: MH^+, 226.0173. $C_9H_7NO_4S$ + H requires 226.0174); λ_{max} (acetonitrile)/nm 203 (log ε 3.88), 232 (3.97), 280 (3.91), 383 (2.78); ν_{max} (KBr)/cm^{-1} 3248, 1691, 1641, 1595, 1524, 1474, 1444, 1315, 1248, 1114, 1064; δ_H (300 MHz; $CDCl_3$) 6.05 (1 H, s, 6-H), 5.06 (2 H, s, CH_2OH), 3.92 (3 H, s, OMe), 2.65 (1 H, bs, OH); δ_C (100 MHz; $CDCl_3$) 182.0 (C), 180.9 (C), 174.7 (C), 161.7 (C), 152.5 (C), 140.4 (C), 108.8 (CH), 63.0 (CH_2), 57.8 (Me); *m*/*z* (CI) 226 (MH^+, 100%), 208 (30).

5-Methoxy-2-methyl-4-nitrobenzothiazole 26

5-Methoxy-2-methylbenzothiazole **21** (4.95 g, 27.65 mmol) was added to a solution of nitric acid (12.5 ml) at −10 °C. The reaction mixture was stirred at room temperature overnight, followed by addition of ice (50 g) and neutralization of the acid with a saturated solution of sodium hydrogen carbonate. The mixture was extracted with dichloromethane, the combined extracts dried over $MgSO_4$, filtered and the filtrate evaporated under reduced pressure. The NMR of the crude product showed a 5 : 2 mixture of the 4- and 6-nitro derivatives. The crude product was purified by chromatography, eluting with dichloromethane, to yield the *title compound* (3.74 g, 60%) as a light yellow crystalline solid, recrystallized from dichloromethane–pentane; mp 153–155 °C (lit.,[38] mp 144–145 °C); (Found: C, 47.9; H, 3.4; N, 12.4. $C_9H_8N_2O_3S$ requires C, 48.2; H, 3.6; N, 12.5%); δ_H (300 MHz; $CDCl_3$) 7.84 (1 H, d, *J* 8.9, ArH), 7.13 (1 H, d, *J* 8.9, ArH), 3.99 (3 H, s, OMe), 2.85 (3 H, s, Me); δ_C (75 MHz; $CDCl_3$) 172.4 (C), 149.7 (C), 145.9 (C), 134.0 (C), 129.0 (C), 123.7 (CH), 110.5 (CH), 57.2 (Me), 20.6 (Me).

4-Amino-5-methoxy-2-methylbenzothiazole

To a suspension of 5-methoxy-2-methyl-4-nitrobenzothiazole **26** (2.00 g, 8.93 mmol) in ethanol (148 ml) was added tin powder (1.07 g, 35.71 g-atom) and hydrochloric acid (3 M; 64 ml). The mixture was stirred and heated under reflux for 1 h. Upon cooling, the reaction mixture was decanted from the excess of tin and neutralized with a saturated aqueous solution of sodium hydrogen carbonate. The suspension obtained was added to an equal volume of water. The precipitate and aqueous layer were filtered through Celite and extracted with dichloromethane. The organic layer was dried over Na_2SO_4 and evaporated under reduced pressure. The crude product was purified by chromatography, eluting with dichloromethane, to yield the *title compound* (1.72 g, 100%) as a light yellow solid; mp 122–124 °C (lit.,[38] mp 118–119 °C); δ_H (300 MHz; $CDCl_3$) 7.08 (1 H, d, *J* 8.6, ArH), 6.92 (1 H, d, *J* 8.6, ArH), 4.40 (2 H, bs, NH_2), 3.90 (3 H, s, OMe), 2.77 (3 H, s, Me); δ_C (75 MHz; $CDCl_3$) 165.4 (C), 144.1 (C), 142.3 (C), 130.1 (C), 128.7 (C), 110.1 (CH), 108.6 (CH), 56.6 (Me), 20.1 (Me)

5-Methoxy-2-methylbenzothiazole-4,7-dione 27

To a solution of 4-amino-5-methoxy-2-methylbenzothiazole (500 mg, 2.58 mmol) in acetone (138 ml) was added a solution of potassium nitrosodisulfonate (2.76 g, 10.31 mmol) in sodium dihydrogen phosphate buffer (0.3 M; 69 ml). The reaction was stirred at room temperature for 1 h. The excess acetone was removed *in vacuo*, the resulting residue extracted with dichloromethane and the combined organic layers evaporated off. The residue obtained was stirred at room temperature in a 1 : 1 mixture of 2 M hydrochloric acid and acetone (280 ml) for 1 h. The acetone was removed *in vacuo* and the resulting residue was extracted with dichloromethane. The organic layer was washed with water, dried over $MgSO_4$, filtered and evaporated under reduced pressure. The crude material was purified by flash chromatography, eluting with ethyl acetate–light petroleum (1 : 1), to yield the *title compound* (436 mg, 81%) as a yellow crystalline solid, recrystallized from dichloromethane–pentane; mp 255 °C (decomp.) (lit.,[38] mp 248–249 °C); (Found: C, 51.7; H, 3.1; N, 6.6. $C_9H_7NO_3S$ requires C, 51.7; H, 3.4; N, 6.7%); λ_{max} (acetonitrile)/nm 228 (log ε 3.96), 268 (3.91), 292 (3.86), 388 (2.94); ν_{max} (KBr)/cm^{-1} 3425, 3041, 2991, 2944, 2848, 1690, 1640, 1598, 1501, 1471, 1440, 1324, 1251, 1101; δ_H (300 MHz; $CDCl_3$) 6.02 (1 H, s, 6-H), 3.91 (3 H, s, OMe), 2.85 (3 H, s, Me); δ_C (100 MHz; $CDCl_3$) 179.4 (C), 173.8 (C), 172.9 (C), 160.0 (C), 150.9 (C), 141.1 (C), 107.9 (C), 57.0 (Me), 20.1 (Me); *m*/*z* (CI) 210 (MH^+, 100%), 180 (2).

Electrochemistry

Electrochemical studies were performed on representative quinones in DMF as solvent with tetra-*n*-butylammonium tetrafluoroborate as supporting electrolyte as previously described.[16] E_{redox} (±0.005 V) values, referenced to ferrocene and calculated as (E_{pc} + E_{pa})/2, are averages of the values determined from voltammograms recorded at potential sweep rates of 50, 100, 200, 300, 400 and 500 mV s^{-1}. E_{pc}, cathodic peak potential; E_{pa}, anodic peak potential.

Biology

HPLC analysis. Reduction of the quinones was followed by HPLC using an Alltech C18 (5 μm, 250 mm × 4.6 mm) column with a Waters HPLC system (2487 Dual λ Absorbance detector, two 515 HPLC pumps, 717plus Autosampler, Millennium32 Chromatography Manager). The solvent program used a linear gradient of 5% to 80% B over 10 min, 80% B for 5 min, then 80% B to 5% B over 5 min (solution A, 10 mM potassium phosphate buffer, pH 6.0; solution B, methanol). Reactions were

run in 25 mM Tris-HCl (pH 7.4) containing 200 μM NADH (Sigma), 50 μM quinone, and recombinant human NQO1 (gift from David Ross, University of Colorado Health Sciences Center, Denver, CO). NADH oxidation was quantified at 340 nm following 30–40 min incubations at 22 °C.

Spectrophotometric method. Quinone reduction by recombinant human NQO1 was also quantified using a modification of an assay that uses cytochrome *c* as the terminal electron acceptor.[17] Reaction mixtures contained 1 mM NADH (Sigma), 25 μM quinone, 70 μM cytochrome *c* (Sigma) and 0.1–3.0 μg ml^{-1} rhNQO1 in 25 mM Tris-HCl (pH 7.4) with 0.07% BSA and 0.1% Tween-20. Reactions were run at least in triplicate at 22 °C in a Beckman DU 7500 spectrophotometer at 550 nm (molar absorptivity 21.1 mM^{-1} cm^{-1} for cytochrome *c*). Initial reduction rates (μmol cytochrome *c* reduced min^{-1} mg^{-1} NQO1) were calculated from the linear portion (0–30 s) of the reaction curves.

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