# SYNTHESIS OF BIOREDUCTIVELY ACTIVATED ALKYLATING HOECHST 33258 ANALOGUES

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Abstract –A series of DNA-interactive bis-benzimidazole analogues of Hoechst 33258 was prepared to explore the potential for anticancer activity mediated for certain of the drugs *via* bioreductive activation by endogenous NADH or NADPH.

The design of DNA sequence-specific cleaving agents has received increasing attention because of their potential as anticancer drugs. One strategy to this goal is by the linkage of a DNA-cleaving moiety to a sequence-specific DNA-binding agent (1). Based on this idea, a number of approaches have been taken to this problem including use of the naturally occurring oligopeptide distamycin as a DNA recognizing moiety (2). The synthetic bis-benzimidazole Hoechst dye 33258 is also known to bind to the minor groove of double-helical DNA and selectively at 5'-AATT sites (3). The interaction of Hoechst 33258 with DNA has been extensively studied by biophysical methods, including footprinting, NMR, and x-ray techniques (4). The flexible nature of this bis-benzimidazole ring system permits the dye to take an optimum conformation and thus binding effectively to double-stranded DNA (5). The bis-benzimidazole Hoechst 33258 has been used widely as a chromosomal staining agent in biochemistry because it has ready access into cells (6).
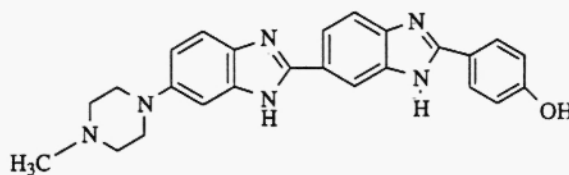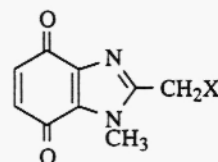

Hoechst 33258
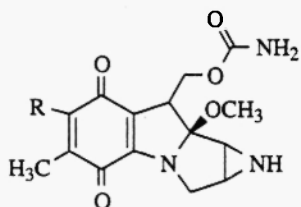
Mitomycin R = NH₂, OCH₃
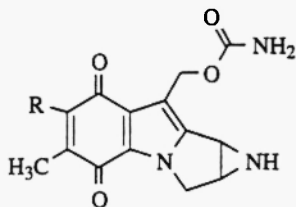
Mitosene R = NH₂, OCH₃

Benzoquinoneimidazole ring system

On the other hand, several naturally occurring quinonoid anticancer agents, e.g. mitomycins and the corresponding mitosene analogues, are capable of bioreductive activation to generate reactive quinone methides that alkylate biomolecules (7). Recently, the benzimidazole ring system has been designed as a reductive alkylator by functionalizing the quinone devivatives of the ring system with -CH$_2$X, where X is a leaving group (8). These derivatives result in quinone methide formation upon reduction (8). This suggests that if we incorporate a quinone methide precursor into the Hoechst 33258 structure it would be effective both as a sequence and site directed groove binder and alkylator. We reported herein the synthesis of such functionalized Hoechst 33258 quinone derivatives and some preliminary biological properties.

Synthesis of the bisbenzimidazole quinone compounds was based on incorporation of our reported procedure (9) for the synthesis of certain Hoechst 33258 analogues and Skibo's method (8) for the generation of the benzimidazole-quinone system. This involves the synthesis of the substituted aldehyde and construction of the benzimidazole heterocycle. The preparation of bis-benzimidazole compounds **5**, **6**, and **7** is shown in Scheme I.

Scheme I
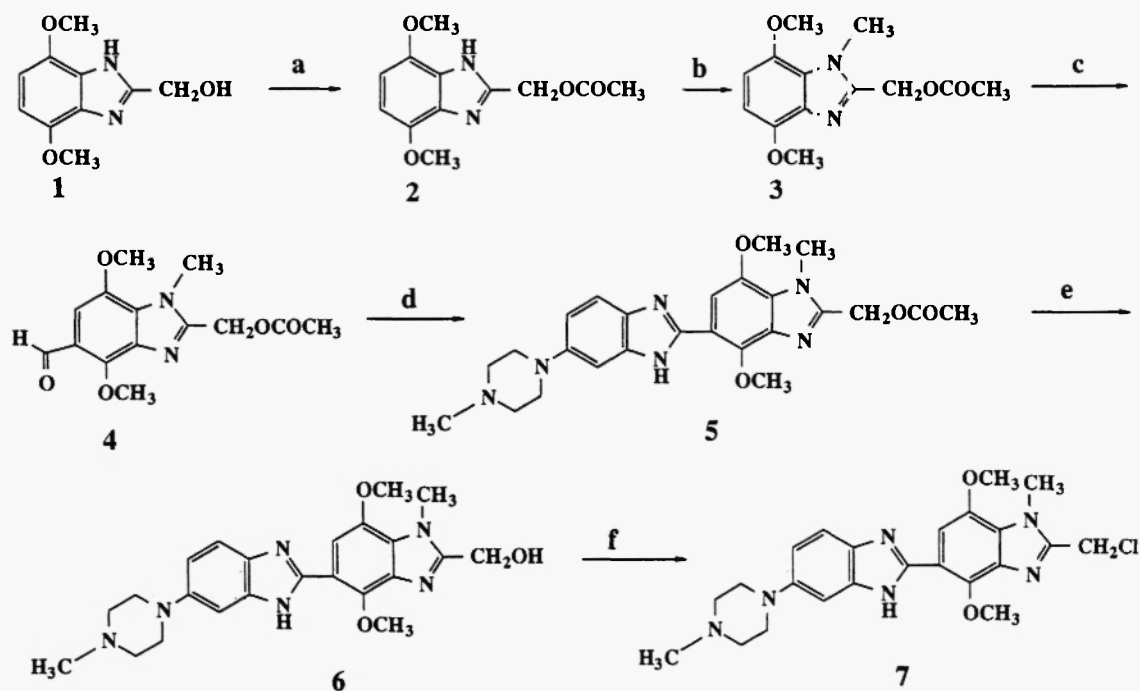
Reagents: a) (CH$_3$CO)$_2$O, THF; b) NaH, CH$_3$I, THF; c) SnCl$_4$, Cl$_2$CHOCH$_3$; d) 4-(4-methyl-1-piperazinyl)-1,2-diaminobenzene, nitrobenzene; e) NaOH, C$_2$H$_5$OH/H$_2$O (1:1); f) PCl$_5$, DMF.

Compound **1** (10) was acetylated in THF with acetic anhydride and the resulting ester compound **2** was subjected to methylation with methyl iodide in the presence NaH to give **3**. Formylation of **3** with TiCl$_4$ and Cl$_2$CHOCH$_3$ gave aldehyde **4**. In order to identify the position of the formyl group, we have carried out an x-ray crystallographic determination. The results of crystallography (see Figure 1, Tables 1 and 2) show that this formyl aldehyde benzimidazole is 1-methyl-5-formyl-2-(acetoxymethyl)-4,7-dimethoxybenzimidazole. Condensation of this aldehyde with 4-(4-methyl-1-piperazinyl)-1,2-

diaminobenzene (11) gave the bis-benzimidazole derivative **5**. The bis-benzimidazole hydroxy compound **6** was obtained by treatment of **5** with NaOH in MeOH and water. Conversion of **6** to bis-benzimidazole chloride compound **7** was accomplished with PCl_5 in DMF at room temperature.

Table 1. Selected interatomic distances (Å) of aldehyde (**4**)

Figure 1. X-ray crystal structure of aldehyde (**4**)

Atom			Atom		
1	2	Distance	1	2	Distance
O1	C9	1.445(3)	N2	C1	1.311(3)
O1	C10	1.341(3)	N2	C2	1.389(3)
O2	C10	1.191(3)	C1	C9	1.494(3)
O3	C3	1.375(3)	C2	C3	1.408(3)
O3	C12	1.399(3)	C2	C7	1.398(3)
O4	C13	1.211(3)	C3	C4	1.382(3)
O5	C6	1.365(3)	C4	C5	1.420(3)
O5	C14	1.424(3)	C4	C13	1.465(3)
N1	C1	1.368(3)	C5	C6	1.371(3)
N1	C7	1.381(3)	C6	C7	1.407(3)
N1	C8	1.459(3)	C10	C11	1.482(3)
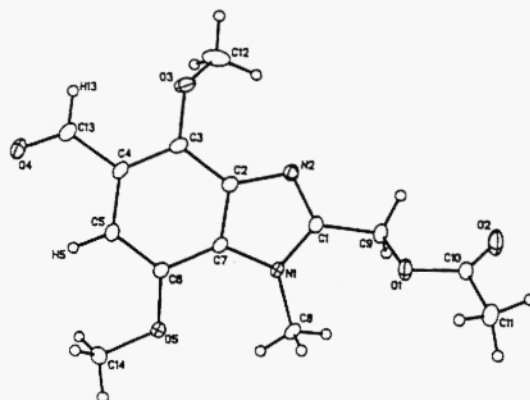
Table 2. Selected interatomic angles (deg) of aldehyde **4**

Atom 1	Atom 2	Atom 3	Angle	Atom 1	Atom 2	Atom 3	Angle
C9	O1	C10	116.3(2)	C3	C4	C5	121.7(2)
C3	O3	C12	116.6(2)	C3	C4	C13	119.5(2)
C6	O5	C14	116.6(2)	C5	C4	C13	118.7(2)
C1	N1	C7	106.3(2)	C4	C5	C6	121.3(2)
C1	N1	C8	125.8(2)	O5	C6	C5	126.3(2)
C7	N1	C8	127.8(2)	O5	C6	C7	117.1(2)
C1	N2	C2	104.2(2)	C5	C6	C7	116.7(2)
N1	C1	N2	113.9(2)	N1	C7	C2	105.2(2)
N1	C1	C9	122.6(2)	N1	C7	C6	131.9(2)
N2	C1	C9	123.5(2)	O2	C7	C6	123.0(2)
N2	C2	C3	130.0(2)	O1	C9	C1	107.3(2)
N2	C2	C7	110.4(2)	O1	C10	O2	122.3(2)
C3	C2	C7	119.5(2)	O1	C10	C11	111.6(2)
O3	C3	C2	122.0(2)	O2	C10	C11	126.1(2)
O3	C3	C4	120.2(2)	O4	C13	C4	125.1(2)
C2	C3	C4	117.7(2)				

The bis-benzimidazole quinone compound was synthesized in a similar manner. Benzimidazole ether **8** was obtained by alkylation of **1** with 3 equivalents of NaH and methyl iodide in THF. The 5-substituted formyl group of **9**, the structure of which is based on the x-ray structure of **4**, was introduced by reaction of **8** with $SnCl_4$ and Cl_2CHOCH_3. Compound **10** was prepared by condensation of **9** with 4-(4-methyl-1-piperazinyl)-1,2-diaminobenzene. One of the dimethyl groups in the benzene ring of **10** was removed selectively in refluxing 48% HBr to give the monohydroxy compound **11** as the only product. The structure of **11** was established by the absence of a NOE signal between the remaining OCH_3 and the N–CH_3 group. However, treatment of **10** with BBr_3 in toluene afforded hydroquinone compound **12** which was oxidized with p-chloranil to corresponding quinone **13**. Treatment of **13** with PCl_5 in DMF gave the final compound **14**. Acetylation of **13** with acetyl chloride afforded **15** as summarized in Scheme II.

Scheme II
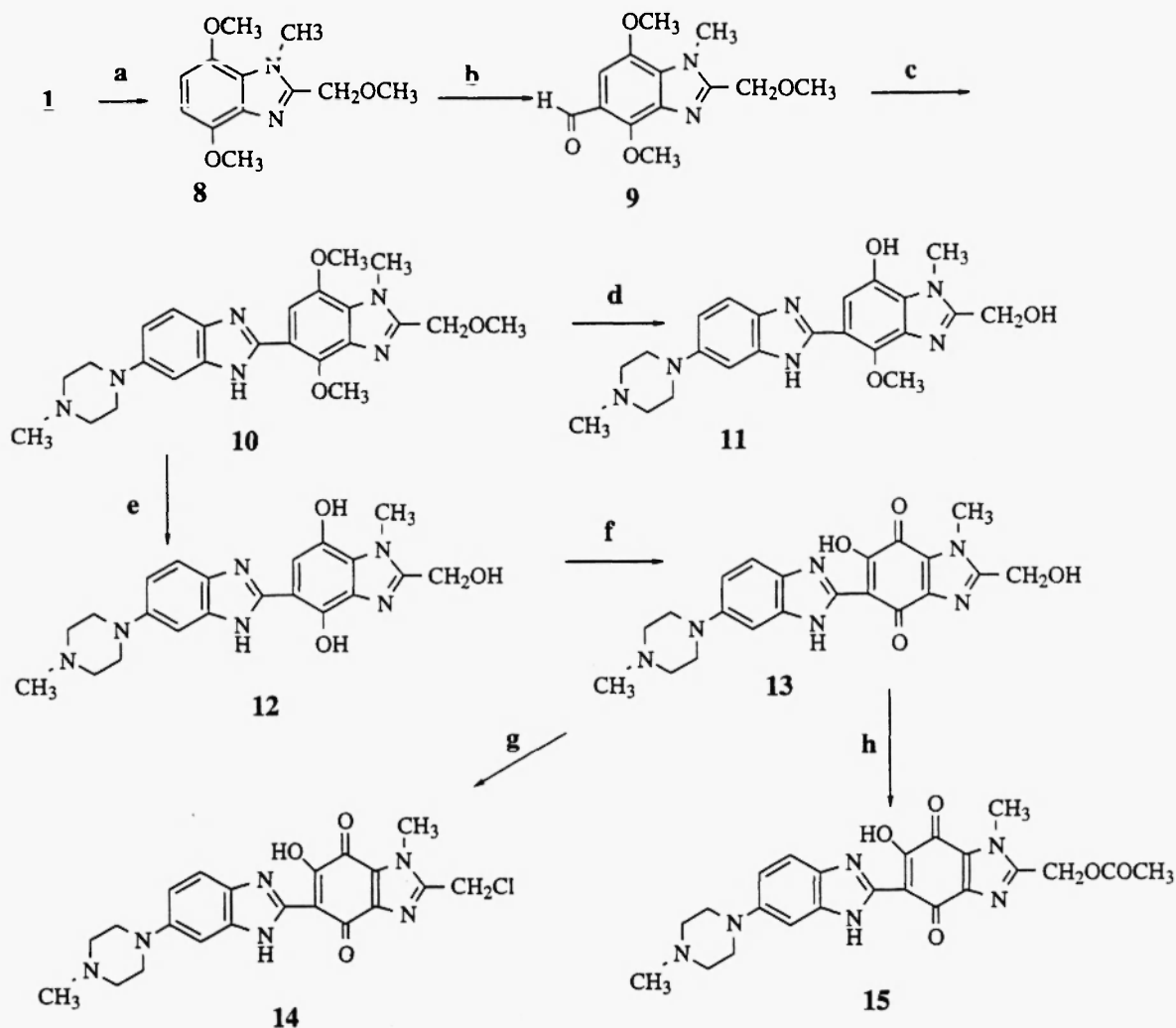
Reagents: a) NaH, CH₃I, THF; b) SnCl₄, Cl₂CHOCH₃; c) 4-(4-methyl-1-piperazinyl)-1,2-diamino-benzene, nitrobenzene, d) 48% HBr: e) BBr₃, toluene; f) *p*-chloranil, CH₃OH, H₂O; g) PCl₅, DMF; h) CH₃COCl, CH₃COOH.

    The interactions between these compounds and supercoiled covalently closed circular pBR322 DNA in the presence of reductant and oxygen were investigated. Preliminary results show that compounds containing a quinone group react with DNA via two possible pathways in the presence of reductants NADH or NADPH: i.e via radical cleavage and also via DNA alkylation. The corresponding dimethoxy compounds, which are not subject to reduction, showed very weak DNA cleaving ability. The extent of the DNA alkylation reaction of the quinone derivatives is, *inter alia*, related to leaving group ability. Furthermore, the quinone compounds preferentially alkylate DNA at 5'-**CG** and **TG** sequences rather than at the AT sites preferred as binding sites of Hoechst 33258 (12).

EXPERIMENTAL SECTION

    Melting points were determined on a Fisher-Johns apparatus and are uncorrected. The ir spectra were recorded on a Nicolet magna 750 spectrophotometer with a Nic-plane microscope. The ¹H nmr

spectra were measured on Bruker WH-200 and WH-40 spectrometer. FAB (fast atom bombardment) mass spectra with glycerol as the matrix were determined on a Associate Electrical Ind. (AEI) MS-9 and MS-50 focusing high resolution mass spectrometers. Kieselgel 60 (9230-400 mesh) of E. Merck and florisil (60-100 mesh) were used for chromatography, and precoated silica gel 60F-254 sheets of E. Merck were used for tlc, with the solvent system indicated in the procedure.

2-(Acetoxymethyl)-4,7-dimethoxybenzimidazole (2). To a solution of 1 (2.08 g, 10 mmol) in THF (50 mL) was added acetic anhydride (0.79 g, 11 mmol), and the reaction mixture was stirred for 2 h at room temperature. The solvent was removed in vacuum and the residue was diluted with water and then extracted with ethyl acetate (3x50 mL). The organic phase was washed with 5% $NaHCO_3$ solution, water, dried (Na_2SO_4), and evaporated to yield an oil, which was crystallized from ethanol to give 2 (2.38 g, 95%). mp 158°C. ^1H NMR (CDCl$_3$): 9.75 (bs, 1H, NH), 6.58 (d, J = 11 Hz, 1H), 6.51 (d, J = 11 Hz, 1H), 5.32 (s, 2H), 3.96 (s, 3H), 3.89 (s, 3H), 2.12 (s, 3H); EIHRms Calcd for $C_{12}H_{14}N_4O_2$ 250.0953, found 250.0954 (M$^+$, 100). Anal. Calcd for $C_{12}H_{14}N_4O_2$: C, 57.59, H, 5.64, N, 11.19. Found: C, 57.54, H, 5.71, N, 11.21. Ir (cast, CHCl$_3$), 3235, 2939, 2980, 1719, 1520, 1290.

1-Methyl-2-(acetoxymethyl)-4,7-dimethoxybenzimidazole (3). To a solution of 2 (2.5 g, 10 mmol) in THF (50 mL) was added NaH (85%) (0.42 g, 15 mmol) and methyl iodide (2.13 g, 15 mmol), and the resulting mixture was stirred for 1 h before concentration in vacuum. The residue was diluted with water and then extracted with ethyl acetate (3x50 mL). The organic phase was washed with water, dried (Na_2SO_4), and evaporated to yield an oil, which was crystallized from ethanol to give 3 (2.56 g, 97%). mp 98°C. ^1H NMR (CDCl$_3$): δ 6.57 (d, J = 11 Hz, 1H), 6.49 (d, J = 11 Hz, 1H), 5.30 (s, 2H), 4.03 (s, 3H), 3.95 (s, 3H), 3.89 (s, 3H), 2.11 (s, 3H); EIHRms Calcd for $C_{13}H_{16}N_2O_4$ 264.1110, found 264.1113 (M$^+$, 100). Anal. Calcd for $C_{13}H_{16}N_2O_4$: C, 59.07, H, 6.11, N, 10.60. Found: C, 59.15, H, 6.07, N, 10.51. Ir (cast, CHCl$_3$), 2950, 1725, 1544,1460, 1250.

1-Methyl-5-formyl-2-(acetoxymethyl)-4,7-dimethoxybenzimidazole (4). A solution of 3 (2.64 g, 10 mmol) and α,α-dichloromethyl methyl ether (8.04 g, 70 mmol) was dissolved in 20 mL of dry methylene chloride, and the solution was cooled to -40 °C under a nitrogen atmosphere. The solution was stirred vigorously as a solution of 8.65 g of titanium tetrachloride in 20 mL of dry methylene chloride was added. The dark green mixture was stirred for 4 h at -40°C then for an additional 4 h at room temperature. The reaction was poured into 200 mL of ice water and the aqueous layer thoroughly extracted with 3x 200 mL of ethyl acetate. The combined extracts were washed with saturated $NaHCO_3$ (200 mL), water (500 mL), and saturated NaCl (450 mL) and dried (Na_2SO_4) and the volatile solvents evaporated in vacuum. The residual yellow solid was recrystallized from ethyl acetate to give 4 (2.19 g, 75%). mp 138°C. ^1H NMR (CDCl$_3$) δ 10.50: (s, 1H), 7.11 (s, 1H), 5.32 (s, 2H), 4.41 (s, 3H), 4.05 (s, 3H), 3.95 (s, 3H), 2.15 (s, 3H); EIHRms Calcd for $C_{14}H_{16}N_2O_5$ 292.1059, found 292.1058 (M$^+$, 100). Anal. Calcd for $C_{14}H_{16}N_2O_5$: C, 57.53, H, 5.52, N, 9.58. Found: C, 57.447, H, 5.50, N, 9.54. Ir (cast, CHCl$_3$), 2950, 1720, 1673, 1540,1464, 1255.

5-(4-methyl-1-piperazinyl)-2-[2'-(acetoxymethyl)-1'-methyl-4',7'-dimethoxybenz-imidazoi-5'-yl]benzimidazole (5). A mixture of 4-(4-methyl-1-piperazinyl)-1,2-diaminobenzene (2.02 g, 10 mmol) and 4 (2.92 g, 10 mmol) in 20 mL of nitrobenzene was heated at 140°C for 24 h. The nitrobenzene was removed under reduced pressure, and the resulting residue was chromatographed on

florisil (EtOAc/MeOH) to give 5 (3.73 g, 78%) as a yellow solid: mp 134. ^{1}H NMR (DMSO-d$_6$) δ 11.00 (bs, 1H, NH), 7.70 (s, 1H), 7.49 (d, J = 9 Hz, 1H), 7.20 (d, J = 2.3 Hz, 1H), 6.92 (d,d, J = 2.3 Hz, 9 Hz, 1H), 5.32 (s, 2H), 4.31 (s,3H), 4.03 (s, 3H), 3.98 (s, 3H), 3.29 (m, 4H, 2xCH$_2$N), 2.60 (m, 4H, 2xCH$_2$N), 2.12 (s, 3H); EIHRms Calcd for C$_{25}$H$_{30}$N$_6$O$_4$ 478.2328, found 478.2320 (M$^+$, 100). Anal. Calcd for C$_{25}$H$_{30}$N$_6$O$_4$: C, 62.75, H, 6.32, N, 17.56. Found: C, 62.66, H, 6.41, N, 17.40. Ir (cast,CHCl$_3$),3440, 2920, 2850, 1700, 1611, 1540,1450.

5-(4-methyl-1-piperazinyl)-2-[2'-(hydroxymethyl)-1'-methyl-4',7'-di–methoxybenz-imidazole-5'-yl]benzimidazole (6). A suspension of 5 (478 mg, 1 mmol) in NaOH solution (100 mL, 1N) was stirred at room temperature overnight. Methanol was evaporated and the remaining aqueous solution was adjusted to pH =7 using 1N HCl., and then extracted with ethyl acetate (3x50 mL). The organic phase was washed with water, dried (Na$_2$SO$_4$), and evaporated to yield a solid, which was chromatographed on silica gel (EtOAc/MeOH ratio (1:1)) to give 6 (414 mg, 95%) as a yellow solid: mp 170 °C. ^{1}H NMR (DMSO-d$_6$): δ 11.00 (bs, 1H, NH), 7.68 (s, 1H), 7.50 (d, J = 9 Hz, 1H), 7.10 (d, J = 2.3 Hz, 1H), 6.90 (d,d, J = 2.3 Hz, 9 Hz, 1H), 5.60 (s, 1H, OH), 4.70 (s, 2H), 4.30 (s,3H), 4.05 (s, 3H), 3.95 (s, 3H), 3.08 (m, 4H, 2xCH$_2$N), 2.49 (m, 4H, 2xCH$_2$N), 2.20 (s, 3H); EIHRms Calcd for C$_{23}$H$_{28}$N$_6$O$_3$ 436.2222, found 436.2215 (M$^+$, 84). Anal. Calcd for C$_{23}$H$_{28}$N$_6$O$_3$: C, 63.29, H, 6.47, N, 19.25. Found: C, 63.11, H, 6.40, N, 19.16. Ir (cast, CH$_3$OH/CHCl$_3$), 3450, 3300, 2920, 2851, 1610, 1480.

5-(4-methyl-1-piperazinyl)-2-[2'-(chloromethyl)-1'-methyl-4',7'-di–methoxybenz-imidazole-5'-yl]benzimidazole (7). To a solution of 6 (43.6 mg, 0.1 mmol) in DMF (200 mL) was added PCl$_5$ (104 mg, 0.5 mmol), and the reaction mixture was stirred for 2 h at room temperature. The solvent was removed in vacuum and the residue was diluted with water and then extracted with ethyl acetate (3x50 mL). The organic phase was washed with 5% NaHCO$_3$, water, dried (Na$_2$SO$_4$), acidified with dry HCl and evaporated to yield a solid, which was crystallized from ethanol/ether to give 7 (420 mg, 70%). mp 198°C. ^{1}H NMR (DMSO-d$_6$): δ 11.05 (bs, 1H, NH), 7.78 (s, 1H), 7.73 (d, J = 9 Hz, 1H), 7.35 (d,d, J = 2.3 Hz, 9 Hz, 1H), 7.26 (d, J = 2.3 Hz, 1H), 5.10 (s, 2H), 4.51 (s,3H), 4.04 (s, 3H), 4.02 (s, 3H), 3.85 (m, 2H), 3.55 (m, 2H, CH$_2$N), 3.20 (m, 2H, CH$_2$N), 2.85 (d, J = 3 Hz, 3H NCH$_3$); EIHRms Calcd for C$_{23}$H$_{27}$N$_6$O$_2$Cl 454.1884, found 454.1899 (M$^+$, 45). Ir(cast, CH$_3$OH/CHCl$_3$), 3325, 2910, 2840, 1620, 1475.

1-Methyl-2-(methoxymethyl)-4,7-dimethoxybenzimidazole (8). To a solution of 1 (2.08 g, 10 mmol mol) in THF (50 mL) was added NaH (85) (0.84 g, 30 mmol) and methyliodide (4.16 g, 30 mmol), and the resulting mixture was allowed to stir for 6 h before concentration in vacuum. The residue was diluted with water and then extracted with ethyl acetate (3x50 mL). The organic phase was washed with water, dried (Na$_2$SO$_4$), and evaporated to yield a solid, which was crystallized from ethanol/ether to give 8 (2.29 g, 97%). mp 78°C. ^{1}H NMR (CDCl$_3$): δ 6.53 (d, J = 10 Hz, 1H), 6.45 (d, J = 10 Hz, 1H), 4.75 (s, 2H), 4.03 (s, 3H), 3.95 (s, 3H), 3.86 (s, 3H), 3.37 (s, 3H); EIHRms Calcd for C$_{12}$H$_{16}$N$_2$O$_3$ 236.1161, found 236.1165 (M$^+$, 100). Anal. Calcd for C$_{12}$H$_{16}$N$_2$O$_3$: C, 60.99, H, 6.83, N, 11.86. Found: C, 61.00, H, 6.75, N, 11.74. Ir (cast, CHCl$_3$), 2940, 1719, 1465, 1240, 1105.

1-Methyl-2-(methoxymethyl)-5-formyl-4,7-dimethoxybenzimidazole (9). Compound **8** was prepared from **8** (2.36 g, 10 mmol) using a similar procedure as that described for **4** in 78% yield: mp 121°C. ^{1}H NMR (CDCl$_3$): δ 10.46 (s, 1H, OCH), 7.12 (s, 1H); 4.75 (s, 2H), 4.40 (s, 3H), 4.09 (s, 3H), 3.96 (s, 3H), 3.42 (s, 3H); EIHRms Calcd for C$_{13}$H$_{16}$N$_2$O$_4$ 264.1110, found 264.1108 (M$^+$, 100). Anal. calcd for C$_{13}$H$_{16}$N$_2$O$_4$ C, 59.07, H, 6.11, N, 10.60, Found C, 59.11, H, 6.23, N, 10.53.

5-(4-methyl-1-piperazinyl)-2-[2'-methoxymethyl-1'-methyl-4',7'-di–methoxybenz-imidazole-5'-yl]benzimidazole (10). Compound **10** was prepared from **9** (2.64 g, 10 mmol), using a similar procedure as that described for **5** in 77% yield: mp 123°C. ^{1}H NMR (DMSO-d$_6$): δ 11.80 (bs, 1H, NH), 7.68 (s, 1H), 7.49 (d, J = 9 Hz, 1H), 7.14 (d, J = 2.3 Hz, 1H), 6.94 (d,d, J = 2.3 Hz, 9 Hz, 1H), 4.69 (s, 2H), 4.32 (s,3H), 4.04 (s, 3H), 3.98 (s, 3H), 3.36 (s, 3H), 3.28 (m, 4H, 2xCH$_2$N), 2.90 (m, 4H, 2xCH$_2$N), 2.50 (s, 3H, NCH$_3$); EIHRms Calcd for C$_{24}$H$_{30}$N$_6$O$_3$ 450.2379, found 450.2384 (M$^+$, 100). Anal. calcd for C$_{24}$H$_{30}$N$_6$O$_3$: C, 63.97, H, 6.72, N, 18.66, Found C, 63.74, H, 6.75, N,18.54. Ir (cast, CHCl$_3$), 3440, 3300, 2915, 2850, 1621, 1475, 1100.

5-(4-methyl-1-piperazinyl)-2-[2'-hydroxymethyl-1'-methyl-4'-methoxy-7'-hydroxybenz-imidazole-5'-yl]benzimidazole (11). Compound **10** (450 mg, 1 mmol) was refluxed for 12 h with 35 mL of 48% hydrobromic acid and ethanol was added to the cooled reaction solution. The resulting precipitate was collected and recrystallized from ethanol: yield **11** (282 mg, 67%). ^{1}H NMR (DMSO-d$_6$): δ 14.00 (bs, 1H, NH), 9.90 (bs, 1H, OH), 7.72 (d, J = 9 Hz, 1H), 7.55 (s, 1H), 7.29 (d,d, J = 2.3 Hz, 9 Hz, 1H), 7.25 (d, J = 2.3 Hz, 1H), 5.36 (bs, 1H, CH$_2$OH), 4.84 (s, 2H), 4.08 (s, 3H), 4.02 (s, 3H), 3.88 (m, 2H, CH$_2$N), 3.62 (m, 2H, CH$_2$N), 3.28 (m, 2H, CH$_2$N), 3.13 (m, 2H, CH$_2$N), 2.90 (s, 3H, NCH$_3$); EIHRms Calcd for C$_{22}$H$_{26}$N$_6$O$_3$ 422.2066, found 422.2057 (M$^+$, 100). Ir (cast, CHCl$_3$), 3550, 3305, 2935, 2855, 1625, 1477.

5-(4-methyl-1-piperazinyl)-2-[2'-hydroxymethyl-1'-methyl-4',7'-dihydroxybenz-imidazole-5'-yl]benzimidazole (12). The title compound was prepared in 63% yield by treating **10** with refluxing BBr$_3$; according to the reported procedure (8). ^{1}H NMR (DMSO-d$_6$): δ 14.00 (bs, 1H, NH), 10.50 (bs, 1H, OH), 9.89 (bs, 1H, OH), 7.68 (d, J = 9 Hz, 1H), 7.28 (d,d, J = 2.3 Hz, 9 Hz, 1H), 7.25 (s, 1H), 7.21 (d, J = 2.3 Hz, 1H), 5.20 (bs, 1H, CH$_2$OH), 4.90 (s, 2H), 4.10 (s, 3H), 3.87 (m, 2H, CH$_2$N), 3.60 (m, 2H, CH$_2$N), 3.20 (m, 2H, CH$_2$N), 3.08 (m, 2H, CH$_2$N), 2.90 (s, 3H, NCH$_3$); EIHRms Calcd for C$_{21}$H$_{24}$N$_6$O$_3$ 408.1910, found 408.1913 (M$^+$, 100). Ir (cast, CHCl$_3$), 3550, 3305, 2935, 2855, 1625, 1477.

5-(4-methyl-1-piperazinyl)-2-[2'-hydroxymethyl-1'-methyl-6'-hydroxybenzimidazole-4',7'-dione-5'-yl]benzimidazole (13). To a solution of **12** (40.8 mg, 0.1 mmol) in MeOH (50 mL) was added chloranil (105 mg, 0.5 mmol) and the resulting mixture was stirred for 2 h before concentration in vacuum. The residue was purified on a silica gel column eluting with CHCl$_3$:MeOH (4:1) to give **13** (22.7 mg, 54%) as a yellow solid. ^{1}H NMR (DMSO-d$_6$): δ 13.02 (bs, 1H), 13.00 (bs, 1H), 7.60 (d, J = 9 Hz, 1H), 7.30 (d, J = 2.3 Hz, 1H), 7.02 (d,d, J = 2.3 Hz, 9 Hz, 1H), 5.65 (bs, 1H, CH$_2$OH), 4.68 (s, 2H), 3.95 (s, 3H), 3.10 (m, 4H, 2xCH$_2$N), 2.51 (m, 4H, 2xCH$_2$N), 2.28 (s, 3H, NCH$_3$); EIHRms Calcd for C$_{21}$H$_{22}$N$_6$O$_4$ 422.1703, found 422.1698 (M$^+$, 46). Ir (cast, CHCl$_3$), 3455, 3301, 2938, 2875, 1659, 1472, 1060.

5-(4-methyl-1-piperazinyl)-2-[2'-chloromethyl-1'-methyl-6'-hydroxybenzimidazole-4',7'-dione-5'-yl]benzimidazole (14). To a solution of **13** (21 mg, 0.05 mmol) in DMF (20 mL) was added PCl_5 (42 mg, 0.2 mmol) and the resulting mixture was stirred for 10 h before concentration in vacuum. The residue was purified on a silica gel column eluting with $CHCl_3$: MeOH(4:1) to give **14** (0.34 mg, 47%) as a yellow solid. ^1H NMR (DMSO-d_6): δ 13.05 (b, 2H), 7.57 (d, *J* = 9 Hz, 1H), 7.25 (d, *J* = 2.3 Hz, 1H), 7.05 (d,d, *J* = 2.3 Hz, 9 Hz, 1H), 5.02 (s, 2H, CH_2Cl), 4.01 (s, 3H), 3.15 (m, 4H, $2xCH_2N$), 2.55 (m, 4H, $2xCH_2N$), 2.24 (s, 3H, NCH_3); EIHRms Calcd for $C_{21}H_{21}N_6O_3Cl$ 440.1363, found 440.1358 (M$^+$, 56). Ir (cast, $CHCl_3$), 3405, 3300, 2928, 2870, 1659, 1475, 1068.

5-(4-methyl-1-piperazinyl)-2-[2'-acetoxymethyl-1'-methyl-6'-hydroxybenzimidazole-4',7'--dione-5'-yl]benzimidazole (15). To a solution of **13** (21 mg, 0.05 mmol) in acetic acid (10 mL) was added acetyl chloride (39.2 g, 0.5 mol) and the resulting mixture was stirred for 10 h before concentration in vacuum. The residue was purified on a silica gel column eluting with $CHCl_3$: MeOH (4:1) to give **15** (11.83 mg, 51%) as a yellow solid. ^1H NMR (DMSO-d_6): δ 13.00 (b, 2H), 7.55 (d, *J* = 9 Hz, 1H), 7.25 (d, *J* = 2.3 Hz, 1H), 7.02 (d,d, *J* = 2.3 Hz, 9 Hz, 1H), 5.30 (s, 2H), 3.92 (s, 3H), 3.10 (m, 4H, $2xCH_2N$), 2.55 (m, 4H, $2xCH_2N$), 2.22 (s, 3H, NCH_3), 2.10 (s, 3H, CH_3CO); EIHRms Calcd for $C_{23}H_{24}N_6O_5$ 464.1801, found 464.1795 (M$^+$, 44). Ir (cast, $CHCl_3$), 3411, 3305, 2930, 2875, 1655, 1470, 1070.

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REFERENCES

1. C. Bailly and J. P. Henichart, *Bioconj. Chem.* 1991, **2**, 379.

2. a) P. B. Dervan, *Science*, 1986, **232**, 464; b) E. Nishiwaki, H. Nakagawa, M. Takasaki, T. Matsumoto, H. Sakurai, and M. Shibuya, *Heterocycles*, 1990, **31**, 1763; c) P. Herfeld, P. Helissey, and S. Giorgi-Renault, *Bioconj. Chem.*, 1994, **5**, 67; d) G. Xie, A. R. Morgan, and J. W. Lown, *Bioorga. Biomed. Chem. Lett.*, 1993, **3**, 1565; e) M. Tokuda, K. Fujiwara, T. Gomibuchi, M. Hirama, M. Uesugi, and Y. Sugiura, *Tetrahedron Lett.*, 1993, **34**, 669; f) S. T. Sigurdsson, S. M. Rink, and P. B. Hopkins, *J. Am. Chem. Soc.*, 1993, **115**, 12633; g) F. M. Arcamone, F. Animati, B. Barbieri, E. Configliacchi, R. D'Alessio, C. Geroni, F. C. Giuliani, E. Lazzari, M. Menozzi, N. Mongelli, N. Mongelli, S. Penco, and M. A. Verini, *J. Med. Chem.*, 1989, **32**, 774; h) M. Lee, A. L. Rhodes, M. D. Wyatt, M. D'Incalci, S. Forrow, and J. A. Hartley, *J. Med. Chem.*, 1993, **36**, 863; i). K. Krowicki, J. Balzarini, E. D. Clercq, R. A. Newman, and J. W. Lown, *J. Med. Chem.*, 1988, **31**, 341.

3. a) W. Muller, and F. Gautier, *Eur. J. Biochem.*, 1975, **54**, 385; b) C. Zimmer, and U. Wahnert, *Prog. Biophys. Mol. Biol.*, 1986, **47**, 31-112; c) V. Murray, and R. F. Matin, *J. Mol. Biol.* 1988, **201**, 437-442.

4. a) K. D., Harshman, and P. B, Dervan, *Nucleic Acids Res.* 1985, **13**, 4825-4835; b) R. E., Dickerson, M. L. Kopka, and P. E., Pjura, In DNA-ligand Interactions from Drugs to proteins. ed. by W. Guschlbauer, and W. Saenger, Plenum: New York, 1987, pp. 45-54.

5. D. Goodsell, and R. E. Dickerson, *J. Med. Chem.* 1986, **29**, 727-733.

6. a) J. Bontempts, C. Houssier, and E. Fredericq, *Nucleic Acids Res.* 1975, **2**, 971-984; b) D. E. Comings, *Chromosoma*, 1975, **52**, 229-243.

7. a) H. W. Moore, *Science* (washington, D.C.) 1977, **197**, 527; b) H. W. Moore, and R. Czerniak. *Med. Res. Rev.*, 1981, **1**, 249.

8. a) E. B. Skibo, *J. Org. Chem.*, 1986, **51**, 522; (b) C. H., Lee, E. B. Skibo, B*iochemistry*, 1987, **26**, 7355; c) R. H. Lemus, C. H. Lee, and E. B. Skibo, *J. Org. Chem.*, 1989, **54**, 3611; d) E. B. Skibo, *J. Org. Chem.*, 1992, **57**, 5874.

9. M. P. Singh, T. Joseph, S. Kumar, Y. Bathini, and J. W. Lown, *Chem. Res. Toxicol.*, 1992, **5**. 597.

10. L. Weinberger, and A. L. Day, *J. Org. Chem.*, 1959, **24**, 1451.

11. Y. Bathini, R. E. Rao, R. G. Shea, and J. W. Lown, *Chem. Res. Toxicol.*, 1990, **3**, 268.

12. L. L Guan, R. Zhao, and J. W. Lown, *Biochem. Biophys. Res. Common.*, 1997, **231**, 94.