SYNTHESIS OF HOECHST 33258 ANALOGUES DESIGNED TO TARGET HUMAN TUMOR HELICASES

Ashok K. Singh and J. William Lown*

Department of Chemistry, University of Alberta ,Edmonton,Canada T6G 2G2

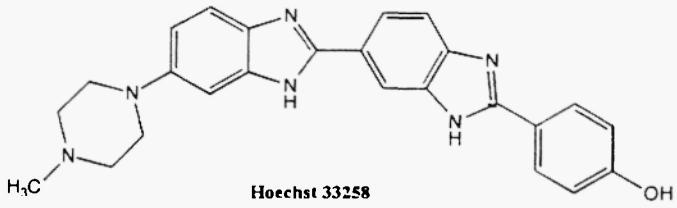
Abstract- The syntheses of certain analogues of the DNA minor groove binding agent Hoechst 33258 designed to explore the potential for bioreductive activation and as selective inhibitors of human tumor helicases are described. The structural modifications include the following: substitution of pyridine for benzene ring of the piperazinyl benzimidazole moiety and replacement of one benzimidazole unit by a imidazoquinone moiety.

Introduction

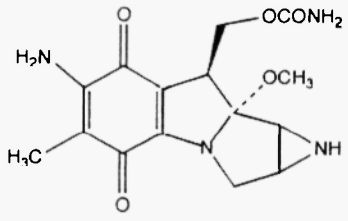
The design of DNA sequence-specific binding agents has received considerable attention because of their potential as anticancer drugs. The synthetic bis-benzimidazole Hoechst dye 33258 is known to bind to the minor groove of double helical DNA selectively at 5' AATT sites (1). The interaction of Hoechst 33258 with DNA has been extensively studied by biophysical methods including footprinting, NMR and X-ray techniques (2).Structural data on Hoechst 33258-DNA complexes from X-ray crystallography (3,4) and NMR studies (5-7) provide evidence for minor groove binding. The flexible nature of the bis-benzimidazole ring system permits the drug to adopt an optimum conformation and thus bind effectively to double stranded DNA (8).

In addition, the quinone class of bioreductive alkylating agents has received increasing attention because of their potential role in cancer therapy. Certain quinones are able to act as bioreductively activated alkylating agents (9). Mitomycin C, a naturally occurring antitumor antibiotic, and the corresponding mitosene analogues bearing a para-quinone moiety, are capable of bioreductive activation to generate reactive quinone methides that alkylate biomolecules (10). Recently, the benzimidazole ring system has been designed as a reductive alkylator by functionalizing the quinone derivative of the ring system with $-CH_2X$, where X is a leaving group (11). These latter types of

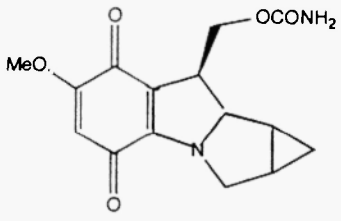
quinone derivatives result in quinone methide formation upon quinone reduction (11) and this suggests that if we incorporate a quinone methide precursor into the Hoechst 33258 structure it should be effective both as a sequence and site directed groove binder as well as selective alkylator. Several indole quinones have recently been studied as bioreductive drugs (12). These studies aroused our interest in this area and the synthetic and biological studies of prototypes of such analogues of Hoechst 33258 bearing a quinone moiety have been reported including their property of sequence selective inhibition of human tumor helicases (13-15).



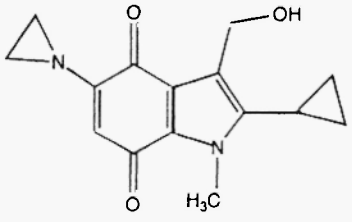
Hoechst 33258



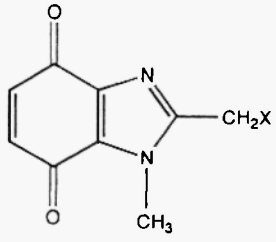
Mitomycin C (MMC)



Cyclopropamitosene (CPM)



Indolquinone ring system



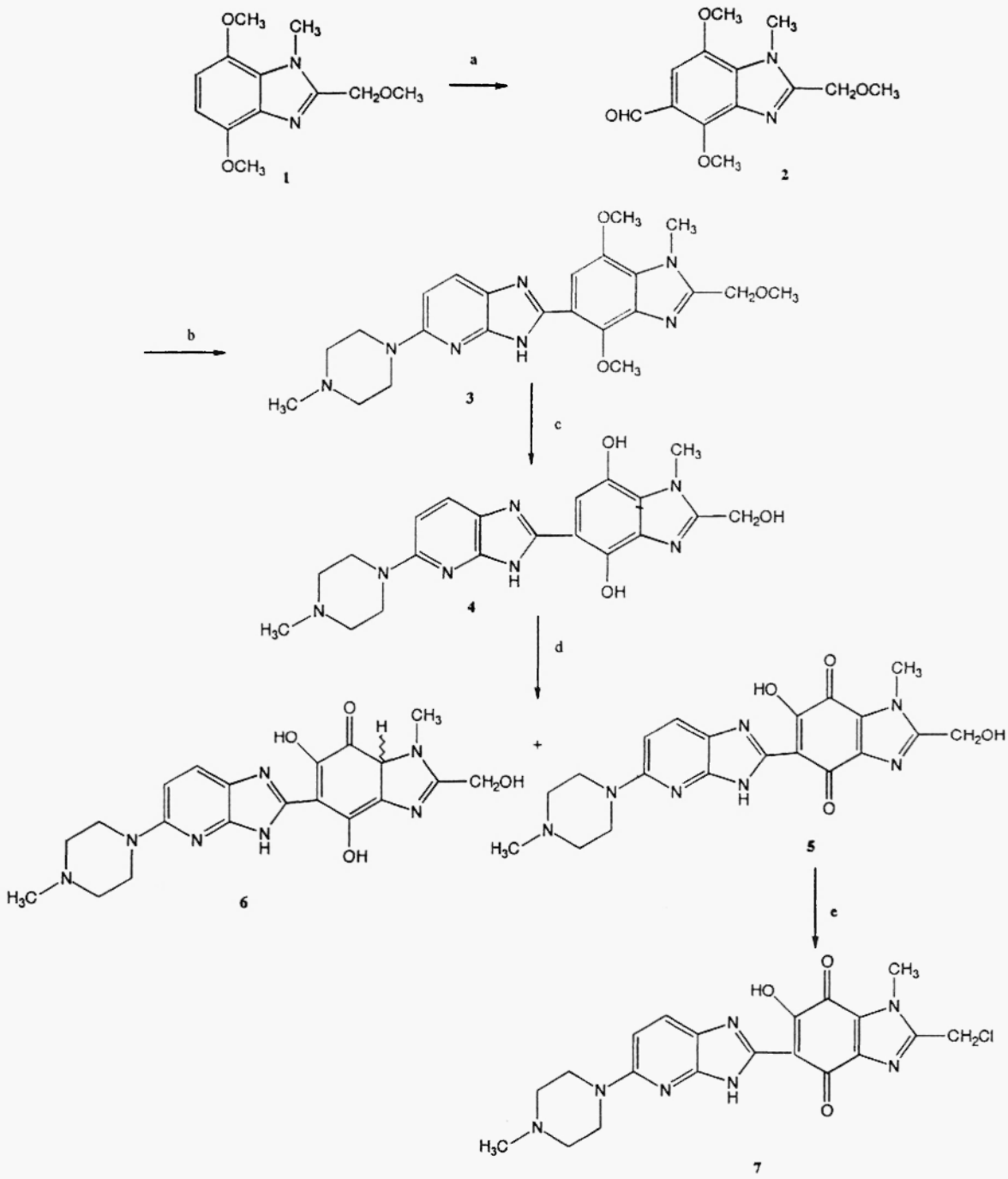
Benzimidazolequinone ring system

As part of our ongoing research on the synthesis of Hoechst 33258 with structural modifications we report on agents consisting of substitution of the benzene ring by pyridine in one of the benzimidazole moieties so as to change the sequence recognition within the minor groove in a predictable manner, and with replacement of another benzimidazole unit by an imidazoquinone moiety

in order to examine synthetically the effect of the structural changes on both biophysical and pharmacological properties, especially their property of inhibiting human tumor helicases (14,15).

Results and Discussion

The present target molecules were obtained by a convergent approach outlined in the **Scheme**. 2-Methoxymethyl-1-methyl-4,7-dimethoxy benzimidazole **1** and its formylated product **2** was obtained by a reported procedure (13). Compound **3** was obtained as reported (16) using nitrobenzene mediated coupling of the appropriate orthodiamine and aldehyde (17). Demethylation of 5-(4-Methyl-1-piperazinyl)-2-[2'-(methoxymethyl)-1'-methyl-4',7'-dimethoxy benzimidazo-5'yl]-3H-imidazo[4,5 b] pyridine **3** was conveniently carried out by adding BBr_3 to a solution of **3** in freshly distilled CH_2Cl_2 under N_2 at -78°C and then allowing the mixture to warm up to room temperature overnight. This procedure afforded 5-(4-methyl-1-piperazinyl)-2-[2'-(hydroxymethyl)-1'-methyl-4',7'- dihydroxy benzimidazo-5'yl]-3H-imidazo[4,5 b] pyridine **4**, the corresponding hydroxy compound which serves as one of the key step in introducing the quinone moiety into the target molecule. It was observed that the use of 1 equiv. of BBr_3 for each MeO group was found to be insufficient since this leads to only partially demethylated products. It appears that one equiv. of BBr_3 for each MeO group is not sufficient to effect demethylation satisfactorily when the other group containing an electron donor element is present in the molecule (18). For the reaction compound **3** and BBr_3, a molar ratio of 1:9 was used to afford a completely demethylated product **4.** The hydroquinone **4** thus obtained was subjected to oxidation using *p* - chloranil in methanol. Oxidations using CAN and Ag_2O were also attempted but were not successful. The reaction with *p*-chloranil however proceeds smoothly at room temperature. Upon oxidation of **4,** two products **5** and **6** were obtained as shown in the scheme, that were isolated by column chromatography on silica gel using $CHCl_3$-MeOH as eluent. Compound **6**, as expected, proved to be a racemic mixture and the specific rotation is zero within experimental limits. Although the structure of compound **6** has not been definitively established, the NMR and mass data support the structure depicted in the scheme above. Detailed structural and mechanistic studies have yet to be performed on compound **6**. Treatment of **5** with PCl_5 in DMF gave the compound 7 (13).Biophysical and pharmacological evaluation of the new agents is underway and will be reported in due course.



Scheme: Reagents a) $TiCl_4$, Cl_2CHOCH_3; b) 2,3-diamino-6-(4-methyl-1-piperazinyl) pyridine, nitrobenzene; c) BBr_3, CH_2Cl_2; d) p-chloranil, CH_3OH; e) PCl_5, DMF

Experimental:

Melting points were determined on an electrothermal melting point apparatus and are uncorrected. All the chemicals used were of reagent grade. Dimethylformamide(DMF) and methanol (MeOH) were of anhydrous grade procured from Aldrich Chemical Co. Freshly distilled dichloromethane was used. The progress of the reactions was monitored by thin layer chromatography using precoated silica gel 60F 254, E. Merck TLC plates visualizing under UV light. ^{1}H NMR spectra were recorded on a Brucker (300 mHZ) spectrometer with tetramethyl silane (TMS) as internal standard on the ppm scale (δ). Multiplicity of resonance peaks are indicated as singlet (s), doublet (d), broad single (brs) and multiplet (m). Mass spectrometric analysis was performed by positive mode elctrospray ionization on Micromass ZapSpec Hybrid Sector -TOF. The IR spectra were recorded on a Nicolet magna

750 spectrophotometer with a Nic-plane microscope.

5-(4-Methyl-1-piperazinyl)-2-[2'-(hydroxymethyl)-1'-methyl-4',7'-dihydroxybenzimidazo-5'-yl]-3H-imidazo[4,5b]pyridine (4)

Compound **4** was obtained by the reported procedure (19) with some modification. Compound **3** (90.2mg) was dissolved in freshly distilled CH_2Cl_2 (40 ml) and cooled to -78 °C, then to it BBr_3 (500 mg) in dry CH_2Cl_2 (20 ml) was slowly added and the mixture was allowed to reach to room temperature overnight under N_2. The reaction mixture was quenched with water and methylene chloride removed in vacuum. After extracting the reaction mixture with ethyl acetate (3 x 20 ml) the water layer was neutralized to pH 7.0 and again extracted with ethyl acetate. The ethyl acetate fractions were combined,dried (Na_2SO_4) and concentrated in vacuum. The residue was dissolved in methanol and precipitated by ether to give 57 mg of the title compound **4** as a dull yellow solid (70% yield) : mp. > 250°C, IR (microscope) (cm^{-1}) 3015, 1631, 1509, 1167, 1079, 668; ^{1}H NMR (DMSO d_6) (δ)13.8 (brs, 1H), 10.55 (brs, 1H), 9.9 (brs, 1H), 8.00(d, 1H), 7.4 (s, 1H), 7.05 (d, 1H), 4.9 (s, 2H), 4.4 (d, 2H), 4.1 (s, 3H), 3.8 (d, 2H), 3.1-3.3 (m, 4H), 2.95 (s, 3H); MS [E S+] calculated for $C_{20}H_{23}N_7O_3$ 409.19 found 410.19 [100 M+H].

5-(4-Methyl-1-piperazinyl)-2-[2'-(hydroxymethyl)-1'-methyl-4',7'-dioxo-6'-hydroxybenzimidazo-5'-yl]-3H-imidazo[4,5b]pyridine (5)

and

5-(4-Methyl-1-piperazinyl)-2-[2'-(hydroxymethyl)-1'-methyl-4'/7'-oxo-7'/4',6'-dihydroxybenzimidazo-8'/9'-H-5'-yl]-3H-imidazo[4,5b]pyridine (6)

The title compounds were obtained by applying the reported procedure (13). To a solution of **4** (40.9 mg, 0.1 mmol) in anhydrous MeOH (50 ml) was added *p*-chloranil (105 mg, 0.5 mmol) and the resulting mixture was stirred for 2 hrs before concentrating in vacuum.Both the products were isolated by column chromatography on silica gel. Elution with $CHCl_3$ - MeOH (7:3) gave **5** (15 mg) and further elution with $CHCl_3$-MeOH(5:5) gave **6** (16 mg) as dark purple solid.

5: mp. > 250°C, IR (microscope) (cm^{-1}) 3330, 1672, 1609, 1558, 1243, 668; ^1H NMR (DMSO d_6) (δ) 13.15 (s, 1H), 12.95 (s, 1H), 7.95 (d, 1H), 7.00 (d, 1H), 4.6 (s, 2H)4.35 (d, 2H), 3.95 (s, 3H), 3.8 (m, 2H), 3.55 (m, 4H), 3.15 (s, 3H); MS [E S+] calculated for $C_{20}H_{21}N_7O_4$ 423.17 found 424.17 [100 M+H]

6 : mp > 250°C, IR(microscope) (cm^{-1}) 3262, 2952, 2694, 1679, 1558, 1237, 723;^1H NMR (DMSO d_6) (δ) 13.15 (s,1H), 12.95 (s,1H), 9.75 (brs, 1H), 7.9 (d, 1H), 7.0 (d, 1H), 4.65 (s, 2H), 4.4 (d, 2H), 4.1 (s, 0.5H), 4.0(s, 0.5H), 3.9 (s, 3H), 3.6 (m, 2H), 3.1-3.3 (m, 4H), 2.85 (s, 3H); MS[ES+] calculated for $C_{20}H_{23}N_7O_4$ 425.2 found 448.2[100M+Na].

5-(4-Methyl-1-piperazinyl)-2-[2'-(chloromethyl)-1'-methyl-4',7'-dioxo-6'-hydroxybenzimidazo-5'-yl]-3H-imidazo[4,5b]pyridine(7)

Compound 7 was prepared from **5** by following the reported procedure (13). To a solution of **5** (42.4 mg 0.1 mmol) in 10 ml of DMF added 84 mg (0.4 mmol) PCl_5 and the reaction mixture was stirred at room temperature for 12 hrs, then filtered and the solvent was removed under vacuum. The residue was triturated with methanol-ether and a product 7 as a reddish purple compound separated out (25 mg, 56% yield).mp. > 250 °C, IR (microscope) (cm^{-1}) 3268,1680, 1608, 1526, 1246, 949,675; ^1H NMR (DMSO d_6) (δ) 13.07 (s, 1H), 12.9 (s, 1H), 7.95 (d, 1H), 7.00 (d, 1H),5.1 (s,2H), 4.35 (d,2H), 4.00 (s,3H) , 3.8 (m, 2H), 3.5 (m, 4H), 3.15 (s, 3H); MS [ES+] calculated for $C_{20}H_{20}N_7O_3Cl$ 441.12 found 464.12 [100 M+Na].

ACKNOWLEDGEMENT

This research was supported by grant (to J.W.L.) from the National Cancer Institute of Canada.

REFERENCES

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

(2) a) K.D. Harshman and P.B. Dervon, *Nucleic Acids Res.* , 13, 4825-4835 (1985);
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 .

(3) P.E. Pjura, I. Grzeskowiak and R.E. Dickerson, *J. Mol. Biol.*, 197, 257 (1987)

(4) M-K. Teng, N. Usman, C.A. Frederick and A.H.J Wang; *Nucleic Acids Res.*, 16, 2671 (1988)

(5) J.A. Parkinson, J. Barber, K.T. Douglas, J. Rosamund and D. Sharpless *Biochemistry*, 29, 10181 (1990)

(6) M.S. Searle, K.J. Embrey, *Nucleic Acids Res.*, 18, 3753 (1990)

(7) A. Fede, A. Labhardt, W. Bannwarth, W. Leupin; *Biochemistry*, 30, 11377 (1991)

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

(9) T. Lin, B. Teicher and A. Sartorelli, *J. Med. Chem.*, 23, 1237 (1980)

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

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

(12) a) S.A. Everett, M.A. Naylor, J. Nolan, K.B. Patel and P. Wardman, *Anti-Cancer Drug Design*, 13, 635-653 (1998);

b) C.J. Moody, C.L. Norton, A.M.Z. Slawin and S. Taylor, *Anti-Cancer Drug Design*, 13, 611-634 (1998)

(13) R. Zhao and J.W. Lown, *Heterocycl. Commun.*, 4, 11-19 (1998)

(14) L. Guan, R. Zhao and J.W. Lown, *Biochem. Biophys. Res. Commun.*, 231, 94-98 (1997)

(15) K-J. Soderlind, B.Sorodetsky, A.K.Singh, N.R.Baucher, G.G.Miller and J.W.Lown, *Anti-Cancer Drug Design, in press* (1998)

(16) A.K. Singh and J.W. Lown, *Synth. Commun.*, 28, 4059-4066 (1998)

(17) Y. Bathini and J.W. Lown, *Synth. Commun.*, 20, 955-963 (1990)

(18) J.F.W. McOmie, M.L. Watts and D.E. West, *Tetrahedron*, 24, 2289-2292 (1968)

(19) Jung S. Kim, Q. Sun, C. Yu, A. Liu, L.F. Liu and E.J.Lavoie, *Bioorg. Med. Chem.*, 6, 163-172 (1998)

Received on November 20, 1998