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Using Bis-Benzimidazole to Synthesis A DNA Minor Groove Binding Agents

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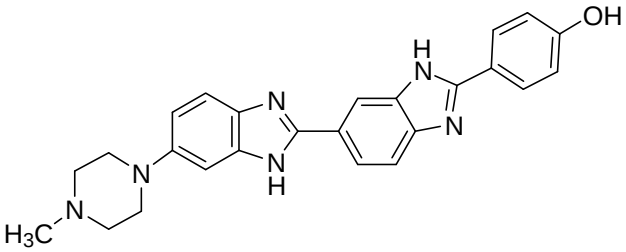
ABSTRACT

*A new series of bis-benzimidazole derivatives was designed and synthesized. In vitro cytotoxity evaluation showed that these compounds exhibited high activity against the selected tumor cells. Among them, compound **8** owned the best potential, its IC_{50} values being 0.58 μ M (mononuclear tumor cell line (U937)) and 0.56 μ M (cervical cancer cell (Hela)), respectively. Molecular modeling, fluorescence and viscometry study showed that compound **8**could bind into the minor groove of DNA.*

Keywords: bisbenzimidazole; DNA minor groove; molecular modeling

INTRODUCTION

Bis-benzimidazoles have been proved to be potent anti-tumor agents *via* DNA minor groove binding, such as Hoechst 33258(**Scheme** 1). Hoechst 33258[1], a fluorescent compound with a head-to-tail bis-benzimidazole structure, was initially found to be active against L1210 murine leukemia. During phase I clinical trials in humans, positive responses were observed in pancreatic cancer. However, a subsequent phase II clinical trials failed to show any objective responses[2]. Due to the synthetic accessibility and high binding affinity of Hoechst 33258, several groups have focused on a strategy to utilize the pharmacophore-like benzimidazole motif derived from Hoechst 33258[3].



1 (Hoechst 33258)

Scheme 1

To our knowledge, most derivatives of Hoechst 33258 kept whole molecules to planar[3]. Little literature related adding the linker between two benzimidazolehas been reported so far. Molecular modeling studies（shown in Fig. 1）suggest that the novel symmetric head-to-head benzimidazole (compound **8**) could effectively bind the DNA minor groovewith a linker between two benzimidazoles. Thereforewe combined two benzimidazole derivatives into one molecule with an oxygen atom. The interaction of compound **8** with CT (calf thymus)-DNA has been investigated using absorption spectroscopy, fluorescence spectroscopy. These results show that compound **8** interacted with the

DNA by binding into the minor groove. All of these compounds were screened for anti-tumor activity in vitro. Among them, the most compound **8** had the good activity for three tumor cell lines (HeLa, HL60 and U937).

EXPERIMENTAL SECTION

2.1. General

All commercially available reagents and solvents were used without further purification unless otherwise specified. Solvents were dried and re-distilled prior to use according to standard methods. Melting points were determined on a Büchi Melting Point B-540 apparatus (BüchiLabortechnik, Flawil, Switzerland) and are uncorrected. ^{1}H-NMR and ^{13}C-NMR spectra were measured in DMSO-d_6 on a Bruker ARX 300 spectrometer (Bruker, Rheinstetten, Germany). Chemical shifts are reported in parts per million (ppm) using tetramethylsilane (TMS) as the internal standard if not specifically mentioned (*J*in Hz). Mass spectra were measured on Waters Micromass Quattro Micro API mass spectrometer (Waters Corporation, Milford, United States).

*2.2. General Procedure for the Synthesis of Substituted Compounds **8-15***

To a stirred acetone (50 ml) solution of 4, 4′-diaminodiphenyl ether (**2**) (5.0 g, 19.6 mmol) at 0 °C, acetic anhydride (10.0 ml, 105.8 mmol) was added dropwise and reactionhad been carried out for 3 h. The reaction was monitored by TLC. After stopping the reaction, triethylamine (25 ml, 179.4 mmol) was added dropwise, neutralizing the solution, to give a white solid, filtrated, washed with acetone, and dried to obtain the compound **3**. And then, the above obtained compound **3** (6.2 g, 16.6 mmol) was dissolved with acetic acid (50 ml) and stirred at 0 °C for 10 minutes. Fuming nitric acid (8 ml, 171.7 mmol) was added dropwise to the above solution within 2 h. After that, the ice-bath was removed and the reaction mixture was further stirred at room temperature for 2 h. The mixture was poured into ice (100 ml), the resulting yellow solid (compound **4**) was filtered, washed with water, dried in vacuum, and dissolved in the mixed water (10 ml) and ethanol (30 ml) solution of potassium hydroxide (7.3 g, 106.7 mmol), and then refluxed for 4 h. After stopped the reaction, the above solution was poured into ice (100 ml). The resulting red solid was filtrated, washed with water, dried to give 5.2 g (17.9 mmol) of compound **5**. And then, it was dissolved in methanol (50 ml), with Pd-C (0.3 g, 10 %) in it, enabling hydrogen gas passing through continuously at a flow rate of 10 ml/min for 3 h. After that, it was filtrated to collect the filtrate, evaporated it under vacuum to give 3.7 g of compound **6**. Finally, compound **6** (0.5 g, 1.8 mmol), benzaldehyde derivatives, sodium hydrogensulfite (0.37 g, 3.6 mmol) were all dissolved in methanol (40 ml) and being refluxed for 8 h. The mixture was evaporated under vacuum to obtain the compounds **8-15**. Compounds **8-15** were purified by silica gel column chromatography ($CHCl_3$:CH_3OH = 50:1).

Compounds **8-15** were characterized as follows.

*5,5'-oxy-bis [2-(4'-methoxyphenyl)-1H-benzimidazole] **(8).*** White solid; Yield: 60.3 %, mp: 313-315℃; ^{1}H-NMR (600 MHz, DMSO-d_6) δ(ppm): 3.91 (s, 6H, -OCH3), 7.22 (dd, 2H, *J*=2.4, 9.0 Hz, Bz-6), 7.29 (d, 4H, *J*=9.0 Hz, Bz-3',5'), 7.32 (d, 2H, *J*=2.4 Hz, Bz-4), 7.72 (d, 2H, *J*=9.0 Hz, Bz-7), 8.27 (d, 4H, *J*=9.0 Hz, Bz-2',6'), 15.09 (s, 2H, Bz-NH). ESI-HRMS m/z: 462.1687 (Calcd for $C_{28}H_{22}N_4O_3$: 462.1692)

*5,5'-oxy-bis [2-(3', 5'-methoxyphenyl)-1H-benzimidazole] **(9).*** White solid; Yield: 62.7 %, mp 136-138℃; ^{1}H-NMR (600 MHz, DMSO-d_6) δ: 3.85 (s, 12H, -OCH3), 7.31 (s, 2H, Bz-4), 6.93 (dd, 2H, *J*=2.4, 8.4Hz, Bz-6), 7.34 (d, 4H, *J*=2.4 Hz, Bz-2', 6'), 7.58 (d, 2H, *J*=8.4Hz, Bz-7),6.60 (s, 2H, Bz-4'),12.74 (d, 2H, Bz-NH). ESI-HRMS m/z: 522.1912 (Calcd for $C_{30}H_{26}N_4O_5$: 522.1903)

*5,5'-oxy-bis [2-(3', 4', 5'-methoxyphenyl)-1H-benzimidazole] **(10).*** White solid; Yield: 69.1 %, mp 196-198℃; ^{1}H-NMR (600 MHz, DMSO-d_6) δ: 3.74 (s, 6H, -OCH3), 3.91 (s, 12H, -OCH3), 6.92(dd, 2H, *J*=8.4 Hz, Bz-6), 7.30 (s, 2H, Bz-4), 7.49 (s, 4H, Bz-2', 6'), 7.57 (d, 2H, *J*=8.4 Hz, Bz-7), 12.74 (s, 2H, -NH). ESI-HRMS m/z: 582.2122 (Calcd for $C_{32}H_{30}N_4O_7$: 582.2114)

*5,5'-oxy-bis [2-(3'-methoxy-4'-hydroxyphenyl)-1H-benzimidazole] **(11).*** White solid; Yield: 75.8 %, mp 181-183℃; ^{1}H-NMR (600 MHz, DMSO-d_6) δ: 3.91 (s, 6H, -OCH3), 5.38 (s, 2H, -OH),7.22 (d, 2H, *J*=2.4Hz, Bz-6), 7.27 (d, 2 H, *J*=9.0 Hz, Bz-2'), 7.29 (d, 2H, *J*=9.0 Hz, Bz-5'), 7.46 (dd, 2H, J= 2.4, 9.0 Hz, Bz-4), 7.72 (d, 2H, *J*=9.0 Hz, Bz-7), 8.27 (dd, 2 H, *J*=9.0 Hz, Bz-6'), 15.09 (s, 2H, -NH). ESI-HRMS m/z: 494.1599 (Calcd for $C_{28}H_{22}N_4O_5$: 494.1590)

*5,5'-oxy-bis (2-phenyl-1H-benzimidazole) **(12).*** White solid; Yield: 64.6 %, mp 237-239℃; ^{1}H-NMR (600 MHz, DMSO-d_6) δ: 6.93 (dd, 2H, *J*=2.4, 9.0 Hz, Bz-6), 7.19 (s, 2H, Bz-4), 7.50 (m, 6H, Bz-3', 4', 5'), 7.56 (d, 2H, *J*=9.0 Hz, Bz-7), 8.15 (d, 4H, *J*=7.2 Hz, Bz-2', 6'), 12.84 (s, 2H, Bz-NH). ESI-HRMS m/z: 402.1472 (Calcd for $C_{26}H_{18}ON_4$: 402.1481)

5,5'-oxy-bis [2-(4'-bromophenyl)-1H-benzimidazole] ***(13).*** White solid; Yield: 59.3 %, mp 143-145℃; ^{1}H-NMR (600 MHz, DMSO-d_6) δ: 7.14 (d, 4H, *J*=9.0 Hz, Bz-3',5'), 7.22 (dd, 2H, *J*=2.4, 9.0 Hz, Bz-6), 7.32 (d, 2H, *J*=2.4 Hz, Bz-4), 7.56 (d, 4H, *J*=9.0 Hz, Bz-2',6'), 7.72 (d, 2H, *J*=9.0 Hz, Bz-7), 15.09 (s, 2H, Bz-NH). ESI-HRMS m/z: 557.9698 (Calcd for $C_{26}H_{16}ON_4Br_2$: 557.9691)

5,5'-oxy-bis [2-(2', 4'-chlorophenyl)-1H-benzimidazole] ***(14).*** White solid; Yield: 51.3 %, mp 381-383℃; ^{1}H-NMR (600 MHz, DMSO-d_6) δ: 7.18 (dd, 2H, *J*=1.8, 9.0 Hz, Bz-5'), 7.22 (dd, 2H, *J*=2.4, 9.0 Hz, Bz-6), 7.32 (d, 2H, *J*=2.4 Hz, Bz-4), 7.34 (d, 2 H, J=1.8 Hz, Bz-3'), 7.36 (d, 2 H, *J*=9.0 Hz, Bz-6'), 7.72 (d, 2H, *J*=9.0 Hz, Bz-7), 15.09 (s, 2H, Bz-NH). ESI-HRMS m/z: 537.9919 (Calcd for $C_{26}H_{14}ON_4Cl_4$: 537.9922)

5,5'-oxy-bis [2-(4'-fluorophenyl)-1H-benzimidazole] ***(15).*** White solid; Yield: 50.2 %, mp 283-285℃; ^{1}H-NMR (600 MHz, DMSO-d_6) δ: 7.22 (dd, 2H, *J*=1.8, 9.0 Hz, Bz-6), 7.32 (d, 2H, *J*=1.8 Hz, Bz-4), 7.36 (d, 4H, *J*=9.0 Hz, Bz-2',6'), 7.50 (d, 4H, *J*=9.0 Hz, Bz-3',5'), 7.72 (d, 2H, *J*=9.0 Hz, Bz-7), 15.09 (s, 2H, Bz-NH). ESI-HRMS m/z: 438.1286 (Calcd for $C_{26}H_{16}ON_4F_2$: 438.1292).

2.3. Biological assays

The anti-tumor properties of all these compouns were tested by the standard MTT assay technique. Tumor cells in RPMI 1640 medium with 10 % fetal bovine serum were plated into 96-well microtiter plates (St. Louis, MO, USA) (4.0×10^4 cells per well) and allowed to adhere at 37 °C with 5 % CO_2 for 4 h. The test compound was then added and the cells were incubated at 37 °C with 5 % CO_2 for 72 h.

2.4. Biological assays

The anti-proliferational effects of tumour cells were tested by the same methods. Tumor cells in RPMI1640 medium with 10 % fetal bovine serum were plated in 96-well microtiter plates (4.0×10^4 cells per well), and allowed to adhere at 37°C with 5% CO_2 for 4 h. The test compound was then added, and the cells were incubated at 37°C with 5 % CO_2 for 72 h. The cell viability was assessed using a standard MTT assay.

2.5. Spectral Measurements

The absorption spectral measurements were recorded on Cary Varian double beam spectrophotometer (Cary BIO 100, Australia). The sample cuvette used was a pair quartz cells of 1.00 cm path length. All scanning parameters were optimized to obtain the best spectra and in general the parameters were scan range 230-300 nm, wavelength step 0.5 and all measurements were carried out at room temperature.

2.6. Fluorescence Measurements

Fluorescence measurements were performed using Spectrofluorimeter model FS920 of Edinburgh Instruments, U.K. equipped with xenon arc lamp. The temperature of the sample holder was regulated with a peltier cooled thermostat. Quartz cuvettes of 3ml capacity, path length 1 cm were used for all measurements.

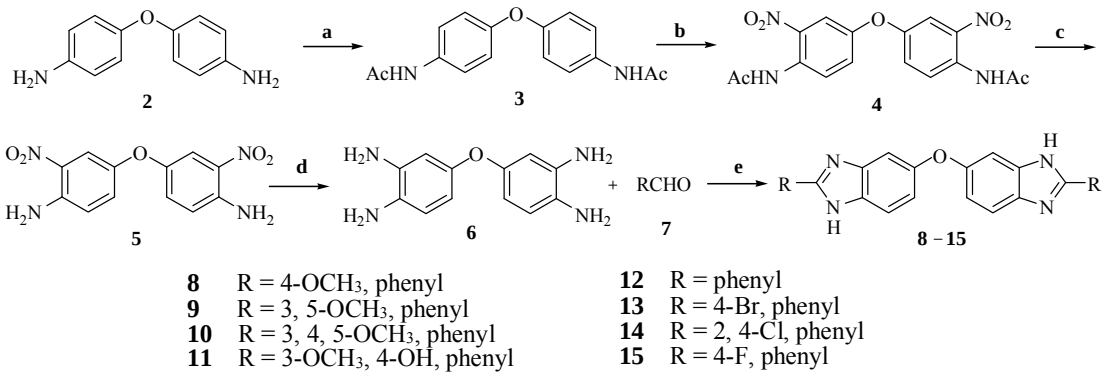
RESULTS AND DISCUSSION

3.1. Synthesis

Compound **8-15** were achieved with an efficient synthetic route (**Scheme 1**). Protection of the amino group of commercially available 4, 4′-diaminodiphenyl ether with acetic anhydride at 0℃ gave4, 4′-diacetamido-diphenyl(**3**). Nitration of compound **3** with nitric acid in acetic acid formed compound **4**. Treatment of compound **4** with a solution of potassium hydroxidein waterand ethanolgave 3, 3′-dinitro-4, 4′-diaminodiphenyl ether (**5**), followed by reduction of the nitro group to afford 3, 3′, 4, 4′-tetraaminodiphenyl ether (**6**). Treatment of compound**6**with various substituted benzaldehyde in methanol at reflux gave the desired compounds.

3.2. Biological evaluation

All the newly synthesized compounds were investigated for their anti-tumor activities in three cancer cell lines using MTT assay[4]. In respect to the in vitro cytotoxic activities listed in **Table1**, almost all of the synthesized compounds exhibited high anti-tumor activity when compared with known DNA minor groove agent Hoechst 33258in **Table1**.



Scheme 2. Reagents and conditions: (a) $(CH_3CO)_2O/NEt_3$, acetone, 0 °C, 2 h, 98%; (b) Conc. HNO_3, acetic acid, 0-50 °C, 5 h, 98%; (c) 42% KOH aq, MeOH, reflux, 2 h, 97%; (d) Pd-C, H_2, MeOH, rt, 6 h, 89%; (e) $NaHSO_3$, methanol,rt,8 h, 54-93%

Table 1 The anti-tumor activities of the compounds (8-15)

Compound	IC_{50} (μM)		
	HeLa	HL60	U937
8	4.96	0.56	0.58
9	8.59	6.25	7.09
10	9.37	7.56	6.92
11	10.61	8.17	5.60
12	18.35	16.47	17.11
13	20.39	17.22	21.67
14	17.24	15.78	20.52
15	22.76	23.19	22.63
5-FU	0.28	0.06	1.07
H. 33258	51.31	32.43	15.42

As shown in **Table1**, compounds **8-11**with electronic-donating substituents (methoxy group) on the benzene ring showed more potent anti-tumor activities than compounds **13-15**, which only contains electronic-withdrawing halogen substituents (Cl, F or Br) on benzene ring. Compounds **8-11** showed low cytotoxicity atconcentration of 10 μM while compounds **13-15** were less potent with IC_{50} values more than 10 μM. Among them, compound **8** was most potent with IC_{50} values of0.56 μM for HL60 tumor cell line and 0.58μM for U937 tumor cell line.

3.3. Molecular modeling

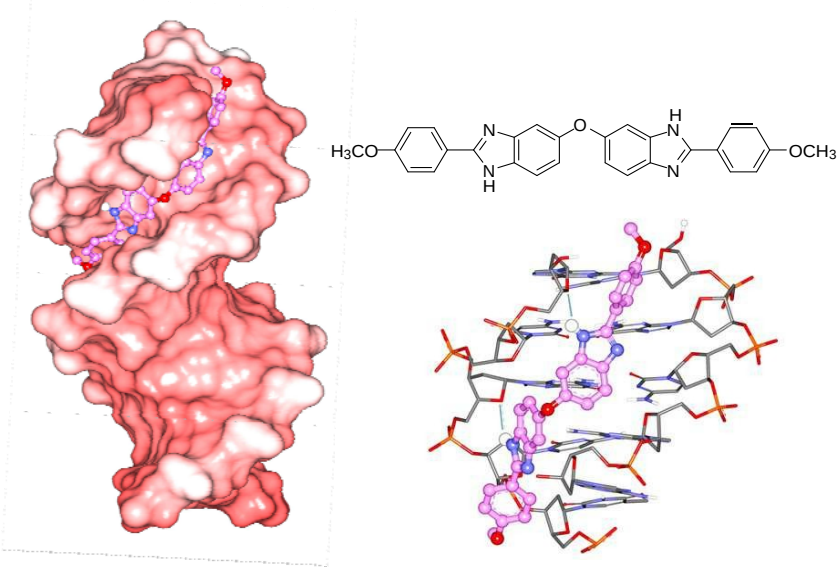


Figure 1.Close-up view of compound 8 binding in the minor groove

In order to determine the binding ability of compound **8** with the DNA minor groove, a molecular modeling study was carried out by the AutoDock 3.05 program(4). In our model, the X-ray crystallographic structure of the DNA dodecamerd(CGCAAATTTGCG) was selected from the Protein Data Bank (PDB code: 2DND) for the docking study[5]. **Figure 1** show that there are van der Waals contacts between compound **8** and the narrow minor groove.

Compound **8** exactly fits into the convex minor groove in the model. In addition, two hydrogen bonds between compound **8** and DNA are formed between two benzimidazole NH group. Molecular modeling studies (shown in Fig. 1) suggest that the novel symmetric bis-benzimidazole derivative (compound **8**) could effectively bind into the DNA minor groove.

3.4. Spectral Studies

UV-Vis absorption spectral was carried out to investigating the binding mode of the compound **8** with DNA. The CT-DNA solution (10 mM) was titrated against compound **8** in 0.1 M Tris-HCl buffer pH 7.4 (**Figure2a**). The absorbance of CT-DNA at 258 nm progressively increased when the concentration of compound **8**solution was increased from 0 to 20 mM. There was a distinct blue shift of DNA-compound **8** complex in the 258 nm region. Hoechst 33258 has the similar increase in absorbance of CT-DNA at 254 nm associated with blue shift[6].So, compound **8** may have the same binding ability with DNA minor groove.

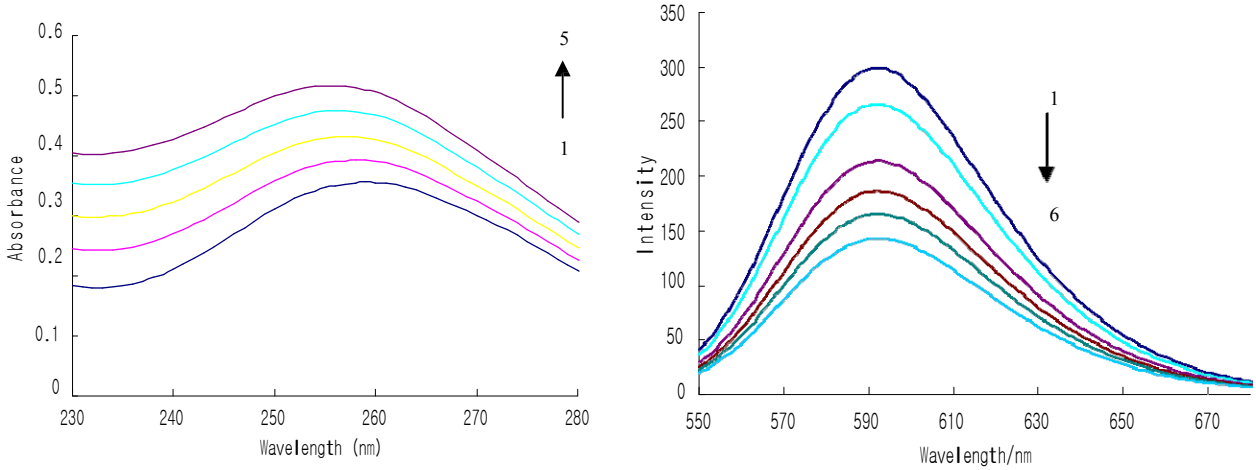


Figure 2. (a) Absorption Spectra of DNA-compound 8 in Tris-HCl Buffer Solution at pH 7.4CT-DNA concentration was kept fixed at 10 mM and compound 10 concentration was varied from(1) 0 to (5) 20 mM; (b) Fluorescence emission spectra (excited at 520 nm) of EB, EB-DNA complexes in the absence (1) and presence (2-6) of increasing concentrations of the compound 8 (2 mmol L^{-1}, 1 L per scan)

Fluorescence quenching measurements is effective to monitor the binding nature of the small molecules to DNA. The molecular fluorophore EB (ethidium bromide) has a conjugate planar structure and its fluorescence intensity is very weak, but it emits intense fluorescence at about 600 nm in the presence of DNA due to its strong intercalation between the adjacent DNA base pairs. Many DNA minor groove agents could quench the intense fluorescence[7]. Similar quenching was observed in the compound **8** (**Figure2b**). Therefore, it is concluded that compound **8** could bind into the minor groove of DNA.

CONCLUSION

In conclusion, we synthesized a new series of bis-benzimidazole derivatives with higher anti-cancer activity than Hoechst 33258. On the other hand,the novel symmetric bis-benzimidazole derivatives could effectively bind into the DNA minor groove. This work enriched the structural types of bis-benzimidazole.

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