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Research Article

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Synthesis of New Series of Bis-Benzothiazole Derivatives as DNA Minor Groove Binding Agents

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ABSTRACT

A series of novel bis-benzothiazole derivaties with head-to head orientation were synthesised, and their anti-proliferative activities on U937, HL60 and HeLa cells were investigated. Most of these new compounds showed significant in vitro anticancer activity when compared to 5-FU. Molecular modeling, fluorescence and viscosimetry study showed that these compounds could bind into the minor groove of DNA.

Keywords: Bis-benzothiazole; DNA minor groove; Molecular modeling; Fluorescence

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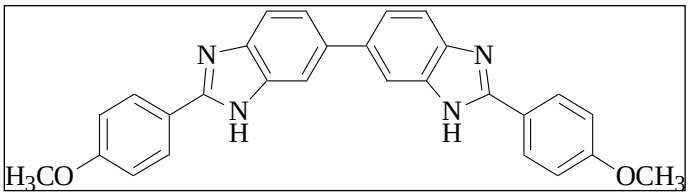
INTRODUCTION

Benzothiazole derivatives play an important role in medicinal chemistry due to their synthetic utility and diversified biological activities, such as antitumor [1-9], antimalarial [10], antidiabetic [11], antitubercular [12], anticonvulsant [13], analgesi and anti-inflammtory [14] activities. In recent years, many researches have focused on developing novel benzothiazole derivatives to improve antitumor activities. However, most investigators focused on single benzothiazole, no literature related bis-benzothiazole has reported so far.

Bis[2-(4'-methoxyphenyl)-1*H*-benzimidazole] (1) [15], a fluorescent compound with a head-to-head bis-benzimidazole structure, was reported to have significant in vitro activity against a group of ovarian carcinoma cell lines.



Scheme 1 Chemical structures of compound 1

Based on these structural studies, molecular modelling has suggested to us that compound **5** could effectively bind into the DNA minor groove (Figure 1). Then, a series of derivatives with a head-to-head bis-benzothiazole structure were synthesized on the basis of modeling results. The interaction of compound **5** with CT (calf thymus)-DNA has been investigated using absorption spectroscopy, fluorescence spectroscopy. All of these compounds were screened for anti-tumor activity in vitro and showed significant antitumor activity.

EXPERIMENTAL SECTION

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 (Büchi Labortechnik, 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 internal standard if not specifically mentioned (*J* in Hz). Mass spectra were obtained on Waters Micromass Quattro Micro API mass spectrometer (Waters Corporation, Milford, United States).

General Procedure for the Synthesis of Substituted Compounds 5-9

Synthesis of [6,6'-Bibenzothiazole]-2,2'-diamine (3):

To 50 mL glacial acetic acid, 18.42g (0.1mol) of Benzidine and 14.58g (0.15mol) of potassium thiocyanate was added and reaction mixture was kept in an ice bath and cooled. The mixture was allowed to cool at 2 ºC up to 1h. Then, in the reaction mixture, bromine in glacial acetic acid (0.15 mol) was added slowly to maintain the temperature of the reaction mixture below 10 ºC and was stirred at room temperature for 2 h to obtain the compound 4 22.68g. Yellow solid; Yield: 76.1 %, mp 356.7-358.9 ℃; ^{1}H NMR (300 MHz, DMSO-d_6) δ: 4.05 (s, 4H, -NH2), 7.74 (d, J = 9.0 Hz, 2H, Bz-5), 8.26 (d, J = 9.0 Hz, 2H, Bz-4), 8.32 (s, 2H, Bz-7). ^{13}C NMR (75 MHz, DMSO-d_6) δ: 121.8, 122.9, 123.7, 124.5, 133.9, 146.8, 174.9. HRMS (ESI$^+$) calculated for $C_{14}H_{10}N_4S_2$ [M+1]$^+$: 299.3860; Found 299.3868.

Synthesis of [1,1'-Biphenyl]-3,3'-dithiol-4,4'-diamine (4):

Compound **3** (14.9 g, 0.05 mol) was dissolved in 300ml CH_3OH. KOH (5.6g, 0.1mol) was added. The solution was heated to 80℃ for 6 h. Then, the mixture was poured into water (100 ml) and filterated to obtained compound **4** 14.9 g. White solid. Yield: 89.2 %. Mp: 276-278 ℃. ^{1}H NMR (300 MHz, DMSO-d_6) δ: 3.13 (s, 2H, -SH), 4.01 (s, 4H, -NH_2), 6.43 (d, *J* = 9.0 Hz, 2H, Bz-5), 7.12 (dd, *J* = 2.4, 9.0 Hz, 2H, Bz-6), 7.14 (d, *J* = 2.4 Hz, 2H, Bz-2). ^{13}C NMR(75 MHz, DMSO-d_6) δ: 115.6, 119.7, 125.2, 126.1, 128.5, 145.9. HRMS (ESI$^+$) calcd. for $C_{12}H_{12}N_2S_2$[M + 1]$^+$: 249.3671; Found 249.3680.

General procedure for the reaction of 4 with substituted benzaldehyde:

Compound **4** (0.5 g, 1.8 mmol), substituted benzaldehyde, sodium hydrogensulfite (0.37 g, 3.6 mmol), methanol (40 ml) were heated to reflux for 8 h. The mixture was evaporated under reduced pressure and obtained the compounds **5-9**. Compounds **5-9** were purified by gel column chromatography ($CHCl_3$:CH_3OH=30:1).

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

Bis(2-phenyl-1H-benzothiazole) (5):

Yellow solid. Yield: 77.9%. mp: 243.1-245.4 ℃. ^{1}H-NMR (300MHz, DMSO-d_6) δ: 7.42 (m, 2H), 7.53 (m, 4H), 7.78 (dd, 2H, *J* = 2.4, 9.0 Hz), 7.82 (d, 2H, *J* = 9.0 Hz), 8.05 (d, 4H, *J* = 9.0 Hz), 8.36 (d, 2H, *J* = 2.4Hz). ^{13}C NMR (75 MHz, DMSO-d_6) δ: 121.2, 122.1, 124.3, 127.6, 128.6, 129.8, 133.6, 134.0, 136.0, 151.9, 171.2. HRMS (ESI$^+$) Calcd for $C_{26}H_{16}N_2S_2$ [M + 1]$^+$: 421.5486; Found 421.5472.

Bis[2-(4'-methylphenyl)-1H-benzothiazole] (6):

Yellow solid; Yield: 79.7%, mp 226.4-228.9 ℃; ^{1}H-NMR (300MHz, DMSO-d_6) δ: 2.36 (s, 6H), 7.30 (d, 2H), 7.68 (d, 2H), 7.78 (dd, 2H, *J* = 2.4, 9.0 Hz), 7.82 (d, 2H, *J* = 9.0 Hz), 8.36 (d, 2H, *J* = 2.4,). ^{13}C NMR (75 MHz, DMSO-d_6)δ: 21.0, 121.2, 122.1, 124.3, 126.2, 129.4, 133.4, 133.6, 134.0, 137.5, 151.9, 171.2. HRMS (ESI$^+$) Calcd for $C_{28}H_{20}N_2S_2$ [M + 1]$^+$: 449.6018; Found 449.6012.

Bis[2-(4'-methoxyphenyl)-1H-benzothiazole] (7):

Yellow solid; Yield: 74.2%, mp 216.3-218.6 ℃; ^{1}H-NMR (300MHz, DMSO-d_6) δ: 3.83 (s, 6H), 7.05(d, 2H, *J* = 9.0 Hz), 7.68 (d, 2H, *J* = 9.0 Hz), 7.78 (dd, 2H, *J* = 2.4, 9.0 Hz), 7.82 (d, 2H, *J* = 9.0 Hz), 8.36 (d, 2H, *J* = 2.4,). ^{13}C NMR (75 MHz, DMSO-d_6) δ: 56.1, 114.5, 121.2, 122.1, 124.3, 128.3, 128.9, 133.6, 134.0, 151.9, 161.9, 171.2. HRMS (ESI$^+$) Calcd for $C_{28}H_{20}N_2O_2S_2$ [M + 1]$^+$: 481.6006; Found 481.6012.

Bis[2-(4'-chlorophenyl)-1H-benzothiazole] (8):
Yellow solid yield: 64.1%. mp 196.7-197.5 ℃. ^{1}H-NMR (300MHz, DMSO-d_6) δ: 7.57 (d, 4H, J = 9.0 Hz), 7.79 (dd, 2H, J = 2.4, 9.0 Hz), 7.84 (d, 2H, J = 9.0 Hz), 8.04 (d, 4H, J = 9.0 Hz), 8.36 (d, 2H, J = 2.4). ^{13}C NMR (75 MHz, DMSO-d_6) δ: 121.2, 122.1, 124.3, 128.2, 129.6, 133.6, 133.8, 134.0, 134.9, 151.9, 171.2. HRMS (ESI$^+$) Calcd for $C_{26}H_{14}Cl_2N_2O_2$ [M + 1]$^+$: 490.4388; Found 490.4383.

Bis[2-(4'-bromophenyl)-1H-benzothiazole] (9):
Yellow solid yield: 63.8%, mp199.8-201.6 ℃; ^{1}H-NMR (300MHz, DMSO-d_6) δ: 7.67 (d, 4H, J = 9.0 Hz), 7.79 (dd, 2H, J = 2.4, 9.0 Hz), 7.84 (d, 2H, J = 9.0 Hz), 7.88 (d, 4H, J = 9.0 Hz), 8.36 (d, 2H, J = 2.4). ^{13}C NMR (75 MHz, DMSO-d_6) δ: 121.2, 122.1, 123.6, 124.3, 128.7, 132.6, 133.6, 134.0, 135.7, 151.9, 171.2. HRMS (ESI$^+$) Calcd for $C_{26}H_{14}Br_2N_2O_2$ [M + 1]$^+$: 578.3408; Found 579.3400.

Molecular modeling
The anti-tumor properties of all these compounds were tested by the standard MTT assay technique. Tumor cells in RPMI 1640 medium with 10 % fetal bovine serum were plated into 96-well microliter 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.

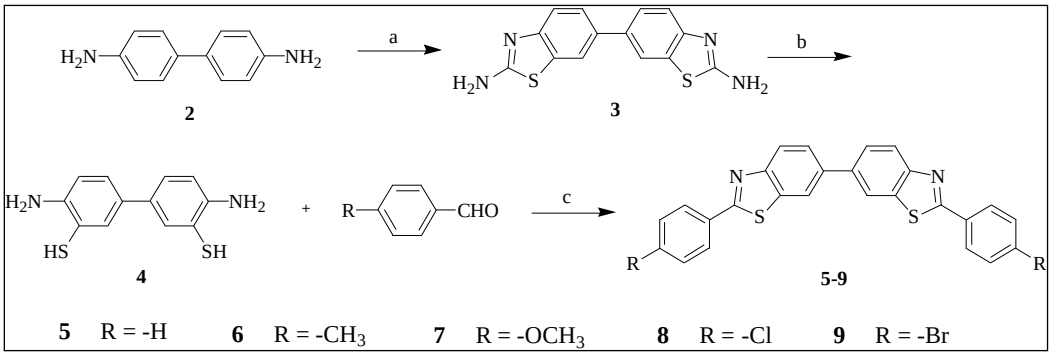
Biological assays
Cytotoxicity assays were performed by MTT method. Tumor cells in RPMI1640 medium with 10 % fetal bovine serum were plated in 96-well microtiter plates (4.0×10^4 cells per well), and allowed to adhere at 37°C with 5% CO_2 for 4 h. The test compound was then added, and the cells were incubated at 37°C with 5 % CO_2 for 72 h.

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 240-290 nm, wavelength step 0.5 and all measurements were carried out at room temperature.

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 3 ml capacity, path length 1 cm were used for all measurements.

RESULTS AND DISCUSSION

Synthesis
The synthesis of these bis-benzothiazoles is outlined in Scheme 2. Benzidine was reacted with Potassium thiocyanate to provide [6,6'-Bibenzothiazole]-2,2'-diamine (3). Treatment of compound 3 with the solution of potassium hydroxide in water gave [1,1'-Biphenyl]-3,3'-dithiol-4,4'-diamine (4) which reacted with various substituted benzaldehyde in methanol at reflux gave the desired compounds 5-9.



Scheme 2: Reagents and conditions: (a) KSCN, Br_2, CH_3COOH, 0 °C, 4 h, 98%; (b) KOH, H_2O, reflux, 6 h, 89%; (c) $NaHSO_3$, methanol, reflux, 8 h, 51-77%

Biological evaluation

To further study the cytotoxic profile, the newly synthesized compounds were investigated for their in vitro anti-tumor activities on three cancer cell lines using MTT assay [16]. Table 1 showed the IC_{50} (μM) values (concentration required to achieve 50% inhibition of the tumor growth) of the tested compounds and the standards. Almost all of the synthesized compounds exhibited the antitumor activity when compared with 5-FU.

Table 1: The anti-tumor activities of the compounds (5 - 9)

Compound	IC_{50} (μM)		
	U937	Hela	HL60
5	9.56	12.57	8.89
6	9.48	9.04	9.24
7	6.76	7.21	8.67
8	28.42	40.36	39.19
9	31.57	37.38	36.48
5-FU	16.72	10.58	13.96

The experiments were performed twice and the average values were obtained from two independent experiments.
As shown in Table 1, compound 6 and 7 with electronic-donating substituents (methyl or methoxyl) on the benzene ring showed more potent antitumor activities than compound 8 and 9, which only contains electronic-withdrawing halogen substituents (Cl or Br) on benzene ring. Compounds 5-7 showed low cytotoxicity at concentration of 20 μM while compounds 8-9 were less potent with IC_{50} values more than 30 μM. Among them, compound 7 was most potent with IC_{50} values of 6.76 μM for U937 tumor cell line, 8.67 μM for HL60 tumor cell line and 7.21 μM for Hela tumor cell line.

Molecular modeling

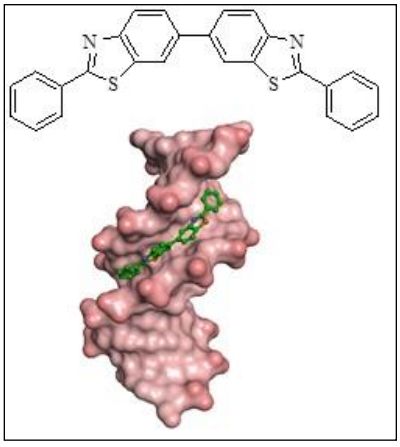


Figure 1: Close-up view of compound 5 binding in the minor groove

In order to determine the binding ability of compound 5 with the DNA minor groove, a molecular modeling study was carried out by the AutoDock 4.2 program [17]. In our model, the X-ray crystallographic structure of the DNA sequence d(CGCGAATTCGCG) was selected from the Protein Data Bank (PDB code: 453D) for the docking study. Figure 1 show that there are van der Waals contacts between compound 5 and the narrow minor groove. Compound 5 exactly fits into the convex minor groove in the model.

Spectral studies

To understand the binding mode of the compound 5 with DNA, UV-Vis absorption spectral was carried out. The CT-DNA solution (10 mM) was titrated against compound 5 in 0.1 M Tris-HCl buffer pH 7.4 (Figure 2a). The absorption maxima at 254 nm is due to the CT-DNA. The absorbance of CT-DNA at 254 nm progressively increased when the concentration of compound 5 solution was increased from 0 to 20 mM. There was a distinct blue shift of DNA-compound 5 complex in the 254 nm region. It was indicated that compound 5 have the binding ability with DNA minor groove.

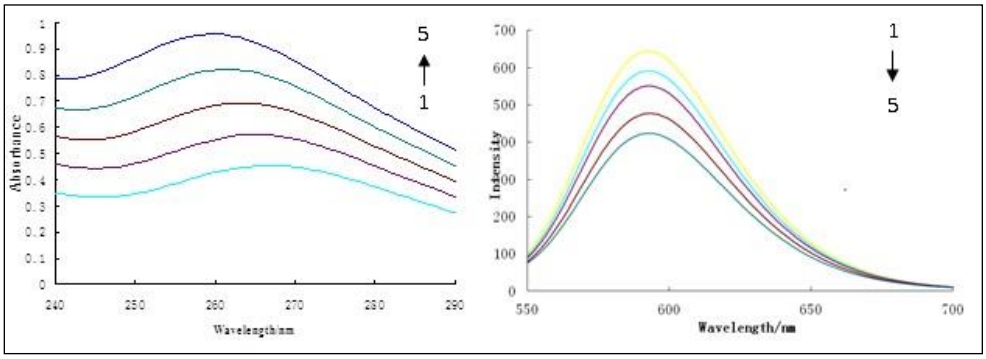


Figure 2: (a) Absorption Spectra of DNA-compound 5 in Tris-HCl Buffer Solution at pH 7.4 CT-DNA concentration was kept fixed at 10 mM and compound 10 concentration was varied from (1) 0 to (5) 20 mM; (b) Fluorescence emission spectra (excited at 520 nm) of EB, EB-DNA complexes in the absence (1) and presence (2-5) of increasing concentrations of the compound 5 (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 [18]. Similar quenching was observed in the compound 5 (Figure 2). Therefore, it is concluded that compound 5 could bind into the minor groove of DNA.

CONCLUSIONS

We synthesized a new series of derivatives with a head-to-head bis-benzothiazole strture and these compounds showed significant anticancer activity compared with 5-FU. On the other hand, the novel compounds could effectively bind into the DNA minor groove.

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