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Research Article

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Synthesis, DNA Interaction and Cytotoxic Activity Studies of a Novel 2,1,3-Benzothiadiazole-Benzimidazole Derivative

Engin Saka[1], Senem Akkoç[2]*, Burak Coban[1]*

[1]Department of Chemistry, Faculty of Arts and Sciences, Zonguldak Bulent Ecevit University, Zonguldak 67100, Turkey
[2]Department of Basic Pharmaceutical Sciences, Faculty of Pharmacy, Suleyman Demirel University, Isparta 32260, Turkey
*Corresponding Authors: senemakkoc44@gmail.com, senemakkoc@sdu.edu.tr, burakcoban@yahoo.com, burakcoban@beun.edu.tr

Abstract Benzimidazoles and benzothiodiazoles are biologically active heterocyclic compounds. In here, a new compound 4-(1*H*-benzimidazol-2-yl)-7-bromo-2,1,3-benzothiodiazole containing both heterocyclic ring systems was prepared. The highest occupied molecular orbital (HOMO) and the lowest unoccupied molecular orbital (LUMO) of the new compound was calculated using the Spartan 10 program. The compound binds to DNA with a moderate strength, and the cytotoxic activity studies show that the relative compound has no antiproliferative activity against cancer cell lines.

Keywords DNA; benzimidazoles; benzothiodiazoles; cytotoxicity

1. Introduction

Anticancer agents have usually some type of mechanisms to interact with DNA, which in turn may impair its function and cause cell death. These interaction types may include intercalation between the base pairs, close contact interactions with DNA grooves (groove binding) or electrostatically external binding. The investigations to develop new DNA binding drugs continue because of the side effects and resistance to current drugs.

Benzothiodiazoles (2,1,3-) (BTD) are precursors of heterocyclic compounds of important substances with applications in various fields such as pharmacological compounds, fluorescent dyes, organic conductors and molecular recognition sensors due to their intense fluorescent properties [1-9]. Many fluorescent BTD bioprobes have been designed to mark some components of the cell. Some 4,7-derivatised BTD compounds have been reported as DNA markers with high fluorescence increase upon binding as intercalators and groove binders could be used for DNA quantification [5-7,10-17].

Benzimidazoles are abundantly present in natural products and also in pharmaceutics [18-19] and show a variety of biological activities including antifungal, antimicrobial, anthelmintic, antiviral, topoisomerase inhibition and anticancer activities [20-26]. Because of these pharmacological properties, great attention has been paid to the synthesis of benzimidazoles [27-28].

To obtain a novel and effective chemotherapeutic agents, a hybrid compound possesses both heterocycles mentioned above: a benzimidazole and a BTD derivative designed with an internal H-bond forcing the whole structure to be an extended flat surfaced aromatic ring system. 4-(1*H*-benzo[*d*]imidazol-2-yl)-7-bromobenzo[*c*][1,2,5]thiadiazole (4-

(1*H*-benzoimidazol-2-yl)-7-bromoBTD) was synthesized and fully characterized by using infrared spectrophotometry, nuclear magnetic resonance spectrometry, and mass spectrometry techniques. DNA binding properties were evaluated and cytotoxicity parameters were studied against human liver hepatocellular carcinoma cell line (HepG2), human breast cancer cell line (MDA-MB-231) and human colorectal adenocarcinoma cell line (DLD-1). The results show that the compound developed has no antiproliferative activity towards these three cell lines.

2. Experimental Section

2.1. Materials and instrumentations

Colorectal adenocarcinoma DLD-1 (ATCC® CCL-221™), MDA-MB-231 human breast cancer (ATCC® HTB-26™) and HepG2 liver hepatocellular carcinoma (ATCC® HB-8065™) cell lines were bought from ATCC (American Type Culture Collection, USA). The chemicals and reagents were supplied from various suppliers and used without purification. DNA solutions were prepared according to the standard procedure [29]. DNA concentrations were determined according to spectrophotometric methods by using ε_{260nm} = 6600 M^{-1} cm^{-1} [29]. The compound was dissolved in DMSO and diluted to desired concentrations by using buffer solution (50 mM ammonium acetate, pH 7.5). Varian Cary 100 spectrophotometer and PerkinElmer LS 55 spectrofluorophotometer were used for titrations. Leco 932 CHNS analyzer was used for CHN analyses. PerkinElmer Spectrum 100 instrument was used for FT-IR spectra. Bruker Ultra Shield Plus ultra-long-hold-time spectrometer was used at 400 MHz at room temperature for nmr spectra, chemical shifts are given relative to TMS as an internal standard. The electronic parameters of this compound were calculated using Spartan 10 program.

2.2. Synthesis of 4-(1H-benzimidazol-2-yl)-7-bromo-2,1,3-benzothiodiazole (1)

The reaction was designed according to the green chemistry procedure of Lin and Yang (2005) (figure 1) [28]. Equal molar amounts of benzene-1,2-diamine (44,3 mg; 0.41 mmol) and aldehyde (100 mg; 0.41 mmol) were dissolved in 25 mL of ethanol, and the mixture was heated to reflux for 16 h in the presence of air. After evaporation of the solvent, the residue was purified by chromatography on silica gel using a mixture of petroleum ether and CH_2Cl_2 (6:1, v/v) as the eluent to give **1** as a colorless liquid, 0.04 g, yield of 30%. Melting point: 198-200 °C. Molecule **1** [$C_{13}H_7BrN_4S$] showed the molecular ion peak at 331.1 and $[C_{13}H_8BrN_4S]^+$ protonated ion peak at 333.12 in the ESI-MS spectra (Suppl.). Found: C, 47.10 %; H, 2.18 %; N, 16.88 %. Calculated: C, 47.15 %; H, 2.13 %; N, 16.92 %. FT-IR (ATR) (ν, cm^{-1}): 3386 (H_2O), 3372, 3050 (C-H_{ar}), 1619, 1590, 1551 (C=N), 1507, 1487, 1472 (C=C), 1445, 1415, 1322, 1276, 1225, 1143, 938, 879, 840, 713 (C-Br) (Suppl.). ^{13}C-NMR (referenced to $CDCl_3$, 100 MHz, ppm): 153.56, 151.38, 147.55, 133.15, 129.64, 122.38, 115.07 (Suppl.). ^{1}H-NMR (referenced to $CDCl_3$, 400 MHz, ppm): s 12.84 (NH, 1H), 8.51 (d, *J* 3.9, 1H, BDT), 8.21 (d, *J* 3.9, 1H, BDT), 7.72 (d, *J* 3.9, 2H, benzimidazole), 7.25 (d, *J* 2.1, 2H, benzimidazole) (Suppl.).

2.3. UV-Vis and fluorescence studies

A solution of **1** (20 µM) in buffer (5 mM ammonium acetate, pH 7.5) was titrated with the CT-DNA stock solution with 1 µL additions until no change was observed. The spectrum was recorded each time DNA was added. The reaction solutions were incubated (10 min, 25 °C) then the spectrum was recorded after each addition. The ex/em wavelengths are 410 and 550 nm for emission intensity measurements (5-nm entrance slit and a 5-nm exit slit).

2.4. Gel electrophoresis

The pBR322 plasmid DNA was used for 1% agarose gel electrophoresis experiments. The mixtures (10 µL) contain 0.05 µg of DNA, varying amounts of **1** (0, 1, 20, 100, 400 µM), and 1 µL of 30% peroxide solution. To run the electrophoresis 35 V potential was applied for 4 h at room temperature. Gels were photographed under UV light after dyed in EB (0.5 mg mL^{-1}) and rinsed in water.

2.5. Cytotoxic activity studies

Literature procedure was followed for the cytotoxic activity studies [30-33]. The cell lines were incubated in DMEM medium containing **1** at following concentrations 200, 100, 50, 20, 10 and 5 µM. After 48 h incubation time to each well was added MTT (50 µL, 5 mg mL^{-1}). Then, the absorbance values were measured at 560 nm.

3. Results and Discussions

3.1. Synthesis

The synthesis of imidazole compound was performed in one step, by reacting substituted BTD-carbaldehyde with phenylenediamine (Figure 1). Imidazole-derived compound was achieved according to the general method [28] in a moderate yield of 30%. The structural characterization of the compound was performed with elemental analysis, FT-IR, ^{1}H- and ^{13}C-NMR (Look at Supplementary Materials). The frequencies of 3386, 3372 and 3050 cm^{-1} correspond to N-H and aryl C–H stretching vibrations, respectively. The frequencies of 1619, 1590, 1551, 1507, 1487 and 1472 cm^{-1} correlate with conjugated C=N and C=C bonds. The frequency of 713 cm^{-1} could be corresponding specific C-Br vibration in the BTD ring. Compound **1** [$C_{13}H_7BrN_4S$] showed the molecular ion peak at 331.1 and the protonated form $[C_{13}H_8BrN_4S]^+$ (calculated 332.96) 333.12 in the ESI-MS spectra proving the structure. The aromatic carbon signals between 155-110 ppm in the ^{13}C-NMR spectrum attribute with the structure. The signals between 155-110 ppm associate with the carbons neighboring nitrogen atoms and the other signals (110-125 ppm) assign to the aromatic carbons of symmetrical benzimidazole carbons. In the ^{1}H-NMR spectrum, a characteristic singlet signal at 12.85 ppm was appeared for the N-H proton of the benzimidazole ring [29]. The doublet at 8.51 and singlet 8.22 ppm correlate with the protons of the BTD ring neighboring Br atom and the one further away, respectively. Integration of the rest of the signals 7.72 and 7.24 correspond to benzimidazole protons (4 protons).

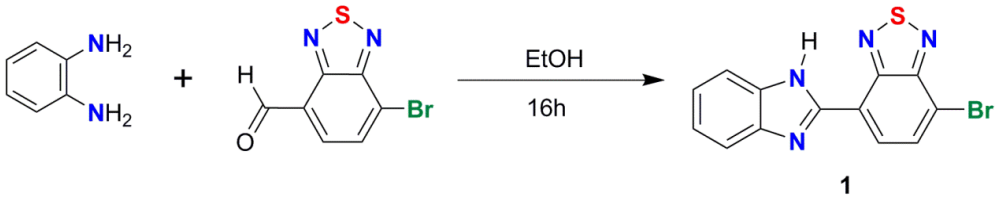

Figure 1: Synthesis scheme of 1

3.2. Theoretical studies

The Hartree-Fock method in 3.21 G basic set in ethyl alcohol was used for calculations. The HOMO and LUMO maps are given in Figure 2. As can be seen in Figure 2, HOMO surfaces of molecule **1** was situated over benzimidazole nucleus and benzene ring in the 4-bromobenzo[*c*][1,2,5]thiadiazole. On the other hand, LUMO map of **1** shows that the surface was settled over 4-bromobenzo[*c*][1,2,5]thiadiazole.

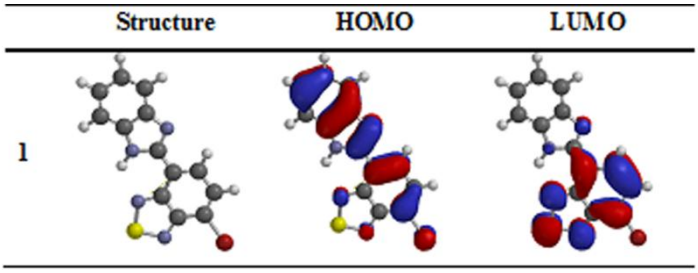

Figure 2: HOMO and LUMO maps of molecule 1

The energy values of HOMO and LUMO were obtained as -0.2950 and 0.0158 eV, respectively. The LUMO - HOMO energy difference was found as 0.3108 eV for **1**. The electronegativity (χ) of this compound was found as 0.14 eV. On the other hand, the chemical hardness (η) of the molecule was calculated and found to be 0.16 eV.

3.3. Absorption and emission titrations

The figure 3 shows the change in absorption spectra of the compound in the absence and presence of CT-DNA. After adding DNA, no red shift was visible but the strong hypochromism was present. When the amount of DNA was upto the half of the compounds concentration, absorption decreased 50%. A quantitative measure maintained by calculating the binding constant K_b of the compound based on the following equation:

$[DNA]/(\varepsilon_A-\varepsilon_f) = [DNA]/(\varepsilon_B-\varepsilon_f) + 1/K_b(\varepsilon_B-\varepsilon_f)$;

$\varepsilon_A = A_{obs}/[compound]$, and ε_f and ε_B are the absorption coefficients of the free and the fully bound compound, respectively. The value of the intrinsic binding constant K_b for compound **1** was found to be 7.8 (± 0.6) × 10^4 M^{-1}. This is a very low binding constant compare to the known DNA binders with biological activities [34, 35]

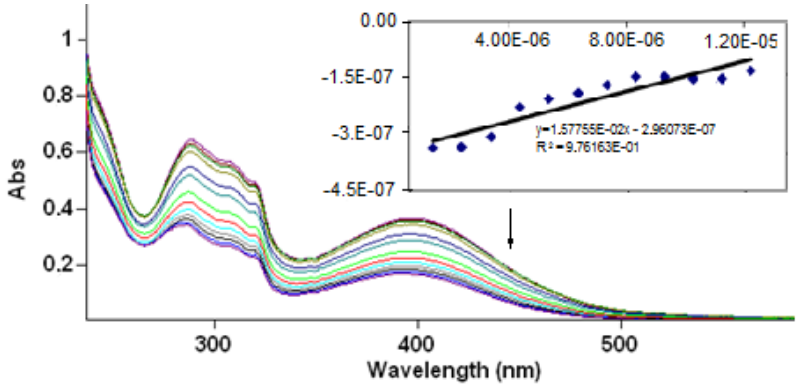

*Figure 3: Absorption spectra of **1**, in buffer (50 mM ammonium acetate) in the absence and presence of calf thymus DNA. [**1**]= 20 µM, [DNA]= 0-12 µM. Arrows indicate the change in absorbance with the addition of DNA. The plot = [DNA]/(ε_B-ε_f) vs [DNA]*

In the absence of DNA **1** can emit a moderate level luminescence at 550 nm in this buffer at room temperature, when excited at 410 nm. The emission intensity drops when DNA added to the solution (Figure 4). This indicates that the compound is efficiently protected by the hydrophobic DNA grooves and the water molecules cannot access to the compound. This leads to a decrease in the vibrational modes of relaxation. However, the increase in intensity of emission is much higher for the classical intercalators. Therefore, the compound binds to DNA as a groove binder.

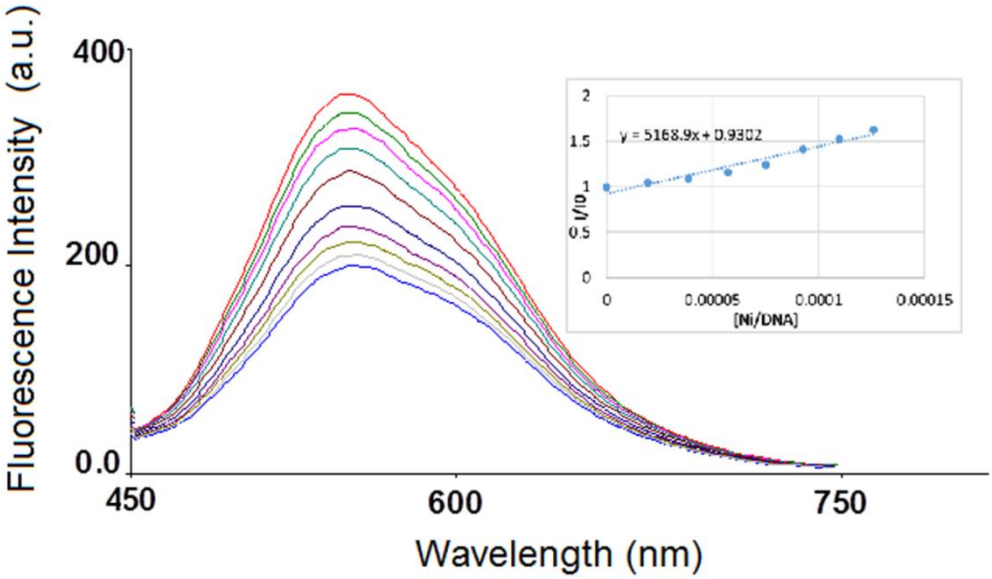

Figure 4: the change in emission spectra of 1 (20 µM, in 2.5 mL 5 mM ammonium acetate buffer, pH= 7.5) when titrated with DNA. [DNA]= 1,25 mM (0-11 µM)

3.4. Cleavage of plasmid DNA

Agarose gel electrophoresis study was used to evaluate the potential of the compound to cleave pBR322. The plasmid DNA was used for the study and it has three different forms as supercoiled DNA (form I) moves the fastest on the gel. However when a damage occurs then the supercoil opens open circular DNA will be generated (form II) which moves slower. Agarose gel electrophoresis separation of pBR322 was shown in Figure 5 in the presence of **1** and hydrogen peroxide. As the concentration of **1** increases then the amount of form I of pBR322 decreases, but the amount of form II raises. This indicates that **1** damages DNA at high concentrations in the presence of peroxide. The DNA damage observed here is very low but similar and more severe results were obtained by other studies [36, 37].

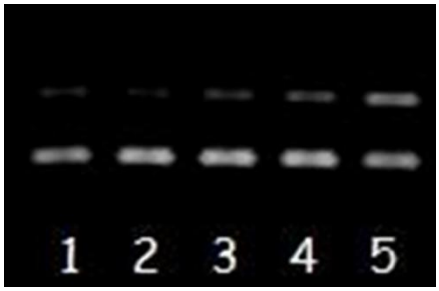

Figure 5: Gel electrophoresis result shows the effect of varying amounts of 1 (0-1-20-100-400 µM) in the presence of peroxide on pBR322 supercoiled plasmid DNA

3.5. Antiproliferative activity studies

A 48 h MTT assay was applied to reaction between **1** as an anticancer agent and three different cancer cells. Under the same experimental conditions, and control drug was cisplatin. The IC_{50} results are given in Table 1.

Table 1: IC_{50} results for **1** and cisplatin against cancer cells

	IC_{50} (µM)		
Compounds	**HepG2**	**DLD-1**	**MDA-MB-231**
1	249.00	231.40	1040
Cisplatin	30.38	60.79	3.78

As can be seen in Table 1, compound **1** has no antiproliferative activity on cancer cell lines, and the half maximal inhibitory concentration (IC_{50}) values were obtained higher than 200 µM. In other word, this compound was found to be biologically inactive for the tested six different concentrations ranging from 5 to 200 µM in liver, breast and colon cancer cell lines. The figure 6 shows the relationship between the concentrations of the compounds and the cell viabilities.

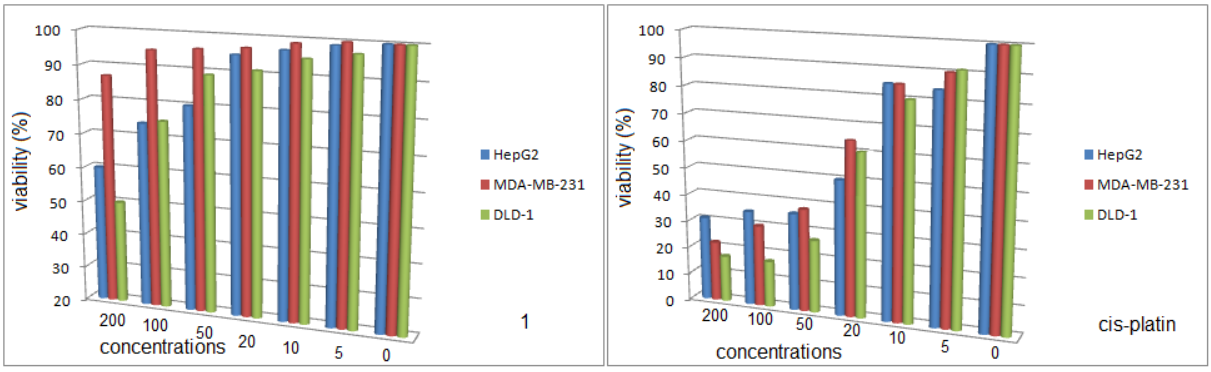

Figure 6: The viability ratios of cancer cell lines were presented as percentages for different concentrations of (left) 1; (right) cisplatin (µM)

The viability ratios of HepG2 and DLD-1 cells are absolutely changed to depending on the concentrations of molecule **1** (Figure 6). Considering its binding constant and DNA damage studies the compound has an expected weak activity level in liver and colon cancer cell lines, and exhibited no cytotoxic activity in breast cancer cell line. On the other hand, cisplatin, as expected, dropped the viability of the cells at concentrations tested (Figure 6). Very similar compounds cationic pip derivative and naphthalimide derivatives (Figure 7) were reported with higher cytotoxicity values against some healthy human cells [36-40].

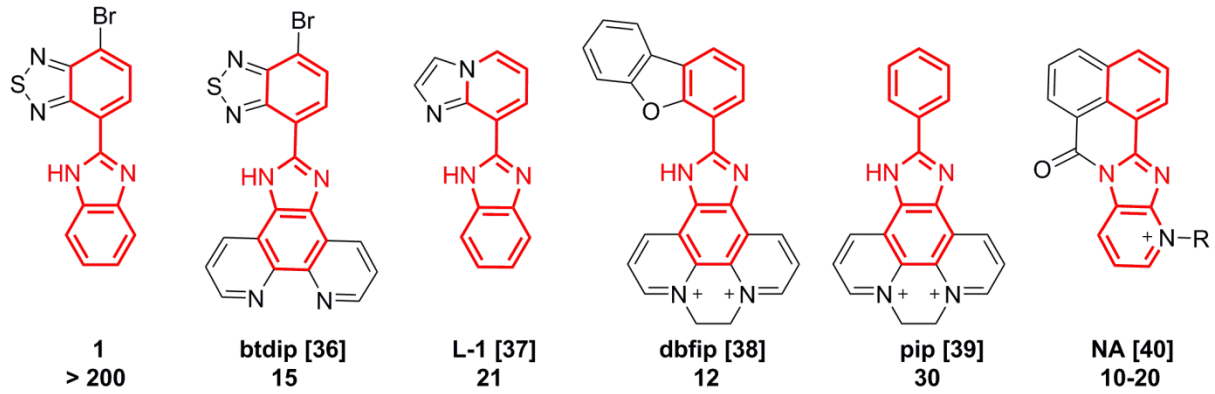

Figure 7: A comparison of IC_{50} values of 1 and similar compounds with from the literature. Similarity is shown by red ink. Names are given by the original authors, references are given in brackets, and IC_{50} values (µM) for DLD-1 cell lines are below the names

4. Conclusions

The search of new drugs to combat cancer has been in progress because of the continuous demand to overcome the side effects and the resistance of the existing anticancer-agents. A new BTD-benzimidazole derivative 4-(1*H*-benzoimidazol-2-yl)-7-bromo(2,3,1)-benzothiadiazole (**1**) was synthesized and characterized. DNA binding ability of the compound was moderate and it possibly binds to DNA via minor grooves. It is a DNA single strand cleaver in the presence of peroxide. As a result of a high energy gap between LUMO and HOMO, a moderate affinity and minor damage to DNA, this compound showed very weak antiproliferative activity against HepG2 and DLD-1 cells. On the other hand, it was found to be inactive against MDA-MB-231.

Disclosure Statement

The author declares that no conflict of interest is associated with this work.

Acknowledgment

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