ORIGINAL ARTICLE

A C G publications

Org. Commun. 9:3 (2016) 65-72

organic communications

Synthesis and cytotoxic activities of novel 2-(1,5-bis(aryl) penta-1,4-dien-2-yl) benzo[d]thiazol derivatives

Betül Şahin[1], Ayşe Şahin Yağlıoğlu[2] and Mustafa Ceylan[*1]

[1]Department of Chemistry, Faculty of Art and Science, Gaziosmanpasa Univesity, 60250, Tokat, Türkiye,
[2]Department of Chemistry, Faculty of Science, Çankırı Karatekin University, 18100 Çankırı, Türkiye

(Received September 30, 2016; Revised October 3, 2016; Accepted October 4, 2016)

Abstract: Novel 2-(1,5-bis(aryl)penta-1,4-dien-2-yl)benzo[d]thiazol (**5a,b**) and 2-(((1S,2S,E)-2-(benzo[d]thiazol-2-yl)-1,5-di-arylpent-4-en-1-yl)thio)aniline (**6a,b**) derivatives were obtained by addition of 2-aminobenzothiol to bis-benzylidinecyclobutanones (**3a,b**). The structures of the obtained compounds were characterized using the spectroscopic methods (NMR, IR, Elemental Analysis). Compounds **5a,b** showed cytotoxic activities against C6 (Rat Brain tumor cells) and HeLa (human uterus carcinoma) in vitro.

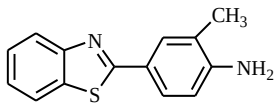
Keywords: 2-Alkylbenzothiazol; Anticancer; C6 and HeLa cell lines.

1. Introduction

Compounds containing benzothiazole nucleus belong to an important class of heterocycles which are known to perform important biological and pharmaceutical activities such as antitumor[1] and anticancer[2] activities. In particular, 2-arylbenzothiazoles have been investigated extensively by organic chemists due to their medicinal properties such as antitumor, antiviral, and antimicrobial drugs.[3,4] For example, 2-(4-amino-3-methylphenyl)- benzothiazole (DF 203) are found to possess potent and selective antitumor activity against panels of human cancer cell lines, and a fluorinated DF 203 prodrug (Phortress) has entered clinical trials for cancer.[5-7] The structurally related 2-(3,4-dimethoxyphenyl)-5-fluorobenzothiazole (PMX 610) has also shown potent and selective in vitro antitumor properties.[8] In addition, (aminophenyl)benzothiazole derivative (Pittsburgh compound-B) has attracted considerable interest in potential diagnosis of Alzheimer's disease,[9,10] progressing to clinical evaluation. Therefore, various benzothiazole compounds are considerably interesting due to their potential for diverse pharmaceutical uses. Although a number of benzothiazole derivatives with antitumor activity are described so far, these are especially 2-arylsubstituted benzothiazole derivatives.[11-22]

In this paper, we reported the synthesis and anti-cancer activities of 2-alkylsubstituted benzothiazole, (2-(1,5-bis(aryl) penta-1,4-dien-2-yl) benzo[d]thiazol), derivatives (**5a,b**) against HeLa (Human Uterus Carcinoma) and C6 (Rat Brain Tumor) cancer cells by using 5-florourasil (5-FU) as standard.

[*] Corresponding author: E-Mail: mustafac.ceylan@gop.edu.tr , Tel: + 90 3562521616; fax: + 90 3562521585.



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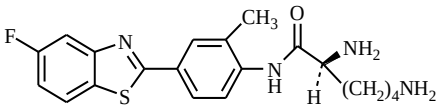
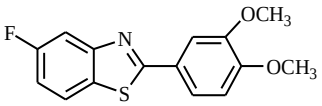
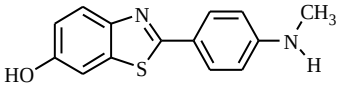


Phortress



PMX 610



Pitsburgh compound-B

Figure1. Some medicinaly important thiazol derivatives

2. Experimental

All the chemicals and solvents employed in the synthesis were supplied by Merck (Germany) and Fluka (Germany) and used without purification. The melting points were measured on an Electrothermal 9100 apparatus. The IR spectrums (KBr disc) were recorded on a Jasco FT/IR-430 spectrometer. The ^{1}H and ^{13}C NMR spectra were recorded on a Bruker Avance DPX-400 instrument. As internal standards served TMS (δ 0.00) for ^{1}H NMR and $CDCl_3$ (δ 77.0) for 13C NMR spectroscopy *J* values are given in Hz. The multiplicities of the signals in the ^{1}H NMR spectra are abbreviated by s (singlet), d (doublet), t (triplet), q (quarted), m (multiplet), br (broad) and combinations thereof. The elemental analyses were obtained from a LECO CHNS 932 Elemental Analyzer.

2.1. Chemistry

2.1.1. General Procedure for Synthesis of 3a-c:

To a solution of cyclobutanone (1) (10 mmol) and corresponding benzaldehyde derivative (2) (20 mmol) in 30 mL of ethanol was added NaOH (20 mmol, 2.5 M) and stirred at room temperature for 4 hours. After completed the reaction, the mixture was poured into ice/water. The participated was washing cold ethanol, dried and crystallized with CH_2Cl_2/diethyl ether.

(*2E,4E)-2,4-Bis(4-metilbenziliden)siklobutanon (3a):* Yellowish crystals, Yield, 78%, M.P. 164-167°C. ^{1}H NMR (400 MHz, $CDCl_3$, ppm): δ = 7.49 (d, *J* = 8.4 Hz, 4H), 7.28-7.23 (m, 6H), 3.85 (t, J = 2.4 Hz, 2H), 2.42 (s, 6H). ^{13}C-NMR (100 MHz, DMSO, ppm): δ = 190.9, 145.1, 140.6, 132.1, 129.9, 129.7, 127.3, 35.7, 21.6. IR (KBr, cm^{-1}): 3025, 2925, 1735, 1643, 1604, 1513, 1099, 813, 516. Anal. Cald. for $C_{20}H_{18}O$: C, 87.56; H, 6.61; Found: C, 87.35; H, 6.32.

(2E,4E)-2,4-bis(4-methoxybenzylidene)cyclobutanone (3b): Yellowish crystals, Yield, 88%; M.P. 183-186°C. **^{1}H NMR (**400 MHz, $CDCl_3$, ppm**)**: δ = 7.55 (d, *J* = 8.4 Hz, 4H), 7.22 (s, 2H); 6.97 (d, *J* = 8.4 Hz, 4H); 3.88 (s, 6H), 3.82 (s, 2H). **^{13}C-NMR (**100 MHz, DMSO, ppm**):** δ= 190.7, 161.1, 143.7, 131.6, 127.6, 126.8, 114.5, 55.5, 35.3. **IR** (KBr, cm^{-1}): 3016, 2946, 2894, 1714, 1598, 1509, 1257, 1174, 1089, 1029, 829, 748. Anal. Cald. for $C_{20}H_{18}O_3$: C, 78.41; H, 5.92; Found: C, 78.63; H, 5.72.

(2E,4E)-2,4-bis(4-bromobenzylidene)cyclobutanone (3c): Colorless crystals, Yield, 44%; M.P. 194-197°C. **^{1}H NMR (**400 MHz, $CDCl_3$, ppm**)**: δ= 7.,51 (d, *J* = 7.2 Hz, 4H), 7.44 (d, *J* = 7.2 Hz, 4H), 7.23 (s, 2H); 3.88 (s, 6H), 3.87 (s, 2H). **^{13}C-NMR (**100 MHz, DMSO, ppm**):** δ = 190.3, 146.1, 136.2, 133.0; 130.8, 129.4; 126.5, 35.7. **IR** (KBr, cm^{-1}): 3023, 2917, 1716, 1666, 1631, 1602, 1509, 1228, 1091, 815, 674, 512. Anal. Cald. for $C_{18}H_{12}Br_2O$: C, 53.50; H, 2.99; Found:C, 53.35; H, 2.79.

2.1.2. General Procedure for Synthesis of 5a,b and 6a,b:

Equimolar amounts of **3** and 2-aminobenzenethiol was dissolved in ethanol (30 mL) in the presence of a catalytic amount of *p*-TsOH and heated at reflux temperature for 5 hours. After the mixture was extracted with ethyl acetate, the organic layer was dried with anhydrous Na_2SO_4 and the solvent was removed under reduced pressure. The crude product was separated on a silica gel column eluting with hexane/ethyl acetate (9:1). While the first fraction was compound **5**, the second fraction was compound **6**.

2-((1Z,4E)-1,5-dip-tolylpenta-1,4-dien-2-yl)benzo[d]thiazol (5a): Yellowish crystals, Yield, 55%, M.P. 171-174°C. **^{1}H-NMR** (400 MHz, $CDCl_3$): δ = 8.03 (d, *J* = 8.0 Hz, 1H), 7.87 (d, *J* = 8.0 Hz, 1H), 7.72 (s, 1H), 7.49 (t, *J* = 7.2 Hz, 1H), 7.45 (d, *J* = 8.0 Hz. 2H), 7.38 (t, *J* = 7.2 Hz, 1H), 7.27-7.7.23 (m, 4H), 7.09 (d, *J* = 8.0 Hz, 2H), 6.55 (d, *J* = 16.0 Hz, 1H), 6.50 (dt, *J* = 16.0, 4.8 Hz, 1H), 3.86 (d, *J* = 4.8 Hz, 2H), 2.41 (s, 3H), 2.32 (s, 3H). **^{13}C-NMR** (100MHz, $CDCl_3$): δ = 170.7, 153.8, 138.3, 136.9, 135.4, 134.8, 134.6, 133.1, 131.2, 129.9, 129.7, 126.2, 126.1, 125.1, 123.1, 121.4, 31.9, 29.7, 21.3. **IR** (KBr, cm^{-1}) 3064, 2938, 2872, 1524, 1436, 1311, 1241, 1108, 966, 759, 728. Anal. Cald. for $C_{26}H_{23}NS$: C, 81.85; H, 6.08; N, 3.67; S, 8.40; Found: C, 81.65; H, 5.98; N, 3.87; S, 8.50.

2-((1Z,4E)-1,5-bis(4-metoxyphenyl) penta-1,4-dien-2-yl) benzo[d]thiazol (5b): Yellowish crystals, Yield, 20%, M.P. 193-197°C. **^{1}H-NMR** (400 MHz, $CDCl_3$): δ = 8.03 (d, *J* = 7.6 Hz, 1H), 7.86 (d, *J* = 7.6 Hz, 1H), 7.70 (s, 1H), 7.53 (d, *J* = 6.8 Hz, 2H), 7.49 (dt, *J* = 7.6, 2.4 Hz, 1H), 7.38 (dt, *J* = 7.6, 1.2 Hz, 1H), 7.31 (d, *J* = 6.8 Hz, 2H), 6.96 (d, *J* = 6.8 Hz, 2H), 6.83 (d, *J* = 6.8 Hz, 2H), 6.52 (d, *J* = 16.4 Hz, 1H), 6.41 (dt, *J* = 16.4, 5.2 Hz, 1H), 3,87 (m, 5H, 3H -OCH_3, 2H –CH_2-), 3,82 (s, 3H). **^{13}C-NMR** (100MHz, $CDCl_3$): δ = 170.9, 159.6, 158.9, 153.9, 135.1, 134.8, 131.9, 130.9, 130.7, 130.3, 128.6, 127.3, 126.1, 125.0, 124.9, 122.9, 121.3, 114.1, 113.9, 55.4, 55.3, 33.3. **IR** (KBr, cm^{-1}): 3041, 2954, 2859, 1606, 1509, 1436, 1249, 1174, 1033, 964, 759, 728, 530. Anal. Cald. for $C_{26}H_{23}NO_2S$: C, 75.52; H, 5.61; N, 3.39; S, 7.75; Found: C, 75.42; H, 5.54; N, 3.57; S, 7.81.

2-(((1S,2S,E)-2-(benzo[d]thiazol-2-yl)-1,5-di-p-tolylpent-4-en-1-yl)thio)aniline (6a): Yellowish crystals, Yield, 60%, M.P. 203-206°C. **^{1}H-NMR** (400 MHz, $CDCl_3$): δ = 8.05 (d, *J* = 7.6 Hz, 1H), 7.89 (d, *J* = 7.6 Hz, 1H), 7.51 (t, *J* = 7.6 Hz, 1H), 7.41 (t, *J* = 7.6 Hz. 1H), 7.19-7.15 (m, 4H), 7,11-7.03 (m, 4H), 6.98 (t, *J* = 7.2 Hz, 1H), 6.78 (d, *J* = 7.2 Hz, 1H), 6.58 (d, *J* = 7.2 Hz, 1H), 6.36 (t, *J* = 7.2 Hz), 6.11 (d, *J* = 15.6 Hz, 1H), 5.92 (dt, J = 15.6, 7.2 Hz, 1H), 4.49 (d, *J* = 11.2 Hz, 1H), 4.18 (brs, 2H, -NH_2), 3.87 (dt, *J* = 11.2, 6.8 Hz, 1H), 2.56 (t, *J* = 6.8 Hz, 2H), 2.36 (s, 3H), 2.29 (s, 3H). **^{13}C-NMR** (100MHz, $CDCl_3$): δ = 172.6, 152.1, 148.1, 137.4, 136.3, 135.5, 134.8, 134.2, 132.4, 129.1, 128.1, 127.4, 126.5, 125.9, 125.0, 124.4, 123.2, 122.9, 122.6, 121.7, 119.6, 117.8, 114.6, 50.2, 46.2, 36.3, 21.3, 21.1. **IR** (KBr, cm^{-1}) 3374, 3054, 3031, 2919, 2852, 1513, 1436, 1311, 1241, 1108, 966, 759, 728. Anal. Cald. for $C_{32}H_{30}N_2S_2$: C, 75.85; H, 5.97; N, 5.53; S, 12.66. Found: C, 75.82; H, 5.99; N, 5.68; S, 12.74.

2-(((1S,2S,E)-2-(benzo[d]thiazol-2-yl)-1,5-bis(4-methoxyphenyl)pent-4-en-1-yl)thio) aniline (6b): Yellowish crystals, Yield, 24%, M.P. 214-217°C. **^{1}H-NMR** (400 MHz, $CDCl_3$): δ = 8.06 (d, *J* = 8.0 Hz, 1H), 7.90 (d, *J* = 8.0 Hz, 1H), 7.52 (dt, *J* = 8.0, 1.2 Hz, 1H), 7.41 (dt, *J* = 8.0, 1.2 Hz, 1H), 7.13 (d, *J* = 6.8 Hz, 2H), 7.10 (d, *J* = 6.8 Hz, 2H), 7,00 (dt, *J* = 8.0, 1.6 Hz, 1H), 6.84 (d, *J* = 6.8 Hz, 2H), 6.80 (dd, *J* = 8.0, 1.6 Hz, 1H), 6.76 (d, *J* = 6.8 Hz, 2H), 6.58 (dd, *J* = 8.0, 1.2 Hz, 1H), 6.38 (dt, *J* = 78.0, 1.2 Hz, 1H), 6.10 (d, *J* = 15.6 Hz, 1H), 5.81 (dt, *J* = 15.6, 7.6 Hz, 1H), 4.50 (d, *J* = 11.2 Hz, 1H), 4.20 (brs, 2H, -NH_2), 3.86 (dt, *J* = 11.2, 6.8 Hz, 1H), 3.82 (s, 3H), 3.77 (s, 3H), 2.57 (t, *J* = 6.8 Hz, 2H). **^{13}C-NMR** (100MHz, $CDCl_3$): δ = 173.4, 161.1, 158.8, 153.1, 149.1, 143.7, 137.4, 132.5, 131.9, 131.6, 130.2, 129.4, 127.6, 127.2, 126.8, 126.0, 124.1, 123.8, 122.8, 121.7, 117.8, 114.5, 113.8, 55.4, 55.2, 50.4, 38.1, 35.3. **IR** (KBr, cm^{-1}) 3412. 3043, 3032, 2923, 2862, 1513, 1436, 1311, 1241, 1108, 966, 759, 728. Anal. Cald. for $C_{32}H_{30}N_2O_2S_2$: C, 71.34; H, 5.61; N, 5.20; S, 11.90. Found: C, 71.45; H, 5.74; N, 5.33; S, 12.12.

2.2. Biologigal Assay

2.2.1. Cell culture

Antiproliferative activities of the compounds **5a,b** were investigated on C6 (Rat Brain tumor cells) and HeLa (human uterus carcinoma) cells using proliferation BrdU ELISA assay.[27,28] The cells were grown in Dulbecco's modified eagle's medium (DMEM, Sigma), supplemented with 10% (v/v) fetal bovine serum (Sigma, Germany) and PenStrep solution (Sigma, Germany) at 37°C in a 5% CO_2 humidified atmosphere.

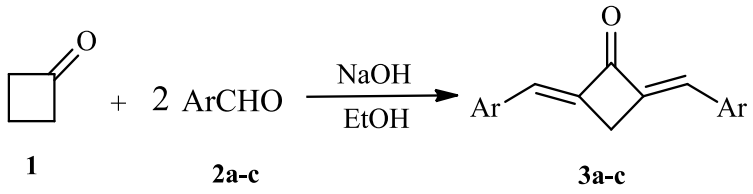
2.2.2. Cell proliferation assays

The cells were plated in 96-well culture plates (COSTAR, Corning, USA) at a densitiy of 3 X 10^4 cells per well. The activities of samples were investigated on three concentrations. 5-FU was used as standard compounds. The cells were then incubated overnight before applying the BrdU Cell Proliferation ELISA assay reagent (Roche, Germany) according to the manufacturer's procedure. The amount of cell proliferation was determined the A450 nm by using a microplate reader (Awareness Chromate, USA). Results were reported as percentage of the inhibition of cell proliferation, where the optical density measured from vehicle-treated cells was considered to be 100% of proliferation. Percentage of inhibition of cell proliferation was calculated as follows: [1 - ($A_{treatments}$ / $A_{vehicle\ control}$)] x 100. The stock solution of the compounds was prepared in dimethyle sulfoxide (DMSO) and diluted with DMEM. DMSO final concentration is below 0.1% in all tests.

3. Results and Discussion

3.1. Chemistry

Cyclobutanone was used as starting material for the synthesis of thiazole derivatives. Addition[23] of two equimolar amounts of aryl aldehydes (**2a-c**) to cyclobutanone in the presence of NaOH in ethanol for 3 hours afforded the bis-benzylidinecyclobutanones (**3a-c**) in good yields (78% and 88%, respectively) (Figure 2). The structures of compounds were explained on basis of spectral data and comparison with their authentic samples (for **3a** and **3b**).[24-25]
The reaction of cyclobutanone with various aryl aldehydes such as 4-Cl-, 4-F-, 4-NO_2- and 2,5-diCl-benzaldydes gave the polymeric products.



1 **2a-c** **3a-c**

Ar = a) 4-CH_3Ph **b)** 4-CH_3OPh **c**) 4-BrPh

Figure 2. Synthesis of compounds **3a-c**

The bis-benzylidinecyclobutanones (**3a-c**) were reacted[26] with 2-aminobenzothiol in the presence of catalytic amount of *p*-TsOH in ethanol at reflux temperature for 5 hours. The TLC and NMR studies are seen that the two products were occurred approximately a ratio of 2:1 (Figure 3). The products were separated on a silica gel column eluting with hexane/ethyl acetate (9:1). After the separation, the structures of compounds were determined by NMR studies and elemental analysis. It was seen that isolated products were thiazole derivatives **5a,b** and **6a,b** instead of expected thiazepine derivatives **7a,b**. The products derived from **5c** and **6c** could not be isolated as adequately pure.

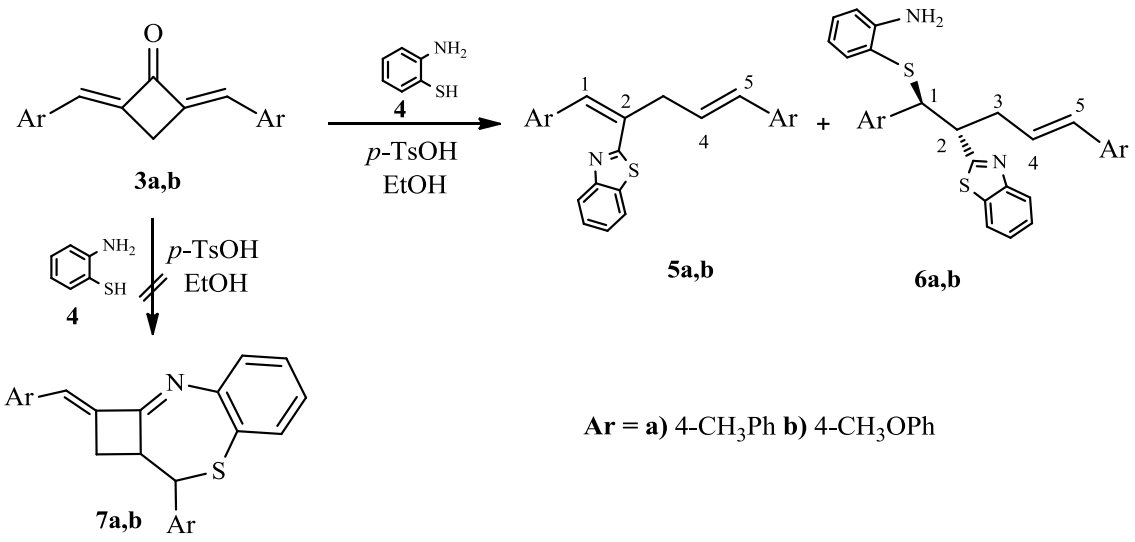


Figure 3. Synthesis of benzothiazole derivatives **5a,b** and **6a,b**

The structure of isolated compounds **5a,b** and **6a,b** were determined on the basis of spectral data. In the ^{1}H NMR spectrum of compound **5a**, the olefinic ptoton H1 resonates as singlet at 7.72 ppm, and the other olefinic protons HC(4) and HC(5) gave a AB system. A part of AB system was shown as a doublet at 6.55 ppm (J = 16.0 Hz), and B part was a triplets of doublet at δ 6.50 (J = 16.0, 8.8 Hz). The coupling constant (J = 16.0 Hz) indicates the *trans* orientation of double bond. Addition, the signals at 8.03 (d, J = 8.0 Hz), 7.87 (d, J = 8.0 Hz), 7.49 (t, J = 7.2 Hz) and 7.38 (t, J = 7.2 Hz) ppm are characteristic for aromatic hydrogens of benzothiazole ring. The methylene protons of $H_2C(3)$ resonate at 3.86 ppm as doublet (J = 4.8 Hz) as expected. Furthermore, a 19 line in ^{13}C NMR spectrum of **5a** are in good agreement with the proposed structures.

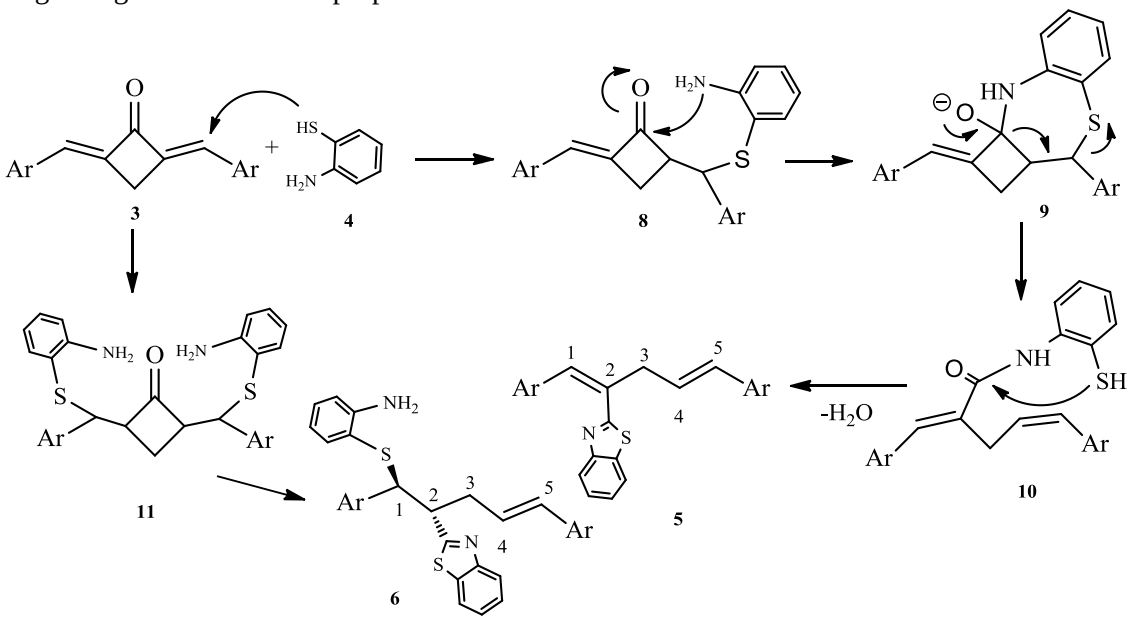


Figure 4. Proposed formation mechanism of benzothiazole derivatives **5a,b** and **6a,b**

In the ^{1}H NMR spectrum of compound **6a**, disappearing the singlet at 7.72 ppm in the spectrum of **5a** and appearing 4 new signal groups in aromatic region, 2 signal groups in aliphatic region and a broad singlet at 4.18 ppm (-NH_2) clearly indicated that the 2-aminothiophenol moiety was connected to the structure. Moreover, the olefinic protons HC(4) and HC(5) gave a AB system, A part of system was shown as a doublet at 6.11 ppm (J = 15.6 Hz), and B part was a triplets of doublet at 5.92 ppm (J = 15.6, 7.2 Hz). The coupling constant (J = 15.6 Hz) indicates the trans orientation of double bond. Aliphatic proton HC(1) resonates as doublet at 4.49 ppm with coupling constant (J = 11.2 Hz) and

adjacent proton HC(2) gave the doublet of triplet at 3.87 ppm with coupling constants (J = 11.2, 6.8 Hz) which indicate HC(1) and HC(2) are neighbored. Addition, 28 signals observed in the ^{13}C NMR spectrum of **6a** confirmed the proposed structures.

The formation of compounds **5** and **6** can be explained as shown Scheme 3. First, thia-Michael additions of 2-aminothiophenol (4) to 3 gives adduct **8**. Adduct **8** converts to intermediate **9** by attack of amino group to carbonyl carbon atom, and following ring opening give compound **10**. Finally, compound **5** was formed by cyclization which occur attack of sulphur group to carbonyl carbon atom and losing of H_2O. Compound **6** was formed by double thia-Michael addition of 2-aminothiophenol to **3** and rearrangement or may be thia-Michael addition 2-aminothiophenol to compound **5** in the reaction medium.

3.2. Biological assay

The compounds **5a** and **5b** were tested for their potential growth inhibitory activity against human uterus carcinoma (HeLa) and rat brain tumor cells (C6) cell line using proliferation BrdU ELISA assay. The tests were performed at 50, 75 and 100 μM concentrations and 5-Fluorouracil (5-FU) was used as standard. The antiproliferative activities of compounds were shown to increase of the activities depending to dose increasing. The results are given in Table 1 and Table 2. According to results, compounds **5a, b** showed activity against both cell lines at high concentration and they were better active against C6 than HeLa cell lines. Addition compound **5b** higher active than compound **5a** against both cell lines. This is attributed to the presence of methoxide group.

Table 1. The antitumor activities of compounds **5a** and **5b**

	HeLa cell			**C6 cell**		
Con. (mM)	**5a**	**5b**	**5-FU**	**5a**	**5b**	**5-FU**
100	66.81±0.86	74.21±0.10	90.71±0.45	77.37±0.37	79.60±0.37	86.11±1.01
75	25.77±1.21	17.30±0.18	88.75±0.78	54.37±1.28	69.63±1.18	85.22±1.99
50	14.69±0.93	7.60±0.08	87.22±1.26	41.86±0.18	35.00±0.22	81.94±0.00

Addition, the IC_{50} and IC_{75} values of tested compounds against HeLa and C6 cells were determined using ED50 plus v1.0 and given at Table 2.

Table 2. IC_{50} and IC_{75} **values** of **5a and 6a**

	HeLa cell		C6 **cell**l	
	IC_{50}	**IC_{75}**	**IC_{50}**	**IC_{75}**
5a	72.74	88.76	47.99	75.22
5b	78.05	91.98	50.77	73.08

4. Conclusion

Novel 2-(1,5-bis(aryl)penta-1,4-dien-2-yl)benzo[d]thiazol (**5a,b**) and 2-(((1S,2S,E)-2-(benzo[d]thiazol-2-yl)-1,5-di-arylpent-4-en-1-yl)thio)aniline (**6a.b**) derivatives were obtained by addition of 2-aminobenzothiol to bis-benzylidinecyclobutanones (**3a-b**). The structures of obtained compounds were characterized using the spectroscopic methods (NMR, IR, Elemental analysis). The compounds **5a** and **5b** were tested for their potential growth inhibitory activity against human uterus carcinoma (HeLa) and rat brain tumor cells (C6) cell lines using proliferation BrdU ELISA assay. According to results, compounds **5a, b** showed activity against both cell lines at high concentration and they were better active against C6 than HeLa cell lines. Addition compound **5b** higher active than compound **5a** against both cell lines. This may be attributed to the presence of methoxide group.

Acknowledgements

The authors are indebted to the TUBITAK (Scientific and Technological Council of Turkey) (Project No: 111T111) for their financial supports.

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