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Synthesis, Characterization and Biological Activities of Novel Chiral Bis 1,2-diolcarbothioamides

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ABSTRACT

2,2'-[(2R,3R)-2,3-dihydroxy-1,4-dioxobutane-1,4-diyl]bis(N-aryl-alkylhydrazine-1-carbothioamide (T1–5) were obtained by the interaction of (2R,3R)-2,3-dihydroxybutanedihydrazide (1) with five different isothiocyanate alkyl(aryl) derivatives. The structures of the final compounds were confirmed by elemental analyses, FT-IR, ^{1}H-NMR and ^{13}C-NMR. The antioxidant, antimicrobial and anticancer properties of the synthesized compounds were also investigated. Three of the triazole derivatives with p-tolyl, benzyl and phenyl substituents (T3–5) displayed good antioxidant and anticancer activity in comparison to the standards.

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1. Introduction

Thioamides exhibit wide range of biological properties such as antifungal [1], antioxidant [2,3] and anticonvulsant [4] activities. Some of them strongly inhibit phosphoglycerate dehydrogenase [5]. Furthermore they also have found wide applications as inter-mediates in organic synthesis for the preparation of heterocycles and many valuable organic building-blocks [6-9]. The chemical properties of thioamides are considered in a review from Jagodzinski [6] in 2003 and by Dyachenko et al., [7] in 2018. At the same time these reactions represent new and effective methods for the synthesis of various heterocyclic compounds, new types of amidines and their vinylogs, enamines, exhibiting various types of biological activity [10,11], and can be used in synthetic organic chemistry as valuable chemical reagents [8,9].

Optically pure 1,2-diols represent valuable building blocks for the production of pharmaceuticals, chemical catalysts, and agrochemicals [12]. Enantiomerically pure 1,2-diols are valuable intermediates in the organic synthesis of biologically active compounds and natural products [13]. They are readily transformed into chiral epoxides [14], aziridines, and amino alcohols [15]. Moreover, the 1,2-diol functionality is found in a number of synthetic [16] and pharmaceutical intermediates [17]. Compounds that have optical activity can change to another compound that has optical activity without breaking of covalent bonds, which is connected with asymmetric carbon atoms. In this change, the configuration stays unchanged. So, our synthesized compound configuration is the same as that of our starting compound.

Natural chiral compounds (from the chiral pool) often offer an alternative to the synthesis of enantiomerically pure products. (*2R,3R*)-2,3-dihydroxybutanedihydrazide **(1)** is one chiral carboxylic acidhydrazide isolated from natural sources. In this study, we aimed to synthesize a new 2,2'-[(*2R,3R*)-2,3-dihydroxy-1,4-dioxobutane-1,4-diyl]bis(*N*-aryl-alkylhydrazine-1-carbothioamide (T1-5) by using (*2R,3R*)-2,3-dihydroxybutanedihydrazide **(1)** as starting compound.

In the present paper, we have investigated the preparation and characterization of various carbothioamide (T1-5) derivatives of novel chiral compounds. In light of the studies conducted on the synthetic chemistry of

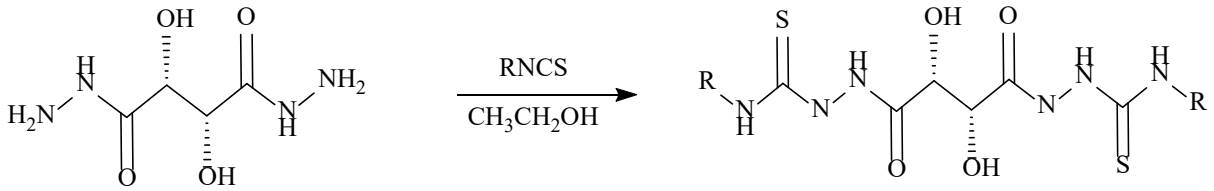
carbothioamide during last 20 years, we have also partly contributed to this progress by developing a new chiral carbothioamide synthesis.

2. Materials and Methods

Melting points were determined on a Thomas Hoover melting point apparatus and use uncorrected, but checked by differential scanning calorimeter (DSC). Specific rotations were recorded on a POLS-1 high-sensitivity polarimeter, with a fixed sodium lamp of wavelength 589 nm. The IR spectra were measured with Perkin–Elmer Spectrum One FTIR spectrophotometer. The ^{1}H, ^{13}C, spectra were taken on Bruker AC-400 NMR spectrometer operating at 400 MHz for ^{1}H, 100 MHz for ^{13}C. Compounds were dissolved in DMSO-d_6 and chemical shifts were referenced TMS, (^{1}H and ^{13}C NMR). Elemental analyses were performed on a LECO-CHNS-938. Starting chemicals were obtained from Merck or Aldrich.

2.1. General procedure for the synthesis of Chiral Bis Carbothioamides (T1-5)

To the reaction flask was added 10 mmol (2*R*,3*R*)-2,3-dihydroxybutanedihydrazide **(1)** and 50 mL of absolute ethyl alcohol. After the reflux process began, 20 mmol of five different isothiocyanate alkyl(aryl) derivatives was added. After about 4 hours, solid 2,2'-[(2*R*,3*R*)-2,3-dihydroxy-1,4-dioxobutane-1,4-diyl]bis(*N*-aryl-alkylhydrazine-1-carbothioamide (T1-5) began to form in the reaction flask. The solid formed was filtered off and dried (the reactions are shown in Scheme 1).



(1) (T1-5)

R:1) phenyl 2) p-tolyl 3) methoxyphenyl 4) allyl 5) ethyl

Scheme 1. Synthesis reaction of (T) compounds.

2.2. Cell culture and cell viability assay

Cell lines were purchased from the American Type Culture Collection (ATCC, Manassas, VA, USA). Human colon adenocarcinoma cell line HT29 cells were maintained in Dulbecco's modified Eagle's medium (DMEM, Invitrogen) supplemented with 10% FBS, 2 mM L-glutamine, 100 U/mL penicillin, and 100 µg/mL streptomycin and kept in a humidified atmosphere at 37°C incubator with 5% CO_2 in air. To determine the cell viability cells were plated onto 96-well plates ($1x10^4$ cells/well). The cells were treated with different concentrations (0 µM, 0.1 µM, 1 µM, 10 µM, 100 µM, 1000 µM) of synthesized compound derivatives and incubated for 24 h. After the incubation cells washed with PBS and added to 100 µL DMEM. 10 µL of the MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) (Vybrant, Invitrogen) labeling reagent was added to each well and incubated for 4h in humidified atmosphere at 37 °C incubator with 5% CO_2 in air. After the incubation 100 µL of the SDS buffer was added into each well for solubilization of formazan precipitate. Then absorbance was measured by microplate reader at 570 nm and was carried out in triplicate of each assay.

2.3. Cell survival assays

In order to investigate the effects of compounds on cell survival of HT29 cells, we treated cells with selected compound at a final concentration of 10 µM in growth medium (without antibiotics) for 24h. After the incubation, methylene blue staining was used for cell survivals. The cells were extracted with 1% SDS in PBS solution and stained with methylene blue solutions. The absorbance was measured at 600 nm with microplate reader.

2.4. Measurement of Caspase-3 Activity

HT29 cells were incubated with 10 µM concentrations of compound at 24 h in 12 well plates. After the incubations, cells were harvested with cell lysis buffer. Caspase-3 activity assay was performed with caspase-3 activity assay kit according to the manufacturer's instructions (Invitrogen).

2.5. Trans-well migration assay

The migration assay, which was used to screen the active compounds from the target products was performed using a 24-well cell culture plate with 8.0 µm pore size membrane inserts without matrigel. Briefly, the lower chamber was filled with 800 µL DMEM containing 10% FBS as a chemoattractant. A total of $1x10^4$ HT29 cells in 200 µL serum-free DMEM were seeded in the upper well and were respectively incubated with 10 µM compounds for 24 h at 37˚C. After 24 h under normoxic conditions, the cells that had migrated were stained with 1% crystal violet. Images were captured using an inverted microscope. The number of migratory cells was then counted and analyzed to determine statistically significant differences. Migrated cells were fixed with methanol and stained.

2.6. 8-hydroxy-2′-deoxyguanosine (8-OHdG) assay

For the measurement of 8-OHdG levels, genomic DNA was extracted in cell lysates by using extraction kit (Invitrogen, USA), and the assays were performed using kits as stated in the manufacturer's instructions (Cell Biolabs, USA).

2.7. Antioxidant activity

The standard antioxidant and synthesized compounds (T1-5) were dissolved in DMSO (for HPLC Grade) at 5000 µM concentration.

2.7.1. $ABTS^{\bullet+}$ Radical Scavenging Activity

The spectrophotometric analysis of $ABTS^{\bullet+}$ radical scavenging capacity was determined according to the method of Re et al. [18] and product absorbance was recorded at 734 nm.

2.7.2. Hydroxyl ($OH^{\bullet}$) Radical Scavenging Activity

The new derivatives inhibitor capabilities over hydroxyl radical-mediated peroxidation were measured as suggested by Halliwell et al. [19]. The level of oxidation 2-deoxyribose underwent was determined by the absorbance of the solution at 532 nm wavelength.

2.7.3. DPPH Radical Scavenging Activity

The DPPH radical scavenging capacity of compounds was measured by 2,2-diphenyl-1-picrylhydrazyl using the method of Brand-Williams et al. [20]. The absorbance of the mixture was measured at 517 nm in a spectrophotometer.

All antiradical tests were repeated three times and the average values were calculated. The radical scavenging activity percentages (RSA%) for each sample were calculated by the following equation [21-24]:

$RSA\% = [(A_0 - A_1)/A_0] \times 100$

A_0: control absorbance; A_1: sample absorbance.

2.8. Antimicrobial activity

For the determination of the antimicrobial activity of derivatives, the *Staphylococcus aureus* ATCC25923, *Klebsiella pneumoniae* ATCC700603, *Escherichia coli* ATCC25322, *Bacillus megaterium* DSM32 and *Candida albicans* FMC17 strains were used in the study. The microorganisms were taken from the culture collection of the Microbiology Laboratory of Firat University. Prepared bacteria, yeast and fungi in the broth culture (10^6 bacteria/mL, 10^4 fungus/mL), and the inoculated plates were incubated at 37±0.1 °C for 24 hours and the yeasts and dermophytes plates were incubated at 25±0.1 °C for 72 hours. Standard discs were used for control (Streptomycin sulfate 10 mg/disk, Nystatin 30 mg/disk). The end of the incubation time, the inhibition zones were evaluated formed on the medium as mm [25].

3. Results and Discussion

3.1. Chemistry

2,2'-[(2*R*,3*R*)-2,3-dihydroxy-1,4-dioxobutane-1,4-diyl]bis(*N*-phenylhydrazine-1-carbothioamide (T1)

Yield: 75%. M.P.: 213–214 °C. IR (KBr) νmax (cm^{-1}): 3352–3236 (hydrogen bonded OH, NH), 3072–3107 (Ar-H), 2870–2914 (C-H), 1677 (NH-C=O), 1267 (C=S); NMR (400 MHz) (DMSO-d6) δ (ppm): 4.43 (d, 2H, 2xCH, J = 4.81 Hz), 6.22 (br, 2H, 2xOH), 7.16 (t, 2H, 2xCH, Ar-H, J = 7.31 Hz), 7.33 (t, 4H, Ar-H, J = 7.67 Hz), 7.57 (d, 4H, Ar-H, J = 7.57 Hz), 9.12 (s, 2H, 2xNH-NH-C=O), 9.83 (s, 2xNH-NH-C=O), 10.27 (s, 2H, 2xNH-C=S); 13C NMR (400 MHz, DMSO-d6) δ (ppm): 73.38, 125.13, 126.28, 128.61, 139.33, 171.67, 180.16. Elemental analysis for $C_{18}H_{20}N_6O_4S_2$ (448): Calculated (%): C: 48.20; H: 4.49; N: 18.74; S: 14.30. Found (%): C: 48.11; H: 4.39; N: 18.69; S: 14.33. [α]D20 -8.462° (*c* 1, ethanol).

2,2'-[(2*R*,3*R*)-2,3-dihydroxy-1,4-dioxobutane-1,4-diyl]bis(*N*-p-tolylhydrazine-1-carbothioamide (T2)

Yield: 70%. M.P.: 221–222 °C. IR (KBr) νmax (cm−1): 3394–3400 (hydrogen bonded OH, NH), 3070–3113 (Ar-H), 2925–2960 (C-H), 1645 (NH-C=O), 1282 (C=S); NMR (400 MHz) (DMSO-d6) δ (ppm): 2.29 (s, 6H, 2xAr-CH3), 4.43 (d, 2H, 2xCH, J = 5.21), 6.19 (br, 2H, 2xOH), 7.13 (d, 4H, Ar-H, J = 8.30 Hz), 7.42 (d, 4H, Ar-H, J = 8.20 Hz), 9.07 (s, 2H, 2xNH-NH-C=O), 9.76 (s, 2xNH-NH-C=O), 10.23 (s, 2H, 2xNH-C=S); 13C NMR (400 MHz, DMSO-d6) δ (ppm): 21.02, 73.41, 123.19, 129.05, 134.55, 136.76, 171.50, 180.26. Elemental analysis for $C_{20}H_{24}N_6O_4S_2$ (476): Calculated (%): C: 50.40; H: 5.08; N: 17.63; S: 13.46. Found (%):C: 50.33; H: 4.99; N: 17.21; S: 13.44. [α]D20 -11.364° (*c* 1, ethanol).

2,2'-[(2*R*,3*R*)-2,3-dihydroxy-1,4-dioxobutane-1,4-diyl]bis(*N*-*p*-methoxyphenylhydrazine-1-carbothioamide (T3)

Yield: 68%. M.P.: 229–230 °C. IR (KBr) νmax (cm^{-1}): 3255–3319 (hydrogen bonded OH, NH), 3130–3160 (Ar-H), 2930–2958 (C-H), 1660 (NH-C=O), 1279 (C=S); NMR (400 MHz) (DMSO-d_6) δ (ppm): 3.75 (s, 6H, 2xAr-OCH_3), 4.43 (d, 2H, 2xCH, *J* = 5.21 Hz), 6.16 (br, 2H, 2xOH), 6,90 (d, 4H, Ar-H, *J* = 8.93 Hz), 7.38 (d, 4H, Ar-H, *J* = 8.88 Hz), 9.06 (s, 2H, 2xNH-NH-C=O), 9.72 (s, 2xNH-NH-C=O), 10.21 (s, 2H, 2xNH-C=S); ^{13}C NMR (400 MHz, DMSO-d_6) δ (ppm): 55.68, 73.39, 113.75, 127.00, 132.16, 157.10, 171.53, 180.55. Elemental analysis for $C_{20}H_{24}N_6O_6S_2$ (508): Calculated (%):C: 47.23; H: 4.76; N: 16.52; S: 12.61. Found (%):C: 47.11; H: 4.56; N: 16.33; S: 12.59. $[\alpha]_D^{20}$ -17.201° (*c* 1, ethanol).

2,2'-[(2R,3R)-2,3-dihydroxy-1,4-dioxobutane-1,4-diyl]bis(N-allylhydrazine-1-carbothioamide (T4)

Yield: 69%. M.P.: 182–186 °C. IR (KBr) νmax (cm^{-1}): 3205–3285 (hydrogen bonded OH, NH), 2842–2893 (C-H), 1647 (NH-C=O), 1255 (C=S); NMR (400 MHz) (DMSO-d_6) δ (ppm): 4.06 (dt, 2H, 2xH_1, *J* = 16 Hz, 5.42 Hz), 4.15 (dt, 2H, 2xH_2, *J* = 16 Hz, 5.42 Hz), 4.37 (d, 2H, 2xCH-OH, *J* = 6.4 Hz), 5.06 (dd, 2H, 2xH_5-cis, *J* = 10 Hz, 1.5 Hz), 5.16 (dd, 2H, 2xH_4-trans, *J* = 16 Hz, 1.5 Hz), 5.82 (m, 2H, 2xH_3), 5.93 (br, 2H, 2xOH), 7.62 (s, 2H, 2xNH-NH-C=O), 9.44 (s, 2xNH-NH-C=O), 10.01 (s, 2H, 2xNH-C=S); ^{13}C NMR (400 MHz, DMSO-d_6) δ (ppm): 46.32, 73.34, 116.02, 135.02, 171.42, 181.57. Elemental analysis for $C_{12}H_{20}N_6O_4S_2$ (376): Calculated (%): C: 38.29; H:

5.35; N: 22.32; S: 17.04. Found (%):C: 38.31; H: 5.33; N: 22.29; S: 16.99. $[\alpha]_D^{20}$ -7.341° (*c* 1, ethanol).

2,2'-[(2*R*,3*R*)-2,3-dihydroxy-1,4-dioxobutane-1,4-diyl]bis(*N*-ethylhydrazine-1-carbothioamide (T5)

Yield: 71%. M.P.: 221–222 °C. IR (KBr) νmax (cm^{-1}): 3253 (hydrogen bonded OH, NH), 2943–2977 (C-H), 1679 (NH-C=O), 1278 (C=S); NMR (400 MHz) (DMSO-d_6) δ (ppm): 1.07 (t, 6H, 2xCH_2-CH_3, *J* = 7.17 Hz), 3.39-3.46 (m, 4H, 2xCH_2-CH_3), 4.34 (d, 2H, 2xCH, *J* = 5.10 Hz), 6.06 (br, 2H, 2xOH), 7.42 (s, 2H, 2xNH-NH-C=O), 9.32 (s, 2xNH-NH-C=O), 9.95 (s, 2H, 2xNH-C=S); ^{13}C NMR (400 MHz, DMSO-d_6) δ (ppm): 14.73, 39.03, 73.42, 171.26, 181.03. Elemental analysis for $C_{10}H_{20}N_6O_4S_2$ (352): Calculated (%):C: 34.08; H: 5.72; N: 23.85; S: 18.26. Found (%): C: 33.99; H: 5.51; N: 23.92; S: 18.12. $[\alpha]_D^{20}$ -6.921° (*c* 1, ethanol).

The new derivatives were prepared following the reaction sequences depicted in Scheme 1. New compounds were prepared in yields ranging from 68 to 75% by the from available (2R,3R)-2,3-dihydroxybutanedihydrazide **(1)** and with five different isothiocyanate alkyl(aryl) derivatives.

The IR spectra of the 2,2'-[(2*R*,3*R*)-2,3-dihydroxy-1,4-dioxobutane-1,4-diyl]bis(*N*-aryl-alkylhydrazine-1-carbothioamide derivatives (T1-5) have hydrogen bonded OH, NH bands at 3400-3205 cm^{-1}, Ar-H stretching bands at 3160-3070 cm^{-1}, NH-C=O bands at 1679-1645 cm^{-1} and C=S stretching bands at 1282-1255 cm^{-1}. The 2,2'-[(2R,3R)-2,3-dihydroxy-1,4-dioxobutane-1,4-diyl]bis(*N*-aryl-alkylhydrazine-1-carbothioamide T1-5 were charcterized by the presence of the NH-C=O, NH-NH, NH-C=S protons at 7.42-10.27 ppm. The IR, ^{1}H-NMR spectra of all synthesized compounds were consistent with the reported values for similar structures. The evaluated ^{13}C-NMR spectra also support the proposed structures.

Bis Carbothioamides are suitable for the production of varied and numerous materials. In the light of these truths, this effort is an extension of study in the using hydrazides in the synthesis of different chiral 1,2-diol carbothioamides compounds as reported. In this article, we would like to report the preparation of some bis chiral 1,2-diol carbothioamides. Thus, hydrazide namely is (2*R*,3*R*)-2,3-dihydroxybutanedihydrazide **(1)** were reacted with five different isothiocyanate alkyl(aryl) derivatives in absolute ethyl alcohol under reflux until exclusion of water to yield the final product chiral bis carbothioamides T1-5. The formation of the final compounds starts by nucleophilic attack of hydrazide followed by thiol-thion equilibrium to give the final products T1-5 as depicted in Scheme 1. The low-cost and simple reaction conditions, commercial obtainability of the substance, and good yields of the products make this method valuable from a preparative point of view. The data of all the compounds are given in the Experimental section.

3.2. Biological Activity

3.2.1. *In vitro* Cytotoxicity

Determination of Cytotoxicity of Expressed Compounds in HT29 Cells

Colon cancer cell HT29 was allowed to incubate for 24 hours by dosing the synthesized compounds at varying concentrations (10-1000 µM). IC_{50} values in cells were calculated in Graphpad program and concentrations of cell deaths were determined as 50%. At these concentrations, the upper limit accepted by the NCBI cancer event was 10 µM. Compounds having an IC_{50} value of 10 µM concentration were selected to investigate the potential effects of anti-cancer. According to our results, anti-cancer studies were continued with this compound due to low IC_{50} values of T4 (7.77 µM) compound.

As seen in Table 1 in cytotoxicity studies, T4 compound has effective anticancer activity on human colon cancer cell HT29. According to Figure 1 and Figure 2, T4 compounds stopped cell growth and their effects on cells increased over time. Considering the migration inhibition levels of the synthesized compound T4, it can be considered as an indicator that they can be effective in advanced tumors as well as their anticancer properties. Experiments have shown that the compound has the potential to cause DNA damage and are effective on cell death. Again, when the Caspase-3 activities of the compounds are examined, it is seen in Figure 4 that the T4 compound significantly increase the caspase-3 levels.

When investigated on HT29 colon cancer cells whether the compounds cause DNA damage on cells and whether they go to apoptosis mechanism with this damage, it is seen that T4 compound significantly increase the levels of 8-OHdG, which is an important marker for DNA damage in the cell, after 24 hours of incubation at 10 µM concentration (Figure 5).

After it was understood that the 2-aminothiadiazole (ATDA) compound had anticancer activity, the synthesis of 1,3,4-thiadiazole compounds increased and it was reported that these new derivatives had similar effects [26-28].

Table 1. IC_{50} levels of compound T4 in HT29 cells

Compounds	**HT29, IC_{50} (µM)**
T1	162.14
T2	13.42
T3	290.25
T4	7.77
T5	12.24

Time-dependent Cell Growth of Synthesized Compounds in HT29 Cells

HT29 colon cancer cells were treated with T4 compound and were incubated in 10 µM concentration for followed by morphological changes. When cell growths were examined morphologically at 24 th hour, it was found that cell-cell interaction decreased and morphologically differed from control cells as seen in T4 (Figure 1)

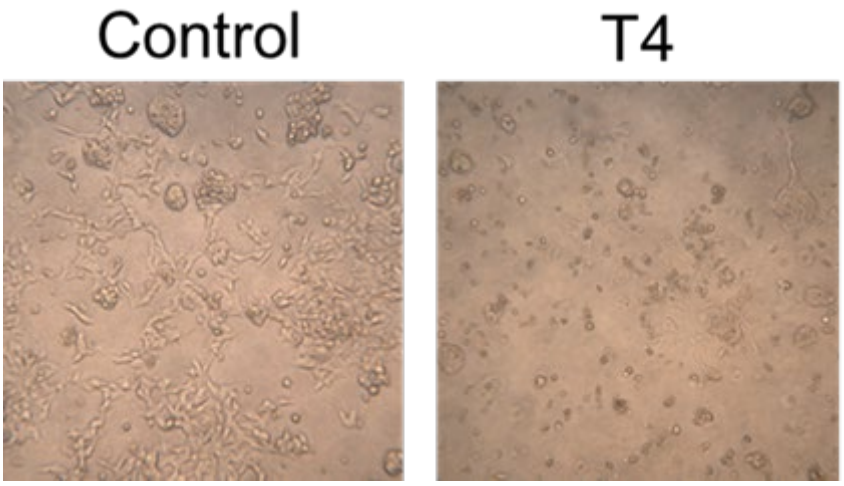


Figure 1. Time-dependent Cell Growth

HT29 cells treated with T4 compound were incubated in 10 µM concentration and cell growth was monitored. When cell growth is compared to the control, it is seen that the cell growth of T4 (+ $p < 0.05$) compound decreases at 12 h. When the 24 h cell growth was compared to the control, it was seen that the cell growth of T4 (+ $p < 0.05$) compound decreased statistically. T4 compound stopped cell growth over time and its effect on cells increased with time (see Figure 2).

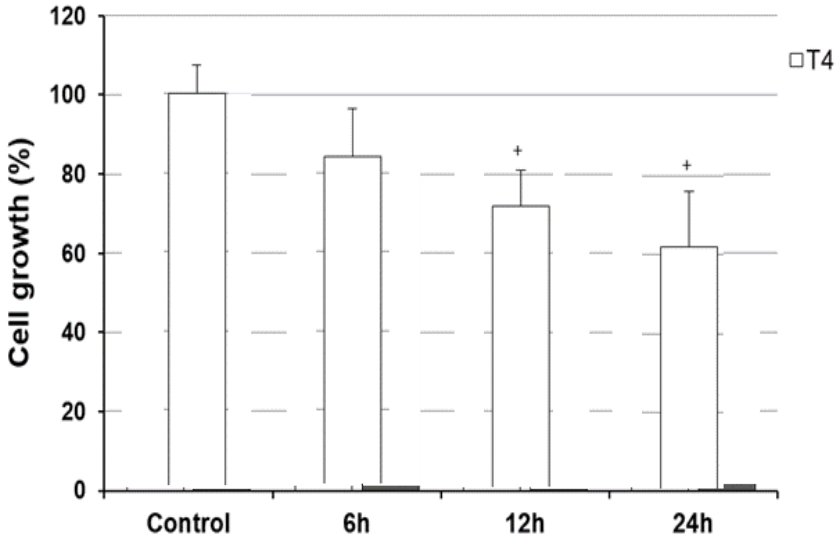


Figure 2. Graph of cell growth of compound T4 in HT29 cells

Trans-well Migration Test

T4 compound on HT29 colon cancer cells was incubated at 10 µM concentration for 24 hours and its effect on cell migration was investigated. In migration studies, cells with high metastatic ability try to go to other places by passing through plates containing special membranes. Trans-well migration assays in cancer are considered a model of metastasis. The more cells visible in the migration study, the greater the migration sees Figure 3. In other words, the cells settle so much in the tissue that they initiate cancer. When the migration results were examined, it was observed that the cells on the membrane were highly stained in the control experimental group, and the migration of the cells decreased with the addition of T4 to the medium.

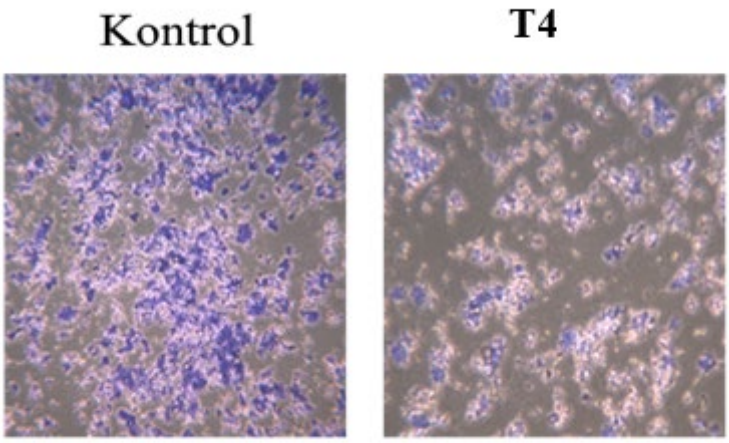


Figure 3. Trans-well Migration test result

Caspase-3 activity of the synthesized compounds in HT29 cells

T4 compound was incubated on colon cancer cells at a concentration of 10 µM for 24 hours to measure caspase-3 levels, an important marker for apoptosis in the cell (Figure 4). When Caspase-3 activitiy of compound was examined, it was seen that T4 compound increased caspase-3 levels significantly. (+++ $p < 0.001$)

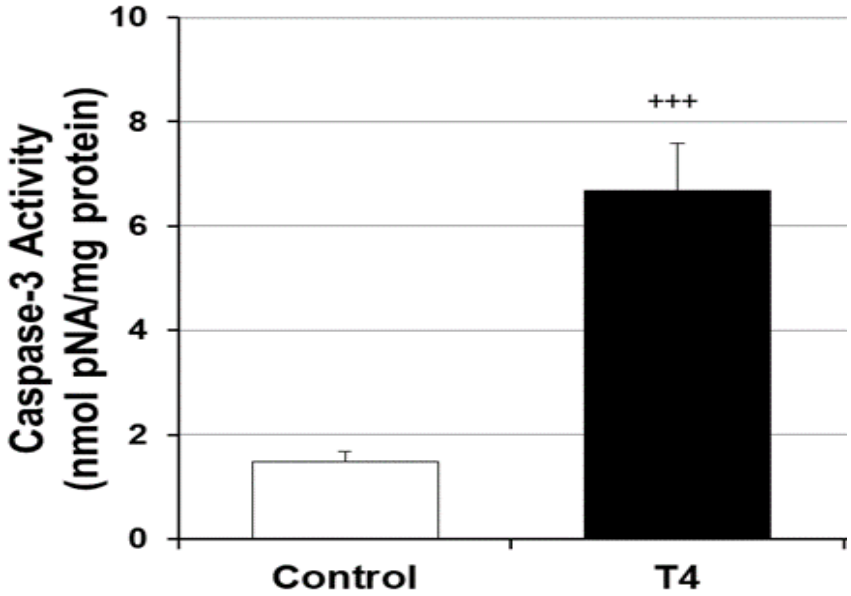


Figure 4. Caspase-3 activity of T4

8-OHdG Levels in HT29 Cells

It has been investigated whether drugs cause DNA damage on cells and whether they go to apoptosis mechanism with this damage. T4 compound was incubated on HT29 colon cancer cells at a concentration of 10 µM for 24 hours, and the levels of 8-OHdG, which is an important marker for DNA damage, were measured in the cell. When 8-OHdG activities of the compound are examined, it is seen that T4 ($p<0.01$) increases 8-OHdG levels significantly. Experiments have shown that the compound has the potential to cause DNA damage and is effective on cell death see Figure 5.

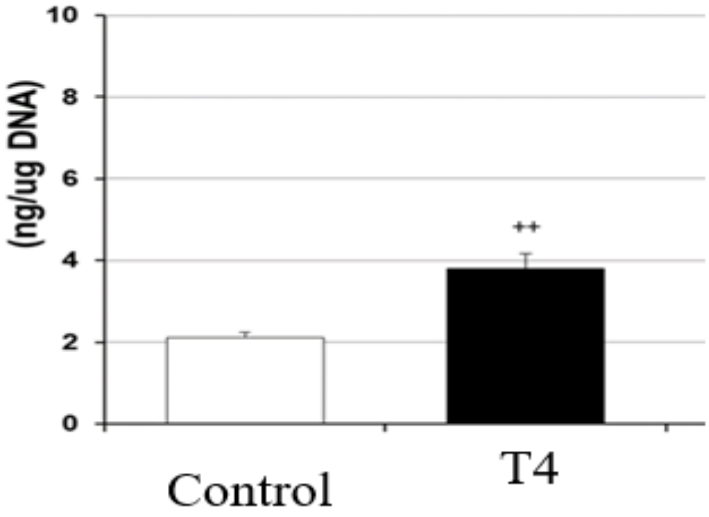


Figure 5. 8-OHdG Levels in HT29 Cells

Antimicrobial Activity Findings

As can be seen from Table 2, the substances have different antimicrobial activities. MIC values of T1 for *Staphylococcus aureus*, *Bacillus subtilis*, *Klebsiella pneumoniae* and *Escherichia coli* were calculated as 0.125, 0.0625, 0.125, 0.0625 mg/mL, respectively, but did not show antimicrobial activity against *C. albicans*. MIC values of T2 for *Staphylococcus aureus*, *Bacillus subtilis*, *Klebsiella pneumoniae* and *Escherichia coli* were calculated as 0.125, 0.0625, 0.250, 0.15625 mg/mL, respectively. It was determined that it did not show any antimicrobial effect against *C. albicans*. MIC values of T3 for *Staphylococcus aureus*, *Bacillus subtilis*, *Klebsiella pneumoniae* and *Escherichia coli* were calculated as 0.0625, 0.03125, 0.125, 0.0625 mg/mL, respectively. It was determined that it did not show any antimicrobial effect against *C. albicans*. The MIC values of T4 for *Bacillus subtilis* and *Klebsiella pneumoniae* were calculated as 0.25 and 0.0625 mg/mL, respectively. It was determined that it did not show any antimicrobial effect against *Staphylococcus aureus*, *Escherichia coli* and *C. albicans*. MIC values of T5 for *Bacillus subtilis*, *Klebsiella pneumoniae* and *Escherichia coli* were calculated as 0.0625, 0.0625, 0.015625 mg/mL, respectively. It was determined that it did not show antimicrobial effect against *Staphylococcus aureus* and *C. albicans*.

The antimicrobial activity of the substances was investigated using *Staphylococcus aureus* ATCC25923, *Klebsiella pneumoniae* ATCC700603, *Escherichia coli* ATCC25322, *Bacillus subtilis* DSM32 and *Candida albicans* FMC17 strains. We can say that MIC values of chemicals are effective on different microorganisms in the range of 0.015625 and 0.0625 mg/mL (Table 2). According to the results obtained, T1 and T2 *Bacillus subtilis* and *Escherichia coli*; T3 against *Staphylococcus aureus*, *Bacillus subtilis* and *Escherichia coli*; against T4 *Klebsiella pneumoniae*; It showed high antimicrobial activity against T5 *Bacillus subtilis*, *Klebsiella pneumoniae* and *Escherichia coli*. Some heterocyclic 4,6-disubstituted-1,2,4-triazole-1,3,4-thiadiazole derivatives have been synthesized and tested *in vitro*. It has been stated that the compounds with intense aromatic group show more effective antibacterial activity [29,30].

Table 2. Antifungal and antimicrobial activity results of test compounds

	S.	***B.***	***K.***	***C.***	***E. coli***
T1	0.125	0.0625	0.125	-	0.0625
T2	0.125	0.0625	0.25	-	0.015625
T3	0.0625	0.03125	0.125	-	0.0625
T4	-	0.25	0.0625	-	-
T5	-	0.0625	0.0625	-	0.015625

Antiradical Activity Results of Compounds

The antiradical activity results of the T series compounds are shown in Table 3. Accordingly, in the $ABTS^{+\bullet}$ radical scavenging test of 1,2-diol and thiadiazole compounds that we code as T1- T5, compounds coded T1, T2, T3, T4, T5 showed higher antiradical activity than the standard antioxidant BHT. In the $OH^{\bullet}$ radical scavenging test, T2 coded compound showed higher antiradical activity than the standard antioxidant BHT. In the $DPPH^{\bullet}$ radical scavenging test, T1, T2, T3 and T5 coded compounds showed higher antiradical activity than the standard antioxidant BHT. Compared with the standard antioxidant BHT, it can be said that especially T2 compound has good antiradical activity. In addition, in another study, the antioxidant properties of these substances were tested on *Saccharomyces cerevisiae* cells and it was determined that the T2 compound decreased the MDA level and increased the vitamin E level in these cells [31]. All literature research has shown that structures containing Carbothioamide nuclei have remarkable effects and therefore have been studied extensively.

Table 3. The $ABTS^{+\bullet}$, $OH^{\bullet}$, $DPPH^{\bullet}$ radical scavenging activity of T series compounds

Compounds (500 µM)	**$ABTS^{+\bullet}$ Scavenging (%)**	**$OH^{\bullet}$ Scavenging (%)**	**$DPPH^{\bullet}$ Scavenging (%)**
T1	99.82	56.85	94.48
T2	99.40	99.36	94.36
T3	99.52	36.27	93.60
T4	99.52	53.38	85.10
T5	99.70	10.55	93.72
BHT	93.25	91.87	93.54

The production of unnatural compounds with antioxidant properties and their effects on biological systems, studies with exogenous and endogenous antioxidants that do not adversely affect living things, delay or prevent the degradation of biomolecules, are increasing day by day and are becoming a great area of interest every day [32,33]. The oxidative stress levels faced by living things increase, especially as a result of the stagnant lifestyle of people, the decrease in natural habitats, the genetic modification of food and beverages, the decrease in consumption of natural foods, the increase in reinforced concrete structures and constant exposure to stress. Recent studies have aimed to reveal the effects of free radicals on the emergence of different and various diseases and the development of the disease. In this context; synthetic or natural antioxidant compounds under laboratory conditions and finding various methods to reduce oxidative stress are among the topics of interest.

Studies have shown that 1,2-diol-carbothioamide compounds have many biological properties such as anti-inflammatory, antihypertensive, antioxidant, antimicrobial, enzyme inhibitor, antituberculosis, anticancer and antidepressant. Because of these different and broad pharmacological properties, these compounds are widely used in medicine, pharmacy, organic chemistry, pharmaceutical chemistry and biochemistry.

4. Conclusion

In this study, new 1,2-diol derivatives were synthesized and analyzed for their biological properties. The obtained results from the investigated compounds exhibited that all compounds have *in vitro* antioxidant and antimicrobial effects. Also, only one compound (T4) exhibited strong anticancer properties *in vitro*. It can be said that such compounds would be useful in the selection and design of molecular models in further studies.

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