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Investigation of the Antioxidant and Antitumour Properties of Some Cyclobutane Ring Containing 2,5-Thiophene Diacyl Compounds

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Abstract: In this research, the effect of thiophene derivatives on the (MDA) concentration which is an indicator of lipid peroxidation, antioxidant vitamins A, E and in vitro antioxidant activity and antitumor activity, were investigated in cell culture media. In the comparison done among groups, it was observed that MDA and vitamin A concentrations were statistically ($P<0.05$) changed. Looking at the measured parameters during the experiment, the number of live cells, the compounds is an effective antitumor activity in the L 1210 cell types. The oxidative stress parameters are used for all compounds.

Keywords: HPLC; thiophene; cyclobutane; MDA; antioxidant activity; antitumour activity.

1. Introduction

Thiophene-derived compounds are interesting in chemistry, biology and pharmacology because of their various pharmacological activities. Complex compounds with metals have also been prepared and studied. It is stated that the structure and conformation of the molecule significantly alter the biological activities [1]. Such studies will help to understand the synthesis of more active compounds or the behavior of natural compounds. Such substances are intensively investigated as herpes simplex virus type 1 replication inhibitors in pharmacological chemistry as inhibitors of antimitotic, cysteine or serine proteases, drug-receptor analgesics and 5-HT antagonists [2].

Different substitutions of benzo [b] thiophenes have been used as estrogen receptor modulators and have been used to treat osteoporosis [3,4]. Benzo [b] Thiophene residues have been identified as an important center for biological activity. Zileuton is used as an anti-asthma medication [5], a potential inhibitor of 5-lipogenesis [6].

Ferreira *et al*. have identified and synthesized benzo [b] thiophene derivatives, analogous to raloxifene, as estrogen receptor modulators thought to reduce the risk of lung cancer in postmenopausal women. At the same time, the in vitro antitumor activity of these compounds was investigated. Some of these agents have been shown to exhibit good affinity for the estrogen receptor and exhibit useful antitumor properties [7]. Diaryl amine skeleton and Benzo [b] thiophene systems are of great interest as biologically active compounds [8].

Pinto *et al*. have investigated antifungal activities by preparing benzo [b] thiophene derivatives of di heteroaryl amines. They found that the presence of hydroxyl group in aryl derivatives is necessary for antifungal activity. It has also been observed that this effect increases in pyridine derivatives [9].

The synthesized new Schiff base derivatives with thiophene and antimicrobial properties have also been studied [10]. Romagnoli *et al*. Have studied the biological properties of thiophenes by preparing chalcone and 2 and 3 amino benzo [b] thiophene derivatives. All compounds were found to inhibit the development of cancer cells at micromolar concentrations [11,12]. The pyrazole-clubbed thiophene derivatives synthesized and evaluated for biological activity and found that they exhibit high anti-inflammatory activity [13]. Synthesized thiophene derivatives of urea analogs incorporating a cyclohepta[b]thiophene scaffold were synthesized and examined their antiproliferative activity on several human cancer cells. The compounds were observed to exhibit good activity [14]. Thiophene-benzothiazole dyad ligand and its Ag(I) complex examined their interaction with DNA and RNA. Some of these compounds have also been shown to exhibit good antibacterial activity simultaneously [15].

Piperizine thiophenes were synthesized, allosteric properties of the compounds that potentiate adenosine A1 receptors were investigated [16]. Thiophenes containing triarylmethane and determined that exhibited antituberculous properties at concentrations of 3.12 to 12.5 micrograms/milliliter [17]. The anti-amoebic activity of thiophene 2 carboxy aldehyde thiosemicarbazone with cyclooctadiene and Ru (II) complexes was determined [18]. Copper-2 complexes of thiosemicarbazone derivatives of thiophene-2-carboxy aldehyde tested their anti-amoebic properties and showed that some of these complexes are more effective than the commercial drug metronidazole [19].

The antileukemic activity of thiosemicarbazone derivatives of thiophene-2-carboxy aldehyde has been the subject of several studies [20]. Synthesized some new azomethine compounds containing thiophene and examined their antibacterial activity. They have found stronger activity [21]. Novel thiophene analogs of kenapullone and investigated their biological activity against lung cancer. Kenpaullon is an inhibitor that is used to inhibit the cyclin-dependent kinase (CDKs) enzyme, which plays an important role in cell division. These and similar compounds have been synthesized and used especially in anticancer studies [22]. Furthermore, antiviral [23], antineoplastic [24], antiproliferative [25] effects of derivatives of the cyclobutane ring with other biologically active compounds and the contribution of the cyclobutane group to the enrichment of these properties are being tried to be explained[8,26].

Cis-diamine-1,1-cyclobutane dicarboxylate platinum (II) complexes may have similar effects to the cis-diamine dichloroplatinate (II) (cis-DDP) complex and that this complex is present in cis-DDP reported less nephrotoxic effect [27].

Some thiosemicarbazide derivatives containing a cyclobutane ring were obtained and the antioxidant and antitumor properties of the synthesized compounds were also investigated. These compounds displayed good antioxidant and antitumor activity in comparison to the standards [28].

Antitumor and antioxidant properties of compounds containing thiophene and cyclobutane groups were investigated *in vitro*. It is aimed to determine antioxidant and antitumor activities by adding compounds containing thiophene and cyclobutane groups to the cell culture medium. It is also aimed to determine whether these substances have superoxide radical reduction and hydroxyl radical capture properties *in vitro* [29,30].

2. Materials and Methods

The compounds synthesized and characterized in the chemistry department of Firat University [29,30] have the structure of compounds containing thiophene and cyclobutane groups as follows (Figure 1-3).

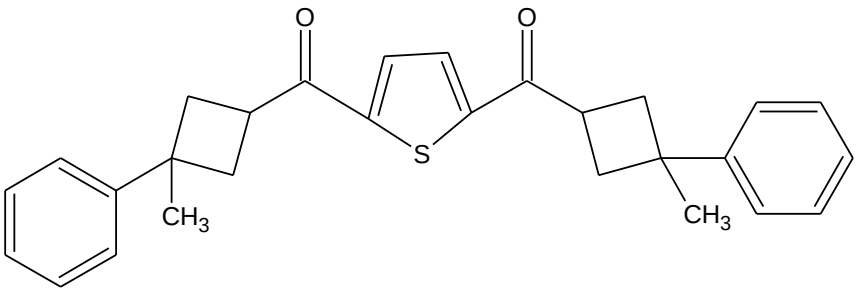

Figure 1. 2,5-di [1-methyl-1 phenylcyclobutanecarboxylate 3-Iloil] thiophene (LI).

$C_{28}H_{28}O_2S$ (Mw=428.00)

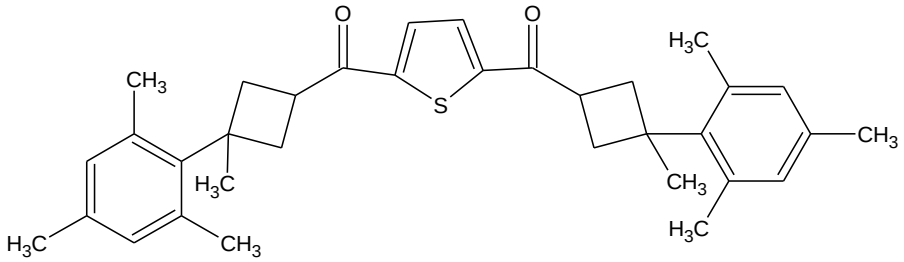

Figure 2. 2,5-Di [1-methyl-1-methylcyclobutan-3-yloyl] thiophene (LII).

$C_{34}H_{40}O_2S$ (M_W=512.00)

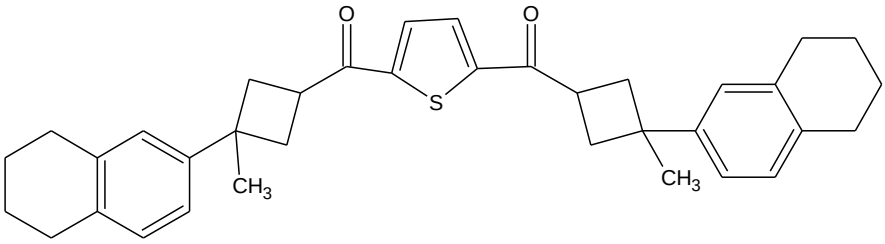

Figure 3. 2,5-Di [1-methyl-1- (2-tetralin) cyclobutan-3-yloyl] thiophene (LIII).

$C_{36}H_{40}O_2S$ (M_W=536.00)

2.1. Applied cells.

L1210 cell culture (rodent leukemia cell) and *Saccharomyces cerevisiae* yeast cells for antioxidant activity were used for antitumor activity determination. *Saccharomyces cerevisiae*, a member of the fungi kingdom, is a single-celled microorganism. It is an asymmetric yeast. Yeast is an asexual (or asexual) breeding or sexually reproducing microorganism, such as budding and transverse cleavage. *Saccharomyces cerevisiae* ferment carbohydrates like other yeasts used in beer making and baking. The high vitamin content of the yeast increases its value as a nutrient. Many *Saccharomyces cerevisiae* species synthesize from vitamins, especially B-vitamin [31].

Saccharomyces cerevisiae malt extracted broth and incubated at 25 °C. Experimental studies were performed by taking 10^6 cells / mL in the exponential phase. These cells are often used as a model for oxidative stress response studies of molecular metabolism [32].

2.2. L 1210 cells.

Frozen L 1210 rodent leukemia cells from the cell culture bank (Sigma, USA) were dissolved at room temperature and transferred into a 75 ml flask. DC5 (25 ml), previously prepared, was added to the flask, and flasks were placed in a Nuaire brand, 5% CO2 medium incubator (Plymouth, MN, USA). On a daily basis, the state of the cells was checked using an inverted microscope of the Soif brand (Soif Optical Inc., China), and at the end of the third day, the DC5 in the flask was withdrawn. This process was repeated repeatedly at intervals of three days. The cells, which continue to increase in number, began to form layers on top of each other by completely covering the flask base. At the end of the 15th day, the medium in the flask was withdrawn and replaced with 3 ml of trypsin. The flasks were shaken slightly for 2-3 minutes to allow the cells to separate from the adhered surface.

After all the cells were separated from the surface of the flask, 12 mL of DC5 was added to the flask and carefully diluted to allow the cells to disperse into the solution homogeneously. Cells were counted using a hemocytometer. A cell suspension was added to each flask as 1×10^5 cells, DC 5 was added thereto, and all flasks were placed in the incubator. The cells' shots, feeding, and experiments were performed in a sterile Class II Laminar Flow (Biolaf, Ankara) [32,33].

2.3. Number of cells to be used and determination of drug dosage.

The L 1210 leukemia cell suspension was centrifuged at 2000 rpm for 5 minutes. Cells were counted using the hemocytometer and the cell number was adjusted to 1x105 / ml cells for L 1210 cell assays. Preliminary experiments were done to determine the doses and the method was used more or less. One ml of cell suspension was transferred to the test tubes and the test agents were added at concentrations of 30, 60 μM. The same amount of serum physiological was added to the negative control tubes, DMSO was added to the Vehicle tubes in the same amount, and the tube incubator was placed. The amount of DMSO in cell suspensions was not more than 1% den. After 24 hours, the tube was removed from the incubator. The cell suspension was counted in 100 cell hemocytometers randomly selected at 1: 1 (v / v) with 0.4% trypan blue. Cell viability was expressed as a percentage. The same procedure was repeated after 48 hours and the experiment was terminated [34].

2.4. In vitro antioxidant activity measurements.

2.4.1. Measurement of *Saccharomyces cerevisiae* specimens.

Chromatographic methods HPLC used to determine the antioxidant properties of the ingredients. For the antioxidant vitamins A, E and MDA measurements, a solution containing 2 ml of cells was placed in each test tube. The materials were dissolved in DMSO and the solutes were prepared at specific concentrations and final concentrations were added to the tubes as 50 μM and 100 μM. For all experiments, additives were added to equal the amount of DMSO in the tubes. Test tubes with an equal volume of DMSO added were used as the control group. Certain periods have waited after the addition of the substance. Compared to the relevant literature, the doses were determined by considering the cell number at [35-37] ml to be 10^6 cells / mL and taking into account the solubility of the substances.

2.4.2. MDA analysis.

The treated cells were shaken for MDA analysis by adding 250 µL of 15% trichloroacetic acid and 750 µL of 0.5 M $HClO_4$ to the treated cells. The cells were separated into small pieces and the lysate was centrifuged at 4500 rpm for 5 minutes and then clarified and analyzed by HPLC [38].

2.4.3. Vitamin A and E analysis.

The A and E Vitamin levels were analyzed liquid-liquid extraction method with small modifications of the definition method by Pan *et al*. using HPLC [39].

2.4.4. Measurement with DPPH radical reduction method.

Another method of determining antioxidant activity was the DPPH radical reduction method [40]. 4 ml of the solution prepared in the form of 5 mg / L DPPH in methanol was taken into each tube individually. Additives were added to the final concentrations of 100, 250, 500 and 1000 µM for each group from the test compounds dissolved in DMSO at a concentration of 4000 µM. The mixture was allowed to stand in the dark at room temperature for 30 minutes so that the reaction would take place. It was decided whether to be read in a spectrophotometer at 517 nm by looking at the color opening. Reading was carried out on the spectrophotometer as it was color expansion.

2.4.5. Capture activity of hydroxyl radical with deoxyribose degradation.

This method is based on the capture of non-enzymatic hydroxyl radicals formed by Deoxyribose degradation [41]. Deoxyribose, hydrogen peroxide, sodium ascorbate and $FeSO_4.7H_2O$ were prepared by mixing in KH_2PO_4 buffer (pH: 7.4). The amount of MDA formed was determined by spectrophotometrically.

2.4.6. Statistical evaluation

All statistical analyzes in this study were done with SPSS statistical program. One-way ANOVA analysis Tukey test was used for the antitumor feature. LSD test was used For analyzes MDA, A and E vitamin. In evaluating the test results, the P values are expressed as meaningful when the significance value is less than 0.05 and (n) values indicate the number of working samples.

3. Results and Discussion

The results obtained were tabulated to show the comparisons of the groups to the control group throughout the application in each of the parameters. The viable cell results of L 1210 cells treated with the test compounds are given in Table 1, Figure 4 and Figure 5. The results of MDA levels of *Saccharomyces cerevisiae* yeast cells treated with the test compounds are given in Table 2 and Figure 6. Free radicals have been the subject of intense research and research in recent years. The cellular sources of free radicals, the reactions play, and the defense of cellular defense mechanisms against free radicals have been tried to clarify the relationship of the molecules with many diseases such as cancer, sugar, heart diseases [42]. Free radicals in biological systems, both as a by-product of normal metabolism and by the action of foreign substances, damage cellular membranes and affect different diseases.

Table 1. Viability states of the L 1210 cells treated with the compound according to the duration and dose.

Group (n=6)	30 µM (24h)	60 µM (24h)	30 µM (48h)	60 µM (48h)
Control	65.00 ± 1.53	61.33 ± 1.20	56.67 ± 1.76	52.33 ± 2.03
LI	42.33 ±1.67^{c}	36.33 ± 1.66^{c}	35.00 ± 1.00^{c}	22.00 ± 2.00^{c}
LII	38.67 ± 1.20^{c}	35.33 ± 0.88^{c}	33.00 ± 1.53^{c}	19.67 ± 0.67^{c}
LIII	42.00 ± 1.16^{c}	35.67 ± 0.88^{c}	32.33 ± 1.86^{c}	17.33 ± 0.67^{c}

a: P<0.05; b: P<0.01; c: P<0.001

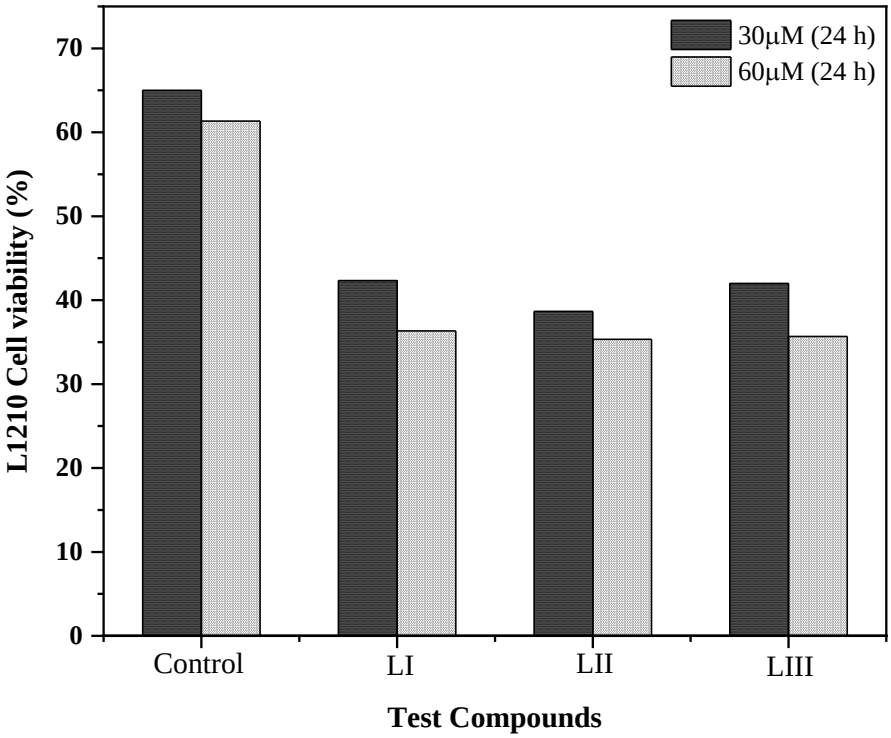

Figure 4. Viability states of the L 1210 cells treated with the compound according to the duration and dose.

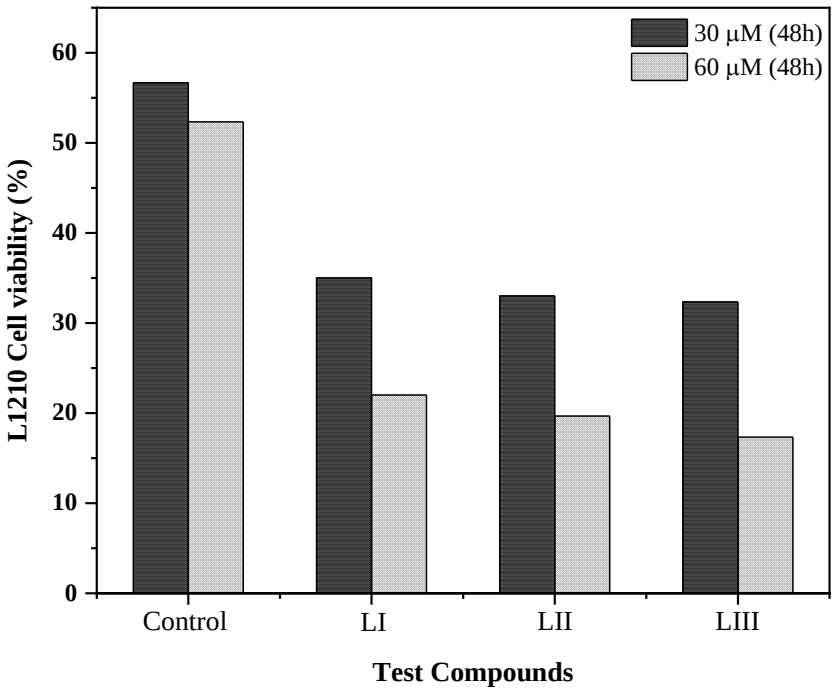

Figure 5. Viability states of the L 1210 cells treated with the compound according to the duration and dose.

These compounds in the organism have defensive systems consisting of small molecular weight radical scavengers and enzymes against harmful effects.

Table 2. The mean values of MDA (mg / 4.10^6 cell) levels of the *Saccharomyces cerevisiae* yeast cells treated with the compound according to the doses.

MDA Groups (n=3)	50µM	100µM
Control	0.602 ± 0.014	0.602 ± 0.014
LI	0.831 ±0.241	0.581 ± 0.133
LII	1.010 ± 0.024[a]	1.083 ± 0.005[a]
LIII	0.673 ± 0.097	0.734 ± 0.210

a: P<0.05; b: P<0.01; c: P<0.001

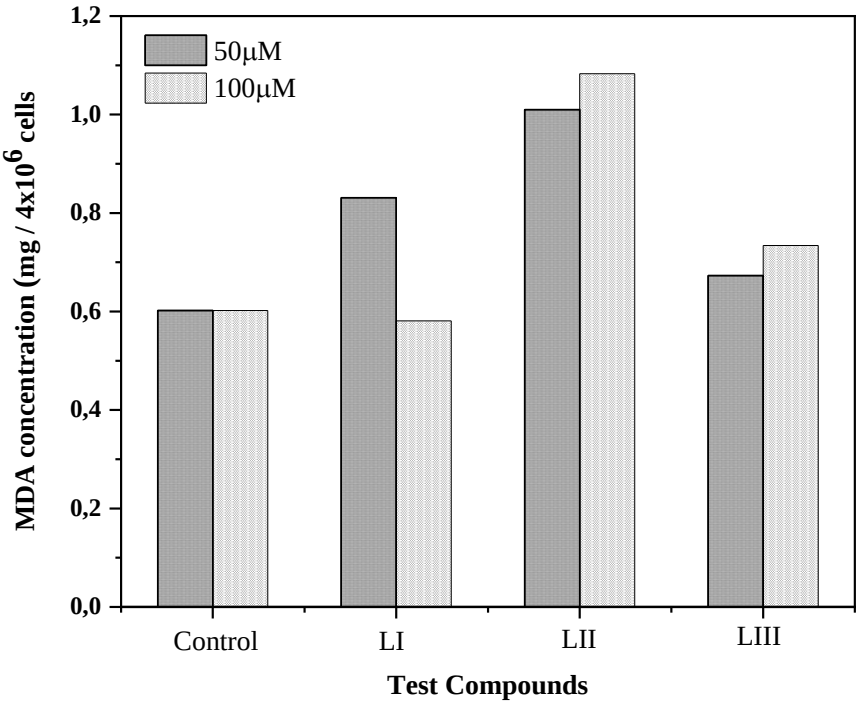

Figure 6. The mean values of MDA (mg / 4.10^6 cell) levels of the *Saccharomyces cerevisiae* yeast cells treated with the compound according to the doses.

Reactive structures of free radicals and their very short longevity make direct determination difficult. For this reason, the investigation of the products and defense systems of free radical reactions is preferred by many researchers [43]. Organic compounds containing 5-membered aromatic and heterocyclic rings are found in many natural structures but play a major role in biochemical events. As a result, it has created a very important and new field of study for chemists [44]. Thiophene derivatives are an important compound in pharmaceuticals sciences due to their aromatic heterocyclic groups [45,46].

Thiophene compounds have been the subject of many studies due to the properties of nematocidal [47], insecticidal [48], antibacterial [49], antifungal [50], antiviral [51] and antioxidant [52]. In their study, Meotti and colleagues investigated the degree of inhibition of lipid peroxidation in brain tissue of thiophene compounds and noted that some compounds exhibit this activity in 90% of low concentrations, such as 10 mM [53]. Süleymanoğlu and his colleagues have synthesized, characterized and examined antitumor properties of some new thiophene compounds. The compounds they synthesized showed good antitumor activity against some tumor cell type s[54].

Basto and colleagues synthesized benzo [b] thiophene compounds and found that these compounds affect mitochondrial lipid peroxidation level and antitumor activity studied. They observed that the compounds they used were inhibitory to lipid peroxidation. There was no cell proliferative effect at various concentrations against tumor cells. They also investigated DPPH radical cleansing and observed a good radical scavenging feature [55].

Diana *et al*. Synthesized indole thiophene derivatives and examined antitumor properties against tumor cells in different cultures. These compounds have shown to be more active against some types of cancer [56].

In this study, it was found that compounds containing thiophene and cyclobutane groups administered in vitro to the L 1210 cell had different effects on duration and the comparison of the dose-dependent antitumor activity with the control group. From Table 1, it can be seen that all the materials used in the study have antitumor activity near each other, which is effective in the L 1210 cell type. In our study, significant and statistically significant differences were observed between the control group and the other groups when the concentrations of the substances were 30 and 60 μM, 24 and 48 hours, respectively.

Taking into account the statistical differences, it can be said that compounds containing thiophene and cyclobutane groups have antitumor activity. This study also found that compounds containing thiophene and cyclobutane groups on the MDA levels applied to Saccharomyces cerevisiae cells had different effects than the control group. Statistical differences in MDA levels were observed between the control group and compound II compared. Table 2 shows that the levels of MDA in all groups of substances used are higher than the controls. This may be an indication that the test substances used have caused lipid peroxidation to cause damage. Table 3 and Table 4 show changes in vitamin A and E levels of *Saccharomyces cerevisiae* cells treated with the substances. As can be seen from the tables, it is seen that there is a statistically decrease in the group of substance II in vitamin A levels.

Table 3. Mean values of vitamin A (mg / 4.106 cell) levels of *Saccharomyces cerevisiae* yeast cells treated with the compound according to doses.

Vit. A (n=3)	**50μM**
Control	2.70 ± 0.67
LI	1.84 ± 0.77
LII	1.93 ± 0.34
LIII	2.36 ± 0.19

P<0.05

Table 4. Mean values of Vitamin E (mg / 4.106 cells) levels of *Saccharomyces cerevisiae* yeast cells treated with the compound according to doses.

Vit. E (n=3)	**50μM**
Control	8.94 ± 0.00
LI	6.33 ± 0.63
LII	8.15 ± 0.54
LIII	6.70 ± 0.02

P<0.05

However, vitamin E levels of the substance groups I and III in Table 4 and the substance groups I, II and III in Table 3 decrease with decreasing statistical significance. Compounds containing thiophene and cyclobutane groups were examined for free radical scavenging activity and hydroxyl radical trapping activity by the DPPH reduction method. As can be seen in Table 5 and Table 6, it can be said that the compounds do not possess antioxidant activity

since they do not show radical clearing and capture properties. Furthermore, when we examine the effects of antitumor activity and MDA levels on *Saccharomyces cerevisiae* cells, we can establish a relationship between MDA levels and the effect of the substances on the viability of the cells.

Table 5. Hydroxyl radical capture activity measurement results (%).

Groups	(5 µg/mL)	(10 µg/mL)
Control	95.6	94.9
LI	19.51	22.36
LII	13.85	15.04
LIII	12.16	11.38

Table 6. Measurement results by DPPH radical reduction method (%).

Groups	1000 µg/mL
Control	88.57
LI	24.21
LII	22.63
LIII	24.66

4. Conclusion

As a consequence of the effects of the compounds in yeast cells, the increase in the amount of MDA and the decrease in antioxidant vitamin levels, as well as the lack of radical cleaning and hydroxyl radical retention, may lead to the idea that the antitumor activity of the substances may be a cytotoxic end effector of an oxidative damage mechanism.

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Conflicts of Interest

The authors declare that there is no conflict of interest.

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