




Antioxidant and prooxidant activity of new 1,2-diols and thiadiazoles derivatives in *Saccharomyces cerevisiae* yeast cells

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Abstract

The present research was undertaken to determine the effect of substituted 2,2'-(2R,3R)-2,3-dihydroxy-1,4-dioxobutane-1,4-diyl]bis(N-R-hydrazine-1-carbothioamide and (1R,2R)-1,2-bis[5-(R-amino)-1,3,4-thiadiazole-2-yl]ethane-1,2-diols on the antioxidant status of the yeast *Saccharomyces cerevisiae* cells. This cell serves a good eukaryotic model system for the study of molecular mechanisms of oxidative stress. The *Saccharomyces cerevisiae* yeast cells were treated a series of 1,2-diols and thiadiazoles compounds and the malondialdehyde (MDA) and antioxidant vitamins (A, E, C) levels in the medium were measured by HPLC-UV. In the comparison done among groups, the MDA which is an indicator of lipid peroxidation and Vitamin E concentrations were showed statistically changed in the samples. Exposure of yeast cells to L9 showed an increase in MDA and decrease in vitamin E levels but L2 and L8 showed decrease in MDA and increase in vitamin E levels. The results showed that compounds L9 caused a considerable oxidative stress and L2 and L8 have antioxidant activity.

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1. Introduction

The natural or chemical materials such as pharmaceuticals, dyes, organic materials and biologically active compounds are containing the nitrogen atom containing heterocycles [1]. The 1,3,4-thiadiazole nucleus is well-known heterocyclic nuclei, which is a functional group of a variety of medicinal agents and natural products. Because of their diverse biological activity, the 1,3,4-thiadiazole groups have become great interest by the researchers as important class of heterocycles. These biological activities are including antimicrobial [2], antituberculosis [3], antioxidant [4], antiinflammatory [5], anticonvulsants [6], antidepressant and anxiolytic [7], antihypertensive [8], anticancer [9] and antifungal activity [10].

The diverse biological activities of the 1,3,4-thiadiazole rings are associated with incorporating a toxophoric -N=C-S linkage of which has been showed in many pesticides [11-14] and therefore 1,3,4-thiadiazoles and their Schiff bases have recently received significant importance [15]. Some natural products include enantiomerically pure 1,2-diols and this molecule is valuable intermediates in the organic synthesis of biologically active compounds [16]. They are readily transformed into chiral epoxides [17] aziridines, and amino alcohols [18]. Moreover, the 1,2-diol functionality is found in a number of synthetic and pharmaceutical intermediates [19].

The production of pharmaceuticals, chemical catalysts, and agrochemicals are need optically pure 1,2-diols [20]. Although the only commercial 1, 2, 4-thiadiazole drug is the antibiotic cefozopram, many drugs such as acetazolamide, methazolamide, megalol are containing 1,3,4-thiadiazole nucleus which are available in the market [2, 21].

As a result of these studies, it is suggested that 1,3,4-thiadiazole derivatives have wide biological effects.

However, the nitrogen containing heterocyclic compounds becomes an important goal in modern organic synthesis for efficient antioxidant activity, nowadays.

The hydrogen peroxide, superoxide anions and hydroxyl radicals, which are known reactive oxygen species (ROS), are generated by aerobic life style and living cells are exposed to these molecules. Free radicals are highly reactive ions or molecules that have unpaired electron [22, 23]. In some special cases, if the amount of reactive oxygen species increases and the antioxidant capacity exceeds, oxidative stress occurs [1]. The extracellular xenobiotics and substances can be generating this stress condition by which cause the loss of cellular integrity [2]. The several lethal diseases such as cancer and neurodegenerative disorders as well as aging related with oxidative stress has been in great focus due to its correlation with a normal and inevitable eukaryotic process [3]. Reactive oxygen species (ROS) which are destroy to DNA via oxidative

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damage, have been associated many health problems such as coronary heart diseases and carcinogenesis [24,25].

The complex physiological response contains both enzymes (e.g., catalase, superoxide dismutase) and protective molecules for resistance to oxidative stress [22, 23] and the non-enzymatic low molecular weight molecules such as ascorbate, tocopherols, flavonoids and glutathione are important in scavenging ROS [23].

The *Saccharomyces cerevisiae* yeast cells that possibility of using genetic approaches are made a common experimental model for studies of stress response at the molecular level. Mammalian and yeast cells activate a wide response involving several defense mechanisms which named adaptive response, to adapt sensitive cells for environmental change such as temperature shifts and increased ROS levels [26].

The aim of this work was to find antioxidant ability new chemical structures 1,2-diols and thiadiazoles by using *Saccharomyces cerevisiae* yeast cells.

2. Materials and Methods

2.1. Materials and chemicals

The vitamin A (all trans-retinol), MDA (1,1,3,3-tetraethoxypropane for standart) were obtained from Sigma (St. Louis, MO, USA). Vitamin C (ascorbic acid), vitamin E (α -tocopherol), HClO_4 , methanol, H_3PO_4 and KH_2PO_4 were purchased from Merck (Darmstadt, Germany). Stock solutions of compounds were prepared as 5000 μM solutions in DMSO (dimethylsulfoxid).

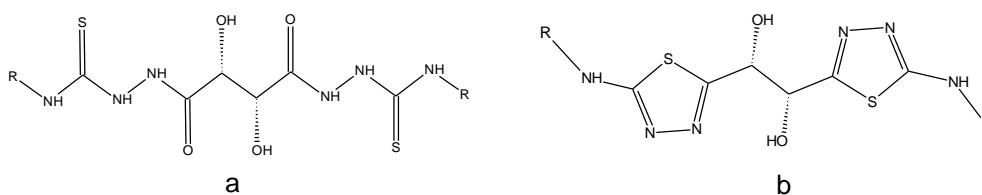


Figure 1. Structure of compounds a) 1,2-diols and b) thiadiazoles **R**: L1,L6 phenyl, L2,L7 p-tolyl, L3,L8 p-methoxyphenyl, L4,L9 allyl and L5,L10 ethyl.

2.3. Test compounds treatment *Saccharomyces cerevisiae* yeast cells for antioxidant prooxidant activity

Saccharomyces cerevisiae yeast cells were added in test tubes at a density of about $1 \times 10^6 \text{ ml}^{-1}$ cell, and incubated for 1 day before the experiment. The test

2.2. Applied cells

Saccharomyces cerevisiae yeast cells were used to determine antioxidant activity. *Saccharomyces cerevisiae*, a member of the fungi kingdom, is a single-celled microorganism. *Saccharomyces cerevisiae* is ascomycetic yeast. The yeasts are contain high vitamin level, therefore increases its value as a nutrient. These cells are often used as a model for molecular responses to oxidative stress metabolism [26].

The dry yeast sample containing the Microorganism *Saccharomyces cerevisiae* was stored at $+4^\circ \text{C}$ during the study. Preparation, cultivation of microorganism culture and the addition and incubation of chemicals to investigate the effect on microorganism were done in the Microbiology Laboratory. For *Saccharomyces cerevisiae* development and reproduction to be used in the experiment, Malt Extract Broth (Difco) was inoculated into and incubated for 48 h. at $25 \pm 1^\circ \text{C}$. The prepared yeast, broth culture is inoculated into YEDP (1 g yeast extract for 100 ml, 2 g bactopectone, 2 g glucose, 2 g agar) into the medium at a rate of 1% (10^4 yeast / ml) at $25 \pm 0.1^\circ \text{C}$ for 48 h. has been incubated.

2.2. Chemical compounds used

The whole of the work will be carried out in the chemistry department of Firat University biochemistry and microbiology cell culture laboratory. The structure of the compounds 2,2'-(2R,3R)-2,3-dihydroxy-1,4-dioxobutane-1,4-diyl]bis(N-R-hydrazine-1-carbothioamide and (1R,2R)-1,2-bis[5-(R-amino)-1,3,4-thiadiazole-2-yl]ethane-1,2-diol to be used in the studies is given below [27].

compounds were prepared at specific concentration by dissolving in DMSO. The final concentrations of test compounds in cells were 50 μM and incubated and stirred 24h.

2.4. Determination of MDA and vitamin C

MDA and vitamin C levels were assayed according to the method of Karatepe, small modifications [28]. Briefly, the cells were collected and were gently rinsed twice with 2 ml of ice-cold Krebs–Ringer–Hepes buffer at the end of the incubation period (128 mM NaCl, 20 mM Hepes, 1.4 mM MgSO₄, 1.4 mM CaCl₂, 1 mM

NaH₂PO₄ and 5.2 mM KCl, pH 7.4). Then the mediums were treated with 0.1 ml of 0,5 M perchloric acid and 0,1 ml water. The cells were scraped from the tubes and the lysates were santrifuged 5 min at ambient temperature. The supernatant were taken and separated 17,5 % methanol (v/v) in 30 M monobmasic potassium phosphate buffer (pH 3,6) mobile phase.

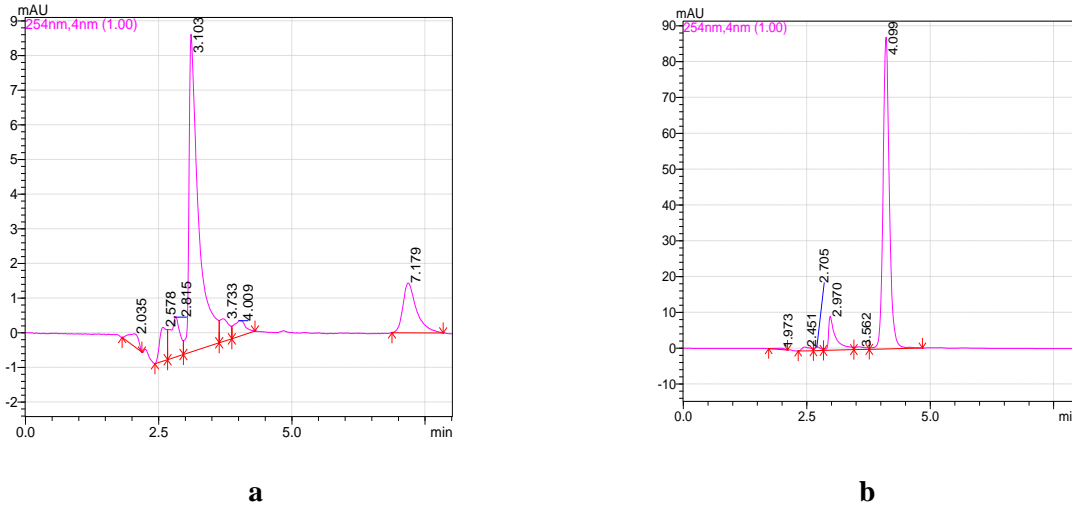


Figure 2. HPLC chromatograms a) MDA (retention time 7.179) b) vitamin C (retention time 4.099).

2.5. HPLC quantification of vitamin E and vitamin A

Lipid soluble vitamins in cells were assayed according to the method of Catignani [29]. After incubation with the compounds in 100 µl of the cells suspensions, 200 µL of Ethanol: Sulfuric acid (99: 1) and 100 µL of water was added for precipitation of proteins. After thorough mixing with vortex, it was centrifuged at 4500 rpm for 5 minutes. Then 100 µL of n-hexane (0.05% butylated hydroxytoluene) was added on the centrifuged samples. With the addition of hexane, the

lipid-soluble vitamins in the medium were extracted into the hexane phase. The tubes were mixed on vortex and centrifuged again. At the end of the centrifuge, the hexane phase was carefully separated and taken into the glass tube. 100 µL of n-hexane was added onto the sample again, mixed and centrifuged, and the n-hexane phase was combined with the hexane phase in the glass tube. The extracted hexane phase was carefully evoperated by using nitrogen gas. The residue from the hexane was dissolved in 100 µl of mobile phase (methanol/acetonitrile/chloroform, 47:42:11, v/v). 20 µL of this solution was taken and injected into HPLC.

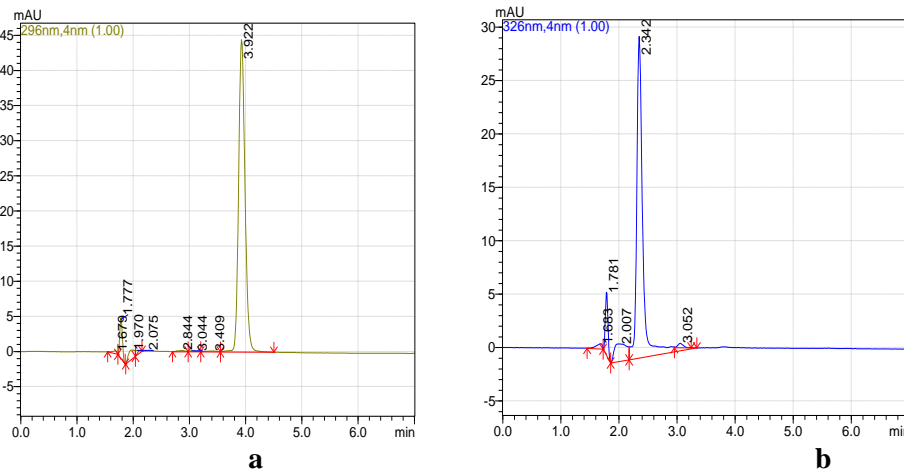


Figure 3. HPLC chromatograms a) vitamin E (retention time 3.922) b) vitamin A (retention time 2.342).

2.6. Apparatus

Liquid chromatographic system consisted of LC-20AD pumps, DGU-20A5 degasser, SIL 20A autosampler, CTO-10AS VP column oven, SPD-M20A DAD system. These apparatus were connected via a communication module (Model CBM-20A), and controlled by a Shimadzu LC Solution Workstation (Shimadzu, Kyoto, Japan). A Shimadzu Shim-pack vp-ODS column (150 L×4.6) was used.

2.7. Statistical analysis

SPSS 22.0 for Windows software (SPSS Inc., New York, IBM, USA) were used for statistical analyses. The statistical significance was set at $P < 0.05$ and results are expressed as mean±S.D. Comparison between mean values for antioxidant parameters were made by independent sample t-test.

3. Results and Discussion

3.1. Selection of working type

Only *in vitro* method cannot be sufficient to determine antioxidant activity, and therefore *in vivo* methods are required to assess antioxidant activity. Due to this fact, antioxidant activities of the new compounds have been tested by using *Saccharomyces cerevisiae* yeast cells as *in vivo* model [30].

3.2. Induction of lipid peroxidation by test compounds

The mean MDA levels of the groups are given in Table 1 and Figure 2a. Significant differences were found in MDA levels. After 24 h incubation of the *Saccharomyces cerevisiae* yeast cells with test compounds, the levels of MDA in L2, L3 and L8 groups statistically decreased and compound L9 induced lipid peroxidation from compared to the controls.

3.3. Effects of the compounds on the vitamins A and E levels of cells

Vitamins E levels are increased by the L2 and L8 and decreased by the L9 compounds treatment (Table 1 and Figure 2b). Vitamin A and C levels were not changed all compounds statistically. The stopping chain reactions, metal ion binding activity, radical capture activity and reducing force factors explain of a possible antioxidant substance which it posses antioxidant activity [31].

This work was carried out in order to verify and to clarify the contribution of the biological side effects and relation of new compounds with reactive oxygen species (ROS). Reactive oxygen species (ROS) are formed and degraded by all aerobic organisms, leading to either physiological concentrations required for normal cell function or excessive quantities, the state called oxidative stress [32]. The malondialdehyde (MDA) [33] and isoprostanes [34], well known lipid peroxidation products, are produced ROS-mediated oxidation of membrane lipids.

Table 1. The mean values of the vitamin A, E, C and MDA of *Saccharomyces cerevisiae* yeast cells treated with the compounds

Groups	C vitamin, ppm	MDA, ppm	Vitamin A, ppm	Vitamin E, ppm
Control	0.79±0.04	22.24±0.57	0.07±0.009	0.87±0.08
L1	0.72±0.05	21.47±1.15	0.07±0.011	0.74±0.05
L2	0.84±0.04	17.34±0.88**	0.08±0.003	0.94±0.05*
L3	0.69±0.06	19.33±1.26*	0.08±0.007	0.74±0.04
L4	0.74±0.07	21.26±2.15	0.07±0.005	0.86±0.04
L5	0.74±0.06	20.84±1.32	0.07±0.004	0.74±0.04
L6	0.75±0.08	20.76±1.23	0.07±0.006	0.75±0.03
L7	0.75±0.07	21.59±1.15	0.07±0.007	0.76±0.03
L8	0.85±0.07	17.66±0.81**	0.08±0.004	0.96±0.05*
L9	0.64±1.00	26.40±0.94*	0.07±0.004	0.72±0.03*
L10	0.74±0.08	21.84±0.53	0.06±0.008	0.79±0.04

* $p < 0.05$; ** $p < 0.01$;

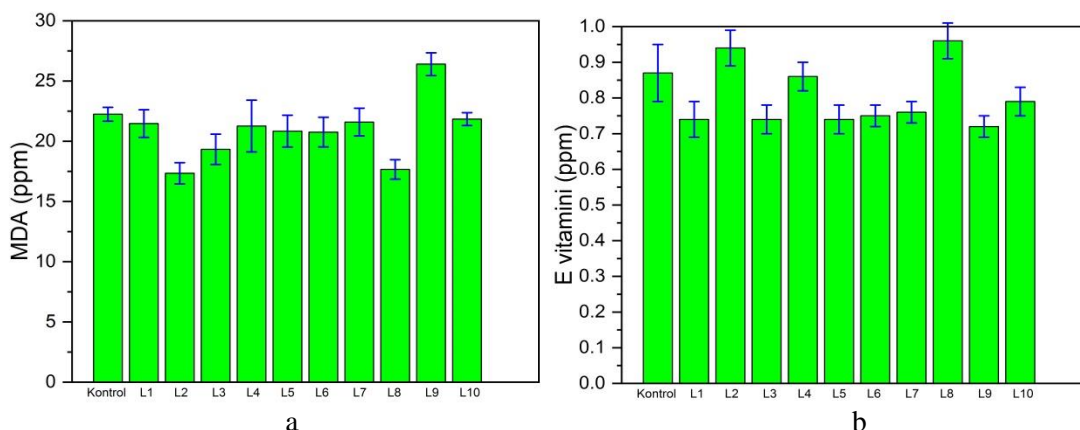


Figure 4. Comparison of the mean concentration of the a) MDA and b) vitamin E compound treated cells, (* $p \leq 0.05$, ** $p \leq 0.01$).

ROS are continuously produced in actively metabolizing cells. However, *Saccharomyces cerevisiae*, like all organisms, contains effective antioxidant defense mechanisms, which detoxify ROS as they are generated and maintain the intracellular redox environment in a reduced state. An oxidative stress is said to occur when ROS overwhelm these defenses, resulting in genetic degeneration and physiological dysfunction, leading eventually to cell death.

The cellular antioxidant systems have two major groups, enzymatic and nonenzymatic [32]. The vitamin C, vitamin E and carotenoids are non enzymatic low molecular weight antioxidant compounds and they are consumed by the metabolism and may fall below normal ranges. For the evaluation of oxidative stress in biological systems, the analyses of levels of antioxidants and MDA are very important. *Saccharomyces cerevisiae*, as a typical eukaryotic model microorganism has some advantages in comparison to humans and animals, such as its simple structure that known genetic background and easy manipulation. Moreover, *Saccharomyces cerevisiae* genome possesses a strikingly high-level of functional conservation within the human genome and other higher eukaryotes. In consideration of these advantages, *Saccharomyces cerevisiae* has become a prominent model for evaluating some cell damages to quickly provide functional clues [30].

In order to eliminate the harmful effects of oxidative stress, many researches are carried out to illuminate the oxidation antioxidant mechanisms and produce solutions. These mechanisms are very important in various living systems, especially human beings. Yeast cells and mammalian cells largely are similar. For this reason, aging, apoptosis and various diseases caused by oxidative stress are important in lighting studies.

Cytotoxicity of xenobiotics could be explained by the impairment of either cellular regulation system, intracellular synthesis of macromolecules or cellular transduction signalling. For the determination of antioxidant and prooxidant activity of test compounds, we choose and consider MDA and antioxidant vitamins [35]. The cell death occurs as a result of membranes that change structure and lose function with lipid peroxidation and increased production of MDA [36]. MDA can be measured by many methods and it is one of the most important indicators of lipid peroxidation [37, 38]. The results show that in the *Saccharomyces cerevisiae* yeast cells, L9 compound induced oxidative damage by generating lipid peroxidation which it increased MDA formation and decreased antioxidant vitamin E levels. The presence of high MDA levels in sample points to the induction of peroxidation following L9 administration.

As seen from the table and figures, L1, L3, L4, L5, L6, L7 and L10 did not affect vitamins A, E, C and MDA levels but L2 and L8 caused significant increase vitamin E and the decrease MDA concentrations statistically which has not been previously described. This indicates that the L2 and L8 supplements were able to spare the vitamin E concentrations with decreasing the lipid peroxidation in the cells.

The Vitamin E antioxidant capacity is very large and high because its antioxidant duty performs by using all mechanisms such as radical destruction, breaking the chain, suppression, repairing broken structures [31, 32].

The more literatures showed that the thiadiazole nuclei have anticonvulsant, anti-leishmanial, antimicrobial, antidepressant, antioxidant, radio protective, anticancer and anti-inflammatory activities.

Kus et al [39] synthesized some novel 5-[(2-(substituted phenyl)-1H-benzimidazole-1-yl)methyl]-methyl-1,3,4- thiadiazole-2-amines and tested for

antioxidant properties by using various *in vitro* systems. They have stated that some compounds prevented lipid peroxidation slightly at 10^{-3} M concentration.

The 6-[3-(4-fluorophenyl)-1H-pyrazol-4-yl]-3-[(2naphthoxy)methyl][1,2,4] triazolo [3,4-b]-[1,3,4] thiadiazole (FPNT) and 6-[3-(4chlororophenyl)-1H-pyrazol-4-yl]-3-[(phenyloxy)methyl]-[1,2,4] triazolo [3,4b][1,3,4] thiadiazole (CPPT) *in vitro* antioxidant activity had investigated by DPPH and ABTS radical scavenging methods as well as by lipid peroxide assay [40]. The results of these assays proved FPNT to be an excellent antioxidant.

The antioxidant activity studies of 1,3,4-thiadiazoles demonstrated that the thiol, thiosulfonic acid and phosphorothioate derivatives of thiadiazoles exhibit evident antioxidant activity. This activity can explain direct link between biological function and thiol containing aromatic ring [41].

The thiol and aminothiols derivative thiadiazoles were synthesized by the Prouillac et al [42] and examined them for their antioxidant ability. The new compounds demonstrated the most scavenging activity for DPPH• and ABTS•+ free radicals.

Conclusion

The increase in free radical formation causes an increase in MDA level and GSH-Px enzyme activity and a decrease in vitamin E level.

As a result of our experimental studies, the groups that the chemical substances used have affected their activities significantly.

In conclusion, we investigated the antioxidant and prooxidant effects of test compounds on *Saccharomyces cerevisiae* yeast cells and the results of this work demonstrate that L9 compound caused oxidative stress and L2 and L8 have antioxidant activity.

The antioxidant activity evaluation results have demonstrated that only aromatic groups exhibit evident antioxidant activity. It seems that our findings are compatible with the literature.

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