Cumhuriyet Science Journal

e-ISSN: 2587-246X
ISSN: 2587-2680

Cumhuriyet Sci. J., 41(4) (2020) 756-763
http://dx.doi.org/10.17776/csj.758957

Biological activity of some pyrimidine derivatives: Cytotoxicity and oxidative stress potential in human lung cancer cell line (A549)

Mahmoud ABUDAYYAK [1, 2, *], ***Fatma Betul SAMLIOGLU*** [1], ***Beyza SELEN*** [1]
Seda FANDAKLI [3, 4], ***Nurettin YAYLI*** [4]

[1] *Karadeniz Technical University, Faculty of Pharmacy, Department of Pharmaceutical Toxicology, Trabzon / Turkey*

[2] *Istanbul University, Faculty of Pharmacy, Department of Pharmaceutical Toxicology, Istanbul / Turkey*

[3] *Avrasya University, Faculty of Health Sciences, Department of Nutrition and Dietetics, Trabzon / Turkey*

[4] *Karadeniz Technical University, Faculty of Pharmacy, Department of Pharmacognosy, Trabzon / Turkey*

Abstract

Compounds with pyrimidine ring in their structure have many biological activities including antimicrobial, antiviral and anticancer. Recently, studies related to their synthesis and so their applications have increased. In a previous study, a solid-phase microwave method was used to synthesized 25 new hydroxy- and methoxy-substituent 4,6-diarylpyrimidin-2 (1H) -ol and 4,6 diarylpyrimidin-2 (1H) –thiol compounds. In the present study, as a preliminary estimation of the anticancer activity, the cytotoxicity and oxidative stress induction potential of 6 derivatives that show highest antibacterial activities was evaluated in human lung epithelial cancer cell line (A549). Results of the 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide (MTT) cytotoxicity test indicate that pyrimidine derivatives caused concentration-dependent cell death that IC_{50} values were calculated between 16.7 and 41.5 µg/mL. additonally, pyrimidine derivatives induced significant changes in malondialdehyde (MDA) and glutathione (GSH) levels and catalase (CAT) activity; oxidative stress could be the mechanism of action of the tested pyrimidine derivatives in the cancerous cells. The results could be used to design the further *in vivo* and *in vitro* detailed studies to appreciate these pyrimidine derivatives anticancer activity, compare this activity with in-use known drugs, elucidate their mechanisms of action and estimate their safety.

Article info

History:
Received: 27.06.2020
Accepted: 06.12.2020

Keywords:
Pyrimidine,
Cytotoxicity,
Oxidative damage,
A549 cells,
Anticancer.

1. Introduction

Pyrimidine is a heterocycle organic aromatic diazine structure, containing nitrogen atom at the 1 and 3 positions of the hexagon ring [1]. Pyrimidine ring widely found in different natural compounds; thymine base, uracil base, and cytosine base in DNA and RNA, vitamin B1, vitamin B2, vitamin B6, and folic acid are examples of compounds with pyrimidine ring in their structures [1,2]. This wide existence of pyrimidine ring in nature and especially in the nucleic acids as DNA bases isosteres, and their ability to make hydrogen bonds and pi bonds suggest that pyrimidine derivatives can easily interact with polymers in the living organisms [1,3], therefore, different studies were modified these structures and evaluate their biological activities. It was shown that pyrimidine derivatives have antimicrobial and antifungal [4], anticancer [5], antiviral [6-8], calcium channel blocker, antimalarials, antihypertensive, antituberculosis, anti-inflammatory, and antiulcerogenic [9,10], anagenetic, anticonvulsant and antioxidant activities [11].

The anticancer activity of pyrimidine derivatives attracts the attention; especially that different pyrimidine nucleoside analogues as forodesin, tegafur, thioguanine, cytarabine, phasarabin and zebularine [12-14]; and DNA polymerase inhibitor as cytarabine are used in haematological malignancies and solid tumours treatment [12].

Pyrimidine derivatives could be synthesis by different methods. Some of these are difficult, waste of time, inefficient or harsh reactions [15-21]. Fandakli et al., (2016; 2018) used a solid-phase microwave method to synthesis 25 new hydroxy- and methoxy-substituent 4,6-diarylpyrimidine-2(1H)-ol and 4,6

*Corresponding author. *e-mail address: abudayyak@ktu.edu.tr*

diarylpyrimidine-2(1H)–thiol pyrimidine compound derivatives and evaluate their gram-negative and gram-positive antibacterial activities, α-glucosidase activity and *in vitro* pancreatic lipase activity. Besides the antilipase activity and the inhibitory effects on α - glucosidase, results showed that some of these original pyrimidine drivetives possess significant activity against *Escherichia faecalis*, *Staphylococcus aureus*, *Bacillus cereus* and *Mycobacterium smegmatis* with minimum inhibitory concentrations between 62.5 and 500 µg/mL [13,14].

The antibiotics are amongst the most important anticancer chemotherapeutic agents, different agents of anthracycline, mitomycin and other families known for ther antineoplastic activity. Doxorubicin, daunorubicin, idarubicin, mitoxantrone are examples of antibiotics that currently in use as anticancer agents [22,23].

Inasmuch as different pyrimidine derivatives have anticancer activity and as antibiotics assume to be important group of antineoplastic agents; It was hypothesized that the previously synthesized [14] pyrimidine derivatives which have antibiotic activity have also anticancer activity. In order to test this hypothesis, the anticancer activity of the 6 pyrimidine derivatives with the highest antibacterial activity in the aforementioned study was evaluated in human lung cancer epithelial cell line (A549) one of the most widely used cell lines in toxicology and new drug development studies. The cytotoxicity was assessed by MTT assay. The MDA and GSH levels and CAT activity were evaluated as endpoints of oxidative stress, one of the most important mechanisms of action of anticancer agents in cancerous cells.

2. Materials and Methods

2.1. Materials

GSH ELISA kit was purchased from Elabscience (Wuhan, China). Fetal bovine serum (FBS), phosphate buffer solution (PBS), DMEM (dulbecco's modified eagle's medium) - F12, Trypsin / EDTA (0.05% Trypsin, 0.53mM EDTA), and antibiotic solution (Penicillin-Streptomycin Solution 100X) were purchased from Multicell (Wisent Bioproducts, Quebec, Canada). Dimethyl sulfoxide (DMSO), bovine serum albumin (BSA), MTT (3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide), thiobarbituric acid, trichloroacetic acid, hydrogen peroxide (H_2O_2), ethyl alcohol, and chloroform, Urea, hydrochloric acid (HCl), sodium (meta) arsenite, Potassium phosphate, Monopotassium phosphate, and Sodium chloride were obtained from Sigma-Aldrich (Missouri, USA).

The tested pyrimidine derivatives (Figure 1) were synthesized according to method of Fandakli et al. [13,14]. Brifely, 15 mL of chloroform was used to dissolve urea (or thiourea), Methoxy- and hydroxy-substituted 1,3-diaryl-2-propene-1-one (4 mmol of each). The solution was completely adsrobed in Celite-$AlCl_3$ (5:2 ratio). The adsorbed metrials were put into milestone micro wave oven and heated for 10 minuts at 85 ºC using fixed power (600 Watt). After the dissolving using methanol, nutralization by HCl (2 N) and evapuration, the residue dissolved in water and extracted by chloroform. hexane and hexane diethyl ether solvent mixture and column chromatography was used to purify the chemicals. Methods and devices as nuclear magnetic resonance (NMR), Fourier-transform infrared spectroscopy (FT-IR), and liquid chromatography with tandem mass spectrometry (LC-MS/MS) were used to confirm the strctures [13, 14].

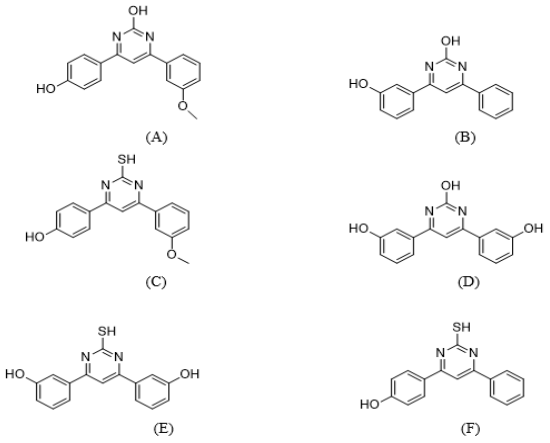

Figure 1. The tested pyrimidine derivatives

(A) 4-(4-hydroxyphenyl)-6-(3-methoxyphenyl)pyrimidin-2-ol(4OHR-U)

(B) 4- (3-hydroxyphenyl) -6-phenylpyrimidin-2-ol (3OH-U)

(C) 4- [2-mercapto-6-(3-methoxyphenyl) pyrimidin-4-yl] phenol(4OHR-T)

(D) 4,6-bis(3-hydroxyphenyl) pyrimidin-2-ol (3,3'-OH-U)

(E) 3.3'-(2-mercaptopyrimidine-4,6-diyl) diphenol (3,3'-OH-T)

(F) 4- (2-mercapto-6-phenylpyrimidin-4-yl) phenol (4OH-T)

2.2. Cell culture and chemical exposure

Human Lung epithelial carcinoma (A549) cell line (CCL-185™) was obtained from American Cell Culture Collection (ATCC). Cells were cultured in DMEM: F12 medium supplied with 10% heat inactivated FBS and 1% antibiotics (Penicillin-Streptomycin Solution 1X). The cells were incubated at 37°C, with 90% humidity, and 5% CO_2. In the cytotoxicity evaluation, 96- well plates were used, while the cells incubated with the synthesized

chemicals in 25 cm^2 flasks for oxidative stress evaluation.

2.3. Cytotoxicity evaluation

The cytotoxicity potential of pyrimidine derivatives was evaluated using MTT assay. In this test, the water-soluble yellow MTT dye metabolized to water-insoluble formazan crystals by succinate dehydrogenase mitochondrial enzyme which is active only in the viable cells [24]. Fort that, cells with density about $1x10^4$ cell/well were treated with concentrations arrange from 12.5 -100 µg/mL for 24 hours. DMSO was used to solve the chemicals; 1% DMSO was used as a solvent (negative) control group and 0.5 %Triton-x-100 was used as positive control. After the exposure period, 25 µL of 5 mg/mL MTT solution was added for each well has cell and incubated for further 2 h. The supernatant discarded and 100 µL DMSO was added to the wells. The Plate reader (Epoch, Erlangen, Germany) was used to measure the optical densities (OD) at 590 nm (reference wavelength of 670 nm). The concentrations caused a 50% inhibition of enzyme activity in the cells (IC_{50}) were calculated from dose response curve using Microsoft Excel computar programme, compared to the negative control group and using the equation 1, Results were expressed as mean (n=12) ± standard deviation (± SD).

Equation 1:

Inhibition (%) = 100 – [(corrected mean OD $_{sample}$ × 100) /corrected mean OD $_{solvent\ control}$]

2.4. Oxidative stress induction

Lipid peroxidation assay: The formation of thiobarbituric acid reactive substances (TBARS) accept as evidence of the existence of MDA which is the end-product of lipid peroxidation[25]. For that, about $1\text{-}2x10^6$ cells/flask were incubated for 24 hours with 1, 5 and 10 µg/mL, and with 1% DMSO or H_2O_2 at 100 µM concentration as negative or positive control, resepectivly. After that, the exposed cells were trypsinized, counted and adjusted to 10^6 cells/mL, the cells were exploded by repeated freeze-thaw three times and centrifuged at 1500 rpm for three minutes. 1 mL of cell supernatant was mixed with 500 µL of trichloroacetic acid- sodium (meta) arsenite solution (28%) and centrifuged at 2000 rpm for 15 minutes. 250 µL of thiobarbituric acid was added to 1 mL of the supernatant, mixed and incubated in a water bath at 90ºC for 15 minutes. After cooling, the absorbance was measured by spectrophotometer (Epoch, Erlangen, Germany) at 532 nm. 1,3,3-tetraethoxypropane was used as the standard, and the standard curve (0.312 – 15 nmol/mL) was used to calculate MDA levels in the cells. Results were expressed as nmol/g protein.

CAT assay: The activity of CAT in the cells homogenates was assayed according to the method of Aebi (1984) [26]. In this method, the rate constant of hydrogen peroxide decomposition by catalase enzyme assessed by determining the decrease in the absorbance at 240 nm, and calculate according to the first-order kinetic equation (equation 2).

Equation 2:

$k=(2.3/t)\ (\log A_0/A_1)$.

K: the rate constant of a first-order reaction.

t: time.

A_0: absorbance of standard tube.

A_1: absorbance of test tube.

For that, cells exposed to pyrimidine derivatives were collected, accounted, adjusted to 10^6 cells/mL, and ruptured using the repeated freeze-thaw method for three times. 20 µL of cellular supernatant was mixed with 2 mL of phosphate buffer and added to a quartz spectrophotometer cuvette. 1 mL of 40 mM H_2O_2 was mixed, and immediately the decrease in absorbance (at 240 nm) of the sample was monitored for 3 minutes at 30-secondes intervals. Specific activity was expressed as k per mg of protein.

Glutathione level: The GSH levels were assessed by ELISA kit (Elabscience, Wuhan -China) according to the manufacturer's instructions. Briefly, After the treatment of cells with 1, 5 and 10 µg/mL pyrimidine derivatives for 24 hours, the cells harvested, accounted, adjusted to 10^6 cell/mL, and ruptured by a freeze-thaw method. The cells were centrifuged at 2000 rpm for 15 minutes and the supernatants were used. 50 µL of supernatants were added to wells with 50 µL of biotinylated detection solution and Incubated at 37°C for 45 minutes. After washing three times, 100 µL of Horseradish Peroxidase (HRP) conjugate solution was added to each well and incubated at 37°C for 30 minutes. Then, the wells were washed, and 90 µl of substrate reagent was added to the wells and incubated at 37°C for 15 minutes. the absorbance at 450 nm was measured directly after adding 50 µL of a ready to use stop solution provieded with kit. The GSH levels were calculated according to the standard curve and the results were expressed as µg/g protein.

Protein amount assessment: Bradford (1976) method [27] was used to evaluate the protein amount in the cell suspensions used in oxidative stress evaluation. For that, in a microplate, 150 µL of cell suspension was mixed with 7.5 µL of Bio-Rad protein assay kit solution. The plate incubated in dark at room

temperatures for 10 minutes. Then the absorbance was measured at 595 nm. Protein amounts were calculated according to the BSA standard curve (0.0075 – 0.5 mg/mL).

2.5. Statistical analysis

Cytotoxicity assays were done in triplicates and repeated in four independent days (n=12). Oxidative damage evaluation assays were done in triplicates in three independent days (n=9). Data are expressed as mean ± standard deviation (SD). The cells exposed to DMSO (1%) were evaluated as negative (solvent) control. The significance of differences between negative control and exposed cells was evaluated using a one-way analysis of variance (ANOVA) and post hoc -Dunnett's test by SPSS version 23.0 for Windows (SPSS, Inc.). p-value less than 0.05 was chosen as the level of significance.

3. Results

3.1. Cytotoxicity evaluation

MTT test was performed to evaluate the cytotoxic potentials of the previously synthesized pyrimidine derivative compounds (concentrations 12.5-100 µg/mL) on the A549 cell line. Results showing that concentration-dependent cytotoxicity are given in Table 1. IC_{50} values for these compounds were calculated to be between 16.7-41.5 µg/mL (Figure 2).

Table 1. MTT test results of pyrimidine derivative.

Compound	Cell death Ratio ± SD (%) at exposure concnetrations			
	12.5 µg/mL	25 µg/mL	50 µg/mL	100 µg/mL
4OHR-U	16.7 ± 1.2 %	75.3 ± 4.7 %	96.1 ± 8.6 %	95.6 ± 5.3 %
3OH-U	5.7 ± 0.8 %	65.0 ± 5.3 %	94.8 ±5.1 %	97.0 ± 2.5 %
4OHR-T	7.6 ± 0.5 %	45.4 ± 4.4 %	93.8 ± 7.3 %	97.1 ± 9.1 %
4OH-T	24.1 ± 1.6 %	93.0 ±7.2 %	96.2 ± 6.8 %	95.6 ± 4.3 %
3,3'-OH-T	23.1 ± 3.5 %	29.5 ± 2.1 %	71.5 ± 3.3 %	94.3 ± 5.2 %
3,3'-OH-U	8.5 ± 1.5 %	66.3 ± 6.1 %	91.0 ± 7.1 %	95.7 ± 8.4 %

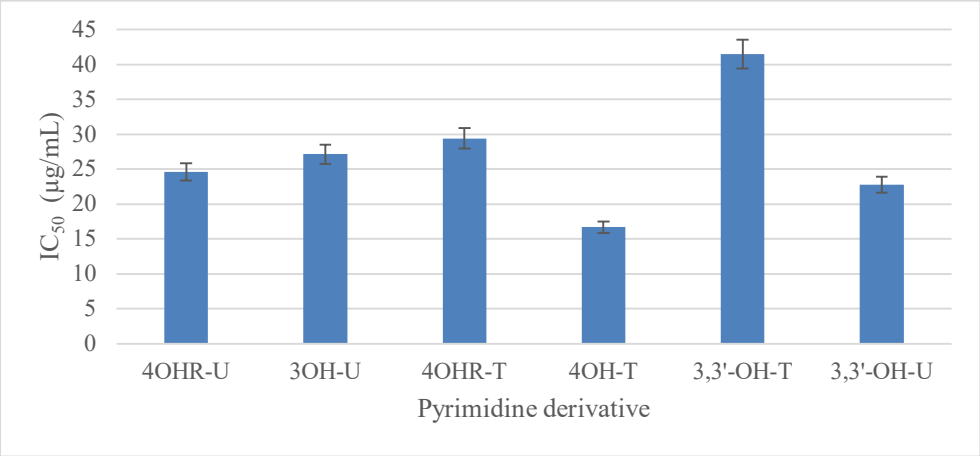

Figure 2: The cytotoxicity activity of pyrimidine derivatives by MTT test.

3.2. Oxidative stress induction

Results indicate that oxidative stress could be a mechanism of the tested pyrimidine derivative; that 4OHR-T significantly ($p \leq 0.05$) induced a change in CAT activity (≤2-folds) and increases the GSH levels (≤1.9-folds) at all test concentrations. 4OH-T similarly induced changes in CAT activity (≤1.8-folds), decreased GSH levels (≤1.4-folds) at 5 and 10 µg/mL groups and increase MDA level at 1 µg/mL group. 3,3'-OH-T induced a significant increase in MDA level (≤4.4-folds) and CAT activity (≤1.3-folds) at all concentrations and decreased GSH level at the highest test concentration (10 µg/mL). Both 3OH-U and 3,3'-OH-U induced a significant increase in MDA level (≤1.3-folds and ≤1.9-folds, respectively) at 5 and 10 µg/mL groups. While 4OHR-U did not significantly change the tested parameters (Table 2).

Table 2. The oxidative stress induction by pyrimidine derivatives.

	Concentration (µg/mL)	MDA (nmol/g protein) ± SD	GSH (µg/g protein) ± SD	CAT (K/g protein) ± SD
Negative Control	0	0.617 ± 0.28	0.280 ± 0.056	6.42 ± 0.55
4OHR-U	1	0.804 ± 0.46	0.313 ± 0.092	5.77 ±0.12
	5	0.745 ± 0.24	0.323 ± 0.086	5.83 ±0.14
	10	0.622 ± 0.11	0.285 ± 0.122	4.52 ±0.28
3OH-U	1	0.616 ± 0.01	0.293 ± 0.053	4.88 ±1.20
	5	0.782 ± 0.06*	0.316 ± 0.096	5.17 ±0.90
	10	0.816 ± 0.155*	0.306 ± 0.038	6.09 ±0.0
4OHR-T	1	0.737 ± 0.054	0.545 ± 0.106*	7.33 ±1.30
	5	0.748 ± 0.223	0.346 ± 0.055*	8.63 ±0.0*
	10	0.620 ± 0.167	0.376 ± 0.094*	12.09 ±0.0*
4OH-T	1	1.171 ± 0.011*	0.321 ±0.110	8.80 ±0.50*
	5	0.635 ± 0.184	0.207 ± 0.087†	9.21 ±0.0*
	10	0.709 ± 0.091	0.232 ± 0.082†	11.45 ±0.0*
3,3'-OH-T	1	1.077 ± 0.212*	0.277 ± 0.064	7.95 ±0.0*
	5	2.689 ± 1.321*	0.327 ± 0.102	8.09 ±0.0*
	10	1.276 ± 0.425*	0.207 ± 0.91†	8.47 ±0.0*
3,3'-OH-U	1	0.624 ± 0.255	0.282 ± 0.077	4.49 ±0.25
	5	1.173 ± 0.397*	0.310 ± 0.052	4.15 ±0.19
	10	0.926 ± 0.245*	0.254 ± 0.150	5.05 ±0.88

† significant decrease compared to the negative control, ($p \leq 0.05$).
* significant increase compared to the negative control, ($p \leq 0.05$).

4. Discussion

Different studies show that pyrimidine derivatives are pharmacologically important promising compounds as anti-cancer, antiviral, and antibacterial agents [1,13]. A previously synthesized new antibacterial active 6 pyrimidine derivatives were evaluated in A549 cells for cytotoxicity and different oxidative stress endpoint. As these compounds did not evaluated previously for cytotoxicity; their activities were compared and discussed according to other pyrimidine derivatives that have similar features. In the study conducted by Altıparmak D. (2018) the cytotoxic effect of nucleoside analogs containing pyrimidine ring was investigated with the National Cancer Institute (NCI) anticancer activity test (sulforodamine B / SRB) using Huh7 liver, HCT116 colon, and MCF7 breast cancer cell lines. The results show that nonsignificant cytotoxicity for these compounds. However, it was observed that 4- (3,4-dichlorophenyl) piperazine-1 (β-D-ribofuranosyl) -5- (methyl) -2 (1H) -pyrimidinone compound showed a cytotoxic effect close to fludarabine in the MCF7 breast cells (IC_{50}: 17.5 µM, Fludarabine IC_{50}: 15.2 µM) [12]. In other study, the cytotoxicity of some new 2,5-disubstituted 1,3,4-oxadiazole derivatives containing pyrimidine ring was evaluated at 3.9-500 mg /mL concentrations in A549 cells. Their results indicated that some of the synthesized compounds as, 1-phenyl-2- [5- (3- (pyrimidin-2-yl) thio) propyl) -1,3,4-oxadiazol-2-yl) thio] ethane-1-on (IC_{50}: 68.33 mg /mL) and 1- (4-methoxyphenyl) -2- [5- (3- (pyrimidin-2-yl) thio) propyl) -1,3,4-oxadiazol-2-yl) thio] ethane-1-on compound (IC_{50}: 95 mg /mL), have important anticancer activities against the A549 cell line [28]. Kahriman et al. (2019) indicated that the new 2,4,6-tri-substituted pyrimidine and their N-alkyl bromide derivatives inhibit the cell proliferation and caused a cytotoxic effect, (IC_{50}: 2-10 µg/mL), in MCF7, A549, Hep3B, C6, Hela, Ht29 and FL cell lines [29]. The cytotoxicity of thiazole-pyrimidine derivatives synthesized as an anti-candid agent was evaluated by Cytotoc-XTT 1 cytotoxicity kit in A549 and NIH3T3 mouse embryonic fibroblast cell lines. Results indicated that while some compounds like 2- (3,4-diphenyl-3H-thiazole-2-ylidene) amino-4,6-dimethylpyrimidine have low cytotoxicity, other compounds like 2- [3-phenyl-4- (4-nitrophenyl) -3H-thiazol-2-ylidene] amino-4,6-dimethylpyrimidine compound have high cytotoxic potential with IC_{50} value 1.27 and 25.11 µg /mL in NIH3T3 and A549 cells, respectively [30]. Gömez-Jeria et al. (2015) evaluate the cytotoxicity of some pyrimidine-benzimidazole derivatives in MCF-7, MGC-803 human stomach cancer, EC-9706 human esophageal cancer, and SMMC-7721 human liver cancer cell lines. The IC_{50} values calculated to be between 0.03-1.83 µM. Besides that, they reported an antiproliferative activity of pyrimidine-benzimidazole derivatives [31]. Similarly, Xie et al. (2009) reported a strong inhibitory activity of 2,4,5-substructured pyrimidine derivatives against the BEL-7402 hepatocellular carcinoma cell line ($IC_{50} < 0.10$ µM) [5].

In the current study, the cytotoxicity of six pyrimidine-derived compounds was evaluated in A549 cells using

MTT test. Our results indicate that the tested pyrimidine-derivatives induce a concentration-dependent cell death with IC_{50} values calculated to be between 16.7-41.5 µg/mL. Of these, the 4OH-T compound with an IC_{50} value of 16.7 µg/mL is the most toxic. While 3,3'-OH-T compound has the lowest cytotoxicity with an IC_{50} value of 41.5 µg/mL. In compare with the previous data which show that antineoplastic agents have different IC_{50} values in A549 cell line, where the IC_{50} calculated to be 0.53 for epirubicin, 3.59 µg/mL for 5-fluorouracil and for cisplatin, 20.56 µg/mL for paclitaxel, while it was calculated to be 41.31 µg/mL for carboplatin and to be 60.24 µg/mL for etoposide [32]; The tested pyrimidine-derivatives show a good cytotoxic activity, and these results indicate that these pyrimidine-derivatives could be developed and studied as antineoplastic compounds.

The potential for oxidative damage related to the compounds containing pyrimidine derivative was not estimated before. In order to evaluate the oxidative damage potentials, MDA, GSH levels, and catalase activity were determined. In general, the tested pyrimidine derivatives, except 4OHR-U, induced significantly oxidative stress either by increasing the level of MDA (0.616 – 2.96 nmol/g protein), increase CAT activity (4.15 – 12.1 K/g protein) or change the level of GSH (0.207 – 0.545 µg/g protein) after 24 hour exposure period.

In conclusion, pyrimidine derivatives are pharmacologically important compounds; The previously synthesized and shown to have high antibacterial activity, have also cytotoxic activity against human lung cancer cells. Besides that, compounds induced oxidative damages in the cells, which could be the mechanism of action of these compounds in the cells. The tested pyrimidine derivatives are promising, our results could be used as preliminary data to design further *in vitro, in vivo* studies to develop these derivatives and evaluate their pharmacological and toxicological activities.

Acknowledgment

This study was supported by TUBITAK 2209-A University Students Research Projects Support Program.

Conflicts of interest

The authors state that there is no conflict of interest.

References

[1] Mercan S., Synthesis and Investigation of Biological Effects of Some New Pyrimidine and Pyridine Derivative Compounds [Bazı Yeni Pirimidin ve Piridin Türevi Bileşiklerin Sentezi ve Biyolojik Etkilerinin İncelenmesi], Master Thesis, Gazi University Institute of Science: Ankara, 2010.

[2] Tunç A., Synthesis of New Mercapto Pyrimidine Ringed Schiff Base and Metal Complexes, [Yeni Merkapto Pirimidin Halkalı Schiff Bazı ve Metal Komplekslerinin Sentezi], Master Thesis, Gaziantep University Institute of Science: Gaziantep, 2014.

[3] Gianolio D. A. and McLaughlin L. W., Synthesis and Triplex Forming Properties of Pyrimidine Derivative Containing Extended Functionality, *Nucleos. Nucleot. Nucl*., 18 (1999) 1751-1769.

[4] Patel A. A. and Mehta A. G., Synthesis of novel heterocyclic compounds and their bioglogical evalution, *Der Pharma Chem*., 2(1) (2010) 215-223.

[5] Xie F., Zhao H., Zhao L., LOU L. and Hu Y., Synthesis and biological evaluation of novel 2,4,5-substituted pyrimidine derivatives for anticancer activity, *Bioorg. Med. Chem. Lett.*, 19 (2009) 275-278.

[6] Hockova D., Holy A., Masojı´dkova M., Andrei G., Snoeck R., Clercq E. and Balzarin J. 5-Substituted - 2, 4- diamino – 6 - [2 (phosphonomethoxy) ethoxy] pyrimidines Acyclic Nucleoside Phosphonate Analogues with Antiviral Activity, *J. Med. Chem.,* 46 (2003) 5064-5073.

[7] Kumar R., Semaine W., Johar M., Tyrrell D. L. J. and Agrawal B., Effect of various pyrimidines possessing the 1-[(2-Hydroxy-1-(hydroxymethyl) ethoxy) methyl] Moiety, able to mimic natural 2¢-Deoxyribose, on wild-type and mutant hepatitis B virus replication, *J. Med. Chem.,* 49 (2006) 3693-3700.

[8] Wanga Y., Chen F., Balzarini J., Clercq E. D. and Pannecouque C., Synthesis and Anti-HIV Activity of 5-Alkyl-6-(1-naphthylmethyl) pyrimidin-4(3H)-ones with a Mono- or Disubstituted 2-Amino Function as Novel *Dihydro-Alkoxy-Benzyl-Oxopyrimidine/ (DABO) Analogues, *Chem. Biodıvers.,* 5 (2008) 168-176.

[9] Amir M., Javed S. A. and Kumar H., Synthesis and biological evaluation of some 4-(1H-indol-3-yl)-6-phenyl-1,2,3,4-tetrahydropyrimidin-2-ones/thiones as potent anti-inflammatory agents, *Acta Pharm.*, 58 (2008) 467–477.

[10] Kappe C. O., 4-Aryldihydropyrimidines via the Biginelli Condensation: Aza-Analogs of Nifedipine-Type Calcium Channel Modulators, *Molecules,* 3 (1998) 1-9.

[11] Baysal K., Synthesis, Characterization, Antibacterial and Antioxidant Properties of Pyrimidine Ringed Schiff Base and Metal Complexes Containing Azo Group [Azo Grubu İhtiva Eden Pirimidin Halkalı Schiff Bazı ve Metal Komplekslerinin Sentezi, Karakterizasyonu, Antibakteriyal ve Antioksidan Özelliklerinin İncelenmesi]. Master Thesis, Yüzüncü Yıl University Institute of Science: Van, 2013.

[12] Altıparmak D., Studies on the Synthesis, Structure illumination and Cytotoxic Activities of Some Newly Synthesized Purine and Pyrimidine Nucleoside Analogue Compounds [Yeni Sentezlenmiş Bazı Pürin, Pirimidin Nükleozit Analoğu Bileşiklerin Sentez, Yapı Aydınlatması ve Sitotolsik Aktiviteleri üzerinde Çalışmalar]. Ph.D. Thesis, Ankara University Institute of Health Sciences, Department of Pharmaceutical Chemistry: Ankara, 2018.

[13] Fandaklı S., Synthesis and Biological Activities of Pyrimidine Derivative Compounds from Calcons by Microwave Method [Pirimidin Türevi Bileşiklerin Kalkonlardan Mikrodalga Yöntemi ile Sentezi ve Biyolojik Aktiviteleri]. PhD Thesis, Karadeniz Technical University, Institute of Science: Trabzon, 2016.

[14] Fandaklı S., Kahriman N., Yücel T. B., Alpay Karaoglu S. and Yaylı N. Biological evaluation and synthesis of new pyrimidine-2(1H)-ol/-thiol derivatives derived from chalcones using the solid phase microwave method, *Turk. J. Chem.,* 42 (2018) 520-535.

[15] Rangappa S. K., Kallappa M. H., Ramya V. S. and Mallinath H. H., Analgesic, anti-pyretic and DNA cleavage studies of novel pyrimidine derivatives of coumarin moiety, *Eur. J. Med. Chem.*, 45 (2010) 2597-2605.

[16] Chaturvedi A. M., Mishra Y. K. and Rajawat V., Synthesis of Pyrimidine 2-ol/thiol Derivatives of Benzimidazole as a Ligand and Their Bi (III) Metal Complexes by Conventional as Well as Microwave Technique. Am. J. Phytomed., *Clin. Ther.*, 3 (2015) 383-393.

[17] Khan S. A., Asiri A. M., Kumar S. and Sharma K., Green synthesis, antibacterial activity and computational study of pyrazoline and pyrimidine derivatives from 3-(3,4-dimethoxy-phenyl-1-(2,5-dimethylthiophen-3-yl)-propenone, *Eur. J. Chem.,* 5 (2014) 85-90.

[18] Patel A. A. and Mehta A. G., Synthesis of novel heterocyclic compounds and their biological evaluation, *Der Pharma Chem.* 2 (2010) 215-223.

[19] Kachroo M., Panda R. and Yadav Y., Synthesis and biological activities of some new pyrimidine derivatives from chalcones, *Der Pharma Chem.* 6 (2014) 352-359.

[20] Mohsin H. F., Synthesis of some New Pyrimidines from Chalcone Containing an Imin Group, *Asian J. Research Chem.,* 6 (2013) 849-854.

[21] Baddar F. G., Al-Hajjar F. H. and El-Rayyes N. R., Acetylenic ketones. Part V†. Reaction of acetylenic ketones with thiourea and some of its derivatives, *J. Heterocyclic Chem.,* 15 (1978) 105-112.

[22] Cragg G. M. and Newman D. J., Ethnomedicine and Drug Discovery In: Maurice M. Iwu M. M. and Wootton J. (Eds). Advances in Phytomedicine. Amsterdam: Elsevier Science, 2002.

[23] Jeswani G. and Paul S. D., Recent Advances in the Delivery of Chemotherapeutic Agents., In: Grumezescu M. A. (Eds). Nano- and Microscale Drug Delivery Systems, Amsterdam: Elsevier Science, 2017.

[24] Alley M. C., Scudiero D. A., Monks A., Hursey M.L., Czerwinski M.J., Fine D.L., Abbott B.J., Mayo J.G., Shoemaker R.H. and Boyd M.R. Feasibility of drug screening with panels of human tumor cell lines using a microculture tetrazolium assay. *Cancer Res.*, 48 (1988) 589–601.

[25] Buege J. A. and Aust S. D. Lactoperoxidase-catalyzed lipid peroxidation of microsomal and artificial membranes. *Biochimica et Biophysica Acta.*, 444 (1976) 192. 26.

[26] Aebi H. Catalase. In Methods of Enzymatic Analysis, Bergmeyer HU (ed.). Academic Press: New York, 1974; 673-677.

[27] Bradford M. Protein reaction with dyes. *Anal. Biochem.,* 72 (1976) 248-251.

[28] Kaya B., Kaplancıklı Z. A., Yurttaş L. and Çiftçi G. A. Synthesis and biological evaluation of some new pyrimidine bearing 2, 5-disubstituted 1, 3, 4-oxadiazole derivatives as cytotoxic agents, *Turkish J. Biochem.*, 42(2) (2016) 131-137.

[29] Kahriman N., Serdaroğlu V., Peker K., Ayın A., Usta A., Fandaklı S. and Yaylı N. Synthesis and Biological evaluation of new 2,4,6-trisubstituted pyrimidines and their N-alkyl derivatives, *Bioorg. Chem.,* 8 (2019) 580–594.

[30] Zitouni-Turan G., Altıntop M. D., Kaplancıklı Z. A., Özdemir A., Demirci F., Ilgın S., Atlı Ö. and Göger G. Synthesis and Evaluation of Thiazole – Pyrimidine Derivatives as New Anticandidal and Cytotoxic Agents, *Pharm. Chem. J.*, 48 (2014) 452-5.

[31] Gömez-Jeria J. S. and Robles-Navvarro A. Quantum-Chemical Study of the Anticancer Activity of Pyrimidine Benzimidazole Hybrids against MCF-7, MGC-803, EC-9706 and SMMC-7721 cell lines, *Res. J. Pharm. Biol. Chem. Sci.*, 6(2) (2015) 775.

[32] Ulukaya E., Ozdikicioglu F., Oral A. Y., Demirci M. The MTT assay yields a relatively lower result of growth inhibition than the ATP assay depending on the chemotherapeutic drugs tested, *Toxicol. In Vitro.*, 22(1) (2008) :232-9.