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CELLULAR AND MOLECULAR BIOLOGY

Genotoxicity evaluation of a new phthalazine substituted β-lactam derivative in human lymphocytes

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Abstract: The aim of present study, to evaluate the genotoxic potential of 1-(4-(3,3-dimethyl-1,6-dioxo-2,3,4,6,11,13-hexahydro-1H-indazolo[1,2b] phthalazine-13yl) phenyl)-2-phenylazetidine-3-yl-acetate which was synthesised assuming that it may be a pharmaceutical raw material and found to inhibit human carbonic anhydrase I, II isozymes. To determine the genotoxic potential of this phthalazine substituted β-lactam compound, chromosomal aberration (CA) and micronucleus (MN) tests were implemented in human peripheral blood lymphocytes. In these tests, lymphocyte cultures were treated with four concentrations (30, 15, 7.5, 3.75 µg/mL) of test compound and simultaneously with negative control (sterile distilled water), solvent control (DMSO) positive control (MMC). According to our results, CA frequencies were significantly increased in two high applied concentrations (30, 15 µg/mL) compared with negative and solvent control. MN frequencies were significantly increased in three applied concentrations (30, 15, 7.5 µg/mL) except lowest concentration (3.75 µg/mL) compared with solvent control. Mitotic indices were also affected by treatment with test compound. The obtained results provide evidence to demonstrate that new phthalazine substituted β-lactam derivative can exert genotoxic and cytotoxic effects in peripheral human lymphocytes especially at high concentrations.

Keywords: chromosomal aberration, carbonic anhydrase, β-lactam, micronucleus, phthalazine, toxicology.

INTRODUCTION

β-lactam ring system is a moiety of commonly used antibacterial molecules such as penicillins, cephalosporins, carbapenems, monobactams (Thomas et al. 2016, Mehta & Pathak 2011). Other than antibacterial activity, β-lactam ring containing compounds has antiviral (Küçükgüzel et al. 1999, Sperka et al. 2005), antidiabetic (Goel et al. 2004), antihyperlipidemic (Leach et al. 2001), anti-inflammatory (Kumar & Rajput 2009), vasopressin V1a antagonist (Guillon et al. 2007), central nervous system activator (Goel et al. 2005), antiparkinsonian (Srivastava et al. 1999) and apoptosis inductor activity (Kazi et al. 2004). Additionally, in recent literatures, β-lactam substrates have been reported as potent inhibitors of some human enzymes such as serine proteases, (Turan et al. 2016), tryptase (Bisacchi et al. 2004), matrix metalloproteases (Cainelli et al. 2003), trombin (Han et al. 1995), chymase (Aoyama et al. 2001) and carbonic anhydrases (Turan et al. 2016, Berber et al. 2015). The broad and strong bioactivities of β-lactam derivatives have established them as one of the biologically prominent scaffolds in pharmaceutical development (Thomas et al. 2016).

Carbonic anhydrase enzyme inhibitory agents also attract attention in pharmaceutical chemistry. The carbonic anhydrase enzymes that show a widespread distribution in human tissues, catalyze the reversible hydration of carbon dioxide: $CO_2 + H_2O \leftrightarrow HCO_3^- + H^+$. This reaction plays role in many important physiological and pathological processes in the human body such as fluid secretion, pH control, ion transport, bone resorption, severeal biosynthetic reactions, calcification, epiloptegenezis and tumoregenesis (De Simone et al. 2013, Supuran & Scozzafava 2000, 2007, Supuran 2001). Untill now, 12 active carbonic anhydrase isozymes (I, II, III, IV, VA, VB, VI, VII, IX, XII, XIII, XIV) have been isolated from different human tissues. Carbonic anhydrase I and II are physiologically abundant and widely distributed isozymes (Supuran 2008, Wilkinsin et al. 2007). Carbonic anhydrase inhibitor (CAI) pharmaceuticals are in clinical use for more than 50 years as diuretic, antiglaucoma and entiepileptic agents. However, since available CAIs don't have isoenzyme selectivity, they cause many systemic side effects on people using these drugs. Therefore, studies are underway to develop new CAIs targeting specific carbonic anhydrase isozymes. In addition, researches on the synthesis of CAI that can be used in the treatment of cancer, obesity, osteoporosis and infections continue (Supuran 2001).

For the last few decades, the synthesis of novel nitrogen-containing heterocyclic compounds has atrracted a great deal of interest in drug development. Among a large variety of nitrogen-containing heterocyclic compounds, the heterocycles containing phthalazine moiety arouses considerable attention due to their various biological activities (Sayyafi et al. 2008, Shaterian et al. 2008). Phthalazine derivatives have been reported to exhibit numerous activities including anticonvulsant (Grasso et al. 2000, Nomoto et al. 1990), vasorelaxant (Watanabe et al. 1998, Demirayak et al. 2004), antiinflammatory (Sharma et al. 2014, Dogruer et al. 2004), antibacterial, antifungal (Sönmez et al. 2006, Sinkkonen et al. 2002), antitumor (Wasfy et al. 2013, Zhang et al. 2010), antihiperglisemic (Davis 2013), antihypertensive (Abd El-Ghaffar et al. 2011), antihistaminic (Tatsumi et al. 1980) and cytotoxic (Kim et al. 2008, Rodriguez-Ciria et al. 2003, Zhai et al. 2008, Zhang et al. 2010, Xue et al. 2014).

Since the beta-lactam and phthalazine derivatives are compounds that have a variety of activities and are the main skeleton of various pharmaceticals used for many years, the compounds carrying these groups in the same structure are likely to exhibit potent biological effects. From this point of view, a new series of phthalazine substituted β-lactam derivatives synthesised at Sakarya University Chemistry Department. Furthermore, their carbonic anhydrase inhibitory activities have been investigated. Among the synthesised compounds, 1-(4-(3,3-dimethyl-1,6-dioxo-2,3,4,6,11,13-hexahydro-1H-indazolo[1,2b] phthalazine-13-yl)phenyl)-2-phenyl-azetidine-3-yl-acetate (IV-b) found most active for human carbonic anydrase I and II enzyme inhibition (Berber et al. 2015). Due to this biological activity, we thought it may be proposed as pharmaceutical raw material. In order to be able to use a synthetic substance as a medicine in the pharmaceutical industry; ligand-receptor interaction studies, pharmacokinetic analyzes, other toxicity tests including genotoxicity should be conducted. In this regard, at present work; we aimed to investigate the genotoxic potential of this new compound with the chromosomal aberration (CA) and micronucleus (MN) tests in human peripheral blood lymphocytes.

MATERIALS AND METHODS

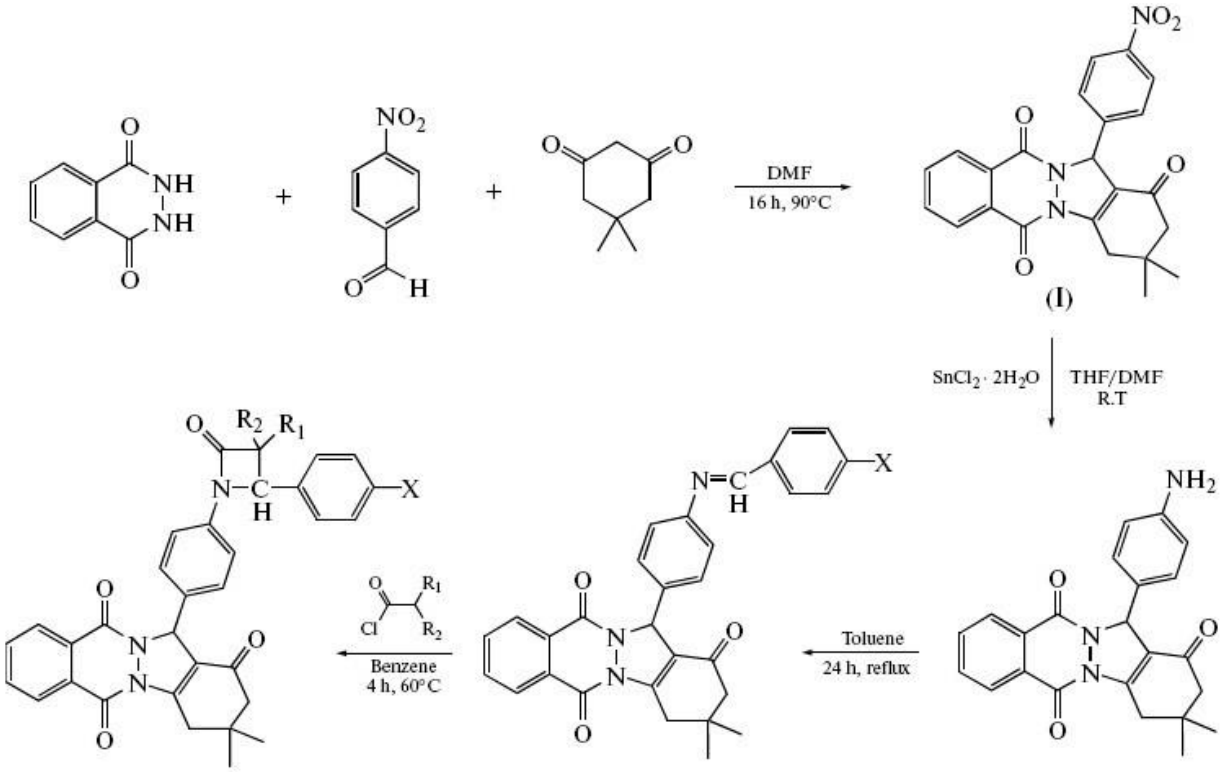
Chemicals

Synthesis, characterization and human carbonic anhydrase I and II inhibitory activity determination of the test compound (IV-b) were performed at Sakarya University Chemistry Department by Berber et al. (2015). The chemical structure and synthesis steps of the test subtance of this research and other derivates are shown in Figure 1. Hereby, we obtained the test substance from Sakarya University, Department of Chemistry.

The other chemicals which were used for genotoxicity tests: Chromosome medium B was obtained from Biochrome (Cas no: F 5023, Berlin, Germany). Mitomycin C (Cas no: 50-07-7), Colchicine (Cas no: 9754), Cytocalasin B (Cas no: 14930-96-2) were obtained from Sigma (St. Louis, MO, USA.).

Collection of Blood Samples

For all genotoxicity tests, peripheral bloods were obtained from 4 healthy humans (non-smokers, aged 20-22 years, 2 male, 2 female) with no known exposure to any drug therapy or mutagenic



	(IVa)	(IVb)	(IVc)	(IVd)	(IVe)	(IVf)	(IVg)	(IVh)	(IVi)	(IVj)
X	–H	–H	$–CH_3$	$–CH_3$	$–NO_2$	$–NO_2$	–OMe	–OMe	–F	–F
R_1	–Cl	$–OCOCH_3$	–Cl	$–OCOCH_3$	–Cl	$–OCOCH_3$	–Cl	$–OCOCH_3$	–Cl	$–OCOCH_3$
R_2	–Cl	$–OCOCH_3$	–Cl	$–OCOCH_3$	–Cl	$–OCOCH_3$	–Cl	$–OCOCH_3$	–Cl	$–OCOCH_3$

Figure 1. Synthesis of phthalazine substituted β-lactam derivatives (Berber et al. 2015).

agent over the past 2 years, with no exposure to ionizing radiation with in the previous 6 months and with no history of chromosome fragility or recent viral infection.

Dose Selection

The applied concentrations were selected according to IC_{50} values which was reported by Berber et al. 2015. The highest applied concentration of test substance was taken as 30 µg/mL in all genotoxicity tests. Other concentrations of test substance were determined as 1/2, 1/4 and 1/8 of the highest concentration. Consequently, 30, 15, 7.5 and 3.75 µg/mL concentrations of test substance were used in both genotoxicity tests. Also, negative, positive and solvent controls were used in both tests.

Chromosomal Aberration Assay

0.2 mL heparinized peripheral blood samples of 4 healthy (2 male and 2 female) donors were cultured in 2.5 mL chromosome medium B and treated with 30, 15, 7.5 and 3.75 µg/mL concentrations of test substance. A negative, a solvent (DMSO; 20 µL) and a positive control (Mytomycin C; 0.2 µg/mL) were also maintained in all treatments. Cells in culture were exposed to test substances for 24 and 48 h. Cultures were incubated for 72 h at 37 °C, and colchicine (final concentration: 0.06 µg/mL) was added to each culture at the 70th h of the incubation. Then, the cells were harvested by centrifugation (1200 rpm for 10 minutes), and the pellet was treated with 0.075 M of KCl for 30 minutes at 37 °C. Cells were centrifuged again and fixed in cold methanol/glacial acetic acid (3:1) solution. The fixation process was repeated three times. Slides were stained with 5 % Giemsa (pH=6.8) in Sorensen buffer for 20–25 minutes, washed in distilled water, dried at room temperature and mounted with entellan.

Micronucleus (MN) Assay

0.2 mL of heparinized venous blood from 4 healty person were added to 2.5 mL of Chromosome Medium B. Human lymphocytes were incubated at 37 °C for 72 h and treated with 30, 15, 7.5 and 3.75 µg/mL concentrations of test substance. A negative, a positive control (mytomycin C; 0.2 µg/mL) and a solvent control (DMSO; 20 µL) were also used in all treatments. After 44 h incubation, Cytocalasin B (5.2 µg/mL) was added to block cytokinesis. Following additional 28 h incubation at 37 °C, cells were harvested by centrifugation (1000 rpm for 10 minutes) and the pellet was treated with hypotonic solution (0.075 M of KCl) for 5 minutes at 4 °C. Then, cells were recentrifuged and fixed three times in cold methanol/glacial acetic acid (3:1). In the last fixative, 1 % formaldehyde was added to preserve the cytoplasm. Slides were prepared by dropping and air-drying. Slides were stained with 5 % Giemsa (pH=6.8) in Sorensen buffer for 13–15 minutes, washed in distilled water, dried at room temperature and mounted with entellan.

Slide Evaluation

100 well-spread first division metaphases including 46±1 chromosomes per donor (total, 400 metaphases per concentration) were analysed for CA assay. The mitotic indices (MI) were also analysed by scoring 3000 cells from each donor (total, 12000 cells per concentration). 1000 binucleated cells per donor (total, 4000 binucleated cells per concentration) were analysed for determining the micronuclei score.

Statistical Analyses

For the statistical analysis of the results, z-test for percentage of abnormal cell, CA/cell, MI, MN (%) were used. Concentration-response relationships were determined from

the regression coefficients for the percentage of abnormal cell, CA/cell, MN (%).

RESULTS

In order to evaluate the genotoxic potential of test compound CA and MN tests were implemented in human peripheral blood lymphocytes. Furthermore, for evaluation of the cytotoxicity, mitotic indices were determined. The results of CAs analysis (number of chromosomal aberration types, abnormal cell % and chromosomal aberration/cell) and mitotic indices are shown in Table I. The test substance has induced three types of structural aberrations both 24 and 48 h applications. These aberrations were observed to as chromatid breaks, chromosome breaks and fragments. Chromatid breaks were the most common type of aberrations. This was followed by the fragments in both application periods.

The test substance has increased the abnormal cell (%) in a dose dependent manner (r=0.99 and r=00.97, negative and solvent control respectively) both 24 h and 48 h treatment periods. In 24 h treatment, this increase was found statistically significant at three high applied concentrations (30, 15, 7.5 µg/mL) compared to negative control. When compared to solvent control, test substance has increased the abnormal cell (%) at only highest applied concentration (30 µg/mL). In 48 h treatment, this increase was found statistically significant at two high applied concentrations (30, 15 µg/mL) compared to negative control. When compared to solvent control, results were same as the 24 h treatment.

Table I. Total chromosomal aberrations in human lymphocytes treated with test substance.

Test substance	Treatment		Aberrations						Abnormal cell ± SE (%)	CAs/Cell ± SE	MI ± SE (%)
	Period (h)	Doses (µg/mL)	ctb	csb	f	scu	cte	dc			
Negative Control	24	0,00	5	2	1	-	-	-	1,75±0,66	0,020±0,007	7,09±0,23
Solvent Control	24	10 µL	10	2	2	-	-	-	3,50±0,92	0,035±0,009	6,28±0,22
Positive Control	24	0,10	113	29	15	1	10	2	29,00±2,27	0,425±0,025	3,27±0,16
IV-b	24	3,75	5	2	3	-	-	-	2,50±0,78	0,025±0,008	5,49±0,21***††
		7,5	11	2	4	-	-	-	4,25±1,01*	0,043±0,010	5,99±0,22***
		15	12	4	7	-	-	-	5,00±1,09*	0,058±0,012**	6,25±0,22**
		30	22	7	8	-	-	-	8,75±1,41***††	0,093±0,015***†††	6,17±0,22**
Negative Control	48	0,00	4	1	-	-	-	-	1,50±0,67	0,013±0,006	5,70±0,21
Solvent Control	48	10 µL	7	-	4	-	-	-	2,75±0,82	0,028±0,008	5,23±0,20
Positive Control	48	0,10	74	41	7	-	10	1	25,75±2,19	0,333±0,024	2,44±0,14
IV-b	48	3,75	4	1	1	-	-	-	1,50±0,67	0,015±0,006	4,81±0,20**
		7,5	8	1	3	-	-	-	3,00±0,85	0,030±0,009	3,56±0,17***†††
		15	11	5	8	-	-	-	5,00±1,09**	0,060±0,012***†	4,48±0,19***††
		30	25	5	11	-	-	-	9,25±1,45***†††	0,103±0,015***†††	4,75±0,19**

Four hundred metaphases were scored for each treatment for CAs and 12000 metaphases were scored for each dose level for the MI. ctb, chromatid break; csb, chromosome breaks; f, fragment; scu, sister chromatid union; cte, chromatid exchanges; dc, dicentric chromosomes; SE, standard error. *Significantly different from the negative control; P<0,05 (z test), **Significantly different from the negative control; P<0,01 (z test), ***Significantly different from the negative control; P<0,001 (z test), †Significantly different from the solvent control; P<0,05 (z test), †† Significantly different from the negative control; P<0,01 (z test), ††† Significantly different from the negative control; P<0,001 (z test).

Test substance has increased the number of CA per cell in a dose dependent manner (r=0.99 and r=00.97, negative and solvent control respectively) both 24 h and 48 h treatment periods. This increase was found stastically significant at two high applied concentrations (30, 15 µg/mL) compared to negative control. Also, this increase was found statistically significant at highest applied concentration (30 µg/mL) in 24 h and at two high applied concentrations (30, 15 µg/mL) in 48 h compared to solvent control.

Test substance has decreased the mitotic index in all concentrations both 24 h and 48 h application periods compared to negative control. But compared to solvent control, test substance has decreased the mitotic index at the concentration of 3.75 µg/mL in 24 h application and at 15 and 7.5 µg/mL concentration in 48 h application. These decreases weren't dose dependent in both exposure times (r=-0.16 ve r=-0.21; r=0.31 ve r=-0.08, 24 h, 48 h, negative and solvent control, respectively).

To evaluate possible clastogenic and/or aneugenic effects the cytokinesis-block MN assay was conducted. The results are reported in Table II. The test substance hasn't generated significant difference in the frequency of micronucleus compared to negative control. Whereas test substance has increased the micronucleus frequency significantly at all concenrations except lowest concentration (3.75 µg/mL) compared to solvent control. However, these increases were dose dependent manner (r=0,83 ve r=0,88, negative and solvent control, respectively).

DISCUSSION

Detecting the biological activities (e.g., enzyme inhibition) of new pharmaceutical raw materials isn't enough to suggest them for drug candidates. In chemotherapy, it is essential to treat patients without creating health risks and the safety of pharmaceuticals is more important than their effectiveness. In this respect, chemical substances that are intended to be offered as pharmaceutical raw material should undergo extensive toxicological investigations before applying to human. In genotoxicity researches which are a stage of toxicological investigations, possible damages of the pharmaceutical candidates on genetic material is evaluated. For this purpose, short-term genotoxicity tests are used in *in vivo* or *in vitro* conditions. Since DNA damages caused by genotoxic agents may lead to serious health problems, implementing

Table II. The MN frequency and CBPI in human lymphocytes treated with test subtance.

Test Substance	Treatment		BN cells Scored	Distrubition of BN cells according to the no of MN			MN(%)±SE(%)
	Period (h)	Doses (µg/mL)		(1)	(2)	(3)	
Negative Control	48	0,00	4000	4	1	-	0,150 ±0,055
Solvent Control	48	0,10	4000	2	-	-	0,050 ±0,035
Positive Control	48	0,10	4000	164	5	1	4,425 ±0,318
IV-b		3,75	4000	2	-	-	0,050 ±0,035
		7,5	4000	10	-	-	0,250 ±0,079†
		15	4000	10	-	-	0,250 ±0,079†
		30	4000	12	1	-	0,350 ±0,089††

BN, binucleated; SE, standard error. †Significantly different from the solvent control, P<0,05 (z test); ††Significantly different from the solvent control P<0,01 (z test).

the genotoxicity tests at the beginning of the pharmaceutical development process is a very important principle (Sen 2018). Since our test substance is a compound with the potential to be a pharmaceutical raw material and due to its ability to inhibit carbonic anhydrase I and II isoenzymes, the investigation of its genotoxic potential is necessary.

The most commonly used test systems which are the structural or numerical chromosome abnormalities may be determined for the evaluation of the genotoxicity of chemical subtances are chromosome abnormality and micronucleus methods. In many scientific studies, it has been reported that the using of a single genotoxicity test isn't sufficient solely to detect genotoxic effects. Because, genotoxicity can be formed by a variety of mechanisms and implimentation of the tests with different methods or organisms may provide different results (Au 2007, Şekeroğlu & Şekeroğlu 2011). On account of this, we have used two test systems (CA and MN test) to determine the genotoxic potentials of new phthalazine substituted β-lactam derivative as carbonic anhydrase inhibitor.

There are many studies in the literature by using the CA test for the genotoxic evaluation of β-lactam ring including antibacterials, such as Cloxacillin, Ampicillin, Amoxicillin, Carbenicillin, Ceftriaxone, Cephalosporin. The majority of these studies have reported positive *in vitro* effects which were seen only at moderate to very high β-lactam concentrations (İstifli & Topaktaş 2010). Zavarise et al. (1984) has researched the chromosomal aberrations in lymphocyte cultures exposed to Cloxacillin at different concentrations. Researchers had reported high concentrations of Cloxacillin were induced chromosomal aberrations in human lymphocyte cultures similar to the results we found in our study.

Stemp et al. (1989) has investigated *in vitro* clastogenic potential of three β-lactam antibiotics (Ampicillin, Carbenicillin and Penicillin VK) using cultured human lymphocytes. Neither Ampicillin nor Carbenicillin test concentrations up to 10 mg/mL were induced significant increases in chromosome damage. On the other hand, *in vitro* Penicillin VK's concentrations down to of 1.25 mg/mL were induced a dose-related increase in chromosome and chromatid gaps and breaks. Jaju & Ahuja (1984) has studied *in vitro* genotoxic effects of Ampicillin and Carbenicillin in human lymphocytes. Both drugs weren't affected the frequency of chromosome aberrations, satellite associations, mitotic index and cell turnover rate at plasma level concentrations. However, all these parameters were affected at higher concentrations. When the concentration values are compared between the above-mentioned studies and our studies, the concentration values we evaluated are much lower. In parallel with these data, the genotoxic profile of this newly synthesized substance may be deduced to be low.

Metovic et al. (2013) has analyzed Ceftriaxone genotoxicity in a 48-hour culture of human peripheral blood lymphocytes by standard CA test. A positive correlation was observed between the increase in the frequency of structural aberrations and ceftriaxone concentrations (0.15, 0.25, 0.50 mg/mL). Fahmy & Diab (2009) has evaluated the genotoxic effect of Cefotaxime (a Cephalosporin derivative) in mouse spermatocytes by using chromosomal aberration test (260, 520 and 1040 mg/kg b.wt for 4, 7 and 10 days.). Significant increases were observed in the percentage of structural and numerical chromosomal aberrations in spermatoids of 520 and 1040 mg cefotaxime/kg b.wt. treated mice. Similarly, Donya (2002) has investigated the ability of the two Cephalosporin antibiotics Cefadroxil and Cefaclor to induce

chromosomal abnormalities in mouse spermatocytes. 40, 80, 160 mg/kg b.wt. of Cefadroxil and 20, 40, 80 mg/kg b.wt. of Cefaclor and samples were taken 24 h after the treatment. The percentage of chromosomal aberrations in diakinesis-metaphase I spermatocytes was increased in a dose dependent manner and found to be statistically significant after high and repeated doses. The results of our studies are consistent with the aforementioned works but in our study, the highest concentration of test substance was 30 µg/mL but concentrations in the studies mentioned above is much higher than our concentrations.

There are also studies on genotoxic evaluation of beta-lactams with MN test. Anlas & Ustuner (2016) has investigated the genotoxicity of Amoxicillin in rainbow trout (*Oncorhynchus mykiss*) erythrocytes and they reported that concentrations of 80 and 160 mg/kg b.wt. amoxicillin weren't caused any genotoxic effects. Otherwise, the highest concentration of Amoxicillin (320 mg/kg b.wt.) was induced micronucleus frequency. Other studies parallel to this study are Isitifli & Topaktaş (2010) and Stemp et al. (1989). In these studies, Stemp et al. (1989) has investigated the clastogenic potential of Ampicillin, Carbenicillin and Penicillin VK by using *in vivo* rat micronucleus assay and the results showed all drugs were found to be inactive in the *in vivo* rat micronucleus test. Isitifli & Topaktaş (2010) has also evaluated the genotoxicity of 400, 600, 800, 1000 µg/mL Amoxicilin in human peripheral blood lymphocytes with SCE, CA and MN tests in the presence and absence of metabolic activator. Amoxicillin weren't induced CAs and formation of MN both in the presence and absence of metabolic activator. Additionally, in 24 h Amoxicillin treated cultures; mitotic index has generally reduced when compared with the negative control but not compared with the solvent control. In present study, we have observed that MN frequencies were significantly increased when compared with the solvent control except lowest concentration. Furthermore, the test substance was significantly decreased mitotic index when compared with the negative control at all treatment times and concentrations. However, when compared with the solvent control, we observed that mitotic index wasn't significantly decreased at all groups.

Since the chromosomal damage formation mechanisms are similar in different tissues, the damage levels in the lymphocytes reflects the damage levels in the cancer-prone tissues. For this, it is appropriate to use the DNA damages in lymphocytes to assess the genotoxicity or cancer risk (Bonassi et al. 2000, Albertini 2003, Norppa et al. 2006). In literature we haven't found any research on genotoxicity of phtalazine derivatives. But there are many researches about cytotoxicity of several phthalazine derivatives. These researches were done on cancer cell lines (Kim et al. 2008, Rodrıguez-Ciria et al. 2003, Zhai et al. 2008, Zhang et al. 2010, Xue et al. 2014). Arif et al. (2006) has evaluated the cytotoxicity of newly synthesized phthalazine derivatives including copper and platinum complexes in human breast cancer cell lines. The cells were incubated with the compounds (100 µM) for 72 h and cytotoxicity, apoptosis and DNA content were measured by flow cytometry. Their results have suggested that the parent (H1-2), copper (C1-2) and platinum (P1-2) derivatized compounds were relatively more active in inducing apoptosis and cell killing in both human breast cancer cell lines, MDA-MB-231 cells being the more sensitive. Other compounds have showed weak or no response towards these parameters except H-5 causing 40 % apoptosis in MDA-MB-231 cells. Kim et al. (2004) has synthesized a series of phthalazine derivatives and evaluated their *in*

vitro cytotoxicity against several human tumor cell lines. Most of the tested compounds were showed significantly higher potential cytotoxic activity than that of the reference compounds. Neftel & Hübscher (1987) has emphasized the antiproliferative effects of β-lactam antibiotics in cultured rat liver, human fibroblast and human lymphoid cells. Marie et al. (1986) has also showed antiproliferative effects of Piperracilin, Ceftazidime and Ceftriaxone and Mezlocilin on granulocytes *in vitro*. In this study, we have also suggested that the test substance was cytotoxic *in vitro* at the concentrations we used on peripheral human lymphocytes.

As a result, all the obtained results provide evidence that this compound can exhibit genotoxic and cytotoxic effects on peripheral human lymphocytes in culture especially at high concentrations. So, we can't say that this new compound safe for therapeutic drugs with these results. Also, its cytotoxic and genotoxic profile must be completely identified on malignat and/or abnormal cell systems and the obtained results should be evaluated together to fully unleash its potential.

REFERENCES

ABD EL-GHAFFAR NF, GHANEM HM & ZAK HM. 2011. Synthesis and biochemical evaluation of some substituted phthalazines. J Am Sci 7(4): 771-781.

ALBERTINI RJ. 2003. Mechanistic insights from biomarker studies: somatic mutations and rodent/human comparisons following exposure to a potential carcinogen. IARC Sci Pub 157: 153-177.

ANLAS C & USTUNER O. 2016. Genotoxic assessment of amoxicillin in rainbow trout (Oncorhynchus mykiss) by comet assay and micronucleus test. Fresenius Environ Bull 25(12): 5358-5364.

AOYAMA Y, UENAKA M, Kİİ M, TANAKA M, KONOİKE T, HAYASAKİ-KAJİWARA Y, NAYA N & NAKAJİMA M. 2001. Design, synthesis and pharmacological evaluation of 3-benzylazetidine-2-onebased human chymase inhibitors. Bioorg Med Chem Lett 9(11): 3065-3075.

ARIF JM, KUNHI M, BEKHIT AA, SUBRAMANIAN MP, AL-HUSSEİN K, ABOUL-ENEIN HY & AL-KHODAIRY FM. 2006. Evaluation of apoptosis-induction by newly synthesized phthalazine derivatives in breast cancer cell lines. Asian Pac J Cancer Prev 7(2): 249-252.

AU WW. 2007. Usefulness of biomarkers in population studies: From exposure to susceptibility and to prediction of cancer. Int J Hyg Environ 210(3): 239-246.

BERBER N, ARSLAN M, BILEN Ç, SACKES Z, GENÇER N & ARSLAN O. 2015. Synthesis and evaluation of new phthalazine substituted β-lactam derivatives as carbonic anhydrase inhibitors. Rus J Bioorg Chem 41(4): 414-420.

BISACCHI GS ET AL. 2004. Synthesis of potent and highly selective nonguanidine azetidinone inhibitors of human tryptase. Bioorg Med Chem Lett 14(9): 2227-2231.

BONASSI S ET AL. 2000. Chromosomal aberrations in lymphocytes predict human cancer independently of exposure to carcinogens. Can Res 60(6): 1619-1625.

CAINELLI G, GALLETTI P, GARBISA S, GIACOMINI D, SARTOR L & QUINTAVALLA A. 2003. 4-Alkylidene-azetidin-2-ones: novel inhibitors of leukocyte elastase and gelatinase. Bioorg Med Chem Lett 11(24): 5391-5399.

DAVIS JL. 2013. Phthalazine-containing antidiabetic compounds. U.S. Patent Application 8,575,166, 5 November.

DE SIMONE G, ALTERIO V & SUPURAN CT. 2013. Exploiting the hydrophobic and hydrophilic binding sites for designing carbonic anhydrase inhibitors. Expert Opin Drug Dis 8(7): 793-810.

DEMIRAYAK S, KARABURUN AC, KAYAGIL I, EROL K & SIRMAGUL B. 2004. Some pyridazinone and phthalazinone derivatives and their vasodilator activities. Arch Pharm Res 27(1): 13-18.

DOGRUER DS, KUPELI E, YESILADA E & SAHIN MF. 2004. Synthesis of New 2-[1 (2H)-Phthalazinon-2-yl] acetamide and 3-[1 (2H)-Phthalazinon-2-yl] propanamide derivatives as antinociceptive and anti-inflammatory agents. Archiv Pharmazie 337(6): 303-310.

DONYA SM. 2002. Cytogenetic studies of some cephalosporines antibiotics on mouse germinal cells. Cytologia 67(1): 33-39.

FAHMY MA & DIAB KA. 2009. In vivo genotoxicity studies of cefotaxime. Cytologia 74(4): 417-425.

GOEL RK, SINGH A, NAIDU PS, MAHAJAN MP & KULKARNI SK. 2005. PASS assisted search and evaluation of some azetidin-2-ones as CNS active agents. J Pharm Sci 8(2): 182-189.

GOEL RK, MAHAJAN MP & KULKARNI SK. 2004. Evaluation of antihyperglycemic activity of some novel monocyclic beta lactams. J Pharm Sci 7(1): 80-83.

GRASSO S, DE SARRO G, DE SARRO A, MICALE N, ZAPPALÀ M, PUJA G, BARALDI M & DE MICHELI C. 2000. Synthesis and anticonvulsant activity of novel and potent 6,7-methylenedioxyphthalazin-1(2H)-ones. J Med Chem 43(15): 2851-2859.

GUILLON CD ET AL. 2007. Azetidinones as vasopressin V1a antagonists. Bioorg Med Chem Lett 15(5): 2054-2080.

HAN WT, TREHAN AK, WRIGHT JJ, FEDERICI ME, SEILER SM & MEANWELL NA. 1995. Azetidin-2-one derivatives as inhibitors of thrombin. Bioorg Med Chem Lett 3(8): 1123-1143.

ISTIFLI SE & TOPAKTAŞ M. 2010. Cytogenetic Genotoxicity of Amoxicillin. Environ Mol Mutagen 51: 222-228.

JAJU M & AHUJA YR. 1984. Evaluation of genotoxicity of ampicillin and carbenicillin on human lymphocytes in vitro: chromosome aberrations, mitotic index, cell cycle kinetics, satellite associations of acrocentric chromosomes and sister chromatid exchanges. Hum Toxicol 3(3): 173-191.

KAZI A, HILL R, LONG TE, KUHN DJ, TUROS E & DOU QP. 2004. Novel N-thiolated β-lactam antibiotics selectively induce apoptosis in human tumor and transformed, but not normal or nontransformed cells. Biochem Pharm 67(2): 365-374.

KIM JS, LEE HJ, SUH ME, CHOO HY, LEE SK, PARK HJ, KIM C, PARK SW & LEE CO. 2004. Synthesis and cytotoxicity of 1-substituted 2-methyl-1H-imidazo [4, 5-g] phthalazine-4, 9-dione derivatives. Bioorg Med Chem 12(13): 3683-3686.

KIM JS, RHEE HK, PARK HJ, LEE SK, LEE CO & PARK CHOO HY. 2008. Synthesis of 1-/2-substituted-[1, 2, 3] triazolo [4, 5-g] phthalazine-4, 9-diones and evaluation of their cytotoxicity and topoisomerase II inhibition. Bioorg Med Chem 16(8): 4545-4550.

KÜÇÜKGÜZEL SG, ROLLAS S, ERDENIZ H & KIRAZ M. 1999. Synthesis, characterization and antimicrobial evaluation of ethyl 2-arylhydrazono-3-oxobutyrates. Eur J Med Chem 34(2): 153-160.

KUMAR A & RAJPUT CS. 2009. Synthesis and anti-inflammatory activity of newer quinazolin-4-one derivatives. Eur J Med Chem 44(1): 83-90.

LEACH CA, HICKEY DM, IFE RJ, MACPHEE CH, SMITH SA & TEW DG. 2001. Lipoprotein-associated PLA 2 inhibition—a novel, non-lipid lowering strategy for atherosclerosis therapy. II Farmaco 56(1): 45-50.

MARIE JP, THEVENIN D & ZITTOUN R. 1986. In vitro inhibition of granulopoiesis by beta-lactam antibiotics, comparison of piperacillin, mezlocillin, ceftriaxone and ceftazidime. Presse Med 15(46): 2358-2361.

MEHTA PD & PATHAK AK. 2011. Antimictobial activity of novel 4, 4'-bis [3-chloro-4-aryl-azetidin-2-one-1-yl] diphenyl sulphones. Bull Pharm Res 1(3): 38-48.

METOVIC A, MACKIC-DJUROVIC M & IBRULJ S. 2013. Analysis of chromosome aberrations contained in vitro human peripheral blood lymphocytes after treatment with ceftriaxone. Med Arch 67(4): 228-232.

NEFTEL KA & HÜBSCHER U. 1987. Effects of beta-lactam antibiotics on proliferating eucaryotic cells. Antimicrob Agents Chemother 31(11): 1657-1661.

NOMOTO Y, OBASE H, TAKAI H, TERANISHI M, NAKAMURA J & KUBO K. 1990. Studies on cardiotonic agents. II.: synthesis of novel phthalazine and 1, 2, 3-benzotriazine derivatives. Chem Pharm Bull 38(8): 2179-2183.

NORPPA H ET AL. 2006. Chromosomal aberrations and SCEs as biomarkers of cancer risk. Mutat Res 600(1): 37-45.

RODRIGUEZ-CIRIA M, SANZ AM, YUNTA MJ, GOMEZ-CONTRERAS F, NAVARRO P, FERNANDEZ I, PARDO M & CANO C. 2003. Synthesis and Cytotoxic Activity of N, N-bis-{3-[N-(4-Chlorobenzo [g]-phthalazin-1-yl)] aminopropyl}-N-methylamine: A New Potential DNA Bisintercalator. Bioorg Med Chem 11(10): 2143-2148.

SAYYAFI M, SEYYEDHAMZEH M, REZAKHAVASI H & BAZGIR A. 2008. One-pot, three-component route to 2H-indazolo [2, 1-b] phthalazine-triones. Tetrahedron Lett 64(10): 2375-2378.

ŞEKEROĞLU ZA & ŞEKEROĞLU V. 2011. Genetic Toxicity Tests. TÜBAV Bilim Dergisi 3: 221-229.

SEN S. 2018. Determination of genotoxic profiles of methylaminobenzene sulfonamide derivatives as carbonic anhydrase inhibitor (PhD Thesis), Sakarya University.

SHARMA D, KUMAR D & BANSAL R. 2014. Synthesis of 6-(4-methanesulphonamidophenyl)-substituted dihydropyridazinone/phthalazinone derivatives as potent anti-inflammatory and analgesic agents. Arch Med Res 1(2015).

SHATERIAN HR, GHASHANG M & FEYZI M. 2008. Silica sulfuric acid as an efficient catalyst for the preparation of 2H-indazolo [2, 1-b] phthalazine-triones. Applied Catalysis A: General 345(2): 128-133.

SINKKONEN J, OVCHARENKO V, ZELENIN KN, PI BEZHAN, BORIS A, CHAKCHIR BA, AL-ASSAR F & PIHLAJA K. 2002. 1H and 13C NMR study of 1-hydrazino-2,3-dihydro-1H-pyrazolo[1,2-a]

pyridazine-5,8-diones and 1H-pyrazolo[1,2-b] phthalazine-5,10-diones and their ring-chain tautomerism. Eur J Org Chem 13: 2046-2053.

SÖNMEZ M, BERBER İ & AKBAŞ E. 2006. Synthesis, antibacterial and antifungal activity of some new pyridazinone metal complexes. Eur J Med Chem 41(1): 101-105.

SPERKA T, PITLIK J, BAGOSSI P & TOZSER J. 2005. Beta-lactam compounds as apparently uncompetitive inhibitors of HIV-1 protease. Bioorg Med Chem Lett 15(12): 3086-3090.

SRIVASTAVA SK, SRIVASTAVA S & SRIVASTAVA SD. 1999. Synthesis of new carbazoyl-thiazol-2-oxo-azetidines antimicrobial, anticonvulsant and anti-inflammatory agents. Indian J Chem B 38(B): 183-187.

STEMP G, PASCOE S & GATEHOUSE D. 1989. In vitro and in vivo cytogenetic studies of three β-lactam antibiotics (penicillin VK, ampicillin and carbenicillin). Mutagenesis 4(6): 439-445.

SUPURAN CT. 2001. Bacterial carbonic anhydrases as drug targets: toward novel antibiotics. Front Pharmacol 2(34): 1-6.

SUPURAN CT. 2008. Carbonic anhydrases: novel therapeutic applications for inhibitors and activators. Nat Rev Drug Discov 7(2): 168-181.

SUPURAN CT & SCOZZAFAVA A. 2000. Carbonic anhydrase inhibitors and their therapeutic potential. Expert Opin Ther Pat 10(5): 575-600.

SUPURAN CT & SCOZZAFAVA A. 2007. Carbonic anhydrases as targets for medicinal chemistry. Bioorg Med Chem 15(13): 4336-4350.

TATSUMI K, OU T, YAMADA H & YOSHIMURA H. 1980 Studies on metabolic fate of a new antiallergic agent azelastine (4-(p-chlorobenzyl)-2-[N-methylperhydroazepinyl-(4)]-1-(2H)-phthalazinone hydrochloride). J Pharmacol Sci 30(1): 37-48.

THOMAS AB, NANDA RK, KOTHAPALLI LP & HAMANE SC. 2016. Synthesis and biological evaluation of Schiff's bases and 2-azetidinones of isonocotinyl hydrazone as potential antidepressant and nootropic agents. Arab J Chem 9(1): 79-90.

TURAN B, ŞENDIL K, ŞENGÜL E, GÜLTEKIN MS, TASLIMI P, GULCIN I & SUPURAN CT. 2016. The synthesis of some β-lactams and investigation of their metal-chelating activity, carbonic anhydrase and acetylcholinesterase inhibition profiles. J Enzym Inhib Med Chem 31(1): 79-88.

WASFY AF, ALY AA, BEHALO MS & MOHAMED NS. 2013. Synthesis of novel series of phthalazine derivatives as potential antitumor agents. Synth 10: 20-32.

WATANABE N, KABASAWA Y, TAKASE Y, MATSUKURA M, MİYAZAKİ K, ISHİHARA H, KODAMA K & ADACHİ H. 1998. 4-Benzylamino-1-chloro-6-substituted phthalazines: synthesis and inhibitory activity toward phosphodiesterase 5. J Med Chem 41(18): 3367-3372.

WILKINSIN LB, BORNAGHI LF, HOUSTON TA, INNOCENTI A, VULLO D, SUPURAN CT & POULSEN SA. 2007. Carbonic anhydrase inhibitors: Inhibition of Isozymes I, II and IX with triazole-linked O-Glycosides of benzene sulfonamides. J Med Chem 50(7): 1651-1657.

XUE DQ ET AL. 2014. Synthesis and anticancer activities of novel 1, 2, 4-triazolo [3, 4-a] phthalazine derivatives. Eur J Med Chem 85: 235-244.

ZAVARISE G, GHIAZZA G, GRILLO G, MORINO P & MONDAVIO M. 1984. Evaluation of the effect of cloxacillin on human lymphocyte chromosomes through the study of karyotypic changes and sister chromatid exchanges. Boll. Soc Ital Biol Sper 60(11): 2143-2148.

ZHAI X, Li J, HE L, ZHENG S, ZHANGA YB & GONGA P. 2008. Synthesis and in vitro cytotoxicity of novel 1, 4-disubstituted phthalazines. Chin Chem Lett 19(1): 29-32.

ZHANG S, ZHAO Y, LIU Y, CHEN D, LAN W, ZHAO Q, DONG C, XIA & GONG P. 2010. Synthesis and antitumor activities of novel 1, 4-disubstituted phthalazine derivatives. Eur J Med Chem 45(8): 3504-3510.

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Aygün B dealt with in the whole test processes mentioned in the study, contributed to the work plan and the idea of work. Berber AA contributed to the experiments carried out in the study and to the writing of the article. Dogancı MA contributed to the selected issues and made appropriate arrangements. Berber N synthesized and supplied test substances. Şen S and Yıldız E contributed to the literature review, tests and writing of the article. Aksoy H was the work supervisor, who guided and revised the manuscript. All authors gave their final approval for submission.