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In vitro Cytogenetic Analysis in Human Cultured Blood Lymphocytes of Newly Synthesized Two Different Benzoic Acid Derivatives [(4-(3-acetyl-2-methyl-4-phenyl-1H-pyrrol-1-yl) Benzoic Acid) and (4-(3-cinamoyl-2-methyl-4-phenyl-1H-pyrrol-1-yl) Benzoic Acid)]

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Abstract

Globally, benzoic acid and its various derivatives are widely used as antibacterial and antifungal agents, as well as in the hygiene, cosmetic, food, and pharmaceutical industries. This study is planned to reveal the cytotoxic and genotoxic identity of the newly synthesized pyrrole derivative molecules [4-(3-acetyl-2-methyl-4-phenyl-1H-pyrrol-1-yl) Benzoic Acid (D085)] and pyrrole-chalcone hybrid derivative [4-(3-cinamoyl-2-methyl-4-phenyl-1H-pyrrol-1-yl) Benzoic Acid (D085-K)]. Experiments were performed using in vitro chromosome aberration (CA) and micronucleus (MN) tests in human peripheral lymphocytes (HPL). The test substance concentrations used in the study were determined via a preliminary study. Based on the genotoxic and cytotoxic effects of both test compounds, the 24-hour treatment was found to be more effective than the 48-hour treatment. The fact that the induced MN frequency is similar to the CA frequency gives a preliminary idea about the clastogenic potential of the test substances. Compound D085 induced a base change mutation, while D085-K induced a frameshift mutation. Furthermore, in this study, high concentrations of the test compounds were generally found to be cytotoxic according to MI and NDI data as indicators of cytotoxicity.

Keywords: 4-(3-acetyl-2-methyl-4-phenyl-1H-pyrrol-1-yl) Benzoic Acid, 4-(3-cinamoyl-2-methyl-4-phenyl-1H-pyrrol-1-yl) Benzoic Acid, Genotoxicity, Chromosome Aberration (CA), Micronucleus (MN), Ames/Salmonella/Microsome Bacterial Reverse Mutation Test

1. Introduction

Environmental pollution, habitue to alcohol and cigarettes, food additives, drugs, ultraviolet (UV) rays, pesticides, and many different harmful factors that adversely affect health are the situations that people are exposed to, either intentionally or unintentionally more and more. Today, more resources should be allocated to health expenditures compared to the past to prevent or eliminate existing or later, onset diseases that may arise due to life and business conditions. To effectively fight existing or potential health problems, medicine, pharmacology, and basic science researchers are searching for new solutions with outstanding efforts. Especially the synthesis of new multifunctional molecules with biological activity produced using organic chemistry, and the research and testing of their possible novel pharmacochemical properties are becoming more important nowadays and also for the

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future. Some notable arguments accept the justification that the novel hybrid drugs may show superior activity than the equivalent drug combination [1]. Two different chemical products, which are newly synthesized pyrrole derivatives, were used in this study as test substances. The first of these chemicals the pyrrole is the simplest member of heterocyclic compounds consisting of one nitrogen (N) and four carbon (C) atoms and shows a stable structure in the five-membered ring. Furan and thiofen are other important members [2]. The second is the pyrrole-chalcone hybrid compound. Chalcones are abundant in edible plants and constitute a subclass of bioactive compounds, flavonoids. Pyrroles are common in nature and their analog forms the core of many natural products. Vitamin B12 [3], hemin [4], chlorophyll [5,6], bile pigments, bilirubin [7], indigo [8], indole structures, porphyrins [9], constitute the essence of many natural products important for life and the structure of synthetic substances [10]. They are extremely important because of their anti-bacterial, anti-viral, anti-tumor and, anti-oxidant activities and their ability to inhibit cytokine-induced diseases [11]. Pyrroles have also attracted great attention in the field of medicine and pharmacy due to their synthetic importance and beneficial biological activities that are used extensively in drug discovery [12].

The second newly synthesized test substance used in this study is similar to the flavonoid group since it contains chalcone in its molecular structure. These compounds can also be valued as pyran ring-opened flavonoids. In the basic structure of flavonoids, the presence of α , β -unsaturated carbonyl group on the propane chain, the presence of a ketone group, and a double bond, form chalcones. Scientific research on chalcone compounds obtained from natural sources is limited, but it is known that chalcone compounds synthesized by synthetic means have a wide spectrum of biological activity [13-19].

Chalcones are a class of anti-cancer agents with promising healing effects for human cancers [20]. As a result of the studies, it has been determined that chalcones have anti-cancer [21], anti-inflammatory [22], anti-tuberculosis, and anti-fungal [23] activities. In addition, they have been reported to be anti-oxidant, anti-malarial, anti-leishmanial, and anti-tumor agents [24]. Some chalcone and flavonoid derivatives have also been reported to show anti-HIV activity [25]. Chalcones are used in many applications such as medical treatment, the food industry, UV-absorption filters in polymers, different optical materials, and holographic recording technology [26]. Especially chalcones containing hydroxyl groups are widely used in many fields [27,28].

Similar to the test items, some of the compounds synthesized by Rida et al [4-[1-(1-1H-benzimidazol-2-yl) alkylamino]-1,5-dihydro-2H-pyrrol-2-one and 3-[1-(1H-benzimidazole)-2-yl) alkyl]-2-4-(3H)-quinazolinone)] have shown antibacterial and antifungal activity in vitro [29]. These compounds have also been found to be devoid of positive activity against P-388 lymphocytic leukemia in mice. Of the newly synthesized 3'-deoxythymidine analogs, only the 3'-pyrrol-1-yl derivative has exhibited potent antiviral activity against the human immunodeficiency virus [30]. The cytotoxic effect of N-substituted pyrrole derivatives on liver cancer cell line (HepG2 and Hepa1-6), colon cancer cell line (HT-29 and Caco-2), cervical cancer cell line (HeLa) in vitro was evaluated [31]. Of those tested, two compounds (5-(1H-pyrrol-1-yl)-1,10-phenanthroline and 1-(phenanthrene-2-yl)-1H-pyrrole) were noted to show good cytotoxicity against some cancer cell lines. Geng et al. [32] have identified a compound consisting of derivatives of 5-hydroxy-1H-pyrrole-2-(5H)-one and possessing potent anti-proliferative activity in multiple cancer cell lines. Cell cycle analysis has revealed that this compound is capable of triggering S-phase cell cycle arrest, and particularly the colon cancer cell line (HCT116) is susceptible to compound-induced apoptosis. Other analyzes have shown that this compound preferentially induces DNA damage and p53 activation in HCT116 cells and that apoptosis is partially p53 dependent. It has also been shown that the compound significantly suppresses tumor growth in xenograft tumor models in vivo. Kumar et al. [33] have determined significant cytotoxicity of a number of indolyl chalcone compounds to three different human cancer cell lines (A549, mia PaCa-2, and PC-3). They identified the compounds with indolyl chalcones as the most potent and selective anticancer agents against the mia PaCa-2 (pancreatic cancer cell line) cell line. In a different study, 12 new pyrazoline derivative piperidyl chalcones were synthesized by condensing 4-piperidin-1-ylbenzaldehyde into various acetylthiophenes. While some of these compounds show anti-HIV activity and cytotoxicity, two have been found to be non-cytotoxic in human cells [34]. According to the results of the research conducted by Maronpot [35], the experimental (chromosome aberration, in vivo micronucleus, and bacterial reverse mutation) results of two physiologically active flavonoid chalcone (4-hydroxydricin and xanthoangelol) of the Angelica keiskei plant did not exhibit possible genotoxic potential.

Today, new derivative compounds, which will be used in many areas both in pharmaceutical production and industry, must pass some toxicology tests and information on their biological activities should be available before being sold [36]. As a result of the rapid increase in the use of chemicals in every field, it is extremely important to determine whether these chemicals have negative effects on the human genome [37]. In this study, the cytotoxic and genotoxic profiles of previously unexamined two different

pyrrole derivative molecules, which will be used as chemical components for various purposes in many industrial fields, were tried to be revealed.

2. Material and Method

In this study, two different newly synthesized pyrrole derivatives [4-(3-acetyl-2-methyl-4-phenyl-1H-pyrrol-1-yl) Benzoic Acid (D085) (Fig. 1)] and pyrrole-chalcone hybrid derivative [4-(3-cinamoyl-2-methyl-4-phenyl-1H-pyrrol-1-yl) Benzoic Acid (D085-K) (Fig. 2)] molecule was used as the test substance. Test substances were dissolved in DMSO. The pyrrole derivative molecules were synthesized in the Cukurova University Science and Letters Faculty Chemistry Department Organic Chemistry Laboratory [38]. As a material, peripheral blood obtained from four healthy individuals of close age who did not smoke and drink alcohol was used in chromosome aberration (CA) test and micronucleus (MN) test in PB-Max karyotyping medium (Gibco 12557-013). For the Ames test, TA98 and TA100 strains developed with in vitro mutations from the commercially available Salmonella typhimurium LT2 ancestral strain were used.

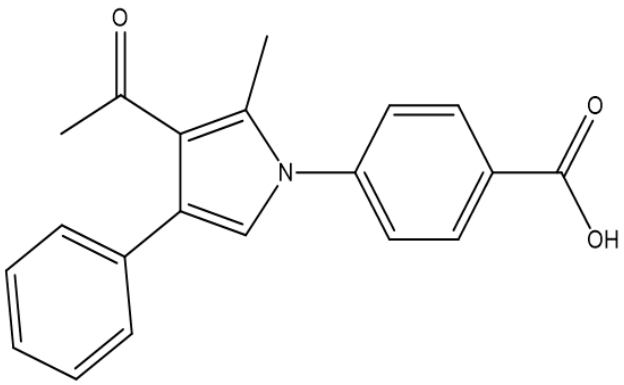

Figure 1. Open formula of D085, chemical structure of D085 drawn with ChemBio Draw Ultra 13.0.

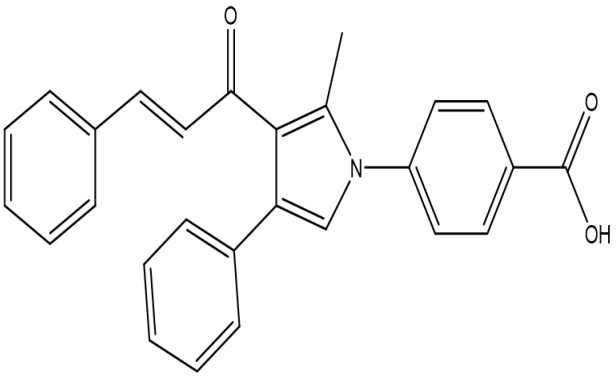

Figure 2. Open formula of D085-K, chemical structure of D085-K drawn with ChemBio Draw Ultra 13.0.

2.1. Ethical Issues Related to Research, Test Concentration, Treatment Time and Statistical Analysis

Ethical permission of the study program was confirmed positively by the Çukurova University Faculty of Medicine Non-Invasive Clinical Research Ethics Committee at its 73rd meeting on January 12, 2018.

In the preliminary study to determine the test doses, 4-(3-acetyl-2-methyl-4-phenyl-1H-pyrrol-1-yl) benzoic acid [D085] has 7.81, 15.62, 31.25 µg/mL and 4-(3- Concentrations of 7.5, 15, 30 µg/mL were determined for cinamoyl-2-methyl-4-phenyl-1H-pyrrol-1-yl) benzoic acid [D085-K]. Treatment time was chosen as 24 or 48 hours, corresponding to 1 or 2 cell cycles in culture. The importance level of all data was checked by Post-Hoc LSD test in One-Way ANOVA analysis using IBM SPSS Statistics 22 program ($P \leq 0.05$).

2.2. Cell Culture to Detect Chromosome Aberration (CA), Microscopic Examination and Mitotic Index (MI)

In this study, cell culture was carried out following the method of Evans [39] to detect CA. 1/10 heparinized blood samples taken from donors (4 healthy/volunteers) were added 6 drops (0.2 mL) [40] to 2.5 mL chromosome medium under sterile conditions. The cell culture was mixed well and incubated in the incubator at 37±1°C for a total of 72 hours.

To examine the effects of two newly synthesized pyrrole derivatives D085 (7.81, 15.62, 31.25 µg/mL) and D085-K (7.5, 15, 30 µg/mL), they were administrated to culture tubes for 24 or 48 hours. Besides, MMC (0.25 µg/mL) was used as a positive control at treatment times of 24 or 48 hours. It was pretreated by adding colchicine solution (0.06 µg/mL) to each tube 2 hours before the end of the culture period (ie, at the 70th hour of the culture) and the harvesting process was started at the 72nd hour of culture.

A total of 100 metaphases (4x100=400 cells) with well-dispersed chromosomes in the preparations prepared separately from each person were examined to determine CA. As a result of the examinations, CA per cell (CA/cell) and total CA number were determined. Chromatid and chromosomal gaps were not considered as abnormalities in the study [41]. To determine the mitotic index (MI), the percentage of metaphase in 3000 cells belonging to each treatment group was calculated.

$$\mathrm{MI} = \frac{\text{Metaphase Number}}{3000} \mathrm{X100}$$

2.3. Cell Culture to Detect Micronucleus (MN) Formation, Microscopic Examination and Nucleus Division Index (NDI)

In the in vitro micronucleus test, Rothfuss et al. the method developed by [42] has been modified and used. Blood was taken from the donors [40] and the cells were incubated at 37 ± 1°C for 68 hours. Two different pyrrole derivatives, D085 (7.81, 15.62, 31.25 µg/mL) and D085-K (7.5, 15, 30 µg/mL), were solved in DMSO and added to culture tubes 20 and 44 hours after the start of the culture. Cytochalasin-B (Sigma-Aldrich 14930-96-2) was added to each tube 44 hours after the beginning of incubation at a final concentration of 6 µg/mL. At the end of the 68th hour, which is the end of the culture period, harvesting was started.

To determine the number of micronuclei, a total of 1000 cells with two nuclei (binuclear) from each treatment group were examined (4x1000=4000 cells) and the micronucleated cells were detected among these binuclear cells (MNBN). Also, the total number of micronucleus (MN per thousand) was determined in the cells examined. In this experiment, the Nucleus Division Index (NDI) was calculated according to the formula written below.

$$\mathrm{NDI} = \frac{\mathrm{M1} + 2 \times \mathrm{M2} + 3 \times \mathrm{M3} + 4 \times \mathrm{M4}}{\mathrm{N}}$$

M1 shows the number of cells with one nucleus, M2 with two nuclei, M3 with three nuclei, M4 with four nuclei. N refers to the total number of cells. For NDI, a total of 1000 cells (4x1000=4000 cells) were examined in randomly selected areas from each person's preparations.

2.4. Ames/Salmonella/Microsome (Bacterial Reverse Mutation) Test

TA98 and TA100 strains developed by Maron and Ames [43] with in vitro mutations were available by donation and used in assays. Test substances concentrations (3.9, 7.81, 15.62, 31.25, 62.5 µg/plate for D085 and 3.25, 7.5, 15, 30, 60 µg/plate for D085-K) and bacterial strains were added separately into the 2 mL top agars. In the continuation, they were transferred to MGA Petri plates under sterile conditions. Then Petri plates were left to incubate for 72 hours in a 37°C incubator. Colonies in the Petri plate were counted after incubation. Control groups were used to evaluate the results to be obtained. 100 µL DMSO as solvent control, as positive control; 100 µL of 4-nitro-o-phenylenediamine (4-NPD) (0.02 ug/uL) for TA98 strain and 100 µL of sodium azide (SA) (0.1 ug/uL) for TA100 strain were used. Mutagenic activities of test compounds were evaluated by counting revertant bacterial colonies [44].

3. Results

3.1. Effects of D085 and D085-K Molecules on Chromosome Aberration (CA) Formation and Mitotic Index (MI)

The effects of test compounds on chromosome abnormalities and cell division were investigated with various parameters. It was observed that all concentrations of D085 coded chalcone-free pyrrole compound applied to cell culture for 24 or 48 hours increased abnormal cell (AC) percentage and chromosome abnormality (CA)/Cell rations to some extent. When the data obtained as a result of the 24-hour treatment of the agent were analyzed, it was determined that the percentage of AC formation increased only at the concentration of 15.62 and 31.25 µg/mL compared to the control. It was also determined that the CA/Cell ratio increased significantly at a concentration of only 31.25 µg/mL compared to the untreated control, solvent, or even positive control ($P \leq 0.05$). Furthermore, this concentration (31.25 µg/mL) was found to be more clastogenic than the positive control (Table 1).

Table 1. Abnormal cell percentage*, CA/Cell and Mitotic Index (MI) Ratios in Human Peripheral Blood Lymphocytes (HPL) Treated for 24 or 48 Hours with D085 and D085-K at Different Concentrations

Test Substance	Treatment		Abnormal Cell % ± SE	CA/Cell ± SE	MI ± SE
	Time (hour)	Cons. (µg/mL)			
Control		-	2.5±0.28	0.025±0.002	6.4±1.14
Solvent Cont.	24	3.7 µL/mL	3.75±0.75	0.037±0.007	5.23±0.19
	48	3.7 µL/mL	3.75±1.79	0.042±0.02	4.33±0.86
Pos. Cont.	24	0.25	9.25±2.8	0.102±0.025	4.51±0.81
	48	0.25	12.75±0.85	0.142±0.02	3.83±0.88
		7.81	8.5±1.93	0.09±0.023	5.23±0.75
D085	24	15.62	14.5±3.92 a_2b_2	0.185±0.063	4.16±0.81
		31.25	14.75±4.21 a_2b_2	0.312±0.137 $a_2b_2c_1$	2.5±0.63 a_2b_1
		7.81	6±1.08 a_1c_3	0.067±0.011 a_1c_3	4.36±0.74
D085	48	15.62	5±1.00 c_3	0.052±0.009 c_3	4.49±0.98
		31.25	4.5±0.95 c_3	0.047±0.011 c_3	4.05±1.08
		7.5	9.5±1.84 a_1b_1	0.095±0.018 a_1	5.2±0.61
D085-K	24	15	9.25±2.01 a_1	0.1±0.020 a_1b_1	5.83±0.60
		30	7.5±2.39	0.087±0.029 a_1	4.78±0.59
		7.5	5.5±1.19 c_3	0.057±0.013 c_3	5.24±0.60
D085-K	48	15	7.25±1.18 a_1c_2	0.077±0.014 a_1c_2	4.14±0.43
		30	7.5±1.25 $a_2b_1c_2$	0.077±0.01 a_1c_2	3.91±0.73 a_1

The difference is statistically important according to the untreated control (**a**), the solvent control (**b**), and the positive control (**c**). $\mathbf{a_1b_1c_1}$: $P \leq 0.05$; $\mathbf{a_2b_2c_2}$: $P \leq 0.01$; $\mathbf{a_3b_3c_3}$: $P \leq 0.001$

*100 metaphases from each preparation and a total of 400 metaphases from 4 donors for each treatment group were evaluated.

In cell cultures treated with pyrrole compound (D085) for 48 hours, increases in abnormal cell percentage and CA/Cell ratio were noticeable, and chromosome aberration values only at the lowest concentration were found to be significantly higher than untreated

control (P≤0.05). Abnormalities detected at other concentrations were found to be close to the untreated control and solvent control levels. An evident decrease was observed in the number of abnormalities caused by the test substance applied to the culture during the 48 hour treatment period compared to the 24-hour application. It was determined that all values observed in this period were significantly lower than the averages of anomalies caused by the positive control (P≤0.001) (Table 1). When the mitotic index (MI) values in pyrrole derivative compound-applied cultures were examined, a significant decrease was found only at the highest concentration within 24 hours of application. The MI reduction at this concentration was found to be significant relative to the untreated control and the solvent control (P≤0.05). MI changes were not found to be significant at 48 hours of treatment (Table 1).

It was observed that all concentrations of D085-K pyrrole-chalcone hybrid compound applied to cell culture for 24 or 48 hours increased AC percentage and CA/Cell ratios slightly. However, when the chromosomal abnormality data obtained as a result of the 24-hour treatment of the agent was analyzed, it was found that the resulting AC percentage increased only at 7.5 and 15 µg/mL concentrations compared to the untreated control (P≤0.05). It increased the CA/Cell ratio at all applied concentrations compared to the untreated control (P≤0.05). Besides, it was found that the treatment data of 7.5 µg/mL in abnormal cell percentage rates and 15 µg/mL in CA/Cell ratios were significantly higher compared to the solvent control (P≤0.05). There was no significant difference between the data obtained in 24 hours with the pyrrole-chalcone hybrid molecule and the positive control data (P>0.05) (Table 1). Significant abnormal cell and CA/Cell ratios were detected in 48 hours of treatment of the hybrid compound relative to the untreated control, except at a concentration of 7.5 µg/mL (P≤0.05). Also, the increase in the abnormal cell at the 30 µg/mL dose was found to be significant compared to both the untreated control and the solvent control (P≤0.05). All of the chromosome abnormality data (Abnormal cell and CA/Cell ratios) detected during this 48 hour application period were found to be statistically significantly lower than the positive control (MMC) (P≤0.01) (Table 1).

The pyrrole chalcone hybrid compound has generally reduced the mitotic index at all concentrations however a significant decrease was only observed at 30 µg/mL (48 hours treatment) compared to the untreated control (P≤0.05). No statistically significant MI differences were detected in all other groups (Table 1). These results have not shown a positive correlation between chromosome aberrations and cytotoxicity.

3.2. Effects of D085 and D085-K Molecules on Micronucleus (MN) Formation and Nucleus Division Index (NDI)

Pyrrole derivative (D085) stimulated the formation of micronucleus in the 24-hour treatment, which was evaluated to be caused by the clastogenic and/or aneugenic effects. However, it was determined that the MN frequency at the concentration of only 31.25 µg/mL increased significantly compared to the untreated control, the solvent control, and the positive control (P≤0.001). It has been found that the formation of MN at this concentration is significantly higher even than the positive control (P≤0.05). Increases in other concentrations were found to be within the confidence interval (Table 2). Besides, the ratio of MN and micronucleated binuclear cells (MNBN) was found to be similar. After the 48-hour D085 treatment of the cell culture, a significant increase in MN formation relative to the untreated control was observed at a concentration of only 31.25 µg/mL (P≤0.01). MN formation at other concentrations was found to be close to controls. Also, the positive control over the 48 hours showed a strong MN stimulation potential (P≤0.001) (Table 2). In this period, the ratio of MN and the ratio of micronucleated binuclear cells were similar, with a few exceptions.

In the 24 hour treatment of the pyrrole compound, the nucleus division index (NDI) was significantly reduced at all concentrations except the lowest one (7.81 µg/mL). Significant NDI reductions were found at the median concentration (15.62 µg/mL) compared to the untreated control, and at higher concentration compared to all controls. At the 48 hours of treatment, the NDI data at a concentration of only 31.25 µg/mL was found to be significantly lower than the untreated control (P≤0.05) (Table 2).

In terms of micronucleus formation, 30 µg/mL concentration of the pyrrole-chalcone hybrid (D085-K) compound during the 24-hour application period significantly increased MN formation compared to the untreated control (P≤0.05). The ratio of micronucleated binuclear cells (MNBN) caused significant increases at concentrations of 15 and 30 µg/mL compared to the untreated control (P≤0.05). The MN values examined during this period were close to those detected in the positive control, except those detected at a concentration of 7.5 µg/mL (Table 2). Both MN and MNBN ratios found in cultures treated with the same compound for 48 hours were close to untreated and solvent control values (P>0.05). On the other hand, all of the MN numbers

examined were found to be significantly lower than the positive control (P≤0.001) (Table 2). It has been determined that the MN ratio is similar to the ratio of micronucleated binuclear cells. Although a slight decrease was observed in the MN ratios due to the increase in treatment time, which also occurred in the treatment of the D085-K compound, this decrease was not found to be significant.

The result of the 24-hour treatment of the compound D085-K was shown that the value detected in NDI at a dose of only 15 µg/mL was significantly reduced compared to the untreated control (P≤0.05). In the 48-hour treatment of this compound, the reduction of NDI at a dose of only 30 µg/mL was found to be significant compared to the untreated and solvent control (P≤0.05) (Table 2). The decrease in NDI inversely proportional to MN formation detected here is noteworthy.

Table 2. Human Peripheral Blood Lymphocytes Treated with Different Concentrations of D085 and D085-K for 24- and 48-Hours Total MN ‰ per Cell, Micronucleated Binuclear Cell ‰ (MNBN) and Nucleus Division Index (NDI) Ratios

Test Substance	Treatment Time (hour)	Treatment Cons. (µg/mL)	MN ‰ ± SE	Micronucleated Bi-nuclear Cell (MNBN) ‰ ± SE	NDI ‰ ± SE
Control		-	4.25±1.1	3.75±0.75	1.765±0.12
Solvent	24	3.7 µL/mL	7.5±2.1	7±1.77	1.680±0.116
Cont.	48	3.7 µL/mL	8.5±1.75	7.75±1.65	1.634±0.1
Positive	24	0.25	10.75±1.75	10±1.68	1.508±0.099
Cont.	48	0.25	28.25±3.22	25.75±3.09	1.424±0.054
		7.81	4.5±0.86 c_2	4.00±0.70 c_2	1.682±0.081
D085	24	15.62	6.25±0.62 c_1	6.25±0.62 c_1	1.490±0.076 a_1
		31.25	17±1.78 $a_3b_3c_2$	13.75±1.03 $a_3b_3c_1$	1.212±0.024 $a_3b_3c_1$
		7.81	5.75±0.47 c_3	5.00±0.70 c_3	1.624±0.039
D085	48	15.62	8.25±1.18 c_3	7.75±0.94 c_3	1.480±0.090
		31.25	12.25±1.79 a_2c_3	11.50±1.55 a_2c_3	1.407±0.084 a_1
		7.5	5.75±0.47 c_1	5.00±0.70 c_1	1.594±0.118
D085-K	24	15	7.75±0.62	7.50±0.50 a_1	1.479±0.095 a_1
		30	9.25±1.49 a_1	9.00±1.47 a_2	1.503±0.065
		7.5	5.25±0.62 c_3	5.25±0.62 c_3	1.623±0.042
D085-K	48	15	6.75±1.10 c_3	6.5±0.95 c_3	1.548±0.037
		30	9±1.41 c_3	8.5±1.19 c_3	1.380±0.044 a_2b_1

The difference is statistically important according to the untreated control (**a**), the solvent control (**b**), and the positive control (**c**). $\mathbf{a_1b_1c_1}$: P≤0.05; $\mathbf{a_2b_2c_2}$: P≤0.01; $\mathbf{a_3b_3c_3}$: P≤0.001

3.3. The Effects of D085 and D085-K Molecules on Revertant Colony Formation (Ames Test Findings)

The test substance (D085) administered at five different concentrations (3.9, 7.81, 15.62, 31.25, and 62.5 µg/plate) did not cause a significant increase in the number of colonies associated with reverse mutation stimulation in the TA98 strain. They were also significantly lower than the number of colonies counted in the positive control (P≤0.001). In the TA100 strain, 3.9, 7.81, 15.62 µg/plate concentrations led to significant increases in revertant colony numbers compared to the untreated control and solvent control (P≤0.01). At concentrations of 31.25 and 62.5 µg/plate, the number of revertant colonies decreased and no statistically significant differences were found compared to all controls. Also, the number of colonies in the TA100 strain treated with the test substance was found close to the number of colonies in the positive control (Table 3).

In order to reveal the effects of the pyrrole-chalcone hybrid (D085-K) molecule on mutant *Salmonella typhimurium* strains, all the concentrations except 3.75 µg/plate applied significantly increased the number of colonies in the TA98 strain compared to the untreated control (P≤0.05). In addition, the number of colonies at a dose of 15 µg/plate was significantly higher compared to both untreated and solvent controls (P≤0.01). The colony numbers at all treatment concentrations determined in the TA98 strain were significantly lower compared to the positive control (P≤0.001) (Table 3). D085-K also did not cause a significant increase in the number of colonies indicative of reverse mutation in the TA100 strain (Table 3).

Table 3. Reverse mutation effect of D085 and D085-K on *Salmonella typhimurium* TA98 and TA100 strains.

Test Substance	Cons (µg/plate)	MRCN*± SE	
		TA98	TA100
Control	-	47.6±0.33	268±3.51
Solvent Cont.	100 µL	57±10.78	278.3±5.17
4-NPD	20	254.6±14.71	-
SA	1	-	315.3±15.89
	3.9	77.6±6.35 c_3	329±4.58 a_2b_2
	7.81	61.6±5.84 c_3	337.3±18.77 a_3b_2
D085	15.62	52±25.23 c_3	338±17.61 a_3b_2
	31.25	86.6±11.05 c_3	286.6±8.08
	62.5	58.3±19.02 c_3	286±3.51
	3.75	51.3±9.9 c_3	284.6±9.93
	7.5	85±15.13 a_1c_3	298.3±12.3
DO85-K	15	95.6±10.39 $a_2b_1c_3$	262±12 c_1
	30	84.6±15.81 a_1c_3	282±22.14
	60	86.3±4.09 a_1c_3	246.6±33.21 c_1

4-NPD: 4-Nitro-o-phenylenediamine; **SA**: SodiumAzide

The difference is statistically important according to the untreated control (**a**), the solvent control (**b**), and the positive control (**c**). $\mathbf{a_1b_1c_1}$: P≤0.05; $\mathbf{a_2b_2c_2}$: P≤0.01; $\mathbf{a_3b_3c_3}$: P≤0.001

***MRCN**: Mean Revertant Colony Number (The study was carried out in triplicate)

Revertant colony numbers observed in mutant bacterial strains also give an idea of cytotoxicity. When the colony numbers of the strains treated with pyrrole compound (D085) and pyrrole-chalcone hybrid compound (D085-K) are examined, there was no remarkable difference observed. However, in the TA100 strain treated with D085-K at a concentration of 60 µg/plate, a slight decrease was detected compared to the untreated control (Table 3). No significant cytotoxic effect was observed in the Ames test (Table 3).

4. DISCUSSION

In this study, the sublethal concentrations of the test agents determined by the preliminary study were applied to the human peripheral lymphocytes (HPL) cell culture for 24 or 48 hours without a metabolic activator. Significant increases in chromosome aberration frequency, which can be associated with the clastogenic potential of the compound, were found at some concentrations and treatment times during which the test substances were applied to the culture. According to the results obtained from the experiments, it was revealed that chromosome abnormality (CA) and micronucleus (MN) frequencies increased significantly, especially in cells with high concentration, with a few exceptions. The underlying reasons for the emergence of this result may be the possible oxidative activity of test substances on DNA, covalent bonding with DNA, and/or topoisomerase-II inhibition. In addition, toxic effects of test substances as nucleotide analogs during DNA replication/repair or on enzymes and/or auxiliary factors involved in this process may cause genotoxicity. In a previous study that confirms this idea, it was found that monofunctional and bifunctional pyrroles increase sister chromatid exchange (SCE), which is an indicator of genotoxicity in human lymphocytes, especially bifunctional pyrroles are more effective in increasing SCE [45]. In a recent study with similar results, genotoxicity variations originated from four commercially available and partially photo-treated pyrrole derivative intermediates [(1H-pyrrole-2-

carboxaldehyde, 6-chloro-2-pyridinecarboxylic acid, 2,3-dichloropyridine, and 2-Pyridinecarbonitrile) 1H-pyrrole-2-carboxaldehyde and 6-chloro-2-pyridinecarboxylic acid] were shown to be highly genotoxic even at very low concentrations. The other solution (2-pyridinecarbonitrile) has been observed to be only cytotoxic [46].

The conformation of any biological molecule is determined by the combination of electrical charges of the atoms that make up that molecule. Unexpected electron or proton shifts in the molecule can affect the standard stability of the molecule unpredictably. This result is likely to affect the molecule directly or indirectly, preventing its essential function. The fact that unstable oxygen species (ROS) produced in aerobic cells, attack all biological molecules, including nucleic acids, and disrupt their electrical dynamics, restrict or completely block the function of the molecule can be considered as an oxidative stress phenomenon. It has long been known that mitochondrial-derived reactive oxygen species are the basis of oxidative damage. Important findings have been reached in many studies investigating the effects of oxidative interactions on the genome. Among these findings; cell cycle arrest or apoptotic cell death has been reported as a result of the disruption of the genomic integrity of the cell and inactivation of the biochemical mechanism, oxidized nucleotide/DNA lesions, and their direct/indirect effects [47-51]. However, no reliable study has been found on the direct oxidative effect of pyrrole derivative compounds. As our findings may be due to a reason other than oxidative effect, a previously unknown oxidative effect of these newly synthesized pyrrole derivative compounds should not be excluded. On the other hand, the second test substance, the pyrrole-chalcone derivative (D085-K) compound is similar to flavonoids. Therefore, the fact that flavonoids are in the group of natural antioxidants somewhat contradicts the argument of oxidative stress-induced genotoxicity we discussed in this section. The hypothesis that pyrrole derivative and pyrrole-chalcone derivatives may affect molecular pathways related to DNA integrity, DNA replication/repair and cell division by other effects other than oxidative stress has been supported by some previous studies. In one of these, the pyrrole-imidazole-polyamide compound, which is a pyrrole derivative compound, reduces the cellular replication potential. It has been suggested that the ionizing radiation used to cure some diseases may play a sensitizing role for the cell to the substance by altering the cellular response to the genotoxic effect [52].

According to the result of a study evaluating the epigenetic effect of pyrrole derivatives, it has been stated that pyrrole-imidazole polyamides are synthetic ligands covalently bound with predetermined sequences in the minor grooves of DNA without causing any genotoxic effect. It has been stated that as a result of the DNA binding properties of these compounds, polyamides can be useful molecules that regulate gene expression by inhibiting DNA-protein interactions in specific DNA sequences. To address this, a well-characterized pyrrole-derived polyamide conjugate has been found to alkylate predetermined regions of the HIV promoter by selectively binding at subnanomolar concentrations and affecting the expression of the gene [53]. Polyamides composed of N-methyl pyrrole (Py) and N-methylimidazole (Im) subunits can bind to the minor grooves of DNA in predetermined sequences with subnanomolar affinity and high specificity. Covalent bonding to the DNA of polymer subunits using a p-aminobutyric acid linker has been shown to increase both the affinity and specificity of polyamides [54]. The joint changes created by the ligands formed by the pyrrole-imidazole polyamides that naturally bind to the minor groove of the DNA and the peptides and proteins that bind to DNA were investigated by the Surface Plasmon Resonance (SPR) method. The formation of different protein-pyrrole-imidazole-polyamide conjugates was successful in that study. However, it has been observed that the DNA affinity for these conjugates is weakened and therefore its practical use is not yet possible [55]. In our study, although there is no direct epigenetic effect data such as gene expression, an indirect effect can be mentioned regarding the change of molecular path dynamics involved in DNA repair and cell division, especially at high concentrations. DNA topoisomerases are cellular enzymes that change the topological state of DNA by breaking down and recombining DNA strands. The in vitro cytotoxic activity of camptothecin derivatives carrying five-membered heterocyclic compounds is more active than those without pyrrole. Single strand breaks have been shown to occur in polyacrylamide gel sequencing electrophoresis with fluorescence-labeled DNA incubated with pyrrole compounds. Agarose gel electrophoresis showed different inhibitory activities of camptothecin analogs against topoisomerase-I mediated DNA cleavage. However, thiophene and furan compounds did not show any inhibitory activity on the DNA cleavage functions of topoisomerase-I [56]. Prodigiosin, the red pigment with apoptotic activity synthesized by Serratia marcescens, is a tripyrrole alkaloid compound. DNA cleavage occurs by binary elimination of topoisomerase-I and II activity, with some preferences for alternative base pairs, but with no distinction between AT or CG sequences [57]. In addition, there are patents received stating that pyrrole derivatives act as DNA ligase and topoisomerase inhibitors and can be used for this purpose [58]. Consistent with the results here, it can be considered that the clastogenic effect determined in our results may be due to topoisomerase enzyme inhibition.

We believe that the test substances cause significant colony increases in some concentrations of Salmonella mutant strains (TA98 frameshift and TA100 nucleotide substitution), which is related to its increase in reverse mutation frequency. The pyrrole derivative compound (D085) in our study increased reverse mutation at low concentrations in the base exchange mutant TA100 strain. Pyrrole-chalcone hybrid compound (D085-K) increased the frequency of reverse mutations at all concentrations except the lowest dose in the TA98 strain, which is a frameshift mutant. This difference is thought to be due to the structural difference of the test items and/or the types of mutations carried by the test strains. In an older study that supports this argument, some of the pyrrole compounds tested [2,3-bishydroxymethyl-1-methylpyrrole (BHMP), 3-hydroxymethyl-1-methyl pyrrole (3HMP), and dehydroretronecine (DHR)] have been suggested to significantly induce reverse mutation in the TA92 strain, a base substitution mutant [45]. No published reliable data on whether the pyrrole-chalcone compound (D085-K) induces base change or frameshift mutation could be found. The findings obtained from our study are also a first in this respect.

In our study, the pyrrole-chalcone hybrid compound caused similar genotoxic and cytotoxic effects to the pyrrole derivative compound. As mentioned earlier, this compound has structural similarities with the flavonoid molecule also found in edible plants. With a short scan, a large number of studies on the antioxidant and protective properties of flavonoids can be reached. However, there is a very limited number of reliable studies conducted by mentioning the name of the pyrrole-chalcone derivative compound. In one of those studies, two different pyrrole chalcone [3-(2,5-dimethoxy-phenyl)-1-(1H-pyrrol-3-yl)-propenone (compound 1) and 1-(1H-pyrrol-2-yl)-3-m-tolylpropenone (compound 2)] was screened for anti-cancer properties by DNA binding analysis by ultraviolet-visible (UV/VIS) absorption spectroscopy. The intrinsic binding constants (Kb) of complexes 1 and 2, which are Veal-thymus DNA (CT-DNA), are $1.08x10^3$ M^{-1} and $5.225x10^3$ M^{-1}, respectively. Due to the methoxyl (-OCH3) group, compound 1 has a higher binding affinity than compound 2. It has been found that there is a better electron-donating group capable of forming intramolecular hydrogen bonding with DNA compared to the methyl (-CH3) moiety. The results from this study will be very useful in understanding the mechanism of interaction of pyrrole-chalcone analogs that bind to DNA and will serve as preliminary information for developments as new potential anti-cancer agents [59].

Antifungal, cytotoxicity and genotoxicity potentials of Pyrrole-based chalcone compounds [1-(1-methyl-1H-pyrrol-2-yl)-3-(5-(4-nitrophenyl) furan-2-yl) prop-2-en-1-one (1-coded), 1-(1-methyl-1H-pyrrol-2-yl)-3-(5-(2-nitrophenyl) furan-2-yl) prop-2-en-1-one (3-coded) , 1-(1-methyl-1H-pyrrol-2-yl)-3-(5-(4-chlorophenyl) furan-2-yl) prop-2-en-1-one (7-coded) and 1-(1-methyl-1H-pyrrol-2-yl)-3-(5- (2,5-dichlorophenyl) furan-2-yl) prop-2-en-1-one (9 coded)] were investigated. As a result of the study, it was revealed that the antifungal activity of some compounds against *Candida krusei* was significantly higher compared to the other compounds and the reference drug (ketoconazole). The same compounds were found to be non-genotoxic to TA98 and TA100, with/without metabolic activation, according to the Ames MPF (microplate format) test.

Besides, the MTT analysis of the 3-coded compound was revealed selective anti-cancer activity against the HepG-2 cell line more than positive carcinogen cisplatin. On the other hand, the compound coded 1 was found to be more effective and more selective than cisplatin in the A549 cell line [60]. In a recent review, chalcones were investigated as multifunctional molecules with promising pharmacological activities. In this review, chalcones have been emphasized that it has anti-cancer, antioxidant, anti-inflammatory, anti-microbial, anti-tuberculosis, anti-leishmanial, anti-malarial, anti-helminthic, osteogenic activities [61].

According to the mitotic index (MI) and nucleus division index (NDI) data obtained from our study, both test substances showed cytotoxic effects, especially at high concentrations. This observed cytotoxic effect is likely to be a direct result of genotoxicity or related to the influence of components involved in the progression of cell division. The genotoxic effect of the test substances in this study at high concentrations also largely explains the cause of the detected cytotoxicity. In summary, it can be said that the current cytotoxicity is due to genotoxicity. However, according to the results of a study investigating the effects of various chalcone derivatives on structures that have important roles in cell division; it has been reported that propenones, a chalcone derivative, show strong toxicity to many cancer cells lines and interact with tubulin at the colchicine binding site. These tubulin-binding molecules interfere with the dynamic balance of microtubules, destroying microtubule reorganization in the M phase and forming abnormal spindles. This causes apoptotic cell death by causing cell cycle arrest [62]. A study investigating the cytotoxicity of pyrrole derivatives was carried out by the National Cancer Institute. In that study, when tested with water-soluble or insoluble conjugates of pyrrole derivatives against 60 human cancer cell panels, it was shown that water-soluble PBD-polyamide compounds exhibited a higher cytotoxic activity than existing natural and synthetic pyrrolo [2,1-c] [1,4] benzodiazepines [20].

When the results are considered in total, some pyrrole and pyrrole-chalcone derivatives showed genotoxic and cytotoxic effects, while some of them did not show the same effects. This creates the impression that the test chemicals may have been due to their toxic effect or their cytotoxic effect due to gene expression.

In this study, the genotoxic and cytotoxic effects of test substances, especially at high concentrations, are striking. With this feature, pyrrole derivatives can give a new perspective to the solution to the cell proliferation problem. Also, focusing on experiments that will reveal the metabolic mechanism of action of test chemicals will enrich the results of this study. By comparing the results of our study with the results of new studies to be carried out from now on, more objective scientific data will be obtained about the use of likely chemicals to be put on the market.

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Disclosure statement

No potential conflict of interest was reported by the authors.

Author Contributions

All authors contributed to the study conception and design. Material preparation, data collection, and analysis were performed by Rumeysa MEŞE and Hasan Basri İLA. The authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

Approval to Participate

Informed consent was obtained from the Non-Invasive Clinical Research Ethics Committee of Çukurova University Faculty of Medicine at its 73rd meeting on January 12, 2018, from all individual participants included in the study.

References

[1] Breen, E.C., Walsh, J.J. (2010). Tubulin-targeting agents in hybrid drugs. Current Medicinal Chemistry; 17: 609-639. https://doi.org/10.2174/092986710790416254

[2] Joule, J.A., Mills, K., Smith, G.F. (2000). Heterocycl-chem. 4th Edition UK: Blackwell Science Publishers.

[3] Danks, T.N. (1999). Microwave assisted synthesis of pyrroles. Tetrahedron Letters; 40(20): 3957-3960. https://doi.org/10.1016/S0040-4039(99)00620-6

[4] Gabriele, B., Salerno, G., Fazio, A., Bossio, M.R. (2001). Palladium-catalyzed cycloisomerization of (Z)-(2-en-4-ynyl)amines: a new synthesis of substituted pyrroles. Tetrahedron Letters; 42(7), 1339-1341. https://doi.org/10.1016/S0040-4039(00)02206-1

[5] Farcas, S., Namy, J.L. (2001). Samarium diiodide promoted reactions of a diphenyl α iminoketone, a new synthesis of some pyrrole derivaties. Tetrahedron; 57(23): 4881-4888.

[6] Deryagina, E.N., Russavakaya, N.V., Vvedenskii, V.Y. (1999). Synthesis of 1-[5-(2, 2'-dithienyl)]-1-ethanone oxime and its reaction with acetylene. Russian Journal of Organic Chemistry; 35(8): 1225-1227.

[7] Ranu, B.C., Hajra, A. (2001). Synthesis of alkyl-substitued pyrroles by three-component coupling of carbonyl compound, amine and nitro-alkane/alkene on a solid surface of silica gel/alumina under microwave irradiation. Tetrahedron; 57(22): 4767-4773.

[8] Xu, X., Zhang, Y. (2002). Synthesis of 3H-pyrroles promoted by samarium/cat. iodine system. Synthetic commun; 32(17): 2643-2650.

[9] Chen, B.C., Guang-Zhi, Z., Katritzky, A.R., Yousaf, T.I. (1986). An H-1, C-13 and N-15 NMR Study of the Paal-Knorr condensation of acetonylacetone with primary amines. Tetrahedron; 42(2): 623-628. https://doi.org/10.1016/S0040-4020(01)87462-7

[10] Dede, B. (2006). Önemli bir beş-üyeli heteroaromatik bileşik: Pirol. Erciyes Üniversitesi, Fen Bilimleri Enstitüsü. Fen Bilimleri Dergisi; 22(1): 121-141.

[11] Nandi, S., Ray, J.K. (2011). Copper catalyzed synthesis of highly substituted pyrrole and isoindole derivatives. Tetrahedron Letters; 52: 6203-6206.

[12] Idhayadhulla, A., Kumar, R.S., Abdul Nasser, A.J. (2011). Synthesis, characterization and antimicrobial activity of new pyrrole derivatives. Journal of the Mexican Chemical Society; 55: 218-223.

[13] Dhar, D.N. (1981). The chemistry of chalcones and related compounds. Wiley-Interscience, New York.

[14] Dimmock, J.R., Elias, D.W., Beazely, M.A., Kandepu, N.M. (1999). Bioactivities of chalcones. Curr. Med. Chem; 6(12): 1125-1149.

[15] Lunardi, F., Guzela, M., Rodrigues, A.T., Correa, R., Eger-Mangrich, I., Steindel, M., Grisard, E.C., Assreuy, J., Calixto, J.B., Santos, A.R.S. (2003). Trypanocidal and leishmanicidal properties of substitution-containing chalcones. Antimicrobial Agents and Chemotherapy; 1449-1451. https://doi.org/10.1128/AAC.47.4.1449-1451.2003

[16] Mahapatra, D.K., Asati, V., Bharti, S.K. (2015a). Chalcones and their therapeutic targets for the management of diabetes: Structural and pharmacological perspectives. European Journal of Medicinal Chemistry; 92: 839-865. https://doi.org/10.1016/j.ejmech.2015.01.051

[17] Mahapatra, D.K., Bharti, S.K., Asati, V. (2015b). Anti-cancer chalcones: Structural and molecular target perspectives. European Journal of Medicinal Chemistry; 98: 69-114. https://doi.org/10.1016/j.ejmech.2015.05.004

[18] Mahapatra, D.K., Bharti, S.K., Asati, V. (2015c). Chalcone scaffolds as anti infective agents: Structural and molecular target perspectives. European Journal of Medicinal Chemistry; 496-524. https://doi.org/10.1016/j.ejmech.2015.06.052

[19] Mahapatra, D.K., Bharti, S.K. (2016). Therapeutic potential of chalcones as cardiovascular agents. Life Sciences; 154-172. https://doi.org/10.1016/j.lfs.2016.02.048

[20] Kumar, R., Lown, J.W. (2003). Design, synthesis and in vitro cytotoxicity studies of novel pyrrolo [2,1][1,4] benzodiazepine-glycosylated pyrrole and imidazole polyamide conjugates. https://doi.org/10.1039/B306685A.

[21] Boulamwini, J.K., Addo, J., Kamath, S., Patil, S., Mason, D., Ores, M. (2005). Small molecule antagonists of the Mdm2 oncoprotein as anticancer agents. Current Cancer Drug Targets; 5: 57-68. https://doi.org/10.2174/1568009053332672.

[22] Herencia, F., Ferrandiz, M.L., Ubeda, A., Dominguez, J.N., Charris, J.E., Lobo, G.M., Alcarez, M.J. (1998). Synthesis and anti-inflammatory activity of chalcone derivatives. Bioorg. and Med. Chem.; 8: 1169-1174. https://doi.org/10.1016/S0960-894X(98)00179-6

[23] Rao, Y.K., Fang, S.H., Tzeng, Y.M. (2004). Differential effects of synthesized 2'-oxigenated chalcone derivatives: Modulation of human cell cycle phase distribution. Bioorganic and Medicinal Chemistry; 12: 2679-2686. https://doi.org/10.1016/j.bmc.2004.03.014

[24] Satyanarayana, M., Tiwari, P., Tripathi, B.K., Srivastava, A.K., Pratap, R. (2004). Synthesis and antihyperglycemic activity of chalcone based arlyoxypropanolamines. Bioorganic and Medicinal Chemistry; 12: 883-889.

[25] Wu, J., Wang, X., Yi, Y., Lee, K. (2003). Anti-AIDS agents 54. A potent anti-HIV chalcone and flavonoids from genus desmos. Bioorganic&Medicinal Chemistry Letters; 13: 1813-1815.

[26] Fayed, T.A., Awad, M.K. (2004). Dual emission of chalcone-analogue dyes emitting in the red region. Chemical Physics; 303: 317-326. https://doi.org/10.1016/j.chemphys.2004.06.023

[27] Mukherjee, S., Kumar, V., Prasad, A.K., Raj, H.G., Bracke, M.E., Olsen, C.E., Jain, S.C., Parmar, V.S. (2001). Synhetic and biological activity evaluation studies on novel 1,3-diarylpropenones. Bioorg. Med. Chem.; 9: 337-345. https://doi.org/10.1016/S0968-0896(00)00249-2

[28] Hu, Z., Liu, J., Dong, Z., Guo, L., Wang, D., Zeng, P. (2004). Synthesis of chalcones catalysed by SOCI2/EtOH. Journal of Chemıcal Research; 158-159.

[29] Rida, S.M., Soliman, F.S., Badawy, E.S. (1986). Novel benzimidazoles with potential antimicrobial and antineoplastic activities. Pharmazie.; 41(8): 563-5.

[30] Wigerinck, P., Van Aerschot, A., Janssen, G., Claes, P., Balzarini, J., De Clercq, E., Herdewijn, P. (1990). Synthesis and antiviral activity of 3'-heterocyclic substituted 3'-deoxythymidines. J. Med. Chem.; 1990 Feb; 33(2): 868-73.

[31] Bandyopadhyay, D., Mukherjee, S., Granados, J.C., Short, J.D., Banik, B.K. (2012). Ultrasound-assisted bismuth nitrate-induced green synthesis of novel pyrrole derivatives and their biological evaluation as anticancer agents. Eur J Med Chem.; 2012 Apr; 50: 209-215. https://doi.org/10.1016/j.ejmech.2012.01.055.

[32] Geng, Y., Wang, X., Yang, L., Sun, H., Wang, Y., Zhao, Y., She, R., Wang, M.X., Wang, D.X., Tang, J. (2015). Antitumor activity of a 5-hydroxy-1H-pyrrol-2-(5H)-one-based synthetic small molecule in vitro and in vivo. PLoS One.; 2015 Jun 4; 10(6): e0128928. https://doi.org/10.1371/journal.pone.0128928

[33] Kumar, D., Kumar, N.M., Akamatsu, K., Kusaka, E., Harada, H., Ito, T. (2010). Synthesis and biological evaluation of indolyl chalcones as antitumor agents. Bioorganic&Medicinal Chemistry Letters; 3916-3919. https://doi.org/10.1016/j.bmcl.2010.05.016

[34] Rizvi, S.U.F., Siddiqui, H.L., Johns, M., Detorio, M., Schinazi, R.F. (2012). Anti-HIV-1 and cytotoxicity studies of pi-peridyl-thienyl chalcones and their 2-pyrazoline derivatives. Med. Chem. Res.; 21: 3741-9.

[35] Maronpot, R.R. (2015). Toxicological assessment of ashitaba chalcone. Food and Chemical Toxicology; 77: 111-119. https://doi.org/10.1016/j.fct.2014.12.021

[36] Akkurt, D. (2014). Mutagenic activities of benzoxazole derivative 12 compounds on Salmonella typhimurium TA98, TA100. Master Thesis, Hacettepe University, Institute of Science, Department of Biology.

[37] Yüzbaşıoğlu, D., Zengin, N., Ünal, F. (2014). Food preservatives and genotoxicity tests. Food; 39(3): 179-186.

[38] Akbaşlar, D. (2018). Synthesis of 1,2,3,4-Tetrasubstituted pyrrole compounds in lactic acid media by one pot-three com-ponent method: heteroaril derivatives of these molecules and ınvestigation antimicrobial activities of these molecules. PhD Thesis, Cukurova University, Instıtute of Natural and Applied Sciences, Department of Chemistry.

[39] Evans, H.J. (1984). Human peripheral blood lymphocytes for the analysis of chromosome aberrations in mutagen tests. Handbook of mutagenicity test procedures. In: Kilbey BJ, Legator M, Nichols W and Ramel C (Eds.), Second edition, Elsevier Science Publishers, BV, pp. 405-427.

[40] Rencüzoğulları, E., Topaktaş, M. (1991). The relationship between quantities of bromodeoxyuridine and human periph-eral blood with determination of the best differential staining of sister chromatids using chromosome medium-B. Fen ve Mühendislik Bilimleri Dergisi; 5(3): 19-24.

[41] Mace Jr, M.L., Daskal, Y., Wray, W. (1978). Scanning electron microscopy of chromosome aberrations. Mutation Res.; 52: 199-206. https://doi.org/10.1016/0027-5107(78)90141-0

[42] Rothfuss, A., Schutz, P., Bochum, S., Volm, T., Elberhard, E., Kreinberg, R., Vogel, V., Speit, G. (2000). Induced micronucleus frequencies in peripheral lymphocytes as a screening test for carries of a BRCA1 mutation in Breast cancer families. Cancer Res.; 60: 390-394.

[43] Maron, D., Ames, B.N. (1983). Revised methods for the Salmonella mutagenicity test. Mutation Research/Environmental Mutagenesis and Related Subjects; 113, (3-4), 173-215, ISSN 0165-1161.

[44] Ames, B.N., McCann, J., Yamasaki, E. (1975). Methods for detecting carcinogens and mutagens with the Salmonella/mammalian-microsome mutagenicity test. Mutat. Res.; 31: 347-364.

[45] Ord, M.J., Herbert, A., Mattocks, A.R. (1985). The ability of bifunctional and monofunctional pyrrole compounds to induce Sister-Chromatid Exchange (SCE) in human lymphocytes and mutations in Salmonella typhimurium. Mutation Research; 149: 485-493. https://doi.org/10.1016/0027-5107(85)90167-8

[46] Skoutelis, C., Antonopoulou, M., Konstantinou, I., Vlastos, D., Papadaki, M. (2017). Photodegradation of 2-Chloropyridine in aqueous solution: Reaction pathways and genotoxicity of intermediate products. Journal of Hazardous Materials.; 753-7635. https://doi.org/10.1016/j.jhazmat.2016.09.058.

[47] Limoli, C.L., Giedzinski, E. (2003). Induction of chromosomal ınstability by chronic oxidative stress. Elsevier; 339-346. https://doi.org/10.1016/S1476-5586(03)80027-1

[48] Cooke, M.S., Evans, M.D., Dizdaroğlu, M., Lunec, J. (2003). Oxidative DNA damage: mechanisms, mutation, and disease. The FASEB Journal.; 0892-6638/03/0017-1195. https://doi.org/10.1096/fj.02-0752rev.

[49] Barzilai, A., Yamamoto, K-I. (2004). DNA damage responses to oxidative stress. Elsevier; DNA Repair 3: 1109-1115. https://doi.org/10.1016/j.dnarep.2004.03.002.

[50] Salmon, T.B., Evert, B.A., Song, B., Doetsch, P.W. (2004). Biological consequences of oxidative stress-induced DNA damage in Saccharomyces cerevisiae. Nucleic Acids Research; 3712-3723. https://doi.org/10.1093/nar/gkh696

[51] Gonzalez-Hunt, C.P., Wadhwa, M., Sanders, L.H. (2018). DNA damage by oxidative stress: Measurement strategies for two genomes. Current Opinion in Toxicology; 7: 87-94. https://doi.org/10.1016/j.cotox.2017.11.001.

[52] Diaz-Perez, S., Kane, N., Kurmis, A.A., Yang, F., Kummer, N.T., Dervan, P.B., Nickols, N.G. (2018). Interference with DNA repair after ionizing radiation by a pyrrole-imidazole polyamide. Research Artıcle. Plos One; 13(5): e0196803. https://doi.org/10.1371/journal.pone.0196803

[53] Wurtz, N.R., Dervan, P.B. (2000). Sequence specific alkylation of DNA by hairpin Pyrrole-imidazole polyamide conjugates. Chemistry&Biology; 7: 153-161. https://doi.org/10.1016/S1074-5521(00)00085-5

[54] Baliga, R., Baird, E.E., Herman, D.M., Melander, C., Dervan, P.B., Crothers, D.M. (2001). Kinetic consequences of covalent linkage of DNA binding polyamides. Biochemistry; 40: 3-8. https://doi.org/10.1021/bi0022339.

[55] Janssen, B.M.G., Van Ommeren, S.P.F.I., Merkx, M. (2015). Efficient synthesis of peptide and protein functionalized pyrrole-imidazole polyamides using native chemical ligation. Int. J. Mol. Sci.; 16: 12631-12647. https://doi.org/10.3390/ijms160612631

[56] Zhao, R., Guan, L.L., Oreski, B., Lown, J.W. (1998). Synthesis, topoisomerase I inhibitory activity and in vitro cytotoxicity of camptothecin derivatives bearing five-membered heterocycle containing 10-substituents. Anticancer Drug Des.; 13(2): 145-57.

[57] Montaner, B., Castillo-Avila, W., Martinell, M., Öllinger, R., Aymami, J., Giralt, E., Perez-Tomas, R. (2005). DNA interaction and dual topoisomerase I and II inhibition properties of the anti-tumor drug prodigiosin. Toxicological Sciences; 85: 870-879. https://doi.org/10.1093/toxsci/kfi149

[58] WO/2006/092599 (08.09.2006) (International Application Number: PCT/GB2006/000742 International Filing Date: 02.03.2006) (https://patentscope.wipo.int/search/en/detail.jsf?docId=WO2006092599&tab=PCTBIBLIO).

[59] http://eprints.lincoln.ac.uk/32123/.

[60] Özdemir, A., Altıntop, M.D., Sever, B., Gençer, H.K., Kapkaç, H.A., Atlı, Ö., Baysal, M. (2017). A new series of pyrrole-based chalcones: Synthesis and evaluation of antimicrobial activity, cytotoxicity and genotoxicity. Molecules; 22(12). https://doi.org/10.3390/molecules22122112

[61] Ovonramwen, O.B., Owolabi, B.J., Oviawe, A.P. (2019). Recent advances in chalcones: Synthesis, transformation and pharmacological activities. Asian Journal of Chemical Sciences; 1-16. https://doi.org/10.9734/ajocs/2019/v6i318996.

[62] Budhiraja, A., Kadian, K., Kaur, M., Aggarwal, V., Garg, A., Sapra, S., Nepali, K., Suri, O.P., Dhar, K.L. (2011). Synthesis and biological evaluation of naphthalene, furan and pyrrole based chalcones as cytotoxic and antimicrobial agents. Med Chem Res; 21(9): 2133–2140. https://doi.org/10.1007/s00044-011-9733-y.