Araştırma Makalesi https://doi.org/10.46810/tdfd.790808 Research Article

Türk Doğa ve Fen Dergisi
Turkish Journal of Nature and Science
www.dergipark.gov.tr/tdfd

Cytotoxic and Genotoxic Effects of Some Azo Dyes in *Allium cepa* Root Tip Cells

Mehmet GÜMÜŞ[1*], H. Erhan EROĞLU[2], Nisa GÜMÜŞ[2], Emre YAĞCI[2]

[1]Yozgat Bozok Üniversitesi, Akdağmadeni Sağlık Yüksekokulu, İş Sağlığı ve Güvenliği Bölümü, Yozgat, Türkiye
[2]Yozgat Bozok Üniversitesi, Fen Edebiyat Fakültesi, Biyoloji Bölümü, Yozgat, Türkiye
Mehmet GÜMÜŞ ORCID No: 0000-0001-9262-7940
H. Erhan EROĞLU ORCID No: 0000-0002-4509-4712
Nisa GÜMÜŞ ORCID No: 0000-0002-5067-3874
Emre YAĞCI ORCID No: 0000-0001-9460-4857

**Sorumlu yazar: mehmet.gumus@bozok.edu.tr*

(Alınış: 05.09.2020, Kabul: 02.12.2020, Online Yayınlanma: 30.12.2020)

Keywords
Azo dye, Enaminone, Keto-hydrazo form, *Allium* test, Chromosome aberration, Mitotic index

Abstract: Azo dyes (**AD-1, AD-2)** were synthesized from the reaction diazonium salts of the aromatic amines salt with the enaminone derivative. The chemical structure of the synthesized novel azo dye (**AD-2**) was characterized by elemental analysis and other spectral techniques (FTIR, ^{1}H NMR and ^{13}C NMR). Azo dyes are used frequently in the industry and pose a great danger especially for water resources. In this respect, azo dyes threaten many products indirectly in agricultural applications. In this study, the cytotoxic and genotoxic effects of potential azo dyes (**AD-1**, **AD-2**) that can be used in industrial applications were determined using *Allium* test system in five different concentrations (6.25, 12.5, 25, 50 and 100 µM). As a result of the cytogenetic analyzes, it was determined that both azo dyes significantly reduced the number of divisions of *A. cepa* cells and caused chromosomal abnormalities in dividing cells. As a result, in this research, it is emphasized that the azo dyes (**AD-1**, **AD-2**), which are potentially used in the industry, cause genotoxic and cytotoxic effects in the living structures.

Allium cepa Kök Ucu Hücrelerinde Bazı Azo Boyarmaddelerin Sitotoksik ve Genotoksik Etkileri

Anahtar Kelimeler
Azo boya, Enaminon, Keto-hidrazo form, *Allium* test, Kromozomal anormallik, Mitotik indeks

Öz: Enaminon türevleriyle aromatik aminlerin diazonyum tuzlarının reaksiyonundan azo boyaları (**AD-1**, **AD-2**) sentezlendi. Sentezlenen yeni azo boyanın (**AD-2**) kimyasal yapısı, elementel analiz ve diğer spektral tekniklerle (FTIR, ^{1}H NMR ve ^{13}C NMR) karakterize edildi. Azo boyaları endüstride sıklıkla kullanılmakta ve özellikle su kaynakları için büyük tehlike oluşturmaktadır. Bu bakımdan azo boyalar tarımsal uygulamalarda birçok ürünü dolaylı olarak tehdit etmektedir. Bu çalışmada, endüstriyel uygulamalarda kullanılabilecek potansiyel azo boyaların (**AD-1**, **AD-2**) sitotoksik ve genotoksik etkileri, *Allium* test sistemi kullanılarak beş farklı konsantrasyonda (6.25, 12.5, 25, 50 ve 100 µM) belirlendi. Sitogenetik analizler sonucunda, her iki azo boyanın da *A. cepa* hücrelerinin bölünme sayısını önemli ölçüde azalttığı ve bölünen hücrelerde kromozomal anormalliklere neden olduğu belirlendi. Sonuç olarak, bu araştırmada, endüstride potansiyel olarak kullanılabilecek azo boyaların (**AD-1**, **AD-2**) canlı yapılarda genotoksik ve sitotoksik etkilere neden olduğu vurgulanmaktadır.

1. INTRODUCTION

Natural dyes have been used for centuries to change the color of materials such as paper and leather [1]. Nowadays, dyes and pigments are preferred in industrial applications to color products. Coloring materials are very important especially in textile, cosmetics and paper industries, as well as in lithography and coating processes. It is estimated that there are more than 100,000 different commercially produced paints each year. One of the most important materials used for this purpose are organic compounds classified as azo dyes [2]. Heterocyclic compounds containing the azo functional group (-N=N-) are used in various fields due to their interesting properties. Azo compounds attract the attention of chemists in the industrial field with some of their properties and the ability to be used as reagents in

many synthesis reactions. These compounds are used in many applications such as textile dyes, pharmaceutical chemistry, indicator synthesis, optical materials [3-4]. Especially, in textile dyes, it is highly preferred due to its easy production, wide color range and durability features. It is possible to develop color and other properties by condensing azo dyes with different hetero structures. Therefore, in recent years, synthesis studies to obtain more functional and useful azo compounds have increased its popularity [5]. Among the azo compounds, especially, α-diazocarbonyl compounds are used as a versatile synthetic intermediate in many organic reactions. Wolff arrangements, cyclopropanation, benzannulation and cyclo addition reactions are some of these [6]. Also, azo compounds are used as carbens precursors in the synthesis of many heterocyclic structures [7-8]. These compounds are called amphiphilic reagents because they can react with nucleophiles and electrophiles. Therefore, it is widely used as a reactant, especially in multicomponent reactions [9]. There are many methods in the literature for the synthesis of azo compounds. Azo compounds can be obtained using reagents such as *N*-sulfonylhydrazones and active methylene compounds [10].

Since azo dyes are used extensively in painting processes in many industrial applications, they cause pollution of water sources as industrial waste [11]. It is estimated that more than half of the paint contamination is caused by azo dyes. Contamination of water resources with dyes affects all living things, including humans, animals and plants. This situation damages ecological balance very much. The fact that the broken products or intermediates of the dyes cannot be biodegraded easily is the main source of the problem. Contamination of water resources with dyestuffs poses great danger for agricultural practices. It is always possible for azo dyestuffs that show toxic and carcinogenic effects to pass indirectly to humans [2]. Due to the chromophore group (-N=N-) it contains, it is difficult to biodegrade. It is also resistant to oxidizing compounds and light. For example, Orange G (OG) is one of the azo dyes commonly used in paper industry (Figure 1) [12]. Also, Mordant Black 17 is one of the aromatic dyes compounds and is widely preferred in the textile industry. [13].

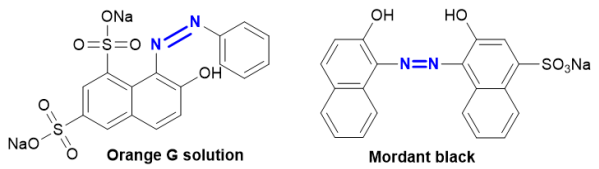

Figure 1. Structures of some important azo dyes

Allium test is an easy to use and inexpensive test and correlates particularly well with mammalian test systems [14]. The results of the *Allium* test may indicate the presence of certain cytotoxic and genotoxic substances that represent direct or indirect risks to living organisms in the environment. In *Allium* test, it is usually explained the reduction of cell division by cytotoxicity and major chromosome aberrations by genotoxicity [15]. If the decrease in the mitotic index (MI) falls below 22% compared to the control, it is accepted as the subletal effect value [16], if it falls below 50% the lethal effect value [17]. These values are cytotoxic breakpoints [18]. Chromosome aberrations (CA) show that chemicals interact with DNA and cause damage and are considered to be a highly reliable assay for evaluating genotoxicity [19-20].

In this study, starting from the enaminone compounds (**EN**), 4-(2-(1,3-dioxo-1-(*p*-tolyl)propan-2-ylidene) hydrazinyl)-*N*-(pyrimidin-2-yl) benzene sulfonamide (**AD-1**) and 4-(2-(1,3-dioxo-1-(*p*-tolyl)propan-2-ylidene)hydrazinyl) benzamide (**AD-2**) were synthesized in two steps. The synthesized new azo compound (**AD-2**) was purified by crystallization method, and their structure was characterized by elemental analysis, FTIR and NMR techniques. The cytotoxic and genotoxic effects of target molecules (**AD-1**, **AD-2**) were studied in five different concentrations in Allium cepa root stem cells (100 µM, 50 µM, 25 µM, 12.5 µM, 6.25 µM).

2. MATERYAL VE METOT

2.1. Materials

The reagents and solvents used in all the syntheses were purchased from commercial suppliers. The monitoring of the all reactions and the purity of the substances were done by thin layer chromatography (TLC) using silica gel plates (60F254 aluminium sheets) and ultraviolet light at 254-366 nm. Melting points of the target products were measured on a Electrothermal 9200 melting point apparatus and are reported uncorrected. Elemental analyzes were performed on a Leco-932 CHNS-O Elemental Analyzer at Yozgat Bozok University. FTIR spectra were obtained with a Perkin Elmer Spectrum Two Model FT-IR Spectrophotometer (ATR method). The ^{1}H and ^{13}C NMR spectra were obtained on a Bruker Avance 400 spectrometer in $CDCl_3$ at 400 and 100 MHz, respectively. Coupling constants (*J*) are reported in Hertz (Hz), and proton multiplicities are expressed as singlet (s), dublet (d) and multiplet (m) using $CDCl_3$ as a solvent. Plant materials (A. cepa, 2n = 16) were obtained from local bazaar (Yozgat, Turkey).

2.2. Synthesis

2.2.1. General procedure for azo dyes containing aldehyde group (AD)

The diazonium salt solution (4.0 mmol) was dropwise added to the solution of **EN** (1.0 equiv, 4.0 mmol) and K_2CO_3 (1.0 equiv, 4.0 mmol) in ethanol. The reaction was carried out in an ice bath. Within fifteen minutes, the yellow crude product which precipitated in the reaction medium was filtered. Target products (**AD1** and **AD2**) were purified by crystallization with butanol solvent [21].

4-(2-(1,3-dioxo-1- (*p*-tolyl) propan-2-ylidene) hyd razinyl) benzamide (AD-2)
Color: Yellow, Yield 1.051 g, 85%, mp 225-226 °C, FT-IR (ATR, cm^{-1}): υ_{max} 3370, 3294 (NH_2), 3160 (NH),

3068-2924 (CH, aromatic and aliphatic), 2888 (C-H, aldehyde), 1644 (C=O, amide); 1624 (C=O, aldehyde); 1618 (C=O, ketone); 1602-1462 (C=N and C=C). ^{1}H-NMR (400 MHz; $CDCl_3$, ppm): δ 14.60 (s, 1H, NH); 10.19 (s, 1H, CHO); 7.92-7.90 (m, part A of the system AA'BB', 2H, H_d); 7.88-7.86 (m, part A of the system AA'BB', 2H, H_b); 7.42-7.39 (m, part B of the system AA'BB', 2H, H_a); 7.33-7.31 (m, part B of the system AA'BB', 2H, H_c); 5.98-5.67 (broad d, 2H, NH_2); 2.48 (s, 3H, CH_3). ^{13}C-NMR (100 MHz; $CDCl_3$, ppm): δ 190.1 (CO, aldehyde), 182.3 (CO, benzoyl), 169.6 (CO, amide), 144.2, 135.7, 133.8, 130.6, 130.3, 129.3, 128.8, 128.2, 116.2 (C=C and C=N), 21.7 (CH_3). Calcd. for $C_{17}H_{15}N_3O_3$ (309.3250): C, 66.01; H, 4.89; N, 13.58. Found: C, 66.26; H, 4.67; N, 13.75 %.

2.3. *Allium* Test

Young bulbs were germinated at room temperature until reach a length of about 0.5 cm. Then, root tips were treated with different concentrations (6.25, 12.5, 25, 50, and 100 µM) of **AD-1** and **AD-2** for a period of 24, 48, and 72 h. Then, root tips were fixed in Carnoy's fixative (acetic acid:alcohol, 1:3 v/v) for 24 h, hydrolyzed in 1N HCl at 60 ºC for 8 min, stained in aceto-orcein for 2h, and squashed in 45% acetic acid.

2.4. Observations and Statistical Analysis

In cytogenetic analysis, six slides for each concentration and time period were observed under research light microscope (Olympus BX53, Japan) and photographed by digital camera (Olympus DP72, Japan). About 3500 cells were analyzed for each concentration and time period in the evaluation of MI and CA. MI and CA were calculated to equation 1 and 2.

$$MI\ (\%) = [Total\ number\ of\ dividing\ cells\ /\ Total\ number\ of\ cells\ (3500)] \times 100 \quad (1)$$

$$CA\ (\%) = [Total\ number\ of\ aberrant\ cells\ /\ Total\ number\ of\ cells\ (3500)] \times 100 \quad (2)$$

The data were calculated by one-way analysis of variance (ANOVA) and differences among groups were determined by the Tukey test ($p < 0.05$) in MS-Excel software.

3. RESULTS AND DISCUSSION

3.1. Chemistry

Some studies by our group on the synthesis of azo dyes, which are target compounds, are available in the literature [21]. Compound **AD-1**, one of the target compounds, was synthesized within the scope of this previous study. Compound **AD-2** is original and the synthesis procedure is the same as our previous study [21]. In the two-step synthesis procedure, the reaction of 4-methylacetophenone (**AP**) with the *N*,*N*-Dimethylformamitdimethylacetal (DMF-DMA) reagent was carried out in the first step of the synthesis, and the enaminone derivative (**EN**) was obtained. Then, two azo compounds (**AD-1**, **AD-2**) were synthesized as target compounds by reaction of the compound **EN** with solutions of diazonium salt of aromatic amines (4-amino-*N*-(pyrimidin-2-yl) benzene sulfonamide, 4-methylaminobenzamide) [Figure 2]. FTIR bands of functional groups in compound **AD-2** and the characteristic NMR signals of all atoms were confirmed by spectroscopic analysis.

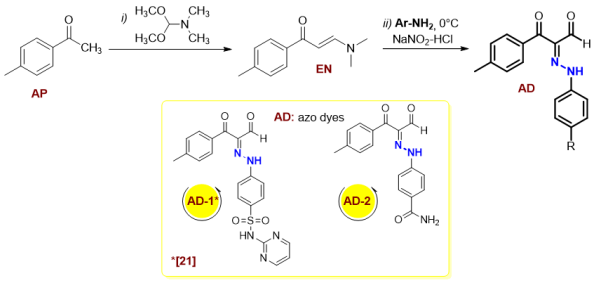

Figure 2. The synthesis route and molecular structures of azo dyes

Among the target compounds, the compound **AD-2** contains amide, aldehyde, ketone and diazo groups. In the FTIR spectrum of **AD-2**, the absorption bands belonging to NH_2 (two bands), NH and carbonyl groups (ketone, aldehyde) stretching vibrations were seen in 3370, 3294, 1644, 1624 and 1618 cm^{-1}, respectively. The absorption bands of aliphatic and aromatic hydrogens in this molecule are identified in the range 3068-2924 cm^{-1}. In addition, the CH stretching vibration belonging to the aldehyde group was characterized in 2888 cm^{-1}. In the ^{1}H NMR spectrum taken in the $CDCl_3$ solvent of compound **AD-2**; NH, CHO (aldehyde), NH_2 groups and CH_3 protons were characterized at 14.60, 10.19, 5.98-5.67 and 2.48 ppm, respectively. Also, the signals of aromatic CH protons in the molecule were seen in the ^{1}H NMR analysis in the range of 7.91-7.32 ppm. In ^{13}C NMR analysis of **AD-2** (in $CDCl_3$ solvent), aldehyde, benzoyl, amide and methyl groups gave characteristic signals at 190.1, 182.3, 169.6 and 21.7 ppm, respectively. The signals belonging to eight aromatic carbon atoms were observed in the range of 144.2-116.2 ppm. FTIR and NMR data showed that the compound **AD-2** is in the form of keto-hydrazo tautomer. The details on spectral findings are given in the supplementary material. The spectral data of this molecule are compatible with the spectroscopic data of azo dyes previously synthesized by our group [21].

3.2. Cytotoxic and Genotoxic Effects

Table 1 shows data of MI and CA in *A. cepa* cells, after exposure to **AD-1**. The MI rates of 48 h time period are 5.96 in 50 µM and 5.77 in 100 µM, and they are considerably lower than the control group (8.98). The MI rates of 72 h time period are 5.28 in 25 µM, 3.98 in 50 µM, and 3.81 in 100 µM, and they are considerably lower than the control group (7.55) ($p < 0.05$). Increasing **AD-1** concentrations have caused decreasing in MI rates at all time periods.

Tablo 1. Cytogenetic analysis of *A. cepa* root tips exposed to different concentrations of **AD-1**.

Concentration (µM)	Bridges	Stickiness	C-mitosis	Lagging	Vagrant	Binucleated	Defected polarization[1]	Chromosome loss	Aberration (%)	Mitotic index (%)
Control (24h)	3	6	5	3	4	2	0	3	0.74	9.03
6.25	2	8	4	3	3	1	2	2	0.71	9.11
12.5	3	4	2	3	7	2	2	2	0.71	8.87
25	6	7	5	4	8	3	4	5	1.20	8.26
50	15	14	15	10	14	9	7	10	2.69*	7.77
100	19	17	16	12	15	11	11	11	3.20*	7.81
Control (48h)	6	6	3	5	6	2	4	2	0.97	8.98
6.25	7	9	5	10	12	3	3	4	1.51	8.56
12.5	6	11	5	8	8	3	3	5	1.40	8.11
25	8	11	12	9	10	8	5	7	2.00	7.03
50	21	26	20	15	19	13	10	13	3.91*	5.96*
100	21	19	19	16	17	15	15	16	3.94*	5.77*
Control (72h)	7	8	3	5	6	2	4	2	1.06	7.55
6.25	8	11	5	8	11	3	2	5	1.51	6.98
12.5	8	11	7	7	9	4	3	5	1.54	7.01
25	13	15	17	14	14	11	9	12	3.00*	5.28*
50	22	29	23	16	21	17	12	16	4.46*	3.98*
100	19	22	20	21	18	20	18	21	4.54*	3.81*

[1] Multipolar and diagonal anaphase/telophase.
* indicate significant differences between control and concentrations. Significance at $p < 0.05$.

Figure 3 shows the normal stages of cell division and CA in meristematic cells of *A. cepa* after exposure to **AD-1**. CA are detected as bridges, stickiness, C-mitosis, lagging, vagrant chromosome, binucleate cell, multipolar and diagonal anaphase/telophase, and chromosome loss. The types and numbers of CA are given in Table 1. The most common chromosomal defects are stickiness, vagrant, and bridges, respectively. The CA rates of 24 h time period are 2.69 in 50 µM and 3.20 in 100 µM, and they are considerably higher than the control (0.74). The CA rates of 48 h time period are 3.91 in 50 µM and 3.94 in 100 µM, and they are considerably higher than the control (0.97). The CA rates of 72 h time period are 4.46 in 50 µM and 4.54 in 100 µM, and they are considerably higher than the control (1.06). Increasing **AD-1** concentrations have caused increasing in CA rates at all time periods ($p < 0.05$).

The compound **AD-1** is a sulfonamide derivative. In literature, there are reports on the cytotoxic and genotoxic potential of sulfonamides. Özkan and Liman showed that penoxsulam as a sulfonamide herbicide had cytotoxic effect by reducing MI and genotoxic effect by increasing CA, which are lagging, bridges, stickiness, polyploidy, and disturbed anaphase/telophase [22]. Badr reported the cytotoxic and genotoxic potential of three sulphonamide derivatives (sulphadimidine, sulphaphenazole, sulphadiazine) [23]. In addition, Leme and Marin-Morales recorded the detailed review regarding *A. cepa* test in environmental monitoring and presented cytotoxicity and genotoxic effects of pesticides, metals, textile dyes, complex mixtures, aromatic hydrocarbons, and other agents as sulfonamide derivatives [24]. The cytotoxic activity of the chiral sulfonamides was evaluated by human hepatocellular carcinoma, glioblastoma, and medulloblastoma cell lines. The compounds were shown to notably reduce cell viability as compared to nonmalignant cells [25]. The cytotoxicity of sulfonamide-based azaheterocyclic schiff base derivatives was performed on human keratinocyte and MCF-7 cell lines. The derivatives were nontoxic and the cytotoxic effects were followed dose-dependent [26]. The cytotoxicity of coumarin-sulfonamide derivatives was evaluated in human colon cancer cells. The results showed that compound 8i could inhibit cellular proliferation [27].

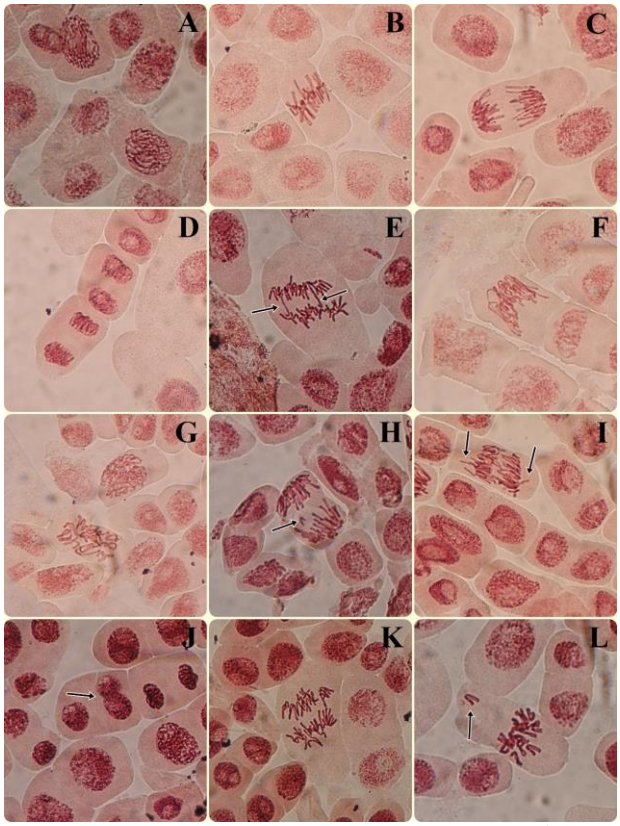

Figure 3. Chromosome aberrations in meristematic cells of *Allium cepa*. A) normal prophase; B) normal metaphase; C) normal anaphase; D) normal telophase; E) anaphase bridges (arrows); F) stickiness; G) C-mitosis; H) lagging (arrow); I) vagrant chromosomes (arrows); J) binucleate cell (arrow); K) defected polarization; L) chromosome loss (arrow). Magnification 400×

Table 2 shows data of MI and CA in *A. cepa* cells, after exposure to **AD-2**. The MI rates of 48 h time period are 4.23 in 50 µM and 4.32 in 100 µM, and they are considerably lower than the control group (6.86). The MI rates of 72 h time period are 2.25 in 50 µM and 2.40 in

100 µM, and they are considerably lower than the control group (6.62) ($p < 0.05$). Increasing **AD-2** concentrations have caused decreasing in MI rates at all time periods.

Figure 3 shows the normal stages of cell division and CA in meristematic cells of *A. cepa* after exposure to **AD-2**. The types and numbers of CA are given in Table 2. The most common chromosomal defects are stickiness, lagging, and vagrant, respectively. The CA rates of 24 h time period are 2.14 in 100 µM, and it is considerably higher than the control (0.74). The CA rates of 48 h time period are 2.46 in 50 µM and 2.69 in 100 µM, and they are considerably higher than the control (0.97). The CA rates of 72 h time period are 3.43 in 50 µM and 3.80 in 100 µM, and they are considerably higher than the control (1.06). Increasing **AD-2** concentrations have caused increasing in CA rates at all time periods ($p < 0.05$).

The compound **AD-2** is a benzamide derivative. In literature, there are reports on the cytotoxic and genotoxic potential of benzamides. Quiwei et al. determined the genotoxic effect of benzamide on chromosomal defects in *Vicia faba* [28]. The results showed that benzamide might induce CA. Maldonado et al. reported that *Allium* root meristem cells post-treated by benzamide after visible light showed high genotoxic potential with increasing sister chromatid Exchange [29]. In addition, it is recorded the genotoxicity newly synthesized *o*-benzoyl benzamide derivatives [30].

Table 2. Cytogenetic analysis of A. cepa root tips exposed to different concentrations of **AD-2**

Concentration (µM)	Bridges	Stickiness	C-mitosis	Lagging	Vagrant	Binucleated	Defected polarization[1]	Chromosome loss	Aberration (%)	Mitotic index (%)
Control (24h)	3	6	5	3	4	2	0	3	0.74	7.26
6.25	2	5	7	5	4	1	0	2	0.74	7.26
12.5	5	6	7	6	6	3	1	4	1.09	7.00
25	3	7	6	5	5	4	0	6	1.03	6.65
50	6	10	6	7	8	4	2	7	1.43	6.01
100	9	12	11	12	10	8	4	9	2.14*	6.11
Control (48h)	6	6	3	5	6	2	4	2	0.97	6.86
6.25	6	7	4	5	5	2	2	4	1.00	6.55
12.5	7	7	6	7	7	4	4	7	1.40	5.99
25	8	5	5	9	6	6	4	9	1.49	5.44
50	12	10	9	15	10	10	7	13	2.46*	4.23*
100	11	12	12	16	15	9	8	11	2.69*	4.32*
Control (72h)	7	8	3	5	6	2	4	2	1.06	6.62
6.25	6	11	5	7	8	2	4	4	1.34	5.91
12.5	8	9	7	8	8	7	3	6	1.60	5.66
25	9	10	11	11	10	9	5	9	2.11	4.00
50	18	15	18	17	15	15	9	13	3.43*	2.25*
100	21	17	17	19	18	17	8	16	3.80*	2.40*

[1] Multipolar and diagonal anaphase/telophase.
* indicate significant differences between control and concentrations. Significance at $p < 0.05$.

4. CONCLUSION

Diazo compounds are widely used in industrial applications with their dye feature, and especially natural water sources are contaminated with industrial wastes containing azo dyes. In this study, two compounds that can be used in industrial applications as azo dyes were synthesized and their cytotoxic and genotoxic effects were evaluated. The aromatic azo dyes (**AD-1**, **AD-2**) containing pyrimidine and amide groups as substituted groups were synthesized by a two-step synthesis procedure. **AD-2**, which is novel from the synthesized azo dyes, was characterized by spectroscopic methods. According to spectral data, compound **AD-2** was found to be in keto-hydrazo form structure. As a result of the cytogenetic analyzes, it was determined that both chemicals significantly reduced the number of divisions of *A. cepa* cells and caused chromosomal abnormalities in dividing cells. As a result, in this research, it is emphasized that the chemicals, which are potentially used in the industry, cause genotoxic and cytotoxic effects in the living structure.

Acknowledgement

This work was supported by Research Fund of Science and Technology Practice & Research Centre of Yozgat Bozok University (Project number: 6602c-ASYO/19-266).

REFERENCES

[1] Ilic DK, Ostafe R, Djurdjevic DA, Popovic N, Schillberg S, Fischer R, et al. Saturation mutagenesis to improve the degradation of azo dyes by versatile peroxidase and application in form of VP-coated yeast cell walls. Enzyme Microb Technol. 2020; 136: 109509.

[2] Bediako JK, Lin S, Sarkar AK, Zhao Y, Choi JW, Song MH, et al. Evaluation of orange peel-derived activated carbons for treatment of dye-contaminated wastewater tailings. Environ Sci Pollut Res Int. 2020; 27: 1053-1068.

[3] Abbas Al-Tahan RA. Synthesis and characterization of azo dyes ligand complexes with

some metal ions. Int J Chemtech Res. 2018; 11(6): 108-113.
[4] Lole BA, Piste PB. Clay mediated one step synthesis of azo dyes with 1,3-benzoxazole moie. J Chem Pharm Res. 2017; 9(12): 53-58.
[5] Demircali A, Karci F, Avinc O, Kahriman AU, Gedik G, Bakan E. The synthesis, characterization and investigation of absorption properties of disperse disazo dyes containing pyrazole and isoxazole. J Mol Struct. 2019; 1181: 8-13.
[6] Ansari MA, Yadav D, Singh MS. Rhodium(II)-Catalyzed Annulative Coupling of β-Ketothioamides with α-Diazo Compounds: Access to Highly Functionalized Thiazolidin-4-ones and Thiazoline. J Org Chem. 2020; 85(13): 8320-8329.
[7] He Y, Lou J, Wu P, Zhou Y, Yu Z. Copper-Catalyzed Annulative Coupling of S,S-Disubstituted Enones with Diazo Compounds to Access Highly Functionalized Thiophene Derivatives. J Org Chem. 2020; 85(2): 1044-1053.
[8] Li X, Ye X, Wei C, Shan C, Wojtas L, Wang Q, et al. Diazo Activation with Diazonium Salts: Synthesis of Indazole and 1,2,4-Triazole. Org Lett. 2020; 22(11): 4151-4155.
[9] Xiong M, Liang X, Gao Z, Lei A, Pan Y. Synthesis of Isoxazolines and Oxazines by Electrochemical Intermolecular [2+1+n] Annulation: Diazo Compounds Act as Radical Acceptors. Org Lett. 2019; 21(23): 9300-9305.
[10] Green SP, Wheelhouse KM, Payne AD, Hallett JP, Miller PW, Bull JA. Thermal Stability and Explosive Hazard Assessment of Diazo Compounds and Diazo Transfer Reagents. Org Process Res Dev. 2020; 24(1): 67-84.
[11] Luna-Alvarado MA, Estrada-Flores M, Manriquez-Ramirez ME, German CMR. Bismuth oxide carbonate structures synthesized by microwave-assisted solvothermal approach and its use as catalyst for the degradation of azo dye in a solution. J Miner Mater Char Eng. 2019; 7(6): 468-479.
[12] Feng T, Bavumiragira JP, Wambui MA, Kabtamu DM, Laszlo K, Wang Y, et al. Hierarchical porous induced competent removal of low concentration azo dye molecules by generating a leachy crystalline structure H-MIL-53(Fe). Chin Chem Lett. 2020; Ahead of Print.
[13] Benkhaya S, M'rabet S, El Harfi A. Classifications, properties, recent synthesis and applications of azo dyes. Heliyon. 2020; 6(1): e03271.
[14] El-Shabhaby OA, Abdel Migid HM, Soliman MI, Mashaly IA. Genotoxicity screening of industrial wastewater using the Allium cepa chromosome aberration assay. Pak J Biol Sci. 2003; 6: 23-28.
[15] Kovalchuk O, Kovalchuk I, Arkhipov A, Telyuk P, Hohn B, Kovalchuk L. The Allium cepa chromosome aberration test reliably measures genotoxicity of soils of inhabited areas in the Ukraine contaminated by the Chernobyl accident. Mutat Res. 1998; 415: 47-57.
[16] Antosiewicz D. Analysis of the cell cycle in root meristem of Allium cepa under the influence on Ledakrin. Folia Histochem Cytobiol. 1990; 28(1-2): 79-95.
[17] Panda BB, Sahu UK. Induction of abnormal spindle function and cytokinesis inhibition in mitotic cells od Allium cepa by the organophosphorus insecticide fensulfotion. Cytobios. 1985; 42: 147-155.
[18] Sharma, CBSR. Plant meristems as monitors of genetic toxicity of environmental chemicals. Curr. Sci. 1983; 52(21): 1000-1002.
[19] Saxena PN, Chauhan LKS, Gupta, SK. Cytogenetic effects of commercial formulation of cypermethrin in root meristem cells of Allium sativum: Spectroscopic basis of chromosome damage. Toxicology. 2005; 216: 244-252.
[20] Liman R, Cigerci IH, Akyıl D, Eren Y, Konuk, M. Determination of genotoxicity of Fenaminosulf by *Allium* and Comet Tests. Pestic Biochem Phys. 2011; 99: 61-64.
[21] Gümüş M, Sert Y, Koca İ. Synthesis, characterization and theoretical studies of novel sulfonamide-aldehydes derivatives having tautomeric forms. Org Commun. 2019; 12(4): 176-187.
[22] Özkan S, Liman R. Cytotoxicity and genotoxicity in *Allium cepa* L. root meristem cells exposed to the herbicide penoxsulam. CBU Journal Of Science. 2019; 15(2): 221-226.
[23] Badr A. Cytogenetic activities of 3 sulphonamides. Mutat Res Lett. 1982; 104(1-3): 95-100.
[24] Leme DM, Marin-Morales MA. *Allium cepa* test in environmental monitoring: A review on its application. Mutat Res. 2009: 682, 71-81.
[25] Samadaei M, Pinter M, Senfter D, Madlener S, Rohr-Udilova N, Iwan D, Kaminska K, Wojaczynska E, Wojaczynski J, Kochel A. Synthesis and Cytotoxic Activity of Chiral Sulfonamides Based on the 2-Azabicycloalkane Skeleton. Molecules. 2020: 13(21), 5010.
[26] Abas M, Rafique H, Shamas S, Roshan S, Ashraf Z, Iqbal Z, Raza H, Hassan M, Afzal K, Rizvanov AA. Sulfonamide-based azaheterocyclic schiff base derivatives as potential carbonic anhydrase inhibitors: synthesis, cytotoxicity, and enzyme inhibitory kinetics. Biomed Res Int. 2020: 5, 1-9.

Attachments

Attachments A. Supplementary material

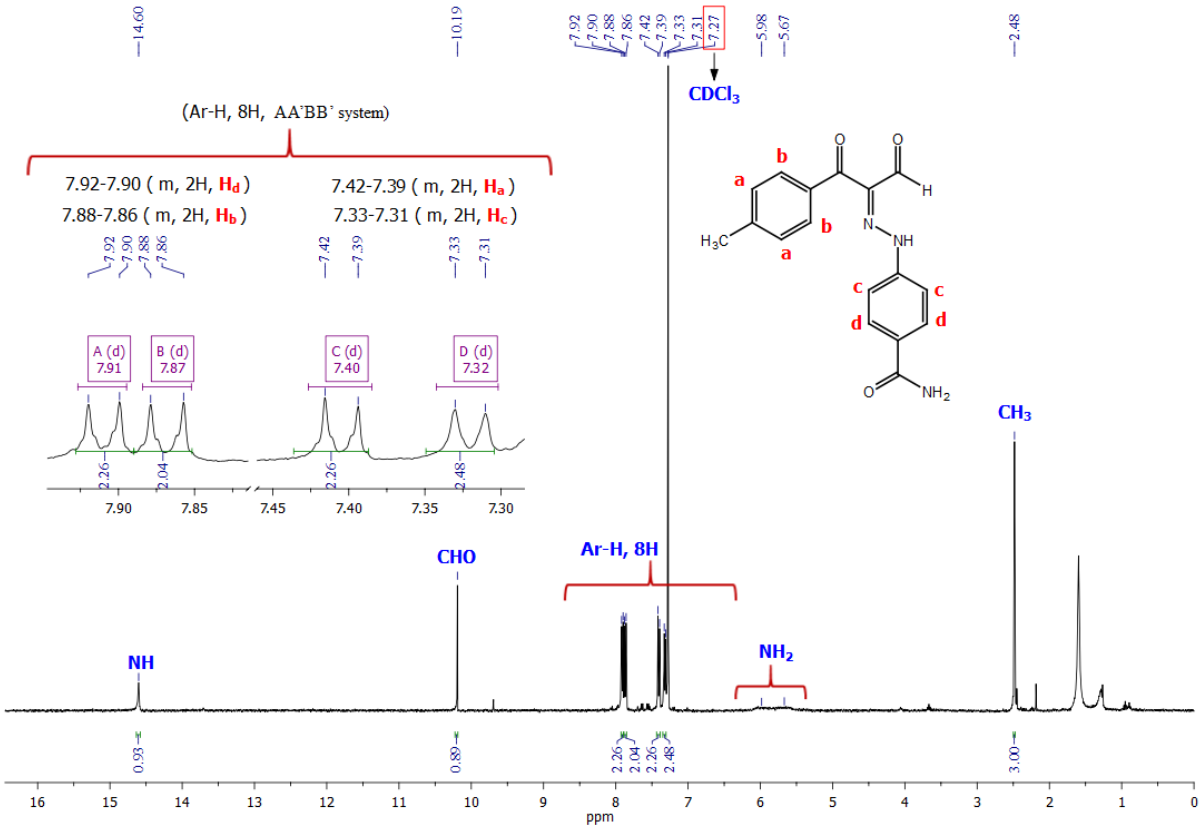

Figure S1: [1]H NMR spectra of **AD-2**

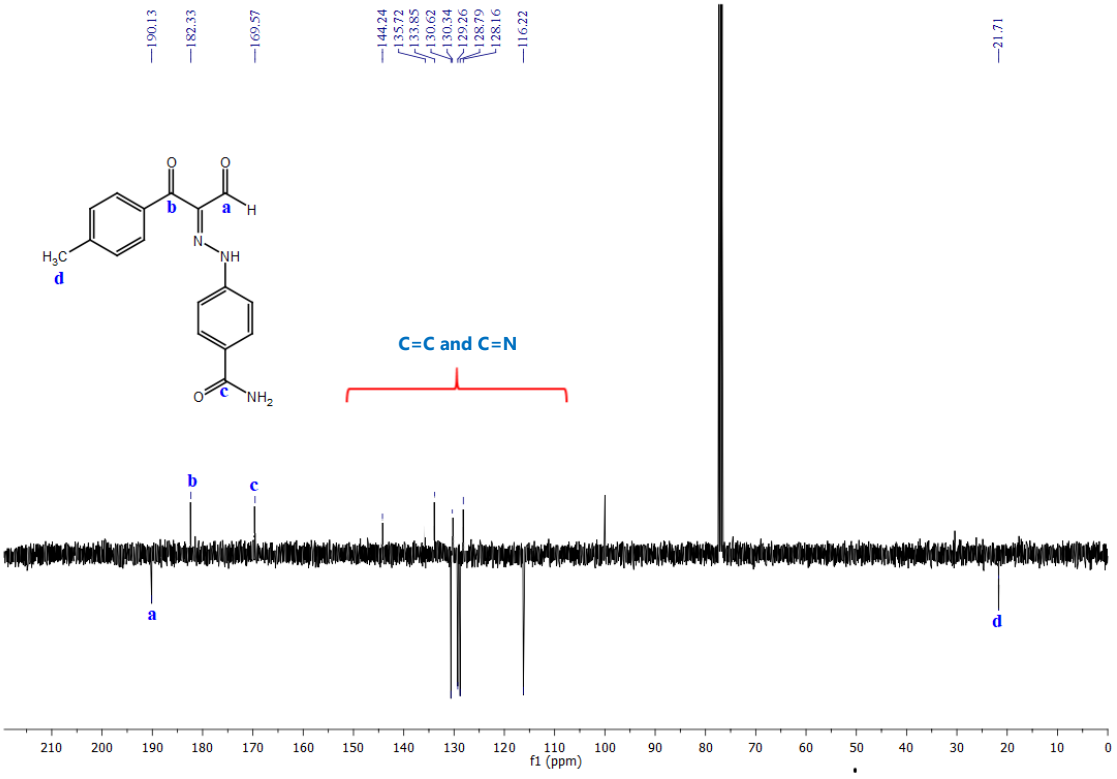

Figure S2: [13]C NMR spectra of **AD-2**