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Research Article

Evulation of Antimutagenic Activity of Ni(II) Complexes with Unsymmetric Schiff Bases

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ABSTRACT

In this work, Ni(II) complexes with unsymmetric Schiff bases (**NiL_1, NiL_2, NiL_3, NiL_4**) were prepared by a two-stage method reported by one of us recently for investigate antimutagenic properties. Sodium azide-induced antimutagenic effect in lymphocytes was determined by sister chromatid exchange (SCE) and micronucleus (MN) methods. It has been determined that the synthesized compounds have antimutagenic properties and reduce the mutagenicity caused by sodium azide (NaN_3) which is used as a positive control.

Keywords: *Unsymmetric diimin, Ni (II) complex, Sodium azide, Antimutagenic.*

Asimetrik Schiff Bazı Ni(II) Komplekslerinin Antimutajenik Aktivitesinin Değerlendirilmesi

ÖZET

Bu çalışmada, asimetrik Ni(II) kompleksleri (**NiL_1, NiL_2, NiL_3, NiL_4**) potansiyel antimutajen özelliklerini incelemek için son zamanlarda grubumuzdan biri tarafından rapor edilen yeni iki aşamalı bir yöntem ile hazırlandı. Lenfositlerdeki sodyum azid kaynaklı antimutagenik etki, kardeş kromatid değişimi (SCE) ve mikronükleus (MN) yöntemleriyle belirlendi. Sentezlenen bileşiklerin antimutagenik özelliklere sahip olduğu ve pozitif kontrol olarak kullanılan sodyum azid (NaN_3)'ün neden olduğu mutajeniteyi azalttıkları belirlenmiştir.

Anahtar Kelimeler: *Asimetrik diimin, Ni(II) kompleksi, Sodyum azit, Antimutajen*

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I. INTRODUCTION

Shiff bases known as azomethine or imine are obtained by the condensation of amines with carbonyl compounds [1]. Schiff bases with their metal complexes are very common investigated for catalysts, dyes, polymer stabilisers, corrosion inhibitors, intermediates, fluorescense properties, electroluminescent properties, antimicrobial, antibacterial, insecticial, anti inflammatory activity, enzyme cofactors [2-8].

Unsymmetric Schiff bases including N_2O_2 have been great interested, due to their catalytic activity, structural versality, antimicrobial activity, magnetic, optic properties [9, 10]. Symmetrical Schiff bases (i.e. –CH=N-aryl-N=CH– or –N=HC-aryl-CH=N–) can be directly synthesized. But, unsymmetrical Schiff bases (i.e. –CH=N-aryl-CH=N–) cannot be synthesized directly due to a reaction formed between $-NH_2$ and -CHO groups in the same aromatic ring. First time, a new two step method for the synthesis of these type diimines with respect to the unsymmetrical nature of the imine bond were reported by one of us [11, 12]. Thus, potentiometric, tautomeric and antimicrobial studies of these type unsymmetric Schiff bases were started to be investigated [13-17].

In people DNA damage occurs as a result of exposure to food and surrounding genotoxic substances [18]. In recent years, an increase in the mutation-associated disease is observed [19]. Mutagens are chemical and physical substances that affect DNA, causing mutations. Mutagens have been determined to cause many genetic diseases such as cancer [20, 21]. Mutagens play a harmful role in living systems by inducing oxidative damage to cell structures and biomolecules [22]. Sodium azide is the mutagenic substance in several organisms including bacteria, plants and animals [7]. It is used in agriculture to bring out resistance in different crops to develop their quality and production features against various pathogens [23]. If sodium azide is toxic in the cell, azide ions bind to Fe^{3+} in hemoglobin and inhibit the respiratory chain [24, 25]. Mutagenicity of NaN_3 is produced by the production of an organic metabolite called L-azidoalanine [26, 27].

Anti-mutagenicity is an elimination of the activity of mutagenic substances by various methods [20]. Antimutagenic agents are important in the treatment of cancer or other diseases related to mutation formation. Antimutagens prevention the negative effects of induced mutations in human by inhibiting the effect of the mutation on genes or inactivating the mutagenic agent [28]. For this reason the discovery of novel antimutagens has been important. Heterocyclic compounds has a great potential to develop preservative negative effects of mutagens [29, 30].

The goal of this work was to examine the antimutagenic activity of four Ni(II) complexes with unsymmetric Schiff bases against sodium azide. Herein, we report the synthesis of Ni(II) complexes with (–CH=N-aryl-CH=N–) type unsymmetric Schiff bases by using a two-stage method. We also calculate the leading SCE and MN frequencies of the sodium azide-induced antimutagenic effect in human peripheral lymphocytes, working with polymeric microspheres.

II. MATERIALS AND METHODS

All materials were supplied from Sigma-Aldrich company.

A. GENERAL PROCEDURE FOR SYNTHESIS OF Ni(II) COMPLEXES WITH UNSYMMETRIC SCHIFF BASES (NiL_1, NiL_2)

Recently reported the synthesis of the Ni(II) complexes with unsymmetric diimines (**NiL_1**, **NiL_2**) by one of us [11]. Firstly, 2-hydroxy-N(2-nitrobenzylidene)aniline (the starting Schiff base) was prepared by reacting of 2-hydroxy aniline with 2-nitro-benzaldehyde in ethanol. Secondly, the unsymmetric Schiff bases were synthesized by using a two-stage method. The starting Schiff base was dissolved in ethanol-

water solution 1:1(v/v). The starting Schiff base's nitro group was reduced to an amino using reducing agent ($Na_2S_2O_4$). In this phase, the $Na_2S_2O_4$ was poured to the mixture during 1 hour. The solution was stirred additional 1 hour at 45-50 °C. Then, 2-hydroxy-5-methylbenzaldehyde (or 2-hydroxy-5-chlorobenzaldehyde) in ethanol was poured to the solution and was heated to reflux for 2 hours at 40-50 °C. The solution was vaporized at room temperature for 3 day. The yellow crystalline material was filtered and recrystallized from ethanol. Finally, the unsymmetric Schiff bases Ni(II) complexes (**NiL_1**, **NiL_2**) were obtained by reaction of equimolar amounts of the obtained unsymmetric diimines and Ni(II) chlorides in ethanol. The stirring prosess was continued in about 4-6 h under reflux and was vaporized approximate 7-10 days. The solution was filtered and purification by washed with hot water, ethanol and ether, respectively.Then, the green solid dried in a vacuum dessicator over anhydrous $CaCl_2$.

B. GENERAL PROCEDURE FOR SYNTHESIS OF Ni(II) COMPLEXES WITH UNSYMMETRIC SCHIFF BASES (NiL_3, NiL_4)

Recently, reported the synthesis of the Ni(II) complexes with unsymmetric Schiff bases (**NiL_3**, **NiL_4**) by one of us [12]. Firstly, 2-hydroxy-N-(5-nitrofurylidene)aniline (the starting Schiff base) was prepared by reacting of 5-nitro-furfural with and 2-hydroxyaniline in ethanol. Secondly, the unsymmetric Schiff bases were synthesized by using a two-stage method. The starting Schiff base was dissolved in ethanol-water solution 1:1(v/v) at 70 °C. The starting Schiff base's nitro group was reduced to an amino using reducing agent ($Na_2S_2O_4$). In this phase, the $Na_2S_2O_4$ was poured to the mixture during 1 hour. The solution was stirred additional 1 hour at 50 °C. Then, 2-hydroxy-5-methylbenzaldehyde (or 2-hydroxy-5-chlorobenzaldehyde) in ethanol was poured to the solution and was heated to reflux for 3 hours at 60 °C. The solution was vaporized at room temperature for 3 day. The yellow crystalline material was filtered and recrystallized from ethanol. Finally, the unsymmetric Schiff bases Ni(II) complexes (**NiL_3**, **NiL_4**) were obtained by reaction of equimolar amounts of the obtained unsymmetric diimines and Ni(II) chlorides in ethanol. The stirring prosess was continued in about 3-5 h under reflux and was vaporized approximate 3-4 days. The solution was filtered and purification by washed with hot water, ethanol and ether, respectively.Then, the green solid dried in a vacuum dessicator over anhydrous $CaCl_2$.

C. DETERMINATION OF ANTIGENOTOXIC PROPERTIES

The antimutagenic activities of the unsymmetric Schiff bases Ni(II) complexes (**NiL_1**, **NiL_2**, **NiL_3**, **NiL_4**) against the sodium azide-induced mutagenicity were investigated by MN and SCE methods.

Peripheral blood samples were obtained from four volunteers non-smokers (two females and two males). Lymphocyte cultures were created as follows: 0.5 mL of heparinized whole blood + RPMI 1640 chromosome medium + 15% heat-inactivated fetal calf serum + 1% streptomycin + 1% penicillin + 2% L-glutamine + 2% phytohemagglutinin. NaN_3 (5 μM) was studied as positive control.

The researches were performed as follows:

Culture 1: Solvent control;
Culture 2: Pozitif control;
Culture 3: Polymeric microspheres (80 μg/mL);
Culture 4: 5 μM NaN_3 + unsymmetric diimine complexes (5 μg/mL);
Culture 5: 5 μM NaN_3 + unsymmetric diimine complexes (10 μg/mL);
Culture 6: 5 μM NaN_3 + unsymmetric diimine complexes (20 μg/mL);
Culture 7: 5 μM NaN_3 + unsymmetric diimine complexes (40 μg/mL);
Culture 8: 5 μM NaN_3 + unsymmetric diimine complexes (80 μg/mL);

In SCE assay, 5-bromo 2-deoxyuridine was added to the cultures at 6 mg / mL and incubated at 37 ° C in the dark for 72 hours. 0.1 mg / mL of colcemide was added to stop mitosis at the metaphase stage. After centrifugation at 1200 rpm for 10 minutes, the supernatant was discarded. The cells were treated and treated with hypotonic solution (0.075 M KCl) for 25 minutes and fixed in a 1: 3 mixture of acetic acid / methanol (v/v). Metaphase chromosomes with bromodeoxyuridine were stained by fluorescence

plus Giemsa technique [31]. SCE results were recorded by selecting 60 metaphases. For each treatment condition, well-distributed second compartment metaphases containing 42-46 chromosomes in each cell were scored and the values obtained were calculated as SCE per cell [32].

In MN method, after 44 hours of incubation, 3 μg / mL cytokalacin B was added to the blood samples and incubated for 72 hours. After centrifugation, the cells were harvested and 6 mL of 0.05 M KCl was added and allowed to incubate for 7 minutes. Then centrifuge again, 6 ml of fresh fixation solution was added to the cells. The cells were then further treated with 1 ml of fixation solution on a microscope slide and then stained with 5% Giemsa dye. Slides were examined under a microscope, 1000 bi-nucleated cells were scored [33].

III. RESULTS AND DISCUSSION

A. CHEMISTRY

The Ni(II) complexes with unsymmetric Schiff bases (**NiL_1, NiL_2, NiL_3, NiL_4**) have previously been characterized by spectroscopic techniques [11, 12].

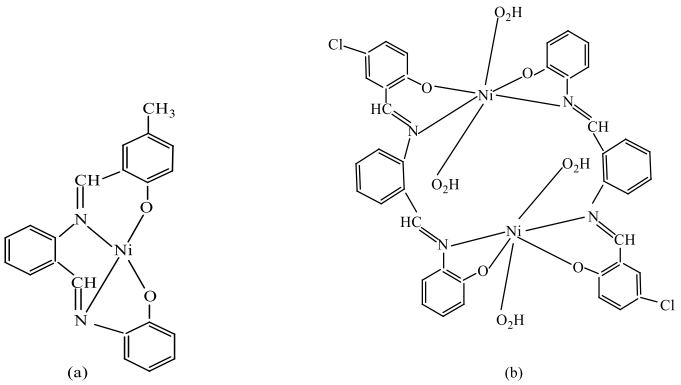

Figure 1. *Unsymmetric Schiff bases Ni(II) complexes (**NiL_1, NiL_2**).*

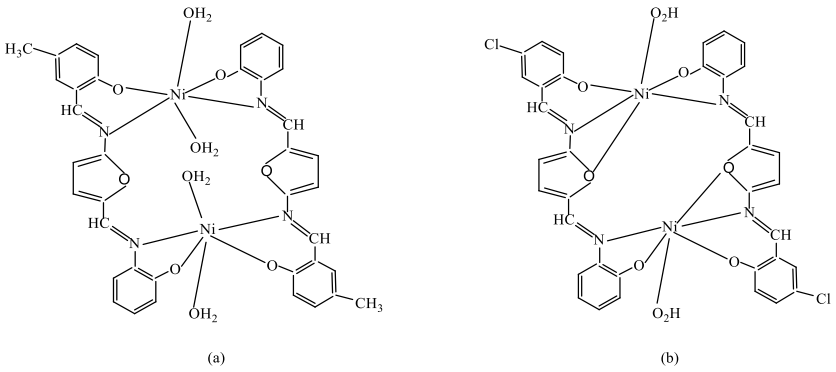

Figure 2. *Unsymmetric Schiff bases Ni(II) complexes (**NiL_3, NiL_4**).*

B. ANTIMUTAGENIC ACTIVITY

The antimutagenic activities for the Ni(II) complexes with unsymmetric Schiff bases (**NiL_1, NiL_2, NiL_3, NiL_4**) are given in Table 1. The antimutagenic activities of different concentrations (5, 10, 20 and 40 μg/mL) of the complexes (**NiL_1, NiL_2, NiL_3, NiL_4**) were investigated against NaN_3 in human lymphocyte cells by MN and SCE tests. NaN_3 is a strong mutagen affecting many organisms. It was determined that NaN_3 caused DNA damage and the increase in MN and SCE frequencies determined in the control group was statistically significant ($p < 0,05$). Comparisons were made between different

concentrations of the Ni(II) complexes with unsymmetric Schiff bases added to the cultures to inhibit the genotoxicity caused by NaN_3.

Table 1. *The effects of Ni(II) complexes with unsymmetric Schiff bases and sodium azide*

Groups	Doses	SCE/Cell ± S.E.	MN numbers ± S.E.
Medium		5.92 ± 0.04^{a}	1.60 ± 0.09^{a}
NaN_3	5 μM	8.22 ± 0.06^{e}	3.18 ± 0.12^{e}
NiL_1	80 μg/mL	6.00 ± 0.04^{a}	1.68 ± 0.02^{a}
$NaN_3 + NiL_1$	5 μM + 5 μg/mL	7.54 ± 0.09^{d}	2.66 ± 0.14^{d}
$NaN_3 + NiL_1$	5 μM + 10 μg/mL	6.96 ± 0.03^{c}	2.30 ± 0.04^{c}
$NaN_3 + NiL_1$	5 μM + 20 μg/mL	6.76 ± 0.07^{b}	2.22 ± 0.06^{bc}
$NaN_3 + NiL_1$	5 μM + 40 μg/mL	6.35 ± 0.14^{ab}	1.78 ± 0.01^{a}
$NaN_3 + NiL_1$	5 μM + 80 μg/mL	7.10 ± 0.04^{c}	2.03 ± 0.09^{b}
NiL_2	80 μg/mL	6.08 ± 0.06^{a}	1.85 ± 0.04^{a}
$NaN_3 + NiL_2$	5 μM + 5 μg/mL	7.75 ± 0.11^{de}	2.74 ± 0.06^{d}
$NaN_3 + NiL_2$	5 μM + 10 μg/mL	7.32 ± 0.04^{d}	2.62 ± 0.04^{d}
$NaN_3 + NiL_2$	5 μM + 20 μg/mL	7.21 ± 0.14^{cd}	2.36 ± 0.04^{c}
$NaN_3 + NiL_2$	5 μM + 40 μg/mL	7.87 ± 0.05^{de}	2.56 ± 0.81^{cd}
$NaN_3 + NiL_2$	5 μM + 80 μg/mL	8.04 ± 0.12^{e}	2.72 ± 0.68^{d}
NiL_3	80 μg/mL	6.02 ± 0.10^{a}	1.74 ± 0.04^{a}
$NaN_3 + NiL_3$	5 μM + 5 μg/mL	7.56 ± 0.06^{d}	2.55 ± 0.07^{cd}
$NaN_3 + NiL_3$	5 μM + 10 μg/mL	7.04 ± 0.31^{d}	2.41 ± 0.02^{c}
$NaN_3 + NiL_3$	5 μM + 20 μg/mL	6.64 ± 0.51^{b}	2.24 ± 0.10^{bc}
$NaN_3 + NiL_3$	5 μM + 40 μg/mL	7.12 ± 0.07^{c}	2.68 ± 0.02^{d}
$NaN_3 + NiL_3$	5 μM + 80 μg/mL	6.68 ± 0.08^{b}	2.86 ± 0.03^{de}
NiL_4	80 μg/mL	5.96 ± 0.03^{a}	1.69 ± 0.05^{a}
$NaN_3 + NiL_4$	5 μM + 5 μg/mL	7.82 ± 0.06^{de}	2.72 ± 0.07^{d}
$NaN_3 + NiL_4$	5 μM + 10 μg/mL	7.19 ± 0.89^{c}	2.40 ± 0.13^{c}
$NaN_3 + NiL_4$	5 μM + 20 μg/mL	7.03 ± 0.74^{c}	2.28 ± 0.09^{c}
$NaN_3 + NiL_4$	5 μM + 40 μg/mL	6.85 ± 0.09^{bc}	1.96 ± 0.03^{b}
$NaN_3 + NiL_4$	5 μM + 80 μg/mL	7.26 ± 0.10^{cd}	2.14 ± 0.08^{b}
$NaN_3 + NiL_1$	5 μM + 40 μg/mL	6.35 ± 0.14^{ab}	1.78 ± 0.01^{a}
$NaN_3 + NiL_1$	5 μM + 80 μg/mL	7.10 ± 0.04^{c}	2.03 ± 0.09^{b}
NiL_2	80 μg/mL	6.08 ± 0.06^{a}	1.85 ± 0.04^{a}
$NaN_3 + NiL_2$	5 μM + 5 μg/mL	7.75 ± 0.11^{de}	2.74 ± 0.06^{d}
$NaN_3 + NiL_2$	5 μM + 10 μg/mL	7.32 ± 0.04^{d}	2.62 ± 0.04^{d}
$NaN_3 + NiL_2$	5 μM + 20 μg/mL	7.21 ± 0.14^{cd}	2.36 ± 0.04^{c}
$NaN_3 + NiL_2$	5 μM + 40 μg/mL	7.87 ± 0.05^{de}	2.56 ± 0.81^{cd}
$NaN_3 + NiL_2$	5 μM + 80 μg/mL	8.04 ± 0.12^{e}	2.72 ± 0.68^{d}
NiL_3	80 μg/mL	6.02 ± 0.10^{a}	1.74 ± 0.04^{a}
$NaN_3 + NiL_3$	5 μM + 5 μg/mL	7.56 ± 0.06^{d}	2.55 ± 0.07^{cd}
$NaN_3 + NiL_3$	5 μM + 10 μg/mL	7.04 ± 0.31^{d}	2.41 ± 0.02^{c}
$NaN_3 + NiL_3$	5 μM + 20 μg/mL	6.64 ± 0.51^{b}	2.24 ± 0.10^{bc}
$NaN_3 + NiL_3$	5 μM + 40 μg/mL	7.12 ± 0.07^{c}	2.68 ± 0.02^{d}
$NaN_3 + NiL_3$	5 μM + 80 μg/mL	6.68 ± 0.08^{b}	2.86 ± 0.03^{de}
NiL_4	80 μg/mL	5.96 ± 0.03^{a}	1.69 ± 0.05^{a}
$NaN_3 + NiL_4$	5 μM + 5 μg/mL	7.82 ± 0.06^{de}	2.72 ± 0.07^{d}
$NaN_3 + NiL_4$	5 μM + 10 μg/mL	7.19 ± 0.89^{c}	2.40 ± 0.13^{c}
$NaN_3 + NiL_1$	5 μM + 40 μg/mL	6.35 ± 0.14^{ab}	1.78 ± 0.01^{a}

Table 1. *(continuation) The effects of Ni(II) complexes with unsymmetric Schiff bases and sodium azide*

NaN_3 + NiL_1	5 μM + 80 μg/mL	7.10 ± 0.04^{c}	2.03 ± 0.09^{b}
NiL_2	80 μg/mL	6.08 ± 0.06^{a}	1.85 ± 0.04^{a}
NaN_3 + NiL_2	5 μM + 5 μg/mL	7.75 ± 0.11^{de}	2.74 ± 0.06^{d}
NaN_3 + NiL_2	5 μM + 10 μg/mL	7.32 ± 0.04^{d}	2.62 ± 0.04^{d}
NaN_3 + NiL_2	5 μM + 20 μg/mL	7.21 ± 0.14^{cd}	2.36 ± 0.04^{c}
NaN_3 + NiL_2	5 μM + 40 μg/mL	7.87 ± 0.05^{de}	2.56 ± 0.81^{cd}
NaN_3 + NiL_2	5 μM + 80 μg/mL	8.04 ± 0.12^{e}	2.72 ± 0.68^{d}
NiL_3	80 μg/mL	6.02 ± 0.10^{a}	1.74 ± 0.04^{a}
NaN_3 + NiL_3	5 μM + 5 μg/mL	7.56 ± 0.06^{d}	2.55 ± 0.07^{cd}
NaN_3 + NiL_3	5 μM + 10 μg/mL	7.04 ± 0.31^{d}	2.41 ± 0.02^{c}
NaN_3 + NiL_3	5 μM + 20 μg/mL	6.64 ± 0.51^{b}	2.24 ± 0.10^{bc}
NaN_3 + NiL_3	5 μM + 40 μg/mL	7.12 ± 0.07^{c}	2.68 ± 0.02^{d}
NaN_3 + NiL_3	5 μM + 80 μg/mL	6.68 ± 0.08^{b}	2.86 ± 0.03^{de}
NiL_4	80 μg/mL	5.96 ± 0.03^{a}	1.69 ± 0.05^{a}
NaN_3 + NiL_4	5 μM + 5 μg/mL	7.82 ± 0.06^{de}	2.72 ± 0.07^{d}
NaN_3 + NiL_4	5 μM + 10 μg/mL	7.19 ± 0.89^{c}	2.40 ± 0.13^{c}
NaN_3 + NiL_4	5 μM + 20 μg/mL	7.03 ± 0.74^{c}	2.28 ± 0.09^{c}
NaN_3 + NiL_4	5 μM + 40 μg/mL	6.85 ± 0.09^{bc}	1.96 ± 0.03^{b}
NaN_3 + NiL_4	5 μM + 80 μg/mL	7.26 ± 0.10^{cd}	2.14 ± 0.08^{b}

Positive controls: sodium azide.

[a, b, c, d, e] Statistically significant differences in the same column ($\alpha = 0.05$).

When the findings obtained from SCE and MN test systems are evaluated, it has been determined that **NiL_1**, **NiL_2**, **NiL_3** and **NiL_4** exhibit antimutagenic properties and reduce the mutagenicity caused by NaN_3 as a positive control. They are the unsymmetric Schiff bases Ni(II) complexes including aromatic or heterocyclic fragments. The inhibitory activities of the compounds including phenolic fragments more effective [34]. In addition, **NiL_1**, **NiL_2**, **NiL_3** and **NiL_4** are also found to be to eliminate the mutagenic effect caused by NaN_3 at 5, 10, 20, 40 and 80 μg / mL concentrations. The protective role of these complexes is related to their concentration. Among the Ni(II) complexes with unsymmetric Schiff bases, especially the most effective results are obtained at a concentration of 80 μg/mL applications. It has been obtained that **NiL_1** containing chlorine has more strongly protective against the toxic effect of NaN_3. The antigenotoxic activities of the unsymmetric diimines complexes can be said to be related to their antioxidant effect or cofactor on the enzymatic system [35].

IV. CONCLUSIONS

Herein, the Ni(II) complexes with unsymmetric Schiff bases were synthesized by using a two-stage method. The inhibitory activities of these unsymmetric Schiff base complexes were examined against the mutagenic effects of NaN_3. Consistent with these findings, it can be concluided that the unsymmetric Schiff bases Ni(II) complexes including aromatic or heterocyclic fragments have significant antimutagenic property. It can be said that the Ni(II) complexes with unsymmetric Schiff bases are antigenotoxic compounds and their concentration have an effect on their protective actions. These complexes may be used in various application in biomedical fields.

V. REFERENCES

[1] C. M. da Silva, D. L. da Silva, L.V. Modolo, R. B. Alves, M. A. de Resende, C. V. B. Martins and A. de Fatima, ‘‘Schiff bases: A short review of their antimicrobial activities,’’ *Journal of Advanced Research*, vol. 2, pp. 1-8, 2011.

[2] B. Katarzyna and E. L. Chruscınska, ‘‘Schiff bases-interesting range of applications in various fields of science,’’ *Chemik International,* vol. 68, no. 2, pp. 129-138, 2014.

[3] A. K. Gupta and R. Pal, ‘‘Dehydroacetic acid based Schiff’s bases and their metal complexes: A review,’’ *World Journal of Pharmaceutical Sciences,* vol. 4, no. 1, pp. 386-425, 2015.

[4] S. Kumar, D. N. Dhar and P. N. Saxena, ‘‘Applications of metal complexes of Schiff bases-a review,’’ *Journal of Scientific and Industrial Research,* vol. 68, no. 3, pp. 181-187, 2009.

[5] P. Anand, V. M. Patil, V. K. Sharma, R. L. Khosa and N. Masand, ‘‘Schiff bases: A review on biological insights,’’ *International Journal of Drug Discovery and Development,* vol. 3, no. 3, pp. 851-868, 2012.

[6] O. A. M. Ali, S. M. El-Medani, M. R. A. Serea and A. S. Sayed, ‘‘Unsymmetrical Schiff base (ON) ligand on complexation with some transition metal ions: Synthesis, spectral characterization, antibacterial, fluorescence and thermal studies,’’ *Spectrochimica Acta, Part A: Molecular and Biomolecular Spectroscopy,* vol. 136, pp. 651-660, 2015.

[7] K. Turhan, S. A. Ozturkcan, Z. Turgut, M. Karadayi and M. Gulluce, ‘‘Protective properties of five newly synthesized cyclic compounds against sodium azide and N-methyl-N0-nitroN-nitrosoguanidine genotoxicity,’’ *Toxicology and Industrial Health,* vol. 28, no. 7, pp. 605-613, 2012.

[8] N. Sarı, N. Pişkin, H. Öğütcü and N. K. Yetim, ‘‘Spectroscopic characterization of novel d-aminoacid-Schiff bases and their Cr(III) and Ni(II) complexes as antimicrobial agents,’’ *Medicinal Chemistry Research,* vol. 22, no. 2, pp. 580-587, 2012.

[9] S. Meghdadi, M. Amirnasr, M. Majedi, M. Bagheri, A. Amiri, S. Abbasi and K. Mereiter, ‘‘Template synthesis, and X-ray crystal structures of copper(II) and nickel(II) complexes of new unsymmetrical tetradentate Schiff base ligands. Electrochemistry, antibacterial properties, and metal ion effect on hydrolysis-recondensation of the ligand,’’ *Inorganica Chimica Acta,* vol. 437, pp. 64-69, 2015.

[10] M. Kalita, K. J. Tamuli, P. Barman, B. Sarma, R. Baruah, H. P. D. Boruah, ‘‘Synthesis, crystal structure, bioactivities of Ni(II), Cu(II), Co(II) and Pd(II) complexes with unsymmetrical thioether donor Schiff base: Phosphine free Pd(II) complex catalyzed Suzuki reaction,’’ *Polyhedron,* vol. 97, pp. 140-147, 2015.

[11] D. Nartop, P. Gürkan, N. Sarı and S. Çete, ‘‘Tetradentate asymmetric Schiff bases and their Ni(II) and Fe(III) complexes,’’ *Journal of Coordination Chemistry,* vol. 61, no. 21, pp. 3516-3524, 2008.

[12] D. Nartop and P. Gürkan, ‘‘Synthesis, characterization and antibacterial activities of unsymmetric diimine Schiff bases and their Fe(III) and Ni(II) complexes,’’ *Chinese Journal of Inorganic Chemistry,* vol. 29, no. 6, pp. 1227-1234, 2013.

[13] Ö. Güngör and P. Gürkan, ‘‘Synthesis and spectroscopic properties of novel asymmetric Schiff bases,’’ *Spectrochimica Acta, Part A: Molecular and Biomolecular Spectroscopy,* vol. 77, no. 1, pp. 304-311, 2010.

[14] Ö. Özdemir, ‘‘Novel symmetric diimine-Schiff bases and asymmetric triimine-Schiff bases as chemosensors for the detection of various metal ions,’’ *Journal of Molecular Structure,* vol. 1125, pp. 260-271, 2016.

[15] D. Nartop, Ö. Özdemir and P. Gürkan, ‘‘Synthesis, characterization and investigation of tautomeric, potentiometric and antimicrobial properties of a novel unsymmetric Schiff base and its Fe(III) and Ni(II) complexes,’’ *Moroccan Journal of Chemistry,* vol. 5, pp. 560-572, 2017.

[16] Ö. Güngör, ‘‘Intramolecular proton transfer equilibrium in salicylidene- and naphthalene-based tetraimine Schiff bases,’’ *Gazi University Journal of Science,* vol. 30, no. 1, pp. 191-214, 2017.

[17] D. Nartop, W. Clegg, R. W. Harrington, R. A. Henderson, C. Y. Will, ‘‘Binding multidentate ligands to Ni^{2+}: Kinetic identification of preferential binding sites,’’ *Dalton Transactions,* pp. 3372-3382, 2014.

[18] W. M. El-Sayed and W. A Hussin, ‘‘Antimutagenic and antioxidant activity of novel 4-substituted phenyl-2,2′-bichalcophenes and aza-analogs,’’ *Drug Design Development and Therapy,* vol. 7, pp. 73-81, 2013.

[19] L. E. S. Fedel-Miyasato, A. S. N. Formagio, S. A. Auharek, C. A. L. Kassuya, S. D. Navarro, A. L. Cunha-Laura, A. C. D. Monreal, M. C. Vieira and R. J. Oliveira, ‘‘Antigenotoxic and antimutagenic effects of Schinus terebinthifolius Raddi in Allium cepa and Swiss mice: a comparative study,’’ *Genetics and Molecular Research,* vol. 13, no. 2, pp. 3411-3425, 2014.

[20] S. A. Öztürkcan, ‘‘Farklı ortamlarda çok bileşenli tek-kap yöntemi ile Mannıch reaksiyonu,’’ Doktora tezi, Kimya Bölümü, Yıldız Teknik Üniversitesi, İstanbul, Türkiye, 2012.

[21] D. E. Levin, L. J. Marnett and B. N. Ames, ‘‘Spontaneous and mutagen-induced deletions: Mechanistic studies in Salmonella tester strain TA102,’’ *Proceedings of the National Academy of Sciences,* vol. 81, no. 14, pp. 4457-4461, 1984.

[22] T. J. Makhafola, E. E. Elgorashi, L. J. McGaw, L. Verschaeve and J. N. Eloff, ‘‘The correlation between antimutagenic activity and total phenolic content of extracts of 31 plant species with high antioxidant activity,’’ *BMC Complementary and Alternative Medicine,* vol. 16, no. 490, pp. 1-13, 2016.

[23] S. Khan, F. Al-Qurainy and F. Anwar, ‘‘Sodium azide: a chemical mutagen for enhancement of agronomic traits of crop plants,’’ *Environment & We an International Journal of Science & Technology,* vol. 4, pp. 1-21, 2009.

[24] H. Shan, Y. Chu, P. Chang, L. Yang, Y. Wang, S. Zhu, M. Zhang and L. Tao, ‘‘Neuroprotective effects of hydrogen sulfide on sodium azide-induced autophagic cell death in PC12 cells,’’ *Molecular Medicine Reports,* vol. 16, no. 5, pp. 5938-5946, 2017.

[25] T. Ishikawa, B. L. Zhu and H. Maeda, ‘‘Effect of sodium azide on the metabolic activity of cultured fetal cells,’’ *Toxicology & Industrial Health,* vol. 22, no. 8, pp. 337-341, 2006.

[26] Z. Ciesla, T. Filutowicz and T. Klopotowski, ‘‘Involvement of the L-cysteine biosynthetic pathway in azide-induced mutagenesis in Salmonella typhimurium,’’ *Mutation Reseach,* vol. 70, no. 3, pp. 261-268, 1980.

[27] W. M. Owais and A. Kleinhofs, ‘‘Metabolic activation of the mutagen azide in biological systems,’’ *Mutation Research,* vol. 197, no. 2, pp. 313-323, 1988.

[28] İ. Şakıyan, M. Anar, H. Öğütcü, G. Agar and N. Sarı, ‘‘Schiff bases attached L-Glutamine and L-Asparagine: First investigation on antimutagenic and antimicrobial analysis,’’ *Artificial Cells, Blood Substitutes, and Biotechnology,* vol. 42, no. 3, pp. 199-204, 2014.

[29] M. Güllüce, G. Agar, O. Barış M. Karadayı, F. Orhan, F. Şahin, ‘‘Mutagenic and antimutagenic eddects of hexane extract of some Astragalus species grown in the Eastern Anatolia region of Turkey,’’ *Phytotherapy Research,* vol. 24, no. 7, pp. 1014-1018, 2010.

[30] Z. Güvenalp, M. Güllüce, M. Karadayı, M, H. Özbek, T. A. Özbek, L. Demirezer, ‘‘Determination of mutagenic and antimutagenic properties of flavonoid compounds isolated from Mentha longifolia ssp. longifolia and Origanum vulgare ssp. vulgare by using AMES test system,’’ *Planta Medica,* vol. 76, no. 12, pp. 1286-1287, 2010.

[31] P. Perry and H. Evans, ‘‘Cytological detection of mutagen–carcinogen exposure by sister chromatid exchange,’’ *Nature,* vol. 258, no. 5531, pp. 121-125, 1975.

[32] S. Çeker, G. Agar, L. Alpsoy, G. Nardemir and H. E. Kızıl, ‘‘Protective role of essential oils of Calamintha nepeta L. on oxidative and genotoxic damage caused by aflatoxin B1 in vitro,’’ *Fresenius Environmental Bulletin,* vol. 22, no. 11, pp. 3258-3263, 2013.

[33] F. Orhan, S. Çeker, M. Anar, G. Agar, T. Arasoglu, M. Gulluce, ‘‘Protective effects of three luteolin derivatives on aflatoxin B-1-induced genotoxicity on human blood cells,’’ *Medicinal Chemistry Research,* vol. 25, pp. 2567-2577, 2016.

[34] V. A. Alexandrova, G. V. Obukhova and D. A. Topchıev, ‘‘Synthesis and antimutagenic properties of novel systems based on poly(quaternized ammonium) salts,’’ *Journal of Bioactive and Compatible Polymers,* vol. 17, no. 5, pp. 321-341, 2002.

[35] S. Koçoğlu, H. Öğütcü, Z. Hayvalı, ‘‘Photophysical and antimicrobial properties of new double-armed benzo-15-crown-5 ligands and complexes,’’ *Research on Chemical Intermediates,*vol. 45, pp.2403-2427,2019.