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 (NiL1, NiL2, NiL3, NiL4) 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 (NaN₃) 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 (NiL1, NiL2, NiL3, NiL4) potansiyel antimutajen özellikleri incelenmek için son zamanlarda grubaaktın rapor edilen yeni bir yöntem ile hazırlanlandı. Lefositlerdeki sodيوم azid kaynaklı antimutagenik etki, kardeş kromatid değişimi (SCE) ve mikronükleus (MN) yöntemleriyle belirlendi. Sentezlenen bileşiklerin antimutagenik özelliklerine sahip olduğu ve pozitif kontrol olarak kullanılan sodيوم azid (NaN₃)'ın neden olduğu mutajeniteyi azalttıkları belirlenmiştir.

Anahtar Kelimeler: Asimetrik diimin, Ni(II) kompleksi, Sodyum azit, Antimutajen
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 N₂O₂ 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; and -CHO groups in the same aromatic ring. First time, a new two step method for the synthesis of these type dimines 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 Fe³⁺ in hemoglobin and inhibit the respiratory chain [24, 25]. Mutagenicity of NaN₃ 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 prevent 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 unsymmeric 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 (NiL₁, NiL₂)

Recently reported the synthesis of the Ni(II) complexes with unsymmetrics diimines (NiL₁, NiL₂) 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 (Na₂S₂O₄). In this phase, the Na₂S₂O₄ 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 (NiL₁, NiL₂) were obtained by reaction of equimolar amounts of the obtained unsymmetric dimines 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 CaCl₂.

B. GENERAL PROCEDURE FOR SYNTHESIS OF Ni(II) COMPLEXES WITH UNSYMERIC SCHIFF BASES (NiL₃, NiL₄)

Recently, reported the synthesis of the Ni(II) complexes with unsymmetric Schiff bases (NiL₃, NiL₄) 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 amino using reducing agent (Na₂S₂O₄). In this phase, the Na₂S₂O₄ 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 (NiL₃, NiL₄) were obtained by reaction of equimolar amounts of the obtained unsymmetric dimines 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 CaCl₂.

C. DETERMINATION OF ANTIGENOTOXIC PROPERTIES

The antimutagenic activities of the unsymmetric Schiff bases Ni(II) complexes (NiL₄, NiL₂, NiL₃, NiL₄) 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. NaN₃ (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 NaN₃ + unsymmetric diimine complexes (5 μg/mL);
Culture 5: 5 μM NaN₃ + unsymmetric diimine complexes (10 μg/mL);
Culture 6: 5 μM NaN₃ + unsymmetric diimine complexes (20 μg/mL);
Culture 7: 5 μM NaN₃ + unsymmetric diimine complexes (40 μg/mL);
Culture 8: 5 μM NaN₃ + 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 binucleated cells were scored [33].

III. RESULTS AND DISCUSSION

A. CHEMISTRY

The Ni(II) complexes with unsymmetric Schiff bases (NiL1, NiL2, NiL3, NiL4) have previously been characterized by spectroscopic techniques [11, 12].

B. ANTIMUTAGENIC ACTIVITY

The antimutagenic activities for the Ni(II) complexes with unsymmetric Schiff bases (NiL1, NiL2, NiL3, NiL4) are given in Table 1. The antimutagenic activities of different concentrations (5, 10, 20 and 40 µg/mL) of the complexes (NiL1, NiL2, NiL3, NiL4) were investigated against NaN3 in human lymphocyte cells by MN and SCE tests. NaN3 is a strong mutagen affecting many organisms. It was determined that NaN3 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 NaN3.

### 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 ± 0.04a   | 1.60 ± 0.09a    |                   |
| NaN3       | 5 µM           | 8.22 ± 0.06c    | 3.18 ± 0.12c      |
| NiL1       | 80 µg/mL       | 6.00 ± 0.04a    | 1.68 ± 0.02a      |
| NaN3 + NiL1| 5 µM + 5 µg/mL | 7.54 ± 0.09d    | 2.66 ± 0.14d      |
| NaN3 + NiL1| 5 µM + 10 µg/mL| 6.96 ± 0.03c    | 2.30 ± 0.04c      |
| NaN3 + NiL1| 5 µM + 20 µg/mL| 6.76 ± 0.07b    | 2.22 ± 0.06b      |
| NaN3 + NiL1| 5 µM + 40 µg/mL| 6.35 ± 0.14ab   | 1.78 ± 0.01a      |
| NaN3 + NiL1| 5 µM + 80 µg/mL| 7.10 ± 0.04c    | 2.03 ± 0.09a      |
| NiL2       | 80 µg/mL       | 6.08 ± 0.06a    | 1.85 ± 0.04a      |
| NaN3 + NiL2| 5 µM + 5 µg/mL | 7.75 ± 0.11de   | 2.74 ± 0.06d      |
| NaN3 + NiL2| 5 µM + 10 µg/mL| 7.32 ± 0.04d    | 2.62 ± 0.04d      |
| NaN3 + NiL2| 5 µM + 20 µg/mL| 7.21 ± 0.14cd   | 2.36 ± 0.04d      |
| NaN3 + NiL2| 5 µM + 40 µg/mL| 7.87 ± 0.05de   | 2.56 ± 0.01cd     |
| NaN3 + NiL2| 5 µM + 80 µg/mL| 8.04 ± 0.12e    | 2.72 ± 0.06d      |
| NiL3       | 80 µg/mL       | 6.02 ± 0.10a    | 1.74 ± 0.04a      |
| NaN3 + NiL3| 5 µM + 5 µg/mL | 7.56 ± 0.06d    | 2.55 ± 0.07cd     |
| NaN3 + NiL3| 5 µM + 10 µg/mL| 7.04 ± 0.31d    | 2.41 ± 0.02c      |
| NaN3 + NiL3| 5 µM + 20 µg/mL| 6.64 ± 0.51b    | 2.24 ± 0.10bc     |
| NaN3 + NiL3| 5 µM + 40 µg/mL| 7.12 ± 0.07c    | 2.68 ± 0.02a      |
| NaN3 + NiL3| 5 µM + 80 µg/mL| 6.68 ± 0.08b    | 2.86 ± 0.03de     |
| NiL4       | 80 µg/mL       | 5.96 ± 0.03a    | 1.69 ± 0.05s      |
| NaN3 + NiL4| 5 µM + 5 µg/mL | 7.82 ± 0.06de   | 2.72 ± 0.07f      |
| NaN3 + NiL4| 5 µM + 10 µg/mL| 7.19 ± 0.89c    | 2.40 ± 0.13c      |
| NaN3 + NiL4| 5 µM + 20 µg/mL| 7.03 ± 0.74c    | 2.28 ± 0.09d      |
| NaN3 + NiL4| 5 µM + 40 µg/mL| 6.85 ± 0.09bc   | 1.96 ± 0.03s      |
| NaN3 + NiL4| 5 µM + 80 µg/mL| 7.26 ± 0.10cd   | 2.14 ± 0.08d      |
| NaN3 + NiL4| 5 µM + 40 µg/mL| 6.35 ± 0.14ab   | 1.78 ± 0.01d      |
| NaN3 + NiL4| 5 µM + 80 µg/mL| 7.10 ± 0.04c    | 2.03 ± 0.09b      |
| NiL2       | 80 µg/mL       | 6.08 ± 0.06a    | 1.85 ± 0.04a      |
| NaN3 + NiL2| 5 µM + 5 µg/mL | 7.75 ± 0.11de   | 2.74 ± 0.06d      |
| NaN3 + NiL2| 5 µM + 10 µg/mL| 7.32 ± 0.04d    | 2.62 ± 0.04d      |
| NaN3 + NiL2| 5 µM + 20 µg/mL| 7.21 ± 0.14cd   | 2.36 ± 0.04d      |
| NaN3 + NiL2| 5 µM + 40 µg/mL| 7.87 ± 0.05de   | 2.56 ± 0.01cd     |
| NaN3 + NiL2| 5 µM + 80 µg/mL| 8.04 ± 0.12e    | 2.72 ± 0.06d      |
| NiL3       | 80 µg/mL       | 6.02 ± 0.10a    | 1.74 ± 0.04a      |
| NaN3 + NiL3| 5 µM + 5 µg/mL | 7.56 ± 0.06d    | 2.55 ± 0.07cd     |
| NaN3 + NiL3| 5 µM + 10 µg/mL| 7.04 ± 0.31d    | 2.41 ± 0.02c      |
| NaN3 + NiL3| 5 µM + 20 µg/mL| 6.64 ± 0.51b    | 2.24 ± 0.10bc     |
| NaN3 + NiL3| 5 µM + 40 µg/mL| 7.12 ± 0.07c    | 2.68 ± 0.02a      |
| NaN3 + NiL3| 5 µM + 80 µg/mL| 6.68 ± 0.08b    | 2.86 ± 0.03de     |
| NiL4       | 80 µg/mL       | 5.96 ± 0.03a    | 1.69 ± 0.05s      |
| NaN3 + NiL4| 5 µM + 5 µg/mL | 7.82 ± 0.06de   | 2.72 ± 0.07f      |
| NaN3 + NiL4| 5 µM + 10 µg/mL| 7.19 ± 0.89c    | 2.40 ± 0.13c      |
| NaN3 + NiL4| 5 µM + 40 µg/mL| 6.35 ± 0.14ab   | 1.78 ± 0.01d      |
Table 1. (continuation) The effects of Ni(II) complexes with unsymmetric Schiff bases and sodium azide

| NaN3 + NiL1 | 5 µM + 80 µg/mL | 7.10 ± 0.04c | 2.03 ± 0.09b |
| NiL2 | 80 µg/mL | 6.08 ± 0.06a | 1.85 ± 0.04a |
| NaN3 + NiL2 | 5 µM + 5 µg/mL | 7.75 ± 0.11de | 2.74 ± 0.06d |
| NaN3 + NiL2 | 5 µM + 10 µg/mL | 7.32 ± 0.04d | 2.62 ± 0.04d |
| NaN3 + NiL2 | 5 µM + 20 µg/mL | 7.21 ± 0.14ed | 2.36 ± 0.04c |
| NaN3 + NiL2 | 5 µM + 40 µg/mL | 7.87 ± 0.05de | 2.56 ± 0.01cd |
| NaN3 + NiL2 | 5 µM + 80 µg/mL | 8.04 ± 0.12e | 2.72 ± 0.68d |
| NiL3 | 80 µg/mL | 6.02 ± 0.10a | 1.74 ± 0.04a |
| NaN3 + NiL3 | 5 µM + 5 µg/mL | 7.56 ± 0.06d | 2.55 ± 0.07cd |
| NaN3 + NiL3 | 5 µM + 10 µg/mL | 7.04 ± 0.31d | 2.41 ± 0.02c |
| NaN3 + NiL3 | 5 µM + 20 µg/mL | 6.64 ± 0.51b | 2.24 ± 0.10bc |
| NaN3 + NiL3 | 5 µM + 40 µg/mL | 7.12 ± 0.07c | 2.68 ± 0.02d |
| NaN3 + NiL3 | 5 µM + 80 µg/mL | 6.68 ± 0.08b | 2.86 ± 0.03de |
| NiL4 | 80 µg/mL | 5.96 ± 0.03a | 1.69 ± 0.05a |
| NaN3 + NiL4 | 5 µM + 5 µg/mL | 7.82 ± 0.06de | 2.72 ± 0.07d |
| NaN3 + NiL4 | 5 µM + 10 µg/mL | 7.19 ± 0.89c | 2.40 ± 0.13c |
| NaN3 + NiL4 | 5 µM + 20 µg/mL | 7.03 ± 0.74c | 2.28 ± 0.09b |
| NaN3 + NiL4 | 5 µM + 40 µg/mL | 6.85 ± 0.09bc | 1.96 ± 0.03b |
| NaN3 + NiL4 | 5 µM + 80 µg/mL | 7.26 ± 0.10ed | 2.14 ± 0.08b |

Positive controls: sodium azide.

a, b, c, d, e Statistically significant differences in the same column (α = 0.05).

When the findings obtained from SCE and MN test systems are evaluated, it has been determined that NiL1, NiL2, NiL3 and NiL4 exhibit antimutagenic properties and reduce the mutagenicity caused by NaN3 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, NiL1, NiL2, NiL3 and NiL4 are also found to be to eliminate the mutagenic effect caused by NaN3 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 NiL1 containing chlorine has more strongly protective against the toxic effect of NaN3. 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 NaN3. Consistent with these findings, it can be concluded 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

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