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Anti-genotoxic Effects of Schiff bases and their Mn(III) Complexes Containing L-Aspartic acid and L-Phenylalanine

Elvan HASANOĞLU ÖZKAN[1,*], Hamit Emre KIZIL[2], İffet ŞAKIYAN[3], Dilek NARTOP[4], Nurşen SARI[1], Güleray AĞAR[5]

[1]*Department of Chemistry, Faculty of Science, Gazi University, 06500 Teknikokullar, Ankara, Turkey*

[2]*Bayburt University, Vocational School of Health Services, Bayburt, Turkey*

[3]*Department of Chemistry, Faculty of Science, Ankara University, 06100 Ankara, Turkey*

[4]*Department Polymer Engineering, Faculty of Technology, Düzce University, Turkey*

[5]*Department of Biology, Faculty of Science, Atatürk University,25240 Erzurum, Turkey*

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Abstract

The purpose of the research was to evaluate the genotoxic and anti-genotoxic properties of Schiff bases and their Mn (III) complexes containing L-aspartic acid, and L-phenylalanine. The anti-genotoxic properties of four compounds in human lymphocytes cells were investigated by sister chromatid exchanges (SCEs) test system against aflatoxin Bı (AFBı). The results showed that compounds have strong anti-genotoxic properties.

1. INTRODUCTION

Aflatoxin Bı (AFBı) is a highly toxic substance produced by *Aspergillus* fungi. According to the research, AFBı in foodstuff occurs with temperatures more than 25 ˚C and relative humidity above 80% [1]. Even, inadequate long-term storage are leading to the presence of *Aspergillus* in foodstuff [2]. AFBı is causing damage mainly in the liver and has been classified as a Group I carcinogen [3]. Due to the increase seen in mutation-related diseases in recent years, there is a need for new anti-mutagenic agent. Antimutagenic is eliminate mutagenic effects of potentially harmful chemicals. Mutagenic agents act through the production of free radicals and free radicals are damage to DNA. Antimutagens agents are capable to deactivate of radicals. So, new compounds demonstrating antimutagenic activities are of great practical importance, especially for cancer therapy. Numerous compounds are synthesized and investigated for their antimutagenic activities each year.

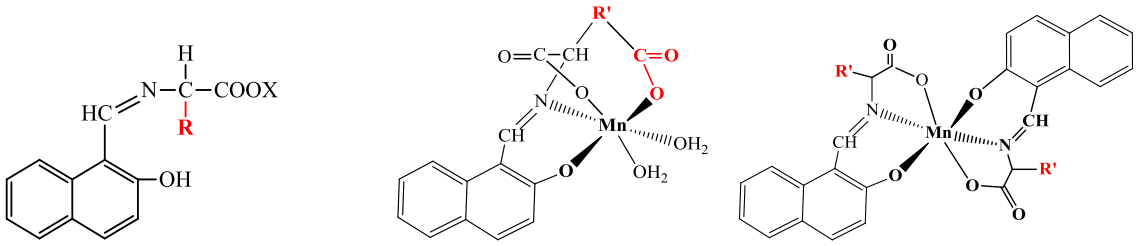
Manganese is an essential metal which can be neurotoxic in some instances. Furthermore, Mn derivatives are used as contrast agents in magnetic resonance images (MRI) [4]. Mn-based metallodrugs are prevalent in clinical practice [5]. So, complexes Manganese-based have been studied as metallodrugs. Mn(III)-bis(salicylaldehyde) ethylenediamine derivatives have been suggested as possible mimetics of enzymes in antioxidant therapy [6].

Schiff base compounds from multidentate ligand exhibit broad biological activity due to sensitivity toward the central metal atom. Also, Schiff bases containing nitrogen and oxygen as donor atoms have an

*Corresponding author, e-mail: elvan.hasanoglu@gazi.edu.tr

important role to be use as anti-cancer and anti-viral activities [7,8]. Investigation on antimutagenic activities of Aflatoxin B1 for Schiff bases-metal complexes started with our group. Our previous research has demonstrated anti-genotoxic activity of derivatives aminoacid-Schiff bases and their Mn(III) complexes [4,9]. Among these compounds the most promising were Mn(III) complexes of aminoacid-Schiff bases. In the present study is the continuation of our previous studies.

We report the preparation of Mn(III) complexes using the [N-(2-hydroxy-1-naphthylidene)aspartic acid] and [N-(2-hydroxy-1-naphthylidene)phenylalanine] [10]. The structures of Mn(III) complexes (Figure 1b) were determined using elemental analyses, electronic spectra, i.r, and magnetic moment measurements.



(a) **(b)**

R	X	Schiff bases	R′	Mn(III) Complexes
$-CH_2-COOH$	-H	(Asp- SchH)	$-CH_2$	[Mn(Asp-Sch)]
$-CH_2-(C_6H_6)$	-Na	(Phe-SchNa)	$-CH_2-(C_6H_6)$	$Na[Mn(Phe\text{-}Sch)_2]$

Figure 1. *Structures and abbreviations of synthesized structures; a) Aminoacid-Schiff bases b) Their Mn(III) complexes*

And then, these compounds were investigated for anti-genotoxic properties against Aflatoxin B1 (AFB1) by the SCE assay in human lymphocyte cell culture in vitro. Results may be compared to previously reported methods [4,9]. The effect size of the complexes (ML and ML_2) on anti-genotoxic properties have been evaluated.

2. EXPERIMENTAL

2.1. Chemicals and Physical Measurements

The amino acids (L-Aspartic acid (Asp) and L-Phenylalanine (Phe)) with 2-hydroxy-1-naphthaldehyde, methanol, and n-heptane were purchased from Sigma-Aldrich. Manganese (III) acetate was prepared according to *Sakiyan et al.*2001 [11]. All melting points were determined in sealed capillaries and are reported without correction. For Mn(III) complexes, elemental analysis were performed with a LECO-CHNS-9320 analyzer while the metal contents were determined by using an Atomic Absorption Spectrophotometer Perkin-Elmer 400. The electronic spectra were recorded on a UV-1800 ENG240V spectrophotometer in ethanol. The IR spectra were recorded on Bruker Vertex-80 FT-IR spectrometer between 4000 and 400 cm^{-1}. The melting points were determined with a Barnstead-Electrothermal-9200 melting point apparatus. Magnetic measurements were performed with a Sherwood Scientific Magnetic Susceptibility Balance (Model No: MK 1) at 23 ± 0.1˚C with $Hg[Co(NCS)_4]$ as a calibration.

2.2. Synthesis of Aminoacid-Schiff Bases

A solution of 2-hydroxy-1-naphthaldehyde (5 mmol, 0.61 g) in methanol (50 mL) was added to an aminoacid (L-Aspartic acid and L-Phenylalanine, 5 mmol) MeOH solution (50 mL). The synthesis was

carried out according to the method we had detailed in an earlier articles [10,11] (Asp-SchH; m.p. 207 ˚C, Phe-SchNa; m.p. 232 ˚C).

2.3. Synthesis of Mn(III) Complexes

Firstly, $Mn(CH_3COO)_3{\cdot}2H_2O$ and aminoacid-Schiff Bases were synthesized according to the method explained in our earlier article [10]. We found that these synthesized compounds were consistent with the literature. Then, we synthesized their Mn(III) complexes in methanol (Figure 1b).

2.4. Cytogenetic Analysis

Peripheral blood lymphocytes were taken from four (two men and two women) non-smoking healthy individuals. Lymphocyte cultures were set up by adding 0.5 mL of heparinised whole blood to RPMI-1640 chromosome medium supplemented with 15% heat-inactivated fetal calf serum, 100 IU/Ml streptomycine, 100 IU/mL penicillin and 1% L-glutamine. Lymphocytes were stimulated to divide by 1% phytohaemaglutinin.

The experiments were designed as six groups for each compound:

Group 1:Control;
Group 2: 5 μM AFB_1;
Group 3: Compound 40 μg/mL;
Group 4: 5 μM AFB_1 + Compound (5 μg/mL);
Group 5: 5 μM AFB_1 + Compound (10 μg/mL);
Group 6: 5 μM AFB_1 + Compound (20 μg/mL);

For SCE (sister chromatid exchanges) demonstration, the cultures were incubated at 37˚C for 72 *h*, and 5-bromo 2-deoxyuridine at 8 mg/mL was added at the initiation of cultures. All cultures were kept in the darkness. Next, 0.1 mg/mL of colcemide was added 3 *h* before harvesting to arrest the cells at metaphase. The cultures were centrifuged at 800 g for 10 min. Cells were harvested and treated for 30 min with hypotonicsolution (0.075M KCl) and fixed in a 1:3 mixture of acetic acid / methanol (v/v). Bromodeoxyuridine-incorporated metaphase chromosomes were stained with fluorescence plus Giemsa technique as described by Perry and Evans 25 [12]. In SCE study, by selecting 60 satisfactory metaphases, the results of SCE were recorded on the evaluationtable. For each treatment condition, well-spread second division metaphases containing 42-46 chromosomes in each cell were scored, and the values obtained were calculated as SCE per cell.

Statistical analysis of SCE values Duncan's multiple range test was used. A value of Plessthan 0.05 was accepted as statistically significant. Results were expressed as mean ± SD. For these procedures, SPSS 15.0 version for Windows (SPSS Inc, Chicago, Illinois, USA) was used.

3. RESULTS AND DISCUSSION

Analytical datas and some of the physical properties of the Schiff bases and their Mn(III) complexes are given in Table 1. The complexes are easily soluble in DMF and DMSO.

3.1. Characterization of Aminoacid–Schiff Bases

(Asp-SchH) ($C_{15}H_{12}O_5N$); m.p. 207 ˚C; yield 50%; Yellow; IR: cm^{-1} = 3325 (-OH), 3240 (-NH), 1714 (-COOH), 1644 (C=O) and (C=N), 1548 (C=C). ^{1}H NMR (300 MHz, $CDCl_3$): δ = 10.9 (s, 1H), 8.85 (s, 1H), 8.1-6.2 (m, 6H), 4.3 (t, 1H), 2.90-2.58 (m, 2H) ppm.

(Phe-SchNa) ($C_{20}H_{17}O_3N$); m.p. 232 ˚C; yield 77 %; Yellow; IR: cm^{-1} = 3410 (-OH), 1625 (-CH=N), 1600 $(-COOH)_{asym}$, 1548 (C=C) and 1395 $(-COOH)_{sym}$. ^{1}H NMR (300 MHz, $CDCl_3$): δ = 10.8 (s, 1H), 8.1-6.7 (m, 10H), 7.82 (s, 1H), 4.4 (t, 1H), 2.90-2.60 (m, 2H) ppm.

3.2. Characterization of Mn(III) Complexes

All of the synthesized compounds were characterized by FT-IR, elemental analyses, magnetic moment. In the IR spectrum of (Asp-SchH), the characteristic ν(C=O) and ν(C=N) stretching absorption band of the keto and imine form was observed between 1633 and 1644 cm^{-1} as an overlap [13]. The elemental analyses can be considered compatible with the chemical formulas of the calculated molecule structure.

The azomethine and carboxylate bands in the IR spectra of the complexes appear in the range 1625/1616, 1600/1600 and 1395-1394 cm^{-1}, somewhat different than observed for the ligands. These indicate that the azomethine nitrogen and the oxygen of the carboxylate group are coordinated to metal ion [11]. Mn (III) complexes show medium broad bands at 575-586 cm^{-1} assigned to ν(M–N) stretching and bands at *ca* 510 assigned to ν(M–O) vibration [14]. In the IR spectra of the manganese complexes of (Asp-SchH), broad absorption bands were detected *ca* between 3400 and 3420 cm^{-1}, which proved the presence of coordinated water molecules in the complexes.

Data of magnetic susceptibility indicated that all the complexes were paramagnetic. For this reason the ^{1}H-NMR spectra of the Mn(III) complexes couldn't be obtained. The magnetic moment measurements show that the complexes have μ_{eff} values which range from *ca* 4.30-4.40 B.M., indicating the presence of four unpaired electrons per Mn(III) ion.

Table 1. *Analytical data and some of the physical properties of Schiff bases attached L- Aspartic acid, L-Phenylalanine and their Mn(III) complexes*

Compounds Formula (Weight)	Colour Mp (°C)	μ_{eff}	Elemental analysis; Found (calculated)%			
			C	H	N	Mn
(Asp-SchH) $C_{15}H_{12}O_5N$ (286)	Yellow 207	-	62.75 (62.94)	4.25 (4.19)	4.62 (4.89)	- -
[Mn(Asp-Sch)] $C_{15}H_{13}O_7NMn$ (374)	Reddish-Brown 251	4.32	49.45 (48.13)	3.11 (3.48)	4.13 (3.74)	14.90 (14.71)
(Phe-SchNa) $C_{20}H_{17}O_3NNa$ (341)	Yellow 232	-	70.42 (70.38)	4.80 (4.99)	4.12 (4.11)	- -
$Na[Mn(Phe\text{-}Sch)_2]$ $C_{40}H_{32}O_6N_2MnNa$ (714)	Orange-Brown 243	4.38	67.86 (67.23)	7.64 (4.48)	3.60 (3.92)	7.92 (7.70)

3.3. The Anti-genotoxic Activities of Aminoacid-Schiff Bases and Their Mn(III) Complexes

SCE frequency significantly decreased after treatment with the different concentrations of [Mn(Asp-Sch)] in DMSO solution and AFB_1. 5 μg/mL concentration of [Mn(Asp-Sch)] in DMSO solution was the most effective against AFB_1. It was also determined that all concentrations of (Asp-SchH) have anti-genotoxic properties (5, 10 and 20 μg/mL) and 10 μg/mL is the most effective concentration. Mutagenic results of SCE assay showed that any concentration of these compounds did not show mutagenic activity.

The results of the SCE analysis also showed that the (Phe-SchNa) and $Na[Mn(Phe\text{-}Sch)_2]$ compounds exhibited antigenotoxicity in a similar manner. All concentrations of both compounds were effective and the highest antigenotoxicity was observed at a concentration of 10 μg/mL ($p < 0.05$). Previous researches have reported antimutagenic activity of some free amino acids [15-17]. Consequently, in the present study, it has been revealed that these compounds are the active inhibitors of anti-genotoxic activity of AFB_1. Antimutagenic effects of these compounds are probably related to their action on the enzymatic activation system. This effect of studied compounds can be attributed primarily to their antioxidant action or cofactor for enzymes system, which is known to protect DNA and other cellular components from damage by oxygen radicals. According to Table 2, ML compound are the more active inhibitors of anti-genotoxic activity of AFB_1 than ML_2. When the *Katsuki* mechanism is considered [4], a coordinate bond may form between AFB_1-epoxide and Mn(III) in ML structure. We may said that it is difficult to coordinate with ML_2 structure.

Table 2. *Comparison of the anti-genotoxic effects of Schiff bases and their Mn (III) complexes containing L-aspartic acid, and L-phenylalanine against the mutagenic effect of AFB1 in human peripheral lymphocytes*

	Metaphase	Range of SCEs	SCE/Cell		Metaphase	Range of SCEs	SCE/Cell
Control	60	3-9	5.23±0.65$^{\mathbf{c}\dagger}$	Control	60	4-8	6.24±0.60^{c†}
AFB1 5 µM	60	5-12	9.42±0.42$^{\mathbf{a}}$	AFB1 5 µM	60	5-14	10.44±0.09^{a}
[Mn(Asp-Sch)] (40µg/mL)	60	4-10	7.25±0.35$^{\mathbf{b}}$	(Asp-SchH) (40µg/mL)	60	3-9	7.28±0.04^{b}
AFB1 (5µM)+ [Mn(Asp-Sch)] (5µg/mL)	60	4-11	6.36±0.51$^{\mathbf{b}}$	AFB1 (5µM)+ Asp-SchH (5µg/mL)	60	5-11	6.40±0.08c
AFB1 (5µM)+ [Mn(Asp-Sch)] (10µg/mL)	60	5-9	7.30±0.50$^{\mathbf{b}}$	AFB1 (5µM)+ Asp-SchH (10µg/mL)	60	4-10	6.34±0.64^{c}
AFB1 (5µM)+ [Mn(Asp-Sch)] (20µg/mL)	60	3-10	9.24±0.65$^{\mathbf{a}}$	AFB1 (5µM)+ Asp-SchH (20µg/mL)	60	4-11	7.30±0.99^{b}
Control	60	3-9	6.10±0.64^{dc†}	Control	60	4-9	6.15±0.9^{e†}
AFB1 5 µM	60	4-11	9.33±0.50^{a}	AFB1 5 µM	60	4-13	12.39±0.50^{a}
Na[Mn(Phe-Sch)$_2$](40µg/mL)	60	4-10	8.13±0.93^{ab}	(Phe-SchNa) (40µg/mL)	60	4-9	8.21±0.24^{c}
AFB1 (5µM)+Na[Mn(Phe-Sch)$_2$] (5µg/mL)	60	5-10	9.24±0.23^{a}	AFB1 (5µM)+ (Phe-SchNa) (5µg/mL)	60	5-11	10.28±0.24^{b}
AFB1 (5µM)+Na[Mn(Phe-Sch)$_2$] (10µg/mL)	60	4-10	6.18±0.99^{d}	AFB1 (5µM)+ (Phe-SchNa) (10µg/mL)	60	4-11	6.27±0.64d^{e}
AFB1 (5µM)+Na[Mn(Phe-Sch)$_2$] (20µg/mL)	60	3-11	7.11±0.78^{bc}	AFB1 (5µM)+ (Phe-SchNa) (20µg/mL)	60	3-10	7.23±0.46^{d}

†: Duncan's multiple range test ($p < 0.05$)

4. CONCLUSION

In summary, compounds attached to amino acid (L-aspartic acid, and L-phenylalanine) have been prepared for preliminary screening as a good anti-genotoxic effect against the mutagenic effects of AFB1. Howewer, The protective role of these compounds is related to their concentration. [Mn(Asp-Sch)] complex in the ML compound was the most effective against AFB1. When the Katsuki mechanism is considered, a coordinate bond may form between AFB_1-epoxide and Mn(III) in ML structure. Binding of the [Mn(Phe-Sch)$_2$] complex in the ML_2 compound to the epoxide is difficult due to the steric effect.

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CONFLICTS OF INTEREST

No conflict of interest was declared by the authors.

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