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ORIGINAL PAPER

The inhibitory effect of the amino acid complexes of Zn(II) on the growth of *Aspergillus flavus* and aflatoxin B_1 production

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Abstract
The zinc(II) complexes with amino acids as the ligands, $Zn(Gln)_2$ (**1**), $[Zn(Arg)_2(OAc)]OAc{\cdot}3H_2O$ (**2**), $Zn(His)_2$ (**3**), $Zn(Gly)_2$ (**4**), $Zn(Met)_2$ (**5**), and $Zn(Cys)_2$ (**6**) (Gln = glutamine, Arg = arginine, His = histidine, Gly = glycine, Met = methionine, and Cys = cysteine) have been synthesized in aqueous solutions and characterized by elemental analysis and spectroscopic methods. In addition, the solid-state structure of **1** and **2** has been determined by single-crystal X-ray crystallography. X-ray analysis revealed that the central Zn(II) atom is six- and five- coordinated in **1** and **2**, respectively. Furthermore, the antifungal activity of the synthesized complexes was investigated against the *Aspergillus flavus* fungus. The aqueous solutions of the zinc(II) amino acid complexes at various concentrations were added to the potato dextrose agar (PDA) medium containing spores of *Aspergillus flavus*, and the mixtures were then incubated at 25–30 °C for 6 days. The aflatoxin B_1 produced in vitro was measured using enzyme-linked immunosorbent assay (ELISA). The increasing concentration of these complexes decreased the growth of *Aspergillus flavus* and aflatoxin B_1 production, consequently. Furthermore, the results showed that the synthesized Zn(II) amino acid complexes are more antifungal active than the zinc(II) ion.

Keywords Zn(II) complexes · Amino acids · *Aspergillus flavus* · Aflatoxin · Potato dextrose agar (PDA) · Antifungal activity

Introduction

The transition metals complexes have been widely studied for their antimicrobial and antifungal properties [1, 2]. They have also attracted considerable attention in modern medicine [3–5]. A literature survey indicates that the zinc(II) complexes of aminobenzolamide derivatives have shown notable antifungal activities [6]. Other antifungal studies have been done on the amino acid-derived compounds [7], zinc(II) carboxylates [8], and zinc complexes of sulfadrugs such as sulfadiazines and sulfamerazines [9].

Mycotoxins are the toxic secondary chemical metabolites, which are produced by the filamentous fungi. Mycotoxins do not belong to a single class of the chemical compounds, and they have different toxicological effects [10, 11]. Among the various dietary approaches to reduce the effects of mycotoxins, the addition of mycotoxin binders to contaminated diets has drawn much attention from researchers [12]. Therefore, many reviews about the mycotoxin binders have been published [13–15]. The binder significantly prevents the animal toxicity, so that counteracts mycotoxins in the feedstuff by binding them strongly enough to avoid the toxic interactions with the consuming animal.

Aflatoxins are the highly toxic secondary metabolites and the main naturally occurring mycotoxins in agricultural products, which are produced by a complex biosynthetic pathway [16]. First time, they discovered and characterized in 1960s, when more than 100,000 turkey poults in England died of evident poisoning from contaminated peanut meal [17, 18]. Aflatoxins are produced by several species of *Aspergillus* genus, especially *Aspergillus flavus, Aspergillus parasiticus*, and *Aspergillus nomius* [19, 20]. They contaminate many commodities used for human food and

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animal feed, which leads to serious economic and health problems worldwide. There are four most common aflatoxins including B_1, B_2, G_1, and G_2, which have been named based on their fluorescence color under ultraviolet light (blue or green) and their relative mobility on a thin-layer chromatographic plate. In addition, there are less common aflatoxins including M_1, B_{2a}, G_{2a}, P_1, and Q_1, which have been defined as the mammalian biotransformation products of the main metabolites [21]. Aflatoxin B_1 (Fig. 1) is the most potent liver toxin [22], which is classified by the International Agency of Research on Cancer as the Group 1 carcinogen [23]. Aflatoxin B_1 exhibits an intense blue fluorescence visible at 450 nm, when excites with the long-wavelength ultraviolet light (365 nm) as the excitation wavelength [24]. *Aspergillus flavus* produces only B aflatoxins, while *Aspergillus parasiticus* and *Aspergillus nomius* produce both B and G aflatoxins [25]. The fungi are more difficult to quantify than bacteria, because they usually grow slowly and often in multicellular forms. Therefore, the complicated experiments are required to evaluate the in vitro or in vivo properties of a promising antifungal agent [26].

The different countries have developed the regulations for aflatoxins in food and feed materials because of the negative effect of aflatoxins on human health. It has been reported that the maximum admissible levels of aflatoxins in food are 10 and 5 μg kg^{-1} for total aflatoxins and aflatoxin B_1, respectively, in more than 75 countries [11]. Therefore, several qualitative and quantitative analytical methods for aflatoxin have been extensively developed, which include thin-layer chromatography (TLC) [27], high-performance liquid chromatography (HPLC) [28], enzyme-linked immunosorbent assay (ELISA) [29], immunoaffinity with fluorescence [30], and liquid chromatography (LC) coupled to tandem mass spectrometry (LC–MS/MS) [31]. As compared to the TLC and HPLC techniques, ELISA method can be used for screening procedures due to its advantages, including high speed, high sensitivity, ease of operation, low-sample-volume requirement, and high-sample throughput with minimum sample clean-up [32]. ELISA is a well-known immunoassay method, which appeared in 1960 [33]. Because of the commercial availability of ELISA test kits for most of the mycotoxins, ELISA is used for the routine analysis of mycotoxins in food and feed [34].

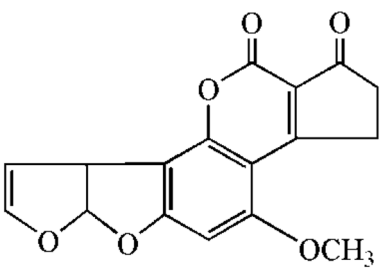


Fig. 1 Molecular structure of aflatoxin B_1

Herein, we report the synthesis and characterization of the Zn(II) amino acid complexes including $Zn(Gln)_2$ (**1**), $[Zn(Arg)_2(OAc)]OAc•3H_2O$ (**2**), $Zn(His)_2$ (**3**), $Zn(Gly)_2$ (**4**), $Zn(Met)_2$ (**5**), and $Zn(Cys)_2$ (**6**) (Gln = glutamine, Arg = arginine, His = histidine, Gly = glycine, Met = methionine, and Cys = cysteine). Furthermore, the antifungal activity of these complexes has been studied on growth of the *Aspergillus flavus* in PDA culture medium along with determination of amount of aflatoxin B_1 produced, using the ELISA method.

Experimental

Materials and methods

All chemicals and solvents were commercially available in analytical grade and were used without further purification. Pure *Aspergillus flavus* was received from the Department of Microbiology, Isfahan University of Technology. Zinc(II) acetate dihydrate, $Zn(OAc)_2·2H_2O$, amino acid ligands, and PDA culture medium were purchased from Merck Company. ELISA kits Ridascreen aflatoxin B_1 (R-Biopharm AG, Germany) were used according to the manufacturer's instructions. Double-distilled water was used in all experiments. Elemental analyses were performed using a Leco CHNS-932 elemental analyzer. Infrared spectra were taken in the 4000–400 cm^{-1} region as KBr pellets using an FT-IR JASCO 680-PLUS spectrophotometer. Electronic absorption spectra were recorded on a JASCO 7580 UV–Vis double-beam spectrophotometer. The cultivation processes were performed in an IKA KS 4000i control shaking incubator. ^1H NMR spectra were recorded on a Bruker-400 MHz spectrometer at room temperature in D_2O.

The white single crystals of (**1**) and (**2**) were chosen for the single-crystal X-ray diffraction study. Data were collected on a Bruker APEX II CCD diffractometer using graphite-monochromated Mo-*K*α radiation ($\lambda = 0.71073$ Å) at 296(2) K. Data collection, cell refinement, data reduction, and absorption correction were performed using multiscan method with Bruker software [35]. The structures were solved by direct method using SIR2004 [36]. The non-hydrogen atoms were refined anisotropically by the full-matrix least-squares method on F^2 using SHELXL [37]. All the hydrogen atoms of the ligands were placed at the calculated positions and constrained to ride on their parent atoms. Details of the data collection and refinement parameters for $Zn(Gln)_2$ are reported in Table 1, and the selected bond lengths and angles of its molecular structure are listed in Table 2. Crystal data of $Zn(Gln)_2$ and $[Zn(Arg)_2(OAc)]$

Table 1 Crystallographic data and structure refinement for $Zn(Gln)_2$

Chemical formula	$C_{10}H_{18}N_4O_6Zn$
Formula weight	355.65
Temperature (K)	296 (2)
Crystal system	Monoclinic
Space group	$P2_1$
a (Å)	9.4226 (4)
b (Å)	5.0517 (2)
c (Å)	14.5028 (6)
α (°)	90.00
β (°)	105.253 (2)
γ (°)	90.00
V (Å^3)	666.02 (5)
Z	2
Calculated density (Mg m^{-3})	1.773
Crystal size (mm)	0.13 × 0.07 × 0.03
F(000)	368
θ range (°)	2.24–25.00
Reflections collected	9721
Independent reflections (R_{int})	2321 (R(int) = 0.0348)
Data/restraints/parameters	2321/2/202
Goodness-of-fit on F^2	1.042
Final R indices	R_1 = 0.0257, wR_2 = 0.0538
R indices (all data)	R_1 = 0.0317, wR_2 = 0.0700
Range of h, k, l	−9/9, −20/20, −13/13

Table 2 Selected bond lengths (Å) and bond angles (°) for $Zn(Gln)_2$

Bond lengths (Å)	
Zn(1)–O(1)	2.0520 (2)
Zn(1)–O(3)	2.0444 (2)
Zn(1)–N(1)	2.0520 (3)
Zn(1)–N(2)	2.0800 (2)
Zn(1)–O(4)	2.5210 (2)
Zn(1)–O(6)	2.3036 (2)
Bond angles (°)	
O(1)–Zn(1)–O(3)	176.98 (9)
N(1)–Zn(1)–N(2)	169.80 (11)
O(1)–Zn(1)–N(1)	82.18 (9)
O(3)–Zn(1)–N(2)	81.02 (9)
O(3)–Zn(1)–N(1)	97.50 (9)
O(1)–Zn(1)–N(2)	98.76 (9)
O(3)–Zn(1)–O(6)	90.67 (8)
O(1)–Zn(1)–O(6)	92.34 (7)
N(1)–Zn(1)–O(6)	91.85 (9)
N(2)–Zn(1)–O(6)	98.25 (8)

OAc•3H_2O have been deposited at the Cambridge Crystallographic Data Center (CCDC nos. 1556782 and 1558139, respectively).

General procedure for synthesis of zinc(II) amino acid complexes

$Zn(OAc)_2{\cdot}2H_2O$ (438.9 mg, 2 mmol) was dissolved in 5 mL of water, and the obtained colorless solution was added slowly to an aqueous solution of amino acid ligand (4 mmol of Gln, Arg, His, Gly, Met, or Cys). The reaction mixture was then vigorously stirred and heated at 40–50 °C for 2 h. The solution was evaporated at room temperature to dryness. The white precipitate of Zn(II) amino acid complex was then washed with acetone and dried at room temperature under vacuum. The complexes (**1**–**6**) were recrystallized by slow diffusion of ethanol into the saturated aqueous solutions of each complex. After 3 weeks at room temperature, single crystals of $Zn(Gln)_2$ (**1**) and $[Zn(Arg)_2(OAc)]OAc{\bullet}3H_2O$ (**2**), suitable for X-ray crystallography, were formed in a yield of 81% and 87%, respectively. In addition, the crystals of other Zn(II) amino acid complexes were separated and washed with ethanol and vacuum dried. However, they were unsuitable for X-ray crystallography.

$Zn(Gln)_2$: Anal. calc. for $C_{10}H_{18}ZnN_4O_6$ (355.66 g mol^{-1}): C, 33.96; H, 4.52; N, 15.83. Found: C, 33.83; H, 4.49; N, 15.97%. UV–Vis (H_2O) λ_{max}/nm (ε/M^{-1} cm^{-1}): 203 (1567). IR (KBr pellet), υ/cm^{-1}: 3408, 3213 (N–H); 1686 (C=O); 1667 (COO); 1162 (C–N).

$[Zn(Arg)_2(OAc)]OAc{\cdot}3H_2O$: Anal. Calc. $C_{16}H_{40}ZnN_8O_{11}$ (585.93 g mol^{-1}): C, 32.79; H, 6.82; N, 19.11. Found: C, 33.08; H, 6.86; N, 19.31%. UV–Vis (H_2O) λ_{max}/nm (ε/M^{-1} cm^{-1}): 209 (2350). IR (KBr pellet), υ/cm^{-1}: 3346 (O–H); 3273 (N–H); 1677 (C=O); 1578 (COO).

$Zn(His)_2$: Anal. calc. for $C_{12}H_{16}ZnN_6O_4$ (373.68 g mol^{-1}): C, 38.77; H, 3.76; N, 22.60. Found: C, 38.61; H, 3.78; N, 22.76%. UV–Vis (H_2O) λ_{max}/nm (ε/M^{-1} cm^{-1}): 226 (4830). IR (KBr pellet), υ/cm^{-1}: 3393 (N–H); 3134 (C–H); 1650 (C=N); 1550 (C=C). ^{1}H NMR (D_2O, δ (ppm)): 7.947 (s, 2H), 7.060 (s, 2H), 3.903 (t, 2H), 3.204–3.079 (dd, 4H).

$Zn(Gly)_2$: Anal. Calc. for $C_4H_8ZnN_2O_4$ (213.51 g mol^{-1}): C, 22.71; H, 2.83; N, 13.24. Found: C, 22.90; H, 2.88; N, 13.31%. UV–Vis (H_2O) λ_{max}/nm (ε/M^{-1} cm^{-1}): 196 (1223). IR (KBr pellet), υ/cm^{-1}: 3262 (N–H); 1654 (C=O); 1137 (C–N). ^{1}H NMR (D_2O, δ (ppm)): 3.404 (s, 4H).

$Zn(Met)_2$: Anal. calc. for $C_{10}H_{20}ZnN_2O_4S_2$ (361.77 g mol^{-1}): C, 33.38; H, 5.00; N, 7.78. Found: C, 33.46; H, 4.97; N, 7.69%. UV–Vis (DMF) λ_{max}/nm (ε/M^{-1} cm^{-1}): 271 (1010). IR (KBr pellet), υ/cm^{-1}: 3245 (N–H); 1607 (COO); 697 (C–S–C). ^{1}H NMR (D_2O, δ (ppm)): 3.740 (s, 2H), 2.617–2.573 (dt, 4H), 2.200–2.014 (m, 4H), 2.080(s, 6H).

$Zn(Cys)_2$: Anal. calc. for $C_6H_{10}ZnN_2O_4S_2$ (305.69 g mol^{-1}): C, 23.73; H, 3.29; N, 9.22. Found: C, 23.89; H, 3.33; N, 9.30%. UV–Vis (DMF) λ_{max}/nm (ε/M^{-1} cm^{-1}): 275 (230). IR (KBr pellet), υ/cm^{-1}: 3411, 3080

(N–H); 1629 (COO). ^{1}H NMR (D_2O, δ (ppm)): 3.864 (t, 2H), 2.693–2.576 (dd, 4H).

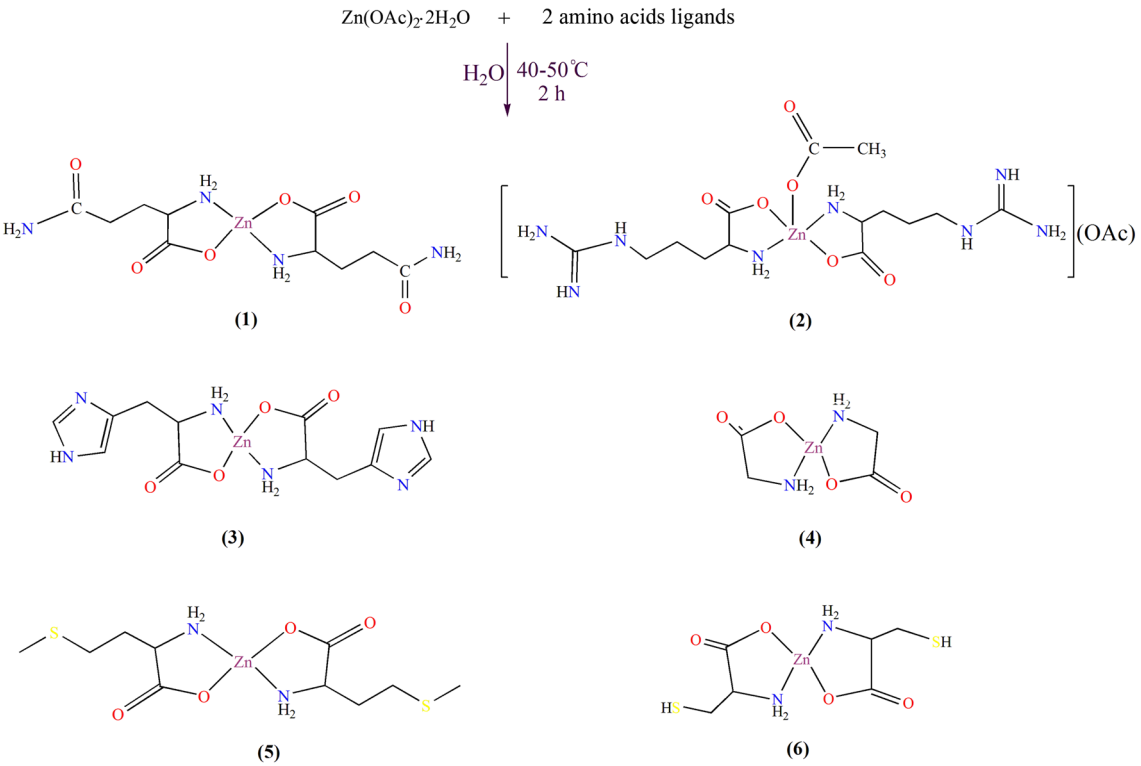
In vitro antifungal activity and minimum inhibition concentration (MIC) of the zinc(II) complexes

Antifungal activity of the Zn(II) ion as $Zn(OAc)_2$•$2H_2O$ and its complexes with amino acid ligands was tested against the *Aspergillus flavus*. The lowest concentration that prevents the growth of fungus is considered as minimum inhibitory concentration (MIC). The MIC of the compounds was evaluated against the *Aspergillus flavus* using a dilution method. In this method, the solutions of Zn(II) compounds at different concentrations (5–50 μg mL^{-1}) were prepared in the sterile test tubes by the serial dilution method. The culture medium of PDA was prepared according to the manufacturer's guideline. PDA (39 g) was dissolved in 1 L of water, and the obtained solution was then autoclaved at 120 °C for 15 min. 20 mL of PDA solution was added to each petri dish. The homogeneous suspension of *Aspergillus flavus* fungus (1 mL), and the solutions of Zn(II) complexes (10 mL) were also added immediately to each petri dish. The plates were incubated at 25–30 °C for 6 days. Four replicates for each concentration of the complexes were investigated simultaneously. Blank assays were done for cultures, which were not treated with the inhibitors to calculate the normal growth of *Aspergillus flavus* fungus.

Results and discussion

Synthesis of the zinc(II) amino acid complexes

All zinc(II) amino acid complexes were synthesized in good yields through the reaction of $Zn(OAc)_2{\cdot}2H_2O$ with the amino acid ligands (1:2 mol ratio) in the aqueous solutions (Scheme 1). The acetate anion can act as a weak base and remove a proton from the free neutral amino acids. The synthesized Zn(II) amino acid complexes were stable in the aqueous solutions for 48 h and no precipitation was observed even after long storage at room temperature, which indicates the stability of these complexes. In biology, the essential metal ions such as Zn(II) most frequently bind to donor ligands according to the hard–soft acid–base theory (HSAB). The overall guidelines for the coordination of Zn(II) ion in proteins have been described in the literatures [38, 39]. According to the Pearson's principle of HSAB, Zn(II) is a borderline metal, so it can bind well to both hard (nitrogen and oxygen) and soft (sulfur) donor atoms. As can be seen in Scheme 1, the amino acid ligands are coordinated



Scheme 1 General synthesis route and molecular structures of the Zn(II) amino acid complexes

to Zn(II) ion as N,O-chelating agents in the synthesized complexes.

IR and UV–Vis spectroscopic studies

The primary information about the zinc ion coordination was obtained by comparison of the IR frequencies of the uncoordinated ligands and the zinc amino acid complexes. The amino acids are as zwitterions in the crystalline state. The predominant vibrations for the free amino acid ligands are related to $\nu(COO^-)$, $\nu(NH_3^+)$, and $\nu(CCN)$. The amino acids coordinate to the metal ion in the N,O-chelating mode, and act as the bidentate ligands. It has been reported that the $\nu(COO^-)$ vibration of the amino acid complexes is affected by bonding of the non-coordinating C=O group with the metal of the neighboring complex, or its hydrogen bonding with the neighboring complex or lattice water molecules [40].

The FT-IR spectra of the Zn(II) amino acid complexes show the absorption patterns in the 4000–400 cm^{-1} region similar to those observed for the free amino acid ligands. Selected vibrations of these complexes and their assignments have been reported in the "Experimental" section. In general, the intense and broad absorption bands, which appear around 3000–3400 cm^{-1}, are ascribable to the ν(N–H) vibrations of the coordinated amino acid ligands. As compared to the free amino acids, these bands are shifted toward higher frequencies, which confirm the involvement of the $–NH_2$ group in the coordination to Zn(II) and complex formation [41]. Because the carboxylic group of amino acid ligands binds to the Zn(II) ion via covalent bonding, the ν(C=O) is metal-sensitive, so that its stretching vibration is shifted toward higher frequencies [42]. Furthermore, the observed absorption band at 2915 cm^{-1} for $Zn(Met)_2$ complex is attributed to the symmetric and asymmetric stretching vibrations of CH_2–S and CH_3–S bonds. This vibration band is not shifted compared to the free methionine ligand (2915 cm^{-1}), indicating that the methionine ligand is not coordinated to Zn(II) by the sulfur atom. In addition, the narrow peak at 1242 cm^{-1} is due to the wagging vibration of the CH_2–S bond for $Zn(Met)_2$ complex [43].

Absorption in the UV–Vis region is related to the electronic transitions within the molecule. The electronic absorption spectra of the synthesized complexes were taken in the aqueous or DMF solutions and data have been presented in the "Experimental" section. Since the Zn(II) complexes have a d^{10} configuration, there is no ligand field transition ($d \rightarrow d$). In general, the UV–Vis spectra of the Zn(II) complexes show the intraligand transitions, including $\sigma \rightarrow \sigma^*$, $n \rightarrow \sigma^*$, $n \rightarrow \pi^*$, and $\pi \rightarrow \pi^*$. The $\sigma \rightarrow \sigma^*$ transitions are observed only in the vacuum UV ($\lambda < 190$), because they are very high energy [44]. In this work, the synthesized Zn(II) amino acid complexes show a high energy absorption band in the UV region around 195–280 nm due to ligand-centered $n \rightarrow \pi^*$ or $\pi \rightarrow \pi^*$ transitions of amino acid ligands, which is slightly shifted compared to the free amino acid ligands.

Crystal structure description of the zinc(II) amino acid complexes

X-ray quality crystals of $Zn(Gln)_2$ and $[Zn(Arg)_2(OAc)]OAc{\cdot}3H_2O$ complexes were obtained by slow diffusion of ethanol into a saturated aqueous solution of each complex, while efforts to obtain the suitable single crystals of other complexes were unsuccessful. A white single crystal of $Zn(Gln)_2$ with dimensions of $0.13 \times 0.07 \times 0.03$ mm was chosen for the single-crystal X-ray diffraction study. An ORTEP view of $Zn(Gln)_2$ is shown in Fig. 2, and the selected bond lengths and angles are listed in Table 2. As shown in Fig. 3, its structure consists of the $[Zn(Gln)_2]$ units, which lie in the crystallographic *a*–*c* planes. In addition, the individual units are linked by long-range Zn–O contacts. Figure 3 shows that each Zn(II) ion is surrounded by four glutamine moieties, while each glutamine ligand is connected to two zinc atoms. The geometry around each Zn(II) ion is roughly octahedral with four short bonds and two long bonds, a configuration similar to that found for hexacoordinated copper complexes [45, 46]. Li and co-workers have also recently reported the same crystal structure for the $[Zn(Gln)_2]$ complex with a little difference in the unit cell parameters [47].

The base plane of the octahedron is formed by nitrogen and oxygen atoms, N(l), O(1), N(2), and O(3), from two independent glutamine ligands. The chelating glutamine ligands are arranged in a *trans*-configuration. The bond lengths are in the range of Zn–O and Zn–N distances usually observed for the other zinc amino acid complexes [48, 49]. Furthermore, the axial sites are occupied by two oxygen atoms of carboxyl groups, O(4) and O(6), from two other molecules of complex. The bond lengths of Zn–O(4) and

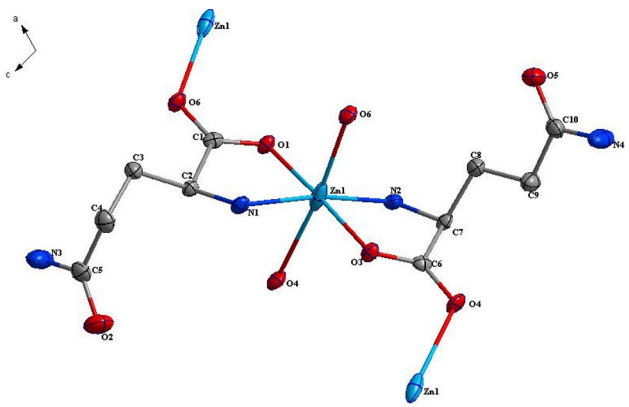


Fig. 2 ORTEP view of $Zn(Gln)_2$ with displacement ellipsoids drawn at the 40% probability level

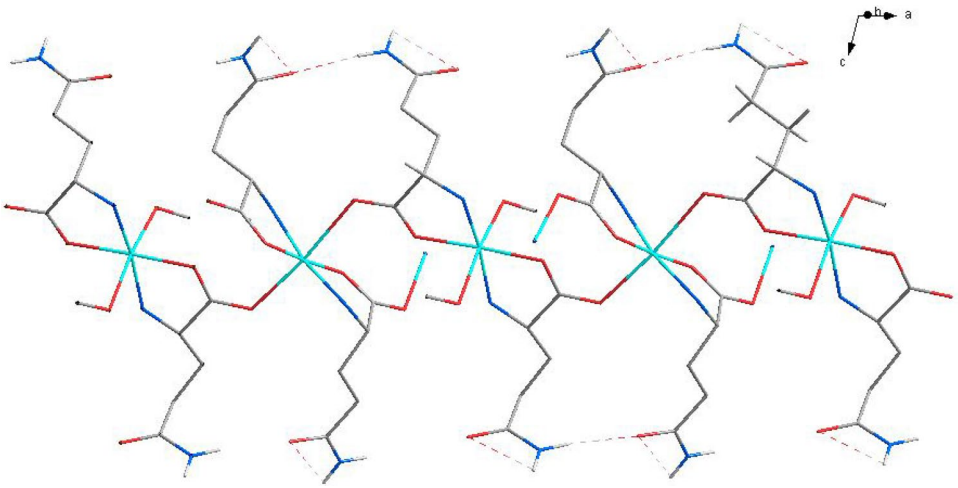


Fig. 3 View of the polymeric nature of $Zn(Gln)_2$ complex

Zn–O(6) are 2.521 and 2.304 Å, respectively, which are comparable to those reported previously for similar complexes, including Zn(L-glutamate) [50], $Zn(L\text{-serine})_2$ [51], and $Zn(DL\text{-histidine})_2$ [52].

As can be seen in Fig. 2, Zn(II) ion displays a slightly distorted octahedral, because the *trans*oid angles are all within the 169.80 (11)°–176.98 (9)° range, and the *cis*oid angles are in the range of 81.02 (9)°–98.76 (9)°. The chelate bite angles of O(3)–Zn–N(2) and O(1)–Zn–N(1) are 81.03 (95)° and 82.18 (93)°, respectively, which force the other "in-plane" angles to differ from 90° and 180°. In addition, the mean planes of two glutamine ligands form the dihedral angle of 9.76 (11) to each other, which confirms that they are not co-planar.

The hydrogen-bonding interactions in $Zn(Gln)_2$ complex are much less extensive than the free glutamine ligand. Many of hydrogen-bonding interactions, which link the individual molecules into sheets in the free glutamine, are eliminated by long- and short-range coordination of the metal atom in $Zn(Gln)_2$ complex. Only one hydrogen atom of $–NH_2$ group shows the evidence of hydrogen bonding.

The $[Zn(Arg)_2(OAc)]OAc{\cdot}3H_2O$ complex was also characterized crystallographically, matching the unit cell parameters reported in the literature [53] (see ESI).

In vitro antifungal activity and minimum inhibition concentration (MIC) of the complexes

A wide range of properties of amino acids and zinc ion promoted us to examine some biological activities of the zinc amino acid complexes. Fortunately, all of these Zn(II) amino acid complexes except $Zn(Cys)_2$, are sufficient soluble in water to investigate their in vitro antifungal activities. Therefore, the inhibitory effect of the synthesized complexes on the growth of *Aspergillus flavus* fungus and aflatoxin B_1 production was studied. In this respect, the aqueous solutions of the compounds at different concentrations including 5, 10, 15, 20, 25, 30, and 50 μg mL^{-1} were added to PDA medium consisting of *Aspergillus flavus* fungus. Each experiment was simultaneously repeated in four times to ensure the accurate results. The produced aflatoxin B_1 was determined using ELISA, after the incubation of the culture media at 25–30 °C for 6 days (Table 3). Furthermore, two blank assays were used for cultures. One of them was considered as a positive control to calculate the normal growth of fungus, without addition of the inhibitors to contaminated PDA medium with spores of *Aspergillus flavus*. The most amount of produced aflatoxin (3233 μg L^{-1}) was found for this blank, in the absence of the Zn(II) amino acid complexes

Table 3 Amount of produced aflatoxin B_1 (μg L^{-1}) in the culture media in the presence of 10 mL of Zn(II) compounds solutions at different concentrations after incubation at 25–30 °C for 6 days

Concentration of Zn(II) compounds solutions (μg mL^{-1})	5	10	15	20	25	30	50
$Zn(OAc)_2{\cdot}2H_2O$	2340	2118	1966	1785	1720	1686	1630
$Zn(Gln)_2$	756	305	123	N. D.	N. D.	N. D.	N. D.
$Zn(His)_2$	930	447	168	N. D.	N. D.	N. D.	N. D.
$Zn(Gly)_2$	1280	831	571	84	N. D.	N. D.	N. D.
$[Zn(Arg)_2(OAc)]OAc{\cdot}3H_2O$	1338	1120	776	138	22	N. D.	N. D.
$Zn(Met)_2$	1645	1336	857	280	154	14	N. D.

N. D. no detectable

as the inhibitors. In the other blank, only PDA medium was used as the negative control. The latest confirmed that the medium was not polluted before the addition of fungus, and it was found to be completely sterilized. As can be seen in Table 3, these water soluble complexes (**1–5**) showed the remarkable inhibitory effect on aflatoxin B_1 production by *Aspergillus flavus* fungus. The results revealed that the measured aflatoxin B_1 content was significantly changed at the different concentrations of these complexes. In general, the growth of *Aspergillus flavus* decreased with increasing concentration of the Zn(II) complexes. As a consequence, the aflatoxin production was decreased. The minimal inhibitory concentration (MIC) of Zn(II) compounds against *Aspergillus flavus* is also shown in Table 4. Therefore, in the presence of the high concentration of these compounds, the amount of aflatoxin B_1 produced in vitro by the fungus was the minimum, so that was no detectable using ELISA. At any certain concentration, $Zn(Gln)_2$ complex exhibited the most inhibitory effect in comparison with the other complexes. These complexes are arranged in order according to the antifungal activity as $Zn(Gln)_2$, $Zn(His)_2$, $Zn(Gly)_2$, $[Zn(Arg)_2(OAc)]OAc{\cdot}3H_2O$, and $Zn(Met)_2$. For example, Fig. 4 shows the images of plates at the different concentration of $Zn(Gln)_2$ as the inhibitor, after 6 days of incubation at 25–30 °C. As can be seen, the growth of the *Aspergillus flavus* has significantly decreased with the increasing concentration of $Zn(Gln)_2$ complex.

To compare, the inhibitory effect of $Zn(OAc)_2{\bullet}2H_2O$ on the growth of *Aspergillus flavus* fungus was also studied under the same experimental conditions (entry 1, Tables 3, 4). The results indicate that the synthesized Zn(II) amino acid complexes are more antifungal active than the zinc ion for the *Aspergillus flavus*. In general, the polarity of the metal ion is noticeably reduced after coordination of the ligand, due to the overlap of its valence orbitals with the ligand orbitals. This effect decreases the positive charge of metal ion and π-electron delocalization in the ligand chelating ring, which would enhance the lipophilic character of the metal ion center. The increased lipophilicity character leads to more diffusion of the complexes into the cell membrane and blocks the metal coordination sites of the enzymes in the fungi structures [54, 55]. Therefore, it is considerable that the complexation of Zn(II) ion with amino acids as the ligands significantly improves its antifungal activity.

Table 4 Minimal inhibitory concentration (MIC) of the Zn(II) compounds against *Aspergillus flavus*

Compounds	MIC (μg mL^{-1})
$Zn(OAc)_2{\cdot}2H_2O$	150
$Zn(Gln)_2$	20
$Zn(His)_2$	20
$Zn(Gly)_2$	25
$[Zn(Arg)_2(OAc)]OAc{\cdot}3H_2O$	30
$Zn(Met)_2$	50

Conclusions

In this paper, synthesis and characterization of Zn(II) amino acid complexes including $Zn(Gln)_2$ (**1**), $[Zn(Arg)_2(OAc)]OAc{\bullet}3H_2O$ (**2**), $Zn(His)_2$ (**3**), $Zn(Gly)_2$ (**4**), $Zn(Met)_2$ (**5**), and $Zn(Cys)_2$ (**6**) are reported. In addition, the $Zn(Gln)_2$ and $[Zn(Arg)_2(OAc)]OAc{\bullet}3H_2O$ complexes have been structurally characterized. The structure of $Zn(Gln)_2$ reveals the distorted octahedral environment around the zinc center. The synthesized Zn(II) amino acid complexes exhibit the

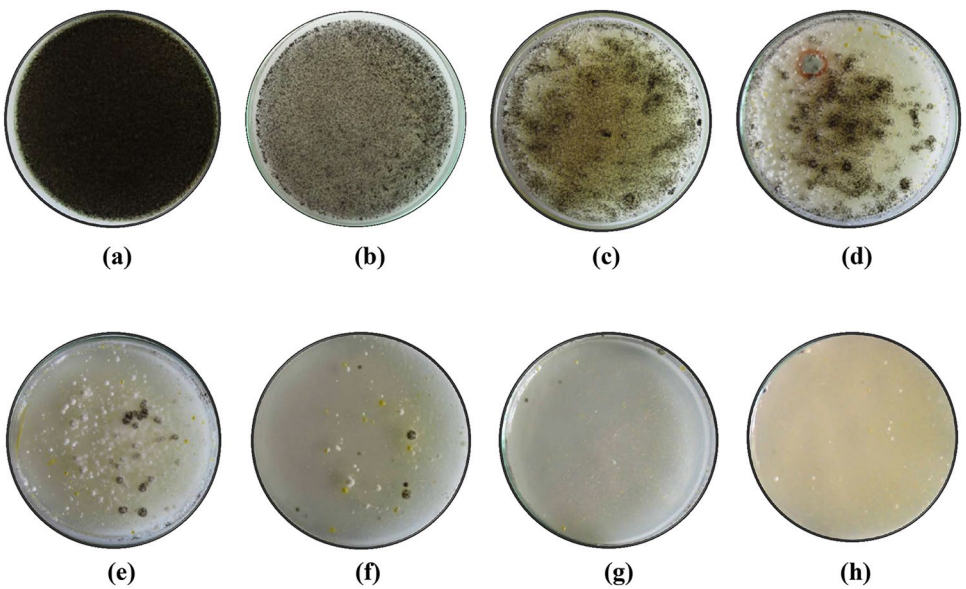


Fig. 4 Images of plates after 6 days of incubation at 25–30 °C in the presence of different concentration of $Zn(Gln)_2$, 0 (positive control blank), 5, 10, 15, 20, 25, 30, and 50 μg mL^{-1}, which are, respectively, shown as **a**–**h** images

intraligand transitions in the UV region. The inhibitory effect of the synthesized Zn(II) complexes on aflatoxin B_1 production was also studied in PDA medium, in the presence of *Aspergillus flavus* spores. The results show that the growth of *Aspergillus flavus* and the amount of toxin produced in vitro consequently decrease with increasing concentration of these Zn(II) complexes. Among the synthesized Zn(II) amino acid complexes, $Zn(Gln)_2$ and $Zn(Met)_2$ showed the most and least of the antifungal activity at any certain concentration, respectively. The minimal inhibitory concentration (MIC) of Zn(II) complexes against *Aspergillus flavus* was also evaluated.

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Supporting information Electronic supplementary material available: CIF files giving the crystal data for $Zn(Gln)_2$ and $[Zn(Arg)_2(OAc)]OAc{\cdot}3H_2O$. CCDC 1556782 and 1558139 contain the supplementary crystallographic data for $Zn(Gln)_2$ and $[Zn(Arg)_2(OAc)]OAc{\cdot}3H_2O$, respectively. These data can be obtained free of charge via http://www.ccdc.cam.ac.uk/conts/retrieving.html, or from the Cambridge Crystallographic Data Centre, 12 Union Road, Cambridge CB2 1EZ, UK; fax: (+44) 1223-336-033; or e-mail: deposit@ccdc.cam.ac.uk.

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