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Design, characterization, teratogenicity testing, antibacterial, antifungal and DNA interaction of few high spin Fe(II) Schiff base amino acid complexes

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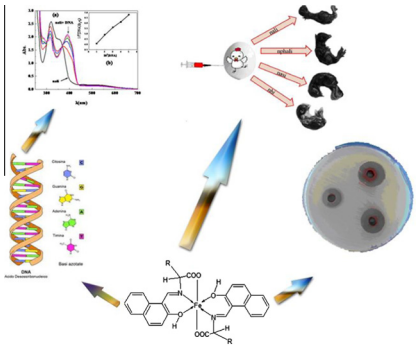
HIGHLIGHTS

- A new Schiff base amino acid ligands and their Fe(II) complexes were prepared.
- They were characterized by different physicochemical and spectral studies.
- The teratogenicity of the studied complexes was tested on chick embryos.
- The interaction between CT-DNA and the complexes was studied.
- The antimicrobial activity has been performed against different strain of organism.

GRAPHICAL ABSTRACT



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ABSTRACT

In this study, new Fe(II) Schiff base amino acid chelates derived from the condensation of o-hydroxynaphthaldehyde with L-alanine, L-phenylalanine, L-aspartic acid, L-histidine and L-arginine were synthesized and characterized via elemental, thermogravimetric analysis, molar conductance, IR, electronic, mass spectra and magnetic moment measurements. The stoichiometry and the stability constants of the complexes were determined spectrophotometrically. Correlation of all spectroscopic data suggested that Schiff bases ligands exhibited tridentate with ONO sites coordinating to the metal ions via protonated phenolic-OH, azomethine-N and carboxylate-O with the general formulae $[Fe(HL)_2]\cdot nH_2O$. But in case of L-histidine, the ligand acts as tetradentate via deprotonated phenolic-OH, azomethine-N, carboxylate-O and N-imidazole ring ($[FeL(H_2O)_2]\cdot 2H_2O$), where HL = mono anion and L = dianion of the ligand. The structure of the prepared complexes is suggested to be octahedral. The prepared complexes were tested for their *teratogenicity* on chick embryos and found to be safe until a concentration of 100 μg/egg with full embryos formation. Moreover, the interaction between CT-DNA and the investigated complexes were followed by spectrophotometric and viscosity measurements. It was found that, the prepared complexes bind to DNA via classical intercalative mode and showed a different DNA activity with the sequence: nhi > nari > nali > nasi > nphali. Furthermore, the free ligands and their complexes are screened for their in vitro antibacterial and antifungal activity against three types of bacteria, *Escherichia coli*, *Pseudomonas aeruginosa* and *Bacillus cereus* and three types of anti fungal cultures, *Penicillium purpurogenium*, *Aspergillus flavus* and *Trichotheium rosium* in order to assess their antimicrobial potential. The results show that the metal complexes are more reactive with respect to their corresponding Schiff base amino acid ligands.

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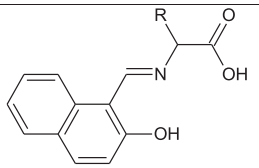
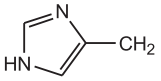
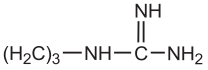
Introduction

Schiff base metal complexes have been studied extensively because of their remarkable chemical and physical properties. Metal complexes of Schiff base phenolates with favorable cell membrane permeability have been exploited in cancer multidrug resistance [1] and used as antimalarial agents [2]. Schiff base amino acid complexes have gained importance from the inorganic viewpoint and due to their physiological and pharmacological activities [3,4]. Moreover, metal chelates of Schiff bases derived from o-hydroxy aromatic aldehydes, e.g. o-hydroxynapthaldehyde and amino acids have some relationship to ligands involved in a variety of biological processes, e.g. transamination, racemization and carboxylation [5]. Furthermore, Schiff base complexes have an extensive importance in many fields such as radiotracers [6], biologically active reagents [7–10], catalysts in a large number of homogeneous and heterogeneous reactions such as oxidation [11–13], epoxidation [14,15], polymerization [16,17] and decomposition reactions [18–20]. Little effort has been expended to prepare Fe(II) amino acid Schiff base complexes [21–23]. Studying the interaction between transition metal complexes and DNA has attracted many interests [24–31] due to their importance in cancer therapy, design of new types of pharmaceutical molecules and molecular biology. On the other hand, few studies were carried out concerning the interaction of DNA with Schiff base amino acid complexes [32].

Therefore, in the present paper describes synthesis few novel Fe(II) Schiff base amino acid complexes and characterization by various physical methods to obtain more information and indicate their structures and behavior. Moreover, the teratogenicity of the studied complexes was tested on chick embryos to check the safety of these compounds in the human body. Moreover, the interaction between native calf thymus deoxyribonucleic acid (CT-DNA) and the investigated complexes was performed by spectrophotometric and viscosity measurements. Furthermore, Biological studies of the investigated compounds were screened against some bacteria and fungi to check how can we use these compounds as chemotherapeutic agent. The aldehyde used in this investigation is 2-hydroxy-1-naphthaldehyde and the amino acids are L-alanine (ala), L-phenylalanine (phala), L-aspartic acid (aspa), L-histidine (his) and L-arginine (arg). The structures of the Schiff base amino acid ligands studied in this investigation are shown in Scheme 1.



Acronym		R
Ligand	Complex	
nal	nali	CH_3
nphal	nphali	$CH_2C_6H_5$
nas	nasi	CH_2COOH
nh	nhi	
nar	nari	

Scheme 1. Structures and abbreviations of the Schiff base ligands and abbreviations of their corresponding complexes.

Experimental

All chemicals used in this investigation such as 2-hydroxy-1-naphthaldehyde, amino acids, the metal salt ($FeSO_4{\cdot}(NH_4)_2SO_4{\cdot}6H_2O$) calf thymus DNA (CT-DNA), Ethidium bromide (EB) and Tris[hydroxymethyl]aminomethane (Tris) were obtained from Sigma–Aldrich and used without further purification. All other reagents and solvents were purchased from commercial sources and were of analytical grade.

Synthesis of Schiff base amino acid ligands

The Schiff base ligands (hydroxynapthylidine amino acid) under investigation were obtained according to the procedure previously described in the literature [33]. 3 mmol of the 2-hydroxy-1-naphthaldehyde was dissolved in 40 ml ethanol and then added to 3 mmol of the amino acid (ala, phala, aspa, his or arg) solution in aqueous-ethanol mixture. The mixture was refluxed for 5 h, the solvent was removed on a rotary evaporator and the residue crystallized at room temperature. After one day, yellow crystals in case of L-alanine, L-phenylalanine and L-aspartic acid were obtained where brown and green crystals were obtained in the case of L-histidine and L-arginine, respectively. The precipitate was recrystallized from ethanol/diethy ether and then dried in vacuo over anhydrous $CaCl_2$.

Synthesis of the complexes

To an ethanolic ligand solution, an equimolar quantity of $FeSO_4{\cdot}(NH_4)_2SO_4{\cdot}6H_2O$ was added drop wise with constant stirring. In order to avoid oxidation of Fe(II), a few drops of glacial acetic acid were added [21]. The resulting solution was stirred magnetically for 8 h under nitrogen at 25 °C then evaporated over night. After that, the resulted solid product was filtered, washed with water and dry ether, respectively and finally dried in desicator.

Physical measurements

Melting or decomposition points were carried out on a melting point apparatus, Gallenkamp, England. Elemental analyses were carried by Elemental analyzer Perkin–Elmer model 240c. Molar conductance was measured in ethanol (10^{-3} M) solutions using JENWAY conductivity meter model 4320 at 298 K. The electronic spectra of the complexes using ethanol as solvent were monitored using 10 mm matched quartz cells on Jasco UV–Visible spectrophotometer model V-530. IR spectra of the metal chelates were monitored using Shimadzu FTIR model 8101 in the region 4000–400 cm^{-1} using dry KBr discs. Room temperature magnetic moment measurements of the investigated complexes were carried out on on a Guoy's balance by making diamagnetic corrections using Pascal's constant [34]. Fast Atom Bombardment (FAB) mass spectra were recorded on a JEOL SX 102/DA-6000 mass spectrometer/data system using argon/xenon (6 kV, 10 mA) as the FAB gas. The values of absorbance of 5×10^{-3} M of each complex were measured at different values of pH obtained by preparing a series of Britton universal buffer [35] was used. pH measurements were carried out using HANNA 211 pH meter at 298 K.

Teratogenicity testing

The toxicity of the prepared complexes at different concentrations (5, 10, 20, 40, 100 and 200 μg/egg) were investigated by

exploration of their administration to developing chicken embryos for 17 days at 37 °C in incubation. All eggs were turned with a quick movement of the wrist and left to lie horizontally 24 h before injection to let the germ cells float free to the top of the yolk and avoid trauma during the injection procedure. The day of injection was denoted as day 0. The egg shell was wiped with a tissue with 70% ethanol, and a 2 mm. hole was drilled over the air chamber, not penetrating the membrane. Then, the eggshell was wiped with 70% ethanol again, and 50 μl of the complex solution was injected into the yolk [36]. Exposure doses were 5, 10, 20, 40, 100 and 200 μg/egg of each complex. Once removed from the incubator, eggs were opened and embryos were sacrificed by decapitation. The embryos were weighed and compared with a reference sample injected only with the solvent. Differences in the uptake of the investigated complexes could not be attributed to differences in egg weight because eggs were weighed and randomly assigned to treatment groups; initial egg weights did not differ between groups. All data were analyzed by one way analysis of variance (ANOVA) by dose. Statistical significance was determined when $P < 0.05$. When ANOVA revealed a statistically significant model, post hoc *t*-tests were performed to determine statistical significance between dose groups.

Interaction with calf thymus DNA (CTDNA)

DNA binding analysis using electronic spectra

The interaction of the prepared complexes with DNA were carried out in Tris–HCl buffer (50 mM, pH 7.2). Calf thymus DNA (CT-DNA) was purified by centrifugal dialysis before use. CT-DNA solution at pH = 7.5 gave a ratio of UV absorbance at 260 and 280 nm of about >1.86:1, indicating that the DNA was sufficiently free from protein contamination [37,38]. The concentration of CT-DNA was determined by monitoring the UV absorbance at 260 nm using $\varepsilon_{260} = 6600\ mol^{-1}\ cm^2$. The stock solution was stored at 4 °C and used within only one day. The spectrophotometeric titration was performed by maintaining the concentration of the complex constant and varying the concentration of CT-DNA in interaction medium. The absorption of free CT-DNA was eliminated by adding an equimolar CT-DNA to pure buffer solution in the reference compartment and the resulting spectra were considered to result from the metal complexes and the DNA-metal complex aggregates. From the absorption data, the intrinsic binding constant (K_b) was determined by plotting [DNA]/($\varepsilon_a - \varepsilon_f$) versus [DNA] according to the following equation:

$$\frac{[DNA]}{(\varepsilon_a - \varepsilon_f)} = \frac{[DNA]}{(\varepsilon_b - \varepsilon_f)} + \frac{1}{[K_b(\varepsilon_b - \varepsilon_f)]}$$

where [DNA] is the concentration of DNA in base pairs, the apparent absorption coefficients ε_a, ε_f and ε_b are correspond to A_{obs}/[complex], the extinction coefficient for the free complex and extinction coefficient for the complex in fully bound form, respectively. The data were fitted to the above equation with a slope equal to $1/(\varepsilon_b - \varepsilon_f)$ and *y*-intercept equal to $1/[K_b(\varepsilon_b - \varepsilon_f)]$ and K_b was obtained from the ratio of the slope to the intercept [39]. The standard Gibb's free energy for DNA binding was calculated from the following relation [31]: $\Delta G_b^o = -RT \ln K_b$.

DNA binding analysis using viscosity measurements

Viscosity measurements were carried out using an Oswald type viscometer, thermostated at 25 ± 1 °C. The flow times of the samples were repeatedly measured with an accuracy of ±0.2 with a digital stopwatch operated timer for different concentrations of the complex (10–250 μM), maintaining the concentration of DNA constant (250 μM). The control sample was carried out on EB by using the same method. The average value of the three measurements was used to determine the viscosity of the samples. The buffer flow time was recorded as $t°$. The relative viscosities for DNA in the presence (η) and absence ($\eta°$) of the complex were calculated using the relation $\eta = (t - t°)/t°$, where t is the observed flow time in seconds. The values of relative viscosity ($\eta/\eta°$) were plotted against $1/R$ (R = [DNA]/[Complex]) [40].

Antibacterial bioassay (in vitro)

The main aim of the production and design of any antimicrobial compound is to inhibit the causal microbe without any side effects on the patients. In addition, it is worthy to stress here on the basic idea of applying any chemotherapeutic agent which depends essentially on the specific control of only one biological function and not multiple ones. The chemotherapeutic agent affecting only one function has a highly sounding application in the field of treatment by anticancer, since most anticancer agents used in the present time affect both cancerous diseased cells and healthy ones which in turns affect the general health of the patients. Therefore, there is a real need for having a chemotherapeutic agent which controls only one function [41].

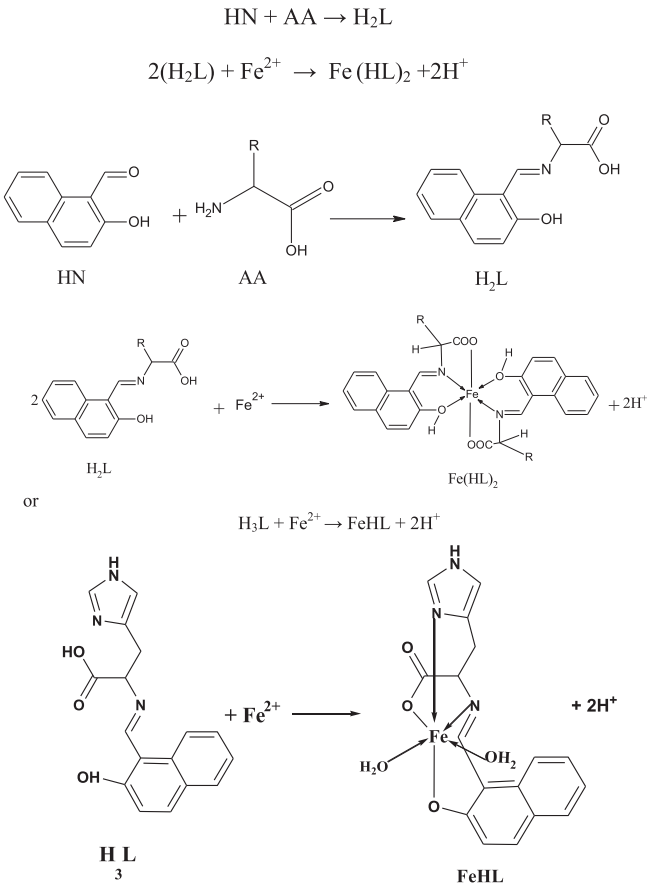
All the synthesized Schiff base ligands and their corresponding Fe(II) complexes were screened in vitro for their antibacterial activity against two Gram-negative (*Escherichia coli*, *Pseudomonas aeruginosa)* and one Gram-positive (*Bacillus cereus*) bacterial strains using the disc diffusion method [42]. Each compound was dissolved in dimethyl sulfoxide at different concentrations (5, 10 and 20 mg/ml). DMSO was used as solvent and also for control. DMSO was found to have no antimicrobial activity against any of the test organisms. 1 cm^3 of a 24 h broth culture containing 106 CFU/cm^3 was placed in sterile Petri-dishes. Molten nutrient agar (15 cm^3) maintained at ca. 45 °C was then poured into the Petri-dishes and allowed to solidify. Then holes of 6 mm diameter were formed in the agar using a sterile cork borer and these holes were completely filled with the test solutions. The plates were incubated for 24 h at 37 °C. The zones of inhibition based upon zone size around the discs were measured.

Antifungal activity (in vitro)

Antifungal activity for the prepared complexes were performed in faculty of pharmacy, El-azhar Assuit University. Antifungal activities of all compounds were studied against three fungal cultures (*Penicillium purpurogenium, Aspergillus flavus, Trichotheium rosium*) using disk diffusion method. The tested fungi were inoculated in Sabouraud dextrose broth medium (Hi-Media Mumbai) and incubated for 48–72 h at 35 °C, and subsequently a suspension of about 1.6×10^4–6×10^4 c.f.u./ml was introduced on the surface of sterile agar plates, and a sterile glass spreader was used for even distribution of the inoculum. The discs measuring 6 mm in diameter were prepared from Whatman No. 1 filter paper and sterilized by dry heat at 140 °C for 1 h. The sterile discs previously soaked in known concentration of the test compounds were placed in Sabouraud dextrose Agar (SDA) lates. The plates were inverted and incubated at 35 °C for 7 days. The susceptibility was assessed on the basis of diameter of inhibition against albicans and non-albicans strains of fungi [41].

Results and discussion

The formation of the investigated Schiff base amino acid ligands and their complexes is represented in Scheme 2 [21].



Scheme 2. Formation of the investigated Schiff base amino acid ligands and their complexes (HN = 2-hydroxy-1-naphthaldehyde, AA = amino acid, H_2L = nal, nphal, nas and nar ligands, H_3L = nh ligand, $Fe(H_2L)_2$ = nali, nphali, nasi, nari complexes and FeHL = nhi complex).

Characterization of the prepared complexes

Microanalysis and molar conductance measurements

The microanalyses results of the prepared Schiff base ligands and their complexes are given in Table 1 and suggested that Schiff base ligands act as tridentate and form complexes in 1:2 ratio metal to ligand in case of (ala, phala, asp, arg) while act as tetradentate and form complexes in 1:1 ratio metal to ligand in case of (his). Thus the general formula of the prepared complexes is suggested to be $[Fe(HL)_2]\cdot nH_2O$ and $[FeL(H_2O)_2]\cdot 2H_2O$. To prove the presence of water as coordinated or crystalline water, the prepared complexes are heated at 110 and 160 °C for about 2 h and the obtained weight loss percent data at 110 and 160 °C (for crystallization and coordination water, respectively) were consistent with the corresponding microanalytical data. The following is a comparison between thermogravimetric data at 110 °C and microanalytical data for crystallization water in the prepared complexes: [nali: 6.20 (6.25)%; nphali: 2.53 (2.35)%; nasi: 2.79 (2.79)%; nhi: 8.25 (8.28)%; nari: 6.95 (7.07)]. Moreover, a comparison between thermogravimetric data at 160 °C and microanalytical data is reported for coordination water, in the case of the complex nhi: 16.65 (16.66)% which is corresponding to four H_2O molecules, two of them are coordinated and the others are hydrated.

The measured molar conducatance values of 10^{-3} molar solutions of the prepared Fe(II) complexes in ethanol were found to be in the range 28.24–47.61 $ohm^{-1}\ cm^2\ mol^{-1}$ (cf. Table 1) indicating that the complexes are nonelectrolytic in nature.

Infrared spectra

The characteristic vibrations of the free ligand were shifted upon complex formation. A shift to lower frequency, relative to the free Schiff base ligands, of about 8–16 cm^{-1} for the —C═N stretching vibration in the complexes showed that coordination occurs through the nitrogen atoms of the azomethine groups [43]. In the IR spectra of the Schiff bases, the strong absorptions at 1528–1597 and 1370–1405 cm^{-1} are attributed to the asymmetric and symmetric υ(COO) bands, respectively. These bands shifted to lower frequency values upon complexation indicating that the azomethine nitrogen and the oxygen atom of the carboxylate group are coordinated to the metal ion [44,45] (cf. Table 2).

The recorded IR spectra of the prepared complexes (cf. Table 2) exhibited broad band at 3440–3405 cm^{-1} which assigned to υ(OH) vibration of water molecules associated with the complexes which are confirmed by the elemental and thermal analyses listed in Table 1 [21,46]. A strong band at 1630–1610 cm^{-1} of the complexes may be evidently due to υ(—C═N) vibration, which indicates the presence of an imine structure [47,48]. In nasi complex, υ(C═O) vibration band of the coordinated β-COOH group of aspartic acid band is obscured under the effect of the strong υ(—C═N) vibration band. The shift of this band to lower frequency than that expected (1700 cm^{-1}) can be presumably ascribed to its interaction with the water of hydration through hydrogen bonding. A band appearing at 1550–1537 cm^{-1} may be assigned to $\upsilon(COO^-)$asym vibrations, while (COO^-)sym vibrations appearing at 1404–1361 cm^{-1}. $\Delta\upsilon(COO^-) \sim 200\ cm^{-1}$ indicates the unidenticity of the carboxylate group [46,49]. It is worth noting that the asymmetric vibration band of COO^- is obscured by the high intensity of the band of C═C stretching vibration at 1462–1448 cm^{-1} [50]. All complexes showed an absorption band in the region of 1315–1294 cm^{-1} which can be attributed to υ(CO) (phenolic) vibration [46,51]. The bands observed in the region 2937–2900 cm^{-1} and the region 3064–3015 cm^{-1} can be assigned to υ(C—H) aliphatic and υ(C—H) aromatic stretching vibrations, respectively [52]. The IR bands at

Table 1
Analytical and physical data of Schiff base amino acid ligands and their Fe(II) complexes.

Schiff base ligands and their complexes	Empirical formula (formula weight)	Yield (%)	Molar conductance Λ_m ($\Omega^{-1}\ cm^2\ mol^{-1}$)	Decom. temp. (°C)	Analysis found (calculated)		
					C	H	N
nal	$C_{14}H_{13}NO_3$ (243.254)	83		162	69.25 (69.12)	5.17 (5.39)	5.61 (5.76)
nali	$C_{28}H_{28}FeN_2O_8$ (576.37)	75	35.12	260	58.50 (58.34)	4.67 (4.90)	4.88 (4.86)
nphal	$C_{20}H_{17}NO_3$ (319.346)	85		170	75.35 (75.22)	5.21 (5.37)	4.45 (4.39)
nphali	$C_{40}H_{34}FeN_2O_7$ (710.54)	83	28.24	>350	67.85 (67.61)	4.57 (4.82)	3.76 (3.94)
nas	$C_{15}H_{13}NO_5$ (287.168)	73		97	62.81 (62.73)	4.63 (4.56)	4.72 (4.88)
nasi	$C_{30}H_{26}FeN_2O_{11}$ (646.38)	72	39.31	185	56.01 (55.74)	3.92 (4.05)	4.21 (4.33)
nh	$C_{17}H_{15}N_3O_3$ (309.32)	85		156	66.13 (66.01)	4.72 (4.89)	13.44 (13.59)
nhi	$C_{17}H_{21}FeN_3O_7$ (435.22)	81	47.61	>350	46.83 (46.91)	4.72 (4.86)	9.58 (9.66)
nar	$C_{17}H_{20}N_4O_3$ (328.37)	86		133	62.29 (62.18)	6.05 (6.14)	17.21 (17.07)
nari	$C_{34}H_{44}FeN_8O_9$ (764.62)	84	32.21	235	53.64 (53.40)	5.72 (5.80)	14.43 (14.66)

Table 2
The infrared absorption frequencies (cm^{-1})[a] of the investigated Schiff base amino acid ligands and their Fe(II) complexes.

Schiff base ligands and their complexes	υ(OH); H_2O	υ(—C=N) stretching	υ(C=C) stretching	υ(C—H) aromatic	υ(C—H) aliphatic	υ(COO^-) symmetric stretching	υ(COO^-) asymm.	υ(C—O) phenolic	υ(Fe—N)	υ(Fe—O)
nal	3403 (w)	1630 (s)	1458 (s)	3081 (w)	2812 (w)	1413 (m)	1528 (w)	1362 (m)	–	-
nali	3439 (m)	1622 (s)	1450 (s)	3061 (w)	2924 (w)	1395 (m)	1548 (w)	1314 (m)	522 (w)	490 (m)
nphal	3466 (w)	1624 (s)	1500 (m)	3121 (w)	2879 (w)	1405 (w)	1597 (m)	1326 (m)	–	-
nphali	3440 (m)	1614 (s)	1448 (m)	3057 (w)	2923 (w)	1394 (w)	1542 (w)	1315 (w)	559 (w)	497 (m)
nas	3440 (m)	1640 (s)	1462 (m)	3062 (w)	2883 (w)	1462 (m)	1590 (w)	1319 (m)	–	–
nasi	3430 (m)	1629 (s)	1463 (s)	3064 (w)	2900 (w)	1404 (w)	1550 (w)	1311 (m)	550 (w)	482 (m)
nh	3421 (m)	1626 (s)	1469 (w)	3025 (w)	2922 (w)	1370 (m)	1531 (w)	1352 (m)	–	–
nhi	3405 (w)	1610 (s)	1459 (m)	3015 (w)	2920 (w)	1360 (m)	1537 (w)	1294 (w)	563 (m)	491 (m)
nar	3413 (w)	1639 (s)	1479 (w)	3058 (w)	2935 (w)	1377 (m)	1577 (w)	1341 (w)	–	–
nari	3421 (w)	1630 (s)	1451 (s)	3039 (sh)	2937 (w)	1361 (w)	1537 (w)	1296 (m)	553 (w)	465 (w)

[a] s = Strong, m = medium, w = weak.

3172 and 3350 cm^{-1} for the nari complex containing arginine moiety are assignable to the terminal guanidinium group [48]. Far-IR of the compounds showed bands in 563–550 and 497–465 cm^{-1} regions which can be attributed to υ(Fe—N) and υ(Fe—O) stretching, respectively [53,54].

Histidine is considered to have significantly different properties compared to the other amino acids because of the presence of an imidazole ring containing two nitrogen atoms, one of which protonates in the biologically significant pH ranges of 6–7. This nitrogen atom can strongly coordinate to transition metal ions [55]. This trend confirmed the participation of protonated nitrogen in coordination in nhi complex.

Electronic spectra

In order to propose a geometrical structure of the designed Fe(II) Schiff base amino acid complexes, the electronic absorption spectra of ethanolic solutions of the ligands and their complexes were recorded at the wavelength range 800–200 nm. The wavelengths at maximum absorption band (λ_{max}) and the molar absorptivity (ε) of the different bands in the recorded spectra of the complexes (cf. Fig. 1) are given in Table 3. The ligand exhibits absorption bands in UV–Vis region around 380 and 430 nm which is assigned to $n \rightarrow \pi^*$ transition originating from the amide or imine function of the Schiff base ligand. Another band observed below 300 nm is assigned to $n \rightarrow \pi^*$ transition. The designed complexes display a characteristic band centered at λ_{max} = 376–390 nm (ε_{max} = 2667–3740 mol^{-1} cm^2). This band could be mainly ascribed to an intramolecular charge transfer transition taking place in the complexed ligand. Moreover, there is a band shown in the region 414–462 nm (ε_{max} = 1113–3488 mol^{-1} cm^2) which can be attributed to charge transfer from ligand to metal. Furthermore, the L → MCT band is followed by a broad band from 494 to 520 nm (ε_{max} = 704–1380 mol^{-1} cm^2). This band could be mainly attributed to d → d transition in an octahedral structure of the prepared complexes [21,56].

Determination of the stoichiometry of the investigated complexes

The stoichiometry of the various complexes formed in solutions via the reaction of Fe(II) with the studied ligands was determined by applying the spectrophotometric molar ratio [57–60] and continuous variation methods [59,60] as shown in Figs. 2 and 3. The curves of continuous variation method (Fig. 2) displayed maximum

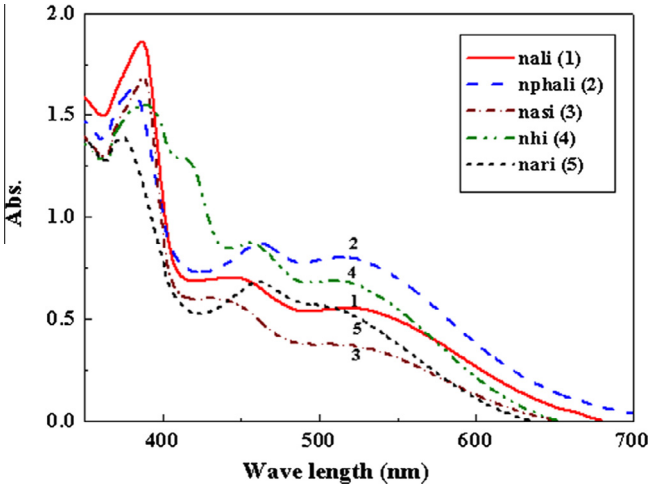


Fig. 1. Molecular electronic spectra of (1) [nali] = 5×10^{-4} mol dm^{-3}, (2) [nphali] = 2.5×10^{-4} mol dm^{-3}, (3) [nasi] = 5.4×10^{-4} mol dm^{-3}, (4) [nhi] = 5×10^{-4} mol dm^{-3}, (5) [nari] = 6×10^{-4} mol dm^{-3}.

absorbance at mole fraction X_{ligand} = 0.56 in case of nhi complex, which indicates the formation of complex with metal ion to ligand ratio 1:1. While in the case of nali, nphali, nasi and nari complexes, the maximum absorbances were obtained at X_{ligand} = 0.65–0.7 indicating the formation of complexes with metal ion to ligand ratio 1:2 as presented in Schemes 3 and 4. Moreover, the data resulted from applying the mole ratio method support the same metal ion to ligand ratio of the prepared complexes (cf. Fig. 3).

Magnetic moment measurements

Magnetic susceptibility measurements showed that the prepared complexes have paramagnetic character and suggested high spin values (4.18–5.12 BM) i.e., the studied Schiff base amino acid ligands are so weak that they exhibited low t_{2g} and e_g d-splitting of the octahedral structures of the complexes [61,62].

Mass spectra

The mass spectrum of the nal Schiff base ligand shows a well-defined molecular ion peak at m/z = 243 (relative intensity = 16%), which coincides with a formula weight of the Schiff base ligand.

Table 3
Molecular electronic spectra of the prepared Schiff base amino acid ligands and their Fe(II) complexes.

Schiff base ligands and their complexes	λ_{max}, nm[a]	ε_{max}, mol^{-1} cm^2	Assignment
nal	256	1614	$\pi-\pi^*$
	292	1600	$\pi-\pi^*$
	392	1690	$n-\pi^*$
	412	932	$n-\pi^*$
nali	390	3740	Intraligand band
	455	1404	LMCT band
	520 (b)	1108	d–d band
nphal	258	1653	$\pi-\pi^*$
	292	1640	$\pi-\pi^*$
	396	1800	$n-\pi^*$
	430	1970	$n-\pi^*$
nphali	380	6520	Intraligand band
	462	3488	LMCT band
	516 (b)	3212	d–d band
nas	256	1650	$\pi-\pi^*$
	292	1630	$\pi-\pi^*$
	349	1770	$n-\pi^*$
	414	1070	$n-\pi^*$
nasi	380	3148	Intraligand band
	430	1113	LMCT band
	502 (b)	704	d–d band
nh	256	1641	$\pi-\pi^*$
	292	1640	$\pi-\pi^*$
	396	1800	$n-\pi^*$
	424	1960	$n-\pi^*$
	456	522	$n-\pi^*$
	480	435	$n-\pi^*$
nhi	388	3200	Intraligand band
	414 (sh), 456	2500, 1746	LMCT band
	508 (b)	1380	d–d band
nar	258	1620	$\pi-\pi^*$
	306	1540	$n-\pi^*$
	398	1728	$n-\pi^*$
	420	1850	$n-\pi^*$
nari	376	2667	Intraligand band
	462	1472	LMCT band
	494 (b)	1288	d–d band

[a] b = Broad, sh = shoulder.

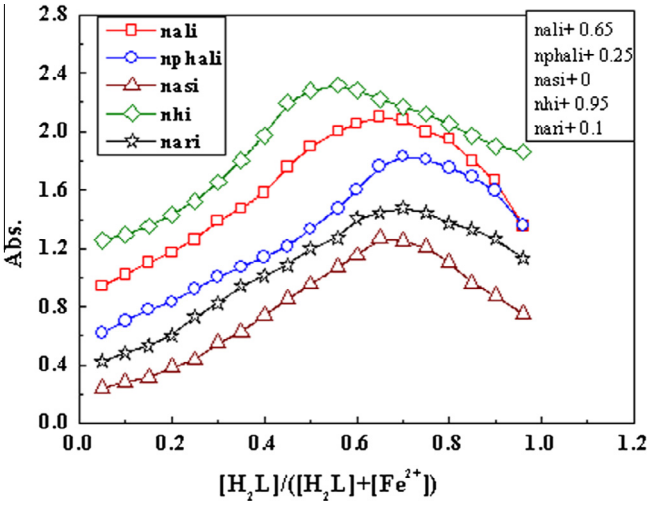


Fig. 2. Continuous variation plots for the prepared complexes in aqueous-alcoholic mixture at [nali] = 3×10^{-3} M, [nphali] = 3×10^{-3} M, [nasi] = 5×10^{-3} M, [nhi] = 2×10^{-3} M, [nari] = 1×10^{-3} M and 298 K.

The spectrum of nal ligand shows a series of peaks at m/z 242 (100%), 198 (58%), 168 (72%), 142 (60%) and 125 (53%) corresponding to its various fragments. The mass spectrum of nali complex shows a molecular ion peak at m/z 540 (18%), 452 (12%), 308 (23%), 164 (28%), 136 (20%) and 108 (22%) respectively, suggesting the complex to be monomeric, confirming the stoichiometry of the metal to ligand ratio to be 1:2 in nali. It is worth mention that the results of mass spectrum are in agreement with CHN data and suggested formula.

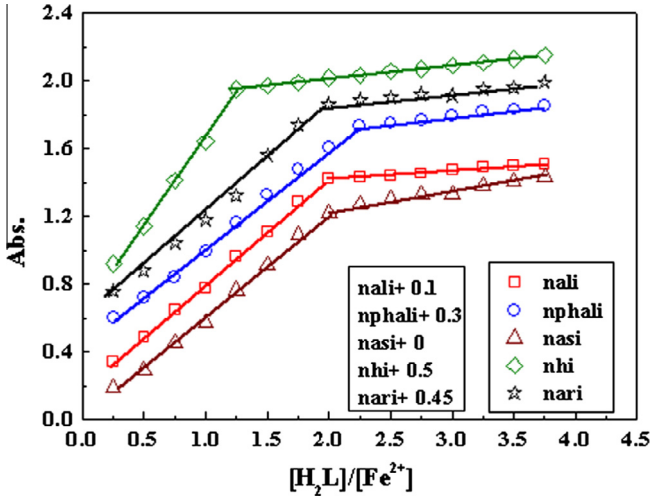
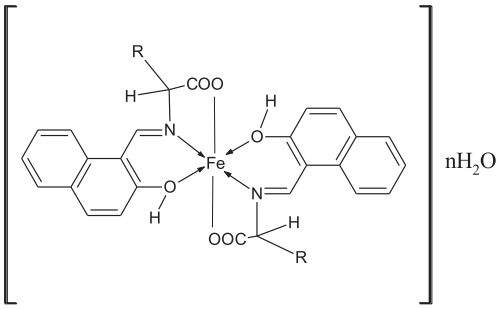
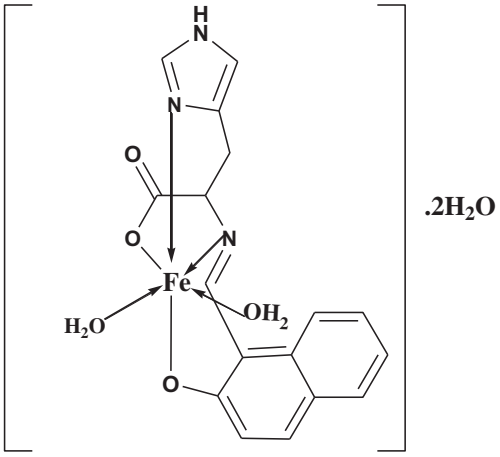


Fig. 3. Molar ratio plots for the studied complexes in aqueous-alcoholic mixture at nali, nphali: [Fe^{2+}] = 6×10^{-4} M; nasi: [Fe^{2+}] = 1×10^{-3} M; nhi: [Fe^{2+}] = 4×10^{-4} M; nari: [Fe^{2+}] = 1×10^{-3} M and 298 K.



Scheme 3. The suggested structures of nali, nphali, nasi and nari complexes, nali: $n = 2$, nphali and nasi: $n = 1$, nari: $n = 3$, (R as shown in scheme 1).



Scheme 4. The suggested structure of nhi complex.

The formation constants of the investigated complexes

The formation constants (K_f) of the studied Fe(II) Schiff base amino acid complexes formed in solution were obtained from

the spectrophotometric measurements by applying the continuous variation method [21,63,64] according to the following relations:

$$K_f = \frac{A/A_m}{(1 - A/A_m)^2 C} \quad \text{in case of } 1:1 \text{ complexes}$$

and

$$K_f = \frac{A/A_m}{4C^2(1 - A/A_m)^3 C} \quad \text{in case of } 1:2 \text{ complexes}$$

where A_m is the absorbance at the maximum formation of the complex, A is the arbitrary chosen absorbance values on either sides of the absorbance mountain col (pass) and C is the initial concentration of the metal. As mentioned in Table 4, the obtained K_f values indicate the high stability of the prepared complexes. The values of K_f for the studied complexes increase in the following order: nhi < nasi < nphali < nail < nari. Moreover, the values of the stability constant (pK) and Gibbs free energy ($\Delta G^{\neq}$) of the investigated complexes are cited in Table 4.

Stability range of the investigated complexes

The pH-profile (absorbance vs. pH) presented in Fig. 4 showed typical dissociation curves and a wide stability pH range (4–10) of the studied complexes. This means that the formation of the complex greatly stabilizes the Schiff base amino acid ligands. Consequently, this pH range is suitable for the different physiological applications of the prepared complexes.

Teratogenicity test of the prepared complexes

The chicken embryo has been used to assess the toxicity and teratogenic effects of a wide variety of chemicals for more than a century by numerous investigators. In these investigations there have been many variations with respect to time and route of administration, length of development, and method of examination or analysis for abnormal occurrences. In control embryos of 18-days old, the growth morphological features appears normal, head; body covered with feathers and appendages are well developed. The used solvent (5% (v/v) ethanol/water) has no effect on embryos and their formation was 100% at this percent (cf. Fig. 7a). Thus the difference in the weight of embryos could be attributed to the effect of the investigated complexes. The recent complexes were found to be safe until a concentration of 100 μg/egg and relatively well developed embryos were noted. In exposed embryos to nphali complex, concentration-dependent embryo resorption was observed. The percentage of resorption was 20% at 200 μg/egg. In addition to embryo resorption that represents a characteristic feature; retarded development of feathers to featherless skin was observed in exposed embryos at 200 μg/egg. From the statistical calculations (cf. Table 5 and Figs. 5 and 6), it was observed that for nali and nhi complexes until a concentration of 20 μg/egg, there is not any significant difference in weight of embryos compared to the control. Moreover, there is a relatively decrease in the weight of embryos at the concentration range from 40 to 200 μg/egg. Furthermore, there is no significant decrease in the weight of embryos at concentrations of 5 and 10 μg/egg for nasi complex and at 5 μg/egg for nphali complex. At the concentration range from 10 to 100 μg/egg for nphali complex, there is a significant decrease in the weight of embryos compared to the control. This behavior was observed for nasi complex at the concentration range from 20 to 200 μg/egg. It is worth noting that the relative effect of the investigated complexes on the weight of embryos could be mainly ascribed to the effect of the function group. Finally, we can say that our investigated compounds are safe and can be used as therapeutic agents.

Table 4
The formation constant (K_f), stability constant (pK) and Gibbs free energy ($\Delta G^{\neq}$) values of the prepared complexes in aqueous-ethanol at 298 K.

Complex	Type of complex	K_f	pK	$\Delta G^{\neq}$ kJ mol^{-1}
nali	1:2	$1.29 \pm 0.02 \times 10^{10}$	10.11	−57.68
nphali	1:2	$7.81 \pm 0.02 \times 10^{9}$	9.89	−55.44
nasi	1:2	$1.14 \pm 0.02 \times 10^{9}$	9.06	−51.67
nhi	1:1	$6.13 \pm 0.02 \times 10^{5}$	5.79	−33.02
nari	1:2	$4.47 \pm 0.02 \times 10^{10}$	10.65	−60.76

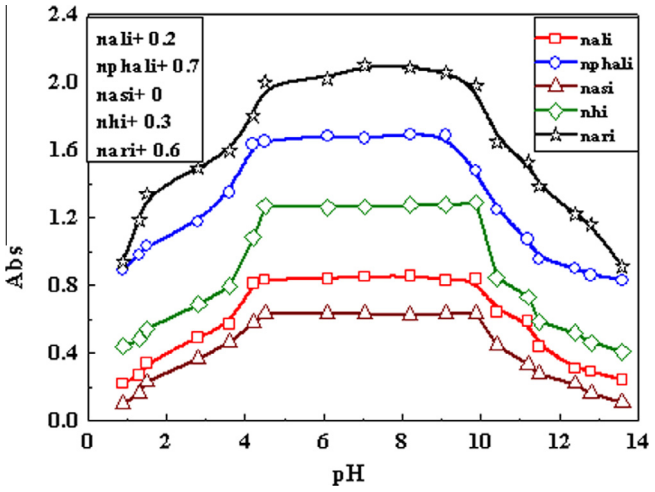


Fig. 4. Dissociation curve of the prepared complexes in aqueous alcoholic mixture at [complex] = 5×10^{-3} M and 298 K.

DNA-binding studies

Titration with electronic absorption spectroscopy is universally employed and an effective method to investigate the binding mode of DNA with a metal complex [65]. The spectra were recorded as a function of the addition of the buffer solutions of pre-treated CT-DNA to the buffer solutions of the complexes. If the binding mode is intercalation, the orbital of intercalated ligand can couple with the orbital of the base pairs, reducing the $\pi-\pi^*$ transition energy and resulting in bathochromism. If the coupling orbital is partially filled by electrons, it results in decreasing the transition probabilities and resulting in hypochromism [66]. The extent of the hypochromism in the metal-to-ligand charge transfer (MLCT) band is commonly consistent with the strength of intercalative interaction [67]. The electronic absorption spectra of nali complex in the absence and presence of different concentration of buffered CT-DNA are given in Fig. 7. By adding of DNA, the absorption intensities of MLCT band gradually increased. Moreover, addition of increasing amounts of CT-DNA resulted in a decrease of absorbance for each investigated complex. Representative spectra illustrating this hypochromicity and the presence of isosbestic points observed for the interaction of nali with CT-DNA (cf. Fig. 7). Table 6 show spectra parameters for DNA interaction with the prepared complexes. It can be realized from the high percent of hypochromicity that the high strength binding of the prepared complexes with DNA. The investigated complexes could bind to DNA via an intercalative mode and showed a different activity with the sequence: nhi > nari > nali > nasi > nphali. The results revealed that the structure difference on the amino acids might lead to obvious difference of DNA binding and intercalation abilities of the complexes. The values of binding constant of the investigated complexes with DNA are smaller than that of reported for typical classical intercalators (EB-DNA, 3.3×10^5 L mol^{-1}) [68].

Spectroscopic data are necessary, but not sufficient to support a binding mode. To further clarify the nature of the interaction be-

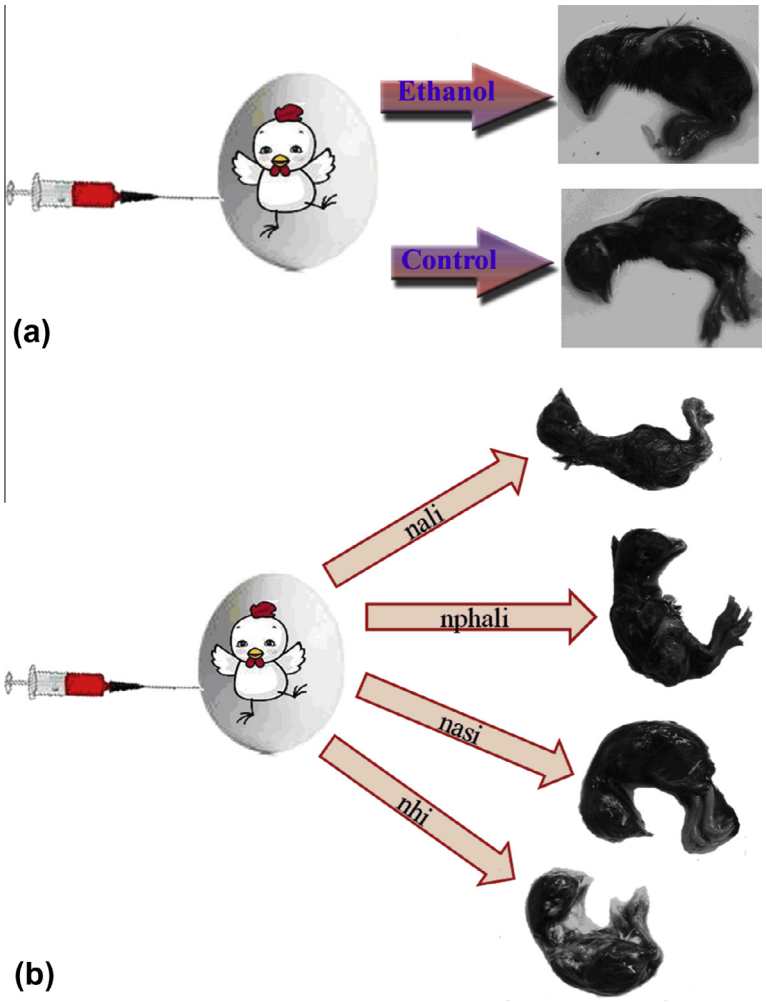


Fig. 5. (a) Photographs of chick embryos exposed to zero% alcohol and 5% alcohol. (b) Photographs of chick embryos exposed to 40 μg/egg of the investigated complexes.

Table 5
P-values[a] for teratogenicity testing of the investigated complexes compared to control and the used solvent.

Concentration μg/egg	Complex			
	nali	nphali	nasi	nhi
5	0.17	0.59×10^{-1}	0.14	0.85×10^{-1}
10	0.85	1.24×10^{-2}	3.85×10^{-1}	22.24×10^{-2}
20	0.67×10^{-1}	0.33×10^{-2}	0.21×10^{-1}	0.11×10^{-2}
40	0.11×10^{-1}	0.99×10^{-3}	0.27×10^{-2}	35.92×10^{-3}
100	0.01×10^{-1}	0.12×10^{-3}	0.43×10^{-3}	3.11×10^{-3}
200	0.11×10^{-2}	20% Embroy resorbed	0.48×10^{-4}	1.92×10^{-4}

[a] $P > 0.05$ (insignificant), $P < 0.05$ (significantly different).

tween the complex and DNA. Hydrodynamic methods such as viscosity measurements, which are sensitive to length increase or decrease of DNA, are regarded as the most effective means of studying the binding mode of complexes to DNA [69].

The relative viscosity of DNA solution increases significantly as the amount of the complex increases, but the increase is less than that observed for the typical intercalator EB which is consistent with the above absorption spectroscopic result, indicating that intercalative as shown in Fig. 8. This may be due to the insertion of aromatic ring in Schiff base ligand into the DNA base pairs and resulting in a bend in the DNA helix, hence, increase in separation

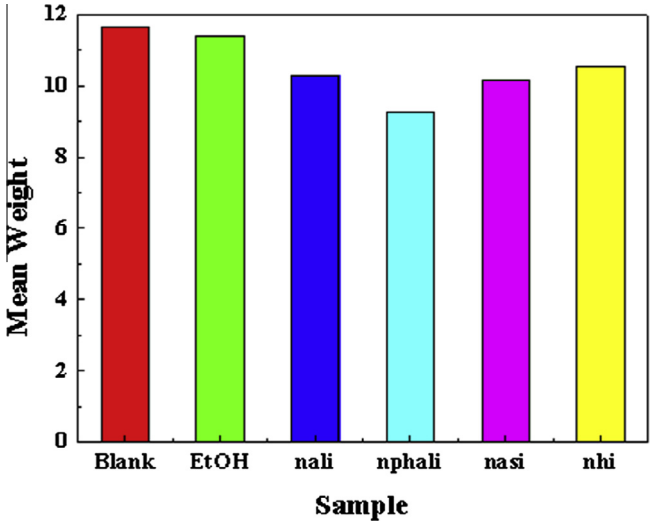


Fig. 6. Mean weight of chick embryos exposed to 100 μg/egg of the investigated complexes.

of the base pairs at the intercalation site, consequently increasing in DNA molecular length. Moreover, the sequence of the observed increase in values of viscosity was correlated the binding affinity to

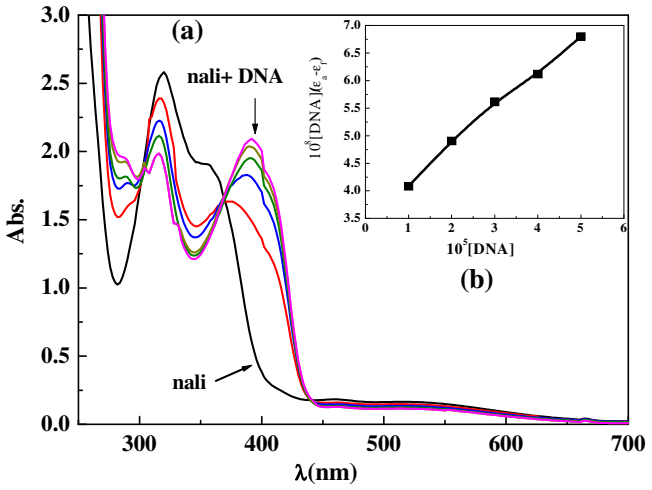


Fig. 7. (a) Spectrophotometer titration of nali complex (10^{-3} M) in 0.01 M Tris buffer (pH 7.5, 25 °C) with CT-DNA (from top to bottom, 0–50 μM DNA, at 10 μM intervals). (b) Plot of [DNA]/($\varepsilon_a-\varepsilon_f$) versus [DNA] for the titration of DNA with nali complex.

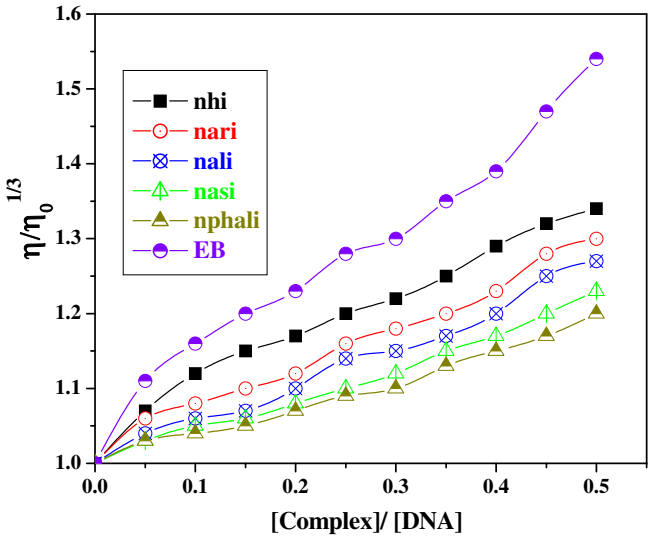


Fig. 8. The effect of increasing the amount of the synthesized complexes on the relative viscosities of DNA at [DNA] = 0.5 mM, [complex] and [EB] = 25–250 μM, and 298 K.

DNA i.e. nhi show the highest binding affinity to DNA and the highest viscosity. The information obtained from this work could be helpful to the understanding of the mechanism of the interaction of small molecules with nucleic acids, and should be useful in the development of potential probes of DNA structure and conformation [70].

Biological activity

The susceptibilities of certain strains of bacteria and fungal cultures to Schiff base amino acid and their complexes were evaluated by measuring the size of the bacteriostatic diameter. The antimicrobial activity data of all synthesized compounds are summarized in Tables 7 and 8 and show that the newly synthesized ligands and their Fe(II) complexes possess biological activity. The antibacterial screening results exhibited marked enhancement in activity on coordination with the metal ions against one or more testing bacterial strains (cf. Figs. 9–11). This enhancement in the activity can be rationalized to the basis of the structures of the ligands by possessing an additional azomethine (C═N) linkage which is important in elucidating the mechanism of transamination and resamination reaction in biological system [71]. It has also been suggested [72] that the ligands with nitrogen and oxygen donor systems might inhibit enzyme production, since the enzymes which require these groups for their activity appear to be especially more susceptible to deactivation by the metal ions upon chelation. The polarity of the metal ion is reduced by chelation [73] and this mainly because of the partial sharing of its positive charge with the donor groups and possibly with the delocalized π – elec-

Table 6
Spectral parameters for DNA interaction with the prepared complexes.

Complex	λ_{max} free (nm)	λ_{max} bound (nm)	Δn (nm)	Chromism (%)[a]	Type of chromism	Binding constant $K_b \times 10^4$ M^{-1}	ΔG^0 kJ mol^{-1}
nali	516	512	4	10.30	Hypo	3.72 ± 0.02	−26.07
	346	372	26	16.06	Hypo		
nphali	522	516	6	9.11	Hypo	2.92 ± 0.02	−25.49
	350	384	34	16.19	Hypo		
nasi	520	518	2	19.85	Hypo	3.51 ± 0.02	−25.93
	382	392	10	10.15	Hyper		
nhi	518	514	4	9.25	Hypo	35.73 ± 0.02	−31.68
	363	384	21	14.53	Hypo		
nari	502	498	4	4.06	Hypo	6.02 ± 0.02	−27.27
	354	390	36	27.16	Hypo		

[a] Chromism (%) = $(A_{free}-A_{bound})/A_{free}$.

Table 7
Results of antibacterial bioassay of the prepared Fe(II) hydroxylnapthylidene amino acid complexes in DMSO.

Bacteria	Compound (zone of inhibition in mm)										
	Conc. (mg/ml)	nal	nali	nphal	nphali	nas	nasi	nh	nhi	nar	nari
P. aeruginosa	5	–	5	–	4	2	7	1	7	1	8
	10	3	8	2	5	6	11	5	10	4	11
	20	6	10	5	9	8	13	8	13	6	14
E. coli	5	–	7	–	6	2	8	3	9	3	10
	10	4	10	3	7	7	12	6	12	5	13
	20	7	12	5	10	9	16	9	17	6	14
B. cereus	5	3	17	5	13	3	9	4	10	3	10
	10	7	22	8	16	5	14	5	14	5	14
	20	11	33	10	25	8	18	7	17	7	15

Table 8
Results of antifungal bioassay of the prepared Fe(II) hydroxylnapthylidene amino acid complexes in DMSO.

Compound	Compound (zone of inhibition)			
	Fungi Conc.	*Penicillium purpurogenium*	*Aspergillus flavus*	*Trichotheium rosium*
nal	20,000	–	–	–
	30,000	–	–	–
	40,000	–	+	+
	50,000	+	+	+
nali	20,000	–	+	–
	30,000	–	++	+
	40,000	+	++	++
	50,000	++	+++	++
nphal	20,000	–	–	–
	30,000	–	–	–
	40,000	–	+	+
	50,000	+	+	+
nphali	20,000	–	+	–
	30,000	–	+	+
	40,000	+	++	++
	50,000	++	+++	++
nas	20,000	–	–	–
	30,000	–	–	–
	40,000	+	+	+
	50,000	++	++	+
nasi	20,000	+	+	+
	30,000	++	++	+
	40,000	++	++	++
	50,000	+++	+++	++
nh	20,000	–	–	–
	30,000	–	–	–
	40,000	+	+	–
	50,000	+	++	+
nhi	20,000	–	–	–
	30,000	+	+	–
	40,000	+	++	+
	50,000	++	+++	++
nar	20,000	–	–	–
	30,000	–	–	–
	40,000	+	+	–
	50,000	+	+	+
nari	20,000	+	+	–
	30,000	+	+	+
	40,000	++	++	++
	50,000	++	+++	+++

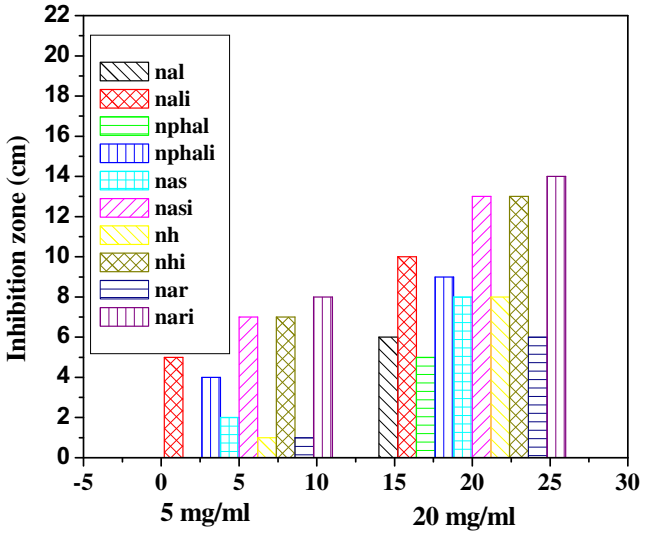


Fig. 9. Antibacterial evaluation of the investigated Fe(II) hydroxylnapthylidene amino acid complexes against *Pseudomonus aeruginosa* bacteria.

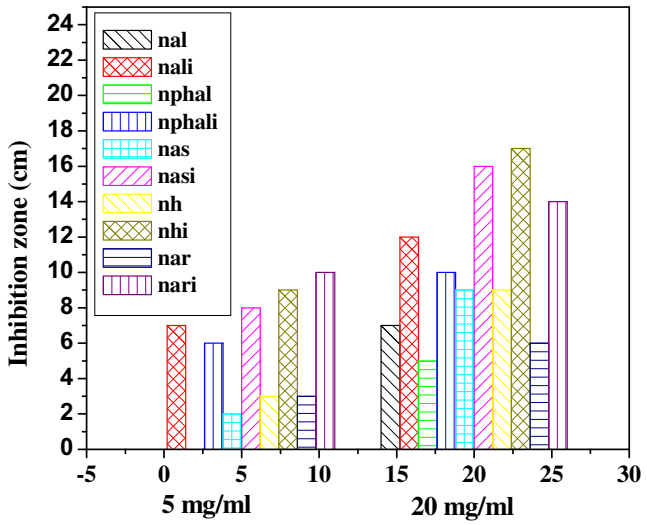


Fig. 10. Antibacterial evaluation of the investigated Fe(II) hydroxylnapthylidene amino acid complexes against *E. coli* bacteria.

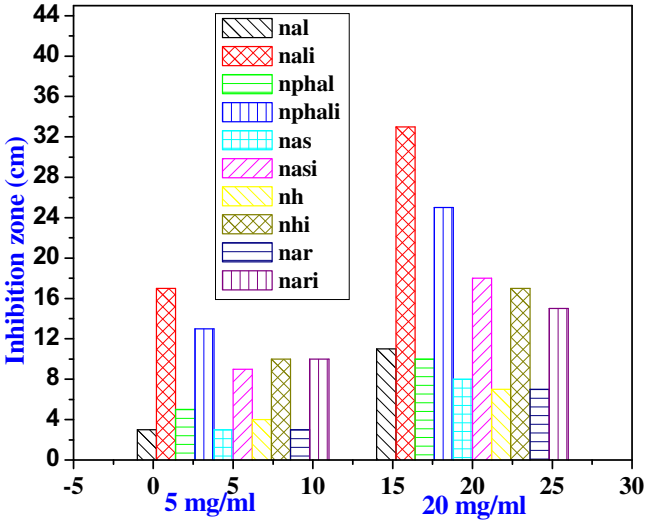


Fig. 11. Antibacterial evaluation of the investigated Fe(II) hydroxylnapthylidene amino acid complexes against *Bacillus cereus* bacteria.

trons within the whole chelation ring, which is formed because of the coordination. This process of chelation increases the lipophilic nature of the central metal atom, which in turn favors its permeation through the lipoid layer of the membrane [74]. This is also responsible for the increasing of the hydrophobic character and liposolubility of the molecules in crossing the cell membrane of the microorganism and hence enhances the biological utilization ratio and activity of the testing drug/compound.

Conclusion

A new series of Fe(II) tridentate Schiff base amino acid complexes have been synthesized. The Schiff bases derived from o-hydroxynaphthaldehyde and L-alanine (nal), L-phenylalanine (nphal), L-aspartic acid (nas) and L-arginine (nar) are monoanionic tridentate ligands. Results of the physical measurements show that Fe(II) ion is coordinated by two phenolic oxygen atoms, two azomethine N atoms and two carboxylate O atoms to form octahedral complexes with the general formula $[Fe(HL)_2]\cdot nH_2O$. Since, Schiff

base ligand of histidine (nh) has an imidazole ring which contains two nitrogen atoms one of which protonates at pH range (6–7), it behaves as dianionic tetradentate and coordinates to Fe(II) to give complex of the general formula $[FeL(H_2O)_2]\cdot 2H_2O$. The prepared complexes have non-electrolytic nature. The suggested formulas were confirmed by applying the molar ratio and continuous variation methods. Moreover, the obtained K_f values indicate the high stability of the prepared complexes and their values increase in the following order: nhi < nali < nasi < nari < nphali. Furthermore, the results of teratogenicity testing indicate that the investigated complexes are safe until the concentration of 100 μg/chick egg and follow the order nhi > nali > nasi > nphali which enlarge the area of the biological application of the considered Fe(II) Schiff base amino acid complexes. According to the spectrophotometric and viscosity measurements, the prepared complexes bind to DNA via an intercalative mode. These findings clearly indicate that transition metal based complexes have many potential practical applications, like the development of nucleic acid molecular probes and new therapeutic reagents for diseases. Also the prepared compounds show antibacterial activity.

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