The impact of synthetic analogs of histidine on copper(II) and nickel(II) coordination properties to an albumin-like peptide. Possible leads towards new metallodrugs.

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**Abstract**

The purpose of our research was to obtain peptidomimetics possessing Cu(II) and Ni(II) binding properties, which would be useful for biomedical applications. In this context we used potentiometry, UV-VIS and CD spectroscopies to characterize the Cu(II) and Ni(II) binding properties of pentapeptide analogs of the N-terminal sequence of histatin 5. The peptides investigated had a general sequence DSXAK-am (am stands for C-terminal amide), with X including His and its three synthetic analogs, (4-thiazolyl)-L-alanine (1), (2-pyridyl)-L-alanine (2), and (pyrazol-1-yl)-L-alanine (3). The heterocyclic nitrogens present in these analogs were significantly more acidic than that of the His imidazole. We found that DSXAK-am peptides were able to bind Cu(II) and Ni(II) and form 4N complexes in a cooperative fashion, with similar affinities. These results indicate that acidic heterocyclic amino acids provide a viable alternative for histidine in peptidomimetics designed for metal ions binding.

Keywords: Copper(II); Nickel(II); peptides; synthetic amino acids; complex formation

**1. Introduction**

Peptides which contain histidine in the third position counting from the N‑terminus (His-3 peptides) bind certain transitions metal ions, e.g. Cu(II), Ni(II), Pd(II), with high affinities, behaving as quadridentate ligands with four nitrogen donor atoms: the N-terminal amine group, two deprotonated peptide nitrogens, and the imidazole nitrogen, forming three fused chelate rings (4N) [1, 2]. It has been demonstrated that the coordination of the metal ion effective in the formation of this chelate system occurs at the π, not the τ nitrogen of the imidazole ring, because of the creation of a favorable 6-membered chelate ring (with the His deprotonated peptide nitrogen) rather than a 7-membered ring. The overall process of triple chelate ring formation is characterized by a high degree of cooperativity and a square-planar arrangement of the 4N set of donor atoms [1-3]. Due to their high stability, complexes of the described type with Cu(II) and Ni(II) ions are spontaneously formed in a wide pH range, including physiological conditions [4].

The imidazole π nitrogen is the primary metal ion anchoring group in His-3 peptides, as evidenced by the preferred formation of the low-pH 1N complexes at this site [5, 6]. This feature is ascribed to the higher acidity of this nitrogen vs. the amino nitrogen. Typical p*K*a values are 6.0-6.5 and 7.0-8.0, respectively [1-6]. We were thus interested in verifying the influence of heterocyclic rings more acidic than imidazole on Cu(II) and Ni(II) coordination to these peptides. In novel peptide analogs studied in this work the His residue was therefore substituted with synthetic amino acids presented in Fig. 1. In (4-thiazolyl)-L-alanine (**1**), the τ nitrogen atom was replaced with a more electronegative sulfur atom. This replacement was expected to affect coordination abilities of the π nitrogen atom by modulating its electronegativity, without bringing in an excessive steric hindrance. Using (2-pyridyl)-L-alanine (**2**) with a larger, 6-membered aromatic ring we aimed to produce a greater steric hindrance in the 4N complex plane, at the same time maintaining the ability of the appropriately positioned nitrogen to bind Cu(II) or Ni(II) in a structure similar to that presented in Fig. 1 (square-planar 4N coordination). In (pyrazol-1-yl)-L-alanine (**3**) we changed the mode of attachment of the heterocyclic ring to the rest of the peptide (using a nitrogen-carbon instead of a carbon-carbon bond), also expecting to modulate the complex formation by changing the localization and number of nitrogen atoms in the ring.

Several His-3 peptides and proteins are present in the human body. Their prominent representative, human serum albumin (HSA) transports the Cu(II) and Ni(II) ions in blood, using its N-terminal sequence DAHK. Similar properties were proposed for other members of this group [7-9]. Histatin 5 is the major representative of the histatin family of cationic salivary peptides contains the N-terminal sequence DSHAK, similar to that of HSA [10]. Histatins are part of human immune system, combating bacteria and fungi in the mouth [10, 11]. The proposed mechanism of their antimicrobial properties is multicomponent, including disruption of the cell membrane, impairment of the electron transport system in mitochondria, deprivation of essential metal ions, copper and zinc, required by pathogens, and reactive oxygen species generation via Cu(II) complexes [10, 12-19]. Histatin 5 is known to bind Ni(II) and Cu(II) strongly at its albumin-like N-terminal sequence [20, 21]. The presence of this binding site may contribute to the antimicrobial activity of histatins.

Despite the potential utility of histatins as therapeutic agents, their low stability against proteolysis makes them unattractive as future drug leads [22]. One possible way to overcome this disadvantage may be to introduce synthetic histidine analogs into the histatin sequence in order to extend their lifetime *in vivo* without compromising the crucial properties of natural peptides. By analogy with our recent Ni(II) study [21], we chose the N-terminal pentapeptide of histatin 5, DSHAK-amide as a simple, but sufficient model to accommodate synthetic analogs presented in Fig. 1 and to study the effects of these substitutions on the Cu(II) and Ni(II) coordination.

**2. Experimental**

*2.1. Peptide synthesis*

All peptides were synthesized in our laboratory on a Prelude™ peptide synthesizer from Protein Technologies, Inc. (Tucson, AZ), according to the Fmoc strategy [23]. Standard Fmoc amino acids were purchased from NovaBiochem. Fmoc-β-(4-thiazolyl)-Ala-OH, Fmoc-β-(2-pyridyl)-Ala-OH and Fmoc-3-(1-pyrazolyl)-Ala-OH were obtained from Sigma-Aldrich. Crude peptides were analyzed and purified by HPLC on an Empower system (Waters) equipped with an ACE 5C18-300 analytical column (5 mm particle size, 4.6 × 250 mm) and a Vydac semi-preparative column (5 mm particle size, 10 × 250 mm), respectively. The mobile phase consisted of (A) 0.1% trifluoroacetic acid (TFA) in water and (B) 0.1% TFA in 90% acetonitrile in water. The pure lyophilized peptides were characterized on a Q-Tof Premier mass spectrometer (Waters), exhibiting correct molecular masses.

*2.2 Potentiometry*

Potentiometric titrations of DSXAK-am peptides were performed on a Titrando 907 automatic titrator (Metrohm), using a combined glass-Ag/AgCl electrode (InLab®Micro, Mettler Toledo, Switzerland), which were calibrated daily by nitric acid titrations [24]. The 0.1 M NaOH (carbon dioxide free) was used as titrant. Sample volumes of 1.0‑1.5 ml were used. The samples contained 0.5–1.0 mM peptides, dissolved in 4 mM HNO3/96 mM KNO3. The Cu(II) complex formation was studied, for the 1.1 stoichiometry, using a 5–10 % excess of peptides over Cu(II), added as Cu(NO3)2. All experiments were performed under argon at 25 C, in the pH range of 2.3 to 12.2. The collected data were analyzed using the SUPERQUAD and HYPERQUAD programs [25, 26]. Three titrations were included simultaneously into calculations, separately for protonation and Cu(II) complexation.

*2.3. UV‑Visible (UV‑VIS) Spectroscopy*

UV‑VIS spectroscopy was used to characterize the Cu(II) and Ni(II) complex formation of DSXAK-am peptides. The UV‑VIS spectra were recorded at 25 °C on a Perkin Elmer spectrophotometer, over the spectral range 230‑900 nm. The optical path for all experiments was 1 cm. In the pH dependent titrations the peptide concentrations were 0.5‑1.0 mM, and the ligand to metal ratios were 1:0.9. The samples containing the peptide and Cu(II) ions were titrated with NaOH by careful manual additions of very small amounts of the concentrated base solution, in the pH range of 2.9‑11.0. In the metal dependent titrations the peptide concentrations used were 0.5‑1.0 mM, and the samples containing the peptide were titrated with Cu(NO3)2 or Ni(NO3)2 by careful manual additions of very small amounts of the concentrated (100 mM) solutions.

*2.4. Circular Dichroism (CD) Spectroscopy*

The CD spectra were recorded at 25°C on a J-815 spectropolarimeter (JASCO), over the spectral range of 250–700 nm. The optical path for all experiments was 1 cm. All samples contained 0.5 mM peptide and 0.45 mM Cu(II) or Ni(II) in a 100 mM Hepes buffer, pH 8.2.

*2.5. Molecular modeling*

Quantum mechanical calculations were carried out for DSX molecules, which represented metal binding portions of the studied peptides. Starting structures were derived from the crystal structures of analogous complexes: the Ni(II) complex of glycylglycyl-α-hydroxy-D, L-histamine [27] and the Cu(II) complex of the DAHK peptide [28]. The peptides were N-methylated on the C-terminus and modifications of the histidine ring were introduced. Amino acid side chains were introduced to match the studied sequence. The correct geometry of modified rings were adjusted by energy minimization according to the Dreiding forcefield in Accelrys Discovery Studio Visualizer 2.0 [29]. Geometry of metal complexes was optimized on the B3LYP/631++G(d,p) level of theory using using the Kohn-Sham theory for Ni(II) and the polarized form of Kohn-Sham theory for Cu(II) [30-32]. The stationary points were confirmed to be the minimum by frequency analysis. Calculations were performed in water, which was described by the polarizable continuum model (PCM) [33] and its integral equation formalism (IEF) version implemented in Gaussian 09 [34-36].

**3. Results and discussion**

We started our study of DSXAK-am peptides by an overall characterization of their Cu(II) binding properties. First, we employed UV-vis spectroscopy to see whether the His substitution affected the general binding mode. Titrations of peptides with Cu(II) ions at pH 7.4 indicated that all formed strong 4N (four nitrogen) copper(II) complexes in a 1:1 stoichiometric ratio, evidenced by a linear increase of their *d-d* absorption bands around 526-530 nm up to the 1:1 molar ratio. Above this ratio, the excessive Cu(II) ions precipitated as Cu(II) hydroxide. Therefore, we limited our further studies to this stoichiometry.

Fig. 2 shows the pH dependence of UV-vis spectra of Cu(II) complexes of the studied peptides. These experiments were performed to find out what kinds of complexes were formed and to establish the pH ranges of their formation. The sole species detected in all cases are typical 4N complexes, as evidenced by spectral parameters, provided in Table 1. Fig. 3 compares the pH dependence of formation of these 4N complexes, indicating that the peptide DS3AK-am was able to bind the Cu(II) ions with the p*K*a of 3.9, ca. 0.3 pH unit lower from that of all other analogs (see Table 2 for the exact values).

Fig. 4 presents the CD spectra of these 4N complexes, recorded at pH 8.2. The spectral patterns of complexes of DSHAK-am, DS1AK-am and DS3AK-am are very similar to each other and typical for complexes of His-3 peptides [4, 8, 37, 38]. The spectrum of DS2AK-am exhibits the same general pattern of alternate *d-d* bands, but the intensities of *d-d* bands and the pattern of charge transfer transitions in the near-UV range are slightly different from all others.

We also used potentiometry to establish protonation constants of the peptides and exact stability constants of their Cu(II) complexes. The results are presented in Table 2. The protonation properties of DSHAK-am were studied by us before in the context of Ni(II) binding [21]. In this study we obtained values that are in a satisfactory agreement with those obtained previously on a different apparatus, deviating by not more than about ±0.1 pH units. The p*K* values for the Lys and N-terminal amino groups of all peptides studied are typical for these groups in oligopeptides [1, 4, 39]. The nitrogens of heterocycles of **1**, **2**, and **3** are significantly more acidic than the imidazole. The representative p*K* values determined previously under similar conditions for free molecules of thiazole, pyridine and pyrazole were 2.68 [40], 5.22 [41], and 2.66 [40], respectively. The p*K* values of these rings measured in the context of larger molecules could be even lower, e.g. 4.64 for 2-pyridylethyleneglycol [42]. The p*K* of the Asp carboxylate was found to be very acidic. We were able to determine its value (~2.0) for DSHAK-am. The HYPERQUAD fitting of potentiometric data yielded very low Asp p*K* values for DS1AK-am and DS2AK-am (~1.0), and no value could be determined for DS3AK-am. A very high acidity of this side chain carboxylate is caused by the formation of a six-membered salt-bridged ring with the protonated N-terminal amino function of this residue [2, 42]. We think that the exact p*K* values of the Asp carboxylate are probably similar to each other in all four compounds studied, but they are at, or even beyond the limit of our potentiometric method. The apparently lower values for derivatives **1**, **2**, and **3** are therefore probably numeric artifacts, resulting from a lesser separation between the deprotonations of the carboxylate and the heterocycle nitrogen in these peptides.

Potentiometric and spectrophotometric results are very well correlated, as depicted on the species distribution plots in Fig. 5. All peptides formed three protonic forms of complexes, namely CuL, CuH‑1L, and CuH‑2L. All have a tetragonal geometry and square-planar 4N coordination of the peptide, as evidenced by stoichiometry and spectroscopic parameters [36-38, 42]. At the physiological pH (7.4) the main complex species is CuH‑1L. The results of quantum mechanical calculations of structures of complexes, shown in Fig. 9, clearly show that the introduced modifications do not disturb the arrangement of nitrogen donors around the Cu(II) ion, known from X-ray structures and spectroscopic studies of His-3 peptides [2, 27, 28].

The results presented above clearly show that substituents **1**, **2** and **3** behave analogically to the imidazole ring in term of formation of Cu(II) complexes. The stoichiometry of Cu(II) binding is 1:1 in all cases, and the pH-dependence of the spectra show the cooperative coordination of all four nitrogen donors (no species with other coordination modes are present in the spectra, except of Cu(II) aqua ion at 816 nm). Also the λmax and εmax of all four complexes are nearly identical, which demonstrates the equivalence of electron-donating abilities of heterocyclic nitrogens of **1**, **2** and **3** with the π nitrogen of the imidazole ring. These nitrogens differ, however, significantly in terms of their proton affinities. As shown in Table 2, their p*K* values in the context of the DSXAK-am sequence are lower from that of the imidazole by 2-3 pH units. This difference has, however, only a minor impact on the Cu(II) binding abilities of DSXAK-am peptides. This fact is seen e.g. in Fig. 3, which shows that the pH profiles of the formation of 4N Cu(II) complexes by the studied peptides do not differ significantly from each other, and in Fig. 5, which shows the pH dependence of formation of all individual complex species determined by potentiometry.

To compare the strength of Cu(II) binding in a detailed fashion we used the competitivity index (CI) approach (see footnote in Table 3 for definition) [43]. The CI calculations are informative when the free metal ion concentration is low, and therefore we performed them for pH 7.4 and pH 6.0. For lower pH values, 3.5 - 5.0, where substantial amounts of Cu2+ aqua ion are present, we used simple calculations of the percentage of Cu2+ complexation. These results are presented in Table 3. At pH 6.0 and higher new analogs bind Cu(II) somewhat less strongly than DSHAK-am, but at pH 5.0 and lower they become slightly more effective. At pH 5.0 this regards only DS3AK-am, at pH 4.5 and 4.0 also DS2AK-am binds Cu(II) stronger than DSHAK-am, and at pH 3.5 this property is shared by all analogs.

Our previous paper reported the Ni(II) binding to the DSHAK-am peptide [21]. In this work we wanted to test whether the synthetic substituents **1**, **2** and **3** would affect Ni(II) binding as well. We used only the UV-Vis spectroscopy for this purpose. The slow rate of reaction between Ni(II) and these DSXAK-am peptides made the potentiometric study impossible, because the pH change resulting from Ni(II) binding could not be discerned from the electrode drift during titrations. Such phenomenon was observed before for many Ni(II) complexes with His-3 peptides [1-4].

Fig. 6 shows the pH dependence of *d-d* absorption spectra of Ni(II) complexes of the novel analogs, confirming that similar diamagnetic 4N complexes were formed in all cases. Fig. 7 presents the CD spectra of these complexes, recorded at pH 8.2. Like in the Cu(II) case, the spectra are all similar to each other and typical of His-3 peptides, with slight differences seen only for the DS2AK-am analog. Parameters of these spectra are collected in Table 4. Fig. 8 shows that, in contrast with Cu(II) complexes, the p*K* of formation of the 4N complex is higher for all substituted analogs than for the original peptide: 5.68(1) for DSHAK-am, 6.28(1) for DS1AK-am, 6.10(2) for DS2AK-am, and 5.84(2) for DS3AK-am (numbers in parentheses denote standard deviations on the last significant digits). Taking into account the fact that substituents **1**, **2**, and **3** have p*K* values lower than the His residue, these values indicate that all three novel peptides bind the Ni(II) ion somewhat less strongly than the original pentapeptide.

In order to gain a better insight into the complex forming properties of the studied analogs, we calculated the structures of their 4N complexes with Cu(II) and Ni(II) ions. These structures are shown in Fig. 9, while important bond lengths and angles are given in Table 5. Clearly, all four complexes are quite similar to each other for both metal ions in terms of the metal ion binding geometry. The only significant differences can be noted for the six-membered ring of **2**. It forms the longest Cu-N and Ni-N bonds, and produces a larger steric crowding than the five-membered rings. These effects may be responsible for slight differences in the CD spectra of complexes of this analog.

In brief, our experiments demonstrated that the higher acidity of the metal anchoring heterocyclic nitrogen has a low impact on the efficacy of metal ion binding to His-3 peptides.

**4. Conclusions**

Peptides containing the His residue in the third position relative to the N-terminus (His-3 peptides) are a well-known class of strong chelating agents for Cu(II) and Ni(II) ions. This property has been considered as a possible basis of antimicrobial activity of histatins. In this work we studied the effect of substituting the His imidazole in the N-terminal sequence of histatin 5 with synthetic heterocyclic rings containing the nitrogen atoms more acidic than the imidazole. The results presented above clearly demonstrate that such analogs were able to bind Cu(II) and Ni(II) ions in the fashion analogous to that of the original His-containing peptide. The histidine residue constituted the best ligand for Cu(II) at “general” physiological pH 7.4 as well as pH 6.0, which is more typical for the oral cavity [44]. Instead, synthetic analogs bind Cu(II) ions stronger at pH 5.0 and lower. The original peptide was the most efficient chelator for Ni(II) ions in the whole pH range. Taken together, our results indicate that synthetic analogs **1**, **2** and, in particular, **3** should retain the antimicrobial activity of histatins related to Cu(II) sequestration, and are expected to have an increased resistance to proteolysis *in vivo*. Therefore, the histatin-like peptidomimetics using these substituents might become drug leads for novel oral cavity antimicrobials.

**5. Abbreviations**

4N - four-nitrogen

5Hst-5 - histatin-5 N-terminal pentapeptide, DSHAK-am

ESI-MS - electrospray ionization mass spectrometry

TFA - trifluoroacetic acid

HEPES - 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid

**1 -** (4-thiazolyl)-L-alanine

**2 -** (2-pyridyl)-L-alanine

**3 -** (pyrazolyl-1-yl)-L-alanine

**X -** any of **1, 2**, **3**

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**Tables**

**Table 1.** Spectroscopic parameters of *d-d* and charge transfer bands of major 4N Cu(II) complexes of N-terminal pentapeptide Histatin 5 and analogs.

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Peptide** | **UV-VIS** | | **CD** | |
| **λmax** | **ε** | **λext** | **Δε** |
| **DSHAK-am** | 526 | 119 | 569 | -0.77 |
| 489 | 0.87 |
| **DS1AK-am** | 530 | 111 | 571 | -0.65 |
| 489 | 0.94 |
| **DS2AK-am** | 530 | 109 | 562 | -0.69 |
| 478 | 0.38 |
| **DS3AK-am** | 526 | 108 | 569 | -0.85 |
| 489 | 0.87 |

**Table 2.** Protonation constants and stability constants (log β and pK values) of Cu(II) complexes of the pentapeptides at I = 0.1 M (KNO3) and 25 °C. Standard deviations on the last digits are given in parentheses. The p*K*s values correspond to the formation of 4N Cu(II)-peptide complexes determined by UV-Vis spectroscopy, n values refer to Hill coefficients of these values.

|  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **Ligand species** | **DSHAK-am** | | | | **DS1AK-am** | | | | **DS2AK-am** | | | | **DS3AK-am** | | | |
| **log β** | | **pK** | | **log β** | | **pK** | | **log β** | | **pK** | | **log β** | | **pK** | |
| **HL** | 10.055(4) | | 10.06a | | 10.284(2) | | 10.28a | | 10.317(3) | | 10.32a | | 10.129(4) | | 10.13a | |
| **H2L** | 17.490(6) | | 7.40b | | 17.584(3) | | 7.30b | | 17.598(5) | | 7.28b | | 17.337(6) | | 7.21b | |
| **H3L** | 23.681(7) | | 6.15c | | 20.586(4) | | 3.00c | | 21.823(5) | | 4.23c | | 20.192(8) | | 2.86c | |
| **H4L** | 25.96(1) | | 1.94d | | 21.71(4) | | 1.12d | | 22.74(7) | | 0.92d | | - | | - | |
| **Complex species** | **DSHAK-am** | | | | **DS1AK-am** | | | | **DS2AK-am** | | | | **DS3AK-am** | | | |
| **log β** | **pK** | | **pKs / n** | **log β** | **pK** | | **pKs / n** | **log β** | **pK** | | **pKs / n** | **log β** | **pK** | | **pKs / n** |
| **CuL** | 13.95(1) |  | | 4.238(5)  2.41(7) | 12.332(7) |  | | 4.28(1)  1.92(8) | 12.689(5) |  | | 4.25(2)  2.7(2) | 12.664(3) |  | | 3.92(1)  1.81(7) |
| **CuH-1L** | 9.721(8) | 4.23d | | 8.024(5) | 4.31d | | 8.317(4) | 4.37d | | 8.397(2) | 4.27d | |
| **CuH-2L** | -0.50(1) | 10.22a | |  | -2.358(7) | 10.38a | |  | -1.865(6) | 10.18a | |  | -1.769(4) | 10.17a | |  |

a Lys side chain group NH3+; b Asp terminal NH3+; c hetrocyclic ring NH+; d Asp side chain COOH.

**Table 3.** Competitivity index (CI)a values at pH 6.0 and pH 7.4 and percentage of Cu(II) bound to respective peptides at pH 3.5 - 5.0, calculated for Cu(II) complexes from stability constants presented in the Table 2. Peptides which are more efficient than DSHAK-am are marked bold.

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| **Peptide** | **CIa** | | **% Cu** | | | |
|  | **pH 7.4** | **pH 6.0** | **pH 5.0** | **pH 4.5** | **pH 4.0** | **pH 3.5** |
| **DSHAK-am** | 14.13 | 9.82 | 97.1 | 78.2 | 18.9 | 0.6 |
| **DS1AK-am** | 12.29 | 8.43 | 94.6 | 76.4 | **33.5** | **4.4** |
| **DS2AK-am** | 12.55 | 8.70 | 95.5 | **79.4** | **31.0** | **2.1** |
| **DS3AK-am** | 12.86 | 9.05 | **97.3** | **87.5** | **56.8** | **14.7** |

a CI was calculated for Cu(II), peptide and ligand Z concentrations of 0.001 M. CI is the value of log KCuZ such as the condition Ʃijk([CuiHjLk])=[CuZ] is fulfilled.

**Table 4.** Spectroscopic parameters of *d-d* bands of major 4N Ni(II) complexes of N-terminal pentapeptide of histatin 5 and analogs. Standard deviations on the last digits are given in parentheses.

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| **Peptide** | **UV-VIS** | | | **CD** | |
| **λmax** | **εmax** | **pKa** | **λext** | **Δε** |
| **DSHAK-am** | 422 | 130 | 5.680(8) | 476 | -2.39 |
| 409 | 2.15 |
| **DS1AK-am** | 424 | 139 | 6.28(1) | 484 | -1.45 |
| 414 | 2.38 |
| **DS2AK-am** | 425 | 156 | 6.10(2) | 476 | -1.28 |
| 409 | 0.86 |
| **DS3AK-am** | 417 | 136 | 5.84(2) | 482 | -2.78 |
| 409 | 2.99 |

**Table 5.** Selected parameters of calculated structures of Cu(II) and Ni(II) complexes containing the investigated X residues presented in Fig. 9. The parameters for the peptide containing the 6-membered ring **2** are marked bold. N1, N2, N3 and Nhet denote the consecutive metal ion binding nitrogen atoms, starting from the N-terminal amine.

|  |  |  |  |
| --- | --- | --- | --- |
| **Cu(II)** | | | |
|  | **planar angle N1-Cu-Nhet** | **dihedral angle N1-N2-N3-Nhet** | **Bond length**  **Cu-Nhet** |
| **DSH** | 100.78 | -1.13 | 1.99 |
| **DS1** | 100.97 | 4.65 | 2.02 |
| **DS2** | **101.40** | **8.63** | **2.04** |
| **DS3** | 100.66 | -0.48 | 2.01 |
| **Ni(II)** | | | |
|  | **planar angle N1-Cu-Nhet** | **dihedral angle N1-N2-N3-Nhet** | **Bond length**  **Ni-Nhet** |
| **DSH** | 97.22 | 2.56 | 1.92 |
| **DS1** | 97.48 | 5.58 | 1.94 |
| **DS2** | **97.83** | **8.56** | **1.95** |
| **DS3** | 96.82 | 3.02 | 1.93 |

**Figure captions**

**Figure 1.** Structures of synthetic histidine analogs: (4-thiazolyl)-L-alanine (**1**),   
(2 piridyl)-L-alanine (**2**), and (pyrazol-1-yl)-L-alanine (**3**). The overall structure of the 4N complex is also shown.

**Figure 2.** Spectra obtained from the pH titrations of Cu(II) complexes of DSHAK-am. DS1AK-am, DS2AK-am, and DS3AK-am. Peptide concentration were 0.5-1.0 mM, with a Cu(II):peptide ratios of 0.9:1.

**Figure 3.** Titration curves obtained from titrations presented in Figure 3, generated by following the absorption of the centre of the *d-d* band: 526 nm for DSHAK-am and DS3AK-am, and 530 nm for DS1AK-am and DS2AK-am.

**Figure 4.** CD spectra of Cu(II) complexes of DSHAK-am. DS1AK-am, DS2AK-am, and DS3AK-am, recorded for 0.5 mM peptides and 0.45 mM Cu(II) at pH 8.2 (100 mM HEPES buffer).

**Figure 5.** Species distribution plots of DSHAK-am, DS1AK-am, DS2AK-am, and DS3AK-am calculated for 1 mM peptide and 0.9 mM Cu(II) ions. Solid lines represent molar fractions of individual complex species calculated using stability constants obtained by potentiometry. Symbols mark *d-d* band intensities at λmax values indicated on the plots.

**Figure 6.** Spectra obtained from the pH titrations of Ni(II) complexes of DSHAK-am. DS1AK-am, DS2AK-am, and DS3AK-am. Peptide concentration were 0.5-1.0 mM, with a Ni(II):peptide ratios of 0.9:1.

**Figure 7.** CD spectra of Ni(II) complexes of DSHAK-am. DS1AK-am, DS2AK-am, and DS3AK-am, recorded for 0.5 mM peptides and 0.45 mM Cu(II) at pH 8.2 (100 mM HEPES buffer).

**Figure 8.** Titration curves obtained from titrations presented in Figure 8, generated by following the absorption of the centre of the *d-d* band: 422 nm for DSHAK-am, 424 nm for DS1AK-am, 425 for DS2AK-am and 417 for DS3AK-am.

**Figure 9.** Optimized structures of Cu(II) complexes (A) and Ni(II) complexes (B) of the metal binding cores of the studied peptides, superposed on amide nitogens. DSH – blue, DS1 – orange, DS2 – green, DS3 – red.

**Fig 1.**

****

**Fig 2.**



**Figure 3.**

****

**Fig 4.**

****

**Figure 5.**

****

**Fig 6.**



**Figure 7.**

****

**Figure 8.**

****

**Figure 9.**

****

**Graphical Abstract**

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Synthetic heterocyclic rings provide a viable alternative to His imidazole in the formation of Cu(II) and Ni(II) complexes of His-3 peptides.