Metal(II) Ion Complexes with 5-(Pyrazin-2-yl)-2,4-dihydro-3*H*-1,2,4-triazole-3-thione; Synthesis, Structural Characterization, Acid-base, and Complexing Properties in Solution

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Abstract. The study reports the synthesis of complexes $Co(HL)Cl_2$ (1), Ni(HL)Cl₂ (2), Cu(HL)Cl₂ (3), and Zn(HL)₃Cl₂ (4) with the title ligand, 5-(pyrazin-2-yl)-1,2,4-triazole-5-thione (HL), and their characterization by elemental analyses, ESI-MS (*m*/*z*), FT-IR and UV/Vis spectroscopy, as well as EPR in the case of the Cu^{II} complex. The comparative analysis of IR spectra of the metal ion complexes with HL and HL alone indicated that the metal ions in **1**, **2**, and **3** are chelated by two nitrogen atoms, N(4) of pyrazine and N(5) of triazole in the thiol tautomeric form, whereas the Zn^{II} ion in **4** is coordinated by the non-protonated N(2) nitrogen atom of triazole in the thione form. pH potentiometry and UV/Vis spectroscopy were used to examine Co^{II}, Ni^{II}, and Zn^{II} complexes in 10/90 (v/v) DMSO/water solution, whereas the Cu^{II} complex was examined in 40/60 (v/v) DMSO/water solution. Monodeprotonation of the thione triazole in solution enables the formation of the L:M = 1:1 species with Co^{II}, Ni^{II} and Zn^{II}, the 2:1 species with Co^{II} and Zn^{II}, and the 3:1 species with Zn^{II}. A distorted tetrahedral arrangement of the Cu^{II} complex was suggested on the basis of EPR and Vis/NIR spectra.

Introduction

Triazoles and thiadiazoles, as well as their derivatives, have drawn a fair degree of scientific attention due to their structural and biological activity. In particular, the 1,2,4-triazoles and their derivatives are very important ligands with *N*,*S*-donor centers giving a wide spectrum of possibilities for the synthesis of subsequent compounds with different specificity or mode of

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action.^[1] It is noteworthy that mercapto-triazoles can exist in two major tautomeric forms; the thione/thiole tautomers exhibit different reactivity, metal complexation, and substitution reactions.^[2] The relationship between their binding behavior towards metal ions, either bidentate- or monodentate, and the position, as well as the nature of the substituent at the triazole ring has allowed 1,2,4-triazoles to find various functions in coordination and bioinorganic chemistry.^[3] It has been revealed that metal chelation is an advantageous factor in increasing the antibacterial and antifungal activities of some triazole derived compounds because it enhances effective penetration through the lipid layer of the cell membrane.^[4]

The triazole rings are known to be pharmacologically important scaffolds in a variety of drug categories.^[5] Their derivatives and metal complexes have been reported as antimicrobial,^[6,7] antiviral,^[8] antitumor,^[9–11] anticonvulsant,^[12] antidepressant,^[13] analgestic,^[14] and anti-inflammatory agents.^[15]

A series of 1,3,4-thiadiazole-2(3H)-thiones, 1,3,4-oxadiazole-2(3H)-thiones, and 4-amino-1,2,4-triazole-5(4H)thiones have been synthesized as potent new inhibitors of tyrosinase: a copper-containing enzyme responsible inter alia for biosynthesis of the melanin pigment in skin, hair, and eyes of humans. Kinetic and active site binding studies attribute their potent inhibition to the monodentate binding of triazole rings to the active site, the dicopper center of tyrosinase, and to the enzyme's hydrophobicity. Substitutions of the triazole ring were found to play a major role in the high binding affinity to tyrosinase.^[16]

1,2,4-Triazole derivatives have also been identified as promising antiacetylcholinesterase (AChE) agents due to their inhibitory effect on AChE.^[17] Cholinesterase inhibitors may be very important in the treatment of Alzheimer's disease.^[18] The capacity of 4R-1,2,4-triazoles derived from amino acids and

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amino-acid esters to coordinate Zn^{II} ions has been exploited for their inhibition of metallo- β -lactamases. The activity recorded for the triazole derivative of methionine against the BcII enzyme showed modest inhibition of the BcII enzyme.^[19]

This paper reports the synthesis and physicochemical evaluation of newly-obtained cobalt(II), nickel(II), copper(II), and zinc(II) complexes of the title triazole derivative. It also describes the interaction of this derivative with the same biometals in solution. The results would highlight the possibility of using, and eventually modifying, the thione compound (also as a ligand coordinated by metal ions) with a pharmacologically important triazole scaffold as a potential enzyme inhibitor.

Results and Discussion

IR Spectroscopic Characterization of Metal Complexes

The most probable structures of complexes 1-4 (Scheme 1) were predicted on the basis of their IR spectra compared with that of the ligand.



Scheme 1. Synthesis of Co $^{\rm II}$ (1), Ni $^{\rm II}$ (2), Cu $^{\rm II}$ (3) and Zn $^{\rm II}$ (4) complexes.

The IR data of the ligand and its metal complexes are listed in Table 1. The ligand shows characteristic bands at 3294 and 3066 cm⁻¹ due to v(N–H) and at 2343 cm⁻¹ due to v(S–H). The absorption band at 1103 cm⁻¹ should be assigned as v(C=S) vibrations.^[6,20,21] These results suggest that the 1,2,4triazole ligand exhibits thione-thiol tautomerism (Scheme 1). The IR spectra of all the metal complexes show signals of stretching vibrations v(N-H) of the triazole/triazolate in the region 3292–3431 cm⁻¹. The presence of a characteristic band at 2359 cm⁻¹ due to v(S-H) stretching and the disappearance of the absorption band at ca 1103 cm⁻¹ corresponding to v(C=S) vibrations, shows that complexes **1**, **2**, and **3** exist in thiol form when in a solid state. The appearance of a C=S stretching band at 1107 cm⁻¹ and the absorption band around 2300 cm⁻¹ (which is connected with the S–H group) suggest that for **4** the ligand coordinates in thione form.^[22]

The spectra of HL and complexes **1–4** also show bands in the range 1575–1628 cm⁻¹ resulting from stretching vibrations v(C=N) and in 1053–1022 cm⁻¹ from stretching vibrations v(N-N).^[1] For the complexes, these characteristic bands are shifted to higher, for v(C=N), and lower, for v(N-N), frequencies in comparison with the spectrum of the ligand. This observation can be explained by the nitrogen atom of the triazole ring coordinating with the metal ions in complexes **1–4**. An intensive band that appears for the ligand at 1172 cm⁻¹ due to the pyrazine ring, is shifted to lower wavelengths and appears in the spectra of complexes **1, 2,** and **3** at about 1157 cm⁻¹, thus indicating complexation of the metal atoms via pyrazine nitrogen.^[23] However, in the spectrum of complex **4**, this band remains unshifted, suggesting that the pyrazine nitrogen is not involved in coordination.^[24]

¹H NMR Spectroscopy of the Zinc(II) Complex

The ¹H NMR spectrum of the Zn^{II} complex was recorded in [D₆]DMSO (Figure S2, Supporting Information) and the data is presented in the Experimental Section. The position and intensity of the signals correspond to reagents used in the synthesis. In the spectra, a signal was observed at $\delta = 1.22$ ppm for the proton of the –SH group. This signal is not shifted as compared to the free ligand (Figure S3, Supporting Information). Signals for the protons from the aromatic ring were found at $\delta = 8.75$ ppm and 9.18 ppm as singlets. The amine protons of the triazoline ring were observed as two singlets at $\delta = 13.90$ and $\delta = 14.12$ ppm. Because of the paramagnetic character of the Ni^{II} complex, the proton NMR spectrum was unattainable (Figure S4, Supporting Information).

ESI-MS Spectrometry

Valuable information about the structure of newly-synthesized complexes 1–4 was obtained by mass spectrometric measurements and is presented in the Experimental Section. The

Table I. FI-IK spectroscopic data /c	lable I. F	spectroscopic data /c	m-1.
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Compound	v(N–H)triazole	v(C=N)triazole	v(N–N)triazole	v(C=N)pyrazine	v(C=S)triazole	v(S–H)triazole
HL	3294, 3066	1575	1053	1172	1103	2343
1	3390, 3373	1616	1039	1157	-	2359
2	3392,3377	1616	1039	1159	-	2359
3	3431, 3397	1618	1047	1155	_	2357
4	3292, 3211	1628	1016	1170	1107	_

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peaks observed in the spectra of **1**, **2**, and **3** were assigned to the ions of parent complexes, whereas for **4**, they were assigned to the $[Zn(HL)_3Cl]^+$ ion. The spectra of complexes **1**– **4** exhibited peaks ascribed to the molecular ions $[ML]^+$ (M = Co^{II} , Ni^{II}, Cu^{II}, Zn^{II}). Additionally, the analysis of the spectra recorded allowed for the identification of the $[Co(HL)Cl]^+$ ion for **1** and the $[Zn(HL)_2Cl]^+$ and $[Zn(HL)_3]^+$ ions for **4**. The peaks at (m/z) 179 and 180 in the spectra of complexes **2** and **3** correspond to the L and L+1H ions, respectively. The obtained results are in good agreement with those derived using the elemental analysis (see Experimental Section).

Potentiometric Equilibrium Studies

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The titrations started to neutralize the excess inorganic acid and ended in an alkali medium. A representative set of titrations for ligand:metal = 2:1 is shown in Figure 1. In the case of Cu^{II} , the share of DMSO in the solvent had to be increased to an optimum 40% (v/v), which led to entire transparency of the forming brown solution. Even so, this did not enable further titration at higher pH due to predominating hydrolytic effects (cf. last part of the curve for Cu in Figure 1). Therefore, the formation constants of the Cu^{II}-HL complexes had to be determined spectrophotometrically, as will be described later (chapter "UV/Vis/NIR Studies").



Figure 1. Potentiometric titration of ligand HL and titrations in presence of the metal ion in an argon atmosphere. The negative values of base equivalent, *a*, correspond to HNO₃ in excess as related to the ligand. Solv. 10/90 (v/v) DMSO/water for Ni^{II}, Co^{II}, Zn^{II} and 40/60 (v/v) DMSO/water for Cu. Ligand-to-metal ratio 2:1. $C_{\rm L} = 4.0 \times 10^{-3}$ M.

It is visible that the base equivalent for the ligand alone comes close to the end-point at ca 0.8, but does not attain 1.0, which is due to precipitation occurring at pH > 7. Such an effect indicates fair DMSO/water 10/90 (v/v) solubility of the title triazole ligand in its monodeprotonated form. In contrast, in the presence of the metals, the titration curves go nearer to the base equivalent 1.0 as a result of the pH falling due to the displacement of hydrogen ions by the metal ions. Moreover, these curves do not exhibit inflection after neutralization of the excessive inorganic acid, which indicates a high complexing affinity of the metal ions to the ligand already in acidic medium. Hence, the refinements for ligand HL unambiguously need to consider the presence of one dissociable proton of HL in the proton balance, irrespective of the protons from the excessive inorganic acid. However, the analysis of all titrations show that the refined number of accessible protons originating

from the ligand was around 10% lower than the number resulting from the total concentration value. Thus, it may be presumed that coordination occurs due to a dissociable proton of one of the tautomeric forms, most likely the thione form usually predominating in non-basic medium.^[25,26] The dissociation constant of the ligand was confirmed with high accuracy, $pK_a = 5.76 \pm 0.01$. The corresponding protonation constant log $\beta_{011} = pK_a$ is found in Table 2.

Table 2. Protonation constants, cumulative $\beta_{mlh} = [M_m L_l H_h]/[M]^m [L]^l [H]^h$ formation constants and UV/Vis/NIR spectroscopic data in the metal(II) - 5-(pyrazin-2-yl)-2,4-dihydro-3*H*-1,2,4-triazole-3-thione systems. For Co^{II}, Ni^{II} and Zn^{II}: solv. 10/90 (v/v) DMSO/H₂O. I = 0.1 (KNO₃), temp. 25 °C. For Cu^{II}: solv. 40/60 (v/v) DMSO/H₂O. Standard deviations in parentheses refer to random errors only.

Species	$\log(\beta_{mlh})$	$\lambda_{\rm max}$ /nm	$\varepsilon_{\rm max}$ /M ⁻¹ ·cm ⁻¹
L ^{a)}	_	319	3.9×10^{3}
LH	5.76(1)	314	6.6×10^{3}
Cu ²⁺ complexes ^{b)}			
CuL	6.24(2)	770 ^{c)}	1.3×10^{2}
CuL ₂	10.31(2)	1050 ^{c)}	5.6×10^{2}
		≈500 sh ^d	1.0×10^{3}
Co ²⁺ complexes			
CoL	4.62(2)	513 ^c	2.0×10^{2}
		351 ^d	9.9×10^{3}
CoL ₂	8.50(3)	≈508 sh ^{c)}	5.1×10^{2}
-		324^{d}	1.3×10^{4}
Ni ²⁺ complexes			
NiL	5.59(3)	648 ^{c)}	3.2×10^{2}
		694 ^{c)}	3.1×10^{2}
		348 ^{d)}	6.0×10^{3}
Zn ²⁺ complexes			
ZnL	3.98(2)	_	_
ZnL ₂	7.84(1)	_	_
ZnL ₃	10.24(6)	-	-

a) $L = L^-$ deprotonated ligand. b) Results obtained spectrophotometrically. c) d-d transition. d) CT transition.

The potentiometric titrations in the presence of the metals were also based on measurements below pH 7. These titration results indicate the formation of L:M = 1:1 species with Ni^{II}; however, for Co^{II}, the refinements showed higher convergence with the model involving 1:1 and 2:1 species of formation constants presented in Table 2. In the case of Zn^{II}, the calculations, particularly at excess of ligand, indicated also a 3:1 species. The species distribution curves are shown in Figure 2.

Noteworthy is the agreement of the first step formation constants series with the Irving-Williams series ($Cu^{II} > Ni^{II} > Co^{II} > Zn^{II}$).

UV/Vis/NIR Spectroscopy

The spectrophotometric titrations of the ligand (Figure 3) were carried out at concentrations one order of magnitude lower than in the potentiometric experiments as the molar absorbances of the two ligand species (HL and $L = L^{-}$) in the UV reached $10^3-10^4 \text{ m}^{-1} \cdot \text{cm}^{-1}$.

Consequently, the lowered concentrations were also used in presence of the metals Co^{II} and Ni^{II}. By comparison with a similar benzene, 1,2,4-triazole derivative described in reference,^[26] the UV bands of both the ligand forms at 310–320 nm





Figure 2. Species distribution curves as a function of pH in the H⁺/ Metal ion/HL systems in 10/90 (v/v) DMSO/water solutions·L:M = 2:1 for Co^{II}, Ni^{II} and L:M = 4:1 for Zn^{II}.



Figure 3. UV/Vis spectrophotometric titration of ligand HL in an argon atmosphere. Fibre optics dip probe 5 mm (i.e. path length 10 mm). Characteristic pH values shown for corresponding curves. $C_{\rm L} = 1.0 \times 10^{-4}$ M. Solv. 10/90 (v/v) DMSO/water.

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are likely due to $\pi \to \pi^*$ and $n \to \pi^*$ chromophore transitions of the thione group (Figure S1, Supporting Information). The lower wavelength part of the total optical density should be considered as an overall contribution of $\pi \to \pi^*$ transitions in the 1,2,4-triazole ring.

During the spectrophotometric titrations in the presence of Co^{II} and Ni^{II}, the absorbance evidently increased, as compared to the ligand alone, around 400 nm due to the rising contribution of the complex species and their CT bands (Figure 4a for Co and Figure 5a for Ni). The absorbance plotted against pH shows buffer zones within the main range of complex formation. For Co^{II}, the absorbance increases more visibly due to the rising contribution of the CoL₂ species (Figure 4b), whereas for Ni^{II}, the absorbance within the buffer zone is due only to the NiL contribution moderately rising starting from pH 3.5 (Figure 5b). The maximum wavelengths and calculated molar absorbances obtained by HypSpec deconvolution are found in Table 2 (both for the ligand and the complexes with Co^{II} and Ni^{II}). The values for the ligand field d-d transitions in the visible part, which are characteristic of pseudo-octahedral symmetry, are also reported in Table 2. The expected lower d-d transition wavelength of the Ni^{II}-L complex around 390 nm was invisible; it was overlapped by the neighboring, strong CT band.



Figure 4. (a) UV/Vis spectra of the H⁺/Co^{II}/HL system in an argon atmosphere recorded by means of a 5 mm dip probe between pH 3.25 and 8.10 (pH values shown for corresponding curves). (b) Absorbance at 400 nm as a function of pH. $C_{\rm Co} = 1.0 \times 10^{-4}$ M, $C_{\rm L} = 2.0 \times 10^{-4}$ M. Solv. 10/90 (v/v) DMSO/water.

As it follows from the potentiometric results for Cu^{II} , the spectrophotometric investigations had to be carried out as batch file titrations at constant pH in 40/60 (v/v) DMSO/water solutions (Figure 6a). The absorption spectra taken at constant metal concentration and varying L:*M* concentration ratio showed broad low-energy absorption bands moving from 800 to 1050 nm, apparently due to d-d transitions, and finally an



Figure 5. (a) UV/Vis spectra of the H⁺/Ni^{II}/HL system in an argon atmosphere recorded by means of a 5 mm dip probe between pH 3.18 and 8.28 (pH values shown for corresponding curves). (b) Absorbance at 400 nm as a function of pH. $C_{\rm Ni} = 1.0 \times 10^{-4}$ M, $C_{\rm L} = 2.0 \times 10^{-4}$ M. Solv. 10/90 (v/v) DMSO/water.

inflection at 500 nm most likely assigned to a LMCT $\pi N \rightarrow$ Cu transition.^[27,28]



Figure 6. (a) UV/Vis spectra of Cu^{II}/HL recorded at various ligandto-metal concentrations (shown for corresponding curves). pH = 2.9– 3.0. $C_{\rm Cu} = 1.0 \times 10^{-3}$ M. Solvent 40/60 (v/v) DMSO/water: (b) Calculated absorption spectra of the individual complex species: 1 - Cu²⁺ solv.; 2 - CuL; 3 - CuL₂.

The UV/Vis/NIR parameters following from the absorption spectra (Figure 6b) of the two individual complex species CuL and CuL₂, accepted by HypSpec, are shown in Table 2. The presence of a radical red shift accompanied by consecutive predomination of CuL₂ indicates a consecutive change in complex structure from a solvated Cu²⁺ ion to a pseudotetrahederal structure of CuL₂.

EPR Studies

The Co^{II} and Ni^{II} complexes appeared to be EPR silent when the conventional X-band frequency (about 9.6 GHZ) was used at 295 and 77 K. The magnetic measurement of Ni^{II} complex gave magnetic moment per individual molecule of 3.09 B.M at 100 K, which corresponds to a high spin (S = 1) 3d⁸ electron configuration, strongly indicating that the geometry of the coordination sphere is close to tetrahedral.

The EPR spectrum of the powdered complex CuLCl₂ reveals a symmetrical, isotropic line at g = 2.12, the same at 295 K and 77 K (Figure 7, spectrum 1). This spectrum feature is unusual for copper(II) complex, which symmetry of coordination sphere must be at least axial leading to the anisotropic spectrum consisting of two signals at different g tensor components $g_x = g_y \neq g_z$. The symmetrical line can be related to averaging of the anisotropic g tensor due to the intramolecular exchange interaction between spin centers at close positions. The isotropic spectrum agrees with an unresolved spectra arising from distorted tetrahedral environment associated with e.g. Cu^{II} sites [(H₂pyimH)[CuCl₄],^[29] and in CunicX₂ complexes (nic = nicotnic acid, X = Cl or Br).^[30]



Figure 7. The EPR spectra of $CuLCl_2$ measured for powdered sample at 77 K (1) and frozen solution in DMF, at 77 K, (2) which is compared with the spectrum (sim2) simulated using the EPR parameters given in the text. The EPR spectrum (3*) was obtained by subtracting (2) from (3) (see text).

The EPR spectra of frozen solutions of CuLCl₂ in DMF or DMSO show poorly resolved splitting due to hyperfine interaction between the spins of unpaired electron and copper nuclei (I = 3/2); the resolution was improved considerably after adding about 30 vol% of ethyl glycol, which efficiently prevents the aggregation of Cu^{II} ions (Figure 7). It is not possible to assign the parameters, $g_{\parallel} = 2.397$, $g_{\perp} = 2.075$ and $A_{\parallel} = 138 \times 10^{-4}$ cm⁻¹, derived by simulation of the experimental EPR spectrum 2 (Figure 7), to axial local symmetry of the central Cu^{II} ion realized in chelating Cu^{II} *xy* plane through two nitrogen donors of one ligand. The A_{\parallel} values are usually much larger and g_{\parallel} much smaller for the typical in-plane Cu^{II} coordination by two nitrogen donors, provided by e.g. one ethylenediamine with $g_{\parallel} = 2.283$, $g_{\perp} = 2.060$, 183×10^{-4} cm⁻¹.^[31]



Hence, the observed EPR spectral parameters suggest a geometry distorted towards tetrahedral in agreement with those found for the frozen solutions of Cu^{II} dihalide adducts with nicotine acid^[30] or bis(2-benzimidazolyl)ethane^[27] being a model of Cu^{II} blue proteins.^[28] The spectrum 2 is close to that obtained for CuCl₂ in DMF/H₂O or DMSO/H₂O frozen solutions, which is expected considering the coordination of only one ligand and replenishment of the remaining coordination sites by solvent molecules and/or counter anions, together with a distorted arrangement. The results of analysis of d-d electronic transitions strongly support this finding (Figure 6b).

The EPR spectrum 3 (Figure 7) was detected for frozen DMSO/water solution containing 2:1 molar ratio of ligand to Cu(ClO₄). It is composed of two spectra, a dominant is similar to 2 and new one is distinctly seen as additional line at the highest magnetic field. By subtracting the spectrum 2 from 3 the resulting one, 3*, with poorly resolved hyperfine splitting may be simulated approximately using $g_{\parallel} = 2.305$, $g_{\perp} = 2.065$, $A_{\parallel} = 160 \times 10^{-4} \text{ cm}^{-1}$. They differ significantly from those $(g_{\parallel} = 2.207, g_{\perp} = 2.040, A_{\parallel} = 198 \times 10^{-4} \text{ cm}^{-1})$ characteristic of a Cu^{II} complex with two ethylenediamines.^[31] This fact again indicates a geometry close to tetrahedral in the formed CuL₂ species, which is in very good agreement with the significant red shift of the d-d transition to about 1050 nm corresponding to CuL₂ complex (Figure 6b). Such low-energy absorption bands are commonly found in pseudotetrahedral copper(II) complexes.^[27,28]

Conclusions

The results indicate that the title ligand displays a high affinity to the transition metals, Co^{II} , Ni^{II} , Cu^{II} , and Zn^{II} . The chelation effect is confirmed by the obtainment of the corresponding solid complex compounds. The ligand creates neutral, pseudotetrahedral complexes of the general type $M(HL)Cl_2$ with Co^{II} , Ni^{II} , Cu^{II} ions. In the case of the Zn^{II} complex, monodentate binding of 1,2,4-triazole-3-thione to the metal ion and formation of the complex $M(HL)_3Cl_2$ are observed. The L:M = 3:1coordination is also fully accepted in the solution equilibrium model. The ligand's exceptionally high affinity towards Cu^{II} in DMSO/water solutions indicates the necessity for further experimental studies on inhibition of the *o*-diphenolase activity of tyrosinase by the 5-(pyrazin-2-yl)-2,4-dihydro-3*H*-1,2,4-triazole-3-thione.

Experimental Section

Materials and Methods of Product Identification: All substances were used without further purification. Cobalt(II) chloride hexahydrate, nickel(II) chloride hexahydrate, copper(II) chloride dihydrate and zinc(II) chloride anhydrous were purchased from Aldrich. Solvents for synthesis of the complexes (acetonitrile, dichloromethane, diethyl ether and methanol) were reagent grade or better and were dried according to standard protocols.^[33] The melting points were determined using an Electrothermal IA9100 apparatus and they are uncorrected. The IR spectra were recorded as KBr pellets with a FT-IR-8400S Shimadzu spectrophotometer. For the new compounds, elemental analyses (C, H and N) were obtained with a Perkin-Elmer PE 2400 CHNS

analyzer. ESI-MS were recorded with a Varian 500-MS LC ion-trap mass spectrometer and a Bruker, AmaZon Speed ETD. The experiments were performed in positive ion mode.

All reagents and solvents used for synthesis of the ligand were purchased from either Lancaster or Sigma Aldrich and used without further purification. Melting points were determined with an Electrothermal IA9100 apparatus and are presented without corrections. Elemental analyses were performed with an AMZ 851 CHX analyzer and the results were within $\pm 0.4\%$ of the theoretical value. The ¹H NMR spectra were recorded with a Bruker Avance 300 MHz instrument using [D₆]DMSO as a solvent and TMS as an internal standard. IR spectra (v in cm⁻¹) were recorded in KBr with a Specord IR-75 spectrophotometer.

Preparation and Spectroscopic Characterization of Metal(II) Complexes: Ligand 5-(pyrazin-2-yl)-2,4-dihydro-3*H*-1,2,4-triazole-3thione (HL) was synthesized according to the procedure described below. The ligand formed solid complexes **1**–**4** in reactions with cobalt(II), nickel(II), copper(II), and zinc(II) salts. The triazole derivative HL reacted with cobalt(II), nickel(II), and copper(II) chloride hexahydrate in a 1:1 molar ratio in dichloromethane/methanol mixture yielding complexes **1**, **2**, and **3** of general formula [*M*LCl₂]. The ligand and anhydrous zinc(II) chloride in acetonitrile/methanol solution formed complex **4** of [*M*(HL)₃Cl₂] type, irrespective of a L:*M* molar ratio (1:1 or 2:1), in which the reaction proceeded.

Preparation of 5-(Pyrazin-2-yl)-1,2,4-triazole-5-thione (HL): 4-Benzoyl-1-(pyrazin-2-ylcarbonyl)-thiosemicarbazide (0.01 mol) was dissolved in 2% sodium hydroxide solution (10 mL) and the mixture was heated under reflux for 4 h. After cooling, the solution was neutralized with dilute hydrochloric acid. The precipitate was filtered off and then crystallized from 95% ethanol. The melting point of the obtained compound is known to be 278–280 °C,^[34] however, our present result was 282–285 °C. Yield: 89%. ¹H NMR ([D₆]DMSO): $\delta = 8.78$ (s, 2 H, 2 × CH); 9.20 (s, 1 H, 1 × CH); 13.96 (s, 1 H, NH); 14.17 (s, 1 H, NH) ppm. **IR** (KBr): $\tilde{v} = 3294$, 3066, 2924, 2850, 2762, 2343, 1641, 1575, 1489, 1216, 1172, 1135, 1103, 1020, 973, 855, 756, 707, 583, 551, 411 cm⁻¹.

Synthesis of Complex 1: Cobalt(II) chloride hexahydrate CoCl₂·6H₂O (43.0 mg, 0.18 mmol) was dissolved in methanol (2.0 mL) and was added dropwise at room temperature to a stirred solution of HL (32.4 mg, 0.18 mmol) in dichloromethane (8.0 mL). The reaction mixture was stirred for 12 h at room temperature. The resulting green powder of **1** was obtained by evaporation under reduced pressure to dryness. Yield: 47.4 mg (74%). M.p. 306.7–307.3 °C. C₆H₅N₅SCoCl₂·2·5H₂O (354.02 g·mol⁻¹): calcd. C 20.36; H 2.85; N 19.78%; found: C 20.19; H 2.51; N 19.98%. **FT-IR** (KBr): $\tilde{v} = 3390$, 3373, 2359, 2330, 1616, 1550, 1489, 1288, 1157, 1060, 856, 761 cm⁻¹. ESI-**MS** (*m*/*z*): [Co(HL)Cl₂ + 2H] 310 (100%), [Co(HL)Cl]⁺ 273 (10%), [Co(HL)]⁺ 240 (70%).

Synthesis of Complex 2: Nickel(II) chloride hexahydrate NiCl₂·6H₂O (51.2 mg, 0.22 mmol) was dissolved in methanol (2.0 mL) and was added dropwise at room temperature to a stirred solution of HL (39.2 mg, 0.22 mmol) in dichloromethane (12.0 mL). The reaction mixture was stirred for 12 h at room temperature. A yellow-orange solid product was obtained, filtered off and dried in vacuo. Yield: 30.0 mg (41%). M.p. dec. > 320 °C. C₆H₅N₅SNiCl₂·1·75H₂O (335.77 g·mol⁻¹): calcd. C 21.46; H 2.40; N 20.86%; found: C 21.39; H 2.68; N 20.33%. **FT-IR** (KBr): $\bar{\nu} = 3392$, 3377, 2359, 2322, 1616, 1550, 1489, 1288, 1159, 1062, 852, 763 cm⁻¹. ESI-MS (*m*/*z*): [Ni(HL)Cl₂ + 1H] 309 (80%), [Ni(HL)]⁺ 238 (100%).

Synthesis of Complex 3: Copper(II) chloride dihydrate CuCl₂·2H₂O (59.8 mg, 0.35 mmol) was dissolved in methanol (2.0 mL) and was added dropwise at room temperature to a stirred solution of HL (62.8 mg, 0.135 mmol) in dichloromethane (15 mL). The reaction mixture was stirred for 12 h at room temperature. A green-brown solid product **3** was obtained, filtered off and dried in vacuo. Yield: 52.7 mg (48%). M.p. 262.3–262.8 °C. C₆H₅N₅SCuCl₂·CH₂Cl₂ (398.53 g·mol⁻¹): calcd. C 21.10; H 1.77; N 17.57%; found: C 20.79; H 1.85; N 17.74%. **FT-IR** (KBr): $\tilde{v} = 3431$, 3397, 3096, 2357, 1618, 1496, 1421, 1384, 1286, 1155, 1047, 856, 756 cm⁻¹. ESI-MS (*m/z*): [Cu(HL)Cl₂–1H] 312 (15%), [Cu(HL)]⁺ 242 (7%), [L] 179 (40%).

Synthesis of Complex 4: Zinc(II) chloride anhydrous ZnCl₂ (54.52 mg, 0.40 mmol) was suspended in methanol (10 mL) and was added dropwise at room temperature to a stirred solution of HL (71.68 mg, 0.40 mmol) in acetonitrile (12.0 mL). The reaction mixture was stirred and refluxed for 6 h. A yellow micro-crystalline product was obtained, filtered off and dried. Yield: 61.3 mg (62%). M.p. 299.0-302.0 °C. $C_{18}H_{15}N_{15}S_3ZnCl_2\cdot0\cdot5H_2O$ (682.76 g·mol⁻¹): calcd. C 31.67; H 2.36; N 30.77 %; found: C 31.91; H 1.88; N 30.52 %. FT-**IR** (KBr): \tilde{v} = 3292, 3211, 3070, 2762, 1575, 1521, 1489, 1384, 1294, 1170, 1051, 889, 856, 756 cm⁻¹. ¹H NMR (300 MHz, [D₆]DMSO): δ = 1.22 (s, 1 H, SH); 2.16 (s, 3 H, CH₃ (Ar); 8.75 (s, 2 H, Ar); 9.18 (s, 1 H, Ar); 13.90 (s, 1 H, NH); 14.12 (s, 1 H, NH) ppm. ESI-MS (m/z): $[Zn(HL)_3Cl]^+$ 638 (20%), $[Zn(HL)_3]^+$ 600 (20%), [Zn(HL)₂Cl]⁺ 459 (30%), [Zn(HL)₂]⁺ 423 (22%), [Zn(HL)]⁺ 242 (100%), [L + 1H] 180 (40%).

Materials and Physical Measurements: All reagents and solvents were purchased from commercial sources and were used as received. The standard solutions of cobalt(II), nickel(II), and copper(II) salts were titrated with disodium salt of EDTA in the presence of murexide. In turn, the standard solution of $Zn(NO_3)_2$ was also determined with EDTA but in the presence of eriochrome black (EBT).

Potentiometric Measurements: pH-Potentiometric equilibrium studies were carried out with a Molspin automatic titration kit equipped with a combined Russell CMAWL/4/5/S7 microelectrode. All the experiments were carried out at a constant temperature of 25.0 ± 0.1 °C. The ligand (insoluble in water) was initially dissolved in of DMSO (1 mL) and added to a 10 mL flask filled with an aqueous solution containing HNO₃ in excess, in regards to the ligand, and KNO₃. Water solutions of the cobalt(II), nickel(II), and zinc(II) nitrates were added to the acidified ligand solution and next titrated with alkali solution. The ionic strength of the final 10/90 (v/v) DMSO/water solution was 0.1 (KNO₃). The addition of copper(II) nitrate to the same ligand solution gave a brown precipitate. A transparent brown solution was obtained only by using a higher share of DMSO in the solvent mixture, optimal at 40/60 (v/v) DMSO/water, however further titration with alkali led to the formation of insoluble hydrolytic products.

The protonation and formation constants were determined by pH-metric titrations of 4.0 mL samples. Alkali (0.1 M NaOH carbonate-free) was added by a 0.500 mL calibrated micro syringe. The measurement cell was calibrated daily with 0.1 M NaOH vs. –log[H⁺] by titration of 0.005 M HNO₃ in 10/90(v/v) DMSO/water solution (containing KNO₃ up to *I* = 0.1). The calculated ionic product for this solution was 14.06 (close to the value 14.04 reported in^[35]), but the ionic product in 40/ 60(v/v) DMSO/water solution amounted to 14.64, quite close to the literature data under similar conditions: 14.98,^[36] 14.42.^[37] The overall concentration formation constants ($\beta_{mLh} = [M_m L_l H_h]/[M]^m [L]^l [H]^h$) were initially calculated by SUPERQUAD^[38] and then by HYPER-QUAD 2013.^[39] The graphical simulation of speciation diagrams on the basis of these determined constants was carried out by HySS 2009.^[40] The total concentration of the ligand in each sample amounted to 4.0×10^{-3} M. The metal–ligand interaction was studied at ligand-to-metal ratios 1:1, 2:1 and 4:1.

UV/Vis/NIR Spectroscopy: For spectrophotometric investigation of the equilibrium systems simultaneously with the potentiometric measurements, the UV/Vis absorption spectra were recorded with a Cary 50 Bio spectrophotometer equipped with a fiber-optic device, in an oxygen-free atmosphere (argon). A fiber-optic probe (5 mm), corresponding to a path length of 1 cm, was dipped directly into the titration vessel (initial volume 25.0 mL, total cobalt concentration 5.0×10^{-5} or 1.0×10^{-4} M, total nickel concentration 1.0×10^{-4} or 2.0×10^{-4} M). For optimum absorbance measurements, the ligand:metal molar ratio was 2:1, and the temperature was 25.0 ± 0.1 °C. To avoid interference from the absorption by the nitrate ion at ca. 300 nm, the UV experiments were carried out with perchlorates (basic electrolyte NaClO₄). Perchlorate salts are potentially explosive and were handled only in small quantities with care. The ionic strength in aqueous phase was 0.5, i.e. higher than in the solely potentiometric measurements, to avoid undesired dilution of samples during titration with 0.1 M NaOH. The used alkali (0.5 M NaOH carbonate-free) was added from a 0.250 mL calibrated micro syringe. After each base increment added, the pH and EMF were recorded with a Molspin titrator with a combined InLab Semi-Micro (METTLER TOLEDO) electrode. The reference half-cell was filled with a polymer XEROLYT®EXTRA electrolyte instead of KCl due to the insolubility of KClO₄. A time delay was given to equilibrate the system. After each point or set of points, the process was paused and the spectrum was recorded at a slow scan (300 nm·min⁻¹). Following the collection of a consecutive curve, new aliquots of base were added and the procedure repeated. It was therefore possible to collect a spectrum at each titration point or at each chosen pH. The molar absorbances of species were calculated after deconvolution by HypSpec (part of Hyperquad 2008 suite, Protonic Software). In the spectrophotometric batch file titrations of the equilibrium Cu^{II}-HL system, the UV/Vis/NIR absorption spectra were read with a Varian Cary 5000 spectrophotometer in sealed 1 cm quartz cuvettes and 10.0 mL flasks for a series of solutions of known concentration.

EPR Spectroscopy and Magnetic Measurement: The EPR spectra were measured with a Bruker ELEXYS E500 spectrometer equipped with an NMR teslameter (ER 036TM) and frequency counter at X-band. The experimental spectra were analyzed using the programs WINEPR SimFonia, version 1.25 and DoubletExact (S = 1/2) written by Dr Andrew Ozarowski from NHMFL, University of Florida, with resonance field calculated by full diagonalization of energy matrix. A magnetic susceptibility measurement at temperature 100 K was performed at a magnetic field of 0.5 T with a Quantum Design SQUID MPMSXL-5 magnetometer. Correction for the sample holder and the diamagnetic corrections to the magnetic susceptibility, which were estimated from the Pascal constants, were applied.

Supporting Information (see footnote on the first page of this article): UV/Vis/NIR studies are presented in Figure S1. ¹H NMR spectra are presented in Figures S2–S4.

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Metal(II) Ion Complexes with 5-(Pyrazin-2-yl)-2,4-dihydro-3*H*-1,2,4-triazole-3-thione; Synthesis, Structural Characterization, Acid-base, and Complexing Properties in Solution

