

## SUPPORTING INFORMATION

### Synthesis, structural, DFT and cytotoxicity studies of Cu(II) and Ni(II) complexes with 3-aminopyrazole derivatives\*

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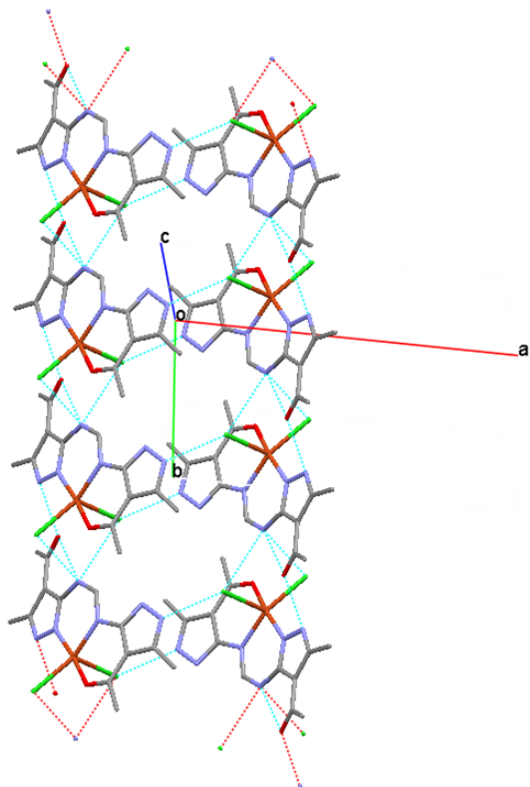
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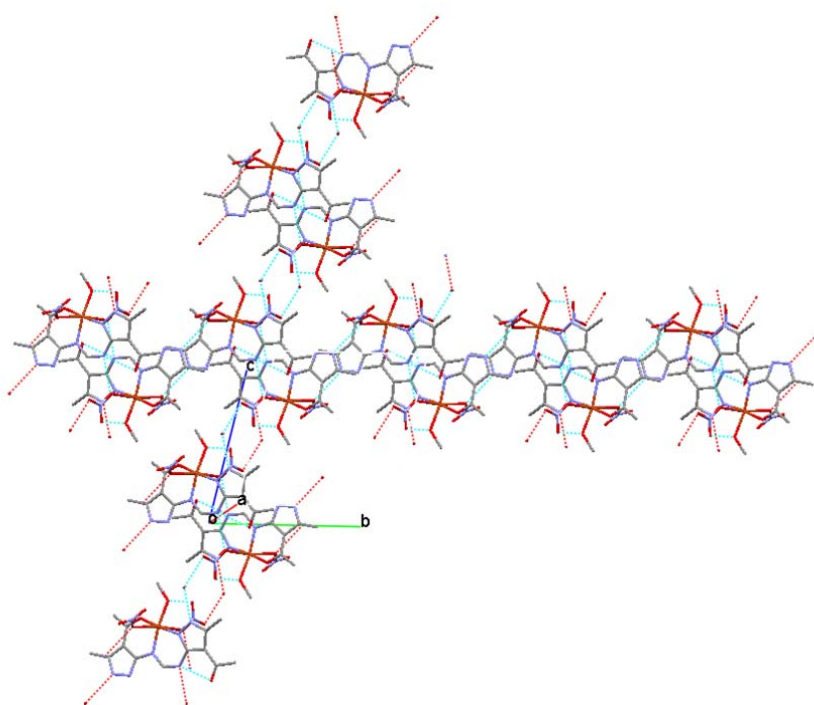
\* Transition metal complexes with pyrazole-based ligands. Part 30.

**Table 1. Intermolecular interactions in structures [Cu(*ampf*)Cl<sub>2</sub>], 1, [Cu(*ampf*)MeOH(NO<sub>3</sub>)<sub>2</sub>]MeOH, 2 and [Ni(*ampf*)(MeOH)<sub>2</sub>NO<sub>3</sub>]NO<sub>3</sub>, 3.**

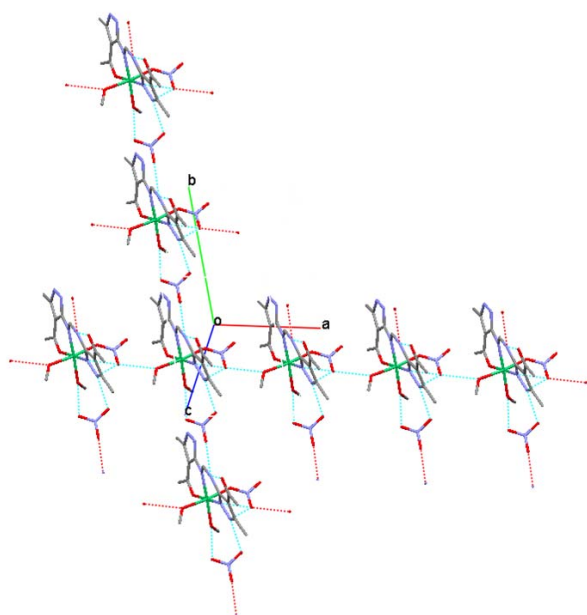
	D-H...A	Type	D-H [Å]	H...A [Å]	D...A [Å]	D-H...A [°]	Symmetry operation
<b>1</b>	N6-H6...O42	intra	0.88	2.20	2.828(5)	127	
	C7-H7...N10	intra	0.95	2.23	2.637(6)	105	
	N2-H2...Cl1	intra	0.88	2.55	3.036(4)	115	
	N11-H11...Cl2	dimer	0.88	2.26	3.131(4)	171	$-x, -y, -z$
	C121-H12A...Cl2	dimer	0.98	2.68	3.654(4)	173	$x, -1/2-y, 1/2+z$
	N2-H2...O42	chain <i>b</i>	0.88	2.45	2.973(4)	119	$x, -1+y, z$
	N6-H6...Cl2	chain <i>b</i>	0.88	2.57	3.218(4)	131	$x, 1+y, z$
	C7-H7...Cl2	chain <i>b</i>	0.95	2.67	3.284(4)	123	$x, 1+y, z$
	C43-H43C...Cl1	intercolumn	0.98	2.71	3.505(5)	138	$1-x, -y, -z$
<b>2</b>	N6-H6...O42	intra	0.86	2.13	2.759(3)	129	
	C7-H7...N10	intra	0.93	2.27	2.667(4)	105	
	N6-H6...O501	dimer	0.86	2.22	2.900(3)	136	$1-x, -y, 2-z$
	N11-H11...O401	chain <i>b</i>	0.86	2.06	2.900(4)	164	$1-x, 1-y, 2-z$
	N11-H11...O403	chain <i>b</i>	0.86	2.51	3.225(4)	141	$1-x, 1-y, 2-z$
	O301-H301...O503	chain <i>c</i>	0.82	2.50	3.159(4)	138	
	O301-H301...O504	chain <i>c</i>	0.82	2.00	2.794(4)	164	
	N2-H2...O301	chain <i>c</i>	0.86	1.96	2.769(3)	156	$1-x, -y, 1-z$
	C121-H12C...O403	intersheet	0.96	2.41	3.339(5)	163	$1+x, y, z$
<b>3</b>	N6-H6...O42	intra	0.88	2.11	2.755(2)	130	
	C7-H7...N10	intra	0.95	2.27	2.665(3)	104	
	N2-H2...O501	in asym unit	0.88	1.98	2.845(4)	167	
	O201-H201...O503	in asym unit	0.84	1.86	2.692(3)	168	
	O301-H301...O404	chain <i>a</i>	0.84	1.99	2.784(2)	157	$-1+x, y, z$
	N6-H6...O504	chain <i>b</i>	0.88	2.21	2.896(3)	135	$x, 1+y, z$
	C7-H7...O504	chain <i>b</i>	0.95	2.55	3.082(3)	115	$x, 1+y, z$
	N11-H11...O401	dimer	0.88	2.30	3.093(3)	151	$2-x, 1-y, 1-z$
	N11-H11...O403	dimer	0.88	2.30	3.074(3)	148	$2-x, 1-y, 1-z$



**Fig. 1a.** Packing arrangement in crystal 1 [1].



**Fig. 1b.** Packing arrangement in crystal 2 [1].



**Fig. 1c.** Packing arrangement in crystal **3** [1].

### Computed geometry and vibrational frequencies

The computed metal-ligand distances are compiled in Table 2. The data reflect the main geometrical differences between the crystal structure obtained from the X-ray study and the isolated complex from the calculations. The agreement is fairly good for the ligand bond lengths. This implies the reliability of the computed IR data of *ampf*, corresponding to most of the bands in the IR spectrum. The agreement is worse for the metal-ligand distances. The M–N1 and M–O15 distances are still in fair agreement, but the M–N8 bonds and the bonds with the NO<sub>3</sub><sup>−</sup> and MeOH ligands deviate up to 0.3 Å. The main reason of the deviations is that the computed geometrical data refer to the isolated complexes, therefore cannot take into account the crystal packing effects and hydrogen bonding interactions between neighboring complexes. The anions are involved in strong hydrogen bonding interactions with the neighboring complex molecules in the crystal. Therefore they occupy an intermediate position between two complexes. In the isolated molecule model of the computations, there is only one complex these ligands can coordinate to, so they can bind more strongly to the metal (by donor-acceptor interactions) and to the other ligands (by hydrogen bonding interactions) resulting in smaller distances.

**Table 2.** Comparison of selected experimental and computed bond distances (Å).

	1		2		3	
	Calculated	X-ray	Calculated	X-ray	Calculated	X-ray
N1=C5	1.318	1.322(5)	1.328	1.332(4)	1.325	1.317(3)
C5–N6	1.372	1.367(5)	1.383	1.372(4)	1.387	1.382(3)
N6–C7	1.345	1.341(5)	1.354	1.340(4)	1.346	1.338(3)
C7=N8	1.302	1.301(5)	1.295	1.302(4)	1.303	1.289(3)
N8–C9	1.413	1.414(4)	1.384	1.412(4)	1.398	1.411(3)
C14=O15	1.254	1.252(4)	1.250	1.247(4)	1.240	1.240(3)
M–N1	2.015	1.962(3)	2.000	1.962(3)	2.074	2.016(2)
M–N8	2.312	2.008(3)	2.252	1.978(3)	2.131	2.050(2)
M–O15	1.983	1.973(2)	1.972	1.955(2)	2.090	2.020(1)
Cu–Cl <sub>eq</sub>	2.381	2.288(1)				
Cu–Cl <sub>ax</sub>	2.354	2.543(1)				
M–O401			2.092	2.381(3)	2.011	2.111(8)
M–O301 <sub>complex3</sub>					2.233	2.089(2)

M–O501 <sub>complex2</sub>	2.058	2.539(3)		
M–O201	2.439	1.974(3)	2.084	2.065(2)

The full list of the IR absorption bands and their assignment are given in Table 3 while the spectra are shown in Figure 2. The assignment of the absorption bands was aided by the computed vibrational frequencies and IR intensities, while the computed normal modes were visualized by the GaussView 4.1 program [2].

The most notable feature is the high frequency of the stretchings of the formally CN and NN single bonds which can be attributed to the partial double-bond character of these bonds due to conjugation of the neighboring  $\pi$  bonds and the lone pairs of nitrogens. These bands appear at very close wavenumber values in the spectra of the three complexes, in agreement with the identical donor-acceptor interaction of the *ampf* ligand with the metals. The most important information gained from the far-IR range are the metal-ligand vibrations, which correlate well with values published for related complexes in Ref. [8].

**Table 3. Characteristic bands<sup>A</sup> of the FT-IR spectra of 1, 2 and 3 and their assignments on the basis of the DFT calculations**

Experimental	Calculated	Intensity	Assignment <sup>B</sup>
[Cu( <i>ampf</i> )Cl <sub>2</sub> ] (1)			
3241s	3657	112	$\nu$ NH
3151m	3477	383	$\nu$ NH
3126m	3411	366	$\nu$ NH
3074m	3171	7	$\nu_{as}$ CH <sub>3</sub>
3040m	3152	8	$\nu$ CH
3020m	3145	7	$\nu_{as}$ CH <sub>3</sub>
3000m	3132	12	$\nu_{as}$ CH <sub>3</sub>
2967m	3118	5	$\nu_{as}$ CH <sub>3</sub>
2919m	3061	21	$\nu_s$ CH <sub>3</sub>
2860m	3058	2	$\nu_s$ CH <sub>3</sub>
1641s	1700	753	$\nu$ C=O
1605s	1681	184	$\nu$ C=N
1578s	1633	362	$\nu$ ring, $\beta$ N6H, $\nu$ C=O
1548s	1606	483	$\nu$ C7–N6
1529m	1587	45	$\nu$ ring
1504s	1571	119	$\nu$ ring, $\beta$ NH <sub>ring</sub>
1466s	1496	464	$\nu$ ring, $\delta_{as}$ CH <sub>3</sub>
1440m	1497	30	$\delta_{as}$ CH <sub>3</sub>
1412s	1468	149	$\delta_{as}$ CH <sub>3</sub> ,
1355s	1397	18	$\delta_{as}$ CH <sub>3</sub> , $\beta$ CH, $\beta$ NH
1312m	1339	88	$\nu$ ring
1212m	1260	50	$\nu$ N–N
1159m	1218	41	$\nu$ N–N, $\nu$ C7–N6
1096s	1118	58	$\nu$ ring, $\nu$ C–CH <sub>3</sub>
1036w	1061	5	$\delta_r$ CH <sub>3</sub>
1005w	1044	2	$\delta_r$ CH <sub>3</sub>
973m	981	46	$\nu$ C–CH <sub>3</sub>
964m	969	48	$\nu$ C–CH <sub>3</sub>
913m	924	16	$\delta$ ring, $\beta$ N–CH–NH
799s	786	26	$\gamma$ N6H
773m	770	32	$\tau$ ring, $\gamma$ N6H
743m	768	26	$\gamma$ N6H, $\tau$ ring
732m	748	40	$\gamma$ NH <sub>ring</sub>
712s	727	32	$\gamma$ NH <sub>ring</sub>
666m	668	35	$\gamma$ NH <sub>ring</sub>
587w	583	15	$\delta$ ring

575w	562	40	$\gamma\text{NH}_{\text{ring}}$ , $\tau\text{ring}$
449w	452	5	$\tau\text{ring}$ , $\gamma\text{C}_{\text{ring}}-\text{N}$
343w	368	13	$\nu\text{Cu}-\text{N}8$ , $\nu\text{Cu}-\text{O}15$ , $\beta\text{C}-\text{C}-\text{CH}_3$
302sh	314	13	$\gamma\text{C}-\text{N}8$
273m	283	51	$\nu\text{Cu}-\text{Cl}$
233w	222	15	$\nu\text{Cu}-\text{Cl}$
172m	178	2	$\beta\text{N}8-\text{Cu}-\text{O}15$
140m	146	9	$\beta\text{N}1-\text{Cu}-\text{N}8$
106w	121	17	$\gamma\text{CuCl}_2$
95sh	100	9	$\tau\text{CH}_3$
70sh	62	11	$\beta\text{Cl}-\text{Cu}-\text{Cl}$

[Cu(ampf)(MeOH)(NO<sub>3</sub>)<sub>2</sub>]MeOH (2)

3485s	3582	714	$\nu\text{NH}$ , $\nu\text{OH}$
3229s	3417	542	$\nu\text{NH}$ , $\nu\text{OH}$
3089sh	3107	56	$\nu_{\text{as}}\text{CH}_3$
3042sh	3152	8	$\nu\text{CH}$
2973sh	2995	107	$\nu_{\text{s}}\text{CH}_3$
1643s	1721	200	$\nu\text{C}=\text{O}42$
1610s	1688	902	$\nu\text{C}=\text{N}8$
1601s	1663	296	$\nu\text{C}=\text{O}15$
1580sh	1636	445	$\nu\text{ring}$ , $\beta\text{N}6\text{H}$ , $\nu\text{C}=\text{O}$
1552s	1598	428	$\nu\text{C}7-\text{N}6$
1529w	1584	124	$\nu\text{ring}$
1504s	1576	352	$\nu_{\text{as}}\text{NO}_3$ , $\beta\text{OH}$
1467s	1543	75	$\nu\text{ring}$
1453m	1506	212	$\nu\text{C}5-\text{N}6$ , $\delta_{\text{as}}\text{CH}_3$
1413m	1492	238	$\nu\text{C}4-\text{C}41$ , $\delta_{\text{as}}\text{CH}_3$
1384vs	1339	541	$\nu_{\text{as}}\text{NO}_3$
1354sh	1406	17	$\nu\text{ring}$
1330sh	1388	22	$\beta\text{C}7\text{H}$
1207m	1255	48	$\nu\text{N}-\text{N}$ , $\nu\text{C}9-\text{N}8$
1159w	1220	16	$\nu\text{N}-\text{N}$ , $\nu\text{C}9-\text{N}8$
1100m	1124	47	$\nu\text{ring}$ , $\nu\text{C}-\text{CH}_3$
1063w	1076	99	$\nu_{\text{s}}\text{NO}_3$ , $\delta_{\text{r}}\text{CH}_3$
1042w	1065	91	$\nu\text{C}-\text{O}$ , $\nu_{\text{s}}\text{NO}_3$ , $\delta_{\text{r}}\text{CH}_3$
1007w	1054	59	$\nu\text{C}-\text{O}$ , $\nu_{\text{s}}\text{NO}_3$ , $\delta_{\text{r}}\text{CH}_3$
975m	989	57	$\nu\text{C}-\text{CH}_3$
914m	926	15	$\beta\text{NH}-\text{CH}-\text{N}$ , $\delta\text{ring}$ , $\nu\text{C}-\text{CH}_3$
850-650vbr	949	207	$\tau\text{OH}$
839w	821	71	$\delta\text{NO}_3$ , $\gamma\text{NH}$
794m	792	18	$\gamma\text{NH}_{\text{ring}}$ , $\delta\text{ring}$ , $\nu\text{C}9-\text{N}8$
773w	778	17	$\beta\text{C}5-\text{N}6-\text{C}7$
703w	746	17	$\tau\text{OH}$ , $\delta\text{NO}_3$ , $\delta\text{ring}$
671m	676	31	$\beta\text{C}-\text{C}=\text{O}$
589m	597	26	$\delta\text{ring}$
581m	572	28	$\gamma\text{NH}_{\text{ring}}$
451m	455	5	$\tau\text{ring}$ , $\gamma\text{C}5-\text{N}6$
340w	374	16	$\nu\text{Cu}-\text{O}$ , $\nu\text{Cu}-\text{N}8$
303w	328	11	$\nu\text{Cu}-\text{N}8$ , $\gamma\text{C}7\text{H}$
279w	308	42	$\nu\text{Cu}-\text{O}_{\text{NO}3}$
253	258	10	$\nu\text{Cu}-\text{O}_{\text{NO}3}$
202m	224	15	$\beta\text{O}-\text{Cu}-\text{O}$ , $\text{twNO}_3$
188	188	9	$\beta\text{N}8-\text{Cu}-\text{O}$ , $\tau\text{CH}_3$
128m	131	6	$\gamma\text{CuO}-\text{CH}_3$

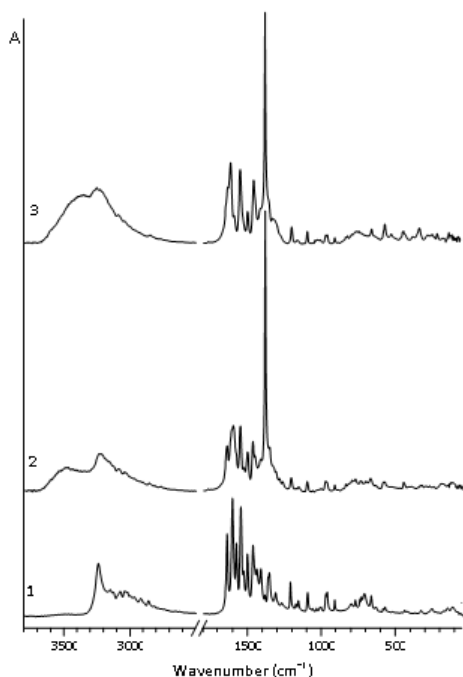
97w	98	12	$\beta\text{O}-\text{Cu}-\text{O}$ , $\tau\text{CH}_3$
56w	52	3	$\text{twNO}_3$

[Ni(ampf)(MeOH)<sub>2</sub>(NO<sub>3</sub>)]NO<sub>3</sub> (3)

3355s,br	3385	509	$\nu\text{NH}$
3254s,br	3010	1901	$\nu\text{NH}$ , $\nu\text{OH}$
3086sh	3172	3	$\nu\text{CH}$
3043sh	3122	29	$\nu_{\text{as}}\text{CH}_3$
3007sh	3057	186	$\nu_{\text{s}}\text{CH}_3$
1650sh	1714	285	$\nu\text{C}=\text{O}42$
1637sh	1707	496	$\nu\text{C}=\text{O}15$
1617s	1677	639	$\nu\text{C}=\text{N}8$
1594sh	1645	127	$\nu\text{ring}$ , $\beta\text{N}6\text{H}$ , $\nu\text{C}=\text{O}$
1553s	1616	509	$\nu\text{C}7-\text{N}6$
1502m	1556	81	$\beta\text{OH}$ , $\nu\text{N}-\text{O}$
1461s	1497	489	$\nu\text{C}4-\text{C}41$ , $\delta_{\text{as}}\text{CH}_3$
1416m	1469	81	$\delta_{\text{as}}\text{CH}_3$
1384s	1365	474	$\nu_{\text{as}}\text{NO}_3$
1359sh	1398	51	$\beta\text{C}7\text{H}$ , $\delta_{\text{s}}\text{CH}_3$
1280w	1311	72	$\nu\text{C}5-\text{N}6$ , $\nu\text{ring}$ , $\beta\text{N}6\text{H}$
1204m	1254	65	$\nu\text{C}9-\text{N}8$ , $\nu\text{N}-\text{N}$
1164w	1214	55	$\nu\text{N}-\text{N}$ , $\nu\text{C}9-\text{N}8$
1098m	1119	62	$\nu\text{ring}$ , $\nu\text{C}-\text{CH}_3$
1045w	1074	69	$\nu_{\text{s}}\text{NO}_3$
1031w	1061	86	$\nu\text{C}-\text{O}_{\text{MeOH}}$
1023w	1055	63	$\nu_{\text{s}}\text{NO}_3$ , $\nu\text{C}-\text{O}_{\text{MeOH}}$
1010w	1036	113	$\nu\text{C}-\text{O}_{\text{MeOH}}$
973w	982	121	$\nu\text{C}-\text{CH}_3$ , $\delta\text{CH}_3$ , $\gamma\text{NH}_{\text{ring}}$ , $\beta\text{NH}-\text{CH}-\text{N}$
962w	976	127	$\gamma\text{NH}_{\text{ring}}$
913w	924	45	$\beta\text{N}-\text{C}-\text{N}$ , $\delta\text{ring}$
870-550vbr	1021	20	$\tau\text{OH}$
839w	843	66	$\gamma\text{N}6\text{H}$
825w	824	33	$\gamma\text{NO}_3$
795w	794	21	$\gamma\text{N}6\text{H}$ , $\delta\text{ring}$ , $\nu\text{C}5-\text{N}6$
768w	777	15	$\beta\text{N}-\text{C}-\text{N}$
663m	663	36	$\beta\text{C}-\text{C}=\text{O}$
582sh	611	217	$\tau\text{OH}_{\text{axMeOH}}$
574w	590	17	$\delta\text{ring}$
529w	567	36	$\gamma\text{NH}_{\text{ring}}$ , $\gamma\text{C}-\text{C}=\text{O}$
444w	455	11	$\tau\text{ring}$ , $\gamma\text{C}5-\text{N}6$
381w	399	24	$\nu\text{Ni}-\text{O}_{\text{eqMeOH}}$
333m	327	19	$\nu\text{Ni}-\text{O}_{\text{axMeOH}}$ , $\nu\text{Ni}-\text{O}_{\text{NO}_3}$ , $\nu\text{Ni}-\text{N}8$
286w	309	27	$\nu\text{Ni}-\text{O}_{\text{axMeOH}}$ , $\nu\text{Ni}-\text{O}_{\text{NO}_3}$
274w	287	37	$\nu\text{Ni}-\text{O}_{\text{axMeOH}}$ , $\nu\text{Ni}-\text{O}_{\text{NO}_3}$
244w	255	27	$\nu\text{Ni}-\text{N}8$
210m	218	18	$\nu\text{Ni}-\text{N}8$
184w	189	11	$\beta\text{N}8-\text{Ni}-\text{O}_{\text{eqMeOH}}$ , $\tau\text{CH}_3$
129m	129	2	$\tau\text{CH}_3$ , $\gamma\text{NiO}-\text{CH}_3(\text{axMeOH})$
97m	93	4	$\gamma\text{C}_{\text{ring}}-\text{CH}_3$ , $\beta\text{N}-\text{Ni}-\text{O}_{\text{eqMeOH}}$
70m	64	2	$\text{twNO}_3$

<sup>A</sup> In  $\text{cm}^{-1}$ . The abbreviations vs, s, sh, m, w, br mean very strong, strong, shoulder, medium, weak and broad, respectively.

<sup>B</sup> Major contributions deduced from visual observation of the computed vibrations. The abbreviations M, as, v,  $\delta$ ,  $\beta$ , tw,  $\gamma$ ,  $\tau$  mean metal (Cu or Ni), asymmetric, stretch, deformation, bend, twist, wag and torsion, respectively.



**Fig. 2.** FT-IR spectra of **1**, **2** and **3**.

The mid-IR range consists of the ligand vibrations, from which the asymmetric stretching of the  $\text{NO}_3^-$  group (in **2** and **3**) gives rise to the highest-intense band at around  $1380\text{ cm}^{-1}$ . The *ampf* ligand provides several strong bands in this region due to CO, CN, NN, NO stretching, and OH and NH bending vibrations, in agreement with the identical donor-acceptor interaction of the *ampf* ligand with the metals.

**Table 4.** Additional natural atomic charges<sup>A</sup> and second-order perturbation energies<sup>B</sup>

		Atomic charges		
Fragment	Atom	1	2	3
$\text{Cl}^-$	Cleq	-0.588		
	Clax	-0.573		
$\text{NO}_3^-$	O501		-0.517	
	O401		-0.544	-0.573
MeOH	O201		-0.810	-0.806
	O301			-0.749

**Charge transfers to the metals**

**$\text{Cl}^- \rightarrow \text{M}$**

$E^{(2)} \text{LP}(\text{Cleq}) \rightarrow \text{M}$	302.3
$E^{(2)} \text{LP}(\text{Clax}) \rightarrow \text{M}$	360.4
$\Sigma E^{(2)} \text{LP}(\text{Cl}) \rightarrow \text{M}$	662.7

**$\text{NO}_3^- \rightarrow \text{M}$**

$E^{(2)} \text{LP}(\text{O401}) \rightarrow \text{M}$	210.7	222.4
$E^{(2)} \text{LP}(\text{O501}) \rightarrow \text{M}$	214.2	



$\Sigma E^{(2)} \text{LP(O)} \rightarrow \text{M}$	424.8	222.4
<b>MeOH <math>\rightarrow</math> M</b>		
$E^{(2)} \text{LP(O201)} \rightarrow \text{M}$	70.1	153.4
$E^{(2)} \text{LP(O301)} \rightarrow \text{M}$		107.1
$\Sigma E^{(2)} \text{LP(O)} \rightarrow \text{M}$	70.1	260.5

<sup>A</sup> The atomic charges are given in electrons.

<sup>B</sup> M means the metal (Cu or Ni) in the complexes. Second-order perturbation energies ( $E^{(2)}$  donor  $\rightarrow$  acceptor, kJ/mol). The abbreviation LP means the lone pairs of the atoms. Items starting with  $\Sigma$  represent the summarized quantities for the species. For the numbering of atoms see Figures 1a – c in the main part of the paper.

### Electronic spectra

Spectra of the complexes were recorded in MeOH in the spectral range 220 – 1400 nm. The complexes **2** and **3** are readily soluble at 1 mmol/dm<sup>3</sup> concentration level, but not **1**. In order to improve that, the complex **1** was mildly heated (to about 40 °C) for app. 15 minutes. The color of solutions of Cu(II) complexes was green and that of the Ni(II) compound was very light blue.

The spectra of Cu(II) complexes are, generally, similar. In the  $\lambda$  range of 220-400 nm three well resolved absorption bands appear. The bands at ~233 nm and ~266 nm, which are present also in the spectrum of the starting ligand *aamp*, belong to  $\pi \rightarrow \pi^*$  transitions (pyrazolyl-based). A slight blue shift of the bands in the spectra of **1** and **2** was observed. This was not the case with [Co(*aamp*)(MeOH)<sub>2</sub>NO<sub>3</sub>]NO<sub>3</sub> complex [3] where the bands in the ligand absorption range are considerably red-shifted and molar absorptivities several times less intense than those of *aamp*. These facts reflect a greater influence of the Co(II) on the coordinated ligand in the complex.

The third band, appearing at about 320 nm for both Cu(II) complexes, can be ascribed to LMCT. Since this band is rather broad and intense, it seems reasonable to assume that it is composed of two close absorptions, probably of  $\pi_1, \pi_2 \rightarrow \text{Cu(II)}$ . Such close transitions are not rare at pyrazole-type Cu(II) complexes [4]. Furthermore, for the complex **1** in this spectral range ( $\lambda \sim 330$  nm) also Cl  $\rightarrow$  Cu(II) band can be expected [5]. Finally, the low-energy bands ( $\lambda > 600$  nm) in the spectra of both complexes can be attributed to *d-d* transitions. Having in mind the observed similarities in the colour and spectral characteristics of the complexes **1** and **2**, and also of conductivity data for **2** which point to a partial dissociation of the NO<sub>3</sub> ligands, it can be supposed that both complexes have a rather distorted, probably square-pyramidal Cu(II) environment.

The spectrum of Ni complex (**3**) in its high energy region is quite similar to that of the Cu(II) complexes and so are the corresponding assignments of the bands. Three other, rather weak absorptions in the range  $\lambda > 590$  nm (*d-d* transitions) and a very light blue colour of their solution may be an evidence of a preserved distorted octahedral Ni(II) environment in methanolic solution.

### Thermal analysis

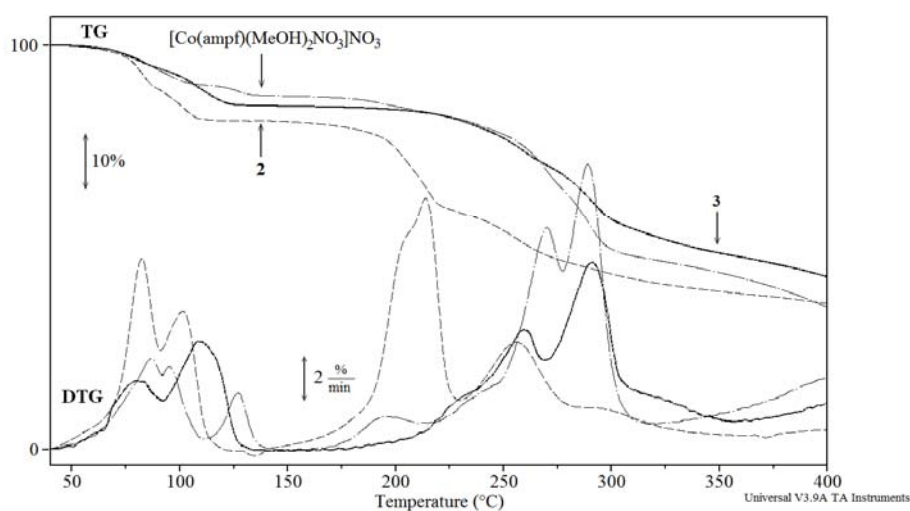
There are few data connecting the structure of the compounds with their thermal behavior in spite of the strong relationship between the decomposition pattern and the structure of the compounds. This is not surprising, because beside the structure of the compound, its macroscopic properties as well as the experimental conditions affect the thermal decomposition.

The thermal stability of the complexes is found to be the same in both gas carriers. The decomposition mechanism in air and nitrogen above 400 °C for **1** and for the nitrate complexes **2** and **3** above 200 °C, takes a different course, being more intensive in air.

Compound **1** is thermally stable up to 255 °C. Its endothermic decomposition begins with a DTG maximum at 266 °C and a DTA minimum at 270 °C and proceeds further by exothermic processes in nitrogen, too. The decomposition of **1** is continuous in the whole temperature range. Even with the SWI technique no intermediates could be isolated. The mass loss up to the first minimum of the DTG curve could fit the loss of HCl or it would correspond to the fragmentation of the side chains of the organic ligand. To decide which process is more likely we performed a qualitative reaction, the gases evolved during the decomposition bubbling through an acidic AgNO<sub>3</sub> solution. As no precipitation was observed, the HCl evolution could be excluded. Hence the decomposition begins with the departing of organic fragments.

In the lack of coupled measurements, only the mechanism of solvent evaporation can be discussed. Usually the decomposition of isostructural compounds is very similar [6,7]. On the other hand, similar decomposition curves do not necessarily belong to isostructural compounds. Sometimes even very small structural differences

may be detected by means of TA. The thermal curves of the MeOH evaporation of **2** and **3** are presented in Fig. 3. As is evident, the mechanism of the solvent loss is almost identical, in spite of the fact that in **3** both methanol molecules belong to the inner coordination sphere while in **2** only one MeOH is coordinated to the metal centre. The evaporation of MeOH begins at 65 °C (**2**) and at 67 °C (**3**) despite of their different crystallographic position. The solvent evaporation is a complex process and takes place in two well separated steps. It is completed at 112 and 128 °C for **2** and **3**, respectively. The mass loss of solvent evaporation to the corresponding DTG minimum, within experimental errors, agrees with the theoretical one (calcd.): **2**, 13.0% (11.24%); **3**, 11.0% (11.97%). The complexes without solvent are not stable and the decomposition proceeds exothermically at a slow, steady rate in both atmospheres. In spite of the similarities in MeOH evaporation, the rate of the process is significantly higher and begins at somewhat lower temperature in **2**. This complex contains MeOH as crystal solvent referring probably to its lower bonding energy, including its stabilization by H-bonds and/or an easier diffusion through the lattice.



**Fig. 3.** TG-DTG curves of **2**, **3** and  $[\text{Co}(\text{ampf})(\text{MeOH})_2\text{NO}_3]\text{NO}_3$  [**3**].

Supporting the complexity of the task to correlate the structural data with the thermal decomposition mechanism, a good example is the unexpected similarity between the mechanisms of the solvent evaporation of **2** and **3**, compounds with similar composition, but different crystal structure. It cannot be explained on the basis of the structural data only. In addition, they do not explain the significant differences in thermal decomposition of isostructural  $[\text{Co}(\text{ampf})(\text{MeOH})_2\text{NO}_3]\text{NO}_3$  [**3**] and **3** complexes. TA data for the cobalt(II) complex were repeated using the equipment and experimental conditions described in this paper. Its decomposition pattern did not change. The slight differences in are due to the better resolution of the instrument and to lower sample mass. The lower solvent content is the consequence of the storage conditions. As the interactions in Co(II) and Ni(II) crystals are similar, the different thermal behavior may be also a consequence of the different redox properties of Co(II) and Ni(II).

### In vitro biological activity

The synthesized compounds and the ligand precursor (*aamp*) were tested for their biological activity against human myelogenous leukaemia K562, colon adenocarcinoma HT29, cervix carcinoma HeLa and normal foetal lung fibroblasts, MRC-5. In vitro cytotoxicity was evaluated by sulforhodamine B (SRB) assay after 48h-treatment of cells. Table 3 shows the cytotoxicity of *aamp* and the complexes against four cell lines for the target compounds including Doxorubicin (Dox) as a reference drug.

**Table 3. *In vitro* cytotoxicity of *aamp*, **1**, **2** and **3****

Compounds	IC <sub>50</sub> , μM <sup>A</sup>			
	K562	HeLa	HT29	MRC-5
<i>aamp</i>	47.3	>100	0.6	>100
<b>1</b>	>100	20.2	89.0	>100
<b>2</b>	50.7	88.3	14.3	>100
<b>3</b>	>100	>100	10.13	>100
<b>Dox</b>	0.4	1.2	0.5	0.3

<sup>A</sup> IC<sub>50</sub> is the concentration of compound required to inhibit the cell growth by 50% compared to an untreated control

The cytotoxic activity of the *aamp* ligand and *ampf* complexes **1**, **2**, **3** against the selected test lines is very different (see data in Table 3). The common feature of all compounds is that their cytotoxicity against human non-tumor MRC-5 cell line is negligible. The cytotoxicity of *aamp* for HT29 is comparable to that of the Doxorubicin, but without cytotoxic effect against MRC-5 cell line. *Aamp* shows low or no activity against K562 and HeLa cell line, respectively. The activity of **1** is moderate against HeLa cell line but negligible against the two other tumor cell lines. This feature of **1** might be a consequence of the absence of the nitrate group. According to literature data <sup>[9]</sup> in some cell lines copper(II) is reduced to copper(I) that induces production of reactive oxygen species (ROS) with significant cytotoxic effect against human tumor cells. This reduction could be prevented by the presence of nitrate group in **2**. However, the reason for the different cytotoxicity of the compounds against HeLa has not yet been determined. For HT29 line the complexes show moderate cytotoxicity, in the increasing order of **1** < **2** < **3**. The activity of the complexes is very low or negligible against K563 tumor cells.

#### *Experimental for cytotoxicity in vitro*

Three human tumor cell lines and one human non-tumor cell line were used in the study: K562 (Chronic myelogenous leukemia), HeLa (Epitheloid carcinoma of cervix), HT29 (Colon adenocarcinoma), and MRC-5 (Lung foetal fibroblasts). The cells were grown in RPMI 1640 (K562 cells) or Dulbecco's modified Eagle's medium (DMEM) with 4.5% of glucose (HeLa, HT29, and MRC-5 cells). Media were supplemented with 10% of fetal calf serum (FCS, NIVNS) and antibiotics: 100 IU/cm<sup>3</sup> of penicillin and 100 μg/cm<sup>3</sup> of streptomycin (ICN Galenika). All cell lines were cultured in flasks (Costar, 25 cm<sup>2</sup>) at 37 °C in the 100% humidity atmosphere and 5% of CO<sub>2</sub>. Only viable cells were used in the assay. Viability was determined by dye exclusion assay with Trypan blue. Cytotoxicity was evaluated by colorimetric SRB assay after Skehan *et al.* <sup>[10]</sup>. Briefly, single cell suspension was plated into 96-well microtitar plates (Costar, flat bottom): 1·10<sup>4</sup> of K562 and 5·10<sup>3</sup> of HeLa, HT29, and MRC5 cells, per 180 cm<sup>3</sup> of medium. Plates were preincubated 24 h at 37 °C, 5% CO<sub>2</sub>. Tested substances at concentration ranging from 10<sup>-8</sup> mol dm<sup>-3</sup> to 10<sup>-4</sup> mol dm<sup>-3</sup> were added to all wells except to the control ones. After the incubation period (48 h /37 °C /5% CO<sub>2</sub>) SRB assay was carried out as follows: 50 μl of 80% trichloroacetic acid (TCA), was added to all wells; an hour later plates were washed with distilled water, and 75 μl of 0.4% SRB was added to all wells; half an hour later plates were washed with citric acid (1%) and dried at room temperature. Finally, 200 μl of 10 mmol TRIS (pH=10.5) basis was added to all wells. Absorbance was measured on the microplate reader (Multiscan MCC340, Labsystems) at 540/690 nm. The wells without cells, containing compete medium only, were taken as blank. Cytotoxicity was calculated according to the formula  $(1 - A_{\text{test}}/A_{\text{control}}) \times 100$  and expressed as a percent of cytotoxicity (CI%).

## References

- [1] C. F. Macrae, I. J. Bruno, J. A. Chisholm, P. R. Edgington, P. McCabe, E. Pidcock, L. Rodriguez-Monge, R. Taylor, J. van de Streek and P. A. Wood, *J. Appl. Cryst.* **2008**, *41*, 466.
- [2] GaussView, Version 4.1, R. Dennington II, T. Keith, J. Millam, Semichem, Inc., *Shawnee Mission*, KS, 2007.
- [3] V. M. Leovac, Z. D. Tomić, A. Kovács, M. D. Joksović, Lj. S. Jovanović, K. Mészáros Szécsényi, *J. Organomet. Chem.* **2008**, *693*, 77.
- [4] B. P. Lever, *Inorganic Electronic Spectroscopy*, Part 1, 1967, pp. 353–362 (Sec. Edition, Elsevier, Russian translation).
- [5] E. I. Solomon, K. W. Penfield, D. E. Wilcox, *Struct. & Bonding* **1983**, *53*, 1.
- [6] Z. Z. Rzączyńska, M. Woźniak, W. Wołodkiewicz, A. Ostasz, S. Pikus, *J. Therm. Anal. Cal.* **2008**, *91*, 951.
- [7] S. A. AbouEl-Enein, *J. Therm. Anal. Cal.* **2008**, *91*, 929.
- [8] K. Nakamoto, *Infrared and Raman Spectra of Inorganic and Coordination Compounds. Part B: Applications in Coordination, Organometallic and Bioinorganic Chemistry*, **1997**, pp. 24–26, 58–59, 87, 184 (Wiley & Sons, New York).
- [9] R. Alemón-Medina, M. Brena-Valle, J. L. Muñoz-Sánchez, M. I. Gracia-Mora, L. Ruiz-Azuara, *Cancer Chemother. Pharmacol.* **2007**, *60*, 219.
- [10] P. Skehan, R. Storeng, D. Scudiero, A. Monks, J. McMahon, D. Vistica, J. T. Warren, H. Bokesch, S. Kenney, M. R. Boyd, *J. Natl. Cancer Inst.* **1990**, *82*, 1107.