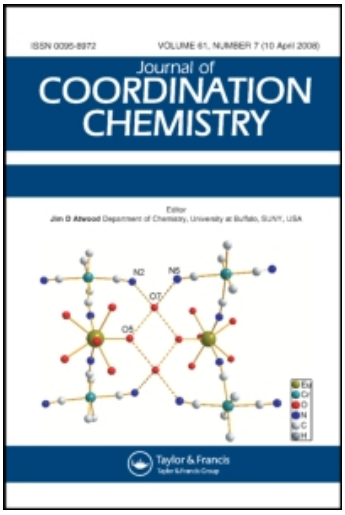


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# Cytotoxic activity of iron(III), cobalt(III), nickel(II), zinc(II), and cadmium(II) complexes of 2-acetylpyrazine thiosemicarbazone: crystal structure of the cobalt(III) complex

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# Cytotoxic activity of iron(III), cobalt(III), nickel(II), zinc(II), and cadmium(II) complexes of 2-acetylpyrazine thiosemicarbazone: crystal structure of the cobalt(III) complex

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Transition metal complexes $[Fe(HL)_2]Cl_3 \cdot 1.5H_2O$ (**1**), $[Co(L)_2] \cdot ClO_4 \cdot H_2O$ (**2**), $Ni(HL)_2(ClO_4)_2 \cdot 2H_2O$ (**3**), $Zn(HL)L \cdot BF_4 \cdot 2H_2O$ (**4**), and $Cd(HL)_2(ClO_4)_2 \cdot 2H_2O$ (**5**), where $HL = C_7H_9N_5S$, 2-acetylpyrazine thiosemicarbazone, have been synthesized. Complex **2** was characterized by elemental analysis, infrared spectra, mass spectra, and single-crystal X-ray diffraction. Preliminary *in vitro* screening showed that **1**, **4**, and **5** exhibit higher antitumor activity than **2** and **3** against K562 leucocythemia cancer cell line.

*Keywords*: 2-Acetylpyrazine; Thiosemicarbazone; Metal complexes; Crystal structure; Cytotoxic activity

## 1. Introduction

Heterocyclic thiosemicarbazones and their transition metal complexes have received considerable attention in chemistry and biology, primarily because of their marked and various biological properties [1–6]. The biological activities of thiosemicarbazones often depend on the parent aldehyde or ketone while metal thiosemicarbazones may differ from those of either the ligands or the metal ions [7–9]. In some cases the highest *in vivo* activity is associated with a metal complex rather than the parent ligand and side effects may decrease upon complexation [10–13]. These observations encourage detailed studies on coordination chemistry involving heterocyclic thiosemicarbazones.

We have been working on the structural and biological properties of heterocyclic thiosemicarbazones and their metal complexes [14]. The results reveal that 2-acetylpyrazine thiosemicarbazone HL (scheme 1) shows significant biological activity *in vitro* against K562 leukemic and lung cancer A549 cell lines [14a, 14b]. Therefore, it seemed useful to compare the biological activity of 2-acetylpyrazine thiosemicarbazone and its iron(III), cobalt(III), nickel(II), zinc(II), and cadium(II) coordination

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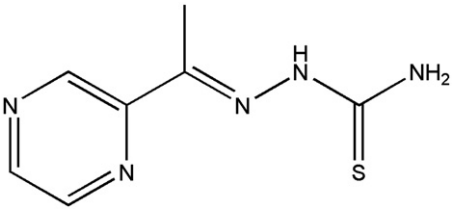


Scheme 1. 2-Acetylpyrazine thiosemicarbazone, HL.

complexes and investigate the influence of the nature of the metal atoms on the biological properties of the complexes.

In the present article, using the screening method of our previous results, we have tested the biological activity of a series of transition metal complexes derived from 2-acetylpyrazine thiosemicarbazone against K562 leukemic cancer cell lines. Our objective is to compare the variation in antitumor activity by changing metal ions with 2-acetylpyrazine thiosemicarbazone and investigate the metal chelation effect on biological properties of the complexes. Our interest in finding more valuable compounds with antitumor activity and also in finding structure–activity relationships led us to evaluate each of these complexes and analyze the differences in antitumor activity. The five complexes show significantly different levels of antitumor activity with iron(III) **1**, zinc(II) **4**, and cadmium(II) **5** complexes particularly effective against the studied cell line with $IC_{50}$ values in the μM range. We also describe the synthesis and single crystal X-ray crystal structure of **2**.



## 2. Experimental

### 2.1. *General procedures*

All chemicals were of reagent grade quality obtained from commercial sources and used without purification. 2-Acetylpyrazine thiosemicarbazone (HL) and **1**, **3**, **4**, and **5** were prepared according to the literature method [14b, 14c, 15]. Instrumentation: elemental analysis of C, H, and N was performed with a Perkin-Elmer 240 analyzer. The infrared (IR) spectra were recorded from KBr discs with a Nicolet 170 FT IR spectrophotometer. The mass spectra (MS) were carried out on an Es-quire 3000 LC-MS mass spectrophotometer. The crystal structure determination was carried out on a Siemens SMART-CCD X-ray diffractometer.

### 2.2. *Synthesis of the complex* $[Co(L)_2] \cdot ClO_4 \cdot H_2O$ *(2)*

An ethanol solution containing $Co(ClO_4)_2 \cdot 6H_2O$ (0.073 g, 0.2 mmol) was added dropwise with constant stirring and slow heating to an ethanol solution (20 mL) of 2-acetylpyrazine thiosemicarbazone (0.065 g, 0.4 mmol). The solution immediately turned deep red. After stirring for 0.5 h, the resultant solution was filtered. Deep-red crystals suitable for X-ray studies were obtained by slow evaporation of

Table 1. Summary of crystal data and refinement for **2**.

| | |
|---|---|
| Empirical formula | $C_{14}H_{18}ClCoN_{10}O_5S_2$ |
| Formula weight | 564.88 |
| Temperature (K) | 293(2) |
| Crystal size ($mm^3$) | $0.20 \times 0.18 \times 0.16$ |
| Crystal system | Orthorhombic |
| Space group | *Pbcn* |
| Unit cell dimensions (Å) | |
| *a* | 10.517(2) |
| *b* | 22.463(4) |
| *c* | 20.555(4) |
| Volume ($Å^3$), *Z* | 4856.0(16), 8 |
| Calculated density ($g\,cm^{-3}$) | 1.545 |
| Absorption coefficient ($mm^{-1}$) | 1.035 |
| $\theta$ range for data collection (°) | 1.98–25 |
| *F*(000) | 2304 |
| Limiting indices | $-12 \le h \le 11$; $-26 \le k \le 25$; $-24 \le l \le 24$ |
| Reflections measured | 4245 |
| Reflections unique | 2985 |
| Independent reflection | $R_{int} = 0.0628$ |
| Parameters | 338 |
| Goodness-of-fit on $F^2$ | 1.008 |
| Final *R* indices [$I \ge 2\sigma(I)$] | $R_1 = 0.0667$, $wR_2 = 0.1840$ |
| *R* indices (all data) | $R_1 = 0.0917$, $wR_2 = 0.1983$ |
| Largest difference peak and hole ($e\,Å^{-3}$) | 1.269 and −0.742 |



an ethanol solution. Anal. Calcd for **2** (%): C, 34.78; H, 4.14; N, 28.98. Found (%): C, 34.92; H, 4.28; N, 28.69.

### 2.3. *Crystal structure determination*

The intensities of the title complex were collected on a Siemens SMART-CCD diffractometer equipped with graphite-monochromated Mo-K$\alpha$ ($\lambda = 0.71073$ Å) radiation using the SMART and SAINT programs. The structure was solved by direct methods and refined on $F^2$ by full-matrix least-squares with SHELXTL *version* 5.1 [16]. All non-hydrogen atoms were refined with anisotropic thermal displacement parameters. Hydrogens were positioned according to the theoretical models. The crystallographic data of the cobalt complex are listed in table 1, selected bond distances and angles are shown in table 2, and hydrogen bond lengths and angles are shown in table 3.

### 2.4. In vitro *cytotoxicity study*

K562, a human leucocythemia cancer cell line (purchased from the Institute of Biochemistry and Cell Biology, SIBS, CAS), was cultured in RPMI-1640 medium supplemented with 10% FBS, $100\,U\,mL^{-1}$ of penicillin, 100 μg (200 μL per well) of streptomycin at 37°C in humid air of 5% $CO_2$. Cell cytotoxicity was assessed by the MTT assay. Briefly, cells were placed into a 96-well plate ($5 \times 10^3$ cells per well). The next day the compound diluted in culture medium at various concentrations was added (200 μL per well) to the wells. Forty-eight hours later 20 μL of MTT ($0.5\,mg\,mL^{-1}$ MTT in PBS) was added and cells were incubated for a further 4 h.

Table 2. Selected bond lengths (Å) and angles (°) for **2**.

| | | | |
|---|---|---|---|
| Co(1)–N(8) | 1.880(3) | Co(1)–N(3) | 1.881(3) |
| Co(1)–N(4) | 1.943(3) | Co(1)–N(9) | 1.949(4) |
| Co(1)–S(1) | 2.211(1) | Co(1)–S(2) | 2.226(1) |
| S(1)–C(1) | 1.727(4) | S(2)–C(8) | 1.746(4) |
| N(1)–C(1) | 1.341(5) | N(2)–C(1) | 1.334(5) |
| N(2)–N(3) | 1.368(4) | N(3)–C(2) | 1.311(5) |
| N(6)–C(8) | 1.336(5) | N(7)–C(8) | 1.311(5) |
| N(7)–N(8) | 1.375(4) | N(8)–C(9) | 1.315(5) |
| N(8)–Co(1)–N(3) | 178.5(1) | N(8)–Co(1)–N(4) | 95.47(13) |
| N(3)–Co(1)–N(4) | 83.14(13) | N(8)–Co(1)–N(9) | 82.54(14) |
| N(3)–Co(1)–N(9) | 96.79(14) | N(4)–Co(1)–N(9) | 91.20(13) |
| N(8)–Co(1)–S(1) | 95.08(9) | N(3)–Co(1)–S(1) | 86.30(10) |
| N(4)–Co(1)–S(1) | 169.5(1) | N(9)–Co(1)–S(1) | 90.06(10) |
| N(8)–Co(1)–S(2) | 86.23(10) | N(3)–Co(1)–S(2) | 94.41(11) |
| N(4)–Co(1)–S(2) | 89.21(10) | N(9)–Co(1)–S(2) | 168.8(1) |
| S(1)–Co(1)–S(2) | 91.60(4) | | |

Table 3. Hydrogen bond lengths (Å) and angles (°) for **2**.

| D–H···A | $d$(H···A) | $d$(D···A) | ∠(DHA) |
|---|---|---|---|
| N(1)–H(1A)···N(5)#1 | 2.36 | 3.016(5) | 132.8 |
| N(1)–H(1B)···O(1W)#2 | 2.05 | 2.906(7) | 177.4 |
| N(6)–H(6B)···S(1)#3 | 2.95 | 3.582(4) | 131.7 |
| N(6)–H(6C)···N(10)#4 | 2.14 | 2.990(5) | 168.3 |

Symmetry transformations used to generate the equivalent atoms: #1: $x$, $-y+1$, $z-1/2$; #2: $-x+1/2$, $-y+3/2$, $z-1/2$; #3: $x-1/2$, $-y+3/2$, $-z+1$; #4: $x-1$, $y$, $z$.



Then, 200 μL of DMSO were added to each culture to dissolve the MTT crystals. The MTT-formazan product dissolved in DMSO was estimated by measuring absorbance at 570 nm with a microplate reader. Then the inhibitory percentage of each compound at various concentrations was calculated and the $IC_{50}$ value determined.

## 3. Results and discussion

### 3.1. *Crystal structure of 2*

Figure 1 shows the molecular structure of **2** along with the atom numbering scheme and the unit cell packing is depicted in figure 2.

Complex **2** crystallizes in orthorhombic system with space group *Pbcn*. As shown in figure 1, **2** contains monomeric entities of six-coordinate cobalt(III), one disordered perchlorate, and one disordered water. The coordination sphere of cobalt(III) can be described as a distorted octahedron. One sulfur, one imine nitrogen, and one pyrazine nitrogen from one ligand and one imine nitrogen from another ligand occupy the basal positions, the two remaining positions in the octahedral geometry are occupied by one sulfur and one pyrazine nitrogen from different ligands. The pseudo-macrocyclic coordination mode of each ligand afford two five-membered

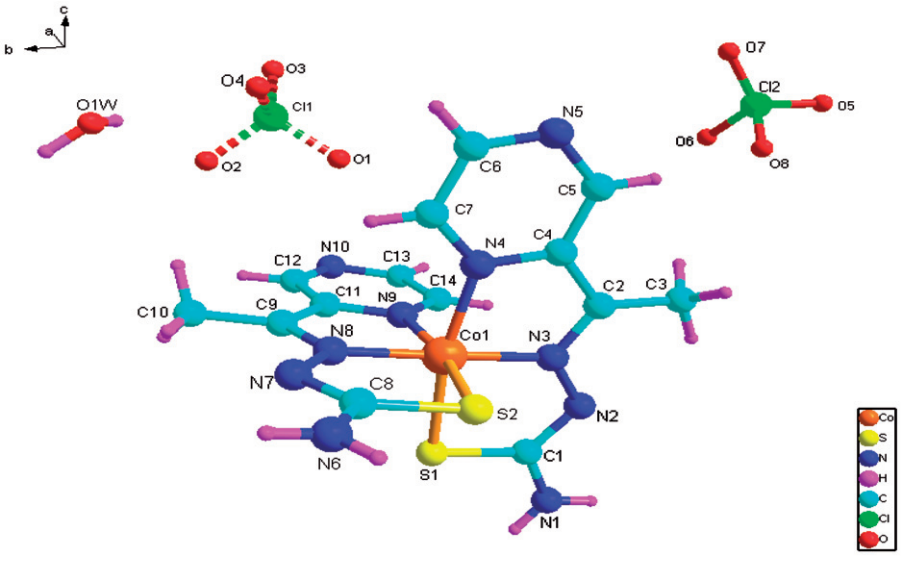


Figure 1. The molecular structure of **2** along with the atom numbering scheme.

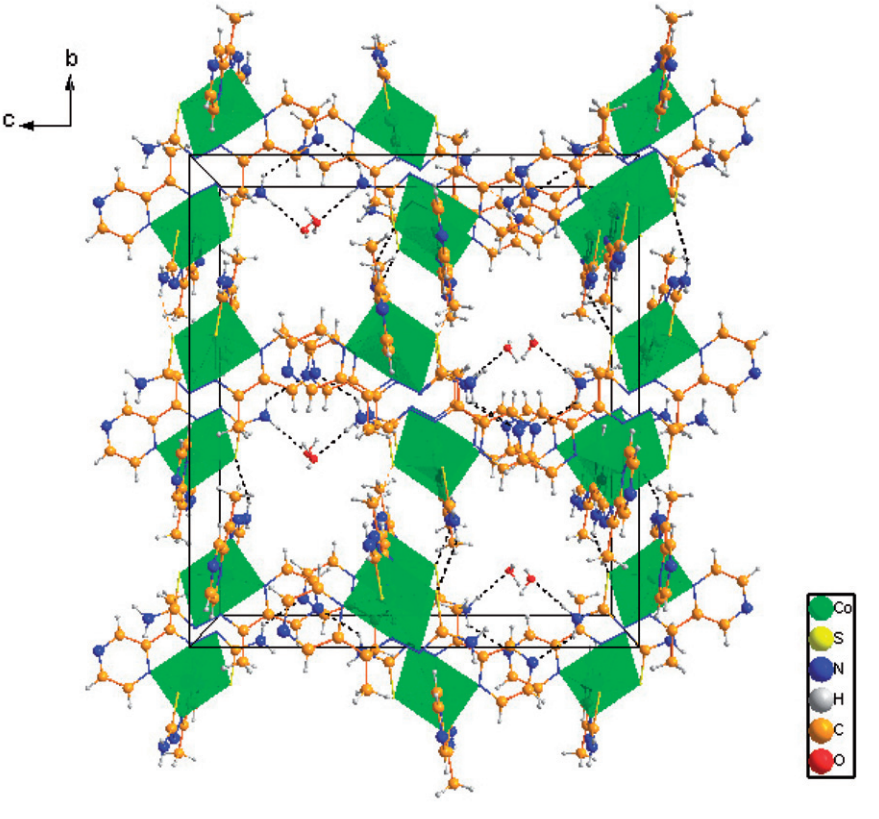


Figure 2. The molecular packing projected along the *a*-axis of the crystal.

chelate rings, which are nearly planar and the dihedral angle between the mean planes are 1.3° and 2.3°, respectively. The two pyrazine rings of two thiosemicarbazone ligands are almost perpendicular with a dihedral angle of 88.0°. The measured C(1)–S(1) and C(8)–S(2) bond distances of 1.727(4) and 1.746(4) Å, respectively, were within the normal range of a C–S single bond, indicating that the ligand adopted a *thiol* tautomeric form as a mononegative ligand [17a]. The C–N and N–N bond distances in $L^-$ were intermediate between the formal single and double bonds, pointing to extensive electron delocalization over the entire molecular skeleton. The shortening of the bond lengths of the Co–N(imine), relative to the bond distances of the Co–N(pyrazine), may be attributed to imine nitrogen being a stronger base than pyrazine nitrogen [17b].

As the thiosemicarbazone moieties have both the hydrogen bond donors and the hydrogen bond acceptors, the molecules are held together in the crystal packing through an extended network of intermolecular hydrogen bonds involving amino nitrogen N(1), N(6), uncoordinated nitrogen of the pyrazine rings N(5), N(10), the oxygen O(1W) of water, and coordinated sulfur S(1) (figure 2, table 3).



### 3.2. *IR and MS spectra*

The difference of the IR bands between the 2-acetylpyrazine thiosemicarbazone ligand and **2** provide significant indications regarding the bonding sites of the ligand. Both the ligand and **2** have three bands around 3172, 3293, and 3438 $cm^{-1}$, suggesting that the amino nitrogens do not participate in the coordination. The $\nu$(C=N) bands of the ligand and **2** are at 1598 and 1560 $cm^{-1}$, respectively. The decrease in frequency of this band in the complex is evidence for coordination *via* the azomethine nitrogen [18]. In 2-acetylpyrazine thiosemicarbazone, a band at 852 $cm^{-1}$ is assigned to $\nu$(C=S), whereas in **2** this band is shifted to lower frequencies (821 $cm^{-1}$), indicating coordination *via* sulfur. The sharp, strong peak at 1090 $cm^{-1}$ is assigned to perchlorate stretching vibrations and the single peak devoid of any splitting gives evidence of non-coordinating perchlorate. These observations have also been confirmed by X-ray single crystal structure analysis.

The MS of **2** show a signal at $m/z = 447.1$ assigned to $[Co(L)_2]^+$. This signal confirms the results of X-ray crystal study that two ligands are deprotonated as ionic NNS tridentate ligands to coordinate with center cobalt(III). The oxidation to cobalt(III) occurs during preparation of the complex. Although the mechanism of this oxidation is not proven we assume that the starting cobalt(II) salt presumably undergoes air oxidation in the presence of the ligand in ethanol [19].

### 3.3. *Cytotoxic activity*

Taking into account that thiosemicarbazone molecules exhibit cytotoxic activity [20], we have tested the ability of the five complexes to inhibit tumor cell growth. In our preliminary screening experiment, $IC_{50}$ values (compound concentration that produces 50% of cell death) in micromolar units were calculated against K562 leukemia cell line; $IC_{50}$ of the parent ligand HL was added in figure 3 for comparison [14a].

As shown in figure 3, complexation with metals has a synergetic effect on antitumor activity which depends upon the type of metal ion. Compounds **1**, **4**, and **5** show lower $IC_{50}$ values (4.64, 31.6, 9.68 μmol) than HL (47.5 μmol), indicating increased antitumor

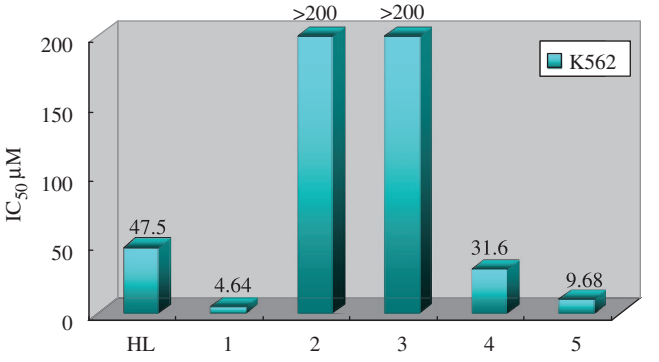


Figure 3. The antitumor activities of HL and its five complexes against K562 leucocythemia cancer cell line.

activities when complexed with iron(III), zinc(II), and cadmium(II). Many studies suggest that iron(III) and zinc(II) complexes have significantly greater antitumor activity than free ligand [21, 22]. Iron(III) and zinc(II) are essential ions, present in certain metalloenzymes [23, 24]. Although cadmium coordination chemistry is already well-known, little work has been done on screening for antitumor efficacy because cadmium compounds are poisonous [25]. It was surprising and not predictable that among these complexes, **5** also has significant antitumor activity. The enhancement of antitumor activity of these metal complexes can be related to an increase in the lipophilicity so they can penetrate into the cells more easily [26]. It has also been suggested that metal complexation may be a vehicle for activation of the ligand as the cytotoxic agent [27]. On the contrary, **2** and **3** exhibited poorer antitumor activity compared with the free ligand. Similar effect was observed upon complexation of other thiosemicarbazones with cobalt(III) and nickel(II) [28].



## 4. Conclusions

These experiments showed that **1** and **4** with important biological properties have potential as antitumor agents for further stages of screening *in vitro* and/or *in vivo*, encouraging further research in this field. Our continuing and detailed studies of the toxicity of these compounds as well as mechanism of action are in process, which could be helpful in designing more potent antitumor agents for therapeutic use.

## Supplementary material

Crystallographic data for the structural analyses reported in this article have been deposited with the Cambridge Crystallographic Data Centre with CCDC number 733518. Copies of this information may be obtained free of charge from The Director, CCDC, 12 Union Road, Cambridge, CB2 1EZ, UK (Fax: +44-1223-336033; E-mail: deposit@ccdc.cam.ac.uk).

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