

Synthesis, structure, and *in vitro* evaluation of biological activity of Cu^{II} furancarboxylates against the non-pathogenic *M. smegmatis* strain

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The reaction of copper(II) acetate with 2-furancarboxylate (Hfur, pyromucate) anions and the N-donor ligands 4-phenylpyridine (phpy) and 3-aminopyridine (NH₂py) in acetonitrile afforded the mononuclear complexes of the composition [Cu(fur)₂(phpy)₂(H₂O)]·phpy (**1**) and [Cu(fur)₂(NH₂py)₂] (**2**), respectively. The structures of the complexes were established by X-ray diffraction. The simultaneous thermal analysis of the thermal behavior of complex **1** showed that this complex is thermally stable up to 125 °C. The *in vitro* biological activity of complexes **1** and **2** was evaluated against the non-pathogenic mycobacterial *Mycobacterium smegmatis* strain.

Key words: copper(II) complexes, 2-furancarboxylic acid, structure, simultaneous thermal analysis, biological activity.

Complexes with bactericidal properties, which can be considered as promising drug candidates, occupy a special place among coordination compounds employed in different fields (analytical chemistry, catalysis, paint and varnish industry, optics, and as magnetic materials).^{1–5} The following complexes are used as drugs: platinum(II) complexes (cisplatin, nedaplatin) for the treatment of certain cancers; iron-containing agents for the treatment of anemia; silver, zinc, and mercury complexes as anti-septics.^{6–10} The field of antituberculosis drug development includes the search for new biologically active compounds to tackle the problem of drug-resistant mycobacterium tuberculosis (MTb, Koch's bacillus).¹¹ One strategy for increasing antimicrobial activity is based on the use of complexes with essential metal ions. It is expected that such compounds would be effective not only against sensitive multi-drug resistant MTb strains but also against dormant mycobacteria.^{12–15} Copper is an essential ion and is present as an important component in many enzymes with various functions in living organisms (for example, redox enzymes involved in the respiratory chain).^{16–20} Recently, the investigation of Elesclomol

(active against MTb H37Rv with the minimum inhibitory concentration (MIC) of 4 mg L⁻¹)²¹ demonstrated that the efficacy of the drug increases by more than 65 times upon the complexation with copper(II). Our previous studies^{22–24} of *in vitro* biological activity of Cu^{II}, Zn^{II}, Co^{II}, and Fe^{III} furoate complexes, in particular those with different N-donor ligands, showed that they are effective against the non-pathogenic mycobacterial *M. smegmatis* strain. It was found that N-donor ligands can both significantly enhance biological activity (4-phenylpyridine, 2,2'-bipyridyl, 1,10-phenanthroline) and decrease it (pyridine).²² In the present study, we synthesized mononuclear copper(II) complexes with 4-phenylpyridine and 3-aminopyridine and evaluated their *in vitro* biological activity against the non-pathogenic mycobacterial *M. smegmatis* strain.

Results and Discussion

As part of our research^{21–23} on the effect of co-ligands on the biological activity, we synthesized copper(II) furoate

complexes with 4-phenylpyridine (phpy) and 3-aminopyridine (NH₂py). The complexes [Cu(fur)₂(phpy)₂·(H₂O)]·phpy (**1**, fur is the 2-furancarboxylate anion) and [Cu(fur)₂(NH₂py)₂] (**2**) were prepared by the replacement of acetate anions in acetonitrile (Cu : fur : L = 1 : 2 : 2) in yields higher than 80%.

According to the single-crystal X-ray diffraction data, complex **1** crystallizes in the triclinic space group *P* $\bar{1}$ as a solvate with one phpy molecule. The Cu²⁺ cation is in a distorted tetragonal-pyramidal coordination environment formed by two monodentate carboxylate groups of 2-furancarboxylic acid, which are nearly perpendicular to each other, two phpy molecules, and one water molecule (Fig. 1, selected bond lengths and bond angles are given in Table 1).

The coordinated water molecule forms intermolecular hydrogen bonds with oxygen atoms of two carboxylate groups of the adjacent molecule of the complex (O \cdots O, 2.727(9), 2.741(7) Å; O—H—O, 147.3(6), 160.4(7)°),

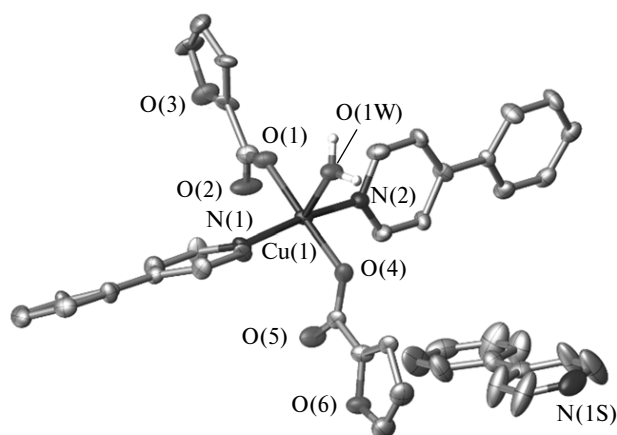


Fig. 1. Molecular structure of complex **1**. Here and in other figures, hydrogen atoms, except OH hydrogens, are omitted for clarity; nonhydrogen atoms are represented by displacement ellipsoids ($p = 50\%$).

Table 1. Selected bond lengths (d) and bond angles (ω) in compounds **1** and **2**

Parameter	1	2
Bond	$d/\text{Å}$	
Cu—O(fur)	1.935(8), 1.942(8)	2.0226(19)
Cu—O(H ₂ O)	2.193(7)	—
Cu—N	2.012(12), 2.027(11)	1.980(4)
Angle	ω/deg	
O—Cu—O	176.4(4), 87.7(3)—92.3(3)	180.00(19)
N—Cu—O	89.0(4)—91.0(4)	90.49(11)
N—Cu—N	167.1(4)	180.00(1)

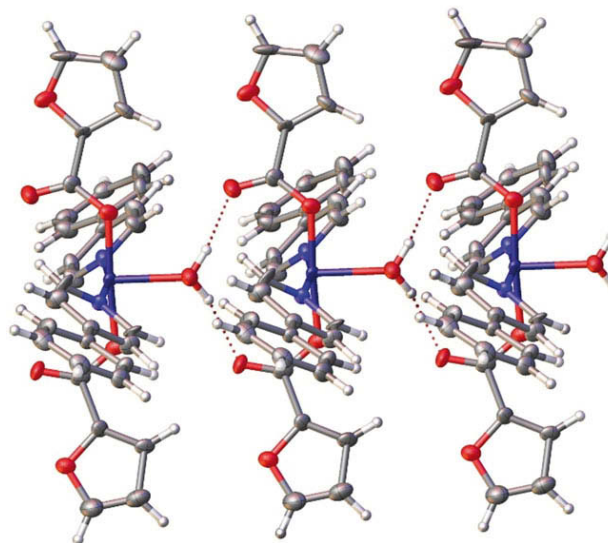


Fig. 2. Fragment of a hydrogen-bonded chain in the crystal of **1**.

resulting in the formation of infinite hydrogen-bonded motifs along the crystallographic a axis (Fig. 2).

The parallel chains are linked to each other by weaker intermolecular interactions, including C—H \cdots O and C—H \cdots N contacts with the shortest C \cdots O and C \cdots N distances of 3.560(16) and 4.168(14) Å, respectively, and the O—H—O and O—H—N angles in the range of 128.7(7)—147.9(7)°. It should be noted that, despite the presence of aromatic moieties in the complex, there are no stacking interactions.

Complex **2** crystallizes in the trigonal space group *R*-3 with a copper(II) ion lying in a special position on an inversion center. In the crystals of **2**, infinite chains are not formed because this complex (Fig. 3) does not contain

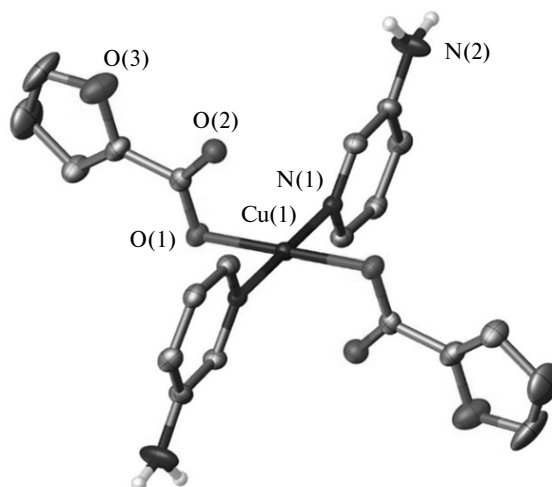


Fig. 3. Molecular structure of complex **2**. The numbering scheme is shown for the asymmetric unit.

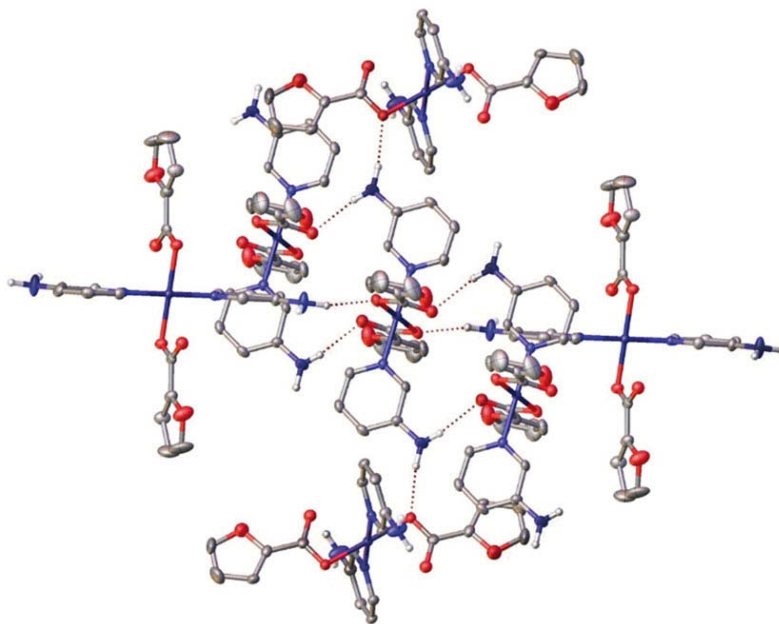


Fig. 4. Fragment of hydrogen bonding of molecules **2** in the crystal.

a water molecule coordinated to the metal atom. The square-planar coordination environment of copper(II) involves two symmetrically equivalent monodentate carboxylate groups, which slightly deviate from the common plane, and two 3-aminopyridine molecules coordinated through the N atoms of pyridine rings (see Table 1, Fig. 3). In the crystal, both amino groups of two coordinated aminopyridine molecules are involved in hydrogen bonding ($N\cdots O$, 2.999(6), 3.005(5) Å; $N-H\cdots O$, 164.4(3), 166.2(2)°) with the carboxylate oxygen atoms of four adjacent molecules of the complex, resulting in the formation of a three-dimensional hydrogen-bonded framework (Fig. 4).

The thermal behavior of **1** was studied by simultaneous thermal analysis (STA) under an argon atmosphere simultaneously recording TG and DSC curves (Fig. 5). An analysis of the TG curve (Fig. 5, *a*) shows that complex **1** is thermally stable up to 125 °C. The first step of the TG curve (125–195 °C, corresponding to desolvation of the outer-sphere phpy molecule) is almost immediately followed by the second step (195–268 °C) and is accompanied by desorption of two coordinated phenylpyridine moieties and elimination of molecular nitrogen ($m_{\text{calc/exp}} = 10.9/10.7\%$) (Fig. 5, *a*). The DSC curve (Fig. 5, *b*) displays the corresponding exothermic peak with a maximum at 209 °C. (In the previous thermal studies of complexes with amines,²⁵ the corresponding effect was observed at 209 °C.) In this temperature range, there is dissociation of hydrogen bonds between coordinated water molecules and furoate anions of adjacent molecules. Hence, the final step is accompanied by significant destruction of the organic moiety of the complex and is character-

ized by a strong endothermic effect with a maximum at 357 °C.

The antibacterial activity of compounds **1** and **2** was evaluated against the non-pathogenic mycobacterial *M. smegmatis* strain. The mycobacterial resistance to chemotherapeutic agents is known to be related to low permeability of the mycobacterial cell wall, with its unusual structure. *Mycobacterium smegmatis* is a rapidly growing non-pathogenic bacterium and, consequently, it is used as a model organism for the slowly growing bacteria *M. tuberculosis* and for the preliminary screening of antituberculosis agents.²⁶ The *M. smegmatis* test system is more resistant to antibiotics and antituberculosis agents

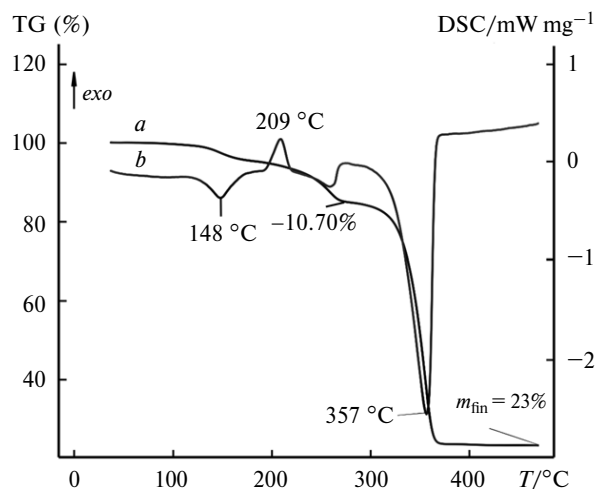


Fig. 5. TG (*a*) and DSC (*b*) curves of compound **1**.

Table 2. Evaluation of *in vitro* antibacterial activity against *Mycobacterium smegmatis*

Compound	MIC ^a /μg disc ⁻¹	Inhibition zone /mm	
		24 h	120 h
1	224	7.0±0.5	7.0±0.5 ^b
2	474	7.0±0.5	7.0±0.5 ^b
[Cu(fur) ₂ (phen)] ²²	2	7±0.5 ^c	7±0.5 ^c
[Zn ₂ (fur) ₄ phpy ₂] ²²	41	6.5±0.5 ^c	6.5±0.5 ^c
[Cu ₂ (fur) ₄ (py) ₂] ²²	146	7±0.5	7±0.5 ^b
[Cu(fur) ₂ (py) ₂ (H ₂ O)] ²²	153	7±0.5	7±0.5 ^b
[Zn ₂ (fur) ₄ py ₂] ²²	366	6.5±0.3 ^c	6.5±0.3 ^c
Hfur	112	—	—
INH	100	7±0.5	6.5±0.5 ^b
Rif	10	6.6±0.4 ^c	6.6±0.4 ^c

^a The diameter of the paper disc was 6 mm, *t* = 24 h.

^b The zone of growth inhibition of *M. smegmatis* mc² 155 is overgrown.

^c A clear zone of growth inhibition, the absence of weak background growth of the culture.

compared to *M. tuberculosis*. Therefore, unlike *M. tuberculosis*,²⁷ the criterion for selection is the concentration <100 nmol disc⁻¹.

All data on the *in vitro* biological activity of the new compounds were compared with the activity of the drugs of the first line for the treatment of tuberculosis, such as isoniazid (INH) and rifampicin (Rif), under these experimental conditions. As is seen in Table 2, complexes **1** and **2** exhibit low biological efficacy. It should be noted that the previously synthesized²² mononuclear copper complex with 1,10-phenanthroline is characterized by very high biological activity, which is five times higher than that of rifampicin and exhibits, like rifampicin, a bactericidal effect (the zone of growth inhibition of the bacteria is not overgrown overnight).

A comparison of **1** and the binuclear zinc complex with the same ligand [Zn₂(fur)₄(phpy)₂]²² shows that copper complex **1** is much less effective. Apparently, this is due to the different structures of the complexes and, as a consequence, different catalyzed/inhibited processes and different biological targets. Complex **2** with 3-aminopyridine displays the lowest biological activity (like compounds with pyridine²²).

To conclude, we demonstrated that Cu²⁺ cations interact with fur⁻ anions and phpy or NH₂py molecules to form mononuclear complexes with a monodentate coordination of N-donor ligands. An analysis of the thermal properties of **1** shows that this complex is thermally stable up to 125 °C, and the destruction of the organic moiety occurs almost completely in one step. The evaluation of the *in vitro* biological activity of **1** and **2** demonstrated that there is a correlation between the additional ligand, the nature of the complex-forming agent, and the bio-

logical efficacy against the non-pathogenic *M. smegmatis* strain.

Experimental

The following commercial reagents and solvents were used as received for the synthesis of the complexes: copper(II) acetate monohydrate (95%, Acros), 2-furancarboxylic acid (pyromucic acid, Acros), 3-aminopyridine (high-purity grade, Khimmed), 4-phenylpyridine (97%, Aldrich), and acetonitrile (special purity grade, Khimmed).

Attenuated total internal reflection (ATR) IR spectra were recorded on a Perkin-Elmer Spectrum 65 Fourier-transform infrared spectrometer in the frequency range of 400–4000 cm⁻¹.

Elemental analysis was performed on a Carlo Erba EA 1108 automated C,H,N analyzer.

Biological activity was evaluated using the *M. smegmatis* mc² 155 test system by the paper disc assay method. The zone of growth inhibition was assessed against the strain by the spot-on-the-lawn method on an agar medium around paper discs loaded with the test compounds at different concentrations. Bacteria, which were transferred from Petri dishes containing the tryptone soya agar medium M290 (Himedia), were grown for 16 h in the liquid medium Lemco-TW (Lab Lemco' Powder 5 g L⁻¹ (Oxoid), Peptone special 5 g L⁻¹ (Oxoid), NaCl 5 g L⁻¹, Tween-80) at 37 °C to the mid-logarithmic growth phase at the optical density OD₆₀₀ = 1.5 and then mixed with the molten agar medium M290 in a ratio of 1 : 9 : 10 (culture : Lemco-TW : M290). The culture was incubated for 24 h at 37 °C. The minimum inhibitory concentration was identified as the lowest concentration of the compound, at which the minimum zone of growth inhibition was observed.

(Aqua)di(furan-2-carboxylato-O)di(4-phenylpyridine)copper(II) 4-phenylpyridine solvate, [Cu(fur)₂(phpy)₂(H₂O)] · phpy (1**).** The compounds Cu(OAc)₂ · H₂O (0.2 g, 1 mmol) and Hfur (0.224 g, 2 mmol) were suspended in MeCN (40 mL), phpy (0.310 g, 2 mmol) was added to the suspension, and the reaction mixture was kept at 70 °C for 3 h. The resulting blue solution was filtered off and concentrated to 20 mL. The blue crystals that formed overnight were separated from the mother liquor by decantation and dried in air. The yield of **1** was 0.62 g (81%). Found (%): C, 67.18; H, 4.61; N, 5.39. CuO₇C₄₃H₃₅N₃. Calculated (%): C, 67.13; H, 4.59; N, 5.46. IR, ν/cm⁻¹: 3283 (w), 3127 (w), 3108 (w), 3079 (w), 3045 (w), 1605 (v.s), 1562 (v.s), 1476 (v.s), 1407 (s), 1389 (v.s), 1357 (v.s), 1225 (m), 1188 (v.s), 1140 (m), 1076 (m), 1045 (w), 1008 (v.s), 927 (m), 883 (m), 839 (s), 825 (m), 790 (v.s), 768 (v.s), 756 (v.s), 743 (v.s), 727 (v.s), 687 (v.s), 624 (v.s), 607 (v.s), 582 (v.s), 560 (v.s), 472 (v.s), 448 (v.s), 423 (v.s).

Di(furan-2-carboxylato-O)di(3-aminopyridine)copper(II), [Cu(fur)₂(NH₂py)₂] (2**).** The compounds Cu(OAc)₂ · H₂O (0.2 g, 1 mmol) and Hfur (0.224 g, 2 mmol) were suspended in MeCN (40 mL), NH₂py (0.188 g, 2 mmol) was added to the suspension, and the reaction mixture was kept at 70 °C for 3 h. The resulting gray-green solution was filtered off and concentrated to 20 mL. The green crystals that formed overnight were separated from the mother liquor by decantation and dried in air. The yield of **2** was 0.41 g (87%). Found (%): C, 50.63; H, 3.77; N, 11.79. CuO₆C₂₀H₁₈N₄. Calculated (%): C, 50.69; H, 3.83; N, 11.82. IR, ν/cm⁻¹: 3399 (w), 3325 (m), 3222 (m), 1639 (m), 1577 (v.s),

Table 3. Crystallographic data and structure refinement statistics for complexes **1** and **2**

Parameter	1	2
Molecular formula	C ₄₃ H ₃₅ CuN ₃ O ₇	C ₂₀ H ₁₈ CuN ₄ O ₆
<i>M</i> /g mol ⁻¹	769.28	473.92
Crystal system	Triclinic	Trigonal
Space group	<i>P</i> $\bar{1}$	<i>R</i> -3
<i>a</i> /Å	5.5903(6)	27.624(3)
<i>b</i> /Å	13.2220(16)	27.624(3)
<i>c</i> /Å	13.2991(15)	7.1446(9)
α /deg	110.748(4)	90
β /deg	108.124(3)	90
γ /deg	92.851(4)	120
<i>V</i> /Å ³	895.76(18)	4721.6(11)
<i>Z</i>	1	9
<i>d</i> _{calc} /g cm ⁻³	1.426	1.500
μ /cm ⁻¹	6.68	10.85
2 θ _{max} /deg	56	55
<i>F</i> (000)	399	2187
<i>R</i> _{int}	0.0725	0.0966
Number of reflections		
total	9132	15273
unique	6961	2295
observed with <i>I</i> > 2 σ (<i>I</i>)	4052	1603
Number of parameters	491	142
GOOF	1.002	1.055
<i>R</i> ₁	0.0859	0.0431
<i>wR</i> ₂ (<i>I</i> > 2 σ (<i>I</i>))	0.1624	0.1209
Residual electron density (ρ _{max} / ρ _{min})/e Å ⁻³	0.598/−0.787	0.467/−0.513

1553 (v.s), 1495 (m), 1480 (v.s), 1450 (s), 1405 (s), 1389 (v.s), 1362 (v.s), 1324 (s), 1281 (s), 1225 (m), 1189 (s), 1140 (m), 1078 (w), 1064 (m), 1010 (s), 930 (m), 885 (m), 814 (v.s), 800 (m), 782 (v.s), 747 (v.s), 699 (v.s), 663 (s), 615 (s), 594 (s), 547 (s), 467 (v.s), 432 (v.s), 415 (v.s).

Single-crystal X-ray diffraction study of complexes **1 and **2****
 was performed on a Bruker ApexII DUO diffractometer equipped with a CCD detector (Mo-*K* α , λ = 0.71073 Å, graphite monochromator) at 150 and 120 K, respectively. The structures were solved with the ShelXT program²⁸ and refined by the full-matrix least-squares method with anisotropic displacement parameters for all nonhydrogen atoms using the Olex2 program.²⁹ The OH and NH hydrogen atoms were located in difference Fourier maps; all other hydrogen atoms were positioned geometrically. All hydrogen atoms were refined isotropically using a riding model. Principal crystallographic data and structure refinement statistics are given in Table 3. The atomic coordinates were deposited with the Cambridge Crystallographic Data Centre (CCDC 2048453 and 2048173 for **1** and **2**, respectively).

Thermal behavior of **1** was studied by STA under an argon atmosphere by simultaneously recording thermogravimetric (TG) and differential scanning calorimetry (DSC) curves. The measurements were performed on a NETZSCH 449 F1 Jupiter STA analyzer in aluminum crucibles with a hole in the lid to maintain the vapor pressure of 1 atm during thermal decomposition of the samples. The heating rate was 5 °C min⁻¹ up to 450 °C. The sample weight was 2.98 mg. The accuracy of temperature measurements was ± 0.7 °C, the accuracy of weight measurements was $\pm 1 \cdot 10^{-2}$ mg. The TG and DSC curves were recorded using the correction file and the temperature and sensitivity

calibration for the specified temperature program and the heating rate.

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No human or animals were involved in this research.

The authors declare no conflict of interest, financial or otherwise.

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