

Copper(II) and Zinc(II) Complexes with Heterocyclic Acid Anions and 3,5-Dimethylpyrazole: Synthesis, Structure, and Biological Properties

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Abstract—The reaction of copper(II) and zinc(II) acetates with 3-furancarboxylic (HFur) and 2-thiophene-carboxylic (HTph) acids with subsequent addition of 3,5-dimethylpyrazole (HDmpz) gave mononuclear complexes $[M(L)_2(HDmpz)_2]$ ($M = \text{Cu(II)}$, $L = \text{Fur}^-$ (**I**), Tph^- (**II**); $Zn(\text{II})$, $L = \text{Fur}^-$ (**III**)). The structures of compounds **I**–**III** were determined by X-ray diffraction. According to X-ray diffraction data, **I** and **II** are isostructural: the central Cu(II) atom occurs in a square planar environment formed by two oxygen atoms of carboxylate anions and HDmpz nitrogen atoms; in **III**, the Zn atom is in the tetrahedral environment of two furoate anions and HDmpz molecules, thus forming the $\{\text{MO}_2\text{N}_2\}$ groups. The complexes are additionally stabilized in the crystal by inter- (**I** and **II**) and intramolecular (**III**) hydrogen bonds. The biological activity of **I**–**III** was determined in relation to the non-pathogenic *Mycobacterium smegmatis*.

Keywords: copper(II) and zinc(II) complexes, 3,5-dimethylpyrazole, 3-furancarboxylic acid, 2-thiophene-carboxylic acid, crystal structure, biological activity

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INTRODUCTION

The design of new antibacterial drugs is one of the fundamental problems of medicine. This problem can be addressed, in particular, by development and antimicrobial assays of new coordination compounds based on biogenic metals. More and more current studies confirm the fact that complex formation can markedly increase the activity of already known organic agents [1–3]. A drawback of metal complexes acting as potential drugs is their possible cytotoxicity. Therefore, it is expedient to use complexes of essential (biologically important) metals, such as copper and zinc. An important factor in the search for biologically active coordination compounds is selection of the organic ligand. In particular, five-membered heterocycles such as thiophene, furan, and pyrazole are parts of many antimicrobial drugs [4–6] and complexes based on them are biologically active [7–11].

The in vitro studies of biological activity of *d*-metal furancarboxylate complexes [12–19] with various N-donor ligands towards a non-pathogenic mycobacterial strain, *Mycobacterium smegmatis*, carried out

by our research team revealed a number of regular trends, in particular, cations to which the mycobacterium is most sensitive and mono- and oligopyridine co-ligands able to enhance/inhibit the biological efficiency were identified. It appeared reasonable to study the biological activity of compounds with a fundamentally different type of co-ligand, 3,5-dimethylpyrazole (HDmpz), the derivatives of which have exhibited antimicrobial, antiviral, and anticarcinogenic properties [20–24]. From the structural standpoint, pyrazole derivatives have a broad coordination potential, as they contain two chemically different nitrogen atoms (pyridine and pyrrole atoms), which cause different types of binding. In particular, the lone electron pair of the pyridine nitrogen atom provides monodentate coordination, while formation of the pyrazolate anion (via pyrrole deprotonation) leads, most often, to bridging coordination. The introduction of donor CH_3 groups into the pyrazole molecule results in increasing basicity of nitrogen atoms (due to the positive inductive effect of substituents); therefore,

HDmpz is coordinated, most often, in the monodentate fashion [25–30].

This paper describes the synthesis and structure of copper(II) and zinc complexes with Fur[−]/Tph[−] anions and HDmpz and presents the results of biological assays of the obtained compounds in relation to the *Mycobacterium smegmatis* strain (model mycobacterial strain for *Mycobacterium tuberculosis*).

EXPERIMENTAL

The complexes were synthesized in air using commercial solvents without further purification: acetonitrile (special purity grade, Khimmed), 3-Furancarboxylic acid (98%, Acros), 2-thiophenecarboxylic acid (98%, Acros), zinc acetate dihydrate (98%, Acros), and copper(II) acetate hydrate (98%, Acros) were commercial chemicals. Elemental analysis was performed on a Carlo Erba EA 1108 automated C, H, N-analyzer. IR spectra were recorded on a Perkin-Elmer Spectrum 65 Fourier transform IR spectrophotometer in the attenuated total reflectance (ATR) mode in the 400–4000 cm^{−1} frequency range.

The biological activity of **I–III** was determined by the paper disc method using *M. smegmatis* mc² 155 strain. The growth inhibition zone of the strain seeded in a lawn on agar medium was measured around paper discs containing the test compounds in various concentrations. The bacteria that were washed from the Petri dishes with Tryptone soya agar M-290 (Himedia) were grown overnight in the Lemco-TW liquid medium (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 up to the mid-logarithmic growth phase at the optical density OD₆₀₀ = 1.5, and mixed with the molten M-290 agar medium in 1 : 9 : 10 ratio (culture : Lemco-TW : M-290). The culture was incubated for 24 h at 37°C. The compound concentration inducing the minimum inhibition zone was taken as the minimum inhibitory concentration (MIC).

X-ray diffraction study of **I–III** was carried out on a Bruker Apex II diffractometer (MoK_α, $\lambda = 0.71073 \text{ \AA}$, graphite monochromator, CCD array detector) at 100 K. A semiempirical absorption correction was applied by the SADABS program [31]. The structures were solved by the direct method using ShelXT software [32] and refined by the full-matrix least-squares method using ShelXL software [33] in the anisotropic approximation for non-hydrogen atoms. The hydrogen atoms of the NH groups were located from difference Fourier maps and included in the refinement in the isotropic approximation without constraints. The positions of other hydrogen atoms were calculated geometrically, and all of H atoms were refined in the isotropic approximation by the riding model with $U_{\text{iso}}(\text{H}) = 1.5U_{\text{eq}}(\text{C})$ for methyl groups and $U_{\text{iso}}(\text{H}) = 1.2U_{\text{eq}}(\text{C})$ for other hydrogen atoms. In the structure of **III**, one of the furan rings was disordered

over two positions with 0.63(1)/0.37(1) occupancy ratio. The main crystallographic data and structure refinement details for compounds **I–III** are summarized in Table 1.

The full set of X-ray diffraction parameters was deposited with the Cambridge Crystallographic Data Centre (CCDC no. 2243672 (**I**), 2243673 (**II**), 2243674 (**III**); deposit@ccdc.cam.ac.uk).

Synthesis of [Cu(Fur)₂(HDmpz)₂] (I). Weighed amounts of Cu(OAc)₂·H₂O (0.2 g, 1 mmol) and HFur (0.33 g, 3 mmol) were dissolved in acetonitrile (10 mL) and refluxed for 1 h. Then HDmpz (0.2 g, 2 mmol) was added to the solution, and the mixture was stirred for 30 min. The resulting dark blue solution was kept for 2 days at room temperature. The blue crystals thus formed were separated from the mother liquor by decantation. The yield was 0.3 g (70%).

For C₂₀H₂₂N₄O₆Cu (**I**)

Anal. calcd., %	C, 50.26	H, 4.64	N, 11.72
Found, %	C, 50.31	H, 4.67	N, 11.74

IR (ATR; ν , cm^{−1}): 3670 s, 3182 s, 3095 s, 2981 m, 2933 m, 2869 m, 2779 s, 1705 s, 1541 w, 1501 m, 1411 w, 1364 m, 1306 m, 1256 s, 1206 m, 1151 m, 1062 m, 1013 m, 970 s, 831 w, 781 w, 736 m, 600 m, 547 s, 463 m, 431 s.

Synthesis of [Cu(Tph)₂(HDmpz)₂] (II). Weighed amounts of Cu(OAc)₂·H₂O (0.2 g, 1 mmol) and HTph (0.39 g, 3 mmol) were dissolved in acetonitrile (10 mL) and refluxed for 1 h. Then HDmpz (0.20 g, 2 mmol) was added to the solution and the mixture was stirred for 30 min. The resulting dark blue solution was filtered and kept for 2 days. The violet crystals thus formed were separated from the mother liquor by decantation. The yield was 0.36 g (72%).

For C₂₀H₂₂N₄O₄S₂Cu (**II**)

Anal. calcd., %	C, 47.10	H, 4.35	N, 10.10
Found, %	C, 47.15	H, 4.42	N, 10.01

IR (ATR; ν , cm^{−1}): 3092 s, 2933 s, 2859 s, 1542 w, 1501 w, 1411 w, 1363 w, 1305 m, 1204 m, 1150 m, 1060 m, 1011 m, 830 w, 780 w, 736 w, 599 m, 546 m, 462 w, 431 m.

Synthesis of [Zn(Fur)₂(HDmpz)₂] (III). Weighed amounts of Zn(OAc)₂·2H₂O (0.22 g, 1 mmol) and HFur (0.33 g, 3 mmol) were dissolved in acetonitrile (10 mL) and refluxed for an hour. Then HDmpz (0.20 g, 2 mmol) was added to the solution, and the mixture was stirred for 30 min. The obtained transparent solution was left for 5 days at room temperature. The resulting transparent crystals were separated from

Table 1. Main crystallographic data and X-ray experiment details for **I**–**III**

Parameter	Value		
	I	II	III
Molecular formula	C ₂₀ H ₂₂ N ₄ O ₆ Cu	C ₂₀ H ₂₂ N ₄ O ₄ S ₂ Cu	C ₂₀ H ₂₂ N ₄ O ₆ Zn
<i>M</i>	477.95	510.07	479.78
<i>T</i> , K	100(2)	100(2)	100(2)
System	Monoclinic	Monoclinic	Triclinic
Space group	<i>P</i> 2 ₁ / <i>n</i>	<i>P</i> 2 ₁ / <i>n</i>	<i>P</i> 1̄
<i>a</i> , Å	5.7312(2)	5.9903(4)	8.3507(3)
<i>b</i> , Å	17.2051(7)	17.4745(11)	11.6130(4)
<i>c</i> , Å	10.4723(4)	10.5606(6)	12.1465(4)
α, deg	90	90	96.1076(12)
β, deg	104.4374(16)	103.5911(19)	101.9847(12)
γ, deg	90	90	107.8456(10)
<i>V</i> , Å ³	1000.02(7)	1074.50(12)	1078.37(6)
<i>Z</i>	2	2	2
ρ(calcd.), g cm ⁻³	1.587	1.577	1.478
μ, cm ⁻¹	11.39	12.46	11.83
2θ _{max} , deg	60.0	60.0	60.0
<i>T</i> _{max} / <i>T</i> _{min}	0.895/0.812	0.907/0.685	0.871/0.789
Number of measured reflections	17296	12273	17613
Number of unique reflections	2912	3132	6274
Number of observed reflections with <i>I</i> > 2σ(<i>I</i>)	2429	2689	5617
Number of refined parameters	148	148	329
GOOF	1.089	1.085	1.045
<i>R</i> ₁ (on <i>F</i> for reflections with <i>I</i> > 2σ(<i>I</i>))	0.0443	0.0385	0.0294
<i>wR</i> ₂ (on <i>F</i> ² for all reflections)	0.1212	0.1028	0.0800
Residual electron density (max/min), e Å ⁻³	1.850/−0.500	1.070/−0.660	0.460/−0.860

the mother liquor by decantation. The yield was 0.33 g (73%).

For C₂₀H₂₂N₄O₆Zn (**III**)

Anal. calcd., % C, 50.07 H, 4.62 N, 11.68
Found, % C, 50.20 H, 4.88 N, 11.74

IR (ATR; *v*, cm⁻¹): 3200 m, 3131 m, 3106 m, 3036 m, 2971 w, 2932 m, 2787 m, 2608 s, 1594 m, 1553 m, 1481 m, 1415 w, 1365 m, 1301 w, 1199 s, 1151 m, 1021 w, 1010 w, 840 w, 777 w, 737 m, 604 s, 477 s, 425 s.

RESULTS AND DISCUSSION

The reactions of copper(II) and zinc acetate hydrates with HFur/HTph in 1 : 2 ratio in acetonitrile afford homogeneous solutions. The addition of HDmpz (2 mol) to these solutions results in the formation of mononuclear complexes: blue [Cu(Fur)₂-(HDmpz)₂] (**I**), violet [Cu(Tph)₂(HDmpz)₂] (**II**), and colorless [Zn(Fur)₂(HDmpz)₂] (**III**) (Scheme 1). For obtaining valid data on the biological activities of complexes **I**–**III**, previously reported complex [Zn(Tph)₂(HDmpz)₂] (**IV**) was prepared [30].

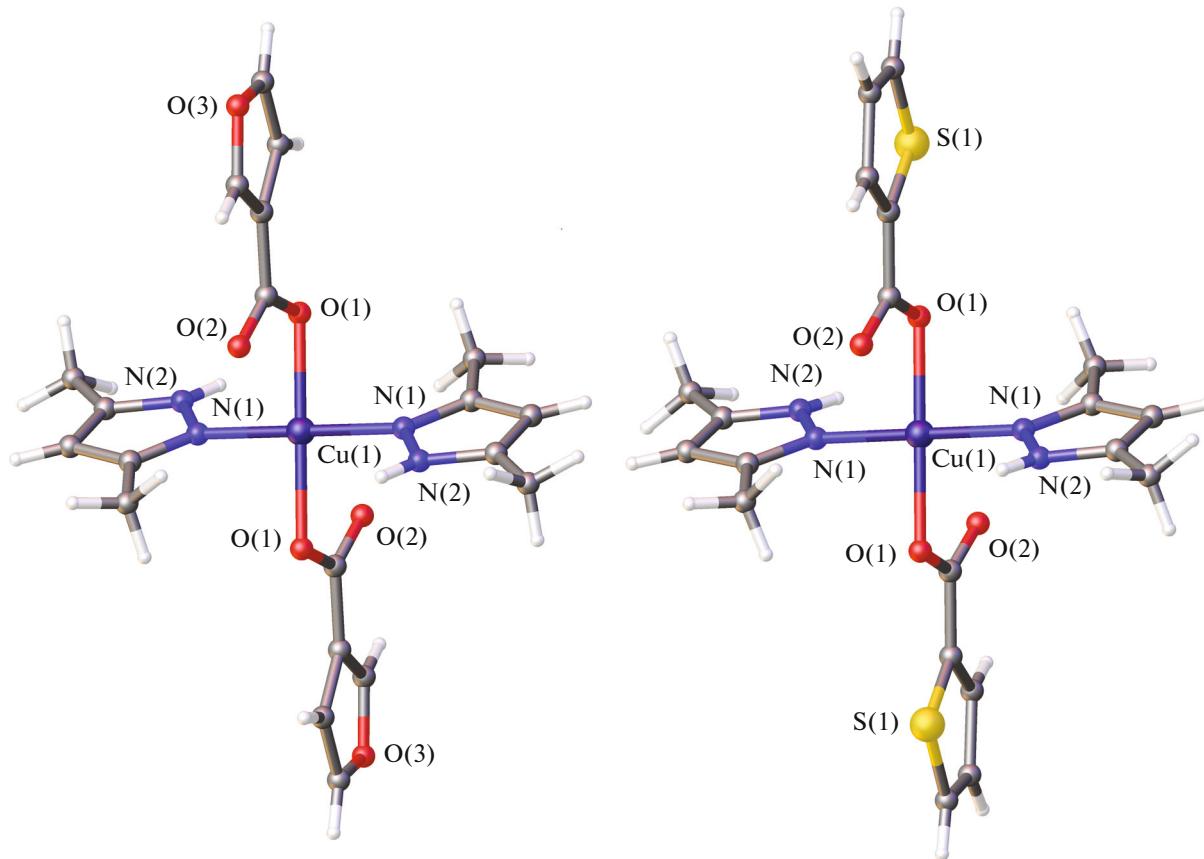
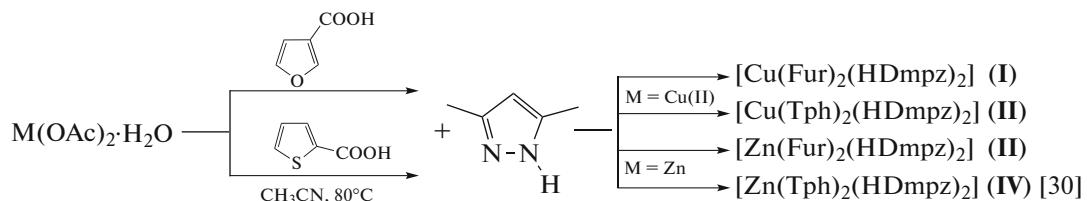


Fig. 1. Molecular structure of complexes (on the left) **I** and (on the right) **II** with atoms represented as thermal ellipsoids (50% probability).



Scheme 1.

The complexes *trans*-[Cu(Fur)₂(HDmpz)₂] (**I**) and *trans*-[Cu(Tph)₂(HDmpz)₂] (**II**) crystallize in space group *P*2₁/*n* with nearly equal unit cell parameters (Table 1). In the crystal, the copper(II) ion is located in a special position at the inversion center and has a square planar environment (Fig. 1) composed of two oxygen atoms of furancarboxylic acid in **I** (Cu(1)–O(1), 2.014(2) Å) or thiophenecarboxylic acid in **II** (Cu(1)–O(1), 1.995(1) Å) and two nitrogen atoms of the monodentate N-donor HDmpz ligand (Cu(1)–N(1) of 1.959(2) Å in **I** and 1.965(2) Å in **II**). The second oxygen atom O(2) of the carboxylate group is located at a much longer distance from Cu²⁺ (Cu(1)...O(2) distance of 2.578(2) Å in **I** and 2.622(2) Å in **II**). The protonated nitrogen atoms of

HDmpz form intermolecular hydrogen bonds with carboxylate oxygen atoms not involved in the strong coordination with the complex-forming metal (Table 2). These hydrogen bonds combine the complexes into chains along the *a* axis of the crystal (Fig. 2). The chains are additionally stabilized by several C–H...O contacts in **I** or C–H...O and C–H...S contacts in **II** (characteristics of these contacts are also given in Table 2). The hydrogen-bonded chains are combined into layers parallel to the *ac* plane of the crystal as a result of π...π stacking interactions between the furan substituents (the distances between the centroids of the neighboring rings are 3.855(2) Å (**I**) and 4.985(1) Å (**II**); the shortest interatomic distances: C(10)...C(10)[#] ([#] 1 – *x*, –*y*, –*z*) is 3.441(3) Å (**I**) and

Table 2. Hydrogen bond parameters for the structures of **I**–**III**

D–H...A	Distance, Å			DHA angle, deg	Coordinates of <i>A</i> atom
	D–H	H...A	D...A		
I					
N(2)–H(2)...O(2)	0.85(3)	1.93(3)	2.752(3)	163(3)	$-x + 2, -y, -z + 1$
C(8)–H(8 <i>A</i>)...O(1)	0.95	2.38	3.281(3)	158	$x + 1, y, z$
C(10)–H(10 <i>A</i>)...O(3)	0.95	2.66	3.582(3)	164	$x - 1, y, z$
II					
N(2)–H(2)...O(2)	0.85(3)	1.94(3)	2.770(3)	167(3)	$-x, -y, -z$
C(8)–H(8 <i>A</i>)...O(1)	0.95	2.62	3.527(3)	161	$x - 1, y, z$
C(9)–H(9 <i>A</i>)...S(1)	0.95	2.88	3.556(3)	129	$x - 1, y, z$
III					
N(2)–H(2)...O(2)	0.85(2)	1.88(2)	2.695(2)	162(2)	
N(4)–H(4)...O(5)	0.81(2)	1.94(2)	2.722(2)	162(2)	
C(19)–H(19 <i>A</i>)...O(2)	0.95	2.21	3.156(2)	171	$x - 1, y - 1, z$

C(8)...C(9)[#] ([#] $-x, -y, 1 - z$) is 3.416(3) Å (II)). No specific interatomic contacts are present between the layers.

Complex **III** crystallizes in the triclinic space group $P\bar{1}$ and occupies a general position in the crystal. The zinc ion has a tetrahedral coordination environment (Fig. 3) composed of two oxygen atoms of monodentately coordinated furoate anions ($Zn-O$, 1.926(1) and 1.952(1) Å) and two nitrogen atoms of two monodentately coordinated HDmpz molecules ($Zn-N$, 2.001(1) and 2.008(1) Å). The bond angles of the zinc atom are in the range of $103.28(5)^\circ$ – $119.09(5)^\circ$. Unlike copper complexes **I** and **II**, in complex **III**, the carboxylate oxygen atoms O(2) and O(5) are not

involved in the additional coordination to the metal (the Zn–O distances to these oxygen atoms are 3.123(1) and 3.301(1) Å). These uncoordinated oxygen atoms are involved in the formation of intramolecular N–H...O hydrogen bonds with protonated N atoms of HDmpz (Table 2). A similar structure was inherent in the previously reported zinc complexes with the 2-furancarboxylate anions $[\text{Zn}(2\text{-Fur})_2(\text{HDmpz})_2]$ [34] and with thiophenecarboxylate anions $[\text{Zn}(\text{Tph})_2(\text{HDmpz})_2]$ (IV) [30]. The square planar and tetrahedral states of the complex cations in **I–III** are generally characterized by the $\{\text{MO}_2\text{N}_2\}$ metal core, in which C.N.(Cu/Zn) is 4. In the crystal of **III**, the molecules of the complex are joined by rel-

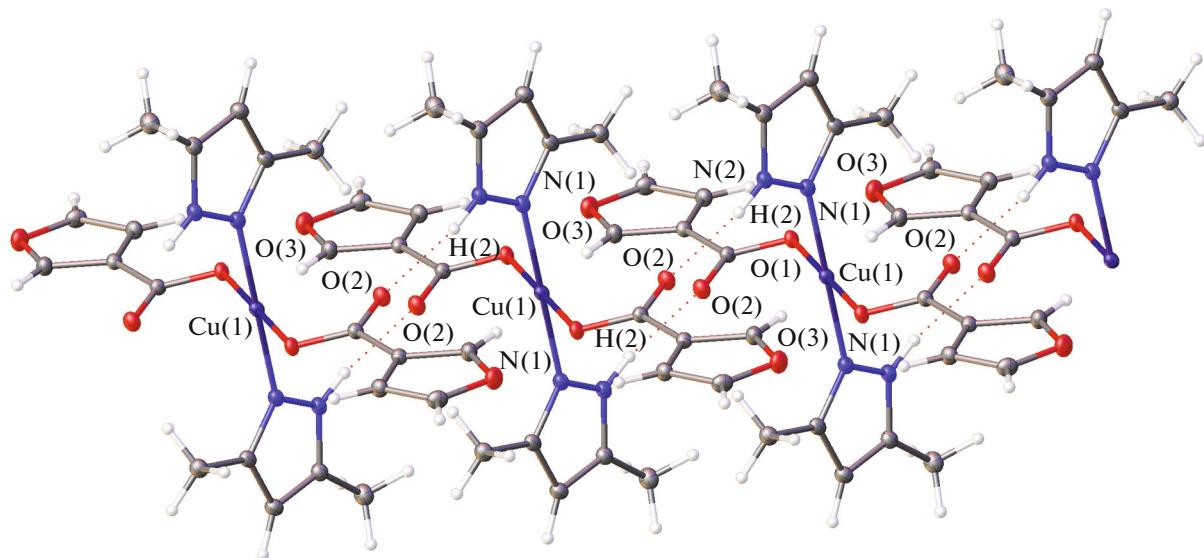


Fig. 2. Fragment of hydrogen-bonded chain in the structure of **I**.

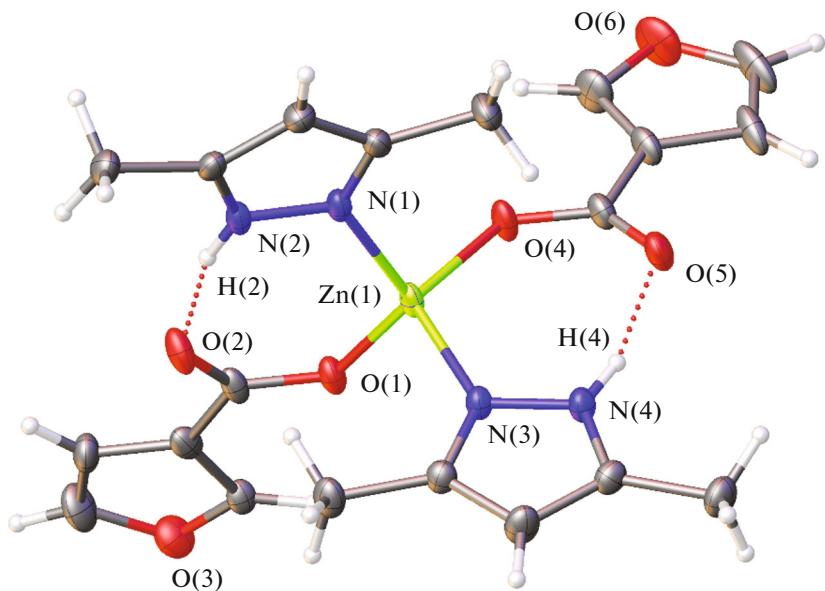


Fig. 3. Molecular structure of complex **III** with atoms represented as thermal ellipsoids (30% probability).

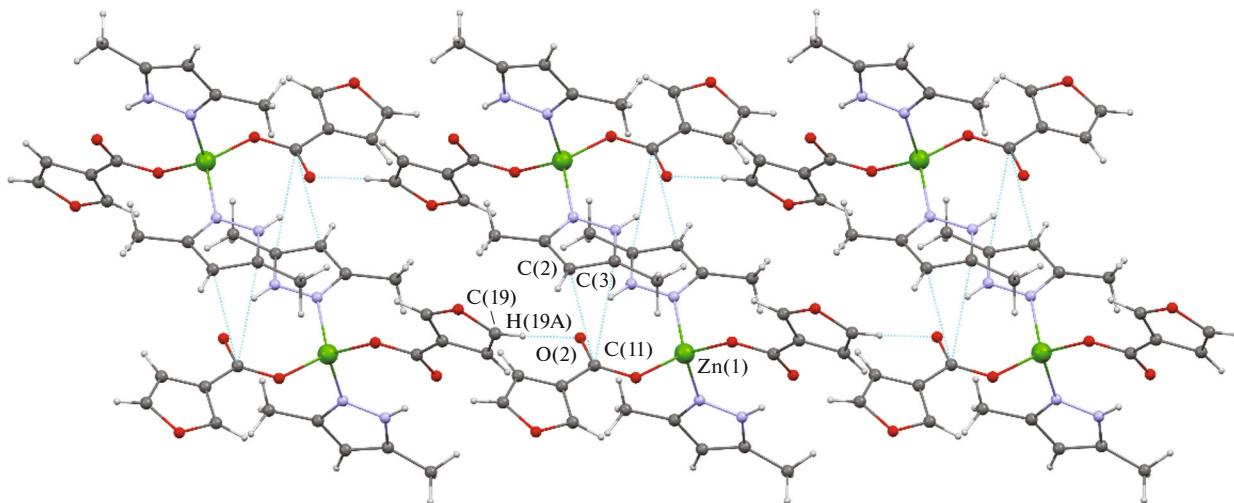


Fig. 4. Fragment of the crystal packing in the structure of **III** illustrating the formation of doubled chains.

atively weak intermolecular interactions to form double chains (Fig. 4) extended along the [1 1 0] crystallographic direction. The chains are formed via C—H...O hydrogen bonds (Table 2) between the furan 4-CH group and the carboxylate oxygen atom not bound to the metal. The parallel chains are doubled via the stacking contacts involving one of the pyrazole rings and the carboxylate group at C(11) (C(11)...C(2) distances are 3.477(2) and C(11)...C(3) distances are 3.500(2) Å). The oxygen atoms of the furan moieties do not participate in the formation of the shortened intermolecular contacts. Only usual van der Waals contacts are present between the doubled chains in the crystal. It should be noted that the crystal packing of

III coincides with that described earlier for $[\text{Zn}(\text{2-Fur})_2(\text{HDmpz})_2]$ [34] (the compounds are isostructural). Meanwhile, the crystal packing of **IV** with thiophene substituents is markedly different due to the formation of shortened C—H...S contacts and stacking interactions involving the thiophene rings [30].

The antibacterial activity of **I**–**IV** was determined against the non-pathogenic *M. smegmatis* strain. The resistance of mycobacteria to chemotherapeutic agents is known to be related to the low permeability and unusual structure of the mycobacterial cell wall. *M. smegmatis* is a fast-growing non-pathogenic bacterium; therefore, it is used to model the slowly growing *M. tuberculosis* bacterium and for the primary screen-

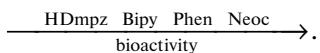
Table 3. Antibacterial activity against *M. smegmatis*

Compound	MIC, nmol/disc	Inhibition zone, mm			Ref.
	24 h	24 h	120 h		
I	300	6.4 ± 0.2*	6.1 ± 0.1		This work
II	500	6.9 ± 0.2	6.5 ± 0.12		"
III	250	6.4 ± 0.1	6.1 ± 0.1		"
IV	200	6.5 ± 0.5*	6.1 ± 0.1		"
[Cu ₂ (5NO ₂ Fur) ₄ (Phen) ₂]	1	6.8 ± 0.29*	6.5 ± 0.1*		[19]
[Zn ₂ (2-Fur) ₄] _n	1000	6.6 ± 0.3	6.5 ± 0*		[18]
[Ni(2-Fur) ₂ (Phen)(H ₂ O) ₂]	484	6.7 ± 0.3	6.7 ± 0.3		[16]
[Ni(2-Fur) ₂ (HPz) ₄]	1200	6.5 ± 0.5	0		[16]
[Zn(2-Fur) ₂ Neoc]	101	7.1 ± 0.3	6.5 ± 0.5		[18]
[Cu ₂ (2-Fur) ₄ (Py) ₂]	200	7 ± 0.5	7 ± 0.5		[12]
[Cu(2-Fur) ₂ (Phen)]	5	7 ± 0.5	7 ± 0.5		[12]
3-HFur	50	7 ± 0.5*	6 ± 0.5*		This work
HDmpz	>3000	**	**		"
HTph	2000	6.4 ± 0.1	**		"
INH	730	7.0 ± 0.5	6.5* ± 0.5		"
Rif	6	6.5 ± 0.5	6.5 ± 0.5		"

* The growth inhibition zone of the bacterial culture, which initially appeared after a few hours of growth, begins to be overgrown over the entire surface.

** No growth inhibition zone is present.

ing of antituberculosis drugs [35]. The *M. smegmatis* test system is more resistant to antibiotics and antituberculosis agents than *M. tuberculosis*. Therefore, the <100 μmol/disc concentration was used as the selection criterion [36]. The results on the biological activity in vitro for the test compounds were compared with the activities of isoniazid (INH) and rifampicin (Rif), first-line drugs for the treatment of tuberculosis, under the same experimental conditions. The compounds were applied onto the discs in various concentrations. The data on the antibacterial activity against the *M. smegmatis* mc² 155 test system and its variation with time for compounds **I–IV** are summarized in Table 3. As follows from the data, the low MIC values for **I–IV** (>200 nmol/disc) attest to low biological activity of the complexes in comparison with the reference drugs INH and Rif (Table 3). Generally, it is evident that HDmpz present in the complexes significantly reduces the effect on the *M. smegmatis* strain. Previously, we found [12–19] that the introduction of oligopyridine (1,10-phenanthroline (Phen), neocuprone (Neoc), 2,2'-bipyridine (Bipy)) into the Cu(II) and Zn(II) furoate complexes can increase the biological activity several-fold (Table 3). Thus, the previously established series of efficiency of N-donor ligands [18] against mycobacteria can be represented in the following way:



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CONFLICT OF INTEREST

The authors of this work declare that they no conflicts of interest.

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