J Therm Anal Calorim (2012) 110:167–176
DOI 10.1007/s10973-011-2173-6

Thermoanalytical investigation and biological properties of zinc(II) 4-chloro- and 5-chlorosalicylates with N-donor ligands

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CEEC-TAC1 Conference Special Issue
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Abstract New zinc(II) 4- and 5-chlorosalicylate complexes of general formula $[Zn(X\text{-sal})_2(L)_n(H_2O)_x]$ (where X-sal = 4-Clsalicylate, 5-Clsalicylate; L = *N*,*N*-diethylnicotinamide, isonicotinamide, theophylline; $n = 1, 2$; $x = 0, 1, 2, 4$) were prepared. The complexes were determined by elemental analysis and characterised by infrared spectroscopy. The thermal behaviour of the complexes was studied by simultaneous TG, DTG and DTA methods under dynamic air conditions. The thermal decomposition is a multi-step process. In the first step of the thermal decomposition, water is released in hydrated compounds. The anhydrous compounds start to decompose by the release of organic ligand, followed by chlorosalicylic acid, chlorophenol and carbon monoxide. The final solid product of the thermal decomposition is zinc oxide. The volatile products of the thermal decomposition were determined by mass spectrometry. The antimicrobial activities of the complexes were evaluated against selected pathogen and probiotic bacteria, yeasts and fungi strains. Bioactivities of the tested compounds are different against bacteria, yeasts and filamentous fungi. It was found that bacteria were more sensitive to the studied zinc(II) complex compounds than yeasts or filamentous fungi.

Keywords Chlorosalicylate · Zinc(II) · Nicotinamide derivatives · Theophylline · Thermal properties · Biological activity

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Introduction

Metal carboxylate complexes have long been the extensively studied class of compounds [1, 2]. Many metal cations play a significant role in a great number of various biological processes, as components of several vitamins and drugs. Particular attention is focused on carboxylate complexes (benzoates, salicylates and nicotinates) of biometals, e.g. zinc [3–5], which are well known to possess antimicrobial activity [6]. Salicylic acid and its derivatives (e.g. acetylsalicylic and 5-chlorosalicylic) are used as antipyretic, analgesic and anti-inflammatory drugs [7, 8]. Zinc salicylate complexes of N-, O- and S-donor ligands attract a considerable attention because of their interesting physicochemical properties, crystal structures and pronounced biological activities [9, 10]. It is well documented that the presence of organic bioactive ligand in the zinc complex can increase its bioactivity [11, 12]. Nicotinic acid (niacin, vitamin B_3) derivatives are widely used in pharmacology; e.g. *N*,*N*-diethylnicotinamide is an important respiration stimulant [13]. Another derivative isonicotinamide possesses strong antitubercular, antipyretic, fibrinolytic and antibacterial effects. Because of its strong pharmacological effects, mixed salts of isonicotinamide find extensive use as drugs in various biological and medicinal processes [14]. Theophylline is a naturally occurring alkaloid found in tea

and cocoa beans. It is well known that theophylline has anti-inflammatory effects in asthma [15]. Theophylline works as a bronchodilator by the relaxation of bronchial smooth muscle. It also has an inhibitory effect on superoxide anion release from human neutrophils [16]. Organic ligands containing N-, S- and O-donor atoms show broad biological activity and are of special interest because of the variety of ways in which they are bonded to metal ions. It is known that the presence of metal ions bonded to biologically active compounds may enhance their activities [17, 18]. From a coordination standpoint, salicylate is a versatile ligand, displaying a variety of bonding modes (e.g. monodentate, bidentate bridging or chelating, etc.) [19, 20].

As a continuation of our previous studies, we report here the synthesis, thermal, spectral and biological properties of zinc(II) 4-chloro- and 5-chlorosalicylates with *N*,*N*-diethylnicotinamide, isonicotinamide and theophylline.

Experimental

The A. R. grade chemicals were used for the preparation of studied compounds: $ZnCl_2$ (Fluka), $NaHCO_3$ (Centralchem), 4-chlorosalicylic acid (Aldrich), 5-chlorosalicylic acid (Aldrich), *N*,*N*-diethylnicotinamide (Aldrich), isonicotinamide (Aldrich) and theophylline (Fluka) (Scheme 1).

Preparation of the complexes

The following compounds were prepared:

$[Zn(4\text{-}Clsal)_2(dena)_2(H_2O)_2]$ (**I**)
$[Zn(4\text{-}Clsal)_2(ina)]$ (**II**)
$[Zn(4\text{-}Clsal)_2(H_2O)_4]\cdot 2tph\cdot 2H_2O$ (**III**)
$[Zn(5\text{-}Clsal)_2(dena)_2(H_2O)_2]$ (**IV**)
$[Zn(5\text{-}Clsal)_2(ina)_2(H_2O)]$ (**V**)

The synthesis may be expressed by these equations:

$$ZnCO_3 + 2ClC_6H_3{-}2{-}(OH)COOH \rightarrow \left[Zn\{ClC_6H_3{-}2{-}(OH)COO\}_2\right] + H_2O + CO_2$$
$$\uparrow \left[Zn\{ClC_6H_3{-}2{-}(OH)COO\}_2\right] + nL \rightarrow \left[Zn\{ClC_6H_3{-}2{-}(OH)COO\}_2L_n\right]$$

$ZnCO_3$ was prepared by the reaction of aqueous solutions of the stoichiometric amounts of $ZnCl_2$ and $NaHCO_3$. Then methanol/aqueous solution of chlorosalicylic acid was added to an aqueous suspension of $ZnCO_3$ under continual stirring in a stoichiometric amount. The reaction mixtures were stirred for 1 h. Further ethanol solution of ligands (*N*,*N*-diethylnicotinamide, isonicotinamide and theophylline), were added to the filtrate in a molar ratio of 2:1 and stirred together for 30 min. Then, the reaction mixtures were reduced to one half of volume in a water bath at 80 °C and left to crystallise at room temperature.

Instrumentation

Elemental analysis (C, H, N and Cl) was performed using a Perkin Elmer 2400 CHN Analyser. The content of zinc was determined complexometrically using Complexone III as an agent and Eriochrome black T as an indicator.

Infrared spectra of prepared compounds were recorded on AVATAR 330 FT-IR Thermo Nicolet using KBr pellets (2 mg/200 mg KBr) in the range of 4,000–400 cm^{-1}.

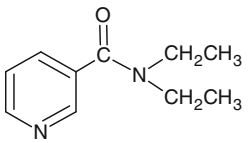
The thermal properties (TG/DTG and DTA) were studied in air atmosphere in ceramic crucibles under dynamic conditions on NETZSCH STA 409 PC/PG Thermoanalyzer (heating rate 9 °C min^{-1}).

A gas chromatograph coupled with a quadrupole mass spectrometer (HP G1800 A GCD system) was used for the determination of volatile intermediate products of thermal decomposition. The analysed sample was dissolved in chloroform/ethanol (2:1) and 1 μL of this solution was injected. The products were separated on a fused-silica capillary column (30 m × 0.25 mm) with a 0.25-μm-thick chemically bonded HP1 and ionized at 70 eV electron impact voltages.

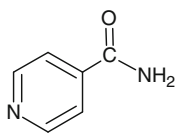
Antimicrobial assay

Antimicrobial activities of the prepared Zn(II) complexes, chlorosalicylic acids, and their sodium salts and used ligands were evaluated by a micro-dilution method [21] using G^+ bacteria *Staphylococcus aureus* CCM 3953 and G^- bacteria *Escherichia coli* CCM 3988. Effects of these

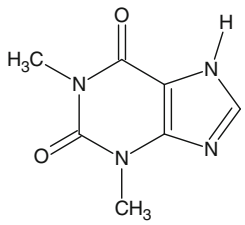
Scheme 1 Structural formulas of N,N-diethylnicotinamide (dena), isonicotinamide (ina) and theophylline (tph)



N,*N*-diethylnicotinamide



isonicotinamide



theophylline

compounds on the growth of yeasts *Candida albicans* were determined by a macro-dilution method in L-shaped tubes adapted for direct measurement of absorbance [22]. The cultures of bacteria (in Mueller–Hinton medium) and yeasts (Sabouraud–glucose medium) were incubated under vigorous shaking. Efficiencies of the prepared derivatives on the growth of filamentous fungi *Rhizopus oryzae, Alternaria alternata*, *Aspergillus fumigatus* CCM F-373 and *Microsporum gypseum* were observed by a macro-dilution technique on Sabouraud's (dermatophytes) and malt agar (other fungi) during static cultivation, and the diameters of growing fungal colonies were measured at intervals [23].

The antimicrobial activity of tested compounds was characterized by the IC_{50} values (concentration of a derivative which in comparison to the control inhibits the growth of microorganisms to 50%) and MIC values (minimal inhibitory concentration of a derivative which inhibits microbial growth by 100%). The IC_{50} and MIC values were read from toxicity curves.

The pure compounds were dissolved in dimethylsulphoxide. Concentration of tested compounds was in the range of 0.10–3.00 mmol dm^{-3} in all experiments.

Biological tests of compounds $[Zn(4\text{-}Clsal)_2(H_2O)_4]\cdot 2tph\cdot 2H_2O$, $[Zn(4\text{-}Clsal)_2(H_2O)_2]$, Na(4-Clsal) and tph were also carried out on microorganisms isolated from piglets, and the achieved results were compared with the results of $[Zn(sal)_2(tph)_2(H_2O)_2]$, $[Zn(sal)_2(H_2O)_2]$ and Na(sal). Zinc salicylates/4-chlorosalicylates and ligands were tested at a concentration of 0.01 M, which corresponds to 653.9 mg of Zn^{2+}/L culture medium. Antimicrobial effect was observed in in vitro conditions for the G^-pathogens (*E. coli* O149: $K88^+ent^+$, *Salmonella enterica* Serovar Düsseldorf SA31, *S. enterica* Serovar Typhimurium) and G^+ pathogens (*St. aureus*) and *Lactobacillus plantarum* CCM 7102. Pathogenic bacteria and lactobacilli were incubated 24 h at 37 °C in PYG broth. Medium pH was adjusted to 6.9–7.2 for pathogens, and for lactobacilli to 5.9–6.2. The numbers of bacteria were collected by plate count, and aerobic pathogens were cultured for 24 h on Mueller–Hinton agar and 48 h of anaerobic lactobacilli on MRS agar. Numbers of cfu were expresed as log cfu/g (cfu = colony forming units) [24].

Results and discussion

The prepared compounds $[Zn(4\text{-}Clsal)_2(dena)_2(H_2O)_2]$ (**I**) and $[Zn(4\text{-}Clsal)_2(ina)]$ (**II**), $[Zn(4\text{-}Clsal)_2(H_2O)_4]\cdot 2tph\cdot 2H_2O$ (**III**) are light brown coloured. Complexes $[Zn(5\text{-}Clsal)_2(dena)_2(H_2O)_2]$ (**IV**) and $[Zn(5\text{-}Clsal)_2(ina)_2(H_2O)]$ (**V**) are white. Studied compounds are stable in air and light, and their solubilities in various solvents are presented in Table 1. Elemental analyses (Table 2) are in good agreement with the calculated ones. The presence of individual functional groups was confirmed by IR spectra (Table 3).

The characteristic IR bands for the prepared compounds are reported in Table 3 and are in good accordance with the literature data [25, 26]. The IR spectra of the prepared compounds indicate the typical carboxylate stretching frequencies for the asymmetric stretching vibration $\nu_{as}(COO^-)$ in the range 1,585–1,579 cm^{-1} and for the symmetric stretching vibration $\nu_s(COO^-)$ at 1,385–1,342 cm^{-1}. The difference between asymmetric and symmetric carboxylate stretching vibrations, Δ, was calculated from ($\Delta = \nu_{as}(COO^-) - \nu_s(COO^-)$). This value was used as a criterion of carboxylate anion coordination to the central zinc atom and compared with the $\Delta_{sodium\ salt}$ of 4- and 5-chlorosalicylic acid. Lewandowski [27] suggested the following order for metal carboxylates: Δ(monodentate) > Δ(ionic) > Δ(bridging) > Δ(chelating). The Δ value for sodium 4-chlorosalicylate is 233 cm^{-1} and for sodium 5-chlorosalicylate 218 cm^{-1}. From the IR spectra, the calculated values Δ are for **I** 216 cm^{-1}, for **II** 200 cm^{-1}, for **III** 237 cm^{-1}, for **IV** 223 cm^{-1} and for **V** 224 cm^{-1}. In accordance with Lewandowski order, for compounds **III**, **IV** and **V,** we propose monodentate-binding mode of carboxylate anion and for **I** and **II** bidentate chelating-binding mode.

The coordination of the bioactive ligand (dena, ina and tph) to the central zinc(II) atom through O or N donor atom

Table 1 Solubility of the prepared compounds

Compound	Solvent							
	H_2O	CH_3OH	C_2H_5OH	$(C_2H_5)_2O$	$CHCl_3$	CCl_4	DMFA	DMSO
$[Zn(4\text{-}Clsal)_2(dena)_2(H_2O)_2]$ (**I**)	Insol	Insol	Insol	Insol	Sol	Insol	Sol	Sol
$[Zn(4\text{-}Clsal)_2(ina)]$ (**II**)	Sol	Sol	Sol	Insol	Insol	Insol	Sol	Sol
$[Zn(4\text{-}Clsal)_2(H_2O)_4]\cdot 2tph\cdot 2H_2O$ (**III**)	Sol	Sol	Sol	Insol	Insol	Insol	Sol	Sol
$[Zn(5\text{-}Clsal)_2(dena)_2(H_2O)_2]$ (**IV**)	Insol	Sol	Sol	Insol	Sol	Insol	Sol	Sol
$[Zn(5\text{-}Clsal)_2(ina)_2(H_2O)]$ (**V**)	Sol	Sol	Sol	Insol	Insol	Insol	Sol	Sol

Sol soluble, *Insol* insoluble

Table 2 The results of elemental analysis

Compound	C%		H%		N%		Cl%		Zn%	
	Exp.	Theor.	Exp.	Theor.	Exp.	Theor.	Exp.	Theor.	Exp.	Theor.
$[Zn(4\text{-Clsal})_2(dena)_2(H_2O)_2]$ (**I**) $C_{34}H_{40}N_4O_{10}Cl_2Zn$ F.W. = 801.00	50.50	50.98	4.99	5.03	6.39	6.99	8.94	8.85	8.48	8.16
$[Zn(4\text{-Clsal})_2(ina)]$ (**II**) $C_{20}H_{14}N_2O_7Cl_2Zn$ F.W. = 530.64	45.47	45.27	3.07	2.67	5.54	5.28	13.67	13.36	12.11	12.32
$[Zn(4\text{-Clsal})_2(H_2O)_4]\cdot 2tph\cdot 2H_2O$ (**III**) $C_{28}H_{32}N_8O_{14}Cl_2Zn$ F.W. = 840.90	40.22	39.99	3.95	3.84	13.48	13.33	8.71	8.43	7.89	7.78
$[Zn(5\text{-Clsal})_2(dena)_2(H_2O)_2]$ (**IV**) $C_{34}H_{40}N_4O_{10}Cl_2Zn$ F.W. = 801.00	50.69	50.98	4.82	5.03	6.84	6.99	8.70	8.85	8.03	8.16
$[Zn(5\text{-Clsal})_2(ina)_2(H_2O)]$ (**V**) $C_{26}H_{22}N_4O_9Cl_2Zn$ F.W. = 670.75	46.73	46.56	3.08	3.31	8.23	8.35	10.52	10.57	9.43	9.75

Table 3 Characteristic absorption bands $\nu[cm^{-1}]$ in IR spectra of **I–V**

Assignment	Compound				
	I	**II**	**III**	**IV**	**V**
$\nu(O-H)_{H_2O}$	3433 vs	–	–	3381 m	3485 s
$\nu_{as}(N-H)$	–	3361 s, 3323 s	3373 s	–	3361 s, 3320 s
$\nu_s(N-H)$	–	3180 s	3128 m	–	3108 vs
$\nu_{ar}(C-H)$	3072 w	3070 m	3005 m	3066 m	3061w
$\nu(C-H)_{-CH_3}$	2983 vs, 2937 s	–	2914 m	2978 m, 2937 m	–
$\nu(N-CH_2)$	2752 m	–	–	2762 w	
$\nu(C=O)$	1636 vs	1697 vs	1701 vs	1638 vs	1697 vs
$\delta(O-H)_{H_2O}$	1612 vs	–	1666 vs	1610 vs	1640 vs
$\delta(N-H)$	–	1625 s	1643 vs	–	1625 vs
$\nu_{as}(COO^-)$	1579 vs	1585 s	1579 s	1585 vs	1585 vs
$\nu_s(COO^-)$	1363 vs	1385 s	1342 m	1362 s	1361 vs
$\delta_{as}(C-H)_{-CH_3}$	1435 vs	–	1421 s	1435 vs	–
$\delta_s(C-H)_{-CH_3}$	1306 s	–	1300 m	1317 m	–
$\nu(C-Cl)$	787 m	781 m	764 m	750 m	781 m
$\gamma(N-H)$	710 s	719 m	721 m	724 m	703 w
$\gamma_{ar}(C-H)$	698 s	705 m	704 m	715 s	694 m
$\delta(COO^-)$	646 m	694 m	675 m	694 s	646 m
$\Delta(COO^-)$	216	200	237	223	224

I $[Zn(4\text{-Clsal})_2(dena)_2(H_2O)_2]$, **II** $[Zn(4\text{-Clsal})_2(ina)]$, **III** $[Zn(4\text{-Clsal})_2(H_2O)_4]\cdot 2tph\cdot 2H_2O$, **IV** $[Zn(5\text{-Clsal})_2(dena)_2(H_2O)_2]$, **V** $[Zn(5\text{-Clsal})_2(ina)_2(H_2O)]$

s strong, *m* medium, *w* weak, *ar* aromatic, *as* asymmetric, *vs* very strong

can be predicted according to the shift of the stretching vibration of carbonyl group ν(C=O) of ligand. In compounds with dena and ina (**I**, **II**, **IV** and **V**) the values of ν(C=O) are shifted to a higher wavenumber (1636, 1697, 1638 and 1697 cm^{-1}) compared to the free ligands ($\nu(C=O)_{dena}$ = 1,632 cm^{-1}, $\nu(C=O)_{ina}$ = 1,666 cm^{-1}). It indicates that the pyridine nitrogen atom of these ligands is involved in coordination with zinc, and therefore, the

electron density is shifted towards the pyridine nitrogen, leading to the increase in the double-bond character of the carbonyl group and the shift of the stretching vibration ν(C=O) to a higher value. This prediction is proved by the solved crystal structure of compound $[Zn(5\text{-Clsal})_2(ina)_2(H_2O)]$ (shift of ν(C=O) towards higher wavenumber by 31 cm^{-1}) [28].

Thermal behaviour

*[Zn(4-Clsal)$_2$(dena)$_2$(H$_2$O)$_2$] (**I**)*

The thermal decomposition of **I** (Fig. 1) starts at 60 °C and water is released, which is shown on the DTA curve as endothermic effect at 100 °C (the experimental mass loss 4.66%; the theoretical mass loss 4.50%). In the next step of the thermal decomposition, *N*,*N*-diethylnicotinamide (*m/z* 178) is liberated, accompanied by endothermic effect at 150 °C (the experimental mass loss 44.35%; the theoretical mass loss 44.50%). Further, the release of 4-chlorosalicylic acid (*m/z* 172 [$C_7H_5O_3Cl^{+\cdot}$], 154 [$C_7H_3O_2Cl^{+\cdot}$], 126 [$C_6H_3OCl^{+\cdot}$] and 63 [$C_5H_3^{+\cdot}$]) accompanied by endothermic effect on the DTA curve at 260 °C takes place (the experimental mass loss 21.17%; the theoretical mass loss 21.54%). In the last step of the thermal decomposition, 3-chlorophenol (*m/z* 128 [$C_6H_5OCl^{+\cdot}$] and 65 [$C_5H_5^{+\cdot}$]) and CO are liberated accompanied by exothermic effect in the temperature range of 400–850 °C (the experimental mass loss 17.92%; the theoretical mass loss 19.48%). The final solid product of the thermal decomposition is ZnO (the experimental mass value 11.95%; the theoretical mass value 10.16%) (Table 4). The following equations are proposed for the process of the thermal decomposition:

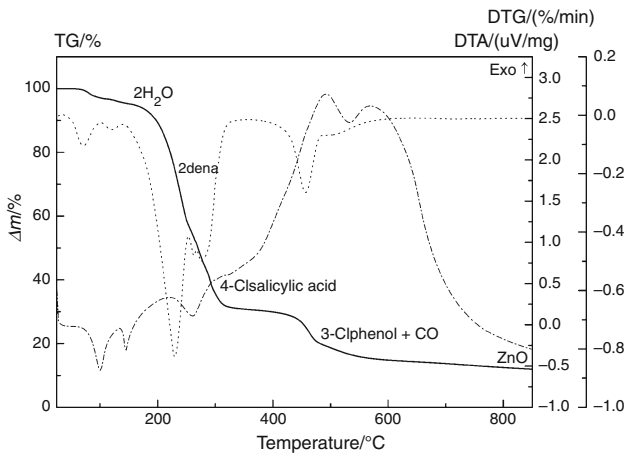


Fig. 1 TG/DTG and DTA curves of $[Zn(4\text{-Clsal})_2(dena)_2(H_2O)_2]$ (**I**)

$$\left[Zn\{4\text{-}ClC_6H_3\text{-}2\text{-}(OH)COO\}_2(dena)_2(H_2O)_2\right] \xrightarrow{60-145\,^{\circ}C} \left[Zn\{4\text{-}ClC_6H_3\text{-}2\text{-}(OH)COO\}_2(dena)_2\right] + 2H_2O$$

$$\left[Zn\{4\text{-}ClC_6H_3\text{-}2\text{-}(OH)COO\}_2(dena)_2\right] \xrightarrow{145-255\,^{\circ}C} \left[Zn\{4\text{-}ClC_6H_3\text{-}2\text{-}(OH)COO\}_2\right] + 2dena$$

$$\left[Zn\{4\text{-}ClC_6H_3\text{-}2\text{-}(OH)COO\}_2\right] \xrightarrow{255-850\,^{\circ}C} 4\text{-}ClC_6H_3\text{-}2\text{-}(OH)COOH + 3\text{-}ClC_6H_4OH + CO + ZnO$$

*[Zn(4-Clsal)$_2$(ina)] (**II**)*

At the beginning of thermal decomposition, **II** (Fig. 2) melts without mass loss, which is shown on the DTA curve as endothermic effect at 100 °C. Above this temperature, mass loss corresponding to the release of isonicotinamide (*m/z* 122) takes place, shown on the DTA curve as endothermic effect at 210 °C (the experimental mass loss 22.03%; the theoretical mass loss 22.99%). The release of 4-chlorosalicylic acid (*m/z* 172 [$C_7H_5O_3Cl^{+\cdot}$], 154 [$C_7H_3O_2Cl^{+\cdot}$], 126 [$C_6H_3OCl^{+\cdot}$] and 63 [$C_5H_3^{+\cdot}$]) takes place accompanied by endothermic effect at 300 °C (the experimental mass loss 33.18%; the theoretical mass loss 32.52%). In the next step of the thermal decomposition, the release of 3-chlorophenol (*m/z* 128 [$C_6H_5OCl^{+\cdot}$] and 65 [$C_5H_5^{+\cdot}$]) and CO takes place, which is shown on the DTA curve as exothermic effect in the temperature range of 325–850 °C (the experimental mass loss 28.34%; the theoretical mass loss 29.40%). The final product of the thermal decomposition is ZnO (the experimental mass value 16.45%; the theoretical mass value 15.34%) (Table 4). The following equations are proposed for the process of the thermal decomposition:

$$\left[Zn\{4\text{-}ClC_6H_3\text{-}2\text{-}(OH)COO\}_2(ina)\right] \xrightarrow{140-245\,^{\circ}C} \left[Zn\{4\text{-}ClC_6H_3\text{-}2\text{-}(OH)COO\}_2\right] + ina$$

$$\left[Zn\{4\text{-}ClC_6H_3\text{-}2\text{-}(OH)COO\}_2\right] \xrightarrow{245-850\,^{\circ}C} 4\text{-}ClC_6H_3\text{-}2\text{-}(OH)COOH + 3\text{-}ClC_6H_4OH + CO + ZnO$$

*[Zn(4-Clsal)$_2$(H$_2$O)$_4$]·2tph·2H$_2$O (**III**)*

The complex compound **III** starts to decompose at 65 °C (Fig. 3) with the release of water, shown on the DTA curve as endothermic effect at 100 °C (the experimental mass loss 11.19%; the theoretical mass loss 12.33%) (Table 4). In the next step of the thermal decomposition, theophylline (*m/z* 180) and 4-chlorosalicylic acid (*m/z* 172 [C_7H_5

Table 4 Thermal decompositions of the prepared compounds

Compounds	Temperature range/DTA/T/°C	Products of the thermal decomposition	Mass loss/%	
			Exp.	Theor.
$[Zn(4\text{-Clsal})_2(dena)_2(H_2O)_2]$	100 endo	$2H_2O$	4.66	4.50
	150 endo	2dena	44.35	44.50
	260 endo	4-Clsalicylic acid	21.17	21.54
	400–800 exo	3-Clphenol + CO	17.92	19.48
	850	ZnO	11.95	10.16
$[Zn(4\text{-Clsal})_2(ina)]$	100 endo	Melting	–	–
	210 endo	ina	22.03	22.99
	300 endo	4-Clsalicylic acid	33.18	32.52
	325–750 exo	3-Clphenol + CO	28.34	29.40
	850	ZnO	16.45	15.34
$[Zn(4\text{-Clsal})_2(H_2O)_4]\cdot 2tph\cdot 2H_2O$	100 endo	$4H_2O$	11.19	12.33
	270 endo	2tph + 4-Clsalicylic acid	61.04	60.77
	415–780 exo	3-Clphenol + CO	18.49	17.86
	800	ZnO	8.72	9.28
$[Zn(5\text{-Clsal})_2(dena)_2(H_2O)_2]$	90 endo	$2H_2O$	4.33	4.50
	225 endo	2dena	44.36	44.50
	315 endo	5-Clsalicylic acid	20.84	21.54
	350–650 exo	4-Clphenol + CO	20.70	19.48
	850	ZnO	9.77	10.16
$[Zn(5\text{-Clsal})_2(ina)_2(H_2O)]$	95 endo	H_2O	2.62	2.69
	195, 235 endo	2ina + 5-Clsalicylic acid	61.42	62.14
	385–700 exo	4-Clphenol + CO	23.83	23.34
	800	ZnO	12.25	12.13

endo endothermic effect, *exo* exothermic effect, *dena N,N*-diethylnicotinamide, *ina* isonicotinamide, *tph* theophylline

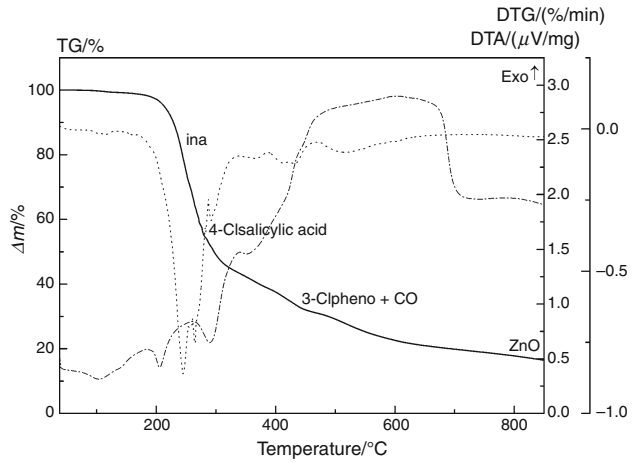


Fig. 2 TG/DTG and DTA curves of $[Zn(4\text{-Clsal})_2(ina)]$ (**II**)

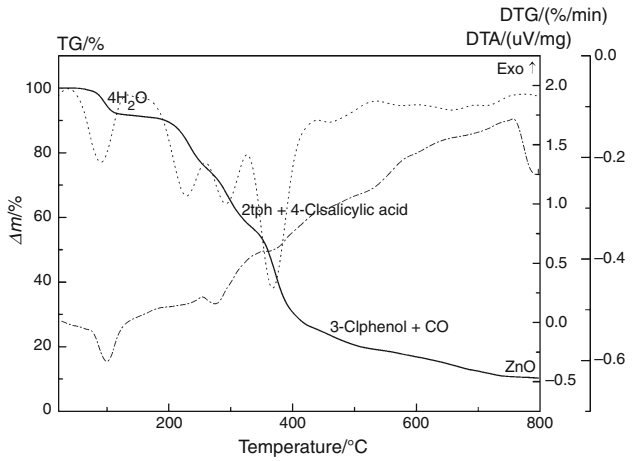


Fig. 3 TG/DTG and DTA curves of $[Zn(4\text{-Clsal})_2(H_2O)_4]\cdot 2tph\cdot 2H_2O$ (**III**)

$O_3Cl^{+\cdot}$], 154 [$C_7H_3O_2Cl^{+\cdot}$], 126 [$C_6H_3OCl^{+\cdot}$] and 63 [$C_5H_3^{+\cdot}$]) are liberated, accompanied by endothermic effect on the DTA curve at 270 °C (the experimental mass loss 61.04%, the theoretical mass loss 60.77%). The thermal decomposition continues in the temperature range of 415–800 °C with the release of 3-chlorophenol (*m/z* 128 [$C_6H_5OCl^{+\cdot}$] and 65 [$C_5H_5^{+\cdot}$]) and CO (the experimental mass loss 18.49%, the theoretical mass loss 17.86%).

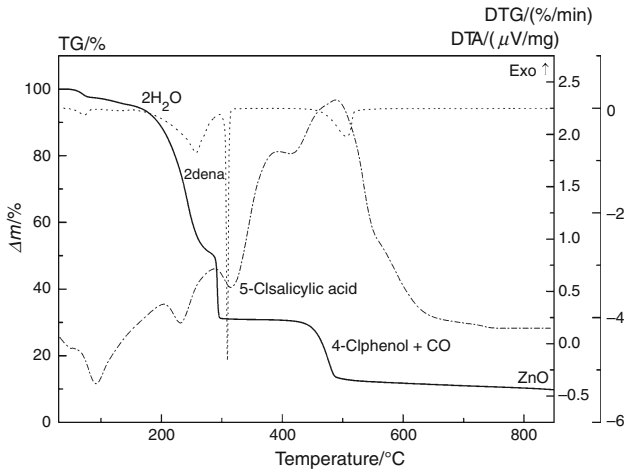


Fig. 4 TG/DTG and DTA curves of $[Zn(5\text{-}Clsal)_2(dena)_2(H_2O)_2]$ (**IV**)

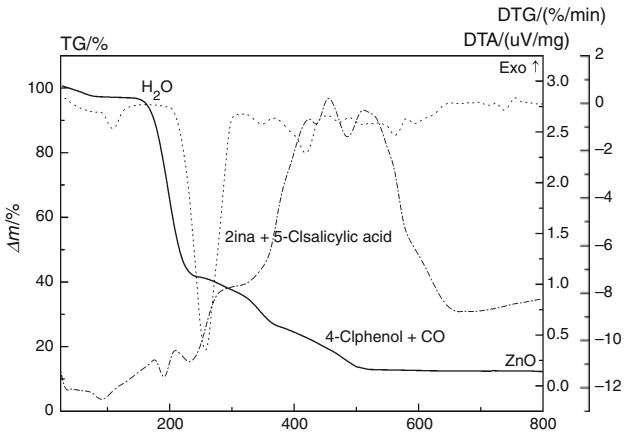


Fig. 5 TG/DTG and DTA curves of $[Zn(5\text{-}Clsal)_2(ina)_2(H_2O)]$ (**V**)

The final product of the thermal decomposition is ZnO (the experimental mass value 9.28%; the theoretical mass value 8.72%). The thermal decomposition can be expressed by the following equations:

$$[Zn\{4\text{-}ClC_6H_3\text{-}2\text{-}(OH)COO\}_2(H_2O)_4]\cdot 2tph\cdot 2H_2O \xrightarrow{65-130\,^{\circ}C} [Zn\{4\text{-}ClC_6H_3\text{-}2\text{-}(OH)COO\}_2]\cdot 2tph + 6H_2O$$

$$[Zn\{4\text{-}ClC_6H_3\text{-}2\text{-}(OH)COO\}_2]\cdot 2tph \xrightarrow{130-780\,^{\circ}C} 2tph + 4\text{-}ClC_6H_3\text{-}2\text{-}(OH)COOH + 3\text{-}ClC_6H_4OH + CO + ZnO$$

*[Zn(5-Clsal)$_2$(dena)$_2$(H$_2$O)$_2$] (**IV**)*

The thermal decomposition of **IV** shown in Fig. 4 starts in the temperature range of 55–135 °C with the release of water, shown on the DTA curve as an endothermic effect at 90 °C (the experimental mass loss 4.33%; the theoretical mass loss 4.50%). In the temperature range of 135–280 °C, the release of *N*,*N*-diethylnicotinamide (*m/z* 178) takes place (the experimental mass loss 44.36%; the theoretical mass loss 44.50%) accompanied by endothermic effect on the DTA curve at 225 °C. In the next step of the thermal decomposition, 5-chlorosalicylic acid (*m/z* 172 $[C_7H_5O_3Cl^{+\cdot}]$, 154 $[C_7H_3O_2Cl^{+\cdot}]$, 126 $[C_6H_3OCl^{+\cdot}]$ and 63 $[C_5H_3^{+\cdot}]$) is liberated, which is shown on the DTA curve as endothermic effect at 315 °C (the experimental mass loss 20.84%; the theoretical mass loss 21.54%). Further, 4-chlorophenol (*m/z* 128 $[C_6H_5OCl^{+\cdot}]$ and 65 $[C_5H_5^{+\cdot}]$) and CO are released, shown on the DTA curve as exothermic effect (the experimental mass loss 20.70%; the theoretical mass loss 19.48%). The final product of the thermal decomposition is ZnO (the experimental mass value 9.77%; the theoretical mass value 10.16%) (Table 4). The thermal decomposition is expressed by the following equations:

$$\left[Zn\{5\text{-}ClC_6H_3-2-(OH)COO\}_2(dena)_2(H_2O)_2\right] \xrightarrow{55-135\,^{\circ}C} [Zn\{5\text{-}ClC_6H_3-2-(OH)COO\}_2(dena)_2] + 2H_2O$$

$$[Zn\{5\text{-}ClC_6H_3-2-(OH)COO\}_2(dena)_2] \xrightarrow{135-280\,^{\circ}C} [Zn\{5\text{-}ClC_6H_3-2-(OH)COO\}_2] + 2dena$$

$$[Zn\{5\text{-}ClC_6H_3-2-(OH)COO\}_2] \xrightarrow{280-650\,^{\circ}C} 5\text{-}ClC_6H_3-2-(OH)COOH + 4\text{-}ClC_6H_4OH + CO + ZnO$$

*[Zn(5-Clsal)$_2$(ina)$_2$(H$_2$O)] (**V**)*

Complex compound **V** starts to decompose at 65 °C with the release of water accompanied by endothermic effect at 95 °C (the experimental mass loss 2.62%; the theoretical mass loss 2.69%) (Fig. 5). In the next step of the thermal decomposition, isonicotinamide and 5-chlorosalicylic acid (*m/z* 172 $[C_7H_5O_3Cl^{+\cdot}]$, 154 $[C_7H_3O_2Cl^{+\cdot}]$, 126 $[C_6H_3OCl^{+\cdot}]$ and 63 $[C_5H_3^{+\cdot}]$) are released in the temperature range of 140–385 °C, shown on the DTA curve as endothermic effects at 195 and 235 °C (the experimental mass loss 61.42%; the theoretical mass loss 62.14%). In the temperature range of 385–700 °C, 4-chlorophenol (*m/z* 128 $[C_6H_5OCl^{+\cdot}]$ and 65 $[C_5H_5^{+\cdot}]$) and CO are released (the experimental mass loss 23.83%; the theoretical mass loss 23.34%). The final product of the thermal decomposition is ZnO (the experimental mass value 12.25%; the theoretical mass value 12.13%). The thermal decomposition is expressed by the following equations:

$$\left[Zn\{5\text{-}ClC_6H_3\text{-}2\text{-}(OH)COO\}_2(ina)_2(H_2O)\right] \xrightarrow{65-140\,^{\circ}C} \left[Zn\{5\text{-}ClC_6H_3\text{-}2\text{-}(OH)COO\}_2(ina)_2\right] + H_2O$$

$$\left[Zn\{5\text{-}ClC_6H_3\text{-}2\text{-}(OH)COO\}_2(ina)_2\right] \xrightarrow{140-700\,^{\circ}C} 2ina + 5\text{-}ClC_6H_3\text{-}2\text{-}(OH)COOH + 4\text{-}ClC_6H_4OH + CO + ZnO$$

Antimicrobial activities

Results of quantitative determination of antimicrobial activity characterized by IC_{50} and MIC values are presented in Table 5 and as log cfu/ml in Fig. 6. Bioactivities of the tested compounds are different against bacteria, yeasts and filamentous fungi. Free carboxylic acids and organic ligands did not affect the growth of model pathogen and probiotic bacteria, yeasts and filamentous fungi ($IC_{50} > 2$ or >3 mmol dm^{-3}), except 5-chlorosalicylic acid against *A. alternata* and tph against *M. gypseum*. Free 4-chlorosalicylic acid has shown antifungal efficiency against all the tested filamentous fungi (IC_{50} = 1.30–2.40 mmol dm^{-3}), the most sensitive of which was *M. gypseum* (IC_{50} = 1.30 mmol dm^{-3}). Unlike the sodium salts of carboxylic acids, which did not influence the growth of model microorganisms (IC_{50}, MIC > 3 mmol dm^{-3}), zinc carboxylates

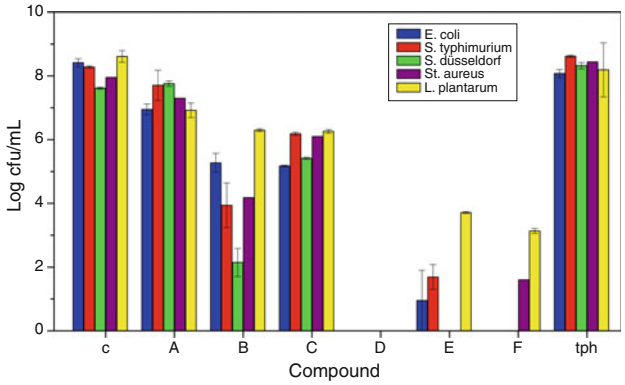


Fig. 6 Antimicrobial efficiency of selected compounds against pathogen and probiotic bacteria. *c* control sample, *A* Na(sal), *B* Na(4-Clsal), *C* $[Zn(sal)_2(H_2O)_2]$, *D* $[Zn(4\text{-}Clsal)_2(H_2O)_2]$, *E* $[Zn(sal)_2(tph)_2(H_2O)_2]$, *F* $[Zn(4\text{-}Clsal)_2(H_2O)_4]\cdot 2tph\cdot 2H_2O$

Table 5 Antimicrobial activities of Zn(II) complexes characterised by IC_{50} and MIC values (mmol dm^{-3})

Compound	Bacteria				Yeasts		Filamentous fungi							
	St. aureus		*E. coli*		*C. albicans*		*R. oryzae*		*A. alternata*		*A. fumigatus*		*M. gypseum*	
	IC_{50}	MIC	IC_{50}	MIC	IC_{50}	MIC	IC_{50}	MIC	IC_{50}	MIC	IC_{50}	MIC	IC_{50}	MIC
I	0.51	3^c	0.53	>3	0.54	3^s	2.00	>3	1.30	3^c	–	–	2.00	3^c
II	0.52	3^s	0.51	3^s	0.39	2^s	1.70	>3	1.40	2^s	–	–	1.30	2^s
III	1.30	2	1.00	2	2.30	>3	2.50	>3	1.60	3^s	1.50	>3	1.20	2^c
IV	0.54	3^s	0.50	3^s	0.52	2^s	1.20	>3	1.50	3^s	–	–	1.00	3^c
V	0.58	2^s	0.78	2^s	1.70	>3	1.60	3^c	1.00	2^c	1.00	3^s	1.00	2^c
VI	>3	>3	>3	>3	>3	>3	>3	>3	>3	>3	–	–	>3	>3
VII	>3	>3	>3	>3	>3	>3	2.40	>3	1.50	>3	1.80	>3	1.30	2^c
VIII	0.80	>3	0.68	3^s	1.10	3^s	–	–	3.00	>3	–	–	>3	>3
IX	0.55	3^s	0.51	3^s	0.45	2^s	2.70	>3	2.40	>3	–	–	>3	>3
X	0.50	3^s	1.10	3^s	0.55	2^s	2.00	>3	2.50	>3	–	–	2.50	>3
XI	0.51	2^s	0.70	3^s	2.30	>3	>3	>3	1.00	>3	1.70	>3	0.60	2^c
XII	>3	>3	>3	>3	>3	>3	>3	>3	>3	>3	–	–	>3	>3
XIII	>3	>3	>3	>3	>3	>3	>3	>3	2.40	>3	>3	>3	>3	>3
XIV	>2	>2	>2	>2	>2	>2	>3	>3	>3	>3	–	–	>3	>3
XV	>2	>2	>2	>2	>2	>2	>3	>3	>3	>3	–	–	>3	>3
XVI	>3	>3	>3	>3	>3	>3	>3	>3	>3	>3	>3	>3	2.30	>3
XVII	>2	>2	>2	>2	>2	>2	>3	>3	>3	>3	–	–	>3	>3

I $[Zn(4\text{-}Clsal)_2(dena)_2(H_2O)_2]$, **II** $[Zn(4\text{-}Clsal)_2(ina)]$, **III** $[Zn(4\text{-}Clsal)_2(H_2O)_4]\cdot 2tph\cdot 2H_2O$, **IV** $[Zn(4\text{-}Clsal)_2(Menia)_2(H_2O)_2]$, **V** $[Zn(4\text{-}Clsal)_2(H_2O)_2]$, **VI** $4\text{-}ClC_6H_3\text{-}2\text{-}(OH)COONa$, **VII** 4-chlorosalicylic acid, **VIII** $[Zn(5\text{-}Clsal)_2(dena)_2(H_2O)_2]$, **IX** $[Zn(5\text{-}Clsal)_2(ina)_2(H_2O)]$, **X** $[Zn(5\text{-}Clsal)_2(Menia)_2(H_2O)_2]$, **XI** $[Zn(5\text{-}Clsal)_2(H_2O)_2]$, **XII** $5\text{-}ClC_6H_3\text{-}2\text{-}(OH)COONa$, **XIII** 5-chlorosalicylic acid, **XIV** *N,N*-diethylnicotinamide (dena), **XV** isonicotinamide (ina), **XVI** theophylline (tph), **XVII** *N*-methylnicotinamide (Menia)

[c] Microbicidal effect

[s] Microbistatical effect

increased the inhibitory activity ($IC_{50} = 0.39$–3.00 mmol dm^{-3}). In general, zinc chlorosalicylates compared with zinc salicylate showed higher antimicrobial activity. The presence of bioactive ligands in zinc complexes increased the antimicrobial activity of zinc salicylates and chlorosalicylates in comparison with free acids and ligands (Table 5; Fig. 6).

Conclusions

In the present article, the thermal decompositions of the following compounds were studied: $[Zn(4\text{-Clsal})_2(dena)_2(H_2O)_2]$, $[Zn(4\text{-Clsal})_2(ina)]$, $[Zn(4\text{-Clsal})_2(H_2O)_4]\cdot 2tph\cdot 2H_2O$, $[Zn(5\text{-Clsal})_2(dena)_2(H_2O)_2]$ and $[Zn(5\text{-Clsal})_2(ina)_2(H_2O)]$. It was found that the thermal decomposition is a multistep process. In hydrated compounds, during the thermal decomposition, water is released in the first step. In the next steps, organic ligand, chlorosalicylic acid, chlorophenol and carbon monoxide are liberated. The final product of the thermal decomposition in all the studied compounds is zinc oxide. The volatile intermediates of the thermal decomposition were determined by mass spectrometry. The thermal stabilities of dehydrated compounds decrease in the following order:

$$\begin{aligned}&\left[Zn(4\text{-Clsal})_2(dena)_2\right](145\,^\circ C) > Zn(4\text{-Clsal})_2(ina)\\&(140\,^\circ C) = Zn(5\text{-Clsal})_2(ina)_2](140\,^\circ C)\\&> \left[Zn(5\text{-Clsal})_2(dena)_2\right](135\,^\circ C) > \left[Zn(4\text{-Clsal})_2\right]\\&\cdot 2tph(130\,^\circ C).\end{aligned}$$

On the basis of the infrared spectra, the Δ values of asymmetric and symmetric stretching vibrations of COO^- anion were calculated, and then the binding mode of carboxylate anion was proposed. For compounds $[Zn(4\text{-Clsal})_2(H_2O)_4]\cdot 2tph\cdot 2H_2O$, $[Zn(5\text{-Clsal})_2(dena)_2(H_2O)_2]$ and $[Zn(5\text{-Clsal})_2(ina)_2(H_2O)]$, we propose monodentate-binding mode of carboxylate anion to the central zinc atom, and for compounds $[Zn(4\text{-Clsal})_2(dena)_2(H_2O)_2]$ and $[Zn(4\text{-Clsal})_2(ina)]$ we propose bidentate chelating carboxylate-binding mode.

Based on the results of antimicrobial activities of the tested compounds, we can conclude that bacteria are more sensitive to the tested zinc(II) compounds than yeasts and filamentous fungi. Furthermore, zinc(II) complexes with/without organic ligands are more effective against the tested microorganisms than free parent acids or free heterocyclic ligands.

Acknowledgements The financial support of the Slovak Ministry of Education (VEGA project No. 1/0122/08) for this study is gratefully acknowledged.

References

1. Melník M, Győryová K, Skoršepa J, Holloway CE. Zinc(II) compounds: classification and analysis of crystallographic and structural data. J Coord Chem. 1995;35:179–279.
2. Győryová K, Szunyogová E, Kovářová J, Hudecová D, Mudroňová D, Juhászová E. Biological and physicochemical study of zinc(II) propionate complexes with N-donor heterocyclic ligands. J Therm Anal Calorim. 2003;72:587–96.
3. Findoráková L, Győryová K, Večerníková E, Balek V. Use of emanation thermal analysis and evolved gas analysis in thermal study of zinc(II) benzoate complex compounds. J Therm Anal Calorim. 2009;98:765–9.
4. Győryová K, Chomič J, Szunyogová E, Piknová L, Zeleňák V, Vargová Z. Thermal study of zinc(II) 4-chlorosalicylate complex compounds with bioactive ligands. J Therm Anal Calorim. 2006;84:727–32.
5. Vargová Z, Zeleňák V, Císařová I, Győryová K. Correlation of thermal and spectral properties of zinc(II) complexes of pyridinecarboxylic acids with their crystal structures. Thermochim Acta. 2004;423:149–57.
6. Krajníková A, Győryová K, Hudecová D, Kovářová J, Vargová Z. Thermal decomposition and antimicrobial activity of zinc(II) 2-bromobenzoates with organic ligands. J Therm Anal Calorim. 2011;105:451–60.
7. Hermann M, Kapiotis S, Hofbauer R, Exner M, Seelos Ch, Held I, Gmeiner B. Salicylate inhibits LDL oxidation initiated by superoxide/nitric oxide radicals. FEBS Lett. 1999;445(1):212–4.
8. Diamantis W, Kohlhepp WC, Haertlein B, Melton J, Sofia RD. Meseclazone, 5-chlorosalicylic acid and acetylsalicylic acid. Comparison of their effects on in vitro and ex vivo platelet aggregation. Thromb Haemost. 1978;40(1):24–36.
9. Bujdošová Z, Győryová K, Kovářová J, Hudecová D, Halás L. Synthesis, biological and physicochemical properties of Zinc(II) salicylate and 5-chlorosalicylate complexes with theophylline and urea. J Therm Anal Calorim. 2009;98:151–9.
10. Krajníková A, Gyepes R, Győryová K. Crystal Structure of $[Zn(2\text{-Bromobenzoato})_2]_n$ and $[Zn(2\text{-Bromobenzoato})_2(N\text{-Methylnicotinamide})]_2$. J Chem Crystallogr. 2010;40:650–5.
11. Zeleňák V, Győryová K, Mlynarčík D. Antibacterial and antifungal activity of zinc(II) carboxylates with/without N-donor organic ligands. Metal Based Drugs. 2002;8(5):269–74.
12. Szunyogová E, Győryová K, Hudecová D, Piknová L, Chomič J, Vargová Z, Zeleňák V. Thermal, spectral and biological properties of Zn(II) complex compounds with phenazone. J Therm Anal Calorim. 2007;88(1):219–23.
13. Bigoli F, Braibanti A, Pellinghelli MA, Tiripicchio A. The Crystal and Molecular Structure of Mono-(*N*,*N*-diethylnicotinamide)cadmium dithiocyanate. Acta Crystallogr. 1972;B28:962–6.
14. Ahuja IS, Prasad I. Isonicotinamide complexes with some metal(II) halides and pseudohalides. Inorg Nucl Chem Lett. 1976;12(10):777–84.
15. Barnes PJ, Drazen JM, Rennard SI, Thomson NC. Asthma and COPD: basic mechanisms and clinical management. 2nd ed. London: Elsevier; 2009. p. 627.
16. Nielson CP, Crawley JJ, Morgan ME, Vestal RE. Polymorphonuclear leukocyte inhibition by therapeutic concentrations is facilitated by cyclic-3′,5′ adenosine monophosphate. Am Rev Respir Dis. 1988;137:25–30.
17. Ferrari MB, Capacchi S, Pelosi G, Reffo G, Tarasconi P, Albertini R, Pinelli S, Lunghi P. Synthesis, structural characterization and biological activity of helicin thiosemicarbazone monohydrate and a copper(II) complex of salicylaldehyde thiosemicarbazone. Inorg Chim Acta. 1999;286:134–41.

18. Bujdošová Z, Győryová K, Hudecová D, Kovářová J, Halás L. Preparation, spectral, thermal, and biological properties of zinc(II) 4-chloro- and 5-chlorosalicylate complexes with methyl-3-pyridylcarbamate and phenazone. Chem Pap. 2010;64(5):584–91.
19. Bujdošová Z, Győryová K, Melník M, Koman M, Kovářová J. Structures and characterization of [Zn(*n*-chlorosalicylato)$_2$(*N*-methylnicotinamide)$_2$(H_2O)$_2$] ($n = 4$ or 5) ligand isomers. J Chem Crystallogr. 2010;41:443–8.
20. Findoráková L, Győryová K, Koman M, Moncoľ J, Melnik M. Crystal structure and physical characterization of [Zn_2(Benzoato)$_4$(Caffeine)$_2$]·2 caffeine. J Chem Crystallogr. 2010;40:145–50.
21. Jantová S, Hudecová D, Stankovský S, Špirková K, Ružeková Ľ. Antibacterial effect of substituted 4-quinazolylhydrazines and their arylhydrazones determined by a modified microdilution method. Folia Microbiol. 1995;40:611–4.
22. Dudová B, Hudecová D, Pokorný R, Mikulášová KM, Palicová M, Segľa P, et al. Copper complexes with bioactive ligands. Folia Microbiol. 2001;46:379–84.
23. Hudecová D, Jantová S, Melník M, Uher M. New azidometalkojates and their biological activity. Folia Microbiol. 1996;41:473–6.
24. Mudroňová D, Nemcová R, Lauková A, Koščová J, Strompfová V, Győryová K, Szunyogová E, Lazar G. Effect of *Lactobacillus fermentum* alone, and in combination with zinc (II) propionate on *Salmonella enterica* serovar Duesseldorf in Japanese quails. Biologia. 2006;61:797–801.
25. Nakamoto K. Infrared and Raman spectra of inorganic and coordination compounds. New York: Wiley; 1997.
26. Miller RGJ, Willis HA. IRSCOT-infrared structural correlation tables and data cards. London: Heyden; 1969.
27. Lewandowski W, Kalinowska M, Lewandowska H. The influence of metals on the electronic system of biologically important ligands. Spectroscopic study of benzoates, salicylates, nicotinates and isoorotates. Review. J Inorg Biochem. 2005;99:1407–23.
28. Bujdošová Z, Győryová K, Růžička A, Melník M, Koman M. Crystal structures of two aromatic zinc(II) carboxylates: [Zn(4-chlorosalicylato)$_2$(H_2O)$_4$]·2theophylline·(H_2O)$_2$ and unique [zn(5-chlorosalicylato)$_2$(isonicotinamide)$_2$(H_2O)]. J Chem Crystallogr. 2011;41:1077–84.