Polyhedron 52 (2013) 106–114

Contents lists available at SciVerse ScienceDirect

Polyhedron

journal homepage: www.elsevier.com/locate/poly

New copper(II) complexes with isoconazole: Synthesis, structures and biological properties

Galina M. Dulcevscaia [a], Victor Ch. Kravtsov [a], Fliur Z. Macaev [b], Gheorghe G. Duca [b], Eugenia P. Stingachi [b], Serghei I. Pogrebnoi [b], Veaceslav V. Boldescu [b], Steliana F. Clapco [c], Janeta P. Tiurina [c], Alexandra A. Deseatnic-Ciloci [c], Janusz Lipkowski [d], Shi-Xia Liu [e], Silvio Decurtins [e], Svetlana G. Baca [a,*]

[a] Institute of Applied Physics, ASM, Academiei 5, MD2028 Chisinau, Republic of Moldova
[b] Institute of Chemistry, ASM, Academiei 3, MD2028 Chisinau, Republic of Moldova
[c] Institute of Microbiology and Biotechnology, ASM, Academiei 1, MD2028 Chisinau, Republic of Moldova
[d] Institute of Physical Chemistry, Polish Academy of Sciences, 01-224 Warsaw, Poland
[e] Department of Chemistry and Biochemistry, University of Bern, Freiestrasse 3, CH-3012 Bern, Switzerland

ARTICLE INFO

Article history:
Available online 6 November 2012

Dedicated to the 100th anniversary of the award of the Nobel Prize in chemistry to Professor Alfred Werner.

Keywords:
Isoconazole
Copper(II) complex
Synthesis
X-ray structure
Biological properties

ABSTRACT

There is an increasing demand for novel metal-based complexes with biologically relevant molecules in technology and medicine. Three new Cu(II) coordination compounds with antifungal agent isoconazole (L), namely mononuclear complexes $[CuCl_2(L)_2]$ (**1**), and $[Cu(O_2CMe)_2(L)_2]\cdot 2H_2O$ (**2**) and coordination polymer $[Cu(pht)(L)_2]_n$ (**3**) (where H_2pht – *o*-phthalic acid) were synthesized and characterized by IR spectroscopy, thermogravimetric analysis and X-ray crystallography. X-ray analysis showed that in all complexes, the isoconazole is coordinated to Cu(II) centres by a N atom of the imidazole fragment. In complex **1**, the square-planar environment of Cu(II) atoms is completed by two N atoms of isoconazole and two chloride ligands, whereas the Cu(II) atoms are coordinated by two N atoms from two isoconazole ligands and two O atoms from the different carboxylate residues: acetate in **2** and phthalate in **3**. The formation of an infinite chain through the bridging phthalate ligand is observed in **3**. The biosynthetic ability of micromycetes *Aspergillus niger* CNMN FD 10 in the presence of the prepared complexes **1**–**3** as well as the antifungal drug isoconazole were studied. Complexes **2** and **3** accelerate the biosynthesis of enzymes (β-glucosidase, xylanase and endoglucanase) by this fungus. Moreover, a simplified and improved method for the preparation of isoconazole nitrate was developed.

1. Introduction

Research in the development of new metal-based compounds with biologically relevant molecules has attracted enormous interest. Such metal coordination complexes have much potential for design of novel therapeutic and diagnostic agents that target specific properties and show reduced side effects, avoidance of resistance, improved selectivity and can be used for treating a wide range of important human diseases; their pharmacological properties as antiviral, antimicrobial, anti-inflammatory, antihyperlipidemic, antitumoral, antidiabetic, diuretic and anticoagulant remedies were emphasized and widely explored (see some recent reviews and books, [1]). Moreover, metal coordination compounds represent an outstanding playground for the creation of the next generation of realistically useful metal-based drugs and diagnostic agents. They allow to combine the features of metals which have a wide range of coordination numbers and geometries, variable oxidation states, structural diversity and ability to bind a variety of organic ligands with the possibility to select or develop the organic ligands possessing electron donating or electron withdrawing groups in an attempt to tune the optimal stability and the biological *in vitro* activity. Furthermore, the coordination compounds may unite in the same molecule metal ion, biologically relevant ligands and some ancillary ligands to create mixed-ligands coordination species to reach the desired properties [2]. Additional challenges are the possibility to better understand the mechanism of action of the small molecules, further evaluation and modulation of the chemical composition and reactivity, and even the development or improvements in the methods for detection of biological activity. From the other side, some metal coordination compounds have been recognized as delivery vehicle for CO and NO molecules in living organisms [3], as powerful probes for DNA-mediated charge transport [4], and enzyme inhibitors [5]. The significant regulatory and biostimulatory effects of coordination compounds in the oriented synthesis of bioactive substances, including the enzymes (cellulase, protease and amylase), by microorganisms have also been emphasized [6].

* Corresponding author. Tel.: +373 22 738154; fax: +373 22 725887.
E-mail address: sbaca_md@yahoo.com (S.G. Baca).

0277-5387/$ - see front matter © 2012 Elsevier Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.poly.2012.10.040

Isoconazole nitrate is a well-known antifungal drug and belongs to a family of N-substituted imidazole derivatives, which showed their potent activity against a variety of fungal and yeast infections and have widely been used in the medical treatments of human and animals [7]. Moreover, the isoconazole nitrate showed a more powerful antifungal activity compared to other imidazole derivative drugs against some pathogenic *Candida* species [8] and has proven efficacy in treating dermatomycoses [9]. Coordination of the isoconazole to metal ions may lead to both enhancing its properties and displaying specific target features. To our knowledge, no coordination complexes with isoconazole have been reported so far. As the Cu ion is an essential element for many biological systems [10] and Cu coordination compounds have recently shown great promises as therapeutic agents with antifungal and anticancer activities [11], three new Cu(II) complexes with the isoconazole (L) have been synthesized. These include two mononuclear complexes $[CuCl_2(L)_2]$ (**1**) and $[Cu(O_2CMe)_2(L)_2]\cdot 2H_2O$ (**2**) and coordination polymer $[Cu(pht)(L)_2]_n$ (**3**) (where H_2pht – *o*-phthalic acid). Moreover, a simplified and improved method for the synthesis of the isoconazole nitrate has also been developed. The compounds were characterized by means of elemental analysis, IR, ^1H, and ^{13}C NMR spectroscopy, thermogravimetric and X-ray analyses. Further, for studying the influence of these compounds on biosynthetic activity of fungal strain, in particular the production of hydrolytic enzymes, and revealing important chemical information that can be implement into more specific structure–activity relationships, the impact of both the prepared coordination compounds **1**–**3** and the isoconazole nitrate has been investigated.

2. Experimental

2.1. Materials and physical measurements

All chemicals were purchased from commercial sources and were used without further purification. All reactions were carried out under aerobic conditions using commercial grade solvents. $[Cu(Hpht)_2(H_2O)_2]$ has been synthesized according to previously reported procedure [12]. Thin-layer chromatography was carried out on Merck aluminum sheets, silica gel 60 F254. Column chromatography was performed on Fluka silica gel 60, 70–230 mesh. Melting points were determined on a Boëtius melting point apparatus (PHMK, VEB Wägetechnik Rapido, Radebeul, Germany) and are uncorrected. ^1H and ^{13}C NMR spectra were acquired on a Bruker Avance III 400 spectrometer operating at 400.13 MHz for ^1H and 100.61 MHz for ^{13}C. Chemical shifts (δ) are given in ppm referring to the signal centre using the solvent peaks for reference: DMSO-d_6 2.50/39.52 ppm. The NMR signals were assigned by two-dimensional ^1H,^1H COSY and ^1H,^{13}C correlation spectra (HSQC, HMBC) using standard pulse sequences. The infrared spectra were recorded on a Perkin-Elmer Spectrum 100 FT-IR in the region 4000–650 cm^{-1} or a Nicolet Avatar 360 FT-IR spectrometer in the region of 4000 – 400 cm^{-1} using KBr pellet technique. TGA measurements were carried out with a Mettler Toledo TGA/SDTA 851 in dry N_2 (60 ml min^{-1}) at a heating rate of 10 K min^{-1}.

2.2. X-ray crystallography

The data for **1**, **2** and **3** were collected at 130, 100 and 293 K on Bruker SMART, Nonius Kappa-CCD, and Xcalibur Oxford Diffraction diffractometers, respectively, all equipped with a CCD area detector and employing graphite monochromatized Mo Kα radiation (λ = 0.71073 Å). Final unit cells dimensions were obtained and refined on entire data set. Though many crystallization experiments have been done, only small and poor diffracting crystals were available for **2** and **3**, which resulted in low resolution data. Crystals of **3** revealed non-merohedral twinning and some contamination of diffraction pattern appeared upon cooling. A two components twin sample with component ratio 0.6196/0.3804 at room temperature has been chosen for the crystal structure investigation in this case. The structures were solved by direct methods and refined by full-matrix least squares on weighted F^2 values for all reflections using the SHELX suite of programs [13]. The HKLF5 dataset for refinement of **3** has been generated by the CrysAlisPro, Agilent Technologies software (Version 1.171.34.49). The non-hydrogen atoms in all structures were refined with anisotropic displacement parameters. The C-bound H atoms were placed in calculated positions and were treated in a riding model approximation with $U_{iso}(H) = 1.2U_{eq}(C)$, while the O-bound H atoms of water molecule in **2** were found from difference Fourier maps and were refined with isotropic displacement parameters $U_{iso}(H) = 1.5U_{eq}(O)$. The Figs. 1 and 2 were produced using the Mercury program [14]. Crystal data and details of the structure refinement are given in Table 1.

2.3. Biological testing

To estimate the biological role of the synthesized coordination compounds **1**–**3** and isoconazole (L) the fungal strain *Aspergillus niger* CNMN FD 10, an active producer of extracellular cellulases and xylanases, was used. The submerged cultivation of the strain was carried out in 0.5 L Erlenmeyer flasks on shakers (180–200 rpm) at 28–30 °C during 6–9 days. The nutrient medium of the following chosen composition (g/L) was used as a control: beet pulp – 20.0; wheat bran – 10.0; apple or grape marc – 10.0; KH_2PO_4 – 1.0; $CaCl_2\cdot 2H_2O$ – 0.1; KCl – 0.1; $MgSO_4\cdot 7H_2O$ – 0.3; $NaNO_3$ – 2.5; $FeCl_3$ – 0.01; pH – 5.5–6.0. The tested compounds were introduced into the nutritive medium in concentrations of 1, 5 and 10 mg/L. Coordination compounds were added to the sterile cultivation medium (after medium autoclaving) in the form of solutions prepared as follows: 100 mg of the compound was dissolved in 20 mL ethylic alcohol (96%) and was completed to 100 mL with sterile distilled water.

Activity of cellulosolytic enzymes in culture filtrates was assayed by measuring the amount of reducing sugars released from the corresponding substrates: 4-nitrophenyl-β-D-glucopyranoside – for β-glucosidases; Na-carboxymethyl cellulose – for endoglucanases; xylan from oat spelt – for xylanases. The determination of glucose liberated from the substrate was measured calorimetrically using the Somogy–Nelson copper method [15].

2.4. Synthesis of $[CuCl_2L_2]$ (**1**)

A solution of $CuCl_2\cdot 2H_2O$ (0.02 g, 0.12 mmol) in water (1 mL) was added to a solution of isoconazole nitrate **15** (0.12 g, 0.25 mmol) in ethanol (4 mL). The dark blue crystals suitable for X-ray analysis were filtered off next day, washed with ethanol and dried in air. Yield 0.062 g, 52%. *Anal.* Calc. for $C_{36}H_{28}Cl_{10}CuN_4O_2$ (966.71): C, 44.73; H, 2.92; N, 5.79; Found: C, 45.02; H, 3.02; N, 5.78%. IR (KBr, cm^{-1}): 3158 (w), 3136 (m), 3082 (w), 3060 (w), 2890 (w), 1585 (m), 1564 (m), 1527 (m), 1513 (w), 1470 (m), 1436 (s), 1376 (m), 1337 (m), 1277 (w), 1259 (w), 1223 (m), 1196 (m), 1142 (w), 1112 (sh), 1097 (s), 1088 (s), 1074 (sh), 1044 (m), 1034 (sh), 1017 (s), 985 (m), 944 (m), 886 (m), 856 (m), 823 (m), 788 (sh), 778 (s), 759 (m), 746 (s), 725 (m), 696 (w), 656 (s), 639 (m), 623 (s), 562 (m), 520 (w), 497 (w), 454 (w), 432 (w).

2.5. Synthesis of $[Cu(O_2CMe)_2L_2]\cdot 2H_2O$ (**2**)

A solution of $Cu(O_2CMe)_2\cdot H_2O$ (0.01 g, 0.05 mmol) and isoconazole nitrate **15** (0.047 g, 0.1 mmol) in 10 mL of MeOH was refluxed

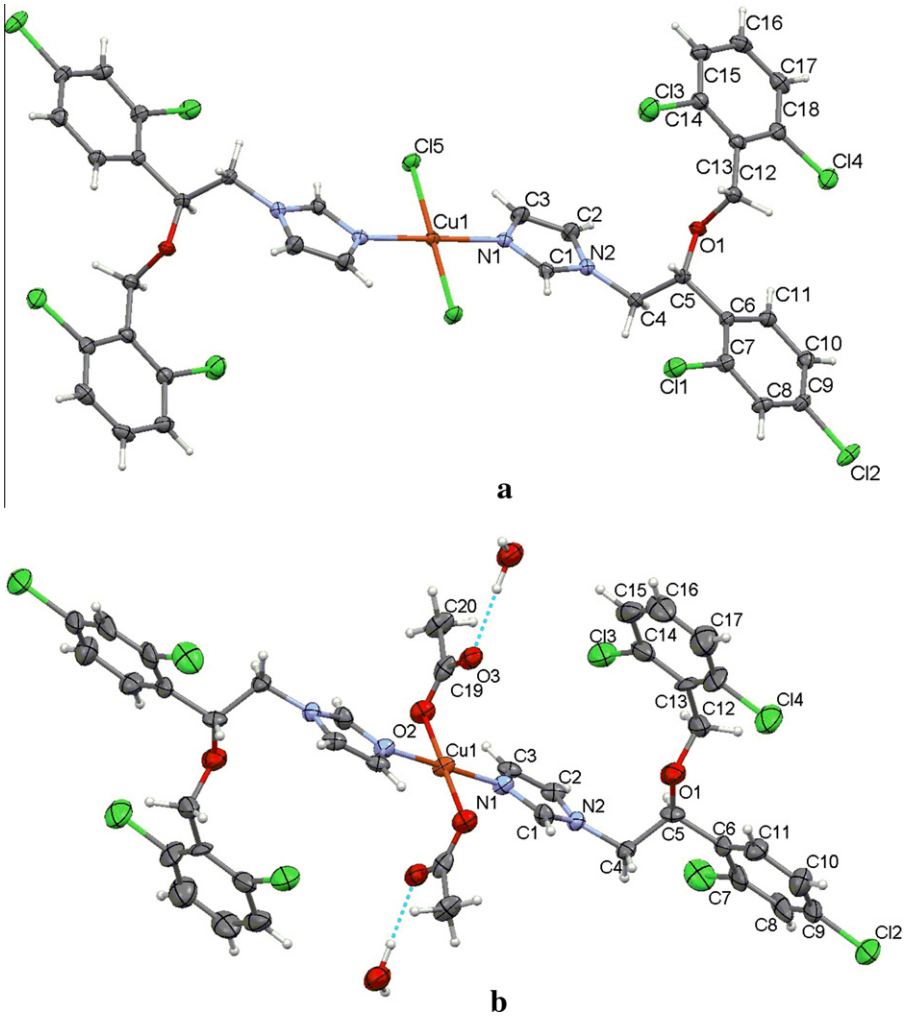


Fig. 1. Molecular structures of **1** (a) and **2** (b) (ORTEP at 50% level) with an atom labeling scheme. Hydrogen bonds are shown as dotted line.

during 40 min. Dark blue crystals of **2** suitable for X-ray analysis were filtered off, washed with MeOH and dried in air. Yield 0.03 g, 58%. *Anal.* Calc. for $C_{40}H_{38}Cl_8CuN_4O_8$ (1049.93): C, 45.76; H, 3.65; N, 5.34. Found: C, 45.68; H, 3.97; N, 4.67%. IR (KBr, cm^{-1}): 3659 (w), 3289 (w), 3195 (w), 3141 (w), 3118 (w), 2931 (w), 2888 (w), 1585 (vs), 1563 (s), 1526 (m), 1467 (m), 1438 (s), 1395 (vs), 1378 (sh), 1336 (m), 1291 (m), 1242 (m), 1218 (m), 1199 (m), 1141 (w), 1107 (s), 1095 (s), 1079 (sh), 1045 (s), 1020 (s), 992 (m), 955 (m), 861 (s), 819 (m), 782 (s), 762 (s), 728 (m), 682 (m), 656 (m).

2.6. Synthesis of $[Cu(pht)L_2]_n$ (**3**)

To a solution of $[Cu(Hpht)_2(H_2O)_2]$ (0.05 g, 0.12 mmol) in 15 mL of MeOH 0.12 g of isoconazole nitrate **15** (0.25 mmol) was added. Dark blue crystals of **3** suitable for X-ray analysis were filtered off after a few days, washed with MeOH and dried in air. Yield 0.10 g, 81%. *Anal.* Calc. for $C_{44}H_{32}Cl_8CuN_4O_6$ (1059.92): C, 49.86; H, 3.04; N, 5.29; Found: C, 49.88; H, 3.40; N, 5.05%. IR (KBr, cm^{-1}): 3136 (w), 3065 (w), 2989 (br.w), 1615 (vs), 1593 (s), 1565 (sh), 1524 (m), 1497 (m), 1467 (m), 1449 (sh), 1437 (sh), 1415 (sh), 1399 (s), 1375 (vs), 1345 (sh), 1278 (w), 1246 (m), 1151 (m), 1096 (s), 1083 (sh), 1041 (m), 1026 (m), 1015 (m), 985 (w), 950 (w), 877 (m), 851 (m), 839 (m), 829 (m), 778 (s), 766 (s), 730 (s), 709 (vs), 653 (m).

2.7. Synthesis of isoconazole nitrate

*Synthesis of tetrabutylammonium 1H-imidazole (**7**).* A solution of NaOH (0.4 g, 10 mmol) in 2-propanol (20 mL) was treated with 1H-imidazole (0.68 g, 10 mmol) followed by refluxing for 20 min. Then, *n*-Bu_4NBr (3.09 g, 10 mmol) was added at room temperature and the resulting mixture was refluxing for 10 min. A solid substance was filtered, solvent was evaporated under vacuum, and the residue was dried over P_2O_5 in vacuum desiccator. Yield 3.02 g, 98%. IR (KBr, cm^{-1}): 3101 (w), 2989 (w), 2958 (s), 2874 (m), 1679 (w), 1587 (s), 1491 (m), 1473 (s), 1465 (s), 1379 (m), 882 (s), 738 (s). ^1H NMR (DMSO-d_6, ppm): δ = 0.93 (t, J = 8.0 Hz, 12 H, 4 Me,), 1.26–1.35 (m, 8 H, 4 CH_2), 1.53–1.61 (m, 8 H, 4 CH_2), 3.18 (t, J = 8.0 Hz, 8 H, 4 CH_2-N,), 6.97 (s, 2 H, im), 7.58 (s, 1 H, im). ^{13}C NMR (DMSO-d_6, ppm): δ = 13.96 (Me x 4), 19.68 (CH_2 x 4), 23.56 (CH_2 x 4), 58.01 (CH_2 x 4), 122.20 (C^4 im, C^5 im), 135.91 (C^2 im).

General procedure for reduction. Procedure for synthesis of halohydrins. $NaBH_4$ (0.21 g, 5.5 mmol) was added (in three portions) to a solution of the corresponding ketone (5.5 mmol) 4 or 5 in 2-propanol (25 mL) at 3–5 °C. The mixture was stirred at room temperature for 2 h. Then, the reaction mixture was poured into water, the solution was extracted with ethyl acetate and dried over anhydrous Na_2SO_4. The solvent was distilled. The obtained light crude product was recrystallized from the appropriate solvent.

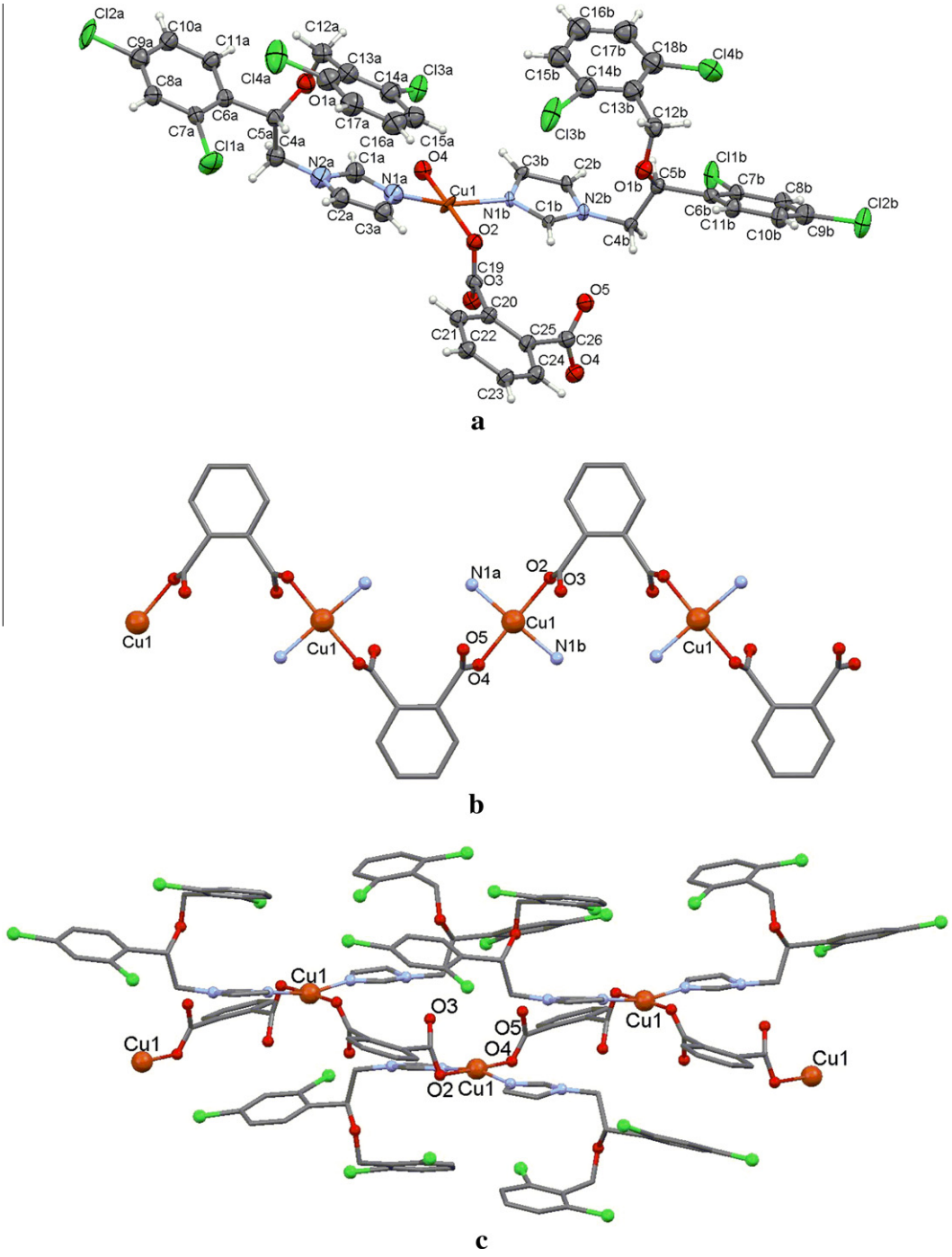


Fig. 2. (a) Asymmetric fragment of a polymeric chain in **3**; only O4 atom is shown twice to illustrate the coordination surrounding of Cu1 (ORTEP at 30% level). (b) Zigzag like polymeric chain in **3**; two L ligands are represented only by nitrogen atoms N1a and N1b of imidazole fragments. (c) A fragment of the chain with the attached isoconazole molecules.

2-Chloro-1-(2,4-dichlorophenyl)ethanol (***11***). Yield 73%. M.p. 53 °C (from acetone). IR (KBr, cm^{-1}): 3600 (w), 3200 (m), 1475 (s), 1100 (m), 815 (s), 750 (m). ^{1}H NMR (DMSO-d_6, ppm): 3.32–3.8 (m, 2 H, $^2CH_2$), 5.0–5.2 (m, 1 H, ^{1}CH), 5.44 (d, J = 10.4 Hz, 1 H, OH), 7.20 (d, J = 1.94 Hz, 1 H, ^{6}CH), 7.31 (d, J = 1.94 Hz, 1 H, ^{5}CH), 7.60 (s, 1 H, ^{3}CH). ^{13}C NMR (DMSO-d_6, ppm): δ = 137.20 (^{2}C), 133.20 (^{5}C), 132.14 (^{6}C), 130.01 (^{3}C), 129.20 (^{4}C), 99.12 (^{6}C), 69.14 (^{7}C), 48.7 (^{8}C). *Anal.* Calc. for $C_8H_7Cl_3O$ (225.5): C, 42.61; H, 3.13; Found: C, 42.62; H, 3.13%.

2-Bromo-1-(2,4-dichlorophenyl)ethanol (***12***). Yield 57%. M.p. 72–74 °C (from hexane). ^{1}H NMR (DMSO-d_6, ppm): 3.39–4.1 (m, 2 H, $^2CH_2$), 5.02–5.25 (m, 1H, ^{1}CH), 5.46 (d, J = 10.4 Hz, 1 H, OH), 7.20 (d, J = 1.94 Hz, 1 H, ^{6}CH), 7.31 (d, J = 1.94 Hz, 1H, ^{5}CH), 7.60 (s, 1H, ^{3}CH). *Anal.* Calc. for $C_8H_7BrCl_2O$ (269.95): C, 35.59; H, 2.61; Found: C, 35.47; H, 2.76%.

(a) *1-(2,4-Dichlorophenyl)-2-(1H-imidazol-1-yl)ethanol* (***13***). A solution of halohydrins (1.0 equiv.) and ionic liquid **7** (1.0 equiv.) in anhydrous MeCN was refluxed for 10–12 h. The reaction has been monitored by TLC. A ¾ volume of solvent was removed under vacuum, the residue poured into water and extracted with ethyl acetate. The organic phase was dried over anhydrous Na_2SO_4, distilled off. The obtained crude product was precipitated in acetone

Table 1
Crystallographic data for complexes **1**–**3**.

	1	2	3
Formula	$C_{36}H_{28}Cl_{10}CuN_4O_2$	$C_{40}H_{38}Cl_8CuN_4O_8$	$C_{44}H_{32}Cl_8CuN_4O_6$
Crystal system	triclinic	triclinic	monoclinic
Space group	$P\bar{1}$	$P\bar{1}$	$P2_1/n$
a (Å)	8.6570(16)	7.8401(8)	15.457(6)
b (Å)	9.0619(17)	8.9852(9)	13.0982(16)
c (Å)	13.797(3)	18.179(2)	22.867(4)
α (°)	107.698(2)	99.399(6)	90
β (°)	107.840(3)	95.422(7)	105.40(3)
γ (°)	92.215(3)	115.234(9)	90
U (Å^3)	971.2(3)	1123.4(2)	4463(2)
R indices	$R_1 = 0.0299$	$R_1 = 0.0925$,	$R_1 = 0.0794$
$[I > 2\sigma(I)]$	$wR_2 = 0.0798$	$wR_2 = 0.2590$	$wR_2 = 0.2624$

as a white solid. Yield 68% from **11**. Yield 40% from **12**. M.p. 142–143 °C. IR (KBr, cm^{-1}): 1512 (m), 1079 (s), 845 (s), 733 (m). ^{1}H NMR (DMSO-d$_6$, ppm): δ = 4.0–4.06 (m, 2 H, ^{2}CH$_2$), 5.06–5.08 (m, 1 H, ^{1}CH), 4.16 (d, J = 10.8 Hz, 1 H, OH), 6.03 (d, J = 4 Hz, 1 H, ^{4}CH of the imidazole ring), 6.83 (d, J = 4 Hz, 1 H, ^{5}CH of the imidazole ring), 7.03 (s, 1 H, ^{2}CH), 7.42–7.46 (m, 2 H, ^{5}CH, ^{6}CH). 7.59 (s, 1 H, ^{3}CH). ^{13}C NMR (DMSO-d$_6$, ppm): δ = 137.14 (^{6}C), 136.20 (^{1}C), 132.99 (^{10}C), 130.11 (^{7}C), 130.01 (^{2}C), 129.68 (^{8}C), 128.04 (^{11}C), 126.12 (^{9}C), 118.44 (^{3}C) 63.88 (^{5}C); 53.22 (^{4}C). *Anal.* Calc. for $C_{11}H_{10}C_{l2}N_2O$ (257.12): C, 51.38; H, 3.92; N, 10.89; Found C, 51.39; H, 3.92; N, 10.90%.

(b) $NaBH_4$ (0.21 g, 5.5 mmol) was added (in three portions) to a solution of the ketone (1.4 g, 5.5 mmol) **8** in 2-propanol (25 mL) at 3–5 °C. The mixture was stirred at room temperature for 2 h (monitored by TLC). Then, 50% of solvent was removed under reduced pressure and 3% solution of HCl (3 mL) was added. The reaction was neutralized with sodium carbonate and poured into water. The mixture was extracted with ethyl acetate and the organic phase was dried over anhydrous Na_2SO_4. The solvent was removed under reduced pressure on a rotary evaporator to yield a viscous gum, which was recrystallized in acetone to give **13**. Yield: 1.19 g, 85%.

*1-[2-(2,6-Dichlorobenzyloxy)-(2,6-dichlorophenyl)ethyl]-1H-imidazole (**10**) and its salt (**15**).* To a suspension of 60% dispersion of NaH in mineral oil (0.25 g, 7.5 mmol) in dry 1,4-dioxane (15 mL) at room temperature under a nitrogen atmosphere was added dropwise a solution of **13** (1.28 g, 5 mmol) in 1,4-dioxane (20 mL). The reaction mixture was refluxed for 1 h. 1,3-Dichloro-2-(chloromethyl)benzene **14** (1 g, 5 mmol) was added dropwise and refluxed for 2.5 h. Solvent was removed in vacuum, and after that the reaction was quenched with water (100 mL). The aqueous solution was extracted with ethyl acetate (3x50 mL), the organic phases were combined, dried over anhydrous Na_2SO_4, and filtered. The solvent was removed by distillation under reduced pressure, giving a crude reaction mixture that was purified followed by crystallization from acetone to give 1.45 g of ester **10**. Yield: 70%. M.p. 115–116 °C.

A solution of base **10** (1.2 g, 2.8 mmol) in acetone (5 mL) was treated with concentrated (86%) HNO_3 (5 mL) followed by stirring for 12 h. The white solid **15** was filtered and dried over P_2O_5 in vacuum desiccator. Yield 1.35 g, 98%. M.p. 203–204 °C. IR (KBr, cm^{-1}): 1381 (w), 1380 (w), 1510 (m), 1150 (m), 810 (s), 720 (s). ^{1}H NMR (DMSO-d$_6$, ppm): δ = 4.40–4.50 (m, 2 H, ^{1}CH), 4.62 (d, J = 10.8 Hz, 2 H, -OCH$_2$), 5.12–5.20 (m, 1 H, ^{2}CH), 7.30–7.42 (m, 3 H, phenyl of 2-(2,6-dichlorobenzyloxy)), 7.48–7.59 (m, 2 H, ^{5}CH, ^{6}CH), 7.60 (s, 2 H, ^{4}CH, ^{5}CH of the imidazole ring), 7.72 (s, 1 H, ^{3}CH) 9.04 (s, 1 H, ^{2}CH of the imidazole ring), 14.6 (s, 1 H, HNO_3). ^{13}C NMR (DMSO-d$_6$, ppm): δ = 138.10 (^{1}C), 137.79 (^{13}C), 135.35 (^{6}C), 133.12 (^{14}C, ^{7}C), 131.88 (^{10}C), 131.22 (^{2}C), 130.00 (^{16}C), 129.41 (^{7}C), 128.21 (^{8}C), 128.16 (^{15}C, ^{18}C) 127.13 (^{11}C), 125.18 (^{9}C), 118.29 (3C), 74.31 (^{5}C), 71.80 (^{12}C), 50.99 (^{4}C). *Anal.* Calc. for $C_{18}H_{15}Cl_4N_3O_4$ (479.14): C, 45.12; H, 3.16; N, 8.77; Found C, 45.40; H, 3.15%.

3. Result and discussion

3.1. Synthesis of isoconazole nitrate

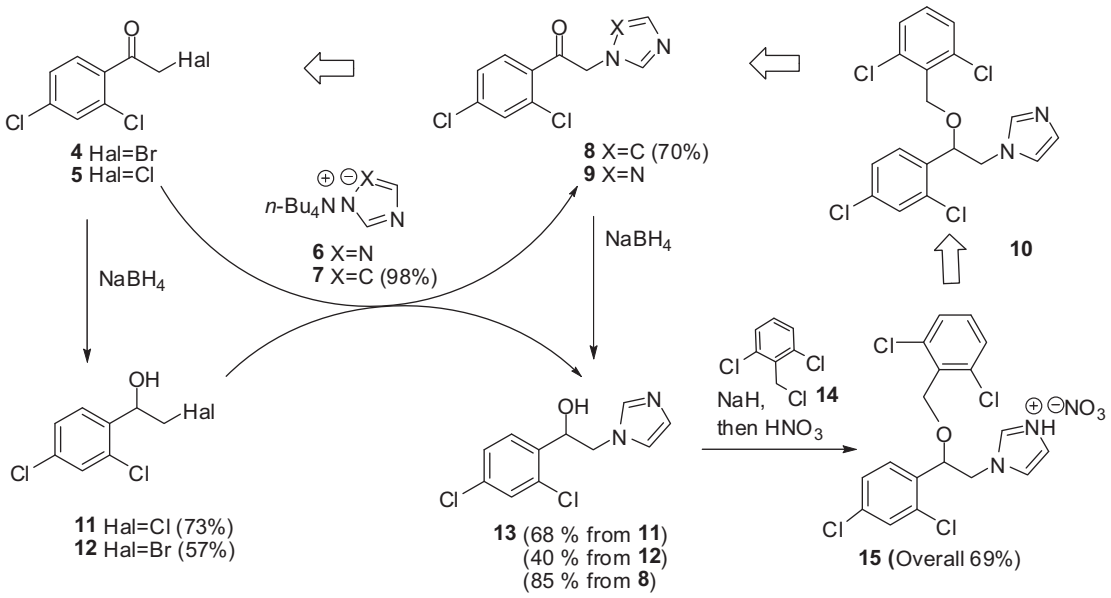
A general method for the preparation of isoconazole **10** consists in the initial formation of the 1-(2,4-dichlorophenyl)-2-(1H-imidazol-1-yl)ethanol **13** via substitution reaction between 1H-imidazole and 2-bromo- or 2-chloro-1-(2,4-dichlorophenyl)ethanones (**4**, **5**) followed the reduction of 1-(2,4-dichlorophenyl)-2-(1H-imidazol-1-yl)ethanone **8** [16–18]. A previous study [19] has shown that an ionic liquid derivative of 1H-1,2,4-triazole **6** can successfully be applied as the synthetic equivalent of the Na (K or Li) salt of 1,2,4-triazole towards 1-(2,4-dichlorophenyl)-2-(1H-1,2,4-triazol-1-yl)ethanone **9** upon treatment with 2-bromo-1-(2,4-dichlorophenyl)ethanone **4** (Scheme 1). A follow-up study [20–24] on the preparation of a remarkably promising class of technologically useful materials called "task-specific ionic liquids" as recyclable reagents for "green chemistry approach" has proved the strategic advantages of using these materials.

Reaction of ketones **4** or **5** with 1 equiv. of ionic liquid **7** in boiling MeCN afforded the substituted imidazole **8** with 50% and 70% yields, respectively. On the other side, the ketones **4** and **5** were transformed into the racemic halohydrins **11** and **12** by the reaction with $NaBH_4$ in 2-propanol. The addition of 2-chloro-1-(2,4-dichlorophenyl)ethanol **11** or 2-bromo-1-(2,4-dichlorophenyl)ethanol **12** to ionic liquid **7** gave directly **13** with 68% and 40% yields, respectively. Finally, the 2,6-dichlorobenzyl ester **10** was prepared via treatment of alcohol **13** and 1,3-dichloro-2-(chloromethyl)benzene **14** with 1.25 equiv. of NaH in refluxing 1,4-dioxane for 1.5 h. Isoconazole **10** can easily be transformed into the corresponding salt **15** upon treatment with an excess of HNO_3 in acetone at room temperature with a nearly quantitative yield. Our methodology offers an easy and alternative way for the procedure developed by others [16,17].

3.2. Synthesis of Cu(II) complexes and preliminary characterization

The reaction of Cu(II) chloride dihydrate with isoconazole nitrate **15** in water:ethanol (1:4) solution at room temperature leads to the crystalline product with the formula $[CuCl_2(L)_2]$ (**1**) in ca. 52% yield. Using Cu(II) acetate hydrate or Cu(II) phthalate dihydrate in the reaction with isoconazole nitrate **15** in methanol results in $[Cu(O_2CMe)_2(L)_2]\cdot 2H_2O$ (**2**) and $[Cu(pht)(L)_2]_n$ (**3**) in ca. 58% and 81% yield, respectively. The materials **1**–**3** were characterized by elemental analysis, IR data, and thermogravimetric and single-crystal X-ray diffraction analyses to confirm the homogeneity of the obtained solids.

The IR spectrum of the free isoconazole ligand shows medium intensity bands at 1582 and 1561 cm^{-1} due to C=N bond stretching of aromatic rings. In **1**, these bands are slightly shifted to higher values of 1585 and 1564 cm^{-1}, indicating the coordination of a imidazole nitrogen atom in the Cu(II) complex. In this region, the IR spectra of compounds **2** and **3** exhibit intense bands at 1615–1563 cm^{-1} due to the asymmetric stretching vibrations of the coordinated carboxylate groups of the acetate and phthalate ligands, that overlap the ν(C=N) stretching vibrations of the imidazole fragment of the isoconazole ligand. The symmetric stretching vibrations of coordinated carboxylate groups are observed in the region 1415–1374 cm^{-1}. IR spectra of all complexes **1**–**3** also show absorptions due to C-H bond stretching of aromatic rings in the range 3289–2817 cm^{-1}. The presence of solvate water molecules



Scheme 1. The synthesis of isoconazole nitrate.

in **2** caused the appearance of absorption bands with maxima at 3659 cm^{-1}.

Thermogravimetric analyses for all complexes were performed in a nitrogen atmosphere in the temperature range of 25–800 °C. The TGA data show that complex **1** is stable up to approximately 185 °C and then it starts to decompose in one step with total weight loss of 65.9% to the final products (observed 34.1%). It is accompanied by an endothermic effect at 196 °C. Coordination polymer **3** is also stable up to approximately 200 °C and then the decomposition of the organic ligands takes place in two unidentified steps with total weight loss of 70.0% to the final products (observed 30.0%). It is accompanied by an endothermic effect at 219 °C. For complex **2**, the loss of solvate water molecules with a weight loss of 4.25% (calculated 3.43%) was observed before 200 °C followed by the decomposition of organic part in two unidentified steps to final products (observed 29.2%).

Table 2
Selected bond lengths [Å] and angles [°] in **1**–**3**.

	1	2	3
Cu1–N1 (N1a)	2.006(1)	1.964(9)	1.972(16)
Cu1–N1b			1.990(12)
Cu1–Cl5 (O2)	2.2564(5)	1.953(8)	1.989(15)
Cu1–O4$^{\#1}$			1.936(17)
N1(N1a)–Cu1–Cl5(O2)	89.90(4)	89.1(3)	88.4(7)
N1a–Cu1–O4$^{\#1}$			93.1(8)
N1b–Cu1–O2			90.6(6)
N1b–Cu1–O4$^{\#1}$			88.6(6)
N1a–Cu1–N1b			163.3(6)
O2–Cu1–O4$^{\#1}$			177.4(7)

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

3.3. X-ray analysis

X-ray crystallographic studies revealed that **1** and **2** contain centrosymmetric mononuclear $[CuCl_2(L)_2]$ and $[Cu(O_2CMe)_2(L)_2]\cdot 2H_2O$ complexes, respectively. Each Cu(II) atom in both structures resides on a crystallographic center of symmetry and coordinates in trans-positions to two isoconazole molecules via a nitrogen atom of a imidazole fragment and two chlorine atoms in **1** or two oxygen atoms from different acetate groups in **2** resulting in a square-planar coordination geometry (Fig. 1). The imidazole fragments form with the coordination plane of the copper atom dihedral angles of 18.31° in **1** and 13.82° in **2**. This difference in dihedral angles as well as the longer Cu1–N1 bond length of 2.006(1) Å in **1** compared with 1.964(9) Å in **2**, Table 2, is related with the different nature of the ligands *cis*–situated with respect to the two imidazole fragments. Solvate water molecules are hydrogen bonded to acetate groups in **2** [O1W–H...O3 = 2.795(12) Å, O1W–H = 0.92(2) Å, H...O3 = 1.87(2) Å, ∠O1W–H...O3 = 178(16)°].

The structure of **3** reveals a $[Cu(pht)(L)_2]_n$ chain coordination polymer. The coordination of Cu(II) atom is square-planar and realized by two nitrogen atoms from two symmetry independent monodentate L ligands [Cu1–N1a = 1.972(16) Å and Cu1–N1b = 1.990(12) Å] and oxygen atoms from two pht^{2-} ligands [Cu–O2 = 1.989(15) and Cu–O4 = 1.936(17) Å], Fig. 2a. The deviation of the donor atoms from the coordination plane is less than 0.165 Å and results in some tetrahedral distortion of the coordination plane. In contrast to **1** and **2**, the isoconazole molecules in **3** reside on the same side of the copper(II) coordination plane and the phthalate ligands are situated on opposite sides of this plane. They serve as exo-bidentate O,O′-ligands and coordinate via carboxylic groups to neighboring Cu(II) atoms, linking them to a zig-zag-like polymeric chain along the crystallographic *b* axis, Fig. 2b. Repeating units in the chain are symmetry related by 2-fold screw axis. Three dihedral angles between the planes of carboxylic groups and benzene fragment are very similar and possess values in the narrow range 59.2–60.8°. Isoconazole molecules attached to copper(II) atoms decorate the polymeric chain, as shown in Fig. 2c.

The analysis of torsion angles reveals a similarity in the conformation of isoconazole molecules in **1**–**3**. In **1** and **2**, isoconazole molecules differ only by reverse orientation of an imidazole fragment with respect to other parts of the molecule; the torsion angle C1–N2–C4–C5 equals 91.58° and −115.3°, respectively, Table 3. The structural conformations of the crystallographically independent isoconazole molecules a and b in **3** are rather similar and close to that found in **2**. The largest difference in the conformation of the isoconazole molecule in **1** and **2** compared to **3** is related with the torsion angle C5–O1–C12–C13, which is *anti* in **1** and **2**, and

Table 3
Selected torsion angles [°] in the isoconazole molecules in **1**–**3**.

	1	2	3a	3b
C1–N2–C4–C5	91.58(18)	−115.3(12)	−100.4(18)	−102(2)
N2–C4–C5–C6	−174.32(13)	−179.6(10)	−170.0(15)	−168.7(13)
C4–C5–C6–C7	79.31(18)	78.0(14)	88.1(19)	78.5(18)
N2–C4–C5–O1	64.76(16)	55.7(13)	63(2)	61(2)
C4–C5–O1–C12	−167.42(13)	−154.0(10)	−157.5(17)	−159.1(13)
C5–O1–C12–C13	169.26(13)	169.9(11)	59(2)	59.6(14)
O1–C12–C13–C14	−85.57(18)	−92.9(14)	71(2)	77.9(7)
C7–C6–C5–O1	−164.77(14)	−160.2(11)	−144.8(14)	−151.2(12)
C6–C5–O1–C12	76.32(16)	83.7(13)	75(2)	71.5(19)

adopts *gauche* conformation in **3**. The opposite sign of O1–C12–C13–C14 torsion angles in **1** and **2** with regard to **3** is also worth to be mentioned. Crystal packing diagrams of **1**–**3** (Figs. S1–S3) do not reveal any specific intermolecular contacts in addition to van der Waals interactions.

3.4. Biological studies

Biological activity of complex compounds can be explained by the presence in their composition as a complexing metal atoms of trace elements Co, Cu, Fe, Mn, Zn, Ni, and Mo, etc. Oligoelements interact with proteins, enzymes, vitamins or hormones and actively take part in the regulation of important biochemical processes in living organisms [25]. Copper is one of the most abundant metallic elements in biological systems [7]. In enzymology, copper is somewhat similar to iron in that it function in a series of oxidases, oxygenases and low-molecular-weight electron transfer proteins that are reminiscent of ferredoxins. Furthermore, one class of superoxide dismutases contains copper, as well as polyphenoloxidases and tyrosinases. Copper is a bioactive metal required for growth of microorganisms since it is a cofactor for numerous enzymes. It is known that in the presence of copper the iron absorption is facilitated as well as its incorporation into the cytochromes [26–28]. From the foregoing, it is apparent that studies on the biological properties of the synthesized copper coordination compounds are very promising as a theoretical and a practical point of view.

The biological activity of coordination compounds **1**–**3** towards the biosynthesis of enzymes (β-glucosidase, xylanase and endoglucanase) by the fungal strain *A. niger* CNMN FD 10 has been tested. Because of the evaluation and explanation of the biological activity of a coordinative compound requires knowledge of the contribution of the metal ion, the ligand and of the integral complex molecule, as well as synergistic action of two or more components, additionally to the coordination compounds **1**–**3** the free isoconazole ligand has also been screened. The results are presented in Tables 4–6. The data show that the addition of both the free isoconazole ligand and coordination compound **1**, in which Cu(II) ions are coordinated to isoconazole and Cl^- groups, to the cultivation medium of micromycete *A. niger* CNMN FD 10 causes a pronounced inhibitory effect on the enzymatic activity of all types of hydrolases (β-glucosidase, endoglucanase and xylanase) as compared to the control (Figs. 3–5). The inhibitory effect increases with increasing concentration of **1**. It may be due to the presence of chloride atoms in the ligand, as well as in the coordination sphere of the metal in complex **1**. Chloride ions and some of their compounds are some of the strongest antimicrobial remedies.

In the case of addition of coordination compounds **2** and **3**, in which chloride ions coordinated to metal were replaced by carboxylic groups, with a concentration of 1.0 mg/L into nutritive medium of *A. niger* CNMN FD 10, a positive effect was observed. The maximum accumulation of the enzymes was detected 24–48 h earlier and it was significantly higher than in the control. Thus, the maximum accumulation of β-glucosidases in the control was 2.35 U/mL on the 7th day of cultivation and 2.36 U/mL (**2**), and 2.38 U/mL (**3**) on the 6th day of cultivation (Fig. 3).

Maximum xylanases biosynthesis in control (38.73 U/mL) was observed on the 8th day of cultivation, and in optimized media – 39.09 U/mL (**2**) and 38.00 U/mL (**3**) on the 7th day of cultivation,

Table 4
The influence of **1**–**3** and isoconazole nitrate (L) concentrations in cultivation culture on β-glucosidase biosynthesis by *Aspergillus niger* CNMN FD 10.

Compounds	Concentration (mg/L)	Enzyme activity (U/mL)			
		6 days	7 days	8 days	9 days
1	1	1.62	1.87	1.30	0.29
	5	0.56	0.92	0.51	0.30
	10	0.36	0.36	0.38	0.28
2	1	**2.38**	2.20	1.58	0.75
	5	**2.31**	2.05	1.71	0.72
	10	0.23	0.15	0.81	0.47
3	1	**2.36**	2.05	1.69	0.20
	5	**2.25**	2.02	1.63	0.56
	10	0.44	0.89	1.42	0.35
L	1	0.22	1.82	1.54	0.06
	5	0.14	0.75	0.54	0.05
	10	0.11	0.61	0.20	0.06
Control	0	2.09	**2.35**	1.75	0.72

The maximum accumulation of enzymes has been highlight in bold.

Table 5
The influence of **1**–**3** and isoconazole nitrate (L) on xylanase biosynthesis by *Aspergillus niger* CNMN FD 10.

Compounds	Concentration (mg/L)	Enzyme activity (U/mL)			
		6 days	7 days	8 days	9 days
1	1	11.42	27.41	22.12	19.00
	5	7.01	23.39	12.87	15.23
	10	5.62	11.78	12.15	8.41
2	1	34.85	**38.00**	21.47	24.14
	5	27.09	35.43	27.99	24.66
	10	18.61	26.76	19.58	22.19
3	1	32.82	**39.09**	21.51	26.11
	5	30.46	31.99	20.16	24.17
	10	10.44	24.44	18.31	22.77
L	1	10.34	15.05	15.77	8.26
	5	5.26	20.67	10.33	7.11
	10	1.63	18.68	8.16	0.00
Control	0	22.55	28.29	**38.73**	33.29

The maximum accumulation of enzymes has been highlight in bold.

Table 6
The influence of **1**–**3** and isoconazole nitrate (L) concentrations in cultivation culture on endoglucanase biosynthesis by *Aspergillus niger* CNMN FD 10.

Compounds	Concentration (mg/L)	Enzyme activity (U/mL)			
		6 days	7 days	8 days	9 days
1	1	3.76	3.94	2.37	2.58
	5	3.41	3.34	2.18	2.48
	10	2.46	3.28	1.47	2.28
2	1	**5.36**	5.09	4.31	3.41
	5	4.67	4.89	3.03	3.55
	10	3.22	4.46	3.20	2.92
3	1	**5.42**	5.11	4.87	3.28
	5	5.07	4.89	4.12	3.38
	10	3.24	4.49	2.27	3.09
L	1	3.24	3.44	2.06	2.31
	5	3.09	3.44	2.01	1.64
	10	2.87	1.74	1.63	1.40
Control	0	3.76	3.84	**5.40**	3.73

The maximum accumulation of enzymes has been highlight in bold.

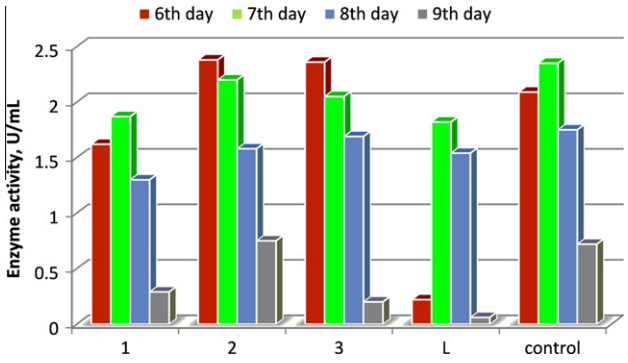


Fig. 3. β-Glucosidase activities obtained with the concentration of compounds of 1.0 mg/L in nutritive medium on the 6th, 7th, 8th and 9th day of cultivation.

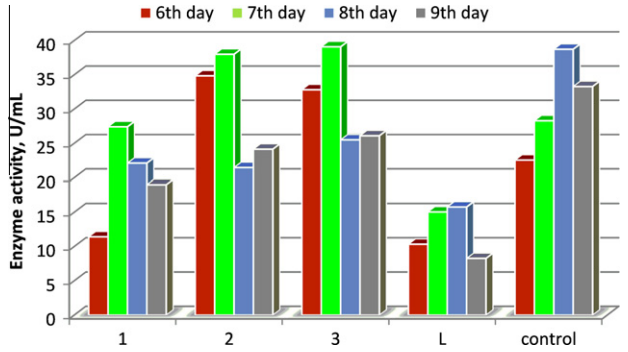


Fig. 4. β-Xylanase activities obtained with the concentration of compounds of 1.0 mg/L in nutritive medium on the 6th, 7th, 8th and 9th day of cultivation.

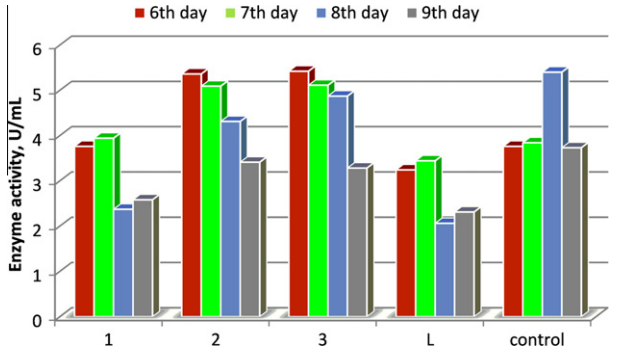


Fig. 5. β-Endoglucanase activities obtained with the concentration of compounds of 1.0 mg/L in nutritive medium on the 6th, 7th, 8th and 9th day of cultivation.

which is 24 h earlier (Fig. 4). Endogluconase's maximum accumulation in the control was 5.4 U/mL (on 8th day of cultivation), but in optimized media it was 5.42 U/mL (complex **2**) and 5.36 U/mL (complex **3**) on 6th day of cultivation, reducing the biological cycle by 48 h (Fig. 5). However, increasing the concentration of coordination compounds up to 10.0 mg/L causes a significant reduction in enzymatic activity of all studied components of the enzymatic complex. Perhaps this phenomenon is caused by the toxicity of tested compounds. The results correlate with literature data and show that toxicity is a function of concentration of the substance. Metals are characterized by a very narrow boundary between the concentrations necessary for vital activity of organisms and those which are toxic to them [29].

Thus, this study showed that Cu(II) coordination compounds **2** and **3** in the selected optimal concentration (1.0 mg/L) can be used to accelerate by 24–48 h the biosynthesis of β-glucosidase, xylanase and endoglucanase from micromycetes *A. niger* CNMN FD 10, which is economically profitable, since it significantly reduces energy costs for industrial production of the mentioned enzymes. The reduction of technologic cycles presents significance for biotechnology, offering several economic advantages – increasing the amount of product per unit of time, reduces energy consumption that contributes to increasing technological profitability and reducing of final product cost. It is worth to mention that enzymes play the most crucial roles as biocatalysts in organic synthesis where they show greater specificities than more conventional forms of organic reactions; in chemical industry they were used for the synthesis of specific chemicals and polymers as well as in developing and producing key pharmaceutical ingredients and new therapeutic agents. [30]. Moreover, enzymes have contributed greatly in the development of more environmentally adapted household and industrial detergents, in textile industry, in oil and gas drilling, in the production of biopolymers and fuel ethanol, in paper processing, and in the food industry [31].

4. Conclusions

The reaction of copper(II) salts with an antifungal agent isoconazole nitrate (L), for which a simplified and improved method of synthesis was developed, affords three new Cu(II) coordination compounds, namely mononuclear complexes $[CuCl_2(L)_2]$ (**1**) and $[Cu(O_2CMe)_2(L)_2]\cdot 2H_2O$ (**2**), and one-dimensional coordination polymer $[Cu(pht)(L)_2]_n$ (**3**). Single-crystal X-ray diffraction analysis showed that in all complexes the isoconazole is coordinated to Cu(II) centres by a N atom of the imidazole fragment. The formation of an infinite zigzag chain through the bridging phthalate ligand was observed in **3**. The investigation of the biosynthetic ability of micromycetes *A. niger* CNMN FD 10 in the presence of the prepared coordination compounds **1**–**3** as well as the antifungal drug isoconazole showed that complexes **2** and **3** accelerate the biosynthesis of enzymes (β-glucosidase, xylanase and endoglucanase) by this fungus and their regulatory and biostimulatory effect on the biosynthesis of enzymes can be explored. Both complex **1** and isoconazole nitrate displayed pronounced inhibitory effect on the enzymatic activity. The potent biological activity of this family of Cu–isoconazole compounds stresses the need for improvements of their solubility properties and for more studies comprising the interaction with other biologically relevant structures. The synthesis of other metal complexes with isoconazole and the investigation of their biological properties are under way.

Acknowledgments

This study was supported by the Supreme Council for Science and Technological Development of R. Moldova (Project 09.836.05.02A) and the Swiss National Science Foundation (SCOPES IZ73Z0_127925).

Appendix A. Supplementary data

CCDC 869079 (**1**), 869077 (**2**), and 869078 (**3**) contain the crystallographic data for **1–3**. These data can be obtained free of charge via http://www.ccdc.cam.ac.uk/conts/retrieving.html, or from the Cambridge Crystallographic Data Centre, 12 Union Road, Cambridge CB2 1EZ, UK; fax: +44 1223 336 033; or e-mail: deposit@ccdc.cam.ac.uk. Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.poly.2012.10.040.

References

[1] (a) Ch. Orvig, M.J. Abrams, Chem. Rev. 99 (1999) 2201;
(b) J. Reedijk, Curr. Opin. Chem. Biol. 3 (1999) 236;
(c) L. Ronconia, P.J. Sadler, Coord. Chem. Rev. 251 (2007) 1633;
(d) P.C. Bruijnincx, P.J. Sadler, Curr. Opin. Chem. Biol. 12 (2008) 197;
(e) K.L. Haas, K.J. Franz, Chem. Rev. 109 (2009) 4921;
(f) M. Navarro, Coord. Chem. Rev. 253 (2009) 1619;
(g) M. Patra, G. Gasser, N. Metzler-Nolte, Dalton Trans. 41 (2012) 6350;
(h) M. Gilen, E.R.T. Tiekink, Metallotherapeutic Drugs and Metal-Based Diagnostic Agents: The Use of Metals in Medicine, John Wiley & Sons, New York, 2005;
(j) J.C. Dabrowiak, Metals in Medicine, John Wiley & Sons, New York, 2010.

[2] Z. Ma, B. Moulton, Cryst. Growth Des. 7 (2007) 196.

[3] (a) M.J. Rose, P.K. Mascharak, Curr. Opin. Chem. Biol. 12 (2008) 238;
(b) D. Crespy, K. Landfester, U.S. Schubert, A. Schiller, Chem. Commun. 46 (2010) 6651.

[4] J.K. Barton, E.D. Olmon, P.A. Sontz, Coord. Chem. Rev. 255 (2011) 619.

[5] G. Gasser, N. Metzler-Nolte, Metal compounds as enzyme inhibitors, in: E. Alessio (Ed.), Bioinorganic Medicinal Chemistry, Wiley-VCH, Weinheim, 2011.

[6] (a) V. Rudic, V. Bulimaga, T. Kiriak, L. Chapurina, Algologia (Kiev) 13 (2003) 322;
(b) I. Filippova, O. Gherco, Yu. Simonov, A. Deseatnic-Ciloci, S. Clapco, J. Tiurina, S. Baca, Polyhedron 29 (2010) 1102;
(c) P.N. Bourosh, E.B. Koropchanu, A.A. Desyatnik, O.A. Bologa, Zh.P. Tyurina, M.V. Stratan, O.A. Chiobenike, Russ. J. Coord. Chem. 35 (2009) 751;
(d) A.A. Deseatnic, N.V. Gerbeleu, E.B. Coropceanu, J.P. Tiurina, S.V. Labliuc, S.I. Clapco, Russ. J. Coord. Chem. 28 (2002) 144.

[7] (a) R.C. Heel, R.N. Brogden, T.M. Speight, A. Carmine, P.A. Morley, G.S. Avery, Drugs 16 (1978) 177;
(b) G. Schar, F.H. Kayser, M.C. Dupont, Chemotherapy 22 (1976) 211;
(c) B.S. Verma, Curr. Ther. Res. 24 (1978) 745;
(d) T. Fredriksson, Curr. Ther. Res. 25 (1979) 590;
(e) D.R. Popkin, Curr. Ther. Res. 3 (1982) 948;
(f) A.K. Gupta, T.R. Einarson, R.C. Summerbell, N.H. Shear, Drugs 55 (1998) 645;
(g) C.A. Oyeka, H.C. Gugnani, Drugs 35 (1992) 357;
(h) L. Cohen, Br. J. Vener. Dis. 60 (1984) 42.

[8] J.M. Hernandez-Molina, J. Llosa, A. Martinez Brocal, A. Ventosa, Mycopathologia 118 (1992) 15.

[9] (a) B. Havlickova, M. Friedrich, Mycoses 51 (2008) 16;
(b) E. Herms, G. Kallischnigg, Z. Hautkr. 63 (1988) 377;
(c) H.C. Gugnani, L.E. Akpata, M.K. Gugnani, R. Srivastava, Mycoses 37 (1994) 39.

[10] (a) M.C. Linder, Biochemistry of Copper, Plenum Press, New York, 1991;
(b) K.H. Thompson, Ch. Orvig, Science 300 (2003) 936.

[11] (a) C. Marzano, M. Pellei, F. Tisato, C. Santini, Anti-Cancer Agents Med. Chem. 9 (2009) 185;
(b) C. Duncan, A.R. White, Metallomics 4 (2012) 127.

[12] M.B. Cingi, C. Guastini, A. Musati, N. Nardelli, Acta Crystallogr., Sect. B 25 (1969) 1833.

[13] G.M. Sheldrick, Acta Crystallogr., Sect. A 64 (2008) 112.

[14] C.F. Macrae, P.R. Edgington, P. McCabe, E. Pidcock, G.P. Shields, R. Taylor, M. Towler, J. van de Streek, J. Appl. Crystallogr. 39 (2006) 453.

[15] N.A. Rodionova, N.A. Tiunova, G.F. Feniksova, Appl. Biochem. Microbiol. 2 (1966) 197.

[16] S.-H. Lee, S. Kim, M.-H. Yun, Y.S. Lee, S.-N. Cho, T. Oh, P. Kim, Bioorg. Med. Chem. Lett. 21 (2011) 1515.

[17] J. Mangas-Sanchez, E. Busto, V. Gotor-Fernandez, F. Malpartida, V. Gotor, J. Org. Chem. 76 (2011) 2115.

[18] K. Hori, A. Sakaguchi, M. Kudon, K. Ishida, Y. Aoyama, Y. Yoshida, Chem. Pharm. Bull. 48 (2000) 60.

[19] G.V. Roytburg, O.M. Radul, Yu.B. Kalyan, E.P. Styngachi, M.Z. Krimer, Buletinul Academiei de Stiinte a R.S.S. Moldovenesti 3 (1990) 43.

[20] F. Makaev, E. Styngach, V. Shargarovskii, L. Bets, L. Vlad, A. Barba, Russ. J. Org. Chem. 46 (2010) 6010.

[21] F. Macaev, L.A. Vlad, L.P. Bets, S.T. Malinovskii, K.N. Gavrilov, M. Gdanets, Chem. Nat. Compd. 4 (2010) 528.

[22] F. Macaev, E. Stingaci, V. Munteanu, in: L. Simeonov, V. Sargsyan (Eds.), Soil Chemical Pollution, Risk Assessment, Remediation and Security, Springer Science, Berlin Heidelberg, New York, 2008, p. 223.

[23] F. Macaev, K. Gavrilov, V. Munteanu, E. Stingaci, L. Vlad, L. Bet, S. Pogrebnoi, A. Barba, Chem. Nat. Compd. 2 (2007) 136.

[24] F. Makaev, E. Styngach, V. Muntyanu, S. Pogrebnoi, Z. Rybkovskaya, A. Barba, Russ. J. Org. Chem. 43 (2007) 1512.

[25] H. Dugas, Bioorganic Chemistry. A Chemical Approach to Enzyme Action, Springer-Verlag, New York, 1996. p. 388.

[26] H. Claus, in: I. Shermati, A. Varma (Eds.), Soil Heavy Metals, Soil Biology, vol. 19, Springer Verlag, Berlin Heidelberg, 2010, p. 281.

[27] D. Lopez-Serrano, F. Solano, A. Sanchez-Amat, Microbiology 153 (2007) 2241.

[28] K. Tsekova, D. Todorova, Z. Naturforsch., C: Biosci. 57 (2002) 319.

[29] C. Cervantes, F. Gutierrez-Corona, FEMS Microbiol. Rev. 14 (1994) 121.

[30] (a) W.-K. Yeh, H.Ch. Yang, J.R. McCarthy, Enzyme Technologies: Metagenomics, Evolution, Biocatalysis, and Biosynthesis, John Wiley & Sons, Hoboken, New Jersey, 2010;
(b) A. Illanes, Enzyme Biocatalysis – Principles and Applications, Springer Verlag, New York, 2008.

[31] (a) T. Nagodawithana, G. Reed, Enzymes in Food Processing, third ed., Academic Press, San-Diego, 1993;
(b) W. Aehle, Enzymes in Industry: Production and Application, third ed., Wiley-VCH, Weinheim, 2007.