Electronic Supplementary Information

Copper(II) complexes with aromatic nitrogen-containing heterocycles as effective inhibitors of quorum sensing activity in *Pseudomonas aeruginosa*

Biljana Đ. Glišić,*[a] Ivana Aleksic,[b] Peter Comba,*[c] Hubert Wadepohl,[c] Tatjana Ilic-Tomic,[b] Jasmina Nikodinovic-Runic*[b] and Miloš I. Djuran[a]

[a]*Department of Chemistry, Faculty of Science, University of Kragujevac, R. Domanovića 12, PO Box 60, 34000 Kragujevac, Serbia*

[b]*Institute of Molecular Genetics and Genetic Engineering, University of Belgrade, Vojvode Stepe 444a, 11000 Belgrade, Serbia*

[c]*Anorganisch-Chemisches Institüt and Interdisciplinary Center for Scientific Computing (IWR), Heidelberg University, Im Neuenheimer Feld 270, 69120 Heidelberg, Germany*

*Corresponding authors. Tel.: +381 34 300 251; fax: +381 34 335 040 (B. Đ. Glišić); Tel.: +49 6221 54-8453; fax: +49 6221 54-6617 (P. Comba); Tel.: +381 11 397 6034; fax: +381 11 397 5808 (J. Nikodinovic-Runic).

E-mail address: bglisic@kg.ac.rs (B. Đ. Glišić); peter.comba@aci.uni-heidelberg.de (P. Comba); jasmina.nikodinovic@gmail.com (J. Nikodinovic-Runic).

Abstract

Five copper(II) complexes **1-5** with aromatic nitrogen-containing heterocycles, pyrimidine (pm, **1**), pyrazine (pz, **2**), quinazoline (qz, **3** and **4**) and phthalazine (phtz, **5**) have been synthesized and structurally characterized by spectroscopic and single-crystal X-ray diffraction techniques. The crystallographic results show that, dependent on the ligand structure, complexes **1-5** are of different nuclearity. The antimicrobial efficiency of the complexes **1-5** has been evaluated against three clinically relevant microorganisms and none of the complexes showed significant growth inhibiting activity, with values of minimum inhibitory concentrations (MIC) in the mM range. Since in many bacteria, pathogenicity and virulence are regulated by intercellular communication processes, quorum sensing (QS), the effect of the copper(II) complexes on bacterial QS has also been examined. The results indicate that the investigated complexes inhibit violacein production in *Chromobacterium violaceum* CV026, suggesting an anti-QS activity. In order to differentiate, which of the QS pathways was affected by the copper(II) complexes, three biosensor strains were used: the PAO1 ΔrhlIpKD-rhlA and the PA14-R3ΔlasIPrsaI lux strain to directly measure the levels of C4-HSL (N-butanoyl-homoserine lactone) and 3OC12-HSL (N-3-oxo-dodecanoyl-homoserine lactone), respectively, and PAO1 ΔpqsA mini-CTX luxPpqsA for the detection of AHQs (2-alkyl-4-quinolones). Complexes **1-5** were shown to be efficient inhibitors of biofilm formation of the human opportunistic pathogen *Pseudomonas aeruginosa* PAO1, with the qz-containing complex **3** being the most active. Finally, the most anti-QS-active complexes **1** and **3** showed synergistic activity against a multi-drug resistant clinical isolate of *P. aeruginosa*, when supplied in combination with the known antibiotics piperacillin and ceftazidime.

Keywords: Copper(II) complexes; N-heterocycles; *Pseudomonas aeruginosa*; Quorum sensing; Synergism

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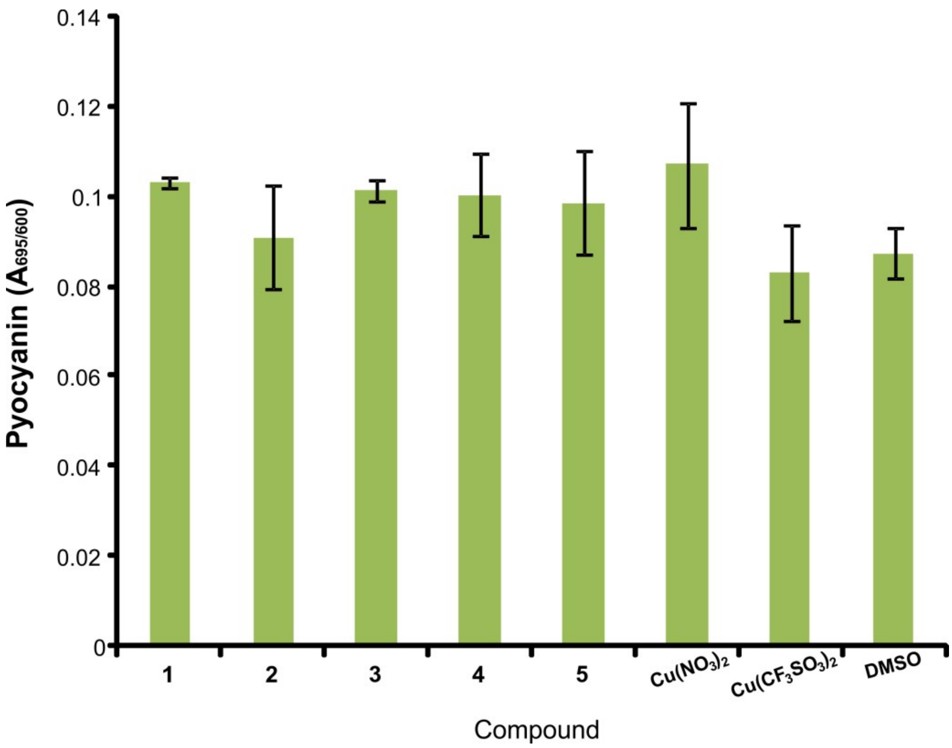

Fig. S1 Effect of the copper(II) compounds on the pyocianin production in *P. aeruginosa* PA14 strain.

Table S1 Hydrogen bond parameters for copper(II) complexes **1-5**

D—H···*A*	*D*—H (Å)	H···*A* (Å)	*D*···*A* (Å)	*D*—H···*A* (º)
		1		
C3—H3···O2^{i}	0.89(2)	2.443(12)	3.0631(14)	127.0(4)
C3—H3···O2^{ii}	0.89(2)	2.443(12)	3.0631(14)	127.0(4)
C1—H1···O4	0.94(2)	2.481(8)	2.9456(12)	110.5(5)
C1—H1···O4^{iii}	0.94(2)	2.481(8)	2.9456(12)	110.5(5)
C2—H2···O3^{iv}	0.921(14)	2.378(14)	3.2658(15)	161.7(12)
O4—H4A···O3^{v}	0.788(18)	1.961(18)	2.7486(13)	178.2(18)
O4—H4A···N2^{v}	0.788(18)	2.693(18)	3.4230(16)	154.9(15)
O4—H4B···O3^{vi}	0.837(19)	1.937(19)	2.7547(13)	165.0(17)
Symmetry codes: (i) −*x*+1, −*y*+2, −*z*+1; (ii) *x*+1/2, −*y*+2, *z*; (iii) −*x*+3/2, −*y*, −*z*+1; (iv) −*x*+1, *y*+1/2, −*z*+1/2; (v) *x*+1/2, −*y*+1, *z*; (vi) *x*+1, *y*, *z*				
		2		
O4—H4A···O21^{i}	0.81(3)	1.95(3)	2.763(2)	174(2)
O4—H4B···O13^{ii}	0.78(3)	2.01(3)	2.781(2)	169(3)
Symmetry codes: (i) *x*−1/2, *y*+1/2, *z*; (ii) *x*, *y*+1, *z*				
		3		
O4—H4A···O3^{i}	0.78(2)	1.97(2)	2.7105(17)	160(2)
O4—H4B···O3^{ii}	0.79(2)	1.93(2)	2.6916(17)	163(2)
Symmetry codes: (i) −*x*, −*y*+1, −*z*; (ii) *x*+1, *y*, *z*				
		4		
O7—H7A···O2^{i}	0.77(2)	2.07(2)	2.8414(16)	178(2)
O7—H7B···N4^{ii}	0.81(2)	2.04(2)	2.8508(17)	178(2)
Symmetry codes: (i) –*x*+1/2, −*y*+3/2, −*z*+1/2; (ii)) –*x*+1, *y*–1/2, −*z*+1/2				
		5		
O1—H1A···O6	0.81	2.07	2.817(3)	153.5
O1—H1B···O6^{i}	0.86	2.17	2.974(3)	150.4
O1—H1B···O6^{ii}	0.86	2.46	3.000(3)	121.3
O2—H2···O5^{iii}	0.70(3)	2.12(3)	2.797(2)	164(3)
Symmetry codes: (i) -*x*+2, -*y*, -*z*; (ii) -*x*+1, -*y*, -*z*+1; (iiii) *x*+1, *y*, *z*				

Table S2 Crystallographic data collection and structure refinement information for copper(II) complexes **1-5**

	1	**2**	**3**	**4**	**5**
Chemical formula	$C_4H_8CuN_4O_8$	$C_{18}H_{18}CuF_6N_8O_7S_2$	$C_{16}H_{16}CuN_6O_8$	$C_{17}H_{15.50}CuN_{6.50}O_7$	$C_{32}H_{30}Cu_3N_{12}O_{16}$
M_r	303.68	700.06	483.89	486.40	1029.30
Crystal system, space group	monoclinic, *I*2/*a*	monoclinic, *C*2/*c*	triclinic, $P\bar{1}$	monoclinic, *I*2/*a*	triclinic, $P\bar{1}$
a (Å)	7.478(3)	24.751(13)	6.497(3)	15.126(7)	6.9579(2)
b (Å)	11.463(4)	8.703(5)	7.540(3)	10.555(5)	10.1787(2)
c (Å)	11.434(4)	24.827(11)	10.882(4)	24.976(11)	13.4465(3)
α (°)			98.788(13)		82.8821(18)
β (°)	102.655(11)	90.181(10)	98.616(10)	101.681(8)	81.365(2)
γ (°)			113.900(14)		73.620(2)
V (Å^3)	956.2(6)	5348(5)	468.3(4)	3905(3)	899.97(4)
F_{000}	612	2824	247	1984	521
Z	4	8	1	8	1
X-radiation, λ /Å	Mo-*K*a, 0.71073	Mo-*K*a, 0.71073	Mo-*K*a, 0.71073	Mo-*K*a, 0.71073	Mo-*K*a, 0.71073
data collect. temperat. /K	100(1)	100(1)	100(1)	100(1)	120(1)
Calculated density (Mg m^{-3})	2.110	1.739	1.716	1.655	1.899
Absorption coefficient (mm^{-1})	2.330	1.070	1.228	1.176	1.854
Crystal size (mm)	0.28 × 0.21 × 0.20	0.25 × 0.14 × 0.10	0.16 × 0.12 × 0.08	0.26 × 0.24 × 0.10	0.15 × 0.06 × 0.05
θ range (°)	2.5 to 32.4	2.3 to 31.5	1.9 to 32.4	1.7 to 32.5	3.3 to 32.8
index ranges *h*,*k*,*l*	-11 ... 11, -17 ... 17, -17 ... 17	-35 ... 36, -12 ... 12, -36 ... 36	-9 ... 9, -11 ... 11, -16 ... 16	-22 ... 22, -15 ... 15, -37 ... 36	-10 ... 10, -15 ... 15, -20 ... 20
No. of collected, independent and observed [$I > 2\sigma(I)$] reflections	11973, 1651, 1577	66620, 8863, 7024	12030, 3148, 2936	48962, 6715, 5776	41600, 6187, 5058
R_{int}	0.0240	0.0569	0.0292	0.0363	0.0616
Transmission factors: max, min	0.6229, 0.5504	0.7464, 0.6925	0.7464, 0.6802	0.7464, 0.6652	0.927, 0.799
Data / restraints / parameters	1651 / 0 / 97	8863 / 0 / 387	3148 / 0 / 168	6715 / 0 / 294	6187 / 0 / 295
Goodness-on-fit on F^2	1.093	1.017	1.086	1.044	1.055
Final *R* indices [$F_o > 4\sigma(F_o)$] $R(F)$, $wR(F^2)$	0.0154, 0.0443	0.0358, 0.0791	0.0267, 0.0657	0.0287, 0.0709	0.0430, 0.1042
Final *R* indices (all data) $R(F)$, $wR(F^2)$	0.0165, 0.0451	0.0529, 0.0867	0.0303, 0.0674	0.0375, 0.0750	0.0570, 0.1112
Difference density: max, min (e Å^{-3})	0.533, -0.427	0.665, -0.446	0.578, -0.284	0.574, -0.532	1.049, -0.864