Supplementary Information

Crystalline pyrazine-2-amidoxime isolated by diffusion method and its structural and behavioral analysis in the context of crystal engineering and microbiological activity

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Notes

The authors declare no competing financial interest.

X-ray Crystallography

Single-crystal specimens of pyrazine-2-amidoxime were selected for the X-ray diffraction experiments at 22 ± 0.1°C (**Tables S1-S4**). Diffraction data were collected on an Oxford Diffraction Gemini R ULTRA Ruby CCD diffractometer with Cu*K*α (λ = 1.54184) radiation. The lattice parameters were obtained by least-squares fit to the optimized setting angles of the reflections collected by means of *CrysAlis CCD.*[61] Data were reduced using *CrysAlis RED* software and applying multi-scan absorption corrections (empirical absorption correction using spherical harmonics, implemented in the SCALE3 ABSPACK scaling algorithm).[61] The structural resolution procedure was carried out using the *SHELX* package.[62] Structures were solved with direct methods that carried out refinements by full-matrix least-squares on F^2 using the *SHELXL-97* program. All H-atoms bounded with O/N atoms were placed geometrically and refined freely with $U_{iso}(H) = 1.5/1.2U_{eq}(O/N)$. All interactions were found using the PLATON program.[63] The ORTEPII[64] and Mercury[65] programs were used to prepare the molecular graphics. The structure in $P2_1$ space group was validated using PLATON and checkCIF. The intensity distribution within the diffraction pattern suggested non-centrosymmetric structure with[66] $<E^2–1 >= 0.790$.

Minimal bactericidal or fungicidal concentrations

Determination of the antimicrobial activity of the tested compounds was performed *in vitro* on selected bacterial strains in appropriate broths: *Enterococcus hirae* ATCC 10541 (Brain Heart Infusion Broth, BHI), *Staphylococcus aureus* ATCC 6538, *Escherichia coli* ATCC 8739, *Proteus vulgaris* ATCC 4635, *Pseudomonas aeruginosa* ATCC 9077 (Mueller-Hinton Broth, MH), and yeast, *Candida albicans* ATCC 10231 (Sabouraud Dextrose Broth), using standard microbroth dilution assay. Minimal inhibitory and minimal bactericidal (fungicidal) concentrations (MIC and MBC/MFC, respectively) were determined in three independent measurements.[67,68] The tested compounds were diluted with a culture medium in geometric progression in 96-well flat-bottomed microculture plates, so that their final volumes were 100 μL. Each well was inoculated with overnight bacteria cultures containing 10^5 cells mL^{-1} diluted with Mueller-Hinton or BHI broth, and yeast cultures containing 10^3 cells mL^{-1} diluted with Sabouraud Dextrose Broth. The plates were incubated at 35-37°C for 18 hours (bacteria) or at 25°C for 24 hours (yeasts). After an incubation time the growth of microorganisms was examined to determine the MIC value, which was taken as the lowest concentration of the tested complexes that inhibited visible growth of bacteria (yeasts). In addition, 100 μL of suspension from each tube without growth was inoculated in a proper agar plate to control bacterial (fungal) viability. The MBC (MFC) was defined as the lowest concentration at which antimicrobial compounds would kill a particular microorganism in the medium after 24 hours of incubation.[69]

Crystallographic results

Table S1. Crystal data and structure refinement parameters for the title compound.

Compound	**pyrazine-2-amidoxime**
Chemical formula	$C_5H_6N_4O$
FW/g mol^{-1}	138.14
Crystal system	monoclinic
Space group	$P2_1$
a/Å	3.780(4)
b/Å	10.572(1)
c/Å	15.039(1)
β/°	94.25(9)
V/Å^3	599.4(9)
Z	4
t/°C	22(0.1)
λ_{Cu}/Å	1.54184
ρ_{calc}/g cm^{-3}	1.531
F(000)	288
μ/mm^{-1}	0.967
2θ range/º	5.12–67.33
Reflections collected	10811
Reflections unique	2162 [R_{int}=0.0534]
Data/restraints/parameters	2162/1/200
Goodness-of-fit on F^2	1.094
Final R_1value ($I > 2\sigma(I)$)	0.0350
Final wR_2 value($I > 2\sigma(I)$)	0.0842
Final R_1value (all data)	0.0413
Final wR_2 value (all data)	0.0898
CCDC number	1474463

Table S2. Bond lengths (d) in [Å] of pyrazine-2-amidoxime A and B. Data obtained from single-crystal X-ray diffraction measurements. The atom labels and numbers in agreement with those in Figure 4.

	d[a] (Å)	
Bond	**A**	**B**
C(6)-C(5)	1.377(3)	1.366(3)
N(4)-C(5)	1.334(3)	1.336(3)
H(5)-C(5)	0.930(3)	0.930
C(3)-N(4)	1.326(3)	1.335(3)
C(2)-C(3)	1.393(3)	1.391(3)
N(1)-C(2)	1.337(3)	1.333(3)
H(3)-C(3)	0.931	0.930
N(1)-C(6)	1.335(3)	1.341(3)
C(7)-C(2)	1.486(3)	1.485(3)
H(6)-C(6)	0.929	0.930
N(8)-C(7)	1.346(3)	1.348(3)
N(9)-C(7)	1.286(3)	1.288(3)
H(8)-N(8)	0.920(3)	0.890(3)
H(8)-N(8)	0.960(3)	0.870(3)
N(9)-O(10)	1.427(2)	1.420(2)
O(10)-H(10)	0.920 (3)	0.990(3)

Table S3. Valence angles in [deg] of pyrazine-2-amidoxime A and B. Data obtained from single-crystal X-ray diffraction measurements. The atom labels and numbers in agreement with those in Figure 4.

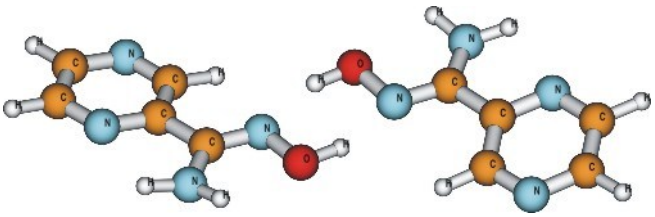
	Angle [degree]	
Bonded atoms	**A·**	**B**
C(5)-N(4)-C(3)	115.90(2)	115.80(2)
N(4)-C(5)-C(6)	121.80(2)	121.70(2)
H(5)-C(5)-C(6)	119.10	119.10
N(1)-C(6)-H(6)	118.70	118.60
N(1)-C(2)-C(3)	121.10(2)	120.90(2)
C(6)-N(1)-C(2)	115.90(2)	116.00(2)
H(3)-C(3)-N(4)	118.70	118.70
N(1)-C(6)-C(5)	122.60(2)	122.90(2)
C(7)-C(2)-C(3)	122.20(2)	122.00(2)
H(6)-C(6)-C(5)	118.70	118.50
N(8)-C(7)-C(2)	117.60(2)	118.20(2)
N(9)-C(7)-C(2)	116.90(2)	115.70(2)
H(8)-N(8)-C(7)	118.00(2)	119.00(2)
C(7)-N(9)-O(10)	108.50(2)	110.90(2)
N(9)-O(10)-H(10)	104.00(2)	103.00(2)

Table S4. Dihedral angles in [deg] of pyrazine-2-amidoxime A and B. Data obtained from single-crystal X-ray diffraction measurements. The atom labels and numbers in agreement with those in Figure 4.

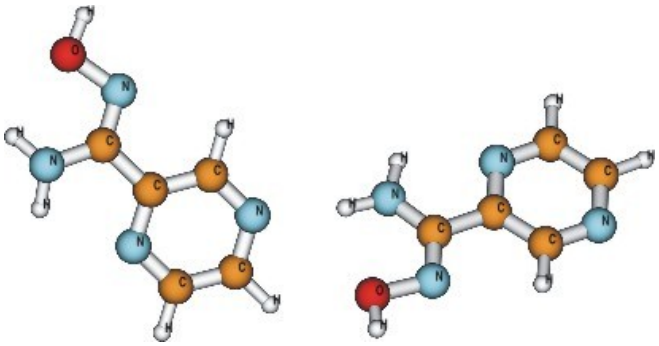
	Dihedral angles (degree)	
Bonded atoms	**A·**	**B·**
C(6)-N(1)-C(2)-C(3)	1.20 (3)	-0.30(3)
C(6)-N(1)-C(2)-C(7)	-176.80 (2)	-179.59(18)
N(1)-C(2)-C(3)-N(4)	-2.80(3)	0.40(3)
C(7)-C(2)-C(3)-N(4)	175.10(2)	-179.70(2)
C(2)-C(3)-N(4)-C(5)	1.30(3)	-0.20(3)
C(3)-N(4)-C(5)-C(6)	1.50(3)	-0.10(3)
C(2)-N(1)-C(6)-C(5)	1.60(3)	0.00(3)
N(4)-C(5)-C(6)-N(1)	-3.10(3)	0.10(4)
N(1)-C(2)-C(7)-N(8)	-9.30(3)	-2.70(3)
C(3)-C(2)-C(7)-N(8)	172.70(2)	178.01(19)
N(1)-C(2)-C(7)-N(9)	168.30(2)	175.29(18)
C(3)-C(2)-C(7)-N(9)	-9.70(3)	-4.0(03)
N(8)-C(7)-N(9)-O(10)	-2.10(3)	-1.40(3)
C(2)-C(7)-N(9)-O(10)	-179.48(16)	-179.15(16)

DFT structural and energetic data for dimers and tetramer

Table S5. Structure, free energy (G in kcal mol^{-1}), and atom cartesian coordinates (in Å) of the **D1-D3** dimers and the **T1** tetramer of pyrazine-2-amidoxime modelled for its crystal structure and calculated at the DFT level.

Structure	Free energy and atom coordinates
D1 	G = -613133.07 N 1.516892 2.928463 5.539512 C 1.148480 4.211384 5.452290 C 0.818383 4.946428 6.602437 H 0.496429 5.977894 6.527915 N 0.877440 4.412054 7.817986 C 1.259106 3.132049 7.896519 H 1.311432 2.689384 8.885997 C 1.570876 2.392700 6.760389 H 1.869429 1.351449 6.830904 C 1.106116 4.789183 4.086559 N 1.254458 3.928893 3.047619 H 1.392647 4.319320 2.129766 H 1.577171 2.998271 3.260971 N 0.914953 6.063025 3.961519 O 0.911106 6.433286 2.607652 H 0.782790 7.406095 2.654139 N 0.397186 12.247285 1.907655 C 0.329418 10.914707 2.001861 C 0.136426 10.120175 0.860132 H 0.056992 9.043017 0.941086 N 0.035136 10.649798 -0.354717 C 0.116747 11.982532 -0.440672 H 0.036718 12.422093 -1.429682 C 0.290474 12.777100 0.687658 H 0.345034 13.858547 0.611484 C 0.461530 10.347065 3.366123 N 0.445830 11.222407 4.402955 H 0.697421 10.876305 5.314497 H 0.544220 12.200976 4.182842 N 0.565569 9.063300 3.492513 O 0.688199 8.706517 4.844470 H 0.780492 7.729599 4.798521

D2



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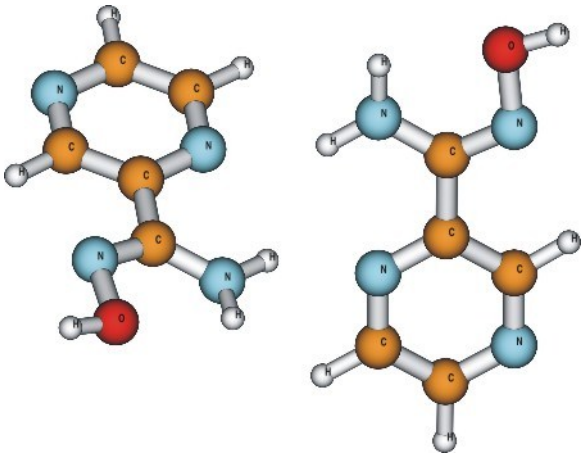
C	1.516374	5.121602	6.623635
H	1.352589	6.186573	6.731901
N	1.839169	4.426694	7.709756
C	2.035254	3.111928	7.547806
H	2.299089	2.537084	8.429844
C	1.908101	2.502690	6.305188
H	2.068792	1.436060	6.182434
C	1.026655	5.279977	4.140942
N	0.937597	4.580852	2.989001
H	0.703053	5.010308	2.099365
H	1.114054	3.590213	3.041494
N	0.833432	6.550044	4.303729
O	0.489230	7.150508	3.062281
H	0.456792	8.084455	3.291681
N	-1.058492	6.322422	-2.268976
C	-0.162582	5.331266	-2.249944
C	0.396366	4.881835	-1.042789
H	1.124020	4.079619	-1.043048
N	0.056450	5.420683	0.123852
C	-0.842753	6.414622	0.101124
H	-1.107510	6.853578	1.056492
C	-1.395487	6.860152	-1.093709
H	-2.123384	7.665005	-1.109150
C	0.201823	4.737614	-3.560442
N	-0.313387	5.328001	-4.669814
H	-0.214202	4.857716	-5.553844
H	-1.040040	6.013294	-4.535830
N	0.995303	3.717330	-3.545347
O	1.224557	3.278958	-4.871547
H	1.804966	2.521027	-4.751049

D3



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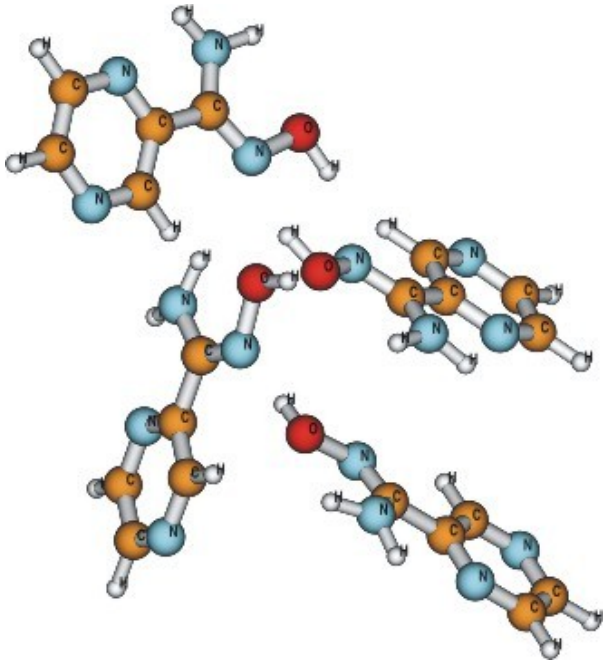
C	-1.731642	5.193409	6.246310
H	-1.771728	6.274272	6.191750
N	-1.088835	4.644282	7.270924
C	-1.045120	3.306557	7.308286
H	-0.519054	2.849492	8.140086
C	-1.644364	2.529694	6.324660
H	-1.605234	1.445358	6.359861
C	-3.063036	5.037528	4.108282
N	-3.349081	4.266616	3.030017
H	-3.949715	4.658543	2.323386
H	-3.194692	3.265543	3.046409
N	-3.346321	6.295282	4.229105
O	-4.019019	6.740765	3.064772
H	-4.217600	7.659555	3.269949
N	-2.882851	1.303072	2.103504
C	-3.466866	0.103075	2.190069
C	-3.345305	-0.834035	1.148864
H	-3.816197	-1.805344	1.236353
N	-2.661459	-0.570228	0.040965
C	-2.080513	0.633587	-0.036751
H	-1.516876	0.856323	-0.936896
C	-2.188771	1.563122	0.990127
H	-1.715160	2.537591	0.921723
C	-4.231789	-0.200685	3.425059
N	-4.048244	0.593779	4.508427
H	-4.665996	0.462725	5.292542
H	-3.500410	1.443755	4.448097
N	-5.015400	-1.230258	3.372403
O	-5.667137	-1.390593	4.619891
H	-6.245767	-2.143420	4.464504

T1



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C	0.683716	-2.158332	2.467431
H	-0.264913	-1.669561	2.652166
N	1.495413	-2.318519	3.509335
C	2.671186	-2.908294	3.272233
H	3.340410	-3.039975	4.116398
C	3.030623	-3.336227	1.997193
H	3.989315	-3.810134	1.812379
C	0.153887	-2.425592	-0.003080
N	0.590278	-2.934423	-1.171621
H	0.106454	-2.657205	-2.010922
H	1.561034	-3.204203	-1.219558
N	-0.995096	-1.833498	0.155427
O	-1.687523	-1.770689	-1.071232
H	-2.593235	-1.510753	-0.793088
N	-7.349998	-0.487109	-0.514837
C	-6.015130	-0.497664	-0.595591
C	-5.351678	0.100655	-1.678650
H	-4.269568	0.120089	-1.724161
N	-6.016295	0.673802	-2.677239
C	-7.351542	0.664936	-2.596923
H	-7.900813	1.128805	-3.409908
C	-8.014461	0.094276	-1.515204
H	-9.097938	0.103905	-1.450140
C	-5.296227	-1.158277	0.522075
N	-6.026004	-1.482798	1.618767
H	-5.598284	-2.072584	2.313776
H	-7.029533	-1.444826	1.531507
N	-4.025459	-1.365308	0.395583
O	-3.512276	-2.000110	1.540335
H	-2.559922	-2.069511	1.336453
N	0.827348	4.971041	1.713017
C	0.725593	3.911382	0.901975
C	1.403551	3.876886	-0.327680
H	1.313027	3.029580	-0.995424
N	2.181880	4.880492	-0.725063
C	2.287407	5.928376	0.098571
H	2.923225	6.748515	-0.219697
C	1.608191	5.974717	1.311423
H	1.691016	6.832587	1.971476
C	-0.148214	2.811656	1.387246
N	-0.906677	3.077496	2.480439
H	-1.381263	2.307665	2.923045
H	-0.682294	3.909614	3.003252
N	-0.160049	1.682813	0.750441
O	-1.042911	0.791063	1.378393
H	-0.970149	-0.049857	0.882069
N	5.391535	-1.055428	-2.554378
C	4.619236	-0.649918	-1.540582
C	5.111913	-0.617505	-0.223428
H	4.472829	-0.286448	0.585995
N	6.353259	-0.986856	0.070785
C	7.121057	-1.389184	-0.951881
H	8.137264	-1.692003	-0.720505
C	6.641540	-1.421006	-2.255322
H	7.270844	-1.747574	-3.077544
C	3.234934	-0.246121	-1.874883
N	2.809377	-0.464121	-3.158526
H	1.983911	0.042423	-3.440510
H	3.537738	-0.609004	-3.842146
N	2.512336	0.249886	-0.925727
O	1.245428	0.589768	-1.408559
H	0.777230	0.966201	-0.624811

Experimental and DFT-calculated IR and Raman spectra

Table S6. Selection of the experimentally observed (exp.) and calculated using DFT(B3LYP)/6-311+G** method FT-IR and Raman bands (in cm^{-1}) for the isolated *PAOX* (monomer), its the most energetically stable dimer (D1) and their general assignments. The theoretical bands were rescaled with a factor equal 0.95.

FT-IR			Raman			
exp.	**monomer**	**D1**	**exp.**	**monomer**	**D1**	**Assignment[a]**
3437	3587	3531	3333	3537	3532	$\nu_{as}(NH_2)$
3331	3414	3410		3415	3410	$\nu_{sym}(NH_2)$
3144	3657	3294	3060	3657	3249	$\nu(OH)$
2816	3016	3017	3059	3037-2999	3017	$\nu(CH_{ring})$
1659	1619	1627	1662	1619	1627	$\nu(C{=}N_{oxime})$
1590	1530	-	1576	1530	1530	$\nu(C{=}N_{ring})$
1520	1510	-	1521	1493	1495	ν_{as} $(C\text{-}C_{ring})$
1483	1472	1426	1491	1432	-	ν_{sym} $(C\text{-}C_{ring})$
1433	-	-	1435	-	1493	$\delta(C\text{-}H)$
1373	1386	1380-1324	1382	1385-1336	1487-1324	$\delta(CH) + \delta(NH) + \nu(C\text{-}C_{ring})$
1297	1278	1062	1293	-	-	$\delta(NH_2)$
1153 - 1018	1128-1043	1130	1188-1056	1134-1020	1130	$\delta(CH)$ in-plane
953	914	940	-	-	-	$\nu(N\text{-}O_{oxime})$
862- 844	825-805	823	845	-	981	$\delta(CH)$ out-of-plane
713	687	746	709	-	-	$\delta(C\text{-}C)_{ring}$ in-plane
479	466-292	398	465	-	-	$\delta(C\text{-}C)_{ring}$ out-of-plane

[a]Abbreviations: ν- stretching, ν_{as} – asymmetric stretching, ν_{sym} - symmetric stretching, δ - bending

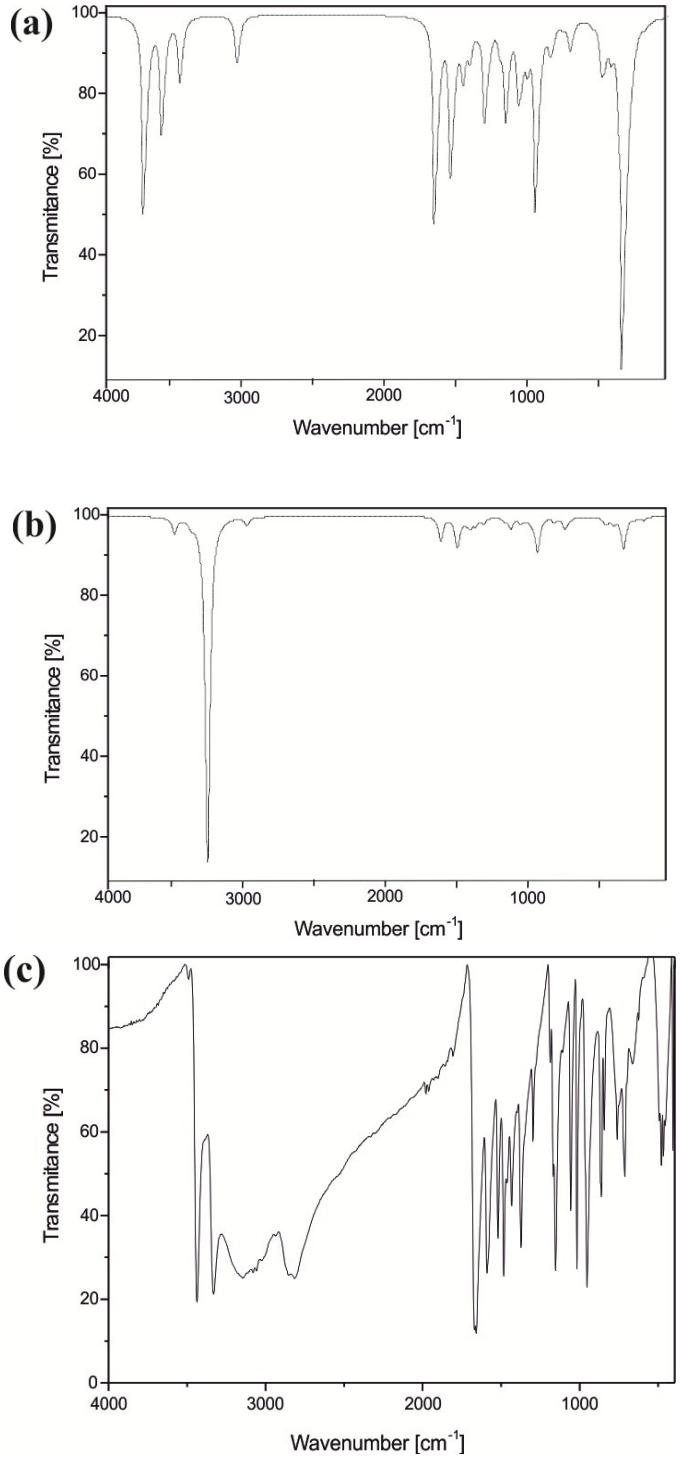

Figure S1. The calculated IR spectra (DFT/B3LYP/6-311+G**) for the isolated monomer of *PAOX* (**a**) and for the most stable dimer D1 (**b**) compared to the experimental IR spectrum for solid *PAOX* in KBr (**c**).

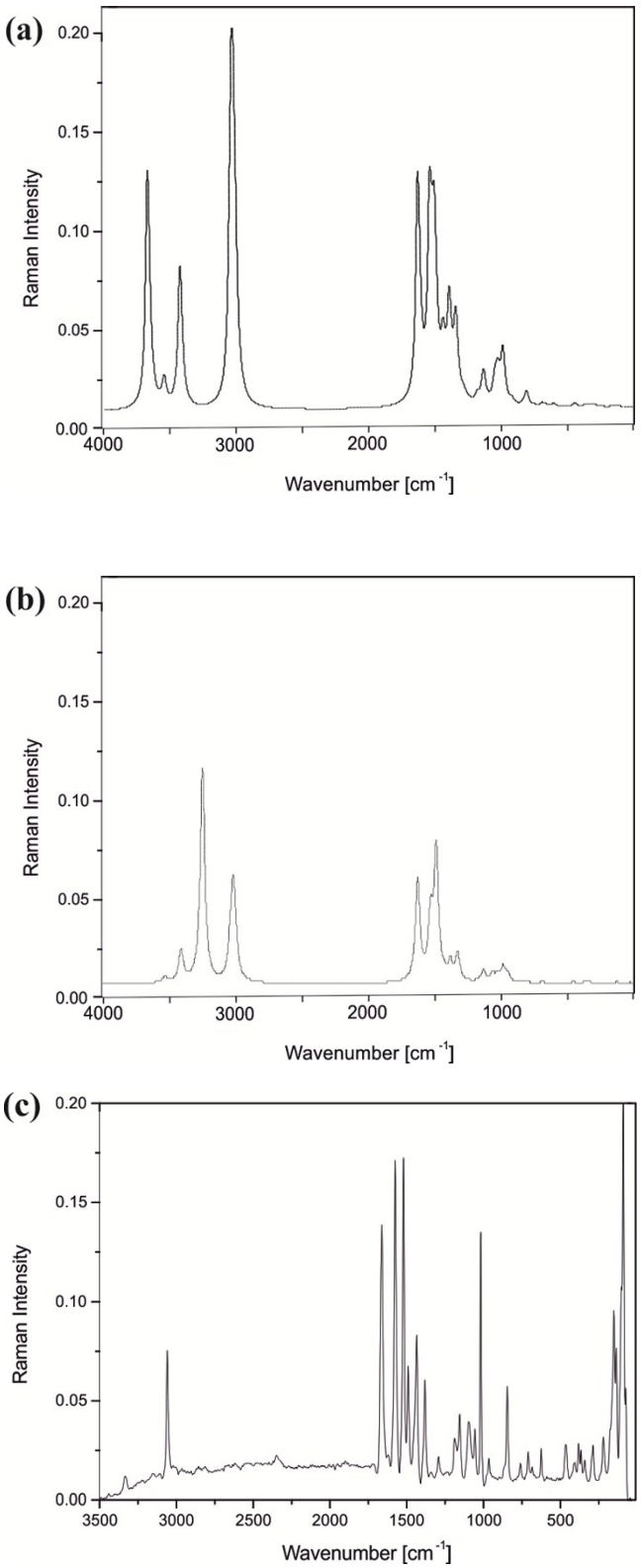

Figure S2. The calculated Raman spectra (DFT/B3LYP/6-311+G**) for the isolated monomer of *PAOX* (**a**) and for the most stable dimer D1 (**b**) compared to the experimental Raman spectrum for solid *PAOX* (**c**).

NMR study

^{1}H NMR spectra were recorded with Brüker AVANCE 700 MHz spectrometer the NMR Laboratory at the Faculty of Chemistry (University of Gdansk). Chemical shifts of NMR spectra were obtained with pyrazine-2-amidoxime in DMSO-d_6 (2.5 ppm) as the standard, relative to tetramethylsilane TMS (0.0 ppm). All measurements were performed at room temperature.

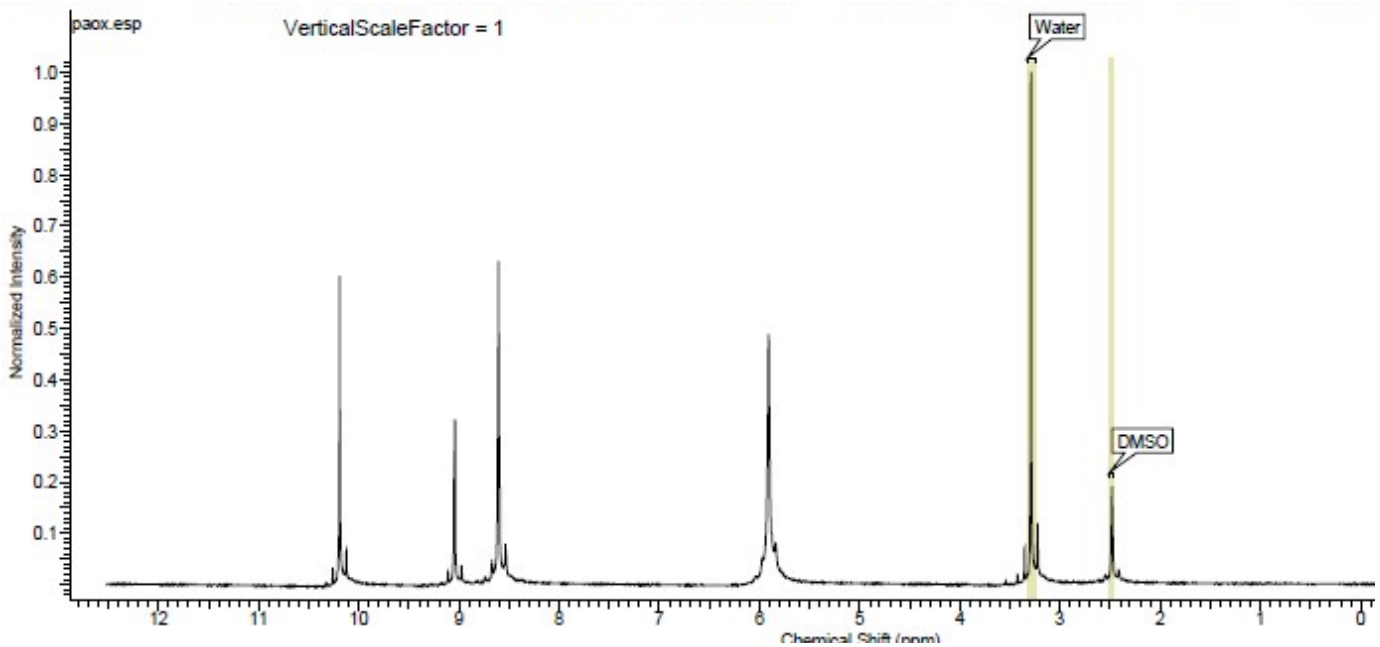

Figure S3. The scan of the original ^{1}H NMR spectra obtained in DMSO-d_6 for *PAOX*.

The numbering for ^{1}H NMR data of examined compounds was shown in Figure S3. Chemical shifts were reported in ppm (δ) and were applied indirectly to tetramethylsilane as a signal of solvent (2.49 for ^{1}H in DMSO-d_6). In the spectrum of *PAOX*, the signal appeared at 9.07 ppm was assigned to H(3) proton. The peaks observed at 8.50 ppm and 8.66 ppm were attributed to H(5) and H(6) aromatic protons, respectively. The ^{1}H NMR spectrum of pyrazine-2-amidoxime shows at 5.97 ppm signal for H protons of amine group (NH_2). The signal appeared at 10.26 ppm was ascribed to H(10) proton of hydroxyl group.

Complexometric properties of PAOX

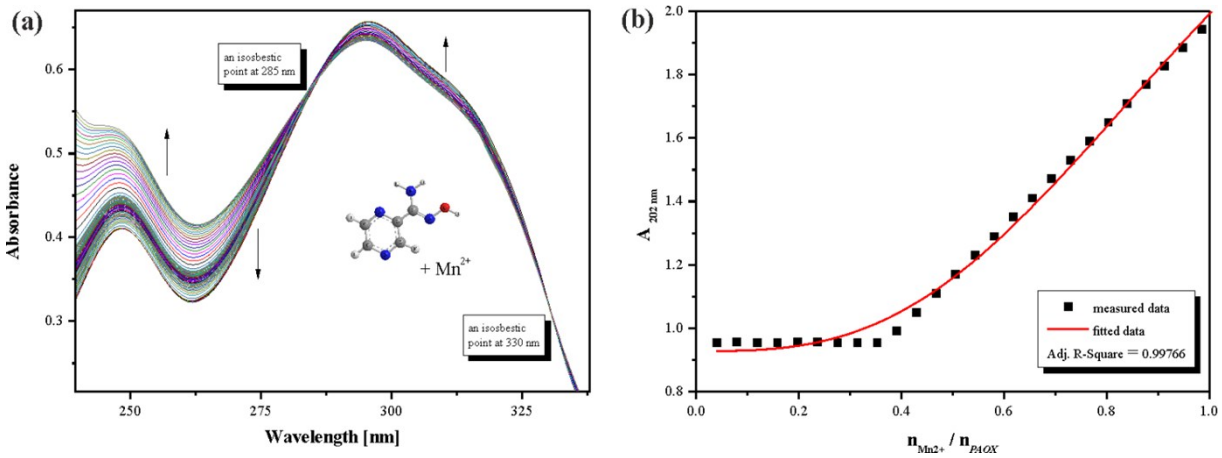

Figure S4. (a) The titration spectra for *PAOX* in acetonitrile with Mn(II): **(a)** full titration curves, **(b)** absorption as a function of metal-ligand molar ratio at 202 nm. For titration conditions, see Section '*Analytical Instrumentations'* presented in manuscript. Arrows indicate the change in absorbance during the titration.

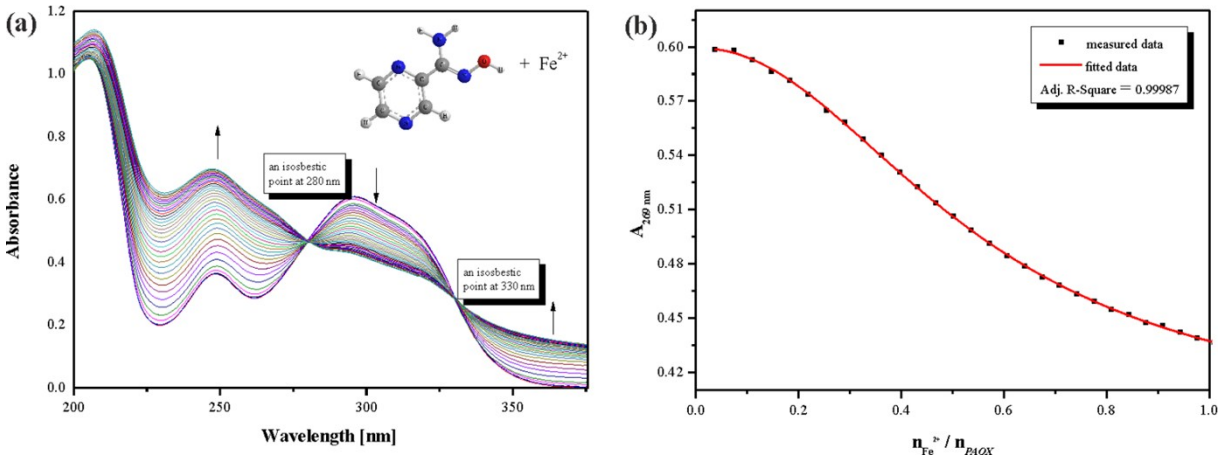

Figure S5. (a) The titration spectra for *PAOX* in acetonitrile with Fe(II): **(a)** full titration curves, **(b)** absorption as a function of metal-ligand molar ratio at 269 nm. For titration conditions, see Section '*Analytical Instrumentations'* presented in manuscript. Arrows indicate the change in absorbance during the titration.

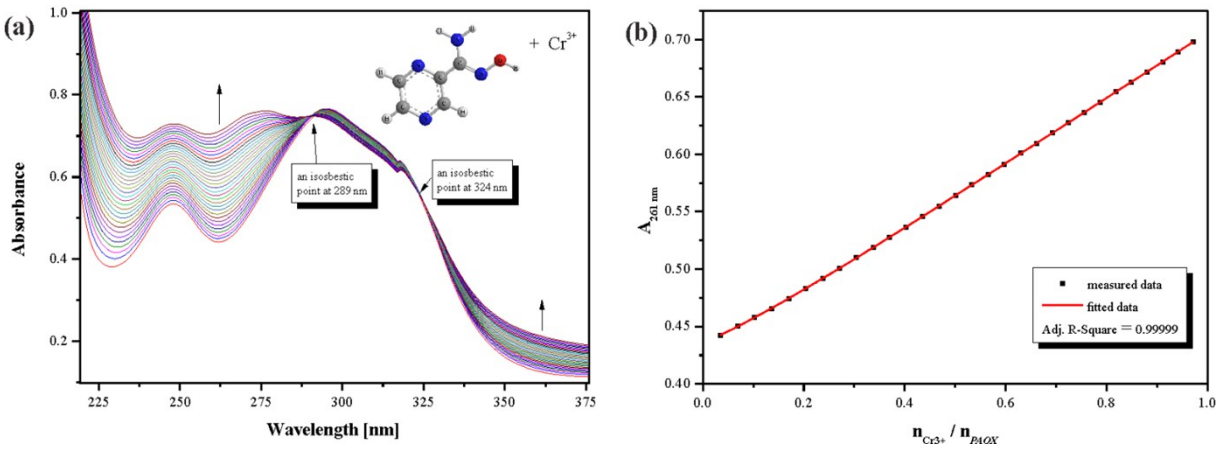

Figure S6. (a) The titration spectra for *PAOX* in acetonitrile with Cr(III): **(a)** full titration curves, **(b)** absorption as a function of metal-ligand molar ratio at 261 nm. For titration conditions, see Section '*Analytical Instrumentations'* presented in manuscript. Arrows indicate the change in absorbance during the titration.

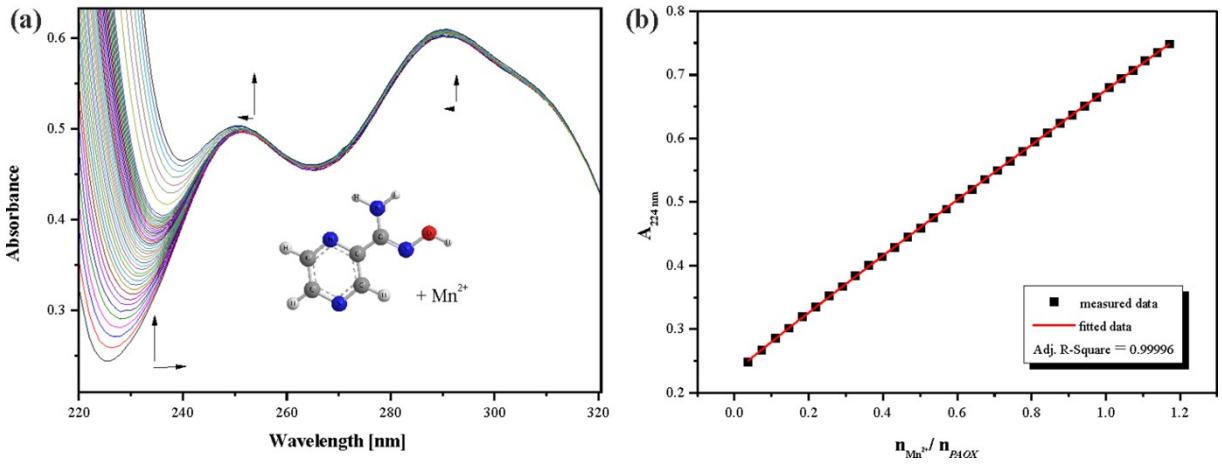

Figure S7. (a) The titration spectra for *PAOX* in water with Mn(II): **(a)** full titration curves, **(b)** absorption as a function of metal-ligand molar ratio at 224 nm. For titration conditions, see Section '*Analytical Instrumentations'* presented in manuscript. Arrows indicate the change in absorbance during the titration.

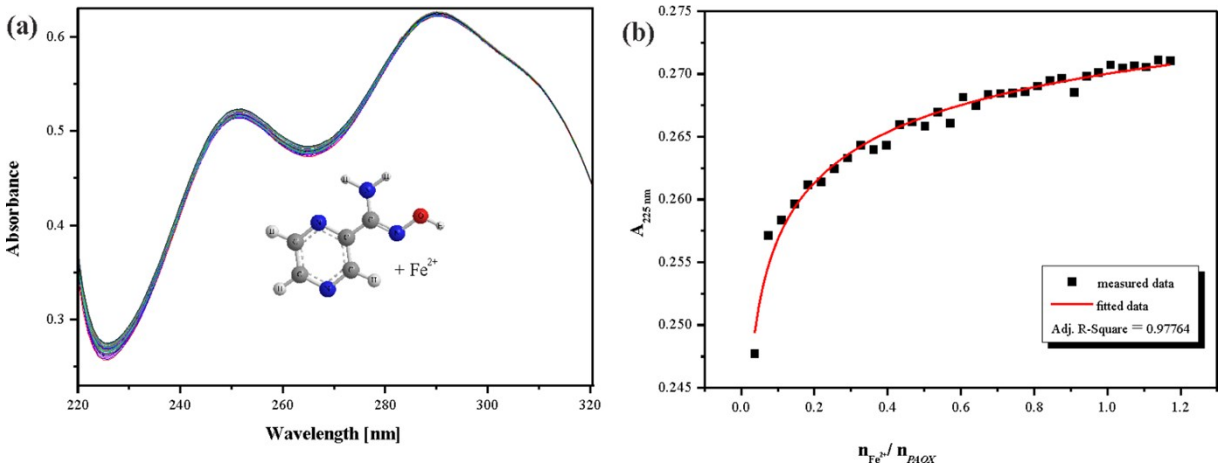

Figure S8. (a) The titration spectra for *PAOX* in water with Fe(II): **(a)** full titration curves, **(b)** absorption as a function of metal-ligand molar ratio at 225 nm. For titration conditions, see Section '*Analytical Instrumentations'* presented in manuscript. Arrows indicate the change in absorbance during the titration.

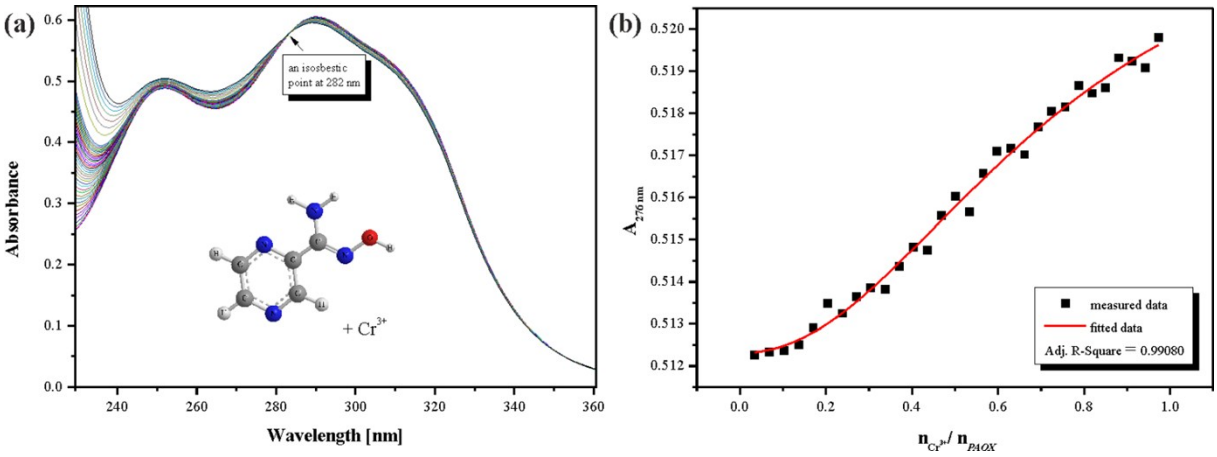

Figure S9. (a) The titration spectra for *PAOX* in water with Cr(III): **(a)** full titration curves, **(b)** absorption as a function of metal-ligand molar ratio at 276 nm. For titration conditions, see Section '*Analytical Instrumentations'* presented in manuscript. Arrows indicate the change in absorbance during the titration.

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