Supplementary data for the article:

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A Comparative Study of In Vitro Antitumor, Antioxidant and Antimicrobial activity of Pt(II), Zn(II), Cu(II) and Co(III) Complexes with *N*-heteroatomatic Schiff Base (*E*)-2-[*N*'-(1-pyridin-2-yl-ethylidene)hydrazino]acetate

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MATHERIALS AND METHODS

Antimicrobial Activity Assay

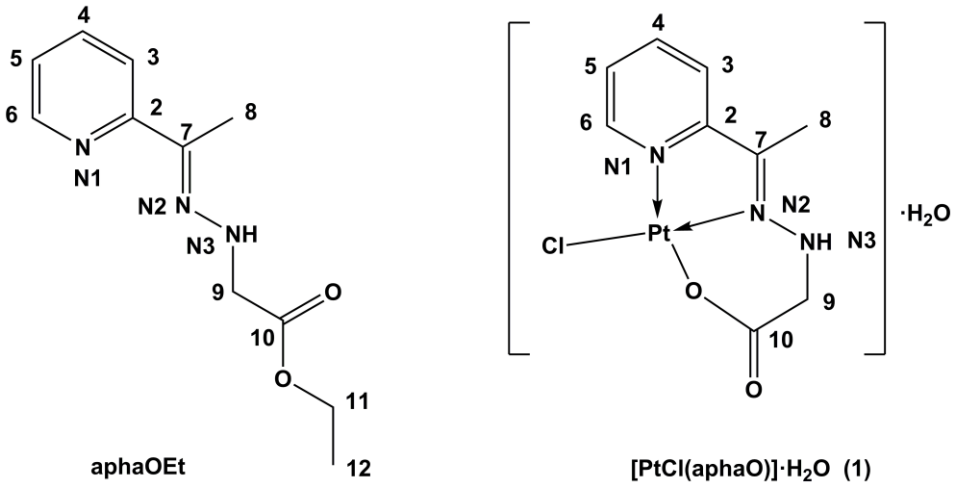
The bacteria cultures were cultivated in the appropriate broth and agar (Table S1, Supporting Information) at 37 ± 1 ºC for 24 h and 50 ± 1 °C for *G. stearothermophylus*, while the yeasts were cultivated at 30 ± 1 ºC for 24 to 48 h. The cultures were diluted with culture agar/broth to contain 10^7 CFU/mL (CFU = colony forming units). The number of live bacteria and yeasts was counted by the plate enumeration method. All cultures were incubated in aerobic conditions. An aliquot of the cultures (100 μL) was evenly spread on the surface of the solidified agar. The disks (6 mm) were impregnated with 10 μL solution of the tested compounds in DMSO. The concentration of the tested compounds was 0.5 mg/10 μL. Disks impregnated with 10 μL of DMSO were used as a negative control. The amount of standard antimicotic nystatin was 100 U/disc. The following antibiotics were used as the positive controls: penicillin (for *L. monocytogenes* and *E. faecalis*; 6 μg/disc), chloramphenicol (for *B. cereus*, *G. stearothermophilus* and *Y. enterocolitic*; 30 μg/disc), gentamycin (for *E. coli* O157:H7, *S. enteritidis* and *S. sonnei*; 30 μg/disc), amoxicillin (for *P. aeruginosa* and and *P. hauseri*; 10 μg/disc) and ampicillin (for *S. aureus*; 10 μg/disc). From each zone of inhibition, a piece of agar was transferred into the appropriate broth which was then incubated. If the broth was blurred, the effect of the compound was referred as microbiostatic, and if the broth remained clear the effect of the compound was referred as microbicidal. The plates were incubated at 37 ± 1 ºC (24 h) for the bacteria, except for *G. stearothermophylus* where the plates were incubated at 50 ± 1°C (24 h). The plates with the yeasts were incubated at 30 ± 1 ºC (48 h).

Table S1. The substrates used for growing of microorganisms

Microorganism	Broth	Solid medium
Cryptococcus neoformans ATCC 76484	Malt Extract Broth, HiMedia	Malt Extract Agar, HiMedia
Saccharomyces cerevisiae ATCC 9763	Malt Extract Broth, HiMedia	Malt Extract Agar, HiMedia
Candida albicans ATCC 24433	Malt Extract Broth, HiMedia	Malt Extract Agar, HiMedia
Bacillus cereus ATCC 10876	Nutrient Broth, Torlak	M 833 *Bacillus cereus* Agar Base + FD 003 + FD 045
Staphilococcus aureus ATCC 25923	Nutrient Broth, Torlak	M 1468 Hi Crome Aureus Agar Base + FD 046
Enterococcus faecalis ATCC 49532	Nutrient Broth, Torlak	Mueller Hinton Agar, Torlak
Geobacillus stearothermophylus ATCC 7953	Nutrient Broth, Torlak	Mueller Hinton Agar, Torlak
Listeria monocytogenes ATCC 19115	TSYEB, Biolab	TSYEA, Biolab
Salmonella enteritidis ATCC 13076	Nutrient Broth, Torlak	Salmonella Shigella Agar, Torlak
Escherichia coli O157:H7 ATCC 35150	TSYEB, Biolab	MacConkey Sorbitol Agar, HiMedia
Shigella sonnei ATCC 29930	Nutrient Broth, Torlak	Salmonella Shigella Agar, Torlak
Pseudomonas aeruginosa ATCC 10145	Nutrient Broth, Torlak	Mueller Hinton Agar, Torlak
Proteus hauseri ATCC 29905	Nutrient Broth, Torlak	Mueller Hinton Agar, Torlak
Yersinia enterocolitic ATCC 23715	Nutrient Broth, Torlak	Mueller Hinton Agar, Torlak

RESULTS

Structure of Pt(II) Complex 1



Scheme S1. Structures of aphaOEt and Pt(II) complex **1** along with atom numbering scheme.

Table S2. ^{1}H and ^{13}C NMR spectral data of the ligand aphaOEt and the complex **1**

compound	Chemical shifs (δ, ppm) and assignments of the signals in ^{1}H NMR spectrum	Chemical shifs (δ, ppm) and assignments of the signals in ^{13}C NMR spectrum
aphaOEt	*δ:* 1.19 (t, 3H, H–C12 , $^{3}J_{12,11}$ = 7.0 Hz), 2.17 (s, 3H, H–C8), 4.01 (s, 2H, H–C9), 4.11 (q, 2H, H–C11, $^{3}J_{11,12}$ = 7.0 Hz), 6.84 (br. s, 1H, H–N3), 7.21 (ddd, 1H, H–C5, $^{3}J_{5,4}$ = 7.0 Hz, $^{3}J_{5,6}$ = 5.0 Hz, $^{3}J_{5,3}$ = 1.0 Hz), 7.67 (td, 1H, H–C4, $^{3}J_{4,3}$ = 8.0 Hz, $^{3}J_{4,5}$ = 7.0 Hz), 7.82 (dt, 1H, H–C3, $^{3}J_{3,4}$ = 8.0 Hz, $^{3}J_{3,5}$ = 1.0 Hz), 8.48 (br. d, 1H, H–C6, $^{3}J_{6,5}$ = 5.0 Hz)	*δ:* 10.3 (C8); 14.0 (C12); 51.6 (C9); 60.0 (C11); 118.3 (C3); 121.9 (C5); 135.8 (C4); 142.4 (C7); 148.0 (C6); 156.1 (C2); 171.3 (C10)
1	*δ*: 2.26 (s, 3H, H–C8), 3.89 (br. d, 2H, H–C9), 7.63 (ddd, 1H, H–C5, $^{3}J_{5,4}$ = 7.7, $^{3}J_{5,6}$ = 5.8; $^{4}J_{5,3}$ = 1.3 Hz), 7.87 (br. d, 1H, H–C3, $^{3}J_{3,4}$ = 7.7 Hz), 8.24 (td, 1H, H–C4, $^{3}J_{4,3}$ = $^{3}J_{4,5}$ = 7.7 Hz, $^{4}J_{4,6}$ = 1.0 Hz;), 8.45 (br. s, 1H, H–N3), 9.14 (dd, 1H, H–C6, $^{3}J_{6,5}$ = 5.8; $^{4}J_{6,4}$ = 1.0 Hz)	*δ*: 12.7 (C8), 55.0 (C9), 124.8 (C3), 125.7 (C5), 140.2 (C4), 150.1 (C6), 151.2 (C7), 158.7 (C2), 169.3 (C10)
Calcd. for **1**[a)]	*δ*: 2.26 (H–C8)[b)], 3.97 (H–C9) [b)], 7.73 (H–C5), 7.75 (H–C3), 8.20 (H–C4), 9.14 (H–C6)	*δ*: 11.0 (C8), 57.0 (C9), 117.5 (C3), 118.7 (C5), 132.2 (C4), 142.7 (C6), 140.1 (C7), 145.7 (C2), 159.9 (C10)

[a)] ^{1}H and ^{13}C NMR chemical shifts for the complex **1** were obtained by the GIAO/WP04 calculations.

[b)] Average value.

Table S3. ^{15}N NMR spectral data (derived from ^{1}H-^{15}N HMBC and HSQC spectra) of the ligand aphaOEt and the complex **1**

aphaOEt	δ: 114.5 (N3), 306.4 (N1), 345.0 (N2)
1	δ: 126.7 (N3), 189.6 (N1), 248.9 (N2)

Free radical scavenging activity

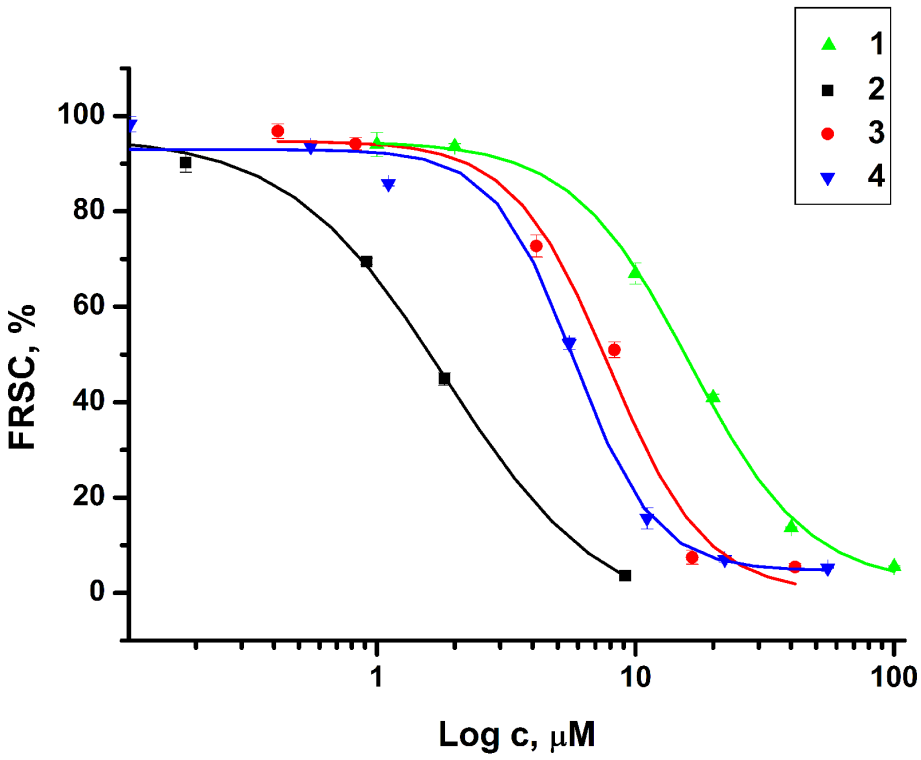


Figure S1. The log sigmoid dose–response curves of free radical scavenging activity of the complexes **1**–**4**.

DNA Cleavage

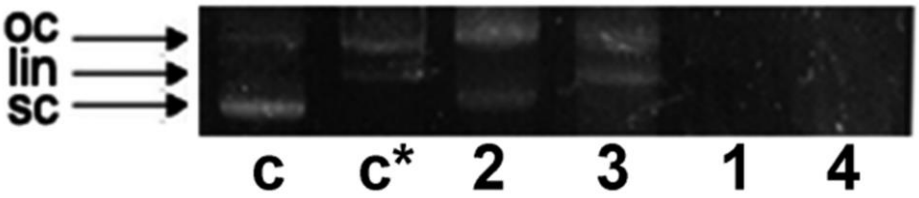


Figure S2. Electrophoregram of open circular (oc), linear (lin) and supercoiled (sc) form of plasmid pET20b treated with *in situ* generated hydroxyl radical in the presence or absence of the complexes (Legend: c = DNA control; c* = DNA + H_2O_2 + UV; 1 = DNA + **1** + H_2O_2 + UV; 2 = DNA + **2** + H_2O_2 + UV; 3 = DNA + **3** + H_2O_2 + UV; 4 = DNA + **4** + H_2O_2 + UV).

Antimicrobial Activity of the Complexes

The complexes **1**–**4**, as well as the metal salts used in syntheses were screened for their antimicrobial activity against a panel of fourteen strains of microorganisms: *C. neoformans*, *S. cerevisiae*, *C. albicans*, *B. cereus*, *S. aureus*, *E. faecalis*, *G. stearothermophylus*, *L. monocytogenes*, *S. enteritidis*, *E. coli* O157:H7, *S. sonnei*, *P.s aeruginosa*, *P. hauseri* and *Y. enterocolitic*. The antimicrobial activities of the tested compounds are summarized in Table S4 and Figure S3. The obtained results showed that starting metal salts did not show the inhibitory effect on the growth of tested strains of microorganisms. Among the tested complexes, only **1** showed moderate antifungal activity, while others were not active.

Table S4. Antimicrobial activities of the investigated compounds tested by the disc diffusion method (inhibition zone size including disc, mm)

	(**1**)	(**2**)	(**3**)	(**4**)	Standard [a)]
C. neoformans	8* [b)]	n.i. [c)]	n.i.	n.i.	20*
S. cerevisiae	10*	n.i.	n.i.	n.i.	25*
C. albicans	8*	n.i.	n.i.	n.i.	19*
B. cereus	n.i.	8	12* + 6	10	11
S. aureus	10* + 10	n.i.	14* + 6	19	21
E. faecalis	10*	n.i.	7*	n.i.	15*
G. stearothemnoph.	22*	16*	14*	n.i.	19*
L. monocytogenes	10*	7*	n.i.	n.i.	15*
S. enteritidis	9*	n.i.	18*	n.i.	30*
E. coli O157:H7	n.i.	n.i.	n.i.	n.i.	26*
S. sonnei	8*	10*	10*	n.i.	32
P. aeruginosa	n.i.	16*	32*	n.i.	40
P. hauseri	10*	5	11	n.i.	16
Y. enterocolitica	14	10	8*	n.i.	28

[a)] The following antimicotic/antibiotics were used as standards: nystatin (all fungi strains; 100 U/disc), penicillin (*L. monocytogenes* and *E. faecalis*; 6 µg/disc), chloramphenicol (*B. cereus*, *G. stearothermophilus* and *Y. enterocolitic*; 30 µg/disc), gentamycin (*E. coli* O157:H7, *S. enteritidis* and *S. sonnei*; 30 µg/disc), amoxycyclin

(*P. aeruginosa and* and *P. hauseri*; 10 μg/disc) and ampicillin (*S. aureus*; 10 μg/disc); [b] * - microbicid; [c] n.i. – no inhibition.

The complexes were generally more active against the Gram-positive than the Gram-negative bacteria. The least sensitive strain was *E. coli* since the tested compounds did not show the activity. The Cu(II) complex **3** exhibited strong bactericidal activity against *B. cereus*, while Pt(II) complex **1** showed strong activity against *G. stearothermophylus*. Both complexes posses greater activity against corresponding strain in comparison to the activity of standard antibiotic (chloramphenicol). Interestingly, although the activity of amoxicillin on *P. aeruginosa* was slightly greater than the activity of **3**, the complex showed microbicidal mode of action.

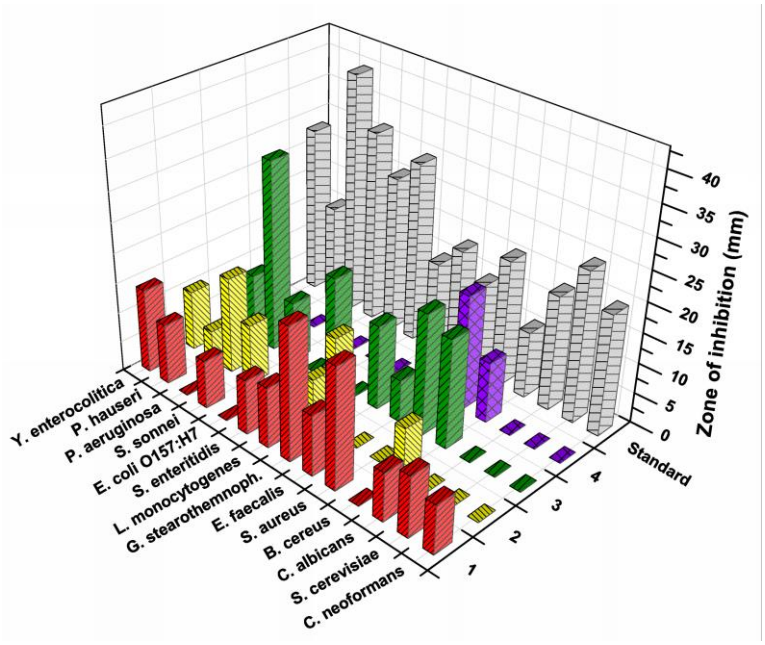


Figure S3. Representative antimicrobial activity plots for the complexes **1**–**4** and standard antimicotic/antibiotics. The following antibiotics were used as standards: nystatin (all fungi strains), penicillin (*L. monocytogenes* and *E. faecalis*), chloramphenicol (*B. cereus*, *G. stearothermophilus* and *Y. enterocolitic*), gentamycin (*E. coli* O157:H7, *S. enteritidis* and *S. sonnei*), amoxycyclin (*P. aeruginosaand* and *P. hauseri*) and ampicillin (*S. aureus*).