Supporting Information

Inert Cationic Iridium(III) Complexes with Phenanthroline-based Ligands: Application in Antimicrobial Inactivation of Multidrug-Resistant Bacterial Strains

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General procedure for the synthesis of Ir(III) complexes

Dimer complex [(Ir(µ-Cl)(C^N)₂]₂

2-Phenyl-1H-benzo[d]imidazole (2.2 mmol) and iridium(III) chloride (1 mmol) were dissolved in 2-ethoxyethanol/deionized H_2O (3:1) in a Schlenk flask. The mixture was stirred and refluxed at 110 °C for 24 h under a nitrogen atmosphere. The mixture was cooled to room temperature, and the resultant solid was collected by filtration. The solid was washed with water and vacuum dried. Reported.¹

Iridium monomers Ir1-Ir5.

The cyclometalated iridium(III) chlorido-bridged dimer (1.0 mmol) and the respective polypyridyl N^N ligand (2.1 mmol) were dissolved in dichloromethane/methanol (2:3, v/v) in a Schlenk flask. The mixture was stirred and refluxed at 58°C for 24 h under a nitrogen atmosphere. After cooling the solution to room temperature, an excess amount of KPF₆ (2.5 mmol) was added, and the mixture was stirred for 30 min. The solvent was removed under reduced pressure, and the product was washed with water. The product was recrystallized from dichloromethane and ether.



Figure S1. ¹H and ¹³C NMR spectra of Ir1 (400 MHz, [D₆]DMSO).



Figure S2. ¹H and ¹³C NMR spectra of Ir2 (600 MHz, [D₆]DMSO).



Figure S3. ¹H and ¹³C NMR spectra of Ir4 (600 MHz, [D₆]DMSO).



Figure S4. ¹H and ¹³C NMR spectra of Ir5 (400 MHz, [D₆]DMSO).



Figure S5.HSQC 2D ¹H-¹³C NMR of Ir5 ([D₆]DMSO).

RP-HPLC purity and stability analyses.

The purity of new Ir(III) complexes was analyzed using an RP-HPLC/MS TOF 6220 equipped with a double binary pump (model G1312A), degasser, autosampler (model G1329A), diode array detector (model G1315D) and mass detector in series Agilent Technologies 1200. Chromatographic analyses were carried out on a Brisa C18 column (150 mm \times 4.6 mm, 5 µm particle size); Teknokroma, Macclesfield, UK. The mobile phase was a mixture of (A) H₂O/HCOOH 0.1% and (B) acetonitrile/HCOOH 0.1%. The flow rate was 0.8 mL/min in a linear gradient starting (see Table S1 in the Supporting Information for the gradient used). Chromatograms were recorded at 280 nm. The HPLC system was controlled by a ChemStation software (MASS HUNTER.). The mass detector was an ion trap spectrometer equipped with a dual source electrospray-APCI. Mass spectrometry data were acquired in the positive ionization mode. The ionization conditions were adjusted at 350 °C and 3 kV for capillary temperature and voltage, respectively. The nebulizer pressure and flow rate of nitrogen were 60 psi and 12 L/min, respectively. The full scan mass covered the range from m/z 100 up to m/z 1000. Samples were dissolved in DMSO.

The stability of Ir1-Ir5 were evaluated by UV/Vis spectrophotometry and NMR spectroscopy. For the stability in DMSO, the complexes were dissolved in $[D_6]DMSO$ and

were measured over at room temperature by NMR. The stability of complexes in water was studied by UV/Vis, dissolving the compounds in 1% DMSO (10 μ M).



Figure S6. HPLC/MS of complex Ir1.



Figure S7. HPLC/MS of complex Ir2.



Figure S7. HPLC/MS of complex Ir3.



Figure S8. HPLC/MS of complex Ir4.



Figure S9. HPLC/MS of complex Ir5.

Table S1. HPLC method

Time (min)	0.1% acid formic in dH ₂ O	0.1% acid formic in CH ₃ CN
0-14	90	10
14-20	10	90
20-25	90	10



Figure S10. Stability of complex Ir2 in DMSO after 3 days.



Figure S11. Stability of complexes in water (1% DMSO).



Figure S12. UV/Vis absorption spectra complexes in acetonitrile (red line) and water (dark line).

Compound	Solvent	$\lambda_{abs},$ nm (ϵ , M^{-1} cm ⁻¹)	λexc/λem, nm
Ir1	H ₂ O ^b	236 (48620), 272 (42210), 303 (29540), 368	370/573
	ACN	(10240)	380/575
		229 (74910), 270 (40760), 303 (32520), 380 (7880)	
Ir2	$\mathrm{H}_2\mathrm{O}^\mathrm{b}$	256 (66520), 302 (40800), 375 (10500)	375/667
	ACN	254 (69340), 296 (41260), 343 (15390), 395 (5000)	395/582
Ir3	$\mathrm{H}_2\mathrm{O}^{\mathrm{b}}$	245 (55640), 282 (57550), 380 (14410)	385/621
	ACN	240 (60640), 279 (82020), 365 (20650), 380 (17160)	375/580
Ir4	$\mathrm{H}_2\mathrm{O}^\mathrm{b}$	245 (66640), 310 (50090), 402 (10120)	402/-
	ACN	245 (66640), 324 (80610), 396 (13130), 420 (9630)	415/567
Ir5	${\rm H_2O^b}$	238 (6514), 303 (5675), 410 (1913)	410/-
	ACN	230 (70704), 304 (69340), 394 (2548), 415 (32550)	420/582

Table S2. UV/vis absorption bands and extinction coefficients for Ir1–Ir5^a

^a[Ir complex] = 10 μ M. ^b 1% DMSO



Figure S13. Emission spectra of complexes in acetonitrile (λ_{exc} max).



Figure S14. A) Fluorescence intensity at 617 nm ($\lambda_{exc} = 535$ nm) of treated MRSA *S.aureus* with the Ir(III) complexes and stained with propidium iodide over time. B) Variation of OD₂₆₀ in the treated and untreated MRSA *S.aureus* filtrates.

References

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