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Luminescent Cyclometalated Iridium(III) Polypyridine Di-2-picolylamine Complexes: Synthesis, Photophysics, Electrochemistry, Cation Binding, Cellular Internalization, and Cytotoxic Activity

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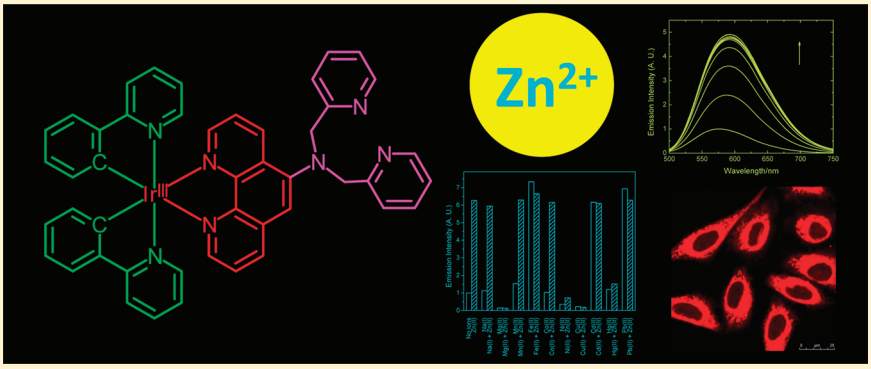
Supporting Information

ABSTRACT:



A series of luminescent cyclometalated iridium(III) polypyridine complexes containing a di-2-picolylamine (DPA) moiety $[Ir(N^{\wedge}C)_2(phen\text{-}DPA)](PF_6)$ (phen-DPA = 5-(di-2-picolylamino)-1,10-phenanthroline) ($HN^{\wedge}C$ = 2-phenylpyridine, Hppy (**1a**), 2-(4-methylphenyl)pyridine, Hmppy (**2a**), 2-phenylquinoline, Hpq (**3a**), 4-(2-pyridyl)benzaldehyde, Hpba (**4a**)) and their DPA-free counterparts $[Ir(N^{\wedge}C)_2(phen\text{-}DMA)](PF_6)$ (phen-DMA = 5-(dimethylamino)-1,10-phenanthroline) ($HN^{\wedge}C$ = Hppy (**1b**), Hmppy (**2b**), Hpq (**3b**), Hpba (**4b**)) have been synthesized and characterized, and their photophysical and electrochemical properties investigated. Photoexcitation of the complexes in fluid solutions at 298 K and in alcohol glass at 77 K resulted in intense and long-lived luminescence. The emission of the complexes has been assigned to a triplet metal-to-ligand charge-transfer (^{3}MLCT) ($d\pi(Ir) \rightarrow \pi^*(N^{\wedge}N)$) or triplet intraligand (^{3}IL) ($\pi \rightarrow \pi^*$) ($N^{\wedge}C$) excited state and with substantial mixing of triplet amine-to-ligand charge-transfer (^{3}NLCT) ($n \rightarrow \pi^*$) ($N^{\wedge}N$) character, depending on the identity of the cyclometalating and diimine ligands. Electrochemical measurements revealed an irreversible amine oxidation wave at ca. +1.1 to +1.2 V vs saturated calomel electrode, a quasi-reversible iridium(IV/III) couple at ca. +1.2 to +1.6 V, and a reversible diimine reduction couple at ca. −1.4 to −1.5 V. The cation-binding properties of these complexes have been studied by emission spectroscopy. Upon binding of zinc ion, the iridium(III) DPA complexes displayed 1.2- to 5.4-fold emission enhancement, and the K_d values determined were on the order of 10^{-5} M. Job's plot analysis confirmed that the binding stoichiometry was 1:1. Additionally, selectivity studies showed that the iridium(III) DPA complexes were more sensitive toward zinc ion among various transition metal ions examined. Furthermore, the cytotoxicity of these complexes toward human cervix epithelioid carcinoma cells have been studied by the 3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyltetrazolium bromide assay and their cellular-uptake properties by inductively coupled plasma mass spectrometry and laser-scanning confocal microscopy.

INTRODUCTION

Divalent zinc ion is an essential trace metal element in many cellular processes; for example, it controls the synthesis of both DNA and RNA, promotes vascular endothelial repair, and participates in epidermal cell division.[1] Depletion of biological zinc level would thus lead to a decrease in wound healing strength as a result of impaired collagen synthesis, in addition to growth retardation and impair immunity.[1] Also, zinc ion is involved in many important biological controls, such as gene expressions,[2] neurotransmission,[3] and bioinorganic catalysis.[4] However, a too high level of zinc is cytotoxic and may lead to skin diseases,[5] diabetes,[6] and prostatic adenocarcinoma.[7] For these reasons, the development of both in vitro and in vivo sensors for zinc ion is of paramount importance. In this context, the design of molecular probes for zinc ion has mainly relied on the use of fluorescent

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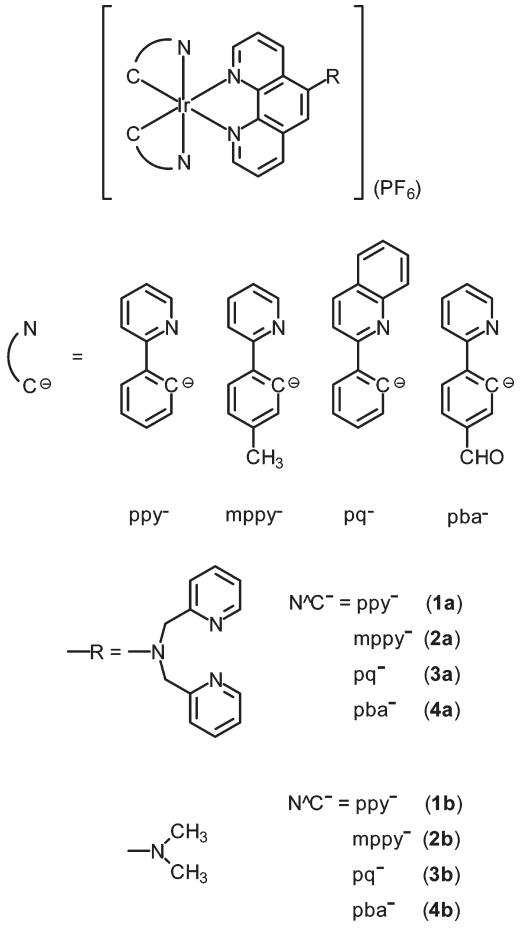
organic dyes,[8] the majority of which employed di-2-picolylamine (DPA) as the recognition unit. Since many luminescent cyclometalated iridium(III) polypyridine complexes exhibit excellent photophysical properties,[9−19] the adoption of these luminophores as the detection unit is anticipated to be an alternative approach. For instance, many luminescent iridium(III) complexes have been functionalized and widely utilized for sensing various biological targets, such as ions,[19] proteins,[20a−c,e−g,j−l] DNA,[20b,f,h,i] liposomes,[20d] sugars,[20j] and cellular components.[17c,20d−l]

Up to now, the development of iridium(III) complexes as zinc ion sensors is relatively limited;[19d,e] for instance, the complex $[Ir(ppy)_2(bpy-C{\equiv}C-C_6H_4-DPA)]^+$ has been reported as a potential probe for zinc ion.[19d] Its emission enhancement upon zinc binding has been ascribed to the conversion of excited-state nature from triplet intraligand (^{3}IL) to an admixture of triplet ligand-to-ligand charge-transfer (^{3}LLCT) and triplet metal-to-ligand charge-transfer (^{3}MLCT). In another study, a luminescent iridium(III) complex equipped with a DPA unit has been designed.[19e] This complex gives a blue-shifted emission band upon ion binding. We believe that the direct attachment of a DPA unit to the diimine ligand of cyclometalated iridium(III) polypyridine complexes would allow the ion-binding event to significantly perturb the electronic structures of the complexes and hence induce more drastic emission changes. Herein we describe the synthesis, characterization, and electronic absorption, photophysical, and electrochemical properties of a series of luminescent cyclometalated iridium(III) polypyridine complexes containing a DPA moiety $[Ir(N^{\wedge}C)_2(phen\text{-}DPA)](PF_6)$ (phen-DPA = 5-(di-2-picolylamino)-1,10-phenanthroline) ($HN^{\wedge}C$ = 2-phenylpyridine, Hppy (**1a**), 2-(4-methylphenyl)-pyridine, Hmppy (**2a**), 2-phenylquinoline, Hpq (**3a**), 4-(2-pyridyl)benzaldehyde, Hpba (**4a**)) and their DPA-free counterparts $[Ir(N^{\wedge}C)_2(phen\text{-}DMA)](PF_6)$ (phen-DMA = 5-(dimethylamino)-1,10-phenanthroline) ($HN^{\wedge}C$ = Hppy (**1b**), Hmppy (**2b**), Hpq (**3b**), Hpba (**4b**)) (Chart 1). Additionally, the cation-binding properties of the complexes have been examined by emission titrations, Job's plot analysis, and ion-selectivity study, and their biological properties have also been investigated by various methods.

Chart 1. Structures of the Iridium(III) Complexes



EXPERIMENTAL SECTION

Materials and Synthesis. All solvents were of analytical grade and purified according to standard procedures.[21] $IrCl_3 \cdot 3H_2O$ (Aldrich), Hppy (Aldrich), Hmppy (Aldrich), Hpq (Acros), Hpba (Aldrich), 5-nitro-1,10-phenanthroline (Acros), palladium (5 wt % on activated carbon) (Aldrich), 2-(bromomethyl)pyridine hydrobromide (Acros), methyl iodide (Aldrich), NaH (60% dispersion in mineral oil) (Aldrich), KPF_6 (Acros), and 3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyltetrazolium bromide (MTT) (Sigma) were used without further purification. Sodium perchlorate hydrate, potassium perchlorate, magnesium perchlorate, manganese(II) perchlorate hexahydrate, iron(II) perchlorate hydrate, cobalt(II) perchlorate hexahydrate, nickel(II) perchlorate hexahydrate, copper(II) perchlorate hexahydrate, zinc(II) perchlorate hexahydrate, cadmium(II) perchlorate hydrate, and mercury(II) perchlorate hydrate were purchased from Aldrich and used as received. $[Ir_2(N^{\wedge}C)_4Cl_2]$[9a] and 5-amino-1,10-phenanthroline[20a] (phen-NH_2) were prepared by reported methods.

Phen-DPA. NaH (60% dispersion in mineral oil) (289 mg, 7.22 mmol) was added to a THF (10 mL) solution of phen-NH_2 (117 mg, 0.60 mmol) that was cooled on an ice bath under an inert atmosphere of nitrogen. The suspension was stirred for 1 h, and 2-(bromomethyl)-pyridine hydrobromide (608 mg, 2.41 mmol) was then added. The mixture was further stirred for 24 h at room temperature, and the reaction was quenched by slow addition of water (5 mL). The product was extracted with CH_2Cl_2 (100 mL × 2), and the organic layer was washed with H_2O (20 mL × 2), dried over $MgSO_4$, and evaporated to dryness. The residual solid was purified by column chromatography on silica gel, and the product was eluted with CH_2Cl_2/MeOH (20:1, v/v) and subsequently isolated as a pink solid. Yield: 106 mg (47%). ^{1}H NMR (300 MHz, methanol-d_4, 298 K, TMS): δ 9.16 (dd, 1H, J = 8.3 and 1.5 Hz, H7 of phen), 9.08 (dd, 1H, J = 4.2 and 1.2 Hz, H4 of phen), 9.07 (dd, 1H, J = 4.2 and 1.5 Hz, H9 of phen), 8.46−8.43 (m, 2H, H6 and H6′ of pyridyl rings), 8.17 (dd, 1H, J = 8.0 and 1.2 Hz, H2 of phen), 7.80 (dd, 1H, J = 8.3 and 4.2 Hz, H8 of phen), 7.69−7.63 (m, 2H, H4 and H4′ of pyridyl rings), 7.60 (dd, 1H, J = 8.0 and 4.2 Hz, H3 of phen), 7.58−7.49 (m, 2H, H3 and H3′ of pyridyl rings), 7.38 (s, 1H, H6 of phen), 7.24−7.20 (m, 2H, H5 and H5′ of pyridyl rings), 4.61 (s, 4H, CH_2). Positive-ion electrospray ionization mass spectrometry (ESI-MS) ion cluster at m/z: 378 $\{phen\text{-}DPA + H^+\}^+$.

Phen-DMA. The synthetic procedure was similar to that of phen-DPA except that methyl iodide (192 μL, 3.08 mmol) was used instead of 2-(bromomethyl)pyridine hydrobromide. The product was isolated as a yellow solid. Yield: 95 mg (84%). ^{1}H NMR (300 MHz, methanol-d_4, 298 K, TMS): δ 9.04 (dd, 1H, J = 4.5 and 1.8 Hz, H7 of phen), 8.89 (dd, 1H, J = 4.5 and 1.8 Hz, H4 of phen), 8.70 (dd, 1H, J = 8.4 and 1.8 Hz, H9 of phen), 8.29 (dd, 1H, J = 8.1 and 1.8 Hz, H2 of phen), 7.74 (dd, 1H, J = 8.4 and 4.5 Hz, H8 of phen), 7.64 (dd, 1H, J = 8.1 and 4.5 Hz, H3 of phen), 7.38 (s, 1H, H6 of phen), 2.95 (s, 6H, CH_3). Positive-ion ESI-MS ion cluster at m/z: 224 $\{phen\text{-}DMA + H^+\}^+$.

Synthesis of the Cyclometalated Iridium(III) Complexes. A mixture of $[Ir_2(N^{\wedge}C)_4Cl_2]$ (0.083 mmol) and the diimine ligand

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phen-DPA or phen-DMA (0.17 mmol) in 20 mL of CH_2Cl_2/MeOH (1:1, v/v) was refluxed under an inert atmosphere of nitrogen in the dark for 4 h. The solution was then cooled to room temperature, and KPF_6 (0.21 mmol) was added to the solution. The mixture was evaporated to dryness, and the solid was dissolved in CH_2Cl_2 and purified by column chromatography on silica gel. The desired product was eluted with CH_2Cl_2/MeOH (20:1, v/v) and subsequently recrystallized from acetone or CH_2Cl_2/diethyl ether.

[Ir(ppy)$_2$(phen-DPA)](PF$_6$) (***1a***). Complex **1a** was isolated as yellow crystals. Yield: 89 mg (74%). ^{1}H NMR (400 MHz, acetone-d_6, 298 K, TMS): δ 9.72 (dd, 1H, J = 8.8 and 1.6 Hz, H7 of phen-DPA), 8.66 (dd, 1H, J = 8.0 and 1.2 Hz, H4 of phen-DPA), 8.58−8.56 (m, 2H, H6 and H6′ of pyridyl rings of phen-DPA), 8.42 (dd, 1H, J = 5.2 and 1.6 Hz, H9 of phen-DPA), 8.25−8.21 (m, 3H, H2 of phen-DPA and H3 of pyridyl ring of ppy), 8.10 (dd, 1H, J = 8.8 and 5.2 Hz, H8 of phen-DPA), 7.96−7.88 (m, 6H, H4 and H4′ of pyridyl rings of phen-DPA and H3 of phenyl ring and H4 of pyridyl ring of ppy), 7.72−7.57 (m, 6H, H3 and H3′ of pyridyl rings, H3, and H6 of phen-DPA and H6 of pyridyl ring of ppy), 7.27−7.24 (m, 2H, H5 and H5′ of pyridyl rings of phen-DPA), 7.09−6.99 (m, 6H, H5 of pyridyl ring and H4 and H5 of phenyl ring of ppy), 6.44 (t, 2H, J = 6.8 Hz, H6 of phenyl ring of ppy), 4.75 (s, 4H, CH_2 of phen-DPA). IR (KBr) ν/cm^{-1}: 844 (s, PF_6^-). Positive-ion ESI-MS ion cluster at m/z: 879 $\{[Ir(ppy)_2(phen\text{-}DPA)]^+\}^+$. Anal. calcd for $C_{46}H_{35}N_7PF_6Ir\cdot H_2O$: C, 53.07; H, 3.58; N, 9.42. Found: C, 52.84; H, 3.62; N, 9.43.

[Ir(ppy)$_2$(phen-DMA)](PF$_6$) (***1b***). Complex **1b** was isolated as yellow crystals. Yield: 77 mg (85%). ^{1}H NMR (400 MHz, acetone-d_6, 298 K, TMS): δ 8.99 (dd, 1H, J = 8.8 and 1.2 Hz, H7 of phen-DMA), 8.68 (dd, 1H, J = 8.4 and 1.2 Hz, H4 of phen-DMA), 8.41 (dd, 1H, J = 5.2 and 1.2 Hz, H9 of phen-DMA), 8.23 (d, 2H, J = 8.4 Hz, H3 of pyridyl ring of ppy), 8.20 (dd, 1H, J = 5.2 and 1.2 Hz, H2 of phen-DMA), 8.04 (dd, 1H, J = 8.8 and 5.2 Hz, H8 of phen-DMA), 7.93−7.88 (m, 5H, H4 of pyridyl ring and H3 of phenyl ring of ppy and H3 of phen-DMA), 7.78 (s, 1H, H6 of phen-DPA), 7.68 (t, 2H, J = 6.4 Hz, H6 of pyridyl ring of ppy), 7.10−6.94 (m, 6H, H5 of pyridyl ring and H4 and H5 of phenyl ring of ppy), 6.45 (t, 2H, J = 7.6 Hz, H6 of phenyl ring of ppy), 3.10 (s, 6H, CH_3 of phen-DMA). IR (KBr) ν/cm^{-1}: 844 (s, PF_6^-). Positive-ion ESI-MS ion cluster at m/z: 724 $\{[Ir(ppy)_2(phen\text{-}DMA)]^+\}^+$. Anal. calcd for $C_{36}H_{29}N_5PF_6Ir$: C, 49.77; H, 3.36; N, 8.06. Found: C, 49.62; H, 3.52; N, 8.09.

[Ir(mppy)$_2$(phen-DPA)](PF$_6$) (***2a***). Complex **2a** was isolated as yellow crystals. Yield: 70 mg (80%). ^{1}H NMR (400 MHz, acetone-d_6, 298 K, TMS): δ 9.70 (d, 1H, J = 7.3 Hz, H7 of phen-DPA), 8.61 (d, 1H, J = 8.3 Hz, H4 of phen-DPA), 8.56 (d, 1H, J = 4.0 Hz, H9 of phen-DPA), 8.42 (d, 2H, J = 3.8 Hz, H6 and H6′ of pyridyl rings of phen-DPA), 8.22 (d, 1H, J = 3.6 Hz, H2 of phen-DPA), 8.19−8.15 (m, 2H, H3 of pyridyl ring of mppy), 8.08 (dd, 1H, J = 8.5 and 5.0 Hz, H8 of phen-DPA), 7.88−7.80 (m, 6H, H3 and H6 of phen-DPA and H4 of pyridyl ring and H3 of phenyl ring of mppy), 7.72−7.68 (m, 2H, H4 and H4′ of pyridyl rings of phen-DPA), 7.65−7.56 (m, 4H, H6 of pyridyl ring of mppy and H3 and H3′ of pyridyl rings of phen-DPA), 7.28−7.25 (m, 2H, H5 and H5′ of pyridyl rings of phen-DPA), 6.97−6.91 (m, 4H, H4 of phenyl ring and H5 of pyridyl ring of mppy), 6.26 (d, 2H, J = 7.4 Hz, H6 of phenyl ring of mppy), 4.74 (s, 4H, CH_2 of phen-DPA). IR (KBr) ν/cm^{-1}: 843 (s, PF_6^-). Positive-ion ESI-MS ion cluster at m/z: 907 $\{[Ir(mppy)_2(phen\text{-}DPA)]^+\}^+$. Anal. calcd for $C_{48}H_{41}N_7PF_6Ir\cdot H_2O$: C, 53.97; H, 3.87; N, 8.92. Found: C, 53.83; H, 4.05; N, 9.15.

[Ir(mppy)$_2$(phen-DMA)](PF$_6$) (***2b***). Complex **2b** was isolated as yellow crystals. Yield: 187 mg (77%). ^{1}H NMR (400 MHz, acetone-d_6, 298 K, TMS): δ 8.99 (dd, 1H, J = 8.5 and 1.2 Hz, H7 of phen-DMA), 8.86 (d, 1H, J = 7.1 Hz, H4 of phen-DMA), 8.42 (dd, 1H, J = 5.0 and 1.2 Hz, H9 of phen-DMA), 8.21 (dd, 1H, J = 5.0 and 1.2 Hz, H2 of phen-DMA), 8.17 (d, 2H, J = 8.2 Hz, H3 of pyridyl ring of mppy), 8.04 (dd, 1H, J = 8.5 and 5.0 Hz, H8 of phen-DMA), 7.93−7.78 (m, 6H, H4 of pyridyl ring and H3 of phenyl ring of mppy and H3 and H6 of phen-DMA), 7.65−7.61 (m, 2H, H6 of pyridyl ring of mppy), 6.97−6.91 (m, 4H, H5 of pyridyl ring and H4 of phenyl ring of mppy), 6.28 (d, 2H, J = 7.7 Hz, H6 of phenyl ring of mppy), 3.10 (s, 6H, CH_3 of phen-DMA). IR (KBr) ν/cm^{-1}: 842 (s, PF_6^-). Positive-ion ESI-MS ion cluster at m/z: 752 $\{[Ir(mppy)_2(phen\text{-}DMA)]^+\}^+$. Anal. calcd for $C_{38}H_{33}N_5PF_6Ir$: C, 50.92; H, 3.82; N, 7.56. Found: C, 50.89; H, 3.71; N, 7.81.

[Ir(pq)$_2$(phen-DPA)](PF$_6$) (***3a***). Complex **3a** was isolated as red crystals. Yield: 90 mg (59%). ^{1}H NMR (400 MHz, acetone-d_6, 298 K, TMS): δ 9.57 (d, 1H, J = 8.4 Hz, H7 of phen-DPA), 8.71 (d, 1H, J = 4.0 Hz, H4 of phen-DPA), 8.58 (d, 1H, J = 8.4 Hz, H9 of phen-DPA), 8.53−8.42 (m, 7H, H6 and H6′ of pyridyl rings, H2, and H9 of phen-DPA and H3 of phenyl ring and H3 of quinoline ring of pq), 8.33−8.27 (m, 2H, H4 of quinoline ring of pq), 8.10 (dd, J = 8.4 and 5.2 Hz, H8 of phen-DPA), 7.89−7.80 (m, 3H, H8 of quinoline ring of pq and H3 of phen-DPA), 7.59−7.54 (m, 3H, H4 and H4′ of pyridyl rings and H6 of phen-DPA), 7.42−7.16 (m, 10H, H3, H3′, H5, and H5′ of pyridyl rings of phen-DPA and H6 of phenyl ring and H5 and H7 of quinoline ring of pq), 6.93−6.85 (m, 4H, H4 of phenyl ring and H6 of quinoline ring of pq), 6.70−6.64 (m, 2H, H5 of phenyl ring of pq), 4.55 (s, 4H, CH_2 of phen-DPA). IR (KBr) ν/cm^{-1}: 846 (s, PF_6^-). Positive-ion ESI-MS ion cluster at m/z: 979 $\{[Ir(pq)_2(phen\text{-}DPA)]^+\}^+$. Anal. calcd for $C_{54}H_{39}N_7PF_6Ir\cdot 2H_2O$: C, 55.95; H, 3.74; N, 8.46. Found: C, 55.81; H, 4.03; N, 8.70.

[Ir(pq)$_2$(phen-DMA)](PF$_6$) (***3b***). Complex **3b** was isolated as red crystals. Yield: 79 mg (85%). ^{1}H NMR (400 MHz, acetone-d_6, 298 K, TMS): δ 8.96 (dd, 1H, J = 8.4 and 1.2 Hz, H7 of phen-DMA), 8.71 (dd, 1H, J = 5.1 and 1.2 Hz, H4 of phen-DMA), 8.59−8.46 (m, 6H, H2 and H9 of phen-DMA and H3 of phenyl ring and H3 of quinoline ring of pq), 8.31 (m, 2H, H4 of quinoline ring of pq), 8.07 (dd, 1H, J = 8.4 and 4.8 Hz, H8 of phen-DMA), 7.92 (dd, 1H, J = 8.4 and 5.2 Hz, H3 of phen-DPA), 7.83 (dt, 2H, J = 8.0 and 1.2 Hz, H8 of quinoline ring of pq), 7.48 (s, 1H, H6 of phen-DMA), 7.43−7.21 (m, 6H, H6 of phenyl ring and H5 and H7 of quinoline ring of pq), 6.96−6.86 (m, 4H, H4 of phenyl ring and H6 of quinoline ring of pq), 6.70−6.65 (m, 2H, H5 of phenyl ring of pq), 2.92 (s, 6H, CH_3 of phen-DMA). IR (KBr) ν/cm^{-1}: 828 (s, PF_6^-). Positive-ion ESI-MS ion cluster at m/z: 824 $\{[Ir(pq)_2(phen\text{-}DMA)]^+\}^+$. Anal. calcd for $C_{44}H_{33}N_5PF_6Ir\cdot(CH_3)_2CO$: C, 54.97; H, 3.83; N, 6.82. Found: C, 54.89; H, 3.90; N, 6.95.

[Ir(pba)$_2$(phen-DPA)](PF$_6$) (***4a***). Complex **4a** was isolated as orange-yellow crystals. Yield: 199 mg (54%). ^{1}H NMR (400 MHz, acetone-d_6, 298 K, TMS): δ 9.79 (s, CHO of pba), 9.74 (dd, 1H, J = 8.5 and 1.3 Hz, H7 of phen-DPA), 8.65 (dd, 1H, J = 8.3 and 1.3 Hz, H4 of phen-DPA), 8.56 (d, 1H, J = 4.9 Hz, H9 of phen-DPA), 8.48−8.42 (m, 4H, H6 and H6′ of pyridyl rings of phen-DPA, H5 of phenyl ring of pba), 8.26 (dd, 1H, J = 5.0 and 1.3 Hz, H2 of phen-DPA), 8.21−8.17 (m, 2H, H4 of pyridyl ring of pba), 8.10−8.02 (m, 3H, H8 of phen-DPA and H3 of pyridyl ring of pba), 7.93 (s, 1H, H6 of phen-DPA), 7.88 (dd, 1H, J = 8.3 and 5.0 Hz, H3 of phen-DPA), 7.85−7.78 (m, 2H, H6 of pyridyl ring of pba), 7.73−7.69 (m, 2H, H4 and H4′ of pyridyl rings of phen-DPA), 7.64−7.60 (m, 2H, H6 of phenyl ring of pba), 7.57 (d, 2H, J = 7.8 Hz, H3 and H3′ of pyridyl rings of phen-DPA), 7.28−7.25 (m, 2H, H5 and H5′ of pyridyl rings of phen-DPA), 7.20−7.13 (m, 2H, H5 of pyridyl ring of pba), 6.95 (d, J = 5.9 Hz, H2 of phenyl ring of pba), 4.74 (s, 4H, CH_2 of phen-DPA). IR (KBr) ν/cm^{-1}: 1686 (s, C═O), 843 (s, PF_6^-). Positive-ion ESI-MS ion cluster at m/z: 935 $\{[Ir(pba)_2(phen\text{-}DPA)]^+\}^+$. Anal. calcd for $C_{48}H_{37}N_7OPF_6Ir\cdot CH_2Cl_2$: C, 51.03.; H, 3.43; N, 8.40. Found: C, 51.17; H, 3.42; N, 8.53.

[Ir(pba)$_2$(phen-DMA)](PF$_6$) (***4b***). Complex **4b** was isolated as orange-yellow crystals. Yield: 299 mg (75%). ^{1}H NMR (400 MHz, acetone-d_6, 298 K, TMS): δ 9.79 (s, 2H, CHO of pba), 9.02 (dd, 1H, J = 8.6 and 1.3 Hz, H7 of phen-DMA), 8.72 (dd, 1H, J = 8.3 and 1.2 Hz, H4 of phen-DMA), 8.48−8.44 (m, 3H, H9 of phen-DMA and H5 of phenyl ring of pba), 8.25 (dd, 1H, J = 5.0 and 1.3 Hz, H2 of phen-DMA),

8.20 (dd, 2H, J = 8.0 and 2.6 Hz), 8.08−8.03 (m, 3H, H4 of pyridyl ring of pba and H8 of phen-DMA), 7.92 (dd, 1H, J = 8.0 and 5.0 Hz, H3 of phen-DMA), 7.85−7.81 (m, 3H, H6 of pyridyl ring of pba and H6 of phen-DMA), 7.63 (d, 2H, J = 8.0 Hz, H6 of phenyl ring of pba), 7.20−7.13 (m, 2H, H5 of pyridyl ring of pba), 6.97 (d, 2H, J = 8.1 Hz, H2 of phenyl ring of pba), 3.11 (s, 6H, CH_3 of phen-DMA). IR (KBr) ν/cm^{-1}: 1686 (s, C═O), 843 (s, PF_6^-). Positive-ion ESI-MS ion cluster at m/z: 781 $\{[Ir(pba)_2(phen\text{-}DMA)]^+\}^+$. Anal. calcd for $C_{38}H_{29}N_5OPF_6Ir\cdot 0.5CH_2Cl_2$: C, 48.31; H, 3.41; N, 7.27. Found: C, 48.61; H, 3.18; N, 7.36.

Physical Measurements and Instrumentation. Instruments for the characterization and photophysical and electrochemical measurements have been described previously.[20f] Luminescence quantum yields were measured using the optically dilute method[22a] with an aerated aqueous solution of $[Ru(bpy)_3]Cl_2$ (Φ_{em} = 0.028) as the standard solution.[22b]

Emission Titrations with Zinc Ion. In a typical procedure, aliquots (5 μL) of $Zn(ClO_4)_2\cdot 6H_2O$ (2 mM) were added to the iridium(III) complex (50 μM) in CH_3CN (2 mL) at 1-min intervals. The steady-state emission spectra of the solution were measured. The dissociation constant, K_d, of the cation M^{2+} from the receptor iridium for the following equilibrium:

$$Ir-M^{2+} \overset{K_d}{\rightleftharpoons} Ir + M^{2+}$$

was obtained by fitting the experimental data to the following equation:[23]

$$y = \left(\frac{I_o}{I_o - I_\infty}\right)\left(\frac{K_d}{[M^{2+}]} + 1\right)$$

where I_o is the emission intensity of the receptor only and I_∞ is the limiting emission intensity. The K_d was determined as the ratio of the slope to the y-intercept of the linear fit of a plot of $I_o/(I_o - I_x)$ vs $[M^{2+}]^{-1}$, where I_x is the emission intensity of the receptor in the presence of the cation at a concentration $[M^{2+}]$.

Job's Plot Analysis. In a typical procedure, stock solutions of $Zn(ClO_4)_2\cdot 6H_2O$ (50 μM) and the iridium(III) complex (50 μM) in CH_3CN were mixed in various ratios such that the total concentration of iridium(III) complex and zinc ion was maintained at 50 μM. The emission intensity of the resultant solution was then measured. The binding stoichiometry was determined as the x-axis value corresponding to the interception of the two linear fits of a plot of $(I_o - I_x)$ vs $([Ir] + [Zn^{2+}])$, where I_o is the emission intensity of the iridium(III) complex only and I_x is the emission intensity of the complex in the presence of the zinc ion at concentration $[Zn^{2+}]$.

Cation Selectivity Studies. Stock solutions of various metal ions (200 μM) in CH_3CN were added to the iridium(III) complex solution (50 μM) in CH_3CN. The emission intensity of the resultant solution (2 mL) was measured. The emission intensity of the solution was measured again after addition of two equivalents (100 μM) of zinc ion.

Inductively Coupled Plasma Mass Spectrometry (ICP-MS). Human cervix epithelioid carcinoma (HeLa) cells were grown in a 60-mm tissue culture dish and incubated at 37 °C under a 5% CO_2 atmosphere for 48 h. The culture medium was then removed and replaced with medium/DMSO (99:1 v/v) containing the iridium(III) complexes at a concentration of 10 μM. After incubation for 30 min, the medium was removed, and the cell layer was washed gently with phosphate-buffered saline (PBS) (1 mL × 3). The cell layer was then trypsinized and added up to a final volume of 2 mL with PBS. The harvested cells were digested with 65% HNO_3 (3 mL) at 70 °C for 2 h and then diluted with Milli-Q water to a final volume of 10 mL. The concentration of iridium was measured using an Elan 6100 DRC-ICP-MS system (PerkinElmer SCIEX Instruments, Waltham, MA) equipped with a peristaltic pump, Meinhard quartz nebulizer, cyclonic spray chamber, nickel skimmer, and sample cones.

Live-Cell Confocal Imaging. HeLa cells (100 000 cells mL^{-1}) were grown on sterile glass coverslips in a 35-mm tissue culture dish and incubated at 37 °C under a 5% CO_2 atmosphere for 48 h. The culture medium was removed and replaced with medium/DMSO (99:1, v/v) containing the iridium(III) complexes at a concentration of 10 μM. After incubation for 30 min, the medium was removed, and the cell layer was washed gently with PBS (2 mL × 3). After washing with PBS, the coverslips were mounted onto slides for measurements. Imaging was performed using a confocal microscope (Leica TCS SPE) with an excitation wavelength at 405 nm. The emission signal was collected from 520 to 800 nm.

Cytotoxicity Assays. HeLa cells were seeded in a 96-well flat-bottomed microplate (10 000 cells/well) in growth medium (100 μL) and incubated at 37 °C under a 5% CO_2 atmosphere for 24 h. The iridium(III) complexes and cisplatin (positive control) were then added, respectively, to the wells with concentrations ranging from ca. 10^{-7} to 10^{-4} M in a mixture of medium/DMSO (99:1, v/v). Wells containing medium without cells were used as blank controls. The microplate was incubated at 37 °C under a 5% CO_2 atmosphere for 48 h. Then, 10 μL of MTT in PBS (5 mg mL^{-1}) was added to each well. The microplate was incubated at 37 °C under a 5% CO_2 atmosphere for another 3 h. The medium was then removed, and DMSO (100 μL) was added to each well. The absorbance of the solutions at 570 nm was measured with a SPECTRAmax 340 microplate reader (Molecular Devices Corp., Sunnyvale, CA, USA). The IC_{50} values of the complexes were determined from dose dependence of surviving cells after exposure to the complexes for 48 h.

RESULTS AND DISCUSSION

Synthesis. The diimine ligands phen-DPA and phen-DMA were synthesized from the substitution reactions of phen-NH_2 with excess 2-(bromomethyl)pyridine hydrobromide and methyl iodide, respectively, and were characterized by ^{1}H NMR spectroscopy and positive-ion ESI-MS. The cyclometalated iridium(III) polypyridine complexes were prepared from the reaction of $[Ir_2(N^\wedge C)_4Cl_2]$ (HN^C = Hppy, Hmppy, Hpq, Hpba) with two equivalents of phen-DPA or phen-DMA in a mixture of CH_2Cl_2 and MeOH, followed by anion exchange with KPF_6 and purification by column chromatography and recrystallization. All the complexes were characterized by ^{1}H NMR spectroscopy, positive-ion ESI-MS, and IR spectroscopy and gave satisfactory elemental analyses.

Electronic Absorption and Emission Properties. The electronic absorption spectral data of the diimine ligands and the iridium(III) complexes are summarized in Table 1. The electronic absorption spectra of the diimine ligands and complexes **1a** and **1b** in CH_3CN at 298 K are shown in Figure 1. Both ligands exhibited intense $\pi \rightarrow \pi^*$ absorption bands and shoulders in the UV region at ca. 264−283 nm (ε on the order of 10^4 dm^3 mol^{-1} cm^{-1}) and less intense $n \rightarrow \pi^*$ absorption shoulders at ca. 324−332 nm.[20a] The more intense absorption of phen-DPA compared to phen-DMA in the UV region is due to the two additional pyridyl rings. As for the complexes, the intense absorption bands and shoulders at ca. 248−395 nm have been tentatively assigned to spin-allowed intraligand (^{1}IL) ($\pi \rightarrow \pi^*$) (N^N and N^C) transitions, whereas the less intense absorption shoulders at ca. 416−485 nm have been attributed to spin-allowed metal-to-ligand charge-transfer (^{1}MLCT) ($d\pi(Ir) \rightarrow \pi^*$(N^N and N^C)) transitions.[9−20] Also, it is noteworthy that the absorption shoulders at ca. 317−338 nm were moderately intense,

Table 1. Electronic Absorption Spectral Data of the Diimine Ligands and the Iridium(III) Complexes at 298 K

ligand or complex	solvent	λ_{abs}/nm (ε/dm^3 mol^{-1} cm^{-1})
phen-DPA	CH_2Cl_2	265 (23 385), 270 (24 180), 283 sh (21 445), 332 sh (6030)
	CH_3CN	264 (26 820), 269 (27 460), 283 sh (22 300), 332 sh (6495)
phen-DMA	CH_2Cl_2	280 (13 045), 325 (4040)
	CH_3CN	278 (18 815), 324 (5710)
1a	CH_2Cl_2	255 (64 155), 269 sh (54 865), 291 sh (35 110), 338 sh (16 370), 358 sh (13 475), 383 sh (10 095), 416 sh (5520), 469 sh (1285)
	CH_3CN	252 (64 740), 271 sh (50 745), 289 sh (34 550), 335 sh (16 345), 382 sh (9410), 419 sh (4385), 469 sh (1030)
1b	CH_2Cl_2	252 (49 490), 270 sh (41 480), 291 sh (29 075), 338 sh (13 275), 360 sh (10 660), 383 sh (8370), 419 sh (4280), 469 sh (1090)
	CH_3CN	250 (49 045), 270 sh (39 750), 288 sh (29 060), 338 sh (12 790), 382 sh (7700), 422 sh (3375), 468 sh (860)
2a	CH_2Cl_2	257 (65 320), 272 sh (58 765), 292 sh (40 490), 341 sh (17 885), 363 sh (13 695), 387 sh (10 530), 473 sh (1210)
	CH_3CN	254 (69 715), 272 sh (60 260), 288 sh (43 630), 338 sh (18 685), 382 sh (10 800), 472 sh (1105)
2b	CH_2Cl_2	254 (53 835), 273 sh (50 615), 291 (38 145), 338 sh (16 485), 362 sh (12 675), 382 sh (10 270), 471 sh (1160)
	CH_3CN	252 (60 625), 272 sh (55 645), 288 (42 805), 322 sh (18 430), 382 sh (10 430), 470 sh (1120)
3a	CH_2Cl_2	262 (59 730), 270 sh (55 680), 282 (49 095), 335 (25 428), 350 sh (22 705), 395 sh (7605), 437 (5557), 520 sh (525)
	CH_3CN	260 (60 195), 269 sh (55 600), 281 (48 690), 333 (24 835), 350 sh (21 945), 378 sh (10 170), 433 (5580), 520 sh (475)
3b	CH_2Cl_2	263 (60 850), 270 sh (58 810), 283 (55 125), 335 (28 920), 350 sh (26 015), 394 sh (8790), 439 (6635), 466 sh (4050), 525 sh (520)
	CH_3CN	260 (56 130), 267 sh (54 085), 278 (50 355), 333 (25 575), 348 sh (23 250), 374 sh (11 560), 439 (5870), 485 sh (1730), 520 sh (435)
4a	CH_2Cl_2	252 sh (64 895), 271 (70 065), 300 (53 850), 321 sh (36 255), 373 sh (13 030), 420 sh (7705), 447 sh (5755)
	CH_3CN	250 sh (62 955), 270 (70 065), 297 sh (54 705), 319 sh (35 935), 369 sh (12 535), 424 sh (6965), 444 sh (5135)
4b	CH_2Cl_2	250 (60 805), 275 (66 450), 296 sh (55 030), 317 sh (38 350), 369 sh (13 620), 421 sh (7845), 449 sh (5670)
	CH_3CN	248 (54 695), 272 (61 230), 296 sh (51 580), 319 sh (33 220), 369 sh (11 780), 421 sh (6845), 446 sh (4810)

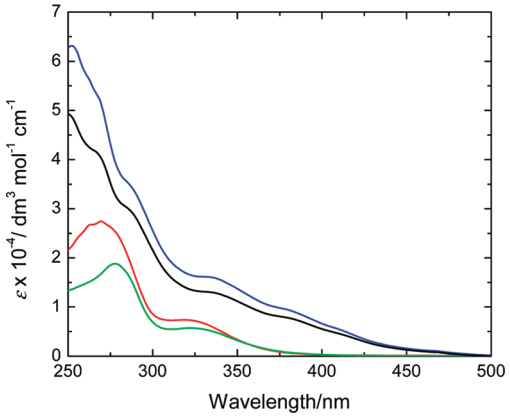

Figure 1. Electronic absorption spectra of phen-DPA (red), phen-DMA (green), and complexes **1a** (blue) and **1b** (black) in CH_3CN at 298 K.

and it is likely that these absorption features are mixed with some spin-allowed amine-to-ligand charge-transfer (^{1}NLCT) ($n \rightarrow \pi^*$) (N^N) character. Additionally, weak absorption tailings were observed in the lower energy region (λ > ca. 450 nm) in all the spectra. These features have been assigned to spin-forbidden ^{3}MLCT (dπ(Ir) $\rightarrow$ π^*(N^N and N^C)) absorption, which probably gained some intensity due to spin-orbit coupling associated with the heavy metal center.

Upon irradiation, all the complexes displayed intense and long-lived greenish-yellow to orange luminescence in fluid solutions at ambient conditions and low-temperature alcohol glass. The photophysical data are summarized in Table 2. The emission spectra of the DPA complexes **1a**, **2a**, **3a**, and **4a** in CH_2Cl_2 at 298 K are shown in Figure 2. On the basis of the emission properties, the complexes can be classified into two groups. In the first group, the ppy (**1a**, **b**) and mppy (**2a**, **b**) complexes showed a broad band with positive solvatochromic properties in fluid solutions at room temperature. With reference to the photophysical studies on related luminescent cyclometalated iridium(III) polypyridine complexes [Ir(N^C)$_2$(N^N)]$^+$,[9−20] the emission of these complexes has been tentatively assigned to a ^{3}MLCT (dπ(Ir) $\rightarrow$ π^*(N^N)) excited state. The occurrence of the emission of the mppy complexes **2a**, **b** at a lower energy than that of their ppy counterparts **1a**, **b** is in accordance with the assignment of a ^{3}MLCT excited state because the electron-donating methyl substituent of the mppy ligand destabilizes the dπ orbitals of the iridium center, thereby lowering the ^{3}MLCT emission energy. It is noteworthy that the lifetimes of the complexes in fluid solutions at 298 K were exceptionally long (ca. 5.03−10.40 μs), and their emission quantum yields were relatively lower (ca. 0.013−0.28) than those of common iridium(III) ^{3}MLCT emitters (Table 2). On the basis of related studies,[9−20] it is conceivable that the excited state of these complexes is mixed with some ^{3}NLCT ($n \rightarrow \pi^*$) (N^N) element, which could be viewed as metal-perturbed ^{3}IL (N^N) character. Hence, the excited state of these complexes is best described as an admixture of ^{3}MLCT and ^{3}NLCT. The DPA complexes **1a** and **2a** showed slightly shorter lifetimes and higher luminescence quantum yields than their DMA counterparts **1b** and **2b**, respectively, in both CH_2Cl_2 and CH_3CN at 298 K. This is probably due to the π-accepting pyridyl groups in these complexes, which reduce the electron-donating ability of the amine moiety, resulting in a lower degree of ^{3}NLCT contribution in the excited state. In low-temperature alcohol glass, all these four complexes displayed structured emission with long emission lifetimes (ca. 4.90−436.18 μs), which can be attributed to a ^{3}MLCT (dπ(Ir) $\rightarrow$ π^* (N^N)) excited state mixed with some ^{3}NLCT ($n \rightarrow \pi^*$)(N^N) character.

In the second group, the pq (**3a**, **b**) and pba (**4a**, **b**) complexes showed rich structured features in their emission bands, and their

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Table 2. Photophysical Data of the Iridium(III) Complexes

complex	medium (T/K)	λ_{em}/nm	τ_o/μs	Φ_{em}
1a	CH_2Cl_2 (298)	564	9.66	0.19
	CH_3CN (298)	575	7.97	0.053
	glass[a] (77)	517, 546 sh	158.33 (82%), 11.95 (18%)	
1b	CH_2Cl_2 (298)	565	10.40	0.055
	CH_3CN (298)	583	8.64	0.013
	glass[a] (77)	493, 567 sh	4.90	
2a	CH_2Cl_2 (298)	574	5.03	0.28
	CH_3CN (298)	584	5.19	0.13
	glass[a] (77)	519, 550 sh	144.70	
2b	CH_2Cl_2 (298)	572	6.10	0.22
	CH_3CN (298)	586	7.03	0.021
	glass[a] (77)	567, 605 sh	436.18	
3a	CH_2Cl_2 (298)	556, 598 sh	4.56 (73%), 2.92 (27%)	0.34
	CH_3CN (298)	559, 598 sh	3.82 (58%), 2.42 (42%)	0.26
	glass[a] (77)	540, 583 sh, 634 sh	4.72	
3b	CH_2Cl_2 (298)	555, 599 sh	10.35 (90%), 7.88 (10%)	0.37
	CH_3CN (298)	559, 601 sh	9.98 (96%), 4.84 (4%)	0.25
	glass[a] (77)	543, 586 sh, 638 sh	28.13 (38%), 5.33 (62%)	
4a	CH_2Cl_2 (298)	534, 571 sh	58.39	0.29
	CH_3CN (298)	536, 571 sh	29.92	0.20
	glass[a] (77)	522 (max), 564, 614 sh	6.91	
4b	CH_2Cl_2 (298)	533 (max), 569	92.19	0.11
	CH_3CN (298)	539 (max), 572	55.49	0.059
	glass[a] (77)	525, 569 (max), 615 sh	473.16	

[a] EtOH/MeOH (4:1 v/v).

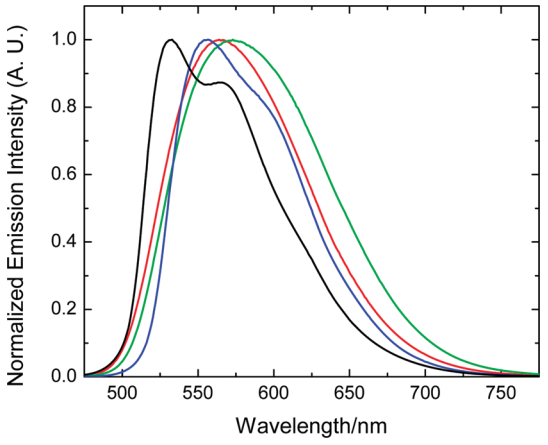

Figure 2. Emission spectra of complexes **1a** (red), **2a** (green), **3a** (blue), and **4a** (black) in CH_2Cl_2 at 298 K.

photophysical properties were much less dependent on the polarity of the solvents (Table 2), suggestive of the heavy involvement of a ^{3}IL ($\pi \rightarrow \pi^*$) (N^C) excited state. Again, the emission lifetimes of these complexes were noticeably longer than those of common iridium(III) pq and pba systems.[20a,d−j,l] It is likely that the excited state also involves ^{3}NLCT ($n \rightarrow \pi^*$)(N^N) character.[20c,l] In alcohol glass at 77 K, the lifetimes of these complexes ranged from ca. 4.72 to 473.16 μs, and the emissive state of the complexes has been ascribed to mixed ^{3}IL ($\pi \rightarrow \pi^*$) (N^C) and ^{3}NLCT ($n \rightarrow \pi^*$)(N^N) nature. On the basis of the photophysical data, we believe that the emissive state of the DPA complexes **3a** and **4a** possessed predominantly ^{3}IL character, whereas that of the DMA complexes **3b** and **4b** probably exhibited a higher degree of ^{3}NLCT character, which is also in accordance with the electron-withdrawing properties of the pyridyl rings in the DPA complexes.

Table 3. Electrochemical Data of the Iridium(III) Complexes[a]

complex	oxidation, $E_{1/2}$ or E_a/V	reduction, $E_{1/2}$ or E_c/V
1a	+1.11,[b] +1.29[c]	−1.41, −2.04,[b] −2.14,[b] −2.27,[b] −2.46,[b] −2.68[b]
1b	+1.23,[b] +1.45[c]	−1.45, −2.07,[b] −2.14,[b] −2.29,[b] −2.40,[b] −2.69[b]
2a	+1.19,[b] +1.22[c]	−1.41, −2.12,[b] −2.26,[c] −2.50[b]
2b	+1.22,[b] +1.37[c]	−1.45, −2.17,[b] −2.34,[b] −2.45[b]
3a	+1.13,[b] +1.34[c]	−1.41, −1.75,[c] −1.96,[c] −2.38, −2.65[b]
3b	+1.13,[b] +1.45[c]	−1.43, −1.75,[c] −1.98,[c] −2.32,[c] −2.61[b]
4a	+1.18,[c] +1.41[c]	−1.37, −1.59,[b] −1.70,[b] −1.92[b]
4b	+1.23,[c] +1.57[c]	−1.40, −1.63,[b] −1.76,[b] −1.90[b]

[a] In CH_3CN (0.1 M TBAP) at 298 K (glassy carbon working electrode, sweep rate = 100 mV s^{-1}, all potentials vs SCE). [b] Irreversible waves. [c] Quasi-reversible couples.

Electrochemical Properties. The electrochemical properties of the iridium(III) complexes have been studied by cyclic voltammetry. The electrochemical data are listed in Table 3. These complexes showed a quasi-reversible couple or an irreversible wave at ca. +1.11 to +1.23 V vs saturated calomel electrode (SCE). These features have been assigned to the oxidation of the tertiary amines of the diimine ligands.[19d,20a] Additionally,

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all the complexes displayed a quasi-reversible oxidation couple at a potential between ca. +1.22 and +1.57 V vs SCE, which has been attributed to a metal-centered iridium(IV/III) oxidation process.[10−20] These potentials follow the orders: **2a** < **1a** < **3a** < **4a** and **2b** < **1b** < **3b** < **4b**, which are in agreement with the descending electron-donating effect of the cyclometalating ligands. Compared with related systems,[10−20] there is reduced reversibility of the iridium(IV/III) couples, which is most likely a consequence of the prior quasi-reversible or irreversible oxidation of the amine moieties at lower potentials. The first reduction couples of all the complexes were reversible in nature and occurred at ca. −1.37 to −1.45 V vs SCE, which have been assigned to the reduction of the diimine ligands.[10−20] This is consistent to the electron-donating effect of the methyl substituents of phen-DMA, which rendered the DMA complexes **1b**, **2b**, **3b**, and **4b** to be reduced at a slightly more negative potential than their DPA counterparts, complexes **1a**, **2a**, **3a**, and **4a**, respectively. Additionally, reduction waves of highly irreversible nature occurred at more negative potentials for all the complexes. These waves have been assigned to the reduction of the cyclometalating ligands and further reduction of the diimine ligands.

Cation-Binding Properties. The zinc-binding properties of the iridium(III) complexes in aerated CH_3CN have been studied by emission titrations. The emission spectral traces of all the complexes and the titration results of complexes **2a** and **2b** are shown in Figures 3 and 4, respectively. Upon addition of zinc perchlorate, the DPA complexes **1a**, **2a**, **3a**, and **4a** showed emission enhancement of ca. 1.2- to 5.4-fold (Table 4), whereas the DMA complexes **1b**, **2b**, **3b**, and **4b** did not exhibit any notable changes. Thus, the change in the emission intensities has been attributed to the specific binding of zinc ion to the DPA unit. It is noteworthy that both complexes **1a** and **2a** showed a bathochromic shift upon addition of zinc ion, but no similar response was observed for complexes **3a** and **4a** (Figure 3 and Table 4). Since the coordination of zinc ion to the amine of the DPA moiety is expected to lead to bathochromic and hypsochromic shifts for ^{3}MLCT and ^{3}NLCT emission, respectively, this red shift for complexes **1a** and **2a** suggests substantial suppression of ^{3}NLCT character and subsequent prevalence of ^{3}MLCT character in their emissive states. As a result, the polarity-dependent ^{3}MLCT state of complexes **1a** and **2a** and the polarity-insensitive ^{3}IL character of complexes **3a** and **4a** become predominant upon the binding event. Fitting of the emission titration data revealed that the dissociation constants of the Ir−Zn adducts ranged from 1.1×10^{-5} to 8.9×10^{-5} M (Table 4). These are about four orders of magnitude larger than those of related transition metal DPA complexes,[19d,e] which may be a consequence of the electron-withdrawing effect as well as the bulkiness of the metal polypyridine core. Additionally, the binding stoichiometry of zinc ion to the iridium(III) complexes has been investigated by Job's plot analysis.[24] The Job's plot for the results of complex **4a** is shown in Figure 5 as an example. All the complexes gave a break point at a molar ratio of ca. 0.5 in the Job's plots, indicating a 1:1 binding stoichiometry. Although similar titration experiments have been performed in aqueous buffer, the emission intensity of the complexes was intense, and no significant changes were observed upon addition of zinc ion. This is due to the lack of ^{3}NLCT ($n \rightarrow \pi^*$)(N^N) character in their emissive states as a result of the hydrogen bonding between their amine group and water molecules in aqueous buffer.

The binding selectivity of the DPA complexes toward the perchlorate salts of different metal ions has been investigated by emission spectroscopy. The corresponding emission data for the results of complexes **1a**−**3a** and **4a** are displayed in Figure S1, Supporting Information and Figure 6, respectively. Upon addition of sodium, manganese(II), cobalt(II), and mercury(II) ions to the DPA complexes, the emission intensity remained essentially

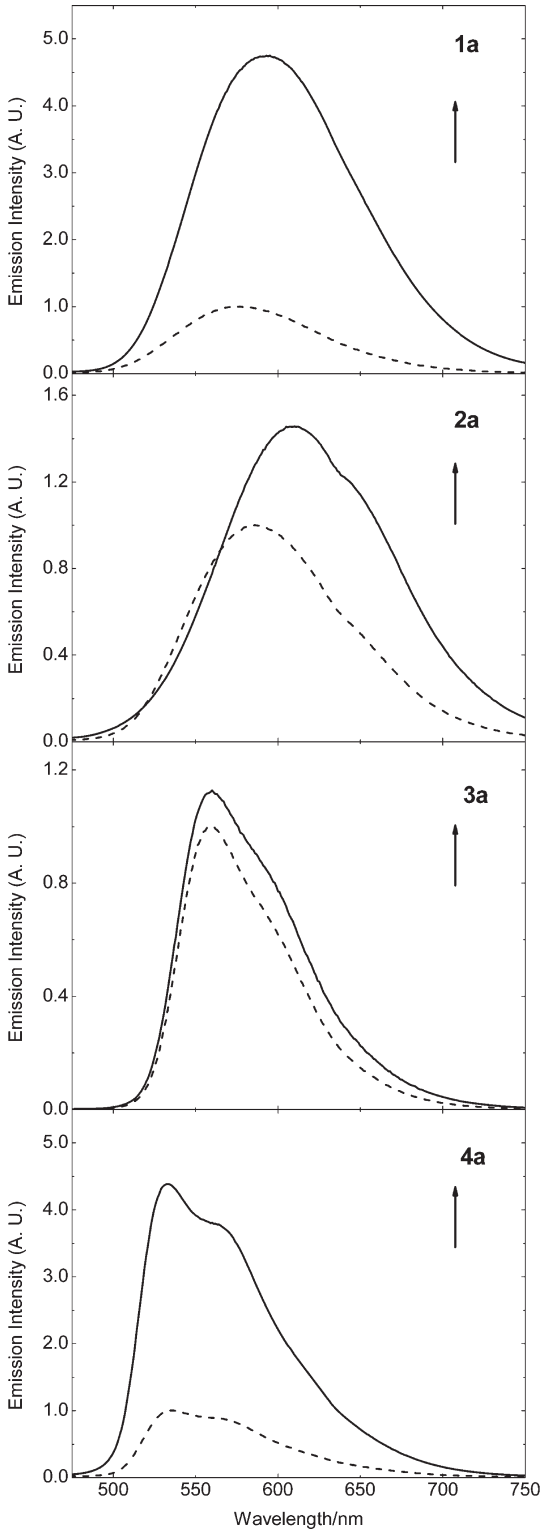

Figure 3. Emission spectral traces of complexes **1a**, **2a**, **3a**, and **4a** (50 μM) in aerated CH_3CN in the absence (dashed) and 100 μM (solid) of Zn^{2+} at 298 K.

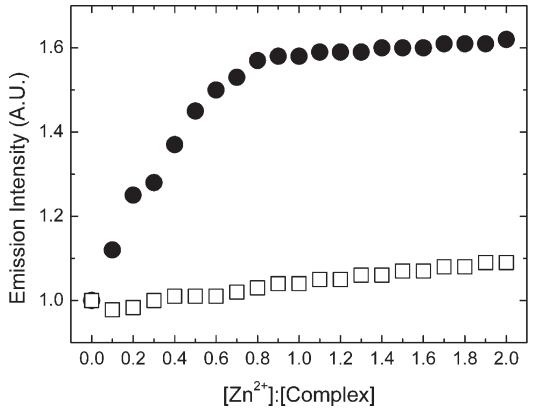

Figure 4. Emission titration curves of complexes **2a** (solid circles) (50 μM) and **2b** (open squares) (50 μM) with Zn^{2+} in aerated CH_3CN at 298 K.

Table 4. Results of Titrations of the Iridium(III) Complexes with Zn^{2+} in CH_3CN at 298 K

complex	I/I_o[a]	λ_o[b]/nm	λ[b]/nm	K_d/M
1a	5.4	574	592	1.1×10^{-5}
2a	1.6	586	609	1.1×10^{-5}
3a	1.2	560	557	8.9×10^{-5}
4a	4.4	535	533	5.1×10^{-5}

[a] I_o and I are the emission intensities of the complexes in the absence and 100 μM of Zn^{2+}, respectively. [b] λ_o and λ are the emission maxima of the complexes in the absence and 100 μM of Zn^{2+}, respectively.

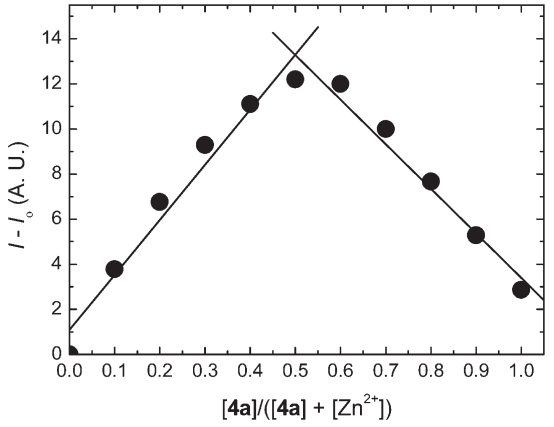

Figure 5. Job's plot for the binding of Zn^{2+} to complex **4a** and the theoretical fit in aerated CH_3CN at 298 K, where [**4a**] + [Zn^{2+}] = 50 μM.

the same. However, magnesium, nickel(II), and copper(II) ions quenched the emission of the complex, which is probably due to a quenching pathway involving the low-lying d−d states in the two latter cases.[8c,g,19d,e] Addition of two equivalents (100 μM) of iron(II), cadmium(II), and lead(II) ions, respectively, induced ca. 6.2- to 7.3-fold emission enhancement. This is not unexpected as similar emission changes have been reported in other DPA-based ion sensors.[8c,h,i,19d] Subsequent addition of zinc ion to a mixture of the DPA complexes and various ions increased the emission intensity, except in the cases of magnesium and mercury(II) ions.

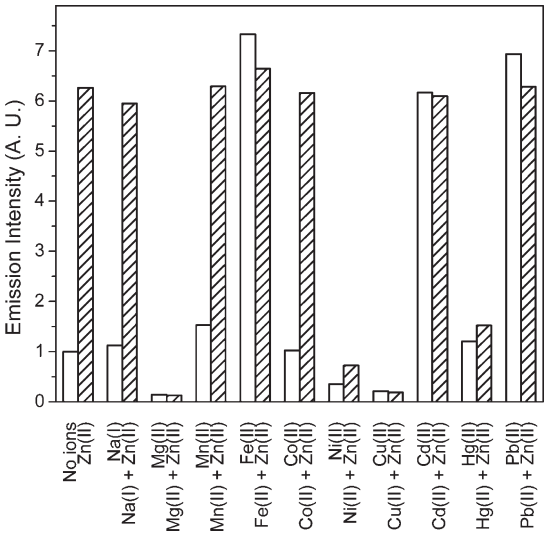

Figure 6. Emission intensities of complex **4a** (50 μM) in aerated CH_3CN solutions at 298 K containing no or different cations (100 μM) (open) and upon subsequent addition of zinc(II) ion (100 μM) (shaded).

Table 5. Cellular Uptake Efficiency and Cytotoxicity (IC_{50}, 48 h) of the Iridium(III) Complexes Toward the HeLa Cell Line

complex	amount of Ir per cell[a]/fmol	IC_{50}[b]/μM
1a	1.3	4.1 ± 0.3
1b	1.4	3.8 ± 0.2
2a	0.87	4.5 ± 0.8
2b	1.5	3.0 ± 0.7
3a	1.5	3.1 ± 0.5
3b	3.2	1.0 ± 0.08
4a	0.34	54.1 ± 5.1
4b	0.36	34.7 ± 7.1

[a] Number of moles of iridium associated with an average HeLa cell upon incubation with the iridium(III) complexes (10 μM) at 37 °C for 30 min as determined by ICP-MS. [b] Cytotoxicity of the complexes toward HeLa cells after incubation for 48 h (IC_{50} of cisplatin = 19.7 ± 1.5 μM).

This illustrates the higher selectivity of the DPA complexes toward zinc ion over these ions.

Cellular Uptake Studies. There is an emerging interest in the cellular studies of luminescent iridium(III) complexes. In this work, we have examined the cellular uptake properties and cytotoxic activity of these complexes. HeLa cells loaded with the iridium(III) complexes (10 μM) at 37 °C for 30 min have been analyzed using ICP-MS and laser-scanning confocal microscopy. The intracellular concentrations ranged from 0.34 to 3.2 fmol iridium in one cell (Table 5), which is comparable to other iridium(III) complexes[20h−l] and in accordance with the hydrophobicity of the cyclometalating ligands. Upon photoexcitation, the cells incubated with the complexes displayed intense emission intensity, indicating efficient cellular internalization of the complex molecules. A typical image of cells treated with complex **3a** is shown in Figure 7 as an example. The complex distribution in the cytoplasm was not even but localized in the perinuclear region, forming luminescent rings surrounding the nuclei. The nuclei showed much weaker emission, indicative of negligible nuclear uptake of the complex. From the image

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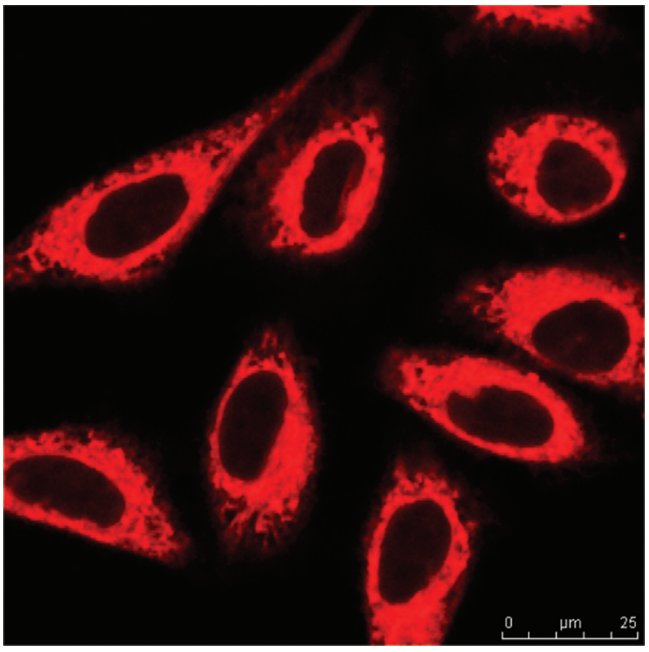

Figure 7. Confocal images of HeLa cells incubated with complex **3a** (10 μM) at 37 °C for 30 min.

(Figure 7), it is likely that the complex binds to hydrophobic organelles, such as Golgi apparatus, endoplasmic reticulum, and mitochondria.[25] Although the direct application of these iridium(III) complexes as intracellular zinc sensors is less effective, the cellular studies show that such complexes with the incorporation of a metal ion-binding unit are still membrane permeable.

We have examined the cytotoxic effect of the cyclometalated iridium(III) polypyridine complexes toward HeLa cells using the MTT assay.[26] The IC_{50} values have been determined from the dose dependence of surviving cells after exposure to the complexes for 48 h. The IC_{50} values of the iridium(III) complexes ranged from 1.0 to 54.1 μM (Table 5), which are on the same order of magnitude as other iridium(III) complexes.[20d−l] The cytotoxicity of the complexes varied positively with the intracellular accumulation of the iridium(III) complexes as determined by ICP-MS; for example, the relatively polar aldehyde complexes **4a** and **4b** showed the lowest uptake and cytotoxic activity (Table 5). Thus, by a judicious structural design of the complexes, we anticipate that the cytotoxicity of the complexes can be modified without affecting their intracellular accumulation and localization properties.

CONCLUSION

A series of new zinc sensors derived from luminescent cyclometalated iridium(III) polypyridine complexes functionalized with a DPA moiety have been designed, and the electronic absorption, photophysical, and electrochemical properties of which have been investigated. Photoexcitation of these iridium(III) complexes resulted in intense and long-lived greenish-yellow to orange luminescence under ambient conditions and in low-temperature alcohol glass, attributable to a ^{3}MLCT ($d\pi(Ir) \rightarrow \pi^*(N^\wedge N)$) or a ^{3}IL ($\pi \rightarrow \pi^*$) (N^C) excited state, with mixing of some ^{3}NLCT ($n \rightarrow \pi^*$) (N^N) character of different degrees. Upon binding to zinc ion, the DPA complexes showed emission enhancement, and the spectral changes have been ascribed to the substantial suppression of the ^{3}NLCT character in the emissive states of the complexes. Additionally, the ion-binding properties of the DPA complexes with various metal ions have been studied, and the results showed that these complexes are sufficiently selective toward zinc ion among most of the metal ions studied. Furthermore, these complexes were efficiently internalized by HeLa cells, and their strong emission was retained in the biological environment of living cells. By suitable modification to their molecular structures, these iridium(III) complexes are anticipated to become potential intracellular zinc sensors.

ASSOCIATED CONTENT

Supporting Information. The cation-binding selectivity of complexes **1a**, **2a**, and **3a**. This material is available free of charge via the Internet at http://pubs.acs.org.

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