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Luminescent Cyclometalated Iridium(III) Polypyridine Indole Complexes—Synthesis, Photophysics, Electrochemistry, Protein-Binding Properties, Cytotoxicity, and Cellular Uptake

Jason Shing-Yip Lau, Pui-Kei Lee, Keith Hing-Kit Tsang, Cyrus Ho-Cheong Ng, Yun-Wah Lam, Shuk-Han Cheng, and Kenneth Kam-Wing Lo*

Department of Biology and Chemistry, City University of Hong Kong, Tat Chee Avenue, Kowloon, Hong Kong, People's Republic of China

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A series of luminescent cyclometalated iridium(III) polypyridine indole complexes, $[Ir(N^C)_2(N^N)](PF_6)$ (HN^C = 2-phenylpyridine (Hppy), N^N = 4-((2-(indol-3-yl)ethyl)aminocarbonyl)-4′-methyl-2,2′-bipyridine (bpy-ind) (**1a**), N^N = 4-((5-((2-(indol-3-yl)ethyl)aminocarbonyl)pentyl)aminocarbonyl)-4′-methyl-2,2′-bipyridine (bpy-C6-ind) (**1b**); HN^C = 7,8-benzoquinoline (Hbzq), N^N = bpy-ind (**2a**), N^N = bpy-C6-ind (**2b**); and HN^C = 2-phenylquinoline (Hpq), N^N = bpy-ind (**3a**), N^N = bpy-C6-ind (**3b**)), have been synthesized, characterized, and their photophysical and electrochemical properties and lipophilicity 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 (λ_{em} = 540−616 nm, τ_o = 0.13−5.15 μs). The emission of the complexes has been assigned to a triplet metal-to-ligand charge-transfer (^{3}MLCT) (dπ(Ir) → π^*(N^N)) excited state, probably with some mixing of triplet intraligand (^{3}IL) ($\pi \rightarrow \pi^*$) (pq) character for complexes **3a**,**b**. Electrochemical measurements revealed that all the complexes showed an irreversible indole oxidation wave at ca. +1.1 V versus SCE, a quasi-reversible iridium(IV/III) couple at ca. +1.3 V, and a reversible diimine reduction couple at ca. −1.3 V. The interactions of these complexes with an indole-binding protein, bovine serum albumin (BSA), have been studied by emission titrations, and the K_a values are on the order of 10^4 M^{-1}. Additionally, the cytotoxicity of the complexes toward human cervix epithelioid carcinoma (HeLa) cells has been examined by the 3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyltetrazolium bromide (MTT) assay. The IC_{50} values of the complexes ranged from 1.1 to 6.3 μM, which are significantly smaller than that of cisplatin (30.7 μM) under the same experimental conditions. Furthermore, the cellular uptake of the complexes has been investigated by flow cytometry and laser-scanning confocal microscopy. The microscopy images indicated that complex **3a** was localized in the perinuclear region upon interiorization. Temperature-dependence experiments suggested that the internalization of the complex was an energy-requiring process such as endocytosis. This has been confirmed by cellular-uptake experiments involving the luminescent conjugates Ir−BSA and Ir−TF (TF = holo-transferrin), which were prepared by conjugation of the proteins with the complex $[Ir(pq)_2(phen\text{-}NCS)](PF_6)$ (phen-NCS = 5-isothiocyanato-1,10-phenanthroline).

Introduction

On the basis of the wide existence and the important physiological activities of many indole derivatives,[1] the isolation and studies of the biological receptors of these compounds have attracted much attention. The most common indole-binding proteins in the circulatory system are serum albumins, which contain six specific substrate-binding sites. Among these, sites I and II are for small heterocyclic or aromatic carboxylic acids, sites III and IV are for long-chain fatty acids, and sites V and VI are for metal ions.[2] Indole and its derivatives are believed to bind to site II.[2] Various methods such as radioactive binding assays,[3] fluorescence titrations and quenching experiments,[4] and enzyme inhibition assays[5] have been used to investigate the binding of indole derivatives to serum albumins. Recently, we have designed luminescent rhenium(I)[6a−c] and ruthenium(II)[6d] polypyridine indole complexes and redox-active ferrocene indole

* To whom correspondence should be addressed. E-mail: bhkenlo@cityu.edu.hk. Phone: (852) 2788 7231. Fax: (852) 2788 7406.

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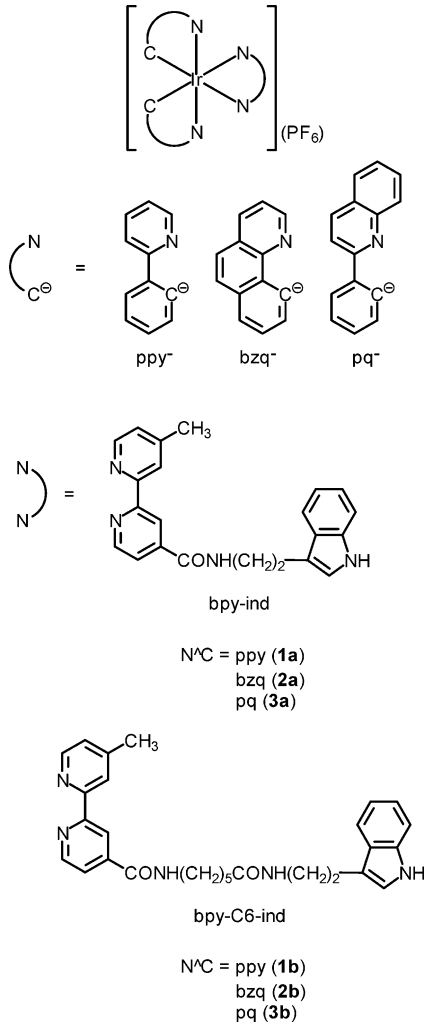
Chart 1. Structures of the Cyclometalated Iridium(III) Indole Complexes



compounds[6e] as probes for bovine serum albumin (BSA). By virtue of their intense and long-lived emission in the visible region,[7−24] luminescent cyclometalated iridium(III) polypyridine indole complexes are anticipated to serve as new biological probes for indole-binding proteins.

Herein we describe the synthesis, characterization, photophysical and electrochemical properties, and lipophilicity of a series of luminescent cyclometalated iridium(III) polypyridine indole complexes, $[Ir(N^C)_2(N^N)](PF_6)$ (HN^C = 2-phenylpyridine (Hppy), N^N = 4-((2-(indol-3-yl)ethyl)-aminocarbonyl)-4′-methyl-2,2′-bipyridine (bpy-ind) (**1a**), N^N = 4-((5-((2-(indol-3-yl)ethyl)aminocarbonyl)pentyl)-aminocarbonyl)-4′-methyl-2,2′-bipyridine (bpy-C6-ind) (**1b**); HN^C = 7,8-benzoquinoline (Hbzq), N^N = bpy-ind (**2a**), N^N = bpy-C6-ind (**2b**); and HN^C = 2-phenylquinoline (Hpq), N^N = bpy-ind (**3a**), N^N = bpy-C6-ind (**3b**)) (Chart 1). The interactions of these complexes with the indole-binding protein BSA have been studied by emission titrations. Additionally, the cytotoxicity of the complexes toward human cervix epithelioid carcinoma (HeLa) cells has been examined by the 3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyltetrazolium bromide (MTT) assay. Furthermore, the cellular uptake of the complexes has been investigated by flow cytometry and laser-scanning confocal microscopy.

Experimental Section

Materials and Synthesis. All solvents were of analytical grade and purified according to standard procedures.[25] All buffer components were of biological grade and used as received. $IrCl_3 \cdot 3H_2O$, Hppy, Hbzq, Hpq, 4,4′-dimethyl-2,2′-bipyridine, tryptamine, 6-aminocaproic acid, *N*-hydroxysuccinimide, *N*,*N*′-dicyclohexylcarbodiimide, and KPF_6 were purchased from Acros and used without further purification. $[Ir_2(N^C)_4Cl_2]$,[7a] succinimidyl-4-carboxy-4′-methyl-2,2′-bipyridine,[26] bpy-ind,[6d] bpy-C6-ind,[6d] and $[Ir(pq)_2(phen-NCS)](PF_6)$[24c] were prepared by reported methods. PD-10 size-exclusion columns and YM-30 microcons were purchased from Pharmacia and Amicon, respectively.

BSA, holo-transferrin (TF), and double-stranded calf thymus DNA from Calbiochem and guanosine (dG) from Sigma were used as received. HeLa cells were obtained from American Type Culture Collection. Dulbecco's modified Eagle's medium (DMEM), fetal bovine serum (FBS), phosphate buffered saline (PBS), trypsin−EDTA, and penicillin/streptomycin were purchased from Invitrogen. The growth medium for cell culture contained DMEM with 10% FBS and 1% penicillin/streptomycin. Sugar-free growth medium (Invitrogen), nocodazole (Acros), colchicine (Acros), carbonyl cyanide 3-chlorophenylhydrazone (CCCP) (Acros), potassium nitrate (Acros), sodium orthovanadate (Acros), sodium molybdate(VI) dehydrate (Acros), and *N*-ethylmaleimide (NEM) (Acros) were used without purification.

Synthesis of the Cyclometalated Iridium(III) Complexes. A mixture of $[Ir_2(N^C)_4Cl_2]$ (0.13 mmol) and the diimine ligand, bpy-ind or bpy-C6-ind (0.26 mmol), in 30 mL of $MeOH/CH_2Cl_2$ (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 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 acetone/CH_2Cl_2 (1:1, v/v) and subsequently recrystallized from acetone/diethyl ether.

$[Ir(ppy)_2(bpy-ind)](PF_6)$ (1a). Complex **1a** was isolated as orange crystals. Yield: 169 mg (65%). ^{1}H NMR (300 MHz, acetone-d_6, 298 K, TMS): δ 10.06 (s, 1H, N*H* of indole), 9.02 (s, 1H, H3 of bpy), 8.75 (s, 1H, H3′ of bpy), 8.34 (t, 1H, J = 5.8 Hz, CON*H*), 8.25 (d, 2H, J = 7.9 Hz, H3 of pyridyl ring of ppy), 8.18 (d, 1 H, J = 5.6 Hz, H6′ of bpy), 8.00−7.84 (m, 7H, H3 of phenyl ring of ppy, H4 and H6 of pyridyl ring of ppy, and H5′ of bpy), 7,82 (d, 1H, J = 0.9 Hz, H5 of bpy), 7.61−7.58 (m, 2H, H4 of bpy and H4 of indole), 7.40 (d, 1H, J = 8.2 Hz, H7 of indole), 7.22−7.12 (m, 3H, H5 of pyridyl ring of ppy and H2 of indole), 7.10−7.02 (m, 3H, H4 of phenyl ring of ppy and H6 of indole), 7.00−6.89 (m, 3H, H5 of phenyl ring of ppy and H5 of indole), 6.36−6.32 (m, 2H, H6 of phenyl ring of ppy), 3.79−3.71 (m, 2H, CONHCH_2), 3.07 (t, 2H, J = 7.2 Hz, CH_2−indole), 2.64 (s, 3H, CH_3 on C4′ of bpy). IR (KBr) ν/cm^{-1}: 3440 (br s, NH), 1610 (s, C=O), 844 (s, PF_6^-). Positive-ion ESI-MS ion cluster at m/z 857 $\{[Ir(ppy)_2(bpy-ind)]^+\}^+$. Anal. Calcd for $C_{44}H_{36}N_6OPF_6Ir \cdot 1/3H_2O$: C, 52.43; H, 3.67; N, 8.34. Found: C, 52.64; H, 3.95; N, 8.49.

$[Ir(ppy)_2(bpy-C6-ind)](PF_6)$ (1b). Complex **1b** was isolated as orange crystals. Yield: 128 mg (44%). ^{1}H NMR (300 MHz, acetone-d_6, 298 K, TMS): δ 10.01 (s, 1H, N*H* of indole), 9.09 (s, 1H, H3 of bpy), 8.83 (s, 1H, H3′ of bpy), 8.30 (t, 1H, J = 5.8 Hz, CON*H*), 8.26−8.16 (m, 3H, H3 of pyridyl ring of ppy and H6′ of bpy), 8.01 (dd, 1H, J = 5.9 and 1.8 Hz, H5′ of bpy), 7.98−7.86 (m, 6H, H3 of phenyl ring of ppy and H4 and H6 of pyridyl ring of ppy),

7.81 (d, 1H, J = 6.0 Hz, H6′ of bpy), 7.57−7.51 (m, 2H, H5′ of bpy and H4 of indole), 7.36 (d, 1H, J = 8.2 Hz, H7 of indole), 7.15−7.08 (m, 5H, H5 of pyridyl ring of ppy, NHC_2H_4−indole, and H2 and H6 of indole), 7.03 (t, 2H, J = 7.3 Hz, H4 of phenyl ring of ppy), 6.95−6.89 (m, 3H, H5 of phenyl ring of ppy and H5 of indole), 6.36−6.32 (m, 2H, H6 of phenyl ring of ppy), 3.49−3.36 (m, 2H, CONHCH_2), 2.89−2.84 (m, 2H, CH_2−indole), 2.60 (s, 3H, CH_3 on C4′ of bpy), 2.17 (t, 2H, J = 7.0 Hz, CH_2CONHCH$_2$CH$_2$−indole), 1.64−1.34 (m, 6H, NHCH$_2$C$_3$-H_6CH$_2$CONH). IR (KBr) ν/cm^{-1}: 3416 (br s, NH), 1655 (s, C═O), 845 (s, PF_6^-). Positive-ion ESI-MS ion cluster at m/z 969 {[Ir(ppy)$_2$(bpy-C6-ind)]$^+$}$^+$. Anal. Calcd for $C_{50}H_{47}N_7O_2PF_6Ir\cdot 2H_2O$: C, 52.17; H, 4.47; N, 8.52. Found: C, 51.98; H, 4.39; N, 8.69.

[Ir(bzq)$_2$(bpy-ind)](PF$_6$) (2a). Complex **2a** was isolated as brown crystals. Yield: 111 mg (47%). ^{1}H NMR (300 MHz, acetone-d_6, 298 K, TMS): δ 10.05 (s, 1H, NH of indole), 9.04 (s, 1H, H3 of bpy), 8.78 (s, 1H, H3′ of bpy), 8.57 (d, 2H, J = 8.2 Hz, H4 of bzq) 8.32 (dd, 1H, J = 5.6 and 1.2 Hz, CONH), 8.25 (dd, 2H, J = 5.6 and 1.2 Hz, H2 of bzq), 8.17 (d, 1H, J = 5.3 Hz, H6 of bpy), 7.99 (d, 2H, J = 8.8 Hz, H5 and H6′ of bpy), 7.92−7.85 (m, 4H, H5 and H6 of bzq), 7.63−7.55 (m, 5H, H3 and H7 of bzq and H5′ of bpy), 7.47 (d, 1H, J = 5.0 Hz, H4 of indole), 7.39 (d, 1H, J = 7.9 Hz, H7 of indole), 7.21−7.15 (m, 3H, H8 of bzq and H2 of indole), 7.08 (t, 1H, J = 7.0 Hz, H6 of indole), 6.95 (dt, 1H, J = 7.9 and 0.9 Hz, H5 of indole), 6.36 (t, 2H, J = 7.0 Hz, H9 of bzq), 3.76−3.69 (m, 2H, CONHCH_2), 3.05 (t, 2H, J = 7.0 Hz, CH_2−indole), 2.62 (s, 3H, CH_3 on C4′ of bpy). IR (KBr) ν/cm^{-1}: 3442 (br s, NH), 1655 (s, C═O), 846 (s, PF_6^-). Positive-ion ESI-MS ion cluster at m/z 904 {[Ir(bzq)$_2$(bpy-ind)]$^+$}$^+$. Anal. Calcd for $C_{48}H_{36}N_6OPF_6Ir\cdot H_2O$: C, 53.98; H, 3.59; N, 7.87. Found: C, 53.86; H, 3.76; N, 7.80.

[Ir(bzq)$_2$(bpy-C6-ind)](PF$_6$) (2b). Complex **2b** was isolated as brown crystals. Yield: 121 mg (40%). ^{1}H NMR (300 MHz, acetone-d_6, 298 K, TMS): δ 10.01 (s, 1H, NH of indole), 9.13 (s, 1H, H3 of bpy), 8.86 (s, 1H, H3′ of bpy), 8.55 (d, 2H, J = 7.9 Hz, H4 of bzq), 8.32−8.30 (m, 2H, H2 of bzq and CONH), 8.25 (dd, 1H, J = 5.6 and 1.2 Hz, H2 of bzq), 8.16 (d, 1H, J = 5.3 Hz, H6 of bpy), 8.01−7.97 (m, 2H, H5 and H6′ of bpy), 7.92−7.86 (m, 4H, H5 and H6 of bzq), 7.60−7.49 (m, 5H, H3 and H7 of bzq and H5′ of bpy), 7.45 (d, 1H, J = 5.6 Hz, H4 of indole), 7.35 (d, 1H, J = 8.2 Hz, H7 of indole), 7.25 (m, 1H, NHC_2H_4−indole), 7.18 (t, 2H, J = 7.3 Hz, H8 of bzq), 7.09−7.01 (m, 2H, H2 and H6 of indole), 6.90 (dt, 1H, J = 7.5 and 1.2 Hz, H5 of indole), 6.35 (t, 2H, J = 6.2 Hz, H9 of bzq), 3.49−3.36 (m, 2H, CONHCH_2), 2.92−2.83 (m, 2H, CH_2−indole), 2.59 (s, 3H, CH_3 on C4′ of bpy), 2.17 (t, 2H, J = 7.3 Hz, CH_2CONHCH$_2$CH$_2$−indole), 1.64−1.33 (m, 6H, NHCH$_2$C$_3$$H_6$CONH). IR (KBr) ν/cm^{-1}: 3421 (br s, NH), 1650 (s, C═O), 840 (s, PF_6^-). Positive-ion ESI-MS ion cluster at m/z 1017 {[Ir(bzq)$_2$(bpy-C6-ind)]$^+$}$^+$. Anal. Calcd for $C_{54}H_{47}N_7O_2PF_6Ir\cdot 2H_2O$: C, 54.08; H, 4.29; N, 8.18. Found: C, 54.07; H, 4.28; N, 8.32.

[Ir(pq)$_2$(bpy-ind)](PF$_6$) (3a). Complex **3a** was isolated as red crystals. Yield: 115 mg (40%). ^{1}H NMR (300 MHz, acetone-d_6, 298 K, TMS): δ 10.06 (s, 1H, NH of indole), 8.58−8.54 (m, 4H, H3 and H3′ of bpy and H3 of quinoline ring of pq), 8.45 (d, 1H, J = 5.9 Hz, H6′ of bpy), 8.27−8.20 (m, 5H, H6′ of bpy, H3 of phenyl ring of pq, H4 of quinoline ring of pq, and CONH), 8.00−7.93 (m, 3H, H5′ of bpy and H8 of quinoline ring of pq), 7.58 (d, 1H, J = 5.0 Hz, H6′ of bpy), 7.52−7.37 (m, 7H, H5 and H7 of quinoline ring of pq, H5 of bpy, and H4 and H7 of indole), 7.22−7.11 (m, 5H, H4 of phenyl ring and H6 of quinoline ring of pq and H2 of indole), 7.06 (t, 1H, J = 7.8 Hz, H6 of indole), 6.84 (m, 3H, H5 of phenyl ring of pq and H5 of indole), 6.57 (dd, 2H, J = 7.3 and 3.8 Hz, H6 of phenyl ring of pq), 3,70−3.64 (m, 2H, CONHCH_2), 3.03 (t, 2H, J = 1.8 Hz, CH_2−indole), 2.51 (s, 3H, CH_3 on C4′ of bpy). IR (KBr) ν/cm^{-1}: 3440 (br s, NH), 1610 (s, C═O), 844 (s, PF_6^-). Positive-ion ESI-MS ion cluster at m/z 956 {[Ir(pq)$_2$(bpy-ind)]$^+$}$^+$. Anal. Calcd for $C_{52}H_{40}N_6OPF_6Ir\cdot 0.5((CH_3)_2CO)$: C, 56.16; H, 3.81; N, 7.56. Found: C, 55.50; H, 3.55; N, 7.56.

[Ir(pq)$_2$(bpy-C6-ind)](PF$_6$) (3b). Complex **3b** was isolated as orange crystals. Yield: 120 mg (38%). ^{1}H NMR (300 MHz, acetone-d_6, 298 K, TMS): δ 10.02 (s, 1H, NH of indole), 8.70 (s, 1H, H3 of bpy), 8.53 (s, 2H, H3′ of bpy and CONH), 8.47−8.41 (m, 4H H3 of phenyl and H3 of quinoline ring of pq), 8.26−8.17 (m, 4H, H4 of quinoline ring of pq and H5 and H6 of bpy), 8.02 (d, 1H, J = 6.2 Hz, H6′ of bpy), 7.92 (d, 1H, J = 7.9 Hz, H8 of quinoline ring of pq), 7,88 (d, 1H, J = 8.5 Hz, H8 of quinoline ring of pq), 7.56 (d, 1H, J = 5.6 Hz, H5 of quinoline ring of pq), 7.49 (d, 1H, J = 7.9 Hz, H4 of indole), 7.43−7.35 (m, 4H, H5 and H7 of quinoline of pq and H7 of indole), 7.19−7.03 (m, 7H, H4 of phenyl ring and H6 of quinoline ring of pq, N$HC_2C_2H_4$−indole, and H2 and H6 of indole), 6.90 (t, 1H, J = 7.9 Hz, H5 of indole), 6.82 (t, 2H, J = 6.7 Hz, H5 of phenyl ring of pq), 6.55 (t, 2H, J = 6.5 Hz, H6 of phenyl ring of pq), 3.46−3.32 (m, 2H, CONHCH_2), 2.97−2.82 (m, 2H, CH_2−indole), 2.46 (s, 3H, CH_3 on C4′ of bpy), 2.17 (t, 2H, J = 7.3 Hz, CH_2CONHCH$_2$CH$_2$−indole), 1.60−1.28 (m, 6H, NHCH$_2$C$_3$$H_6CH_2$CONH). IR (KBr) ν/cm^{-1}: 3421 (br s, NH), 1655 (s, C═O), 840 (s, PF_6^-). Positive-ion ESI-MS ion cluster at m/z 1069 {[Ir(pq)$_2$(bpy-C6-ind)]$^+$}$^+$. Anal. Calcd for $C_{58}H_{51}N_7O_2PF_6Ir\cdot 2H_2O$: C, 55.67; H, 4.43; N, 7.84. Found: C, 55.62; H, 4.37; N, 7.72.

Physical Measurements and Instrumentation. Instruments for the characterization and photophysical and electrochemical measurements have been described previously.[24b] Luminescence quantum yields were measured using the optically dilute method[27a] with an aerated aqueous solution of [Ru(bpy)$_3$]Cl$_2$ (Φ_{em} = 0.028) as the standard solution.[27b]

Preparation of the Luminescent Bioconjugates Ir−BSA and Ir−TF. [Ir(pq)$_2$(phen-NCS)](PF$_6$) (1 mg) in anhydrous DMSO (20 μL) was added to BSA or TF (2 mg) dissolved in 50 mM carbonic buffer pH 10.0 (980 μL). The mixture was gently stirred at 4 °C for 24 h. The precipitate was removed by centrifugation. The filtrate was then loaded to a PD-10 size-exclusion column that had been equilibrated with 50 mM phosphate buffer at pH 7.4. The same buffer was used as the mobile phase, and the first band with strong orange-yellow emission was collected and concentrated with

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a YM-30 microcon. The solution was further purified using HPLC equipped with a size-exclusion column (Waters Protein Pak Glass 300SW). The eluted fractions were collected and concentrated. It was filtered using a nitrocellulose membrane (0.25 μm) and stored at 4 °C before use.

Determination of Lipophilicity. The lipophilicity of the complexes, which is referred to as log $P_{o/w}$ ($P_{o/w}$ = *n*-octan-1-ol/water partition coefficient), was determined from the log k'_w values (k'_w = chromatographic capacity factor at 100% aqueous solution). Detailed procedures for the determination of lipophilicity have been described previously.[28]

Emission Titrations with BSA. To the iridium(III) indole complex (22 μM) in a mixture of 50 mM potassium phosphate buffer pH 7.4 and MeOH (2 mL, 9:1, v/v) were added 10 μL aliquots of BSA (1.5 mM) in 50 mM potassium phosphate buffer pH 7.4 at 1-min intervals. The solution was excited at 350 nm, and the emission spectra were measured.

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) indole complexes and cisplatin (positive control) were then added to the wells, with concentrations ranging from ca. 10^{-7} to 10^{-4} M in a mixture of growth medium/DMSO (99:1, v/v). Wells containing growth 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. Solubilization solution (100 μL) containing 10% SDS in 2-propanol/0.04 M HCl (1:1, v/v) was then added to each well, and the microplate was further incubated for 24 h. The absorbance of the solutions at 570 nm was measured with a SPECTRAmax 340 microplate reader (Molecular Devices Corp., Sunnyvale, CA). The IC_{50} values of the complexes were determined from the dependence of the survival of HeLa cells after exposure to the complexes for 48 h.

Flow Cytometry. HeLa cells in growth medium (100000 cells mL^{-1}) were seeded in a 35-mm tissue culture dish and incubated

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Table 1. Electronic Absorption Spectral Data of the Cyclometalated Iridium(III) Indole Complexes at 298 K

complex	medium	λ_{abs}/nm (ε/dm^3 mol^{-1} cm^{-1})
1a	CH_2Cl_2	257 (58520), 269 sh (55870), 291 sh (39090), 319 sh (19320), 339 sh (10740), 360 sh (9060), 382 (8310), 416 sh (3640), 469 sh (1050)
	CH_3CN	254 (54260), 267 (52370), 291 sh (34470), 319 sh (18030), 340 sh (10390), 360 sh (8810), 380 (7600), 407 sh (4050), 466 sh (1070)
1b	CH_2Cl_2	258 (51735), 269 sh (49565), 290 sh (35270), 319 sh (18045), 338 sh (10045), 360 sh (8370), 380 (7680), 411 sh (4035), 469 sh (1010)
	CH_3CN	254 (48850), 268 sh (46520), 280 sh (38000), 290 sh (31290), 318 sh (16685), 338 sh (9320), 357 sh (7940), 378 sh (6890), 411 sh (3390), 469 sh (1015)
2a	CH_2Cl_2	258 (50175), 291 sh (26705), 319 sh (18260), 360 sh (10550), 384 sh (6000), 420 sh (4575), 495 sh (620)
	CH_3CN	258 (48410), 289 sh (26335), 317 sh (17840), 361 sh (13030), 416 sh (5815), 495 sh (775)
2b	CH_2Cl_2	259 (59205), 284 sh (34325), 290 sh (32450), 319 sh (21925), 360 sh (12385), 419 sh (5255), 387 sh (6210), 498 sh (640)
	CH_3CN	253 (55165), 282 sh (33715), 290 sh (30540), 318 sh (21910), 354 sh (12925), 415 (4880), 498 sh (450)
3a	CH_2Cl_2	260 (51230), 282 (53270), 348 (20750), 395 sh (5240), 438 (4850), 450 sh (4230)
	CH_3CN	259 (53130), 281 (53810), 316 sh (23330), 338 (22300), 393 sh (5450), 435 (5140), 450 sh (4270)
3b	CH_2Cl_2	260 sh (52880), 281 sh (54350), 318 sh (23925), 395 sh (5690), 439 (5025), 525 sh (605)
	CH_3CN	259 sh (51230), 281 sh (51080), 317 sh (22695), 342 sh (21175), 434 (4820), 524 sh (510)

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) indole complexes at a concentration of 5 μM. After incubation for 1 h, the medium was removed, and the cell layer was washed gently with PBS (1 mL × 3). The cell layer was then trypsinized and added up to a final volume of 3 mL with PBS. The samples were analyzed by a FACSCalibur flow cytometer (Becton, Dickinson and Co., Franklin Lakes, NJ) with excitation at 488 nm. The number of cells analyzed for each sample was between ca. 9000 and 10000.

Live-Cell Confocal Imaging. HeLa cells were grown on sterile glass coverslips in a 35-mm tissue culture dish. The sample preparation procedure was similar to that of the flow cytometry. After washing with PBS, the coverslips were mounted onto slides for measurements. Imaging was performed using a confocal microscope (Carl Zeiss, LSM510) with an excitation wavelength at 488 nm. The emission was measured using a long-pass filter at 505 nm.

Results and Discussion

Synthesis. The cyclometalated iridium(III) polypyridine indole complexes were prepared in moderate yields from the reaction of $[Ir_2(N^\wedge C)_4Cl_2]$ (HN^C = Hppy, Hbzq, or Hpq) with 2 equivalents of bpy-ind or bpy-C6-ind in a mixture of MeOH and CH_2Cl_2, followed by anion exchange with KPF_6, 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 Luminescence Properties. The electronic absorption spectral data of the iridium(III) polypyridine indole complexes are summarized in Table 1. The electronic absorption spectra of complexes **1a**−**3a** in CH_2Cl_2 are shown in Figure 1. All the complexes showed intense spin-allowed intraligand (^{1}IL) ($\pi \rightarrow \pi^*$) (N^N and N^C) absorption bands and shoulders in the UV region at ca. 253−340 nm (ε on the order of 10^4 dm^3 mol^{-1} cm^{-1}) and less intense spin-allowed metal-to-ligand charge-transfer (^{1}MLCT) (dπ(Ir) → π^*(N^N and N^C)) absorption bands and shoulders at ca. 338−525 nm.[7−10,11c,12,13,14a,c,16a,c,17,18,19b,20,23a,b,24] In addition, all the complexes displayed weak absorption tailing (>525 nm) that has been ascribed to spin-forbidden ^{3}MLCT (dπ(Ir) → π^*(N^N and N^C)) transitions.[7−10,11c,12,13,14a,c,16a,c,17,18,19b,20,23a,b,24]

Upon irradiation, all the complexes displayed intense and long-lived orange to greenish-yellow luminescence. The photophysical data are summarized in Table 2. The emission spectra of complexes **1a** and **3a** in degassed CH_2Cl_2 at 298 K and in alcohol glass at 77 K are shown in Figures 2 and 3, respectively. The emission of the ppy and bzq complexes **1a**,**b** and **2a**,**b** in fluid solutions at 298 K has been assigned to a ^{3}MLCT (dπ(Ir) → π^*(N^N)) excited state.[7,9b,c,10−12,14−16,17a,18,20,21,22b,24a−d,f−j] The emission maxima of these complexes appeared at lower energy compared with those of their 2,2′-bipyridine counterparts.[24d] This observation is in accordance with an assignment of a ^{3}MLCT excited state because the electron-withdrawing amide substituent of the ligands, bpy-ind and bpy-C6-ind, stabilizes the π^* orbitals,

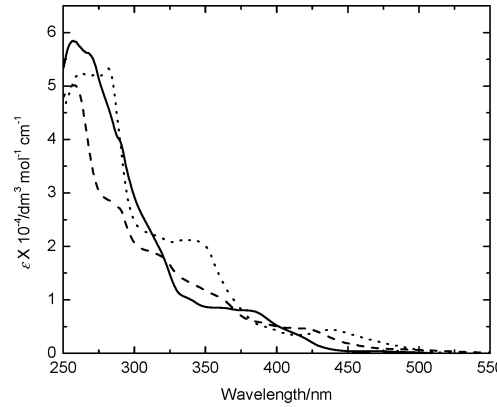

Figure 1. Electronic absorption spectra of complexes **1a** (—), **2a** (----), and **3a** (····) in CH_2Cl_2 at 298 K.

Table 2. Photophysical Data of the Cyclometalated Iridium(III) Indole Complexes[a]

complex	medium (T/K)	λ_{em}/nm	τ_o/μs	Φ
1a	CH_2Cl_2 (298)	609	0.48	0.16
	CH_3CN (298)	614	0.28	0.072
	buffer[b] (298)	600	0.15	0.0032
	glass[c] (77)	540	4.83	
1b	CH_2Cl_2 (298)	608	0.43	0.14
	CH_3CN (298)	615	0.24	0.059
	buffer[b] (298)	594	0.36	0.0081
	glass[c] (77)	541, 578 sh	4.98	
2a	CH_2Cl_2 (298)	609	0.40	0.074
	CH_3CN (298)	613	0.21	0.046
	buffer[b] (298)	596	0.13	0.0010
	glass[c] (77)	545	4.25	
2b	CH_2Cl_2 (298)	612	0.35	0.095
	CH_3CN (298)	616	0.17	0.032
	buffer[b] (298)	595	0.30	0.0039
	glass[c] (77)	545	4.14	
3a	CH_2Cl_2 (298)	550 sh, 595	0.80	0.23
	CH_3CN (298)	580	0.51	0.14
	buffer[b] (298)	556 sh, 595	0.16	0.0049
	glass[c] (77)	543 (max), 586, 635 sh	5.15	
3b	CH_2Cl_2 (298)	560 sh, 593	0.76	0.23
	CH_3CN (298)	560 sh, 596	0.46	0.12
	buffer[b] (298)	574 sh, 615	0.20	0.0089
	glass[c] (77)	543 (max), 585, 640 sh	5.00	

[a] Excitation wavelength = 350 nm. [b] 50 mM potassium phosphate buffer pH 7.4/DMSO (3:1, v/v). [c] EtOH/MeOH (4:1, v/v).

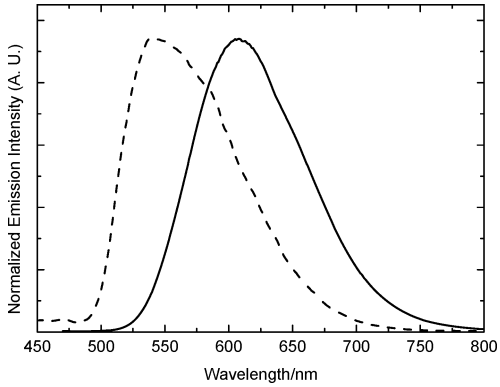

Figure 2. Emission spectra of complex **1a** in CH_2Cl_2 at 298 K (—) and in EtOH/MeOH (4:1, v/v) at 77 K (----).

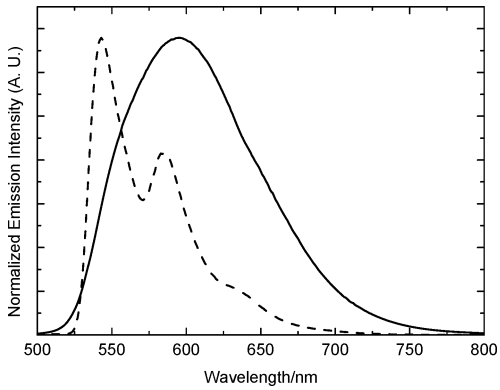

Figure 3. Emission spectra of complex **3a** in CH_2Cl_2 at 298 K (—) and in EtOH/MeOH (4:1, v/v) at 77 K (----).

thereby lowering the ^{3}MLCT (dπ(Ir) → π*(N^N)) emission energy. The pq complexes **3a**,**b** showed an emission shoulder at ca. 556−574 nm and an emission maximum at ca. 580−615 nm in fluid solutions (Table 2 and Figure 3). The higher-energy emission of the pq complexes, compared with those of the ppy and bzq complexes, could be due to the fact that pq, being a stronger π-acceptor, stabilizes the dπ orbitals of the metal center more effectively, resulting in a higher ^{3}MLCT (dπ(Ir) → π*(N^N)) emission energy. However, the emission lifetimes of the pq complexes are noticeably longer than those of their ppy and bzq counterparts (Table 2), suggesting that the ^{3}MLCT (dπ(Ir) → π*(N^N)) excited states of these complexes are significantly mixed with some ^{3}IL (π → π*) (pq) character,[24c,d,h,j] which could also explain their higher emission energy. The bpy-ind complexes **1a**−**3a** displayed a decrease of emission lifetimes upon changing the solvent from less polar CH_2Cl_2 to more polar CH_3CN and buffer (Table 2), which has been commonly observed in other cyclometalated iridium(III) polypyridine ^{3}MLCT emitters. However, the bpy-C6-ind complexes **1b**−**3b** did not display a similar dependence of emission lifetimes on the polarity of the solvents. In particular, the emission lifetimes of these complexes in buffer solutions were longer than expected. We believe that this can be attributed to the longer spacer arm, which provides a more nonpolar local environment to these complexes, especially in aqueous buffer.[24i,j]

Upon cooling the samples to 77 K, the emission maxima of all the complexes exhibited a blue shift (Table 2). This finding reflects the charge-transfer character of the emissive states of the complexes. The emission lifetimes of the ppy and bzq complexes **1a**,**b** and **2a**,**b** are ca. 4.1−5.0 μs, and the emission has been assigned to a ^{3}MLCT (dπ(Ir) → π*(N^N)) state in nature. On the contrary, the pq complexes **3a**,**b** showed a relatively small blue shift in the emission maxima and rich vibronic structures in the emission bands upon cooling to 77 K. It is likely that the emission of these complexes originates from an excited state of predominantly ^{3}IL (π → π*) (pq) character, although the existence of charge-transfer character cannot be totally ruled out.

When excited at 280 nm, all the complexes showed an additional emission band at ca. 350−390 nm. A similar emission band has been observed in other luminescent transition metal polypyridine indole complexes, and it has been assigned to the fluorescence of the appended indole unit.[6a,b,d] In this work, the intensities of this indole fluorescence are ca. 99−208 times weaker than that of unmodified indole in CH_3CN ($A_{280\,nm}$ = 0.1). We have attributed the difference to the fact that the iridium(III) polypyridine moieties also absorbed at the excitation wavelength (Table 1), and, thus, the indole pendant did not absorb all the excitation power. Also, all the complexes exhibited quite intense absorption at the emission wavelength of indole, resulting in efficient resonance-energy transfer from the indole unit to the iridium(III) polypyridine core and, hence, weaker indole fluorescence.

Electrochemical Properties. The electrochemical properties of the cyclometalated iridium(III) polypyridine indole complexes have been studied by cyclic voltammetry. The electrochemical data are listed in Table 3. The cyclic voltammograms of complex **1b** are displayed in Figure 4 as an example. All the complexes showed an irreversible oxidation wave at ca. +1.06 to +1.09 V versus SCE and a

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

complex	oxidation, $E_{1/2}$ or E_a/V	reduction, $E_{1/2}$ or E_c/V
1a	+1.06,[b] +1.25[c]	−1.29, −1.82,[b] −2.23[c]
1b	+1.06,[b] +1.25[c]	−1.28, −1.82,[b] −2.22[c]
2a	+1.08,[b] +1.21[c]	−1.27, −1.80,[b] −1.98,[b] −2.38[c]
2b	+1.09,[b] +1.22[c]	−1.27, −1.79,[b] −1.90,[b] −2.20[c]
3a	+1.07,[b] +1.28[c]	−1.29, −1.73,[b] −1.92,[b] −2.21[c]
3b	+1.07,[b] +1.29[c]	−1.30, −1.74,[b] −1.99,[b] −2.27[c]

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

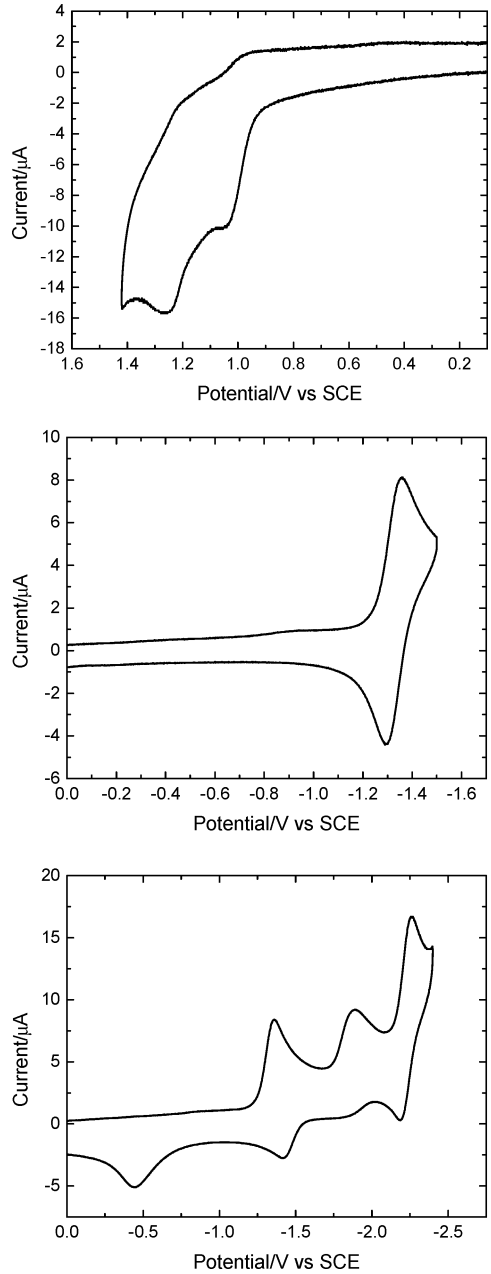

Figure 4. Cyclic voltammograms of complex **1b** in CH_3CN (0.1 M TBAP) at 298 K in various scanning windows.

quasi-reversible couple at ca. +1.21 to +1.29 V, which have been assigned to the oxidation of the indole moiety[6] and the iridium(IV/III) couple, respectively.[8,9b,10,11,12c,d,13,14b,c,16a,c,17a−c,20b,21,22b,c,23c,24a−h,j] With reference to previous electrochemical studies of related cyclometalated iridium(III) polypyridine complexes,[8,9b,10,11,12c,d,13,14b,c,16a,c,17a−c,20b,21,22b,c,23c,24a−h,j] the first reduction couple of all the complexes at ca. −1.3 V has been assigned to the reduction of the diimine ligands. Additionally, all the complexes displayed more reduction waves at more negative potentials, which have been assigned to the reduction of the diimine and cyclometalating ligands.[8,9b,10,11,12c,d,13,14b,c,16a,c,17a−c,20b,21,22b,c,23c,24a−h,j]

Table 4. Log $P_{o/w}$ Values of the Cyclometalated Iridium(III) Indole Complexes

complex	log $P_{o/w}$
1a	1.30
1b	1.37
2a	2.61
2b	2.95
3a	2.72
3b	3.40

Lipophilicity. The cellular and in vivo tissue-uptake selectivity and characteristics of probes and therapeutic reagents can be estimated by their lipophilicity. This is commonly referred to as the *n*-octan-1-ol/water partition coefficients (expressed in log $P_{o/w}$) of the compounds, which are readily determined by reversed-phase HPLC.[29] The log $P_{o/w}$ values of the iridium(III) indole complexes are listed in Table 4. The lipophilicity of the complexes was slightly higher when incorporated with a longer spacer arm, as indicated by the larger log $P_{o/w}$ values of the bpy-C6-ind complexes **1b**−**3b**, compared with those of their bpy-ind counterparts **1a**−**3a**. In addition, the log $P_{o/w}$ values of the complexes followed the order: ppy < bzq < pq, which is in accordance with the hydrophobic character of the ligands. Interestingly, the indole moiety also renders the complexes more lipophilic because the log $P_{o/w}$ values of complexes **1a** (1.30) and **3a** (2.72) are higher than those of their indole-free counterparts, $[Ir(ppy)_2(bpy\text{-}CONH\text{-}Et)](PF_6)$ (log $P_{o/w}$ = 0.44) (bpy-CONH-Et = 4-(ethylaminocarbonyl)-4′-methyl-2,2′-bipyridine) and $[Ir(pq)_2(bpy\text{-}CONH\text{-}Et)](PF_6)$ (log $P_{o/w}$ = 2.01), respectively.[24j] Thus, the use of longer spacer arms, hydrophobic cyclometalating ligands, as well as an indole unit may facilitate the tissue and cellular uptake of these complexes.

Emission Titrations. Since serum albumins are well known to bind small aromatics including indole, the possible binding interactions of the cyclometalated iridium(III) polypyridine indole complexes with BSA have been investigated by emission-titration experiments. The emission intensities and lifetimes of all the complexes were increased in the presence of BSA (Table 5). The emission spectral traces and emission titration curve for complex **3a**, upon addition of BSA, are shown in Figures 5 and 6, respectively. Similar emission enhancement and lifetime elongation have been observed for related luminescent transition metal polypyridine indole complexes.[6a−d] We have ascribed these changes to the binding of the indole moiety of the complexes to a hydrophobic pocket of BSA, resulting in an increase of the hydrophobicity and rigidity of the local surroundings of the

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Table 5. Results of Titrations of the Cyclometalated Iridium(III) Indole Complexes with BSA in Aerated 50 mM Potassium Phosphate Buffer pH 7.4/MeOH (9:1, v/v) at 298 K

complex	I/I_o[a]	$\tau/\mu s$[b]	$\tau_o/\mu s$[b]	K_a/M^{-1}	n
1a	2.4	0.12	0.28	5.3×10^4	1.4
1b	2.2	0.14	0.27	6.3×10^4	0.9
2a	3.0	0.11	0.25	3.1×10^4	0.4
2b	2.3	0.19	0.28	4.3×10^4	0.8
3a	12.6	0.28	0.67	3.3×10^4	0.4
3b	4.6	0.29	0.78	4.2×10^4	0.2

[a] I_o and I are the emission intensities of the complexes in the absence and in the presence of 1 mM of BSA, respectively. [b] τ_o and τ are the emission lifetimes of the complexes in the absence and in the presence of 1 mM of BSA, respectively.

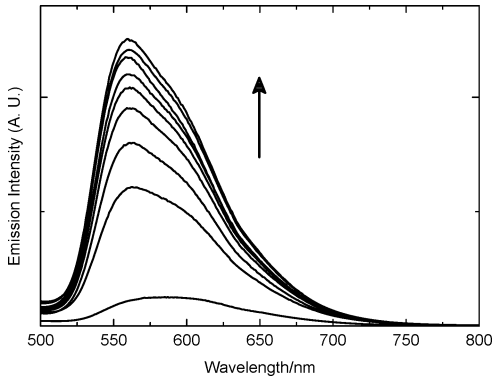

Figure 5. Emission spectral traces of complex **3a** (22 μM) in 50 mM potassium phosphate buffer pH 7.4/MeOH (9:1, v/v) at 298 K in the presence of 0, 59, 115, 170, 222, 272, 321, 391, and 561 μM BSA.

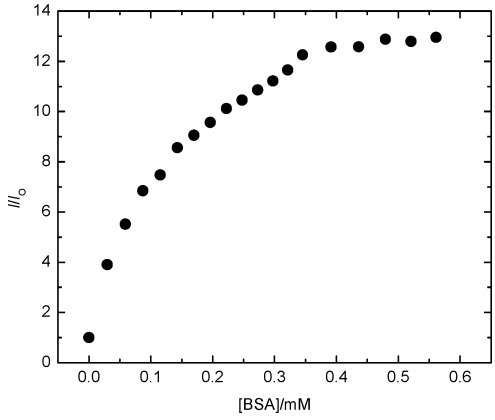

Figure 6. Emission titration curve of complex **3a** with BSA. I_o and I are the emission intensities of the complex in the absence and presence of BSA, respectively. The emission intensity of complex **3a** was monitored at 561 nm.

complexes. Interestingly, the emission enhancement factors of the bpy-C6-ind complexes **1b**−**3b** are smaller than those of complexes **1a**−**3a**. This is probably a result of the flexible C6 spacer arm that renders the iridium(III) polypyridine units more exposed to the polar bulk solution after binding to the protein. The binding parameters of the cyclometalated iridium(III) polypyridine indole complexes to BSA have been determined by the Scatchard analyses.[30] The K_a values of the complexes ranged from ca. 3.3×10^4 to 6.3×10^4 M^{-1} (Table 5), which are of the same order of magnitude as those of the binding of tryptamine to BSA ($K_a = 1.1 \times 10^4$ M^{-1})[31] and the other rhenium(I) ($K_a = 0.9 \times 10^4$ to 3.5×10^4 M^{-1})[6a−c] and ruthenium(II) ($K_a = 6.6 \times 10^4$ to 9.5×10^4 M^{-1})[6d] indole complexes we reported previously. The BSA-binding strengths of the bpy-C6-ind complexes **1b**−**3b** are similar to those of the bpy-ind complexes **1a**−**3a** (Table 5), indicative of insignificant effects of the C6 spacer arm.

In view of the interesting DNA-binding properties of indole derivatives,[32a,b] we have preliminarily studied the possible binding of complex **3a** to double-stranded calf thymus DNA. Upon addition of DNA, the complex revealed very small emission enhancement (ca. 1.1 fold at [DNA]:[Ir] = 0.3), which was followed by gradual reduction in emission intensity (I/I_o = ca. 0.9 at [DNA]:[Ir] = 3.5). The small initial increase could be due to intercalation of the indole unit of the complex to the DNA molecule and a certain degree of polyelectrolyte-induced aggregation of the complex molecules.[32c] However, importantly, the subsequent decrease may be a result of photoreactions of the complex with calf thymus DNA. Additionally, we have studied the emission behavior of the same complex in the presence of dG. Interestingly, the emission was quenched by dG (I/I_o = ca. 0.74 at [dG]:[Ir] = 8.3). Since the excited-state reduction potential of complex **3a**, $E°[Ir^{+}*/Ir^{0}]$, was estimated to be ca. +1.00 V versus SCE (E_{00} = ca. +2.29 eV from the emission data), it is likely that the emission quenching is a result of the oxidation of dG ($E°[dG^{+}/dG^{0}]$ = +0.948 V versus SCE)[33] by the excited complex. More detailed investigations are required to elucidate the quenching mechanism.

Cytotoxicity. We have used the MTT assay to examine the cytotoxic effect of the cyclometalated iridium(III) polypyridine indole complexes toward HeLa cells.[34] The IC_{50} values have been determined from the dependence of the survival of HeLa cells on the dose of the complexes for 48 h. The relationship between the cell viability and concentration of complex **1a** is shown in Figure 7 as an example. The IC_{50} values of the cyclometalated iridium(III) polypyridine indole complexes ranged from 1.1 to 6.3 μM (Table 6), which are significantly smaller than that of cisplatin (30.7 μM) under the same experimental conditions, indicative of high cytotoxicity. These IC_{50} values are smaller than those of related indole-free complexes (3.8 to 18.1 μM),[24j] suggesting that the indole unit renders the complexes more cytotoxic to HeLa cells. In general, the cytotoxicity of the complexes is similar to those of the rhenium(I) diphosphine complexes, $[Re(CO)_3(diphosphine)Br]$,[35a] and much higher than those of $[Re(CO)_3(2\text{-appt})Cl]$ (2-appt = 2-amino-4-phenylamino-6-(2-pyridyl)-1,3,5-triazine) (IC_{50} = ca. 50 μM) and [Ru(*t*-

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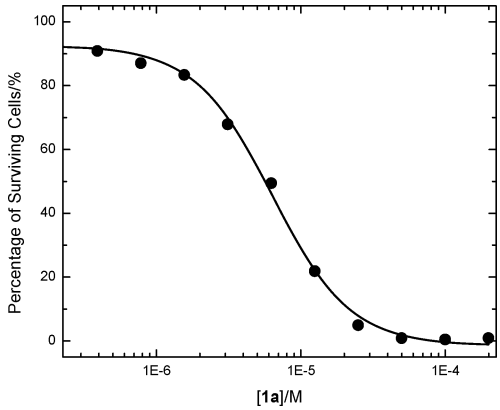

Figure 7. Dependence of the survival of HeLa cells on the dose of complex **1a**.

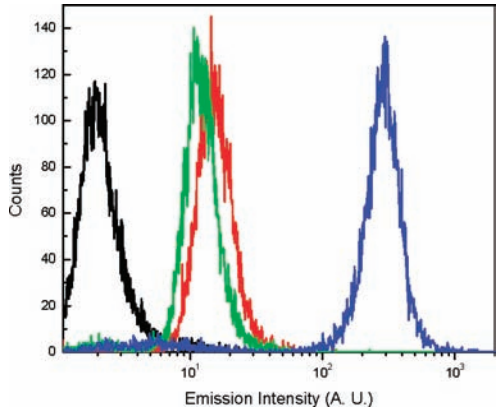

Figure 8. Cytometric results of HeLa cells incubated with blank medium (black), **1a** (red), **2a** (green), and **3a** (blue) at 37 °C for 1 h.

Bu_2-bpy)$_2$(2-appt)]$^{2+}$ (*t*-Bu_2-bpy = 4,4′-di-*tert*-butyl-2,2′-bipyridine) (IC_{50} = 59.7 μM), both of which can bind to the minor groove of double-stranded DNA.[35b] In general, the iridium(III) complexes in this work are relatively cytotoxic compared with the organometallic ruthenium arene complexes [(η^6-arene)Ru(ethylenediamine)(X)]$(PF_6)_n$ (X = substituted pyridines and halides), some of which exhibit fast hydrolysis kinetics and high cytotoxicity toward the human ovarian cancer cell line A2780.[36]

Flow Cytometry and Laser-Scanning Confocal Microscopy Studies. HeLa cells loaded with the iridium(III) polypyridine complexes (5 μM) for 1 h have been analyzed using flow cytometry. The results for complexes **1a**−**3a** are shown in Figure 8. Upon excitation, all the cell samples incubated with the complexes displayed higher emission intensities, compared with the autofluorescence of untreated HeLa cells, indicating efficient cellular internalization of the complexes. The emission intensity of HeLa cells treated with complex **3a** is higher than those treated with complexes **1a** and **2a** (Figure 8), which is in accordance with the luminescence quantum yields and emission wavelengths of the free complexes (Table 2). Another possible reason is that complex **3a** is more effectively interiorized by the cells.

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Table 6. Cytotoxicity (IC_{50}, 48 h) of the Cyclometalated Iridium(III) Indole Complexes and Cisplatin toward the HeLa Cell Line

complex	$IC_{50}/\mu M$
1a	6.3 ± 0.4
1b	6.0 ± 0.3
2a	3.3 ± 0.4
2b	2.4 ± 0.3
3a	2.4 ± 0.2
3b	1.1 ± 0.1
cisplatin	30.7 ± 0.7

The cellular-uptake properties of complex **3a** have been studied in more detail using laser-scanning confocal microscopy. The microscopy images of HeLa cells treated with complex **3a** at a concentration of 5 μM for 1 h are illustrated in Figure 9. The complex distribution in the cytoplasm was not even, but localized in the perinuclear region, forming very sharp luminescent rings surrounding the nuclei. However, the nuclei showed much weaker emission, indicative of negligible nuclear uptake of the complex. From the image, it is likely that the complex binds to hydrophobic organelles such as Golgi apparatus, endoplasmic reticulum, and mitochondria.[37] Interestingly, incubation of the HeLa cells with complex **3a** at 4 °C resulted in much reduced cellular-uptake efficiency, as revealed by confocal microscopy (data not shown) and flow cytometry results (Figure 10). This suggests that the interiorization of the complex was an energy-requiring process such as endocytosis.[38] Since the uptake of both BSA and TF by HeLa cells occurs via receptor-mediated endocytosis,[38b,c] we have exploited the use of luminescent derivatives of these two proteins to gain more insights into the cellular uptake of the iridium(III) indole complexes. Thus, we have labeled BSA and TF with an amine-specific luminescent cyclometalated iridium(III) polypyridine isothiocyanate complex, [Ir(pq)$_2$(phen-NCS)](PF_6) (phen-NCS = 5-isothiocyanato-1,10-phenanthroline),[24c] that displays absorption and emission characteristics that are similar to those of complex **3a**. The resultant Ir−BSA and Ir−TF bioconjugates have been isolated and purified carefully to ensure the absence of unreacted complexes. HeLa cells were then incubated with these bioconjugates under the same experimental conditions. Confocal microscopy images of the treated cells revealed localization of the luminescent bioconjugates in the perinuclear region (Figure 11), which is indistinguishable to the case of complex **3a**. These results strongly suggest that complex **3a** was internalized to HeLa cells in a similar fashion, probably by endocytosis.

The effects of different reagents such as cytoskeletal, metabolic, and ATPase inhibitors on the cellular uptake of complex **3a** have been investigated by flow cytometry and confocal microscopy. First, we have treated HeLa cells with nocodazole (30 μM) and colchicine (10 μM), which are

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(38) (a) Reaven, E.; Tsai, L.; Azhar, S. *J. Biol. Chem.* **1996**, *271*, 16208. (b) Weaver, D. J., Jr.; Voss, E. W., Jr. *Biol. Cell* **1998**, *90*, 169. (c) Dautry-Varsat, A.; Ciechanover, A.; Lodish, H. F. *Proc. Natl. Acad. Sci. U.S.A.* **1983**, *80*, 2258.

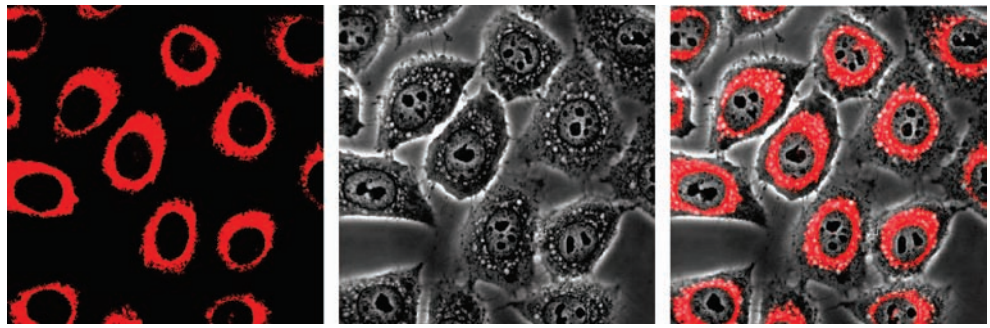

Figure 9. Fluorescence (left), brightfield (middle), and overlaid (right) images of HeLa cells incubated with complex **3a** (5 μM) at 37 °C for 1 h.

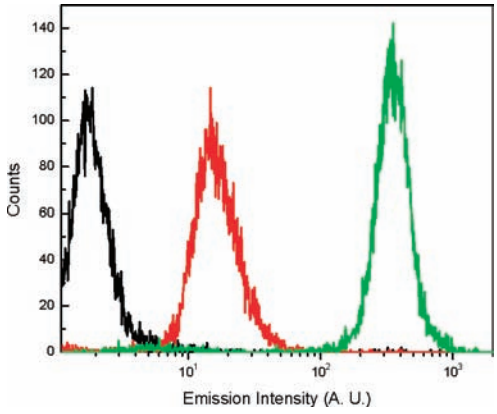

Figure 10. Cytometric results of HeLa cells incubated with blank medium (black) and complex **3a** at 4 °C (red) and 37 °C (green) for 1 h.

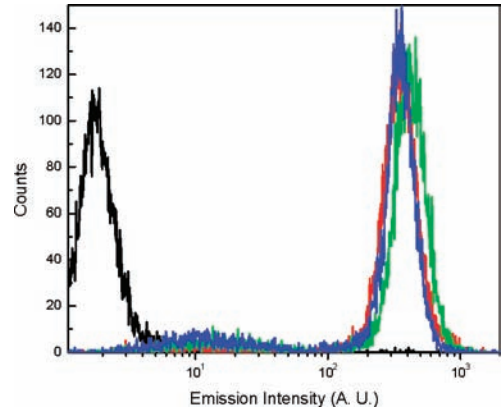

Figure 12. Cytometric results of HeLa cells incubated with blank medium (black) and complex **3a** (red) after preincubation of nocodazole (green) and colchicine (blue) at 37 °C for 1 h.

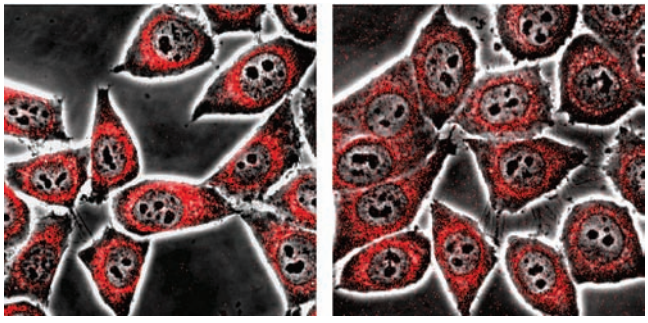

Figure 11. Confocal images of HeLa cells incubated with Ir−BSA (left) and Ir−TF (right) at 37 °C for 1 h.

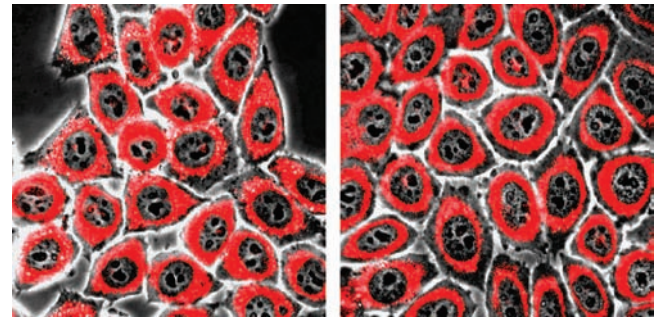

Figure 13. Confocal images of HeLa cells incubated with complex **3a** after preincubation of nocodazole (left) and colchicine (right) at 37 °C for 1 h.

typical cytoskeletal inhibitors that disrupt the microtubule assembly and the Golgi membranes, respectively,[38] before incubation with complex **3a**. Interestingly, the emission intensity of the loaded HeLa cells was very similar to those of the cells untreated with these two reagents (Figure 12), and the luminescent images also revealed perinuclear rings (Figure 13). This indicates that cytoskeleton plays a minor role in the cellular uptake and intracellular transport of complex **3a** and that the complex may not bind to the Golgi apparatus. Second, incubation of HeLa cells with CCCP (20 μM), a metabolic inhibitor, at 37 °C in a glucose-free medium, before treatment with complex **3a**, led to a substantial decrease of cellular-uptake efficiency (Figure 14). Since CCCP inhibits oxidative phosphorylation, resulting in decreased ATP production and, hence, a lowered metabolic rate, the observed decrease of cellular uptake is in accordance with the argument that internalization of complex **3a** occurred via energy-requiring endocytosis. Additionally, ATPase inhibitors including potassium nitrate (50 mM), sodium orthovanadate(V) (50 μM), and sodium molybdate(VI) dehydrate (250 μM) did not result in any observable changes in the cellular uptake and intracellular migration of complex **3a** (data not shown).[38a] However, it is interesting to note that incubation of HeLa cells with NEM (1 mM), before complex **3a** was loaded, gave rise to significant nuclear uptake (Figure 15). The origin of this enhanced nuclear uptake requires more detailed investigations, but it is possible that the nuclear accumulation of this compound is caused by an increased nuclear entry as a result of NEM-induced nuclear pore malformation[39a] or a reduced nuclear export

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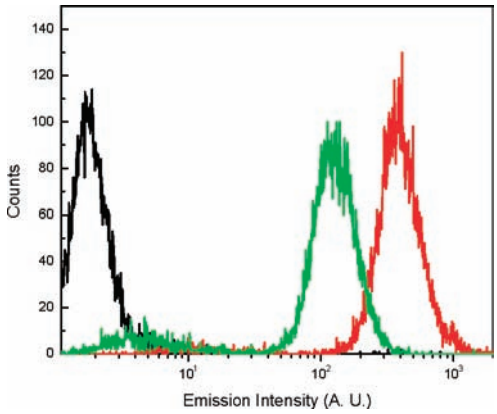

Figure 14. Cytometric results of HeLa cells incubated with blank medium (black) and complex **3a** (red) after preincubation of CCCP (green) at 37 °C for 30 min under a glucose-free condition.

due to the inhibitory effect of NEM on nuclear export pathways.[39b]

Conclusions

We report herein the synthesis, characterization, photophysical and electrochemical properties, and lipophilicity of a series of luminescent cyclometalated iridium(III) polypyridine indole complexes. The interactions of these complexes with the indole-binding protein BSA have been studied by emission titrations. All the complexes showed emission enhancement and lifetime extension upon binding to BSA, attributable to the increased hydrophobicity and rigidity of the local environments of the complexes. The cytotoxicity of these complexes toward HeLa cells was relatively high compared with cisplatin in a 48-h incubation period. Flow cytometry and laser-scanning confocal microscopy studies revealed efficient uptake of complex **3a** by HeLa cells and

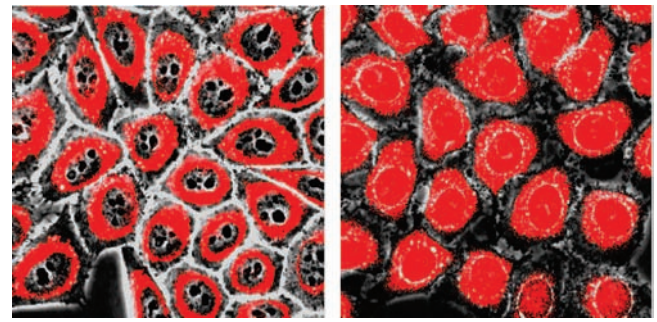

Figure 15. Confocal images of HeLa cells incubated with complex **3a** (left) after preincubation of NEM (right) at 37 °C for 20 min.

subsequent localization in the perinuclear region. Results from various experiments indicated that the interiorization mechanism was endocytic in nature, and the intracellular migration of the complex did not appear to be mediated by cytoskeleton. The highly efficient internalization of the complex at low concentration in a relatively short incubation time suggests that these luminescent cyclometalated iridium(III) polypyridine indole complexes are promising candidates as live-cell imaging reagents.

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