Imidazole and Benzimidazole Modified Half-Sandwich Iridium\textsuperscript{III} N-Heterocyclic Carbone Complexes: Synthesis, Anticancer Application, and Organelle Targeting

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Herein, we report the synthesis, characterization and anticancer activity of a series of half-sandwich iridium\textsuperscript{III} imidazole and benzimidazole N-heterocyclic carbene (NHC) anticancer complexes, and the general formula of which can be expressed as \[ \left[ \eta^5-Cp^x \right] \text{Ir}(C^\wedge N)\text{Cl} \text{Cl} \] (Cp\textsuperscript{x}: pentamethylcyclopentadienyl (Cp\textsuperscript{∗}) or biphenyl derivatives (Cp\textsuperscript{xbiph}); C^\wedge N: imidazole and benzimidazole NHC chelating ligands). Compared with cis-platin, these complexes showed interesting antitumor activity against A549 cells. Complexes could bind to bovine serum albumin (BSA) by means of static quenching mode, catalyze the oxidation of nicotinamide adenine dinucleotide (NADH) and increase the levels of reactive oxygen species (ROS). Meanwhile, these complexes could arrest the cell cycles of A549 cells and influence the mitochondrial membrane potential significantly. Due to the inherent luminescence property, laser confocal test show that complexes could enter cells followed an energy-dependent mechanism and effectively accumulate in lysosome (the value of Pearson’s co-localization coefficient is 0.70 after 1 h), further destroy lysosome integrity and induce apoptosis.

Keywords: iridium(III) compounds, anticancer, N-heterocyclic carbene, organelle targeting, imidazole

INTRODUCTION

When cell growth is uncontrollable and abnormal proliferation occurs, malignant tumors are formed (Valastyan and Weinberg Robert, 2011; Grossi et al., 2016). Most forms of disseminated tumors are currently untreatable, although developments in chemotherapy over the past years have led to the treatment of various cancers (Thota et al., 2018). Cis-platin exerts good and broad-spectrum anticancer effects (Deubel and Lau, 2006; Romero-Canelón and Sadler, 2013; Muhammad and Guo, 2014), but, when used extensively, platinum-based drugs may promote drug resistance and instability; become insoluble in biological media; and become toxic to blood and gastrointestinal tract (Ma et al., 2014; Liu et al., 2015). Thus, various organometallic anticancer drugs, such as tin, iridium, ruthenium, and osmium anticancer complexes, have been developed (Sun and Che, 2009; Lu et al., 2015; Zeng et al., 2017; Ge et al., 2019; Li et al., 2019).
Ir\textsuperscript{III} complexes are inert, half-sandwich Ir\textsuperscript{III} complexes (a type of \{(Cp\textsuperscript{x})Ir(L\textsuperscript{N}L')Z\}X) show good anticancer activities (Richens, 2005), especially for tumors resistant to platinum-based drugs (Singh et al., 2010). These complexes are composed of cyclopentadienyl ligands (Cp\textsuperscript{x}), chelating ligands (L\textsuperscript{N}L'), leaving groups (Z), and counter ions (X), each of which has an inherent effect on the anticancer activity of these complexes (Liu et al., 2011a). The types and positions of substituents on L\textsuperscript{N}-chelating ligands are the most studied and finely regulated for the effective modulation of targeted sites, the lipid solubility and even the anticancer activities of these complexes (Yellol et al., 2013; Zamora et al., 2015; Hao et al., 2019). The anticancer activity of a simple half-sandwich Ir\textsuperscript{III} complex increases nearly 10 times when the bipyridine chelating ligand (N\textsuperscript{N}N) is replaced by a phenyl pyridine ligand (C\textsuperscript{N}N) (Liu and Sadler, 2014). The IC\textsubscript{50} value (the concentration at which the growth of 50% of the cells is inhibited) of Ir\textsuperscript{III} complex can be reduced by nearly 100 times by controlling the binding mode of bipyridine chelate ligand substituents and the number of triphenylamine (TPA) units (He et al., 2019).

As a chelating ligand, N-heterocyclic carbene (NHC) provides an s-donor, which not only ensures the high stability of a metal complex, but also prevents its hydrolyzation in various physiological media. Transition metal complexes containing NHC have been widely used in the field of catalysis (Powell et al., 2010; Schuh et al., 2012; Pellei et al., 2015). These complexes affect different catalytic modes and even change the progress of the cyclic metal complex catalytic process (Zou et al., 2018). Metal iridium complexes containing NHC targets lysosomes and mitochondria selectively, induces lysosome damage, alters mitochondrial membrane potential, blocks the cell growth cycle, and promotes apoptosis (Han et al., 2018). In the present study, a series of half-sandwich Ir\textsuperscript{III} NHC complexes were synthesized and characterized in detail, and NHC pro-ligands were coordinated with metal iridium in the mode of C\textsuperscript{N}N (Figure 1). MTT assay was used to assess the anticancer activity of Ir\textsuperscript{III} NHC complexes in A549 cells lines. The anticancer mechanism of these complexes were also studied by flow cytometer and laser scanning confocal microscope. The results indicated that half-sandwich Ir\textsuperscript{III} NHC complexes may be promising candidate for anticancer drugs and broaden the research scope in this field.

RESULTS AND DISCUSSION

Synthesis and Characterization

NHC pro-ligands and a series of half-sandwich Ir\textsuperscript{III} NHC complexes of the type \{(Cp\textsuperscript{x})Ir(C\textsuperscript{N}N)Cl\}Cl (1A-3B) containing Cp ring and its biphenyl derivative (Scheme 1), were synthesized and characterized for the first time. Then, 1-diphenylmethylimidazole, 1-benzylbenzimidazole and 1-benzhydrylbenzimidazole were synthesized with imidazole and benzimidazole as raw materials and then reacted with 2-(chloromethyl)-1-methylbenzimidazole (Figure S1). The products were named L\textsubscript{1}, L\textsubscript{2}, and L\textsubscript{3}, respectively. The target complexes were obtained by catalyzing the reaction between the chelating ligands (L\textsubscript{1}-L\textsubscript{3}) and dimers (Cp\textsuperscript{x} and Cp\textsuperscript{abiph}) in dichloromethane with the use of silver oxide (Ag\textsubscript{2}O) at ambient temperature and good yields of 59–69%. The target complexes were isolated as Cl salt and characterized by \textsuperscript{1}H NMR (Figure S2), \textsuperscript{13}C NMR (Figure S3), mass spectroscopy (Figure S4) and elemental analysis. The \textsuperscript{1}H NMR spectra and MS spectral data are provided in the support information, as shown in Tables S1, S2. At the same time, we characterized the carbon in the complex, in which the peak shift of C-Ir is between 148.66 and 167.58 ppm, and the peak shift of NCN is between 138.56 and 150.24 ppm. After simple purification, the complexes were isolated as powdered and non-hygrosopic solids. The complexes were highly soluble in dichloromethane, chloroform, and dimethyl sulfoxide; partially soluble in methanol; and insoluble in ether, petroleum ether, and n-hexane.

M-OH\textsubscript{2} complexes usually have higher anticancer activities than M-Cl complexes (Liu et al., 2011b). Hydrolysis behavior of complex 3B in 50% CD\textsubscript{3}OD-d\textsubscript{4}/50% D\textsubscript{2}O (v/v) and 50% MeOH/50% H\textsubscript{2}O (v/v) was monitored by \textsuperscript{1}H NMR (Figure S5) and ultraviolet-visible spectrophotometry (UV-Vis; Figure S6) at 298 K, respectively. No obvious changes were observed in the \textsuperscript{1}H NMR and UV-Vis spectra, which indicating that Ir\textsuperscript{III} NHC complexes did not hydrolyze and the structure was stable high water content. Thus, the Ir\textsuperscript{III} NHC complexes are sufficiently stable for the bioassay.

Cytotoxicity Test

Lung cancer has a high mortality rate in developed and developing countries, therefore, A549 cells were selected as the model of biological research (Jemal et al., 2011). The antiproliferative activities of half-sandwich Ir\textsuperscript{III} complexes [(Cp\textsuperscript{x})Ir(L\textsuperscript{N}L')Z]Cl at was affected by changes in cyclopentadienyl...
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Scheme 1: Synthesis of chelating ligands L1–L3 and IrIII NHC complexes 1A–3B.

Table 1: IC50 values of complexes 1A–3B and cis-platin against A549 cancer cells after 24h exposure.

| Complexes                                      | IC50 (µM) |
|------------------------------------------------|-----------|
| [η5-C5Me5]Ir(L1)Cl | 18.2 ± 0.1 |
| [η5-C5Me5]Ir(L2)Cl | 15.6 ± 2.0 |
| [η5-C5Me5]Ir(L3)Cl | 12.3 ± 2.3 |
| [η5-C5Me4C6H4C6H5]Ir(L1)Cl | 8.9 ± 0.1 |
| [η5-C5Me4C6H4C6H5]Ir(L2)Cl | 6.7 ± 0.7 |
| [η5-C5Me4C6H4C6H5]Ir(L3)Cl | 5.9 ± 0.2 |
| Cis-platin                                       | 21.3 ± 1.7 |

Protein Binding Assay

Serum albumin (SA) has significant binding properties and is an important drug delivery medium in vivo (Chen et al., 2016; Esteghamat-Panah et al., 2017). Bovine serum albumin (BSA) has the structural homology with human serum albumin (HSA), can be easily purified, and shows good stability and is thus an economical and effective protein binding model (Naz et al., 2017). The interactions of complexes with BSA were analyzed by using UV-Vis absorption and fluorescence spectrum (Figure 3).

As shown in Figure 3A and Figure S7A, the maximum absorption at 228 nm (the absorption of BSA) decreased when the concentrations of complexes 1B–3B increased. The decrease indicated that the complexes combined with BSA through...
alpha-helical interference. The absorption peaks increased gradually at 278 nm. This result demonstrated that the complexes changed the microenvironments of the three aromatic acid residues (tryptophan, tyrosine and phenylalanine) in BSA (Zhang et al., 2015; Baral et al., 2017).

The molecular environment information near the fluorophore molecule was obtained by synchronous fluorescence spectroscopy at a low concentration (Jayabharathi et al., 2011). The use of $\Delta \lambda = 15 \text{ nm}$ and $\Delta \lambda = 60 \text{ nm}$ corresponds to the spectral characteristics of tyrosine residues and tryptophan residues, respectively. As shown in Figure S8, with the increase of the complexes, the synchronous fluorescence intensity decreased gradually. When $\Delta \lambda = 15 \text{ nm}$, the emission wavelength red shifted to 1~3 nm (285 nm). Meanwhile, no change was observed when $\Delta \lambda = 60 \text{ nm}$. Thus, tyrosine was more affected than tryptophan by the binding of Ir$^{III}$ NHC complexes to BSA.

The properties of the bonds between the complexes and BSA were further examined by fluorescence emission spectrum. The fluorescence spectra were calibrated for “internal filter” effect correction (Pacheco and Bruzzone, 2013). As shown in Figure 3B and Figure S7B, the fluorescence intensity of BSA (~343 nm) was quenched obviously when the concentrations of complexes 1B–3B increased. The possible quenching mechanism can be interpreted by using the Stern-Volmer equation and the Scatchard equation (Chatterjee and Mukherjee, 2014), $K_q$ (quenching rate constant), $K_b$ (banding constant), and $n$ (number of banding site) were then calculated. The $K_q$ values of complexes 1B–3B were $1.82 \times 10^{12}$, $2.34 \times 10^{12}$ and $2.66 \times 10^{12} \text{ M}^{-1} \text{ s}^{-1}$ (Figure S9 and Table S3), respectively, which were about two orders of magnitude higher than the value of a pure dynamic quenching mechanism ($2.0 \times 10^{10} \text{ M}^{-1} \text{ s}^{-1}$). The results indicated that the half-sandwich Ir$^{III}$ NHC complexes interacted with BSA in a static quenching mode. Additionally, 3B has the largest $K_b$ ($1.13 \times 10^{-4} \text{ M}^{-1}$) and $n$ (1.31), which were consistent with the results of MTT assay that complex 3B has better best anticancer activity than 1B–3B. The results indicate that Ir$^{III}$ NHC complexes can be effectively combined with BSA, and thus, BSA can be considered as an excellent carrier for delivering these anticancer complexes in vivo.

Apoptosis Assay
The effect of Ir$^{III}$ complexes inducing apoptosis was determined by exposing the A549 cells to complex 3B with a concentration of $0.5 \times IC_{50}$, $1.0 \times IC_{50}$ and $2.0 \times IC_{50}$ for 24h. The cells were stained with AnnexinV/ Propidium Iodide, then detected by flow cytometry (Rubbiani et al., 2011). As shown in Figure 4 and Table S4, complex 3B induced apoptosis in a concentration-dependent manner, and this condition was mainly observed in early apoptosis and late apoptosis. At $2.0 \times IC_{50}$, 97.6% (early apoptosis 56.2% + late apoptosis 41.4%) of the cells underwent apoptosis, and no considerable increase in increased necrotic population was detected. These results indicated that the Ir$^{III}$ NHC complexes induced apoptosis effectively and exhibited favorable anticancer activity.

Reactive oxygen species (ROS) are mainly produced and stored by the mitochondria and play an important role in the regulation cell apoptosis and represent a pathway for oxidation anticancer mechanisms (Trachootham et al., 2009). After the 24h treatment using complex 3B, the ROS levels in the A549 cells changed (Figure S10). When the concentration of complex 3B changed from $0.25 \times IC_{50}$ to $0.5 \times IC_{50}$, the ROS levels in the A549 cells were 1.3 and 1.6 times of the ROS level of the negative control (Table S5). This result is consistent with the result of the nicotinamide adenine dinucleotide (NADH) catalytic test. NADH (reduced coenzyme) can provide hydrides for Ir$^{III}$ complexes and lead to the production of ROS, which providing a possibility anticancer mechanism of oxidation. To investigate the catalytic ability of complex for NADH, the reactions of complex 3B (ca. 1 μM) with NADH (100 μM)
in 60% MeOH/40% H2O (v/v) were monitored by UV-Vis spectrum at 298 K (Figure S11). The conversion of NADH to NAD+ was detected by measuring the changes at 339 and 259 nm (the absorption peak of NADH and NAD+, respectively). As shown, with the increase of the concentration of 3B, there is a noticeable reduce and increase at 339 and 259 nm, respectively, which confirmed the catalytic activity of these complexes.

The relationship between anticancer mechanism and cell cycle arrest was determined by analyzing the cell cycle arrest of complex 3B in A549 cells through flow cytometry. Compared with the untreated cells, the A549 cells were mainly arrested in the sub-G1 and G2/M, and the cell population increased from 52.0 to 66.8% and from 11.1 to 20.1%, respectively, when the concentration of complex 3B increased from 0.25 × IC50 to 2.0 × IC50 (Figure S12 and Table S6). The results suggested that complex 3B may blocked the synthesis of RNA, ribosomes, and several proteins (Senese et al., 2014). The 6.9% increase in the percentage of cells in the S phase indicated that complex 3B inhibited the synthesis of DNA, histone, and some DNA replication-related enzymes and that IrIII NHC complexes arrested tumor cell cycle at multiple stages and induced apoptosis (Sabharwal and Schumacker, 2014).

The mitochondria are the main energy-producing sites in cells and play an essential role in apoptosis. Once the mitochondrial membrane is damaged, releases signals, and the damage leads to mitochondrial dysfunction and then induces apoptosis (Nichi et al., 2016). The degree of mitochondrial dysfunction can be evaluated by measuring the changes in mitochondrial membrane potential (MMP; Gómez-Sintes et al., 2016). The change in the green/red fluorescence intensity ratio for JC-1 (an ideal fluorescent probe widely used for detecting MMP) reflects the depolarization of MMP. As shown in Figure 5 and Table S7, the population of mitochondrial membrane depolarized cells changed from 11.0 to 77.4% with the increase of complex 3B (from 0.25 × IC50 to 2.0 × IC50). Increase in the ratio of JC-1 green/red fluorescence intensity (Figure 5B) confirmed that the IrIII NHC complexes affect the integrity of the mitochondrial membrane and induce apoptosis.

### Cellular Localization and Uptake Mechanism

The co-localization of IrIII NHC complexes was observed by laser confocal microscopy. Lyso Tracker Red DND-99 (LTRL) and Mito Tracker Deep Red (MTDR) were used. As shown in Figure 6, complex 3B was assimilated effectively by the A549 cells, and the complex mainly targeted lysosomes. The Pearson's co-localization coefficients (PCCs) after 1 h and 6 h were 0.70 and 0.81, respectively, which for mitochondria were 0.08 and 0.13. As the waste disposal center, lysosomes play an important role in many physiological processes and signal transduction pathways (Boya and Kroemer, 2008). These organelles are acidic (pH 4.5–5.5) and contain abundant nitrogen atoms (containing lone electron pairs). Thus, NHC pro-ligands provide convenient conditions for the lysosomes targeting. Interestingly, the IrIII NHC complex did not lead immediately induced A549 cells death. This feature is convenient for observing the effects the complexes on lysosome morphology.

The destruction of the lysosomal membrane destroys the integrity of the lysosome and induces cell death (Saftig and Klumperman, 2009; Zhitomirsky and Assaraf, 2017). Acridine orange (AO), an effective staining material for acidic organelles, was used to detect lysosomal damage induced by complex 3B. Then, whether the damage is the result of lysosome targeting specificity was determined. Green fluorescence represents the binding of AO to RNA in the nucleus or cytoplasm, whereas red fluorescence shows aggregation in the lysosome (Yamabe
et al., 2016). As shown in Figure 7, A549 cells subjected to AO alone showed significant red fluorescence in their lysosomes, but the intensity of the red fluorescence decreased quickly after the cells were exposed to complex 3B with concentrations of 1.0 × IC₅₀ and 2.0 × IC₅₀ for 6 h, which was the result of lysosomal damage, which was mainly attributed to the introduction of nitrogen-containing ligands into the complexes. The ligands increased the total alkalinity of the molecules, which easily accumulated and damaged the integrity of the acidic lysosomes (Daum et al., 2017). Additionally, the intracellular iridium contents of 3B in the cytosol, nucleus, nuclear chromatin and cytoskeleton fractions isolated from A549 cells were determined by inductively coupled plasma mass spectrometry (ICP-MS) after 24 h exposure. 3B is mainly accumulated in the cytosol (Table S8), which further confirm the results of lysosomal damage. Meanwhile, all these explains why these complexes did not target the mitochondria, but the fact that mitochondrial dysfunction occurred, including the elevation of intracellular ROS levels and the depolarization of MMP.

results indicated that Ir₃⁺ NHC complexes can induce apoptosis by lysosomal damage.

Small molecule drugs permeate cell membranes through different modes of action, mainly including energy-independent and energy-dependent mechanisms (Li et al., 2011). When the A549 cells were pretreated at 4°C or with carbonyl cyanide 3-chlorophenyl-hydrazone (CCCP, a metabolic inhibitor), their uptake efficiency decreased considerably after they were incubated with complex 3B (Figure 8), although no considerable change was observed in the level for complex 3B after pretreatment at 37°C or with chloroquine (an endocytosis inhibitor, which could inhibit endoderm acidification). Therefore, we conclude that the target Ir₃⁺ complex enters cells through an energy-dependent mechanism, including hydrophobic factors, which are also important in entering cells (Ye et al., 2017).

CONCLUSIONS

A series of half-sandwich Ir₃⁺ NHC complexes [(η⁵-C₅H₅)Ir(C=NC)(Cl)]Cl were synthetized and characterized. Each C=N chelating ligand formed a six-member ring with a metal center (Ir(III)), and the existence of benzene ring connected imidazole ring at different locations can effectively enhance the anticancer activity of the complexes. All the complexes
showed potential anticancer activity toward A549 cells, and had better anticancer activity than cis-platin (the clinical anticancer drug). Moreover, the complexes interacted with BSA by static quenching mode, catalyzed the conversion of NADH to NAD$^+$, induced ROS production, changed mitochondrial membrane potential, disrupted the cell cycle, and eventually induced apoptosis. The laser confocal test showed that complexes entered the cells followed through an energy-dependent mechanism, effectively targeted lysosomes, further destroyed the integrity of the lysosomes, and led to cell death. These results prompted us to further explore the medicinal value of the half-sandwich Ir$^{III}$ complex with additional NHC.

**EXPERIMENTAL SECTION**

**General Procedure**

The imidazole, benzimidazole, diphenylmethyl chloride, benzyl chloride, N-methyl-1, 2-phenylenediamine, diphenyl methane, diterbutyl peroxide and the appropriate dimer $\left(\eta^5-C_5Me_5\right)\text{IrCl}_2$ includes $\left(\eta^5-C_5Me_5\right)\text{IrCl}_2$ and $\left(\eta^5-C_5Me_4-C_6H_4C_6H_5\right)\text{IrCl}_2$. 2-(chloromethyl)-1-methyl benzimidazol, 1-Diphenyl-methylimidazol, 1-Benzylbenzimidazol, 1-Benzhydryl-benzimidazol were prepared according to literature procedures (specific synthesis steps and experimental data in support information) (Nobre and Monteiro, 2004; Corberán et al., 2006). Nitrogen was used as the inert gas filling.

**Synthesis of Chelating Ligands**

1-diphenylmethylimidazole (1.17 g, 5.0 mmol) was added to a solution of 2-chloromethyl-1-methylbenzenimidazole (0.48 g, 2.5 mmol) in acetonitrile. The reaction solution was distilled to 10 mL by vacuum rotary evaporation after reflex for 48 h, and 10 mL ether was added to precipitate the product. Then white powder product ($L_1$) was filtered, washed with ether three times and dried in air. Yield: 76.6%. $^1$H NMR (500 MHz, DMSO-$d_6$) $\delta$ 9.37 (s, 1H, NC$_H$N), 7.97 (s, 1H, imidazole-H), 7.84 (s, 1H, imidazole-H), 7.61 (dd, $J = 7.6$, 5.4 Hz, 2H, Ar-H), 7.47 (dq, $J = 14.4$, 7.1 Hz, 6H, Ar-H), 7.30 (dd, $J = 14.5$, 7.5 Hz, 6H, Ar-H), 7.23 (t, $J = 7.6$ Hz, 1H, NCH), 5.89 (s, 2H, NCH$_2$C), 3.85 (s, 3H, NCH$_3$).

Chelating ligands $L_2$ and $L_3$ were synthesized using 1-benzylbenzimidazol and 1-benzhydryl-benzimidazol with 2-chloromethyl-1-methylbenzimidazol by the same method, respectively, the data were as follows: $L_2$: Yield: 72.1%. $^1$H NMR (500 MHz, DMSO-$d_6$) $\delta$ 10.22 (s, 1H, NCHN), 8.14–8.10 (m, 1H, Ar-H), 8.05–8.00 (m, 1H, Ar-H), 7.69–7.66 (m, 2H, Ar-H), 7.64 (d, $J = 8.1$ Hz, 1H, Ar-H), 7.56 (d, $J = 7.1$ Hz, 2H, Ar-H), 7.53 (d, $J = 8.0$ Hz, 1H, Ar-H), 7.45 (t, $J = 7.1$ Hz, 2H, Ar-H), 7.33–7.30 (t, $J = 7.8$ Hz, 2H, Ar-H), 7.23 (t, $J = 7.4$ Hz, 1H, NCH), 6.09 (s, 2H, NCH$_2$C), 3.52 (s, 3H, NCH$_3$).
7.3 Hz, 2H, Ar-H), 7.40 (d, J = 7.2 Hz, 1H, Ar-H), 7.32–7.28 (m, 1H, Ar-H), 7.22–7.18 (m, 1H, Ar-H), 6.28 (s, 2H, NCH2C), 5.91 (s, 2H, NCH2C), 3.97 (s, 3H, NCH3).

L2: Yield: 72.3%.1H NMR (500 MHz, DMSO-d6) δ 5.69 (s, 1H, NCHN), 8.13 (d, J = 8.1 Hz, 1H, NCH), 7.78 (d, J = 8.1 Hz, 1H, Ar-H), 7.73 (s, 1H, Ar-H), 7.70–7.61 (m, 3H, Ar-H), 7.52 (t, J = 7.9 Hz, 5H, Ar-H), 7.49–7.42 (m, 6H, Ar-H), 7.28 (t, J = 7.7 Hz, 1H, Ar-H), 7.20 (t, J = 7.6 Hz, 1H, Ar-H), 6.23 (s, 2H, NCH2C), 3.91 (s, 3H, NCH3).

**Synthesis of the Complexes (1A–3B)**

General method: In a round bottom flask, silver oxide (2.4 eq) and (L1–L3) (2.0 eq) were added to a solvent of dichloromethane. After 8h, the mixture was filtered through celite. The combined filtrates were added dropwise to a solution of dichloromethane containing Dimer ([(η5-Cp)IrCl]2, 1.0 eq) (Weaver et al., 2011). The solution was stirred for 8h at room temperature, filtered with celite and washed with dichloromethane for three times. The solvent was removed by rotary evaporator and yellowish solids were obtained by crystallization in the solution of 10% dichloromethane/50% n-hexane. The data were listed as follows:

[(η5-Cp)Ir(L1)]Cl (1A): Yield: 65.4%.1H NMR (500 MHz, DMSO-d6) δ 7.83–7.78 (m, 2H, imidazole-H), 7.73 (d, J = 7.9 Hz, 1H, NCH), 7.53–7.40 (m, 8H, Ar-H), 7.32 (d, J = 2.1 Hz, 1H, Ar-H), 7.24 (dd, J = 5.1, 1.7 Hz, 3H, Ar-H), 7.20 (d, J = 7.4 Hz, 2H, Ar-H), 6.00 (d, J = 16.6 Hz, 1H, NCH2C), 5.06 (d, J = 16.6 Hz, 1H, NCH2C), 4.08 (s, 3H, NCH3), 1.44 (s, 15H, Cp*-H).13C NMR (126 MHz, DMSO) δ 154.03 (C-1), 149.42 (NCN), 140.79, 139.79, 138.37, 135.27, 130.09, 129.13, 128.72, 128.53, 127.75, 124.76, 124.37, 123.73, 123.27, 119.18, 112.71, 90.97, 64.96, 45.04, 31.87, 9.45 ppm. Elemental analysis: Found: C, 54.13; H, 4.79; N, 7.24%. Calculated for C33H37ClIrN4: C, 54.12; H, 4.80; N, 7.21%. ESI-MS (m/z): [IrCl(C33H37ClIrN4)+] = 741.01.

[(η5-Cp)Ir(L2)]Cl (1B): Yield: 63.7%.1H NMR (500 MHz, DMSO-d6) δ 8.25 (d, J = 8.4 Hz, 1H, Ar-H), 7.84 (d, J = 8.3 Hz, 1H, Ar-H), 7.76–7.64 (m, 7H, Ar-H), 7.59 (d, J = 8.2 Hz, 1H, Ar-H), 7.56–7.36 (m, 6H, Ar-H), 7.32 (t, J = 7.6 Hz, 1H, Ar-H), 7.22–7.10 (m, 3H, Ar-H), 6.97–6.90 (m, 2H, Ar-H), 6.32 (d, J = 17.0 Hz, 1H, NCH3), 5.80 (d, J = 15.7 Hz, 1H, NCH2), 5.42 (d, J = 15.8 Hz, 1H, NCH2C), 5.35 (d, J = 17.0 Hz, 1H, NCH2C), 4.22 (s, 3H, NCH3), 1.90 (s, 2H, Cp*-H), 1.83 (d, J = 11.3 Hz, 3H, Cp*-H), 1.74 (s, 3H, Cp*-H), 1.70 (d, J = 9.8 Hz, 4H, Cp*-H).13C NMR (126 MHz, DMSO) δ 167.58 (C-1), 148.68 (NCN), 138.49, 136.40, 135.28, 135.09, 133.85, 128.67, 128.03, 124.81, 123.83, 119.19, 112.84, 112.59, 92.56, 91.70, 52.30, 42.04, 32.08, 31.43, 22.54, 14.45, 9.39 ppm. Elemental analysis: Found: C, 59.41; H, 4.66; N, 6.29%. Calculated for C44H8Cl2IrN4: C, 59.45; H, 4.65; N, 6.30%. ESI-MS (m/z): [IrCl(C44H8Cl2IrN4)+] = 853.26.

[(η5-Cp)Ir(L3)]Cl (1C): Yield: 69.0%.1H NMR (500 MHz, DMSO-d6) δ 8.31 (d, J = 8.5 Hz, 1H, Ar-H), 7.86 (d, J = 8.2 Hz, 1H, NCH), 7.81 (d, J = 7.2 Hz, 2H, Ar-H), 7.73 (dd, J = 13.9, 6.8 Hz, 6H, Ar-H), 7.54 (t, J = 7.7 Hz, 2H, Ar-H).4.73 (ddt, J = 28.1, 12.8, 7.4 Hz, 5H, Ar-H), 7.27 (t, J = 7.4 Hz, 1H, Ar-H), 7.18 (dd, J = 8.8, 4.6 Hz, 1H, Ar-H), 7.14 (d, J = 4.3 Hz, 3H, Ar-H), 7.05 (dd, J = 15.6, 7.7 Hz, 3H, Ar-H), 6.58 (dd, J = 8.0, 4.2 Hz, 3H, Ar-H), 6.32 (d, J = 16.9 Hz, 1H, NCH2C), 5.28 (d, J = 16.8 Hz, 1H, NCH2C), 4.24 (s, 3H, NCH3), 1.89 (s, 3H, Cp*-H), 1.69 (s, 3H, Cp*-H), 1.39 (s, 3H, Cp*-H), 1.33 (s, 3H, Cp*-H).13C NMR (126 MHz, DMSO) δ 165.72 (C-1), 149.11 (NCN), 140.46, 139.88, 138.92, 138.21, 136.57, 135.55, 135.16, 134.01, 131.11, 130.74, 129.66, 129.26, 128.90–128.18, 127.99, 127.65, 127.45, 127.15, 124.86, 123.87, 119.26, 114.68, 113.22, 112.79, 103.22, 98.12, 97.38, 85.56, 65.56, 63.28, 42.16, 32.28, 31.43, 22.54, 11.11, 9.81 ppm. Elemental analysis: Found: C, 62.24; H, 4.69; N, 5.84%. Calculated for C50H45Cl2IrN4: C, 62.23.
H, 4.70; N, 5.81%. ESI-MS (m/z): calc for C_{50}H_{45}ClIrN_{4}: 929.24 [M-Cl]^{-}; Found: 929.40.

**DATA AVAILABILITY STATEMENT**

All datasets generated for this study are included in the article/Supplementary Material.

**AUTHOR CONTRIBUTIONS**

XL, YH, and XG conceived the research and conducted the experiments. ZL directed the project and co-wrote the paper.

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**SUPPLEMENTARY MATERIAL**

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fchem.2020.00182/full#supplementary-material
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Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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