29th Symposium on Chemistry Postgraduate Research in Hong Kong

Photofunctional Cyclometalated Iridium(III) Polypyridine Methylsulfone Complexes as Sulfhydryl-Specific Reagents for Bioconjugation, Bioimaging, and Photocytotoxic Applications

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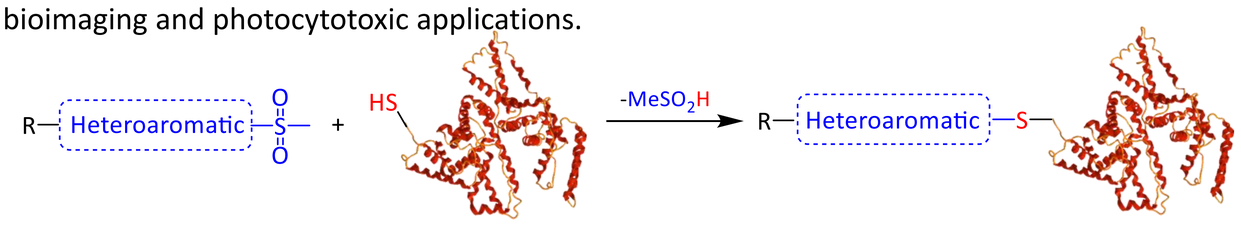
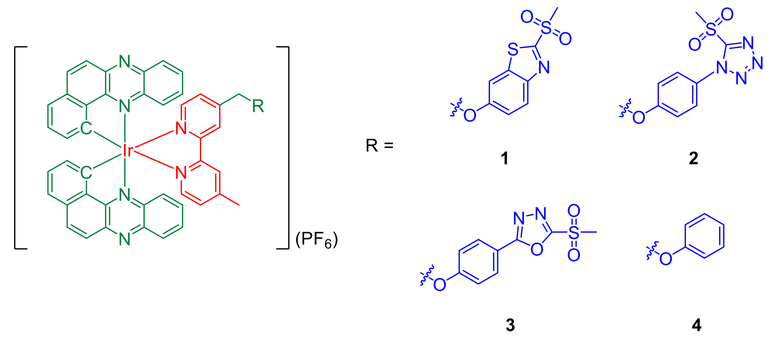
Introduction

Heteroaromatic methylsulfones have emerged as a new class of electrophiles for selective sulfhydryl modification via nucleophilic aromatic substitution, forming a heteroaryl–thiol linkage in proteins. In this work, we report near-infrared (NIR)-emitting cyclometalated iridium(III) complexes bearing a heteroaromatic methylsulfone moiety as sulfhydryl-specific reagents. One of the complexes was conjugated to cysteine and cysteine-containing peptides and proteins for bioimaging and photocytotoxic applications.



Stuctures of Iridium(III) Methylsulfone Complexes



The ligand benzo[*a*]phenazine (Hbpz) with a high degree of π-conjugation was selected as a cyclometalating ligand to tune the emission of the complexes to the NIR region.

Reactivity and Stability of the Complexes

Reaction kinetics of the three methylsulfone complexes toward L-cysteine:

3 > **2** > **1**

Stability of the complexes in aqueous solution:

1 ~ **2** >> **3**

Complex **2** displayed a delicate balance between the reactivity and stability.

Photophysical Properties of Complex 2

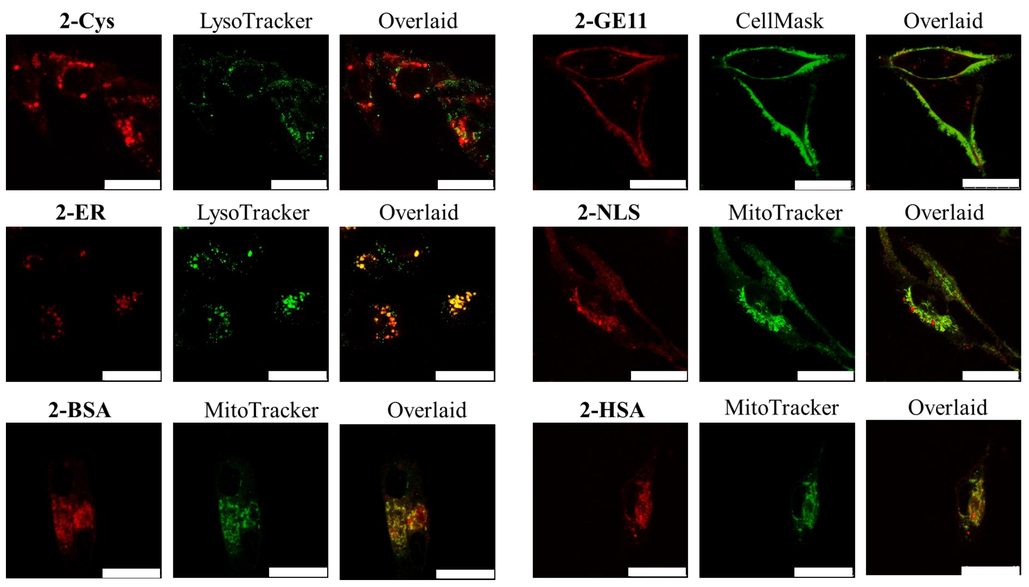
Medium (*T*/K)	λ_{em}/nm	τ_o/μs	Φ_{em}	$\Phi_\Delta{}^a$
CH_2Cl_2 (298)	664	5.63	0.085	0.79
CH_3CN (298)	668	3.26	0.044	-
Glass (77)	663, 726 sh	8.30	-	-

[a] $[Ru(bpy)_3]Cl_2$ in aerated MeOH was used as a reference (Φ_Δ = 0.73)

Predominant ^{3}IL ($\pi \rightarrow \pi^*$) (bpz) emissive state

Confocal Microscopy Images

Complex **2** was selected as the model complex to react with L-cysteine, cysteine-modified epidermal growth factor receptor (EGFR)-targeting peptide, endoplasmic reticulum-targeting peptide, and nuclear localization sequence, cysteine-containing proteins bovine serum albumin (BSA) and human serum albumin (HSA) to afford conjugates **2-Cys**, **2-GE11**, **2-ER**, **2-NLS**, **2-BSA** and **2-GE11**, respectively, for cellular studies.



Confocal images of MDA-MB-231 cells incubated with **2-Cys**, **2-GE11**, **2-ER**, **2-NLS**, **2-BSA**, and **2-HSA** (10 μM, 4h) and then with an organelle stain (LysoTracker Green, 100 nM, 20 min; MitoTracker Green, 100 nM, 20 min; CellMask Deep Red, 5 μg mL^{-1}, 10 min) at 37°C. Scale bar = 25 μm.

(Photo)cytotoxicity

	MDA-MB-231			A431			HEK-293T		
Compound	$IC_{50,dark}$/μM	$IC_{50,dark}$/μM	PI	$IC_{50,dark}$/μM	$IC_{50,dark}$/μM	PI	$IC_{50,dark}$/μM	$IC_{50,dark}$/μM	PI
2-Cys	> 20	0.07 ± 0.01	> 270	> 20	0.12 ± 0.01	> 166	> 20	0.44 ± 0.10	> 46
2-GE11	> 20	1.01 ± 0.08	> 20	> 20	0.49 ± 0.01	> 40	> 20	10.09 ± 1.85	> 2
2-ER	> 20	0.52 ± 0.07	> 39	> 20	0.25 ± 0.01	> 82	> 20	3.08 ± 0.10	> 6
2-NLS	> 20	0.11 ± 0.01	> 187	> 20	0.13 ± 0.01	> 156	> 20	1.27 ± 0.12	> 16
2-BSA	46.21 ± 3.74	0.28 ± 0.06	168	28.36 ± 3.40	0.17 ± 0.01	169	49.65 ± 6.04	0.24 ± 0.06	204
2-HSA	53.05 ± 2.99	0.44 ± 0.09	120	24.91 ± 0.20	0.13 ± 0.01	186	74.90 ± 3.29	0.43 ± 0.07	174
4	0.69 ± 0.05	0.02 ± 0.005	41	0.49 ± 0.04	0.005 ± 0.001	98	0.94 ± 0.12	0.02 ± 0.008	47

(Photo)cytotoxicity (IC_{50}) of the conjugates of complex **2** and the methylsulfone-free complex **4** toward MDA-MB-231, A431, and HEK-293T cells. The cells were first incubated with the complexes in the dark for 4 h, then washed thoroughly with PBS, incubated in the dark or irradiated at 450 nm (10 mW cm^{-2}) for 20 min, and subsequently incubated in the dark for 24 h. Photocytotoxicity index (PI) = $IC_{50,dark}/IC_{50,light}$.

All the conjugates displayed low cytotoxicity toward the three cell lines in the dark. However, the cytotoxicity was substantially enhanced upon light irradiation. Higher photocytotoxicity of the conjugates toward the cancer cells (MDA-MB-231 and A431) than the normal cells (HEK-293T) was observed.

Conclusions

In conclusion, three NIR-emissive iridium(III) polypyridine complexes bearing a heteroaromatic methylsulfone moiety were designed, synthesized, and characterized. Their methylsulfone-free counterpart was also prepared for comparison studies. Complex **2** with a delicate balance of high reactivity and stability, was selected as the model complex for the bioconjugation studies. The modification of L-cysteine and cysteine-bearing peptides or proteins with complex **2** afforded conjugates that exhibited rich photophysical properties. The resulting conjugates displayed different intracellular localization, and (photo)cytotoxic properties.

Acknowledgement

We thank the Hong Kong Research Grants Council (Project No. CityU 11300019, CityU 11302820, CityU 11301121, T42-103/16-N, C7075-21GF and N_CityU104/21) for financial support. We also thank the funding support from "Laboratory for Synthetic Chemistry and Chemical Biology" under the Health@InnoHK Program launched by Innovation and Technology Commission, The Government of Hong Kong SAR, P. R. China.

Reference

Huang, L.; Leung, P. K.-K.; Lee, L. C.-C.; Xu, X.-X.; Lam, Y.-W.; Lo, K. K.-W. *Chem. Commun.* **2022**, *58,* 10162 – 10165.