29th Symposium on Chemistry Postgraduate Research in Hong Kong

Bioorthogonal Control of the Phosphorescence and Singlet Oxygen Photosensitization Properties of Iridium(III) Tetrazine Complexes

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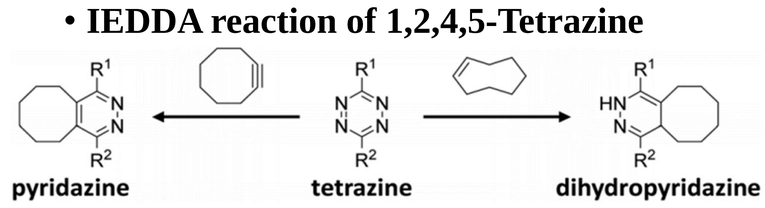
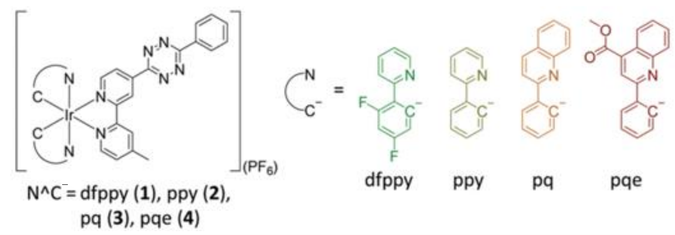
Introduction

Fluorogenic probes are indispensable tools for the detection and visualization of biomolecules and their biological processes. They display intense emission only upon reaction with their targets, enabling highly sensitive detection without the requirement of stringent washing. 1,2,4,5-Tetrazine, a commonly used bioorthogonal functionality, is a fluorescence quencher that undergoes IEDDA reaction with dienophiles including strained alkynes and alkenes to give pyridazine and dihydropyridazine derivatives, respectively. Despite the structural similarity between the pyridazine and dihydropyridazine products, the overall emission enhancement can be very different since the dihydropyridazine unit is known to suppress the fluorescence of the products. Herein, we present a new family of iridium(III) polypyridine complexes conjugated with a tetrazine unit $[Ir(N^C)_2(bpy\text{-}Tz\text{-}Ph)](PF_6)$ (HN^C = Hdfppy (**1**), Hppy (**2**), Hpq (**3**) and Hpqe (**4**)) as bioorthogonal phosphorogenic probes and $^1O_2$ photosensitizers.

- **IEDDA reaction of 1,2,4,5-Tetrazine**



- **Structure of complexes**



N^C = dfppy (**1**), ppy (**2**), pq (**3**), pqe (**4**)

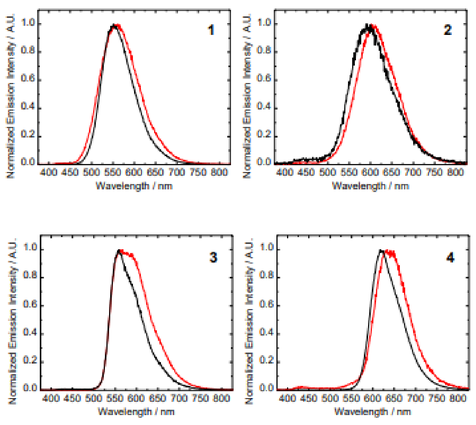
Photophysical Properties

- **Photophysical data**

Complex	Medium (T/K)	λ_{em}/nm	Φ_{em}	τ_o/μs
1	CH_2Cl_2 (298)	551	0.0142	1.03
	CH_3CN (298)	557	0.0074	0.61
	Buffer (298)[a]	556	0.0049	0.49
	Glass (77)[b]	486 (max), 528, 565 sh		
2	CH_2Cl_2 (298)	594	0.0044	0.41
	CH_3CN (298)	606	0.0017	0.18
	Buffer (298)[a]	601	0.0008	0.16
	Glass (77)[b]	548, 589 sh		
3	CH_2Cl_2 (298)	558, 599 sh	0.0111	1.16
	CH_3CN (298)	561, 596 sh	0.0077	1.06
	Buffer (298)[a]	561, 596 sh	0.0062	0.98
	Glass (77)[b]	543 (max), 584, 638 sh		
4	CH_2Cl_2 (298)	619	0.0202	1.33
	CH_3CN (298)	637	0.0033	0.24
	Buffer (298)[a]	647	0.0013	0.15
	Glass (77)[b]	593, 643 sh		

[a] PBS (1X, pH 7.4)/CH_3CN (1:1, *v*/*v*). [b] EtOH/MeOH (4:1, *v*/*v*).

- **Normalized emission spectra**



Complexes **1**, **2**, and **4**: ^{3}MLCT mixed with some ^{3}LLCT character.

Complex **3**: ^{3}IL.

Efficient emission quenching of the complexes by both FRET and PET.

Reactivity and Phosphorogenic Responses

- **Second-order rate constants (k_2)**

Compound	$k_2/M^{-1}\ s^{-1}$	
	BCN-OH	**TCO-OH**
1	23.8 ± 0.6	676.8 ± 18.5
2	20.9 ± 0.4	634.6 ± 7.4
3	74.2 ± 2.0	1313.1 ± 34.7
4	83.1 ± 2.4	1471.3 ± 39.6
bpy-Tz-Ph	29.1 ± 0.9	188.0 ± 5.6

- **Emission enhancement factors (I/I_o) and lifetimes (τ)**

Complex	BCN-OH		TCO-OH		BCN-BSA		TCO-BSA		BSA	
	I/I_o[a]	τ/μs	I/I_o[a]	τ/μs	I/I_o[a]	τ/μs	I/I_o[a]	τ/μs	I/I_o[a]	τ/μs[b]
1	97.7	0.33	2.1	0.28	254.8	0.51	1.9	0.22	1.2	N.D.
2	105.3	0.05	1.8	0.04	383.3	0.37	4.3	0.11	1.4	N.D.
3	88.7	0.12	5.6	0.04	291.6	1.08	6.4	0.25	0.9	N.D.
4	51.7	0.19	13.5	0.05	76.4	0.29	17.5	0.22	2.0	N.D.

[a] I_o and I are the emission intensities of the complexes in the absence and presence of the dienophiles or proteins, respectively.

[b] The emission lifetimes were too short to be determined accurately.

Incubation with BCN-OH and BCN-BSA shows substantial emission enhancement and lifetime extension while TCO-OH and TCO-BSA gave much smaller changes.

Singlet Oxygen Generation

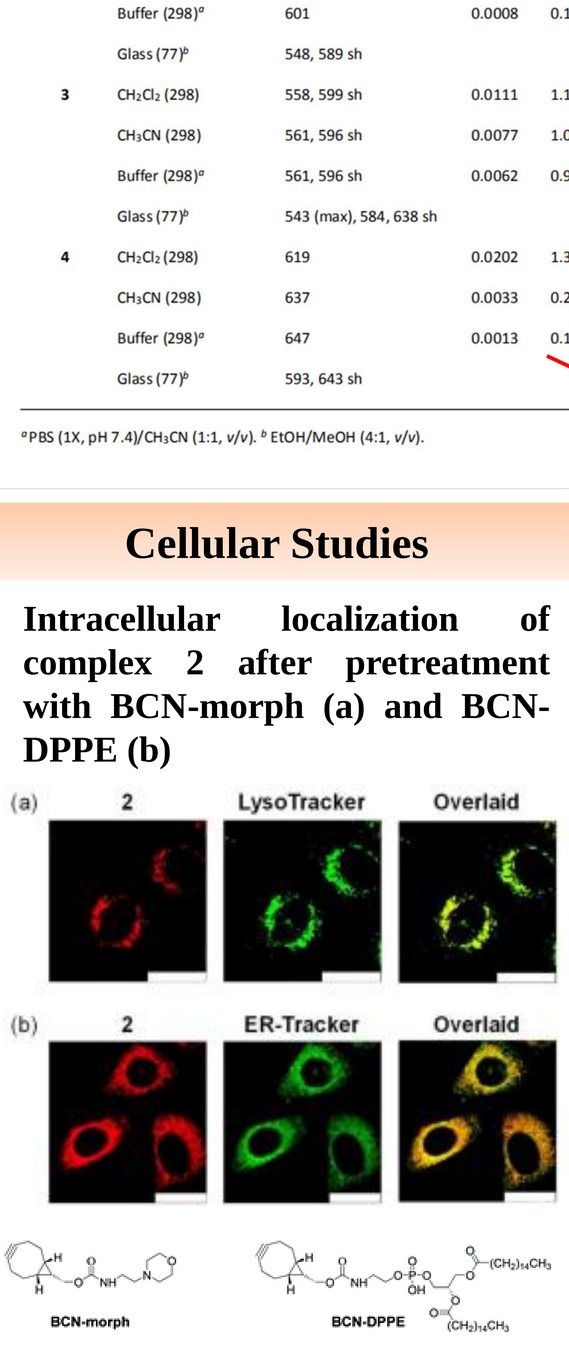
- **$^1O_2$ generation quantum yields**

Complex	Φ_Δ[a]	Φ_Δ[b]
1	0.82	0.42
1a	0.76	0.32
1b	0.36	0.12
2	0.63	0.43
2a	0.46	0.31
2b	0.27	0.23
3	0.66	0.66
3a	0.31	0.47
3b	0.23	0.33
4	0.61	0.73
4a	0.56	0.65
4b	0.26	0.34

The $^1O_2$ photosensitization of the iridium(III) tetrazine complexes can be manipulated by different bioorthogonal reaction partners.

Cellular Studies

- **Intracellular localization of complex 2 after pretreatment with BCN-morph (a) and BCN-DPPE (b)**

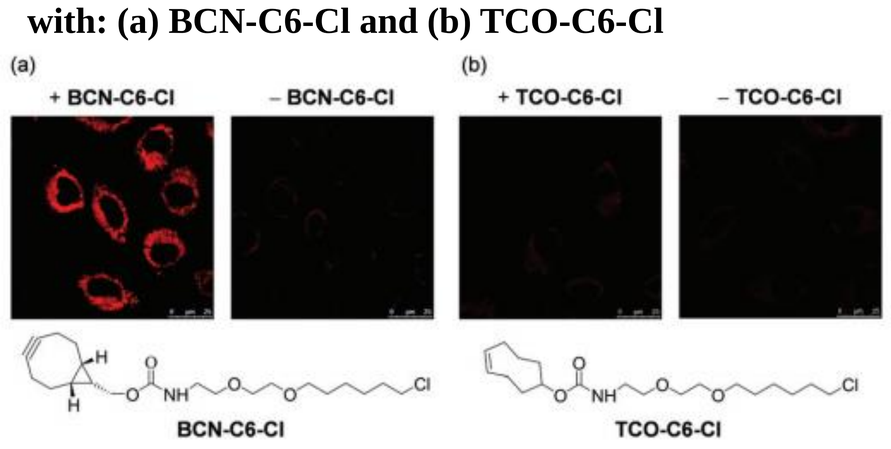


High Pearson's correlation coefficients.

BCN-morph: 0.88

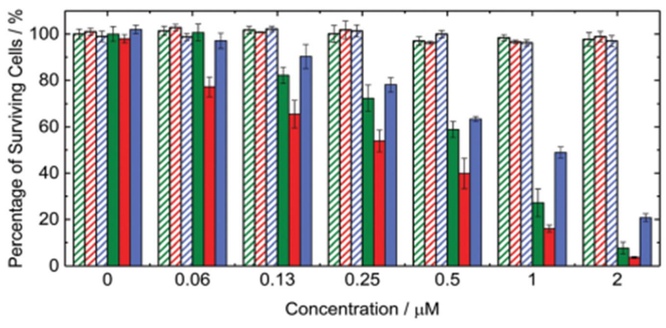
BCN-DPPE: 0.91

- **Transfected HeLa cells with and without pretreatment with: (a) BCN-C6-Cl and (b) TCO-C6-Cl**



Transfected cells pretreated with **BCN-C6-Cl**: Significant emission intensity upon incubation with complex **2**.

- **Dark cytotoxicity (shaded) and photocytotoxicity (solid)**



Enhancement of the photocytotoxicity of complex **2** by **BCN-C6-Cl**.

- **Cellular uptake**

Complex	Amount of iridium/fmol[a]		
	Complex only	+ **BCN-C6-Cl**[b]	+ **TCO-C6-Cl**[c]
1	0.489 ± 0.006	0.645 ± 0.012	0.641 ± 0.012
2	0.716 ± 0.010	1.159 ± 0.010	1.078 ± 0.006
3	0.742 ± 0.010	1.533 ± 0.007	1.406 ± 0.017
4	0.897 ± 0.007	1.976 ± 0.024	1.963 ± 0.025

[a] Amount of iridium of complexes **1** – **4** associated with an average HeLa cell upon incubation with the complexes (10 μM) at 37 °C for 1 h, as determined by ICP-MS. [b] Pretreatment of **BCN-C6-Cl** (50 μM) for 1 h, followed by washing with PBS (1 mL × 3). [c] Pretreatment of **TCO-C6-Cl** (50 μM) for 1 h, followed by washing with PBS (1 mL × 3).

Transfected cells pretreated with **BCN-C6-Cl** and **TCO-C6-Cl**: Higher accumulation of the iridium(III) tetrazine complexes.

Conclusion

New iridium(III) tetrazine complexes were designed and shown to exhibit different phosphorogenic responses and photosensitization properties upon bioorthogonal reactions with strained alkynes and alkenes. With a judicious design of bioorthogonal substrates, the photophysical, photochemical, cellular uptake and (photo)cytotoxicity of transition metal complexes can be further exploited.

Acknowledgements

We acknowledge the funding support from "Laboratory for Synthetic Chemistry and Chemical Biology" under the Health@InnoHK Programme launched by Innovation and Technology Commission, The Government of Hong Kong SAR, P. R. China. We also thank the Hong Kong Research Grants Council (Project No. CityU 11302820 CityU 11300019, CityU 11300318, CityU 11300017 and T42-103/16-N) for financial support.

Reference

Leung, P. K.-K.; Lee, L. C.-C.; Yeung, H. H-Y.; Io, K-W.; Lo, K. K.-W. *Chem. Commun.* **2021**, *57*, 4914 – 4917.