Anticancer Gold (III) Pincer Complexes Containing N-Heterocyclic Carbene Ligands

Wong Kwan Yuen

Supervised by Professor Chi-Ming Che

Department of Chemistry, Faculty of Science, University of Hong Kong

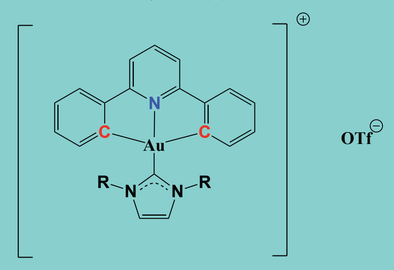
Name: Wong Kwan Yuen
University No.: 3035606499
Student's Major: Chemistry (Intensive)
Poster No.: B6

Research Colloquium for
Science UG Students 2021-22

Introduction

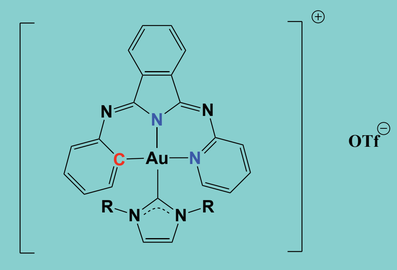
Cyclometalated gold(III) complexes are a promising class of anti-cancer agents displaying potent in vitro and in vivo activities[1][2][3][4]. In recent years, Che and colleagues demonstrated that $[(C\hat{}N\hat{}C)Au^{III}(NHC)]^+$ (CH^N^CH = 2,6-diphenylpyridine; NHC = N-heterocyclic carbene) **A** has a distinct pincer cyclometalated metal–NHC scaffold that can engage multiple molecular targets for combating drug-resistant cancer[2][3][4]. It is conceived that modification of the three-dimensional scaffold can provide new directions in metal-based anti-cancer agents. In this work, a series of $[(C\hat{}N\hat{}N)Au^{III}(NHC)]^+$ complexes (HC^HN^N = (1-phenylimino-3-pyridylimino)isoindoline) **B**, **C**, **D** were proposed, featuring a fused 6,-6 membered metallacycle with an extended pincer structure. Amidst the novel complexes, those with lipophilic NHC modification was found to offer heightened anticancer activity and cellular uptake. Results of thermal proteome profiling suggested that the change in pincer structure led to alternative cellular targets.

Previously Reported



R = *n*-Bu (**A**)

Newly Proposed



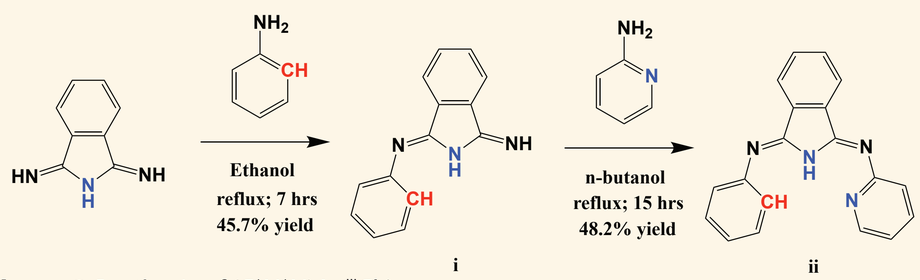
R = Me (**B**)
n-Bu (**C**)
Bz (**D**)

Fig. 1 Molecular structures of the cyclometalated gold complexes.

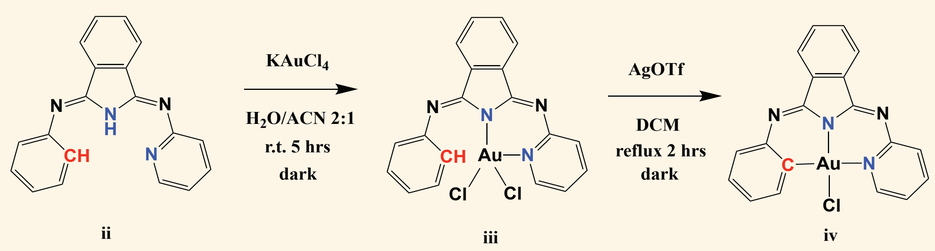
Synthesis

The $[(C\hat{}N\hat{}N)Au^{III}(NHC)]OTf$ complexes **B**, **C**, **D** were prepared from precursors **i**-**v** according to **Scheme 1** to **3**. The complexes were characterized by ^1H NMR, ^{13}C NMR, ^{19}F NMR and ESI-MS spectroscopies. $[KAu^{III}Cl_4]$, $[Ag^{I}CF_3SO_3]$, 1,3-diiminoisoindoline and 2-aminopyridine were received without further purification. Aniline was distillated before use. Imidazolium salt were prepared according to the literatures[2][3][4][5]. Generally, they were synthesized by reacting imidazole with the corresponding alkyl/benzyl bromide in THF at 100˚C in basic condition. Asymmetrical 1-phenylimino-3-pyridyliminoisoindoline HC^HN^N ligand **ii** was synthesized according to **Scheme 1**[6]. $(C\hat{}N\hat{}N)Au^{III}Cl$ iv was synthesized according to **Scheme 2**. $[(C\hat{}N\hat{}N)Au^{III}(NHC)]CF_3SO_3$ **B**, **C**, **D** was synthesized according to **Scheme 3**. The previously proposed $[(C\hat{}N\hat{}C)Au^{III}(NHC)]CF_3SO_3$ **A** was synthesized according to the literature[7].

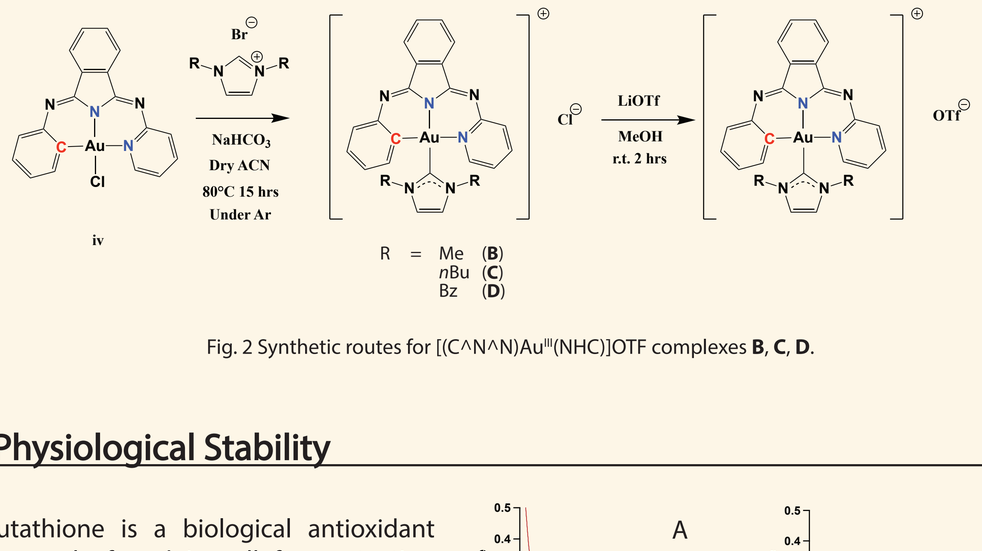
Scheme 1 Synthesis of asymmetrical (1-phenylimino-3-pyridylimino)isoindoline HC^HN^N ligand **ii**.



Scheme 2 Synthesis of $(C\hat{}N\hat{}N)Au^{III}Cl$ **iv**.



Scheme 3 Synthesis of $[(C\hat{}N\hat{}N)Au^{III}(NHC)]CF_3SO_3$ **B**, **C**, **D**.



R = Me (**B**)
*n*Bu (**C**)
Bz (**D**)

Fig. 2 Synthetic routes for $[(C\hat{}N\hat{}N)Au^{III}(NHC)]OTF$ complexes **B**, **C**, **D**.

Physiological Stability

Glutathione is a biological antioxidant commonly found in cell for protection against oxidation of cellular components[8]. It was reported that in some drug resistance cancers, the GSH level was upregulated to generate a reducing environment. This could lead to deactivation of metal-based complex and disrupt drug activity[9]. To ensure the high valent gold(III) complexes can remain intact in the cellular environment and engage with its intended targets, the complexes are treated with 100-fold excess GSH and monitored by UV-vis absorption.

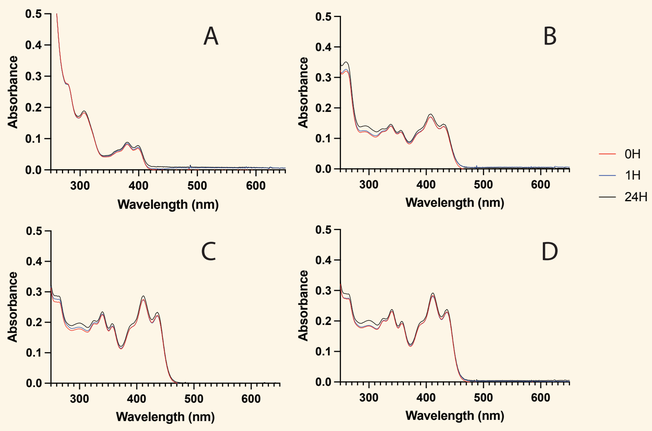


Fig. 3 UV–vis absorption spectra of complexes (20μM) in PBS:DMSO (95:5, v/v) with 100-fold excess GSH (2 mM) at 37 °C in 0h, 1h and 24h.

All complexes demonstrated no significant change absorption spectrum after 24 hours, indicating no reactions between GSH and the complexes.

Anticancer Activity

In vitro Cytotoxicity

To determine their *in vitro* anticancer activity, the cyclometalated gold complexes (**A**-**D**) together with 2 FDA approved anticancer drugs (**E**: cisplatin and **F**: sorafenib) were treated to 4 human cancer cell lines, including lung carcinoma (NCI-H460), hepatocellular carcinoma (MHCC97-L, HepG2), cervical epitheliod carcinoma (LO2) and 1 normal cell derived lung fibroblast cell line (CCD19-Lu). The 24h, 48h and 72h IC_{50} values were determined by the NBB assay.

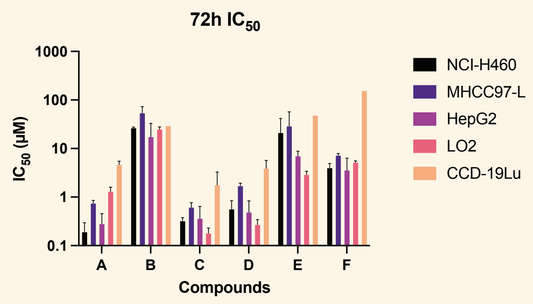


Fig. 4 The 72 hours IC_{50} values of the cyclometalated gold complexes A-D and 2 FDA approved anticancer drugs

Complex **A**, **C** and **D** were showing at least 10-fold higher toxicity than the FDA approved cisplatin and sorafenib activity across the cancer cell lines. They were also slightly less toxic in the normal fibroblast, indicating selectivity. Complex B with a less lipophilic NHC demonstrated lower toxicity. The newly proposed complexes did not outperform the previous one in terms of toxicity nor selectivity.

Lipophilicity and Cellular Uptake

The drastic difference in the IC_{50} values between **B**, **C** and **D** could be accounted by its cellular uptake. The time dependent and concentration dependent Au uptake in NCI-H460 was determined by ICP-MS. **C** and **D** was showing significantly higher uptake compared to **B**.

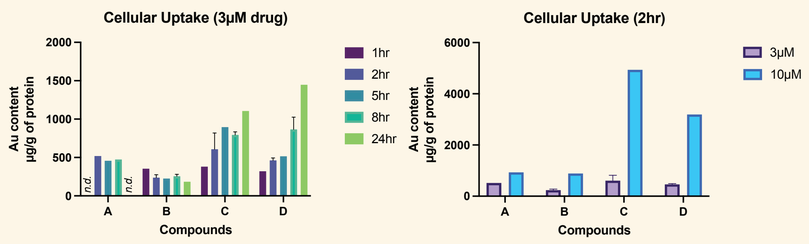


Fig. 5 The time-dependent (left) and concentration-dependent (right) cellular uptake of the cyclometalated gold complexes **A**-**D** in NCI-H460.

These findings can be attributed to the lipophilicity as lipophilic compounds tend to penetrate through the lipid bilayer membrane more readily. The lipophilicity (log P) of **B**, **C** and **D** were determined to be -0.18, 0.53 and 0.71 respectively, which increases reasonably with the carbon chain length on the NHC. The lipophilicity aligns with the trend of the cellular uptake.

Target Identification

To identify the cellular targets of these complexes, thermal proteome profiling was performed for compound **C** in the lung cancer cell line NCI-H460. 123 proteins were identified with increase in melting temperature greater than 1.5˚C and 42 proteins with decrease in melting temperature less than -1.5˚C.

Given the structural similarity between **A** and **C**, their molecular targets were compared. Fung[7] has identified several targets of compound A by photo-affinity probe and molecular docking model. Two of them (PRDX1: +4.78˚C; HSP60: -7.14˚C) had change in melting temperature upon treatment of **C**. The other targets of **A** were not identified. The significant drop in melting temperature of HSP60 indicated that **C** might induce its anticancer activity by engaging with alternative cellular targets.

Pathway analysis of the 165 proteins with change in melting temperature was performed by Cytoscape[10]. Among the pathways with FDR < 0.005 (Table 1), ESR-mediated signaling was reported to be associated with the promotion and growth of breast cancer[11]. Inhibition of estrogen would deprive the cancer from supporting hormones and lead to regression.

Pathway	FDR
Processing of Capped Intron-Containing Pre-mRNA(R)	2.54E-10
Spliceosome(K)	4.66E-08
tRNA Aminoacylation(R)	6.56E-07
Selenoamino acid metabolism(R)	1.12E-03
Aminoacyl-tRNA biosynthesis(K)	1.39E-03
HSP90 chaperone cycle for steroid hormone receptors (SHR) in the presence of ligand(R)	1.45E-03
ESR-mediated signaling(R)	3.05E-03

Table 1 Pathways analysis of the 165 proteins with change in melting temperature. Selected pathways with FDR < 0.005

6 proteins involved in the ESR-mediated signaling were found to have significant change in melting temperature upon treatment of **C**. They were KPNA2 (+2.06˚C), CBFB (+2.05˚C), POLR2C (+3.66˚C), POLR2E (+3.20˚C), PTGES3 (+3.74˚C) and CARM1 (-3.13˚C). The identity and importance of these proteins will be further investigated.

Conclusion

In conclusion, the newly proposed structure did not significantly improve the *in vitro* toxicity, selectivity nor uptake on cancer cells. However, thermal proteome profiling revealed that they might share different targets despite having high structural similarity. This could provide insight in alternative targets for future anticancer agents.

Reference

[1] Tong, K., Lok, C., Wan, P., Hu, D., Fung, Y., & Chang, X. et al. (2020). An anticancer gold(III)-activated porphyrin scaffold that covalently modifies protein cysteine thiols. Proceedings Of The National Academy Of Sciences, 117(3), 1321-1329. doi: 10.1073/pnas.1915202117
[2] Fung, S., Zou, T., Cao, B., Lee, P., Fung, Y., & Hu, D. et al. (2017). Cyclometalated Gold(III) Complexes Containing N-Heterocyclic Carbene Ligands Engage Multiple Anti-Cancer Molecular Targets. Angewandte Chemie, 129(14), 3950-3954. doi: 10.1002/ange.201612583
[3] Tong, K., Hu, D., Wan, P., Lok, C., & Che, C. (2020). Anticancer Gold(III) Compounds With Porphyrin or N-heterocyclic Carbene Ligands. Frontiers In Chemistry, 8. doi: 10.3389/fchem.2020.587207
[4] Yan, J., Chow, A., Leung, C., Sun, R., Ma, D., & Che, C. (2010). Cyclometalated gold(iii) complexes with N-heterocyclic carbene ligands as topoisomerase I poisons. Chemical Communications, 46(22), 3893. doi: 10.1039/c001216e
[5] Wai-Yin Sun, R., Lok-Fung Chow, A., Li, X., Yan, J., Sin-Yin Chui, S., & Che, C. (2011). Luminescent cyclometalated platinum(ii) complexes containing N-heterocyclic carbene ligands with potent in vitro and in vivo anti-cancer properties accumulate in cytoplasmic structures of cancer cells. Chemical Science, 2(4), 728. https://doi.org/10.1039/c0sc00593b
[6] Bröring, M., Kleeberg, C., & Köhler, S. (2008). Palladium(II) Complexes of UnsymmetricalCNNPincer Ligands. Inorganic Chemistry, 47(14), 6404-6412. https://doi.org/10.1021/ic800507k
[7] Fung, S. K. [馮善琪]. (2017). Anti-cancer N-heterocyclic carbene complexes of platinum(II) and gold(III) : luminescence probes for mismatched DNA, target(s) identification and anti-angiogenesis agents. (Thesis). University of Hong Kong, Pokfulam, Hong Kong SAR.
[8] Noctor, G., Queval, G., Mhamdi, A., Chaouch, S., & Foyer, C. H. (2011). Glutathione. The Arabidopsis Book/American Society of Plant Biologists, 9.
[9] Greenwood, H. E., McCormick, P. N., Gendron, T., Glaser, M., Pereira, R., Maddocks, O. D., ... & Witney, T. H. (2019). Measurement of tumor antioxidant capacity and prediction of chemotherapy resistance in preclinical models of ovarian cancer by positron emission tomography. Clinical Cancer Research, 25(8), 2471-2482.
[10] Marc Gillespie, Bijay Jassal, Ralf Stephan, Marija Milacic, Karen Rothfels, Andrea Senff-Ribeiro, Johannes Griss, Cristoffer Sevilla, Lisa Matthews, Chuqiao Gong, Chuan Deng, Thawfeek Varusai, Eliot Ragueneau, Yusra Haider, Bruce May, Veronica Shamovsky, Joel Weiser, Timothy Brunson, Nasim Sanati, Liam Beckman, Xiang Shao, Antonio Fabregat, Konstantinos Sidiropoulos, Julieth Murillo, Guilherme Viteri, Justin Cook, Solomon Shorser, Gary Bader, Emek Demir, Chris Sander, Robin Haw, Guanming Wu, Lincoln Stein, Henning Hermjakob, Peter D'Eustachio, The reactome pathway knowledgebase 2022, Nucleic Acids Research, 2021;, gkab1028, doi: 10.1093/nar/gkab1028
[11] Haldosén, L. A., Zhao, C., & Dahlman-Wright, K. (2014). Estrogen receptor beta in breast cancer. Molecular and cellular endocrinology, 382(1), 665-672.