GERMANY/HONG KONG JOINT RESEARCH SCHEME
THE PROJECT REPORT
(for Project Completion)

Project Number: G_HK025/12

Title

Medicinal Chemistry of Gold Complexes
金類配合物的醫藥化學

Particulars

	Hong Kong team	German team
Name of Project Co-ordinator (with title)	Prof. Chi-Ming CHE	Prof. Ingo OTT
Name of Co-Investigator (if any)	Dr. Raymond Wai-Yin SUN	Dr. Riccardo RUBBIANI Dr. Riccardo was initially planned as a team member. However, at the beginning of the project he was awarded as postdoc fellowship and had to leave the group to join Univ. Zurich.
Institution or Institutional affiliation	[ ] CityU [√] HKU [ ] CUHK [ ] HKUST [ ] HKBU [ ] LU [ ] HKIEd [ ] PolyU	[ ] Technische Universität Braunschweig [ ] Others: _____
Other project team members (if any)	Dr. Jingjing Zhang Dr. Taotao Zou Dr. Annie F.M. Siu Dr. Chun-Nam Lok	Dr. Annika Gross Mr. Vincent Andermark

Funding Period

	1st year	2nd year (if applicable)
Start Date	1 Jan 2013	1 Jan 2014
Completion Date	31 Dec 2013	31 Dec 2014

Objective(s) as per original application

This project aims at the investigation of gold(I) and gold(III) complexes as prospective drug candidates for the treatment of cancer. Gold complexes have been increasingly studied in inorganic medicinal chemistry over the past decade, mainly driven by their intriguing biochemical properties and promising preclinical results. Nevertheless, the molecular target(s) of various classes of anti-cancer active gold complexes have not been fully established. Moreover, structure-activity relationship studies for this type of compounds are limited. The long term goal of this project is to resolve the challenges faced though a multidisciplinary approach involving inorganic and medicinal chemistry, cell biology and animal studies.

Details of Report [Please attach relevant document(s)]

i) Outline of proposed research and results obtained

The research aims to investigate the biochemical mechanisms of anti-cancer gold complexes as well as to elucidate the structure-activity relationships. The gold complexes examined show potent anti-cancer activities with a possible application in cancer chemotherapy. The inhibition of the anticancer molecular target thioredoxin reductase by different types of gold complexes together with their cellular uptake and cytotoxicity. These gold complexes were developed at **HKU** and **Technische Universität Braunschweig (TUBS).** Some of these gold complexes have been examined in animal models and showed promising anti-tumor properties.

During the project duration (**1 Jan 2013 to 31 Dec 2014**), the **PIs Prof. C.M. Che** of HKU and **Prof I. Ott** of **TUBS** have been collaborating on the design of new bioactive gold(I) complexes supported by phosphine and N-heterocyclic carbene ligands. They held regular meetings (four at HKU and one at TUB) to discuss the progress of the projects and the issues of metal compounds in Medicine with the team members from both institutions. Dr. **Raymond W.Y. Sun** (Co-I of the project, currently Professor of Chemistry, Shantou University) helped to supervise two post-graduate students from Prof. Che's laboratory (Miss **Jingjing Zhang** and Mr **Taotao Zou**) and the visitors from TUB in the synthesis and characterization of the gold compounds. He visited TUBS in June 2013 to present initial findings on anticancer activities of the newly synthesized gold compounds.

Miss **Jinging Zhang** paid visit to TUBS in 2013 for comparative studies on the inhibition of thioredoxin reductase by gold compounds developed at HKU and TUB.

Mr. **Taotao Zou** paid visit to TUBS in May 2014 to study thiol interaction and cellular uptake of cytotoxic gold compounds, and to learn techniques for testing anti-angiogenic agents in zebrafish models developed by Prof. Ott's team members. He also helped to organize the visits of Prof. Ott and the TUBS team members to HKU.

** *In the proposed travel plan, the PI Prof. Che planned to accompany his post-graduate student Mr Taotao Zou together to visit **TUBS** in May 2014. However, owing to very stringent time period of travel visa of Mr Taotao Zou (as a student holding PR China visa to Hong Kong) and the very busy several research conferences that Prof Che had to chair and speak in the time May 2014 and also the schedule of Prof. Ott , Professor Che and Mr Zhou had to visit TUBS in separate periods (5-14 & 22-28 May 2014 for Mr. Zou and 4-7 June for Prof. Che) after considering all alternatives.*

Two members from TUBS (Dr. **Annika Gross** and Mr. **Vincent Andermark**) had visited Prof. Che's laboratory to learn animal experiments for testing anticancer compounds and to share their experiences in liposomal formulation of gold compounds.

The two post-graduate students Miss Jingjing Zhang and Mr Taotao Zou and TUB members from Ott's group have benefited from this joint research scheme. Dr. Jingjing Zhang has subsequently been awarded a Humboldt postdoctoral fellowship to work at Prof. Ott's laboratory in Technische Universität Braunschweig. Dr. Taotao Zou has been granted a postdoctoral fellowship to work at HKU to continue medicinal inorganic chemistry. He is also a visiting research fellow working in Prof. Peter J Sadler (FRS) 's laboratory at University of Warwick, UK.

ii) Significance of research results

Gold compounds have been known to exhibit anti-cancer activities in association with inhibition of thiol-containing enzyme activities. The findings on potent inhibition of cellular thioredoxin reductase induction of cancer cell cytotoxicity and *in vivo* anti-tumour activities in mice model of cancer by gold complexes as demonstrated in the current project are indicative of promising application of gold complexes for treatment of cancer.

1. Caffeine derived platinum(II) N-heterocyclic carbene complexes with multiple anti-cancer activities. **J. J. Zhang, C. M. Che, I, Ott.** *J. Organometallic Chemistry.* 2015, *782*, 37.
   Organometallic platinum(II) terpyridine complexes with a caffeine derived *N*-heterocyclic carbene (NHC) ligand were investigated as possible anti-cancer drugs in comparison to analogs, which contain *N*-coordinated theobromine. In general the carbene complexes displayed significantly higher biological activity than the theobromine analogs. In particular, the caffeine derived organometallic platinum(II) complex [Pt^{II}(2,2':6',2"-terpyridine) (1,3,7,9-tetramethylxanthine-8-ylidene)]$(PF_6)_2$ (**1**) displayed very promising cytotoxic activity towards several cancer cell lines, and showed *in vitro* anti-angiogenic effects at sub-cytotoxic concentrations. Moreover, it induced neural differentiation-like morphological changes in breast adenocarcinoma (MCF-7) cells.

2. A binuclear gold(I) complex with mixed bridging diphosphine and bis(N-Heterocyclic Carbene) ligands shows favorable thiol reactivity and effectively inhibits tumor growth and angiogenesis *in vivo*. **T. Zou**, C. T. Lum, C. N. Lok, W. P. To, K. H. Low and **C. M. Che.** *Angewandte Chemie, International Edition*, 2014, 53, 5810
   In the design of anticancer gold(I) complexes with high *in vivo* efficacy, tuning the thiol reactivity to achieve stability towards blood thiols yet maintaining the thiol reactivity to target cellular thioredoxin reductase (TrxR) is of pivotal importance. We have developed a dinuclear gold(I) complex (**1**-PF_6) utilizing a bridging bis(N-heterocyclic carbene) ligand to attain thiol stability and a diphosphine ligand to keep appropriate thiol reactivity. Complex **1**-PF_6 displays a favorable stability that allows it to inhibit TrxR activity without being attacked by blood thiols. *In vivo* studies reveal that **1**-PF_6 significantly inhibits tumor growth in mice bearing HeLa xenograft and mice bearing highly aggressive mouse B16-F10 melanoma. It inhibits angiogenesis in tumor models and inhibits sphere formation of cancer stem cells *in vitro*. Toxicology studies indicate that **1**-PF_6 does not show systemic anaphylaxis on guinea pigs and localized irritation on rabbits.

3. A dinuclear cyclometalated gold(III)-phosphine complex targeting thioredoxin reductase inhibits hepatocellular carcinoma *in vivo*.
   R. W. Sun, C. N. Lok, T. T. H. Fong, C. K. L. Li, Z. F. Yang, **T. Zou**, A. F. M. Siu and **C. M. Che.** *Chemical Science*, 2013, 4, 1979-1988.
   A stable gold(III)–phosphine complex [(C^N^C)$_2$Au$_2$(μ-dppp)](CF$_3$SO$_3$)$_2$ [**Au3**, HC^N^CH =2,6-diphenylpyridine; dppp = bis(diphenylphosphino)propane] displays potent *in vitro* cytotoxicity towards various cancers with sub-micromolar range cytotoxic IC_{50} values, and is significantly more potent than its structural and iso-electronic platinum(II) analog [(C^N^N)$_2$Pt$_2$(μ-dppp)](CF$_3$SO$_3$)$_2$ (HC^N^N = 6-phenyl-2,2′-bipyridine) and gold(III)–carbene complexes. Complex **Au3** displays promising inhibition on tumor growth in animal models, and its acute and sub-chronic toxicities have been examined in mice and beagle dogs. Transcriptomic and connectivity map analyses have revealed that the transcriptional profile of **Au3** is similar to those of inhibitors of thioredoxin reductase (TrxR) and inducers of endoplasmic reticulum (ER) stress. As we found that **Au3** is also a nanomolar inhibitor of TrxR, a model of ER stress-induced cell death mediated by inhibition of TrxR is

proposed. The transcriptomic analysis also leads to the identification of TRAIL, a ligand for death receptor 5 (DR5), as a synergistic agent of the anti-tumor activity of **Au3**. Collectively, our results demonstrate that the gold(III) complex **Au3** effectively inhibits tumor growth *in vivo*, and displays promising cytotoxicity towards cancer cells in association with the inhibition of TrxR, induction of ER stress and also a death-receptor-dependent apoptotic pathway.

iii) Research output

Joint Publications of Prof. Che and Prof. Ott

1. A joint research paper "Caffeine derived platinum(II) N-heterocyclic carbene complexes with multiple anti-cancer activities" **J. J. Zhang,; C. M. Che,; I. Ott,** *J. Organometallic Chemistry* **2015**, *782*, 37. (Special Issue on International Symposium on Bioorganometallic Chemistry)
2. A joint research paper "Alkynyl gold(I) phosphane complexes: evaluation of structure-activity-relationships of the phosphane ligands, effects on key signaling proteins and preliminary in-vivo studies" V. Andermark, K. Göke, M. Kokoschka, M. Abu el Maaty, M. Lum, T. Zou, R. W. Sun, L. Oehninger, L. Rodriguez, H. Bunjes, S. Wölfl, C.M. Che, I. Ott. **2015**, is under preparation and would be submitted for consideration of publication soon.

Publications of HKU team members related to this project on Medicinal Chemistry of Gold Complexes

3. L. He, T. Chen, Y. You, H. Hu, W. Zheng, W.-L. Kwong, **T. Zou**, **C. M. Che**, A Cancer-Targeted Nanosystem for Delivery of Gold(III) Complexes: Enhanced Selectivity and Apoptosis-Inducing Efficacy of a Gold(III) Porphyrin Complex. *Angewandte Chemie, International Edition*, **2014**, 53, 12532
4. **T. Zou**, C. T. Lum, C. N. Lok, W. P. To, K. H. Low and **C. M. Che**, A binuclear gold(I) complex with mixed bridging diphosphine and bis(n-heterocyclic carbene) ligands shows favorable thiol reactivity and effectively inhibits tumor growth and angiogenesis in vivo. *Angewandte Chemie, International Edition*, **2014**, 53, 5810
5. **T. Zou**, J. Liu, C. T. Lum, C. Ma, R. C.-T. Chan, C. N. Lok, W. M. Kwok, **C. M. Che**, Luminescent cyclometalated platinum(II) complex forms emissive intercalating adducts with double-stranded dna and rna: differential emissions and anticancer activities. *Angewandte Chemie, International Edition*, **2014**, 53, 10119
6. C.N. Lok, **T. Zou**, **J.J. Zhang,** I.W. Lin, **C.M. Che**, Controlled release systems for metal based nanomedicine: encapsulated/self-assembled nanoparticles of anticancer gold(III)/platinum(II) complexes and antimicrobial silver nanoparticles. *Advanced Materials*, 2014, 26, 5550
7. C. T. Lum, **R. W. Sun, T. Zou**, and **C. M. Che**, Gold(III) complexes inhibit growth of cisplatin-resistant ovarian cancer in association with upregulation of proapoptotic PMS2 gene. *Chemical Science*, **2014**, 5, 1579
8. **J. J. Zhang**, K. M. Ng, C. N. Lok, **R. W. Sun** and **C. M. Che**, Deubiquitinases as potential anti-cancer targets for gold(III) complexes, *Chemical Communications*, **2013**, 49, 5153-5155.
9. **R. W. Sun**, C. N. Lok, T. T. H. Fong, C. K. L. Li, Z. F. Yang, **T. Zou**, A. F. M. Siu and **C. M. Che**, A dinuclear cyclometalated gold(III)-phosphine complex targeting thioredoxin reductase inhibits hepatocellular carcinoma in vivo, *Chemical Science*, **2013**, 4, 1979-1988.
10. C. T. Lum, A. S. Wong, M. C. Lin, **C. M. Che** and **R. W. Sun**, A gold(III) porphyrin complex as an anti-cancer candidate to inhibit growth of cancer-stem cells, *Chemical Communications*, **2013**, 49, 4364-4366.

Ph.D. thesis

11. **J.J. Zhang**, 2014 "The anti-cancer properties of cyclometalated gold(III) complexes and organogold(III) supramolecular polymers". Dr. J.J. Zhang is currently a postdoctoral fellow working in Prof. Ott's laboratory in Technische Universität Braunschweig.
12. **T. Zou**, 2015 "Anti-cancer N-heterocyclic carbene complexes of gold(III), gold(I) and platinum(II): thiol "switch-on" fluorescent probes, thioredoxin reductase inhibitors and endoplasmic reticulum targeting agents". Dr. T. Zou is currently a postdoctoral fellow in Prof. Che's laboratory.

Awards

The PI Prof. **C.M. Che** of HKU team has received the 2013 Royal Society Chemistry Centenary Prize (inspiring contributions including therapeutic applications of metal compounds) and the 2013-2014 MIT Davison Lectureship for his works on Anti-Cancer Metal Compounds of Gold and Platinum. He has registered as Foreign Associate of the National Academy of Sciences of USA in 2014.

Dr. **Jingjing Zhang**, has been awarded a Humboldt postdoctoral fellowship to work at Prof. Ott's laboratory in Technische Universität Braunschweig

Dr. **Taotao Zou** is the winner of 2014 Young Scientist Awards established by Hong Kong Institution of Science. He is a finalist of 2015 Reaxys PhD Prize and will compete with other internationally renowned finalists in the Reaxys PhD Prize Symposium on the 7th and 8th of September.

iv) Potential for or impact on further research collaboration

- The gold(I) complexes developed by Prof. Ott exhibit strong *in vitro* cytotoxicity to different cancer cells and *in vivo* anti-tumor activities are anticipated. The gold(III) and Pt(II) complexes developed by Prof. Che display potent *in vitro* and *in vivo* anti-cancer activities and detailed mechanistic studies are in progress. Collaborations will be continued for *in vivo* anti-tumor studies in Hong Kong for the gold(I) complexes developed by Prof. Ott and mechanistic studies on the gold(III) and other metal complexes developed by Prof. Che in Germany
- Prof. Ott and Prof. Che will collaborate on nano-formulation of anticancer gold compounds for therapeutic applications.
- A graduated PhD student from HKU team, Dr. Jingjing Zhang, has been awarded a Humboldt postdoctoral fellowship to work at Prof. Ott's laboratory in Technische Universität Braunschweig; Dr. Zhang is presently working on collaborative research projects in the area of anticancer metal compounds.
- Another graduated PhD student from HKU team, Dr. Taotao Zou, is currently a postdoctoral fellow in Prof. Che's laboratory and a visiting scientist in Peter Sadler's Laboratory in University of Warwick, UK. He will participate in collaborative projects on molecular structural analysis by NMR and X-ray crystallography.