RGC Ref.: N_HKUST621/13
NSFC Ref. : 81361168002
(please insert ref. above)

The Research Grants Council of Hong Kong
NSFC/RGC Joint Research Scheme
Joint Completion Report

(Please attach a copy of the completion report submitted to the NSFC by the Mainland researcher)

Part A: The Project and Investigator(s)

1. Project Title

Development of triterpenoid natural product derivatives as new antiviral drugs directly blocking the receptor binding site of influenza virus

2. Investigator(s) and Academic Department/Units Involved

	Hong Kong Team	Mainland Team
Name of Principal Investigator *(with title)*	Prof. Zhihong Guo	Prof. Demin Zhou
Post	Associate Professor	Professor
Unit / Department / Institution	Chemistry/HKUST	State Key Laboratory of Natural and Biomimetic Drugs, School of Medicine, Peking University
Contact Information	Department of Chemistry, The Hong Kong University of Science and Technology, Clear Water Bay, Kowloon, Hong Kong	State Key Laboratory of Natural and Biomimetic Drugs, School of Pharmaceutical Sciences, Peking University, 38 Xueyuan Road, Beijing 100191, China
Co-investigator(s) *(with title and institution)*	N.A.	Dr. Maorong Yu

3. Project Duration

	Original	Revised	Date of RGC/ Institution Approval *(must be quoted*)
Project Start date	1-1-2014		
Project Completion date	31-12-2017		
Duration *(in month)*	48		
Deadline for Submission of Completion Report	31-12-2018		

Part B: The Completion Report

5. Project Objectives

5.1 Objectives as per original application

1. To crystallize the complexes of two hemagglutinins in different phylogenic groups with **Q9** and determine their structure in high-resolution.
2. To determine the specificities of the anti-influenza activity of **Q9** by determining its binding affinity for various hemagglutinin subtypes and its antiviral activities towards influenza strains carrying the corresponding subtypes of hemagglutinin.
3. To carry out multiple rounds of **Q9** analog synthesis and activity evaluation to maximize the antiviral potency and therapeutic index, as guided by cell-based antiviral assays and the structure-activity relationships acquired from the biochemical and structural studies.

4. To test the efficacy of the optimized lead compound in mice challenged with highly virulent strains of influenza virus such as H5N1 and H1N1.

5.2 Revised Objectives

Date of approval from the RGC: _____

Reasons for the change: _____

_____

1.
2.
3. ....

6. Research Outcome

Major findings and research outcome
(maximum 1 page; please make reference to Part C where necessary)

This research project is focused on the development of new antif-influenza drugs that blocks the entry of the viral pathogens into the host cells. It has achieved several important findings as listed below:

(1) The structure-activity relationships of the hit compound of a galactose conjugate of orleanolic acid (**Q9**) have been successfully elucidated after multiple rounds of analogue synthesis and activity determination.

(2) The structure of the hit compound has been optimized to obtain several analogs with significantly enhanced anti-influenza activity, including **Q8**, **Y3** and **Y4** (Yu *et al. J. Med. Chem*. **2014**, *57*, 10058–10071).

(3) The hit and optimized triterpene-sugar conjugates have been successfully demonstrated to bind the receptor binding pocket of hemaglutinins through photoactivated crosslinking and protein mass spectroscopy, despite the failure to determine the crystal structures of the hemagglutinins in complex with the small molecule inhibitors.

(4) The original hit compound **Q9** and the optimized analogs have been found to be active against a broad-spectrum of influenza viruses, demonstrating the general applicability of targeting the receptor binding site of the viral pathogens.

(5) The entry inhibitors have been shown to be provide protection against infection of influenza viruses in Balb/C mice, demonstrating their *in vivo* efficacy (Yu *et al. J. Med. Chem*. **2014**, *57*, 10058–10071).

These findings validate the receptor recognition site of the influenza hemagglutinin as a new molecular target for development of anti-influenza drugs and provide a new venue to counter the widespread resistance to the few currently available anti-influenza drugs. In addition, the optimized triterpene-sugar conjugates are readily available drug candidates with a new mode of action mechanism that may be subjected to clinical trials.

Potential for further development of the research and the proposed course of action *(maximum half a page)*

This research project has successfully established the receptor binding site of the influenza hemagglutinin as a new molecular target to block the entry of the viral pathogen into the human host cells. This has paved the way forward to develop the viral entry inhibitors as a new class of anti-influenza drugs, which are very promising drug candidates to counter the widespread drug resistance of the influenza viruses. The optimized triterpene-sugar conjugates are currently under evaluation for clinical trials in an attempt to develop them into clinical drugs to treat influenza infections.

7. The Layman's Summary

(describe in layman's language the nature, significance and value of the research project, in no more than 200 words)

Seasonal and pandemic influenza infections pose a constant threat to public health, claiming hundreds of thousands of lives worldwide every year. A valid therapeutic intervention to reduce mortality is to disrupt the virus life cycle in the acute phase of the viral infections, such as blocking the detachment of nascent virions from host cells by Tamiflu. In this research project, we have successfully developed new anti-influenza drug candidates with a new mode of action to block the entry of the viral pathogens into the human host cells. Specifically, we have elucidated the structure-activity relatioships for a galactose conjugate of orleanolic acid, a triterpene natural product, and optimized its structure to obtain several analogues with significantly enhanced entry inhibition activities. In addition, these optimized triterpene-sugar conjugates are found to exhibit broad-spectrum activity against different clinical strains of influenza viruses of both A and B types and to protect mice from the viral infections. Moreover, the optimized conjugates are shown to bind to the receptor binding site of the viral hemagglutinin protein via photoactivated crosslinking and protein mass spectroscopy. These findings not only provide several promising drug candidates to treat influenza infections but have also have successfully established the receptor binding site of the influenza hemagglutinin as a new molecular target for the development of anti-influenza drugs.

Part C: Research Output

8. Peer-reviewed journal publication(s) arising directly from this research project

(Please attach a copy of each publication and/or the letter of acceptance if not yet submitted in the previous progress report(s). All listed publications must acknowledge RGC's funding support by quoting the specific grant reference.)

The Latest Status of Publications				Author(s)	Title and Journal/ Book	Submitted to RGC	Attached to this report	Acknowledged the support of this Joint Research Scheme	Accessible from the institutional repository
Year of publication	Year of Acceptance *(For paper accepted but not yet published)*	Under Review	Under Preparation *(optional)*	***bold** the authors belonging to the project teams and denote the corresponding author with an asterisk*)*	*(with the volume, pages and other necessary publishing details specified)*	*(indicate the year ending of the relevant progress report)*	*(Yes or No)*	*(Yes or No)*	*(Yes or No)*

2014				Maorong Yu*, Longlong Si, Yufei Wang, Yiming Wu, Fei Yu, Pingxuan Jiao, Yongying Shi, Han Wang, Sulong Xiao, Ge Fu, Ke Tian, Yitao Wang, Zhihong Guo, Lihe Zhang, and Demin Zhou*	Discovery of pentacyclic triterpenes as potential entry inhibitors of influenza viruses. *J. Med. Chem.* **2014**, *57*, 10058–10071.	2015	No	Yes	Yes
2015				Yaozong Chen, Yueru Sun, Haigang Song, and Zhihong Guo*	Structural basis for the ATP-dependent configuration of adenylation active site in *Bacillus subtilis* *o*-succinylbenzoyl-CoA Synthetase. *J. Biol. Chem.* **2015**, *290*, 23971–23983.	2015	No	Yes	Yes
2016				Ju-Xian Song, Yue-Ru Sun, Ivana Peluso, Yu Zeng, Xing Yu, Jia-Hong Lu, Zheng Xu, Ming-Zhong Wang, Liang-Feng Liu, Ying-Yu Huang, Lei-Lei Chen, Siva Sundara Kumar Durairajan, Hong-Jie Zhang, Bo Zhou, Hong-Qi Zhang, Aiping Lu, Andrea Ballabio, Diego L. Medina*, Zhihong Guo*, and Min Li*	A novel curcumin analog binds to and activates TFEB in vitro and in vivo independent of MTOR inhibition. *Autophagy* **2016**, *12*, 1372–1389.	2018	Yes	Yes	Yes
2016				Haigang Song, Chen Dong, Mingming Qin; Yaozong Chen, Yueru Sun, Jingjing Liu, Wan Chan, Zhihong Guo*	A thiamine-dependent enzyme utilizes an active tetrahedral intermediate in vitamin K biosynthesis. *J. Am. Chem. Soc.* **2016**, *138*, 7244–7247.	2018	Yes	Yes	Yes

2016				Yaozong Chen, Yiping Jiang, Zhihong Guo*	Mechanistic insights from the crystal structure of Bacillus subtilis o-*succinylbenz*oyl-CoA synthetase complexed with the adenylate intermediate. *Biochemistry* **2016**, *55*, 6685–6695.	2018	Yes	Yes	Yes
2017				Yaozong Chen, Tin Lok Li, Xingbang Lin, Xin Li, Xiang David Li, Zhihong Guo*	Crystal structure of the thioesterification conformation of *Bacillus subtilis* *o*-succinylbenzoyl-CoA synthetase reveals a unique substrate recognition mode. *J. Biol. Chem.* **2017**, *292*, 12296–12310.	2018	Yes	Yes	Yes
2018				Mingming Qin, Haigang Song, Xin Dai, Chi-Kong Chan, Wan Chan, Zhihong Guo*	Single turnover kinetics reveal a distinct mode of thiamine diphosphate-dependent catalysis in vitamin K biosynthesis. *ChemBioChem* **2018**, *19*, 1514–1522.	2018	Yes	Yes	Yes

2018				Mingming Qin, Haigang Song, Xin Dai, Yaozong Chen, Zhihong Guo*	*. Two active site arginines are critical determinants of substrate binding and catalysis in MenD, a thiamine-dependent enzyme in menaquinone biosynthesis. *Biochem. J.* **2018**, *475*, 3651–3667.	2018	Yes	Yes	Yes

9. Recognized international conference(s) in which paper(s) related to this research project was/were delivered

(Please attach a copy of each delivered paper. All listed papers must acknowledge RGC's funding support by quoting the specific grant reference.)

Month/Year/ Place	Title	Conference Name	Submitted to RGC *(indicate the year ending of the relevant progress report)*	Attached to this report *(Yes or No)*	Acknowledged the support of this Joint Research Scheme *(Yes or No)*	Accessible from the institutional repository *(Yes or No)*
Nov/2016/ Southern University of Science and Technology, Shenzhen, China	Structural and kinetic investigation of the thiamine-dependent MenD from the Escherichia coli menaquinone biosynthetic pathway	The Fourth South China Structural Biology Symposium	2018	Yes	Yes	Yes
Nov/2016/ Southern University of Science and Technology, Shenzhen, China	Structural and mechanistic investigation of the membrane associated phosphate acyltransferase PlsX	The Fourth South China Structural Biology Symposium	2018	Yes	Yes	Yes
Nov/2016/ Southern University of Science and Technology, Shenzhen, China	Conformational intermediates in the domain-alteration catalysis of an adenylating enzyme in vitamin K biosynthesis	The Fourth South China Structural Biology Symposium	2018	Yes	Yes	Yes
Nov/2016/ Southern University of Science and Technology, Shenzhen, China	Structural basis of substrate recognition of Bacillus subtilis BioF in biotin biosynthesis	The Fourth South China Structural Biology Symposium	2018	Yes	Yes	Yes

Nov/2017/ Fuzhou University, Fuzhou, Fujian, China	Structural basis for peripheral sensing of membrane regions of increased fluidity	The Fifth South China Structural Biology Symposium	2018	Yes	Yes	Yes
Nov/2017/ Fuzhou University, Fuzhou, Fujian, China	α-Synuclein senses unsaturated fatty acid in vitro to form lipid-associated nonfibrillar α-helical oligomers	The Fifth South China Structural Biology Symposium	2018	Yes	Yes	Yes
Nov/2017/ Fuzhou University, Fuzhou, Fujian, China	Generality of the novel thiamine-dependent catalysis medicated by an active tetrahedral intermediate in menaquinone biosynthesis	The Fifth South China Structural Biology Symposium	2018	Yes	Yes	Yes
Nov/2017/ Fuzhou University, Fuzhou, Fujian, China	Structural and mechanistic investigation of the UbiE monotopic membrane localization in coenzyme Q and vitamin K biosynthesis	The Fifth South China Structural Biology Symposium	2018	Yes	Yes	Yes
Nov/2017/ Fuzhou University, Fuzhou, Fujian, China	Role of two conserved arginine residues in the new thiamine catalysis mediated by a tetrahedral intermediate in menaquinone biosynthesis	The Fifth South China Structural Biology Symposium	2018	Yes	Yes	Yes

10. Student(s) trained *(Please attach a copy of the title page of the thesis.)*

Name	Degree registered for	Date of registration	Date of thesis submission/ graduation
Yaozong Chen	Ph.D	Sept. 1, 2012	Jan. 31, 2017

11. Other impact *(e.g. award of patents or prizes, collaboration with other research institutions, technology transfer, etc.)*

N.A.