BIOGRAPHICAL SKETCH

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NAME: Asquith, Christopher Robert Michael

eRA COMMONS USER NAME (credential, e.g., agency login):

POSITION TITLE: Research Assistant Professor

EDUCATION/TRAINING *(Begin with baccalaureate or other initial professional education, such as nursing, include postdoctoral training and residency training if applicable. Add/delete rows as necessary.)*

INSTITUTION AND LOCATION	DEGREE *(if applicable)*	Completion Date MM/YYYY	FIELD OF STUDY
University of Southampton, United Kingdom	BSc	06/2010	Chemistry
University of Southampton, United Kingdom	MSc	02/2012	Chemistry
University College London, United Kingdom	PhD	03/2016	Medicinal Chemistry
University of North Carolina at Chapel Hill, NC	Postdoctoral	05/2019	Medicinal Chemistry
University of North Carolina at Chapel Hill, NC	Res. Assist Prof.	Present	Medicinal Chemistry

A. Personal Statement

I have a keen interest in medicinal chemistry and the exploration of new chemical space. Throughout the PhD and early years of my career, I was focused on antiviral zinc abstractors and novel zinc binding matrix metalloproteinase (MMP) inhibitors. I have since specialized in kinase inhibitor design for the last 5 years since working at UNC-CH, recently developing a number of high potency, narrow kinome spectrum chemical series probing different disease relevant kinases. I am well versed in chemical synthesis and modern techniques, purification, analysis with a good understanding of biochemical assays. In my current work, I have taken an active role in the synthesis and design of all compounds, concurrently developing and implementing cell-based assays in a multi-site effort. I have also worked many types of simulation software and used these to inform the next stage of development through in-silico modelling, Quantitative Structure-Activity Relationship (QSAR) and WaterMap. This combination has enabled me to be a successful leader of multi-faceted projects, at the level required in this application. I am currently the lead medicinal chemist on the Illuminating the Druggable Genome (IDG) working with the Structural Genomics Consortium (SGC) to advance chemical probes for understudied kinases to provide tools and resources for the wider scientific community. I have a track record of delivering chemical series that are considered promiscuous across the kinome (oxindole and quin(az)oline) and generating chemical tools and probes for some of the toughest kinase targets.

B. Positions and Honors

Positions and Employment

2015 Research associate, University of Cyprus, Cyprus
2016 Post-doctoral research associate, University College London, United Kingdom
2016 Post-doctoral research associate, University of North Carolina at Chapel Hill, NC
2019 Research Assistant Professor, School of Pharmacology, University of North Carolina at Chapel Hill, NC

Professional Memberships

2007- Associate Member of the Royal Society of Chemistry (AMRSC)
2012- Member of Science and the Chemical Industry (SCI)
2014- Member of the American Chemical Society (ACS)

Honors

2007 Chemistry Award - Peter Symonds College, Winchester, UK
2007 RSC Chemistry Olympiad - Certificate of Commendation, - Peter Symonds College, Winchester, UK
2010 R.E. Parker Project Prize for excellence in a BSc 3rd year project, University of Southampton, UK
2014 Selected from 1000s of applications to present at SET for Britain to over 100 UK Members of Parliament

C. Contribution to Science (*senior author)

1. **Development of novel, non-toxic, potent antiviral zinc abstractors**. The early stage of my career was focused on designing, synthesizing and testing novel series of compounds targeting the nucleocapsid protein of Feline Immunodeficiency Virus (FIV) as a model for HIV (eg. **1a-d**, **2a-b**). In addition to mentoring students and coordinating with collaborators. I tested, developed and optimized several chemical series most containing at least one disulfide bridge required for zinc abstraction. This research revealed several high potency analogs with excellent toxicity profiles (**1a-b**, **2b**). This led to my interest in the medicinal chemistry of understudied high-density sulphur nitrogen heterocycles and their potential as therapeutics. This work was the culmination of an international collaboration spanning the UK, Switzerland, Finland and Russia.

a. Asquith C. R. M.*, Laitinen, T., Konstantinova, L. S., Poso, A., Rakitin, O. A., Hofmann-Lehmann, R., Hilton, S. T.* Investigation of the pentathiepin functionality as an inhibitor of Feline Immunodeficiency Virus (FIV) *via* a potential zinc ejection mechanism, as a model for HIV infection *ChemMedChem.* **2019**, *14*, 454-461.
b. Asquith C. R. M., Konstantinova L. S., Meli M. L., Laitinen T., Poso A., Rakitin O. A., Hofmann-Lehmann R., Hilton S. T.* Evaluation of Substituted 1,2,3-Dithiazoles as Inhibitors of the Feline Immunodeficiency Virus (FIV) Nucleocapsid Protein *via* a Proposed Zinc Ejection Mechanism *ChemMedChem* **2016***, 11,* 2119-2126.
c. Asquith C. R. M., Meli M. L., Konstantinova L. S., Laitinen T., Poso A., Rakitin O. A., Hofmann-Lehmann R., Allenspach K., Hilton S. T.* Novel fused tetrathiocines as zinc abstractors of the nucleocapsid protein of Feline Immunodeficiency Virus (FIV) as a model of HIV infection. *Bioorg. Med. Chem. Lett.* **2015**, *25*, 1352-1355.
d. Asquith C. R. M., Meli M. L., Konstantinova L. S., Laitinen T., Peräkylä M., Poso A., Rakitin O. A., Allenspach K., Hofmann-Lehmann R., Hilton S. T.* Evaluation of the antiviral efficacy of *bis*[1,2]dithiolo[1,4]thiazines and *bis*[1,2]dithiolopyrrole derivatives against the nucleocapsid protein of the Feline Immunodeficiency Virus (FIV) as a model for HIV infection. *Bioorg. Med. Chem. Lett.* **2014**, *24*, 2640-2644.

2. **Therapeutic investigation of neglected/under-investigated sulphur/nitrogen heterocycles as potent antivirals and/or antifungals.** I have a continued interest in novel sulfur heterocyles/chemistry and their therapeutic potential. I have investigated some of the rarest heterocycles known, including asymmetric pentathiepins and 1,2,3-thiaselenazoles. I have demonstrated the first biological evaluation of the ultra-rare 1,2,3-thiaselenazole useing it as an antiviral against FIV (**2a**) and as an anti-bacterial. This discovery was coulpled to several other first in class demonstrations of highly active molecules including, pentathiepins being used to treat Sporothrix brasiliensis an emerging fungal infection (**2b**) among others. We were only the second group to demonstrate that the epidithiodiketopiperazine core can by tuned to be non-toxic and still active on targets of biological interest. This was achieved by pendant arm modification, where we can now design compounds that are both a highly active antiviral but also non-toxic to the host cell (**2c**). I have also investigated a significant amount of C-H activated chemistry, using the DABCO/S_2Cl_2 complex as a multi-functional reagent (sulfonating, oxidation, reduction, etc) under carefully controlled conditions (**1a**, **1c-d**, **2d**).
I have worked with sulfur/nitrogen heterocyclic chemistry specialists at the University of Cyprus, in the laboratory of Prof. Panayiotis A. Koutentis; where I was able to advance the intermediate starting material to the final stages of a novel natural product scaffold that was previously inaccessible. In addition, I have with other world leading sulfur chemists including Prof. Oleg A. Ratikin at the Zelinsky Institute of Organic Chemistry in Moscow, Russia to advance several unique scaffolds (**1a-d**, **2a-b, 2d**). The combination of this work is a culmination of an international collaboration spanning the UK, Switzerland, Finland, Brazil, Cyprus and Russia. The work has been highlighted in Russian media.

a. Asquith C. R. M.*, Meili T., Laitinen, T., Baranovsky, I. V., Konstantinova, L. S., Poso, A., Rakitin, O. A.,

Hofmann-Lehmann, R. Synthesis and comparison of substituted 1,2,3-dithiazole and 1,2,3-thiaselenazole as inhibitors of the feline immunodeficiency virus (FIV) nucleocapsid protein as a model for HIV infection. *Bioorg. Med. Chem. Lett.* **2019**, *29*, 1765-1768.

b. Asquith, C. R. M.*, Machado, A. C. S., de Miranda, L. H. M., Konstantinova, L. S., Almeida-Paes, R., Rakitin, O. A., Pereira S. A. Synthesis and Identification of a Pentathiepin-Based Inhibitors of Sporothrix brasiliensis *Antibiotics* **2019**, *8*, 249.
c. Asquith C. R. M.*, Sil B. C., Laitinen, T., Tizzard G. J., Coles S. J., Poso, A., Hofmann-Lehmann R., Hilton S. T.* Novel epidithiodiketopiperazines as anti-viral zinc ejectors of the Feline Immunodeficiency Virus (FIV) nucleocapsid protein as a model for HIV infection *Bioorg Med Chem.* **2019**, *27*, 4174-4184.
d. Asquith C. R. M.*, Konstantinova, L. S., Tizzard, G. J., Laitinen, T., Coles, S. J., Rakitin, O. A., Hilton, S. T.* Exploration of a C-H activated route to access the [1,2]dithiolo[4,3-*b*]indole-3(4*H*)-thione core and related derivatives *Synlett* **2019**, *30*, 156-160.

3. **Development of chemical probes and tools for investigation of understudied kinases.** Since joining the University of North Carolina at Chapel Hill first as part of the Structural Genomics Consortium (SGC) and then the School of Pharmacology. I have been integrally involved in several projects to develop chemical probes for the under-studied kinases including GAK, AAK1, BIKE, STK10, SLK, CaMKK2, ULK3, MAP4K4, TLK2 and PKMYT1. The ultimate aim of these efforts is the generation of research tools to enable the elucidation of the biological functions of these kinases. One case study of this work is GAK, where we first investigated GW577382 as lead compound (**3a**) and optimized potency, cellular activity and kinome specificity to identify SGC-GAK-1 and the negative control SGC-GAK-1N (**3b-c**) as a probe set. This is a high-quality chemical probe set, where there is no other kinase inhibited within 50-fold of GAK and potency for of IC_{50} = >50 nM in cells, allows for direct investigation of GAK as a direct target with this inhibitor set. The 4-anilinoquin(az)oline scaffold is synonymous with EGFR inhibition, with a number of compounds being used in the clinic of this exact scaffold including gefitinib, erlotinib and lapatinib. I have showed a method of tuning EGFR and GAK activities both in and out of the scaffold while maintaining a narrow kinome profile in one of the first experiments of its kind (**3c**). I then went on to show direct relevant to a rare cancer – chordoma. I also showed a method of optimizing SGC-GAK-1 for use *in vivo* by structural modification of the aniline and co-administration with 1-aminobenzotriazole (ABT) (**3d**). The work forms the precursor to on aim of this application. Where a series of modifications to the core heterocycle in addition to several back up series will allow use access to potent *in vivo* GAK inhibitor.

a. Asquith C. R. M., Laitinen T., Bennett J. M., Godoi P. H., East M. P., Tizzard G. J., Graves L., Johnson G. L., Dornsife R. E., Wells C. I., Elkins J. M., Willson T. M., Zuercher W. J.* Identification and Optimization of 4-Anilinoquinolines as Inhibitors of Cyclin G Associated Kinase *ChemMedChem* **2018**, *13,* 48-66
b. Asquith C. R. M., Berger, B. T., Wan, J., Bennett, J. M., Capuzzi, S. J., Crona, D. J., Drewry, D. H., East, M. P., Elkins, J. M., Fedorov, O., Godoi P. H., Hunter D. H., Knapp S., Müller S., Torrice, C. D., Wells, C. I., H. Earp, S., Willson T. M., Zuercher W. J.* SGC-GAK-1: a chemical probe for cyclin G associated kinase (GAK) *J Med Chem.* **2019**, *62*, 2830-2836
c. Asquith C. R. M.*, Naegeli N., East M. P., Laitinen T., Havener T. M., Wells C. I., Johnson G. L., Drewry D. H., Zuercher W. J., Morris D. C.* Design of a cyclin G associated kinase (GAK)/epidermal growth factor receptor (EGFR) inhibitor set to interrogate the relationship of EGFR and GAK in chordoma *J Med Chem.* **2019**, *62,* 4772-4778. *Featured as front cover* - 10.1021/acs.jmedchem.9b00350
d. Asquith, C. R. M.*, Bennett J. M., Su L., Laitinen T., Elkins J. M., Pickett J. E., Wells C. I., Li Z., Willson T. M., Zuercher W. J.* Towards the Development of an In vivo Chemical Probe for Cyclin G Associated Kinase (GAK) *Molecules* **2019**, *22*, 4016. *Feature Paper*

4. **Understanding solvent dynamics in kinase design and the exploration of targeting the water network.** Water networks within kinase inhibitor design and more widely within drug discovery are generally poorly understood. The successful targeting of these networks prospectively has great promise for all facets of inhibitor design, including potency and selectivity on target. This particularly relevant for kinase inhibitors where there is a set of x-ray structures of proteins all binding the same biological target - ATP. I have been seeking to develop a method to target the water network using WaterMap and existing x-ray crystal structures to prospectively target individual higher energy water molecules (**4a**). This can be applied to different targets and more specifically to access differences between kinases and kinase family members ATP binding sites (**3a**, **4a-d**, **5a-b**). I have shown that a 10-fold boost in cellular potency (or more) is possible using this method in the correct scenario (**4a**). This targeting of the water network holds for a series of different kinases including GAK (**3a, 4a**) STK10/SLK

(**4b**), EGFR (**4c**) and TNNi3K (**4d**). The understanding of the solvent expose section of the ATP memetic has always been a secondary consideration, usually probed by a rational array. We hope to use this technique further understand this part of inhibitor binding. This will enable a narrow, targeted selection of the substitution pattern of the solvent exposed region and we plan to utilize this method in the current application.

a. Asquith C. R. M.*, Tizzard G. J., Bennett J. M., Wells C. I., Elkins J. M., Willson T., Poso A., Laitinen T. Targeting the water network in cyclin G associated kinase (GAK) with 4-anilino-quin(az)oline inhibitors. *ChemMedChem.* **2020***, 15,* 1200-1215.
b. Asquith C. R. M.*, Laitinen T., Bennett J. M., Wells C. I., Elkins J. M., Zuercher W. J., Tizzard G. J., Poso A. Design and analysis of the 4-anilino-quin(az)oline kinase inhibition profiles of GAK/SLK/STK10 using quantitative structure activity relationships. *ChemMedChem.* **2020***, 15, 26-49. Featured as the front cover - 10.1002/cmdc.201900691*
c. Asquith C. R. M.*, Maffuid K. A., Laitinen T., Torrice C. D., Tizzard G. J., Crona D. J., Zuercher W. J.* Targeting an EGFR water network using novel 4-anilinoquin(az)olines inhibitors for chordoma *ChemMedChem* **2019**, *14*, 1693-1700.
d. Asquith, C. R. M.*, Laitinen T., Wells C. I., Tizzard G. J., Zuercher W. J. New insights into 4-anilinoquinazolines as inhibitors of cardiac troponin I–interacting kinase (TNNi3K). *Molecules*. **2020**, *25*, 1697.

5. Understanding of kinase inhibitor design in order to expand coverage of the kinome, chemical space and disease links. The crossover of different techniques provides opportunities to both expand chemical space/utility, but also further the understanding of kinase design (**5a-d**). One example of this is where in the search for novel chemical matter, I templated a series of promiscuous anilinopyrimidine inhibitors from the literature (inhibiting 100-200 kinases each) and overlaid the electronic isostere of the 1,2,6-thiadiazinone. This led to the identification of a potent narrow spectrum inhibitor of Calcium/Calmodulin-Dependent Protein Kinase Kinase 2 (CaMKK2) and the first ever co-crystallization of a 1,2,6-thiadiazinone in a protein (**5a**). We have also used a broad panel screening approach to identify potent lead compounds which we have then optimized for the Salmonella PhoP/PhoQ signal transduction system (**5b**) and Mycobacterium tuberculosis (*Mtb*) (**5c**). In addition, we have investigated the use of kinome mini-panels to enhance kinome specificity during lead optimization (**5d**). We have many other unpublished results, all of which will be utilized in the advancement of this application.

a. Asquith C. R. M.*, Godoi P. H., Couñago R. M., Laitinen T., Scott J. W., Langendorf C. G., Oakhill J. S., Drewry D. H., Zuercher W. J., Koutentis P. A., Willson T. M., Kalogirou A. S.* 1,2,6-Thiadiazinones as Novel Narrow Spectrum Calcium/Calmodulin-Dependent Protein Kinase Kinase 2 (CaMKK2) Inhibitors. *Molecules.* **2018**, *23* (5), E1221.
b. Carabajal M. A.[/], Asquith C. R. M.[/], Laitinen, T., Tizzard, G. J., Yim, L., Rial, A., Chabalgoity, J., Zuercher W. J., Véscovi E. G.* Quinazoline-based anti-virulence compounds that selectively target Salmonella PhoP/PhoQ signal transduction system *Antimicrob. Agents Chemother.* **2019**, *64*, pii: e01744-19. [/]equal contribution
c. Asquith C. R. M., Fleck N., Torrice C. D., Crona, D. J., Grundner C.*, Zuercher W. J.* Anti-tubercular activity of novel 4-anilinoquinolines and 4-anilinoquinazolines *Bioorg. Med. Chem. Lett.* **2019**, *18*, 2695-2699.
d. Asquith C. R. M.*, Treiber D. K., Zuercher W. J.* Utilizing comprehensive and mini-kinome panels to optimize the selectivity of quinoline inhibitors for cyclin G associated kinase (GAK) *Bioorg. Med. Chem. Lett.* **2019**, *29*, 1727-1731.

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