SRF VACATION SCHOLARSHIP REPORT 2022

The form below should be completed by the student, then forwarded to the supervisor for approval and submission to srf@conferencecollective.co.uk within 8 weeks of completing the project.
Please submit the form as a PDF document and save it as: First name, Last name and 'VS'.

A maximum of one figure (with legend of less than 100 words) may be appended if required.

Please note: excerpts from this form may be published on the SRF website, so please ensure content is suitable for website publication, and does not compromise future dissemination of data in peer-reviewed papers etc. The SRF reserves the right to edit responses to ensure suitability for publication on the website, newsletter or in promotional material.

Student's Name:	Hanh Duyen Tran	**Student's Institution/University:**	St George's University of London
Degree Title and year of study:	BSc Biomedical Science Year 2		
Supervisor's Name:	Dr Thomas Hopkins	**Supervisor's Department and Institution:**	Department of Women's and Children's Health, King's College London
Project Title:	Identification of potential follicle-stimulating hormone receptor antagonists		

Briefly outline the background and aims of the project *(max 200 words)*

Background: The follicle-stimulating hormone receptor (FSHR) is a glycoprotein hormone receptor, belonging to the family of class A G protein-coupled receptors. Interaction with FSH, activates a network of signalling pathways, namely the $G_{\alpha s}$/cAMP/PKA signalling pathway, to mediate physiological events that support ovarian follicle growth and survival. Recent studies propose a link between menopausal elevation of FSH levels and age-related physiological changes (bone loss, increased visceral adiposity, neurodegeneration…). Thus, selective inhibition of FSHR may provide a novel approach to combat the aforementioned post-menopausal co-morbidities and present a non-steroidal method for contraception.

Aims: To screen and identify potential FSHR antagonist compounds in HEK293 heterologous cell lines.

Methods: HEK293 cells were cultured over 3–4-day period and were transfected with HA-FSHR, cre-luciferase, and *renilla*-luciferase plasmids. For screening, cells were pre-treated with 100µM of each compound for 30 minutes at at 37°C, before stimulation with 100ng/ml of FSH for 4-6 hours at at 37°C. For dose-response experiments, transiently-expressing cells were pre-treated with series of antagonist dilutions (0-100 µM), then stimulated with 100ng/ml of FSH for the same incubation conditions as previously described. Following this, the Dual-Luciferase® Reporter Assay System (Promega) was performed following the manufacturer's instructions to measure cAMP-induced cre-luciferase luminescence readings.

Did the project change from that proposed in the application? If so, what changes were made and why? *(max 100 words)*

Yes, the project initial aim was altered due to problems with the breeding of animals within the in-house breeding facility. This reduced sample numbers for experimentation. Therefore, we pivoted slightly and looked at another reproductive GPCR, the FSHR, using different samples. With the aim of looking to understand the effects of inhibiting the receptor in the context for new potential therapeutics.

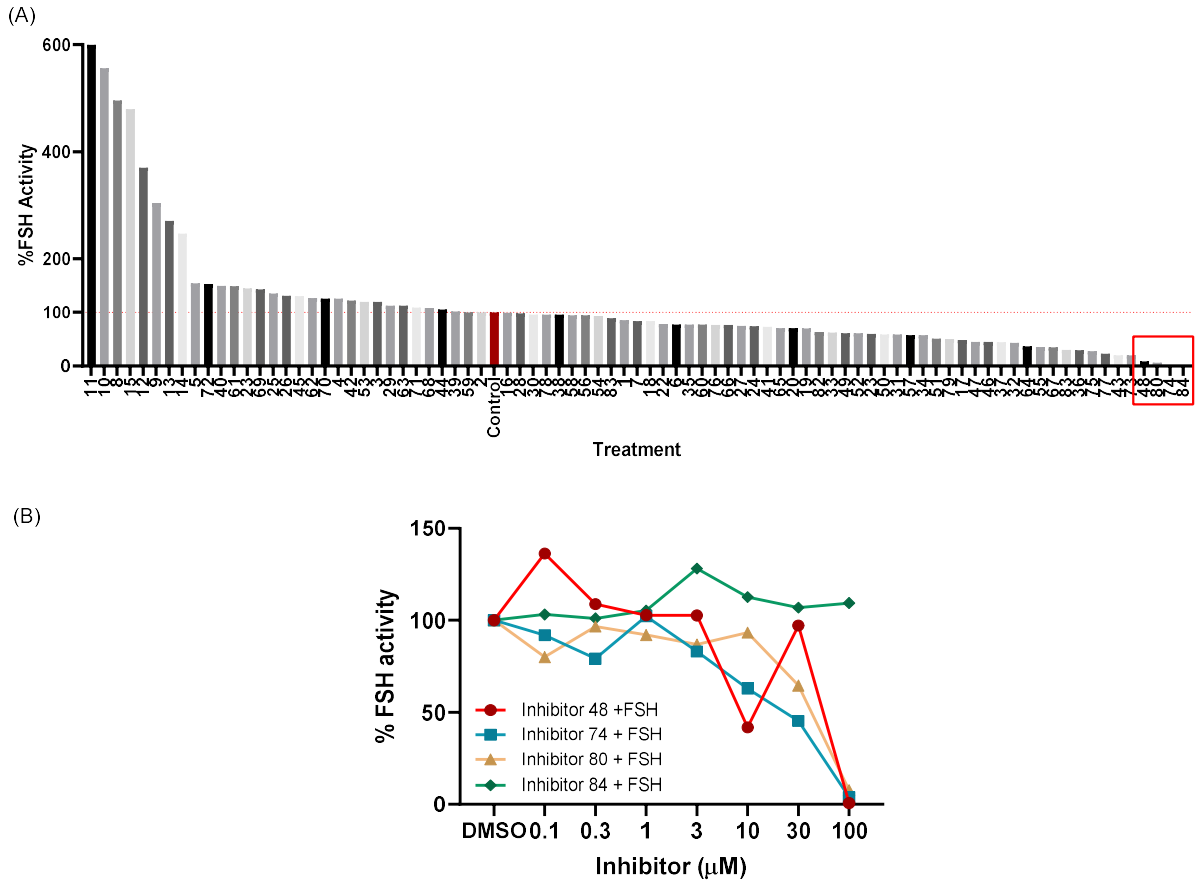
What were the main results/findings of the project? *(max 300 words)*

Our results show that 56 out of 84 compounds were identified as inhibitors, with only 18 compounds displaying more than 50% inhibition of FSH activity (Fig. 1). Interestingly, the rest of compounds showed stimulation of FSH activity. Four most prominent inhibitors were identified as follows: inhibitor 48, inhibitor 74, inhibitor 80, and inhibitor 84. Dose-response experiments using these latter inhibitors were conducted. Inhibitors 48, 74, and 80 show concentration-dependent inhibition of FSH activity, with 30-100 µM

exhibiting highest decrease of FSH activity (Fig. 2), whereas inhibitor 84 doesn't seem to inhibit FSHR to the same degree.

Fig.1

Cell-based dose-response assessment following high-throughput screening of 84 potential inhibitor compounds. (A) Cre-luciferase assays were performed to determine compounds with properties to inhibit FSH-mediated cAMP production. Results are expressed in percentage of FSH activity in descending order, with control (100%) represented in red. Compounds with measurements above the red line are activators, and those below the red line are inhibitors. (B) Dose-response assays were then performed using the four most prominent inhibitors to assess their concentration-dependent effect on FSH activity. Each data point represents the mean for triplicate cells from one independent experiment.



What do you conclude from your findings? *(max 150 words)*

Our findings have identified inhibitor 48, 74, and 80 as effective inhibitors of FSH-mediated $G_{\alpha s}$/cAMP/PKA signalling pathway. The results of our study show that higher concentrations of these inhibitors exhibited notable inhibition of cAMP-mediated cre-luciferase activity, compared to lower concentrations. Despite showing high inhibitor activity in the initial screening, inhibitor 84 does not demonstrate this inhibition. Further repeats and investigation may provide better understanding of the results observed and give a better insight into the molecular mechanism by which these inhibitors exert their effects.

How has this experience influenced your thinking regarding your future/ongoing studies, and/or career choice? *(max 150 words)*

With little prior lab experience (mostly due to COVID-19 restrictions), this opportunity enabled me to engage and get a first-hand experience into the work of a research lab. It was an incredible experience to be a part of a lab that tackles some of the great challenges/questions in reproduction and fertility. My project has helped me grow professionally and has given me the motivation to work with initiation and reliance—qualities I may not have had initially. I enjoyed learning about new laboratory techniques, expanding my knowledge in the field, and further develop my skills. Without such a dynamic, encouraging workplace, it would not have been feasible for me to work with such level of maturity and drive. With this valuable and

positive experience, I hope to use what I have learned moving forward in my final year of my BSc degree, as well as consider pursuing a PhD soon.

Please use the space below to add any other comments/thoughts about the SRF Vacation Scholarship *(max 100 words)*

Student: The Summer Vacation Scholarship was an amazing opportunity for me to gain important lab work experience, enabling me to gain a deeper insight to not only research, but also the professional working environment. It enabled me to challenge myself for self-development, put my academic knowledge into practice, and further develop my skills.

Supervisor: The summer vacation scheme is a fantastic opportunity for students to experience the lab working environment and academic research. I believe the scheme is perfectly setup to support labs and students to undertake work. This allowed myself as well to have experience of supporting a student, paving the way for my further career prospects.