Sphingosine kinase (SPHK), a new drug target for treating schistosomiasis

Ziada Kiwanuka, Bismark Dankwa, Mary Evans, Benjamin Hulme, Sarah Davey, Kristin Lees, Iain Chalmers, Gabriel Rinaldi, Josephine Forde-Thomas, Karl Hoffmann

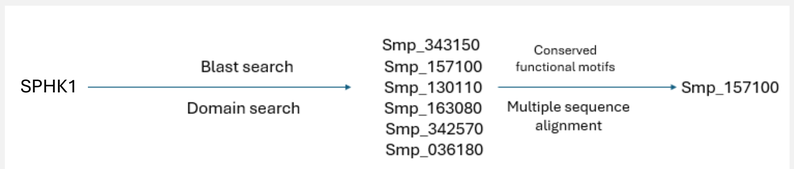
1872 Prifysgol Aberystwyth University

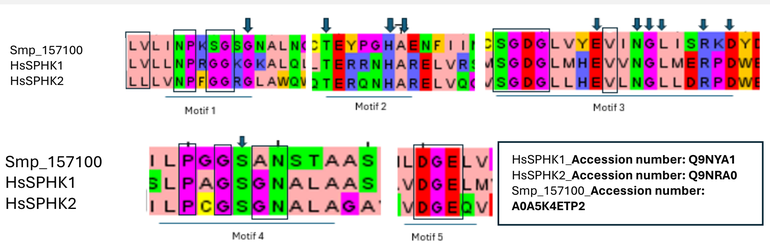
Barrett Centre for Helminth Control

Introduction

- Schistosomiasis is a neglected tropical disease (NTD) affecting 230 million people, and 779 million are at risk of becoming infected globally.
- There are two types of Schistosomiasis infections. Urogenital schistosomiasis caused by *Schistosoma haematobium,* and intestinal schistosomiasis caused by either *Schistosoma mansoni* or *Schistosoma japonicum*.
- *Schistosoma mansoni* is a major species responsible for infecting approximately 54 million people annually.
- Praziquantel **(PZQ)** is the only World Health Organization's recommended drug for treating all *Schistosoma* species worldwide
- In absence of vaccine and documented evidence indicating possible resistance of PZQ, new chemical matter needs to be brought into the drug discovery pipeline to sustain schistosomiasis control.

Bioinformatic Searches

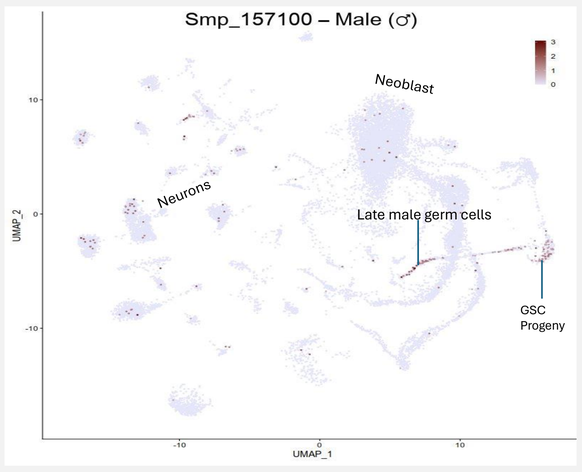


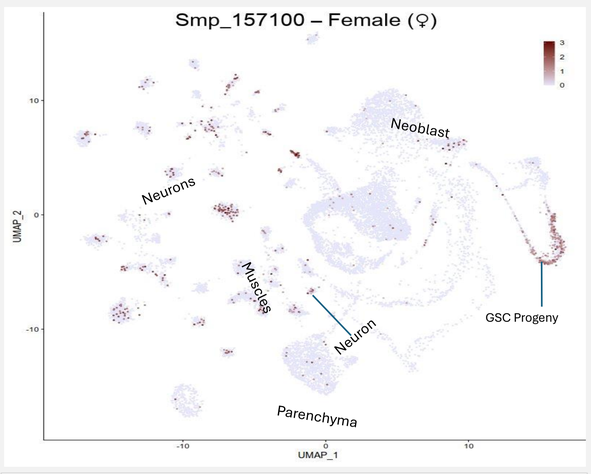


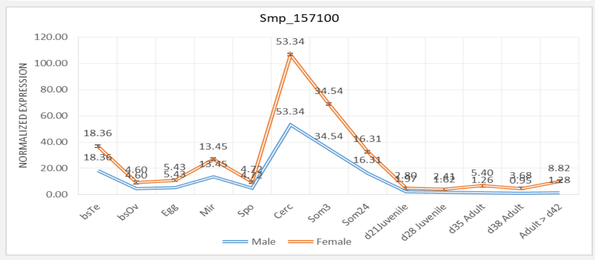
HsSPHK1_**Accession number: Q9NYA1**
HsSPHK2_**Accession number: Q9NRA0**
Smp_157100_**Accession number: A0A5K4ETP2**

- Analysis of aligned sequence was performed using Jal view 2.11.3.2.
- Human Sphingosine Kinase 1 contains five motifs that are functionally important.
- Residues highlighted in boxes are those that are highly conserved within the diacylglycerol kinase putative catalytic domain family.
- Residues pointed by arrows are highly conserved within the sphingosine kinases (Wang et al., 2013) (Pitson et al., 2002).
- Based on conservation of these residues Smp_157100 is the most likely *S. mansoni* protein target that is functionally important as human sphingosine kinase 1.

Single cell expression







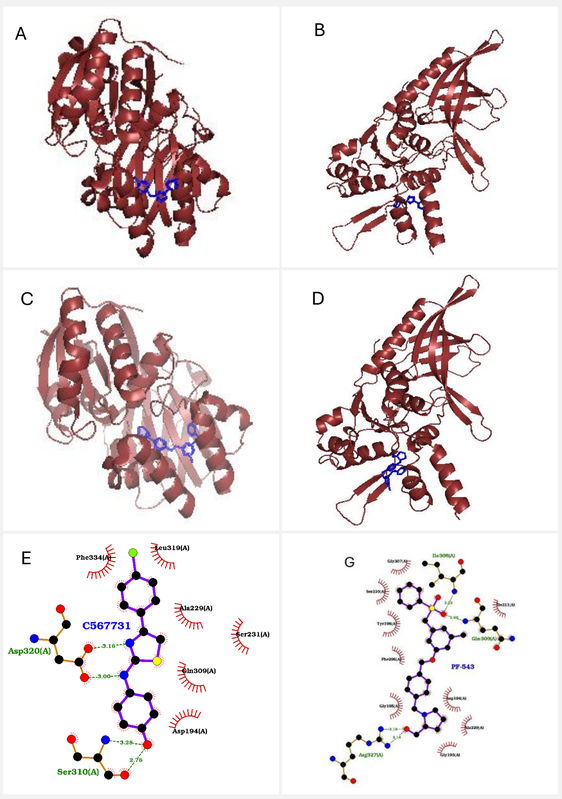
Gene expression throughout the life cycle

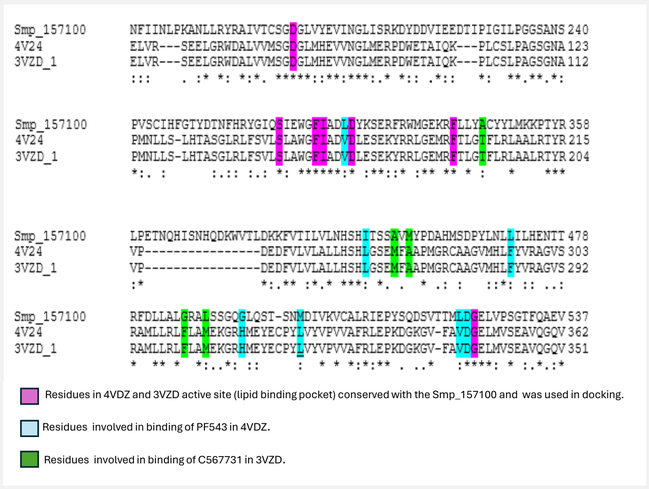
- Highly expressed in cercaria and schistosomula (Lu et al., 2016)(Protasio et al., 2017)
- Single-cell RNA sequencing (RNA-seq) from adult schistosomes, where this transcript shows enriched expression in neurons, germinal stem cell progenies (GSCs) and male germ cells (Wendt et al., 2020).

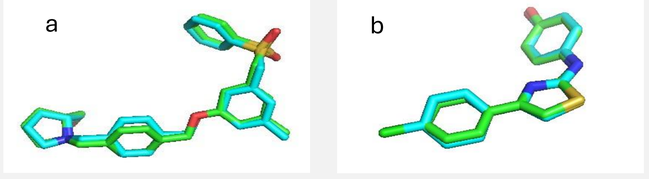
Structural Protein Analysis

- This analysis predicts the binding of PF543 and C567731 to *S. mansoni* SPHK using the known crystallographic structures for human sphingosine kinase 1 co-complexed with the compounds (PDB 4V24 – PF543 & 3VZD – C567731) (J. Wang et al., 2014) (Z. Wang et al., 2013).
- Validation of our analysis tools – Repeating the docking of the known human protein (SPHK) crystal structure and the compounds to produce the same pose of compound
- In Auto Dock Vina, the ligands were energy minimized before docking was carried out. The protein's active site was placed with grid box dimensions set at X=12.65 Å, Y=13.4757 Å, and Z=-11.1428 Å for the centre while box size dimensions were set at X=27.0291 Å, Y=23.3081 Å, and Z=23.4367 Å.

Figure A & B shows the crystal structure of 4V24 & Smp_157100 homology respectively and the binding of compound PF543
Figure C & D shows the crystal structure of 3VZD & Smp_157100 homology respectively and the binding of compound C567731







Validation of our analysis tool

a) Compound position Alignment of our docking results; **PF543: RMS – 0.382**

b) Compound position Alignment of our docking results; **C567731: RMS – 1.088**

- Ligplot was used to observe the interactions of the residues and the compound of our docking results, Hydrogen bond interactions are coloured in green which are Asp 320 and Ser 310 in **compound C567731 (Figure E**), These compounds are both conserved with the human protein.
- **In compound PF543 (Figure F),** Ile 308, Gln 309 and Arg 327 shows the hydrogen bond interaction which are also conserved with human protein, and the residues coloured black are the Hydrophobic bonds.

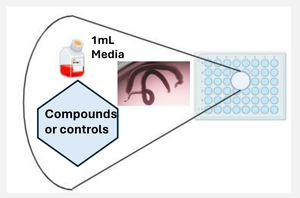
(Dankwa et al., 2022)(Z. Wang et al., 2013)(J. Wang et al., 2014)(Whatley et al., 2019)

Objective

- This study aims to characterise the role of Sphingosine Kinase in the parasite *S. mansoni* and assess its suitability as a potential drug target.
- SPHK is an enzyme that phosphorylates the sphingolipid sphingosine to sphingosine 1 phosphate (S1P).
- S1P is involved in the regulation of various cellular processes, such as cell survival, proliferation, migration, and differentiation. Dysregulation of S1P has been implicated in several human diseases, including cancer, inflammation, and autoimmune disorders (Zheng et al., 2019).
- In human diseases, Sphingosine kinase inhibitors have been primarily studied as a potential therapeutic agent for cancer and inflammatory conditions.
- They have shown promise in preclinical studies and early clinical trials for their ability to suppress tumor growth, induce apoptosis in cancer cells, and inhibit angiogenesis (Zheng et al., 2019). Thus, the importance of screening these compounds against schistosome parasite.
- More argumentation of using these compounds is evidenced by the structural protein (3D) structure - see figure D, E, F, and G showing binding of the compounds to the *S. mansoni* protein.

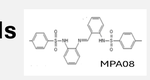
Adult worm screening of Human Sphingosine Kinase 1 inhibitors

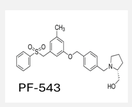
- 3 chemically distinct compounds known to inhibit HsSPHK were screened against adult *S. mansoni* Compounds were screened against worm pairs in triplicate on 3 separate occasions
- Praziquantel (10 µM PZQ) and Auranofin (10 µM AUR) were included as positive controls and DMSO was included as a negative control.
- First experiment was performed at a final concentration of 50 µM followed by 2 dose response titrations of 25 µM, 12.5 µM, and 6.25 µM concentrations.
- Worms were scored by microscopy at 24, 48 and 72h using the WHO-TDR scoring metric described in the table below.

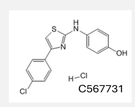


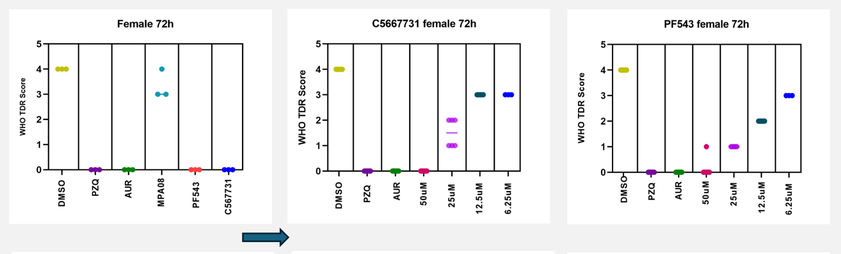
50 µM concentration → **Compound titrations; 50 µM-6.25 µM**

Compounds









WHO – TDR SCORE

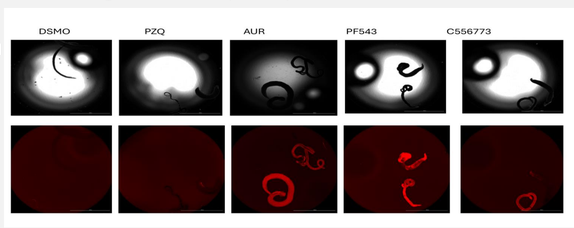
0	No movement
1	Movement of the suckers only and slight contraction of the body
2	Movement at the anterior and posterior regions only
3	Full body movement but sluggish
4	Normal movement

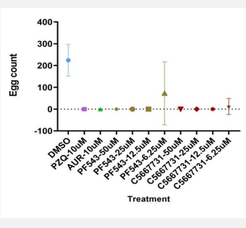
PI staining, egg count, EC_{50} measurements

- At 72h, 15µg/ml of propidium iodide was added to those worms that showed no movement (i.e. scored 0) to distinguish the compounds that caused worm death and those that only immobilized the worms.
- Worms that stained red upon the addition of propidium iodide were confirmed as dead.
- Eggs present in each well were counted using a Cytation 7. Egg production was inhibited in wells treated with compounds when compared to the negative control, (DMSO)

Bright field images

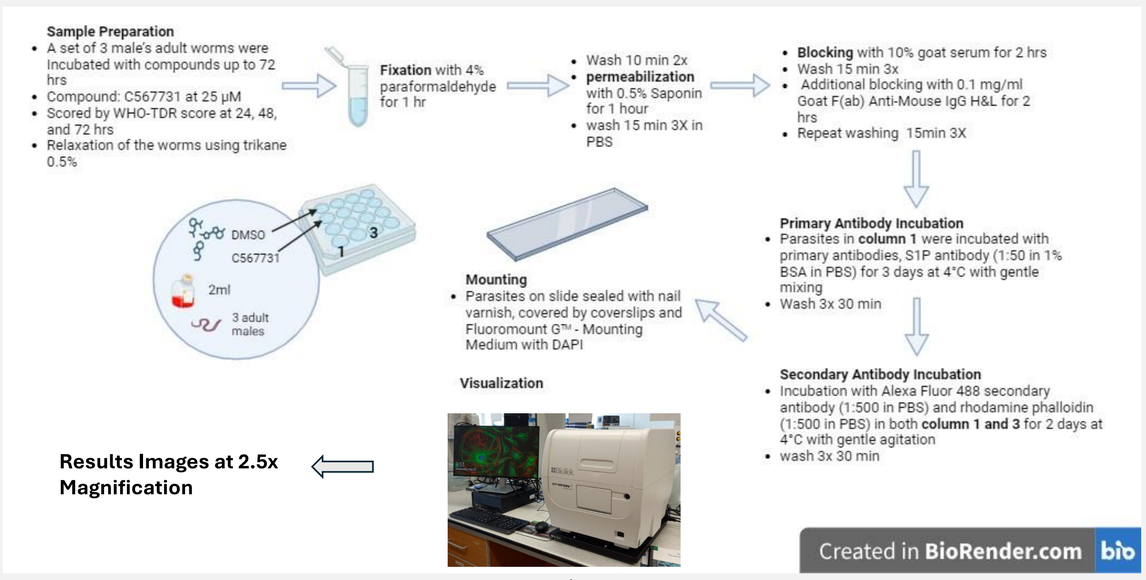
PI images





Compound at 72 hrs	EC50	95% CI
PF543 (males)	12.4	11.48 - 13.33
PF543 (females)	17.94	13.02 - 23.40
C567731 (males)	18.77	16.82 - 20.82
C567731 (females)	21.14	18.35 - 24.73

Immunolocalization of S1P in adult worms



Ongoing activities

- S1P is not detected in the worms treated with SPHK inhibitors, however, further experiments are to be performed to clearly see the localization of S1P on worms
- Currently performing long-term culture on schistosomula treated with compounds to observe if SPHK inhibition affects worm development

Future directions

- Further titration of the compounds until full recovery of the worms is observed
- Knock-down of Smp_157100 using RNA interference

References

1. Dankwa, B., Broni, E., Enninful, K. S., Kwofie, S. K., & Wilson, M. D. (2022). Consensus docking and MM-PBSA computations identify putative furin protease inhibitors for developing potential therapeutics against COVID-19. Structural Chemistry, 33(6), 2221–2241. https://doi.org/10.1007/s11224-022-02056-1
2. Lu, Z., Sessler, F., Holroyd, N., Hahnel, S., Quack, T., Berriman, M., & Grevelding, C. G. (2016). Schistosome sex matters: A deep view into gonad-specific and pairing-dependent transcriptomes reveals a complex gender interplay. Scientific Reports, 6. https://doi.org/10.1038/srep31150
3. Pitson, S. M., Moretti, P. A. B., Zebol, J. R., Zareie, R., Derian, C. K., Darrow, A. L., Qi, J., D'Andrea, R. J., Bagley, C. J., Vadas, M. A., & Wattenberg, B. W. (2002). The nucleotide-binding site of human sphingosine kinase 1. Journal of Biological Chemistry, 277(51), 49545–49553. https://doi.org/10.1074/jbc.M206687200
4. Protasio, A. V., van Dongen, S., Collins, J., Quintais, L., Ribeiro, D. M., Sessler, F., Hunt, M., Rinaldi, G., Collins, J. J., Enright, A. J., & Berriman, M. (2017). MiR-277/4989 regulate transcriptional landscape during juvenile to adult transition in the parasitic helminth Schistosoma mansoni. PLoS Neglected Tropical Diseases, 11(5). https://doi.org/10.1371/journal.pntd.0005559
5. Wang, J., Knapp, S., Pyne, N. J., Pyne, S., & Elkins, J. M. (2014). Crystal structure of Sphingosine Kinase 1 with PF-543. ACS Medicinal Chemistry Letters, 5(12), 1329–1333. https://doi.org/10.1021/ml5004074
6. Wang, Z., Min, X., Xiao, S. H., Johnstone, S., Romanow, W., Meininger, D., Xu, H., Liu, J., Dai, J., An, S., Thibault, S., & Walker, N. (2013). Molecular basis of sphingosine kinase 1 substrate recognition and catalysis. Structure, 21(5), 798–809. https://doi.org/10.1016/j.str.2013.02.025
7. Whatley, K. C. L., Padalino, G., Whiteland, H., Geyer, K. K., Hulme, B. J., Chalmers, I. W., Forde-Thomas, J., Ferla, S., Brancale, A., & Hoffmann, K. F. (2019). The repositioning of epigenetic probes/inhibitors identifies new anti-schistosomal lead compounds and chemotherapeutic targets. PLoS Neglected Tropical Diseases, 13(11). https://doi.org/10.1371/journal.pntd.0007693
8. Wendt, G., Zhao, L., Chen, R., Liu, C., O'donoghue, A. J., Caffrey, C. R., Reese, M. L., & Iii, J. J. C. (2020). A single-cell RNAseq atlas of Schistosoma mansoni identifies a key regulator of blood feeding HHS Public Access. Science, 369(6511), 1644–1649. https://doi.org/10.5061/dryad.0k6djh9xk
9. Zheng, X., Li, W., Ren, L., Liu, J., Pang, X., Chen, X., Kang, D., Wang, J., & Du, G. (2019). The sphingosine kinase-1/sphingosine-1-phosphate axis in cancer: Potential target for anticancer therapy. In Pharmacology and Therapeutics (Vol. 195, pp. 85–99). Elsevier Inc. https://doi.org/10.1016/j.pharmthera.2018.10.011

Special thanks to my supervisor Prof Karl Hoffmann and his laboratory team

Much thanks to the British Society of Parasitology for the travel award grant

1872 Prifysgol Aberystwyth University | Adran Y Gwyddorau Bywyd Department of Life Sciences

The Queen's Anniversary Prizes 2023

Commonwealth Scholarships