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Identifying potential drug targets in the kinomes of two monogenean species

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Summary

Protein kinases are enzymes involved in essential biological processes such as signal transduction, transcription, metabolism, and the cell cycle. Human kinases are targets for several drugs approved by the US Food and Drug Administration. Therefore, the identification and classification of kinases in other organisms, including pathogenic parasites, is an interesting subject of study. Monogeneans are platyhelminths, mainly ectoparasites, capable of causing health problems in farmed fish. Although some genomes and transcriptomes are available for monogenean species, their full repertoire of kinases is unknown. The aim of this study was to identify and classify the putative kinases in the transcriptomes of two monogeneans, *Rhabdosynochus viridisi* and *Scutogyrus longicornis*, and then to predict potential monogenean drug targets (MDTs) and selective inhibitor drugs using computational approaches. Monogenean kinases having orthologs in the lethal phenotype of *C. elegans* but not in fish or humans were considered MDTs. A total of 160 and 193 kinases were identified in *R. viridisi* and *S. longicornis*, respectively. Of these, 22 kinases, belonging mainly to the major groups CAMK, AGC, and TK, were classified as MDTs, five of which were evaluated further. Molecular docking analysis indicated that dihydroergotamine, ergotamine, and lomitapide have the highest affinity for the kinases BRSK and MEKK1. These well-known drugs could be evaluated in future studies for potential repurposing as anti-monogenean agents. The present study contributes valuable data for the development of new antiparasitic candidates for finfish aquaculture.

Keywords: Ergotamine; aquaculture; fish parasites; genomics; Diplectanidae; Dactylogyridae

Introduction

Monogeneans are ectoparasitic flatworms that cause health problems in farmed fish, increasing production costs. Monogenean treatments in aquaculture are somewhat ineffective, predominantly relying on the use of formalin in immersion baths (Morales-Serna *et al.,* 2020). Other treatments are recommended, but they are either only partially effective or are toxic to fish. Therefore, the discovery of new drugs against these parasites is imperative; however, as with neglected diseases, there is little market incentive.

To find new treatments, it is crucial to identify biomolecular targets (typically proteins) that can be modulated through their interactions with a drug. Kinases are a group of proteins most commonly targeted by therapeutic drugs owing to their central role in signal transduction in eukaryotic cells and their control of other processes such as transcription, metabolism, and the cell cycle (Manning *et al*., 2002). There are two kinase superfamilies: eukaryotic protein kinases (ePKs) and atypical protein kinases (aPKs), both with biochemical kinase activity, that is, to phosphorylate a protein at a specific residue. Most ePKs can be classified into nine major

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groups that share a conserved catalytic domain (Manning *et al*., 2002). Evidence from schistosomes—flatworms that cause disease in humans—suggests that kinases are potential drug targets since their inhibition reduces reproduction and survival of the parasite (Dissous & Grevelding, 2011). To combat schistosomiasis, several kinase inhibitor drugs have been proposed through computational tools (Giuliani *et al*., 2018); however, no similar studies have been performed in monogeneans.

Modern computational tools such as molecular docking have been used to discover new treatments for diseases of medical and veterinary importance (Giuliani *et al*., 2018; Pushpakom *et al*., 2019). Particularly, molecular docking has been used for drug repurposing, which is a viable alternative for combating neglected diseases. In this approach, new therapeutic applications are found for existing drugs beyond their original medical indications. This strategy has some advantages, including a lower risk of failure, since the repositioned drug has already undergone preclinical and phase I and II clinical testing in humans—it is safe and development times and costs are minimized (Pushpakom *et al*., 2019).

The present study aimed to identify and classify putative kinases in the transcriptomes available for two monogenean species, and then identify kinase targets and their possible inhibitors using molecular docking methods. The two monogeneans are *Scutogyrus longicornis*, commonly found on farmed Nile tilapia (*Oreochromis niloticus*), and *Rhabdosynochus viridisi*, which is considered a potential threat to the culture of the Pacific white snook (*Centropomus viridis*) (Morales-Serna *et al*., 2020).

Material and Methods

Defining the kinomes of R. viridisi and S. longicornis

Kinomes were identified in the predicted proteins from the transcriptomes of *R. viridisi* and *S. longicornis* (Caña-Bozada *et al*., 2022) by using an integrated bioinformatics pipeline, similar to Giuliani *et al*. (2018), with modifications (Fig. 1A). To that end, a preliminary group of kinases was identified and classified using Kinannote 1.0 software (Goldberg *et al*., 2013), with the parameter "-m" of metazoan. Incomplete sequences detected in the proteins of *S. longicornis* were processed with the CAP3 program (Huang & Madan, 1991) to obtain longer sequences. Then, another preliminary group of kinases was identified and classified by retrieving *R. viridisi* and *S. longicornis* kinases with orthology to *Schistosoma japonicum* (Giuliani *et al*., 2018), *Schistosoma mansoni* (Andrade *et al*., 2011), *Taenia asiatica*, and *Taenia saginata* (Wang *et al*., 2016) using Orthologous MAtrix (OMA) Standalone 2.5.0 software (Altenhoff *et al*., 2019). To improve the annotation, the kinase sequences identified from Kinannote and OMA were merged, and their classifications were compared to detect possible misanno-

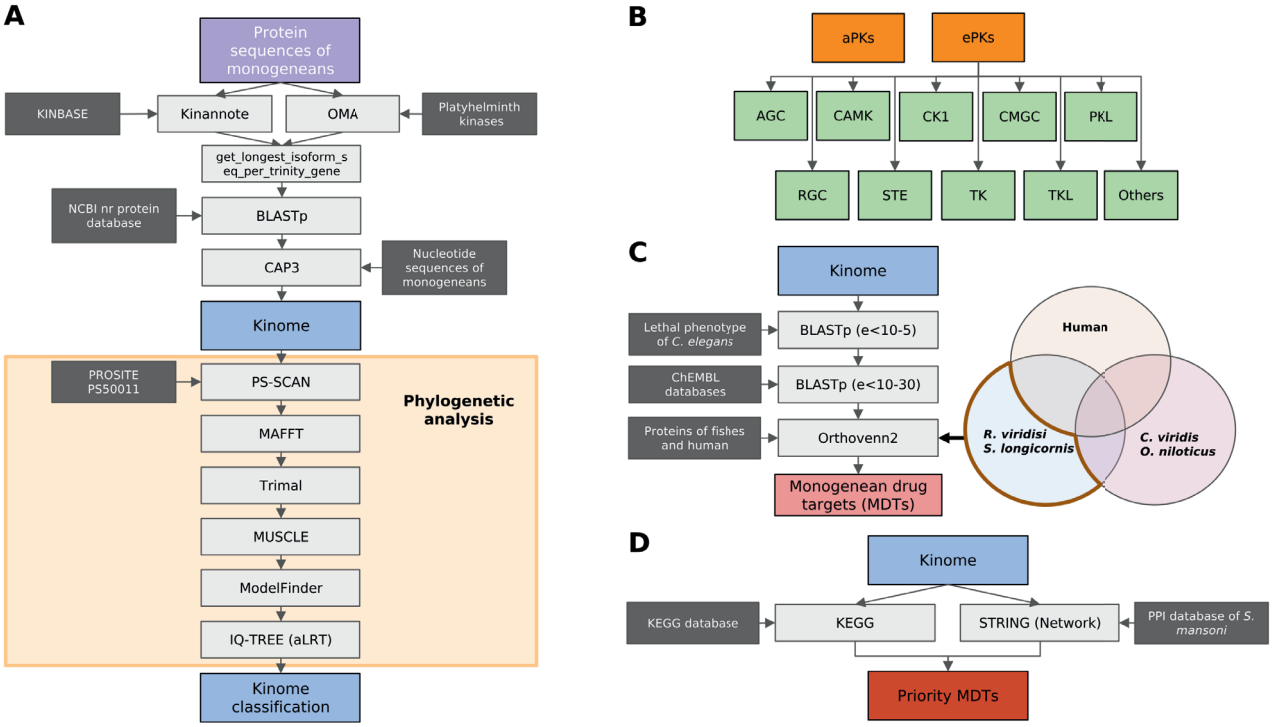

Fig. 1. Bioinformatic pipeline for the identification, classification, and selection of potential drug targets. A) Pipeline for the identification and classification of monogenean kinases. B) Classification of protein kinases. C) Pipeline used for the identification of drug targets. D) Pipeline used for the prioritization of monogenean drug targets (MDTs). ePKs, eukaryotic protein kinases; aPKs, atypical protein kinases; AGC, cAMP-dependent protein kinase/protein kinase G/protein kinase C extended family; CAMK, calcium/calmodulin-dependent kinase; CK1, cell kinase 1; CMGC, cyclin-dependent kinases and other close relatives; PKL, protein kinase-like; RGC, receptor guanylate cyclase; STE, MAP kinase cascade kinases; TK, protein tyrosine kinase; TKL, tyrosine kinase-like.

tations. These sequences were aligned against the NCBI nonredundant protein database version 5 (Sayers *et al*., 2022) using BLASTp 2.12.0 (e-value < 0.05) (Camacho *et al*., 2009) to eliminate non-kinase sequences and possible contamination of bacteria, viruses, fungi, and fish. To avoid overrepresentation of genes, the longest isoform per gene was extracted using the Trinity script "get_longest_isoform_seq_per_trinity_gene.pl".

In addition, the proteins were classified by aligning the kinase sequences against the database of Kinbase (Manning *et al*., 2022) using BLASTp. Sequences with an e-value > $10e^{-5}$ were removed. The sequences obtained from Kinannote, OMA, and BLASTp were used to classify the putative kinases by group, family, and subfamily, according to KinBase, which is a database containing information on human kinases and their homologs in other eukaryotes (Fig. 1B). Then, this classification was confirmed by phylogenetic analysis, as described below.

Phylogenetic analysis

Phylogenetic analysis was performed for each major group of kinases using their conserved catalytic domain (PROSITE domain type PS50011), which was predicted using PS-SCAN 2.0 (de Castro *et al*., 2006). The catalytic domain sequences were aligned using MAFFT 7.31 (Katoh & Standley, 2013) with the parameters "--localpair" and "maxiterate 1000", and then the gaps were removed with Trimal (Capella-Gutiérrez *et al*., 2009) using the parameter "-gappyout". The alignment was refined in MUSCLE 3.8.31 (Edgar, 2004). The analysis included reference kinases from *Homo sapiens*, *Drosophila melanogaster*, *Saccharomyces cerevisiae*, and *Caenorhabditis elegans* obtained from the KinBase database.

The tree was constructed with IQ-TREE 1.6.12 (Nguyen *et al*., 2015), using the Shimodaira–Hasegawa-like approximate likelihood ratio test (SH-aLRT; 1000 replicates). The best evolutionary models were selected according to the Bayesian Information Criterion (BIC) with the ModelFinder program (Kalyaanamoorthy *et al*., 2017), which is integrated into IQ-TREE. The trees were visualized and annotated in FigTree 1.4.2 (http://tree.bio.ed.ac.uk/software/figtree/). Nodes with support values ≥ 80 % were considered functional predictions with support.

Functional annotation based on Gene Ontology terms and the KEGG pathway

Putative kinases were functionally annotated using Gene Ontology (GO) terms and the Kyoto Encyclopedia of Genes and Genomes (KEGG) (Kanehisa & Goto, 2000). The PANNZER2 server (Törönen *et al*., 2018) was used to retrieve GO terms. The KEGG Automatic Annotation Server (KAAS 2.0) (Moriya *et al*., 2007) and BlastKOALA 3.0 (Kanehisa *et al*., 2016) were used to retrieve KEGG Orthology (KO) terms and then map to the KEGG terms using the KO database as reference (https://www.genome.jp/keggbin/get_htext?ko00001.keg; accessed May 6, 2021).

Inferring potential targets

Monogenean drug targets (MDTs) were selected from predicted kinomes according to homology (Fig. 1C). To that end, the *R. viridisi* and *S. longicornis* kinases with orthology to the *Caenorhabditis elegans* lethal RNAi phenotype, which represent potential targets for parasite control (Gahoi *et al*., 2019), were retrieved using BLASTp (e-value < $10e^{-5}$). Then, the retrieved monogenean sequences were aligned against the ChEMBL database (Gaulton *et al*., 2017) to identify potential kinase drug targets using BLASTp (e-value < $10e^{-30}$). Finally, the retained sequences were submitted to the Orthovenn2 web platform (Xu *et al*., 2019) to identify kinases shared between the monogeneans, the fish hosts *C. viridis* (NCBI SRA: SRP165941) and *O. niloticus* (NCBI Assembly: GCA_001858045.2), and humans. Potential target kinases of monogeneans were considered MDTs if they had orthologs in neither fish nor humans but did in the lethal phenotype of *C. elegans*. Subsequent analyses included only MDTs that we deemed priorities, which were selected as follows. Particular attention was paid to KEGG pathways involving only one or two kinases. If those kinases corresponded to an MDT by manual inspection, they were assigned priority (Fig. 1D). The rationale here was based on the assumption that an essential biological process is easier to inhibit if it depends on only a few proteins instead of several (Stroehlein *et al*., 2018). In addition, priority MDTs were also selected through a network analysis of protein–protein interactions (PPIs) performed for each monogenean species. To that end, each kinome was submitted to the STRING server (Snel *et al*., 2000) using the PPI database for *S. mansoni* and the parameters "high confidence cutoff of 0.7" and "zero node addition". The interaction network was exported, visualized, and analyzed within the Cytoscape 3.9.1 platform (Shannon *et al*., 2003). The cytoNCA 2.1.6 plugin (Tang *et al*., 2015) in Cytoscape was used to calculate the betweenness centrality, which is a value representing the participation of nodes in multiple pathways and that presents a positive correlation with its degree (Rai *et al*., 2021). MDTs with better centrality indices were assigned priority.

Protein Structural Modeling and Ligand Docking

Priority MDTs were mapped against the Protein Data Bank (PDB) database (www.rcsb.org) (Burley *et al*., 2017), using BLASTp (e-value < $10e^{-5}$), to identify those with known 3D structures. The molecular structures of MDTs were generated using the SWISS-MODEL server (Schwede *et al*., 2003) and downloaded in PDB file format. For each of the five target kinases selected, only the best model predicted by SWISS-MODEL was used in posterior analyses. The best predicted structures were selected based on the sequence identity and the evidence of the template used (Crystal structure of apo murine Nf-kappaB inducing kinase (NIK), Crystal structures of human p70S6K1-T389A (form II), Crystal structures of human p70S6K1-T389E Discovery of a Novel, Potent and Selective Inhibitor of 3-Phosphoinositide Dependent Kinase (PDK1), and Structure of the N-terminal domain of SAD). The predicted

structure for each candidate MDT was used to perform molecular docking against all US Food and Drug Administration (FDA)-approved drugs from the ZINC20 database (Sterling & Irwin, 2015). The drug database included the 3D structure in Mol2 file format of approximately 2000 drugs. The prepare_ligand4.py script of AutoDock tools (Morris *et al.*, 2009) was used to translate each PDB file, of both MDT and drugs, into Protein Data Bank Partial Charge & Atom Type (PDBQT) format. The 3D structures of the modeled proteins along with model information are available in Supplementary File S1. For each of the combinations (receptor-ligand) of the five receptors and 2000 ligands, we explore the whole receptor space by generating a custom script to perform the molecular docking with AutoDock Vina 1.1.2 (Trott & Olson, 2010) in a 3-dimensional grid of 50x50x50 Angstroms and exhaustiveness of 16 (as recommended in the AutoDock Vina Manual, https://vina.scripps.edu/manual/).

Ethical Approval and/or Informed Consent

This article does not contain any studies with human participants or animals.

Results

Prediction of kinomes

Putative kinases of *R. viridisi* and *S. longicornis* were identified and classified using an integrative bioinformatic pipeline. The curated kinome of *R. viridisi* is composed of 159 ePKs and 1 aPK (Table 1), distributed across 64 families and 69 subfamilies. The curated kinome of *S. longicornis* comprises 191 ePKs and 2 aPKs (Table 1), distributed into 74 families and 80 subfamilies. More information about the results of each step in the bioinformatic pipeline is given in Supplementary Table S1 and the sequences in Fasta format are available in Supplementary File S2.

The kinase group having the most members was CMGC, followed by AGC, whereas the groups least represented were RGC, PKL, and aPKs (Table 1). The most represented families were CDK and CAMKL (Supplementary Table S1).

The classification of kinases described above was well supported (bootstrap ≥ 80 %) by the phylogenetic analysis, except for 13 kinases of *R. viridisi* and 18 of *S. longicornis* (Supplementary Figs. S1 – S9). The 31 kinases that had low bootstrap support were grouped with kinases from subfamilies or families that differed from the classification obtained using Kinannote, OMA, and BLASTp; thus, those kinases were only classified to the level supported by phylogenetic analysis (major group or family; Supplementary Table S1).

Functional annotation

Of the 160 kinases in *R. viridisi*, 106 were mapped to 1109 GO terms, whereas of the 193 *S. longicornis* kinases, 122 were mapped to 1125 GO terms (Supplementary Table S2). The GO term distribution was similar in both species. For the two species of monogeneans, the most represented GO terms were ATP binding (GO:0005524), protein kinase activity (GO:0004672), and protein serine/threonine kinase activity (GO:0004674) within of the Molecular Function category; protein phosphorylation (GO:0006468), peptidyl-serine modification (GO:0018209), and intracellular signal transduction (GO:0035556) within the Biological Process category; and integral component of membrane (GO:0016020), nucleus (GO:0005634), and cytoplasm (GO:0005737) within the Cellular Component category (Supplementary Fig. S10A).

Kinases from the two monogenean species mapped to 224 KEGG pathways (Supplementary Table S3). According to the KEGG database, the kinases mainly belonged to Brite Hierarchies (142 and 164 members in *R. viridisi* and *S. longicornis*, respectively) and the Environmental Information Processing category (57 and 75 members, respectively) (Supplementary Fig. S10B and Supplementary

Table 1. Classification of kinases in various platyhelminths.

Group		AGC	CAMK	CK1	CMGC	PKL	Other	RGC	RGC/CAMK	STE	TK	TKL	Unknown	Atypical	**Total general**
Monogenea	*R. viridisi*	29	17	10	30	5	25	2	-	13	19	9	-	1	**160**
	S. longicornis	32	27	11	34	4	29	2	-	13	26	13	-	2	**193**
Cestoda	*E. multilocularis*	43	35	11	44	-	30	5	-	25	46	26	-	-	**265**
	E. granulosus	41	35	11	43	-	30	5	-	24	42	22	-	-	**253**
	T. solium	56	36	12	61	-	37	11	1	28	45	23	-	-	**310**
	H. microstoma	48	33	13	64	-	30	6	-	28	33	18	-	-	**273**
Trematoda	*S. mansoni*	34	38	9	44	-	39	3	-	27	34	19	5	-	**252**
	S. haematobium	39	41	9	51	4	40	3	-	27	31	20	4	-	**269**
	S. japonicum	27	33	8	41	6	35	4	-	23	31	13	-	1	**222**
	F. gigantica	44	55	13	66	-	45	0	-	35	34	16	-	-	**308**
	F. buski	33	42	10	47	-	40	1	-	23	39	15	-	-	**250**

Information taken from Andrade *et al*. (2011), Young *et al*. (2011), Tsai *et al*. (2013), Stroehlein *et al*. (2015), Giuliani *et al*. (2018), and the present study.

Table 2. Kinases of *Rhabdosynochus viridisi* and *Scutogyrus longicornis* predicted to be monogenean drug targets (MDTs).

Species	ID	Classification (group/family/subfamily)	Priority for molecular docking ***
R. viridisi	Contig3487.p1	(CK1/CK1/CK1-A)	-
R. viridisi	Contig474.p1	(AGC)	-
R. viridisi	Contig130.p1	(AGC/RSK/RSKp70)	1**
R. viridisi	TRINITY_DN128_c1_g2_i2.p1	(TK/Ack)	-
R. viridisi	TRINITY_DN430_c0_g1_i4.p1	(TK/Fer)	-
R. viridisi	Contig2641.p1	(AGC/PKG)	-
R. viridisi	TRINITY_DN4663_c0_g2_i1.p1[a]	(CAMK/CAMKL/BRSK)	-
R. viridisi	Contig4408.p1	(CAMK/MLCK)	-
R. viridisi	Contig5219.p1	(CMGC/GSK)	-
R. viridisi	TRINITY_DN1044_c0_g2_i2.p2	(STE/STE11/MEKK1)	3*
R. viridisi	Contig3286.p1	(CAMK/CAMKL/MARK)	-
S. longicornis	Contig1825.p1	(TK/Ack)	-
S. longicornis	TRINITY_DN2502_c0_g3_i3__g.25108	(TK)	-
S. longicornis	TRINITY_DN770_c0_g1_i4__g.58217	(TK/Fer)	-
S. longicornis	Contig3492.p1	(AGC/PKG)	-
S. longicornis	TRINITY_DN1812_c0_g1_i2__g.5503	(AGC/RSK/RSKp70)	1*
S. longicornis	TRINITY_DN7486_c0_g6_i1__g.37596	(CAMK/MLCK)	-
S. longicornis	TRINITY_DN1065_c0_g1_i1__g.51110	(CMGC/GSK)	-
S. longicornis	TRINITY_DN14095_c0_g1_i1__g.60752	(CK1/CK1/CK1-A)	-
S. longicornis	Contig772.p1[a]	(CAMK/CAMKL/BRSK)	2**
S. longicornis	Contig773.p1[a]	(CAMK/CAMKL/BRSK)	2**
S. longicornis	Contig3764.p1	(CAMK/CAMKL/MARK)	-

*Prioritized by KEGG; **prioritized by network; ***same numbers indicate orthologous groups; [a]TRINITY_DN4663_c0_g2_i1.p1, had two orthologous paralogues in *S. longicornis*: Contig772.p1 and Contig773.p1.

Table S4). In *R. viridisi*, the predominant terms included protein kinases [BR:ko01001], membrane trafficking [BR:ko04131], chromosome, and associated proteins [BR:ko03036], pathways in cancer [PATH:ko05200], PI3K-Akt signaling pathway [PATH:ko04151]. Meanwhile, *S. longicornis* exhibited a similar profile with protein kinases [BR:ko01001], MAPK signaling pathway [PATH:ko04010], pathways in cancer [PATH:ko05200], and membrane trafficking [BR:ko04131] as the most abundant terms.

Drug target prediction

Using the bioinformatics pipeline, 152 kinases of *R. viridisi* were identified as orthologous with the *C. elegans* lethal RNAi phenotype. Of these, 128 mapped to the ChEMBL database of target associations; 10 had equivalents in *S. longicornis* but were absent in the fish hosts *C. viridis* and *O. niloticus*. In addition, we found one kinase specific to *R. viridisi*. In *S. longicornis*, 182 kinases mapped to the *C. elegans* lethal RNAi phenotype. Of these, 157 mapped to the ChEMBL database and 11 had equivalents in *R. viridisi* but not with *C. viridis* and *O. niloticus* (Supplementary Table S5; one kinase in *R. viridisi*, TRINITY_DN4663_c0_g2_i1.p1, had two orthologous paralogues in *S. longicornis*).

These 11 kinases of *R. viridisi* and the 11 of *S. longicornis*, which we assigned as MDTs (Table 2 and Supplementary Table S5), belong to the major groups CK1, AGC, TK, CAMK, STE, and CMGC, and are listed in the KEGG BRITE categories metabolism (09181), signal transduction (09132), genetic information processing (09182), cellular community—eukaryotes (09144), endocrine system (09152), and immune system (09151). According to the analysis carried out with Orthovenn2, all the kinases classified as MDTs turned out to be homologous to proteins with known 3D structures. Of the 22 MDTs mentioned above, two were unique kinases involved in two separate KEGG pathways (Supplementary Table S5), and three had high values of betweenness centrality (betweenness ≥ 168; Supplementary Table S6). Therefore, these five MDTs were considered priorities for the molecular docking analysis (Table 2 and Supplementary Fig. S11).

Table 3. The highest binding affinities (≤ −9.0 kcal/mol) obtained from molecular docking using a set of FDA-approved drugs.

Drug	Species	Receptor id	Binding affinity (kcal/mol)
Dihydroergotamine[a]	*S. longicornis*	Contig773.p1 and Contig772.p1	−9.7
Lomitapide[b]	*R. viridisi*	TRINITY_DN1044_c0_g2_i2.p2	−9.5
Dihydroergotamine[a]	*R. viridisi*	TRINITY_DN1044_c0_g2_i2.p2	−9.4
Ergotamine[c]	*S. longicornis*	Contig773.p1 and Contig772.p1	−9.2
Bicalutamide[d]	*R. viridisi*	Contig130.p1	−9.1
Piroxicam[e]	*R. viridisi*	TRINITY_DN1044_c0_g2_i2.p2	−9.0
Suvorexant[f]	*R. viridisi*	TRINITY_DN1044_c0_g2_i2.p2	−9.0

Description according DrugBank (Wishart et al., 2018): [a]dihydroergotamine (ZINC ID: ZINC3978005) is an ergot alkaloid used in the acute treatment of migraine and cluster headaches; [b]lomitapide (ZINC ID: ZINC27990463) is a microsomal triglyceride transfer protein inhibitor used to lower cholesterol associated with homozygous familial hypercholesterolemia, reducing the risk of cardiovascular events such as myocardial infarction and stroke; [c]ergotamine (ZINC ID: ZINC52955754) is an α1-selective adrenergic agonist vasoconstrictor used to treat migraines with or without aura and cluster headaches; [d]bicalutamide (Casodex, ZINC ID: ZINC538564) is an androgen receptor inhibitor used to treat stage D2 metastatic carcinoma of the prostate; [e]piroxicam (ZINC ID: ZINC51133897) is an NSAID used to treat the symptoms of osteoarthritis and rheumatoid arthritis; [f]suvorexant (ZINC ID: ZINC49036447) is an orexin receptor antagonist used to treat insomnia characterized by difficulties with sleep onset and/or sleep maintenance.

Molecular docking

The molecular docking was performed using FDA-approved drugs from the ZINC database and five priority MDTs. The MDTs used for the molecular docking analysis are shown in Table 2. Dihydroergotamine was found to have the highest affinity (ΔG = −9.7 kcal/mol) interactions with kinases of both *S. longicornis* and *R. viridisi* (Table 3). The scores obtained from the molecular docking are shown in Supplementary Table S7.

Discussion

Kinomes of R. viridisi and S. longicornis

The bioinformatic pipeline, the inspection of each protein, and the phylogenetic analysis let us identify and classify the kinomes of *S. longicornis* and *R. viridisi* with a high degree of confidence. The number of kinases in *R. viridisi* and *S. longicornis* was lower compared to other metazoan organisms (Stroehlein *et al.*, 2018), including parasitic helminths such as cestodes, trematodes, and other flatworms. Given that our identification relied on transcriptomic data from adult organisms, unlike the genomic data used in previous studies, the observed decrease in the number of kinases in monogeneans may be attributed to the discrepancy in data sources. All the major kinase groups were represented by fewer members in monogeneans than in other platyhelminths, except for CK1, which was represented similarly in monogeneans, trematodes, cestodes, and other metazoans (Stroehlein *et al.*, 2018).

In both monogenean species studied here, members of CMGC and AGC were the most abundant kinases. Similarly, CMGC is the principal group in most cestodes and trematodes, followed by AGC in the case of cestodes. In the trematode *S. mansoni*, the abundance of CMGC kinases has been linked to essential processes such as control cell proliferation, organelle replication, and segregation (Andrade *et al.*, 2011), whereas in the cestode *T. solium*, these have been associated with hermaphroditism (Arora *et al.*, 2020). This dominance of CMGC and AGC among platyhelminth species is distinct from other metazoa (Stroehlein *et al.*, 2018). Therefore, higher representation of these major groups of kinases might tie them to crucial roles in platyhelminths.

Kinase drug targets and molecular docking

The workflow facilitated the identification of MDTs. Although the transcriptomes of *R. viridisi* and *S. longicornis* were obtained from adult organisms, it is essential to acknowledge the lack stage-specific transcriptomes, which prevents definitive conclusions about the expression of the predicted MDT kinases at all stages of development. However, it is notable that most kinases play fundamental roles in crucial signaling processes throughout the parasite life cycle, such as in *S. haematobium*, where 214 of 258 transcribed kinases show expression in all sexes and life stages studied (Stroehlein *et al.*, 2015), underscoring the fundamental functions these kinases likely play throughout various stages of parasite development.

CAMK, AGC, and TK were the most represented major groups in the 22 MDTs. This is consistent with the results of Giuliani *et al.* (2018), whose bioinformatics pipeline also found CAMK and AGC kinases as the most represented drug targets. Coincidentally, members of the CAMKL family were the most abundant in both studies—five in ours, four in the aforementioned study. The kinase GSK (group CMGC) of *R. viridisi* and *S. longicornis* was proposed here as an MDT, which is consistent with the result of a compar-

ative chemogenomics study in *S. mansoni* (Caffrey *et al.,* 2009). Members of GSK are involved with cell proliferation, regulation of glycogen synthase, and participation in the Wnt signaling pathway, which is an integral component of embryonic development (Grimes & Jope, 2001). Inhibition of GSK3 stops *S. mansoni* laying eggs (Morel *et al*., 2014). Therefore, the GSK family members represent potential drug targets in monogeneans.

To find candidate drugs to be repurposed against these parasites, we performed molecular docking analysis with 2000 FDA-approved drugs and the five priority MDTs. The drug–kinase pairs with the highest affinities were dihydroergotamine–BRSK and dihydroergotamine–MEKK1. Dihydroergotamine is used to treat migraine through vasoconstriction in the cranial vascular bed by its agonist activity at 5-HT, α-adrenergic, and dopaminergic receptors (Ramírez-Rosas *et al*., 2013). It actively inhibits the growth of the parasite *Plasmodium falciparum* (Weisman *et al.,* 2006). Although experimental studies in mammals indicate that dihydroergotamine acts by activating α-adrenergic and 5-HT receptors, our cheminformatic study showed that in monogeneans, the kinases BRSK and MEKK1 might be targets of this drug. Therefore, the efficacy of dihydroergotamine against these parasites should be tested in future experiments.

The interaction between ergotamine and BRSK kinase was one of the strongest in our study. Ergotamine is an α_1-selective adrenergic drug used to treat migraine and has affinity for a wide variety of receptors, such as 5-HT, dopamine, and noradrenaline receptors, and induces contraction of the peripheral, pulmonary, cerebral, and coronary arteries (Dahlöf & Maassen Van Den Brink, 2012). In a study performed by Chan *et al*. (2018), ergotamine demonstrated remarkable efficacy in combating *S. mansoni* infection. In that study, schistosome-infected mice exhibited reduced parasite load and egg production when treated with ergotamine. Moreover, in the host, the drug mitigated the organ damage induced by the infection and lowered mortality rates. Although the repeated use of ergotamine in humans is not feasible due to secondary effects (Chan *et al.,* 2018), it is unknown whether its mechanism of action would lead to similar side effects in fish. Therefore, future research should be directed toward validating the effect of ergotamine as a possible anti-monogenean agent. The activity of ergotamine on BRSK kinases, in addition to its known activity at G-protein-coupled receptors, and its selectivity, remains to be biochemically determined.

Another of the strongest interactions was between lomitapide and MEKK1 kinase. Lomitapide inhibits lipid transfer by directly binding to the microsomal triglyceride transfer protein in the liver and intestines, and is used to treat homozygous familial hypercholesterolemia in adults (Rader & Kastelein, 2014). Experimental evaluation of lomitapide against malaria parasites demonstrated its inhibition of β-haematin formation and parasite growth (de Sousa *et al*., 2020). In addition, this drug inhibits hemozoin formation in the parasite, the same mechanism by which traditional antimalarial drugs work (de Sousa *et al*., 2020).

This is the first study aimed at predicting and classifying kinases in monogeneans. From our bioinformatics pipeline, 22 kinases were identified as MDTs, of which five were proposed as leads. We suggest that drugs with conceivable anti-monogenean activity—as hinted at by a molecular docking analysis—could be experimentally evaluated for repurposing, particularly dihydroergotamine, ergotamine, and lomitapide given their high affinity with the monogenean kinases BRSK and MEKK1. Further research in this field is essential to enhance the control of parasitic diseases in finfish aquaculture.

Data availability

Supplementary Tables, Supplementary Figures, and Supplementary Files are available in the Mendeley Data repository https://doi.org/10.17632/6b9r75m9ft.1.

Conflict of Interest

Authors state no conflict of interest.

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