Characterisation of *Trichomonas vaginalis* ENT-family nucleoside transporters by heterologous expression in ΔENT *Trypanosoma brucei* and *Leishmania mexicana* strains.

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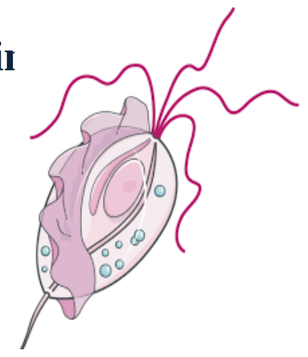


INTRODUCTION

Trichomonas vaginalis is a highly prevalent human urogenital protozoan parasite and the causative agent of trichomoniasis, the most common non-viral sexually transmitted infection (STI) globally. Chronic infection of the reproductive tract leads to an increased risk of secondary infections, such as human immunodeficiency virus and other STIs. As with all protozoan parasites, *T. vaginalis* lacks *de novo* synthesis of purines. It is also unable to synthesise pyrimidine nucleotides, instead relying on salvage pathways as the main source of nutrients from the host. The salvage of nucleosides and nucleobases is believed to be dependent on the equilibrative nucleoside transporter (ENT) family, marking these proteins out as potential drug targets to disrupt the uptake of nutrients by the parasite. An ideal strategy to explore this further would be to express *T. vaginalis* ENT genes (TvENTs) by cloning them into a compatible heterologous system, where they can be studied individually to characterise the transport of nucleosides. All nine TvENT genes were successfully sub-cloned into the vector pHD1336 for expression in *Trypanosoma brucei brucei*, from which the adenosine transporter 1 had been deleted (TbAT1-KO). Transport assays established that TVENT3 transported cytidine the most efficiently, and TvENT6 transported uridine. However, high background levels of uridine and of purine nucleoside transport by P1-type trypanosome transporters confounded the results, prompting the search for a more suitable expression system. As such, a Leishmania mexicana strain from which all the nucleoside transporter genes (NT1.1, NT1.2, NT2) were deleted was constructed using CRISPR/cas9. So far, the nucleoside transport *null* line of *L. mexicana* has been confirmed for uptake of adenosine, uridine, thymidine, guanosine and inosine, and three TvENTs have been introduced in this cell line for characterisation. As we have recently identified adenosine analogues with powerful antitrichomonal activity the identification and characterisation of the adenosine transporters is expected to contribute to this emerging drug strategy.

BACKGROUND

TREATMENT AND THERAPY

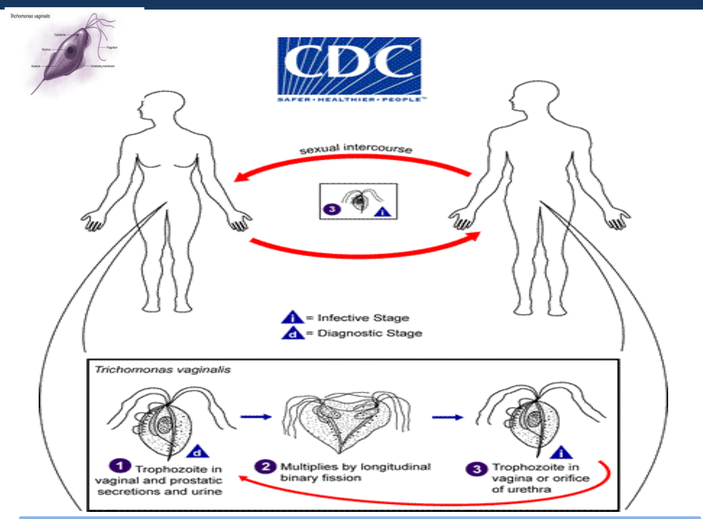
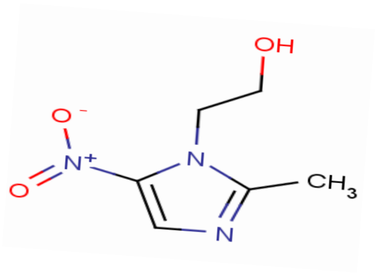


Figure 3: The life cycle of T. vaginalis. Infection occurs through vaginal or prostatic secretions during sexual intercourse between humans. Once the parasite enters a new host, it binds to epithelial cells and replicates by longitudinal binary fission and can be passed on to infect others (CDC 2017). Adapted from https://www.cdc.gov/dpdx/trichomoniasis/index.html

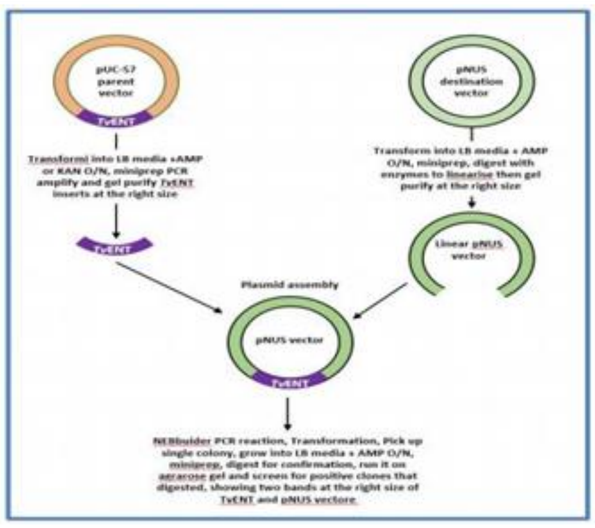


Structure of Metronidazole, it is a member of class imidazoles substitute

MOLECULAR ASPECT

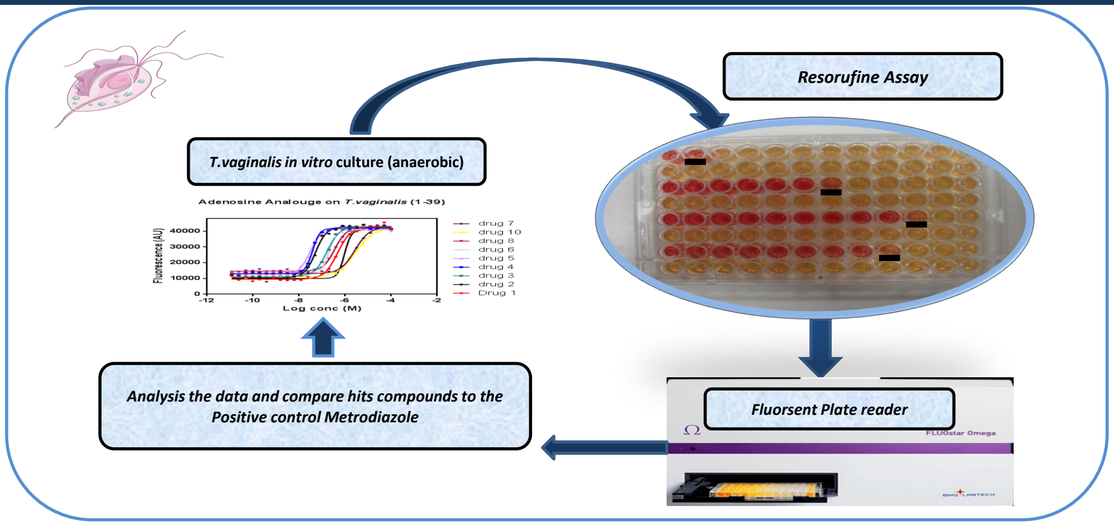
No.	ENT ID genes (Tricdb.org)	Size (bp)
1	ENT 1-TVAG_166380	1388
2	ENT2-TVAG_192810	1023
3	ENT3-TVAG_271560	1275
4	ENT4-TVAG_441760	1215
5	ENT5-TVAG_483030	1212
6	ENT6-TVAG_053320	1278
7	ENT7-TVAG_101510	1218
8	ENT8-TVAG_271570	1299
9	ENT9-TVAG_341290	1239

Table 2: Synthetic TvENT genes present in the pUC57-Kan and pUC57-Amp vector.



Schema for subcloning the TvENT genes into the pNUS vector.

MATERIAL & METHODS

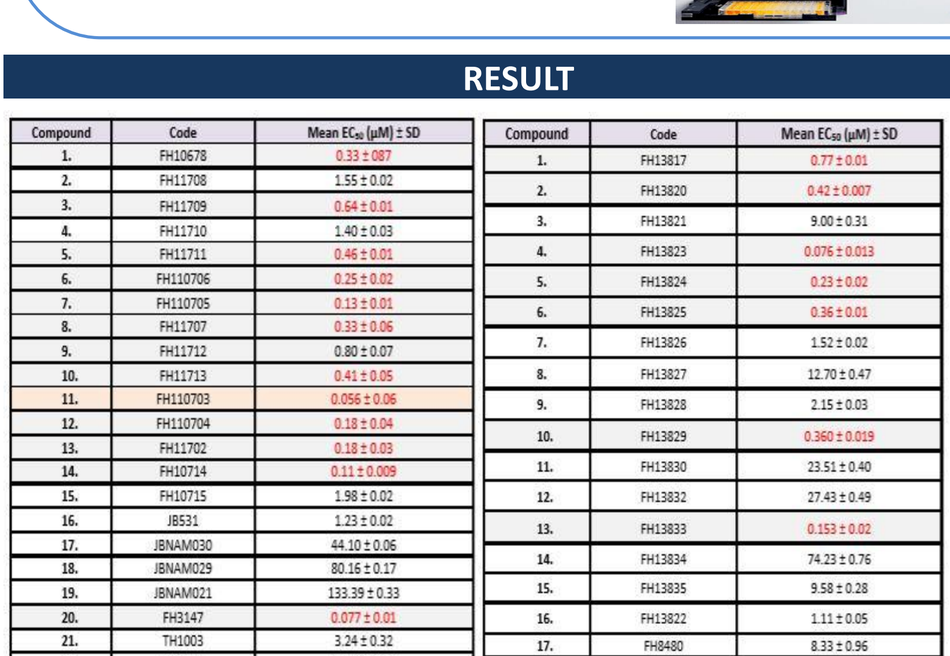


RESULT

Compound	Code	Mean EC_{50} (µM) ± SD
1.	FH10678	0.33 ± 087
2.	FH11708	1.55 ± 0.02
3.	FH11709	0.64 ± 0.01
4.	FH11710	1.40 ± 0.03
5.	FH11711	0.46 ± 0.01
6.	FH110706	0.25 ± 0.02
7.	FH110705	0.13 ± 0.01
8.	FH11707	0.33 ± 0.06
9.	FH11712	0.80 ± 0.07
10.	FH11713	0.41 ± 0.05
11.	FH110703	0.056 ± 0.06
12.	FH110704	0.18 ± 0.04
13.	FH11702	0.18 ± 0.03
14.	FH10714	0.11 ± 0.009
15.	FH10715	1.98 ± 0.02
16.	JB531	1.23 ± 0.02
17.	JBNAM030	44.10 ± 0.06
18.	JBNAM029	80.16 ± 0.17
19.	JBNAM021	133.39 ± 0.33
20.	FH3147	0.077 ± 0.01
21.	TH1003	3.24 ± 0.32
22.	FH5319	55.41 ± 0.24
23.	JB588	0.715 ± 0.05
24.	JB602	12.70 ± 0.37
25.	FH13749	15.83 ± 0.19
26.	FH13778	3.997 ± 0.23
27.	FH13779	1.378 ± 0.18
28.	FH13783	4.091 ± 0.44
29.	FH13809	0.330 ± 0.03
30.	FH13808	8.073 ± 0.47
31.	AF1012	8.288 ± 0.76
32.	JB609	0.357 ± 0.02
Control	Metronidazole	0.520 ± 0.03

Compound	Code	Mean EC_{50} (µM) ± SD
1.	FH13817	0.77 ± 0.01
2.	FH13820	0.42 ± 0.007
3.	FH13821	9.00 ± 0.31
4.	FH13823	0.076 ± 0.013
5.	FH13824	0.23 ± 0.02
6.	FH13825	0.36 ± 0.01
7.	FH13826	1.52 ± 0.02
8.	FH13827	12.70 ± 0.47
9.	FH13828	2.15 ± 0.03
10.	FH13829	0.360 ± 0.019
11.	FH13830	23.51 ± 0.40
12.	FH13832	27.43 ± 0.49
13.	FH13833	0.153 ± 0.02
14.	FH13834	74.23 ± 0.76
15.	FH13835	9.58 ± 0.28
16.	FH13822	1.11 ± 0.05
17.	FH8480	8.33 ± 0.96
18.	FH8481	1.54 ± 0.03
Control	Metronidazole	0.54 ± 0.01

- **Table 1**: EC5O values (mean ± SD) for the initial screen of 32 nucleoside analogue compounds with EC50 <1 µM are highlighted in red. Of the 15 hit compounds, 13 had greater inhibitory activity vs. T. vaginalis compared with the current first-line treatment metronidazole



-**Figure 1**:Sample sigmoidal curve outputs from the fluorescence reading of the resorufin drug-sensitivity assay for a selection of adenosine analogue compounds and metronidazole

RESULT

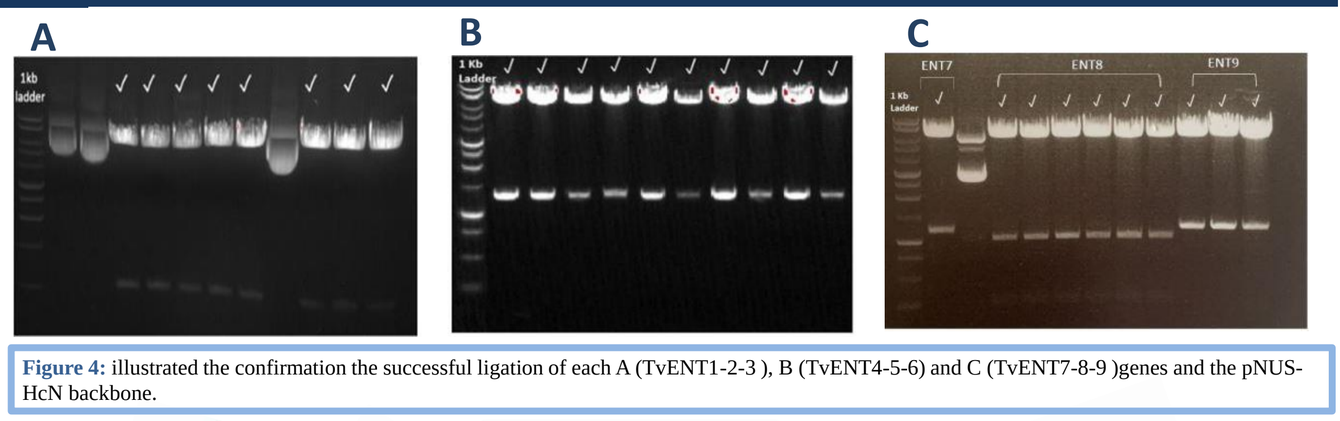
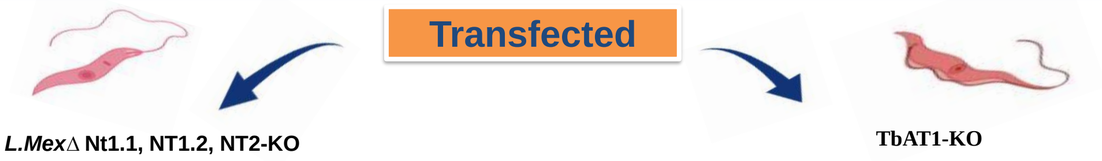


Figure 4: illustrated the confirmation the successful ligation of each A (TvENT1-2-3), B (TvENT4-5-6) and C (TvENT7-8-9)genes and the pNUS-HcN backbone.



for a more suitable expression system. As such, a Leishmania mexicana strain from which all the nucleoside transporter genes (NT1.1, NT1.2, NT2) were deleted was constructed using CRISPR/cas9. So far, the nucleoside transport *null* line of *L. mexicana* has been confirmed for uptake of adenosine, uridine, thymidine, guanosine and inosine, and three TvENTs [TvENT3, TvENT6,TvENT8] have been introduced in this cell line for characterisation.

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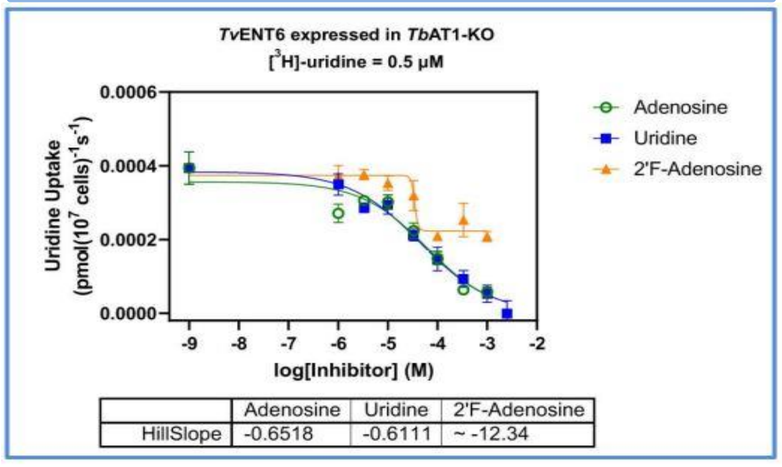

Figure 3 : Inhibitor profile of dose-dependent uptake assay of [3H]-uridine uptake of TvENT6 expressed in TbAT1-KO using vector pHD1336

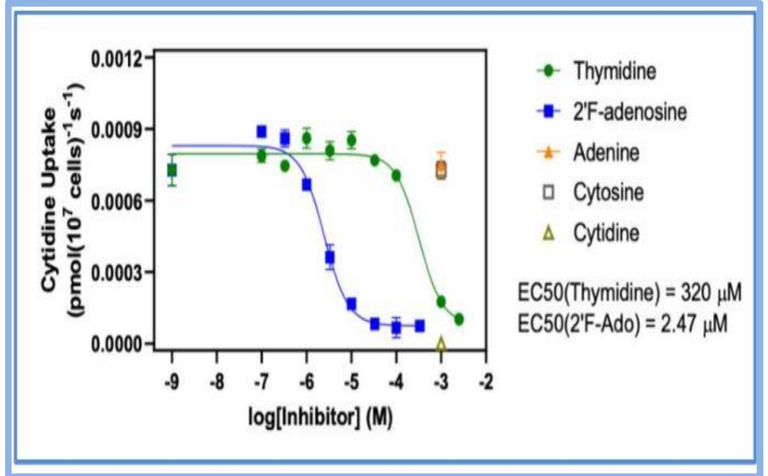

Figure 2: Inhibitor profiles of [H]-cytidine uptake of TvENT3 expressed in TbAT1-KO using vector Phd1336

CONCLUSIONS

- There is a clear need for new and effective treatments for trichomoniasis, and the development of a novel, high-throughput drug-screening assay specific for T. vaginalis will speed up the search for candidate drugs.
- Since T. vaginalis is unable to synthesise purine nucleotides de novo the parasite relies on a nucleoside salvage pathway mediated by the enzymes PNP and PNK, Inhibiting the enzymatic action of PNK may result in the inhibition of trophozoite growth and lead to cell apoptosis
- the most promising mechanism of action for potential new treatments is to exploit a relatively simple pathogenic pathway that is both essential and unique to T. vaginalis infection

FUTURE WORK

- Growth curve analysis will be performed for each of the hit compounds shown to be highly effective at inhibiting the activity of T. vaginalis compared with metronidazole
- g fluorescence slides will also be investigated in order to examine the effects of the hit compounds on *T. vaginalis* morphology, specifically on cell nuclei and membranes.
- evaluate the toxicity of the hit compounds on mammalian cell lines (e.g. human embryonic kidney cells) and determine the selectivity index, to help guide decisions on further drug development.
- use the resorufin-based high-throughput drug-sensitivity assay to screen nucleoside analogues vs. T. foetus and against *T. Gallinae* and compare the EC50 values with those obtained for *T. vaginalis.*
- conduct a metabolomics experiment and RNAseq with *T. vaginalis* TH1012-Res (strain resistant to nucleoside analogue TH1012, under construction) and control in the presence and absence of TH1012. The aim is to determine whether the nucleoside analogue will be phosphorylated, aand whether other changes in nucleotide levels can be detected.
- Continue transfection of all TvENT's into 'Super-KO' of L. mexicana and study characterisation of nucleobase transporter.

AKNOWLEDGMENT: