Non-natural myristate analogues: Synthesis and their potent, selective activity upon bloodstream *Trypanosoma brucei*

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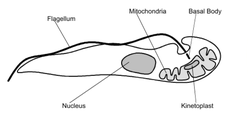
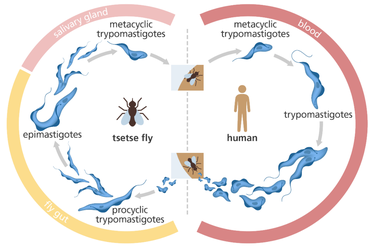
INTRODUCTION

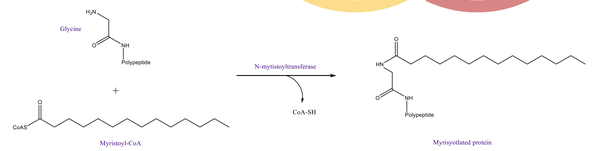
Inadequate and antiqued drugs for treating sleeping sickness, a neglected tropical disease caused by the protozoan *Trypanosoma brucei* (*T. brucei*), remains a persistent problem across many developing countries.

One chemotherapeutic target, the N-myristoyltransferase (NMT) has received significant attention in recent years and has been validated as drug target against T. *brucei*; ablating the NMT gene led to cell death and is thus essential for parasite survival. NMT is responsible for the attachment of myristate from myristoyl-CoA to the N-terminal glycine residue of specific proteins. Myristoylation is also required for the remodelling and exchange steps in their essential glycosylphosphatidylinositol (GPI) anchor biosynthesis.[2,3]

By synthesizing trypanocidal myristate analogues, it is hoped that these structural mimics will be taken up and utilized by *T. brucei*, leading to the interference and disruption of their essential downstream metabolic pathways.

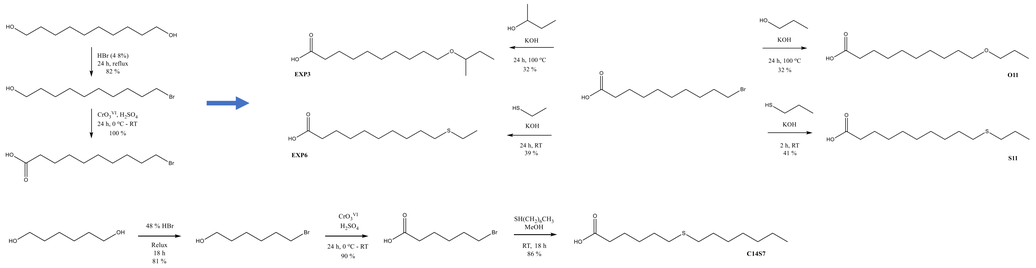






SYNTHESIS and TOXICITY

The synthesis of myristate analogues with an EC_{50} value (10 % foetal bovine serum) ≤30 µM is shown below. Most analogues were synthesized via and base catalyzed S_N2 reaction except for C11-G which was synthesized via an EDC/HOBt coupling reaction.



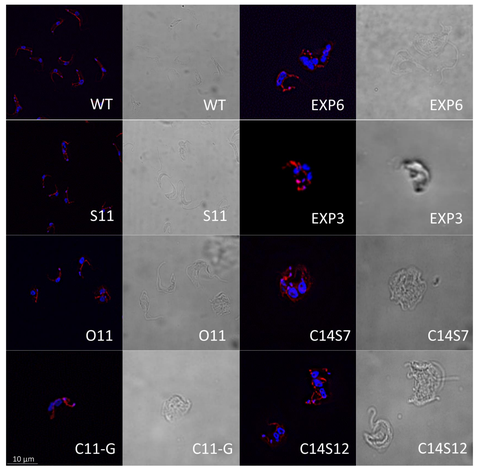
All compounds aside from EXP6 showed significantly lower EC_{50} values in lower FBS environments. All compounds also showed a pleasing selectivity towards *T. brucei* with all EC_{50} values being >50 times greater for the comparative experiments on HeLa cell lines.

Sulfoxide analogues of S11, EXP6, C14S7 and C14S12 were synthesized to compare oxidative states, however these compounds had far higher EC_{50} values than their reduced counterparts.

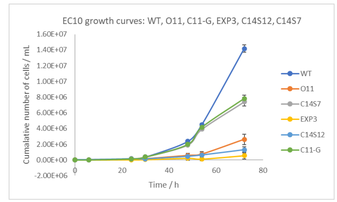
A C15 chain analogues of S11 similarly gave a much higher EC_{50} value, highlighting the importance of a C14 chain in these trypanocidal compounds.

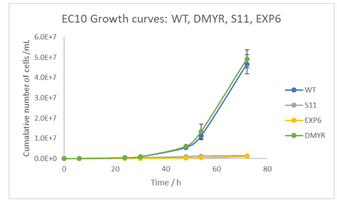
Compound	BSF *T. brucei* (10 % FBS) EC_{50} / µM	BSF *T. brucei* (5 % FBS) EC_{50} / µM	PCF *T. brucei* EC_{50} / µM	*L. Major* EC_{50} / µM	HeLa EC_{50} / µM	Methylated analogue BSF *T. brucei* (5 % FBS) EC_{50} / µM
S11	3.44 ± 0.43	1.09 ± 0.13	> 250	16.40 ± 0.90	>250	2.35 ± 0.27
O11	30.73 ± 1.84	5.10 ± 1.38	230.16 ± 62.35	84.21 ± 5.88	>250	67.93 ± 8.84
EXP6	16. 33 ± 0.98	21.65 ± 1.59	>250	31.06 ± 2.48	>250	50.52 ± 16.85
EXP3	14.22 ± 1.49	4.64 ± 0.47	>250	39.83 ±1.22	>250	16.00 ± 1.24
C11-G	12.06 ± 0.67	8.21 ± 0.65	36.15 ± 2.27	134.39 ± 8.38	>250	>250
C14S7	1.86 ± 0.28	0.45 ± 0.03	57.25 ± 4.76	48.28 ± 9.78	>50	4.09 ± 0.22
C14S12	22.95 ± 2.33	7.11 ± 0.40	>250	213.50 ± 33.57	>250	48.13 ± 7.93

EXPERIMENTAL RESULTS



(BSF, 10% FBS) Cells were incubated with EC_{10} concentration of drug for 48 h.

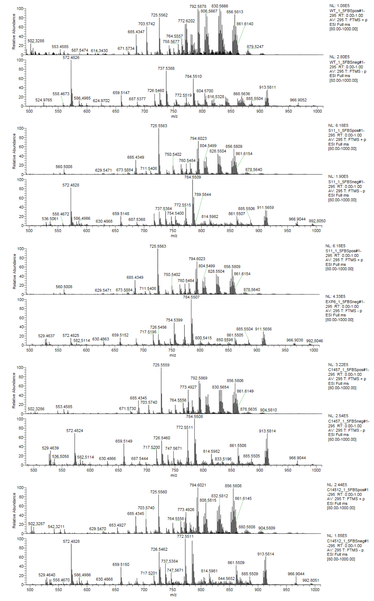




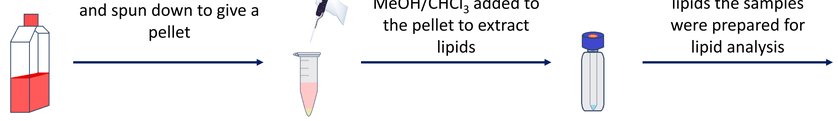
Cells were incubated in HMI-11 media (10% FBS) with EC_{10} concentration of drugs. The cells were maintained so that their concentration never exceeded 1 x 10^6 cells / mL. Results are made from triplicate experiment.

Microscope imaging of the parasites after 48 h incubation with EC_{10} concentrations of compounds highlighted several morphological changes including 'Big Eye' phenotype in addition to large numbers of nuclei and kinetoplast in parasites.

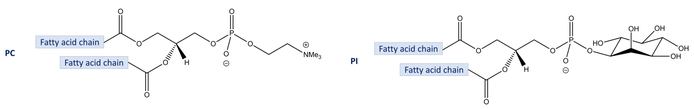
Growth curves showed a significant change to the cumulative number or parasites over a 72 h period. DMYR was used as a deuterated myristate control and was expected to have little-to-no effect on parasite growth rate.



Lipid analysis:



Initial analysis of mass spectra suggests significant changes to some PC and PI species



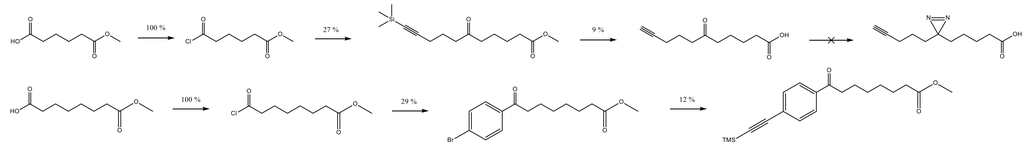
Fatty acid methyl ester (FAME) analysis: The same extraction method was used, and the extracted lipids were methylated before being prepared for FAME GC-MS analysis. The relative abundance of fatty acid species was subsequently analyzed. Lysing the cells with water was then used before the MeOH/$CHCl_3$ step to try and increase the intensity of the myristate peak following difficulty in detecting it.

CONCLUSIONS AND FUTURE WORK

To determine precisely which lipid species have changed, the next step is to analyze fragmentation patterns from the lipid mass spectra. This will also identify if and where my compounds are being incorporated into fatty acid chains.

^3H labelled myristate experiments may also help to quantify the amount of myristate analogues being taken up/ how much they block the uptake of ^3H labelled myristate.

Another topic of interest is the synthesis of a bifunctional fatty acid, which incorporated a terminal alkyne and a diazirine group. The synthesis of this will allow us to further understand the targets of these myristate analogues. While the synthesis is on-going, low yielding or unsuccessful reactions requires further research to optimize such reactions.



References:
1. https://www.yourgenome.org/facts/what-is-african-sleeping-sickness (accessed June 2021)
2. T. Bayliss et al., J. Med. Chem., 2017, 60, 9790-9806
3. Y. Hong, T. Kinoshita, Korean J. Parasitol., 2009, 47 (3), 197-204
4. https://commons.wikimedia.org/wiki/File:Trypanosome_Cell_Structure.png (accessed June 2021)