CBCS PROJECT PROPOSAL FORM (example)

PROJECT TITLE **Novel inhibitors of Trypanosoma brucei survival**

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Date of Submission 2012-03-30

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Project Abstract

(Please provide a brief summary of the proposed project and it's primary goals, max 1500 characters)

New drugs for treatment of Human African Trypanosomiasis (HAT) are urgently needed. Due to the lack of extensive market potential of developed therapies, this process is best suited for development in an academic environment. Our laboratories have developed a number of relevant in vitro proliferation and killing assays based on detection of oxidative metabolites for the evaluation of trypanocidal compounds with concomitant selectivity panels available to evaluate parasite-mammalian selective toxicity patterns. Predictive acute and chonic murine in vivo models are available for efficacy evaluation of discovered compounds in stage 1 and stage 2 infections. We wish to screen the CBCS compound collection in the hopes of discovering novel leads for the development of trypanocidal therapies. The ultimate goal of the collaboration will be to identify compounds with novel mechanisms of action and to evaluate these mechanisms as new avenues towards HAT therapy. Compounds suitable for further advancement towards drug development will be progressed into more advanced models of HAT in collaboration with our colleagues at the University of Rhodesia (Prof Fly). Assays for monitoring morphological and phenotypic alterations of parasites will be developed in collaboration with CBCS. The tentative publication strategy includes dislcosure of novel in vitro assays in concert with developed chemical probes from screen, novel developed in vivo efficacy models, and at least one publication/patent applications for new chemotypes showing promise as novel leads for the treatment of HAT.

Biological Background (max 2 pages)

(Please describe the underlying biology, data supporting biological relevance of proposed study, biological and chemical novelty in literature, application of potential discoveries. Include relevant literature references. Include figures and relevant tables).

African trypanosomiasis or African sleeping sickness is a disease with deadly outcome caused by the parasite *Trypanosoma brucei*. The parasite adopts several different life forms to ensure survival in the gut of the Tsetse fly, which serves as the vector for transfer to man, through transport to the salivary glands of the insect and following transfer to the bloodstream of the mammalian target upon insect biting (see illustration below). Two out of three *Trypanosoma brucei* sub-species can infect man (*gambiense* and *rhodesiense*), whereas the third sub-species *brucei* is infective in animals. The reason humans are resistant to the *brucei* sub-species is the presence of the human-specific serum protein, apolipoprotein L-1, which causes lysis of the *brucei* sub-species.[1] For this reason this sub-species is commonly used as a model organism in laboratories.

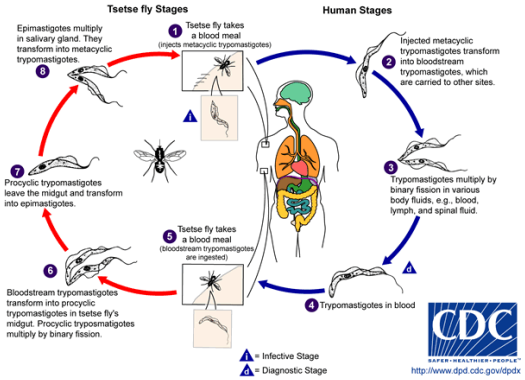
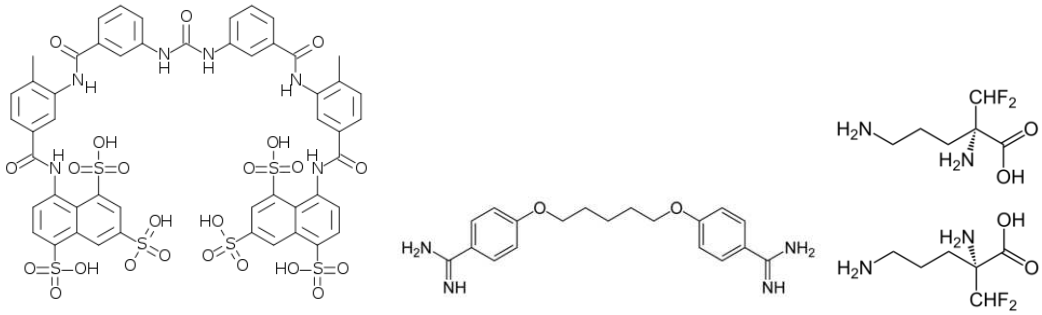


Illustration taken from http://www.dpd.cdc.gov/dpdx/HTML/TrypanosomiasisAfrican.htm

African trypanosomiasis occurs in two stages, referred to as the hemolymphatic and neurologic phases, respectively. The hemolymphatic phase is characterized by fever, headache and extreme swelling of lymph nodes. If left untreated it will over time cause more extensive damage including anaemia and organ dysfunctions. The neurological phase appears following parasite penetration of the blood-brain-barrier, which results in the symptoms that have given the disease the term sleeping sickness (*e.g.* confusion and irregular sleep cycles). Damage that occurs during the neurological phase is irreversible. The course of the disease depends on the infecting sub-species, with *rhodesiense* causing an acuter development than does *gambiense*.

If left untreated the disease is invariably fatal. There are treatments available and these depend on sub-species as well as on disease stage. Pentamidine isethionate (*gambiense*) and suramin (*rhodesiense*) are used to treat the hemolymphatic stage, whereas melarsoprol is used for late stage neurological disease. Also protocols involving nifurtimox and eflornithine and combination treatments with melarsoprol are used during the second stage. Unfortunately these treatments are not sufficiently effective and new approaches are urgently needed, particularly for the neurological phase. A significant number of efforts have been published lately, but few of these are effective in animal models of late stage disease.[2,3] There are some encouraging examples of promising compounds also in late stage models,[4,5] but until these have demonstrated efficacy and safety in man there is a very good rationale for additional efforts.



This coat enables an infecting T. brucei population to persistently evade the host's immune system, allowing chronic infection. The two properties of the VSG coat that allow immune evasion are:

- Shielding - the dense nature of the VSG coat prevents the immune system of the mammalian host from accessing the plasma membrane or any other invariant surface epitopes (such as ion channels, transporters, receptors etc.) of the parasite. The coat is uniform, made up of millions of copies of the same molecule; therefore the only parts of the trypanosome the immune system can 'see' are the N-terminal loops of the VSG that make up the coat.[5]
- Periodic antigenic variation - the VSG coat undergoes frequent stochastic genetic modification - 'switching' - allowing variants expressing a new VSG coat to escape the specific immune response raised against the previous coat.

Sequencing of the T. brucei genome has revealed a huge VSG gene archive, made up of thousands of different VSG genes. All but one of these are 'silent' VSGs, as each trypanosome expresses only one VSG gene at a time. VSG is highly immunogenic, and an immune response raised against a specific VSG will rapidly kill trypanosomes expressing this VSG. This can also be observed in vitro by a complement-mediated lysis assay. However, with each cell division there is a possibility that one or both of the progeny will switch expression to a silent VSG from the archive (see below). The frequency of such a switch has been measured to be approximately 1:100. This new VSG will likely not be recognised by the specific immune responses raised against previously expressed VSGs. It takes several days for an immune response against a specific to develop, giving trypanosomes which have undergone VSG coat switching some time to reproduce (and undergo further VSG coat switching events) unhindered. Repetition of this process prevents extinction of the infecting trypanosome population, allowing chronic persistence of parasites in the host. The clinical effect of this cycle is successive 'waves' of parasitaemia (trypanosomes in the blood).

We have preliminary results indicating an unknown transcription factor (named tbTR1) critical to the expression of VSG proteins in the virulent stages of infection. RNAi studies targeting this transcription factor have shown that inhibition of the protein causes massive loss of the parasites ability to adapt to the hosts adaptive immunity response. Targeting this molecular target with small molecules may provide a unique method of action for pharmacological modulation. We wish to further explore this opportunity, and other molecular targets in collaboration with CBCS.

References:

1) Fly, T T, (2010) Nature Chemical Biology, 234, 4561-4567.
2) Bugs, B. (2009) Science, 123, 456-459.

Project Description (max 2 pages)

(Please provide information regarding the proposed project plan, milestones, short- and long-term goals of the project, publication strategy, eventual IP interests and tentative timelines. Also disclose which aspects of the project require input/resources from CBCS).

We wish to screen the CBCS compound collection for novel inhibitors of parasite proliferation in the virulent phase of stage 1 infection AND for inhibition of the aforementioned transcription factor tBTR1. The short-term goals are to develop new insights into T brucei infection and pathophysiology ultimately leading to the long-term goals of curing this precarious disease.

In order to format the assay for HTS, the proliferation assay needs to be transferred from its current 6-well format to 384-well format and the incubation times adjusted to optimize fluorfluorafluor signal level with respect to controls. We have had some indications that DMSO concentrations in the assay may affect compound efficacy and/or parasite susceptibility and/or fluorescence readout. This issue need to be resolved before proceeding to screen. We also need to make sure that we consistently get signal levels with sufficient intensity to be able to conclusively distinguish inhibitors from background. The appropriate parasite strains, reagents and media can be provided by PI. However, the assay formatting, statistics and adjustments to protocol require the assistance of CBCS. The number of compounds to be screened and the selection of these compounds need to be decided on based on the outcome of this work. We believe this work will take ca 4-6 months prior to screen.

The screen should be conducted at the CBCS facilities in order to provide the appropriate levels of compound and liquid-handling automation. However, all preparation of parasite stock and incubation needs to be performed in our cell labs at MBB. Confirmed positives form the screen will be evaluated in dose-response at CBCS and also counter-screened at our facility using our newly developed orthogonal assay. Novel chemotypes and a number of identified compounds can at this point be published with our proprietary readout in focus. Completion of the screen, DR verification and publication ca 6 months after screen completed.

Compounds with favorable IP status will be further developed by chemical optimization using an already established CRO contact, under the guidance of CBCS Medicinal Chemistry. Developed compounds can be assessed at the CRO where our fluorfluorafluor assay is already up and running. Preliminary in vitro ADME/PK data should be obtained before proceeding in vivo which is planned to be performed by CBCS at UDOPP in Uppsala.

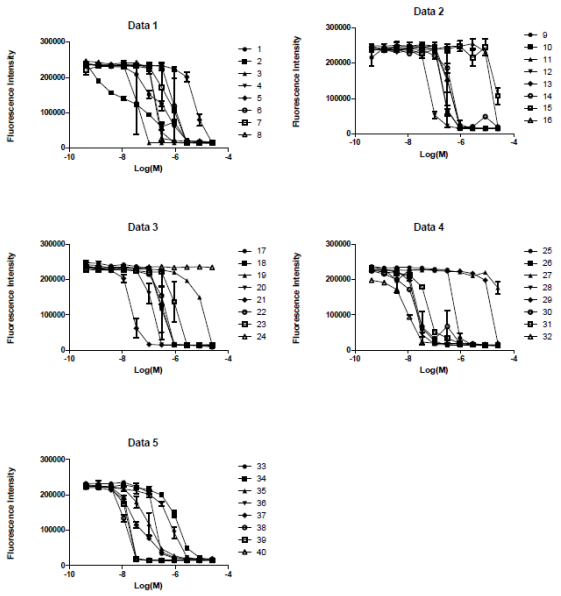
In vivo studies of novel inhibitors in the acute and chronic models will be performed in joint collaboration with suitable developed compounds (ca 12 months). CBCS will provide sufficient materials for in vivo studies either through in house chemistry or CRO. Late-stage studies of selected compound effect will be performed at Prof Fly's facilities in Rhodesia.

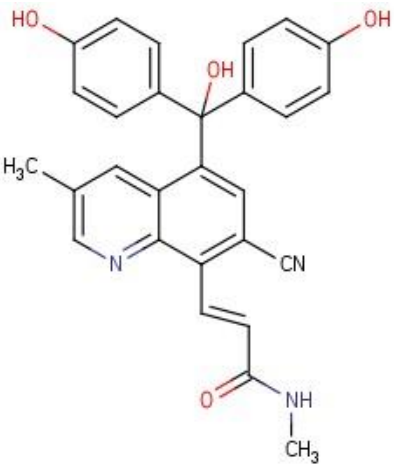
We foresee the possibility to publish at least three manuscripts and at least one patent for novel compounds discovered assuming that we get a reasonable hit rate and efficacy of compounds.

Technical Feasibility (max 2 pages)

(Please provide information as to the availability of recombinant protein materials, cell lines, primary in vitro assays, secondary assays, cellular assays, suitability of formatting existing assays for screening purposes, available in vivo models etc.

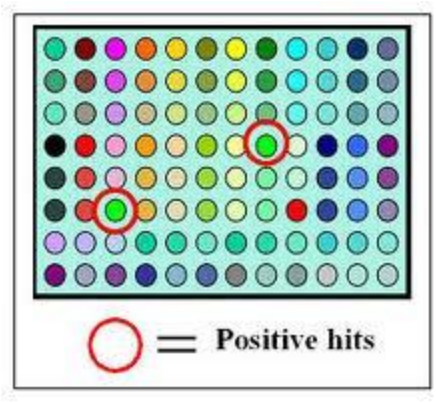
We have developed a novel assay for the detection of oxidative metabolites as a sign of parasite proliferation and infection increase. Our system biuilds on the use of an enzyme X-coupled fluorescence assay using fluorfluorafluor as an indicator. The use of this system has several advantages over pure ATP-detection kits in that they are selective to both life-cycle forms of T brucei which are both proliferating AND virulent. (See representative curves below).



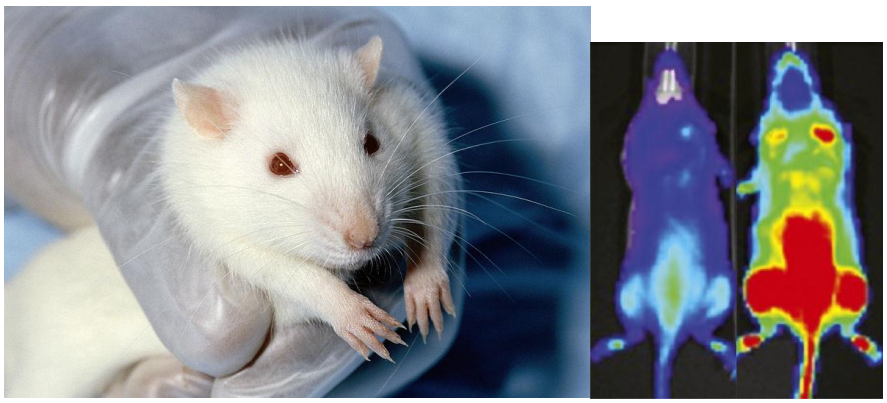


This is a much more relevant model than just identifying all forms of proliferation as these are not relevant in the infectious stage.

We have recently published the discovery of a novel compound, Crackalackalore, which efficiently clears parasite infection in the above described models (Bugs, 2010). This compound is, however, not effective in stage 2 infections due to rapid metabolism by Cyp 123. However, due to its high efficacy and stability in the assays systems, it is a suitable control for the development of high throughput screening protocols and formatting (see below).



We have developed two distinct animal models to evaluate the effects of developed compounds in vivo. These reflect both the acute primary infection stage which is haemolymphatic in character as well as the secondary stage of infection which is CNS-penetrant in character. Survival studies of stage one infected mice can be done in collaboration with the existing animal facilities and higher stage in vivo models will be available through our ongoing collaboration with Prof TseTse Fly at the University of Rhodesia. These models provide insight into the underlying biology behind infection and offer histopathological information as to the compounds mechanisms of action and distribution to affected tissues (see bleow).



In conclusion, we believe we have all the critical tools for the successful identification and development of novel leads for the elucidation of unique mechanisms for the treatment of Human African Trypanosomiasis.

Deliverables & Timelines

(Please fill in the Table below according to the template to the best of your ability. Additional comments can be made in the next section)

The scope of the projects varies widely and we appreciate and value this, but our ambition is to view the projects as either:

Phase 1 projects: prior to a screen, *i.e.* when no compounds are available for biological studies. A common example here is assay development efforts and the conductance of a screening campaign, including hit confirmation and the first follow-up studies to characterize the value of identified hits. For projects approved by PRC we generally commit to take the project through to dose-response characterization of existing hits and analogs. For extended studies including the synthesis of hits and new analogs a new PRC application will be required.

Phase 2 projects: post a screening campaign or another means to identify small molecule modulators, *i.e.* when compounds are available for biological studies. Examples here include the need to chemically optimize or modify compounds to suit the needs of the investigator, structure activity relationship studies

The background to this is that the different activities require different competences and resources from within CBCS so we need to categorize the projects in this way to ensure we can deliver support to all projects that PRC approves.

Goal / Activity	Timeline	Tentative patent/ publication	Who
Phase 1 project activities (eg assay development, assay formatting, screening, hit confirmation etc)			
Assay formatting of fluorfluorafluor assay to 384	3 months	-	CBCS/PI
DMSO tolerance studies	3 months	-	CBCS/PI
Growth optimization study	3 months	-	PI's group
Screen	4 months	-	CBCS
Hit confirmation and DR	6 months	Publication 1	CBCS
		Choose an item.	Choose an item.
		Choose an item.	Choose an item.
Phase 2 project activities (eg hit optimization, enabling chemistry, target identification, efficacy models, in vivo etc)			
Hit optimization of promising hits	10 months	Patent 1	CRO
Hit optimization of promising hits	10 months	Patent 1	CBCS
In vivo acute	12 months	Publication 2	PI's group
In vivo chronic model	14 months	Publication 2	CBCS/PI
Pathophysiological studies	18 months	Publication 3	Co-PI
Target identification studies	18 months	Additional Publication	CBCS/PI
		Choose an item.	Choose an item.
Ultimate project goal: **To cure Human African Trypanosomiasis through the discovery of novel mechanisms of action and novel compounds with high levels of efficacy and safety.**			

Additional Comments

(Please enter any additional comments you see fit in order to clarify your proposal)

NOTE TO AUTHORS!

Please note that project proposals in general are classified as "Phase 1" OR "Phase 2" applications (see section above) for evaluation internally. If the intention of the collaboration is eg. to develop a screenable assay and perform a focused or high-throughput screen on an insolated target or system (ie. Phase 1), followed by development of novel chemistry based on hits and target identification activities (ie. Phase 2), PRC will (in general) only approve CBCS involvement in the Phase I activities initially.

This does not exclude that long-term development plans and goals are included as a part of the project description. In fact, we encourage authors to include long-term goals of the collaboration to give the proper perspective on the project. It does, however, imply that CBCS may only commit to a limited number of activities (Phase 1) outlined in the project description. Depending on the outcome of these studies and resource requirements, additional (Phase 2) activities in the project may be initiated pending PRC approval of an updated application.

Please convert the finalized project proposal form as a pdf and email to a representative of CBCS.