1. Sagan, C. & Mullen, G. *Science* **177,** 52–56 (1972).
2. Rosing, M. T., Bird, D. K., Sleep, N. H. & Bjerrum, C. J. *Nature* **464,** 744–747 (2010).
3. Holland, H. D. *Phil. Trans. R. Soc. B* **361,** 903–915 (2006).
4. Kuhn, W. R. & Atreya, S. K. *Icarus* **37,** 207–213 (1979).
5. Owen, T., Cess, R. D. & Ramanathan, V. *Nature* **277,** 640–642 (1979).
6. Walker, J. C. G., Hays, P. B. & Kasting, J. F. *J. Geophys. Res.* **86,** 9776–9782 (1981).
7. von Paris, P. *et al. Planet. Space Sci.* **56,** 1244–1259 (2008).
8. Haqq-Misra, J. D., Domagal-Goldman, S. D., Kasting, P. J. & Kasting, J. F. *Astrobiology* **8,** 1127–1137 (2008).
9. Kiehl, J. T. & Dickinson, R. E. *J. Geophys. Res.* **92,** 2991–2998 (1987).
10. Rondanelli, R. & Lindzen, R. S. *J. Geophys. Res.* **115,** doi:10.1029/2009JD012050 (2010).
11. Goldblatt, C. *et al. Nature Geosci.* **2,** 891–896 (2009).
12. Knauth, L. P. *Palaeogeogr. Palaeoclimatol. Palaeoecol.* **219,** 53–69 (2005).
13. Ohmoto, H., Watanabe, Y. & Kumazawa, K. *Nature* **429,** 395–399 (2004).
14. Rye, R., Kuo, P. H. & Holland, H. D. *Nature* **378,** 603–605 (1995).
15. Sheldon, N. D. *Precambrian Res.* **147,** 148–155 (2006).
16. Gough, D. O. *Solar Phys.* **74,** 21–34 (1981).

DRUG DISCOVERY

Fat-free proteins kill parasites

George A. M. Cross

The addition of a fatty acid to certain proteins is vital for the survival of protozoa that cause sleeping sickness and of their mammalian hosts. Compounds that target this process in the protozoa are now reported.

No safe and effective drugs exist for the treatment of human African trypanosomiasis (HAT, also known as sleeping sickness; Fig. 1), the fatal disease caused by *Trypanosoma brucei* protozoa and their relatives. More than a century of study has left the impact of HAT on individuals almost undiminished. To make matters worse, the disease has received little attention recently amid the many woes that afflict equatorial Africa, home of the tsetse flies that act as the main agent of transmission. But on page 728 of this issue, Frearson *et al.*[1] describe a giant step towards improving this situation with their report of a new class of compounds that cures trypanosomiasis in mice.

The *T. brucei* parasite is a small, unicellular organism with many biological similarities to its human host cells, but which has taken different routes in the evolution of certain critical cellular pathways. It also has some unique cellular pathways. There is therefore widespread optimism that it should be possible to develop treatments for HAT by identifying drug targets that are specific to the organism — assuming that the financial resources are available. What's more, *T. brucei* lives outside its host's cells (unlike its distant cousins *Trypanosoma cruzi* and the many varieties of *Leishmania*), which should facilitate drug development. The human immune system cannot eradicate *T. brucei* because the parasite has developed an apparently insurmountable capacity for antigenic variation. This allows HAT infections to persist until (usually) the death of the infected person, following the coma that occurs once the parasite invades the patient's brain.

In recent years, a myriad of processes have been discovered through which proteins are chemically modified after their translation, resulting in the attachment of groups that influence the proteins' properties in important ways. Frearson *et al.*[1] targeted the modification known as *N*-myristoylation[2], in which an unsaturated fatty acid (myristic acid) is attached to one end of a small subset of cellular proteins. Discovered[3] in the early 1980s, *N*-myristoylation enables many proteins with essential signalling functions to associate with cell membranes.

The enzyme responsible for this modification — *N*-myristoyltransferase, NMT — is found in the cells of trypanosomes[4,5] and mammals[2], and is necessary for their survival. Because the signals associated with *N*-myristoylated proteins are sometimes disrupted by viral infections or cancer-causing mutations, NMT has been widely studied. Crucially, this has resulted in the preparation of libraries of potential NMT inhibitors, and has facilitated detailed comparisons of the trypanosome and mammalian enzymes, and of their interactions with potential drugs.

Frearson and colleagues' landmark discovery[1] is the development and validation of a potent and selective NMT inhibitor that cures trypanosome infections in mice at low oral doses. They began by screening 62,000 chemical structures, a process that identified several moderately active NMT inhibitors. These included some pyrazole sulphonamide compounds (see Fig. 1a on page 729), which can be synthesized relatively easily in a modular manner — a fact that made them attractive candidates for optimization of their pharmacological properties.

The team went on to prepare more than 200 additional sulphonamide derivatives, including some that were not only active at 1,000-fold lower concentrations than the compounds identified in the screening, but that also showed selectivity for trypanosome NMT over the human variant of the enzyme. One such structure, DDD85646, killed trypanosomes in culture at very low (nanomolar) concentrations. Notably, these concentrations were 200-fold lower than those at which the compound killed mammalian cells in culture.

Frearson *et al.* obtained several lines of evidence to show that the trypanocidal activity of their sulphonamides was specifically due to their ability to inhibit NMT. For example, they observed that the compounds were less lethal

A. CRUMP, TDR, WHO/SPL

EYE OF SCIENCE/SPL

Figure 1 | A forgotten disease. These teenagers in Uganda have sleeping sickness, a potentially fatal disease caused by the protozoan *Trypanosoma brucei* (inset). Frearson *et al.*[1] have identified compounds that kill trypanosomes in mice.

for trypanosomes that had been genetically manipulated to over-express NMT. The authors also performed studies in which radiolabelled myristic acid was administered to drug-treated trypanosomes. They found that although the microorganisms continued to make proteins at a normal rate in the short period before they died, radioactive myristic acid was no longer transferred to those proteins that would normally be myristoylated.

When the authors administered DDD85646 orally to mice at the virulent stage 1 of trypanosomiasis, the drug persisted for at least ten hours in the bloodstream at concentrations lethal to the parasites, curing the mice of infection. The ultimate objective of trypanocidal drug development, however, is to find compounds that are active against stage 2 of HAT, which occurs after trypanosomes have invaded the patient's central nervous system. The sulphonamides tested so far are not effective against this stage of trypanosomiasis, so further optimization of the compounds will be necessary to achieve this goal.

In fact, it might not be possible to find one perfect compound that combines all the desirable properties for the treatment of HAT, at least in the short term. For example, although it is much more preferable for drugs to be taken by mouth, rather than by injection, intravenous injection could be an acceptable interim compromise for a non-toxic drug that is rapidly effective against stage 2 of the most virulent form of HAT. An intravenous drug — melarsoprol — already exists for this form and stage of the disease, but its side effects are unacceptable.

Frearson and colleagues[1] used biochemical studies and X-ray crystallography to identify the molecular interactions between DDD85646 and NMT. This information will guide future chemical modifications to the sulphonamides aimed at improving the potency, selectivity and bioavailability of the compounds. Intriguingly, the authors note that DDD85646 shows only a small window of biochemical selectivity between trypanosome and human NMT — its half-maximum inhibitory concentration (IC_{50}) for the trypanosome enzyme is only half that of the human enzyme. They therefore suggest that the observed hypersensitivity of trypanosomes to NMT inhibition is due to the uniquely high rates at which *T. brucei* recycles membrane proteins[6] — a process that depends on myristoylation.

Although NMT is one of the few trypanosome proteins that has been comprehensively validated as a 'druggable' enzyme, it might not ultimately have the ideal profile for a drug target. To produce new leads of similar promise to Frearson and colleagues' sulphonamides, we need to find more targets and study them in the depth and detail of the current report[1].

Drug development is risky and expensive. The next stages in validating DDD85646 or related compounds will be no exception. But the omens are encouraging, especially given the approach taken by the authors of the present paper[1], many of whom are based at the Dundee Drug Discovery Unit[7]. I was involved in a review process that endorsed the establishment of the unit, where decisions about what targets to pursue[8] are the prerogative of drug-development professionals, but are informed by neighbouring researchers at the University of Dundee College of Life Sciences. This creates a sound basis for the effective translation of academic research into practical applications, especially for drug discovery aimed at finding treatments for neglected tropical diseases. ■

George A. M. Cross is at The Rockefeller University, New York, New York 10065-6399, USA.
e-mail: george.cross@rockefeller.edu

1. Frearson, J. A. *et al. Nature* **464,** 728–732 (2010).
2. Resh, M. D. *Nature Chem. Biol.* **2,** 584–590 (2006).
3. Henderson, L. E., Krutzsch, H. C. & Oroszlan, S. *Proc. Natl Acad. Sci. USA* **80,** 339–343 (1983).
4. Price, H. P. *et al. J. Biol. Chem.* **278,** 7206–7214 (2003).
5. Price, H. P., Guther, M. L. S., Ferguson, M. A. J. & Smith, D. F. *Mol. Biochem. Parasitol.* **169,** 55–58 (2010).
6. Engstler, M. *et al. Cell* **131,** 505–515 (2007).
7. www.drugdiscovery.dundee.ac.uk/index.html
8. Frearson, J. A., Wyatt, P. G., Gilbert, I. H. & Fairlamb, A. H. *Trends Parasitol.* **23,** 589–595 (2007).