Nature Reviews Drug Discovery | Published online 31 May 2017; doi:10.1038/nrd.2017.93

INFECTIOUS DISEASE

Peroxin inhibitor treats *Trypanosoma* infection

Insect-borne *Trypanosoma* spp. parasites transmitted predominantly by the tsetse fly infect humans as well as livestock, which causes devastating diseases, such as human African trypanosomiasis (HAT) and Chagas disease. Current treatments for trypanosomiases are limited, can cause serious side effects and require long treatment schedules. Writing in *Science*, Dawidowski *et al.* now identify a small-molecule inhibitor of the peroxisomal membrane protein PEX14 that effectively reduces parasitaemia in a mouse model of *Trypanosoma* infection.

The PEX proteins play a crucial part in the biogenesis and function of trypanosomal glycosomes, which are peroxisome-related organelles containing enzymes that are required for glucose metabolism and are therefore essential for parasite survival. Given this, Dawidowski *et al.* set out to explore the therapeutic potential of blocking glycosome assembly by disrupting the interaction between two PEX proteins, PEX5 and PEX14, which is required for the import of matrix proteins into glycosomes.

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First, the authors created a 3D pharmacophore model mimicking the binding mode of specific PEX5 peptide motifs to the respective pockets of the PEX14 amino-terminal domain. Using this model, they carried out *in silico* 3D pharmacophore-based screening followed by 3D docking. This led to the identification of a drug-like pyrazolo[3,4-c]pyridine, termed derivative 1, which exhibited moderate affinity to PEX14 and disrupted the PEX14–PEX5 interaction.

Derivative 1 was shown to be toxic to the bloodstream form of T*rypanomsoma brucei* (which causes HAT), and displayedlow toxicity in mammalian cell lines. Using NMR-based fragment screening, Dawidowski *et al.* further optimized the molecule to increase its affinity towards trypanosomal PEX14. This ultimately led to the more selective and potent PEX14–PEX5 interaction inhibitor, derivative 5, which contained hydrophobic features identified by NMR as preferred by *T. brucei* PEX14. The mode of binding was confirmed by high-resolution crystal structures of PEX14 bound to the inhibitors.

Next, the authors tested derivative 5 in cell-based assays against different *Trypanosoma* spp.. The inhibitor showed strong cellular potency against the livestock pathogen *T. brucei brucei* and the human-infecting *T. brucei rhodesiense* subspecies. Notably, the activity of derivative 5 against the intracellular form of *Trypanosoma cruzi* (which causes Chagas disease) was twofold greater than the reference anti-parasitic drug benznidazole.

Mechanistically, derivative 5 was shown to prevent compartmentation of glycolytic enzymes in glycosomes of *T. brucei brucei*, with all tested glycosomal enzymes being mislocalized to the cytosol, accompanied by a significant reduction in ATP levels.

Finally, the authors tested the therapeutic potential of their inhibitors using an *in vivo* disease model. To increase the available concentration of free PEX14 inhibitor in serum, they synthesized derivative 6, which exhibited significantly reduced plasma protein binding. Oral application of derivative 6 twice a day for 5 days to mice infected with *T. brucei brucei* reduced or stalled parasitaemia levels without adverse effects.

In summary, these findings identify trypanosomal protein–protein interactions as promising drug targets and support further clinical development of PEX14 inhibitors.

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ORIGINAL ARTICLE Dawidowski, M. *et al.* Inhibitors of PEX14 disrupt protein import into glycosomes and kill *Trypanosoma* parasites. *Science* **355**, 1416–1420 (2017)