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Invited Review

Aminoacyl-tRNA synthetases as drug targets in eukaryotic parasites ☆

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ABSTRACT

Aminoacyl-tRNA synthetases are central enzymes in protein translation, providing the charged tRNAs needed for appropriate construction of peptide chains. These enzymes have long been pursued as drug targets in bacteria and fungi, but the past decade has seen considerable research on aminoacyl-tRNA synthetases in eukaryotic parasites. Existing inhibitors of bacterial tRNA synthetases have been adapted for parasite use, novel inhibitors have been developed against parasite enzymes, and tRNA synthetases have been identified as the targets for compounds in use or development as antiparasitic drugs. Crystal structures have now been solved for many parasite tRNA synthetases, and opportunities for selective inhibition are becoming apparent. For different biological reasons, tRNA synthetases appear to be promising drug targets against parasites as diverse as *Plasmodium* (causative agent of malaria), *Brugia* (causative agent of lymphatic filariasis), and *Trypanosoma* (causative agents of Chagas disease and human African trypanosomiasis). Here we review recent developments in drug discovery and target characterisation for parasite aminoacyl-tRNA synthetases.

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1. Introduction – the need for new antiparasitic drugs

The prevalence and persistence of parasitic infections are both remarkable and troubling phenomena. Approximately one billion people harbour at least one worm infection (nematodes and platyhelminths) (Lustigman et al., 2012) and many individuals are simultaneously infected with multiple parasites from distantly related eukaryotic phyla (Fevre et al., 2008; Gething et al., 2011; Nacher, 2012). These parasites cause diseases that impose a serious burden to the health and economic development of affected countries, and are therefore the subject of many varied prevention and control strategies. No human-licensed vaccine exists for any eukaryotic disease, therefore drugs are a major component of intervention against most parasitic diseases (Prichard et al., 2012). Drug based strategies include treatment of verified infections, mass drug administration to presumptive infected communities or at risk individuals (e.g. pregnant mothers), and sporadic prophylaxis for individuals. In many cases existing drug-based programs are at risk from parasites developing resistance, and therefore rendering ineffective our affordable and effective drugs. Some antiparasitic drugs have already had their effective usage severely restricted in regions due to the development of widespread drug resistance (Baird, 2005; Croft and Olliaro, 2011). The development of future control strategies is threatened by the impending and inevitable emergence of resistance to additional drugs (Geerts and Gryseels, 2000). To deal with existing and future shortcomings of antiparasitic drugs, multiple classes of new drugs are urgently needed for many parasitic diseases.

Parasites cause diverse types of disease, requiring drug treatments that address varying causes of pathogenesis. Apicomplexan parasites include *Plasmodium* spp., *Toxoplasma gondii* and *Cryptosporidium*. All parasites in this phylum are obligate intracellular parasites, but their host range and disease type varies immensely. *Plasmodium* species cause generally acute disease through proliferation within and destruction of erythrocytes. Most existing antimalarial drugs work by killing this proliferative intra-erythrocytic stage, though action against the parasite forms that initially infect humans (sporozoites) and the forms that are transmitted to mosquitoes (gametocytes) is highly desirable in addition to disease control purposes (Burrows et al., 2013). *Toxoplasma gondii* parasites infect many diverse animals and many cell types. In humans, *Toxoplasma* is normally pathogenic only in immunocompromised individuals or in the human foetus. Drugs are needed to arrest the faster growing tachyzoite stages of *Toxoplasma*, as well as the latent bradyzoite stages that form cysts in the brain and other organs (Rodriguez and Szajnman, 2012). *Cryptosporidium* infects epithelial cells of the intestine, causing potentially severe and chronic diarrhea. As with *Toxoplasma*, the most severe *Cryptosporidium* cases are in immunocompromised individuals, and the need for drugs is more pressing for treatment of such cases (Rossignol, 2010).

Typanosomatid parasites also cause a broad spectrum of diseases. *Trypanosoma brucei*, spread by the bite of the tsetse fly, causes human African trypanosomiasis, also known as sleeping sickness. These parasites proliferate extra-cellularly in the bloodstream and lymphatic system and later infect the central nervous system (CNS) (Barrett et al., 2007). This disease is fatal within months to years if not treated, and most existing treatments are difficult to administer, toxic or ineffective. New drugs must overcome the additional challenge of crossing the blood brain barrier to treat parasites in the CNS. *Trypanosoma cruzi* infections are the cause of the chronic and potentially fatal Chagas disease. Existing drugs to treat *T. cruzi* are ineffective if not administered early during infection and are highly toxic. *Leishmania*, the second medically important genus of trypanosomatid parasites, includes species that also cause a range of serious human diseases. In humans, *Leishmania* parasites invade and grow within phagocytic cells. As with other trypanosomatid parasites, existing drugs are generally toxic, difficult to deliver and subject to parasite resistance (Stuart et al., 2008). Although trypanosomatid parasites kill fewer people than malaria, the lack of effective and safe drugs arguably makes discovery of new drugs even more pressing for these parasites.

Three parasites whose anaerobic metabolism distinguishes them from most other eukaryotes are the extracellular parasites *Giardia*, *Trichomonas*, and *Entamoeba.* In these parasites the mainstays for treatment are the nitroimidazole drugs, which are activated by the parasites' unusual pyruvate:ferredoxin oxidoreductase enzymes (Ali and Nozaki, 2007). In each of these parasites, resistance to nitroimidazol is possible through altered metabolism and alternative drugs are scarce or ineffective (Upcroft and Upcroft, 2001).

The final parasite discussed below in the context of tRNA synthetase targets is the helminth parasite *Brugia*. *Brugia malayi* is a nematode spread between humans by mosquitoes and is one of several parasites to cause human filariasis. Lymphatic filariasis is caused by immunological reaction to the adult worms and the thousands of transmissive microfilaria they produce. Drug discovery against nematodes introduces the added difficulty of selective inhibition between the bilaterian animal parasites and their hosts, although *Brugia*'s dependence on its bacterial *Wolbachia* symbiont may offer other potential drug targets (Bandi et al., 2001).

2. Protein translation as a drug target

One biological pathway that has been thoroughly validated as a target for anti-infective compounds in a wide range of microbes is the process of protein translation. Most antibiotics that target protein translation interact with microbial ribosomes themselves—binding directly to the rRNA or ribosomal subunit proteins. However, additional molecules within the broader process of protein translation can act as targets for drugs. One such target for existing and future antimicrobial therapeutics is the aminoacyl-tRNA synthetase (aaRS) family. This family of enzymes catalyses the attachment of amino acids to their cognate tRNAs to produce the aminoacyl tRNAs (also aa-tRNA or charged tRNA) that are the substrates for translation (reviewed by Ibba and Soll, 2000). The aaRSs enzymes are not only responsible for producing the raw materials for translation, but also for ensuring the fidelity of translation from nucleic acid to amino acid information. Disruption of aaRSs therefore interrupts or poisons the process of protein translation. Compounds that inhibit aaRSs have been successfully exploited, with at least one antibacterial drug, mupirocin, currently in clinical use for the topical treatment of *Staphylococcus aureus*, that acts through the inhibition of the isoleucyl-tRNA synthetase (IleRS) of gram-positive bacteria (Nakama et al., 2001). The pursuit of diverse other aminoacyl-tRNA synthetases has yielded specific aaRS inhibitors (Rock et al., 2007), some of which are currently in clinical trials as antimicrobials (de Jonge et al., 2006; Koon et al., 2011).

Besides the excellent precedence for druggability in bacteria, there are several reasons to support protein translation in general, and aaRSs specifically, as a useful antiparasitic target. First is the dependence of many parasites on abundant protein translation in fast growing cells. Because many parasites constitutively undergo active and continuous proliferation they are heavily reliant on efficient protein translation and may be sensitive to disruptions to the translation machinery. Other parasites pass through quiescent life-stages with relatively little cellular proliferation—these stages (such as the bradyzoite stages of *Toxoplasma gondii*) are likely to have a reduced requirement for protein turnover and may be less sensitive to translation inhibitors. Such stages present a general problem for chemotherapy, though it is noteworthy that inhibition