SMGBs as novel *in vitro* and *in vivo* anti-infective agents for *Acanthamoeba* spp. infections.

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Acanthamoeba spp. are causative agents of a painful and severe sight-threatening corneal infection that can lead to blindness known as *Acanthamoeba* keratitis and a subacute disease in the brain which is usually fatal known as granulomatous amoebic encephalitis. Over the last few years, there has been a notorious increase in the number of infections due to *Acanthamoeba* spp. Poor diagnosis, problems of side effects and toxicity of the current drug treatment contribute to a high mortality rate. Strathclyde Minor Groove Binders (S-MGBs), compounds that bind to the minor groove of the DNA that designed and synthesised at University of Strathclyde were evaluated as potential alternative inhibitors against *Acanthamoeba* infections. Through cell viability microplate alamarBlue assays 42 S-MGBs were screened from which a library of 6 showed potent active inhibitory effect with half maximal inhibitory concentration (IC_{50}) below 1 µM. S-MGB 235 showed the most potent inhibitory effect with IC_{50} in the nanomolar range against five *Acanthamoeba* isolates after 24 h and 96 h incubation. Confocal microscopy of trophozoites labelled with fluorescent S-MGB 363 (analogue of S-MGB 235) showed this compound in the nucleus, nucleolus and distributed over the granuloplasm causing cell lysis, supporting the potent effect observed *in vitro* by S-MGB 235. Furthermore, conditions were standardised to establish *Galleria mellonella* larvae as a new *in vivo* infection model for *A. castellanii* Neff infections to assess the efficacy and toxicity of voriconazole, miltefosine and S-MGB 235. Voriconazole and miltefosine did not protect larvae from trophozoite infection, however S-MGB 235 significantly protected larvae when compared with the negative control. Preliminary results show S-MGB 235 as cysticidal with minimum inhibitory concentration (MIC) ranging 0.5-1 µM in most of the strains, which is a potent inhibitory effect in low concentrations compared to hexamidine and PHMB, both drugs widely used in the current clinical treatment. Given the above, we suggest S-MGB-235 as a relevant novel compound that might complement aromatic diamidines in the current combination therapies for *Acanthamoeba* spp. diseases.