Development of antimicrobials against *Acanthamoeba castellanii*

Alemao Carpinteyro[1], Fraser J. Scott[2], Colin J. Suckling[3], Craig W. Roberts[1]

[1]Strathclyde Institute of Pharmacy and Biomedical Sciences, University of Strathclyde G4 0RE, Glasgow UK

[2]School of Applied Sciences, University of Huddersfield, Queensgate, UK

[3]Department of Pure and Applied Chemistry, University of Strathclyde G4 0RE, Glasgow UK

Contact: alemao.carpinteyro-sanchez@strath.ac.uk

Abstract

Acanthamoeba is a free-living amoeba widely distributed in the environment[1] which exists as two stages in their life cycle: a motile, trophic and replicating trophozoite and a resistant cyst stage. In recent years, the incidence of infections due to *Acanthamoeba* spp. has shown a remarkable increase. This parasite is the causative agent of a sight-threatening infection of the cornea known as *Acanthamoeba* keratitis (AK) and a fatal disease of the central nervous system known as Granulomatous Amoebic Encephalitis (GAE) mainly in immunocompromised patients[2]. Most drugs target the trophozoite however, at present there are no effective treatment that can eliminate the cyst form. A particular set of compounds known as Minor Grove Binders (MGBs) have the characteristic of binding specifically to minor groove region of double-stranded DNA. The main effect of these MGBs is their ability to interfere with DNA-centric processes such as transcription machinery and inhibition of DNA enzymes resulting in cell death[3]. These molecules have received great attention since they can be empirically screened and iteratively refined via chemical synthesis to target various entities such as tumours, bacteria, viruses and parasites[4]. 12 compounds synthesized by our Strathclyde research group (SMGBs) have been tested *in vitro* using a colorimetric alamarBlue viability cell assay against *Acanthamoeba castellanii* Neff strain. To date, 2 compounds: SMGB 1 and SMGB 11 were able to target trophozoites with IC_{50}s of 25 µM and 6.25 µM, respectively at 96 hours.

Research Aim

- To determine the sensitivity of *Acanthamoeba castellanii* Neff strain to 12 SMGBs.

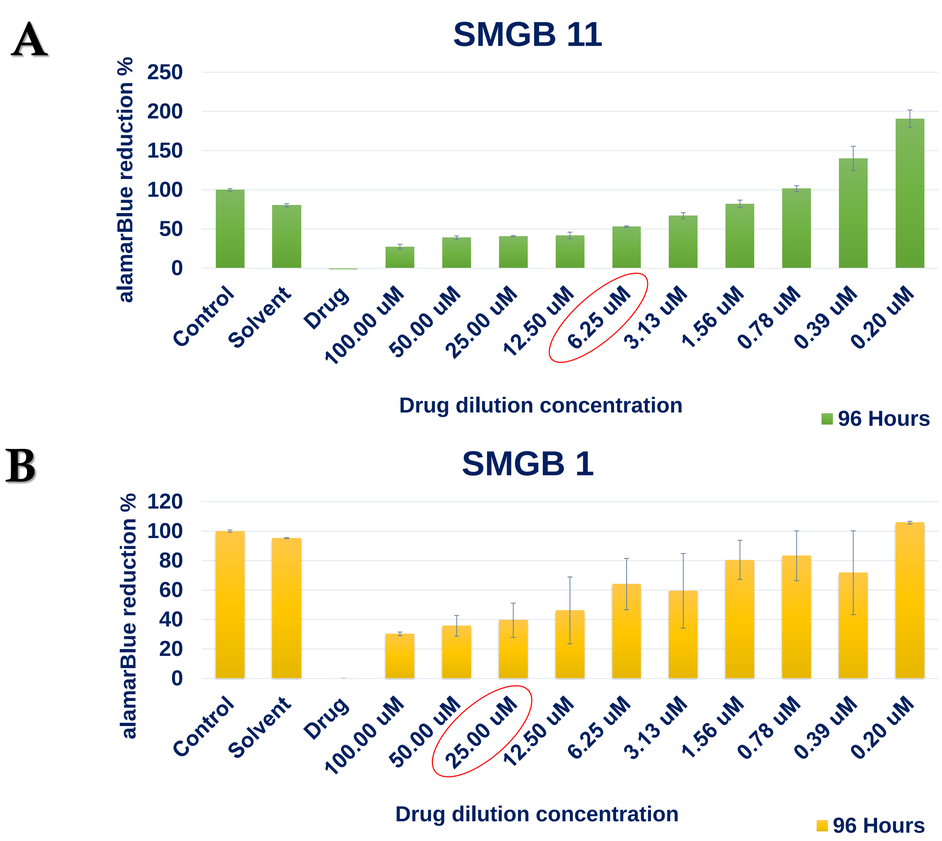
Material and Methods

The schematic overview of alamarBlue drug sensitivity assay[5]:

1) Cultivation and growth of *A. castellanii* trophozoites.
2) *A. castellanii* passage and preparation for drug sensitivity assay.
3) Cell enumeration in a Neubauer haemocytometer.
4) SMGBs serial dilution preparation with trophozoites culture in 96-well plates.
5) Addition of alamarBlue dye to measure the effect caused by SMGBs.
6) AlamarBlue reduction determined by spectrophotometry.

Principle of alamarBlue dye reaction on this assay[6]:
Resazurin is metabolized by live cells to resorufin and the fluorescent signal generated is proportional to the number of live cells. Thus, the assay measures cell number rather than detecting cell lysis and low cell number indicates inhibition of cell cycle or lethal effects of the S-MGB on trophozoites population.

Results and Discussion



Graph 1. Efficacy of SMGB 11 (A) and 1 (B) against *A. castellanii* Neff strain trophozoites showing an IC_{50}= 6.25 and 25 µM, respectively (highlighted in red).

Table 1. Half maximal inhibitory concentration (IC_{50}) of remaining SMGBs tested against *A. castellanii* Neff strain by means of alamarBlue drug sensitivity assays.

Strathclyde Minor Groove Binders (SMGBs)	Half maximal inhibitory concentration (IC_{50})
2	>200 µM
3	>200 µM
4	>200 µM
5	>200 µM
6	200 µM
7	200 µM
8	100 µM
9	>200 µM
10	200 µM
12	200 µM

- In this study, cell seeding concentration against the SMGBs batch was optimized to 2x10[3] trophozoites. *A. castellanii* owns an impressive and fast metabolic machinery[2], this would explain the need to use a lower quantity of cell density compared to other protozoa including *Trypanosoma*[7] and *Leishmania*[8].
- SMGB 11 and 1 contain an N-alkyl pyrrole which binds preferentially to AT sites instead of a C-alkyl thiazole (a common change in SMGBs) which couples with GC sites. Interestingly this is inconsistent with the high concentration of GC (86%) in *Acanthamoeba* DNA[9].
- From all the SMGBs studied so far, strong affinity for DNA with nuclear localization has been observed in other parasites[10] and the interference with DNA machinery events seem to be the most important. Nevertheless, its suggested that indirect MGBs mechanisms against pathogenic microorganisms such as the inhibition of complex DNA-protein or DNA-enzyme exist[3].

Conclusions

- ✓ From the 12 SMGBs library tested, only 2 hit-compounds: 1 and 11 were identified against *A. castellanii* trophozoites.
- ✓ The tertiary amine tail in SMGB 11 and morpholine tail in SMGB 1 could play an important possible role in the antiparasitic activity.

Future Work

i. Analyse the physicochemical properties of SMGBs that gave the best performance on this study to re-synthesise them therefore, enhance their potency and efficacy against *A. castellanii* Neff trophozoites.

ii. Design and standardise a protocol to study a new *in vivo* model infection for SMGBs drug assays.

iii. Identify the specific targets of the effective SMGBs by nuclear magnetic resonance (NMR) spectroscopy.

Acknowledgments

- To **Professor Craig W. Roberts** for your faith, let me continue researching in *Acanthamoeba* field and so making my dreams come true.
- To **Dr. Fraser J. Scott** and **Prof. Colin J. Suckling** for gently donate their S-MGBs compounds.
- To my fellow **Scott Thomson** for your training and knowledge on this technique.
- To **Consejo Nacional de Ciencia y Tecnología (CONACYT)**, my Mexican sponsorship.
- To **Microbiology Society**, for your grant contribution and make possible to be here.

References

1. Marciano-Cabral & Cabral (2003) Clin. Microbiol. Rev. 16(2):273.
2. Khan (2015) Caister Academic Press 2nd Ed.
3. Khalaf et al. (2016) Acta Chim Slov 63, 689-704
4. Barrett et al. (2013) Pharmacol Ter. 139(1):12-23
5. McBride et al. (2005) J Clin Microbiol 43(2): 629–634
6. O'Brien et al. (2000) Eur J Biochem. 267(17):5421-6
7. Räz et al. (1997) Acta Trop. 68, 139-147.
8. Mikus & Steverding (2000) Parasitol Int. 48(3):265-9
9. Byers (1986) Int Rev Cytol 99:311-41
10. Scott et al. (2016) Bioorg Med Chem Lett. Aug 1;26(15):3478-86