Oligochitosan conjugates of the antimalarials dihydroartemisinin and lumefantrine: synthesis, stability, cell viability, and antiplasmodial studies

William Matshe[1], †Sindisiwe Mvango[1,2], Rudzani Malabi[1], Asongwe Tantoh[3], Charlene Andraos[4], Ibukun Famuyide[5], Lyndy McGaw[5], Sooraj Baijnath[6], Lynne Pilcher[2], and Mohammed Balogun[1]*

1. Biopolymer Modification and Advanced Therapeutics Laboratory, Centre for Nanostructured and Advanced Materials, Council for Scientific and Industrial Research, P. O. Box 395, Pretoria 0001, South Africa.
2. Department of Chemistry, University of Pretoria, Pretoria 0002, South Africa.
3. Pharmaceutical Technologies, Chemicals cluster, Council for Scientific and Industrial Research, P. O. Box 395, Pretoria 0001, South Africa.
4. Toxicology and Biochemistry department, National Institute for Occupational Health, National Health Laboratory Service, P. O. Box 4788, Johannesburg 2000, South Africa.
5. Phytomedicine Programme, Department of Paraclinical Sciences, Faculty of Veterinary Science, University of Pretoria, Private Bag X04, Onderstepoort, Pretoria 0110, South Africa.
6. Wits Integrated Molecular Physiology Research Initiative, Wits Health Consortium, School of Physiology, Faculty of Health Sciences, University of the Witwatersrand, Private Bag 3. WITS 2050, Johannesburg, South Africa.

*Corresponding author: mohammedbalogun@tuks.co.za

†Deceased: 26 April 2024

Mass determination of oligochitosan

Mass analysis of oligochitosan (OC) was performed using a MassTech atmospheric pressure matrix assisted laser desorption ionisation (AP-MALDI) (MassTech, CA, USA) source coupled to a ThermoExactive Orbitrap High Resolution mass spectrometer (ThermoScientific, Waltham, USA). A saturated solution of OC in H_2O was deposited as 1.00 µL droplets onto a MALDI optitof steel plate. Individual spots were coated with hydroxycinnamic acid (HCCA) matrix and left to dry at room temperature (20 °C – 25 °C). Mass spectrum was obtained by analysing each spot with a laser energy of 2%.

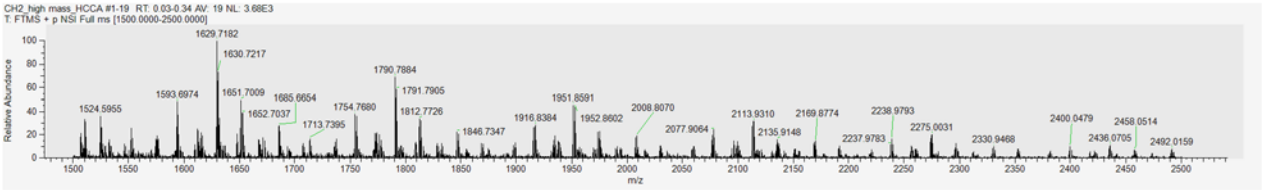

Figure S1. MALDI mass spectrum of OC. m/z: 1.5kDa – 2.5kDa.

^{1}H NMR spectroscopic analysis of OC-Lumefantrine

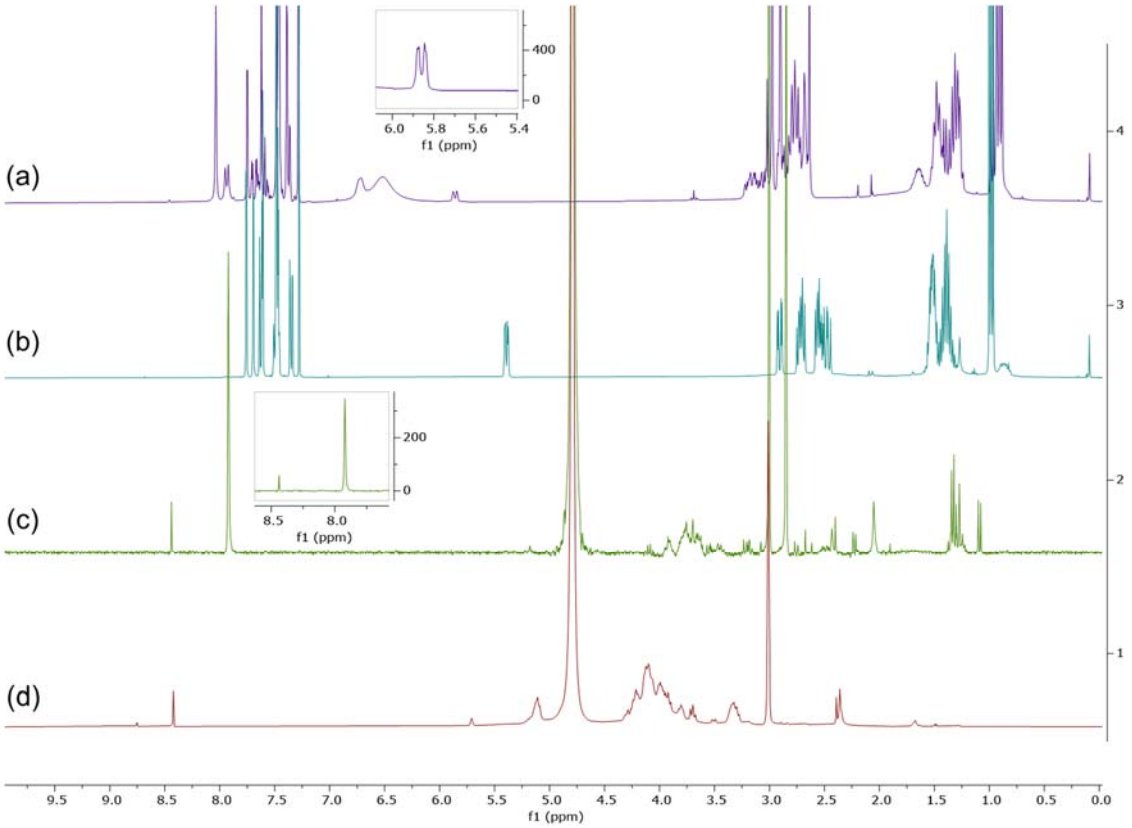

Figure S2. ^{1}H NMR of (a) Lumf-Suc, (b) Lumf, (c) OC-Lumf conjugate, and (d) OC. Spectra (a) and (b) were run in $CDCl_3$ while (c) and (d) were run in D_2O.

Spectrophotometric analysis of lumefantrine

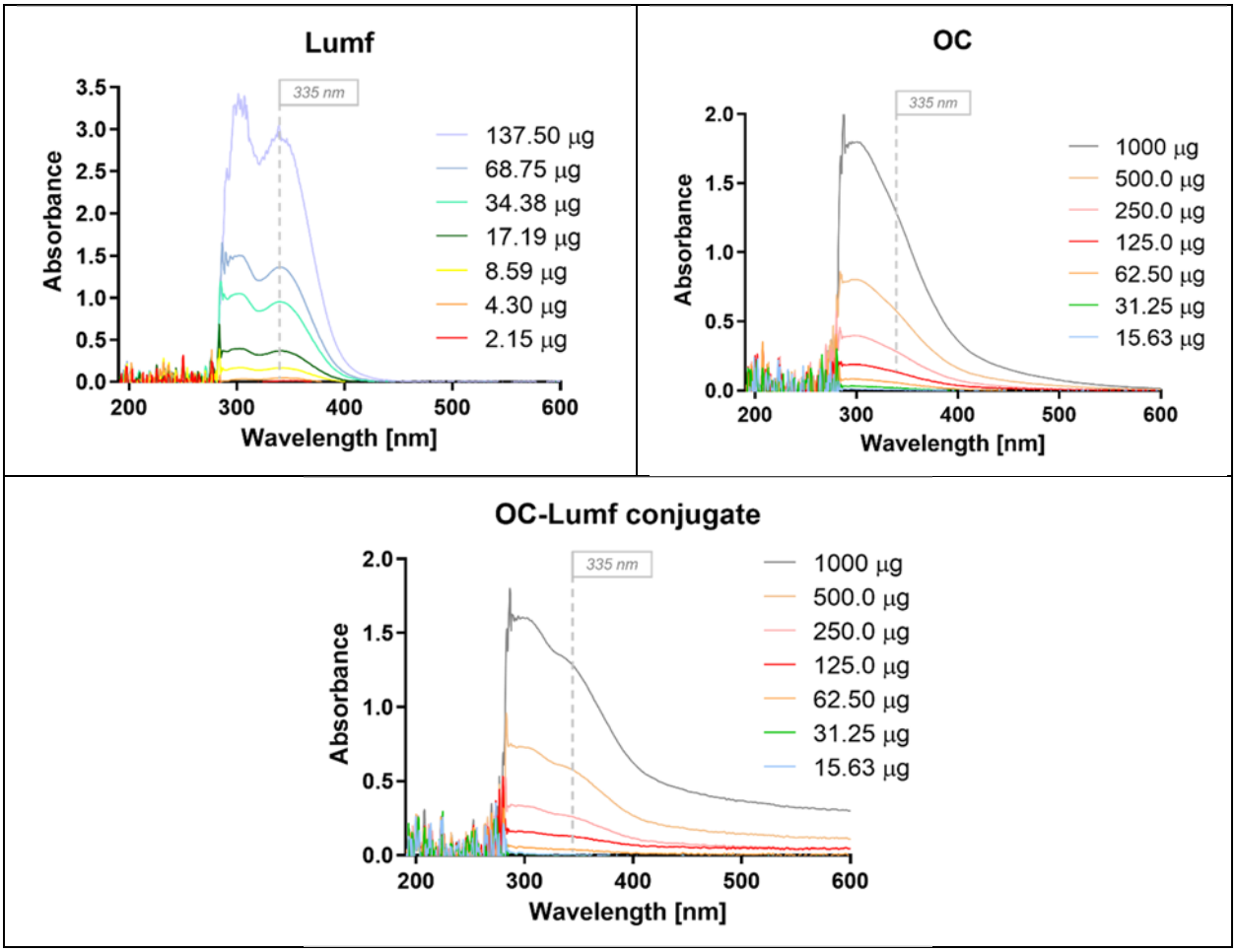

Figure S3. Spectrophotometric analysis of unconjugated lumefantrine (Lumf), unmodified OC, and OC-Lumf. Lumf absorbance readings were taken at 335 nm.

Immunofluorescent imaging of Caco-2 cells exposed to free antimalarial drugs

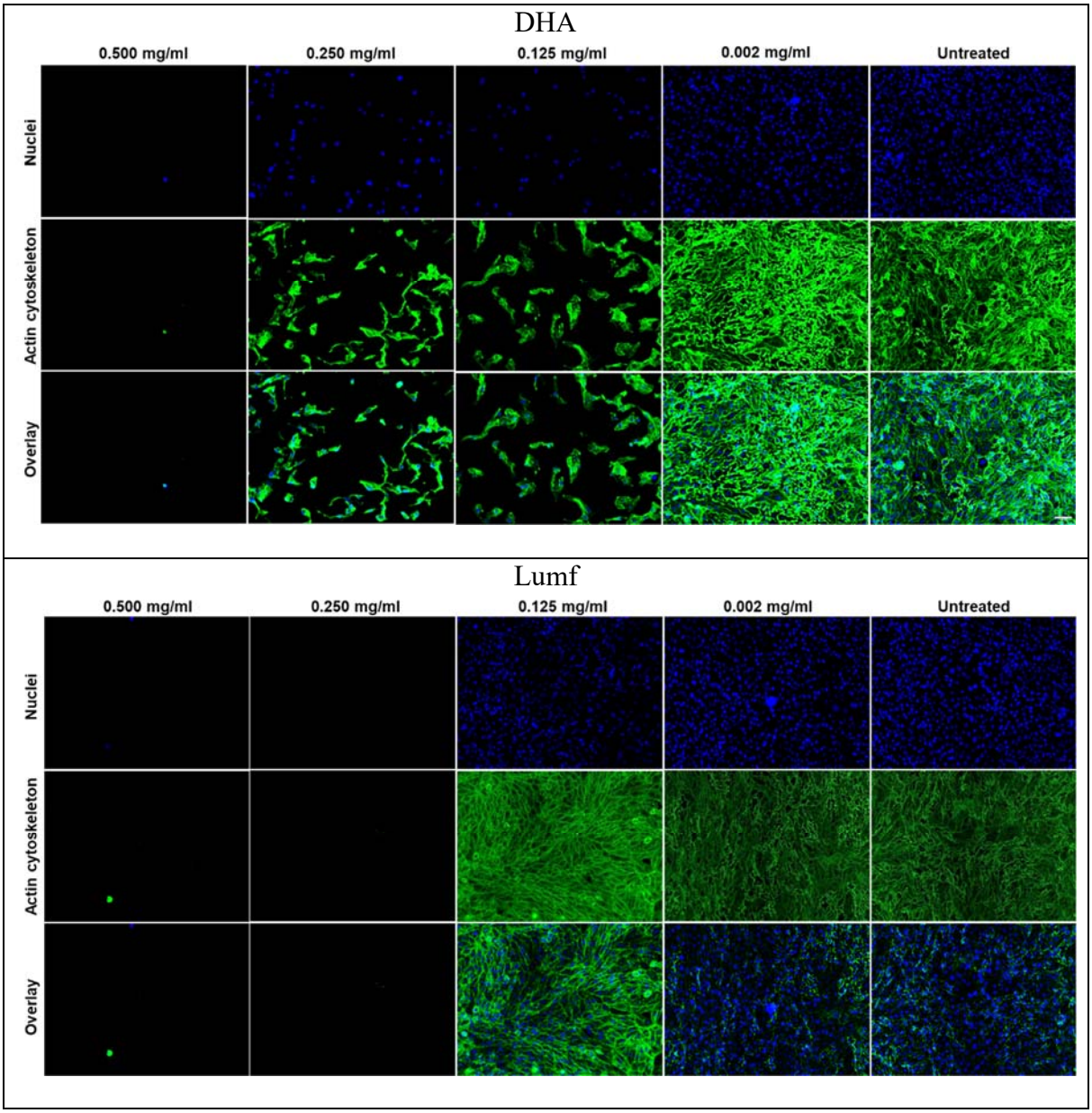

Figure S4. Immunofluorescent imaging of Caco-2 cell actin cytoskeleton and nuclei after 72-hour exposure to DHA) and Lumf. Scale: 100 µm.

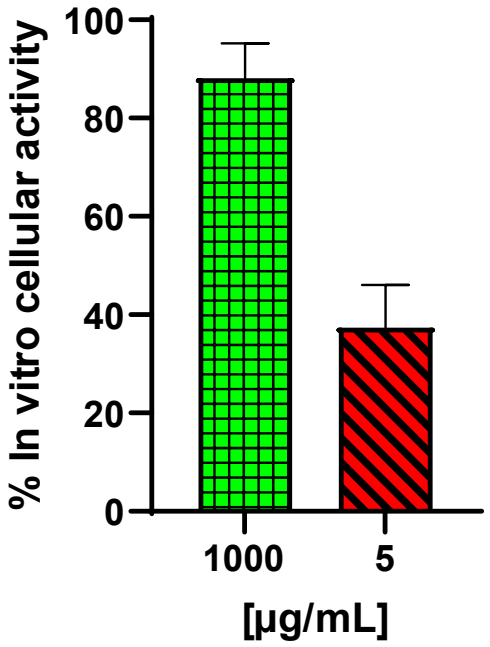

Figure S5. Cytotoxicity of pristine OC against Caco-2 cells (green) and *P. falciparum* (red). Cellular activity and viability were largely unperturbed in Caco-2 cells whereas there was a significant antiplasmodial activity even at a concentration exposure 200 times less than that of the mammalian cells.