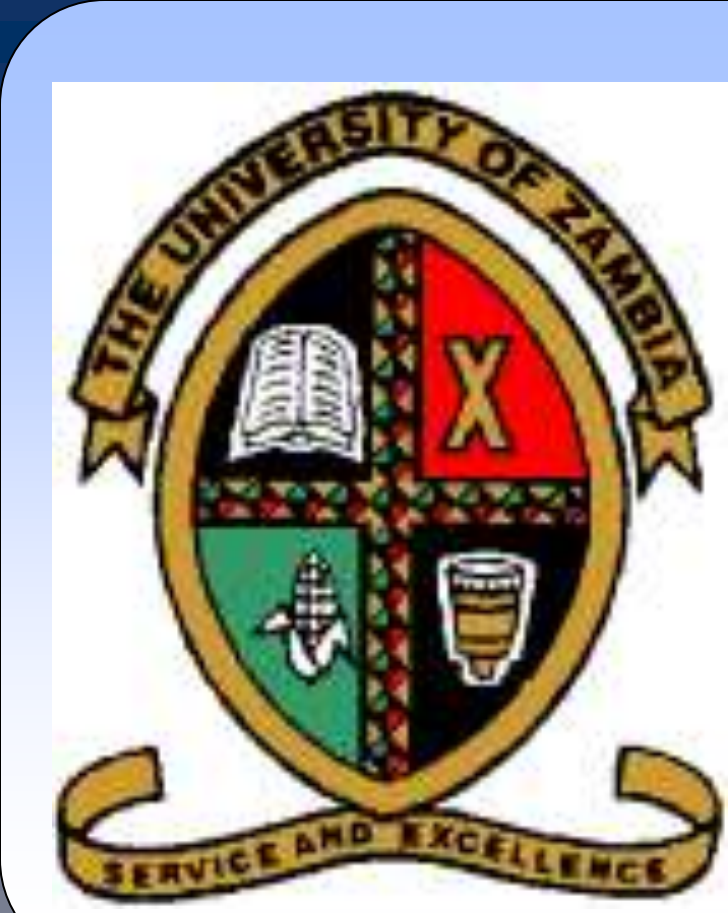




Synthesis and Antischistosomal Structure-Activity Relationship Profiling of *N*-Pyridazin-3-Ylbenzamides by In Silico and In Vitro Approaches

Harrison Banda*, Peter M. Cheuka** and Evelyn Funjika***

Department of Chemistry, School of Natural Sciences, University of Zambia

NB: *Principal Investigator and Corresponding Author 2018248812@student.unza.zm ; **Principal Supervisor; ***Co-supervisor



Ministry of
Technology and
Science

Introduction

Background

In 2021, WHO reported 240 million schistosomiasis (SCH) cases worldwide with 700 million people either living with it or at risk with a mortality rate of 300,000 deaths p.a. After malaria, it is the 2nd most prevalent tropical disease (TD) (an NTD but not an orphan disease). The approximate economic loss to SCH is US\$641,790,130 p.a., globally while *S. haematobium*-associated bladder cancer results in US\$20 million loss worldwide. Loss from SCH-disability in Africa is US\$445, 866, 945 p.a.; i.e. 70 % of the global cost with over 150 000 deaths p.a. attributed to *S. haematobium* and over 130 000 deaths resulting from *S. mansoni*. (1)

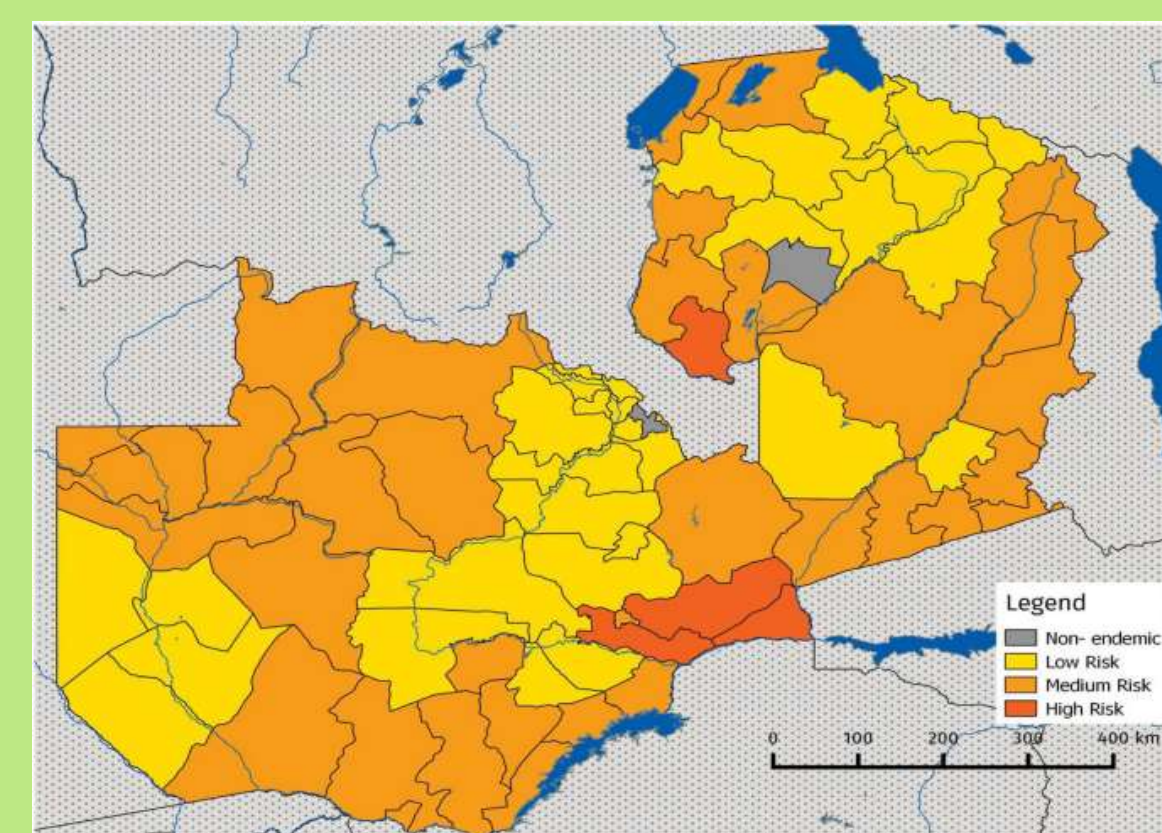
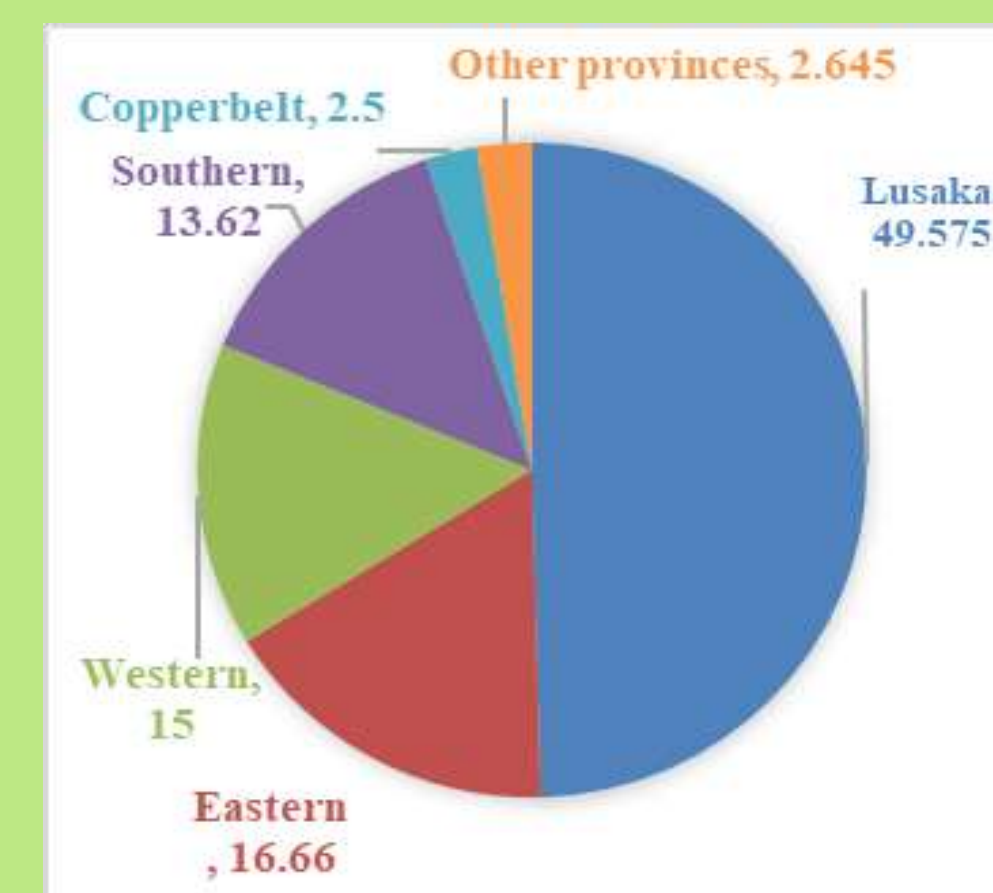


Figure 1: Schistosomiasis prevalence in Zambia: mapping (L); pie chart (R) (2)



Statement of the Problem

Although this drug PZQ is safe, effective, has a broad spectrum of activity, is orally administrable; but it has shown drug resistance and tolerance. Besides, it is ineffective against immature parasites, needs repetitive treatment since it does not prevent reinfection. Though considered cheap (to the patient as subsidized), the cost is US\$17.92 million p.a. globally. (2) Zambia has no available PZQ brand since 2nd August 2023.(3)

Aim

To synthesize and profile antischistosomal SARs of novel *N*-PdzbAs

Objectives

1. To design and synthesize novel *N*-PdzbA target compounds (TCs)
2. To characterize TCs via HPLC-MS, IR, ¹H-NMR and ¹³C-NMR
3. To study *in vitro* antischistosomal SARs on *Sm* NTS & adult worms
4. To profile the most potent derivatives wrt solubility and cytotoxicity

Rationale

The pyridazine moiety is expected to contribute more to the solubility.

Significance of the Study

This study, has added insights to the search for cheaper, multi-stage, less toxic, more efficacious anti-SCH drug leads.

Literature Review

Schistosoma (bilharzia) and *Plasmodium* (malaria) share a common parasitology of hemozoin production from heme detoxification.(4) Over the past 12 yrs, the Medicines for Malaria Venture (MMV) had released 400 compound (cpd) list which were first screened *in vitro* on *S. mansoni* (common to Zambia (2)), also tested against adult worms from which, 11 hits were identified most notably **MMV687807**, an *N*-phenylbenzamide (*N*-PhBA).(6) Kanyanta recently (2020-23) did SARs on **MMV687807** and made potent *N*-PhBAs (7) which unfortunately might face solubility issues in drug development based on *in silico* predictions just as other *N*-PhBAs from the MMV library have experimentally been poorly soluble (5).

Materials and Methods

Design and Synthesis

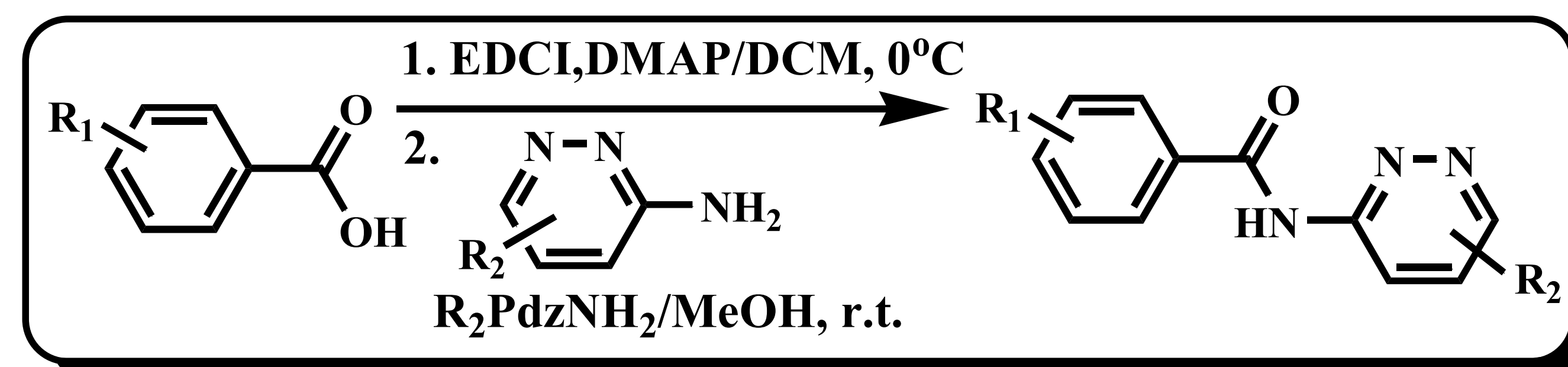


Figure 2: *N*-PdzbA EDCI/DMAP 1-pot synthetic design (L); rxn setup pictures (R)

Results and Discussion

Characterization

HPLC-MS & IR Analysis

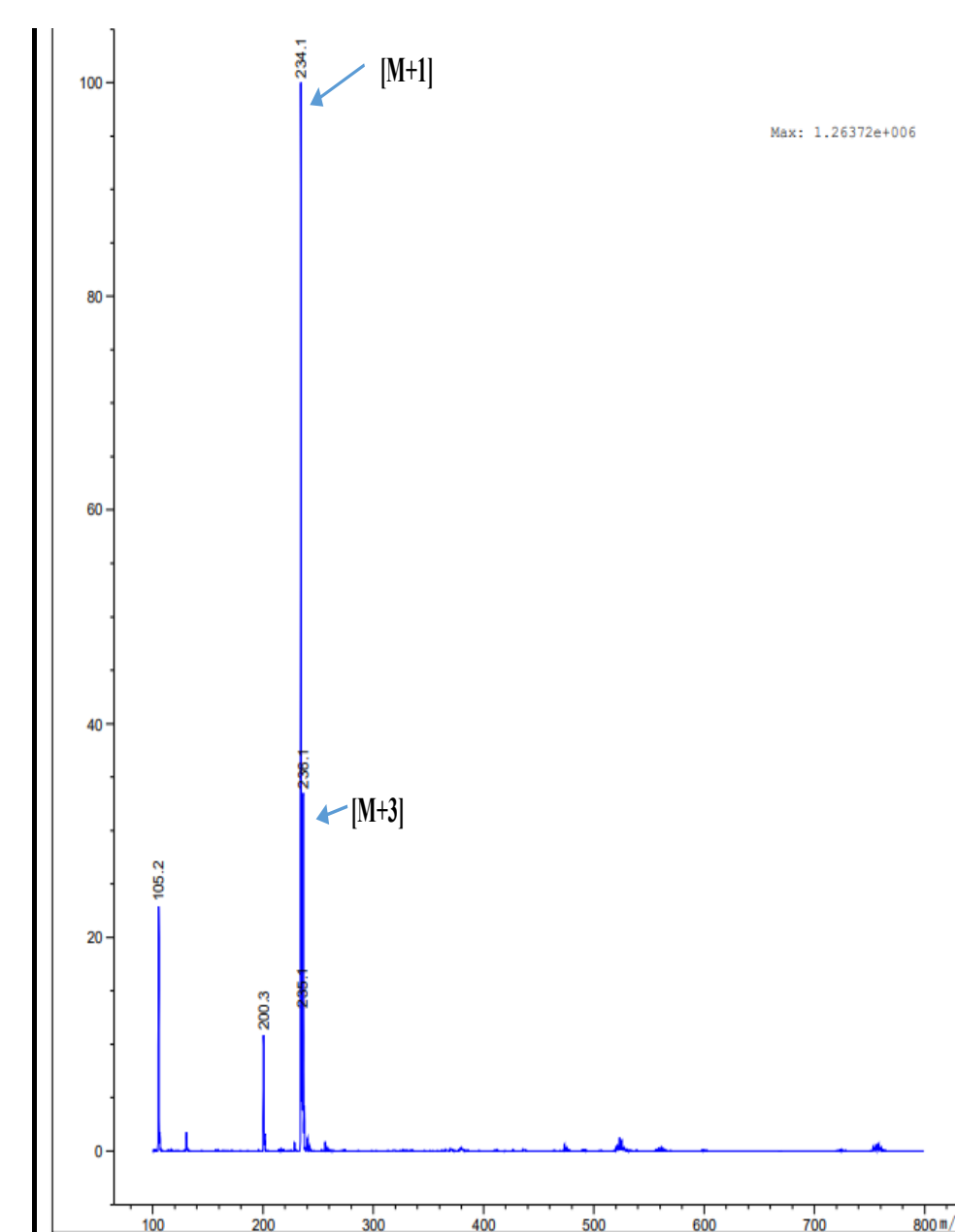


Figure 3: HPLC chromatogram 280nm (top); MS spectrum (bottom) of HB1-03

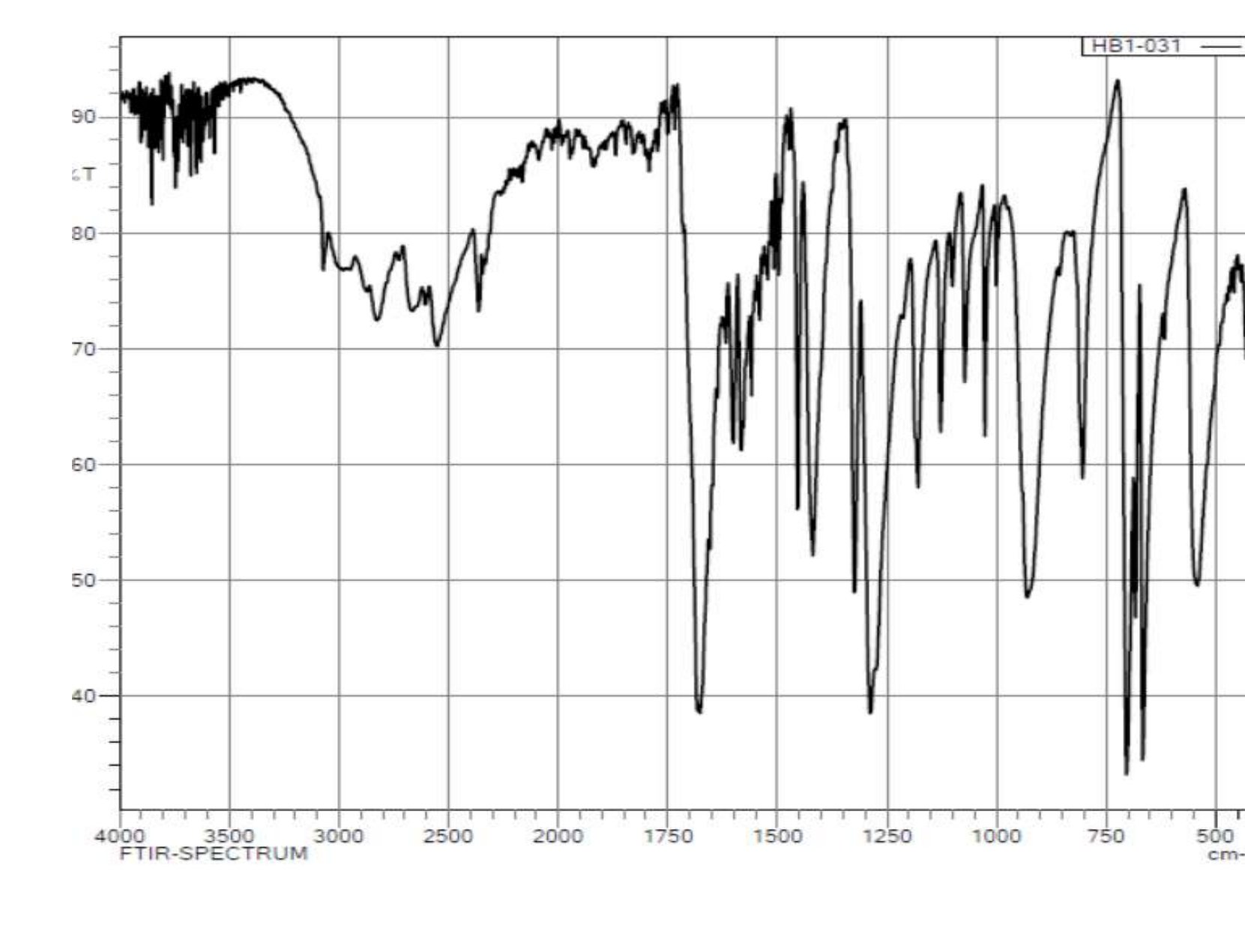


Figure 4: HPLC-MS, IR spectrometer (top) IR spectrum of HB1-03 (bottom), YashLife (Z)

¹H-NMR Spectroscopic Analysis

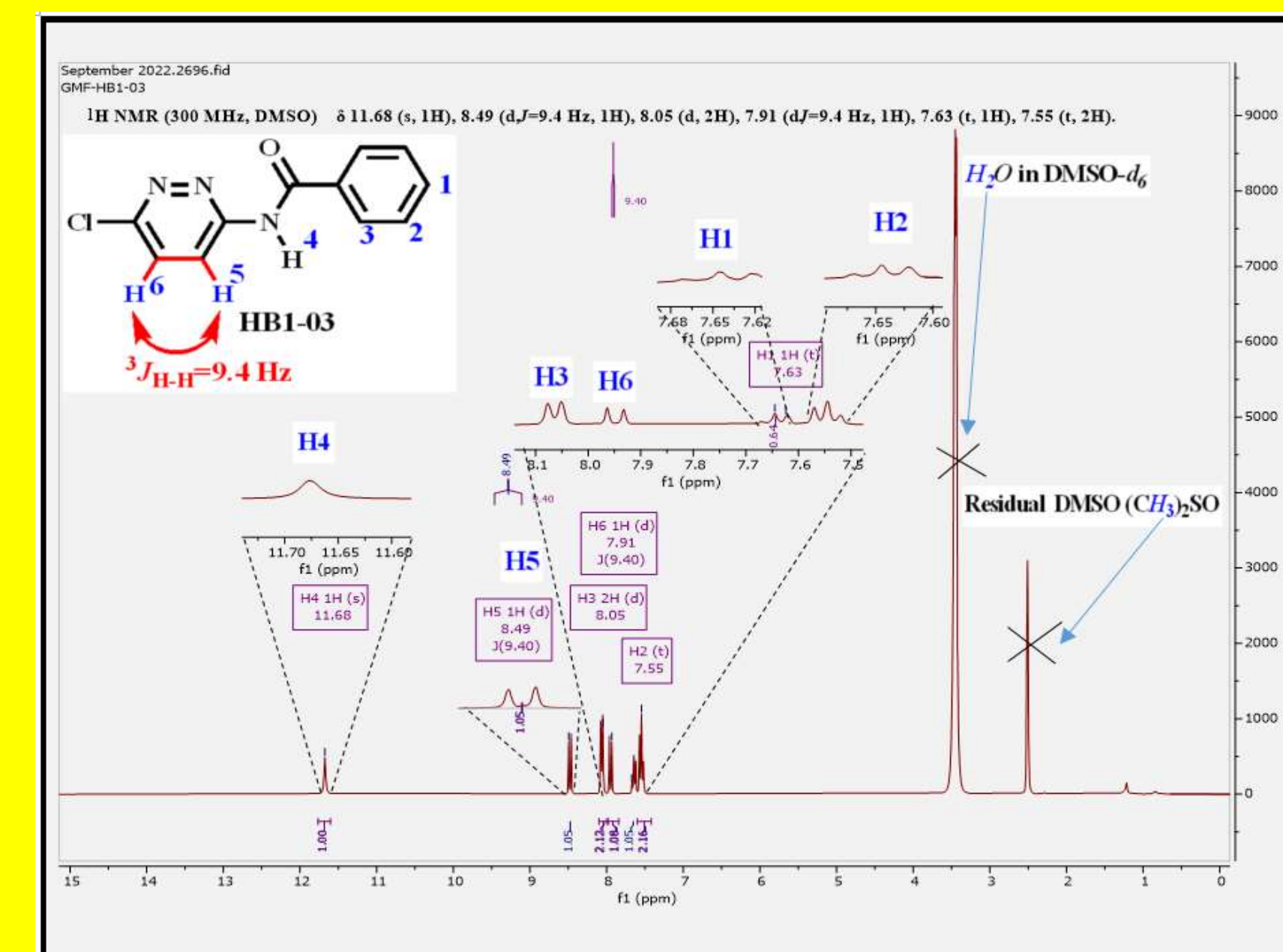


Figure 5: ¹H-NMR: spectrum (DMSO-*d*₆) of HB1-03 (L); spectrometer (R), UCT-RSA

¹³C-NMR Spectroscopic Analysis

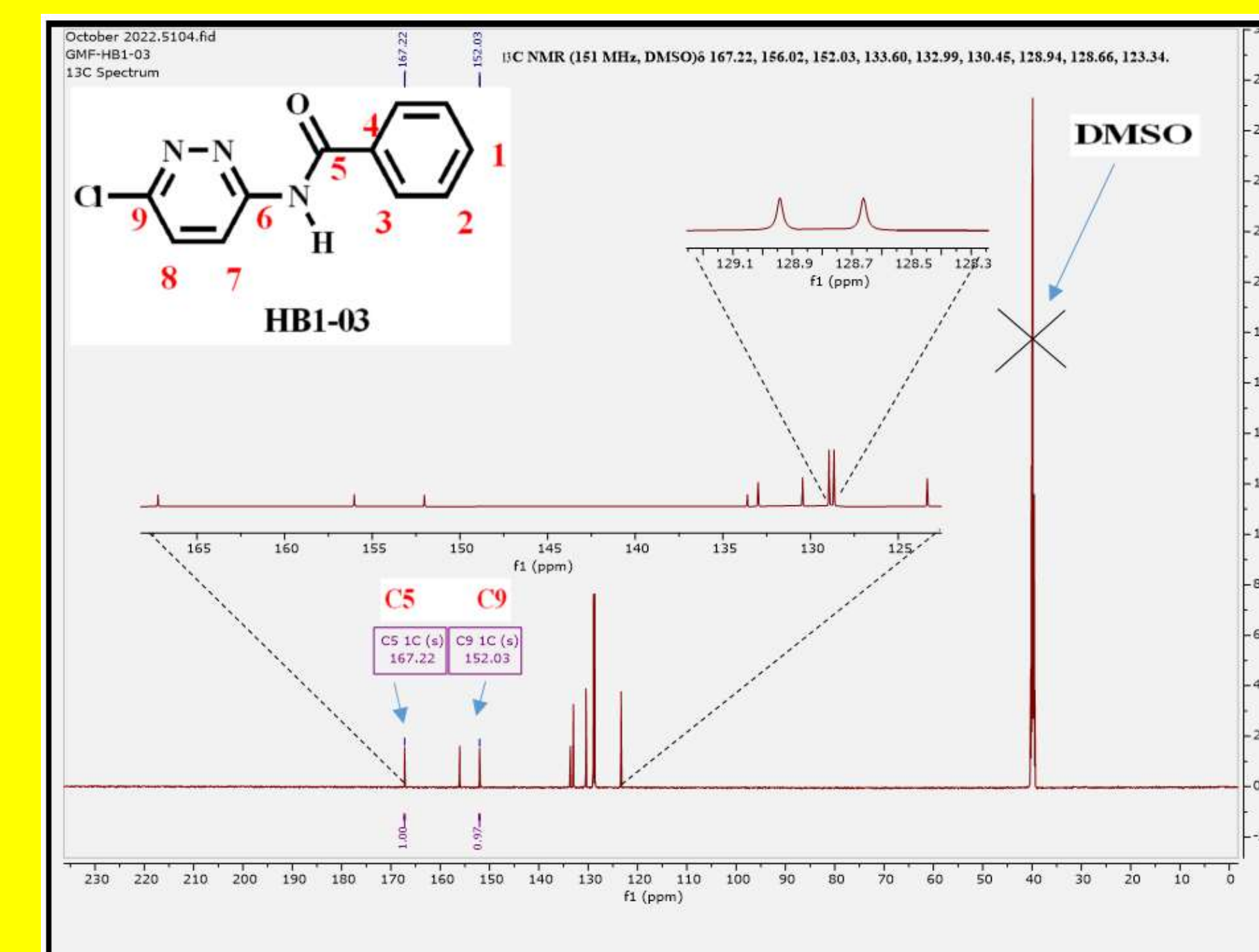


Figure 6: ¹³C-NMR: spectrum (DMSO-*d*₆) of HB1-03 (L); spectrometer (R), UCT-RSA

Pharmacological Testing

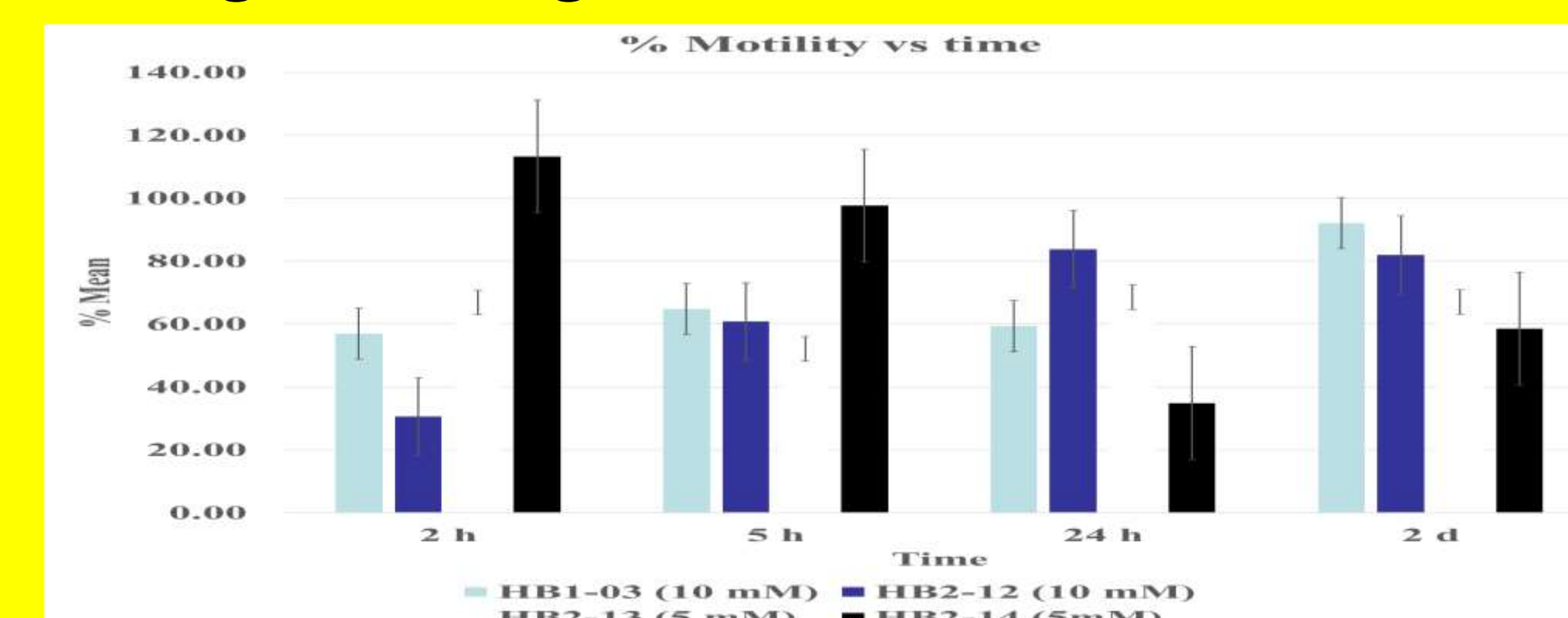


Figure 7: Histogram of *Sm* adult motility (UCSD, USA)

Table 1: *S. mansoni* assay *in vitro*: effect (% dead, 72h), SD (Swiss TPH)

	NTS				<i>S. mansoni</i> adult	
CPD	100µg/mL	50µg/mL	10µg/mL	1µg mL	50µg/mL 1 round	10µg/mL 1 round
HB1-03	48.33 (1.7)	ND	ND	ND	ND	ND

Conclusions and Recommendations

Figs. 3-5: Six (6) TCs had purities of >95%. All candidates were tested on *Sm* adult worms (Fig 6-7) with fair potency; favourable CC50≥20µM and better cLogP (and solubility) but only one (HB1-03) underwent *Sm* NTS assays with 48.33% dead in 72 h activity (Table 1).

Acknowledgements

- **Supervisor:** Dr P.M. Cheuka and Dr E. Funjika
- **Sponsor:** Ministry of Technology & Science, GRZ
- **Collaborating Labs:** University of Cape Town (Prof K. Chibale); UCSD (Prof C.R. Caffrey); Swiss TPH (Prof J. Keiser).

References

- 1.WHO. *Schistosomiasis (bilharzia)*. www.who.int (accessed 2023-08-25).
- 2.Global Atlas of Helminth Infections. www.thiswormyworld.org (accessed 2023-08-25)
3. <https://www.znbc.co.zm/news/zamra-recalls-indian-firms-medical-supplies-from-market/> (accessed September 11, 2023)
- 4.Weber, C. J. *et al. Trop Med Infect Dis* **2019**, 4 (1). <https://doi.org/10.3390/tropicalmed4010011>.
- 5.Ingram-Sieber, K. *et al. PLoS Negl Trop Dis* **2014**, 8 (1), 30. <https://doi.org/10.1371/journal.pntd.0002610>.
- 6.Pasche, V. *et al. Early ACS Infect Dis* **2018**, No. 2016, 1–9. <https://doi.org/10.1021/acsinfecdis.8b00220>.
- 7.Kanyanta, M. *et al. Bioorg Med Chem Lett* **2023**, 82, 129164. <https://doi.org/10.1016/j.bmcl.2023.129164>.