Characterization of the Structure-Function Relationship of a Host-Targeted anti-*Salmonella* Agent

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Background

- Typhoid fever is caused by the enteric pathogen *Salmonella enterica* serovar Typhi
- It is a major global health problem with over 22 million new infections as well as over 200,000 fatalities annually
- Infections are a result of consuming contaminated or uncooked food and water

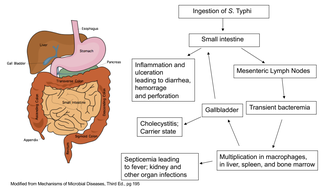


Fig.1 | Pathological effects of *Salmonella* infection

- Few vaccines for Typhoid fever, including a typhoid conjugate vaccine, have been developed
- There are antibiotic treatment options, however, increase in multi-drug resistant strains calls for alternate therapeutic options
- 3-(methoxycarbonyl)benzyl2-hydroxy-4-quinoline carboxylate (KH-2) was shown to inhibit *Salmonella* growth inside macrophages in previous cell-based screen assays **(Figure 5a)** and in a typhoid fever mouse model **(Figure 4)**. However, it does not have direct anti-*Salmonella* killing *in vitro* **(Figure 5b)**
- A weak ester link between the KH-2 carboxylate and quinoline groups raised the possibility that the carboxylic acid released from KH-2 as a result of intracellular metabolic activities of the infected macrophages is what causes the anti-*Salmonella* phenotype **(Figure 2)**

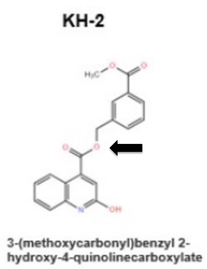
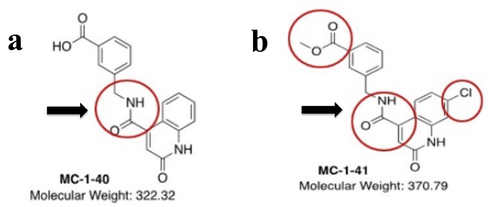


Fig.2 | Structure of KH-2

Objectives

- Is there a direct linkage between the efficacy of the compound in macrophages if the labile ester linkage is changed to a more stabile amide linkage between the carboxylate and quinolone groups?

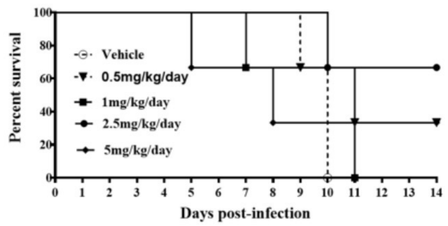
Fig.3 | Structures of KH-2 analogs **a.** MC-1-40 and **b.** MC-1-41



Methods

- The weak ester link was structurally replaced with a more stable amine linkage between the carboxylate and quinoline groups
- Two KH-2 analogs, MC-1-40 and MC-1-41, were formed as a result **(Figure 3)**
- *In vitro* toxicity studies were conducted alongside colony forming unit analyses **(Figures 6 & 7)**

Fig.4 | Survival Rates of BALB/c mice infected with *S.* Typhimurium (causing Typhoid fever) and treated for 10 days with KH-2 (i.p.)



Results

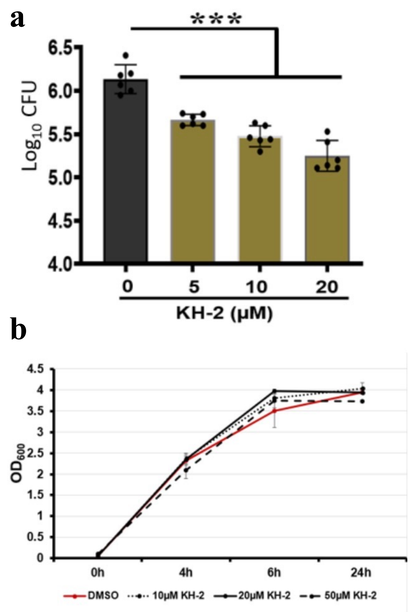


Fig.5 | Effects of KH-2 on *Salmonella* growth in **a.** macrophages and **b.** LB Broth

Fig.6 | LDH Assay of compounds at 20μM and 40μM to test toxicity at **a.** 24h and **b.** 48h

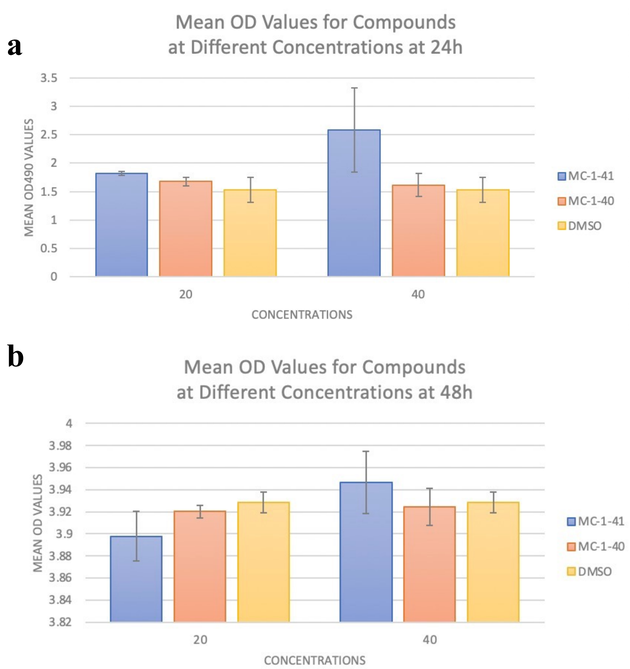
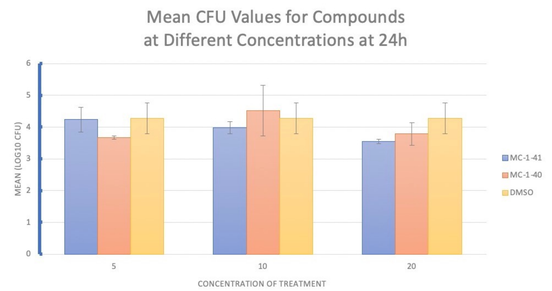


Fig.7 | Colony forming units after macrophage infection with *Salmonella* and treatment with compounds MC-1-40 and MC-1-41



Conclusions

- *In vitro* examination of toxicity showed both MC-1-40 and MC-1-41 to display a similar non-toxic effect on J774.1 macrophages as their parent compound KH-2 **(Figure 6)**
- Colony forming unit analyses showed that structural modification of MC-1-40 and MC-1-41 doesn't affect the anti-*Salmonella* activity such that bacterial intramacrophage survival is similar to that observed with KH-2 **(Figure 7)**

Future Directions

- This study shows insight into the structure-function relationship of KH-2 for the development of a lead compound for host-targeted therapy to control Typhoid fever

References

1. Typhoid, G.B.D. and C. Paratyphoid, The global burden of typhoid and paratyphoid fevers: a systematic analysis for the Global Burden of Disease Study 2017. Lancet Infect Dis, 2019. 19(4): p. 369-381.
2. Syed, K.A., et al., Review on the Recent Advances on Typhoid Vaccine Development and Challenges Ahead. Clin Infect Dis, 2020. 71(Supplement_2): p. S141-S150
3. Britto, C.D., et al., A systematic review of antimicrobial resistance in Salmonella enterica serovar Typhi, the etiological agent of typhoid. PLoS Negl Trop Dis, 2018. 12(10): p. e0006779.

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