Author’s response to reviews

Title: Identification of cryptolepine metabolites in rat and human hepatocytes and metabolism and pharmacokinetics of cryptolepine in Sprague Dawley rats

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RESPONSE TO REVIEWER COMMENTS

Reviewer 1

Comment 1: It would be important to stress that this research was a "mere proof of concept", due to the extremely low sample size (2+2 rats!)

Response: The fifth line of the fifth paragraph reiterates that this study was a mere proof of concept hence the low sample size for the various route of drug administration. A more in depth radiolabelled cryptolepine and higher dose studies allowing analysis of plasma and excreta over a longer timeframe similar to mass balance excretion studies as described in literature has been recommended for further studies.
Comment 2: The lackness of a formal sample size determination has to be justified: moreover, why 2+2 rats?

Response: As discussed above, the PK investigation was a basic proof of concept study. In our experiments, the main aim was a qualitative investigation of drug metabolites, which was achieved with the sample size selected.

Comment 3: Stat and pk methods are partially lacking, no stat tests were used? which pk package was used? oral vs. iv data were different? and so on!

Response: Non compartmental pharmacokinetic analysis was performed using Phoenix 6.3 (Certara, St Louis, MO, USA) to determine the pharmacokinetic parameters. Because of the low sample size used it was not possible to determine meaningful statistical parameters and therefore no statistical evaluations were performed. The IV and oral route had slightly different PK profiles because of the dose and the route of administration, and are otherwise not substantially different. Due to the metabolism focus of this paper, the low PK sample size and the variability of the data, extensive discussion of the PK parameters was omitted.

Comment 4: Mind several undefined abbreviations, like i.e. NVP-TAA501, D2O, CH3OD, NIBR-CA IACUC: all of them have to be properly defined.

Response: All abbreviations have been defined or removed as appropriate.

Comment 5: Mind some typos here and there, like Cremaphor

Response: Cremaphor EL was misspelled. The correct spelling (Cremophor EL) has been replaced in the manuscript.
Response: The changes recommended by reviewer 2 with regards to the in-text referencing above has been effected according and highlighted in the text.

Comment 2: Line 35-37

Based on this data and our new rat pharmacokinetic data, cryptolepine is likely to extensively distribute in human,

Based on this data and our new rat pharmacokinetic data, cryptolepine and its metaboliteproducts are likely to extensively distribute in human,

Response: The cited data and our current study only investigated PK/distribution of the parent compound cryptolepine and do not allow conclusions on the distribution of metabolites. It could be expected that metabolites would distribute less extensively than cryptolepine as they are relatively polar.

Comment 3: Most probably the antimalarial activity of cryptolepine is related to its active metabolites

References 1, 14 and 24
Instead of et al at least 5 Authors should be written

Page 13 line 3

Response: All names for the authors of reference 1, 2, 14 and 24 have been provided as suggested.

Comment 4:

Note:

ADF, CA, CR, DP and AC performed the statistical analysis. However no statistical analysis have been shown in the manuscript?

Response: As stress in the manuscript, this research was a mere proof of concert hence the low sample size for the in vivo experiment. ADF, CA, CR, DP and AC participated in the study design, carried out the experiments and drafted the manuscript.