Summary
The protozoan parasites *Trypanosoma* and *Leishmania* infect humans as well as the livestock animals and against these infections no safe and effective drugs are currently available. Glycosomes are peroxisome-like organelles that are essential for the parasites. Therefore disruption of the glycosome biogenesis is an attractive drug target. In this study, a protein essential for glycosome biogenesis was identified and structure-based small molecule inhibitors of the glycosome biogenesis were designed and characterized.

Identification and functional characterization of Trypanosomatid PEX16
PEX3, PEX16 and PEX19 are essential proteins in different organisms for the peroxisomal membrane protein import and *de novo* peroxisome formation from the endoplasmic reticulum (ER). Of these three proteins, only PEX19 is known in trypanosomes. Using bioinformatics approach, we identified a candidate PEX16 homolog in Trypanosomatid parasites. *T. brucei* PEX16 is an integral membrane protein localized to the glycosomes. RNA interference (RNAi) knockdown of PEX16 expression led to disruption of glycosome biogenesis. The glycosome number was drastically reduced and the glycosomal enzymes were mislocalised to the cytosol. Consequently, a strong reduction in the cellular ATP levels and defects of the normal flagella-driven motility was observed. The few remaining glycosomes were distributed unevenly in a trypanosome. Opposite effects on the glycosome distribution in a trypanosome were seen upon downregulation or overexpression of PEX16. These observations suggest that *de novo* pathway plays major role in the glycosome biogenesis and regulated transport of glycosomes likely exists in trypanosome. Upon heterologous expression, parasite PEX16 proteins localized to the peroxisomes in normal yeast and human cells, but failed to rescue the function of defective human PEX16 in patient cells. Due to the low sequence similarity and the functional differences with human protein, Trypanosomatid PEX16 provides a novel drug target for the specific disruption of the glycosome biogenesis.

Identification and characterization of the inhibitors of PEX5-PEX14 interaction (Generated by structure-based design)
Di-aromatic pentapeptide motifs in PEX5 interact with the well-conserved N-terminal domain of PEX14. Based on the known 3D structure of human complex, homology modeling of the *Trypanosoma* PEX5-PEX14 complex revealed differences in the binding site. Structure-based design of small molecules, synthesis and *in vitro* inhibition studies (by collaborators in Helmholtz Zentrum München) provided an early stage competitive inhibitor of *Trypanosoma* PEX5-PEX14 binding. During this thesis, a medium-throughput assay was established for screening the activity of these inhibitors against trypanosomes and human
cells. Using this assay, compounds with promising anti-trypanosomal activity and low human cell cytotoxicity were identified and were further subjected to the chemical structure optimization. Multiple cycles of bio-activity assays followed by modification of compound and \textit{in vitro} inhibition tests were performed. More than 200 compounds were screened during activity-guided optimization which rapidly yielded highly potent inhibitors with trypanocidal activity in nanomolar range and very low toxicity to human cells. Despite of some suboptimal pharmacokinetic properties, the treatment of trypanosome-infected mice with the inhibitor led to the partial cure of parasite load in the blood. A direct correlation between \textit{in vitro} PEX5-PEX14 inhibition and anti-trypanosomal activity was observed, which is an indicator of the compounds acting on target \textit{in vivo}. The most active compounds allowed the validation of the action of the inhibitors on biogenesis of glycosomes (on-target). Successful inhibition of the glycosomal protein import was shown and corroborated by the inhibitor-induced glucose toxicity and ATP depletion in trypanosomes.