# Discovery of Paroxetine as a Potent Anti-Schistosomal Compound: Integration of High-Content Screening and Molecular Modeling Methods

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## Abstract

Here we report the *ex vivo* activity of paroxetine (PAR) against adult *S. mansoni* worms. Further, we explored structural basis for PAR activity to design more potent and selective analogues.

## Introduction

Recently, we reported an *in silico* chemogenomics approach[1] that predicted PAR, as a *Schistosoma mansoni* serotonin transporter (*Sm*SERT) inhibitor, and consequently, a new anti-schistosomal drug candidate. In this study, we validated the anti-schistosomal activity of PAR on adult *S. mansoni* worms using a new automated image-based assay to accurately measure worm motility. Further, to guide the optimization of new anti-schistosomal compounds, we explored the interactions of PAR with binding sites of *Sm*SERT and its respective human counterpart (*h*SERT).

## Results and Discussion

Individual adult male and female *S. mansoni* worms were incubated in culture medium DMEM with 0.01-200 µM PAR for various time periods up to 72h. *Ex vivo* drug sensitivity was determined using motility measurements obtained with a newly developed anti-helminthic high-content screening platform. This method is based on sequential pairwise comparison of 100 time-lapse images captured every 250-300 ms using an automated bright-field microscope with a 2x objective lens (ImageXpress Micro XLS, Molecular Devices, CA). Analysis of images was then carried out in our most recent custom-developed pipeline built with open-source CellProfiler software v. 2.1.2 in order to detecting changes in parasite motility. We observed for low drug concentrations (i.e., <10 µM in females and <20 µM in males) an increase in worm motility of up to 9-fold in females and 2-fold in males immediately after drug exposure. This delayed effect would be consistent with the timing of serotonin receptor internalization, a protective mechanism to avoid excitotoxicity. After, $EC_{50}$ values determined for PAR at different incubation times indicate that after 24h an inhibitory effect on motility is fully developed with values varying from 2.7 to 5.1 µM for male worms and 9.9 to 11.9 µM for female worms. Our results indicate that male worms are slightly more sensitive to PAR action, since they showed on average $EC_{50}$ values 2-3 times lower than those determined in females. In order to explore structural basis for PAR activity in schistosome motility, homology models were built for *Sm*SERT and *h*SERT in SWISS-MODEL server and extensively validated in SAVES webpage. To investigate the binding mode of PAR with both targets, we performed docking studies using FRED software available in OEDocking suite v. 3.0.1. Results showed that the aromatic rings of PAR interact with the hydrophobic pocket whereas the protonated nitrogen of piperidine is able to form two hydrogen bonds with the carbonyl groups of Phe81 and Asp84 of *Sm*SERT. The analysis of the modeled proteins revealed differences in the hydrophobicity of the two binding sites. Three amino acid residues (Thr158, Phe81 and Ala506) of *Sm*SERT were substituted in *h*SERT by Ile172, Tyr95 and Thr497, respectively. It therefore appears that the binding site of *Sm*SERT can accommodate bulkier ligands. These key differences may be useful to design and synthesize more potent and selective structural analogues.

## Conclusion

In conclusion, we identified potent anti-schistosomal activity of PAR against adult *S. mansoni* worms. This drug offers a new biochemical pathway to kill schistosomes by disrupting serotonin signaling and its downstream events. In addition, homology modeling and docking studies with *Sm*SERT and *h*SERT revealed insights into the chemical basis of PAR anti-schistosomal activity. These results provide guidance for further studies to optimize PAR in terms of potency and selectivity.

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