

Discovery of New Anti-Schistosomal Hits Using Integrated Medicinal Chemistry Approaches

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Abstract

We here report the discovery of six new anti-schistosomal hits by integration of QSAR-based virtual screening (VS) of *S. mansoni* Thioredoxin Glutathione Reductase (*SmTGR*) inhibitors and High-Content Screening (HCS).

Introduction

Schistosomiasis, a debilitating neglected tropical disease, is caused by trematode flatworms of *Schistosoma* genus. The treatment relies on a single drug, praziquantel (PZQ),¹ making urgent the discovery of new drugs that possess different mechanisms of action. In this study, we developed a QSAR-based VS workflow for the identification of new *SmTGR* inhibitors, a validated target for the treatment of schistosomiasis.² Then, the prioritized virtual hits were experimentally evaluated on live schistosomula and adult worms using two distinct HCS platforms.

Results and Discussion

The QSAR models were developed according to OECD principles. A large dataset of compounds with inhibition data for *SmTGR* (PubChem Bioassay ID: 485364) was prepared according to following steps: inconclusive biological data were removed; a threshold value was defined to discriminate between inhibitors ($\leq 10 \mu\text{M}$) and non-inhibitors ($> 10 \mu\text{M}$); chemical structures were standardized, and duplicates identified. In addition, a linear under-sampling strategy was developed for reducing the number of samples in the non-inhibitors set to make it equivalent in size to the inhibitors set. Next, QSAR models were developed by combining eight different machine learning methods and five types of descriptors, and rigorously validated using 5-fold external cross-validation strategy. This approach led to highly predictive (CCR ranging between 0.81 – 0.85 and coverage of 0.62 – 0.77), and mechanistic interpretable models.

After modeling, developed models were used in the most conservative way (i.e., in consensus fashion and with the strictest applicability domain criteria) for VS of ChemBridge database. As a result, 29 compounds were purchased and experimentally evaluated against schistosomula using a HCS platform. Six compounds showed significant mobility and phenotypic scores in 20 μM concentration. For comparison with standard drugs, four hits were more potent inhibitors of schistosomula motility than PZQ while two hits showed inhibitory effect (i.e. EC_{50} values ranging between 2.62 – 3.23 μM for motility and 3.41 – 6.60 for phenotype) equivalent to oltipraz (OLT), a known *SmTGR* inhibitor.² Schistosomula exposed to the compounds showed a phenotypic profile similar to the ones observed after *SmTGR* gene knockout and exposure to OLT. In addition, most of hits showed negligible to tolerable cytotoxicity against human cells (WSS-1). Further, we investigated the gender- dose- and time-dependent drug effect of the schistosomula hits on adult *S. mansoni* worms using motility related Overlap_RandIndex. After 24-72h of incubation at 100 μM , three hits almost completely halted female worms motility (76-98% inhibition), an effect that was statistically equivalent to PZQ.

Conclusion

We have developed robust, predictive, and experimentally validated QSAR models for VS of new anti-schistosomal hits. To the best of our knowledge, this is the first study integrating QSAR-based VS and HCS methods to discover new anti-schistosomal agents.

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