Target-based molecular modeling strategies for schistosomiasis drug discovery

Schistosomiasis, a neglected tropical disease caused by worms from the class Trematoda (genus *Schistosoma*), is a serious chronic condition that has been reported in approximately 80 countries. Nearly 250 million people are affected worldwide, mostly in the sub-Saharan Africa. Praziquantel, the mainstay of treatment, has been used for 30 years, and cases of resistance have been reported. The purpose of this perspective is to discuss current target-based molecular modeling strategies in schistosomiasis drug discovery. Advances in the field and the role played by the integration between computational modeling and experimental validation are also discussed. Finally, recent cases of the contribution of modern approaches in computational medicinal chemistry to the field are explored.

Neglected tropical diseases

Neglected tropical diseases (NTDs) are defined as a group of 17 health conditions, highly prevalent in tropical areas, mainly in low-income countries [1]. The humanitarian burden caused by NTDs reaches approximately one and a half billion people around the world, who are mostly concentrated in rural areas and degraded urban zones where precarious sanitary conditions are a determinant for the proliferation of vectors [2]. Because of international migration, the worldwide distribution of NTDs has been extended to developed countries, especially North America, Europe and Asia [3]. In spite of this globalization, among the 402 new chemical entities (NCEs) registered between 2000 and 2013, none targeted NTDs. In general, the available drugs have low efficacy and cause severe side effects, usually consisting of drugs developed to treat other diseases [4].

Recently, investments in basic and applied research have increased, essentially through the implementation of public–private partnerships (PPPs) [5]. Because drug development is extremely risky and expensive, the PPP model is highly attractive for NTDs as the associated costs are shared among the

different partners and the goals are settled to meet public health issues [5].

These multilateral efforts are usually formulated and implemented by nonprofit organizations in association with private companies and public research institutions – generally global centers of excellence in specific areas of expertise [6]. Integrated drug discovery may be considered the current standard model for NTDs because it privileges cooperativeness, improves cost–effectiveness and increases the probability of success [7].

Schistosomiasis

Schistosomiasis is a highly prevalent and chronic NTD caused by flat worm parasites of the genus *Schistosoma*, affecting almost 250 million people worldwide in tropical and subtropical regions [8]. The disease puts approximately 800 million people at risk of infection and causes approximately 300,000 deaths annually [9]. It is found in 78 countries in Africa, the Caribbean, South America, the Middle East and Asia, with more than 70% of all cases occurring in ten countries of Africa [10].

The larval form of the parasite, called cercaria, is transmitted through water infested with its intermediate snail hosts during

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agricultural labor, and domestic, occupational and recreational activities. Children are especially vulnerable to infection because of poor hygiene habits and swimming and fishing in infested water. Prevalence is higher in children from 5 to 14-years-old, who suffer the most consequences of the disease, such as poor growth and defective cognitive development [10]. Schistosomiasis also contributes to malnutrition and interruption in school attendance [11].

The standard approach for the treatment and control of the disease is massive administration of praziquantel (PZQ) (Figure 1). The compound – a pyrazinoisoquinoline derivative – is a 30-year-old drug with an unknown mechanism of action, which is administered to millions of people each year [12]. The last WHO epidemiological record reports that 42 million people received PZQ as preventive chemotherapy in 2012 [10]. The β subunits of calcium channels have been speculated as a possible target for PZQ. The drug affects the influx of calcium, causing muscle contraction, paralysis and disintegration of the tegument [13]. Praziquantel is also used against other helminths, including *Taenia saginata*, *Taenia solium*, *Diphyllobothrium latum*, *Dipylidium caninum*, *Hymenolepis nana*, *Clonorchis sinensis*, *Heterophyes heterophyes*, *Fasciolopsis buski*, *Opisthorchis viverrini*, *Metagonimus yokogawai* and *Paragonimus westermani* [13].

The effectiveness of PZQ is approximately 60–90% in ideal conditions; however, the drug needs the host's immune response to be effective, and immature parasites are insensitive. Although rare, resistance has been found in the three main species that cause schistosomiasis (*Schistosoma mansoni*, *S. haematobium* and *S. japonicum*) [14]. These drawbacks have given rise to the search for novel chemotherapeutic alternatives.

Strategies in drug discovery

Current pharmaceutical R&D productivity has declined in the past two decades. The rate of NCEs entering the market has remained approximately constant, but the costs of developing a new drug and attrition rates have increased [15]. Therapeutic innovation has become more challenging partly because of the proliferation of potential macromolecular targets resulting from advances in genomics, molecular and structural

biology. Additionally, current R&D investments focus on high-risk and complex therapeutic areas, such as multifactorial diseases, which are characterized by outstanding uncertainty and difficulty [16]. This context has demanded a strong emphasis on highly integrated molecular and computational strategies, which have been employed in parallel with more traditional approaches, such as phenotypic screening and the synthesis of analogs of active compounds [17]. The current panorama of pharmaceutical R&D emphasizes the iterative integration of phenotypic, genomic, molecular and computational methods, mainly in the early preclinical stages of discovery [18].

Bioinformatics plays a central role in the discovery process today. Pharmaceutical companies have enhanced their ability to find novel molecular targets by exploring the information provided by genomic data [19]. As the knowledge of the molecular basis of biological systems evolve, the number of tools for storing and analyzing these data increases as well. This is exemplified by the wide variety of databases currently available, which contain information on **transcriptomes**, proteomes, metabolomes, protein sequence and structure, as well as protein-ligand interactions [20–24]. The analysis of such data has been used by industry and academia to predict and prioritize novel drug targets. In the current scenario, bioinformatics has a synergic relationship with medicinal chemistry, molecular modeling and drug discovery.

Computational methods in medicinal chemistry are broadly classified into **structure-based drug design** (SBDD) and **ligand-based drug design** (LBDD) [25]. R&D programs employ SBDD methods when sufficient structural information about the molecular target, mainly obtained using x-ray crystallography and nuclear magnetic resonance (NMR), is available [26]. SBDD explores the 3D structural data from molecular targets, simulating critical intermolecular events between small-molecule ligands and macromolecules. This information is applied with different methods, such as structure-based virtual screening (SBVS); molecular docking, which predicts binding conformations; and molecular dynamics, which evaluates conformational stability and flexibility [27–29]. Macromolecular databases, such as the Protein Data Bank (PDB), have been of pivotal importance for SBDD. The PDB contains the 3D coordinates of more than 100,000 biological macromolecule structures, among which 92% are proteins [30].

LBDD methods focus on the investigation of small-molecule compounds with known activity. They extract molecular features essential for biological activity, producing rules and patterns usually represented as pharmacophoric hypotheses, classification models,

Figure 1. Structure of praziquantel, the standard treatment for schistosomiasis adopted by WHO.

quantitative structure-activity and structure-property relationships (QSAR and QSPR, respectively) [31,32]. These models are employed to predict biological activity, to design novel compounds and for use in ligand-based virtual screening (LBVS) [33,34]. Ligand databases used in LBVS incorporate millions of small-molecule compounds containing valuable information, such as structure, physicochemical properties and biological activity data. The ZINC database and PubChem are examples of such compound repositories [35,36]. Handling this large amount of data requires constant development of new tools in chemo- and bioinformatics to convert the available information into useful relationships and knowledge.

Schistosomiasis drug discovery

Drug discovery for schistosomiasis is historically based on phenotypic assays, such as *in vitro* and *in vivo* screening [14]. All current marketed anthelmintics were discovered using these methods [37]. Limitations such as poor knowledge of the molecular biology of the worms and lack of **functional genomics** tools contributed to impair, until recently, the development of molecular and target-based approaches for schistosomiasis. This background resulted in the current small number of thoroughly validated molecular targets, which is the case for all NTDs [38].

The publication of the genomes of *S. mansoni*, *S. japonicum* and *S. haematobium* was a major advance in the field, providing new information on the parasites' biology and the molecular apparatuses responsible for their growth, nutrition and interaction with hosts [39–41]. Schistosomes take advantage of various signaling pathways of their hosts for their own metabolism. For instance, they are unable to *de novo* synthesize fatty acids, sterols, purines and some essential amino acids [40]. Their genome encodes many transporters, including nuclear receptors, apolipoproteins and neuropeptide receptors, able to interact with key metabolites obtained from hosts [41].

The disclosure of the genome was responsible for the increasing amount of investigations on potential molecular targets for schistosomiasis [42]. Proteins such as G-protein-coupled receptors, ion channels, kinases, reductases, acetylases and proteases were identified to be potential points of vulnerability, emerging as new frontiers to be explored as putative drug targets. Currently, 114 protein structures from schistosomes are registered in the PDB, most of them obtained via x-ray crystallography, and 73% of them obtained after the publication of the genomic data. The majority of the available structures are from *S. mansoni*, followed by *S. japonicum* and *S. haematobium* (Figure 2).

Parallel with macromolecular and ligand databases, genome repositories are now available to be used in schistosomiasis drug discovery projects. SchistoDB and GeneDB are examples of these platforms available for accessing the genomic data of *Schistosoma* species [43,44]. These databases offer several tools, such as genomic data mining, similarity searches and identification of gene type. Additionally, it is possible to predict gene function and properties of the encoded proteins.

This knowledge brought the drug discovery for schistosomiasis into the molecular age and opened many new possibilities for the field. Today, molecular modeling methods are used alongside drug repositioning studies, structure-based drug design and target-focused compound screening in schistosomiasis drug discovery programs [45–48].

Molecular modeling approaches in schistosomiasis drug discovery

Medicinal chemistry approaches in schistosomiasis drug R&D have evolved alongside the progress observed in NTD drug discovery. The amount of *in silico* studies significantly increased, stimulated by the detailed knowledge of the *Schistosoma* species at the molecular level and by the advances in chemo- and bioinformatics. Recent cases covering different methodologies of such molecular modeling strategies are discussed next.

Prediction of molecular targets

Computational metabolic prediction captures key information on the biochemical transformations of specific organisms, based on genomic, proteomic and

Key terms

Transcriptome: Collection of RNA molecules (transcripts), including mRNA, rRNA, tRNA and other noncoding RNA produced in a given organism or specific cell.

Structure-based drug design: Medicinal chemistry methods used to simulate and predict the binding mode of a small molecule ligand in the binding site of a macromolecule. SBDD methods require the knowledge of the 3D structure of the molecular target under investigation.

Ligand-based drug design: Medicinal chemistry strategies that explore the chemical and biological data available for a set of ligands. The main goal is to identify key molecular descriptors that determine pharmacodynamic or pharmacokinetic properties. LBDD methods do not consider the 3D structure of the molecular target.

Functional genomics: Molecular biology tools employed to understand gene function and gene interactions. Functional genomics is dedicated to the dynamic aspects of genes, such as transcription, translation and protein–protein interactions.

Figure 2. Number of protein structures of *Schistosoma* species available in the protein data bank and the relative interspecies distribution.

metabolomic data [49]. These methods have been developed over the previous 10 years, and the number of such studies has increased proportionally to the number of sequenced whole genomes. Diverse studies have performed biochemical mapping to predict metabolic bottlenecks in schistosomes [39,50].

One of these studies identified approximately a hundred enzymes as metabolic chokepoints, including novel targets and those that are known drug targets in other organisms [39]. For instance, the enzymes dihydrofolate reductase, the target for the antibiotic sulfamethoxazole, and HMG-CoA reductase, the

target for statins – the widely used class of cholesterol lowering drugs – were labeled as metabolic bottlenecks in *S. mansoni*. Attesting to the usefulness of such modeling studies, HMG-CoA reductase was recently confirmed through chemical and genetic methods to be a validated drug target for statins (Figure 3) in *S. mansoni* [51].

The study also detected, through sequence alignment, several matches of *S. mansoni* macromolecules with human and microbial targets. Potent compounds with good bioavailability are marketed for some of these proteins. For example, human tubulin, a molecular target for cancer, was found to be related to the schistosomal **ortholog**. This information could be useful in repositioning and structure-based studies for drugs targeting human tubulin – vinblastine, colchicine, vincristine, docetaxel and paclitaxel. Additionally, anthelmintic drugs such as albendazole, mebendazole and thiabendazole, have been demonstrated to target tubulin. These findings gave rise to diverse **chemogenomic** studies aimed at drug repositioning for schistosomiasis, an approach that is, in view of the obvious convenience, tending to grow significantly [39,42,51].

Figure 3. Structure of statins, inhibitors of human HMG-CoA reductase used in the therapy of hypercholesterolemia. The enzyme was recently validated to be a molecular target for schistosomiasis. The five illustrated statins are active against somules and mature *Schistosoma mansoni* worms.

Tyrosine kinases

Recently, a study aimed at drug repositioning for imatinib, a drug used in cancer therapy, was published [52]. The authors described a computational approach involving homology modeling and molecular docking.

Protein kinases (PKs) are enzymes involved in signal transduction, acting in processes such as cell growth and differentiation. Tyrosine kinases, such as Abl-kinases (Abl-PKs), are among the most investigated PKs [53]. Imatinib, a competitive inhibitor of Abl-PK, which binds to the adenosine triphosphate (ATP) binding cavity of the enzyme, is widely employed in cancer therapy [54].

Research on PKs has demonstrated their importance for growth and development of schistosomes [55]. The two isoforms, Abl1-PK and Abl2-PK, from *S. mansoni* exhibit high sequence similarity compared with human Abl-PKs. Additionally, structural investigations have shown that *S. mansoni* and human Abl-PKs possess a high conserved binding cavity for imatinib [56,57].

A drug repositioning study reports the activity of imatinib on Abl-PKs and the treatment of adult schistosomes with the drug, which led to the death of the parasites. Enzymatic activity was completely suppressed at nanomolar and low-micromolar concentrations of the compound (Figure 4).

Human Abl1-PK and Abl2-PK were used as templates for building homology models of *S. mansoni* Abl-PKs. Molecular docking of imatinib, carried out on the homology models, resulted in appropriate structural agreement with the crystallographic conformation of the drug in complex with human Abl2-PK (Figure 5). Essential residues of human Abl-PK interacting with imatinib were found to be conserved in the docking complexes of *S. mansoni* Abl-PKs.

The docking studies have indicated that the compound has the structural requirements to bind to the parasite's Abl-PKs. This finding was confirmed as the experimental assays showed that imatinib inhibits both Abl1-PK and Abl2-PK of *S. mansoni*. Despite the drawbacks associated with the administration of imatinib in children, such as growth deceleration and gastrointestinal toxicity, the results show promising perspectives for repositioning of this drug and the role of Abl-PKs as molecular targets for schistosomiasis.

Purine nucleoside phosphorylase

Crystallography has been one of the most active fields since the genomic data for the species of *Schistosoma* were published. Purine nucleoside phosphorylase (PNP) is a key enzyme in the purine salvage pathway of schistosomes [58]. The parasites lack the *de novo* pathway for purine biosynthesis and depend on the salvage pathway for their purine demand for the synthesis of nucleic acids. In this context, PNP has been employed

Figure 4. Structure of imatinib, a human Abl-PK inhibitor used in cancer therapy. The compound is currently being studied as an antischistosomal agent. The drug completely suppressed GVBD (germinal vesicle breakdown) at 0.1 μM for Abl2-PK and 1 μM for Abl1-PK.

Figure 5. Binding conformation and interactions of imatinib within the catalytic tyrosine domain of human Abl2-PK (PDB 3GVU, 2.05 Å). The protein backbone is represented as a cartoon. The ligand and residues in the active site are shown in stick representation. Water is shown as a purple sphere, and hydrogen bonds are indicated as dashed lines.

Figure 6. Structure and activity of purine nucleoside analogs identified using structure-based drug design to be *Schistosoma mansoni* purine nucleoside phosphorylase inhibitors.

Key terms

Ortholog: Genes or gene products retaining the same function in different species. Orthologs evolved from a common ancestry by speciation.

Chemogenomics: Drug discovery approach that integrates ligand-based methods with information on drug targets to understand molecular recognition between a large small-molecule chemical diversity and all possible drug targets of a given biological system.

as an attractive target for molecular modeling studies in the search for novel schistocidal agents [59].

A series of potent inhibitors of PNP from *S. mansoni* was identified via the integration of protein crystallography and SBDD studies [60]. The crystallographic structure of the enzyme was used in molecular docking simulations of a series of deazaguanine derivatives and other purine bases.

The investigation identified potent compounds, including inhibitors having IC_{50} values in the nanomolar range (Figure 6). To evaluate the selectivity of the inhibitors, kinetic assays were performed on the human PNP. Three compounds were found to be selective for the parasite's enzyme. The crystallographic

Figure 7. Crystallographic structure of *Schistosoma mansoni* purine nucleoside phosphorylase in complex with a nucleoside analog (PDB 3DJF, 1.45 Å). The protein backbone is represented as a cartoon. The ligand and residues in the active site are shown in stick representation. Water is shown as a purple sphere, and hydrogen bonds are indicated as dashed lines.

Figure 8. Structure and activity of thioxothiazolidinone derivatives identified using pharmacophore-based virtual screening to be *Schistosoma mansoni* purine nucleoside phosphorylase inhibitors.

complex obtained for one of the inhibitors revealed key structural patterns responsible for the activity of these inhibitors (Figure 7). Further exploration of these data can be used in the optimization of purine-based analogs as inhibitors of PNP from *S. mansoni*.

PNP was also selected as the molecular target for pharmacophore modeling studies. A series of thioxothiazolidinone-based inhibitors of PNP from *S. mansoni* was discovered using pharmacophore-based virtual screening [61]. The pharmacophoric model was constructed based on the analyses of protein-ligand interactions of several crystallographic structures of PNP-inhibitor complexes. Subsequently, the structure-based 3D pharmacophore was applied to virtually screen a commercially available database previously filtered using Lipinski's rule of 5. Given that the 12 structures of *S. mansoni* PNP available in the PDB are in complex with ligands, the study illustrates how SBDD and LBDD can be balanced to fulfill specific 3D structural requirements.

The effort resulted in the identification of compounds with IC_{50} values in the low micromolar range (Figure 8). These PNP inhibitors are suitable starting points for further medicinal chemistry work to discover new schistocidal compounds.

3-Oxoacyl-ACP reductase

In schistosomes, the enzymes of lipid metabolism lack overall sequence homology with the mammalian orthologs, and are considered attractive molecular targets for drug design [62]. The enzyme 3-oxoacyl-ACP reductase (OAR), predicted to be a metabolic chokepoint in schistosomes, belongs to the class of short-chain reductases, which are also prospective drug targets for the treatment of tuberculosis and malaria [63,64].

Inhibitors of OAR were identified by integrating virtual screening and homology modeling [63]. Molecular docking of a small-molecule database filtered for drug-likeness properties was performed against a homology model of *S. japonicum* OAR. Next, the selected virtual hits were assayed for their inhibition activity on the recombinant enzyme. The study resulted in the identification of two irreversible inhibitors of OAR (Figure 9) having antischistosomal activity in whole cell assays and low cytotoxicity.

This research introduced a successful approach in schistosomiasis drug design, in which homology modeling and molecular docking were integrated and experimentally validated. These compounds, particularly the most potent one, may be explored in further molecular optimization studies. The study illustrates a case in which irreversible inhibitors were identified, an interesting alternative to the most typical strategy of developing reversible enzyme inhibitors.

Histone deacetylases

Histone modifying enzymes catalyze posttranslational modifications, such as ubiquitination, methylation, phosphorylation and acetylation. The addition of these groups to the structure of histones affects the overall organization of chromatin filaments, thereby maintaining the equilibrium of transcriptional activity [65]. Histone deacetylases (HDACs) catalyze the deacetylation of lysine residues of histones and have been reported to be drug targets in different therapeutic areas, including cancer [66]. Recent studies have investigated the role of HDACs in the growth of *S. mansoni*. Among these studies, histone deacetylase 8 (HDAC8) has emerged as a promising molecular target for schistosomiasis [67].

Inhibitors of *S. mansoni* HDAC8 were identified through an *in silico* approach, combining homology modeling, molecular dynamics and SBVS [68]. A homology model of HDAC8 was generated based on the available crystallographic structures of the human ortholog. Next, molecular dynamics simulations were employed to refine and evaluate the structural flexibility of the residues at the active site of the *S. mansoni* HDAC8 model. The refined homology model was then used to virtually screen the drug-like subset of the ZINC database. At this stage, the authors employed an interesting strategy to delimitate the ligand database using the presence of zinc binding moieties as the search query, based on information derived from human HDAC8-inhibitor complexes.

The virtual hits were selected from their docking scores and the interaction with the zinc ion located at the enzyme's binding site. A series of compounds was then tested for its activity on human and *S. mansoni* HDAC8, resulting in the identification of low-micromolar hydroxamates and sulfonamide-thiazole derivatives as inhibitors of the parasite's HDAC8 (Figure 10). Crystallographic structures of the enzyme in complex with inhibitors identified using SBVS were further obtained (Figure 11). Analyses of the x-ray structural data, including protein conformation and ligand binding mode, confirmed the reliability of the generated homology models and docking poses.

The study also investigated structural aspects that could lead to selective inhibitors. Although the active site

Figure 9. Inhibitors of *Schistosoma japonicum* OAR identified through homology modeling and SBVS.

is highly conserved, one single replacement of a hydrophobic residue (Met274) with a polar one (His292) generates conformational changes that may be essential in designing *S. mansoni* HDAC8-selective inhibitors. Lead-like compounds suitable for further optimization were identified as potent inhibitors of the parasite's HDAC8.

Quantum mechanics in schistosomiasis drug discovery

Covalent enzyme inhibitors have proved to be successful for various therapeutic indications (e.g., tuberculosis and cancer). Covalent drugs inactivate their molecular targets and restoration of the impaired biological activity requires protein resynthesis. Consequently covalent drugs require less frequent dosing. However, because of adverse effects, they are usually avoided in most drug discovery projects [69]. Toxicity of covalent drugs is associated with the lack of target specificity and high reactivity, which lead to deleterious effects such as cellular damage, metabolic imbalances and production

Figure 10. *Schistosoma mansoni* HDAC8 inhibitors identified using homology modeling, molecular dynamics and structure-based virtual screening. Values of IC_{50} for the human enzyme: (1) 0.97 μ M; (2) 3.09 μ M; (3) 1.33 μ M and (4) 0.62 μ M.

Figure 11. Crystallographic structures of *Schistosoma mansoni* HDAC8 in complex with inhibitors identified through molecular modeling studies. (A) *S. mansoni* HDAC8 in complex with compound 1 (PDB 4BZ8, 2.21 Å); (B) *S. mansoni* HDAC8 in complex with compound 2 (PDB 4BZ9, 2.00 Å). The protein backbone is represented as a cartoon. The ligands and residues in the active site are shown in stick representation. The zinc ion is shown as a blue sphere, and water is shown as a purple sphere. Hydrogen bonds are indicated as dashed lines.

of autoantibodies. Although this concept has now been reconsidered, computational tools for designing covalent inhibitors are much less developed than those for their noncovalent counterparts [70]. Structure-based

Figure 12. Crystallographic structure of *Schistosoma mansoni* CB1 in complex with the inhibitor K11017 (PDB 3S3Q, 1.80 Å). The protein backbone is represented as a cartoon. The ligand and residues in the active site are shown in stick representation. Water is shown as purple spheres, and hydrogen bonds are indicated as dashed lines.

discovery of new covalent drugs requires molecular modeling tools that effectively answer the field's requisites, mainly because of the specificities concerning binding thermodynamics.

The first step of noncovalent binding can be predicted well with current molecular mechanics (MM) algorithms. However, as MM is not able to describe the formation and breaking of chemical bonds, the second step, covalent-bond formation, requires the use of quantum mechanical methods (QM) to explore the whole reaction mechanism [71]. QM methods are useful for describing both steps of ligand binding: the formation of the noncovalent complex and the generation of the covalent bond [72]. The binding process of a series of vinyl sulfone derivatives acting as covalent inhibitors of the peptidase cathepsin B1 of *S. mansoni* (CB1) was studied using a QM-based scoring function [73]. Among the six crystallographic structures of CB1 from *S. mansoni* available in the PDB, the x-ray structure of the protease covalently bound to the vinyl sulfone K11017 was used as the reference to analyze covalent bond breakage and formation (Figure 12).

The individual interactions of the inhibitors with the enzyme's active site and the correlation of the calculated binding affinities with the experimental binding data were also investigated. Good correlation coefficients were obtained when the experimental binding values were compared with the covalent scores. This method allowed the recognition of essential binding interactions and provided insights into the molecular basis of covalent binding for this series of CB1 inhibitors.

A new computational approach for the evaluation and design of covalent inhibitors was proposed by the authors, in which individual terms of the scoring function provides an estimation of the specific contributing forces. Another advantage of the QM-based approach is its ability to analyze individual molecular fragments. Certainly, potential applications and limitations of QM-based procedures in the analysis of covalent protein-ligand complexes need to be deeply explored in all fields of drug discovery, including schistosomiasis drug R&D.

Conclusion

Although schistosomiasis affects millions of people worldwide, its treatment has relied on a single drug for over 30 years. Resistance to PZQ has been found in several cases in which repeated standard treatment fails to eliminate the worms. As the mechanism of action of PZQ is unknown, the components of drug resistance remain unclear. Given this context, novel drug discovery approaches have been adopted to develop therapeutic alternatives. As a common feature, these modern strategies are based on the integration between

in silico and experimental medicinal chemistry methodologies. The current panorama of computational drug design for schistosomiasis has been driven mainly by structure-based strategies, an aspect that was strongly motivated by the publication of the parasite's genome. Homology modeling, virtual screening and molecular docking are among the most used molecular modeling methods for schistosomiasis. However, the computational toolbox also includes target prediction, pharmacophore modeling, molecular dynamics and quantum mechanics. Extraordinary progress has been made in the knowledge of the biomolecular machinery of schistosomes and the small-molecule diversity that interacts with known and potential targets. Molecular modeling of these data has been essential to shape novel strategies for schistosomiasis drug R&D.

Future perspective

Further advances in the field depend on the investigation of successful cases of drug discovery programs using molecular modeling strategies. From such an analysis, trends to support knowledge-based decisions will emerge. Considering the recent opportunities to investigate novel targets, these data will certainly contribute to the strengthening of structure-based molecular modeling as a tool for schistosomiasis drug design.

Given the current R&D panorama, careful selection of promising targets is paramount to enhance the probability of success of discovery projects.

Major progress in the field depends on proper validation of the investigated molecular targets. The rising amount of putative targets will require full evaluation to identify those more capable of yielding suitable drug candidates. The process of discovery of new lead compounds with the desired properties will need to overcome the target validation barrier. Full chemical and genetic target validation, as well as the evaluation of drugability, will be required to diminish the high attrition rates usually found in current pharmaceutical R&D. In a prospective scenario, the integration of molecular modeling and structure-based design will certainly grow as an effective approach for identifying new drug candidates for schistosomiasis.

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Executive summary

- Schistosomiasis affects almost 250 million people worldwide in tropical and subtropical regions.
- Praziquantel, the only available drug for schistosomiasis, has faced cases of resistance.
- The current panorama of drug R&D relies on the strong integration of phenotypic, genomic, molecular and computational methods.
- Publication of the genomic data of *Schistosoma* species was fundamental in stimulating the development of molecular modeling for schistosomiasis.
- Drug repositioning and structure-based drug design are promising strategies in the discovery of new antischistosomal compounds.
- Target validation is paramount to increase the probability of success in schistosomiasis drug R&D programs.

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