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Is Praziquantel a lead compound for its amides and N-alkyl or aryl derivatives? – A molecular modeling insight at the binding site

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Abstract

Praziquantel (PZQ) has been used for over four decades as a first-line monotherapy against schistosomiasis, demonstrating high clinical efficacy. However, scientific evidence suggests reduced sensitivity in certain parasite species, highlighting the need for a deeper understanding of its molecular mechanism of action and its intrinsic structural limitations. In this study, a computational screening analysis was performed to identify the structural determinants that define the lead optimization space for PZQ. Additionally, the viability of PZQ as a lead compound in the rational design of new antiparasitic agents was evaluated by analyzing derivatives previously reported in the scientific literature. Two hundred PZQ derivatives with reported *in vitro* biological activity data were compiled. From these, eight representative molecules were selected for structure-activity relationship (SAR) analysis and molecular docking studies. The set included compounds without antischistosomal activity, as well as those with activity similar to, equal to, or superior to PZQ. The results revealed that the stability of the $Sm.TRP_{PZQ}$–ligand (PZQ and derivatives) complex depends on a cooperative equilibrium between critical hydrogen bonding and hydrophobic interactions, which ensure precise conformational orientation within the $Sm.TRP_{PZQ}$ binding site. Alterations in these properties significantly compromise biological activity, thus restricting the chemical space for optimization. Collectively, these findings indicate that PZQ represents an exceptionally well-balanced anti-schistosomal scaffold, whose structural characteristics suggest a conformational equilibrium that is difficult to surpass, explaining why extensive optimization efforts have not yielded superior clinical alternatives.

1. Introduction

Praziquantel (PZQ) belongs to the class of anthelmintics and has been used in first-line antischistosomiasis pharmacotherapy for more than 40 years. Schistosomiasis, also known as bilharziasis, is a subtropical and tropical infectious disease caused by trematode worms (flukes), primarily three species: *Schistosoma mansoni, S. japonicum*, and *S. haematobium*. During the infectious cycle, the parasites adopt different life stages in various hosts, invade human patients through the skin, and can affect the blood, tissues, and organs [1]. Because there are no therapeutic alternatives with comparable efficacy to PZQ for treating patients at risk of developing drug resistance, reliance on this drug as monotherapy is a critical limitation in the control of schistosomiasis [2–4]. In this context, the World Health Organization has classified schistosomiasis as a neglected tropical disease and estimates that it affects more than 250 million people worldwide [5].

The high epidemiological burden, coupled with the possibility of decreased sensitivity to PZQ, underscores the urgent need to develop new therapeutic strategies. These may include the identification of alternative compounds, combination regimens, or the structural optimization of derivatives with greater biological activity [4]. Following the withdrawal of hycanthone (HYC) and oxamniquine (OXA), only PZQ remains in clinical use as monotherapy [6, 7].

The scientific literature indicates that the chemical derivatization of PZQ has been proposed as a structural optimization strategy aimed at identifying analogs with improved pharmacodynamic and pharmacokinetic profiles, with the goal of developing candidate molecules capable of replacing PZQ as first-line monotherapy. This approach has sought not only to increase antiparasitic potency and selectivity but also to reduce the risk associated with therapeutic dependence on a single active ingredient [4, 6, 8–11].

However, despite numerous efforts over several decades to optimize the pharmacological profile of PZQ through chemical derivatization, to date, no analogue has been developed whose improvements in terms of biological activity, therapeutic efficacy, or pharmacokinetic properties consistently surpass those of PZQ [4, 6, 8–11]. To address this limitation, this study employed structure–activity relationship (SAR) analysis, molecular docking studies and molecular modeling approaches. These were used to comparatively analyze ligand (PZQ and derivatives)–receptor ($Sm.TRP_{PZQ}$) interactions and the physicochemical properties of PZQ and its structural derivatives. The objective was to justify, from a structural and physicochemical perspective, the reasons for the lack of commercially available compounds with superior activity (Fig. 2).

1.1 PZQ activity

PZQ has a chiral structure due to one asymmetric carbon atom on its scaffold. Although the drug is administered to patients as the (R,S)-racemate, only its R-enantiomer is pharmacologically active. This observation hints at a stereoselective step during drug delivery or action [12–16]. This fact also indirectly reflects that PZQ itself is the active form, rather than a metabolite generated upon cleavage of the asymmetric center. In theory, pharmacokinetic reasoning does not exclude the possibility of carrier-protein-based absorption and distribution, nor metabolism, where PZQ could act as an inactive prodrug requiring a stereoselective biotransformation step into its true active form. HYC and OXA are prodrugs that are activated in the worm by a sulfotransferase whose crystal structure has been elucidated (PDB entries: 6UUX, 6UUY) [7]. PZQ has one chiral center: an asymmetric carbon atom in the tetrahydroisoquinoline ring. Recent pharmacodynamic findings have unraveled the stereoselective differences: the eutomer is the (R)-enantiomer of PZQ, while the (S)-enantiomer constitutes the distomer, as the latter is less active [13, 15]. The target protein belongs to the family of transient receptor potential (TRP) channels [13, 17–19].

As long as the identification of the schistosomal target molecule remained elusive, not target-structure-based but ligand-based drug design (LBDD) has guided academic research and development of PZQ congeners (Table 1). Nowadays, it is believed that PZQ acts through modulation of the parasite's ion channels, similar to other investigational and commercial drugs with no structural similarities to PZQ, such as pyrantel, derquantel, piperazine, levamisole, monepantel, emodepside, tribendimidine, and macrocyclic lactones (e.g., ivermectin) [13, 17, 20–22].

Table 1
Provides a comprehensive inventory of PZQ derivatives reported in the literature, organized by source and number of compounds

Published derivatives (how many?)	References	Published derivatives (how many?)	References
10	[23]	32	[8]
7	[24]	10	[9]
8	[25]	3	[10]
7	[26]	3	[27]
17	[28]	28	[11]
23	[29]	1	[30]
19	[31]	19	[32]

The advent of experimental target identification and structural elucidation of the PZQ-target binding site at atomic scale will usher a new area for exploring active conformations and chemical space at the binding site to design new active derivatives, which was not the case during the time of this study.

On theoretical grounds, we studied published PZQ derivatives to determine whether PZQ constitutes a lead compound for its amide (> N–C(= O)-alkyl/aryl) or reduced N–C(H_2)-alkyl/aryl derivatives. The expected results will be embedded into the broader context of ongoing drug research on anti-infective lead compounds, such as isoniazid, nitazoxanide, and dapsone [33–35].

2. Materials and Methods

2.1 Homology modeling of the Sm.TRP$_{PZQ}$ receptor

Since no crystallographic structure of the *Schistosoma mansoni* Sm.TRP$_{PZQ}$ channel is available, a structural model was constructed using homology modeling. The amino acid sequence was obtained from WormBase ParaSite [36], under the identifier Smp_246790, comprising 2268 amino acids.

Template selection and model generation were performed using the SWISS-MODEL server [37]. The Smp_246790 sequence was used as input, initially identifying 50 potential templates. These were evaluated based on: (i) belonging to the TRP channel family, (ii) TRPM subfamily, and (iii) presence of a co-crystallized ligand, to select a functionally relevant structural conformation for subsequent molecular docking studies (Fig. 1).

Four structures belonging to TRPM receptors met the initial criteria: PDB codes 6NR3 [38], 6MIX [39], 8DDS [40] and 8SR8 [19], obtained from the RCSB Protein Data Bank [41]. After comparative analysis, only structure 6NR3 was selected as the template for homology modeling, due to the presence of a co-

crystallized ligand (icilin), a known TRPM channel agonist, which confers a functionally active conformation of the channel — a feature particularly relevant for subsequent molecular docking studies.

To validate the homology model, it was compared with the artificial intelligence-predicted structure available in the AlphaFold Protein Structure Database [42]. Structural alignment showed good superposition of the transmembrane domain and ligand-binding site, with low RMSD values in the active site region. No significant variations in the binding pocket architecture were observed; therefore, the homology-generated model was selected for subsequent docking analyses, as it corresponds to an experimentally validated active conformation.

2.2 Phylogenetic analysis

To evaluate the evolutionary conservation of the Sm.TRP_{PZQ} channel across different species and to identify residues potentially critical for function, a sequence similarity search was performed using BLAST at the National Center for Biotechnology Information [43]. This search identified 106 homologous sequences. From these, 14 representative sequences from different species were selected based on criteria including sequence identity, length, and belonging to the TRP channel family and TRPM subfamily. The specific selection thresholds were: (i) > 50% identity and > 1000 amino acids for high-identity sequences, and (ii) < 50% identity and ≤ 800 amino acids for divergent sequences (Fig. 1 **and Table S1**).

The selected 14 sequences included representatives from diverse taxonomic groups, such as choanoflagellates, trematodes, cestodes, mammals, and birds, to assess the evolutionary conservation of the voltage sensor–like domain (VSLD) (**Fig. S1 and** Fig. 5a-b) across distantly related species. The selected sequences, together with those corresponding to the four template structures (6NR3, 6MIX, 8DDS, and 8SR8) (**Table S1 and Fig. S1**), were used to perform multiple sequence alignment using BioEdit version 7.0.9 [44]. Among the four structures, TRPM8 structure (PDB: 6NR3) was selected as the final template for homology modeling because it contains a co-crystallized ligand (icilin), which stabilizes an active conformation of the channel – a critical feature for subsequent molecular docking studies of PZQ and its derivatives. The alignment enabled analysis of structural conservation, as well as identification of conserved and variable regions. From this analysis, a consensus sequence was determined, which facilitated the evaluation of residue conservation potentially relevant to channel function.

2.3 Generation of 3D ligand structures

The three-dimensional structures of the ligands were built using VEGA ZZ version 3.2.4.29 [45], generating the initial Cartesian coordinates for subsequent refinement. Geometric optimization and partial atomic charge calculation were performed using Avogadro version 1.2.0 [46], employing the program's default parameters.

Subsequently, the ligand structures were aligned with the icilin ligand present in the TRPM-icilin crystallographic complex (PDB code 6NR3) using Swiss-PdbViewer [47]. This procedure spatially

oriented the ligands within the receptor's active site, ensuring appropriate initial configurations for subsequent molecular docking analyses.

2.4 Molecular docking

Prior to molecular docking, the 6NR3 crystallographic structure was prepared by removing water molecules, the co-crystallized ligand, and non-essential ions. Polar hydrogens were then added, and partial charges were assigned using the Gasteiger method [48] implemented in MDL-Tools 1.5.6 [49].

Molecular docking simulations were performed using AutoDock version 4.2.6 [49], employing the Lamarckian Genetic Algorithm (LGA) for conformational sampling and energy optimization. For each ligand–protein complex, energy maps were generated using the AutoGrid algorithm with a grid spacing of 0.375 Å. The grid dimensions were adjusted to the binding site previously defined by the coordinates of the icilin [50].

Icilin was not subjected to further geometric optimization to preserve the experimentally determined crystallographic conformation. Only partial atomic charges were calculated using the Gasteiger method, ensuring compatibility with the docking algorithm and avoiding alterations to the original binding geometry. The validation was carried out by docking icilin into its original binding site within 6NR3, evaluating the procedure's ability to reproduce the experimentally reported crystallographic pose. The obtained structural concordance confirmed the robustness of the docking protocol.

Subsequently, blind docking of PZQ and its eight representative derivatives was performed on the structural model of Sm.TRP_{PZQ} using the binding site defined by the icilin coordinates. The obtained poses were compared with the reference icilin interaction profile, evaluating positional and energetic consistency, as well as the conservation of interactions previously reported in the literature [13, 14, 18, 50, 51]. The docking grid was defined as a box centered at coordinates (172.707, 137.326, 168.285) Å, with a grid spacing of 0.375 Å. The Lamarckian Genetic Algorithm (LGA) implemented in AutoDock 4 was used with a population size of 150, 2,500,000 energy evaluations, and 27,000 generations per run.

Analysis of non-covalent interactions (hydrogen bonds, electrostatic interactions, and hydrophobic contacts) of the different ligand–protein complexes was performed using Discovery Studio 25.1.0 [52].

3. Results

3.1 Ligand-based analysis: activity

A ligand-based quantitative activity relationship (LB-QAR) study was carried out for all PZQ derivatives. To this end, the ligands activities were sorted in decreasing order and displayed graphically as a function of their ID numbers (Fig. 3). It can be concluded by eyesight, that only a few amide derivatives have as strong an activity as reference drug PZQ (Fig. 2). In analogy to a classical two-dimensional quantitative structure – activity relationships study the analysis was carried out without taking into account the

structural information: formally, QSAR – “S” = QAR; or: 2D QSAR – 1D = 1D QSAR. The following Fig. 3 shows the EC_{50} values reported by Sprague et al. (2025), comparing the effective concentration 50 (EC_{50}) of PZQ derivatives with that of the reference drug PZQ. Very few of the 50 derivatives require an EC_{50} equal to that of PZQ (large circle), and none exhibit an EC_{50} lower than PZQ.

3.2 Analysis of relative biological potencies compared to the reference agent PZQ.

Using the dataset published by DaSilva et al. (2019), which includes 200 PZQ derivatives with their respective *in vitro* biological activity values, we performed a comparative structural analysis focused on the structure-activity relationship (SAR) **(**Fig. 2**)**. This analysis systematically examined the chemical modifications introduced and their impact on the reported biological activity.

The PZQ scaffold is a heterocyclic pyrazinoisoquinoline system **(**Fig. 4). It contains two amide functional groups within the heterocyclic ring, whose carbonyl groups act as hydrogen bond acceptors (HBAs). The molecule also features a cyclohexylcarbonyl substructure, characterized by a carbonyl group joined to a cyclohexyl ring. This cyclohexyl ring has a single attachment point to the carbonyl group, which defines the exocyclic amide side chain. Overall, the PZQ scaffold can be divided into three key domains: (i) a rigid aromatic tetrahydroisoquinoline core, responsible for π-π stacking interactions; (ii) a piperazinone ring (a cyclic diamide) that presents carbonyl groups capable of forming hydrogen bonds; and (iii) an exocyclic cyclohexanecarboxamide side chain, which acts as a conformational anchor essential for proper ligand orientation within the binding site.

Based on this analysis, eight representative structures were selected **(**Fig. 4). The selection criteria included:

1. availability of reported biological activity (either *in vivo* or *in vitro*),
2. the type of chemical modification in three key structural regions of the molecule — amide group, cyclohexyl substituent, and fused aromatic ring — and,
3. physicochemical properties such as electron-donating or electron-withdrawing capacity, aromaticity, and molecular volume. These eight structures summarize the main modification patterns present in the total set of 200 derivatives. This selection allows for a more detailed and rational analysis of the structural trends associated with variation in biological activity.

Comparative analysis between PZQ and the series of evaluated analogs reveals clear patterns in the SAR that identify critical structural determinants of antiparasitic activity. First, the presence of the cyclohexyl group and the aliphatic amide (cyclohexanecarboxamide), characteristic of PZQ, is essential for antiparasitic activity. Molecules 01 and 13, which exhibit substantial modifications in these fragments or lack them entirely, showed a complete loss of activity (0% kill at 25 μM), suggesting that these groups contribute significantly to the interaction and correct orientation of the lactam nucleus at the $Sm.TRPM_{PZQ}$ binding site.

Furthermore, the integrity of the rigid isoquinoline-type aromatic backbone appears to be essential. Molecule 13 (which also features a distinct and less rigid aromatic system) showed no detectable activity, reinforcing the hypothesis that the planarity and rigidity of the aromatic core are likely necessary to maintain optimal binding to the receptor. In contrast, modifications to this core, such as those observed in compounds 23, 9, and 34, allow for the retention of moderate to high activity (87–100%), indicating that certain electronic or steric variations can be tolerated as long as the overall architecture of the isoquinoline core is maintained.

A particularly interesting finding was observed with molecule 36. Although it exhibited 100% *in vitro* activity (equivalent to PZQ under the same conditions), its *in vivo* activity in rats was significantly lower (44.3% worm burden reduction) compared to PZQ (62.4%). This result suggests that, despite high *in vitro* potency, pharmacokinetic factors (absorption, metabolism, or bioavailability) could be limiting the *in vivo* efficacy of this derivative, highlighting the importance of evaluating both types of assays in the structural optimization process.

In light of the more than 200 PZQ derivatives documented by DaSilva et al. (2019) and the SAR analysis presented above (Fig. 3), several critical questions emerged that required systematic evaluation. Is PZQ an appropriate lead compound for further optimization? Which derivatives exhibit the strongest and weakest anthelmintic activities? Do these activity differences correlate with distinct structural features? Can structure–activity relationships be described qualitatively (SAR) or quantitatively (QSAR)? Do the observed relationships hint at hidden activity differences due to chemical or biochemical instabilities (e.g., prodrug activation or metabolite formation)?

To address these questions, the structural complexity was dissected into electronic and steric components. Spatial requirements (conformations, steric hindrance, etc.) were analyzed using target structure-based studies with molecular mechanics methods (force field and docking). Experimental conditions were scrutinized to identify physical or chemical factors that could assist the cleavage of PZQ and its derivatives (catalysis) as well as enzymatic biotransformation (biocatalysts).

3.3 Structure-based analysis: sequences for homology modeling of target receptor $SmTRPM_{PZQ}$

In parasites, TRP channels (see Fig. 5a-b) are sensors of cellular and environmental signals and are essential for the normal functioning of neuromuscular and other tissues [17]. Despite this fact, only a few classes of ion channels in schistosomes and other helminths have been evaluated for their pharmacological properties or their role in parasite physiology. *Schistosoma mansoni* contains 15 genes that encode TRP channels, grouped into 5 subfamilies (TRPC, TRPA, TRPM, TRPP, TRPML) [17]. TRP channels have been observed to share a common core structure (**Fig. S1**), but are highly diverse in their activation mechanisms and ion selectivity [50, 53]. The limited information available to date indicates that these channels in helminths also exhibit diverse activation mechanisms.

Despite this limitation, the MSA performed in this work (Fig. 5) shows that the VSLD domain is highly conserved across different species and classes of organisms (see **Table S1**). The VSLD domain in groups as diverse as choanoflagellates, trematodes, cestodes, mammals, and birds has conserved amino acids that could be involved in ligand positioning (orientation) and the activation/inactivation of these channels. In particular, the residues highlighted in green in Fig. 5 (panel C were identified by molecular docking analysis as critical for proper ligand orientation and subsequent channel activation. The presence of these conserved residues from primitive organisms (choanoflagellates) to vertebrates (consensus line in panel C) suggests that they play an essential role in receptor conformation and ligand orientation, supporting the use of structural homologs such as avian TRPM8 as a template to model the PZQ binding site in Sm.TRPM$_{PZQ}$.

One of the main reasons why PDB entry 6NR3 was chosen as a 3D template for protein homology modeling from the target sequence of Sm.TRPM$_{PZQ}$ was because the crystal shows a liganded complex. We compared the ligand icilin to PZQ and find structural and chemical similarities (Fig. 6). Other small organic compounds have been experimentally determined as strong target binders, too, namely: N-benzamidoquinazolinone (BQZ) and meclonacepam (MCLZ) [54, 55].

3.4 Ligand – target analysis

Icilin at the TRPM8 binding site (PDB: 6NR3) reproduced the crystallographic pose, confirming the robustness of the docking protocol (RMSD < 2.0 Å). Although icilin has been experimentally shown not to activate the Sm.TRPM$_{PZQ}$ receptor [13], its binding site on TRPM8 was used to map the amino acids involved in ligand-receptor interactions within the VSLD cavity, given the high structural homology between the two channels. Analysis of the interactions in the TRPM8–icilin complex showed that the ligand binds primarily to aromatic residues. Specifically, π-π interactions were observed with F838, hydrogen bonds with Y1004 and N741, and π-alkyl interactions with Y745 and R841 (the latter also participating in a π-cation interaction) (Fig. 7).

Once the protocol was validated, PZQ and its eight derivatives were docked at the same binding site. Comparative analysis of the obtained poses allowed the identification of critical structural determinants for antischistosomal activity, as well as correlating chemical modifications with variations in biological activity (Fig. 4).

3.5 Blind docking at the binding site of experimentally unknown complexes (PZQ, derivatives, MCLZ and BZQ)

All ligands (see Fig. 4) were generated from the coordinates of icilin, used as a reference for molecular docking at the VSLD binding site. Docking was performed with PZQ, its derivatives, MCLZ, and BZQ, and the receptor was the structural model of Sm.TRPM$_{PZQ}$. Subsequently, the binding positions and interactions of each complex were analyzed.

For the analysis of PZQ– Sm.TRPM$_{PZQ}$ interactions, different types of non-covalent contacts that promoted the stability of the complex were identified (Fig. 8). The ligand formed three hydrogen bonds (HBs) with residues R1514, Y1678, and R1681, which played a fundamental role in the orientation and anchoring of the compound within the active site. Van der Waals interactions were also observed with residues N1388, T1389, Q1432, W1451, N1452, L1515, T1518, and D1677, contributing to steric complementarity and the spatial fit of the ligand within the Sm.TRPM$_{PZQ}$ cavity. Furthermore, π-π stacking interactions with Y1392 and a π-alkyl interaction with Y1517 were observed, both of which reinforce hydrophobic associations and stabilize the aromatic region of the ligand. Taken together, the presence of polar and hydrophobic interactions suggested stable and specific anchoring, consistent with a significant affinity of the ligand for the binding site on Sm.TRPM$_{PZQ}$.

The interactions of PZQ and those of the other ligands under scrutiny (PZQ derivatives, MCLZ, and BZQ) are all on an equal footing: SAR analysis, *in vitro* activity assays, and *in vivo* efficacy assays in a rat model lend insight into the critical structural determinants for antiparasitic activity (see Fig. 9 **and Fig. S2**). Importantly, these findings explain why structural optimization efforts for PZQ have failed to produce a clinically superior analogue.

4. Discussion

4.1 Analysis of the amide group as an essential conformational anchor

Our results demonstrate that the aliphatic amide group (cyclohexanecarboxamide) is not merely a substituent, but rather an essential conformational anchor for the correct three-dimensional orientation of the entire molecule within the binding site. Several lines of evidence support this conclusion. Derivative 13 (see Fig. 4), in which the amide group has been converted into a tertiary amine, is completely inactive (*in vitro*: 0% death) despite possessing a favorable binding energy (ΔG = -8.34 kcal/mol) and establishing interactions with key residues such as Y1392, Y1517, and Y1678. This apparent paradox demonstrates that mere occupation of the binding site is insufficient: the rigidity and directionality of the amide bond, with its dual capacity to be both a hydrogen bond donor and acceptor, are fundamental for establishing a precise conformation that is recognized by the receptor as an agonist. The tertiary amine of derivative 13, which is more flexible and has a different hydrogen bonding chemistry, fails to achieve this conformational anchoring.

Similarly, derivative 01 (see Fig. 4**)** exhibits the least favorable binding energy of the entire series (ΔG = -6.48 kcal/mol) and is inactive. The loss of this key interaction point results in a less favorable binding energy, which directly correlates with the overall loss of activity. Conversely, derivative 09, which replaces the cyclohexane ring with a phenyl ring (aromatic amide), is active (87.5%), suggesting that the amide carbonyl group is responsible for a crucial interaction (likely hydrogen bonding with R1514).

These findings are consistent with those reported in the literature [51], which demonstrated that residues such as Y1517, Y1678, and R1681 are critical for PZQ activity. Our docking model correctly predicts that PZQ interacts with these residues, validating the model accuracy.

4.2 Analysis of hydrogen bond interactions

A key finding of our study is that the specific pattern of interactions, rather than the number, determines whether a compound is an effective agonist. Derivative 13 (see Fig. 4**)** forms hydrogen bonds with R1388 and Y1678, and π-π interactions with Y1392 and Y1517, yet it is inactive. In contrast, derivative 07, which establishes a different pattern of interactions (HBs with R1388 and R1514; π-alkyl with Y1392), is active (100% *in vitro*). This finding has profound implications: channel activation is not a binary (bound/unbound) process, but an allosteric process in which the ligand must induce a specific conformational change. A ligand can bind with high affinity (like derivative 13) but in an orientation that does not trigger the conformational change necessary for channel opening and parasite death. The secret of PZQ lies not in the strength of its binding, but in the quality of its orientation and the specificity of its interaction pattern.

4.3 *In vitro*/*in vivo* activity analysis

The most significant finding of our study is the marked dissociation between the high in vitro activity and the poor *in vivo* efficacy observed for derivatives 36 and 43 (see Fig. 4). This phenomenon is key to answering the central question of why these analogs do not outperform PZQ.

Derivative 36, which incorporates a bromine atom, is a paradigmatic case. Its excellent *in vitro* activity (100%) and its binding energy (-9.00 kcal/mol), practically the same as that of PZQ, would suggest that it is an optimal candidate. However, its *in vivo* efficacy (54.3%) is significantly lower. This can be explained by molecular docking analysis: the bulky and polarizable bromine atom forces the molecule to adopt an alternative binding orientation, losing critical interactions with Y1517 and R1681. While this orientation is compatible with channel activation under the static and controlled conditions of the in vitro assay (where the ligand is always present at high concentration), it is likely that, in the dynamic physiological environment in living cells, this binding conformation is weaker and could facilitate its recognition as a xenobiotic and, therefore, its rapid metabolism by hepatic cytochrome P450 (CYP450) enzymes, preventing it from reaching sustained therapeutic concentrations. Furthermore, derivative 43 represents the most extreme example of this phenomenon. Its acetate group ($–OCOCH_3$) introduces an ester group into the molecule. Esters are well known in medicinal chemistry as metabolically labile groups, as they are classic substrates of esterases, a superfamily of ubiquitous hydrolytic enzymes present in plasma, liver, and intestine. Esterases rapidly and efficiently hydrolyze the ester bond, generating an acidic metabolite that is predictably inactive and likely to be rapidly excreted [56, 57]. This drastic metabolic instability, coupled with its poor interaction profile (based primarily on Y1392 and R1681, with loss of critical interactions with Y1517 and Y1678), explains why derivative 43, despite being an *in vitro* agonist as potent as PZQ (100%), is practically ineffective *in vivo* (13.6% worm reduction). This way, derivative 43

is the perfect example of a "laboratory drug": very potent under ideal conditions, but completely useless in a living.

4.4 Pharmacokinetics of PZQ

PZQ is a drug with complex pharmacokinetics. It is rapidly absorbed but undergoes extensive first-pass metabolism in the liver, resulting in low and variable bioavailability. The main metabolic pathway is hydroxylation, mediated by cytochrome P450 isoenzymes, with CYP3A4 playing a central role. This rapid hepatic metabolism constitutes the main pharmacokinetic barrier limiting drug exposure to the parasite [58]. The therapeutic success of PZQ lies in a delicate balance: despite its rapid metabolism, its structure allows it to be a sufficiently good substrate for CYP450 enzymes to be absorbed and reach the bloodstream, but it is not such a good substrate that it is completely eliminated before reaching the parasite. The derivatives we analyzed, in their quest to increase *in vitro* potency, have disrupted this balance: derivative 36, with its alternative orientation, has likely become an excellent substrate for CYP450 enzymes, being recognized and eliminated much more rapidly than PZQ. Derivative 43, by incorporating the ester group, ceases to depend solely on the CYP450 system and becomes a perfect target for the ubiquitous and immediate action of esterases. The first time the molecule comes into contact with plasma or the liver, esterases inactivate it, preventing any possibility of a therapeutic concentration reaching the parasite.

4.5 MCLZ, BZQ and triazole derivatives: a new optimization paradigm

The studies by Park et al. (2024) and Sprague et al., (2024), together with the recent work by Paes Silva Junior et al. (2025), provide essential context for interpreting our results and positioning our contribution within the current research landscape.

MCLZ has been shown to activate a different TRPM channel ($TRPM_{MCLZ}$), not the same one activated by PZQ ($Sm.TRPM_{PZQ}$). Our docking shows that MCLZ can indeed bind to the VSLD site of $Sm.TRPM_{PZQ}$, forming interactions with key residues (Y1392, R1514, Y1678). However, MCLZ does not actívate $Sm.TRPM_{PZQ}$ because it does not interact with the critical residue Y1517 within the PZQ binding site. This result is an indirect validation of our model and our central hypothesis: the $Sm.TRPM_{PZQ}$ binding site is ultra-specific and has extremely stringent structural requirements. The chemical scaffold of MCLZ (benzodiazepine) does not meet these requirements.

Conversely, evidence indicates that surpassing PZQ by scaffold hopping is possible: a molecule with a new scaffold, N-benzamidoquinazolinone (BZQ), has been designed and shown to activate $Sm.TRPM_{PZQ}$ in multiple parasite species, including *Fasciola* (against which PZQ is inactive) [55]. Our docking shows that BZQ establishes more interactions with key residues than PZQ and its derivatives. Crucially, BZQ preserves critical interactions with R1514, Y1517, and Y1678 (unlike derivative 36) and does not incorporate metabolically labile groups (unlike derivative 43, see Fig. 4).

Conversely, evidence indicates that surpassing PZQ by derivatization is also possible: a further example of this new paradigm is the recent study by Paes Silva Junior et al. (2025), who reported eight new 1H-1,2,3-triazole derivatives of PZQ [59] (see Table 1**).** Unlike classical derivatization approaches, these compounds were designed based on the structural knowledge of the Sm.TRPM$_{PZQ}$ binding site. Importantly, molecular modeling studies from this work suggest that the PZQ binding site on Sm.TRPM$_{PZQ}$ may accommodate extended substituents, due to conformational flexibility of the Y1517 side chain. This finding is consistent with our own observations regarding the critical role of Y1517 in ligand orientation and channel activation.

Therefore, surpassing PZQ is not achieved through minor modifications to its scaffold, but rather through the design of a new chemical scaffold that preserves and enhances PZQ's native binding mode. BZQ and the triazole derivatives represent proof of concept that it is possible to design superior agonists. However, this requires abandoning the "derivatization of PZQ" strategy and adopting a rational de *novo* design strategy based on the binding site structure, as these recent studies demostrate.

5. Conclusions

Traditionally, PZQ has been considered a lead compound for the development of new anti-schistosomal agents, given that its core structure is amenable to chemical modification. However, the results of this study necessitate a reevaluation of this assertion. Our analysis demonstrates that PZQ is not a promiscuous scaffold that tolerates a wide variety of structural modifications, but rather an exquisitely tuned drug that has reached a sweet spot where *in vitro* potency, metabolic stability, and *in vivo* efficacy are in delicate equilibrium. The analyzed derivatives, despite being active *in vitro*, have disrupted this equilibrium by adopting alternative binding orientations (derivative 36) or by incorporating metabolically labile groups (derivative 43), invariably resulting in lower *in vivo* efficacy than PZQ.

Therefore, we conclude that PZQ is not a lead compound in the classical sense of the term. The chemical space for optimization is extremely limited: modifications that increase potency *in vitro* are achieved at the expense of pharmacokinetics, and modifications that improve pharmacokinetics likely decrease receptor affinity. High *in vitro* potency is an illusion that does not correlate with *in vivo* efficacy.

Implications for future development:

Despite these limitations, our data allow us to define a set of principles that should guide any future optimization efforts:

1. Preserve the aliphatic amide (cyclohexanecarboxamide) intact, as it is an indispensable conformational anchor.
2. Maintain the native hydrogen bond network with Y1517, Y1678, and R1681, avoiding modifications that force alternative binding orientations.

3. Avoid introducing metabolically labile groups (esters, primary amides, etc.) that are substrates for esterases or glucuronosyltransferases.
4. Explore conservative modifications in peripheral regions that do not affect the overall orientation (e.g., substitution of the aromatic ring with heteroaromatics, as in derivative 23, or the introduction of metabolic shield groups such as fluorine).

In conclusion, PZQ is an exceptionally well-balanced drug, but its very structural intricacy makes it a difficult target to surpass. Successfully designing a superior analog will require not only maintaining its interaction pattern but simultaneously improving its pharmacokinetic profile without compromising ligand orientation—a challenge that the analyzed derivatives failed to overcome.

CRediT authorship contribution statement

Itzel Gutiérrez-Aztatzi: Writing – review and editing, Writing – original draft, Methodology, Investigation, Formal analysis, Data curation, Visualization. T. Scior: Writing – review and editing, Writing – original draft, Methodology, Investigation, Validation, Conceptualization, Supervision. Israel Quiroga Montes: Methodology, Formal analysis, Investigation, Supervision. Luis Javier Martinez-Morales: Funding acquisition, Resources, Investigation, Project administration, Supervision.

Declarations

CRediT authorship contribution statement

Itzel Gutiérrez-Aztatzi: Writing – review and editing, Writing – original draft, Methodology, Investigation, Formal analysis, Data curation, Visualization. T. Scior: Writing – review and editing, Writing – original draft, Methodology, Investigation, Validation, Conceptualization, Supervision. Israel Quiroga Montes: Methodology, Formal analysis, Investigation, Supervision. Luis Javier Martinez-Morales: Funding acquisition, Resources, Investigation, Project administration, Supervision.

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Declaration of Competing Interest

-The PZQ work has no need to declare competing interests.

-The work was carried out by four researchers at universisties, BUAP and UPAEP in Mexico, without any other interests than scientific ones.

Data availability

The authors declare that the data supporting the findings of this study are available within the paper and its Supplementary Information files. Should any raw data files be needed in another format they are available from the corresponding author upon reasonable request. Source data are provided with this paper.

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Author Contribution

I.G.A.: Writing – review and editing, Writing – original draft, Methodology, Investigation, Formal analysis, Data curation, Visualization. T.S.: Writing – review and editing, Writing – original draft, Methodology, Investigation, Validation, Conceptualization, Supervision. I.Q.M.: Methodology, Formal analysis, Investigation, Supervision. L.J.M-M.: Funding acquisition, Resources, Investigation, Project administration, Supervision.

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Figures

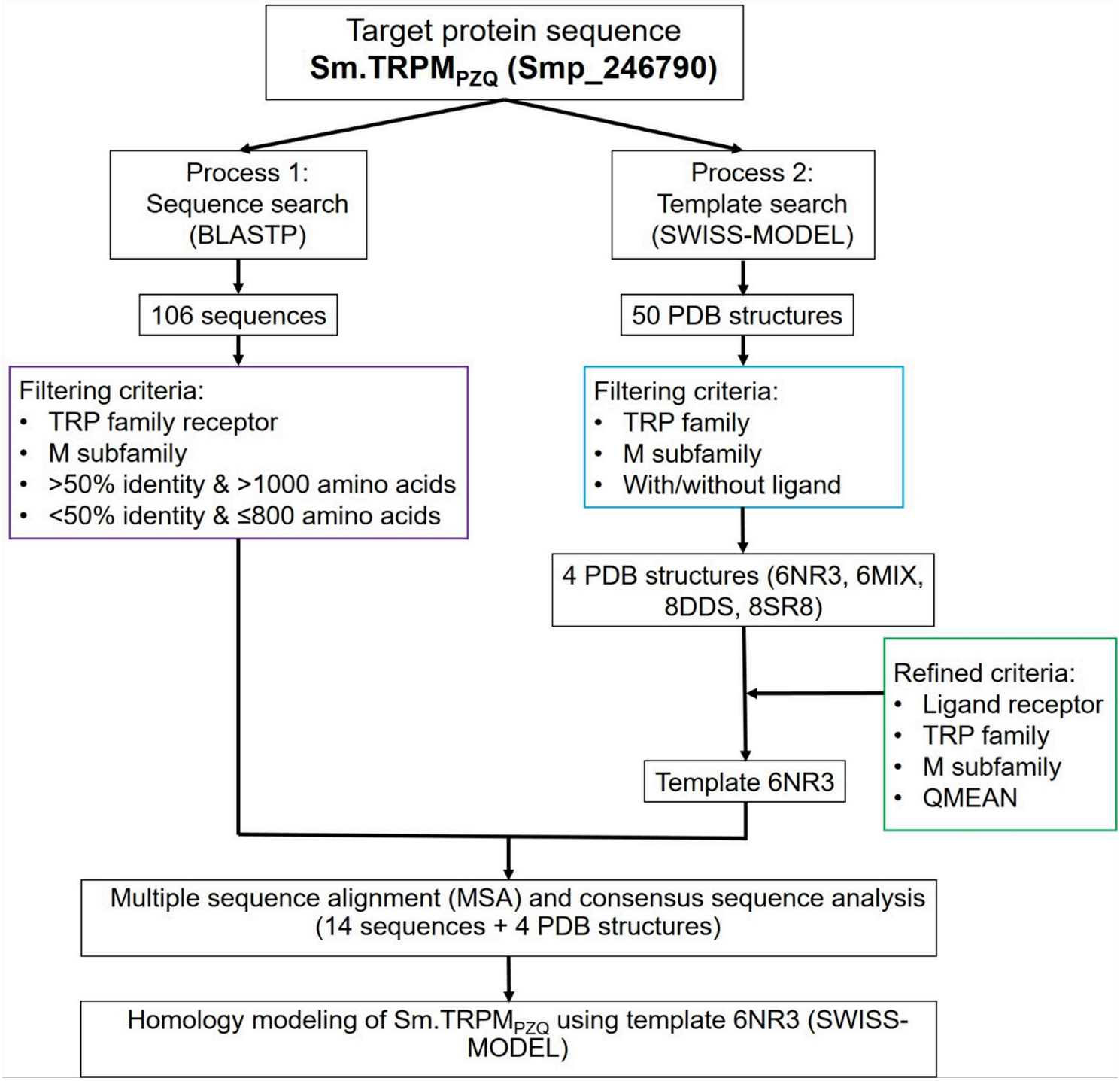


Figure 1

Workflow for sequence analysis and homology modeling of Sm.TRPM$_{PZQ}$. The workflow consists of two parallel processes starting from the target protein sequence (Smp_246790). Left column (Process 1): BLASTP search identifies 106 homologous sequences, which are filtered according to criteria in the purple box, resulting in 14 representative sequences used for multiple sequence alignment (MSA). Right column (Process 2): SWISS-MODEL identifies 50 potential PDB templates, filtered by criteria in the blue box to yield 4 PDB structures (6NR3, 6MIX, 8DDS, 8SR8). A second refinement (green box) selects 6NR3 as the final template based on ligand presence, TRP family, M subfamily, and QMEAN identity. The 4 PDB

structures (right) together with the 14 sequences (left) are used for MSA, followed by homology modeling of Sm.TRPM$_{PZQ}$ using template 6NR3

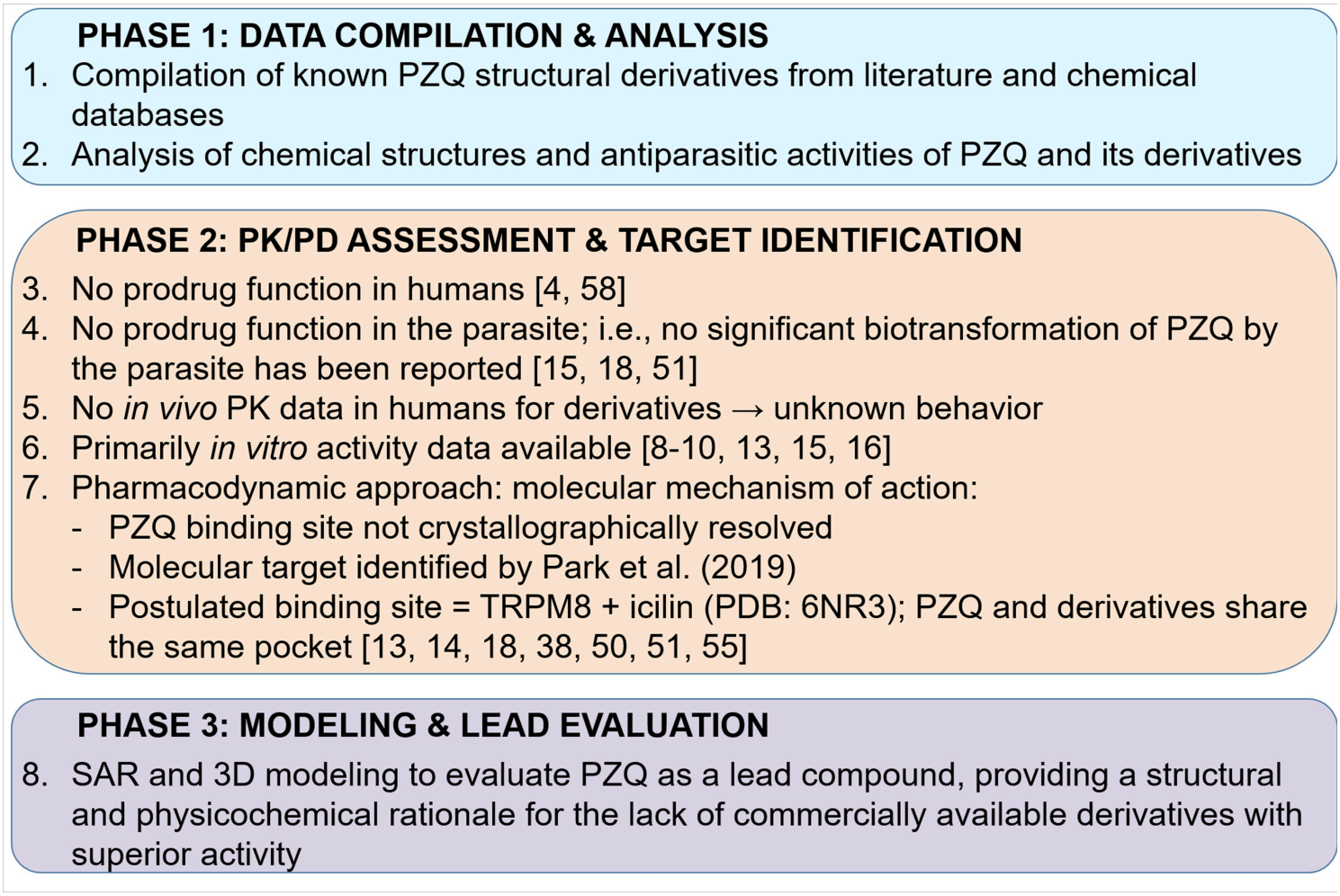


Figure 2

Workflow for the structural and pharmacodynamic and pharmacokinetic analysis of PZQ and its derivatives. The diagram illustrates the sequential phases of the study, from data compilation and analysis (Phase 1) through pharmacokinetic (PK) and pharmacodynamic (PD) assessment (Phase 2) to computational modeling and lead evaluation (Phase 3). The postulated binding site is based on the TRPM8–icilin complex (PDB: 6NR3). PZQ, praziquantel; SAR, structure–activity relationship

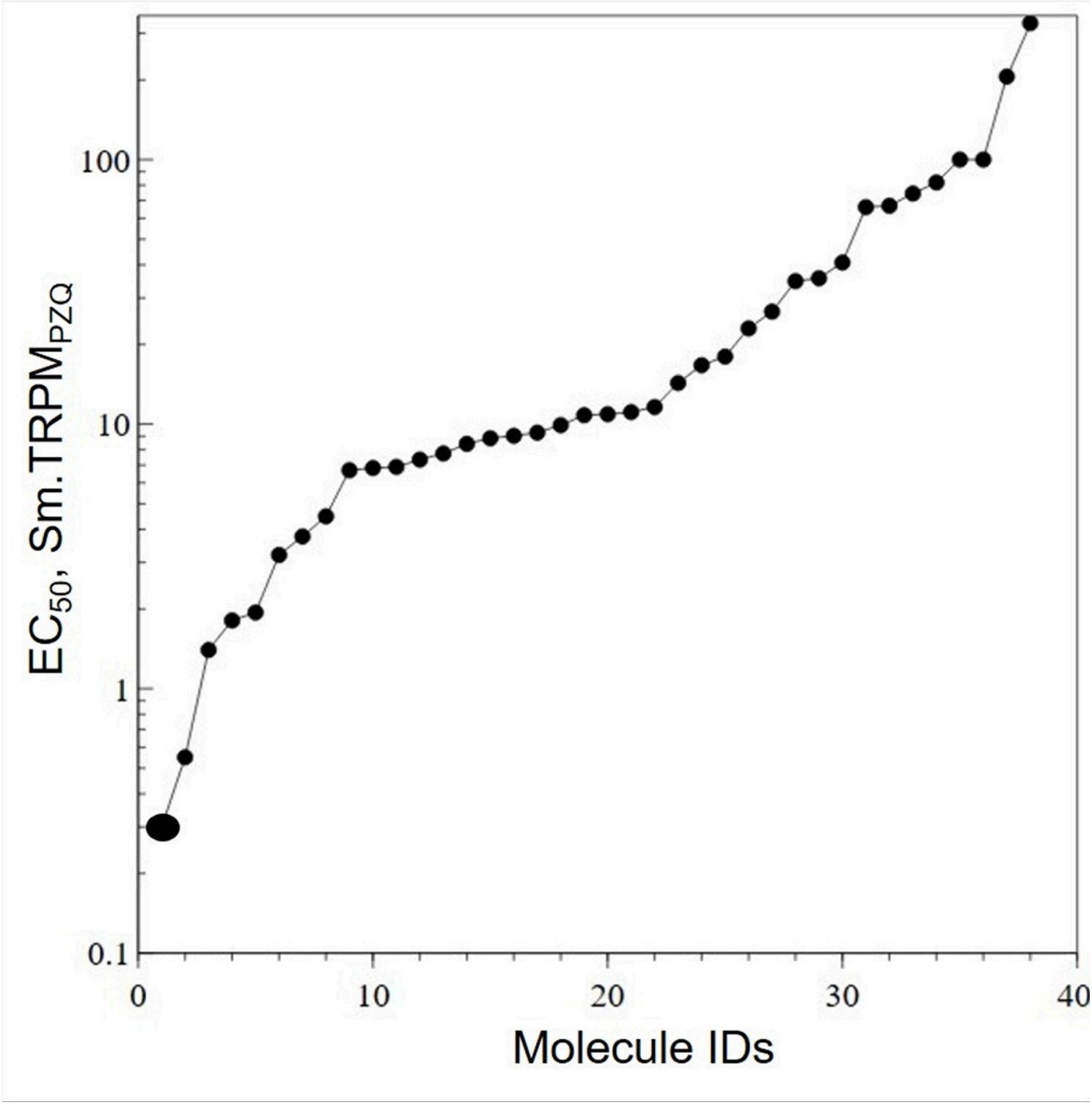


Figure 3

Analysis of relative biological potencies compared to the reference agent PZQ. Each point on the graph represents the activity value (y-axis) for the corresponding PZQ derivative (x-axis). Symbols: large dot, PZQ; small dots, derivatives. All values are expressed in micromolar (μM). Data and numering scheme were obtained from the Supplemental Material of Sprague et al. (2025), which originally reported 50 molecules. Of these, 38 are shown in the graph; 12 derivatives were excluded because their EC_{50} values were zero (no detectable activity)

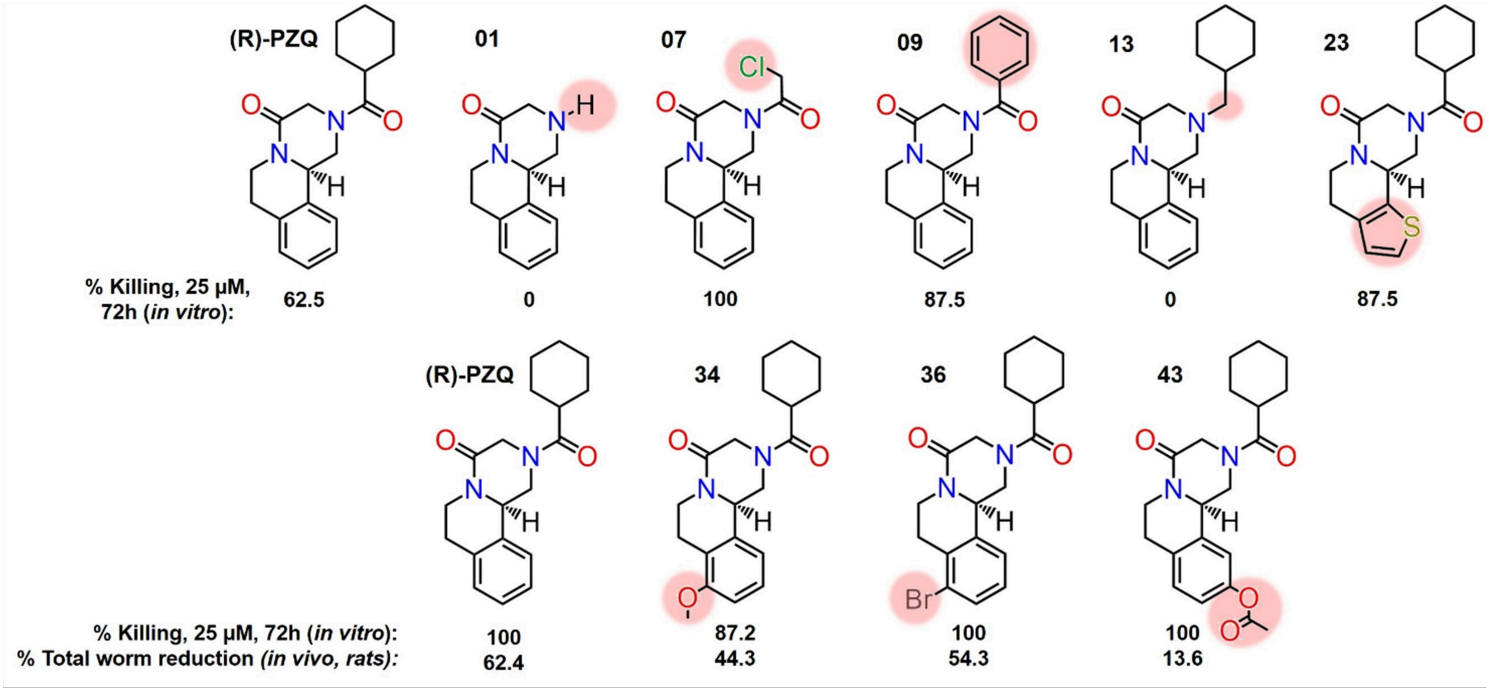


Figure 4

Biological activity and SAR of selected PZQ derivatives. (**A**) *In vitro* activity against *S. mansoni*(25 µM, 72 h) [8]. (**B**) *In vitro* (25 µM, 72 h) and *in vivo*(rats) activity against *S. japonicum* [9]. (R)-PZQ is shown as reference in both panels. Red shading indicates structural modification relative to (R)-PZQ. Of note: numering scheme from (DaSilva et al., 2019)

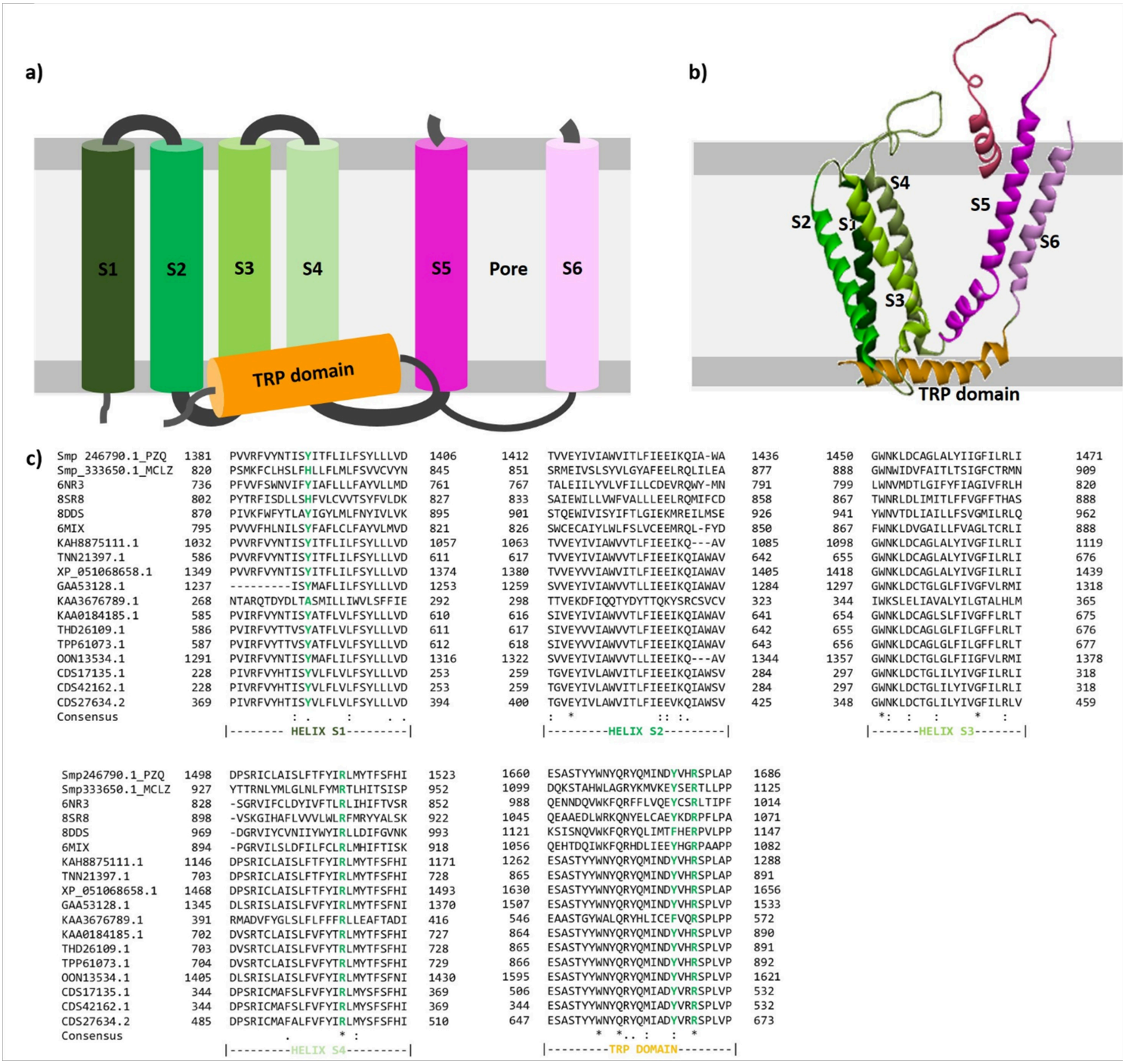


Figure 5

Topological scheme of the TRPM8 channel and multiple sequence alignment of the VSLD domain. a) Topological scheme of the TRPM8 channel. The cylinders represent the transmembrane helices (S1–S6); gray tones indicate the lipid bilayer. Helices S1–S4 form the voltage sensor–like domain (VSLD), which includes the PZQ binding site (Park et al., 2019), while helices S5–S6 form the pore domain responsible for Ca^{2+} influx. The TRP domain is also indicated. **b)** Spatial arrangement of helices S1–S6 (modelo $Sm.TRP_{PZQ}$). **c)** Multiple sequence alignment of the VSLD domain (14 species, choanoflagellates to birds; see **Table S1**). Consensus symbols: (*) identical; (:) similar properties; (.) low similarity. Green

residues are critical for ligand docking and channel activation based on our docking/SAR analysis, and overlap with previously reported essential residues [51]

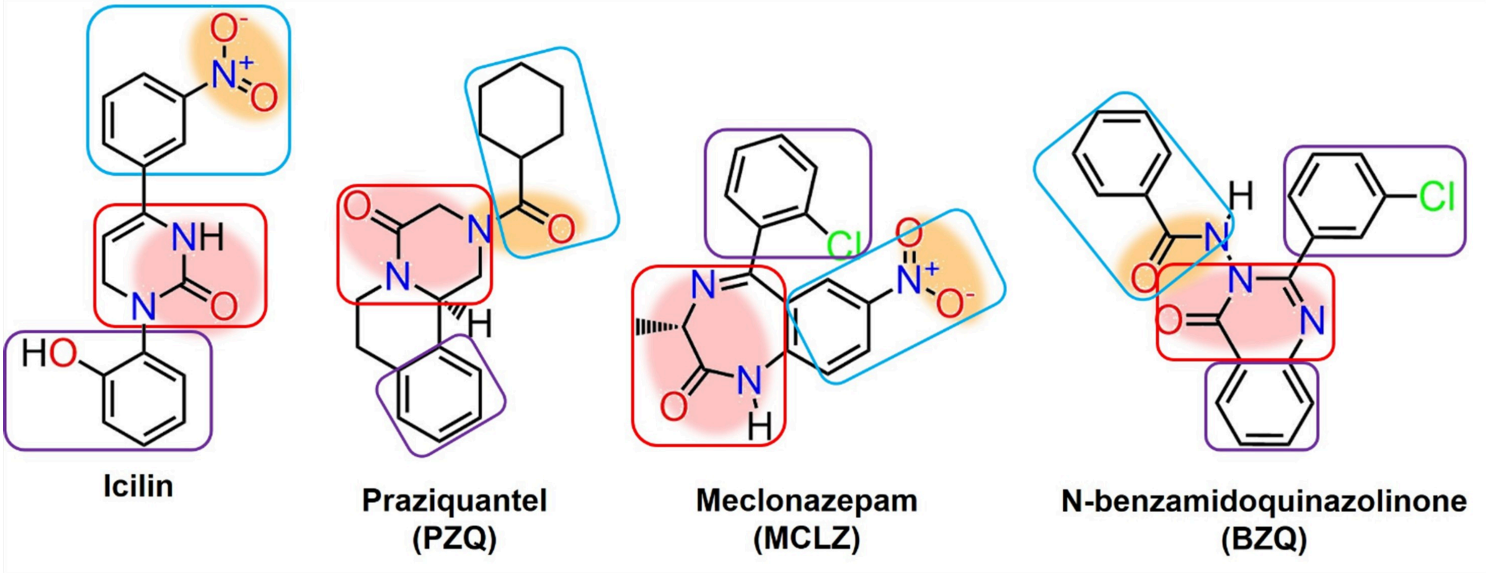


Figure 6

Structural and chemical relatedness for four known strong binders to target: Icilin, PZQ, MCLZ and BQZ. Four related topological features are present in each structure: 1) Peripheral polar moiety (blue box): Nitrogen (N) and oxygen (O) atoms attached to a six-membered carbocyclic ring. 2) Central heterocyclic core (red box): Two nitrogen (N) atoms within a heterocycle, bearing one exocyclic oxygen (O) atom. 3) Aromatic counter-pole (purple box): A peripheral aryl ring attached to a heteroatom. 4) Hydrogen-bond accepting capacity: All heteroatoms can function as hydrogen-bond acceptors (HBAs) due to prototropy in mesomeric systems. The keto-amine ↔ enol-imine conversion, observed in icilin and MCLZ, represents a case analogous to the well-known keto-enol tautomerism. This tautomerism is not present in PZQ or BZQ

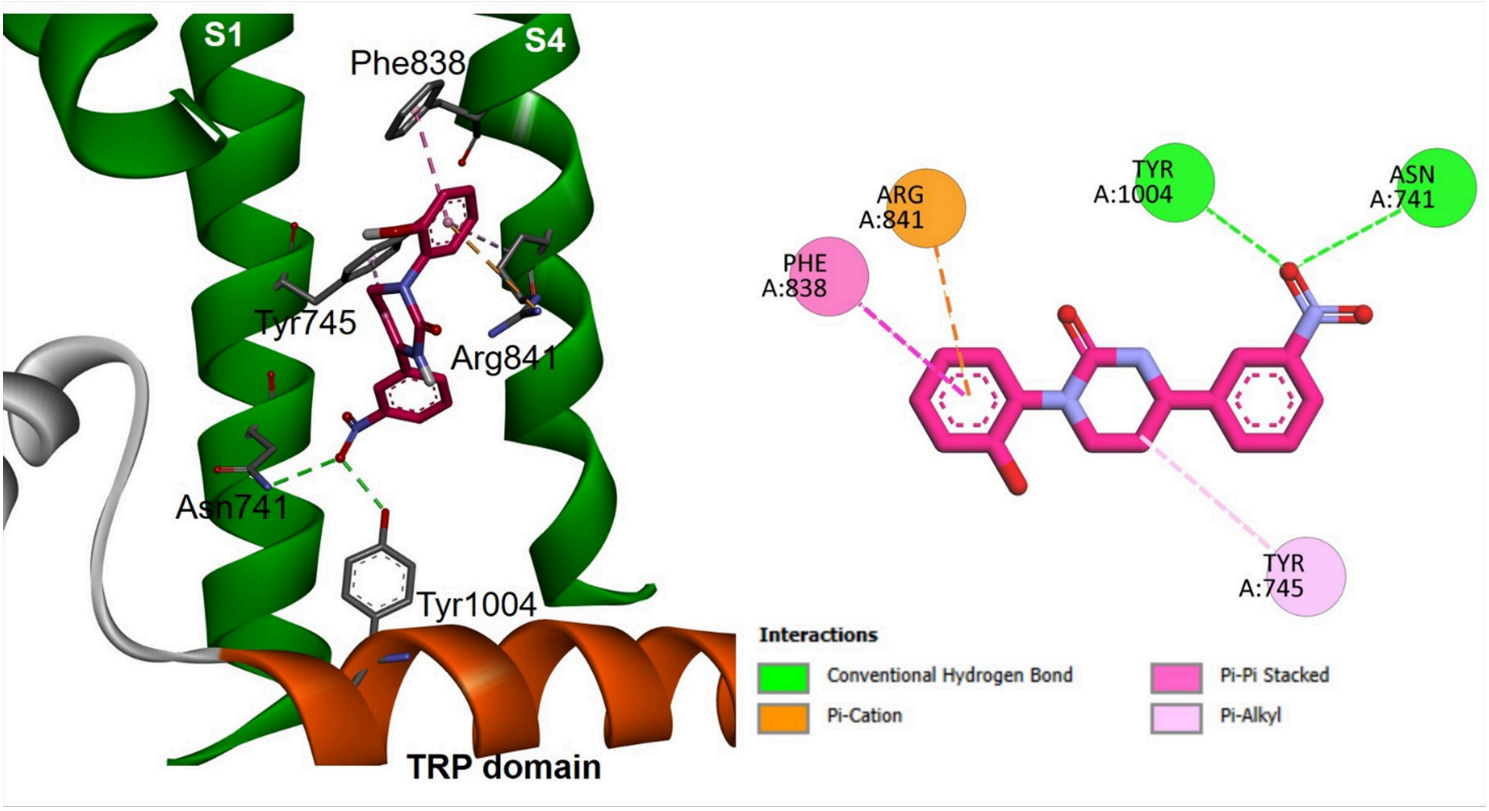


Figure 7

Icilin interactions at the TRPM8 VSLD site (PDB: 6NR3). Left: VSLD cavity (S1–S4 + TRP domain) with residues that chemically interact with icilin. For clarity, helices 2 and 3 are not shown to improve visualization of ligand position and orientation. Icilin interacts mainly with aromatic residues: hydrogen bonds (Tyr1004, Asn741); π-alkyl (Tyr745, Arg841); π-cation (Arg841); π-π (Phe838). **Right:** 2D diagram of the interactions

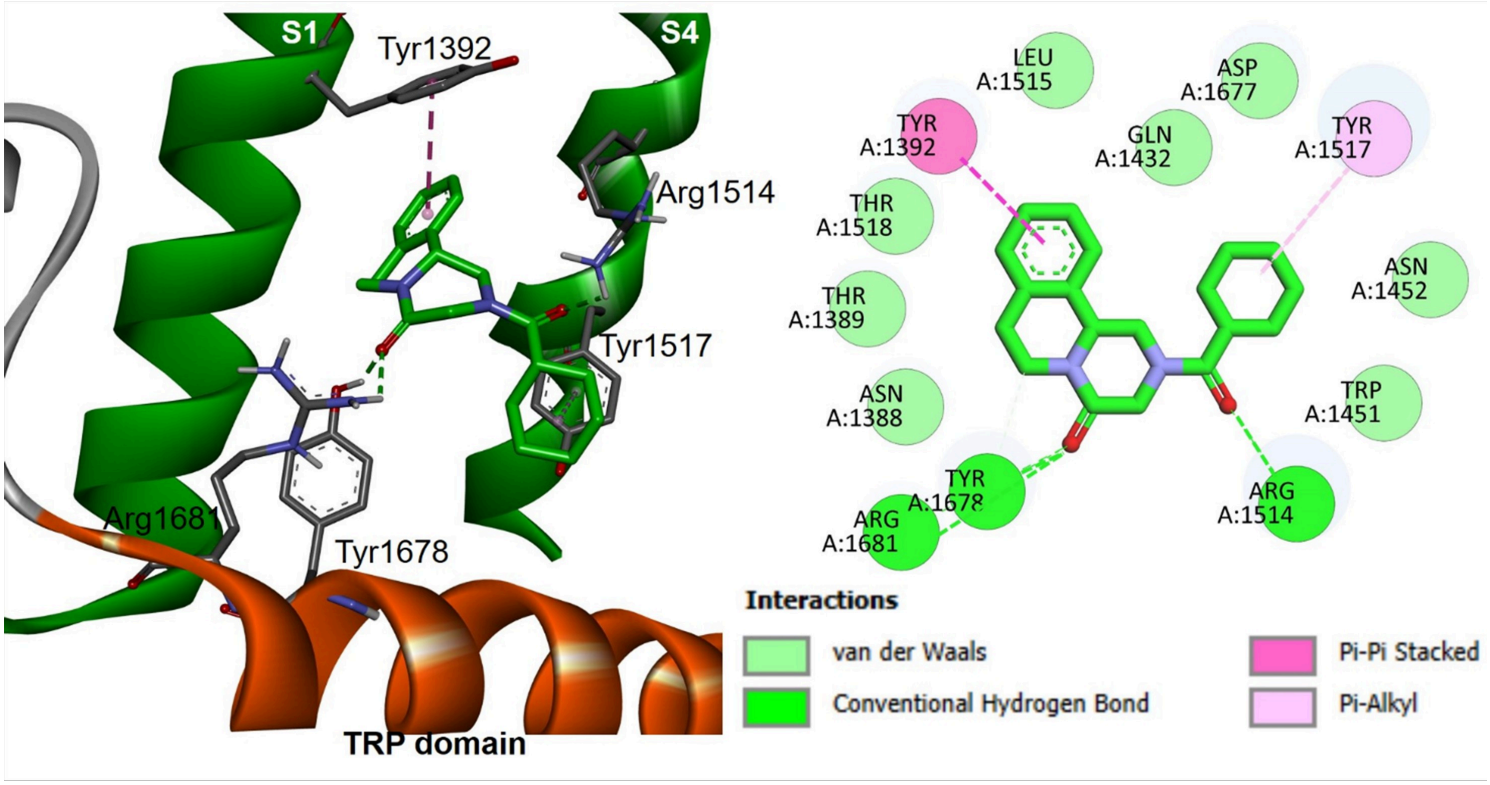


Figure 8

PZQ interactions at the Sm.TRPM$_{PZQ}$ VSLD site. Left: Receptor model (S2/S3 omitted for clarity). S1/S4 (green) and TRP domain (orange) contain interacting residues. PZQ forms H-bonds with Arg1514, Tyr1678, Arg1681; π-π stacking with Tyr1392; and π-alkyl with Tyr1517. **Right:** 2D interaction diagram

Mol	ΔG (Kcal/ mol)	% killing, 25μM, 72h	% Total worm reduction *(in vivo, rats)*	F 1385	R 1388	Y 1392	I 1393	Q 1432	R 1514	L 1515	Y 1517	Y 1678	R 1681	P 1686
(R)-PZQ	-9.07	100				π-π stacked			HB		π-alkil	HB	HB	
02	-6.48	0	----						HB		π-alkil			
07	-7.01	100	----		HB	π-alkil			HB					
09	-8.83	87.5	----			π-π stacked					π-π T-shaped	HB		
13	-8.34	0	----		HB	π-π stacked / π-alkil					π-alkil	HB		
23	-8.60	87.5	----		HB	π-π stacked / π-alkil / π-Sulfur					π-alkil			
34	-8.30	88	44.3			π-π stacked / π-alkil	Alkil		π-alkil	Alkil	π-alkil	HB		
36	-9.00	100	54.3			π-π T-shaped / π-alkil			π-alkil / Amide-π stacked	Alkil / Amide-π stacked		HB		
43	-8.92	100	13.6			π-π stacked		HB					HB	
BZQ	-9.58	----	----		HB	π-π stacked			Alkil, Halogen		π-Alkil	HB		π-Alkil
(S)-MCLZ	-8.48	---	---	π-π T-shaped		π-π T-shaped			π-Alkil			HB		

Figure 9

Interaction profile of PZQ, derivatives, MCLZ, and BZQ at the Sm.TRPM$_{PZQ}$ VSLD site. First column: molecule. First row: amino acid (single-letter code + position). Shading indicates interaction; interaction type is noted within each shaded cell (HB, π-alkyl, etc.). Empty cells without data entries = no interaction. Biological activity data (% killing at 25 μM, 72 h; % total worm reduction *in vivo*, rats) are from external sources (see [8, 9, 11]). Dashed lines: no biological data reported

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- 046PZQderivativesSupplM.pdf