Synthesis of oxadiazole-2-oxide derivatives as potential drug candidates for schistosomiasis targeted on SjTGR

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Abstract

Background: Schistosomiasis is a chronic parasitic disease that affects millions of people's health worldwide. Since the increasing drug-resistant to praziquantel (PZQ), which is the primary drug for schistosomiasis, developing new drugs to treat schistosomiasis is crucial. Oxadiazole-2-oxides have been identified as a potential anti-schistosomiasis reagent targeted to thioredoxin glutathione reductase (TGR).

Methods: In this work, one of the oxadiazole-2-oxides derivatives furoxan was used as the lead compound to exploit series of novel furoxan derivatives for studying inhibitory activity against both recombinant *Schistosoma japonicum* TGR containing selenium (rS*j*TGR-Sec) and soluble worm antigen protein (SWAP) containing wild type *Schistosoma japonicum* TGR (wtS*j*TGR) to develop new leading compound for schistosomiasis. Thirty nine novel derivatives were prepared to test their activity to both two enzymes. The docking method was used to detect the bind sites between active molecule and S*j*TGR; The structure-activity relationship (SAR) of these novel furoxan derivatives was preliminarily analyzed.

Results: It was founded that several new derivatives such as compounds **6a-6d**, **9ab**, **9bd**, **9be** have a better activity to rS*j*TGR-Sec or SWAP containing wtS*j*TGR than furoxan. Interestingly, all intermediates bearing hydroxy (**6a-6d**) showed excellent inhibitory activity to both two enzymes. In particular, compound **6d** with trifluoromethyl on pyridine ring has been found to have much higher inhibition to both rS*j*TGR-Sec (half-maximal inhibitory concentration, IC_{50},7.5nM) and SWAP containing wtS*j*TGR (IC_{50} 55.8nM) than furoxan. Additionally, the docking method revealed the matching sites between **6d** and *Schistosoma japonicumi* TGR (S*j*TGR), which theoretically lends support to the inhibitory activity of **6d**.

Conclusion: The data obtained herein showed preliminarily that **6d** with trifluoromethyl on pyridine ring could be a valuable leading compound for further study.

Background

Schistosomiasis is one of the most widely spread parasitic diseases in the world, as well as is one of the main tropical endemic disease in Asia, Africa and South America [1]. It is estimated that there are approximately 779 million individuals around the world bearing the risk of schistosomes infection [2]. Schistosomiasis is caused by five species of the trematode flatworms, *Schistosoma japonicum (S. japonicum), Schistosoma mansoni (S. mansoni)*, *Schistosoma haematobium*, *Schistosoma mekongi* and *Schistosoma intercalatum*. When a human body is infected, the worms will persist in the liver, hepatic portal system or the urinary tract system, leading to inflammation and obstructive disease [3]. In China, schistosomiasis is mainly caused by *Schistosoma japonicum* and more than 77 thousand people are infected [4].

The most effective drug for the treatment of schistosomiasis is PZQ (**1**), which has been extensively used since the 1980s [5] in the clinic without any back-up drugs. The overuse of praziquantel has caused and accelerated the emergence of drug resistance. Furthermore, PZQ is very effective against the adult forms of all schistosome species, but it is weak for juvenile forms of all schistosomes, which might be the

reason that PZQ can not cure schistosomiasis completely. Although R-PZQ was approved by the FDA as an orphan drug in 2018, it is also at risk of resistance and there still no relative drug against the migratory juvenile and sub-adult worms [6]. Therefore, it is very important and urgent to look for potential reagent to discover novel alternatives to PZQ for the treatment of schistosomiasis.

It has been proved that instead of separate thioredoxin reductase (TrxR) and glutathionereductase (GR) enzymes of mammalian hosts, a single multifunctional selenocysteine-containing flavoenzyme designated TGR in both *S. mansoni* TGR (S*m*TGR) and *S. japonicum* TGR (S*j*TGR) play a critical role in maintaining proper redox balance for parasite survival [1, 7] (**Figure 1**). The analysis showed that schistosome TGR is similar to mammalian TrxR and GR with amino acid sequence and domain structure distribution. The protein sequence contains the binding domain of nicotinamide adenine dinucleotide phosphate (NADPH) and flavin adenin dinucleotide (FAD), the transformation center between –SH and disulfide, and the dimer interface that in contact with pyridine nucleotide disulfide oxidoreductase. A carboxyl-terminal GCUG active site motif exists in the schistosome TGR that has the functions of both TR and GR. Moreover, schistosome TGR also has an additional extension of glutaredoxin (Grx) domain, it's made up of approximately 110 amino acids, with typical CPYC activity [7d, 8].

Figure 1: Redox pathways in mammals and S. *mansoni*.

The study of the anti-oxidative protection mechanism of *S. mansoni* indicated that schistosomes and mammalian hosts have obvious biochemical differences in the characteristics of reactive oxygen species (ROS) defense. The specificity of schistosomes in the ROS defense causes the biochemical differences of REDOX pathway between schistosomes and hosts (mammalian). Schistosome TGR is in a critical position in the REDOX pathway [9] and is inferred to be a potential target for new drug design.

In previous studies [1-2, 7], some oxadiazole-2-oxide analogues have been shown to inhibit S*m*TGR and S*j*TGR effectively, even had good worm killing activities in vivo. Importantly, unlike PZQ, some oxadiazole-2-oxides showed good juvenile and adult *S.japonica* killing activities in vitro [2]. Modifications around oxadiazole-2-oxide skeleton have demonstrated to be a valuable strategy for discovering potential antischistosomal agents. The application of bioisosteres is an effective strategy in drug design. The previous researchers mainly substituted on the benzene ring and changed in the CN moiety when designing the compound [1-2, 7], but did not pay attention to modification of benzene ring and other moieties in the furoxan structure with corresponding bioisosteres. Therefore, in this work, the strategy of the application of bioisosteres was exploited to design novel oxadiazole-2-oxide derivatives.

On the other hand, the prokaryotic expression system has been used extensively for protein expression due to its rapid growth rate, capacity for continuous fermentation, and relatively low cost. Due to the lack of the post-translational modifications of this expression system, the bioactivity, function, structure, solubility and so on will be affected to the expressed functional products. Although eukaryotic expression systems can resolve this problem, just like a two-edged sword, some disadvantages such as low yield, high demanding culture conditions and higher cost cannot be avoided totally [10]. Thus, the special SWAP containing wtS*j*TGR extracted from the host cells might be a better alternative target for studying

the bioactivity. It would reflect the actual situation in advance when Schistosoma japonicum is treated with chemical compounds. Therefore, the target compounds herein were also applied to test the inhibition activity on SWAP containing wtS*j*TGR besides rS*j*TGR-Sec.

In this work, two series of novel furoxan derivatives (**7** and **9**) are obtained through two ways, one is to replace the pheny moiety of furoxan with its bioisosteres pyridine or substituted pyridine, the other is to modify the cyano moiety into an ester or amine group. Besides, fluorine atom possesses a strong electron-withdrawing effect and high lipophilicity, appropriate introduction of fluorine atom into molecules can improve their biofunctions [11]. Therefore, several fluorine-containing derivatives were also intentionally synthesized. The target compounds (**7** and **9**) and their corresponding intermediates (**6**) were tested the inhibition activity on TrxR from rS*j*TGR-Sec and SWAP containing wtS*j*TGR respectively. Based on the result obtained, the SAR of these novel furoxan derivatives was preliminarily analyzed.

Figure 2. The structure of PZQ and furoxan and the general structure of compounds **6**, **7** and **9**.

Methods

Reagent

All chemical reagents involved in synthetic procedure were purchased commercially from J&K Chemicals. The chemical reagents used in activity measurement, such as DMSO, EDTA, DTNB, MTT, were purchased from Sigma-Aldrich Inc..

Synthesis of Oxadiazole-2-oxide derivatives

The synthesis of compounds **6a-d** commenced with the synthesis of α, β-unsaturated esters (**4a-d**) from substituted nicotinaldehydes (**3a-d**) by wittig reaction [12]. Then compounds **4a-d** were reducted using Diisobutylaluminum hydride (DIBAL-H) [13] to produce alcohols **5a-d**. Subsequent sodium nitrate-mediated cyclization [13] gave oxadiazole-2-oxides (**6a-d**) (**Scheme 1**).

In **Scheme 2**, compound **7** was readily prepared by esterification of alcohol **6a-d** with various acyl chlorides using triethylamine and dimethylaminopyridine (DMAP) as base and catalyst respectively. The synthesis of target compounds **9** began with the treatment of compounds **6a-d** with benzene sulfonyl chloride to generate unstable intermediates **8a-d,** which were aminated in situ to afford various target amines **9**. As a result, total 39 novel oxadiazole-2-oxide derivatives were synthesized and their structure were determined by NMR (nuclear magnetic resonance) and HRMS (high resolution mass spectrometer). The procedures for the preparation of compounds are detailed in **Additional file 1**

Crystallography

Normally, two isomers **6** and **6'** were obtained (**Scheme 1**) and one of them was main product after cyclization. In order to confirm the conformation of main product, its crystal was selected and was measured on a SuperNova, Dual, Cu at zero, EosS2 diffractometer. The crystal was kept at 150 K during

data collection. Using Olex2, the structure was solved with the Superflip. Structure solution program using Charge Flipping and refined with the ShelXL. Refinement package used Least Squares minimisation.

Preparation of the rS*j*TGR-Sec and the SWAP containing wtS*j*TGR

The rS*j*TGR-Sec was expressed and harvested as described previously [7d, 15]. Most of rS*j*TGR-Sec was expressed in *E. coli* as a soluble His-tagged fusion protein when bacterial growth occurred at 24 °C. The rS*j*TGR-Sec was purified by His•Mag™ Agarose Beads according to manufacturer's instructions. The concentration of purified rS*j*TGR-Sec was determined using a Bradford assay kit (Sangon, China), and the protein was identified by sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) and stored at 4 °C [16].

The soluble worm antigen protein (SWAP) containing wtS*j*TGR was prepared from adult schistosomes. The rabbits were infected with cercariae of *S. japonicum* by exposing their abdominal skin, were sacrificed at 42 day after infection to collect the adult schistosomes by perfusion. The worms were washed three times with phosphate buffered saline (PBS). After sufficient grinding of the adult schistosomes in PBS, suitable amount of benzoyl sulfonyl fluoride (PMSF) solution until its final concentration to 1 mM were added and the homogenate was centrifuged at 4°C, 12,000×g for 20 min[1, 7a]. The concentration of supernatant containing wtS*j*TGR was determined by Bradford assay kit (Sangon, China).

Compounds Inhibition of rS*j*TGR-Sec and SWAP containing wtS*j*TGR

The novel furoxan derivatives in the paper were dissolved in DMSO (dimethylsulfoxide) with the storage solution concentration of 64 millimoles per liter. The TrxR activities of SWAP containing wtS*j*TGR and rS*j*TGR-Sec was performed based on the methods described previously [17], and the inhibitory activities of the compounds against the TrxR activity of SWAP containing wtS*j*TGR and rS*j*TGR-Sec described in this paper were performed based on the method described previously [2]. The reaction was performed in 0.1 M potassium phosphate buffer (pH 7.0) containing 10 mM EDTA (Ethylene Diamine Tetraacetic Acid), 100 μM NADPH, 0.2 μg supernatant contained SWAP containing wtS*j*TGR or 0.6 μg rS*j*TGR-Sec and the compounds with different concentrations at 25℃ in a final volume of 200 μL and initiated by adding 3 mM dithionitrobenzoic acid (DTNB). The increase of the absorbance at 412 nm was monitored, and the IC_{50} values of the compounds were calculated by GraphPad Prism v6.0c software, and the results were reported in **Table 1**. All assays were done in triplicate.

Docking study

The crystal structure of S*j*TGR is a homodimer, and we retained chain B for docking studies [18]. The docking was conducted by AutoDock 4 and the results were displayed in **Figure 4** and **Figure 5**. The results were further processed and displayed by PyMOL2.3.2. The hydrophobic effect between inhibitors and protein were analyzed by LigPlot^{+}v.2.1 and the result was showed in **Figure 5.**

Results And Discussion

Synthesis and Crystallography

Totally, thirty nine novel oxadiazole-2-oxide derivatives were synthesized following **Scheme 1** and **Scheme 2**. The structures of these compounds were detailed in **Table 1**. During synthesis of the important intermediate **6**, two isomers were inevitably formed when the oxadiazole-N-oxide ring was constructed by sodium nitrate-mediated cyclization. Gasco and co-workers reported [19] that intramolecular hydrogen bond played a crucial role on the formation of the main isomer during cyclization and could stabilize the structure of the isomer a (**Scheme 3**).

For our case, two isomers **6** and **6'** were obtained (**Scheme 1**) and one of them was the main product after cyclization. To confirm the position of N-oxide moiety, is the main product **6** or **6**'? The crystal of the main product was obtained. It was interesting that the X-ray confirmed that the main product had a similar conformation with the isomer a in **Scheme 3**, and specifically, the main product was **6a** (**Scheme 1**). The crystal structure showed that there were two independent molecules in an asymmetric unit (**Figure 3a**). One molecule's nitrogen atom was labeled with N1, N2 and N3, and the other molecule's nitrogen atom was labeled with N4, N5 and N6. However, intermolecular hydrogen bonds instead of intramolecular hydrogen bonds were found in the crystal of **6a**. The molecular labeled N1, N2 and N3 of nitrogen atoms formed a hydrogen bonds helix chain (**Figure 3b**), in addition, the molecular labeled N4, N5 and N6 of nitrogen atoms formed a hydrogen bonds zigzag chain (**Figure 3c**). This result also indicated that after the pyridine replaced the benzene, the intramolecular hydrogen bond showed in **Scheme 3** might not be necessary for the formation of main isomer **6a-d**.

Figure 3: (a) Crystal structure of **6a**; (b) Hydrogen bond helix chain formed by molecules labeled N1, N2 and N3 of nitrogen atoms; (c) Hydrogen bond zigzag chain formed by molecules labeled N4, N5 and N6 of nitrogen atoms.

Activity assessments of TGR

With furoxan as the positive control, oxadiazole-2-oxides (**6, 7, 9**) were evaluated for their inhibitory activities against both rS*j*TGR-Sec and SWAP containing wtS*j*TGR under different concentrations (**Table 1**). As shown in **Table 1**, all intermediates bearing hydroxy (**6a-6d**) showed better inhibitory activity against both two enzymes than furoxan. The pyridine ring in the structure of compound **6a** had a stronger electron withdraw effect on the oxadiazole-2-oxide ring than compound **6b**, and **6a** (IC_{50} 70nM of SWAP containing wtS*j*TGR, IC_{50} 36nM of rS*j*TGR-Sec) had better inhibitory activity on both two enzymes than **6b** (IC_{50} 0.14μM of SWAP containing wtS*j*TGR, IC_{50} 0.39μM of rS*j*TGR-Sec). Compared with **6b**, the electron-donating methoxy group at the ortho position of pyridine (like compound **6c**) decreased the inhibitory activity (**6c**, IC_{50} 1.1μM of SWAP containing wtS*j*TGR, IC_{50} 2.4μM of rS*j*TGR-Sec). Notably, electron-withdrawing group trifluoromethyl made **6d** exhibited excellent inhibitory effect (IC_{50} 7.5nM of

SWAP containing wtS*j*TGR, IC_{50} 55.8nM of rS*j*TGR-Sec). These data indicated that the electron-withdrawing substituents very possibly provided potency enhancements of activity.

To further investigate the relationship between the structure and bioactivity, modification of hydroxyl group into the ester group and amine, respectively afforded compounds **7** and **9**. It was found that the ester group could not effectively improve the inhibitory effect, except the ethyl ester (**7ca**), chloracetyl ester (**7ae, 7be**), benzoyl ester (**7af, 7cf**) and acryloyl ester (**7bj**) exhibited inhibitory effects similar to furoxan. Only **7ca** (IC_{50} 34.77μM of SWAP containing wtS*j*TGR) showed inhibitory effect against SWAP containing wtS*j*TGR, but not as good as furoxan. Among chloracetyl esters, maybe is because the pyridine ring of **7ae** had a stronger electron-withdrawing effect, **7ae** (IC_{50} 4.23μM of SWAP containing wtS*j*TGR) had a slight advantage over **7be** (IC_{50} 16.40μM of SWAP containing wtS*j*TGR, IC_{50} 14.82μM of rS*j*TGR-Sec). However, **7ae** did not show an effective inhibitory effect on rS*j*TGR-Sec. As for phenyl derivatives, **7af** (IC_{50} 8.87μM of SWAP containing wtS*j*TGR, IC_{50} 3.23μM of rS*j*TGR-Sec) and **7cf** (IC_{50} 12.51μM of SWAP containing wtS*j*TGR) did not differ significantly in inhibiting SWAP containing wtS*j*TGR, but **7cf** did not show an inhibitory effect on rS*j*TGR-Sec. Among acrylate compounds, only **7bj** showed inhibitory activity against SWAP containing wtS*j*TGR (IC_{50} 5.80μM).

Some amine derivatives (**9aa**, **9ab**, **9bd**, **9be**, **9da**) showed inhibitory activity, among which **9bd** had an inhibitory effect against SWAP containing wtS*j*TGR at the nanomolar level (IC_{50} = 42nM). Compared with phenyl amino derivative **9aa**, **9ba**, **9ca** and **9da**, the pyridine with strong electron-withdrawing ability improved the activity, such as, compounds **9aa** (IC_{50} 21.57μM to SWAP containing wtS*j*TGR) and **9da** (IC_{50} 11.62μM to SWAP containing wtS*j*TGR, IC_{50} 3.73μM to rS*j*TGR-Sec) showed better inhibition effect than **9ba** (IC_{50}>50μM) and **9ca** (IC_{50}>50μM). Similar to phenyl amino derivatives, 4-trifluoromethroxyphenyl amino derivative **9ab** (IC_{50} 0.62μM to SWAP containing wtS*j*TGR, IC_{50} 1.52μM to rS*j*TGR-Sec) showed better inhibitory effect than **9bb** (IC_{50}>50μM both to SWAP containing wtS*j*TGR and rS*j*TGR-Sec). However, among piperazine substituted derivatives (**9ac**, **9ad** and **9bd**), **9bd** exhibited good inhibitory activity against SWAP containing wtS*j*TGR (IC_{50} 0.042μM). Interestingly, compound **9be** is an intermediate of a synthetic target molecule, but it displayed much better inhibitory effects (IC_{50} 0.63μM to SWAP containing wtS*j*TGR, IC_{50} 0.22μM to rS*j*TGR-Sec) than furoxan Perhaps the sulfamide or Si-O-N moieties in molecule **9be** has some influence on enzyme inhibition ability, this result might provide a new direction for more compounds design.

Docking studies

To rationalize the obtained bioactivity data and to understand how the synthesized inhibitors interact with schistosomal proteins, the selected compounds with high activity **6d**, **7af** and **9ab** were docked to the available crystal structure of S*j*TGR (thioredoxin glutathione reductase from *Schistosoma japonicumi*, PDB ID:4LA1). The crystal structure of S*j*TGR is a homodimer, and we retained chain B for docking studies. The results were displayed in **Figure 4** and **Figure 5**.

Compound **6d** formed four hydrogen bonds with Asp433, Ser117 and Thr153, and a π-cation interaction with Arg393(**Figure 4a)** and two strong hydrogen bonds existed between the hydroxyl moiety of **6d** and S*j*TGR. These results could explain the highest activity of **6d**. Compound **7af** formed two hydrogen bonds with Cys154 and Asp433, and a π-cation interaction with Arg393(**Figure 4b)**, while the pyridine ring and oxadiazole ring didn't form any type of interactions with the residues of Arg393. Compound **9ab** formed one hydrogen bond with Ser276, a π-cation interaction with Arg393 and a π-π stacking interaction with Tyr296. It's noticed that the oxadiazole ring participated in the formation of chemical bonds, to constitute more interaction bonds. The distance between the oxadiazole ring and other ring -shaped conjugated structures should be taken into consideration. After superimposing three compounds (**6d**,**7af** and **9ab**) together (**Figure 4d**), each of them formed one π-cation interaction with Arg393, therefore, Arg393 was speculated to play a role of anchor during the binding process.

Figure 4: In **(a)**, **(b)** and **(c),** the protein (S*j*TGR) is shown as ribbons, the synthesized inhibitors are shown in orange sticks, and the residues that can interact with inhibitors are shown as green sticks. **(a)** Compound **6d** forms a π-cation interaction (red dashed line) with Arg393 and hydrogen bonds (yellow dashed lines) with Ser117, Thr153 and Asp433. **(b)** Compound **7af** forms a π-cation interaction with Arg393 and hydrogen bonds with Cys154, and Asp433. **(c)** Compound **9ab** forms a π-cation interaction with Arg393, a π-π stacking interaction (blue dashed line) with Tyr296, and a hydrogen bond with Ser276. **(d)** Superposition of docking poses of compounds **6d** (blue), **7af** (purple), **9ab** (green) in the binding pocket of *S. japonicum* TGR.

On the other hand, the hydrophobic effect was showed by red arcs with spokes radiating toward the atoms involved (**Figure 5**). The numbers of the residues that had hydrophobic contacts with the inhibitors were associated negatively with the enzyme inhibitory activities. Among three compounds (**6d**, **7af** and **9ab**) compound **7af** had the most of hydrophobic contacting residues and it had the lowest activity, compound **6d** had the least of hydrophobic contacting residues and it exhibited the highest activity.

Figure 5: LigPlot^{+} generated two-dimensional schematic overview of molecular interactions between S*j*TGR and compound **6d**, **7af** and **9ab**. Hydrogen bonds are indicated by green dashed lines with corresponding distances between the atoms given in Å. Hydrophobic contacts are shown by red arcs with spokes radiating toward the atoms involved.

Conclusions

Using furoxan as the leading compound, series of novel esters and amine derivatives have been synthesized by modifying the cyano moiety into an ester or amine group and replacing pheny moiety with its bioisosteres pyridine or substituted pyridine. Moreover, the structure of the key intermediate for target compounds was confirmed by crystallography method. The inhibitory activity of all title compounds and key intermediates were evaluated against the TrxR activity of both rS*j*TGR-Sec and SWAP containing wtS*j*TGR for the first time. Several new derivatives, **6a-d**, **9ab**, **9bd**, **9be** have a better activity to rS*j*TGR-Sec or SWAP containing wtS*j*TGR than furoxan. The SAR of these novel furoxan derivatives was preliminarily

analyzed. Pyridine ring with strong electron-withdrawing ability might be beneficial to enhance the inhibitory effect. Docking studies have shown that hydroxyl moiety can form two strong hydrogen bonds with S*j*TGR, which is of great help to enhance the inhibitory activity of compounds. Compound **6d** with trifluoromethyl on pyridine ring has the highest activity and is a good S*j*TGR inhibitor and could be potent antischistosomal agents

Declarations

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Availability of data and materials

Data supporting the conclusions of this article are included within the article and its additional file.

Competing interests

The authors declare that they have no competing interests.

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Authors' contributions

DS, YH and GL designed the experiments and drafted the manuscript. GL, HC and WZ prepared and identified the compound. QG and SL performed the biological experiments and conducted the data analysis.

CF performed the docking study. GL, QG and CF drafted the initial version of the manuscript. DS, YH and GL drafted the final version of the manuscript. All authors read and approved the final manuscript.

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Abbreviations

PZQ: praziquantel; TGR: thioredoxin glutathione reductase; S*j*TGR: *Schistosoma japonicumi* TGR; S*m*TGR: *Schistosoma mansoni* TGR; rS*j*TGR-Sec: recombinant *Schistosoma japonicumi* TGR containing selenium ; SWAP: soluble worm antigen; wtS*j*TGR: wild type S*j*TGR; SAR: structure-activity relationship; TrxR: thioredoxin reductase; GR: glutathionereductase; NADPH: nicotinamide adenine dinucleotide phosphate; FAD: flavin adenin dinucleotide; ROS: reactive oxygen species; NMR: nuclear magnetic resonance; HRMS: high resolution mass spectrometer; SDS-PAGE: sodium dodecyl sulfate polyacrylamide gel electrophoresis; PBS: hosphate buffered saline; DMSO: dimethylsulfoxide; EDTA: ethylene diamine tetraacetic acid; DTNB: dithionitrobenzoic acid ; DIBAL-H: Diisobutylaluminum hydride; DMAP: dimethylaminopyridine; PMSF: benzoyl sulfonyl fluoride.

References

1. Song L, Li J, Xie S, Qian C, Wang J, et al. Thioredoxin Glutathione Reductase as a Novel Drug Target Evidence from Schistosoma japonicum. PloS one. 2012;7:e31456.
2. Song LJ, Luo H, Fan WH, Wang GP, Yin XR, et al. Oxadiazole-2-oxides may have other functional targets, in addition to SjTGR, through which they cause mortality in Schistosoma japonicum. Parasites & Vectors. 2016;9:1-12.
3. Gryseels B, Polman K, Clerinx J, Kestens L. Human schistosomiasis. Lancet. 2006;368:1106-18.
4. Zhang LJ, Xu ZM, Qian YJ, Dang H, Lv S, et al. Endemic status of schistosomiasis in People's Republic of China in 2015. Chin J Schisto Control. 2016;28:611-7.
5. Thetiot-Laurent SAL, Boissier J, Robert A, Meunier B. Schistosomiasis chemotherapy. Angewandte Chemie. 2013;52:7936-56.
6. (a) Cioli D, Pica-Mattoccia L, Basso A, Guidi A. Schistosomiasis control: praziquantel forever? Molecular and biochemical parasitology. 2014;195:23-9; (b) Guglielmo S, Cortese D, Vottero F, Rolando B, Kommer VP, et al. New praziquantel derivatives containing NO-donor furoxans and related furazans as active agents against Schistosoma mansoni. European journal of medicinal chemistry. 2014;84:135-45; (c) Sadhu PS, Kumar SN, Chandrasekharam M, Pica-Mattoccia L, Cioli D, et al. Synthesis of new praziquantel analogues: potential candidates for the treatment of schistosomiasis. Bioorganic & medicinal chemistry letters. 2012;22:1103-6; (d) Zheng Y, Dong L, Hu C, Zhao B, Yang C, et al. Development of chiral praziquantel analogues as potential drug candidates with activity to juvenile Schistosoma japonicum. Bioorganic & medicinal chemistry letters. 2014;24:4223-6.
7. (a) Sayed AA, Simeonov A, Thomas CJ, Inglese J, Austin CP, et al. Identification of Oxadiazoles as New Drug Leads for the Control of Schistosomiasis. Nature Medicine. 2008;14:407; (b) Rai G, Sayed AA, Lea WA, Luecke HF, Chakrapani H, et al, Inglese J. Structure mechanism insights and the role of nitric oxide donation guide the development of oxadiazole-2-oxides as therapeutic agents against schistosomiasis. Journal of Medicinal Chemistry. 2009;52:6474-83; (c) Bonilla M, Denicola A, Novoselov SV, Turanov AA, Protasio A, et al. Platyhelminth mitochondrial and cytosolic redox homeostasis is controlled by a single thioredoxin glutathione reductase and dependent on selenium and glutathione. J Biol Chem. 2008;283:17898-907; (d) Kuntz AN, Davioud-Charvet E, Sayed AA,

Califf LL, Dessolin J, et al. Thioredoxin glutathione reductase from Schistosoma mansoni: an essential parasite enzyme and a key drug target. Plos Medicine. 2007;4:e206.

8. Hu W, Yan Q, Shen DK, Liu F, Zhu ZD, et al. Evolutionary and biomedical implications of a Schistosoma japonicum complementary DNA resource. Nature Genetics. 2003;35:139
9. (a) Angelucci F, Sayed AA, Williams DL, Boumis G, Brunori M, et al. A. Inhibition of Schistosoma mansoni Thioredoxin-glutathione Reductase by Auranofin: STRUCTURAL AND KINETIC ASPECTS*. Journal of Biological Chemistry. 2009;284:28977; (b) Sharma M, Khanna S, Bulusu G, Mitra A. Comparative modeling of thioredoxin glutathione reductase from Schistosoma mansoni: a multifunctional target for antischistosomal therapy. Journal of Molecular Graphics & Modelling. 2009;27:665-75.
10. Yin J, Li G, Ren X, Herrler G. Select what you need: A comparative evaluation of the advantages and limitations of frequently used expression systems for foreign genes. Journal of Biotechnology. 2007;127:335-47.
11. Eric P G, Kyle J E, Matthew D H, David J D, Nicholas A M. Applications of Fluorine in Medicinal Chemistry. Journal of medicinal chemistry. 2015;58:8315-59.
12. Sano S, Yokoyama K, Fukushima M, Yagi T, Nagao Y. New reaction mode of the Horner–Wadsworth–Emmonsreaction using Sn(OSO2CF3)2 andN-ethylpiperidine. Chemical Communications. 1997;28:559-60.
13. Rai G, Thomas CJ, Leister W, Maloney DJ. Synthesis of Oxadiazole-2-oxide Analogues as Potential Antischistosomal Agents. Tetrahedron Letters. 2009;50:1710-3.
14. V. Dolomanov LJB, R.J. Gildea, J. A. K. Howard, H. Puschmann. OLEX2: a complete structure solution, refinement and analysis program. J. Appl. Crystallogr. 2009;42:339-41.
15. Rengby O, Johansson L, Carlson LA, Serini E, Vlamis-Gardikas A, et al. Assessment of production conditions for efficient use of Escherichia coli in high-yield heterologous recombinant selenoprotein synthesis. Applied and environmental microbiology. 2004;70:5159-67.
16. Alger HM, Williams DL. The disulfide redox system of Schistosoma mansoni and the importance of a multifunctional enzyme, thioredoxin glutathione reductase. Molecular & Biochemical Parasitology. 2002;121:129-39.
17. Han Y, Zhang M, Hong Y, Zhu Z, Li D, et al. Characterization of thioredoxin glutathione reductase in Schiotosoma japonicum. Parasitology international. 2012;61:475-80.
18. Peng Y, Wu Q, Huang F, Chen J, Li X, et al. Crystal structure of SjTGR complex with FAD. RCSB PDB. http://www.rcsb.org/structure/4LA1. Accessed 15 Oct 2019.
19. Gasco AM, Fruttero R, Sorba G, Gasco A. Unsymmetrically Substituted Furoxans, XIII. Phenylfuroxancarbaldehydes and Related Compounds. Liebigs Ann. Chem. 1991:1211-3.

Tables

Due to technical limitations, Table 1 is only available as a download in the supplementary files section.

Figures

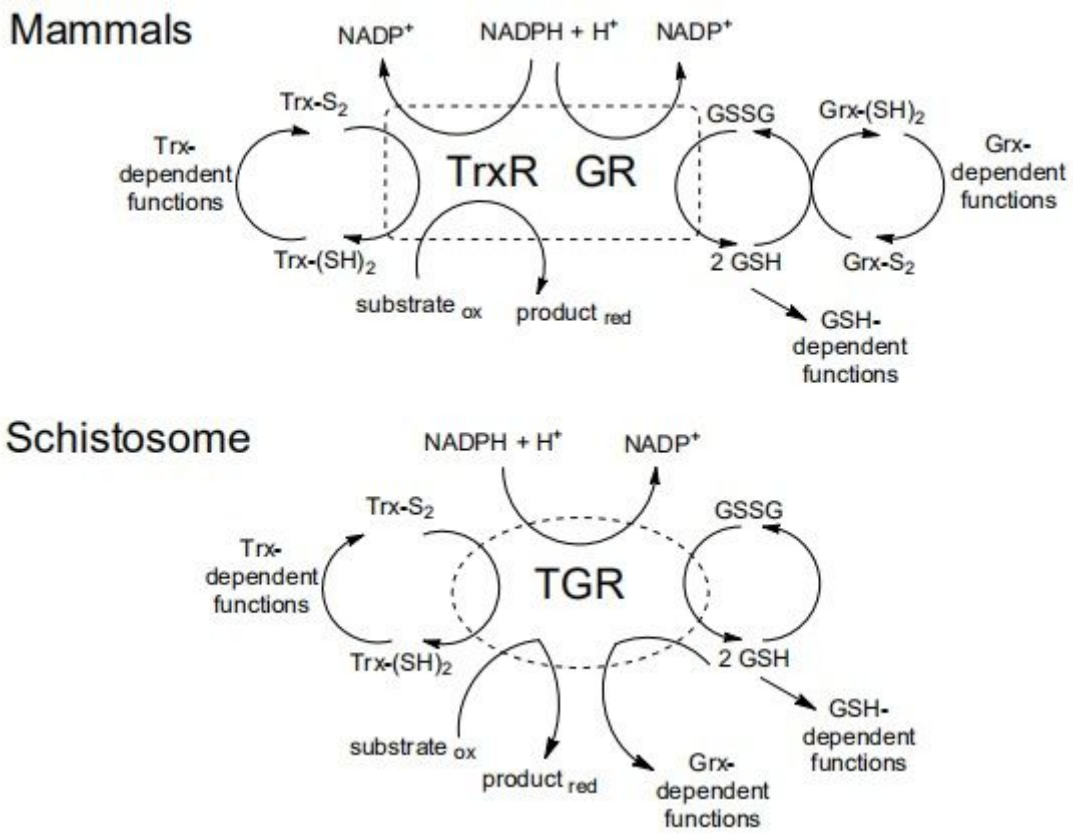

Figure 1

Redox pathways in mammals and S. mansoni.

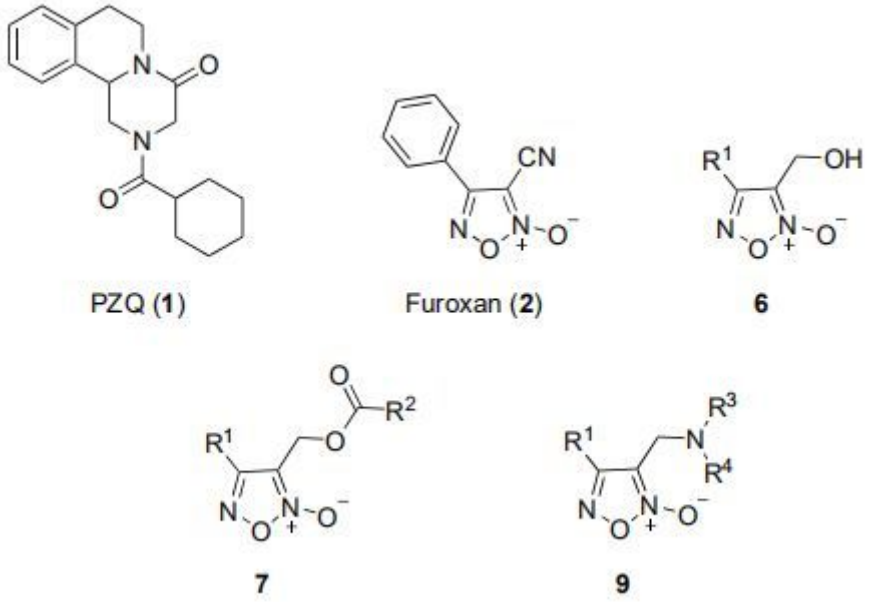

Figure 2

The structure of PZQ and furoxan and the general structure of compounds 6, 7 and 9.

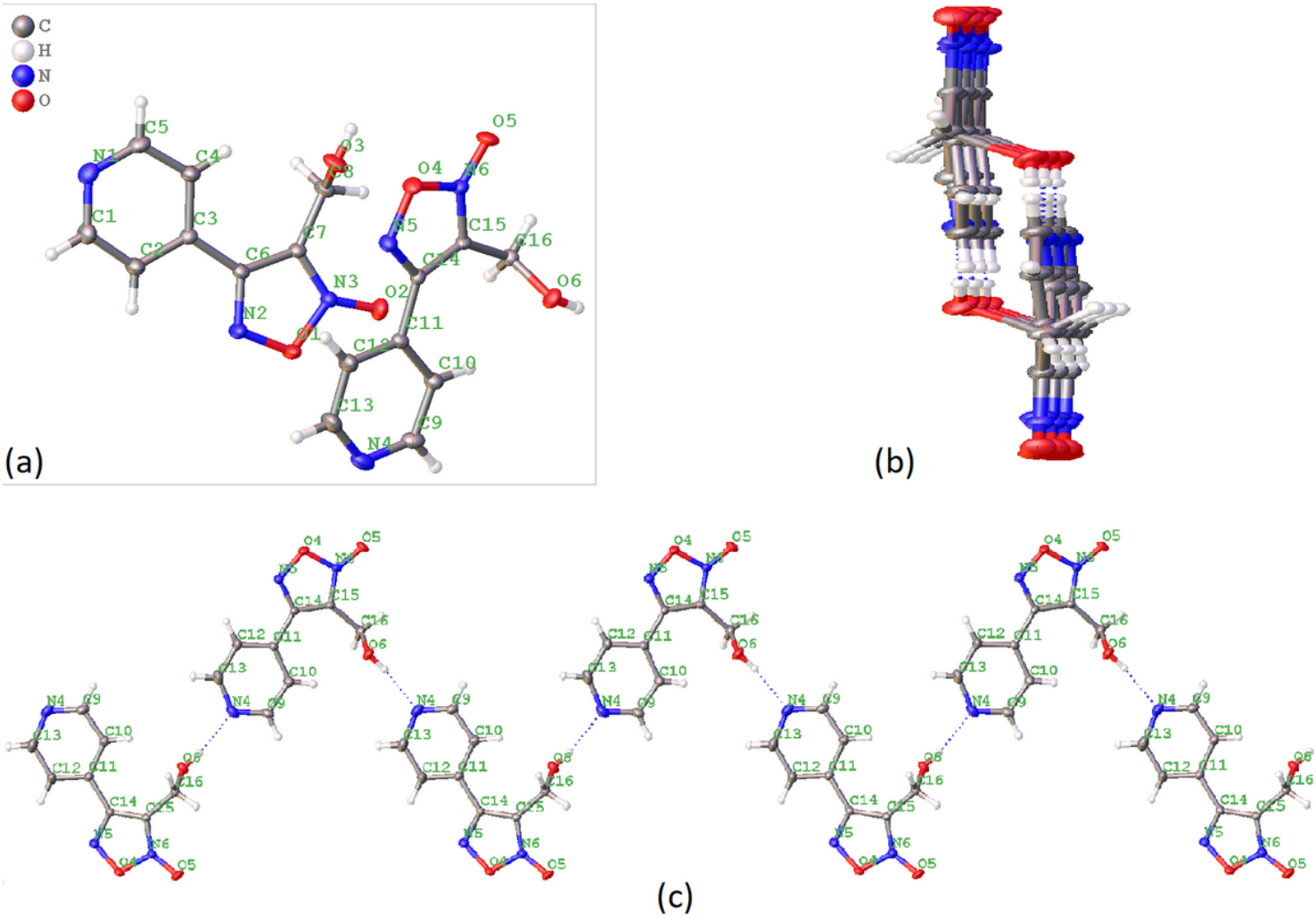

Figure 3

(a) Crystal structure of 6a; (b) Hydrogen bond helix chain formed by molecules labeled N1, N2 and N3 of nitrogen atoms; (c) Hydrogen bond zigzag chain formed by molecules labeled N4, N5 and N6 of nitrogen atoms.

Supplementary Files

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

- Additionalfile1.docx
- Table1.docx