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Privileged structure-guided synthesis of quinazoline derivatives as inhibitors of trypanothione reductase

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ABSTRACT

Novel quinazoline-type compounds were designed as inhibitors of the parasite specific enzyme trypanothione reductase (TR), and their biological activities were evaluated. Some of our compounds inhibited TR, showed selectivity for TR over human glutathione reductase, and inhibited parasite growth in vitro. We propose that the quinazoline framework is a privileged structure that can be purposely modified to design novel TR inhibitors. Furthermore, the use of privileged motifs might emerge as an innovative approach to antiparasitic lead candidates.

Neglected tropical diseases (NTD) do not have high visibility in Western societies. Their name is also related to the fact that they are often neglected when health agendas and budgets are set, and at the level of research and development.[1] This is despite the one billion people worldwide suffering from NTD, and the inappropriateness of the currently available drugs, which have problems with safety, administration, cost, and, increasingly, resistance.[2] NTD, such as different forms of leishmaniasis and trypanosomiasis, remain difficult to cure and often lead to fatalities. There are several current medicinal chemistry strategies for tackling this problem. Among others, an exciting opportunity has emerged from the genome-sequencing projects of these parasites.[3] The knowledge of the total genomes offers the possibility of identifying novel drug targets that are vital to the pathogens but are absent in their hosts, and that are open to selective inhibition.[4,5]

Trypanothione ($T[SH]_2$; bis-(glutathionyl)-spermidine), is unique to members of the order Kinetoplastida. $T[SH]_2$ is the main low molecular mass thiol in these parasitic protozoa and is responsible for maintaining the intracellular reducing environment. $T[SH]_2$ is kept reduced by the essential flavoenzyme trypanothione reductase (TR, E.C. 1.6.4.8).[6] TR is an FAD-disulfide oxidoreductase. The catalyzed NADPH-dependent reduction of trypanothione disulfide (TS_2) to $T[SH]_2$ is homologous to the reduction of glutathione disulfide (GSSG) by glutathione reductase (GR) in other organisms, such as in cells of the mammalian host. Both enzymes have three residues that are directly involved in catalysis, the redox active dithiol/disulfide (Cys53-Cys58 in *Trypanosoma cruzi* TR) and a His residue (His461′ in *T. cruzi* TR), which is provided by the second subunit of the homodimer.[7,8] Although TR and GR share 40% of all residues, two major characteristics account for the selectivity of inhibitors against the two enzymes. Because of the presence of an extra protonated amino group in $T[SH]_2$, TR possesses a negatively charged disulfide substrate binding site whereas that of human GR carries an overall positive charge.[8,9] Moreover, TR has a larger active site to accommodate its bulkier substrate. This distinct profile, together with the availability of the coordinates of several crystal structures, makes TR a validated target in the search for antiparasitic drugs.[10–17]

The aim of the present study was to develop, by investigating the active site topology of TR, novel quinazoline-based ligands as selective TR inhibitors. We started from the consideration that a scaffold protonated at physiological pH is a prerequisite for ensuring TR/GR selectivity. However, antiparasitic molecules with positively charged groups, such as amidine or guanidine moieties, are in general poorly orally available.[18] Therefore, we searched for new chemical entities with superior intrinsic drug-like properties. Privileged structures with their inherent affinity for diverse

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biological targets, represent an ideal source of core scaffolds for the design of libraries targeted at various receptors.[19] In addition, the likely drug-like properties of privileged structures and substructures might produce more drug-like compound libraries and leads.[20] Properly substituted 2,4-diamino-quinazolines are privileged sub-structures protonated at physiological pH.[21] Numerous quinazoline-based libraries have been synthesized, and have proven to be extremely powerful tools to the rapid discovery and optimization of ligands for a wide variety of targets,[22] also in the field of antiparasitic drugs.[23] On these bases, a series of 2-piperazin-1-yl-quinazolin-4-ylamine derivatives (**1**–**7**) was, for the first time, designed and tested against TR. Docking of the quinazoline core 1-(4-(4-amino-6,7-bis(2-(dimethylamino)ethoxy)quinazolin-2-yl)piperazin-1-yl)ethanone showed that such a scaffold was able to interact with the TR active site. Then, all compounds were designed in such a way as bearing structural features of typical TR inhibitors, namely the overall positive charge together with extended hydrophobic parts.[24] Different moieties, such as methoxy

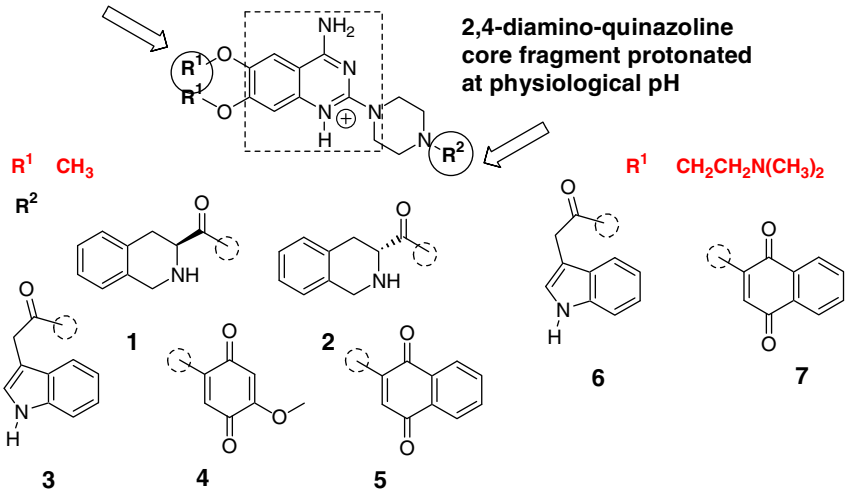


Figure 1. Design strategy for compounds **1**–**7**.

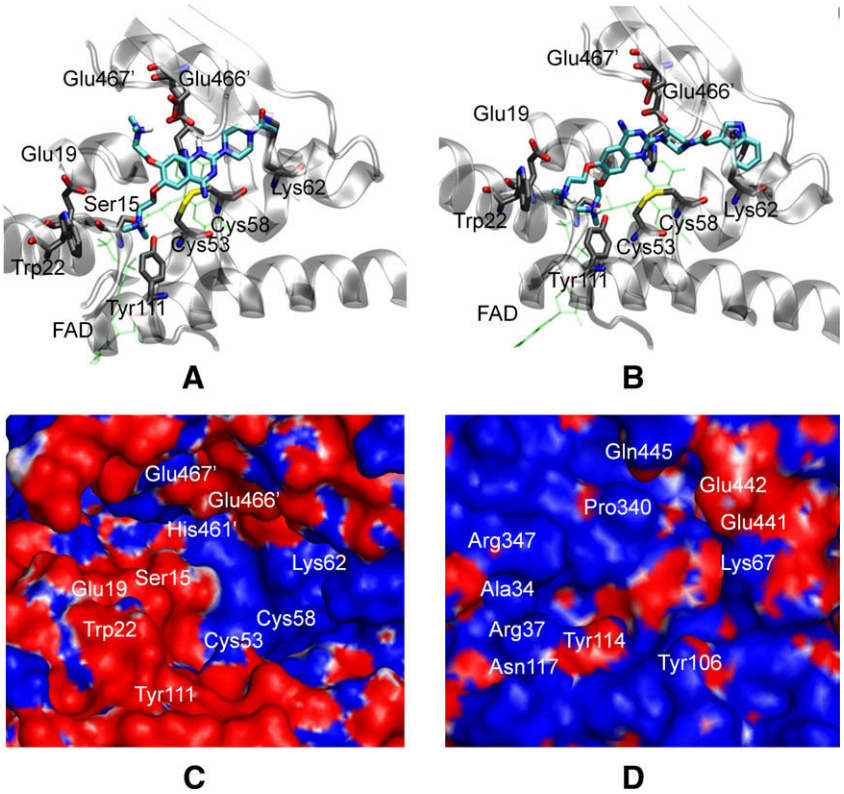


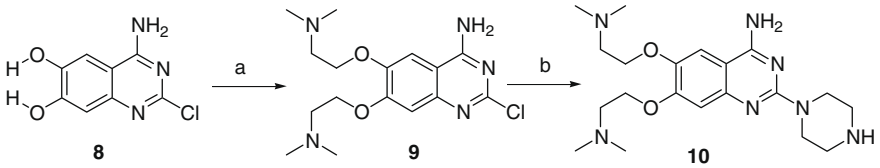
Figure 2. (A) Binding mode of a statistically populated cluster of 1-(4-(4-amino-6,7-dimethoxyquinazolin-2-yl)piperazin-1-yl)ethanone (carbon atoms cyan) at the TR binding site. The key amino acids responsible for the binding are explicitly reported. (B) Binding mode of **6** at the TR binding site. The electrostatic potential of TR (C) and GR (D), calculated by the APBS plug-in of Pymol, is shown: negative and positive electrostatic potential is red and blue, respectively. The electrostatic potential helps explaining the rather good TR/GR selectivity of **6**.

and dimethylaminoethoxy groups in R^1, and indole, quinone, and tetrahydroquinoline moieties in R^2, were attached to the quinazoline core to provide additional interactions with the target (Fig. 1).

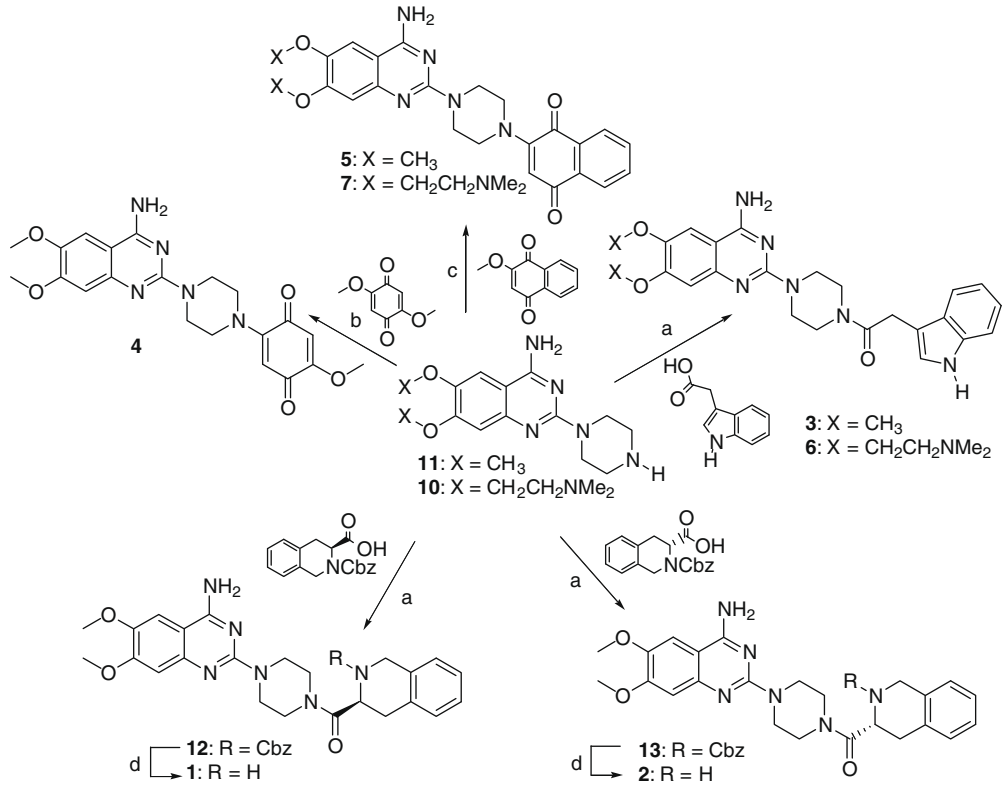
Figure 2A shows the suggested binding mode of the starting fragment 1-(4-(4-amino-6,7-bis(2-(dimethylamino)ethoxy)quinazolin-2-yl)piperazin-1-yl)ethanone. An electrostatic interaction could be identified between the protonated N1 nitrogen of the quinazoline moiety and Glu466′. The substituents on position R^1 were required to capture additional interactions with Glu19 and Trp22 that could confer selectivity versus the GR enzyme. Then, structural modifications on position R^2 could be envisaged to capture further interactions with Lys62. The binding mode of **6**, which can be considered the end point of the above described design strategy, is reported in Figure 2B. Indeed, a cation-π interaction was detected between the indole ring of **6** and Lys62, as well an electrostatic interaction between the protonated nitrogen of the 2-dimethylaminoethoxy chain and both Glu19 and Trp22. A further interaction was observed between the chain and the OH group of Tyr111. In Figure 2C and D, the electrostatic surfaces of TR and GR, respectively, are depicted. While TR bears a negative electrostatic potential, GR has positive values for the potential at the same region of the active site. This electrostatic feature could account for the TR/GR selectivity of **6** and parent molecules of this series. Further docking simulations were carried out, being however the binding mode of quinone containing compounds not univocally determined. Indeed, such molecules could bind to TR enzyme at different sites, thus accounting for their non-competitive or mixed inhibition mechanisms. This has been already reported in the literature for similar quinone derivatives.[24]

The novel substituted 2-piperazin-1-yl-quinazolin-4-ylamines **1**–**6** were synthesized using the readily accessible methodologies developed for this class of compounds, whereas the synthesis of **7**[25] was previously reported.[25,26] The only new chemistry concerned the insertion of the dimethylaminoethyl chain in positions 6 and 7 of the quinazoline ring of **8**,[27] to afford **9**: the nucleophilic substitution strictly required the use of cesium carbonate as a base (Scheme 1, complete synthetic details and spectroscopic analysis are available online in Supplementary data). Afterwards, the protocol proceeded by allowing **9** to react with excess piperazine. Then, intermediates **10** and **11** were reacted with quinone, indole, and tetrahydroisoquinoline derivatives, affording directly, or after a deprotection step, the final compounds **1**–**7** (Scheme 2).

The assays contained NADPH, TR, and 100 μM of each inhibitor and TS_2 which corresponds to a substrate concentration of $5 \times K_m$. All of the quinazolines proved to be inhibitors of TR, except **1** and **2**. The inhibition ranged from 23% to 76%, with the naphthoquinone



Scheme 1. Reagents and conditions: (a) $CsCO_3$, (2-chloroethyl)-dimethylamine, N_2, DMF, reflux, 2 h, 10%; (b) piperazine, isoamylic alcohol, 160 °C, 30 h, 34%.



Scheme 2. Reagents and conditions: (a) EDCI, HOBt, DMF, rt, overnight, 24–61%; (b) $CHCl_3$, reflux, 96 h, 4%; (c) EtOH, reflux, 96 h, 47%; (d) MeOH, 10 % Pd/C, 90–95%.

derivatives **5** and **7** being the most effective inhibitors (Table 1). From these results, we conclude that the 2,4-diaminoquinazoline scaffold is a good motif for TR recognition. However, the presence of a suitable capping fragment is fundamental for activity. In fact, the tetrahydroisoquinolines **1** and **2** did not inhibit the enzyme, whereas quinones-bearing molecules **4**, **5** and **7**, by virtue of the known quinone ability to interact with TR, display a more pronounced inhibition.[28] For **4**, **5**, and **7** the inhibitor constants were determined. The naphthoquinone derivatives **5** and **7** inhibited TR with K_i-values of about 10 μM, not dissimilar to that of 4,4′-bis(4-benzyloxy-3-methoxybenzimidoylamino)di-cyclohexylmethane (**14**, see SI Fig. 2), used as positive control.[24] The degree of inhibition is comparable to that exerted by other known TR inhibitors such as mepacrine (K_i 19 ± 5 μM)[29] and clomipramine (K_i 6.5 ± 0.6 μM).[30] These results suggest that **5** and **7** are a good starting point for a medicinal chemistry program aimed at identifying derivatives with higher TR inhibitory potency. Inhibition of TR was not purely competitive, confirming that quinone-bearing compounds might bind to multiple sites.

Compounds **5** and **7** were also tested against human GR. At 10 μM, the highest concentration soluble in the assay buffer, compound **5** did not show any inhibition of GR in the presence of 100 μM GSSG. 125 or 250 μM of compound **7** resulted in about 10 and 20% inhibition, respectively. Taking into account that 100 μM GSSG corresponds to only 1.5 × K_m, inhibition of the host GR is negligible when compared to that of the parasite TR. Thus, both inhibitors displayed selectivity for the parasite enzyme, confirming the design rationale.

Table 1
Inhibition of *T. cruzi* TR by quinazoline derivatives

No.	[Inhibitor] (μM)	% inhibition of TR at		K_i (μM)	Inhibition type
		100 μM (TS_2)	50 μM (TS_2)		
1	100	0	0	nd[a]	
2	100	0	0	nd	
3	100	23	13	nd	
4	100	75	69	32 ± 4	Non competitive
5	25	57	53	7.5 ± 2	Mixed-type, K_i' 22 ± 6 μM
6	100	33	44	nd	
7	40	76	79	11 ± 3	Non competitive
14	100	97	94	2	Mixed-type, K_i' 16 μM

[a] Not determined.

Table 2
Antiparasitic activity of compounds **1**–**7** in whole cell assays. IC_{50}-values (μM) for amastigote forms of *T. cruzi* in L6 cells, bloodstream trypomastigote forms of *T. brucei rhodesiense*, axenic amastigote form of *Leishmania donovani*, and the intra-erythrocytic form of *Plasmodium falciparum* are reported. Activity for mammalian L6 cells (cytotoxicity) is also shown. **15**, **16**, **17**, **18** and **19** are the reference compounds benznidazole, melarsoprol, miltefosine, chloroquine, and podophyllotoxin, respectively

Compd	*T. cruzi*	*T. b. rhodesiense*	*L. donovani*	*P. falciparum*	L6
1	14.3	1.81	>67	2.10	14.2
2	27.6	5.37	>67	1.24	37.2
3	10.2	2.21	42.2	1.35	19.6
4	35.6	1.98	45.7	3.01	52.5
5	3.68	0.12	1.78	0.53	2.76
6	>50	21.8	>54	2.19	>161
7	10.5	2.54	67.1	0.19	5.25
15	1.48				
16		0.01			
17			0.34		
18				0.22	
19					0.012

The anti-parasitic potential of all compounds was analyzed in light of the WHO/TDR criteria, as previously described.[31] Table 2 summarizes the activity of compounds **1**–**7** against *Trypanosoma brucei rhodesiense*, *T. cruzi*, *Leishmania donovani*, and *Plasmodium falciparum*, as well as their cytotoxicity against L6 cells. The results show good correlation between enzyme inhibition and trypanocidal activity for compound **5**, which is a TR inhibitor and the most active against the different trypanosomatids. On the other hand, compounds **1** and **2**, inactive at 100 μM against TR, also interfered with parasite growth, confirming the potential of the quinazoline scaffold for antiparasitic activity. As a drawback, some derivatives showed cytotoxicity towards L-6 myoblast cells. However, against the extracellular African trypanosomes compounds **1**–**5** were 7–27-fold more active than against the mammalian control cells. The mammalian cytotoxicity proved to be highest for the naphthoquinone-containing quinazolines **5** and **7**. It can, therefore, be argued that toxicity can be tuned down by properly modifying or replacing the naphthoquinone moieties. It is encouraging that **11** itself was not found to modify cell proliferation.[25] It is also important to emphasize that all the designed compounds are active in the context of the cell environment and therefore are able to penetrate cells and reach the intracellular target.[32] However, we can not exclude that other proteins are (also) modulated by our privileged quinazoline compounds. In this respect, we were pleased to note that all seven derivatives exhibited remarkable activity against *P. falciparum*, with IC_{50}-values in the micromolar and submicromolar range, although *P. falciparum* does not have the trypanothione metabolism.

The quinazoline ring is a heterocyclic motif that meets the requirements of a privileged structure that is, according to Evans' original definition, 'a single molecular framework able to provide ligands for diverse receptors'.[33] The inherent promiscuity of privileged scaffolds might also be a drawback, as selectivity might become an issue. However, several reports confirm that small modifications in and around the core cause difference in activity, giving raise to the desired selectivity.[34] Compounds based on this motif might have favourable pharmacokinetic features, a high degree of drug-likeness.[35] In antiparasitic drug discovery, where economic reality raises the need for inexpensive chemotherapeutics, the importance of using pre-validated chemical scaffolds might acquire relevance. Cost of goods is a crucial issue because the drugs will have to be cheap to produce and distribute. The drugs should also be orally available since other routes of administration are problematic in rural settings.[18] In this scenario, this small library holds promise for the further exploration of quinazoline substructure for interaction with TR, with the potential to generate, through iterative medicinal chemistry and DMPK (Drug Metabolism and Pharmacokinetics) network activities, preliminary SAR and in vivo activities. All these considerations, especially those related to economic issues, indicate that the development of quinazoline- or other privileged structure-based antiparasitic compounds might have potential for the research and development of new drugs for NTD.

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Supplementary data

Supplementary data (the detailed synthetic and biological procedures, as well as full spectral characterization of the new com-

pounds are given) associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2009.04.060.

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