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Original article

Antileishmanial activity of quinazoline derivatives: Synthesis, docking screens, molecular dynamic simulations and electrochemical studies

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ABSTRACT

A series of quinazoline-2,4,6-triamine were synthesized and evaluated in vitro against *Leishmania mexicana*. Among them, N^6-(ferrocenmethyl)quinazolin-2,4,6-triamine (**H2**) showed activity on promastigotes and intracellular amastigotes, as well as low cytotoxicity in mammalian cells. Docking and electrochemical studies showed the importance of both the ferrocene and the heterocyclic nucleus to the observed activity. **H2** is readily oxidized electrochemically, indicating that the mechanism of action probably involves redox reactions.

1. Introduction

The search for antiparasitic molecules has recently become important because of the emergence of drug resistant strains, the toxicity of the known molecules, increased poverty and the percentage of the affected population [1]. Parasitic tropical diseases affect hundreds of millions of people worldwide, however, it has been neglected to develop drugs against these diseases because they primarily affect people in poor regions of the world [1]. Leishmaniasis, African trypanosomiasis, and Chagas disease are vector-borne diseases caused by parasites of the kinetoplastida order [2]. Leishmaniasis is a set of devastating diseases caused by the obligate intracellular protozoa parasites of the *Leishmania* genus, which are transmitted by a group of 50 species and subspecies of phebotomine insects. About 1.5 million of new cases of cutaneous leishmaniasis and 500 000 new cases of visceral disease occur each year. Cutaneous leishmaniasis is endemic in more than 70 countries worldwide [3]. A major emerging problem is co-infection of *Leishmania* with human immunodeficiency virus, especially because there is no effective treatment for these patients [3]. Conventional chemotherapy relies on multiple parenteral administrations of pentavalent antimonials that are considerably toxic and induce resistance. Second-line drugs, such as amphotericin B and its lipid formulations, are either too toxic or too expensive for routine use in developing countries [4]. Recently, miltefosine, a phosphocholine analogue originally developed as an anticancer agent, was introduced as a drug against visceral leishmaniasis, but its effectiveness has still not been conclusively determined and there have already been reported cases of resistance [5,6]. Because chemotherapy for leishmaniasis is still inefficient, there is an urgent need for the development of new efficient and safe drugs.

Abbreviations: PTR1, Pteridine reductase 1; DHFR, Dyhydrofolate reductase; IC_{50}, Half inhibitory concentration; TAQ, Quinazoline-2,4,6-triamine; PDB, Protein Data Bank.

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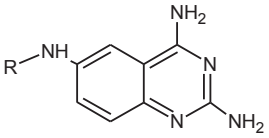
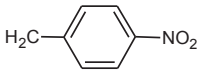
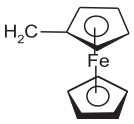
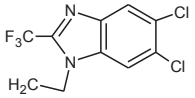
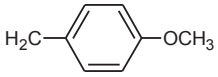
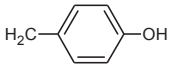
In order to find new drugs against leishmaniasis, the computational design of new drugs against parasites has been based on the knowledge and availability of new information from molecular targets [7,8]. With the goal of following this strategy, we chose pteridine reductase (PTR) as the target of the study, which is a protein that appears to be important to the resistance mechanism of *Leishmania*. This enzyme is NADPH-dependent with oxide reductase activity, and pterins are its natural substrate [7]. However, under conditions of cellular stress or when folate metabolism is brought down by molecules that inhibit dihydrofolate reductase (DHFR), PTR may reduce folate, therefore allowing the production of DNA through the salvage route, causing parasite resistance [9–11]. New approaches in the design of antifolates should consider the importance of PTR to discover new molecules capable of inhibiting DHFR and/or PTR through rational design [7].

The isoform PTR1 of *Leishmania major* was co-crystallized with quinazoline-2,4,6-triamine (**TAQ**) [12]. By analyzing the interactions of **TAQ** with the active site of PTR1, the chemical modification of **TAQ** at position 6 was considered because the reception site near this position is large and possesses both polar and non-polar regions (Fig. 1). Berman and co-workers, showed the importance of the lipophilic substituents at position 6 of the quinazoline ring, decreasing the ED_{50} against *L. major*. In addition, they have shown that, by increasing the size of the substituent, the ED_{50} increased [13]. In this case, the selection of the chemical substituent at position 6 was based on its potential to function as an anti-protozoal scaffold. With this in mind, nitrobenzene, ferrocene, benzimidazole, anisole and phenol moieties were selected as the substituents in **H1**, **H2**, **H3**, **H4** and **H5**, respectively (Table 1). Ferrocene is present in ferroquine, an anti-malarial drug that is currently under clinical evaluation and has got a great potential [14]. A number of studies have shown that the introduction of the ferrocene core may significantly enhance the molecule's (desirable) bioactive properties. The ferrocene unit might act as a hydrophobic spacer and/or lipophilicity/bioavailability enhancer (enabling an easier way through cell membranes) [15]. It is also known that ferrocene Fe^{+2}/Fe^{+3} redox chemistry might contribute to the bioactivity of ferrocene derivatives [16]. Benzimidazole is a privileged structure in antiparasitic molecules [17]; particularly, in a compound prepared in our group, 5,6-dicloro-2-(trifluoromethyl)-1*H*-benzimidazole, denoted in this work as **G2** [18]. Nitrobenzene, anisole and phenol groups are present in several anti-parasitic drugs [19,20].

With the aim of exploring PTR1 binding site surfaces of the protein, we employed docking studies using **TAQ** as ligand and attempted to reproduce this ligand is in the previously reported crystal structure [12]. Next, we performed a molecular coupling of the proposed molecules (**H1**–**H5**) into active site of *Leishmania* PTR1 and DHFR. The affinity of all the target compounds to both enzymes was higher than the affinity of **TAQ**. Subsequently, the proposed compounds were synthesized and once their structure was elucidated by x-ray crystallographic, they were tested in vitro against the promastigote form of *Leishmania mexicana*. The most active was **H2**. For this reason, chemical modifications were carried out to the **H2** structure (Fig. 2) in order to explore changes in biological responses that could cause these structural modifications. Finally, the reduction potential for this group of compounds was carried out using DMSO as aprotic solvent. The data in aprotic solvent did not only describe the situation in that way but it also allowed us to obtain biological significance of interpretation.

Table 1
Derivatives of **TAQ** with antiparasitic moieties.



Compound	R
H1	
H2	
H3	
H4	
H5	

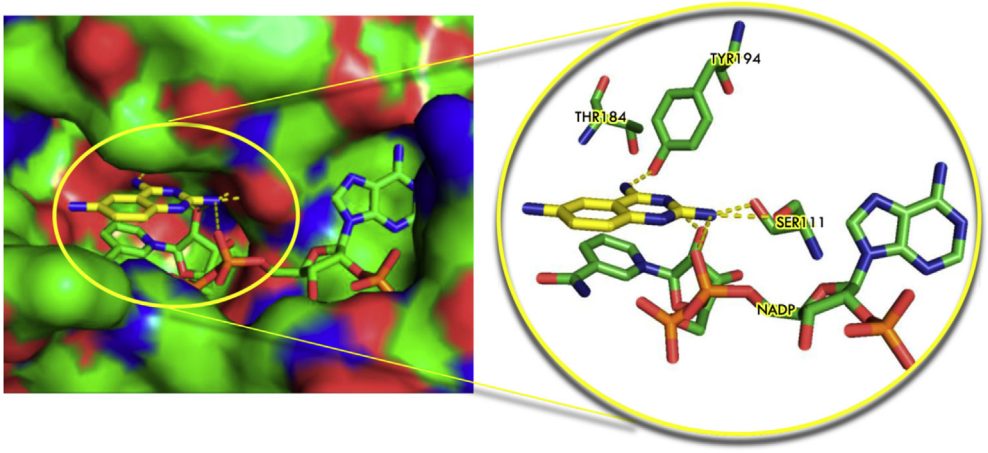


Fig. 1. Interaction of **TAQ** with PTR1 (ID-PDB: 1WOC).