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Thiadiazoles: the appropriate pharmacological scaffolds with leishmanicidal and antimalarial activities: a review

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ABSTRACT

Leishmaniasis and malaria are serious public health problems in tropical and sub-tropical regions worldwide. Development of drug-resistant strains has disrupted efforts to control the spread of these diseases in the world. The conventional antiparasitic chemotherapy still suffers from side effects and drug resistance. Therefore, the development of novel antimalarial and leishmanicidal drugs remains a critical topic to combat against these diseases. Five-membered heterocyclic systems have possessed antiparasitic activity such as thiadiazole scaffold which is a prevalent and an important heterocyclic ring. For this purpose, the authors introduce a series of synthetic thiadiazole derivatives with antileishamanial activity. Also, the authors searched a number of sources and articles to find thiadiazole derivatives with antileishamnial and antimalarial activity. Then all of the findings were reviewed. 5-nitroheteroaryl-1,3,4-Thiadiazole derivatives with different substituents at position 2 of the thiadiazole ring (8, 10-11) presented the best antileishmanial activity with low toxicity compared with reference drug. Also, 1,3,4-thiadiazole-2-sulfonamide derivative (18) showed excellent inhibitory activity against *pf*CA as a special enzyme in *Plasmodium falciparum.* Thiadiazole scaffold has the suitable physicochemical and pharmacokinetic properties and still stays as a therapeutic target for the development of a novel lead in the medicinal chemistry. Therefore, the current review provides a brief summary of medicinal chemistry of thiadiazole ring and introduces novel leads possessing this nucleus with antimalarial and antileishmanial activities.

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Introduction

In many tropical and sub-tropical regions, protozoan parasites can cause severe diseases, such as malaria, leishmaniasis, and trypanosomiasis. Due to the infection with *Plasmodium spp.*, malaria is one of the most destructive diseases (1) and transmitted to humans through the bite of female *Anopheles* mosquitoes. The disease is caused by five different species of malaria parasite from the genus Plasmodium, i.e. *P. falciparum*, *P. ovale*, *P. vivax*, *P. malariae*, and *P. knowlesi*. Among these species, *P. falciparum* is the most virulent and deadly strain. During their life cycle, *Plasmodium* parasites are dependent on 2 hosts: the sexual cycle in the *Anopheles* mosquito and the asexual cycle in the human (Figure 1). In humans, the parasites grow and multiply in the liver cells (liver stage) and then in the red blood cells (blood stage). In mosquitoes, gametocytes are picked up during a blood meal and started sexual cycle.

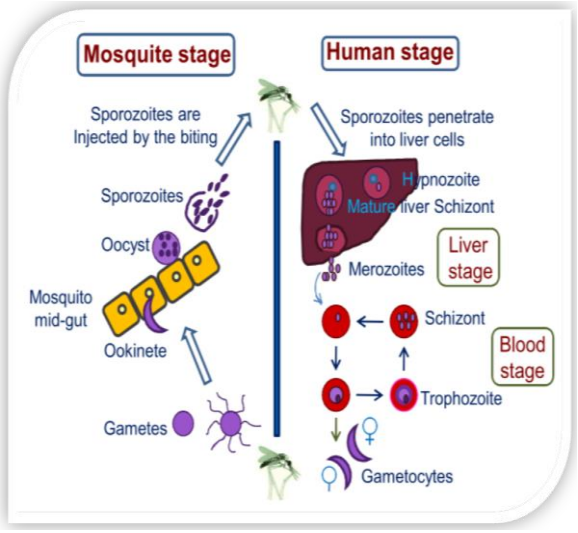

Figure 1. Life cycle of the malaria parasite

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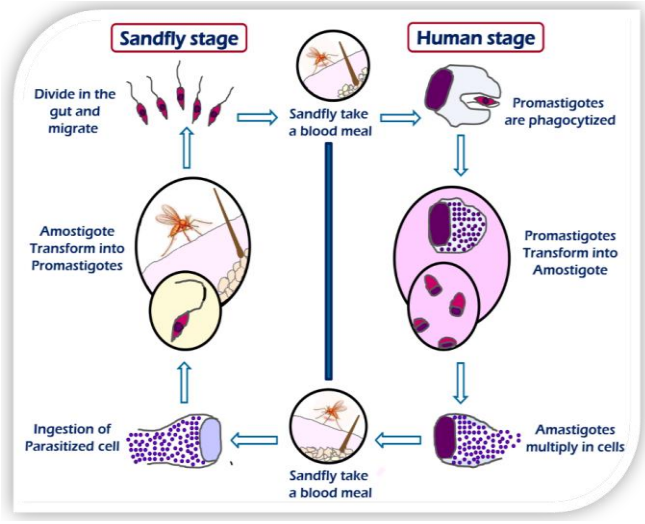

Figure 2. Life cycle of the leishmania parasite

Leishmaniasis is induced by parasitic protozoa of the genus Leishmania, and humans are infected via the bite of phlebotomine sandflies. There are three main types of the disease: visceral leishmaniasis (VL), cutaneous leishmaniasis (CL), and mucocutaneous leishmaniasis, and VL are almost always fatal if not treated (2). Leishmanial parasite has two hosts: sandfly and mammalian hosts (Figure 2). Promastigotes are delivered into human by the bite of sandfly vector. Promastigotes are transformed into amastigotes and replicated. In sandfly, parasitized macrophages are picked up during a blood meal from infected person and promastigotes are developed.

In 2015, 95 countries had ongoing malaria transmission with an estimated 3.2 billion people at risk of malaria and there were an estimated 214 million new cases of malaria and 438000 deaths. Leishmaniasis is endemic in 98 countries and territories, approximately 310 million people are at risk of contracting leishmaniasis, and 1.3 million new cases and 20,000 to 30,000 deaths occur annually (2, 3). It has been estimated that approximately 0.2 to 0.4 million new VL cases and 0.7 to 1.2 million new CL cases occur worldwide each year.

In 2015, WHO aims to launch a new technical strategy for elimination and eradication of malaria from 2016 to 2030. Accordingly, the RBM Partnership's Global Malaria Action Plan 2, will focus on the technical strategies for malaria elimination in order to reduce the incidence of infection to zero in a defined geographical area. Development of resistance to the main antimalarial drugs such as chloroquine and cross resistance of other drugs with quinolone scaffold, plasmodial dihydrofolate reductase inhibitors like pyrimethamine, and controlled use of artemisinin analogs especially as artemisinin-based combination therapy have created an urgent need to discover new antimalarial agents (2).

On the other hand, more than 90% VL that is caused by *Leishmania donovani* occurs in India, Bangladesh, Sudan, South Sudan, Ethiopia, and Brazil (3). The VL elimination program was launched in 2005, and its target was set to reduce the number of cases at level below one case per 10,000 people by 2015 (4, 5). Unfortunately, as the initial situation in 2005 and the current epidemiological situation are not well captured; many drugs currently being used to treat leishmaniasis as the antimony derivative glucantime, the bisamidines, pentamidine, or the glycol macrolide amphotericin B are old, expensive, and toxic. More importantly, resistance to the current drugs is so extensive that it threatens the success of control measures as a serious problem (6, 7). Whereas no vaccine exists against this disease, an urgent need for development of safe, inexpensive, and orally available treatments is necessary (7, 8). Unfortunately the design and synthesis of new antiparasitic drugs are difficult because in many cases, the mechanism of resistance is not fully understood. The importance of the development of novel drugs with a new mode of action against these diseases has been confirmed as a solution, and the research groups have synthesized different compounds with heterocyclic substituents and evaluated their antiparasitic activity.

The heterocyclic nuclei have special importance in medicinal chemistry, and the thiadiazole rings with four isomeric forms (1,2,3-, 1,2,4-, 1,2,5-, and 1,3,4-thiadiazole) are the versatile scaffolds in medicinal chemistry and exhibit substantial therapeutic potential (Figure 3). The mesoionic character of these five-membered heterocyclic rings by different regions of positive and negative charges enables thiadiazole rings to cross easily cellular membranes and interacts strongly with biological targets (9).

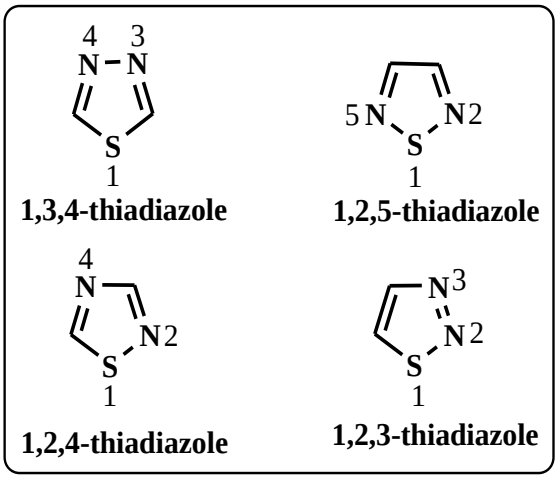

Figure 3. Isomers of thiadiazole

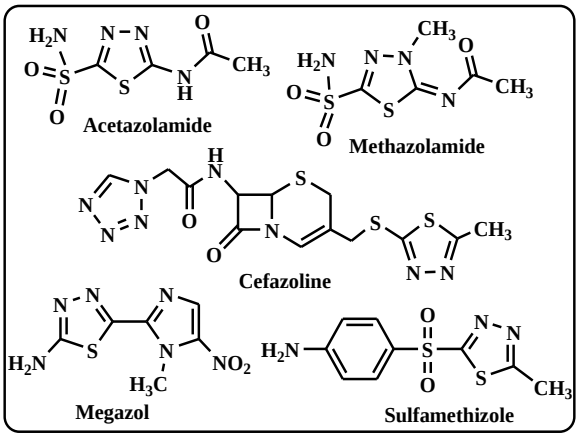

Figure 4. Structure of drugs with thiadiazole scaffold

In addition, the presence of sulfur atom causes high liposolubility as well as improves pharmacokinetic properties and the biological activity of compounds with thiadiazole ring. The synthetic compounds containing thiadiazole rings exhibit a broad spectrum of biological activities, such as antibacterial (10), anticancer (11), antiparasitic (12) and antiviral (13) actions. Moreover, there are many drugs containing thiadiazole ring in the market, such as carbonic anhydrase inhibitors, acetazolamide, and methazol amide as diuretic drugs, cefazolin as the first generation cephalosporin, sulfamethizole as an antimicrobial sulfonamide, and the antiparasitic drug, megazol (Figure 4). Regarding to the importance of this heterocyclic scaffold, here we introduce some derivatives containing thiadiazole rings with antileishmanial and antimalarial activities that synthesized by different research groups.

Leishmanicidal compounds with thiadiazole substituent

The development of drug resistance in leishmaniasis is a serious problem in some regions. Thus, the development of new drug for the treatment of leishmaniasis is imperative. Accordingly, scientists focused on compounds with different scaffold in comparison with current antileishmanial drugs.

The antiparasitic property of 1,3,4-thiadiazoles has been well recorded, and their attachment to other heterocycles scaffolds often ameliorate or diminish the bioresponses, depending on position of the attachment and the substituent type (14-29) (Figure 5, Table 1). Rodrigues *et al.* compared the effect of 4-phenyl-5-(4- or 3-*R*-cinnamoyl)-1,3,4-thiadiazolium-2-phenylamine mesoionic compounds with diversity in the nature and position of the group in the 5-cynnamoyl ring against three different species of Leishmania (*L. amazonensis*, *L. braziliensis*, and *L. chagasi*). The compound (1) with methoxy substituent at 4 position presented more potent activity against promastigotes of *L. amazonensis*, *L. braziliensis*, and

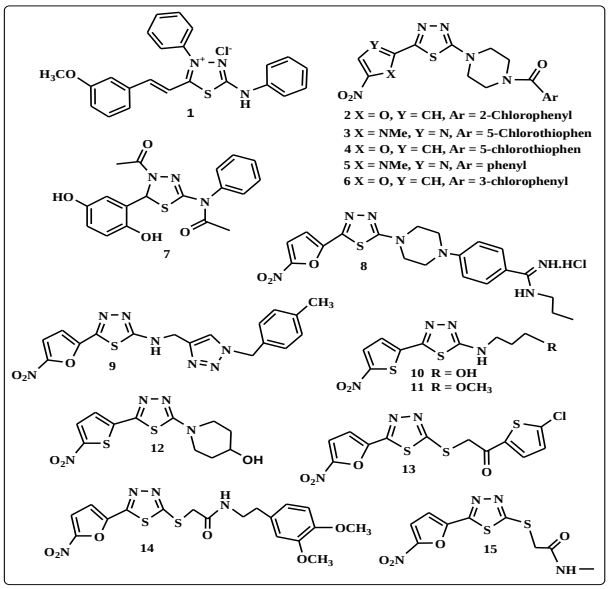

Figure 5. Antileishmanial compounds with thiadiazole scaffold

L. chagasi with ED_{50} values of 0.04, 30.64, and 4.75 µM, respectively. Also, the cytotoxicity of compound was assayed on murine peritoneal macrophages (TD_{50}= 0.61 µM) (14). The mesoionic derivatives were capable of producing nitric oxide in macrophage cultures. In continuation, Rodrigues and coworker (14) evaluated the *in vivo* efficacy of compound (1) on the *L. amazonensis* cutaneous infection in a mouse model in *L. amazonensis*-infected CBA/J mice with reference drug glucantime, which presented significant activity relative to untreated control. No apparent hepatic or renal toxicity was found in these mesoionic compounds.

A series of 1-[5-(5-nitroaryl-2-yl)-1,3,4-thiadiazol-2-yl]-4-aroylpiperazines (15) and 1-[5-(1-methyl-5-nitro-1*H*-imidazol-2-yl)-1,3,4-thiadiazol-2-yl]-4-aroylpiperazines (16) were synthesized. Compounds 2-5 exhibited potent activity (IC_{50} =10.73, 9.35, 11.75, and 10.39 µM, respectively) against *L. major* promastigotes of vaccine strain. These compounds were also assessed for their activity against the amastigote form of *L. major* in marine peritoneal macrophages, and compounds 3-5 were found to be more efficient in reducing the infectivity indices and the percentage of infected macrophages (17). Also, compounds 3 and 4 had more efficient activity against intracellular form of the parasite with IC_{50} values of 2.7 and 2.8 µM for amastigote form and selectivity index (SI: macrophage IC_{50}/intracellular amastigote IC_{50} > 14.7). However, compounds 2 and 5 (with IC_{50} value of 8 and 4.2 µM for amastigote form, respectively) showed a higher SI (28.6 and 31.9, respectively). The results indicated that these compounds in the IC_{50} concentrations (against promastigote form of the *L. major*) were not remarkably toxic towards marine peritoneal macrophage cells. In addition, three wild-type strains of Leishmania were treated with these analogs and

Table 1. *In vitro* activity, cytotoxicity and selectivity index of the thiadiazole derivatives against *leishmania major* promastigote and amastigote

Comp*	IC_{50} (µM) Promastigote form of *L. major*	SI** (promastigote form)	IC_{50} (µM) Amostigote form of *L. major*	SI** (amostigote form)	CC_{50}*** (µM)	Ref
2	10.73	7.5	8	28.6	80	
3	9.35	>10.7	2.7	>14.7	> 100	
4	11.75	>8.5	2.8	>14.7	> 100	16, 18
5	10.39	8.3	4.2	31.9	86	
6	13.19	> 7.6	6.8	>14.7	> 100	
8	0.08	78.5	-	-	785	21
9	12.2	-	-	-	-	25
10	3	14.05	-	-	42.16	26
11	3	12.60	-	-	37.8	
12	27	2.35	-	-	63.55	27
13	114.98	-	-	-	-	28
14	19.1	2.93	-	-	55.95	29
15	19.5 µM	3.06	-	-	59.72	

*The compound (1) showed anti- promastigote activity against of *L. amazonensis*, *L. braziliensis*, and *L. chagasi* with ED_{50} values of 0.04, 30.64, and 4.75 µM (14). The compound (7) had IC_{50} value of 0.495 µM against *L. donovani* (19)
**SI = Selectivity Index (CC_{50}/IC_{50}-24 hr)
*** CC_{50} = cytotoxic concentration for 50% inhibition

evaluated by MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) assay. The compounds 2, 3, and 6 with IC_{50} values of 8.34, 11.68, and 9.54 µM were the best compounds against *L. tropica*, *L. major* and *L. infantum*, respectively. The mechanism of action of these 5-nitroaryl analogs of N-substituted-piperazinyl-1,3,4-thiadiazoles was studied against Leishmania species by Poorrajab *et al* (17). The findings showed that at least a part of leishmaniacidal effect of the compounds could be attributed to disruption DNA-relaxed activities of topoisomerases I and II and cleavable complex formation. The analog 3 bearing 5-chlorothiophen-2-ylcarbonyl substitution and the analog 5 containing benzoyl substitution on the piperazine ring displayed the most *L. major* Top I catalytic activity at their IC_{50} dose with 62 and 73% inhibition activity, respectively. However, compound 4 was the most potent inhibitor against *L. major* kinetoplast Top II with 83% inhibition activity.

These effective compounds were investigated further by the measurement of acid phosphatase (AcP) activity as an indicator of cell viability in the promastigotes with calorimetric assay (18). AcP is an essential enzyme for survival of the parasite, and AcP assay of *L. major* promastigotes showed that 1,3,4-thiadiazole derivatives were more effective on stationary than logarithmic phase. Also, active compounds (2-6) displayed activity (AcP activity of control sample/AcP activity of drug-treated sample) ≥ 2.0 for logarithmic phase and ≥ 2.9 for stationary phase. Nevertheless, reference drug (glucantime) showed lower effect on stationary phase.

Al-Qahtani *et al* .(19) synthesized and tested a series of 1,3,4-thiadiazole analogs for their antileishmanial activity against *L. donovani* promastigotes. The most active compound was (7) at low concentration (50 µM) with IC_{50} value of 0.495 µM. However, the reference drug, amphotericin B, at this concentration showed IC_{50} value of 0.381 µM. Different mechanisms have been reported about antileishmanil activity of 1,3,4-thiadiazole analogs. For example, these compounds could permeate through the cell plasma membrane and damage nucleic acids and/or proteins inside the cell (20). Also, the antileishmanial effect of these compounds could be related to the functional groups capable of formation of free radicals such as 1,4-dihydroxyphenyl group. On the other hand, these compounds may apply antiproliferative effects through the production of nitro radicalanions.

A novel set of nitrofuryl-1,3,4-thiadiazoles containing piperazinyl-benzamidine substituents were synthesized and evaluated for their *in vitro* antileishmanial effect against both the amastigote and promastigote forms of *L. major* (21). The outcomes indicated that the propyl substitution on the benzamidine group in compound (8) prepared the best effect in 72 hr with IC_{50} value of 0.08 µM against promastigotes of *L. major*. The compound also displayed a CC_{50} value of 785 µM whith the highest SI=78.5 against macrophages which confirm low level of toxicity. Also, the compounds having benzyl and butyl substitutions presented remarkable activity against amastigotes form of *L. major* and significantly decreased the number of amastigotes per macrophage, the percentage of macrophage infectivity and infectivity indices. Furthermore, 1,3,4-thiadiazole analogs might exert their effect through the modification of sulfhydryl groups of cysteine residues in some essential enzymes and other important proteins as shown to be strong sulfhydryl-modifying agents (22). Marznaki and coworkers showed AcP activity of nitrofury1-thiadiazoles containing benzamidine substituents (23). Active

compound (8) decreased the activity of both logarithmic and stationary promastigotes to 3.6 and 2.3, respectively. Also, the activity of these piperazinyl-benzamidine analogs (21) on reactive oxygen species production in lymphocyte and the induction of nitric oxide in macrophage were evaluated, and the results exhibited a decrease in the viability of the parasite and an increase in reactive oxygen species and nitric oxide production in lymphocyte and macrophage, respectively. Furthermore, these compounds could stimulate parasite killing using two methods, by directly decreasing the parasite viability and by indirectly displaying a considerable increase in immune system.

Antileishmanial activity of 5-(5-nitroheteroaryl-2y1)-1,3,4-thiadiazole derivatives were predicted based on quantitative structure activity relationship (QSAR) analyses (MLR- and ANN- models) (24). Both models were found to be successful in predicting the activity of these compounds. Also, molecular modeling studies were conducted based on DNA topoisomerase I (TOP I) as a target enzyme. The result presented hydrogen bonding and hydrophobic interactions between the ligands and the binding site of *L. major* TOP IB. Therefore, the potent antileishmanial activity of the thiadiazole derivatives was related to inhibit *L. major* TOP IB by these interactions.

Also, a new set of 5-(5-nitrofuryl)-1,3,4-thiadiazole containing benzyltriazolylmethyl moiety via click chemistry was synthesized (25). Most derivatives showed promising leishmanicidal effect against *L. major* promastigotes. and 4-methyl benzyl analog (9) was the best compound against promosti-gotes with IC_{50} value of 12.2 μM.

The other series of 5-(5-nitroaryl-2-yl)-1,3,4-thiadiazole-2-amines bearing acyclic amine at C-2 position of thiadiazole ring were evaluated *in vitro* against promastigote and amastigote forms of *L. major* (26). The structure-activity relationship studies of the compounds exhibited that 5-(5-nitrothiophen-2-yl)-1,3,4-thiadiazole analogs with hydroxylpropyl- and methoxypropyl substituents (analogs 10 and 11) were the most active derivatives with IC_{50} values of 3 μM compared to the reference drugs; glucantime (IC_{50} = 68.44 mM) and fluconazole (IC_{50}= 941.1 μM). The toxicity of compounds was assessed against mouse peritoneal macrophages, and the most active leishmanicidal derivatives (10 and 11) exhibited tolerable toxicity with CC_{50} value of 42.16 and 37.8 μM against macrophages, respectively and also the best selectivity index (SI >12). Analog of compound 10 containing cyclic amine was synthesized and exhibited less antipromastigote and antiamastigote activity with IC_{50} value of 27 μM (27). The experimental and computational studies confirm lower activity of the derivative (12) compared with its analog (10) due to steric hindrance.

Adibi *et al.* (28) synthesized novel derivatives of 1-(5-halo-2-thienyl)-2-[5-(5-nitroheteroaryl)- 1,3, 4-thiadiazol-2-ylthio)ethanone. The results indicated that the nitrofuran analog (13) with 5-chlorothiophen-2-yl substitution was the best compound against promastigote form of *L. major* (IC_{50}= 114.98 μM) as compared with fluconazole (FLUCZ) as a reference drug (IC_{50}= 980 μM). It is remarkable that the nitrofuran analogs possess more activities than FLUCZ. Vosooghi and coworkers (29) prepared a novel series of 2-(5-(5-nitrofuran-2-yl)-1,3,4-thiadiazol-2-ylthio)acetamides and assayed against extracellular promastigotes of *L. major*. The antipromastigote activity of these derivatives was extremely depen-dent on the type of substitutes positioned on the nitrogen of acetamides. In addition, the most potent compounds against the promostigotes were *N*-(3,4-dimethoxyphenethyl)-2-(5-(5-nitrofuran-2-yl)-1,3,4-thiadiazol-2-ylthio)acetamide (14) and 2-(5-(5-nitrofuran-2-yl)-1,3,4-thiadiazol-2-ylthio)-*N*-propyla cetamide (15) with 3,4-dimethoxyphenethyl and methyl moiety on the nitrogen of acetamides and IC_{50} values of 19.1 and 19.5 μM, respectively. The *in vitro* cytotoxicity of these derivatives against mouse peritoneal macrophages exhibited CC_{50} values of 55.95 and 59.72 μM, respectively, with the highest SI of 2.93 and 3.06. These compounds were also evaluated against three human cancer cell lines and methyl analog 14 displayed the maximum activity against HepG2 cells with IC_{50} values of 10.43 μM. The QSAR study of these compounds indicated that topological and two-dimensional auto-correlation are influential parameters in the antipromastigote activity. It is noticeable that compounds 9 - 11, 13 and 15, that were the most effective derivatives against promastigotes, also showed potent activity against amastigotes in various terms (% macrophage infectivity, infectivity index, and number of amastigotes per macrophage).

As a result, these research projects indicated that 1,3,4-thiadiazole analogs are promising as anti-leishmanial agents and provided useful models for further structural optimization. The best compound (8) containing 5-nitrofuran thiadiazole with propyl benzamidine substituent showed IC_{50} value of 0.08 μM, 72 hr after treatment against promastigotes of *L. majo*r with low toxicity against macrophages. Whereas, the compounds (10-11) containing 5-nitrothiophen thiadiazole with propanol and methoxypropyl substituent showed IC_{50} value of 3 μM, 24 hr after treatment which is a considerable point. On the other hand, the compounds 5 with nitro imidazole substituent (IC_{50} value of 4.2 μM for amastigote form) showed a highest SI (31.9). The compound was not remarkably toxic towards marine peritoneal macrophage cells. It highest SI (31.9). The compound was not remarkably toxic towards marine peritoneal macrophage cells. It

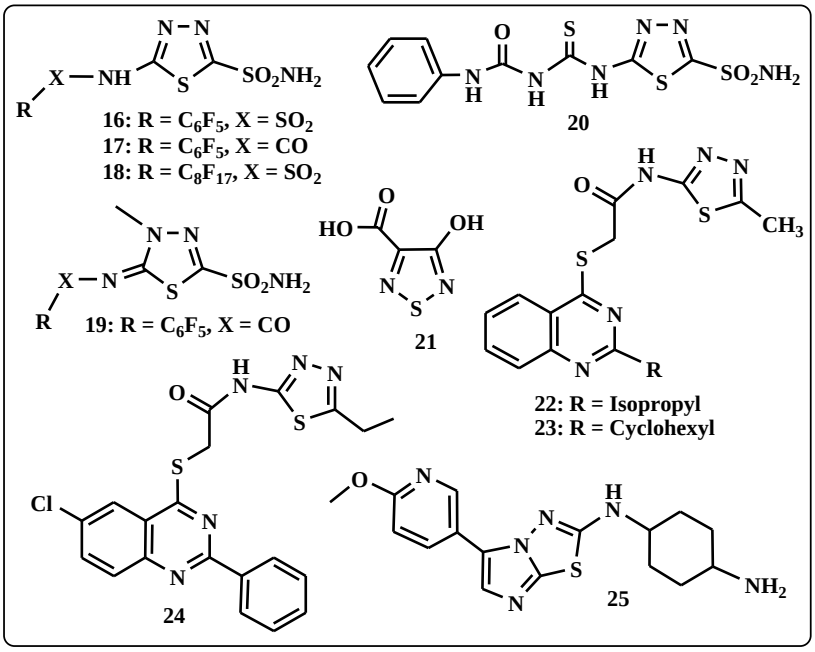

Figure 6. Antimalarial compounds with thiadiazole scaffold

seems that, these compounds have suitable potential for the next development stages.

This is noticeable that there are different methods for synthesis of thiadiazole derivatives but all these methods have some drawbacks such as low yield, long reaction period, and use of toxic organic solvents which are harmful for environment. Therefore, high cost of synthesis route, moderate toxicity and side effects are the most important disadvantage of these derivatives and further research is needed to improve these weakness in the future.

Antimalarial compounds with thiadiazole substituent

The current antimalarial drugs have different mode of actions (30). 4-aminoquinoline (CQ and AQ) and aryl-amino alcohol drugs (MQ and HAL) inhibit the heme polymerization. Artemisinin and its derivatives also interact with heme or iron by their endoperoxide bond. While, the antifolate drugs inhibit either dihydropteroate synthase (DHPS) (sulfadoxine) or dihydrofolate reductase (DHFR) (cycloguanil, pyrimethamine). Today, drugs resistant variants showed reduced affinity toward anti-malarial drugs. Actually, drug resistance is consider-ed as an important global health problem in malaria prophylaxis and treatment. In this situation, the development of new, cheap, efficient, and safe drugs for the treatment of this disease is imperative.

Recently, the research groups have focus on the synthesis of new antimalarial compounds especially with a different mechanism of action compared with current drugs (Figure 6, Table 2). For instance; enzymatic activity of carbonic anhydrase in *P. falciparum* and in the mouse parasite *P. berghei* has been demonstrated in various studies (31-33). Krungkral *et al.* evaluated acetazolamide as a standard carbonic anhydrase inhibitor (CAI) (Figure 4). Acetazolamide showed weak antimalarial activity for monitoring growth of *P. falciparum* in *in vitro* (IC_{50} value of 20 µM) (30) and a strong *PfCA* inhibitory activity with K_I of 0.32 µM (31). When the human red cells were pretreated with acetazolamide, the host enzyme activity wholly abrogated, present no pronounced effect on the parasite invasion. On the other hand, *in vivo* studies showed complete inhibition of *P. berghei* carbonic anhydrase (*pb*CA) by acetazolamide. Whereas, it was ineffective against mice infected with *P. berghei*.

Indeed, the *P. falcipaum* carbonic anhydrase (*pf*CA) is one of the enzymes that involve in the sequential steps in the path–way leading to DNA/RNA synthesis in Plasmodium. Furthermore, the inhibitors of *pf*CA can prevent this biosynthetic pathway with different mechanism compared with current antimalarial drugs. Krungkrai *et al.* (2008) has reported the *in vitro pf*CA inhibition studies with a library of sulfonamide compounds. In this study 1,3,4-thiadiazole- and 1,3,4-thiadiazoline-2-sulfonamide derivatives 16-20 exhibited the inhibition of *pf*CA with *Kıs* in the range of 0.192–6.867 µM (33). The variation of the inhibitory activity of these compounds depends on different substitution on thiadiazole ring. For instance, in the thiadiazoline 19 (K_I = 6.867 µM), substituted methyl group on thiadiazole ring has been led to a two-fold increase in the inhibitory activity of 19 compared to 17 (K_I = 3.441 µM). Also,

Table 2. *In vitro* activity and mode of action of the thiadiazole derivatives against *Plasmodium falciparum*

Comp*	IC_{50} , EC_{50} µM				Mode of action	Ref
	3D7	K1	W2	NF54		
21	75.3	144	-	-	Inhibition of *pf*LDH	34
22	-	-	> 10	-		
23	-	-	>10	-	Inhibition of FP	35
24	-	-	>10	-		
25	297	-	-	284	-	36

*The compounds (16-20) showed the inhibitory activity against *pf*CA with K_I values of 2.583, 3.441, 0.192, 6.867 and 6.730 µM, respectively (33). EC_{50} value was calculated for the compound (25)

replacement of the perfluoro-phenyl sulfonyl moiety of compound 16 (K_I = 2.583 µM) with the corresponding perfluoro-phenyl carbonyl moiety present in compound 19 resulted in increased inhibitory activity 19 (K_I = 6.867 µM) compared to 16. In fact, compound 18 (K_I = 0.192 µM) with perfluoro-octyl sulfonyl moiety was the most potent *pf*CA inhibitor with thiadiazole scaffold in compared with acetazolamide as a standard carbonic anhydrase inhibitor with a K_I of 0.315 µM (33). Compound 20 (K_I = 6.730 µM) possessing phenyl ureido-thiourea moiety showed a less effective inhibitor than compounds 16-18. In the following, all compounds indicated low *ex vivo* anti *P. falciparum* activity with IC_{50} > 50 µM when compared with reference drug acetazolamide, which inhibited the *ex vivo* growth of *P. falciparum* with an IC_{50} of 20.0 µM. As a result, maybe other analogues of these compounds can have potential in development of novel antimalarial drugs because of inhibiting malaria parasite carbonic anhydrase, which is the first step of pyrimidine nucleotide biosynthesis.

P. falciparum lactate dehydro-genase (*pf*LDH) can consider as another target for antimalarial drug discovery. The parasite *P. falciparum* lacks a functional Kreb's cycle (34). Therefore, for energy production, it is dependent on glycolysis. LDH is the latest enzyme in the glycolytic pathway in *P. falciparum* that converts pyruvate to lactate and simultaneously converts NADH to NAD^+. Moreover, this enzyme can be considered as a potential molecular target for antimalarial drugs as selective inhibitors of *pf*LDH. The inhibition of LDH is expected to stop the production of adenosine triphosphate, and subsequently *P. falciparum* cell death. Cameron and co-workers (34) synthesized a new class of heterocyclic, azole-based compounds, as selective inhibitors of the *pf*LDH that exhibited antimalarial activity. Using high-throughput enzymatic assay of several compounds, they found that azoles family was active against *pf*LDH, among thiadiazole compound including 3-hydroxyl and 4-carboxyl moieties. X-ray crystallographic and steady-state kinetic analyses showed the interaction of these compounds with *pf*LDH. The crystallographic analysis of enzyme-thiadiazole inhibitory complex between *pf*LDH and 4-hydroxy-1,2,5-thiadiazole-3-carboxylicacid (TDA1, 21) showed an amino acid interaction network at the active site of *pf*LDH, such as a hydrogen bond between sulfur atom in the thiadiazole ring with the side chain at serin 245 of enzyme. It also demonstrated that the formation of hydrogen bonds between carboxyl and hydroxyl groups with argenins 109 and 171 from the enzyme is vital for inhibition activity.

The study of antimalarial activity of compound (21) against *P. falciparum*-infected erythrocytes in both K1 chloroquine-resistant (IC_{50} value of 144 µM) and 3D7 chloroquine-sensitive (IC_{50} value of 75.3 µM) strains was parallel with its activity against *pf*LDH with IC_{50} value of 0.14 µM (34). Activity of compound (21) against the human LDH (*hs*LDH) isoforms showed IC_{50} value of 10.27 µM. The selectivity of this compound against *hs*LDH is rather less than malaria LDH. However, the inhibitory activity of these azole-based compounds was measured in a dose-response experiment against *P. berghei* in BALB/c mice, and TDA1 (21) showed the efficacy in killing the parasite. Although, TDA1 (21) was less effective than the chloroquine as a control drug. In the pharmacokinetic studies (34), TDA1 (21) has been exhibited to have good plasma level at 30 min after oral administration with a peak level of 35.2 µg/ml and after IP administration with peak level of 41.0 µg/ml. However, its half-life was short in the circulation that justifies the lack of complete suppression of parasitemia at dose of 100 mg/kg/day with different route of administration. The complex of *Pf*LDH with NADH and TDA1 has been also reported with PDB ID:1T26 (34).

For the development of antimalarial chemotherapy with different mode of action, researchers also focused on Falcipains (FPs) as validated targets. The hemoglobinases of *P. falciparum*, which are called FP, degrade hemoglobin during the blood stage of *P. falciparum* and lead the parasite growth. With composition of structure and ligand - based virtual screening, a set of 28 potent inhibitors of FP were recognized, then in each sets were carefully assessed their structure-activity relationship (35). Analogs with the quinazoline core and thiadiazole substitution

exhibited inhibitory activity against FP II. The type of substitution at position R_2 in the quinazoline ring is important for its activity. With aliphatic substitutions at this position, the activity of compound 22 was maintained (FP II IC_{50}= 5.93 µm). However, cyclohexyl substitution in compound 23 resulted in a reduced activity against FP II FP II IC_{50} > 50 µm) in comparison to its analog (22). These compounds were also evaluated against the *P. falciparum* CQ-resistant strain W2 and showed IC_{50} values of > 10 µM against cultured parasites. The compound 24 with chlorine insertion at position 6 and phenyl at position 2 in quinazoline ring didn't show any activity against FP II at a test concentration up to 50 µM and presented a weak affinity (>10 µM) against W2 strain of *P. falciparum*.

A large chemical library has been assessed against blood-stage *P. falciparum* with growth inhibition assay for oral drug discovery (36). Originally, *in vitro* assays (cytotoxicity, dose-response, and spot test) as well as *in silico* and *in cerebro* analyses are involved in the choice and the validation of novel antiplasmodial hits. The chemical properties and the diversity of a set of hits were recognized using the computational analysis. Eventually, new chemotypes with favorable properties were synthesized and evaluated by a *P. falciparum* growth inhibition assay. At first, the screening campaign caused to the selection of 178 compounds with EC_{50} < 1 µM and selectivity >10. Cluster 25 with a imidazo-[2,1-b]-[1,3,4]-thiadiazole core and its six analogs were selected from 178 compounds. This cluster showed EC_{50} values of 297 and 284 against 3D7 and NF54 strains of *P. falciparum*, respectively and also indicated good physicochemical properties.

As a result, these researches showed that the synthesized compounds had moderate activity against *P. falciparum*. Meanwhile, the compound (18) showed excellent inhibitory activity against *pf*CA compared to acetazolamide (33). This thiadiazole analog have excellent potential in development of novel antimalarial drugs because of inhibiting malaria parasite carbonic anhydrase as a key enzyme in the first step of pyrimidine nucleotide biosynthesis. Although, continuing research on the improving of activity is needed for this analoge. This is considerable that the analog has different mechanism of action compared with current antimalarial drugs which can be its advantage relative to compounds with similar mechanism.

Nevertheless, with all advantages of thiadiazole scaffold in medicinal chemistry, their toxicity still remains a major concern and further exploration on desired synthesis methods is necessary which contribute to development of new drugs.

Conclusion

Malaria and leishmaniasis are serious public health problems in the new world. With considering drug resistance development globally, the achievement of novel, inexpensive, efficient, and safe drugs for the treatment of these parasitic diseases is imperative. On the other hand, delay in the treatment of these diseases can cause serious or even fatal consequences, especially in children and pregnant women as sensitive groups, who should receive drugs without any side effect. Moreover, new drugs should be effective against different drug-resistant strains to resolve the problem of drug resistance. In this field, the various research groups have focused on designing and synthesis of new compounds with a novel mode of action to overcome this problem. Substituted five-member heterocyclic rings have diverse pharmaceutical activities. Thiadiazole rings are one of the most important classes of these heterocyclic scaffolds in medicinal chemistry. Thiadiazole derivatives possess a versatile type of biological activities, such as analgesic, antiviral, antiparasitic, antidepressant, antifungal, anti-microbial activities and so on. Due to the suitable chemical, physical and pharmacokinetic properties of thiadiazole, this scaffold still stays as a therapeutic target for the development of a novel lead in the medicinal chemistry, and it is one of the best scaffolds despite of the increasing levels of drug resistance in the today's world. However, more detailed studies are required to confirm quality of the thiadiazole derivatives as the new class of antiparasitic agents. Also, novel drug delivery systems can be used to improve antiparasitic activity of these thiadiazole derivatives in the future studies.

Moreover, the design of new drugs with this appropriate heterocyclic nucleus is a good candidate for parasitic diseases such as leishmaniasis and malaria to achieve elimination and possibly eradication of these diseases.

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