

## 2-Amino-1,3,4-thiadiazoles as prospective agents in trypanosomiasis and other parasitoses

GEORGETA SERBAN

*Pharmaceutical Chemistry  
Department, Faculty of Medicine  
and Pharmacy, University of Oradea  
410028 Oradea, Romania*

Parasitic diseases are a serious public health problem affecting hundreds of millions of people worldwide. African trypanosomiasis, American trypanosomiasis, leishmaniasis, malaria and toxoplasmosis are the main parasitic infections caused by protozoan parasites with over one million deaths each year. Due to old medications and drug resistance worldwide, there is an urgent need for new antiparasitic drugs. 1,3,4-Thiadiazoles have been widely studied for medical applications. The chemical, physical and pharmacokinetic properties recommend 1,3,4-thiadiazole ring as a target in drug development. Many scientific papers report the antiparasitic potential of 2-amino-1,3,4-thiadiazoles. This review presents synthetic 2-amino-1,3,4-thiadiazoles exhibiting antitrypanosomal, antimalarial and antitoxoplasmal activities. Although there are insufficient results to state the quality of 2-amino-1,3,4-thiadiazoles as a new class of antiparasitic agents, many reported derivatives can be considered as lead compounds for drug synthesis and a promise for the future treatment of parasitosis and provide a valid strategy for the development of potent antiparasitic drugs.

*Keywords:* 2-amino-1,3,4-thiadiazoles, antiparasitic activity, antitrypanosomal activity, antimalarial activity, antitoxoplasmal activity, inhibitory concentration

Accepted October 24, 2019  
Published online November 18, 2019

### INTRODUCTION

Human parasitic diseases are caused by organisms of different sizes and shapes, from unicellular organisms (protozoa) to large-sized worms. Amoebiasis, giardiasis, leishmaniasis, malaria, toxoplasmosis, trichomoniasis, trypanosomiasis, scabies, pediculosis and helminth infections are some of the many types of human parasitic infections that can lead to host's illness or death. Contaminated food or water, insect bites, and domestic animals are involved in the transmission of parasites (1, 2). Parasitic diseases are a serious public health problem affecting hundreds of millions of people worldwide. Most of these diseases are common in low and middle-income countries of tropical and subtropical areas, among the indigent with limited treatment means. In such situations, the cost of health care is the dominant factor in treating the patient. The combination of climate and poverty contributes to the transmission of parasitic infections in these regions (2).

---

\* Correspondence, e-mail: getaserban\_2000@yahoo.com

Some of these illnesses have become known as “neglected tropical diseases” (NTDs). NTDs represent a group of chronic diseases caused by bacteria, viruses, protozoa and helminths. Most of them are parasitic infections that can last for years or even decades and have a high prevalence in developing countries of Africa, Asia and Latin America (3, 4). NTDs can cause severe pain and long-term consequences (*e.g.*, blindness, disfigurement, deformity, cancer, neurological problems) (5). Multiple infections in a single individual are common (3). In 2005, the World Health Organization’s (WHO) NTDs department was established. One year later, the United States Agency for International Development launched its NTDs program (6). Tropical diseases are currently issues of global concern. Malaria, leishmaniasis and trypanosomiasis are the protozoan parasitic diseases that are targeted for prevention, control, elimination and eradication by the WHO’s Division of Control of Tropical Diseases (7).

Nitrogen-containing heterocycles are found in a wide range of natural products and biologically active synthetic compounds (8–15). In addition, nitrogen-containing heterocycles constitute a common structural unit of many marketed drugs (16, 17). There are five small molecules containing nitrogen-heterocycles (*i.e.*, lisinopril, atorvastatin, amlodipine, omeprazole and losartan) ranked in the top ten of the 200 most prescribed drugs in the US in 2019 (Fig. 1) (18, 19). Moreover, nitrogen-containing heterocycles are found in many drugs used for the treatment of parasitic diseases. The nitroimidazole derivatives, metronidazole and tinidazole, are widely known as antibacterial and antiprotozoal medications. These derivatives are very effective for giardiasis, trichomoniasis, amoebic liver abscesses, amoebic dysentery and anaerobic bacterial infections (20). Albendazole, mebendazole, levamisole and pyrantel pamoate are the main drugs used for the treatment of soil-transmitted helminthiases, the benzimidazole derivatives albendazole and mebendazole being the most commonly used drugs during the helminths infection prevention campaigns (Fig. 2) (21). Antimalarial drugs have been intensively studied over the last few decades. Although the artemisinin-based combination therapy (ACT) is currently the main strategy for treating malaria, drugs such as chloroquine (1), primaquine (2), natural-occurring

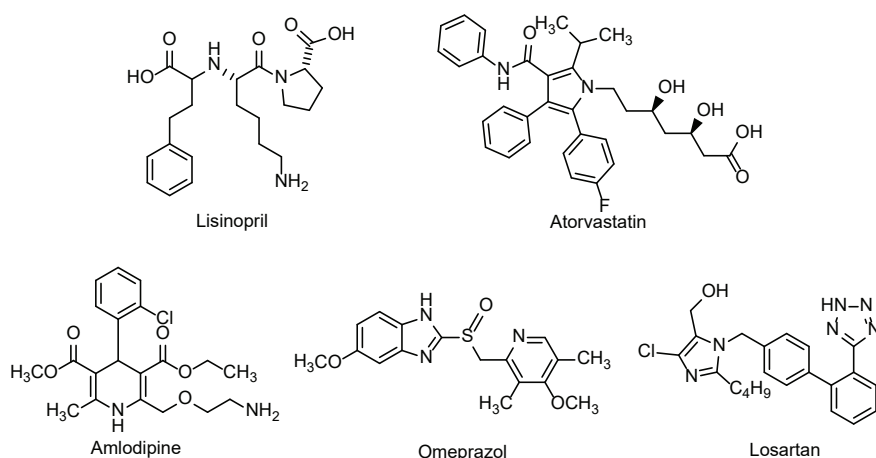


Fig. 1. Nitrogen-heterocyclic drugs ranked in the US top ten of the most prescribed drugs in 2019 (18).

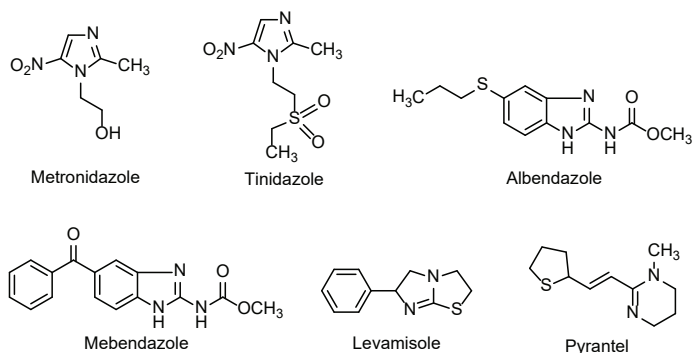


Fig. 2. Nitrogen-heterocyclic drugs used in the treatment of parasitosis.

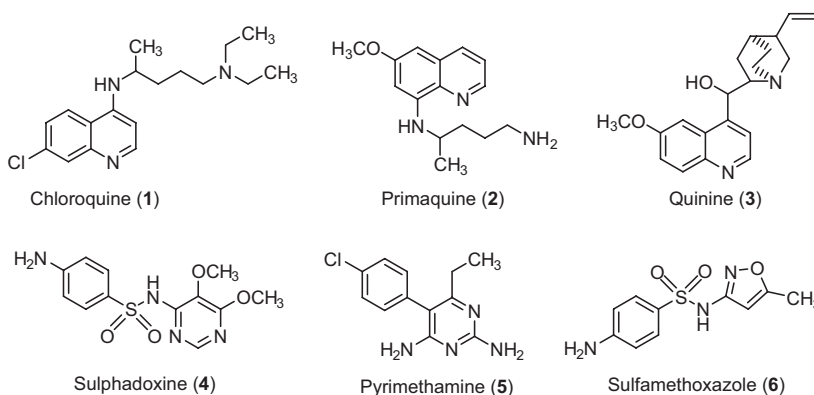


Fig. 3. Nitrogen-heterocyclic drugs used in the treatment of parasitosis.

quinine (3), sulfadoxine (4) or pyrimethamine (5) still retain their value when ACT is not available (21, 22). In addition, pyrimethamine (5) in combination with sulfonamide derivatives such as sulfamethoxazole (6) is a good treatment for toxoplasmosis (Fig. 3) (23).

Heterocycles containing both nitrogen and sulfur atoms are an important class of compounds in medicinal chemistry due to their interesting and wide biological applications (24). Five-membered nitrogen heterocycles, such as thiadiazole, are known as biologically active compounds. Among the four thiadiazole isomeric forms (*e.g.*, 1,2,3-thiadiazole, 1,2,4-thiadiazole, 1,2,5-thiadiazole and 1,3,4-thiadiazole), 1,3,4-thiadiazole ring is the most prevalent in medically significant synthetic compounds. A number of 1,3,4-thiadiazole-containing drugs are currently on the market: acetazolamide and methazolamide as carbonic anhydrase inhibitors, cefazolin, cefazedone and sulfamethizole as antimicrobial drugs, and megalol – a known antitrypanosomal agent (25–27).

The 1,3,4-thiadiazole moiety is also found in derivatives that have demonstrated biological activity and are currently under investigation. BMS-341 (7) is a glucocorticoid receptor modulator with improved pharmacokinetic properties, which has shown oral

activity in a chronic model of adjuvant-induced arthritis in rats, may have potential in treating human disease as a replacement for traditional glucocorticoid medicines (28, 29). 1-[5-(6-Bromopyridin-2-ylamino)-1,3,4-thiadiazol-2-yl]-1-(4-methylthiazol-2-yl)ethanol (**8**) is a potent nanomolar inhibitor of the enoyl acyl carrier protein reductase (InhA) which showed excellent antimycobacterial activity *in vitro* and promising *in vivo* efficacy against *Mycobacterium tuberculosis* strains (Fig. 4) (29, 30).

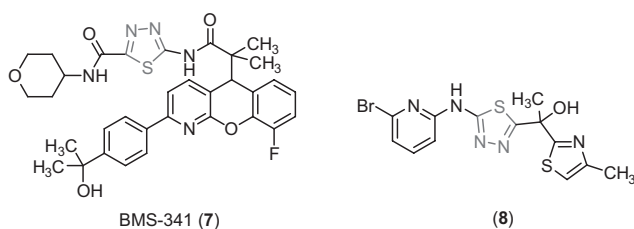


Fig. 4. New 1,3,4-thiadiazole derivatives under biological investigation.

In the last two decades, the number of scientific publications concerning the synthesis and biological investigation of 1,3,4-thiadiazoles has considerably increased (27). A look at the reference works shows that 1,3,4-thiadiazole has been investigated more than the other isomers. Indeed, 1,3,4-thiadiazole derivatives have been widely studied for medical, agricultural and industrial applications (1, 16, 31, 32).

The rationale behind drug design is to incorporate heterocycles with favorable physicochemical properties into the structure of a biologically active molecule. Lipophilicity, polarity and water solubility are properties that can be improved and can influence the possible mechanisms of action of biologically active compounds (17). In addition, the bioisosteric replacement of a homocyclic ring with a heterocycle makes possible the synthesis of different analogs that interact more with the receptors (27). The 1,3,4-thiadiazole ring is a weak base due to the inductive effect of the sulfur atom and possesses relatively high aromaticity although lower compared to 1,2,5-thiadiazole due to the relative positions of the heteroatoms in the ring (32, 33). It is thermally stable and is relatively stable in acidic aqueous solutions but can undergo ring cleavage under basic conditions. The ring is also very electron-deficient due to the electron-withdrawing effect of the nitrogen atoms and relatively inert to electrophilic substitution but susceptible to nucleophilic attacks (32). The sulfur atom increases lipophilicity and offers great stability to the three-dimensional structure within the molecule (24). 1,3,4-Thiadiazoles carrying mercapto, hydroxyl and amino substituents can exist in tautomeric forms. On the other hand, 1,3,4-thiadiazole derivatives can make mesoionic systems (34). The mesoionic character of thiadiazoles gives good oral absorption

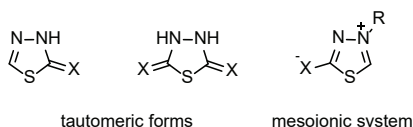


Fig. 5. Tautomeric forms and mesoionic systems of 1,3,4-thiadiazole derivatives.

and good cell permeability, resulting in good bioavailability. Moreover, the mesoionic nature of 1,3,4-thiadiazoles enables these compounds to have strong interactions with biomolecules (e.g., DNA, proteins) (Fig. 5) (1, 27, 35).

2-Amino-1,3,4-thiadiazole and certain structurally related compounds have been known for 50 years as compounds with antitumor activity (24, 25, 36–44). Some compounds in this class also exhibit uricogenic properties (24, 45). Both antitumor and uricogenic activities can be prevented or reversed by nicotinamide (24, 46, 47). The cytostatic properties of 2-amino-1,3,4-thiadiazole and the antitrypanosomal activity of megalzol are evidence of the biological potential of a 2-amino-1,3,4-thiadiazole moiety (27). In addition, many papers present 2-amino-1,3,4-thiadiazoles exhibiting antibacterial, antifungal, antimycobacterial (27) and antileishmanial activities (48). Moreover, due to the structural properties of 2-amino-1,3,4-thiadiazoles, they can participate in several chemical reactions and have significant use as intermediates for the synthesis of nitrogen-containing heterocyclic compounds (24). Due to our interest in the synthesis and biological activities of thiadiazoles (27, 48–54), the purpose of this review is to highlight some antiparasitic properties exhibited by derivatives having the 2-amino-1,3,4-thiadiazole moiety in their structure.

#### SOME PARASITOSEs: STATE OF THE ART AND ANTIPARASITIC ACTIVITIES OF 2-AMINO-1,3,4-THIADIAZOLE SYSTEM

##### *Antitrypanosomal activity*

*Trypanosoma parasites and trypanosomiases.* – Eighteen NTDs have been identified by the WHO (55). It is estimated that over 1 billion people are infected with NTDs, with another 1 billion people at risk (5, 56). Among the NTDs, the parasitic infections caused by trypanosomatids have a huge impact on human health. Trypanosomatids (*Euglenozoa* phylum, *Kinetoplastea* class) are unicellular eukaryotic parasites responsible for serious diseases in humans and animals (57). Three major NTDs are caused by kinetoplastid infections: human African trypanosomiasis, American trypanosomiasis and various forms of leishmaniasis (58), resulting in more than 60,000 human deaths per year and the loss of approximately 5 million disability-adjusted life years (DALY's) (48, 56, 57, 59–61).

The flagellated protozoans of the *Trypanosoma* genus are mainly transmitted to humans through the insects of the *Triatominae* subfamily (kissing-bugs, *Hemiptera*, *Reduviidae* family) and *Glossina* species (tsetse fly, *Diptera*, *Glossinidae* family) (62, 63). Human African trypanosomiasis (sleeping sickness, HAT) with the two forms – West African and East African trypanosomiasis, is caused by *Trypanosoma brucei gambiense* and *Trypanosoma brucei rhodesiense*, resp., and is transmitted by the bite of blood-feeding tsetse fly (59). The infective trypomastigote forms of the parasite penetrate into mammalian cells and convert into proliferative amastigotes, which are the mammalian replicative forms of the parasite. Rupture of these cells leads to the liberation of parasites and the proliferation of the infection (64, 65). Infectious trypomastigotes present in the salivary fluid of the flies produce a primary lesion in the skin known as a trypanosomal chancre that occurs 5–15 days after the initial bite. The parasites multiply into the lymph and the blood of the person bitten and then disseminate throughout the body (66–68). During the early stage of the infection, patients experience unspecific symptoms such as headaches, fever and weakness,

pain in the joints, lymphadenopathy and stiffness. Infected people may or may not show signs of illness immediately but over time the parasites cross the blood-brain barrier (69). The late stage of HAT is defined by the entry of trypanosomes into the central nervous system causing neurological changes such as sleep disorder – including nocturnal insomnia and daytime somnolence giving rise to the name “sleeping sickness”, sensory, motor and psychiatric disorders. Patients inevitably progress to coma and death in the absence of proper medication (66, 69). *T. brucei gambiense* is responsible for more than 95 % of cases and causes chronic infections with the onset of symptoms after a prolonged incubation period of months or even years. *T. brucei rhodesiense* causes acute infections that can rapidly lead to central nervous system damage due to parasites crossing the blood-brain barrier. African trypanosomiasis threatens the lives of approximately 60 million people in sub-Saharan Africa and is fatal if not treated (59, 66, 70).

American trypanosomiasis (Chagas disease) is caused by *Trypanosoma cruzi* and is a serious health concern in 21 countries of Latin America. Due to population mobility between Latin America and the rest of the world, Chagas disease has been increasingly detected in traditional non-endemic countries of America, such as the United States and Canada and some European and Western Pacific countries. WHO estimates that 6-8 million people worldwide are infected with *T. cruzi* and 65 million people in the Americas live in areas of exposure and are at risk of contracting this disease (62, 71). Chagas disease is transmitted by the bite of blood-feeding kissing-bugs and consists of an initial acute phase that is followed by a chronic phase. The acute phase can remain asymptomatic for many years but eventually, it progresses into its chronic phase (59). Although mortality has significantly declined (about 12, 000 deaths per year), the disease can cause irreversible and chronic damage to heart leading to cardiac arrhythmias and cardiac dilatation (20–30 %), digestive system damage leading to megaesophagus and megacolon (5–10 %), and autonomic nervous system damage (70, 72).

*Current pharmacotherapy for trypanosomiasis.* – Despite significant advances in understanding cell biology, etiology, the pathophysiology of parasitic infections and parasitic genome, there is currently no available vaccine for trypanosomiasis. It is due to the fact that parasitic infections do not stimulate immune system (73). In the absence of an available vaccine for trypanosomiasis, antiparasitic chemotherapy remains the only option for both clinical management and control of these diseases (59, 73). Effective drugs should be able to treat both stages of the disease or at least one of them. Pentamidine (9) and suramin (10) are used in early stages of African trypanosomiasis, whereas eflornithine (12) or the combination nifurtimox (13)-eflornithine (12) is approved for second-stage treatment. Melarsoprol (11), an arsenical drug with high toxicity, is also approved in later stages (Fig. 6) (58, 59). Chagas disease treatment currently depends on two nitroheterocyclic prodrugs: nifurtimox (13) and benznidazole (14). These two drugs are very effective against the circulating form of the parasite (trypomastigotes) if given soon after infection (acute phase), but not during the chronic stage of the disease (74).

However, current therapies of trypanosomiasis have proven to be unsatisfactory because of limited efficacy, serious side-effects in long-term therapy, and difficulties in administration. There are also differences in sensitivity between the different strains of the parasite. cofactor, the time required to complete the treatment and the associated cost remain extremely limiting. Treatment failures are not uncommon and drug-resistant parasites have been identified. Given the deficiencies of the current treatment options,

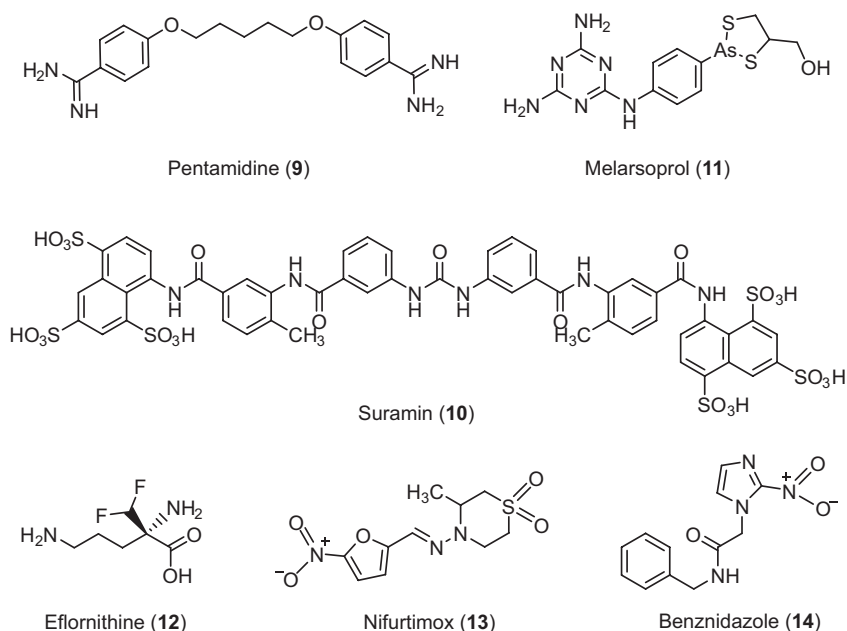


Fig. 6. Marketed drugs for the treatment of trypanosomiasis.

there is a clear and urgent need for the development of improved drugs that are safe, inexpensive and orally available (58, 59, 74, 75).

**Megazol and megazol derivatives.** – Megazol (5-(1-methyl-5-nitroimidazol-2-yl)-1,3,4-thiadiazol-2-amine) (15) is a nitroheterocyclic derivative containing the 2-amino-1,3,4-thiadiazole moiety. Megazol showed antibacterial and antiparasitic activities, particularly against *T. cruzi* and *T. brucei*, as well as drug-resistant strains of *Trypanosoma* (74, 75). Megazol exhibited a trypanocidal effect *in vitro* against *T. brucei brucei* ten-times stronger than that of suramin with half of the maximally effective concentration ( $EC_{50}$ ) value of  $0.01 \mu\text{g mL}^{-1}$  (76). *In vitro* studies on bloodstream trypomastigote form of *T. brucei* showed similar activity of megazol ( $EC_{50}$  value of  $4 \times 10^{-5} \text{ mmol L}^{-1}$ ) compared with that of suramin ( $EC_{50}$  value of  $2 \times 10^{-5} \text{ mmol L}^{-1}$ ) (77). *In vivo* studies made on *T. brucei brucei* infected mice showed that a single intraperitoneal injection of  $20 \text{ mg kg}^{-1}$  of megazol cured the acute disease. However, megazol administered alone was not able to cure the mice carrying a subacute infection with central nervous system involvement (76, 77). The combination of megazol with suramin or melarsoprol in the treatment of *T. brucei* infections potentiates its trypanocidal effect and these results confirm the interest in testing drugs in combinations (74, 77).

Many pharmacological studies have focused on revealing the mechanisms that mediate the antiparasitic activity of megazol. Some of the suggested mechanisms include: the activation of megazol inside the parasite by nitroreductase enzymes – possibly *via* a second one-electron reduction (78), to a nitroso derivative which can bind to intracellular



macromolecules of epimastigotes (74); megalzol can decrease the thiols and acts as thiol scavenger for trypanothione T(SH)<sub>2</sub> in *T. cruzi*, the cofactor of the trypanothione reductase (TryR) – the detoxification enzyme which has been shown to be essential for the survival of the trypanosome parasites (74); megalzol mediates DNA damage in *T. brucei* mutants lacking RAD51 – the main enzyme involved in eukaryotic DNA repair (79); megalzol can block the nucleic acids and protein synthesis (74, 76). Although it is 3–4 times more effective against *T. cruzi* than nifurtimox (**13**) (74) and benznidazole (**14**), resp., megalzol promoted chromosomal aberrations in mammalian cells. Due to its mutagenic and genotoxic properties, megalzol is not used clinically, but it has been extensively used as a lead compound in the search for nitroaromatics with antitrypanosomal activity (61, 75).

Therefore, three megalzol derivatives **16–18** obtained by substitution at the 4-position of the imidazole ring by electron-donating or electron-withdrawing groups (Fig. 7), were tested for the trypanocidal effect *in vitro* and *in vivo*. All derivatives were less active than the parent compound, megalzol. The most active compound, 5-(4-bromo-1-methyl-5-nitro-1*H*-imidazol-2-yl)-1,3,4-thiadiazol-2-amine (**17**) was ten times less active ( $EC_{50}$  value 0.15  $\mu\text{g mL}^{-1}$ ) than megalzol ( $EC_{50}$  value 0.01  $\mu\text{g mL}^{-1}$ ). The methyl derivative (**16**) and trifluoromethyl derivative (**18**) were not trypanocidal even when the dose was elevated to 10  $\mu\text{g mL}^{-1}$  (76). An interesting feature of megalzol, which is important for bioactivity, is its high bioavailability due to lipophilicity. Several studies have indicated that megalzol enters the parasite by passive diffusion and the positive value of the partition coefficient ( $\log P$ ) has great influence (74, 75). Megazol also binds to the P2-aminopurine transporter, which also carries pentamidine, melarsoprol, adenine and adenosine. The carrier binds the substances through the  $-\text{N}=\text{C}-\text{NH}_2$  moiety, and the amine group must, therefore, be free (80). Since derivatives **16–18** appear to be more lipophilic than megalzol, it should be easier for these derivatives to pass through the biological membranes and penetrate into parasites. They also possess a free amine group. However, no correlation between the lipophilicity and antiparasitic effect was observed. These results show the relationship between the hydrogen substitution at position 4 of the imidazole ring and the decrease or loss of trypanocidal activity (76, 77). Consistent with this observation was the result of biological tests performed on isomer 4-nitro-megazol. The location of the nitro group at position 4 made the compound totally inactive (77).

Other analogues of megalzol were also prepared, but the biological experiments indicated that megalzol was the most active compound against *T. brucei* (100 % inhibition at concentration of 1.5  $\text{mmol L}^{-1}$ ), *T. cruzi* (99 % inhibition at concentration of 3.1  $\text{mmol L}^{-1}$ ) and *Leishmania infantum* (95 % inhibition at concentration of 6.2  $\text{mmol L}^{-1}$ ), with no cytotoxicity to macrophages at active concentrations. However, it is worth considering two derivatives (Fig. 8). Although lower efficacy, activity was found against *T. brucei* (89 %

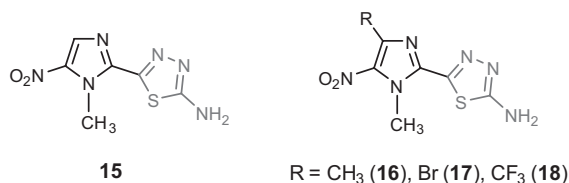


Fig. 7. Megazol derivatives **16–18**.



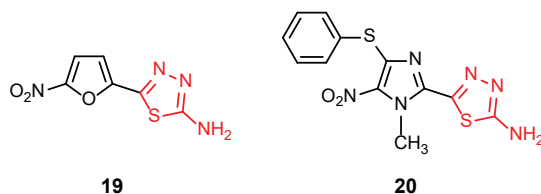


Fig. 8. Megazol derivatives **19** and **20**.

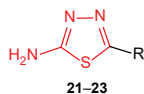
inhibition at concentration of 3.1 mmol L<sup>-1</sup>) and *T. cruzi* (95 % inhibition at concentration of 6.2 mmol L<sup>-1</sup>) for the 5-(5-nitrofuran-2-yl)-1,3,4-thiadiazol-2-amine (**19**) and against *T. cruzi* cultures (60 % inhibition at concentration of 12.5 mmol L<sup>-1</sup>) for the 5-(1-methyl-5-nitro-4-(phenylthio)-1H-imidazol-2-yl)-1,3,4-thiadiazol-2-amine (**20**) (77).

The efficacy of megazol in combination with suramin in *T. brucei gambiense* infected primates, including late-stage central nervous system infections, was also evaluated. Complete recovery was observed in five studied monkeys, without relapse of parasitemia during a 2-year follow-up (77).

*2-Amino-1,3,4-thiadiazole derivatives as pteridine reductase-1 inhibitors.* – As mentioned in a previous paper on antileishmanial properties of 2-amino-1,3,4-thiadiazole derivatives (48), one successful approach for the treatment of several microbial and parasitic infections is the use of drugs that attack essential enzymes in the biosynthetic pathway of folates (58). Folic acid and related pteridines, *e.g.*, dihydrobiopterin (H<sub>2</sub>B) and tetrahydrobiopterin (H<sub>4</sub>B), are essential co-factors in all forms of life, playing critical roles in a variety of metabolic pathways such as DNA and RNA synthesis and amino acids metabolism (81). Two enzymes that are of particular interest in these pathways are thymidylate synthase (TS) and dihydrofolate reductase (DHFR). In trypanosomatids and other protozoans, DHFR is encoded as a fusion protein with TS (DHFR-TS), with the DHFR domain on the amino terminus and TS domain on the carboxy terminus (66, 81). The inhibition of DHFR does not cause the death of parasites by itself, but due to another key enzyme, pteridine reductase-1 (PTR1). PTR1 is a short-chain NADPH dependent dehydrogenase/reductase with an essential role in reducing conjugated (folate) and unconjugated (biopterin) pterins and is much less sensitive to inhibition by antifolates. PTR1 is responsible for the reduction of biopterin to its derivatives (*e.g.* H<sub>2</sub>B and H<sub>4</sub>B) and for the biosynthesis of 10 % tetrahydrofolate required for cellular metabolism and its activity is enhanced when the parasitic DHFR is drug-inhibited. PTR1 provides reduced pteridines and reduced folic acid required for the survival of the parasites and, therefore contributes to the failure of the treatment (58, 82). It seems that the role of PTR1 in *Leishmania major* and *T. brucei* is considerably different. *Leishmania* mutants lacking the PTR1 enzyme can be rescued *in vitro* by supplements with H<sub>2</sub>B or H<sub>4</sub>B and retain their infectivity *in vivo*. In contrast, knockdown of PTR1 levels in *T. brucei* abolishes the infectivity of the parasite *in vivo* and is lethal *in vitro* when the parasites cannot be rescued by H<sub>2</sub>B or H<sub>4</sub>B supplements. Thus, PTR1 is a promising drug target for the treatment of African trypanosomiasis (82, 83).

Some 2-amino-1,3,4-thiadiazole derivatives were identified as selective *L. major* PTR1 (*Lm*PTR1) inhibitors and antileishmanial agents in the combination with DHFR inhibitors (84). *T. brucei* PTR1 (*Tb*PTR1) shares 50 % amino acid sequence identity with *Lm*PTR1, and

Table I. Structural details and biological profile of compounds 21–23 (58)



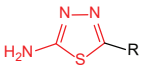
| Compd. | R | Inhibition <i>Tb</i> PTR1<br>(0.05 mmol L <sup>-1</sup> )<br>(%)<br><i>IC</i> <sub>50</sub> (mmol L <sup>-1</sup> ) | Inhibition <i>T. brucei</i><br>(0.05 mmol L <sup>-1</sup> )<br>(%)<br><i>EC</i> <sub>50</sub> (mmol L <sup>-1</sup> ) | <i>IC</i> <sub>50</sub> against<br>mammalian<br>cells<br>(mmol L <sup>-1</sup> ) |
|--------|---|---|---|--|
| 21     |   | 15 %<br><i>K</i> <sub>i</sub> 0.053   | –   | –  |
| 22     |   | 10 %<br><i>K</i> <sub>i</sub> 0.088   | 67 %<br>0.170   | 0.0515   |
| 23     |   | 18 %<br><i>K</i> <sub>i</sub> 0.048   | –   | –  |

*K*<sub>i</sub> – inhibition constant

studies have shown that the architecture of the co-factor binding site and the enzyme's catalytic center are highly conserved. However, there are some differences in the placement of certain amino acids in the active site that significantly reduce the size of the substrate-binding site of *Tb*PTR1 and modify the chemical properties compared to *Lm*PTR1 (85). Taking into account the morphology of *Leishmania* and *Trypanosoma* parasites, the 2-amino-1,3,4-thiadiazole scaffold was further studied as a source of potential antitrypanosomal agents. A total of 57 compounds based on 2-amino-1,3,4-thiadiazole moiety, synthesized by Linciano *et al.* (58), were studied as potential *Tb*PTR1 inhibitors. The *in vitro* antiparasitic activity against cultured *T. brucei* was also investigated. Some previously studied compounds, such as 3-(5-amino-1,3,4-thiadiazol-2-yl)pyridin-4-amine (**21**), 5-(1*H*-benzo[*d*] [1,2,3]triazol-5-yl)-1,3,4-thiadiazol-2-amine (**22**) and 3-(5-amino-1,3,4-thiadiazol-2-yl)-1-(thiophen-2-yl)propan-1-one (**23**), have shown competitive inhibition of *Lm*PTR1 (48, 84), but have behaved as weak inhibitors of *Tb*PTR1. However, derivative **22** showed promising *T. brucei* growth inhibition (67 % inhibition) and this observation guided the design of the newly 57 thiadiazoles in a structure-based approach (Table I) (58).

Previous docking studies have shown that the thiadiazole ring can be inserted between the nicotinamide ring of NADP<sup>+</sup> and Phe113 of *Lm*PTR1 (corresponding to Phe97 of *Tb*PTR1) (58, 86). Indeed, the structure of the ternary complex of *Tb*PTR1-NADP<sup>+</sup>-2-amino-1,3,4-thiadiazole derivative (**21**, **22** and **23**, resp.) showed that the thiadiazole ring is sandwiched between the nicotinamide ring of co-factor NADP<sup>+</sup> and the phenyl ring of Phe97 of *Tb*PTR1. A particular role plays the amino group at the 2-position of the thiadiazole derivative, which overlaps with the amino group at the 2-position of the pteridine ring, making hydrogen bonds with both *Tb*PTR1 and NADP<sup>+</sup>. In addition, a series of polar interactions and hydrogen bondings stabilize the complex. Comparison between the binding modes of compounds **21** or **23** and folate (*Tb*PTR1-NADP<sup>+</sup>-2-amino-1,3,4-thiadiazole derivative complex and

Table II. Structural and pharmacological details of compounds 24–26 (58)



**24–26**


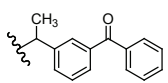
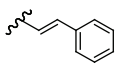
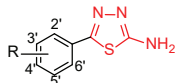
| Compd. | R   | Inhibition <i>Tb</i> PTR1<br>(0.05 mmol L <sup>-1</sup> )<br>(%) | Inhibition <i>T. brucei</i><br>(0.05 mmol L <sup>-1</sup> )<br>(%)<br><i>EC</i> <sub>50</sub> (mmol L <sup>-1</sup> ) |
|--------|---|--|---|
| 24     |  | 8 (at 0.1 mmol L <sup>-1</sup> )                                 | 90<br>35  |
| 25     |  | 47.2 ± 0.4   | 88<br>20  |
| 26     |  | 34.3 ± 1.2   | 82.7<br>58  |

Table III. Structural and pharmacological details of compounds 27–32 (58)



**27–32**

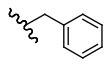
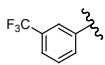
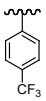
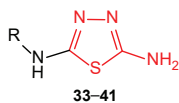
| Compd. | R2'   | R3' | R4'                             | R5'   | R6' | Inhibition <i>Tb</i> PTR1<br>(0.05 mmol L <sup>-1</sup> )<br>(%)<br><i>IC</i> <sub>50</sub> (mmol L <sup>-1</sup> ) | Inhibition <i>T. brucei</i><br>(0.05 mmol L <sup>-1</sup> )<br>(%)<br><i>EC</i> <sub>50</sub> (mmol L <sup>-1</sup> ) |
|--------|---|-----|---------------------------------|---|-----|---|---|
| 27     | H   | H   | CH <sub>2</sub> NH <sub>2</sub> | H   | H   | 7.7 ± 0.6<br>–  | 93<br>0.8   |
| 28     | H   | OH  | OH                              | H   | H   | 16.7 ± 1.0<br>–   | 78.6<br>18.3  |
| 29     | H   | OH  | OH                              | OH  | H   | 44.9 ± 0.9<br>41  | 100<br>1.0  |
| 30     |  | H   | H                               | H   | H   | 9.1 ± 0.5<br>–  | 84<br>47  |
| 31     | H   | H   | H                               |  | H   | 4.6 ± 1.0<br>–  | 94.8<br>37  |
| 32     | H   | H   | H                               |  | H   | 6.6 ± 0.6<br>–  | 92.1<br>42  |

Table IV. Structural and pharmacological details of compounds 33–41 (58)



| Compd. | R | Inhibition <i>TbPTR1</i><br>(0.05 mol L <sup>-1</sup> ) |  | Inhibition <i>T. brucei</i><br>(0.05 mmol L <sup>-1</sup> ) |  |
|--------|---|---|--|---|--|
|        |   | (%)   | IC <sub>50</sub> (mmol L <sup>-1</sup> ) | (%)   | EC <sub>50</sub> (mmol L <sup>-1</sup> ) |
| 33     |   | 48.5 ± 2.1  | 31                                       | –   |  |
| 34     |   | 37.7 ± 0.5  | 48                                       | –   |  |
| 35     |   | 34.6 ± 3.0  | 70                                       | –   |  |
| 36     |   | 31.0 ± 1.3  | 56                                       | 95.6  | 16                                       |
| 37     |   | 73.0 ± 1.1  | 16                                       | 6.6   |  |
| 38     |   | 79.8 ± 0.4  | 25                                       | 37.9  |  |
| 39     |   | 36.7 ± 2.0  | 62                                       | 4.0   |  |
| 40     |   | 50.6 ± 2.6  | 112                                      | –   |  |
| 41     |   | 30.0 ± 1.9  | 57                                       | –   |  |

*TbPTR1*-NADP<sup>+</sup>-folate complex, resp.) shows that the thiadiazole ring overlaps the 2-amino-4-oxypyrimidine moiety in the folic acid structure. Thus, while maintaining the 2-amino-1,3,4-thiadiazole scaffold that forms key bonds with the enzyme and the cofactor, various types of substituents designed to interact with the binding site residues were introduced at the position 5 (e.g., derivatives 24–26, 27–32 and 33–41) (58). Some derivatives, such as 25, 26, 29, 33–41, exhibited an inhibition of *TbPTR1* of over 30 % at a concentration of 0.05 mmol L<sup>-1</sup>

with half of the maximal inhibitory concentration ( $IC_{50}$ ) values in the range of 0.016–0.112 mmol L<sup>-1</sup> showing that the inhibition was improved by two to 12-fold compared to the first studied compounds **21**, **22** and **23** ( $IC_{50} > 0.2$  mmol L<sup>-1</sup>) (Tables II–IV). Taking into account that both *TbPTR1* and *TbDHFR* use dihydrofolic acid as a substrate, the 1,3,4-thiadiazole derivatives were also tested as *TbDHFR* inhibitors. However, the compounds did not show any potential as *TbDHFR* inhibitors (58).

The 2,5-diamino-1,3,4-thiadiazole moiety proved to be a good scaffold, and derivatives **33–41** were the most active inhibitors. *N*<sup>2</sup>-([1,1'-biphenyl]-4-ylmethyl)-1,3,4-thiadiazol-2, 5-diamine (**37**) and *N*<sup>2</sup>-[2-(thiophen-2-yl)ethyl]-1,3,4-thiadiazol-2,5-diamine (**38**) showed the best  $IC_{50}$  values (0.016 and 0.025 mmol L<sup>-1</sup>, resp.). In these structures, the thiadiazole ring is usually linked to an aromatic ring in the side chain. These two aromatic domains are connected by a flexible chain of 2–3 atoms, the nitrogen atom in position 5 being one of them. The second amino group at C-5 of thiadiazole ring also plays a role in the stability of the complex, allowing the formation of additional bonds with both the cofactor and the enzyme. Molecular docking studies have shown that larger compounds, which are also more active, are locked in the complex in a binding orientation very similar to that of folic acid. The authors concluded that there is a higher probability of enzyme inhibition for bulky derivatives, such as (**37**), capable of mimicking the orientation of folic acid into the complex (58).

The *in vitro* antiparasitic activity against cultured *T. brucei* was also investigated (Tables II–IV). Some derivatives such as **24**, **25**, **26**, **27–32** and **36** caused over 75 % of parasite inhibition. However, these compounds exhibited low enzyme inhibition (< 50 % inhibition of *TbPTR1* at 0.05 mmol L<sup>-1</sup>) and possibly have a different target. On the other hand, there are the derivatives **37** and **38** that showed the highest *TbPTR1* inhibition (73 and 79.8 %, resp.), but poor antiparasitic activity against *T. brucei*. It seems that there is no correlation between *TbPTR1* inhibition and antiparasitic activity (58).

Previous studies have shown that simultaneous inhibition of two parasitic enzymes (DHFR and TS) ensures parasite survival due to increased activity of PTR1. Simultaneous inhibition of parasitic DHFR and PTR1 is probably an alternative that could lead to the destruction of parasites (48, 87, 88). Therefore, the best *TbPTR1* inhibitors **37** and **38** were evaluated in combination with a *TbDHFR* inhibitor (58). When the derivative **37** was combined with equimolar amounts of methotrexate (MTX), a *TbDHFR* and *TbPTR1* inhibitor, the activity of MTX against *T. brucei* was improved ( $EC_{50}$  of MTX alone ~0.035 mmol L<sup>-1</sup>,  $EC_{50}$  of MTX with **37** ~8.6 mmol L<sup>-1</sup>), suggesting that PTR1 inhibitors enhance antiparasitic activity of DHFR inhibitors, probably by decreasing the amount of reduced folates required for parasitic metabolism. A similar result was obtained for the combination of derivative **38** with MTX, when the  $EC_{50}$  value of MTX was reduced two times ( $EC_{50}$  of MTX with **38** ~0.0175 mmol L<sup>-1</sup>) (58).

The toxicity of derivatives at a concentration of 0.01 mmol L<sup>-1</sup> was evaluated by some preliminary tests, such as cytotoxicity (e.g., WI-38, human lung fibroblasts), mitochondrial toxicity, cardiotoxicity (*hERG* inhibition), and inhibition of enzymes (e.g., cytochrome CYP450 and Aurora B kinase) (58). Generally, thiadiazole derivatives showed a safe profile (< 30 % inhibition for mitochondrial toxicity, cardiotoxicity, CYP isoforms and Aurora B kinase and > 70 % for growth of WI-38 cells). Exceptions (e.g., cardiotoxicity, CYP2D6 inhibition, WI-38 toxicity) were observed in the 2,5-diamino-1,3,4-thiadiazole derivative series (compounds **33–41**), suggesting that the second amino group at C-5 of thiadiazole ring can induce toxicity (Table V) (58).

Table V. Toxicological details of the studied compounds (58)

| Compd.    | Inh <i>t</i> ERG<br>(0.01 mmol L <sup>-1</sup> )<br>(%) | Inh CYP1A2<br>(0.01 mmol L <sup>-1</sup> )<br>(%) | Inh CYP2C9<br>(0.01 mmol L <sup>-1</sup> )<br>(%) | Inh CYP2C19<br>(0.01 mmol L <sup>-1</sup> )<br>(%) | Inh CYP2D6<br>(0.01 mmol L <sup>-1</sup> )<br>(%) | Inh CYP3A4<br>(0.01 mmol L <sup>-1</sup> )<br>(%) | Cell growth WI-38<br>(0.01 mmol L <sup>-1</sup> )<br>(%) |
|-----------|---|---|---|--|---|---|--|
| <b>25</b> | 16.0 ± 6.3  | -14.2 ± 7.1                                       | 21.9 ± 4.3  | 39.3 ± 3.2   | -12.7 ± 6.2                                       | 94.5 ± 0.8  | 127.2 ± 21.4   |
| <b>26</b> | 58.7 ± 4.9  | 25.2 ± 1.1  | 4.1 ± 5.9   | 7.2 ± 9.8  | -22.1 ± 8.0                                       | 45.9 ± 5.5  | 110.9 ± 42.0   |
| <b>29</b> | 3.3 ± 2.2   | 71.4 ± 6.9  | 38.2 ± 5.4  | 67.4 ± 7.3   | -6.0 ± 7.3  | 67.7 ± 3.1  | 110.9 ± 12.9   |
| <b>33</b> | 75.6 ± 4.4  | 29.1 ± 3.4  | 17.57 ± 0.9                                       | 36.8 ± 0.9   | 99.0 ± 0.6  | 27.4 ± 6.8  | 68.5 ± 18.8  |
| <b>34</b> | 69.9 ± 2.3  | 24.6 ± 2.5  | 19.5 ± 1.3  | 11.9 ± 6.6   | 72.4 ± 3.8  | 7.8 ± 9.8   | 80.9 ± 23.6  |
| <b>35</b> | 82.6 ± 11.8   | 17.1 ± 4.4  | 15.0 ± 1.4  | -2.0 ± 6.3   | 62.4 ± 2.9  | 7.2 ± 12.7  | 106.9 ± 24.9   |
| <b>36</b> | 107.9 ± 9.6   | 15.9 ± 1.1  | 33.5 ± 1.7  | 45.9 ± 0.8   | 105.2 ± 0.1                                       | 87.7 ± 0.4  | 128.9 ± 14.1   |
| <b>37</b> | 52.6 ± 11.6   | 60.4 ± 4.5  | 46.9 ± 4.4  | 22.5 ± 4.7   | 53.6 ± 0.6  | 25.6 ± 6.3  | 95.9 ± 18.6  |
| <b>38</b> | 95.4 ± 8.0  | -2.9 ± 3.4  | 3.1 ± 4.8   | -20.9 ± 6.1  | 81.2 ± 1.2  | 30.4 ± 4.5  | 96.7 ± 13.5  |
| <b>39</b> | 91.4 ± 6.2  | 22.7 ± 1.9  | 30.4 ± 2.9  | 29.2 ± 6.6   | 103.0 ± 0.2                                       | 25.1 ± 4.3  | 66.1 ± 3.1   |
| <b>40</b> | 87.3 ± 5.7  | -3.2 ± 2.9  | -7.7 ± 4.7  | -13.9 ± 18.0                                       | 27.4 ± 5.7  | -2.6 ± 4.5  | 96.3 ± 7.6   |
| <b>41</b> | 81.1 ± 11.6   | 22.1 ± 2.7  | 18.6 ± 4.5  | 17.3 ± 4.6   | 100.2 ± 0.4                                       | 24.2 ± 4.7  | 111.0 ± 43.7   |

Inh – inhibition (%).

The cytotoxicity of compounds **37** and **38** at 0.1 mmol L<sup>-1</sup>, alone (~ 45 % of cell viability for compound **37**, ~ 93 % of cell viability for compound **38**, and ~ 48 % of cell viability for MTX, resp.) and in combination with equimolar MTX (~ 44 % of cell viability for the combination of **37** with MTX and ~ 60 % of cell viability for the combination of **38** with MTX, resp.), was evaluated using THP-1 macrophage-like cells (58).

These results showed that 2-amino-1,3,4-thiadiazoles have potential as future antitrypanosomal agents and the combination of thiadiazole derivatives with DHFR inhibitors provides a good strategy for the future development of potent antiparasitic agents with synergistic effect (58).

*2-Amino-1,3,4-thiadiazole derivatives as trypanothione reductase inhibitors.* – A promising target for new antitrypanosomal drugs is the enzyme trypanothione reductase (TryR). Poisoning and selective inhibition of this enzyme can be the molecular mechanism of action for useful antitrypanosomatid agents, as it was shown that TryR is essential for the parasite survival by protecting them against oxidative stress (66, 89). Therefore, many researchers focus on the importance of the trypanothione pathway for the survival and infectivity of parasites (66, 90, 91). Trypanothione (*N*<sup>1</sup>,*N*<sup>8</sup>-bis(glutathionyl)spermidine) with the two forms, TS<sub>2</sub> – oxidized form and T(SH)<sub>2</sub> – reduced form of trypanothione, is a thiopeptide essential for parasites. Trypanothione synthesis is catalyzed by two key enzymes: trypanothione synthetase (TryS) and trypanothione reductase (TryR). TryS is responsible for the synthesis of trypanothione, while TryR maintains trypanothione in its reduced form T(SH)<sub>2</sub> (Fig. 7) (48, 66, 92). Thiol-dependent redox metabolism is one of the unique metabolic traits that distinguish trypanosomatids from humans. A genetic feature of trypanosomatids is the lack of genes encoding glutathione reductase (GR) and thioredoxin reductase, the main enzymes in the redox systems of most living organisms (48, 92). In trypanosomatids, T(SH)<sub>2</sub>, T(SH)<sub>2</sub>-dependent antioxidant system (tryparedoxin TXN/tryparedoxin peroxidase system TXNPx) and TryR replace the antioxidant functions performed by glutathione (GSH), GSH-dependent antioxidant enzymes and glutathione reductase (GR) in most cells (90, 91, 93). T(SH)<sub>2</sub> provides the reduction potential to several redoxin proteins that control metabolic functions. In addition, T(SH)<sub>2</sub> is involved in the neutralization of xeno- and endobiotics, the coordination of iron-sulfur complexes and the reduction of ascorbate (92). Therefore, T(SH)<sub>2</sub> is used by TXN/TXNPx system to reduce hydrogen peroxide, alkyl-hydroperoxide and other reactive oxygen species (Fig. 9) (90, 91, 93). Due to the specific features of the antioxidant physiology of these parasites, different genetic strategies to validate the enzymatic pathway of T(SH)<sub>2</sub> as drug targets in *T. brucei* have been studied. At 80–100 % down-regulation, most of the target enzymes were found to be essential for parasite survival, infectivity, or management of oxidative stress (94).

Some mesoionic derivatives such as **42**, **43**, **44** and **45** have already been studied to determine their antiparasitic activity and very potent or potent antileishmanial activities have been shown for all derivatives against three *Leishmania* species (*L. amazonensis*, *L. braziliensis* and *L. chagasi*) as compared to pentamidine as a standard drug (48, 95, 96). Their effect on the TryR activity of three *Leishmania* species was also investigated and the nitro-derivative (**45**) provided very promising results (89). Since the protozoans of the genus *Leishmania* and *Trypanosoma* belong to the same family (*Trypanosomatidae*), this study was extended to the parasite that causes Chagas' disease, *T. cruzi*. The effects of mesoionic derivatives **42**, **43**, **44** and **45** (Fig. 10) against TryR from parasites extracts and against recombinant enzyme from *T. cruzi* were evaluated. TryR is an FAD-dependent



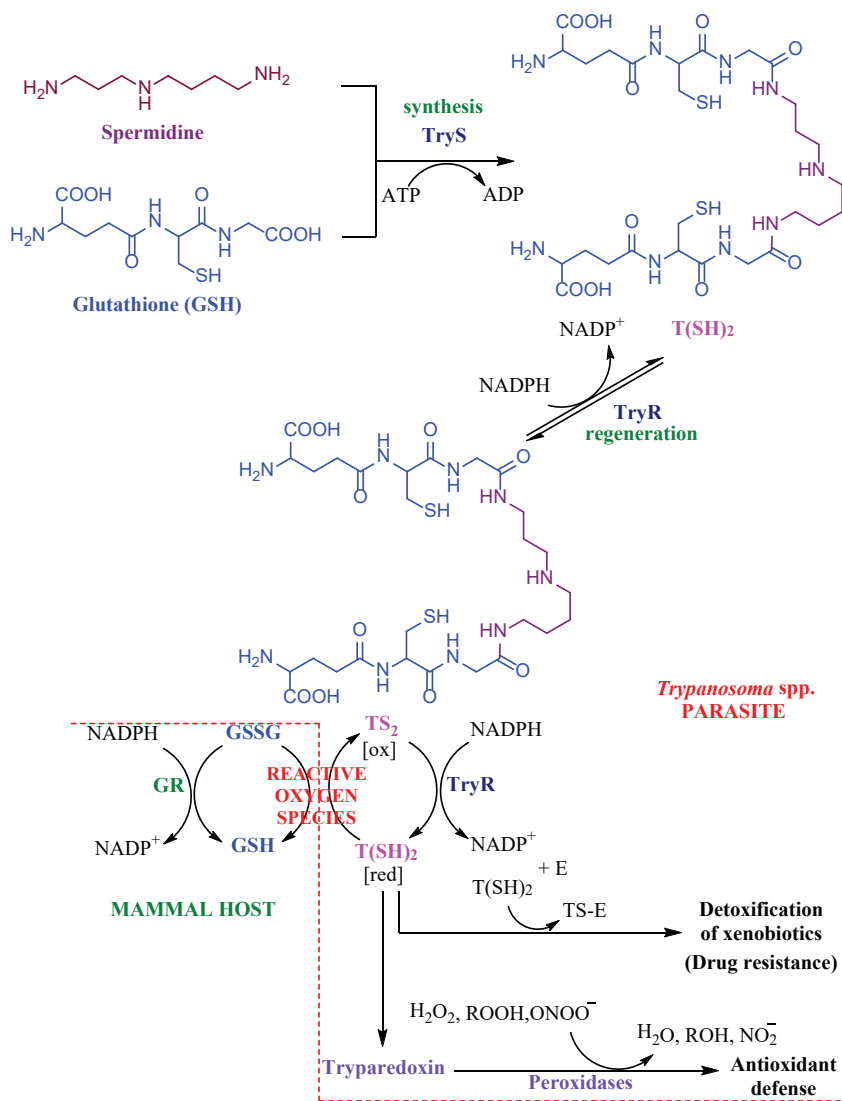


Fig. 9. Redox systems in mammals and trypanosomatids. GSSG – glutathione disulfide, H<sub>2</sub>O<sub>2</sub> – hydrogen peroxide, ROOH – alkyl-hydroperoxide, ONOO<sup>-</sup> – peroxynitrite, ROH – alcohol, NO<sub>2</sub><sup>-</sup> – nitrite, E – electrophilic species, TS-E – trypanothione-electrophile adduct (adapted from refs. 48, 91, 92).

oxidoreductase that uses NADPH as an electron donor and contains binding sites for FAD and NADPH, besides the substrate-binding site (89). Only 4-phenyl-5-(4-nitrostyryl)-1,3,4-thiadiazolium-2-phenylamine chloride (**45**) inhibited TryR in the parasite extract. Moreover, derivative **45** inhibited 83 % of NADPH consumption by TryR in *T. cruzi* at 1 mmol L<sup>-1</sup> compared with the control. On the other hand, preincubation of 1 mmol L<sup>-1</sup> of nitro-

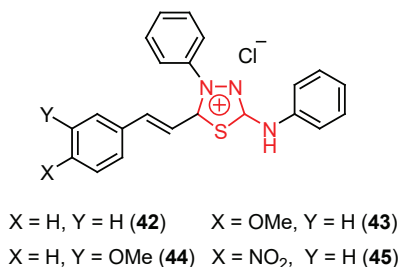


Fig. 10. Mesoionic derivatives **42–45**.

derivative **45** with the recombinant enzyme from *T. cruzi* – *TcTryR* showed a 69 % inhibition of *TcTryR*, indicating that TryR could be a molecular target for derivative **45**. The addition of 1 mmol L<sup>-1</sup> of derivative **42** did not alter NADPH consumption in comparison with the control (89). Although the TryR assay identified derivative **45** as the only active enzyme inhibitor, all these mesoionic compounds have demonstrated antiparasitic activities. It is possible that several metabolic pathways in parasites are involved and other mechanisms of action independent of TryR inhibition could be identified by future studies (89).

However, the inconvenience is that TryR has a structural similarity to human GR, and the designing of selective TryR inhibitors can be difficult (66). Therefore, TryS remains the most promising target enzyme since it is a low-abundance and essential enzyme in *Trypanosomatidae* parasites with no human homologs (93). Genetic and pharmacological tests on *T. brucei brucei* and *L. infantum* parasites have demonstrated the importance of TryS activity for the viability of the parasites (92). A kinetic model of T(SH)<sub>2</sub> metabolism in *T. cruzi* predicted that, in order to diminish the T(SH)<sub>2</sub> synthesis by 50 %, TryS inhibition by 63 % or TryR inhibition by over 98 % is required. Highly potent and specific TryR inhibitors are needed to affect the antioxidant capacities of the parasite, while moderate inhibition of TryS may be a promising target in drug development (94). In addition, TryS has several advantages as a molecular target candidate: it is encoded by a single copy gene, TryS has been shown to provide metabolic control to the trypanothione pathway in *T. cruzi* and kinetic information is available for multiple TryS (48, 92, 94, 97–99).

*Mesoionic 1,3,4-thiadiazolium-2-phenylamine salts of natural piperine.* – Piperine is a natural amide and the main secondary metabolite in *Piper nigrum*. Promising results concerning the *in vitro* activity of piperine against promastigote forms of *L. donovani* and *in vivo* studies on *L. donovani*-infected hamsters were the starting point for the synthesis of hybrid mesoionic salts containing the piperine moiety and the 1,3,4-thiadiazolium-2-phenylamine skeleton (100). The toxic effects of mesoionic derivatives were evaluated against different evolutive forms of *T. cruzi* (epimastigotes, trypomastigotes and amastigotes) and the toxicity of the most active compounds was evaluated against the host cells (murine macrophages). Among the derivatives that exhibited the lowest *IC*<sub>50</sub> values on epimastigotes (e.g., **46**, **47**, **48** and **49**, Fig. 11), 4-phenyl-5-[4-(3,4-methylenedioxyphenyl)-1(*E*)-3(*E*)-butadienyl]-1,3,4-thiadiazolium-2-phenylamine chloride (**46**) possesses the best activity profile (*IC*<sub>50</sub> value of 10.83 mmol L<sup>-1</sup> against epimastigotes, 6.70 mmol L<sup>-1</sup> against trypomastigotes and 1.35 mmol L<sup>-1</sup> against amastigotes, resp.), showing an activity comparable to that of the reference drug, benznidazole (*IC*<sub>50</sub> value of 2.21 mmol L<sup>-1</sup> against epimastigotes, 6.61 mmol L<sup>-1</sup> against trypomastigotes and 2.51 mmol L<sup>-1</sup> against amastigotes, resp.) (Table VI) (100). Thus,

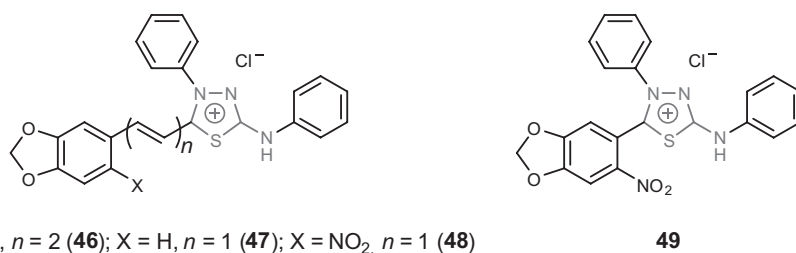


Fig. 11. The structural formula of compounds **46–49**.

derivative **46** can be considered a prototype in the development of new trypanocidal agents with good antiparasitic activity and low cytotoxicity. The higher antitrypanosomal activity exhibited by derivatives **46**, **47** and **48** suggests that the conjugation between the mesoionic and methylenedioxyphenyl rings is an important structural feature for the activity of these compounds (100). Although derivatives **47**, **48** and **49** exhibited significant toxic effects against *T. cruzi* epimastigotes, subsequent investigations were limited due to their toxicity to host murine macrophages. However, these results highlight the potential of natural piperine as a precursor of new molecules with antitrypanosomal activity that could be useful in the treatment of Chagas disease (100).

### Antimalarial activity

*Malaria and plasmodium parasites.* – Malaria is one of the most destructive human diseases caused by protozoan parasites. Found in tropical and subtropical regions of the world, the malaria parasites belonging to the *Plasmodium* genus (*Plasmodiidae* family) are transmitted to humans through the bites of infected *Anopheles* mosquitoes (101, 102). There are five *Plasmodium* species responsible for malaria in humans: *P. falciparum*, *P. malariae*, *P. ovalae*, *P. vivax* and *P. knowlesi*. *P. falciparum* and *P. vivax* are the most infective species to humans (59, 101, 102). *Plasmodium* parasites have a complex life cycle and are dependent on

Table VI. Pharmacological and toxicological details of derivatives **46–49** (100)

| Compd.       | <i>T. cruzi</i> /IC <sub>50</sub> (mmol L <sup>-1</sup> ) |                     |             | Cytotoxicity<br>LD <sub>50</sub> (mmol L <sup>-1</sup> ) |
|--------------|---|---------------------|-------------|--|
|              | Epimastigotes   | Trypomastigotes     | Amastigotes |  |
| Piperine     | 7.31 ± 1.5  | > max conc. allowed | 4.91 ± 1.1  | 20.01 ± 3.35   |
| <b>46</b>    | 10.83 ± 2.2   | 6.70 ± 1.7          | 1.35 ± 0.95 | 38.56 ± 4.6  |
| <b>47</b>    | 4.13 ± 1.2  | > max conc. allowed | –           | 1.95 ± 0.5   |
| <b>48</b>    | 0.64 ± 0.14   | > max conc. allowed | –           | 1.08 ± 0.23  |
| <b>49</b>    | 13.42 ± 3.0   | > max conc. allowed | –           | 6.62 ± 2.3   |
| Benznidazole | 2.21 ± 0.85   | 6.61 ± 2.4          | 2.51 ± 0.7  | –  |

> max conc. allowed – IC<sub>50</sub> values are higher than the maximum concentration allowed.

two hosts: *Anopheles* mosquitoes and humans (101). Malaria infection is established in humans following the injection of the sporozoite form of the parasite by female anopheline mosquitoes (103). In humans, parasites follow their asexual cycle of life when they grow and multiply in liver cells (hepatic stage), followed by the release of parasites into the bloodstream and the invasion of red blood cells (blood-stage). Replication within these cells and their subsequent rupture leads to the clinical manifestations of malaria (101, 103). Mosquitoes take parasites as gametocytes during a blood meal and the parasite will begin the sexual cycle of life (101). The usual incubation period, from the time of infection with *P. falciparum* to the initial symptoms, is 8–14 days in non-immune individuals. The incubation period can be much longer for patients with a certain degree of immunity. Symptoms of *P. falciparum* infection can range from mild fever (which makes it difficult to clinically distinguish from other similar illnesses) to a life-threatening disease with coma, respiratory distress, severe anemia or circulatory shock. Symptoms can change in 24 hours from a mild condition to life-threatening disease in young children and non-immune adults (103).

In 2017, *P. falciparum* was the cause of 99.7 % of malaria cases in the African region, while *P. vivax* was the predominant parasite in South America causing 74.1 % of cases of malaria (102). According to WHO, 219 million new cases of malaria and 435,000 malaria-related deaths in 90 countries were estimated in 2017. The African region has a disproportionately high rate in all cases of malaria worldwide. In 2017, the region was home to 92 % of malaria cases and 93 % of malaria deaths (102, 104). Children under the age of five in sub-Saharan Africa are particularly vulnerable, accounting for about two-thirds of all global deaths caused by malaria (105). Substantial progress in malaria control has been achieved by African countries using basic tools to prevent diseases such as insecticide-treated mosquito nets and indoor residual spraying with insecticides as well as antimalarial drugs for treating patients. However, malaria and malaria-related deaths remain high, which raises the need for new tools to combat the disease (105, 106).

*First malaria vaccine.* – A first-generation vaccine, also known as RTS, S/AS01 (RTS, S), was developed by GlaxoSmithKline (GSK) over 30 years of research, including through a collaboration launched in 2001 with Malaria Vaccine Initiative (MVI) and a network of African research centers (106). RTS, S is the first and the only vaccine that demonstrates partial protection against malaria among young African children, the population most affected by the disease. RTS, S acts against *P. falciparum*, the most prevalent malaria parasite in Africa (105). Infection is prevented by inducing humoral and cellular immunity with high antibody titers that block the parasite from infecting the liver (107). The European Medicines Agency (EMA) announced in 2015 that the benefits of TRS, S in preventing malaria outweigh the potential risks (105, 108). WHO announced the launch of the first malaria vaccine by the Government of Malawi in a pilot program on 23 April 2019. It will be followed by Ghana and Kenya in the near future (109). However, RTS, S is only moderately effective and its introduction is unlikely to diminish the demand for antimalarial drugs (110). Therefore, the vaccine is a complementary malaria control tool that can be added to the recommended WHO measures for malaria prevention (*e.g.*, the use of insecticide-treated bed nets, insecticide spray), malaria prompt diagnosis, and effective antimalarial treatment (103, 105, 106, 109).

*Current pharmacotherapy for malaria.* – Current malaria treatment still depends on chemotherapy. According to WHO, all uncomplicated malaria infections caused by

*P. falciparum* or *P. vivax* should be treated with artemisinin-based combination therapy (ACT). In areas where chloroquine-resistant *P. vivax* is not found, the infection can be treated with chloroquine (1). Primaquine (2) should be added to antimalarial treatment in order to reduce the transmission of the infection and to prevent relapses. Severe malaria should be treated with quinine (3) or with injectable artemisinin derivatives – artemether or artesunate, followed by ACT (102). The WHO currently recommends three malaria prevention strategies for high risk groups: intermittent preventive treatment in pregnancy (IPTp) with sulfadoxine (4)-pyrimethamine (5); intermittent preventive treatment in infants (IPTi) with sulfadoxine (4)-pyrimethamine (5); and seasonal malaria chemoprevention (SMC) with amodiaquine (50) (Fig. 12) plus sulfadoxine (4) – pyrimethamine (5) for children aged 3–59 months in areas of highly seasonal malaria transmission (59, 102, 104). The majority of these drugs are blood schizonticides with different mechanisms of action that target the erythrocytic asexual stages of malaria parasites and thereby terminate clinical attacks of malaria (111). Although significant progress has been made in understanding the life cycle of the parasite and the mode of transmission, there are still challenges in the treatment and eradication of malaria for which new therapeutic agents, as well as the control of parasite transmission, will be needed (112). Unfortunately, all drugs currently available for the treatment of malaria have been associated with decreased efficacy and the emergence of drug-resistant parasites. Therefore, new antimalarial drugs with new modes of action and efficacy against multi-drug resistant parasites should be designed (59).

*2-Amino-1,3,4-thiadiazole derivatives as potential antimalarial agents.* – A large chemical library containing more than 250,000 compounds was evaluated for the discovery of oral antiplasmodial drugs in a blood-stage *P. falciparum* growth inhibition assay (112). The selection process involved a three-step *in vitro* assay (spot test, dose-response and cytotoxicity) followed by *in silico* analysis of the confirmed hits and *in cerebro* evaluation of the selected chemical structures. In addition, chemical diversity and physicochemical properties were also evaluated through computational analysis. Finally, the new chemotypes with the desired properties were synthesized and tested in a *P. falciparum* growth inhibition assay. One hundred seventy-eight compounds with  $EC_{50} < 0.001$  mmol

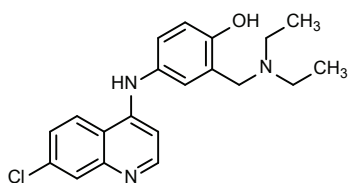


Fig. 12. The structural formula of amodiaquine (50).

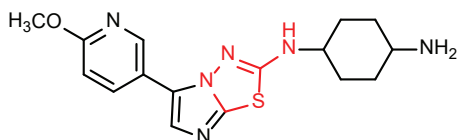


Fig. 13. The structural formula of compound 51.

L<sup>-1</sup> and selectivity index SI >10 were selected. Among them, N<sup>1</sup>-[5-(6-methoxypyridin-3-yl)imidazo[2,1-b][1,3,4]thiadiazol-2-yl]cyclohexane-1,4-diamine (**51**) (Fig. 13.) and six of its analogues exhibited promising antiplasmodial activity (112).

Derivative **51** has shown consistent antimalarial activity in both high throughput screening (HTS) method and after synthesis with EC<sub>50</sub> values of 0.297 mmol L<sup>-1</sup> and 0.284 mmol L<sup>-1</sup> against *P. falciparum* strain 3D7 and *P. falciparum* strain NF54, resp. In addition, this compound indicated good physicochemical properties and a high ligand efficiency index (LEI) value of 19 and therefore provided a good chemical starting point (112).

### *Antitoxoplasmosis activity*

*Toxoplasma parasites and toxoplasmosis.* – *Toxoplasma gondii* is an apicomplexan protozoan in the *Sarcocystidae* family that causes the toxoplasmosis, one of the most prevalent parasitic infections in humans and domestic animals. *T. gondii* is an intracellular parasite with widespread distribution in both developed and developing countries and the seropositive rates ranging from less than 10 to over 90 % (113, 114). It is estimated that one-third of the world's population is infected by this parasite, with more than 2 million people affected every year in the European region. In addition, *T. gondii* can infect more than 200 warm-blooded animal species causing toxoplasmosis in them (115, 116). According to WHO, it is estimated that 95 % of people with an immunocompetent system will not develop clinical symptoms when they are infected with *T. gondii*, or they may experience mild influenza-like symptoms such as fever, headache or myalgia that quickly pass (116). However, *T. gondii* can cause serious pathologies such as ocular disorders (e.g., eye infection, decreased visual acuity, retinochoroiditis) and severe neurological disorders (e.g., toxoplasmic encephalitis, mental retardation, necrotic lesions of central nervous system, epilepsy, and schizophrenia) in neonates and immune-suppressed individuals such as HIV-AIDS positive, cancer or organ transplant patients (113, 117, 118).

*T. gondii* has a complex life cycle consisting of a sexual phase and an asexual phase. The sexual phase generates fertilized gametes (oocysts) from sexual replication and occurs in the small intestine of definitive hosts, the members of *Felidae* family (domestic cats and their relatives). The oocysts are shed in the cats' feces and thus contaminate the water, food and environment. The excreted oocysts can last in the environment for 18 months. When ingested by humans, the sporozoites residing within oocysts or the bradyzoites residing in animal tissue cysts are released and are rapidly converted into the tachyzoite stage (asexual phase). Tachyzoites are the stage of rapid multiplication of the parasite and they invade almost any kind of cell, multiplying at intervals of six hours. Tachyzoites can be detected in the host's leukocytes or in the bloodstream where they circulate freely. These tachyzoites localize in brain, liver and muscle tissue and develop into bradyzoite tissue cysts (115, 118, 119).

*T. gondii* is an important foodborne pathogen. Humans usually become infected by horizontal transmission through ingestion or handling of undercooked or raw meat containing *T. gondii* cysts, through ingestion of food or water contaminated by oocysts from feline feces or through direct contact with cats or infected soil (114, 116). The parasite can be transmitted vertically as well. Thus, when women acquire the disease during or just before pregnancy, the protozoan can be transmitted trans-placentally (from mother to

fetus) and fetal death or congenital defects can result. In very rare cases, the disease can also be transmitted through blood or transplanted organs (116, 118).

*Current pharmacotherapy for toxoplasmosis.* – The ideal treatment for toxoplasmosis is the inhibition of different stages in the life cycle of the parasite. Compared to mammals that extensively use exogenous folate, *Toxoplasma* mainly relies on folate synthesis pathways to produce most of the folate needed for metabolism (113). Therefore, the folate synthesis pathway is the best-known therapeutic target against *Toxoplasma*. Two main enzymes are targeted by the pharmaceutical products, dihydropteroate synthetase and dihydrofolate reductase (DHFR). The dihydropteroate enzyme is not found in mammalian cells and provides a specific target for selective antitoxoplasmosis agents (113).

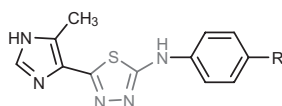
The current treatment for toxoplasmosis consists in the use of pyrimethamine (5) (DHFR inhibitor) and sulfonamides such as sulfamethoxazole (6) or sulfadiazine (dihydropteroate synthetase inhibitors), supplemented with folinic acid. The effectiveness of this treatment is limited by the large amounts of necessary drugs and serious side-effects such as hypersensitivity, haematological toxicity, teratogenicity, allergic reactions, bone marrow suppression and liver toxicity that occur in long-term treatment (117, 120). The treatment is also ineffective in removing the bradyzoite form of parasites located in the central nervous system (113, 117, 120). In order to reduce the transmission of parasite from mother to fetus, pregnant women can be treated with spiramycin, a macrolide antibiotic with low toxicity but limited activity against the parasite (59, 113). Additional drugs available include clindamycin, co-trimoxazole, azithromycin or atovaquone, but each of these drugs has their own inconvenience (117). In addition, *Toxoplasma's* resistance to antitoxoplasmosis drugs has been reported particularly in neonates and pregnant women whose treatment requires special medication (32).

*2-Amino-1,3,4-thiadiazole derivatives as potential antitoxoplasmosis agents.* – Considering that compounds containing an imidazole ring exhibit antiprotozoal activity, Liesen *et al.* (121) synthesized some 2-amino-1,3,4-thiadiazole derivatives having a methyl-imidazolyl group structurally similar to megalozol. *In vitro* studies against intracellular *T. gondii* showed complete morphological disorganization of tachyzoites following 24 hours incubation with

Table VII. *In vitro* anti-toxoplasma activity of derivatives 52–55 (121)

| Compd.       | Infected cells (%)  |                                 |         |           | Mean number of intracellular parasites |                                 |           |          |
|--------------|---------------------|---------------------------------|---------|-----------|--|---------------------------------|-----------|----------|
|              | Untreated (control) | Treated (mmol L <sup>-1</sup> ) |         |           | Untreated (control)                    | Treated (mmol L <sup>-1</sup> ) |           |          |
|              |                     | 0.1                             | 1       | 10        |  | 0.1                             | 1         | 10       |
| 52           | 72 ± 7              | 13 ± 4                          | 10 ± 1  | 0.3 ± 0.2 | 780 ± 76                               | 103 ± 32                        | 6 ± 1.6   | 2 ± 1.9  |
| 53           | 56 ± 8              | 28 ± 9                          | 14 ± 4  | 0         | 444 ± 75                               | 194 ± 40                        | 79 ± 16   | 0        |
| 54           | 70 ± 7              | 12 ± 2                          | 8 ± 1   | 1 ± 0.3   | 595 ± 45                               | 147 ± 46                        | 42 ± 12   | 11 ± 2.5 |
| 55           | 56 ± 4              | 22 ± 7                          | 6 ± 2   | 3 ± 0.5   | 507 ± 56                               | 139 ± 52                        | 34 ± 15   | 13 ± 1.2 |
| Hydroxyurea  | 59 ± 9              | 53 ± 7                          | 4 ± 0.3 | 1 ± 0.3   | 487 ± 69                               | 452 ± 58                        | 6 ± 1.1   | 3 ± 2.8  |
| Sulfadiazine | 79 ± 11             | 64 ± 11                         | 48 ± 15 | 36 ± 18   | 716 ± 191                              | 570 ± 101                       | 259 ± 114 | 115 ± 68 |



R = H (**52**); OCH<sub>3</sub> (**53**); Cl (**54**); F (**55**)Fig. 14. Structures of thiadiazole derivatives **52–55**.

the thiadiazole derivatives **52–55** (Fig. 14). This resulted in a significant decrease in the percentage of infected cells as well as in the average number of tachyzoites per cell at concentrations of 0.1, 1 and 10 mmol L<sup>-1</sup>, compared to hydroxyurea and sulfadiazine as standard drugs (Table VII) (121).

Thiadiazole derivatives **52–55** were very active against *T. gondii* at a concentration of 0.1 mmol L<sup>-1</sup> and showed low cytotoxicity in uninfected cells (median lethal dose  $LD_{50} > 10$  mmol L<sup>-1</sup>) and hence provided an interesting chemical scaffold for the discovery of drugs useful in the treatment of toxoplasmosis. These derivatives showed  $LD_{50}$  values between 0.07 and 0.6 mmol L<sup>-1</sup> for infected cells and 0.05 mmol L<sup>-1</sup> for parasites, indicating a higher activity than standard drugs (Table VIII) (121).

5-(5-Methyl-1H-imidazol-4-yl)-N-phenyl-1,3,4-thiadiazol-2-amine (**52**) was the most selective compound against intracellular parasites (13 % infected cells at a concentration of 0.1 mmol L<sup>-1</sup> compared to hydroxyurea – 53 % and sulfadiazine – 64 %, resp.). According to SAR results, the electron-withdrawing or electron-donating substituents on the phenyl moiety did not substantially influence the anti-*Toxoplasma* activity (121).

Table VIII.  $LD_{50}$  values of derivatives **52–55** (121)

| Compd.       | $LD_{50}$ (mmol L <sup>-1</sup> ) |                |                         |
|--------------|-----------------------------------|----------------|-------------------------|
|              | Uninfected cells                  | Infected cells | Intracellular parasites |
| <b>52</b>    | > 10                              | 0.6            | 0.05                    |
| <b>53</b>    | > 10                              | 0.1            | 0.05                    |
| <b>54</b>    | > 10                              | 0.08           | 0.05                    |
| <b>55</b>    | > 10                              | 0.07           | 0.05                    |
| Hydroxyurea  | > 10                              | > 10           | 0.5                     |
| Sulfadiazine | > 10                              | > 10           | 6                       |

## CONCLUSIONS

Parasitic diseases have a huge impact on human health, as well as on social and economic life, especially in developing and economically disadvantaged regions of the world (59). Many of these diseases are caused by protozoan parasites, unicellular eukaryotic organisms belonging to the Protista kingdom. The main parasitic infections in humans are due to protozoan of *Leishmania*, *Trypanosoma* and *Plasmodium* genus. Other protozoan

parasites, such as *Toxoplasma gondii*, *Trichomonas vaginalis*, *Giardia lamblia* and *Entamoeba histolytica* cause frequent infections with widespread distribution in both developing and developed countries (122).

Many people in tropical areas suffer from poor nutrition, poor living conditions and, therefore, poor health. As a result, they suffer from diseases that affect humans around the world. In addition, they must endure the serious consequences of diseases specific to their geographical areas: the so-called tropical diseases. Diseases such as African sleeping sickness, Chagas disease, leishmaniasis, malaria or toxoplasmosis cause pain and suffering from deformities to internal organs disorders, heart and brain damage and death. Delayed treatment of these diseases can cause serious or even fatal consequences, especially in sensitive groups such as children and pregnant women, which should receive medication as soon as possible and without any side-effects. Some of these diseases are no longer limited to the tropics. Tourism, trade, business travel and immigration have brought cases of these diseases to the industrialized world, where health systems are not used to diagnose them. Therefore, tropical diseases are currently issues of global concern. Leishmaniasis, malaria and trypanosomiasis are protozoan parasitic diseases that are targeted for prevention, control, elimination and eradication by the WHO's Division of Control of Tropical Diseases (3, 7).

Although significant advances have been made in the treatment of parasitic diseases over the last decade, currently available chemotherapy is far from satisfactory. The lack of an approved vaccine for any human parasitic disease, combined with the insufficient efficacy and excessive toxicity of the drugs, as well as the increased resistance, has spurred research to find new alternatives (59, 101). Several research groups belonging to pharmaceutical companies or academia are involved in the synthesis of heterocyclic compounds with potential antiparasitic activity. In order to alleviate the increasing drug resistance, a good solution is the production of new compounds with different mechanisms of action and several groups of scientists have done research not only in the field of new antiparasitic drugs synthesis but also in molecular parasitology to discover molecular targets in parasitosis therapy. It has been shown that the thiadiazole ring is a structural component of biologically active natural, as well as synthetic compounds. Among the four isomers, 1,3,4-thiadiazole derivatives are the most studied due to the wide spectrum of pharmacological activities, proving a significant utility in the development of biologically active compounds. Literature surveys report the antiparasitic properties of 2-amino-1,3,4-thiadiazole derivatives. Taking into account the reactivity of the amino group in the derivatization process, 2-amino-1,3,4-thiadiazole moiety can be an excellent scaffold for future antiparasitic derivatives. The amino group attached to the 1,3,4-thiadiazole ring will probably be the focus of future research and will take full advantage of its special properties. The 1,3,4-thiadiazole ring is a very stable scaffold and the introduction of different kinds of substituents is a challenging approach to obtain antiparasitic agents with improved potency and less toxicity. In addition, the chemical, physical and pharmacokinetic properties recommend the 1,3,4-thiadiazole ring as a target in the development of new drugs. Inhibition of several enzymes essential for the survival of parasites appears to be the molecular mechanisms of action for promising antitrypanosomal, antimalarial, antitoxoplasmosis and antileishmanial drugs, and several 2-amino-1,3,4-thiadiazole derivatives have shown these effects. Although there are insufficient results to state the quality of 2-amino-1,3,4-thiadiazoles as a new class of antiparasitic agents, these derivatives can be considered a great promise for the treatment of parasitic diseases

and provide a valid strategy for the development of potent antiparasitic drugs. Modifications to improve the potential of these compounds through structural diversification are under progress in many laboratories around the world.

#### REFERENCES

1. Y. Li, J. Geng, Y. Liu, S. Yu and G. Zhao, Thiadiazole – a promising structure in medicinal chemistry, *ChemMedChem* 8 (2013) 27–41; <https://doi.org/10.1002/cmdc.201200355>
2. T. L. Lemke, *Antiparasitic Agents*, in *Foye's Principles of Medicinal Chemistry* (Eds. T. L. Lemke, D. A. Williams, V. F. Roche and S. W. Zito), 7<sup>th</sup> ed., Lippincott Williams and Wilkins, Baltimore 2013, pp.1126.
3. World Health Organization, *Neglected Tropical Diseases. Prevention, Control, Elimination and Eradication*, Sixty-six world health assembly A66/20, Provisional agenda item 16.2, 15 March 2013; [https://www.who.int/neglected\\_diseases/A66\\_20\\_Eng.pdf](https://www.who.int/neglected_diseases/A66_20_Eng.pdf); last access date: March 27, 2019
4. P. J. Hotez, *The Neglected Tropical Diseases and the Neglected Infections of Poverty: Overview of Their Common Features, Global Disease Burden and Distribution, New Control Tools, and Prospects for Disease Elimination*, in *Institute of Medicine (US) Forum on Microbial Threats. The Causes and Impacts of Neglected Tropical and Zoonotic Diseases: Opportunities for Integrated Intervention Strategies*, National Academies Press, Washington (DC) 2011, A7; last access date March 27, 2019
5. D. Molyneux, Neglected tropical diseases, *Community Eye Health J.* 26 (2013) 21–24.
6. T. Furst, P. Salari, L. M. Llamas, P. Steinmann, C. Fitzpatrick and F. Tediosi, Global health policy and neglected tropical diseases: then, now and in the years to come, *PLoS Negl. Trop. Dis.* 11 (2017) e0005759; <https://doi.org/10.1371/journal.pntd.0005759>
7. F. Pourrajab, S. K. Forouzannia and S. A. Tabatabaee, Novel immunomodulatory function of 1,3,4-thiadiazole derivatives with leishmanicidal activity, *J. Antimicrob. Chemother.* 67 (2012) 1968–1978; <https://doi.org/10.1093/jac/dks144>
8. J. A. Joule, *Natural Products Containing Nitrogen Heterocycles – Some Highlights 1990-2015*, in *Advances in Heterocyclic Chemistry: Heterocyclic Chemistry in the 21st Century – A Tribute to Alan Katritzky* (Eds. E. F. V. Scriven and C. A. Ramsden), 1<sup>st</sup> ed., Academic Press, Cambridge (MA) 2016, Vol. 119, pp. 81–106.
9. S. B. A. M. W. Van den Broek, S. A. Meeuwissen, F. L. van Delft and F. P. J. T. Rutjes, *Natural Products Containing Medium-Sized Nitrogen Heterocycles Synthesized by Ring-Closing Alkene Metathesis*, in *Metathesis in Natural Product Synthesis: Strategies, Substrates and Catalysts* (Eds. J. Cossy, S. Arseniyadis and C. Meyer), Wiley-VCH, Weinheim 2010, pp. 45–85.
10. F. Diaba, J. A. Montiel, G. Serban and J. Bonjoch, Synthesis of normorphans through an efficient intramolecular carbamoylation of ketones, *Org. Lett.* 17 (2015) 3860–3863; <https://doi.org/10.1021/acs.orglett.5b01832>
11. G. Serban, H. Abe and Y. Takeuchi, Synthetic studies of substituted pyridine aldehydes as intermediates for the synthesis of toddaquinoline, its derivatives and other natural products, *Heterocycles* 83 (2011) 1989–2000; <https://doi.org/10.3987/COM-11-12239>
12. G. Serban, H. Abe, Y. Takeuchi and T. Harayama, A new approach to the benzopyridoxepine core by metal mediated intramolecular biaryl ether formation, *Heterocycles* 75 (2008) 2949–2958; <https://doi.org/10.3987/COM-08-11443>
13. G. Serban, Y. Shigeta, H. Nishioka, H. Abe, Y. Takeuchi and T. Harayama, Studies toward the synthesis of toddaquinoline by intramolecular cyclization, *Heterocycles* 71 (2007) 1623–1630; <https://doi.org/10.3987/COM-07-11062>
14. D. Sole, F. Diaba and J. Bonjoch, Nitrogen heterocycles by palladium-catalyzed cyclization of amino-tethered vinyl halides and ketone enolates, *J. Org. Chem.* 68 (2003) 5746–5749; <https://doi.org/10.1021/jo034299q>

15. B. Bradshaw, C. Parra and J. Bonjoch, Organocatalyzed asymmetric synthesis of morphans, *Org. Lett.* **15** (2013) 2458–2461; <https://doi.org/10.1021/ol400926p>
16. P. K. Shukla, A. Verma and P. Mishra, *Significance of Nitrogen Heterocyclic Nuclei in the Search of Pharmacological Active Compounds*, in *New Perspective in Agricultural and Human Health* (Eds. R. P. Shukla, R. S. Mishra, A. D. Tripathi, A. K. Yadav, M. Tiwari and R. R. Mishra), Bharti Publication, New Delhi 2017, pp. 100–126.
17. P. Martins, J. Jesus, S. Santos, L. R. Raposo, C. Roma-Rodrigues, P. V. Baptista and A. R. Fernandes, Heterocyclic anticancer compounds: recent advances and the paradigm shift towards the use of nanomedicine's tool box, *Molecules* **20** (2015) 16852–16891; <https://doi.org/10.3390/molecules200916852>
18. The Top 200 Drugs of 2019; <https://clincalc.com/DrugStats/Top200Drugs.aspx>; last access date March 29, 2019
19. A. V. Fuentes, M. D. Pineda and K. C. N. Venkata, Comprehension of top 200 prescribed drugs in the US as a resource for pharmacy teaching, training and practice, *Pharmacy* **6** (2018) 43–52; <https://doi.org/10.3390/pharmacy6020043>
20. *Antiparasitic Drugs (Antiprotozoal Drugs, Nitazoxanide and Ivermectin)*; <https://www.tm.mahidol.ac.th/pediatrics/?q=Antiparasitic-drugs>; last access date July 12, 2019
21. J. Keiser, K. Ingram and J. Utzinger, Antiparasitic drugs for paediatrics: systemic review, formulations, pharmacokinetics, safety, efficacy and implications for control, *Parasitology* **138** (2011) 1620–1632; <https://doi.org/10.1017/S0031182011000023>
22. F. Castelli, L. R. Tomasoni and A. Matteelli, Advances in treatment of malaria, *Mediterr. J. Hematol. Infect. Dis.* **4** (2012) e2012064; <https://doi.org/10.4084/MJHID.2012.064>
23. S. Rajapakse, P. Weeratunga, C. Rodrigo, N. L. de Silva and S. D. Fernando, Prophylaxis of human toxoplasmosis: a systematic review, *Pathog. Glob. Health* **111** (2017) 333–342; <https://doi.org/10.1080/20477724.2017.1370528>
24. M. Yoosefian, Z. J. Chermahini, H. Raissi, A. Mola and M. Sadeghi, A theoretical study on the structure of 2-amino-1,3,4-thiadiazole and its 5-substituted derivatives in the gas phase, water, THF and DMSO solutions, *J. Mol. Liq.* **203** (2015) 137–142; <https://doi.org/10.1016/j.molliq.2015.01.002>
25. K. M. Dawood and T. A. Farghaly, Thiadiazole inhibitors: a patent review, *Expert Opin. Ther. Pat.* **27** (2017) 477–505; <https://doi.org/10.1080/13543776.2017.1272575>
26. S. Haider, M. S. Alam and H. Hamid, 1,3,4-Thiadiazoles: a potent multi targeted pharmacological scaffold, *Eur. J. Med. Chem.* **92** (2015) 156–177; <https://doi.org/10.1016/j.ejmech.2014.12.035>
27. G. Serban, O. Stanasel, E. Serban and S. Bota, 2-Amino-1,3,4-thiadiazole as a potential scaffold for promising antimicrobial agents, *Drug Des. Devel. Ther.* **12** (2018) 1545–1566; <https://doi.org/10.2147/DDDT.S155958>
28. M. G. Yang, T. G. M. Dhar, Z. Xiao, H. Y. Xiao, J. J. W. Duan, B. Jiang, M. A. Galella, M. Cunningham, J. Wang, S. Habte, D. Shuster, K. W. McIntyre, J. Carman, D. A. Holloway, J. E. Somerville, S. G. Nadler, L. Salter-Cid, J. C. Barrish and D. S. Weinstein, Improving the pharmacokinetic and CYP inhibition profiles of azaxanthene-based glucocorticoid receptor modulators – Identification of (S)-5-(2-(9-fluoro-2-(4-(2-hydroxypropan-2-yl)phenyl)-5H-chromeno[2,3-b]pyridin-5-yl)-2-methylpropan amido)-N-(tetrahydro-2H-pyran-4-yl)-1,3,4-thiadiazole-2-carboxamide (BMS-341), *J. Med. Chem.* **58** (2015) 4278–4290; <https://doi.org/10.1021/acs.jmedchem.5b00257>
29. Y. J. Wu, *Five-membered ring systems: with N and S atom*, in *Progress in Heterocyclic Chemistry* (Eds. G. W. Gribble and J. A. Joule), Elsevier, Amsterdam 2017, Vol. 29, pp. 315–335.
30. R. Sink, I. Susic, M. Zivec, R. Fernandez-Menendez, S. Turk, S. Pajk, D. Alvarez-Gomez, E. M. Lopez-Roman, C. Gonzales-Cortez, J. Rullas-Triconado, I. Angulo-Barturen, D. Barros, L. Ballell-Pages, R. J. Young, L. Encinas and S. Gobec, Design, synthesis and evaluation of new thiadiazole based direct inhibitors of enoyl acyl carrier protein reductase (InhA) for the treatment of tuberculosis, *J. Med. Chem.* **58** (2015) 613–624; <https://doi.org/10.1021/jm501029r>

31. F. Hipler, M. Winter and R. A. Fischer, N-H··S hydrogen bonding in 2-mercapto-5-methyl-1,3,4-thiadiazole. Synthesis and crystal structures of mercapto functionalized 1,3,4-thiadiazoles, *J. Mol. Struct.* **658** (2003) 179–191; [https://doi.org/10.1016/S0022-2860\(03\)00386-7](https://doi.org/10.1016/S0022-2860(03)00386-7)
32. Y. Hu, C. Y. Li, X. M. Wang, Y. H. Yang and H. L. Zhu, 1,3,4-Thiadiazole: synthesis, reactions and applications in medicinal, agricultural, and materials chemistry, *Chem. Rev.* **114** (2014) 5572–5610; <https://doi.org/10.1021/cr400131u>
33. A. T. Balaban, D. C. Oniciu and A. R. Katritzky, Aromaticity as a cornerstone of heterocyclic chemistry, *Chem. Rev.* **104** (2004) 2777–2812; <https://doi.org/10.1021/cr0306790>
34. G. Kornis, *Five-membered Rings with More than Two Heteroatoms and Fused Carbocyclic Derivatives*, in *Comprehensive Heterocyclic Chemistry II* (Eds. A. R. Katritzky, C. W. Rees and E. F. V. Scriven), Elsevier, Oxford 1996, Volume 4, pp. 379–408.
35. A. Senff-Ribeiro, A. Echevarria, E. F. Silva, C. R. C. Franco, S. S. Veiga and M. B. M. Oliveira, Cytotoxic effect of a new 1,3,4-thiadiazolium mesoionic compound (MI-D) on cell lines of human melanoma, *Br. J. Cancer* **91** (2004) 297–304; <https://doi.org/10.1038/sj.bjc.6601946>
36. M. M. Ciotti, S. R. Humphreys, J. M. Venditti, N. O. Kaplan and A. Goldin, The antileukemic action of two thiadiazole derivatives, *Cancer Res.* **20** (1960) 1195–1201.
37. M. Juszcak, J. Matysiak, W. Brzana, A. Niewiadomy and W. Rzeski, Evaluation of antiproliferative activity of 2-(monohalogenophenylamino)-5-(2, 4-dihydroxyphenyl)-1,3,4-thiadiazoles, *Arzneim. Forsch. Drug Res.* **58** (2008) 353–357; <https://doi.org/10.1055/s-0031-1296519>
38. J. Matysiak, Evaluation of antiproliferative effect in vitro of some 2-amino-5-(2, 4-dihydroxyphenyl)-1,3,4-thiadiazole derivatives, *Chem. Pharm. Bull.* **54** (2006) 988–991; <https://doi.org/10.1248/cpb.54.988>
39. J. Matysiak and A. Opolski, Synthesis and antiproliferative activity of *N*-substituted 2-amino-5-(2,4-dihydroxyphenyl)-1,3,4-thiadiazoles, *Bioorg. Med. Chem.* **14** (2006) 4483–4489; <https://doi.org/10.1016/j.bmc.2006.02.027>
40. W. Rzeski, J. Matysiak and M. Kandefer-Szerszen, Anticancer, neuroprotective activities and computational studies of 2-amino-1,3,4-thiadiazole based compound, *Bioorg. Med. Chem.* **15** (2007) 3201–3207; <https://doi.org/10.1016/j.bmc.2007.02.041>
41. R. Asbury, J. A. Blessing and D. Moore, A phase II trial of aminothiadiazole in patients with mixed mesodermal tumors of the uterine corpus: a gynecologic oncology group study, *Am. J. Clin. Oncol.* **19** (1996) 400–402.
42. P. L. Elson, L. K. Kvols, S. E. Vogl, D. J. Glover, R. G. Hahn and D. L. Trump, Phase II trials of 5-day vinblastine infusion (NSC 49842), L-alanosine (NSC153353), acivicin (NSC 163501), and aminothiadiazole (NSC 4728) in patients with recurrent or metastatic renal cell carcinoma, *Invest. New Drugs* **6** (1988) 97–103.
43. P. F. Engstrom, L. M. Ryan, G. Falkson and D. G. Haller, Phase II study of aminothiadiazole in advanced squamous cell carcinoma of the esophagus, *Am. J. Clin. Oncol.* **14** (1991) 33–35.
44. G. Y. Locker, L. Kilton, J. D. Khandekar, T. E. Lad, R. H. Knop, K. Albain, R. Blough, S. French and A. B. Benson, High-dose aminothiadiazole in advanced colorectal cancer. An Illinois Cancer Center phase II trial, *Invest. New Drugs* **12** (1994) 299–301.
45. I. H. Krakoff, Purine metabolism in the chick embryo: influence of 2-substituted thiadiazoles, *Biochem. Pharmacol.* **13** (1964) 449–459; [https://doi.org/10.1016/0006-2952\(64\)90165-0](https://doi.org/10.1016/0006-2952(64)90165-0)
46. H. F. Oettgen, J. A. Reppert, V. Coley and J. H. Burchenal, Effects of nicotinamide and related compounds on the antileukemic activity of 2-amino-1,3,4-thiadiazole, *Cancer Res.* **20** (1960) 1597–1601.
47. D. M. Shapiro, M. E. Shils, R. A. Fugmann and I. M. Friedland, Quantitative biochemical differences between tumor and host as a basis for cancer chemotherapy IV. Niacin and 2-ethylamino-1,3,4-thiadiazole, *Cancer Res.* **17** (1957) 29–33.
48. G. Serban, Future prospects in the treatment of parasitic diseases: 2-amino-1,3,4-thiadiazoles in leishmaniasis, *Molecules* **24** (2019) 1557–1578; <https://doi.org/10.3390/molecules24081557>
49. G. Serban, 5-Arylamino-1,3,4-thiadiazol-2-yl acetic acid esters as intermediates for the synthesis of new bisheterocyclic compounds, *Farmacia* **63** (2015) 146–149.

50. T. Horvath, G. Serban and S. Cuc, Synthesis of new 2-phenylamino-5-[( $\alpha$ -acylamino)-p-X-stiryl]-1,3,4-thiadiazole compounds, *Farmacia* **62** (2014) 422–427.
51. G. Serban, A. Suciuc, M. Coman and E. Curea, Synthesis and physical-chemical study of some 3-(5-arylamino-1,3,4-thiadiazol-2-yl)coumarins, *Farmacia* **50** (2002) 50–54.
52. G. Serban, M. Coman and E. Curea, Synthesis of some heterocyclic nitrocoumarins by Knoevenagel condensation, *Farmacia* **53** (2005) 78–84.
53. G. Serban, D. Matinca, O. Bradea, L. Gherman, M. Coman and E. Curea, The study of the biological activity of some heterocyclic coumarins, *Farmacia* **53** (2005) 91–99.
54. G. Serban, M. Coman, E. Curea and L. Proinov, Synthesis and description of some heterocyclic coumarins, *Farmacia* **49** (2001) 45–52.
55. World Health Organization, *Integrating Neglected Tropical Diseases Into Global Health and Development: Fourth WHO Report on Neglected Tropical Diseases*, WHO, Geneva, 19 April 2017, licence: CC BY-NC-SA 3.0 IGO; <https://apps.who.int/iris/bitstream/handle/10665/255011/9789241565448-eng.pdf;jsessionid=9AA10810B00430B8A67751281F4AEFDD?sequence=1>; last access date March 27, 2019
56. World Health Organization, WHO Dept. of Control of Neglected Tropical Diseases, *Working to Overcome the Global Impact of Neglected Tropical Diseases: First WHO Report on Neglected Tropical Diseases*, WHO Press, Geneva 2010; [https://apps.who.int/iris/bitstream/handle/10665/44440/9789241564090\\_eng.pdf;jsessionid=FADC468AEF33A190CEC5CAB713DAAB9F?sequence=1](https://apps.who.int/iris/bitstream/handle/10665/44440/9789241564090_eng.pdf;jsessionid=FADC468AEF33A190CEC5CAB713DAAB9F?sequence=1); last access date September 25, 2017
57. J. A. Chandler and P. M. James, Discovery of trypanosomatid parasites in globally distributed *Drosophila* species, *PLoS ONE* **8** (2013) e61937; <https://doi.org/10.1371/journal.pone.0061937>
58. P. Linciano, A. Dawson, I. Poohner, D. M. Costa, M. S. Sa, A. Cordeiro-da-Silva, R. Luciani, S. Gul, G. Witt, B. Ellinger, M. Kuzikov, P. Gribbon, J. Reinshagen, M. Wolf, B. Behrens, V. Hannaert, P. A. M. Michels, E. Nerini, C. Pozzi, F. di Pisa, G. Landi, N. Santarem, S. Ferrari, P. Saxena, S. Lazzari, G. Cannazza, L. H. Freitas-Junior, C. B. Moraes, B. S. Pascoalino, L. M. Alcantara, C. P. Bertolacini, V. Fontana, U. Wittig, W. Muller, R. C. Wade, W. N. Hunter, S. Mangani, L. Costantino and M. P. Costi, Exploiting the 2-amino-1,3,4-thiadiazole scaffold to inhibit *Trypanosoma brucei* pteridine reductase in support of early-stage drug discovery, *ACS Omega* **2** (2017) 5666–5683; <https://doi.org/10.1021/acsomega.7b00473>
59. K. T. Andrews, G. Fisher and T. S. Skinner-Adams, Drug repurposing and human parasitic protozoan diseases, *Int. J. Parasitol. Drugs Drug Resist.* **4** (2014) 95–111; <https://doi.org/10.1016/j.ijpddr.2014.02.002>
60. World Health Organization, *Neglected Tropical Diseases*; [http://www.who.int/neglected\\_diseases/diseases/en/](http://www.who.int/neglected_diseases/diseases/en/); last access date July 21, 2018
61. S. Patterson and S. Wyllie, Nitro drugs for the treatment of trypanosomatid diseases: past, present, and future prospects, *Trend Parasitol.* **30** (2014) 289–298; <https://doi.org/10.1016/j.pt.2014.04.003>
62. World Health Organization, *Chagas Disease in the Americas: A Review of the Current Public Health Situation and a Vision for the Future. Report: Conclusions and Recommendations*, Washington, D.C., 3–4 May 2018; [https://www.paho.org/hq/index.php?option=com\\_content&view=article&id=14399:enfermedad-chagas-en-americas-revision-de-situacion-vision-futuro&Itemid=72315&lang=en](https://www.paho.org/hq/index.php?option=com_content&view=article&id=14399:enfermedad-chagas-en-americas-revision-de-situacion-vision-futuro&Itemid=72315&lang=en); last access date July 17, 2018
63. C. J. Schofield and J. P. Kabayo, Trypanosomiasis vector control in Africa and Latin America, *Parasit. Vectors* **1** (2008) Article ID 24 (7 pages); <https://doi.org/10.1186/1756-3305-1-24>
64. A. K. Jain, S. Sharma, A. Vaidya, V. Ravichandran and R. K. Agrawal, 1,3,4-Thiadiazole and its derivatives: a review on recent progress in biological activities, *Chem. Biol. Drug Des.* **81** (2013) 557–576; <https://doi.org/10.1111/cbdd.12125>
65. S. Tomlinson, F. Vandekerckhove, U. Frevert and V. Nussenzweig, The induction of *Trypanosoma cruzi* trypomastigote transformation by low pH, *Parasitology* **110** (1995) 547–554; <https://doi.org/10.1017/S0031182000065264>
66. A. S. Nagle, S. Khare, A. B. Kumar, F. Supek, A. Buchynskyy, C. J. N. Mathison, N. K. Chennamaneni, N. Pendem, F. S. Buckner, M. H. Gelb and V. Molteni, Recent developments in drug discovery for



- leishmaniasis and human African trypanosomiasis, *Chem. Rev.* **114** (2014) 11305–11347; <https://doi.org/10.1021/cr500365f>
67. Centers for Disease Control and Prevention, *Parasites, African trypanosomiasis*; <https://www.cdc.gov/parasites/sleepingsickness/biology.html>; last access date May 9, 2019
  68. *Parasites in humans, Trypanosoma brucei – sleeping sickness*; <http://www.parasitesinhumans.org/trypanosoma-brucei-sleeping-sickness.html>; last access date May 9, 2019
  69. World Health Organization, *Human African Trypanosomiasis, Symptoms, Diagnosis and Treatment*; [http://www.who.int/trypanosomiasis\\_african/disease/diagnosis/en/](http://www.who.int/trypanosomiasis_african/disease/diagnosis/en/); last access date November 1st, 2018
  70. World Health Organization, *Trypanosomiasis*; <http://www.who.int/ith/diseases/trypanosomiasis/en/>; last access date November 1, 2018
  71. World Health Organization, *Chagas Disease (American Trypanosomiasis)*, 1 February 2018; [http://www.who.int/news-room/fact-sheets/detail/chagas-disease-\(american-trypanosomiasis\)](http://www.who.int/news-room/fact-sheets/detail/chagas-disease-(american-trypanosomiasis)); last access date July 24, 2018
  72. World Health Organization, *Chagas Disease: Control and Elimination*, Sixty-third World Health Assembly, 22 April 2010; [http://apps.who.int/gb/ebwha/pdf\\_files/WHA63/A63\\_17-en.pdf](http://apps.who.int/gb/ebwha/pdf_files/WHA63/A63_17-en.pdf); last access date November 1, 2018
  73. S. Pund and A. Joshi, *Nanoarchitectures for Neglected Tropical Diseases: Challenges and State of the Art, in Nano- and Microscale Drug Delivery Systems: Design and Fabrication* (Ed. A. M. Grumezescu), 1<sup>st</sup> ed., Elsevier, Amsterdam 2017, pp. 449.
  74. J. D. Maya, S. Bollo, L. J. Nunez-Vergara, J. A. Squella, Y. Repetto, A. Morello, J. Perie and G. Chauviere, Trypanosoma cruzi: effect and mode of action of nitroimidazole and nitrofurans derivatives, *Biochem. Pharmacol.* **65** (2003) 999–1006; [https://doi.org/10.1016/S0006-2952\(02\)01663-5](https://doi.org/10.1016/S0006-2952(02)01663-5)
  75. A. Silva de Carvalho, K. Salomao, S. Lisboa de Castro, T. R. Conde, H. P. da Silva Zamith, E. R. Caffarena, B. S. Hall, S. R. Wilkinson and N. Boechat, Megazol and its bioisostere 4H-1,2,4-triazole: comparing the trypanocidal, cytotoxic and genotoxic activities and their *in vitro* and *in silico* interactions with the *Trypanosoma brucei* nitroreductase enzyme, *Mem. Inst. Oswaldo Cruz* **109** (2014) 315–323; <https://doi.org/10.1590/0074-0276140497>
  76. B. Bouteille, A. Marie-Daragon, G. Chauviere, C. de Albuquerque, B. Enanga, M. L. Darde, J. M. Vallat, J. Perie and M. Dumas, Effect of megazol on *Trypanosoma brucei brucei* acute and subacute infections in Swiss mice, *Acta Tropica* **60** (1995) 73–80; [https://doi.org/10.1016/0001-706X\(95\)00109-R](https://doi.org/10.1016/0001-706X(95)00109-R)
  77. G. Chauviere, B. Bouteille, B. Enanga, C. de Albuquerque, S. L. Croft, M. Dumas and J. Perie, Synthesis and biological activity of nitro heterocycles analogous to megazol, a trypanocidal lead, *J. Med. Chem.* **46** (2003) 427–440; <https://doi.org/10.1021/jm021030a>
  78. S. R. Wilkinson and J. M. Kelly, Trypanocidal drugs: mechanisms, resistance and new targets, *Expert Rev. Mol. Med.* **11** (2009) e31; <https://doi.org/10.1017/S1462399409001252>
  79. B. Enanga, M. R. Ariyanayagam, M. L. Stewart and M. P. Barrett, Activity of megazol, a trypanocidal nitroimidazole, is associated with DNA damage, *Antimicrob. Agents Chemother.* **47** (2003) 3368–3370; <https://doi.org/10.1128/AAC.47.10.3368-3370.2003>
  80. H. B. Leites, F. S. Damasceno, A. M. Silber, R. Z. Mendonca and C. N. Albuquerque, Synthesis and evaluation of trypanosomicidal activity of new derivatives of megazol, *Pharm. Biol. Eval.* **5** (2018) 40–51.
  81. T. J. Vickers and S. M. Beverley, Folate metabolic pathways in *Leishmania*, *Essays Biochem.* **51** (2011) 63–80; <https://doi.org/10.1042/bse0510063>
  82. N. Sienkiewicz, H. B. Ong and A. H. Fairlamb, *Trypanosoma brucei* pteridine reductase 1 is essential for survival *in vitro* and for virulence in mice, *Mol. Microbiol.* **77** (2010) 658–671; <https://doi.org/10.1111/j.1365-2958.2010.07236.x>
  83. H. B. Ong, N. Sienkiewicz, S. Wyllie and A. H. Fairlamb, Dissecting the metabolic roles of pteridine reductase 1 in *Trypanosoma brucei* and *Leishmania major*, *J. Biol. Chem.* **286** (2011) 10429–10438; <https://doi.org/10.1074/jbc.M110.209593>



84. S. Ferrari, F. Morandi, D. Motiejunas, E. Nerini, S. Henrich, R. Luciani, A. Venturelli, S. Lazzari, S. Calo, S. Gupta, V. Hannaert, P. A. M. Michels, R. C. Wade and M. P. Costi, Virtual screening identification of nonfolate compounds, including a CNS drug, as antiparasitic agents inhibiting pteridine reductase, *J. Med. Chem.* **54** (2011) 211–221; <https://doi.org/10.1021/jm1010572>
85. A. Dawson, F. Gibellini, N. Sienkiewicz, L. B. Tulloch, P. K. Fyfe, K. McLuskey, A. H. Fairlamb and W. N. Hunter, Structure and reactivity of *Trypanosoma brucei* pteridine reductase: inhibition by the archetypal antifolate methotrexate, *Mol. Microbiol.* **61** (2006) 1457–1468; <https://doi.org/10.1111/j.1365-2958.2006.05332.x>
86. D. Spinks, H. B. Ong, C. P. Mpamhanga, E. J. Shanks, D. A. Robinson, I. T. Collie, K. D. Read, J. A. Frearson, P. G. Wyatt, R. Brenk, A. H. Fairlamb and I. H. Gilbert, Design, synthesis and biological evaluation of novel inhibitors of *Trypanosoma brucei* pteridine reductase 1, *Chem. Med. Chem.* **6** (2011) 302–308; <https://doi.org/10.1002/cmdc.201000450>
87. B. Nare, J. Luba, L. W. Hardy and S. Beverley, New approaches to *Leishmania* chemotherapy: pteridine reductase 1 (PTR1) as a target and modulator of antifolate sensitivity, *Parasitology* **114** (1997) S101–S110.
88. A. Cavazzuti, G. Paglietti, W. N. Hunter, F. Gamarro, S. Piras, M. Loriga, S. Allecca, P. Corona, K. McLuskey, L. Tulloch, F. Gibellini, S. Ferrari and M. P. Costi, Discovery of potent pteridine reductase inhibitors to guide antiparasite drug development, *Proc. Natl. Acad. Sci. USA* **105** (2008) 1448–1453; <https://doi.org/10.1073/pnas.0704384105>
89. R. F. Rodrigues, D. Castro-Pinto, A. Echevarria, C. M. dos Reis, C. N. Del Cistia, C. M. R. Sant’Anna, F. Teixeira, H. Castro, M. Canto-Cavalheiro, L. L. Leon and A. Tomas, Investigation of trypanothione reductase inhibitory activity by 1,3,4-thiadiazolium-2-aminide derivatives and molecular docking studies, *Bioorg. Med. Chem.* **20** (2012) 1760–1766; <https://doi.org/10.1016/j.bmc.2012.01.009>
90. G. Colotti, P. Baiocco, A. Fiorillo, A. Boffi, E. Poser, F. Di Chiaro and A. Ilari, Structural insights into the enzymes of the trypanothione pathway: Targets for antileishmaniasis drugs, *Future Med. Chem.* **5** (2013) 1861–1875; <https://doi.org/10.4155/fmc.13.146>
91. M. O. F. Khan, Trypanothione reductase: A viable chemotherapeutic target for antitrypanosomal and antileishmanial drug design, *Drug Target Insights* **2** (2007) 129–146; <https://doi.org/10.1177/1177392807002000007>
92. D. Benítez, A. Medeiros, L. Fiestas, E. A. Panozzo-Zenere, F. Maiwald, K. C. Prousis, M. Roussaki, T. Calogeropoulou, A. Detsi, T. Jaeger, J. Šarlauskas, L. P. Mašič, C. Kunick, G. R. Labadie, L. Flohé and M. A. Comini, Identification of novel chemical scaffolds inhibiting trypanothione synthetase from pathogenic trypanosomatids, *PLoS Negl. Trop. Dis.* **10** (2016) e0004617 (25 pages); <https://doi.org/10.1371/journal.pntd.0004617>
93. A. Ilari, A. Fiorillo, I. Genovese and G. Colotti, An update on structural insights into the enzymes of the polyamine-trypanothione pathway: targets for new drugs against leishmaniasis, *Future Med. Chem.* **9** (2017) 61–77; <https://doi.org/10.4155/fmc-2016-0180>
94. V. Olin-Sandoval, Z. Gonzalez-Chavez, M. Berzunza-Cruz, I. Martinez, R. Jasso-Chavez, I. Becker, B. Espinoza, R. Moreno-Sanchez and E. Saavedra, Drug target validation of the trypanothione pathway enzymes through metabolic modeling, *FEBS J.* **279** (2012) 1811–1833; <https://doi.org/10.1111/j.1742-4658.2012.08557.x>
95. R. F. Rodrigues, E. F. da Silva, A. Echevarria, R. Fajardo-Bonin, V. F. Amaral, L. L. Leon and M. Canto-Cavalheiro, A comparative study of mesoionic compounds in *Leishmania* sp. and toxicity evaluation, *Eur. J. Med. Chem.* **42** (2007) 1039–1043; <https://doi.org/10.1016/j.ejmech.2006.12.026>
96. R. F. Rodrigues, K. S. Charret, E. F. da Silva, A. Echevarria, V. F. Amaral, L. L. Leon and M. Canto-Cavalheiro, Antileishmanial activity of 1,3,4-thiadiazolium-2-aminide in mice infected with *Leishmania amazonensis*, *Antimicrob. Agents Chemother.* **53** (2009) 839–842; <https://doi.org/10.1128/AAC.00062-08>
97. D. Spinks, L. S. Torrie, S. Thompson, J. R. Harrison, J. A. Frearson, K. D. Read, A. H. Fairlamb, P. G. Wyatt and I. H. Gilbert, Design, synthesis and biological evaluation of *Trypanosoma brucei* trypanothione synthetase inhibitors, *Chem. Med. Chem.* **7** (2012) 95–106; <https://doi.org/10.1002/cmdc.201100420>

98. A. F. Sousa, A. G. Gomes-Alves, D. Benitez, M. A. Comini, L. Flohe, T. Jaeger, J. Passos, F. Stuhlmann, A. M. Tomas and H. Castro, Genetic and chemical analyses reveal that trypanothione synthetase but not glutathionylspermidine synthetase is essential for *Leishmania infantum*, *Free Radic. Biol. Med.* **73** (2014) 229–238; <https://doi.org/10.1016/j.freeradbiomed.2014.05.007>
99. P. K. Fyfe, S. L. Oza, A. H. Fairlamb and W. N. Hunter, Leishmania trypanothione synthetase- amidase structure reveals a basis for regulation of conflicting synthetic and hydrolytic activities, *J. Biol. Chem.* **283** (2008) 17672–17680; <https://doi.org/10.1074/jbc.M801850200>
100. W. da Silva Ferreira, L. Freire-de-Lima, V. Barbosa Saraiva, F. Alisson-Silva, L. Mendonca-Previato, J. O. Previanto, A. Echevarria and M. E. Freire de Lima, Novel 1,3,4-thiadiazolium-2-phenylamine chlorides derived from natural piperine as trypanocidal agents: chemical and biological studies, *Bioorg. Med. Chem.* **16** (2008) 2984–2991; <https://doi.org/10.1016/j.bmc.2007.12.049>
101. A. Tahghighi and F. Babalouei, Thiadiazoles: the appropriate pharmacological scaffolds with leishmanicidal and antimalarial activities: a review, *Iran. J. Basic Med. Sci.* **20** (2017) 613–622; <https://doi.org/10.22038/IJBMS.2017.8828>
102. World Health Organization, *World Malaria Report 2018*, World Health Organization, Geneva 2018, Licence: CC BY-NC-SA 3.0 IGO, ISBN 978-92-4-156565-3; <https://apps.who.int/iris/bitstream/handle/10665/275867/9789241565653-eng.pdf>; last access date November 29, 2018
103. World Health Organization, *Malaria Vaccine: WHO Position Paper – January 2016*, *Weekly Epidemiological Record* **91** (2016) 33–52; <https://www.who.int/wer>; last access date June 8, 2019
104. World Health Organization, *Malaria*, 19 November 2018; <http://www.who.int/en/news-room/factsheets/detail/malaria>; last access date November 29, 2018
105. World Health Organization, *First Malaria Vaccine in Africa: A Potential New Tool for Child Health and Improved Malaria Control*, WHO/CDS/GMP/2018.05; <https://www.who.int/malaria/publications/atoz/first-malaria-vaccine/en/>; last access date June 8, 2019
106. World Health Organization, *Short Overview of the Malaria Vaccine Implementation Programme*, April 2019; <https://www.who.int/malaria/media/malaria-vaccine-overview/en/>; last access date June 8, 2019
107. L. Foquet, C. Hermsen, G. J. van Gemert, E. Van Braeckel, K. Weening, R. Sauerwein, P. Meuleman and G. Leroux-Roels, Vaccine-induced monoclonal antibodies targeting circumsporozoite protein prevent *Plasmodium falciparum* infection, *J. Clin. Invest.* **124** (2014) 140–144; <https://doi.org/10.1172/JCI70349>
108. World Health Organization, *Malaria Vaccine Implementation Programme (MVIP)*; [https://www.who.int/immunization/diseases/malaria/malaria\\_vaccine\\_implementation\\_programme/about/en/](https://www.who.int/immunization/diseases/malaria/malaria_vaccine_implementation_programme/about/en/); last access date June 8, 2019
109. World Health Organization, *Malaria Vaccine Pilot Launched in Malawi. Country First of Three in Africa to Roll Out Landmark Vaccine*, Geneva 23 April 2019; <https://www.who.int/news-room/detail/23-04-2019-malaria-vaccine-pilot-launched-in-malawi>; last access date June 8, 2019
110. E. A. Ashley and A. P. Phyto, Drugs in development for malaria, *Drugs* **78** (2018) 861–879; <https://doi.org/10.1007/s40265-018-0911-9>
111. P. B. Bloland and H. A. Williams, *Malaria Control During Mass Population Movements and Natural Disasters*, National Academies Press, Washington (DC) 2002, pp. 145–150.
112. V. M. Avery, S. Bashyam, J. N. Burrows, S. Duffy, G. Papadatos, S. Puthukkuti, Y. Sambandan, S. Singh, T. Spangenberg, D. Waterson and P. Willis, Screening and hit evaluation of a chemical library against blood-stage *Plasmodium falciparum*, *Malar. J.* **13** (2014) Article ID 190 (12 pages); <https://doi.org/10.1186/1475-2875-13-190>
113. E. G. Severance, J. Xiao, L. Jones-Brando, S. Sabuncuyan, Y. Li, M. Pletnikov, E. Prandovszky and R. Yolken, *Toxoplasma gondii* – a gastrointestinal pathogen associated with human brain diseases, *Int. Rev. Neurobiol.* **131** (2016) 143–163; <https://doi.org/10.1016/bs.irn.2016.08.008>
114. P. R. Torgerson and P. Mastroiacovo, The global burden of congenital toxoplasmosis: a systematic review, *Bull. World Health Organ.* **91** (2013) 501–508.

115. M. Pan, C. Lyu, J. Zhao and B. Shen, Sixty years (1957-2017) of research on toxoplasmosis in China – an overview, *Front. Microbiol.* 8 (2017) 1–16; <https://doi.org/10.3389/fmicb.2017.01825>
116. World Health Organization, *Toxoplasmosis: Greater Awareness Needed*; <http://www.euro.who.int/en/health-topics/disease-prevention/food-safety/news/news/2016/11/toxoplasmosis-greater-awareness-needed>; last access date November 23, 2018
117. K. Dzitko, A. Paneth, T. Plech, J. Pawelczyk, P. Staczek, J. Stefanska and P. Paneth, 1,4-Disubstituted thiosemicarbazide derivatives are potent inhibitors of *Toxoplasma gondii* proliferation, *Molecules* 19 (2014) 9926–9943; <https://doi.org/10.3390/molecules19079926>
118. J. M. Furtado, J. R. Smith, R. Belfort, D. Gattey and K. L. Winthrop, Toxoplasmosis: a global threat, *J. Global Infect. Dis.* 3 (2011) 281–284; <https://doi.org/10.4103/0974-777X.83536>
119. R. P. Tenorio, C. S. Carvalho, C. S. Pessanha, J. G. de Lima, A. R. de Faria, A. J. Alves, E. J. T. de Melo and A. J. S. Goes, Synthesis of thiosemicarbazone and 4-thiazolidinone derivatives and their *in vitro* anti-*Toxoplasma gondii* activity, *Bioorg. Med. Chem. Lett.* 15 (2005) 2575–2578; <https://doi.org/10.1016/j.bmcl.2005.03.048>
120. T. M. de Aquino, A. P. Liesen, R. E. A. da Silva, V. T. Lima, C. S. Carvalho, A. R. de Faria, J. M. de Araujo, J. G. de Lima, A. J. Alves, E. J. T. de Melo and A. J. S. Goes, Synthesis, anti-*Toxoplasma gondii* and antimicrobial activities of benzaldehyde 4-phenyl-3-thiosemicarbazones and 2-[(phenylmethylene)hydrazono]-4-oxo-3-phenyl-5-thiazolidineacetic acids, *Bioorg. Med. Chem.* 16 (2008) 446–456; <https://doi.org/10.1016/j.bmc.2007.09.025>
121. A. P. Liesen, T. M. de Aquino, C. S. Carvalho, V. T. Lima, J. M. de Araujo, J. G. de Lima, A. R. de Faria, E. J. T. de Melo, A. J. Alves, E. W. Alves, A. Q. Alves and A. J. S. Goes, Synthesis and evaluation of anti-*Toxoplasma gondii* and antimicrobial activities of thiosemicarbazides, 4-thiazolidinones and 1,3,4-thiadiazoles, *Eur. J. Med. Chem.* 45 (2010) 3685–3691; <https://doi.org/10.1016/j.ejmech.2010.05.017>
122. L. Monzote and A. Siddiq, Drug development to protozoan diseases, *Open Med. Chem. J.* 5 (2011) 1–3; <https://doi.org/10.2174/1874104501105010001>