Chapter

Thiazolidinone-Related Heterocyclic Compounds as Potential Antitrypanosomal Agents

Anna Kryshchyshyn, Danylo Kaminskyy, Philippe Grellier and Roman Lesyk

Abstract

Human African trypanosomiasis (HAT) and Chagas disease are neglected tropical diseases (NTDs) due to parasite protists from the *Trypanosoma* genus transmitted by insect vectors. Trypanosomiases affect mostly poor populations in the developing countries, and the development of new antitrypanosomal drugs is underinvested by governments and the pharmaceutical industry. In this chapter, we described the development of 4-thiazolidinone and thiazole derivatives with heterocyclic fragments which exhibit good inhibition of trypanosome growth and might constitute potential candidates for the development of new drugs against trypanosomiasis. Antitrypanosomal design, mainly within structure-based design, led to the synthesis of 5-ene-4-thiazolidinone-3-alkanecarboxylic acids; 2,3-disubstituted 4-thiazolidinones; thiazolidinone-pyrazoline, phenylindole-thiazolidinone, and imidazothiadiazole-thiazolidinone hybrids; as well as 4-thiazolidinone-based fused heterocycles, especially thiopyrano[2,3-*d*]thiazoles, and non-thiazolidinone compounds–namely, isothiocoumarine derivatives. Moreover, antitrypanosomal 4-thiazolidinones are of special interest in the search for new antimalarial and antileishmanial agents. Also many active anticancer agents among the abovementioned 4-thiazolidinones have been discovered.

Keywords: sleeping sickness, Chagas disease, antitrypanosomal drugs, thiazolidinone derivatives, hybrids

1. Introduction

Trypanosomatid infections belong to the neglected tropical diseases (NTDs)–a group of communicable diseases spread in 149 countries in the tropical and subtropical regions of the globe and affecting more than 1 billion people [1]. These vector-borne parasitic diseases are associated with poverty, contact with infectious vectors, as well as limited accesses to health services [2]. Human trypanosomiasis is caused by kinetoplastids, flagellated protists of *Trypanosoma* genus transmitted by an insect vector [3].

Trypanosoma brucei gambiense (*T.b. gambiense*) and *Trypanosoma brucei rhodesiense* (*T.b. rhodesiense*) are transmitted by the tsetse fly and cause two forms of

human African trypanosomiasis (HAT) known as sleeping sickness when neurological manifestations associated with presence of parasites in the brain become apparent [4]. *T.b. gambiense* accounts for more than 98% of reported cases; *T.b. rhodesiense* is responsible for an acute infection and represents under 2% of reported cases [5]. Other *Trypanosoma* species (e.g., *T. vivax*, *T. congolense*, and *T. evansi*) affect cattle, causing animal African trypanosomiasis (Nagana) and contributing to livestock losses. Cattle are also a reservoir of infection for human trypanosomes [6]. Therefore, the necessity to control animal trypanosomiasis should not be underestimated within the concept of "one health" [7]. There had been several devastating HAT epidemics during the twentieth century, the last one occurred in the late 1990s with estimated near 300,000 cases. Thanks to the coordinated work of the WHO and governmental and nongovernmental organizations to combat NTDs, the number of cases reported in 2009 has dropped below 10,000 for the first time in 50 years. This trend persists, and in 2019 there were less than 1000 incidences of HAT, although the estimated number of people being at risk of infection is near 65 million. First signs and symptoms of HAT are observed a few weeks after infection. During the first hemolymphatic stage, trypanosomes invade the human host and locally multiply spreading via the lymph and blood to various peripheral organs. The following meningoencephalitic stage develops when the parasites invade the brain parenchyma crossing the blood-brain barrier. The second stage of HAT is characterized by neurological disturbances and neuropsychiatric and sleep disorders [4]. If left untreated, the disease leads to coma and death [8]. Vector control is an important issue in the efforts taken to eliminate HAT. This is evidenced by the elimination of trypanosomiasis in Zanzibar Island due to tsetse clearance. This approach is still difficult to implement on a continent; therefore chemotherapy remains the main tool in the HAT management [9]. Difficulty of vaccine development because of the antigenic variation of the parasite surface proteins has been one more unsolved problem [10].

Chagas disease (American trypanosomiasis) caused by *Trypanosoma cruzi* (*T. cruzi*) is a devastating human disease with about 8 million infected people mostly in Latin and South America. Over the past decades, due to migration and population mobility, Chagas disease cases were reported in Europe, the United States, and Canada [11]. It is transmitted to man during the bite of a bloodsucking triatomine bug, via its feces or urine through skin breaks or mucous membranes, and occasionally causing outbreaks through contaminated food. Transmission through blood transfusion and pregnancy is also possible and, less frequently, through organ transplantation or laboratory accidents [12–14]. Once the parasite reaches the human host, it multiplies in the host's cells in the amastigote form that differentiates into the infective trypomastigote form, which is released after the host cell rupture, causing inflammatory reactions and leading to megaesophagus, megacolon, and cardiac conduction disturbances [15, 16]. Since Chagas disease was discovered in 1909, numerous studies have been carried out to investigate the pathogenesis of acute and chronic phases of the disease [11]. While the acute phase is often asymptomatic or characterized by non-specific symptoms, except sometimes occurring chagoma or Romaña sign, the chronic phase can be subdivided into an asymptomatic indeterminate phase and a symptomatic determinant phase [17]. Between 60% and 70% of serologically positive patients have no manifestation of the disease; in the remaining 30–40%, cardiac and gastrointestinal complications develop, indicating a symptomatic determinant phase [18]. If earlier autoimmune reactions were thought to be the primary factors leading to the lesions associated with the chronic stage, recent investigations showed that the persistence of parasites also contribute to the inflammatory processes, leading to cardiac or gastrointestinal complications. Therefore treatment success depends greatly on the elimination of *T. cruzi* from the organism [16].

1.1 Treatments of trypanosomiasis

1.1.1 HAT

Suramin, pentamidine, melarsoprol, and eflornithine have been used to treat HAT for decades [19, 20] (**Figure 1**). An important advance was the development of the nifurtimox-eflornithine combination therapy (NECT), which has now became the standard first-line treatment for the second stage of *T.b. gambiense* HAT [20, 21]. Choice of the drug as well as duration of treatment depends on the stage of the disease and the parasite subspecies. Pentamidine isethionate is the first-line treatment for the first stage of *T.b. gambiense* disease, while suramin is used in the treatment of first stage of HAT caused by *T.b. rhodesiense.* Intravenous treatment with suramin, although usually effective, especially when given early in the disease, can result in potential complications such as renal failure, skin lesions, anaphylactic shock, bone marrow toxicity, and neurological complications. Pentamidine, administered by the intramuscular route or intravenously, despite non-negligible undesirable effects (hypoglycemia, prolongation of the QT interval on electrocardiogram, hypotension, and gastrointestinal features), is in general well tolerated by patients and is usually effective [22, 23]. NECT, being the first-line treatment for the second stage of *T.b. gambiense* disease, consists of nifurtimox delivered orally and eflornithine delivered intravenously. In the case of contraindications to nifurtimox, eflornithine may be given as a monotherapy for *T.b. gambiense* HAT (meningoencephalitic stage), but it is not recommended for *T.b. rhodesiense* disease [4, 24]. Melarsoprol is restricted to the treatment of the second stage of *T.b. rhodesiense* HAT because of severe adverse drug reactions, such as an encephalopathy syndrome that occurs in 5–18% of all treated cases and may be fatal [25–27]. The only indication of

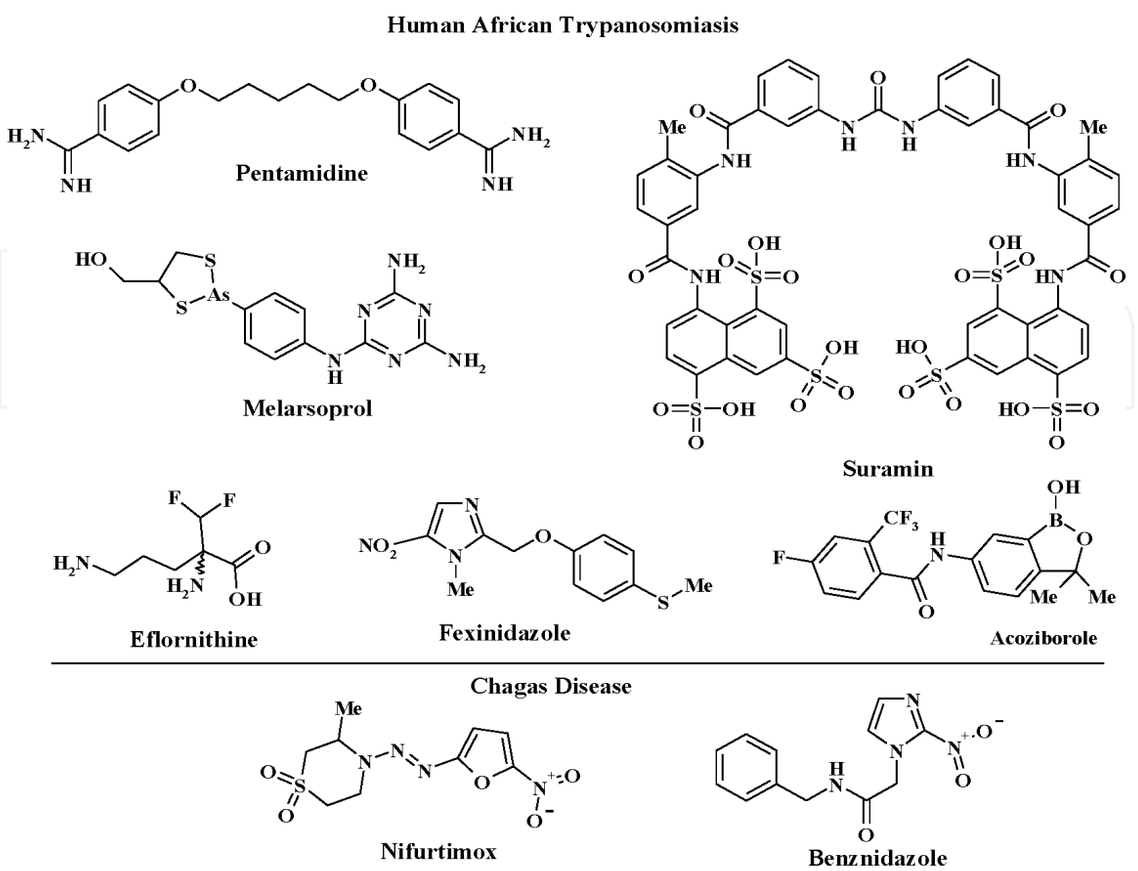

Figure 1.
Drugs used for human trypanosomiasis treatment.

melarsoprol for the treatment of *T.b. gambiense HAT* appears in the case of disease relapse after administering NECT or eflornithine monotherapy.

New effective oral monotherapy of HAT with fexinidazole has been developed and approved, so in 2018 the European Medicines Agency's (EMA) Committee for Medicinal Products for Human Use issued a positive opinion for fexinidazole treatment of *T.b. gambiense* HAT [28–30]. According to new WHO guidelines, under particular conditions, fexinidazole may replace pentamidine as first-line treatment in patients with the first stage of *T.b. gambiense* HAT and replace nifurtimox-eflornithine combination therapy as first-line treatment in patients with the second stage of *T.b. gambiense* HAT with fewer than 100 cerebrospinal fluid white blood cells per μL. These recommendations cannot be applied for the treatment of patients younger than 6 years or with a bodyweight less than 20 kg [31, 32]. One more new oral compound developed for treatment of all stages of *T.b. gambiense* HAT is acoziborole being at late Phase II/III of clinical trials [31].

1.1.2 Chagas disease

Only two drugs are currently available, nifurtimox and benznidazole (**Figure 1**), that both are active in the acute stage of the disease (up to 80% efficacy), though of limited efficacy against the established chronic stage of the disease [14, 33]. Benznidazole is a nitroimidazole, which generates radical species in aerobic and anaerobic conditions [34], and is the agent of choice for monotherapy of Chagas disease because of its extensive security and efficacy profile. Generalized adverse effects [17] as well as occasionally reported resistance to benznidazole make nifurtimox usage an alternative treatment. Both drugs produce important adverse reactions, especially in adults, because newborn, nursing, and small children tolerate these drugs better [35, 36]. In the acquired acute period, 70% of the cases are cured, and in newborn and nursing children with congenital Chagas disease, 98–100% cure is obtained. On the one hand, there is evidence about efficiency of benznidazole in early chronic infections [33], but on the other hand, the expediency of antitrypanosomal treatment in the chronic stages remains controversial, because of significant toxicity profiles and the unproven role in preventing the cardiomyopathy progression. Therefore, therapy for the majority of patients suffering from chronic Chagas disease consists mostly in nonetiologic treatments [11]. New effective and safety drugs are needed, especially for the chronic stage treatment [37].

1.2 Drug discovery strategies

As American and African trypanosomiases affect mostly poor population in the low- and middle-income countries and have not been interesting for the big pharmaceutical companies for years, a number of public and private institutions, partnerships, and consortia were initiated. For example, the Special Programme for Research and Training in Tropical Diseases of WHO (WHO/TDR), the European Commission [38] as a government agency, or the international Drugs for Neglected Diseases Initiative (DNDi) [39] had emerged. The work of these organizations has had an undeniable positive impact on the development of novel therapies and for the elimination of trypanosomiasis.

In general, three known major approaches to novel drug development, including antitrypanosomals, may be outlined: (i) ligand-based approach, (ii) target-based drug discovery [40], and (iii) phenotype-based drug discovery [41]. Different types of compounds, namely, thiosemicarbazones, thiazolidines, triazole- and furan-based compounds, benzofuran derivatives, peptidyl compounds, peptidomimetics acyl- and arylhydrazones, etc. have been studied as novel antitrypanosomal agents [42].

In ligand-based approaches, already known active synthetic and natural compounds or approved drugs are used as starting scaffolds to develop novel agents [43]. For example, development of pentamidine analogues resulted in the lead compound DB289 that underwent preclinical and clinical studies [44]. Other examples of the abovementioned approach are label extension or search for the new indications of existing drugs [45].

Target-based approaches involve screening of drug libraries with established targets, within target repurposing strategy, or screening libraries of novel compounds against a definite protein target. The structures of identified hit compounds are often optimized in order to increase their selectivity and pharmacokinetic properties or decrease their toxicity [46]. It should be mentioned that the target validation status used in the antitrypanosomal drug discovery often has not been clear. WHO/TDR Target Prioritization Network helps the scientists in the rational drug design of antiparasitic agents including antitrypanosomal drugs. The TDR Targets database, developed by this organization, contains information on validated, essential, as well as putative targets; it also can serve as a tool for prioritization of targets in whole genomes [47, 48].

1.3 Examples of targets used in novel antitrypanosomal agent development

1.3.1 Trypanosomatid peptidases

Numerous studies showed that intra- and/or extracellular trypanosomatid peptidases play important roles in different cell functions including invasion, intracellular survival, replication, differentiation, infectivity, immune evasion, and nutrition. "Validated" trypanosomatid peptidases belong to the endopeptidases and include cruzipain, prolyl oligopeptidases (POPs; *T. cruzi*), congopain (*T. congolense*), rhodesain (*T.b. rhodesiense*), and brucipain (*T.b. brucei*) [49]. For example, the cysteine peptidase cruzipain being differentially expressed in the different stages of *T. cruzi*, along with other peptidases, is responsible for parasite survival, differentiation, and growth. Cruzipain is a sulfated glycoprotein, which is investigated not only as a drug target but also as a candidate for vaccine development [50]. Selective inhibitors of this peptidase arrest metacyclogenesis in vitro and block the proliferation of both extracellular epimastigotes and intracellular amastigotes. The main lysosomal cysteine peptidases *rhodesain*, *brucipain*, and *congopain* are cathepsin L-like proteases [49]. They may play a role in anemia and immunosuppression due to infection, and conversely, anti-cysteine peptidase antibodies may modulate the trypanosome-induced pathology [51]. Oligopeptidases B and Tc80 are serine protease representatives of the prolyl oligopeptidase family [49]. Oligopeptidase B is involved in the mammalian host cell invasion by the trypomastigotes [52]. It retains full catalytic activity when released into the host bloodstream providing anomalous degradation of host peptide hormones that reinforces the importance of its protein-processing activity [53]. POP Tc80 has been detected in all the developmental stages of *T. cruzi* but is secreted by the trypomastigotes. POP Tc80 was shown to exhibit the unusual property of cleaving collagens I and IV, fibronectin, and peptide hormones. POP TC80 inhibitors block the host cell invasion by trypomastigotes; selectivity between parasitic and human POPs toward inhibitors could be expected [54, 55].

1.3.2 Nitroreductases

Nitroreductases are mainly associated with the nifurtimox mode of action. The activity of type I nitroreductase is believed to be "oxygen-insensitive" as it does not

involve oxygen in the reduction process and therefore does not cause the reactive oxygen species production. In contrast, the activity of type II nitroreductase results in the production of superoxide anions, so it is considered "oxygen-sensitive." Nifurtimox selectivity toward parasites was associated with the expression of type I nitroreductase. But, considering that nifurtimox-treated trypanosome extracts contain superoxide anions and nitro anion radicals, an oxidative stress with a type II nitroreductase involving is generally accepted to be the main trypanocidal mode of its action [56].

1.3.3 Dolicholphosphate mannose synthase

Dolicholphosphate mannose synthase is a mannosyltransferase critically involved in glycoconjugate biosynthesis in *T. brucei*. Variant surface glycoprotein (VSG) dimers, covering the surface of the parasite and undergoing constant antigenic variation, act as a physical diffusion barrier for components of the innate immune system as the parasite switches between many immunologically distinct VSG genes. All VSG variants are linked to the plasma membrane via glycosylphosphatidylinositol (GPI) anchors. The biosynthesis of GPI anchor was shown to be essential for viability of the bloodstream form of *T. brucei*, thus validating it as a drug target against HAT [57].

1.3.4 Dihydrofolate reductase

Dihydrofolate reductase (DHFR) is a key enzyme of the folate metabolism, deeply studied in the design of a number of anticancer, antibacterial, and antimalarial agents [58]. Detailed structural analysis of *T. brucei* and *T. cruzi* DHFRs showed their differences from the human enzyme, indicating them as attractive targets for the development of selective antitrypanosomals. Well-known DHFR inhibitors, as trimethoprim and pyrimethamine, are weakly active against *T. brucei* and *T. cruzi* DHFR unlike methotrexate being reported to inhibit *T. cruzi* enzyme in nanomolar concentrations [59].

1.3.5 Trypanothione reductase

Trypanothione reductase (TryR)–an enzyme of the NADPH-dependent flavoprotein oxidoreductase family–converts trypanothione disulfide into the physiologically relevant reduced dithiol. TryR is essential for growth of trypanosomatids as in the absence of catalase and glutathione peroxidase, the trypanothione system is involved in response to an oxidative stress. To some extent, trypanothione disulfide serves as glutathione in mammalian cells. Although mammalian glutathione reductase is homologous to parasite TryR, there are significant differences in their active sites [60, 61].

1.3.6 Kinases

The genomic analysis of *T. brucei* and *T. cruzi* revealed 156 and 171 eukaryotic protein kinases (PKs) in the parasite genomes. Atypical PKs representing four families, RIO, alpha, PIKK, and PDK, had also been discovered. Such an amount of PKs that are key mediators of signal transduction indicates the important role they play in trypanosomatid life cycles [62]. The differences in structure between trypanosomatid PKs and mammalian PKs as well as the evidence that some trypanosomatid PKs are vital for the parasite make these enzymes suitable for the antitrypanosomal drug search [63].

1.3.7 Triosephosphate isomerase

Triosephosphate isomerase (TIM) catalyzes the interconversion between glyceraldehyde 3-phosphate and dihydroxyacetone phosphate in the glycolytic pathway [64]. The presence of TIM in both human and parasite (68–74% of identity between both enzymes) makes targeting this enzyme problematic [65]. The structures of *T. brucei* and *T. cruzi* TIMs are also quite similar, except the structural differences that influence their different sensitivity to sulfhydryl reagents. *T. cruzi* TIM showed the highest sensitivity, constituting a good target for the development of selective therapeutics for the Chagas disease [66].

1.3.8 Farnesyl diphosphate synthase

Farnesyl diphosphate synthase (FPPS) catalyzes isopentenyl diphosphate and dimethylallyl diphosphate condensation resulting in the formation of geranyl diphosphate and subsequently farnesyl diphosphate that are precursors for the biosynthesis of isoprenoid derivatives (e.g., dolichols, sterols) and for protein prenylation. Bisphosphonates, such as alendronate and risedronate, are considered to be ligands for *T. cruzi* FPPS [67]. FPPS is an attractive target for antichagasic drug development as it is essential for parasite's growth and proliferation [68, 69].

1.3.9 Cyclic nucleotide-specific phosphodiesterases

Cyclic nucleotide-specific phosphodiesterases (PDEs) are also shown to be promising antitrypanosomal drug targets [70]. There are four distinct PDE families encoded in the genome of *T. brucei* [71].

Kinase inhibitors [72], such as human *Aurora kinase* inhibitors, typified by danusertib [73], and human *epidermal growth factor receptor* (EGFR) inhibitors lapatinib and canertinib [74] are examples of successful implementations of the target repurposing strategy when pathogen targets are matched with known homologous human targets.

One more variation of target-based drug design is the screening of known drug libraries in order to establish new pharmacological profile. For example, screening of a library of bioactive compounds against TryR [75] led to identification of a new class of TryR inhibitors based on indatraline, a nonselective monoamine reuptake inhibitor [76].

1.3.10 Lanosterol 14α-demethylase

Lanosterol 14α-demethylase or CYP51, which belongs to the family of cytochrome P450s, is one of the most promising antitrypanosomal targets. This enzyme is involved in the ergosterol biosynthesis, taking part in the production of components of the plasma membranes and serving as precursors for regulatory molecules that modulate growth, division, differentiation, and development processes [77, 78]. Fungicides as well as clinically used antifungal azoles inhibit CYP51 that along with the resemblance of sterol biosynthesis in trypanosomatids to such in fungi [79], makes *lanosterol 14α-demethylase* an attractive target for the design of antitrypanosomal agents.

In the era of target therapy, phenotypic screening that lies in pharmacological screening of chemical libraries against whole-cell or biological system should not be neglected [80–82]. This approach is particularly advantageous in the search of antitrypanosomals [83, 84], as the success strongly depends on the penetration properties of the drug into the parasite as well as on the crossing of the blood-brain barrier.

Sometimes, high-affinity ligands toward validated trypanosomal targets were shown ineffective *in vivo* against the parasite because not crossing the membranes, that is one more argument in favor of the whole-cell phenotypic assays [42]. Target resolution from phenotypic hits may also contribute to drug discovery process [84].

It should be mentioned that the parasites of *Leishmania* genus belong to the same order *Kinetoplastida* as *Trypanosoma* ssp. sharing some phylogenetic similarities [85]. Similar structural and biochemical features include, for example, special organelles (kinetoplast (mitochondrion with a discrete structured DNA body), glycosomes (involved in glycolysis)), a sub-pellicular microtubular corset, and a unique thiol metabolism [10, 86]. Interesting is that hit compounds found in antitrypanosomal screening may be used for the design of agents against *Leishmania* ssp. [87, 88] or *vice versa*.

2. 4-Thiazolidinone frame in the design of antitrypanosomals

4-Thiazolidinones are well-known class of azoles, which have been investigated for many decades as useful tools for the design and development of new drugs [89–93]. 4-Thiazolidinone scaffolds (2,4-thiazolidinedione, rhodanine (2-thioxo-4-thiazolidinone), 2-alkyl(aryl)-substituted and 2-amino(imino)-substituted 4-thiazolidinones) (**Figure 2**) are used as privileged structures and substructures in the modern medicinal chemistry [94–98] for the design of new anti-inflammatory, antitumor, antimicrobial, antidiabetic, antibacterial agents, etc. The synthetic approaches for these heterocycles are well known and described [96].

Majority of the 4-thiazolidinone-based hit and lead compounds, drug-like molecules, and approved drugs belong to derivatives containing the exocyclic double bond at C5 position—5-ene-4-thiazolidinones [96, 97]. These compounds, especially rhodanine derivatives, are possible Michael acceptors and are claimed as frequent hitters or pan-assay interference compounds (PAINS), being treated as useless in the drug discovery process because of their possible/predicted insufficient selectivity [99]. This statement should not be regarded as a general knockout criterion that excludes such screening hits from further development and should be studied in more detail [96, 97, 100, 101]. Therefore, "4-thiazolidinones and related scaffolds should not be regarded as problematic or promiscuous binders *per se*" [95], while "positive" properties of Michael acceptors should be effectively used [95, 97]. For instance, Michael acceptors are among the most effective activators of Nrf2 through the Keap1 modification, which open new perspectives in the treatment of inflammation, cancer, etc. [102]. Moreover, Michael acceptor properties are often not confirmed in experimental studies [103, 104] under conditions similar to physiological ones.

The search for new antimicrobial and antiparasitic agents based on 4-thiazolidinone cores is one of the earliest directions of biological studies of 4-thiazolidinones. The structural similarity of 4-azolidinones with penicillin antibiotics was the stimulus to the study of such type of activity [90, 105–107]. However,

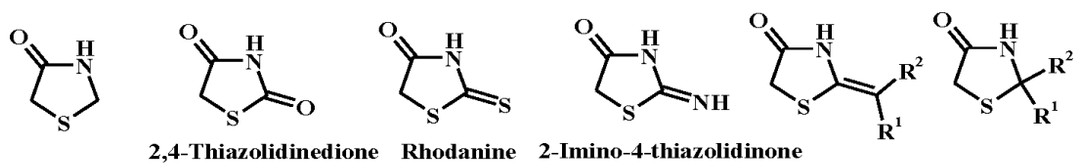

Figure 2.
Main 4-thiazolidinone-based scaffolds.

currently the effects of 4-thiazolidinones are not related to the "penicillin" mode of action [91, 96].

In the field of antiprotozoal agent search, the design of antitrypanosomal agents based on thiazolidinone scaffolds is of special interest [42, 108]. Data on the search for new antitrypanosomal agents among 4-thiazolidinone derivatives present mostly investigations on the inhibition of parasite growth (phenotype screening) mainly within a privileged substructure-based design. A much smaller number of publications are devoted to the study of the mechanism of action or the design of high-affinity ligands to "validated" targets [42, 96].

One of the arguments for the study of 4-thiazolidine-based compounds as antitrypanosomal agents is the thesis that thiazoles, especially 4-thiazolidinones, are considered as thioureas/thiosemicarbazones' cyclic analogues and biomimetics [42, 96, 108, 109]. Different (thio)ureas/(thio)semicarbazides were reported as inhibitors of the trypanosome proliferation [110–112] and had shown high affinity to the antitrypanosomal targets: cruzain and rhodesain [109, 113], cysteine proteases [114], etc. Different classes of "drug-like" molecules based on a thiazolidinone scaffold have been designed and synthesized in the process of search for antitrypanosomals [42, 115–119]. One of the most prominent directions is the conjugation of the thiazolidinone core with other different molecular fragments (mainly privileged substructures) [120, 121] that proves the efficiency of a molecular hybridization approach and a hybrid pharmacophore approach for the design of new antitrypanosomals [122–124].

Combination of 4-thiazolidinone and pyrazoline cores led to the synthesis of rows of promising trypanocidal agents (**1–4**) (**Figure 3**) with sub-micromolar activity levels against *T.b. brucei* and *T.b. gambiense* [121, 125–127] and low toxicity levels against mammalian cells.

Compounds with an enamine linker **5**, **6** (**Figure 4**) were designed based on the early hits **1**, **2** (4-thiazolidinone and pyrazoline cores are bonded without additional linker). Most active compounds from these series, 5-[5-(4-methoxyphenyl)-3-naphthalen-2-yl-4,5-dihydropyrazol-1-ylmethylene]-3-methyl-2-thioxothiazolidin-4-one (IC_{50} = 0.6μM) and 5-[5-(2-hydroxyphenyl)-3-(4-methoxyphenyl)-4,5-dihydropyrazol-1-ylmethylene]-3-(3-acetoxyphenyl)-2-thioxothiazolidin-4-one (IC_{50} = 0.7μM), possess sub-micromolar activities and high selectivity indexes [121]. Elongation of the enamine bearing linker group (compounds **6**) led to a decrease of the activity, and modification of the N3 position of thiazolidinone core (compounds

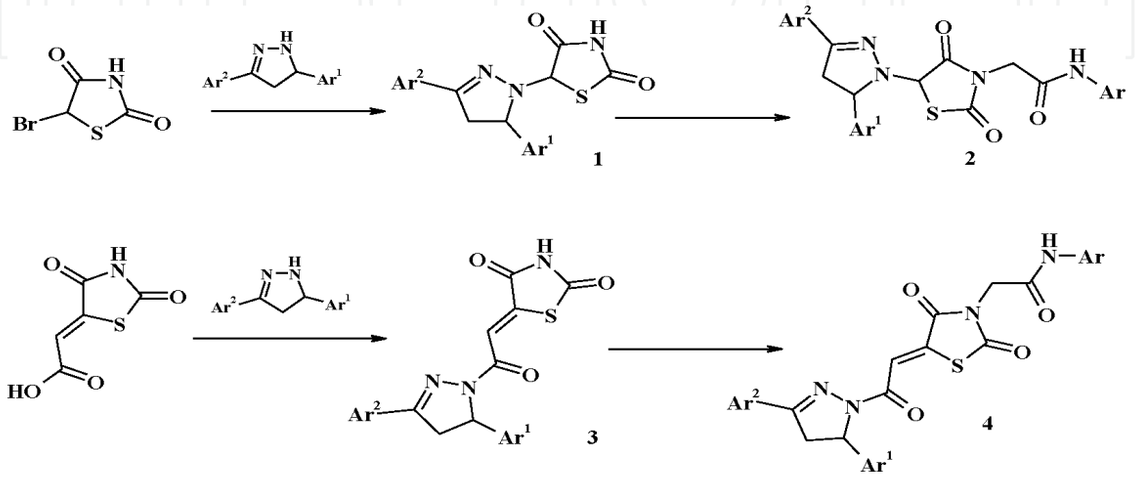

Figure 3.
Thiazolidinone-pyrazoline conjugate synthesis.

5 as well as compounds **2** and **4**) was considered as crucial for the trypanocidal activity (methyl or small aryl fragments are desirable) [127].

It should be noted that mentioned compounds are considered as prominent anticancer agents [127] and compounds **5** showed a strong antileukemic activity with an apoptotic-related mitochondria-dependent mode of action with a prooxidant action [128].

Related 4-thiazolidinone-pyrazoline conjugates **7** (**Figure 5**) synthesized based on an isorhodanine (4-thioxo-2-thiazolidinone) core [129, 130] were also studied in vitro against *T.b. brucei*, and compounds with a micromolar activity were identified [126].

A moderate antitrypanosomal activity of pyrimidine-thiazolidine-4-one hybrids **8** (**Figure 6**) was reported against bloodstream forms of *T.b. brucei* (IC_{50} = 25–100 μM) [131].

Related 2,3-substituted 4-thiazolidinones **9** with simple aromatic substituents at the position C2 and N3 also possessed low to moderate levels of activity against *T.b. brucei* and *T.b. gambiense* [132]. The synthetic methods for their obtaining are based on the one-pot three-component reaction of amine, oxocompound, and thioglycolic acid or its derivatives [133, 134]. It should be noted that the abovementioned derivatives of thioglycolic acids, namely, 2-mercaptoacrylic acids, can be easily synthesized or formed via a metabolic transformation based on simple 5-arylidenerhodanines (**Figure 7**) and possess similar pharmacological profiles [135].

Moreover, simple 5-ene-2,4-thiazolidinones were proposed as possible scaffolds for the design of new antitrypanosomal agents as pteridine reductase 1 inhibitors [136].

5-Arylidenerhodanine-3-acetic acids **10** (**Figure 8**) as one of the most studied types of thiazolidinones were reported to inhibit the activity of the dolicholphosphate mannose synthase and the GPI anchor synthesis and exhibited trypanocidal activity against the bloodstream forms of *T.b. brucei* (ED_{50} = from 96 to 492 μM) [57]. Structure optimization of 4-thiazolidinone-carboxylic acids, including compounds with anticancer properties [137, 138], allowed to obtain a series of 2-(5-aminomethylene-4-oxo-2-thioxothiazolidin-3-yl)-3-phenylpropionic acid ethyl esters **11**. Among them, several hit compounds (2-{5-[(5-chloro-2-methoxyphenylamino)-methylene]-4-oxo-2-thioxothiazolidin-3-yl}-3-phenylpropionic acid ethyl ester, 2-(5-{[2-methyl-5-(morpholine-4-sulfonyl) phenylamino]-methylene}-4-oxo-2-thioxothiazolidin-3-yl)-3-phenylpropionic acid ethyl ester, and 4-{[3-(1-ethoxycarbonyl-2-phenylethyl)-4-oxo-2-thioxothiazoli-

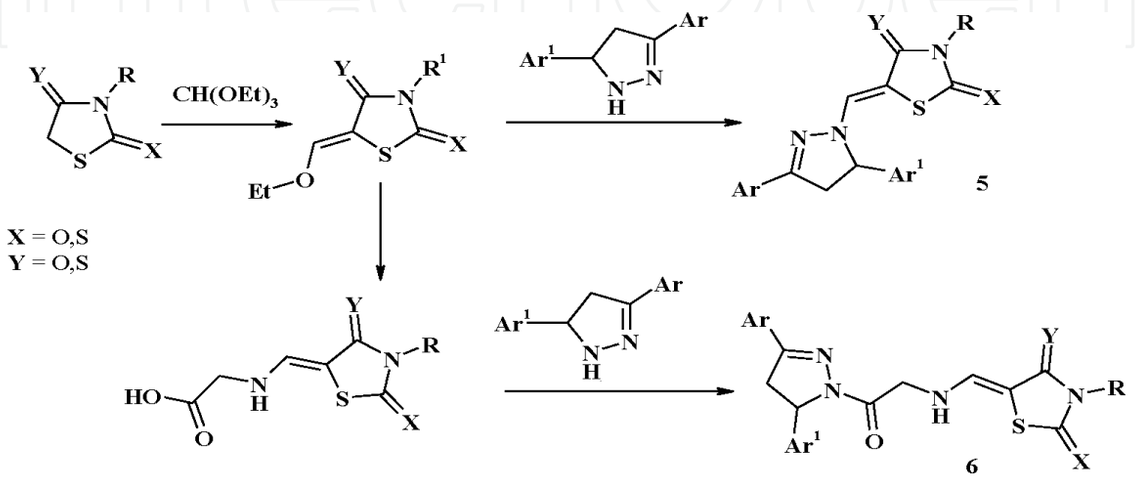

Figure 4.
5-Enamine 4-thiazolidinone-pyrazoline conjugates.

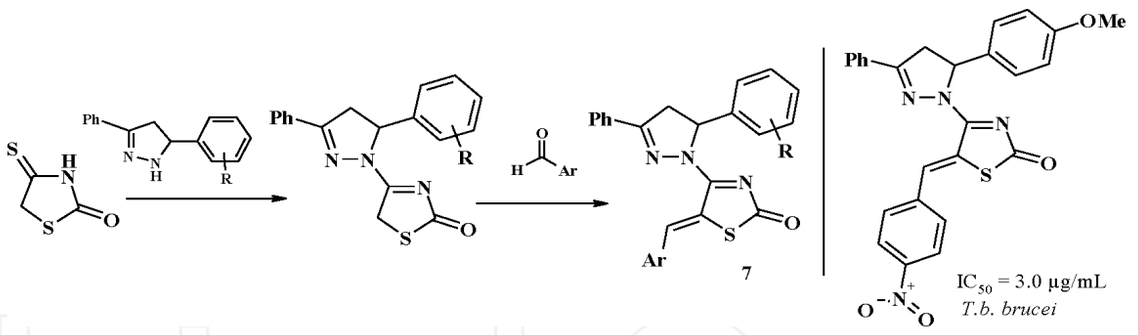

Figure 5.
4-Substituted 2-thiazolidinone synthesis.

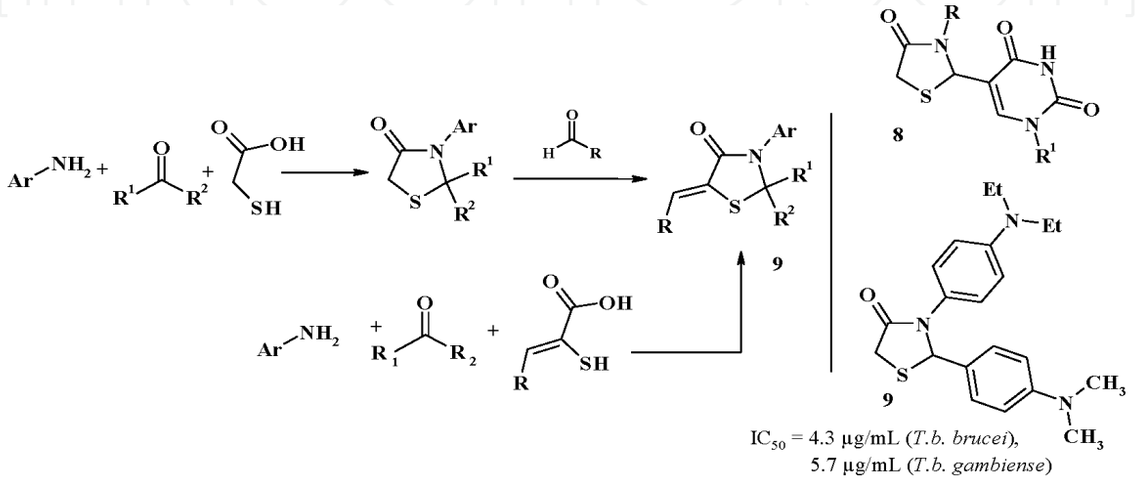

Figure 6.
2,3-Disubstituted 4-thiazolidinone synthesis.

din-5-ylidenemethyl]-amino}-benzoic acid ethyl ester) inhibited the in vitro growth of *T.b. brucei* and *T.b. gambiense* at nano- and sub-micromolar concentrations (IC_{50} = 0.027–1.936 µM), and significant selectivity indices (SI = 108–1396) were calculated [139].

Screening of a focused kinase inhibitor library against cultures of *T.b. brucei* allowed identifying a series of active compounds based on 2,4-diaminothiazoles, some of them possessing antitrypanosomal activity at the nanomolar range [140]. Combination of thiazolidine scaffold with a thiophene moiety yielded thiophen-2-iminothiazolidine hybrids that showed trypanocidal activity in vitro against *T. cruzi* (amastigote and trypomastigote forms) and cruzain inhibition activity [115].

One of the directions for the design of new antitrypanosomal agents using a molecular hybridization approach is the utilization of hydrazone fragments (**Figure 9**) as the linker group for the connection of the thiazole/4-thiazolidinone scaffold with the other molecular fragments [117, 141–147].

Screening of 4-thiazolidinone-hydrazones against *T. cruzi* yielded active and non-cytotoxic compounds **12** (**Figure 10**) [148, 149]. The 2-hydrazolyl-4-thiazolidinone-5-carboxylic acid derivatives **13** have shown promising activity on the cruzipain protease. Compounds were selected based on a virtual screening of 500,000 chemical structures (ZINC5 database). Structurally related compounds **14** (with exocyclic double bond at C5 position) showed the highest antiproliferative activity when screened on *T. cruzi* epimastigotes but were inactive toward cruzipain [127]. 5-Alkyl-4-thiazolidinone-2-hydrazones **15** tested in a cruzain inhibition assay and against cultures of the epimastigote and trypomastigote forms (*T. cruzi*, Y strain) inhibited the cruzain activity and showed an antiproliferative activity

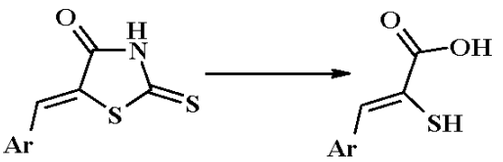

Figure 7.
Thiazolidinone-based approach to 2-mercaptoacrylic acid formation.

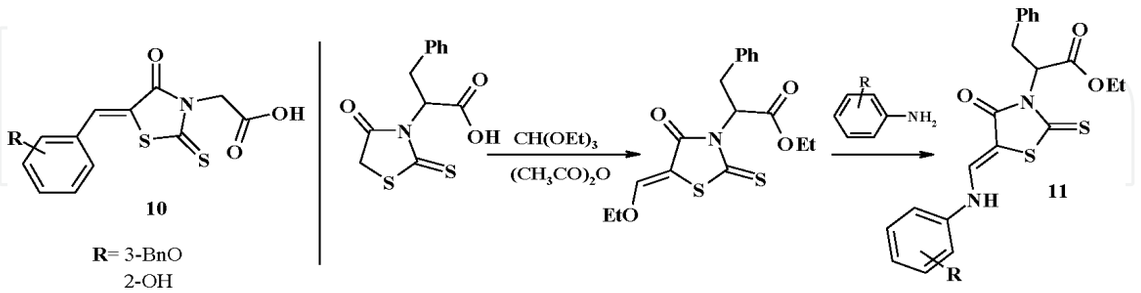

Figure 8.
5-Ene-4-thiazolidinone-3-carboxylic acid synthesis.

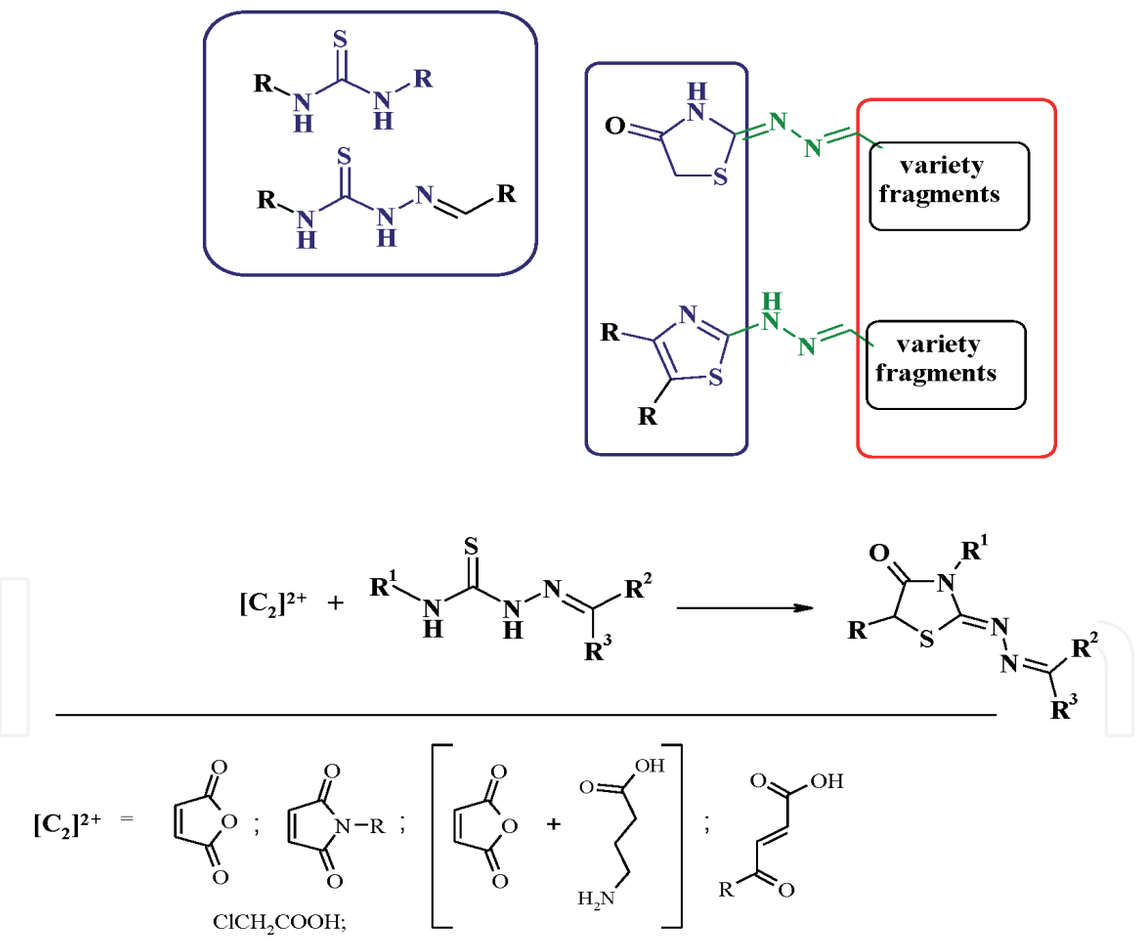

Figure 9.
2-Hydrazono-4-thiazolidinone synthesis.

at non-cytotoxic concentrations [150]. Study of analogues, namely, 2-imino-1,3-thiazoles, showed that the bioisosteric replacement of thiazolidine cycle with thiazole led to loss of the cruzain inhibitory activity and a significant reduction of the trypanocidal activity. The most potent cruzain inhibitor 2-((1-phenoxypropan-2-ylidene)hydrazono)-3-phenyl-5-isopropylthiazolidine-4-one also impaired intracellular trypomastigote development and attenuated trypomastigote invasion

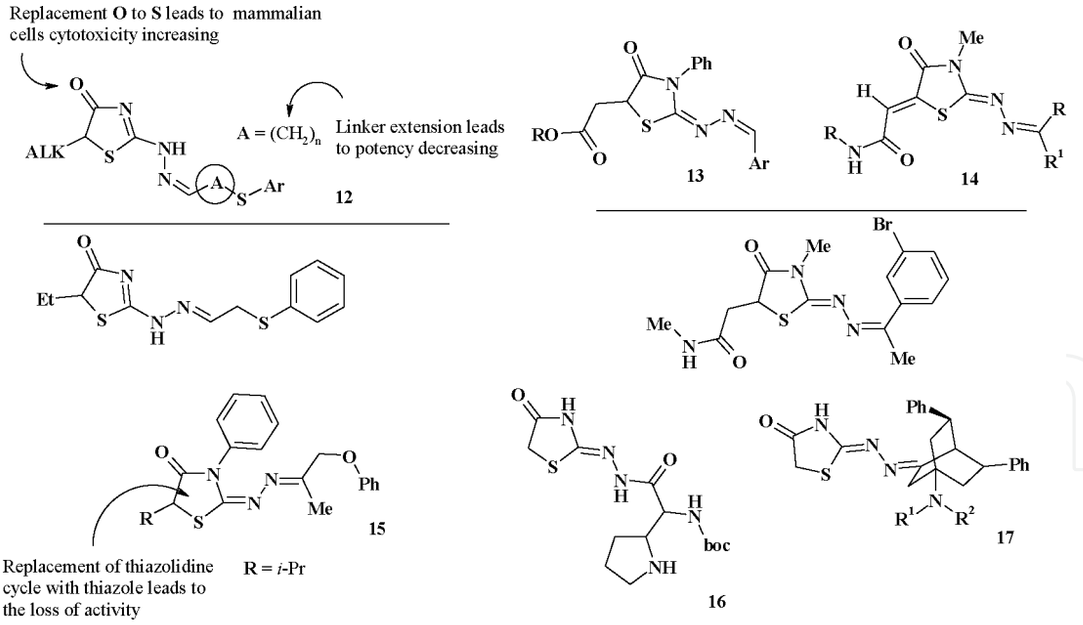

Figure 10.
4-Thiazolidinone-hydrazones as trypanocidal agents.

of macrophages; however it did not eradicate parasite in mice [150]. 2-Aminoacyl-4-thiazolidinone derivatives also showed good trypanocidal properties against *T. cruzi*; the proline derivative **16** showed differences of efficiency according to the parasite strains tested (Y strain vs Colombian strain). Docking analysis to *T. cruzi* cruzain that corroborated the experimental IC_{50} data and analysis of the binding characteristics of tested ligands revealed important interactions, which explain the affinity of such derivatives to cruzain [42, 151]. Combination of 4-dialkylaminobicyclo[2.2.2]octane fragment with the 5-unsubstituted 4-thiazolidinone core led to compounds **17** with weak to moderate activity against *T.b. rhodesiense* [152].

Molecular hybridization of the thiazole ring with a pyridine moiety through a hydrazine bridge led to identification of selective *N*-[3-phenyl-3*H*-thiazol-2-ylidene]-*N*′-(1-pyridin-2-yl-ethylidene)-hydrazines inducing the parasite death via an apoptotic mechanism [153]. Combination of a thiazole core with fused [6+5] or [6+6] scaffolds turned out to be especially interesting, leading to highly active and selective antitrypanosomal agents. Synthesized indanone-thiazole hybrids **18** (**Figure 11**) provide good trypanocidal properties against *T. cruzi* (IC_{50} within 0.09–1.35 μM, Tulahuen 2 strain); these compounds were also characterized by low mammalian cell cytotoxicity [154].

Development of optimization directions of thiazolidinone-hydrazone structures led to new hybrid molecules bearing thiazolidinone/thiazole and 2-phenylindole/6-phenyl-imidazo[2,1-*b*][1, 3, 4]thiadiazole cores with hydrazone linkers **19**, **20** [155]. Compounds with sub-micromolar levels of trypanocidal activity toward bloodstream forms of *T.b. brucei* and *T.b. gambiense* and relatively low cytotoxicity upon human primary fibroblasts were identified, as well as some aspects of SAR (**Figure 12**) were derived.

Compounds with a 2-arylindole fragment were more active than 6-aryl-imidazo[2,1-*b*][1, 3, 4]thiadiazole analogues. For the compounds without phenyl ring attached to the indole fragment, no significant antitrypanosomal activity was found as well as for the compounds with a C5-ene-fragment in the 4-thiazolidinone core [155].

The main features of the molecular structure of thiazolidinone-hydrazone-based compounds can be outlined as the following: (i) thiazole core (position C4, small aryl or alkyl substituent; C5 position, unsubstituted or small alkyl fragment;

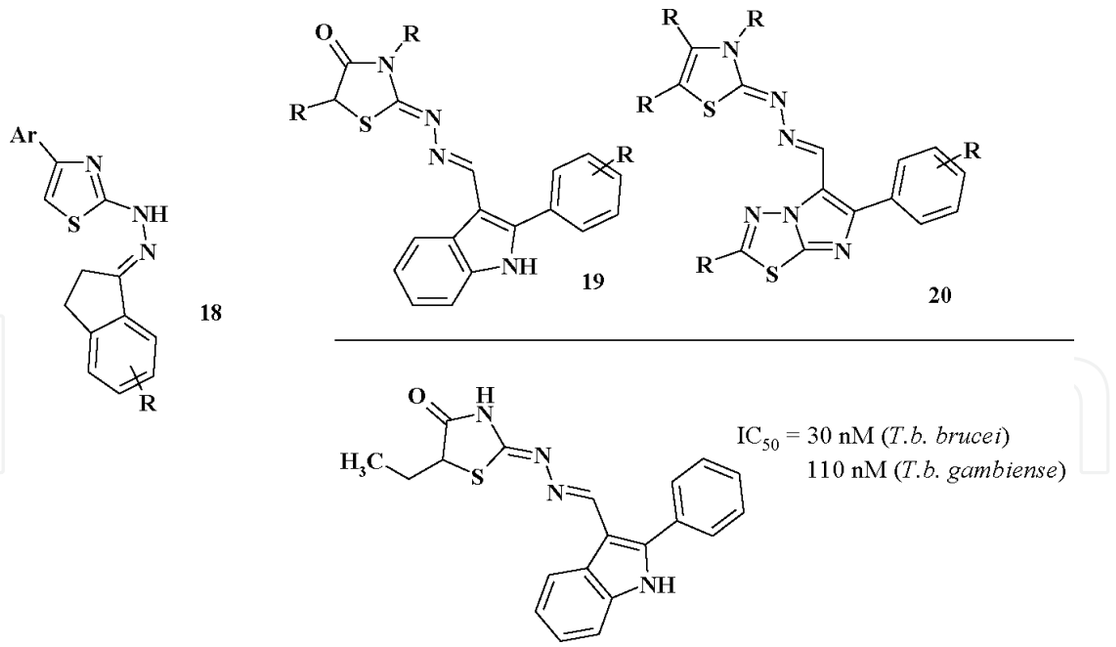

Figure 11.
Thiazolidinone-indanone/indole/imidazothiadiazole hybrids.

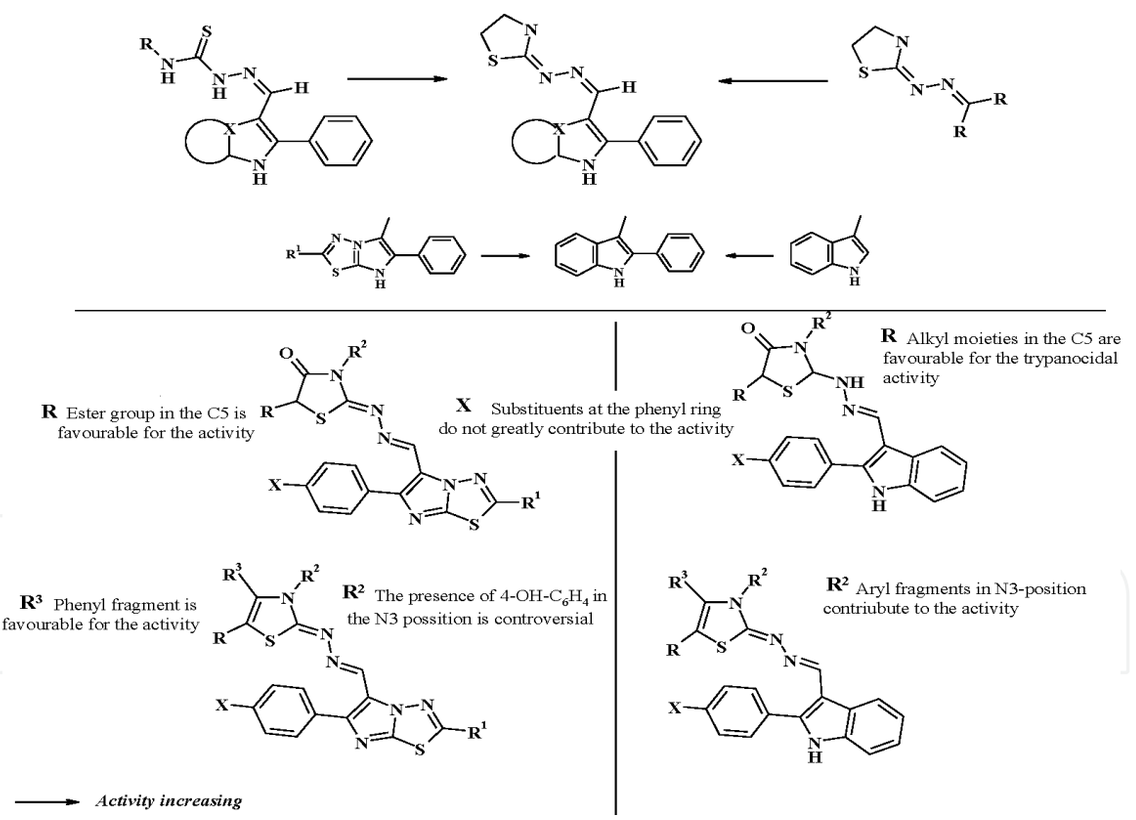

Figure 12.
SAR of indole/imidazothiadiazole-thiazolidinone/thiazole hybrids.

N3 position, variety of substituents) or 4-thiazolidinone core (C5 position, unsubstituted or small alkyl fragment); (ii) hydrazone linker in the C2 position of the main core; (iii) additional molecular fragment, diverse substituents (from simple alkyl(aryl)ydene fragment to privileged heterocyclic cores); and (iv) target compounds imitating the thiosemicarbazones with trypanocidal activity [147, 153, 155].

The "fixation" of the hydrazone fragment in a pyrazoline core (**Figure 13**) as one of the methods of such compound optimization has been also described for the synthesis of active compounds **21**, **22** [126, 127].

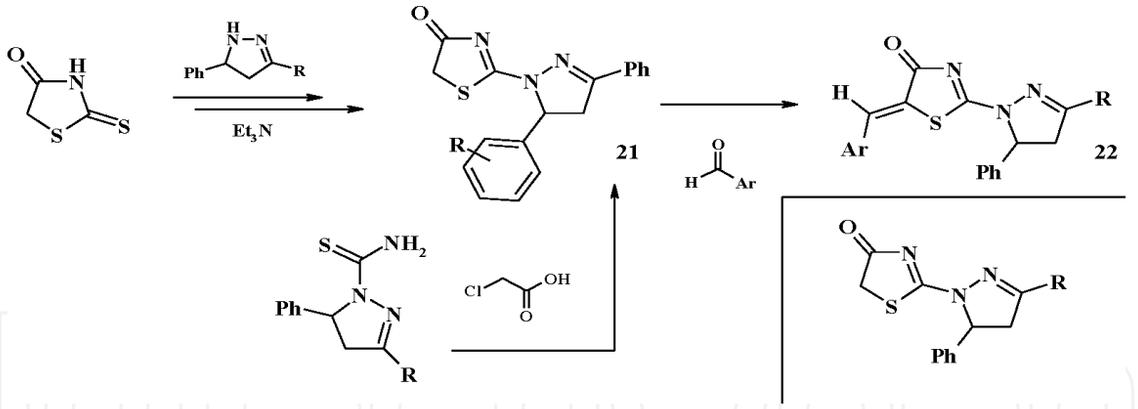

Figure 13.
"Fixation" of hydrazone fragment for thiazolidinone-pyrazoline hybrid synthesis.

The hit compound from thiazolidinone-pyrazoline hybrids **21** showed inhibitory activity on the in vitro growth of *T.b. rhodesiense* (IC_{50} = 12 μg/mL) and *Leishmania donovan*i (IC_{50}> 30 μg/mL) and a higher influence on *Plasmodium falciparum* (IC_{50} > 5 μg/mL) with cytotoxicity level CC_{50}> 90 μg/mL.

3. Fused heterocyclic molecules based on the core 4-thiazolidinone

Thiopyranothiazoles that frequently are synthesized in *hetero*-Diels-Alder reaction starting from 5-ene-thiazolidinones are considered as their fused mimetics, without Michael acceptor properties, though with saved pharmacological profiles (**Figure 14**) [90, 96, 156, 157].

So, various thiopyranothiazoles serve as a fruitful source of drug-like molecules that, unlike their precursors 5-ylidene-4-thiazolidinones, cannot be claimed as PAINS [99]. This class of fused thiazolidinone derivatives is characterized by a number of different biological activities [158], the most studied being the antitumor activity [96, 157, 159, 160]. Recently, antiparasitic properties of these polycyclic compounds have been also reported.

A series of N-substituted thiopyrano[2,3-*d*]thiazoles showed excellent inhibitory activity of *T.b. brucei* (bloodstream form) at the concentration of 10 μg/mL in vitro. The most promising compounds were 3-[2-(4-fluoro(chloro)phenyl)-2-oxoethyl]-3,5a,6,11b-tetrahydro-2*H*,5*H*-chromeno[4′,3′:4,5]thiopyrano[2,3-*d*] thiazol-2-ones and N-(4-chloro(ethylcarboxy)phenyl)-2-(2-oxo-5a-methyl-(5aRS,11bSR)-3,5a,6,11b-tetrahydro-2*H*,5*H*-chromeno[4′,3′:4,5]thiopyrano[2,3-*d*] thiazol-3-yl)-acetamides **23** (**Figure 15**) that inhibited more than 95% of parasite growth in the above concentration and near quarter at the concentration of 1 μg/mL [132].

Development of novel synthetic protocols for the thiopyrano[2,3-*d*]thiazoles and their modifications led to the synthesis of new spiro thiopyrano[2,3-*d*]thiazoles. A hit compound rel-(6′R,7′R)-7′-(3,4-dimethoxyphenyl)-1-(4-chlorophenyl)-3′,7′-dihydro-2*H*,2′*H*,5*H*-spiro[pyrolidin-3,6′-thiopyrano[2,3-*d*]thiazol]-2,2′,5-trione **24** (**Figure 16**), inhibiting growth of *T.b. brucei* and *T.b. gambiense* with the IC_{50} values of 0.26 μM and 0.42 μM, respectively, was identified [161].

Effective and feasible method of functionalized thiazolothiopyrane core synthesis has been the utilization of norbornene as a dienophile with 5-ylidene-isorhodanines as heterodienes in the *hetero*-Diels-Alder reaction. Obtained 9-aryl(heteryl)-3,7-dithia-5-azatetracyclo[9.2.1.$0^{2,10}$.$0^{4,8}$]tetradecen-4(8)-ones-6 and their N-arylidene substituted analogues **25** (**Figure 17**) showed moderate trypanocidal activity. The most active representatives possessed IC_{50} within 3.7–4.1

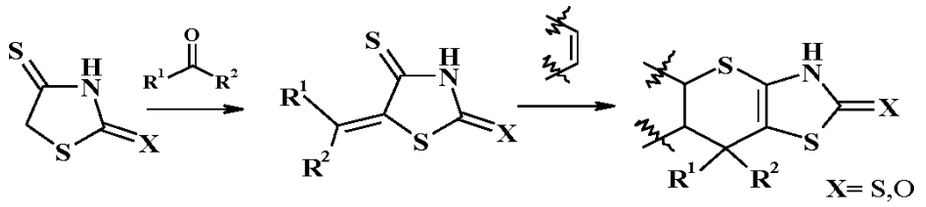

Figure 14.
General scheme of thiopyranothiazole core formation.

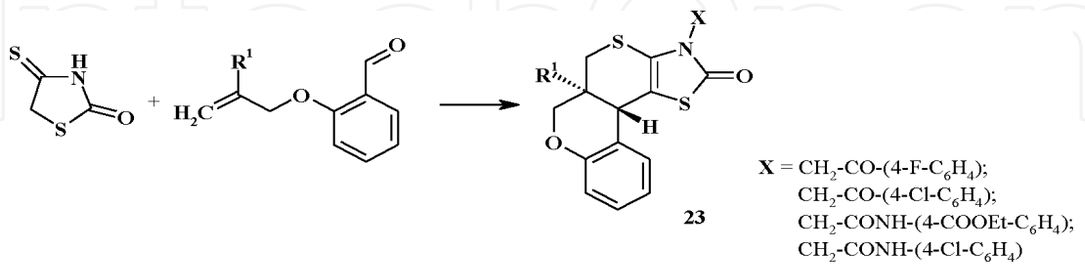

Figure 15.
Chromeno-thiopyrano-thiazolidinones as trypanocidal agents.

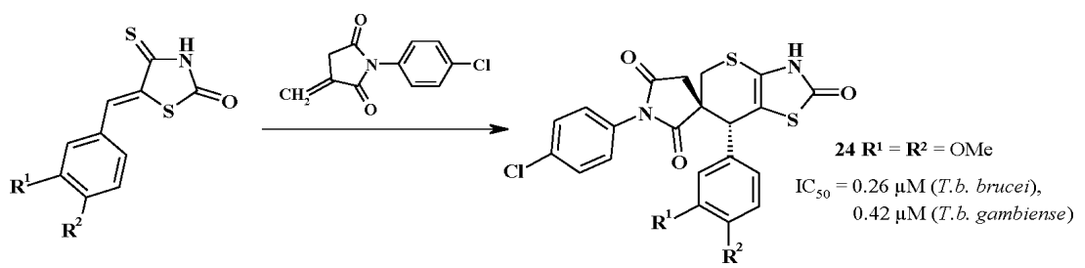

Figure 16.
*Synthesis of spiro thiopyrano[2,3-*d*]thiazole derivatives as trypanocidal agents.*

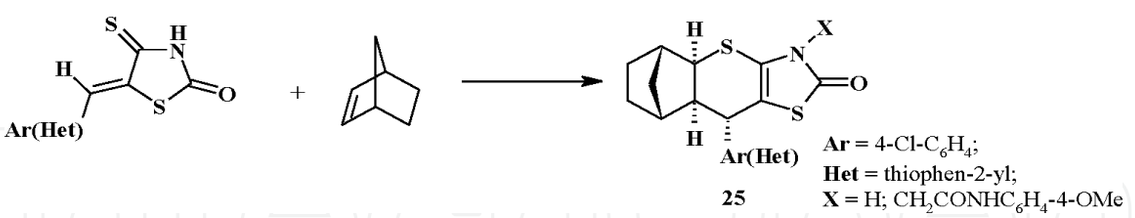

Figure 17.
*Thiopyrano[2,3-*d*]thiazoles bearing norbornane moiety as antitrypanosomal agent.*

μM against *T.b. brucei*. Interesting was the dual antileukemic and trypanocidal effects observed for some thiopyranothiazoles bearing norbornane moiety that may be used for establishing the molecular mode of action for this class of compounds [118].

Comparable antitrypanosomal activity was observed for a series of isothiochromeno[4a,4-*d*][1,3]thiazoles **26** (**Figure 18**) in vitro against bloodstream forms of *T.b. brucei*. It should be mentioned that SAR analysis revealed the positive influence of N3-substituent for the trypanocidal activity. The same trend was found for the abovementioned tetracyclic thiopyrano[2,3-*d*]thiazoles **23** and thiopyranothiazoles with norbornane core **25**. Good trypanocidal properties along with a low acute toxicity in mice (LD_{50}: 240–480 mg/kg) for the isothiochromeno[4a,4-*d*][1,3] thiazole hits make such fused systems based on the thiazolidinone core attractive scaffolds for the discovery of antitrypanosomals [162].

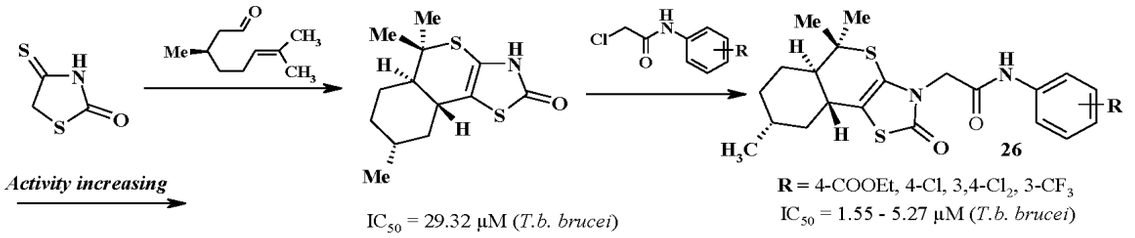

Figure 18.
*Isothiochromeno[4a,4-*d*][1,3]thiazoles as antitrypanosomal agents.*

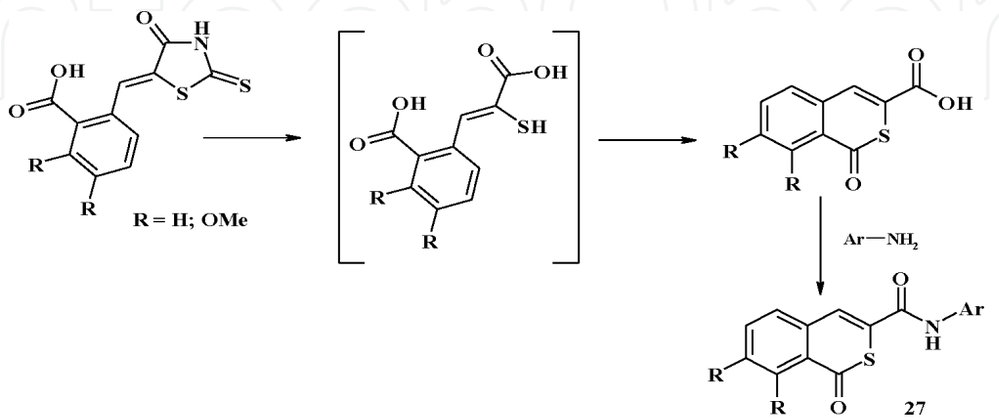

Figure 19.
Rhodanine-based isothiocoumarine derivative synthesis.

One more class of polycyclic fused molecules based on the thiazolidinone scaffold, being tested against *T.b. brucei*, were different 1-oxo-*1H*-2-benzothiopyran-3-carboxylic acids. The latter were synthesized in a result of heterocyclization of intermediates obtained in the hydrolysis reaction of 5-arylidenerhodanines with substituent in *ortho* position (**Figure 19**). Investigated amides did not exhibit significant antitrypanosomal effects except 1-oxo-1H-isothiochromene-3-carboxylic acid naphthalen-1-ylamide and 7,8-dimethoxy-1-oxo-1H-isothiochromene-3-carboxylic acid (4-sulfamoyl-phenyl)-amide **27** that inhibited growth of *T.b. brucei* bloodstream forms [119].

4. Conclusion

Thus, 4-thiazolidinone derivatives, especially thiazolidinone-bearing hybrids, as well as fused analogues are efficient compounds for the design of new antitrypanosomal agents within different drug design strategies. Thiazolidinone derivatives are more active than the known thiosemicarbazone analogues. Moreover, they can be used as starting compounds for the design and development of non-thiazolidinone compounds with trypanocidal activity. In addition, there are many active anticancer agents among 4-thiazolidinones with trypanocidal properties, and some active antitrypanosomal 4-thiazolidinones can be interesting for the search for new antimalarial and antileishmanial agents.

Conflict of interest

The authors declare no conflict of interest.

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