Specific Molecular Targets to Control Tropical Diseases

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Abstract: Chagas' disease or American trypanosomiasis is considered by the Word Health Organization to be one of the important tropical parasitic diseases worldwide together with malaria and schistosomiasis. The etiologic agent of this illness is the kinetoplastid protozoon *Trypanosoma cruzi*. The present chemotherapy for the treatment of Chagas' disease remains unsolved. The drugs currently in use are old, ineffective and toxic. Bearing in mind the metabolic differences between the parasite and the mammalian host, some attractive interesting molecular targets for drug design are presented.

INTRODUCTION AND OVERVIEW

Chagas' disease or American trypanosomiasis is a major health problem in Central and South America. It is produced by the kinetoplastid protozoan *Trypanosoma cruzi*. This illness is an important cause of morbidity and mortality from Southern United States to Southern Argentina, and is considered by the World Health Organization as one of the major parasitic diseases worldwide [1]. In fact, the number of infected people with the etiologic agent for Chagas' disease was estimated to be close to 18 million with 90 million at risk. 2-3 million individuals develop the characteristic symptoms of this disease that produces almost 50,000 deaths each year [2].

In rural areas this illness is transmitted by reduviid bugs such as *Rhodnius prolixus* or *Triatoma infestans* as a consequence of blood-sucking activity among these Chagas' disease vectors and humans and other mammals [3]. Similar to other kinetoplastid parasites, *T. cruzi* has a complex life cycle: It multiplies within the insect gastrointestinal track as a noninfective epimastigote form, which differentiates to the nondividing highly infecting metacyclic trypomastigotes. This form is released within the insect excrements and the circulatory system is reached *via* the wounds produced by the blood-sucking activity of the vector. Once in the mammalian host, *T. cruzi* invades different kind of tissues with preference for the cardiac muscle and the gastrointestinal track. After invasion the parasite differentiates to the amastigote form, which proliferates intracellularly. Finally, this form redifferentiates to the nondividing trypomastigote form, which is liberated to the bloodstream [3-5]. The bloodstream trypomastigote form can either invade other tissues or can infect the respective Chagas' disease vectors closing the cycle [3]. Transmission of Chagas' disease could also occur *via* the placenta or by transfusion of contaminated blood [6]. This latter mechanism is responsible for the occurrence of Chagas' disease in countries where the disease is not endemic [7,8].

In the last few years a large amount of work on the biochemistry and physiology of *T. cruzi* has been done. However, the corresponding chemotherapy to control Chagas' disease remains unsolved [9]. It is based on old and fairly unspecific drugs such as nifurtimox ((4-([5-nitro-furfurylidene]-amino)-3-methylthiomorpholine-1,1-dioxide compound **1**) and benznidazole (*N*-benzyl-2-nitro-1-imidazoleacetamide; compound **2**).[3] Both of these drugs are able to cure at least 50% of recent infections according to the disappearance of symptoms and to give rise to negativization of parasitemia and serology in most

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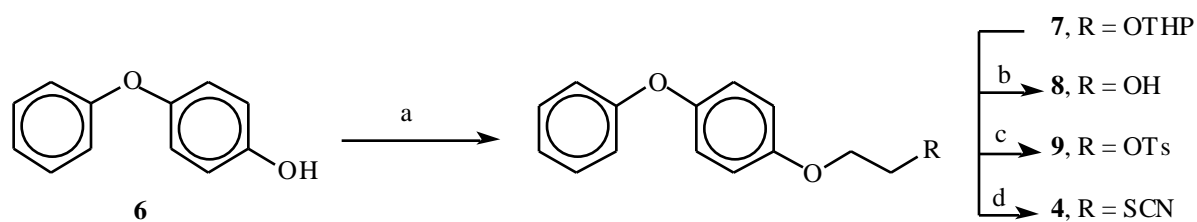
Scheme 1. Reagents and conditions: (a) KOH, DMSO; BrCH$_2$CH$_2$OTHP, rt, 16 h; (b) PPTs, MeOH, rt; (c) ClTs/py, 0 °C, 4 h; (d) KSCN, DMF, 100 °C, 6 h.

cases [10-12]. Nevertheless, these drugs suffer from serious drawbacks: (a) in acute infections, therapy has not been consistent among distinct geographical areas, presumably due to selective drug sensitivity on different *T. cruzi* strains [10,11]; (b) both drugs produce serious side effects including vomiting, anorexia, peripheral neuropathy, allergic dermopathy, etc [13]; (c) long term treatment is another disadvantage since these agents have to be administered for extended periods [13]. Therefore, it exits an urgent need of having new chemotherapeutic agents that are effective against all strains of *T. cruzi*, and with less or no side effects than those currently available [14]. Some questions have been raised

Fig. (1). Chemical Structures of the Drugs Currently in use for the Treatment of Chagas' Disease and Blood Sterilization.

about the value of these drugs for parasitological cure in the indeterminate or chronic stage of the infections. Certainly, after treatment, serology remains positive in most cases even when parasitemia is absent [15]. The only drug available to prevent blood transmission of Chagas' disease is Gentian Violet (*N*-{4-bis[[4-(dimethylamino)-phenyl]methylene]-2,5-cyclohexadien-1-ylidene} *N*-methylammonium chloride, compound **3**), a dye discovered for that purpose many years ago (Figure 1) [16]. However, the innocuousness of this drug is in doubt because it is carcinogenic in some animal models [17].

Most pharmaceutical companies do not carry out serious research and developmental programs because is not commercially attractive. In fact, this illness is closely related to poverty and bad housing quality and the impetus to design and develop new drugs has fallen largely to academic institutions. The above arguments stress the need of having new antiparasitic agents that are effective against all strains of *T. cruzi*, and with less or no side effects than those currently available [14,17]. The rational chemotherapeutic approaches are focused on the metabolic differences between this pathogenic microorganism and mammals. Then, it can be anticipated that the selective inhibition of a biosynthetic pathway that leads to a crucial metabolite for parasite survival would not have any significant toxic effect for the host. In the present review article, some particular molecular targets with potential utility from the chemotherapeutic point of view are presented.
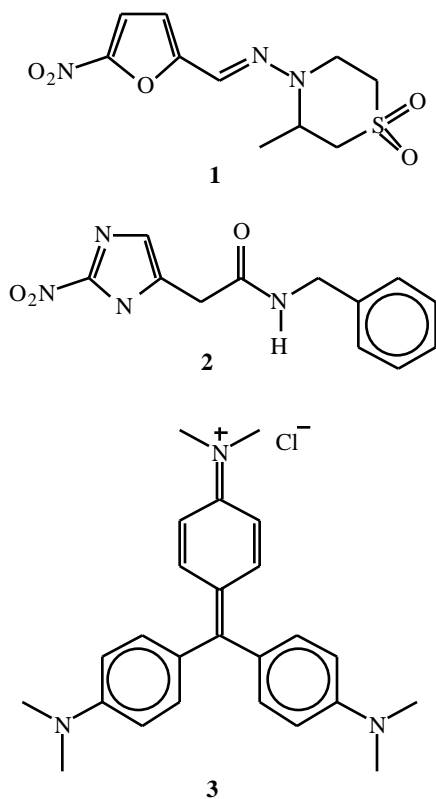
Inhibitors of Ergosterol Biosynthetic Pathway at an Early Stage

The drug 4-phenoxyphenoxyethyl thiocyanate (compound **4**) represents a new class of antiparasitic agents (Scheme 1) [18]. This drug was extremely potent with an IC$_{50}$ = 2.2 µM, four

times more active than nifurtimox (IC$_{50}$ = 8.6 μM, under similar assay conditions), one of the drugs clinically in use to control this disease which also presents severe side effects. This sulfur-containing derivative also exhibited very potent inhibitory action against amastigotes, which is the clinically more relevant form of the parasite. The concentration required to inhibit 50% of growth and also to reduce the number of infected cell to a half was the low nanomolar level [18]. The mode of action of **4** was preliminary studied. There is strong evidence to believe that the target of 4-phenoxyphenoxy derivatives is the ergosterol biosynthetic pathway [19]. When treated *T. cruzi* cells with this thiocyante derivative, accumulation of low-molecular weight metabolites from mevalonate to squalene is observed compared with untreated cells [19]. Compound **4** was rationally designed based on the chemical structure of the well known insect growth regulator fenoxycarb (*N-*{2-[(4-phenoxyphenoxy)ethyl]} ethyl carbamate, compound **5**) [20]. This type of molecule can be divided into two fragments: the polar one, represented by the carbamate moiety, and the nonpolar one represented by the 4-phenoxyphenoxy skeleton (Figure 2). The lead
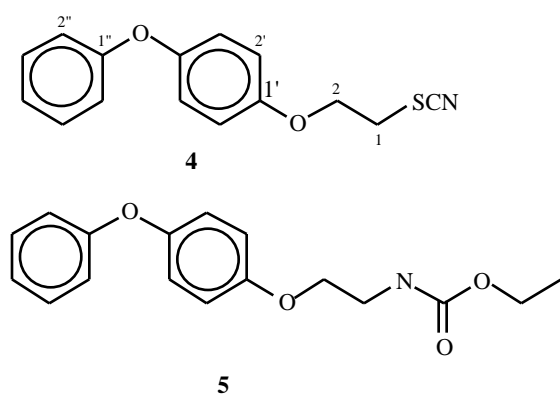

Fig. (2). Chemical Structures of 4-Phenoxyphenoxyethyl Thiocyanate taken as Lead Drug and the well known Insect Growth Regulator Fenoxycarb.

structures were discovered while working on the design and synthesis of insects' juvenile hormone analogues (JHAs) to be evaluated against the Chagas' disease vector like *T. infestans* and *R. prolixus* [21]. These hormones are isoprenoid derivatives that are crucial for maintaining larval stages and maturation of the reproductive system in the female [21]. The chemical structures of the naturally occurring juvenile hormone of insects are

illustrated in Figure 3. The rationale for the preparation of metabolically stable JHAs had been the introduction of the ethyl carbamate of fenoxycarb and other related functional groups like carbonates, thiolcarbonates, etc, onto an isoprenoid unit. The synthetic JHAs had exhibited a variable degree of potencies against these bugs and, even some of them had resulted to be more
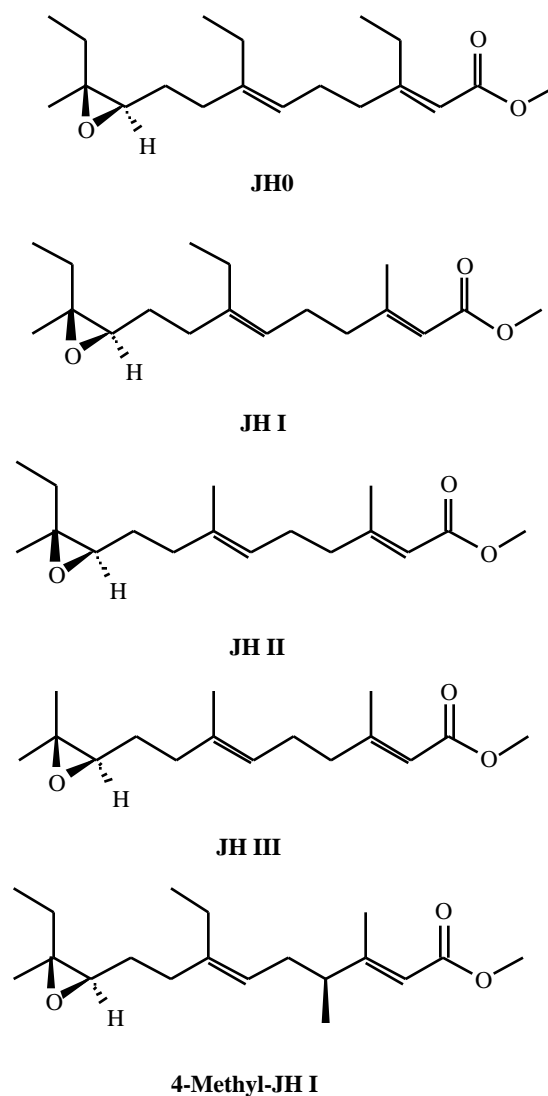

Fig. (3). Chemical Structures of Naturally Occurring Juvenile Hormones of Insects.

potent than the naturally occurring juvenile hormones [22,23]. Taking into account that these insects, after treatment with JHAs, were less susceptible to natural infections with the parasite, *T. cruzi*, than normal non-treated vectors [24], the designed JHAs were tested against the epimastigote forms of *T. cruzi*. Very surprisingly,
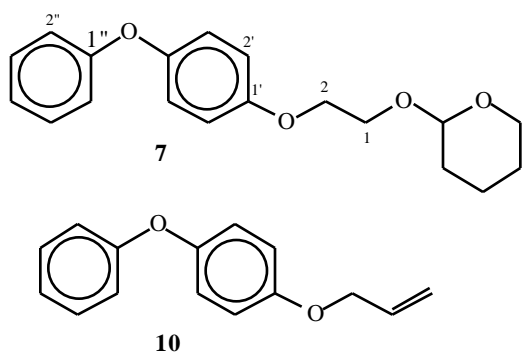
Fig. (4). Chemical Structures of 4-Phenoxyphenoxyethyl Allyl Ether and 4-Phenoxyphenoxyethyl Tetrahydro-2*H*-pyran-2-yl Ether Formerly Employed as Lead Drugs.

these compounds exhibited a variable degree of biological response, some of these JHAs being moderately potent agents in inhibiting cell proliferation of the parasite [25,26]. These results were quite encouraging, because these drugs, previously designed to behave as juvenile hormone analogues of insects, became cell growth inhibitors

[25,26]. At the beginning, fenoxycarb was employed not only as lead drug but also as standard control because it behaved as a highly potent agent against egg eclosion and as a juvenile hormone mimic towards nymphal stages of Chagas' disease vectors. Nevertheless, some changed structures containing the 4-phenoxyphenoxy moiety were found to be more potent than fenoxycarb against *T. cruzi* growth. 4-Phenoxyphenoxy allyl ether and 4-phenoxyphenoxy allyl tetrahydro-2*H*-pyran-2-yl ether, compounds **7** and **10**, respectively, were the primary structures that led to the development of compound **4** (Figure 4).

Preliminary studies of the mode of action of these drugs have indicated the blockage of the sterol biosynthesis within the cells according to the experiments observed in Leydig tumor cells [27,28]. Tumor and *T. cruzi* cells present many coincidences because of their rapid multiplication. Certainly, some anti-tumor agents have proven to
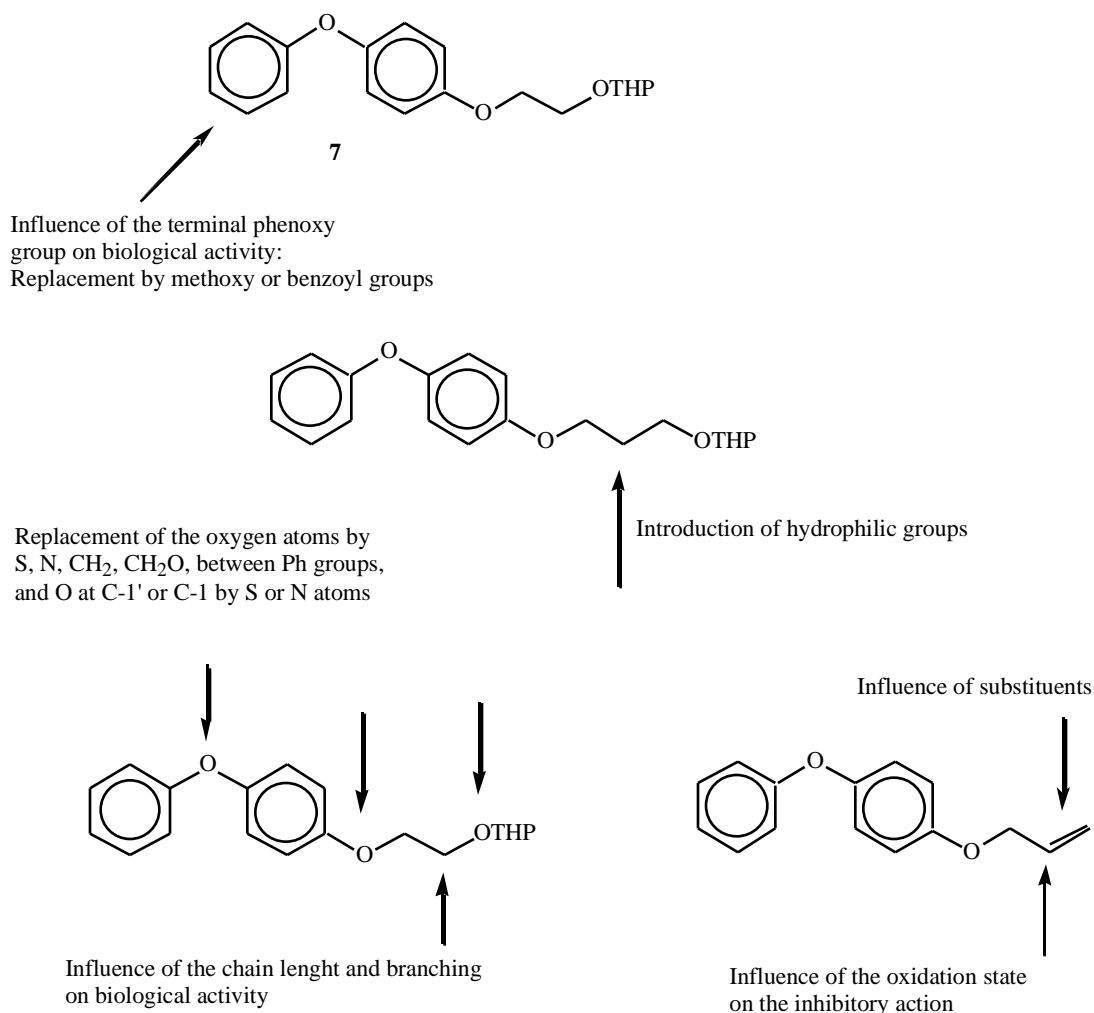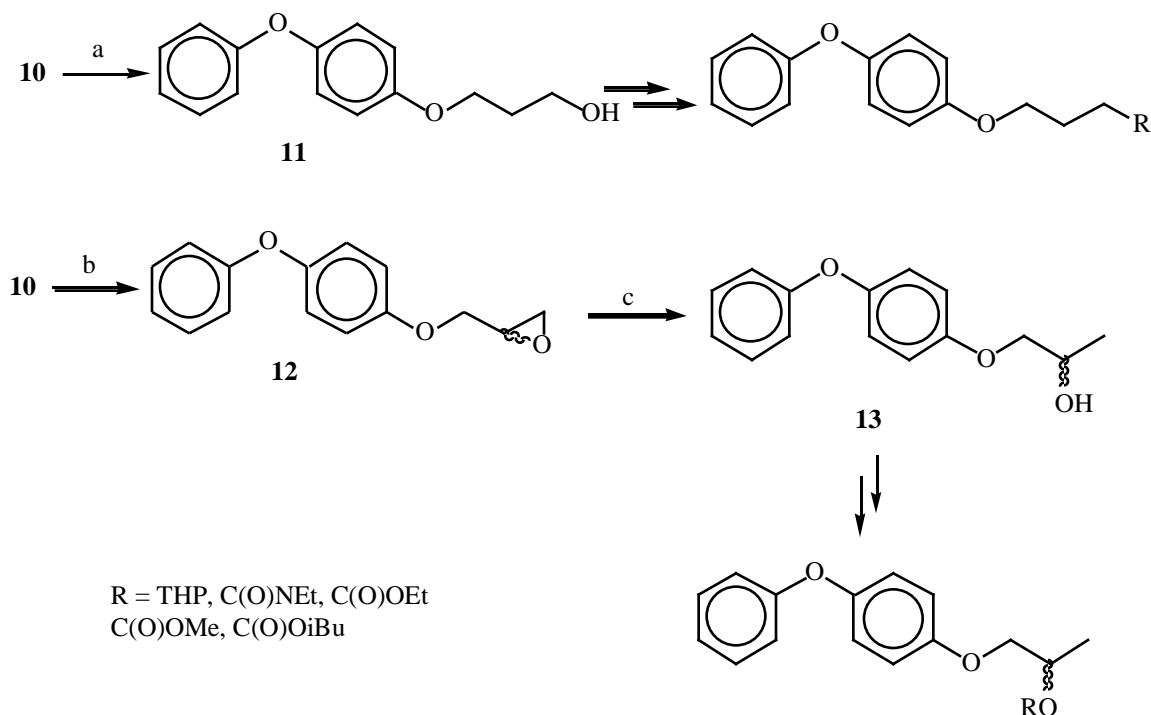

Fig. (5). Different Structural Variations to Optimize 4-Phenoxyphenoxy Derivatives.

R = THP, C(O)NEt, C(O)OEt
C(O)OMe, C(O)OiBu

Scheme 2. Reagents and conditions: (a) i. BH_3-THF/THF, rt; ii. H_2O_2, 46%; (b) *m*-CPBA, Cl_2CH_2, rt, 61 %; (c) $LiAlH_4$/THF, rt, 88%

act as trypanocidal drugs [29-31]. Compound **7** taken as a representative member of this new class of antiparasitic agent proved to be very active against the intracellular form of the parasite [32] and, as it occurs with other sterol biosynthesis inhibitors, it was devoid of activity against the non-dividing highly infective bloodstream trypomastigotes [33].

Once the preparation and biological data for compounds **7** and **10** were at hand a careful

chemical structure/biological activity relationship (SAR) was investigated as it is outlined in Figure 5 [18, 34-37]. Therefore, the aromatic nonpolar skeleton as well as the polar terminal end were optimized. Although many isoprenoid compounds resulted to be potent inhibitors of the parasite growth, this kind of drugs will not be discussed due to they were not stable compounds on standing. The chain length of the aliphatic portion of these molecules were homologated in one carbon *via* hydroboration of allyl ether **10**. The resulting
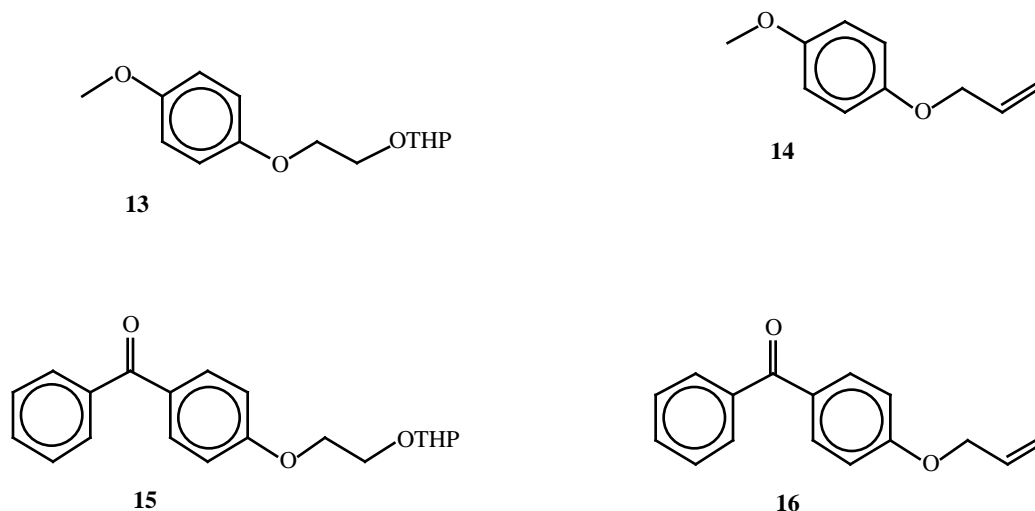

Fig. (6). Representative allyl and Tetrahydro-2*H*-pyran-2-yl ethers in which lack the terminal phenoxy moiety.

alcohol **11** resulted to be a useful synthetic intermediate to prepare these homologated drugs. The preparation of branched derivatives was also carried out from **10**. On treatment with *m*-chloroperbenzoic acic followed by selective ring opening with liyhium aluminium hydride **10** was transformed into the desired secondary alcohol **12** intermediate for branched derivatives at C-1. The influence of the oxidation degree between positions C-2 and C-3 on the biological activity was also investigated. Neither of these variations produced a significant improvement on the effectiveness of the designed drugs [34] (Scheme 2).

The replacement of the terminal phenoxy group by a methoxy group or a benzoyl group reduced to a large extent the biological action [35]. The introduction of the benzoyl moiety was envisioned to align the phenyl groups in the space, that is, in order to have potentially planar drugs [35]. Representative members of these compounds (**13-16**) are illustrated in Figure 6.

An interesting structural variation was the replacement of the aromatic hydrogen atoms at C-2' position by some atoms or functional groups finding that the drug potency increases as the substituent size increases. On the other hand, substitutions of the hydrogen at C-4" position were not satisfactory in terms of the biological activity, less potent drugs were obtained in most cases [18]. The selection of the C-2' and C-4" positions for structural variations was based on the ability of 4-phenoxyphenol (compound **6** was currently employed as starting material) for electrophilic aromatic substitution at those

positions: C-2' is the more reactive position but the acetyl derivative deactivates this position to favor the respective C-4" position [18].

A very interesting structural modification was the replacement of the different oxygen atoms present in the lead drug **7** by heteroatoms such as nitrogen and sulfur: (a) the replacement of the oxygen atom by a sulfur atom at the C-1 position brought about an extremely potent drug, especially when a thiocyanate was employed as a polar extreme (compound **4**) [18]; (b) the replacement of the oxygen at C-1' produced also an ultrapotent drug against epimastigotes but resulted to be toxic for myoblasts when tested against the intracellular form of the parasite (compound **17**) [36], while the introduction of a nitrogen atom at C-1' afforded moderately active drugs such as compound **18** [36]; (c) the replacement of the oxygen atom between the phenyl groups by a sulfur atom produced a poorly potent drug [18]. The influence of the sulfur atom at the C-1 position is so marked that several of these sulfur-containing derivatives were very potent against both diving forms of the parasites, amastigotes and epimastigotes [36,37]. In addition, several other groups such as the benzyl group, the benzyloxy group, the hydrogen atom, etc, replaced the phenoxy unit. In any case an improvement in the potency was observed, but when a chlorine atom replaced the phenoxy terminal group another ultrapotent drug was obtained (compound **19**) [18,36]. The episulfide **20** was another ultrapotent drug against epimastigotes but with toxic effect against myoblasts when are the host for intracellular amastigotes [37] (Figure 7).
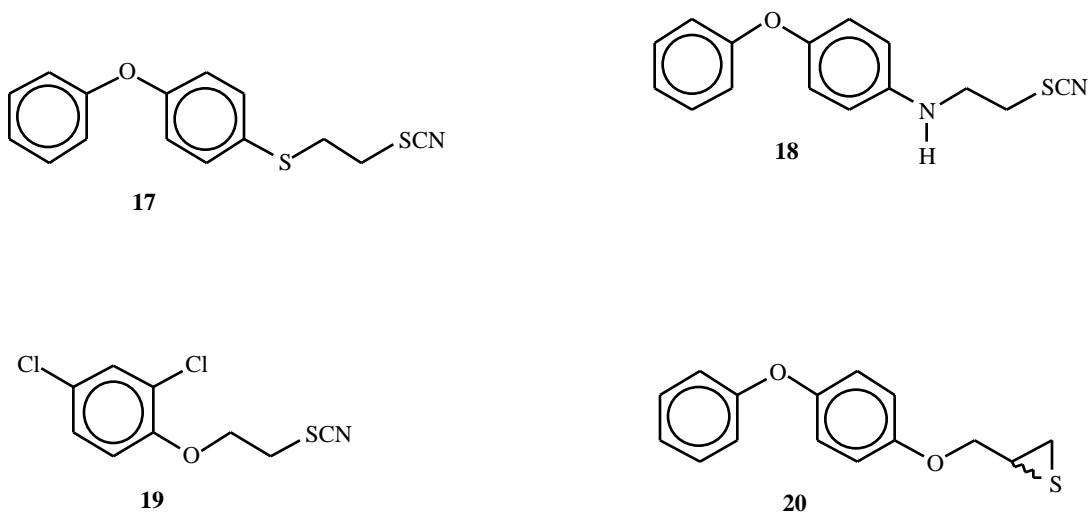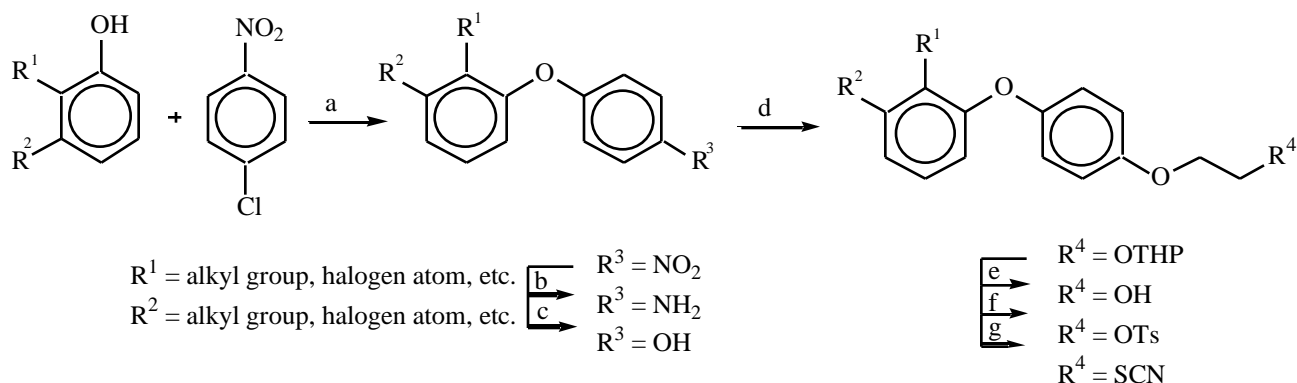

Fig. (7). Chemical Structures of Potent Sulfur-containing Derivatives that are Potent Inhibitors of *T. cruzi* Growth.

Scheme 3. Reagents and conditions: (a) KOH, DMSO, CuCl, ; (b) H_2, Pd/C, rt; (c) $NaNO_2$, H_2SO_4, 0 °C, urea; (d) KOH, DMSO, $BrCH_2CH_2OTHP$, rt; (e) PPTs, MeOH, rt; (f) ClTs, py, 0 °C; (g) KSCN, DMF, 100 °C, 5 h.

The preparation of substituted derivatives at C-2" and C-3" positions to study their influence on the biological activity was not straightforward. In order to prepare these derivatives, a nucleophilic aromatic substitution was required. Then, an *ortho* or a *meta* substituted phenol was coupled with 4-chloronitrophenol to afford the corresponding 4-phenoxynitrobenzene derivative [36]. This compound was transformed into the phenol intermediate that was further converted into the desired thiocyanate derivative after four synthetic steps. These transformations are outlined in Scheme 3. As an example, compounds **21** and **22** resulted to be as potent as **4** against epimastigotes but both of these drugs were almost devoid of activity against the intracellular form of the parasite [36]. A similar synthetic approach was

employed to prepare conformationally restricted analogues structurally related to **4**. These rigid analogues were inactive in inhibiting the parasite growth. For example, compound **23** presented IC_{50} values close to 50 μM, that is almost 25 fold less potent than **4**. The introduction of branching at the aliphatic side chain to incorporate a stereogenic center such as compounds **24** and **25** also impaired the potency of the drugs (Figure 8).

In conclusion, a big synthetic effort has been done to rationally prepare hundreds of compounds from which compounds **4** and **19** arise as principal members of aryloxyethyl thiocyanate derivatives. The 4-phenoxyphenoxy motif has been found in other structurally related drugs as it is the case of matrix metalloprotease inhibitors [38], and also in
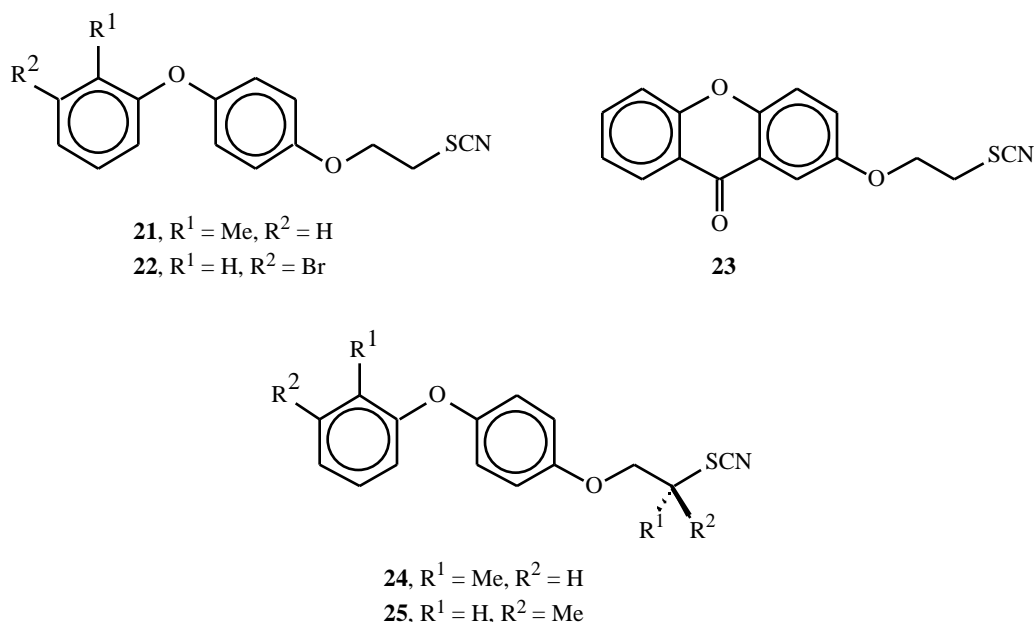
Fig. (8). Interesting Structural Variation on the Lead Drug 4-Phenoxyphenoxyethyl Thiocyanate.

a number of other drugs [39]. In addition and surprisingly, it has been found that juvenile hormone mimics that act against bugs other than Chagas' disease vectors such as *Tenebrio molitor*, *Galleria mellonella*, *Dysdercus cingulatos*, and *Pyrrhocoris apterus* were moderately inhibitors of *T. cruzi* proliferation [40]. Moreover, preliminary results in *in vivo* assays in the acute phase of Chagas' disease on Swiss mice indicated that 4-phenoxyphenoxyethyl thiocyanate also was able to reduce parasitemia [41], and for that reason, this drug presents very good prospective not only as a lead drug but also as a potential chemotherapeutic and chemoprophylactic agent.

The Surface *Trans*-Sialidase as a Potential Molecular Target for Drugs Design

In the last years, a significant amount of work has been conducted to study the chemical structure of *T. cruzi* surface membrane on the basis that these elements might be involved in a key role for parasite-host cell interactions. Sialic acid is a crucial metabolite for parasite survival (trypomastigotes) biosynthesized in higher eukaryotic cells and present at the parasite cell surface bonded as terminal glycoconjugates. Sialic acid are referred as a family of monosaccharides widespread in mammals, a number of bacteria, and parasites mainly as terminal sugar of oligosaccharides and glycoconjugates bonded (2 3) or (2 6) to galactose. Sialic acid may bond to another sialic acid molecule *via* an (2 8) glycosidic bond. *N*-acetylneuraminic acid (compound **26**) arises as the principal member of sialic acids. These naturally occurring carboxylated sugar derivatives are also observed substituted at all different hydroxylated position, that is, C-4, C-5, C-7, C-8 and C-9 (Figure 9) [42-44]. *T. cruzi* does not synthesize sialic acid but present a *trans*-sialidase activity that catalyzes the transferring of sialic acid from host cell-surface and serum glycoproteins to mucin-like acceptor molecules present in the parasite plasma membrane [45]. The surface membrane *trans*-sialidase catalyzes reversibly the transferring of a terminal molecule of sialic acid (2 3) linked to a -galactose to an appropriate acceptor, a glycoconjugate with terminal -galactose, excluding -galactose. In the absence of a suitable acceptor of sialic acid the enzyme act as a hydrolase (sialidase activity) transferring a molecule of sialic acid to water

[44,46]. The process of sialyltransferase is faster than the hydrolysis of terminal sialic acids but reasons for this preference is still unknown. This *trans*-sialidase activity is very different from that found in the Golgi complex which only employ CMP-sialic acid as the donor molecule [47]. Pereira was the first to identify sialidase activity in trypomastigotes [48]. This activity was attribute to a polymorphic family of molecules ranging in M_r from 160 to > 220 kDa [44,46,49]. The *trans*-sialidase activity is observed primarily in the trypomastigote forms of the parasite and in the epimastigote forms. In fact, the sialidase activity in tissue cultures of trypomastigotes is several times higher than that found in the epimastigote forms of the parasite [44]. There is no enzymatic activity in the intracellular form of the parasite. This *trans*-sialylation reaction is associated with the invasion process to the host cell [50-54]. Another function is the protection of trypomastigotes from lysis by the alternative pathway of complement as well as circulation of parasites in the mammalian host [49,55]. Actually, sialidase-treated trypomastigotes give rise to parasites not only susceptible to recognition by the alternate pathway of complement but also their uptake by macrophages is increased [56]. On the other hand, it has been described that removal of sialic acid from mucin-like surface molecules of *T. cruzi* metacyclic trypomastigotes increased host cell invasion [57].
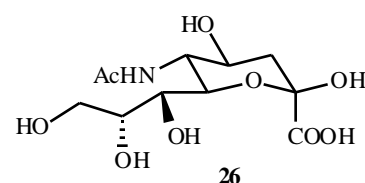

Fig. (9). Chemical structure of the most abundant sialic acid: *N*-acetylneuraminic acid.

As this *trans*-sialidase is unique for *T. cruzi* and bearing in mind that there is no analogue in mammalian cells, its selective inhibition constitutes an attractive approach for drug design. The *trans*-sialidase protein family is encoded by around 140 genes [47,58, 59]. The enzymes express by the trypomastigote forms are anchored by glycosylphosphatidylinositol [60] to the surface membrane. This family of proteins presents two main region: an *N*-terminal catalytic domain and a C-terminal region with repeats of 12 amino acids named SAPA repeats (shed-acute phase antigen) [61,62]. Several of these enzymes lack the *trans*-

sialidase activity due to a single mutation of Tyr342 by His [63]. It is worth to point out that inactive member of *trans*-sialidase family recognize -galactose units similarly to active *trans*-sialidases. The epimastigotes also express trans-sialidase activity, these enzymes do not present the SAPA repetitive domain. As these epimastigote enzymes are active ones, the SAPA domain is not required for activity. Very recently, the crystal structure of a sialidase from *T. rangely* as well as the complex with its inhibitor 2-deoxy-2,3-didehydro-*N*-acetylneuraminic acid has been reported [61]. The crystal structure of the protein alone was determined at 2.2 Å, while the complex with its inhibitor was determined at 2.9 Å [59]. The overall crystal structure shows the enzyme folds into two domains: a canonical -propeller corresponding to the active site and a *C*-terminal domain these domains are connected by a long -helix. Another -helix is found at the *C*-terminal. This work open very good prospective for the rational design of new drugs. In addition, a modeled structure of *T. cruzi trans*-sialidase has been studied together with mutagenesis experiments identifying the amino acids within the active site as well as the kind of enzyme-inhibitor interactions [61].

Finally, the chemical structures of a number of mucins (acceptors of sialic acid) have been accomplished [64,65].

Trypanosoma Cruzi Dihydrofolate Reductase as a Potential Target for Drug Design

The enzyme dihydrofolate reductase (DHFR) that catalyses the transformation of dihydrofolate into tetrahydrofolate (THF) has been targeted for mechanism-based enzyme inhibition for a number of diseases as antitumor, antibacterial and

antiprotozoal agents [66]. The structure of the enzymes, especially the active site fluctuates significantly among different species. Therefore, a selective inhibition of *T. cruzi* DHFR would lead to growth impairing of the parasite because *T. cruzi* do not have a mechanism of transport of this cofactor from the host. In this case, a highly selective inhibition of *T. cruzi* enzyme compared with the host DHFR will have a vanishing toxic effect. Very recently, potential inhibitors of this *T. cruzi* DHFR has been designed and synthesized employing a structure-based approach [67]. The structure of *Leishmania major* DHFR has been solved [68], and employing these data a homology model of *T. cruzi* DHFR was developed [69]. The major differences at the active site are: residues Gli 20 and Asp 21 for human DHFR change to Arg 39 and Ser 40 in *T. cruzi*. In addition, Phe 31, Gln 35 and Asp 64 change to Met 49, Arg 53 and Phe 88 in *T. cruzi* [67]. The well known DHFR inhibitor methotrexate (compound **27**) was used as lead drug and as a positive control for enzyme assays and for parasite intracellular inhibition. Docking studies suggested that a carboxylic group linked at the N-10 position of the pteridine ring with four or six methylene groups would overlap residues 20 and 21 by charge repulsion giving place to a better selectivity between human and *T. cruzi* enzymes. Although the designed compounds **28**-**30** were less potent than **27** against amastigotes, they presented a good selectivity being **28** the most effective drug with a selectivity close to 20. In addition, it was found that the potency decreases as the chain length increases [67].

Glyceraldehyde-3-Phosphate Dehydrogenase

This is another interesting enzyme to be employed as target for drug design. There is strong evidence to believe that glycolysis is one of the
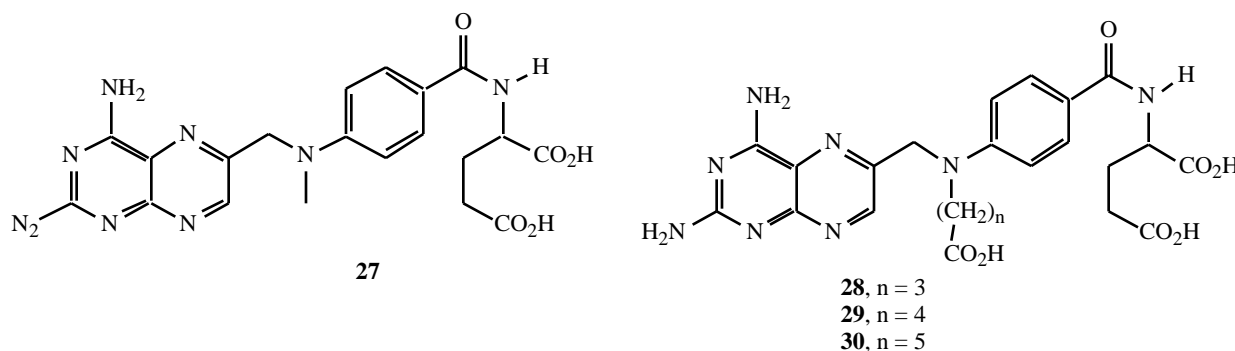

Fig. (10). Chemical structures of the dihydrofolate reductase inhibitor methotrexate and other closely related compounds.

principal energy sources for amastigotes. Selective inhibition of parasite glycolytic enzymes over the mammalian host ones would provide an interesting approach for new drugs, not only for *T. cruzi* but also for *T. brucei* and *Leishmania spp* [70,71]. Adenosine was employed as lead drug bearing in mind the structure of the cofactor NAD$^+$ [70]. The *L. mexicana* enzyme expressed in *Escherichia coli* was employed for docking studies. Substitutions at the N-6 position of the adenosine and at C-2' position at the furanose ring increase significantly the efficacy of the lead drug with no effect for the mammalian enzyme. Certainly, N^6-(1-naphtalenmethyl)-2'-(3-chlorobenzamido)adenosine present IC$_{50}$ at the low micromolar level against amastigotes [70]. The same activity is observed against bloodstream *T. brucei* [70].

CONCLUSIONS

The aim of this review was the presentation of several attractive targets for rational design of drugs. Other interesting molecular targets are presented in this issue and they deserve a predominant place for their potential utility.

ACKNOWLEDGMENTS

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