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Review

Prodrugs for the Treatment of Neglected Diseases

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Abstract: Recently, World Health Organization (WHO) and Medicins San Frontieres (MSF) proposed a classification of diseases as global, neglected and extremely neglected. Global diseases, such as cancer, cardiovascular and mental (CNS) diseases represent the targets of the majority of the R&D efforts of pharmaceutical companies. Neglected diseases affect millions of people in the world yet existing drug therapy is limited and often inappropriate. Furthermore, extremely neglected diseases affect people living under miserable conditions who barely have access to the bare necessities for survival. Most of these diseases are excluded from the goals of the R&D programs in the pharmaceutical industry and therefore fall outside the pharmaceutical market. About 14 million people, mainly in developing countries, die each year from infectious diseases. From 1975 to 1999, 1393 new drugs were approved yet only 1% were for the treatment of neglected diseases [3]. These numbers have not changed until now, so in those countries there is an urgent need for the design and synthesis of new drugs and in this area the prodrug approach is a very interesting field. It provides, among other effects, activity improvements and toxicity decreases for current and new drugs, improving market availability. It is worth noting that

it is essential in drug design to save time and money, and prodrug approaches can be considered of high interest in this respect. The present review covers 20 years of research on the design of prodrugs for the treatment of neglected and extremely neglected diseases such as Chagas' disease (American trypanosomiasis), sleeping sickness (African trypanosomiasis), malaria, sickle cell disease, tuberculosis, leishmaniasis and schistosomiasis.

Keywords: Tropical neglected diseases, Prodrug design, American trypanosomisasis, African trypanosomiasis, Malaria, Sickle cell disease, Tuberculosis, Leishmaniasis, Schistosomiasis.

Introduction

Billions of dollars are invested each year in research and development (R&D) of drugs for conditions that affect people who can pay, which include obesity, baldness, and ageing. However, the World's poor are left completely marginalized. Tropical infectious diseases, including protozoan infections, helminths, as well as other diseases such as sickle cell disease are known as neglected diseases [1,2]. Recently, these diseases were officially classified as neglected and/or extremely neglected diseases by the World Health Organization and Medicins San Frontieres. They affect areas of extreme poverty and are themselves poverty promoting. For this very reason, they fail to attract attention from the drug developers, who do not consider this group of diseases as a lucrative target [3,4]. The market logic is: no profit, no investment [5].

Only 10% of global health research is related to diseases that account for 90% of the global disease burden, which urgently need new drugs. From 1975 to 1999, 1,393 new drugs were launched in the market, but only 16 (1%) were for the treatment of tropical diseases [3]. Science has advanced in biology, molecular biology and also genetics of the etiologic agents that cause those diseases. However, this knlowledge has not been used for drug design [6] and this is not acceptable.

Different basic approaches to drug discovery for tropical diseases included combinations of existing drugs, new indications for existing drug, improvements to known drugs and compound classes and focused sample collections [7]. The prodrug approach was developed to overcome mainly pharmacokinetics problems of many therapeutic classes of drugs [8;9]. A prodrug is an inactive drug carrier form that requires a metabolic activation step before it exhibits its pharmacological effects in a target tissue. It can provide reduced toxicity, improved therapeutic index, slow release of the parent drug and also improved selectivity towards the target tissue or cell. In this work we have focused in prodrugs for the following neglected diseases: Chagas'disease, sleeping sickness, leishmaniasis, malaria, schistosomiasis, tuberculosis and sickle cell disease.

Chagas'disease

In 1909 Carlos Chagas discovered American trypanosomiasis, commonly known as Chagas´ disease, which is a parasitosis caused by *Trypanosoma cruzi*. The disease is mostly spread throughout Latin America, affecting 21 countries [10]. It is estimated that some 16 to 18 million people are infected by the parasite and 50,000 people die annually as a consequence of the illness [11]. Chagas' disease remains essentially incurable. The pharmaceutical industry has had limited interest in developing new antichagasic drugs, due principally to a lack of commercial incentives [12]. Benznidazole and nifurtimox are the available drugs for the treatment of this parasitosis. However, both drugs are toxic nitroheterocyclic derivatives and almost only effective in the acute phase of the disease. They have been used as long-term treatment [13]. These drugs cure only a very low percentage of chronic patients [12] and natural resistance of *T. cruzi* to nitro derivatives has been suggested as an important factor to explain the low rates of cure detected in chagasic patients [12]. Thus, a search for new drugs against *T. cruzi* is of utmost importance and must be emphasized.

Sleeping sickness

Human African Trypanosomiasis (HAT), or sleeping sickness, is caused by infection with parasitic protozoa of the *Trypanosoma brucei* (*T. brucei*) subspecies, which are introduced to the human bloodstream by the bites of infected tsetse flies in the inter-tropical regions of Africa. *Trypanosoma brucei gambiense*, found in West and Central Africa, leads to a chronic form of the disease [14]. Over 60 million people living in 36 countries are at risk of acquiring sleeping sickness and the estimated number of people affected is between 300,000 and 500,000 [15].

Chemotherapy of African trypanosomiasis is still based on old drugs and some of these show toxic side effects. Suramin and pentamidine are the drugs of choice against the Rhodesian and Gambian forms of the disease, respectively, before the onset of central nervous system symptoms. Late-stage disease is treated with the melaminophenyl arsenical drug melarsoprol. In the case of the disease caused by *T. b. gambiense*, eflornithine (DFMO) is also used [16]. New drugs are urgently needed for human African trypanosomiasis and the emergence of drug resistance is likely to be a challenge that must be faced.

Leishmaniasis

Parasites from *Leishmania* genus are the causative agents of leishmaniasis, a group of protozoan diseases transmitted to mammals, including human beings, by phlebotomine sandflies. Globally, there are an estimate of 350 million people that are at risk of infection and disease [17]. Among all parasitic infections, it is considered the second most important from the socioeconomics point of view. It affects 12 million people in 88 countries, there are 1.5–2 million new cases and 70,000 deaths each year, and 350 milllion are under the risk of contamination [17]. Only malaria and schistosomiasis, respectively, present more expressive numbers than leishmaniasis [18, 19].

The clinical forms of the disease range from cutaneous leishmaniasis to the most severe visceral infection (known as kala-azar in India), fatal if untreated [20]. Chemotherapy for leishmaniasis is

scarce and limited to pentavalent antimonials, pentamidine, amphotericin B, miltefosine, fluconazole and few other drugs at various stages of their development process [21-23], drugs with undesirable side effects. The latter and the emergence of drug resistance have been the driven force to researchers to search for new potential compounds [24-26].

Malaria

Malaria is a life-threatening parasitic infection caused by parasites of genus *Plasmodium* -- *P. falciparum*, *P. vivax*, *P. ovale* and *P. malariae* – from which *P. falciparum* is the only one capable of producing fatal complications. Over three billion people live under the threat of malaria and it has been estimated that there are 300 to 500 million new cases of malaria resulting in approximately two million deaths per year. The majority of these cases occur in the poorest countries [27]. With the absence of an effective vaccine and global emergence of multidrug resistance, malaria continues to be a worldwide epidemic. Resistant strains of malaria have been identified for most of the antimalarial drugs in clinical use today [28]. Many drugs, alone or in combination – chloroquine, primaquine, mefloquine, halofantrine, artemisinin, atovaquone, among others – have been used for malaria chemotherapy. However, drug or multi-drug resistance has been a challenge for chemotherapy effectiveness [29]. The need for drugs against tecidual forms as well as the emergence and spread of resistance against chloroquine and other major antimalarial drugs have brought the urgency to develop a new generation of safe and effective agents [1].

Schistosomiasis

Schistosomiasis is caused by a trematode of the genus *Schistosoma* and five major species, *S. mansoni, S. japonicum, S. haematobium, S. intercalatun* and *S. mekong,* infect humans. It is estimated that two billion people are chronically infected with soil transmitted helminths and schistosomes, many suffering from severe morbidity. World Health Organization has estimated that some 200 million people are infected, a further 600 million people are at risk of infection and more than 200,000 deaths per year ocurr just from schistosomiasis [30]. It ranks second only to malaria considering the extent of endemic areas and the number of infected people. It is distributted in around 52 countries in South America, Caribbean, Africa and East Mediterranean. The intermediate host of the parasite is a snail widespread in fresh water, and it is skin contact with this water that permits infection. Schistosomiasis represents both public health and socioeconomics challenges.

Chemotherapy has become the most effective means of endemic disease control and therapeutic drugs available are: metrifonate, oxamniquine and praziquantel. In some countries only oxamniquine and praziquantel are used. The latter has been the drug of choice as it is effective against all human schistosomose. However, there is considerable concern about the development of resistance. Oxamniquine is used as second drug of choice, due to its side effects, mainly those associated to CNS [30]. Since vaccine development is still far from practical application, the design of new schitosomicide drugs are urgently needed. However, as in other neglected diseases, this is too expensive and is not economically attractive to industry [31].

Tuberculosis

Parallel to the tropical endemic diseases, tuberculosis (TB) appears as great challenge for the world-wide organizations and related groups of research involved in health. TB is the most serious infectious lung disease in the world and the leading cause of death from a single infectious organism. It is spreaded worldwide but affects mainly African regions. It was estimated that it maintains hidden in two billion people [32] and between 2005 and 2020, one billion people will be newly infected, over 125 million people will get sick, and 30 million will die of TB if control is not further strengthened [33]. HIV infection, which compromises cell-mediated immunogenicity [34], increases to 50% the percentage of people infected with *M. tuberculosis* who would develop active TB during a shortened lifetime [35]. This and the growing TB emergence of drug resistant strains of *Mycobacterium tuberculosis* [36] has been the major challenges and claim, urgently, for new and better drugs.

Sickle cell disease

Sickle cell disease (SCD), an inherited disorder of haemoglobin synthesis, is a single point mutation that substitutes valine for glutamic acid in the β-globin subunit [37].It causes deformation of the red blood cells that can result in vaso-occlusive events [38], ischaemia and tissue and organ damage as well [39,40] and can be fatal. This condition is prevalent mainly in tropical areas and predominates in Africa. Millions of people throughout the world have been affected with this disease, mainly from Sub-Saharan Africa, Latin America, United States, Saudi Arabia, India, and Mediterranean countries as Turkey, Greece, and Italy. The sickle gene has however have a genetic advantage: it protects heterozygous carriers from succumbing to endemic *Plasmodium falciparum* malaria infection [41]. Hydroxyurea is the only US Food and Drug Administration (FDA)-approved drug for the treatment of SCD. Some compounds have been studied for sickle cell treatment like: butyric acids, aldehydes, decitabine, clotrimazole, L-arginine, zileuton [42-44].

For most of the neglected diseases the improvement of pharmaceutical, pharmacokinetic or even pharmacodinamic profile of the existant drugs using molecular modification processes is a recommended alternative. From those methods, prodrug design is one of the most promising [9,45]. We describe, in this paper, the main results of 20 years of research into prodrug design against some neglected diseases.

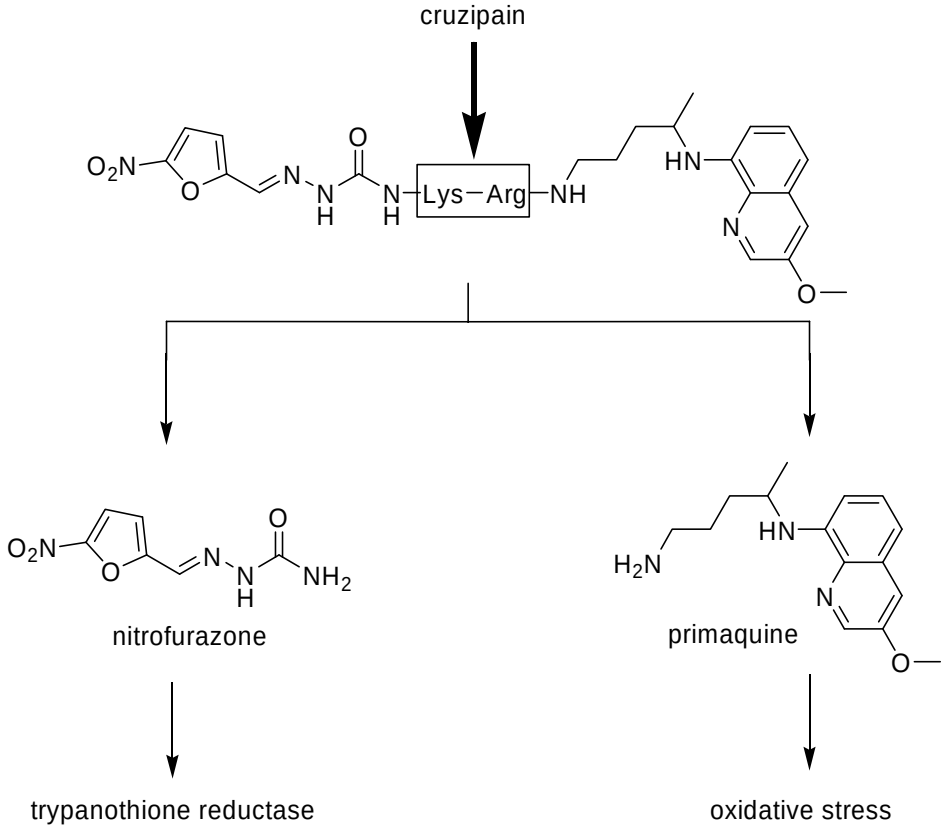
Antichagasic prodrugs

Prodrugs of primaquine and nitrofurazone

Chung, in 1996, synthesized mutual prodrugs of primaquine (PQ) and nitrofurazone (NF) using dipeptides as spacer groups. These peptides are selectively cleaved by cruzipain, a cysteinyl-protease found only in *T. cruzi* [46]. The rationale for this approach was the mechanism of the drugs: while primaquine increases the oxidative stress into the parasite, nitrofurazone (NF), as a trypanothione reductase inhibitor [47], does not allow the protective action of the enzyme, provoking the increase in

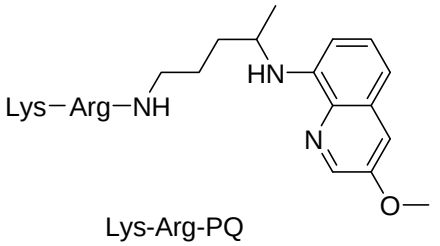
the intracellular oxidative stress. The compound in which Lys-Arg was the spacer group has been the most active (Figure 1).

Figure 1. Chemical structure of the mutual prodrug of nitrofurazone and primaquine and its conversion to the parent drugs by cruzipain.

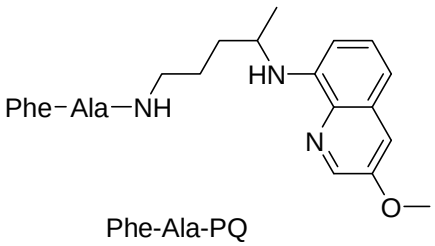


Dipeptide prodrugs of primaquine as synthesis intermediates were also shown to be active on LLC-MK2 cell culture infected with trypomastigotes forms of *T. cruzi*. It was demonstrated that Lys-Arg-PQ is active on *T. cruzi* development inside host cells, probably by interfering in the initial steps of trypomastigote-amastigote transformation. It was found that Lys-Arg-PQ is more active than Phe-Ala-PQ and Phe-Arg-PQ, suggesting that the specific cleavage has an important role in the release of PQ. The dipeptide Lys-Arg, putatively specific for cruzipain, was a good carrier for PQ and has potential to be used as a spacer group for the development of other PQ prodrugs in the future (Figure 2). Thus, Lys-Arg-PQ is a very promising compound for *in vivo* tests [48].

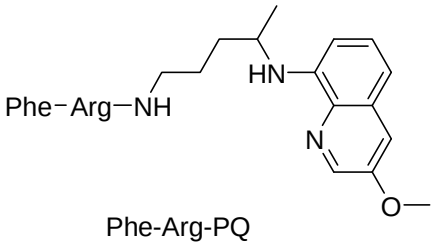
Figure 2. Chemical structures of dipeptide prodrugs of primaquine.



Lys-Arg-PQ



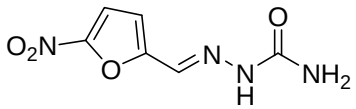
Phe-Ala-PQ



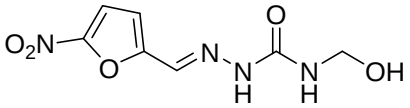
Phe-Arg-PQ

T. cruzi trypanothione reductase has been considered a key enzyme in the oxidative metabolism of the parasite [47] and nitrofuran derivatives have shown to produce irreversible inactivation of that enzyme under anaerobic as opposed to aerobic conditions. Hydroxymethyl derivatives are prodrugs whose main goal is to provide more hydrophilic derivatives than the parent compound. In general, NH acidic drugs such as amides, imides and ureides are potential targets to *N*-hydroxymethylation [49]. Chung synthesized the nitrofurazone hydroxymethyl derivative (NFOH) (Figure 3), as a synthesis intermediate of mutual prodrugs of primaquine and nitrofurazone, potentially active in Chagas' disease. This compound was shown to be highly active in cell cultures infected with *Trypanosoma cruzi* and much less toxic than the drug, when subtmitted to Ames' mutagenicity test [50].

Figure 3. Chemical structures of nitrofurazone and its prodrug hydroxymethyl nitrofurazone.



nitrofurazone (NF)



hidroxymethyl nitrofurazone (NFOH)

The chemical hydrolysis at pH 1.2 and 7.4 showed that NFOH was completed converted to NF within 16 hours; however the recovery of NF occurs just 1 hour after hydrolysis. This fact suggests that the conversion of NFOH into NF is carried out by a two-step pathway, in which there is the formation of an intermediate compound. The half-life at pH 1.2 was about 1.5 hours while at pH 7.4 it takes 134 hours to be 50% hydrolyzed with no formation of an intermediate compound. These facts suggest the great stability of this compound at physiological pH [50].

Later, the group extended their studies with NFOH and dipeptidic prodrugs to other fields. Molecular modeling studies have been performed to evaluate possible interactions between the prodrugs and cruzain, one of the possible molecular targets of these derivatives [51]. The AM1 study suggests that the aminoacid nature and dipetide sequence are determinant for the approximation and nucleophilic attack of cruzain, as observed in the irreversible inhibitors model. Steric and electrostatic interactions in the double prodrugs indicate the spacer group AlaPhe as the most favorable sequence relative to PheAla. In addition, the LysArg spacer group seems to be the best substrate for the enzyme.

Semi-empirical AM1 studies were applied to the mutual dipeptide prodrugs of primaquine and nitrofurazone to understand the release of the drugs by cruzain. The work suggested that the aminoacid sequence is determinant to this release. The volume and electronic nature of the aromatic nucleus of Phe, comparatively to those of Ala, and the possibility of steric and electrostatic interactions between the side chains of the aminoacids and the drugs lead to different rearrangements that allow or avoid cruzain approximation ant attack [52].

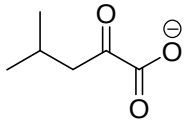
Also, in order to find the reason of higher activity of NFOH than NF, the voltametric behaviour of the drug and its prodrug was also determined by Scalea and co-workers [53]. No differences have been detected in the reduction capability of the nitrogroup.

Prodrugs of N-isopropyl oxamate

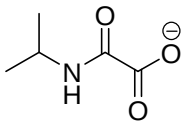
Trypanosoma cruzi possesses an enzyme similar to the lactate dehydrogenase isozyme-x (LDH-C4) from mammalian spermatozoa [54]. This enzyme has two molecular forms (HADH-isozyme I and HADH-isozyme II). Isozyme I is responsible for the weak lactate dehydrogenase activity found in *T. cruzi* extracts [55], while isoenzyme II is not active against pyruvate but on many linear and branch chain substrates, especially α-ketoisocaproate [56]. Its similarity to LDH-C4 leads to analogous functions in *T. cruzi* [57]. These enzymes display a role in supplying energy for the motility of the flagellum and the survival of the parasite [56;57] This hypotheses was advanced based on the structural similarity between *N*-isopropyl oxamate (NIPOx) and the substrate α-ketoisocaproate (Figure 4).

Elizondo and co-workers (2003) have investigated and demonstrated that NIPOx was a selective inhibitor of HADH-isozyme II from *T. cruzi.* However, this compound did not show any activity when tested on V2R *T. cruzi* epimastigote culture, unless it was transformed in its prodrug, the ethyl ester of *N*-isopropyl oxamate (Et-NIPOx). Actually, it was even better than nifurtimox and benznidazol [58]. A hypothesis advanced to explain this effect was the existance of carboxylesterases, aliphatic or non-specific esterases, in *T. cruzi* epimastigotes [59].

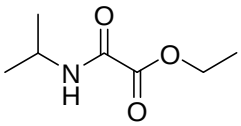
Figure 4. Chemical structures of α-ketoisocaproate, NIPOx and Et-NIPOx.



α-ketoisocaproate



N-isopropyl oxamate (NIPOx)



Ethyl-N-isopropyl oxamate (Et-NIPOx)

Increased effectiveness of Et-NIPOx most probably resulted from their better absorption by this parasite and its efficient hydrolysis by carboxylesterases in situ into the active HADH inhibitor [58]. This was later also confirmed *in vivo* [60], when Et-NIPOx was tested on mice parasitaemia, exhibiting trypanocidal activity in the five tested *T. cruzi* strains. It is worth to note that benznidazol and nifurtimox, submitted to the same test, were active only against three out of five tested *T. cruzi* strains, probably due to natural resistance of the parasite to nitroderivatives [61].

Prodrugs for Sleeping Sickness

ADEPT approach and mAb-drug conjugate for treatment of African trypanosomiasis

Trypanosomes protect themselves from host immune responses by continuously changing the VSGs that cover their entire membrane, lowering the possibility of developing a conventional vaccine [62-64]. Thus, it is important to search for chemotherapeutics to treat sleeping sickness (African trypanosomiasis).

Antibody-Directed Enzyme Prodrug Approach, ADEPT, is one of the highest mean to accomplish selectivity in the prodrug design field [65]. It requires monoclonal antibodies and enzymes that are not common in the mammalian host. The system requires two phases: first, an interaction of the conjugate enzyme-antibody with the antigen and second, the relase of drug from classical prodrug by the enzyme of the previous conjugate. One of the most used enzymes in the conjugate has been β-lactamase along with cephalosporins as the carrier for the second phase. Pratt [66] has shown that when cephalosporins are hydrolysed by a β-lactamase the C-3' substituent is expelled depending on its living-group reactivity (Figure 5).

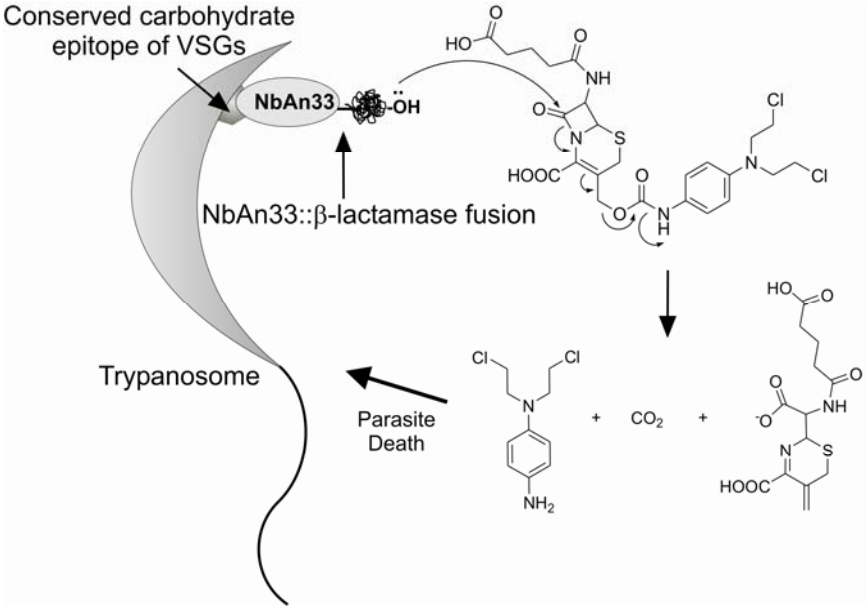
In the case of African trypanosomiasis, isolation of a monoclonal antibody reactive toward multiple VSGs on viable parasites has been a challenge, as the conserved epitopes become cryptic upon the assembly of VSGs on the membrane of the parasite. To circumvent this problem, Stijlemans and co-workers developed an ADEPT approach potentially useful for the treatment of African

trypanosomiasis using a nanobody named *NbAn33,* a *mAb* specific for the conserved carbohydrate epitope of VSGs fused to a β-lactamase enzyme for activation of a cephalosporin mustard prodrug [67,68]. Nanobodies were introduced by Stijlemans and co-workers [69] and are antigen-binding fragment with a molecular mass of only 15 kDa, coming from the serum of *Camelidae* which contains a high proportion of functional antibodies with no light chains [70]. The *in vitro* assay was carried out with sub-lethal doses of 0.5 to 10 μM of the prodrug following the incubation with the *NbAn33*::β-lactamase conjugate and succeeded.

Recently a human-specific serum protein, apoL-I, was found to be the trypanolytic factor of normal human serum (NHS) [71;72]. Also, it was discovered that trypanosomes become resistant to NHS after expression of the apoL-I-neutralizing serum resistance-associated (SRA) protein [73]. Based on these findings, a Tr-apoL-I, which cannot be neutralized by SRA and thus is capable of lysing both NHS-sensitive and NHS-resistant *T. b. rhodesiense* [74], was obtained. This compound is a natural trypanolytic agent that can be useful to cure *T. b. rhodesiense* infections providing its targeting Tr-apoL-I to the parasite surface could improve its effectiveness.

According to this and considering the nanobody approach and the use of *NbAn33* as a trypanosoma monoclonal antibody [69], Baral and co-workers [75] tested the performance of *NbAn33*-Tr-apoL-I in mouse models of African trypanosomiasis and the promising results allowed them to conclude a new trypanocidal modality for the human parasitosis treatment had been developed. The successful use of single domain antibody derivatives to combat trypanosomiasis could also be repeated to various other infectious agents.

Figure 5. ADEPT approach for African trypanosomiasis.

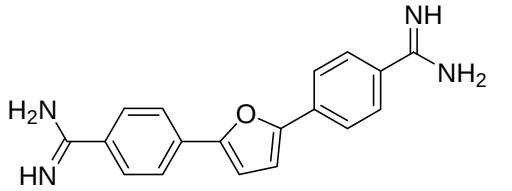


Prodrugs of aromatic diamines

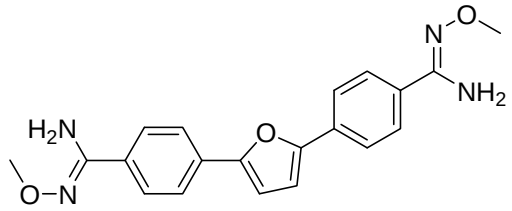
Aromatic diamines represent a class of compounds with broad-spectrum antimicrobial activity. DB75 [2,5-bis(4-amidenophenyl)furan] is an important aromatic diamine that is active against *Trypanosoma* sp *in vitro* [76;77] and DB289 [2,5-*bis*-(4-amidinophenyl)furan *bis*-*O*-methyl-

amidoxime] is its orally active amidoxin prodrug (Figure 6)**.** The latter is in phase IIb human clinical trials for treatment of early-stage human African trypanosomiasis, *Pneumocystis jiroveci carinii* pneumonia and malaria. Charlotte and co-workers found that mitochondrion is a cellular target of DB75 in yeast cell. This finding can aid in the target-based design of new antimicrobial aromatic diamines [62].

Figure 6. Chemical structures of DB75 and its prodrug DB289.



DB75



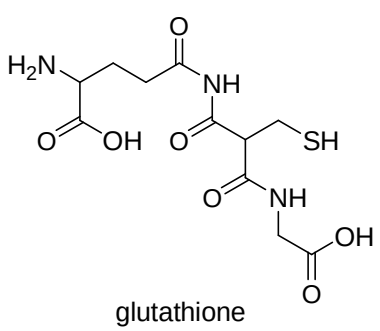
DB289

Prodrugs for Leishmaniasis

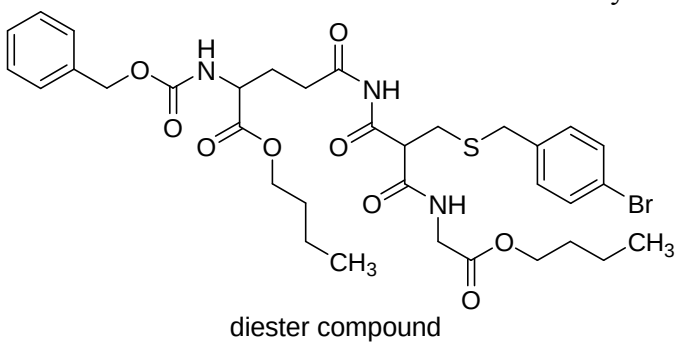
Glutathione prodrugs

Trypanotione plays a central role in the metabolism of trypanosomatids, like *T. brucei*, *T. cruzi* and *L. donovani*, acting both as a source of reducing equivalents and as a defense against oxidative stress [78]. Diester compounds *per se* were considered to be ineffective inhibitors of trypanotione metabolism, suggesting that these compounds might act as prodrugs, increasing the membrane penetration of glutathione derivatives prior to cleavage into possible gluthatione derivatives, which includes combinations of monoesters, free acids, and amines, some of which are inhibitors of trypanothione metabolism [78]. The dibutyl ester compound presented in Figure 7 shows moderate activity against *L. donovani* in an *in vitro* assay, and a higher activity against *T. brucei*.

Figure 7. Diester prodrug of glutathione derivative with antileishmaniasis activity.



glutathione



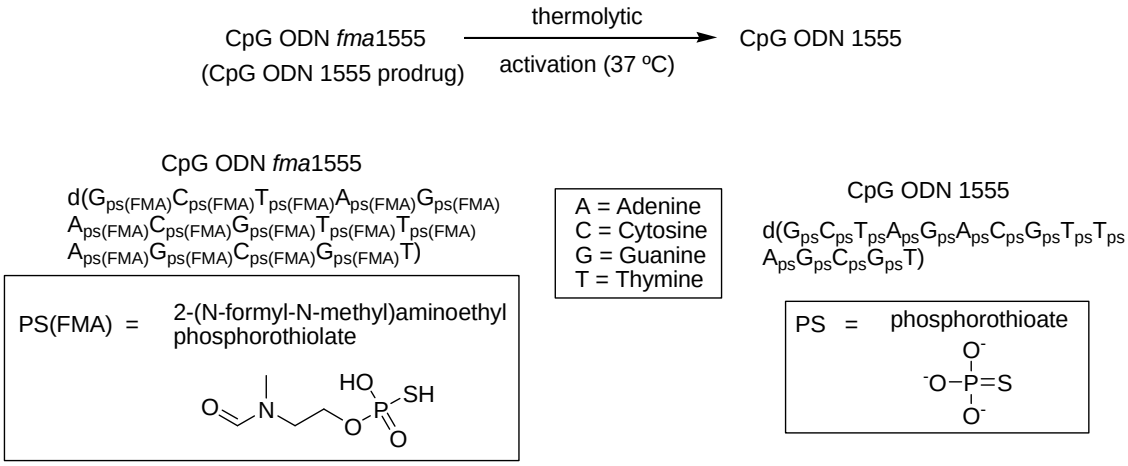
diester compound

Thermolytically activated oligonucleotide prodrugs

Oligonucleotides such as those containing unmethylated CpG dinucleotides are known to trigger vertebrate imune system through the activation of Toll-like receptor 9 (TLR-9) [79,80]. The resulting innate immune response limits the early spread of infectious organisms, while promoting the development of adaptative immunity. These dinucleotides show promise as vaccine adjuvants and to improve clinical outcome in *rhesus* macaques challenged with *L. major* [79,80]. The administration of such compounds can improve the host response to parasitic, bacterial and viral infection in animal models [80-82]. Prodrugs of those oligonucleotides have been designed to facilitate cellular uptake, allowed them to have increased resistance to hydrolytic nucleases.

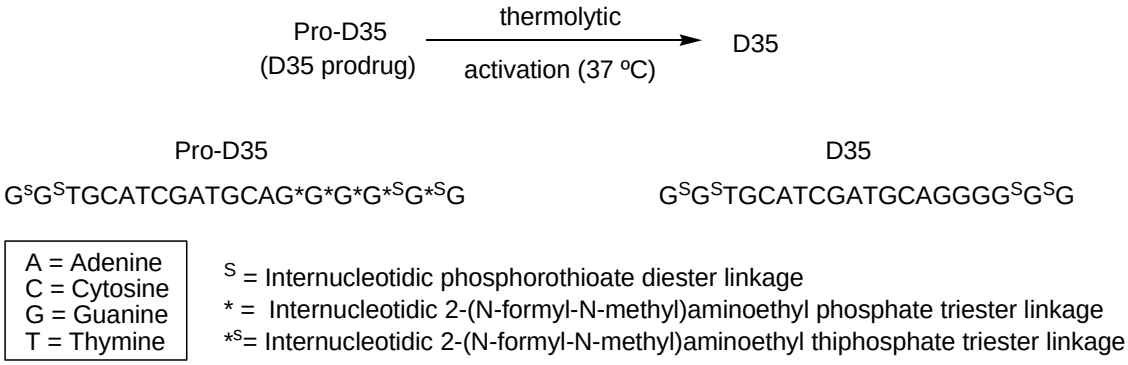
Grajowski and co-workers [83] reported the synthesis and characterization of a CpG ODN functionalized with thermolytic 2-(*N*-formyl-*N*-methyl)aminoethyl (*fma*) phosphorothioate triesters (Figure 8). Those compounds are activated only by temperature (37 ^{o}C). The half-life of thiophosphate deprotection was estimated to be 73 h and the complete deprotection was achieved within 600 h. Studies in mice infected with *Leishmania major* metacyclic promastigotes [83] prove that CpG ODN *fma*1555 was as effective as that of CpG ODN 1555 in reducing the size of *Leishmania* lesions in local intradermal administration.

Figure 8. Thermolytic activation of CpG ODN *fma*1555



Previous studies demonstrated that administration of ODN type D, which has a 3´ and poli(G) motif [84] – a group of gaunine ribonucleotide with phosphate residues acting as bridges to form diester linkages between the ribose moieties – improves its performance in healthy and immunocompromised non-human primates challenge with *Leishmania major* [85;86]. However, this structure can lead to product polymorphisms, aggregation and precipitation, which negates their clinical applications [87-89]. A prodrug form of CpG ODN type D (Pro-D ODN) with *fma* groups was developed by Puig and co-workers [90]. The resultant prodrug (pro-D35, Figure 9) demonstrated the immunoprotective activity of pro-D ODN when applied to macaques infected with *L. major*.

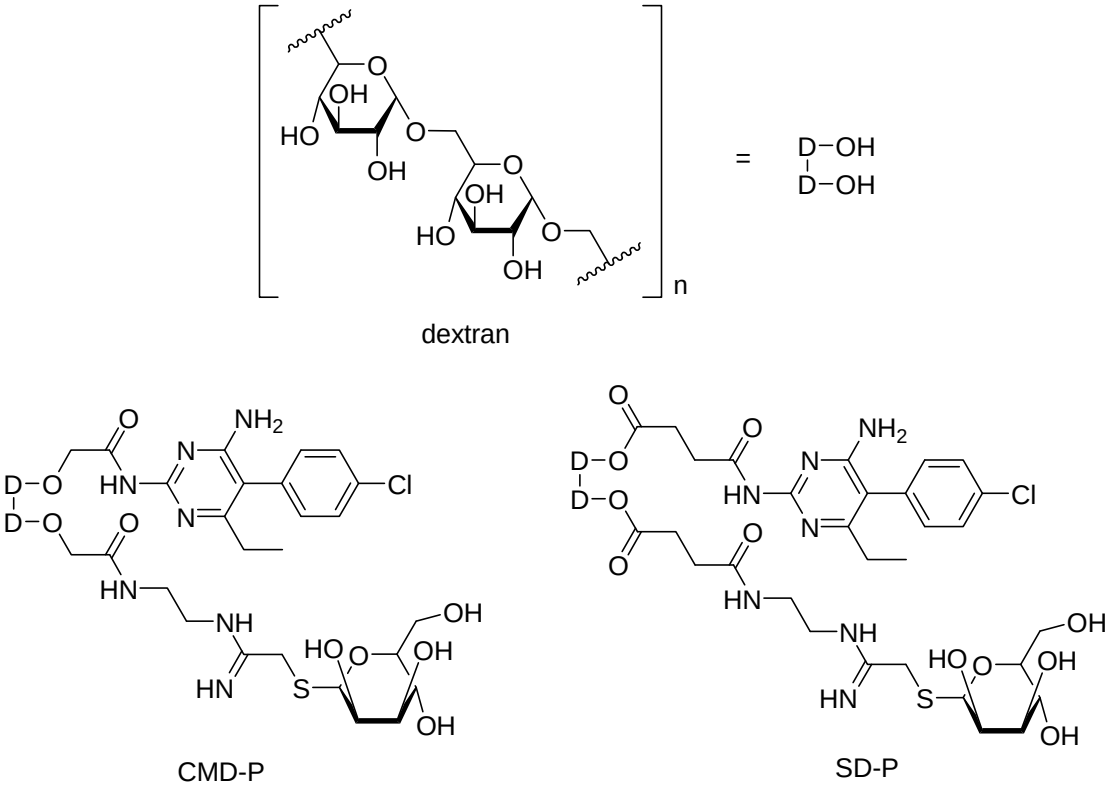
Figure 9. Thermolytic activation of Pro-D35.



Pyrimethamine polymer prodrugs

Although classic antifolate drugs, such as pyrimethamine, have been used with success in most protozoan diseases, some of them have not been effective when applied to leishmaniasis [91]. Pterins are a chemical class that plays an important role in mammalian folate pathways. Bello and co-workers [92] identified a *L. major* pteridine reductase enzyme (PTR1) that reduces pterins to their tetrahydro derivatives, reducing folic acid to tetrahydrofolic acid. This enzyme is an alternative to the folate pathway, which explains the parasites resistance to the classic DHFR inhibitors [93]. This represents a new and potential target to be considered. Pyrimethamine, an antimalarial drug, was found to be able to inhibit both enzymes (DHFR-TS and PTR-1) of the leishmanial folate pathway, although this effect *in vivo* appears only in relatively high concentrations [94].

Figure 10. Chemical structure of the prodrugs CMD-P and SD-P.

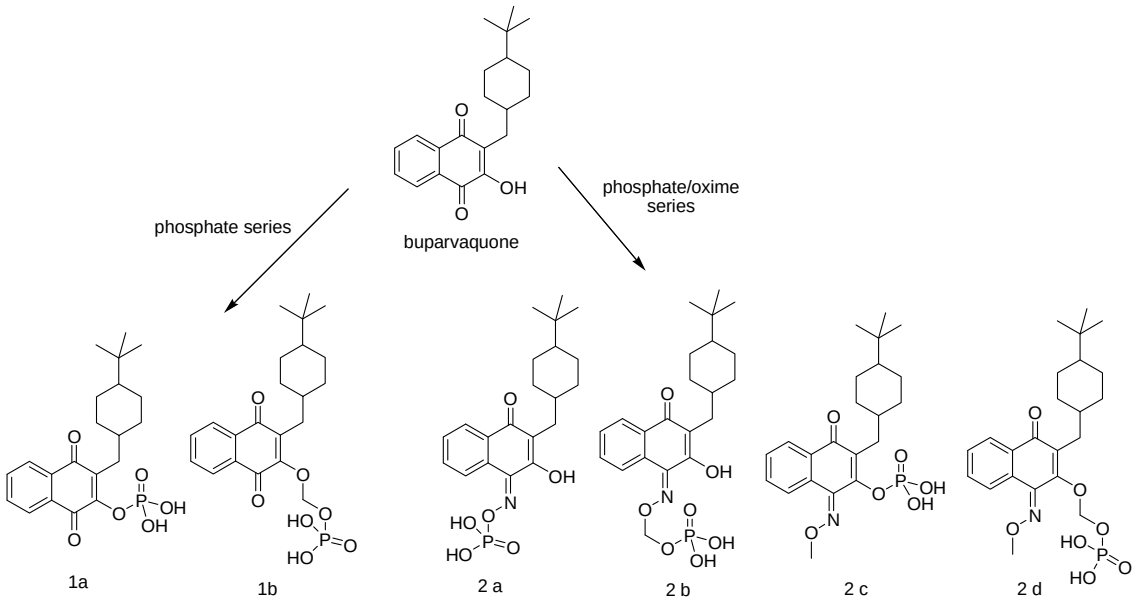


Based on that finding and on *Leishmania* amastigotes living inside mammal's macrophages [95], where mannose receptors are found on the surface membrane [96], Carvalho and co-workers [94] have synthesized targeted drugs of pyrimethamine, carboxymethyldextran-thiomannopyranoside-pyrimethamine (CMD-P), and succinyldextran-thiomannopyranoside-pyrimethamine (SD-P) (Figure 10), in order to release the drug into those cells. The targeted drugs were tested against macrophages infected with *L. (L.) amazonensis* amastigotes in the presence or absence of CMD-P and SD-P at concentrations ranging from 100 to 200 μg/mL for 24 h. After treatment with a 200 μg/mL CMD-P dosis a 46,4% reduction of the infection rate was observed and similar results were verified with a lower dosis of CMD-P (100 μg/mL). However, SD-P in similar doses did not show good results, probably due to the release of succinylpyrimethamine, still a prodrug, instead of the free drug. Further studies will test the *in vivo* effects of CMD-P prodrug.

Prodrugs of buparvaquone

Various hydroxynaphtoquinones have high antileishmanial activity *in vitro* against species that cause both cutaneous (CL) and visceral leishmaniasis (VL) [97;98]. Buparvaquone is a hydroxynaphtoquinone derivative (Figure 11) more active against *in vitro* than *in vivo L. donovani* amastigotes, after subcutaneous administration [97]. Two factors can account for this lack of *in vivo* activity: poor distribution from the site of injection to the intracellular target, when administered subcutaneously, and a combination of low aqueous solubility and high lipophilicity, that lowers the drug bioavailability [99].

Figure 11. Chemical structures of buparvaquone and its prodrugs.

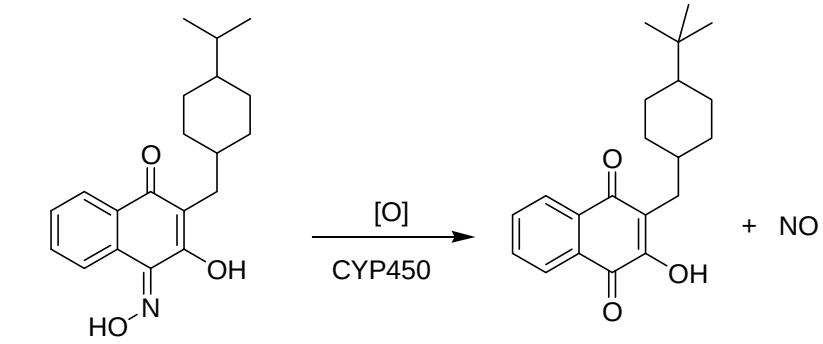


Mäntylä and co-workers [99,100] have synthesized two series of phosphate prodrugs (Figure 11) with the objective to enhance the drug bioavailability, increasing its aqueous solubility. This goal can be reached by the introduction of a phosphate promoiety in a hydroxyl group [101]. Neverthless, a phosphate carrier protecting a hydroxyl group is not always cleaved enzymatically due to steric

hindrance. Phosphonooxymethyl prodrugs [102,103] can have more lability, due to a two-step hydrolysis reaction, one with enzyme participation and the other with a spontaneous release of the parent drug [99]. Compounds **1a** and **1b** presented a rapid buparvaquone release in alkaline phosphate solution, while only **1b** was able to release the drug in human skin homogenate. These prodrugs had the same potency as buparvaquone against the promastigote and amastigote form of most species investigated. They also presented improved efficacy in *in vivo Leishmania major* and *Leishmania donovani* infections [104].

Oxime derivatives **2a-2d** (Figure 11) have also been evaluated *in vitro* [100]. All prodrugs showed increased water solubilities and released corresponding oxime derivative in alkaline phosphate solution. The prodrug phosphonooxymethyl-buparvaquone-oxime (**2b** in Figure 11) was the most interesting, not only because of its high aqueous solubility and chemical stability over a pH range of 3.0 to 7.4, but also due to a quick alkaline phosphatase hydrolysis to the lipophilic parent buparvaquone-oxime. The mechanism of its conversion to buparvaquone (Figure 12) takes place in the body after absorption, probably by a oxidation cytochrome P450 mediated thorugh the formation nitrogen oxides formation, including NO, and the corresponding parent compounds containing a C=O group. The generation of NO in addition to the drug may account for the elimination of *Leishmania* [105,106]. This is an interesting example of mixed prodrug-bioprecursor that can be useful to improve physicochemical properties and bioavailability of the drug, besides releasing other therapeutic agent, NO in this case.

Figure 12. The conversion of buparvaquone-oxime into buparvaquone by enzymatic oxidation and release of NO.



buparvaquone-oxime buparvaquone

Antimalarial prodrugs

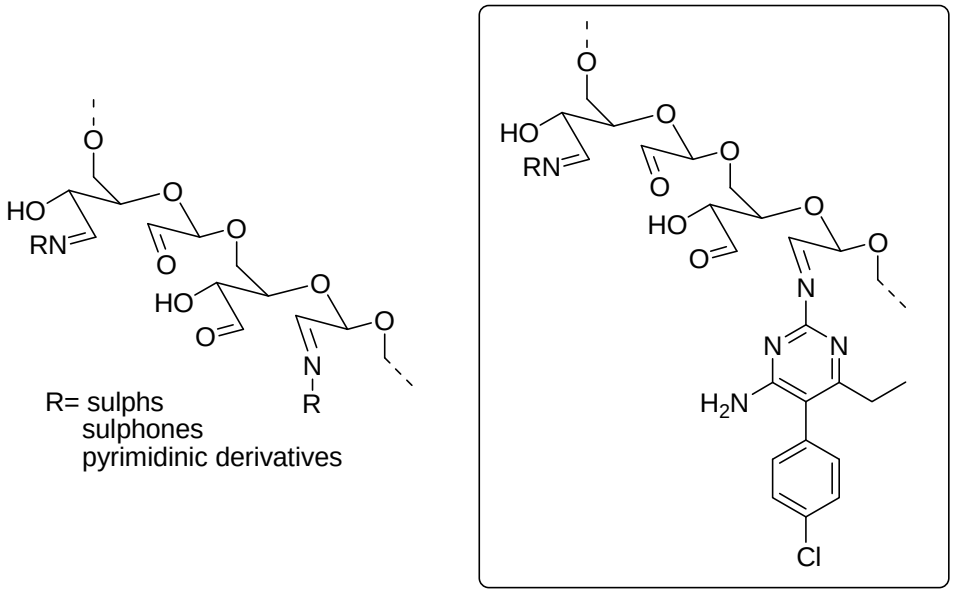
Polymeric antimalarial prodrugs

Current antimalarial chemotherapy consists of several compounds mainly active against the bloody form of *Plasmodium* parasites. Nonetheless, the need for drugs against tecidual forms as well as the emergence and spread of resistance against chloroquine and other major antimalarial drugs have brought the urgency to develop a new generation of safe and effective agents [107].

Ferreira and co-workers used polysaccharides as polymeric drug carriers to develop slow releasing prodrugs. The group developed several prodrugs using oxidized polysaccharides of antimalarial drugs

as sulphadoxine, sulphadiazine, dapsone as well as some pyrimidinic derivatives, for instance, pyrimethamine and trimethoprim (Figure 13) [108-110].

Figure 13. Oxidized polysaccharides applied at the development of prodrugs.



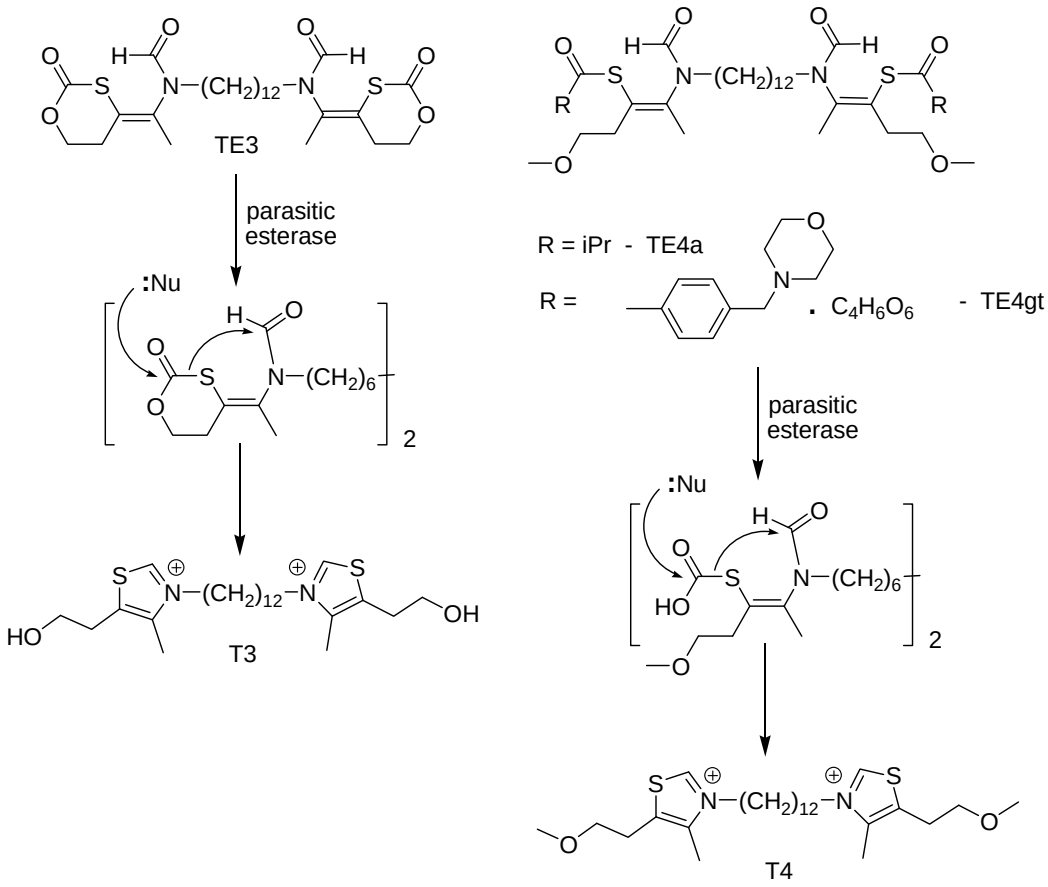
These polymeric prodrugs can also present better bioavailability profile and low toxicity when compared to the drug by itself whose problems with bioavailability is questioned as being related to the parasite resistant emergence.

Prodrugs of bisthiazolium salts

Among the targets available for chemotherapeutic design, phospholipid metabolism has been considered as an attractive biochemical step for developing new malaria chemotherapy. It is important for the parasite, is absent from normal mature human erythrocytes [111], and these cells have their content increased by around 500% because of the parasite metabolic activity. Because phosphatidylcholine is the major phospholipids, some monoquartenary and bisquartenary ammonium compounds have been used for malaria chemotherapy, showing exceptional *in vitro* and *in vivo* antimalarial proprieties, with no mutagenic activity [112,113]. Nevertheless, these compounds, structuraly similar to choline, present a cationic group which impairs their oral absorption [114]. Thus, prodrugs that mask the ionizable groups through thioester function were designed and synthesized (Figure 14) [112,113].

Prodrugs TE3, TE4a and TE4gt showed promising results: IC_{50} < 10 nM against 3D7 and Nigerian chloroquine (CQ)-sensitive strains and FCB1 and FCM29 CQ-resistant strains; rapid release to the active compounds in plasma and high effectiveness *in vivo,* with ED_{50} < 4 mg/kg after intraperitonial administration. Prodrugs TE4a and TE3 had higher activity (ED_{50} of 11 and 5 mg/kg, respectively) [113].

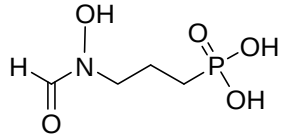
Figure 14. Chemical structures of bisthiazolium salt prodrugs.



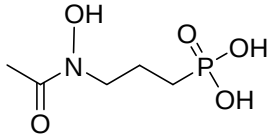
Prodrugs of fosmidomycin and FR900098

Fosmidomycin (Figure 15), involved in the inhibition of 1-deoxy-D-xylulose 5-phosphate (DOXP) reductoisomerase, an important and specific pathway in the isoprenoid synthesis [115], was shown to be active in recent clinical trials conducted in Gabon and Thailand with uncomplicated *P. falciparum* malaria [116]. Its acetyl derivative is around twice as active *in vitro* against *P. falciparum* [117] and in a *P. vinckei* mouse model [118]. Besides, it inhibited the growth of multidrug resistant *P. falciparum* strains *in vitro* [117].

Figure 15. Chemical structures of fosmidomycin and FR900098.



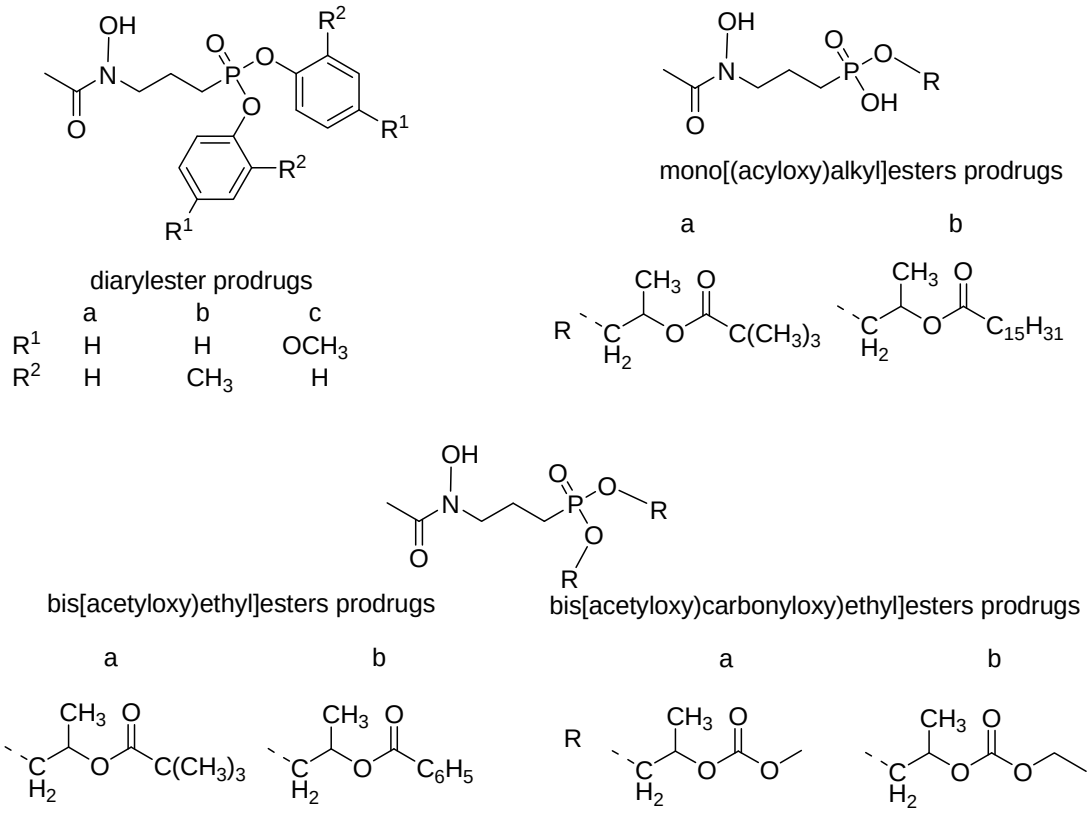
fosmidomycin



FR900098

The problem for both compounds is their poor oral biovailability, probably because of their low lipophilicity provoked to the high ionization of the phosphono moiety at physiological pH values [118]. In order to overcome this unwanted effect, Reichenberg and co-workers synthesized diaryl ester prodrugs of the acetyl derivative (Figure 16) [119]. The diaryl prodrugs, although more bioavailable in *P. vinckei* infected mice, could release toxic phenol derivatives. Therefore, a series of acyloxyalkyl esters expected to be hydrolysed by non-specific esterases was prepared [120] (Figure 16) and the *bis*[(acetyloxy)ethyl]ester was twice more active than the prototype.

Figure 16. Chemical structures of FR900098 ester prodrugs.



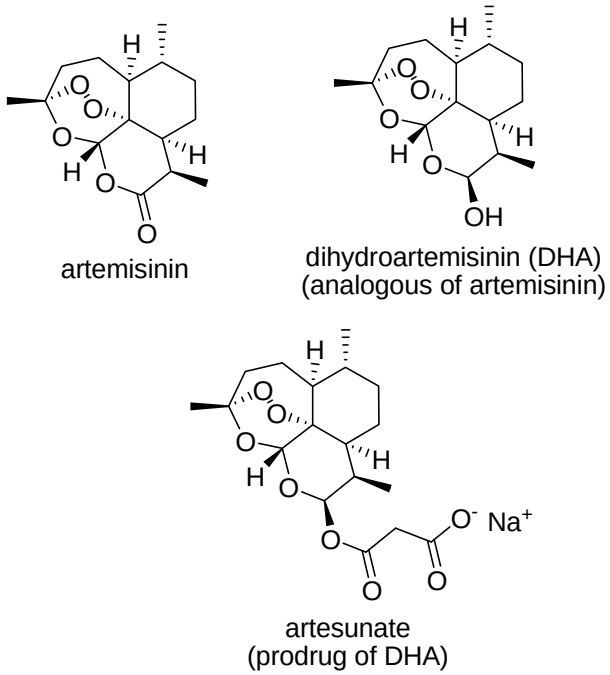
Also mono[(acyloxy)alkyl] and *bis*[(alkoxycarbonyloxy)ethyl] esters were prepared and were significantly more active than the parent compound [112,119-121].

Prodrugs of trioxane and dioxane derivatives (endoperoxidic agents)

Artemisinin (qinghaosu) is a sesquiterpene lactone, a 1,2,4-trioxane, which has been used clinically in China for the treatment of multidrug-resistant *P. falciparum* malaria [122]. It is believed that it acts through an interaction with heme, or ferrous ions, in the acidic parasite food vacuole, generating radical species responsible for membrane lipoperoxidation.

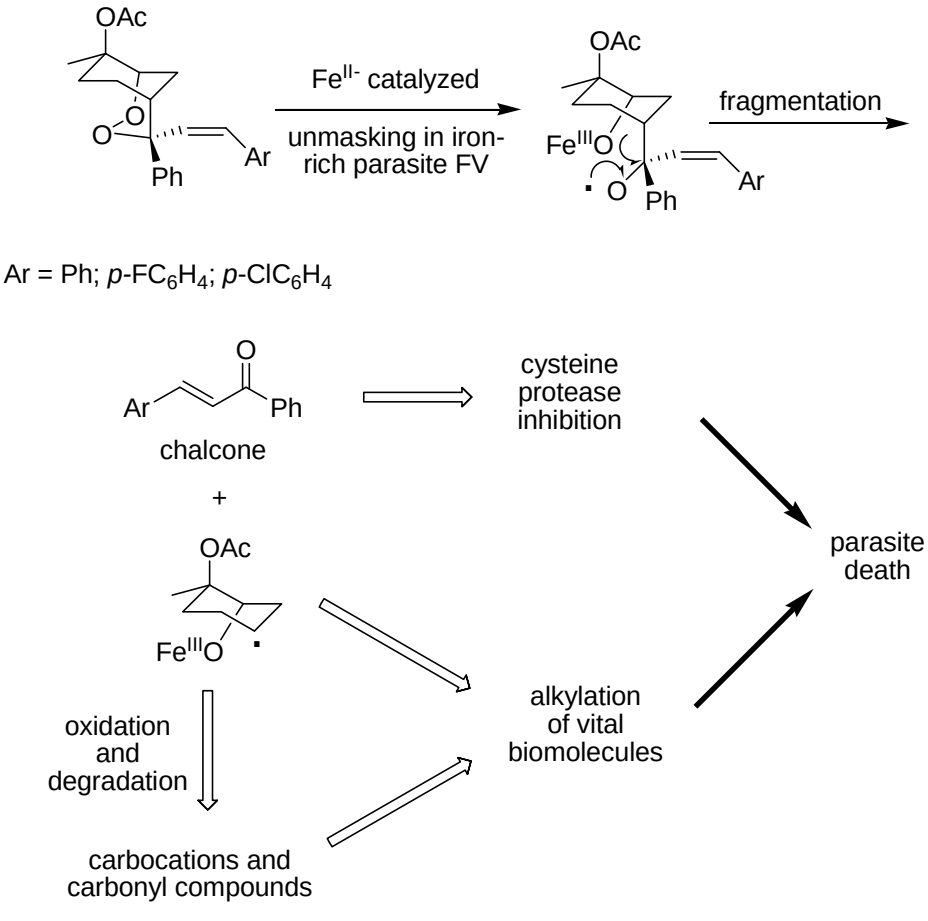
Despite the high activity of artemisinin, its clinical use has been limited by the inadequate pharmacokinetic profile. Prodrugs of its active metabolite, dihydroartemisinin, have been designed to improve its properties (Figure 17) [122,123].

Figure 17. Chemical structures of artemisinin, dihydroartemisinin and artesunate sodium salt.



The sodium salt of artesunic acid is a water soluble prodrug, able to reduce parasitemia and to recover comatose cerebral malaria patients [124]. Other artemisinin prodrugs are artemether and arteether, more lipophilic than the parent drug, and also have been used in malaria chemotherapy.

Figure 18. Fe^{II}-catalysed degradation of endoperoxide prodrugs.

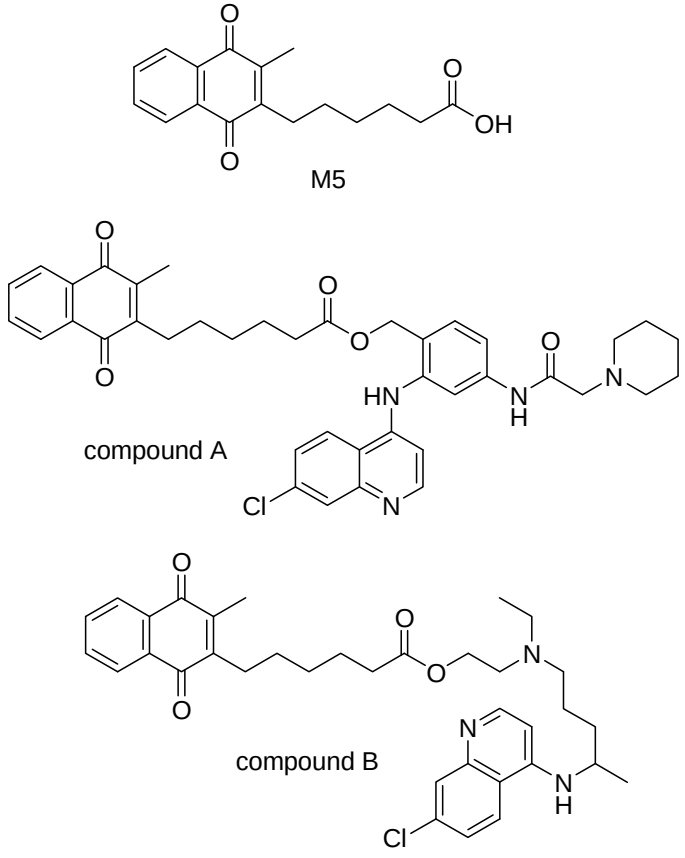


Compounds containing two antimalarial pharmacophores, such as a 2,3-dioxane and a cysteine protease inhibitor within the same molecule are part of new approaches using endoperoxide derivatives [125]. O'Neill and co-workers designed dual mechanism [125-128] prodrugs composed of a bicyclo endoperoxide prototype [126] in which the substituents at position 4 in prodrug prototype generates chalcone, through iron (II) mediation, alongside additional noxious species as shown in Figure 18. There are species that look like those generated by artemisinin and related trioxanes and presumably kill the parasite through a similar mode of action, and chalcones, that are considered to be effective antimalarial cisteine protease inhibitors [129].

Prodrugs of 4-aminoquinoline

The glutathione-dependent heme modification is inhibited by chloroquine (CQ) and other related 4-aminoquinolines. This suggest glutatione (GSH) is implied in the development of CQ resistance [130]. Glutathione reductase (GR) displays an important role in glutatione disulfide production and parasite detoxification, and GR inhibitors were proposed to protect from malaria and to reverse the CQ-resistance [131-133].M_5 is one of GR inhibitors that has a carboxilate function. Prodrugs of 4-aminoquinoline derivatives and M_5 were prepared via an ester bond (Figure 19), that showed to be very stable in biological compartments but releases the drug in the food vacuole of the parasite [132]. The IC_{50} value of compound **A** in Figure 19 was 23.1 nM and that of the compound B was 107 nM against FeB1R strain of *P. falciparum* [134]. Compound **A** presented ED_{50} values around 30 nM on average. It was equally active against CQ-sensitive and CQ-resistant *P. falciparum* strains.

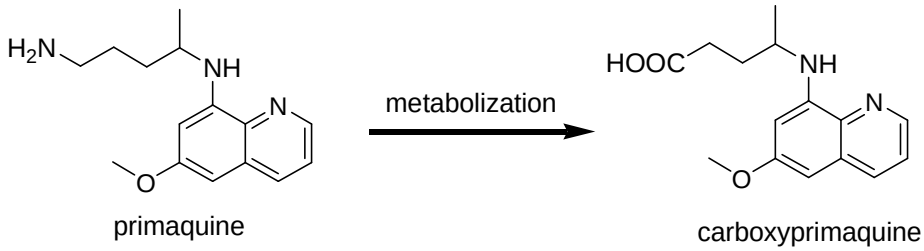
Figure 19. Chemical structures of M5 and 4-aminoquinoline derivative prodrugs.



Prodrugs of 8-aminoquinoline

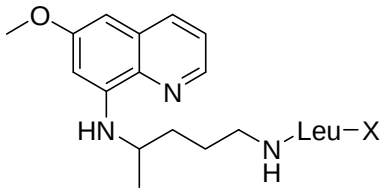
Primaquine is the only currently available drug as tissue schizonticidal agent. It is active against both the latent liver forms of the relapsing malaria caused by *P. vivax* and *P. ovale* and the gametocytes from all species of parasite causing human malaria including CQ-resistant *P. falciparum* [135]. Although it has been useful for those malaria form, its rapid metabolic inactivation to form carboxyprimaquine (Figure 20) together with methemoglonbinemia, a serious blood toxicity it provokes, prevent its wide use as antimalarial [136]. Several prodrugs with the goal of reducing toxicity and metabolic oxidative deamination have been synthesized.

Figure 20. Metabolism of primaquine.



Trouet and co-workres [137] reported the preparation, antimalarial activity and toxicity of several amino acid and peptide derivatives of primaquine. The activity was assessed against *P. berghei* in mice and the toxicity was established by determining the LD_{50}. Compounds **A** and **B** in Figure 21 were found to be less toxic and more active than primaquine.

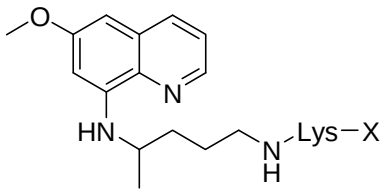
Figure 21. Chemical structures of (A) alanylleucylprimaquine and (B) alanylleucylalanylleucylprimaquine.



(A) - X=Ala
(B) - X=Ala-Leu-Ala

Philip and co-workers [138] have synthesized other three peptide derivatives of primaquine (Figure 22). All prodrugs showed activity against *P. cynomolgi* greater than the parent compound. The toxicity of compound **A** in Figure 22 was lower than that of primaquine in mice.

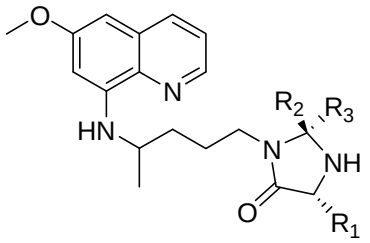
Figure 22. Chemical structures of (A) D-valylleucyllysylprimaquine, (B) D-alanylleucyl-lysylprimaquine and (C) valylleucyllysylprimaquine.



(A) - X=Leu-D-Val
(B) - X=Leu-D-Ala
(C) - X=Leu-Val

The problem of the peptide prodrugs, in general, is their instability [139,140], and this also happens with peptide primaquine prodrugs. They are rapidly hydrolyzed to primaquine by aminopeptidases and endopeptidases [141,142]. This suggests an extensive hydrolysis in the intestinal lumen when the prodrugs are orally administered, therefore, more stable peptide derivatives were needed [146]. One example is the use of imidazolidin-4-one formation (Figure 23) to protect the *N*-terminal amino acid residue of di-tri- and penta peptides against aminopeptidases-catalyzed hydrolysis [143].

Figure 23. Chemical structures of imidazolin-4-one derivatives of primaquine.



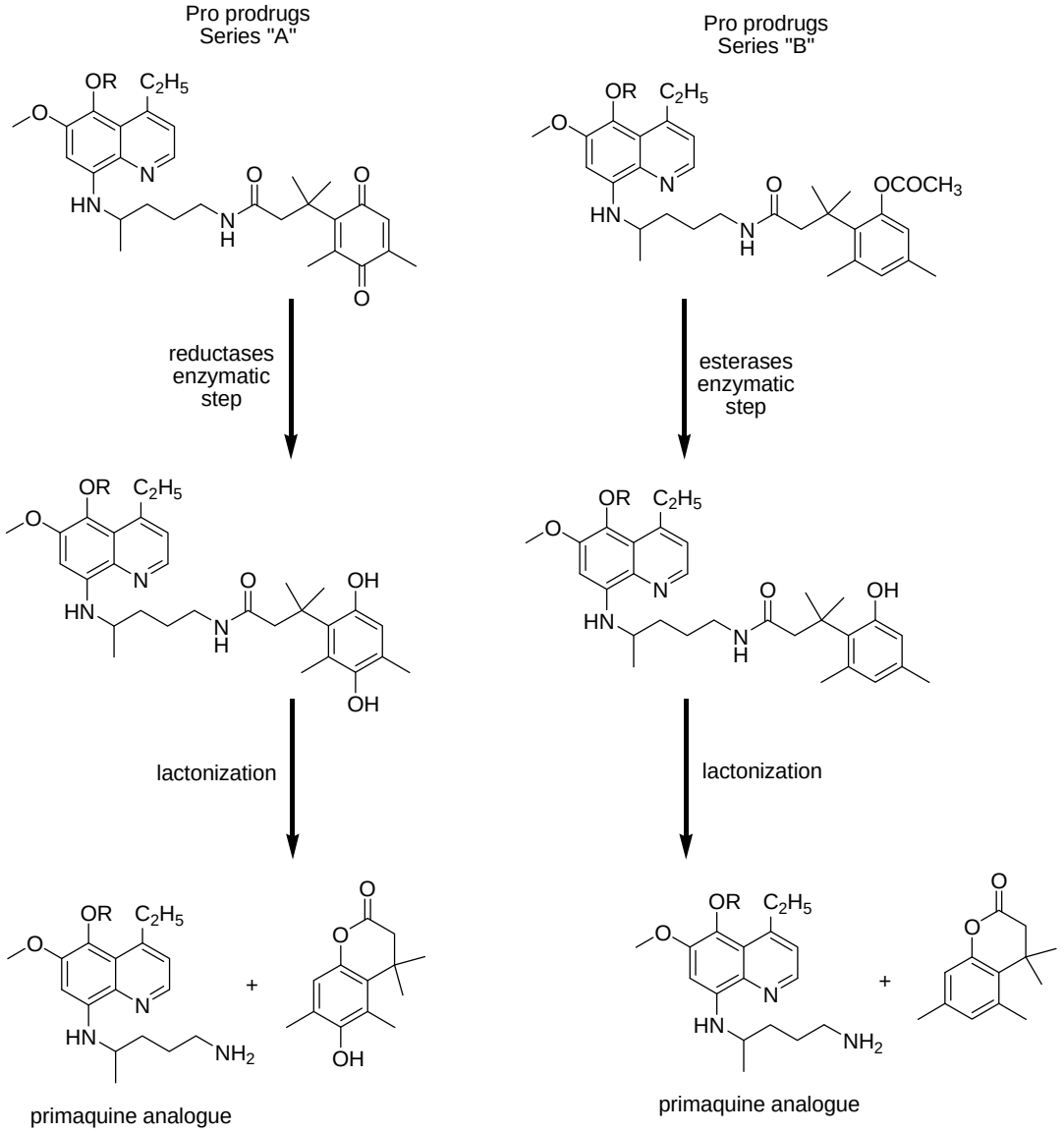
imidazolin-4-one derivatives

R_1, R_2 and R_3 = (cyclo)alkyl or benzyl group

These primaquine prodrugs were synthesized as potential double prodrugs of primaquine and may be useful as slow-release forms of the parent amino acid derivatives. These compounds showed to be highly potent as gametocytocidal against *P. berghei* [144].

Vagapandu and co-workers [145] have used modified 8-aminoquinoline compounds with increased blood-schizontocidal activity and activity against drug-resistant strains. Attempts to improve the properties of 8-quinolinamine antimalarial agents through prodrug approach indicated, however, that a single chemical modification is not sufficient to achieve the desired alteration in the biological properties to enhance blood-schizontocidal activity [138]. Two series of "double prodrug" or "pro-prodrug" were therefore proposed (Figure 24). They need two independent reactions in order to regenerate the parent drug.

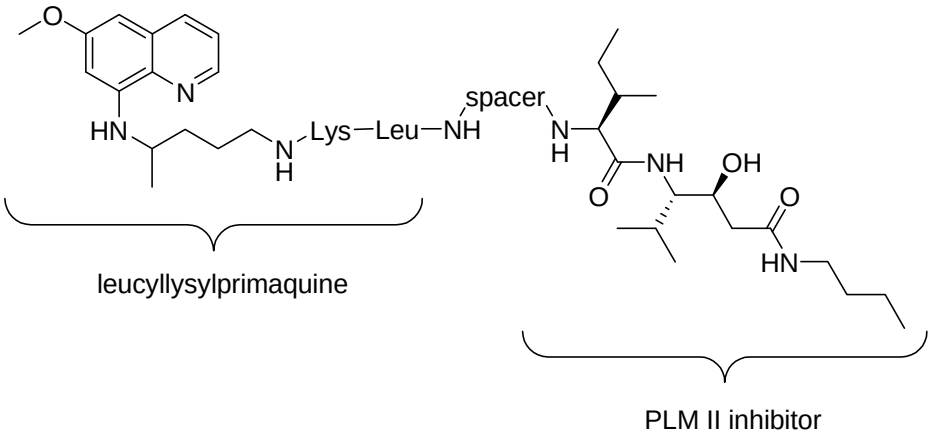
Figure 24. Pro-prodrug series of primaquine analogue (R=C_5H_{11} or C_7H_{15}), and the regeneration of parent drug.



Pro-prodrug from series "A", where R=C_5H_{11} or C_7H_{15}, and from series "B", where R=C_5H_{11}, have shown curative activity at initial test dosis of 100 mg/kg. The most potent compound of series "A", where R= C_5H_{11} produced suppressive effects at the dose of 50 mg/kg, and was the most potent compound from the pro-prodrug series, leading to antimalarial activity comparable to its parent drug [145].

Pro-prodrugs, mutual prodrugs in the following examples, with statine-based inhibitor of protease plasmepsinII (PLMII) and primaquine peptide derivative have been linked by an alkyl diacid or an aromatic diacid (Figure 25) [146]. They have been designed based on the fact that inhibition of leads to starvation of the parasite. This enzyme is a potential target for antimalarial agents. The most active compound was 4,4'-oxybis(benzoic acid) and the primaquine derivative was leucyllysylprimaquine. Its IC_{50} was 0.59 nM in in vitro test against *P. falciparum* [147].

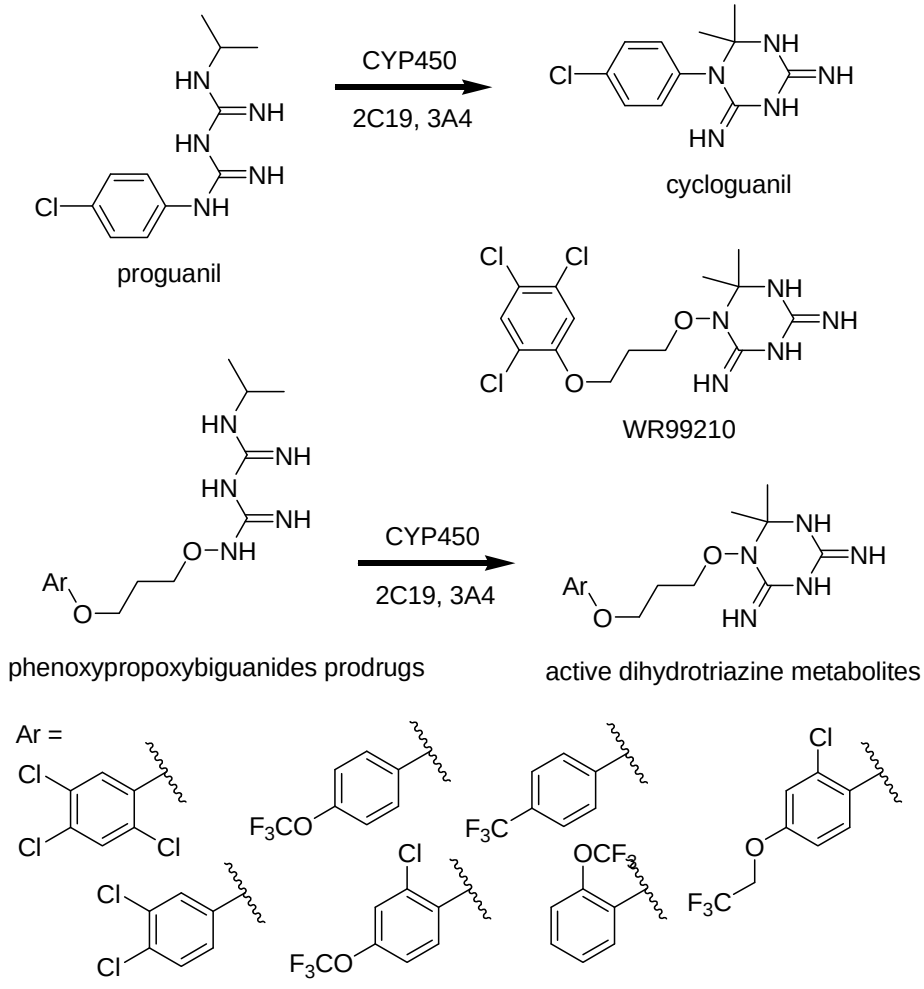
Figure 25. Primaquine-statine pro-prodrugs.



Prodrugs of dihydrotriazine

Malaria parasites have shown drug resistance for most of the antimalarials currently used. They include antifolate drugs such as cycloguanil, the active metabolite of proguanil (Figure 26).

Figure 26. Chemical structures of proguanil, cycloguanil, WR99210, phenoxypropoxy-biguanides prodrugs and their active metabolites.



Mutations on the *P. falciparum* dihydrofolate reductase thymidilate synthase (*pf*DHFR-TS) enzyme lead to a decreased binding affinity for the drugs [148]. Rieckmann, in 1973 [149], demonstrated compound WR99210 to be potent *in vitro* against a CQ- and pyrimethamine-restistant strain of malaria and high level of efficacy against mutant *pf*DHFR-TS enzymes and antifolate resistant isolates of the malaria parasite [150]. Nevertheless, this interesting compound provokes significant gastrointestinal intolerance in animal studies and poor biovailability.

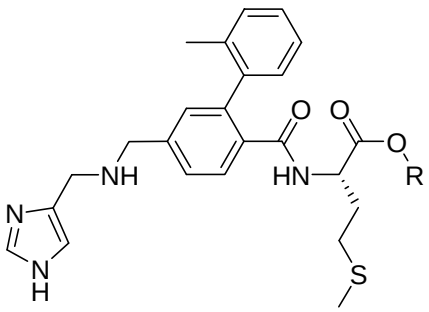
Phenoxypropoxybiguanide prodrugs, including the prodrug of WR99210 (Figure 26), were then synthesized. They have similar metabolization to that of proguanil [151,152]. Shearer and co-workers [153] have studied these prodrugs and prove that the active dihydrotriazines metabolites exhibited IC_{50} values less than 0.04 ng/mL against the CQ/pyrimethamine- sensitive (D6) and CQ/pyrimethamine-resistant DHFR mutant strain (W2).

Prodrugs of protein farnesyltransferase inhibitors

Another potential target for the design of new antimalarial drugs is protein farnesyltransferase (PFT) [154]. PFT catalyzes the covalent transfer of a farnesyl group to cysteine residues near to the C-terminus of protein substrates. It displays a critical role in cellular signaling and other regulatory paths [155].

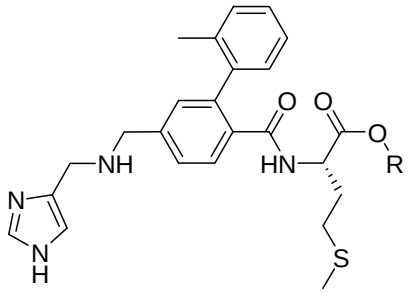
Ohkanda and co-workers [156] have tested a series of peptidomimetics PFT inhibitors against *P. falciparum in vitro*. In this study, compound FTI-2148 has no activity against *P. falciparum* (ED_{50}>30 μg/mL), despite being a potent mammalian PFT inhibitor (IC_{50}=1 nM). On the other hand, the methyl ester prodrug of FTI-2148 (Figure 27) inhibited *P. falciparum* growth with an ED_{50}=2 μg/mL. Other prodrugs are depicted in Table I and resulted from further work of Carrico and co-workers [157]. Although not active in the *P. falciparum* PFT, they were able to cross biological barriers due to their higher hydrophobicity and membrane permeability when compared to the free acid. Not only the hydrophobicity may affect the delivery of the compound into the parasite cell. Other factors, such as size and conformation of the ester side chain also display a role in the activity.

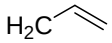
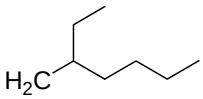
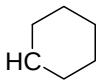
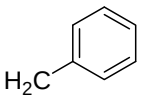
Figure 27. Chemical structures of compound FTI-2148 and its methyl ester prodrug FTI-2153.



R= H FTI-2148
R = CH_3 FTI-2153

Table 1. Inhibition activity of FTI-2148 prodrugs against *P. falciparum* PFT and *P. falciparum* infected red blood cell

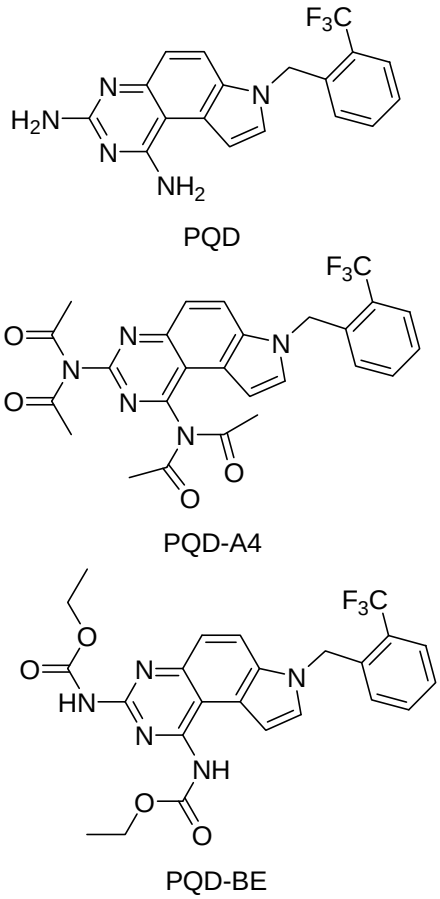


R	Log*P*	*P. falciparum* PFT IC_{50}(nM)	*P. falciparum* infected RBC[a] ED_{50} (nM)
CH_3 (FTI-2153)	4.44	>1000	3300
C_2H_5	4.88	750	1900
	5.19	>1000	1800
	5.19	>1000	800
	7.09	>1000	700
	4.94	>1000	350
	6.09	1000	150
H (FTI-2148)	1.77	15	>66,000

Prodrugs of pyrroloquinazolinediamine

Pyrroloquinazolinediamine (PQD) and its derivatives possess antimalarial among other activities [158]. They are very active antimalarial agents *in vivo* and *in vitro* [159-161], but are also highly toxic. Several prodrugs were prepared and two of these derivatives, tetraacetamido-pyrroloquinazolinediamine (PQD-A4) and *bis*-ethylcarbamyl pyrroloquinazolinediamine (PQD-BE), were more potent and less toxic than the parent compound (Figure 28) [162]. They show high *in vitro* activity against *P. falciparum*, with a IC_{50}=0.01 ng/mL, and against *P. berghei* in a rodent model, with a 100% curative oral dose between 0.1 and 4 μg/kg [162]. These two derivatives were also highly active in *Aotus* monkeys (oral curative doses, 1 mg/kg) and no toxicity was reported to the mammalian host. The high activity of PQD-A4 together with its high safety account for its interest as a promising candidate for antimalarial treatment [163].

Figure 28. Chemical structures of PQD, PQD-A4 and PQD-BE.

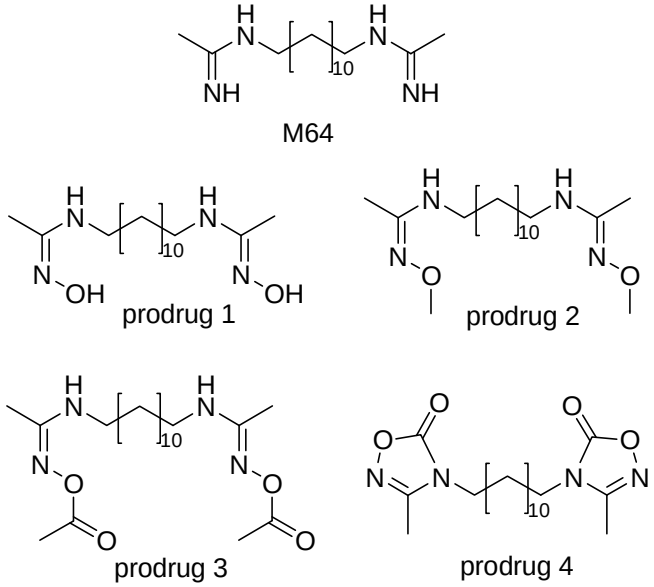


Prodrugs of aromatic amidine

Due to the potential interest of benzamidine groups found in many drugs, bezamidoxime and derivatives or carbamates are more lipophilic and less basic prodrugs with decreased pKa, overcoming the low oral biovailability of this group of compounds [164-168]. These modifications improved biovailability up to 30-fold and oral activity by 20-fold [164,168,169] and reduced adverse effects [164,168], although little effect was seen on the half-life eliminations of these compounds [164]. The conversion of benzamidoxime groups to benzamidine, both *in vitro* and *in vivo*, is performed by a microsomal reductase system present in all mammalian species [170].

Ouattara and co-workers [171] have synthesized a series of prodrugs of the lead compound 1,12-bis(*N*,*N'*-acetamidinyl)dodecane (M64, Figure 29). These prodrugs were tested in mice infected with *P. vinckei* and were administered intraperitoneally and orally once daily for four consecutive days. Prodrug 2 was not very effective probably due to its low conversion to drug. Prodrugs 1 and 3 showed to be effective and prodrug 4 was even more potent by oral route with an activity at least 3 times higher than the parent drug M64 and the best after oral administration.

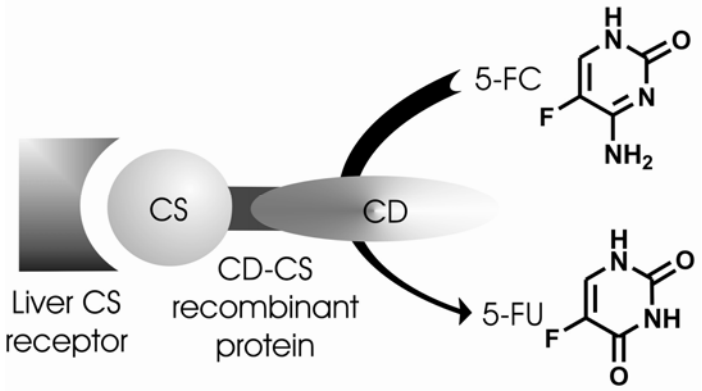
Figure 29. Chemical structures of compound M64 and its prodrugs.



CD-CS approach

Cerami and co-workers [172] demonstrated that CS protein (malaria circunsporozoite) [173] found in the outer surfaces of sporozoites and responsible for the liver-specific invasion of malaria parasite could be used as a delivery vehicle to introduce recombinant DNA into hepatocytes. They used adenovirus as an endosomal lysing agent. On the other hand, Lin-Lee and co-workers [174] used nonviral vectors for the same purpose. They studied a receptor-mediated delivery strategy using recombinant fusion protein consisting of CS protein as a ligand and bacterial cytosine deaminase (CD). This enzyme catalyzes the production of 5-fluorouracil (5-FU) from its prodrug 5-fluorocytosine (5-FC) (Figure 30). Receptor for CS protein is distributed predominantly at the basolateral domain of hapatocytes [175]. This CD-CS system could be cell type-specific and in the presence of exogenous 5-FC would be prone to kill the parasites [174].

Figure 30. CD-CS approach. Conversion of 5-fluorocytosine (5-FC) into 5-fluorouracil (5-FU) by cytosine deaminase (CD).

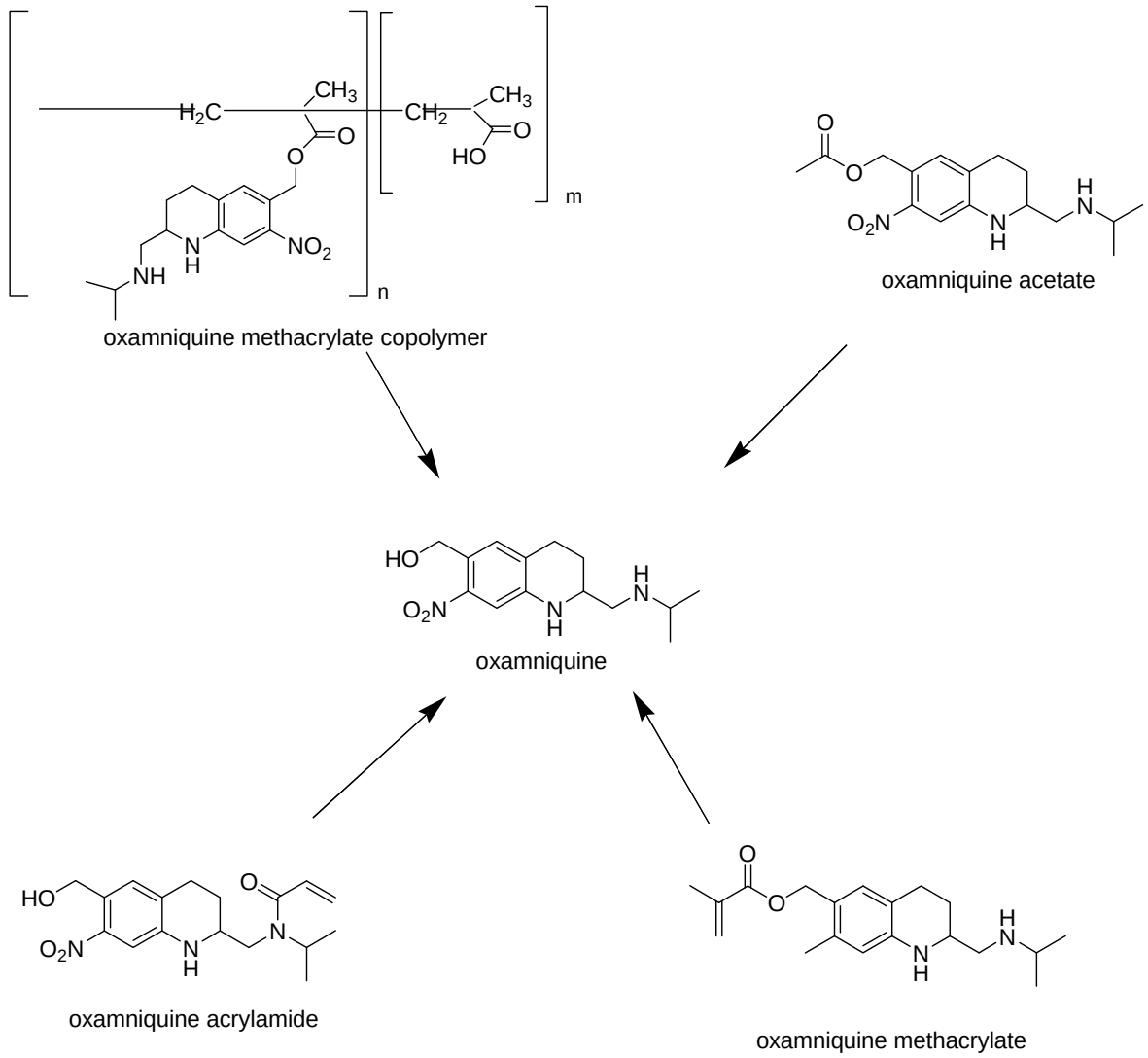


Prodrugs for Schistosomiasis

Prodrugs of oxamniquine

With the purpose of obtaining new and better oxamniquine derivatives, polymeric prodrugs have been designed. Polymethacrylic and polyacrylic are known to anchor drugs, promoting improved bioavailability, prolonged action and reduced adverse effects. Thus, Parise Filho and co-workers [176] selected methacrylic and acrylic monomers as carriers and synthesized oxamniquine methacrylate, acrylamide and methacrylate copolymer (Figure 31).

Figure 31: Structures and hydrolysis of prodrugs of oxamniquine.



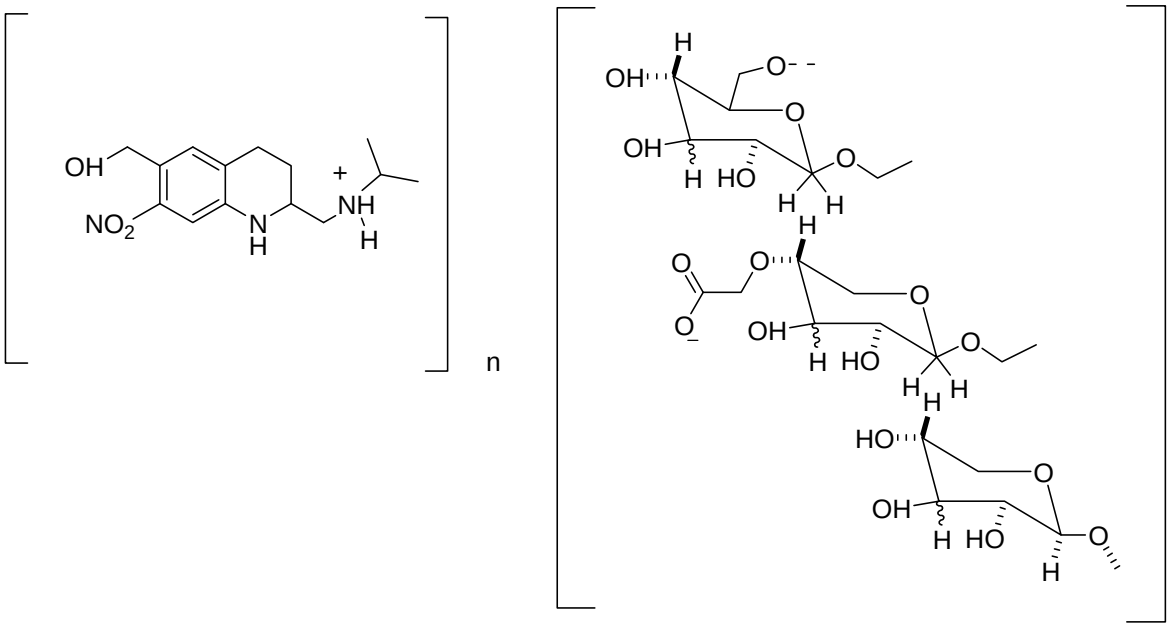
Preliminary *in vivo* evaluation showed that oxamniquine methacrylate and oxamniquine acrylamide have prolonged action profiles and reduced worm charge. Concerning parasite charge, similar profiles to oxamniquine were observed. Otherwise, for the compound oxamniquine methacrylate copolymer, a significant increase in the number of worms was observed, suggesting that the methacrylate copolymer did not exhibit antiparasitic activity. This behavior may be related to

several factors such as: the compound might not have achieved the effective dosis in the studied period; problems of drug release from the polymeric matrix, due to its complexity, and also to the high molecular weight of the prodrugs obtained, which may have inhibited the endocytosis process.

Almeida and co-workers synthesized an oxamniquine polymeric prodrug [177], using dextran T-70 (Figure 32) as a carrier, in order to improve the oxamniquine action and minimize its side effects. The preliminary tests of biological activity of this compound showed similar activity of the polymeric prodrug when compared to the commercial oxamniquine (Mansil®).

Based on the oxamniquine mechanism of action – enzyme esterification to a reactive ester that acts as an alkylating agent and binds covalently to schistosomal DNA -- El-Hamouly synthesized oxamniquine acetate (Figure 31) and studied the effect on the survival of hycanthone-sensitive and hycanthone-resistant strains of *S. mansoni* [178]. In addition, the effect on schistosome [^{3}H]uridine incorporation was determined, since it has been shown that there is a good correlation between this effect and schistosomicidal activity. The prodrug inhibited [^{3}H]uridine incorporation and caused death in the hycanthone-sensitive strain, but no effective in hycanthone-resistant strain.

Figure 32: Chemical structure of the dextran-methylcarboxylate prodrug of oxamniquine.



Prodrugs of dihydroartemisinin

Derivatives of artemisinin, which are already widely and effectively used in the treatment of malaria, also display antischsitosomal properties [179]. Artesunate acts as a prodrug of dihydroartemisinin (DHA) is rapidly hydrolysed to DHA in presence of water (Figure 17). Utzinger and co-workers showed this prodrug is highly active against the juvenile stages of *S. mansoni*, whereas adult worms are considerably less susceptible [180]. Another study [181] lead to the conclusion that artesunate is more active than praziquantel in causing damage to the tegument of adult *S. mekongi.*

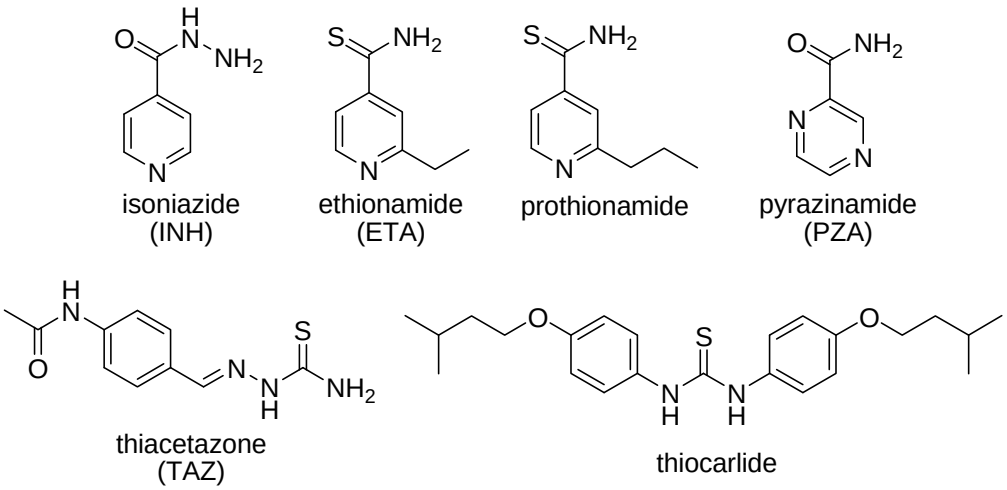
Further studies are needed to answer whether *S. mekongi* has the same typical stage specific susceptibilities to artesunate or artemether as previously observed in *S. mansoni* and *S. japonicum.*

Tuberculostatic prodrugs

Bioprecursors for tuberculosis chemotherapy

Several antituberculosis compounds are bioprecursor prodrugs that require activation by *Mycobacteriu*m enzymes to acquire bacterial toxicity (Figure 33). These include pyrazinamide (PZA), isoniazid (INH) and ethionamide (ETA). PZA is activated by the mycobacterial pyrazinamidase (PncA) to pyrazinoic acid, that lowers the pH enhancing the intracellular accumulation of the latter. This is unable to diffuse across the mycobacterial cell wall, leading to the disruption of membrane transport and energy depletion [182,183]. Because no pyrazinoic acid efflux mechanism exists, this accumulation process causes a remarkable susceptibility of *M. tuberculosis* to pyrazinamide. Mutations in the gene encoding pyrazinamidase/nicotinamidase (*pncA*), cause resistance to the antituberculous drug pyrazinamide in tubercle bacillus [184]. Both isoniazid and the structurally analogous thioamide, ethionamide, act as inhibitors of InhA (enoyl-acyl carrier protein reductase). However, the large majority of isoniazid-resistant strains remain full susceptible to ethionamide [185]. This is due the fact that INH and ETA are activated by different mechanisms; thereby there is no cross-resistance [186,187].

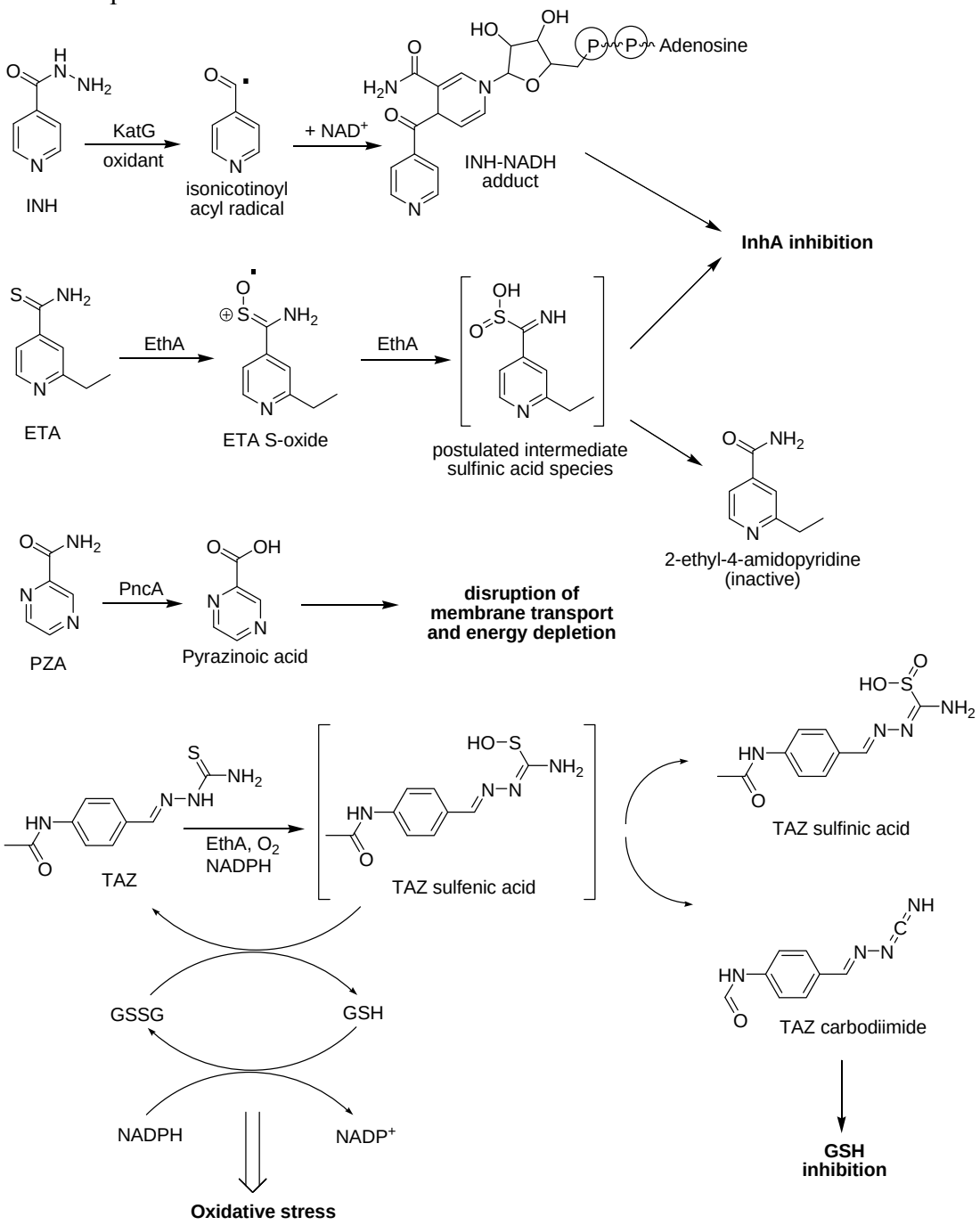
Figure 33. Chemical structures of some antimycobacterial agents.



The INH is activated by the catalase/peroxidase (KatG) [187;188], generating an isonicotinoyl radical, which then couples to NADH [189], giving an INH-NADH adduct, which is a potent inhibitor of InhA. Monooxygenase (EthA)-mediated conversion of ETA results in the formation of the corresponding sulfoxide product (Figure 34). This has to be activated in a subsequent reaction and the second activation step was also found to be catalyzed by EthA yielding 2-ethyl-4-amidopyridine as final product with no antitubercular activity [190]. This indicates that the key toxic species is formed as an unstable reactive product intermediate [186]. It has been suggested that the initial sulfinate

product formed after two consecutive EthA-mediated sulfoxidations of the parent drug decomposes to form a labile toxic intermediate [191]. Besides ethionamide, several others thioamide prodrugs and probably thiacetazone (TAZ) are activated by EthA. However, the final target for these antitubercular prodrugs differ for each one, although ethionamide and several others thioamide prodrugs but not TAZ affect the synthesis of short-chain fatty acids [186,190,192,193].

Figure 34. Activation pathway of the bioprecursors for tuberculosis and targets of the active compounds.



Oxidation of TAZ by EthA at basic pH favors formation of the carbodiimide, whereas neutral or acidic conditions lead to the sulfinic acid. These metabolites derive from an initial sulfenic acid

intermediate. The sulfenic acid and carbodiimide metabolites, but not the sulfinic acid product, readily react with glutathione. These reactions may be involved in the antitubercular activity of TAZ (Figure 34) [194].

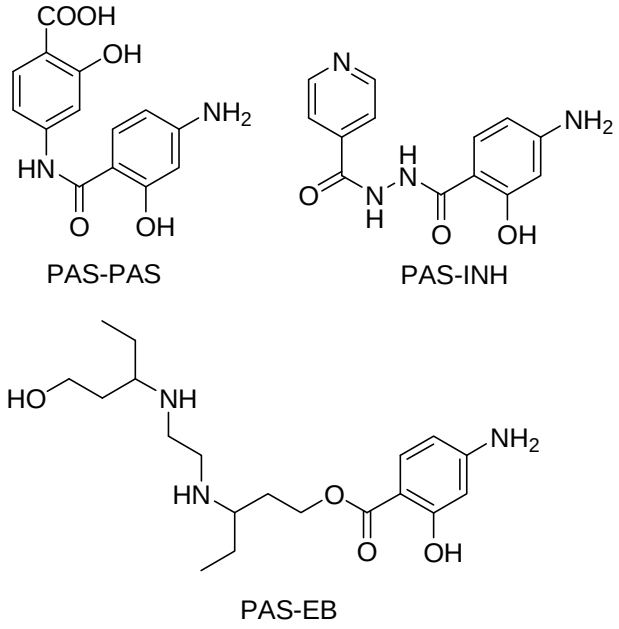
Mutual prodrug of isoniazid, para-aminosalicylic acid, and ethambutol

Antitubercular therapy is given for a long time and involves combination of drugs as 4-amino-salicylic acid (PAS), isoniazid (INH), ethambutol (EB) along with rifampicin, which is marketed in combination dosage form.

The free acid carboxylic group in PAS accounts for its gastrointestinal irritation effects. This drug is extensively metabolized by acetylation of the amino group and conjugation with glucuronic acid and glycine at the carbonyl group. Since its half-life for the metabolism is one hour, large doses are needed to maintain a minimum effective level of the PAS. INH is readily absorbed on oral administration, extensively metabolized to inactive metabolics (diacetylhydrazide, acetylhydrazide, *N*-acetylisoniazid and hydrazine) [195] and when co-administered with PAS, this drug is found to reduce its acetylation increasing its plasma level

EB is a water-soluble, bacteriostatic agent which is readily absorbed (75–80%) following oral administration. Most of the administered EB is excreted unchanged, with not more than 50% appearing in urine in oxidized form of aldehyde or carboxylic acid [196,197]. Since this drug can be used in combination with PAS and INH, Rawat and co-workers synthesized mutual prodrugs. The objectives were the reduction of gastrointestinal toxicity of PAS; the reduction of intestinal acetylation of isoniazid and the increase in duration of action, besides decreasing the dose of drugs (Figure 35) [198].

Figure 35. Chemical structures of the mutual prodrugs (PAS = *para*-aminosalicylic acid; INH = isoniazid; EB = ethambutol).



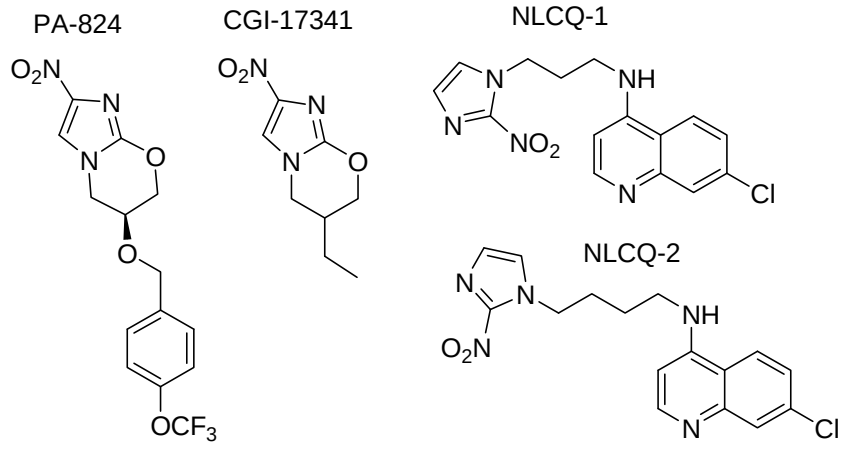
Those mutual prodrugs were absorbed unhydrolyzed. *In vivo* studies were also performed to confirm the release profile of these prodrugs and greater serum concentrations of EB, PAS and INH than their concentrations when given alone were found. Therefore, those mutual prodrugs eliminate significantly the problem of catabolism by acetylation in gastrointestinal tract and also PAS and INH toxicity [198].

Prodrugs of nitroimidazoles

One-third of the world population is currently infected with latent TB associated with the presence of the latent, non-replicating and resistant to conventional antimycobacterium drugs forms of the bacterium [199,200]. The latent bacilli have adapted to anaerobiosis and can maintain viability for extended periods of time. The first compound to show selective activity against those forms of *M. tuberculosis* was metronidazole. It undergoes reduction of its nitro group to reactive species that causes DNA damage and subsequent cell death [201]. However, metronidazole failed to show activity in an *in vivo* mouse model [202].

Based on the activity of nitroimidazoles, a series of 6-substituted-2-nitroimidazo[2,1-*b*][1,3]oxazimes prodrugs (Figure 36**)** were synthesized, which require bioreductive activation of an aromatic nitro group to exert antitubercular effects. PA-284 showed significative antitubercular activity against sensitive, mono-resistant and multidrug-resistant strains of *M. tuberculosis*, as well as against latent bacilli. It was also active against *M. tuberculosis* in *in vivo* mouse models [203]. This prodrug is in phase I clinical trials sponsored by the Global Alliance. However, *M. tuberculosis* mutant relatively to a specific protein, a F_{420}-dependent glucose-6-phosphate dehydrogenase, that metabolizes PA-824 and also CGI17341, showed to be resistant those compounds [204;205].

Figure 36. Chemical structures of nitroimidazole prodrugs.



Compounds NLCQ-1 and NLCQ-2 hydrochloride (Figure 36) were tested against dormant *M. tuberculosis* $H_{37}Rv$ [204]. Both compounds demonstrated significant activity, comparable with PA-824. They showed Minimal bactericidal concentrations (MBCs) of 3.1-18.4 and 4.9-9.8 μg/mL, respectively while PA-824 MBC was 6.4-12.8 μg/mL. These compounds, mainly NLCQ-1 are

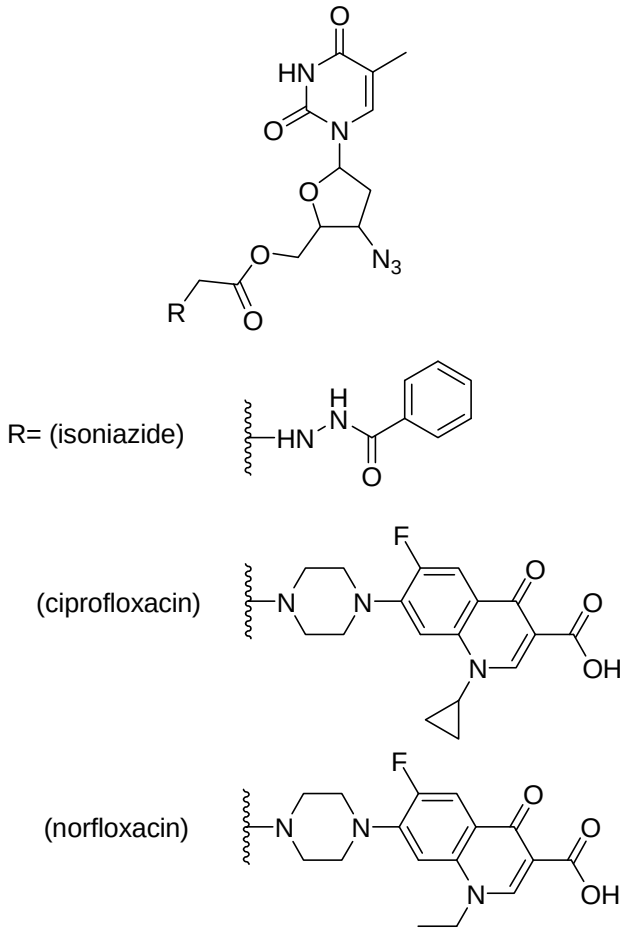
activated by different reductive enzymes (cytochrome P450 reductase and cytochrome B_5 reductase) and therefore resistance to those prodrugs does not appear in *M. tuberculosis* $H_{37}Rv$ mutant [205,206].

Nucleoside analogues prodrugs

Co-infection of AIDS with TB is now the leading cause of death for one out of three people who die with the former. For this very reason, an ideal drug for HIV/AIDS patients should have anti HIV activity while treating TB [207-209].

Shriram and co-workers developed and evaluated mutual prodrugs composed of an antimycobacterial agent, as ciprofloxacin, norfloxacin and isoniazid, and a anti HIV nucleoside analogues, as zidovudine, stavudine and lamivudine [209-212].

Figure 37. Ester prodrugs of zidovudine.



Mutual prodrugs of zidovudine (Figure 37) with INH, ciprofloxacin and norfloxacin were tested against *M. tuberculosis* strain $H_{37}Rv$ at a single concentration, 6.25 μg/mL. The prodrugs bearing ciprofloxacin or norfloxacin were the most active, showing 99% inhibition while isoniazid containing prodrug showed 90% inhibition [213]. Prodrugs of stavudine containing a fluoroquinolone moiety (Figure 38) showed 100% inhibition, while INH containing prodrug presented 90% inhibition [210]. Lamivudine prodrugs with fluoroquinolones (Figure 39) showed 92–100% inhibition against $H_{37}Rv$ *M. tuberculosis* strains [213].

Figure 38. Ester prodrugs of stavudine.

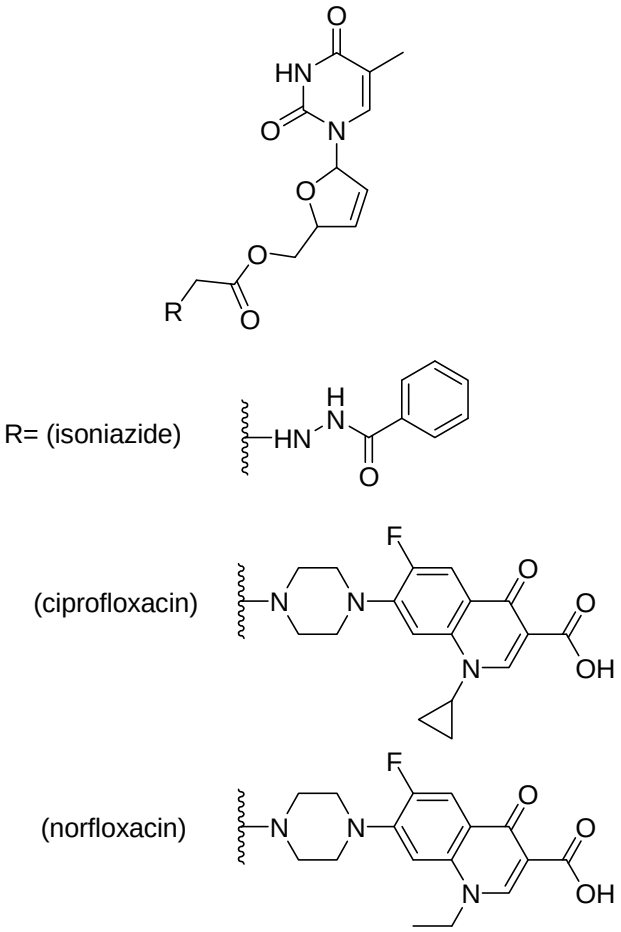
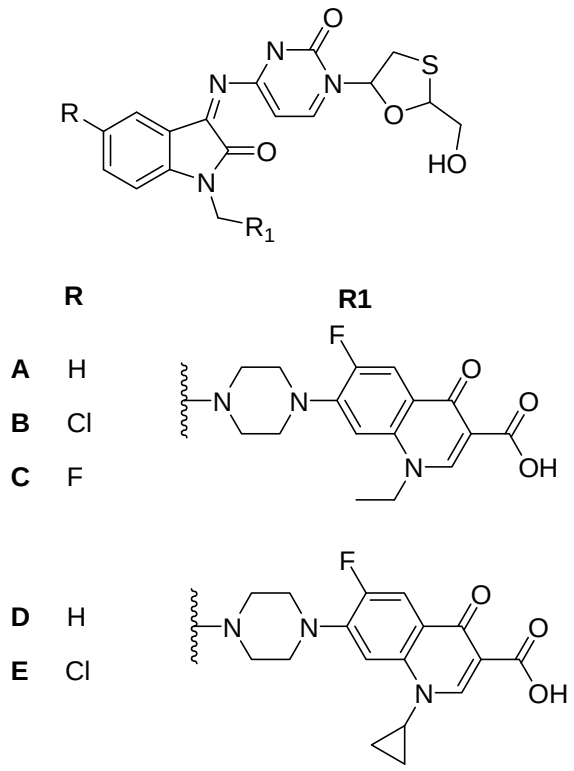


Figure 39. Prodrugs of lamivudine.



The stability for these mutual prodrugs concerning the transport across the cell membrane and their intracellular reversion to the parent compound, especially in the virally infected cells, display a determinant role in their interest as antituberculous agents in co-infection TB and HIV. Those prodrugs were hydrolized in the range of 20 to 240 min, and 120 to 240 for lamivudine prodrugs. The lamivudine prodrug C (Figure 39) inhibited HIV-1 replication and was responsible for 100% inhibition of *M. tuberculosis* and those properties account for its effective treatment of HIV/AIDS co-infection [213].

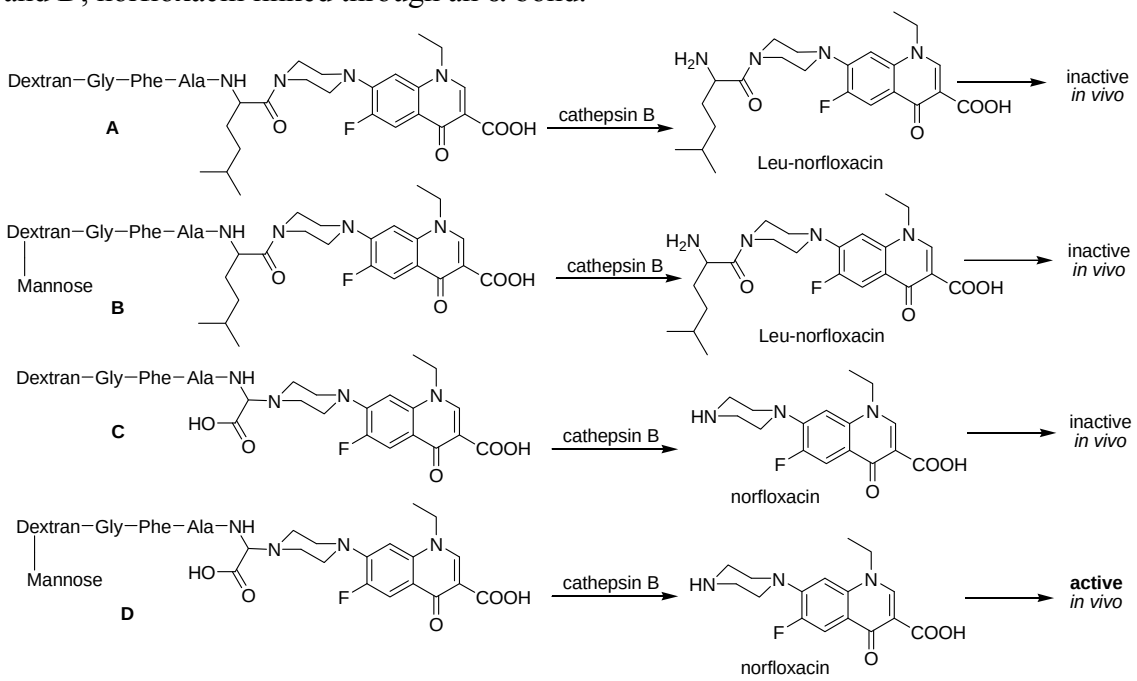
Polymeric prodrugs of norfloxacin

Mycobacteria are facultative intracellular pathogens and scape killing during phagocytosis by blocking phagosome-lysosome fusion. Intracellular mycobacteria are also largely protected against drugs and in this condition, they are difficult to be destroyed [214].

To be effective against intracellular bacteria, the drugs must penetrate the cells. Drug delivery systems capable of specifically targeting the drug into the macrophages can help to face this challenge [215]. Drugs linked to a homing device, that interact with a specific cell receptor, through a macromolecular carrier could induce the endocytosis of the drug, improving its effectiveness and imparting a selective action. Mannosyl ligands are director groups that can be used to target macrophages [216].

Covalent linking of norfloxacin to a polymeric carrier is one approach to improve its pharmacological properties (Figure 40). The prodrug is uptaken by cells, enters through endocytosis, and since the conjugate is large enough to impair renal filtration, the drug delivery and consequently its effectiveness are improved [217-219].

Figure 40. Schematic representation of the polymeric prodrugs and the product of enzymatic hydrolysis activity. A and B, norfloxacin linked through amide bond and C and D, norfloxacin linked through an α bond.

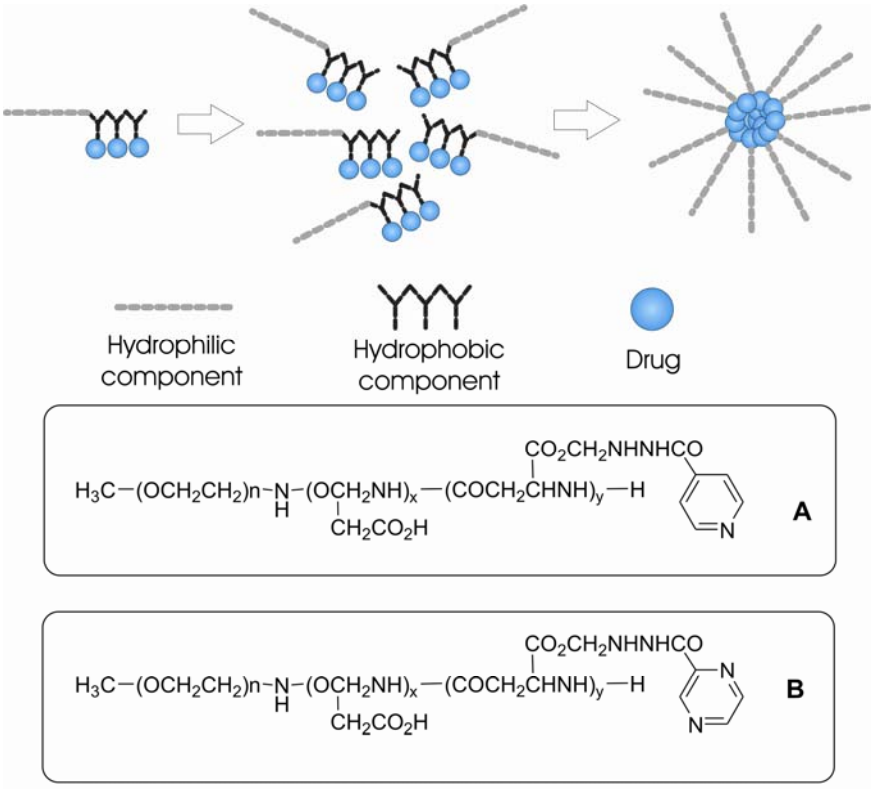


In this example, two different peptide spacer arms, Gly-Phe-Ala-Leu and Gly-Phe-Gly-Gly were used to link norfloxacin to dextran. In the former, Leu-norfloxacin was released in the presence of the lisosomal enzyme cathepsin B, instead of free norfloxacin. On the other hand, more than 60% of norfloxacin was released at pH 5.5 in presence of cathepsin B if the peptide was Gly-Phe-Gly-Gly (α-norfloxacin)-O-methyl linked to dextran with or without mannose [217]. The first conjugate is 20 times less active than the other conjugate, when tested *in vitro* against *M. bovis* BCG. The test in mice infected with *M. bovis* BCG prove that only the conjugate containing both an α bond and mannose was as active as isoniazid. This conjugate was able to deliver norfloxacin directly into macrophages and controls micobacterial growth in the liver, where isoniazid is inactive due to the low pO_2, which turns *M. tuberculosis* insensitive to isoniazid and rifampicin [220].

Polymeric prodrug of isoniazid and pyrazinamide

Micellar systems of polyethyleneglycol-poly(aspartic acid) copolymer were used by Silva and co-workers [221] as carrier to the tuberculostatic drug isoniazid, which was covalently bounded to the system (Figure 41A).

Figure 41. Micelles of PEG-PAS covalently linked to drugs. A. isoniazide; B: pyrazinamide.



This polymeric prodrug envisioned not only the prolonged delivery of the drug, but also the lowering of toxicity and extension of the period between two consecutive doses, so patients would be more willing to follow the treatment schedule.

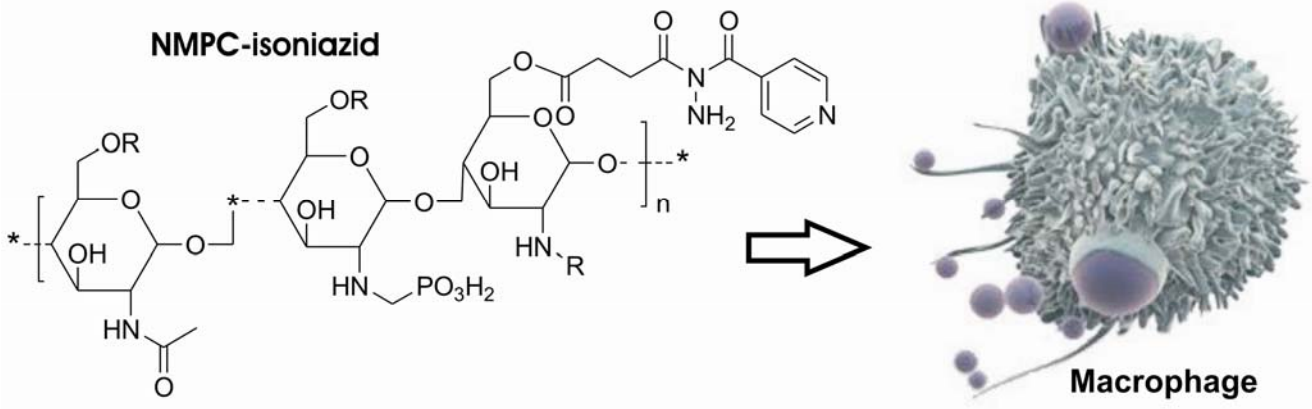
Later on, the same group extended their work to another antitubercular polymeric prodrug, derived from a poly(ethyleneglycol)-poly(aspartic acid) block copolymer by substituting pyrazinamide on the aspartate free carboxylic groups (PEG-PASP-PZA) (Figure 41B) [222]. The substituted polymer forms a micelle, with a hydrophobic core consisting of the drug-ligand group and a hydrophilic outer coat [221,222].

The advantage of this micellar carrier over others lies in the ability to control the particle size, its structural stability and good solubility in water. In addition, this structure allows a controlled rate of delivery of the drug, reduced toxicity and selective action on the chosen target [223,224]. The potential for prolonged drug action at low toxicity level means that this system could lead to greater patient approval. This micelle-forming polymeric prodrug offers the advantages of reducing toxicity and immunogenicity.

The activity of pyrazinamide is pH-dependent and is limited to slow-growing bacilli. Owing to its toxic effect on the liver, it has to be used under closed medical supervision, accompanied by regular tests of liver function. Thereby, this polymeric prodrug can be very useful to reduce pyrazinamide toxicity to the liver. Furthermore, the PEG-PASP-PZA derivative, when assayed for its anti-*Mycobacterium* activity, exhibited stronger activity than the simple drug [222].

In 2004, Rando and co-workers. [225] used *N*-methylene phosphonic chitosan (NMPC) to obtain a polymeric prodrug of isoniazid, defined as chitosan-isoniazid hemisuccinate. This polymeric system aimed, not only to slow the drug delivery as proposed by Silva and co-workers [221;222], but also to obtain a specific action (Figure 42).

Figure 42. Chitosan-Isoniazid hemisuccinate.



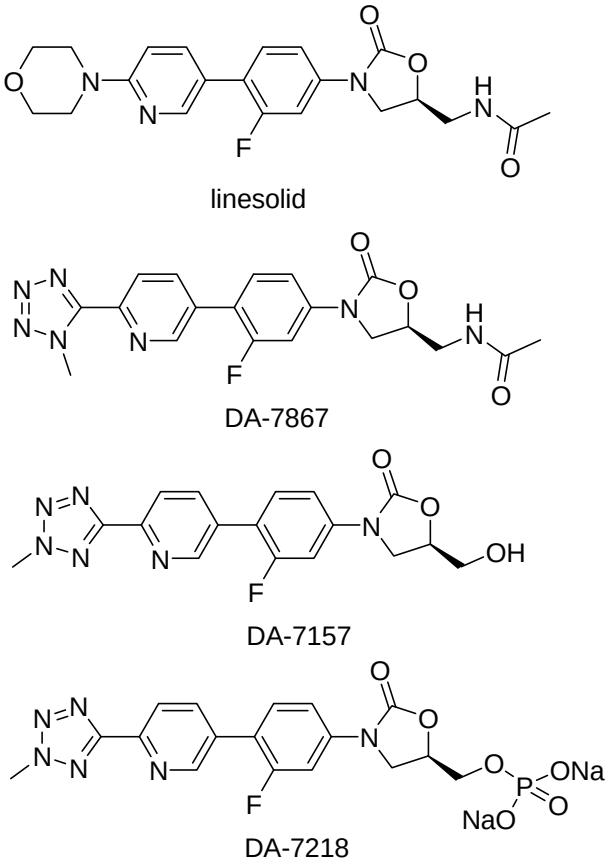
Generally, chitosan is a largely used carrier because of the presence of free NH_2 group that provides a reactional center either to modification or drug linkage [225]. This derivative is almost atoxic and has immunopotentiating effect by stimulating macrophages and increasing the humoral response [226]. Since the macrophages are the host cell of *M. tuberculosis*, prodrugs with chitosan as carrier could act as a targeted prodrug.

The *N*-methylene phosphonic group account for the higher hydrosolubility of the resulting prodrug which can be easily handled and intravenously administrated [227].

DA-7218 prodrugs

Oxazolidinones are a new, unique class of synthetic antibacterial agents effective against many gram-positive bacteria, including aerobic pathogenic actinomycetes of the genera *Mycobacterium, Nocardia,* and *Actinomadura* [228-232]. Linesolid (Figure 43) was the first oxazolidinone introduced in the market. It was active in animal models and also in clinical trials with patients infected with *M. tuberculosis* [233]. Oxazolidinones selectively inhibit bacterial protein synthesis by avoiding the formation of a functional 70S initiation complex [234]. DA-7157 was shown to be more active than linesolid in *in vitro* test against several gram-positive species [235].

Figure 43. Chemical structures of oxazolidinone derivatives.



This compound is generated by the metabolism of DA-7218, a highly hydrophilic prodrug (Figure 43). Both compounds were tested against clinical isolates of *Mycobacterium tuberculosis*, their MIC_{90}s against *M. tuberculosis* was about 0.5 μg/mL [235]. DA-7218 is an excellent candidate to be used in humans, although the *in vivo* test has not been performed yet. It has good water solubility, which allows high availability and perfusion in the tissues.

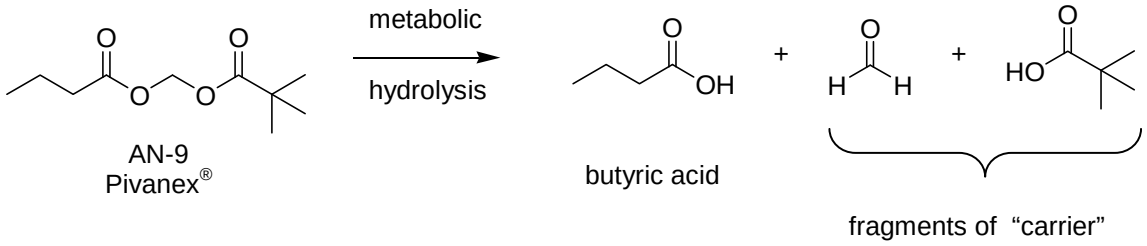
Prodrugs for Sickle Cell Disease

Butyric acid prodrugs

Recently, butyric acid (BA) and derivatives [236-238] were proposed to be useful in the treatment of β-globin disorders such as sickle cell disease and β-thalassemia. Their effect is associated with an increase in HbF production that leads to the minimization of the clinical symptoms of this disease [239]. However, BA is poorly absorbed in human gastrointestinal tract. In order to solve this problem, a series of butyric acid prodrugs were designed [238]. BA prodrugs, which also display low toxicity, may have significant advantages for the treatment of β-thalassemia and sickle cell anemia, when compared to the cytotoxic agent hydroxyurea approved for the chronic treatment of the latter [240].

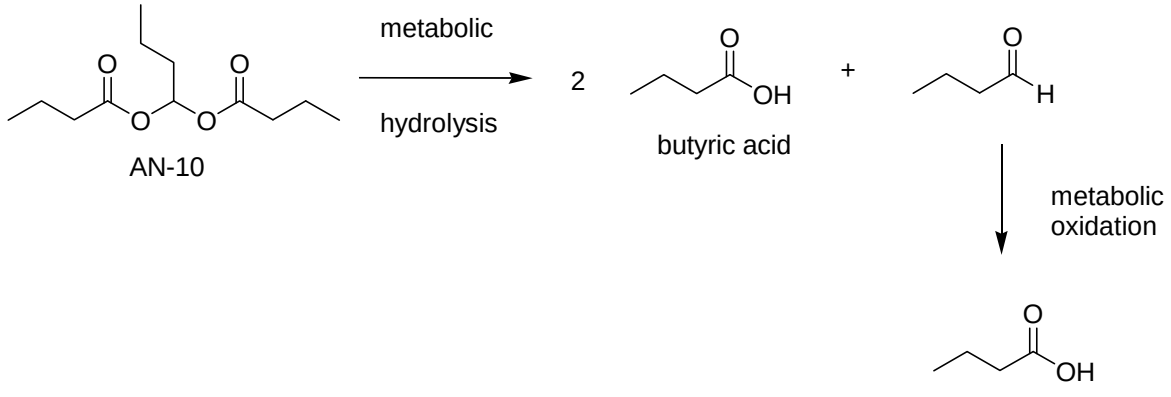
BA possesses a carboxylic acid function and there are suitable prodrugs for this functional group [9]. Esterification of BA improves its permeability across cell membranes and imparts this acidic compound with an efficient delivery to a subcellular target. Therefore, a series of acyloxyalkyl esters prodrugs designed with the aim of increasing stability and lipofilicility [238] was designed. Those compounds release equimolar amounts of BA, an aldehyde and a second acid. This is mainly biologically inactive and acts as "carrier" moieties. An example of this strategy was the compound AN-9 (Pivanex®) that after metabolic hydrolysis releases one molecule of butyric acid and fragments of carrier represented by pivalic acid and formaldehyde (Figure 44).

Figure 44. Metabolic hydrolysis of Pivanex®.



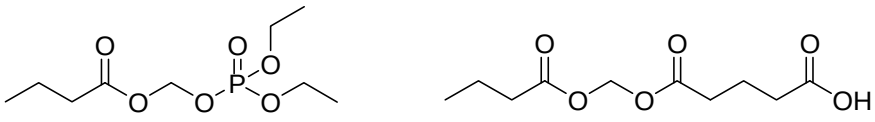
The most active prodrug was the butylidene dibutyrate (AN-10), that releases 2 equivalents of BA and 1 of butyraldehyde. This undergoes further in vivo oxidation to BA (**Fig 45**).

Figure 45. Metabolic hydrolysis of AN-10.



Although AN-9 (Figure 44) and AN-10 (Figure 45) are highly lipophilic derivatives that better cross cell membranes than BA, they have poor solubility in water, with leads to a non-aqueous media for clinical formulation. To solve this problem, a series of acid, basic and neutral prodrugs was obtained. The neutral and the acid prodrugs 1 and 2, respectively, in Figure 46, could induce fetal hemoglobin. They increase hemoglobin levels and showed lower toxicity than BA [239;241].

Figure 46: Neutral (1) and acid (2) derivatives of butyric acid.



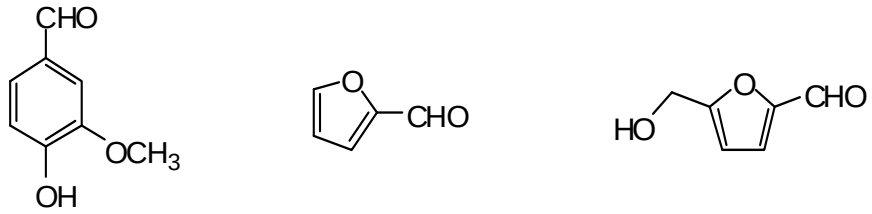
1 2

Vanillin prodrugs

Vanillin (Figure 47), obtained from vanilla beans, increases oxygen affinity by hemoglobin S (Hb S), when sickle erythrocytes (SS cells) is incubated with this flavouring agent [243]. This compound reacts covalently with Hb S and shifted the oxygen equilibrium curve (OEC) towards the left which inhibits cell sickling.

Other aldehydes like five-membered heterocyclic aldehydes (Figure 47) increase the oxygen affinity of hemoglobin (Hb), inhibiting the sickling of homozygous sickle red blood (SS). This aldehyde group form Schiff base-adducts with Hb, through a specific binding to the N-terminal αVal1 of Hb T state, leading to opposite allosteric shifts [242,243].

Figure 47. Some aldehyde derivatives with potent antisickling effect.



vanillin furfural 5-hydroxymethyl-2 furfural

Vanillin did not have a significant anti-sickling effect when it was administered orally because of its gastrointestinal (GI) unstability, due to aldehyde chemical and metabolic labiality, what hinders its use in the therapeutics [244;245]. A vanilin prodrug, MX-1520, is more stable and improve the bioavailability of the parent compound [246].

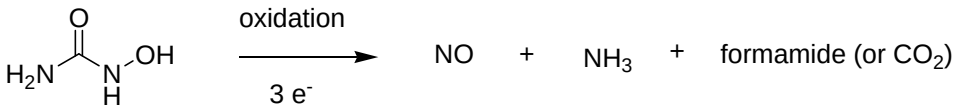
Hydroxyurea and nitrate derivatives

Hydroxyurea (HU) is the only US Food and Drug Administration (FDA)-approved drug for the treatment of SCD [40,41]. It inhibits ribonucleoside diphosphate reductase, the enzyme that converts

ribonucleotides into deoxyribonucleotides (dNTPs), involved in DNA synthesis and repair [247,248]. It increases the level of fetal hemoglobin (HbF), a genetically distinct hemoglobin (Hb) that inhibits the polymerization of deoxygenated sickle cell hemoglobin [249,250]. HU display other mechanism of action [249,251,252], being also considered as a source of nitric oxide (NO). This plays an important role in the maintenance of normal blood pressure and flow, and has drawn considerable interest as a sickle cell disease treatment.

Hydroxyurea could be consider a bioprecursor prodrug because after its oxidation, it may decompose into NO, ammonia and formamide or carbon dioxide, depending on the conditions and type of oxidant (Figure 48).

Figure 48: Hydroxyurea oxidation to nitric oxide (NO).



Nitrate derivatives

Nitrate organic compounds have vasodilatador acivity and are effective against stable angina. They could be metabolized into nitric oxide (NO) through bioactivation catalyzed by various enzymes such as glutathione S-transferase, cytochrome P-450, and possibly esterases [253]. Since NO is unstable, the prodrug approach can solve this problem, through more stable NO-donors, as nitrate prodrugs. The compounds NO-donors have vasodilatation and plalet aggregation inhibition activities among others [254].

Recently nitrate prodrugs with NO-donor properties were developed in our laboratory. These compounds increased gama-globin expression in K562 cell culture and presented important analgesic and anti-inflammatory activity that could be useful to the treatment of the SCD [255].

Conclusions

Prodrug approaches are interesting and promising alternatives to solve problems presented by current drugs or also by those that are under development. We have shown valuable examples of their use as a molecular modification tool to improve mainly pharmacokinetics properties of existing drugs, or also to experimental compounds, for the treatment of some neglected diseases.

Although many examples have been presented herein, prodrug design could be more explored by researchers in order to minimize the lack of suitable chemotherapeutic agents for the diseases considered in this review. It is worth noting that improving drug properties can, generally, lead to better alternatives associated with low cost and less time-consuming processess. These characteristics are of utmost importance for drug development mainly considering people affected by the neglected diseases, their needs and also the limited interest of pharmaceutical companies due to the low profit involved.

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List of abbreviations used

5-FC: 5-fluorocytosine
5-FU: 5-fluorouracil
ADEPT: antibody-directed enzyme prodrug therapy
AN-10: butylidene dibutyrate
apoL-I: apolopoprotein L1
BA: butyric acid
CD-CS: recombinant fusion protein consisting of CS protein as a ligand and bacterial CD: cytosine deaminase
CL: cutaneous leishmaniasis
CMD-P: carboxymethyldextran-thiomannopyranoside-pyrimethamine
CpG ODN: CpG oligodeoxynucleotides
CpG: a dinucleotide constituted by a cytosine followed by guanine separated by a phosphate
CQ: chloroquine
CS: malaria circunsporozoite
DFMO: eflornithine
DHA: dihydroartemisinin
DHFR: dihydrofolate reductase
DHFR-TS: dihydrofolate reductase thymidilate synthase
dNTPs: deoxyribonucleotides
DOXP: 1-deoxy-D-xylulose 5-phosphate
EB: ethambutol
ETA: ethionamide
EthA: a flavin monooxygenase found to catalyze the Baeyer-Villiger reaction
Et-NIPOx: ethyl ester of *N*-isopropyl oxamate
fma: 2-(*N*-formyl-*N*-methyl)aminoethyl
GI: gastrointestinal
GR: glutathione reductase
GSH: glutatione
HADH-isozyme: α-hydroxyacid dehydrogenase-isozyme
HAT: Human African Trypanosomiasis
Hb: hemoglobin
Hb F: fetal hemoglobin
Hb S: hemoglobin S
HU: hydroxyurea
INH: isoniazid

InhA: enoyl-acyl carrier protein reductase
KatG: catalase/peroxidase
LDH-C4: lactate dehydrogenase isozyme-x
mAb: monoclonal-antiboby
mAb-drug: monoclonal-antiboby drug conjugate
MBCs: minimal bactericidal concentrations
NbAn33: a mAB specific for the conserved carbohydrate epitope of VSGs
NF: nitrofurazone
NFOH: nitrofurazone hydroxymethyl derivative
NHS: normal human serum
NIPOx: *N*-isopropyl oxamate
NMPC: *N*-methylene phosphonic chitosan
OEC: oxygen equilibrium curve
PAS: 4-aminosalicylic acid
PEG-PAS polyethyleneglycol-poly(aspartic acid)
PEG-PASP-PZA: poly(ethyleneglycol)-poly(aspartic acid) block copolymer by substituting pyrazinamide on the aspartate free carboxylic groups
***pf*DHFR-TS**: *P. falciparum* dihydrofolate reductase thymidilate synthase
PFT: protein farnesyltransferase
PLMII: plasmepsinII
PncA: mycobacterial pyrazinamidase
PQ: primaquine
PQD: pyrroloquinazolinediamine
PQD-A4: tetra-acetamido pyrroloquinazolinediamine
PQD-BE: *bis*-ethylcarbamyl pyrroloquinazolinediamine
Pro-D ODN: a prodrug form of CpG ODN type D
Pro-D35: D-35 prodrug
PTR1: pteridine reductase enzyme
PZA: pyrazinamide
SCD: sickle cell disease
SD-P: succinyldextran-thiomannopyranoside-pyrimethamine
SRA: serum resistance-associated
SS cells: sickle erythrocytes
TAZ: thiacetazone
TB: tuberculosis
TLR-9: Toll-like receptor 9
Tr-apoL-I: truncated apoL-I
VL: visceral leishmaniasis
VSG: variant surface glycoprotein

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