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Pyrimidine Metabolism in Schistosomes: A comparison with Other Parasites and the Search for Potential Chemotherapeutic Targets

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Abstract

Schistosomes are responsible for the parasitic disease schistosomiasis, an acute and chronic parasitic ailment that affects more than 240 million people in 70 countries worldwide. It is the second most devastating parasitic disease after malaria. At least 200,000 deaths per year are associated with the disease. In the absence of the availability of vaccines, chemotherapy is the main stay for combating schistosomiasis. The antischistosomal arsenal is currently limited to a single drug, Praziquantel, which is quite effective with a single-day treatment and virtually no host-toxicity. Recently, however, the question of reduced activity of Praziquantel has been raised. Therefore, the search for alternative antischistosomal drugs merits the study of new approaches of chemotherapy.

The rational design of a drug is usually based on biochemical and physiological differences between pathogens and host. Pyrimidine metabolism is an excellent target for such studies. Schistosomes, unlike most of the host tissues, require a very active pyrimidine metabolism for the synthesis of DNA and RNA. This is essential for the production of the enormous numbers of eggs deposited daily by the parasite to which the granulomas response precipitate the pathogenesis of schistosomiasis. Furthermore, there are sufficient differences between corresponding enzymes of pyrimidine metabolism from the host and the parasite that can be exploited to design specific inhibitors or “subversive substrates” for the parasitic enzymes. Specificities of pyrimidine transport also diverge significantly between parasites and their mammalian host. This review deals with studies on pyrimidine metabolism in schistosomes and highlights the unique characteristic of this metabolism that could constitute excellent potential targets for the design of safe and effective antischistosomal drugs. In addition, pyrimidine metabolism in schistosomes is compared with that in other parasites where studies on pyrimidine metabolism have been more elaborate, in the hope

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This paper is dedicated to the memory of Alfred W. Senft, a teacher, a colleague, and a dear friend. His enthusiasm and pioneering work in the field of nucleotide metabolism in schistosomes have inspired us and many others to pursue the study of this fascinating facet of parasitology.

of providing leads on how to identify likely chemotherapeutic targets which have not been looked at in schistosomes.

Keywords

Schistosomes; parasites; pyrimidine; enzymes; transport; chemotherapy

1. Introduction

Six species of the trematode Schistosoma (*Schistosoma mansoni, S. japonicum*, *S. haematobium*, *S. mekongi*, *S. guineensis* and *S. intercalatum)* are the causative agents of the parasitic disease schistosomiasis. Schistosomiasis is an acute and chronic parasitic ailment with a wide range of clinical manifestations that have plagued mankind since ancient times. The clinical disease dates as early as 1500 B.C. Calcified Schistosoma eggs have been identified in Egyptian mummy tissues from the twentieth dynasty (1200 to 1090 B.C.). In China, there are records of schistosomiasis of comparable antiquity. Schistosoma infections in the New World are more recent in origin, probably beginning with African slave trade to the Americas during the sixteenth and seventeenth centuries. At the present time, schistosomiasis affects more than 240 million people in 70 countries worldwide. Most endemic rural populations will have a 40–60% prevalence rate at any one time; but almost everyone (i.e., 95%) has had an infection sometime during his life. These estimates, superimposed on approximately 1.5 billion humans in schistosome endemic areas, easily qualify schistosomiasis as one of the major world public health problems. Indeed, schistosomiasis is the second most devastating parasitic disease after malaria. At least 200,000 deaths per year are associated with the disease.

Since antischistosomal vaccines are not yet available, chemotherapy still is the main stay to control this disease. However, only a small number of effective drugs are currently available. Classical antischistosomal drugs fall into four major groups: the antimonials, the nitrothiazoles, the thioxanthones and the organophosphates. Each of these drugs can be effective against some species of human schistosomes, but none is highly effective against all. In addition, for each of these chemotherapeutic agents, there are known contraindications and/or severe side effects. Thus, none of the classical drugs fulfill the requirements of an ideal antischistosomal compound. The antischistosomal arsenal is currently limited to a single drug, Praziquantel, which has been the drug of choice for the treatment of schistosomiasis for more than 35 years. Praziquantel is orally effective against all six species of schistosomes with a single-day treatment and virtually no toxicity towards the host. Nevertheless, the question of reduced of Praziquantel efficacy was raised recently. Therefore, the search for alternative antischistosomal agents continues to merit the study of new approaches of chemotherapy.

The search for antischistosomal drugs has a long and tortuous history. Most of the currently available drugs were discovered by empirical methods. Rational design of a drug based on biochemical and physiological differences between the pathogen (e.g., cancer, bacteria, parasites, etc.) and the host has been the goal of many investigators for decades. In general,

rational design of antischistosomal drugs is lacking because of the paucity of information about the biochemistry, physiology, molecular biology, etc. of this parasite. However, the great phylogenic separation between schistosomes and their host renders the biology and biochemistry of the parasite amenable to better chances of discovering exploitable differences between the host and schistosomes.

The pathogenesis of schistosomiasis (e.g., hepatic fibrosis, portal hypertension, bladder cancer, etc.) results from the granulomas response to the unending accumulation of deposited eggs ranging from approximately 400–4000 eggs/female/day depending on the species. Considering the number of ovipositing worm pairs can reach 2,000 pairs/patient (Gryseels and De Vlas, 1996), the parasites can produce a tremendous number of eggs (800,000–8,000,000 eggs/day/patient). The production of such enormous number of eggs requires a highly active DNA and RNA synthesis which involves a dynamic production of purine and pyrimidine nucleotides, the building blocks of DNA and RNA. Therefore, interference with purine and pyrimidine synthesis in schistosomes would result in the cessation of oviposition and the pathogenesis of schistosomiasis as a result of blocking RNA and DNA synthesis.

The synthesis of purines and pyrimidines can proceed via the de novo and/or the salvage pathways. The de novo pathway utilizes simple compounds for the synthesis of the various purines and pyrimidines. The salvage pathways, on the other hand, are reutilization routes by which the cell can satisfy its purine and pyrimidine requirements from endogenous and/or exogenous preformed purines and pyrimidines. Searching for differences between the parasites and their host in these metabolic pathways could provide highly selective targets for anti-schistosomal chemotherapy. Indeed, one of the most striking differences between schistosomes and its mammalian host is that schistosomes differ significantly from their host with respect to purine metabolism. Senft and co-workers at Brown University (Senft et al., 1972, 1973a and 1973b; Stegman et al., 1973; Crabtree and Senft, 1974; Miech et al., 1975; Senft and Crabtree, 1983) established that schistosomes are incapable of de novo purine biosynthesis and are dependent on the salvage pathway to meet their purine requirements. This was quite surprising since the production of enormous numbers of eggs by the schistosomes requires a highly active purine metabolism for RNA and DNA synthesis. The importance of these differences in nucleotide metabolism between the parasite and its hosts was further highlighted by the discovery that potent nucleoside transport inhibitors of mammalian systems do not significantly inhibit the uptake of nucleosides in *S. mansoni* (el Kouni et al., 1983a; el Kouni and Cha, 1987). Based on these findings, a successful antischistosomal chemotherapeutic regimen was developed. The highly toxic purine analogues tubercidin and nebularine were made selectively toxic against *S. mansoni*, *S. japonicum* and *S. haematobium* by simultaneous administration of a nucleoside transport inhibitor as an antidote for the host but not the parasite (el Kouni et al., 1983a, 1985, 1987 and 1989; Bear et al., 1988; Baer, 1989; el Kouni, 1991, 1992 and 2003).

Pyrimidines, like purines, are required by all living organisms for the synthesis of DNA, RNA and other metabolites. Studies on the biological, chemical and pharmacological aspects of pyrimidine metabolism in various organisms, from prokaryotes to mammalian systems, are numerous and have been discussed in several reviews by O'Donovan and

Neuhard (1970), Henderson and Paterson (1973), Levine et al. (1974), Jaffe (1975), Hurst (1980), Jones (1980), Kensler and Cooney (1981), Munch-Peterson (1983), Hammond and Gutteriddge (1984), Hassan and Coombs (1988), Evans and Guy (2004), Hyde (2007), Garavito et al. (2015), and Krungkrai and Krungkrai (2016). However, in contrast to the relatively extensive work on purine metabolism in schistosomes (Senft et al., 1972, 1973a and 1973b; Stegman et al., 1973; Crabtree and Senft, 1974; Miech et al., 1975; Levy and Read, 1975a and 1975b; Senft and Crabtree, 1983; el Kouni et al., 1983a, 1985, 1987 and 1989; Dovey, et al., 1984 and 1985; el Kouni and Cha, 1987; Baer et al., 1988; el Kouni, 1991; Craig III et al., 1991; Yuan et al., 1993; Kanaaneh et al., 1994: Kanaani et al., 1995 and 1997; Foulk et al., 2002; Pereira et al., 2003, 2005, 2010a and 2010b; da Silveira et al., 2004; Yang et al., 2007; Castilho et al., 2010; D'Muniz-Pereira et al., 2011; Postigo et al., 2010; Marques-Ide et al., 2012; de Moraes et al., 2013; Romanello et al., 2013 and 2017; Saverese and el Kouni, 2014; Torini et al., 2016; Zeraik et al., 2017), little information is available on pyrimidine metabolism in this parasite.

Contrary to their inability to synthesize purines de novo, schistosomes are capable of de novo pyrimidine synthesis in addition to their capacity of pyrimidine salvage. The possession of both the biosynthesis and salvage routes would appear to make pyrimidine metabolism an unattractive drug target. Nevertheless, many of the enzymes involved in these pathways are essential for survival and, unlike purine metabolism, there is little redundancy in the pathways of pyrimidine metabolism. This paper will attempt to review the broad aspects of pyrimidine metabolism in schistosomes and compare them to their mammalian host in an effort to shed light on similarities and differences between this parasite and their host. Elucidation of pyrimidine metabolism in schistosomes not only contributes to the general knowledge of metabolism in this parasite, but may also reveal potential targets for the treatment of schistosomiasis with one or more of the already available chemotherapeutic pyrimidine analogues. Studies on pyrimidine metabolism in other parasites have been more elaborate and sophisticated. Therefore, pyrimidine metabolism in schistosomes is compared with that in other parasites in the hope of providing leads on how to identify likely chemotherapeutic targets.

De novo pyrimidine nucleotide biosynthesis is defined as the formation of UMP (uridine 5′-monophosphate) which is considered the "focal point" of pyrimidine metabolism since all other pyrimidine nucleotides can be synthesized from this compound. Fig. 1 summarizes the state of knowledge regarding the de novo biosynthesis of UMP as compiled from studies on various organisms. De novo UMP biosynthesis consists of six sequential enzymatic steps (Fig. 1) which are practically universal in all systems studied from prokaryotes to mammals and have remained intact throughout evolution, although the primary structures of the enzymes responsible for pyrimidine biosynthesis may deviate significantly among prokaryotes, parasitic protozoa, fungi, animals, and mammals including humans.

The first step in the pathway begins with the synthesis of carbamyl phosphate from glutamine, bicarbonate (HCO_3^-) and 2 moles of ATP and ends by six enzymatic steps later as UMP. The activities of all six enzymes involved in de novo UMP biosynthesis were demonstrated in extracts of *S. mansoni* by Aoki and Oya (1979), Hill, et al. (1981b), el Kouni et al. (1983b), Iltzsch et al. (1984) and *S. japonicum* by Huang et al. (1985). These

studies established that the parasite is capable of de novo pyrimidine biosynthesis. This is in contrast to the inability of the worms to synthesize purines de novo (Senft et al., 1972, 1973a and 1973b; Stegman et al., 1973; Crabtree and Senft, 1974; Miech et al., 1975; Senft and Crabtree, 1983).

De novo pyrimidine biosynthesis has been detected in most parasites studied except *Eimeria tenella* (Hill et al., 1981b), *Giardia intestinalis* (Lindmark and Jarroll, 1982; Aldritt et al., 1985; Jarroll et al., 1989), *Trichomonas vaginalis* (Hill et al., 1981b; Miller and Lindstead, 1983; Heyworth et al., 1984; Wang and Cheng, 1984a), *Tritrichomonas foetus* (Wang et al., 1983; Hassan and Coombs, 1988), *Cryptosporidium* (Striepen et al., 2004), and are thus pyrimidine auxotrophs. It was reported previously that *Entamoeba histolytica* is able to de novo synthesize pyrimidines (Reeves, 1984). Surprisingly, however, none of the enzymes of the pyrimidine synthesis pathway can be identified within the genome (Anderson and Loftus, 2005).

It is interesting to note that de novo pyrimidine biosynthesis is required for virulence of *Toxoplasma gondii* (Fox and Bzik, 2002) and *Trypanosoma brucei* (Ali et al., 2013b; Ong et al., 2013) in vivo. Furthermore, *T. brucei* mutants that are deficient in de novo UMP synthesis regain virulence when maintained in tissue culture for a long time. Uptake studies showed that these mutants increased their transport of uracil from the media to overcome the inhibition of the de novo pathway (Ong et al., 2013).

2. Enzymes of de novo pyrimidine biosynthesis

2.1. Carbamyl phosphate synthetase II (EC 6.3.5.5), aspartate transcarbamylase (EC 2.1.3.2) and dihydroorotase (EC 3.5.2.3)

Carbamyl phosphate synthetase II, aspartate transcarbamylase and dihydroorotase are the first three enzymes in de novo pyrimidine biosynthesis. Carbamyl phosphate synthetase II catalyzes the following reaction.

$$\text{L- Glutamine} + \text{HCO}_3^- + 2\,\text{ATP} \rightleftharpoons \text{Carbamyl phosphate} + \text{L- Glutamate} + 2\,\text{ADP} + P_\text{i}$$

Carbamyl phosphate synthetase II is specific for pyrimidine biosynthesis and differs from carbamyl phosphate synthetase I (EC 6.3.4.16) which utilizes *N*-acetyl-L-glutamate, instead of L- glutamine (or ammonia), as a nitrogen donor for the synthesis of the carbamyl phosphate that becomes the precursor for arginine but not pyrimidine biosynthesis.

In the next step, aspartate transcarbamylase catalyzes the condensation of carbamyl phosphate with L-aspartate to form carbamyl aspartate as follows:

$$\text{Carbamyl phosphate} + \text{L- Aspartate} \varpi N\text{- Carbamyl- L- Aspartate} + P_\text{i}$$

Cyclization of the carbamyl aspartate to produce the pyrimidine ring is catalyzed by dihydroorotase. Dihydroorotase in a reversible condensation reaction eliminating one

molecule of water from carbamyl aspartate and close the pyrimidine ring to form 5,6-dihydroorotic acid as follows:

$$N\text{- Carbamyl- L- Aspartate} \rightleftharpoons \text{L- 5, 6- Dihydroorotate} + H_2O$$

The three enzymes, carbamyl phosphate synthetase II, aspartate transcarbamylase and dihydroorotase have been isolated and partially purified from *S. mansoni* by Aoki and Oya (1979). These enzymes occur in the cytosol (Aoki and Oya, 1979; el Kouni et al., 1983b). The relative activities of carbamyl phosphate synthetase II, aspartate transcarbamylase and dihydroorotase are 1:45:5 (Aoki and Oya, 1979). The three enzymes are also present in *Ascaris suum, Angiostrongylus cantonensis* (Aoki et al., 1980), *Paragonimus ohirai, Clonorchis sinensis* (Kobayashi et al., 1978), *Toxoplasma gondii* (Schwartzman and Pfefferkorn, 1981; Hill et al., 1981b; Asai et al., 1983), *Plasmodium berghei* (Hill et al., 1981a), *P. falciparum* (Hill et al., 1981b; Gero et al., 1984), *Crithidia fasciculata*, *Trypanosoma cruzi*, *Leishmania major*, *Fasciola gigantica*, *Hymenolepis diminuta*, *Nippostrongylus brasiliensis* and *Trichuris muris*, but not *Eimeria tenella* (Hill et al., 1981b). In *Trichomonas vaginalis*, only carbamyl phosphate synthetase II activity was detected (Hill et al., 1981b) explaining why this parasite is devoid of de novo pyrimidine biosynthesis.

The three enzymes, carbamyl phosphate synthetase II, aspartate transcarbamylase and dihydroorotase from *Plasmodium falciparum* were cloned and expressed and shown to contain unusual protein inserts when compared with sequences of these enzymes from other organisms (Christopherson et al., 2004). Aspartate transcarbamylase (Mejias-Torres and Zimmermann, 2002) and dihydroorotase (Robles Lopez et at., 2006) from *Toxoplasma gondii* were cloned, purified and characterized.

In Schistosomes, carbamyl phosphate synthetase II is inhibited by uridine 5′-di- and 5′-triphosphates (Aoki and Oya, 1979). Similar results were found in *Paragonimus ohirai, Clonorchis sinensis* (Kobayashi et al., 1978), *Ascaris suum, Angiostrongylus cantonensis* (Aoki et al., 1980), and *Plasmodium falciparum* (Gero et al., 1984). Thus, the enzyme seems to be a rate limiting step of pyrimidine de novo biosynthesis in these parasites.

Schistosomal carbamyl phosphate synthetase II, aspartate transcarbamylase and dihydroorotase appear to exist as a multienzyme complex (Aoki and Oya, 1979), as is the case in *Ascaris suum* (Aoki et al., 1980) and mammalian systems (c.f. Jones, 1980). The significance of this complex is that the intermediate metabolites are “channeled” from the catalytic site of one enzyme to the other without being diluted in the cell. The result is that these intermediates are protected from degradation by enzymes such as the carbamyl phosphate phosphatases (Black and Jones, 1984), hence, a highly efficient metabolic process. This contrasts with carbamyl phosphate synthetase II, aspartate transcarbamylase and dihydroorotase of *Plasmodium berghei* (Krungkrai et al., 1990), *Crithidia fasciculata* (Aoki and Oya, 1987; Krungkrai et al., 1990), *Leishmania donovani* (Mukherjee et al., 1988), *L. mexicana,* and *Trypanosoma cruzi* (Nara et al., 1998; Gao et al., 1999), where these three enzymes are not covalently linked and are present in three discrete monofunctional proteins. Nevertheless, a tri-enzyme complex can be formed in *Trypanosoma cruzi* from direct interactions between the three enzymes (Nara et al., 2012). It

is unclear whether or not this is the case in *Plasmodium berghei*. Hill et al. (1981a and 1981b) reported that carbamyl phosphate synthetase II and aspartate transcarbamylase is a bifunctional enzyme in *P. berghei*; whereas, Krungkrai et al. (1990) were able to separate the three enzymatic activities by gel filtration chromatography.

2.2. Dihydroorotate dehydrogenase (EC 1.3.3.1)

Dihydroorotate dehydrogenase is usually mitochondrial and connected to the respiratory chain in mammals. It catalyzes the fourth step in de novo pyrimidine biosynthesis, which involves the ubiquinone-mediated oxidation of dihydroorotate to form orotate in the following manner:

$$\text{Dihydroorotate} + O_2 \rightleftharpoons \text{Orotate} + H_2O_2$$

The enzyme is present in extracts of *Schistosoma mansoni* as a membrane-bound enzyme (el Kouni et al., 1983b). In *Toxoplasma gondii* (Asai et al., 1983; Hortua Triana et al., 2012), *Plasmodium knowlesi*, *P. berghei*, *P. gallinaceum* (Gutteridge et al., 1979; Krungkari et al., 1991) and *P. falciparum* (Gutteridge et al., 1979; Gero et al., 1984; Krungkrai, 1995), the enzyme is particulate and probably mitochondrial. The physiological electron acceptor for the schistosome enzyme has not yet been identified but most probably it uses coenzyme Q as an electron acceptor (M. H. el Kouni unpublished). This is the case in *Babesia bovis*, *B. bigemina* (Gero et al., 1983), *Plasmodium falciparum* (Krungkrai, 1995), and *Toxoplasma gondii* (Hortua Triana et al., 2012), where the enzyme is intimately connected to the electron transport chain to which it passes electrons directly, probably at the ubiquinone level. In contrast, in certain species of *Leishmania* (Gero and Coombs, 1980; Hammond and Gutteridge, 1982; Feliciano et al., 2006), *Crithidia fasciculata* (Pascal Jr. et al., 1983) and *Trypanosoma* (Gutteridge et al., 1979; Hammond and Gutteridge, 1982 and 1984; Pascal Jr. et al., 1983; Takashima et al., 2002; Annoura et al., 2005), dihydroorotate dehydrogenase is located in the cytosol, and utilizes fumarate as an electron acceptor (Takashima et al., 2002; Feliciano et al., 2006).

The enzymes from *Toxoplasma gondii* (Sierra-Pagan and Zimmermann, 2003), *Leishmania major* (Feliciano et al., 2006) and *Trypanosoma brucei* (Arakaki et al., 2008), were cloned, expressed, and characterized. Dihydroorotate dehydrogenase in *Toxoplasma gondii* is not only essential for de novo pyrimidine biosynthesis but also for mitochondrial function that is not directly dependent on the enzyme activity (Hortua Triana et al., 2016). Similarly, enzyme knockouts of *Trypanosoma cruzi* could not survive even in the presence of pyrimidine nucleosides, suggesting a vital role of dihydroorotate dehydrogenase activity in the regulation of cellular redox balance (Annoura et al., 2005).

Among the dihydroorotate dehydrogenase inhibitors, atovaquone, an ubiquinone analogue, leflunomide, an antirheumatic drug, brequinar, an immunosuppressive, and triazolopyrimidines, significantly inhibit enzyme activity as well as the growth of *Toxoplasma gondii* (Araujo et al., 1991; Moshkani and Dalimi, 2000; Hortua Triana et al., 2012; Ferreira et al., 2012), *Plasmodium falciparum* (Seymour et al., 1994; Basco et al., 1995; Gujjar et al., 2011), *Babesia microti* (Wittner et al., 1996; Gray and Pudney, 1999;

Lawres et al., 2016), and *B. bovis* (Kamyingkird et al., 2014) in vitro. Knockout of dihydroorotate dehydrogenase in *Trypanosoma brucei* greatly reduced growth in pyrimidine depleted medium (Arakaki et al., 2008). If a similar case exists in schistosomes, dihydroorotate dehydrogenase could be a target for the development of drug for the treatment of schistosomiasis. Nevertheless, despite the availability of potent dihydroorotate dehydrogenase inhibitors, no study has evaluated the effect of these inhibitors on schistosomal dihydroorotate dehydrogenase. Therefore, the assessment and characterization of schistosomal dihydroorotate dehydrogenase and assessment of its potential as a new drug target for schistosomes remains to be evaluated.

2.3. Orotate phosphoribosyltransferase (EC 2.4.2.10) and orotidine 5′-monophosphate decarboxylase (EC 4.1.1.23)

Orotate phosphoribosyltransferase is the fifth enzyme in the de novo pyrimidine biosynthesis pathway. It catalyzes the transfer of a ribosyl phosphate group from PRPP (α-D-phosphoribosylpyrophosphate) to orotate leading to the formation of OMP (orotidine 5′-monophosphate) as follows:

$$\text{Orotate} + \text{PRPP} \rightleftharpoons \text{OMP} + PP_{\text{i}}$$

OMP decarboxylase is the last enzyme in de novo pyrimidine biosynthesis and catalyzes the decarboxylation of OMP to UMP as follows:

$$\text{OMP} \varpi \text{UMP} + \text{CO}_2$$

Orotate phosphoribosyltransferase and OMP decarboxylase are present in the cytosol of schistosomes (Hill et al, 1981b; el Kouni et al., 1983b; Iltzsch et al., 1984; Huang et al., 1985; el Kouni and Naguib, 1990). However, *Schistosoma mansoni* has two distinct phosphoribosyltransferases (Iltzsch et al., 1984). These two enzymes differ from one another in their molecular weights as well as substrate and inhibitor specificities. The enzyme with the higher molecular weight, is non-specific for the substrates, as it catalyzes the conversion of orotate, 5-fluorouracil, and uracil to their respective nucleoside 5′-monophosphate. The other enzyme, which has a slightly lower molecular weight, appears to be specific for orotate. Both the specific and non-specific enzymes are inhibited by 5-azaorotic acid (Iltzsch et al., 1984), as is the case with orotate phosphoribosyltransferase from *Plasmodium berghei* (O'Sullivan and Ketley, 1980) and *Toxoplasma gondii* (Asai et al., 1983; Javaid et al., 1999), but only the "orotate-specific" enzyme is inhibited by 4,6-dihydroxypyrimidine (Iltzsch et al., 1984). The exact physiological role(s) of these two phosphoribosyltransferases is unclear. It would appear that both enzymes are capable of catalyzing de novo UMP biosynthesis. It is possible, however, that the primary function of the orotate-specific enzyme may be de novo UMP biosynthesis; whereas, the non-specific enzyme may function as a salvage enzyme for uracil by converting it to UMP (See below *6.7.*).

Study of orotate metabolism revealed significant differences between *Schistosoma mansoni* and mammalian enzymes (Iltzsch et al., 1984). In mouse liver, the major products of orotate metabolism were UMP and uridine with a small amount of OMP and no detectable orotidine

(Fig. 2). This is due to the fact that in mammalian cells, orotate phosphoribosyltransferase and OMP decarboxylase exist as a multienzyme complex (Jones, 1980). Therefore, OMP is "channeled" directly to OMP decarboxylase to form UMP (Traut and Jones, 1977; Traut, 1980; Reyes, 1977). As a result, no OMP or orotidine are detected in the cell (Janeway and Cha, 1977). Furthermore, in mammalian cells, OMP decarboxylase activity is greater than orotate phosphoribosyltransferase activity (Kavipurau and Jones, 1977; Brown and O'Sullivan, 1977; Levinson et al., 1979; McClard et al., 1980) presumably to facilitate the "channeling effect". Iltzsch et al. (1984) showed that this relationship between the two enzyme activities is also present in both *S. mansoni* and mouse liver. Nevertheless, in contrast to mammalian system, orotidine and uridine were the predominant products of orotate metabolism in *S. mansoni* (Fig. 2). These results led Iltzsch et al. (1984) to suggest that orotate phosphoribosyltransferase and OMP decarboxylase in *S. mansoni* may exist as separate enzymes as in most prokaryotes and lower eukaryotes rather than as a multienzyme complex typical of mammalian cells (Jones, 1980), and *Trypanosoma cruzi* (Gao et al., 1999). However, attempts to separate these two enzymes in *Schistosoma mansoni* were unsuccessful (Iltzsch et al., 1984). While failure to separate these enzymes does not prove that they are indeed part of a multienzyme complex, it does suggest that orotate phosphoribosyltransferase activity may be associated with OMP decarboxylase activity in *S. mansoni*.

The pattern of product formation of orotate metabolism over time in *S. mansoni* (Fig. 2) resembles that in *Plasmodium falciparum*, where OMP accumulates more than UMP at the initial stages of the time course (Rathod and Reyes, 1983). However, orotidine and uridine did not accumulate in *P. falciparum* as in *Schistosoma mansoni*. This contradiction may be explained by the fact that *Plasmodium falciparum* have little OMP phosphatase (EC none) activity (Rathod and Reyes, 1983), as well as the nature of orotate phosphoribosyltransferase and OMP decarboxylase in *P. falciparum.* The genes encoding the two enzymes in *P. falciparum* are distinct and located on different chromosomes. The two enzymes are produced separately, but they get cross-linked later and appear as a multienzyme complex with different kinetic properties from the host bifunctional protein complex (Christopherson et al., 2004; Krungkrai et al., 2004 and 2005; Krungkrai and Krungkrai, 2016). Proteomic data and structural modeling showed that an insertion of a low complexity amino acid sequence is responsible for this interaction of orotate phosphoribosyltransferase and OMP decarboxylase in *P. falciparum* (Imprasittichail et al., 2014). This structural complex of the parasite enzymes provides an efficient functional kinetic advantage. Therefore, it appears that orotate phosphoribosyltransferase and OMP decarboxylase from *P. falciparum* have unique structural and functional properties, sharing characteristics of the monofunctional pyrimidine-metabolizing enzymes in prokaryotes and bifunctional complexes in eukaryotes (Krungkrai et al., 2005). A similar situation may exist in *Schistosoma mansoni* and can explain the lack of "channeling" effect along with inability to separate orotate phosphoribosyltransferase and OMP decarboxylase from one another observed by Iltzsch et al. (1984). Regardless of whether or not these enzymes comprise a multienzyme complex in *S. mansoni*, there does not appear to be a "channeling effect" as seen for mouse liver and other mammalian cells (Traut and Jones, 1977; Reyes, 1977; Traut, 1980).

The subcellular location of phosphoribosyltransferase activity in the cytosol of *S. mansoni* is similar to many other organisms (see Jones, 1980) as well as most parasites (Hill et al., 1981a and 1981b; Gero and Coombs, 1982; Miller and Linsdtead, 1983; Wang et al., 1983; Asai et al., 1983). In contrast, the enzyme is associated with the glycosomes in *Crithidia fasciculata* (Gero and Coombs, 1980; Hammond and Gutteridge, 1982), *C. luciliae* (Pragobpol et al., 1984), certain species of *Leishmania* (Gutteridge et al., 1979; Hammond et al., 1981; Gero and Coombs, 1980; Hammond and Gutteridge, 1982), and *Trypanosoma* (Gutteridge et al., 1979; Hammond et al., 1981; Hammond and Gutteridge, 1982 and 1984).

Both orotate phosphoribosyltransferase and OMP decarboxylase activities are present in *Toxoplasma gondii* (Hill et al., 1981b; Asai et al., 1983), *Plasmodium berghei* (O'Sullivan and Ketley, 1980; Hill et al., 1981a), *P. falciparum* (Hill et al., 1981b; Reyes et al., 1982; Gero et al., 1984), *Angiostrongylus cantonensis* (So et al., 1992). *Crithidia fasciculata*, *Trypanosoma cruzi*, *Leishmania major*, *Eimeria tenella*, *Plasmodium berghei*, *Fasciola gigantica*, *Hymenolepis diminuta*, *Nippostrongylus brasiliensis* and *Trichuris muris*, but are missing from *Trichomonas vaginalis* (Hill et al., 1981b) explaining why this parasite is devoid of de novo pyrimidine biosynthesis. Orotate phosphoribosyltransferase and OMP decarboxylase are the only enzymes of the de novo pathway that are present in *Eimeria tenella* (Hill et al., 1981b) which is incapable of de novo pyrimidine biosynthesis. It is interesting to note that in *Trypanosoma brucei*, orotate phosphoribosyl transferase and OMP decarboxylase are essential for the virulence and survival of the parasite in vivo (Ali et al., 2013b; Ong et al., 2013).

A large number of orotate analogues have been systematically tested against toxoplasma orotate phosphoribosyltransferase and structural features necessary for strong binding were defined (Javaid, et al., 1999). 1-Deazaorotic, 6-iodouracil, 5-bromoorotic acid and 2-methylthioorotic acid were identified by Javaid, et al. (1999) as better ligands of the parasite enzyme than the mammalian counterpart studied earlier by Niedzwicki et al. (1984). The orotic acid analogue, 1,6-dihydro-6-oxo-2-pyrazinecarboxylic acid 4-oxide also known as carboxyemimycin (Fig. 3), exhibited marked anticoccidial activities against *Eimeria tenella*, *E. necatrix*, *E. acervulina*, and *E. maxima* (Matsuno et al., 1984). In *Plasmodium falciparum*, which are completely dependent on de novo pyrimidine metabolism, another orotate analogue, 5-fluoroorotate (Fig. 3), was shown to inhibit chloroquine-resistant clones in vitro with an IC_{50} of 6 nM (Gómez and Rathod, 1990). Mammalian cells are far less sensitive to 5-fluoroorotate, particularly in the presence of uridine. 5-Fluoroorotate, in combination with uridine, cured mice infected with *P. yoelii* without obvious host-toxicity. The mice were immune to subsequent challenge with a potentially lethal inoculum of *P. yoelii* (Gómez and Rathod, 1990). The activity of 5-fluoroorotate is mediated through its metabolism to FdUMP (5-fluoro-2′-deoxyuridine 5′-monophosphate), a suicide inhibitor of thymidylate synthase (Santi and McHenry, 1972) and DNA synthesis (see below *2.5.*), or by undergoing processing to FUTP (5-fluorouridine 5′-triphosphate) that may inhibit carbamyl phosphate synthetase II (Seymour et al., 1994).

2.4. Orotidine 5′-monophosphate phosphatase (EC none)

In *Schistosoma mansoni*, the intermediate metabolite OMP is dephosphorylated to orotidine by a phosphatase (Iltzsch et al., 1984). It is unclear whether OMP phosphatase activity in *S. mansoni* is due to an OMP-specific enzyme, as in microsomal extracts of mouse liver (el Kouni and Cha, 1982), or to a non-specific phosphatase. Although such a phosphatase is present in mammalian cells, excessive OMP degradation does not occur because of the "channeling mechanism". *Plasmodium falciparum*, on the other hand, appear to have little OMP phosphatase activity (Rathod and Reyes, 1983). Cytosolic OMP phosphatase in *Schistosoma mansoni* may be different from that in mouse liver as it is inhibited by 6-azaUMP; whereas in mouse liver it is not inhibited at all (Iltzsch et al, 1984). Since there are no salvage pathways for orotidine in *S. mansoni*, the accumulation of orotidine in this parasite would appear to be inefficient metabolism when compared to other organisms.

2.5. Thymidylate synthase (EC 2.1.1.45) and dihydrofolate reductase (EC 1.5.1.3)

Thymidylate synthase is a key enzyme in DNA synthesis. This enzyme, together with dihydrofolate reductase, are involved in the biosynthesis of dTMP (thymidine 5′-monophosphate). Thymidylate synthase is the sole de novo source of dTMP. It catalyzes the conversion of dUMP (2′-deoxyuridine 5′-monophosphate) and N^5,N^{10}-methylenetetrahydrofolate to dTMP and 7,8-dihydrofolate, respectively, as follows:

$$\mathrm{dUMP}+N^5,N^{10}\text{- Methylenetetrahydrofolate} \varpi \mathrm{dTMP}+7,8\text{- Dihydrofolate}$$

Subsequently, dihydrofolate reductase reduces 7,8-dihydrofolate by NADPH to generate 5,6,7,8- tetrahydrofolate. The reaction is NADPH dependent. NADH will also work, albeit less efficiently.

$$7,8\text{- Dihydrofolate}+\mathrm{NADPH}+\mathrm{H}^+ \rightleftharpoons 5,6,7,8\text{- Tetrahydrofolate}+\mathrm{NADP}^+$$

Serine hydroxymethyltransferase (EC 2.1.2.1) then catalyzes the reversible transfer of one carbon unit from L-serine to regenerate N^5,N^{10}-methylenetetrahydrofolate as follows:

$$5,6,7,8\text{- Tetrahydrofolate}+\text{L- Serine} \rightleftharpoons N^5,N^{10}\text{- Methylenetetrahydrofolate}+\mathrm{Glycine}$$

Fig. 4 depicts the dTMP synthesis cycle showing the sequential reactions and metabolic relationship of thymidylate synthase, dihydrofolate reductase, and serine hydroxymethyltransferase.

The structure and function of thymidylate synthase and dihydrofolate reductase in parasites have been extensively studied. They are popular chemotherapeutic targets as inhibition of either enzyme will block DNA synthesis. Indeed, as seen in Fig. 4, inhibitors of dihydrofolate reductase (e.g., methotrexate, trimethoprim, aminopterin, pyrimethamine, etc.) and thymidylate synthase (e.g., 5-fluorouracil or 5-fluoroorotate as precursors of FdUMP), are useful in treating several human ailments including parasitic diseases.

Earlier reports indicated that no thymidylate synthase activity was found in schistosomes or filaria (Jaffe, 1971; Jaffe et al., 1972). However, later reports indicated the presence of the enzyme in *Dirofilaria* and *Brugia pahangi* (Jaffe and Chrin, 1980). In addition, uracil and uridine were shown to be incorporated into the DNA in filaria and schistosomes (Jaffe et al., 1972; el Kouni and Naguib, 1990), suggesting the presence of the enzyme in these parasites. Thymidylate synthase is also found in the nematodes; *Angiostrongylus cantonensis* (So et al., 1992), *Trichinella spiralis* and *T. pseudospiralis* (Dabrowska et al., 1996; Rode et al., 2000). Comparative studies on inhibition of purified *T. spiralis* and rat thymidylate synthases by substrate analogues, 4-thio-5-fluoro-dUMP, 2-thio-5-fluoro-dCMP and N^4-hydroxy-dCMP, indicated that only dUMP analogues show weak selectivity towards the parasite enzyme (Rode et al., 2000).

The presence of thymidylate synthase activity has been reported also in the protozoa; *Crithidia fasciculata, C. oncopelti,* the blood forms of *Trypanosoma brucei, T. congolense*, *T. lewisi*, and blood, intracellular and culture forms of *T. cruzi* (Walter et al., 1970). Enzyme activity in the cytosol fractions of these parasites were compared with mammalian enzymes. The parasite enzymes have apparent molecular weights in the range 175,000–200,000, as determined by molecular sieving on Sephadex G-200, which are about three times higher than those of mammalian enzymes. The trypanosomatid enzymes have higher apparent K_m values for substrate (dUMP) and cofactor (N^5,N^{10}-methylenetetrahydrofolate). No evidence was obtained for the regulation of the parasite enzyme, either by the product, dTMP, or by dTDP (thymidine 5′-diphosphate) or dTTP (thymidine 5′-triphosphate). The trypanosomal enzymes are inhibited by Mg^{2+}, and are more sensitive to mercaptoethanol. Their activities are sensitive to inhibition by fluorinated pyrimidines, a property they share with thymidylate synthases from all sources. The parasite enzymes are also markedly more sensitive to inhibition by suramin (IC_{50} ~1.8×10^{-6} M) than foetal rat liver enzyme (IC_{50} 1.1×10^{-3} M). The trypanosomal enzyme is, therefore, a possible target for chemotherapeutic attack, either on its own or in combination with a dihydrofolate reductase inhibitor (Al Chalabi and Gutteridge, 1977a).

Dihydrofolate reductase is present in *Schistosoma mansoni* (Jaffe, 1971; Jaffe et al., 1972; Serrão et al., 2017b), and the adult filarial worms; *Dirofilaria immitis, Litomosoides carinii, Dipetalonema witei, Brugia pahangi*, *Onchocerca volvulus* (Jaffe, 1971; Jaffe et al., 1972; Jaffe and Chrin, 1980), the nematodes; *Aphelenchus Avenae*, *Nippostrongylus brasiliensis* (Platzer, 1974a), *Trichinella spiralis*, *T. pseudospiralis* (Rode et al., 2000), and the protozoa *Plasmodium lophurae* (Platzer, 1974b). The enzyme from of *P. lophurae* differed from the host enzyme in greater molecular weight, pH optimum, substrate, cofactor specificity, stimulation by salts (Platzer, 1974b), and higher sensitivity to pyrimethamine inhibition (Platzer, 1974b; Bilsland et al., 2011). The schistosomal and filarial dihydrofolate reductases, on the other hand, closely resemble the enzyme from rat liver. The molecular weight of each of the three enzymes is around 20,000. The three enzymes have strong preference for dihydrofolate over folate as substrate, and NADPH over NADH as a cofactor. The apparent K_m of dihydrofolate for the parasite enzymes is much higher than that of the rat liver enzyme. There is less of a difference between the apparent K_{NADPH} of the parasite and rat liver enzymes. Both schistosomal and filarial dihydrofolate are less sensitive or equisensitive to inhibition by folate analogues than the mammalian enzyme (Jaffe, 1971;

Jaffe et al., 1972; Bilsland et al., 2011). This suggests that it is unlikely that these compounds could be selectively toxic as antischistosomal or antifilarial agents. This suggestion is further supported by recent biochemical, kinetics and structural studies on recombinant schistosomal dihydrofolate reductase (Serrão et al., 2017b). The gathered information showed high structural similarity and the conservation of interactions at the folate and $NADP^+$ binding sites of the parasite and host enzymes which would make it difficult to infer that a specific dihydrofolate reductase inhibitor can be found.

Dihydrofolate reductase and thymidylate synthase usually are distinct monofunctional enzymes in most organisms. In protozoa, however, the two enzymes coexist as a bifunctional protein (Ferone and Roland, 1980; Coderre et al., 1983; Garrett et al., 1984). Though sharing the same protein, the two enzymatic activities are not interdependent. When the gene portion which encodes dihydrofolate reductase is expressed, the protein functions normally (Sirawaraporn et al., 1993). It may be interesting here to note that the bifunctional protein is used as a criterion for identifying protozoa among microorganisms of uncertain taxonomy. For example, by using this distinction, it was decided that *Pneumocystis carinii* is a fungus rather than a protozoan (Edman et al., 1989).

The bifunctional enzyme dihydrofolate reductase-thymidylate synthase from *Cryptosporidium parvum* contains novel residues at several positions analogous to those at which point mutations have been shown to produce antifolate resistance in other dihydrofolate reductases (Vásquez et al., 1996). Thus, *C. parvum* dihydrofolate reductase may be intrinsically resistant to inhibition by some dihydrofolate reductase inhibitors which may explain why cryptosporidiosis is refractory to treatment with the clinically common antibacterial and antiprotozoal antifolates.

Not all parasites have thymidylate synthase and dihydrofolate reductase. Neither enzyme could be detected in *Tritrichomonas foetus* (Wang et al., 1983), *Trichomonas vaginalis* (Heyworth et al., 1984; Wang and Cheng, 1984a), *Entamoeba histolytica*, *E. invadens* (Garrett et al., 1984), and *Giardia intestinalis* (Aldritt et al., 1985). These parasites rely on the activities of nucleoside phosphotransferases (see below *6.2.*) to salvage exogenous deoxyribosides, including, thymidine, for the synthesis of DNA.

The significance of dihydrofolate reductase-thymidylate synthase in various protozoa has been and still is the focus of intensive research efforts to identify inhibitors of this bifunctional enzyme using different methodologies including: design, synthesis, and evaluation of analogues, the use of crystal structure of wild-type and mutant enzymes as templates for designing novel drugs against resistant-mutant parasites, selective inhibitors interfering with interdomain interactions, molecular dynamics simulation of interactions between rigid and flexible antifolates of wild-type and mutant enzymes, in silico screening for allosteric inhibitors at the interface between the two domains, etc. The discussion of these studies is beyond the scope of the current work and has been reviewed recently by Nyíri and Vértessy (2017).

2.6. Cytidine 5′-triphosphate synthetase (CTP synthetase, 6.3.4.2)

This enzyme carries out the de novo synthesis of CTP (cytidine 5′-triphosphate), the last committed step in pyrimidine nucleotide biosynthesis, and the sole source of CTP from both the de novo and uridine salvage pathways. It is a rate-limiting enzyme. The enzyme catalyzes the ATP (adenosine 5′-triphosphate)-dependent amination of UTP (uridine 5′-triphosphate) to CTP with either L-glutamine or ammonia as a source of nitrogen as follows:

$$\mathrm{UTP+ATP+L\text{-}\ Glutamine \rightarrow CTP+ADP+}P_{\mathrm{i}}\mathrm{+L\text{-}\ Glutamate}$$

There is no direct evidence that CTP synthetase is present in schistosomes. However, incorporation studies in *Schistosoma mansoni* (el Kouni and Naguib 1999) showed that uridine and uracil are incorporated into cytidine nucleotides suggesting the existence of the enzyme in these parasites. Similar results were shown in *Angiostrongylus cantonensis* (So et al., 1992). The enzymes from *Giardia intestinalis* (Jimenez and O'Sullivan, 1994; Lim et al., 1996), *Trypanosoma brucei gambiense*, *T. b. rhodesiense* (Fijolek et al., 2007), and *Plasmodium falciparum* (Hendriks et al., 1998; Yuan et al., 2005) were cloned, expressed, purified and characterized. The enzyme in *Plasmodium falciparum* is the largest CTP synthetase found in any organism due to the presence of two novel sequences which are part of the continuous open reading frame and are not introns (Hendriks et al., 1998; Yuan et al., 2005). These features distinguish the parasite enzyme from that of the host making it an attractive target for structure based drug design.

3-Deazauridine (Fig. 3) as well as the glutamine analogues, DON (6-diazo-5-oxo-l-norleucine) and acivicin (α-amino-3-chloro-4,5-dihydro-5-isoxazoleacetic acid) are known inhibitors of CTP synthetase. Acivicin irreversibly inhibits the enzyme from trypanosomes and is trypanocidal in vitro and in vivo (Hofer et al., 2001; Fijolek et al., 2007).

2.7. Ribonucleotide reductase (EC 1.17.4.1)

Ribonucleotide reductase provides the only de novo means of synthesizing both pyrimidine and purine 2′-deoxyribotides, essential precursors for DNA replication and repair. Therefore, the enzyme is highly and allosterically regulated to maintain balanced quantities of the 2′-deoxyriboside 5′-triphosphates required for DNA synthesis. The reaction involves a reduction at the 2′-carbon of the 2′-ribonucleoside 5′-diphosphates as follows:

$$\mathrm{Ribonucleoside\,5'\text{-}\ diphosphate+Reduced\,Thioredoxin \rightarrow}$$
$$\mathrm{2'\text{-}\ Deoxyribonucleoside\,5'\text{-}\ diphosphate+Thioredoxin\,disulfide+H_2O}$$

This reduction is initiated with the generation of a free radical. Following a single reduction, ribonucleotide reductase requires electrons donated from the dithiol groups of the protein thioredoxin. Regeneration of thioredoxin occurs when NADPH provides two hydrogen atoms that are used to reduce the disulfide groups of thioredoxin as follows:

$$\mathrm{Oxidized\,Thioredoxin+NADPH+H^+ \rightarrow Reduced\,Thioredoxin+NADP^+}$$

In *Trypanosoma brucei*, the thioredoxin gene is expressed throughout the life cycle of *T. brucei*, however, the protein concentration in the parasites is unusually low and the trypanothione/tryparedoxin system seems to be the main donor of reducing equivalents for the parasite synthesis of 2′-deoxyribotides (Dormeyer et al., 2001).

There is no report on the activity of ribonucleotide reductase in schistosomes. Nevertheless, genome analysis indicates the presence of the enzyme in *Schistosoma mansoni* (Berriman et al., 2009). Furthermore, it was observed that DNA synthesis in male and female *S. mansoni* was inhibited by hydroxyurea, a potent and selective inhibitor of ribonucleotide reductase, suggesting the presence of active enzyme (Den Hollander and Erasmus, 1984). It should be noted here that, although hydroxyurea has been used to treat a variety of human diseases with few side effects (Parker and Parker, 2004), there are no reports of treating schistosomiasis with this drug.

Hydroxyurea also inhibited the growth and DNA synthesis in *Toxoplasma gondii* grown in vitro suggesting the presence of ribonucleotide reductase in this parasite (Kasper and Pfefferkorn, 1982). The enzyme was found in the genomes of *Entamoeba invadens* and *E. moshkovskii*, but not in the close relatives, *E. histolytica* and *E. dispar*, suggesting a recent loss from *E. histolytica* and *E. dispar* (Anderson and Loftus, 2005). *Giardia lamblia* (Baum et al., 1989) and *Trichomonas vaginalis* (Wang and Cheng, 1984b) also lack ribonucleotide reductase and appear to depend on the transport of deoxyribosides, and activities of nucleoside phosphotransferases (see below *6.2.*) to provide deoxyribotides for DNA synthesis.

The genes coding for ribonucleotide reductase in *Plasmodium falciparum* (Chakrabarti et al., 1993; Rubin et al., 1993), *Leishmania mexicana amazonensis* (lye et al., 1997), *Trypanosoma brucei* (Dormeyer et al., 1997), and *Cryptosporidium parvum* (Akiyoshi et al., 2002) were cloned, sequenced and expressed. The sequences of full-length clones from *Plasmodium falciparum* showed significant identity with other ribonucleotide reductase sequences in the data base. The gene from *Leishmania mexicana amazonensis* showed 62% similarity in nucleotide sequence to human and 48.5% to *Plasmodium falciparum* gene (Lye et al., 1997). Antisense oligodeoxynucleotides against the *P. falciparum* gene inhibited its growth at concentrations below 0.5 μM (Barker Jr. et al., 1996).

All known ribonucleotide reductases have a quaternary structure where enzymatic activity is dependent on the complex between a large subunit (Rl) dimer and a small subunit (R2) dimer. The interaction between Rl and R2 seems entirely accounted for by *C*-terminal sequences of R2. The gene coding for the small subunit of ribonucleotide reductase in the nematode *Haemonchus contortus* was cloned, sequenced, and expressed (Chen et al., 2005). A synthetic oligopeptide corresponding to the *C*-terminal 7 residues of the small subunits in *Plasmodium falciparum* inhibited malarial enzyme at concentrations approximately 10-fold lower than that predicted to inhibit the mammalian subunit (Rubin et al., 1993). Furthermore, a recent comparative, annotated, structure-based, multiple-sequence alignment of R2 subunits, identified a clade of R2 subunits unique to Apicomplexa. This novel apicomplexan R2 subunit may be a promising candidate for chemotherapeutic-induced

inhibition as it differs greatly from known eukaryotic host ribonucleotide reductases and may be specifically targeted (Munro et al., 2013).

Iron chelators used as ribonucleotide reductases inhibitors were shown to cure *Plasmodium berghei* malaria in mice (Klayman et al., 1984 and 1986) and are effective against of *P. falciparum* growth in culture (Pradines et al., 1996; Holland et al., 1998).

2.8. 2′-Deoxyuridine 5′-triphosphate pyrophosphatase (dUTPase, EC 3.6.1.23)

The enzyme dUTPase is a highly active and specific pyrophosphatase that hydrolyze dUTP (2′-deoxyuridine 5′-triphosphate) to dUMP in the following manner:

$$\mathrm{dUTP}+\mathrm{H_2O} \rightleftharpoons \mathrm{dUMP}+PP_\mathrm{i}$$

The nucleotide dUTP is formed by the phosphorylation of dUDP (2′-deoxyuridine 5′-monophosphate), the direct product of ribonucleotide reductase reaction, by nucleoside 5′-diphosphate kinase (Fig. 6). dUTP is a good substrate for DNA polymerase from different species and could end up incorporated into DNA. To prevent such incorporation of dUTP into DNA, dUTPase degrades dUTP to dUMP. The enzyme is quite active resulting in intracellular pools of dUTP at/or less than 0.3 fmol/10^6 cells and making the incorporation of dUTP into DNA a very rare event (Goulian et al., 1980). Thus, dUTPase has a dual function. First, it produces dUMP, the precursor of dTMP and ultimately dTTP. The second, is preserving the integrity of DNA by removing dUTP from the 2′-deoxyribotide pool, thus reducing the probability of the incorporation of this nucleotide into DNA. Lack or inhibition of dUTP activity leads to harmful perturbations in the nucleotide pool resulting in increased uracil content of DNA that activates a hyperactive futile cycle of DNA repair (Vértessy and Tóth, 2009). Hence, dUTPase is considered an excellent target for chemotherapy.

Activity of dUTPase has not been studied in *Schistosoma mansoni*, but genome analysis indicates the presence of the enzyme in this parasite (Berriman et al., 2009). Enzyme activity of dUTPase has been reported in the nematodes; *Trichinella spiralis* and *T. pseudospiralis* (Rode et al., 2000), and the protozoa; *Plasmodium falciparum* (Whittingham et al., 2005), *Leishmania major* (Camacho et al., 1997), *Trypanosoma cruzi* (Hidalgo-Zarco and González-Pazanowska, 2001; Bernier-Villamor et al., 2002) and *T. brucei* (Castillo-Acosta et al., 2008). The protozoan enzymes were subjected to detailed analysis and characterization.

Currently, there are two recognized major families of dUTPases in protozoa which are unrelated in sequence or structure: dimeric as in *Trypanosoma cruzi*, *T. brucei, Leishmania major*, and trimeric as in *Plasmodium falciparum*. The trimeric dUTPases, including the human enzyme, possess five conserved sequence motifs. These cluster form the substrate recognition site and reaction center, bestowing high selectivity toward dUTP to the exclusion of dCTP (2′-deoxycytidine 5′-triphosphate), dTTP, and UTP. This specificity is achieved through hydrogen bonding patterns that favor the binding of the uracil nucleobase, and intimate interactions with the ribose moiety and the nucleobase that exclude the 2′-hydroxyl group of the pentose sugar and the 5-methyl group of thymine (Persson et al., 2001). The trimeric dUTPase from *Plasmodium falciparum* has been cloned, overexpressed, and

characterized. It has relatively low sequence similarity with its human ortholog (28.4% identity) (Whittingham et al., 2005) making it a suitable drug target for chemotherapy.

The significance of dUTPase in thymidylate biosynthesis in *P. falciparum*, is enhanced by the lack of salvage pathways in this parasite. Therefore, the enzyme has been and still is the focus of intense research efforts in targeting this enzyme using different methodologies including design, synthesis, and evaluation of analogues (Nguyen et al., 2005 and 2006; Whittingham et al., 2005; McCarthy et al., 2009; Baragaña et al., 2011; Ruda et al., 2011; Hampton et al., 2011), and high throughput searches (Crowther et al., 2011). These studies established that the uracil ring is of utmost importance in binding of ligands to the active site, whereas more variations are allowed at the 3′- and 5′-positions (Recio et al., 2011). Recent ongoing research is focusing on QSAR models (Quantitative Structure–Activity Relationship) to facilitate the design of novel compounds (de Araújo et al., 2015). For a more detailed discussion on the search for inhibitors of *P. falciparum* dUTPase see review by Nyíri and Vértessy (2017).

Dimeric dUTPases are members of the all-α NTP pyrophosphohydrolase family and represent promising drug targets due to their unique properties regarding substrate specificity and product inhibition as well as significant different structural and biochemical properties which bears no resemblance to typical eukaryotic trimeric dUTPases (Harkiolaki et al., 2004). The catalytic mechanism of dimeric dUTPases was elucidated by Hemsworth et al. (2013).

The enzyme from *Leishmania major* is encoded by a single gene, and differs significantly from trimeric dUTPases. None of the characteristic five amino acid motifs that are common to all currently known trimeric dUTPases were readily identifiable, and the sequence encoded a larger polypeptide with a molecular weight of 30.4 kDa. The enzyme hydrolyzes both dUTP and dUDP, but not other nucleotides (Camacho et al., 1997 and 2000), is highly dependent on Mg^{2+} concentrations, and markedly sensitive to the phosphatase inhibitor, NaF (Camacho et al., 2000). Kinetic parameters for dUTP hydrolysis are comparable to that of the human enzyme. However, the binding of dUDP and dUMP suggest differences in the structure of the active sites when compared with the human enzyme (Hidalgo-Zarco et al., 2001). The enzyme was crystalized in complex with substrate analogues, the product dUMP, and a substrate fragment (Hemsworth et al., 2011)

In *Trypanosoma brucei*, the enzyme is a nuclear enzyme. Down-regulation of its activity by RNAi greatly reduces cell proliferation, causes lethality, and increases the intracellular levels of dUTP. dUTPase-depleted cells presented hypersensitivity to methotrexate, a drug that increases the intracellular pools of dUTP. The knockdown of activity produces numerous DNA strand breaks and defects. It also produced parasites with a single enlarged nucleus as well as an enhanced population of anucleated cells. Defects in growth could be partially reverted by the addition of exogenous thymidine (Castillo-Acosta et al., 2008). Adding uracil, uridine or deoxyuridine could not rescue this phenotype (Castillo-Acosta et al., 2013). Therefore, it appears that dimeric dUTPases are strongly involved in the control of dUTP incorporation into DNA, and that adequate levels of enzyme are indispensable for efficient cell cycle progression and normal DNA replication.

In *T. cruzi,* dUTPase was cloned and characterized (Bernier-Villamor et al., 2002). The deduced amino acid sequence was similar to that of *Leishmania major* dUTPase, although it exhibits an amino acid insertion that is sensitive to protease inactivation. The enzyme is a dimer and detailed kinetic characterization showed that it is highly specific for dUTP and dUDP. Crystal structures of *T. cruzi* dUTPase showed major differences between the substrate binding pocket of dimeric and trimeric dUTPases (Harkiolaki et al., 2004). Asp80Ala substitution in the enzyme induces only a slight conformational change in the active site, yet results in a significant alteration of nucleotide binding and modifies the ability of the enzyme to discriminate between dUTP and dUMP when magnesium is present (Téllez-Sanz et al., 2007). It was also observed for the first time that ligand binding induces large conformational change in dimeric dUTPases (Harkiolaki et al., 2004). Inhibitor designed based on in silico docking were synthesized and tested against *T. cruzi* dUTPase. However, none of the compounds produced inhibited the enzyme at a concentration of 1 mM. Neither did the compounds inhibit parasite growth at the maximum concentrations studied. It was suggested that the failure of this approach is a result of not considering the flexibility of the protein (Mc Carthy et al., 2006)

3. Uptake of pyrimidine nucleobases and nucleosides

As shown in Table 1, schistosomes are capable of salvaging pyrimidine nucleobases and nucleosides (Levy and Read, 1975a; Nollen et al., 1976; Mattoccia et al., 1981; Mattoccia and Ciolo, 1983; el Kouni and Naguib, 1990). Thymine, cytosine and orotidine are transported into adult *S. mansoni*, but not incorporated into their nucleic acids (el Kouni and Naguib, 1990). Uridine, cytidine, deoxycytidine, thymidine and uracil were converted to their respective 5′-mono-, di- and triphosphate nucleosides as well as their hexose nucleotides and ultimately incorporated into the nucleic acids of the parasite (Mattoccia et al., 1981; Mattoccia and Cioli, 1983; Huang et al., 1984, el Kouni and Naguib, 1990). Cytidine is the most efficiently utilized pyrimidine. Cytidine, uridine and uracil were incorporated into the worm's nucleic acids better than orotate by 11-, 4- and 3-fold, respectively, (Table 1) suggesting that the salvage pathways may be more important than the de novo pathway in providing the pyrimidine requirements of the parasite. Orotate, uridine and uracil are incorporated into the various pyrimidines nucleotides; whereas, thymidine, cytidine and deoxycytidine are exclusively incorporated into their respective nucleotides. No incorporation of cytidine or deoxycytidine was detected in uracil nucleotides (el Kouni and Naguib, 1990).

The nematode *Angiostrongylus cantonensis* can utilize uracil, uridine, and cytidine, but not cytosine, thymine and thymidine for the synthesis of RNA and DNA (So et al., 1992). *Trichomonas vaginalis* incorporate uridine, cytidine, deoxycytidine, thymidine and uracil, but not thymine or cytosine, into their nucleic acids (Heyworth et al., 1984; Wang and Chen, 1984a). *Eimeria tenella* incorporate cytidine and uridine, but not thymidine (Ouellette et al., 1973). In contrast, *Plasmodium berghei* (Van Dyke et al., 1970) and *P. falciparum* (Krungkrai and Krungkrai, 2016) have little ability to salvage preformed pyrimidine nucleobases and nucleosides from the host cell and plasma, but rely mostly on nucleotide synthesis through the de novo pathway. Pyrimidine salvage was shown not to be an essential function for bloodstream *Trypanosoma brucei brucei*. However, Trypanosomes lacking de

novo pyrimidine biosynthesis are completely dependent on an extracellular pyrimidine source, strongly preferring uracil, and display reduced infectivity (Ali et al., 2013b).

4. Transport of pyrimidines

Transport across the cell membrane is the first step in the uptake of exogenous pyrimidines. An understanding of the mechanisms of transport and membrane function in parasites as well as differences in their properties as compared to those of their mammalian hosts may provide the foundation for rational antiparasitic drug development. Indeed, differences in the properties of nucleoside transport between mammalian and parasitic cells were the basis of a combination therapy approach involving the use of cytotoxic purine nucleoside analogues and host-protecting mammalian nucleoside transport inhibitors (Ogbunude and Ikediobi, 1982; el Kouni et al., 1983a, 1985, 1987, and 1989; el Kouni and Cha, 1987; Gati et al., 1987; Baer et al., 1988; Gero et al., 1989; el Kouni, 1991, 1992 and 2003; Aoki et al., 2009). Pyrimidine transporters, may also be helpful in gene therapy by delivering drugs to particular cells. For example, expression of a trypanosomal adenosine transporter in *Saccharomyces cerevisiae* rendered yeast hypersensitive to melarsen oxide (Mäser et al., 1999).

Studies of pyrimidine nucleoside and nucleobase transport in mammalian cells are numerous and have been discussed in several reviews, most recently by Griffith and Jarvis (1996), Buolamwini (1997), Cass et al. (1999), de Koning and Diallinas (2000), Kong et al. (2004), Young et al. (2008 and 2013), and Molina-Arcas el al. (2009). A great deal of the significant progress in understanding nucleoside transport in mammalian cells can be attributed to the discovery and synthesis of a number of highly specific inhibitors of nucleoside transport. The most notable and frequently used among these inhibitors are NBMPR (nitrobenzylthioinosine), dilazep and dipyridamole. The chemical structures of these compounds are shown in Fig. 5. In addition to passive diffusion, there are at least three main classes of nucleoside transporters in mammalian cells. The first (*es*) is an equilibrative non-concentrative carrier mediated transporter sensitive to NBMPR inhibition and is inhibited by all three nucleoside transport inhibitors mentioned above. The second (*ei*) is also an equilibrative non-concentrative carrier mediated transporter but insensitive to NBMPR inhibition although inhibited by dipyridamole. The third class is sodium ion driven concentrative nucleoside transporters insensitive to all three inhibitors of the equilibrative nucleoside transport systems. The mammalian nucleoside transporters have broad specificity and vary in their ability to recognize both purine and pyrimidine nucleosides. Therefore, each class can be divided accordingly to several families, the discussion of which is beyond the scope of this review. Nucleobase transport in mammalian cells occurs by passive diffusion or sodium ion dependent transporters (Kraup and Marz, 1995; Griffith and Jarvis, 1996; de Koning and Diallinas, 2000) that are inhibited by papaverine, dilazep or dipyridamole (Griffith and Jarvis, 1993 and 1996; Kraup and Marz, 1995).

Few studies have addressed pyrimidine uptake in parasites except where purine transporters were competitively inhibited by some pyrimidines. Pyrimidines are transported into parasites by carriers mediated nucleoside and nucleobase transporters or by passive diffusion. Substrate specificities, inhibition, and affinities for ligands among transporters from

parasites are quite different from their host. In addition, none of the parasite transporters have been shown to be a sodium dependent.

4.1. Pyrimidine nucleoside transport

Only one of three nucleoside transporters in *Schistosoma mansoni* is reported to transport uridine but can also transport adenosine. The second transporter is specific for adenosine. The third transporter has high affinity for adenine but also transports adenosine (Levy and Read, 1975a). Uridine uptake is saturable. At high concentrations, uridine is absorbed, in part, through simple diffusion. Mediated uridine uptake was inhibited completely by adenosine (Pappas and Read, 1975). In another trematode, *Fasciola hepatica*, uridine uptake occurs only by simple diffusion (Anderson et al., 1993). Studies on three species of cestodes; *Hymenolepis diminuta*, *H. citelli*, and *H. microstoma*, indicated that there is at least one locus involved in the transport of pyrimidine nucleosides. No significant difference was observed in their affinity for ribo- or deoxyribosides (Page 3rd and MacInnis, 1975). In *Hymenolepis diminuta*, uridine transport is saturable (MacInnis and Ridley, 1969; Pappas and Read, 1974) and diffusion was negligible (Pappas and Read, 1974). The uptake of uridine was stimulated by thymine, unaffected by hypoxanthine or uracil (MacInnis and Ridley, 1969), and inhibited by uridine, adenosine, thymidine, deoxyuridine, cytidine, inosine, AMP (adenosine 5′-monophosphate), and ATP (MacInnis and Ridley 1969; Pappas and Read, 1974). However, thymidine was slightly less effective as an inhibitor of riboside transport than uridine (Page 3rd and MacInnis, 1975). Thymine has no effect on uridine transport in the three cestodes (Page 3rd and MacInnis, 1975).

To the best of our knowledge, there are no other studies on the transport of pyrimidine nucleosides in a multicellular parasites, except these mentioned above in the trematodes; *Schistosoma mansoni* (Levy and Read, 1975a; Pappas and Read, 1975) and *Fasciola hepatica* (Uglem and Levy, 1976), and the cestodes; *Hymenolepis diminuta*, *H. citelli*, and *H. microstoma*, (MacInnis and Ridley, 1969; Pappas and Read, 1974; Page 3rd and MacInnis, 1975). Most of the transport studies in parasites were performed on the protozoa because of the ease of growing them in culture.

The protozoa; *Toxoplasma gondii* (Chiang et al., 1999; Al Safarjalani et al., 2003; de Koning et al., 2003), *Plasmodium falciparum* (Carter et al., 2000; Downie et al., 2006), *Crithidia fasciculata* (de Koning et al., 2000), and *Plasmodium vivax* (Deniskin et al., 2015), all appear to have a single broad-specificity transporter for pyrimidine nucleosides that can also transport purine nucleosides. The nucleoside transporter in *P. falciparum* was localized to the parasite plasma membrane (Rager et al., 2001). *Giardia intestinalis* can takeup uridine, cytidine, and thymidine (Jarroll et al., 1987). This parasite has two facilitative transporters for pyrimidine nucleosides; one specific for thymidine (Davey et al., 1991), a second less specific that transport all pyrimidine and purine ribosides and 2′- or 3′-deoxyriboside (Davey et al., 1992; Baum et al., 1993). *Trichomonas vaginalis* also have two separate nucleoside transporters that can transport pyrimidine nucleosides. The first is non-specific as it takes all pyrimidine and purine nucleosides (Harris et al., 1988). The second transports uridine as well as adenosine, guanosine. In *Trypanosoma brucei brucei* (Gudin et al., 2006), *Leishmania donovani* (Ogbunude et al., 1991; Iovannisci et al., 1984; Aronow et al., 1987;

Ghosh and Mukherjee, 2000), *L. major* (Baer et al., 1992; Alzahrani, et al., 2016a), and *L. mexicana* (Alzahrani, et al., 2016a), uridine is taken up by at least two transporters. One of which also transports thymidine, 5-fluoro-2′-deoxyuridine as well as the nucleobases, uracil and 5-fluorouracil. However, their efficiency for the nucleobases transport are much higher than those for the corresponding nucleosides. The other transporter mediate the uptake of uridine, adenosine, thymidine, 5-fluoro-2′-deoxyuridine and thymidine. Stein et al. (2003) reported that equilibrative pyrimidine nucleoside transporters from *Leishmania donovani* are nucleoside/proton symporters.

It should be noted here that nucleoside transporters in parasites are not inhibited by NBMPR, dilazep and to a lesser extent dipyridamole at the concentration required to inhibit NBMPR-sensitive mammalian nucleoside transporters (for references see review by el Kouni, 2003). In fact, NBMPR is a permeant in *Plasmodium falciparum* (Gero et al., 1989), and *Toxoplasma gondii* (el Kouni et al., 1999; Al Safarjalani et al., 2003; el Kouni 2003 and 2007).

4.2. Pyrimidine nucleobase transport

The uptake of pyrimidine nucleobases (uracil, thymine, and cytosine) by *Schistosoma mansoni* (Levy and Read, 1975a), and the cestode *Taenia crassiceps* (Uglem and Levy, 1976) occurs solely by simple diffusion. The uptake rate of each of these pyrimidines was linear with respect to concentration (Levy and Read, 1975; Uglem and Levy, 1976). In the cestode *Hymenolepis diminuta,* thymine and uracil are transported through a single locus where uracil transport is a mediated system (MacInnis et al., 1965). Both purines and pyrimidines inhibited the uptake of labeled uracil (MacInnis et al., 1965). In addition, pyrimidine nucleobases stimulated or inhibited uracil uptake depending on the inhibitor (I)/substrate (S) ratio. At low I/S ratios, uptake of uracil is inhibited by uracil, thymine, and 5-bromouracil, followed by stimulation of uptake at higher 1/S ratios (MacInnis et al., 1965; Pappas et al., 1973). Thymine stimulated uracil transport in *Hymenolepis citelli*, but not in *H. microstoma*, (MacInnis et al., 1965; Pappas et al., 1973; Page 3rd and MacInnis, 1975).

The nature of the chemical group occupying *C*5 and *C*6 of the uracil ring was shown to be important in determining the effects of uracil derivatives on uracil uptake by *H. diminuta* (MacInnis and Ridley, 1969). Uptake of thymine, uracil or 5-bromouracil was unaffected by cytosine, 5-methylcytosine, alloxan, 5-carboxyuracil, thymidine, and uridine. Uracil transport was stimulated by uracil and 5-substituted uracil derivatives, but inhibited by 6-methyluracil (MacInnis and Ridley 1969). Amino acids with ring structures similar to purines and pyrimidines had no effect on the uptake of uracil by *H. diminuta* (Pappas et al., 1973).

Pyrimidine nucleobase transport mechanisms in protozoa may also be distinct from those in mammalian systems. *Tritrichomonas foetus* has a pyrimidine nucleobase transporter but can also transport xanthine (Hedstorm and Wang, 1989). *Giardia intestinalis* has a facilitative transporter specific for uracil/thymine, but not cytosine derivatives (Ey et al., 1992). *Leishmania major* and *L. mexicana* have a highly selective, high-affinity uracil transporter which also transports 5-fluorouracil as well as the nucleosides uridine, adenosine, and 5-fluoro-2′-deoxyuridine (Papageorgiou et al., 2004; Alzahrani et al., 2016a). *Trypanosoma*

brucei brucei has a highly selective, high-affinity cytosine transporter (Gudin et al., 2006) as well as a high-affinity uracil transporter (de Koning and Jarvis, 1998; Gudin et al., 2006) that is also capable of transporting orotate, uridine, and deoxyuridine, but with lower affinities (Ali et al., 2013a). In contrast to mammalian cells, the *T. brucei brucei* nucleobase transporter is a nucleobase/proton symporter, and not a sodium ion dependent transporter (de Koning and Jarvis, 1997a, 1997b and 1980). For a more detailed discussion on the characterization of pyrimidine nucleobase transport in *T. brucei brucei* see review by Bellofatto (2007)

5. Pathways of pyrimidine salvage

UMP, the focal metabolite in pyrimidine metabolism, can be synthesized by the de novo pathway in almost all organisms but it can also be synthesized by the salvage pathways. These salvage pathways utilize endogenous pre-existing nucleotides and their derivatives or exogenous sources of these compounds. However, unlike the universality of de novo pyrimidine synthesis, differences do exist in the salvage pathways between various organisms (c.f. Munch-Peterson, 1983). Fig. 6 illustrates the reactions of the salvage pathways by which UMP and other pyrimidine nucleobase, nucleoside and nucleotides may be interconverted, as determined from various organisms.

The incorporation of uracil into the nucleic acids of schistosomes (Table 1) takes place via the formation of UMP. As depicted in Fig. 6, the conversion of uracil to UMP can occur either by the sequential reactions of uridine phosphorylase and uridine kinase or by the direct conversion of uracil to UMP by a phosphoribosyltransferase (Iltzsch et al., 1984; el Kouni et al., 1988; el Kouni and Naguib, 1990; Naguib and el Kouni, 2014). As mentioned below (*6.7.*), schistosomes have a relatively non-specific phosphoribosyltransferase that can utilize uracil under physiological conditions and could be the principal pathway for the conversion of uracil to UMP (Iltzsch et al., 1984).

Thymidine and thymine incorporation into nucleic acids was observed in *S. mansoni* (Table 1). However, neither the cleavage of thymidine to thymine nor the synthesis of thymidine from thymine was detected in vivo (el Kouni and Naguib, 1990) in spite of the fact that such activities occur in vitro (el Kouni et al., 1988; el Kouni and Naguib, 1990; Naguib and el Kouni, 2014). The utilization of orotate by schistosomes confirms the existence of at least the last part of the de novo UMP biosynthesis (Iltzsch et al., 1984; el Kouni and Naguib, 1990). However, the relatively better efficiency of cytidine, uridine, and uracil utilization suggest that the contribution to pyrimidine metabolism by the salvage pathways may be of greater importance to schistosomes than the de novo pathway. This apparent reliance on the salvage pathway may have evolved from the selective adaptation of these parasites to their environment. Plasma concentration of cytidine and uridine are in the micromolar range in mammals (Rustum, 1978; Moyer et al., 1981; Pellwein et al., 1987) and probably can satisfy the schistosomes requirements for pyrimidines.

6. Enzymes of pyrimidine salvage pathways

Assays of enzyme activities in *Schistosoma mansoni* cell free extracts can better prove the routes, summarized in Fig. 6, by which pyrimidine nucleobases and nucleosides are metabolized. In the following are the description and characteristics of the enzymes involved in pyrimidine salvage in schistosomes as well as a comparison with these enzymes from other species.

6.1. Alkaline phosphatase (EC 3.1.3.1)

Alkaline phosphatase is ubiquitous and widely distributed enzyme among different organisms. In humans, it is present in almost all tissues throughout the entire body. It is a non-specific hydrolase which is important in the salvage of pyrimidines. The enzyme allows the uptake of impermeable negatively charged nucleotides by removing their organic phosphate group and convert them to the more permeable nucleosides as follows:

$$\text{Nucleoside}\,5'\text{- monophosphate}+\text{H}_2\text{O} \varpi \text{Nucleoside}+P_{\text{i}}$$

Extracts of adult *Schistosoma mansoni* have high levels of phosphatase activities capable of dephosphorylating pyrimidine nucleoside 5′-monophosphates (Nimmo-Smith and Standen, 1963; Levy and Read, 1975a and 1975b; Cesari et al., 1981; el Kouni and Naguib, 1990). These activities in schistosomes are correlated with activities towards β-glycerophosphate (Table 2) indicating that the dephosphorylation of pyrimidine nucleoside 5′-monophosphates in schistosomes are due to alkaline phosphatases rather than the more specific 5′-nucleotidases (EC 3.1.3.5) (Nimmo-Smith and Standen, 1963; Levy and Read, 1975a and 1975b; Cesari et al., 1981; el Kouni and Naguib, 1990). These activities are associated with the particulate fraction of the parasite which is constituted mainly of the tegument of the parasite (Nimmo-Smith and Standen, 1963; Levy and Read, 1975a and 1975b; Wheater and Wilson, 1976; Ernst, 1976; Simpson et al., 1981; Cesari et al., 1981; el Kouni and Naguib, 1990). The tegument of the adult schistosome is delimited by a double membrane that completely surrounds the parasite (Hockley and McLaren, 1973). The outer of the two membranes plays an important role in nucleotide metabolism as it contains high levels of phosphatase activities that can dephosphorylate pyrimidine nucleoside 5′-monophosphates. Indeed, by the use of various methodologies, alkaline phosphatase was identified on the external surface of the tegument of adult *S. mansoni*. (Dusanic 1959; Morris and Threadgold, 1968; Levi-Schaffer et al., 1984; Cesari, 1974; Roberts et al., 1983; Payares et al., 1985; van Balkom et al., 2005; Braschi et al., 2006). Therefore, alkaline phosphatase activity is used as marker enzyme in the isolation of surface fragments from *S. mansoni* (Wheater and Wilson, 1976; Simpson et al., 1981). An immunoglobulin G fraction from sera of mice chronically infected with *S. mansoni* partially inhibited the parasite alkaline phosphatase activity (Cesari et al., 1981). Alkaline phosphatase from *S. mansoni* is expressed not only in the tegument but in internal tissues and in life stages outside the mammalian host (Dusanic, 1959; Robinson, 1961; Halton, 1967; Bhardwaj and Skelly, 2011). Alkaline phosphatase activities were also identified in cercariae of *S. mansoni* (Conde-del Pino et al., 1968; Sodeman Jr. et al., 1968), *S. haematobium*, and *S. japonicum* (Sodeman Jr. et al., 1968), but its tissue localization differed among the three species.

Nevertheless, the activity appears to be related to the excretory system and the penetration glands. (Sodeman Jr. et al., 1968)

Alkaline phosphatase was extracted from adult *S. mansoni* membranes and purified by Payares et al. (1984) who also concluded that the enzyme is not exposed at the schistosome's surface, and is probably buried in the tegumental membrane network. The enzyme was cloned, expressed and characterized. The cloned enzyme potentially encodes a 536 amino acid protein of ~60 kDa, a pI of 5.92, and possesses six potential *N*-linked glycosylation sites (Bhardwaj and Skelly, 2011).

Alkaline phosphatases are also found in the trematodes; *Dicrocoelium lanceatum*, *Eurytrema*, *Paragonimus westermani* (Yamao, 1952a and 1952b), *Clonorchis sinensis* (Yamao, 1952a), *Fasciola hepatica* (Halton, 1967), the cestodes; *Moniezia expansa* (Rogers, 1947; Erasmus, 1957b), *Taenia pisiformis* (Erasmus, 1957a), *Cysticercus tenuicollis* (Erasmus, 1957b), *Hymenolepis microstoma* (Bogitsh, 1963), *H. diminuta* (MacInnis, et al., 1965; Lumsden et al., 1968), the nematode *Ascaris lumbricoides* (Rogers,1947), and the protozoa *Toxoplasma gondii* (Iltzsch, 1993). On the other hand, the nematode *Trichinella spiralis* has a specific 5′-nucleotidase (EC 3.1.3.5) (Gounaris, 2002), and the protozoa *Leishmania tropica* has a broad substrate specificity nucleotidase (EC 3.1.3.31) (Pereira and Königk, 1981) which convert nucleotides to their respective nucleosides.

6.2. Nucleoside phosphotransferases (EC 2.7.1.77)

These enzymes phosphorylate nucleosides to their respective nucleoside 5′-monophosphates. Using low energy phosphate esters (e.g., various nucleoside 5′-monophosphates, *p*-nitrophenylphosphate, β-glycerophosphate, etc.) as phosphate donors as follows:

$$\text{Nucleoside}_1 + \text{Nucleoside}_2\ 5'\text{- monophosphate} \rightleftharpoons \text{Nucleoside}_1\ 5'\text{- monophosphate} + \text{Nucleoside}_2$$

Adult *Schistosoma mansoni* do not have nucleoside phosphotransferases (el Kouni and Naguib, 1990; Naguib and el Kouni, 2014). No activity was detected when AMP, β-glycerophosphate or *p*-nitrophenylphosphate was used as a phosphate donor in the extracts of *S. mansoni* (el Kouni and Naguib, 1990; Naguib and el Kouni, 2014). A similar situation exists in *Angiostrongylus cantonensis* (So et al., 1992), and *Toxoplasma. gondii* (Iltzsch, 1993). In contrast, pyrimidine nucleoside phosphotransferases are found in *Tritrichomonas foetus* (Wang et al., 1983), *Trichomonas vaginalis* (Wang and Cheng, 1984a and b), *Giardia intestinalis* (Aldritt et al., 1985), and *Tetrahymena pyriformis* (Bols and Zimmerman, 1977; Yuyama et al., 1978 and 1979; Fink, 1980).

6.3. Pyrimidine nucleoside kinases [EC 2.7.1.-)

Nucleoside kinases are similar to the nucleoside phosphotransferases as they phosphorylate pyrimidine ribo- and 2′-deoxyribosides to their respective nucleoside 5′-monophosphates. The difference is that the kinase reaction requires a high energy phosphate ester (e.g., ATP) as a phosphate donor as follows:

$$\text{Pyrimidine nucleoside} + \text{ATP} \varpi \text{Pyrimidine nucleoside } 5' \text{- monophosphate} + \text{ADP}$$

Among the enzymes involved in pyrimidine salvage, the nucleoside kinases play a pivotal role in the utilization of pyrimidine nucleosides. They are responsible for the first step in the anabolism of pyrimidine nucleosides. Extracts of *S. mansoni* phosphorylate all pyrimidine nucleosides (except orotidine), in the presence of ATP, to their respective nucleoside 5′-monophosphates, indicating the presence of nucleoside kinases (el Kouni and Naguib, 1990; Naguib and el Kouni, 2014). Competition studies and column chromatography demonstrated that adult *S. mansoni* contain three cytosolic nucleoside kinases that can phosphorylate pyrimidine nucleosides (Naguib and el Kouni, 2014). The first is a non-specific deoxyriboside kinase (EC 2.7.1.145) which indiscriminately phosphorylates all five naturally occurring pyrimidine (thymidine, 2′-deoxyuridine, and 2′-deoxycytidine) and purine (2′-deoxyguanosine and 2′-deoxyadenosine) deoxyribosides. The second is a kinase specific solely for uridine. The third is a distinct cytidine kinase which differs from uridine kinase. Other than these three nucleoside kinases, the only other nucleoside kinase that is reported in schistosomes is adenosine kinase (EC 2.7.1.20) which is specific for adenosine and its analogues (Senft and Crabtree, 1983; el Kouni et al., 1983a and 1987; Dovey et al., 1984; el Kouni and Cha, 1987).

S. mansoni uridine kinase is a tightly regulated enzyme and is strongly inhibited by its substrate. Unless the appropriate assay conditions are used, this activity cannot be detected (Naguib and el Kouni, 2014). Various nucleoside 5′-triphosphates act as phosphate donors for the three kinases albeit to different degrees. GTP (guanosine 5′-monophosphate) was the best phosphate donor for the kinase reaction when uridine or cytidine, but not when thymidine, was used as a substrate. UTP was the best phosphate donor for the activity towards thymidine. The kinetic parameters of the three nucleoside kinases were characterized as shown in the Table 3. The mechanism of the three kinases is ping pong (Naguib and el Kouni, 2014).

Other than *S. mansoni,* a non-specific deoxyriboside kinase activity was reported so far only in insects; *Drosophila melanogaster* (Munch-Petersen et al., 1989), silk worm *Bombyx mori* (Knecht et al., 2002), and the mosquito *Anopheles gambiae* (Knecht et al., 2003). The presence of separate cytidine and uridine kinases in schistosomes, is a conspicuous difference between pyrimidine metabolism in schistosomes and their mammalian host where uridine and cytidine are phosphorylated by a single uridine-cytidine kinase (EC 2.1.7.48). The presence of a distinctive cytidine kinase along with the absence of cytidine deaminase and phosphorylase can be manipulated for chemotherapy. In this regard, various available cytidine analogues can be used as antischistosomal drugs. These compounds can be phosphorylated to the nucleotide level in the parasites without being degraded by the sequential reaction of cytidine deaminase and uridine phosphorylase (Fig. 6) as is the case in the host.

No pyrimidine nucleoside kinase activity was detected in *Plasmodium falciparum* (Reyes et al., 1982), *Tritrichomonas foetus* (Wang et al., 1983), or *Toxoplasma gondii* (Iltzsch, 1993)

indicating that these protozoa lack the ability to directly phosphorylate pyrimidine nucleosides. No uridine kinase was detected in culture promastigote form of *Leishmania mexicana amazonensis*, the blood trypomastigote and the culture epimastigote of *Trypanosoma brucei*, blood trypomastigote and intracellular amastigote forms of *T. cruzi* (Hammond and Gutteridge, 1982), *Giardia intestinalis* (Aldritt et al., 1985; Vitti et al., 1987), or *Leishmania donovani* (Wilson et al., 2012). There is a controversy about the presence of uridine kinase in *Trichomonas vaginalis*. Miller and Lindstead (1983) reported the presence of the activity while Wang and Cheng (1984a) could not detect it.

Activity of uridine-cytidine kinase was detected in *Angiostrongylus cantonensis* (So et al., 1992) and uridine kinase in *Crithidia luciliae* (Tampitag and O'Sullivan, 1986). Phosphorylation of uridine and thymidine was observed in *Paragonimus ohirai* and *Clonorchis sinensis* (Kobayashi et al., 1978), but it was not clear whether these two nucleosides were phosphorylated by nucleoside kinases or a phosphotransferases. An early report of the existence of uridine kinase in *Giardia intestinalis* (Lindmark and Jarroll, 1982) was subsequently shown to be incorrect (Vitti et al., 1987). Uridine-cytidine kinase from *Entamoeba histolytica* was cloned, expressed and purified (Lossani et al., 2009). Striepen et al. (2004) reported the presence of a unique uridine-cytidine kinase activity in *Cryptosporidium parvum* where the activity is fused with uracil phosphoribosyltransferase in a single bifunctional enzyme.

Thymidine kinase is apparently lacking in the protozoa *Trichomonas vaginalis* (Wang and Cheng,1984a), the nematodes; *Angiostrongylus cantonensis* (So et al., 1992), and *Ascaris lumbricoides,* but found in the cestode *Hymenolepis diminuta*, the acanthocephalan *Moniliformis dubius* (Farland and MacInnis, 1974), and the protozoa *Cryptosporidium parvum* (Striepen et al., 2004). The presence of thymidine kinase in *C. parvum* is unique within the phylum Apicomplexa. Phylogenetic analysis suggests horizontal gene transfer of thymidine kinase from a proteobacterium (Striepen et al., 2004). *Tetrahymena pyriformis* has both nucleoside kinase and phosphotransferase activities towards thymidine (Bols and Zimmerman, 1977; Yuyama et al., 1978 and 1979; Fink, 1980). It was suggested that at high thymidine concentrations, nucleoside phosphotransferase is the dominant phosphorylating enzyme while at low thymidine concentrations both nucleoside phosphotransferase and kinase activities can be detected (Fink, 1980).

Thymidine kinases from *Trypanosoma brucei rhodesiense* (Chello and Jaffe, 1972a and 1972b), *Brugia pahangi*, *Dirofilaria immitis* (Jaffe et al., 1982), *Giardia intestinalis* (Lindmark and Jarroll, 1982; Aldritt et al., 1985; Vitti et al., 1987; Laoworawit et al., 1993), *Cryptosporidium parvum* (Sun et al., 2010), and *Leishmania major* (Timm et al., 2015) were partially purified and characterized. The enzyme from *Giardia intestinalis* can also phosphorylates deoxycytidine (Laoworawit et al., 1993) while that from *Leishmania major* can phosphorylate uridine, but to a much lower extent (Timm et al., 2015). Thymidine kinases from *Entamoeba histolytica* (Lossani et al., 2009), and *Trypanosoma brucei* (Ranjbarian et al., 2012) were cloned, expressed, and characterized. The trypanosomal enzyme is a tandem protein consisting of two homologous parts, domain 1 and domain 2. The two domains are fused into a single open reading frame. Domain 1 is catalytically inactive but improves substrate binding by domain 2. Furthermore, the enzyme is less

discriminative against purines than its human counterpart with the following order of efficiency: thymidine > deoxyuridine ≫ deoxyinosine> deoxyguanosine (Ranjbarian et al., 2012). Knockdown of *T. brucei* thymidine kinase leads to cell death that could not be rescued by exogenous pyrimidines. Metabolite profiling of the thymidine kinase depleted cells revealed a buildup of pyrimidine deoxyribosides (Leija et al., 2016). These findings indicate that thymidine kinase is the sole route for de novo synthesis of thymine nucleotides in *T. brucei*. Trypanosomatids lack alternative routes found in other eukaryotic cells to synthesize these nucleotides (Leija et al., 2016). For these reasons thymidine kinase was found to be essential to establish infection in mice and, hence, constitutes a strong potential chemotherapeutic target.

6.4. Pyrimidine nucleoside 5′-monophosphate (EC 2.7.4.-) and nucleoside diphosphate (EC 2.7.4.6) kinases

Pyrimidine nucleoside 5′-monophosphate kinases catalyze the reversible phosphorylation of various nucleoside pyrimidine 5′-monophosphates in the presence of a nucleoside 5′-triphosphate, usually ATP, to their respective pyrimidine nucleoside 5′-diphosphate as follows:

$$\text{Pyrimidine nucleoside} + 5'\text{- monophosphates} + \text{ATP} \rightleftharpoons \text{Pyrimidine nucleoside } 5'\text{- diphosphate} + \text{ADP}$$

There are two pyrimidine nucleoside 5′-monophosphate kinases. The first is a relatively non-specific pyrimidine nucleoside 5′-monophosphate kinase (EC 2.7.4.14) which phosphorylates all pyrimidine nucleoside 5′-monophosphates except dTMP. The other is dTMP kinases (EC 2.7.4.9) which is specific for the phosphorylation of dTMP and probably dUMP. Activity of dTMP kinase is the only known pathway to synthesize dTDP and ultimately dTTP (Fig. 6). Inhibition of dTMP kinase could be very effective in blocking both the de novo and salvage pathways of dTTP production and hence DNA synthesis.

Nucleoside 5′-diphosphate kinases catalyze the exchange of terminal phosphates between various NDP (nucleoside 5′-diphosphates) and NTP (nucleoside 5′-triphosphates) in a reversible manner to produce nucleotide 5′-triphosphates. Many NTP are donors of the phosphate group but the principal donor is ATP.

$$\text{N}_1\text{DP} + \text{N}_2\text{TP} \rightleftharpoons \text{N}_1\text{TP} + \text{N}_2\text{DP}$$

Schistosoma mansoni contains appreciable levels of nucleoside 5′-monophosphate kinase(s) towards UMP, CMP, dCMP, dUMP and dTMP. Further phosphorylation of nucleoside 5′-diphosphates was also detected, indicating the presence of pyrimidine nucleoside 5′-diphosphate kinases in *S. mansoni* (el Kouni and Naguib, 1990). A similar situation exists in *Plasmodium falciparum* (Reyes et al., 1982), and *Angiostrongylus cantonensis* (So et al., 1992). In *Giardia intestinalis,* activities were observed towards uridine and cytidine nucleotides (Aldritt et al., 1985). However, in all these cases, there was no information about the nature and specificity of the nucleoside 5′-monophosphate kinase responsible for the

phosphorylation of each of the pyrimidine nucleoside 5′-monophosphates. On the other hand, the nucleoside diphosphate kinase from the parasitic nematode *Brugia malayi* was identified, cloned and characterized. Molecular modeling of the enzyme showed several regions surrounding the conserved catalytic site that may be important in the design of drugs specific for the disruption of NTP synthesis in filarial parasites (Ghosh et al., 1995). A soluble nucleoside diphosphate kinase from epimastigote forms of *Trypanosoma cruzi* was purified and characterized. The enzyme is a hexamer composed of ~16.5 kDa monomers (Ulloa et al., 1995), and is predominantly localized in the nucleus (Hunger-Glaser et al., 2000). Nucleoside diphosphate kinase was found also in the secretome of *Leishmania amazonensis*. Activity of the released enzyme prevented ATP-induced cytolysis of infected macrophages. Hence, it appears that, beside its role in nucleotide metabolism in trypanosomatid protozoa, nucleoside diphosphate kinase has other functions related to preserving host-cell integrity for the benefit of the parasite survival (Kolli et al., 2008). Due to their importance for housekeeping functions in the parasite and prolonging the life of host cells in infections with intracellular parasites, nucleoside diphosphate kinase should be considered an attractive target for chemotherapy.

6.5. Uridine phosphorylase (EC 2.4.2.3)

Extracts of schistosomes are capable of cleaving both uridine and thymidine to uracil and thymine, respectively. The activity towards thymidine was approximately 16% of that towards uridine (el Kouni et al., 1988; el Kouni and Naguib, 1990).

There are two different pyrimidine nucleoside phosphorylases that can perform such reversible phosphorolysis: uridine phosphorylase and thymidine phosphorylase (EC 2.4.2.4) as follows:

$$\text{Pyrimidine (deoxy)riboside} + P_i \rightleftharpoons \text{Pyrimidine base} + \text{(Deoxy)ribose- 1- phosphate}$$

Substrate specificities of both enzymes vary greatly dependent on the source of the enzymes (el Kouni et al., 1993). In general, however, uridine phosphorylase cleaves primarily pyrimidine ribosides, but is relatively non-specific as it can also cleave pyrimidine 2′- and 5′-deoxyribosides. Thymidine phosphorylase, on the other hand, is more specific for pyrimidine 2′- and 5′-deoxyribosides.

In *S. mansoni*, the activity towards thymidine is not due to a distinct thymidine phosphorylase but to a non-specific uridine phosphorylase (el Kouni et al., 1988). Uridine phosphorylase is the only pyrimidine nucleoside cleaving activity that can be detected in extracts of *S. mansoni*. The enzyme is distinct from purine nucleoside phosphorylases (EC 2.4.2.1) and adenine nucleoside phosphorylase (EC none), the two other nucleoside phosphorylases in this parasite (el Kouni et al., 1988; Savarese and el Kouni, 2014; Torini et al., 2016). Although uridine is the preferred substrate, *S. mansoni* uridine phosphorylase can also catalyze the reversible phosphorolysis of 2′-deoxyuridine and thymidine, but not cytidine, 2′-deoxycytidine, 6-azauridine or orotidine (el Kouni et al., 1988, el Kouni and Naguib, 1990). It should be noted, however, that only the phosphorolysis and reverse phosphorolysis of uridine, but not thymidine, was observed in vivo (el Kouni and Naguib,

1990). The reverse phosphorolysis of uridine in vivo indicates that endogenous ribose-1-phosphate is in adequate amounts for such reaction. The lack of detectable in vivo thymidine cleavage could be due to the competition between the added thymidine and endogenous uridine since schistosomes have only uridine phosphorylase which reacts six-fold better with uridine than thymidine (el Kouni et al., 1988). Such competition by uridine, the better substrate, in addition to the relatively higher ratio of kinase/phosphorylase activity towards thymidine (0.4) than uridine (0.1) (el Kouni and Naguib, 1990) could result in thymidine cleavage activity below the sensitivity of the assay. The competition between the kinases and phosphorylase activities can be further accentuated by the relative intracellular concentrations of co-factors required for their activity, i.e. ATP and phosphate. Similarly, the synthesis of thymidine from thymine may be below the level of detection because of the low intracellular concentration of deoxyribose-1-phosphate.

Schistosomal uridine phosphorylase was purified 170-fold to a specific activity of 2.76 nmol/min/mg protein with a 16% yield. It has a M_r of 56,000 as determined by molecular sieving on Sephadex G-100. The mechanism of uridine phosphorylase is sequential. When uridine was the substrate, the $K_{uridine}$ = 13 μM and the $K_{Phosphate}$ = 535 μM. When thymidine was used as a substrate the $K_{thymidine}$ = 55 μM and the $K_{Phosphate}$ = 760 μM. The V_{max} with thymidine was 53% that of uridine. Thymidine was a competitive inhibitor when uridine was used as a substrate. The enzyme showed substrate inhibition by uridine, thymidine (>0.125 mM) and phosphate (>10 mM) (el Kouni et al., 1988).

A recent study (da Silva Neto et al., 2016) demonstrated that *S. mansoni* genome has two isoforms of uridine phosphorylase genes (*Sm*UPa and *Sm*UPb) that share 92% sequence identity. Both *Sm*UP isoforms were successfully cloned, expressed, purified, and crystallized (da Silva Neto et al., 2016). Both enzymes present an overall fold and active site structure similar to other known uridine phosphorylases. They are composed of two monomers related by a two-fold symmetry axis with a large interface. The homodimer has two active sites, each of which include residues from both monomers. The active site is composed of nucleobase, ribose and phosphate binding sites (da Silva Neto et al., 2016). Furthermore, the study showed conclusively that *Sm*UPa is an active uridine phosphorylase while *Sm*UPb has no detectable activity. This is quite strange given the high level of sequence identity between the two isoforms. It is probably the result of the significant loss of available space and hydrogen bonding to the nitrogen pyrimidine ring in the nucleobase binding site of *Sm*UPb rendering this enzyme incapable of binding pyrimidine nucleosides, and consequently incapable of the reversible phosphorolysis of uridine/thymidine (da Silva Neto et al., 2016). It is noteworthy that *Sm*UPa is continuously expressed through the *S. mansoni* life cycle while *Sm*UPb is mainly expressed in adult parasite phase (da Silva Neto et al., 2016). The reason for the maintenance of the two *Sm*UP genes in the *S. mansoni* genome remains unknown.

A non-specific uridine-phosphorylase has also been reported in *Giardia intestinalis* (Vitti et al., 1987; Lee et al., 1988), *Toxoplasma gondii* (Iltzsch, 1993), the tapeworm *Hymenolepis diminuta* (Drabikowska, 1996), and *Plasmodium falciparum* (Krungkrai et al., 2003). The primary structure of the *P. falciparum* uridine phosphorylase suggests that both uridine phosphorylase and purine nucleoside phosphorylase (EC 2.4.2.1) activities are on the same

polypeptide (Kicska et al., 2002). Phosphorolysis of uridine and thymidine was reported in *Trichinella pseudospiralis* (Rode et al., 2000) but it was not clear whether these activities were due to one or two phosphorylases. A more specific uridine phosphorylase that cannot cleave thymidine was identified in *Trichomonas vaginalis* (Wang and Cheng, 1984a).

All trypanosomatids investigated; *Herpetomonas muscarum muscarum*, *H. m. ingenoplastis*, procyclic and trypomastigotes of *Trypanosoma brucei brucei,* culture forms of *Crithidia fasciculata,* amastigotes and cultured promastigotes of *Leishmania mexicana,* cultured promastigotes of *L. m. amazonensis*, but not *L. donovani* and *L. tarentolae,* are able to convert uracil to uridine in the presence of ribose-l-phosphate (Hammond and Gutteridge, 1982; Hasaan and Coombs, 1986), suggesting that their uridine phosphorylase activities are reversible. It should be emphasized here that since no uridine kinase activity was found in the homogenates of any of these species (Hammond and Gutteridge, 1982). Therefore, it appears that uridine salvage in these parasites is routed via a uridine phosphorylase and uracil phosphoribosyltransferase as shown in Fig. 6. Indeed, uracil phosphoribosyltransferase activity was detected in all organisms tested except the intracellular amastigote form of *Trypanosoma cruzi* (Hammond and Gutteridge, 1982). It should also be noted that *T. brucei brucei* can adjust its uridine phosphorylase expression levels to accommodate growth on uridine as its sole pyrimidine source, whether these cells are pyrimidine auxotrophs or prototrophs, (Ong et al., 2013; Ali et al., 2013b). The enzyme from *T. brucei* was purified, crystalized and characterized. Beside uridine, no other pyrimidine nucleoside can be used as a substrate except 2′-deoxyuridine (Larson et al., 2010).

Thymine was converted to thymidine in vitro by *Herpetomonas* spp., *Trypanosoma brucei brucei,* and *Crithidia fasciculata,* but not by several Leishmania species (Hasaan and Coombs, 1986). The very high catabolic activity of thymidine phosphorylase in *C. fasciculata* and culture forms of *Trypanosoma cruzi* prevented incorporation of thymidine into their DNA (Al Chalabi and Gutteridge, 1977b). In the nematode *Angiostrongylus cantonensis*, uridine, deoxyuridine, thymidine, and surprisingly cytidine were converted to their respective nucleobases by a phosphorylase reaction. However, only uracil and thymine, but not cytosine, could form nucleosides in the reverse reaction (So et al., 1992). It was not clear whether the phosphorolysis of these pyrimidine nucleosides was carried by one or more enzymes. It is also curious that cytidine can be subject to phosphorolysis in *A. cantonensis*. To the best of our knowledge there is no other report of a cytidine phosphorylase in the literature.

5-(Benzyloxybenzyloxybenzyl)acyclouridine was identified as a potent and specific inhibitor of *S. mansoni* (K_i = 0.98 μM), but not the host uridine phosphorylase (el Kouni et al., 1988). Other benzylacyclouridines were also identified as inhibitor of *S. mansoni* uridine phosphorylase but were not specific for the parasite enzyme (el Kouni et al., 1988). The difference in degree of inhibition by the benzylacyclouridines as well as the ranking of the potencies of these inhibitors (Table 4) suggest that there are subtle differences between the binding sites of uridine phosphorylase from mammals and that of the parasite. Structure-activity relationship studies in *S. mansoni* (el Kouni et al., 1988), *Giardia intestinalis* (Jiménez et al., 1989), *Toxoplasma gondii* (Iltzsch and Klenk, 1993; el Kouni et al., 1996),

and *Hymenolepis diminuta* (Drabikowska, 1996) suggest that uridine phosphorylase from these different parasite have common features. All have a hydrophobic pocket adjacent to the 5-position of the pyrimidine ring. This hydrophobic pocket in *S. mansoni* is larger than that of the mammalian enzyme (el Kouni et al., 1988). The most effective inhibitors were analogues of acyclouridines and 2,2′-anhydrouridines substituted at the *C5* position with electron withdrawing (e.g., nitro or halogens) or hydrophobic (e.g., ethyl or benzyl) groups. The inhibitory effect at the 5-position appeared to be further enhanced by substitution at the *C6* position with electron releasing groups. The parasite but not the mammalian host uridine phosphorylase can participate in hydrogen bonding with *N*3 of the pyrimidine ring of nucleoside ligands. Furthermore, the binding of ligands to the enzyme is in the *syn*/high *syn* conformation around the *N*-glycosyl bond. These differences may be useful in designing specific inhibitors for the parasite uridine phosphorylase which will interfere selectively with nucleic acids synthesis in this parasite. Such inhibitors may be used alone or in combination with other pyrimidine analogues as antischistosomal chemotherapeutic agents to interfere selectively with nucleic acids synthesis in this parasite.

6.6. Cytosine and its nucleoside and nucleotide deaminases (EC 3.5.4.-)

Three enzymes, cytosine deaminase (EC 3.5.4.1), (2′-deoxy)cytidine deaminase (EC 3.5.4.5), and (d)CMP deaminase (EC 3.5.4.12) catalyze the reversible hydrolytic deamination of cytosine nucleobases, nucleosides and nucleotides to their uracil derivatives in the following manner:

$$\mathrm{Cytosine}+\mathrm{H_2O} \rightleftharpoons \mathrm{Uracil}+\mathrm{NH_3}$$
$$(2'\text{- deoxy})\mathrm{Cytidine}+\mathrm{H_2O} \rightleftharpoons (2'\text{- deoxy})\mathrm{Uridine}+\mathrm{NH_3}$$
$$(\mathrm{d})\mathrm{CMP}+\mathrm{H_2O} \rightleftharpoons (\mathrm{d})\mathrm{UMP}+\mathrm{NH_3}$$

As seen in Fig. 6, there is a fourth cytosine nucleotide deaminase, dCTP deaminase (EC 3.5.4.13). This deaminase has been reported, so far only in bacteria. Hence, it will not be discussed further in this review.

Among these deaminases, (d)CMP deaminase is of particular importance. The enzyme is one of only two enzymes allosterically regulated enzymes in pyrimidine metabolism. The other is ribonucleotide reductase (see above *2.7.*). (d)CMP deaminase is activated by dCTP and inhibited by dTTP. As shown in Fig. 6, deamination of dCMP leads to the synthesis of dUMP, the substrate for thymidylate synthase and precursor of dTMP and the only de novo source of dTTP. However, dCMP is also a precursor of dCTP via nucleoside 5′-mono- and diphosphate kinases (Fig. 6). It is assumed that accumulation of dCTP would activate dCMP deaminase to enable the synthesis of thymidine nucleotides. On the other hand, dTTP accumulation can shut off its own synthesis by inhibiting dCMP deaminase. This scenario can explain the allosteric activation of dCMP deaminase activity by dCTP and inhibition by dTTP, and how the necessary balance between the two pyrimidine deoxyribotides required for normal DNA synthesis is achieved.

el Kouni and Naguib (1990) showed that *Schistosoma mansoni* incorporate cytidine and deoxycytidine exclusively into their respective nucleotides. In addition, enzyme assays

indicated that the parasites are incapable of deaminating cytosine, cytidine, deoxycytidine, CMP and dCMP to their respective uracil derivatives. The absence of deoxycytidine deaminase was also observed by Senft et al. (1973a). However, the *S. mansoni* genome analysis (Berriman et al., 2009) indicates the presence of (d)CMP deaminase. Furthermore, (d)CMP deaminase activity was recently identified in *S. mansoni* (Serrão et al., 2017a). The enzyme has 175 amino acids and molecular weight of 19.5 kDa with 58% sequence identity to human (d)CMP deaminase, and a K_{dCMP} of 22 μM and k_{cat}, of 54^{s-1}. Its activity, like other known (d)CMP deaminases, requires the presence of dCTP and two metals (Zn^{2+} and Mg^{2+}), and is feedback inhibited by dTTP (Serrão et al., 2017a). The absence of (d)CMP deaminase activity reported by and el Kouni and Naguib (1990) could due to the use of EDTA in their assay buffer. EDTA is a chelator of metal ions (e.g., Zn^{2+}) required for the activity of cytosine nucleoside/nucleotide deaminases. The same argument could explain the absence of (deoxy)cytidine and cytosine deaminases. However, the *S. mansoni* genome does not codify for these two enzymes (Berriman et al., 2009). Hence, it will be maintained that *S. mansoni* lack both (deoxy)cytidine and cytosine deaminases until proven otherwise..

Cytidine and deoxycytidine also are not cleaved by uridine phosphorylase in *S. mansoni* (el Kouni et al., 1988; Naguib and el Kouni, 2014). Therefore, cytidine and deoxycytidine appear to be exclusively incorporated into their respective nucleotides. The absence of cytidine deaminase and phosphorylase, along with the fact that cytidine is the most efficiently utilized pyrimidine by schistosomes (el Kouni and Naguib, 1990) can be manipulated for chemotherapy. Various available (deoxy)cytidine analogues may be used as antischistosomal drugs. These compounds could be phosphorylated efficiently by the parasites specific cytidine kinase or the non-specific deoxyriboside kinase (Naguib and el Kouni, 2014) without being degraded by sequential reaction of cytidine deaminase and uridine phosphorylase as is the case in the host. Deamination of cytosine nucleoside analogues is the major obstacle in their use in cancer chemotherapy. Cytidine deaminase is found in the post-infective L3 stage of the filarial nematode, *Brugia pahangi* (Anant et al., 1997). It was also shown that *Plasmodium falciparum* (Reyes et al., 1982)*, Tritrichomonas foetus* (Wang et al., 1983)*, Crithidia fasciculata* (Kidder, 1984; Hassan and Coombs, 1986*)*), *Trypanosoma cruzi* (Kidder, 1984), *Trichomonas vaginalis* (Wang and Cheng, 1984a), *Giardia intestinalis* (Aldritt et al., 1985), *Angiostrongylus cantonensis* (So et al., 1992), and *Toxoplasma gondii* (Iltzsch, 1993) can deaminate cytidine but not cytosine. Similar results were reported for amastigotes and cultured promastigotes of *Leishmania mexicana mexicana*, cultured promastigotes of *L. m. amazonensis, L. donovani*, *L. tarentolae,* culture forms of *Crithidia fasciculata*, *Herpetomonas muscarum muscarum*, *H. m. ingenoplastis,* and procyclic trypomastigotes of *Trypanosoma brucei brucei*, (Hassan and Coombs, 1986). However, deamination of deoxycytidine was observed only in *Crithidia fasciculata,* and cultured promastigotes of *Leishmania mexicana mexicana* (Hassan and Coombs, 1986). Although, *Trypanosoma brucei* has cytidine deaminase (Leija et al., 2016), it lacks dCMP deaminase (Castillo-Acosta et al., 2013). Knockdown of cytidine deaminase in *T. brucei* leads to thymidine/deoxyuridine auxotrophy and cell death despite the fact that the parasite does not require exogenous pyrimidines for growth (Leija et al., 2016).

6.7. Orotate/uracil phosphoribosyltransferase (EC 2.4.2.-)

As mentioned above (*2.3.*), schistosomes have two phosphoribosyltransferases (Iltzsch et al., 1984). The first is an orotate-specific phosphoribosyltransferase. The second is a non-specific orotate/uracil phosphoribosyltransferase that utilizes orotate, uracil, and 5-fluorouracil as substrates (Iltzsch et al., 1984; el Kouni and Naguib, 1990). Its K_m for orotate (2.7 uM) (Iltzsch et al., 1984) is like those of similar enzymes from other parasites (O'Sullivan and Ketley, 1980; Asai et al., 1983; Hammond and Gutteridge, 1982 and 1984), and mammalian cells (Kasbekar et al., 1964; Kessel et al., 1972; Kavipurapu and Jones, 1976; Levinson et al., 1979; Krooth et al., 1979). The K_m values for uracil (111 μM) (Iltzsch et al., 1984) is low enough such that uracil may indeed be a substrate for the schistosomal enzyme under physiological conditions. In contrast, the K_m values of uracil for the mammalian enzymes (≃6500 μM) are so high that uracil would not serve as a substrate under physiological conditions, particularly in the presence of the strongly competing substrate orotate ($K_m \leq 1$ μM). (Kessel et al., 1972; Iltzsch et al., 1984).

Therefore, the orotate/uracil phosphoribosyltransferase reaction could be the principal pathway for the conversion of uracil to UMP in schistosomes because of its relatively higher activity with uracil as a substrate (92 ρmol/min/mg protein) (el Kouni and, Naguib 1990) over those of uridine phosphorylase (synthetic direction = 38 ρmol/min/mg protein) (el Kouni et al., 1988) and uridine kinase (16 ρmol/min/mg protein) (el Kouni and, Naguib 1990). The metabolism of uridine to UMP may also proceed via the same pathway for the same reasons. Uridine phosphorylase (catabolic direction = 118 ρmol/min/mg protein) and orotate/uracil phosphoribosyltransferase (92 ρmol/min/mg protein) activities are higher than that of uridine kinase (16 ρmol/min/mg protein) (el Kouni and Naguib, 1990) which is a tightly regulated and strongly inhibited by its substrate (Naguib and el Kouni, 2014). Therefore, it is probable that uridine salvage proceeds by the indirect route via the sequential activities of uridine phosphorylase and orotate/uracil phosphoribosyltransferases as shown in Fig. 6. This contention may also be supported by the similar pattern of incorporation of radioactive uracil and uridine into the nucleotide pool of this parasite (el Kouni and Naguib, 1990). In the final analysis the predominance of one pathway over the other for the salvage of uracil may depend on the efficiency (V_{max}/K_m) of the various enzymes as well as the availability and intracellular concentrations of the co-factors. Thus, unlike mammalian cells, phosphoribosyltransferase activities in *S. mansoni* may play a role in both de novo UMP biosynthesis as well as in the salvage of uracil and uridine. A similar situation seems to exist in the nematode *Angiostrongylus cantonensis* (So et al., 1992).

The results of Iltzsch et al. (1984) clearly indicate that the schistosomal orotate/uracil phosphoribosyltransferase differs significantly from the mammalian enzymes in inhibitor specificities. The ratios of apparent K_i values between mouse liver and *S. mansoni* differ considerably for various compounds (Table 5). These differences in inhibition constants of various drugs between the *S. mansoni* and mouse liver enzymes, as well as the difference in orotate metabolism between the two species suggest that this enzyme might be a good target for chemotherapy. In this respect, 5-azaorotic acid may be a potential antischistosomal agent, since its apparent K_i value for the host (mouse) enzyme is 50-fold higher than for the parasite enzyme (Table 5). Furthermore, both orotate/uracil phosphoribosyltransferase and

the specific orotate phosphoribosyltransferase are inhibited by 5-azaorotic acid (Iltzsch et al., 1984). Therefore, inhibitors of phosphoribosyltransferases of orotate in schistosomes (e.g., 5-azaorotate) should be selectively toxic against the parasite by blocking the synthesis of UMP by both the salvage and the de novo pathways. Even when an inhibitor blocks orotate phosphoribosyltransferases of both the host and the parasite, selective toxicity can still be achieved by co-administration of uridine. As mentioned above (*6.2.*), *S. mansoni* uridine kinase is a tightly regulated enzyme and is strongly inhibited by its substrate (Naguib and el Kouni, 2014). Therefore, the high doses of uridine required to alleviate the inhibition by 5-azaorotate will inhibit the *S. mansoni*, but not the host, uridine kinase, allowing the host to salvage uridine via the uridine kinase pathway and overcome the block of de novo UMP biosynthesis. Alternatively, co-administration of 5-benzylacyclouridine with 5-azaorotate may rescue the host, but not the parasite, from the possible toxicity of orotate phosphoribosyltransferase inhibitors. 5-Benzylacyclouridine is a potent inhibitor of uridine phosphorylase from various species (Niedzwicki et al., 1982; Park et al., 1986; Naguib et al., 1987; el Kouni et al., 1988; el Kouni et al., 1996), and can alleviate the inhibition of de novo UMP biosynthesis by increasing the plasma levels and salvage of uridine only in the host (Monks et al., 1983; Darnowski and, Handschumacher, 1985; Martin, et al., 1989; Davis et al., 1993; Sommadossi et al., 1995) to overcome pyrimidine starvation. Furthermore, 5-benzylacyclouridine may act synergistically with 5-azaorotate on schistosomes by inhibiting the salvage of uridine by the worms via uridine phosphorylase.

The non-specific *S. mansoni* orotate/uracil phosphoribosyltransferase is different from the specific uracil phosphoribosyltransferase (EC 2.4.2.9) reported in several parasites that cannot synthesize pyrimidine de novo such *Giardia intestinalis* (Lindmark and Jarroll,1982; Aldritt et al., 1985; Jarroll et al., 1989), *Tritrichomonas foetus* (Wang et al., 1983; Hassan and Coombs, 1988), and *Cryptosporidium* (Striepen et al., 2004) or the parasites that lack uridine kinase like *Toxoplasma gondii* (Pfefferkorn, 1978; Iltzsch, 1993), *Plasmodium falciparum* (Reyes et al., 1982); culture promastigote form of *Leishmania mexicana amazonensis*, the blood trypomastigote and the culture epimastigote form of *Trypanosoma brucei*, blood trypomastigote and intracellular amastigote forms of *T. cruz*i (Hammond and Gutteridge, 1982), *Giardia intestinalis* (Aldritt et al., 1985; Vitti et al., 1987), *Tritrichomonas foetus* (Wang et al., 1983), and *Leishmania donovani* (Wilson et al., 2012). In these parasites UMP synthesis from uridine salvage occurs via the phosphoribosyltransferase reaction (Fig. 6). It should also be mentioned that uracil phosphoribosyltransferase activity in *Leishmania mexicana* and *Trypanosoma brucei*, as well as the epimastigote and trypomastigote forms of *T. cruzi,* is a cytoplasmic enzyme and is quite distinct from the glycosomal orotate phosphoribosyltransferase (Hammond and Gutteridge, 1982). The specific uracil phosphoribosyltransferase was purified from *Giardia intestinalis* and was found to be a dimer. GTP and dGTP caused a dramatic increase in enzyme activity but not from other eukaryotes (Dai et al., 1995). Genomic analysis indicated that *Cryptosporidium parvum* has two different uracil phosphoribosyltransferases with one fused with a uridine-cytidine kinase (Striepen et al., 2004). Such a bifunctional uracil phosphoribosyltransferase/uridine-cytidine kinase enzyme has not been previously reported in any parasite or mammalian system.

Pyrimidine auxotrophs of *Toxoplasma gondii* (Fox and Bzik, 2002), *Leishmania donovani* (Wilson et al., 2011), *Trypanosoma cruzi* (Hashimoto el al., 2012), and *T. brucei* (Ali et al.,

2013b; Ong et al., 2013) can grow on medium supplemented with uracil or uridine with a marked preference for uracil. However, such auxotrophs exhibit hypersensitivity to uracil doses which does not affect wild type parasites. Furthermore, that hypersensitivity to uracil is not observed with any of the ribonucleosides (Ali et al., 2013b; Ong et al., 2013). This could be due to substrate inhibition of uracil phosphoribosyltransferase. The enzyme from *Leishmania donovani* is substrate inhibited (Soysa et al., 2013).

7. Pyrimidine analogues as potential antischistosomal drugs

The literature on the effects of pyrimidine nucleoside analogues in other organisms is voluminous. The majority of these compounds have been studied with regard to their therapeutic and toxic effects. Nevertheless, very few systematic structure activity relationship studies of pyrimidine analogues as ligands of pyrimidine metabolizing enzymes in parasites have been performed, e.g., schistosomes (Iltzsch et al., 1984; el Kouni et al., 1988) as well as toxoplasma (Iltzsch and Klenk, 1993; Iltzsch and Tankersley, 1994; el Kouni et al., 1996; Javid et al., 1999) and some potential chemotherapeutic agents were identified.

Pyrimidine analogues were used sporadically by a number of workers on parasites. With the exception of 5-fluorinated pyrimidines (Jaffe, 1975; Pfefferkorn and Pfefferkorn, 1977; Howells et al., 1981; Gómez, and Rathod, 1990; Papageorgiou et al., 2004; Arakaki et al., 2008; Ali et al., 2013a and 2013b; Alzahrani et al., 2016a), and carboxyemimycin (1,6-dihydro-6-oxo-2-pyrazinecarboxylic acid 4-oxide) (Matsuno et al., 1984) none of the pyrimidine analogues tested were found to be effective against parasites. These results, however, should not discourage the testing of other and newer pyrimidine analogues as antiparasitic agents. Recently, it was shown that analogues of 5′-norcarbocyclic uridine displayed antiparasitic activity in the low to mid-micromolar range against protozoan parasites, specifically the major pathogens *Leishmania mexicana* and *Trypanosoma brucei* (Alzahrani et al., 2016b). It is interesting to note that although these 5′-norcarbocyclic nucleoside are pyrimidine analogues they do not act directly on enzymes of pyrimidine metabolism (Alzahrani et al., 2016b), but more likely on S-adenosylhomocysteine hydrolase (EC 3.3.1.1), an enzyme involved in many biological methylation reactions. The chemical structures of these effective antiparasitic pyrimidine analogues are shown in Fig. 3.

In general and in spite of the fact that pyrimidine metabolism in parasites has not been studied with the same degree of thoroughness as has their purine metabolism, the information discussed in this review indicates that parasites are versatile in their ability to synthesize pyrimidine nucleotides. In addition, significant differences exist between different parasites, even among members of the same subgroup with respect to the efficiency of pyrimidine de novo and salvage pathways as well as to the types of enzymes involved in pyrimidine metabolism. These differences may have evolved depending on the availability and accessibility of nutrients in the particular environment in which each parasite developed.

In schistosomes, the presence of a phosphoribosyltransferase that can utilize uracil under physiological conditions (Iltzsch et al., 1984), the presence of a distinctive cytidine kinase (Naguib and el Kouni, 2014), the active metabolism of cytidine, absence of deamination, and

lack of phosphorolysis of cytidine derivatives (el Kouni et al., 1988; el Kouni and Naguib, 1990), raises the possibility of using cytidine analogues for the selective treatment of schistosomiasis. Deamination of cytidine analogues is the major obstacle in their use in cancer chemotherapy. Hence the use of pyrimidine analogues as antiparasitic agents in general, and antischistosomal agents in particular remains feasible. Some of the most potent pyrimidine analogues have been synthesized and studied in this laboratory. Acyclouridine and its congeners, the most potent uridine phosphorylase inhibitors (Niedzwicki et al., 1981 and 1982; Naguib et al., 1987 and 1993; el Kouni et al., 1988 and 2000; Al Safarjalani et al., 2005), are potential antischistosomal agents. These compounds have little toxicity to the host, but they may be quite deleterious to parasites which are dependent, or can be made dependent, on the salvage of preformed pyrimidines for their survival. The recent encouraging results with 5′-norcarbocyclic uridines in protozoa (Alzahrani et al., 2016b) tempts the testing of these compound on schistosomes and other parasites. Furthermore, if antischistosomal activity is found in any of the toxic pyrimidine nucleoside analogues, it may still be administered safely. We have demonstrated that even a very toxic nucleoside (e.g., tubercidin) may be used safely as an antischistosomal drug by coadministration of a nucleoside transport inhibitor (el Kouni et al., 1983a, 1985, 1987 and 1989; el Kouni 1991).

8. Conclusions and prospects

It is quite clear that parasites differ from their host in various aspects of pyrimidine metabolism including: substrate specificity of various enzymes, the presence of special enzymes exclusively in the parasites but not the host, nature and type of pyrimidine nucleobase and nucleoside transport, insensitivity of nucleoside transport to inhibitors of mammalian nucleoside transport, etc. It is precisely these marked differences in pyrimidine metabolism between parasites and the host that make pyrimidine metabolism a potential and logical target for antiparasitic chemotherapeutic interventions. However, optimism in this endeavor should be accompanied by caution. In certain situations, multiple independent pathways as well as transport systems for pyrimidine metabolism in parasites may exist. In this case, the design of new antiparasitic chemotherapeutic regimens aimed at blocking pyrimidine metabolism would require inhibiting all these alternative pathways. Therefore, to continue to exploit pyrimidine metabolism in schistosomes and other parasites as a tool of a rational approach to chemotherapy, all the major steps in pyrimidine metabolism by a parasite must first be identified and each subjected to thorough structure-function analysis. An inhibitor against each of these steps must be identified and two or more of these inhibitors should be combined for an effective chemotherapy against the parasite. Indeed, strategies aimed at interfering with pyrimidine metabolism in parasites should be directed towards attacking two or more independent pathways to successfully overcome the ingenuity of the parasites in evading blockage of their pyrimidine metabolism by a single agent.

A final note, this review emphasizes that the ideal drug target is a protein that is essential for the parasite and does not have homologues in the host. The entry of parasitology into the post-genomic age raises hopes for the identification of such novel kinds of drug targets and in turn, new treatments for parasitic diseases. However powerful this functional genomics approach, it will miss some of the attractive targets for the chemotherapy of parasites as

many essential proteins tend to be more highly conserved between species than non-essential ones.

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Abbreviations

AMP	adenosine 5′-monophosphate
ADP	adenosine 5′-diphosphate
ATP	adenosine 5′-triphosphate
CMP	cytidine 5′-monophosphate
CDP	cytidine 5′-diphosphate
CTP	cytidine 5′-triphosphate
dCMP	2′-deoxycytidine 5′-monophosphate
dCTP	2′-deoxycytidine 5′-triphosphate
dTMP	thymidine 5′-monophosphate
dTDP	thymidine 5′-diphosphate
dTTP	thymidine 5′-triphosphate
dUMP	2′-deoxyuridine 5′-monophosphate
dUDP	2′-deoxyuridine 5′-diphosphate
dUTP	2′-deoxyuridine 5′-triphosphate
dUTPase	deoxyuridine 5′-triphosphate pyrophosphatase
FdUMP	5-fluorodeoxyuridine 5′-monophosphate
IC_{50}	concentration for 50% inhibition
NBMPR	nitrobenzylthioinosine
OMP	orotidine 5′-monophosphate
UMP	uridine 5′-monophosphate
UTP	uridine 5′-triphosphate

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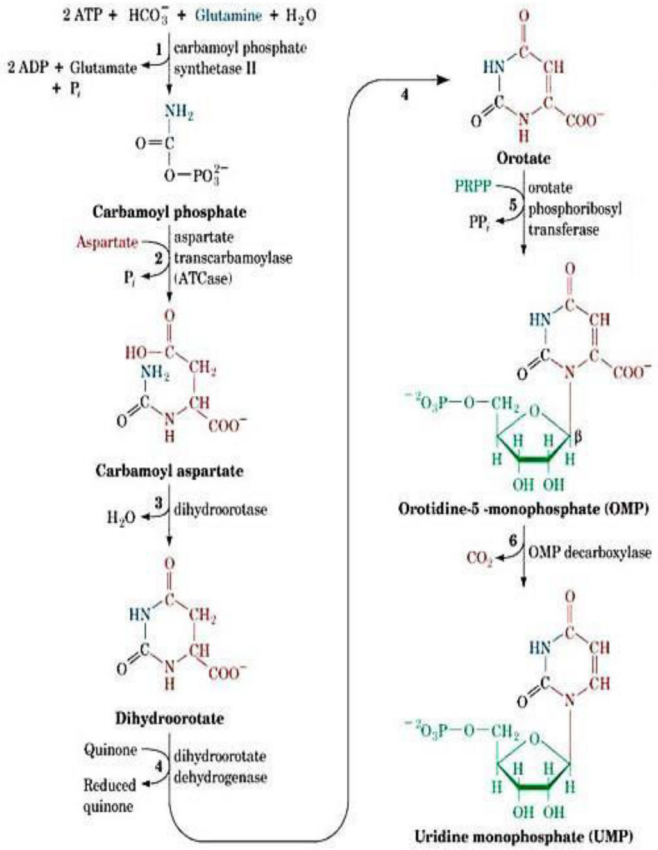

Fig. 1.
Pyrimidine de novo pathway (After Voet and Voet, 2004).

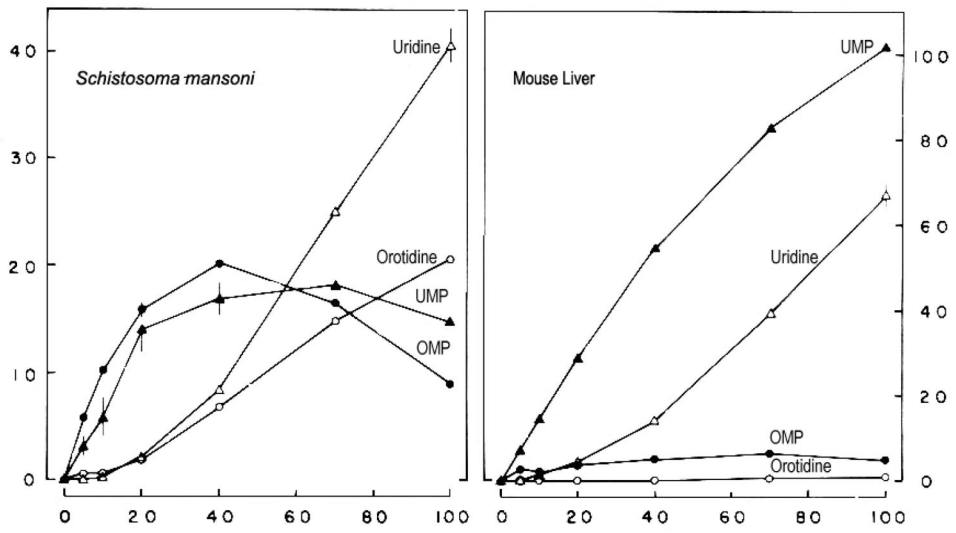

Fig. 2.

Time course of orotate metabolism by extracts of *Schistosoma mansoni* (21.8 mg protein/mL) or mouse liver (8.3 mg protein/ml). The amount of extract used was 325 μL for *Schistosoma mansoni* and 375 μL for mouse liver. (●) OMP; (▲) UMP; (○) orotidine; (△) uridine. (After Iltzsch et al., 1984)

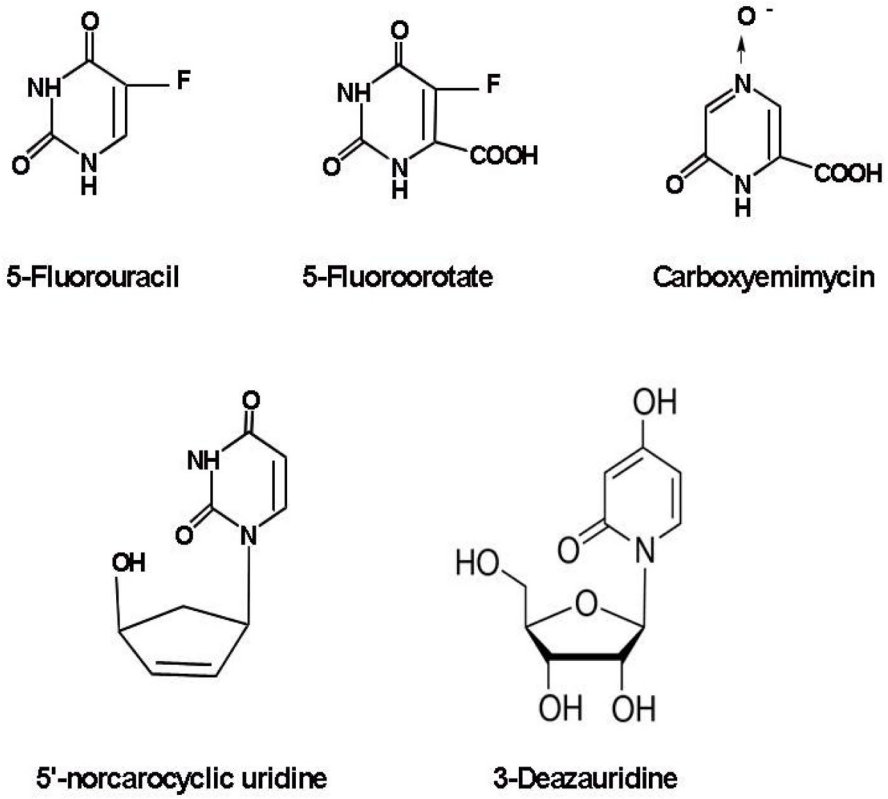

Fig. 3.
Chemical structures of various pyrimidine analogues that exhibited antiparasitic activity.

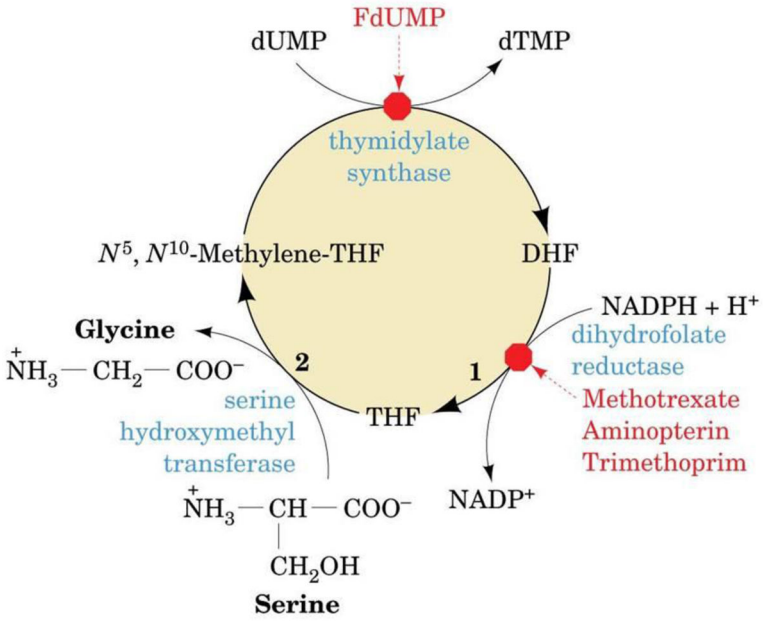

Fig. 4.

The sequential reactions and metabolic relationships of dihydrofolate reductase and thymidylate synthase for the cycle of dTMP synthesis. DHF, dihydrofolate; THF, tetrahydrofolate. (After Voet and Voet, 2004).

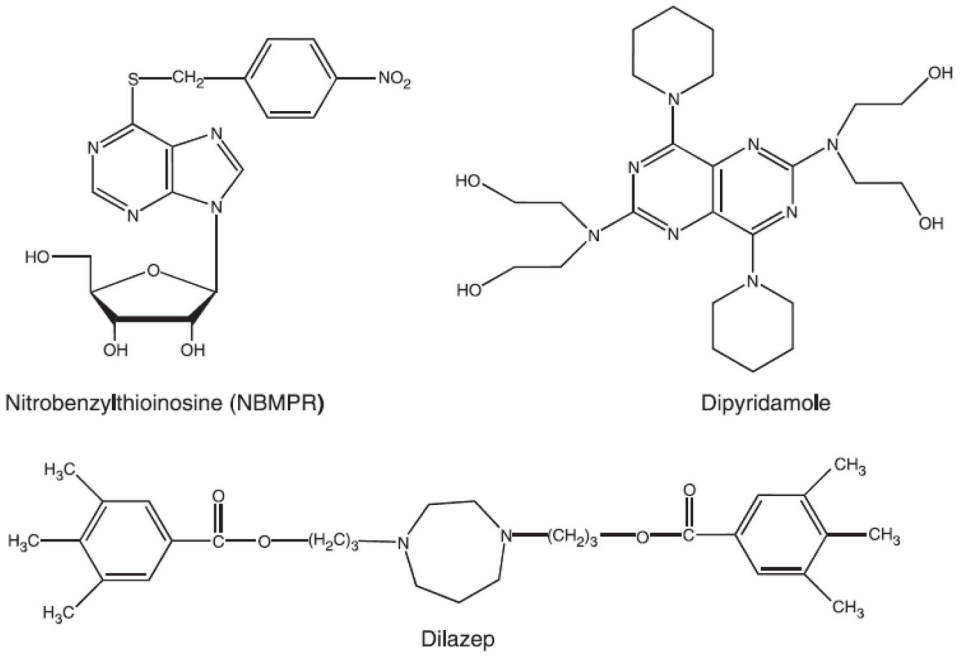

Fig. 5.
Chemical structures of 6-nitrobenzylthioinosine (NBMPR), dipyridamole and dilazep.

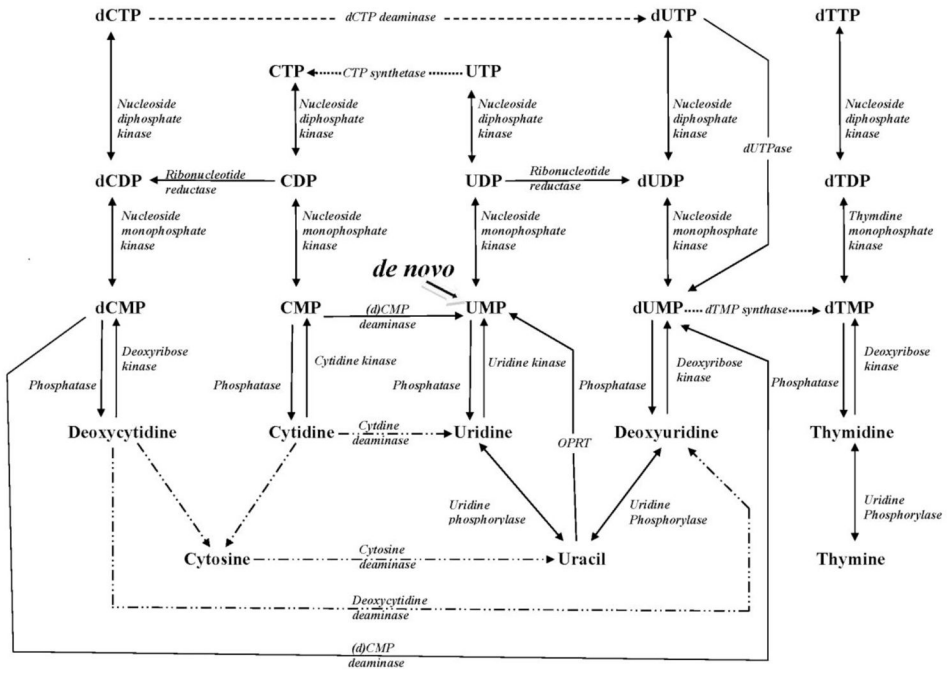

Fig. 6.

Pyrimidine salvage pathways as gathered from various organisms. In *Schistosoma mansoni,* solid line, reactions that have been established enzymatically or by genome analysis; dashed lines, reactions that have not been studied yet; dotted lines, reactions that are suggested to exist as inferred from metabolic studies; dotted/dashed lines, reactions that could not be established.

Table 1

Incorporation of radiolabeled pyrimidine nucleobases and nucleosides into nucleic acids of *Schistosoma mansoni*

Compound (45 μM)	Specific activity (Ci/mol)	Incorporation (pmol/10 worm pairs)	Ratio
Orotate	56.2	18.4	1
Uracil	55.2	69.3	3.8
Cytosine	4.6	-[a]	-
Thymine	55.3	-[a]	-
Orotidine	50.0	-[a]	-
Uridine	56.0	56.3	3.1
Cytidine	51.0	210	11
Thymidine	56.0	19.4	1.1
Deoxycytidine	56.0	18.9	1.0

[a]Below the sensitivity of the assay (<1 pmol/10 worm pairs). (After el Kouni and Naguib, 1990)

Table 2

Alkaline phosphatase activities (μmol/min) in various fractions of *Schistosoma mansoni* towards different substrates

	30,000 × g		105,000 × g		
Substrate (1 mM)	**Homogenate (39.61 mg)[a]**	**Supernatant (17.75 mg)**	**Pellet (19.35 mg)**	**Cytosol (12.40 mg)**	**Microsomes (2.59 mg)**
CMP	1.90 ± 0.15(100)[b]	0.26 ± 0.09(100)	1.12 ± 0.19(100)	0.10 ± 0.03(100)	0.12 ± 0.02(100)
UMP	1.79 ± 0.15(94)	0.25 ± 0.09(93)	1.19 ± 0.00(106)	0.06 ± 0.01(60)	0.12 ± 0.03(104)
OMP	1.50 ± 0.14(79)	0.22 ± 0.07(83)	0.09 ± 0.03(80)	0.08 ± 0.03(80)	0.10 ± 0.02(88)
dTMP	1.71 ± 0.07(90)	0.22 ± 0.09(84)	1.04 ± 0.15(93)	0.09 ± 0.03(90)	0.10 ± 0.02(84)
dCMP	1.77 ± 0.25(93)	0.24 ± 0.08(89)	1.10 ± 0.10(98)	0.08 ± 0.03(85)	0.12 ± 0.03(100)
dUMP	1.78 ± 0.32(94)	0.25 ± 0.08(95)	1.16 ± 0.10(103)	0.10 ± 0.03(99)	0.12 ± 0.03(99)
β-Glycerophosphate	1.45 ± 0.20(76)	0.20 ± 0.06(75)	1.08 ± 0.08(96)	0.09 ± 0.02(91)	0.11 ± 0.01(92)

[a] Total amount of protein from 10.8 mL of packed worms.

[b] Percent activity relative to that towards CMP in parenthesis. (After el Kouni and Naguib, 1990)

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Table 3

Kinetic Parameters of *S. mansoni* Various Nucleoside Kinases.

[^{14}C]Substrate	K_m ± S.E.		V_{max} ± S.E. (pmol/min/mg)	Competing Nucleoside	K_{is} ± S.E. (μM)
	Nucleoside (μM)	ATP (mM)			
Uridine	83[a]			Cytidine	-[b]
Cytidine	73 ± 13	2.1 ± 0.01	43 ± 1.3	Uridine	-[b]
Thymidine	5.2 ± 0.001	2.8 ± 0.001	24 ± 0.2	Cytidine	-[b]
				Uridine	-[b]
				2′-Deoxyadenosine	54 ± 8.2
				2′-Deoxyguanosine	61 ± 12
				Adenosine	-[b]
2′-Deoxyuridine	54 ± 0.06	13 ± 0.008	52 ± 1.0	Thymidine	6.3 ± 1.2
				2′-Deoxycytidine	21 ± 6.4
				Cytidine	-[b]
				Uridine	-[b]
2′-Deoxycytidine	13 ± 0.005	2.7 ± 0.001	26 ± 0.2	Thymidine	4.4 ± 0.9
				Cytidine	-[b]
				Uridine	-[b]

[a] K_m value was estimated from $V_{0.5}$ value because of the allosteric nature of the enzyme.

[b] No competition. (After Naguib and el Kouni, 2014)

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Table 4

Apparent K_i values for various inhibitors of uridine phosphorylase activity in *Schistosoma mansoni* and mouse liver.

Compound	Apparent K_i (μM) ± S.E.E.[a]		Ratio[b]
	S. mansoni	Mouse	
5-Benzylacyclouridine	5.2 ± 0.20	3.12 ± 0.21	0.60
5-(Benzyloxybenzyl)acyclouridine	4.1 ± 0.25	1.25 ± 0.10	0.30
5-(Benzyloxybenzyloxybenzyl)acyclouridine	3.5 ± 0.23	519 ± 34	148

[a] Standard error of estimation,

[b] Ratio of mouse liver apparent K_i to S. mansoni apparent K_i (data from el Kouni et al, 1988).

Table 5

Apparent K_i values for selected pyrimidine nucleobase analogues as inhibitors of phosphoribosyltransferase activity towards 5-fluorouracil (100 μM). in cytosol extracts of *Schistosoma mansoni* and mouse liver

Compound	Apparent K_i (μM) ± S.E[a]		Ratio[b]
	S. mansoni	**Mouse Liver**	
5-Azaorotic acid (Oxonic acid)	0.9 ± 0.2	45 ± 16	50
Barbituric acid	35 ± 4	23 ± 3	0.66
5-Azabarbituric acid (Cyanuric acid)	392 ± 59	212 ± 34	0.54
6-Carboxymethyluracil	539 ± 86	156 ± 28	0.29
4,6-Dihydroxypyrimidine	8090 ± 1220	15 ± 1	0.002

[a] S.E. = standard error,

[b] Ratio of mouse liver apparent K_i to *S. mansoni* apparent K_i. (data from Iltzsch et al., 1984).