Original Article

Anti-trypanosomal Activity of Potential Inhibitors of Trypanosoma Brucei Glycolytic Pathway Enzymes Selected by Docking Studies

C. Musanabaganwa[1*], F. Byiringiro[2]

[1] *Rwanda Biomedical Center, Medical Research Center, Kigali, Rwanda*
[2] *Rwanda Neurosurgery Center, Kigali University Teaching Hospital, Rwanda*

ABSTRACT

Human African trypanosomiasis (HAT), a potentially fatal protozoan infection caused by tsetse-fly mediated transmission of Trypanosoma brucei (T. Brucei), is largely recognized as a neglected disease. The repertoire of drugs that is effective against the infection is limited and all drugs have several drawbacks including high level of toxicity, difficult administration regimens, and the resurgence of resistance. At present the biology of the parasite is well studied and a number of technologies are now available which can aid in the identification of potential drug targets. This review identifies putative inhibitors of trypanosomal glycolytic enzymes.

Keywords: Human African trypanosomiasis - Trypanosoma brucei - glycolytic pathway enzymes

RESUME

La Trypanosomiase humaine africaine est une zoonose potentiellement mortelle mais tres largement méconnue et causée par Trypanosoma Brucei (T. Brucei). Le schéma thérapeutique est très limité; et parmi eux le plus efficace suscite beaucoup d'effets secondaires avec une toxicité remarquablement élevée, d'où une mauvaise observance; et par conséquent l'apparition de plusieurs résistances de la maladie au traitement. Actuellement, la biologie et la pathogénie du parasite sont connues; et les plus récentes innovations thérapeutiques facilitent l'identification des molécules plus spécifiques contre le germe. La présente revue littéraire mets en évidence les inhibiteurs putatives des enzymes glycolytiques trypanosomiales.

Mots-clés: Trypanosomiase humaine africaine - Trypanosoma brucei - Voie des enzymes glycolytiques

INTRODUCTION

Human African trypanosomiasis (HAT), also known as African sleeping sickness is a potentially fatal condition caused by Tsetse-fly mediated transmission of Trypanosoma brucei (T. Brucei) [1, 2]. HAT pathology is characterized by an initial hemolymphatic stage characterized by mild symptoms including fever, malaise, headache, weight loss, arthralgia, and pruritus. Late chronic stage of the disease is characterized by neurological involvement with attendant symptoms including severe headaches, nocturnal insomnia, mental and psychological disturbances, coma, and death if untreated3.

The disease is endemic in specific geographical foci in sub-Saharan Africa where the vector tsetse fly and the parasite subspecies – T. b. gambiense (West Africa) and T. b. rhodesiense (East Africa) are present [4, 3]. It is estimated that between 50,000 and 70,000 people living in sub-Saharan Africa are affected by HAT, with an additional 60 million potentially at risk [4, 5]. The World Health Organization also estimates that 30,000 new cases are recorded in the Sub-Saharan Africa annually and a morality rate of 8000 [4]. In East Africa, the disease is endemic in Uganda, Kenya (western and Nyanza province), and Tanzania [6]. In Rwanda, no cases have been reported [7] - a surprising fact given its proximity to Democratic Republic of Congo, a high endemic region. The related disease in cattle, cattle trypanosomiasis or Nagana, caused by T.b. brucei, T. congolense and T. evansi also represents a major health concern due to its significant health and economic impact on African families (according to WHO, the annual economic loss associated with the disease is estimated at approximately US $ 4 billion) [8]. Analyzed in terms of disability-adjusted life years (DALY), it has been argued that the total burden of trypanosomiasis (DALY=1598000) is on a par with known diseases such as malaria and tuberculosis [8].

Successful control and management of HAT is dependent on early case detection and treatment of those diagnosed positive [9]. Early case detection of HAT disease is via parasitological tests: microscopic examination of both the venous blood (using the haematocrit method) [10] and lymph node aspirates. The stage of the disease is determined in accordance with the recommendations by World Health Organization [11] where cerebral spinal fluid (CSF) is collected via a lumbar puncture and examined for the presence of trypanosomes and the number of white cells. However, current treatment options are complicated by many factors.

Pentamidine and Suramin, indicated for early hemolymphatic stage disease for infections associated with T. b. gambiense and T. b. rhodesiense subspecies respectively have several drawbacks including high toxicity level, difficult administration regimens and emerging drug resistance [8, 9]. In late stage disease, where the parasite cross the blood brain barrier (BBB) and invade the central nervous system (CNS), the treatments options are limited to melarsoprol, eflornithine ornithine decarboxylase inhibitor (difluoromethylornithine - DFMO) and the recently introduced nifurtimox/eflornithine combination therapy (NECT) [12,9]. The arsenical melarsoprol is extremely toxic and causes encephalopathy resulting in the death

* *Correspondence to:* *Clarisse MUSANABAGANWA*
RBC/MRC, B.P 7162 Kigali-Rwanda
E-mail: musaclasse2007@yahoo.fr
Tel: +(250) 784010813

of 1 out of every 20 recipients and is associated with a treatment failure rate of about 30% in some places [13]. Eflornithine is ineffective against the T. b. rhodesiense subspecies8 and has unpleasant side effect including seizures, fever, and infections, although NECT alleviates some of these adverse effects [14].

At present, vaccination is not an option for the control of T. brucei. This is because the parasite undergoes antigenic variation by altering its surface glycoprotein coat - variable surface antigen (VSA), thereby evading the immune system [15, 43].

As drugs that target infections prevalent in the developing world are largely unprofitable, the development of new therapeutic agents for these diseases has largely been neglected by pharmaceutical companies [3]. Of the drugs listed above, for instance, one, DFMO and to some extent NECT, have been developed since the late 1940s [3]. Admittedly, and courtesy of renewed effort of public private partnerships (PPP), there has been some renewed focus on the need to develop new drugs for HAT. The first success of this effort was the development of NECT [6]. In addition, the dimethoxyamidine prodrug pafuramidine (DB289), a pentamidine-like compound developed by the Consortium for Parasitic Drug Development (CPDD), became the first oral drug to enter phase III clinical trials for 1st stage HAT [6]. However, development of the drug was terminated due to high incidence of renal toxicity, a finding which was demonstrated after an extended phase I clinical trial [15]. At the same time, recent research on plant phytocompounds, and identification of novel drugs through whole cell assays, while showing some promise, has not yielded much [16, 17, 42].

In light of the foregoing, there is an argent need to develop new anti-trypanosomal drugs. To be ideal, and according to WHO, the new drugs should have the following requirements: It should achieve complete parasitological cure in both early and late stages of the disease; should be affordable, should be effective in single or few doses; should be easy to administer and should have no/or minimal adverse effects (collateral or teratogenic effects) [8].

The current drug pipeline is not only expensive but is also time-consuming since it takes a lot of time from lead identification to pre-market approval. It also involves use of experimental techniques that are laborious to use, are too costly, and which are unable to generate high order information. Bioinformatics or in silico approaches help to shorten the lead to market time, are less expensive, and help to reduce the failure rate of drugs since they help to identify only those targets and leads that have a high chance of succeeding.

DISCUSSION

PUTATIVE TARGETS OF TRYPANOSOMA BRUCEI

Older antimicrobial identification strategies that were successful at the dawn of the antibiotic age have not yielded additional useful classes of compounds in recent years. As a result, novel ways to identify new therapeutic targets for the discovery or design of novel inhibitors have been sought. One aspect has been the continuing attempt to catalog the entire metabolic machinery of microbes to identify essential functions that might serve as starting points for the search for novel inhibitors. This exercise has been aided greatly by the presence of a complete and annotated genome of T. brucei. Additionally, a number of molecular biology techniques are also available to evaluate gene function and essentiality [17]. The biology of the parasite has been studied in details mainly because these organisms harbor several peculiarities [23]. For example, trypanosomatids are diploid organisms and knockouts of non-essential genes can be readily generated via homologous recombination [17]. According to the same authors, regulated conditional knockout of essential genes in the parasite is also feasible and is achieved by integration of a gene copy under the control of the tetracycline-inducible system prior to knockout of the endogenous alleles.

Using these methods, putative targets have been identified in a number of metabolic pathways.

Energy Metabolism

The T. brucei bloodstream form (BSF) is entirely dependent on host glucose for as a source of carbon and for energy generation via the glycolytic pathway – fructose, mannose and glycerol can also be metabolized [23]. Whereas in most eukaryotic cells the glycolytic pathway occurs in the cytosol, in the trypanosome the first seven enzymes of the glycolytic pathway are localized sub-cellular compartment related to plant peroxisomes named glycosomes17, 23. Because of their localization in the glycosome; these enzymes share limited homology with host – enzymes making them good drug candidates [23, 24, 25]. At present, all the enzymes in the glycolytic pathway have been described, isolated and purified, either through classical or recombinant means, allowing a comprehensive understanding of the kinetics and flux of the pathway to be developed (figure 1) [17].

Hexokinase (TbHK1)

Hexokinase (HK) is the first enzyme of the glycolytic pathway, and catalyzes glucose phosphorylation. The enzyme has a low sequence homology (36%) with human HK which enhances the chances of developing specific inhibitors – human enzyme is also twice as large owing to possible gene duplication [23]. The genome of T. brucei contains two hexokinases (TbHK1 and TbHK2) which are nearly identical and differ primarily in the C-terminus. TbHK has also been shown to have relatively low specificity towards sugars [26] and is not regulated by glucose 6-phosphate and glucose 1, 6 -bisphosphate, like human HKs. In addition, studies have shown that the enzyme also exhibits low specificity towards nucleotides including ATP, Uridine Triphosphate (UTP) and Cytidine Triphosphate (CTP) [23]. According to these authors, the lack of specificity may render the nucleotide-binding pocket of this enzyme an interesting target for drug development. The glucose-binding pocket offers an interesting target as well. Moreover, RNAi studies have TbHK is essential for the parasites survival [27]. Indeed, these researchers demonstrated that Lodamine, an inhibitor of TbHK1 is trypanocidal.

Phosphoglucose isomerase (TbPGI) and Phosphofructokinase (TbPFK)

Phosphoglucose isomerase (TbPGI) catalyzes the isomerization of G6P into F6P while Phosphofructokinase (TbPFK) mediates a phosphate group transfer from ATP to form Fructose bisphosphate. Recently the structural characteristics of the two enzymes were been elucidated via X-ray crystallography [17, 27]. These studies have revealed unique features of the two enzymes relative to mammalian orthologs and provide opportunities for design of species-specific inhibitor [17]. The synthesis and evaluation of 5-phospho-D-arabinonate, a potent transition state analog, for TbPGI and a series of 2, 5-anydro-D-mannitol derivatives for TbPFK has been described [23,27].

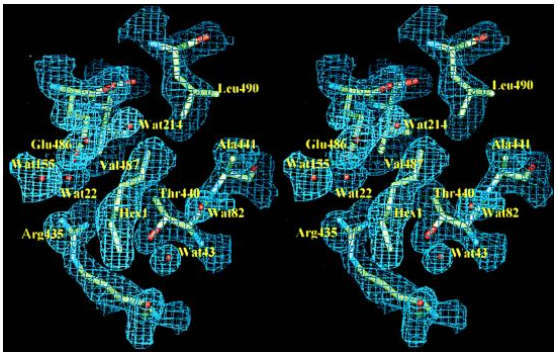

Figure 1 : Crystal structure of T. cruzi as determined by Gao et al. (1999)

Fructose-1, 6-Bisphosphate aldolase

This enzyme belongs to the class I aldolases that effects reversible aldol cleavage of fructose 1, 6 -bisphosphate via a Schiff base, into dihydroxyacetone phosphate (DHAP) and D-glyceraldehyde-3-phosphate (GA3P). Using the crystal structure of Aldolase, Chudzik and colleagues employed structure based drug design to develop inhibitors based on the 1,6-dihydroxy-2-naphthaldehyde and 2,5-dihydroxybenzaldehyde scaffolds and showed that these agents were selective for the T. brucei enzyme [28]. Similar studies were undertaken by Dax and colleagues with similar results. While phosphyorylated inhibitors did not exhibit activity against T. brucei, presumably due to poor membrane permeability, use of phosphate ester prodrugs, showed modest activity in a whole cell assays [17].

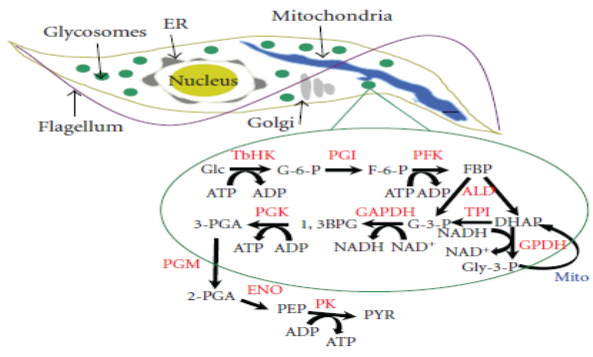

Figure 1: Glycolysis and glycosomes in the bloodstream form African trypanosome.

Abbreviations: ALD: aldolase; DHAP: dihydroxyacetone phosphate; 1,3BPGA: 1,3-bisphosphoglycerate; ENO: enolase; F-6-P: fructose-6-phosphate; FBP: fructose 1,6-bisphosphate; G-3-P: glyceraldehyde 3-phosphate; G-6-P: glucose-6-phosphate; GAPDH: glyceraldehyde-3-phosphate dehydrogenase; Glc: glucose; Gly-3-p: glycerol-3-phosphate; GPDH: glycerol 3- phosphate dehydrogenase; Mito: mitochondrial enzymes; PEP:Phosphoenolpyruvate; 2-PGA: 2-phosphoglycerate; 3-PGA: 3- phosphoglycerate; PGI: glucose-6-phosphate isomerase; PGM: phosphoglycerate mutase; PFK: phosphofructokinase; PGK: phosphoglycerate kinase; PK: pyruvate kinase; PYR: pyruvate; TbHK: T.brucei hexokinase 1 and/or 2; TPI: triose-phosphate isomerase17.

Phosphglycerate Kinase and Enolase

Phosphoglycerate kinase (TbPFK), which catalyses the conversion of 1, 3-bisphosphoglycerate to 3-phosphoglycerate, is not as well characterised as the other enzymes in the parasites glycolytic pathway. However, a study by Drew and colleagues reported that the adenosine analog tubercidin is a potential inhibitor [39]. The penultimate enzyme in the glycolytic pathway, T. brucei Enolase (TbENO), is present in the cytosol and has received little attention for this reason [17]. However, recent studies structural studies by de ASNMV and colleagues which were based on TbENO -2-fluoro-2-phosphonoacetohydroxamate complex, demonstrated the enzymes active site is flexible, a development which may allow for the design of larger inhibitors [17,30].

Polyamine and trpanothione biosynthesis

Other pathways which have yielded potential drug target include: polyamine and trpanothione biosynthesis pathways. The polyamines pathway of T. brucei which differs from that of humans is essential for growth in the parasite. As a target, the pathway has yielded at least one clinically useful drug, eflornithine. This, according to Jacobs and colleagues, is a strong indication that the pathways can be a source of additional drugs. Indeed, research on S-adenosylmethioine (AdoMet), which catalyzes the first step in polyamines biosynthesis pathway and S-adenosylmethioine decarbolyase (AdoMetD), Trypanothione reductase (TrpRed) and Trypanothione Synthase (TrpSyn) indicates that these enzymes are druggable targets [31, 32, 17].

Purine Metabolism

Due to the absence of enzymes for synthesis of purines, T. brucei depends on salvage the salvage pathways [17]. To mediate this process, T. brucei BSF utilizes a set of transporters to transports these purines from host and interconvert them into essential cellular nucleotides [33]. Programs to exploit parasite purine salvage rely on inhibition of these transporters [17] or the targeting of transporters to accumulate toxic analogues within the parasite [33]. Indeed, an in vivo and in vitro activity study by Baliani and colleagues which used melamine linked nitroheterocycles demonstrated good trypanocidal activity. However, research on these targets still requires further exploration [40].

Pteridine Metabolism

T. brucei like other trypanosomatids cannot synthesize pteridines (folate and pterins) de novo and rely on salvage from the host [34]. Pteridine salvage depends

on folate and biopterin-specific transporters (FT1 and BT1, respectively) and at least two pteridine reductases – a bifunctional dihydrofolate reductase-thymidylate synthase (DHFR-TS) specific for folate and a pteridine reductase 1 (PTR1) which reduces folate and biopterin [17]. The tow enzymes exhibits marked dissimilarity with human orthologs and have been proposed as putative drug targets given their central role in parasite metabolism [34]. According to Jacobs and colleagues, available validation data is sufficient to warrant further investigation of the two enzymes as drug targets.

DNA Topoisomerases

Like in other organisms, T. brucei DNA topoisomerases which catalyse changes in the linking number of DNA are essential for nucleic acid replication and transcription. Type I enzymes catalyze single strand breaks and type II make double stranded breaks on DNA [35]. The parasites Type I B, a subclass of Type I enzyme, has elicited considerable interest as a potential drug target. The potential of topoisomerase inhibitors including antibacterial fluoroquinolones such as KB5426, ofloxacin, and ciprofloxacin and camptothecin and non-camptothecin classes of topoisomerase inhibitors have shown trypanocidal activity [17]. However, most of the tested compounds have limited parasite selectivity. Limited structural information on these enzymes has been cited as a hindrance in this regard.

Fatty Acid Biosynthesis

The fatty acid biosynthesis pathway is critical for the life cycle of T. brucei. According to Jacobs and colleagues, the uniqueness of these pathways in the parasite may allow for the development of species-specific drugs. Recent studies which have validated several candidate proteins in these pathways have added credibility to this suggestion [36]. An important target molecule are the series of microsomal elongases which synthesize myristate, a key component in the glycosyl phosphatidylinositol (GPI) which anchors the variable surface glycoprotein (VSG) coat of the bloodstream form parasite [37]. Several mitochondrial type II fatty acid synthase and attendant carrier proteins have also been shown to be essential via RNAi approaches [38]. Smith et al provide a comprehensive review [36].

CONCLUSION

Based on the scientific report and results from clinical trials conducted on medicines of Human African Trypanosomiasis (HAT), this Scientific paper reveals the potential putative targets to focus on in the development of new drugs for the treatment of African Sleeping Sickness as it has been reported to be a burden in African population.

REFERENCES

1. Barrett MP, Boykin DW, Brun R, Tidwell RR. Human African trypanosomiasis: pharmacological re-engagement with a neglected disease. Br J Pharmacol. 2007; 152(8):1155–1171.
2. Kennedy PG. The continuing problem of human African trypanosomiasis (sleeping sickness). Ann Neurol. 2008; 64(2):116–126.
3. Durrant J. Urbaniak M, Ferguson M, McCammon (2010),
4. Computer-Aided Identification of Trypanosoma brucei Uridine Diphosphate Galactose 40-Epimerase Inhibitors: Toward the Development of Novel Therapies for African Sleeping Sickness, J. Med. Chem. 2010; 53, 5025-532.
5. WHO. Control and surveillance of African trypanosomiasis. World Health Organization Technical Report series No. 881. 2005; WHO Geneva, Switzerland.
6. Gehrig, S.; Efferth, T. (2008). Development of drug resistance in Trypanosoma brucei rhodesiense and Trypanosoma brucei gambiense. Treatment of human African trypanosomiasis with natural products (Review). Int. J. Mol. Med. 2008; 411-419.
7. Brun, R., Blum, J., Chappuis, F. and Burri, C. Human African Trypanosomiasis. The Lancet. 2009; 6736(09): 60829-1
8. Thuita JK, Wang MZ, Kagira JM, Denton CL, Paine MF, et al.. Pharmacology of DB844, an Orally Active aza Analogue of Pafuramidine, in a Monkey Model of Second Stage Human African Trypanosomiasis. PLoS Negl Trop Dis. 2012; 6(7), 212-224.
9. Zhou L, Stewart G., Rideau E., Wesatwood N., and Smith T. A Class of 50 Nitro-2-furancarboxylamides with Potent Trypanocidal Activity against Trypanosoma brucei in Vitro, J. Med. Chem. 2013; 56, 796-806.
10. Baker, N.; Alsford, S.; Horn, D. Genome-wide RNAi screens in African trypanosomes identify the nifurtimox activator NTR and the eflornithine transporter AAT6. Mol. Biochem. Parasitol.2011; 176, 55-57.
11. Kennedy PG. Human African Trypanosomiasis of the CNS: current issues and challenges. Clinical Investigation. 2004; 113: 496–504.
12. WHO. Control and surveillance of African trypanosomiasis. WHO Technical Report Series No. 881, World Health Organization, Geneva, 1998. 113.
13. Delespaux, V.; de Koning, H. P. Drugs and drug resistance in African trypanosomiasis. Drug Resistance Updates, 2007;10, 30-50.
14. Wilkinson, S. R.; Taylor, M. C.; Horn, D.; Kelly, J. M, Cheeseman, I. A mechanism for cross-resistance to nifurtimox and benznidazole in trypanosomes. Proc. Natl. Acad. Sci. U. S. A., 2008; 105, 5022−5027.
15. Priotto,G.; Kasparian, S.;Mutombo, W.;Ngouama,D.; Ghorashian, S.; Arnold, U.; Ghabri, S.; Baudin, E.; Buard, V.; Kazadi-Kyanza, S.; Ilunga, M.;Mutangala,W.; Pohlig, G.; Schmid, C.; Karunakara, U.; Torreele, E.; Kande, V. Nifurtimox-eflornithine combination therapy for second-stage African Trypanosoma brucei gambiense trypanosomiasis: a multicentre, randomised, phase III, non-inferiority trial. Lancet, 2009; 374, 56–64.
16. Paine MF, Wang MZ., Generaux CN, Boykin DW, Wilson WD et al.. Diamidines for human African trypanosomiasis. Curr Opin Invest. Drugs, 2010; 11: 876–883.
17. Barker RH Jr, Liu H, Hirth B, Celatka CA, Fitzpatrick R, Xiang Y, Willert EK, Phillips MA,Kaiser M, Bacchi CJ, Rodriguez A, Yarlett N, Klinger JD, Sybertz E. Novel Sadenosylmethionine decarboxylase inhibitors for the treatment of human African trypanosomiasis. Antimicrob Agents Chemother. 2009; 53(5):2052–2058
18. Jacobs R.T., Nare B., Phillips MA. State of the Art in African Trypanosome Drug Discovery.Curr Top Med Chem. 2011; 11(10): 1255–1274
19. Darren et al.,
20. Terstappen GC and Reggiani A. (2001). In Silico Research in Drug Discovery. Trend. Pharm. Sci.2001 22.1.
21. Liao C and Nicklous MC. Computer Tools in the discovery of HIV-1 Intergrase Inhibitors. Future Med Chem. 2010. 2 (7): 1123-1140.
Skjevik AA, Teigen K, Martinez A. Overview of Computational Tools employed in early stage drug discovery. Future Med. Chem, 2009; 1 (1):49-63.
22. Tanrikulu Y, Schneider G. Pseudoreceptor models in drug design: bridging ligand- and receptorbased virtual screening. Nat. Rev. Drug Discov. 2008; 7(8):667–677.
23. Opperdoes and Michel. Aerobic protists – typanosomatidae . In: Marr jj. Nilsen TW, Komuniecki , editors. Molecular Parasitology. London: Academic Press; 2008. p. 123- 154.
24. Verlinde CLMJ, Hannaert V, Blonski C, Willson M, Périé JJ, Fothergill-Gilmore LA, Opperdoes FR, Gelb MH, Hol WGJ, Michels PAM. Glycolysis as a target for the design of new antitrypanosome drugs. Drug Resistance Updates. 2001; 4(1):50–65.
25. Lakhdar-Ghazal F, Blonski C, Willson M, Michels P, Perie J. Glycolysis and Proteases as Targets for the Design of New Anti- Trypanosome Drugs. Curr. Topics in Med. Chem.2000; 2(5):439.
26. Chambers JW, Kearns MT, Morris MT, Morris JC. Assembly of Heterohexameric Trypanosome Hexokinases Reveals That Hexokinase 2 Is a Regulable Enzyme. J. Biol. Chem. 2008; 283(22): 14963–14970.
27. Arsenieva D, Appavu BL, Mazock GH, Jeffery CJ. Crystal structure of phosphoglucose isomerase from Trypanosoma brucei complexed with glucose-6-phosphate at 1.6 A resolution. Proteins. 2009; 74(1):72–80.
28. Chudzik DM, Michels PA, de Walque S, Hol WG. Structures of type 2 peroxisomal targeting signals in two trypanosomatid aldolases. J Mol Biol200; 300(4):697–707.
29. Dax C, Duffieux F, Chabot N, Coincon M, Sygusch J, Michels PAM, Blonski C. Selective Irreversible Inhibition of Fructose 1,6-Bisphosphate Aldolase from Trypanosoma brucei. J. Med. Chem.2006; 49(5):1499–1502.
30. Clark DE. What has computer-aided molecular design ever done for drug discovery? Expert Opin. Drug Discov. 2006; 1(2):103–110.
31. Wyllie S, Oza SL, Patterson S, Spinks D, Thompson S, Fairlamb AH. Dissecting the essentiality of the bifunctional trypanothione synthetase-amidase in Trypanosoma brucei using chemical and genetic methods. Mol Microbiol. 2009
32. Creek DJ, Anderson J, Malcolm JM, Barret M, Metabolic analysis of trypanosomid protozoa. Mol. Biochem Parasit. 2012; 181, 73-84.
33. Barrett MP, Gilbert IH. Targeting of toxic compounds to the trypanosome's interior. Adv Parasitol.; 2006. 63:125–183.
34. Nare B, Garraway LA, Vickers TJ, Beverley SM. PTR1-dependent synthesis of tetrahydrobiopterin contributes to oxidant susceptibility in the trypanosomatid protozoan parasite Leishmania major. Curr Genet. 2009; 55(3):287–299.
35. Nenortas EKT, Burri C, Shapiro TA. (2003). Antitrypanosomal activities of fluoroquinolones with pyrrolidinyl substitutions. Antimicrobial Agents and Chemotherapy. 2003; 47(9)
36. Smith TK, Butikofer P. Lipid metabolism in Trypanosoma brucei. Mol. Biochem. Parasit. 2010. 172, 66-79.
37. Lee SH, Stephens JL, Paul KS, Englund PT. Fatty acid synthesis by elongases in trypanosomes. Cell. 2006; 126(4):691–699.
38. Autio Autio KJ, Guler JL, Kastaniotis AJ, Englund PT, Hiltunen JK. The 3-hydroxyacyl-ACP dehydratase of mitochondrial fatty acid synthesis in Trypanosoma brucei. FEBS Lett; 2008. 582(5):729–733.
39. Drew ME, Morris JC, Wang Z, Wells L, Sanchez M, Landfear SM, Englund PT. The Adenosine Analog Tubercidin Inhibits Glycolysis in Trypanosoma brucei as Revealed by an RNA Interference Library. J. Biol. Chem. 2003; 278(47):46596–46600.
40. Baliani A, Bueno GJ, Stewart ML, Yardley V, Brun R, Barrett MP, Gilbert IH. Design and synthesis of a series of melamine-based nitroheterocycles with activity against Trypanosomatid parasites. J Med Chem. 2005; 48(17):5570–5579.
41. Chambers JW, Fowler ML, Morris MT, Morris JC. The anti-trypanosomal agent lonidamine inhibits Trypanosoma brucei hexokinase 1. Mol. Biochem. Parasit.2001; 158(2):202–207.
42. Kayser, O.; Kiderlen, A. F.; Croft, S. L. Natural products as antiparasitic drugs. Parasitol. Res. 2003; 90 (2), S55–S62.
43. Cross, G. A. Antigenic variation in trypanosomes: secrets surface slowly. Bioessays 1996, 18, 283–291.
44. Ellis JA, Fish WR, Sileghem M, McOdimba F. A colorimetric assay for trypanosome viability and metabolic function. Vet Parasitol. 1993, 50(1-2):143-9.