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Review

Aminoacyl tRNA Synthetases: Implications of Structural Biology in Drug Development against Trypanosomatid Parasites

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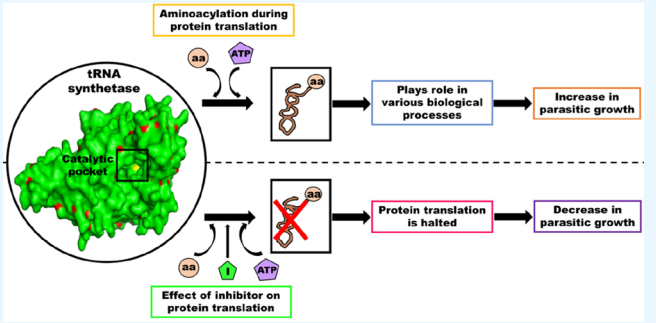
ABSTRACT: The ensemble of aminoacyl tRNA synthetases is regarded as a key component of the protein translation machinery. With the progressive increase in structure-based studies on tRNA synthetase-ligand complexes, the detailed picture of these enzymes is becoming clear. Having known their critical role in deciphering the genetic code in a living system, they have always been chosen as one of the important targets for development of antimicrobial drugs. Later on, the role of aminoacyl tRNA synthetases (aaRSs) on the survivability of trypanosomatids has also been validated. It became evident through several gene knockout studies that targeting even one of these enzymes affected parasitic growth drastically. Such successful studies have inspired researchers to search for inhibitors that could specifically target trypanosomal aaRSs, and their never-ending efforts have provided fruitful results. Taking all such studies into consideration, these macromolecules of prime importance deserve further investigation for the development of drugs that cure spectrum of infections caused by trypanosomatids. In this review, we have compiled advancements of over a decade that have taken place in the pursuit of devising drugs by using trypanosomatid aaRSs as a major target of interest. Several of these inhibitors work on an exemplary low concentration range without posing any threat to the mammalian cells which is a very critical aspect of the drug discovery process. Advancements have been made in terms of using structural biology as an important tool to analyze the architecture of the trypanosomatids aaRSs and concoction of inhibitors with augmented specificities toward their targets. Some of the inhibitors that have been tested on other parasites successfully but their efficacy has so far not been validated against these trypanosomatids have also been appended.



1. INTRODUCTION

The order Trypanosomatida comprises singly flagellated intracellular parasites that are classified under the phylum Euglenozona and class Kinetoplastea. The corkscrew-like motion present in some of its members forms the basis of its nomenclature (trypano: borer, soma: body), and they can either have a monoxenous or dixenous lifecycle. In the monoxenous mode, only one host is needed to complete their lifecycle, while in the dixenous mode, two hosts are involved. Although most of the trypanosomatids are known to follow monoxenous lifestyle by infecting insects, e.g., *Leptomonas* sp., the dixenous forms have also been reported. For instance, *Phytomonas* sp. get transmitted from phytophagous insects to plants,[1] and *Leishmania* sp. spread by hematophagous insects such as sand flies to vertebrates.[2] When it comes to humans, there are three majorly known diseases caused by trypanosomatids, viz. leishmaniasis, known to be caused by *Leishmania* sp., African trypanosomiasis, caused by *Trypanosoma brucei gambiense* and *Trypanosoma brucei rhodesiense*, and American trypanosomiasis which is caused by *Trypanosoma cruzi*. Each of these parasites is transmitted by distinct insect vectors. The female sandflies of the genus *Lutzomyia* and *Phlebotomus* of subfamily Phlebotominae are primary hosts of *Leishmania* sp., the tsetse flies are carriers of *Trypanosoma brucei*, and *Trypanosoma cruzi* is transmitted by triatomine bugs. Thus, these parasites can attack healthy hosts in diverse ways. In leishmaniasis, the infective stage (promastigotes) of *Leishmania* sp. is injected into the secondary hosts by sandflies which are phagocytosed by macrophages and other mononuclear phagocytic cells. Later on, these promastigotes transform into amastigotes and spread infection to other mononuclear cells. On the other hand, metacyclic trypomastigotes causing African trypanosomiasis are injected into skin tissue of its mammalian host by tsetse flies. The parasites at first enter the lymphatic system and then pass into the bloodstream where they get converted to bloodstream trypomastigotes which can be further carried to other sites of the body. Then upon breaching the blood brain barrier, they enter the central nervous system.[3]

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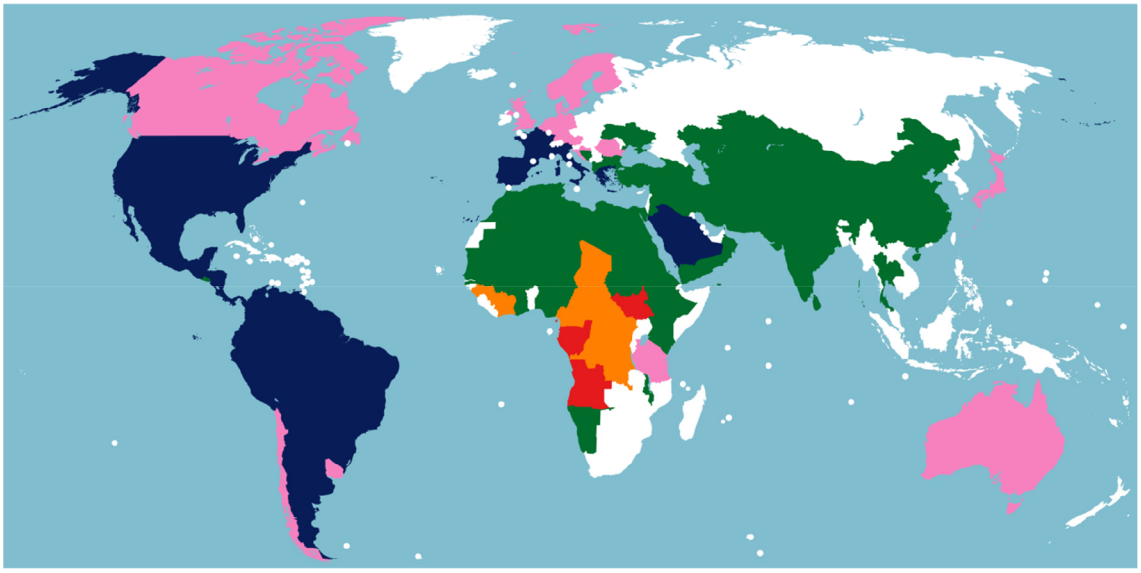

Figure 1. Global distribution of infections caused by trypanosomatids. Regions recognized for a particular trypanosomatid-related infection have been demarcated based on the technical health data published by WHO in the year 2018 for American trypanosomiasis and 2021 for leishmaniasis and human African trypanosomiasis. The green, red, and pink colors represent the localization of leishmaniasis, human African trypanosomiasis, and American trypanosomiasis, respectively. The orange color denotes regions where both leishmaniasis and human African trypanosomiasis are prevalent, while the blue color delineates regions where both leishmaniasis and American trypanosomiasis are found. The global map was generated using MapChart online server (https://www.mapchart.net/).

In American trypanosomiasis, the infected triatomine bug leaves metacyclic trypomastigotes in its faeces that enter the human mucosal membranes through the site of the bite wound. These parasites can infect many kinds of tissues and get transformed into intracellular amastigotes at every newly infected site. Unlike African trypanosomes, they do not replicate in the bloodstream.

Infections that are caused by the trypanosomatids are usually associated with bad housing, migration, and weak immunity, often affecting rural people involved in animal husbandry or agriculture. Even though tryrpanosomatid parasites infecting millions globally, no effective vaccine is available till date. Some of the proposed approaches to exploit proteinaceous and immunogenic components of the pathogen have failed to meet the expected outcomes. For example, the surface-expressed glycoprotein leishmanolysin, also known as gp63, is believed to protect *Leishmania* in macrophages. Although several immunization regimes have employed gp63 as a possible vaccine candidate, its ability to stimulate T-cell response has been a matter of controversy. This is supported by the differential mode of gp63 action in its native and recombinant forms. Erstwhile, the failure of rgp63 (recombinant form) in rendering protection against cutaneous leishmaniasis was documented.[4] However, a latter investigation showed that although the native form was found to be incompetent, rgp63 succeeded in eliciting requisite T-cell responses.[5] When it comes to development of therapeutics, the treatments so far available to combat such infections are known to be ineffective and expensive. Thus, the search for a potent drug should not decline at the present moment. Being familiar with the essentiality of protein synthesis for the survival of an organism, the protein translation machinery has been exploited thoroughly for the development of antimicrobial drugs. Owing to the relevance of aaRSs in protein synthesis, several potent inhibitors have been developed that target these enzymes.[6,7] In this article, reports from over a decade have been presented that propose aaRSs as one of the suitable drug targets against infections caused by trypanosomatids.

2. DISEASES CAUSED BY TRYPANOSOMATIDS

The World Health Organization (WHO) enlists 20 diverse bacterial, parasitic, fungal, viral, and noncommunicable conditions as neglected tropical diseases (NTDs). Of them, the infections caused by trypanosomatids are of serious concern as they are endemic to countries with poor socio-economic backgrounds and limited access to healthcare facilities. Moreover, overlapping of some of these endemic regions results in their coinfection and thus further adding to the woes. The global spread of leishmaniasis and trypanosomal infections has been depicted in Figure 1.

2.1. Leishmaniasis. As per records, about 20 species of *Leishmania* are pathogenic for humans. The severity of this disease can be rated on a scale of high to low depending on the species transmitted by sand flies. On the basis of clinical manifestations, they can be categorized into three broad groups. The cutaneous form of leishmaniasis (CL) chiefly develops a chronic skin ulcer at the site of the sand fly bite, which often takes months to heal. The mucocutaneous leishmaniasis initially causes skin ulcers similar to that of cutaneous form that heal. However, the lesions reappear subsequently in mucous tissue of the nose and mouth leading to massive tissue damage. Lastly, visceral leishmaniasis is a rather serious condition that leads to swelling of visceral organs such as the spleen, liver, lymph nodes, bone marrow, etc., which if neglected is highly fatal. Of late, new forms of leishmaniasis such as diffused cutaneous leishmaniasis (DCL) and post kala-azar dermal leishmaniasis (PKDL) have evolved from cutaneous and visceral leishmaniasis, respectively.[8,9] The disease is mostly reported from Asia, Africa, and Latin America where about 98 countries are endemic for leishmaniasis.

2.2. Human African trypanosomiasis (Sleeping sickness). On the basis of etiology and epidemiology, human

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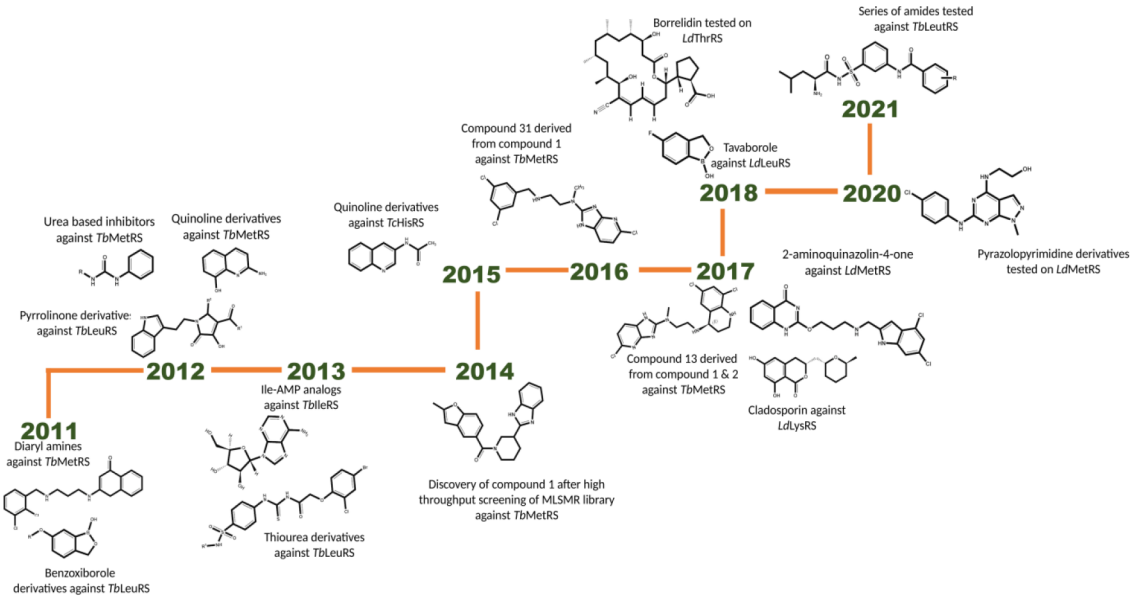

Figure 2. Evolution of drugs against trypanosomatid aaRSs in recent years. Target-specific inhibitor designing in order to attain enriched potency against trypanosomatid aaRSs has been mostly done for LeuRS, MetRS, and HisRS. Also, drugs like borrelidin and cladosporin which are well-known inhibitors of ThrRS and LysRS have shown promising results in the reduction of parasitic growth.

African trypanosomiasis (HAT) exists in two forms: slow-progressing and fast-progressing. The slow-progressing HAT is caused by *T. brucei gambiense* and is transmitted by *Glossina fuscipes* and *Glossina palpalis*. Cases have been mainly reported from west and central Africa which account for 95% of the total number of cases of sleeping sickness. The causal agent of fast-progressing HAT is *T.brucei rhodesiense* that is transmitted by *Glossina morsitans* and *Glossina pallidipes*.[10] The cases of fast-progressing HAT have been chiefly reported from eastern and southern Africa that account for less than 5% of the total cases of HAT. While the slow-progressing HAT results in a chronic infection, the fast-progressing HAT causes an acute infection. Sleeping sickness is manifested by two stages, viz. hemolymphatic and meningoencephalitic. In the hemolymphatic stage, the parasite circulates in the peripheral blood and lymph nodes, while in the later stage, the parasites reach the central nervous system (CNS) upon breaching the blood brain barrier.

2.3. American trypanosomiasis (Chagas disease). American trypanosomiasis is caused by *Trypanosoma cruzi* and is prevalent in the endemic areas of Latin America. In the wake of globalization, increasing incidents of American trypanosomiasis is becoming noticeable in other locations than the hotspot regions for the disease. Known to affect about 6−7 million people worldwide, the 72nd World Health Assembly recommended commemorating 14th of April as World Chagas Disease Day. A healthy individual gets infected when the exposed area of skin/mouth/nose comes in contact with faeces and/or urine of an infected triatomine bug or by consumption of food or water contaminated with waste from an infected bug. This infection can also be transmitted congenitally through blood transfusions as well as organ transplantations (https://www.who.int/health-topics/chagas-disease/). The end of 1−2 weeks of exposure marks the beginning of an acute phase, wherein the parasites can be detected microscopically in blood samples. The acute phase lasts for about 8−12 weeks which if left untreated gets transformed into the chronic form which is characterized by Chagas cardiomyopathy and Chagas digestive disease.[11]

3. AVAILABLE CHEMOTHERAPEUTICS AND THEIR LIMITATIONS

Despite affecting as many as half a million people worldwide annually, remarkable progress in development of drugs against trypanosomatids that are cost-effective and at the same time render a complete cure is not very evident. The remedies which are currently employed for the treatment of these diseases such as arsenals and antimonials have low efficacy, high toxicity, and become ineffective due to drug resistance among parasites. Furthermore, in some cases, the recurrence of infections has also been observed. The disadvantages of some of the current remedies have been listed in Table 1 of the Supporting Information.

However, these diseases are curable if a timely diagnosis complexed with a proper as well as cost-effective regimen is established. In order to combat the rapid spread of infections caused by trypanosomatids, several attempts have been made to immunize the hosts via inducing a passive immunity by injecting dead or weakened parasite. For instance, leishmanization is the inoculation of a mixture of live and dead *Leishmania major* to generate a mild cutaneous leishmaniasis that can avert future infection by the parasite. Some of the licensed vaccines such as Leishmune, CaniLeish, Leishtech, Letifend, etc. are available in a few countries to prevent canine visceral leishmaniasis. Recently, a subunit vaccine was shown to render immunity toward *Trypanosoma vivax*, a pathogen responsible for causing animal trypanosomiasis. However, such strategies have not been adopted against human visceral leishmaniasis.[12]

Trypanosomatids are known to ensure their pathogenicity by defying the host's cellular environment, especially protein translation mechanisms. *Leishmania* sp. has been shown to shut off translation mechanisms happening inside the macrophages of their host by using a GPI-anchored surface metalloproteinase, gp63, or leishmanolysin that cleaves mTOR (rapamycin) leading to dephosphorylation of 4E-BP1.[13] It has been reported that not all targets/pathways perform well in the drug trial sessions, some of them being cytochrome P450 family 51 (CYP51), cytochrome *b*, etc. One of the key reasons

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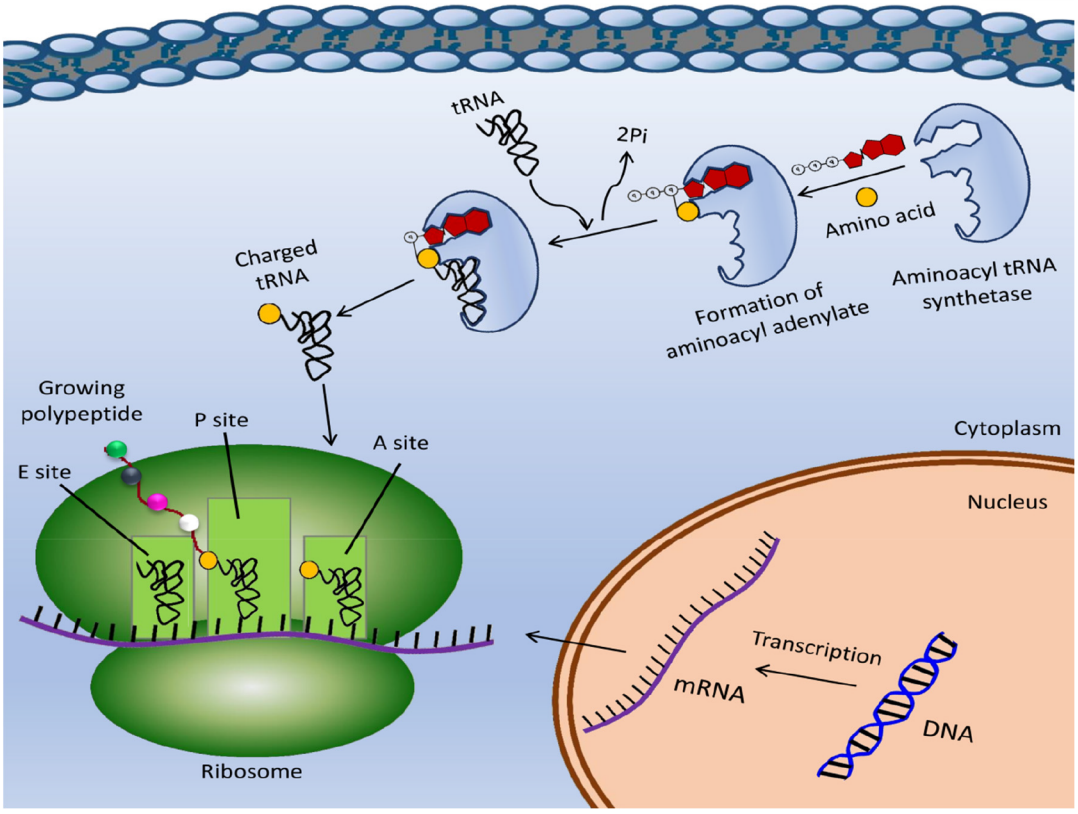

Figure 3. Schematic representation of a cell showing the process of aminoacylation. In the initial steps of aminoacylation, the tRNA synthetase is bound by ATP and its cognate amino acid that results in the formation of aminoacyl adenylate intermediate upon release of a pyrophosphate molecule. In the sequential steps, the cognate tRNA binds to this tRNA synthetase via its anticodon domain and the amino acid is transferred to its CCA arm resulting in the formation of adenylated tRNA (charged tRNA). On the release of charged tRNA as well as AMP, the tRNA synthetase is now ready for another cycle of aminoacylation.

is the presence of limited targets within a pathway that further scale down the scope for combinatorial therapy. On the other hand, the protein translational machinery is a broad umbrella harboring several suitable drug targets that are crucial for the parasite's survival. Lately, the quest for the development of novel antitrypanosomal drugs has brought researchers closer toward targeting aaRSs based on which inhibitors were designed that subsequently showed a post-treatment decrease in parasite growth (Figure 2). Strategies used to concoct each of these inhibitors are discussed in detail later in this review.

4. HOW SIGNIFICANT IS A PARASITE'S PROTEIN SYNTHESIZING MACHINERY FOR DEVISING DRUGS?

The "Central Dogma" of molecular biology elucidates the process by which the genetic code is carefully decoded and converted into a functional product in a living system. Two important phenomena are fundamental to Central Dogma, viz. transcription of mRNA from DNA and mRNA translation into protein. Several macromolecules such as tRNA, aaRSs, ribosomes, auxiliary factors etc., integrate to form the protein synthesizing machinery, all of which are tightly regulated. Since regulation of gene expression at the translational level is associated with cell adhesion and migration, axes development in embryo, etc., dysregulation of any of these constituents can lead to aberrant functioning or even termination of some of the most important metabolic processes happening inside a cell. A rapidly growing cell demands an abundant as well as synchronized pattern of protein synthesis, and protozoan parasites proliferating continuously inside their host are no exception to it. A global map describing the cross-talk between proteins and their complexes happening inside *T. brucei* has also been documented.[14] Antibiotics such as chloramphenicol, tetracycline, erythromycin, kanamycin, etc. are all known to inhibit bacterial growth by terminating the elongation of nascent polypeptides. It has also been reported that inhibitors targeting the translation process can be used to study gene expression by ribosome profiling apart from being used in cell culture techniques.[15] Many of these inhibitors such as anisomycin, trichothecene mycotoxins, narciclasine, A201A, etc. affect polypeptide elongation by binding to the A-site of ribosome thereby destabilizing aminoacyl-tRNA binding. Some of them like bactobolin A and blasticidin S bind to the P-site and promote inhibition by a similar mechanism. Another inhibitor puromycin mimics the CCA-end of tRNA and on entering the ribosome causes untimely termination of polypeptide synthesis. Likewise, with the enumeration of multifaceted roles of aaRSs, these enzymes are being considered as new drug targets on the table. Possessing at least three important sites for their proper functioning viz. a tRNA binding site, ATP binding domain, and a site to incorporate the correct amino acid, aaRSs have been considered as a potent target for drug development.[16−19]

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5. AMINOACYL TRNA SYNTHETASES AS DRUG TARGET

Fidelity is a prerequisite for deciphering the genetic code, and this high degree of fidelity is rendered by aaRSs that are responsible for the ligation of amino acids to their cognate tRNAs. The precision in the mode of action is dependent upon several factors such as stereospecificity of aaRSs toward their respective amino acids and tRNA[20] and the presence of editing domains that remove erroneous amino acids, if incorporated.[21,22] The formation of aminoacyl-tRNA is a two-step enzymatic mechanism[23] (Figure 3). Although the ultimate goal of aaRSs is to catalyze a reaction that leads to attachment of an amino acid with its cognate tRNA, based on the architectural differences of their catalytic sites, the known 23 aaRS can be classified into two groups.

Class I enzymes possess Rossmann fold motif with HIGH (His-Ile-Gly-His) and KMSKS (Lys-Met-Ser-Lys-Ser) conserved sequences just like dehydrogenases and some kinases that have an expanded five stranded parallel beta-strands connected by alpha.[24] Whereas a rare structural fold is observed in the class II enzymes that consists of six antiparallel beta-strands and is elsewhere reported in biotin synthetase holoenzyme.[25] Transfer of aminoacyl adenylate happens on either the 2′ or 3′ hydroxyl group of the terminal adenosine residue present in the 3′ terminal of the tRNA molecule. The class I aaRSs prefer the 2′ hydroxyl group, while the class II aaRSs transfer the aminoacyl adenylate on the 3′ hydroxyl group of the tRNA molecule. Further classification of aaRSs can be made on the basis of the site at which the reaction is taking place, i.e., cytosolic or mitochondrial. The general nomenclature of aaRSs begins with the uppercase alphabet representing the amino acid followed by "RS". Also, a numerical "2" is appended to the mitochondrial version of the synthetase, for example, in aspartyl tRNA synthetase, the cytosolic form is abbreviated as DRS1 and the mitochondrial form as DRS2. It is noteworthy that unlike other eukaryotes that possess at least two genes for most of the cytosolic and mitochondrial aaRSs, the trypanosomatids encode only one gene except for a few, such as AspRS, TrpRS, and LysRS.[26,27] In trypanosomatids, 26 aaRSs have been recognized.[28] In reference to *Trypanosoma*, a study revealed that alternate trans-splicing of an immature RNA results in formation of isoforms, the one with mitochondria targeting sequence (MTS) is directed toward mitochondria while the one devoid of MTS is retained within cytosol.[29] Novel antibiotics having aaRSs as their target have drawn a great deal of interest, some of which are REP8839 and AN-2690 that have successfully stepped forward for clinical trials. In fact, mupirocin marketed as Bactroban by GSK is a well-known drug approved by the FDA. It is isolated from *Pseudomonas fluorescens* and targets the active site of isoleucine tRNA synthetase of *E. coli*.[30] Consecutively, methods that enable high-throughput screening of inhibitor libraries have been developed exclusively for trypanosomatid-based drug targets. Although, the resazurin assay has been used to measure the cytotoxicity of inhibitors toward parasites, there exist several limitations such as false negatives, long incubation period, etc., which hindered the process of drug discovery.[31] Moreover, a SYBR green-based semiautomated assay has been developed that could possibly replace the resazurin test to check the cytotoxicity posed by the potent inhibitors to parasites. This test could be used by the researchers in the times to come as it is cost-effective with minimal background noise.[32]

6. RESEARCH ACCOMPLISHED ON TRYPANOSOMATID AMINOACYL TRNA SYNTHETASES SO FAR

6.1. Leucyl tRNA Synthetase (LeuRS/LRS). The leucyl tRNA synthetase belongs to class I aaRSs and like other aaRSs possesses an error correction mechanism to enhance its fidelity.[33] The catalytic connective polypeptide (CP1) domain of class I aaRSs was earlier recognized for its post-transfer editing mechanisms of mischarged aminoacyl tRNAs.[34] The editing mechanisms of LeuRS have been better understood by comparing the structures of *Neisseria gonorrheae* in apo and ligand bound forms wherein the formation of the Leu-AMP intermediate led to the repositioning of the CP1 domain and movement of the editing domain toward the active site. Furthermore, the movement of the CP1 hairpin was observed to be highly dependent on the binding of leucine and AMP.[35] Thus, the importance of LeuRS in the survivability of trypanosomatids has been proven through gene knockout studies. For example, gene replacement resulted in reduction of infectivity in mouse macrophages infected with *L. donovani*, suggesting that removal of LeuRS led to reduction of the parasitic load. Multiple sequence alignment of LeuRS proteins reveals that the leishmanial enzymes are closer to plant homologues while that of mammals are closer to insect LeuRS1.[36]

Having known its importance, inhibitors have been designed to target LeuRS of various microbes and parasites in order to prevent their pathogenicity. Several inhibitors have also been designed previously targeting LeuRS of trypanosomatids as described ahead. The recent years have gained some additional information on this pursuit. The compound 5-fluoro-1,3-dihydro-1-hydroxy-benziborole (AN2690) is a well-known inhibitor of LeuRS. Having proved its role as an antifungal agent, AN2690 is being tested through clinical trials.[16] The efficacy of this inhibitor has also been tested against apicomplexans.[37] Moreover, the efficiency of AN2690 has also been demonstrated by the oral bioavailability of this inhibitor in murine models infected with *Trypanosoma brucei*.[38] Keeping a note of this, several modified versions of benzoxaborole and N-(4-sulfamoylphenyl)thiourea compounds have been tested and were found with increased inhibitory potential toward trypanosomatid LeuRS.[39−41] Interestingly, AN2690 could inhibit *Leishmania donovani* growth both in amastigote and promastigote stages *in vitro* as well as *in vivo* (BALB/c mice) while rendering low toxicity to mammalian cells. Post AN2690 treatment, the aminoacylation activity got reduced by 2.8-fold, suggesting that AN2690 inhibits parasite growth by targeting LeuRS.[36] A new study demonstrated that CP1 domain of *Ld*LeuRS plays a pivotal role in aminoacylation activities as well. Moreover, with the help of isothermal titration calorimetry, the high binding affinity of AN2690 for CP1 domain could be noticed.[42] With the aforementioned knowledge, it could be deduced that the CP1 domain is essential for the proper functioning of LeuRS. Of the inhibitors designed against trypanosomatid LeuRS, the compounds carrying the thiourea group have posed some of the major drawbacks such as its low solubility and toxicity. However, the replacement of the thiourea group with an amide group led to the development of a series of acetamide inhibitors against *Tb*LeuRS. Interestingly, one of the

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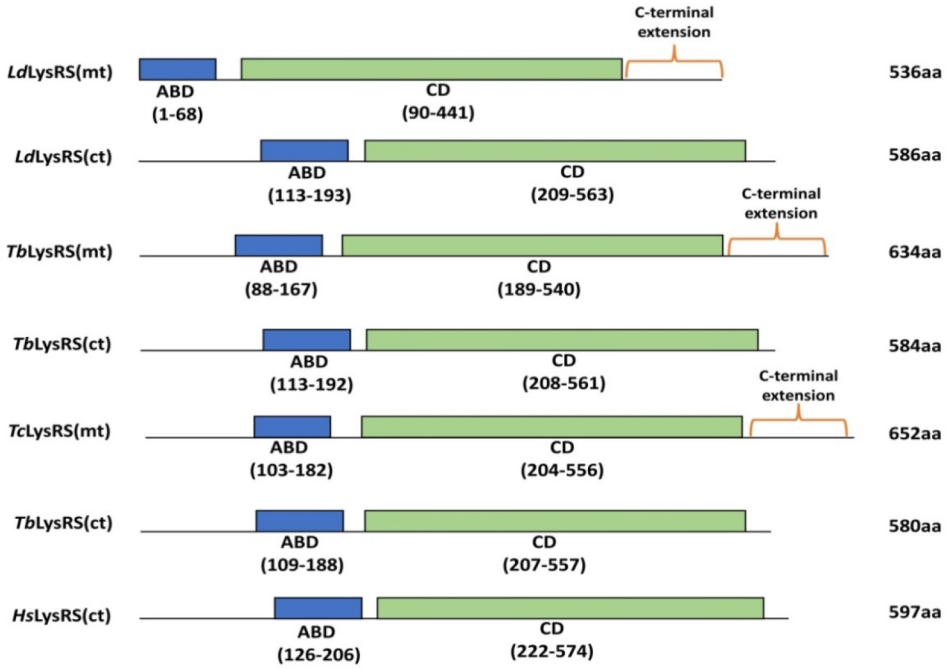

Figure 4. Domain arrangement of trypanosomatid vs *Homo sapiens* LysRS. The trypanosomatid mitochondrial (mt) LysRS contain a C-terminal extension ranging between 90 and 115 amino acids, while the cytosolic (ct) ones have an N-terminal extension of nearly equal lengths.

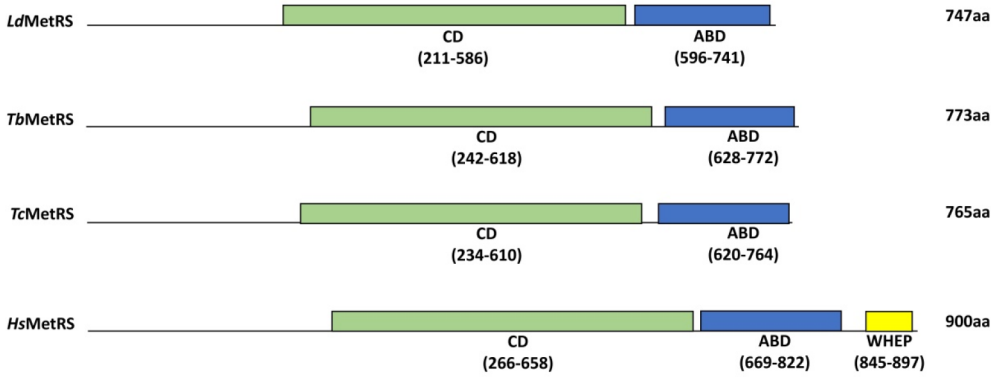

Figure 5. Domain arrangement of cytosolic MetRS from trypanosomatid and human. Comparison of MetRS domains depicts the absence of the C-terminal dimerization domain in humans as well as trypanosmatids that is present elsewhere.

compounds showed up to 250-fold better efficacy with an IC_{50} of 0.70 μM.[43] Further, replacement of the thiourea group along with its acetoxyl group by the amide group has enhanced its inhibitory potential with an IC_{50} of 0.24 μM.[44]

6.2. Lysyl tRNA Synthetase (LysRS/KRS). Lysyl tRNA synthetases are unusual in the sense they can either belong to class I or class II enzymes. Even though prominently found as class II enzymes, they also occur as class I enzymes. Trypanosomatids on the other hand are known to harbor two lysyl tRNA synthetases, both of which are class II type. In *T. brucei*, the lysyl tRNA synthetases are located in mitochondria as well as cytosol, and these isoforms are coded by distinct genes.[45] The mitochondrial LysRS has a C-terminus extension of 92−96 amino acids[46] (Figure 4). In humans, LysRS was found to have a very strong interaction with multi-aaRSs complex (MARS). The strong bond is the result of interaction between p38 and (α2)2, a structure exclusive to LysRS.[47]

Unlike the bacterial homologue, the human LysRS has an additional lysine rich N-terminal extension which is known to be the tRNA binding domain and consists of ELR motif that possesses chemokine activity.[48] Consistent with this observation, sequence alignment of trypanosomatid LysRS have shown the presence of ELR motifs hinting toward their possible role in chemokine activities (Figure 4). However, the chemokine activity from *Ld*LysRS could not be reported, and the function of C-terminal extension remains to be validated. Phylogenetic analysis has revealed that *Ld*LysRS1 is closer to LysRS found in apicomplexans and humans, while *Ld*LysRS2 is a closer homologue of bacterial LysRS.

A known inhibitor of LysRS, cladosporin and its homologue isocladosporin were shown to retard parasite growth with IC50 of 4.19 and 1.09 μM, respectively. The efficacy of cladosporin against *Pf*LysRS could be seen in an early document.[49] There is an extreme conservation seen in the ATP binding pocket of *Leishmania* species and other eukaryotes except two residues (Q308 and S324) which forms the basis of selective inhibition by cladosporin and its isoform. Though not many reports targeting trypanosomatid LysRS are available in the database, this enzyme is known to possess multiple roles in any living

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organism's survival. Thus, it can be exploited for the development of antitrypanosomal drugs, keeping in mind the differences present in the two residues present in the ATP binding pocket. Furthermore, cladosporin could retard parasite growth proving that the LysRS gene is indeed essential for its survival.[50]

6.3. Methionyl tRNA Synthetase (MetRS/MRS). The translation of protein starts with incorporation of methionine, and thus, the importance of MetRS is unquestionable. On entering the nucleolus, it plays a critical role in biogenesis of rRNA which is involved in protein synthesis in the cytosol.[51] Unlike bacteria, the human mitochondrial and *Leishmania major* lack a C-terminal dimerization region[52,53] (Figure 5). Further, a WHEP domain is present toward the C-terminus of *Hs*MetRS that is absent in trypanosomatids. WHEP domains are helix-turn-helix motifs that help in tRNA binding.

Moreover, it was found that there are substantial differences near the ATP and substrate binding regions of *Lm*MetRS to that of human mitochondrial and cytosolic MetRS which form the basis of selective inhibition of parasite MetRS domains.[53] Several inhibitors have been tried and tested in order to inhibit MetRS. Diaryl amines which are known to be the potent inhibitors of prokaryotic MetRS could also inhibit the MetRS bloodstream form of *T. brucei*.[54] Furthermore, urea-based inhibitors (UBIs) have also been found to be effective against *Tb*MetRS.[55,56] The "conformational selection theory" states that a protein is present in various conformational states, and ligands bind any one of these available states based on best fit.[53] Interestingly, unlike other inhibitors previously reported, UBIs could permeate the blood-brain barrier. Also, UBI could inhibit growth of blood stage *T. brucei* with an IC_{50} as low as 150 nM with limited toxicity to mammalian cells. Addition of 5-fluoroimidazole[4,5-*b*]pyridine into UBI inhibitors enhanced their bioavailability to CNS (central nervous system) amidst increasing their efficacy. However, this increase in efficacy and bioavailability is not universal to all the inhibitors and demands a thorough structure−activity relationship study.[57] High-throughput screening has led to identification of at least 19 potent inhibitors of *Tb*MetRS with very low IC_{50} values.[58] Researchers also identified DDD806905 (MetRS01) based on a high-throughput compatible biochemical assay that was shown to inhibit methionine competitively and showed high levels of potency in various *Leishmania* cell-based viability assays and *in vitro* translational assays.[59] However, this inhibitor failed to prove its efficacy in the *in vivo* studies pertaining to its dibasic nature. Subsequently, the same group successfully identified a series of 4,6-diamino-substituted pyrazolopyrimidine based inhibitors toward *L. donovani* MetRS with the help of high-throughput screening. Also, they discovered that the new set of inhibitors could bind to an allosteric site previously never reported, and the mode of action was noncompetitive inhibition. On the basis of homology modeling of *L. donovani* and *T. cruzi* MetRS, the key differences in the allosteric pocket were confirmed where the inhibitor is targeted. This sheds light on what could be the possible reason behind the differences in inhibition activities when the same inhibitor molecule is used. Given the high degree of homology between human MetRS with that of its trypanosomatid counterpart, use of an inhibitor that specifically targets an allosteric pocket which is unique to the parasite enzyme seems to be a suitable approach. The GST-like domain of about 210 amino acids at the N-terminus of trypanosomatid MetRS has 25% sequence identity with that of GST-like domain of human cytosolic MetRS.[60]

6.4. Threonyl tRNA Synthetase (ThrRS/TRS). ThrRS belongs to Class IIa aaRSs. The atomic arrangements of *E. coli* ThrRS evince the presence of Zn^{2+} in its active site that forms a pentacoordinate intermediate with both amino group as well as side chain hydroxyl group of threonine. The sequence analysis depicts the presence of the second additional domain (SECO) which is known to contain perpendicularly oriented antiparallel beta sheets surrounding an alpha helical core, whereas the TGS (ThrRS, GTPase, and SpoT) domain mostly encompasses beta sheets and is present in regulatory proteins.[61] ThrRS, in *Plasmodium*, is localized into the three compartments active in translation, viz. cytosol, mitochondria, and apicoplast, and thus, borrelidin blocks aminoacylation activity of ThrRS in all compartments.[62] Likewise, ThrRS is localized in cytosol and also possibly in mitochondria of trypanosomatids, and treatment with borrelidin could inhibit aminoacylation of *Ld*ThrRS and the growth of parasites ceased.[63,64] The growth arrest on administration of borrelidin was also observed in *Tb*ThrRS.[63] In the same study, it was demonstrated that knockdown of ThrRS led to rapid cell death and morphological changes such as detachment of flagella from cells, leading to cessation of motility. The screening of the marine natural products (MNP) library led to the identification of extracts possessing tremendous inhibitory potential toward *L. major* ThrRS.[65] However, the high degree of sequence similarity between *Ld*ThrRS and *Hs*ThrRS might allow the inhibitors to be cytotoxic toward mammalian cells. However, this result is in contradiction to the reports that show a high druggability score for *Tb*ThrRS.[63] Having known this, it is worth noticing that an analogue of borrelidin retained antimalarial activity but was very less toxic toward mammalian embryonic cells.[66] Similar strategies might be adopted for devising inhibitors for the development of antitrypanosomal drugs.

6.5. Tryptophanyl tRNA Synthetase (TrpRS/WRS). One of the earliest studies on prokaryotic TrpRS stated that it might be an "unexpected homologue" of tyrosyl tRNA synthetase. This hypothesis was further proved by its structural homology to TyrRS in terms of a dinucleotide-binding fold, helical domain, and a dimer interface.[67] Further studies of *Hs*TrpRS have shown that apart from the full-length enzyme, there are three N-terminus truncated isoforms, the shortest being the most angiostatic in nature.[68] TrpRSs generally exist as cytosolic and mitochondrial isoforms. In trypanosomatids, both of its isoforms, TrpRS-1 as well as TrpRS-2, are of cytosolic origin and the latter is transported to mitochondria.[69] Thus, TrpRS-2 is capable of aminoacylating both $tRNA^{Trp}$ (CCA) and $tRNA^{Trp}$ (UCA). Silencing of *T. brucei* TrpRS-1 and -2 isoforms led to rapid decrease in cell growth and finally complete growth arrest. However, TrpRS-2 has been indicated as a better target because of its lesser sequence identity with that of the human counterpart and its ability to aminoacylate both types of tRNA.[63,70] Furthermore, the similarity in the catalytic domains of *Tb*TrpRS-1 and the cytosolic human TrpRS has been proved with the crystal structure of *Tb*TrpRS-1.[71] Inhibitors specifically targeting apicomplexans are available in the database that can be further investigated for their potency in the inhibition of trypanosomatid TrpRSs.[72] Thus, researchers might venture into this aspect of drug targeting in order to discover potent and selective inhibitors of trypanosomatid TrpRSs.

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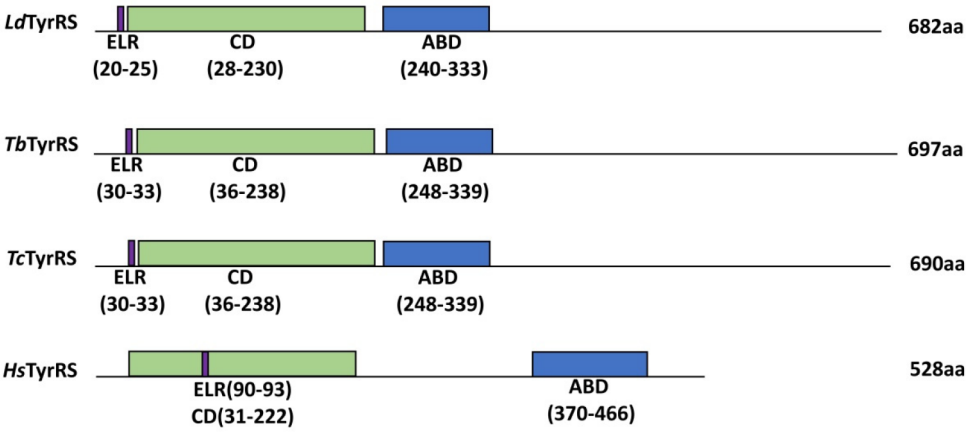

Figure 6. Domain arrangement of trypanosomatid vs human cytosolic TyrRS. All of the trypanosomatids TyrRS harbor an ELR motif which might play a role in chemotaxis.

6.6. Tyrosyl tRNA Synthetase (TyrRS/YRS). Tyrosyl tRNA synthetase is a functional dimer protein of the class I aaRS, and earlier work on the *H. sapiens* tyrosyl tRNA synthetase (*Hs*TyrRS) has led to the revelation that proteolytic cleavage of *Hs*TyrRS results in two distinct fragments (mini TyrRS) with cytokine activities. The full-length TyrRS, however, lacks the ability to stimulate chemotaxis of endothelial cells *in vitro* or initiate angiogenesis in the *in vivo* animal models. From structural analysis, it was reported that removal of C-domain after proteolytic cleavage leads to the exposure of the ELR (Glu-Leu-Arg) motif present in the Rossmann fold catalytic domain activating its cytokine function.[73,74] Thus, like other aaRSs, addition of noncanonical functions does not hinder its own aminoacylation activity. The trypanosomal genome contains a single copy encoding the TyrRS protein which is twice the length of TyrRS present in other organisms. Each half of the protein is barely 17% identical to one another leading to the formation of a pseudo-dimer.[74] Multiple sequence alignment of the amino acid residues shows the presence of ELR motif in all trypanosomatids reflecting its possible role in chemotaxis (Figure 6). While the homologues of mini TyrRS present in prokaryotes and lower eukaryotes such as bacteria and yeast lack the ELR motif, *Pf*TyrRS, on the other hand was reported to trigger proinflammatory response in humans via its ELR motif causing enhanced secretion of TNF-α and IL-6 that are detrimental to the host.[75] On similar lines, *L. donovani* tyrosyl tRNA synthetase (*Ld*TyrRS) was found to be involved in chemokine signaling. It has been proven that *Ld*TyrRS on migrating to the extracellular environment acts as a neutrophil chemoattractant wherein the binding of the ELR peptide motif with the CXCR2 receptor of host macrophages mediate secretion of proinflammatory cytokines such as TNF-α and IL-6 in *Leishmania* infections, thus highlighting its immune-modulating roles. The study further documented slower growth kinetics and virulence for heterozygous mutants of *L. donovani.* Moreover, TyrRS appeared to be an essential enzyme for the parasite as the chromosomal null mutant did not survive..[76]

Structural analysis of *Ld*TyrRS in complex with tyrosyl adenylate analog (TyrSA) have shown the two pseudo-monomers, each with two domains, and the presence of an extra pocket (EP) near to the adenine binding pocket which is absent in *Hs*TyrRS.[77] Apart from *L. donovani*, the essentiality of TyrRS has been reported for normal growth of *Trypanosoma brucei* survival by inducible RNAi. *Tb*TyrRS, like *Lm*TyrRS, forms a pseudo-dimer and is capable of aminoacylating both mitochondrial as well as cytosolic $tRNA^{Tyr}$. Furthermore, the deletion of 36 amino acid residues either from the N- or C-termini abolished the *Tb*TyrRS function, raising the question of what could be the possible role of these amino acid residues in its catalysis.[78] Some of the previously proclaimed antiparasitic plant derivatives such as polyphenolic compounds and alkaloids have been shown to specifically target the active pocket of leishmanial TyrRS with the help of molecular docking analysis.[79,80]

6.7. Aspartyl tRNA Synthetase (AspRS/DRS). AspRS is a class II aaRSs which is found in both discriminating and nondiscriminating forms with regard to differences in their anticodon binding domains.[81] In trypanosomatids, there are two different isoforms of AspRS: cytosolic and mitochondrial. While the cytosolic form has an insertion of 33 amino acids, the mitochondrial version has a long insertion of 70 amino acids.[44] Moreover, a long N-terminal extension of AspRS is found in trypanosomatids and is different from the one found in other eukaryotic AspRSs. The leishmanial AspRS has been characterized at various dimensions as well. On the basis of the gel-filtration chromatogram, the cytosolic *Ld*AspRS was found to be a homodimer.[82] While the *T. brucei* mtAspRS aminoacylates both mitochondrial as well as cytosolic $tRNA^{Asp}$, the ctAspRS is only capable of aminoacylating its cytosolic $tRNA^{Asp}$.[83] On the other hand, one of the reports states that *in vivo* degradation of microcin C (a ribosome synthesized heptapeptide) releases a modified aspartyl-adenylate, an acylphosphoramidate linkage which strongly inhibits *Francisella tularensis* AspRS.[84] Furthermore, enamide derivatives have shown strong inhibition of *Mycobacterium tuberculosis* mitochondrial AspRS.[85]

6.8. Isoleucyl tRNA Synthetase (IleRS/IRS). The isoleucyl tRNA synthetases are the enzymes that constitute long stretches of amino acid residues. Its editing domain renders top precision by hydrolyzing the mis-synthesized intermediates.[86] Inhibitors specifically targeting the pathogen IleRS are well-known, for example, mupirocin, isoleucyl-AMS, etc.[87] It has been demonstrated that alternative trans-splicing results in the formation of two proteins with different lengths of N-terminus. The longer N-terminal containing protein is exclusively found in mitochondria while the shorter segment is found in the cytosol.[88] In another study, the induction of RNAi

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toward IleRS in *Trypanosoma brucei* was found to be detrimental *in vitro* as well as *in vivo*. Analogues of Ile-AMP, i.e., Ile-AMS ([isoleucinyl]-N-[adenosyl]-diaminosufone) could inhibit aminoacylation of *Tb*IleRS by competing for its ATP binding pocket *in vitro* and could cure infected mice proving their efficacy *in vivo*. Interestingly, these analogues could cross the blood brain barrier which is imperative in curing the stage-II human African trypanosomiasis.[89]

6.9. Histidyl tRNA Synthetase (HisRS/HRS). The crystal structure of *Escherichia coli* HisRS revealed that it exists as a homodimer, and its asymmetric unit comprises of two such homodimers.[90] This report is in conjunction with the structures of trypanosomatids, nonetheless they differ in terms of their ligand binding mechanisms[91] as described in section 7. Moreover, N-terminal domain of trypanosomatid HisRS is extensively lysine rich which is not found in the case of humans and may be exploited as an important factor for targeting drugs (Figure 7). It is noteworthy that the lysine rich regions have been observed to play an important role in tRNA binding.[46] The human HisRS also contains a WHEP domain in its N-terminal which could not be seen in trypanosomatids. The essentiality of histidyl tRNA synthetase for the survival of *T. brucei* is documented with the help of RNAi knockdown of the gene that led to a severe decrease in parasite growth rate.[63] Subsequently, quinoline derivatives were docked against the *T. cruzi* HisRS and later on validated for their inhibitory potential.[92,93]

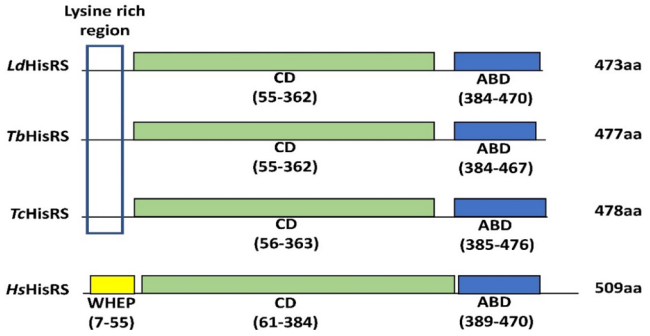

Figure 7. Domain arrangement of cytosolic HisRS from trypanosomatid and human. The extensively lysine-rich N-terminal region is shown in the Pfam domains of trypanosomatids that is absent in the human form.

6.10. Prolyl tRNA Synthetase (ProRS/PRS). Prolyl tRNA synthetase (ProRS) has always been the clinically important aaRS and halofuginone (HF), a less toxic form of febrifugine inhibits ProRS activity of bifunctional human glutamate-prolyl tRNA synthetase (EPRS) by activating the amino acid response (AAR) pathway which subsequently inhibits TH17 cell differentiation. Consequently, HF and its derivatives are well-known to exert their inhibitory effects on *Plasmodium falciparum* prolyl tRNA synthetases.[94,95] The importance of ProRS for the survivability of trypanosomes has been checked in both *L. donovani* as well as *T. brucei*. Further, a quinazolinone-based inhibitor was found to inhibit ProRS in *L. major* as well as other protozoan parasites,[96] whereas its potency was validated through *in vitro* parasite growth inhibition and aminoacylation assays. Moreover, addition of bromide on the quinazoline ring preserved antimalarial efficacy while lowering cytotoxicity to host cells. However, the protein sequence alignment reveals conservation in trypanosomatids, apicomplexans, and humans. Even though the structures of HF-bound *Pf*ProRS and *Hs*ProRS are identical, there are substantial differences in the interaction of some of the residues such as Thr478, terminal phosphate group of AMPPNP, water molecule, etc., which suggest that inhibitors of *Pf*ProRS, like quinazolinone, might also inhibit trypanosomal ProRS. Both cytosolic and mitochondrial ProRS isoforms in *Leishmania* contain the Ybak/ProX domain at the N-terminus (Figure 8), which is a prokaryotic-type editing domain and helps in the rectification of mischarged tRNAs.[28,46]

6.11. Other tRNA Synthetases. The aaRSs, by and large are an intriguing target to work upon. A study focused on inhibition of wide range of aaRSs and their relative effect on parasite growth.[63] As expected, ablation of any of these enzymes contributed toward reduction in parasite growth and thus propagation. However, a differential effect could be observed for each one of them that accompanied drastic morphological changes. Inhibition of PheRSβ, ArgRS, ThrRS, and AsnRS was found to be more effective in retarding cell growth than knockdown of PheRSα, GluRS, ValRS, and TrpRS-1. Phenotypically, cells became more round in shape with detachment of flagella when AsnRS, ArgRS, and ThrRS were ablated. On the other hand, PheRSβ knockdown strains became enlarged, and not much change was observed in morphology when PheRSα, ValRS, and GluRS were inhibited. A study validated the importance of tRNA:aaRS interactions for the development of antitrypanosomal drugs via computational approaches.[65] Of the several inhibitors screened, they were successful in identifying three major inhibitors of

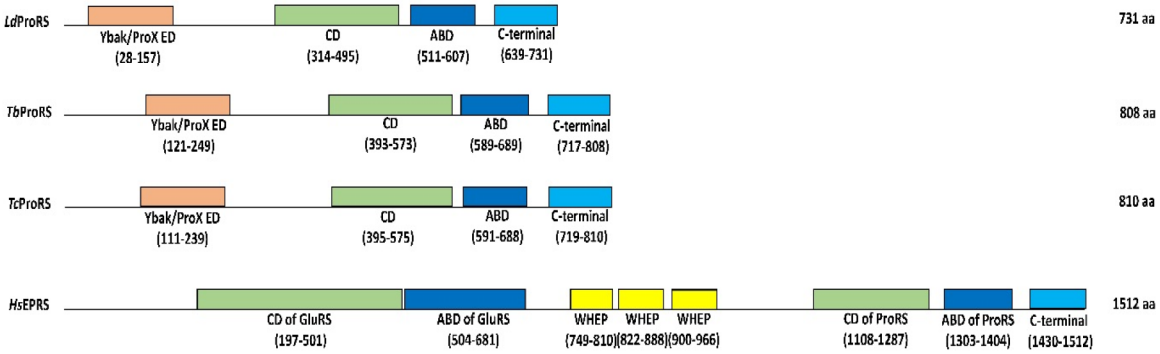

Figure 8. Domain arrangement of trypanosomatid vs *Homo sapiens* cytosolic ProRS. Pfam domain comparison shows the absence of Ybak/ProX domain at the N-terminus of human EPRS when compared to the trypanosomatid ProRS.

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*Lm*AlaRS. Interestingly, these inhibitors were also effective in inhibiting aminoacylation activity of *T. cruzi* AlaRS with an inhibitory efficiency of up to 99%, and no inhibition was observed when the human homologue was targeted. This indicates the existence of conserved architecture of aaRS within the species of trypanosomatids. They further emphasized on the use of antagonistic combination of chemotherapies that might allow lesser tendency for the parasites to acquire resistance than the synergistic ones. For instance divergence in terms of amino acid activation of trypanosome AlaRS has also changed the way by which they interact with their tRNA substrates.[65] Recently, a series of ureidosulfocoumarin compounds was tested for their inhibitory potential against *L. donovani* seryl tRNA synthetase (*Ld*SerRS). Of the three compounds that demonstrated greater specificity toward *Ld*SerRS than its human counterpart, Comp 5l was found to be competing for the ATP binding site of *Ld*SerRS.[97]

As mentioned earlier, some of trypanosomatid tRNA synthetases have extensions/insertions which are very peculiar in nature. Leishmanial and trypanosomal ArgRS have an insertion of about 110 amino acids, which is conserved in both genera. It should be noted that unlike the previous reports on ArgRS that have ruled out the possibility of aminoacylation in the absence of $tRNA^{Arg}$, the leishmanial enzyme was catalytically active even in the absence of $tRNA^{Arg}$. In order to understand the potential role of this insertion in the aminoacylation activity of ArgRS, we removed this stretch of 110 amino acids with the help of splicing by the overlapping extension PCR method. To our surprise, the enzyme lost its activity by almost 90% (unpublished data). Some other insertions/extensions are extensively lysine-rich and occur in the CP1 editing domain of IleRS, anticodon binding domain of GluRS, and N-terminal catalytic domain of HisRS, etc.

7. ROLE OF STRUCTURAL BIOLOGY IN DESIGNING INHIBITORS AGAINST TRYPANOSOMATID AARSS

The use of structural biology in drug discovery has made it possible for researchers to design inhibitors with enriched specificities toward their corresponding targets.[98] These macromolecular structures can be retrieved from PDB which is a database comprising of information related to atomic coordinates of proteins that are determined by scientists across the globe. PDB is used on a regular basis for the derivation of inhibitors after retrieving atomic coordinates of target proteins, and some of the aaRSs structures from parasites other than trypanosomatids have been used to set a framework for designing drugs.[99,100] With regard to the structures obtained through experimental approaches, we have cited such references, wherein the atomic coordinates of the trypanosomatid aaRSs were helpful in the formulation of novel inhibitors.

Fisetin, a flavonoid compound, was cocrystallized with *Leishmania major* tyrosyl tRNA synthetase (*Lm*TyrRS).[74] Fisetin was seen to interact with the protein in the same way as reported for tyrosinol (a tyrosine analog). Although fisetin itself could not retard the parasite growth, it was postulated that binding modes of this compound toward its target might be beneficial in designing inhibitors with better specificities. In a related study, structural analysis of *Ld*TyrRS in complex with a camelid nanobody (NbA) and tyrosyl adenylate analog (TyrSA) could reveal binding modes of the protein with its ligands. The inhibitor TyrSA bound into the active site of catalytic domain in the *Ld*TyrRS crystal structure, which has also been verified through the aminoacylation assay presenting an IC_{50} value of 0.69 nM. A further reduction of loop flexibility inside the protein was seen in the complex. An extra pocket (EP) is present near to the adenine binding pocket (ABP) of the *Ld*TyrRS active site; however, it is absent in *Hs*TyrRS (Figure 9). Presence of EP has been reported from other

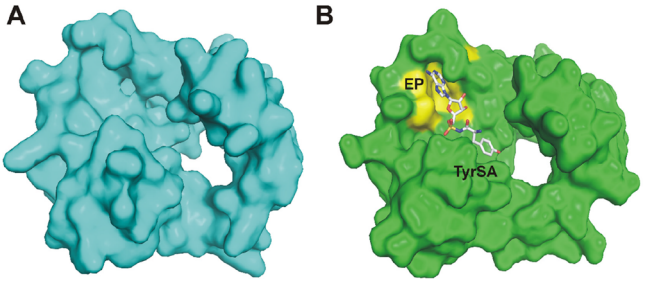

Figure 9. Surface view representation of TyrRS from human and *L. donovani*. (A) Human and (B) leishmanial TyrRS is compared to highlight the location of the extra pocket (EP) present in *Ld*TyrRS. The residues of *Ld*TyrRS EP are indicated in yellow color, while the ligand is displayed in white sticks.

trypanosomatids as well as apicomplexans. A comparison of amino acid sequences revealed that EP and its closer variants are present in almost all protozoans. Thus, the absence of EP in human TyrRS and its presence in protozoan counterparts can be exploited for the development of drugs against other parasites as well.[77]

In trypanosomatid HisRSs, the H-state (histidine bound state) and F-state (ligand free state) structures of *Hs*.cHisRS (where c indicates the cytosolic isoform of *Hs*HisRS) and *Tc*HisRS helped the investigators to design structure-based drugs against *T. cruzi*.[92] Most of the residues were seen to interact identically with histidine except Arg314 and Arg156 of *Tc*HisRS. Unlike *Hs*.cHisRS•His (substrate-bound state), they do not form hydrogen bonds with the carboxylate group of histidine. Also, Arg156 in *Tc*HisRS adopts a different rotamer configuration than that of Arg157 of *Hs*.cHisRS. Furthermore, the presence of a deep pocket extending from the S1 loop to the bound histidine in *Tc*HisRS and its absence in *Hs*.cHisRS is intriguing. When the F-states of *Hs*.cHisRS and *Tb*HisRS were compared, it was observed that the F-state of *Hs*.cHisRS is disordered while that of *Tb*HisRS is ordered and yet in an open conformation. In the pursuit of identifying novel inhibitors, the same research group adopted the fragment-based drug designing approach for about 15 quinoline fragments toward the H-pocket discovered[92] and later on crystallized them in order to understand the molecular interaction.[93] However, to their surprise, none of the inhibitor fragments bound to the H-pocket. Rather, their interaction was seen with another deep vent adjacent to the H-pocket that was earlier never reported. Also, on comparing with *Hs*.cHisRS, the pocket was found to be absent. Further, it was noticed that these new inhibitors interacted with Cys365, which is only present in trypanosomatids and is analogous to a conserved Glu387 in other eukaryotic HisRS.

It has been suggested that binding of inhibitors to *Tb*MetRS is dependent upon the conformational selection theory with a small number of structural adaptations during early stages of binding. However, it should be also noted that *T. brucei* parasites grown in the presence of inhibitors showed an increased expression of MetRS mRNA by a factor of 35 when

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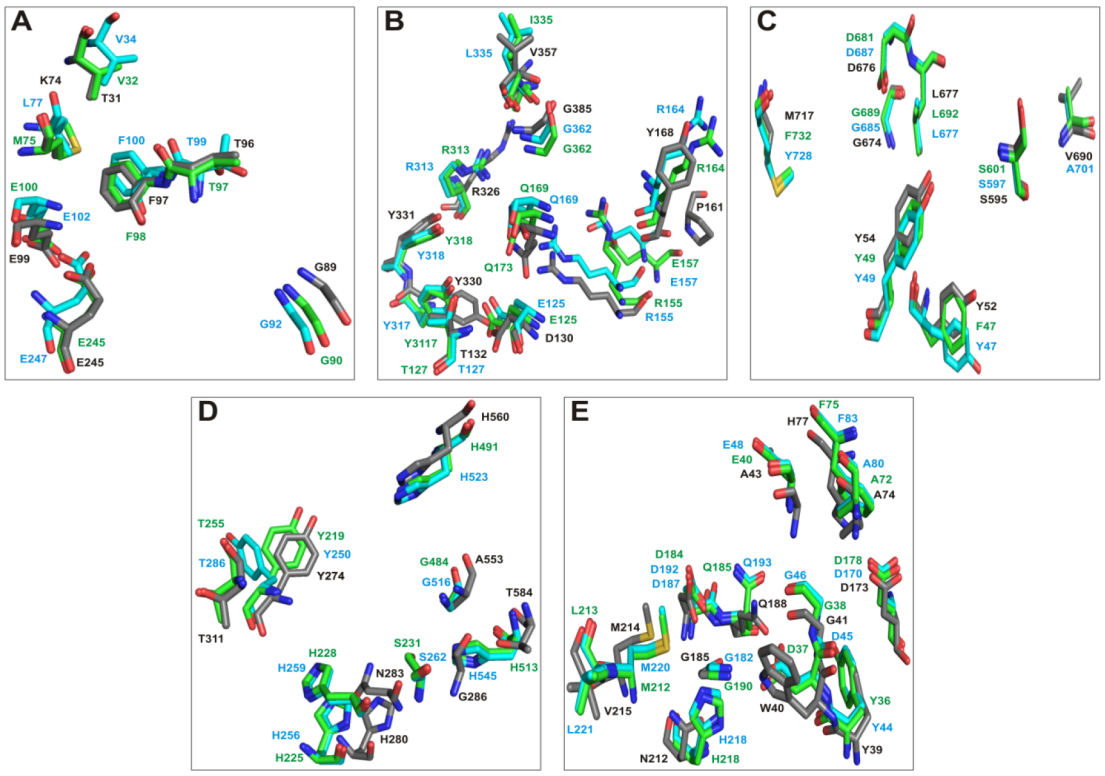

Figure 10. Comparison between catalytic pockets of human and trypanosomatid aaRSs: the structural superimposition between the catalytic pockets of human and trypanosomatids has been shown for (A) AlaRS (corresponding RMSD of *Ld*AlaRS and *Tb*AlaRS from *Hs*AlaRS is 0.28 and 0.35 Å), (B) HisRS (corresponding RMSD of *Ld*HisRS and *Tb*HisRS from *Hs*HisRS is 1.05 and 1.08 Å, respectively), (C) LeuRS (corresponding RMSD of *Ld*LeuRS and *Tb*LeuRS from *Hs*LeuRS is 0.22 and 0.56 Å, respectively), (D) MetRS (corresponding RMSD of *Ld*MetRS and *Tb*MetRS from *Hs*MetRS is 1.1 and 0.82 Å, respectively), and (E) TyrRS (corresponding RMSD of *Ld*MetRS and *Tb*MetRS from *Hs*MetRS is 0.39 and 0.35 Å, respectively). The residues of catalytic pockets are presented as sticks with black, green, and cyan for human, *L. donovani*, and *T. brucei* aaRSs, respectively.

compared to the parental strain in order to develop resistance against the drugs.[101] The crystal structures of the *Tb*MetRS●inhibitor●AMPPCP complex revealed that the inhibitor does not replace ATP from its binding site, and rather, the interaction between inhibitor and ATP is through hydrogen bonds. This means that both inhibitor and ATP can bind simultaneously, and there is no competitive inhibition exhibited by the inhibitor.[102] Consequently, a group of researchers optimized their previously identified compound1[58] using structure-guided approaches which led to derivation of two novel compounds each belonging to cyclic and linear linker series, respectively. The replacement of the 3,5-dichlorobenzyl group of linker series compound with 6,8-dichlorotetrahydroquinoline resulted in a compound that exhibited EC_{50} of 4 nM and targets the enlarged methionine pocket formed as a result of conformational changes. The 3,5-dichlorophenyl moiety of the inhibitor was found to occupy the enlarged methionine pocket, while the benzimidazole moiety is bound to the auxiliary pocket that opens in the presence of the inhibitor.[103,104] Additionally, the potent 5-fluoroimidazole[4,5-*b*]pyridine-based inhibitors as described in section 6.3 are also the result of a structure-guided drug-designing approach.[52] Also, two series of compounds were devised that contained aliphatic and aromatic linkers which subsequently increased the inhibitors potency and selectivity. However, the inhibitors failed to cross the blood brain barrier which lay the groundwork for further optimization.[105] In another study, the virtual screening of about 5 million compounds from the $ZINC_{15}$ database against the *L. infantum* methionyl tRNA synthetase (*Li*MetRS) structure led to identification of 10 probable hit candidates. Further screening of these lead molecules was done by analyzing their interaction with the residues present in the catalytic pocket of *Li*MetRS. Subsequent analysis of pharmacokinetic properties identified two compounds with better stability when bound to the leishmanial enzyme.[106]

Pyrimidine derivatives have been previously proven as competitive inhibitors of *E. coli* aspartyl tRNA synthetases.[107] In this report, it was observed that active moiety of these compounds mimic the aminoacyl-adenylate intermediate which enables them in successfully replacing the canonical purine group of ATP and presents the competitive mode of inhibition. Thus, the structure of *L. donovani* aspartyl tRNA synthetase (*Ld*AspRS) was used in order to determine the affinity of these compounds toward the leishmanial enzyme using an *in silico* approach. The docking of the target-pyrimidine derivative led to the revelation of higher binding affinity for the inhibitors when bound to the leishmanial target than its cofactor, i.e., ATP. This suggests that there might be formation of a much more stable complex between the inhibitor and *Ld*AspRS, which can be further validated through *in vitro* studies.[82] The PDB harbors many more crystal structures of tRNA synthetases belonging to trypanosomatids that can be utilized for structure-based selective inhibition of targets, e.g., *Tb*TrpRS,[71] etc.

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8. COMPARISON OF HUMAN VS TRYPANOSOMATID AARSS STRUCTURES

It is also possible to generate modeled structures of proteins using *ab initio* techniques, comparative modeling or through highly advanced online software that employ artificial intelligence such as AlphaFold (AlphaFold2.ipynb - Colaboratory (google.com), Robetta (https://robetta.bakerlab.org), etc. With the advent of technology, architectural information obtained from the protein structure has been used for fragment-based drug screening as well as *de novo* synthesis of inhibitors. In continuation of the ongoing saga on the role of structural biology methods in drug discovery, we have tabulated the overall as well as catalytic pocket differences of trypanosomatid aaRSs when compared to the human counterparts by retrieving their respective modeled structures from the AlphaFold database (Table 2 of the Supporting Information). In order to achieve this, a sequence independent pairwise structure alignment was employed using the TM-align online tool (https://www.rcsb.org/docs/tools/pairwise-structure-alignment), which is sensitive to global topology. The RMSD (root mean square deviation) values evince that trypanosomatid aaRSs have considerable structural differences from their human equivalent enzymes. Some of the human aaRSs have N-terminal extension such as ValRS, TrpRS, and GlnRS, while LeuRS, IleRS, MetRS, TyrRS, and SerRS have C-terminal extension. On the other hand, some of the trypanosomatid aaRSs were found to harbor insertions in them like N-terminal extension in LysRS, insertion within the catalytic core of ArgRS, and insertion with the anticodon binding domain (ABD) of AspRS, etc. It is noteworthy that the folding of trypanosomatid MetRS and HisRS was found to be very different, especially in HisRS where the deviation was higher for its catalytic pocket than its ABD. Similarly, the helical region formed by the residues 50−175 in trypanosomatid MetRS was significantly different from the human enzyme. Moreover, in humans, the prolyl and glutamyl tRNA synthetase is present as a bifunctional enzyme because of which the structure becomes quite larger than the individual enzymes of trypanosomatids.

A glance at the overall RMSD values clearly indicates that all of the trypanosomatid aaRSs have considerable topological divergence from their human equivalent enzymes. However, the overall RMSD scores might not always be an appropriate parameter based on which the differences in their catalytic pockets can be determined. On the other hand, a closer look at the residues present in the catalytic core gives a clear picture of their variation as well as orientation. Some of the catalytic pockets demonstrating a high degree of deviation in terms of residues and orientation have been shown (Figure 10). For example, although the overall RMSD for SerRS, CysRS, and AspRS are very high, their catalytic residues were found to be identical and present at the same position. On careful observation, it was found that the higher range of RMSD was contributed by regions other than the catalytic core, viz. the N-terminal region of SerRS formed by residues 40−103 and 180−190, the helical region beyond catalytic pocket in CysRS conferred by residues 660−755, whereas a loop region was made by the residues 165−170 between the catalytic core and ABD in AspRS apart from its deviation in the N-terminal domain. On the contrary, aaRSs that showed much lower RMSD scores like TrpRS, ArgRS, LysRS, ThrRS, and ValRS were found to be the enzymes of significance as they contain differences at a moderate level in their catalytic core. In LysRS and GluRS, the orientation for most of the residues was found to be similar despite the presence of some different residues. In contrast, some other aaRSs (IleRS and TrpRS) showed differences in the orientation while having identical residues, except LeuRS wherein Y52 of human enzyme has been replaced with phenylalanine in the leishmanial analogue. Such was also the case for ValRS, ProRS, and GlnRS in which the orientation was found to be different only for a few residues. In these enzymes, the overall RMSD is also contributed by the shift in residue conformations present in the catalytic pocket apart from deviations present in other regions. Also, aaRSs such as AlaRS, MetRS, ThrRS, TyrRS, ArgRS, and HisRS showed differences in the residues as well as their orientation for the ones present at the active site.

9. COMPOUNDS TESTED AGAINST OTHER MICROBIAL AARSS

Throughout the evolution, it is a well-known fact that the enzymes considered crucial for metabolic processes typically remain conserved with minute changes at their catalytic sites across different domains of the taxonomic hierarchy. In this regard, the compounds that have been tested elsewhere can be further used to validate comparative inhibitions between trypanosomatid and their corresponding human enzymes. Table 3 of the Supporting Information consists of a list of inhibitors that have been analyzed in the past decade and were successful in inhibiting the aaRSs in other microorganisms but have so far not been tested in trypanosomatids. Apart from the *in vitro* data, the binding affinity of potential compounds toward their respective targets derived through *in silico* approaches can be pivotal in understanding their efficiencies when tested *in vitro*. For instance, the high degree of specificity of pyrimidine analogues reported toward *Ld*AspRS[82] can be further investigated for their potency in retarding the aminoacylation activity of the enzyme.

10. CONCLUSIONS

Trypanosomatids affect millions of people every year, hence there is an urgent need to identify novel drug targets and design their inhibitors that can successfully replace the ongoing therapies which are not suitable because of several limitations posed by them. Aminoacyl tRNA synthetases are the crucial guarantors of fidelity and have been selected as suitable drug targets to cure other parasitic diseases. In this regard, many compounds have been reported as potent inhibitors of trypanosomatid aaRSs, and the field of structure biology has been pivotal in developing some of these potent inhibitors. Since several aaRSs undergo aminoacylation simultaneously during protein synthesis, a cocktail of aaRSs inhibitors targeting multiple tRNA synthetases at the same time is expected to render fewer evolutionary advantages in parasites as earlier seen in the case of protein kinase inhibitors. This review congregated studies from nearly a decade that used trypanosomatid aaRSs as a major target of interest for devising drugs, and the role of structural biology in the derivation of inhibitors with improved specificities has been emphasized. Altogether, the data presented here highlights the relevance of aaRSs as suitable drug targets that can be further used for designing novel therapeutics to cure the diseases caused by trypanosomatids.

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■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acsomega.3c00826.

Additional tables showing drugs for trypanosomatids, comparative structural analysis, and compounds tested against aminoacyl tRNA synthetases along with references (PDF)

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Notes

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