CHAPTER 1

Leishmaniasis Infection, Current Treatment Methods, And Emerging Interventions

Abstract*

Leishmaniasis, one of the Neglected Tropical Diseases (NTDs), is commonly recognised as 'kala-azar' in India. Leishmaniasis is a disease caused by the parasite *Leishmania*, a protozoan, propagated by the bite of an infected female phlebotomus. Worldwide, more than 7,00,000 new cases of Leishmaniasis are reported annually. Among various kinds of *Leishmania* infection, Visceral leishmaniasis (VL) is of special concern as it is the most lethal, if not treated in time. Although chemo-treatments like miltefosine, liposomal amphotericin B, paromomycin, and antimonials are currently in practice, they have their limitations. This leads to an urgent need for new and efficient treatment, which includes new drug molecule discovery and vaccine development. In the present work, the objective is to discover a new drug molecule against VL. One of the paramount molecules for cell survival is Coenzyme A, which is a vital cofactor in numerous metabolic pathways, including energy production, protein modification, membrane trafficking, and cell differentiation. The last enzyme involved in Coenzyme A biosynthesis in *Leishmania donovani* is the Dephospho-Coenzyme A Kinase (DPCK). DPCK could be a potential target for new drug molecule discovery against VL.

* Part of the review is submitted for the publication.

1. Introduction to Leishmaniasis

Leishmaniasis is a neglected tropical disease caused by protozoan parasites of the genus *Leishmania*, transmitted to humans through the bite of infected phlebotomine sandflies (Figure 1). Among its various forms, visceral leishmaniasis (VL), also known as kala-azar, represents the most severe manifestation, responsible for the highest mortality if left untreated (Figure 2) . VL is predominantly caused by *Leishmania donovani* and *Leishmania infantum*, with endemicity concentrated in regions of Asia, East Africa, and the Mediterranean basin (Figure 3). The disease primarily affects marginalised populations, with children comprising a significant proportion of cases in high-burden area (Zhang et al., 2025).

Upon infection, *Leishmania* promastigotes are injected into the host's skin and are rapidly engulfed by macrophages and other mononuclear phagocytes. Within these cells, the parasites transform into their amastigote stage, enabling persistent intracellular survival and proliferation (Kima, 2007). They disseminate via the bloodstream to internal organs such as the liver, spleen, and bone marrow, where they exploit host immune mechanisms to establish chronic and often asymptomatic infection. Clinical progression to overt VL is marked by irregular fever, anaemia, hepatosplenomegaly, and immune suppression, underscoring the parasite's capacity to manipulate host-pathogen interactions (Costa et al., 2023).

Visceral leishmaniasis (VL), or kala-azar, is a life-threatening disease caused predominantly by *Leishmania donovani* and *Leishmania infantum*, transmitted through the bite of infected phlebotomine sandflies. After entry into the human host, *Leishmania* promastigotes are phagocytosed by immune cells, mainly macrophages. Inside these cells, they transform into the amastigote form, enabling persistent intracellular replication and dissemination to vital organs such as the spleen, liver, and bone marrow (Chaparro et al., 2022).

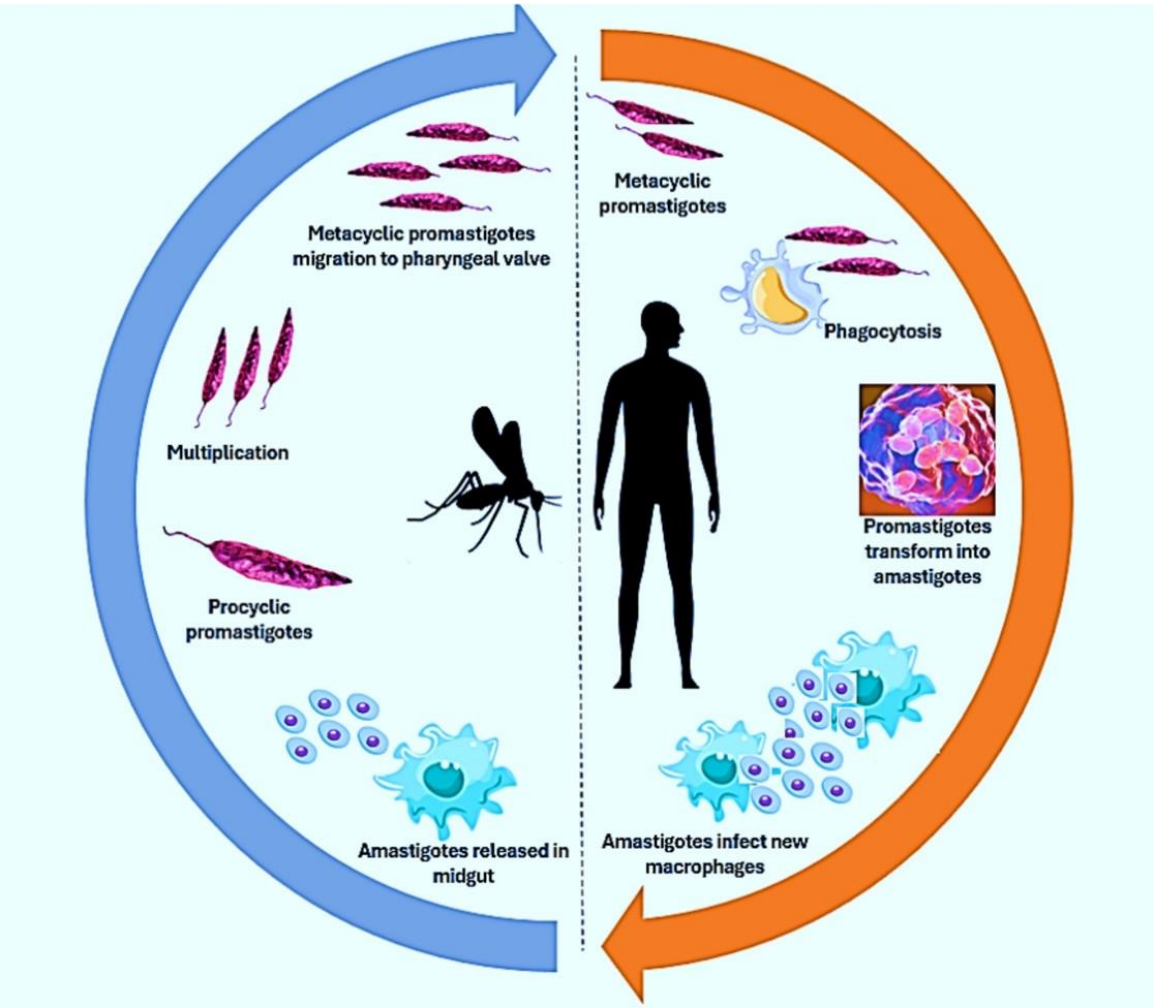

Figure 1: Schematic representation of the *Leishmania* life cycle, highlighting its developmental stages during transmission between the female sandfly vector and the human host.

The pathogenesis of VL hinges on the parasite's sophisticated evasion of host defences. Key parasite molecules, including lipophosphoglycan and GP63, interact with host receptors such as TLR2, TLR4, and TLR9 (Shadab and Ali, 2011). This interaction leads to suppression of the proinflammatory Th1 immune response and increased production of regulatory cytokines like IL-10, IL-4, and TGF-β, which promote expansion of regulatory T cells (Tregs) and T cell anergy, ultimately facilitating persistent infection and disease progression. Meanwhile, successful containment of the parasite requires a robust cell-mediated response, chiefly activation of macrophages by IL-12 to induce strong Th1-type immunity and IFN-γ production, which can effectively clear the parasite (Bhattacharya et al., 2016). Immunosuppression is a

hallmark of active VL patients, who often have reduced cell-mediated immunity, evidenced by negative leishmanin skin tests and impaired PBMC reactivity to *Leishmania* antigens.

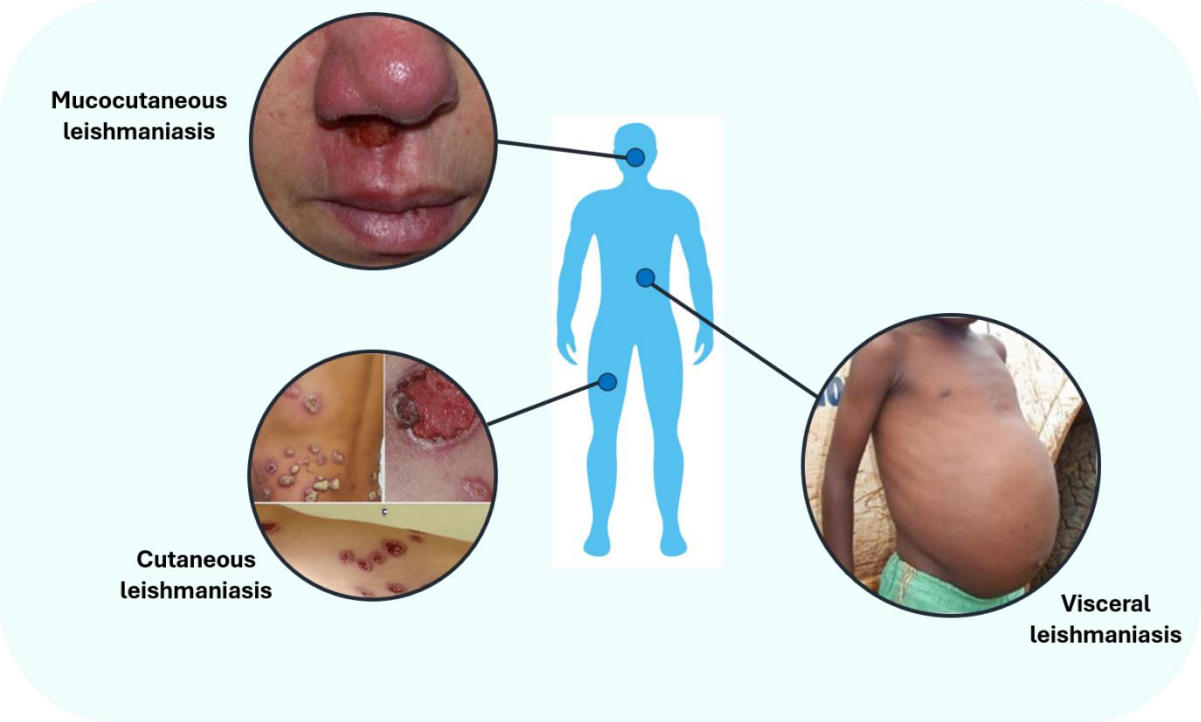

Figure 2: Diagram illustrating the different clinical forms of Leishmaniasis infection affecting humans.

Current treatment strategies for VL have evolved considerably due to widespread resistance and toxicity issues associated with older drugs. The mainstay therapies today include single-dose liposomal amphotericin B, highly effective and with fewer side effects, plus oral miltefosine and injectable paromomycin, often given in combination regimens to increase efficacy and curb resistance (Chakravarty and Sundar, 2019a). Ongoing research is focused on developing shorter, safer regimens and novel drug candidates to improve outcomes and address emerging resistance. There is currently no effective vaccine for visceral leishmaniasis.

Leishmania cell death mechanisms are an area of growing research interest, as they are important for both understanding disease pathogenesis and therapeutic targeting. *Leishmania* parasites are capable of undergoing programmed cell death with features reminiscent of apoptosis, such as DNA fragmentation and phosphatidylserine exposure (Lee et al., 2002). This

process can be triggered by stressors, including heat shock or nitric oxide, and appears to be distinct from metazoan apoptosis, primarily occurring without the involvement of caspases. In addition, *Leishmania* manipulate host cell death pathways: they inhibit host macrophage apoptosis and pyroptosis, forms of cell death that, if allowed, could help clear infection, thereby prolonging their intracellular survival (Fernandes and Zamboni, 2024). By interfering with pyroptosis (an inflammatory cell death pathway) and necroptosis (another lytic form of cell death), *Leishmania* ensure sustained infection of phagocytic cells and persistence in the hosts. Overall, understanding the immunopathogenesis of VL, advancements in treatment, and the intricacies of cell death in both host and parasite is essential for designing next-generation therapies (Barbosa et al., 2018).

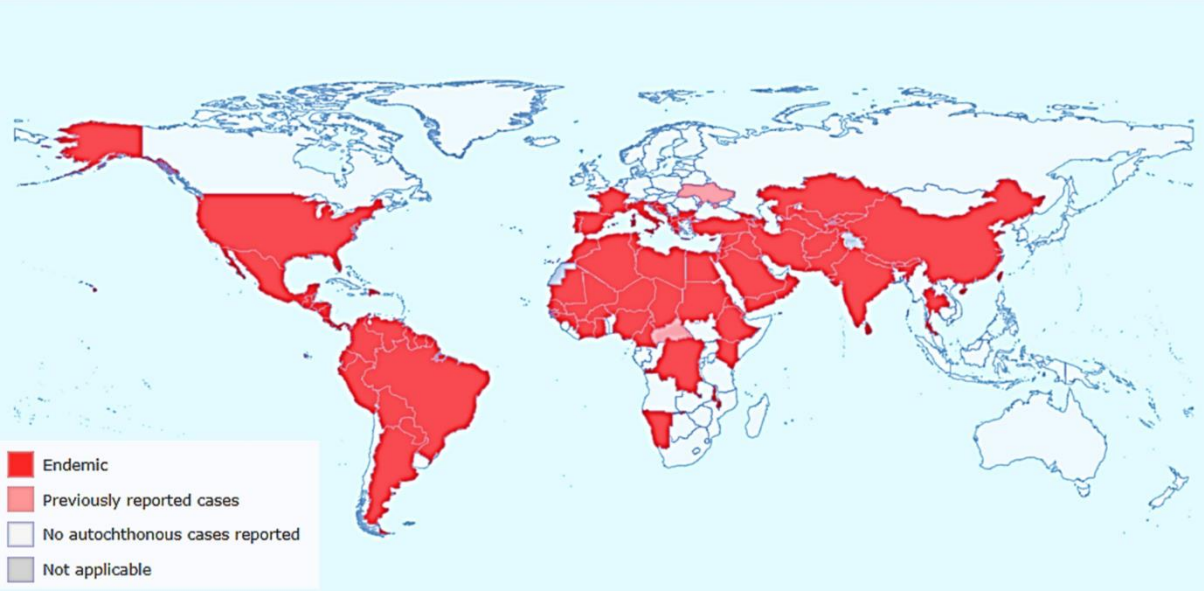

Figure 3: The global burden of Leishmaniasis in 2022, as reported by the World Health Organisation (adapted from https://apps.who.int/neglected_diseases/ntddata/leishmaniasis/leishmaniasis.html).

1.2 Coenzyme A

Andreas Kirschning hypothesised the origin and evolution of coenzymes. The simple, small, and non-protein chemical structure of coenzymes, often including nucleotides, can be thought

of as existing in the prebiotic era, playing a key role in metabolic networks. Coenzyme could have been involved in self-propagation and autocatalysis, or cycles of complex metabolic pathways involving the flux of primitive building blocks (Kirschning, 2021).

Often, proteins involved in site-specific oxidation/reduction and group-modification reactions are inactive without a coenzyme associate. The reverse is also true, as the coenzymes alone do not exert catalytic properties. A protein-coenzyme complex is required to complete enzymatic reactions like acylation and phosphorylation (Kirschning, 2021).

Around 9% of all known enzymes contributing to above 3,500 metabolic reactions use CoA or one of its forms as a metabolic cofactor. The ubiquitous nature of CoA makes it a crucial cofactor in nearly all organisms. The resultant component formed after CoA synthesis regulates the fundamental cellular processes, including gene expression, protein acylation, energy formation, autophagy, cell growth, differentiation, signal trafficking, cell death, etc (Cavestro et al., 2023; Nurkanto et al., 2018b; Sibon and Strauss, 2016).

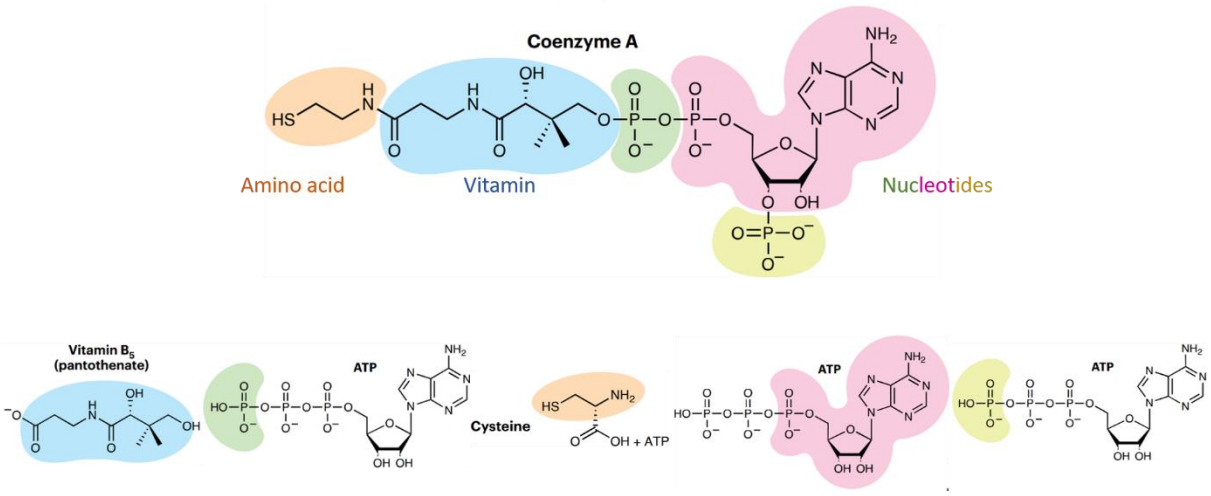

Figure 4: Coenzyme A structure derived from three essential substrates- vitamin B5, Cysteine, and ATP (adapted with permission from Barritt et al., 2024).

The structure of CoA is constituted by three substrates: the vitamin (Pantothenic acid), which acts as an essential precursor, the amino acid (L-cysteine) from which the thiol group and the nucleotides (ATPs), which are the energy donor (Figure 4) (Nurkanto et al., 2018b). Many archaea, bacteria, fungi and plants are able to synthesise Pantothenate *de novo* from aspartate and valine as precursors, but protozoans like *Leishmania spp*., *Trypanosoma spp*., and *Plasmodium spp*. lack the *de novo* Pantothenate synthesis pathway. Once inside the protozoan, pantothenate undergoes a series of events to convert into CoA, involving five steps. The first step is the phosphorylation of pantothenate to 4' -phosphopantothenate using ATP by pantothenate kinase (PanK), which is the first rate-limiting step in CoA biosynthesis. The second and third steps involve ATP-dependent addition of cysteine to form 4' -phosphopantothenoylcysteine by 4' -phosphopantothenoylcysteine synthase (PPCS), and decarboxylation to 4' -phosphopanthetheine by 4' -phosphopantothenoylcysteine decarboxylase (PPCDC). The fourth step involves adenylation to form 4' -dephosphocoenzyme A using ATP by 4' -phosphoadenylyltransferase (PPAT). The fifth and final rate-limiting step is the phosphorylation of CoA using ATP by dephosphocoenzyme A kinase (DPCK) (Figure 5). Unlike in mammals, where the final step is catalysed by a single bifunctional enzyme, CoA synthase (COASY), in protozoans, it is manifested by monofunctional PPAT and DPCK enzymes (Walia et al., 2011).

In protozoans, the salvage pathway for CoA has not been characterised, but of late, it has been hypothesised that there exist partial salvage mechanisms, comprising pantetheine and CoA degradation products. A typical salvage pathway allows the cells to bypass the actual *de novo* biosynthesis pathway by utilising the intermediate or degraded products. If the cell is able to utilize pantetheine, a CoA degradation product, it would bypass the two enzymes PPCS and PPCDC and would be directly phosphorylated by PanK to convert to 4’-phosphopantetheine and undergo further processing to get CoA (Nurkanto et al., 2018c).

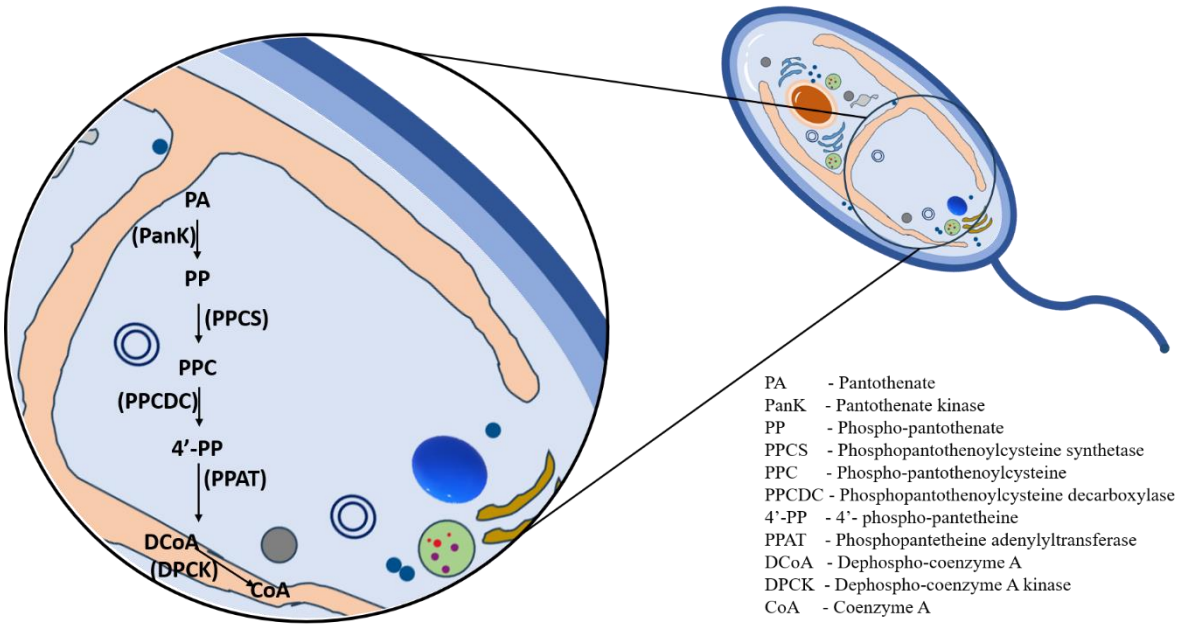

Figure 5: Biosynthetic pathway and subcellular localisation of Coenzyme A synthesis enzymes and intermediates in *Leishmania donovani*.

1.3 CoA-associated metabolic pathways

Multiple catabolic and anabolic pathways are associated with the CoA molecule. (1) CoA is required for the formation of Acetyl-CoA and Succinyl-CoA, which participate in the TCA cycle in the mitochondria. The Acetyl-CoA acts as a carbon donor in the TCA cycle, which supports energy production under nutrient-deficient conditions. (2) Malonyl-CoA and Acetyl-CoA are the precursors of fatty-acid synthesis and elongation pathways, which are essential for building cell membranes, especially during differentiation in the cytosol and mitochondria. (3) The conversion of pyruvate into acetate is further assisted by acetyl-CoA synthetase to form Acetyl-CoA, which is linked to the TCA cycle. (4) Fatty Acyl-CoA are involved as a substrate for lipid remodelling, like acylation, in surface proteins and signalling proteins for membrane localisation and protein trafficking. (5) Acetyl-CoA acts as the acetyl donor for histone acetylation, catalysed by histone acetyltransferase, which regulates the gene expression

plasticity. (6) In protozoa, DNA replication, antioxidant mechanisms and cell proliferation require spermidine, trypanothione and putrescine, present in kinetoplasts (Mignani et al., 2021). Transportation of these compounds requires acetyl from Acetyl-CoA (Table 1).

Table 1: Summary of various metabolic pathways and the diverse roles played by Coenzyme A in cellular function (Barritt et al., 2024).

Pathways	Function	Form
TCA Cycle	Oxidation of acetyl units for energy and intermediate generation in promastigotes	Acetyl-CoA, Succinyl-CoA
Fatty Acid Synthesis	Synthesis of membrane lipids and elongation of fatty acids	Acetyl-CoA, Malonyl-CoA
Acetate Metabolism	Acetate conversion to acetyl-CoA for biosynthesis and energy during glucose starvation	Acetyl-CoA
Protein Myristoylation & Palmitoylation	Acylation of surface and signaling proteins (GPI-anchored, signaling)	Fatty acyl-CoA
Histone Acetylation	Gene expression regulation via chromatin remodelling	Acetyl-CoA
Polyamine Metabolism	Synthesis of surface glycoconjugates for host interaction and immune evasion	Acetyl-CoA
Glycan & GPI Anchor Synthesis	Remodeling of phospholipids and synthesis of storage/structural lipids	Fatty acyl-CoA

1.4 Effect of inhibition of Coenzyme A on cell survival and proliferation

Inhibition of Coenzyme A biosynthesis leads to an increased abundance of long - and medium-chain fatty acids inside the cellular matrix. The accumulation of fatty acids can impair the integrity of membranes and may lead to cell death (Lamont et al., 2020).

The CoA to Acetyl-CoA ratio is decided by the transfer of the Acyl-group between intracellular compartments, stabilising CoA concentration in the cellular matrix. This ratio regulates the

acetylation of protein, which is involved in cell survival mechanisms like cell growth, autophagy and cell death (Sibon and Strauss, 2016).

Deleting PPAT and DPCK genes in *Saccharomyces cerevisiae* led to a lethal phenotype, which was reversed by re-expression. The depletion of COASY expression and subsequent decrease in CoA concentration in Zebrafish resulted in abnormal embryo development and mortality (Cavestro et al., 2023).

Gene silencing of the two isotopes of DPCK in *Entamoeba histolytica* (*ECOASY*1 and *ECOASY*2) resulted in intracellular CoA depletion and growth retardation in the protozoan parasite. Also, a reduction in the concentration of metabolites associated with amino acid, nucleic acid, glycogen metabolism, and chitin and polyamine synthesis. The reduced availability of these metabolites may govern the synthesis, packing and stability of DNA and RNA, which can further alter gene regulation, signalling, cellular trafficking and energy production (Nurkanto et al., 2018b).

1.5 Localisation of Coenzyme A

Coenzyme A is localised across multiple subcellular compartments in protozoa. The pool of free CoA and its intermediates is observed in different organelles such as the cytosol, mitochondria, and glycosomes (peroxisome-like organelle in kinetoplast). The multiple ionizable sites on Coenzyme A make it a charged molecule, which is membrane-impermeable. Therefore, it needs transporters to translocate to subcellular locations. Although organelle-specific transporters have not been extensively studied in protozoa, but the chemical nature of CoA suggests organelle-specific transporters, indicating affinity towards CoA and its intermediates between distinct cellular compartments (Sibon and Strauss, 2016). However, detailed quantification of CoA across different organelles has not been much studied in

protozoa; the available evidence in protozoa indicates the presence of CoA and its intermediates in not limited to a single location.

To date, organelle-specific isoforms of CoA biosynthesis enzymes have not been described in protozoa as reported in mammals. In protozoa, the early steps of CoA biosynthesis have been studied in the cytosol and later steps in organelles. Especially in *Leishmania*, PanK, PPCS and PPCDC are observed in the cytosol, and PPAT and DPCK are examined in organelles.

1.6 Rationale behind choosing DPCK as a drug target

Inherited disorders of Coenzyme A biosynthesis have been documented in humans. The first and the last enzymes of Coenzyme A biosynthesis have been associated with neurodegenerative disorders: pantothenate kinase-associated neurodegeneration (PKAN) and COASY protein-associated neurodegeneration (CoPAN). The third and fourth enzymes have been associated with rapidly fatal dilated cardiomyopathy (Cavestro et al., 2023).

The first and the last enzymes of the CoA synthesis pathway, PANK and DPCK (COASY in humans) are the rate-determining steps. The CoA and its derivative version inhibit the activity of PANK and DPCK, controlling the entire process by a feedback mechanism. Both of the kinases use ATP for phosphorylation (Cavestro et al., 2023; Nurkanto et al., 2018b).

Some organisms follow the salvage pathway, where the CoA intermediate, pantetheine, is used as a precursor for CoA synthesis, requiring only PPAT and DPCK activity (Butman et al., 2020).

The structural comparison between *ECOASY* and *M. musculus* COASY has indicated that, despite having a minimal sequence identity, they have better structural similarity. However, the CoA binding region, the P-loop region, and the LID region had significant structural dissimilarities, which could be manoeuvred to design specific DPCK inhibitors (Nurkanto et al., 2018b). The modelled structure of *Leishmania donovani* DPCK has revealed unique active

site features that distinguish it from the human homolog. These structural differences can be exploited to design selective inhibitors. The active site of *Leishmania donovani* DPCK contains distinct pockets that bind DCoA and ATP, making it possible to design compounds that competitively inhibit substrate binding or disrupt catalysis (Menpadi et al., 2023).

1.7 DPCK enzyme structure and mechanism

DPCK is identified as a NTP hydrolase superfamily. The class of these enzymes share structural similarity despite having low sequence similarity throughout many organisms. In general, the topology of DPCK is similar to nucleotide monophosphate-binding kinase, a member of the P-loop kinases. The three-dimensional structure of the enzyme consists of three domains: the substrate-binding site in α-helix, the nucleotide-binding site in β-sheet and the catalytic site (Butman et al., 2020; Leonardi et al., 2005; Nurkanto et al., 2018b; Walia et al., 2011). The established frame of DPCK consists of parallel β-sheets flanked by α-helices. This superfamily of enzymes is known for its highly conserved sequence, the Walker A sequence or the P-loop sequence (G_ _ _ _ GKT/S), which is the location of the nucleotide-binding site (Nurkanto et al., 2018b).

The mechanism of catalysis of the DPCK enzyme has been extensively studied in bacterial models and shows that the leading substrate, DCoA, binds first to the enzyme, which provides favourable conformational changes for the binding of the ATP molecule, which leads to phosphorylation to form the final product, CoA (Butman et al., 2020; Leonardi et al., 2005).

Only a few eukaryotes encode more than a single DPCK isotype, as observed in *Entamoeba histolytica* (*ECOASY*1 and *ECOASY*2) (Nurkanto et al., 2018b). In *Mycobacterium tuberculosis*, the DPCK enzyme exists as a mixture of monomer and trimer in equilibrium. It has been observed that the trimers transition into monomers in the presence of DCoA but not in the presence of ATP. Also, DCoA facilitates the binding of ATP to the enzyme. Inside

Mycobacterium, the DPCK enzyme exists in an inactive trimeric state, which converts to an active monomeric state in the presence of the primary substrate, DCoA, which assists the binding of the secondary substrate, ATP (Walia and Surolia, 2011).

Table 2: Comparative analysis of the biochemical and biophysical features of recombinant DPCK protein from different organisms.

Organism	Yield	Optimum pH	Purity	Molecular weight	Amino acid length	References
Entamoeba histolytica	0.6 mg/L	8	~ 90-95%	23.9 kDa (*ECOASY*1) 23.1 kDa (*ECOASY*2)	206 (*ECOASY*1) 204 (*ECOASY*2)	(Nurkanto et al., 2018a)
Plasmodium falciparum	-	8	90-95%	31.9 kDa (PfDPCK)	274	(Nurkanto et al., 2022)
Corynebacterium ammoniagenes	~36% yield	8.5	~2800-fold enriched	22.6 kDa	206	(Mishra et al., 2001)
Thermococcus kodakarensis	-	8	-	~20 kDa	-	(Shimosaka et al., 2019a)
Mycobacterium tuberculosis	-	8	Purified	47 kDa	211	(Walia et al., 2009)

Table 3: Kinetic characterisation of recombinant DPCK enzymes derived from multiple organisms.

Organism	**Substrate**	**K_m (µM)**	**V_{max} (µmole/min/mg)**	**K_{cat} (min^{-1})**	**Reference**
Mycobacterium tuberculosis	Dephospho CoA	34.9 ± 3.2	-	1.758	(Walia et al., 2009)
	ATP	56.8 ± 4.8	-	2.886	
Entamoeba histolytica (*ECOASY*1)	Dephospho CoA	114 ± 19	3.71 ± 0.43	88.9 ± 10.3	(Nurkanto et al., 2018b)
	ATP	19.6 ± 1.2	3.54 ± 0.09	84.8 ± 2.4	
Entamoeba histolytica (*ECOASY*2)	Dephospho CoA	57.9 ± 6.07	2.48 ± 0.15	57.5 ± 3.5	(Nurkanto et al., 2018b)
	ATP	15.0 ± 2.4	2.71 ± 0.17	62.9 ± 3.7	
Plasmodium falciparum	Dephospho CoA	105.3 ± 10.2	4.91 ± 0.38	-	(Nurkanto et al., 2022)
	ATP	88.14 ± 11.03	5.18 ± 0.29	-	
Thermococcus kodakarensis	Dephospho CoA	140	17.0 ± 0.8	5.57 s^{-1}	(Shimosaka et al., 2019a)
	ATP	260	20.4 ± 1.0	6.68 s^{-1}	

1.8 Inhibition of DPCK

Inhibition of DPCK would prevent the synthesis of CoA, leading to the accumulation of toxic intermediates, such as dephospho-CoA, and blocking critical metabolic processes. Alteration of CoA abundance would disrupt the TCA cycle, fatty acid metabolism, and the synthesis of acetyl-CoA, which is crucial for both energy production and biosynthesis. The lack of CoA would also impede the post-translational modifications of proteins required for cell survival, including acetylation and acylation (Table 4) (Menpadi et al., 2023).

Table 4: List of DPCK-targeting compounds and their mechanism of inhibition in multiple organisms.

DPCK inhibitors	Mechanism of Action	Selectivity	Preclinical studies	Target organism	Reference
CTP (cytidine triphosphate)	Mimics ATP, binds the kinase site	Moderate	Effective in vitro	*Mycobacterium tuberculosis*	(Walia et al., 2009; Walia and Surolia, 2011)
Multiple PfDPCK-specific HTS hits ("A-15", etc.)	Mimics ATP, binds the kinase site	Moderate	In vitro enzyme + cell assays	*Plasmodium falciparum*	(Nurkanto et al., 2022)
Hupehenine	Competitive binding to the active site	Moderate	Effective in vitro	*Leishmania donovani*	(Menpadi et al., 2023)
Veratramine	Competitive binding to the active site	Moderate	Effective in vitro	*Leishmania donovani*	(Menpadi et al., 2023)