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RECENT ADVANCEMENTS IN DUAL TARGETING STRATEGIES IN THE TREATMENT OF TUBERCULOSIS

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ABSTRACT:

An extensive review of tuberculosis(TB) treatment examines the difficulties in using regular DOTS therapy, particularly when dealing with drug-resistant strains of the disease(MDR-TB and XDR-TB). Polypharmacology's importance in tackling these issues is emphasised in theintroduction. Emphasis on dual inhibitors against fatty acid biosynthesis (which target the enzymes FabG4 and HtdX), dual targeting of Protein Tyrosine Phosphatase B and GroEL/ES chaperonin, and CTP and CoA biosynthesis suppression (which targets PyrG and PanK concurrently) (disrupting protein homeostasis, granuloma formation, and intracellular survival). An intelligent approach to improved medication delivery is shown by the combination of isoniazid and rifampicin encapsulated in liposome's. The review highlights the critical role that multi-targeted medications play in resolving tuberculosis issues. It also highlights the importance of further research and the possibility of reduced drug resistance, shorter treatment durations, and enhanced efficacy. All things considered, the information supplied provides a thorough and organised investigation of multi-targeted medications for the treatment of tuberculosis, skilfully merging scientific understanding with real-world uses.

KEYWORDS: Tuberculosis (TB), Dual targeting, Drug-resistant tuberculosis, Ant tubercular drugs, Drug development, Polypharmacology.

INTRODUCTION:

Mycobacterium tuberculosis (MTB) germs are the primary cause of tuberculosis (TB), an extremely deadly infectious disease [1]. Even though the lungs are the main organ affected, coughing, sneezing, or spitting can spread MTB-laden respiratory droplets to other organs. Even a minimal number of these germs can lead to tuberculosis in an individual.Symptoms of active tuberculosis include fever, sweats at night, weight loss, and a chronic cough that produces blood in the mucus [2].Two forms of TB exist: latent TB, recognized by the immune system without outward symptoms, and active TB disease, a multiorgan illness that can be fatal if untreated [1]. Current treatment strategies involve therapeutic regimens like Ethambutol, isoniazid, rifampicin, and pyrazinamide, provided free under government initiatives [3]. However, multidrug-resistant tuberculosis (MDR-TB) poses challenges due to limited medications [4]. Individuals with MTB often control the disease with their immune systems, but ongoing research focuses on vaccines or immunotherapies for vulnerable populations [3]. HIV-positive people, diabetics, underweight people, and people with compromised immune systems are more likely to have tuberculosis. The emergence of drug-resistant strains and co-infections with HIV have contributed to the recent rise in the incidence of tuberculosis[1].The problem is addressed by the current standard treatments, also known as DOTS therapy, which is a lengthy and complicated course of treatment that involves high dosages of bioactive(s) that are closely correlated to side effects. When DOTS chemotherapy is administered orally or intravenously, there are adverse effects associated with bioactivity when most of the bioactive moieties remain in the body instead of reaching their intended targets. This is the primary clinical problem with DOTS chemotherapy. Rapid

clearance and a brief plasma half-life are two other factors that restrict the bioactive(s)(3).By creating new, clever strategic drug delivery modules that may successfully get around and handle the drawbacks of traditional anti tubercular therapy, non-conventional drug delivery modules may be able to solve these clinical issues [2]. Polymeric implants, liposomes, vesosomes, micro particles, and polymeric microspheres are examples of bioactive carrier systems with significant promise for bioactive and efficient distribution [5].To address these clinical challenges, innovative drug delivery modules are being developed to overcome the drawbacks of traditional antitubercular therapy [3]. Non-conventional drug delivery systems, such as polymeric implants, liposome's, vesosomes, micro particles, and polymeric microspheres, show significant promise in enhancing the bio active's distribution and efficiency [6].

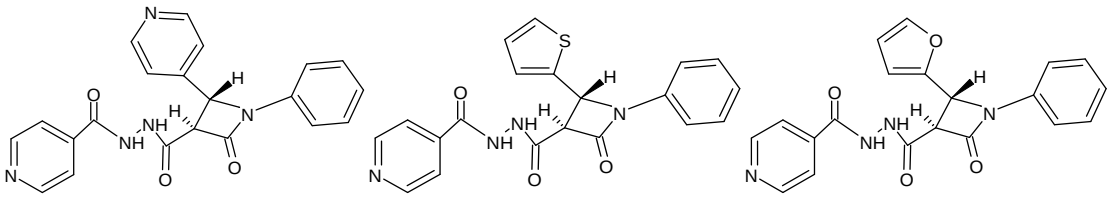
POLYPHARMACOLOGY MAY ASSIST IN THE FIGHT AGAINST MDR-TB:

In the fight against strains of MDR and XDR TB, drug resistance mechanisms pose a serious challenge [7].In fact, a single base mutation is typically all that is needed for a pathogen to become resistant to medication or treatment. One potential solution to get around this issue is the creation of a single pharmacological molecule that targets several locations. "Polypharmacology" is the term used to describe this type of multitargeting [8]."Polypharmacy" is the use of multiple medications, each with a strong affinity for a single target, given together as mixes or multi-component therapies, as opposed to polypharmacology [9]. Even if both approaches prove successful, polypharmacology has notable characteristics to emphasise [10]. First off, using a single molecule as opposed to multiple ones may have fewer adverse effects and cytotoxicity. Furthermore, it is anticipated that polypharmacology would yield greater therapeutic success than the traditional method, which emphasizes on one ideal target at a time [11].Additionally, because multitarget medications target multiple targets simultaneously, they exhibit synergistic or cumulative effects, which allows them to control complicated disorders faster and with lesser doses. Finally, they might be able to avoid the issue of drug interactions. All of these qualities have the potential to enhance the patient's quality of life. These factors make polypharmacology a new therapeutic approach in the creation of medications for cancer [9], neurological disorders [12], and synergistic bacterial illnesses [9]. Despite being a relatively new topic, the development of multitarget medicines has already led to various classifications based on their structures or mechanisms of action. Based on its mechanisms of action, a drug that collaborates in "vertical targeting" affects multiple targets within the same metabolic

pathway. A "series inhibition" occurs when two targets are related because they are consequential or follow the same path, as opposed to a "parallel inhibition," which happens when two targets are unrelated yet should share a similar substrate that can be replicated. Using vertical targeting is one tactic to combat the resurgence of some resistance mechanisms, such as mutations. By acting through "network targeting," a medication can prevent adaptive resistance and compensatory homeostatic responses by hitting multiple targets in distinct pathways [12].

DUAL TARGETING COMPOUNDS AGAINST M. TUBERCULOSIS:

Dual Inhibitors Targeting Fatty Acids Seek to Obstruct Biosynthetic Routes Because the cell wall of Mycobacterium TB is so thick, which is essential to its survival during host infection, provides it with some resistance against molecular diffusion within the cell.Fatty acids are synthesised primarily by the fatty acid synthesis (FAS) pathway, constituting one of the primary components of the cell wall [7]. The FAS-I system, which Produces long-chain fatty acids; FAS-II is a multidomain enzyme that catalyses the production of short-chain fatty acids, are the two main routes of MTB.Other fatty acids, however, must be avoided since they depend on other metabolic pathways. One such mechanism is the metabolism of linked CoA-dependent fatty acids, which may offer a therapeutic target for novel antitubercular medications. Here, Banerjee et al. chose FabG4 and HtdX, two enzymes that are encoded on a single locus to produce distinct multitargeting compounds. As the final result of the reaction that FabG4 catalysed, the reduced ketoacyl is handled by -ketoacyl CoA reductase by the first enzyme and 3(R)-hydroxyacyl CoA dehydratase by the second enzyme [13].Given that both enzymes are involved in successive phases of a metabolic pathway and are reportedly necessary for MTB survival, Anticipate synergistic effects from a common inhibitor.For this purpose, the authors used a ligand- and structure-based design technique. Based on the catalytic sites' structural features, they first selected the β-lactam and isoniazid scaffolds as pharmacophores, in addition to a few aromatic rings. Following their combination, the pharmacophores formed a small library that was utilised in docking studies to identify putative inhibitors. After doing this investigation, seven scaffolds were chosen, and three of them shown notable activity against both enzymes [7].**(Figure 1)**



(1) (2) (3)

Figure 1:FabG4 and HtdX dual inhibitors

Description: Banerjee et al. created dual inhibitors that target the enzymes FabG4 and HtdX, which are expressed on a single locus, in order to interfere with the fatty acid biosynthesis pathways in Mycobacterium tuberculosis (MTB) .In the CoA-dependent fatty acid metabolism, HtdX performs the role of a 3(R)-hydroxyacyl CoA dehydratase and FabG4 as a -ketoacyl CoA reductase. It is believed that concurrent inhibition of both enzymes will have synergistic effects, affecting critical stages of the metabolic pathway essential to MTB survival.β-lactam and isoniazid scaffolds were chosen as part of the design strategy, and aromatic rings were included as pharmacophores. Three of the seven scaffolds found through docking studies had notable activity against the FabG4 and HtdX enzymes [13].

A) INHIBITION OF CTP AND COA BIOSYNTHESIS:

The simultaneous inhibition of two distinct but connected pathways is one benefit of dual-targeting drugs, and this should have complementary effects. This is true for PyrG and PanK, two inhibitors[14].In a high throughput phenotypic screening study, the two chemical compounds, 5-methyl-N-(4-nitrophenyl) thiophene-2-carboxamide and 3-phenyl-N-[(4-piperidin-1-ylphenyl) carbamothioyl] propanamide, were initially found to be inhibitors of the CTP synthetase PyrG [15].It was shown that both compounds were prodrugs, requiring the activation of monooxygenase EthA to exhibit antimycobacterial activity—but only to the particular active sulfone product that was discovered. It was discovered through additional research that these compounds can also block pantothenate kinase PanK, indicating that they may have at least one other target.

The initial stage of the production of coenzyme A (CoA) is facilitated by this enzyme, which transforms pantothenate (vitamin B5) into 40-phosphopantothenate. Additionally, it was shown that two other compounds that had been found through target-based screenings against PyrG inhibitors in vitro and in silico, respectively, similarly inhibited PanK activity [16]. Because CoA is a necessary cofactor for numerous important enzymes involved in various

metabolic processes, including the production and catabolism of fatty acids, reducing its bioavailability offers an appealing therapeutic approach [17].**(Figure 2)**

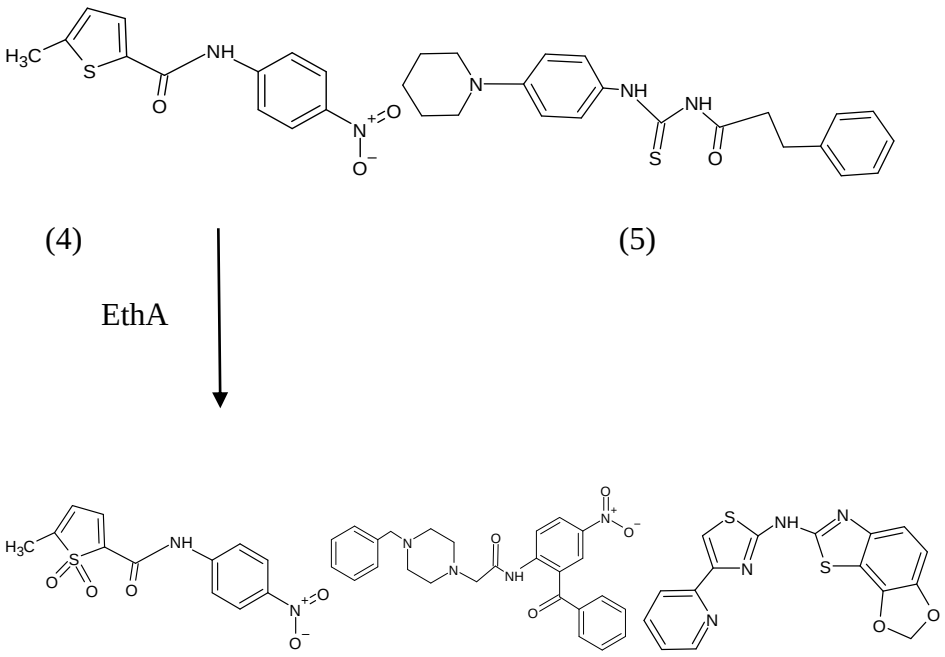


Figure 2: Dual PyrG-PanK inhibitors.

Description Dual-targeting drugs have great therapeutic promise since they simultaneously block related pathways and produce synergistic effects by inhibiting both pantothenate kinase (PanK) and CTP synthetase PyrG.Initially described as PyrG inhibitors, 3-phenyl-N-[(4-piperidin-1-ylphenyl) carbamothioyl] and 5-methyl-N-(4-nitrophenyl) thiophene-2-carboxamidefor propanamide to be effective against mycobacteria, it must function as a prodrug and be activated by monooxygenase O. [18]. These inhibitors are noteworthy for their ability to block PyrG and PanK, demonstrating dual-targeting. Additional compounds found through target-based screens target PyrG, which is in charge of the first stage of coenzyme CTP synthesis. Some of these compounds also exhibit concurrent inhibition of PanK [17].Because PanK is essential to CoA biosynthesis and transforms pantothenate (vitamin B5) into 4'-phosphopantothenate, its suppression is especially noteworthy. Consequently, considering that CoA is crucial for many metabolic activities, such as the synthesis of fatty acids and catabolism, a potential treatment approach is the decrease in CoA bioavailability.

Dual-targeting drugs have multiple benefits when it comes to treating mycobacterial infections. One such benefit is the activation of inhibitors by the monooxygenase EthA [18].

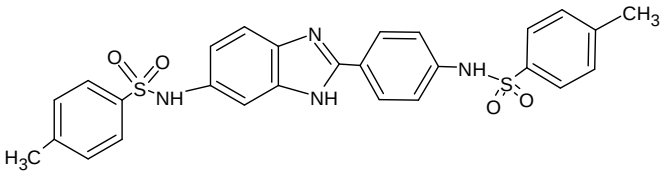
B) COMBINED TARGETING OF PROTEIN TYROSINE PHOSPHATASE B AND GROEL/ES CHAPERONIN:

Molecular chaperones and other protein homeostasis mechanisms are becoming popular targets for antibacterial drug discovery. Two different chaperonins for GroEL/ES (GroEL1 and GroEL2), one of which aids in protein folding and the other in proper protein breakdown, are found in M. tuberculosis [19]. They preserve the equilibrium of proteins within cells in conjunction with proteases. GroEL1 is essential for granuloma formation, whereas only GroEL2, out of the two chaperonins with a sequence identity of only 61%, is required for MTB survival. Thus, to prevent both latent and active tuberculosis, it should be effective to simultaneously suppress these two proteins. Because of this, Johnson et al2014.'s biochemical screen of 700,000 small molecules yielded 235 compounds that block GroEL/GroES-mediated refolding. To date, 22 of these compounds have undergone antibacterial activity testing [20]. Beginning with the benzimidazole-based molecule, the authors produced a range of derived benzoxazoles that demonstrated significant activity against Gram-positive bacteria and varied sulfonamide end caps [21].

These results have led to the testing of these compounds against MTB; however, "half-molecules" (containing a single sulfonamide end-capping on either the left or right side of the molecule) have also been generated in an attempt to ascertain whether the cytotoxicity of these inhibitors could be reduced by simple analysis. The persistent superiority of the "full-molecules" relative to the "half-molecules" suggested that both aryl-sulfonamide moieties were critical for inhibition [22].

However, the researchers found that there is interaction between the protein and the two (oxalylamino-methylene)-thiophene sulfonamide (OMTS) molecules. Tyrosine phosphatase B's active site in a direction that is essentially similar (PtpB) [23].Specifically, PtpB's crystal structure in complex with the inhibitor revealed that, in accordance with the compounds, the sulfonamide moiety of each OMTSmolecule was located approximately 11–12 Å away, implying that they may bind to the proteinand bridge the proximal and distal regions of the active site [19].In the cytoplasm of macrophages, the MTB phospho-tyrosine phosphatase PtpB is produced and interacts with Erk1/2, p38-mediated IL-6 production, and Akt signalling. This interferes with the macrophages' immunological response and aids in intracellular survival. Given this information, a dual targeting drug that can inhibit PtpB and

the GroEL/ES chaperonin systems ought to be a successful treatment for tuberculosis at all phases [21]. To achieve this, two compounds were found and described that contained a primary amine on the left or right side of the structure and a 5-chlorothiophene. These substances demonstrated a moderate level of antitubercular action, but they also exhibited strong selectivity against the target enzymes' human counterparts, making them a suitable beginning point for the creation of more effective multitargeting inhibitors [23]. (**Figure 3)**



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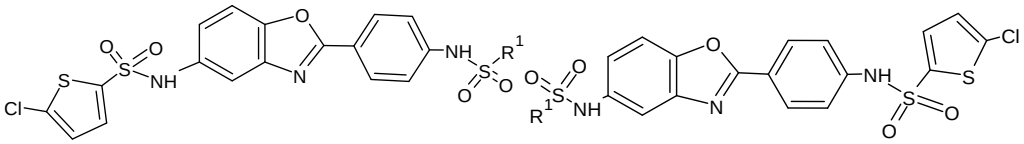

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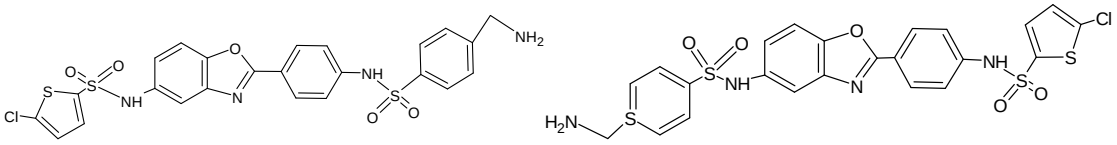

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Figure 3 : Structure of the GroEL/ES chaperonins (GroEL/GroES) and protein tyrosine phosphatase B(PtpB) dual inhibitor

Description: With an emphasis on Mycobacterium tuberculosis, molecular chaperones and protein homeostasis mechanisms are becoming vital targets for the development of antibacterial drugs (MTB). In order to preserve cellular protein homeostasis, MTB has two unique GroEL/ES chaperonins, GroEL1 and GroEL2, which are involved in protein folding and breakdown, respectively. Proteases are also present. Although GroEL1 is crucial for the development of granulomas, GroEL2 is necessary for MTB survival, indicating that dual inhibition may be an effective strategy for treating both latent and active tuberculosis [24]. 235 compounds were found using a biochemical screen to block GroEL/GroES-mediated

refolding; these compounds were then examined for their antibacterial activity. Benzimidazole-based compounds and other derived benzoxazoles with various sulfonamide end caps have significant efficacy against Gram-positive bacteria.The necessity of both aryl-sulfonamide moieties for inhibition was demonstrated by a further investigation that involved the creation of "half-molecules" to evaluate cytotoxicity mitigation [19]. The virulence factor protein tyrosine phosphatase B (PtpB), which is produced by MTB, was notably engaged by two molecules of (oxalylamino-methylene)-thiophene sulfonamide (OMTS) [25]. Potential bridging of proximal and distal regions of the active site was suggested by the binding capacity of the OMTS sulfonamide moieties as indicated by the crystal structure of PtpB. To support MTB's intracellular survival, PtpB impedes the immune responses of macrophages. Drugs that target both PtpB and GroEL/ES chaperonins, such as those that include 5-chlorothiophene and a primary amine, are examples of dual-targeting medications that exhibit great selectivity against human equivalents while still exhibiting a moderate antitubercular effect. A promising foundation for the creation of potent multitargeting inhibitors is provided by these substances [19].

C) COMBINATION OF RIFAMPICIN AND ISONIAZID:

In a study, Shrivastava et al. showed that it is possible to successfully incorporate two antitubercular drugs—Isoniazid and Rifampicin—into liposome's[26,27]. The hydrophobic drug is intercalated in the lipid domains of the liposome's, while the hydrophilic drug is entrapped in the aqueous domains. The hydrophilic drug is confined in the aqueous domains of the liposomes, while the hydrophobic drug intercalates in the lipid domains. First-line antitubercular medications include isoniazid and rifampicin; nevertheless, despite their notable and specific antitubercular effect, they are linked to significant liver metabolism, short biological half-lives, and contraindicated symptoms [27,28].

The drug(s) delivered by the generated, publicly disclosed mannosylated Liposome's enter the cellular structures of pathogenic agents, such Mycobacterium TB.The residual bacterial count assessed as a colony-forming unit (CFU) in macrophages when mannosylated liposome's are utilised instead of the free drug indicates the targeted delivery of antitubercular medication(s) to the macrophages and their cell-specific accumulation(s) [29]. Comparing drug-loaded mannosylated liposome's to non-mannosylated liposome's, infected Balb/C mice showed the greatest in vivo antitubercular activity. Furthermore, it was discovered that the activity was more noticeable than the free drug that was free (s). Both active and passive absorption of the liposomal preparations by macrophages led to their

tendency to accumulate [30]. Mannosylated liposome'scan target alveolar macrophages with a combination of antitubercular drugs, according to an in vivo investigation. The antitubercular activity seen in vitro and in vivo exhibits a strong correlation [29].

Description:

Liposome's, which are small spherical vesicles composed of lipids, are an effective way to deliver antitubercular medicines to macrophages infected with Mycobacterium TB [26]. Both hydrophobic and hydrophilic interactions are used in this liposomal delivery system: hydrophobic rifampicin intercalates in the lipid domains, while hydrophilic isoniazid is entrapped in the aqueous domains [26, 27]. Medications classified as first-line antituberculars have several drawbacks, including high liver metabolism, brief half-lives, and possible adverse effects [29]. In order to get around these problems, mannose-containing liposome's have been created [31]. These liposome's have been shown to have better macrophage targeting and increased antitubercular efficacy in both in vitro and in vivo investigations [29].

D) MULTITARGETING OF THE FOLATE PATHWAY:

Only the folate metabolic route can produce folate, which is necessary for the production of methionine, N-formyl methionyl-tRNA, glycine, serine, pantothenate, purines, thymidine, and deoxythymidine monophosphate (dTMP) [32].The conversion of dihydrofolate (DHF) to tetrahydrofolate is catalysed by DHFR, an enzyme that is crucial to the folate pathway (THF). The folA gene encodes it [33]. **(Figure 4)**

By producing 5,10-methylene tetrahydrofolate, a one-carbon donor for the synthesis of purines, methionine, histidine, and deoxythymidine monophosphate (dTMP), the folate pathway contributes significantly to cell viability. If this process is disrupted, there will be a significant shortage of these important molecules, which would limit DNA replication and ultimately result in cell death [34]. An essential component of the folate system, dihydrofolate reductase (DHFR) is what converts dihydrofolate (DHF) to tetrahydrofolate in a NADPH-dependent manner (THF).DHFR is not currently used in TB therapy, despite being a recognised therapeutic target for protozoal and bacterial diseases. Methotrexate, pyrimethamine, and trimetrexate are potent inhibitors of the MtbDHFR enzyme but do not prevent Mtb from developing, despite being clinically approved antifolates [35,36]. This is probably because they cannot penetrate the lipid-rich cell wall. One promising approach to TB medication discovery and development is the creation of antifolate chemicals that both suppress the growth of live Mtb and the function of the MtbDHFR enzyme [32].

A different folate route found in Mtb was also studied by Hajian et al. in relation to these chemicals. In this alternative method, THF and dTMP are produced by flavin-dependent thymidylate synthase (FDTS), which is encoded by thyX, and Rv2671, a second DHFR identified in Mtb (Figure 1). The identification of these enzymes has significant consequences for the development of antifolate drugs since they give the organism a means of easily developing resistance to antifolates [32]. thyX or Rv2671 overexpression has been associated with resistance to PAS and DHFR inhibitors.. It is unclear, nevertheless, whether inhibiting these compensatory enzymes alone or in combination with both pathways increases the antimycobacterial drugs' ability to kill live bacilli [36]. **(Figure 4)**

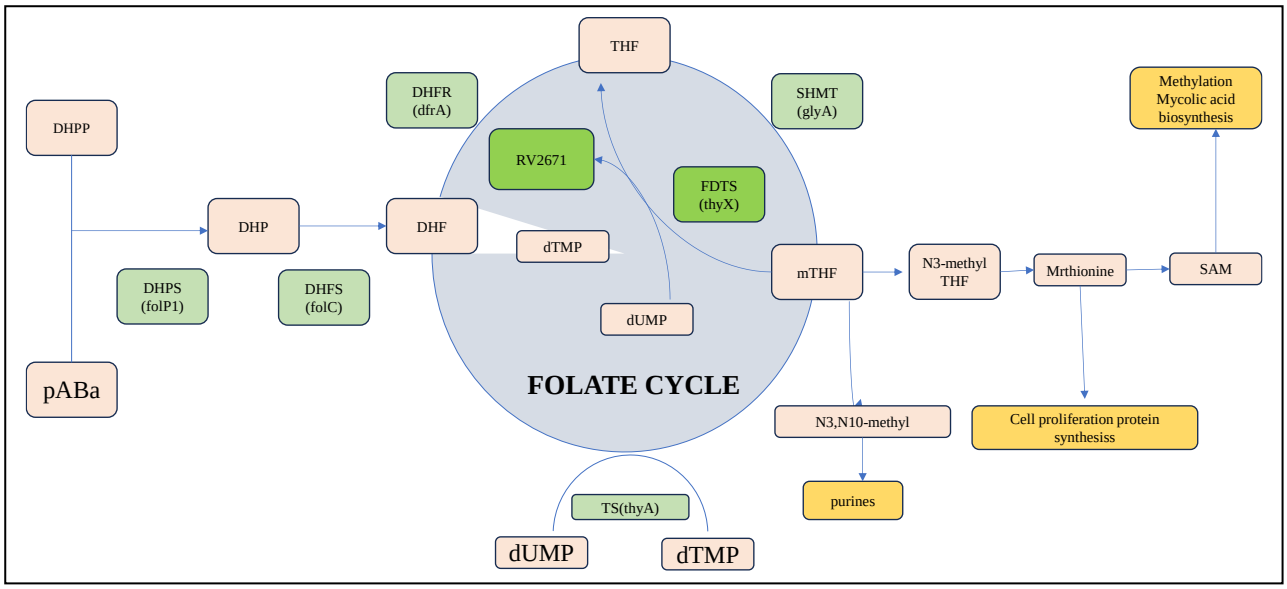

Figure 4: Folate Pathway of Mycobacterium tuberculosis

The enzymes that make up the canonical route are (in light green): DHFS, dihydrofolate synthase; DHFR, dihydrofolate reductase; TS, thymidylate synthase; and SHMT, serine hydroxyl methyltransferase. The (dark green) enzymes of the alternative route are flavin-dependent thymidylate synthase (FDTS) and Rv2671.Here, Hajian et al. demonstrate how certain INCAs exhibit potent dual inhibition of both Rv2671 and Mtb DHFR, which amplifies their efficacy against Mtb cells.Lengthy target occupancy is produced by the INCA inhibitors' slow rate of dissociation from DHFR, which is an advantageous property for antibacterials. When compared to INCAs, PAS metabolite (PAS-M) was shown to be a mild Mtb DHFR with no discernible inhibitory action on Mtb Rv2671 [32]. X-ray crystallography was employed to elucidate the drug-target interactions that underpin the efficaciousness of

PAS-M and INCAs [34].They discovered, via further investigation, that FDTS is an important secondary target for PAS-M, suggesting that PAS has multiple targets for action.Additionally, they showed that the synthesis of mycolic acids is significantly disrupted when Mtb cells are treated with INCAs or PAS. Taken together, our findings suggest that multi-targeted antifolates provide viable alternatives for the development of innovative ant tubercular medications [32].

Description:

To disrupt the vital folate metabolic route in Mycobacterium tuberculosis, multitargeting of the folate pathway is a strategic method in tuberculosis (TB) treatment development (Mtb) [36]. The production of folate, a cofactor required for several cellular functions such as DNA synthesis and cell survival, depends on this system. An essential component of this system is dihydrofolate reductase (DHFR), which converts dihydrofolate to tetrahydrofolate [35]. Methotrexate and pyrimethamine, two therapeutically licenced antifolates, suppress Mtb DHFR but are unable to effectively reduce Mtb growth, possibly because they have difficulty penetrating the lipid-rich cell wall. To tackle this issue, scientists are exploring substances that not only impede MtbDHFR but also specifically target other enzymes involved in the folate pathway, like flavin-dependent thymidylate synthase and Rv2671 (FDTS). Improved effectiveness against Mtb cells is demonstrated by certain drugs, such as INCAs, which dual block Mtb DHFR and Rv2671. The utilisation of a multitargeted strategy augments the effectiveness of antifolates, as gradual dissociation rates guarantee continuous target occupancy [33]. Furthermore, the possibility of multitargeted antifolates in the development of novel antitubercular drugs is further highlighted by the interruption of mycolic acid formation in Mtb cells [32].

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