2. Literature Review

2.1. Why New Antitubercular Drugs?

First-line oral drugs *i.e*. isoniazid, pyrazinamide, rifampicin, and ethambutol are being given in combination for the first two months of TB treatment and continued with isoniazid and rifampicin for additional four months. The longer duration of treatment has resulted in patient noncompliance and ultimately leading to emergence of drug-resistant tuberculosis [17]. Thus, there is definite need for novel drugs with highest potency to shorten the treatment period and thereby, improving the patient compliance, distribution cost and also programme supervision. Under the nutrient deprived and anaerobic conditions, *Mycobacterium tuberculosis* (*Mtb*) develops latent infection that in turn transform into active disease at any moment in lifetime. [17]. Hence, dormant/latent TB needs to be cured before its conversion into active disease [18]. The treatment of resistant TB is also a challenging task. The *Mycobacteria* develop resistance to the first-line drugs *i.e*., rifampicin and isoniazid in case of MDR-TB, whereas all the fluoroquinolones and at least one of the three injectable second-line drugs *i.e*., capreomycin, kanamycin, or amikacin develop resistance along with rifampicin and isoniazid in XDR-TB (**Figure 2-1**). The second-line and third-line anti-TB drugs are bound with high toxicity [19]. The efficacy is also reduced in immune compromised HIV patients. Therefore, the developed novel drug molecule should also be compatible with antiretroviral agents [20, 21]. The treatment has improved with the approval of Bedaquiline in 2013 [22], but it developed the first case of resistance in the following year [23] [24]. The rapid development of resistance is a huge set back in control of the disease. The drugs used in the present treatment regimen are inadequate to

address latent, MDR and XDR-TB. Therefore, novel and potent drugs are definite necessity for effective control of the complex disease.

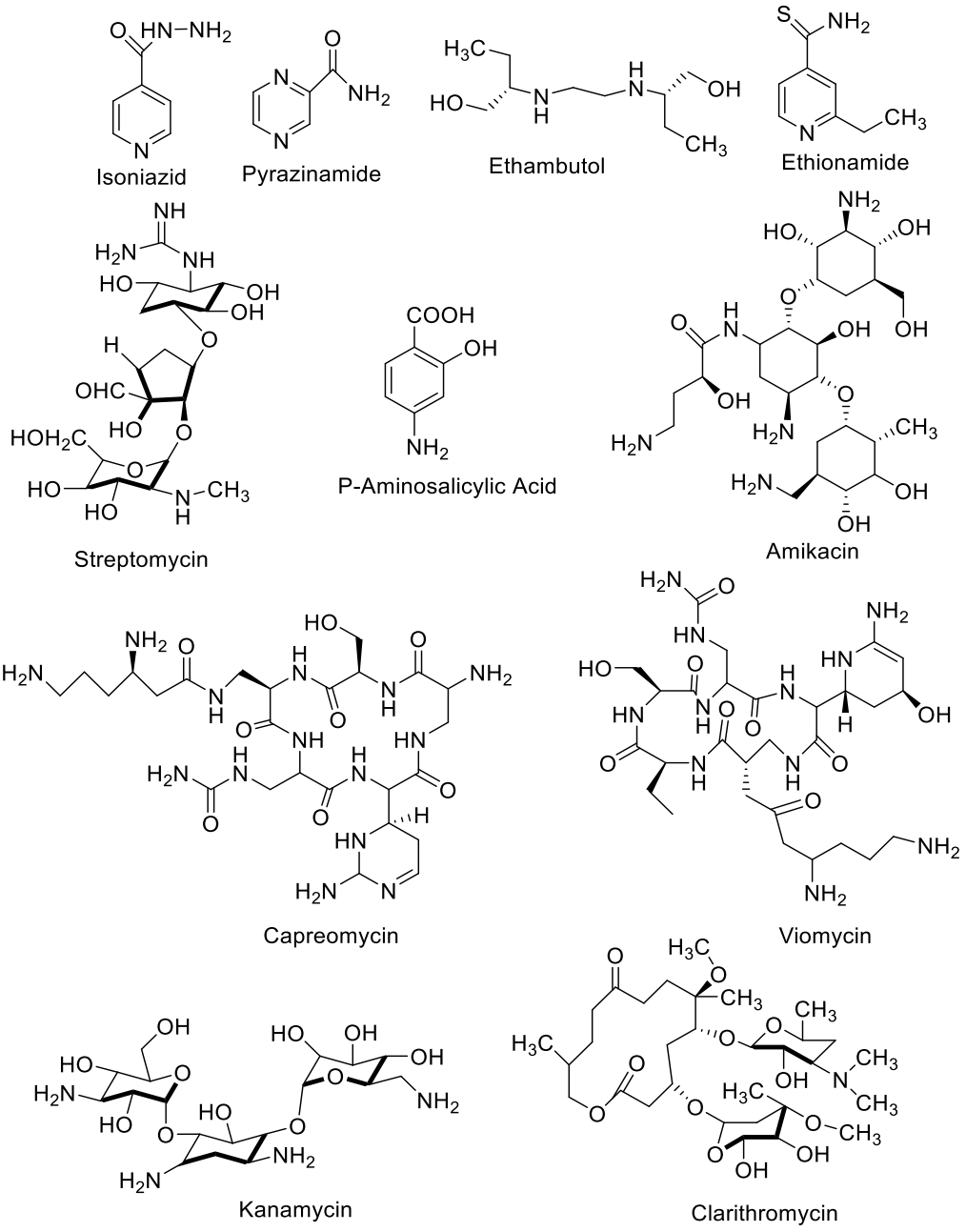


Figure 2-1:Drugs used in the management of Tuberculosis

2.2. Why Phenothiazine Scaffold Against Tuberculosis?

Paul Ehrlich proclaimed a phenothiazine dye called methylene blue by the end of 19th century. It was found to render the bacteria immobile more effectively [25, 26], but the antipsychotic activity had surpassed the antimicrobial efficacy [27, 28]. Since then,

phenothiazines are used in the treatment of psychosis. Chlorpromazine (CPZ) is the first phenothiazine drug used in the management of psychosis. It also displayed promising activity against several bacteria, mycobacteria, protozoa and viruses [29]. Hence, phenothiazines can be optimized to develop as antimicrobial agents. Further, the mildest antipsychotic phenothiazine Thioridazine was found to be active against both susceptible and resistant strains [17, 30, 31]. Therefore, optimization of the phenothiazine scaffold may be fruitful in the development of antitubercular drugs.

2.2.1. Repurposing of Psychotropic Phenothiazine Drugs

The drugs used in the treatment of a particular disease may also produce a secondary therapeutic effect by interacting with unrelated target(s). The secondary effect may become efficacious to cure other ailments [32]. Phenothiazines were primarily used as antipsychotic drugs but were also reported for secondary antitubercular activity. The first neuroleptic phenothiazine drug Chlorpromazine was reported to show promising antitubercular activity against *Mtb* H37Rv [33], but the extrapyramidal side-effects (EPS) at plasma concentration required for antitubercular activity prevented its transformation as an antitubercular drug. Phenothiazines exhibited promising activity against both drug-susceptible and drug-resistant strains of *Mtb*. They also exhibited synergism with the drugs in first-line treatment. Therefore, Phenothiazines can be used as an adjuvant to reduce the dose and toxicity of first-line agents [34]. The approach of repositioning the older agents/drugs for the treatment of TB is well recognized across the globe by researchers [35]. There is a renewed interest in repositioning the neuroleptic phenothiazines for treatment of TB. Chlorpromazine was found effective against intracellular mycobacteria at concentrations of 0.23 to 3.6µg/mL [30] [36]. It acted through blocking the function of efflux pumps in the cytoplasmic membrane [37]. It also exhibited potent *in-vivo* activity against both susceptible and

resistant *Mtb* strains. When chlorpromazine was used as an adjuvant at a concentration that was least effective against MDR TB, the activity of rifampicin and streptomycin steeply increased [38]. 7-hydroxychlorpromazine also showed synergism with spectinomycin, 25-desacetylrifampicin, streptomycin and kanamycin with fractional inhibitory concentration index (FICI) in the range of 0.19–0.5 [32]. Greatest synergy is noted between chlorpromazine and spectinomycin with FICI of 0.31 [17]. Thioridazine was comparatively more effective than chlorpromazine with less EPS side-effects, but the adverse cardiac side-effects limited its usage [39]. The *levo* form of thioridazine was found to be free from all such side effects [40]. Thioridazine was effective against isoniazid, streptomycin, rifampicin, pyrazinamide and ethambutol resistant strains. It also showed synergism with streptomycin and rifampicin [38]. Trifluoperazine produced potent antitubercular activity against *Mtb* H37Rv and isoniazid-resistant strains with MIC in the range of 5 to 8µg/mL. It also exhibited potent activity against both susceptible and resistant *Mtb* strains [41, 42]. Levomepromazine is a low potent antipsychotic drug that produced antitubercular activity with MIC of 10µg/mL. Diethazine (anticholinergic) and Promethazine (antihistaminic) produced promising activity against *Mtb* H37Rv with MIC of 20µg/mL [43] (**Figure 2-2** & **Table 2-1**).

2.2.2. Phenothiazine Derivatives Possessing Antitubercular Efficacy

Dunn *et al* used alkyl triphenyl phosphonium (alkylTPP) cation as a drug functionality to improve the intracellular delivery of phenothiazine derivatives. The incorporation of alkylTPP cations exceptionally increased the drug concentration at the biological membrane and bound to Type-2 NADH dehydrogenase (NDH-2) enzyme present within the mycobacterial membrane. Eventually, the potency of phenothiazines increased up to 130-fold [44].

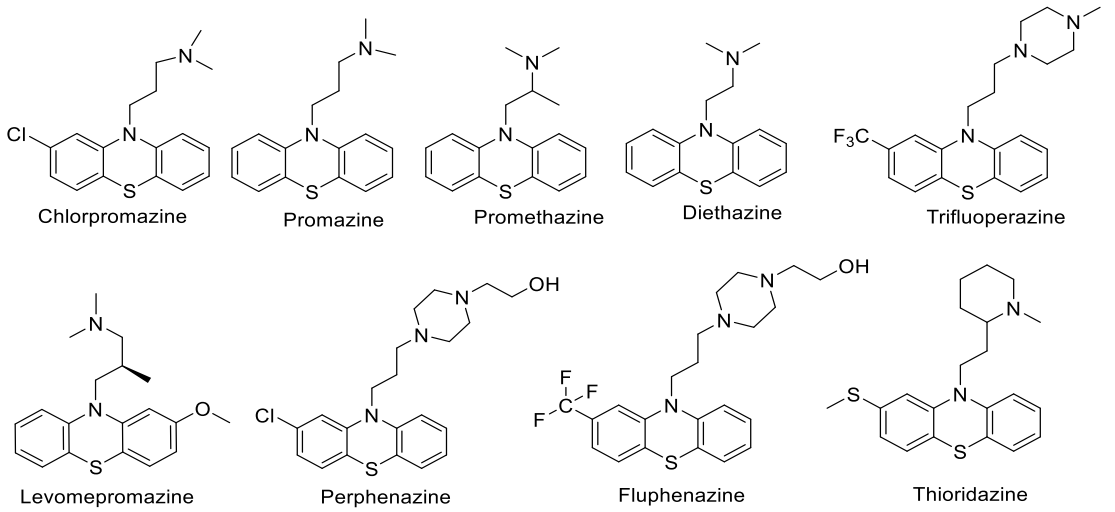


Figure 2-2: Neuroleptic phenothiazines in management of Tuberculosis

Phenothiazines	**MIC(µg/mL)**	**System**	**Reference**
Chlorpromazine	25	ADT	[34]
Chlorpromazine	12	ADT, Cfu	[30],[45]
Chlorpromazine	10	ADT, Cfu	[17], [45]
Chlorpromazine	12.5	ADT, Cfu	[31], [45]
Chlorpromazine	0.9	Mac	[44]
Chlorpromazine	4–32	Bactec	[27], [45]
Chlorpromazine*	10	Bactec	[17]
Chlorpromazine**	20–30	Bactec	[17]
Chlorpromazine	1–16	ADT	[31]
Chlorpromazine sulfoxide	>100	ADT	[31]
Levomepromazine	10	ADT, Cfu	[31], [45]
Levomepromazine	25	ADT	[30]
Promethazine	20	ADT, Cfu	[31], [45]
Diethazine	20	ADT	[31]
Trifluoperazine	5–8	ADT, Cfu	[46], [45]
Trifluoperazine	2.5–7.5	ADT	[47]
Trifluoperazine	8–32	ADT	[32]
Methildiazine	5–12.5	ADT	[48]
Methildiazine	5-15	Cfu	[45]
Promazine	16 to >32	ADT	[31]
Thioridazine	2–16	ADT	[31]
Thioridazine	8-32	Bactec	[32], [45]
Thioridazine*	15	Bactec	[17]
Thioridazine**	20–30	Bactec	[17]
Thioridazine	4	ADT	[17]
(S)-Thioridazine	4–16	ADT	[31]
(R)-Thioridazine	4–16	ADT	[31]

Table 2-1: Psychotropic Phenothiazine drugs and their MIC against *Mtb*; ADT- Agar Diffusion Test. Mac- macrophage; Bactec- generation of $^{14}CO_2$ in Bactec 460 System, 12B vials; *MIC observed against sensitive strains of M. *tuberculosis*; **MIC observed against multidrug resistant strains of *M. tuberculosis.*

Phenothiazine-alkylTPP cation conjugate (**P-3a**) is represented in **Figure 2-3** [46]. Addla *et al.,* synthesized some triazole fused trifluperazine derivatives by click chemistry. Compounds **P-13c**, **P-13l** and **P-13o** produced potent *in-vitro* anti-tubercular activity against *Mtb* H37Rv with MIC of 6.25 µg/mL and selectivity index (SI) value >10 (**Figure 2-3**) [49]. Seelam *et al.,* reported some thiazolidinone tethered phenothiazine derivatives for significant anti-tubercular activity against *Mtb* H37Rv. Compounds **P-14b** and **P-14g** were found to be significant in comparison to the standard drug Isoniazid (**Figure 2-3**) [50]. Trivedi *et al.,* reported pyrazole [3,4-d]pyrimidine fused phenothiazine derivatives for *in-vitro* anti-tubercular activity. Compounds **P-16f**, **P-16d**, and **P-16b** exhibited good anti-tubercular activity with percentage inhibition of 96, 93, and 91 respectively at MIC <6.25µg/mL. Compounds **P-16a**, **P-16e**, **P-16h**, and **P-16g** exhibited moderate activity with percentage inhibition of 75, 74, 68, 63, and 54 respectively at MIC <6.25µg/mL (**Figure 2-3**) [51]. Rajasekaran *et al.,* reported azetidinone based phenothiazine derivatives for excellent antitubercular activity. Compound **P-15j** was found to be the most significant with activity at 10µg/mL concentration. Compound **P-15d** and **P-15f** were active only at MIC of 100µg/mL (**Figure 2-3**) [52]. Ritu *et al.,* studied the potency of phenothiazine based 2-oxo-azetidine derivatives for antimycobacterial activity. Among the reported derivatives, N-[2- (10H- phenothiazinyl) ethyl] -4- (3- nitrophenyl) -3- chloro- 2-oxo - 1-imino azetidine and N-[2- (10H-phenothiazinyl) ethyl]-4 (4-nitrophenyl)-3-chloro-2-oxo-1-imino azetidine inhibited *Mtb* at a concentration of 2.50µg/mL [46]. Salie *et al.,* substituted the N-alkylamine side chain of antipsychotic drugs that is essential for CNS activity, with an aim to repurpose them as antitubercular drugs. The compounds exhibited MICs in the range of 12.5 to 25µg/mL against *Mtb* H37Rv [53]. *Amani AM* synthesized some phenothiazine-based piperazine derivatives and reported for

antitubercular activity at 1µg/mL concentration [54]. He *et al.,* synthesized numerous phenothiazine derivatives with substitutions at 2nd and 10th positions of the phenothiazine core. Among them, compound **P-8e** was found most effective against the virulent strain of *Mtb* with MIC_{90} of 4µg/mL [55]. *Jardine and Jacobs* synthesized some N-alkylsulfonates of phenothiazine and reported for significant activity in direct killing assays against *Mtb* H37Rv with MIC_{50} of ~6-12µg/mL [56]. Structure-activity relationship (SAR) is crucial for future design and development of phenothiazine based anti-tubercular agents. Therefore, SAR is established for these reported compounds in the following section (**Section: 2.2.3**).

2.2.3. Structure Activity Relationship of the Reported Phenothiazines

The antipsychotic phenothiazine drugs such as trifluoperazine, chlorpromazine, thioridazine, triflupromazine, fluphenazine and aceto-promazine (**Figure 2-2**) were reported to inhibit virulent strain of *Mtb*. Among them, trifluoperazine was found to be the most effective with MIC of 5µg/mL. Triflupromazine, thioridazine, and chlorpromazine were active only at MIC of 20µg/mL. Acetopromazine and fluphenazine were found to be inactive at 20µg/mL concentration. Methyl piperazinyl propyl group at 10th position and trifluoromethyl group at 2nd position of the phenothiazine nucleus were found essential for antitubercular activity of trifluoperazine [30]. Ramprasad *et al*., reported SAR of thiadiazole based phenothiazine derivatives. The n-propyl, methyl, and phenyl (4-Cl/4-F/4-methyl) substitutions in the thiadiazole nucleus were found essential for improved activity of the compounds [47]. Lipophilicity of alkylTPP cation fused phenothiazines and their build-up at the biological membrane were studied in *M. smegmatis* and *Mtb*. The lipophilicity of various alkyl TPPcations (**P-1a to P-1l**) was found varying with logP values ranging from 3.41 to 8.64 (**Figure 2-3**). Among them, logP value of propyl TPPcation (**P-1b**)

was found to be optimum at 4.29. Hence, it was used as a drug functionality for improving the permeability of compounds across the cell membrane of *Mtb.* Varying R_1 and retaining R_2 as H provided interesting results in compounds (**P-2a to P-2e**) (**Figure 2-3**). Introduction of electron withdrawing groups such as trifluromethane (CF_3) and chlorine (Cl) increased the LogP value and consequently the anti-TB activity. Introduction of a bulky substitution (tridecane group) at R_2 position increased LogP value, but introduction of N,N-dimethylpropan-1-amine substitution significantly decreased LogP value. He *et al.,* modified the side chain at 10^{th} position and also the amino group at one end of the side chain. Further modifications were also introduced on tricyclic phenothiazine ring. Compound **P-8e** showed encouraging activity with MIC_{90} of 4µg/mL against *Mtb* H37Rv (**Figure 2-3**) and it did not produce cytotoxicity against normal human cells at 32µg/mL concentration. The phenothiazine derivatives (**P-4 to P-8b**) having N-substitution with carboxyl, hydroxyl, alkoxy and phenoxy group through an alkyl linker did not produce improved activity (**Figure 2-3**). On modifying the phenothiazine core (**P-9a, P-9b, P-11 & P-12**), no improvement in anti-tubercular activity was observed (**Figure 2-3**). Modification of the side chain at the N-group of phenothiazine did not produce improvement in activity (MIC >32µg/mL). Introduction of a hydrophobic benzene ring at R, produced a pronounced decrease in MIC (**P-8e**). No change in activity was observed on changing the R substitution (**P-11**). But, bulky group substitutions (fluoro-benzene and tertiary amine) produced steep decrease in activity (**P-12**) (**Figure 2-3**) [55]. *He et al.,* observed that modification of phenothiazine nucleus with 9H-thioxanthene did not produce apparent increase in anti-TB activity. Moreover, introduction of non-basic aryl substituents at 10^{th} position of phenothiazine nucleus were found essential to retain anti-TB potency and also to eliminate the unwanted anti-psychotic side effects. Substitutions at 2^{nd} position of phenothiazine core and N,N-

dimethyl amino group at N-substituted side chain were found imperative for neuroleptic effect, but have no role in bringing anti-TB activity [55].

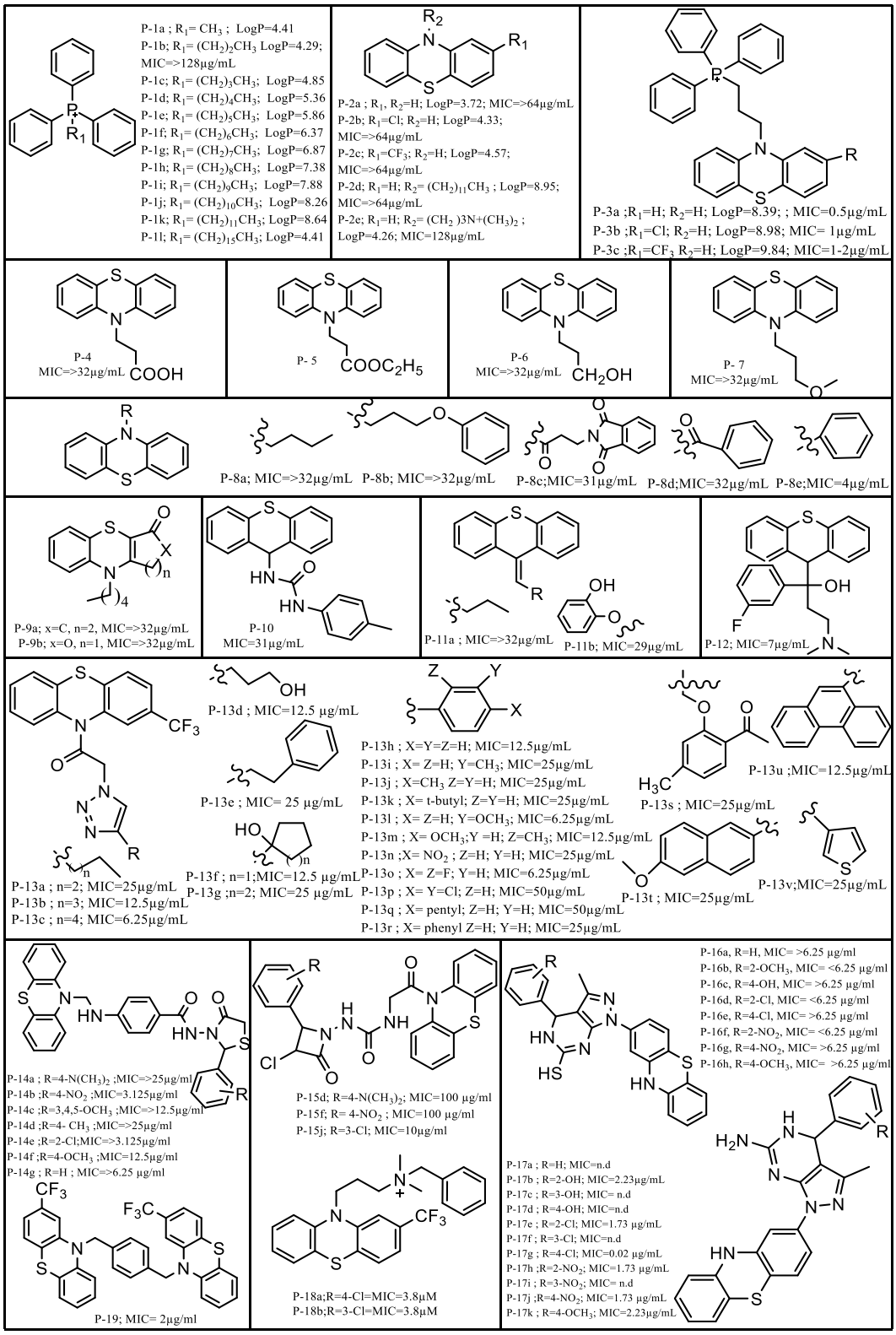


Figure 2-3: Phenothiazine derivatives possessing antitubercular efficacy

Anti-TB activity of some triazole-fused phenothiazine derivatives (**P-13a to P-13c**) improved by increasing the length of alkyl chain attached to 1,2,3-triazole nucleus (**Figure 2-3**). But, it was reduced when the alkyl chain was substituted with a hydroxyl (**P-13d**) and phenyl groups (**P-13e**). Among the phenyl substituted 1,2,3-triazole based phenothiazine derivatives (**P-13h to P-13v**), compounds having electron donating methoxy (**P-13l**) and fluoro substitutions (**P-13o**) on the phenyl nucleus showed improved activity (**Figure 2-3**) [49]. In a series of some pyrazole [3,4-*d*]pyrimidine based phenothiazine derivatives, substitutions on the phenyl ring profoundly affected the activity. Compounds with methoxy (**P-16b**), chloro (**P-16d**), and nitro substitutions (**P-16f**) at 2nd position exhibited excellent anti-TB activity with MIC <6.25µg/mL and percentage inhibition of 93, 91, and 96, respectively (**Figure 2-3**). Moreover, remarkable reduction in activity was observed on changing the position of the substitutions on the phenyl nucleus [51]. Among the dihydro pyrazolo[3,4-*d*]pyrimidine based phenothiazine derivatives reported by Siddiqui *et al*, compound **P-17g** was found to be the most active against *Mtb* with MIC of 0.02µg/mL. Different substitutions on the phenyl ring of dihydro pyrazolo[3,4-*d*]pyrimidine nucleus have greatly affected the activity. The presence of electron-withdrawing halogen and nitro groups at the *ortho* and *para* positions of the phenyl ring in compounds **P-17b, P-17e, P-17g, P-17h** and **P-17j** increased the activity. Further reduction in activity was observed in compound **P-17k** having an alkoxy substitution (**Figure 2-3**) [57]. Bate *et al.,* reported quaternization of triflupromazine, chlorpromazine, and promethazine derivatives and screened them against virulent strains of *Mtb*. Compounds **P-18a** and **P-18b** were found to be active at 3.8µM concentration. The 4- or 3-chlorobenzyl substitution on triflupromazine was found essential in producing the activity (**Figure 2-3**) [58]. Salie *et al.,* made some modifications on phenothiazine nucleus to understand their inhibitory effect on *Mtb*. No

major improvement in *Mtb* inhibition was observed but the unwanted dopamine and serotonin receptor binding was found reduced [53]. Madrid *et al.,* synthesized some derivatives of psychotropic phenothiazine drugs and studied their anti-TB potency. Compound **P-19** produced the most promising activity with MIC of 2µg/mL (**Figure 2-3**). The presence of two phenothiazine nuclei in compound **P-19** was found to be essential for the activity. SAR of the reported phenothiazines is illustrated in **Figure 2-4.** The synthetic derivatives of phenothiazine drugs produced comparatively less binding towards serotonin and dopamine receptors than trifluoperazine and chlorpromazine [59]. Therefore, these synthetic derivatives can be well exploited to treat both normal and resistant strains of *Mtb* with reduced CNS side effects.

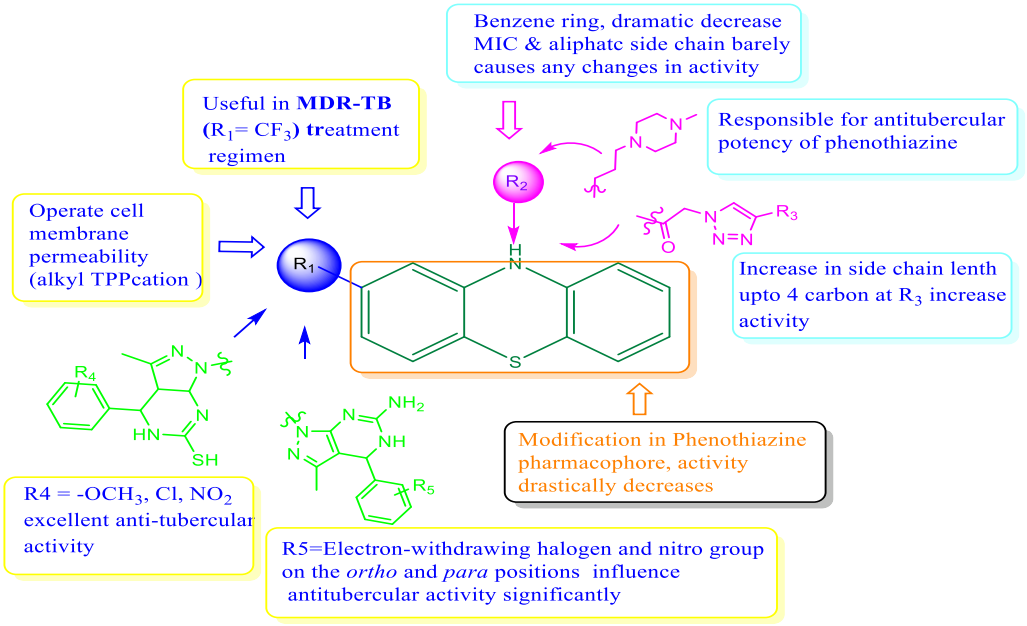


Figure 2-4: Structure activity relationships of reported Phenothiazine derivatives

2.3. Why Carbazole Scaffold Against Tuberculosis?

Carbazole, a tricyclic structure, is generally referred to 9H-carbazole having a pyrrole nucleus flanked by two benzene rings. Carbazole derivatives gain more significance in drug discovery as the nucleus has sources from medicinal plants, coal tar, and synthetic chemistry. Its was first reported from coal tar [60] and then from *Murraya koenigii*

Spreng as Murrayanine alkaloid [61]. The carbazole analogues were reported for different biological activity, such as antibacterial [62, 63], antifungal [63, 64], antitubercular [65-68], anti-alzheimer [69, 70] and antitumor [71, 72]. The potential of the carbazole scaffold was found underutililized in discovery of anti-TB drugs. Therefore, the scaffold has huge potential in development of novel drugs to treat different *Mtb* strains [73].

2.3.1. Marketed and Late-Stage Developmental Drugs Containing Carbazole Scaffold

Several marketed drugs *viz.* Carvedilol, Carprofen, Carazolol, Frovatriptan, and Latrepirdine were found to have the carbazole scaffold. Carvedilol is a non-selective β-1, and β-2 adrenergic blocker approved for treating cardiac failure. [74]. Carprofen is a non-steroidal anti-inflammatory drug that targets cyclooxygenase-1 (COX-1) and cyclooxygenase-2 (COX-2) to relieve pain and inflammation in animals [75]. Carazolol is a β-adrenergic blocker used to treat cardiovascular disorders in non-human mammals [76]. Frovatriptan is a 5-hydroxytryptamine (5-HT) receptor agonist, used to treat migraine disorders [77]. Latrepirdine is a non-selective antihistaminic drug, used to treat allergic reactions until 1983 [78], later it was repurposed as anti-Alzheimer's drug, but failed in phase 3 clinical trials [79]. Drug candidates in late-stage development such as Flucindole, Ciclindole, Rimcazole, Pirlindole, Girinimbine, NMP-7, P7C3 and Pyr were also found to contain this scaffold. Flucindole and Ciclindole are tetrahydrocarbazolamine group of compounds reported for potential antipsychotic efficacy [80]. Rimcazole is a sigma receptor antagonist reported for enormous potential to treat psychosis [81]. Pirlindole is found almost similar in structure with Flucindole and Ciclindole, but it is reported for good antidepressant activity. Mahanimbine and Girinimbine are carbazole alkaloids isolated from *Murraya koenigii* and are reported for potent antidiabetic and anticancer activities respectively [82, 83]. NMP-7 is a

cannabinoid receptor (CB_1 & CB_2) agonist reported to cure neuropathic pain and also reported to block T-type calcium channel for treating epilepsy [84]. P7C3 is an investigational neuroprotective agent and Pyr1 is also an investigational drug in the pipeline to treat cancer (**Figure 2-5**) [85]. These indicate that the carbazole nucleus has ernormous potential in the development of novel drug molecules. No major toxicity and bioavailability issues are associated with these derivatives. Therefore, modifying the linker and also the substituents at different positions on the carbazole core with solid evidences based SAR could be fruitful in further development of potential lead candidates against TB.

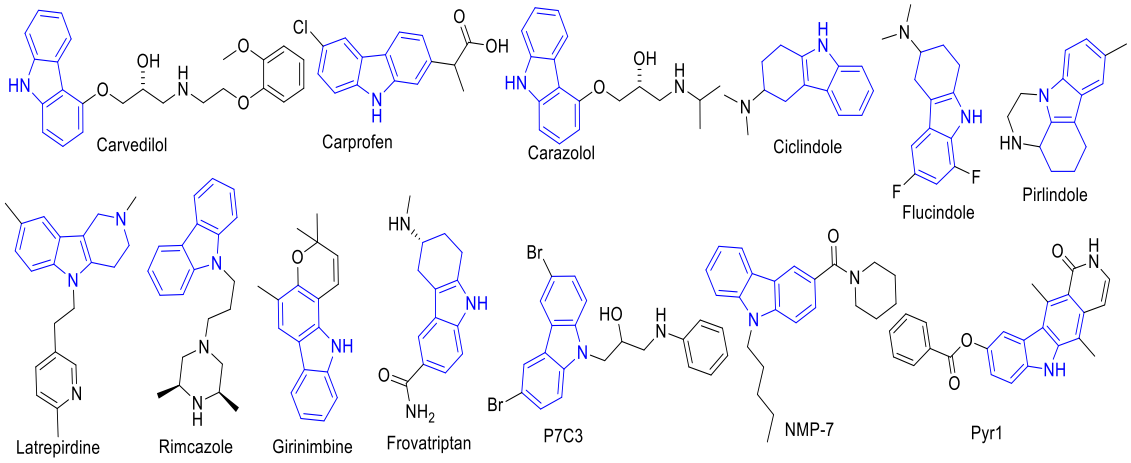


Figure 2-5: Marketed and late-stage developmental drugs containing carbazole scaffold

2.3.2. Carbazole Alkaloids Possessing Antitubercular Efficacy

Several carbazole alkaloids were reported for antitubercular activity (**Figure 2-6**). Sunthitikawinsakul *et al.,* reported various carbazole alkaloids such as 3-formyl carbazole (**C-1a**), 3-methoxy carbonyl carbazole (**C-1b**), 2-hydroxy-3-formyl-7-methoxy carbazole (**C-1c**), clauszoline J (**C-1d**), mukonal/ 2-hydroxy-3-formyl carbazole (**C-1e**), and murrayanine/ 1-methoxy 3-formyl carbazole (**C-1f**) from the shrub *Clausena excavata*. Compounds **C-1a, C-1b, C-1c, C-1d,** and **C-1e** were found to be active against *Mtb* H37Ra with MIC of 100, 50, 100, 100 and 200µg/mL,

respectively. Compound **1f** was reported to be inactive with MIC >200µg/mL. Compounds **C-1a, C-1b, C-1c,** and **C-1e** were also shown to produce antifungal activity against *Candida albicans* with IC_{50} of 13.6, 9.5, 2.8 and 29.3µg/mL respectively. No cytotoxicity was reported against human breast cancer (BC-1) and human epidermoid carcinoma (KB) cells [86]. Ma *et al.,* isolated several alkaloids *viz*. 3-formyl-5-(3-methylbut-2-en-1-yl)-6-hydroxy carbazole (**C-2a**), 2-hydroxy-3-formyl-6-methoxy carbazole (**C-2b**), 3-methyl carbazole (**C-2c**), 3-methyl carboxylate carbazole (**C-2d**), 3-formyl carbazole (**C-2e**) and 3-formy-6-methoxy carbazole (**C-2f**) from the flowering plant *Micromelum hirsutum*. All the compounds produced promising antitubercular activity with MIC of 31.5, 14.3, >128, >128, 42.3 and 15.6µg/mL respectively against the virulent strain of *Mtb*. The compounds were further evaluated for toxicity against VERO cells and were found to produce no cytotoxicity with IC_{50} >100µg/mL [87]. Maneerat *et al*., reported a dimeric carbazole alkaloid Clausenwallines A (C-3) from the roots of *Clausena wallichii* for good anti-TB activity with MIC of 12.50µg/mL. The compound was also reported to be effective against *Plasmodium falciparum* and breast cancer cells with IC_{50} of 2.46 and 25.43µg/mL respectively (**Figure 2-6**) [88].

2.3.3. Synthetic Carbazole Derivatives Possessing Antitubercular Efficacy

Carbazole alkaloids have huge potency as antitubercular drug, but their tedious isolation is challenging. Hence, synthetic derivatives were prepared for better antitubercular activity (**Figure 2-7**). Patpi *et al.,* synthesized some triazole-fused dibenzofuran, dibenzothiophene and carbazole derivatives. The triazole-fused dibenzothiophene derivatives (**C-4c** & **C-5c**) produced most promising activity against *Mtb* H37Rv with MIC of 1.56 and 0.78µg/mL respectively. The triazole-fused dibenzofuran derivatives (**C-4b** & **C-5b**) produced good activity with MIC of 6.25µg/mL. Triazole-fused carbazole derivatives (**C-4a** & **C-5a**) also produced significant activity with a MIC of

12.5µg/mL [89]. Surineni *et al.,* reported some pyrazole-clubbed carbazole compounds for antitubercular activity against *Mtb* H37Rv by agar dilution method. Compounds **C-6a** to **C-6f** exhibited excellent activity with MIC of 50, 3.13, 6.25, 12.5, 25, 6.25µg/mL, respectively. The activity of compound **C-6b** was comparable to the standard drug Ethambutol [90]. Börger *et al.,* synthesized some oxygenated derivatives of carbazole (**C-7, C-8** & **C-9**) and screened them against *Mtb* H37Rv. The compounds exhibited good inhibition against *Mtb* H37Rv with MIC of 6.2, 14.4, and 11.8µM, respectively. They were further screened against VERO cells and were found to produce cytotoxicity with IC_{50} of >50, 74.1 and 33.7µM, respectively. Selectivity index was calculated by MIC/IC_{50} and was found to be >8.1, 5.1 and 2.9, respectively [65]. Surineni *et al.,* reported some triazole-clubbed carbazole derivatives for anti-TB activity. Compounds **C-10d** and **C-10e** inhibited *Mtb* with MIC of 6.25µg/mL, whereas compounds **C-10a** to **C-10c, C-11** and **C-12** with MIC of 12.5µg/mL. Compounds **C-10d** and **C-10e** were further screened against Human Embryonic Kidney (HEK-293T) cell line. Their percentage inhibition at 50µg/mL concentration was found to be 29.03 and 12.36%, respectively [66]. Taj *et al*., reported an efficient one-pot synthesis of pyrazoline-fused carbazole derivatives (**C-13a** to **C-13k**) for antitubercular activity against *Mtb* H37Rv. Compounds **C-13d, C-13g** and **C-13i** were excellent against *Mtb* H37Rv with MIC of 3.5µg/mL. Compounds **C-13c** and **C-13f** also produced excellent activity with MIC of 4.5µg/mL. The activity was better over the standard drugs Streptomycin (7.5µg/mL) and Pyrazinamide (10µg/mL). Rest of the compounds (**C-13a, C-13e, C-13j** and **C-13k**) produced significant activity with MIC around 10µg/mL. The compounds exhibited only partial inhibition against human kidney cancer (A498) and human lung cancer cells (A549) with LC_{50} and TGI (Total Growth Inhibition) values >70µg/mL [67]. Guo *et al.,* synthesized some indole-fused carbazole derivatives (**C-14a to C-14i**)

for anti-TB activity. Compound **C-14d** produced interesting result against *Mtb* with MIC of 15µM, while **C-14c, C-14e**, and **C-14f** produced comparatively lesser activity with MIC of 44.1, 56.6, 60.6µM, respectively. Remaining derivatives (**C-14a, C-14b, C-14g, C-14h** & **C-14i**) produced only medium activity with MIC > 128µM [68]. Shaikh *et al.,* designed some thiazole-tethered carbazole derivatives (**C-15a** to **C-15k**) through molecular hybridization approach and synthesized them for antitubercular activity. Compound **C-15j** was found to be the most active against *Mtb* H37Rv with MIC of 21µM. It was followed by compounds **C-15k, C-15a,** and **C-15f** with MIC of 31, 32 and 37µM, respectively. IC_{90} and IC_{50} of compound **C-15j** were determined for better understanding of its inhibitory potential and were found to be >58 and 28µM respectively. The compound produced very low cytotoxicity against VERO cells, leading to high selectivity index [91]. Balamurali *et al.,* synthesized some tetrahydroindolo derivatives of carbazole (**C-16a** to **C-16h**), by reaction of various 1-oxo-1,2,3,4-teterahydrocarbazoles with phenylhydrazine. These derivatives were further screened against *Mtb* by tube dilution method. Compound **C-16d** produced significant activity with MIC of 50µg/mL, followed by **C-16h** and **C-16f** with MIC of 75 and 100µg/mL respectively. Rest of the derivatives were inactive at MIC of 150µg/mL. Compound **C-16d** exhibited good antimicrobial activity against several other bacterial strains *viz E. coli*, *P. aeruginosa*, *B. subtilis* and *S. aureus* with MIC of 3, 6, 6 and 12.5µg/mL respectively. With this potent activity against different bacterial and mycobacterial strains, it can be optimized as a broad-spectrum antibiotic [92]. Filitis *et al.,* synthesized some hexahydropyrazino derivatives of carbazole (**C-17a** to **C-17g**) and screened them against *Mtb* H37Rv for antitubercular activity. Compounds **C-17d** and **C-17f** produced promising result with MIC of 4µg/mL. Compounds **C-17a** and **C-17b** produced activity with MIC of 8µg/mL, followed by **C-17c**, **C-17e** and **C-17g** with

MIC of 16µg/mL [93]. Kantevari *et al.,* synthesised some isomeric pyranoquinoline derivatives of carbazole through Povarov reaction of 9-methyl-9H-carbazole-3-carbaldehyde with different aromatic amines. The compounds inhibited *Mtb* H37Rv with MIC ranging from 3.13 to 25µg/mL. The *trans* isomeric analogs **C18a** to **C-18f** exhibited two-fold increase in activity than the *cis* isomers. Compound **C-18f** produced comparable activity to that of standard drug Ethambutol with a MIC of 3.13µg/mL. Compounds **C-18d** and **C-18e** also produced better activity with MIC of 6.25µg/mL. Compounds **C-18a** and **C-18c** produced comparatively lesser activity with MIC of 12.5 and 25µg/mL respectively [94]. Chirke *et al.,* synthesized some carbazole and dibenzo[b,d]furan derivatives by 1,3-dipolar cycloaddition reaction. Compounds **C-19a** and **C-19b** exhibited very excellent activity against *M. smegmatis* with MIC of 0.12µg/mL. The activity was comparatively better over dibenzo[b,d]furan derivatives (0.5µg/mL). LogP of the compounds was determined to understand their lipophilicity. The compound **C-19b** exhibited higher LogP value of 7.0 and the higher LogP affected the druglikeness [95]. Choi *et al.,* synthesized several carbazole derivatives (**C-20a to C-20e, C-21, C-22a, C-22b, C-23, C-24a** and **C-24b**) and performed microplate Alamar-blue assay (MABA) to understand their antitubercular efficacy. Compounds **C-20e, C-21,** and **C-24b** were found to be the most promising with MIC of 11, 8, and 9µM respectively. They also showed no cytotoxicity against VERO cells with IC_{50} >128µM. Remaining derivatives in the series were moderately active with MIC values > 50µM [96]. Sellamuthu *et al.,* designed cycloalkane derivatives of carbazole (**C-25a** to **C-25f**) with an aim to reduce blood-brain barrier (BBB) penetration of antipsychotic phenothiazine drugs and increase their therapeutic index as antitubercular drug. Compound **C-25d** produced good inhibitory activity with MIC of 12.5µg/mL, followed by compounds **C-25a, C-25b, C-25c** and **C-25e** with MIC of 25µg/mL [97].

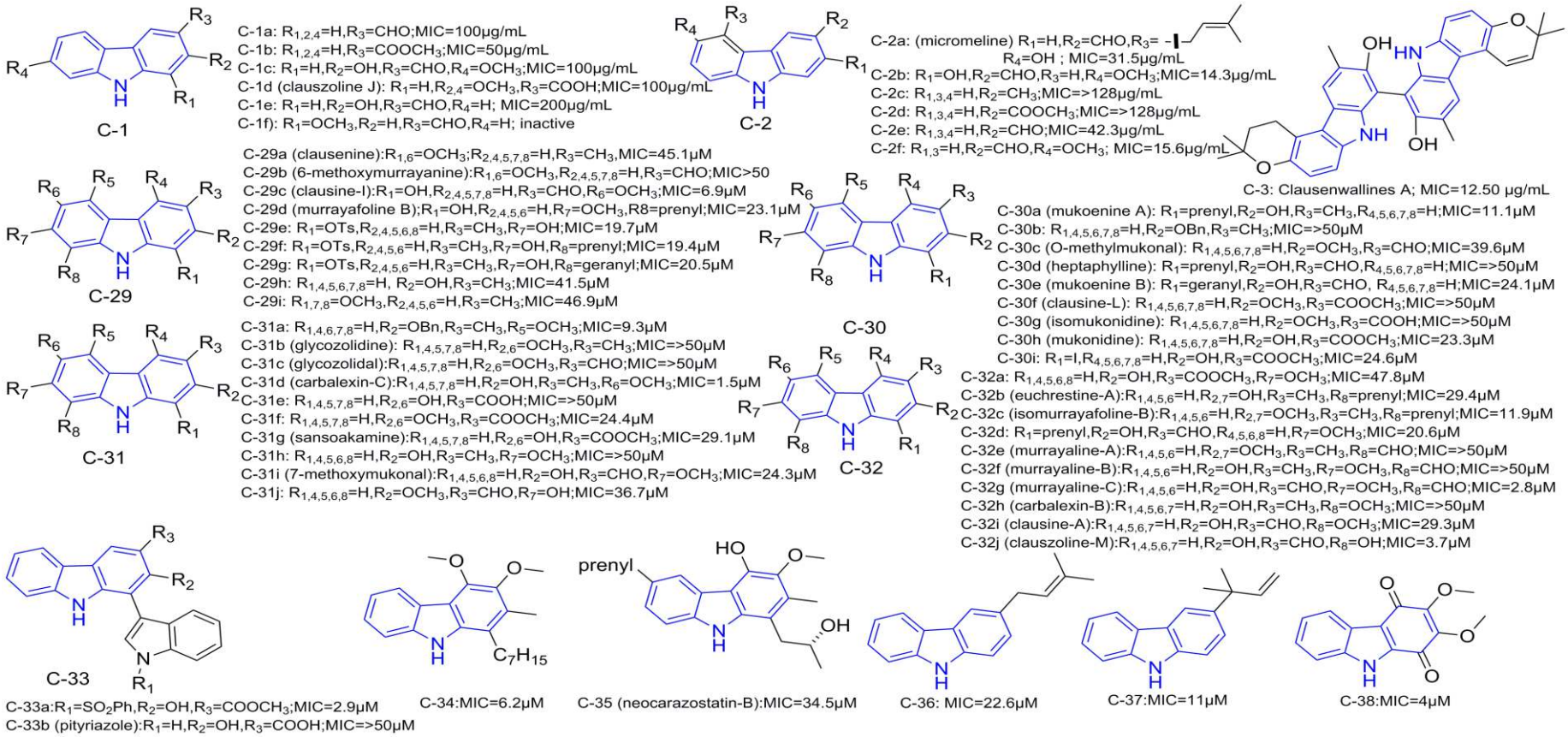


Figure 2-6: Carbazole alkaloids possessing antitubercular activity

Their BBB permeability was found reduced in Parallel artificial membrane permeability assay (PAMPA) in comparison to the standard neuroleptic drug Chlorpromazine. Moreover, there was concomitant increase in antitubercular activity. Scalacci *et al.,* synthesized some carbazole derivatives (**C-26a** & **C-26b**) and screened against various susceptible and resistant strains of *Mtb* by Resazurin Microtiter Assay (REMA). Compound **C-26b** was coparatively better active than **C-26a** with MIC_{90} of 60, 64 and 39µg/mL against H37Rv, mc27000 and CF73 susceptible strains, respectively. It also exhibited potent activity against MDR strains of *Mtb*, *viz*. CF81 and CF104 with of MIC_{90} of 46, and 48µg/mL respectively [98]. Kinjo *et al.,* studied the inhibitory potential of a carbazole derivative (**C-27**) against *M. smegmatis* and compared its efficacy with some diphenyl ether derivatives. The activity was comparable to other diphenyl ether derivatives at 50µM concentration (**Figure 2-7**) [99].

2.3.4. Structure-activity Relationship of Reported Carbazole Derivatives

Carbazole alkaloids obtained from *Clausena excavata* without substitution at C-1 position (**C-1a, C-1b, C-1c, C-1d,** and **C-1e)** exhibited potent antitubercular activity. The methoxy substitution at C-1 position (**C-1f**) was found to have no role in producing the activity, whereas the same substitution at C-3 position (**C-1b**) showed better activity over the other derivatives (**C-1a**, **C-1c**, **C-1d** and **C-1e**). It is obvious that the C-3 substitution is essential over the C-1 substitution and also the methoxy group over the formyl and carboxyl groups [86]. Several carbazole alkaloids (**C-2a to C-2f**) isolated from *Micromelum hirsutum* exhibited very promising results. Compound **C-2b** was found to be the most active with MIC of 14.3µg/mL and that was followed by the compound **C-2f** with MIC of 15.6µg/mL. 3-formyl and 6-methoxy substitutions in **C-2b** and **C-2f** were found highly essential in improving the activity. 2-hydroxy

substitution was also found important for the better activity of **C-2b** over **C-2f** [87]. Compounds **C-4a** and **C-5a** from the reports of Patpi *et al.,* were found active with a MIC of 12.5µg/mL. Replacement of the nitrogen atom with an oxygen atom in the carbazole scaffold produced improved activity with MIC of 6.25µg/mL in compounds **C-4b** and **C-5b**. The same nitrogen atom when replaced with a sulphur atom, the activity has further increased in compounds **C-4c** and **C-5c** with MIC of 1.56 and 0.78 µg/mL respectively [89]. Surineni *et al.,* reported pyrrole-fused carbazole derivatives for more significant antitubercular activity. Compounds **C-6a to C-6f** with aryl group substitution at pyrrole nitrogen produced better activity than the compounds with benzyl group substitution. Compound **C-6b** with 4-fluoro substitution was found to be the most active with a MIC of 3.13µg/mL. It was followed by the 4-chloro (**C-6c**) and 4-bromo (**C-6d**) substituted compounds with MIC of 6.25 and 12.5 µg/mL respectively [90]. Surineni *et al.,* reported triazole-clubbed carbazole derivatives for antitubercular activity. Benzyl-triazole derivatives (**C-10a** to **C-10e**) produced better activity than their simple phenyl derivatives with MIC < 12.5µg/mL. Compounds **C-10d** and **C-10e** inhibited *Mtb* with MIC of 6.25 µg/mL and their potency was attributed to the presence of fluoro substitutions on benzyl ring fused to the triazole ring. Compounds **C-10a** and **C-10b** with electron donating methoxy groups also produced significant antitubercular activity. Compounds **C-11** and **C-12** with aliphatic chain substitution on the triazole ring showed activity with MIC of 12.5µg/mL [66]. Taj *et al.,* reported pyrazoline based carbazole derivatives (**C-13a** to **C-13k**) for promising antitubercular activity. Compounds **C-13b, C-13c, C-13d, C-13f, C-13g**, and **C-13i** inhibited *Mtb* with MIC < 5µg/mL. Inhibitory potential of these compounds was attributed to the presence of electron donating groups such as hydroxy and chloro groups. Compounds **C-13e** and **C-13j** with electron withdrawing groups such as nitro and methoxy group produced

comparatively lesser activity with MIC of 10µg/mL [67]. Guo *et al.,* reported some indolocarbazoles (**C-14a** to **C-14i**) for potent antitubercular activity. Compounds **C-14b**, **C-14e** and **C-14g** with alkyl group substitution at R_1 position produced comparatively lesser activity than compounds **C-14a, C-14d,** and **C-14h**. The same substitution at R_3 position in compounds **C-14a, C-14b** and **C-14c** produced lesser activity than compounds **C-14d, C-14e,** and **C-14f**. The alkyl group substitution at R_2 position was found to have no role in producing the activity. Compound **C-14d** with no $R_1/R_2/R_3$ substitution produced maximum activity with MIC of 15µM. Shaikh *et al.,* reported some thiazole-clubbed carbazole derivatives (**C-15a** to **C-15k**) as antitubercular agents. Compound **C-15j** with 3,4-dimethoxy substitutions and compounds **C-15f** & **C-15k** with hydroxyl substitutions produced excellent activity with MIC of 21, 31, 37 µM, respectively. The halogen or nitro substituted compounds produced no interesting activity with MIC > 200µM. It can be implicated that the electron-donating groups are essential for the improved activity of the compounds than the electron-withdrawing groups [91]. Balamurali *et al.,* reported some tetrahydroindolo derivatives of carbazole (**C-16a** to **C-16h**) for promising antitubercular activity with MIC in the range of 50 to 200µg/mL. Compounds **C-16d, C-16f** and **C-16h** with 3-chloro, 3-bromo and 1-methoxy substitutions exhibited significant activity with MIC of 50, 100 and 75µg/mL, respectively. Compounds (**C-16a** to **C-16c)** with methyl-substitution were the least active. It was inferred that the electron donating methoxy substitution at first position or electron withdrawing chloro substitution at third position on the indolo-benzene ring was essential for the activity [92]. Kantevari *et al.,* reported some pyrano-quinoline class of carbazole derivatives (**C-18a** to **C-18f**) for antitubercular activity. Compounds **C-18d, C-18e,** and **C-18f** with halogen substitutions were found to be most active with MIC <6.25µg/mL.

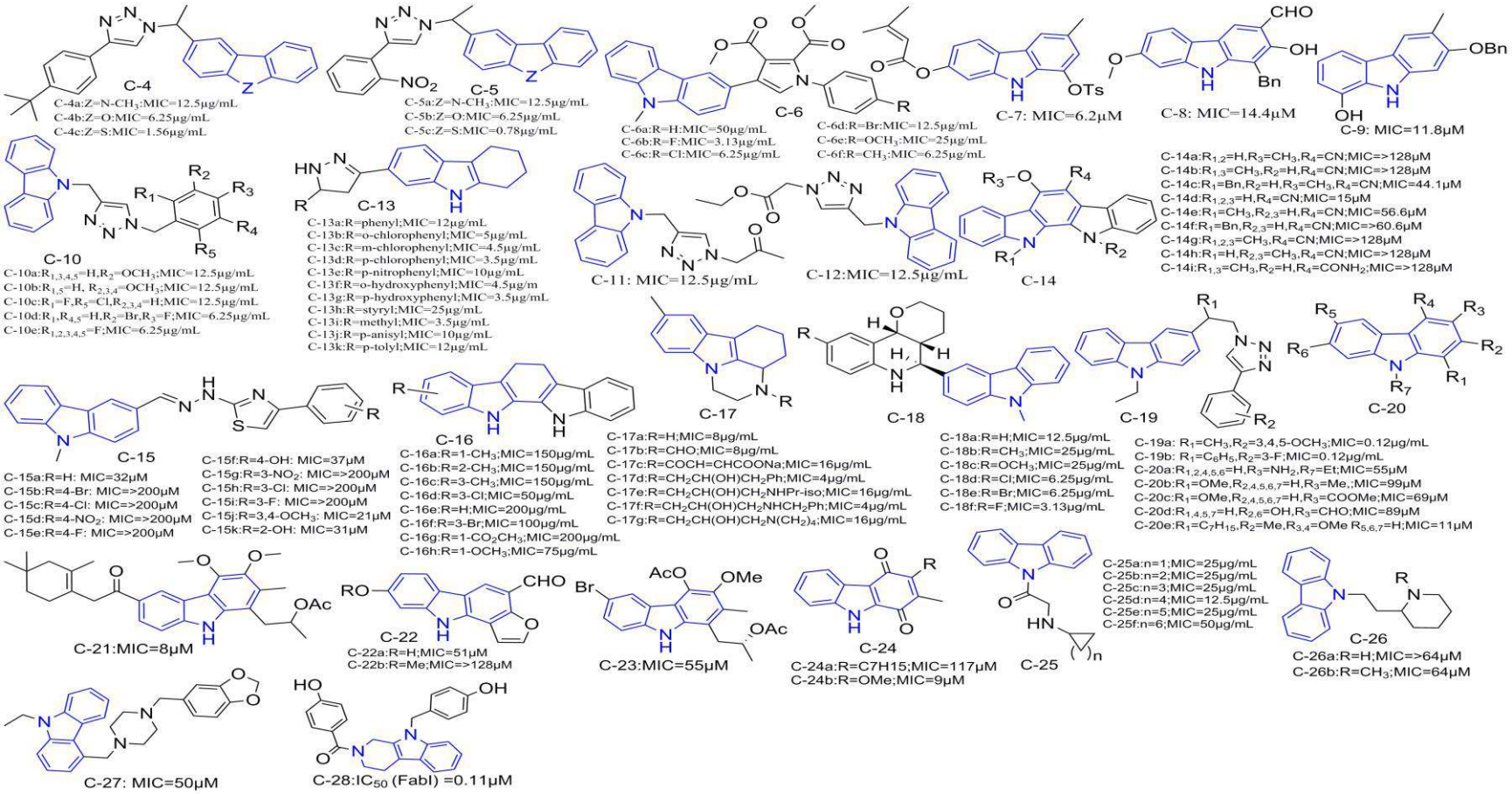


Figure 2-7: Synthetic carbazole derivatives possessing antitubercular activity

Compound **C-18a** with no substitution at quinoline ring exhibited activity with MIC of 12.5µg/mL. Introduction of electron donating methyl and methoxy substituents in compounds **C-18b** and **C-18c** decreased the activity with MIC of 25µg/mL. It was concluded that the electron withdrawing halogen substitution was essential over electron donating methyl and methoxy substitutions [94]. Choi *et al.,* reported some carbazole alkaloids (**C-20a** to **C-20e, C-21, C-22a, C-22b, C-23, C-24a** & **C-24b**) for excellent anti-TB activity. The dione derivatives **C-24a** and **C-24b** were found more promising with MIC of 117 and 9µM, respectively. Compound **C-24b** with methoxy substitution on the dione ring exhibited improved activity than the compound **C-24a** with heptyl ring substitution. The other derivatives **C-20e**, **C-21**, and **C-23** were also found active with MIC of 11, 8, and 55µM, respectively (**Figure 2-7**). The alkaloid furoclausine-A (**C-22a**) exhibited promising activity with MIC of 51µM, whereas the corresponding methyl ether derivative **C-22b** exhibited least activity with MIC value > 128µM [96]. Börger *et al.,* reported some oxygenated carbazole alkaloids (**C-29a** to **C-38**) for anti-TB activity (**Figure 2-6**). The carbalexin-C alkaloid (**C-31d**) exhibited excellent antitubercular activity with MIC of 1.5µM. Murrayaline-C (**C-32g**) and clauszoline-M (**C-32j**) also exhibited very good activity with MIC of 2.8 and 3.7µM, respectively. In all the three alkaloinds, the R_2 substitution was a hydroxyl group. The R_3 substitution was a methyl group in compound **C-31d** and it was a formyl group in **C-32g** and **C-32j**. Whereas, the R_6 substitution was a methoxy group in **C-31d** and it was a formyl group in **C-32g.** An additional methoxy group substitution was observed at R_7 position in compound **C-32g**. No substitutions were noticed at R_6 and R_7 positions in **C-32j**, but a hydroxyl substitution was observed at R_8 position. It is imperative that methoxy substitution at R_3 position and hydroxyl substitution at R_2 position are essential for the activity. When the methoxy substituent was replaced with an ester or a carboxyl group

at R_3 position (**C-30f**, **C-30g**, **C-30h**, **C-30i**, **C-31e**, **C-31f**, **C-31g** and **C-32a**), a drastic reduction in activity was noticed (**Figure 2-6**) [65]. Compounds with substitutions on 'A' and 'B' rings are essential for antitubercular activity. Moreover, the position and type of substitutions are critical in producing the activity. The compounds with 'B' ring modification to fused heterocyclic rings (carbazole, benzofuran, bezoquinone and hydroquinoline) exhibited good activity. Whereas, the compounds with N-substitution produced comparatively lesser activity. Compounds with intact carbazole core exhibited more activity than the compounds with modified core-ring (**Figure 2-8**). Therefore, the carbazole scaffold needs to be retained and substitutions on the scaffold can be manipulated in the design of novel molecules to obtain more potent antitubercular agents.

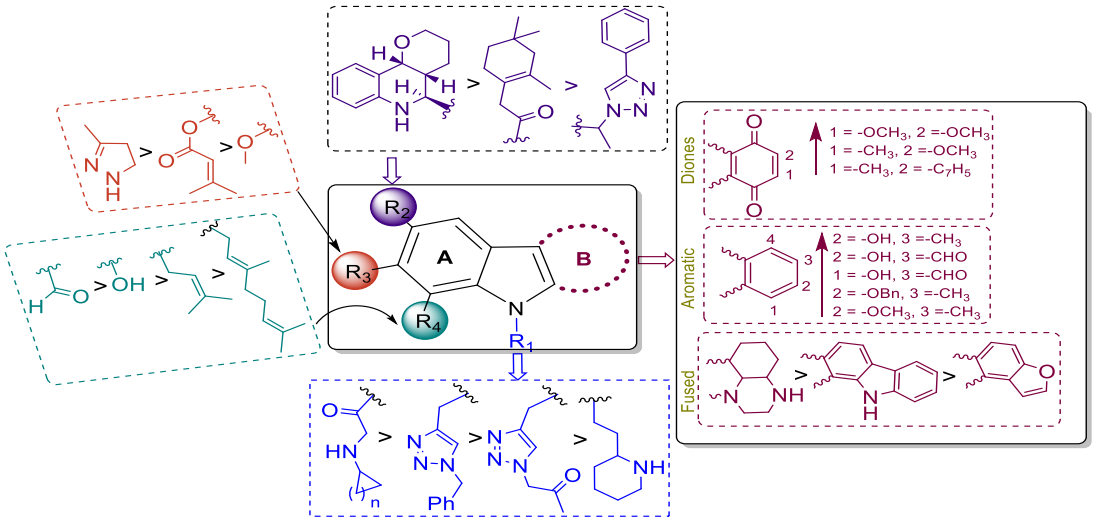


Figure 2-8: Structure antitubercular-activity relationship of reported carbazole derivatives. ↑-indicates increase in activity; >-indicates greater activity.

2.4. Essential components in respiratory production of ATP

Bacteria obtain ATP through oxidative phosphorylation and substrate level phosphorylation. Most bacteria depend on substrate level phosphorylation for energy production and the oxidative phosphorylation seems to be non-essential for the survival

of most bacterial species [100, 101]. Whereas, oxidative phosphorylation is the chief source of energy in *Mtb*. The newly approved drug Bedaquiline acts through this pathway [102]. The oxidative phosphorylation system consists of an electron transport chain that produces proton motive force (PMF) and activates ATP synthase in ATP production. Several bacteria compensate this oxidative phosphorylation and produce ATP from fermentable carbon sources through substrate-level phosphorylation [103]. However, in mycobacteria, there is indispensable need for respiratory production of ATP on fermentable carbon sources. Electrons within *Mtb* enter the electron transport chain through type-2 NADH dehydrogenase (NDH-2), where it oxidise NADH to NAD^+ with concomitant reduction of quinone to quinol. Later, cytochrome aa3-type and cytochrome bd-type terminal oxidase reoxidise quinol to quinone [104]. The whole process led to production of PMF and couples with ATP synthase in the process of ATP production.

2.4.1. Type-2 NADH dehydrogenase (NDH-2)

NDH-2 plays an essential role in respiratory metabolism, where it oxidizes NADH and reduces quinones in production of PMF (**Figure 2-9 & Figure 2-10**) [105]. Inhibition of NDH-2 collapse the production of PMF and lead to death of *Mtb* [105]. The enzyme is present in various organisms *viz*. *Mycobacterium tuberculosis* [106], *Plasmodium falciparum* [107] and *Trypanosoma brucei* [108], but it is absent in human counterpart. These attributes make NDH-2 as a promising target in design and discovery of potent antitubercular drugs.

2.4.1.1. Crystal structure of Type-2 NADH Dehydrogenase (NDH-2)

Heikal *et al* reported for the first time the crystal structure of NDH-2 from *C. thermarum* [109]. It contains a β-sheet structure and embedded a FAD molecule through hydrogen bond interactions with the nitrogen of G12 and carbonyl oxygen of V81. The

tricyclic isoalloxazine ring of FAD was located at the region formed by three-way junction of quinone-binding tunnel, FAD-binding tunnel, and NADH-binding cleft. Hydrogen bonds were also noticed between the side-chain of K376 and main chain nitrogens of A316 and Q317 to position the isoalloxazine ring against the membrane-anchoring domain. Channel extending from the membrane anchoring domain and *Si* side of the FAD forms the quinone binding site (**Figure 2-11**) [109]. Sena *et al*, reported crystal structure of NDH-2 from *S. aureus*. It constitutes 4 monomers, 4 FAD, 77 water molecules and 6 chloride ions [110]. A complete NDH-2 consists of a membrane attachment domain and two Rossmann-like domains. FAD binding domain is the first domain, where the FAD binds in its extended conformation through non-covalent interactions. [110]. No crystal structure of NDH-2 from *Mtb* was reported, but efforts are in progress to solve the structure.

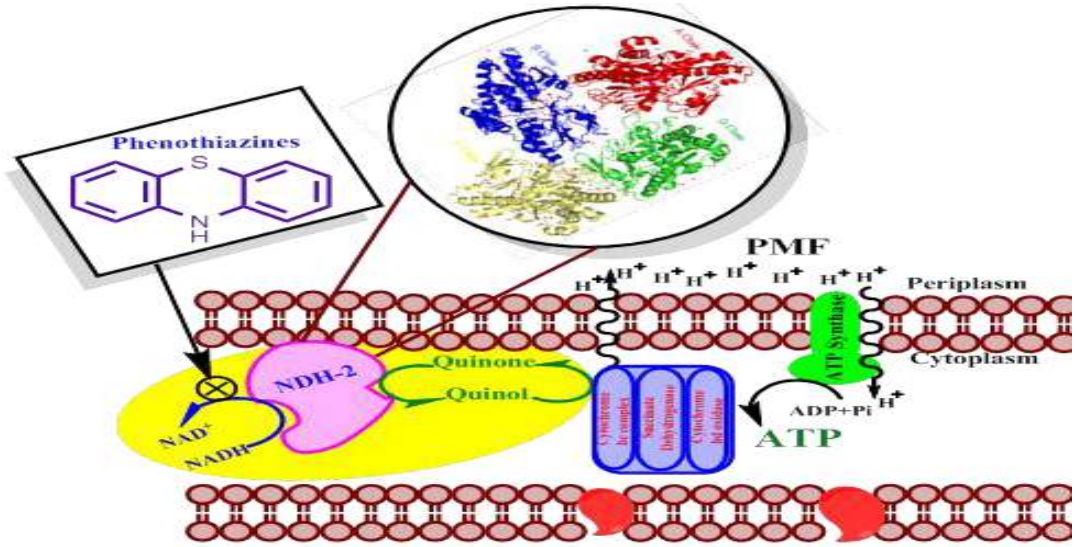


Figure 2-9: Role of NDH-2 and ATP synthase in ATP synthesis

2.4.1.2. Inhibitors of type-2 NADH dehydrogenase

Several phenothiazines produced promising antimycobacterial activity against *Mtb* H37Rv [111-115]. They also exhibited potent activity against isoniazid, rifampin, pyrazinamide, streptomycin and ethambutol resistant strains of *Mtb* [116, 117].

Trifluoperazine (TPZ) exhibited synergism with isoniazid and rifampicin in a macrophage model of *Mtb* H37Rv infection [118, 119]. It significantly reduced the synthesis of ATP in *M. leprae* [120]. The level of ATP has decreased drastically on addition of 5μg/mL of TPZ to the growth media. Many phenothiazines were also reported to be active in mouse model of TB infection [121]. NDH-2 in the electron transport chain of *Mtb* was then identified as the target of phenothiazines. It present in two isoforms *viz*. Ndh and NdhA. Phenothiazines inhibited both isoforms of the enzyme [122]. 10mM Potassium cyanide was added to block the electron-transport chain and the rate of oxidation of NADH was recorded in presence and absence of the phenothiazine in a concentration-dependent manner to determine the activity of phenothiazines [123]. Flavone is a weak inhibitor of NDH-2 that inhibited NADH oxidation with IC_{50} of 750 μM. Pyridaben, rotenone, and piericidin A are type-1 NADH dehydrogenase inhibitors, produced no inhibition against Ndh from *Mtb*. [123]. However, phenothiazines were also reported to act through other mechanisms such as inhibition of calcium-binding proteins and efflux pumps [124, 125]. NDH-2 mechanism of phenothiazines was further confirmed with level of oxygen consumed by cytoplasmic membranes of *Mtb* during the process of respiration. Consumption of oxygen started all of a sudden in a linear manner on addition of NADH to membrane particles of *Mtb*. Oxygen consumption stopped suddenly on addition of TPZ (1mM). Hence, it was suggested that phenothiazines could inhibit NDH-2 activity [126]. NADH:unquinone-2 oxidoreductase activity was studied to assess the specificity of phenothiazines against two isoforms of NDH-2. Interestingly, CPZ inhibited both isoforms with IC_{50} of ~10μM. [122]. Direct correlation was observed between anti-TB activity and NDH-2 inhibition under both aerobic and anaerobic conditions.

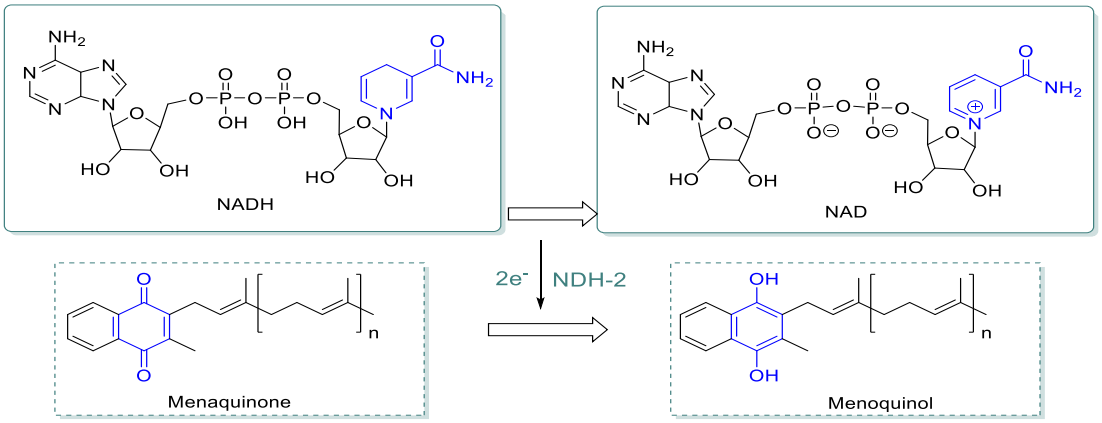
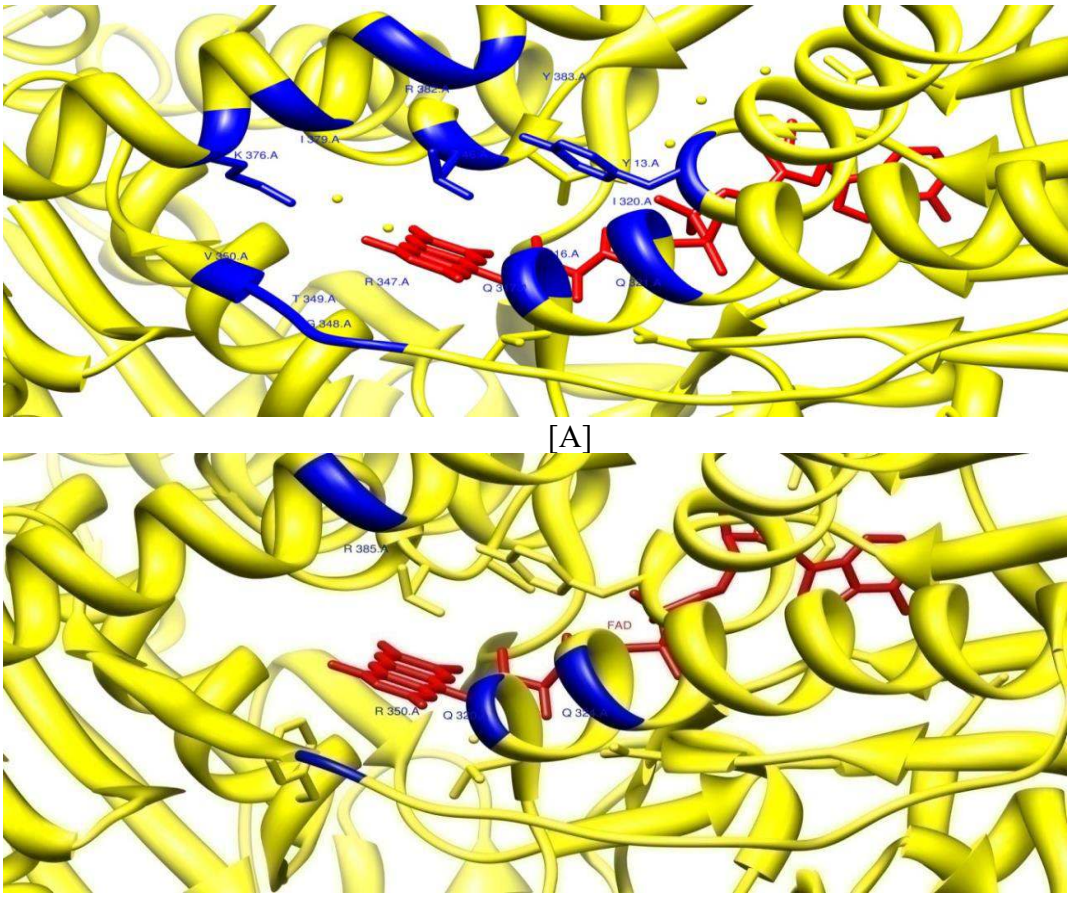


Figure 2-10:Reaction catalyzed by type-2 NADH dehydrogenase (NDH-2)



[A]

[B]

Figure 2-11: Quinine binding sites around FAD of NDH-2 from *C. thermarum* and *S. aureus*; [A] NDH-2 from *C. thermarum* displaying the Quinone binding residues Y13, T46, A316, Q317, I320, Q321, R347, V350, K376, I379, R382, Y383 (blue color) and FAD (red color); [B] NDH-2 from *S. aureus* displaying the Quinone binding residues Q320 and Q324 from AQxAxQ motif and A350 and R385 at the pocket entrance (blue color), and FAD (red color).

However, there was slight increase in activity of phenothiazines in a hypoxia model over that in aerobic model [105, 127]. NDH-2 may be having essential role in survival of dormant *Mtb* and phenothiazines targeting NDH-2 could have increased the activity in hypoxia model. CPZ, TPZ, and Thioridazine (TZ) were reported to inhibit NDH-2 from *S. aureus* with IC_{50} of 10 to 30µM (**Figure 2-12**) [128]. Dunn *et al* incorporated alkylTPP cation **(3)** to phenothiazine **(4)** to improve its inhibitory potential against *Mtb*. The compound **2** obtained by the process, inhibited NADH oxidation with IC_{50} of 15.14µM in the presence of *M. smegmatis* membranes. The activity was 4-fold higher than TPZ (60.07µM). Phenothiazine alone **(4)** or alkylTPP cation alone **(3)** produced no significant response on NADH oxidation (**Figure 2-12**) [129].

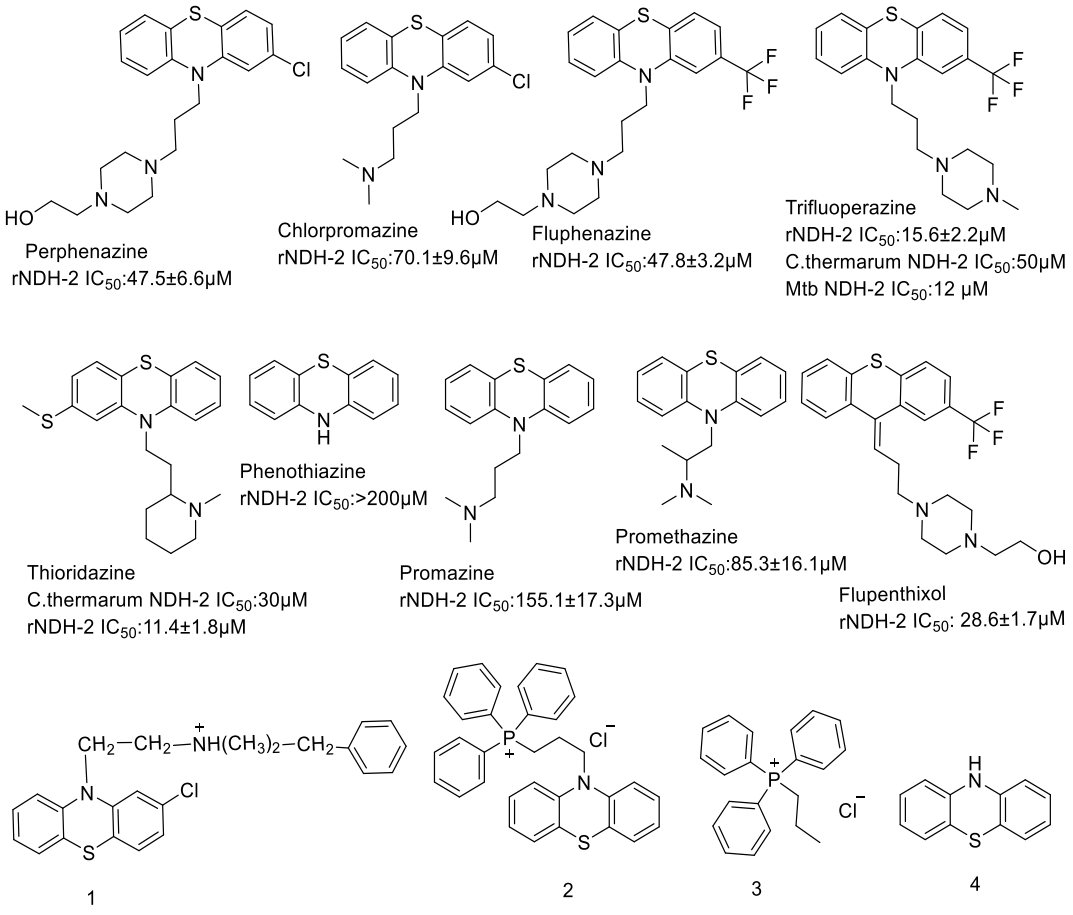


Figure 2-12: Phenothiazine derivatives against type-2 NADH dehydrogenase

2.4.2. ATP synthase

ATP synthase is a macromolecular protein complex found embedded in the mitochondrial membrane and undergoes a rotary mechanism for the conversion of adenosine diphosphate (ADP) to adenosine triphosphate (ATP) by utilizing the PMF or transmembrane electrochemical ion (H^+ or Na^+) gradient.

2.4.2.1. Inhibitors of ATP synthase

Bedaquiline, a diarylquinoline class of drug, inhibits ATP synthase to reduce cellular ATP levels. The Food and Drug Administration authority of United States Health and Human Services department (US-FDA) has approved the use of bedaquiline in adult MDR-TB patients, as a part of combination therapy on 28th December 2012. It is the first drug to be approved after a span of 40 years to treat TB. Bedaquiline is recommended for directly observed therapy (DOT), along with standard MDR-TB drugs in the regimen. A daily dose of 400 mg for two weeks, followed by thrice weekly dose of 200 mg for 22 weeks is recommended. After the completion of bedaquiline therapy for 24 weeks, national TB treatment guidelines should be followed with MDR-TB regimen. Bedaquiline produces many interactions with the 'c ring' of ATP synthase to cover the entire ion-binding sites in c-ring. Thus, it could stop the ion shuttle function of ATP synthase.

2.4.2.2. Mechanism of ATP synthase inhibition

The protons in periplasm enter the membrane through an entry pore in 'subunit a' of ATP synthase and are transported to membrane-spanning region of 'subunit c'. Then the 'subunit c' oligomer gets rotated to almost 360° relative to 'subunit a', and release the proton into cytosolic portion of the membrane through an exit pore in 'subunit a'. During the catalytic function of enzyme, subunit c, ε and γ rotate relative to subunits $\alpha_3\beta_3\delta_{ab}$ for coupling of protons in ATP production. The administered Bedaquiline

reaches the membrane-exposed region of ATP synthase and approaches toward the ion-binding sites of 'subunit c'. During which, the conformation of Phe 69 gets changed to avoid steric classes and also to afford better hydrophobic binding for bedaquiline. A hydrogen bond interaction was formed between the dimethyl amino group of bedaquiline and Glu65. The interactions produced by bedaquiline with the transmembrane oligomeric 'subunit c' of ATP synthase blocked the ion shuttle to inhibit respiratory production of ATP [130-132]. Oligomycin and dicyclohexyl-carbodiimide are classical inhibitors of 'subunit c' of ATP synthase but they are greatly toxic and not selective. Oligomycins are macrolide antibiotics, obtained from *Streptomyces diastatochromogeness.* It forms hydrogen bond interaction with the carboxylic acid group of Glu 59 in 'subunit c' of ATP synthase, which is vital for proton translocation. N, N-Dicyclohexylcarbodiimide (DCCD), a typical inhibitor of ATP synthase, binds to the carboxylic acid group of 'subunit c' to block proton translocation.

2.4.2.3. Crystal structure of ATP synthase

ATP synthase has a membrane anchoring domain consisting of two subunits *viz*. 'subunit a' and 'subunit c'. Both the units are involved in rotary mechanism for translocation of ions from periplasm into cytoplasm. Bedaquiline binds to the rotor ring and totally covers the ion-binding sites of 'subunit c'. This leads to prevention of rotor ring acting as an ion shuttle and stalls the function of ATP synthase. Finally, the process of ATP production is halted. Polymorphisms of amino acids in the membrane-spanning part of 'subunit c' are the key determinants for putative binding of drug molecules. Replacement of a smaller residue in mycobacterial ATP synthase with a bulkier one in ATP synthases from *E. coli* or human mitochondrial homologue imposes steric hindrance on drug binding [133]. Hydrophilic parts of bedaquiline interacts with the essential acidic residues in helix 2 with improved specificity, while the hydrophobic

bulk of bedaquiline interacts with the nearby unipolar residues through Van der Waals contacts or π–π interactions with enhanced free binding energy [101]. Stator stalk, an extended and largely hydrophilic structure was found located at the periphery of the ATP synthase complex. It involved in connecting the non-rotating parts of F_o (subunit a) and F_1 ($\alpha_3\beta_3$) and prevent unproductive rotation of the whole ATP synthase complex during catalysis.

2.5. NDH-2 and ATP synthase: vulnerable targets in latent TB

Dormant *Mtb* does not perform many biosynthetic activities, and therefore, show only very little susceptibility towards the current antibacterials in use [105], [134, 135] [136]. The *Mtb* reside in human alveoli, where they may face low oxygen and nutrition supply and leading to an unconditional challenge for ATP production [137, 138]. It definitely reduces the metabolism and allows the mycobacteria to enter a dormant stage, leading to down-regulation of nucleic acid and protein syntheses, loss of cell replication, and increase in cell-wall thickness. With these repercussions, dormant *Mtb* adapts to the situation and depends on lipid metabolism for energy production [139] [140]. More PMF was generated in the process and consequently sufficient ATP was synthesized [135] [141] . -110mV PMF was reported with *Mtb*, but it was ~-200 mV in other bacteria [105]. The increased PMF level in *Mtb* indicates adaptation to low nutrient environment [105] [102]. Targeting any rate limiting enzyme involved in the production of PMF may be rewarding in handling latent *Mtb*. Thus, targeting NDH-2 could be effective approach. Depletion of ATP was found lethal to non-growing latent *Mtb*. Bedaquiline, the inhibitor of ATP synthase produced higher bactericidal activity in dormant *Mtb* than in replicating *Mtb*, indicating that ATP synthase is also a vulnerable target in latent TB [102].