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Reviews

Mycothiol: A promising antitubercular target

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ABSTRACT

Tuberculosis (TB) is the world's second commonest cause of death next to HIV/AIDS. The increasing emergence of multi drug resistance and the recalcitrant nature of persistent infections pose an additional challenge for the treatment of TB. Due to the development of resistance to conventional antibiotics there is a need for new therapeutic strategies to combat *M. tuberculosis*. One such target is Mycothiol (MSH), a major low molecular-mass thiol in mycobacteria, an important cellular anti-oxidant. MSH is present only in actinomycetes and hence is a good target.

This review explores mycothiol as a potential target against tuberculosis and various research ongoing worldwide.

Contents

1. Introduction

Tuberculosis has been one of the deadliest diseases over the past few decades affecting nearly one-third of the world's population [1] with new infection occurring at 1% of population each year [2]. According to WHO studies, in 2011, there were 8.7 million new cases of TB (13% co-infected with HIV) and 1.4 million people died from TB including one million HIV negative people [3]. The occurrence of Multiple-Drug Resistant (MDR) and Extensive-Drug Resistant (XDR) raised the concern for current regimen [4–9]. Recently it has been suggested that there is an existence of Totally Drug Resistant (TDR) tuberculosis [5,10–11]. Hence the development of new targets and molecules is of utmost importance.

Our main focus in this review is Mycothiol (MSH) an important component for the growth and survival of *Mycobacterium tuberculosis*. Bacterial cells are prone to reactive oxygen species and require protection from their damaging effects [12]. The major cellular components such as nucleic acid, proteins and lipids get affected by oxidation [13]. Internally toxic oxidants are generated by bacteria during respiration and the host cell produces reactive oxygen species (ROS) as a component of their anti-microbial response. MSH is the major low molecular-mass thiol in mycobacteria, an important cellular anti-oxidant [14]. MSH is found only in actinomycetes and is present at high levels in *M. tuberculosis* [15,16]. Mycobacteria lack glutathione, (low-molecular-mass thiol) in

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many other bacteria and hence MSH is required for its anti-oxidant activity [17]. MSH is composed of cysteine group linked with an acetylated amino group [18]. The heavy metal-catalyzed autoxidation of cysteine is much more rapid than glutathione [19–21]. The enzymes involved in the biosynthesis of mycothiol have been studied and their mechanism has been proposed by Fan et al. [22]. Mycothione is reduced to mycothiol with the help of enzyme flavoprotein mycothione reductase [16,23–25].

2. A potent antitubercular target

MSH also acts as a protective substance against various other harmful compounds in addition to their anti-oxidant activity [14]. MSH thought to act as a cofactor for formaldehyde dehyrogenase in the detoxification of formaldehyde in Amycolatopsis methanolica. The alkylating agents are converted to S-conjugate of MSH by mycothiol and thus detoxify them [26]. Apart from this, the sensitivity of *Mycobacterium smegmatis* to antibiotics such as rifampin and isoniazid is also influenced by MSH [27–29]. MSH provides growth in an oxygen-rich environment and establishes the pattern of resistance to isoniazid and rifampin in *M. Tuberculosis* [17]. Furthermore, recent reports describes high density mutagenesis in *M. tuberculosis* have provided further evidence that MSH is essential for the growth and survival of this pathogen [30,31]. So, targeting MSH can achieve *M. tuberculosis* inhibitory activity constituting Mycothiol as an attractive and effective antitubercular target.

3. Biosynthesis of MSH

Isolation and structure elucidation of MSH (1-O-[2-[[(2R)-2-(acetylamino)-3-mercapto-1-oxopropyl]amino]-2-deoxy-α-D-glucopyranosyl]-D-myo-inositol) has been done from Streptomyces strains [32,33] and *Mycobacterium bovis* [29]. The conformation of mycothiol-*S*-bimane in aqueous solution was done by Bewley and coworkers by nuclear magnetic resonance [35]. MSH biosynthesis is accomplished in five steps starting from 1L-myo-inositol-1-phosphate (1L-Ins-1-P or Ins-P), which is produced from glucose-6-phosphate (Glc-6-P) by inositol phosphate synthase (Ino1) (Fig. 1) and was first elaborated in mycobacteria [36,37].

The intermediate 1-O-(2-acetamido-2-deoxy-D-glucopyranosyl) D-myo-inositol-3-phosphate (GlcNAc-Ins-P), produced from UDP-GlcNAc and Ins-P by the glycosyltransferase MshA, is the first intermediate in MSH biosynthesis [38,39]. The next step is dephosphorylation of GlcNAc-Ins-P catalyzed by MshA2. The third step is the deacetylation of 1-O-(2-acetamido-2-deoxy-D-glucopyranosyl)-D-myo-inositol (GlcNAc-Ins) by the metalloprotein MshB [37]

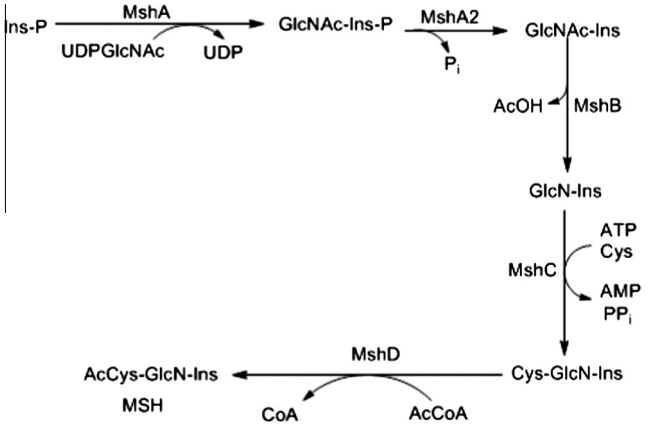

Fig. 1. Biosynthesis of MSH.

to yield 1-O-(2-amino-2-deoxy-D-glucopyranosyl)-D-myo-inositol (GlcN-Ins) and is the major intermediate found in extracts of mycobacteria [17,29,40]. Steenkamp and Spies [34] were the first to identify the substrate as well as the products involved in the fourth and the fifth step of MSH biosynthesis and which was further confirmed by Anderberg et al. [41]. Recent studies showed that MshB follow general acid–base pair mechanism in which side chain of Asp-15 acts as a general base catalyst while His-144 acts as a general acid catalyst [42]. Automated docking study showed that hydrophobic cavity near the active site of MshB is responsible for substrate specificity [43].

The fourth step along the pathway is the ATP-dependent ligation of cysteine with GlcN-Ins by MshC, a homolog of Cys-tRNA synthetase, to produce 1-O-[2-[[(2R)-2-amino-3-mercapto-1-oxopropyl]amino]-2-deoxy-D-glucopyranosyl]-D-myo-inositol (Cys GlcN-Ins) [44]. In the final step of MSH biosynthesis the amino group of cysteine in Cys-GlcN-Ins is acetylated by acetyl-CoA under catalysis by a GCN5 acetyl-transferase, MshD, to synthesize MSH [45]. Initially, in MSH biosynthesis, there is a formation of the pseudo-disaccharide phosphate GlcNAc-Ins-P by MshA, a glycosyl-transferase identified by Tn5 mutagenesis of M. smegmatis [38]. MshA orthologs are found in the complete genomes of many Actinobacteria. The enzymes involved in mycothiol synthesis in *Mycobacterium tuberculi* are shown in Table 1 below [18].

4. Regulation of Msh biosynthesis

Studies showed that there is a transcriptional regulator which precedes MshA in *M. tuberculosis* (Rv0485) and MshD in *M. tuberculosis* (Rv0818). The mRNA levels of biosynthetic enzymes were found relatively constant in *M. tuberculosis* as none of these genes have been identified in studies examining changes in mRNA levels under varying conditions, such as stationary phase and low oxygen [46], exposure to palmitic acid and hydrogen peroxide, growth within macrophages [47], and exposure to nitric oxide [48]. The Concentrations of mycothiol and mycothiol precursors in *M. tuberculosis* is given in Table 2 [17].

5. Msh drug targets in *M. tuberculosis*

5.1. Bromotyrosine-derived natural and Synthetic products as inhibitors of mycothiol-S-conjugate amidase

MSH-dependent detoxification enzyme mycothiol-S-conjugate amidase (MCA) was characterized by Newton et al. [27]. MCA was found to cleave the amide bond linking cysteine and glucosamine in MSH-S-conjugates to form D-glucosamine- α(1-1)myo-D-inositol, having specificity for S-conjugates. It can be then recycled into MSH biosynthesis [37], and the N-acetyl-cysteinyl-S-conjugate, which can be excreted from the cell (Fig. 2) [27]. MCA is present only in actinomycetes and shares no sequence homology with eukaryotic enzymes. Hence it represents a novel target for new classes of antimycobacterials [49]. Following figure shows the role of mycothiol S-conjugates amidase (MCA) in mycothiol dependent detoxification.

Among the six (**1–6**) natural analogues studied, **1** was the most potent analogue having IC_{50} of 3 μM against Mt MCA. Compound **1**, maintains its spirocyclic system while the next potent compound **6**, contains reduced bromophenyl oximinoamide moiety (see Fig. 3).

Seven **(8–14)** analogues were synthesized and studied. The natural products, Psamma-plysins A **3** and B **4** yielded the similar IC_{50} values (20–30 μM) as that of synthetic compounds **13** and **14** (35 μM). These values help to prove the rationale that the spirocyclic ring system is dispensable, but suggest that the

Table 1
Enzymes involved in mycothiol synthesis and their Gene content.

Sr no	Name of the enzyme	Abbrevation	Gene	Reactant	Product	M.tuberculosis
1	MSH Glycosyltransferase	MshA	mshA	1L-Ins-1-p	GlcNAc-Ins-P	Rv0486
2	MSH Phosphatase	MshA2	–	GlcNAc-Ins-P	GlcNAc-Ins	–
3	MSH Deacetylase	MshB	mshB	GlcNAc-Ins	GlcN-Ins	Rv1170
4	MSH Ligase	MshC	mshC	GlcN-Ins	Cys-GlcN-Ins	Rv2130
5	MSH Synthase (MSH Acetyltransferase)	MshD	mshD	Cys-GlcN-Ins	AcCys-GlcN-Ins (Mycothiol, MSH)	Rv0819

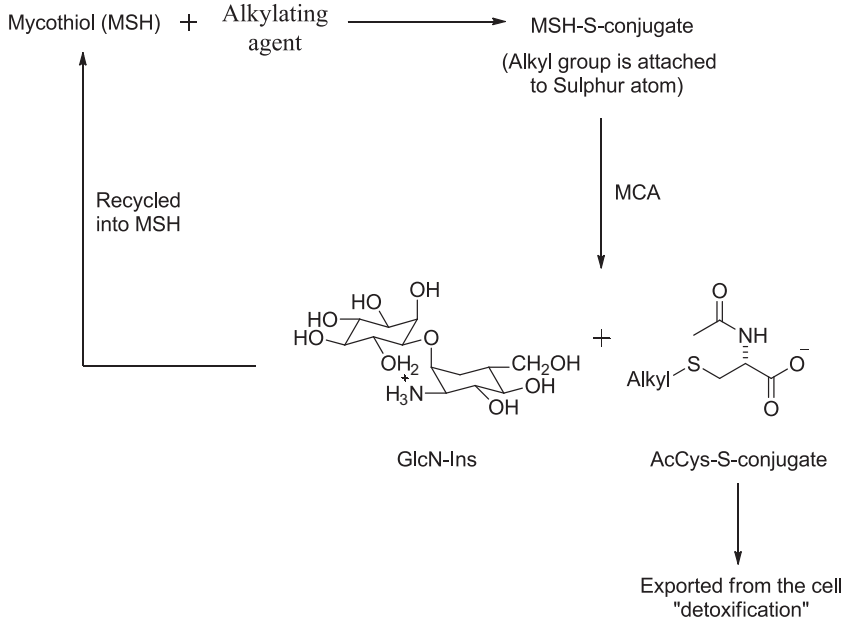

Fig. 2. The role of mycothiol-S-conjugate amidase (MCA) in mycothiol dependent detoxification.

spiro[4,5]decatriene system may be more effective than the spiro[4,6]undecatriene structure present in **3** and **4** [49].

5.2. 2-Deoxy-2-C-alkylglucosides of myo-Inositol as possible inhibitors of a N-deacetylase enzyme

Biosynthetic procedure for the synthesis of mycothiol involves the intermediate called 1-D-1-O-(2-acetamido-2-deoxy-α-D-glucopyranosyl)-myo-inositol. Two new analogues of this 1-D-1-O-(2-acetamido-2-deoxy-α-D-glucopyranosyl)-myo-inositol were synthesized. Both the 2-deoxy-2-C-(2′-hydroxypropyl)-D-glucoside **15**, and the 2-deoxy-2-C-(2′-oxopropyl)-D-glucoside **16** showed to inhibit the incorporation of [^{3}H] inositol by whole cells of *M. smegmatis* into a number of metabolites, which contain inositol [50].

Modifications at C-2 of the glucosamine unit act as inhibitors to the N-deacetylase enzyme. Thus, it prevented the production of mycothiol and the survival of *M. tuberculosis*. Compounds **15** and **16** did not inhibit the growth of *M. smegmatis in vitro*. It was observed that incorporation of [^{3}H] inositol into inositol containing metabolites was inhibited by whole cells [50] (see Fig. 4).

5.3. NTF1836 as an inhibitor of MshC

M. smegmatis mutants defective in MshC were isolated and found that they were devoid in MSH production. This suggests that MshC is essential for *M. tuberculosis* [28]. There are reports on successful expression and affinity purification of three fusion protein constructs of *M. tuberculosis* MshC in *M. smegmatis* [50,51]. NTF1836 (**17**), was the first MSH biosynthesis inhibitor identified and found in a screen for inhibitors of the native *M. Tuberculosis* MshC [52]. Compound **17** was found to be a strong inhibitor of MshC having IC50 of 0.085 ± 0.010 mM. Two quinaldinium derivatives **18** and **19** (Fig.5) were identified by a screen using *M. tuberculosis* MPB-MshC as micro molar inhibitors of MshC [53].

39 Analogues were synthesized with modifications in three positions R1, R2 and X (Fig. 5). The first (motif A, Table 1) focused on variation with the R1 and X groups. The presence of a tri-substituted amine at the terminus of R1 played a key role. Replacement of the amine with a sulfide led to a complete loss of measurable activity. Sulfoxides (X = SO) were found to be better inhibitors by 5- to 15-fold than their corresponding sulfones (X = SO2). The oxidation state of the sulfur atom also affects the activity. For motif A, best compound found was **20** having IC_{50} 0.08 ± 0.02 mM [54]. Removal of the chloro substituent from R2 increased the IC_{50} value about four fold in the sulfoxide series (**21**). For motif B, best compound **22** was found to be best in series having IC_{50} of 0.25 ± 0.10 mM [54].

5.4. Substrate-mimic mycothiol analogs

The substrate-mimic mycothiol analogs were synthesized, tested against the mycobacterial detoxification enzyme mycothiol-S-conjugate amidase (MCA) and were found to be modest inhibitors. The analogues were found to incorporate quinic acid template to replace the inositol ring of natural MSH [55]. Synthesized molecules were tested for inhibition of recombinant MCA with the help of Mycothiol bimane or its cyclohexyl thioglycoside analogue [56] as substrate [57] (see Fig. 6).

Compounds **23** and **24** were found to be the most active against MCA at 50 μM. The inhibitory activity was calculated as % inhibi-

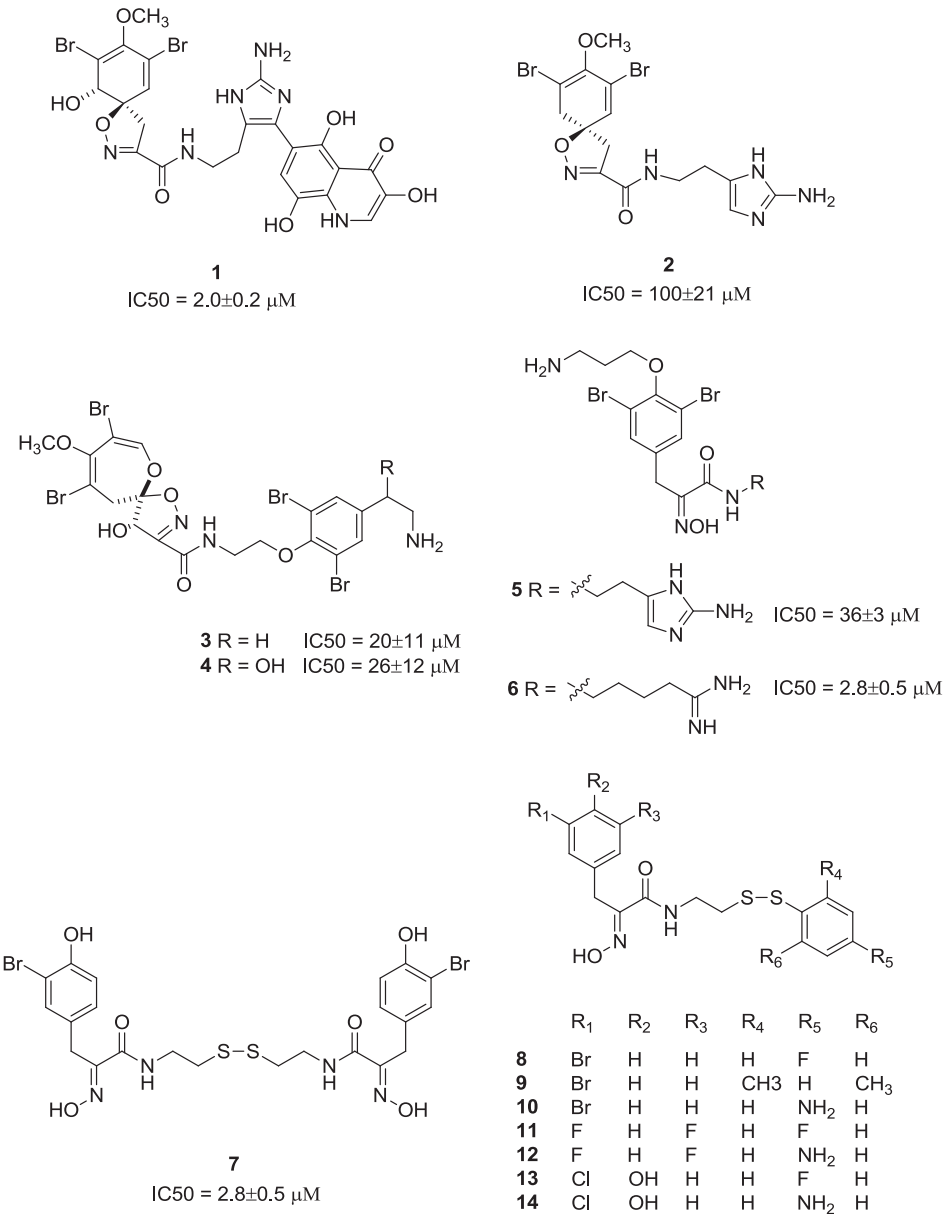

Fig. 3. Bromotyrosine-derived natural and synthetic products.

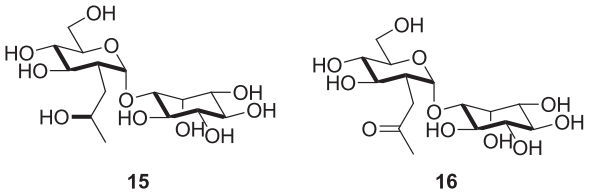

Fig. 4. Deoxy-2-C-alkylglucosides of myo-inositol.

tion of MCA. Most potent analogues were found to be **23** and **24** having MCA inhibition of 46% and 44% resp.

5.5. Conjugates of plumbagin and phenyl-2-amino-1-thioglucoside

2,4-Naphthoquinones, were synthesized as potential inhibitors of MshB using a strategy similar to that reported by Salmon-Chemin et al. [58]. Naphthoquinones were found to exhibit competitive inhibition. Inhibition constants for **25**, **27** and **28** were found to be 167 ± 15 μM, 94 ± 11 μM and 16.8 ± 1.9 μM, respectively. As the length of the spacer increases in the naphthoquinone derivatives that there is an increase in binding affinity. The percentage inhibition is shown in Table 3 (see Fig. 7).

Percentage inhibition of MCA and MshB by substituted naphthoquinones was determined at substrate and inhibitor concentrations of 250 μM each for MCA and 500 μM each for MshB. To get a clear idea, Maynes et al. docked the naphthoquinones with 2 carbons and 5 carbon spacers, **25** and **28** to the crystal structure of MshB [59]. Results showed that due to the longer carbon chain of **26**, the molecule was extended such that to allow interaction of the naphthoquinone group with a hydrophobic dipeptide of Val 184 and Leu 185, while the carbonyl group of amide link is positioned such as to interact with the active site Zn atom. A similar inhibition pattern of was observed for Mca as longer spacers were also found to be better inhibitors of Mca [60].

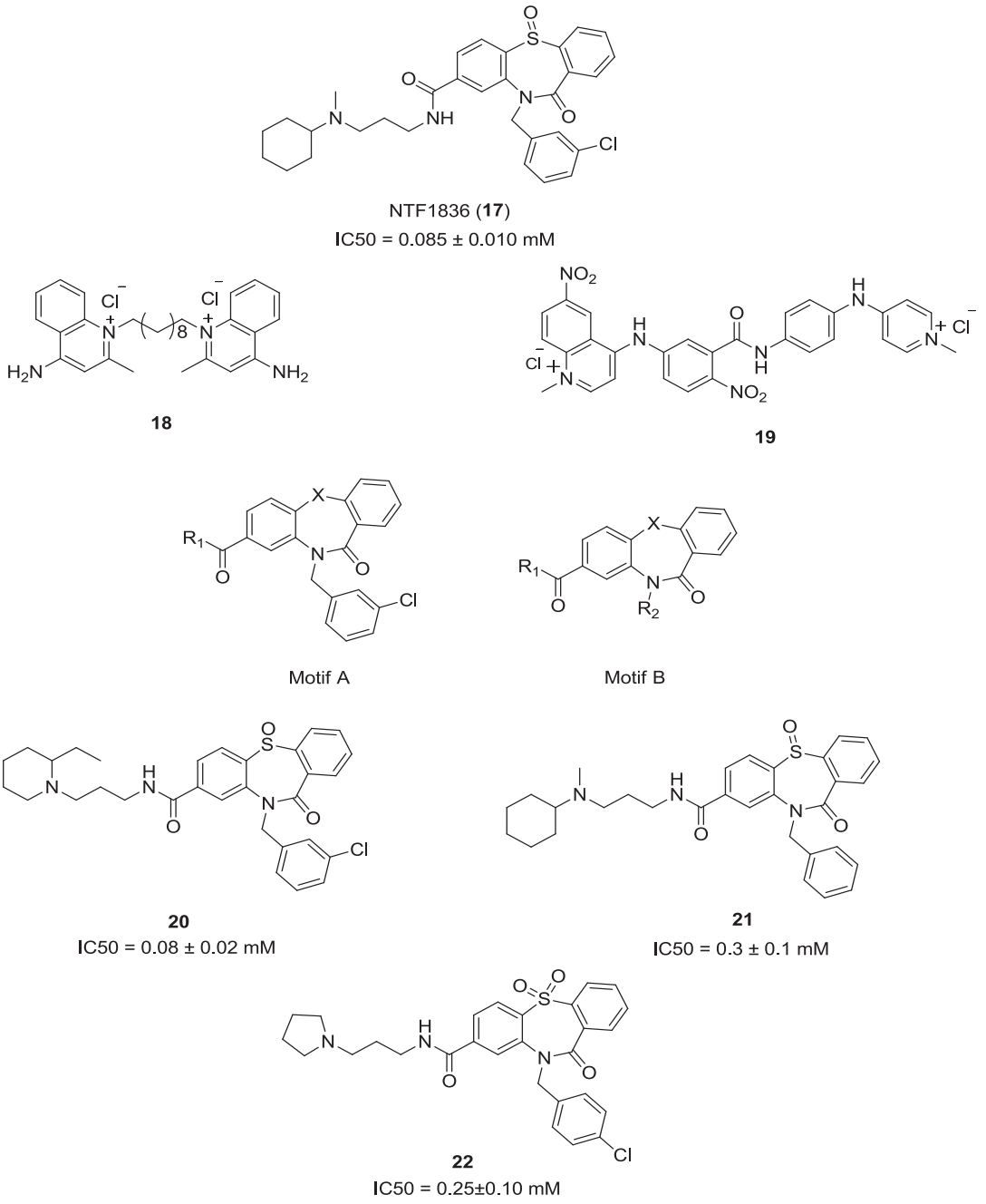

Fig. 5. NTF1836 and its derivatives.

Table 2
Concentrations of mycothiol and mycothiol precursors in M. tuberculosis.

Strain	Mycothiol (nmol/10^9 cells)	GlcNAc-Ins (nmol/10^9 cells)	GlcN-Ins (nmol/10^9 cells)
Wild type	14 ± 3.0	0.86 ± 0.32	6.5 ± 0.8
Rv1170 mutant	2.9 ± 0.3	43 ± 1	0.22 ± 0.02
Complemented mutant	21 ± 3	1.3 ± 0.7	6.4 ± 0.6
Mutant + empty vector	4.9 ± 0.4	52 ± 3	0.07 ± 0.03

5.6. 7-Methyljuglone derivatives

MSH is found to play an important role in oxidative stress management and is oxidized to the symmetrical disulfide (MSSM) in the process. The intracellular environment is maintained in reduced state by NADPH-dependent enzyme mycothiol disulfide reductase (Mtr) by reducing MSSM back to MSH [23]. It was found that MSH is essential for the growth of *M. tuberculosis* [61] and increased sensitivity to oxidative stress was exhibited by MSH-deficient mycobacteria [28,29] and hence this redox pathway can be a good target for novel antitubercular chemotherapies.

Naphthoquinones were found to show various pharmacological properties like anticancer [62], antimalarial [63,64], antiviral [65], antibacterial [66,67], trypanocidal [68], antifungal [58] activity.

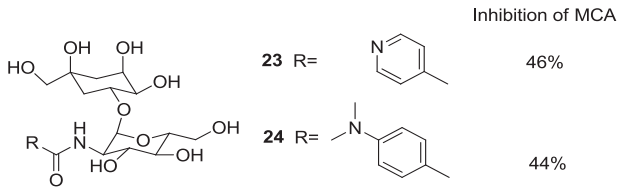

Fig. 6. Substrate-mimic mycothiol analogs.

Table 3
Percentage inhibition of Mca and MshB by substituted naphthoquinones.

Substrate	MCA (%)	MshB (%)
25	28.8	57.4
26	37.8	81.6
27	23.2	81.4
28	44.5	94.8

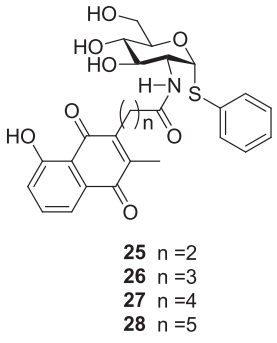

Fig. 7. Naphthoquinones derivatives.

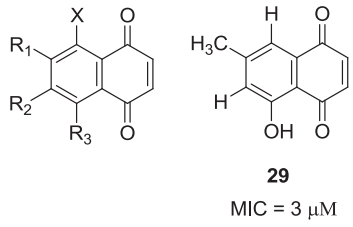

Fig. 8. 7-Methyljuglone derivatives.

Many naphthoquinones are known to operate as subversive substrates with flavoprotein disulfide reductases such as mycothiol disulfide reductase (Mtr) [23].Among the ten naphthoquinone derivatives reported, compound **29** is most active with MIC 3 μM against *Mycobacterial tuberculi* [69]. Mono or dihydroxy substitution has shown better activity than parent 1,4-naphthoquinone because of the increased efficiency of redox cycling [70] (see Fig. 8).

6. Conclusion

Tubercular infections pose a continuous and serious threat to human health and life in recent years. There has been an increased use of antibuercular agents and has resulted in the development of resistance. This has given rise to search for molecules acting on a novel target or a multi targeted combination therapy. MSH is present at high levels in *M. tuberculosis*. Recent reports describes that MSH is essential for growth and survival of this pathogen. So, targeting MSH can achieve *M. tuberculosis* inhibitory activity constituting Mycothiol as an attractive and effective antitubercular target.

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