Vol. 15(2), pp. 41-xx, July-December 2023
DOI: 10.5897/JIDI2020.0199
Article Number: 2D392BC71171
ISSN 2141-2375

http://www.academicjournals.org/JIDI

ACADEMIC JOURNALS
expand your knowledge

Journal of Infectious Diseases and Immunity

Review

Molecular mechanism of Clofazimine resistance in tuberculosis

Sumaia Khatun[1], Sadia Afrin[2] and Shah Alam[3,4]*

[1]Department of Botany, Faculty of Faculty of Biology, Chuadanga Govt. College-0801, National University of Bangladesh, Bangladesh.
[2]Department of Genetic Engineering and Biotechnology, Faculty of Life and earth sciences, University of Rajshahi, Rajshahi-6205, Bangladesh.
[3]Department of Biochemistry and Molecular Biology, Guangzhou Institutes of Biomedicine Science and Health (GIBH), Chinese Academy of Sciences, Guangzhou, China.
[4]Department of Biochemistry, Faculty of Biomedicine Science and Health, University of Chinese Academy of Science (UCAS), Beijing 100049, China

Received 27 February 2020; Accepted 9 June 2020.

Drug resistance which is at present the main impendence to global tuberculosis (TB) control and drug-resistant (DR) activity of *Mycobacterium tuberculosis* in various strains have become the major challenge worldwide. Nowadays, many researchers use different antibiotics for treatment of DR-TB which are often not well tolerated and not adequately efficient. A large group study from various research published in different time described a treatment regimen for multidrug-resistant tuberculosis (MDR-TB) including Clofazimine (CFZ) as high incidence against MDR-TB; whereas the therapeutic ways are still limited, the main strategy for treatment of DR-TB is to repurpose existing anti-mycobacterial agents. CFZ is one such drug that has recently devoted interest against DR-TB. CFZ is a fat-soluble riminophenazine dye used in the treatment of TB, HIV, and leprosy co-infected people worldwide. Clofazimine has shown action against MDR TB and which is now recommended by the WHO to treat drug resistant tuberculosis as a therapeutic agent with "unclear efficacy". Although the mode of action and molecular mechanism of CFZ are not yet entirely understood, it has been exposed that outer membrane is its primary action site, an extensive number of mutant resistant to clofazimine and found mutations in rv0678 to be the most prominent mechanism of clofazimine resistance and the respiratory chain and ion transporters are the putative targets. This study discussed the comprehensive report, action and molecular reactivity of CFZ, and provides new acuteness into the clinical conduct of this drug.

Key words: Clofazimine, background, mode of action, molecular mechanism.

INTRODUCTION

Tuberculosis (TB) is the most crucial bacterial infectious disease and public health concern worldwide caused by *Mycobacterium tuberculosis* that naturally infects lungs and other organs, such as the kidney, spine, or brain.

*Corresponding author. E-mail: shahalam.geb@mails.ucas.ac.cn.

The increase of multidrug-resistant TB (MDR-TB)/ extensively drug-resistant tuberculosis (XDR-TB), with resistance to isoniazid and rifampin, poses major clinical challenges to treatment with present available anti- TB drugs. The crisis is enhanced by the increasing emergence of extensively drug-resistant (XDR) strains of *M. tuberculosis*, which cause inability to treat diseases effectively with subsisting elements (Zumla et al., 2013). According to the latest World Health Organization (WHO) report, there were an estimated 10 million people infected with TB in 2018 and 1.5 million deaths were imputed to the disease. One million children infected with the disease, including 251000 persons who died were reported among HIV-infected persons (WHO, 2019). However, even more disturbing is the emergence of drug resistance. In 2017, there were an estimated 450,000 cases of MDR-TB and 170,000 deaths were as a result. The treatment outcome of MDR-TB and XDR-TB patients is often poor and unsuccessful in the absence of an optimal number of active drugs (Belachew T et al., 2022; Seung KJ et al., 2015). MDR- TB is a disease caused by bacterial strains of *M. tuberculosis* that are resistant to at least rifampicin and isoniazid, two main drugs in the treatment of the disease. Depending on the present situation, it is widely acknowledged that we need to develop new beneficial drug combination for TB and that these new regimens should be tested at the preclinical stage, rather than testing a series of single drugs separately, wherein compensate the TB drug development pipeline more effectively . In addition to being MDR are also resistant to any antibiotics that are commonly used to treat a variety of illnesses or fluoroquinolone and to at least one of the most injectable second-line drugs: Clofazimine, kanamycin, capreomycin or amikacin (Gandhi et al., 2006). Moreover, clofazimine which was currently exhibited to reduce the duration of TB in a mouse model of TB (Tyagi et al., 2015; Ivan et al., 2019). Early identification of all forms of drug resistance in TB is a main factor to reduce and contain the spread of these resistant strains. A better knowledge of the mechanisms of different anti-TB drugs and the development of drug resistance will allow identifying new drug targets and better findings to detect drug resistance. This present investigation will review the background, diagnosis, mode of action and resistance mechanisms of the main anti-TB drugs as well as Clofazimine drugs on recent details described with anti-TB activity. New tuberculosis drug which is used for leprosy and multidrug-resistant tuberculosis prevention (Olayanju et al., 2018), is also active against *Masoud Abessi* and shows synergistic action when given together with amikacin (Singh et al., 2014; Shen et al., 2010).

Clofazimine is a significant antimicrobial force which has activity *in vitro* against mycobacteria. It is a riminophenazine drug which was initiated as an anti-TB agent in the 1950s, (Barry et al., 1957) but is also mostly used to treat leprosy (Browne et al., 1962). Although this drug has showed good reaction against *M. tuberculosis in vitro*, it was not used in the treatment of pulmonary TB MDR- TB, there has recently been interest in the use of clofazimine to treat MDR-TB (Zumla et al., 2013) and its use might shorten MDR-TB treatment to 9 months. Clofazimine, commonly known as B663, which was first synthesized in 1954 by a group study of scientists (Barry et al., 1957) at Trinity College, Dublin, was originally intended as an anti-tuberculosis drug but was substantiated ineffective. In 1959, a researcher named Y. T. Chang destines its essential effectiveness against leprosy. Clofazimine was approved in the United States in 1986 for medical purpose use (Dagron, 2016). It is on the World Health Organization's List of Essential Medicines, the most functionalize and safe medicines needed in a health system in the world (WHO, 2015). Inconsistent results in animal models hindered its development for tuberculosis, but it was licensed for treatment of leprosy in 1969 (Reddy et al., 1999). Clofazimine has a biological shelf-life of about 70 days. Common side effects include abdominal pain, itchiness, dry skin diarrhea and change in color of the skin of human (Van et al., 1982). This may also cause swelling of the lining of gastrointestinal problem, increased blood sugar level, and sensitivity to the sun. Autopsies performed on those who have died while on clofazimine show crystal-like aggregates in the intestinal mucosa, liver, spleen, and lymph nodes (Baik and Rosania, 2011). It is a drug suggested to work by interfering with DNA. The immunosuppressive important action of clofazimine was immediately observed when applied in animal model. Macrophages were first studied to be inhibited due to the stabilization of lysosomal membrane by clofazimine. Clofazimine also showed a dosage-dependent inhibition of neutrophil motility, lymphocyte, transformation (Gartner et al., 1982), mitogen-induced PBMC proliferation (Van et al., 1982) and complement-mediated solubilization of pre-formed immune complexes in vitro condition (Kashyap et al., 1992). Clofazimine has also been sporadically reported with some success in other autoimmune diseases such as psoriasis, Miescher's granulomatous s cheiliti (Chuaprapaisilp and Piamphongsant, 1978). In recent years, as the global prevalence of drug-resistant TB has increased, researchers have sought to re-evaluate the chemotherapeutic role of CFZ as an anti-tuberculosis drug. Tuberculosis (TB) infected early humans in Africa 70,000 years ago and was carried worldwide by human out-of-Africa migrations, according to new research investigation into the genome sequences of *M. tuberculosis*, the bacteria responsible for the serious disease. Tuberculosis (TB) remains a leading cause of morbidity and mortality in the world health condition (Podmore and Burrows, 1986). Despite years of research, no vaccine currently exists that protects reliably against pulmonary TB in adults, which is the most transmissible form of the disease on condition to another (WHO, 2019). History suggests that TB appeared about

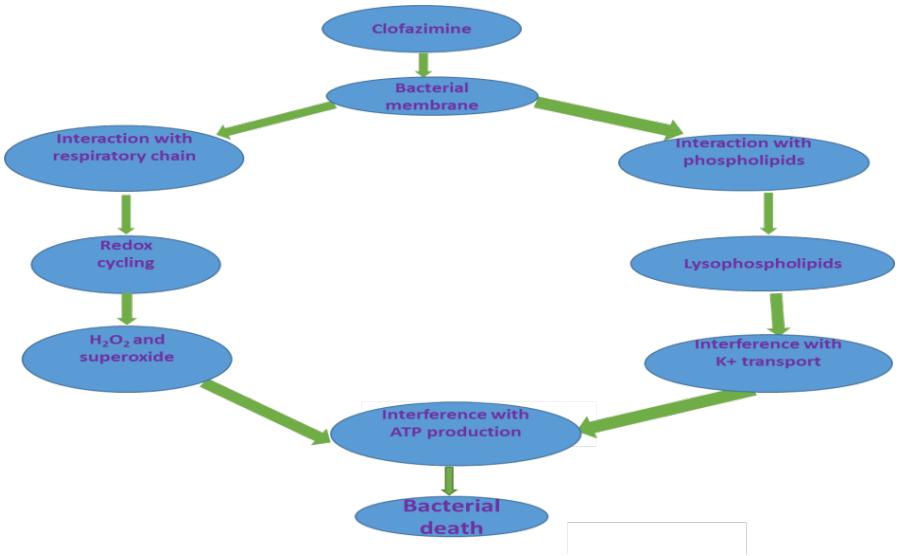

Figure 1. Mode of action of Clofazime.

70 000 years ago and has remained in a sporadic state up to the 18th century. Last of all, it became epidemic during the industrial revolution, owing to the increased population density and the unfavorable living conditions (Copin et al., 2014). Epidemiological data and the work by Comas et al. (2013) on the comparison of mitochondrial DNA from humans and from MTBC race suggest that the different race might have specifically adjusted to the different human populations.

MODE OF ACTION OF CLOFAZIME

The mechanism(s) of antimicrobial action of CFZ (Figure 1) is not well understood. However, it has been exposed that the initial action site of this antibiotic appears to be the outer site of the membrane. In this mechanism, the mycobacterial respiratory chain and ion transporters are the putative targets and CFZ objects by inhibiting these targets (Lange et al., 2019). Another study (Arbiser and Moschella, 1995) suggests that the mechanism of action was primarily thought that CFZ works by binding to the guanine bases of bacterial DNA, thereby inhibiting bacterial proliferations. However this theory has been replaced by the belief that it acts through outcome on intracellular redox cycling (Gopal et al., 2013) and membrane destabilization condition (Gopal et al., 2013) (Figure 2). Some previous studies investigated that CFZ which selectively binds to guanine base of DNA, and it is possible that this drug has a selective performance on DNA functions target microorganism in *M. tuberculosis* (Oliva et al., 2004). Also, CFZ can increase the outcome of bacterial phospholipase A2 and release lysophospholipids, the enzymatic hydrolysis products which are mostly toxic to *M. tuberculosis*, leading to underpin the anti-mycobacterial effect of this drug (Parker et al., 2007). Through increasing reactive oxidant species, CFZ is a drug which may act to enhance killing of antibiotic tolerant *M. tuberculosis* persisted organisms (Grant et al., 2012). In addition other antimicrobial action studies suggested that, the drug has other pharmacological activities such as prooxidative, anti-inflammatory and other immune-pharmacological properties (Reddy et al., 1999). Synergistic effects of interferon gamma and CFZ, as observed by Parak and Wadee (1991) may play an important role to the anti-tuberculosis effect of the drug. CFZ system reverses the inhibitory effect of *M. tuberculosis*; these are derived factors on phagocyte intracellular killing mechanisms which may also exploit to enhance *M. tuberculosis* killing (Wadee et al., 1988). Whereas study on the phenazine molecules is auto-oxidizable compounds, they could work as artificial electron acceptors molecule. Consequently, respiratory system oxidizes CFZ instead of NADH, because of a reduction in the amount of ATP available for all type of active cellular processes (Murugesan et al., 2018), have also shown a potential synergistic effect of CFZ with pyrazinamide(PZA) (Tasneen et al., 2011) and with clarithromycin (CLM) (Lu et al., 2010) in M. tuberculosis killing. Due to highly lipophilicity characters and redox potential of CFZ, the anti-mycobacterial action of this drug is followed by an oxidation of reduced CFZ, leading to the production of reactive oxygen species. Ammerman et al. (2016) have investigated that CFZ has time delayed anti-M. The tuberculosis action was due to its mechanism of action. This team have focused that although in the first week of administration, CFZ did not show any bactericidal activity at any concentration neither *in vitro* nor *in vivo* condition, it showed concentration-dependent antimicrobial action during the second week

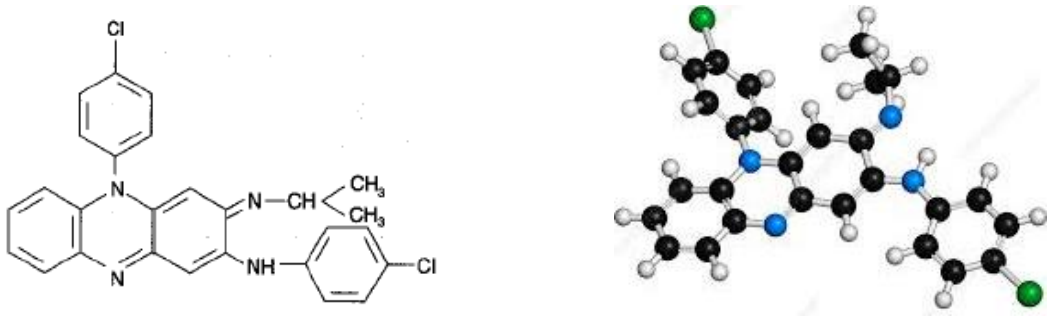

Figure 2. Chemical and 3D structure of Clofazime (hydrogen-white, carbon-black, nitrogen-blue, chlorine-green).
Source: Science photo.

which expose both *in vitro* and *in vivo* condition (Ammerman, et al., 2016).

MOLECULAR MECHANISM OF CLOFAZIMINE

The antileprosy drug clofazimine is also of highly interest for the treatment of multidrug-resistant tuberculosis. Recent study, due to the spread of multidrug-resistant *M. tuberculosis* strains there has been renovated interest in the use of clofazimine for treatment of multidrug-resistant tuberculosis disease (Dalcolmo et al., 2017). It is a phenazine dye and is regarded to act by interfering with DNA (Kaufman, 2013). Clofazimine is an antibiotic which has been clearly elucidated in M. tuberculosis to be a prodrug; which is reduced by NADH dehydrogenase (Ndh2), and then upon spontaneous reoxidation by O2, to release reactive oxygen species (ROS) (Murugesan et al., 2018). As there is no specific target state for ROS, resistance to clofazimine is rare. However, the mechanisms of resistance to clofazimine in *M. tuberculosis* are not still well characterized. rv0678 is a regulatory gene which is clearly an emergent genetic locus involved in such resistance, as it is generally found in both laboratory selective and clinically isolated mutants of M. tuberculosis (Almeida et al., 2016; Hartkoorn et al., 2014). Another study also found clofazimine resistant mutants isolated in-vitro analysis from *M. tuberculosis* H37Rv and found various new mutations in the rv0678 gene that had not been previously studied (Zhang et al., 2015) (Tables 1 and 2). In addition, new mutations were found in two new genes, rv1979c and rv2535c, which are importantly associated with clofazimine resistance in *M. tuberculosis*. InDels that cause frame shift and nonsense or point mutations that result in stop codons were responsible for large proportion (70%) of all mutations in rv0678 (Zhang et al., 2015). BLAST which was used to find homologous involvement traits proteins with more than 40% identities and 90% query coverage for Rv1979c, Rv2535c and Rv0678 (Altschul et al., 1990). On the contrary, functional uniformity between homologous trait proteins is less predictable, which Pearson (2013) investigated to infer functional similarity by orthologues.

Recently, some studies reported that mutations in *rv0678* and *pepQ* regulate the significant mechanisms of clinical resistance to bedaquiline and clofazimine (Hameed et al., 2018; Islam et al., 2017). Rv0678 is well agreed upon as a transcriptional repressor proteins that binds to a sequence located in the intergenic region between this gene locus and the neighboringmmpL5 and mmpS5gene (Hartkoorn et al., 2014). Clofazimine *in vitro* mutants due to mutation in the Rv0678 regulatory gene turn out for up regulation of the MmpL5 efflux pump gene. In a previous study (Milano et al., 2009) many mutations in Rv0678 were found that led to de-repression of mmpL5 gene. The MmpL protein family has the suppositional function of transporting lipids, and an essential proportion of these membrane related proteins are conserved within the genome of *Mycobacterium leprae*. This makes sense because all the mutations that abolish the repressor activity of Rv0678 will cause the overexpression of efflux pump MmpL5, which in turn causes clofazimine resistance. The crystal structure of Rv0678 demonstrated that it forms a dimer and binds to a 2-stearoylglycerol ligand (Radhakrishnan et al., 2014). This finding suggests that Rv0678 may be subject to regulation by metabolic products of the bacteria, which may react as the efflux activity of MmpL5 and susceptibility to clofazimine. The mutation information identified in this study will be important for designing new molecular tests for rapid detection of clofazimine resistance and its cross resistance to bedaquiline in future, as it is more commonly used for treating TB and MDR-TB. However, this study provides new insight into mechanisms of resistance to clofazimine by identifying two new genes (rv1979c and rv2535c) (Table 1), which was previously not well known to be associated with clofazimine resistance. Consequently these mycobacterial proteins possibly have tributary roles in the virulence and drug

Table 1. Catalog of previously published mutations from clofazimine-resistant in vitro and in vivo isolates.

Mutation			Approach	Notes	MIC (µg/mL)	Reference
Rv0678	atpE	Rv2535c			CFZ	
	G187C		Serial passage	Fully Susceptible strain		Ismail et al. (2018)
C189A	-	-	Spontaneous	23 isolates	1.25	Hartkoorn et al. (2014)
T461C	A83G			Isoniazid-resistant strain		
201_206del	A83C			Kanamycin-resistant strain		
	G183T			Pyrazinamide-resistant strain		
	A83G					
A63T	A83G			Rifampicin-resistant strain		
G74A				Fully susceptible strain	4	
T131C				Isoniazid-resistant strain	> 4	
T407C				Kanamycin-resistant strain	4	
C204A				Pyrazinamide-resistant strain	> 4	
T131C				Rifampicin-resistant strain	4	
	A83T		Spontaneous	Fully susceptible strain	-	
C403G				Pyrazinamide-resistant strain		
	A83G			Pyrazinamide-resistant strain		
	G187C			Pyrazinamide-resistant strain		
193delG				Fully susceptible strain	2	
193delG				Fully susceptible strain	4	
A65T				Fully susceptible strain	4	
T407C				Pyrazinamide-resistant strain	1	
C214T				Pyrazinamide-resistant strain	2	
G137A				Pyrazinamide-resistant strain	4	
A97G						
		CinArg271		Mice Treated with BDQ only		
		+CinAla14				
			In vivo		0.5-1	Hartkoorn et al. (2014)
		+CinAla14		Mice treated with BDQ and CFZ		
		L44P				
A413G	WT		*In vitro* mutants	Mutants derived from H37Rv		Koul et al. (2007)
G281A	WT					
A202G	WT					
Ins G192-193	166M			Mutants derived from MDR *M. tuberculosis* clinical strain		
IS6110 nt 272	WT					
Ins A 38-39	WT					

Table 1. Contd.

	A95T	Mutagenesis	M smegmatis		
	C198G G187C	Spontaneous	M tuberculosis		Petrella et al. (2006)
	G183T		2 isolates from MDR strain		
	G187C		3 isolates from MDR strain		
	A83T		1 isolates from MDR strain		
	C198G	Spontaneous	1 isolates from MDR strain		
	A83G		1 isolates from MDR strain		
	G183T		3 isolates from MDR strain		
	G187C		4 isolates from MDR strain		
	G187C A95T	Spontaneous			Andries et al. (2005)
			23 isolates		Segala et al. (2012)
C400T			21isolates	1.25	
G193 deletion		Spontaneous	11 isolates	All≤1	Zhang et al. (2015)
G193 insertion			5 isolates		
C466T			5 isolates		
C364 insertion			2 isolates		
A202G			1 isolates		
T2C			1 isolates		
G58T			1 isolates		
C107T			1 isolates		
G125A			1 isolates		
T29 insertion			1 isolates		
C98A			2 isolates		
T128G			1 isolates		
G137A			1 isolates		
A152G			1 isolates		
C158T			1 isolates		
C176T			2 isolates		
G188A			1 isolates		
G194A			1 isolates		
G197T			1 isolates		
C226T			1 isolates		
C226T			1 isolates		
C251A			1 isolates		
A292 deletion			1 isolates		

Table 1. Contd.

G304A			1 isolates		
C305T			2 isolates		
T341C			1 isolates		
T365C			1 isolates		
CGCTGGGC371-378			1 isolates		
CG444-445 deletion			1 isolates		
G193 insertion			1 isolates		
			3 isolates		
		G265T	1 isolates		
	A63P		Mutants from H37Rv reference strain	-	Segala et al. (2012)
	D28G		Mutant from *M. tuberculosis* clinical isolates		
	E61D				
	L59V				
	166M				

Mutations related in rv0678, atpE and rv2535c genes. A dash (–) is used to indicate where data was unavailable. WT-wild type, no variants detected. CFZ-Clofazimine

Table 2. Catalog of previously published mutations from bedaquiline- and clofazimine-resistant clinical isolates.

Mutation			Notes	MIC (lg/ml)		Reference
rv0678	atpE	rv1979c		BDQ	CFZ	
T124C	–	–	Clinical strains from BDQ trial	0.25		
T1052C		V351A			-	Zhang et al. (2015)
A97C				0.5	–	
C107T				0.5		
Del C 212				0.5		
Ins IS6110 nt 272				0.5		
Ins C 141–142				0.25		
2T4C	WT	–	fMet1Ala-relapse isolate after BDQ compassionate use	0.5	4	
T437C	WT	WT	XDR	0.78	1.2	
G5T	WT	WT	Pre-XDR	0.73	4	
C158T	WT	WT	Pre-XDR	0.39	2.09	
T350G	WT	WT	XDR	1.54	4.16	
WT	WT	A155C	Pre-XDR	0.08	1.2	
Del gg 18–19	–	–	MDR isolates	0.5	–	
Ins G140				0.25		
M139T				0.25		
198–199 Ins G	–	–	Mix: WT þ rv0678 mutant	0.24;0.48;1	–	
274–275 Ins A				1		

Table 2. Contd.

C148T, A187G			intergenic mutation, rv0678 mutant	0.48	
G334C, (-13) Ins IS6110				0.48	
C185T				0.48	
C155T				0.48	
C176T				0.48	
224–225 Ins A				0.24	
T(-44)C			Mix: WT þ rv0678 mutant	0.24	
A263G				0.12	
T116C			Mix: WT þ rv0678 mutant (silent mutation)	0.12	
T124C				0.12	
C45T				0.12	
G256A				0.12	
[Ins139g]	WT	–		0.12–0.25	–
L142R	WT		Baseline and post-treatment BDQ isolates from BDQ clinical trials	0.25	
L142R	A63V			0.25–1	
[Del198G] [Del212C] [G233C, G78A]	WT			0.12	
[G66W] [Del198G] [Ins263A] [Del435T]	WT			0.12	
[Del198G] [Ins466C]	WT			0.25	
[Del435T]	WT			0.25	
[E113K] [Del198G] [Del435T]	WT			0.25	
[Del435T]	WT			0.25	
G121E	WT			0.25	
[L40S] [Del291C] [Ins386C]	WT			0.25	
Del291C	WT			0.25	
[S53P] [Del198G] [Del336C]	WT			0.25	
rv0678	atpE	rv1979c		BDQ	CFZ
M23L Ins142C	WT			0.12	
M23L [Ins142C] [Ins419G]	WT			0.12	
M23L Ins419g	WT			0.12	
[Del19G] [E49stop] [Del198G] [Ins468GA]	WT			0.12	
-[V85A] [R135W]	WT			0.12	
V85A	WT			0.12	
Ins44A	WT			0.06	
[Ins144C]	WT			0.12–0.25	
Ins421G	WT			0.12–0.25	
Del32G	WT			0.06; 0.25	
[Y26stop] [L122P]	WT			0.12	
L122P	WT			0.12	
[Del214C] [Del198G]	WT			0.06	
[F79S] [Ins137G]	WT			0.12	
[Del19G] [Del198G]	WT			0.12	

Table 2. Contd.

A98V	WT			0.12–0.25		
WT	D28N			0.12		
[Ins139G] þ [Ins318CG]	WT			0.12		
[Del274–283] [Ins139TG]	WT			0.12		
[C46R] [Ins139TG] [L40S]	WT			0.12		
Ser53Pro	WT	–	2 XDR isolates	0.5	2–4	Pang et al.(2017)
Ser53Leu			1 XDR isolate	0.25	2	
Tyr157Asp			1 XDR isolate	0.125	2	
WT			1 XDR isolate	0.5	2	
WT	WT	G1226A	3 XDR isolates: Culture negative at 6 months	0.25–1 (MGIT) 0.06–0.125 (BMD)	0.5 (MGIT)	Ismail et al. (2018)
136_137insG		G1226A	XDR: : Culture positive at 6 months	2 (MGIT) 0.25 (BMD)	0.5 (MGIT)	
138_139insG		G1226A	XDR: Culture positive at 6 months	2 (MGIT) 0.5 (BMD)	2 (MGIT)	
141_142insC		G1226A	2 XDR isolates: Culture positive at 6 months	4 (MGIT) 0.25–0.5 (BMD)	0.5–1 (MGIT)	
T200G		G1226A	XDR: Culture positive at 6 months	4 (MGIT) 0.5 (BMD)	2 (MGIT)	
345delG		G1226A	XDR: Culture positive at 6 months	4 (MGIT) 0.5 (BMD)	1 (MGIT)	
-11C4A	WT	–	Fully susceptible clinical isolate	0.016	–	Martinez et al. (2018)
D5G	WT		Fully susceptible clinical isolate	0.016		
M23V	WT		STR resistant clinical isolate	0.063		
D47fs	WT		XDR clinical isolate	0.5		
E55D	WT		Fully susceptible clinical isolate	0.063		
G87R	WT		Fully susceptible clinical isolate	0.063		
R96Q	WT		INH resistant	0.25		
L117R	WT		Fully susceptible clinical isolate	0.016		
WT	-53G4A		Fully susceptible clinical isolate	0.125		
WT	-72T4C		RIF and INH resistant clinical isolate	8		
WT	-138T4C		3 RIF and INH resistant clinical isolates	0.031		
WT	183G4A		Fully susceptible clinical isolate	0.063		
WT	I66V		Fully susceptible clinical isolate	0.125		

Mutations related in rv0678, atpE and rv1979c genes. A dash (–) is used to indicate where data was unavailable. WT-wild type, no variants detected. MGIT- MGIT960 platform used to determine MIC. BMD- indicate Broth Micro Dilution method. BDQ-Bedaquiline. CFZ-Clofazimine.

resistance of the pathogens in difficult situation. Since Rv0678 is a transcriptional repressor protein of MmpS5 and MmpL5, mutations in this gene lead to an increased expression level of this efflux pump (Milano et al., 2009). Mutations of Rv1979c were found in *in vitro* selected clofazimine-resistant isolates without Rv0678 mutations. It is of interest to note that the clofazimine-resistant strain previously studied here (Phelan et al., 2016; Xu et al., 2017) 011873, had a mutation in Rv1979c and did not display cross-resistance to bedaquiline.

Zhang et al. (2015) also found that another orthologue of Rv1979c is Mb2001c (in *Mycobacterium bovis*), but it is absent in Bacillus Calmette–Guerin (BCG) due to deletion effect, and its role in altering clofazimine susceptibility in BCG is thus unknown. The molecular function of Rv1979c is believed to be that of amino acid transmembrane transporter with permease activity. The role of Rv1979c is annotated as a possible conserved permease that might be involved in amino acid transport. It is possible that Rv1979c is involved in clofazimine transport or uptake that

alters the physiology of the bacteria (Zhang et al., 2015). Although the molecular function of Rv253c has not been fully characterized, it is likely to be that of a peptidase activity. He found that only one mutant except the common rv0678 mutation had a T1052C mutation in a new gene, rv1979c (Table 2), due to the amino acid change V351A. Rv1979c is a putative permease that might be attached in amino acid transport. It is possible that Rv1979c is associated with clofazimine transport or uptake, either directly or indirectly, to alter the physiology of the bacteria so as to be less susceptible to the effect of clofazimine. In addition, it is of interest to note that Rv1979c is localized in the RD2 region and its deletion has been shown to be involved in virulence alleviation in BCG (Kozak et al., 2011). It remains is to be tested whether resistance to clofazimine alters the virulence of *M. tuberculosis*. It is intriguing that he also found a stop codon mutation at E89 in a second new gene, rv2535c, associated with clofazimine resistance, inactivating the function of this protein. The exact function of Rv2535c is unknown. Orthologues of Rv2535c include Mb2564c (in *M. bovis*) and MMAR_2180 (in *Mycobacterium marinum*). Other previous study showed the main mechanism of clofazimine resistance in *Mycobacterium abscessus*. Mutations in MAB_2299c encode the transcriptional repressor of efflux pump are analogous to Rv0678 mutations in causing clofazimine resistance in *M. tuberculosis*. Other new genes associated with clofazimine resistance in *M. abscessus* seem to affect transcription or transport functions. Yuan Chen et al. identification of MAB_2299c, MAB_1483, and MAB_0540 mutations in most clofazimine-resistant mutants has implications for rapid molecular discovery of clofazimine-resistant *M. abscessus* (Chen et al., 2018). Contrary to what is generally assumed, clofazimine resistance in *M. tuberculosis, M. absessus* has been reported, though detailed information of the underlying molecular mechanisms is lacking (Ebenezer et al., 2002). Extended term of treatment with clofazimine, especially on a highly remittent basis, say once monthly (as per WHO guidelines on treatment of multibacillary tuberculosis), may increase the propensity for resistance in *M. tuberculosis* to this riminophenazine. If a person with tuberculosis also has latent infection or subclinical disease due to *M. tuberculosis*, it would not be hard to take into consideration the possible development of dual bacillary resistance in both mycobacterial species, following clofazimine treatment for the former disease, with involvement of very similar molecular mechanisms. Such a review may explain, at least partially, primary clofazimine resistance in M. tuberculosis. Although clofazimine is not generally included in the regimens for treatment of non-tuberculosis mycobacterial infections, it sometimes finds a space for such treatment under specific settings, with the attendant risk of development of mycobacterial resistance. In addition, while horizontal transfer of genetic materials between *M. abscessus,* *Mycobacterium leprae* and *M. tuberculosis* has not been reported, such a possibility cannot be totally excluded in other mycobacteria, as revealed by the recent research findings concerning genomic islands which showed a wide spectrum of genes, including those annotated as phagic plasmid genes, inclinical system and environmental *Mycobacterium avium* sub sp. Hominissuis (Sanchini et al., 2016). The relevance of these observations for clofazimine resistance in different mycobacterial species appears; however, future research work is warranted for better descriptive note.

CONCLUSION

This study reviews an extensive number of mutants resistant to clofazimine and found mutations in rv0678 to be the most prominent mechanism of clofazimine resistance. The present findings of mutation properties associated with clofazimine resistance grant valuable information for the next study of new molecular tests for the rapid discovery of clofazimine resistance in the clinical management. Also, the study discovered two some new genes such as rv1979c, rv2535c and also in *M. abscessus*MAB_2299c, MAB_1483, and MAB_0540 associated with clofazimine resistance. Further researches are needed to identify the nature of rv1979c, rv2535c and also in *M. abscessus* MAB_2299c, MAB_1483, and MAB_0540 in clofazimine resistance and to clearly understand the whole mechanisms of clofazimine.

The growing outbreak of MDR- and XDR-TB worldwide spread the emergent essential for extra eff ective drugs and also therapeutic state, especially in TB, HIV, and leprosy co-infected people with high mortality rates. Though therapeutic state for DR-TB is still limited, a complementary approach is to repurpose existing antibiotics. Different research that investigated the available warrant, recommends CFZ-containing combinations as an additional promising treatment option for DR-TB, even though the duration of conduct and optimal dimensions of CFZ necessitate further investigations (Figure 1).

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

ACKNOWLEDGMENT

The authors appreciate the Chuadanga Govt. College Laboratory, National University of Bangladesh, Institute of Biological Science, Rajshahi University and Health (GIBH), Chinese Academy of Sciences, Guangzhou, China Guangzhou Institutes of Biomedicine, and

University of Chinese Academy of Science (UCAS), Beijing 100049, China for their support.

REFERENCES

Almeida D, Ioerger T, Tyagi S, Li SY, Mdluli K, Andries K, Grosset J, Sacchettini J, Nuermberger E (2016). Mutations in pepQ confer low-level resistance to bedaquiline and clofazimine in Mycobacterium tuberculosis. Antimicrobial Agents and Chemotherapy 60(8):4590-4599. doi:10.1128/AAC.00753-16

Altschul SF, Gish W, Miller W, Myers EW, Lipman DJ (1990). Basic local alignment search tool. Journal of Molecular Biology 215(3):403-410. doi:10.1016/S0022-2836(05)80360-2

Ammerman NC, Swanson RV, Tapley A, Moodley C, Ngcobo B, Adamson J, Singh SD (2016). Clofazimine has delayed antimicrobial activity against Mycobacterium tuberculosis both in vitro and in vivo. Journal of Antimicrobial Chemotherapy dkw417. doi: 10.1093/jac/dkw417

Andries K, Verhasselt P, Guillemont J, Gohlmann H W, Neefs JM, Winkler H, Van Gestel J, Timmerman P, Zhu M, Lee E, Williams P (2005). A diarylquinoline drug active on the ATP synthase of Mycobacterium tuberculosis. Science 307(5707):223-227. doi: 10.1126/science.1106753.

Arbiser JL, Moschella SL (1995). Clofazimine: a review of its medical uses and mechanisms of action. Journal of the American Academy of Dermatology *32*(2):241-247. doi:10.1016/0190-9622(95)90134-5

Baik J, Rosania GR (2011). Molecular imaging of intracellular drug–membrane aggregate formation. Molecular Pharmaceutics *8*(5):1742-1749. https://doi.org/10.1021/mp200101b.

Barry VC, Belton JG, Conalty ML, Den-steny JM, Edward DW, Van JF, Winder F (1957). A new series of phenazines (rimino-compounds) with high antituberculosis activity. Nature 179(4568):1013-1015. doi: 10.1038/1791013a0.

Belachew T, Yaheya S, Tilahun N, Gebrie E, Seid R, Nega T, Biset S. Multidrug-Resistant Tuberculosis Treatment Outcome and Associated Factors at the University of Gondar Comprehensive Specialized Hospital: A Ten-Year Retrospective Study. Infection and Drug Resistance 2891-2899.

Browne SG, Hogerzeil LM (1962). " B 663" in the treatment of leprosy. Preliminary report of a pilot trial. Leprosy Review 33(1):6-10.

Chen Y, Chen J, Zhang S, Shi W, Zhang W, Zhu M, Zhang Y (2018). Novel mutations associated with clofazimine resistance in Mycobacterium abscessus. Antimicrobial agents and Chemotherapy *62*(7):e00544-18. doi: 10.1128/AAC.00544-18

Chuaprapaisilp T, Piamphongsant T (1978). Treatment of pustular psoriasis with clofazimine. British Journal of Dermatology 99(3):303-305. https://doi.org/10.1111/j.1365-2133.1978.tb02001.x

Comas I, Coscolla M, Luo T, Borrell S, Holt KE, Kato-Maeda M, Parkhill J, Malla B, Berg S, Thwaites G, Yeboah-Manu D, Bothamley G, Mei J, Wei L, Bentley S, Harris SR, Niemann S, Diel R, Aseffa A, Gao Q, Young D, Gagneux S (2013). Out-of-Africa migration and Neolithic coexpansion of Mycobacterium tuberculosis with modern humans. Nature genetics 45(10):1176-1182.

Copin R, Coscollá M, Efstathiadis E, Gagneux S, Ernst JD (2014). Impact of in vitro evolution on antigenic diversity of Mycobacterium bovis bacillus Calmette-Guerin (BCG). Vaccine *32*(45):5998-6004.doi: 10.1016/j.vaccine.2014.07.113

Dagron S (2016). Review of the national tuberculosis programme in Belarus 8-18

Dalcolmo M, Gayoso R, Sotgiu G, D'Ambrosio L, Rocha JL, Borga L, Fandinho F, Braga JU, Galesi VM, Barreira D, Sanchez DA (2017). Effectiveness and safety of clofaziminein multidrug-resistant tuberculosis: a nationwide report from Brazil. European Respiratory Journal 49(3):1602445[https://doi.org/10.1183/13993003.02445-2016].

Dey T, Brigden G, Cox H, Shubber Z, Cooke G, Ford N (2012). Outcomes of clofazimine for the treatment of drug-resistant tuberculosis: a systematic review and meta-analysis. Journal of antimicrobial chemotherapy 68(2):284-293 https://doi.org/10.1093/jac/dks389

Ebenezer GJ, Norman G, Joseph GA, Daniel S, Job CK (2002). Drug resistant-Mycobacterium leprae-results of mouse footpad studies from a laboratory in south India. Indian journal of leprosy 74(4):301-312.

Gandhi NR, Moll A, Sturm AW, Pawinski R, Govender T, Lalloo U, Friedland G (2006). Extensively drug-resistant tuberculosis as a cause of death in patients co-infected with tuberculosis and HIV in a rural area of South Africa. The Lancet 368(9547):1575-1580. Doi: 10.1016/S0140-6736(06)69573-1.

Gartner E, Anderson R, Van Rensburg CE, Imkamp FMJH (1982). The in vitro and in vivo effects of clofazimine on the motility of neutrophils and transformation of lymphocytes from normal individuals. Leprosy review 53(2):85-90.

Gopal M, Padayatchi N, Metcalfe JZ, O'Donnell MR (2013). Systematic review of clofazimine for the treatment of drug-resistant tuberculosis. The International journal of tuberculosis and lung disease 17(8):1001-1007.

Grant SS, Kaufmann BB, Chand NS, Haseley N, Hung DT (2012). Eradication of bacterial persisters with antibiotic-generated hydroxyl radicals. Proceedings of the National Academy of Sciences 109(30):12147-12152. doi:10.1073/pnas.1203735109

Hameed A, MM Islam, Chhotaray C, Wang C, Y Liu, Y Tan, X Li, Tan S, Delorme V, Yew WW, Liu J, Zhang T (2018). Molecular Targets Related Drug Resistance Mechanisms in MDR-, XDR-, and TDR-*Mycobacterium tuberculosis* Strains. Frontiers in cellular and infection microbiology 8:114. doi:10.3389/fcimb.2018.00114

Hartkoorn RC, Uplekar S, Cole ST (2014). Cross-resistance between clofazimine and bedaquiline through upregulation of MmpL5 in Mycobacterium tuberculosis. Antimicrobial agents and chemotherapy 58(5):2979-2981. doi:10.1128/AAC.00037-14 https://doi.org/10.1016/j.cmi.2018.11.010

inhibit the complement-mediated solubilization of pre-formed immune complexes in vitro. International Journal of Immunopharmacology 14(2):269-273. doi:10.1016/0192-0561(92)90039-n

Islam MM, Hameed HA, Mugweru J, Chhotaray C, Wang C, Tan Y, Liu J, Li X, Tan S, Ojima I, Yew WW, Nuermberger E, Lamichhane G, Zhang T (2017). Drug resistance mechanisms and novel drug targets for tuberculosis therapy. Journal of genetics and genomics 44(1):21-37. doi:10.1016/j.jgg.2016.10.002

Ismail N, Omar SV, Ismail NA, Peters RP (2018). In vitro approaches for generation of Mycobacterium tuberculosis mutants resistant to bedaquiline, clofazimine or linezolid and identification of associated genetic variants. Journal of microbiological methods 153:1-9. doi:10.1016/j.ebiom.2018.01.005

Ivan M, Petrenko T, Keiser O, Estill J, Revyakina O, Felker I, Raviglione MC, Krasnov V, Schwartz Y (2019). Variations in tuberculosis prevalence, Russian Federation: a multivariate approach. Bulletin of the World Health Organization 97(11):737.

Kashyap A, Sehgal VN, Sahu A, Saha K (1992). Anti-leprosy drugs inhibit the complement-mediated solubilization of pre-formed immune complexes in vitro. International journal of immunopharmacology 14(2):269-273.

Kaufman MB (2013). 2012 American Society of Health-System Pharmacists Midyear Clinical Meeting & Exhibition. Pharmacy and Therapeutics 38(2):119.

Koul A, Dendouga N, Vergauwen K, Molenberghs B, Vranckx L, Willebrords R, Bald D (2007). Diarylquinolines target subunit c of mycobacterial ATP synthase. Nature chemical biology 3(6):323-324.. doi:10.1038/nchembio884

Kozak RA, Alexander DC, Liao R, Sherman DR, Behr M (2011). Region of difference 2 contributes to virulence of Mycobacterium tuberculosis. Infection and immunity *79*(1):59-66. doi: 10.1128/IAI.00824-10

Lange CD, Chesov J, Heyckendorf (2019). Clofazimine for the treatment of multidrug-resistant tuberculosis Clinical Microbiology and Infection 25(2):128-130.

Lu Y, Wang B, Zhao WJ, Zheng MQ, Li P, Fu L, Liang BW (2010). A study on the activity of clofazimine with antituberculous drugs against Mycobacterium tuberculosis. Zhonghua jie he he hu xi za zhi=Zhonghua jiehe he huxi zazhi= Chinese journal of tuberculosis and respiratory diseases 33(9):675-678.

Martinez E, Hennessy D, Jelfs P, Crighton T, Chen SC, Sintchenko V

(2018). Mutations associated with in vitro resistance to bedaquiline in Mycobacterium tuberculosis isolates in Australia. Tuberculosis 111:31-34. doi:10.1016/j.tube.2018.04.007.

Milano A, Pasca MR, Provvedi R, Lucarelli AP, Manina G, Ribeiro LDJL, Riccardi G (2009). Azole resistance in Mycobacterium tuberculosis is mediated by the MmpS5-MmpL5 efflux system. Tuberculosis 89(1):84-90. doi:10.1016/j.tube.2008.08.003

Murugesan D, Ray PC, Bayliss T, Prosser GA, Harrison JR, Green K, Warner DF (2018). 2-Mercapto-Quinazolinones as Inhibitors of Type II NADH Dehydrogenase and Mycobacterium tuberculosis: Structure-Activity Relationships, Mechanism of Action and Absorption, Distribution, Metabolism, and Excretion Characterization. *ACS* infectious diseases *4*(6):954-969.doi:10.1021/acsinfecdis.7b00275

Olayanju O, Limberis J, Esmail A, Oelofse S, Gina P, Pietersen E, Fadul M, Warren R, Dheda K (2018). Long-term bedaquiline-related treatment outcomes in patients with extensively drug-resistant tuberculosis from South Africa European Respiratory Journal 51(5):1800544. DOI: 10.1183/13993003.00544-2018

Oliva B, O'Neill AJ, Miller K, Stubbings W, Chopra I (2004). Anti-staphylococcal activity and mode of action of clofazimine. Journal of Antimicrobial Chemotherapy *53*(3):435-440. https://doi.org/10.1093/jac/dkh114

Pang Y, Zong Z, Huo F, Jing W, Ma Y, Dong L, Huang H (2017). *In vitro* drug susceptibility of bedaquiline, delamanid, linezolid, clofazimine, moxifloxacin, and gatifloxacin against extensively drug-resistant tuberculosis in Beijing, China. Antimicrobial agents and chemotherapy 61(10):e00900-17. doi: 10.1128/AAC.00900-17

Parak RB, Wadee AA (1991). The synergistic effects of gamma interferon and clofazimine on phagocyte function: Restoration of inhibition due to a 25 kilodalton fraction fromMycobacterium tuberculosis. Biotherapy *3*(3):265-272. doi:10.1007/bf02171691

Parker SK, Curtin KM, Vasil ML (2007). Purification and characterization of mycobacterial phospholipase A: an activity associated with mycobacterial cutinase. Journal of bacteriology 189(11):4153-4160. doi: 10.1128/JB.01909-06

Pearson WR (2013). An introduction to sequence similarity ("homology") searching. Current protocols in bioinformatics 42(1):3-1.

Petrella S, Cambau E, Chauffour A, Andries K, Jarlier V, Sougakoff W (2006). Genetic basis for natural and acquired resistance to the diarylquinoline R207910 in mycobacteria. Antimicrobial agents and chemotherapy *50*(8):2853-2856. doi:10.1128/AAC.00244-06

Phelan J, Coll F, McNerney R, Ascher DB, Pires DE, Furnham N, Ramsay A (2016). Mycobacterium tuberculosis whole genome sequencing and protein structure modelling provides insights into anti-tuberculosis drug resistance. BMC medicine 14(1):1-13. https://doi.org/10.1186/s12916-016-0575-9

Podmore P, Burrows D (1986). Clofazimine-an effective treatment for Melkersson-Rosenthal syndrome or Miescher's cheilitis. Clinical and experimental dermatology 11(2):173-178. https://doi.org/10.1111/j.1365-2230.1986.tb00443.x

Radhakrishnan A, Kumar N, Wright CC, Chou TH, Tringides ML, Bolla JR, Edward WY (2014). Crystal structure of the transcriptional regulator Rv0678 of Mycobacterium tuberculosis. Journal of Biological Chemistry 289(23):16526-16540. doi:10.1074/jbc.M113.538959

Reddy VM, O'Sullivan JF, Gangadharam PR (1999). Antimycobacterial activities of riminophenazines. Journal of Antimicrobial Chemotherapy 43(5):615-623. https://doi.org/10.1093/jac/43.5.615.

Sanchini A, Semmler T, Mao L, Kumar N, Dematheis F, Tandon K, Lewin A (2016). A hypervariable genomic island identified in clinical and environmental Mycobacterium avium subsp. hominissuis isolates from Germany. International Journal of Medical Microbiology 306(7):495-503.doi:10.1016/j.ijmm.2016.07.001

Segala E, Sougakoff W, Nevejans-Chauffour A, Jarlier V, Petrella S (2012). New mutations in the mycobacterial ATP synthase: new insights into the binding of the diarylquinoline TMC207 to the ATP synthase C-ring structure. Antimicrobial agents and chemotherapy 56(5):2326-2334. doi:10.1128/AAC.06154-11

Seung KJ, Keshavjee S, Rich ML. Multidrug-Resistant Tuberculosis and Extensively Drug-Resistant Tuberculosis. Cold Spring Harbor perspectives in medicine 5(9).

Shen GH, Wu BD, Hu ST, Lin CF, Wu KM, Chen JH (2010). High efficacy of clofazimine and its synergistic effect with amikacin against rapidly growing mycobacteria. International journal of antimicrobial agents 35(4):400-404. doi: 10.1016/j.ijantimicag.2009.12.008

Singh S, Bouzinbi N, Chaturvedi V, Godreuil S, Kremer L (2014). In vitro evaluation of a new drug combination against clinical isolates belonging to the Mycobacterium abscessus complex. Clinical Microbiology and Infection *20*(12):01124-01127. doi: 10.1111/1469-0691.12780

Tasneen R, Li SY, Peloquin CA, Taylor D, William K N, Andries K, Nuermberger EL (2011). Sterilizing activity of novel TMC207-and PA-824-containing regimens in a murine model of tuberculosis. Antimicrobial agents and chemotherapy *55*(12):5485-5492 doi: 10.1128/AAC.05293-11

Tyagi S, Ammerman NC, Li SY, Adamson J, Converse PJ, Swanson RV, Almeida DV, Grosset JH (2015). Clofazimine shortens the duration of the first-line treatment regimen for experimental chemotherapy of tuberculosis. Proceedings of the National Academy of Sciences of the United States of America 112(3):869-874.

Van Rensburg CE, Gatner EM, Imkamp FM, Anderson R (1982). Effects of clofazimine alone or combined with dapsone on neutrophil and lymphocyte functions in normal individuals and patients with lepromatous leprosy. Antimicrobial agents and chemotherapy 21(5):693-697. doi: 10.1128/aac.21.5.693

Wadee AA, Anderson R, Rabson AR (1988). Clofazimine reverses the inhibitory effect of Mycobacteriwn tuberculosis derived factors on phagocyte intracellular killing mechanisms. Journal of antimicrobial chemotherapy 21(1):65-74. https://doi.org/10.1093/jac/21.1.65

World Health Organization (WHO) (2015). The Selection and Use of Essential Medicines: Report of the WHO Expert Committee, 2015 (including the 19th WHO Model List of Essential Medicines and the 5th WHO Model List of Essential Medicines for Children) (Vol. 994). World Health Organization.

World Health Organization (WHO) (2019). Global tuberculosis report 2018. World Health Organization..http://www.who.int/selection_medicines/committees/subcommittee/2/eeb1098%5b1%5d.pdf

Xu J, Wang B, Hu M, Huo F, Guo S, Jing W, Lu Y (2017). Primary clofazimine and bedaquiline resistance among isolates from patients with multidrug-resistant tuberculosis. Antimicrobial agents and chemotherapy 61(6):10-1128. doi: 10.1128/AAC.00239-17

Zhang S, Chen, Cui P, Shi W, Zhang W, Zhang Y (2015). Identification of novel mutations associated with clofazimine resistance in Mycobacterium tuberculosis. Journal of Antimicrobial Chemotherapy 70(9):2507-2510. doi:10.1093/jac/dkv150

Zumla A, Nahid P, Cole ST (2013). Advances in the development of new tuberculosis drugs and treatment regimens. Nature reviews Drug discovery 12(5):388-404. doi:10.1038/nrd4001