Tackling efflux pumps in *Mycobacterium tuberculosis* to combat antimicrobial resistance

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Content

I. Tuberculosis and the five types of efflux pumps

II. Bedaquiline and the efflux pump

III. Efflux pump inhibitors

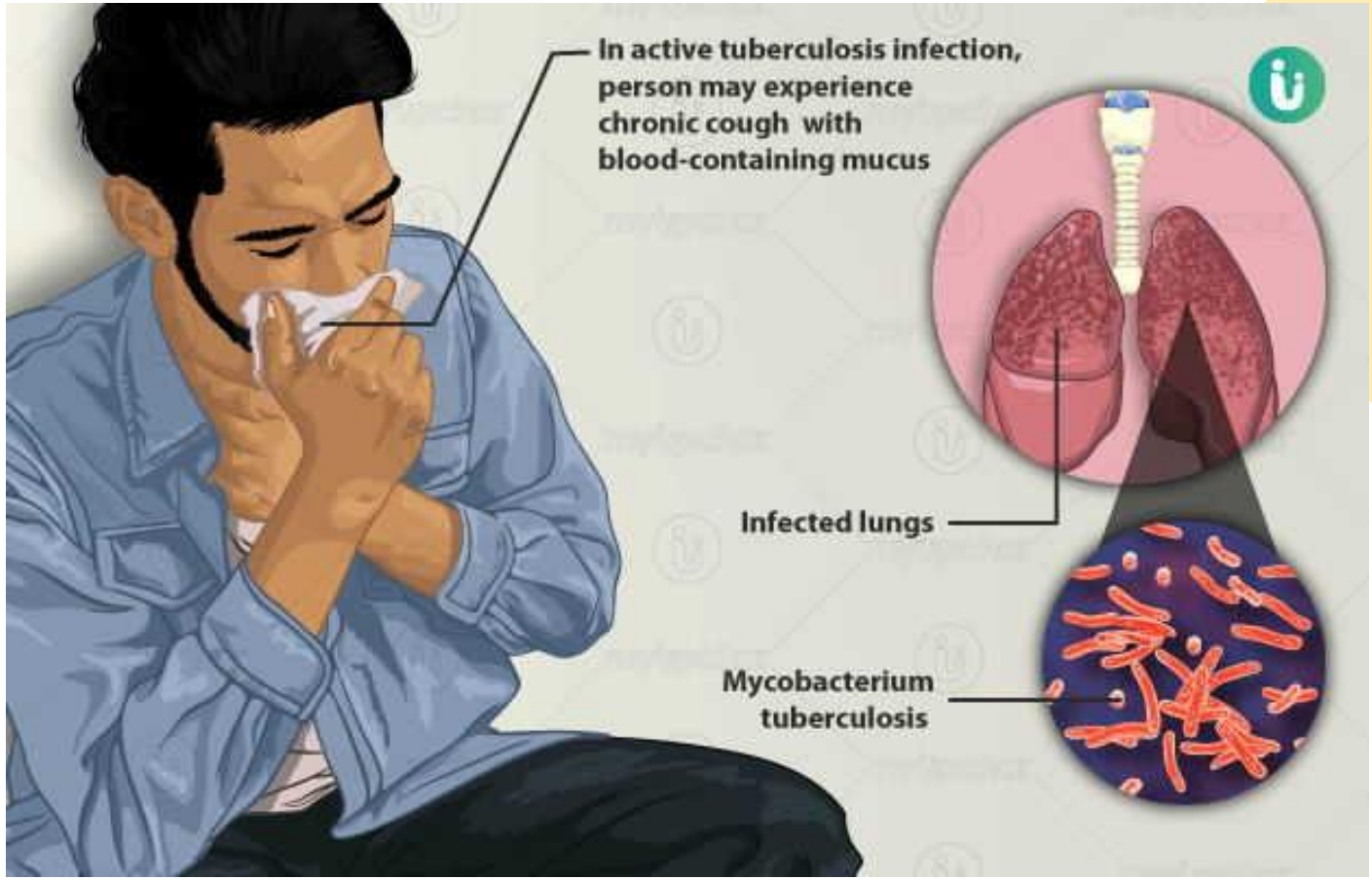
M. tuberculosis

M. tuberculosis

- Most often attacks the lungs.

- Symptoms: prolonged cough, fatigue, fever, night sweats, weight loss, and chest pain.

- Treatment takes 6 months with 4 antibiotics.
  - Hong Kong
    - First two months: isoniazid, rifampicin, pyrazinamide, and ethambutol.
    - Subsequent 4 months: isoniazid and rifampicin.



(AIIMS), D. A. M. (2020, March 6). *Tuberculosis (TB): Symptoms, causes, treatment, medicine, prevention, diagnosis*. myUpchar. Retrieved December 7, 2021, from https://www.myupchar.com/en/disease/tuberculosis-tb.

Oral antituberculosis drugs. Drug Office - Oral Antituberculosis Drugs. (n.d.). Retrieved December 7, 2021, from https://www.drugoffice.gov.hk/eps/do/en/consumer/news_informations/dm_32.html#b.
World Health Organization. (n.d.). *Tuberculosis*. World Health Organization. Retrieved December 7, 2021, from https://www.who.int/health-topics/tuberculosis#tab=tab_1.

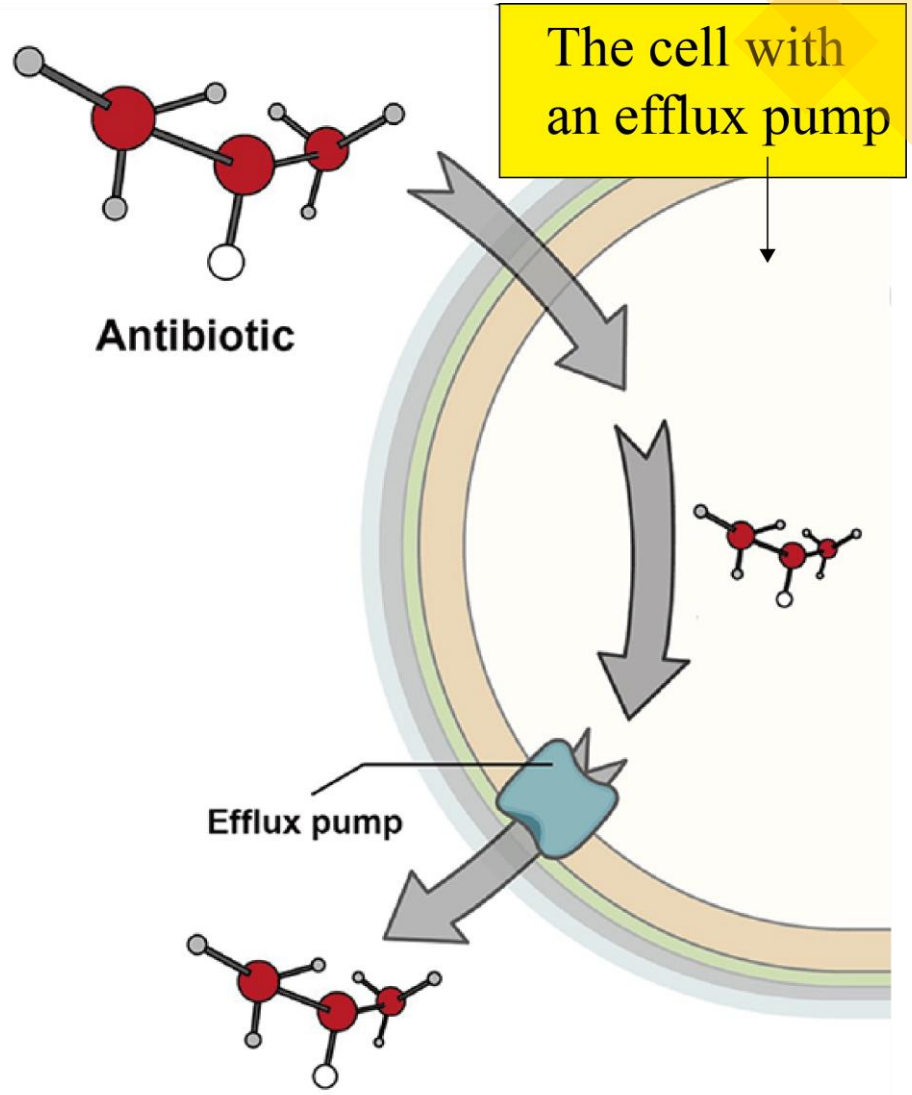
MDR-TB: Multidrug-resistant tuberculosis
XDR-TB: Extensively drug-resistant tuberculosis

Prince, C. (2021, March 24). *New oral MDR-TB treatment shows positive trial results - potential to change clinical practice & save lives*. Health Policy Watch. Retrieved December 7, 2021, from https://healthpolicy-watch.news/mdr-tb-trial/.

World Health Organization. (n.d.). *Tuberculosis deaths rise for the first time in more than a decade due to the COVID-19 pandemic*. World Health Organization. Retrieved December 7, 2021, from https://www.who.int/news/item/14-10-2021-tuberculosis-deaths-rise-for-the-first-time-in-more-than-a-decade-due-to-the-covid-19-pandemic.

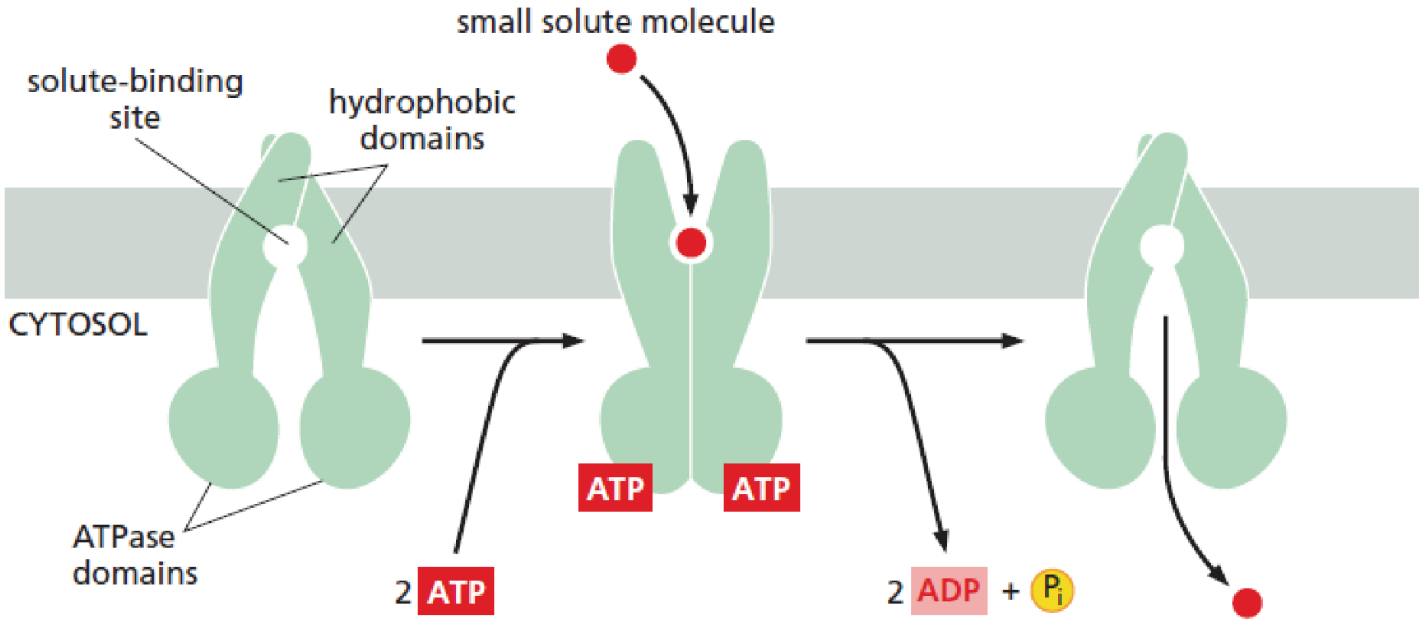
Mechanisms underlying drug resistance

1. Modify antibiotics.
2. Mutate the antibiotic target.
3. Decrease membrane permeability.
4. **Overproduce efflux pumps.**

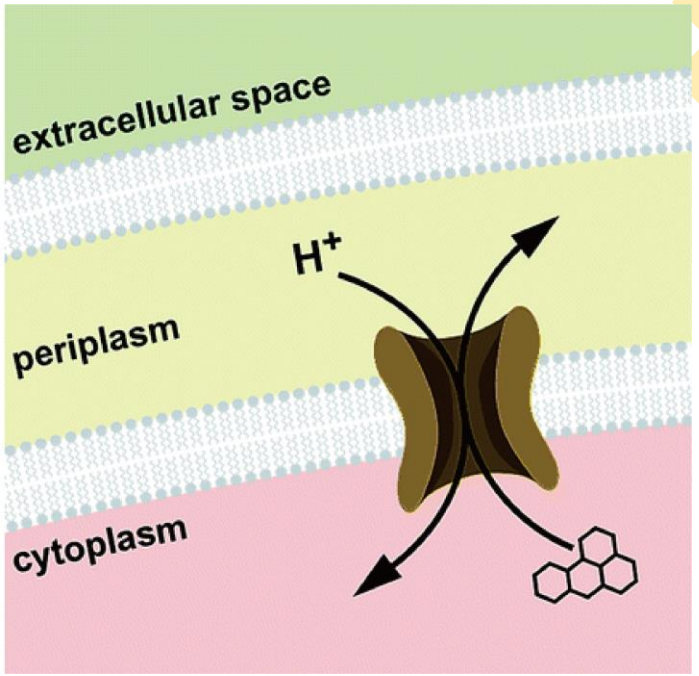


Laws M, Jin P, Rahman KM. Efflux pumps in Mycobacterium tuberculosis and their inhibition to tackle antimicrobial resistance. Trends Microbiol. 2021 May 26:S0966-842X(21)00123-2. doi: 10.1016/j.tim.2021.05.001. Epub ahead of print. PMID: 34052094.

ATP Binding Cassette (ABC) superfamily



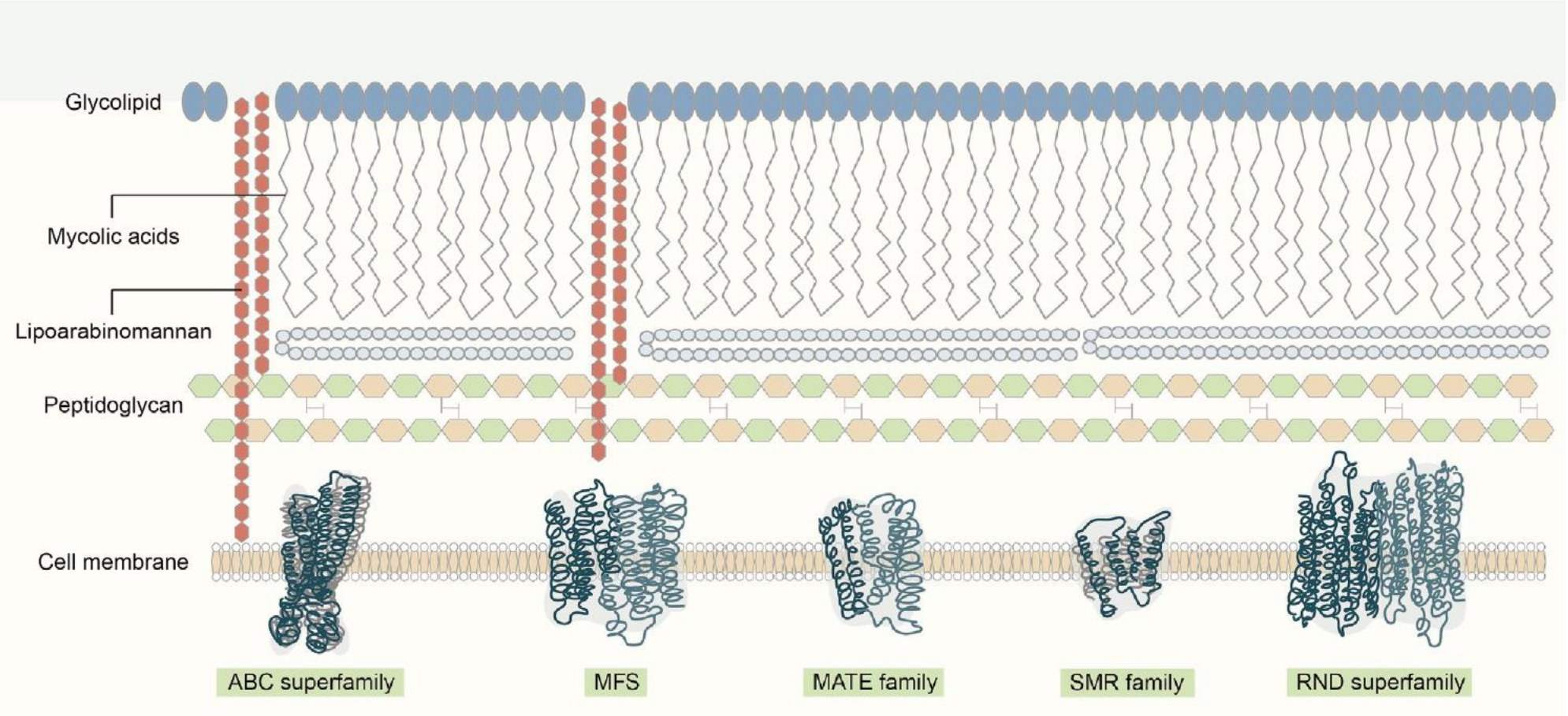
Major Facilitator Superfamily (MFS)



- Two largest families that contribute to rifampicin and isoniazid resistance.
- ABC transporters: encoded by approximately 2.5% MTB genome.
- MFS: 19 MFS in MTB are correlated to resistance.

Mousa JJ, Bruner SD. Structural and mechanistic diversity of multidrug transporters. Nat Prod Rep. 2016 Oct 26;33(11):1255-1267. doi: 10.1039/c6np00006a. PMID: 27472662.
Alberts, B., Johnson, A., Lewis, J., Morgan, D., Raff, M., Roberts, K., Walter, P., Wilson, J. and Hunt, T., 2015. *Molecular Biology of The Cell 6th edition*. Garland Science.

1. **ATP-binding cassette (ABC) transporter**
2. **Major facilitator superfamily (MFS)**
3. Multidrug and toxic compound extrusion family (MATE)
4. Resistance-nodulation-cell-division superfamily (RND)
5. Small multidrug resistance family (SMR)



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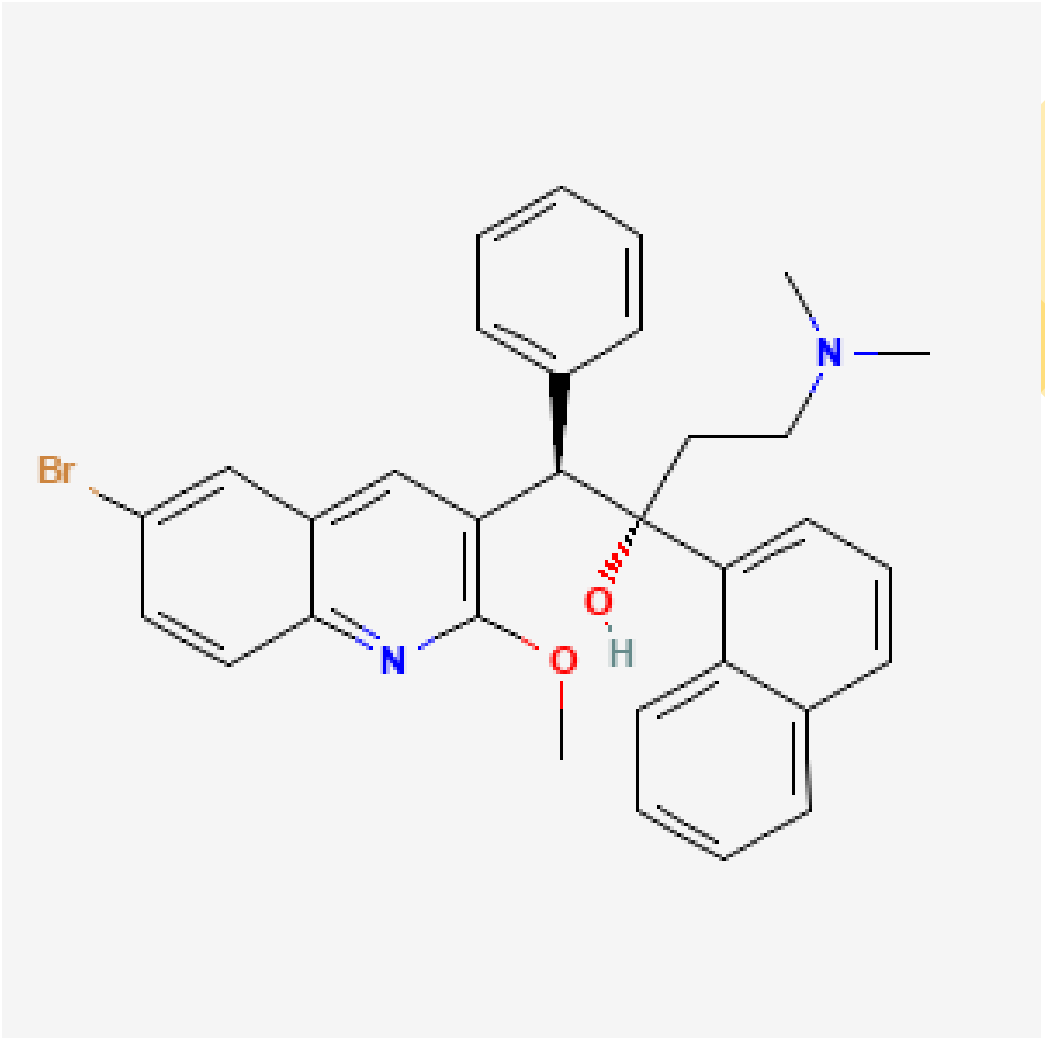
Bedaquiline and the efflux pump

Bedaquiline

- Developed by Janssen Pharmaceuticals and approved by FDA in 2012.
- The first anti-TB drug targeting ATP synthase encoded by *atp*E.
- Over ~2500 patients had been received by the end of 2015.
- Cases of resistance were initially correlated to *Rv0678* mutations that upregulated the expression of *mmpS5* and *mmpL5*.

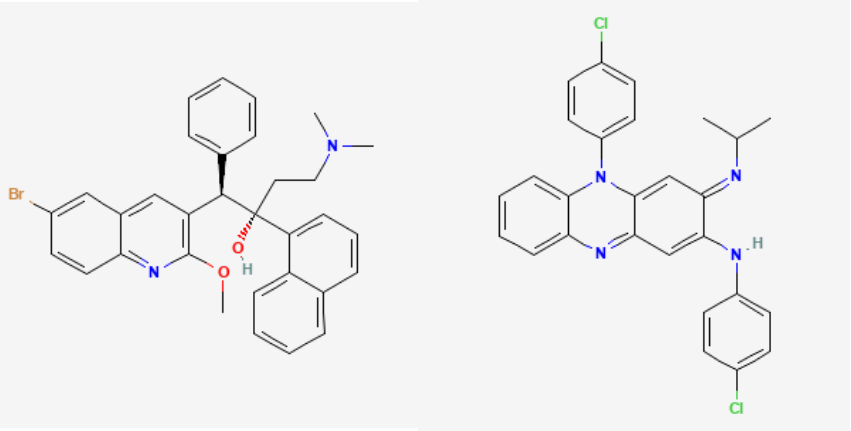


U.S. National Library of Medicine. (n.d.). *Bedaquiline*. National Center for Biotechnology Information. PubChem Compound Database. Retrieved December 7, 2021, from https://pubchem.ncbi.nlm.nih.gov/compound/Bedaquiline.

Veziris N, Bernard C, Guglielmetti L, Le Du D, Marigot-Outtandy D, Jaspard M, Caumes E, Lerat I, Rioux C, Yazdanpanah Y, Tiotiu A, Lemaitre N, Brossier F, Jarlier V, Robert J, Sougakoff W, Aubry A; CNR MyRMA and the Tuberculosis Consilium of the CNR MyRMA; CNR MyRMA and Tuberculosis Consilium of the CNR MyRMA. Rapid emergence of *Mycobacterium tuberculosis* bedaquiline resistance: lessons to avoid repeating past errors. Eur Respir J. 2017 Mar 22;49(3):1601719. doi: 10.1183/13993003.01719-2016. PMID: 28182568.

Mutations conferring BDQ resistance

Genes	Gene Function	MIC Increase
*atp*E	Coding for a transmembrane protein of the ATP synthase, target of Bdq	8- to 133-fold increase in Bdq MIC
Rv0678	Regulating the expression of the MmpS5-MmpL5 efflux pump	2- to 8-fold increase in Bdq MIC and 2- to 4-fold increase in clofazimine MIC
*pep*Q	Unclear	4-fold increase in Bdq and clofazimine MICs



Bedaquiline Clofazimine

U.S. National Library of Medicine. (n.d.). *Clofazimine*. National Center for Biotechnology Information. PubChem Compound Database. Retrieved December 12, 2021, from https://pubchem.ncbi.nlm.nih.gov/compound/Clofazimine.
U.S. National Library of Medicine. (n.d.). *Bedaquiline*. National Center for Biotechnology Information. PubChem Compound Database. Retrieved December 7, 2021, from https://pubchem.ncbi.nlm.nih.gov/compound/Bedaquiline.

Nguyen TVA, Anthony RM, Bañuls AL, Nguyen TVA, Vu DH, Alffenaar JC. Bedaquiline Resistance: Its Emergence, Mechanism, and Prevention. Clin Infect Dis. 2018 May 2;66(10):1625-1630. doi: 10.1093/cid/cix992. PMID: 29126225.

In vitro pathway to acquire resistance

AMERICAN SOCIETY FOR MICROBIOLOGY | Antimicrobial Agents and Chemotherapy®

MECHANISMS OF RESISTANCE

In Vitro Study of Stepwise Acquisition of *rv0678* and *atpE* Mutations Conferring Bedaquiline Resistance

Nabila Ismail,[a,b] Nazir A. Ismail,[a,b,c] Shaheed V. Omar,[b] Remco P. H. Peters[a,d]

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[c]Faculty of Health Sciences, University of the Witwatersrand and the National Health Laboratory Service, Johannesburg, South Africa

[d]Department of Medical Microbiology, School Care and Public Health Research Institute (CAPHRI), Maastricht University, Maastricht, The Netherlands

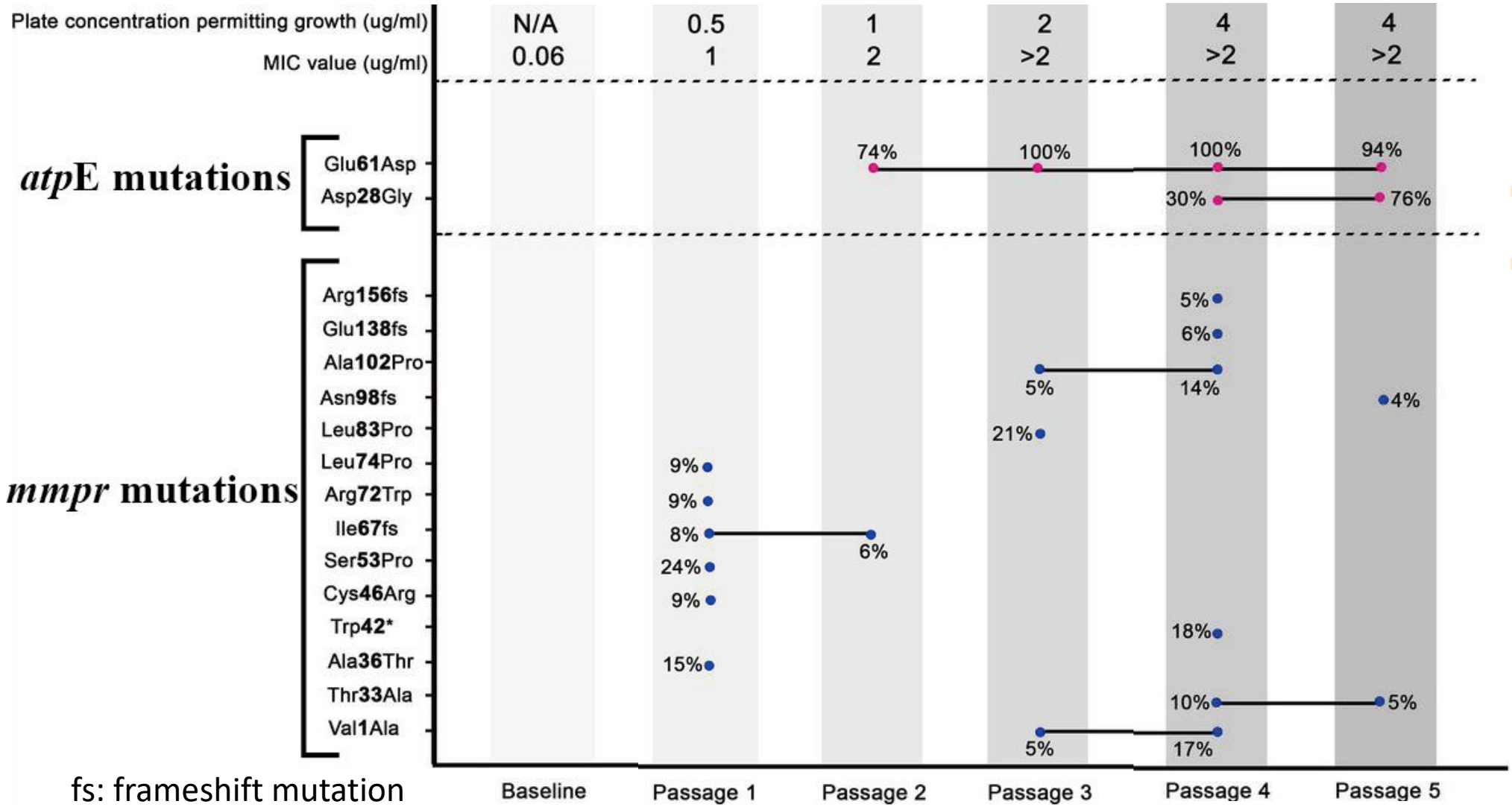
1st passage:
M. tuberculosis ATCC 35828 culture grown in 7H10 plates at 37°C for 21 days

↓

2nd passage:
Used the colonies from the plate with the highest drug concentration permitting growth

Published in 2019.

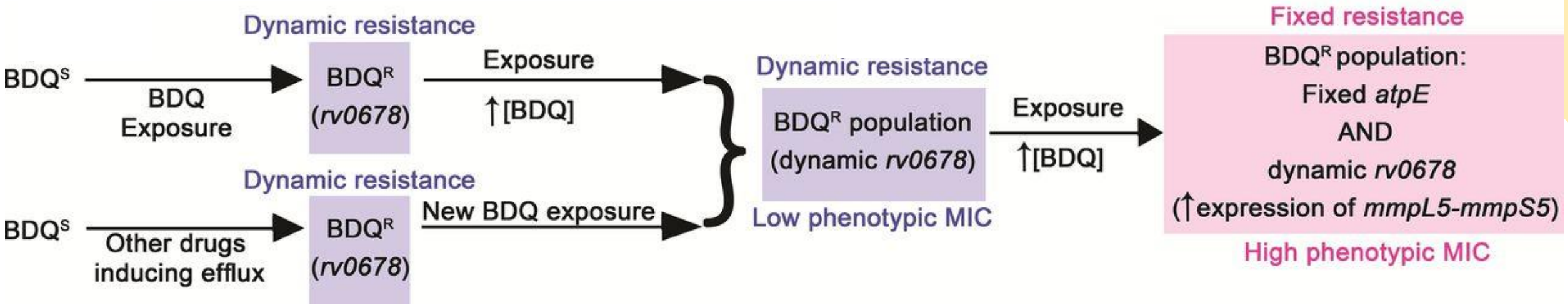
Ismail N, Ismail NA, Omar SV, Peters RPH. *In Vitro* Study of Stepwise Acquisition of *rv0678*and *atpE* Mutations Conferring Bedaquiline Resistance. Antimicrob Agents Chemother. 2019 Jul 25;63(8):e00292-19. doi: 10.1128/AAC.00292-19. PMID: 31138569; PMCID: PMC6658778.



fs: frameshift mutation

Ismail N, Ismail NA, Omar SV, Peters RPH. *In Vitro* Study of Stepwise Acquisition of *rv0678*and *atpE* Mutations Conferring Bedaquiline Resistance. Antimicrob Agents Chemother. 2019 Jul 25;63(8):e00292-19. doi: 10.1128/AAC.00292-19. PMID: 31138569; PMCID: PMC6658778.

Hypothesis of bedaquiline resistance development

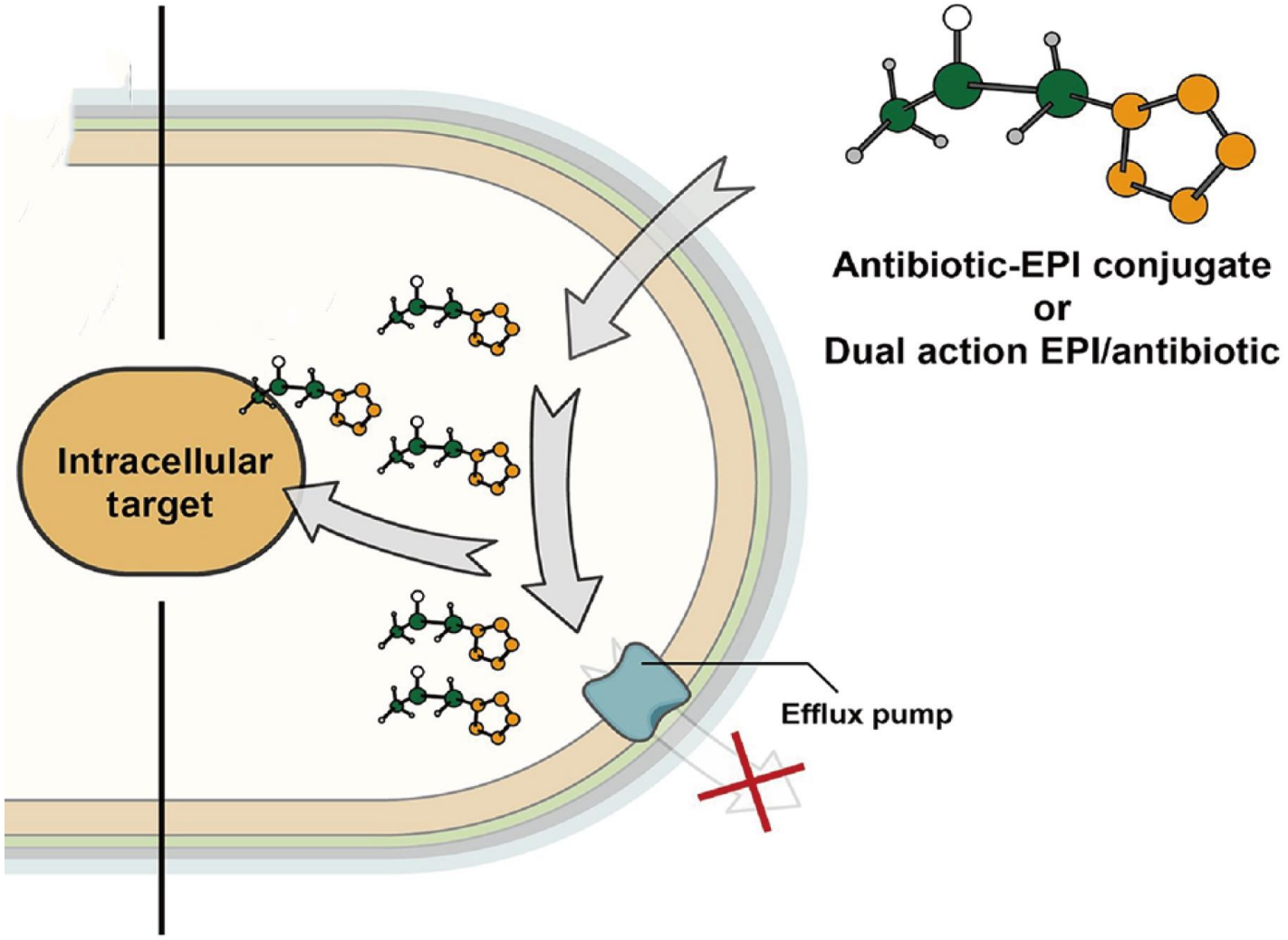


BDQ: Bedaquiline
BDQ^{S}: Bedaquiline-sensitive
BDQ^{R}: Bedaquiline-resistant

Ismail N, Ismail NA, Omar SV, Peters RPH. *In Vitro* Study of Stepwise Acquisition of *rv0678*and *atpE* Mutations Conferring Bedaquiline Resistance. Antimicrob Agents Chemother. 2019 Jul 25;63(8):e00292-19. doi: 10.1128/AAC.00292-19. PMID: 31138569; PMCID: PMC6658778.

Efflux pump inhibitors/EPIs

Efflux pump inhibitors / EPIs



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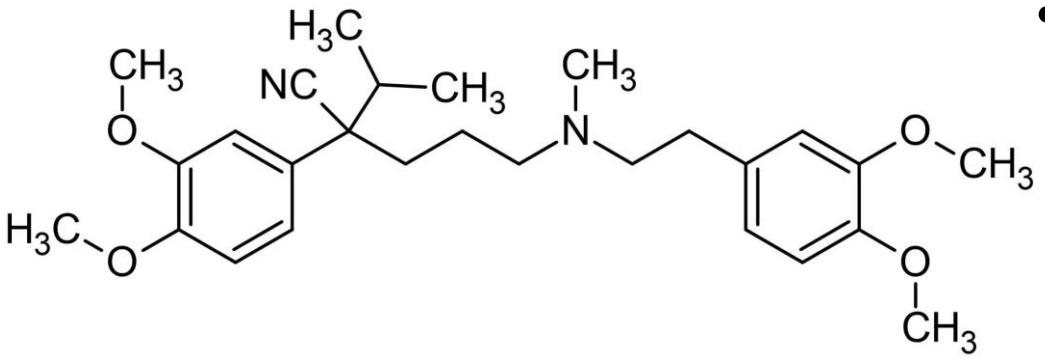
Verapamil



- A Ca^{2+} channel blocker to treat hypertension.
- Showed inhibition on the efflux pumps of ABC, MFS, SMR, and RND families.

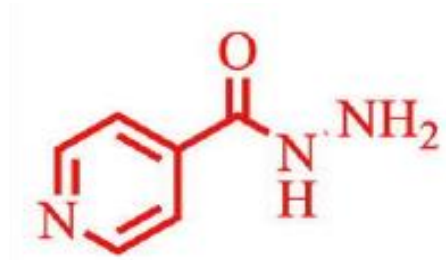
	MIC (μg/ml) for[a]:		
Drug	Drug only	Drug + verapamil	Fold change in MIC
Bedaquiline	0.0625	0.0075	8
Clofazimine	0.25	0.03125	8

MICs of *M. tuberculosis* H37RV.

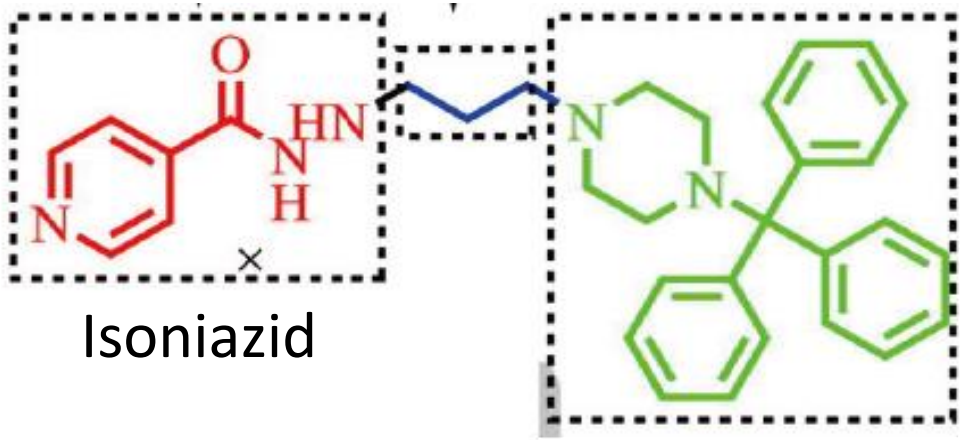
U.S. National Library of Medicine. (n.d.). *(-)-verapamil*. National Center for Biotechnology Information. PubChem Compound Database. Retrieved December 7, 2021, from https://pubchem.ncbi.nlm.nih.gov/compound/92305.

Gupta S, Cohen KA, Winglee K, Maiga M, Diarra B, Bishai WR. Efflux inhibition with verapamil potentiates bedaquiline in Mycobacterium tuberculosis. Antimicrob Agents Chemother. 2014;58(1):574-6. doi: 10.1128/AAC.01462-13. Epub 2013 Oct 14. PMID: 24126586; PMCID: PMC3910722.

Antibiotic-EPI conjugates



Isoniazid



Isoniazid

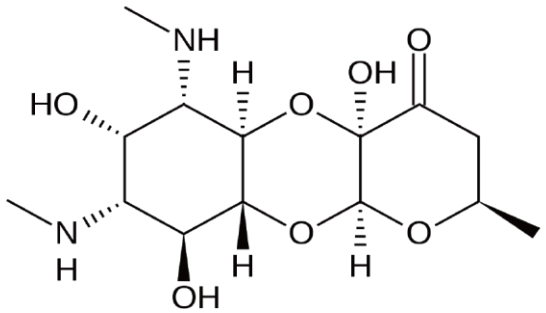
Triphenylmethane

Drugs	MIC (μM)
Isoniazid	>10
Hybrid isoniazid	2.5

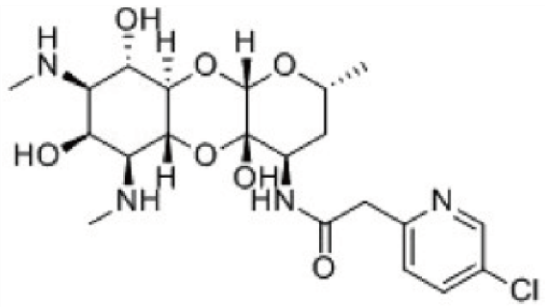
MICs against *M. tuberculosis* X_60, the extensively drug-resistant strain.

Kumar M, Singh K, Ngwane AH, Hamzabegovic F, Abate G, Baker B, Wiid I, Hoft DF, Ruminski P, Chibale K. Reversed isoniazids: Design, synthesis and evaluation against Mycobacterium tuberculosis. Bioorg Med Chem. 2018 Feb 15;26(4):833-844. doi: 10.1016/j.bmc.2017.12.047. Epub 2017 Dec 29. PMID: 29373270.

Engineered antibiotics - spectinamide



Spectinomycin



Spectinamide

- Tap: An intrinsic efflux pump to remove spectinomycin.
- Modified to avoid the interaction with Tap.

	IC_{50} of ribosome inhibition (µg/mL)	**MIC (µg/mL) against H37Rv**
Spectinomycin	0.53	100
Spectinamide	0.36	0.8

Liu, J., Bruhn, D. F., Lee, R. B., Zheng, Z., Janusic, T., Scherbakov, D., Scherman, M. S., Boshoff, H. I., Das, S., Rakesh, Waidyarachchi, S. L., Brewer, T. A., Gracia, B., Yang, L., Bollinger, J., Robertson, G. T., Meibohm, B., Lenaerts, A. J., Ainsa, J., Böttger, E. C., … Lee, R. E. (2017). Structure-Activity Relationships of Spectinamide Antituberculosis Agents: A Dissection of Ribosomal Inhibition and Native Efflux Avoidance Contributions. *ACS infectious diseases*, *3*(1), 72–88. https://doi.org/10.1021/acsinfecdis.6b00158

Summary

- The rapid emergence of efflux-mediated resistance to bedaquiline highlights the need to consider efflux during the development of new anti-TB drugs.

- An alternative to developing drugs with the new mode of action: dual-action EPI-antibiotics.

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Thank you!

Q&A