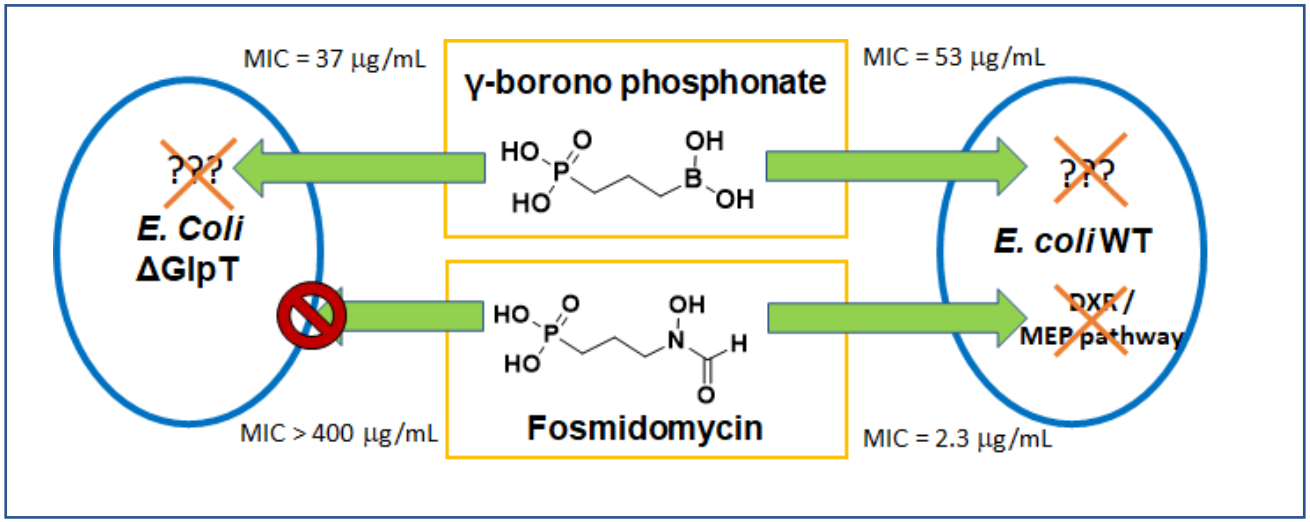
Synthesis and Antimicrobial Evaluation of γ-borono phosphonate analogs against *E. coli* and *M. smegmatis*



ACS national meeting

Giulia Mancini

Advisor : Dr. John Tomsho

3/22/2020

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Mancini, G.; Bouda, M.; Gamrat, M. J.; Tomsho, W. J. *ACS Omega*, **2019**, 4, 11, 14551-14559.

Combating Drug Resistance

- Antimicrobial drug resistance is a worldwide threat to public health
- Two million people contract antibiotic resistant infections and 23,000 die every year in the U.S. alone
- Tuberculosis (TB) is an infectious disease that affects people worldwide
- The development of drug-resistant and multidrug-resistant strains of *Mycobacterium tuberculosis* (Mtb) requires the continuous development of new drugs and the discovery of new targets

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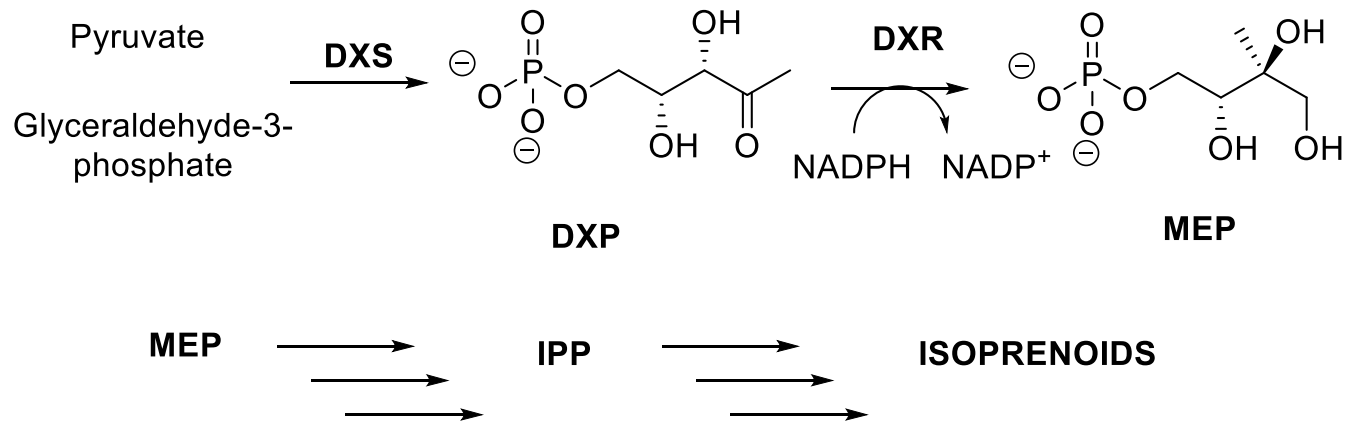
World Health Organization, *Global Tuberculosis Report*, **2018**
Koch, A. et all. *Curr. Opin. Pharmacol.* **2018**, 42, 7-15

Non – Mevalonate (MEP) Pathway



- The MEP pathway leads to the synthesis of isoprenoids in bacteria (e.g. *E. coli* and *Mtb*) and parasites (e.g. malaria)
- The MEP pathway is not present in humans thus making the enzymes in this pathway attractive drug targets

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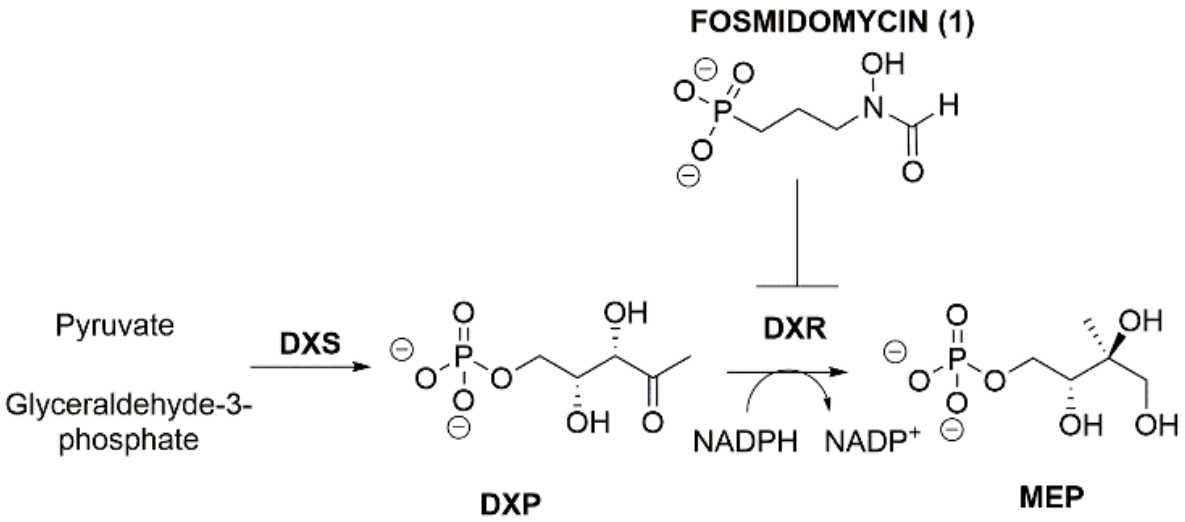
Hale, I. et All. *Med. Chem. Commun.* **2012**, 3,418- 433.

Fosmidomycin is a Potent Inhibitor of the MEP Pathway



- Fosmidomycin is a natural product with antibacterial and antimalarial activity
- It was determined to be a low nanomolar inhibitor of 1-deoxy-D-xylulose-5-phosphate reductoisomerase (DXR or IspC) (IC_{50} = 32 nM)
- DXR is the first committed step in the MEP pathway

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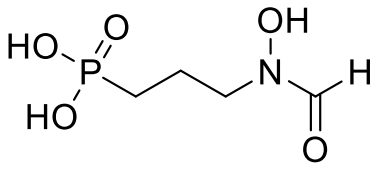
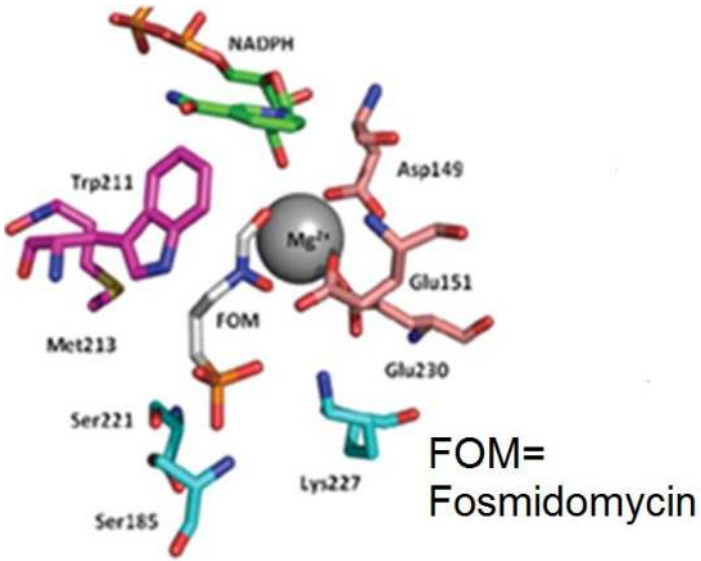
Mechanism of Action for Fosmidomycin

FOSMIDOMYCIN (1)





FOM= Fosmidomycin

Within the DXR active site:

- The phosphonate group of fosmidomycin interacts through noncovalent interactions with serine and lysine residues
- The retrohydroxamic acid portion is coordinated in a bidentate fashion with the divalent metal Mg^{2+}
- Although it shows potent in vitro activity, the polar character of fosmidomycin renders it passively membrane impermeable.
- However… 

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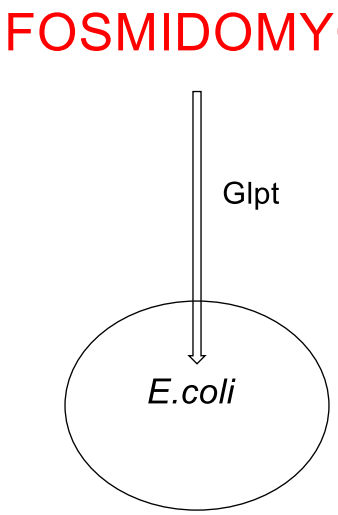
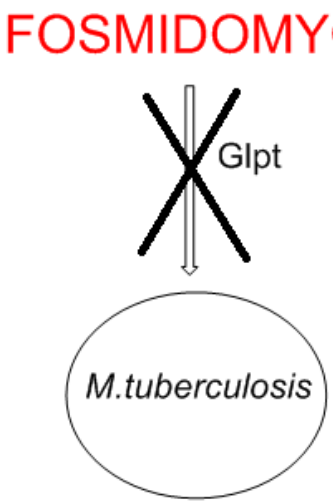
Hale, I. et All.. *Med. Chem. Commun.* **2012**, 3,418- 433.

Fosmidomycin is a Substrate for an Active Transporter

- In *E. coli*, the active uptake of fosmidomycin is mediated by the glycerol-3- phosphate transporter (GlpT)
- Fosmidomycin resistance in *E. coli* can be caused by a mutation in GlpT

- Fosmidomycin is inactive against mycobacteria species
- Fosmidomycin effectively inhibits mycobacterial DXR
- Absence of an analogous transporter renders these organisms inherently resistant





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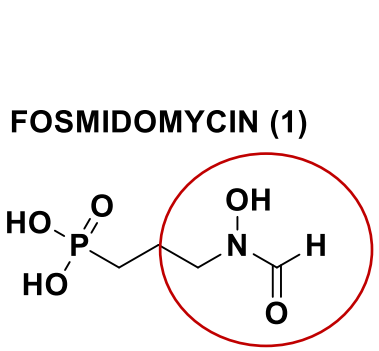
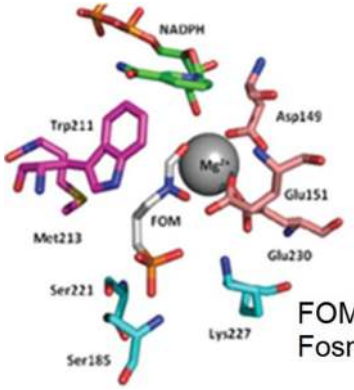
G. S. Jose et All. *Medchemcomm,* **2013** ,1099-1104.
K. B.Cher et All. *J.Med. Chem.*, **2015,** 58,2025-2035.
Mckenney, E. S. et All. *PLoS One* **2012**, 7, No. e38167.
Sakamoto, Y. et All. *Biosci., Biotechnol.Biochem*. **2003**, 67, 2030−2033.

Introduction of a Boronic Acid Moiety to Increase Passive Membrane Permeability

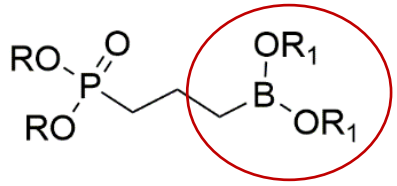
- Isosteric replacement of the retrohydroxamic acid group with a boronic acid
- Boronic acids are known metal chelators
- With these boronic acid-containing compounds, we have the ability of introducing a double prodrug by masking both the highly charged phosphonate moiety and the boronic acid group

γ-Borono phosphonates





FOM= Fosmidomycin



4 : R = H, R_1 = H

5 : R= Bn, R_1= H

6 : R= H, R_1= MIDA

7 : R= POM, R_1= MIDA

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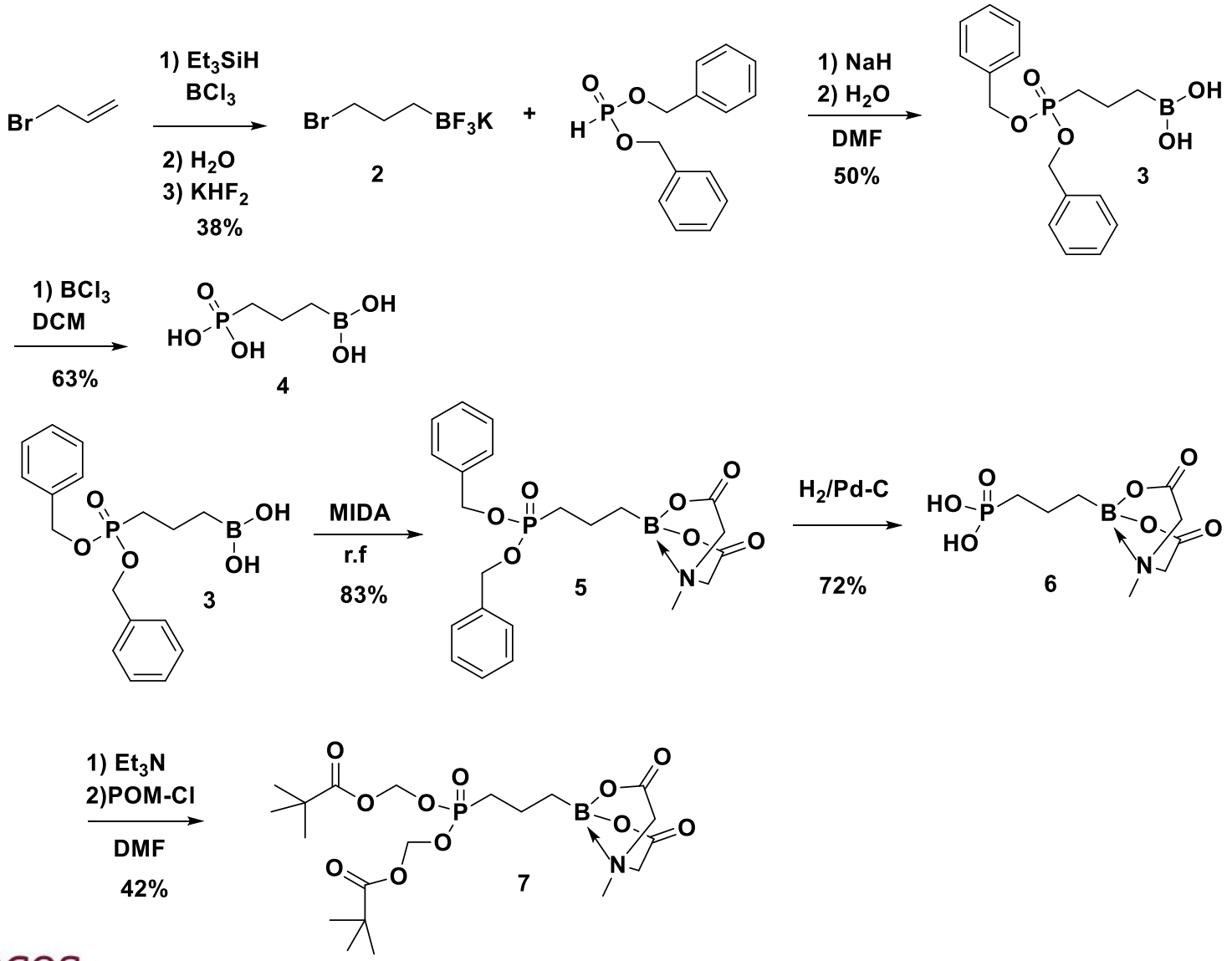
Hale, I. et All. *Med. Chem. Commun.* **2012**, 3,418- 433.
Mancini, G. et All. *ACS Omega*, **2019**, 4, 11, 14551-14559.

Synthesis of γ-Borono Phosphonate Analogs



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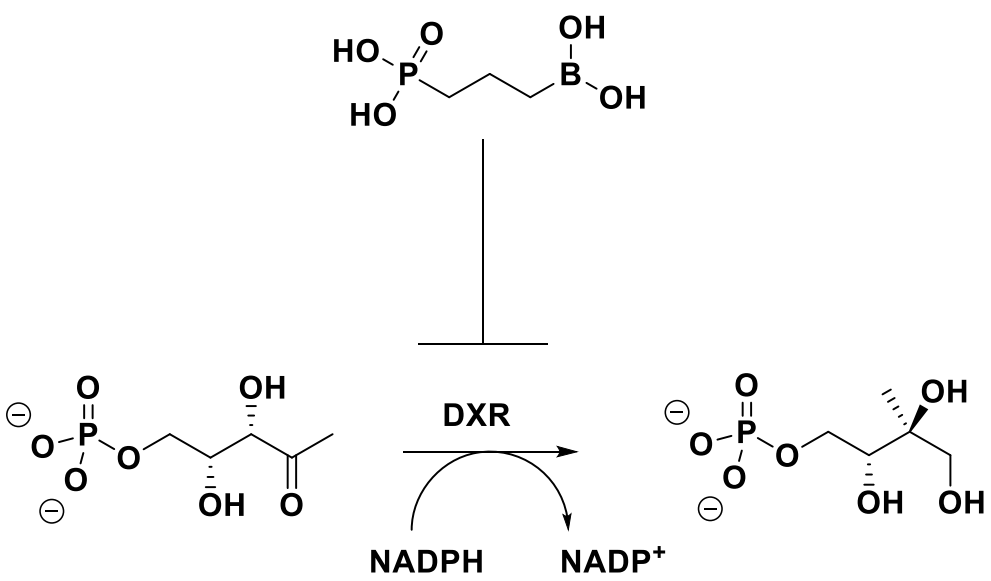
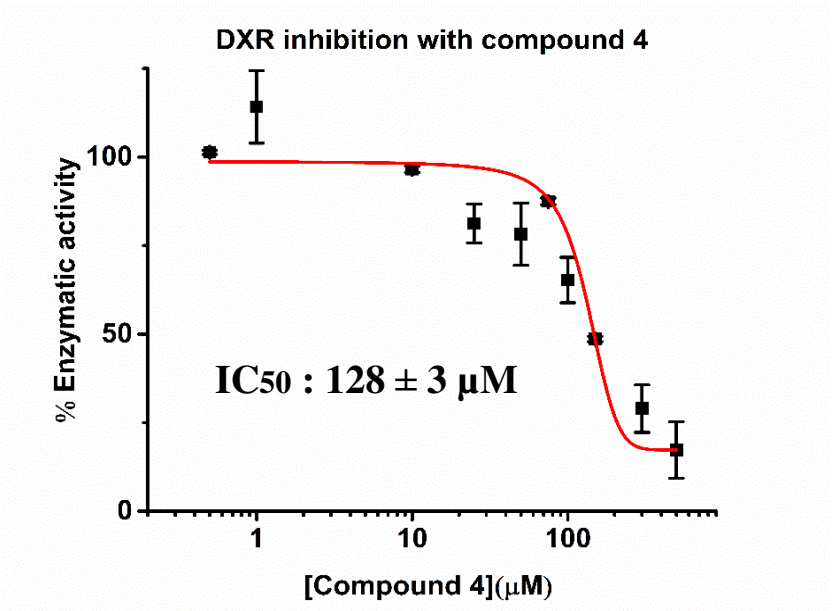
γ-Borono Phosphonate (4) is a Relatively Poor Inhibitor of *E. coli* DXR

γ-Borono Phosphonate (**4**)





- Fosmidomycin IC_{50} was determined to be 0.049 ± 0.007 µM

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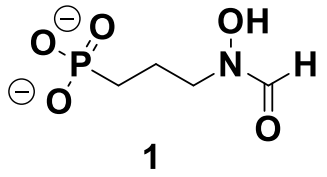
γ-Borono Phosphonate (4) and the MIDA-prodrug (6) Exhibited Significant Antimicrobial Activity



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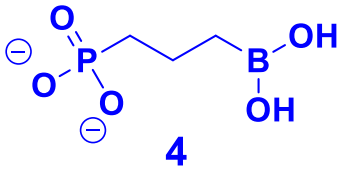

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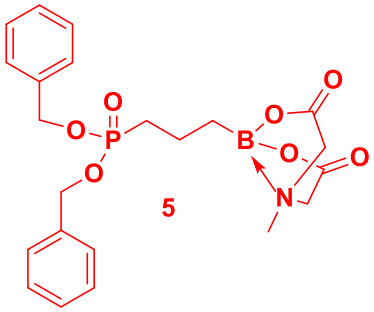
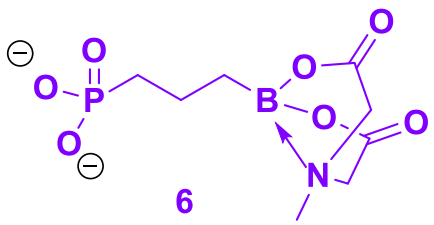


5

MIC90	*E. coli* (μg/ml)		*M. smegmatis* (μg/ml)	*cLogP*
	WT	ΔGlpT		
1	2.3 ± 5	> 400	> 400	-2.21
4	53 ± 6	37 ± 4	> 1000	-1.53
5	>1000	>1000	> 1000	4.02
6	63 ± 13	52 ± 6	>500	-0.99
7	> 750	>750	>750	4.28
POM-FR900098	>200	N. D	50-100[a]	2.44



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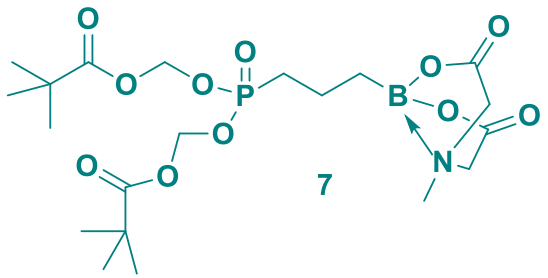

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Interpretation of Antimicrobial Activity Profile

- Our results show that **4** and **6** inhibited in vitro growth of both *E. coli* WT and *E. coli* ΔGlpT, unlike fosmidomycin. *E. coli* ΔGlpT is inherently resistant to fosmidomycin due to compromised membrane transport, yet this strain is not resistant to **4** or **6**.

- Despite these promising results, **4** and **6** are inactive against *M. smegmatis*, probably due to a remaining inability to cross the mycobacterial cell wall

- Pro-drugs **5** and **7**, with no significant activity against *E. coli* WT, *E. coli* ΔGlpT, or *M. smegmatis*, are likely suffering from efflux pump effect or a complete inability to release the active compound under the assay conditions

Given the low affinity of **4** against DXR and the structural similarity of our compound to other metabolites within the MEP pathway, we wanted to independently evaluate compound **4** as a potential inhibitor for any step within the MEP pathway

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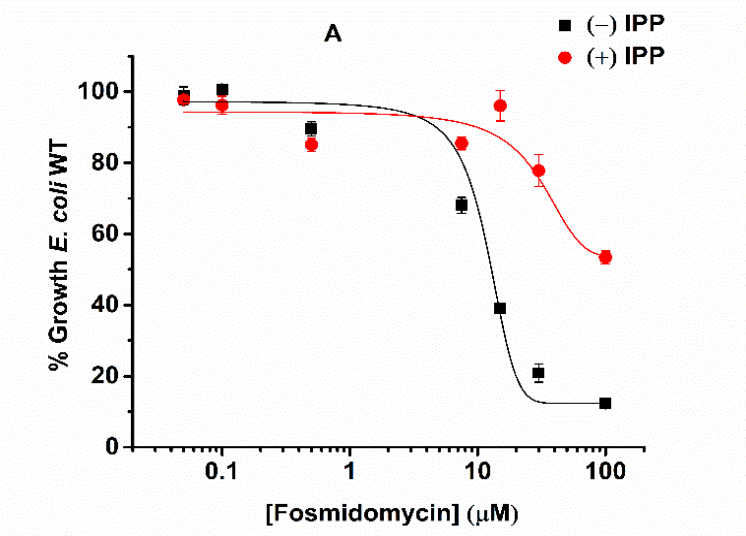
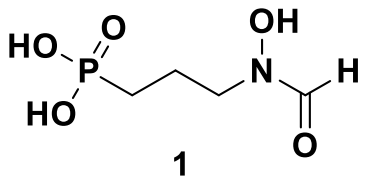
Exogenous IPP Relieves MEP-Pathway Inhibition in *E. coli*





- We determined that fosmidomycin-induced *E. coli* growth inhibition can be relieved via chemical rescue with IPP added to the growth media

- This finding provides an experiment to assess whether compounds inhibit the MEP pathway

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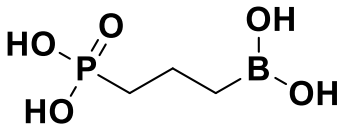
E. coli Chemical Rescue Experiments Reveal that the γ-Borono Phosphonate Does Not Inhibit the MEP Pathway



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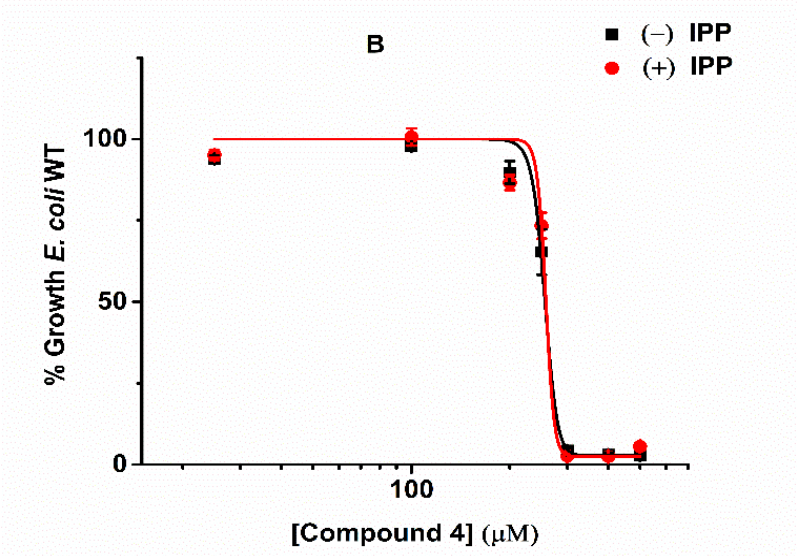
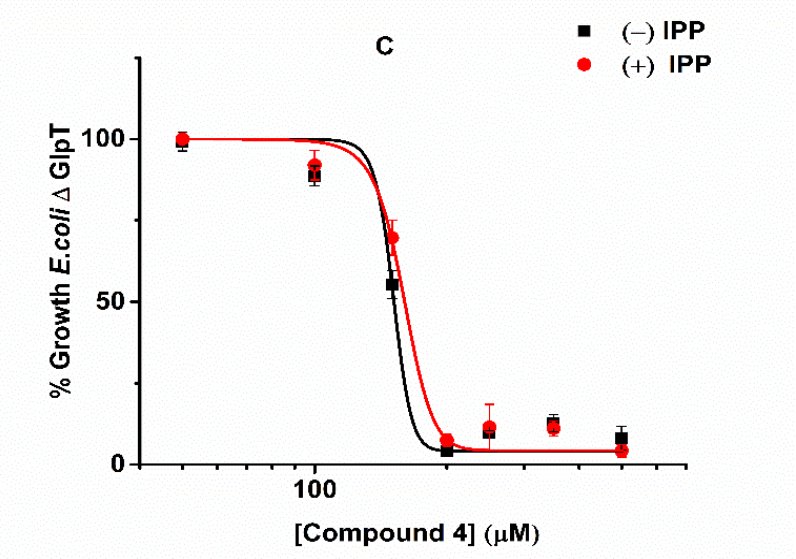
Compound 4 against *E. coli* WT



Compound 4 against *E. coli* ΔGlpT



- The addition of IPP to the media did not restore growth. These results indicate that the target of **4** is not any of the enzymes that are involved in the MEP pathway. Similar results were obtained for the MIDA-borono prodrug **6**
- Similar results were found with *E. coli* ΔGlpT indicating independence from this transporter necessary for the bioactivity of fosmidomycin

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Conclusions

- This work describes the synthesis and the antimicrobial evaluation of a novel class of antimicrobial agents, γ-borono phosphonates, and it is the first report of evaluating a boronic acid as an isostere of the metal-chelating retrohydroxamate moiety

- DXR inhibition by **4** was considerably weaker than with fosmidomycin, indicating that this alkyl boronic acid group is suboptimal in this application, which requires strong metal chelation

- Our antimicrobial evaluation of these compounds against *E. coli* WT and *E. coli* ΔGlpT showed that they have a reasonable activity against both strains and a mechanism of action that is unique from fosmidomycin

- We have proven that these compounds are acting on a target outside of the non-mevalonate pathway and are independent of the glycerol-3-phosphate transporter

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Acknowledgements

Giulia Mancini

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