

# Over 40 years of fosmidomycin drug research: A comprehensive review and future opportunities

## - Supporting information -

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**Table S1:** Antiparasitic and antibiotic data of fosmidomycin (1) and FR900098 (2) obtained from enzyme assays and growth inhibition assays

	Fosmidomycin (1)		FR900098 (2)	
	DXR IC <sub>50</sub> <sup>a</sup>	Whole-cell MIC <sup>b</sup>	DXR IC <sub>50</sub> <sup>a</sup>	Whole-cell MIC <sup>b</sup>
<i>Plasmodium falciparum</i> Dd2	0.16 µM <sup>*1</sup>	IC <sub>50</sub> = 0.81 µM <sup>1</sup>	0.015 µM <sup>*1</sup>	IC <sub>50</sub> = 0.16 µM <sup>1</sup>
<i>Plasmodium falciparum</i> 3D7		IC <sub>50</sub> = 0.88 µM <sup>1</sup>		IC <sub>50</sub> = 0.16 µM <sup>1</sup>
<i>Toxoplasma gondii</i>	K <sub>i</sub> = 0.090 µM <sup>*2</sup>	32% growth inhibition at 2.5 mM <sup>3</sup>	K <sub>i</sub> = 0.048 µM <sup>*2</sup>	
<i>Eimeria tenella</i>		30% growth inhibition at 3.3 mM <sup>3</sup>		
<i>Synechocystis</i> sp.	K <sub>i</sub> = 4 nM <sup>**4</sup>		K <sub>i</sub> = 2 nM <sup>**4</sup>	
<b>Gram (+)</b>				
<i>Bacillus anthracis</i>		0.78 µg/ml <sup>5</sup>		50 µg/ml <sup>5</sup>
<i>Bacillus subtilis</i> (wild strain)		8 mM <sup>6</sup>		
<i>Enterococcus faecalis</i>		> 200 µg/ml <sup>5</sup>		> 200 µg/ml <sup>5</sup>
<i>Mycobacterium tuberculosis</i>	0.31 µM <sup>7</sup>	> 500 µg/ml <sup>5</sup>	2.91 µM <sup>8</sup>	> 500 µg/ml <sup>5</sup>
<i>Staphylococcus aureus</i> (MSSA)		> 200 µg/ml <sup>5</sup>		> 200 µg/ml <sup>5</sup>
<i>S. aureus</i> (MRSA)		200 µg/ml <sup>5</sup>		50 µg/ml <sup>5</sup>
<i>Staphylococcus epidermidis</i>		n.i. <sup>10</sup>		
<i>Staphylococcus ludgenensis</i>		n.i. <sup>10</sup>		
<i>Staphylococcus pseudintermedius</i>		0.5 – 1 µg/ml <sup>10</sup>		
<i>Staphylococcus schleiferi</i>		0.5 -8 µg/ml <sup>10</sup>		
<b>Gram (-)</b>				
<i>Acinetobacter baumannii</i>	46.8 nM <sup>11</sup>	> 512 µg/ml <sup>11</sup>	23.9 nM <sup>11</sup>	128 - > 512 µg/ml <sup>11</sup>
<i>Burkholderia cepacia</i> LMG 1222		> 512 µg/ml <sup>12</sup>		256 µg/ml <sup>12</sup>
<i>Burkholderia multivorans</i> LMG 13010		> 512 µg/ml <sup>12</sup>		256 µg/ml <sup>12</sup>
<i>Burkholderia cenocepacia</i> LMG 16656		> 512 µg/ml <sup>12</sup>		> 512 µg/ml <sup>12</sup>
<i>E. coli</i> K12	0.03 µM <sup>*13</sup>	> 12.5 µg/ml <sup>5</sup>	0.03 µM <sup>*13</sup>	200 µg/ml <sup>5</sup>
<i>E. coli</i> tolC		> 6.25 µg/ml <sup>5</sup>		12.5 µg/ml <sup>5</sup>
<i>Francisella tularensis</i>	247 nM <sup>14</sup>		230 nM <sup>14</sup>	
<i>Francisella</i>		136 µM <sup>14</sup>		254 µM <sup>14</sup>

<i>novicida</i>				
<i>Pseudomonas aeruginosa</i>	150 nM <sup>9</sup>		150 nM <sup>9</sup>	
<i>Klebsiella pneumoniae</i>	20.2 nM <sup>11</sup>	64 – 128 µg/ml <sup>11</sup>	23.1 nM <sup>11</sup>	256 µg/ml <sup>11</sup>
<i>Yersinia pestis</i>	710 nM <sup>15</sup>	128 µg/ml <sup>16</sup>	231 nM <sup>15</sup>	

<sup>a</sup> IC<sub>50</sub> values for enzyme assays are given unless denoted otherwise

<sup>b</sup> MIC (minimum inhibitory concentration) values for whole cell assays are given unless denoted otherwise

\* recombinant enzyme

\*\* listed value obtained by preincubation studies

n.i. = no inhibition

**Table S2:** Existing co-crystal structures of DXR enzymes

Organism	PDB ID	Co-crystallized elements	Publication year
<i>Mycobacterium tuberculosis</i>	4OOF	NADPH, <b>1</b> , Mn <sup>2+</sup>	2014
	3ZHZ	DXRi,	2013
	3ZI0	DXRi, Mn <sup>2+</sup>	
	3ZHX	DXRi, Mn <sup>2+</sup>	
	3ZHY	DXRi, NADPH, Mn <sup>2+</sup>	
	2Y1C	Mn <sup>2+</sup>	2011
	2Y1D	DXRi, Mn <sup>2+</sup>	
	2Y1E	Mn <sup>2+</sup>	
	2Y1F	NADPH, DXRi, Mn <sup>2+</sup>	
	2Y1G	DXR, Mn <sup>2+</sup>	
	2JCV	NADPH, <b>1</b>	2007
	2JCX	NADPH, <b>1</b>	
	2JCY	None	
	2JD0	NADPH	
	2JD1	NADPH, Mn <sup>2+</sup>	
	2JD2	Mn <sup>2+</sup>	
	2C82	None	2006
	4AIC	NADPH, <b>1</b> , Mn <sup>2+</sup>	2012
	4RCV	NADPH, Mn <sup>2+</sup>	2015
	4A03	NADPH, <b>2</b> , Mn <sup>2+</sup>	2012
	3RAS	NADPH, DXRi, Mn <sup>2+</sup>	2011
<i>Plasmodium falciparum</i>	3AU8	Mn <sup>2+</sup> , NADPH	2011
	3AU9	Mg <sup>2+</sup> , NADPH, <b>1</b>	
	3AUA	Mg <sup>2+</sup> , NADPH, <b>2</b>	
	4Y67	DXRi, Mn <sup>2+</sup>	2015
	4Y6S	DXRi, Mn <sup>2+</sup>	
	4Y6R	DXRi, Mn <sup>2+</sup>	
	4Y6P	DXRi, Mn <sup>2+</sup>	
	3WQS	NADPH, DXRi, Mg <sup>2+</sup>	2014
	3WQR	NADPH, DXRi, Mg <sup>2+</sup>	
	3WQQ	NADPH, DXRi, Mg <sup>2+</sup>	
	5JMP	DXRi, Mn <sup>2+</sup>	2016
	5JO0	DXRi, Mn <sup>2+</sup>	
	5JNL	DXRi, Mn <sup>2+</sup>	
	5JBI	DXRi, Mn <sup>2+</sup>	
	5JC1	DXRi, Mn <sup>2+</sup>	
	5JAZ	DXRi, Mn <sup>2+</sup>	
	5JMW	DXRi, Mn <sup>2+</sup>	
	4QJB	Mg <sup>2+</sup>	2014
	4GAE	DXRi, NADPH, Mn <sup>2+</sup>	2013
	4KP7	DXRi, NADPH, Mn <sup>2+</sup>	2013
	4QOX	Mg <sup>2+</sup> , DXRi	2014

Organism	PDB ID	Co-crystallized elements	Publication year
<i>Escherichia coli</i>	2EGH	NADPH, <b>1</b> , Mg <sup>+2</sup>	2007
	3ANL	NADPH, DXRi	2011
	3ANM	NADPH, DXRi	
	3ANN	NADPH, DXRi	
	1Q0L	NADPH, <b>1</b>	2004
	1Q0Q	NADPH, DXP	
	1Q0H	NADPH, <b>1</b>	
	1T1R	DXRi	2004
	1T1S	DXRi, Mg <sup>2+</sup>	
	1K5H	None	2002
	3R0I	DXRi, Mn <sup>2+</sup>	2011
	1JVS	NADPH	2002
<i>Zymomonas mobilis</i>	1R0K	None	2004
	1R0L	NADPH	
<i>Thermotoga maritima</i>	3A06	NADPH, <b>1</b> , Mg <sup>2+</sup>	2010
	3A14	NADPH, Mg <sup>2+</sup>	
<i>Yersinia pestis</i>	3IIE	Mg <sup>2+</sup>	2009
	5DUL	NADPH	2015
<i>Staphylococcus schleiferi</i>	6MH4	None	2020
	6MH5	<b>1</b>	
<i>Brucella abortus</i>	3UPY	<b>1</b> , Mg <sup>2+</sup>	2012
	3UPL	Mg <sup>2+</sup>	
<i>Acinetobacter baumannii</i>	4ZN6	None	2015
	7S04	NADPH, Mg <sup>2+</sup> , <b>2</b>	2021
<i>Moraxella catarrhalis</i>	4ZQE	None	2016
	4ZQF	Mg <sup>2+</sup> , <b>1</b>	
	4ZQH	NADPH, Mg <sup>2+</sup> , <b>1</b>	
	4ZQG	NADPH, Mg <sup>2+</sup> , <b>1</b>	
<i>Vibrio vulnificus</i>	5KRR	Mn <sup>2+</sup>	2017
	5KS1	Mn <sup>2+</sup>	
	5KRV	Arginine	
	5KRY	None	
	5KQO	None	

## References

- (1) Brücher, K.; Gräwert, T.; Konzuch, S.; Held, J.; Lienau, C.; Behrendt, C.; Illarionov, B.; Maes, L.; Bacher, A.; Wittlin, S.; et al. Prodrugs of Reverse Fosmidomycin Analogues. *Journal of Medicinal Chemistry* **2015**, *58* (4), 2025-2035. DOI: 10.1021/jm5019719.
- (2) Cai, G.; Deng, L.; Xue, J.; Moreno, S. N. J.; Striepen, B.; Song, Y. Expression, characterization and inhibition of *Toxoplasma gondii* 1-deoxy-d-xylulose-5-phosphate reductoisomerase. *Bioorganic & Medicinal Chemistry Letters* **2013**, *23* (7), 2158-2161. DOI: <https://doi.org/10.1016/j.bmcl.2013.01.097>.
- (3) Clastre, M.; Goubard, A.; Prel, A.; Mincheva, Z.; Viaud-Massuau, M. C.; Bout, D.; Rideau, M.; Velge-Roussel, F.; Laurent, F. The methylerythritol phosphate pathway for isoprenoid biosynthesis in coccidia: presence and sensitivity to fosmidomycin. *Exp Parasitol* **2007**, *116* (4), 375-384. DOI: 10.1016/j.exppara.2007.02.002 From NLM.
- (4) Woo, Y.-H.; Fernandes, R. P. M.; Proteau, P. J. Evaluation of fosmidomycin analogs as inhibitors of the *Synechocystis* sp. PCC6803 1-deoxy-d-xylulose 5-phosphate reductoisomerase. *Bioorganic & Medicinal Chemistry* **2006**, *14* (7), 2375-2385. DOI: <https://doi.org/10.1016/j.bmc.2005.11.012>.
- (5) Uh, E.; Jackson, E. R.; San Jose, G.; Maddox, M.; Lee, R. E.; Lee, R. E.; Boshoff, H. I.; Dowd, C. S. Antibacterial and antitubercular activity of fosmidomycin, FR900098, and their lipophilic analogs. *Bioorg Med Chem Lett* **2011**, *21* (23), 6973-6976. DOI: 10.1016/j.bmcl.2011.09.123 PubMed.
- (6) Sivy, T. L.; Fall, R.; Rosenstiel, T. N. Evidence of isoprenoid precursor toxicity in *Bacillus subtilis*. *Biosci Biotechnol Biochem* **2011**, *75* (12), 2376-2383. DOI: 10.1271/bbb.110572 From NLM.
- (7) Dhiman, R. K.; Schaeffer, M. L.; Bailey, A. M.; Testa, C. A.; Scherman, H.; Crick, D. C. 1-Deoxy-D-Xylulose 5-Phosphate Reductoisomerase (IspC) from *Mycobacterium tuberculosis*: towards Understanding Mycobacterial Resistance to Fosmidomycin. *Journal of Bacteriology* **2005**, *187* (24), 8395-8402. DOI: doi:10.1128/JB.187.24.8395-8402.2005.
- (8) San Jose, G.; Jackson, E. R.; Haymond, A.; Johnny, C.; Edwards, R. L.; Wang, X.; Brothers, R. C.; Edelstein, E. K.; Odom, A. R.; Boshoff, H. I.; et al. Structure-Activity Relationships of the MEPicides: N-Acyl and O-Linked Analogs of FR900098 as Inhibitors of Dxr from *Mycobacterium tuberculosis* and *Yersinia pestis*. *ACS Infectious Diseases* **2016**, *2* (12), 923-935. DOI: 10.1021/acsinfecdis.6b00125.
- (9) Altincicek, B.; Hintz, M.; Sanderbrand, S.; Wiesner, J.; Beck, E.; Jomaa, H. Tools for discovery of inhibitors of the 1-deoxy-D-xylulose 5-phosphate (DXP) synthase and DXP reductoisomerase: an approach with enzymes from the pathogenic bacterium *Pseudomonas aeruginosa*. *FEMS Microbiology Letters* **2000**, *190* (2), 329-333. DOI: 10.1111/j.1574-6968.2000.tb09307.x (accessed 1/10/2022).
- (10) Misis, A. M.; Cain, C. L.; Morris, D. O.; Rankin, S. C.; Beiting, D. P.; Fey, P. D. Divergent Isoprenoid Biosynthesis Pathways in *Staphylococcus* Species Constitute a Drug Target for Treating Infections in Companion Animals. *mSphere* **2016**, *1* (5), e00258-00216. DOI: doi:10.1128/mSphere.00258-16.
- (11) Ball, H. S.; Girma, M. B.; Zainab, M.; Soojhawon, I.; Couch, R. D.; Noble, S. M. Characterization and Inhibition of 1-Deoxy-d-Xylulose 5-Phosphate Reductoisomerase: A Promising Drug Target in *Acinetobacter baumannii* and *Klebsiella pneumoniae*. *ACS Infectious Diseases* **2021**, *7* (11), 2987-2998. DOI: 10.1021/acsinfecdis.1c00132.
- (12) Messiaen, A. S.; Verbrugghen, T.; Declerck, C.; Ortmann, R.; Schlitzer, M.; Nelis, H.; Van Calenbergh, S.; Coenye, T. Resistance of the *Burkholderia cepacia* complex to fosmidomycin and fosmidomycin derivatives. *Int J Antimicrob Agents* **2011**, *38* (3), 261-264. DOI: 10.1016/j.ijantimicag.2011.04.020 From NLM.
- (13) Haemers, T.; Wiesner, J.; Poecke, S. V.; Goeman, J.; Henschker, D.; Beck, E.; Jomaa, H.; Calenbergh, S. V. Synthesis of  $\alpha$ -substituted fosmidomycin analogues as highly potent *Plasmodium falciparum* growth inhibitors. *Bioorganic & Medicinal Chemistry Letters* **2006**, *16* (7), 1888-1891. DOI: <https://doi.org/10.1016/j.bmcl.2005.12.082>.
- (14) McKenney, E. S.; Sargent, M.; Khan, H.; Uh, E.; Jackson, E. R.; Jose, G. S.; Couch, R. D.; Dowd, C. S.; van Hoek, M. L. Lipophilic Prodrugs of FR900098 Are Antimicrobial against *Francisella novicida* In Vivo and In Vitro and Show GltP Independent Efficacy. *PLOS ONE* **2012**, *7* (10), e38167. DOI: 10.1371/journal.pone.0038167.
- (15) Haymond, A.; Johnny, C.; Dowdy, T.; Schweibenz, B.; Villarroel, K.; Young, R.; Mantooth, C. J.; Patel, T.; Bases, J.; San Jose, G.; et al. Kinetic characterization and allosteric inhibition of the *Yersinia pestis* 1-deoxy-D-xylulose 5-phosphate reductoisomerase (MEP synthase). *PLoS One* **2014**, *9* (8), e106243. DOI: 10.1371/journal.pone.0106243 From NLM.
- (16) Ball, H. S.; Girma, M.; Zainab, M.; Riley, H.; Behrendt, C. T.; Lienau, C.; Konzuch, S.; Avelar, L. A. A.; Lungerich, B.; Soojhawon, I.; et al. Inhibition of the *Yersinia pestis* Methylerythritol Phosphate Pathway of Isoprenoid Biosynthesis by  $\alpha$ -Phenyl-Substituted Reverse Fosmidomycin Analogues. *ACS Omega* **2020**, *5* (10), 5170-5175. DOI: 10.1021/acsomega.9b04171.