Published in final edited form as:
Bioorg Med Chem Lett. 2011 December 1; 21(23): 6973–6976. doi:10.1016/j.bmcl.2011.09.123.

Antibacterial and Antitubercular Activity of Fosmidomycin, FR900098, and their Lipophilic Analogs

Eugene Uh[a,b], **Emily R. Jackson**[a], **Géraldine San Jose**[a], **Marcus Maddox**[c], **Robin E. Lee**[c], **Richard E. Lee**[c], **Helena I. Boshoff**[d], and **Cynthia S. Dowd**[a,*]

[a]Department of Chemistry, George Washington University, Washington DC 20052

[c]Chemical Biology and Therapeutics, St. Jude Children's Research Hospital, Memphis, TN 38105

[d]Tuberculosis Research Section, Laboratory of Clinical Infectious Diseases, National Institute of Allergy and Infectious Diseases, NIH, Bethesda, MD 20892

Abstract

The nonmevalonate pathway (NMP) of isoprene biosynthesis is an exciting new route toward novel antibiotic development. Inhibitors against several enzymes in this pathway are currently under examination. A significant liability of many of these agents is poor cell penetration. To overcome and improve our understanding of this problem, we have synthesized a series of lipophilic, prodrug analogs of fosmidomycin and FR900098, inhibitors of the NMP enzyme Dxr. Several of these compounds show improved antibacterial activity against a panel of organisms relative to the parent compound, including activity against *Mycobacterium tuberculosis* (Mtb). Our results show that this strategy can be an effective way for improving whole cell activity of NMP inhibitors.

There continues to be a pressing need for drugs with novel mechanisms of action. Most clinically useful anti-infectives target only a few of the hundreds of potential targets in metabolism, and global rates of drug resistant bacteria are on the rise.[1,2] For example, in 2010, 2 million people died from tuberculosis, caused by *M. tuberculosis* (Mtb), including approximately 150,000 individuals who died from one of several multi-drug resistant strains.[3,4] Without new therapeutics working through unique targets, drug resistance and decreased drug susceptibility will continue to be a public health concern.[1,2]

Recently, the nonmevalonate pathway (NMP) has been examined as a novel route against bacteria and parasites.[5–10] The role of the NMP is to synthesize activated five carbon units that the cell will elaborate into more complex structures. Humans use the mevalonate pathway to biosynthesize the same isoprenoid units. As such, the enzymes found in the NMP are not found in humans, leading to the interest in this pathway for antibacterial drug targeting. While many of the enzymes in the NMP have been examined, 1-deoxy-D-xylulose 5-phosphate reductoisomerase (Dxr or IspC) has been studied to the greatest extent.[5,6] This enzyme is responsible for reducing and isomerizing 1-deoxy-D-xylulose 5-phosphate (DXP

*Corresponding author: 725 21st Street NW, Corcoran 107, George Washington University, Washington DC 20052, +01-202-994-8405 (ph), +01-202-994-5873 (fax), cdowd@gwu.edu.
[b]Present Address: Tuberculosis Research Section, Laboratory of Clinical Infectious Diseases, National Institute of Allergy and Infectious Diseases, NIH, Bethesda, MD 20892 USA

or DOXP) to 2-C-methyl-D-erythritol 3-phosphate (MEP). Several crystal structures of Dxr from various bacteria have been reported.[11,12] Most of the work developing inhibitors against Dxr has been in the context of *Plasmodium falciparum*[13–30] although more recent studies have targeted Dxr from Mtb.[31,32]

Two well-known inhibitors of Dxr, fosmidomycin (**1**) and FR900098 (**2**), are natural products and mimic Dxr's substrate DXP (Figure 1). Both compounds are quite potent inhibitors against Dxr from a variety of organisms.[33,34] Interestingly, however, while fosmidomycin is active against some bacteria and *P. falciparum*, it is inactive against Mtb.[35,36] Mtb relies on Dxr for isoprene production[35] but does not have a GlpT transporter used by many organisms for fosmidomycin uptake.[37] This, combined with the polar nature of fosmidomycin and the lipophilic tuberculosis cell wall, is thought to be the underlying reason for the lack of effect of fosmidomycin against Mtb.[35,36] Our work aims to develop novel inhibitors of Dxr and use them as tools to examine this enzyme for its potential as an antibacterial target. As a proof of this concept and expanding on prior work from the malaria field,[13–30] we present a series of analogs of compounds **1** and **2** designed to understand if added lipophilicity increases cell penetration and antibacterial activity. It is expected that these prodrugs enter the cell and are hydrolyzed by nonspecific, cellular esterases to reveal the phosphonate which then inhibits Dxr.[29] The compounds have the general structure shown in Figure 1.

There are several published synthetic routes for fosmidomycin derivatives.[13–22,24–28,30–32] We combined and modified these routes to yield the best overall synthetic schemes. The syntheses of the target compounds are shown in Schemes 1–3. Diethyl fosmidomycin (**10**)[25] was prepared using the route shown in Scheme 1. Diethyl phosphite (**3**) was combined with *O*-benzylhydroxylamine (**4**) to yield phosphoramidate (**5**).[38] This compound was combined with diethyl 3-bromopropylphosphonate (**6**) to yield compound **7**.[39] The phosphoramide of **7** was cleaved using acidic conditions to yield compound **8**.[39] Compound **8** was formylated[21] and debenzylated[25] to give compounds **9** and **10**, respectively.

Two routes were used in the preparation of lipophilic esters of FR900098 (Schemes 2 and 3). Scheme 2 begins with the lipophilic moieties installed on the phosphonate (compounds **12**). To prepare ethyl analog **15**, *O*-benzylhydroxylamine was acetylated[40] and combined with **12a** (prepared using microwave conditions[41]) to yield compound **14a**.[25] For isopropyl analog **16**, Boc-protected **11** was alkylated with compound **12b** to give **13**. Removal of the Boc group, followed by acetylation yielded compound **14b**. Hydrogenation to remove the benzyl group gave target compounds **15**[42] and **16**.[43]

Most of the lipophilic esters were prepared using the route shown in Scheme 3.[25] This scheme uses a shorter, but more costly, path to compound **8**. Diethyl acetal-protected diethyl phosphonate ester **17** was deprotected to give aldehyde **18**. This compound underwent reductive amination with *O*-benzylhydroxylamine and $NaBH_3CN$ to yield compound **8**. Acetylation yielded compound **14a**. Removal of the diethyl ester using TMSBr gave common intermediate **19**. Interestingly, despite its use by many groups, the TMSBr deprotection step is not well described. In our hands, the addition of water after deprotection does not yield a solid. The solid, deprotected product is isolated only after complete removal of water or with the addition of THF to facilitate precipitation. Intermediate **19** was alkylated with several different alkyl groups to give compounds **20a–g**. Subsequent hydrogenation yielded target compounds **21–27**. The analytical data for compounds **22**, **24**, and **27** matches literature values.[25] Experimental details are given for new compounds **21**[44], **23**[45], **25**[46], and **26**[47].

The compounds were examined for antibacterial and antitubercular activities using methods previously reported.[48,49] The activities are shown in Table 1. The two parent compounds, fosmidomycin (**1**) and FR900098 (**2**) show activity against Gram (−) strains but this activity is less pronounced against the panel of Gram (+) organisms. The exception is fosmidomycin's potent activity against *B. anthracis* (0.78 μg/mL). As has been demonstrated by others[35,36], fosmidomycin does not have antitubercular activity (MIC >500 μg/mL) and this is also the case for its acetyl derivative, FR900098, which is inactive.

Several of the lipophilic esters showed improved activity relative to fosmidomycin, particularly for Gram (+) bacteria. While diethyl fosmidomycin (**10**) showed little activity against the panel, as the size of the lipophilic ester increased, activity against these organisms generally increased as well. For example, **24** and **26** showed better antitubercular activity compared with compounds **10**, **15** and **16**. Interestingly, analogs with a secondary ester (**22**, **25** and **27**) did not outperform their primary counterparts. A possible explanation for this could be that the cellular esterases needed to reveal the phosphonate do not tolerate branching within the ester. Lack of activity of this family of esters in certain strains may be due to efflux mechanisms or inefficient cleavage of the ester.

Our results suggest that lipophilic esters of fosmidomycin and FR900098 improve cell activity and antibacterial activity for certain organisms. While fosmidomycin takes advantage of GlpT for cell entry, these lipophilic esters are not reliant on this transporter.[50] As *glpT* mutation is the only documented path toward fosmidomycin resistance, we expect these compounds to avoid such a resistance pathway. Dxr inhibitors optimized for both cell penetration and the enzyme's active site could provide an important tool for target validation on the road toward development of a novel therapeutic.

Acknowledgments

This work was supported by funding from the GWU Department of Chemistry, the GWU University Facilitating Fund, the Intramural Research Program of the NIAID (NIH), the American Lebanese Syrian Associated Charities (ALSAC), and NIH (AI086453 to CSD).

References and Notes

1. Payne DJ, Gwynn MN, Holmes DJ, Pompliano DL. Nat Rev Drug Discov. 2007; 6:29. [PubMed: 17159923]
2. Silver LL. Clin Microbiol Rev. 2011; 24:71. [PubMed: 21233508]
3. http://www.who.int/mediacentre/factsheets/fs104/en/index.html.
4. http://www.who.int/mediacentre/factsheets/fs194/en/index.html.
5. Eoh H, Brennan PJ, Crick DC. Tuberculosis (Edinb). 2008
6. Proteau PJ. Bioorg Chem. 2004; 32:483. [PubMed: 15530989]
7. Rohdich F, Bacher A, Eisenreich W. Bioorg Chem. 2004; 32:292. [PubMed: 15381396]
8. Rohdich F, Bacher A, Eisenreich W. Biochem Soc Trans. 2005; 33:785. [PubMed: 16042599]
9. Singh N, Cheve G, Avery MA, McCurdy CR. Current Pharmaceutical design. 2007; 13:1161. [PubMed: 17430177]
10. Testa CA, Brown MJ. Curr Pharm Biotechnol. 2003; 4:248. [PubMed: 14529427]
11. Henriksson LM, Unge T, Carlsson J, Aqvist J, Mowbray SL, Jones TA. J Biol Chem. 2007; 282:19905. [PubMed: 17491006]
12. Mac Sweeney A, Lange R, Fernandes RP, Schulz H, Dale GE, Douangamath A, Proteau PJ, Oefner C. J Mol Biol. 2005; 345:115. [PubMed: 15567415]
13. Devreux V, Wiesner J, Goeman JL, Van der Eycken J, Jomaa H, Van Calenbergh S. J Med Chem. 2006; 49:2656. [PubMed: 16610809]

14. Devreux V, Wiesner J, Jomaa H, Rozenski J, Van der Eycken J, Van Calenbergh S. J Org Chem. 2007; 72:3783. [PubMed: 17428097]
15. Devreux V, Wiesner J, Jomaa H, Van der Eycken J, Van Calenbergh S. Bioorg Med Chem Lett. 2007; 17:4920. [PubMed: 17583502]
16. Haemers T, Wiesner J, Busson R, Jomaa H, Van Calenbergh S. Eur J Org Chem. 2006:3856.
17. Haemers T, Wiesner J, Van Poecke S, Goeman J, Henschker D, Beck E, Jomaa H, Van Calenbergh S. Bioorg Med Chem Lett. 2006; 16:1888. [PubMed: 16439126]
18. Jomaa H, Wiesner J, Sanderbrand S, Altincicek B, Weidemeyer C, Hintz M, Turbachova I, Eberl M, Zeidler J, Lichtenthaler HK, Soldati D, Beck E. Science. 1999; 285:1573. [PubMed: 10477522]
19. Kuntz L, Tritsch D, Grosdemange-Billiard C, Hemmerlin A, Willem A, Bach TJ, Rohmer M. Biochem J. 2005; 386:127. [PubMed: 15473867]
20. Kurz T, Behrendt C, Kaula U, Bergmann B, Walter RD. Aust J Chem. 2007; 60:154.
21. Kurz T, Behrendt C, Pein M, Kaula U, Bergmann B, Walter RD. Arch Pharm (Weinheim). 2007; 340:661. [PubMed: 17994605]
22. Kurz T, Schluter K, Kaula U, Bergmann B, Walter RD, Geffken D. Bioorg Med Chem. 2006; 14:5121. [PubMed: 16679022]
23. Kurz T, Schluter K, Pein M, Behrendt C, Bergmann B, Walter RD. Arch Pharm (Weinheim). 2007; 340:339. [PubMed: 17611943]
24. Ortmann R, Wiesner J, Reichenberg A, Henschker D, Beck E, Jomaa H, Schlitzer M. Bioorg Med Chem Lett. 2003; 13:2163. [PubMed: 12798327]
25. Ortmann R, Wiesner J, Reichenberg A, Henschker D, Beck E, Jomaa H, Schlitzer M. Arch Pharm (Weinheim). 2005; 338:305. [PubMed: 15996004]
26. Ortmann R, Wiesner J, Silber K, Klebe G, Jomaa H, Schlitzer M. Arch Pharm (Weinheim). 2007; 340:483. [PubMed: 17806130]
27. Reichenberg A, Wiesner J, Weidemeyer C, Dreiseidler E, Sanderbrand S, Altincicek B, Beck E, Schlitzer M, Jomaa H. Bioorg Med Chem Lett. 2001; 11:833. [PubMed: 11277531]
28. Schluter K, Walter RD, Bergmann B, Kurz T. Eur J Med Chem. 2006; 41:1385. [PubMed: 17055117]
29. Schultz C. Bioorg Med Chem. 2003; 11:885. [PubMed: 12614874]
30. Wiesner J, Ortmann R, Jomaa H, Schlitzer M. Arch Pharm (Weinheim). 2007; 340:667. [PubMed: 17994601]
31. Andaloussi M, Henriksson LM, Wieckowska A, Lindh M, Bjorkelid C, Larsson AM, Suresh S, Iyer H, Srinivasa BR, Bergfors T, Unge T, Mowbray SL, Larhed M, Jones TA, Karlen A. Journal of medicinal chemistry. 2011; 54:4964. [PubMed: 21678907]
32. Deng L, Diao J, Chen P, Pujari V, Yao Y, Cheng G, Crick DC, Prasad BV, Song Y. Journal of medicinal chemistry. 2011; 54:4721. [PubMed: 21561155]
33. Kojo H, Shigi Y, Nishida M. The Journal of antibiotics. 1980; 33:44. [PubMed: 7378171]
34. Okuhara M, Kuroda Y, Goto T, Okamoto M, Terano H, Kohsaka M, Aoki H, Imanaka H. J Antibiot (Tokyo). 1980; 33:13. [PubMed: 6768704]
35. Brown AC, Parish T. BMC Microbiol. 2008; 8:78. [PubMed: 18489786]
36. Dhiman RK, Schaeffer ML, Bailey AM, Testa CA, Scherman H, Crick DC. J Bacteriol. 2005; 187:8395. [PubMed: 16321944]
37. Sakamoto Y, Furukawa S, Ogihara H, Yamasaki M. Biosci Biotechnol Biochem. 2003; 67:2030. [PubMed: 14519998]
38. Zwierzak A, Osowska K. Synthesis. 1984:223.
39. Blazewska K, Gajda T. Tetrahedron. 2003; 59:10249.
40. Haemers T, Wiesner J, Giessmann D, Verbrugghen T, Hillaert U, Ortmann R, Jomaa H, Link A, Schlitzer M, Van Calenbergh S. Bioorganic & medicinal chemistry. 2008; 16:3361. [PubMed: 18158249]
41. Villemin D, Simeon F, Decreus H, Jaffres PA. Phosphorus, Sulfur and Silicon. 1998; 133:209.
42. Fokin AA, Yurchenko AG, Rodionov VN, Gunchenko PA, Yurchenko RI, Reichenberg A, Wiesner J, Hintz M, Jomaa H, Schreiner PR. Organic letters. 2007; 9:4379. [PubMed: 17887769]

43. Ohler E, Kanzler S. Synthesis. 1995:539.

44. **21**. A solution of compound **20a** (0.29 g, 0.63 mmol) in dry EtOH (50mL) was hydrogenated over 10% Pd/C (112.3 mg) overnight. After filtration and evaporation, the crude product was purified by column chromatography using ethyl acetate to give 0.18 g (0.48 mmol, 76%) of the desired product as a light yellow viscous oil. ^{1}H-NMR ($CDCl_3$, 200MHz), δ (ppm): 0.90 (t, 6H); 1.22–1.50 (m, 12H); 1.57–2.12 (m, 8H); 2.17 (s, 3H); 3.76 (t, 2H); 3.98 (q, 4H). ESI-MS m/z 366 ([M+H]+), 388 ([M+Na]+).

45. **23**. Prepared from **20c** in 65% yield as described above. ^{1}H-NMR ($CDCl_3$, 200MHz), δ (ppm): 1.49 (s, 18H); 1.99–2.13 (m, 4H); 2.17 (s, 3H); 3.77 (t, 2H); 4.51 (d, 4H); 8.93 (bs, 1H). ESI-MS m/z 426 ([M+H]+).

46. **25**. Prepared from **20e** in 43% yield as described above. ^{1}H-NMR ($CDCl_3$, 200MHz), δ (ppm): 1.50 (s, 18H); 1.58 (d, 6H); 1.73–1.94 (m, 4H); 1.98 (s, 3H); 3.33 (q, 2H); 6.27–6.42 (m, 2H). ESI-MS m/z 470([M-CH3]+).

47. **26**. Prepared from **20f** in 59% yield as described above. ^{1}H-NMR ($CDCl_3$, 200MHz), δ (ppm): 1.85–2.10 (m, 4H); 2.15 (s, 3H); 3.62–3.77 (m, 2H); 5.88 (s, 2H); 5.94 (s, 2H); 7.40–7.67 (m, 6H); 7.98–8.20 (m, 4H). ESI-MS m/z 466 ([M+H]+).

48. Kim P, Zhang L, Manjunatha UH, Singh R, Patel S, Jiricek J, Keller TH, Boshoff HI, Barry CE 3rd, Dowd CS. Journal of medicinal chemistry. 2009; 52:1317. [PubMed: 19209889]

49. Yendapally R, Hurdle JG, Carson EI, Lee RB, Lee RE. Journal of medicinal chemistry. 2008; 51:1487. [PubMed: 18281930]

50. McKenney ES, Sargent M, Khan H, Couch RD, Uh E, Jackson ER, San Jose G, Dowd CS, Van Hoek ML. manuscript submitted.

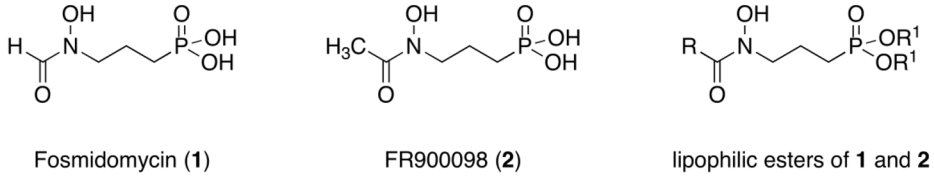
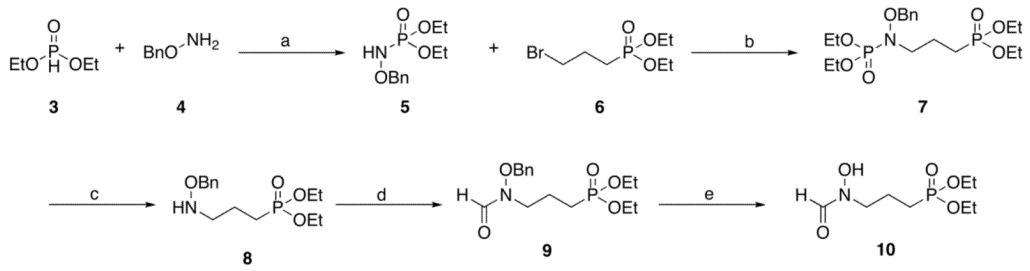
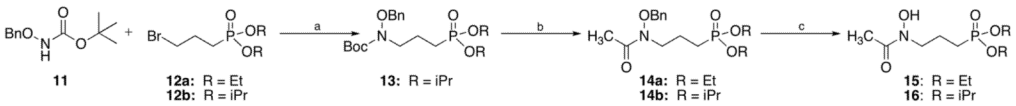


Figure 1.
Structures of parent Dxr inhibitors (**1** and **2**) and lipophilic esters.



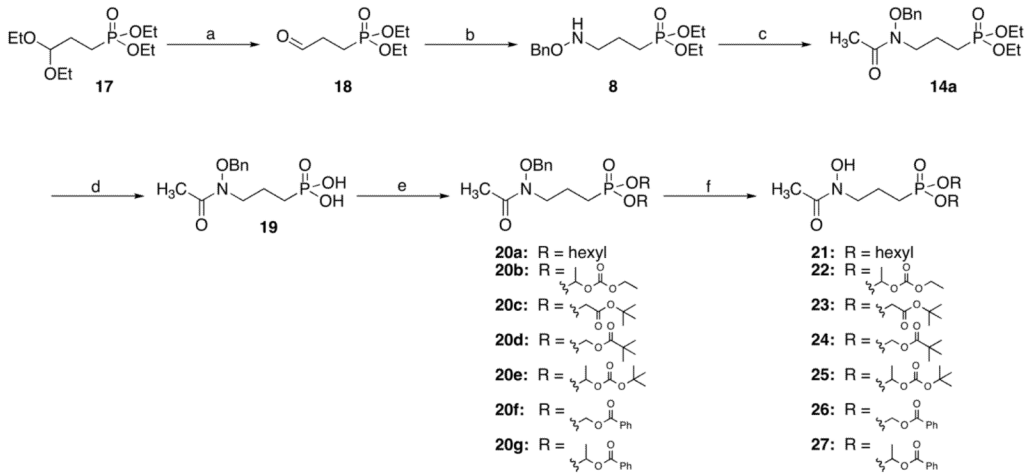
Scheme 1.

Reagents and conditions: (a) TBABr, CCl_4, $KHCO_3$, K_2CO_3, CH_2Cl_2; (b) NaH, TBABr, NaI, THF; (c) HCl, EtOH; (d) $(CH_3CO)_2O$, HCO_2H; (e) H_2, 10% Pd/C.



Scheme 2.

Reagents and conditions: (a) NaH, NaI, DMF; (b) i: TFA, CH_2Cl_2, ii: TEA, AcCl, CH_2Cl_2, 18 h; (c) H_2, 10% Pd/C, MeOH, 2 h.

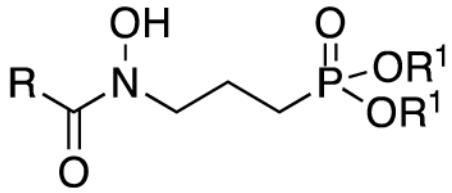


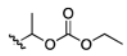
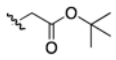
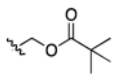
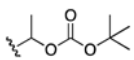
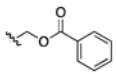
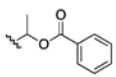
Scheme 3.

Reagents and conditions: (a) HCl, 80 °C, 1 h; (b) i: $BnONH_2$, MeOH, reflux, 1 h; ii: $NaBH_3CN$, MeOH, HCl, rt, 1 h; (c) AcCl, TEA, CH_2Cl_2, rt, 18 h; (d) i: TMSBr, CH_2Cl_2, 0 °C, 30 min; ii: H_2O, rt, 18 h; (e) RBr or RCl, TEA, DMF or DMPU, 60 °C, 6 h; (f) H_2, 10% Pd/C, MeOH.

Table 1

Antibacterial activities of compounds **1**, **2**, **10**, **15**, **16** and **21–27**.[a]



Compound	R	R[1]	Gram (+)					Gram (−)		
			B. anthracis	*E. faecalis*	*S. aureus* (MSSA)	*S. aureus* (MRSA)	*M. tuberculosis* (H37Rv)	Acineto-bacter	*E. coli* k12	*E. coli* tolc
Fosmidomycin (1)	H	H/Na[b]	0.78	>200	>200	200	>500	100	12.5	6.25
FR900098 (2)	CH_3	H/Na[b]	50	>200	>200	50	>500	50	200	12.5
10	H	Et	>200	>200	>200	>200	400	>200	>200	>200
15	CH_3	Et	200	>200	>200	200	200–400	>200	>200	>200
16	CH_3	iPr	>200	>200	>200	>200	400	>200	>200	>200
21	CH_3		ND[c]	ND	ND	ND	100	ND	ND	ND
22	CH_3		>200	>200	>200	>200	400	>200	>200	200
23	CH_3		>200	>200	100	25	400	>200	>200	>200
24	CH_3		200	>200	100	50	50–100	>200	>200	100
25	CH_3		200	>200	100	50	400	>200	>200	>200
26	CH_3		100	>200	>200	>200	25–100	200	>200	50
27	CH_3		50	>200	>200	>200	100–200	100	50	50

[a] Minimum Inhibitory Concentrations (MIC) in μg/mL.

[b] Compounds 1 and 2 used as the monosodium salts.

[c] ND = not determined.