ICAAC 2011

E-111

Comparative *In Vitro* Activity of GSK2251052, a Novel Boron Leucyl-tRNA Synthetase Inhibitor, Against 916 Anaerobic Organisms

D. M. CITRON, E. J. C. Goldstein

R. M. Alden Research Laboratory

Contact information
Diane M. Citron
R. M. Alden Research Laboratory
Culver City, CA 90230
310.641.8340
d.m.citron@rmaldenresearch.com

Abstract

Background: GSK2251052, a novel boron leucyl-tRNA synthetase inhibitor, is currently being developed for the treatment of hospital-acquired Gram-negative bacterial infections. To further define its spectrum of activity versus Gram-negative and Gram-positive anaerobes, we determined the activity of GSK2251052 versus 916 clinical anaerobic isolates.

Methods: All MICs were tested according to CLSI procedures using agar dilution except for *Bacteroides fragilis* group, which were tested using the microbroth dilution method. Isolates were primarily of intestinal origin; comparator drugs were metronidazole (MTZ), moxifloxacin (MXF), imipenem (IMI) and piperacillin-tazobactam (P-T).

Results: $MIC_{50/90}$ (µg/mL) for GSK2251052 were as follows:

Organism (n)	MIC_{50}	MIC_{90}
B. fragilis (200)	2	4
B. thetaiotaomicron (102)	4	8
B. fragilis group (63)	4	8
Prevotella spp. (50)	2	4
Porphyromonas spp. (51)	0.5	1
Bilophila wadsworthia (54)	1	1
Fusobacterium (50)	0.5	2

Organism (n)	MIC_{50}	MIC_{90}
Clostridium spp. (52)	4	>32
C. difficile (102)	4	4
Eggerthella lenta (27)	0.5	1
Eubacterium group (60)	1	2
Bifidobacterium spp. (27)	0.25	0.5
Lactobacillus spp. (27)	4	8
Anaerobic GP cocci (51)	4	8

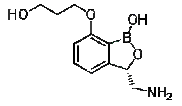
The GSK2251052 $MIC50_{/90}$ for all anaerobes was 2/4 µg/mL. Although most *Clostridium* species were inhibited by ≤4 µg/mL all *C. perfringens*, *C. cadaveris*, *C. paraputrificum*, and *C. tertium* had MIC of >32 µg/mL. A large percentage (30.6%) of *Bacteroides* were resistant to MXF, while five strains of *B. fragilis* were resistant to IMI, 3 to P-T and 2 to MTZ. There was no relationship between non-susceptibility for the comparators and activity of 052.

Conclusion: GSK2251052 had overall good *in vitro* activity against the anaerobic isolates

Introduction

GSK2251052 (formerly AN3365), a novel boron leucyl-tRNA synthetase inhibitor, is currently being developed for the treatment of hospital-acquired Gram-negative bacterial infections. GSK2251052 is active *in vitro* against Enterobacteriaceae with pre-existing resistance mechanisms including those with ESBL and KPC beta-lactamases and shows good *in vitro* activity against aerobic Gram-negative bacteria, including those found in intra-abdominal infections, but very little data exists on its *in vitro* activity against anaerobic organisms. This study determined the *in vitro* activity of GSK2251052 against 916 unique strains of a variety of clinical isolates of anaerobic organisms. Comparator antimicrobials included moxifloxacin, metronidazole, imipenem and piperacillin-tazobactam

GSK2251052 Structure



Methods

Bacterial strains: The organisms were recovered from clinical samples, mostly intra-abdominal sources, with over 75% recovered during the past 5 years, identified by standard methods (2) or by partial sequencing of the 16S gene (3) and then stored in 20% skim milk at –70oC. They were taken from the freezer and transferred at least twice on supplemented Brucella agar to ensure purity and good growth.

Broth dilution tests: Broth microdilution trays for testing the *Bacteroides fragilis* group were prepared in-house using the Quick-Spense apparatus (Sandy Spring Instrument Co. Inc., Germantown, MD.) at double strength, 50µl/well, and frozen at -70oC until used. GSK2251052 was tested in duplicate. Cell paste from 48h hour cultures was suspended in saline and further diluted in supplemented Brucella broth with addition of 50 µl to the wells to achieve a final concentration of ~1 X 106 CFU/mL. Colony counts were determined on every 10th panel.

Agar dilution tests: For testing of all organisms, other than *B. fragilis* group, supplemented Brucella agar deeps were obtained from Anaerobe Systems (Morgan Hill, CA). Defibrinated sheep blood (Hardy Diagnostics, Santa Maria, CA) was frozen and thawed to produce laked blood. On the day of testing, laked blood and the antimicrobial agents were added to the tubes of molten agar before pouring the agar dilution plates. The strains were applied to the plates using a Steers multipronged inoculator for a final concentration of approximately 105 CFU/spot. After 44h incubation at 36 °C in the anaerobic chamber incubator, the plates were examined for growth, and the MICs interpreted. The MIC was the lowest concentration that completely inhibited growth or resulted in a marked reduction of growth compared to the drug-free growth control. All testing was conducted according to procedures in the CLSI M11-A7 document (1).

Table 1. GSK2251052 MIC frequency distribution (%). Highlighted values reflect the mean MIC.

	MIC (µg/mL)									
Organism (no.)	0.125	0.25	0.5	1	2	4	8	16	32	>32
B. fragilis (200)				5.5	**76.5**	17.5	0.5			
B. thetaiotaomicron (102)					6.9	**82.4**	10.8			
B. fragilis group, other (63)				4.8	**47.6**	38.1	7.9	1.6		
Bilophila wadsworthia (54)			24.1	**70.4**	5.6					
Fusobacterium spp. (50)		**34**	24	28	8	6				
Prevotella spp. (50)			2	24	**50**	22	0	2		
Porphyromonas spp. (51)	3.9	11.8	**51**	27.5	5.9					
C. difficile (102)					37.3	**52.9**	9.8			
C. perfringens (11)										**100**
Clostridium spp., other (41)				17.1	14.6	**43.9**	4.9	4.9	2.4	12.2
Eggerthella lenta (27)		7.4	**81.5**	11.1						
Eubacterium group, other (60)		20	21.7	**41.7**	13.3	3.3				
Anaerobic gram-positive cocci (51)			9.8	15.7	21.6	**37.3**	7.8	5.9	2	
Bifidobacterium spp. (27)		**59.3**	40.7							
Lactobacillus spp. (27)			7.4	14.8	11.1	**40.7**	25.9			

Figure 1. GSK2251052 MIC frequency distribution (%) for 916 anaerobic organisms.

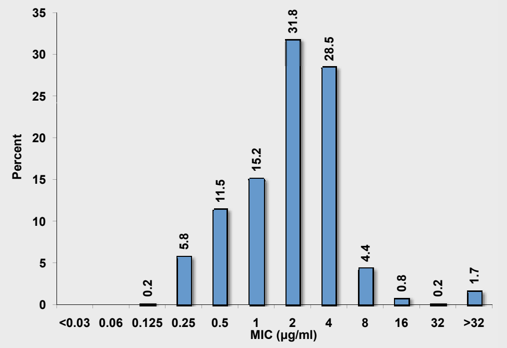


Figure 2. GSK2251052 MIC frequency distribution (%) for *B. fragilis* group organisms.

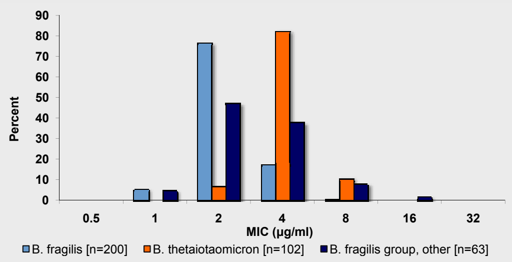


Table 2. *In vitro* activities of GSK2251052 and comparator antimicrobial agents against 916 strains of anaerobic bacteria.

Organism (No.)	Antimicrobial Agent	MIC(µg/mL) Range	MIC_{50}	MIC_{90}	% S	% R
B. fragilis (200)	GSK2251052	1–8	2	4		
	Moxifloxacin	0.125–>32	1	16	62	30.5
	Metronidazole	0.06–>32	1	4	99	1
	Imipenem	0.03–>32	0.125	1	97.5	2.5
	Piperacillin-tazobactam	≤0.015–>128	0.25	2	97	1.5
B. thetaiotaomicron (102)	GSK2251052	2–16	4	8		
	Moxifloxacin	1–>32	2	32	60.8	24.5
	Metronidazole	0.125–4	1	2	100	0
	Imipenem	0.06–2	0.25	1	100	0
	Piperacillin-tazobactam	0.25–>128	4	16	99	1
B. fragilis group, other (63) [1]	GSK2251052	1–16	4	8		
	Moxifloxacin	0.25–>32	4	32	46	39.7
	Metronidazole	0.125–4	1	1	100	0
	Imipenem	0.06–2	0.25	1	100	0
	Piperacillin-tazobactam	≤0.015–16	2	8	100	0
Bilophila wadsworth ia (54)	GSK2251052	0.5–2	1	1		
	Moxifloxacin	≤0.06–4	0.25	1	98	0
	Metronidazole	≤0.06–0.25	0.125	0.25	100	0
	Imipenem	0.125–>32	0.25	>32	61	33.3
	Piperacillin-tazobactam	16–>128	32	>128	51.8	42.6
Fusobacterium spp.(50) [2]	GSK2251052	0.25–4	0.5	2		
	Moxifloxacin	0.125–16	0.25	0.5	90	10
	Metronidazole	≤0.03–2	0.125	0.5	100	0
	Imipenem	≤0.015–0.25	0.06	0.25	100	0
	Piperacillin-tazobactam	≤0.015–0.125	0.03	0.06	100	0
Prevotella spp. (50) [3]	GSK2251052	0.5–16	2	4		
	Moxifloxacin	0.125–64	2	0.5	74	20
	Metronidazole	0.06–4	1	2	100	0
	Imipenem	≤0.015–0.25	0.06	0.125	100	0
	Piperacillin-tazobactam	≤0.015–1	≤0.015	0.06	100	0
Porphyromonas spp. (51) [4]	GSK2251052	0.125–2	0.5	1		
	Moxifloxacin	≤0.03–16	0.5	1	96	2
	Metronidazole	≤0.03–1	0.125	0.5	100	0
	Imipenem	≤0.015–0.125	0.03	0.06	100	0
	Piperacillin-tazobactam	≤0.015–0.03	<0.015	<0.015	100	0
C. difficile (102)	GSK2251052	2–8	4	4		
	Moxifloxacin	0.5–>32	1	16	67.6	30.4
	Metronidazole	0.25–2	0.5	1	100	0
	Imipenem	2–16	8	16	47.1	10.8
	Piperacillin-tazobactam	8–64	8	16	99	1
Clostridium spp. (52) [5]	GSK2251052	1–>32	4	>32		
	Moxifloxacin	0.25–32	1	8	67.3	25
	Metronidazole	≤0.03–2	0.25	2	100	0
	Imipenem	0.03–2	0.25	2	100	0
	Piperacillin-tazobactam	≤0.015–16	0.25	4	100	0
Eggerthella lenta (27)	GSK2251052	0.25–1	0.5	1		
	Moxifloxacin	0.25–>32	0.5	32	59.3	29.6
	Metronidazole	0.25–0.5	0.5	0.5	100	0
	Imipenem	0.25–0.5	0.25	0.25	100	0
	Piperacillin-tazobactam	8–32	16	32	100	0
Eubacterium group. (60) [6]	GSK225105	0.25–4	1	2		
	Moxifloxacin	0.06–8	0.5	1	96.7	1.7
	Metronidazole	0.125–4	0.5	2	100	0
	Imipenem	≤0.015–0.5	0.06	0.25	100	0
	Piperacillin-tazobactam	≤0.015–1	0.25	1	100	0
Anaerobic gram-positive cocci (51) [7]	GSK2251052	0.5–32	4	8		
	Moxifloxacin	≤0.06–16	0.5	2	90.2	5.9
	Metronidazole	0.25–2	0.5	2	100	0
	Imipenem	≤0.015–0.25	0.06	0.125	100	0
	Piperacillin-tazobactam	≤0.015–0.25	0.03	0.125	100	0
Bifidobacterium spp. (27) [8]	GSK2251052	0.25–0.5	0.25	0.5		
	Moxifloxacin	0.06–16	1	8	81.5	11.1
	Metronidazole	0.5–>32	16	>32	37	37
	Imipenem	≤0.015–2	0.06	0.5	100	0
	Piperacillin-tazobactam	≤0.015–2	0.06	1	100	0
Lactobacillus spp. (27) [9]	GSK2251052	0.5–8	4	8		
	Moxifloxacin	0.25–16	0.5	8	85.2	11.1
	Metronidazole	2–>32	>32	>32	3.7	96.3
	Imipenem	0.03–16	0.5	4	96.3	3.7
	Vancomycin	≤0.015–4	1	4	100	0

[1] *B. caccae* (8), *B. ovatus* (12), *B. uniformis* (13), *B. vulgatus* (13), *Parabacteroides distasonis* (11), *P. goldsteinii* (4), *P. merdae* (2)
[2] *F. necrophorum* (10), *F. nucleatum* (40)
[3] *P. bergensis* (2), *P. bivia* (15), *P. buccae* (9), *P. denticola* (2), *P. heparinolytica* (1), *P. intermedia*.(1), *P. loescheii* (2), *P. melaninogenica* (7), *P. nanciensis* (4), *P. oralis* (1), *P. oris* (1), *P. timonensis* (4)
[4] *P. asaccharolytica* (20), *P. endodontalis* (2), *P. gingivalis* (4), *P. gulae* (1), *P. levii* (1), *P. somerae* (17), *P. uenonis* (4)
[5] *C. aminovalericum* (1), *C. butyricum* (3), *C. cadaveris* (2), *C. celerecrescens* (2), *C. clostridioforme* (7), *C. hathewayi* (2), *C. innocuum* (10), *C. paraputrificum* (2), *C. perfringens* (11), *C. ramosum* (7), *C. scindens* (1), *C. sordellii* (1), *C. symbiosum* (2), *C. tertium* (1)
[6] *Atopobium parvulum* (4), *A. rimae* (6), *Collinsella aerofaciens* (4), *Eubacterium cylindroides* (1), *E. limosum* (13), *Mogibacterium neglectum* (1), *M. timidum* (3), *Olsenella uli* (4), *Pseudoramibacter alactolyticus* (9), *Slackia exigua* (9), *Solobacterium moorei* (6)
[7] *Anaerococcus vaginalis* (2), *Finegoldia magna* (17), *Parvimonas micra* (16), *Peptoniphilus harei* (5), *P. asaccharolyticus* (7), *Peptostreptococcus anaerobius* (3)
[8] *B. adolescentis* (6), *B. angulatum* (1), *B. animalis* (1), *B. bifidum* (3), *B. breve* (4), *B. catenulatum* (1), *B. dentium* (2), *B. longum* (7), *B. pseudocatenulatum* (1), *B. ruminatum* (1)
[9] *L. casei* (4), *L. catenaformis* (1), *L. crispatus* (3), *L. fermentans* (3), *L. gasseri/johnsonii* (5), *L. oris* (1), *L. rhamnosus* (9), *L. vaginalis* (1)

Figure 3. GSK2251052 MIC frequency distribution (%), for other Gram-negative organisms .

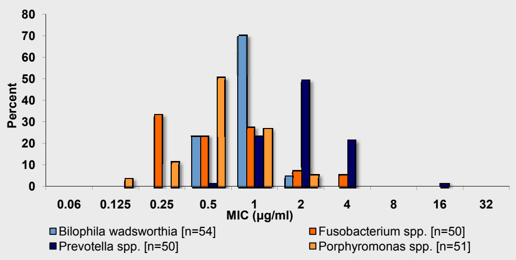


Figure 4. GSK2251052 MIC frequency distribution (%) for *Clostridium* species.

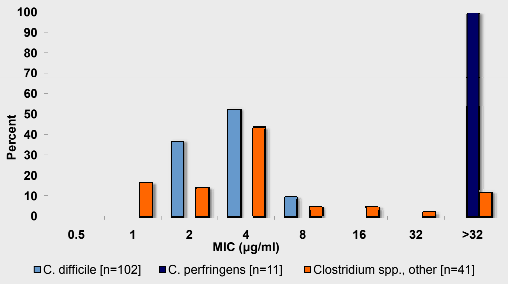
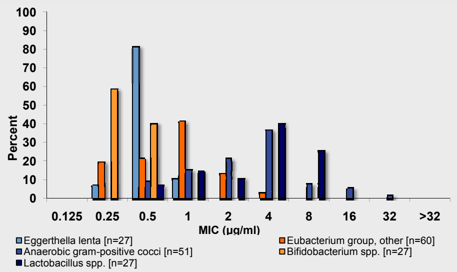


Figure 5. GSK2251052 MIC frequency distribution (%) for Gram-positive non-sporeforming species.



Results

- Table 1 shows the GSK2251052 MIC frequency distribution for the various organisms and organism groups. The mean values are highlighted.
- Table 2 shows the MIC range and 50/90 for GSK2251052 and the % susceptibility for the comparator agents
  - *B. fragilis* group
    - The GSK2251052 MIC_{90} against *B. fragilis* was 4 µg/mL .
    - A large percentage (30.5%) of *Bacteroides* were resistant to moxifloxacin at a breakpoint of ≥8µg/mL, while five strains of *B. fragilis* were also resistant to imipenem, three to piperacillin-tazobactam and two unusual isolates were resistant to metronidazole.
  - Other Gram-negative organisms
    - Among the *Prevotella* species, one strain of *P. bivia* had a GSK2251052 MIC of 16 µg/mL, while the remainder were inhibited by GSK2251052 concentrations of 1 to 4 µg/mL.
    - Resistance to moxifloxacin was common in all *Prevotella* species.
  - *Clostridium* spp.
    - Although most *Clostridium* species were inhibited by ≤4 µg/mL, all *C. perfringens*, *C. cadaveris*, *C. paraputrificum* and *C. tertium* strains had GSK2251052 MICs of >32 µg/mL.
    - Three *C. butyricum* strains had GSK2251052 MICs of 16–32 µg/mL.
    - Forty four percent of the *C. difficile* strains were the hyper- virulent REA type BI, and the GSK2251052 MIC_{90} for all *C. difficile* strains was 4 µg/mL, independent of their REA types.
  - Gram-positive non-sporeforming species
    - The GPAC species showed variable susceptibility to GSK2251052; the MIC_{90} for 16 strains of *P. micra* was 16 µg/mL, while the other species were much more susceptible.
    - The GSK2251052 MIC_{90} for the *Eubacterium* group and bifidobacteria was 2 and 0.5 µg/mL with little variation amongst the species.
  - There was no relationship between resistance to any of the comparators and the *in vitro* activity of GSK2251052.
- Figures 1-5 show the GSK2251052 MIC frequency distribution for the various organisms and organism groups.

Conclusion

GSK2251052 is a new boron-containing antibacterial agent with good *in vitro* activity against a broad spectrum of anaerobic organisms, which warrants further evaluation in clinical trials.

Acknowledgments

This study was funded by GlaxoSmithKline

References

1. **Clinical and Laboratory Standards Institute.** 2007. Methods for antimicrobial susceptibility testing of anaerobic bacteria; approved standard-seventh edition. CLSI document M11-A7. CLSI, Wayne, Pa.
2. **Jousimies-Somer, H. R., P. Summanen, D. M. Citron, E. J. Baron, H. M. Wexler, and S. M. Finegold**. 2002. Wadsworth-KTL Anaerobic Bacteriology Manual. Star Publishing, Belmont, CA.
3. **Warren, Y.A, K.L. Tyrrell, D.M. Citron, E.J.C. Goldstein**. 2006. *Clostridium aldenense* sp. nov. and *Clostridium citroniae* sp. nov. isolated from human clinical specimens. J Clin Microbiol 44:2416-2422.

51st Annual Interscience Conference on Antimicrobial Agents and Chemotherapy Meeting, 17-20 September 2011, Chicago, IL, USA