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Evaluation of Resistance Development of the Novel Antimicrobial MBN-101 Against Gram-Positive and Gram-Negative Pathogens

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Abstract

MBN-101 is a novel antimicrobial, antibiofilm agent with broad-spectrum activity against Gram-positive (GP) and Gram-negative (GN) aerobic and anaerobic bacteria, including resistant pathogens. MBN-101 is in clinical development for the topical/local treatment of diabetic foot ulcer (DFI) and orthopedic-device related infections, and in preclinical development for the treatment of pulmonary infection. In this study, resistance development of MBN-101 was evaluated in spontaneous mutation frequency (SMF) and serial passage assays with key GP and GN pathogens.

MBN-101 susceptibility testing was conducted using CLSI M7 methods. Clinical isolates tested for SMF at 4-, 8- or 16-X the MIC for MBN-101 and comparator antibiotics included 6 *S. aureus* (SA; 4 MRSA), 2 *S. pyogenes* (SP), 2 *S. agalactiae* (GBS), 4 *E. faecalis* (EF; 2 VRE), and 2 each of *E. coli* (EC), *K. pneumoniae* (KP), *P. aeruginosa* (PA), and *A. baumannii* (AB). Broth serial passage resistance development studies were conducted with 1 isolate each of EC, KP, PA, AB, and SA. Agar gradient serial passage studies were conducted with 1 isolate each of EC, PA, and SA.

MBN-101 had agar dilution MIC values of 0.06-0.25 µg/mL against SA, SP, GBS and 1-4 µg/mL against EF, EC, KP, PA and AB. Spontaneous mutation frequency values of $<10^{-9}$ – $<10^{-11}$ were recorded for MBN-101 for all isolates tested which were similar to or lower than the comparators, including vancomycin against GP and gentamicin against GN. Exposure of bacterial pathogens to 4-, 8-, or 16-X the respective MIC of MBN-101 during spontaneous mutation frequency assays generated very few colonies with low-level resistance. Further characterization revealed that a single MRSA isolate produced colonies with MIC values that were 4- to 8-X higher than the parental MIC value. Exposure of 1 isolate each of EC, KP, PA, AB, and SA through 15 broth serial passages resulted in a 4- to 8-X increase in the MBN-101 MIC for most isolates, though re-testing demonstrated either no change or at most a 4-X increase in MIC, indicating that even this low-level resistance was not stable. Finally, agar gradient serial passage yielded similar results in that a 4- to 8-X increase in the MBN-101 MIC was recorded for a single SA isolate.

Resistance to MBN-101 is difficult to develop in vitro for a broad range of GP and GN pathogens (no stable resistant mutants have been generated to date, despite repeated efforts). This is an important property for a novel antimicrobial therapeutic, especially when antibacterial resistance to current therapies is a growing concern.

Introduction

- **MBN-101 is an aqueous suspension of a novel bismuth-thiol compound (“BisEDT”) currently undergoing clinical development for the topical treatment of chronic wound and tissue infections including diabetic foot ulcer infections (DFI) and orthopedic device infections.**
- **Acute DFI and orthopedic infections typically involve Gram-positive cocci, while moderate and severe tissue infections are often polymicrobial involving Gram-negative bacilli and anaerobic bacteria.**
- **Antibiotic resistant pathogens are becoming increasingly common and pathogens (e.g. ESKAPE pathogens) for which there are limited therapeutic options have emerged.**
- **Although the emergence of resistance to any antimicrobial agent is inevitable, agents where the potential for resistance is low are preferred.**
- **As a result, the continued development of new agents with broad-spectrum activity that covers resistant pathogens and limited potential for resistance development is necessary to address treatment of complicated infections such as DFI and orthopedic device related infections.**

Objective

- **To evaluate the development of resistance *in vitro* for BisEDT (the active pharmaceutical ingredient (API) in MBN-101 aqueous suspension formulation) and comparators against target Gram-positive cocci and Gram-negative bacilli by assessing the spontaneous mutation frequency (SMF) and development of resistance during serial passage.**

Materials and Methods

- **MIC testing was conducted according to CLSI M7, using agar dilution MIC values to calculate X-fold MIC drug concentrations for SMF testing and broth microdilution testing to evaluate spontaneous mutants.**
- **For SMF, isolates were exposed to 4-, 8-, or 16-fold the MIC of BisEDT or the comparator drugs.**
- **Broth serial passage resistance development involved testing by broth microdilution according to CLSI M7, utilizing growth at the drug concentration immediately below the MIC as the inoculum for the subsequent passage.**
- **Agar serial passage was conducted by pouring Mueller-Hinton Agar plates with various BisEDT concentrations below and above the MIC. Cultures were streaked, incubated for up to 72 hr at 35ºC, and a single colony was picked for transfer to a subsequent passage with BisEDT at a slightly higher concentration.**
- **Isolates were obtained from hospital clinical microbiology laboratories or from the American Type Culture Collection.**

Results

SPONTANEOUS MUTATION FREQUENCY

- **As shown in Table 1 and 2, BisEDT had MIC values of 0.06-0.25 µg/mL against key GP pathogens and 1-4 µg/mL against GN pathogens.**
- **SMF values for BisEDT varied from $<10^{-9}$ to $<10^{-11}$, which was similar to the comparator agents (GP: vancomycin and linezolid; GN: gentamicin, cefotaxime, ciprofloxacin). As expected SMF values for rifampin were high.**
- **Broth microdilution MIC testing of putative spontaneous mutants revealed no or at most a 2-fold elevation in the BisEDT MIC, with the exception of a single *S. aureus* MMX 778 isolate where increases 4- to 8-fold above the MIC were observed.**

BROTH SERIAL PASSAGE

- **As shown in Figures 1-5, broth microdilution MIC testing was utilized to conduct serial passage studies, utilizing sub-MIC growth as the inoculum for the next passage.**
- **Study was conducted with SA, EC, KP, PA, and AB clinical isolates through 15 serial passages.**
- **During serial passage, BisEDT MIC values increased a maximum of 8-fold, and repeat broth microdilution testing of isolates from each serial passage revealed only a 4-fold increase in the BisEDT MIC.**

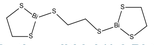
AGAR SERIAL PASSAGE

- **Since SMF and serial passage studies failed to produce BisEDT isolates with at least 8-fold elevation in MIC, agar serial passage was conducted using drug concentrations that were ≤2-fold serial dilutions, evaluating EC, PA, and SA isolates.**
- **Despite serial passage resulting in growth on agar containing up to 16-fold the MIC, broth microdilution testing of serial passaged isolates once again revealed a modest 4-8-fold increase in BisEDT MIC.**

Table 1. Spontaneous mutation frequencies for BisEDT and comparators against Gram-positive cocci

Organism	Isolate/Type	Drug	Agar MIC (µg/mL)	Mutation Frequency		
				4X MIC	8X MIC	16X MIC
S. aureus	MMX 753/ MSSA	**BisEDT**	**0.25**	**$<7.17 \times 10^{-11}$**	**$<7.17 \times 10^{-11}$**	**$<7.17 \times 10^{-11}$**
		VAN	1	8.70×10^{-10}	$<7.17 \times 10^{-11}$	1.45×10^{-10}
		RIF	0.015	TNTC	4.22×10^{-8}	2.91×10^{-8}
	MMX 2063/ MSSA	**BisEDT**	**0.25**	**$<1.14 \times 10^{-10}$**	**$<1.14 \times 10^{-10}$**	**$<1.14 \times 10^{-10}$**
		VAN	1	7.51×10^{-9}	$<1.14 \times 10^{-10}$	$<1.14 \times 10^{-10}$
		RIF	0.004	TNTC	4.52×10^{-8}	3.34×10^{-8}
	MMX 2053/ HA-MRSA	**BisEDT**	**0.12**	**3.05×10^{-9}**	**$<2.16 \times 10^{-10}$**	**$<2.16 \times 10^{-10}$**
		VAN	1	9.80×10^{-9}	$<2.16 \times 10^{-10}$	4.36×10^{-10}
		RIF	0.004	TNTC	TNTC	TNTC
	MMX 778/ HA-MSSA	**BisEDT**	**0.06**	**1.41×10^{-8}**	**$<3.45 \times 10^{-10}$**	**$<3.45 \times 10^{-10}$**
		VAN	1	$<3.45 \times 10^{-10}$	1.03×10^{-9}	$<3.45 \times 10^{-10}$
		RIF	0.004	7.24×10^{-8}	2.96×10^{-8}	3.07×10^{-8}
	MMX 2202/ CA-MRSA	**BisEDT**	**0.25**	**$<1.79 \times 10^{-10}$**	**9.06×10^{-10}**	**$<1.79 \times 10^{-10}$**
		VAN	1	1.01×10^{-8}	$<1.79 \times 10^{-10}$	$<1.79 \times 10^{-10}$
		RIF	0.004	TNTC	TNTC	TNTC
	MMX 2142/ CA- MRSA	**BisEDT**	**0.06**	**3.65×10^{-10}**	**9.97×10^{-10}**	**$<1.11 \times 10^{-10}$**
		VAN	0.5	TNTC	3.43×10^{-9}	$<1.11 \times 10^{-10}$
		RIF	0.004	TNTC	5.26×10^{-8}	4.50×10^{-8}
E. faecalis	MMX 740/ VSE	**BisEDT**	**1**	**$<5.88 \times 10^{-10}$**	**$<5.88 \times 10^{-10}$**	**$<5.88 \times 10^{-10}$**
		LZD	2	$<5.88 \times 10^{-9}$	$<5.88 \times 10^{-9}$	$<5.88 \times 10^{-9}$
		RIF	4	TNTC	1.49×10^{-7}	1.06×10^{-7}
	MMX 796/ VSE	**BisEDT**	**2**	**$<1.06 \times 10^{-9}$**	**$<1.06 \times 10^{-9}$**	**$<1.06 \times 10^{-9}$**
		LZD	4	$<4.95 \times 10^{-10}$	$<4.95 \times 10^{-10}$	$<4.95 \times 10^{-10}$
		RIF	2	TNTC	TNTC	1.16×10^{-7}
	MMX 847/ VRE	**BisEDT**	**1**	**1.35×10^{-9}**	**$<4.46 \times 10^{-10}$**	**$<4.46 \times 10^{-10}$**
		LZD	2	$<5.44 \times 10^{-10}$	$<5.44 \times 10^{-10}$	$<5.44 \times 10^{-10}$
		RIF	1	TNTC	TNTC	1.04×10^{-8}
	MMX 850/ VRE	**BisEDT**	**2**	**$<5.82 \times 10^{-10}$**	**$<5.82 \times 10^{-10}$**	**$<5.82 \times 10^{-10}$**
		LZD	1	$<5.82 \times 10^{-10}$	$<5.82 \times 10^{-10}$	$<5.82 \times 10^{-10}$
		RIF	1	TNTC	TNTC	TNTC
S. pyogenes	MMX 714	**BisEDT**	**0.12**	**$<6.33 \times 10^{-10}$**	**$<6.33 \times 10^{-10}$**	**$<6.33 \times 10^{-10}$**
		VAN	0.5	$<6.33 \times 10^{-10}$	$<6.33 \times 10^{-10}$	$<6.33 \times 10^{-10}$
		RIF	0.06	1.52×10^{-7}	8.67×10^{-8}	5.69×10^{-8}
	MMX 2570	**BisEDT**	**0.12**	**$<2.71 \times 10^{-10}$**	**$<2.71 \times 10^{-10}$**	**$<2.71 \times 10^{-10}$**
		VAN	0.25	$<2.71 \times 10^{-10}$	$<2.71 \times 10^{-10}$	$<2.71 \times 10^{-10}$
		RIF	0.06	8.08×10^{-8}	4.72×10^{-8}	3.31×10^{-8}
S. agalactiae	MMX 736	**BisEDT**	**0.12**	**$<1.74 \times 10^{-10}$**	**$<1.74 \times 10^{-10}$**	**$<1.74 \times 10^{-10}$**
		VAN	0.5	$<1.74 \times 10^{-10}$	$<1.74 \times 10^{-10}$	$<1.74 \times 10^{-10}$
		RIF	0.5	TNTC	3.94×10^{-7}	2.95×10^{-7}
	MMX 743	**BisEDT**	**0.25**	**$<1.47 \times 10^{-10}$**	**$<1.47 \times 10^{-10}$**	**$<1.47 \times 10^{-10}$**
		VAN	0.5	$<1.47 \times 10^{-10}$	$<1.47 \times 10^{-10}$	$<1.47 \times 10^{-10}$
		RIF	0.12	3.95×10^{-8}	2.17×10^{-8}	2.17×10^{-8}

MSSA: methicillin-susceptible *S. aureus*, MRSA: methicillin-resistant, HA: healthcare-associated, CA: community-associated, VSE: vancomycin-susceptible enterococci, VRE: vancomycin-resistant enterococci, TNTC: too numerous to count, VAN: vancomycin, RIF: rifampin, LZD: linezolid, MMX: Micromyx



Bismuth-1,2-ethanedithiol (1:3 Bi:thiol molar ratio)

Table 2. Spontaneous mutation frequencies for BisEDT and comparators against Gram-negative bacilli

Organism	Isolate	Drug	Agar MIC (µg/mL)	Mutation Frequency		
				4X MIC	8X MIC	16X MIC
E. coli	MMX 1394	**BisEDT**	**2**	**6.37×10^{-8}**	**3.70×10^{-10}**	**3.70×10^{-10}**
		GM	2	6.30×10^{-9}	3.70×10^{-10}	$<1.11 \times 10^{-9}$
		CTX	0.06	TNTC	TNTC	TNTC
	MMX 1396	**BisEDT**	**2**	**2.98×10^{-10}**	**$<2.98 \times 10^{-10}$**	**$<2.98 \times 10^{-10}$**
		GM	0.5	TNTC	3.66×10^{-8}	$<2.98 \times 10^{-10}$
		CTX	0.06	TNTC	TNTC	TNTC
K. pneumoniae	MMX 1339	**BisEDT**	**4**	**$<2.87 \times 10^{-10}$**	**$<2.87 \times 10^{-10}$**	**$<2.87 \times 10^{-10}$**
		GM	0.25	6.90×10^{-9}	$<2.87 \times 10^{-10}$	$<2.87 \times 10^{-10}$
		CIP	0.25	$<2.87 \times 10^{-10}$	$<2.87 \times 10^{-10}$	$<2.87 \times 10^{-10}$
	MMX 2266	**BisEDT**	**4**	**$<4.35 \times 10^{-10}$**	**$<4.35 \times 10^{-10}$**	**$<4.35 \times 10^{-10}$**
		GM	1	TNTC	3.48×10^{-9}	$<4.35 \times 10^{-10}$
P. aeruginosa	MMX 1380	**BisEDT**	**2**	**$<4.17 \times 10^{-9}$**	**$<4.17 \times 10^{-9}$**	**$<4.17 \times 10^{-9}$**
		GM	4	$<4.17 \times 10^{-9}$	$<4.17 \times 10^{-9}$	$<4.17 \times 10^{-9}$
		CIP	8	$<4.17 \times 10^{-9}$	$<4.17 \times 10^{-9}$	$<4.17 \times 10^{-9}$
	MMX 1475	**BisEDT**	**2**	**7.62×10^{-10}**	**$<1.90 \times 10^{-10}$**	**$<1.90 \times 10^{-10}$**
		GM	8	TNTC	9.37×10^{-8}	1.10×10^{-8}
		CIP	8	TNTC	1.31×10^{-7}	6.17×10^{-8}
A. baumannii	MMX 2584	**BisEDT**	**2**	**$<2.69 \times 10^{-10}$**	**$<2.69 \times 10^{-10}$**	**$<2.69 \times 10^{-10}$**
		RIF	2	TNTC	TNTC	TNTC
	MMX 2601	**BisEDT**	**1**	**7.67×10^{-9}**	**$<2.92 \times 10^{-10}$**	**$<2.92 \times 10^{-10}$**
		GM	1	2.92×10^{-9}	$<2.43 \times 10^{-10}$	$<2.43 \times 10^{-10}$
		CIP	0.25	TNTC	TNTC	$<2.43 \times 10^{-10}$

TNTC: too numerous to count, GM: gentamicin, CTX: cefotaxime, CIP: ciprofloxacin, RIF: rifampin, MMX: Micromyx

Table 3. Agar serial passage selection for BisEDT resistance: Broth MIC values of parental and passaged isolates.

Organisms	MIC (µg/mL)					
	BisEDT	Imipenem	Gentamicin	Ceftazidime	Ciprofloxacin	Tetracycline
S. aureus ATCC 29213 Parent	**0.12**	0.015	0.5	16	0.5	1
S. aureus ATCC 29213-Passaged	**1**	0.06	0.5	16	0.25	0.5
E. coli ATCC 25922 Parent	**0.5**	0.12	2	0.25	0.008	1
E. coli ATCC 25922-Passaged	**0.5**	0.06	0.5	0.5	0.008	1
P. aeruginosa ATCC 27853 Parent	**1**	2	1	8	0.25	16
P. aeruginosa ATCC 27853-Passaged	**2**	2	1	4	0.25	64

Figure 1. Serial passage of *S. aureus* MRSA MMX 3267 - broth

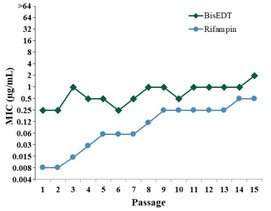


Figure 2. Serial passage of *E. coli* ATCC 25922 - broth

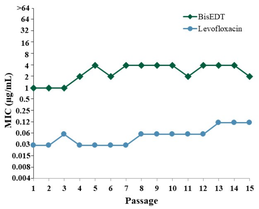


Figure 3. Serial passage of *K. pneumoniae* BAA-1705 - broth

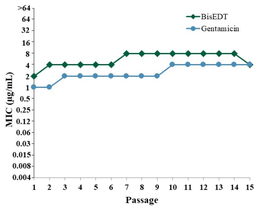


Figure 4. Serial passage of *P. aeruginosa* MMX 2556 - broth

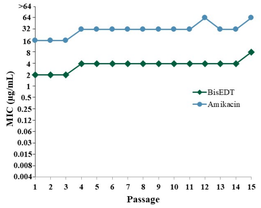
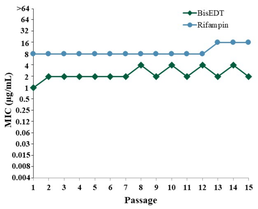


Figure 5. Serial passage of *A. baumannii* MMX 4402 - broth



Conclusions

- **BisEDT has broad spectrum activity against GP and GN pathogens**
- **SMF values are low, and very few colonies were isolated**
- **Spontaneous mutants had modest 4-8-fold increase in MIC relative to the parental strain**
- **Broth serial transfer through 15 passages with 5 different isolates also resulted in only an 8-fold elevation in the BisEDT MIC**
- **Agar serial passage studies failed to produce significantly resistant isolates**
- **BisEDT resistance is difficult to achieve with a variety of pathogens using three different methods of drug exposure.**
- **The low propensity for resistance development suggests that BisEDT formulated as MBN-101 for topical/local administration or in formulations for other modes of delivery may have long-term benefits for the treatment of infections caused by a broad spectrum of bacterial pathogens.**

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