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NOTE

Bis-imidazolinylindoles are active against methicillin-resistant *Staphylococcus aureus* and multidrug-resistant *Mycobacterium tuberculosis*

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The increasing prevalence of methicillin-resistant *Staphylococcus aureus* (MRSA)[1] and multi/extensively drug-resistant *Mycobacterium tubcerulosis* is a major global health problem.[2,3] The development of a new class of antibacterials based on a novel chemotype or mechanism of action, and with efficacy against both susceptible and resistant strains, would be an extremely useful addition to current therapeutic options.

Previously, we identified four novel bis-imidazolinylindole compounds MBX 1066, MBX 1090, MBX 1113 and MBX 1128, that represent a new antibacterial chemotype with potent activity against a broad spectrum of both Gram-positive and Gram-negative bacterial species.[4] While these compounds are rapidly bactericidal, their precise bacterial target is unknown, but studies suggest that they bind to DNA and inhibit DNA synthesis. *In vitro*, these compounds exhibit potent activity against antibiotic-resistant strains such as ciprofloxacin-resistant *B. anthracis* Ames, MRSA and vancomycin-resistant *enterococci*. Members of this class of compounds also exhibit activity *in vivo* in mouse models of lethal infection with *B. anthracis, Y. pestis* and methicillin-susceptible *S. aureus*.[4]

A particularly important feature of this new chemotype is the very low potential for development of mutations to resistance. Specifically, no mutations to resistance were observed for MBX 1066 and MBX 1113 and MBX 1162, even after 20 days of growth of *S. aureus*[4,5] and *Escherichia coli* (unpublished data) in sublethal concentrations. However, for MBX 1090, mutant clones were able to grow in concentrations up to 16× the compound MIC.[4] Interestingly, the mutants were not cross-resistant to the other members of this class of compounds and the mutations responsible for MBX 1090 resistance were demonstrated to cause elevated production of an efflux pump for which other compounds in this class are not a substrate.[5] In this study, we extended the analysis of the activities of the bis-imidazolinylindole class of antibacterials against two clinically important antibiotic-resistant pathogens.

As our previous studies demonstrated that the bis-imidazolinylindole compounds MBX 1066, MBX 1090, MBX 1113 and MBX 1128 (see Table 1 for the structures) are active against *M. smegmatis*,[4] we investigated their effect on one susceptible (H37Rv) and seven multidrug-resistant isolates of *M. tuberculosis.* Table 1 summarizes the *in vitro* activities of the four compounds. MICs ranged from <0.02 to $1.3\,\mu g\,ml^{-1}$. The compounds MBX 1066 and MBX 1113 exhibited more potent antimycobacterial activity than did MBX 1090 and MBX 1128. Next, the antimycobacterial activity of MBX 1066 was compared with its structural analog, MBX 1162 (see Table 2 for the structure), which has previously shown to exhibit potent activity against Gram-positive and Gram-negative bacteria.[6] As shown in Table 2, MBX 1162 was highly active against all the multidrug-resistant isolates of *M. tuberculosis* and compared favorably with MBX 1066. MBX 1162 was assayed for cytotoxicity against HeLa cells as described previously[4] and exhibited slightly increased toxicity ($CC_{50} = 4\,\mu g\,ml^{-1}$) compared with its parent MBX 1066 ($CC_{50} = 33\,\mu g\,ml^{-1}$).[4]

Previously, we reported that the four bis-imidazolinylindole compounds examined herein are quite active *in vitro* against MRSA, with MIC values ranging from 0.2 to $5\,\mu g\,ml^{-1}$.[4] To investigate the *in vivo* efficacy of these compounds, ICR/Swiss Webster mice ($n = 10$) were challenged via the i.p. route with *S. aureus* strain NRS384, a community-associated methicillin-resistant (cMRSA) clinical isolate. One hour post infection, mice were treated via the i.v. route with a single dose of compound MBX 1066 (1 or $10\,mg\,kg^{-1}$), MBX 1090 (1 or $10\,mg\,kg^{-1}$) or MBX 1162

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Table 1 Comparison of the MICs of bis-imidazolinylindole compounds against antibiotic-sensitive and multidrug-resistant isolates of *M. tuberculosis*

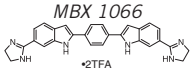
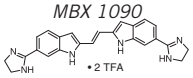
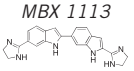
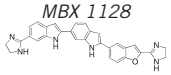
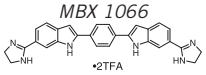
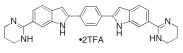
		MIC ($\mu g\,ml^{-1}$)			
		MBX 1066 	MBX 1090 	MBX 1113 	MBX 1128 
M. tuberculosis strain number	*Antibiotic resistance designation/profile*				
H37Rv	Antibiotic sensitive	<0.02	0.3	0.08	0.2
Clinical isolate 1	Isoniazid	<0.02	0.2	0.08	0.2
Clinical isolate 2	Rifampicin	<0.02	0.3	0.04	0.3
Clinical isolate 3	Isoniazid and rifampicin	0.08	0.6	0.08	0.6
Clinical isolate 4	Isoniazid, rifampicin, ethambutol	0.04	0.6	0.20	0.6
Clinical isolate 5	Isoniazid, rifampicin, capreomycin	<0.02	0.3	0.08	0.6
Clinical isolate 6	Isoniazid, rifampicin, ethambutol, streptomycin, pyrazinamide	0.04	0.3	0.02	1.3
Clinical isolate 7	Isoniazid, rifampicin, kanamycin, amikacin	0.08	1.3	0.30	1.3

Table 2 MICs of the two most-potent bis-imidazolinylindole compounds against Beijing antibiotic-sensitive and multidrug-resistant clinical isolates of *M. tuberculosis*

		MIC ($\mu g\,ml^{-1}$)	
		MBX 1066 	MBX 1162 
M. tuberculosis strain number	*Antibiotic resistance designation/profile*		
H37Rv Beijing	Antibiotic sensitive	<0.05	<0.05
Clinical isolate 1	XDR/isoniazid, rifampicin, ethambutol, kanamycin, ofloxacin, capreomycin	<0.05	<0.05
Clinical isolate 2	MDR/isoniazid, rifampicin, ethambutol, pyrazinamide, rifabutin, thiacetazone	0.05	<0.05
Clinical isolate 3	MDR/isoniazid, rifampicin, ethambutol, streptomycin, para-aminosalicylic acid	0.10	<0.05
Clinical isolate 4	MDR/isoniazid, rifampicin, ethambutol, rifabutin	0.05	<0.05
Clinical isolate 5	XDR/isoniazid, rifampicin, ethambutol streptomycin, para-aminosalicylic acid, ofloxacin	0.05	<0.05
Clinical isolate 6	MDR/isoniazid, rifampicin, ethambutol, rifabutin	0.05	<0.05
Clinical isolate 7	MDR/isoniazid, rifampicin, ethambutol, pyrazinamide, streptomycin, rifambutin, para-aminosalicylic acid	0.05	<0.05
Clinical isolate 8	MDR/isoniazid, rifampicin, ethambutol, streptomycin, para-aminosalicylic acid	0.10	<0.05
Clinical isolate 9	Antibiotic sensitive	0.10	<0.05

Abbreviations: MDR, multi drug-resistant; XDR, extensively drug-resistant.

Table 3 *In vivo* efficacy of bis-imidazolinylindole compounds against community-associated methicillin-resistant *S. aureus*

Treatment	*Dose ($mg\,kg^{-1}$)*	*% Survival (n = 10 mice per group)*	*Log reduction in CFU from untreated control*
Vehicle control	*0*	*30*	—
MBX 1066	1	90	3.9
MBX 1066	10	80	3.7
MBX 1090	1	90	3.6
MBX 1090	10	100	4.3
MBX 1162	1	90	3.5
MBX 1162	10	70	2.1
Daptomycin	10	100	4.6

(1 or 10 $mg\,kg^{-1}$). A 70–100% survival rate was observed in the compound-treated mice (Table 3). The protective effects were similar to those observed with the reference antibiotic daptomycin. Furthermore, sampling of the spleen for bacterial load revealed a 2–4 log reduction in CFU (per tissue) in each of the groups compared with the untreated control (Table 3). Thus, the compounds are active *in vivo* against cMRSA infection.

In summary, we have shown that several members of the bis-imidazolinylindole compounds exhibit very potent *in vitro* activity against both susceptible and multidrug-resistant isolates of *M. tuberculosis* as well as *in vivo* activity against cMRSA. A newer analog, MBX 1162, demonstrated equivalent potency and slightly increased mammalian cell toxicity compared with MBX 1066. Optimization of the scaffold proceeds with the goal of lessening toxicity while

maintaining potency. These compounds represent a promising new scaffold for the treatment of drug-resistant bacterial pathogens.

EXPERIMENTAL PROCEDURE

Bacterial strains

The *M. tuberculosis* clinical isolates tested in this study are maintained at an in-house repository at the Southern Research Institute (Table 1) and the National Institute of Allergy and Infectious diseases[7] (Table 2). The drug resistance profiles for each clinical isolate are listed in their respective tables. *S. aureus* NRS384, a cMRSA clinical isolate representative of the USA300 clone, was used in the *in vivo* efficacy studies. The isolate is both staphylococcal chromosomal cassette mec type IV positive and Panton–Valentine leucocidin positive. The isolate was obtained from the Network on Antimicrobial Resistance in *S. aureus* (NARSA, Herndon, VA, USA).

In vitro growth inhibition studies

MIC was determined using the broth microdilution method.[8] Briefly, compounds were dissolved in dimethyl sulfoxide and then diluted with Middlebrook 7H9 broth (BD, Sparks, MD, USA). Twofold serially diluted compounds (0.1 ml per well) were added to 96-well microtiter plates. The plates were inoculated with appropriate bacterial cultures that were standardized turbidimetrically to a concentration of 5×10^5 per well. The plates were incubated at 37 °C for 7 days. MIC was determined visually as the lowest amount of drug that completely inhibited growth and was scored by comparing the growth in each well with growth in the negative-control wells (bacteria only) and positive-control wells (isoniazid).

In vivo efficacy in the mouse model of methicillin-resistant *S. aureus* infection

Female ICR/Swiss mice (Charles River Laboratories, Wilmington, MA, USA) were challenged i.p. with 7.4×10^7 CFU of cMRSA. After 1 h, mice were treated via i.v. route with 1 or 10 mg kg^{-1} of compounds. On day 2, mice that survived challenge were euthanized and spleen harvested. Homogenized spleen was serially diluted in sterile phosphatase-buffered saline and appropriate concentrations plated on Trypticase Soy Agar plates. The number of CFU per tissue was determined by quantitative colony counts.

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