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In Vitro Activity of Benzimidazole (SPR719) Against Clinical Isolates of Nontuberculous Mycobacteria With and Without Clarithromycin or Amikacin Resistance

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Limited data are available regarding the *in vitro* activity of SPR719, a derivative of benzimidazole, against diverse nontuberculous mycobacteria (NTM) species. We investigated the minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of SPR719 against clinical NTM isolates, including clarithromycin- and amikacin-resistant strains. NTM isolates were obtained from patients with NTM-pulmonary disease caused by various NTM species, including *Mycobacterium avium* complex, *M. abscessus* (subspecies *abscessus* and *massiliense*), *M. kansasii*, and *M. fortuitum*. Regardless of clarithromycin or amikacin resistance, the MIC and MBC values of SPR719 were comparable among these major pathogenic NTM species. In over 70% of the isolates, the MIC values were ≤2 μg/mL with MBC values of ≤4 μg/mL. The MIC and MBC values of *M. kansasii* were relatively lower than those of the other species with little difference between them, demonstrating the bactericidal properties of SPR719. The *in vitro* activity of SPR719 against major clinical NTM species suggests that SPR719 can serve as a novel treatment option for NTM-pulmonary disease.

Key Words: Amikacin, Anti-bacterial agents, Benzimidazoles, Clarithromycin, Lung diseases, Microbial sensitivity tests

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The prevalence of nontuberculous mycobacteria (NTM)-pulmonary disease (PD) is increasing worldwide [1]. Major pathogens causing NTM-PD include the slowly growing mycobacteria (SGM) species *Mycobacterium avium* complex (MAC, mainly composed of *M. avium* and *M. intracellulare*) and *M. kansasii*, as well as the rapidly growing mycobacteria (RGM) species *M. abscessus* subspecies *abscessus* (hereafter, *M. abscessus*), *M. abscessus* subspecies *massiliense* (hereafter, *M. massiliense*), and *M. fortuitum* [2, 3].

Treatment guidelines for NTM-PD recommend macrolide (mainly clarithromycin)-based multidrug therapy [4, 5]. In the treatment of infections with SGM, especially MAC or *M. kansasii*, ethambutol and rifampicin can be used as adjunctive agents. For RGM, especially *M. abscessus* or *M. massiliense*, parenteral antibiotics, including amikacin, imipenem, and tigecycline, should be used for several months. RGM are usually highly drug-resistant and few drugs are effective. Clarithromycin is a cornerstone agent for NTM-PD treatment, while amikacin is crucial as a parenteral agent for NTM-PD and as salvage therapy for refractory NTD-PD caused by MAC.

Benzimidazole, a heterocyclic compound with imidazole and benzene rings, has gained attention for its biological properties in infectious disease treatment. [6]. Several benzimidazole derivatives have shown efficacy against *M. tuberculosis*, and their pharmaco-toxicological properties have been reported [7, 8]. The benzimidazole derivative SPR719 (formerly known as VXc-486) blocks mycobacterial gyrase ATPase and exhibits *in vitro* activity against some NTMs [9], but its effect on clinical NTM isolates or strains resistant to major antibiotics is unknown. We evaluated the *in vitro* activity of SPR719 against NTM clinical isolates, including clarithromycin- and amikacin-resistant isolates, from patients with NTM-PD.

We obtained 325 clinical NTM isolates from patients newly diagnosed as having NTM-PD at Samsung Medical Center, Seoul, Korea between 2010 and 2018 who did not have a history of antibiotic treatment for NTM-PD. These isolates belonged to six major species and/or taxonomic groups of pathogenic NTM (63 *M. avium*, 58 *M. intracellulare*, 58 *M. kansasii*, 65 *M. abscessus*, 67 *M. massiliense*, and 14 *M. fortuitum*). We also examined a separate set of 57 clarithromycin-resistant isolates (10 *M. avium*, 17 *M. intracellulare*, 13 *M. abscessus*, and 17 *M. massiliense*) and 44 amikacin-resistant isolates (12 *M. avium*, 17 *M. intracellulare*, 9 *M. abscessus*, and 6 *M. massiliense*), which were confirmed to have *rrl* (encoding 23S rRNA) and *rrs* (encoding 16S rRNA) mutations related to clarithromycin and amikacin resistance, respectively [10]. These drug-resistant isolates were obtained from patients who had previously been diagnosed as having NTM-PD and were treated with antibiotics or identified as having refractory NTM-PD, including infections caused by NTM strains reported in our previous studies [11-15]. To confirm clarithromycin and amikacin resistance in the clinical strains, we performed two susceptibility testing procedures. Initially, the Korean Institute of Tuberculosis (Seoul, South Korea) conducted susceptibility testing using the broth microdilution method for patient care diagnosis. Subsequently, separate susceptibility testing of SPR719 was conducted on clinical isolates in our laboratory. The isolates were recultured to obtain a single colony, and strain DNA was extracted. Finally, gene (16S rRNA, ropB, hsp65) sequencing was used for identification, and the isolates were preserved for research purposes. All data were obtained from an Institutional Review Board-approved observational cohort study performed at Samsung Medical Center (ClinicalTrials.gov identifier: NCT00970801).

In vitro susceptibility testing of SPR719 was performed using the broth microdilution method according to the CLSI guidelines [16, 17]. Cation-adjusted Mueller–Hinton broth (CAMHB) (Difco Laboratories, Detroit, MI, USA) was used for RGM; CAMHB with 5% oleic–albumin–dextrose–catalase (CAMHB-OADC) was used for SGM. SPR719 dissolved in dimethyl sulfoxide was serial-diluted and dispensed into CAMHB or CAMHB-OADC in 96-well plates; a 0.5-McFarland standard bacterial suspension was diluted 1:200 and inoculated in the same way.

The minimum inhibitory concentration (MIC) was determined after incubation in a 36°C, 75%–80% humidity incubator; RGM isolates were incubated for 3–5 days, whereas SGM isolates were incubated for >10 days. After MIC measurement, aliquots above the MIC were taken from the 96-well plates and inoculated on 7H10 agar without SPR719, followed by incubation of RGM (3–4 days) and SGM (≥10 days). After incubation, the minimum bactericidal concentration (MBC) was determined, using M. peregrinum ATCC 700686, *M. abscessus* ATCC 19977, *M. avium* ATCC 700898, and *M. kansasii* ATCC 12478 as controls.

The *in vitro* activity of SPR719 against the 325 treatment-naive NTM isolates is summarized in Table 1, including the MIC range, minimum concentration required to inhibit 50% of the bacteria (MIC_{50}), minimum concentration to inhibit 90% of bacteria (MIC_{90}), MBC range, minimum concentration to kill 50% of the bacteria (MBC_{50}), and minimum concentration to kill 90% of the bacteria (MBC_{90}) analyzed according to NTM species, including SGM (*M. avium*, *M. intracellulare*, and *M. kansasii*) and RGM (*M. abscessus*, *M. massiliense*, and *M. fortuitum*) (Table 1).

The MIC of SPR719 was ≤2 μg/mL in 70% of clinical *M. avium* isolates. Both the MIC and MBC ranged from 0.125 to 16 μg/mL. The MIC_{90} and MBC_{90} were 4 and 16 μg/mL, respectively, which were comparable to the values obtained with *M. intracellulare*. The MIC of SPR719 was ≤1 μg/mL for most of the *M. kansasii* isolates (95%). The MIC and MBC of SPR719 for *M. kansasii* ranged from 0.031 to 16 μg/mL; the MIC_{90} and MBC_{90} were 0.25 and 0.5 μg/mL, respectively, and thus much lower than the values determined for the MAC isolates.

The MIC of SPR719 was ≤2 μg/mL in 72% of clinical *M. abscessus* isolates. The MIC and MBC ranges were 0.125–8 and 0.5–16 μg/mL, and the MIC_{90} and MBC_{90} were 4 and 8 μg/mL, respectively. The MIC of SPR719 for most *M. massiliense* isolates (95%) was also ≤2 μg/mL; the MIC_{90} and MBC_{90} were 2 and 4 μg/mL, respectively, and thus lower than the respective values against the *M. abscessus* isolates. The MIC of SPR719 for 64% of the *M. fortuitum* isolates was ≤2 μg/mL. The MIC range, MBC range, MIC_{90}, and MBC_{90} were comparable to the values determined for *M. abscessus*.

The *in vitro* activity results of SPR719 against clarithromycin-resistant SGM and RGM isolates (N=57) are shown in Table 2.

Table 1. MIC and MBC data for SPR719 against treatment-naive clinical NTM isolates (N=325)

NTM species	N of isolates with indicated MIC (μg/mL)											MIC range (μg/mL)	MIC_{50} (μg/mL)	MIC_{90} (μg/mL)
	0.031	0.062	0.125	0.25	0.5	1	2	4	8	16	>16			
Mycobacterium avium (N=63)			1 (2)			10 (16)	33 (52)	14 (22)		2 (3)	3 (5)	0.125–16	2	4
M. intracellulare (N=58)				1 (2)	11 (19)	32 (55)	5 (9)	7 (12)	2 (3)			0.25–8	1	4
M. kansasii (N=58)	16 (28)	30 (52)	4 (7)	2 (3)	2 (3)	1 (2)		2 (3)			1 (2)	0.031–16	≤0.062	0.25
M. abscessus (N=65)*			1 (2)		2 (3)	15 (23)	29 (44)	16 (25)	2 (3)			0.125–8	2	4
M. massiliense (N=67)		6 (9)		10 (15)	17 (25)	21 (31)	10 (15)	3 (5)				0.062–4	1	2
M. fortuitum (N=14)				1 (7)	1 (7)	4 (29)	3 (21)	3 (21)	2 (15)			0.25–8	2	8
	N of isolates with indicated MBC (μg/mL)											MBC range (μg/mL)	MBC_{50} (μg/mL)	MBC_{90} (μg/mL)
	0.031	0.062	0.125	0.25	0.5	1	2	4	8	16	>16			
M. avium (N=63)			1 (2)			8 (13)	32 (50)	11 (18)	3 (5)	4 (6)	4 (6)	0.125–16	2	16
M. intracellulare (N=58)				1 (2)	3 (5)	18 (31)	23 (39)	5 (9)	3 (5)	5 (9)		0.25–16	2	8
M. kansasii (N=58)	11 (19)	24 (41)	12 (20)	4 (7)	3 (5)	1 (2)			1 (2)	1 (2)	1 (2)	0.031–16	0.062	0.5
M. abscessus (N=65)*					1 (2)	4 (6)	19 (29)	31 (47)	9 (14)	1 (2)		0.5–16	4	8
M. massiliense (N=67)		1 (2)	1 (2)		3 (4)	16 (23)	30 (45)	15 (22)	1 (2)			0.062–8	2	4
M. fortuitum (N=14)					2 (14)	2 (14)	4 (29)	1 (7)	5 (36)			0.5–8	2	8

Data are presented as number (%). *42 of 65 *M. abscessus* isolates showed inducible resistance in phenotypic drug susceptibility testing.
Abbreviations: MIC, minimum inhibitory concentration; MIC_{50}, minimum concentration required to inhibit 50% of bacteria; MIC_{90}, minimum concentration to inhibit 90% of bacteria; MBC, minimum bactericidal concentration; MBC_{50}, minimum concentration required to kill 50% of bacteria; MBC_{90}, minimum concentration required to kill 90% of bacteria; NTM, nontuberculous mycobacteria.

The MIC of SPR719 was ≤2 μg/mL for 80% of clarithromycin-resistant *M. avium* isolates. The MIC and MBC of SPR719 ranged from 0.5 to 8 μg/mL; both the MIC_{90} and MBC_{90} were 8 μg/mL. These values were roughly comparable to those determined for *M. intracellulare*. The MIC of SPR719 was also ≤2 μg/mL for 85% of the clinical isolates of clarithromycin-resistant *M. abscessus*. The MIC and MBC ranges were 0.062–4 and 2–8 μg/mL, and the MIC_{90} and MBC_{90} were 4 and 8 μg/mL, respectively. The MIC of SPR719 was ≤2 μg/mL for 88% of clarithromycin-resistant *M. massiliense* isolates, and the MIC range and MIC_{90} were the same as determined in *M. abscessus*. The MBC range and MBC_{90} were 0.25–4 and 4 μg/mL, respectively, and thus slightly lower than the values determined in *M. abscessus* (Table 2).

The SPR719 *in vitro* activity against 44 amikacin-resistant SGM and RGM strains is also shown in Table 2. The MIC of SPR719 was ≤2 μg/mL for 92% of the amikacin-resistant *M. avium* isolates. Both the MIC and MBC ranged from 1 to 8 μg/mL. The MIC_{90} and MBC_{90} were 2 and 4 μg/mL, respectively, which were roughly comparable to the values determined for *M. intracellulare*. The MIC of SPR719 was ≤2 μg/mL for 89% of the clinical amikacin-resistant *M. abscessus* isolates. The MIC and MBC ranges were 1–4 and 2–8 μg/mL, and the MIC_{90} and MBC_{90} were 4 and 8 μg/mL, respectively. The MIC of SPR719 was ≤2 μg/mL for all amikacin-resistant *M. massiliense* isolates. The MIC and MBC ranges were 0.5–2 and 1–2 μg/mL, respectively, and both the MIC_{90} and MBC_{90} were 2 μg/mL, which was slightly lower than the values determined for *M. abscessus*.

Overall, our results show that the MIC and MBC of SPR719 are similar for the major pathogenic NTM species, including clarithromycin- and amikacin-resistant clinical isolates from patients newly diagnosed as having NTM-PD without prior antibiotic exposure. The exception was *M. kansasii*, as the MIC and MBC values of SPR719 were lower for these isolates than for other species and were quite similar among isolates within the species, demonstrating the bactericidal properties of SPR719. The MIC_{90} and MBC_{90} of SPR719 against *M. kansasii* were very low (8- to 32-fold lower than those obtained with the other tested NTM species, respectively), indicating the potential of SPR719 as a new therapeutic agent for NTM-PD caused by *M. kansasii*. There was also little difference between the MIC and MBC values of SPR719 against the other tested NTM species, including clarithromycin- and amikacin-resistant strains. The comparable *in vitro* activity of SPR719 for SGM and RGM, regardless of drug-resistance status, suggests that this drug could be used in the treatment of NTM-PD caused by several different NTM species. However, additional research focusing on clinical applications is

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Table 2. MIC and MBC data for SPR719 against clarithromycin-resistant and amikacin-resistant clinical NTM isolates

NTM species	N of isolates with indicated MIC (μg/mL)											MIC range (μg/mL)	MIC_{50} (μg/mL)	MIC_{90} (μg/mL)
	0.031	0.062	0.125	0.25	0.5	1	2	4	8	16	>16			
Clarithromycin-resistant isolates (N=57)														
Mycobacterium avium (N=10)					3 (30)	2 (20)	3 (30)	1 (10)	1 (10)			0.5–8	1	8
M. intracellulare (N=17)		1 (6)			3 (18)	6 (35)	3 (17)	2 (12)	2 (12)			0.062–8	1	8
M. abscessus (N=13)		1 (8)			2 (15)	6 (47)	2 (15)	2 (15)				0.062–4	1	4
M. massiliense (N=17)		2 (12)	1 (6)	4 (23)	4 (23)	2 (12)	2 (12)	2 (12)				0.062–4	0.5	4
Amikacin-resistant isolates (N=44)														
M. avium (N=12)						7 (59)	4 (33)		1 (8)			1–8	1	2
M. intracellulare (N=17)					4 (24)	6 (35)	5 (29)	2 (12)				0.5–4	1	4
M. abscessus (N=9)						1 (11)	7 (78)	1 (11)				1–4	2	4
M. massiliense (N=6)					3 (50)	2 (33)	1 (17)					0.5–2	0.5	2
	N of isolates with indicated MBC (μg/mL)											MBC range (μg/mL)	MBC_{50} (μg/mL)	MBC_{90} (μg/mL)
	0.031	0.062	0.125	0.25	0.5	1	2	4	8	16	>16			
Clarithromycin-resistant isolates (N=57)														
M. avium (N=10)					2 (20)	2 (20)	4 (40)	1 (10)	1 (10)			0.5–8	2	8
M. intracellulare (N=17)				1 (6)	2 (12)	5 (29)	5 (29)	2 (12)	1 (6)	1 (6)		0.25–16	1	8
M. abscessus (N=13)							3 (23)	6 (46)	4 (31)			2–8	4	8
M. massiliense (N=17)				4 (23)	2 (12)	3 (18)	3 (18)	5 (29)				0.25–4	1	4
Amikacin-resistant isolates (N=44)														
M. avium (N=12)						6 (50)	4 (34)	1 (8)	1 (8)			1–8	1	4
M. intracellulare (N=17)					1 (6)	6 (35)	7 (41)	1 (6)	2 (12)			0.5–8	2	8
M. abscessus (N=9)							1 (11)	7 (78)	1 (11)			2–8	4	8
M. massiliense (N=6)						4 (67)	2 (33)					1–2	1	2

Data are presented as number (%).
Abbreviations: MIC, minimum inhibitory concentration; MIC_{50}, minimum concentration required to inhibit 50% of bacteria; MIC_{90}, minimum concentration to inhibit 90% of bacteria; MBC, minimum bactericidal concentration; MBC_{50}, minimum concentration required to kill 50% of bacteria; MBC_{90}, minimum concentration required to kill 90% of bacteria; NTM, nontuberculous mycobacteria.

crucial as our study was solely based on *in vitro* results.

Most *M. abscessus* strains have inducible resistance to macrolide antibiotics due to a specific sequence in the erm41 gene region, resulting in high-level resistance after 14 days, according to *in vitro* activity measurements [10]. Although most (65%, 42/65) of the treatment-naïve *M. abscessus* strains exhibited clarithromycin-induced resistance, the lack of a significant difference between the SPR719 MIC and MBC values suggests the possibility of some bactericidal effect. However, it is necessary to evaluate the efficacy of SPR719 by analyzing a large number of *M. abscessus* strains with induced resistance to clarithromycin.

Our results are in line with a recent study showing the effectiveness of SPR719 against NTM *in vitro* [18]. However, we analyzed only a few important SGM and RGM strains and not many other strains. Another study reported the *in vitro* activity of SPR719 against other NTM species such as *M. ulcerans*, *M. marinum*, and *M. chimaera*, with MIC values ranging from 0.125 to 4 μg/mL [19]. A first-in-human phase 1 study of SPR720 (a phosphate prodrug of SPR719) was conducted in 2021, which evaluated the safety, tolerability, and pharmacokinetics of SPR720/SPR719 [20]. The results demonstrated that once-daily dosing of SPR720 was well-tolerated and had acceptable pharmacokinetics, supporting its use to treat NTM-PD. Our results and those of other studies thus support the use of SPR719 as a therapeutic agent in the treatment of NTM-PD, especially for infections caused by *M. kansasii*, although further confirmatory testing is warranted.

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AUTHOR CONTRIBUTIONS

Kim DH, Kim SY, and Jhun BW contributed to the study conception and design; Kim DH and Kim SY were responsible for conducting the experiments; Kim DH, Zo S, and Jhun BW drafted the manuscript; and Jhun BW supervised the study. All authors read and approved the final manuscript.

CONFLICTS OF INTEREST

None declared.

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