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ORIGINAL RESEARCH

In vitro Activity of the Novel Tetracyclines Derivative, Zifanocycline Against *Mycobacterium abscessus*

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Objective: This study aimed to evaluate the in vitro antibacterial activity of zifanocycline against *Mycobacterium abscessus*.

Methods: From 2019 to 2024, *Mycobacterium abscessus* isolates from the respiratory tract of patients were collected at a tertiary hospital in Jiaxing City. All isolates were identified for species, subtypes, and the erm 41 gene polymorphism using whole-genome sequencing (WGS). Susceptibility to zifanocycline and 13 comparators was tested using the broth microdilution method, and the combined effects of zifanocycline and seven antibacterial drugs were evaluated.

Results: A total of 67 strains of *Mycobacterium abscessus* were collected and subjected to whole-genome sequencing. Genomic analysis identified 60 strains of *Mycobacterium abscessus* subsp. *abscessus* and 7 strains of *Mycobacterium abscessus* subsp. *massiliense*. Among the *Mycobacterium abscessus* subsp. *abscessus* strains, 57 exhibited the erm41 T28 genotype, whereas the remaining three showed the erm41 C28 genotype. In our study, zifanocycline (MIC_{50}/MIC_{90}, 0.06/0.25 mg/L) demonstrated a 2-fold lower MIC_{50} and MIC_{90} compared to tigecycline (MIC_{50}/MIC_{90}, 0.12/0.5 mg/L), and a 4-fold and 2-fold lower MIC_{50} and MIC_{90}, respectively, compared to omadacycline (MIC_{50}/MIC_{90}, 0.25/0.5 mg/L). In addition to amikacin and linezolid, zifanocycline demonstrated significantly superior antibacterial activity compared with ciprofloxacin, moxifloxacin, trimethoprim–sulfamethoxazole, cefoxitin, doxycycline, imipenem, and clarithromycin. The combination of zifanocycline and the seven antibacterial drugs used for the treatment of *Mycobacterium abscessus* showed no significant interactions.

Conclusion: Zifanocycline exhibits positive antibacterial activity against *Mycobacterium abscessus* in vitro and has potential as an alternative agent in multidrug combination therapy regimens for the treatment of this pathogen.

Keywords: *Mycobacterium abscessus*, in vitro, tetracycline, zifanocycline

Introduction

M. abscessus is a non-tuberculous mycobacterium that can be classified into three subspecies: *M. abscessus* subsp. *abscessus, M. abscessus* subsp. *bolletii*, and *M. abscessus* subsp. *massiliense*.[1] There is a growing report of *M. abscessus* infections occurring in populations with cystic fibrosis, as well as those without it, around the world.[2–4] *M. abscessus* exhibits a high level of intrinsic resistance to most classes of antibiotics, rendering the infections often difficult to treat, and displays poor tolerance to the limited available antibiotic options.[5] A comprehensive analysis of results from treatments for pulmonary disease caused by *M. abscessus* showed that the cure rate was merely 45%, with *M. abscessus* subsp. *massiliense* (57%), demonstrating a higher cure rate than *M. abscessus* subsp. *abscessus* (33%).[6] Consequently, the threat posed by *M. abscessus* to human health is escalating, highlighting the urgent need for the discovery or development of new anti-infective agents.

Zifanocycline represents a significant advancement as a broad-spectrum third-generation tetracycline that is specifically classified as aminomethylcycline. This antibacterial agent is currently in the developmental phase to address several critical medical conditions, including community-acquired bacterial pneumonia (CAP), acute bacterial skin and skin

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structure infections (ABSSSI), and complicated intra-abdominal infections (cIAI).[7,8] Zifanocycline effectively overcomes many common tetracycline resistance mechanisms by inhibiting the normal functioning of bacterial ribosomes. The 3-methyl-3-azabicyclo[3.1.0]hexane side chain that is unique to KBP-7072 could play a role in overcoming some forms of tetracycline resistance, comparable to that of Tigecycline´s C9 extension, which restricts the access of ribosomal protection proteins (RPP), like Tet(M), to the primary tetracycline binding site.[9] The unique C9 extension of this compound results in a specific interaction pattern with the 30S ribosomal subunit and the ability to form direct, stable interactions with the phosphate groups of 16S rRNA, setting it apart from the third-generation tetracycline such as tigecycline.[9,10] This differentiation not only highlights the unique structural features of the compound but also serves to expand the range of potential interactions within the primary tetracycline-binding pocket.[10] Such distinct interactions could lead to variations in the efficacy and development of novel mechanisms of action against bacterial targets, which could enhance the therapeutic application of this compound in the treatment of antibiotic-resistant infections.

Zifanocycline has completed Phase 1 clinical development as of December 2015, which evaluated its safety, tolerability, pharmacokinetics, and multiple ascending doses in healthy subjects.[11,12] The pharmacokinetic/pharmacodynamic (PK/PD) index, particularly the area under the curve of concentration over time (AUC) the minimum inhibitory concentration (MIC), has a strong correlation with effectiveness.[13] The pharmacokinetic results obtained from animal studies align with the findings from both single- and multiple-ascending-dose trials conducted with healthy subjects, thereby confirming the effectiveness of zifanocycline for once-daily administration via oral and intravenous routes in clinical investigations.[14,15] However, the antibacterial activity of zifanocycline against *M. abscessus* has not been systematically evaluated. In this study, we evaluated the in vitro efficacy of zifanocycline and 13 other antimicrobial compounds against 67 unique clinical isolates of *M. abscessus* that were sourced from the Affiliated Hospital of Jiaxing University between 2019 and 2024.

Materials and Methods

Collection and Culture Conditions of M. abscess Isolates

The Affiliated Hospital of Jiaxing University serves as the designated tuberculosis hospital in the region. This study involved sputum and bronchoalveolar lavage fluid samples from patients exhibiting clinical symptoms of lower respiratory tract infections who visited the hospital between 2019 and 2024. Each sample was incubated at 37°C in Middlebrook 7H10 broth enriched with 10% oleic acid-albumin-dextrose-catalase (Becton Dickinson and Company, USA). Non-tuberculous mycobacteria (NTM)-positive cultures were identified using the MODI-TOF MS mass spectrometer (Mérieux, France) and were preliminarily classified as strains of *M. abscessus*. A total of 67 strains of *M. abscessus* were collected for this study, with duplicate strains (ie, multiple strains from the same patient) excluded. These isolates were obtained through standard diagnostic tests and patient care processes and were not specifically isolated for this study, thus exempting the need for additional patient-informed consent. This study has received approval from the Institutional Ethics Committee of the Jiaxing University Affiliated Hospital (Ethics Approval Number: 2024-LY-400).

Identification of M. abscessus Subspecies and Determination of the erm41 Gene Polymorphism

Genomic DNA was extracted from positive cultures of M. abscessus strains using a magnetic bead-based bacterial genomic DNA extraction kit (Sangon Biotech, Shanghai, China). Whole-genome sequencing was conducted using the same 150-bp paired-end Illumina NovaSeq X Plus platform. The sequence data were subjected to de novo assembly using SPAdes software,[16] which is specifically designed for assembling short sequencing reads into longer contigs. ANI Calculator (https://www.ezbiocloud.net/tools/ani) was used to determine Average Nucleotide Identity (ANI) values. For the ANI comparison, the reference genomes included *M. abscessus subsp. massiliense* strain GO 06 (GenBank: GCA_000277775.2), *M. abscessus subsp. bolletii* BD (GenBank: GCA_003609715.1), and *M. abscessus subsp. abscessus* ATCC 19977 (GenBank: GCA_000069185.1). Typically, the ANI value for the same species is >99%. The ResFinder database was used to analyze *M. abscessus*, leading to the identification of erm41.[17] Using the erm41 gene sequence from *M. abscessus* subsp. *abscessus* ATCC 19977 as a reference, all strains were aligned with SnapGene software to determine the C/T polymorphism at the 28th nucleotide of the erm41 gene.

Antimicrobial Susceptibility Tests

Susceptibility testing for the drug was performed according to the guidelines outlined in the 2018 CLSI M24 document.[18] Single colonies of *M. abscessus* were selected from M7H10 agar plates, diluted to a McFarland standard of 0.5 using sterile saline, and further adjusted to a bacterial burden of 1×10^5 to 5×10^5 CFU/mL with cation-adjusted Mueller-Hinton broth (CAMHB). Amikacin, moxifloxacin, linezolid, bedaquiline, and clofazimine were purchased from Adooq Biosciences (Adooq Bioscience, Nanjing, China). Ciprofloxacin, trimethoprim–sulfamethoxazole, cefoxitin, tigecycline, omadacycline, zifanocycline, doxycycline, imipenem, and clarithromycin were purchased from MedChemExpress (MCE, Shanghai, China). Zifanocycline was dissolved and diluted with deionized water. Bedaquiline and clofazimine were first solubilized with dimethyl sulfoxide (DMSO) and then diluted with deionized water. For other drugs, dissolution and dilution were performed in accordance with the operating procedures specified in CLSI M100.[19] All antibacterial agents were serially diluted in a 1:2 ratio in a 96-well microtiter plate using CAMHB, and 100 μL of the bacterial suspension in CAMHB was added to each well. The final concentrations ranged from 0.015 to 32 mg/L for zifanocycline, tigecycline, omadacycline, bedaquiline, clofazimine, and trimethoprim-sulfamethoxazole; 0.03 to 64 mg/L for doxycycline, moxifloxacin, ciprofloxacin, and clarithromycin; 0.06 to 128 mg/L for amikacin, linezolid, and imipenem; and 0.12 to 256 mg/L for cefoxitin. The drug-susceptible plates were incubated at 37°C for 3–4 days until noticeable growth was observed in the control wells. In this study, *Mycobacterium smegmatis* strain ATCC 700686 and *Staphylococcus aureus* strain ATCC 29213 were used as reference strains to ensure the quality of the tested pharmaceutical products. The MICs of clarithromycin were evaluated after incubation periods of 3 and 14 days to determine the presence of inducible resistance to clarithromycin. *M. abscessus* ATCC 19977 was used as the reference strain for MIC comparison. Quality control and result interpretation for all drugs were conducted according to breakpoints established in the 2018 CLSI M62 guidelines.[20]

Synergistic Antimicrobial Susceptibility Tests

The in vitro combined effects of zifanocycline with seven commonly used antimycobacterial drugs—namely, clarithromycin, cefoxitin, linezolid, bedaquiline, amikacin, imipenem, and moxifloxacin—were evaluated using the broth microdilution checkerboard assay. This study utilized five clinical isolates of *M. abscessus* that were chosen randomly. A 96-well sterile microplate was used, with each antimicrobial agent starting at twice the MIC and serially diluted in sterile CAMHB, typically across eight dilutions. Fifty microliters of each dilution was arranged in rows and columns of the plate. Subsequently, 100 μL of the bacterial suspension was carefully added to a sterile microplate to ensure a final concentration of 5×10^5 CFU/mL. The microplate was then incubated for 72 h. At the end of the incubation period, the MIC was determined as the concentration that successfully inhibited bacterial growth. The fractional inhibitory concentration index (FICI) was calculated based on the difference in MIC values before and after drug combination. The formula for FICI was expressed as follows: FICI = [(MIC of zifanocycline used in combination/MIC of zifanocycline alone) + (MIC of the second antibiotic used in combination/MIC of the second antibiotic when used alone)]. If the FICI is ≤0.5, synergy is indicated; if it falls between 0.5 and 4, it is considered indifference; and if it is greater than 4, it suggests antagonism.

Statistical Analysis

All data were presented using Microsoft Excel 2021. Descriptive statistical methods were used to calculate the resistance rates and related variables of *Mycobacterium abscessus* (*M. abscessus*) isolates. The results were expressed as counts and percentages. All statistical analyses were performed using SPSS Version 20 (IBM Corp., Armonk, NY, USA).

Result

In vitro Activity of Zifanocycline and Comparators Against 67 M. abscessus Isolates

Zifanocycline exhibited potent antibacterial activity against 67 isolates of *M. abscessus*, with MIC_{50} as 0.06 mg/L and MIC_{90} as 0.25 mg/L, respectively. At a concentration of 0.5 mg/L, zifanocycline inhibited the growth of all tested isolates (Figure 1).

In comparison to other broad-spectrum tetracycline antibiotics, zifanocycline (MIC_{90}, 0.25 mg/L) demonstrated a 2-fold lower MIC_{90} than both tigecycline (MIC_{90}, 0.5 mg/L) and omadacycline (MIC_{90}, 0.5 mg/L). Additionally, the

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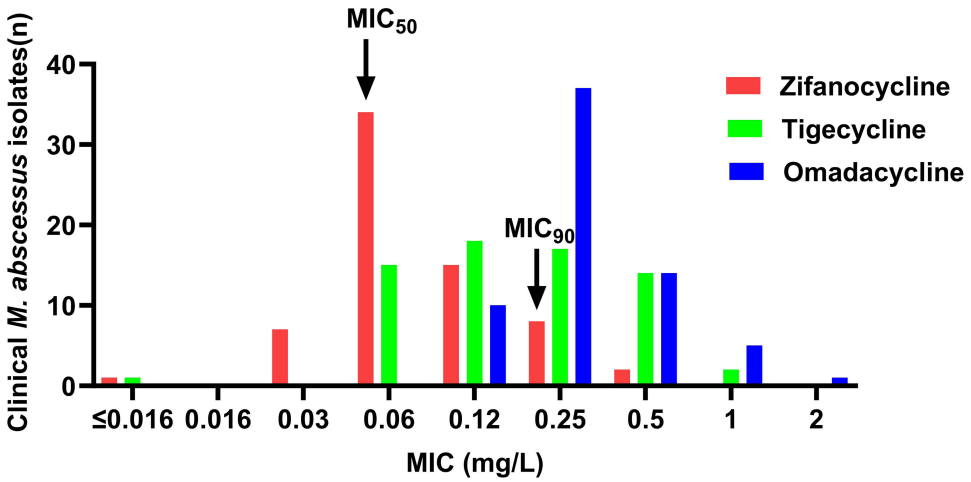

Figure 1 MIC distribution of zifanocycline, tigecycline, and omadacycline for 67 *M. abscessus* isolates. The column indicated by the MIC_{50} arrow shows the MIC_{50} result for zifanocycline, and the column indicated by the MIC_{90} arrow shows the MIC_{90} result for zifanocycline.

MIC_{90} values for both bedaquiline and clofazimine against *M. abscessus* isolates were found to be 0.5 mg/L, indicating antibacterial activity comparable to that of tigecycline and omadacycline. Amikacin (100% susceptibility) and linezolid (100% susceptibility) exhibited excellent antibacterial activity against *M. abscessus* isolates in vitro with MIC_{50} and MIC_{90} values of 2 mg/L, 4 mg/L, and 2 mg/L, 8 mg/L, respectively. The tested isolates demonstrated moderate susceptibility to cefoxitin (52.24%susceptibility), according to CLSI breakpoints. However, ciprofloxacin (4.48% susceptibility), moxifloxacin (25.37% susceptibility), trimethoprim-sulfamethoxazole (35.82% susceptibility), doxycycline (2.99% susceptibility), and imipenem (0% susceptibility) displayed comparatively low antibacterial activities against the tested isolates based on CLSI breakpoints (Table 1).

The MIC_{50} and MIC_{90} of zifanocycline for 57 isolates from the *M. abscessus* subsp. *abscessus* erm41 T28 genotype, which confers induced resistance to clarithromycin, were consistently 0.06 mg/L and 0.25 mg/L (Table 2).

Table 1 In vitro Activities of Zifanocycline and Comparators Against 67 *M. abscessus* Isolates

Antimicrobial Agents	MIC (mg/L)				Resistance (%)	Susceptibility (%)
	MIC Ranges (min-max)[b]	MIC_{50}[c]	MIC_{90}[c]	Mode		
Zifanocycline	<0.016–0.5	0.06	0.25	0.06	NA	NA
Tigecycline	<0.016–1	0.12	0.5	0.12	NA	NA
Omadacycline	0.12–2	0.25	0.5	0.25	NA	NA
Doxycycline	0.25->64	>64	>64	>64	88.06	2.99
Clarithromycin (3 day) [a]	<0.03–64	0.12	1	0.12	4.48	95.52
Clarithromycin (14 day) [a]	0.06->64	32	64	32	76.12	17.91
Moxifloxacin	0.5-16	4	8	4	53.73	25.37
Ciprofloxacin	1-32	4	8	8	77.61	4.48
Amikacin	0.5-8	2	4	2	0	100
Imipenem	32->128	128	>128	128	100	0
Linezolid	0.5-8	2	8	4	0	100
Cefoxitin	1-128	16	64	16	4.48	52.24
Sulfamethoxazole/Trimethoprim	0.25-16	4	16	4	64.18	35.82
Bedaquiline	0.06-1	0.5	0.5	0.5	NA	NA
Clofazimine	0.12-32	0.5	0.5	0.5	NA	NA

Notes: [a]Read the susceptibility results of clarithromycin after 3 days and 14 days. [b]Minimum and maximum MIC values obtained from all *M. abscessus* isolates drug susceptibility tests. [c]Concentrations that inhibited 50% and 90% of the tested clinical isolates are referred to as MIC50 and MIC90, respectively.
Abbreviation: NA, not applicable.

Table 2 In vitro Antimicrobial Activity of Zifanocycline, Tigecycline, and Omadacycline Against *M. abscessus* with Different erm41 Genotypes

Antimicrobial Agent and Species[a] (n=67)	Erm41 Genotype	MIC (mg/L)			
		MIC Ranges (min-max)[b]	MIC_{50}[c]	MIC_{90}[c]	Mode
Zifanocycline					
MAB (n=57)	T28	0.03–0.5	0.06	0.25	0.06
MAB (n=3)	C28	<0.015–0.25	0.03	0.25	NA
MMA (n=7)	Deletion	0.03–0.5	0.06	0.5	NA
Tigecycline					
MAB (n=57)	T28	<0.016-1	0.125	0.5	0.125
MAB (n=3)	C28	0.25-1	0.5	1	NA
MMA (n=7)	Deletion	0.125-1	0.25	1	NA
Omadacycline					
MAB (n=57)	T28	0.12-2	0.25	0.5	0.25
MAB (n=3)	C28	0.5-1	1	1	NA
MMA (n=7)	Deletion	0.25-2	0.5	2	NA

Notes: [a]MAB, *M. abscessus* subsp. *abscessus*; MMA, *M. abscessus* subsp. *massiliense*. [b]Minimum and maximum MIC values obtained from all M. abscessus isolates drug susceptibility tests. [c]Concentrations that inhibited 50% and 90% of the tested clinical isolates are referred to as MIC_{50} and MIC_{90}, respectively.
Abbreviation: NA, not applicable.

Zifanocycline is Compatible with the Most Commonly Utilized Drugs for Treating M. abscessus Infections

The compatibility of zifanocycline with seven commonly used antimycobacterial drugs—namely imipenem, clarithromycin, moxifloxacin, linezolid, cefoxitin, bedaquiline, and amikacin—was evaluated in vitro through the broth microdilution checkerboard titration method and five randomly selected clinical isolates of *M. abscessus*. Indifference effects were observed between zifanocycline and the aforementioned antimycobacterial drugs (Table 3).

Table 3 The Index of Fractional Inhibitory Concentration for Zifanocycline Used Alongside Drugs That are Frequently Employed in the Clinical Treatment of *M. abscessus* Infections

Combination	*M. abscessus* Isolates (n=5) [a]		Interaction
	Average FICI[b]	Range (min-max)[c]	
Zifanocycline/clarithromycin	1.7	1-2	Indifference
Zifanocycline/cefoxitin	1.9	1.5–2	Indifference
Zifanocycline/imipenem	1.625	0.625–2	Indifference
Zifanocycline/amikacin	2	2-2	Indifference
Zifanocycline/moxifloxacin	2	2-2	Indifference
Zifanocycline/linezolid	1.625	0.625-2	Indifference
Zifanocycline/bedaquinoline	1.1	0.75-2	Indifference

Notes: [a]Randomly selected clinical isolates. [b]The formula for the Fractional Inhibitory Concentration Index (FICI) was expressed as follows: FICI = [(MIC of zifanocycline used in combination/MIC of zifanocycline alone) + (MIC of the second antibiotic used in combination/MIC of the second antibiotic when used alone)]. If the FICI is ≤ 0.5, synergy is indicated; if it falls between 0.5 and 4, it is considered indifference; and if it is greater than 4, it suggests antagonism. [c]Minimum and maximum FICI values obtained from the FICI of randomly selected *M. abscessus* isolates.

Discussion

M. abscessus is the second most common non-tuberculous mycobacterial (NTM) pathogen responsible for pulmonary diseases and is widely recognized as one of the most antibiotic-resistant mycobacteria.[5,21] Research reports indicate an overall increase of 64.7% in infections caused by *M. abscessus*, along with a 25% increase in disease incidence.[21] The cumulative mortality rates for patients with *M. abscessus* pulmonary disease at 5, 10, and 15 years are 11.4%, 29.8%, and 50.0%, respectively, representing an exceptionally high mortality rate for a disease that is not typically classified as an infectious disease.[22]

The existing multidrug chemotherapy regimens containing macrolides have demonstrated limited efficacy in the treatment of *M. abscessus*, underscoring an urgent need for the development of novel therapeutic strategies.[23] To date, several innovative agents that may exhibit antibacterial activity against *M. abscessus* have been evaluated, including the quinolone sitafloxacin and the oxazolidinones linezolid, tedizolid, and contezolid. Sitafloxacin has shown superior antibacterial activity against *M. abscessus* (MIC_{50}/MIC_{90}, 1/2 mg/L) compared to other quinolones.[24] Although linezolid has been proven to possess good in vitro and in vivo activity against *M. abscessus*, its use as a long-term oral treatment option is limited due to side effects such as myelosuppression and peripheral neuropathy.[25,26] Although tedizolid appears to be safer regarding myelosuppression and neurotoxicity, and contezolid in terms of myelosuppression,[27–29] further clinical research is necessary to confirm their safety for long-term use.

Recent studies have demonstrated that the novel tetracycline derivatives tigecycline, omadacycline, and eravacycline demonstrate more significant antibacterial activity against *M. abscessus* isolates.[30,31] One of the newest tetracycline derivatives, zifanocycline, exhibits superior in vitro antimicrobial activity against *Acinetobacter baumannii* compared to tigecycline and omadacycline.[7,8] However, its antimicrobial activity against *M. abscessus* remains unexplored. This study evaluated the in vitro antibacterial activity of zifanocycline and its comparator agents against 67 strains of *M. abscessus*. The results demonstrated that zifanocycline exhibited significant advantages over other comparator drugs, including β-lactams, fluoroquinolones, and sulfonamides. Zifanocycline exhibited a MIC_{50} of 0.06 mg/L, which is 4-fold lower than that of omadacycline and 2-fold lower than that of tigecycline; its MIC_{90} is 0.25 mg/L, which is 2-fold lower than those of both omadacycline and tigecycline. Compared with omadacycline, zifanocycline exhibited superior in vitro antibacterial activity against *M. abscessus*. Additionally, zifanocycline and omadacycline are available in both oral and intravenous formulations, and the oral formulation provides an advantage in improving patient compliance for the treatment of *M. abscessus*-related diseases that require long-term administration to achieve therapeutic efficacy. In clinical investigations for the treatment of *M. abscessus* infections, over 75% of patients experienced clinical improvement when treated with chemotherapy regimens that included omadacycline, with a median duration of relatively safe continuous use extending up to 6–8 months.[32,33] The primary adverse reactions attributed directly to this treatment were nausea and vomiting; however, a subset of patients tolerated these side effects well.[33] In a brief pharmacokinetic investigation, it was determined that zifanocycline is safe and well-tolerated when administered at daily doses of up to 200 mg.[34] However, there is a notable lack of clinical research regarding its long-term safety.

A notable intrinsic resistance determinant of *M. abscessus* is the inducible macrolide erm41 gene, which encodes a methylase that modifies the ribosome, the target of clarithromycin and azithromycin, the cornerstone drugs for treating *M. abscessus* infections.[35] The erm41 gene is non-functional in *M. abscessus* subsp. *massiliense*; however, in *M. abscessus* subsp. *bolletii* and *M. abscessus* subsp. *abscessus*, the 28th nucleotide of this gene exhibits a (C/T) polymorphism, with only the erm41 T28 variant mediating macrolide resistance.[1,36] Furthermore, *M. abscessus* demonstrates both intrinsic and acquired resistance to numerous antimicrobial agents, which are challenging to overcome.[5] Consequently, infections caused by *M. abscessus* typically necessitate a combination of multiple drugs.[37] Nevertheless, multiple studies have indicated that the recommended multidrug therapy regimen, primarily consisting of macrolides, amikacin, and β-lactams, results in sputum culture conversion to negative in only 25% to 26% of patients with non-cystic fibrosis *M. abscessus* subsp. *abscessus* infections, followed by a high recurrence rate of 17% to 55%.[38–40] The MIC_{50} and MIC_{90} values of zifanocycline against *M. abscessus* subsp. *abscessus* with the erm41 T28 genotype were determined to be 0.06 mg/L and 0.25 mg/L, respectively, which aligns with the results obtained from the MIC_{50} and MIC_{90} of all isolates. Thus, macrolide-induced resistance does not compromise the in vitro antibacterial activity of zifanocycline.

Synergistic antimicrobial susceptibility tests observed indifference effects between zifanocycline and seven other commonly used anti-mycobacterial drugs, which facilitates flexible adjustment in anti-mycobacterial treatment regimens to achieve optimal therapeutic effects.

Conclusion

In conclusion, zifanocycline shows considerable antibacterial effectiveness against *M. abscessus* and observes indifference effects when combined with other anti-*M. abscessus* treatments in vitro. Thus, zifanocycline could be a promising option for incorporation into new therapeutic regimens for *M. abscessus*.

Data Sharing Statement

The data supporting the findings of this study may be obtained upon reasonable request from the corresponding author, Wang Wei. Furthermore, the raw sequence reads from our study can be found on the NCBI website, cataloged under BioProject accession number PRJNA1257363. We encourage other researchers to use our data to facilitate further scientific research.

Ethics Approval

This study was conducted in compliance with the ethical standards established in the Declaration of Helsinki, which sets forth guidelines for conducting research involving human participants. Furthermore, the study obtained the necessary ethical clearance from the Ethics Committee of the Affiliated Hospital of Jiaxing University. The approval reference number for this authorization was 2024-LY-400, indicating that all protocols were scrutinized and met the required ethical considerations for such investigations.

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Disclosure

We would like to emphasize that this research was free from any conflicts of interest. All authors have confirmed that they possess no financial or personal affiliations that may affect the results of this study.

References

1. Bastian S, Veziris N, Roux AL, et al. Assessment of clarithromycin susceptibility in strains belonging to the Mycobacterium abscessus group by erm (41) and rrl sequencing. *Antimicrob Agents Chemother*. 2011;55(2):775–781. doi:10.1128/aac.00861-10
2. Bryant JM, Grogono DM, Rodriguez-Rincon D, et al. Emergence and spread of a human-transmissible multidrug-resistant nontuberculous mycobacterium. *Science*. 2016;354(6313):751–757. doi:10.1126/science.aaf8156
3. Pierre-Audigier C, Ferroni A, Sermet-Gaudelus I, et al. Age-related prevalence and distribution of nontuberculous mycobacterial species among patients with cystic fibrosis. *J Clin Microbiol*. 2005;43(7):3467–3470. doi:10.1128/JCM.43.7.3467-3470.2005
4. Prevots DR, Shaw PA, Strickland D, et al. Nontuberculous mycobacterial lung disease prevalence at four integrated health care delivery systems. *Am J Respir Crit Care Med*. 2010;182(7):970–976. doi:10.1164/rccm.201002-0310OC
5. Johansen MD, Herrmann JL, Kremer L. Non-tuberculous mycobacteria and the rise of Mycobacterium abscessus. *Nat Rev Microbiol*. 2020;18 (7):392–407. doi:10.1038/s41579-020-0331-1

6. Kwak N, Dalcolmo MP, Daley CL, et al. Mycobacterium abscessus pulmonary disease: individual patient data meta-analysis. *Eur Respir J*. 2019;54(1):1801991. doi:10.1183/13993003.01991-2018
7. Han R, Ding L, Yang Y, et al. In vitro activity of KBP-7072 against 536 acinetobacter baumannii complex isolates collected in China. *Microbiol Spectr*. 2022;10(1):e01471–21. doi:10.1128/spectrum.01471-21
8. Huband MD, Mendes RE, Pfaller MA, et al. In vitro activity of KBP-7072, a novel third-generation tetracycline, against 531 recent geographically diverse and molecularly characterized acinetobacter baumannii species complex isolates. *Antimicrob Agents Chemother*. 2020;64(5):e02375–19. doi:10.1128/AAC.02375-19
9. Arenz S, Nguyen F, Beckmann R, Wilson DN. Cryo-EM structure of the tetracycline resistance protein TetM in complex with a translating ribosome at 3.9-Å resolution. *Proc Natl Acad Sci*. 2015;112(17):5401–5406. doi:10.1073/pnas.1501775112
10. Kaminishi T, Schedlbauer A, Ochoa-Lizarralde B, et al. The third-generation tetracycline KBP-7072 exploits and reveals a new potential of the primary tetracycline binding pocket. *bioRxiv*. 2018:508218. doi:10.1101/508218
11. KBP Biosciences. A randomized, double-blind, placebo-controlled, sequential parallel group, single ascending dose study in healthy subjects to evaluate the safety, tolerability and pharmacokinetics of KBP-7072 following oral administration. clinicaltrials.gov; 2024. Available from: https://clinicaltrials.gov/study/NCT02454361. Accessed April 9, 2025.
12. KBP Biosciences. A randomized, single-blind, placebo-controlled, sequential parallel-group and multiple ascending dose (MAD) study to evaluate the safety, tolerability and pharmacokinetics of KBP-7072 in healthy subjects. clinicaltrials.gov; 2024. Available from: https://clinicaltrials.gov/study/NCT02654626. Accessed April 9, 2025.
13. Lepak AJ, Zhao M, Liu Q, et al. Pharmacokinetic/pharmacodynamic evaluation of a novel aminomethylcycline antibiotic, KBP-7072, in the neutropenic murine pneumonia model against staphylococcus aureus and streptococcus pneumoniae. *Antimicrob Agents Chemother*. 2019;63(3):e02404–18. doi:10.1128/AAC.02404-18
14. Tan X, Zhang M, Liu Q, et al. Nonclinical pharmacokinetics, protein binding, and elimination of KBP-7072, an aminomethylcycline antibiotic, in animal models. *Antimicrob Agents Chemother*. 2020;64(6):e00488–20. doi:10.1128/AAC.00488-20
15. Li L, Tan X, Zhou T, et al. *In vivo* efficacy and PK/PD analyses of zifanocycline (KBP-7072), an aminomethylcycline antibiotic, against *Acinetobacter baumannii* in a neutropenic murine thigh infection model. *J Infect Chemother*. 2024;30(1):34–39. doi:10.1016/j.jiac.2023.09.010
16. Bankevich A, Nurk S, Antipov D, et al. SPAdes: a new genome assembly algorithm and its applications to single-cell sequencing. *J Comput Biol*. 2012;19(5):455–477. doi:10.1089/cmb.2012.0021
17. Bortolaia V, Kaas RS, Ruppe E, et al. ResFinder 4.0 for predictions of phenotypes from genotypes. *J Antimicrob Chemother*. 2020;75(12):3491–3500. doi:10.1093/jac/dkaa345
18. Clinical and Laboratory Standards Institute. *Susceptibility Testing of Mycobacteria, Nocardia Spp. and Other Aerobic Actinomycetes*. 3rd ed. Wayne, PA: Clinical and Laboratory Standards Institute; 2018. CLSI standard M24.
19. Clinical and Laboratory Standards Institute. *Performance Standards for Antimicrobial Susceptibility Testing*. 34st ed. Wayne, PA: Clinical and Laboratory Standards Institute; 2024. CLSI supplement M100.
20. Clinical and Laboratory Standards Institute. Performance standards for susceptibility testing of mycobacteria, nocardia spp. and other aerobic actinomycetes. 1st ed. CLSI supplement M62. Wayne, PA: Clinical and Laboratory Standards Institute; 2018.
21. Cristancho-Rojas C, Varley CD, Lara SC, Kherabi Y, Henkle E, Winthrop KL. Epidemiology of Mycobacterium abscessus. *Clin Microbiol Infect off Publ Eur Soc Clin Microbiol Infect Dis*. 2024;30(6):712–717. doi:10.1016/j.cmi.2023.08.035
22. Jhun BW, Moon SM, Jeon K, et al. Prognostic factors associated with long-term mortality in 1445 patients with nontuberculous mycobacterial pulmonary disease: a 15-year follow-up study. *Eur Respir J*. 2020;55(1):1900798. doi:10.1183/13993003.00798-2019
23. Pasipanodya JG, Ogbonna D, Ferro BE, et al. Systematic review and meta-analyses of the effect of chemotherapy on pulmonary Mycobacterium abscessus outcomes and disease recurrence. *Antimicrob Agents Chemother*. 2017;61(11):e01206–17. doi:10.1128/AAC.01206-17
24. He S, Guo Q, Zhao L, et al. Sitafloxacin expresses potent anti-Mycobacterium abscessus activity. *Front Microbiol*. 2022;12:779531. doi:10.3389/fmicb.2021.779531
25. Li H, Tong L, Wang J, et al. An intensified regimen containing linezolid could improve treatment response in Mycobacterium abscessus lung disease. *BioMed Res Int*. 2019;2019(1):8631563. doi:10.1155/2019/8631563
26. Cheng LP, Zhang Q, Lou H, et al. Effectiveness and safety of regimens containing linezolid for treatment of Mycobacterium abscessus pulmonary disease. *Ann Clin Microbiol Antimicrob*. 2023;22(1):106. doi:10.1186/s12941-023-00655-2
27. Katsarou A, Tzikopoulou M, Papadopoulos D, Palioura S, Falagas ME. Optic and peripheral neuropathy associated with short and prolonged administration of tedizolid: a review. *Expert Rev Anti Infect Ther*. 2025;23(1):49–65. doi:10.1080/14787210.2024.2448143
28. Mensa Vendrell M, Tasias Pitarch M, Salavert Lletí M, et al. Safety and tolerability of more than six days of tedizolid treatment. *Antimicrob Agents Chemother*. 2020;64(7):10.1128/aac.00356–20. doi:10.1128/aac.00356-20
29. Zhao X, Huang H, Yuan H, Yuan Z, Zhang Y. A Phase III multicentre, randomized, double-blind trial to evaluate the efficacy and safety of oral contezolid versus linezolid in adults with complicated skin and soft tissue infections. *J Antimicrob Chemother*. 2022;77(6):1762–1769. doi:10.1093/jac/dkac073
30. Zhang T, Du J, Dong L, et al. In Vitro antimicrobial activities of tigecycline, eravacycline, omadacycline, and sarecycline against rapidly growing mycobacteria. *Microbiol Spectr*. 2023;11(1):e0323822. doi:10.1128/spectrum.03238-22
31. Li A, He S, Li J, Zhang Z, Li B, Chu H. Omadacycline, eravacycline, and tigecycline express anti-Mycobacterium abscessus activity in vitro. *Microbiol Spectr*. 2023;11(3):e0071823. doi:10.1128/spectrum.00718-23
32. El Ghali A, Morrisette T, Alosaimy S, et al. Long-term evaluation of clinical success and safety of omadacycline in nontuberculous mycobacteria infections: a retrospective, multicenter cohort of real-world health outcomes. *Antimicrob Agents Chemother*. 2023;67(10):e00824–23. doi:10.1128/aac.00824-23
33. Mingora CM, Bullington W, Faasuamalie PE, et al. Long-term safety and tolerability of omadacycline for the treatment of Mycobacterium abscessus infections. *Open Forum Infect Dis*. 2023;10(7):ofad335. doi:10.1093/ofid/ofad335
34. Yang F, Wang Y, Wang P, Hong M, Benn V. Multiple ascending dose safety, tolerability, and pharmacokinetics of KBP-7072, a novel third-generation tetracycline. *Open Forum Infect Dis*. 2017;4(suppl_1):S291. doi:10.1093/ofid/ofx163.662
35. Nash KA, Brown-Elliott BA, Wallace RJ. A novel gene, erm(41), confers inducible macrolide resistance to clinical isolates of Mycobacterium abscessus but is absent from Mycobacterium chelonae. *Antimicrob Agents Chemother*. 2009;53(4):1367–1376. doi:10.1128/AAC.01275-08

36. Yoshida S, Tsuyuguchi K, Suzuki K, et al. Further isolation of Mycobacterium abscessus subsp. abscessus and subsp. bolletii in different regions of Japan and susceptibility of these isolates to antimicrobial agents. *Int J Antimicrob Agents*. 2013;42(3):226–231. doi:10.1016/j.ijantimicag.2013.04.029
37. Daley CL, Iaccarino JM, Lange C, et al. Treatment of nontuberculous mycobacterial pulmonary disease: an official ATS/ERS/ESCMID/IDSA clinical practice guideline. *Eur Respir J*. 2020;56(1):2000535. doi:10.1183/13993003.00535-2020
38. Koh WJ, Jeon K, Lee NY, et al. Clinical significance of differentiation of Mycobacterium massiliense from Mycobacterium abscessus. *Am J Respir Crit Care Med*. 2011;183(3):405–410. doi:10.1164/rccm.201003-0395OC
39. Park J, Cho J, Lee CH, Han SK, Yim JJ. Progression and treatment outcomes of lung disease caused by Mycobacterium abscessus and Mycobacterium massiliense. *Clin Infect Dis*. 2017;64(3):301–308. doi:10.1093/cid/ciw723
40. Lyu J, Kim BJ, Kim BJ, et al. A shorter treatment duration may be sufficient for patients with Mycobacterium massiliense lung disease than with Mycobacterium abscessus lung disease. *Respir Med*. 2014;108(11):1706–1712. doi:10.1016/j.rmed.2014.09.002