Original Article

Trends in Pharmaceutical Sciences and Technologies 2025: 11(4):349-356.

Synthesis of Some novel α-substituted Phosphonic Acids by Nano-$SnCl_4/SiO_2$ as Antimicrobial Agents

Leila Emami [1];Ph.D, Zahra Rezaei[2];Ph.D, Kamiar Zomorodian[3,4];Ph.D Leila Zamani[1];Ph.D, Soghra Khabnadideh[1,2*];Ph.D

[1]Pharmaceutical Sciences Research Center, Shiraz University of Medical Sciences, Shiraz, Iran
[2]Department of Medicinal Chemistry, School of Pharmacy, Shiraz University of Medical Sciences, Shiraz, Iran
[3]Department of Medical Mycology and Parasitology, School of Medicine, Shiraz University of Medical Sciences, Shiraz, Iran
[4]Basic Researches in Infectious Diseases Center, Shiraz University of Medical Sciences, Shiraz, Iran

Abstract

α-Substituted phosphonic acids exhibit promising antifungal activity by targeting key fungal metabolic pathways. Their structural versatility allows for enhanced binding affinity to fungal enzymes, inhibiting growth and virulence. These compounds demonstrate broad-spectrum efficacy against resistant strains, including Candida and Aspergillus species. Further optimization could lead to novel antifungal agents with reduced toxicity and improved pharmacokinetics. In this study, a series of α-substituted phosphonic acids (4a-4c) were synthesized using Nano-$SnCl_4/SiO_2$ as a catalyst in an ethanolic solution under reflux conditions with good yields. The chemical structures of the new compounds were confirmed by spectroscopic methods, including ^{1}H-NMR, ^{13}C-NMR, and ^{31}P-NMR. The synthesized compounds were evaluated for their antimicrobial activities. The broth microdilution method, as recommended by the Clinical and Laboratory Standards Institute, was used to determine the antifungal and antibacterial activities of the new compounds. The antimicrobial evaluation was tested against six different species of yeasts and four species of bacteria. Fluconazole and ciprofloxacin were used as reference drugs. The results demonstrated that the presence of an electronegative group on the phenyl ring had a positive effect on antimicrobial potency.

Keywords: Synthesis; Nano SnCl4/SiO2; Antimicrobial activity; Phosphonic acid.

Please cite this article as: Emami L, Rezaei Z, Zomorodian K, Zamani L, Khabnadideh S. Synthesis of some novel α-substituted phosphonic acids by Nano-$SnCl_4/SiO_2$ as antimicrobial agents. Trends in Pharmaceutical Sciences and Technologies. 2025;11(4):349-356. doi: 10.30476/tips.2025.107987.1309

1. Introduction

Alpha-substituted phosphonic acids are a class of organophosphorus compounds characterized by the presence of a substituent at the alpha (α) position relative to the phosphonic acid group (–$PO(OH)_2$) (1-3). These compounds can be synthesized via multicomponent reactions involving dimedone, aromatic aldehydes, and triethyl phosphite, often catalyzed by nano zinc oxide or other catalysts to yield novel derivatives (1, 4). The α-position can be substituted with various groups such as hydroxyl (α-hydroxymethylene), halogens (α-chloromethylene, α-bromomethylene), or alkyl/aryl groups, which affect the compound's biological activity and receptor interactions (3, 4). These compounds often serve as stable analogues of phosphate esters, being resistant to enzymatic hydrolysis, which makes them useful in drug design and biochemical studies

Corresponding Author: Soghra Khabnadideh, Pharmaceutical Sciences Research Center, Shiraz University of Medical Sciences, Shiraz, Iran
E-mail: khabns@sums.ac.ir

Received: 16/04/2025; Accepted: 01/09/2025

(5). Substituted phosphonic acids exhibit diverse biological activities, including anticancer (6-8), antibacterial (9, 10) antiviral (11), enzyme inhibitors (12) and HIV protease inhibitors (13).

Multi-component reactions (MCRs) constitute one of the most efficient tools in modern synthetic organic chemistry for the preparation of highly functionalized organic compounds (14). They play an important role in organophosphorus chemistry, where phosphorus reagents are used as substrates for the synthesis of a wide range of phosphonates (15). The presented examples underline the greatest advantage of multi-component reactions, which is to create diverse bioactive compounds from simple building blocks in only one step. These methodologies will have a bright future in drug design and synthesis (16).

Nano metal oxides are nanoparticles composed of metal oxides with particle sizes typically ranging from 1 to 100 nanometers. These nanomaterials exhibit unique physical, chemical, electrical, and optical properties that differ significantly from their bulk counterparts due to their high surface-area-to-volume ratio and quantum size effects (17, 18). Nano-$SnCl_4/SiO_2$ is a heterogeneous, reusable, and eco-friendly catalyst widely used in organic synthesis. It is prepared by supporting tin tetrachloride ($SnCl_4$) on nano-sized silica (SiO_2), which enhances its catalytic activity and surface area (19, 20). Nano-$SnCl_4/SiO_2$ acts as a mild Lewis acid catalyst, promoting one-pot synthesis reactions under relatively mild and green conditions. It enables high yields, short reaction times, and easy work-up procedures, making it cost-effective and environmentally friendly (19). A simple and green synthesis of some novel phosphonic acids has been achieved from a three-component reaction between various aldehydes, dimedone, and triethyl phosphite using Nano-$SnCl_4/SiO_2$ as a catalyst in an alcoholic solution under reflux conditions. The chemical structures of the novel compounds were confirmed by different spectroscopic methods. The antimicrobial activities of the synthesized compounds were also evaluated using the broth microdilution method against different yeasts and bacteria. Fluconazole and ciprofloxacin were used as positive controls.

2. Materials and Methods

All chemicals were obtained from Merck (Germany), Fluka (Switzerland), and other chemical companies. All yields refer to isolated products after chromatography or other indicated purification methods. Infrared (IR) spectra were run on a Shimadzu FTIR-8300 spectrophotometer. The ^{1}H-NMR, ^{13}C-NMR, and ^{31}P-NMR spectra were run on a Bruker Avance DPX-250

2.1. General procedure for synthesis of α-substituted phosphonic acids

According to the optimized conditions, a mixture of 1 mmol of aldehyde (1), 1 mmol of dimedone (2), 1.1 mmol of triethyl phosphite (3), and 0.3 mmol (0.02 g) of Nano-$SnCl_4/SiO_2$ was added to ethanol (3 mL). The reaction mixture was refluxed, and the progress of the reaction was checked by TLC. After the completion of the reaction, the mixture was centrifuged and filtered. To achieve the pure product, column chromatography with n-hexane and ethyl acetate was applied (Figure 1).

2.2. Spectra data

M((4-bromophenyl)(4,4-dimethyl-2,6-dioxocyclohexyl)methyl)phosphonic acid (4a)

1H-NMR (400 MHz, CDCl3, δ/ppm): 1.10 (s, 6H, CH3),1.59 (brs. 2H, OH), 2.14-2.25 (AB q, *J1*=*J2*=16.4 Hz, 4H, CH2), 4.69 (s, 1H, -CO-CH-CO), 5.52-5.54 (d, J= 8.9 Hz, 1H, -CH-PO-), 7.15-7.18 (m, 2H, aromatic), 7.31-7.34 (m, 2H, aromatic). 13C-NMR (63 MHz, CDCl3, δ/ppm): 31.1 (C(CH3)2), 31.3 (d, 1JCP=44.0 Hz, -CH-PO-), 46.3 (-CH2-CO-), 47.0 (-CO-CH-CO-), 120.6, 121.7, 131.6, 133.7, 150.5, 169.3 (C=O), 170.4 (C=O). 31P-NMR (162 MHz, CDCl3, δ/ppm, 85% aqueous H3PO4 as external reference) -1.5.

((4,4-dimethyl-2,6-dioxocyclohexyl) (4-fluorophenyl)methyl)phosphonic acid (4b)
1H-NMR (400 MHz, CDCl3, δ/ppm): 0.93 (s, 6H, CH3),1.61 (brs. 2H, OH), 2.142.45 (AB q, *J1=J2*=35.64 Hz, 4H, CH2), 4.72 (s, 1H, CO-CH-CO-), 5.36 (d, 1*J*CP =10.92 Hz, 1H, -CH-PO-), 6.86-6.89 (m, 2H, aromatic), 7.10-7.23 (m, 1H, aromatic), 7.23-7.34 (m, 1H, arom.). 13C-NMR (100 MHz, DMSO-d6, δ/ppm): 28.5 (-C(CH3)2), 36.6 (-CH-PO-), 47.0 (-CH2-CO-), 48.4 (-CO-CH-CO-), 122.0, 128.4, 132.8, 149.4, 159.6 (C=O), 161.2 (C=O). 31P-NMR (162 MHz, CDCl3, δ/ppm, 85% aqueous H3PO4 as external reference): -1.5.

((4,4-dimethyl-2,6-dioxocyclohexyl) (4-methoxyphenyl)methyl)phosphonic acid (4c)
1H-NMR (400 MHz, CDCl3, δ/ppm): 1.27 (s, 6H, CH3),1.68 (brs. 2H, OH), 2.30-2.44 (m, 4H, CH2), 3.49 (s, 1H, -CO-CH-CO-),3.73 (s, 3H, OCH3), 4.69-4.73 (d, *J*= 9.0 Hz, 1H, -CH-PO-), 7.06 (m, 2H, aromatic), 7.52 (m, 2H, aromatic). 13C-NMR (100 MHz, DMSO-d6, δ/ppm): 28.0 (-C(CH3)2), 38.0 (d, 1*J*CP=121 Hz, -CH-PO-), 46.4 (-CH2-CO-), 47.6 (-CO-CH-CO-), 52.2 (-OCH3), 113.8, 125.1, 137.3, 155.7, 160.7, 162.5 (C=O), 163.4 (C=O). 31P-NMR (162 MHz, CDCl3, δ/ppm, 85% aqueous H3PO4 as external reference): -1.5.

2.3. Antimicrobial Assessment

The broth microdilution method was used to determine the antimicrobial activities of the α-substituted phosphonic acids. Various American Type Culture Collection (ATCC) strains of fungi and four standard species of bacteria were used for this purpose. The Minimum Inhibitory Concentration (MIC) values of all compounds were examined using the broth microdilution method (Clinical and Laboratory Standards Institute method) with some modifications (22, 23). Serial dilutions of the synthetic compounds (1–256 µg/mL) were prepared in 96-well microtiter plates using RPMI-1640 media (Sigma, St. Louis, MO, USA) buffered with MOPS (Sigma). A stock inoculum was prepared from three colonies of the examined yeast in 5 mL sterile 0.85% NaCl, and then the turbidity of the inoculum was adjusted to 0.5 McFarland standards at 530 nm wavelength. Aspergillus spp. and dermatophytes conidia were cultured on potato dextrose agar using a wetting loop with Tween-20. Their turbidity was adjusted to OD=0.09-0.11, which yields 0.4-5×10^6 conidia/mL. A working suspension was prepared by making a 1/50 and 1/1000 dilution with RPMI of the stock suspension for molds and yeasts, respectively. The working inoculum (0.1 mL) was added to the microtiter plates, which were incubated in a humid atmosphere at 30 ºC for 24–48 h. Uninoculated medium (200 µL) was included as a sterility control (blank). In addition, growth controls (medium with inoculum but without antibiotics or the synthetic compounds) were also examined. The growth in each well was compared with that of the growth in the control well.

3. Results and Discussion

Following our studies on the development of green synthesis of biologically active compounds using nano-catalysts (21), we utilized the valuable nanoparticle catalyst Nano-$SnCl_4/SiO_2$ for the synthesis of α-substituted phosphonic acids. A simple one-pot, three-component reaction involving an aldehyde, dimedone, and triethyl phosphite was conducted in the presence of Nano-$SnCl_4/SiO_2$ under reflux. This approach successfully yielded the corresponding ((4,4-dimethyl-2,6-dioxocyclohexyl)(phenyl)methyl)phosphonic acids (4a–4c) in good yields (Figure 1).

3.1. Preparation and Characterization of Nano-$SnCl_4/SiO_2$ Catalyst

Nano $SnCl_4/SiO_2$ was prepared by supporting tin tetrachloride on activated nano silica following a modified procedure based on the referenced method. Nano silica was dried

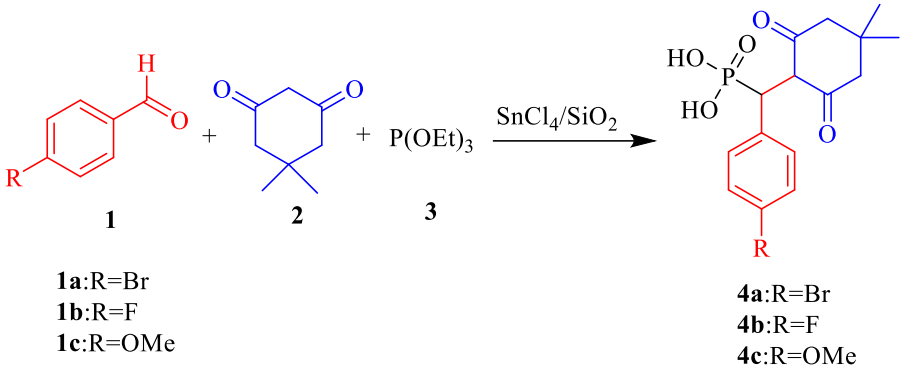


Figure 1. Synthesis of ((4,4-dimethyl-2,6-dioxocyclohexyl)(phenyl)methyl)phosphonic acids (4a–4c).

at 150 °C, suspended in dry n hexane, and treated dropwise with a solution of $SnCl_4$ under nitrogen. After 24 h of stirring at room temperature, the solid was filtered, washed with n hexane, and dried to afford a stable white powder. FT IR analysis confirmed catalyst formation. The spectrum displayed the characteristic Si–O–Si stretching band near 1100 cm^{-1} and Si–OH absorption around 700 cm^{-1}. A distinct band at approximately 900 cm^{-1} indicated the presence of Sn–O–Si bonds, consistent with successful immobilization of $SnCl_4$ on the silica surface (Figure S1). The catalyst thus obtained acts as a mild, reusable solid Lewis acid and was employed efficiently in the synthesis of α substituted phosphonic acids in this study (20).

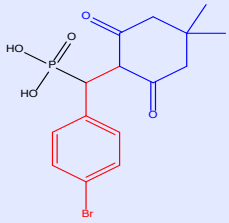
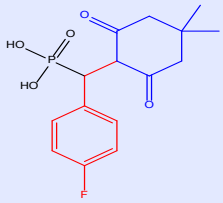
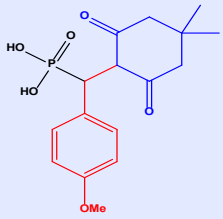
3.2. Optimization of reaction conditions for synthesis of α-substituted phosphonic acids

To find the optimum conditions for the synthesis of α-substituted phosphonic acids, the reactions between 4-bromobenzaldehyde (1a), dimedone (2), and triethyl phosphite (3) were selected for initial screening experiments as a model reaction. Various solvents were examined (Table 1, entries 1-4). The best results were obtained in ethanol. Then, different temperatures were also examined (Table 1, entries 4-6), and the results showed that the optimal temperature for this reaction was 80 °C. To achieve the optimal mmol amount of Nano-$SnCl_4/SiO_2$, various mmol quantities including 0.4, 0.3, and 0.2 mmol were tested. We obtained the best yield with 0.3 mmol (Table 1, entries 7-9). After initial optimization for the synthesis of compound 4a, the optimal conditions were determined to be in ethanol, at 80 °C, and with 0.3 mmol of the catalyst (Table 1, entry 9).

Table 1. Optimization of the reaction conditions for synthesis of ((4,4-dimethyl-2,6-dioxocyclohexyl)(4-bromophenyl)methyl)phosphonic acids (4a)

Entry	Solvent	Temp.(oC)	Time (h)	mmol of the catalyst	Yield(%)
1	CH3Cl	80	24	0.2	65
2	CH2Cl2	80	24	0.2	10
3	CH3OH	80	48	0.2	5
4	EtOH	80	12	0.2	80
5	EtOH	50	12	0.2	20
6	EtOH	90	12	0.2	70
7	EtOH	80	12	0.5	62
8	EtOH	80	12	0.4	70
9	**EtOH**	**80**	**12**	**0.3**	**90**

Table 2. Chemical structure and physical properties of α-substituted phosphonic acid derivatives (4a-4c).

Entry	Structure	M.P. (°C)	Yield (%)	M.W.
4a		112-115	78	389
4b		117-119	81	328.2
4c		130-135	79	340.11

3.3. Catalytic activity of Nano SnCl4/SiO2 in the synthesis of α-substituted phosphonic acids

A multi-component reaction of different aldehydes, dimedone, and triethyl phosphite in the presence of Nano-$SnCl_4/SiO_2$ was carried out to prepare phosphorus compounds (4a-4c) (Figure 1). According to the results in Table 1, the desired compounds were synthesized in ethanol in the presence of 0.3 mmol of catalyst at 80 °C. The synthesized compounds were characterized by ^{1}H-NMR, ^{13}C-NMR, and ^{31}P-NMR spectroscopic methods (Supplementary Information). The chemical structures of the synthesized compounds are shown in Table 2.

3.4. Antimicrobial Assessment

The antifungal activities of the synthesized compounds were evaluated, and the results are summarized in Table 3. The antifungal evaluation was carried out against *C. albicans*, *C. tropicalis*, *C. krusei*, *C. glabrata*, *C. dubliniensis*, and *C. parapsilosis*. The results showed that compound 4a had moderate inhibitory potency on the growth of all studied yeasts in the range of 32-128 µg/mL values, except for *C. tropicalis* and *C. parapsilosis*. The growth of *C. glabrata* and *C. dubliniensis* was inhibited by 4a at a concentration of 32 µg/mL. Compound 4b showed inhibitory activity towards *C. krusei*, *C. glabrata*, and *C. dubliniensis*, ranging from 64-128 µg/mL values. Furthermore, compound 4c had no inhibitory effect on the growth of any studied yeast. As

Table 3. IC_{50} values (µg/mL) of the α-substituted phosphonic acids against the examined yeasts.

Entry	*C. albicans*	*C. tropicalis*	*C. krusei*	*C. glabarata*	*C. dubliniensis*	*C. parapsilosis*
4a	128	>256	64	32	32	>256
4b	>256	>256	128	64	128	>256
4c	>256	>256	>256	>256	>256	>256
Fluconazole	4	1	16	4	1	2

Table 4. IC_{50} values (µg/mL) of the α-substituted phosphonic acids against the examined bacteria.

Entry	*S. aureus*	*E. faecalis*	*E. coli*	*P. aeruginosa*
4a	128	64	G*	G*
4b	>256	128	G*	G*
4c	G*	G*	G*	G*
Ciprofloxacin	0.5	0.025	----	2

a result, derivatives featuring electronegative substitution on the phenyl moiety showed an enhanced effect on the activity in the order of Br > F. It was also noted that the replacement with an electropositive substitution (OMe) in compound 4c on the phenyl ring led to diminished activity.

The antibacterial activities of the synthesized compounds were evaluated, and the results are summarized in Table 4. The antibacterial evaluation was carried out against *S. aureus, E. faecalis*, *E. coli*, and *P. aeruginosa*. The results showed that compound 4a exhibited inhibitory activity towards *S. aureus* and *E. faecalis* with MIC values of 128 and 64 µg/mL, respectively. Also, compound 4b showed an inhibitory effect against *E. faecalis* with an MIC value of 128 µg/mL. The structure-activity relationship indicated that compound 4a with a bromo moiety at the para position was the most active compound.

4. Conclusion

The synthesis of some α-substituted phosphonic acids using an appropriate and green catalyst was reported. The simple workup, high yield, and mild conditions are the advantages of this method. The antifungal studies of α-substituted phosphonic acid derivatives show moderate results *in vitro* toward *C. krusei*, *C. glabrata*, and *C. dublinensis* yeasts. Our results show that the existence of an electron-withdrawing group at the para-position of the phenyl moiety could improve antifungal activities.

Acknowledgements

Financial assistance from the Shiraz University of Medical Sciences by way of grant number 11357 is gratefully acknowledged.

Conflict of Interest

The authors declare that they have no conflict of interest.

References

1. Shaabani Y, Ghassamipour S. Design and synthesis of novel α-substituted phosphonic acids catalyzed by Nano zinc oxide. *Phosphorus Sulfur Silicon Relat Elem.* 2016;191(6):898-903.
2. Huang Y, Zhang Y, Pan L, Wu Q, Li N, Shi E, *et al*. CAMDOL-enabled diastereoselective synthesis of α-substituted phosphonates. *Chem Commun*. 2024;60(14):1924-7.
3. Jiang G, Xu Y, Fujiwara Y, Tsukahara T, Tsukahara R, Gajewiak J, *et al*. α-Substituted phosphonate analogues of lysophosphatidic acid (LPA) selectively inhibit production and action of LPA. *Chem Med Chem*. 2007;2(5):679-90. doi:10.1002/cmdc.200600280
4. Sevrain CM, Berchel M, Couthon H, Jaffrès P-A. Phosphonic acid: preparation and applications. *Beilstein J Org Chem.* 2017;13(1):2186-213.
5. Reich D, Noble A, Aggarwal VK. Facile Conversion of α-Amino Acids into α-Amino Phosphonates by Decarboxylative Phosphorylation using Visible-Light Photocatalysis. *Angew Chem Int Ed Engl.* 2022;134(37):202207063. doi: 10.1002/anie.202207063.
6. Kung HH. Transition metal oxides: surface chemistry and catalysis: Elsevier; 1989.
7. Henrich VE, Cox PA. The surface science of metal oxides: Cambridge university press; 1994.
8. Noguera C. Physics and chemistry at oxide surfaces. Cambridge University Press, Cambridge. *Acta Cryst A*. 1997;53:855-6.

9. Kumar BV, Naik HSB, Girija D, Kumar BV. ZnO nanoparticle as catalyst for efficient green one-pot synthesis of coumarins through Knoevenagel condensation. *J Chem Sci*. 2011;123:615-21.
10. Azzam SHS, Siddekha A, Pasha M. One-pot four-component synthesis of some novel octahydroquinolindiones using ZnO as an efficient catalyst in water. *Tetrahedron lett*. 2012;53(46):6306-9.
11. Balakumar C, Rajendran R, Ahamed HA, Jayakumar S. Size Controlled Synthesis of MgO Nanoparticles and its Fabrication on Textiles for Microbe Resistance. *Int J Curr Res*. 2016;8(5):31234-38.
12. Battez AH, González R, Viesca J, Fernández J, Fernández JD, Machado A, et al. CuO, ZrO2 and ZnO nanoparticles as antiwear additive in oil lubricants. *Wear*. 2008;265(3-4):422-8.
13. Firouzabadi H, Iranpoor N, Sobhani S, Ghassamipour S. Aluminium triflate [Al (OTf) 3] as a recyclable catalyst for the conversion of α-hydroxyphosphonates, alcohols and phenols to their corresponding O-silylated products with hexamethyldisilazane (HMDS). *Synthesis*. 2005;2005(04):595-9.
14. S Bekheit M, A Kamel A. Multi-Component Reactions in the Preparation of α-and β-Substituted Phosphonates. *Curr Organ Chem*. 2017;21(10):923-38.
15. Jiang B, Rajale T, Wever W, Tu SJ, Li G. Multicomponent reactions for the synthesis of heterocycles. *Chem Asian J*. 2010;5(11):2318-2335. doi:10.1002/asia.201000310
16. Ugi I, Dömling A, Hörl W. Multicomponent reactions in organic chemistry. *Endeavour*. 1994;18(3):115-22.
17. Ashik U, Kudo S, Hayashi J-i. An overview of metal oxide nanostructures. In: Synthesis of inorganic nanomaterials: advances and key technologies. Duxford, UK: Woodhead Publishing. 2018:19-57.
18. Negrescu AM, Killian MS, Raghu SN, Schmuki P, Mazare A, Cimpean A. Metal oxide nanoparticles: review of synthesis, characterization and biological effects. *J Funct Biomater.* 2022;13(4):274. Published 2022 Dec 5. doi:10.3390/jfb13040274
19. Zamani L, Faghih Z, Zomorodian K, Mirjalili BBF, Jalilian A, Khabnadideh S. Nano-SnCl4. SiO2, an efficient catalyst for synthesis of benzimidazole drivatives as antifungal and cytotoxic agents. *Res Pharm Sci*. 2019;14(6):496-503.
20. Mirjalili B, Bamoniri A, Mirhoseini M. Nano-SnCl4.SiO2: An efficient catalyst for one-pot synthesis of 2, 4, 5-tri substituted imidazoles under solvent-free conditions. *Sci Iran*. 2013;20(3):587-91.
21. Amiri-Zirtol L, Khabnadideh S. A novel heterogeneous biocatalyst based on graphene oxide for synthesis of pyran derivatives. *Sci Rep.* 2024;14(1):6957.
22. Wiegand I, Hilpert K, Hancock REW. Agar and broth dilution methods to determine the minimal inhibitory concentration (MIC) of antimicrobial substances. *Nat Protoc.* 2008;3(2):163-75.
23. Clinical and Laboratory Standards Institute (CLSI). Methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically. 12th ed. Wayne, PA: Clinical and Laboratory Standards Institute. 2024. (CLSI standard M07).