Schiff bases containing β-lactam rings: synthesis, characterization, and biological activity

Elvin Ibrahimli[1,2*], Aygun Israyilova[3], Ulviyya Hasanova[4], Abdulsaid Azizov[2], Mushgunaz Akhundova[5]

[1]Scientific Research Institute "Geotechnological Problems of Oil, Gas and Chemistry", Ministry of Science and Education of Azerbaijan, Dilara Aliyeva Street 227, AZ1010 Baku, Azerbaijan
[2]High Molecular Compound Chemistry Department, Baku State University, 23 Academic Zahid Khalilov Street, Baku 1148, Baku, Azerbaijan
[3]Laboratory of Microbiology and Virology, Baku State University, 23 Academic Zahid Khalilov Street, Baku 1148, Baku, Azerbaijan
[4]ICESCO Biomedical Materials Department, Baku State University, 23 Academic Zahid Khalilov Street, Baku 1148, Baku, Azerbaijan
[5]Organic Chemistry Department, Baku State University, 23 Academic Zahid Khalilov Street, Baku 1148, Baku, Azerbaijan

Abstract
The aim of this study is to synthesize biologically active Schiff bases and evaluate their antimicrobial activity of synthesized compound. Schiff bases are synthesized by the condensation reaction of primary amines with aldehydes or ketones. In this research, the synthesis process involves a room-temperature condensation reaction between o-vanillin and ampicillin, a β-lactam containing antibiotic commonly used to treat bacterial infections. As a solvent for the procedure of the Schiff base synthesis used methanol under mild conditions.
Following the synthesis, the structural characterization of the obtained Schiff base is conducted using a combination of spectroscopic techniques, including proton nuclear magnetic resonance (^{1}H NMR) and carbon-13 nuclear magnetic resonance (^{13}C NMR) spectroscopy, as well as high resolution mass spectrometry (HRMS). These analytical methods assist in confirming the successful formation of the target compound and provide insights into its molecular structure and purity.
Subsequently, the antimicrobial activity of the synthesized Schiff base is assessed through in vitro antibacterial assays against *Staphyloccoccus aureus* ATCC6538, *Staphylococcus aureus* ATCC 25923, *Escherichia coli* ATCC 25922 and clinical isolate *Escherichia coli* BDU32 The antibacterial efficacy of the Schiff base is compared to that of the parent antibiotic, ampicillin, to ascertain whether the modification augments, diminishes, or maintains its antimicrobial potential. This comparative analysis furnishes valuable information on the structure-activity relationship and the potential of Schiff bases as novel antibacterial agents.
The outcomes of this study contribute to the ongoing research in medicinal chemistry, particularly in the development of Schiff base derivatives as potential drug candidates with enhanced pharmacological properties.

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*Corresponding author: Tel. +994556068800
E-mail address: elvinibraqimli7@gmail.com

1. Introduction

Schiff bases were first described by Hugo Schiff approximately 160 years ago and have since been named after him. While their role in coordination chemistry was recognized nearly a century ago, they remain among the most widely used organic compounds, particularly as essential ligands [1]. Schiff bases are the derivative of a ketone or aldehyde, where the carbonyl group is substituted with an imine or azomethine group. It is typically produced by the condensation reaction of a primary amine with an aldehyde.[2] Simplicity of synthesizing and complexing Schiff bases has garnered significant interest from researchers due to their remarkable thermal, mechanical, electrical, and optical properties. Over the past decades, extensive studies have been conducted on conjugated Schiff base polymers and oligomers.[3] Schiff bases are among the most often used organic chemicals, acting as pigments, dyes, catalysts, organic synthesis intermediates, and polymer stabilizers. Schiff bases have been intensively investigated for their different biological functions, with promising results in a variety of medicinal applications. According to research findings, these chemicals have antifungal, antibacterial, antimalarial, antiproliferative, anti-inflammatory, antiviral, and antipyretic activities. Their wide-ranging bioactivity emphasizes their significance in medicinal chemistry, making them excellent candidates for drug research and development. [4]

Imines were first synthesized in the 19th century by Schiff in 1864. Since that time, numerous methods for their preparation have been developed and reported [5]. The traditional synthesis described by Schiff involves the condensation reaction between a carbonyl compound and an amine.[6] The Schiff base reaction results in residual reactive groups, such as aldehydes and amines, within the multilayer films. These remaining groups can undergo further reactions with other substances, allowing for the modification of the product's properties for a wide range of applications.[7]

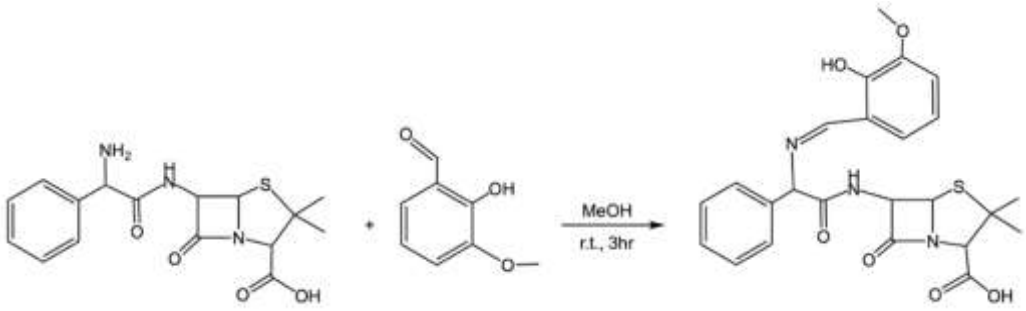
As microorganisms continuously evolve and develop resistance to antibiotics, it is crucial to advance research and improve antibiotics to counteract bacterial resistance. However, there is a notable lack of studies on the synthesis of ampicillin Schiff base derivatives and their metal complexes, as well as the assessment of their antibacterial properties. The antibacterial efficacy of ampicillin is primarily linked to the presence of the imine functional group.[8]

2. Material and methods

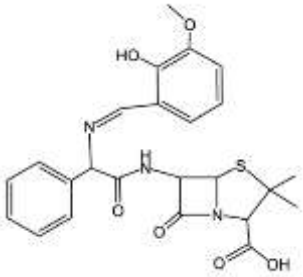
Chemicals. All the chemicals used in this study were of high analytical grade. O-vanillin was purchased from Aldrich and used without further purification. Ampicillin acquired from PanReac AppliChem.

Synthesis. **6-(2-((2-hydroxy-3-methoxybenzylidene)amino)-2-phenylacetamido)-3,3-dimethyl-7-oxo -4-thia-1-azabicyclo [3.2.0] heptane-2-carboxylic acid.** Ampicillin (0.349 g, 1mmol) was dissolved in 5ml methanol and o-vanillin (0.152 g, 1mmol) was dissolved and added. The reaction was continued at room temperature for 3 hours with magnetic stirring. To monitor the progress of the reaction, a sample taken from the mixture was analyzed by Thin Layer Chromatography (TLC). The obtained material was isolated by vacuum evaporation, then purified with diethyl ether. Finally, the structure of the material was confirmed by Nuclear Magnetic Resonance (NMR) spectroscopy. (Scheme 1) Yield 89%. M.p 211^{o}C. **^{1}H NMR spectrum** of targeted compound: (DMSO-d6, δ, ppm), 1.5 s (6H, 2CH_3), 3.73 s (3H, OCH_3), 3.89 s (2H, 2CH), 5.45 s (2H, 2CH), 6.7-7.21 m (3H, Ar), 7.25-7.55 s (5H, Ar), 8.63

s (1H, CH=N) (Figure 1). **^{13}C NMR spectrum** of targeted compound (DMSO-d6, δ, ppm): 27.72 ($2CH_3$), 56.39 (OCH_3), 64.74 (CH), 67.17 ©, 67.39-74.15 (CH), 119.41 ($2CH_{Ar}$), 123.23 (C_{Ar}), 177.75 ($2CH_{Ar}$), 127.94 (CH_{Ar}), 139.27 (CH_{Ar}), 139.28 (CH_{Ar}), 148.49 (C_{Ar}), 150.95 (C_{Ar}), 151.1 (C_{Ar}), 169.97(COO), 170.42 (CH=N), 172,85 (C=O), 173.14 (C=O) (Figure 2). HRMS (ESI- MS): 484.1544 [M+H] + (Figure 3-5).



Scheme 1. Synthesis of o-vanillin based Schiff base.



Scheme 2. Targeted compound.

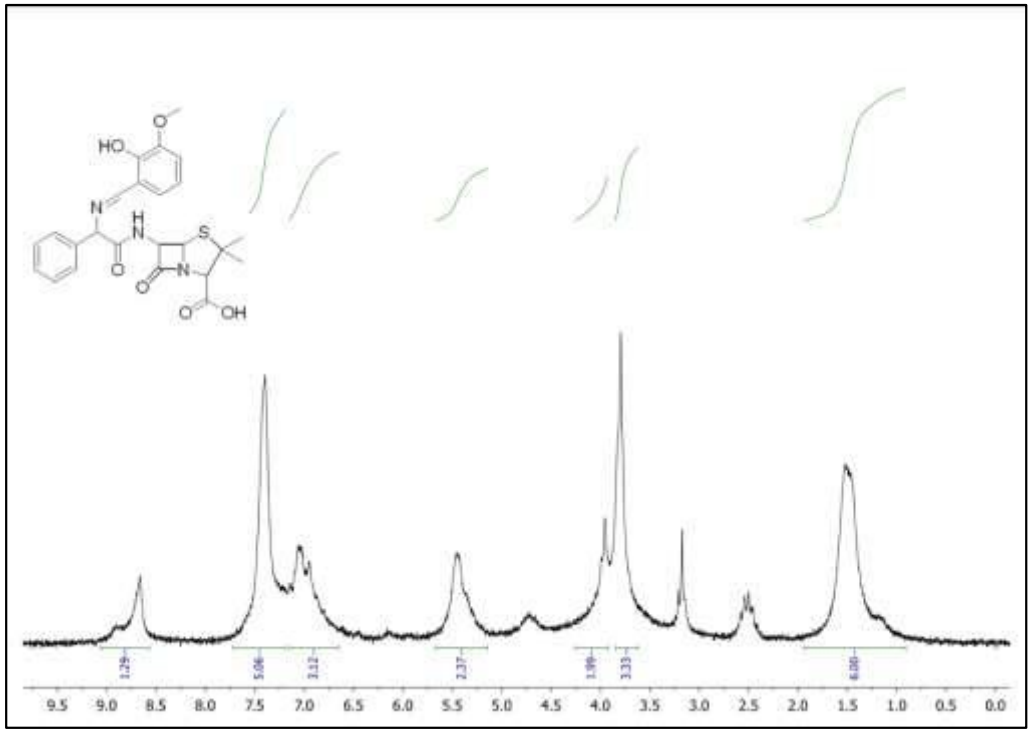


Figure 1. ^{1}H NMR spectrum of compound.

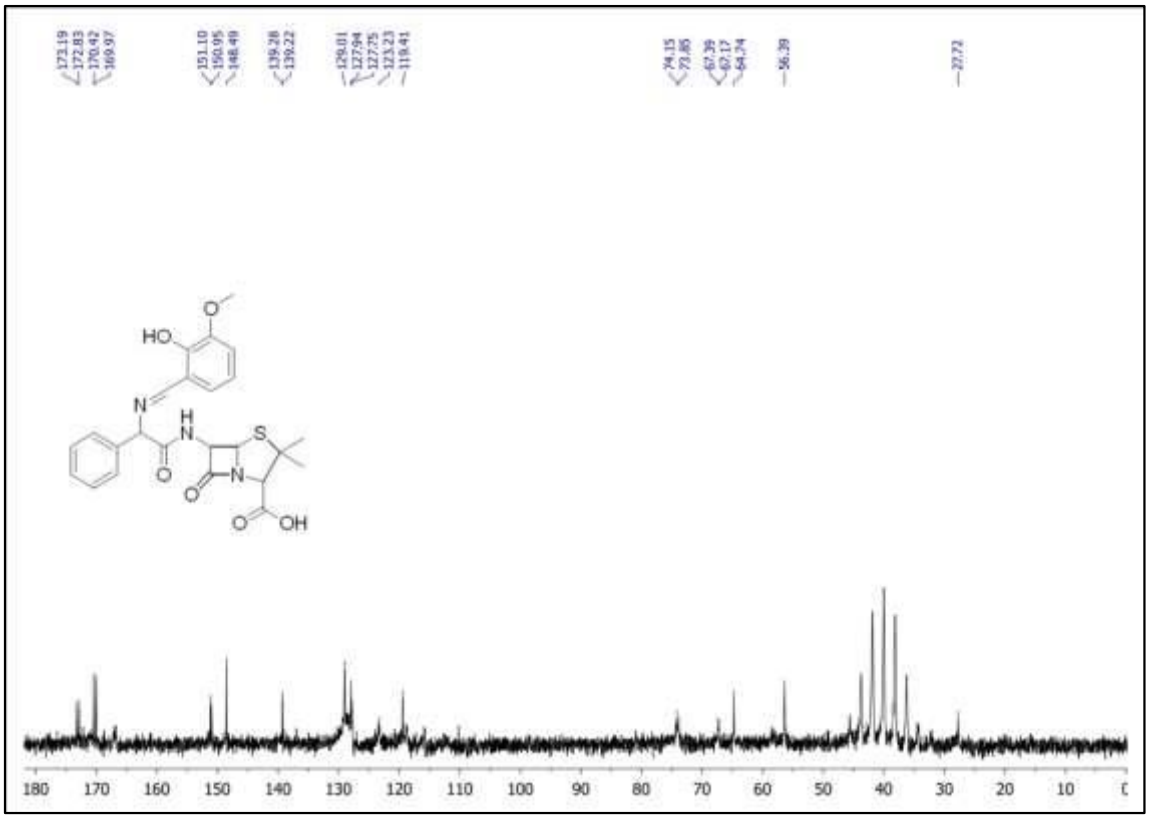


Figure 2. ^{13}C NMR spectrum of compound.

NMR experiments. The NMR experiments were performed on a BRUKER FT NMR spectrometer AVANCE 300 (Bruker, Karlsruhe, Germany) (300 MHz for 1H and 75 MHz for 13C) with a BVT 3200 variable temperature unit in 5 mm sample tubes using Bruker Standard software (TopSpin 3.1). Chemical shifts are given in ppm (δ) and are referenced to internal tetramethylsilane (TMS). Multiplicities are declared as follows: s (singlet), d (doublet), t (triplet), and m (multiplet). Coupling constants J are given in Hz. The experimental parameters for 1H are as follows: digital resolution = 0.23 Hz, SWH = 7530 Hz, TD = 32 K, SI = 16 K, 90° pulse-length = 10 ms, PL1 = 3 dB, ns = 1, ds = 0, d1 = 1 s and for 13C as follows: digital resolution = 0.27 Hz, SWH = 17985 Hz, TD = 64 K, SI = 32 K, 90° pulse-length = 9 ms, PL1 = 1.5 dB, ns = 300, ds = 2, d1 = 3 s. NMR-grade DMSO-d6 (99.7%, containing 0.3% H2O) was used to solubilize the synthesized compound.

MS experiments. High-resolution mass spectrometry (HRMS) was performed using electrospray ionization (ESI) in positive-ion detection mode.

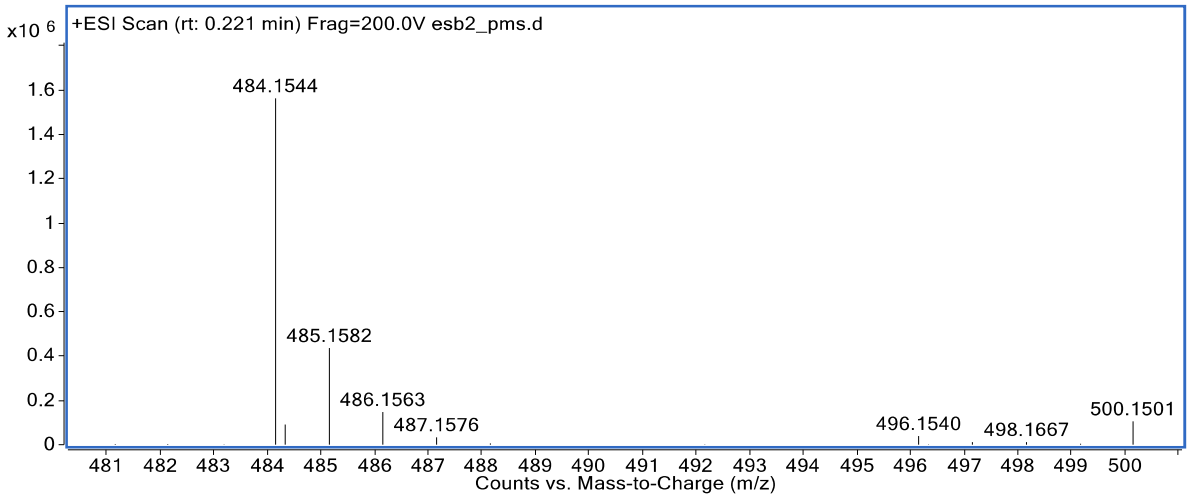


Figure 3. MS spectrum of compound

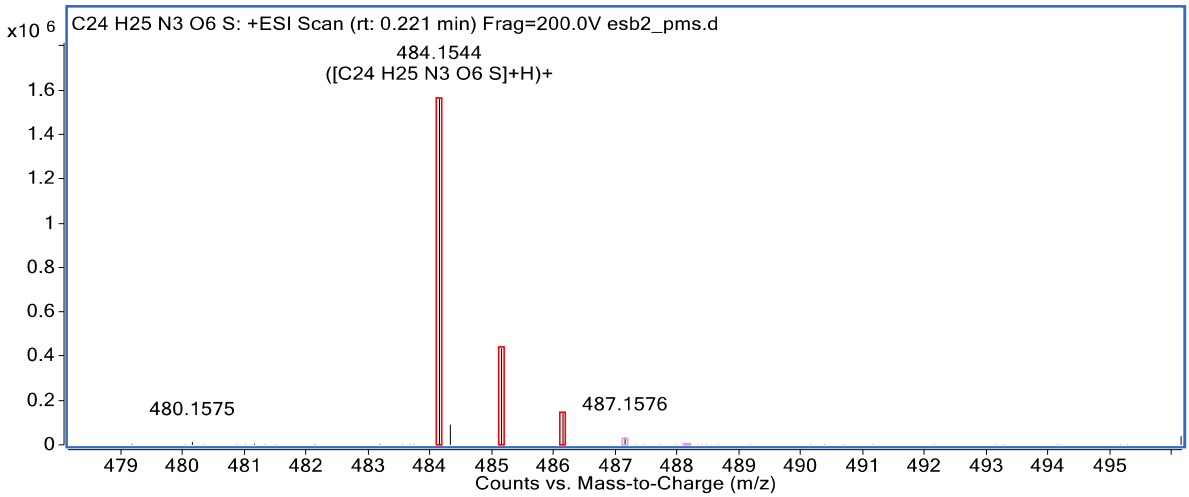


Figure 4. Isotope distribution of compound

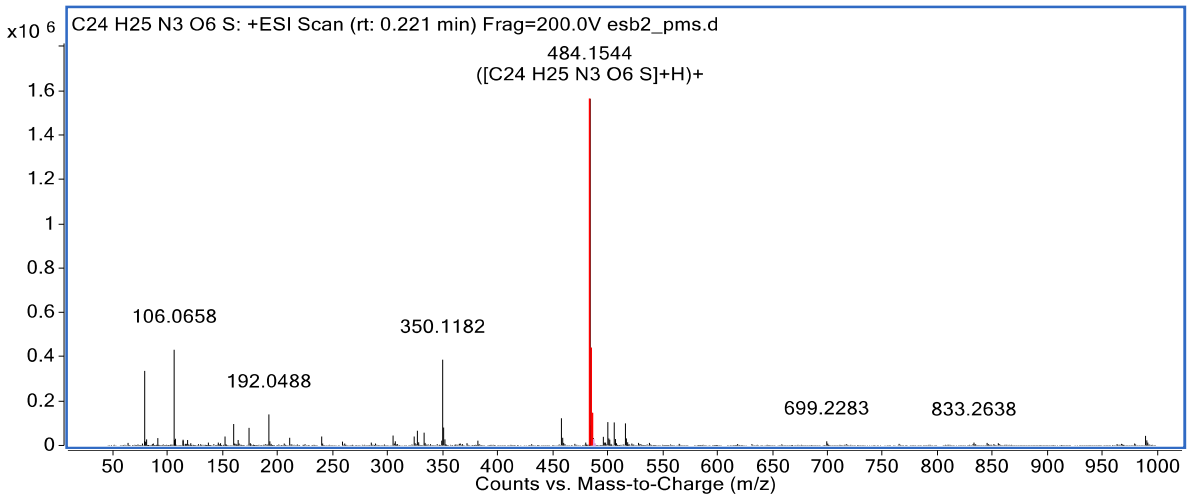


Figure 5. MS spectrum of compound with molecular formula

2.1. Antibacterial activity assay. The antibacterial efficacy of the compound was evaluated against *Staphylococcus aureus* ATCC 6538, *Staphylococcus aureus* ATCC 25923, *Escherichia coli* ATCC 25922, and the clinical isolate *Escherichia coli* BDU32 using a two-fold microdilution assay [9-11]. The assay was developed in accordance with CLSI guidelines. The bacterial strains utilized in the microbiological research were sourced from the culture collection of Baku State University in Azerbaijan. The chemical was prepared in DMSO and followed by dilution in 96-well microtiter plates using Muller Hinton Broth (MHB) from "Liofilchem." The chemical concentration varied from 512 to 4 μg/mL, and the final density of the test cultures was calibrated to 0.5 McFarland using a digital densitometer. Following dilution, bacterial strains were introduced into each well, and the plates were incubated for 24 hours at 37°C. Following incubation, 30 μL of resazurin dye (0.01%) (Sigma Aldrich) was introduced to each well, and the microplates were subsequently returned to the incubator for a further 3-4 hours. The transition from blue to pink is regarded as indicative of bacterial proliferation. The minimum inhibitory concentration (MIC) is defined as the concentration beyond which the tested substances fail to suppress the colour change. The MIC of the investigated chemical was compared with the MICs of ciprofloxacin and ampicillin.

3. Results and discussion

The targeted product **6-(2-((2-hydroxy-3-methoxybenzylidene) amino)-2-phenyl acetamido) -3,3-dimethyl-7-oxo -4-thia-1-azabicyclo [3.2.0] heptane-2-carboxylic acid synthesized by the condensation reaction ampicillin with o-vanillin and as a solvent methanol used. The synthesized product analysed by NMR spectroscopy and high-resolution mass spectrometry. As an NMR spectra** successful formation of the Schiff base linkage (-C=N-). Characteristic proton signal of imine group was observed in 8.63 s (1H, CH=N). The disappearance of the aldehyde proton signal from o-vanillin further confirmed the condensation reaction. Due to the molecular weight in mass spectrometry seen that 484.15 appeared in [M+H] +.

Determination of antibacterial activity of the targeted compound. The minimum inhibitory concentration (MIC) of the targeted compound against the test cultures (*Staphylococcus aureus* ATCC 6538, *Staphylococcus aureus* ATCC 25923, *Escherichia coli* ATCC 25922) was determined using the microdilution method and the resazurin dye [9-12]. The results were compared to those obtained with the control antibiotics such as ciprofloxacin and ampicillin. Interestingly, the bacterial strains were more sensitive to compound than to the controls (Table). *S. aureus* ATCC6538 was the most susceptible, with a MIC value of 4 μg/mL when tested with the targeted compound. The MIC value was 8 μg/mL and 16 μg/mL in case of *S. aureus* ATCC25923 and *E. coli* clinical isolate, respectively. *Escherichia coli* ATCC 25922 was less susceptible to the test compound compare with the ciprofloxacin. However, the MIC value of the ampicillin was 128 μg/mL in case of *E. coli* ATCC 25922 higher than the test compound.

Table 1. Minimum Inhibitory Concentration value (µg/mL) for compound against bacterial strains

Samples	Bacterial strains			
	S. aureus ATCC6538	*S. aureus* ATCC25923	*E. coli* ATCC25922	*E. coli* cl. isolate
Compound	4	8	32	16
Ampicillin	16	16	128	64
Ciprofloxacin	/	/	4	4

The results obtaining that revealed compound is active against *S. aureus* strains compare with the ampicillin, while the MIC value of the antibiotic was 16 µg/mL for both bacterial strains. *E. coli* strains showed resistance against ampicillin, and it is already known that gram negative bacteria are not susceptible against beta lactam antibiotics [12]. In conclusion, the test compound demonstrated greater antibacterial activity against Gram-positive bacteria compared to Gram-negative bacteria. These findings suggest that the compound holds promise for further investigation, particularly in elucidating its mode of action against *Staphylococcus aureus* bacterial strains.

Conflict of interest

The authors declare that they have no conflict of interest in relation to this research.

References

1. Ambike, V., Adsule, S., Ahmed, F., Wang, Z., Afrasiabi, Z., Sinn, E., & Padhye, S. Copper conjugates of nimesulide Schiff bases targeting VEGF, COX and Bcl-2 in pancreatic cancer cells. Journal of Inorganic Biochemistry, 2007, vol. 101, no. 10, pp. 1517–1524. https://doi.org/10.1016/j.jinorgbio.2007.06.028

2. Dalia, S.A., Afsan, F., Hossain, M.S., Khan, M.N., Zakaria, C., Zahan, M.E., & Ali, M. A short review on chemistry of Schiff base metal complexes and their catalytic application. International Journal of Chemical Studies, 2018, vol. 6, no. 3, pp. 2859–2867. https://www.chemijournal.com/archives/?ArticleId=2840&issue=3&vol=6&year=2018

3. Soroceanu, A., & Bargan, A. Advanced and biomedical applications of Schiff-base ligands and their metal complexes: A review. Crystals, 2022, vol. 12, no. 10, pp. 1436. https://doi.org/10.3390/cryst12101436

4. Przybylski, P., Huczynski, A., Pyta, K., Brzezinski, B., & Bartl, F. Biological properties of Schiff bases and azo derivatives of phenols. Current Organic Chemistry, 2009, vol. 13, no. 2, pp. 124–148. https://doi.org/10.2174/138527209787193774

5. Zheng, Y., Ma, K., Li, H., Li, J., He, J., Sun, X., & Ma, J. One pot synthesis of imines from aromatic nitro compounds with a novel Ni/SiO_2 magnetic catalyst. Catalysis Letters, 2009, vol. 128, pp. 465–474. https://doi.org/10.1007/s10562-008-9774-0

6. Abd El-Wahab, H. Synthesis and characterisation of the flame retardant properties and corrosion resistance of Schiff's base compounds incorporated into organic coating. Pigment and Resin Technology, 2015, vol. 44, no. 2, pp. 101–108. https://doi.org/10.1108/PRT-05-2014-0042.
7. Bergbreiter, D.E., & Liao, K.S. Covalent layer-by-layer assembly-an effective, forgiving way to construct functional robust ultrathin films and nanocomposites. Soft Matter, 2009, vol. 5, no. 1, pp. 23–28. https://doi.org/10.1039/B810852H
8. Abbas, A.M., Fisal, S.R., Radwan, A.S., Makhlouf, M.M., & Orabi, A.S. Novel action for ampicillin derivative and its complexes: Physicochemical, thermal analysis, DNA interaction, docking with FabH protein, in silico, and in vitro studies. Journal of Molecular Liquids, 2022, vol. 351, pp. 118333. https://doi.org/10.1016/j.molliq.2021.118333.
9. Huseynzada, A. E., Jelch, C., Akhundzada, H. V. N., Soudani, S., Nasr, C. B., Israyilova, A., & Freccero, M. Synthesis, crystal structure and antibacterial studies of dihydropyrimidines and their regioselectively oxidized products. RSC Advances, 2021, vol. 11, no. 11, pp. 6312–6329. https://doi.org/10.1039/D0RA10255E
10. Israyilova, A., Shoaib, M., Ganbarov, K., Huseynzada, A., Hajiyeva, S., & Ismiyev, A. Antimicrobial activity and time kill curve study of newly synthesized dialkyl carboxylate cyclohexane derivative; A novel anti-Pseudomonas aeruginosa compound. Acta Scientiarum: Technology, 2022, vol. 44, no. 1, pp. 58868.
https://doi.org/10.4025/actascitechnol.v44i1.58868
11. Maia, M.R.G., Marques, S., Cabrita, A.R.J., Wallace, R.J., Thompson, G., Fonseca, A.J.M., & Oliveira, H.M. Simple and versatile turbidimetric monitoring of bacterial growth in liquid cultures using a customized 3D printed culture tube holder and a miniaturized spectrophotometer: application to facultative and strictly anaerobic bacteria. Frontiers in Microbiology, 2016, vol. 7, no. 1, pp. 1381. https://doi.org/10.3389/fmicb.2016.01381
12. Wang, X., Li, H., Chen, Y., Meng, X., Dieketseng, M.Y., Wang, X., & Zheng, G. A neglected risk of nanoplastics as revealed by the promoted transformation of plasmid-borne ampicillin resistance gene by Escherichia coli. Environmental Microbiology, 2022, vol. 24, no. 10, pp. 4946–4959. https://doi.org/10.1111/1462-2920.16178