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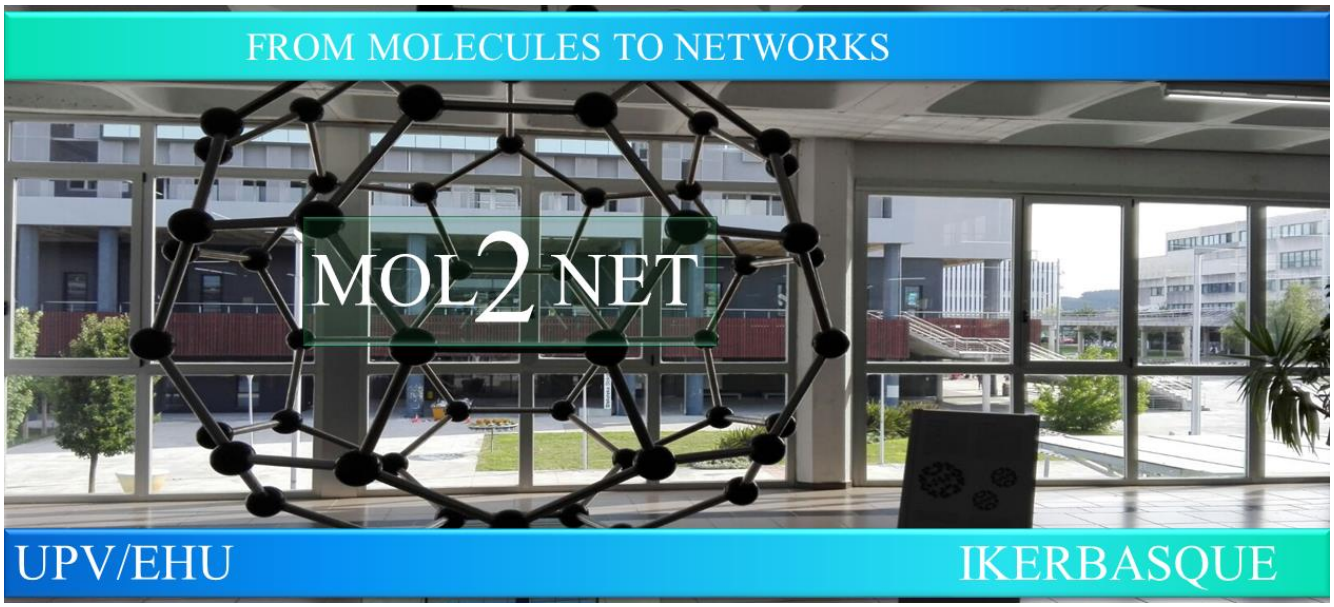

Measurements of the Antibacterial Effect of New Synthetic Aminonitriles

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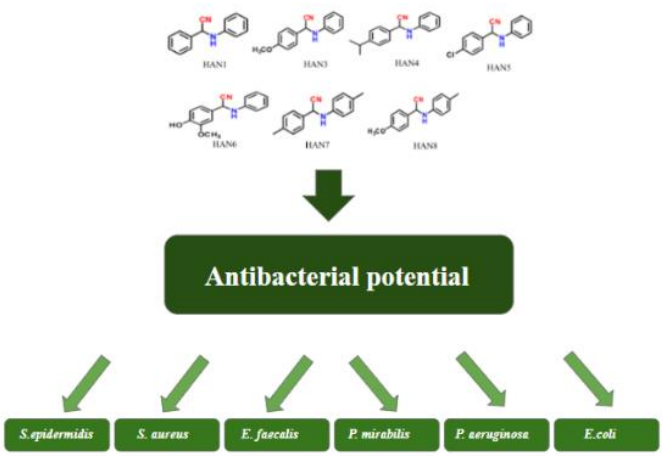
Graphical Abstract



Abstract.

Aminonitriles represent a segment of compounds of natural origin frequently used in synthesis reactions of various organic molecules, having an important role in the acquisition of certain drugs. This characteristic raised the hypothesis that there was intrinsic therapeutic potential to these structures that could be explored scientifically. In this context, studies have identified that the action of this group of molecules goes beyond its participation only as an intermediary for new pharmacological formulations and demonstrates antibiotic activity. Furthermore, reports in the literature show that the potential of aminonitriles includes combating fungi, parasites, bacteria and even tumors. From this perspective, the objective of the research is to screen the antibacterial activity of synthetic aminonitriles against standard bacterial strains. Therefore, the disk-diffusion method was used to carry out the experiments and the tests were carried out using the following bacterial strains: gram-positive *Staphylococcus epidermidis* ATCC 12228, *Staphylococcus aureus* ATCC 25923 and *Enterococcus faecalis* ATCC 29212, and the gram-negative *Pseudomonas aeruginosa* ATCC 27853, *Proteus mirabilis* ATCC 25933 and *Escherichia coli* ATCC 25922. For the test, 7 samples of new aminonitriles were used: HAN1, HAN3, HAN4, HAN5, HAN6, HAN7 and HAN8 at a concentration of 1024 µl and the formation of halos of inhibition of bacterial growth was evaluated. In this context, no antibacterial effect of the substances was identified, since there was no halo formation for any microorganism for the concentration used in the test. Finally, given the relevance of aminonitriles as an intermediate in reactions, there is a need to continue experiments on the antimicrobial potential of these compounds using more sensitive techniques in order to confirm the absence of antibacterial activity.

INTRODUCTION

Antimicrobials have significant prominence in the health sector, since their development, even with penicillin, was a milestone for public health. From this perspective, over the years, these substances have become indispensable for the management of the various current infections. The health context has evolved and with it pathogenic microorganisms, concomitantly, the inadequate use of antibacterials has contributed to the modification of the strain profile and the development of resistance. This circumstance contributed to the intensification of a health problem, since bacteria demonstrated resistance to pioneering drugs, highlighting the need to discover new molecules to combat these microorganisms (1).

In the meantime, non-compliance with therapeutic protocols established in hospital institutions, combined with the COVID-19 pandemic, led to the indiscriminate use of various medications, a circumstance that greatly contributed to the worsening of the microbial resistance health crisis. Faced with this worrying scenario, the need to explore different therapeutic strategies is identified in order to find viable alternatives to combat resistant organisms (2).

Concomitant to these situations, an escalation in the complexity of medications prescribed on an outpatient basis was observed, as substances previously used only in cases of hospital infections began to be administered routinely, further aggravating the current situation of resistance. This situation can be proven by observing evidence of the impact of multi-resistant bacteria demonstrated by the decrease in the use of antibiotics such as Ceftriaxone (50%) followed by the increase in the prescription of drugs such as Amikacin (230%) and Meropenem (131%) (3).

From this perspective, the choice of aminonitriles occurred due to their broad potential as an intermediary in chemical reactions responsible for the formation of versatile substances in the health sector. In this context, the constant presence of aminonitriles in these reactions raised doubts about their therapeutic potential and led to the development of research. In addition, studies have identified the antibacterial, antitumor, antiparasitic and antiprotozoal potential of specimens from this group (4). Therefore, the present work aimed to verify the antibacterial effect of new synthetic aminonitriles.

METHODOLOGY

Materials

To carry out the experiments, DMSO, distilled water, Muller Hinton Agar, BHI and the standard antibiotic were used. To evaluate the activity and sterility of the medium, antibiotic discs were used, gentamicin 10 μg, which was obtained from the company CECON (São Paulo, SP, Brazil).

Bacterial strains

Gram-positive strains *Staphylococcus epidermidis* ATCC 12228, *Staphylococcus aureus* ATCC 25923 and *Enterococcus faecalis* ATCC 29212, and the gram-negative *Pseudomonas aeruginosa* ATCC 27853, *Proteus mirabilis* ATCC 25933 and *Escherichia coli* ATCC 25922.

Bacterial inoculum

To prepare the inoculum, the bacteria were sown in BHI broth and kept in the greenhouse for around 24 hours at a temperature of 35 ± 2°C. After incubation, this sample was seeded in a sterile petri dish containing Muller Hinton Agar and kept in the oven for 24 hours. Then, small bacterial samples were added and homogenized in a saline solution until they reached a concentration of 0.5 McFarland (1×10^8 CFU / mL), which was checked on the turbidimeter.

Antibacterial Activity Screening

According to the method adopted, disk diffusion, a filter paper disk, impregnated with the test substance, was used on the plate sown with the bacterial strain in order to evaluate the formation of the bacterial growth inhibition halo. For this, 6mm diameter discs added with a 10 µL aliquot of aminonitrile were used, at concentrations of 1024, 512, 256, 128 µg/mL. In addition, a 10 µg gentamicin disc was placed on the plate used as a control for the experiment. Culture media were prepared using Muller-Hinton Agar and BHI broth. The results were observed based on the assessment of halo formation.

This method is designed by fixing each disc in the petri dish at an equivalent distance from the center to evaluate the possible formation of halos. After arranging the discs on the plate, it would remain in the oven for another 24-48 hours at a temperature between 35 + 2°C, after which the reading would be done and the presence or absence of halos would be observed in order to evaluate the antimicrobial performance of the compounds. tested.

Statistical analyzes

All experiments were performed in triplicate. The results were subjected to statistical treatment using GraphPad Prism® 9.5.1 software (GraphPad Software, Inc., San Diego, CA). The data obtained were subjected to analysis of variance (ANOVA) and expressed as the mean ± standard deviation.

RESULTS

During screening to determine antibacterial activity, the formation of microbial growth inhibition halos was not identified for any of the bacterial strains, as shown in Table 1.

Table 1. Sensitivity of bacterial strains to aminonitriles using the disk diffusion method

Microrganism	Growth Inhibition Halo Diameter (mm)								
	Aminonitriles (µg/mL)							Gentamicin 10 µg	C*
	HAN 1	HAN 3	HAN 4	HAN 5	HAN 6	HAN 7	HAN 8		
S. aureus ATCC 25923	U.	U.	U.	U.	U.	U.	U.	21,3 ± 0,54	U.
P. mirabilis ATCC 25933	U.	U.	U.	U.	U.	U.	U.	22 ± 1	U.
P. aeruginosa ATCC 27853	U.	U.	U.	U.	U.	U.	U.	23,6 ± 2,3	U.
E. faecalis ATCC 29212	U.	U.	U.	U.	U.	U.	U.	18,6 ± 2,6	U.
S. epidermidis ATCC 12228	U.	U.	U.	U.	U.	U.	U.	33 ± 1	U.
E. coli ATCC 25922	U.	U.	U.	U.	U.	U.	U.	22 ± 1	U.

*C – solvent/diluent control: Discs impregnated with DMSO solution (10%). U: it was not possible to visualize the formation of the bacterial growth inhibition halo in the concentration of the substance used in the disc-diffusion method.

The antibiotic gentamicin was used as a control in the study, and sensitivity was observed for the strains *S. aureus* ATCC 25923, *S. epidermidis* ATCC 12228, *E. faecalis* ATCC 29212, *E. coli* ATCC 25922, *P. mirabilis* ATCC 25933 and *P. aeruginosa* ATCC 27853. A negative control containing a 10% DMSO solution was used, and the formation of halos of inhibition of microbial growth was not observed.

DISCUSSION

Bacterial resistance went beyond the intense concern of researchers and presented itself as a worrying reality in the public health environment, which is aggravated by the reduced effective therapeutic options for eliminating bacteria. It is important to highlight that the evolution of antimicrobials is significantly slow in the face of the emergence of resistant strains. In this context, aminonitriles emerge as a therapeutic possibility to combat pathogenic bacteria (5).

The choice of aminonitriles, as compounds to be studied, occurred due to the versatility of these substances, which were primarily used as mediators of chemical reactions, such as in the development of peptides, proteins and different drugs. They are multifaceted heterocyclic substances that gained prominence due to their constant presence as intermediates and, therefore, aminonitriles

were investigated as an active ingredient. From this perspective, given the scenario of saturation of therapeutic strategies, these compounds emerge as an alternative in the potential combat against strains of the main microorganisms that cause infections (6).

The importance of aminonitriles is justified in the composition and acquisition of various chemical molecules, a fact that highlights their potential as a possible antibiotic. In this sense, it is possible to highlight its role in combating bacteria and co-participation in the synthesis of some antibiotics, as in the case of quinolones. Studies have demonstrated the effectiveness of this group against Staphylococcus aureus and Escherichia coli. Furthermore, a study with α-aminonitriles evaluated antibacterial activity against human pathogenic strains and revealed an effect ranging from moderate to excellent. Examples of the aminonitrile class studied in the literature, in experiments in which the microdilution technique was used, demonstrated the best antibacterial effect, with a Minimum Inhibitory Concentration (MIC) of 3.9 μg/mL against *S. aureus* ATCC 25923 and 7, 8 μg/mL against *E. coli* ATCC 25922 and *S. typhi* ATCC 27870 (7, 8).

According to the research outlined by Khidre *et al*. (2011), when performing the disk diffusion test, it was found that some aminonitrile derivatives showed significant antibacterial activity compared to standard antibiotics, penicillin G and streptomycin. These compounds demonstrated efficacy against *S. aureus* (RCMB 000106) and *B. subtilis* (RCMB 000107), *P. aeruginosa* (RCMB 000102) and *E. coli* (RCMB 000103). Its notable effect in combating *S. aureus* and *E. coli* stands out, with zones of inhibition ranging from 24.4 to 26.4 mm (9).

Although the literature presents favorable results on the antibiotic potential of aminonitriles, the present study did not identify this action in the molecules used. This situation can be elucidated by the possibility of different physicochemical characteristics and interactions of the compounds observed in the present study. The disk-diffusion method, through processes such as the dispersion of substances in agar, may have presented itself as a second limiting factor, which explains the need to apply these compounds to more sensitive tests.

CONCLUSIONS

The results did not reveal sensitivity of standard strains of *E. coli*, *S. aureus*, *S. epidermidis*, *E. faecalis*, *P. mirabilis* and *P. aeruginosas* to aminonitrile samples when tested using the disk diffusion method. There was no formation of an inhibition halo by any of the 7 samples tested against the bacterial strains. In conclusion, aminonitriles have potential as effective antibacterial agents in the literature and open new perspectives to combat bacterial resistance, so it is necessary to continue exploring these compounds with more sensitive evaluation methods and techniques.

Acknowledgments

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References

1. Jo JH, Harkins CP, Schwardt NH, Portillo JA; NISC Comparative Sequencing Program; Zimmerman MD, Carter CL, Hossen MA, Peer CJ, Polley EC, Dartois V, Figg WD, Moutsopoulos NM, Segre JA, Kong HH. Alterations of human skin microbiome and expansion of antimicrobial resistance after systemic antibiotics. Sci Transl Med. 2021 Dec 22;13(625):eabd8077. doi: 10.1126/scitranslmed.abd8077. Epub 2021 Dec 22. PMID: 34936382; PMCID: PMC8878148.
2. Kariyawasam, Ruwandi M et al. "Antimicrobial resistance (AMR) in COVID-19 patients: a systematic review and meta-analysis (November 2019-June 2021)." *Antimicrobial resistance and infection control* vol. 11,1 45. 7 Mar. 2022, doi:10.1186/s13756-022-01085-z
3. Rizvi SG, Ahammad SZ. COVID-19 and antimicrobial resistance: A cross-study. Sci Total Environ. 2022 Feb 10;807(Pt 2):150873. doi: 10.1016/j.scitotenv.2021.150873. Epub 2021 Oct 8. PMID: 34634340; PMCID: PMC8500695.
4. Chaturvedi Devdutt, et al. A Novel Approach to the Synthesis of α-Aminonitriles Using Triphenyl- phosphine Dibromide under Solvent-Free Conditions. SYNLETT [Internet]. 2012 Nov 02 [cited 2022 Apr 5]; DOI 10.1055/s-0032-1317690. Available from: https://www.thieme-connect.com/products/ejournals/abstract/10.1055/s-0032-1317690
5. Almeida, H.M.D.S.; Oliveira, I.d.; Ferreira, S.B. Aminonitrile Potential in Terms of Pharmacological and Clinical Applicability, in Proceedings of the MOL2NET'21, Conference on Molecular, Biomedical & Computational Sciences and Engineering, 7th ed., 25 January 2021–30 January 2022, MDPI: Basel, Switzerland, doi:10.3390/mol2net-07-09258 Available from: https://sciforum.net/manuscripts/9258/manuscript.pdf]
6. Alkhzem, Abdulaziz H et al. "Practical Synthesis of Polyamine Succinamides and Branched Polyamines." *ChemistryOpen* vol. 11,10 (2022): e202200147. doi:10.1002/open.202200147
7. Chaturvedi Devdutt, et al. A Novel Approach to the Synthesis of α-Aminonitriles Using Triphenyl- phosphine Dibromide under Solvent-Free Conditions. SYNLETT [Internet]. 2012 Nov 02 [cited 2022 Apr 5]; DOI 10.1055/s-0032-1317690. Available from: https://www.thieme-connect.com/products/ejournals/abstract/10.1055/s-0032-1317690
8. Shaikh Irfan, et al. Design, synthesis, and evaluation of new α-aminonitrile-based benzimidazole biomolecules as potent antimicrobial and antitubercular agents. Arch Pharm [Internet]. 2018 Jan 22 [cited 2022 Mar 8]; DOI 10.1002/ardp.201700205. Available from: https://pubmed.ncbi.nlm.nih.gov/29356105/.
9. Khidre, Rizk E., et al. "Synthesis and Anti-Microbial Activity of Some 1- Substituted Amino-4,6-Dimethyl-2-Oxo-Pyridine-3-Carbonitrile Derivatives." *European Journal of Medicinal Chemistry*, vol. 46, no. 10, Oct. 2011, pp. 5057–5064, https://doi.org/10.1016/j.ejmech.2011.08.018 . Accessed 6 Jul. 2023.