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ANTIBACTERIAL ACTIVITY OF NEW β-LACTAM COMPOUND

Bonomo, M.G.[1*, **]; Calabrone, L.[1*]; Saturnino, C.[1]; Sinicropi, M.S.[2]; Capasso, A.[3]; Salzano, G.[1]

[1]Università degli Studi della Basilicata, Dipartimento di Scienze, Viale dell'Ateneo Lucano 10, 85100 Potenza, Italy

[2]Università della Calabria, Dipartimento di Farmacia e Scienze della Salute e della Nutrizione, Via Pietro Bucci, 87036 Arcavacata di Rende (Italy)

[3]*Dipartimento di Farmacia, Università degli Studi di Salerno - Via Giovanni Paolo II 132, 84084 Fisciano (SA), Italy*

[*]*these authors contributed equally to this work*

[*]mariagrazia.bonomo@unibas.it

Abstract

A new β-lactam molecule A1 was synthesized and formulated to introduce an innovative solution in order to circumvent the bacterial resistance and their defenses against antibiotics. This innovative molecule was tested towards representative Gram positive and Gram negative strains of antibiotic-resistant pathogenic bacteria to evaluate its antibacterial activity and to analyze the action spectrum and the antibacterial effectiveness. Results showed that the new compound A1 was synthesized with a yield of 55% and demonstrated the antimicrobial activity against all tested bacteria, except *Pseudomonas aeruginosa*, and all Gram-positive bacteria were more sensitive with a good antimicrobial activity. Moreover, as the determined MIC_{90}, both strains of the *Streptococcus* genus were inhibited at a concentration of 16 µg/ml of the compound A1, while *Staphylococcus aureus* resulted sensitive to an inhibitory concentration of 64 µg/ml. The Gram-negative bacteria, instead, required an inhibitory concentration of 64 µg/ml for *Escherichia coli* and 128 µg/ml for *Klebsiella pneumoniae*. In conclusion, compound A1 is a promising molecule to develop a new class of safe and active antibiotics, with a predicted good inhibitory capacity not only against Gram positive, but also Gram negative microorganisms.

Keywords: *β-lactam compound; chemical synthesis; antibacterial activity; minimum inhibitory concentration*

Introduction

Over the last decades, the decline in the discovery of novel antimicrobial molecules and the irresponsible misuse/overuse of traditional antibiotics has led to the selection of multidrug resistant bacteria, that are now considered as a main threat for global public health [1]. The continuous emergence and proliferation of pathogenic bacteria resistant to many or all current antibiotics is a major public health concern and one of the particular importance in clinical settings. Despite many initiatives to deal with this problem, new resistance mechanisms are emerging and spreading globally, resulting in a worldwide health crisis of global dimensions and infections caused by multidrug-resistant bacteria are becoming particularly difficult to treat. Therefore, there is the urgency to focus the research either on the development of new antimicrobial molecules able to escape to the bacterial mechanisms of antibiotic resistance, or to counteract the resistance against the most commonly used antibiotics [2- 7].

β-Lactam antibiotics are still the most used class of antibacterial agents for the treatment of a wide range of human bacterial diseases, because of their pharmacological advantages, such as potent activity combined with relatively low toxicity, and also for their ease of delivery and low production costs. [5; 8]. Their targets are the penicillin-binding proteins (PBPs), a large family of enzymes not present in eukaryotes, which are involved in the synthesis and maintenance of the peptidoglycan, the main component of the bacterial cell wall. All PBPs share a common DD-peptidase activity that could be either a DD-transpeptidase, a DD-carboxypeptidase or a DD-endopeptidase activity, catalyzed by a common domain (the so-called penicillin-binding (PB) domain), made of two subdomains with the active site lying at the interface between them. The reactions catalyzed by the PB domain follow a three-step mechanism with the formation of an acyl-enzyme covalent intermediate through a Ser residue conserved in all the PBPs. There are many classes of these enzymes, depending on their molecular mass, structure and different additional activities held by other domains possibly present in their structures, and there are many different PBPs in each bacterial strain [9-12]. The beta lactam antibiotics resemble to the natural substrate of PBPs (the D-Ala-D-Ala dipeptide that ends the pentapeptide precursors of the peptidoglycan and that forms the crosslinks between the glycan strands of the peptidoglycan), thus they act as suicide inhibitors for these enzymes. Indeed, the catalytic Ser residue attacks the carbonyl of the beta lactam ring, with the formation of a covalent acyl-enzyme complex that is hydrolyzed at a very low rate, therefore preventing further reactions. Blocking enzyme activity has a lethal effect because it produces the inhibition of the growth of the bacteria, or their lysis via several mechanisms that involve a global imbalance of the cell wall metabolism [10; 13; 14].

Since the discovery of benzylpenicillin in the 1920s, thousands of new penicillin derivatives and related β-lactam classes of cephalosporins, cephamycins, monobactams, and carbapenems have been developed, either to broaden their spectrum of action, so as to include additional bacterial species, or to improve some pharmacological features and then to address specific resistance mechanisms that have arisen in the targeted bacterial population. The most common way by which bacteria defend themselves from beta lactam antibiotics is the synthesis of β-lactamases, a family of hydrolytic enzymes whose genes are mainly located on easily transferable plasmids and, as a consequence, have rapidly disseminated through the different bacterial species [15]. These enzymes catalyze the cleavage of the β-lactam ring destroying its antibacterial properties; the intact β-lactam core is essential either to mimic the structure of the D-Ala-D-Ala dipeptide, thereby facilitating the binding to the active site of PBPs, and to increase the reactivity of these molecules towards linear amides, allowing them to compete efficiently with the natural substrate for the reaction of acylation. [5; 15-17].

While the efforts to find new antibacterial drugs proceed, it is essential to develop new molecules belonging to the existing classes of antibiotics, trying to introduce innovative solutions in order to bypass the bacterial defenses against them [15; 18-20]. The newest effort to circumvent resistance is the development of novel broad-spectrum β-lactamase inhibitors that work against many problematic β-lactamases, including cephalosporinases and serine-based

carbapenemases, which severely limit therapeutic options [13; 21-23].
Currently, an alternative approach that sounds effective to combat drug-resistant strains is to combine new compounds with certain existing antibacterial drugs. The key of combination therapies is to search for synergistic effects of the compounds with different mode of action, which has been widely used to treat infectious diseases. For example, b-lactamase inhibitors such as clavulanic acid and tazobactam have been successfully combined with b-lactam antibiotics to treat drug-resistant bacterial infections. [15 ; 24].
In this study, a new antibacterial drug was synthesized, a new way of formulating a molecule has been conceived by putting the antibiotic and the beta-lactamase inhibitor on the same molecule. This innovative molecule was tested towards representative strains of antibiotic-resistant pathogenic bacteria to evaluate its antibacterial activity and to analyze the action spectrum and the antibacterial effectiveness.

Methods

General chemical procedure

Commercial reagents were purchased from Sigma-Aldrich (St. Louis, MO) and used without additional purification. Melting points were determined by open capillary using the digital melting point apparatus and are uncorrected. Silica gel 60 (300-400 mesh, Merck, Kenilworth, NJ) was used for flash chromatography. Preparative TLC was performed on 20x20 cm glass plates coated with a 2mm layer of silica gel PF254 Merk. ^{1}HNMR and ^{13}CNMR spectra were recorded on a Bruker Avance 400 spectrometer (Bruker Corporation, Billerica, MA). Chemical shifts are expressed in parts per million downfield from tetramethylsilane as an internal standard. Multiplicities are represented by s (singlet), d (doublet), t (triplet), q (quartet) and m (multiplet). Coupling constants (J) are reported in Hertz (Hz). The new synthesized compound (label A1) was dissolved in dimethyl sulfoxide (DMSO) to obtain a stock 10 µg/ml solution.

Yield and spectral data of the compound

Pale yellow color, semi solid; Yield: 55%; 1H-NMR: 8.00 s (2H,HAr); 7.20-7.33 m (2H,HAr); 5.45 s (1H,CH); 5.10 s (1H,CH-S); 3.50-3.45m (2H, CH2-N); 3.20-3.30 m (2H,CH2-N); 3.10 s (2H, CH2-S); 2.22-2.26 m (2H,CH2-CO-NH-); 2.02s (3H,CH3);1.59-1.55 m (6H,CH2);1.32 s (6H,2CH3); 1.28 m (2H,CH2).

Antibacterial activity assay

Bacterial strains and growth conditions

A1 was tested for in vitro antibacterial activity against representative Gram positive and Gram negative strains of antibiotic-resistant pathogenic bacteria: *Staphylococcus aureus* (ATCC25923), *Streptococcus pyogenes* (ATCC19615), *Streptococcus pneumoniae* (ATCC6303), *Escherichia coli* (ATCC25922), *Klebsiella pneumoniae* (ATCC13883), *Pseudomonas aeruginosa* (ATCC27853). *Staphylococcus aureus, Escherichia coli* and *Pseudomonas aeruginosa* strains were cultured in tryptone soya yeast extract medium, while the other strains in blood agar medium. All strains were cultivated at 34-37°C for 24 h under aerobic conditions before the assay.

Agar well diffusion assay and Minimum Inhibitory Concentration

Antimicrobial activity was determined by standard agar well diffusion assay [25]. For each strain, a subculture in a specific broth was obtained from the active stock culture by 1% (v/v) inoculum and incubated overnight at the corresponding culture temperature. 200 µl of each subculture was used to inoculate the agar media (to achieve a final concentration of 10^{8} CFU/ml) and distributed into Petri plates. 60 µl of A1 was poured into wells (6 mm diameter) bored in the agar plates and then the plates were incubated at optimal growth conditions for each strain. The synthesized compound A1 was dissolved in DMSO (Sigma) and the evaluated concentration was 10 µg/ml in the total solution composition. Drug-free control, sterility control and control consisted of DMSO alone were included.
The experiment was performed in triplicate and the antimicrobial activity of the compound was expressed in terms of zone of inhibition diameter mean (in mm) produced after 24 h of incubation. Then, A1 was screened to determine minimum inhibitory concentrations (MICs) in order to evaluate the antimicrobial effectiveness of compound against different bacterial strains [20]. Each specific medium inoculated with the strain subculture was added with different concentrations of the new

compound, ranging from 0.25 µg/ml to 256 µg/ml, and incubated for 24 h. After incubation, 200 µl of each subculture was transferred to 96-well plate to measure OD by microplate reader (PerkinElmer multilabel counter) and the MIC was calculated as the lowest concentration of the extract inhibiting the growth of bacterial strains. The MIC values were done in triplicate. The results are summarized in Table 1. Moreover, Table 2 and Table 3 show the comparison between the A1 and the most common cephalosporins used as antibiotics.

Results

Chemistry

The new compound was synthesized by a initial reaction of equivalent amounts of an amine and a ketone in dioxane, that reacted for 1 h in the presence of hot pyridine (dioxane boiling temperature). When the reaction has taken place, the solvent was evaporated to dryness, washed with water, left to dry and resumed in the minimum amount of DMF; then it was added with Na_2CO_3 and heated to 140°C for 1 h. The azetidinone obtained was solubilized in anhydrous acetone at 0°C, then TEA was added and stirred at room temperature. After 30 minutes 7-ACA, solubilized in anhydrous acetone, was added and the mixture was stirred at 0°C for 2 hours, obtaining the final compound (Fig.1). The final product was recovered by filtration and purified by crystallization in anhydrous solvent with a yield of 55%.

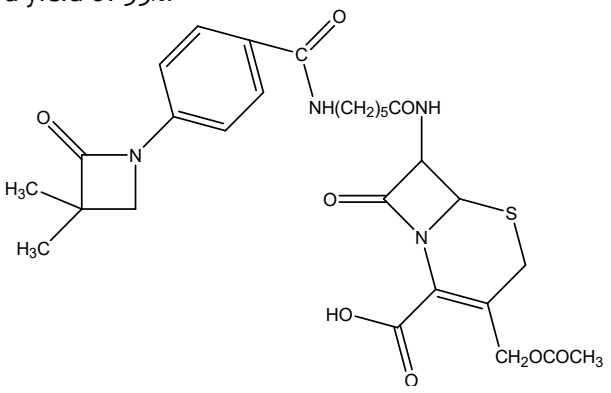

Fig. 1 Structure of the new compound A1

Antibacterial activity

The antimicrobial activity and the MIC of A1 were evaluated by using the agar well diffusion assay against selected bacterial strains, representative of the most common antibiotic-resistant pathogenic bacteria that have shown resistance to the most common antibiotics. Gram-negative and Gram-positive bacteria were employed as preliminary screening microorganisms to determine the antimicrobial effect, the action spectrum and the antimicrobial effectiveness of the new synthesized compound.

Results showed that A1 demonstrated the antimicrobial activity against all tested bacteria, except *Pseudomonas aeruginosa*, and all Gram-positive bacteria were more sensitive with a good antimicrobial activity. Among Gram-positive bacteria, the most sensitive strain was *Staphylococcus aureus* with an inhibition zone of 25.34 mm, whereas for the other gram-positive strains, *Streptococcus pyogenes* and *Streptococcus pneumoniae*, an slightly lower activity with an inhibition zone of 24.42 and 24.58 mm, respectively, was observed (Table 1). Instead, among the Gram-negative bacteria, *Escherichia coli* was the most sensitive strain with an inhibition zone of 26.11 mm; while for *Klebsiella pneumoniae* a slightly lower activity was observed, with 23.32 mm inhibition zone, and then for *Pseudomonas aeruginosa* no activity was detected (Table 1).

Moreover, as the determined MIC_{90}, both strains of the *Streptococcus* genus were inhibited at a concentration of 16 µg/ml of the compound A1, while *Staphylococcus aureus* resulted sensitive to an inhibitory concentration of 64 µg/ml. The Gram-negative bacteria, instead, required an inhibitory concentration of 64 µg/ml for *Escherichia coli* and 128 µg/ml for *Klebsiella pneumoniae*.

The comparison of the new compound with the most common cephalosporins was also carried out and the results were shown in Table 2 and Table 3. The table shows the antibacterial activity and the MIC_{50} and MIC_{90} of each compound valued as the concentration that inhibits 50% and 90% of the isolates, respectively.

Discussion

A new antibacterial drug was synthesized and characterized, a new β lactam molecule has been thought and formulated to introduce an innovative solution in order to circumvent the bacterial resistance and their defenses against antibiotics. This innovative molecule was tested towards

representative strains of antibiotic-resistant pathogenic bacteria to evaluate its antibacterial activity and to analyze the action spectrum and the antibacterial effectiveness.

Among clinically important Gram-negative bacteria, the production of β-lactamases is the most frequent factor contributing to β-lactam resistance [26]. The molecular classification of β -lactamase is based on the amino acid sequence and divides β-lactamases into class A, C and D enzymes that use a serine residue as the nucleophilic species to attack the lactam carbonyl group, forming an acyl enzyme intermediate before hydrolysis [27]. A new group of class A extended-spectrum β-lactamases (ESBLs), called CTX-M enzymes, has emerged worldwide and hydrolyses not only the penicillin, but also the first-, second and third-generation cephalosporins and the current lack of effective treatment options is the reason for increasing number of invasive bacterial infections [7].

Of great concern is the increasing capacity of the bacteria to frequently host multiple β-lactamases with different substrate profiles, making practically all β-lactam antibiotics ineffective.

Moreover, β-lactamases have also evolved resistance to inhibitors and this has motivated several research groups to search for new compound and new β-lactam/ β-lactamase inhibitor combinations with a broad-spectrum inhibition profile [28, 7].

Our scope was to achieve a new β-lactam compound having a suicidal side-chain inhibitor in order to counteract the β-lactamases that are among the major responsible for inactivating the azetidinone ring with a wider spectrum of action, possibly covering also multidrug resistant bacterial strains. This compound proved to have an antibacterial activity towards Gram positive bacteria, at concentrations comparable or even better than those obtained for the reference cephalosporin antibiotics widely used in clinics. Noteworthy, compound A1 showed better antimicrobial activity compared with cefixime against *Staphylococcus aureus*, one of the main pathogens responsible for a number of infections in hospital settings, with considerable morbidity and mortality.

This preliminary assessment will allow to discover the interaction with the target and to understand how to modulate this new combination and so its affinity towards the target.

In conclusion, compound A1 is a promising molecule to develop a new class of safe and active antibiotics, with a predicted good inhibitory capacity not only against Gram positive, but also Gram negative microorganisms.

References

1. World Health Organization (WHO). Antimicrobial resistance: global report on surveillance. Geneva: WHO Press; 2014
2. Wright, G.D., Antibiotics: a new hope. Chem Biol 2012;19:3-10
3. vanDuin, D., Bonomo, R.A., Ceftazidime/Avibactam and Ceftolozane/Tazobactam: Second-generation β-Lactam/β-Lactamase Inhibitor Combinations. Clin Infect Dis 2016;63:234-241
4. Fang, Z., Ban, L., Li, Y., et al., A quinoline-based FtsZ inhibitor for the study of antimicrobial activity and synergistic effects with β-lactam antibiotics. J Pharmacol Sci 2018;137:283-289
5. Verdino, A., Zollo, F., De Rosa, M., et al., Computational analysis of the interactions of a novel cephalosporin derivative with β-lactamases. BMC Struct Biol 2018;18:13
6. Saturnino C., Caruso, A., Iacopetta, D., et al., Inhibition of human Topoisomerase II by new N,N,N-trimethylethanammonium iodide alkylcarbazole derivatives. Chem Med Chem, 2018;13:2635-2643
7. Skŏcibušíc, M., Odžak, R., Ramíc, A., et al., Novel imidazole aldoximes with broad-spectrum antimicrobial potency against multidrug resistant gram-negative bacteria. Molecules 2018;23:1212
8. Bush, K., Bradford, P.A., β-Lactams and β-Lactamase Inhibitors: An Overview. Cold Spring Harb Perspect Med 2019;6:a025247

9. Saturnino, C., Buonerba, M., Martini, S., et al., "Azetidinone structures: Antibacterial activity" Biomed Res 2004;15(2):100-102

10. Bush, K., Jacoby, G.A., Updated functional classification of beta-lactamases. Antimicrob Agents Chemother 2010;54:969-976.

11. Bonomo, M.G., Milella, L., Martelli, G., Salzano, G., Stress response assessment of *Lactobacillus sakei* strains selected as potential autochthonous starter cultures by flow cytometry and nucleic acid double staining analyses. J Appl Microbiol 2013;115(3):786-795

12. King, D.T., Sobhanifar, S., Strynadka, N.C.J., One ring to rule them all: current trends in combating bacterial resistance to β-lactams. Protein Sci 2016;25:787-803

13. Bush, K., Bradford, P.A., β-Lactams and β-Lactamase Inhibitors: An Overview. Perspect Med 2019;1-23

14. Cafaro, C., Bonomo, M.G., Guerrieri, A., et al., Assessment of the genetic polymorphism and physiological characterization of indigenous *Oenococcus oeni* strains isolated from Aglianico del Vulture red wine. Folia Microbiol 2016;61:1-10

15. Drawz, S.M., Bonomo, R.A., Three decades of beta-lactamase inhibitors. Clin Microbiol Rev 2010;23:160-201.

16. Verdino, A., Vigliotta, G., Giordano D, et al., Synthesis and biological evaluation of the progenitor of a new class of cephalosporin analogues, with a particular focus on structure-based computational analysis. PLoS ONE 2017;12(7): e0181563.

17. Bonomo, M.G., Cafaro, C., Guerrieri ,G., et al., Flow cytometry and capillary electrophoresis analyses in ethanol-stressed *Oenococcus oeni* strains and changes assessment of membrane fatty acids composition. J Appl Microbiol 2017;122(6):1615-1626

18. Paesano, N., Marzocco, S., Vicidomini, C., et al., Synthesis and biological evaluation of 3-benzyl-1-methyl-and 1-methyl-3-phenyl-isotioureas as potential inhibitors of iNOS Bioorganic Med Chem Lett 2005;15:539-543

19. Saturnino, C., Buonerba, M., Capasso, A., Synthesis and biological activity of new class of azetidinones compounds as potential HLEInhibitors Lett Drug Des Disc 2007;4:484-486

20. Bonomo, M.G., Di Tomaso, K., Calabrone, L., Salzano, G., Ethanol stress in *Oenococcus oeni*: transcriptional response and complex physiological mechanisms. J Appl Microbiol 2018;125:2-15

21. De Rosa, M., Vigliotta Giovanni, Palma, G., Saturnino, C., Soriente, A., Novel penicillin-type analogues bearing a variable substituted 2-azetidinone ring at position 6: synthesis and biological evaluation. Molecules 2015;20:22044-22057

22. De Rosa, M., Bonomo, M.G., Vassallo, A., et al., Linezolid and its derivatives: the promising therapeutic challenge to multidrug-resistant pathogens. PharmacologyOnLine 2018;2:134-148

23. Napoli, M., Saturnino, C., Cianciulli, E.I., Silver(I)N-heterocyclic carbine complexes: Synthesis, characterization and antibacterial activity. J Organomet Chem 2013;725: 46-53

24. Fang, Z., Ban, L., Li, Y., et al., A quinoline-based FtsZ inhibitor for the study of antimicrobial activity and synergistic effects with β-lactam antibiotics. J Pharmacol Sci 2018;138:283-289

25. Bonomo, M.G., Cafaro, C., Russo, D., et al., Antimicrobial activity, antioxidant properties and phytochemical screening of *Aesculus hippocastanum* mother tincture against food-borne bacteria. Letters in Drug Design & Discovery, 2019 (ahead of print)

26. Boucher, H.W., Talbot, G.H., Benjamin, D.K.Jr., et al., 10x'20 Progress—development of new drugs active against gram-negative bacilli: An update from the Infectious Diseases Society of America. Clin Infect Dis 2013;56:1685-1694

27. Bush, K., Alarming β-lactamase-mediated resistance in multidrug-resistant Enterobacteriaceae. Curr Opin Microbiol 2010;13:558-64

28. Lagacé-Wiens, P.R.S., Tailor, F., Simner, P., et al., Activity of NXL104 in combination with β-lactams against genetically characterized *Escherichia coli* and *Klebsiella pneumoniae* isolates producing class A extended-spectrum β-Lactamases and class C β-Lactamases. Antimicrob Agents Chemother 2011;55:2434–2437

Bacteria	Inhibition zone (diameter in mm)	Concentration of A1 (µg/ml)[a]											MIC_{90} (µg/ml)[b]
	A1	0.25	0.5	1	2	4	8	16	32	64	128	256	
Gram-positive bacteria													
Staphylococcus aureus ATCC 25923	25.34 ± 1.23	-	-	-	-	-	+	+	+	+	+	+	64 ± 1.76
Streptococcus pyogenes ATCC 19615	24.42 ± 0.99	-	-	-	-	-	-	+	+	+	+	+	16 ± 1.33
Streptococcus pneumoniae ATCC 6303	24.58 ± 1.07	-	-	-	-	-	-	+	+	+	+	+	16 ± 0.82
Gram-negative bacteria													
Escherichia coli ATCC 25922	26.11 ± 0.12	-	-	-	-	-	-	-	-	+	+	+	64± 0.36
Klebsiella pneumoniae ATCC 13883	23.32 ± 0.28	-	-	-	-	-	-	-	-	-	+	+	128 ± 1.08
Pseudomonas aeruginosa ATCC 27853	NI												

NI: no inhibition zone was observed. Values of the observed zone of inhibition (in mm diameter) including the diameter of well (6 mm) after 24 h of incubation against different bacterial species. Assay was performed in triplicate and results are the mean of three values ± Standard Deviation.
[a] Different concentrations of the new compound evaluated as described by Bonomo et al. (2018).
All values are expressed in µg/ml; (–) represents "no inhibition observed"; (+) represents "inhibition observed".
[b] Values are presented as mean ± standard deviation.

Table 1. Antibacterial activity and minimum inhibitory concentration of A1 compound.

Bacteria / compounds	Inhibition zone (diameter in mm)					
	Staphylococcus aureus ATCC 25923	*Streptococcus pyogenes* ATCC 19615	*Streptococcus pneumoniae* ATCC 6303	*Escherichia coli* ATCC 25922	*Klebsiella pneumoniae* ATCC 13883	*Pseudomonas aeruginosa* ATCC 27853
A1	25.34 ± 1.23	24.42 ± 0.99	24.58 ± 1.07	26.11 ± 0.12	23.32 ± 0.28	NI
Ceftazidime	23.30 ± 0.88	30.66 ± 0.98	28.56 ± 1.02	27.87 ± 0.41	20.67 ± 0.08	27.33 ± 0.82
Cefotaxime	27. 57± 0.76	32.89 ± 0.68	33.90 ± 0.26	30.74 ± 0.98	32.32 ± 0.99	22.78 ± 0.38
Cefixime	NI	27.58 ± 0.79	24.77 ± 0.59	26.55 ± 0.44	34.54 ± 0.22	NI

NI: no inhibition zone was observed. Values of the observed zone of inhibition (in mm diameter) including the diameter of well (6 mm) after 24 h of incubation against different bacterial species.
Assay was performed in triplicate and results are the mean of three values ± Standard Deviation.

Table 2. Antibacterial activity of A1 compound and of the most common cephalosporins used as antibiotics.

Bacteria / compounds	MIC (µg/ml)									
	Staphylococcus aureus ATCC 25923		*Streptococcus pyogenes* ATCC 19615		*Streptococcus pneumoniae* ATCC 6303		*Escherichia coli* ATCC 25922		*Klebsiella pneu* ATCC 138	
	MIC_{50}	MIC_{90}	MIC_{50}	MIC_{90}	MIC_{50}	MIC_{90}	MIC_{50}	MIC_{90}	MIC_{50}	M
A1	8	64	4	16	2	16	32	64	64	
Ceftazidime	2	8	0.25	0.5	0.25	0.5	0.25	2	4	
Cefotaxime	2	4	0.5	1	0.5	1	0.5	4	1	
Cefixime	NI	NI	0.25	0.5	0.25	0.5	0.25	1	0.5	

NI: no inhibition zone was observed.
Assay was performed in triplicate and results are the mean of three values ± Standard Deviation.

Table 3. MICs of A1 compound and of the most common cephalosporins used as antibiotics.