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Article

Synergistic antibacterial effect of statins with the complex {[1-(4-bromophenyl)-3-phenyltriazene N_3-oxide-κ^2 N^1,O^4] (dimethylbenzylamine-κ^2 C^1,N^4)palladium(II)}

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The treatment of infections caused by resistant microorganisms represents a big challenge in healthcare due to limited treatment options. For this reason, the discovery of new active substances which are able to perform innovative and selective actions is of great impact nowadays. Statins and triazenes (TZC) have consolidated as a promising class of compounds, characterized by the expressive biological activity, especially antimicrobial activities. The aim of this study was to assess the in vitro synergistic antibacterial effect of the association of statins and a new TZC complex {[1-(4-bromophenyl)-3-phenyltriazene N_3-oxide-κ^2 N^1,O^4](dimethylbenzylamine-κ^2 C^1,N^4)palladium(II)} (**Pd(DMBA)LBr**) against American Type Culture Collection (ATCC) strains and clinical isolates. The complex and the statins showed bacterial activity of all tested strains and clinical isolates, evidencing that TZC complexion with metals can be promising. Simvastatin showed synergy when associated to the complex (FICI≤0.5), being the minimum inhibitory concentration (MIC) of 16 μg mL^{-1} found in 6 samples. Thus, it is possible to infer that the association between **Pd(DMBA)LBr** and simvastatin consists of an alternative to increase the pontential of these compounds, since statins have low toxicity.

Keywords: Statins/antimicrobial activity. Triazenide. Atorvastatin. Simvastatin.

INTRODUCTION

Antimicrobial resistance is a serious public health issue worldwide. Multi-drug resistant microorganisms (MDR) are an increasing concern since they show low susceptibility to different classes of antimicrobials that are commonly prescribed in hospitals (WHO, 2014; Tzialla *et al*., 2015). It has also become a challenge for healthcare professionals because therapy options for the treatment of some infections caused by MDR are more and more restrict due to the fast emergence and dissemination of these microorganisms (Azevedo, Silva, 2012; Thangamani *et al*., 2015; Karam *et al*., 2016).

This resistance occurs due to several reasons, mainly the indiscriminate use of these agents, thus decreasing the amount of drugs available for the treatment of such infections (Azevedo, Silva, 2012; Karam *et al*., 2016). Therefore, there is the urge to discover new drugs with antibacterial properties, in addition to associations in the search of synergistic effects (Kalaivani, 2012; Thangamani *et al*., 2015; Tizotti *et al*., 2016).

Statins are a class of drugs which have shown a promising antibacterial activity against several bacterial species, being used for the reduction of lipids in patients with high cholesterol levels as well as showing anti-inflammatory activity (Almog *et al*., 2004; Lopez-

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Cortes *et al*., 2013; Kozarov, Padro, Badimon, 2014; Thangamani *et al*., 2015). Also, other substances worth noticing are triazenes (TZC), which contain an aliphatic chain composed by three nitrogen atoms interconnected in sequence (N=N-N), responsible for their biological properties (Moore, Robinson, 1986). These substances show wide pharmacological versatility such as antifungal, antileukemia and antibacterial activity, making them the focus of several studies (Hörner *et al*., 2008; Domingues *et al*., 2010; Mohammadi, 2014; Tizotti *et al*., 2016).

Also, in order to increase the biological activity and stability of TZC in medicines, there is a growing interest in associating these compounds with metals (Sreedhara, Cowan, 2001; Karami *et al*., 2017). Compounds that contain palladium (Pd(II)) are worth highlighting, mainly regarding antitumor activity, since they have more stability and less toxicity when compared to platin-based anticancer compounds, due to their similar structural behaviour (Dupont, Consorti, Spencer, 2005; Massai *et al*., 2016; Karami *et al*., 2017). Also, their potential antibacterial activity can have their action significantly increased up to 16 times when associated to antimicrobials than the free drug (Guerra *et al*., 2005).

The aim of this study was to assess the in vitro synergistic antibacterial effect of the association between statins and a new TZC complex {[1-(4-bromophenyl)-3-phenyltriazene N_3-oxide-κ^2 N^1,O^4](dimethylbenzylamine-κ^2 C^1,N^4)palladium(II)} (**Pd(DMBA)LBr**), their ligands *N,N'*-dimethylbenzylamine (**DMBA**), 1-Phenyl-3-(4-bromophenyl)triazene N_1-hydroxide (**HLBr)**, and precursor (**[Pd(DMBA)(μ-Cl)]$_2$**) against American Type Culture Collection (ATCC) strains and clinical isolates.

MATERIAL AND METHODS

Chemical compounds

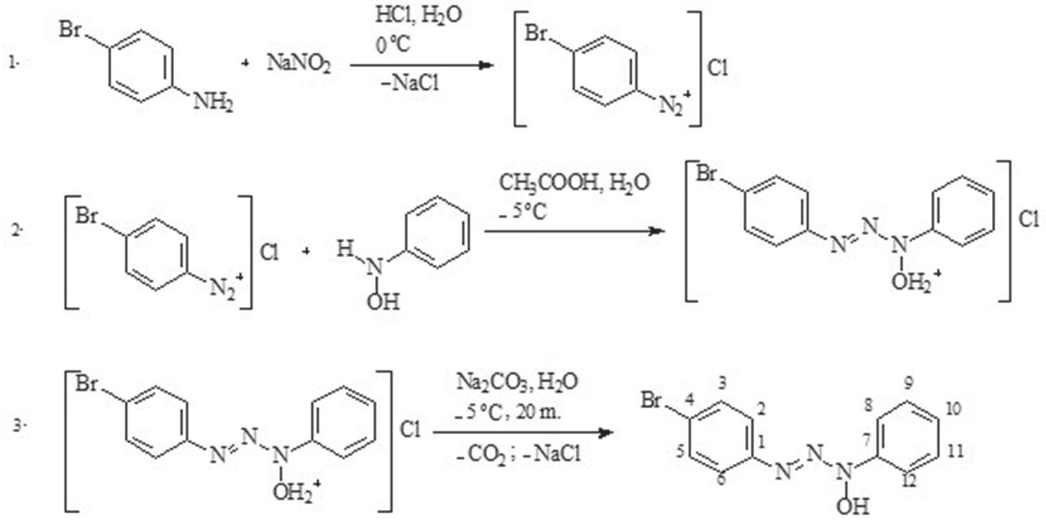
The TZC were previously synthesized and chemically characterized in the Núcleo de Investigação de Triazenos e Complexos (*NiTriCo*) of Universidade Federal de Santa Maria (UFSM). Statins were purchased commercially in the form of their active principle (atorvastatin, formula: $C_{66}H_{68}CaF_2N_4O_{10}.3H_2O$, PM = 1209.4; simvastatin, formula: $C_{25}H_{38}O_5$, PM = 418.57).

Experimental

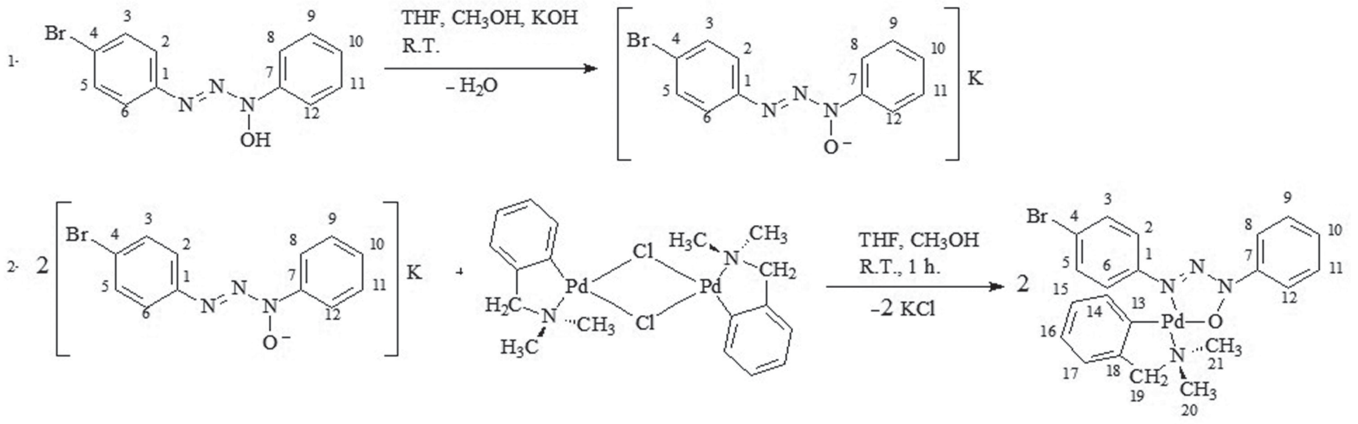
The synthesis of the ligand **HLBr** was realized according the literature and based on Scheme 1, while the synthesis of the complex **Pd(DMBA)LBr** from the ligand **HLBr** and the precursor complex **[Pd(DMBA)Cl]$_2$** followed according Scheme 2 (Martins *et al*., 2017).

Synthesis of {[1-(4-bromophenyl)-3-phenyltriazene N_3-oxide-κ^2 N^1,O^4](dimethyl-benzylamine-κ^2 C^1,N^4)palladium(II)} (Pd(DMBA)LBr)

To obtain the complex **Pd(DMBA)LBr** a solution of the protonated ligand **HLBr** (0.05 g; 17.12 mmol) in 20 mL of tetrahydrofuran was prepared. To this transparent pale-yellow solution five drops of a concentrate solution of KOH in methanol were added under continuous stirring at room temperature. The reaction mixture changes to intense yellow indicating the presence of the deprotonated free



SCHEME 1 - Reaction scheme of the synthesis of the ligand **HLBr**.



SCHEME 2 - Reaction scheme of the synthesis of the complex **Pd(DMBA)LBr** from the ligand **HLBr** and the complex **[Pd(DMBA)Cl]$_2$** as precursors.

ligand anion **[LBr]**$^-$. A solution of the precursor complex **[Pd(DMBA)(μ-Cl)]$_2$** (0.053 g; 0.097 mmol) dissolved in 10 mL of tetrahydrofuran was added under stirring at room temperature - **[Pd(DMBA)(μ-Cl)]$_2$** was previously prepared according Cope and Friedrich (1968). The reaction mixture was stirred 1 h while the color changes to opaque yellow. The reaction mixture was filtered. After evaporation of the solvent mixture at room temperature, transparent yellow crystals with lozenge shape were obtained. Yield: 50% (0.049 g) based on 1-Phenyl-3-(4-bromophenyl)triazene N_1-hydroxide. Anal. Calc. for $C_{21}H_{21}BrN_4OPd$: C, 47.43; H, 3.98; N,10.54; Found: C, 45.9; H, 3.93; N,10.19. IR ν_{max}/cm^{-1}, KBr pellet: 3040 - 2833 (νC-H); 1588 (νC=C); 1480 (δN-H); 1417 (νN=N); 1314 (N-N-N); 1278 (νN→O); 665 (C_{ar}-Br); 1163 (N-N). NMR ^{1}H (600 MHz DMF-d_7) δ =7.94 ppm (*d*, *J* = 8.54 Hz, 2H, *Ar*H); δ=7.53-7.33 ppm (*m*, 7H, Ar*H*); δ =7.00 ppm (*d*, *J* =7,27 Hz, 1H, Ar*H*); δ =6.88 ppm (*t*, *J* =7,27 Hz, 1H, Ar*H*); δ=6.61 ppm (*t*, *J* = 7.42 Hz, 1H, Ar*H*); δ =6.31 ppm (*d*, *J* = 7.66 Hz, 1H, Ar*H*); δ =4.09 ppm (*s*, *J* = 19.34 Hz , 2H, CH_2); δ =3.49 ppm (*s*, CH_3, 6H). NMR ^{13}C (600 MHz DMF-d_7) δ =149.07 ppm (C_{18}); δ =148.91 ppm (C_1); δ =148.44 ppm (C_{13}); δ =141.19 ppm (C_7); δ =134.42 ppm (C_{14}); δ =131.42 ppm (C_3, C_5); δ =129.22 ppm (C_9,C_{11}); δ =127.40 ppm (C_{10}); δ =126.62 ppm (C_2,C_6); δ =124.37 ppm (C_{15}); δ =123.57 ppm (C_{16}); δ =121.76 ppm (C_{17}); δ =118.61 ppm (C_8,C_{12}); δ =117.42 ppm (C_4); δ =72.39 ppm (C_{19}); δ =51.69 ppm (C_{21}, C_{22}).

In vitro antibacterial activity

The *in vitro* antibacterial activity was evaluated against different strains ATCC, including *Bacillus cereus* ATCC 14579, *Enterobacter hormaechei* ATCC 700323, *Enterococcus casseliflavus* ATCC 700327, *Enterococcus faecalis* ATCC 29212, *Enterococcus faecalis* ATCC 51299, *Escherichia coli* ATCC 25922, *Escherichia coli* ATCC 35218, *Klebsiella pneumoniae* ATCC 700603, *Micrococcus luteus* ATCC 7468, *Pseudomonas aeruginosa* ATCC 27853, *Salmonella typhimurium* ATCC 14028, *Salmonella* spp. ATCC 52117, *Staphylococcus aureus* ATCC 25923, *Staphylococcus aureus* ATCC 29213, *Staphylococcus aureus* BAA 1026, *Staphylococcus aureus* BAA 976, *Staphylococcus aureus* BAA 977, *Staphylococcus epidermidis* ATCC 12228 and against ten coagulase-negative staphylococci isolates in newborn blood cultures in 2014. Clinical isolates were identified through by automated system Vitek® 2 (bioMérieux, France).

Determination of the Minimum Inhibitory Concentration (Mic)

Bacterial isolates and ATCC strains, stored in 15% glycerol at -80 °C, were pre-activated using the agar trypticase soy medium (TSA) for 24 h at 35 ± 2 °C. Evaluation of the antibacterial activity of the compounds was performed using the conventional method of broth microdilution for Minimum Inhibitory Concentration (MIC) based on the Clinical and Laboratory Standards Institute guidelines (CLSI, 2012a). The test compounds was diluted in ethanol at a concentration of 20.480 µg mL^{-1} and then successive dilutions were made with concentrations from 1.024 to 1 µg mL^{-1}, with ethanol concentration 5% to 0.0048%. The bacterial inoculum was prepared using a 0.5 McFarland scale, so that each well contained 5 x 105 CFU mL^{-1}. Plates were incubated for 24 h at 35 ± 2 °C and after this period, the MIC

was determined visually as the lowest concentration that completely inhibited growth of microorganisms in dilution wells. For control and comparison MIC was determined as a broad spectrum antibacterial drug used in therapeutics: Tigecycline (Tygacil® - Wyeth). The drug was dissolved in physiological solution in the same manner as the compounds, but at a lower concentration (5.120 μg mL^{-1}). After successive dilutions were performed at concentrations of 256 to 0.25 μg mL^{-1}. Tests were also conducted using only ethanol to demonstrate that it did not interfere with the activity.

Statistical analysis

The analysis of the combination of TZC and statins was obtained by calculating the Fractional Inhibitory Concentration Index (FICI). The FICI was interpreted as "synergic" (FIC≤0.5); "no interaction" (FIC>0.5 and ≤4.0) and "antagonism" (FIC>4.0) (Odds, 2003; Konaté *et al*., 2012).

Ethical considerations

This study was approved by the Research Ethics Committee (CEP) of UFSM, under the certificate number of presentation for ethical consideration (CAAE) 38850614.4.0000.5346.

RESULTS

All compounds showed antibacterial activity against the strains tested. The MIC of ATCC strains against ligands, precursor and complex, associated or not to statins, free palladium(II), tigecycline and fici values, are shown in Table I; and it is possible to observe the same parameters in Table II, however against the 10 clinical isolates. Simvastatin showed an activity similar or better than atorvastatin in all ATCC strains and clinical isolates analyzed, with the lowest MIC (=16 μg mL^{-1}) found in the ATCC strain of *S. aureus* BAA 976. Also, *S. aureus* BAA 977, *Micrococcus luteus* 7468 and the isolates 8 showed MIC=32 μg mL^{-1}. It has also been possible to observe that the best activity occurred in Gram-positive bacteria.

Regarding TZC, the **DMBA** ligand and the precursor **($[Pd(DMBA)(\mu\text{-}Cl)]_2$)** showed MIC≥128 μg mL^{-1} for all the microorganisms analyzed. The ligand **HLBr** showed good activity in ATCC strains, with MIC=64 μg mL^{-1} for *E. faecalis* ATCC 51299 and *S. aureus* ATCC 25923, and for the clinical isolates the lowest MIC was 128 μg mL^{-1}. As for the palladium(II) complex, **(Pd(DMBA)LBr)** showed greater antibacterial potential in nearly all microorganisms when compared to the ligand and the precursor, resulting in MIC=64 μg mL^{-1} for some clinical isolates and MIC=32 μg mL^{-1} for the ATCC strain of *E. faecalis* 51299.

When TZC was associated with simvastatin, it was possible to observe a decrease of MIC values in both ATCC strains and clinical isolates. The **DMBA** associated with simvastatin showed synergy (FICI≤0.5) against strains of *B. cereus* ATCC 14579, *E. faecalis* ATCC 51299, *S. aureus* ATCC 25923, *S. aureus* 29213, and *S. aureus* BAA 1026. The precursor **($[Pd(DMBA)(\mu\text{-}Cl)]_2$)** showed synergy against these strains as well as *Salmonella* ATCC 52117 and isolate 2.

The ligand 2 (HLBr), when associated to simvastatin, showed MIC=64 μg mL^{-1} for *Salmonella* ATCC 52117; MIC=32 μg mL^{-1} for *B. cereus*, *S. aureus* 2923, *S. aureus* BAA 1026, isolates 4 and 6; MIC=16 μg mL^{-1} for *E. faecalis* ATCC 51299 and *S. aureus* 25923, with FICI<0.5 against these samples. The complex **(Pd(DMBA)LBr)** was twice more active than its free ligands and precursor when associated with simvastatin, showing synergy against the strains *E. casseliflavus* ATCC 700327, *E. coli* ATCC 25922, *P. aeruginosa* ATCC 27853 and *S. aureus* 29213, *S. aureus* BAA 1026, isolates 1, 2, 3, 4, 5, 6 and 7, with FICI≤0.5. As for the association of TZC with atorvastatin, no synergy was evidenced, but MIC values were similar to the ones obtained when the compounds were separately tested, except for a few strains.

DISCUSSION

The emergence of antimicrobial resitance shows the need for searching new drugs, as well as their association (Masadeh *et al.*, 2012). Some studies have evidenced a possible antibacterial effect of this class of drugs, associated to antimicrobials or not in order to reduce morbidity and mortality of several infectious diseases (Masadeh *et al.*, 2012; Ajrouche *et al.*, 2013; López-Cortés *et al.*, 2013; Kozarov, Padro, Badimon, 2014; Graziano *et al*., 2015; Thangamani *et al*., 2015).

In this study, it was possible to observe that statins are able to induce variable degrees of antibacterial activity, with simvastatin being more potent than atorvastatin. A study developed in 2012 comparing the antibacterial activity of atorvastatin, simvastatin and rosuvastatin showed that the two first statins were the most potent in Gram-positive microorganisms, with MIC=166.67±72.16 μg mL^{-1} for atorvastatin and MIC=104.17±36.08 μg mL^{-1} for simvastatin in *E. faecalis* 51299 strains, a result similar to the one found in our study. However, below-average results were found for *E. coli* 35218 (MIC=26.04±9.02 μg mL^{-1} and

TABLE I - Minimum Inhibitory Concentrations (MIC) of ligands, precursor and complex, associated or not with statins against standard bacteria

Microorganism	µg mL^{-1}																							
	A	**S**	**L_1**	**L_1+A**	**FICI**	**L_1+S**	**FICI**	**L_2**	**L_2+A**	**FICI**	**L_2+S**	**FICI**	**P**	**P+A**	**FICI**	**P+S**	**FICI**	**C**	**C+A**	**FICI**	**C+S**	**FICI**	**Pd livre**	**Tig**
Bacillus cereus ATCC 14579	256	256	256	128	1	64	0.5	128	128	1.5	32	0.4	256	128	1	64	0.5	64	64	1.3	32	0.6	256	*
Enterobacter hormaechei ATCC 700323	256	128	256	256	2	128	1.5	256	256	2	128	1.5	256	128	1	64	0.8	128	128	1.5	64	1	128	*
Enterococcus casseliflavus ATCC 700327	512	256	128	128	1.3	64	0.8	128	128	1.3	64	0.8	128	128	1.3	64	0.8	128	128	1.3	32	0.4	256	<0.25
Enterococcus faecalis ATCC 29212	256	64	256	256	2	128	2.5	256	128	1	32	0.6	256	128	1	32	0.6	128	128	1.5	32	0.8	256	<0.25
Enterococcus faecalis ATCC 51299	256	128	256	128	1	32	0.4	64	64	1.3	16	0.4	128	64	0.8	32	0.5	32	32	1.1	16	0.6	256	<0.25
Escherichia coli ATCC 25922	512	256	256	256	1.5	128	1	256	256	1.5	128	1	256	256	1,5	128	1	256	128	0.8	64	0.5	256	<0.25
Escherichia coli ATCC 35218	512	256	256	256	1.5	128	1	256	256	1.5	128	1	256	256	1.5	128	1	256	256	1.5	128	1	256	<0.25
Klebsiella pneumoniae ATCC 700603	256	128	256	256	2	128	1.5	256	256	2	128	1.5	256	128	1	64	0.8	128	128	1.5	64	1	256	0.5
Micrococcus luteus 7468	256	32	128	128	1.5	64	2.5	256	256	2	128	4.5	128	128	1.5	64	2.5	128	128	1.5	64	2.5	256	<0.25
Pseudomonas aeruginosa ATCC 27853	256	128	128	128	1.5	64	1	128	128	1.5	64	1	128	128	1.5	64	1	128	64	0.8	32	0.5	256	2
Salmonella typhimurium ATCC 14028	256	128	256	256	2	128	1.5	128	128	1.5	64	1	256	128	1	64	0.8	128	128	1.5	64	1	128	<0.25
Salmonella ATCC 52117	512	256	256	256	1,5	128	1	256	128	0.8	64	0.5	256	128	0.8	64	0.5	128	128	1.3	64	0.8	256	*
Staphylococcus aureus ATCC 25923	512	256	256	128	0.8	64	0.5	128	64	0.6	16	0.2	256	128	0.8	64	0.5	64	64	1.1	32	0.6	256	<0.25
Staphylococcus aureus 29213	512	256	512	256	1	32	0.2	256	128	0.8	32	0.3	256	128	0.8	32	0.3	128	128	1.3	16	0.2	512	<0.25
Staphylococcus aureus BAA 1026	256	128	256	256	2	32	0.4	256	128	1	32	0.4	256	128	1	32	0.4	128	128	1.5	16	0.3	256	<0.25
Staphylococcus aureus BAA 976	512	16	512	512	2	128	8.3	512	256	1	128	8.3	512	128	0.5	64	4.1	256	256	1.5	128	8.5	256	<0.25
Staphylococcus aureus BAA 977	256	32	512	256	1.5	128	4.3	256	256	2	128	4.5	512	256	1.5	128	4.3	128	128	1.5	32	1.3	256	<0.25
Staphylococcus epidermidis ATCC 12228	256	128	512	128	0.8	64	0.6	256	256	2	128	1.5	512	256	1.5	64	0.6	128	128	1.5	64	1	256	<0.25

A = Atorvastatin; S = Simvastatin; L_1= Ligand 1 N,N'-dimethylbenzylamine (**DMBA**); FICI= Fractional Inhibitory Concentration Index; L_2 = Ligand 2 1-Phenyl-3-(4-bromophenyl)triazene N_1-hydroxide (**HLBr**); P = Precursor **[Pd(DMBA)(μ-Cl)]$_2$**; C = Complex {[1-(4-bromophenyl)-3-phenyltriazene N_3-oxide-κ^2 N^1, O^4](dimethylbenzylamine-κ^2 C^1, N^4)palladium(II)} (**Pd(DMBA)LBr**); Pd = palladium(II); Tig = Tigecycline; * = Test not realized.

TABLE II - Minimum Inhibitory Concentrations (MIC) of ligands, precursor and complex, associated or not with statins against CoNS

Micro-organism	µg mL^{-1}																						
	A	S	L_1	L_1+A	FICI	L_1+S	FICI	L_2	L_2+A	FICI	L_2+S	FICI	P	P+A	FICI	P+S	FICI	C	C+A	FICI	C+S	FICI	Pd livre
Isolate 1	256	128	512	256	1.5	64	0.6	256	256	2	64	0.8	256	256	2	64	0.8	128	128	1.5	32	0.5	256
Isolate 2	256	128	256	256	2	64	0.8	128	128	1.5	64	1	256	128	1	32	0.4	64	64	1.25	16	0.4	256
Isolate 3	256	128	256	256	2	64	0.8	128	128	1.5	64	1	256	128	1	64	0.8	64	64	1.25	16	0.4	256
Isolate 4	256	128	256	128	1	128	1.5	256	128	1	32	0.4	256	128	1	128	1.5	128	128	1.5	32	0.5	256
Isolate 5	128	64	128	128	2	64	1.5	128	128	2	32	0.8	128	128	2	64	1.5	64	64	1.5	16	0.5	256
Isolate 6	256	128	256	256	2	128	1.5	128	128	1.5	32	0.5	128	128	1.5	128	2	64	64	1.25	16	0.4	256
Isolate 7	128	64	256	256	3	32	0.6	128	128	2	32	0.8	256	128	1.5	64	1.3	64	64	1.5	16	0.5	256
Isolate 8	128	32	128	128	2	32	1.3	128	128	2	64	2.5	128	128	2	64	2.5	64	64	1.5	16	0.8	256
Isolate 9	256	128	512	256	1.5	64	0.6	128	128	1.5	64	1	256	128	1	64	0.8	64	64	1.25	32	0.8	256
Isolate 10	128	64	128	128	2	64	1,5	128	128	2	64	1.5	128	128	2	64	1.5	64	64	1.5	32	1	256
MIC^{50}	256	128	256	256	-	64	-	128	128	-	64	-	256	128	-	64	-	64	64	-	16	-	256
MIC_{90}	256	128	512	256	-	128	-	256	128	-	64	-	256	128	-	128	-	128	128	-	32	-	256

A = Atorvastatin; S = Simvastatin; L_1= Ligand 1 N,N'-dimethylbenzylamine (**DMBA**); FICI= Fractional Inhibitory Concentration Index; L_2 = Ligand 2 1-Phenyl-3-(4-bromophenyl)triazene N_1-hydroxide (**HLBr**); P = Precursor **[Pd(DMBA)(μ-Cl)]$_2$**; C = Complex {[1-(4-bromophenyl)-3-phenyltriazene N_3-oxide-κ^2 N^1, O^4](dimethylbenzylamine-κ^2 C^1, N^4)palladium(II)} (**Pd(DMBA)LBr**); Pd = Palladium; Tig = Tigecycline; * = Test not realized. Isolates 1, 10 = *Staphylococcus hominis*; Isolates 2, 3, 4, 5, 7, 9 = *Staphylococcus epidermidis*; Isolate 6 = *Staphylococcus saprophyticus*; Isolate 8 = *Staphylococcus capitis*.

MIC=58.08±18.04 µg mL^{-1}), and *S. epidermidis* 12228 (MIC=26.04±9.02 µg mL^{-1}), respectively (Masadeh *et al.*, 2012).

Graziano *et al*. has detected that simvastatin was the only statin with antibacterial activity against clinical isolates and ATCC strains of *S. aureus* susceptible (MSSA) and resitant to methycillin (MRSA), with *S. aureus* 29213 showing MIC=15.65 µg mL^{-1} (Graziano *et al*., 2015). The difference of MIC between these studies can be attributed to the different design of the study performed (Ting, Whitaker, Albandar, 2016). We suppose that it could have been due to the different methodology used in the solubility of statins, since the active principles used in our study were dilluted in ethanol, whereas other researchers used dimethyl sulphoxide (DMSO). Thus, we performed the MIC of these strains by using the same methodology used in other studies, and results remained the same as when principles were dilluted in ethanol. However, it is known that according to the document M100-S22 from CLSI, DMSO can inactivate DNA of microorganisms when used in doses higher than 1%, interfering with the antibacterial activity (CLSI, 2012b).

All statins induce their antihyperlipidemic activity through the same mechanism of action in eukaryotic cells, completely inhibiting the Class I 3-hydroxi-3-methyl-glutaryl-coenzyme to reductase (HMG-CoA), hindering the formation of mevalonate of HMG-CoA, and leading to a decrease in biosynthesis of cholesterol and an increase in the removal of circulation of low density lipoproteins (LDL) (Shitara, Sugiyama, 2006; Masadeh *et al.*, 2012; Graziano *et al*., 2015). However, studies have shown that it is unlikely that the antibacterial activity shown by this class of drugs is related to this action mechanism, since mechanisms of antimicrobial effects of statins are yet to be elucidated (Masadeh *et al.*, 2012; Ting, Whitaker, Albandar, 2016). A possible mechanism which can be related would be due to promoting apoptosis in microbial cells or the hydrofobic nature of statins, leading to the rupture of the bacterial membrane, resulting in cell death (Bergman *et al*., 2011; Tapia-Perez *et al*., 2011; Masadeh *et al.*, 2012).

Other researchers have revealed that simvastatin inhibits the multiple biosynthetic pathways and the cellular processes in bacteria, including the selective interference of bacterial proteic synthesis, aiding the ability of this drug to suppress the production of some bacterial toxins such as α-hemolysin and Panton-Valentine leucocidin (Thangamani *et al*., 2015). Furthermore, Thangamani *et al*. (2015) have shown that simvastatin has an excellent activity against biofilm-forming bacteria in *Staphylococcus.*

In terms of the difference of MIC in statins, it can be due to the difference in their chemical structure as well as their production. Simvastatin is the semisynthetic form which derives from lovastatin, a product of *Penicillium citrinum*, with higher intrinsic antibacterial activity. Atorvastatin, on the other hand, is the pure synthetic form (Mason *et al*., 2005; Jerwood, Cohen, 2008; Ting,

Whitaker, Albandar, 2016). These two are lipophilic statins, thus simvastatin probably goes through the cell membrane more easily, causing the inhibition of bacteria, depending on the dose. Atorvastatin has not shown significant antimicrobial activity, although it is lipophilic, and it is justified because this statin is not derived from funghi (Mason *et al*., 2005; Graziano *et al*., 2015). However, more studies regarding statins structure are needed in order to ellucidate their effect against bacteria (Graziano *et al*., 2015).

Regarding TZC, several studies using these compounds have shown that they have antimicrobial activity (Hörner *et al*., 2008; Domingues *et al*., 2010; Ombaka, Muguna, Gichumbi, 2012; Mohammadi, 2014; Paraginski *et al.,* 2014; Tizotti *et al*., 2016), being proposed that their action mechanism occurs due to the chelating activity to metallic ions from the bacterial cell wall, inhibiting stages of bacterial synthesis, leading to cell death (Hörner *et al*., 2008; Ombaka, Muguna, Gichumbi, 2012; Yeo *et al*., 2013).

The highest antibacterial activity was detected in Gram-positive microorganisms, and could be justified by the difference in the cell wall structure of these bacteria, which is less complex and has a thick layer of peptidoglycan. Gram-positive bacteria need this layer for their protection and the maintenance of osmotic pressure, thus, antibacterial activity may be related to the inhibition of the synthesis of the peptidoglycan, leading to cell death (Yeo *et al*., 2013).

The DMBA ligands and the precursor **[Pd(DMBA)(μ-Cl)]$_2$** showed MIC≥128 μg mL^{-1}. TZC complexed with palladium(II) showed better activity against the strains tested when compared to free ligands and precursor, proving to be an alternative for a new class of drugs with antibacterial activity. This activity can be justified since TZC complexed with transition metals such as Pd(II) can interact and cause damage to the DNA, providing a potent antibacterial activity, in addition to blocking cancer cells division leading to cell death (Hecht, 2000; Song *et al*., 2006; Paraginski *et al.*, 2014). Therefore, complementary studies must be performed for the assessment of other biological parameters, their toxicity and therapeutic efficacy (Nunes *et al*., 2014).

When associating TZC with atorvastatin or simvastatin, simvastatin has shown synergy. It is estimated that a third of American adults over 45 years old make use of these drugs routinely (Wang *et al*., 2016). These drugs show a good safety profile with limited secondary effects, with low toxicity, allowing a frequent use in patients with high cholesterol levels (Thangamani *et al*., 2015; Ting, Whitaker, Albandar, 2016). Also, studies show that patients who make use of this class of drugs have a lower risk of acquiring bacterial infections, proving the correlation of the use of statins and a lower incidence of sepsis and mortality related to these infections (Almog *et al*., 2004; Ajrouche *et al.*, 2013; López-Cortés *et al.*, 2013).

Thus, according to MIC and FICI values obtained, we can infer that TZC complexed with palladium(II) significantly increases the antibacterial activity, and worked even better when associated with simvastatin. The documentation of this synergistic effect is of great impact for the treatment of MDR bacteria together with the low toxicity perfomance of simvastatin.

CONFLICTS OF INTERESTS

The authors declare no conflicts of interest.

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