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PII: S0223-5234(18)30541-5

DOI: [10.1016/j.ejmech.2018.06.050](https://doi.org/10.1016/j.ejmech.2018.06.050)

Reference: EJMECH 10520

To appear in: *European Journal of Medicinal Chemistry*

Received Date: 8 May 2018

Revised Date: 19 June 2018

Accepted Date: 21 June 2018

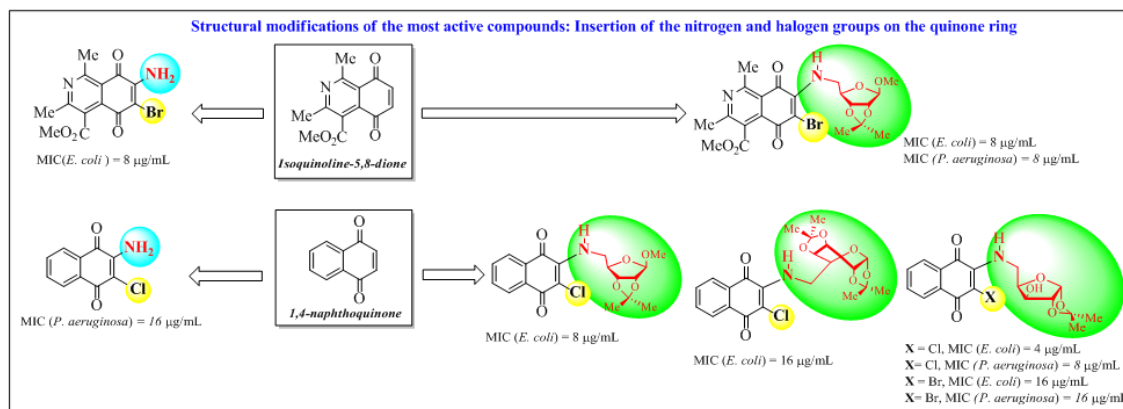


Please cite this article as: F.R.F. Dias, J.S. Novais, T.A. do Nascimento Santos Devillart, W.A. da Silva, M.O. Ferreira, R.d.S. Loureiro, Viní.R. Campos, V.F. Ferreira, M.C.B.V. de Souza, H.C. Castro, A.C. Cunha, Synthesis and antimicrobial evaluation of amino sugar-based naphthoquinones and isoquinoline-5,8-diones and their halogenated compounds, *European Journal of Medicinal Chemistry* (2018), doi: 10.1016/j.ejmech.2018.06.050.

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## Graphical Abstract

This work describes the synthesis and *in vitro* antimicrobial evaluation of carbohydrate-based isoquinoline-5,8-diones and 1,4-naphthoquinones and their halogenated derivatives against Gram-positive and Gram-negative bacteria. Several halogenated quinones exhibited promising MIC and MBC values (MIC=MBC = 4-16  $\mu\text{g/mL}$ ) against two Gram Negative bacteria strains of clinical importance.



# Synthesis and antimicrobial evaluation of amino sugar-based naphthoquinones and isoquinoline-5,8-diones and their halogenated compounds

Flaviana R. F. Dias<sup>a</sup>, Juliana S. Novais<sup>b</sup>, Talita A. do Nascimento Santos Devillart<sup>c</sup>, Wanderson Amaral da Silva<sup>d</sup>, Matheus O. Ferreira<sup>a</sup>, Raquel de S. Loureiro<sup>b</sup>, Vinícius R. Campos<sup>a</sup>, Vitor F. Ferreira<sup>e</sup>, Maria C. B. V. de Souza<sup>a</sup>, Helena C. Castro<sup>b</sup> and Anna C. Cunha<sup>a,\*</sup>

<sup>a</sup>Universidade Federal Fluminense, Instituto de Química, Departamento de Química Orgânica, Programa de Pós-Graduação em Química, 24020-141, Niterói, Rio de Janeiro, Brazil.

<sup>b</sup> Universidade Federal Fluminense, PPBI, Instituto de Biologia, Departamento de Biologia Celular e Molecular, 24020-150, Niterói, Rio de Janeiro, Brazil.

<sup>c</sup> Universidade Federal Fluminense, Departamento de Patologia, Programa de Pós-Graduação em Patologia, Hospital Universitário Antônio Pedro, 24033-900, Niterói, Rio de Janeiro, Brazil

<sup>d</sup>Centro Federal de Educação Tecnológica Celso Suckow da Fonseca, Coordenação de Licenciatura em Física, 25620-003, Petrópolis, Rio de Janeiro, Brazil

<sup>e</sup>Universidade Federal Fluminense, Departamento de Tecnologia Farmacêutica, Faculdade de Farmácia, Santa Rosa, 24241-002, Niterói, Rio de Janeiro, Brazil.

\*Corresponding authors: Anna C. Cunha (Telephone number: +552126292148; e-mail: annac@vm.uff.br) and Helena C. Castro (Telephone number: +5526299954; e-mail: hcastrorangel@yahoo.com.br).

## Abstract

Antibiotic resistance has emerged as a serious global public health problem and lately very few antibiotics have been discovered and introduced into clinical practice. Therefore, there is an urgent need for the development of antibacterial compounds with new mechanism of action, especially those capable of evading known resistance mechanisms. In this work two series of glycoconjugate and non-glycoconjugate amino compounds derived from of isoquinoline-5,8-dione and 1,4-naphthoquinone and their halogenated derivatives were synthesized and evaluated for antimicrobial activity against Gram-positive (*Enterococcus faecalis* ATCC 29212, *Staphylococcus aureus* ATCC 25923, *S. epidermidis* ATCC 12228, *S. simulans* ATCC 27851) and Gram-negative bacteria (*E. coli* ATCC 25922, *Proteus mirabilis* ATCC 15290, *K. pneumoniae*

ATCC 4352 and *P. aeruginosa* ATCC 27853) strains of clinical importance. This study revealed that glycoconjugate compounds derived from halogeno-substituted naphthoquinones were more active against Gram-negative strains, which cause infections whose treatment is even more difficult, according to the literature. These molecules were also more active than isoquinoline-5,8-dione analogs with minimum inhibitory concentration (MIC = 4-32  $\mu\text{g/mL}$ ) within Clinical and Laboratory Standard Institute MIC values (CLSI 0.08-256  $\mu\text{g/mL}$ ). Interestingly the minimal bactericidal concentration (MBC) values of the most active compounds were equal to MIC classifying them as bactericidal agents against Gram-negative bacteria. Sixteen compounds among eighteen carbohydrate-based naphthoquinones tested showed no hemolytic effects on health human erythrocytes whereas more susceptibility to hemolytic cleavage was observed when using non-glycoconjugate amino compounds. *In silico* Absorption, Distribution, Metabolism, Excretion and Toxicity (ADMET) evaluation also pointed out that these compounds are potential for oral administration with low side effects. In general, this study indicated that these compounds should be exploited in the search for a leading substance in a project aimed at obtaining new antimicrobials more effective against Gram-negative bacteria.

**Keywords:** Quinone, Isoquinoline-5,8-dione, Antimicrobial, Glycoconjugate.

## Introduction

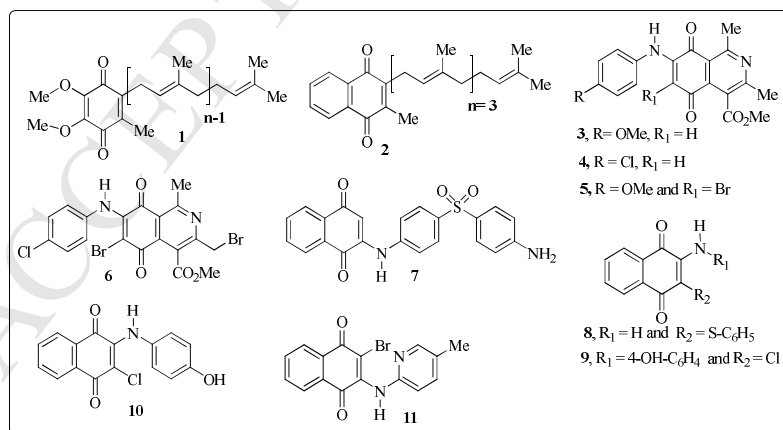
In the past few decades, the discovery and development of antibiotics have successfully led to the drastic reduction in human mortality.<sup>[1]</sup> However, the overuse and incorrect prescription of antimicrobials have resulted in hospital and community-acquired multi-drug resistant pathogens.<sup>[2]</sup> The rising drug resistance against first line drugs had requested more costly second and third line drugs that are often unaffordable or unavailable to most of developing and poor countries.<sup>[3]</sup> Recently, bacterial resistance to oral ciprofloxacin was associated to the use of substandard and spurious quality drugs in the developing countries.<sup>[4]</sup>

According to the Infectious Diseases Society of America (IDSA), few substances have been discovered and/or developed against resistant Gram-negative bacteria, such as *Enterobacter* spp., *Pseudomonas aeruginosa*, *Klebsiella pneumoniae* and *Acinetobacter* spp.<sup>[5]</sup> The development of antimicrobial agents is far from being

considered profitable for the pharmaceutical industries, different from those used to treat such as chronic illness (e.g., diabetes, high blood pressure, psychiatric disorders and asthma).<sup>[6]</sup>

Important antibiotic-resistance genes currently involve *K. pneumoniae* carbapenemase (KPC), methicillin-resistant *Staphylococcus aureus* (MRSA), carbapenemase-producing *Enterobacteriaceae* (CPE), penicillin-resistant *Streptococcus pneumoniae* (PRSP), vancomycin-resistant *enterococci* (VRE) and extended-spectrum  $\beta$ -lactamase producing *Enterobacteriaceae* (ESBL).<sup>[7,8]</sup> These bacteria are responsible for causing serious life-threatening infections such as hospital-acquired pneumonia, bloodstream infections, urinary tract infections from catheters, abdominal infections and cerebro-spinal meningitis.<sup>[7,9,10]</sup> The prevalence of infections caused by Gram-negative bacteria has increased worldwide. They are more resistant to several or all antibacterial agents currently available than Gram-positive bacteria<sup>[11-13]</sup> this resistance profile due to outer membrane of the Gram-negative bacterial cell wall that acts as a barrier to many substances, including antibiotics.<sup>[14,15]</sup>

Quinones are widely distributed in different families of plants, fungi and some animals. Many of them have been described as cytotoxic agents for anticancer therapy<sup>[16-19]</sup> whereas others play a role in vital biochemical processes.<sup>[20,21]</sup> Ubiquinone (**1**, UQ), also called coenzyme Q, and menaquinone (**2**, MK), a fat-soluble vitamin K, are essential components of the electron-transfer bacterial pathway (Figure 1).<sup>[22-25]</sup>



**Figure 1.** Examples of quinone derivatives as antibacterial agents.

The quinones can be cytotoxic through several mechanisms of action, including redox cycle that affects the electron transport systems in cells.<sup>[19]</sup> As part of our research

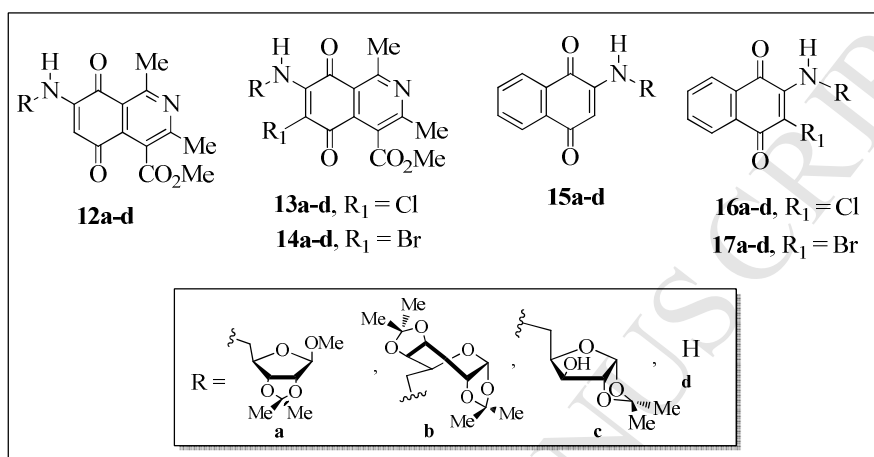
program on the synthesis of structurally modified quinones with biologically antibacterial activity for medical research<sup>[26]</sup>, we explored the synthesis and antibacterial evaluation of sugar-based naphthoquinones and isoquinoline-5,8-diones. According to the literature, representative examples of biologically active naphthoquinones include amino compounds **3-4** (Figure 1)<sup>[26]</sup>, which showed promising antimicrobial profile with Minimum Inhibitory Concentration (MIC) and Minimal Bactericidal Concentration (MBC) values in range of 1.0 to 2.0 µg/ml against *Escherichia coli* ATCC25922 and *P. aeruginosa* ATCC27853, two Gram-negative strains of clinical importance. Mono- and di-brominated aminoquinones **5** and **6** were also effective in preventing *E. coli* growth (MIC = MBC = 2-4 µg/mL).<sup>[26]</sup> Other classes of aminoquinone analogs **7-8**<sup>[27,28]</sup> and halogenated derivatives **9-11**<sup>[29,30]</sup> (Figure 1) were also reported to exhibit significant antimicrobial activity against pathogenic bacteria.

The current literature pointed the generation of active oxygen species (ROS) by redox cycling, intercalation in the DNA double helix or alkylation of biomolecules as inhibitory mechanisms of quinone compounds.<sup>[19,31-33]</sup> In all of them, the biological profile requires bio-reduction of the quinone nucleus as the first activating step.<sup>[19]</sup> The reduction process of the quinones to hydroquinones involving their redox properties is very important in many biological systems and may be related to efficacy and selectivity profiles of these antibiotics.<sup>[33]</sup> Quinones and related analogues are also known by their irreversibly complexation with nucleophilic amino acids in proteins, leading to inactivation and loss of proteins function. In this case, their biological activity is probably due to their ability to interact with cellular polypeptides, membrane-bound enzymes and surface-exposed adhesins of pathogenic bacteria.<sup>[34,35]</sup>

Aminoglycosides represent another class of antimicrobial agents that has been extensively studied. Carbohydrate-based antibiotics such as streptomycin and epistreptomycin<sup>[36]</sup> are representative members of this family. Carbohydrates are important as signaling molecules and for cellular recognition events, being explored for the development of new biologically active sugar-based compounds.<sup>[37,38]</sup>

Based on our experience in the field of synthetically modified quinones<sup>[16-18,26]</sup> we present the synthesis of two classes of quinones containing amino sugar groups (**12a-c** and **15a-c**), related halogen compounds (**13a-c**, **14a-c**, **16a-c** and **17a-c**) and the antimicrobial evaluation of these substances against Gram-positive and Gram-negative bacteria of clinical importance (Figure 2).

We also explored the synthesis and antibacterial profile of the non-halogenated amino compounds **12d** and **15d** and of the halogenated derivatives **13d**, **14d**, **16d** and **17d**, to evaluate the effect of the sugar chain attached to the naphthoquinone ring on their biological activity. Finally, we also determined the *in silico* ADMET parameters and *in vitro* hemocompatibility of the most active compounds.

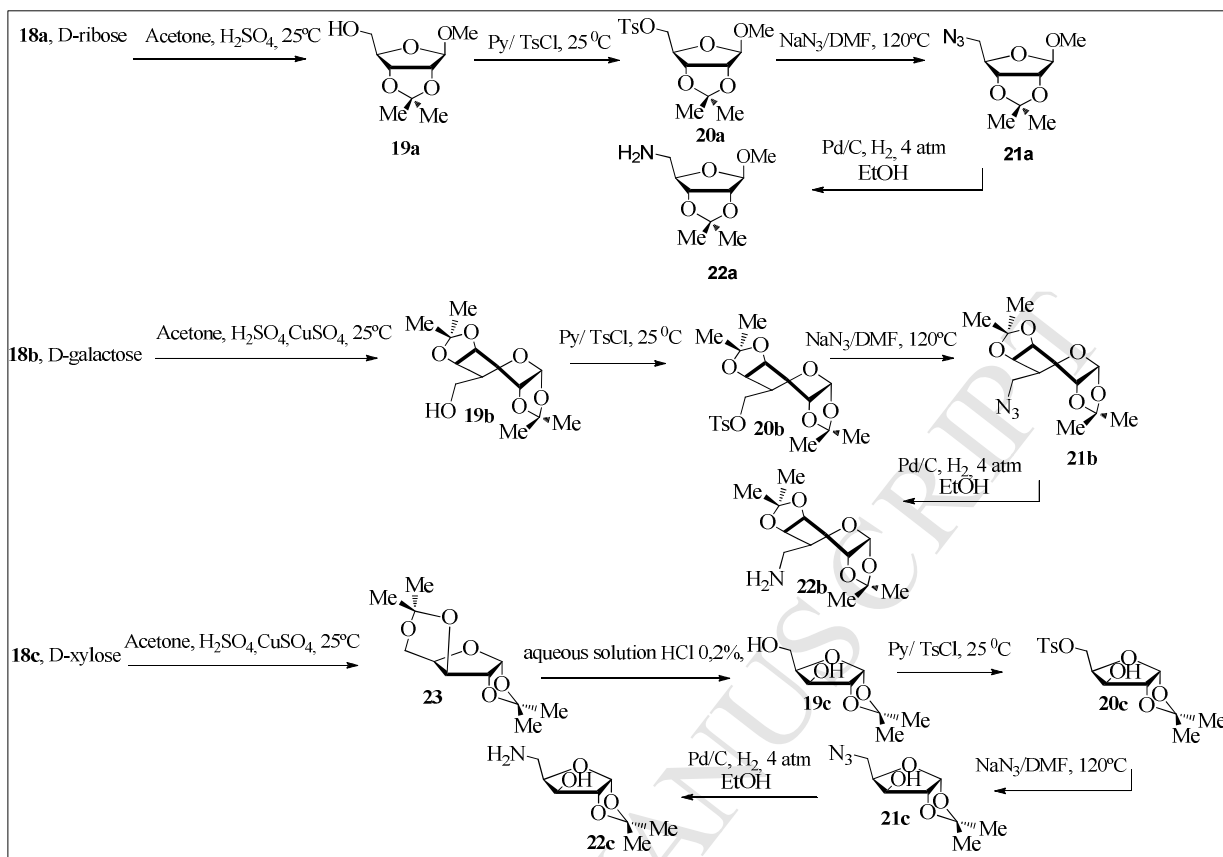


**Figure 2.** Structures of amino sugar-based isoquinoline-5,8-diones **12a-d** and naphthoquinones **15a-d** and their halogenated compounds **13a-d**, **14a-d**, **16a-d** and **17a-d**.

## Results and Discussion

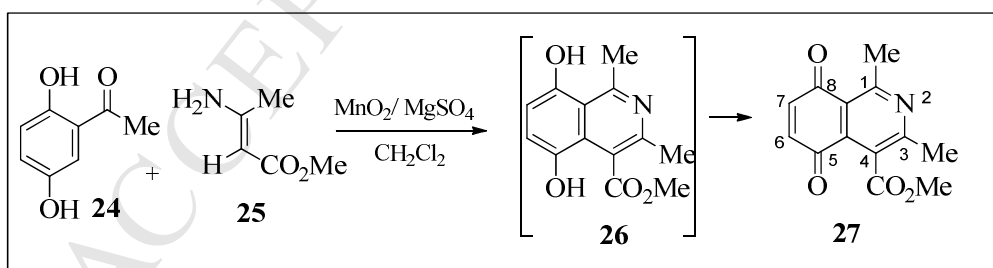
### Chemistry

The synthesis of the derivatives **22a-c** was performed as shown in the following scheme (Scheme 1).<sup>[16]</sup> The compounds **18a-c** were converted to the acetonides **19a-b** and **23** by reaction with acetone under acidic conditions.<sup>[39]</sup> Selective desprotection of the 3,5-acetonide moiety of **23**, in aqueous solution of hydrochloric acid, afforded the monoacetonide structure **19c**.<sup>[40]</sup> Reaction of the carbohydrate derivatives **19a-c** with tosyl chloride, in pyridine, furnished the corresponding tosyl esters **20a-c** in good yields.<sup>[41,42]</sup> The latter derivatives were transformed into the corresponding azide carbohydrates **21a-c** by reaction with sodium azide in DMF. Catalytic reduction ( $H_2$ , 10% Pd/C) of sugar-azides **21a-c** gave the aminocarbohydrates **22a-c**<sup>[43-45]</sup>, as outlined in Scheme 1.



**Scheme 1.** Preparation of the amino-compound **22a-c**.

The reaction between 2,5-dihydroxyacetophenone (**24**) and methyl 3-aminocrotonate (**25**) (Scheme 2) furnished 5,8-dihydroxy-1,3-dimethyl-isoquinoline-4-carboxylate (**26**), which was subsequently oxidized to isoquinoline-5,8-dione (**27**) with  $\text{MnO}_2$  in  $\text{CH}_2\text{Cl}_2$ .<sup>[46]</sup>

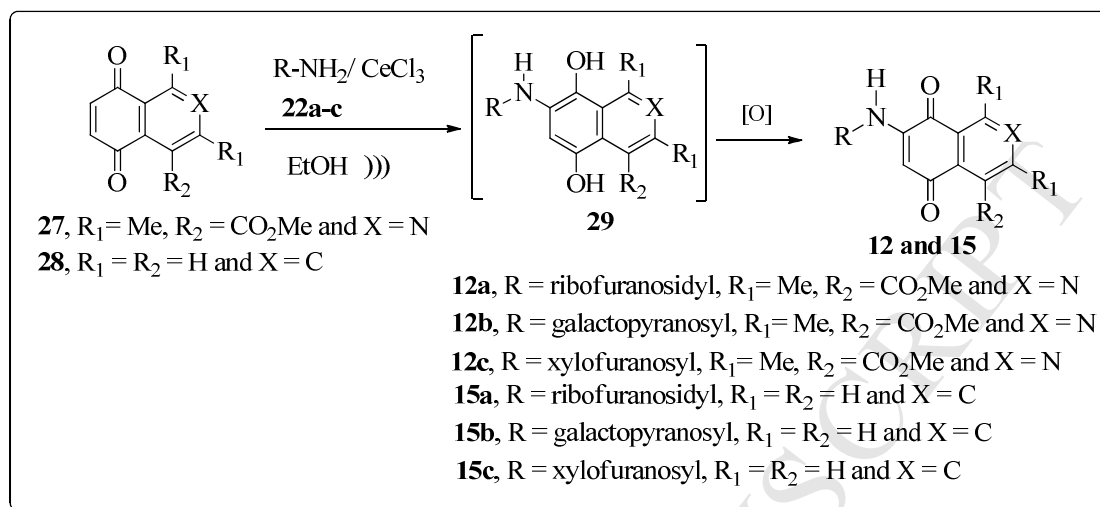


**Scheme 2.** Preparation of functionalized isoquinolinedione **27**.

The Michael addition reaction, under ultrasound, between the aminocarbohydrates **22a-c** and the quinones **27-28** led to the formation of the respective sugar-based quinones **12a-c** and **15a-c**, as shown in Scheme 3. These compounds were purified by silica gel column chromatography and their structures were confirmed using



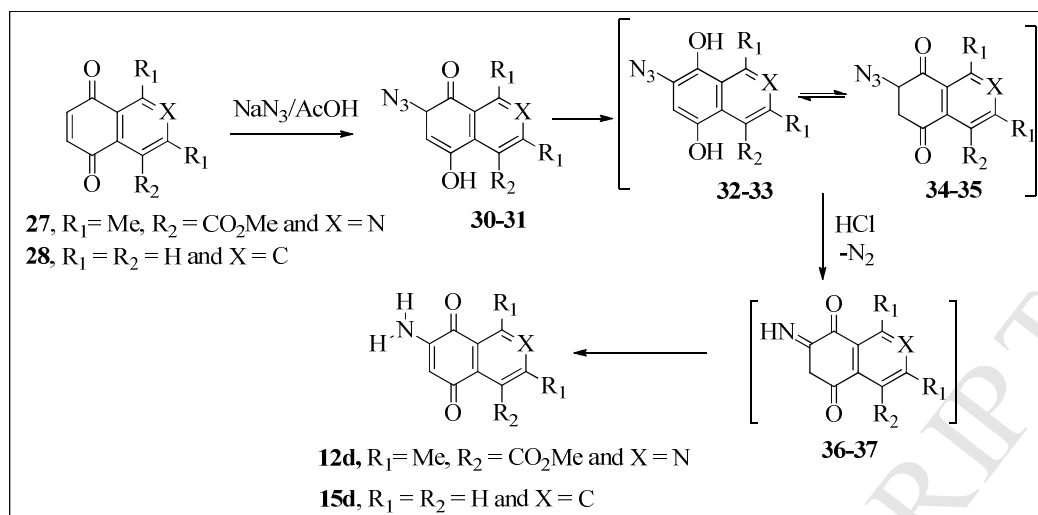
one- and two-dimensional NMR techniques [ $^1\text{H}$ ,  $^{13}\text{C}$ -APT, COSY- $^1\text{H}$  x  $^1\text{H}$ , HSQC and HMBC  $^n\text{J}_{\text{CH}}$  ( $n = 2$  and  $3$ )], IR spectroscopy and elemental analysis.



**Scheme 3.** Synthesis of amino sugar quinones **12a-c** and **15a-c**.

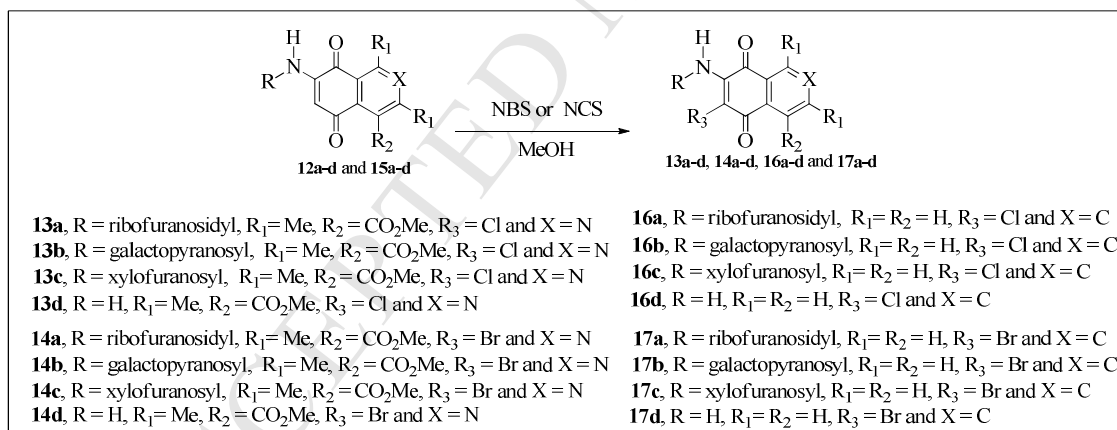
Major 2D HMBC correlations observed in the spectra of **27** and **12a** confirmed the regioselectivity of the addition reaction. In the spectrum of isoquinoline-5,8-dione **27** long range correlations from the methyl protons (3.04ppm) led to the assignments of C-1 (159.9ppm) and C-8a (121.0ppm). It was also observed long-range connectivity from H-7 (7.26ppm) to C-8a. In both spectra there were observed long range correlations from H-6 (5.70ppm, 7.26ppm) to C-4a (138.2ppm, 135.4ppm), C-8 (181.4ppm, 185.3ppm) and C-5 (180.6ppm, 184.4ppm). The resonances of the carbonyl carbons, C-5 and C-8, were assigned on the basis of analysis of the resonance effect of the amino group attached to the C-7 position of the quinone ring of **12a-d**, which renders the C-5 carbon less electrophilic.

In the Scheme 4, the addition reaction of sodium azide to unsaturated carbonyl compounds **27** and **28** gave the corresponding azidoquinones **30** and **31**, which were converted into the desired amino compounds **12d** and **15d**.<sup>[47]</sup>



**Scheme 4:** Synthesis of non-substituted aminoquinones **12d** and **15d**.

The reactions of the quinones **12a-d** and **15a-d** with *N*-chlorosuccinimide (NCS) and *N*-bromosuccinimide (NBS) gave the corresponding chlorinated compounds **13a-d**, **16a-d** and the brominated derivatives **14a-d**, **17a-d**, in good yields (Scheme 5).



**Scheme 5:** Preparation of quinone derivatives **13a-d**, **16a-d**, **14a-d** and **17a-d**.

## Biological analysis

Aminoquinones **12a-d**, **13a-d**, **14a-d**, **15a-d**, **16a-d** and **17a-d** were initially screened for antimicrobial activity against Gram-positive (*Enterococcus faecalis* ATCC 29212, *Staphylococcus aureus* ATCC 25923, *S. epidermidis* ATCC 12228, *S. simulans* ATCC 27851) and Gram-negative bacteria (*E. coli* ATCC 25922, *Proteus mirabilis*

ATCC 15290, *K. pneumoniae* ATCC 4352 and *P. aeruginosa* ATCC 27853) of clinical importance. The disc diffusion test is a qualitative susceptibility assay that revealed 14 active compounds among the 24 tested, with inhibition zones against both Gram-positive and Gram-negative bacteria (7-30 mm) similar to those of ciprofloxacin and vancomycin (Table 1). Interestingly, within the homologous series of 7-amino-isoquinoline-5,8-diones (**12a-d**), only quinone glycoconjugates **12b-c** were active (Table 1). These results suggested that the two types of sugar groups, galactopyranosyl and xilofuranosyl, respectively, are an important requirement for the selective antimicrobial activity of these compounds against *S. epidermidis* ATCC 12228 strain.

Compounds **13b-d**, resulting from the incorporation of chlorine substituent in the structure of their respective analogues **12b-d**, showed different antibacterial profiles (Table 1). The aminoquinone derivative **13b** was inactive against all microorganisms tested and **13c** produced a discrete halo of inhibition against the Gram-positive bacterium *S. epidermidis* ATCC 12228. Whereas **13d** showed growth inhibitory halos against all bacteria of the genus *Staphylococcus* similar to vancomycin. Brominated glycoconjugate derivative **14a** and non-glycosylated compound **14d** showed broad spectrum of activity against Gram-positive and Gram-negative strains. Interestingly, similar to vancomycin and ciprofloxacin, **14d** was active against four Gram-positive bacteria (*S. aureus* ATCC 25923, *S. epidermidis* ATCC 12228, *S. simulans* ATCC 27851 and *S. aureus* MRSA (BMB9393), and two Gram-negative bacteria (*E. coli* ATCC 25922 and *P. aeruginosa* ATCC 27853).

**Table 1:** Results of antimicrobial evaluation of compounds **12a-d**, **13a-d**, **14a-d**, **15a-d**, **16a-d** and **17a-d** against Gram-positive and Gram-negative bacterial strains of clinical importance using disc diffusion test.

Compound	Inhibition zone (mm)								
	Gram-positive					Gram-negative			
	<i>E. faecalis</i>	<i>S. aureus</i>	<i>S. epidermidis</i>	<i>S. simulans</i>	<i>S. aureus</i>	<i>E. coli</i>	<i>P. mirabilis</i>	<i>K. pneumoniae</i>	<i>P. aeruginosa</i>
	ATCC 29212	ATCC 25923	ATCC 12228	ATCC 27851	MRSA (BMB9393)	ATCC 25922	ATCC 15290	ATCC 4352	ATCC 27853
<b>12a</b>	0	0	0	0	0	0	0	0	0
<b>12b</b>	0	0	<b>9±1</b>	0	0	0	0	0	0
<b>12c</b>	0	0	<b>10±2</b>	0	0	0	0	0	0
<b>12d</b>	0	0	0	0	0	0	0	0	0
<b>13a</b>	0	0	0	0	0	0	0	0	0
<b>13b</b>	0	0	0	0	0	0	0	0	0
<b>13c</b>	0	0	<b>8±1</b>	0	0	0	0	0	0
<b>13d</b>	0	<b>15±1</b>	<b>18±2</b>	<b>16±1</b>	<b>12±2</b>	0	0	0	0
<b>14a</b>	0	<b>13±</b>	<b>11±2</b>	0	<b>12±2</b>	<b>17±1</b>	0	0	<b>17±2</b>
<b>14b</b>	0	0	0	0	0	0	0	0	0
<b>14c</b>	0	0	7	0	0	0	0	0	0
<b>14d</b>	0	<b>23±1</b>	<b>22±1</b>	<b>17±2</b>	<b>22±1</b>	<b>30±1</b>	0	0	<b>28±2</b>
<b>15a</b>	0	0	0	0	0	0	0	0	0
<b>15b</b>	0	0	0	0	0	0	0	0	0
<b>15c</b>	0	0	0	0	0	0	0	0	0
<b>15d</b>	0	<b>15±1</b>	<b>17±1</b>	<b>18±2</b>	<b>17±1</b>	<b>20±1</b>	0	0	<b>12±2</b>
<b>16a</b>	0	0	<b>9±1</b>	<b>7±1</b>	0	<b>10±2</b>	0	0	<b>9±1</b>
<b>16b</b>	0	<b>9±1</b>	<b>8±1</b>	0	0	<b>12±1</b>	0	0	<b>13±1</b>
<b>16c</b>	0	<b>14±1</b>	<b>15±1</b>	<b>13±2</b>	0	<b>17±1</b>	0	0	<b>21±3</b>
<b>16d</b>	0	<b>12±1</b>	<b>21±1</b>	<b>14±1</b>	<b>20±1</b>	<b>10±1</b>	0	0	<b>15±1</b>
<b>17a</b>	0	<b>8±1</b>	<b>10±1</b>	<b>8±1</b>	0	<b>16±2</b>	0	0	<b>13±1</b>
<b>17b</b>	0	0	0	0	0	0	0	0	0
<b>17c</b>	0	<b>8±1</b>	<b>10±1</b>	<b>9±1</b>	0	<b>12±1</b>	0	0	<b>16±1</b>
<b>17d</b>	0	<b>10±1</b>	<b>12±1</b>	<b>17±</b>	<b>16±1</b>	<b>8±1</b>	0	0	<b>14±1</b>
DMSO	0	0	0	0	0	0	0	0	0
Cip	-	-	-	-	-	35±2	28±1	27±1	36±1
Van	16±1	16±1	15±2	16±1	14±1	-	-	-	-

Cip = ciprofloxacin and Van = vancomycin (positive controls); DMSO = dimethyl sulphoxide (negative control).

None strains were sensitive to naphthoquinone glycoconjugates **15a-c** in contrast to four Gram-positive and two Gram-negative bacteria that showed sensitivity to the related non-substituted amino compound **15d** (Table 1).

The replacement of a hydrogen atom by electronegative halogen substituents (Cl and Br) in inactive molecules **15a-c** had a positive effect on the antimicrobial activity of corresponding chlorinated compounds **16a-c** and brominated compound **17a** (Table 1). These substances showed higher inhibition zones against Gram-positive and Gram-negative strains (7-21 mm). Based on the positive biological results of the chlorinated derivatives **16a-c** as well as of the brominated compound **17a** which have different monosaccharide units in their structures and also in the fact that the non-halogenated precursors **15a-c** have been inactive against the bacterial strains employed in the biological evaluation, we suggest that the presence of the halogen substituent is essential for the antimicrobial profile of these substances. The aminonaphthoquinone derivative **15d**, which lacks the sugar moiety in its structure, and its halogenated analogues **16d** and **17d** showed similar activity profiles against most bacterial strains. Interestingly, these molecules showed activity against *S. aureus* MRSA (16-20mm), which causes severe infection and is a difficult to treat strain (Table 1).

After disk diffusion analyses, the active quinone derivatives were submitted to MIC assays to determine the lowest concentration capable of inhibit bacterial visible growth. Promisingly, according to our MIC evaluation, the compounds **13c-d**, **14a-d**, **15d**, **16a-d** and **17a-d** presented an activity level (4-256 µg/mL) within the CLSI (CLSI 2015) range (0.08-256 µg/mL) (Table 2).

The quantitative analysis revealed that among isoquinoline-5,8-dione derivatives **12b-c** and their halogenated analogues **13c-d** and **14a-d**, only chlorinated amino compound **13c** exhibited a good active profile against Gram-positive bacteria, *S. aureus* ATCC 25923, *S. epidermidis* ATCC 12228 and *S. simulans* ATCC 27851 (MIC = 32 to 64 µg/mL). Interestingly, two brominated compounds, **14a** and **14d**, inhibited the growth of two Gram-negative bacteria, *E. coli* ATCC 25922 (8 and 8 µg/mL) and *P. aeruginosa* ATCC 27853 (8 and 32 µg/mL). These results suggested that the ribofuranosyl ring present in the **14a** structure contributed to the increase of the antibacterial activity in relation to *P. aeruginosa*.

**Table 2.** Comparison of Minimum Inhibitory Concentration (MIC) of quinone compounds **12b-c**, **13c-d**, **14a-d**, **16a-c** and **17a** against Gram-positive and Gram-negative bacteria.

Compound	MIC ( $\mu\text{g/mL}$ )					
	Gram-positive				Gram-negative	
	<i>S. aureus</i> ATCC 25923	<i>S. epidermidis</i> ATCC 12228	<i>S. simulans</i> ATCC 27851	<i>S. aureus</i> MRSA (BMB9393)	<i>E. coli</i> ATCC 25922	<i>P. aeruginosa</i> ATCC 27853
<b>12b</b>	ND	<b>256</b>	ND	ND	ND	ND
<b>12c</b>	ND	<b>256</b>	ND	ND	ND	ND
<b>13c</b>	ND	<b>32</b>	ND	ND	ND	ND
<b>13d</b>	<b>64</b>	<b>32</b>	32	ND	ND	ND
<b>14a</b>	ND	ND	ND	ND	<b>8</b>	<b>8</b>
<b>14c</b>	ND	<b>64</b>	ND	ND	ND	ND
<b>14d</b>	ND	<b>32</b>	ND	ND	<b>8</b>	<b>32</b>
<b>15d</b>	<b>64</b>	<b>128</b>	<b>64</b>	<b>64</b>	<b>32</b>	<b>64</b>
<b>16a</b>	ND	ND	ND	ND	<b>8</b>	<b>32</b>
<b>16b</b>	ND	ND	ND	ND	<b>16</b>	<b>32</b>
<b>16c</b>	ND	ND	ND	ND	<b>4</b>	<b>8</b>
<b>16d</b>	<b>64</b>	ND	<b>128</b>	<b>64</b>	<b>128</b>	<b>16</b>
<b>17a</b>	ND	ND	ND	ND	<b>32</b>	<b>64</b>
<b>17c</b>	ND	ND	ND	ND	<b>16</b>	<b>16</b>
<b>17d</b>	<b>64</b>	<b>128</b>	<b>128</b>	<b>64</b>	<b>64</b>	<b>32</b>
Cip	ND	ND	ND	ND	0.25	0.25
Van	2	2	2	2	ND	ND

Cip = ciprofloxacin and Van = vancomycin (positive controls). ND = no detected activity at 256  $\mu\text{g/mL}$ .

The structural analysis based on the antibacterial profile of the non-halogenated 1,4-naphthoquinones **15a-d** pointed to the direct relationship of the selectivity with the free amino group in the quinonoid ring, since only **15d** inhibited bacterial strains Gram-positive and Gram-negative. The correlated chlorinated compounds, **16a-c** and **17a-c**, showed promising effects on two Gram-negative bacteria, *E. coli* ATCC 25922 and *P. aeruginosa* ATCC 27853, with MIC in the range 4-128 µg/mL, within the values of CLSI. The introduction of a halogen substituent on the naphthoquinone ring appears to affect the selective inhibitory profile of compounds **16a-c** and **17a-c** against Gram-negative bacteria. These data are in accordance with previous results which indicate the halogen substituent as a modulator parameter for the antibacterial profile.<sup>[26]</sup> The compounds **16d** and **17d**, containing free amino group, exhibited antibacterial profile against Gram-positive strains, including *S. aureus* ATCC 25923 and *S. aureus* MRSA (BMB9393). The antibacterial profile of compounds **16d** and **17d** against MRSA contrasted with the absence of activity of their glycoconjugate analogs **16a-c** and **17a-c** which suggested that the presence of the carbohydrate moiety is important to these results. The sensitivity of MRSA to quinones **15d**, **16d** and **17d** is very important as this strain is a major public health problem worldwide over the past 20 years causing several serious diseases such as toxic shock syndrome, endocarditis, soft tissue infections, and necrotizing pneumonia.<sup>[48]</sup> All naphthoquinones tested (**15d**, **16a-d** and **17a-d**) displayed a significant antibacterial profile against two Gram-negative bacteria (*E. coli* and *P. aeruginosa*). Interestingly, the halogenated derivatives containing xylofuranosil group **16c** and **17c** were selective and 2- to 32-fold more potent than their corresponding parent compounds **16a-b** and **17a-b**. Apparently the enhanced antimicrobial activity of these derivatives can be related to the chemical structure of the furanose side chain (*e.g.*, conformation and intermolecular interactions). Reports in the literature<sup>[30]</sup> indicate that 2-halo-1,4-naphthoquinones are capable of inhibiting the growth of Gram-positive and Gram-negative bacteria. They suggest that the antimicrobial effect occurs through a competitive electron transport pathway with vitamin K or menaquinones<sup>[49]</sup>, which are essential naphthoquinone structures for the survival of several bacteria. Therefore, in the case of the new molecules here shown, possibly the sensitive strains initially recognized these naphthoquinone derivatives as quinones of their own biological systems, leading to a negative interference in the metabolic pathways involved with bacterial growth.

The analysis of the antibacterial profile of compounds **12b-c**, **13c-d**, **14a**, **14c-d**, **15d**, **16a-c** and **17c** also included evaluation of Minimum Bactericidal Concentration

(MBC) (Table 3). This parameter defines the lowest concentration of an antibacterial agent needed to kill the bacteria. The MBC/MIC ratio indicates the antibacterial (bacteriostatic and/or bactericidal) profile of the compound. The MBC / MIC ratio  $\leq 2$  indicates bactericidal activity, while the ratio MBC / MIC  $\geq 4$  defines the bacteriostatic effect.<sup>[50]</sup> According to our data, the MBC and MIC values of the most active compounds **14a**, **14d**, **16a-b**, **16c** and **17a** were the same, classifying them as bactericidal agents against Gram-negative bacteria (Table 3). This is an interesting and promising profile since this type of bacteria causes more difficult to treat infections hard to eradicate.<sup>[51]</sup>

In this work, the naphthoquinone derivatives (**15d**, **16a-d**, **17a** and **17c-d**) were more active than those derived from the isoquinoline-5,8-diones (**14a** and **14d**) against Gram-negative bacteria *E. coli* ATCC 25922 and *P. aeruginosa* ATCC 27853. The naphthoquinone derivatives **15d**, **16d** and **17d** showed promising antibacterial activity against two important gram positive bacteria *S. aureus* ATCC 25923 and *S. aureus* MRSA (BMB 9393). Thus these results indicate that this type of naphthoquinone compounds may contribute for the rational planning of the synthesis of new, more potent molecules against bacteria of great clinical importance.

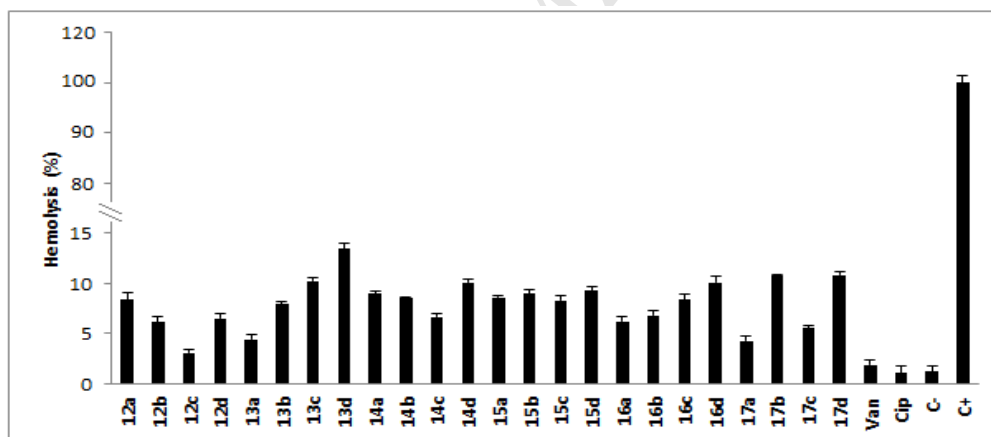


**Table 3.** Bactericidal and / or bacteriostatic profiles of active compounds **12b-c**, **13c-d**, **14a**, **14c-d**, **15d**, **16a-d**, **17a** and **17c-d** based on the calculation of the Minimal Bactericidal Concentration (MBC) / Minimum Inhibitory Concentration (MIC)

Compound	<i>S. aureus</i> ATCC 25923		<i>S. epidermidis</i> ATCC 12228		<i>S. simulans</i> ATCC 27851		<i>S. aureus</i> MRSA (BMB9393)		<i>E. coli</i> ATCC 25922		<i>P. aeruginosa</i> ATCC 27853	
	MBC (µg/mL)	MBC /MIC	MBC (µg/mL)	MBC /MIC	MBC (µg/mL)	MBC/ MIC	MBC (µg/mL)	MBC/ MIC	MBC (µg/mL)	MBC/ MIC	MBC (µg/mL)	MBC /MIC
<b>12b</b>	ND	-	256	1	ND	-	ND	-	ND	-	ND	-
<b>12c</b>	ND	-	256	1	ND	-	ND	-	ND	-	ND	-
<b>13c</b>	ND	-	32	1	ND	-	ND	-	ND	-	ND	-
<b>13d</b>	64	1	32	1	32	1	ND	-	ND	-	ND	-
<b>14a</b>	ND	-	ND	-	ND	-	ND	-	8	1	8	1
<b>14c</b>	ND	-	64	1	ND	-	ND	-	ND	-	ND	-
<b>14d</b>	ND	-	32	1	ND	-	ND	-	8	1	32	1
<b>15d</b>	64	1	128	1	64	1	64	1	32	1	64	1
<b>16a</b>	ND	-	ND	-	ND	-	ND	-	8	1	32	-
<b>16b</b>	ND	-	ND	-	ND	-	ND	-	16	1	32	1
<b>16c</b>	ND	-	ND	-	ND	-	ND	-	4	1	8	1
<b>16d</b>	64	1	ND	-	128	1	64	1	128	1	16	1
<b>17a</b>	ND	-	ND	-	ND	-	ND	-	32	1	34	1
<b>17c</b>	ND	-	ND	-	ND	-	ND	-	16	1	16	1
<b>17d</b>	64	1	128	1	128	1	64	1	64	1	32	1

ND = no detected activity at 256 µg/mL. MBC/MIC $\leq$ 2 = bactericidal activity; MBC/MIC $\geq$ 4 = bacteriostatic activity.

This study also investigated the hemocompatibility of the quinone derivatives **12a-d**, **13a-d**, **14a-d**, **15a-d**, **16a-d** and **17a-d** using the hemolysis test (Graphic 1). These compounds (200  $\mu\text{g/mL}$ ) were added to the solution of healthy human erythrocytes to check whether or not erythrocyte membrane lysis would occur, with consequent release of hemoglobin. Among the 24 molecules tested, the quinone compounds containing free amino group **12d** and **15d** and glycoconjugated derivatives **12a-d**, **13a-b**, **14a-c**, **15a-c**, **16a-b**, **17a** and **17c** showed a percentage of lysis of erythrocytes less than 10% which classified them as nonhemolytic, according to the literature.<sup>[52]</sup> The results showed that non-halogenated isoquinoline-5,8-diones **12b-c** were less toxic than their chlorinated **13b-c** and brominated compounds **14a-c**. An opposite effect occurred with the naphthoquinone series where the halogenated derivatives **16a-c**, **17a** and **17c** were less toxic than their non-halogenated analogues of quinone. The quinone compounds containing free amino group **13d**, **14d**, **15d**, **16d** and **17d** and three glycoconjugated derivatives **13c**, **16c** and **17b** showed a discrete hemolytic profile (10.08-14.43%).



**Graphic 1.** Hemocompatibility profile of glycoconjugate and non-glycoconjugate amino compounds derived from of isoquinoline-5,8-dione and 1,4-naphthoquinone through hemolytic assay. Non-hemolytic  $\leq 10\%$ . Van = vancomycin; Cip = ciprofloxacin; C- = negative control (1% DMSO) and C+ = positive control (1% Triton X-100).

Finally, we analyzed the theoretical Absorption, Distribution, Metabolism and Excretion (ADMET) profiles of the derivatives **12a-d**, **13a-d**, **14a-d**, **15a-d**, **16a-d** and **17a-d** and the results revealed all with high gastrointestinal (GI) absorption profile. This is an important an expected parameter for oral administration medications.<sup>[53]</sup> According to the *in silico* data, only non-glyconconjugate amino compounds derived from 1,4-naphthoquinone (**15d**, **16d** and **17d**) showed cross blood-brain barrier (BBB) features. This result suggested that the sugar groups may influence the polarity, lipophilicity and volume of these molecules, leading to the reduction in the ability to overcome the barrier.<sup>[54]</sup> BBB is known as avoiding the brain uptake of most pharmaceuticals<sup>[55]</sup> including some antibiotics of clinical use such as ciprofloxacin, vancomycin, nitrofurantoin and cefoxitin (Table 4). Molecules that overcome this barrier may be used in the treatment of nervous system infections (*e.g.*, meningitis).<sup>[56,57]</sup>

Cytochrome P4503A4 is the most commonly isoform in human liver, responsible for metabolization about 60% of the therapeutics drugs.<sup>[58]</sup> The interaction with CYP3A4 can interfere in drug metabolism and enhance toxicological effects. Antibiotics such as macrolides (*e.g.*, erythromycin and clarithromycin), used to treat respiratory tract infections, skin infections and syphilis, are moderate/potent inhibitors of CYP3A4. This feature increases the risk of drug-drug interactions and severe toxicity when co-administrated with CYP3A4 substrates, (*e.g.*, sildenafil, midazolam and omeprazole).<sup>[59,60]</sup> In this work we analyzed whether the derivatives could act as inhibitors of the CYP3A4 isoenzyme by using the software SwissADME.

**Table 4.** Absorption, Distribution, Metabolism and Excretion (ADME) and theoretical toxicity parameters, calculated by SwissADME and admetSAR software, respectively. Comparison among glycoconjugate and non-glycoconjugate amino compounds and antibiotics (ciprofloxacin, vancomycin, nitrofurantoin and cefoxitin) of clinical use.

Compounds	ADMET parameters				
	GI	BBB	CYP3A4 inhibitor	Carcinogenic	Mutagenic
<b>12a-c</b>	High	-	-	-	-
<b>12d</b>	High	-	-	-	+
<b>13a-d</b>	High	-	-	-	-
<b>14a-d</b>	High	-	-	-	-
<b>15a-c</b>	High	-	-	-	-
<b>15d</b>	High	+	+	-	+
<b>16a-c</b>	High	-	-	-	-
<b>16d</b>	High	+	+	-	+

<b>17a-c</b>	High	-	-	-	-
<b>17d</b>	High	+	+	-	+
<b>Ciprofloxacin</b>	High	-	-	-	-
<b>Vancomycin</b>	Low	-	-	-	-
<b>Nitrofurantoin</b>	High	-	+	-	+
<b>Cefoxitin</b>	Low	-	+	-	-

GI = Gastrointestinal; BBB = Blood-brain barrier; CYP3A4 = Cytochrome P450 3A4.

The results predicted that 7-amino-isoquinoline-5,8-dione glycoconjugates **12a-c**, **13a-c** and **14a-c** and non-glycoconjugate derivatives **12d**, **13d** and **14d** did not inhibit CYP3A4. However, the non-glycoconjugate compounds derived from 1,4-naphthoquinone (**15d**, **16d** and **17d**) showed inhibitory activity suggesting that the presence of carbohydrate substituent in the parent structures **15a-c**, **16a-c** and **17a-c** seems to be important to avoid enzyme inhibition.

Theoretical toxicity analysis predicted glycoconjugate quinones **12a-c**, **13a-c**, **14a-c**, **15a-c**, **16a-c** and **17a-c** with no carcinogenic or mutagenic profiles, similar to ciprofloxacin, vancomycin and cefoxitin (Table 4). The results highlighted the importance of the sugar groups in contributing to the non-mutagenic effect observed for these derivatives, since those non-glycoconjugated compounds **12d**, **15d**, **16d** and **17d** presented a risk of mutagenic effect. It is important to note that far from assuming the absence of risk and side effects profiles, the *in silico* data reinforced the promising potential of carbohydrate-conjugated quinones for further exploring *in vitro* and *in vivo* studies and to prioritize the molecules for these evaluations.

## Experimental Section

### *In vitro* biological assay

#### *Bacterial strains*

The species used in this study were supplied by Fiocruz and obtained by the American type culture collection (ATCC), of which 5 Gram-positives: *Enterococcus faecalis* INCQS 00234 (ATCC 29212), *S. aureus* INCQS 00015 (ATCC 25923), *S. epidermidis* INCQS 00016 (ATCC 12228), *S. simulans* INCQS 00254 (ATCC 27851), and 4 Gram-negative, *Proteus mirabilis* INCQS 00095 (ATCC 15290), *Pseudomonas*

*aeruginosa* INCQS 00099 (ATCC 27853), *E. coli* INCQS 00033 (ATCC 25922), *Klebsiella pneumoniae* INCQS 00083 (ATCC 4352). We also used a multiresistant MRSA strain, BMB9393, from the lineage ST239 of multilocus sequencing type (MLST), which is highly disseminated in Brazilian hospitals. This variant was isolated in 1993 from a case of nosocomial bloodstream infection in Rio de Janeiro<sup>[61]</sup>, and its complete genome was sequenced by Costa and coworkers.<sup>[62]</sup> All strains were kept in Müller Hinton Broth (MHB) supplemented with 10% (v/v) glycerol at -70°C and were routinely cultivated on MHB.

#### *Disc Diffusion Test*

The screening of the antibacterial activity of quinones was performed by the method initially describes by Kirby-Bauer and according by the Clinical and Laboratory Standards Institute CLSI.<sup>[63]</sup> Bacterial strains were carried out in Mueller Hinton medium (HIMEDIA) and adjusted to  $10^8$  UFC/mL (0.5 McFarland standard) and was uniform spread with swab at petri plate containing Agar Mueller Hinton (HIMEDIA). The compounds were dissolved in dimethyl sulfoxide (DMSO, Sigma-Aldrich) 99.9% and applied in paper disks (Laborclin) in the concentration of 5mg/mL. After the incubation period at 37°C between 18-24h the interpretation of result occurred by the measurement of the inhibition zone forming around the disk. The negative control used was DMSO. The positive controls utilized were vancomycin (by Gram-positive strains) and ciprofloxacin (by Gram-negative strains). The assays were realized in three independent triplicates.

#### *Minimum Inhibitory Concentration (MIC) using microdilution method*

The MIC is defined as the lowest concentration of the substance that prevents visible bacterial growth. The active molecules detected in disk diffusion method were diluted in a 96-well microplate with 100  $\mu$ L of liquid growth medium in geometrically increasing concentrations (0.125  $\mu$ g/mL to 256  $\mu$ g/mL). Then 100  $\mu$ L of bacteria suspension which the inoculum containing  $10^6$  UFC/mL was added in each well. The highest DMSO concentration used in this system was 5%, which had no effect on bacterial growth. Ciprofloxacin and vancomycin were used as positive control against Gram-negative and Gram-positive strains, respectively. After 24 hours of incubation at 37°C, the presence of turbidity or a sediment indicates growth of microorganism and the MIC was

determined by the lower concentration that led to no turbidity. Experiments were performed in triplicate.

#### *Minimum bactericidal concentration – MBC*

The MBC assay was performed through the transference of the culture medium of each well in MIC microplate with no visible growth (10  $\mu$ L) to agar plates. These plates were incubated during 24 h at 37°C and the MBC was determined as the minimum concentration of the compounds capable of inhibiting 99.9% of bacterial growth. The experiment was carried out in triplicate. A colorimetric assay using resazurin was also performed and compared to the conventional plate method to reinforce the quantitative data. An aliquot of 20  $\mu$ L of 0.01% resazurin (Sigma-Aldrich) was added into each well of the 96-well microtiter plates used for the MIC assay. The cells metabolically active were identified by changing the color of the resazurin from blue to pink after incubation for 2 hours at 37°C. The ratio of MBC/MIC was used to classify if the mechanism of action of the derivatives was bactericidal ( $\text{MBC/MIC} \leq 2$ ) or bacteriostatic ( $\text{MBC/MIC} \geq 4$ ).<sup>[50]</sup>

#### *Hemocompatibility – hemolysis assay*

The blood samples were donated from healthy human subjects in compliance with ethics committee and institutional guidelines, document number 621196 approved in May 2014 with an expiration date of May 2018. The blood was donated after reading and signing the consent form terms. Erythrocytes were washed collected in citrate tube and washed 3 times with PBS (pH 7.4) by centrifugation and suspended in the same buffer. All derivatives (200  $\mu$ g/mL) were incubated with the erythrocyte suspension for 3 h at 37°C. The interaction with the erythrocytes membrane and possible release of hemoglobin was quantified by spectrophotometric reading of the supernatant at 545 nm. The experiments were performed three times in independent way. Complete hemolysis (positive control) was determined by using 1% Triton X-100. Hemolysis less than 10% represented hemocompatibility and non-toxicity against erythrocyte membranes, as described elsewhere.<sup>[52]</sup>

## ***In silico* ADMET properties**

### *Toxicological profile*

The *in silico* analysis involved toxicology parameters of two series of glycoconjugate and non-glycoconjugate amino compounds derived from isoquinoline-5,8-dione and 1,4-naphthoquinone and their halogenated derivatives calculated by using admetSAR@LMMD software (lmmd.ecust.edu.cn:8000/). The data were compared with the profiles calculated for four antibacterial (cefoxitin, ciprofloxacin, nitrofurantoin and vancomycin). The carcinogenic and mutagenic properties of the molecules were calculated based on a data bank with over 210,000 pieces of toxicological information for more than 96,000 compounds with 45 ADMET-associated properties in scientific literature.<sup>[64]</sup>

### *ADME parameters*

The Absorption, Distribution, Metabolism and Excretion (ADME) properties were calculated using SwissADME web tool ([www.swissadme.ch](http://www.swissadme.ch)). The analysis was based on molecular fingerprint that uses a sequence of bits that consider all fragments of the molecular structure, allowing to access models for ADME behaviors in a large virtual screening. In this work, we analyzed interaction with blood-brain barrier (BBB), gastrointestinal absorption and inhibition of CYP450 3A4 (CYP3A4).<sup>[64]</sup>

## **Chemistry**

Melting points were determined with a Fisher-Johns/Melting Point Apparatus instrument and are uncorrected. Infrared (IR) spectra were recorded on a Perkin-Elmer FT-IR, model 1600 series spectrometer in KBr pellets, and frequencies were expressed in  $\text{cm}^{-1}$ . NMR spectra were recorded on a Varian Unity Plus 300 MHz or 500 MHz spectrometer, in the specified solvents. Chemical shifts ( $\delta$ ) are reported in ppm, and the coupling constants (J) are expressed in Hertz. Mass spectra were recorded on a high-resolution TOF apparatus – QTOF/Micro from Waters-MicroMass. The fragments are described as the relation between atomic mass units and the load (m/z). Column chromatography was performed on silica gel flash from Acros. The reactions were routinely monitored by thin layer chromatography (TLC) on silica gel pre-coated F254

Merck plates. The developed chromatograms were viewed under ultraviolet light at 254 nm.

*Synthesis of halogenates 2-amino-naphthoquinones derivatives 16a-c and 17a-c*

Aminocarbohydrate derivatives **23a-c** (1.4 mmol) and *N*-halosuccinimide (1.35 mmol) were dissolved in 12.5 mL of methanol was kept at room temperature for 24 hours. After the solvent was evaporated under reduced pressure, the products were purified by column chromatography on silica gel using hexane:ethyl acetate (9:1) as eluent to yield chlorinated and brominated compounds **16a-c** and **17a-c**, respectively.

**3-chloro-2-(methyl-5'-deoxy-2',3'-*O*-isopropylidene- $\beta$ -D-ribofuranosid-5'-yl)-amino-1,4-naphthoquinone (16a):** yield: 91% as a brown solid; m.p.: 165-166 °C; **IR**  $\nu_{\max}$  (cm<sup>-1</sup>; **KBr**): 3266 (N-H); 1681 and 1586 (C=O), 1564 (C=C); **<sup>1</sup>H NMR (500.00 MHz, CDCl<sub>3</sub>)  $\delta$  (ppm):** 1.32 (s, 3H, CH<sub>3</sub>); 1.49 (s, 3H, CH<sub>3</sub>); 3.86-3.91 (m, 1H, H-5''); 3.43 (s, 3H, OCH<sub>3</sub>); 4.13-4.18 (m, 1H, H-5'); 4.44-4.47 (m, 1H, H-4'); 4.65 (d, 1H, *J* = 6.0 Hz, H-3'); 4.68 (d, 1H, *J* = 6.0 Hz, H-2'); 5.05 (s, 1H, H-1'); 6.46 (s, 1H, N-H); 7.63 (td, 1H, *J* = 7.5 and 1.0 Hz, H-6); 7.72 (td, 1H, *J* = 7.5 and 1.0 Hz, H-7); 8.04 (dd, 1H, *J* = 8.0 and 1.0 Hz, H-5); 8.14 (dd, 1H, *J* = 7.5 and 1.0 Hz, H-8); **<sup>13</sup>C NMR (125.00 MHz, CDCl<sub>3</sub>)  $\delta$  (ppm):** 25.10 (CH<sub>3</sub>); 26.59 (CH<sub>3</sub>); 47.82 (C-5'); 55.80 (OCH<sub>3</sub>); 82.04 (C-3'); 85.46 (C-4'); 85.71 (C-2'); 110.06 (C-1'); 112.98 (-OCO-); 127.01 (C-8 and C-5); 130.35 (C-8a); 132.72 (C-6); 132.68 (C-4a); 135.06 (C-7); 149.22 (C-2); 170.72 (C-4); 179.19 (C-1); HRMS-ESI: *m/z*: [M + H]<sup>+</sup> calcd for C<sub>19</sub>H<sub>20</sub>ClNO<sub>6</sub>, 393.0979; found: 394.1046.

**3-Chloro-2-(6'-Deoxy-1',2':3',4'-di-*O*-isopropylidene-D-galactopiranos-6'-yl)-amino-1,4-naphthoquinone (16b):** yield: 83% as a brown solid; m.p.: 93-95 °C; **IR**  $\nu_{\max}$  (cm<sup>-1</sup>; **film**): 3330 (N-H), 1602 and 1677 (C=O), 1572 (C=C). **<sup>1</sup>H NMR (500.00 MHz, CDCl<sub>3</sub>)  $\delta$  (ppm):** 1.31 (s, 3H, CH<sub>3</sub>); 1.36 (s, 3H, CH<sub>3</sub>); 1.45 (s, 3H, CH<sub>3</sub>); 1.50 (s, 3H, CH<sub>3</sub>); 3.89-3.94 (m, 1H, H-6''); 4.03-4.05 (m, 1H, H-5'); 4.21-4.24 (m, 1H, H-6'); 4.27 (dd, 1H, *J* = 8.0 and 1.5 Hz, H-4'); 4.33 (dd, 1H, *J* = 5.0 and 2.5 Hz, H-2'); 4.63 (dd, 1H, *J* = 8.0 and 2.5 Hz, H-3'); 5.54 (d, 1H, *J* = 5.0 Hz, H-1'); 6.52 (s, 1H, N-H); 7.61 (td, 1H, *J* = 7.5 and 1.5 Hz, H-6); 7.71 (td, 1H, *J* = 7.5 and 1.5 Hz, H-7); 8.03 (dd, 1H, *J* = 8.0 and 1.0 Hz, H-5); 8.13 (d, 1H, *J* = 8.0 and 1.0 Hz, H-8). **<sup>13</sup>C NMR**



(125.00 MHz, CDCl<sub>3</sub>)  $\delta$  (ppm): 24.46 ( $\underline{\text{CH}}_3$ ); 25.03 ( $\underline{\text{CH}}_3$ ); 26.12 ( $\underline{\text{CH}}_3$ ); 45.25 (C-6'); 66.98 (C-5'); 70.68 (C-2'); 71.04 (C-4'); 71.65 (C-3'); 96.53 (C-1'); 108.99 (-O $\underline{\text{CO}}$ -); 109.90 (-O $\underline{\text{CO}}$ -); 126.92 (C-5); 126.99 (C-8); 130.55 (C-8a); 132.59 (C-6); 132.76 (C-4a); 134.94 (C-7); 147.21 (C-2); 181.79 (C-4); 189.42 (C-1). HRMS–ESI:  $m/z$ : [M + H]<sup>+</sup> calcd for C<sub>22</sub>H<sub>24</sub>ClNO<sub>7</sub>: 449.8815; found: 450.1302.

**3-chloro-2-(5'-deoxy-1',2'-*O*-isopropylidene-*D*-xilofuranos-5'-yl)-amino-1,4-naphthoquinone (16c):** yield: 80% as a brown solid; m.p.: 99-101<sup>0</sup>C; IR  $\nu_{\text{max}}$  (cm<sup>-1</sup>; film): 3307 (N-H), 1598 and 1676 (C=O), 1566 (C=C). <sup>1</sup>H NMR (500.00 MHz, CDCl<sub>3</sub>)  $\delta$  (ppm): 1.32 (s, 3H,  $\underline{\text{CH}}_3$ ); 1.50 (s, 3H,  $\underline{\text{CH}}_3$ ); 4.10-4.17 (m, 2H, H-5' and H-5''); 4.34 (d, 1H,  $J$  = 2.5 Hz, H-3'); 4.42-4.44 (m, 1H, H-4') 4.55 (d, 1H,  $J$  = 4.0 Hz, H-2'); 6.00 (d, 1H,  $J$  = 3.5 Hz, H-1'); 7.62 (td, 1H,  $J$  = 7.5 and 1.5 Hz, H-6); 7.70 (td, 1H,  $J$  = 7.5 and 1.0 Hz, H-7); 8.00 (dd, 1H,  $J$  = 7.5 and 1.0 Hz, H-5); 8.12 (dd, 1H,  $J$  = 8.0 and 1.0 Hz, H-8). <sup>13</sup>C NMR (125.00 MHz, CDCl<sub>3</sub>)  $\delta$  (ppm): 26.37 ( $\underline{\text{CH}}_3$ ); 26.91 ( $\underline{\text{CH}}_3$ ); 62.44 (C-5'); 75.65 (C-3'); 79.25 (C-4'); 85.71 (C-2'); 104.77 (C-1'); 112.22 (-O $\underline{\text{CO}}$ -); 127.12 (C-5); 127.78 (C-8); 130.06 (C-8a); 132.34 (C-4a); 135.03 (C-7); 135.64 (C-6); 146.30 (C-2); 171.55 (C-4); 176.94 (C-1). HRMS–ESI:  $m/z$ : [M + H]<sup>+</sup> calcd for C<sub>18</sub>H<sub>18</sub>ClNO<sub>6</sub>: 379.0823; found: 380.0883.

**3-bromo-2-(methyl-5'-deoxy-2',3'-*O*-isopropylidene- $\beta$ -*D*-ribofuranosid-5'-yl)-amino-1,4-naphthoquinone (17a):** yield: 85% as a brown solid; m.p.: 124-125<sup>0</sup>C; IR  $\nu_{\text{max}}$  (cm<sup>-1</sup>; KBr): 3065 (N-H); 1683 and 1581 (C=O), 1505 (C=C). <sup>1</sup>H NMR (500.00 MHz, CDCl<sub>3</sub>)  $\delta$  (ppm): 1.32 (s, 3H,  $\underline{\text{CH}}_3$ ); 1.49 (s, 3H,  $\underline{\text{CH}}_3$ ); 3.83-3.88 (m, 1H, H-5'); 3.42 (s, 3H, O $\underline{\text{CH}}_3$ ); 4.18-4.23 (m, 1H, H-5''); 4.45-4.47 (m, 1H, H-4'); 4.66 (d, 1H,  $J$  = 6.0 Hz, H-3'); 4.68 (d, 1H,  $J$  = 6.0 Hz, H-2'); 5.05 (s, 1H, H-1'); 6.45 (s, 1H, N-H); 7.63 (td, 1H,  $J$  = 7.5 and 1.0 Hz, H-6); 7.71 (td, 1H,  $J$  = 7.5 and 1.0 Hz, H-7); 8.03 (dd, 1H,  $J$  = 8.0 and 1.0 Hz, H-5); 8.14 (dd, 1H,  $J$  = 8.0 and 1.0 Hz, H-8). <sup>13</sup>C NMR (125.00 MHz, CDCl<sub>3</sub>)  $\delta$  (ppm): 25.10 ( $\underline{\text{CH}}_3$ ); 26.59 ( $\underline{\text{CH}}_3$ ); 48.21 (C-5'); 55.82 (O $\underline{\text{CH}}_3$ ); 82.05 (C-3'); 85.46 (C-4'); 85.55 (C-2'); 110.11 (C-1'); 112.97 (-O $\underline{\text{CO}}$ -); 127.07 (C-5); 127.22 (C-8); 130.10 (C-8a); 132.66 (C-6); 132.37 (C-4a); 134.95 (C-7); 146.77 (C-2); 176.64 (C-4); 180.22 (C-1). HRMS–ESI:  $m/z$ : [M + 2H]<sup>+</sup> calcd for C<sub>19</sub>H<sub>20</sub>BrNO<sub>6</sub>, 438.2709; found: 440.0523.

**3-bromo-2-(6'-Deoxy-1',2':3',4'-di-*O*-isopropylidene-D-galactopiranos-6'-yl)-amino-1,4-naphthoquinone (17b):** yield: 88% as a brown solid; m.p.: 108-110 °C; **IR**  $\nu_{\max}$  ( $\text{cm}^{-1}$ ; **film**): 3335 (N-H), 1599 and 1677 (C=O), 1567 (C=C).  **$^1\text{H}$  NMR (500.00 MHz,  $\text{CDCl}_3$ )  $\delta$  (ppm):** 1.31 (s, 3H,  $\text{CH}_3$ ); 1.35 (s, 3H,  $\text{CH}_3$ ); 1.45 (s, 3H,  $\text{CH}_3$ ); 1.49 (s, 3H,  $\text{CH}_3$ ); 3.89-3.95 (m, 1H, H-6'); 4.04-4.05 (m, 1H, H-5'); 4.25-4.27 (m, 1H, H-6''); 4.28 (dd, 1H,  $J = 8.0$  and  $1.5$  Hz, H-4'); 4.33 (dd, 1H,  $J = 5.0$  and  $2.5$  Hz, H-2'); 4.64 (dd, 1H,  $J = 8.0$  and  $2.5$  Hz, H-3'); 5.52 (d, 1H,  $J = 5.0$  Hz, H-1'); 6.53 (s, 1H, N-H); 7.57 (td, 1H,  $J = 14.4$  and  $8.0$  Hz, H-6); 7.70 (td, 1H,  $J = 14.5$  and  $8.0$  Hz, H-7); 8.02 (d, 1H,  $J = 7.5$ , H-5); 8.13 (d, 1H,  $J = 7.5$  Hz, H-8).  **$^{13}\text{C}$  NMR (125.00 MHz,  $\text{CDCl}_3$ )  $\delta$  (ppm):** 24.44 ( $\text{CH}_3$ ); 25.04 ( $\text{CH}_3$ ); 26.15 ( $\text{CH}_3$ ); 45.55 (C-6'); 66.86 (C-5'); 70.70 (C-2'); 71.04 (C-4'); 71.64 (C-3'); 96.52 (C-1'); 108.98 (-OCO-); 109.88 (-OCO-); 127.02 (C-5); 127.14 (C-8); 130.11 (C-8a); 132.52 (C-6); 133.69 (C-4a); 134.83 (C-7); 148.41 (C-2); 176.58 (C-4); 180.11 (C-1). **HRMS-ESI:  $m/z$ :**  $[\text{M} + 2\text{H}]^+$  calcd for  $\text{C}_{22}\text{H}_{24}\text{ClNO}_7$ : 494.0824; found: 496.0806.

**3-bromo-2-(5'-Deoxy-1',2'-*O*-isopropylidene-D-xilofuranos-5'-yl)-amino-1,4-naphthoquinone (17c):** yield: 68% as a brown solid; m.p.: 85-87 °C; **IR**  $\nu_{\max}$  ( $\text{cm}^{-1}$ ; **film**): 3312 (N-H), 1601 and 1660 (C=O), 1571 (C=C).  **$^1\text{H}$  NMR (500.00 MHz,  $\text{CDCl}_3$ )  $\delta$  (ppm):** 1.32 (s, 3H,  $\text{CH}_3$ ); 1.49 (s, 3H,  $\text{CH}_3$ ); 4.09-4.14 (m, 1H, H-5'); 4.20-4.23 (m, 1H, OH); 4.33-4.35 (m, 1H, H-3'); 4.35-4.38 (m, 1H, H-5''); 4.42-4.44 (m, 1H, H-4') 4.55 (d, 1H,  $J = 4.0$  Hz, H-2'); 6.00 (d, 1H,  $J = 3.5$  Hz, H-1'); 7.62 (td, 1H,  $J = 8.0$  and  $1.5$  Hz, H-6); 7.70 (td, 1H,  $J = 7.0$  and  $1.0$  Hz, H-7); 8.01 (d, 1H,  $J = 7.5$  Hz, H-5); 8.12 (d, 1H,  $J = 7.5$  Hz, H-8).  **$^{13}\text{C}$  NMR (125.00 MHz,  $\text{CDCl}_3$ )  $\delta$  (ppm):** 26.36 ( $\text{CH}_3$ ); 26.92 ( $\text{CH}_3$ ); 43.81 (C-5'); 75.66 (C-3'); 79.10 (C-4'); 85.69 (C-2'); 104.96 (C-1'); 112.19 (-OCO-); 127.07 (C-5); 127.17 (C-8); 130.13 (C-8a); 132.36 (C-4a); 132.63 (C-6); 134.93 (C-7); 147.10 (C-2); 177.24 (C-4); 180.26 (C-1). **HRMS-ESI:  $m/z$ :**  $[\text{M} + 2\text{H}]^+$  calcd for  $\text{C}_{18}\text{H}_{18}\text{BrNO}_6$ , 424.2415; found: 426.0382.

## Conclusion

In this work, we synthesized the amino sugar-based isoquinoline-5,8-diones **12a-c** and the naphthoquinones **15a-c**, their corresponding halogen compounds **13a-c**, **14a-c**, **16a-c** and **17a-c**, 7-amino-isoquinoline-5,8-diones **12d**, **13d** and **14d** and naphthoquinone derivatives **15d**, **16d** and **17d**. These substances were evaluated for their antimicrobial activity against Gram-positive and Gram-negative strains and the isoquinoline-5,8-dione **14a** and the naphthoquinone derivatives **16a-c**, **17a** and **17c** exhibited promising bactericidal activity (MIC and MBC values in the range of 4-64 µg/mL) against Gram-negative bacteria responsible for infections whose treatment is very difficult.

Only non-glycoconjugate naphthoquinones **15d**, **16d** and **17d** exhibited important antimicrobial activity against two Gram positive strains *S. aureus* ATCC 25923 and *S. aureus* MRSA (BMB9393). The compounds **14a**, **16a-c**, **17a**, **17c** and **15d** did not present a hemolytic profile when tested in erythrocyte cultures and constitute promising molecules for the rational synthetic design of novel antimicrobial agents against bacterial strains of clinical importance.

## Acknowledgements

This work was supported by the Brazilian agency FAPERJ. Fellowships granted to UFF, by CAPES, CNPq, CNPq-PIBIC and FAPERJ are gratefully acknowledged.

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**Highlights**

- Amino sugar based quinones were synthesized with antimicrobial activity.
- Quinones were tested against bacteria of clinical importance.
- Several quinone derivatives exhibited promising activity against Gram-negative bacteria.
- Halogen compounds were classified as bactericidal agents against Gram-negative bacteria.