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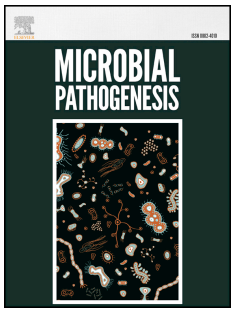

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IN SILICO EVALUATION OF THE ANTIBACTERIAL AND MODULATORY ACTIVITY OF LAPACHOL AND NOR-LAPACHOL DERIVATES

Fernando Gomes. Figueredo [a,d] ; Ingrid T.L. Ramos;[b] Josinete A. Paz; [b] Tania M.S.Silva; [b] Celso A. Camara [b], Cícera Datiane de Morais Oliveira-Tintino[c], Saulo Relison Tintino[c], Pablo Antônio Maia de Farias [d], Henrique Douglas Melo Coutinho[c] *, Marta Maria de F. Fonteles[a]

a Development and Technological Innovation in Medicines, Universidade Federal do Ceará – UFC- CEP 60.430-370 - Fortaleza - CE, Brazil.

b Department of Molecular Sciences, Universidade Federal Rural de Pernambuco, Recife, PE, Brazil

c Department of Biological Chemistry, Regional University of Cariri, Crato, CE, Brazil

d Department of Microbiology, Faculdade de Medicina Estácio de Juazeiro do Norte- CEP: 63048-080, Juazeiro do Norte- CE, Brazil.

∗ Corresponding author: Henrique D.M. Coutinho

Laboratory of Microbiology and Molecular Biology, Department of Biological Chemistry, Regional University of Cariri – URCA, Crato- CE, Brazil, Rua Cel. Antônio Luís 1161, Pimenta, 63105-000, Brazil.

E-mail addresses: hdmcoutinho@gmail.com, hdmcoutinho@urca.br (H.D.M. Coutinho).

Abstract

The aim of this research was to investigate the pharmacological properties of 2-(2-Hydroxyethylamine)-3-(3-methyl-2-butenyl)-1,4-dihydro-1,4-naphthalenedione, 2-(2-Hydroxy-ethylamine)-3-(2-methyl-propenyl)-[1,4]naphthoquinone and 2-(3-Hydroxy-propylamine)-3-(3-methyl-2-butenyl)-[1,4]naphthoquinone using computational prediction models, in addition to evaluating the *in vitro* antibacterial and modulatory activity of these compounds against bacterial ATCC strains and clinical isolates. The substances were synthesized from 2-hydroxy-quinones, lapachol and nor-lapachol obtaining the corresponding 2-methoxylated derivatives via dimethyl sulfate alkylation in a basic medium, these then reacted chemoselectively with 2-ethanolamine and 3-propanolamine to form the corresponding amino alcohols. The antibacterial activity and modulatory activity of the substances were assayed by broth microdilution method to determine the Minimum Inhibitory Concentration (MIC). The molecular structures were analyzed using the ChEMBL database to predict possible pharmacological targets, which pointed to the molecule 2- (2-Hydroxy-ethylamine)-3-(2-methyl-propenyl)-[1,4]naphthoquinone as a probable antibacterial agent for the proteins Replicative DNA helicase and RecA. The compounds had a low molecular weight and a small number of rotatable bonds. The MICs of the substances were not clinically significant, however, the association with gentamicin and amikacin reduced the MICs of these antibiotics. In conclusion, the combination of these substances with aminoglycosides may be a therapeutic alternative to bacterial resistance and the reduction of side effects.

Keywords: Computer Simulation; Antibacterials; Modulation; Lapachol, Norlapachol; Derivatives

1. Introduction

The emergence of new multiresistant microbial strains over the last few years has led to an increase in mortality and morbidity, as well as increased the pharmacological treatment costs of microbial infections [1]. This scenario has

made the research for new bioactive compounds into a target of great scientific interest in the search for therapeutic alternatives for microbial infections [2].

Among the Gram-negative bacteria, *P. aeruginosa* stands out since in addition to being associated with infections in immunocompromised patients, it also affects patients who have had invasive procedures, burns and surgical wounds [3]. *Escherichia coli* belonging to the Enterobacteriaceae family is a Gram-negative bacterium [4], responsible for 80 to 90% of urinary tract infections. Its contamination ascends from the intestinal microbiota reaching the urethra, passing through to the bladder and eventually the urinary tract [5]. Among the gram-positive bacteria involved in nosocomial and community infections, *S. aureus* is one of the most important, due its wide environmental dissemination and its association with severe opportunistic infections [6].

The combined use of antimicrobials, named poliantibiotc therapy, is commonly used due the possibility that one of the antibiotic agents be active against the microorganism by an additive or synergistic effect, reducing the effect of the bacterial mechanisms of antibiotic resistance, being natural products an interesting alternative to this approach [7-9]. Thus, substances with plant origin and their derivatives have become a viable and efficient alternative [10,11], since a drug's antimicrobial activity can be amplified or reduced by the action of natural products [12]

In this context, naphthoquinones are versatile organic compounds that are part of an important class of natural products known as quinones, structurally characterized by their presentation as conjugated cyclic dienones with the 1,4 and 1,2-naphthoquinone isomers being the most common [13,14].

Naturally derived Naphthoquinones [15] represent a wide and varied family of secondary metabolites and are of vital importance to plants, fungi, lichens and algae. The highest occurrence of quinones is found in plants from the Bignoniácea family, more precisely from the *Tabebuia* (*Tecoma*) genus [16]. In addition to being important intermediates in organic synthesis for obtaining numerous natural or synthetic compounds, its participation in several biological activities such as antitumor, antifungal, antibiotic, antibacterial [17,18] and molluscicide [19] activities is relevant.

Lapachol is a functional naphthoquinone with natural origin, easily obtained through extracted from a number of species of *Tabebuia* sp.

(Bignoniaceae) [13,14], popularly known as “ipê”, being abundant in Brazil and South America [19]. Lapachol possesses several biological activities such as: action against esophageal cancer cells [20], antimicrobial activity, trypanocidal [14], including others [21]. Norlapachol is a semi-synthetic derivative of natural lapachol, known to exhibit excellent antitumor and other biological activities [19,22].

Clinical and pre-clinical studies are essential to obtain the necessary informations to the liberation for use of any substance. However, these assays are expensive. By this fact, the usual computational models are useful to give an experimental accurate direction to these assays, informing pharmacological and physico-chemical characteristics of these substances [23].

This study aimed to perform an *in silico* analysis of the sampangine alkaloid analogues 2-(2-Hydroxyethylamine)-3-(3-methyl-2-butenyl)-1,4-dihydro-1,4-naphthalenedione, 2-(2-Hydroxy-ethylamine)-3-(2-methyl-propenyl)-[1,4] naphthoquinone and 2-(3-Hydroxy-propylamine)-3-(3-methyl-2-butenyl)-[1,4] naphthoquinone, derived from lapachol and norlapachol, and to evaluate the *in vitro* antibacterial and modulatory activity of these compounds against ATCC bacterial strains and clinical isolates.

2. Materials and methods

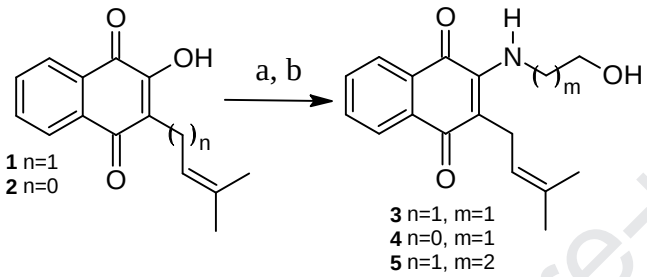
2.1 Synthesis of substances

2.1.1 General Information

Air- and moisture-sensitive reactions were carried out under argon atmosphere. Reagents were purchased from Sigma-Aldrich, Dinamica or Vetec and distilled or used without further purification. Reactions were monitored by TLC analysis on precoated silica gel plates (Merck, Kieselgel 60 GF_{254}) and compounds were visualized with UV light. Column chromatography was performed on silica gel 60 (70-230 mesh, Merck). Melting points were measured in open capillary tubes in a QUIMIS apparatus and are uncorrected. The infrared spectra were recorded on an IFS66 Bruker spectrophotometer using KBr discs or Varian Mercury 640IR with ATR. HRMS analyses were performed on a MALDI-TOF/TOF Autoflex III 10, using positive reflected mode. NMR (^{1}H

at 400 MHz and ^{13}C at 100 MHz) spectra were recorded on a Varian Unity Plus-400 spectrometer, 200 MHz Varian Mercury, using $CDCl_3$ or DMSO-d_6 as solvents, and calibrated for the solvent signal. Chemical shifts are expressed in parts per million (ppm) and coupling constants are given in Hz. Compounds lapachol [22] and the corresponding 2-methoxy derivative, norlapachol [19] and the corresponding 2-methoxy derivative were obtained by previous published procedures (Figure 1).

Figure 1. Schema of synthesis of 2-aminoalquil derivatives 3-5



Reagents and conditions: a) Me_2SO_4, K_2CO_3, acetone, r.t.; b) 2-amino-ethanol or 3-amino-1-propanol in MeOH, r.t.

2.1.2 Synthesis of 2-aminoalquil derivatives 3-5

1 mmol of the 2-methoxy derivative dissolved in 10 mL of MeOH was slowly added to 1.5 mmol of the appropriate amine (2-aminoethanol or 3-amino-1-propanol) in the same solvent (40 ml) with continuous stirring. After reaction completion by inspection in CCD analysis, the solvent was removed under vacuum and the residue submitted to flash chromatography on silica gel and ethyl acetate/hexane with increasing polarity.

2-(2-Hydroxyethylamino)-3-(3-methyl-2-butenyl)-1,4-dihydro-1,4-naphthalenedione (3)

Obtained in 88 % yield as red crystals, mp 80–81℃; ^1H NMR (200 MHz, $CDCl_3$) 1.68 (s, 3H), 1.74 (s, 3H), 2.3 (br, 1H), 3.37 (d, 2H, *J* 5.9 Hz), 3.71 (m, 2H), 3.85 (m, 2H), 5.07 (t, 1H, *J* 5.9 Hz), 6.01 (l, 1H), 7.57 (m, 1H), 7.57 (m, 1H), 7.93 (d, 1H, *J* 7.6 Hz), 8.05 (d, 1H, *J* 7.6 Hz); ^{13}C NMR (50 MHz, $CDCl_3$), 18.2, 23.8, 25.8, 47.2, 62.1, 122.7, 126.1, 126.3, 130.6, 132.0, 132.8, 133.4, 134.5, 146.2, 183.1, 183.2; IR (KBr) (*v* max., cm^{-1}) 3391, 3321, 1678, 1599, 1555, 1513; MS

(rel int) m/z 285 (M+, 57), 270 (100), 198 (70). HRMS found: 285.13649. Calcd for $C_{17}H_{19}NO_3$: 285.13649.

2-(2-Hydroxy-ethylamino)-3-(2-methyl-propenyl)-[1,4]naphthoquinone (4)

Obtained in 87 % yield as red crystals (mp 77–78.5 ℃) in 80% yield. ^{1}H NMR ($CDCl_3$, 200 MHz) 1.47 (d, 3H, *J* 1.0 Hz), 1.89 (d, 3H, *J* 1.6 Hz), 2.46 (br s, 1H), 3.37 (q, 2H, *J* 5.4 Hz), 3.73 (t, 2H, *J* 5.4 Hz), 6.06 (dd, 1H, *J* 1.0/1.6 Hz), 6.25 (br t, 1H, *J* 5.4 Hz), 7.51 (dt, 1H, *J* 1.4/7.6/7.6 Hz), 7.61 (dt, 1H, *J* 1.4/7.6/7.6 Hz), 7.90 (dd, 1H, *J* 1.4/7.6 Hz), 7.99 (dd, 1H, *J* 1.4/7.6 Hz). ^{13}C NMR ($CDCl_3$, 50 MHz) 20.1, 25.4, 46.1, 61.3, 113.6, 117.7, 125.9, 126.1, 130.3, 131.9, 133.3, 134.4, 139.0, 144.8, 182.7, 183.4. IR (KBr) *v* max, 3457, 3349, 3268, 2940, 2874, 1675, 1598, 1563, 1511, 1354, 1335 cm^{-1}. HRMS Calcd for $C_{16}H_{17}NO_2$, 271.1208; found: 271.1169.

2-(3-Hydroxy-propylamino)-3-(3-methyl-2-butenyl)-[1,4]naphthoquinone (5**)**

Obtained in 75% yield as red crystals (m.p. 69-70 ^{o}C) . ^{1}H NMR ($CDCl_3$, 400 MHz) 1.68 (s, 3H), 1.74 (s, 3H). 1.88 (q, 2H, *J* 6.2, 6.16 Hz), 3.39 (d, 2H), 3.69 (t, 2H, *J* 6,6 Hz), 3.80 (t, 2H, *J* 6.2, 5.5 Hz), 5.08 (t, 2H, *J* 5.8), 7.54 (t, 1H, *J* 7.5 Hz), 7.65 (td, 1H, *J* 7.4 Hz), 7.94 (d, 1H), 8.04 (d, 1H); ^{13}C NMR ($CDCl_3$,100 MHz) 17.8, 23.3, 23.4, 32.8, 42.4, 60.3, 115.3, 122.7, 125.6, 125.9, 130.1, 131.5, 132.1, 133.1, 134.0, 145.6, 182.74, 182.76; IR (KBr) *v* max, 3446, 3334, 1672, 1598, 1557, 1527, 1361, 1276, 1473, 728 cm^{-1}. HRMS Calcd for $C_{18}H_{21}NO_3$, 299.1527; found: 299.1501.

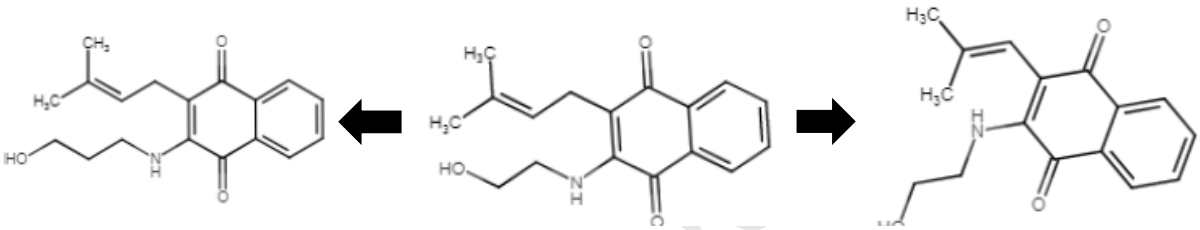
2.2 Prediction of the pharmacological activity of substances (in silico studies)

2.2.1 Obtaining the three-dimensional molecular structure of substances

The three chemical structures that were the object of this work were designed in free ChemSketch® software, version 2015.2.5 and their three-dimensional structure was determined by 3D Viewer software, both for Windows®, produced by Advanced Chemistry Development, Inc. (ACD / Labs). In Avogadro software, version 1.1.1. For MAC OS systems, the most stable conformation of the molecules was determined considering the force field

MMFF94 (Merck Molecular Force Field 94) using a steepest descent algorithm or the Cauchy method as a gradient method for lengths of the bonds and angles of the molecule (Figure 2 and Table 1).

Figure 2. Chemical structures of compounds



5 **3** **4**

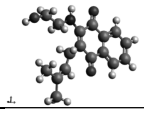
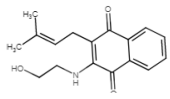
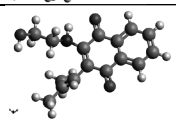
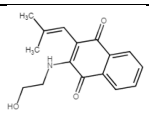
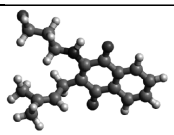
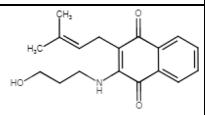
2.2.1 Pharmacological Screening

All three molecules were pharmacologically screened using the ChemProt version 3.0 software from the Technical University of Denmark (Chemprot-3.0, 2019) (http://potentia.cbs.dtu.dk/ChemProt/#)[24].

ChemProt is a publicly available compilation of annotation features on the chemical-protein-disease relationship that enables the study of small-molecule system pharmacology across multiple layers of complexity, from molecular to clinical levels. ChemProt Version 3.0 provides the analysis of over 1.7 million compounds, with 7.8 million bioactivity measurements for 19,504 proteins [25].

The Molinspiration software was used to determine the properties of the molecules (https://www.molinspiration.com), with which the structures were analyzed for agreement with the Rule of Five, data presented in Table 1 [26].

Table 1. Molecular characteristics of the three compounds. MF- Molecular Formula; MW - Molecular weight.

Code	SMILE	Observations	Estructure	MF	MW
3	CC(C)=CCC1=C(NCCO)C(=O)C2=CC=CC=C2C1=O			$C_{16}H_{17}NO_3$	271.311
4	CC(C)=CC1=C(NCCO)C(=O)C2=CC=CC=C2C1=O			$C_{16}H_{17}NO_3$	271.311
5	CC(C)=CCC1=C(NCCCO)C(=O)C2=CC=CC=C2C1=O			$C_{18}H_{21}NO_3$	299.364

2.3 Preparation of the test solution

To prepare the test solution, the extract was dissolved in Dimethyl sulfoxide (DMSO) in the following proportion: 10mg of the extract for each 1mL of DMSO. This solution was diluted in distilled water, obtaining an initial concentration of 2048 µg / mL.

2.4 Microrganisms

The microorganisms used in the tests were provided by the National Institute of Health Quality Control (INCQS) of the Oswaldo Cruz Foundation, Rio de Janeiro, Brazil. Four bacterial strains were used, including standard strains of *Peudomonas aeruginosa* ATCC 27853 and *Staphylococcus aureus* ATCC 25923 and multiresistant strains (clinical isolates) of *P. aeruginosa* 31 and *S. aureus* 358 (Table 2).

Table 2. Resistance profile of the bacteria used in the tests.

Bacterium	N	Collection Site	Resistance Profile
Staphylococcus aureus	SA358	Surgical wound	Oxa, Gen, Tob, Ami, Can, Neo, Para, But, Sis, Net
Pseudomonas aeruginosa	P31	Nose	Pol, Cpm, Ctz, Ptz, Ami, Imi, Cip, Lev, Mer

. Resistance Profile: Ami = amikacin; Cip = ciprofloxacin; Lev = levofloxacin; Ctz = ceftazidime; Pol = polymyxin; Imi = imipenem; Mer = meropenem; Ptz = piperacillin; Can = kanamycin; Tob = tobramycin; Oxa = oxacillin; Gen = gentamicin; Neo = neomycin; Para = paramomycin; But = butyrosine; Sis = sisomycin; Net = netilmicin. The microorganisms used in this research were acquired from the Laboratory of Mycology of the Federal University of Paraíba—UFPB and kindly provided by the Regional University of Cariri—URCA.

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2.5 Culture media

Heart Infusion Agar - HIA (Difco Laboratories Ltd.) and Brain Heart Infusion Broth - BHI (Acumedia Manufacturers Inc.) were prepared according to the manufacturer's specifications. Brain Heart Infusion Broth - BHI at 10% concentration was also used in the assays.

2.6 Preparation and Standardization of Bacterial Inocula

Bacterial cultures were maintained at 4° C in Heart Infusion Agar (HIA). Prior to the tests, the strains were transferred to the BHI medium and incubated at 35 °C for 24 h. Then, they were diluted in sali ne to the concentration of 10^5 cells / mL, which equivalent yo the 0.5 in the McFarlan scale. The pre-standard bacterial suspensions were diluted in BHI broth (1:10) to obtain the final concentration of 10^4 cells / mL [27].

2.7 Determination of the Minimum Inhibitory Concentration (MIC)

The determination of the MIC of the compounds was carried out through the Broth Microdilution Method, using concentrations ranging from 1024 to 16 µg / mL [27].

2.7.1 Execution and Readings of the Assays

The samples were prepared in a folded concentration (2048 µg / mL) relative to the initial concentration and then, serially diluted 1: 2 in 10% BHI broth. Each well was added with 100 µL of the culture medium containing a 1:10 diluted bacterial suspension sample.

Negative controls (culture medium), positive controls (medium + inoculum) and the compounds at concentrations ranging from 1024 to 1 µg / mL were included in the assays. The filled plates were incubated at 35 °C for 24 hours [27].

To determine the MIC, a solution of resazurin sodium (Sigma) in sterile distilled water at the concentration of 0.01% (w / v) was prepared. After incubation, 20 µL of the indicator solution was added into each well and the plates were incubated for 1 h at room temperature. All tests were performed in triplicate.

2.8 Evaluation of the Interference of the Compounds on the Resistance to Aminoglycoside Antibiotics

To evaluate the antibiotic-modulating activity of the compounds, the MICs of aminoglycoside antibiotics (amikacin and gentamicin) were determined in the presence and absence of the extract in sterile microplates. All antibiotics were obtained from Sigma.

The extract was used at subinhibitory concentration (MIC / 8), which was obtained through dilution in 10% BHI broth. The antibiotic solutions were prepared in a folded concentration (2048 µg / mL) relative to the initial concentration with the addition of sterile distilled water. Serial dilutions (1: 2) were performed using in 10% BHI broth. Each well was added with 100 µL of the culture medium containing a 1:10 diluted bacterial suspension sample. The same controls used in the evaluation of MIC of the extract were used [11]. The filled plates were incubated at 35 °C for 24 h and the readings were performed after addition of resazurin sodium as described above.

2.9 Data Analysis

The data were obtained in triplicate and expressed as geometric mean. Differences were analyzed by ANOVA (two-way) with Bonferroni's post-test. The results with values of $p < 0.05$ were considered significant.

3. Results

After the preparation of a drug, its active ingredient must be suitable for use by the chosen route of administration. The oral rout is among the preferred

routes as it is the most convenient for most drugs and patients, thus drugs must be able to cross a series of obstacles until reaching their target area [28].

For a drug to complete its trajectory to its target, it needs to meet molecular requirements for the viability of its use as a drug to occur. While studying the characteristics of drug molecules, Lipinski [29] identified some characteristics often observed in 2,245 new chemical species collected from the World Drug Index (WDI) and presented what became known as the Rule of Five for a molecule to become a drug, these being:

1. Molecular mass is less than or equal to 500 Da;
2. Number of hydrogen bond acceptor groups is less than or equal to 10 (expressed as the sum of N and O atoms);
3. Number of hydrogen bond donor groups is less than or equal to 5 (expressed as the sum of OH and NH in the molecule);
4. Log P is less than or equal to 5.

The reason for their denomination as the “Rule of Five” is because each parameter is defined by a value that, coincidentally, is a multiple of five [29]. The molecular properties of the substances were determined using the online software Molinspiration according to the presentation in Table 3.

Table 3 - Molecular properties of the compounds obtained from the Molinspiration software (http://www.molinspiration.com/cgi-bin/properties)

Substances	LogP	ALH	DLH	MM	RBN
3 CC(C)=CCC1=C(NCCO)C(=O)C2=CC=CC=C2C1=O	2.6	4	2	285.34	5
4 CC(C)=CC1=C(NCCO)C(=O)C2=CC=CC=C2C1=O	2.45	4	2	271.32	4
5 CC(C)=CCC1=C(NCCCO)C(=O)C2=CC=CC=C2C1=O	2.88	4	2	299.37	6

MM: molecular mass; ALH: Hydrogen bond acceptors; DLH: Hydrogen bond donors; number of rotatable bonds.

No violation of the rule of five was identified with the three studied molecules. Particularly, the structure of number 4 obtained a lower molecular weight and fewer rotatable bonds than the other structures, which provides better chemical characteristics for structures with pharmacological activities.

After pharmacological screening, possible therapeutic targets for substance 4 were observed, however, no targets were identified for the other substances Table 04. ChemProt version 3.0 software.

Table 4. Possible therapeutic targetsfor substance 4 as identified by the ChemProt software.

Access to UniProt of the target	Possible therapeutic targets	Species
P10253	70 kDa lysosomal alpha-glucosidase	Human
O14727	Apoptotic protease-activating factor 1	Human
Q9UIF8	Bromodomain adjacent to zinc finger domain protein 2B	Human
P42574	Caspase-3 subunit p12	Human
P55211	Caspase-9 subunit p10	Human
P04637	Cellular tumor antigen p53	Human
P83916	Chromobox protein homolog 1	Human
Q13951	Core-binding factor subunit beta	Human
Q9UNA4	DNA polymerase iota	Human
P27695	DNA-(apurinic or apyrimidinic site) lyase	Human
P28563	Dual specificity protein phosphatase 1	Mouse
Q64346	Dual specificity protein phosphatase 6	Rat
Q96KQ7	Histone-lysine N-methyltransferase EHMT2	Human
P02545	Lamin-A/C	Human
Q9Y468	Lethal(3)malignant brain tumor-like protein 1	Human
P08659	Luciferin 4-monooxygenase	*Photinus pyralis*
O75164	Lysine-specific demethylase 4ª	Human
B2RXH2	Lysine-specific demethylase 4D-like	Human
P10636	Microtubule-associated protein tau	Human
P28482	Mitogen-activated protein kinase 1	Human
P84022	Mothers against decapentaplegic homolog 3	Human
P30305	M-phase inducer phosphatase 2	Human
Q9NR56	Muscleblind-like protein 1	Human
Q16236	Nuclear factor erythroid 2-related factor 2	Human
P0A5U4	Protein RecA	*Mycobacterium tuberculosis*
O97447	Putative fructose-1,6-bisphosphate aldolase	*Giardia*

		intestinalis
P71715	Replicative DNA helicase	*Mycobacterium tuberculosis*
P00352	Retinal dehydrogenase 1	Human
Q01196	Runt-related transcription factor 1	Human
Q9GZR1	Sentrin-specific protease 6	Human
Q9BQF6	Sentrin-specific protease 7	Human
Q96LD8	Sentrin-specific protease 8	Human
P11473	Vitamin D3 receptor	Human

These data corroborate with the literature in that the studied compounds are sampangine alkaloid analogues derived from lapachol and norlapachol. According to Muhammad *et al.,* [30], sampangine possesses antimalarial, antifungal and cytotoxic activities, as well as being a potent inhibitor of leukemic cell proliferation [31]. Research conducted through biological assays reveals a great potential for sampangine against human ovarian cancer cell lines, while also possessing activity against human lung cancer cells [32].

The results presented in Table 3 were fundamental for the planning and performance of the assays used to investigate the presence of a possible antibacterial activity in view of the possible targets: RecA and Replicative DNA helicase, which are associated with bacterial DNA maintenance and replication [33]. According to [34], sampangine possesses an effective action against fungi and mycobacteria.

In the antibacterial activity evaluation of the substances against the *Escherichia coli*, *Staphylococcus aureus* and *Pseudomonas aeruginosa* strains, the substances obtained MIC values ≥ 1024 µg/mL, which are clinically irrelevant values, MIC values greater than 1000 µg/mL are considered to lack direct antibacterial activity for clinical practice [35]. The minimum inhibitory concentration (MIC) can be defined as the lowest concentration capable of completely inhibiting growth in microdilution wells [36].

The data in the present study disagree with the work by Oliveira et al., [37] where several 1,4-naphthoquinone derivatives containing a hydrazine group as a side chain were synthesized from 3-diazo-naphthalene-1,2,4-trione and were evaluated as potential antibacterial agents. In the aforementioned study, naphthoquinone derivatives showed higher antibacterial activity at the preliminary disk diffusion test level than lapachol, a 1,4-naphthoquinone well

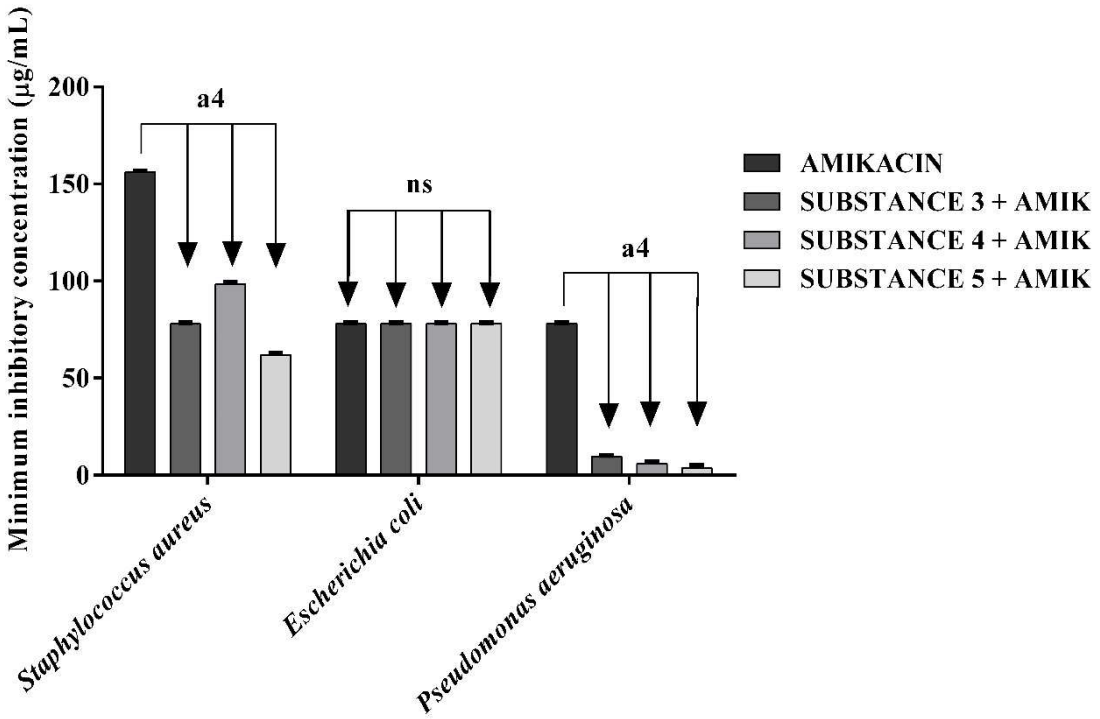
known for its varied biological activities. As for a study on the minimum inhibitory concentration (MIC) of lapachol against *Staphylococcus aureus*, one report showed the 2-[(3-hydroxy-1,4-dioxo-1,4-dihydro-naphthalen-2-yl)-hydrazone] ethyl malonate presented twice as much activity as lapachol. Similarly, an optical density culture study with *S. aureus* and this substance showed an activity similar to that of vancomycin [37].

The activity of several lapachol-derived analogues against sensitive (MSSA) and methicillin resistant *Staphylococcus aureus* (MRSA) strains were verified, where lapachol derivatives presented antibacterial activity against MSSA ATCC [37]. MRSA clinical isolates were susceptible to naphthoquinone derivatives, however, these were resistant to some commercially available antibacterials with the exception of vancomycin. Most naphthoquinone compounds presented MIC values between 30 mg/L and 125 mg/L, while other derivatives obtained MIC values > 500 mg/L. The minimum bactericidal concentrations were > 500 mg/L, demonstrating the tested naphthoquinones exhibited only a bacteriostatic activity against clinical MRSA strains [38].

Recent studies have shown a series of 12 new 2-hydroxy-3-phenylsulfanylmethyl- [1,4] naphthoquinone analogs have been synthesized by the addition of a thiol group with different substituents to a de-quinone methane using microwave irradiation. The compounds were tested against Gram positive and negative bacteria, where ten compounds presented antimicrobial activity, especially against Gram negative strains, in addition to presenting biofilm formation inhibition [39].

Figures 3 and 4 demonstrate the aminoglycoside modulatory activity of substances 3, 4 and 5, when associated with gentamicin and amikacin at sub-inhibitory concentrations (1/8 MIC), where a significant ($p < 0.0001$) MIC reduction was obtained against *S. aureus* and *P. aeruginosa* strains, characterizing this as resistance inhibition. However, no antibiotic activity interference was observed against the *E. coli* strain.

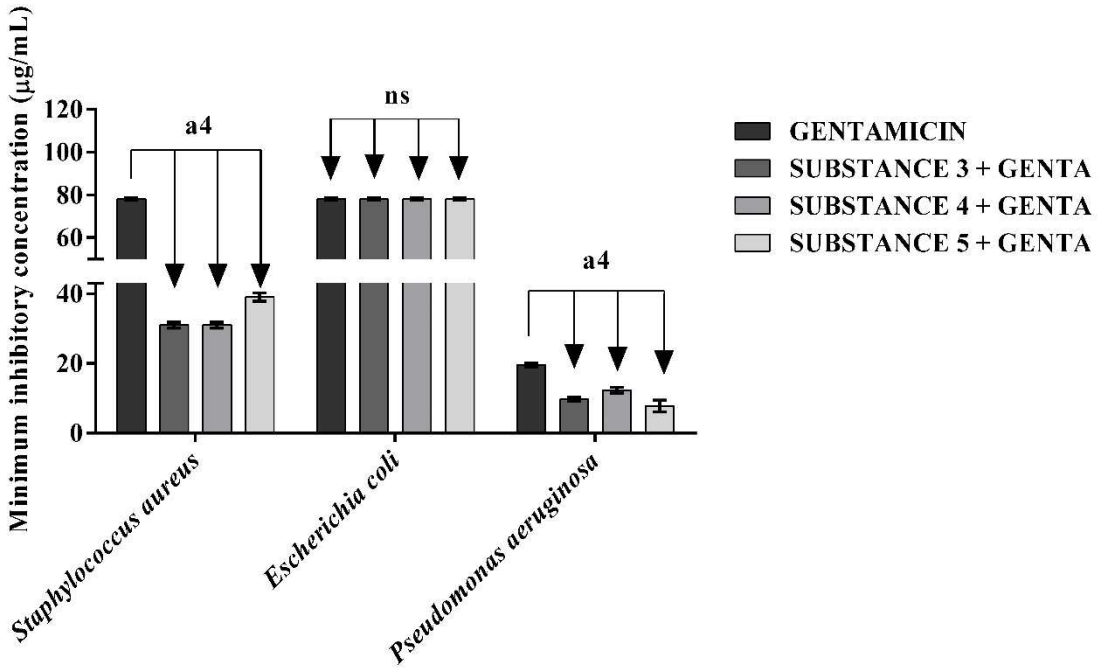
Figure 3. Effect of substances 3, 4 and 5 on the antibacterial action of amikacin against *Staphylococcus aureus*, *Escherichia coli* and *Pseudomonas aeruginosa* strains.



399

400 The values represent the geometric mean ± S.E.M. (standard error of mean). Two-way
401 ANOVA, followed by the Bonferroni test. a4: $p < 0.0001$ vs control of antibiotic; ns: not
402 significant; Amik: Amikacin.

403 Figure 4. Effect of substances 3, 4 and 5 on the antibacterial action of
404 gentamicin against *Staphylococcus aureus*, *Escherichia coli* and *Pseudomonas*
405 *aeruginosa* strains.



406

407 The values represent the geometric mean ± S.E.M. (standard error of mean). Two-way
408 ANOVA, followed by the Bonferroni test. a4: $p < 0.0001$ vs control of antibiotic; ns: not
409 significant; Genta: Gentamicin

410 The aminoglycoside modulatory activity of substances 3, 4 and 5 may be
411 related to molecular characteristics of these substances, for being sampangine
412 alkaloid analogues. The possible mechanism of action of quinones is not fully
413 known, however two proposals for how this may work exist. The first is
414 associated with the generation of reactive oxygen species (H_2O_2, $O_2^{\cdot-}$, $OH^{\cdot}$) in
415 the intracellular environment, leading to the denaturation of membrane proteins
416 [15], such as the efflux pumps that are essential for bacterial resistance.

417 The second possibility is through the inhibition of topoisomerase I and II
418 enzymes. These are nuclear enzymes that aid DNA replication and are part of
419 the proper functioning of any cell [15]. In this context, topoisomerases II are
420 usually targets in antibacterial therapy, for being viable options as they are
421 essential for bacterial cell division and replication. These enzymes have a
422 higher specificity/selectivity for prokaryotic enzymes, which is at least three
423 times greater than for eukaryotic enzymes, thus decreasing the likelihood of
424 adverse effects [40] The generation of reactive oxygen species (ROS) due to

bioreduction in the region quinolinic by specific enzymes and the interaction with topoisomerase is, until the moment, a possible antibacterial action.

Aminoglycosides presents adverse effects, particularly nephrotoxicity nefrotoxicidade [41,42], ototoxicity and neurotoxicity [43,44], side effects which should be considered before prescribing these antibiotics. In this context, the combination of substances with aminoglycosides may be a therapeutic alternative to bacterial resistance and the reduction of side effects, given that a synergism with significant MIC reduction was observed.

According to Oliveira et al [45], the combined use of natural products with antibiotics may be an alternative to minimize the adverse effects caused by the use of aminoglycosides, since lower drug concentrations and doses are required for therapeutic effectiveness, especially in cases of multiresistant strain infections.

4. Conclusion

The *in silico* study of the sampangine alkaloid analogues derived from lapachol and norlapachol suggested possible activities for the 2-(2-Hydroxy-ethylamine)-3-(2-methyl-propenyl)-[1,4] naphthoquinone molecule as a potential antibacterial agent over Replicative DNA helicase and RecA proteins, highlighting the presence of other targets that could be useful for pharmacological research. The compounds reduced the MICs of gentamicin and amikacin when used in association, against *S. aureus* and *P. aeruginosa* strains. In this context, the combination of these substances with aminoglycosides can be a therapeutic alternative to face the bacterial resistance to antibiotics.

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HIGHLIGHTS

1 - The *in silico* study indicated that 2-(2-Hydroxy-ethylamine)-3-(2-methyl-propenyl)-[1,4] naphthoquinone is a potential antibacterial agent;

2 – The putative mechanism is inhibit the Replicative DNA helicase and RecA proteins;

3 - The compounds used with gentamicin and amikacin considerably decreased the MICs of these antibiotics.

Declaration of interests

☒ The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

☐The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: