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ADMET study, spectroscopic characterization and effect of synthetic nitro chalcone in combination with norfloxacin, ciprofloxacin, and ethidium bromide against *Staphylococcus aureus* efflux pumps

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Abstract

Chalcones are present in a wide variety of plants, having in their structure two aromatic rings that are linked together by a chain composed of three carbon atoms with α, β-unsaturated to carbonyl system. Bacteria have several drug resistance mechanisms, among them the efflux pump; this mechanism, when active, is able to expel different compounds from inside bacterial cells. Several efflux pumps have already been identified for *Staphylococcus aureus* bacteria, including MepA and NorA. Many chalcones have been isolated and identified with various activities, such as antimicrobial. In view of this, this article aimed to evaluate the antibiotic modifying effect of chalcone (E)-1-(2-hydroxyphenyl)-3-(3-nitrophenyl)prop-2-en-1-one against *S. aureus* carrier of NorA and MepA efflux pump. Regarding the antibiotic, there was a synergism when associated with ciprofloxacin in SA-K2068 strain, showing this chalcone as an alternative to reverse the resistance to this medicine. The physicochemical properties calculated were fundamental in the description of the predicted pharmacokinetic properties. Despite the mutagenic risk caused by the metabolic activation of nitrochalcone, it is possible to notice a pharmacological principle in a longer half-life for the performance of biological activities. The compound has a good bioavailability, as it is highly absorbed in the intestine and easily transported by plasma proteins, in addition to not presenting neurotoxic, hepatotoxic, and cardiotoxic damage.

Abbreviations: ADMET, absorption, distribution, metabolism, excretion, and toxicity properties.; ANOVA, analysis of variance; ATP, adenosine triphosphate; BHI, brain heart infusion; CCCP, carbonyl cyanide m-chlorophenylhydrazone; $CDCl_3$, chloroform-d; CPZ, chlorpromazine; DMSO, dimethylsulfoxide; EPI, efflux pump inhibitors; EtBR, ethidium bromide; hERG, human ether-à-go-go-go-related gene; MIC, minimum inhibitory concentration; NMR, *nuclear magnetic resonance*; Resazurin, 7-hydroxy-10-oxidophenoxazin-10-ium-3-one; TLC, *thin-layer chromatography*.

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KEYWORDS
Chalcone, efflux pump, infections, *Staphylococcus aureus*

1 | INTRODUCTION

The use of plants for medicinal purposes has been used for a long time to treat, among other diseases, infectious diseases. Many of the biological actions of natural products come from their secondary metabolites, including flavonoids. Both medicinal plants and flavonoids have already had their biological applications studied in the literature, presenting several activities, such as antibacterial, antidiarrheal, anxiolytic, anticancer, antidepressant, anti-inflammatory, among others [1–6].

Chalcones are present in a wide variety of plants, having in their structure two aromatic rings that are linked together by a chain composed of three carbon atoms with α, β-unsaturated to carbonyl system. They have important biological activities and participate in the biosynthesis of secondary metabolites flavonoid as an intermediary derived from phenylalanine. Many chalcones have been isolated and identified with various activities, such as antimicrobial. Its effects against bacteria are given the ability of these compounds to bind covalently and irreversibly to microbial enzymes [7, 8].

Bacteria have several drug resistance mechanisms, among them the efflux pump; this mechanism, when active, is able to expel different compounds from inside bacterial cells. Several efflux pumps have already been identified for *Staphylococcus aureus* bacteria, including MepA and NorA. The NoraA efflux pump offers resistance, for example, to norfloxacin, ciprofloxacin, which belong to the fluoroquinolone antibiotic class; this pump is also resistant to ethidium bromide and quaternary ammonium compounds [9–11].

The bacterial efflux pumps act on intracellular communication, pathogenicity, and the elimination of antibiotics, making this mechanism an important target for inhibiting the action of these microorganisms. There are already reports in the literature regarding the antibacterial activity of chalcones, where this activity is associated with the reactivity of α, β-unsaturated ketone function [12–14].

In view of this, this article aimed to evaluate the antibiotic modifying effect of chalcone (*E*)-1-(2-hydroxyphenyl)-3-(3-nitrophenyl)prop-2-en-1-one against *S. aureus* carrier of NorA and MepA efflux pump.

2 | MATERIALS AND METHODS

2.1 | Spectroscopic methods

The chemical reagents were purchased from Sigma-Aldrich. ^{1}H and ^{13}C NMR spectra were obtained using a Bruker Spectrometer, model Avance DPX-500, operating at a frequency of 500 MHz for hydrogen and 125 MHz for carbon, respectively. The spectra were measured in $CDCl_3$, and chemical shifts are reported as δ values in parts per million (ppm) relative to $CDCl_3$.

2.2 | Synthesis of the chalcone

The compounds 2-hydroxyacetophenone (2 mmol) and 3-nitrobenzaldehyde (2 mmol) were placed in a volumetric flask (25 ml). Then 5 ml of ethanolic NaOH (50%) solution was added and mixed with stirring for 48 h at room temperature. The progress of the reaction was checked by TLC (n-hexane: ethylacetate, 2:1). After 48 h, the reaction mixture was neutralized with dilute HCl (10%) and ice water added. The product was obtained as a yellow solid filtered under reduced pressure, washed with cold water, and recrystallized from ethanol (Scheme 1).

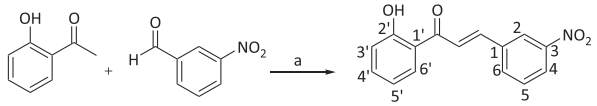
2.3 | Substances

The products used to carry out the microbiological tests were chlorpromazine (CPZ), carbonyl-m-chlorophenylhydrazone cyanide (CCCP), and ethidium bromide (EtBr); all substances were purchased by Sigma Aldrich Co. Ltd. Chalcone (E)-1-(2-hydroxyphenyl)-3-(3-nitrophenyl)prop-2-en-1-one and the antibiotics norfloxacin and ciprofloxacin were also used.

Both antibiotics and chalcone were diluted in dimethylsulfoxide (DMSO) and in sterile water, until a concentration of 1024 μg/ml. Chlorpromazine (CPZ) and ethidium bromide (EtBr) were dissolved in distilled sterile water, while CCCP was dissolved in methanol/water (1:3, v/v). All substances were diluted to a standard concentration of 1024 μg/ml.

2.4 | Bacterial strains

The strains used were *S. aureus* SA 1199B, which overexpresses NorA and *S. aureus* SA-K2068, which



SCHEME 1 Preparation of chalcone. (a) NaOH 50% w v^{-1}, ethanol, room temperature, 48 h

expresses MepA. The strains were provided by Prof. S. Gibbons (University of London), kept on blood agar (Laboratórios Difco Ltda., Brazil), and grown for 24 h at 37°C in solid Brain Heart Infusion (BHI)-Agar (BHI, Acumedia Manufacturers Inc.) before experiments.

2.5 | Determination of minimum inhibitory concentration (MIC)

To perform the determination of the MIC followed the methodology proposed by Clinical and Laboratory Standards Institute (CLSI) [15] with modifications. Before carrying out the tests, the inoculants were seeded 24 h before the test and then a bacterial drag of the strains used was diluted in saline, and their turbidity was compared to the MacFarland scale. Subsequently, eppendorf tubes were filled with 900 μl of BHI solution and 100 μl of bacterial inoculum were added. Then 100 μl of the solution was deposited in a 96-well microdilution plate. The microdilution was done by serial dilutions with chalcone until the penultimate cavity (1:1), the last one being used as growth control. The concentration of the compound ranged from 512 to 8 μg/ml. Then the plates were taken to the oven for 24 h at 37°C. After this period, the reading was done by adding Resazurin (7-hydroxy-10-oxidophenoxazin-10-ium-3-one). All tests were done in triplicate.

2.6 | Evaluation of NorA and MepA efflux pump inhibition

The inhibition of the efflux pump was evaluated according to the methodology proposed by Oliveira-Tintino et al. [16]. In order to observe if the chalcone in study acts as an inhibitor of the NorA and MepA efflux pump, a comparison was made between it and the standard efflux pump inhibitors (EPI) (CPZ and CCCP), evaluating the ability to reduce the bromide MIC of ethidium and the antibiotics norfloxacin and ciprofloxacin. To assess the inhibition of the efflux pump, chalcone and PPE were used in a subinhibitory concentration (CIM/8). A bacterial haul of the strains tested was diluted in saline, corresponding to 0.5 of the McFarland scale, and then placed in 150 ml eppendorf tubes of the inoculum, chalcone, and PPE (CIM/8) and completed with BHI. Then 100 μl of the solution was transferred to microdilution plates and then serial microdilution was performed with 100 μl of the antibiotic or EtBr in serial dilutions (1:1) that ranged from 512 to 0.5 μg/ml. The plates were incubated at 37°C for 24 h and bacterial growth was evaluated using resazurin. MIC was defined as the lowest concentration at which there was inhibition of bacterial growth, both with norfloxacin, ciprofloxacin and EtBr. The MIC of the controls was evaluated using only antibiotic plates and ethidium bromide. All tests were performed in triplicate.

2.7 | ADMET study

Initially, the nitrochalcone was designed in the molecular editor Marvin JS, deployed on the ChamAxon © online server (https://disco.chemaxon.com/calculators/demo/plugins/) for the theoretical calculation of the physicochemical properties of ionization constant (pKa), lipophilicity (logP), distribution (logD), and polarity (PSA), used as pharmacokinetic descriptors.

Then, the molecule was subjected to a virtual screening of pharmacokinetic properties of the pkCSM server similarity test (http://biosig.unimelb.edu.au/pkcsm/) [17], by the models absorption, distribution, metabolism, excretion and toxicity (ADMET), following the applicable domain of the molecular descriptors calculated on the ChamAxon © server.

To emphasize the toxicological profile, the compound was subjected to the similarity test of the eMolTox online database (http://xundrug.cn/moltox), relating the nitrochalcone's molecular fragments to the toxicological models of mutagenicity and carcinogenicity, and to Pred-hERG 4.2 tool from the LabMol online server (http://predherg.labmol.com.br/) to evaluate cardiotoxic fragments by inhibiting hERG ion channels.

2.8 | Molecular docking

The NorA protein model was created using the same procedure as described in Oliveira et al. [18]. The model was then uploaded to the Molprobity [19] service for protonation.

For the docking procedure, which was carried out using the Autodock Vina [20] software, with the grid box defined as a 80 Å × 80 Å × 80 Å box around the geometrical center of the model. Partial Gasteiger charges were added to the protein, and to the ligand atoms, nonpolar hydrogen atoms were mixed while all other parameters were kept at their default values. Docking pose was chosen based on the best binding score.

2.9 | Statistical analysis

The data were analyzed using a two-way ANOVA test, using the geometric mean of the triplicates as the central data and the standard deviation, using the statistical program GraphPad Prisma 5.0. Then, a post hoc Bonferroni test was performed (where $P < 0.05$ and $P < 0.0001$ are considered significant and $P > 0.05$ is not significant).

TABLE 1 ^{1}H and ^{13}C NMR data of chalcone (*E*)-1-(2-hydroxyphenyl)-3-(3-nitrophenyl)prop-2-en-1-one in $CDCl^3$. The chemical shifts in δC and δH are in ppm

C	δ_C	δ_H
1′	120.4	
2′	163.8	
3′	118.9	7.04 (d, $J = 8.3$ Hz)
4′	136.2	7.48 (t, $J = 7.7$ Hz)
5′	119.2	6.98 (t, $J = 7.6$ Hz)
6′	129.9	7.92 (d, $J = 7.2$ Hz)
C=O	193.2	
1	137.1	
2	122.6	8.42(s)
3	148.0	
4	125.1	8.28 (d, $J = 7.3$ Hz)
5	129.9	7.53 (t, $J = 7.2$ Hz)
6	134.7	7.63(d, $J = 7.9$ Hz)
C_α	123.2	7.76 (d, $J = 15.5$ Hz)
C_β	142.4	7.95 (d, $J = 16.3$ Hz)

TABLE 2 Effect of minimum inhibitory concentration (MIC) in μg/ml against the strain of *S. aureus* carrying a NorA efflux pump

Substance	SA-1199B
Chalcone	≥1024 μg/ml
Norfloxacin	128 μg/ml
Ethidium bromide	512 μg/ml

TABLE 3 Effect of minimum inhibitory concentration (MIC) in μg/ml against the strain of *S. aureus* with MepA efflux pump

Substance	SA-K2068
Chalcone	≥1024 μg/ml
Ciprofloxacin	25.4 μg/ml
Ethidium bromide	32 μg/ml

3 | RESULTS AND DISCUSSION

3.1 | NMR data

In the spectrum of 1H NMR (Figures S1 and S2) you can see the signals in 7.76 (d, $J = 15.5$ Hz) and 7.95 (d, $J = 16.3$ Hz) are attributed to two doublets referring to the hydrogens α, β unsaturated, whose coupling constant (*J*) confirms the stereochemistry E of the double bond. The signals at 7.92 (d, $J = 7.2$ Hz, H6′), 7.48 (t, $J = 7.7$ Hz, H4′), 7.04 (d, $J = 8.3$ Hz, H3′), and 6.98 (t, $J = 7.6$ Hz, H5′) refer to aromatic hydrogens in ring A. While the signals at 8.42 (s, H2), 8.28 (d, $J = 7.3$ Hz), 7.53 (t, $J = 7.2$ Hz), and 7.63(d, $J = 7.9$ Hz) refer to the aromatic hydrogens of ring B. In the 13C NMR spectrum (Figures S3 and S4), there is the signal referring to carbonyl α, β unsaturated in 193.2. The ketone absorbs in 203.8; however, the presence of α, β unsaturation causes a displacement to high field and the probable cause is the displacement of charge by the benzene ring or by the double bond that makes the carbonyl carbon less electron deficient. The olefinic carbons α and β are observed in 142.4 and 123.2, respectively. At 163.8 (C-2′), 136.2 (C-4′), 129.9 (C-6′), 120.4 (C-1′), 119.2 (C-3′), and 119.2 (C-5′), there are the signals referring to the carbons present in ring A. While the signals in 148.0 (C-3), 134.7 (C-6), 137.1 (C-1), 125.1 (C-4), 122.6 (C2), and 129.9 (C-5) refer to ring B carbons (Table 1).

3.2 | Antibacterial and antibiotic modifying activity

Chalcone (E) -1- (2-hydroxyphenyl) -3- (3-nitrophenyl) prop-2-en-1-one did not show a direct antibacterial activity against the *S. aureus* strains carrying the NorA and MepA efflux pumps, demonstrated by the MIC value that was greater than or equal to 1024 μg/ml, thus presenting an MIC value that is not clinically relevant, as can be seen in Tables 2 and 3. According to Rios and Recio [21], an isolated substance has an inhibitory effect considered satisfactory when it has a MIC value less than 100 μg/ml.

Regarding the bacterium SA-1199B that overexpresses the NorA pump, there was a decrease in the MIC of norfloxacin when used with the pump inhibitors chlorpromazine and CCCP. However, when chalcone was used in association with norfloxacin, there was an antagonism; there was an increase in the MIC of the antibiotic, requiring a higher concentration of norfloxacin to obtain the same result used alone.

The efflux pump is a bacterial resistance mechanism that consists of expelling substances from its intracellular medium from the bacterial cell to the extracellular medium. This mechanism is responsible for the extrusion of ethidium bromide from the bacterial intracellular medium, and when this occurs, it is one of the ways to show that this mechanism is present in the bacterium. Once there is a reduction in the MIC of ethidium bromide, it can then be suggested that the efflux pump inhibition is taking place. The reduction in the MIC of ethidium bromide (BrEt) can be observed when used together with the chalcone in study, which can be seen in Figure 1, where there was a 50% reduction when used concomitantly.

For the bacterium SA-K2068, the antibiotic ciprofloxacino was used and it was observed that there was a reduction in the MIC when it was used with the chalcone (E)-1-(2-hydroxyphenyl)-3-(3-nitrophenyl) prop-2-en-1-one, thus resulting in synergism, with results from 25.39842 to 16 μg/ml, that is, 37%, when

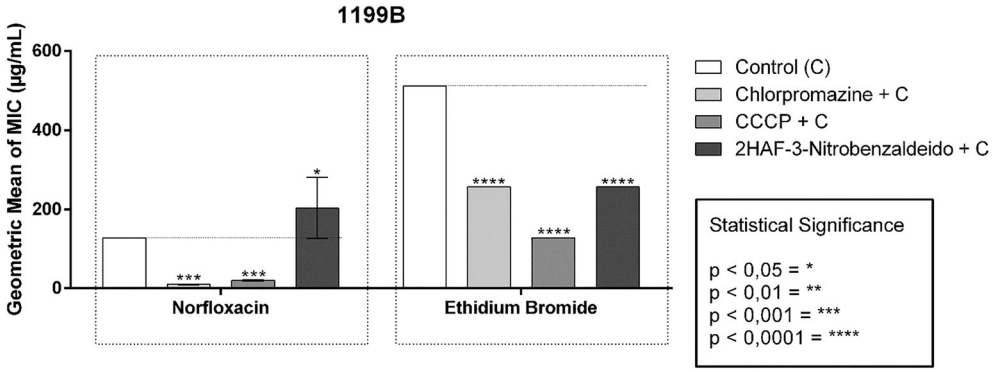


FIGURE 1 Evaluation of antibiotic and ethidium bromide (EtBr) modification by chalcone against *Staphylococcus aureus* 1199B. Legend: Control (C): refers to the antibiotic Norfloxacin on the left and ethidium bromide on the right. Chlorpromazine + C corresponds to the standard inhibitor plus the control, be it the antibiotic (left) or ethidium bromide (right). CCCP + C: corresponds to the carbonyl cyanide m-chlorophenylhydrazone standard inhibitor plus the following controls. 2HAF-3-nitrobenzaldehyde + C: corresponds to the use of chalcone and the respective controls

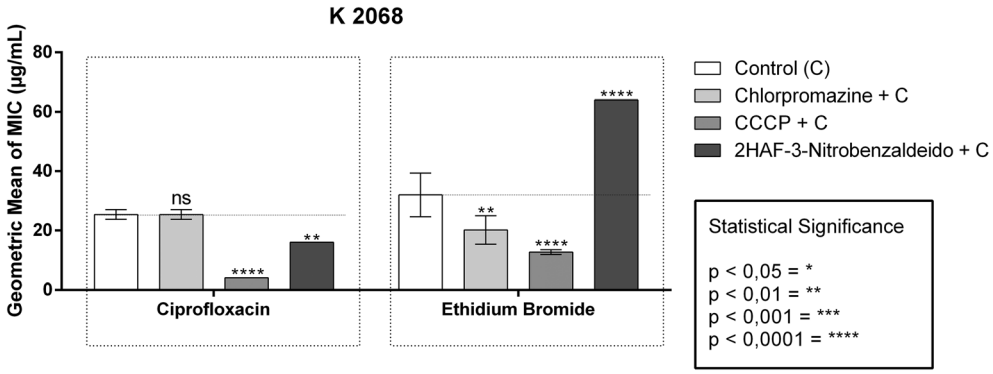


FIGURE 2 Evaluation of antibiotic and ethidium bromide (EtBr) modification by chalcone against *Staphylococcus aureus* K2068. Legend: Control (C): refers to the antibiotic ciprofloxacin on the left and ethidium bromide on the right. Chlorpromazine + C corresponds to the standard inhibitor plus the control, be it the antibiotic (left) or ethidium bromide (right). CCCP + C: corresponds to the carbonyl cyanide m-chlorophenylhydrazone standard inhibitor plus the following controls. 2HAF-3-nitrobenzaldehyde + C: corresponds to the use of chalcone and the respective controls

used concomitantly. Synergism was also observed when the CCCP standard inhibitor and the antibiotic were used. A different result was found when the chalcone was associated with etidio bromide, where there was an antagonism; that is, the MIC of the bromide increased when used together with chlacone. However, when bromide was associated with inhibitors of the standard pump CCCP and chlorpromazine, there was a reduction in MIC, confirming the presence of an efflux pump in this bacterium (Figure 2).

In the present work, a synthetic chalcone, an (E)-1-(2-hydroxyphenyl)-3-(3-nitrophenyl)prop-2-en-1-one, however, studies demonstrate that both natural compounds and synthetics are studied and tested against strains that have an efflux pump resistance mechanism associated with ethidium bromide and when these tests reduce bromide MIC, it indicates that a tested substance acts as an efflux pump inhibitor. Competition with adenosine triphosphate (ATP) and energy exhaustion and interference with the proton gradient caused by natural and/or synthetic compounds are potential mechanisms for inhibiting the efflux pump. The activated NorA efflux pump expels ethidium bromide from the intracellular to the extracellular medium; this same process also occurs with other substances toxic to bacteria, such as antibiotics [10, 22, 23].

It can be observed in the work of Silva et al. [24], who also used chalcones that had nitro groups in their chemical structure, as well as ours; these chalcones that were used by the author were able to synergistically modulate the resistance of the bacterial strain *S. aureus* 1199B when associated with ethidium bromide; however, when it associated the chalcone in study with the antibiotic norfloxacin, it was able to antagonically modulate; this result corroborates with the present study.

Studies report that substances that have nitro group in their molecule, depending on the molecular structure, can be therapeutic agents with great potential, due to

the biotransformation of the nitro group, which causes instability in the membrane structures of many microorganisms [25].

3.3 | ADMET study

Table 4 shows the physicochemical properties used as molecular descriptors of the pharmacokinetic behavior of nitrochalcone. These are the properties that determine the bioavailability of an oral drug candidate: Ionization constant (pKa), lipophilicity (logP), distribution coefficient (logD), and polarity (PSA); used in most deug-like criteria in drug discovery. As an applicable domain, compounds that are not very lipophilic and essentially neutral are preferable [26–30].

When analyzing the microspecies of the molecular system surrounding the nitrochalcone, it is possible to observe that the calculated pKa value in the order of 6694, associated with the phenolic hydroxyl, indicates a chemical balance between the neutral species (microspecie_1) and the ionized species (microspecie_2), which moves towards the predominance of the ionized species at pH > 6.69, the main microspecies being at physiological pH (approximately 7.4) (Figure 3a).

Most of the drug-like rules use the lipophilicity limit in the order of logP <5, as the ideal lipophilicity range of candidates for oral drug. Thus, a very lipophilic compound (logP >5) has a decrease in its bioavailability, as it is poorly absorbed in the intestine [28, 31, 32]. Directly related to lipophilicity, the distribution

TABLE 4 Calculated molecular descriptors in the MarvinSketch software

Molecular descriptor	Predicted value	Unit	Source
Ionization constant	6.694	pKa	ChamAxon
Lipophilicity	4.176	logP	Consensus
Distribution at pH 7.4	3.408	$logD_{7.4}$	Consensus
Polar surface area	80.44	$Å^2$	ChemAxon

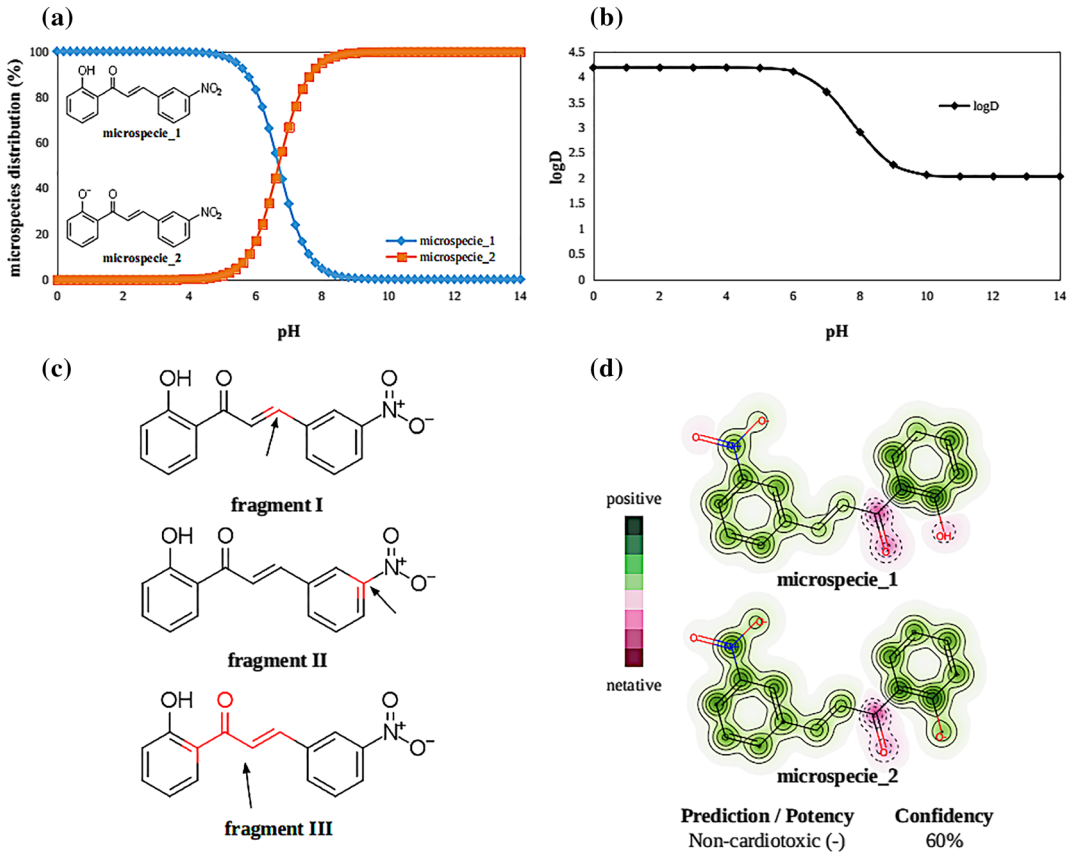


FIGURE 3 (a) Graph showing the distribution of nitrochalcone microspecies as a function of pH; (b) graph of the variation of the distribution coefficient (logD) of the nitrochalcone as a function of pH; (c) molecular fragments of mutagenicity: (I) covalent bind with protein by the isolated alkene; (II) covalent bind with DNA by the quaternary carbon of the benzene bonded to the nitrile group; (III) genotoxic carcinogenicity mutagenicity; (d)

coefficient (logD) shows the distribution index of a molecule at different pH levels. Therefore, logD values at pH 7.4 greater than 3 are associated with a balance between lipophilicity and solubility and can provide information on the absorption and permeability of a substance [27, 33]. Thus, the calculated value of logP in the order of 4176 suggests that the compound is not very lipophilic, meeting the standards established for good absorption. The graph in Figure 3b shows the variation of the distribution coefficient as a function of the pH variation, where it is possible to observe that the reached logD value at pH 7.4 in the order of 3408 suggests that the nitrochalcone is slightly permeable (Table 4). Due to the fact that the chalcone in study is considered a low lipophilic substance, this can be associated with the fact that it does not have a direct antibacterial action, since it hinders its passage through the bacterial plasma membrane.

Thus, it is verifiable that in the pharmacokinetic prediction the compound has moderate permeability in colon adenocarcinoma cells (Caco-2), with a logPapp value in the order of 0.872 (in 10^{-6} cm/s), with human intestinal absorption (HIA) > 90%. In addition, the compound is a substrate for intestinal P-glycoprotein, resulting in a moderate volume of distribution (VD), with logVDss rated at −0.031, following an order of 0.71 L/kg < VDss < 2.81 L/kg, with 100% of the bioavailable fraction bound to plasma proteins. The compound is poorly permeable in the blood–brain barrier (BBB), with a logBB value of −0.111, which suggests that the compound is poorly distributed in the brain and with little activity in the central nervous system (CNS) (Table 5).

Metabolism and toxicological effects by oral administration of a drug candidate can be assessed by predicting activity with cytochrome P450 (CYP450) isoenzymes. Among the main precursors of phase I drug metabolism, CYP1A2 is responsible for molecular biotransformations by metabolic activation, while CYP3A4 is responsible for redox reactions, constituting more water-soluble compounds and susceptible to excretion [34–38]. Despite inhibitions to CYP1A2,

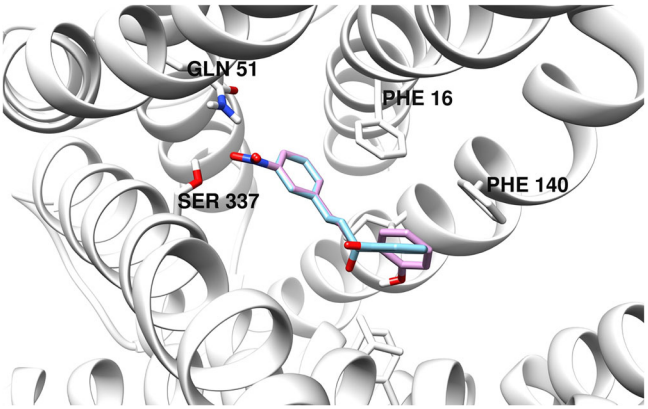


FIGURE 4 The two nitrochalcone microspecies (microspecies 1 in pink, 2 in blue) docked to the binding site of a NorA model

TABLE 5 Predicted pharmacokinetic properties by the ADMET models of pkCSM server

Property	Model name	Predicted value	Unit
Absorption	Caco2 permeability	0.872	Numeric (logPapp in 10^{-6} cm/s)
Absorption	Intestinal absorption (human)	91.586	Numeric (% absorbed)
Absorption	P-glycoprotein substrate	Yes	Categorical (yes/no)
Absorption	P-glycoprotein I inhibitor	No	Categorical (yes/no)
Distribution	VDss (human)	−0.031	Numeric (log L/kg)
Distribution	Fraction unbound (human)	0	Numeric (Fu)
Distribution	BBB permeability	−0.111	Numeric (logBB)
Distribution	CNS permeability	−2.092	Numeric (logPS)
Metabolism	CYP2D6 substrate	No	Categorical (yes/no)
Metabolism	CYP3A4 substrate	Yes	Categorical (yes/no)
Metabolism	CYP1A2 inhibitor	Yes	Categorical (yes/no)
Metabolism	CYP2C19 inhibitor	Yes	Categorical (yes/no)
Metabolism	CYP2C9 inhibitor	Yes	Categorical (yes/no)
Metabolism	CYP2D6 inhibitor	No	Categorical (yes/no)
Metabolism	CYP3A4 inhibitor	No	Categorical (yes/no)
Excretion	Total Clearance	0.105	Numeric (log ml/min/kg)
Toxicity	AMES toxicity	Yes	Categorical (yes/no)
Toxicity	hERG I inhibitor	No	Categorical (yes/no)
Toxicity	Oral Rat Acute Toxicity (LD50)	3.11	Numeric (mol/kg)
Toxicity	Hepatotoxicity	No	Categorical (yes/no)

CYP2C9, and CYP2C19, nitrochalcone is a potential substrate for CYP3A4, which means that the acute oral dose assessed at 3.11 mol/kg does not present a risk of hepatotoxicity or cardiotoxic risk, with renal elimination assessed at 0.105 log ml/min/kg.

Mutagenic activity is detectable in predictions to determine irreversible activity in proteins and DNA structures. It is possible to determine this activity induced by molecular fragments [39–41]. The toxicological prediction showed that the unsaturation of the nitrochalcone aliphatic chain has an electrophilic potential to form a covalent bond with biological receptors, while the quaternary carbon of benzene linked to the nitrile group has the potential to intercalate in DNA structures, resulting in mutagenic toxicity. In addition, the resonance effect between the isolated alkene and the

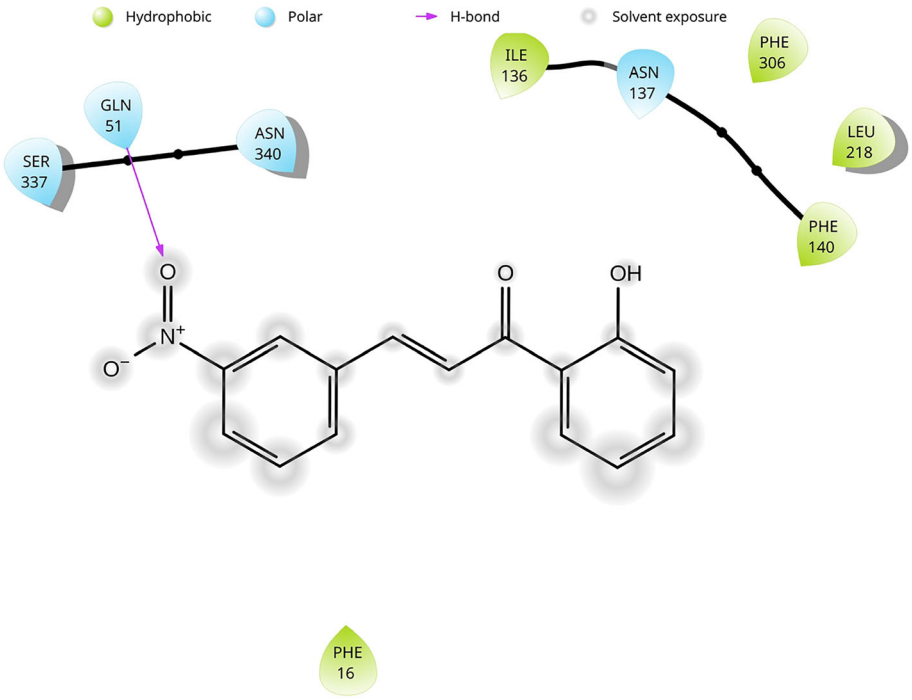


FIGURE 5 A 2D protein-ligand interaction diagram of microspecies 1 docked on the binding site of the NorA model

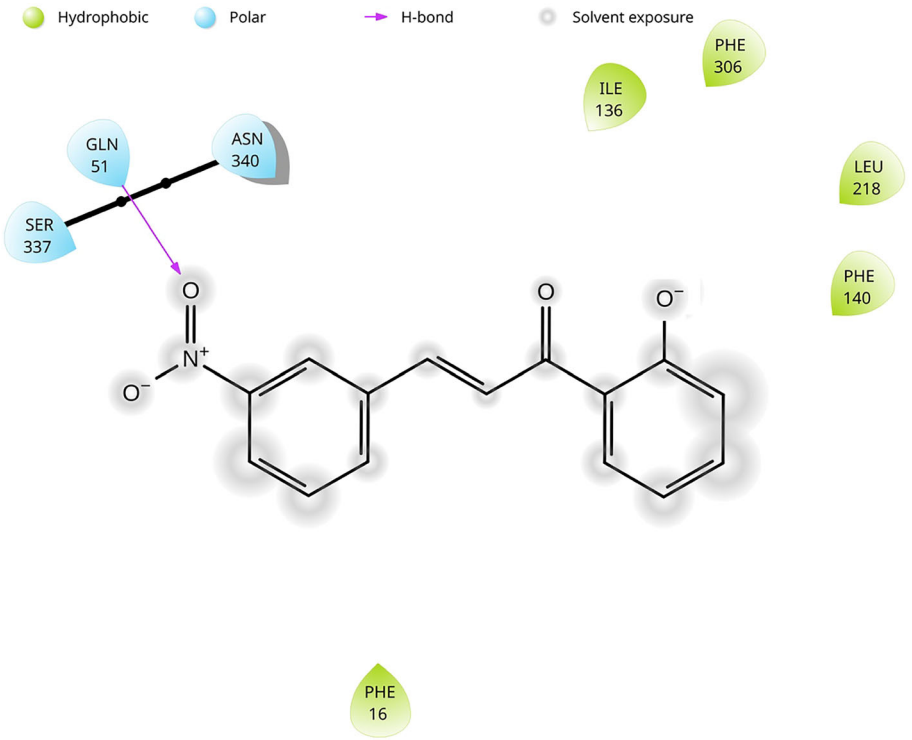


FIGURE 6 A 2D protein-ligand interaction diagram of of microspecies 2 docked on the binding site of the NorA model

carbonyl in the aliphatic chain can result in mutagenicity of genotoxic carcinogenicity. The toxic fragments can be seen in Figure 3c.

In addition, cardiotoxic activity can be determined by inhibiting the hERG ion transport channel (human Ether-à-go-go-go-Related Gene). Molecular fragments may have biological activity for this toxicity model [40, 41]. With the prediction, it is possible to observe that the neutral species of the nitrochalcone (microspecies_1) has negative contributions from the carbonyl and hydroxyl groups (pink region), but that does not overlap the positive contributions (green region). When the ionized species of the molecule (microspecie_2) is evaluated, the generated charge forms a nucleophilic region that does not present inhibitory activity of the hERG channels that, with a 60% reliability degree of the predictive test, does not present a cardiotoxic risk (Figure 1d).

3.4 | Molecular Doking

As previously discussed, at physiological pH, there are two microspecies of the nitrochalcone. We carried out two docking essays on the NorA model, one for each microspecies. The best pose of each microspecie docked on the binding site of the NorA model can be seen in Figure 4. Both microspecies bind to the same region of the binding site and both act as Gln51 through a hydrogen bond. The nitro group of both species also make close contacts with residue Ser337, Met338, Gly339, and Asn340. Hydrogen bonding appears to be a common feature of NorA inhibiting chalcones. On the other hand, the loss of the hydroxyl proton on microspecie 2 changes the positioning of the hydroxyphenyl ring and its interaction with the protein. Thus, while microspecies 1 interacts with residues Asn137, Ser138, and Gly139, microspecie 2 does not. Both, however, appear to bind to the binding site of the NorA model and could act as competitor inhibitors of the efflux pump. A 2D diagram depicting these protein–ligand interactions can be seen as Figures 5 and 6.

4 | CONCLUSIONS

Bacterial resistance to traditionally used drugs has been growing in an exacerbated way and thus the search for potential products that can reverse this resistance becomes increasingly necessary, and these products may be natural, semisynthetic, or synthetic. In this sense, chalcone (E)-1-(2-hydroxyphenyl)-3-(3-nitrophenyl)prop-2-en-1-one, a synthetic substance, showed results against the strains of *S. aureus* that carry the NorA and MepA. Synergistically when associated with ethidium bromide against strain SA-1199B carrying the NorA Pump and synergistic modulation with the antibiotic ciprofloxacin against strain SA-K2068, this way the chalcone is being studied as a possible alternative for reversing resistance to this. However, new tests are needed to better evaluate its effect. The physicochemical properties calculated were fundamental in the description of the predicted pharmacokinetic properties, despite the mutagenic risk caused by the metabolic.

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CONFLICT OF INTEREST

The authors have no conflict of interest.

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