Microbial Pathogenesis 177 (2023) 106058

Contents lists available at ScienceDirect

Microbial Pathogenesis

journal homepage: www.elsevier.com/locate/micpath

In vitro and *in silico* antibacterial evaluation of coumarin derivatives against MDR strains of *Staphylococcus aureus* and *Escherichia coli*

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ARTICLE INFO

Keywords:
Coumarin derivatives
ADME
Antibacterial
Antibiotic resistance
Staphylococcus aureus
Escherichia coli

ABSTRACT

The increase in antibiotic resistance rates has attracted the interest of researchers for antibacterial compounds capable of potentiating the activity of conventional antibiotics. Coumarin derivatives have been reported to develop effective antibacterials with possible new mechanisms of action for treating infectious diseases caused by bacteria with a profile of drug resistance. In this context, the aim of the present study we have now prepared one variety of new synthetic coumarins evaluating the pharmacokinetic and chemical similarity in silico, their antimicrobial activity against *Staphylococcus aureus (*ATCC 25923) and *Escherichia coli* (ATCC 25922), and potential for the modulation of antibiotic resistance against *Staphylococcus aureus (SA10)* and *Escherichia coli (EC06)* clinical isolate bacteria by in vitro assay. The antibacterial activity and antibiotic-enhancing properties were evaluated by the broth microdilution method and pharmacokinetically characterized according to the Lipinsk rule of 5 and had their similarity analyzed in databases such as ChemBL and CAS SciFinder. The results demonstrated that only compound C13 showed significant antibacterial activity (MIC $\leq$256 μg/mL), and all other coumarins did not display relevant antibacterial activity (MIC $\geq$1024 μg/mL). However, they did modulate the antibiotics activities to norfloxacin and gentamicin, except, compound C11 to norfloxacin against *Staphylococcus aureus (SA10)*. The in silico properties prediction and drug-likeness results demonstrated that all coumarins presented a good drug-likeness score with no violations and promising in silico pharmacokinetic profiles showing that they have the potential to be developed into an oral drug. The results indicate that the coumarin derivatives showed good in vitro antibacterial activity. These new coumarin derivatives also demonstrated the capacity to modulate antibiotic resistance with potential synergy action for current antimicrobials assayed, as antibiotic adjuvants, to reduce the emergence of antimicrobial resistance.

1. Introduction

The introduction of Antibiotic resistance has significantly increased in the last years [1–3] and evidence has indicated that this public health problem might have been aggravated by the COVID-19 pandemic [4]. In the search for new therapeutic options, scientists have made significant efforts to find new antibacterial substances, as well as identify compounds capable of potentiating the activity of antibiotics, which may represent a promising strategy in the fight against antibiotic resistance [5,6].

Consistent evidence has demonstrated the antibacterial potential of medicinal plants, indicating that their secondary metabolites can interfere with the metabolism and growth of strains that cause human infections [7]. In addition, research indicates that the combination of compounds may result in an antimicrobial response superior to that obtained with the use of a single compound, especially in the treatment

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https://doi.org/10.1016/j.micpath.2023.106058
Received 3 October 2022; Received in revised form 1 March 2023; Accepted 3 March 2023
Available online 5 March 2023

of infections caused by resistant bacteria [8–11], which encourages the combined use of natural products and conventional antibiotics.

The natural bioactive compounds and/or their metabolites or the association with an antibiotic can promote significant alteration in the intestinal microbiota associated with immunomodulatory activities, which are responsible for allergies and infectious diseases [12]. However, research carried out by our group has found that combining plant extracts with antibiotics often results in synergic effects in cultures of different multidrug-resistant (MDR) bacteria, with low impact under the microbiota bacterial [13–16]. This strategy assumes that the combination of extracts or substances isolated with each other and with antibiotics can affect a more significant number of therapeutic targets and, therefore, result in synergistic interactions that potentiate the antibacterial properties of the individual pharmacological agents [9,17–20].

Literature data show that synthetic compounds from different classes have promissory importance in discovering new antibacterial agents with the potential of reducing antimicrobial resistance profile. Derivatives of the benzimidazoles class have an efficient antibacterial capability to fight against Methicillin-resistant *Staphylococcus aureus* (MRSA) involving different mechanisms of action such as DNA binding, promoting the enzyme inhibitors, anti-biofilm activities, and synergistic effect with available antibiotics [21]. Pyrazole derivatives or the introduction of pyrazole groups was demonstrated to be excellent growth inhibitors of MRSA against diverged bacterial strains influencing protein synthesis inhibition, affecting other biomolecules (RNA, DNA), and interfering in the biosynthesis pathways as fatty acid biosynthesis [22]. Oxadiazole analogs that present substituents with different electronic effects played crucial roles as potential methicillin-resistant with varying mechanisms of action, e.i. Involvement in the ribosome rescue mechanism, LTA biosynthesis inhibition, and others [23].

Ravindar et al., 2018 showed that synthetic analogs of aryl fluorosulfate have excellent activity against the four bacterial strains tested: two Gram-positive strains, *Staphylococcus aureus* and *Bacillus subtilis*, as well as two Gram-negative strains, *Escherichia coli,* and *Klebsiella pneumonia* demonstrating that donors of electrons from the (OCH_3) and sulfonylfluoride (-OSO_2F) groups present in the phenyl ring increase the antibacterial activity, while the electron-withdrawing groups (Cl, F, Br, and NO_2) showed less antibacterial activity [24]. Synthesized compounds derivatives of amino acids conjugated with quinazolinones exhibited significate antimicrobial activities against Gram-negative strains *S. typhimurium, E. aerogenes, E. coli*, and *K. pneumoniae* and proline (Pro) has a better activity decurrent of the hydrophobic nature and the acid-base character of indole nucleus [25]. On the other hand, synthetic derivatives from Quinazolinone-Schiff's promising antimicrobial against gram-positive bacteria *C. staphylococcus* and gram-negative bacteria *E. coli* show that both electron-withdrawing and electron-donating groups increase the potent antimicrobial of Schiff's bases agents [26]. Complex organometals containing benzodioxane midst piperazine associated with chitosan silver nanoparticles (BP*C@AgNPs) show significant biocidal interactions with biofilm protein controlling the MRSA biofilm formation [27].

Due to the structural complexity of synthetic class or bioisosteric group derivates, natural products are less susceptible to microbial adaptation [7,28–31], and as such may be attractive drug candidates in the antibiotic resistance scenario. Moreover, medicinal chemistry and biotechnology strategies have been employed to carry out structural modifications of these compounds, which often results in the improvement of their pharmacological and biopharmaceutical properties, as observed in homologous series of compounds with antimicrobial potential such as coumarins [13,30,32–37].

Coumarin is naturally synthesized from the amino acid phenylalanine, which undergoes enzymatic deamination by the action of phenylalanine ammonia-lyase, generating cinnamic acid, which in turn undergoes hydroxylation of the side chain by the enzyme *trans*-cinnamate-4-hydroxylase, forming the acid o-coumaric, which, by o-glycosylation followed by isomerization of the side chain double bond and lactonization, generates coumarin [38,39].

Previous research has shown that coumarin derivatives have significant antibacterial activity, especially against Gram-positive bacteria such as *Bacillus subtilis* and *Staphylococcus aureus* [30,40]. In addition, natural coumarin derivatives formed from 4-hydroxycoumarin [41] showed effectiveness against pathogenic bacteria such as *Staphylococcus aureus, Bacillus atrophaeus*, *Bacillus subtillus, Pseudomonas aeruginosa*, and *Escherichia coli* [42,43].

In this context, the present study carried out an in silico pharmacokinetic characterization and evaluated in vitro the antibacterial properties of new synthetic coumarin derivatives against Gram-positive and Gram-negative bacterial strains.

2. Results

2.1. In silico analysis of similarity

The search for similarities in the UniChem database identified molecules with 100% similarity for all coumarins included in this study (Chambers, 2013). Accordingly, the search for publications reporting similar molecules in CAS SciFinder showed that, except for C11, the coumarin derivatives have been reported to present antibacterial activity against different strains (Table 1).

Table 2 presents the physicochemical properties that directly influence the probability of a molecule becoming an oral drug. The results demonstrate that none of the molecules showed any violation of Lipinski's rule of five [44], since they are small molecules, with few hydrogen acceptors or donors, small polar surface area, and low partition coefficients.

The ChEMBL database was used to identify possible targets for the coumarins under study. Considering the total number of organisms, eukaryotic organisms were the most likely to present a molecular target for the compounds, with bacteria ranking second in the number of likely targets (Table 3).

2.2. Antibacterial activity

Among the coumarin derivatives evaluated by this study, only the C13 showed a clinically relevant antibacterial activity against *Staphylococcus aureus* ATCC 25923 (MIC $\leq$128 μg/mL) and *Escherichia coli* ATCC 25922 (MIC $\leq$256 μg/mL). Since the other compounds presented minimum inhibitory concentration (MIC) values above 1,024 μg/mL, it is recognized that they do not present a clinically relevant antibacterial activity against these strains. In order to evaluate the ability of the compounds to modulate the activity of aminoglycosides and fluoroquinolones, antibiotics of these classes were combined with subinhibitory concentrations of the coumarins against the multidrug resistant strains *S. aureus* 10 and *E. coli* 06. The association of C10 with either gentamicin or norfloxacin showed modulatory effect with reduction of MIC for both antibiotic against both *E. coli* and *S. aureus* strains (Fig. 1).

The association of norfloxacin and gentamicin with C11 (Fig. 2) or C13 (Fig. 3) caused a significant reduction in the antibiotic MIC against the resistant strains, except for associations with norfloxacin to *S. aureus*, that shows which shows an indifference to the MIC value. As observed to C13 compound, the C14 also reduced the MIC for both antibiotics against both *E. coli 06* and *S. aureus 10* (Fig. 4).

Based on the results, it's possible stabilish the structure—activity relationship (SAR) for the synthetic 4-hydroxycoumarin derivatives present in this work. About the intrinsic antibacterial activity (MIC), it's observed that only the compound C13 presents a MIC value with clinical relevance; this fact can be related to alterations on the benzyl ring, as the presence of withdrawing groups (methyl ketone), proximate a free hydroxyl group present in position 7 and major capacity to release hydrogen from this hydroxyl group. Compound C14 is the pharmacophoric group of synthetic coumarins present in this work without change in the benzylic ring. So, although this compound (C14) also have a free

Table 1
Pharmacological activities of the coumarin derivatives according to the literature.

Compound	IUPAC Name[a]	Similar molecule code (% similarity)[b]	Reported activity	Reference[c]
C10	7-(Allyloxy)-4-methyl-2H-chromen-2-one	ZINC1676264 (100%)	Antioxidant (c) Anticholinesterase (d) Antibacterial (e)	(c) Vianna et al., 2012 (d)(e) Zayane et al., 2016 (e) Tătărîngă et al., 2018
C11	7-(alpha-D-Galactopyranosyloxy)-4-methyl-2H-1-benzopyran-2-one	ZINC4282211 (100%)	Carbonic anhydrase inhibitor (f)	(f) Touisni, 2011
C13	8-Acetyl-7-hydroxy-4-methylcoumarin	ZINC40588 (100%)	Antibacterial (g)	(g) Konc et al., 2011
C14	7-Hydroxy-4-methyl-2H-chromen-2-one	ZINC58121(Hymecromone) (100%)	Anticancer (h) Antibacterial (i)	(h) Piccioni et al., 2015 (i) Shi e Zhou, 2011 (i) Kawasea, 2001

[a] Source: PubChem, National Institutes of Health (NIH).
[b] Reference data bank of similar molecules: zinc.docking.org.
[c] The search was carried out at the CAS SciFinder.[n].

Table 2
Physicochemical properties of coumarins derivatives.

Compound	Molecular formula	Molecular mass (g/mol)	NLR	NHBA	NHBD	TPSA (Å^2)	LogP	Consensus Log $P_{o/w}$[a]
C10	$C_{13}H_{12}O_3$	216.23	3	3	0	39	2.67	2.78
C11	$C_{16}H_{18}O_8$	338.31	3	8	4	129	−0.72	0.00
C13	$C_{12}H_{10}O_4$	218.21	1	4	1	67	2.01	1.75
C14	$C_{10}H_8O_3$	176.17	0	3	1	50	1.81	1.81
Gentamicin	$C_{21}H_{43}N_5O_7$	477.603	7	7	8	200	−3.327	−2.15
Norfloxacin	$C_{16}H_{18}FN_3O_3$	319.336	3	5	1	72.9	1.268	0.98

NLR: Number of rotatable links. NHBA: Number of hydrogen bond acceptor regions. NHBD: Number of hydrogen bond donor regions. TPSA: Topological polar surface area. NVL: Number of violations of Lipinski's rule of five.
[a] Consensus Log Po/w - average of all prediction of caluled Log Po/w (iLOGP); Log Po/w (XLOGP3); Log Po/w (WLOGP); Log Po/w (MLOGP); Log Po/w (SILICOS-IT).

Table 3
Estimated number of target organisms for study coumarins (ChEMBL).

Predicted target organism	C10	C11	C13	C14
Eukaryotes	3	6	–	118
Bacteria	–	2	–	9
Fungi	–	–	–	9
Virus	–	–	–	2
No data available	2	1	–	5

hydroxyl group, they did not show direct activity against the bacteria tested, also modulate the resistance againts both antibiotics. This actions may be related to a lower capacity to release hydrogen from the hydroxyl group. On the other two coumarins, C10 and C11, the hydroxyl group is protected; this can be related to the loss of activity against the bacteria tested. Corroborating our results, data from other coumarins in the literature showed that the antibacterial activity is directly related to the presence of a hydroxyl group on the benzyl ring.

Except for compounds C11 on *S. aureus* for norfloxacin, all other 4-hydroxycoumarin derivatives demonstrate the capacity for antibiotic modulation of antibiotic resistance to both gram-negative and positive bacteria. As observed, alterations on phenyl ring containing electron-donating/withdrawing groups can be promote significant transformation of the modulation of antibacterial activity (MIC value). However, diverse data from literature to synthetic coumarins indicate that the oxygenation of the phenyl ring contributes to an increase of antibacterial activity, principaly, when a free hydroxyl group at position C-7 as present on compounds C13 and C14. However, the presence methyketone group on C13 promote the encrease of lipophilicity when compare with compound C14 (logP 2.01 vesus 1.81). The results do not suggest that the increase in the size or Molecular weight modifies the antimicrobial activity of these coumarins. However, the hydrophobic

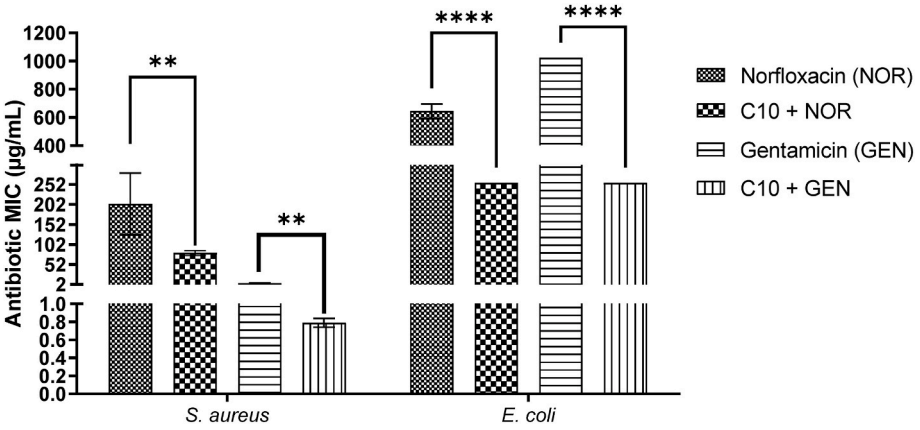


Fig. 1. Minimum inhibitory concentrations (µg/mL) of norfloxacin and gentamicin in association with C10 against *Staphylococcus aureus* 10 and *Escherichia coli* 06. **p < 0,01; ****p < 0,0001.

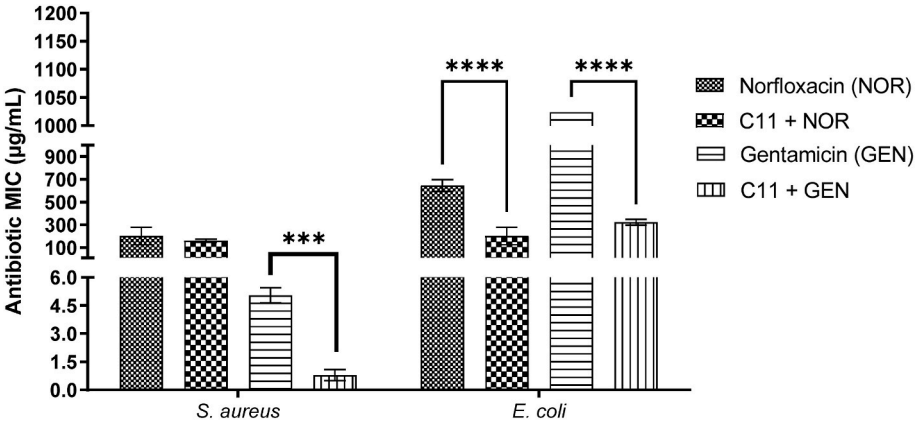


Fig. 2. Minimum inhibitory concentrations (µg/mL) of norfloxacin and gentamicin in association with C11 against *Staphylococcus aureus* 10 and *Escherichia coli* 06. ****p < 0,0001.

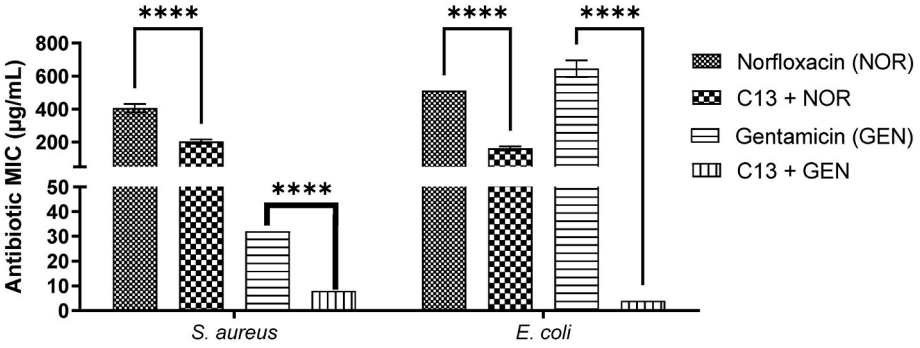


Fig. 3. Minimum inhibitory concentrations (µg/mL) of norfloxacin and gentamicin in association with C13 against *Staphylococcus aureus* 10 and *Escherichia coli* 06. ****p < 0,0001.

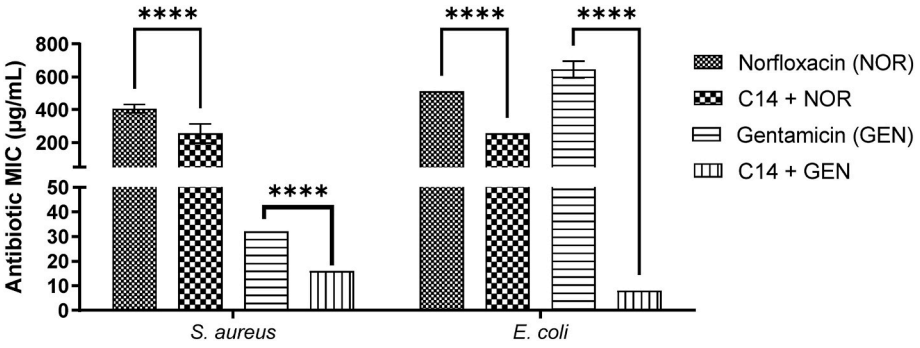


Fig. 4. Minimum inhibitory concentrations (µg/mL) of norfloxacin and gentamicin in association with C14 against *Staphylococcus aureus* 10 and *Escherichia coli* 06. ****p < 0,0001.

characteristic promotes modulations that can affect the permeability and fluidity of the cell envelope, facilitating the penetration of antibiotics and reduction of MIC value.

3. Discussion

The development of a new drug is a lengthy and expensive process, taking at least 12 years and costing around US$ 1.8 billion. Therefore, the use of research strategies to optimize this process has been increasingly valued. In this context, in silico research has been recognized as an important approach to optimizing drug development, as it can reduce both the time and costs associated with this process [45]. However, for the first time, new synthetic derivates of coumarins were synthesized, demonstrating relevant antimicrobial activities displayed a modulatory effect of bacterial resistance against antibiotics norfloxacin and gentamicin to Gram-positive and gram-negative bacteria. Based on these results also, it was possible to promote the correlations between the chemical structures of the coumarins and their antimicrobial activities.

The present in silico analysis demonstrated that the coumarin derivatives have promising physicochemical and pharmacokinetic properties, indicating that, from a computational point of view, they have the potential to be used in the development of oral drugs, especially because they did not violate the Lipinski's rule of five [44]. The in-silico predictions also indicated that these coumarins have potential molecular targets in a great variety of organisms, among which bacteria stand out, encouraging the investigation of the antibacterial activities of these substances.

Previous research has demonstrated that the coumarin derivatives investigated in this study have antibacterial activity against Gram-positive and Gram-negative bacterial strains [32,37,41,46,47]. However, the present study demonstrated that only the C13 derivative showed a clinically effective antibacterial activity. This observation is corroborated by the study of Trykowska Konc et al. who showed that the substance 8-Acetyl-7-hydroxy-4-methyl coumarin was active the Gram-positive bacterial strains *S. aureus* ATCC 6538, *B. cereus* ATCC 11778, *B. subtilis* ATCC 6633, and *M. luteus* ATCC 9341 [48]. However, unlike C13, this substance had no significant activity against the Gram-negative strain (*E. coli* ATCC 10536).

As among the tested compounds, only the C13 coumarin derivative presented intrinsic antibacterial activity, it is hypothesized that such biological property may be due to the presence of a ketone group next to the ring. The carbonyl group in the C-8 position next (in the ortho position) to the hydroxyl in the C-7 position could promote a high electron density field, contributing to the scavenging of cation ions and thus, the compound could function as a metal-chelating agent, which could affect bacterial survival. A study evaluating organic compounds containing carbonyl and phenol groups on their iron-binding capacity in different valences for cluster formation showed that the phenol group, when deprotonated, forms the phenoxy anion, increasing the nucleophilic capacity of the molecule, providing greater iron attraction. Additionally, the carbonyl group can form bridges with metals, and evidence indicates that the iron-attraction capacity is increased when the carbonyl group is in the ortho position with the phenol [49].

While most coumarin derivatives presented clinically ineffective antibacterial activity, all of them showed the ability to potentiate the activity of standard antibiotics (norfloxacin or gentamicin or both) against MDR strains of *S. aureus* and *E. coli*, indicating that these compounds could reverse antibiotic resistance in vitro. While studies reporting the antibiotic-modulating activity of coumarin derivatives are scarce, a study by Araújo et al. demonstrated that these compounds can act as efflux pump inhibitors in *S. aureus* strains [30]. Moreover, it was demonstrated that the combination of coumarin and aminoglycosides (such as gentamicin) and fluoroquinolones (such as levofloxacin), resulted in an enhanced antibacterial effect against Methicillin-resistant *Staphylococcus aureus* (MRSA) [10,11,13,14,20].

With regard to the potential antibacterial mechanisms of coumarin derivatives, electron microscopy analysis indicated that 7-methoxycoumarin caused significant lesions in the bacterial membrane of *Ralstonia solanacearum*, a pathogenic bacterium that infects plants [50]. Despite the structural differences of the compounds and the fact that this bacterium is not pathogenic for humans, the findings of the reported research suggest that the coumarin derivative could affect the integrity of bacterial membranes, increasing the penetration of antibiotics and thus potentiating their antibacterial effects.

The possibility that coumarin compounds promote an additive effect when associated with fluoroquinolone antibiotics is supported by studies demonstrating that coumarins can bind the B subunit of DNA gyrase in bacteria, inhibiting DNA supercoiling, which results in a bactericidal effect [33,51]. As a similar mechanism is played by fluoroquinolone antibiotics, such additive effect could justify the synergism observed from the association of C10, C13, and C14 with norfloxacin against the MDR strain of *S. aureus,* as well from the association of C10, C11, C13, and C14 with fluoroquinolones against MDR *E. coli*.

The compound C13 is more lipophilic when compare with C14 supporting that the lipophilicity is played a key role for their inhibitory activities to gram-positive bacteria [52]. The SAR studies relationate the free hydroxyl group at position C-7 of phenyl ring is crucial for antibacterial activity [46]. Structural charateristic of compounds substitution on phenyl ring containing electron-donating/withdrawing groups as –CH3, –OH and, –OMe groups on aryl ring may be in association with increased antibacterial activity with direct relations with lipophilicity that contribute for penetration by the cell membrane of these bacterias [42,53].

The literature correlates the antibacterial compounds' activity differences between Gram-negative and Gram-positive bacteria with the ideal logP around 4 to Gram-negative bacteria and against Gram-positive around 6 [54]. Physico-chemical properties of antibiotics targeting Gram-negative bacteria showed a greater polar surface area (PSA) or topological polar surface area (TPSA) (~165 Å2) [55], perhaps explaining the activity observed in C13 that presented activity modulate favorable against *S. aureus*. The substituents on the benzyl ring that contain a heterocyclic ring (e.g., imidazole, piperazine, fluoro-isoquinoline, azole, and thiazole) linked with pharmacophore group of coumarin played a key role in the transformation of the antibacterial activity [22–26,33,56].

A study that integrated a coumarin structural fraction to quinolone-based compounds in the same molecule showed that the resulting compound presented an increased antibacterial activity against Gram-positive and Gram-negative bacteria, in comparison to the parent compounds, providing a perspective of using coumarins to obtain compounds with improved antibacterial activity [37].

The speed at which bacteria are developing mechanisms of resistance to existing antibacterial drugs is significantly greater than the speed at which science can develop more potent drugs to combat antibiotic resistance [39]. Thus, the discovery of compounds capable of increasing the activity of conventional antibiotics may significantly contribute to antimicrobial therapy, reducing the required antibiotic dose and consequently causing less toxicity, providing thus increased benefits to the patients. Thus, it is expected that, due to the antibiotic-enhancing properties of coumarin derivatives, research targeting these compounds will contribute to antibacterial drug development.

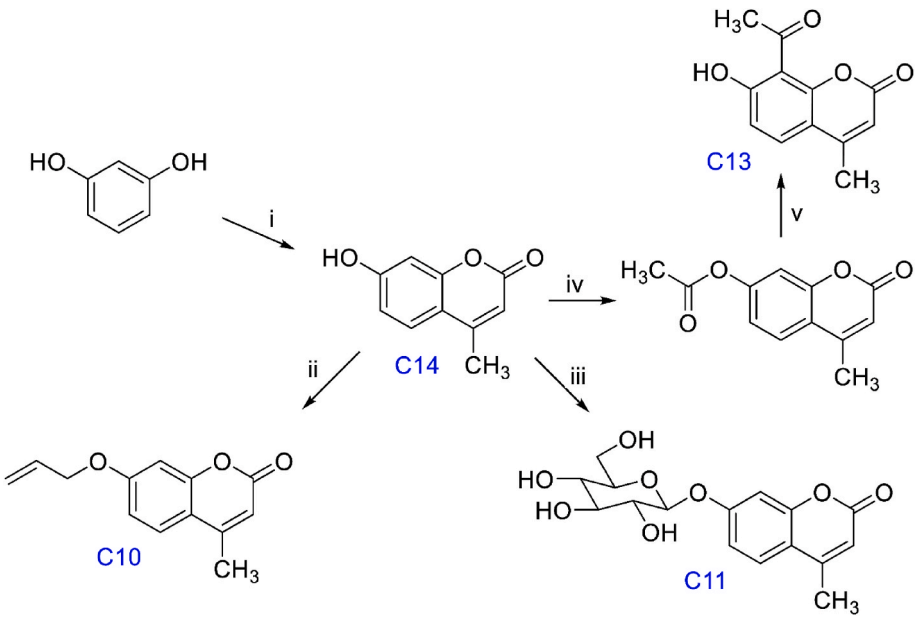
4. Materials and methods

4.1. Obtention of coumarins

The coumarins evaluated in this study were synthesized and provided by the Pharmaceutical Chemistry Research Laboratory (LQFar), Faculty of Pharmaceutical Sciences, Federal University of Alfenas (UNIFAL-MG). These compounds were obtained following classical protocols of organic synthesis, as previously described in the literature (Scheme 1). Briefly, the base coumarin (C14) was prepared by Pechmann reaction between ethyl acetoacetate and resorcinol, under acid catalysis [57]. Coumarin C10 was obtained by a nucleophilic substitution reaction of C14 with allyl bromide in a basic medium and in the presence of polar aprotic solvent [58]. The C11 glycosylated coumarin was prepared by adapting the procedure proposed by Ref. [59] in two steps, starting from the treatment of C14 with the glycosylating agent glucopyranosyl bromide, followed by transesterification with sodium methoxide. C13 coumarin was prepared in two steps by O-acetylation of C14 with acetic anhydride, followed by Fries rearrangement with aluminum chloride [60]. The spectrometric and chromatographic analyzes used in their characterization provided results that attest to their identity and purity.

4.2. In silico analysis by similarity of pharmacokinetic and pharmacological properties (ADME) of coumarins

The molecular structures of coumarin derivatives had the similarity to other molecules compared through the ChEMBL database (EBI, https://www.ebi.ac.uk/chembl/), a strategic grant from the Wellcome Trust and the European Molecular Biology Laboratory (EMBL), for the field of chemogenomic. The ChEMBL is an open database manually fed from journals on small molecules [61,62]. In addition to ChEMBL, the small molecule dictionary ChEBI (Chemical Entities of Biological Interest) was used for a unified search in various databases using the UniChem 2.0 software, beta version [63]. This compound identifier unifies resources of the EMBL-EBI and maintains cross-references between the chemistry resources of the EBI and other databases such as the



Scheme 1. Synthesis of coumarins. Experimental conditions: i) ethyl acetoacetate, perchloric acid, 25 °C, 80%; ii) allyl bromide, sodium carbonate, acetone, 70 °C, 60% iii) a: α-D-glucopyranosyl bromide, sodium hydroxide, water, acetone, 25 °C; b: sodium methoxide, methanol, 0 °C; 15% (2 steps); iv) acetic anhydride, N,N-dimethyl aminopyridine (cat.), dichloromethane, 25 °C, 82%; v) anhydrous aluminum chloride, 150 °C, 40%.

Gene Expression Atlas and the PDBe (Protein Data Bank in Europe). For the analysis of the physicochemical parameters (molecular weight, lipophilicity, water-solubility, number of hydrogen acceptor and donor regions, and number of rotatable bonds), drug-likeness (a prediction of how viable a given molecule is to become a drug, considering the context of bioavailability) and pharmacokinetics (ADME - absorption, distribution, metabolism, and excretion) this study used the SwissADME software, developed and maintained by the Molecular Modeling Group of the SIB (Swiss Institute of Bioinformatics) [64]. SwissADME is a web-based tool that provides access to a set of fast and robust predictive models for physicochemical and pharmacokinetic properties and drug-likeness analysis using medicinal chemistry, including features such as BOILED-Egg, iLOGP, bioavailability radar, and synthetic accessibility score. The search for references on the molecules reported in this study was carried out by consulting their chemical structures in the CAS SciFinder^{n} [65].

4.3. Antibacterial activity analysis

Standard (*Escherichia coli* ATCC 25922 and *Staphylococcus aureus* ATCC 25923) and multidrug-resistant (MDR - *Staphylococcus aureus* 10 and *Escherichia coli* 06) strains were supplied by the Laboratory of Microbiology and Molecular Biology (LMBM) of the Regional University of Cariri (URCA). The aminoglycoside antibiotic gentamicin (GEN) and the fluoroquinolone norfloxacin (NOR) were dissolved in sterile distilled water and used at a concentration of 1024 μg/mL. Antibacterial susceptibility analysis was performed in triplicate using the broth microdilution method in 96-well plates. Test solutions were prepared using 10 mg of the compounds solubilized in 9,265 μL of distilled water and 500 μL of DMSO, achieving a concentration of 1,024 μg/mL, whose serial dilution resulted in a concentration range of 512–8 μg/mL [66].

Bacterial strains were kept on stock agar medium under refrigeration and cultured in brain heart infusion (BHI) broth and incubated at 37 °C for 24 h. The inoculum was diluted in 10% BHI (100 μL of inoculum + 900 μL of BHI) to obtain a concentration of 10^5 CFU/ml. A volume of 100 μL of this solution was distributed in each well on the plate, followed by the addition of 100 μL of the compounds at varying concentrations obtained by serial dilution, as previously described. After 24 h, the wells were added with resazurin (20 μg/mL) and bacterial growth was observed. The minimum inhibitory concentration (MIC) was defined as the lowest concentration at which no bacterial growth was observed [66].

To evaluate the modulating effect of the action of antibiotics, the compounds, in concentrations equivalent to the MIC/8, were combined with antibiotics (in concentrations obtained by serial dilution) and the MIC of these drugs was evaluated in the presence or absence of the compounds as previously described [67]. Wells containing antibiotic, medium, or inoculum in the absence of compounds were used as controls.

4.4. Statistical analysis

The results from the assays were performed in triplicates and expressed as geometric means. A one-way ANOVA followed by Bonferroni's post hoc test was used as the statistical analysis test, using the GraphPad Prism 9.0 software

5. Conclusions

All of the coumarin derivatives investigated in the present research have antibiotic-enhancing properties that stimulate their use in drug development targeting the combat of antibacterial resistance involving both Gram-positive and Gram-negative strains. Additionally, the C13 derivative demonstrated clinically relevant antibacterial effects, which stimulate its use as a prototype drug in the development of compounds with both antibacterial and antibiotic-potentiating effects, which would have a significant impact on modern antibiotic therapy. While the mechanisms underlying the antibacterial effects of coumarin derivatives remain unclear, in silico analysis indicates that these compounds have physicochemical properties, pharmacokinetic profiles, and drug-likeness characteristics that stimulate their use as oral drugs, confirming their potential to be used in antibiotic drug development.

Funding statement

This work was supported by grant from the Fundação de Amparo à Pesquisa do Estado de Minas Gerais – FAPEMIG (APQ-00686-2018). The funder had no role in study design, data collection and analysis, decision

to publish, or preparation of the manuscript.

CRediT authorship contribution statement

Ana Luíza A.R. Martin: Conceptualization. **Irwin R.A. De Menezes:** Methodology. **Amanda K. Sousa:** Investigation. **Pablo A.M. Farias:** Formal analysis. **Francisco A.V. dos Santos:** Writing – original draft. **Thiago S. Freitas:** Software. **Fernando G. Figueredo:** Investigation. **Jaime Ribeiro-Filho:** Writing – original draft. **Diogo T. Carvalho:** Resources. **Henrique D.M. Coutinho:** Supervision, Project administration. **Marta M.F. Fonteles:** Supervision.

Declaration of competing interest

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.

Data availability

Data will be made available on request.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.micpath.2023.106058.

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