DOI: 10.55905/rdelosv18.n68-067
ISSN: 1988-5245

Originals received: 5/9/2025
Acceptance for publication: 6/4/2025

Molecular profile of natural compounds with potential antioxidant and antimicrobial activities in comparison to (E)-3-(2-(4-cyanostyryl)-4-oxoquinazolin-3(4H)-yl)benzoic Acid

Perfil molecular de compostos naturais com potencial atividades antioxidante e antimicrobiano em comparação ao Ácido (E)-3-(2-(4-cianostiril)-4-oxoquinazolin-3(4H)-il)benzóico

Perfil molecular de compuestos naturales con potencial antioxidante y antimicrobiano en comparación con el ácido (E)-3-(2-(4-cianoestiril)-4-oxoquinazolin-3(4H)-il)benzoico

Aderaldo Viegas da Silva
Master in Health Sciences
Institution: Universidade Federal do Amapá
Address: Macapá – Amapá, Brazil
E-mail: aderaldosilva14@gmail.com

Kelton Luis Belém dos Santos
PhD in Biodiversity and Biotechnology
Institution: Universidade Federal do Pará
Address: Macapá – Amapá, Brazil
E-mail: kelton.belem@unifap.br

Francy Mendes Nogueira Cardoso
Master in Health Sciences
Institution: Universidade Federal do Amapá
Address: Macapá – Amapá, Brazil
E-mail: francy.cardoso@unifap.br

Njogu Mark Kimani
PhD in Phytochemistry
Institution: University of Münster
Address: Embu – Kenya
E-mail: njogu.mark@embuni.ac.ke

Cleydson Breno Rodrigues dos Santos
PhD in Biodiversity and Biotechnology
Institution: Universidade Federal do Amazonas
Address: Macapá – Amapá, Brazil
E-mail: breno@unifap.br

ABSTRACT
The study performed quantum chemical calculations of four flavonoids (Chalcone, Flavone, Isoflavone and Aurone) and a synthetic derivative (Isoxazolone) to evaluate their antioxidant and antimicrobial potentialities in comparison to the compound (E)-3-(2-(4-cyanostyryl)-4-oxoquinazolin-3(4H)-yl)benzoic acid, QNZ, known for its activity against antibiotic-resistant *Staphylococcus aureus*. The calculations were performed using Density Functional Theory (DFT) on the B3LYP/6-311G(d,p) and B3LYP/6-311++G(2d,2p) bases. The descriptors obtained included HOMO, LUMO, GAP and Ionization Potential (IP). The results indicated that Aurone presented the lowest GAP, suggesting high reactivity, while QNZ stood out for its electron donation (HOMO) and electron acceptance (LUMO) capacity, presenting results similar to flavonoids. The Tanimoto Index revealed low structural similarity between QNZ and the other compounds, except for Isoxazolone. The results suggest that flavonoids and Isoxazolone may be potential antioxidants and antimicrobial candidates, justifying new *in vitro* assays to evaluate the antimicrobial activity against resistant strains.

Keywords: flavonoids, antioxidant, DFT (Density Functional Theory), antimicrobials.

RESUMO
O estudo realizou cálculos químico-quânticos de quatro flavonoides (Chalcona, Flavona, Isoflavona e Aurone) e um derivado sintético (Isoxazolone) para avaliar suas potencialidades antioxidantes e antimicrobianas em comparação ao composto Ácido (E)-3-(2-(4-cianostiril)-4-oxoquinazolin-3(4H)-il)benzóico, QNZ, conhecido por sua atividade contra *Staphylococcus aureus* resistente a antibióticos. Os cálculos foram realizados usando a Teoria do Funcional de Densidade (DFT) nas bases B3LYP/6-311G(d,p) e B3LYP/6-311++G(2d,2p). Os descritores obtidos incluíram HOMO, LUMO, GAP e Potencial de Ionização (IP). Os resultados indicaram que a Aurone apresentou o menor GAP, sugerindo alta reatividade, enquanto o QNZ se destacou pela capacidade de doação de elétrons (HOMO) e aceitação de elétrons (LUMO), apresentando resultados semelhantes aos flavonoides. O Índice de Tanimoto revelou baixa similaridade estrutural entre o QNZ e os demais compostos, exceto pela Isoxazolone. Os resultados sugerem que os flavonoides e a Isoxazolone podem ser potenciais candidatos antioxidantes e antimicrobianos, justificando novos ensaios *in vitro* para avaliação da atividade antimicrobiana contra cepas resistentes.

Palavras-chave: flavonoides, antioxidante, DFT (Density Functional Theory), antimicrobianos.

RESUMEN
El estudio realizó cálculos químico-cuánticos de cuatro flavonoides (Chalcona, Flavona, Isoflavona y Aurona) y un derivado sintético (Isoxazolone) para evaluar sus potencialidades antimicrobianas en comparación con el compuesto QNZ, conocido por su actividad contra *Staphylococcus aureus* resistente a antibióticos. Los cálculos se realizaron utilizando la Teoría del Funcional de Densidad (DFT) en las bases B3LYP/6-311G(d,p) y B3LYP/6-311++G(2d,2p). Los descriptores obtenidos incluyeron HOMO, LUMO, GAP y Potencial de Ionización (IP). Los resultados indicaron que Aurona presentó el menor GAP, lo que sugiere una alta reactividad, mientras que QNZ destacó por su capacidad de donación de electrones (HOMO) y aceptación de electrones (LUMO), mostrando resultados similares a los flavonoides. El Índice de Tanimoto reveló baja similitud estructural entre QNZ y los demás compuestos, excepto por Isoxazolone. Los hallazgos sugieren que los flavonoides e Isoxazolone pueden ser candidatos antimicrobianos

potenciales, justificando nuevos ensayos *in vitro* para evaluar la actividad antimicrobiana contra cepas resistentes.

Palabras clave: flavonoides, antioxidante, DFT (Density Functional Theory), antimicrobianos.

1 INTRODUCTION

Identifying and developing drugs is a complex task that demands high costs, time, laboratory tests, clinical trials and, above all, scientific and technological innovations. Advances in the fields of chemistry and biology make it possible to understand metabolic pathways and identify new drug candidates more efficiently, with computational studies being essential in optimizing the time to identify potential new drugs (Guido; Oliva, 2009).

Since the 1970s, the evolution of theoretical chemistry for studies of bioactive compounds has formed a new field of study called quantum pharmacology. This set of computational tools allows the use of theoretical calculations to establish drug design strategies through their quantum-chemical properties and physical-chemical aspects, in addition to the knowledge of biological systems and the functioning of enzymes and proteins that are involved in drug/target interaction processes, with this knowledge being developed through Molecular Modeling approaches (Cohen *et al*, 1990; Guido; Oliva; Andricopulo, 2008).

Molecular Modeling allows the generation, manipulation and representation of molecular structures, in addition to the quantitative acquisition of molecular data on the associated physicochemical properties. The objective of this research area is to analyze pharmacophoric groups, functional groups, relate structure and biological activity and even predict the biological activities of new drug candidates by comparing their chemical characteristics to commercial compounds with proven activity (Carvalho *et al.*, 2003).

In this sense, important chemical and pharmacological properties are calculated with the help of computational software, such as: heats of formation, electronic energies, HOMO energies (*Highest Occupied Molecular Orbital*), LUMO (*Lowest Unoccupied Molecular Orbital*), ionization energies, atomic electron densities, net atomic charges, frontier orbital electron densities (HOMO and LUMO), bond orders and dipole moments (Barreiro *et al.*, 1997; Rodrigues, 2001).

Regarding the interaction of drugs with their biological targets, an important aspect to be

highlighted is how chemical bonds are formed, which are directly linked to the electronic properties of chemical compounds and the capacities to donate/receive electrons involved in this process. A concept used in quantum chemical calculations is the Molecular Orbital (MO), which describes chemical behaviors such as charge transfer, photo-excitation, magnetism and molecular electronics, and it is quite significant to extract this information from molecular behavior based on properties of Molecular Orbitals (Zhang; Musgrave, 2007).

In the molecular orbital regions, there is the HOMO orbital region, which is related to the compounds' electron-donating capacity (nucleophilicity); the LUMO region is the electron-accepting region (electrophilicity). Higher HOMO energy is related to a high electron-donating capacity, and a lower LUMO energy is related to a lower resistance to accepting electrons. (Santos *et al.*, 2014). These values are excellent descriptors for investigations of the antioxidant reactivity of a drug candidate (Prasad *et al.*, 2014). Finally, there is the GAP, which consists of the difference in energies of the HOMO-LUMO orbitals. The GAP represents an indicator of the kinetic stability of molecules, in which a low GAP indicates reactivity, and a high value indicates stability of the molecule (Aihara, 1999; Santos *et al.*, 2014). These tools are crucial for the need to develop and plan new drugs, since problems such as bacterial resistance and new viral epidemics are a warning factor on a global scale.

A very interesting alternative in the search for new drugs to solve these problems on a global scale are Natural Products, compounds derived from plants, animals, bacteria, fungi and other living organisms, to which, since the beginning of time, humanity has resorted to natural products in order to treat infections, pain, gastrointestinal diseases, skin irritations and other pathologies (Viegas Jr; Bolzani; Barreiro, 2006). Among the numerous natural products that have already been identified, flavonoids stand out, phenolic compounds widely distributed in the Plantae kingdom (Panche; Diwan; Chandra, 2016). Biological activities attributed to flavonoids have been identified in plants, such as defense against insects, fungi, bacteria, viruses; attraction of pollinators; protection against oxidative stress; enzyme inhibitors. In addition to these, in the area of biological sciences, antiviral, antiallergenic, anti-inflammatory, hepatoprotective, cytoprotective and anticarcinogenic activities have been identified, with these biological activities being related to their antioxidant properties. (Seyoum; Asres; El-Fiky, 2006; Mendes *et al.*, 2012; Borges, 2015).

In the Brazilian context and in the immense diversity of the Brazilian Amazon, several

species will present Flavonoids in their composition, making these plants attractive for scientific investigations to identify potential drug candidates. (Santos, *et al.,* 2014). Thus, the objective of this study was to perform calculations of the quantum chemical descriptors of the Molecular Orbitals to predict the potential antioxidant and antimicrobial activity of four flavonoids and an isoxazolone (synthetic derivative of a flavonoid) in comparison to the compound (E)-3-(2-(4-cyanostyryl)-4-oxoquinazolin-3(4H)-yl)benzoic acid, which already has antimicrobial activity against antibiotic-resistant *Staphylococcus aureus* strains, according to studies by Bouley *et al.* (2015).

2 METHODOLOGY

2.1 SELECTION OF COMPOUNDS

The compounds selected for this study correspond to four flavonoids, namely: Chalcone, Flavone, Isoflavone, Aurora; and a synthetic isoxazolone derivative compound, extracted from the study of Santos *et al.* (2021). These compounds have biological activity, mainly anti-inflammatory, reported in scientific literature, and activity is attributed to the excellent antioxidant profile that these compounds have.

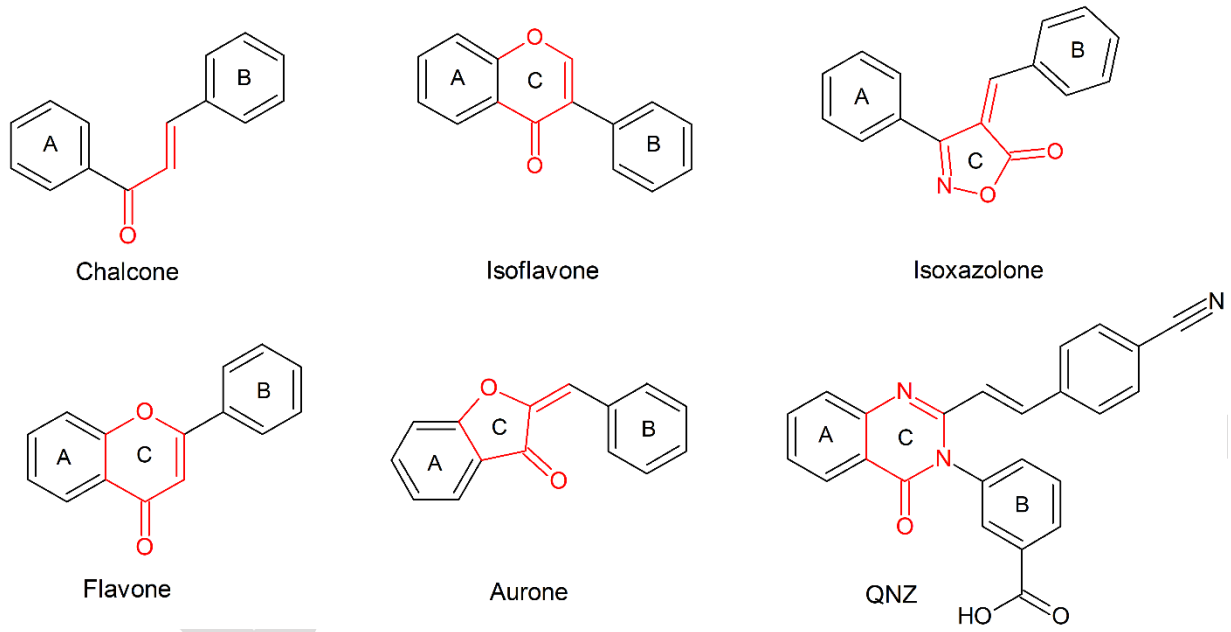
The compound (E)-3-(2-(4-cyanostyryl)-4-oxoquinazolin-3(4H)-yl)benzoic acid was extracted from the findings of Bouley *et al.* (2015). For reading optimization, the compound will be abbreviated to QNZ. The QNZ molecule has a biological activity of inhibiting protein *Penicilin Binding Protein 2a* (PBP2a) a protein that has low affinity for β-lactam antibiotics, conferring resistance to several genera of bacteria, among which the *S. aureus*.

As a selection criterion for the compounds investigated in this study, the structural similarity of their carbon ring systems was considered (Figure 1). This decision was grounded in the well-established principle that structurally related molecules often display analogous biological activities, a concept central to structure–activity relationship (SAR) studies. The notable resemblance between flavonoid compounds and QNZ, a known bioactive molecule, serves as the basis for the hypothesis that such structural congruence may translate into similar modes of interaction with biological targets.

This similarity suggests that flavonoids may exhibit inhibitory potential against the

PBP2a enzyme, a critical determinant of β-lactam resistance in *S. aureus*. Given their natural origin, chemical diversity, and capacity to interact with a wide range of biomolecular targets, flavonoids and their derivatives stand out as promising scaffolds for the development of novel antimicrobial agents. Therefore, the structural overlap with QNZ not only supports the rationale for their inclusion in this study but also highlights the potential of flavonoid-based compounds as a rich source of new therapeutic candidates to combat antibiotic resistance.

Figure 1. Compounds selected for this study



Source: Santos *et al.* (2021); Bouley *et al.* (2015).

2.2 PREPARATION AND QUANTUM CHEMICAL CALCULATIONS OF COMPOUNDS

All selected compounds were designed and pre-optimized in Molecular Mechanics with the aid of Chemsketch Software (ACD/Labs, 2022). Then, the compounds were optimized in three dimensions in the semi-empirical method based on PM3 (Stewart, 1989) with the aid of HyperChem software (Chemplus, 2000). Finally, with the Gaussian 09 package (Frisch, 2009), calculations were performed using the Density Functional Theory (DFT) method on the B3LYP/6-311G(d,p) and B3LYP/6-311++G(2d, 2p) bases, with the B3LYP method (Becke, Lee, Yang and Parr) being a hybrid method, where part of the functional is obtained by quantum mechanics and part is parameterized by correlation functionals (Foresman; Frisch, 1993). The DFT method was chosen because it is the most successful in calculating the electron density of

molecular orbitals, when considering the instantaneous interactions of electron pairs with opposite spins (Leach, 1996). The limiting factor that stands out for the DFT method is the high computational demand required.

The calculation environment was in the Linux Ubuntu operating system, with hardware consisting of a Ryzen 9 5950x Processor (16 cores and 32 threads) and 64Gb of DDR4 RAM at 4600Mhz.

2.3 DATA PROCESSING

After completing the quantum calculations stage, the extraction of the orbital topologies and the HOMO and LUMO values were performed with the aid of the GaussView software (Dennington, 2016). The Ionization Potential (IP) was obtained through the energy difference between the neutral compound and its respective cation (molecule without the presence of electrons), according to Equation 1:

$$IP = Eflav^{\bullet+} - Eflav \qquad (1)$$

where,

IP = Ionization Potential
Eflav•+ = Formation Energy of the molecule in the neutral state
Eflav = Formation Energy of the molecule in the cation state

Finally, the GAP was obtained through the energy difference (measured in electron-volts - eV), using Equation 2:

$$GAP = |ELumo - EHomo| \qquad (2)$$

where,

GAP = Absolute difference between the Lumo Orbital Energy and Homo Orbital Energy
ELumo = Lumo Orbital Energy
EHomo = Homo Orbital Energy

2.4 CALCULATION OF TANIMOTO INDEX AND HIERARCHICAL GROUPING

The Tanimoto index is based on the XYZ coordinates of the atoms, resulting in a similarity percentage from 0 to 1 (0% to 100%) between the analyzed compounds (Bajusz; Rácz; Héberger, 2015). The Tanimoto index of the selected compounds was obtained using the ChemMine Tools web server with the "Similarity Workbench" tool (https://chemminetools.ucr.edu/similarity/). Hierarchical Clustering consists of generating a data set that estimates the hierarchical similarity between the selected compounds, demonstrating which molecules are similar to each other by generating hierarchical groups. Hierarchical Clustering was generated with ChemMine Tools through the "Cluster" tool (https://chemminetools.ucr.edu/tools/launch_job/Clustering/) (Backman; Cao; Girke, 2011).

2.5 PREDICTION OF ANTIBACTERIAL ACTIVITY *IN SILICO*

For the prediction of antibacterial activity *in silico*, the AntiBac Pred tool from the PASS Online WebServer (http://www.way2drug.com/antibac/) will be used. This tool indicates the degree of confidence of the inhibition of a compound against different strains of bacteria (Filimonov *et al.*, 2014; Pogodin *et al.*, 2018). This analysis will be crucial to provide an analysis for continuation of *in vitro* and *in vivo* laboratory tests.

3 RESULTS AND DISCUSSIONS

Table 1 presents the data of the quantum chemical descriptors obtained for the 6 compounds in the bases B3LYP/6-311G(d,p) and B3LYP6/6-311++G(2d,2p).

Table 1. Quantum chemical descriptors of selected compounds

COMPOUNDS	**B3LYP/6-311G(d,p)**				**B3LYP/6-311++G(2d,2p)**			
	HOMO (eV)	LUMO (eV)	GAP (eV)	IP (kcal/mol)	HOMO (eV)	LUMO (eV)	GAP (eV)	IP (kcal/mol)
Chalcone	-6.56	-2.36	4.20	184.09	-6.66	-2.51	4.15	185,08
Flavone	-6.60	-2.11	4.49	185.88	-6.71	-2.26	4.45	187,39
Isoflavone	-6.37	-1.80	4.57	178.92	-6.48	-1.97	4.51	180,26
Aurore	-6.15	-2.62	3.53	174.29	-6.25	-2.75	3.50	175,57
Isoxazolone	-6.72	-2.98	3.74	182.50	-6.84	-3.08	3.76	184,02
QNZ	-6.52	-2.91	3.61	175.97	-6.51	-2.89	3.61	175.51

Source: Prepared by the authors.

When comparing the results obtained in the two selected bases, a variation in the results of the descriptors is noted. Table 2 illustrates the percentages of differences obtained between the chemical-quantum values of both bases.

Table 2. Difference in descriptor values of bases B3LYP/6-311G(d,p) and B3LYP6/3-311++G(2d,2p)

COMPOUNDS	HOMO Diff (%)	LUMO Diff (%)	GAP Diff (%)	IP Diff (%)
Chalcone	1.50	5.98	1.20	0.53
Flavone	1.64	6.64	0.90	0.81
Isoflavone	1.70	8.63	1.33	0.74
Aurore	1.60	4.73	0.86	0.73
Isoxazolone	1.75	3.25	0.53	0.83
QNZ	0.15	0.69	0.00	0.26

Source: Prepared by the authors.

These variations are expected between the bases of the DFT method since the bases have different calculation levels, being able to provide adjustments of the computational time and information of the data obtained relative to experimental data when there is crystallographic data available of chemical compounds, thus, making it possible to select the best calculation base that approaches the experimental values, being able to base the choice on the base that required less computational power for completion (Ozela, 2017).

The B3LYP/6-311++(2d,2p) base provides orbital polarization parameters, but with diffusion (represented by "++") and doubled polarization (2d,2p). This level provides an excellent description of electronic interactions and is especially useful for charged species or electronic transition states that occur in the neutralization of radicals. Therefore, in a virtual screening study, where potential new drug candidates are sought using a molecule as an initial template, the B3LYP/6-311G(d,p) base is excellent for an initial screening of many compounds, as it has lower computational power required, and the B3LYP6/3-311++G(2d,2p) base is intended for a greater refinement of the best compounds obtained, mainly due to the high degree of assertiveness of B3LYP6/3-311++G(2d,2p) when compared to results from experimental data for antioxidant activity (Dehkordi *et al.,* 2022; Ma; Wang, 2023).

Considering this difference between the data obtained in both bases B3LYP/6-311G(d,p) and B3LYP6/6-311++G(2d,2p), it is noted that the classification regarding the values obtained between the compounds was maintained. Aurone was the one that obtained the highest HOMO value (-6.15/-6.25 eV) followed by Isoflavone (-6.37/-6.48 eV), demonstrating that these compounds are the best in terms of nucleophilicity. The compound QNZ, which has proven

activity against resistant strains of *S. aureus*, presented the third best HOMO value (-6.52/-6.51 eV), thus demonstrating a good capacity to donate electrons when analyzed together with the other compounds.

The value obtained by QNZ when compared to the compounds Chalcone, Flavone and Isoxazolone, compounds that have findings in the scientific literature that their biological activity is justified by their excellent antioxidant activity, can be prospected for a possible antimicrobial activity against resistant organisms based on the proximity of the results of the antioxidant capacity obtained by the flavonoid compounds and the derivative Isoxazolone when compared to the compound with antimicrobial activity QNZ (Mendes *et al.*, 2012; Bouley *et al.*, 2015; Xue *et al.*, 2018). Our findings can guide future in vitro tests with these Natural Products to test their bacteriostatic or bactericidal capacity against resistant bacterial strains, not limited to *S. aureus*.

Regarding LUMO, the compounds with the best electrophilic capacity were Isoxazolone and QNZ, with values of -2.98/-3.08 eV and -2.91/-2.89 eV, respectively. These values demonstrate a lower capacity to accept electrons when compared to the compounds Isoflavone (-1.80/-1.97 eV), Flavone (-2.11/-2.26 eV), Chalcone (-2.36/-2.51 eV), and Aurone (-2.62/-2.75 eV). Therefore, the best electron donor in our findings is Aurone (HOMO energy -6.15/-6.25 eV) and the best electron acceptor is Isoflavone (LUMO energy -1.80/-1.97 eV). This occurs because LUMO is the lowest energy empty molecular orbital. A LUMO with less negative energy is more energetically accessible to receive external electrons, while HOMO with less negative energy means that electrons are less strongly bound to the molecule, making it easier to remove them and thus the molecule behaves as a good electron donor (Hashemi *et al.*, 2019).

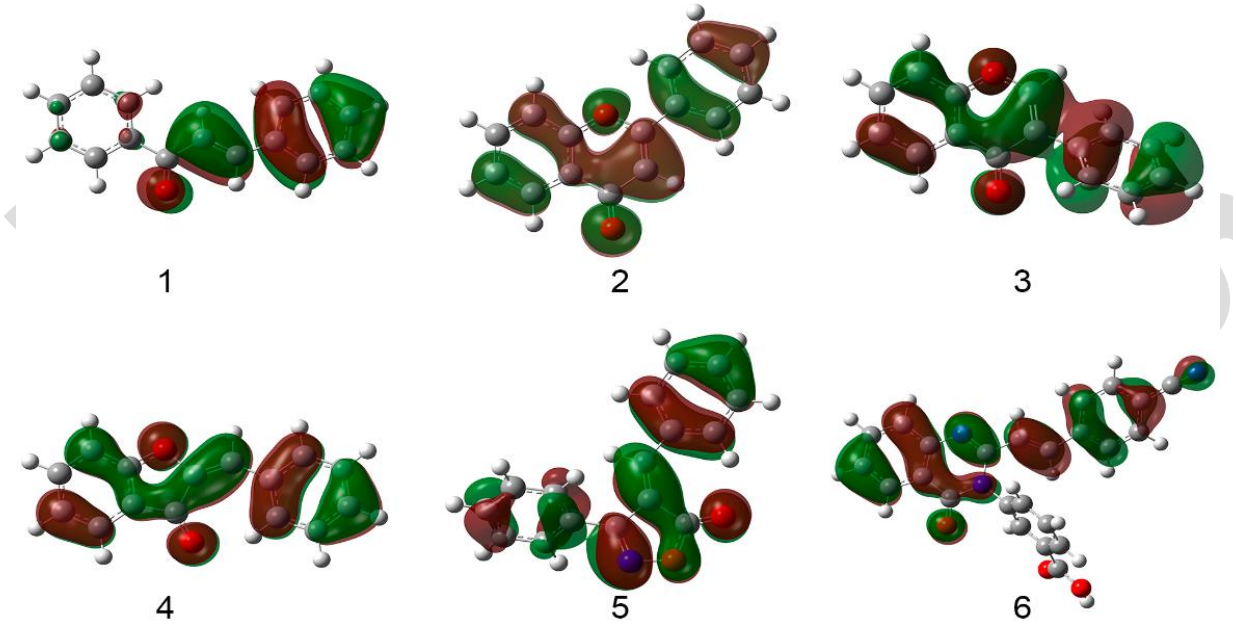
For low GAP values, it means the reactivity capacity of the compounds, while a high value indicates the stability of the molecule. Therefore, the most reactive molecule was Aurone (3.53/3.50 eV), followed by QNZ (3.61/3.61 eV); while the most stable were Isoflavone (4.57/4.51 eV) and Flavone (4.49/4.45 eV). This prediction is fundamental for the development of new computational methodologies for the study of the interaction of these compounds with bacterial targets (Aihara, 1999; Santos *et al.*, 2014).

For the Ionization Potential (IP), a relationship with the HOMO energy values is observed, where the higher the HOMO, the lower the IP value, thus confirming that the energy to remove electrons from the molecule will be lower. It is noteworthy that Aurone has the lowest IP energy, with 174.29/157.57 kcal/mol, followed by QNZ with 175.97/175.51 kcal/mol,

corroborating the HOMO energy values and demonstrating a good potential for electron donation of these compounds.

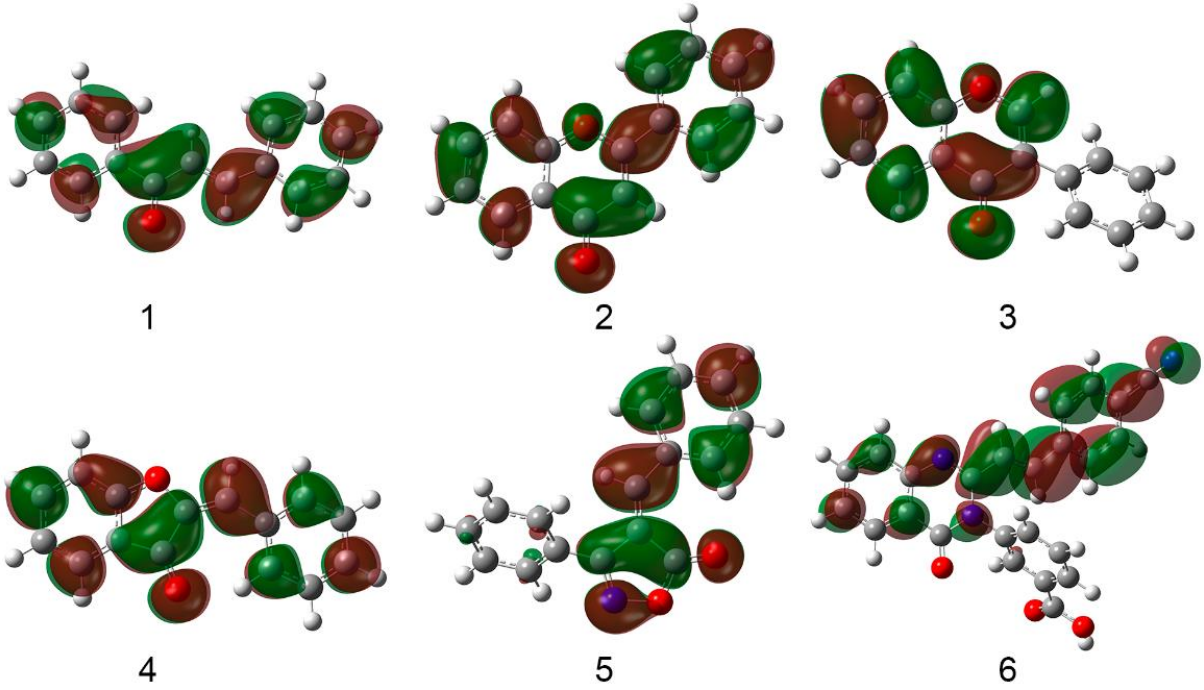
Figures 2 and 3 illustrate the topology of the HOMO and LUMO orbitals, respectively, thus providing a visualization of which regions of the molecules will be involved in the donation and acceptance of electrons. It is noteworthy that the values obtained in this computational study are related to the antioxidant capacity of the compounds. An analysis that involves the study of the chemical mechanisms of the functional groups will be essential for future analyses of the interaction of the target ligands, even enabling the design of study strategies with analogous compounds to identify anti-inflammatory, antimicrobial, antifungal, anticancer and antiparasitic biological activities.

Figure 2. HOMO of the selected compounds



1 - Chalcone; 2 - Flavone; 3 - Isoflavone; 4 - Aurone; 5 - Isoxazolone; 6 – QNZ
Source: Prepared by the authors.

Figure 3. LUMO of the selected compounds



1 - Chalcone; 2 - Flavone; 3 - Isoflavone; 4 - Aurone; 5 - Isoxazolone; 6 – QNZ
Source: Prepared by the authors.

Table 3 shows the Tanimoto Index values among the studied compounds. The Tanimoto Index analysis is widely used to assess the structural similarity between compounds, being an essential tool in the context of medicinal chemistry and in the development and identification of drug candidates. A widely used premise in Medicinal Chemistry is that "similar compounds have similar biological activity" (Borges *et al.*, 2019).

Table 3. Tanimoto index of Selected Compounds

COMPOUNDS	Aurone	Chalcone	Flavone	Isoflavone	Isoxazolone	QNZ
Aurone	1	0.6	0.76	0.74	0.65	0.26
Chalcone	0.6	1	0.59	0.54	0.54	0.25
Flavone	0.76	0.59	1	0.83	0.61	0.26
Isoflavone	0.74	0.54	0.83	1	0.57	0.26
Isoxazolone	0.65	0.54	0.61	0.57	1	0.31
QNZ	0.26	0.25	0.26	0.26	0.31	1

Source: Prepared by the authors.

The compound QNZ, whose biological activity against resistant strains of *S. aureus* was confirmed (Bouley *et al.*, 2015), had its Tanimoto index calculated and compared with the set of flavonoid compounds and the isoxazolone derivative. The results show that QNZ presents low similarity indices with all the compounds analyzed, with values ranging from 0.25 to 0.31. The

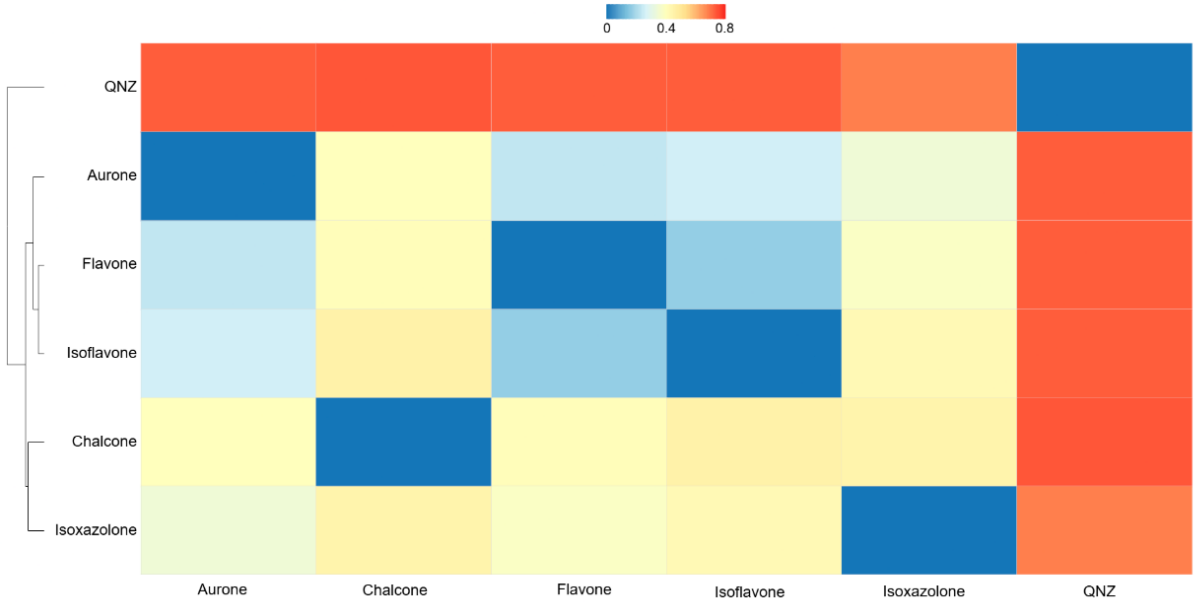
highest index was observed between QNZ and Isoxazolone (0.31), suggesting the presence of some common substructures, although the overall similarity is still considered low. This result indicates that, structurally, QNZ shares few molecular characteristics with the other compounds evaluated. In contrast, the similarity of QNZ with flavonoids (Aurone, Chalcone, Flavone, Isoflavone) remained below 0.26, evidencing significant structural differences.

The low structural similarity observed may suggest that the antimicrobial activity of QNZ against resistant *S. aureus* is not directly associated with structural features shared with flavonoids or the isoxazolone derivative. It is plausible that the biological activity is more related to specific interactions with molecular targets, involving physicochemical properties that were not captured by the binary descriptors used in the Tanimoto calculation.

However, regarding the obtained quantum chemical descriptors, it is noted that the antioxidant activity of QNZ falls within values close to those obtained by the flavonoid compounds and isoxazolone, precisely because the antioxidant potential of these compounds is the attribute of their biological activity. Analyzing Figure 4, the heatmap of the hierarchical analysis lists which compounds are structurally related based on the Tanimoto Index analysis, with QNZ being an outgroup molecule to all flavonoids and isoxazolone.

From the point of view of the development of new derivatives, the low similarity with flavonoids indicates that the modification of the QNZ structure to incorporate flavonoid characteristics may be a strategic approach to be explored in the future. Furthermore, investigations into the potential antimicrobial activity of flavonoid compounds are future objectives of this study, justified mainly by the problem of antibiotic resistance that is growing worldwide and the need to identify new drug candidates.

Figure 4. Heat map of the tanimoto index of the studied compounds



Source: Prepared by the authors.

Conversely, the modest structural similarity observed between Isoxazolone and QNZ offers a strategic starting point for future investigations aimed at rationally optimizing the QNZ framework. Targeted structural modifications could enhance biological efficacy, improve selectivity, and mitigate toxicity, thereby advancing the compound's therapeutic potential. This approach highlights the value of structure-based drug design and refinement of lead compounds for antimicrobial applications.

Furthermore, this similarity prompts the exploration of natural products beyond flavonoids that share key structural motifs with QNZ, broadening the scope for discovering novel bioactive molecules with potent antimicrobial properties. Expanding this chemical landscape may facilitate the identification of diverse candidates capable of overcoming bacterial resistance mechanisms.

Supporting this perspective, the AntiBac-Pred computational model predicted that all five compounds evaluated—Chalcone, Flavone, Isoflavone, Aurone, and Isoxazolone—demonstrate significant inhibitory potential against both Gram-positive and Gram-negative bacteria. Their predicted activities are comparable to QNZ, which showed a confidence score of 0.5909 against *Staphylococcus haemolyticus* (Table 4). These in silico results substantiate the prospect that both natural products and the synthetic Isoxazolone may serve as effective inhibitors of resistant bacterial strains.

Collectively, these findings justify the advancement of these compounds into comprehensive in vitro and in vivo studies to validate their antimicrobial efficacy, evaluate safety, and elucidate mechanisms of action. Integrating computational predictions with experimental validation is essential to expedite the development of novel therapeutics addressing the critical challenge of antibiotic resistance.

Chalcones have been shown to be potent against *S. aureus* and *Mycobacterium* spp., with mechanisms involving DNA gyrase inhibition and cell membrane disruption (Oliveira, *et al.*, 2024). Intermediate predictions of ~0.19–0.24 confidence for *Mycobacterium marinum* and *S. aureus* are in agreement with studies indicating Chalcones activity against methecillin-resistant *Staphylococcus aureus* (MRSA) strains and have the ability to be an adjuvant and increase the efficacy of conventional antibiotics (Bozic; Milenkovic; Ivkovic; Cirkovic, 2014).

Flavones exhibit broad action against *S. aureus*, *Bacillus anthracis* and *Helicobacter pylori*, acting via inhibition of nucleic acid synthesis and increased membrane permeability (SHAMSUDIN *et al.*, 2022). The confidence for *Yersinia pestis* was 0.5183, indicating good potential; experimental studies have already demonstrated MICs in the range of 10–50 µg/mL against *S. aureus* (Yuan *et al.*, 2021). The structural versatility of the flavonoid allows optimizations that maximize lipophilicity and interaction with bacterial targets (Shamsudin *et al.*, 2022).

Isoflavones have shown preferential activity against Gram-positive strains, interfering with the incorporation of nutrients by the bacterial membrane (Mukne; Viswanathan; Phardatare, 2011). In addition, the ability of isoflavones to block the invasion of bacteria into mammalian epithelial cells has been reported in scientific literature for quite some time (Hong; Landauer; Foriska; Ledney, 2006).

Aurones have selective action against Gram-positive bacteria, with a favorable therapeutic index and a mechanism linked to membrane permeabilization (Olleik *et al.*, 2019). The confidence of 0.4638 for *Kocuria rhizophila* and 0.3685 for *Y. pestis* suggests good activity, comparable to studies that reported MICs of 4–32 µg/mL against several strains of *S. aureus*, in addition to proposing changes in the molecular structure that aim to optimize the antibacterial activity of the compounds (Olleik *et al.*, 2019; Szepe *et al.*, 2023).

Table 4. Antibacterial Activity via Antibac-Pred

Structure	Bacterial Strain	Confidence	ChEMBL ID
Chalcone	*Mycobacterium marinum*	0.2393	CHEMBL614987
	Staphylococcus aureus subsp. *aureus* RN4220	0.1907	CHEMBL2366906
	Mycobacterium	0.1703	CHEMBL614981
	Mycobacterium aurum	0.1607	CHEMBL612952
Flavone	*Yersinia pestis*	0.5183	CHEMBL614597
	RESISTANT *Staphylococcus aureus* subsp. *aureus* MW2	0.2508	CHEMBL612531
	Mycobacterium	0.1962	CHEMBL614981
	Mycobacterium aurum	0.1864	CHEMBL612952
	Bacillus anthracis	0.1376	CHEMBL613904
	Helicobacter pylori SS1	0.1278	CHEMBL613197
Isoflavone	*Yersinia pestis*	0.4278	CHEMBL614597
	Mycobacterium	0.1766	CHEMBL614981
	Mycobacterium aurum	0.1577	CHEMBL612952
	Mycobacterium smegmatis	0.1390	CHEMBL613088
	Helicobacter pylori SS1	0.1367	CHEMBL613197
	RESISTANT *Staphylococcus aureus* subsp. *aureus* MW2	0.1138	CHEMBL612531
	Mycobacterium avium	0.1009	CHEMBL614982
Aurone	*Kocuria rhizophila*	0.4638	CHEMBL1075349
	Yersinia pestis	0.3685	CHEMBL614597
	Mycobacterium	0.1639	CHEMBL614981
	Mycobacterium aurum	0.1365	CHEMBL612952
	RESISTANT *Staphylococcus aureus*	0.1095	CHEMBL352
	Helicobacter pylori SS1	0.0767	CHEMBL613197
Isoxazolone	RESISTANT *Staphylococcus simulans*	0.8475	CHEMBL612425
	RESISTANT *Staphylococcus aureus* subsp. *aureus* RN4220	0.6776	CHEMBL2366906
	RESISTANT *Streptococcus agalactiae*	0.3809	CHEMBL614622
	Yersinia pestis	0.3164	CHEMBL614597
	Mycobacterium tuberculosis	0.2577	CHEMBL360
	RESISTANT *Staphylococcus hominis*	0.2562	CHEMBL614423
	Streptococcus pneumoniae R6	0.2314	CHEMBL2366794
	RESISTANT *Mycobacterium tuberculosis*	0.2222	CHEMBL360
	Mycobacterium	0.1507	CHEMBL614981
	Staphylococcus aureus subsp. *aureus* RN4220	0.1380	CHEMBL2366906
	RESISTANT *Staphylococcus haemolyticus*	0.1371	CHEMBL612507
QNZ	*Staphylococcus haemolyticus*	0.5909	CHEMBL612507
	Staphylococcus aureus	0.0422	CHEMBL352
	Salmonella enterica subsp. *enterica*	0.0308	CHEMBL613044

Source: Prepared by the authors.

Isoxazolones are compounds that have already demonstrated potential activity against *Staphylococcu simulans* (confidence 0.8475) and resistant *S. aureus* (0.6776), possibly by inhibiting essential cell wall synthesis enzymes (Alshamari *et al.*, 2020).

The QNZ compound, whose antibacterial activity was validated in vitro (Bouley *et al.*, 2015), showed a confidence of 0.5909 against *S. haemolyticus* and 0.0422 against *S. aureus*. Although some compounds demonstrated higher confidence values, the prediction via AntiBac-Pred demonstrates confidence levels that justify experimental investigations, especially

considering the multitarget profile of Quinazolinone compounds (QNZ class) proven by laboratory tests (Masri *et al.*, 2019).

Finally, molecular modeling and in silico studies are essential for identifying potential new drugs, as well as analyzing large libraries of compounds made available online and identifying those that may be targeted for specific biological activities. Computational theoretical studies are indispensable in preclinical stages, demonstrating great advantages in terms of compound screening, cost reduction by targeting the purchase of those compounds with the best in silico results and suggesting improvements and structural modifications in natural products to optimize pharmacological activities (De Quadros *et al.*, 2023; Duarte *et al.*, 2024).

5 CONCLUSION

Through the quantum chemical descriptors of 4 flavonoids and an isoxazolone, it was possible to perform a computational theoretical analysis with the molecule (E)-3-(2-(4-cyanostyryl)-4-oxoquinazolin-3(4H)-yl)benzoic acid (QNZ) and predict potential antimicrobial activity of the Flavonoid Natural Products with the compound QNZ, addressing the similarity of the antioxidant capacity of the compounds.

The compound QNZ demonstrated good results of HOMO, LUMO, GAP and PI, presenting values close to and even better than the Flavonoid compounds that have biological activity reported in the scientific literature due to their antioxidant potential. The results obtained in this study were satisfactory and the predictions of AntiBac-Pred support the potential of Chalcones, Flavones, Isoflavones, Aurones and Isoxazolones as new antibacterial entities, with profiles complementary to QNZ. It is recommended to advance in vitro MIC assays and toxicity studies to validate these computational hypotheses.

New computational studies will be essential for the identification and selection of Natural Products with chemical properties similar to the QNZ compound and Natural Products. Steps such as Pharmacokinetic Descriptors, Molecular Docking and Molecular Dynamics will provide results for selecting compounds with low toxicity and good intermolecular interactions with biological targets, such as proteins and enzymes. It is noteworthy that structural modifications and synthesis of synthetic derivatives using Flavonoids as a model compound can guide

possibilities for the development of bioactive compounds for various therapies, as well as patent registrations.

REFERENCES

AIHARA, J. Weighted HOMO-LUMO energy separation as an index of kinetic stability for fullerenes. **Theoretical Chemistry Accounts**, 102, 1-6, 134-138, 1999. Disponível em <https://doi.org/10.1007/s002140050483>. Acesso em: 20 de Fevereiro de 2025.

ALSHAMARI, A.; AL-QUDAH, M.; HAMADEH, F.; AL-MOMANI, L.; ABU-ORABI, S. Synthesis, Antimicrobial and Antioxidant Activities of 2-Isoxazoline Derivatives. **Molecules**, v. 25, p. 4271, 2020. DOI: 10.3390/molecules25184271.

BACKMAN, T. W.; CAO, Y.; GIRKE, T. ChemMine tools: an online service for analyzing and clustering small molecules. **Nucleic Acids Research**, v. 39, Web Server issue, p. W486-W491, jul. 2011.

BAJUSZ, D.; RÁCZ, A.; HÉBERGER, K. Why is Tanimoto index an appropriate choice for fingerprint-based similarity calculations?. **J Cheminform**, 2015. Disponível em: <https://doi.org/10.1186/s13321-015-0069-3>. Acesso em: 20 de Fevereiro de 2025

BARREIRO, E.; RODRIGUES, C. R.; ALBUQUERQUE, M. G.; Sant'Anna, C. M. R.; ALENCASTRO, R. B. Modelagem Molecular: Uma Ferramenta para o Planejamento Racional de Fármacos em Química Medicinal. **Química Nova**, v.20, n.3, p. 1-11, 1997. Disponível em: <https://doi.org/10.1590/S0100-40421997000300011>. Acesso em 5 de Março de 2025

BORGES, R. S.; OLIVEIRA, J. P.; MATOS, R. F.; CHAVES NETO, A. M. J.; CARNEIRO, A. S.; MONTEIRO, M. C. Involvement of electron and hydrogen transfers through redox metabolism on activity and toxicity of the nimesulide. **Journal of Molecular Modeling**, 21:166, 2015. DOI: 10.1007/s00894-015-2712-3

BORGES, R. S.; PALHETA, I. C.; OTA, S. S. B.; MORAIS, R. B.; BARROS, V. A.; RAMOS, R. S.; SILVA, R. C.; COSTA, J. D. S.; SILVA, C. H. T. P.; CAMPOS, J. M.; *et al*. Toward safer phenylbutazone derivatives by exploration of toxicity mechanism. **Molecules**, v. 24, p. 143, 2019. DOI:10.3390/molecules24010143.

BOULEY, R.; KUMARASIRI, M.; PENG, Z.; OTERO, L.H.; SONG, W.; SUCKOW, M.A.; SCHROEDER, V.A.; WOLTER, W.R.; LASTOCHKIN, E.; ANTUNES, N.T.; *et al*. Discovery of antibiotic (E)-3-(3-Carboxyphenyl)-2-(4-cyanostyryl)quinazolin-4(3H)-one. **J. Am. Chem. Soc**. 2015, 137, 1738–1741.

BOŽIĆ, D. D.; MILENKOVIĆ, M.; IVKOVIĆ, B.; ĆIRKOVIĆ, I. Antibacterial activity of three newly-synthesized chalcones & synergism with antibiotics against clinical isolates of methicillin-resistant *Staphylococcus aureus*. **Indian Journal of Medical Research**, v. 140, n. 1, p. 130-137, jul. 2014.

CARVALHO, I.; PUPO, M.T.; BORGES A. D. L.; BERNARDES, L. S. C. Introdução a modelagem molecular de fármacos no curso experimental de química farmacêutica. **Química Nova** [online], 26, 3, 428-438, 2003. Disponível em: <http://dx.doi.org/10.1590/S0100-40422003000300023>. Acesso em 15 de Março de 2025.

CHEMPLUS. **Modular Extensions to HyperChem**, Release 8.0, Molecular Modeling for Windows; Hyper, Inc.: Gainesville, FL, USA, 2000.

ChemSketch, version 2022.1.2, **Advanced Chemistry Development, Inc.** (ACD/Labs), Toronto, ON, Canada, Disponível em: <www.acdlabs.com>. Acesso em 15 de Fevereiro de 2025.

COHEN, N. C.; J. M. BLANEY; C. HUMBLET; P. GUND; D. C. BANY. Molecular Modeling Software and Methods for Medicinal Chemistry. **Journal of Medicinal Chemistry**, v. 33, p. 883-894, 1990. DOI: 10.1021/jm00165a001

DE QUADROS, L. H. B.; LOBO , L. G. G.; BARBOSA, E. M.; DOS SANTOS, H. L.; DOS SANTOS, D. L.; DE SOUZA, S. G. H.; DOS SANTOS, T. B. In silico analysis of superoxide dismutase family genes in Ipomoea trifida L. **REVISTA DELOS**, *[S. l.]*, v. 16, n. 48, p. 3223–3239, 2023. DOI: 10.55905/rdelosv16.n48-017.

DEHKORDI, M. M.; ASGARSHAMSI, M. H.; FASSIHI, A.; ZBOROWSKI, K. K. A comparative DFT study on the antioxidant activity of some novel 3-hydroxypyridine-4-one derivatives. **Chemistry & Biodiversity**, v. 19, n. 3, e202100703, 2022. DOI: 10.1002/cbdv.202100703.

DENNINGTON, R.; KEITH, T. A.; MILLAM, J. M. **GaussView, Version 6**. Shawnee Mission: Semichem Inc., 2016.

DUARTE, G. D.; FERREIRA, M. D. L.; OLIVEIRA, B. H. M. de; SILVA, D. L. S.; FAHEINA-MARTINS, G. V.; BARBOSA FILHO, J. M.; RODRIGUES, L. C. Síntese de chalcona quinolínica e investigação in silico de suas propriedades farmacológicas. **REVISTA DELOS**, *[S. l.]*, v. 17, n. 62, p. e3291, 2024. DOI: 10.55905/rdelosv17.n62-152.

FILIMONOV, D. A. *et al*. Prediction of the biological activity spectra of organic compounds using the PASS online web resource. **Chemistry of Heterocyclic Compounds**, v. 50, n. 3, p. 444-457, 2014.

FORESMAN, J. B.; FRISCH, A. E. **Exploring Chemistry with Electronic Structure Methods: A Guide to Using Gaussian.** Gaussian, Inc, USA, 1993.

FRISCH, M. J. *et al*. *Gaussian 09, Revision D.01*. Wallingford: Gaussian Inc., 2009.

GUIDO, R. V. C.; OLIVA, G. Structure-based drug design for tropical diseases. **Current Topics in Medicinal Chemistry**, 9, 9, p.824-43, 2009. Disponível em: <https://doi.org/10.2174/156802609789207064>. Acesso em: 03 de Março de 2025.

GUIDO, R. V. C.; OLIVA, G.; ANDRICOPULO, A. D. Virtual screening and its integration with modern drug design technologies. **Current Medicinal Chemistry**, v.15, n.1, p.37-46, 2008. Disponível em: <https://doi.org/10.2174/092986708783330683>. Acesso em: 03 de Março de 2025.

HASHEMI, D; MA, X; ANSARI, R; KIM, J; KIEFFER, J. Design principles for the energy level tuning in donor/acceptor conjugated polymers. *Physical Chemistry Chemical Physics*, v. 21, n. 2, p. 789-799, 2019. The Royal Society of Chemistry. Dispónivel em: <http://dx.doi.org/10.1039/C8CP03341B>. Acesso em: 10 de Março de 2025

HONG, H.; LANDAUER, M. R.; FORISKA, M. A.; LEDNEY, G. D. Antibacterial activity of the soy isoflavone genistein. **Journal of Basic Microbiology**, v. 46, n. 4, p. 329-335, 2006. DOI: 10.1002/jobm.200510073.

LEACH, A. R., Molecular Modelling Principles and Applications. **Longman**, Essex, 1996.

MA, P.; WANG, Z. Density functional theory study on antioxidant activity of three polyphenols. **Journal of Fluorescence**, v. 33, n. 3, p. 933-944, 2023. DOI: 10.1007/s10895-022-03104-z.

MASRI, A.; ANWAR, A.; KHAN, N. A.; SHAHBAZ, M. S.; KHAN, K. M.; SHAHABUDDIN, S.; SIDDIQUI, R. Antibacterial Effects of Quinazolin-4(3H)-One Functionalized-Conjugated Silver Nanoparticles. **Antibiotic***s*, v. 8, p. 179, 2019. DOI: 10.3390/antibiotics8040179.

MENDES, A. P. S.; BORGES, R. S.; CHAVES NETO, A. M. J.; MACEDO, L. G. M.; SILVA, A. B. F. The basic antioxidant structure for flavonoid derivatives. **J. Mol. Model**., 18, 4073– 4080, 2012. Disponível em: <https://doi.org/10.1007/s00894-012-1397-0> Acesso em: 20 de Março de 2025

MENDES, A. P. S.; BORGES, R. S.; CHAVES NETO, A. M. J.; MACEDO, L. G. M.; SILVA, A. B. F. da. The basic antioxidant structure for flavonoid derivatives. **Journal of Molecular Modeling**, v. 18, p. 4073-4080, 2012. DOI: 10.1007/s00894-012-1397-0.

MUKNE, A. P.; VISWANATHAN, V.; PHADATARE, A. G. Structure pre-requisites for isoflavones as effective antibacterial agents. **Pharmacognosy Reviews**, v. 5, n. 9, p. 13-18, jan. 2011. DOI: 10.4103/0973-7847.79095.

OLIVEIRA, A. S. de; CENCI, A. R.; GONÇALVES, L.; THEDY, M. E. C.; JUSTINO, A.; BRAGA, A. L.; MEIER, L. Chalcone derivatives as antibacterial agents: an updated overview. **Current Medicinal Chemistry**, v. 31, n. 17, p. 2314-2329, 2024. DOI: 10.2174/0929867330666230220140819.

OLLEIK, H.; YAHIAOUI, S.; ROULIER, B.; COURVOISIER-DEZORD, E.; PERRIER, J.; PÉRÈS, B.; HIJAZI, A.; BAYDOUN, E.; RAYMOND, J.; BOUMENDJEL, A.; MARESCA, M.; HAUDECOEUR, R. Aurone derivatives as promising antibacterial agents against resistant Gram-positive pathogens. **European Journal of Medicinal Chemistry**, v. 165, p. 133-141, 1 mar. 2019. DOI: 10.1016/j.ejmech.2019.01.022.

OZELA, P. F. **Validação De Método Computacional Aplicado À Galantamina No Planejamento De Candidatos A Fármacos Para Tratamento Da Doença De Alzheimer**. Dissertação de Mestrado. Universidade Federal do Amapá, Brasil, 2017.

PANCHE, A. N.; DIWAN, A. D.; CHANDRA, S. R. Flavonoids: an overview. **Journal of Nutritional Science**, 5(47):1-15, 2016. Disponível em: <https://doi.org/10.1017/jns.2016.41>. Acesso em: 02 de Abril de 2025.

POGODIN, P. *et al*. How to achieve better results using PASS-based virtual screening: case study for kinase inhibitors. **Frontiers in Chemistry**, v. 6, p. 133, 2018.

PRASAD, A. A.; MUTHU, K.; MEENATCHI, V.; RAJASEKAR, M.; AGILANDESHWARI, R.; MEENA, K.; VIJILA MANONMONI, J.; MEENAKSHISUNDARAM, S. P. Optical, vibrational, NBO, first-order molecular hyperpolarizabilityand Hirshfeld surface analysis of a nonlinear optical chalcona. **Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy**, 140, 311-327, 2014. Disponível em: <http://dx.doi.org/10.1016/j.saa.2014.12.011>. Acesso em: 02 de Abril de 2025.

RODRIGUES, C. R. Processos modernos no desenvolvimento de fármacos: modelagem molecular. **Cadernos Temáticos de Química Nova na Escola**, n. 3, 2001.

SANTOS, C. B. R.; LOBATO, C. C.; SOUSA, M. A. C.; MACÊDO, W. J. C.; CARVALHO, J. C. T. Molecular Modeling: Origin, Fundamental Concepts and Applications Using StructureActivity Relationship and Quantitative Structure-Activity Relationship. **Reviews in Theoretical Science**, 2 (2), 1-25, 2014. Disponível em: https://doi.org/10.1166/rits.2014.1016. Acesso em: 04 de Abril de 2025

SANTOS, K. L. B.; QUEIROZ, A. N.; LOBATO, C. C.; *et al.* A comparative theoretical mechanism on simplified flavonoid derivatives and isoxazolone analogous as Michael system inhibitor. **Journal of Molecular Modeling**, v. 27, p. 26, 2021. DOI: 10.1007/s00894-020-04647-3.

SANTOS, P. M. DOS; *et al.* Perfil de flavonoides e avaliação do potencial antioxidante e citotóxico de *Bauhinia purpurea* (Fabaceae) da região amazônica. **Química Nova**, v. 37, n. 1, p. 89–94, 2014.

SEYOUM, A; ASRES, K.; EL-FIKY, F. K. Structure-radical scavenging activity relationships of flavonoids. **Phytochem,** 67, 2058–2070, 2006. 10.1016/j.phytochem.2006.07.002

SHAMSUDIN, N. F.; AHMED, Q. U.; MAHMOOD, S.; SHAH, S. A. A.; KHATIB, A.; MUKHTAR, S.; ALSHARIF, M. A.; PARVEEN, H.; ZAKARIA, Z. A. Antibacterial effects of flavonoids and their structure-activity relationship study: A comparative interpretation. **Molecules**, v. 27, p. 1149, 2022. DOI: 10.3390/molecules27041149.

STEWART, J. J. P. Optimization of parameters for semiempirical methods I. Method. **Journal of Computational Chemistry**, 10:209, 1989. DOI: 10.1002/jcc.540100208

VIEGAS JR, C.; BOLZANI, V. DA S.; BARREIRO, E. J.. Os produtos naturais e a química medicinal moderna. **Química Nova**, v. 29, n. 2, p. 326–337, mar. 2006.

XUE, Y.; LIU, Y.; ZHANG, L.; WANG, H.; LUO, Q.; CHEN, R.; LIU, Y.; LI, Y. Antioxidant and spectral properties of chalcones and analogous aurones: theoretical insights. **International Journal of Quantum Chemistry**, e25808, 2018. DOI: 10.1002/qua.25808.

YUAN, G.; GUAN, Y.; YI, H. *et al*. Antibacterial activity and mechanism of plant flavonoids to gram-positive bacteria predicted from their lipophilicities. **Scientific Reports**, v. 11, p. 10471, 2021. DOI: 10.1038/s41598-021-90035-7.

ZHANG, G.; MUSGRAVE, C. B. Comparison of DFT Methods for Molecular Orbital Eigenvalue Calculations, **Journal of Physical Chemistry A**. 111, 1554-1561, 2007. DOI: 10.1021/jp061633o