Synthesis of the New Chemical Marker, [Eu(DBM)3(Lap), and Design Via Molecular Docking for Use in Recognition of *Candida auris* Polymerase

Andrei Marcelino Sá Pires Silva[1*], Levy Ribeiro de Souza Bispo[2], Jorge Fernando Silva de Menezes[2,3], Edna Aparecida Faria de Almeida[4], Ananias Freire da Silva[5], Hélcio Silva dos Santos[6], Emmanuel Silva Marinho[7], Aluísio Marques da Fonseca[3,5]

[1]Santa Cruz Stadual University; Ilhéus, Bahia; [2]Federal University of Recôncavo of Bahia, Center for Teacher Education; Amargosa, Bahia; [3]INCT - Energy and Environment, Federal University of Bahia; Salvador, Bahia; [4]Paula Souza State Center for Technological Education, ETEC Júlio de Mesquita; São Paulo, São Paulo; [5]University of International Integration of the Afro-Brazilian Lusophony; Acarape, Ceará; [6]Department of Biological Chemistry, Regional University of Cariri; Crato, Ceará; [7]Dom Aureliano Matos College of Philosophy - FAFIDAM, State University of Ceará; Limoeiro do Norte, Ceará, Brazil

The recent emergence of infections caused by *Candida auris* has raised significant global concern. Efforts to develop structure-based drugs using secondary metabolites from plant species offer promising strategies to reduce the virulence of these fungal pathogens. Lapachol, a member of the 1,4-naphthoquinone family, has shown potential as a metal complex ligand and is a candidate for such therapeutic applications. In the present study, we report the extraction of lapachol using a novel purification process from a natural source—the wood of *Tabebuia impetiginosa* (purple ipê)—and the synthesis of a unique complex of the type [Eu(DBM)3(Lap)]. Additionally, we present a molecular docking simulation of the synthesized lapachol-based complex, evaluating its potential as an inhibitory agent targeting *Candida auris* polymerase.

Keywords: Lapachol. Europium. Naphthoquinone. Lanthanides. Superfungus.

Candida auris and Computational Studies

Candida auris is a yeast species notable for its multidrug resistance, and it has emerged as a significant fungal pathogen due to its capacity to cause invasive infections and outbreaks in healthcare settings that are difficult to manage and treat [1].

Since its emergence, cases of *C. auris* have been reported on five continents as a cause of nosocomial infections, including instances identified in the external auditory canal of patients [2]. Of the nearly 150 *Candida* species described in the literature, only about 10% are known to cause human infections (candidiasis). *Candida* species and other yeasts are part of the human microbiota, commonly found on the skin, mucosal surfaces, the female genital tract, and the gastrointestinal tract [1,3].Infections caused by *C. auris* are associated with high mortality rates and often exhibit resistance to multiple classes of antifungal agents. These infections range from mild and localized conditions (such as vaginitis) to severe and life-threatening systemic infections, including candidemia [4].Despite the rapid global dissemination of *C. auris*, determining the actual burden of infection remains challenging, as standard laboratory identification techniques frequently fail to identify this species [5–8] correctly.

Naphthoquinones: Pharmacological Properties

Lapachol is a naturally occurring organic compound classified as a 1,4-naphthoquinone. It contains a hydroxyl group attached to carbon 2 and a branched 3-methyl-2-butenyl side chain attached to carbon 3. The most stable tautomeric form features carbonyl groups at positions 1 and 4, although a less stable resonance form with carbonyls at positions 1 and 2 also exists (Figures 1a–1b). Lapachol is primarily found in the heartwood of trees belonging to the *Bignoniaceae* family, particularly in the

Received on 15 December 2024; revised 26 February 2025.
Address for correspondence: Andrei Marcelino Sá Pires Silva. Avenida Nestor de Melo Pita, 535 - Centro. Amargosa, Bahia, Brazil. Zipcode: 45300000. E-mail: amspsilva.ppgquim@uesc.br

J Bioeng. Tech. Health 2025;8(2):120-126

Tabebuia genus [9]. These trees, commonly called pau d'arco, are native to the Amazon region.

Pharmacological studies on decoctions made from the sawdust of these trees have identified lapachol as one of the key bioactive components [10]. However, despite its biological potential, lapachol exhibits significant toxicity when ingested in large quantities. To mitigate its cytotoxic effects, several studies have explored the complexation of lapachol with metal ions [11,12], which has been shown to enhance its bioavailability and reduce toxicity.

Lanthanides: General Considerations

The IUPAC Commission on Nomenclature of Inorganic Chemistry recommends using the term "rare earth metals" for the elements Sc, Y, and La–Lu. This designation arises not from their scarcity but because these elements are typically found in nature as mixtures of oxides, or "earth," which require complex separation processes.

Rare earth elements exhibit predominantly ionic chemistry, primarily influenced by the size of the ionic radius. A remarkable phenomenon in this group is the lanthanide contraction, which results from the imperfect shielding effect of the $4f^h$ electrons. This poor shielding increases the effective nuclear charge, reducing the size and volume of the entire $4f^h$ configuration. Consequently, the entire ion or atom contracts - which is significant enough to impact chemical properties such as coordination number, acid strength, and the stability of the 3^+ oxidation state [13–15].

Lanthanides have many applications, including in optical materials, catalysts, magnets, ceramics, and pharmaceuticals [14]. In this study, we synthesized a new material, [Eu(DBM)3(Lap)], using a hydrated europium precursor complex and lapachol extracted from purple ipe sawdust. We investigated this compound's interaction potential and inhibitory activity against *Candida auris* super fungus through *in silico* assays. The objective was to generate new data for this system and explore the potential application of europium complexes as pharmacological agents.

Materials and Methods

Preparation of Lapachol

Approximately 200 g of bow wood sawdust was mixed with 1 L of saturated sodium bicarbonate, producing an intense red solution. The mixture was stirred for 45 minutes and then filtered, discarding the solid residue. Subsequently, approximately 50 mL of hydrochloric acid (37%) was slowly added to the filtrate until the red solution turned yellow, forming a precipitate. The liquid was then vacuum-filtered and discarded.

At this stage, lapachol was obtained, although still containing impurities derived from the sawdust. The crude lapachol was dissolved in ethyl acetate and subjected to a simple filtration. Recrystallization was achieved by allowing the solvent to evaporate at approximately 25 °C (Figure 2).

Figure 1. Lapachol with carbonyls in position for (1,4) (a) and ortho (1,2) (b).

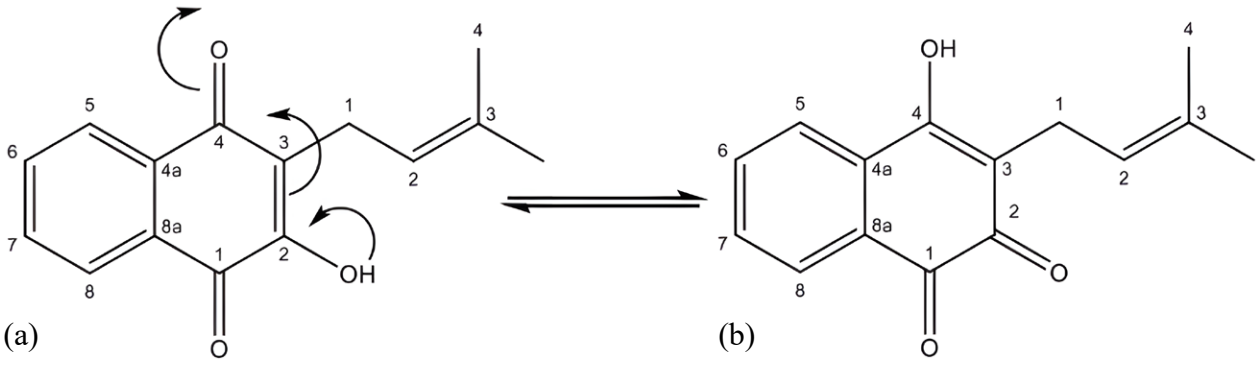
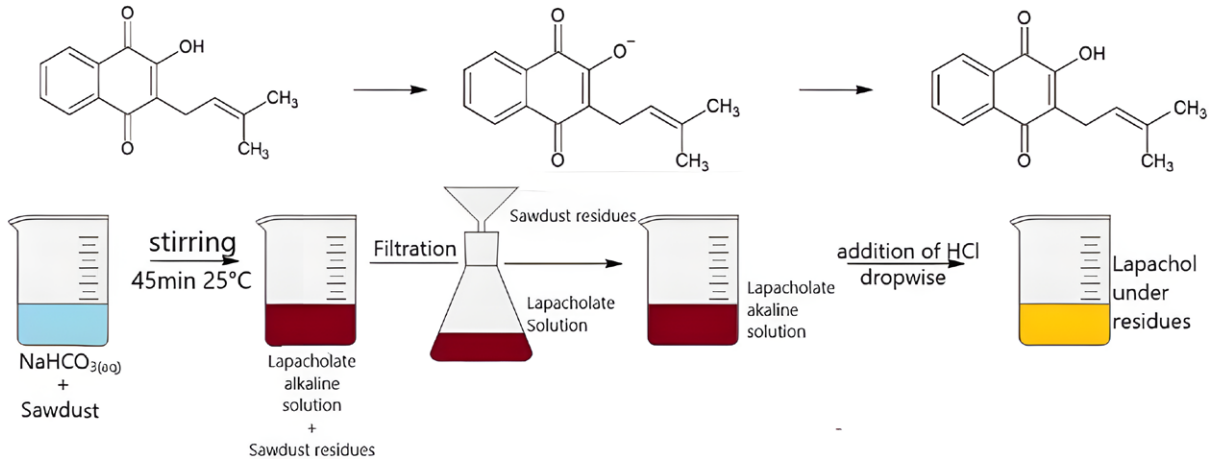


Figure 2. Lapachol extraction process from the sawdust of pau d'arco.



Synthesis of the [Eu(DBM)3(Lap)] Complex

It dissolves about 0.2 mmol of [Eu(DBM)3(Lap)] in approximately 30 mL of ethanol and 0.4 mmol of lapachol. After the salt and ligand dissolve, they are mixed, and agitation begins. The suspension is stored for ten days. Parts of the supernatant are removed, and the crystals are grouped in a well-defined way.

Washing was done using the same solvent as a reaction medium, as shown in Figure 3.

Methods

Melting point data were obtained using a Gehaka fusion point meter, model PF1000, with a temperature accuracy of ±0.3 °C up to 100 °C. For thin-layer chromatography (TLC), chromatoplates with Silica gel 60G F254 (Merck®) were used as the stationary phase. The mobile phase consisted of a mixture of hexane and ethyl acetate in a 3:7 ratio.

Infrared (IR) spectra were recorded on a PerkinElmer Frontier FT-IR spectrometer using KBr pellets. Each sample underwent 32 scans at a resolution of 4 cm^{-1}. Electronic absorption spectroscopy data were collected using a dual-beam UV-VIS spectrophotometer (Thermo Scientific GENESYS 10S) in methanol, with a sample concentration of 0.05 mg/mL.

Molecular Docking

Molecular docking was carried out using AutoDock Vina, applying the Lamarckian genetic algorithm (GA) and a grid-based energy estimation model. Gasteiger partial charges were assigned to the ligands, while Kollman charges were added to the protein. Non-polar hydrogen atoms were merged, and torsional degrees of freedom were assigned to the ligands.

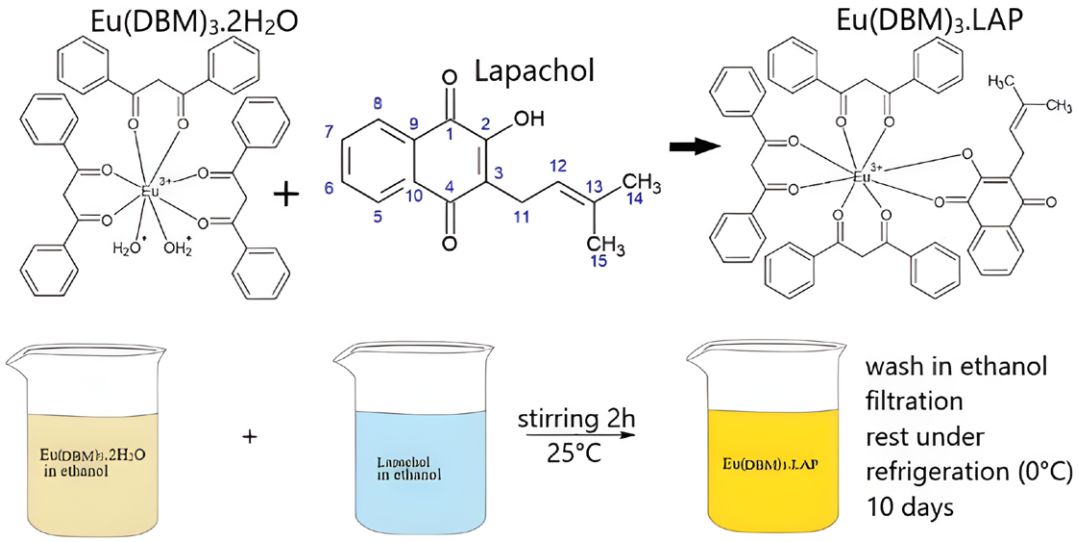
The docking grid box was set to dimensions of 25 Å × 25 Å × 25 Å, centered at x = –28.152, y = 24.208, and z = –1.140. Based on the three-dimensional structure of the target protein, this region, containing residues Leu94 (97) and Phe91 (94), was selected as a potential binding pocket.

Results and Discussion

New Purification Method

The literature typically recommends recrystallization in ethanol for lapachol purification following extraction from sawdust. However, in this study, purification was achieved via dissolution in ethyl acetate. This method proved simpler and more efficient, reducing the steps required to isolate the compound. The resulting lapachol appeared as yellow crystals. Melting point analysis of the purified sample yielded a range of 141 °C–143 °C,

Figure 3. New complex synthesis route of [Eu(dbm)3.(lap)].



closely aligning with the literature values of 139.5 °C–140.2 °C, confirming the compound's purity.

[Eu(DBM)3(Lap)] Complex

The newly synthesized complex, [Eu(DBM)3(Lap)], was obtained as a dark purple powder, indicating the presence of the naphthoquinone moiety in the form of a lapacholate ion. The complex exhibited good solubility in organic solvents such as ethyl acetate, hexane, and ethanol but limited solubility in water, likely due to the non-polar ligands present in the first coordination sphere of the metal center.

Thin Layer Chromatography

TLC analysis in this study aimed to validate the purity of lapachol synthesized via the new route and to identify the components of the complex [Eu(DBM)3(Lap)]. The TLC analysis of pure lapachol yielded an R_f value of 0.51 (Figure 4a). Comparative TLC (Figure 4b) involving the complex [Eu(DBM)3(Lap)] (C), lapachol (L), and HDBM (D) demonstrated successful complexation. The chromatographic profile of the complex (C) contained signals corresponding to L and D, confirming the incorporation of both constituents into the complex without excess unreacted material.

Infrared Spectroscopy

The infrared spectrum of the complex containing lapachol purified using the new method exhibits an intense broadband corresponding to the O–H stretching vibration, with a maximum of 3427.84 cm^{-1}. In contrast, the IR spectrum of pure lapachol shows a narrower band at 3357 cm^{-1}, which may suggest the presence of more accessible hydroxyl groups in the complex. Upon complexation, several bands characteristic of the free ligand (LAP) are either shifted or absent, indicating successful coordination. The primary coordination sites in LAP are the carbonyl group (C1=O1) and the hydroxyl group. In the IR spectrum of the metal complex (Figure 5), the band attributed to the carbonyl stretch is shifted from 1643 cm^{-1} in free LAP to 1549.47 cm^{-1} in the complex. This shift to a lower frequency suggests coordination of the carbonyl oxygen to the metal center, which weakens the double bond character and is consistent with a reduction in bond order upon coordination.

Figure 4. (a) Lapachol sample (L) in chromate plate after performing TCL; (b) Samples of [Eu(DBM)3(Lap)] (C), Lapachol (L), and Dibenzoilmethane (D), respectively, in Chromate plate after TLC revelation.

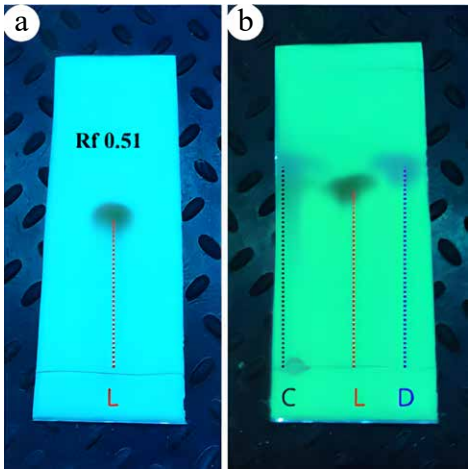


Figure 5. The infrared spectrum of pure lapachol and [Eu(DBM)3(Lap)] complex.

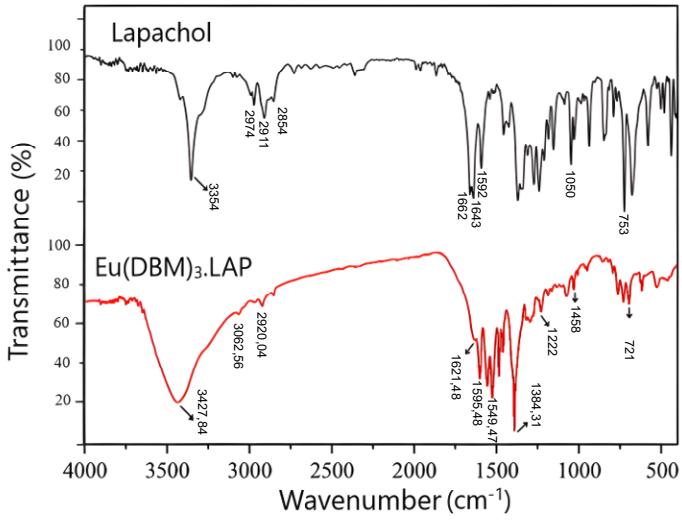
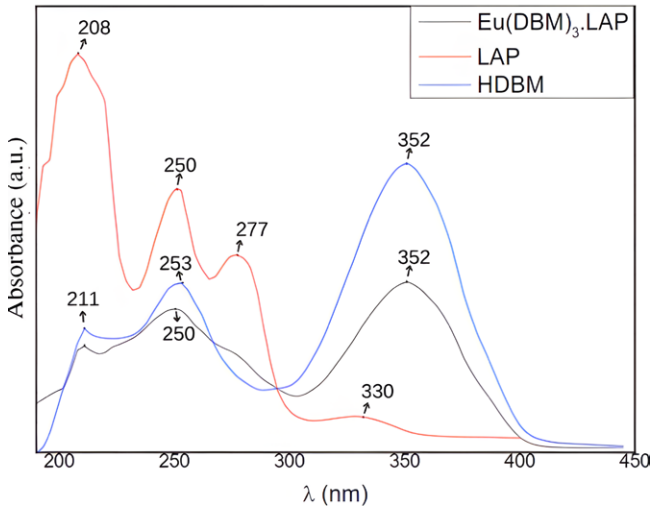


Figure 6. Complex UV-vis Spectrum of [Eu(DBM)3(Lap)], LAP and HDBM.



Furthermore, the IR data suggest that the phenolic oxygen of lapachol may also coordinate with the europium ion. These spectral changes support the forming of a new chemical species through ligand-to-metal complexation.

UV-Vis

Figure 6 shows the ultraviolet absorption curve of free ligands, lapachol (LAP) and dibenzoylmethane (HDBM), and the novel complex, [Eu(DBM)3(Lap)].

Electronic Absorption

In the UV-Vis absorption spectrum of lapachol, the most intense peaks are observed in the 200–280 nm region, a characteristic interval for π→π* electronic transitions. These transitions are associated with the high density of C=C bonds in the molecule's structure, extending from the bicyclic aromatic ring of the naphthoquinone core to the unsaturated side chain. In contrast, the less intense peaks correspond to n→π* transitions, which arise from non-bonding electron pairs on the C=O groups (ketones).

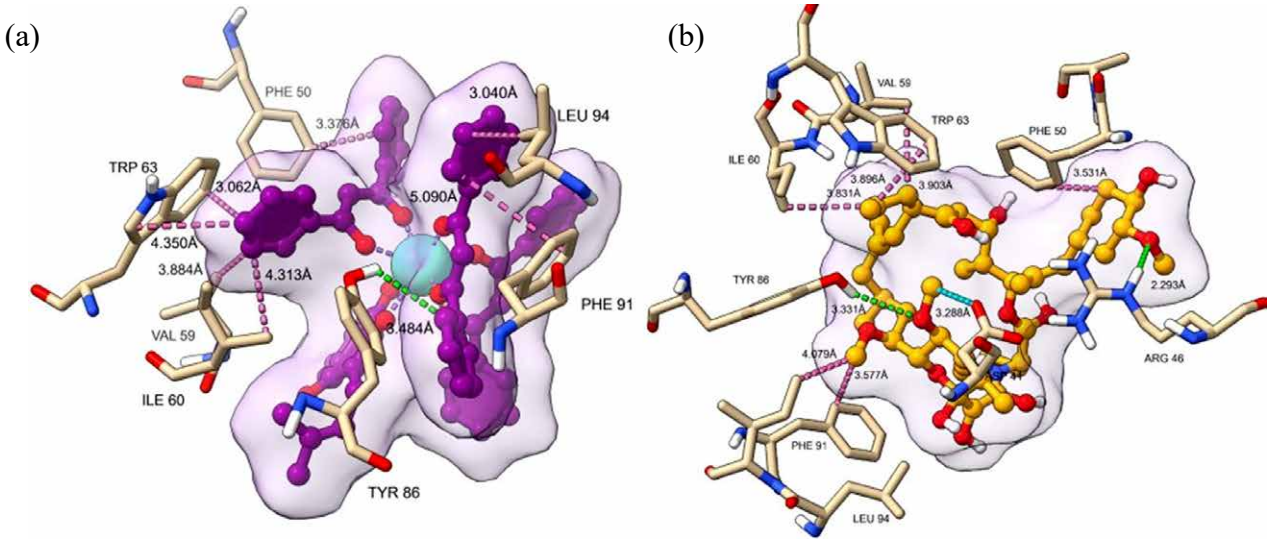
The absorption bands observed at 208, 250, and 277 nm correspond to other chromophoric regions within the LAP molecule. In the absorption spectrum of the novel material [Eu(DBM)3(Lap)], a more substantial influence from HDBM is evident. This is due to HDBM's β-diketone structure, which has a high affinity for europium and is present in greater proportion within the complex relative to lapachol. These spectroscopic findings are consistent with the infrared results, reinforcing the conclusion that both ligands are successfully coordinated to the central metal ion.

Interaction with Protein Residues

The molecular docking analysis revealed that the europium complex [Eu(DBM)3(Lap)] exhibited potential inhibitory activity through hydrophobic interactions within the active site of the target protein. Several amino acid residues were consistently involved in the binding interactions: Phe50, Gly57, Val59, Ile60, Trp63, Tyr86, Phe91, and Leu94.

Figure 7 illustrates the main interactions between [Eu(DBM)3(Lap)] and the protein. All observed interactions were hydrophobic. Among them, two π-alkyl interactions were identified: one with Phe50 (3.37 Å) and another with Val59 (3.88 Å). Previous studies [14,47] have highlighted the importance of Phe91 (5.09 Å) and Leu94 (3.04 Å) in mediating biological activity, particularly when interacting with inhibitory ligands. This study also observed

Figure 7. 3D interactions of receptor-ligand (a) with complex; (b) native ligand.



these interactions involving both the europium complex and the reference compound FK-506.

Additionally, the complex exhibited alkyl interactions with Ile60 (4.31 Å and 4.89 Å) and with Trp63 (3.06 Å and 4.35 Å), as well as a π-donor hydrogen bond with the Tyr86 residue (3.48 Å). These findings suggest a potent and specific binding mode, supporting the potential of the europium complex as a pharmacological agent targeting the protein.

Conclusion

This study presents the novel complex [Eu(DBM)3(Lap)], validated through IR spectroscopy, UV-Vis absorption, melting point determination, and TLC analysis. The data obtained indicate the effective coordination of the ligands HDBM and LAP to the europium ion (Eu^{3+}), confirming the successful synthesis of the complex. The findings suggest that this material holds promise for future applications, particularly as a chemical marker or inhibitor of the multidrug-resistant fungus *Candida auris*, given the known pharmacological potential of lapachol derivatives. This preliminary *in silico* investigation lays the groundwork for future *in vitro* studies to validate the compound's biological activity further.

Acknowledgments

The authors gratefully acknowledge the support of the following Brazilian funding agencies: Instituto Nacional de Ciência e Tecnologia (INCT) – Energia & Ambiente; Fundação Cearense de Apoio ao Desenvolvimento Científico e Tecnológico (FUNCAP); Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq); and Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES), for their fellowships and financial support.

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