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REGULAR ARTICLE

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Enantioselective ene-reduction of *E*-2-cyano-3-(furan-2-yl) acrylamide by marine and terrestrial fungi and absolute configuration of (*R*)-2-cyano-3-(furan-2-yl) propanamide determined by calculations of electronic circular dichroism (ECD) spectra

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Abstract

This work reports the green organic chemistry synthesis of *E*-2-cyano-3(furan-2-yl) acrylamide under microwave radiation (55 W), as well as the use of filamentous marine and terrestrial-derived fungi, in the first ene-reduction of 2-cyano-3-(furan-2-yl) acrylamide to (R)-2-cyano-3-(furan-2-yl)propanamide. The fungal strains screened included *Penicillium citrinum* CBMAI 1186, *Trichoderma* sp. CBMAI 932 and *Aspergillus sydowii* CBMAI 935, and the filamentous terrestrial fungi *Aspergillus* sp. FPZSP 146 and *Aspergillus* sp. *FPZSP* 152. A compound with an uncommon CN-bearing stereogenic center at the α-C position was obtained by enantioselective reactions mediated in the presence of the microorganisms yielding the (*R*)-2-cyano-3-(furan-2-yl) propanamide **3a**. Its isolated yield and e.e. ranged from 86% to 98% and 39% to 99%, respectively. The absolute configuration of the biotransformation products was determined by time-dependent density functional theory (TD-DFT) calculations of electronic circular dichroism (ECD) spectra. Finally, the tautomerization of 2-cyano-3-(furan-2-yl) propanamide **3a** to form an achiral ketenimine was observed and investigated in presence of protic solvents.

KEYWORDS

biocatalysis, electronic circular dichroism, enoate reductase, Knoevenagel condensation, marine- and terrestrial-derived fungi

1 | INTRODUCTION

The use of enzymes (homogenous or heterogeneous catalysts) as mediators in organic reactions is widely known as biocatalysis, and it has become a powerful tool for development of organic synthetical protocols. Moreover, the presence of biocatalysts can promote reactions that introduce a chiral center in the molecule with high chemoselectivity, regioselectivity, and enantioselectivity at mild reaction conditions.[1-6] Numerous organic reactions have reported the use of biocatalytic protocols, such as hydrolytic reactions of esters and amides,[7] transesterification reactions,[8] oligosaccharide syntheses,[9-11] acylation of chlorohydrins,[12] nitrile biodegradations,[13] Baeyer-Villiger oxidations,[14] aldol condensations,[15] polymerizations,[16] industrial applications,[17] and Knoevenagel condensations.[18]

A special attention has been given for the Knoevenagel condensation reactions as an effective tool to increase the carbon chain, as well as its functionalization[19] being applied in the synthesis of bioactive organic compounds,[20] polymers,[21] and synthetic intermediates.[22] On this reaction, an adduct of carbon-carbon double bond are formed by reaction between a carbonyl compound and a compound containing an active methylene group.[19] The reactivity of the active methylene groups is directly attached to electron magnetic groups, for example, CN, COOR, COR (R = aliphatic, aromatic, amide, ester, nitrile).[23]

Our research group have been employed with success the biocatalytic protocols for different purposes such as the asymmetric reduction of isatin,[24] the biodegradation of pentachlorophenol,[25] the acylation of chlorohydrins,[26] the resolution of cyanohydrins,[27] the bioreduction of α,β,γ,δ-unsatured ketones,[28] and the hydrogenation of bis-α,β-unsatured enones.[29] Recently, Jimenez et al[18] reported for the first time the biocatalytic ene-reduction of aromatic benzylidenemalononitriles.

On the basis of these findings, this paper presents a new reaction step for the synthesis of 2-cyano-3-(furan-2-yl) acrylamide by microwave radiation followed by bioreduction of the Knoevenagel adduct using marine-derived and terrestrial fungi. In addition, the absolute configuration of (*R*)-2-cyano-3-(furan-2-yl) propanamide **3a** obtained by biotransformation was determined by time-dependent density functional theory (TD-DFT) calculations of electronic circular dichroism (ECD) spectra.

Finally, the tautomerization of 2-cyano-3-(furan-2-yl) propanamide **3a** to form an achiral ketenimine was observed and investigated in presence of protic solvents.

2 | MATERIALS AND METHODS

2.1 | General methods

All manipulations involving the filamentous fungi were carried out under sterile conditions in a Veco laminar flow hood. A Technal TE-421 orbital shaker was used for biocatalytic experiments.

Gas chromatography-mass spectrometry (GC-MS), a Shimadzu GC2010 Plus gas chromatography system coupled to a mass-selective detector (Shimadzu MS2010 Plus) in electron ionization mode (70 eV) equipped with a DB-5MS fused silica column (Agilent J & W Advances 30 × 0.25 × 0.25 μm) was used. Helium was used as a drag gas with a constant flow of 0.75 mL/min at 46.5 kPa, and the injection volume was 0.5 μL of solution, with concentrations of 1.0 to 3.0 mg/mL. The method of analysis initially consisted of maintaining the column at 90°C for 4 minutes and then on a heating ramp of 6°C/min until reaching the temperature of 280°C, which column was maintained for 5 minutes. The injections were performed in split mode at a 1:20 ratio. Ion fragments were detected in the range of 40 to 500 Da.

Liquid chromatography (LC) analyses were performed in a Shimadzu equipment model LC-20 AT (Shimadzu Co., Japan) with a SPD-M20A detector (λ=215 nm) connected to a CHIRALPAK AD-H chiral column (0.46 × 25 cm). The mobile phase was composed of hexane and ethanol (60:40) with a flow rate of 0.5 mL/min; the oven temperature was set at 30°C and the injection volume of 10 μL.

Fourier Transform Infrared (FT-IR) spectra were recorded on a Shimadzu IRAffinity spectrometer, and the samples were prepared as KBr disks in the 4000 to 400 cm^{-1} region.

^{1}H Nuclear Magnetic Resonance (NMR) and ^{13}C NMR spectra were recorded on an Agilent Technologies 500/54 Premium Shielded or Agilent Technologies 400/54 Premium Shielded spectrometer, with CD_3OD and $CDCl_3$ as deuterated solvents and tetramethylsilane (TMS) as the internal standard. Unless otherwise noted, the chemical shifts given in ppm, and coupling constants (*J*) values were reported in Hz. The chemical shifts (δ) were expressed in parts per million (ppm) and referenced to the internal standard (TMS) signal and the deuterated solvents used, ie, $CDCl_3$ (δ_H 7.26, δ_C 77.16) and CD_3OD (δ_H 4.87, 3.31, δ_C 49.00).

The microwave irradiation experiment was performed using a Discover System from CEM Corporation at a 2.45-GHz frequency with maximal power output of about 200 W. with internal magnetic stirring. For the reactions, the power of the microwave reactor used was 55 W (30 min at 80 °C).

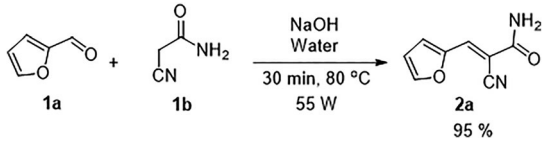
The electronic circular dichroism (ECD) spectrum of each enantiomer eluting from the chiral LC system was measured in a Jasco CD-2095 detector by trapping in a 2.5-cm quartz cell through a switching valve. The spectra were average computed over three instrumental scans, and the intensities were presented in terms of ellipticity values (mdeg). The ECD spectra were baseline corrected by subtraction from a measurement obtained for the same solvent used *n*-hexane and EtOH (60:40).

2.2 | Chemical reagents

Furfural (99%) **1a**, cyanoacetamide (99%) **1b** were purchased from Sigma-Aldrich and used without any further purification. The salts used for preparation of the artificial sea, NaOH (97%), acetone, absolute ethanol, and THF were purchased from Vetec and Synth (Brazil). Malt extract was purchased from Acumedia (São Paulo, Brazil). The deuterated solvents, CD_3OD (99.9%) and $CDCl_3$ (99.8%), were purchased from Cambridge Isotope Laboratories. The thin layer chromatography (TLC) utilized was DC-Fertigfolien ALUGRAM XTra SIL G/UV_{254} (layer: 0.20-mm silica gel 60 with fluorescent indicator UV_{254}).

2.3 | Knoevenagel condensation under microwave irradiation

A mixture of furfural **1a** (1.0 mmol, 96.08 mg), cyanoacetamide **1b** (1.0 mmol, 84.08 mg), and 2 drops of NaOH (1 M) in water (3 mL) were put in the microwave reactor (55 W) for 30 minutes at 80°C. The reaction was monitored by TLC. After completion, the reaction was extracted with ethyl acetate (3×10 mL). The combined organic phases were concentrated under vacuum until total evaporation of the solvent, and the Knoevenagel adduct **2a** was recrystallized in a mixture of ethanol and THF (1:1) to yield 95% of the pure compound. The product **2a** was characterized by ^{1}H NMR and ^{13}C NMR, FT-IR, LC, and GC–MS (Supporting Information). Supplementary Material Scheme 1 illustrates the synthesis of the Knoevenagel adduct **2a** under microwave irradiation.



SCHEME 1 Synthesis of adduct **2a** by Knoevenagel condensation under microwave irradiation

2.4 | Synthesis of racemic 2-cyano-3-(furan-2-yl)propanamide 3a

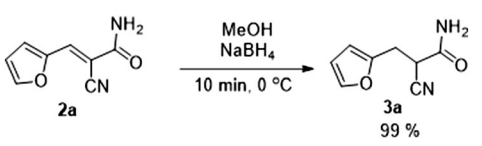
The *E*-2-cyano-3-(furan-2-yl)acrylamide **2a** (50 mg, 0.308 mmol) was placed into the reaction vial with capacity of 25 mL and dissolved in MeOH. The $NaBH_4$ (80 mg, 2 mmol) was then added at 0°C. The reaction was monitored by TLC (in a mixture containing hexane and ethyl acetate 8:2). The TLC plates were revealed with sublimated iodine-impregnated on silica gel. After 10 minutes, all *E*-2-cyano-3-(furan-2-yl)acrylamide **2a** was reduced to the product **3a**. The reaction was extracted with ethyl acetate (3×10 mL). The aqueous phases were discarded and the organic phases combined and concentrated by vacuum until total evaporation of the solvent and the residue was purified by wash with heat hexane. The product **3a** was characterized by GC–MS, LC, FT-IR, and ^{1}H NMR and ^{13}C NMR (Supporting Information). Scheme 2 illustrates the reduction of the Knoevenagel adduct **2a** with $NaBH_4$.

2.5 | Composition of culture media and artificial seawater

Composition of culture medium: 20 g/L malt extract (Acumedia) in artificial seawater. Composition of artificial seawater: $CaCl_2.2H_2O$ (1.36 g/L), $MgCl_2.6H_2O$ (9.68 g/L), KCl (0.61 g/L), NaCl (30.0 g/L), Na_2HPO_4 (0.014 mg/L), Na_2SO_4 (3.47 g/L), $NaHCO_3$ (0.17 g/L), KBr (0.1 g/L), $SrCl_2.6H_2O$ (0.040 g/L), and H_3BO_3 (0.030 g/L) at pH 8 adjusted with KOH solution (0.1 mol/L).

2.6 | Isolation and preservation of the marine-derived fungus

Marine-derived fungus *Penicillium citrinum* CBMAI 1186 was isolated from the marine alga *Caulerpa* sp.; *Trichoderma* sp. CBMAI 932 was isolated from the sponge *Geodia corticostylifera*, and *Aspergillus sydowii* CBMAI 935 was isolated from the marine sponge *Chelonaplysilla erecta*. The alga and sponges were collected by Prof. R.G.S. Berlinck in São Sebastião City, on the coast of the State of São Paulo, Brazil.[30] The fungi were identified using both conventional and molecular



SCHEME 2 Synthesis of reduced compound **3a** (2-cyano-3-(furan-2-yl)propanamide) in the presence of $NaBH_4$

methods at the Chemical, Biological and Agricultural Pluridisciplinary Research Center (CPQBA) at the University of Campinas, SP, Brazil. The fungi *P. citrinum* CBMAI 1186, *Trichoderma* sp. CBMAI 932 and *A. sydowii* CBMAI 935 were deposited in the Brazilian Collection of Environmental and Industrial Microorganisms (CBMAI – http://webdrm.cpqba.unicamp.br/cbmai/). The terrestrial fungi *Aspergillus* sp. FPZSP 146 and *Aspergillus* sp. FPZSP 152 were collected by Prof. Suzan P. de Vasconcellos from the Unit of Organic Composting Production (UOCP) belonging to the São Paulo Zoo Park Foundation (Fundação Parque Zoológico de São Paulo – FPZSP).

2.7 | Microorganism cultivation

The marine fungi *P. citrinum* CBMAI 1186, *Trichoderma* sp. CBMAI 932, and *A. sydowii* CBMAI 935 were cultivated on Petri plates containing 2% of malt solid culture medium using artificial seawater. The terrestrial fungi *Aspergillus* sp. FPZSP 146 and *Aspergillus* sp. FPZSP 152 were cultivated on Petri plates containing 2% of malt solid culture medium using distilled water with the following composition: 20 g/L malt extract in distilled water. The pH was adjusted to 7. Culture mediums were sterilized in autoclave (Phoenix, 121°C, 20 min, 1.5 kPa) and incubated for 7 days at 32°C and 3 days at 32°C, for the marine and terrestrial fungi, respectively.

2.8 | Bioreduction of E-2-cyano-3-(furan-2-yl)acrylamide by filamentous fungi

The mycelia of the fungi *P. citrinum* CBMAI 1186, *Trichoderma* sp. CBMAI 932, *A. sydowii* CBMAI 935, *Aspergillus* sp. FPZSP 146, and *Aspergillus* sp. FPZSP 152 were harvested via Buchner filtration. In addition, 5 g (wet weight) of mycelium from each fungi was suspended in 100 mL of phosphate buffer solution (Na_2HPO_4/KH_2PO_4, pH=7, 0.1 mol/L) and distributed in 250 mL Erlenmeyer flasks. The *E*-2-cyano-3-(furan-2-yl)acrylamide **2a** (50 mg, 0.308 mmol) was previously dissolved in dimethylsulfoxide (300 μL) and added to the culture media. The reactions were incubated for 3 days in an orbital shaker at 32°C and 130 rpm. The reaction was monitored by collecting 2.0 mL aliquot, which was extracted by stirring with ethyl acetate (2.0 mL) followed by centrifugation (6.000 rpm, 6 minutes) and analyzed by TLC and GC-MS every 24 hours. The product **3a** was isolated and identified (Section 2.9). All biotransformations were performed in triplicates.

2.9 | Isolation of product 3a obtained by marine-derived and terrestrial fungi

After 3 days of reaction, the liquid was filtered in a Buchner apparatus and the mycelial mass obtained was transferred to a 50 mL Erlenmeyer flask and suspended in 20 mL of water and ethyl acetate (1:1). This biphasic mixture was submitted to magnetic stirring for 30 minutes and filtered again by a Buchner funnel. The filtrate was extracted with ethyl acetate (3×50 mL). The organic phase was dried over anhydrous Na_2SO_4, filtered, and evaporated under vacuum. The extract obtained from the filtrate was purified by chromatography column (CC) over silica gel (230-400 μm) and eluting with *n*-hexane and ethyl acetate (7:3). Yield obtained for the product **3a** with the fungi *Penicillium citrinum* CBMAI 1186, *Trichoderma* sp. CBMAI 932, *Aspergillus sydowii* CBMAI 935, *Aspergillus* sp. FPZSP 146, and *Aspergillus* sp. FPZSP 152 were 98%, 92%, 86%, 90%, and 94%, respectively. The compound **3a** was characterized by GC-MS, LC, FT-IR, ^{1}H NMR and ^{13}C NMR.

2.10 | Calculations

All density functional theory (DFT) and TD-DFT calculations were carried out at 298 K in the gas phase using Gaussian 09 Software.[31] Calculations were performed for the arbitrarily chosen (*R*)-2-cyano-3-(furan-2-yl) propanamide **3a**. The conformational search was carried out at the molecular mechanics level of theory with the Monte Carlo algorithm employing the MM^+ force field incorporated in HyperChem 8.0.10 software package. Nine conformers with relative energy (rel E.) within 6 kcal mol^{-1} of the lowest-energy conformer were selected and further geometry optimized at the B3LYP/6-31G(d) level. The six conformers with rel E. < 1.9 kcal mol^{-1}, which corresponded to more than 95% of the total Boltzmann distribution, were selected for UV and ECD spectral calculations. Vibrational analysis at the B3LYP/6-31G(d) level resulted in no imaginary frequencies, confirming the considered conformers as real minima. Geometry optimization and frequency calculations were also performed at the B3PW91/6-311G(d, p) level. TD-DFT was then employed to calculate excitation energy (in nm) and rotatory strength *R* in dipole velocity form (R_{vel} in cgs units: 10^{-40} esu^2 cm^2), at the CAM-B3LYP/TZVP and wB97XD/aug-cc-pVDZ levels.

The calculated rotatory strengths from the first 30 singlet → singlet electronic transitions were simulated into an ECD curve using Gaussian bands with bandwidth σ 0.25 eV. The predicted wavelength transitions were multiplied with a scaling factor of 1.05, determined by the

best agreement between experimental and calculated UV spectra. The final spectra were generated as the Boltzmann average of the lowest energy conformers identified and plotted using Origin 8 software.

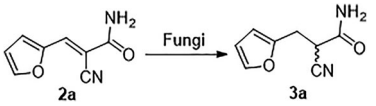
3 | RESULTS AND DISCUSSION

The bioreduction of 2-(furan-2-ylmethylene) malononitrile and the biotransformation of 2-(furan-2-ylmethyl)malononitrile into 2-cyano-3-(furan-2-yl) propanamide by marine fungi *P. citrinum* CBMAI 1186 have already been demonstrated by Jimenez et al[18] (Scheme 3).

Such biotransformation followed a multienzymatic sequence of cascade reactions,[18] in which two steps were involved. On the first step, the bioreduction of compound **1c** to compound **1d** occurred possibly by the action of enoate reductases, whereas on the second step, the hydrolysis of the nitrile group on compound **1d** yielding the compound **3a** was catalyzed by nitrile hydratase present in the culture media. The concomitant bioreduction and biotransformation occurred on the CN group demonstrate the importance of cascade reactions driven by multienzymatic media present on the fungi in order to obtain chiral compounds.

On the basis of the work by Jimenez et al,[18] a new path of reaction has been proposed here to yield the compound **3a** (Scheme 4). In this system, the production of enantiomerically enriched α-cyanoamide **3a** can be obtained from *E*-2-cyano-3-(furan-2-yl) acrylamide **2a** using microorganisms to reduce the carbon-carbon double bond (Scheme 4). The compound **2a** was synthetized using furfuraldehyde **1a** and cyanoacetamide **1b** under microwave irradiation.

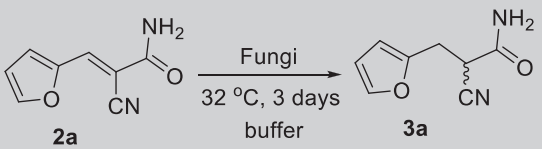
The adduct **2a** illustrated in Scheme 1 was prepared via Knoevenagel condensation using furfural **1a** and cyanoacetamide **1b** in the presence of NaOH under microwave irradiation with 95% yield. The reaction mechanism is based on the removal of acidic hydrogen of cyanoacetamide **1b** by NaOH affording a carbanion as intermediate. The formation of Knoevenagel adduct **2a** occurs by the nucleophilic attack of the carbanion into the carbonyl group of furfural generating an intermediate that suffers a dehydration reaction. The marine-derived fungi (*P. citrinum* CBMAI 1186, *Trichoderma* sp. CBMAI 932, and *A. sydowii* CBMAI 935) and terrestrial fungi (*Aspergillus* sp. FPZSP 146 and *Aspergillus* sp. 152) were used for the bioreduction of *E*-2-cyano-3-(furan-2-yl) acrylamide **2a**. All reactions were monitored over 3 days by TLC. After the extraction and purification process, the products were identified by LC analysis.



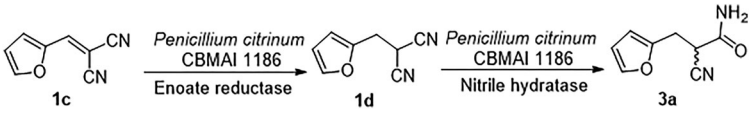
SCHEME 4 Bioreduction of *E*-2-cyano-3-(furan-2-yl) acrylamide **2a** by fungi

The biotransformation reaction of compound **2a** by marine-derived and terrestrial fungi promoted the formation of a single compound **3a** with 86% to 97% of yield (Table 1), which was obtained by action of ene reductases (ERs) on the activated C=C double bond. The mechanism established for the ERs enzyme action occurs by nucleophilic attack of a hydride ion from the flavin cofactor onto a β-carbon atom of the C=C double bond. Subsequently, a tyrosine residue delivers a proton to the α-carbon atom, typically on the alkene opposite face, affording anti-hydrogen addition.[32,33]

TABLE 1 Bioreduction of *E*-2-cyano-3-(furan-2-yl) acrylamide **2a** with marine and terrestrial fungi



Fungi	Isolated Yield(%) 3a	Enantiomeric ratio (%) /Absolute Configuration 3a
Penicillium citrinum CBMAI 1186	98	99/*S*
Aspergillus sydowii CBMAI 935	92	97/*R*
Aspergillus sp. FPZSP 152	86	91/*R*
Aspergillus sp. FPZSP 146	90	39/*R*
Trichoderma sp. 932	94	98/*R*



SCHEME 3 Bioreduction and biotransformation of 2-(furan-2-ylmethylene)malononitrile (adapted from Jimenez et al[18])

The enantioselectivity of reduced product **3a** was determined by enantioselective LC analysis. It can be observed by LC chromatograms that the bioreduction in the presence of all fungi yielded the compound 2-cyano-3-(furan-2-yl)propanamide **3a** with good enantiomeric ratio (Table 1 and Figure 1). The presence of bioreduced product was confirmed by the analysis of racemic **3a**, previously synthetized and purified.

The LC results showed that the fungi *A. sydowii* CBMAI 935, *Aspergillus* sp. FPZSP 152, *Trichoderma* sp. 932 presented enantiomeric ratio up to 95% to *R*-configuration, and only the fungi *P. citrinum* CBMAI 1186 with high *e.*

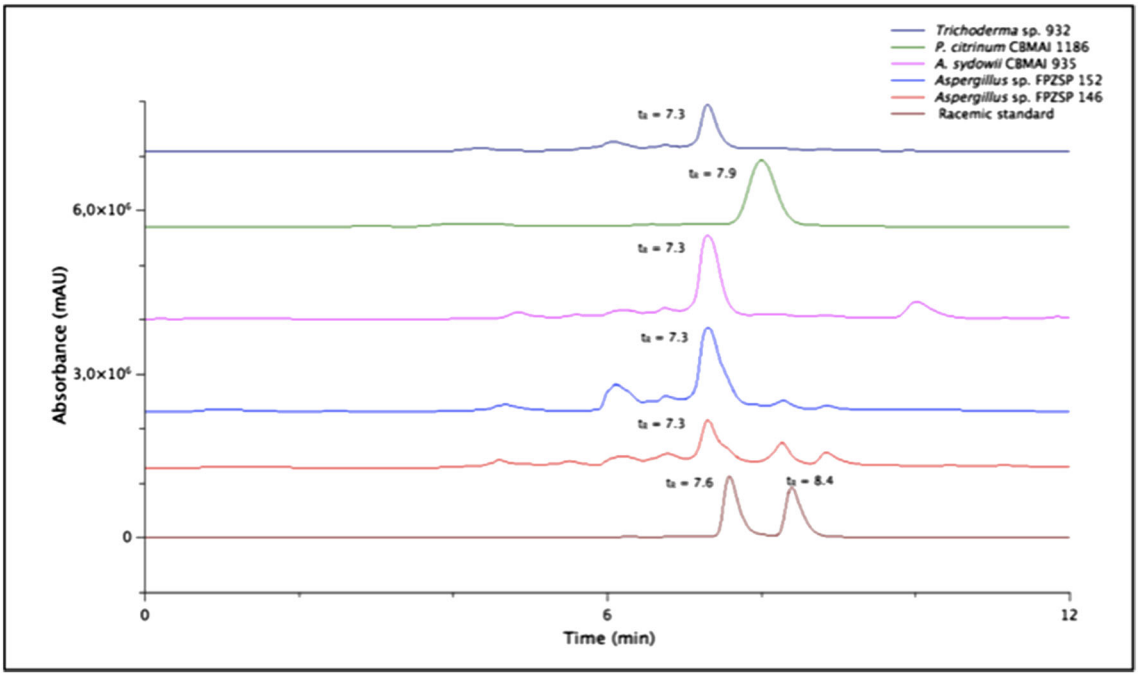


FIGURE 1 LC—chiral chromatograms of compound **3a** obtained by different fungi

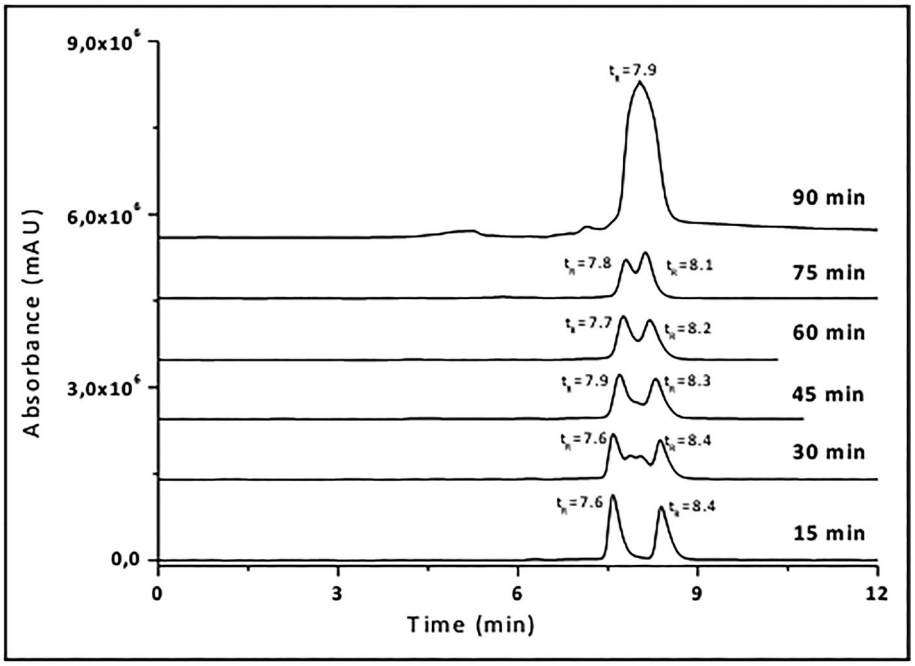


FIGURE 2 Chromatograms of (±)-2-cyano-3-(furan-2-yl) propanamide **3a** at different times of analyses (15, 30, 45, 60, 75 and 90 minutes). Chromatographic conditions: CHIRALPAK AD-H chiral column (0.46 × 25 cm), mobile phase: Hexane/ethanol (60:40), flow rate: 0.5 mL min^{-1}, λ =215 nm). *R*-enantiomer **3a**: t_R~7.7 minutes and *S*-enantiomer **3a**: t_R ~8.3 minutes

e. up to 95% to *S*-configuration. Finally, the fungi *Aspergillus* sp. FPZSP 146 showed low value of *e.e.* (39%) to *R*-configuration.

It was observed that for (±)-2-cyano-3-(furan-2-yl) acrylamide **3a** loss of chromatographic resolution for the racemic compound occurred after successive analysis onto LC system (Figure 2).

A tautomeric equilibrium was proposed for the (±)-2-cyano-3-(furan-2-yl) propanamide **3a** in the presence of polar solvents (MeOH, EtOH). The formation of a ketenimine derivative could justify the loss of chromatographic resolution since it bears no stereogenic center. Basically, this equilibrium occurs between the nitrile and prototropic ketenimine forms (Scheme 5), and it is common in compounds containing cyano groups with one or more acidic hydrogens on the α-C position.

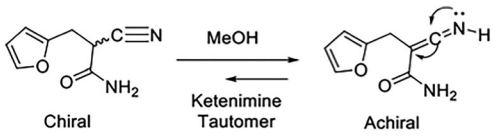
In the equilibrium, the cyano forms generally arises as the major compound.[34] The ketenimine tautomer has been previously reported by Kasturi et al[35] in the presence of polar solvents such as water and methanol at 240 nm (malononitrile) and around 350 nm (extensive conjugation) bands. In apolar solvents, eg, hexane, the formation of ketenimine tautomer was not detectable by Ultraviolet (UV) absorption.

In our study, biotransformation with fungi (Figure 1) did not showed the equilibrium of ketenimine because extraction reactions were performed with ethyl acetate. Thus, it was possible under these conditions to obtain the compound **3a** in the chiral form with their respective enantiomeric ratio.

However, when the samples of compound **3a** obtained of biotransformation reactions were placed in the presence of methanol, the equilibrium to ketenimine form was observed by chiral LC analysis.

It is also worth observed by LC analysis that both the samples obtained by the fungi and the racemic synthesis, after the removal of methanol, the chiral racemic form prevailed.

The formation of the ketenimine tautomer was confirmed by ^{1}H NMR and ^{13}C NMR analyses in $CDCl_3$ (aprotic solvent). The 2-cyano-3-(furan-2-yl) propanamide (**3a**) showed a signal at 3.70 ppm (dd, $J = 8.0$ and 5.4 Hz, 1H), which corresponds to a double doublet from a hydrogen adjacent to the stereogenic center. Also, between 3.40 and 3.23 ppm (m, 2H), a multiplet assigned to two methylene protons adjacently attached to the



SCHEME 5 Tautomerization of 2-cyano-3-(furan-2-yl) propanamide **3a** in protic solvent

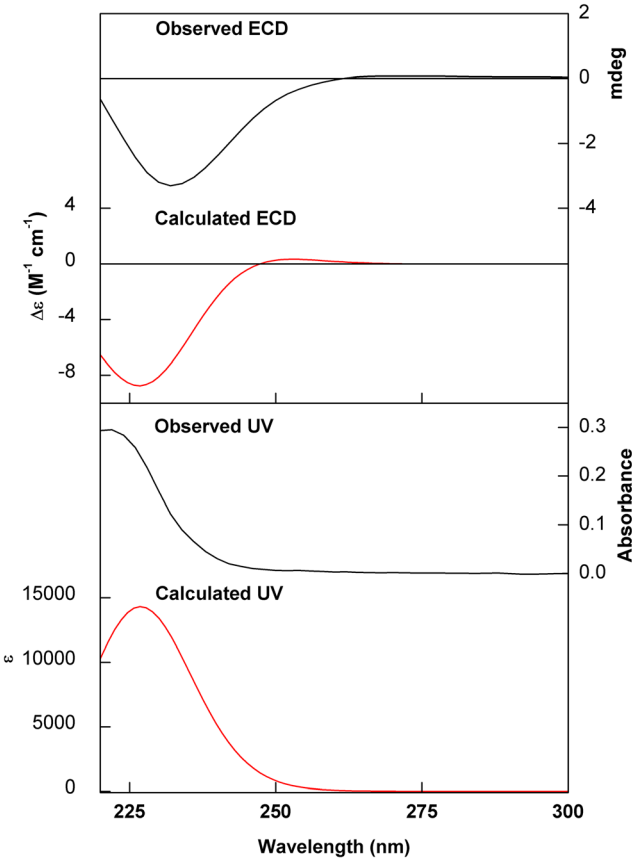


FIGURE 3 Comparison of the observed UV and electronic circular dichroism (ECD) spectra of (±)-**3a** with the calculated [CAM-B3LYP/TZVP//B3LYP/6-31G(d)] UV and ECD spectra of the Boltzmann average of the lowest energy conformers identified for (*R*)-**3a** (Supporting Information for lowest energy conformers)

furan ring was observed (Supporting Information). From the ^{13}C NMR spectra analysis, it was observed a signal at 165.7 ppm, which corresponds to the carbonyl amide group, as well as a methine carbon at 37.5 ppm (stereogenic center) and a methylene carbon at 28.4 ppm signal. These data were in accordance with those in the literature[18] (Supporting Information).

From the ^{1}H NMR analyses for the compound **3a** in CD_3OD (protic solvent) it was not observed the signal at 3.70 ppm (dd, $J = 8.0$ and 5.4 Hz, 1H); however, a signal at 3.36 ppm (s, 2H) was observed, which corresponds to two methylene protons adjacent to the furan ring (Supporting Information). Signals at 165.7 and 28.4 ppm was observed for the ^{13}C NMR spectra analysis; however, the signal at 37.5 ppm corresponding to the stereogenic methine carbon was not observed (Supporting Information).

These findings supported the formation of the ketenimine tautomer (Scheme 5) in the presence of polar solvents (eg, MeOH, EtOH). The signals referent to the stereogenic center were presented in both ^{1}H and ^{13}C NMR spectra recorded in aprotic deuterated solvents

(eg, $CDCl_3$) but was not observed when polar protic deuterated solvents (eg, CD_3OD) (Supporting Information).

In order to assess the selectivity in the biotransformation of **2a** by marine and terrestrial fungi, the absolute configuration of the enantiomers of the product **3a** was assigned by ECD spectroscopy and TD-DFT calculations. The racemic synthetic **3a** was used, and each peak eluting from the chiral column was trapped into the ECD detector.

The experimental ECD spectra of both enantiomers were then compared with those calculated at the CAM-B3LYP/TZVP and wB97XD/aug-cc-pVDZ (Supporting Information) levels of theory. The very good agreement between observed and theoretical data at both levels of theory (Figure 3 and Supporting Information) allowed the unambiguous determination of the absolute configuration of the first eluting peak as *R*-enantiomer **3a** and, consequently, that of the last eluted peak as *S*-enantiomer **3a**.

4 | CONCLUSION

The use of marine-derived and terrestrial fungi on the enantioselective ene-reduction of a Knoevenagel product emerges as alternative tools for green chemistry and biocatalysis. The ene-reduction of *E*-2-cyano-3-(furan-2-yl) acrylamide **2a** to 2-cyano-3-(furan-2-yl)propanamide **3a** was achieved with 39% to 99% *e.e* with the (*R*)-enantiomer **3a** being produced predominantly. A tautomeric equilibrium of chiral 2-cyano-3-(furan-2-yl)propanamide **3a** leading to the formation of an achiral ketenimine was observed by chiral chromatography in the presence of polar protic solvents. The absolute configuration of the enantiomers of the product **3a** was assigned by ECD spectroscopy and TD-DFT calculations and showed very good agreement between observed and theoretical data.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of the article.

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