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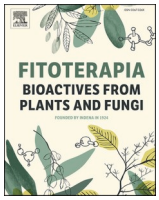

New *bis*-norditerpene by biotransformation of favelines from *Cnidoscolus quercifolius* using *Umbelopsis isabellina* as biocatalyst

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ABSTRACT

Favelines, tricyclic benzylcycloheptene diterpenes predominantly found in *Cnidoscolus quercifolius*, have demonstrated significant cytotoxic activity against human cancer cell lines, including melanoma. In this study, we employed a combinatorial approach to screen microbial strains for biotransformation, focusing on regioselective oxidation of the cyclohexyl ring in the faveline core structure. This strategy, applied directly to the crude plant extract, enabled the rapid identification of *Umbelopsis isabellina* as the active strain. Subsequent preparative biotransformation yielded a novel *bis*-norditerpene, 14-hydroxy-isofavelol (**3**). The new compound exhibited moderate cytotoxic activity (IC_{50} = 68.8 μM) against chemoresistant human melanoma A2058 cells, which are resistant to dacarbazine but sensitive to vemurafenib. Compound **3** enhanced the cytotoxic effect of vemurafenib, reducing its IC_{50} from 24.8 to 9.63 μM. This work demonstrates the utility of microbial biotransformation for generating new bioactive natural product derivatives and highlights the potential of 14-hydroxy-isofavelol as a chemosensitizing agent in melanoma therapy.

1. Introduction

Favelines are tricyclic benzylcycloheptene diterpenes (Fig. 1) predominantly found in *Cnidoscolus quercifolius* [1,2], a medicinal plant native to Brazil. Isolated from the crude extract of its stem bark, favelines have demonstrated significant cytotoxic activity against various human tumor cell lines, including melanoma [3,4], promyelocytic leukemia, lung carcinoma and breast adenocarcinoma [5–10]. Recently, we reported the anti-melanoma potential of phyllacanthone, the major *bis*-norditerpene from *C. quercifolius*, in chemoresistant human melanoma A2058 cells carrying the oncogenic $BRAF^{V600E}$ mutation [3]. Phyllacanthone induced cytotoxicity by promoting tubulin depolymerization, as confirmed through immunoassays and molecular docking studies showing strong interactions with the colchicine-specific binding site. These findings motivated the exploration of new phyllacanthone analogues with anti-melanoma activity. As an initial step, regioselective oxidation of the cyclohexyl ring was targeted, with microbial transformation identified as the most suitable approach.

Biotransformation refers to the use of biological systems, such as enzymes or microorganisms, to perform chemical modifications on molecules [11]. This process is highly valued for its exceptional chemical, regio- and stereoselectivity, which often surpasses the limitations of traditional chemical methods that tend to be less selective [12,13]. Purified enzymes offer a precise and controlled approach but face significant challenges, such as the difficulty of isolation, high costs and the requirement for complex enzymatic systems to accomplish some reaction steps. As an alternative, the use of whole microorganisms proves advantageous, especially due to their natural ability to perform cascade reactions, providing a greater molecular diversity. Moreover, microorganisms facilitate process scalability and enable mild reaction conditions, such as moderate temperatures and aqueous media, avoiding toxic organic solvents and reducing operational costs. These benefits make microbial transformation an indispensable tool in semi-synthesis of bioactive natural compounds.

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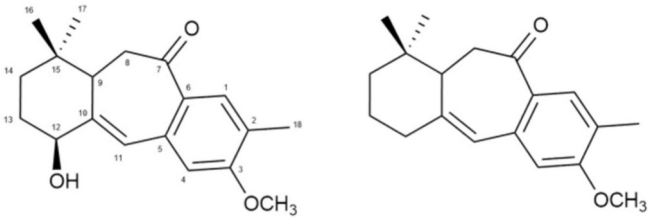

Isofavelol (**1**) Phyllacanthone (**2**)

Fig. 1. Major favelines from the *C. quercifolius* stem-barks extract.

One of the most significant challenges in biotransformation using whole microorganisms is the selection of an active strain capable of performing the desired reaction. Screening an entire microbial library can be a labor-intensive and time-consuming process, as each microorganism's ability to catalyze the specific biotransformation must be individually tested. This involves cultivating the microorganism under sterile conditions, incubating the biomass with the substrate and monitoring the formation of the target product [14]. To streamline this process, several optimization methods have been developed, including combinatorial approaches. They involve testing microorganisms in mixtures, with some strains included in multiple mixtures while others are not [15,16]. When the mixed biomasses are exposed to a substrate, if a single mixture proves to be active, it becomes easier to identify which strain is responsible for the bioconversion. These approaches leverage systematic and parallel testing strategies to efficiently identify active strains, significantly reducing the number of attempts needed and accelerating the discovery of effective biocatalysts for specific bioconversion steps.

In this report, we describe, for the first time, the biotransformation of major favelines (phyllacanthone and isofavelol) from *C. quercifolius* into a new *bis*-norditerpene, using a combinatorial approach. To optimize time and resources, we introduced an original method by initially screening bacterial and fungal strains directly on crude extracts and further confirming their biotransformation ability with the isolated compounds. This rational strategy focused on identifying strains capable of selectively oxidizing the cyclohexyl ring of the favelines' core structure, leading to the production of new compounds with anti-melanoma activity.

2. Material and methods

2.1. Chemicals and general procedures

2.1.1. Materials

Cyclohexane (C-Hex), ethyl acetate (EtOAc), ethanol (EtOH), methanol (MeOH), acetonitrile (ACN) and formic acid were of analytical or HPLC grade (Carlo Erba reagents SSA, Val-de-Reuil, France). Ultrapure water was obtained from the Milli-Q system (Millipore, Burlington, Massachusetts, USA). Thin layer chromatography (TLC) was carried out on silica gel F254 plates (Merck, Darmstadt, Germany). Vanillin for TLC spraying was purchased from Sigma-Aldrich (Darmstadt, Germany). All culture medium ingredients for microbiological assays were purchased from Condalab® (France). Culture medium, antibiotics and fetal calf serum for cytotoxicity assays were from ThermoFisher, while vemurafenib and dacarbazine were procured from Selleckchem® and Sigma-Aldrich®, respectively.

2.1.2. UHPLC-DAD monitoring

During biotransformation experiments, all samples were monitored in a UHPLC-DAD ThermoScientific Dionex U3000 (Thermo-Dionex, Les Ulis, France), equipped with a quaternary pump (LPG-3400 SD), an auto-sampler thermostat (WPS-3000TSL), a column thermostat (TCC-3000SD) and a Diode Array Detector (DAD-3000) on-line. The analytical column was an Acclaim™ Polar Advantage C-18 (250 × 2.1 mm, 3 µm) from Thermo Scientific, heated to 35 °C. Before analyses, samples were filtered on a 0.2 µm nylon filter. Formic acid (0.1 % *v*/v, in water – solvent A) and acetonitrile (solvent B) were employed as mobile phase for a gradient elution as follows: 0–5 min (B, 5 %), 5–55 min (B, 5–95 %), 55–60 min (B, 95 %), 60–65 min (B 95–5 %), 65–70 min (B, 5 %). Isofavelol (**1**) and phyllacanthone (**2**) previously isolated from *C. quercifolius* were used as analytical standards.

2.1.3. Preparative HPLC

Biotransformation products were purified using a preparative HPLC system (Shimadzu, Kyoto, Japan) consisting of an LC-40 delivery system, a Rheodyne manual injection valve, a FRC-40 fraction collector and a SPD-M40 detector (acquisition at 190–400 nm) connected to a SCL-40 control unit. Before injection, samples were dissolved in 1.2 mL of MeOH and filtered on a 0.2 µm nylon filter. After purification on a Nucleodur π^2 biphenylpropyl (250 mm × 32 mm, 5 µm) column (Macherey Nagel), using a gradient mobile phase composed of MeOH in water (60–70 % in 30 min) at a flow rate of 35 mL/min. Recovered fractions were pooled and the solvent was evaporated under reduced pressure.

2.1.4. NMR and HRMS/MS experiments

Nuclear magnetic resonance (NMR) experiments were acquired using $CDCl_3$ or CD_3OD (Eurisotop®, Saint Aubin, France) and conducted on a Bruker™ 400 MHz AVANCE III spectrometer, equipped with a 5 mm direct detection probe and z-axis field gradient. NMR spectra were recorded at 400 MHz for ^1H and two-dimensional (2D) experiments, and at 100 MHz for ^{13}C NMR. Fourier transform, integration, and peak picking were carried out with Bruker TopSpin® 3.6.5 and MestReNova®. ^1H NMR chemical shifts were adjusted according to solvent signals and coupling constants (*J*) were expressed in Hz.

Molecular formulas were determined in a UHPLC-HRMS/MS with an Ultimate 3000-RSLC system (Thermo Scientific) connected to an electrospray ionization – quadrupole – time of flight (ESI-Q-TOF) instrument (Maxis II ETD, Bruker Daltonics), calibrated using a sodium formate solution. The MS detection was carried out in positive mode in the range *m/z* 50–1300. Analyses were performed using collision-induced dissociation in data dependent auto-MS/MS mode. ESI source parameters were set as follows: source voltage at 3500 V, nebuliser pressure at 35 psi, drying gas flow rate at 8 L.min^{-1}, drying gas temperature at 200 °C. MS^2 parameters were as follows: cycle time 3 s, an absolute threshold of 500 counts and MS/MS speed spectra acquisition between 4 and 2 Hz. Selected parent ions were fragmented at collision energy value of between 30 and 50 eV.

2.2. Plant material and extraction

Stem barks of *C. quercifolius* were collected in a typical Caatinga area, located in Petrolina, state of Pernambuco, Brazil (Coordinates: 09°19′43"S / 40°33′09"W). The botanical identification was performed by comparison with a voucher specimen (n° 19,202) previously deposited at the *Herbário Vale do São Francisco* (HVASF), *Universidade Federal do Vale do São Francisco* (UNIVASF). All procedures for access to genetic patrimony and associated traditional knowledge were carried out and the project was registered in SisGen (Register #A65F584). The plant material was dried in an oven with air circulation (40 °C, for 72 h), pulverized (900 g) and then submitted to an exhaustive maceration using ethanol as solvent. The extractive solution was filtered and concentrated using a rotary evaporator, yielding 61 g of ethanolic extract (Cq-EE).

2.3. Purification of favelines

Cq-EE was fractioned by flash liquid chromatography, using an

Interchim Puriflash PF-5-250 system, equipped with DAD, MS and ELSD detectors. Cq-EE (400 mg) was solubilized in methanol and added to 2 g of normal phase silica (30 μm, Sigma-Aldrich®, France). The mixture was homogenized manually until complete solvent evaporation and then placed in a dry-load cartridge. The dry-load cartridge containing Cq-EE-adsorbed silica was coupled on the top of a PF-30SIHP-F0080 column (normal phase, 80 g, 30 μm) and eluted with a mobile phase composed of a binary solvent gradient: A (C-Hex) and B (AcOEt). The gradient flow program was set as follows: 0–8 min, 97.7–96.9 % A; 8–19.5 min, 96.9–93.7 % A; 19.5–37.5 min, 93.7–77.3 % A; 37.5–47.3 min, 77.3–62 % A; 47.4–62 min, 62 % A. The flow rate was 34 mL/min and elution was monitored at 254 nm, with an automatic collector (20 mL per tube). Peaks with λ_{max} typical of favelines (255–260 nm) and presenting *m/z* 284 (phyllacanthone, **2**, Rt 11.20 min) and 300 (isofavelol, **1**, Rt 48.30 min) were collected, analyzed by TLC and characterized by NMR.

2.4. Biotransformations

2.4.1. Screening of active strains

Screening experiments were carried out in resting cells conditions, directly on the *C. quercifolius* extract (Cq-EE). Bacteria and fungi (Table 1) were tested using our method based on combinatory library of microorganisms (CLM) [16]. Cultures were prepared in Erlenmeyer flasks (250 mL) filled with YMS culture medium (100 mL) containing (in g/L): yeast extract 4, malt extract 10, soybean peptone 5 and glucose 16. Microorganisms were grown at 26 °C on rotary shaker (160 rpm). After 72 h, biomasses were recovered by filtration for filamentous fungi and centrifugation for bacteria. Biomasses of bacteria and fungi were suspended in 70 mL of citrate buffer (0.1 M, pH 5.0) and four mixtures (M1–4) were prepared, as described in Table 1. Cq-EE (70 mg, in DMF) was added to each mixture (Cq-EE final concentration of 1 mg/mL) and then kept under incubation conditions for 7 days. A strain control (M1–4 without Cq-EE) and an extract control (without any strain) were prepared under the same conditions. Aliquots from each experimental (M1–4) and control conditions were collected every 24 h for UHPLC-DAD monitoring and TLC analysis. A mixture was considered active when new peaks were detected on chromatograms.

2.4.2. Preparative biotransformation by Umbelopsis isabellina

U. isabellina was cultured in YMS medium (500 mL) as described above. After harvest, the biomass (90 g) was resuspended in citrate buffer (0.1 M, pH 5.0) and Cq-EE (500 mg, solubilized in DMF) was added in the flask to be incubated in the same conditions as in screening experiments. After 24 h of incubation, the reactional medium was centrifuged (10.000 rpm, 25 °C, 5 min) and the cell supernatant and pellet were separated. Biotransformation products were extracted from the cell supernatant by successive liquid-liquid partition using EtOAc (3 × 250 mL) as solvent, while the cell pellet was submitted to ultrasound-assisted extraction (15 min, 3 × 250 mL). Organic fractions were treated with Na_2SO, filtered and then the solvent as evaporated under reduced pression. Both fractions (cell supernatant and pellet) were analyzed by TLC and UHPLC-DAD and then purified by preparative HPLC to give **3** (7.5 mg).

Table 1
Distribution of fungal and bacterial strains in different mixtures (M1–4), using the combinatory library of microorganisms (CLM) approach.

Strain	CLM			
	M1	M2	M3	M4
Streptomyces griseus NRRL B150	X			X
Nocardia corallina ATCC 4273	X			X
Rhizopus arrhizus ATCC 11145	X			
Mucor plumbeus CBS 110–16	X	X		
Aspergillus niger ATCC 9142	X	X		
Cunninghamella echinulata ATCC 9245		X		
Nocardia opaca ATCC 4276		X	X	
Rhodococcus erythropolis ATCC 4277		X	X	
Fusarium roseum ATCC 14717			X	
Curvularia lunata NRRL 2380			X	X
Bipolaris cactivora [14]			X	X
Umbelopsis isabellina NRRL 1757				X

2.4.3. Analytical biotransformation on purified favelines

Biotransformation by *U. isabellina* was performed on the major favelines isolated from Cq-EE. After incubation in the culture conditions detailed before (2.4.1. topic), biomass (1.0 g) was transferred into two flasks containing citrate buffer (0.1 M, pH 5.0, 30 mL). Phyllacantone (10 mg) and isofavelol (10 mg) dissolved in DMF were separately added to the flasks and kept at 26 °C on a rotatory shaker for 24 h. The reactional medium was then centrifuged and the cell pellet was submitted to ultrasound-assisted extraction with AcOEt (15 min, 3 × 25 mL). The extracts were submitted to UHPLC-DAD analysis to determine the presence of compound **3**.

2.5. Anti-melanoma activity

Compound **1** was evaluated on A2058 cells (ATCC® CRL-11147™), expressing the $BRAF^{V600E}$ oncogenic mutation. These are highly invasive and metastatic human melanoma cells, chemoresistant to some conventional anticancer drugs, such as dacarbazine [17]. Cells were grown in 75 cm^2 flasks containing DMEM culture medium supplemented with 10 % fetal calf serum (FCS) and 1 % antibiotics (penicillin-streptomycin) (ThermoFischer, France). Cells were kept at 37 °C in a 5 % CO_2 humidified atmosphere during all experiments. Samples were solubilized in DMSO and then diluted in the cell culture medium. Initially, melanoma cells (2000/well) were added into 96-well microplates and exposed to increasing concentrations of each compound (1–100 μM) for 72 h. The final DMSO concentration was equal to or lower than 1 % and tested as the negative control. After treatment, cell viability was determined by the MTT assay as previously described [18,19] and the IC_{50} value was calculated from at least three independent measurements.

To evaluate the ability of compound **3** to sensitize A2058 cells to chemotherapy, several concentrations (1–100 μM) of vemurafenib (BRAF inhibitor, Selleckchem®, France) and dacarbazine (DNA alkylating agent, Sigma-Aldrich®, France) were tested alone or combined with a compound **3** sub-toxic concentration (30 μM, $<½IC_{50}$). After 72 h of treatment, cell viability was measured by the MTT assay and results were expressed as IC_{50}.

2.6. Statistical analysis

Data were expressed as mean ± standard error of the mean and analyzed by unpaired Student's *t*-test ($n = 3$ or more) using the software GraphPad Prism® 8.0.1. Values of $p < 0.05$ were considered statistically significant. IC_{50} values were calculated by nonlinear regression analysis.

3. Results & discussion

3.1. Screening of active strains

The CLM method was evaluated for screening active strains capable of biotransforming favelines from *C. quercifolius* into targeted compounds with an oxidized cyclohexane ring. In this initial step, four pre-established mixtures (M1-M4) were tested directly on the crude extract, which is rich in favelines, particularly isofavelol (**1**) and phyllacanthone (**2**). These two markers were identified in the extract by comparing their retention times and UV spectra to analytical standards under identical UHPLC-DAD conditions. Testing the mixtures directly on the crude extract enabled the identification of active mixtures prior to conducting bioconversion experiments on purified compounds, thereby optimizing time and resource usage.

As shown in Fig. 2A, the plant extract remained stable under biotransformation conditions (citrate buffer, pH 5.0, 26 °C) for 7 days, with no evidence of spontaneous degradation. However, exposure to mixture M4 produced new peaks with λ_{max} values characteristic of favelines (~260 nm) [4] between 7 and 15 min, indicating that this mixture was the only one capable of converting favelines into new and more polar compounds (Fig. 2B). Notably, as summarized in Table 2, *U. isabellina* was the only fungal strain unique to M4, suggesting its likely role as the active biocatalyst. To confirm this hypothesis and isolate the new biotransformation products, a preparative biotransformation step was conducted using *U. isabellina* as the sole biocatalyst.

3.2. Biotransformation by U. Isabellina

The incubation of Cq-EE in the presence of *U. isabellina* biomass was performed over 24 h. As shown in Fig. 3, a chemical profile similar to that of M4 incubated with Cq-EE (Fig. 2B) was observed, confirming that *U. isabellina* was the strain responsible for the biotransformation reaction. The same major biotransformation compound (**3**) was detected in both cell supernatant and pellet (Rt 10.3 min), while the peaks initially attributed to *C. quercifolius* favelines were completely consumed. This compound was subsequently purified using preparative HPLC, as described in the Materials and Methods section (2.1.3), yielding 7.5 mg of an amorphous white powder.

Compound **3** exhibited a characteristic UV absorption profile for favelines [4], with λ_{max} values at 257 and 303 nm. The HR-MS/MS spectrum revealed an *m/z* ion at 317.1741 $[M + H]^+$ (Δ = 3.78 ppm compared to the theoretical ion), consistent with the molecular formula $C_{19}H_{24}O_4$ (316.1674 g/mol). This data suggests an isofavelol-like structure, incorporating an additional hydroxyl group. The presence of two consecutive *m/z* M-18 losses further supports this hypothesis.

^1H and ^{13}C NMR spectra of **3** exhibited typical signals for a 2-methyl-3-methoxy benzocycloheptene core [10], such as the signals for H-1 (δ 7.63 s, 1H), C-1 (δ 130.9), H-4 (δ 6.89 s, 1H), C-4 (δ 113.8), H-9 (δ 2.91 t, 1H), C-9 (δ 42.9), H-8 (δ 3.05–3.08 m, 2H), C-8 (δ 41.2), H-18 (δ 2.20 s, 3H), C-18 (δ 14.3) and and -OCH_3 (δ_H 3.93 s, 3H, δ_C 54.7). A signal at δ_H 4.43 m (1H), typical of the isofavelol 12-OH group [7], along with another signal at δ_H 3.78 dd (1H, *J* = 4.0 and 12.0 Hz), was detected.

Table 2
Cytotoxic activity of compound **3** (14-OH-Iso), vemurafenib (Vemu), dacarbazine (Daca) and combined treatments (Vemu +14-OH-Iso and Daca +14-OH-Iso). Data are expressed as IC_{50} values and 95 % confidence interval (CI), from at least three measurements (*n* = 3). For combined treatments, increasing concentrations of vemurafenib or dacarbazine (1–100 μM) were associated to subtoxic concentration (<½IC_{50}) of 14-OH-Iso (30 μM).

Monotherapy	IC_{50} (μM, CI)	Combined therapy	IC_{50} (μM, CI)
14-OH-Iso (**3**)	68.8 (60.2–72.1)	–	–
Daca	> 100	Daca +14-OH-Iso (30 μM)	> 100
Vemu	24.8 (20.1–30.5)	Vemu +14-OH-Iso (30 μM)	9.63 (7.4–12.5)

Both were attributed to protons linked to oxidized carbons in the cyclohexane ring. HMBC correlations, shown in Fig. 4A, enabled the assignment of the hydroxyl group positions at C-12 and C-14, while NOESY correlations (Fig. 4B) helped propose their absolute configuration. Together, these data led to the unequivocal identification of compound **3** as 14-hydroxy-isofavelol, reported here for the first time in the literature. All spectra (NMR, MS and UV) are available in supplementary material.

To the best of our knowledge, this is the first report of a combinatorial microbial screening approach being successfully applied to the biotransformation of target compounds directly within a crude plant extract. This method enabled the identification of *U. isabellina* as an active strain capable of selectively oxidizing the faveline core structure without the need for prior purification of individual components. The crude extract of *C. quercifolius* represents a chemically complex matrix, often challenging for selective microbial bioconversion due to the presence of multiple potentially competing substrates. Nevertheless, the combinatorial logic-based methodology allowed efficient strain selection by narrowing down the active strain from a small set of mixed cultures. This streamlined approach significantly reduced time and resource demands while preserving the selectivity and complexity of the biotransformation environment, highlighting its potential as a practical strategy for the semi-synthesis of natural product derivatives from complex plant matrices.

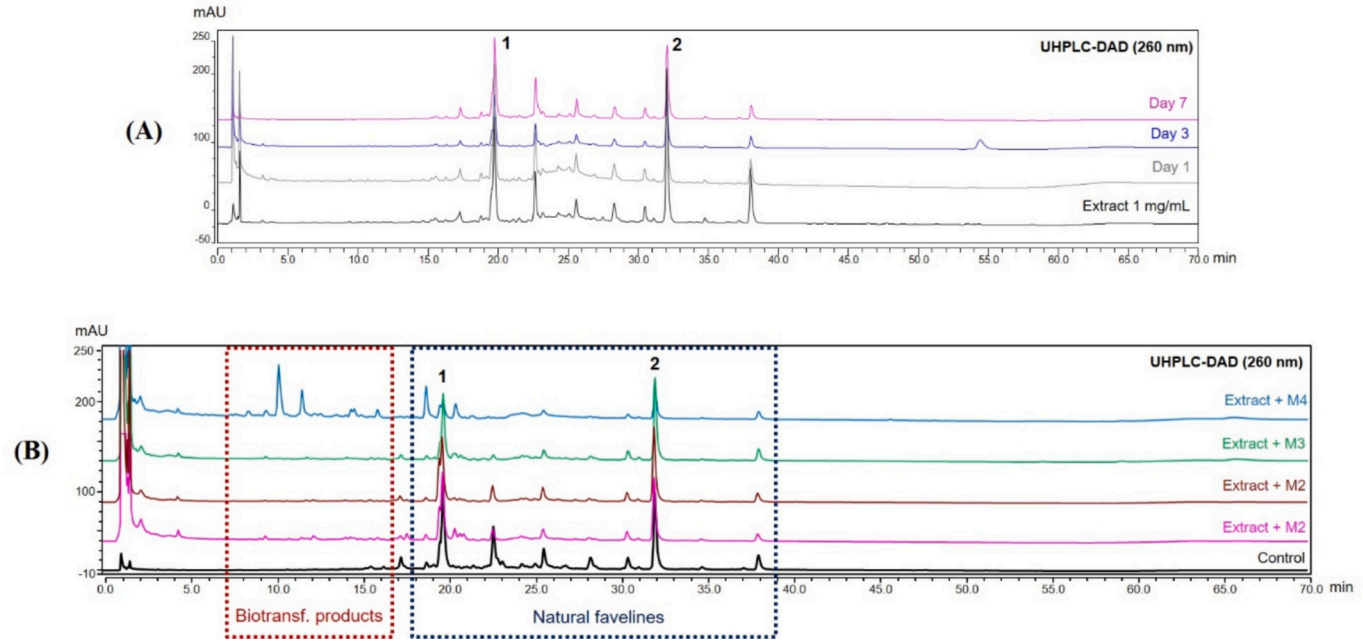

Fig. 2. UHPLC-DAD monitoring of biotransformation steps of Cq-EE (plant extract) in citrate buffer (pH 5.0) alone (A) or in the presence of microorganisms mixtures (M1-M4) (B) after 7 days of incubation (26 °C, 160 rpm). **1** and **2** correspond to the major favelines in Cq-EE: isofavelol and phyllacanthone, respectively. Control (B) represents the plant extract (Cq-EE) under reactional conditions without any microbial strain.

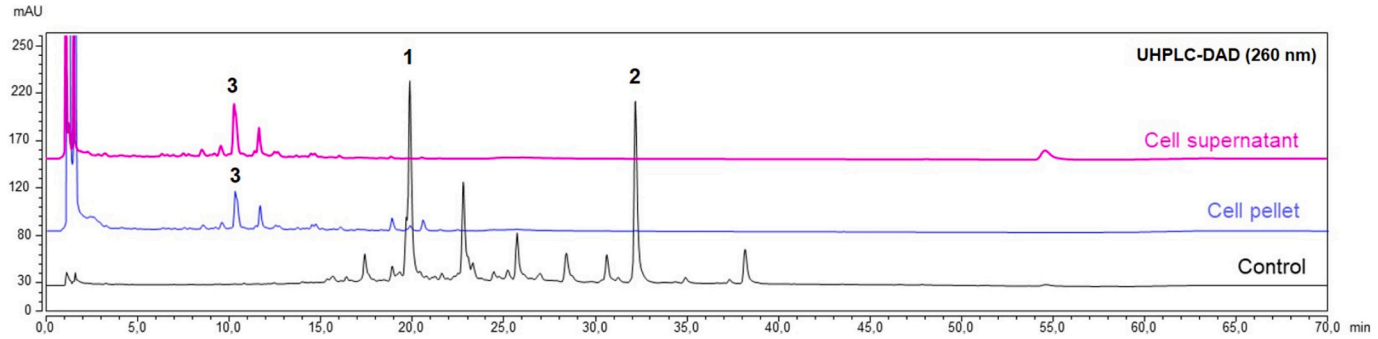

Fig. 3. UHPLC-DAD monitoring of the biotransformation of Cq-EE in citrate buffer (pH 5.0) was conducted either alone (control) or in the presence of *U. isabellina* after 24 h of incubation (26 °C, 160 rpm). Compounds **1** and **2** represent the major favelines in Cq-EE (isofavelol and phyllacanthone, respectively), while compound **3** corresponds to the primary biotransformation product detected in both the cell supernatant and the pellet.

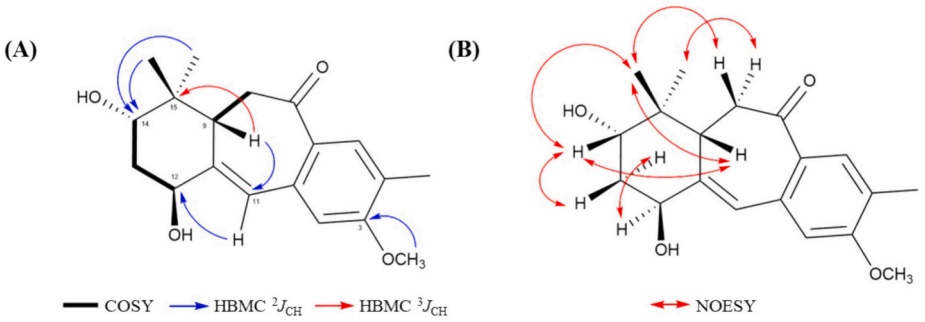

Fig. 4. Key COSY, HMBC (A) and NOESY (B) correlations of 14-hydroxy-isofavelol (**3**).

3.3. Biotransformation on isofavelol (1) and phyllacanthone (2)

To identify which faveline from Cq-EE was being used as a substrate by *U. isabellina*, we performed an analytical biotransformation on isofavelol (**1**) and phyllacanthone (**2**). These compounds were selected because they represent the major components of the crude extract. Subsequently, a purification step was carried out using flash chromatography, as described in section 2.3.

As shown in Fig. 5, a multi-detector (MS-UV-ELSD) flash chromatography separation allowed the fractionation of Cq-EE into ten fractions (F1–F10). Due to their differing polarities, phyllacanthone (F2, 10 mg) and isofavelol (F10, 21.2 mg) were successfully isolated in a single run. Their chemical structures were confirmed by ^{1}H and ^{13}C NMR experiments in comparison with literature data [7,10].

Both favelines were then incubated individually (as controls) and in the presence of the fungus for 24 h, under the same reaction conditions

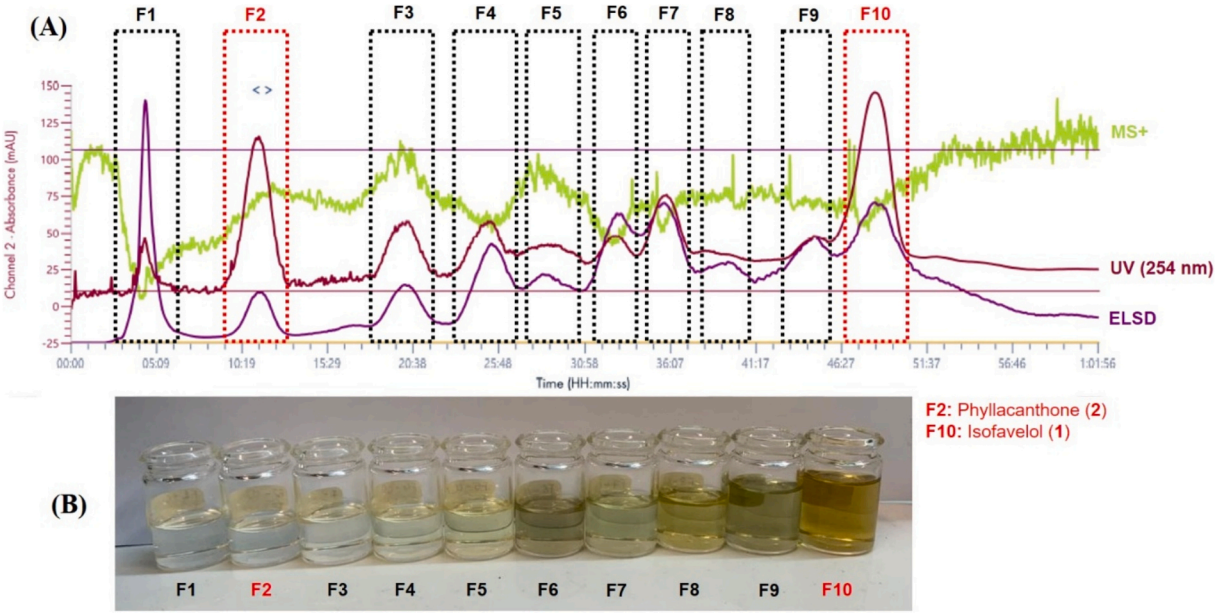

Fig. 5. MS(+)-UV-ELSD chromatogram obtained from the fractionation of Cq-EE by flash chromatography. Fractions F2 and F10 were pure and corresponded to phyllacanthone (**2**) and isofavelol (**1**), respectively.

previously described (section 2.4.3). UHPLC-DAD chromatograms of the extracts from cell pellets (Fig. 6) confirmed the formation of the same major compound (**3**) by the *U. isabellina*, demonstrating that the strain can recognize the basic faveline skeleton as a substrate and selectively convert it into the same product: 14-hydroxy-isofavelol (**3**).

3.4. 14-hydroxy-isofavelol (3) sensitizes melanoma cells to vemurafenib

Finally, we evaluated the anti-melanoma potential of compound **3**. Favelines have demonstrated promising cytotoxic activity against various cancer cell lines, including chemoresistant human melanoma cells. The new *bis*-norditerpene exhibited moderate cytotoxic activity after 72 h of treatment, with an IC_{50} of 68.8 μM (Table 2). A2058 cells were resistant to treatment with dacarbazine (IC_{50} > 100 μM) but sensitive to the cytotoxic effects of vemurafenib. Both drugs are clinically used to treat metastatic melanoma [21,22].

To investigate a potential chemosensitizing effect, cells were exposed to vemurafenib or dacarbazine in combination with a sub-toxic concentration (<½IC_{50}) of compound **3**. As shown in Fig. 7A and Table 2, compound **3** effectively sensitized melanoma cells to vemurafenib, reducing its IC_{50} (9.63 μM *vs.* 24.8 μM). Photomicrographs revealed a marked decrease in cell density compared to vemurafenib treatment alone, along with significant morphological changes induced by the combined therapy, such as cell shrinkage and rounding. In contrast, no significant enhancement of the antiproliferative effect was observed with the combination of dacarbazine and 14-OH-Iso (**3**) (Table 2).

The identification of **3** as a novel *bis*-norditerpene obtained *via* microbial biotransformation expands the chemical diversity of favelines and underscores the potential of biocatalysis to generate structurally novel and pharmacologically relevant derivatives. The selective oxidation of the cyclohexyl ring by *U. isabellina* demonstrates the practicality of microbial catalysis for regioselective modification of complex natural products, using a scalable and eco-friendly approach. Although compound **3** showed only moderate cytotoxicity as a single agent, it exhibited a significant chemosensitizing effect in BRAFV600E-mutated

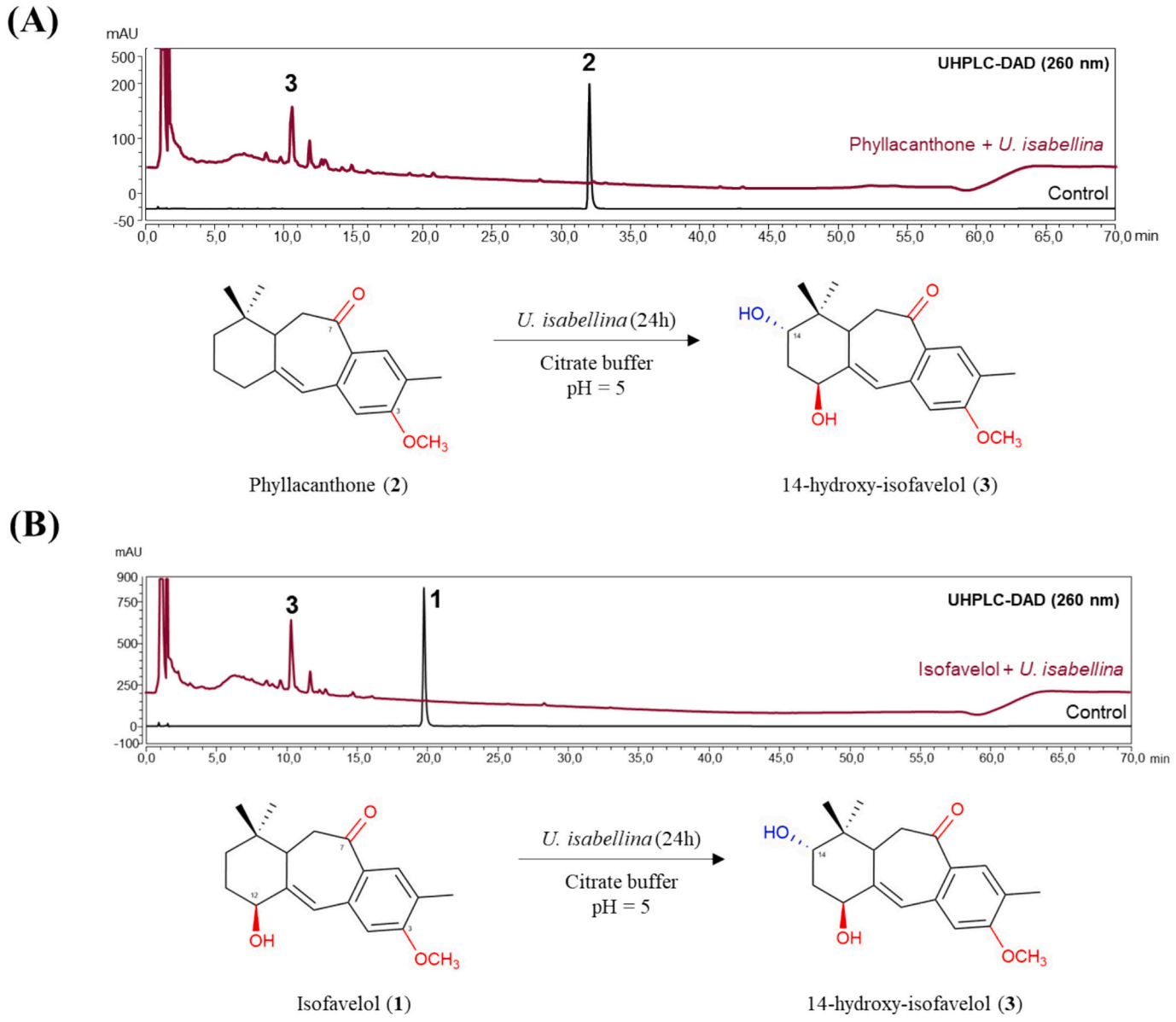

Fig. 6. UHPLC-DAD monitoring of the biotransformation of phyllacanthone (**2**, A) and isofavelol (**1**, B) in citrate buffer (pH 5.0) was conducted either alone (control) or in the presence of *U. isabellina* after 24 h of incubation (26 °C, 160 rpm). Compound **3** corresponds to the major biotransformation product detected in the cell pellet.

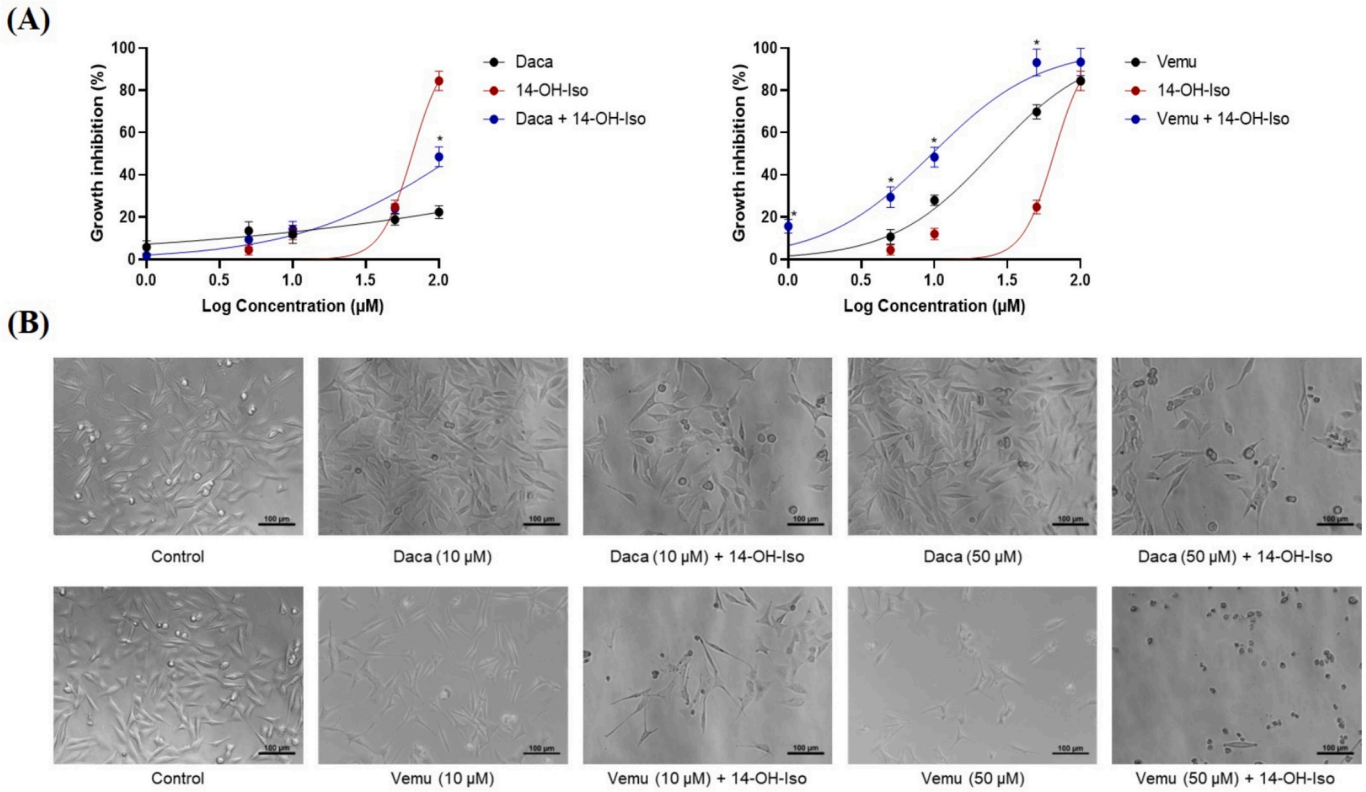

Fig. 7. Cytotoxic activity of 14-OH-Iso (**3**), dacarbazine (Daca), vemurafenib (Vemu) and the respective combined treatments in the MTT assay (A). A2058 cells were grown for 72 h with increasing concentrations of the anti-melanoma drugs (1–100 μM) in the absence or presence of 14-OH-Iso (<½IC_{50}, 30 μM). Photomicrographs (B) show reduction of cell density promoted by combined therapies compared to monotherapies and control untreated cells. Data are presented as mean ± SEM, *$p <$ 0.05 according to unpaired Student's *t*-test, from at least three measurements.

melanoma cells, reducing the IC_{50} of vemurafenib and suggesting a synergistic interaction. This points to **3** as a promising adjuvant candidate to enhance the efficacy of existing targeted therapies in drug-resistant tumors. Overall, these findings highlight the translational value of faveline scaffolds and reinforce the utility of microbial transformation as a strategic tool in anticancer drug discovery.

4. Conclusions

This study highlights the effectiveness of a combinatorial screening approach for identifying active microbial strains capable of specific biotransformations. By applying this strategy directly to the crude extract of *C. quercifolius*, we efficiently identified *U. isabellina* as the optimal strain for regioselective oxidation of the cyclohexane ring in the faveline skeleton, reducing the time and resources required compared to traditional methods. The biotransformation yielded a new *bis*-norditerpene, 14-hydroxy-isofavelol. This compound demonstrated moderate cytotoxic activity against chemoresistant melanoma cells and significantly enhanced the efficacy of vemurafenib, a key drug in melanoma treatment. The findings not only underscore the utility of microbial biotransformation in generating bioactive natural product derivatives but also validate the combinatorial method as a powerful tool for strain selection in complex biocatalytic processes.

CRediT authorship contribution statement

Sovannychloé Nai: Writing – original draft, Methodology, Investigation, Formal analysis, Data curation. **Thomas Gaslonde:** Writing – original draft, Methodology, Investigation, Formal analysis, Data curation. **Julia Krystyańczuk:** Methodology, Investigation, Formal analysis, Data curation. **Séverine Amand:** Writing – original draft, Investigation, Formal analysis. **Laurent Picot:** Writing – review & editing, Resources, Methodology, Investigation, Data curation. **Carlos Arthur Gouveia Veloso:** Methodology, Investigation, Formal analysis, Data curation. **Didier Buisson:** Writing – review & editing, Visualization, Validation, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Marie-Christine Lallemand:** Writing – review & editing, Validation, Supervision, Resources, Project administration, Investigation, Conceptualization. **Raimundo Gonçalves de Oliveira Junior:** Writing – review & editing, Writing – original draft, Supervision, Project administration, Methodology, Investigation, Funding acquisition, Formal analysis, Data curation, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.

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