Supporting Information

Benzoquinones and Terphenyl Compounds as Phosphodiesterase-4B Inhibitors from a Fungus of the Order Chaetothyriales (MSX 47445)

Tamam El-Elimat, Mario Figueroa, Huzefa A. Raja, Tyler N. Graf, Audrey F. Adcock, David J. Kroll, Cynthia S. Day, Mansukh C. Wani, Cedric J. Pearce, and Nicholas H. Oberlies

Producing Organism and Fermentation

Experimental Protocol for the Phosphodiesterase Inhibitor Assay

Figure S1. UPLC chromatograms of compounds **1-3**.

Figure S2. ^{1}H NMR of betulinan C (**3**) [500 MHz, $CDCl_3$].

Figure S3. ^{13}C NMR of betulinan C (**3**) [125 MHz, $CDCl_3$].

Figure S4. Comparison between the binding position of rolipram within the crystal structure (grey) and the binding mode predicted by Glide (orange).

Figure S5. Two-dimensional interaction map of the optimized docking model of compound **1** (a), **2** (b), and rolipram (c) in the cAMP binding pocket of PDE4B. Amino acid residues within 4.5 Å of the ligand are displayed. Blue and green arrows indicate hydrogen bonding to amino acid side chain and main chain atoms, respectively.

Figure S6. Phylogram of the most likely tree (-lnL = 4401.180) from a RAxML analysis of 53 taxa based on partial region of the 28S large subunit nrDNA (1296 bp). Numbers refer to RAxML bootstrap support values ≥ 70% based on 1000 replicates. MSX 47445 is shown in bold.

Table S1. Cytotoxicity and Antimicrobial Activities of Compounds (**1-3**)

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The culture was stored on a malt extract slant and was transferred periodically. A fresh culture was grown on a similar slant, and a piece was transferred to a medium containing 2% soy peptone, 2% dextrose, and 1% yeast extract (YESD media). Following incubation (7 d) at 22 °C with agitation, the culture was used to inoculate 50 mL of a rice medium, prepared using rice to which was added a vitamin solution and twice the volume of rice with H_2O, in a 250 mL Erlenmeyer flask. This was incubated at 22 °C until the culture showed good growth (approximately 14 d). The scale-up culture was grown in a 2.8 L Fernbach flask containing 150 g of rice and 300 mL of H_2O and was inoculated using a seed culture grown in YESD medium. This was incubated at 22 °C for 14 d.

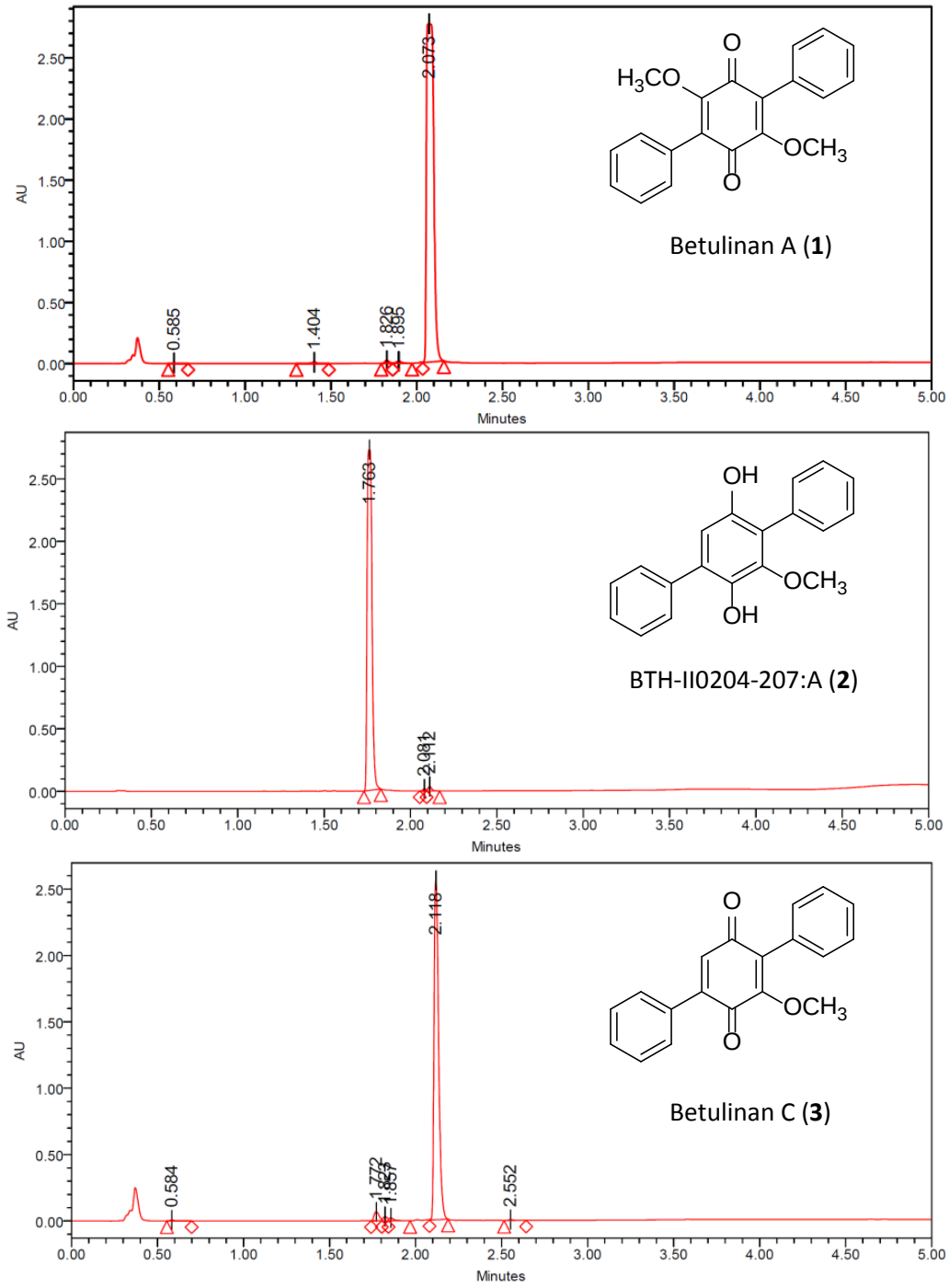
For extraction of genomic DNA of fungal strain MSX 47445, mycelium from axenic cultures grown in YESD broth was scraped with a sterile scalpel and ground to a fine powder in liquid N_2 using a mortar and pestle. Approximately 400 µL of AP1 buffer from the DNAeasy Plant Mini Kit (QIAGEN Inc.) was added to the mycelial powder, and DNA was extracted following the manufacturer's instructions. The DNA was eluted in approximately 25–30 μL distilled H_2O. The complete ITS region, along with the partial region of divergent domains D1/D2 of the large subunit of the 28S nuclear ribosomal DNA (LSU), were amplified with ITS1F and LR3 by PCR using puReTaq™ Ready-To-Go PCR beads (Amersham Biosciences Corp.). The PCR products were sequenced subsequently in a 11 μL sequencing reaction with BigDye® Terminators v3.1 (Applied Biosystems) using ITS primers ITS1F and ITS4[1,2] and LSU primers LROR and LR3.[3,4] For PCR, the following protocol was used: initial denaturation at 95 °C for 5 min, followed by 35 or 40 cycles of 95 °C for 30 s, 41 or 50 °C for 15 s, and 72 °C for 1 min with a final extension step of 72 °C for 10 min. To enhance the PCR reactions, 2.5 μL of BSA (bovine serum albumin,

New England Biolabs) and/or 2.5 μL of DMSO were added. The PCR products were purified to remove excess primers, dNTPs, and nonspecific amplification products with the QIAquick PCR Purification Kit (QIAGEN Inc.). Sequences were generated on an Applied Biosystems 3730XL high-throughput capillary sequencer at the University of Illinois Urbana-Champaign Biotech facility.

The complete ITS sequence, including both spacers and the 5.8S region of MSX 47445 (~644 base pairs), was compared with GenBank's database[5] using the Blastn search.[6] The Blast search revealed *Cyphellophora eucalypti* Cheew & Crous (GQ303274) as the closest match (query coverage of 100%, and a sequence similarity of 89%). To be considered conspecific based on ITS data, studies of Ascomycota fungi have used a $\geq$ 97–98% or 99% cut off as a proxy for species level identification.[7,8] Since, *C. eucalypti*, a member of Chaetothyriales, (Chaetothyriomycetidae, Eurotiomycetes, Ascomycota) was the closest match in the GenBank and shared 89% sequence similarity with the ITS sequence, MSX 47445 had affinities only to the level of the order Chaetothyriales. The variable D1/D2 regions of LSU of MSX 47445 (~ 600 bp) were subjected to a Blast search in GenBank. Blast search with the D1/D2 region also revealed *C. eucalypti* (GQ303305) as the closest match with MSX 47445 (query coverage of 96% and a sequence similarity of 99%). These sequences were downloaded and a Maximum Likelihood (ML) analysis was performed to determine the phylogenetic affinities of MSX 47445 with members of the Chaetothyriales. Multiple sequence alignment and phylogenetic analysis was performed following programs reviewed previously.[9] Results of the ML phylogenetic analysis indicated that MSX 47445 shared phylogenetic affinities with *C. eucalypti* but without significant bootstrap support values (See Supplementary Figure S6).

Experimental Protocol for the Phosphodiesterase Inhibitor Assay.

The PDE inhibitor assay was performed at BPS Bioscience Inc. A series of dilutions of the test compounds were prepared with 10% DMSO in assay buffer, and 5 μL of the dilution were added to a 50 μL reaction so that the final concentration of DMSO was 1% in all of the reactions. The enzymatic reactions were conducted at rt for 60 min in a 50 μL mixture containing PDE assay buffer, 100 nM FAM-cAMP, PDEB2 enzyme and the test compound. After the enzymatic reaction, 100 μL of a binding solution (1:100 dilution of the binding agent with the binding agent diluent) were added to each reaction, and the reaction was performed at rt for 60 min. Fluorescence intensity was measured at an excitation of 485 nm and an emission of 528 nm using a Tecan Infinite M1000 microplate reader.



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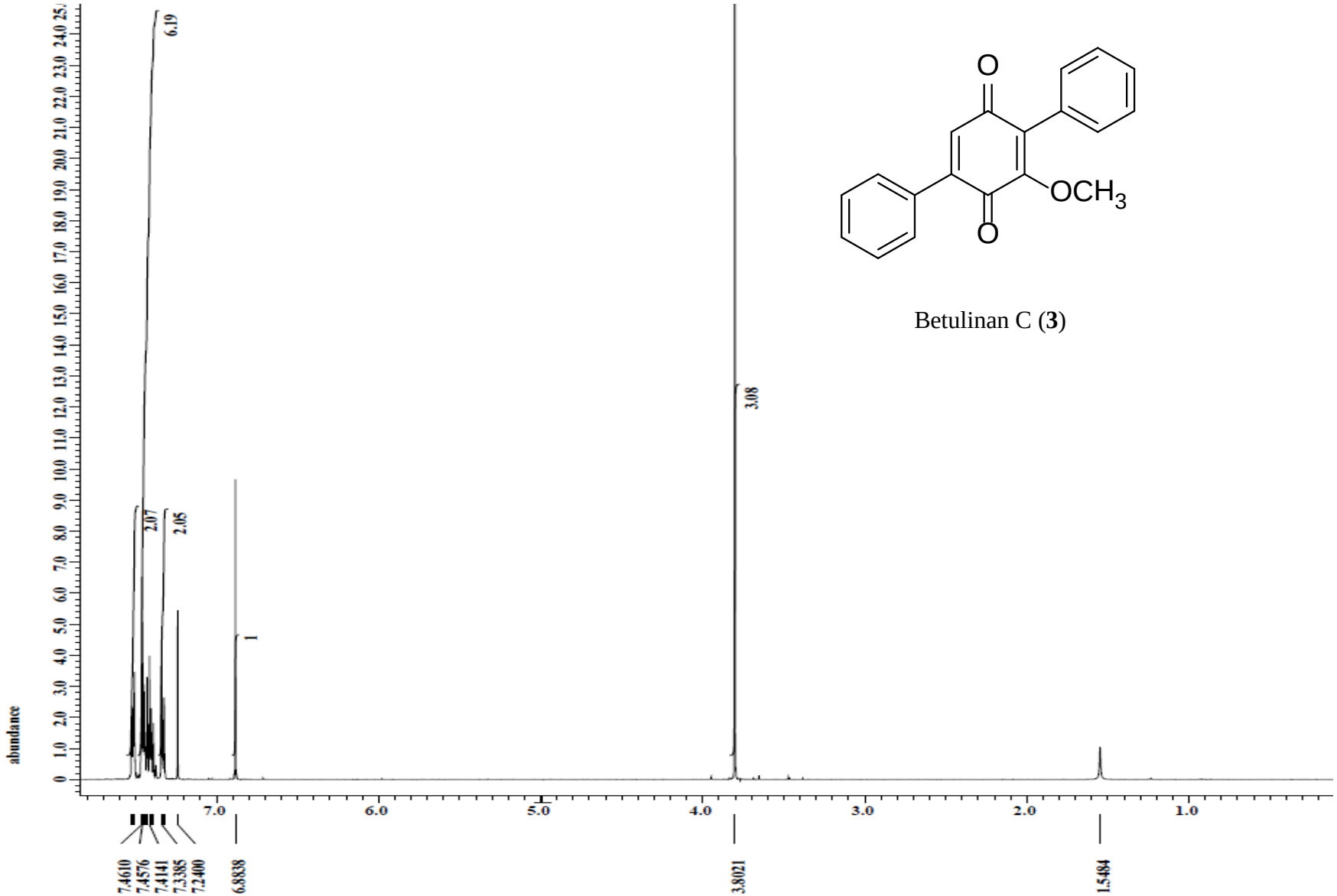


Betulinan C (**3**)

Figure S2. ^{1}H NMR (500 MHz, $CDCl_3$) of betulinan C (**3**).

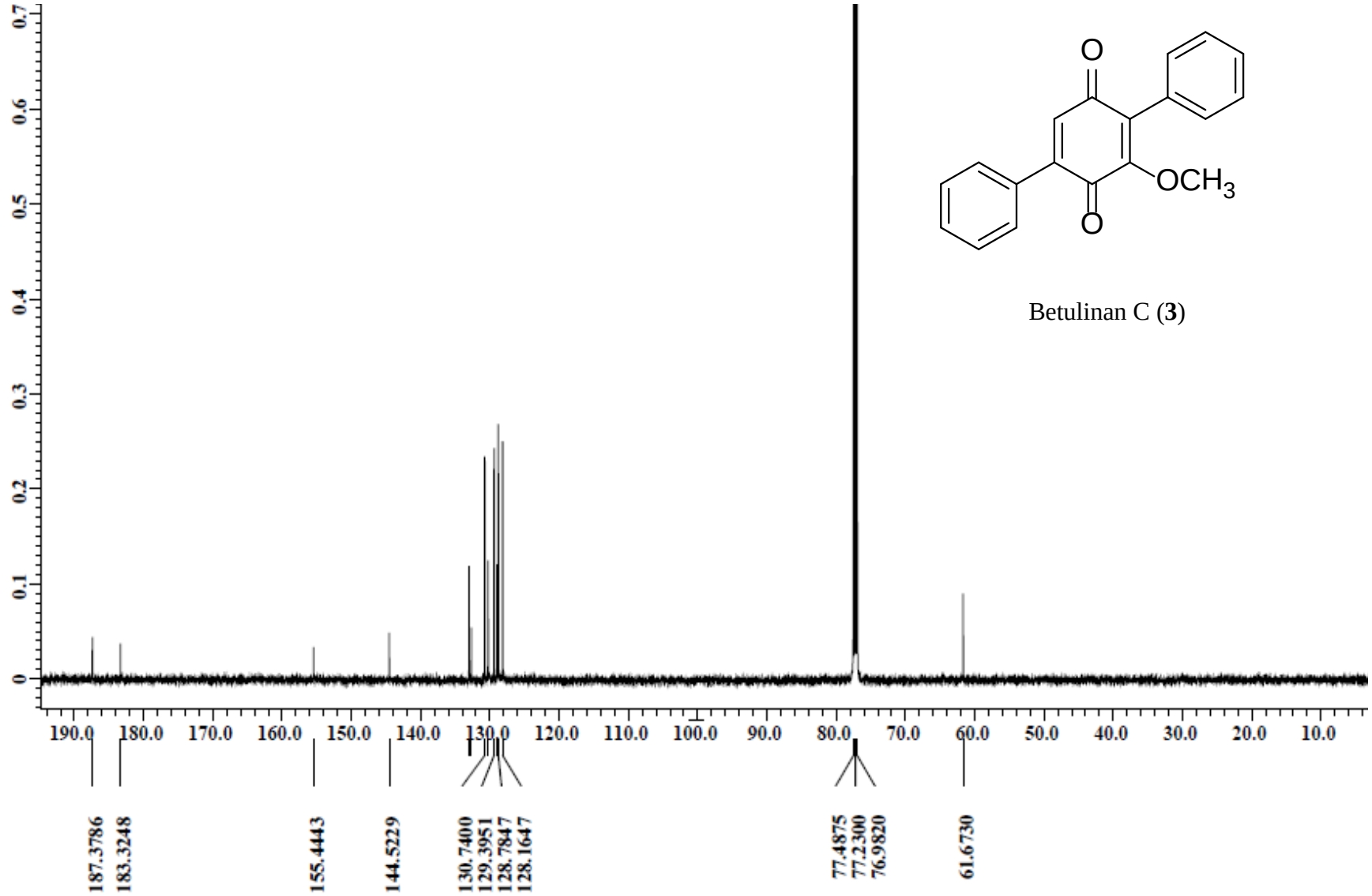
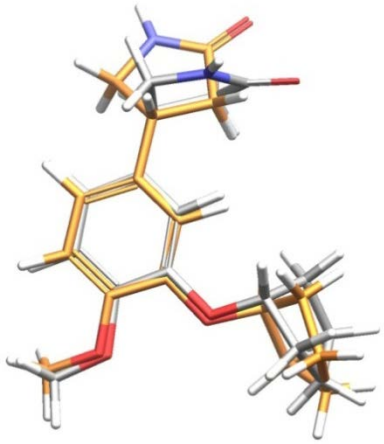


Figure S3. ^{13}C NMR (125 MHz, $CDCl_3$)of betulinan C (**3**).



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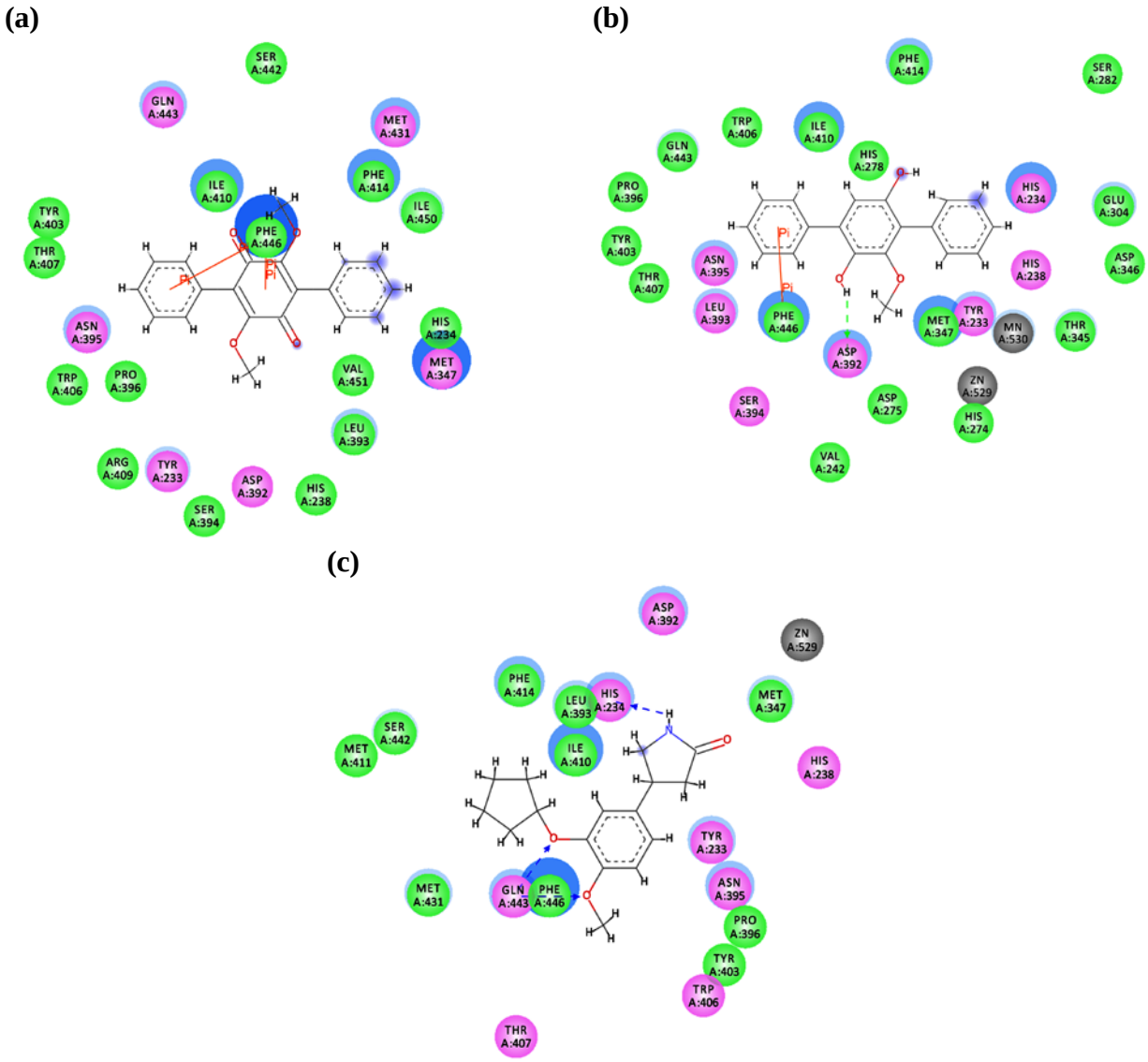

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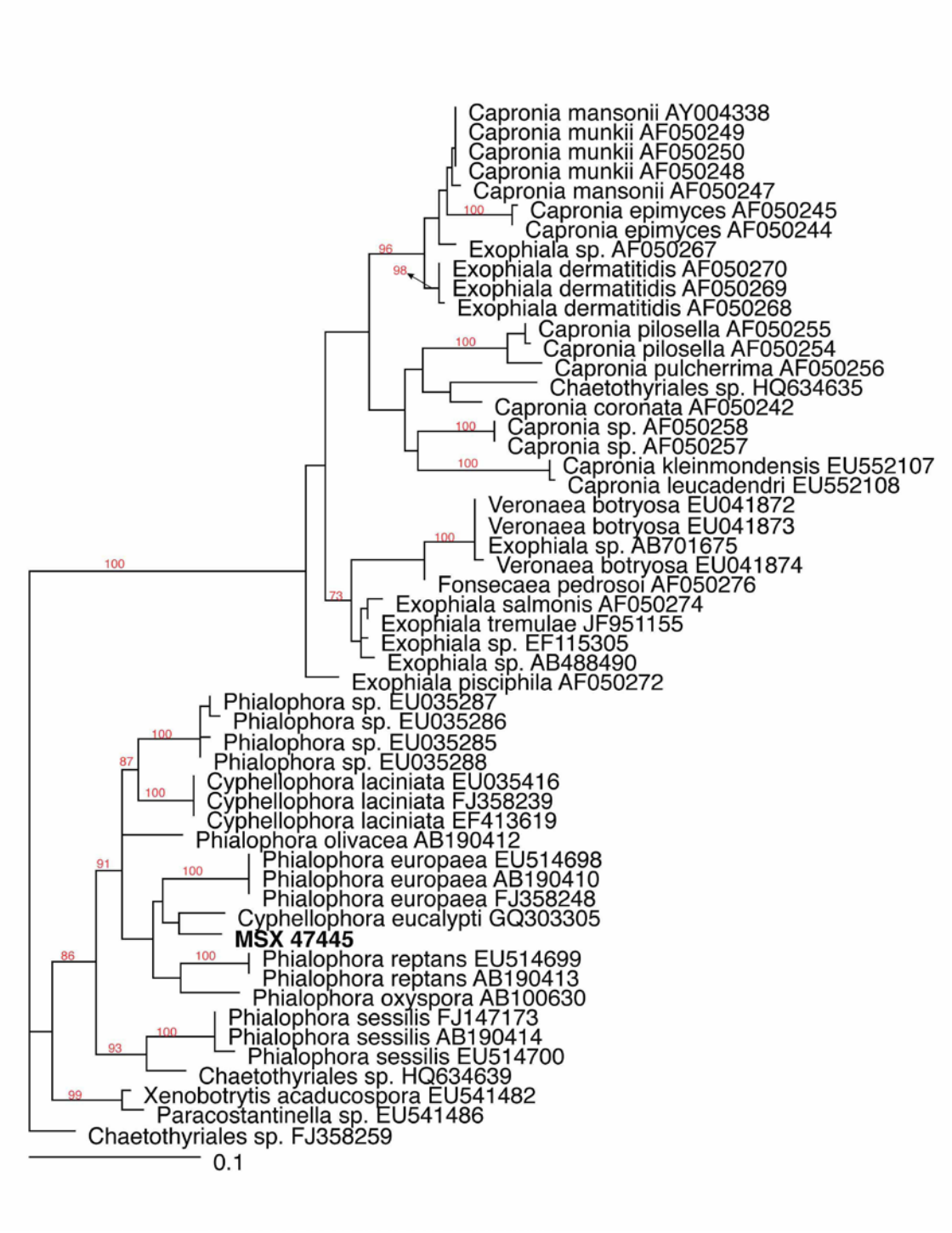

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Note: MSX 47445 shows phylogenetic affinities to *Cyphellophora eucalypti* (GQ303305) but without significant bootstrap support.

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Compound	Cytotoxicity IC_{50} (μM)			MIC (μg/mL)	
	MCF-7	H460	SF268	*C. albicans*	*S. aureus*
betulinan A (**1**)	100.0	101.2	79.7	>150	>150
BTH-II0204-207:A (**2**)	39.0	21.8	38.8	>150	25
betulinan C (**3**)	26.1	19.5	32.8	100	25
camptothecin[a]	0.1	0.0	0.0	---	---
amphotericin B[a]	---	---	---	<0.025	---
novobiocin[a]	---	---	---	---	<0.025

[a]positive controls

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