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POLYKETIDE-DERIVED SECONDARY METABOLITES FROM *DOTHIDEOMYCETES* SP

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*Discovering alternative compounds for treating antibiotic-resistant infections is critical to alleviate the current antibiotic resistance crisis. Endophytic fungi are a source of numerous recently approved antibiotics particularly polyketide-derived molecules. This study aimed to isolate and identify antimicrobial compounds from Dothideomycetes sp. 11144 as well as molecular docking study for their affinity to different Methicillin-resistant Staphylococcus aureus (MRSA) selected proteins. Herein, we describe the isolation of four polyketide-type metabolites, a new natural α-pyrone derivative, (R)-6-(2-hydroxypropyl)-4-methoxy-2H-pyran-2-one (**1**) along with three known anthraquinone derivatives, chrysophanol (**2**), emodin (**3**), and coniothyrinone B (**4**) from Dothideomycetes sp. 11144 liquid culture. The extracts and isolated compounds were screened for their antimicrobial activity. (R)-6-(2-hydroxypropyl)-4-methoxy-2H-pyran-2-one (**1**) and emodin (**3**) exhibited antibacterial activity against MRSA with IC_{50} values of 61.0 and 24.4 µM, respectively. They were screened virtually via molecular docking for their affinity to different MRSA essential proteins compared to conventional inhibitors. Emodin demonstrated high affinity to the penicillin-binding protein with a pose score of -13.57 kcal/mol (1MWT). The emodin architecture could serve as a framework for the development of potent anti-MRSA antibiotics.*

Keywords *antimicrobial; anti-MRSA; pyrone; Anthraquinones*

INTRODUCTION

Polyketides are an important class of compounds frequently found as fungal metabolites and they have structural diversity that accounted for their role in natural product drug discovery [1-3]. *Dothideomycetes* represents the largest Pezizomycotina fungi class, with a wide range of species, functional traits, and ecological modes [4]. Diverse *Dothideomycetes* have been reported to produce novel bioactive polyketide derived compounds [5,6].

Polyketides are a large class of natural products that biosynthetically originate from carbon atoms obtained from small carboxylic acids and produced through condensation of activated intermediates from the acetate pathway [7].

The U.S. Food and Drug Administration (FDA) has approved numerous antibiotic medications derived from polyketides such as erythromycin A[8]. Polyketide-derived medications frequently exhibit immunosuppressive, anticancer, antifungal, and cholesterol-lowering properties, as well as

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antiviral activities [8-10]. Fungi have been shown to present prolific sources of several interesting bioactive polyketides[9]

Many anthraquinone derived compounds have been shown to have potential antimicrobial properties against a variety of bacteria, fungi, and viruses [11]. Coniothyrinone B was found to have activity against *Microbotryum violaceum*, *Septoria tritici*, *Escherichia coli*, and *Bacillus megaterium* in an agar diffusion assay [12].

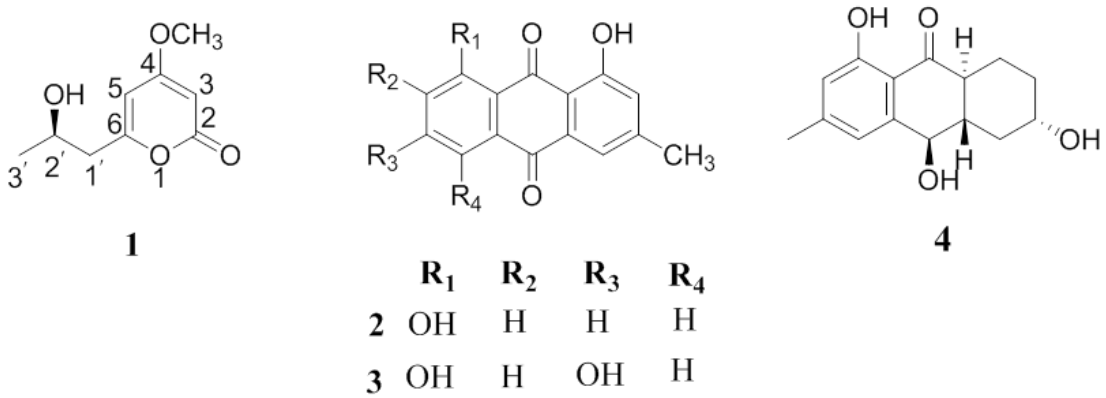
Currently, drug design and discovery are being advanced through using computational techniques as molecular docking simulation tools as an efficient and fast way to evaluate and predict the type of interaction and binding affinity of a ligand with targeted binding sites and exploring underlying mechanisms [13,14].

In order to continue our search for bioactive natural products from endophytic fungi, strain 11144 from the *Dothideomyctes* lineage was tested through a battery of antimicrobial organisms. The current study describes the phytochemical analysis and isolation of four compounds (**1**-**4**) from *Dothideomycetes* sp.11144 **(Fig.e 1)** as well as screening of the antimicrobial activity of the extracts and isolated compounds. In addition, virtual screening via molecular docking simulation for their antimicrobial activity against MRSA selected targets was also conducted.

MATERIALS AND METHODS

General Experimental Procedures

Optical rotation was measured on an Autopol IV Automatic Polarimeter (Rudolph, Hackettstown, NJ, USA). 1D and 2D NMR spectra were acquired on Varian AS 400 and 500 MHz spectrometers. Bruker BioApex spectrometer was used to measure High-resolution mass spectrometry (HRMS) data. Column chromatography (CC) was performed using silica gel (60-120 mesh, Merck) and Sephadex LH-20 (Mitsubishi Kagaku, Tokyo, Japan). Cartridges of solid phase extraction (SPE) (supelco, silica; 20 gm and C_{18}; 5, 10, and 20 gm) were used under vacuum. Diaion® HP-20 (250 μm, Sigma Aldrich) was used to separate metabolites from the liquid broth. The analytical High performance liquid chromatography (HPLC) analysis was carried out on a Waters HPLC Delta Prep 4000 connected to Waters 2487 dual λ absorbance detector and Polymer Laboratories Evaporative Light Scattering (PL-ELS 1000) detector. The column used was Luna 5 μ C_{18} (2) 100 Å, 150 x 4.6 mm with gradient systems; (a) from H_2O:MeOH (95:5) to H_2O:MeOH (30:70) at a flow rate of 0.8 mL/min over a 45 min time span, (b) from H_2O:MeOH (70:30) to H_2O:MeOH (40:60) at a flow rate of 0.8 mL/min over a 45 min time span. Preparative HPLC was used for scale up procedure using a Luna 5 μ C_{18} (2), 100 Å, 250 x 21.2 mm column at a flow rate of 28.3 mL/min with the same solvent systems used for the analytical analysis. Precoated aluminum sheets [silica 60 F254, 0.25 mm (Merck, Darmstadt, Germany)] were used for monitoring fractions from CC, then the spots were detected by UV light (254 and 366 nm) and spraying with 1% vanillin-H_2SO_4 reagent followed by heating at 105 °C for 5-10 min. Incubator shakers (New Brunswick Scientific, innova 4430) were used for fungal incubation.



1

	R_1	R_2	R_3	R_4
2	OH	H	H	H
3	OH	H	OH	H

4

Fig. 1: Structures of the isolated metabolites **1- 4.**

Fungal Material

Dothideomycetes sp. 11144 was isolated from fresh, healthy photosynthetic tissue of a mature individual of *Cupressus arizonica* (Arizona cypress, Cuppresaceae) as described by Hoffman et. al., 2010 [15]. Fresh photosynthetic tissue was surface-sterilized and cut into small pieces, one of which yielded strain 11144 on 2% malt extract agar [15]. The isolate was characterized by sequencing the fungal barcode locus (nuclear ribosomal internal transcribed spacers and 5.8S nrITS) and a portion of the adjacent nuclear ribosomal large subunit (nrLSU). The sequence is accessioned at GenBank under accession GQ153117. The strain was identified as a strain in the *Dothideomycetes* by BLAST [16] with affiliation for Dothioraceae, but there was no known species with high affinity for the strain and its identity could not be determined more accurately in the absence of reproductive structures. The strain was accessioned at the Robert L. Gilbertson Mycological Herbarium at the University of Arizona, accession 11144.

Culture Media

The fungus *Dothideomycetes* sp. 11144 was grown at room temperature ~ 28 °C on potato dextrose agar (PDA) plates for 14 days that were kept in a refrigerator and used when needed. The strain was cultured in potato dextrose broth (PDB) medium using 2.8 L Erlenmeyer flasks and incubated at 30 °C with continuous shaking (160 rpm) for two weeks.

Extraction and Isolation

After the incubation period, vacuum filtration through sterile cotton was performed to filter the fungal cells then the filtrate extracted with activated ion exchange resin (Diaion® HP-20) (100 g / L) with continuous shaking overnight. The contents of the flasks were filtered and the resin was washed with distilled water to eliminate sugars and salts, then eluted with MeOH (5 L) followed by acetone (2 L). The combined MeOH and acetone eluents were dried under vacuum to yield a viscous residue that was successively fractionated by liquid-liquid chromatography to afford *n*-hexane (1.1 g), CH_2Cl_2 (1.8 g) and EtOAc (5 g) extracts.

The *n*-hexane fraction (1.1 g) was subjected to solid phase separation using an SPE cartridge (silica, 20 g) under vacuum. Stepwise gradient elution using gradient of *n*-hexane and EtOAc mixtures (100:0, 1:3, 1:1, 3:1, 0:100 v/v; 500 mL each) afforded five fractions [H1-H5]. The H1 fraction (eluted with 100% *n*-hexane, 424 mg), which was subjected to silica gel CC (60-120 mesh, 12 g, 63 x 1.5 cm) using *n*-hexane and EtOAc (100:0, 99:1, 98:2, 95:5, 9:1 v/v; 250 mL each) gradient elution, where similar fractions were combined according to their TLC profiles to afford four main subfractions [H1A-H1D]. The H1B subfraction was re-chromatographed by silica gel CC with 1% EtOAc in *n*-hexane as isocratic mobile phase to afford compound **2** (5 mg). The H2 fraction was subjected to silica gel CC (60-120 mesh, 9 gm, 41 x 0.7 cm) gradient elution using *n*-hexane and EtOAc (100:0, 99:1, 98:2, 95:5, 9:1, 8:2 v/v, 100 ml each) to afford six main fractions [H2A-H2F]. Isocratic elution of the H2E fraction over silica gel CC eluted with 10% EtOAc in *n*-hexane yielded compound **3** (3.8 mg). The CH_2Cl_2 fraction (1.8 g) was subjected to solid phase separation via C_{18}, 20 g SPE cartridge under vacuum with H_2O and MeOH gradient elution (4:1, 3:2, 2:3, 1:4, 0:10 v/v, 500 mL each) to give five main fractions [D1-D5]; Solid phase separation was performed on group D2 (eluted with 40% MeOH in H_2O, 200 mg) using C_{18}, 5 g SPE cartridge under vacuum with H_2O and MeOH gradient elution (100:0, 19:1, 9:1, 4:1, 7:3, 3:2, 1:1 v/v, 200 mL each) to afford four fractions [D2A-D2D] according to their thin layer chromatography (TLC) profiles. Fraction D2B (eluted with 20% MeOH in H_2O, 100 mg) was subjected to preparative HPLC (Luna 5 μ C_{18} (2) 100 Å, 250 x 21.2 mm i.d.) purification with gradient system (a) yielding compound **1** (t_R 20.4 min, 1.7 mg). Fraction D3 (eluted with 60% MeOH in H_2O, 380 mg) was subjected to solid phase separation using C_{18}, 10 g SPE cartridge under vacuum with H_2O and MeOH gradient (7:3, 3:2, 13:11, 1:1, 2:3 v/v, 300 mL each) to afford five main fractions [D3A-D3E]. Further purification of fraction D3B (eluted with 40% MeOH in H_2O, 55 mg) on HPLC (Luna 5 μ C_{18} (2) 100 Å, 250, 21.2 mm) and eluted with gradient system (b) yielding **4** (t_R 14.7 min, 3 mg).

(*R*)-6-(2-hydroxypropyl)-4-methoxy-2*H*-pyran-2-one (**1**)**:** yellowish residue (MeOH); 1.7 mg; $[\alpha]^{20}_{D}$ +8 (*c* 0.1, MeOH); UV (MeOH) λ_{max}(log ε) 210 (3.59) nm and 280 (3.57) nm; ^{1}H NMR (methanol-d_4, 400 MHz) see **Table 1**; ^{13}C NMR (methanol-d_4,100 MHz) see **Table 1**; HR-ESI-MS *m/z* 185.0837 $[M+H]^+$ (calcd for $C_9H_{13}O_4$, 185.0814).

Determination of the Absolute ConFig.ation of Compound 1

A modified Mosher esterification method was used to determine the absolute conFig.ation by preparing the diastereomeric (*R*)- and (*S*)-α-methoxy-α-trifluoromethylphenylacetyl (MTPA) esters of compound **1** in NMR tubes. Compound **1**, in pyridine-d_5, was separately mixed with (*R*)- and (*S*)- MTPA-Cl in two different NMR tubes, in a molar ratio of 1:1, respectively. The mixtures were warmed to 60-70 ºC for 2-3 min, then the ^{1}H NMR spectra were acquired for both the diastereoisomeric MTPA esters. Their chemical shift differences [i.e., $\Delta\delta^{SR}$ = δ(S-MTPA ester - δ(R-MTPA ester)] were analyzed and calculated[17].

(*R*)-6-(2-hydroxypropyl)-4-methoxy-2H-pyran-2-one (1) ^{1}H NMR (pyridine-d_5, 400 MHz) δ 1.35 (3H, d, J = 6 Hz, H-3′), 2.64 (2H, d, J = 6.4 Hz, H-1′), 3.60 (3H, s, OCH_3), 4.38 (1H, m, H-2′), 5.6 (1H, s, H-3), 6.10 (1H, s, H-5).

Antimicrobial bioassay

The crude extract, fractions and isolated pure compounds were tested for antimicrobial activity against the fungi, *Candida glabrata* ATCC 90030 (Cg), *Candida albicans* ATCC 90028 (Ca), *Candida krusei* ATCC 6258 (Ck), and *Aspergillus fumigates* ATCC 90906 (Af), and the bacteria MRSA ATCC 33591, *Staphylococcus aureus* ATTC 29213 (Sa), *Cryptococcus neoformans* ATTC 90113 (Cn), *Pseudomonas aeruginosa* ATCC 27853 (Pa), *Escherichia coli* ATCC 35218 (Ec), and *Mycobacterium intracellulare* ATCC 23068 (Mi) [18]. Amphotericin B (ICN Biomedicals, Ohio) and ciprofloxacin (ICN Biomedicals, Ohio) were respectively used as positive controls for fungal and bacterial bioassays.

Molecular Docking

Docking experiments were carried out using the PyRx software [19]. The RCSB Protein Data Bank (https://www.rcsb.org/, last visited on 4 December 2022) was used to retrieve the 3D structures of isoleucyl-tRNA synthetase (PDB ID: 1JZS), penicillin-binding protein (PDB ID: 1MWT), DNA gyrase (PDB ID: 2XCQ) [20], and Pyruvate Kinase [21]. The 3D, 3D protonated, partial charge corrected, and energy minimized structures were obtained using the Merck molecular force field (MMFF94). Flexible ligand-fixed receptor docking parameters were used for the molecular docking analysis. As a control, either the complexed ligand or a commercially available inhibitor was used. The most stable affinity binding interactions were chosen, and the pose score, as well as the 2D and 3D interactions, were recorded [22]. Biovia discovery studio (v21.1) was used for 2D and 3D interactions and visualization [23].

Table 1: NMR Spectroscopic Data of compound **1.**

Position	δ_C, type	δ_H (J in Hz)	HMBC	^{1}H-^{1}H COSY
2	164.9,C	-		
3	88.7,CH	5.55, br s	5, 4	
4	174.0,C	-		
5	103.4,CH	6.06, br s	3	
6	168.0,C	-		
1′	44.2,CH_2	2.58, dd (7.6, 10.4)	5, 2′	2′
2′	66.4,CH	4.15, m		1′, 3′
3′	23.7,CH_3	1.22, d (6.4)		2′
O-CH_3	57.2,CH_3	3.85 s	4	

Data were recorded in methanol-d_4 (400 MHz for ^{1}H, 100 MHz for ^{13}C, δ in ppm)

RESULTS AND DISCUSSION

Results

Identification of Isolated Compounds

Compound **1**, was obtained as a yellowish residue (MeOH) and displayed a protonated molecule $[M+H]^+$ at *m/z*185.08370 (calcd *m/z* 185.08139) in the (+) HR-ESI-MS data, that in conjunction with the ^{13}C NMR corresponded to a molecular formula of $C_9H_{12}O_4$. The ^1H and ^{13}C NMR spectra **(Table 1)** revealed the presence of two olefinic methine protons [δ_C 88.7, δ_H 5.55 (1H, br s) and δ_C 103.4, δ_H 6.06 (1H, br s)] and one methoxy group [δ_C 57.2, δ_H 3.85 (3H, s)]. The presence of a lactone carbonyl (δ_C 164.9, C-2) and two oxygenated aromatic carbons (δ_C 174.0, C-4 and δ_C 168.0, C-6) was confirmed by the DEPT spectrum (Fig.e S4). The aforementioned signals suggested the presence of a 4,6 disubstituted α-pyrone nucleus. Also, the ^1H and ^{13}C NMR spectra revealed the presence of a side chain moiety with an oxygenated methine signal [δ_C 66.4/δ_H 4.15 (1H, m)], a methylene group [δ_C 44.2 /δ_H 2.58 (2H, dd, J = 7.6, 10.4 Hz)], and an aliphatic methyl group [δ_C 23.7 /δ_H 1.22 (3H, d, J = 6.4 Hz)]. The side chain structure was confirmed as a 2′-hydroxypropyl group by the ^1H NMR signal of the aliphatic methyl group (δ_H 1.22) which appears as a doublet (J = 6.4 Hz), and the doublet of doublets of the methylene group (J = 10.4, 7.6 Hz) in addition to the ^1H-^1H COSY correlations **(Table 1, Fig.e S5)**; from H_3-3′ (δ_H 1.22) to H-2′ (δ_H 4.15), from H_2-1′ (δ_H 2.58) to H-2′ (δ_H 4.15), and from H-2′ (δ_H 4.15) to H_3-3′ (δ_H 1.22) and H_2-1′ (δ_H 2.58).

The ^3J HMBC correlation **(Table 1, Fig.e S7)** from H_2-1′ (δ_H 2.58) with C- 5 (δ_C 103.4) confirmed the attachment of the side chain at C-6. The full assignment of the compound was confirmed by the ^2J and ^3J HMBC correlations. H-3 (δ_H 5.55) showed ^3J HMBC correlation with C-5 (δ_C 103.4) and ^2J HMBC correlation with C-4 (δ_C 174.0); H-5 (δ_H 6.06) showed ^3J HMBC correlation with C-3 (δ_C 88.7); H_2-1′ (δ_H 2.58) showed ^2J HMBC correlation with C-2′ (δ_C 66.4); and the methoxy protons (δ_H 3.85) showed ^3J HMBC correlation with C- 4 (δ_C 174.0).

By comparing the NMR data of **1** with those of closely related known α-pyrones such as verrucosapyrone B, PC-2, LL-P880γ, [24] and pestalotiopyrones A, D, and E, [25] indicated that they differ in the C-6 side chain. Therefore, the structure of **1** was deduced as the new natural 6-(2-hydroxypropyl)-4-methoxy-2*H*-pyran-2-one. The (2′*R*) absolute conFig.ation was determined, using the Mosher esterification method, [26] by preparing the diastereoisomeric esters separately with (*R*-)- and (*S*)-*α*-methoxy-*α*-trifluoromethylphenylacetyl chloride (MTPA-Cl), followed by analyzing their ^1H-NMR chemical shift differences [i.e., $\Delta\delta^{SR}$ = δ(*S*-MTPA ester - δ(*R*-MTPA ester)]. The ^1H NMR $\Delta\delta^{SR}$ values of the MTPA esters (pyridine-d_5, 400 MHz) (Fig. S10-12) were assigned for H_3-3′ (+0.09 Hz) and H_2-1′ (-0.08 Hz). Thus, the structure of compound **1** was unequivocally assigned (*R*)-6-(2-hydroxypropyl)-4-methoxy-2*H*-pyran-2-one as new natural compound, previously identified as synthetic one[27].

Three known anthraquinones were identified for the first time from *Dothideomycetes* sp. 11144: chrysophanol (**2**),[28] emodin (**3**),[28] and coniothyrinone B (**4**)[12]. The structures of the known compounds were confirmed upon comparing their spectroscopic and spectrometric data with reported data.

Investigation of the Antimicrobial Activity

Several extracts of 11144 and its isolated metabolites were evaluated for their antimicrobial activity against different fungal and bacterial strains including *Candida albicans*, *Candida krusei*, *Candida glabrata*, and *Aspergillus fumigates*, MRSA, *Staphylococcus aureus*, *Cryptococcus neoformans*, *Pseudomonas aeruginosa*, *E. coli*, and *Mycobacterium intracellulare*.

The total culture extract exhibited strong antibacterial activity against MRSA, *C. neoformans* and *S. aureus.* The dichloromethane (DCM) extract revealed moderate activity against MRSA and *S. aureus* with IC_{50} values of 33.8 and 44.0 μg/mL, respectively while the *n*-hexane extract exhibited weak activity against MRSA with an IC_{50} value of 131.9 μg/mL. The new compound **1** exhibited moderate activity against MRSA with an IC_{50} value of 61.0 μM and weak activity against *S. aureus* with an IC_{50} value of 113.7 μM. Compound **3** showed moderate antimicrobial activity against MRSA with an IC_{50} value of 24.4 μM, compared to the control

ciprofloxacin (IC_{50} values of 0.3 μM against MRSA and 0.4 μM against *S. aureus*) **(Table 2)**. The observed antibacterial activity of the total culture extract as well as the DCM and *n*-hexane fractions of the fungus *Dothideomycetes* sp. 11144 against *S. aureus* and MRSA might be due to the additive effects of different compounds. None of the isolated compounds showed antifungal activity nor antibacterial activity against *C. neoformans*, *P. aeruginosa*, *E. coli*, and *M. intracellulare* emphasizing their selectivity.

Molecular Docking of the Isolated Compound Against MRSA Selected Targets

In vitro antimicrobial testing of the identified compounds against MRSA revealed that compounds **1** and **3** had considerable antibacterial effects, with IC_{50} values of 61.0 and 24.4 μM, respectively. To better understand the mechanism of action of **1** and **3** against MRSA, molecular docking was implemented to simulate these compounds in view of fundamental *S. aureus* life cycle protein target in order to assess potential interactions with these targets. Penicillin-binding protein, isoleucyl-tRNA synthetases, DNAgyrase, and Pyruvate Kinase were chosen as targets. Furthermore, as control inhibitors and ligands for the targets, we used mupirocin (TyRS), norfloxacin (DNA Gyr), oxacillin (PBP), and *cis*-3,4-dihydrohamacanthin B. The binding energies of the pose score of the tested compounds are displayed in **Table 3. Fig. 2-5** depicts the interactions of the active compounds with the selected targets.

The pose scores agreed with the *in vitro* studies, indicating that compound **3** has higher activity due to its affinity for specific targets. The docking results showed promising affinity of both compounds to the selected receptors as compared with the results of the control inhibitors **(Table 3)**. Compound **3** demonstrated high affinity to the penicillin-binding protein with a pose score of -13.57 kcal/mol (1MWT). The compound formed conventional hydrogen bonds with TYR 600, SER 598, and THR 600, as well as interactions with GLY 599 and SER 403 via pi-donor hydrogen bonds. Further, it depicts a carbon-hydrogen bond interaction with ASN 464, as well as hydrophobic and Van der Waals interactions **(Fig.e 3)**.

The results of molecular docking suggested that the mechanism of the antimicrobial activity of compound **3** may be related to its ability to target and inhibit penicillin-binding protein.

Table 2: Antimicrobial Activities of Different Extracts of *Dothideomycetes* sp.11144 and Compounds **1** and **3**, IC_{50} (μM).

extract/compound	MRSA IC_{50} (μM)[a]	*S. aureus* IC_{50} (μM)[a]
DCM extract	33.87[b]	44[b]
n-hexane extract	131.9[b]	NA
Compound **1**	61	113.7
Compound **3**	24.4	NA
Ciprofloxacin	0.33	0.39

[a]IC_{50} is the test concentration that affords 50% inhibition of microbial cell growth. [b]Expressed in μg/mL. NA, not active. Test concentrations are 20, 4, and 0.8 μg/mL.

Table 3: Binding Energy (kcal/mol) of the Active Compounds Towards Fundamental *S. aureus* Life Cycle Protein Target

Name	**Pose scores (kcal/mol)**			
	1JZS	**1MWT**	**2XCQ**	**3T07**
Compound **1**	-8.32	-9.50	-6.80	-5.21
Compound **3**	-10.85	-13.57	-9.58	-6.11
Mupirocin	-11.11	-	-	-
Oxacillin	-	-12.84	-	-
Norfloxacin	-	-	-10.55	
cis-3,4-Dihydrohamacanthin B	-	-	-	-6.68

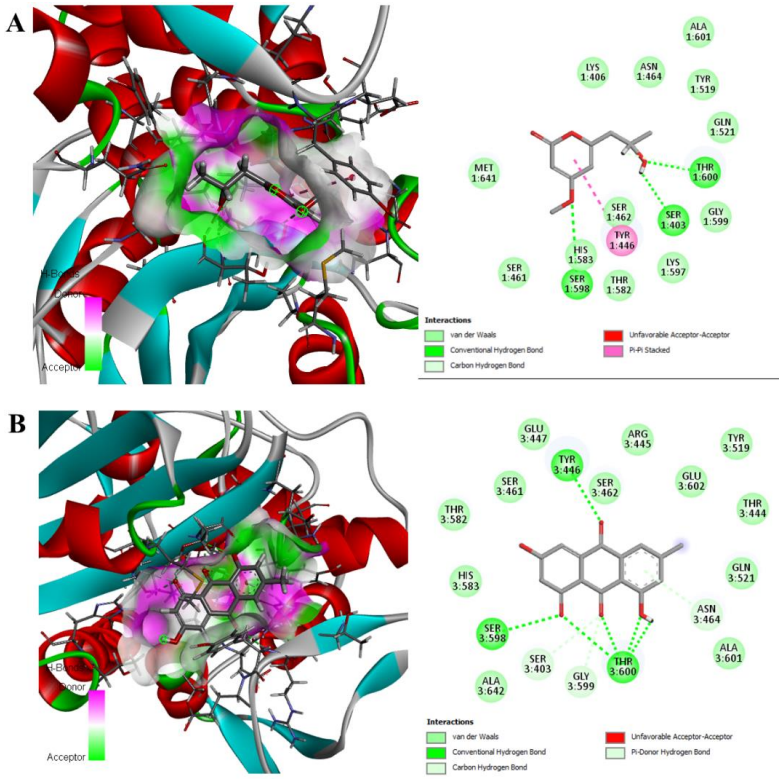


Fig. 2: 2D(right) and 3D(left) diagrams for compound **1**(A) and **3**(B) interactions with penicillin-binding protein.

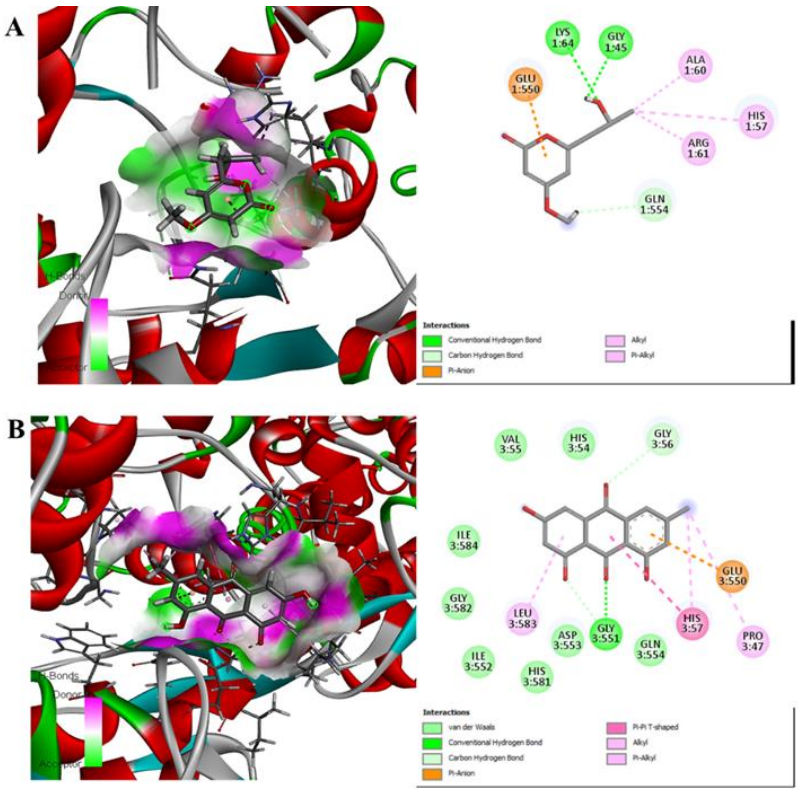


Fig. 3: 2D(right) and 3D(left) diagrams for compound **1**(A) and **3**(B) interactions with isoleucyl-tRNA synthetase (PDB ID: JZS) protein.

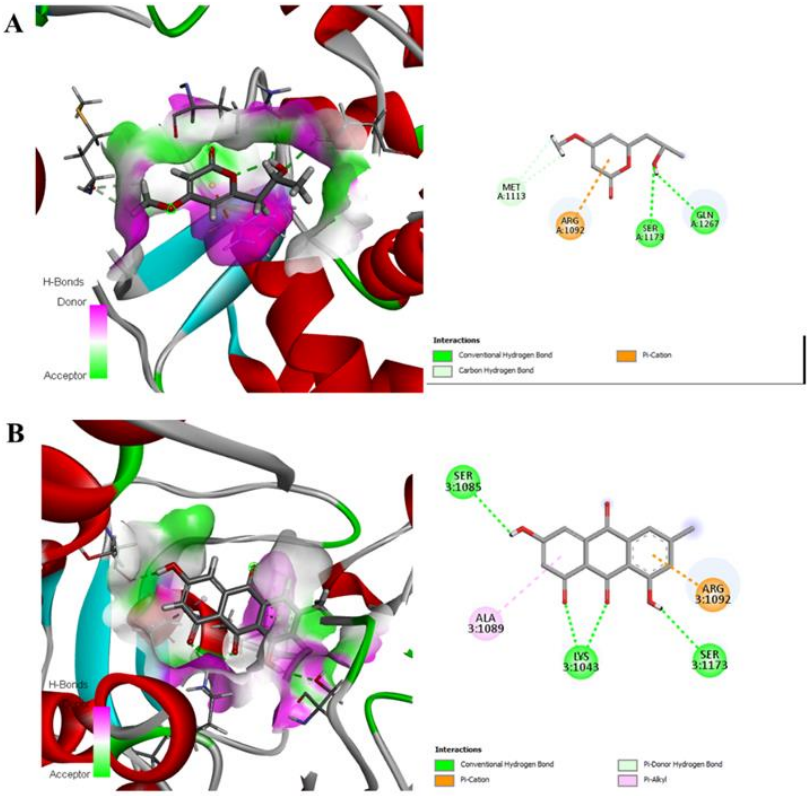


Fig. 4: 2D(right) and 3D(left) diagrams for compound **1**(A) and **3**(B) interactions with DNAgyrase protein.

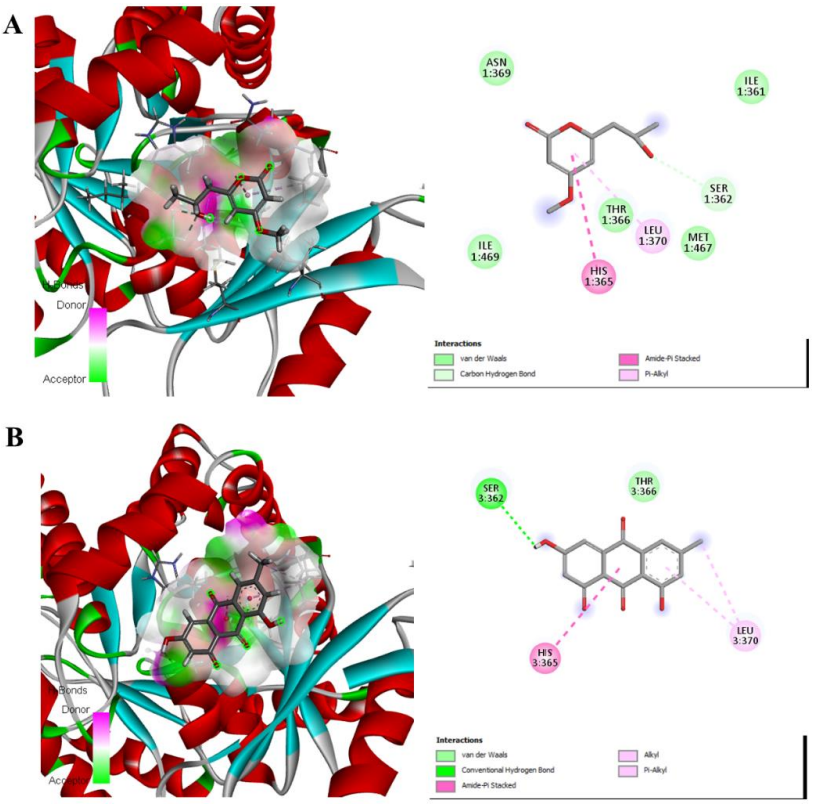


Fig. 5: 2D (right) and 3D(left) diagrams for compound **1**(A) and **3**(B) interactions with Pyruvate Kinase protein.

Discussion

The discovery of novel antimicrobial agents especially anti-MRSA is crucial. In the current study, we focused on the isolation of antimicrobial leads from the fungus *Dothideomycetes* sp. 11144. Four metabolites were isolated and identified as (*R*)-6-(2-hydroxypropyl)-4-methoxy-2*H*-pyran-2-one (**1**), chrysophanol (**2**), emodin (**3**), and coniothyrinone B (**4**). Compound **1**, a novel α-pyrone derivative, and compound **3**, emodin, have demonstrated considerable antimicrobial activity, supporting that they could serve as leads for developing new antibiotic agents. The anti-MRSA potential of compounds **1** and **3** was further evaluated by molecular docking studies via simulating interactions with essential protein targets in the MRSA life cycle. Emodin (**3**) showed ability to inhibit penicillin-binding protein, which plays a critical role in bacterial cell wall synthesis and has been established as a target for β-lactam antibiotics. Interestingly, polyketides, especially anthraquinones, have been recognized to possess antimicrobial, anticancer, and anti-inflammatory properties. However, the isolation and identification of compound **1** as a novel α-pyrone derivative with antimicrobial properties offers a new scaffold in antibiotics development.

Conclusion

The urgent need to discover new antibiotic drug in reasonable time and low cost, prompted us to investigate mycoendophytic derived polyketide metabolites as antimicrobial leads. Four polyketide-type metabolites were isolated from the broth extract of the fungus *Dothideomycetes* sp. 11144. They were identified as the new α-pyrone derivative, (*R*)-6-(2-hydroxypropyl)-4-methoxy-2*H*-pyran-2-one (**1**) together with three known anthraquinone derivatives, chrysophanol (**2**), emodin (**3**), and coniothyrinone B (**4**). Compounds **1** and **3** exhibited moderate activities against MRSA and *S. aureus*. Compounds **1** and **3** were subjected to a molecular docking analysis to determine their potential targets against MRSA by simulating these compounds with selected fundamental protein targets in the MRSA life cycle. The mechanism of antimicrobial activity of compound **3** was suggested to be through its ability to target and inhibit the penicillin-binding protein.

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المستقلبات الثانوية المشتقة من البوليكيتيد من فطر الدوثيديومايسيتس

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يعد اكتشاف مركبات بديلة لعلاج الالتهابات المقاومة للمضادات الحيوية أمرًا بالغ الأهمية للتخفيف من أزمة مقاومة المضادات الحيوية الحالية. تعتبر الفطريات الداخلية مصدرًا للعديد من المضادات الحيوية المعتمدة مؤخرًا وخاصة الجزيئات المشتقة من البوليكيتيد.وقد هدفت هذه الدراسة إلى عزل والتعرف على المركبات المضادة للميكروبات لفطر Dothideomycetes sp. 11144 بالإضافة إلى دراسة الالتحام الجزيئي لتقاربها مع بروتينات مختارة من المكورات العنقودية الذهبية المقاومة للميثيسيلين (MRSA). فى هذه الدراسة، نقوم بوصف عزل أربعة مركبات من نوع بوليكيتيد؛ (R)-6-(2-hydroxypropyl)-4-methoxy-2H-pyran-2-one (1) وهو مشتق جديد من ألفا بيرون، إلى جانب ثلاثة مركبات معروفة؛ الكريسوفانول (٢) والإيمودين (٣) والكونيوثيرينون ب (٤) من الزراعة السائلة لفطر Dothideomycetes sp. 11144. وايضا تم فحص المستخلصات والمركبات المعزولة لمعرفة نشاطها المضاد للميكروبات. أظهر مركبى (R)-6-(2-hydroxypropyl)-4-methoxy-2H-pyran-2-one (1)وإيمودين (٣) نشاطًا مضادًا للبكتيريا ضد MRSA بقيم IC50 تبلغ ٦١,٠ و٢٤,٤ ميكرومولر، على التوالي. وتم فحصهم عبر الالتحام الجزيئي للتأكد من مدى ارتباطهم بالبروتينات الأساسية المختلفة لـــ MRSA مقارنة بالمثبطات التقليدية. أظهر إيمودين تقاربًا عاليًا للبروتين المرتبط بالبنسلين مع درجة وضعية قدرها -١٣,٥٧ سعرة حرارية/مول (MWT1). وبذلك يمكن أن تكون بنية الإيمودين بمثابة إطار لتطوير مضادات حيوية قوية مضادة لبكتيريا MRSA