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ORIGINAL PAPER

Trichoderone, a novel cytotoxic cyclopentenone and cholesta-7, 22-diene-3β, 5α, 6β-triol, with new activities from the marine-derived fungus *Trichoderma* sp.

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Abstract The historical paradigm of the deep ocean as a biological 'desert' has shifted to one of a 'rainforest' owing to the isolation of many novel microbes and their associated bioactive compounds. To explore the potential of the bioactive compounds in our marine microbial natural product library, we screened it for the selective cytotoxicity of six different cancer cell lines to human normal lung fibroblast cell line HLF. The crude extract from a marine-derived fungal strain showed notable selectivity against cancer cell lines. For a bioactivity-guided fractionation and purification, a novel cyclopentenone, (−)-(4R^*, 5S^*)-3-ethyl-4,5-dihydroxycyclopent-2-enone (**1**, trichoderone), and a known compound with new activity, cholesta-7,22-diene-3β,5α,6β-triol (**2**), were identified from a marine *Trichoderma* sp. that was isolated from the deep sea sediment of the South China Sea. Their structures were determined by NMR and MS data analyses. Trichoderone (**1**) displayed potent cytotoxicity against a panel of six cancer cell lines, whereas it did not show much cytotoxicity against normal human lung fibroblast cell line HLF even at a concentration of 7.02 mM. The selectivity index (SI) value for **1** was greater than 100. To the best of our knowledge, both compounds were isolated from marine fungi for the first time. They also exhibited bioactivities against HIV protease and Taq DNA polymerase. Optimization of the compounds would shed new light on treating cancer and infectious diseases.

Keywords Trichoderone · Cyclopentenone · Marine microbial natural product library · Selective cytotoxicity

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Introduction

Marine microbes represent a potential source for commercially important bioactive compounds, and their bioremediation capabilities are also remarkable [26]. We have developed a proprietary natural product library consisting of fermentation products of 4,000 marine microbes. The library currently contains ~23,000 samples, consisting of 15,000 crude extracts from 2,500 strains fermented under different physiological growth conditions, 6,000 fractionated samples from 1,000 of these strains, and 900 compounds that were isolated to purity. Of these purified compounds, 30% have novel structures [3]. More specifically, fungi from the marine environment have shown great potential because of the high diversity of secondary metabolites [1, 4, 20]. Marine fungi can sense, adapt and respond quickly to diverse environments and can compete for defense and survival by producing unique secondary

metabolites [27]. A marine microbial extract library has been established and screened for various biological activities [3]. Identifying beauvericin as an antifungal potentiator is a good example of the use of the library for drug discovery [28].

Cancer is a leading cause of death worldwide [23, 24]. As many anticancer drugs cannot discriminate between cancer cells and non-cancer cells, many normal cells are also killed during the process of chemotherapy. Developing new anticancer drugs with a higher potency and specificity against cancer cells has therefore become an important goal in biomedical research.

We focused on discovering novel anti-tumor compounds with selective cytotoxicity towards cancer cells. Using cytotoxicity against human lung cancer cell line A549 and normal human lung fibroblast cell line HLF as a pilot screen, the crude extract of a marine-derived fungus GIBH-Mf082 characterized as *Trichoderma* sp. from our library showed remarkable selectivity against A549. Guided by the selective cytotoxicity, a novel cytotoxic compound, (−)-(4R^*, 5S^*)-3-ethyl-4,5–dihydroxy-cyclopent-2-enone (**1**, trichoderone) with a high selectivity index (SI) value together with a known compound, cholesta-7,22-diene-3β,5α,6β-triol (**2**), was isolated and identified from the this fungus. Trichoderone (**1**) was evaluated for cytotoxic effect on a panel of six cancer cell lines A549, NCI-H460 (non-small-cell lung cancer), MCF-7, MDA-MB-435s (breast cancer), HeLa-229 (cervical cancer), DU-145 (prostate cancer) cell lines and normal human lung fibroblast cell line HLF. Compound **2,** a known triterpenoids, has been isolated from the marine scallop *Patinopecten yessoensis* and the bryozoan *Myriapora truncateI* [6, 13]. To the best of our knowledge, both compounds were isolated from marine fungi for the first time. In this paper, we describe the isolation, structure elucidation and biological activities of compounds **1** and **2**.

Materials and methods

Fungal isolation, identification and fermentation

Strain GIBH-Mf082 was isolated from marine sediment in the South China Sea in January 2005. The fungal strain is stored in the China Center for Type Culture Collection (CCTCC, no. CCTCC M208080) and China General Microbiological Culture Collection Center (CGMCC, no. CG MCC 4.5580). The total deoxyribonucleic acid (DNA) of marine-derived fungus GIBH-Mf082 was extracted using the EZNA kit (Omega). The internal transcribed spacers (ITS) of ribosomal DNA (rDNA) were amplified employing the combination of a conserved forward primer ITS1 (5′- TCCGTAGGTGAACCTGCGG-3′) and reverse primer ITS4 (5′- TCCTCCGCTTA TTGATATGC-3′). The polymerase chain reaction product is about 0.7 kb. The purified ITS rDNA was sequenced at ShangHai Sheng-Gong Bio. The sequence data have been submitted to GenBank with an accession number of FJ532469. The sequences were aligned manually using CLUSTAL X version 1.8 with sequences of representative strains of the genus Trichoderma retrieved from the DNA Data Bank of Japan/European Molecular Biology Laboratory/GenBank databases. Phylogenetic trees were produced by using the neighbor-joining algorithms from the PHYLIP package version 3.5c. Evolutionary distance matrices were generated following the method of our previous publication [25]. For metabolite production, the fungus was grown in 250-ml flasks containing 30 ml solid medium M22 (unhusked rice 20 g, yeast extract 0.1 g and glucose 0.1 g in 30 ml distilled water) for a period of 10 days at 27°C.

Compound extraction and purification

After 10 days of fermentation, the fermented whole broth (10 l) was harvested and extracted with 95% ethanol and dried. The resulting syrup was diluted by 2L·H_2O then split in four fractions by partition with petroleum ether, chloroform, ethyl acetate and n-butanol. The dried extract of the $CHCl_3$ fraction (3.4 g) was fractionated by column chromatography on Si gel with a stepwise gradient of $CHCl_3$/MeOH (100/0, 98/2, 95/5, 90/10, 80/20, 0/100). The $CHCl_3$/MeOH(95/5) fraction (20 mg), which contained the cytotoxicity with a high SI value, was fractionated further on a reversed-phase silica gel (RP-18) column eluted with 90% MeOH in H_2O to afford compound **2**, and the surplus was purified by Sephadex LH-20 column again to obtain compound **1** (trichoderone).

Chemical characterization of these active compounds

NMR spectra were recorded in $CDCl_3$ on a Bruker AV 400 spectrometer. The HREIMS spectrum was measured on a high-resolution mass spectrometer (Thermo Finnigan Trace GC/MAT 95XP). Optical rotations were measured on a Perkin-Elmer model 124 polarimeter.

Cytotoxicity MTT assay

Cytotoxicity on A549 (human non-small-cell lung cancer), NCI-H460 (human non-small-cell lung cancer), MCF-7, MDA-MB-435s (human breast cancer), HeLa -229 (human cervical cancer), DU-145 (prostate cancer), and HLF (normal human lung fibroblast) cells was measured. Cells were grown in RPMI 1640 medium supplemented with 10% heat-inactivated newborn calf serum, penicillin (50 units/ml), and streptomycin (50 μg/ml) in a humidified

incubator of 5% CO_2 at 37°C. The cells were seeded in 96-well microplates (1–2 × 10^4 cells/well) and cultured for 12 h. The medium was replaced with that containing test compounds, and the cells were further cultured at 37°C. To evaluate the IC_{50}, the cells were exposed to the test compounds at various concentrations. After incubation for 48 h, 10 µl of the 3-(4,5-dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide MTT solution (Sigma) was added into each well, and the cells were incubated under the same conditions for 4 h until a purple precipitate was visible. Then 200 µl of dimethyl sulfoxide (DMSO) was added, and the optical density at 570 nm was measured in a microplate reader (Bio-Tek Synergy HT). Cisplatin and DMSO served as positive and negative controls, respectively.

Calculation of the selectivity index (SI)

The selectivity index (SI) corresponded to the IC_{50} value determined for activity of fungus extracts on normal human lung fibroblast cell line HLF divided by that of cancer cell lines. IC_{50} values were derived from multiple data points each performed in duplicate, and nonlinear regression was performed using GraphPad Prism Software (GraphPad Software, San Diego, CA).

Taq DNA polymerase assay

The activity of Taq DNA polymerase was measured by PCR-based methods [18], amplifying the bacterial 16S rDNA by using the universal primer 1492R (5′-GGTTA CCTTGTTACGACTT-3′) and 27F (5′-AGAGTTTGATC CTGGCTCAG-3′). The Taq DNA polymerase reaction was performed in 25-µl reaction mixture containing PCR buffer (Takara), 10 ng of *Escherichia coli* genomic DNA as template, 0.5 µl primer, 2 µl DMSO or 2 µl compound solution (dissolved in DMSO) and 0.5 µl Taq DNA polymerase.

HIV-1 protease assay

The activity of HIV-1 protease was measured by ProAssay HIV-1 Protease Kit (ProteinOne Inc., USA). The assay was performed according to the users' manual. The HIV-1 protease reaction was performed in 25 µl reaction mixture with 0.5 µl DMSO or 0.5 µl compounds.

Antimicrobial assay

Antimicrobial efficiency was assessed on three test strains (*Staphylococcus aureus* ATCC 6538, *Escherichia coli* ATCC 8739 and *Pseudomonas aerugimosa* ATCC 9027), two fungi (*Candida albicans* Tw07228 and *C. parapsilosis* ATCC 22019) and protozoa (*Plasmodium falciparum* 3D7). MICs (minimal inhibitory concentrations) were evaluated as described elsewhere [11, 21, 28]. The test compounds were reconstituted in DMSO unless specified.

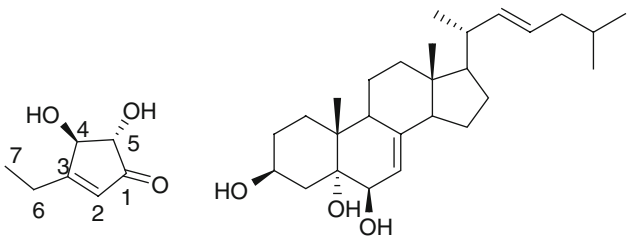
Results

Initial screening for growth inhibition in vitro

The in vitro anti-cancer activity of the marine microbial extract library was screened using the MTT assay [3]. Two cell lines, A549 and NCI-H460, representing human lung malignancy were cultured with microbial extracts to be tested at a concentration of 1% (V/V) for 48 h, and cell viability was determined. About 2% of them showed more than 80% growth inhibition against these two cancer cell lines. We subsequently evaluated the activity of these positive extracts in HLF cell line representing human normal lung cell line. Seven of them showed no inhibition against HLF at a concentration of 1% (V/V). The extracts were produced by seven different strains, including *Streptomycete* sp., *Micromonospora* sp., *Penicillium* sp., *Aspergillus* sp., *Trichoderma* sp. and some other fungi. The IC_{50} values for A549 and HLF of the seven extracts were measured. The extracts of strain GIBH-Mf082 showed the highest selectivity indices with SI >20 on various cell lines. The bioactive components of GIBH-Mf082 were isolated to purity.

Extraction, isolation and purification of active compounds

As illustrated in Methods, compounds were extracted from GIBH-Mf082 broth using 95% EtOH. After extraction by four organic solvents, the $CHCl_3$ fraction with cytotoxicity and high SI value was purified through normal phase Si gel and reversed-phase silica gel columns, and finally purified by Sephadex LH-20 columns to afford compounds **1** (5 mg) and **2** (10 mg) (Fig. 1). NMR and MS analyses indicated that compound **1** was a novel compound.



1 **2**

Fig. 1 Structures of compounds **1** and **2**

Table 1 ^{1}H and ^{13}C NMR data of **1** in $CDCl_3$

Position	δ_H^a (J in Hz)	δ_C^b
1		202.8 s
2	5.99, s	125.6 d
3		180.1 s
4	4.66, brs	77.7 d
5	4.23, brs	81.8 d
6α	2.42, m	23.0 t
6β	2.63, m	
7	1.20, t (7.6)	10.9 q

[a] Recorded at 400 MHz

[b] Recorded at 100 MHz

Structure elucidation

Compound **1** was obtained as an amorphous solid. Its molecular formula was determined as $C_7H_{10}O_3$ by HREIMS, which gave a molecular ion peak at *m/z* 142.0625 (calculated. 142.0624). The ^{1}H, ^{13}C NMR (Table 1) and HMQC spectra indicated the presence of two oxygenated methines [δ_H 4.66 (1H, br s, H-4) and 4.23 (1H, br s, H-5); δ_C 77.7 (d, C-4) and 81.8 (d, C-5)], a methyl group [δ_H 1.20 (3H, t, $J = 7.6$ Hz, H_3-7); δ_C 10.9 (q, C-7)], a tri-substituted vinyl group [δ_H 5.99 (1H, s); δ_C 180.1(s) [2] and 125.6 (d)], a methylene group [δ_H 2.42 and δ 2.63 (each 1H, m, H_2-6); δ_C 23.0 (t)] as well as a keto carbonyl carbon [δ_C 202.8 (s, C-1)]. These functionalities were assembled by interpretation of the ^{1}H-^{1}H COSY and HMBC spectra (Fig. 2). It should be noted that δ_C (180.1) of the C-3 double bond in 1 was significantly shifted downfield, similar to the carbonyl resonances δ_C (173.6) of the C-3 for 2-hydroxymethyl-3-methylcyclopent-2-enone [7].

The ^{1}H-^{1}H COSY spectrum showed correlations between H_2-6 and H_3-7, indicating the presence of an ethyl group. In addition, the correlations of H-2 with H_2-6 and H-4 due to $^{4}J_{HH}$ couplings and of H-4 with H-5 were observed in the COSY spectrum. In the HMBC spectrum, the correlations from H-6 to C-2, C-3 and C-4 and from H-7 to C-3 revealed that C-2, C-4 and C-6 were connected to C-3. Key HMBC correlations from H-2 to C-1 to C-5 indicated that C-1 was directly attached to C-2 and C-5. As shown in Fig. 1, the structure of **1** was very similar to that of dihydroterrein. The n-propanyl group in dihydroterrein was replaced by an ethyl group in **1**. The *erythro* relationship between C-4 and C-5 in **1** was determined by comparing the ^{13}C NMR chemical shift of C-4, C-5 and ^{1}H NMR coupling constant between H-4 and H-5 with those in diacetyldihydroterrein [14]. Therefore, the structure of **1** was deduced as (4*R**,5*S**)-3-ethyl-4,5-dihydroxycyclopent-2-enone and named trichoderone.

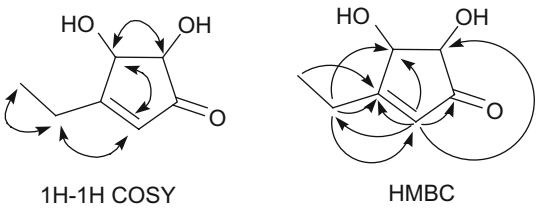


Fig. 2 ^{1}H-^{1}H COSY and key HMBC correlations of compound **1** (trichoderone)

Compound **2** was identified as cholesta-7,22-diene-3β,5α,6β-triol by comparison of its mass and NMR spectral data with those reported in the literature [17]. It has been isolated from the marine scallop *Patinopecten yessoensis* and *Myriapora truncate* [6]. This is the first report on the isolation of **2** as fungal metabolite.

Physical and chemical profile of the compounds

(−)-(4*R,5*S**)-3-ethyl-4,5-dihydroxycyclopent-2-enone (1,**trichoderone**):**amorphous solid, HREIMS: 142.0625 (calculated. 142.0624), $[\alpha]_D^{20} = -4.838$ ($c = 0.93$ CH_3 Cl). ^{1}H and ^{13}C NMR(400Mz, $CDCl_3$, TMS): see Table 1.

Cholesta-7,22-diene-3β,5α,6β-triol (2): white crystal, ^{1}H NMR(400Mz, $CDOD_3$, TMS) δ: 3.98 (m, H-3), 3.55 (brd, $J = 4.0$ Hz, H-6), 5.22 (m, H-7), 0.54 (s, H-18), 0.90 (s, H-19), 0.88 (d, $J = 7.0$ Hz, H-21), 0.80 (d, $J = 6.5$ Hz H-26,27). ^{13}C NMR ($CDOD_3$, TMS) δ: 23.0 (t, C-1), 36.7 (t,C-2), 66.4 (d, C-3), 39.4 (t C-4), 74.9 (s, C-5), 72.6 (d, C-6), 119.9 (d, C-7), 140.1 (s, C-8), 42.7 (d, C-9), 37.1 (s, C-10), 21.8 (t, C-11), 39.1 (t, C-12),43.4 (s, C-13), 54.6 (d, C-14), 31.6 (t, C-15), 28.1 (t, C-16), 55.8 (d, C-17), 12.5 (q, C-18), 18.1 (q, C-19), 42.4 (d, C-20), 17.6 (q, C-21), 135.8 (d, C-22), 131.8 (d, C-23), 40.5 (t, C-24), 32.9 (d, C-25), 20.2 (q, C-26), 19.9 (q, C-7).

The biological activities

Trichoderone (**1**) was tested for cytotoxic effect on six cancer cell lines (A549, NCI-H460, MCF-7, MDA-MB-435 s, Hela and DU-145) representing four types of human malignancies along with a normal human cell line (HLF) by the MTT method. Trichoderone (**1**) showed moderate cytotoxicity toward the six cancer cell lines, and the cytotoxicity was cell type-dependent (Table 2). More importantly, trichoderone (**1**) did not exhibit cytotoxicity toward non-cancer cell line HLF at concentrations up to 7.02 mM. The selectivity index was more than 100. The selective cytotoxicity to cancer cells of trichoderone (**1**) was more remarkable than that of cisplatin. The effect of trichoderone (**1**) on A549 and HLF cells was observed by microscope. After exposure to 50 μM trichoderone (**1**) for 48 h, A549 cells displayed a variety of morphological changes, including cell shrinkage and blebbing, which

Table 2 Cytotoxicities of trichoderone (**1**) against a panel of six cancer cell lines and a normal human lung cell line

Cell line	Cell type	IC50 (μM)	
		Trichoderone (**1**)	Cisplatin
A549	Lung cancer	50.2	17.5
NCI-H460	Lung cancer	164	20.4
MCF-7	Breast cancer	63.5	85.1
MDA-MB-435s	Breast cancer	617	67
HeLa	Cervical cancer	85.6	–
DU-145	Prostate cancer	43.2	–
HLF	Normal lung fibroblast	>7,020	15.4

were the characters of apoptosis (Fig. 3). It suggested that trichoderone (**1**) probably inhibited the growth of cancer cell lines by inducing apoptosis. However, HLF cells did not display any morphological change.

Many members of triterpenes have been found to exhibit diverse biological activities [10, 12, 18]. Nevertheless, there has been no report about the biological activity of compound **2** yet. Therefore, several biological activities of compound **2** were evaluated. It showed weak cytotoxicity with IC_{50} values of 0.29 mM toward the lung cancer cell line A549. It inhibited Taq DNA polymerase completely at a concentration of 4.8 mM with the IC_{50} value of 0.45 mM (Fig. 4). It also exhibited moderately inhibitive activity against HIV-1 protease (17.61% inhibition at 0.24 mM), but did not inhibit the HIV-1 induced cytopathic effect in MT-2 cells at 0.2 mM. The antimicrobial actions of compound **2** were assessed with six test microbes, including three bacteria (*Staphylococcus aureus* ATCC 6538, *Escherichia coli* ATCC 8739 and *Pseudomonas aeruginosa* ATCC 9027), two fungi (*Candida albicans* Tw07228 and *C. parapsilosis*) and a protozoa (*Plasmodium falciparum* 3D7). But compound **2** could inhibit none of them at 2.4 mM.

Biological character of GIBH-Mf082

The producer of trichoderone (**1**) and **2**, marine-derived fungus GIBH-Mf082, was identified to be a strain of *Trichoderma* sp. by traditional morphological criteria and rDNA-ITS sequences (FJ532469). By comparison with other ITS rDNA sequences in GeneBank, the phylogenetic tree was constructed and shown in Fig. 5. Strain GIBH-Mf082 and *Trichoderma koningii* (gi:65332019), *T. atroviride* (gi:126013522) and *T. viride* (gi:111610691) produced a consistent cluster with a high bootstrap value. The number of scored nucleotide identities and the overall percentage of similarity calculated by pairwise analysis indicates a sequence similarity of 99% between these organisms. An unrooted neighbor-joining tree based on nearly complete ITS rDNA sequences showed the position of strain GIBH-Mf082 in the Trichoderma tree (Fig. 5).

Since it was isolated from marine sediment, the salt tolerance of strain GIBH-Mf082 was evaluated. GIBH-Mf082 could grow in the medium with 3% NaCl as fast as in the medium without NaCl, but hardly grow in the medium with 10% NaCl. Three percent of NaCl inhibited the formation of spores. Strain GIBH-Mf082 could not

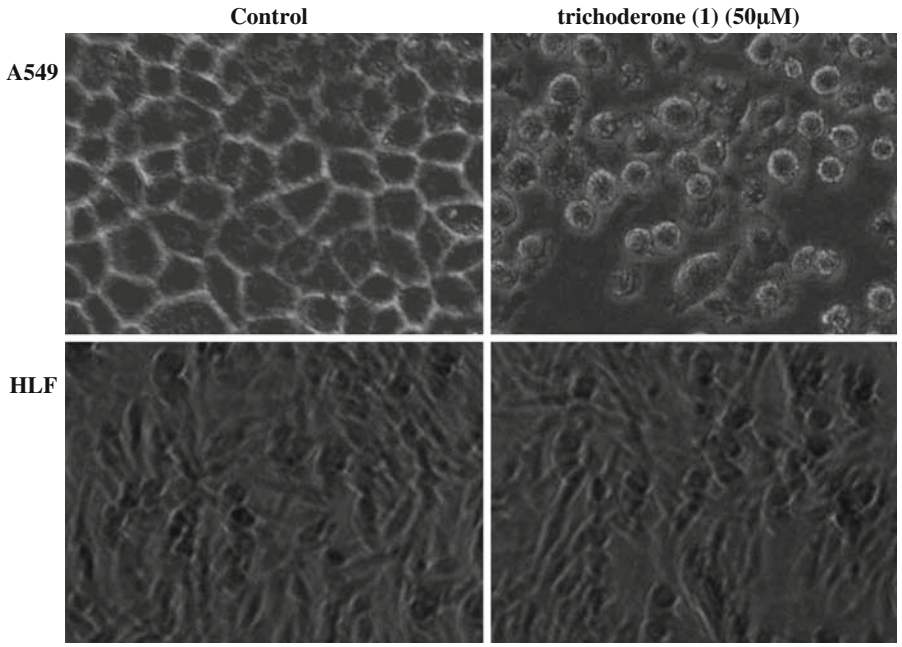


Fig. 3 Morphological appearance of A549 and HLF cells after exposure to trichoderone (**1**). The A549 and HLF cells were incubated in 96 wells in a medium without/with trichoderone (**1**) for 48 h. The magnification is ×400. The data shown are typical fields representative of two independent experiments

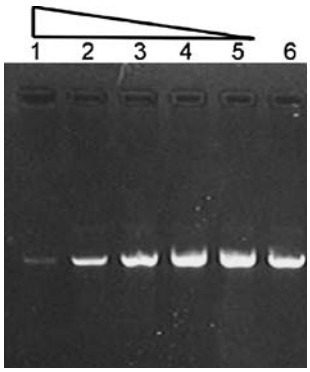


Fig. 4 Inhibitory effect of compound **2** on Taq DNA polymerase. Photograph of gels stained with Sybr Green. Compound **2** was mixed with Taq DNA polymerase. *Lanes* 1–6, compound **2** at concentrations of 1, 0.3, 0.1, 0.037, 0.012 and 0 μg/μl, respectively. This experiment was reproduced three times

only tolerate high salt concentrations, but also adapt to low salt concentrations. The biosynthesis of trichoderone (**1**) and (**2**) did not depend on a high salt concentration. This property will be good for further industrial fermentation and extraction.

Discussion

In this work, a novel cytotoxic cyclopentenone was discovered from a marine microbial natural product library that contained more than 10,000 extracts. It suggested that screening the natural product library by evaluating the cytotoxicity against a cancer cell line versus non-cancer cell line was a good strategy to discover new cytotoxic compounds. Natural products are an attractive source of anticancer agent discovery. The use of natural products as anticancer agents has a long history, and over 50% of the drugs in clinical trials for anticancer activity were isolated from natural sources or are related to them [8]. However, how to dereplicate and discover novel cytotoxic compounds efficiently become new problems. Principally, novel discovery strategies would lead to novel bioactive compounds. Since crude natural product extracts are not well suited for molecular-based high throughput screening, we chose cell-based assay [5]. In order to discover novel small-molecule inhibitors with specificity for tumor cells, especially for lung, we chose two lung cell lines, A549 and HLF, representing cancer cells and non-cancer cells, respectively.

Although the cytotoxicity of trichoderone (**1**) was moderate, it still possessed several properties of promising anticancer agents. First, selectivity cytotoxicity is a useful attribute for potential anticancer agents [23]. The current studies demonstrated that the cytotoxicity of trichoderone (**1**) is selective for cancer compared with non-cancer lines. Of the seven cell lines tested, only cancer cells responded to trichoderone (**1**) treatment. The normal epithelial cell line was not sensitive to trichoderone (**1**)'s toxicity. Second, trichoderone (**1**) has activity in a broad spectrum of human cancer cells, including lung, breast, prostate and cervical cancer. Third, the cytotoxicity of trichoderone (**1**) was cell type-dependent. It suggested that trichoderone (**1**) did not target the biochemical reaction or signal pathway that existed in most cells, and the target may be single. This is why the cytotoxicity of trichoderone (**1**) was specific to cancer cell. Having a single target is an advantage. In some opinions, the drugs with multiple targets are dirty drugs. Furthermore, it will provide us with some clues to discovering the target. For example, both MDA-MB-435s and MCF-7 are breast cancer cell lines, but the IC_{50} of MDA-MB-435s was 9.7-fold higher than MCF-7. As we know, MDA-MB-435s express persistently activated Stat3, a

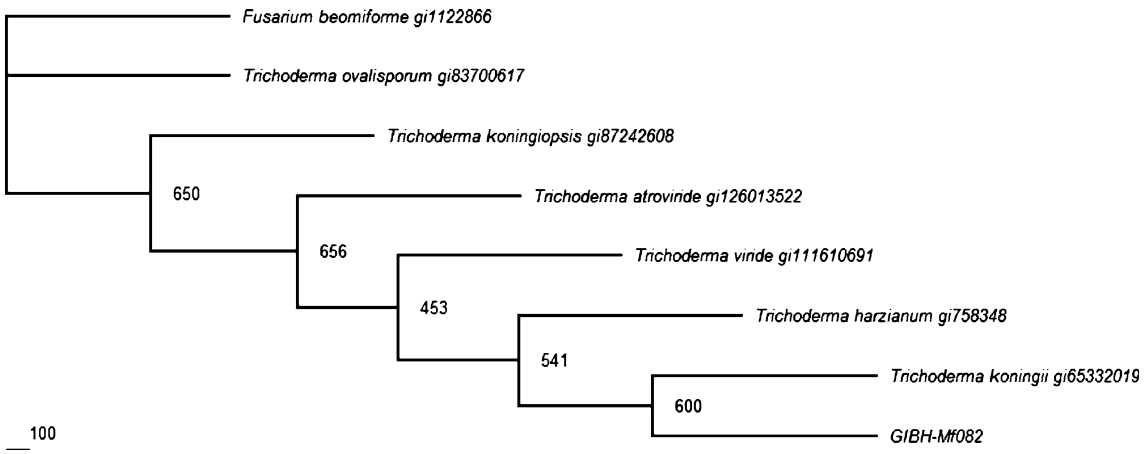


Fig. 5 Unrooted neighbor-joining tree based on nearly complete ITS rDNA sequences, showing the position of strain GIBH-Mf082 in the Trichoderma tree. Numbers at nodes are bootstrap values (%) based on 1,000 resampled datasets; only values greater than 50% are given

signal protein that related to cancer, whereas MCF-7 does not [21]. It is suggested that trichoderone (**1**) may be targeting the related signal pathway. Fourth, trichoderone (**1**) probably inhibited the growth of cancer cell lines by inducing apoptosis. The ability to escape suicide (apoptosis) is a hallmark of most cancer cells and often correlates with tumor aggressiveness and resistance to traditional anticancer drug treatments [9]. Consequently, academic and industrial laboratories are engaged in a herculean effort to develop new molecules that reactivate the apoptotic program in tumor cells.

There are many fungal cyclopentenones with bioactivities. Terrein has been found in *Penicillium* sp., which was reported to show plant growth inhibition, antibacterial activities, moderate cytotoxic activity, the inhibition of melanin formation in melanocyte Mel-Ab cells and the inhibition of the epidermal proliferation of skin equivalents [15, 16, 19]. Dihydroterrein and trichodenones were isolated from the marine-derived fungus *Emericella variecolor* and *Trichoderma harzianum*, respectively [20]. Some cyclopentenones were isolated from endophytic fungi, such as 2-hydroxymethyl-3-methylcyclopent-2-enone [7].

This is the first report on the isolation of compound **2** from *Trichoderma* sp., indicating that fungi may provide a novel source of this compound. As the resource of natural products, one of the advantages of microorganisms is that they can be adapted to artificial culture conditions, thus avoiding problems of collection and supply [1]. Compared with protozoan, fungus GIBH-Mf082 can be a promising producer of compound **2**. Although the bioactivity of compound **2** that has been evaluated was not promising, it may be used as potential raw material for some active triterpenes. Many analogues of compound **2**, such as ganoderiols, cerevisterol and ergostanoids gymnastatins, have been found to exhibit diverse biological activities, e.g., the cytotoxic, antiviral and DNA polymerase inhibitor, DNA topoisomerase inhibitor and the moderately active HIV-1 protease inhibitor [10, 12, 18]. Some of them were isolated from mushroom, such as *Ganoderma lucidum* and *Fomitella fraxinea*, which grow more slowly than fungi.

It is worth exploiting marine *Trichoderma* sp. The terrestrial species of Trichoderma are well-known soil inhabitants and have been studied intensely for many years. Some of them have been used as potent biocontrol agents for a variety of soil-borne phytopathogenic fungi. However, the Trichoderma species from the marine environment have not been exploited enough as a bioactive natural product resource, perhaps due to lack of effective discovery strategies. Hitherto, only 13 novel compounds have been isolated from 5 marine-derived *Trichoderma* species [4, 20, 22].

In conclusion, trichoderone (**1**) is a promising anticancer agent that exhibits high selective toxicity to cancer cells and *Trichoderma* sp. GIBH-Mf082 was a potential source of compound **2**.

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