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Dicentrine and Dicentrinone Isolated from *Stephania tetrandrae* Radix Suppress HepG2 Proliferation through Inhibiting PDI Activity

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Abstract: Inhibition of protein disulfide isomerase (PDI) has been attempted as a promising anti-cancer strategy. However, there is still no currently available PDI inhibitors approved for clinical use. Here, we isolated seven high yield alkaloids from *Stephania tetrandrae* Radix (STR), a medical herb frequently prescribed in anti-tumor condition, and identified two potent natural PDI inhibitors, dicentrine and dicentrinone. Among the seven alkaloids isolated, dicentrinone (**1**), dicentrine (**2**), tetrandrine (**4**), and fangchinoline (**5**) could significantly reduce cell viability in a dosage dependent manner detected by MTT assay in human hepatoma cells. To examine whether the candidate compounds are potent PDI inhibitors, we performed insulin turbidity assay and found dicentrine and dicentrinone, but not tetrandrine and fangchinoline, could effectively inhibit PDI activity, with IC_{50} of 56.70 μM and 43.95 μM respectively. Meanwhile, dicentrine and dicentrinone failed to further reduce the cell number index when co-treated with siRNA of PDI, suggesting the compounds behave as PDI inhibitors. Furthermore, dicentrinone and dicentrine have been successfully docked to the active pocket of PDI (PDB #3UEM) by molecular docking, suggesting the existence of physical interaction between compounds and PDI. Our results suggested that dicentrine and dicentrinone may be developed into safe PDI inhibitors.

Keywords: *Stephania tetrandrae* Radix; dicentrine; dicentrinone; HepG2; PDI.

1. Introduction

Natural products have long been considered as promising sources of anti-tumor agents with advantages in little side effects due to the co-evolution with biological systems for millions of years [1]. Currently, a list of natural products originally isolated from herbs has been clinically proven as safe and effective, such as paclitaxel [2], vincristine [3], camptothecin [4] and so on. *Stephaniae tetrandra* Radix (STR) is a traditional Chinese medical herb prescribed for various medical conditions including tumor, rheumatism, cardiovascular disease, malaria, fever and so on [5]. Alkaloids,

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especially tetrandrine [6-15] and fangchinoline [16-22], are considered as the major active ingredients. Tetrandrine, originally identified as calcium channel blocker, has broad beneficial effects on the suppression of tumor cells growth via cell death signaling in various cell lines, including HT-29 [23], U87 [11], Hep-2 ($CD133^+$) [24], HCC [25] as well as primary cultured cancer cells[26]. Fangchinoline was also a natural product with wide bio-activities including anti-inflammatory, anti-oxidant, and anti-cancer. In term of anti-cancer, fangchinoline could effectively suppress cell proliferation in HepG2 [17], K562 [27], A549 [18], SPC-A-1 [19],AGS [28], T24 [20] via cell death signaling or cell cycle arrest. Studies on other alkaloids isolated from STR still remained unclear.

Protein disulfide isomerase (PDI) is a 57-kDa ER localized chaperone engaging in process of protein folding [29]. PDI expression level was elevated in various types of human cancers, including lung cancers [30], acute myeloid leukemia [31], prostate [32], melanoma [33, 34], ovarian [35, 36], glioma [37] and so on. Increased expression of PDI was a pro-survival signal for cancer cells [38]. Concordantly, inhibition of PDI should led to increased amount of misfolded or unfolded protein resulting in cell death eventually [39]. By now, several PDI inhibitors have been identified recent years, including PACMA31 for ovarian cancer [36], LOC14 for Huntington disease[40], juniferdin for HIV infection [41], CCF642 for multiple myeloma [42], rutin for thrombus [43], and BAP2 for glioblastoma [44]. However, there is still no available effective and safe PDI inhibitors of clinical use by now. We previously reported alkaloids isolated from *Nelumbinis Plumula* could inhibit PDI activity *in vitro* [45]. In this study, we reported STR, another famous anti-cancer herb collected by *Chinese Pharmacopeia* may also suppress tumor growth through PDI inhibition by two alkaloids, dicentrine or dicentrinone.

2. Materials and Methods

2.1. Material

Stephania tetrandrae Radix (STR) were obtained from Yanghetang Pharmaceutical Co., Ltd. (Shanghai, China) and authenticated by Professor Zhang Dafang. A voucher sample (# STR-CC-2019061702) was reserved in Changchun Humanities and Sciences College. HepG2 cell line was gift from Professor Lijing Li, College of Pharmacy, Changchun University of Chinese Medicine. DMEM medium (# 11995), FBS (# 10091), and penicillin/streptomycin (# 15140) were all obtained from Gibco ThermoFisher. Thiazolyl Blue Tetrazolium Bromide (MTT, # M2128), DTT (# 43819), bovine insulin (# I4011), bovine PDI (# P3818), were obtained from Sigma. Silica gel (Tsingtao Ocean Chemical, China), Sephadex LH-20 (GE Bio-Science, Sweden), and ODS (Silicycle, Quebec City, Canada) were used for isolation of compounds. EtOH, $CHCl_3$ and MeOH were all obtained from Jintai Biotechnology Co. (Jilin, China).

2.2. Extraction and Isolation

5.0 kg of dried STR were smashed (120 mesh) and extracted with 70% EtOH for 5hrs 3 times (50 L each time) at 55±3℃. Removed EtOH from the combined extracts and achieved the final volume of 650mL extracts. Resolved the extracts with 4L ditilled H_2O and adjusted the pH to 3 using 2M HCl. Applied the solution to 0.45μm microporous filters and adjusted the pH to 10 using 5% $NaHCO_3$ followed by chloroform extraction and obtained 110g total alkaloids. The total alkaloids (110g) was added to silica gel column chromatography, followed $CHCl_3$-MeOH elution with stepwise addition of MeOH (100:0-0:100 v/v) and obtained 5 fractions eluted by $CHCl_3$-MeOH 75:1, 50:1, 25:1, 15:1 and 5:1 respectively.

Compound **1** (3mg) has been obtained from Fraction 1 or Fr. 1 (CHCl3-MeOH 75:1). Fr.2 (50:1) was subjected to silica gel again (100:0-25:1) and obtained Fr.2-1(100:0-100:1), Fr.2-2(50:1)and Fr.2-3 (25:1). Fr.2-1 was then subjected to SephadexLH-20 eluted by $CHCl_3$-MeOH 1:1 and achieved compound **2** (8g). Fr.2-2 was administrated to ODS (MeOH-H2O 20:80) and obtained compound **3** (12mg). Compound **4** (12 g) has been obtained from Fr.2-3. Fr.3 (25:1) was administrated to silica gel again (50:1) and performed recrystallization to obtain compound **5** (5 mg). Fr.4 (15:1) was added to

silica gel again (25:1) and obtained compound **6** (14 mg). Fr.5 (5:1) was administrated to silica gel again (15:1) and SephadexLH-20 eluted by CHCl3-MeOH 1:1 and obtained compound **7** (5 mg).

2.3. Preparation of Test Compounds

Compounds **1-7** were dissolved into 0.1M stock solution by DMSO and stored in -20℃ before use. For MTT assay, three concentrations of 1.0 μ m, 10 μ m and 50 μ m were diluted by cell culture medium. Blank control group was 0.1% DMSO (v/v) diluted by cell culture medium.

2.4. MTT Proliferation Assay

HepG2 cells were cultured in DMEM(1% PS;10% FBS) in 37°C. Cell viabilities of compounds on HepG2 had been measured by MTT proliferation assay. HepG2 cells were seeded and cultured in 96 well plates with a density of 5×10^3 cells each well in 37℃. After 24 hours, cells were treated by different concentrations of compounds (1.0μm, 10μm and 50μM) for another 48h. Removed the medium and followed 3 time of wash by PBS buffer. Added 20μL of 5mg/mL MTT solution to each well and incubated for another 4h. Remove the supernatant and added 50 μL DMSO to dissolve the formazan crystals with 10 minutes shaking. The absorbance value at 490nm was measured by Model 50 (Bio-Rad, USA). % cell viability can be calculated as value of OD_{490} of test wells divided by control wells.

2.5. Measurement of PDI Activity

As we previously described, PDI activity could be evaluated by insulin turbidity assay [45] and details of solutions preparation and experimental procedures were provided in Table 1. The absorbance values were read by Bio Tek Microplate Monitor at 620nm every 2 minutes for 80 minutes. In control 1 (Ctl 1) group, PDI is not added, which equals to 100% inhibition (0% activity). In control 2 (Ctl 2), no potential inhibitors has been added and PDI could function normally (100% activity), which equals to 0% inhibition. PDI activity (%) = OD_{620}(Test wells–Ctl 1)/ OD_{620} (Ctl 2-Ctl 1)*100%. IC_{50} values were achieved through nonlinear regression analysis.

Table 1. Solutions preparation and experimental procedures of insulin turbidity assay

Solutions Preparation					
Insulin stock solution (20*)		13mM bovine insulin ,0.1M HCl			
Buffer A		0.1M KH_2PO4, 2mM EDTA			
DTT stock solutions (200*)		0.5M DTT			
PDI stock solution (1000*)		1mM PDI in Buffer A			
PDI solution		1μM PDI in Buffer A			
Insulin solution(2.5mM DTT)		0.65mM insulin, 5mM HCl, 2.5mM DTT in Buffer A			
Experimental Procedures	**1**	**2**	**3**	**4**	**5**
Ctl 1/No PDI (100% inhibition)	77.5μL Buffer A	2.5μL DMSO	Incubation 1hr,@37℃	20μL Insulin solution(2.5mM DTT)	Reading 2/min, 80min, 620nm
Ctl 2 /PDI (no inhibition)	77.5μL PDI solution	2.5μL DMSO	Incubation 1hr,@37℃	20μL Insulin solution(2.5mM DTT)	Reading 2/min, 80min, 620nm
PDI+Compounds (? inhibition)	77.5μL PDI solution	2.5μL Compounds in DMSO	Incubation 1hr,@37℃	20μL Insulin solution(2.5mM DTT)	Reading 2/min, 80min, 620nm

2.6. siRNA Transfection

PDI siRNA(sc-44319, Santa Cruz) and control siRNA(sc-44236, Santa Cruz) were dissolved in DMSO and stored in -20℃ before use. Transfected HepG2 cells with 80nM PDI siRNA and control siRNA in 24 wells plates. mRNA expression level of PDI was determined by RT-PCR 48h after siRNA transfection (Fig S15). Stained HepG2 cells with crystal violet solution and imaged by Osteoplan II microscope (Carl Zeiss, NY). Cell number index were used to quantify the cell numbers and normalized by DMSO group.

2.7. Molecular Docking

Molecular docking were performed using Schrödinger Maestro (2015-2, Schrödinger, USA). 3UEM download from Protein Data Bank (https://www.rcsb.org/) is the structure of 2.29 Å human PDI bb'a' domains and has been used for docking of PACMA 31 into PDI active pocket by Xu et al [36]. Structure of 3UEM has been optimized by GLIDE software (v.6.7, Schrödinger, USA) with constraint set of 0.30 Å RSD. AutoDock Vina 1.1.2 was adopted to docking and PyMOL v 1.7.2.1 was used to analyze the interaction between compounds and 3UEM.

2.8. Statistical Analysis

Data analysis were performed using SPSS 25.0 package (SPSS Inc, Chicago, IL). ANOVA and LSD were performed for the comparisons between two groups.

3. Results and Discussion

3.1. Extraction and Isolation

The structures of high yield compounds **1**-**7** were all shown in Figure 1. After analysis of physicochemical properties, ESI-MS, NMR (Figure S1-S14) and comparison with known compounds reported in literatures, compounds 1-7 were identified as dicentrinone (**1**) [46], dicentrine (**2**) [47], oxytetrandrine (**3**) [48], tetrandrine (**4**) [25], fangchinoline (**5**) [49], stephanthrine (**6**) [48], and fenfangjine D (**7**) [50].

*Dicentrinone (**1**)*:Yellow needle-like powder, insoluble in water, soluble in pyridine and chloroform, slightly soluble in methanol and acetone, and appears orange-red in contact with bismuth potassium iodide reagent.mp193~197℃, ESI-MS *m/z*:335.0794,^{1}H NMR (500MHz, $CDCl_3$) δ 8.87 (1H, d, *J* = 8.0 Hz, H-3), 7.92 (1H, dd, *J* = 7.9, 2.3Hz, H-4), 7.71 (1H, s, 7-OH), 7.15 (2H, d, *J* = 9.2 Hz, H-10′,14′), 7.06 (2H, d, *J* = 7.9 Hz, H-11′, 13′), 7.86 (1H, s, H-13), 6.85 (1H, s, H-14), 6.80 (1H, s, H-10), 6.72 (1H, s, H-8′), 6.62 (1H, s, H-5′), 4.08 (3H, s, 2-NCH_3), 3.89 (6H, s, 12, 6′-OCH_3), 3.83 (3H, s, 6-OCH_3),3.43(1H, t, *J* = 8.5 Hz, H-1′), 2.35 (3H, s, 2′-NCH_3).

*Dicentrine (**2**)*:White solid, mp168~170℃,the reaction of ferric chloride-potassium ferricyanide is negative, and the reaction of potassium iodide is positive. The compound is a basic compound. ESI-MS *m/z* 340.1544, ^{1}H NMR (500MHz, $CDCl_3$), δ 7.95 (1H, s, H-11), 6.74 (1H, s, H-8), 6.52 (1H, s, H-6), 6.05 (1H, d, *J* = 12.3Hz, 2-H) and 5.98 (1H, d, *J* = 12.4 Hz, 2-H), 3.86 (3H, s, 9-OCH_3), 3.84 (3H, s, 10-OCH_3), 3.05 (1H, t, *J* = 6.8 Hz, H-7), 2.73 (1H, m, H-5), 2.46 (3H, d, *J* = 3.5 Hz, H-6).

*Oxofangchirine (**3**):*White solid, mp:183~184℃, the reaction of ferric chloride-potassium ferricyanide is negative, and the reaction of potassium iodide is positive. The compound is a basic biologically compound. ESI-MS *m/z*: 618.2366. ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.88 (1Hs, H-10′), 8.79 (1H, d, *J* = 4.9 Hz, H-3′), 8.00 (2H, d, *J* = 8.3 Hz, H-10′, 14′), 7.72 (1H, s, H-4′), 7.47 (1H, d, *J* = 2.3 Hz, H-5′), 7.17 (2H, d, *J* = 8.3 Hz, H-11′,13′), 6.86 (1H, d, *J* = 8.1 Hz, H-13), 6.79 (1H, d, *J* = 9.2 Hz, H-14), 6.57 (1H, s, H-10), 6.53 (1H, s, H-5), 3.89 (6H, d, *J* = 8.7 Hz, 12, 6′-OCH_3), 3.84 (6H, d, *J* = 10.9 Hz, 6, 7-OCH_3), 2.35 (3H, s, 2-NCH_3).

*Tetrandrine (**4**):* White solid, mp217~218°C, the reaction of ferric chloride-potassium ferricyanide is negative, and the reaction of potassium iodide is positive. The compound a basic compound. ESI-MS *m/z*: 622.7601, ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.16 (1H, d, *J* = 18.6 Hz, H-10′), 7.07 (1H, s, H-11′), 6.86 (1H, d, *J* = 8.1 Hz, H-13′), 6.73 (1H, s, H-8′), 6.62 (1H, s, H-5′), 6.57 (1H, s, H-10), 6.53 (1H, s, H-5), 3.90 (6H, d, *J* = 5.1 Hz, 12, 6′-OCH_3), 3.84 (6H, d, *J* = 10.8 Hz, 6, 7-OCH_3), 3.43 (1H, t, *J* = 8.5 Hz, H-1′), 2.35 (6H, m, 2′, 2-NCH_3).

*Fangchinoline (**5**):*White solid, mp237~239°C,the reaction of ferric chloride-potassium ferricyanide is positive, indicating that there are phenolic hydroxyl groups in the compound. The reaction of potassium iodide is negative, indicating that the compound is a biologically basic compound. ESI-MS *m/z*: 608.7286, ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.99 (1H, s, 7-OH), 7.13 (2H, dt, *J* = 7.6, 1.0 Hz, H-10′, 14′), 6.86 (2H, m, H13, -14), 6.75 (1H, s, H-8′), 6.71 (1H, s, H-5′), 6.55 (2H, d, *J* = 17.7 Hz, H-5,10), 3.90 (6H, d, *J* = 5.1 Hz, 12,6′-OCH_3), 3.86 (3H, s, 6-OCH_3), 3.07 (1H, dd, *J* = 8.6, 4.0 Hz, H-1), 2.40 (3H, s, 2′-NCH_3), 2.35 (3H, s, 2-NCH_3).

*Stephanthrine (**6**):*White needle shape,mp234~236°C,the reaction of ferric chloride-potassium ferricyanide is positive,indicating that there are phenolic hydroxyl groups in the compound.The reaction of potassium iodide is negative,indicating that the compound is a biologically basic compound. ESI-MS *m/z* : 293.1398, ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.03 (1H, d, *J* = 3.7 Hz, H-7), 8.01 (1H, d, *J* = 2.9 Hz, H-10), 7.87 (1H, d, *J* = 8.2 Hz, H-5), 7.81 (1H, d, *J* = 8.6 Hz, H-6), 7.57~7.52 (2H, m, H-8, 9), 6.77 (1H, s, H-3), 6.11 (2H, s, H-1), 2.98 (2H, t, *J* = 6.0 Hz, H-4), 2.86 (2H, t, *J* = 5.7 Hz, H-5), 2.38 (6H, s,5- $N(CH_3)_2$).

*Fenfangjine D (**7**):*White solid,mp109.0~110°C,the reaction of ferric chloride-potassium ferricyanide is positive,indicating that there are phenolic hydroxyl groups in the compound.The reaction of potassium iodide is negative,indicating that the compound is a biologically basic compound. ESI-MS *m/z* : 605.2653, ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.87 (1H, d, *J* = 8.0 Hz, H-3), 7.92 (1H, dd, *J* = 7.9, 2.3 Hz, H-4), 7.71 (1H, s, 7-OH), 7.15 (2H, d, *J* = 9.2 Hz, H-10′,14′), 7.06 (2H, d, *J* = 7.9 Hz, H-11′, 13′), 6.86 (1H, s, H-13), 6.85 (1H, s, H-14), 6.80 (1H, s, H-10), 6.72 (1H, s, H-8′), 6.62 (1H, s, H-5′), 4.08 (3H, s, 2-NCH_3), 3.89 (6H, s, 12, 6′-OCH_3), 3.83 (3H, s, 6-OCH_3), 3.43 (1H, t, *J* = 8.5 Hz, H-1′), 2.35 (3H, s, 2′-NCH_3).

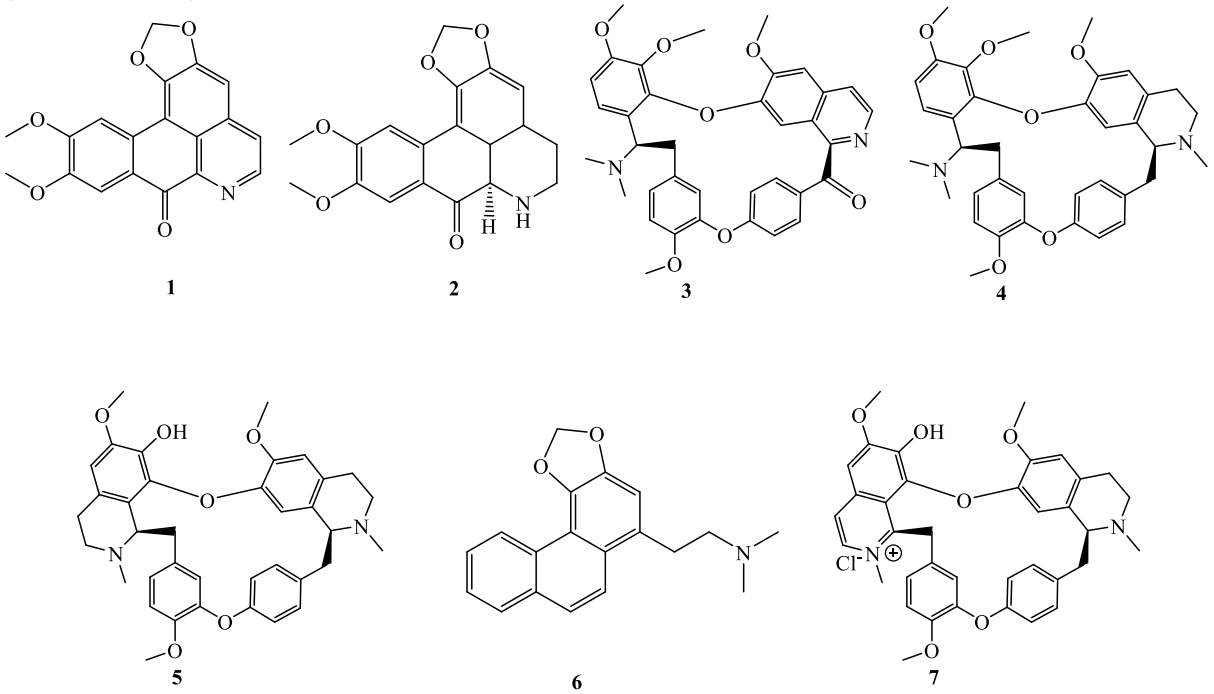
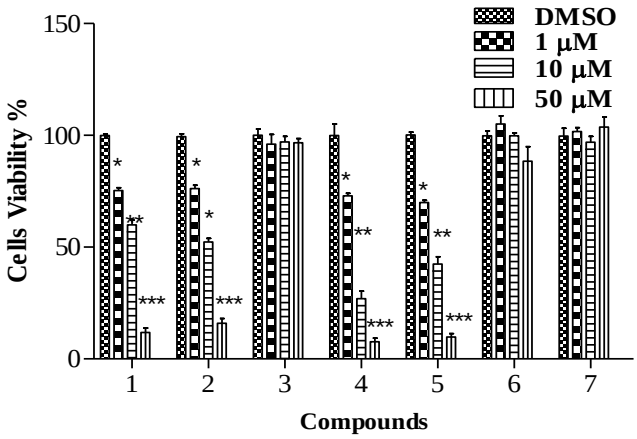


Figure 1. Chemical structures of high yield compounds isolated from STR (**1-7**)

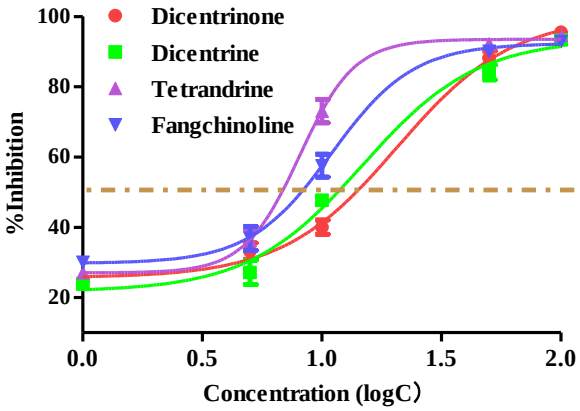
3.2. Effects of Compounds on the Cell Viability of HepG2 Cells by MTT

To identify potential cytostatic agents isolated from STR, a traditional anti-tumor Chinese medicine, we performed MTT assay in HepG2 cells starting with 1μM, 10μM, and 50μM of the 7 high yield compounds respectively (Figure 2). Comparing to DMSO groups, compound **1** had obvious cytostatic effect on HepG2 cells proliferation, with cell viability of 75.29%，59.98% and 11.79% in different concentration of 1μM, 10μM, and 50μM respectively. Similarly, compounds **2**, **4** and **5** also show cytostatic capacity on HepG2 cells in dosage dependent manners as shown in Figure 2. Compounds **3**, **6** and **7** shown no effects. To calculate the CC_{50} (50% cytotoxic concentration) of compounds **1**, **2**, **4** and **5,** we repeated the MTT assay in HepG2 cells using five concentrations of 1μM, 50μM, 10μM, 50μM and 100μM. As shown in Fig2B and 2C, CC_{50} of compounds **1**, **2**, **4** and **5** are 20.70-, 14.80-, 8.07- and 10.97μM.

A.



B.



C.

CC_{50} μM	
Dicentrinone(**1**)	20.70 (15.87-27.01)
Dicentrine(**2**)	14.80 (11.04-19.85)
Tetrandrine(**4**)	8.07 (7.30-8.91)
Fangchinoline(**5**)	10.97 (9.34-12.88)

Figure 2. Effects of seven compounds on HepG2 cell viability by MTT assay

(A) HepG2 cells were treated by three concentration of each compound, low concentration (1μM), medium concentration (10 μM) and high concentration (50μM). For blank control group of each compound, same volume of DMSO was added into the cell medium. Comparing to DMSO group, *, **, and *** stands for $P < 0.05$, 0.01 and 0.005 respectively. (**B**) and (**C**), CC_{50} of compounds **1**, **2**, **4** and **5.** Curves were generated from mean values (BAR, SEM) of treated by compounds of 1μM, 50μM, 10μM, 50μM and 100μM for 48hrs. Each experiments had 6 replicates. Values of CC_{50} (C) were represented as mean (maximum value-minimum value).

3.3. Dicentrinone and Dicentrine Inhibit PDI Activity by Insulin Turbidity Assay

To examine whether the growth inhibitory effects of candidate compounds were through PDI inhibition**,** we performed insulin turbidity assay by using 3μM, 10μM, 30μM, 100μM and 300μM of compounds. For compounds **1** and **2** (Fig 3A and 3B), slopes of the curves of different concentrations decreased from 3μM to 300μM, suggesting inhibitory effects of compounds **1** and **2** on PDI are concentration-dependent. For compounds **4** and **5** shown in Fig 3C and 3D, curves of different concentrations all merged with the control 1 (100% inhibition), suggesting compounds **4** and **5** have no inhibitory effects on PDI activity. To calculate the IC_{50} of compounds **1** and **2** on PDI inhibition, we performed non-linear regression analysis and plotted in Fig 3E. The IC_{50} value of compounds **1** and **2** were 56.70 μM and 43.95 μM.

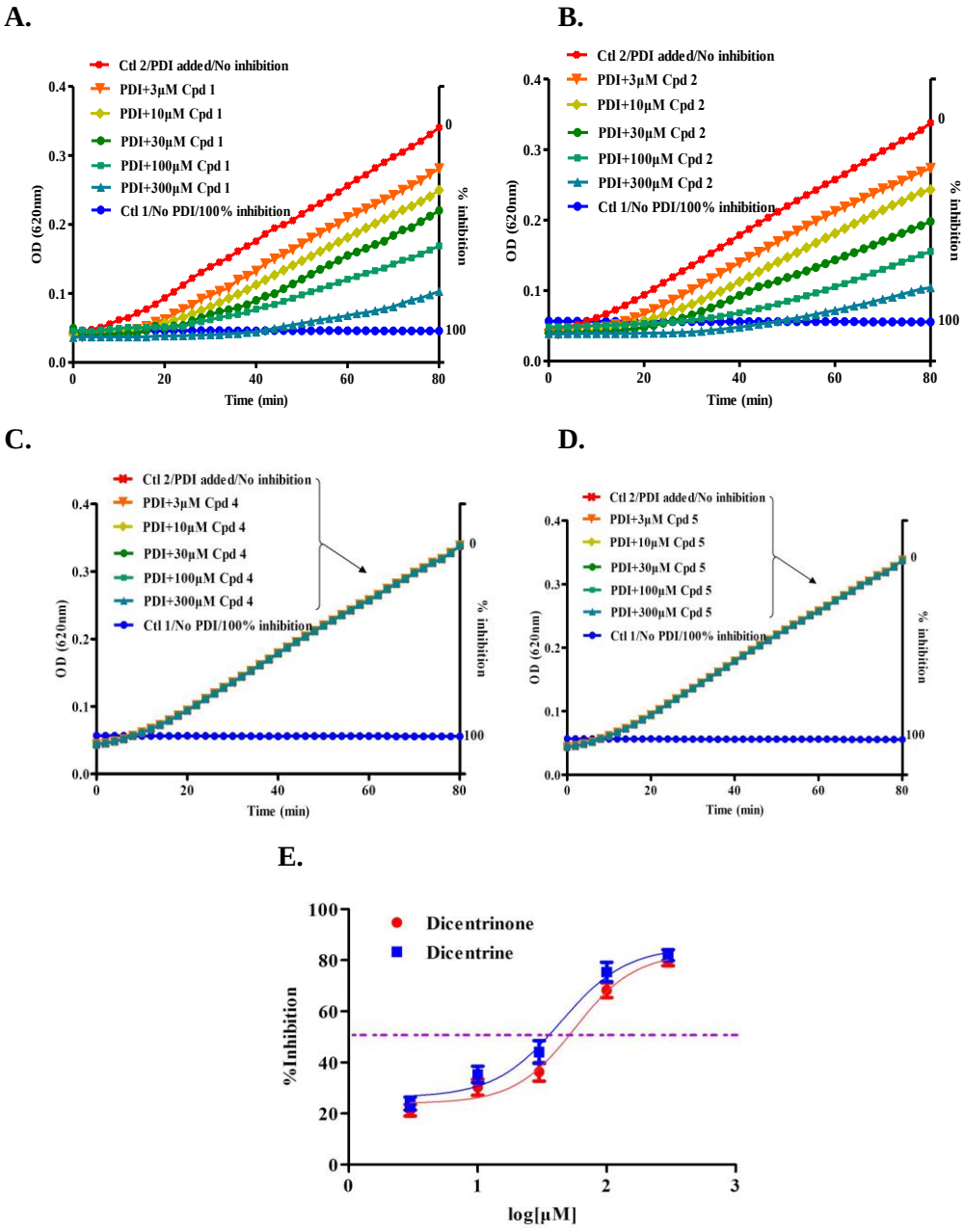


Figure 3. Effects of compounds on PDI activity by insulin turbidity assay

The absorption curves at 620nm of PDI treated by different concentrations of compounds **1**, **2**, **4** and **5** were shown in **A**-**D** respectively. Right Y axis of **A**-**D** stands for the inhibition percentage of each compounds on PDI activity. (**E**) The inhibition of PDI activity by dicentrinone and dicentrine. Groups were treated by compounds of 3μM, 10μM, 30μM, 100μM and 300μM for 48hrs. The dashed line indicated the IC_{50} values of the two compounds plotted by GraphPad.

3.4. Dicentrinone and Dicentrine Suppressed Cell Viability of HepG2 through PDI

To investigate whether the molecular mechanism of cytostatic capacity of dicentrinone and dicentrine on HepG2 were through PDI, we co-treated the HepG2 cell with siRNA of PDI (siPDI) and 56.70 μM dicentrinone (IC_{50} of dicentrinone) or 43.95 μM dicentrine (IC_{50} of dicentrine). To quantify the cell viability after crystal violet staining, we calculated the cell number index and normalized the control group (siCtl) as 1.00 (Fig 4B). Consistently with Fig 2A, cell number index treated by 56.70 μM dicentrinone (siCtl+ dicentrinone group) or 43.95 μM dicentrine (siCtl+ dicentrine group) reduced to 0.54 (p<0.01) and 0.38 (p<0.005) respectively. siPDI alone group, with a reduction of 19.6% in mRNA level of PDI detected by RT-PCR (Figure S15), could reduce the cell number index significantly to 0.22 (p<0.005). However, combination of siPDI with dicentrine (siPDI+ dicentrine group) or dicentrinone (siPDI+ dicentrinone group) failed to further reduce the cell number index (both p >0.05), suggesting dicentrinone and dicentrine suppressed cell viability of HepG2 through PDI.

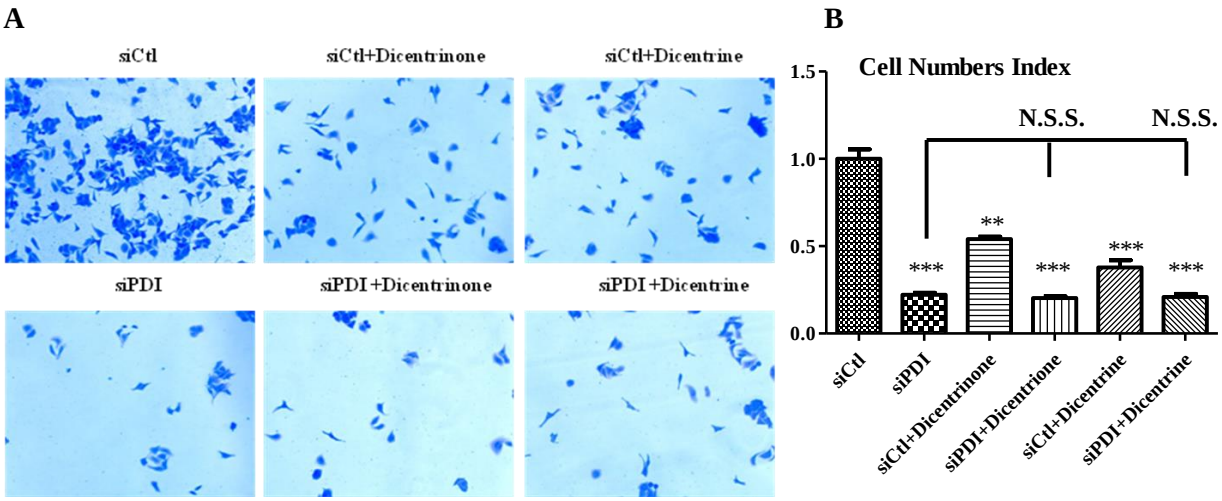


Figure 4. Crystal violet staining of HepG2 cells after transfection of siRNA
(**A**) Morphology of HepG2 cells stained by crystal violet of the six groups. (**B**) Cell number index (crystal violet positive cell numbers in each group normalized by cell numbers siCtl group). (Comparing to siCtl group, ** and *** stands for P < 0.01 and 0.005 respectively. Comparing to siPDI group, "N.S. S." stands for no statistical significance.)

3.5. Dicentrinone and Dicentrine Fit the Active Pocket of PDI by Molecular Docking

The insulin turbidity assay and siPDI transfection assay both suggested the interaction between PDI and dicentrinone or dicentrine. To further confirm the directly physical interaction between PDI and dicentrinone or dicentrine, we performed molecular docking using 3UEM, crystal structure of human PDI bb'a' domains. As shown in Figure 5, dicentrinone and dicentrine fit the active pocket of PDI well and the binding affinity between PDI and dicentrinone or dicentrine were -9.02 and -8.84 kcal/mol respectively, which suggested dicentrinone and dicentrine were the potential inhibitors of PDI.

PDI inhibition has been a promising therapeutic strategy against thrombosis[43], HIV infection [41], neurodegeneration disease [40], cancers [39] and so on. However, there is still no available effective and safe PDI inhibitors used in clinical by now. Natural products has long been considered as valuable source for anti-cancer drugs [51], while little studies on PDI inhibitors isolated from natural products has been reported. In our present study, we isolated compounds from STR, a well-known anti-cancer herb, and found in addition to the previously reported anti-cancer compounds tetrandrine and fangchinoline, dicentrine and dicentrinone could suppress HepG2 Proliferation. Moreover, we found dicentrine and dicentrinone , but not tetrandrine and fangchinoline, could both inhibit the PDI enzyme activity and suppress HepG2 cell proliferation thriough PDI inhition. Our

present study updated the anti-cancer mechanism of STR and provided two promising safe PDI inhibitors derived from natural products.

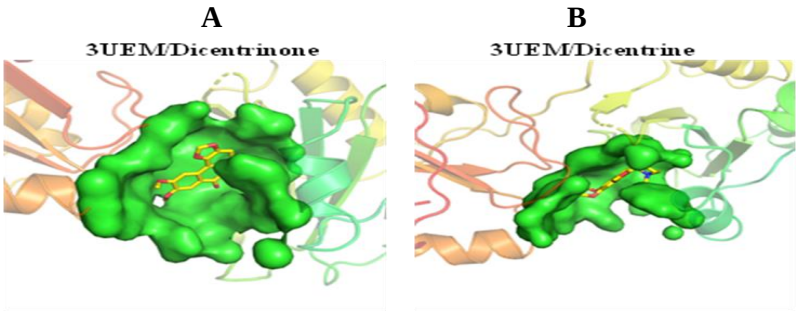


Figure 5. Dicentrinone and dicentrine could be docked into the active pocket of PDI. Green pocket indicated the binding sites of PDI (PDB # 3UEM) with dicentrinone (**A**) and dicentrine(**B**). Structure optimization of 3UEM were constraint to 0.30 Å RSD and the grid box was restricted to the size of 2 nm at the active site. Graphs were plotted by PyMol (Schrödinger, USA).

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Supporting Information

Supporting information accompanies this paper on http://www.acgpubs.org/journal/records-of-natural-products

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