國立嘉義大學生命科學院

學生學術研究成果優良海報評選獲獎名單

時間:106 年 5 月 24 日

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Tunicamycin加強Oxaliplatin誘導人類大腸癌細胞DLD-1細胞凋亡之研究

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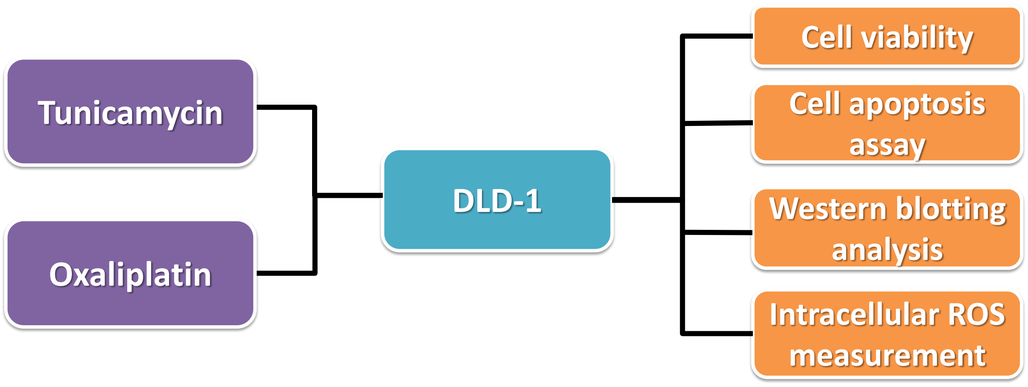
Abstract

現今臨床上治療大腸癌是以化療藥物為主，然而臨床化療藥物的使用也存在許多問題，像是強大的副作用以及抗藥性的問題，因此目前臨床上常會合併使用兩種以上的藥劑作為治療，此種方式可增加治療效果，同時可降低具有強大副作用的藥物使用，並解決了部分的抗性問題。Tunicamycin (Tuni)為鏈黴菌產生之抗生素，會造成細胞的醣蛋白折疊錯誤，導致內質網壓力的產生，進而使細胞走向凋亡。Oxaliplatin (Oxa)為新型platinum之抗癌藥物，可藉由阻斷細胞DNA複製和轉錄，從而導致癌細胞的死亡與細胞週期停滯，在臨床上多與其他藥物搭配使用，但效果依然有限。本研究目的將使用低濃度Tunicamycin與Oxaliplatin合併治療大腸癌細胞株DLD-1，觀察藥物合併使用能否加強細胞凋亡作用，並闡明其基礎作用機轉。實驗首先以MTT試驗評估Oxaliplatin與Tunicamycin對大腸癌細胞株DLD-1的細胞毒性，進一步利用TUNEL Assay觀察細胞凋亡的情形，以Western blot檢測與細胞凋亡有關的蛋白質表現量，以流式細胞儀分析細胞內活性氧物質(ROS)的變化。結果顯示，當DLD-1細胞經Tunicamycin與Oxaliplatin藥物共同處理後，細胞的生長有更明顯的抑制情形，且兩藥物合併下會加強促進細胞凋亡相關蛋白PARP-1活性裂解，並增加細胞DNA斷裂的程度，進而造成細胞凋亡，故推測合併藥物是藉由加強誘導細胞凋亡而造成細胞死亡。機轉路徑方面，推測Tunicamycin與Oxaliplatin合併下可能是透過上調p53、Bax/Bcl-2比例與p38 MAPK蛋白質表現，以及加強細胞內ROS產生的方式造成細胞凋亡。

Materials and Methods



Results

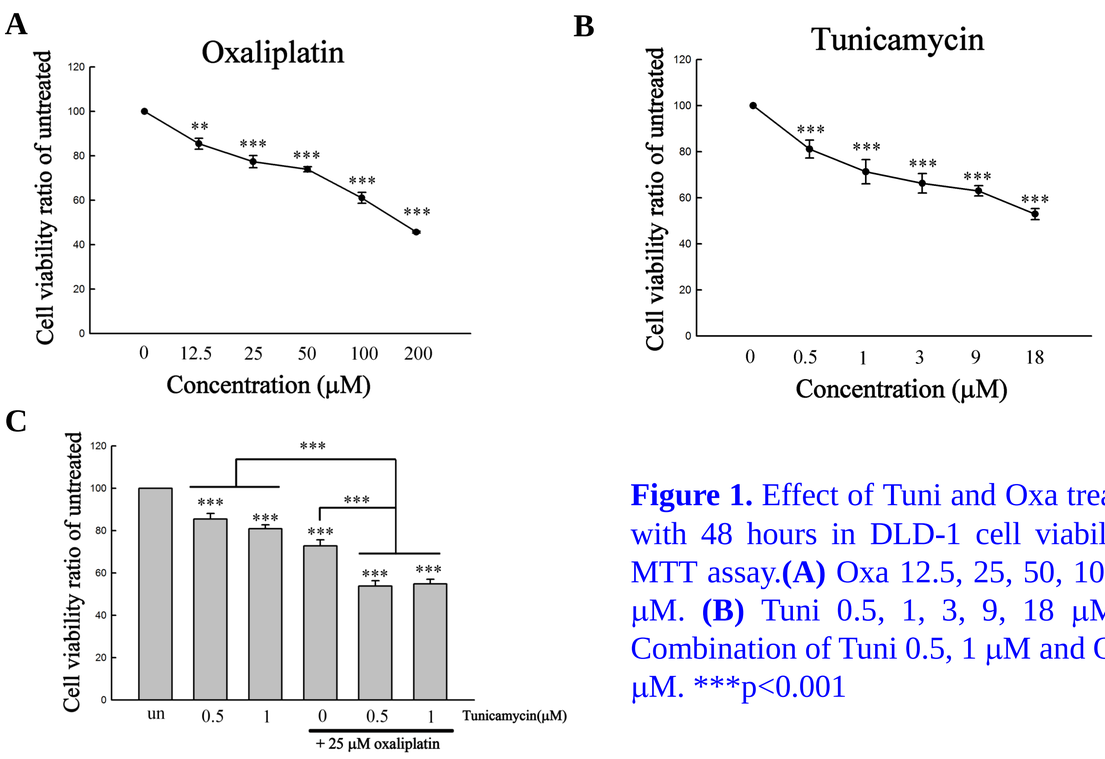


Figure 1. Effect of Tuni and Oxa treatment with 48 hours in DLD-1 cell viability by MTT assay.**(A)** Oxa 12.5, 25, 50, 100, 200 μM. **(B)** Tuni 0.5, 1, 3, 9, 18 μM. **(C)** Combination of Tuni 0.5, 1 μM and Oxa 25 μM. ***p<0.001

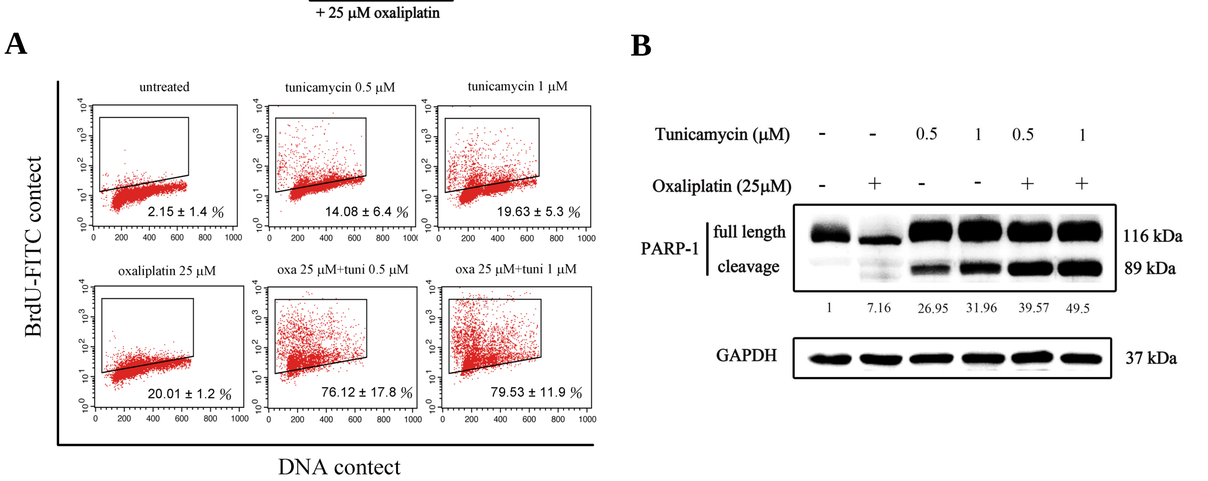
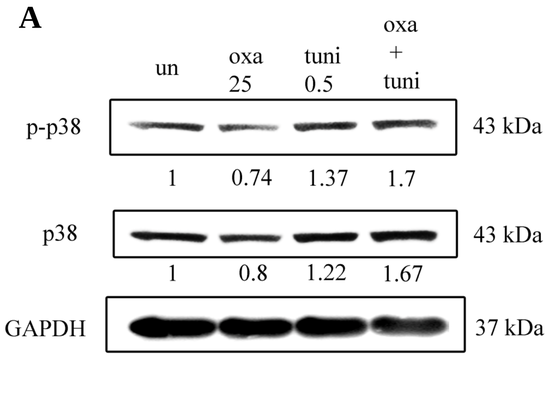


Figure 2. Combination treatment with Tuni and Oxa suppresses the growth of DLD-1 cells by inducing of apoptosis. **(A)** Cell apoptosis was detected using TUNEL assay by flow cytometry after Oxa and/or Tuni treatment with 48 hours. **(B)** Expression levels of apoptotic protein (PARP-1 cleavage) was analyzed by Western blotting after Oxa and/or Tuni treatment with 48 hours.



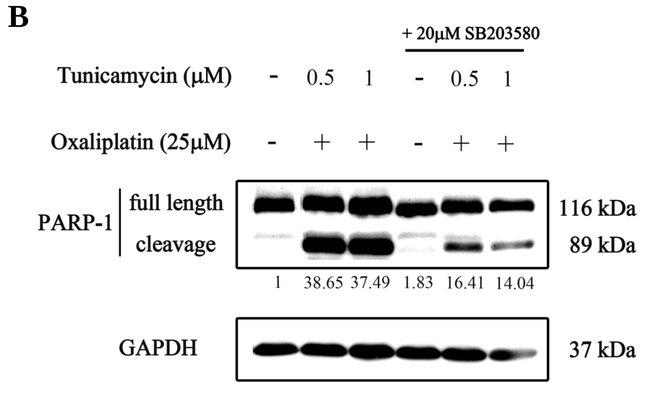
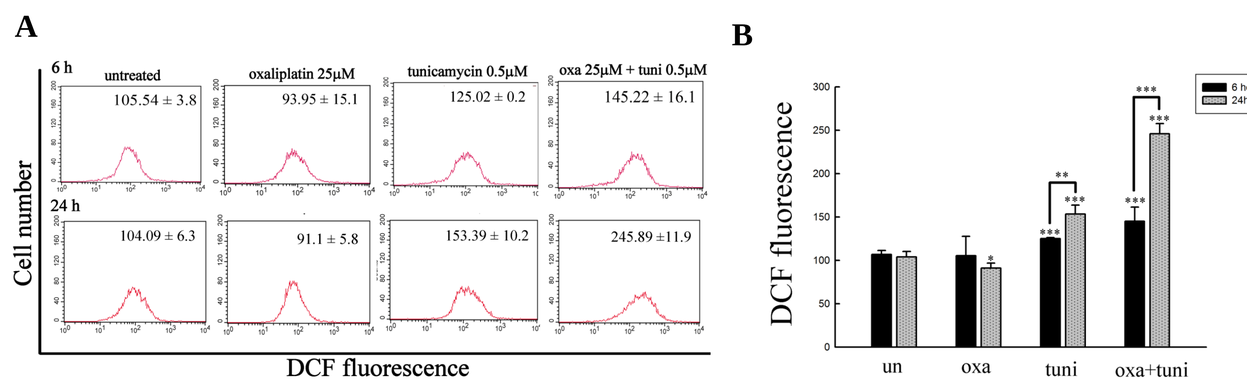


Figure 3. Combination treatment with Tuni and Oxa induced cell apoptosis through p38 MAPK pathway. **(A)** Expression levels of p-p38, p38 protein was analyzed by Western blotting after Oxa and/or Tuni treatment with 1 minute. **(B)** Expression levels of PARP-1 cleavage treated with Oxa and Tuni for 48 hours in the presence of p38 inhibitor (SB203580) ,



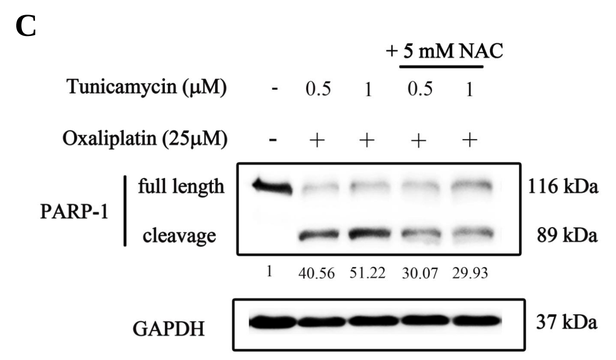


Figure 4. Combination treatment with Tuni and Oxa induced cell apoptosis through increasing the production of reactive oxygen species (ROS). **(A, B)** Intracellular ROS level was detected using DCFH-DA assay by flow cytometry after Oxa and/or Tuni treatment with 6 h and 12 h. **(C)** Expression levels of PARP-1 cleavage treated with Oxa and Tuni for 48 hours in the presence of ROS scavenger (N-Acetyl-cysteine, NAC). *, p<0.05 ; **, p<0.01 and ***, p<0.001.

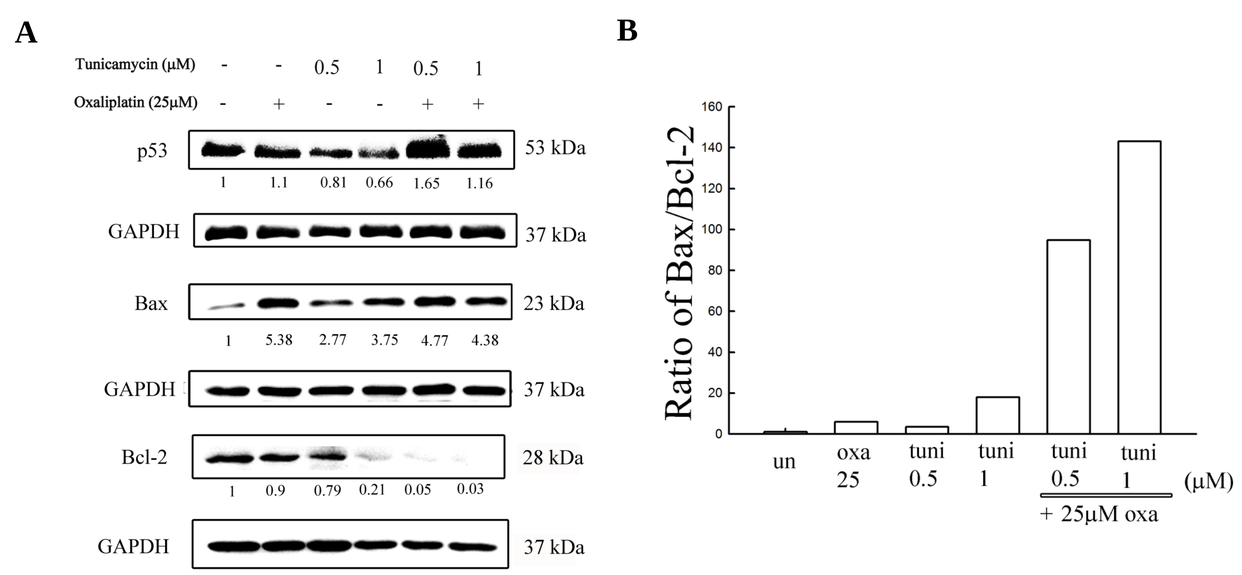
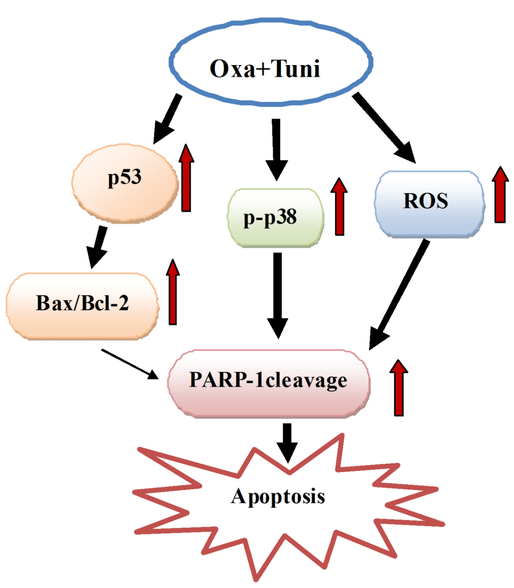


Figure 5. Combination treatment with Tuni and Oxa increases p53 and Bax/Bcl-2 ratio in DLD-1 cell. **(A)** Expression levels of p53, Bax and Bcl-2 was analyzed by Western blotting (Bax and Bcl-2 were treated with Oxa and/or Tuni after 48 hours ; p53 treated with 24 hours). **(B)** The ratio of Bax/Bcl-2 is represented by columngraph.

Conclusion

在本篇研究中，我們發現Tunicamycin與Oxaliplatin的合併模式對大腸癌DLD-1細胞有更好的毒殺效果。經細胞凋亡的分析後，推測合併治療下是藉由加強誘導細胞凋亡而造成細胞死亡的增加，且此種合併治療所誘導的細胞凋亡可能是透過p53/Bax/Bcl-2、p38 MAPK訊息傳遞路徑，以及加強產生細胞內ROS的方式所導致。綜上所述，Tunicamycin與Oxaliplatin的合併使用可作為新型且具有潛力的治療方式。



Synthesis and photocytotoxicity of novel 9-/13-lipophilic substituted berberine derivatives as potential anticancer agents

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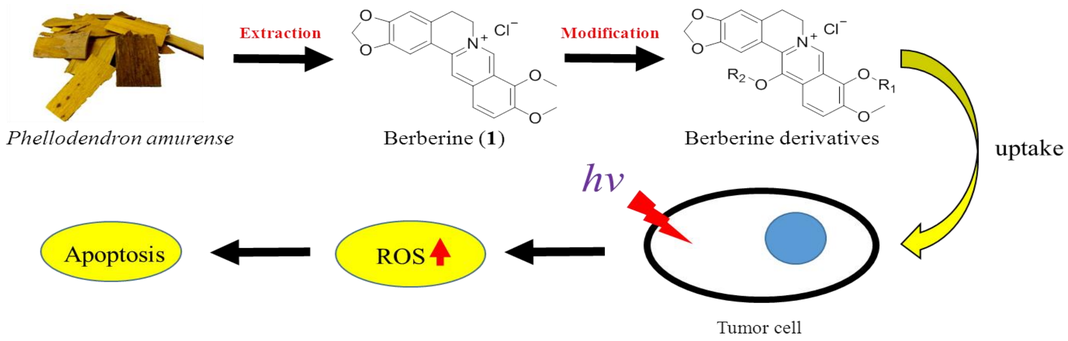
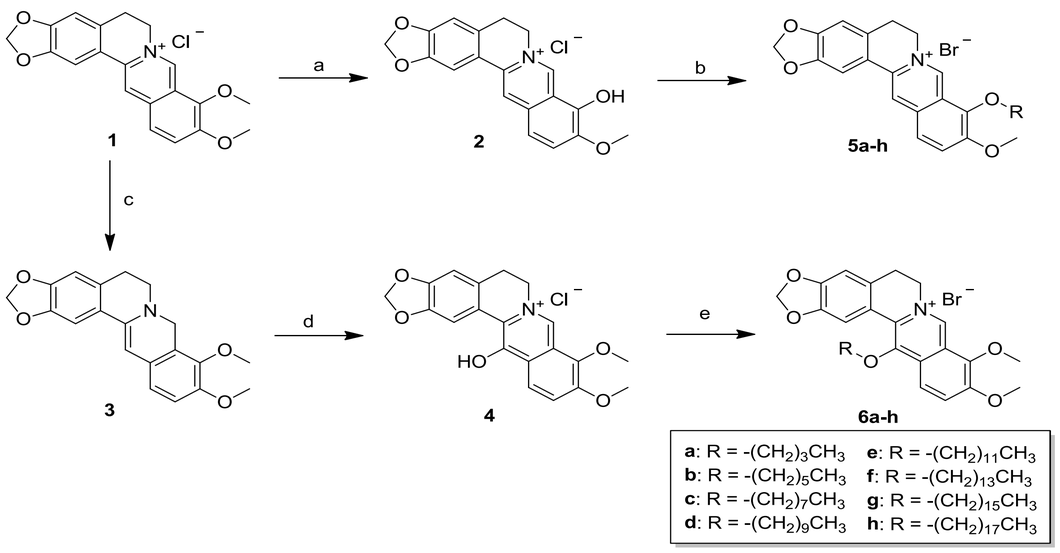
INTRODUCTION

Berberine is a quaternary ammonium salt from the protoberberine group of isoquinoline alkaloids. It has a wide range of biochemical and pharmacological effects. The aim of this study was to synthesize lipophilic 9-/13-substituted berberine derivatives and evaluate the photocytotoxic activity against human colon cancer cell lines using MTT assay *in vitro*. In this report, our results revealed that the longer lipophilic substituents can increase both the cellular uptake and the inhibition of cell growth on two human colon cancer cell lines. These results suggested that the presence of lipophilic substituents with moderate sizes might be crucial for the optimal anticancer activity. In conclusion, it is confirmed berberine derivatives at 9-/13-position bearing long chain *n*-alkyl group as anti-cancer agents and also as potential adjuvant of photocytotoxic or chemotherapeutic drugs. The present work can be the evidence that berberine derivatives have potent anti-cancer activities against human colon cancer cells.



MATERIALS AND METHODS

Scheme 1: Synthesis of 9-/13-substituted berberine derivatives.



Reagents and conditions: (a) 190°C, 20-30 mmHg, 30-60 min; (b) *n*-alkyl bromide, K_2CO_3, CH_3CN, reflux, 16-24 h; (c) $NaBH_4$, K_2CO_3, MeOH, 0°C, 2 h; (d) MCPBA, CH_2Cl_2, -20 ~ -30°C, 2 h; then Na_2SO_3, rt, 1 h; (e) *n*-alkyl bromide, NaI, Et_3N, MeOH, reflux, 16-24 h.

RESULTS

Table 1. IC_{50} values and lipophilicity of berberine and its derivatives on the growth of two human colon cancer cell lines for 24 h

Compd	R	clog *P*	IC_{50} (μM)	
			SW480	DLD-1
1	9-*O*-methyl	-0.77	8.22 ± 4.50	> 40
5a	9-*O*-butyl	0.82	1.14 ± 0.61	4.51 ± 1.07
5b	9-*O*-hexyl	1.87	1.73 ± 0.56	4.53 ± 1.40
5c	9-*O*-octyl	2.93	0.39 ± 0.06	0.38 ± 0.04
5d	9-*O*-decyl	3.99	0.31 ± 0.09	0.28 ± 0.07
5e	9-*O*-dodecyl	5.05	0.16 ± 0.05	0.13 ± 0.02
5f	9-*O*-tetradecyl	6.11	0.27 ± 0.13	0.20 ± 0.02
5g	9-*O*-cetyl	7.16	0.37 ± 0.15	0.22 ± 0.03
5h	9-*O*-octadecyl	8.22	2.08 ± 0.62	0.62 ± 0.09
6a	13-*O*-butyl	0.95	25.30 ± 12.82	> 20
6b	13-*O*-hexyl	2.01	2.90 ± 0.00	2.51 ± 0.74
6c	13-*O*-octyl	3.07	2.61 ± 0.33	1.24 ± 0.20
6d	13-*O*-decyl	4.12	0.77 ± 0.32	0.70 ± 0.35
6e	13-*O*-dodecyl	5.18	0.43 ± 0.04	0.63 ± 0.35
6f	13-*O*-tetradecyl	6.24	0.67 ± 0.38	0.81 ± 0.11
6g	13-*O*-cetyl	7.30	0.74 ± 0.13	0.84 ± 0.16
6h	13-*O*-octadecyl	8.36	1.53 ± 0.14	1.82 ± 0.08

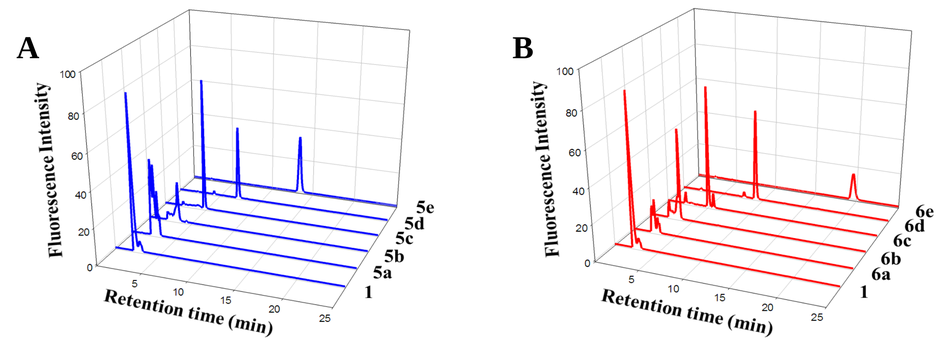


Figure 1. HPLC analysis of compounds (A) **1** and **5a−5e** and (B) **1** and **6a−6e**.

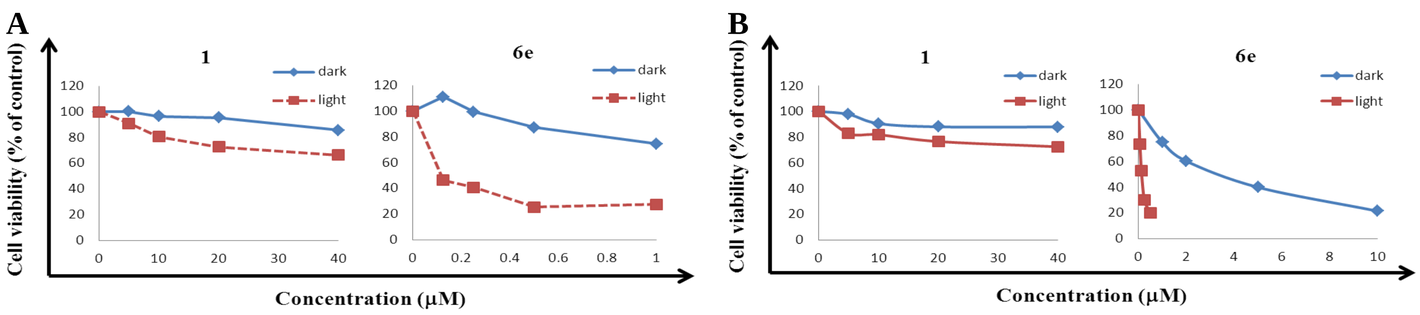


Figure 2. Dark and photocytotoxicity of (A) SW480 and (B) DLD-1 treated with compounds **1** and **6e** after irradiation (420 nm, 5.6 mW/cm^2, 10 min).

Table 2. IC_{50} values of compounds **1** and **6e** on DLD-1 cells in the dark and after irradiation for 24 h

Compd	IC_{50} (μM)					
	SW480			DLD-1		
	Dark	Light	PIF (Dark/Light)	Dark	Light	PIF (Dark/Light)
1	> 40	> 40	1	> 40	> 40	1
6e	> 1	0.11 ± 0.01	> 9.09	2.47 ± 0.74	0.18 ± 0.05	15.30

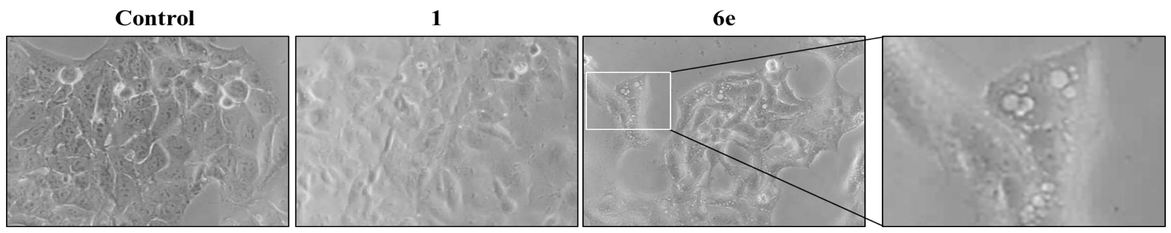


Figure 3. Cell morphological changes of DLD-1 cells treated with compounds **1** (40 μM) and **6e** (0.25 μM) for 24 h.

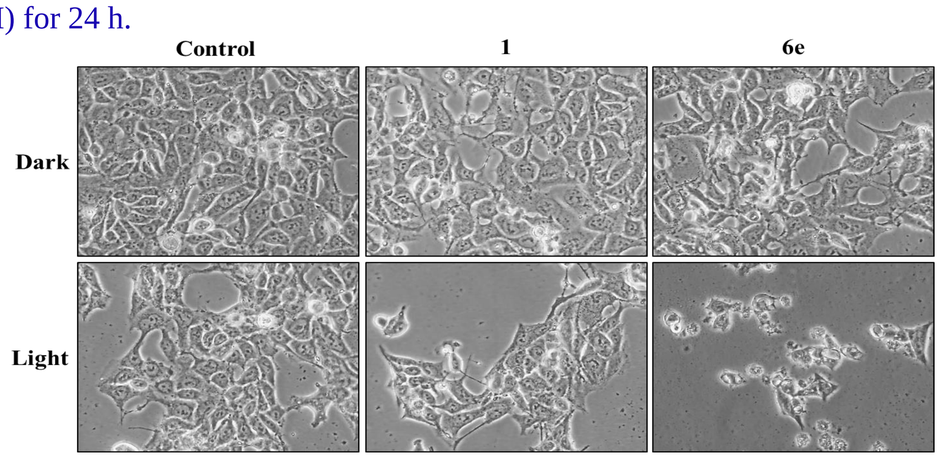


Figure 4. Cell morphological changes of DLD-1 cells exposure without (control) or with **1** (5 μM) and **6e** (0.5 μM) in the dark and after irradiation (420 nm, 5.6 mW/cm^2, 10 min) for 24 h.

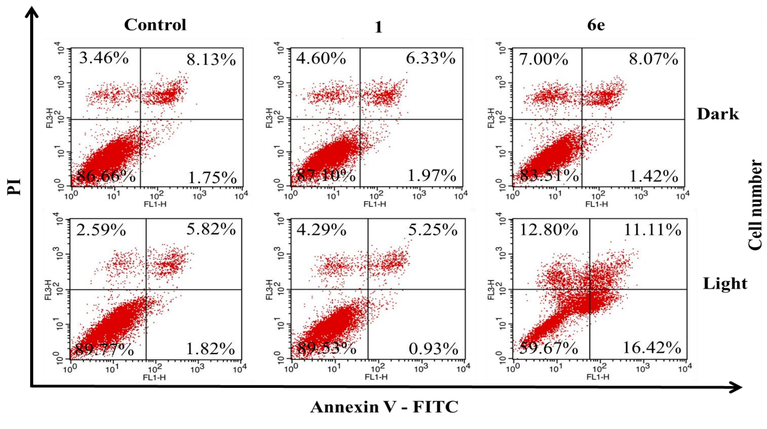


Figure 5. The percentage of apoptotic cell changed by treatment with **1** (5 μM) and **6e** (0.5 μM) on DLD-1 cells for 24 h after irradiation.

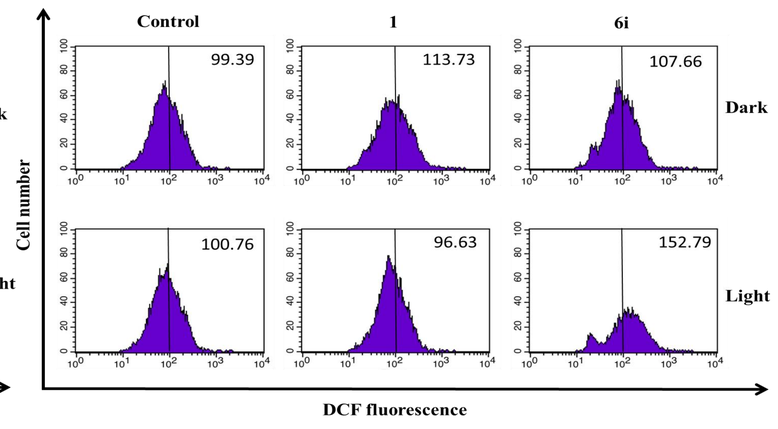


Figure 6. The effects of DLD-1 treated with **1** (5 μM) and **6e** (0.5 μM) for 24 h after irradiation on percentage distribution of ROS by DCFH-DA staining.

CONCLUSIONS

The introduction of long chain *n*-alkyl groups at the 9-/13-position of berberine was to evaluated anticancer activity and the lipophilicity of berberine derivatives was analyzed by HPLC. The photocytotoxic test confirmed that compounds **1** and **6e** increased the cytotoxicity after irradiation. Vacuoles appeared in the cytoplasm after treated with compound **6e** and the number of cells decreased after irradiation showed in the image and apoptosis analysis proved compound **6e** induce cell apoptosis after irradiation. We found that **6e** increased the content of ROS generation in the DLD-1 cells after visible light irradiation. In the present study, the berberine derivatives can to be developed as newly photocytotoxic anticancer agents.