Semi-synthesis and in vitro anti-tumor effects evaluation of novel xanthohumol derivatives

Xiaolong Sun
School of Pharmacy, Bengbu Medical College, 2600 Donghai Road, Bengbu 233030, Anhui
Jie Chen
School of Pharmacy, Bengbu Medical College, 2600 Donghai Road, Bengbu 233030, Anhui
Di Huang
School of Pharmacy, Bengbu Medical College, 2600 Donghai Road, Bengbu 233030, Anhui
Feng Ding
School of Pharmacy, Bengbu Medical College, 2600 Donghai Road, Bengbu 233030, Anhui
Long Zhao
School of Pharmacy, Bengbu Medical College, 2600 Donghai Road, Bengbu 233030, Anhui
Hong-Mei Li
School of Pharmacy, Bengbu Medical College, 2600 Donghai Road, Bengbu 233030, Anhui
Xiang-Shu Wang
School of Laboratory Medicine, Bengbu Medical College, 2600 Donghai Road, Bengbu 233030, Anhui
Yu-Xin Zhang
Anhui Province Engineering Technology Research Center of Biochemical Pharmaceutical, Bengbu 233030, Anhui
Cheng-Zhu Wu (wuchengzhu0611@bbmc.edu.cn)
School of Pharmacy, Bengbu Medical College, 2600 Donghai Road, Bengbu 233030, Anhui

Research Article

Keywords: Xanthohumol derivatives, semi-synthesis, breast cancer cell, migration, invasion, apoptosis

Posted Date: April 4th, 2023

DOI: https://doi.org/10.21203/rs.3.rs-2761204/v1

Additional Declarations: No competing interests reported.

Version of Record: A version of this preprint was published at Molecular Diversity on December 8th, 2023. See the published version at https://doi.org/10.1007/s11030-023-10706-7.

Abstract

Xanthohumol (Xn) is a chalcone compound isolated from *Humulus lupulus Linn.* and has various biological activities. In this study, eight Xn derivatives were synthesized by Williamson, Mannich, Reimer-Tiemann, and Schiff base reactions, and five cancer cell lines (MDA-MB-231, MCF-7, CNE-2Z, SMMC-7721, H1975) were evaluated for *in vitro* cytotoxic activity. Among these, 2-((*E*)-2,4-dihydroxy-5-((*E*)-3-(4-hydroxyphenyl)acryloyl)-6-methoxy-3-(3-methylbut-2-en-1-yl)benzylidene)hydrazine-1-carboximidamide (**8**) exhibited the best potent cytotoxic activity against the five cancer cells, with IC_{50} values ranging from 4.87 to 14.35 μM. Wound-healing and transwell assays also showed that compound **8** could better inhibit the migration and invasion of MDA-MB-231 cells, and western blotting assays showed that it could reduce protein expression of HIF-1α, MMP-2 and MMP-9. In addition, flow cytometry assays showed that compound **8** could induce apoptosis in MDA-MB-231 cells by up-regulation of Bax and down-regulation of Bcl-2 and Akt expression.

1. Introduction

Malignant tumor is one of the major diseases that seriously threaten human health. The number of new cancers and cancer deaths continues to rise every year[1]. Of these, breast cancer is the most common and fatal disease that affects women's lives and mental health [2]. Triple negative breast cancer (TNBC), especially, is extremely difficult to treat due to it is negative for the estrogen receptor, the progesterone receptor and the human epidermal growth factor receptor-2 [3]. Besides, TNBC accounts for 10% of invasive cases in the latest American breast cancer statistics alone [4]. Currently, chemotherapy is still one of the important treatment methods for TNBC. However, some patients did not achieve significant effects because their inferior sensitivity and numerous adverse reactions to chemotherapy drugs [5, 6]. Therefore, finding anti-TNBC drugs with efficient and less adverse reactions has become a significant research in recent years.

Natural products have some advantages, including a wide range of sources, diverse structures and low toxicity, which could provide efficient drugs to reversal of tumor cell resistance and reduction of side effects [7–10]. Xanthohumol (Xn), a major prenylated in *Humulus lupulus Linn.*, has various biological activities such as anti-cancer [11], anti-oxidation [12], anti-bacterial [13] etc. Katagiri 's [14] research had shown that Xn can selectively inhibit BIG3-PHB2 interaction to inhibit MCF-7 cell proliferation. Ferrari's [15] team had found that Xn can down-regulating FAK/AKT/NF-κB signaling DU145 and PC3 cell cycle progression and reduce cell migration and invasion. Furthermore, the study of Li [16] and Calhau [17] had shown that Xn through the NADPH oxidase induced ROS production to cause HL-60 cell cycle arrest and apoptosis and it could also inhibit the formation of estrogen in JAR cells.

At present, the structural modification of Xn is mainly the modification of phenolic hydroxyl, benzene ring and α, β-unsaturated carbonyl, and the preliminary studies have shown that phenolic hydroxyl and α, β-unsaturated carbonyl are the active groups of Xn [18–21]. Xn has attracted more and more attention due to its diverse biological activities and low adverse reactions. However, it is difficult to improve its

biological activity and solve its problems of low water solubility, low stability and easy isomerization [22–25]. Therefore, in this study, a series of derivatives were obtained by modifying the structure of Xn, increasing its water solubility and improving its lipid-water partition coefficient. The cytotoxic activity of five tumor cells, the inhibition of migration and invasion of MDA-MB-231 cells and the induction of apoptosis of MDA-MB-231 cells were investigated.

2. Results And Discussion

2.1 Chemistry

Xn was modified by Williamson, Mannich, Reimer-Tiemann and Schiff base reactions to give eight derivatives (Scheme 1). Compounds **1–2** obtained by substitution of phenolic hydroxyl, compound **3** obtained by Intramolecular oxa-Michael addition and compounds **4–8** obtained by substitution on benzene ring through Mannich reactions (compounds **4–5**), Reimer-Tiemann reactions (compounds **6–7**) and Schiff base reactions (compound **8**), respectively. The structures of all Xn derivatives were confirmed by ^{1}H-NMR ^{13}C-NMR and HR-ESI-MS (see supplementary materials).

2.2 *MTT assay*

The cytotoxic activity of the compounds **1–8** against five tumor cells was detected by MTT assay. As shown in Table 1, compounds **4** and **8** showed potent cytotoxic activity against multiple tumor cells with IC_{50} values of 7.54–12.74 μM and 4.87–14.35 μM, respectively. Among these, compound **8** had the best cytotoxic activity against MDA-MB-231 (5.90 μM), SMMC-7721 (4.78 μM) and CNE-2Z (5.17 μM) cells, which was approximately twice as high as Xn.

Table 1
Cytotoxic activity in *vitro* of compounds **1–8**.

Compound	IC_{50} Values (μM), 72 h				
	MDA-MB-231 [a]	MCF-7 [b]	SMMC-7721 [c]	CNE-2Z [d]	H1975 [e]
1	38.73 [f]	14.61	15.02	> 50 [g]	36.10
2	> 50	> 50	16.09	> 50	> 50
3	27.30	41.17	25.33	30.86	> 50
4	7.54	8.74	12.74	9.10	8.96
5	15.46	23.33	28.71	16.80	16.81
6	19.37	18.5	17.36	17.46	> 50
7	32.11	25.96	19.18	22.19	33.6
8	5.90	6.12	4.87	5.17	14.35
Xn	11.94	6.31	9.86	11.87	8.34

[a] MDA-MB-231: human breast cancer cells; [b] MCF-7: human breast cancer cells; [c] CNE-2Z: human nasopharyngeal carcinoma cells; [d] SMMC-7721: human breast cancer cells; [e] H1975: human lung adenocarcinoma cells; [f] No inhibitory activity at 50 μM; [g] The half maximal inhibitory concentration.

The preliminary structure-activity relationship (SAR) analysis indicated that the activity was significantly reduced after 4, 4'-OH methylation (compounds **1–2**) or α, β-unsaturated carbonyl modification (compound **3**) or phenolic hydroxyl ortho-position introduction of aldehyde group (compounds **6–7**). Thus, phenolic hydroxyl and α, β-unsaturated carbonyl may be the active groups of Xn to maintain the cytotoxic activity. However, the Mannich base (compound **4**) had good cytotoxic activity against MDA-MB-231 and CNE-2Z cells, the cytotoxicity of guanidino-substituted Xn at C5 (compound **8**) also was significantly enhanced. Furthermore, druggability assays showed that the equilibrium solubility and lipid-water partition coefficient of compound **8** were significantly improved. (**Figure S0, Table S1**).

2.3 Anti-Proliferative Activity of Compound 8 for MDA-MB-231 cells *in vitro*

The proliferation inhibitory activity of compound **8** on MDA-MB-231 cells at 24 h, 48 h and 72 h was detected by MTT assay (Fig. 1). It was found that compound **8** had a significant inhibitory effect on MDA-MB-231 cells in a concentration and time-dependent manner. The IC_{50} at 24 h, 48 h and 72 h were 16.58 μM, 11.44 μM and 7.05 μM, respectively.

2.4 Compound 8 suppress MDA-MB-231 cells migration and invasion

To evaluate the effect of compound **8** on the migration of tumor cells, the inhibitory effect of compound **8** on the migration of MDA-MB-231 cells was studied by wound-healing assays. The result showed that compound **8** has a powerful inhibitory effect on the migration of MDA-MB-231 cells, the number of migrated cells gradually decreased with the increase of drug concentration (Fig. 2A, B**)**.

In addition, the inhibitory effect of compound **8** on the migration and invasion of MDA-MB-231 cells was evaluated by transwell assays. The results showed that the cell penetration rate decreased significantly with the increase of compound **8** concentrations, it indicated that compound **8** could inhibit the migration and invasion of MDA-MB-231 cells (Fig. 3A, B**)**.

Further, to explore the mechanism of the inhibitory effect of compound **8** on the migration and invasion of MDA-MB-231 cells, the effect of compound **8** on the expression of migration and invasion proteins was evaluated by Western blotting. It showed that compound **8** reduced the expression of migration and invasion proteins HIF-1α, MMP-2 and MMP-9 (Fig. 4A, B). Hence, compound **8** inhibited the migration and invasion of MDA-MB-231 cells by down-regulating the expression of HIF-1α, MMP-2 and MMP-9.

The migration and invasion of tumor cells are a biological process of interaction between tumor cells and host cells, and approximately 90% of cancer deaths are relate to the migration and invasion of cancer cells. Tumor migration and invasion is a continuous, multi-factor regulated biological event. Inhibition of tumor migration and invasion is one of the key links in anti-tumor therapy [26]. Therefore, the effect of compound **8** on the migration and invasion of MDA-MB-231 cells was evaluated. It was found that compound **8** could inhibit the migration and invasion of MDA-MB-231 cells at low concentrations, and the number of migrated and invaded cells gradually decreased with the increase of drug concentration.

HIF-1α and its downstream proteins MMP-2 and MMP-9 play an important role in tumor migration and invasion [27, 28]. Western Blotting assays showed that the expression of HIF-1α, MMP-2 and MMP-9 decreased gradually with the increase of compound **8** concentration, indicating that compound **8** could inhibit the migration and invasion of MDA-MB-231 cells by inhibiting the expression of HIF-1α and affecting the expression of downstream MMP-2 and MMP-9.

2.5 Compound 8 induces apoptosis of MDA-MB-231 cells

In addition, the effect of compound **8** on the apoptosis of MDA-MB-231 cells was detected by flow cytometry. As shown in Fig. 5A and 5B, the apoptosis rate of the control group was 5.73%. However, the apoptosis rates of MDA-MB-231 cells treated with compound **8** (5, 10 and 20 μM) were 17.63%, 31.65% and 56.10%, respectively, indicating that compound **8** could induce apoptosis to inhibit cell proliferation.

The effects of compound **8** on the expression of apoptosis proteins Bax, Bcl-2 and Akt were detected by Western blotting. The results are shown in Fig. 5C and 5D, with the concentration increase of compound

8, Bax protein expression increased, while Bcl-2 and Akt protein expression decreased, indicating that compound **8** induced apoptosis by up-regulating Bax and down-regulating the expression of Bcl-2 and Akt proteins.

Apoptosis is a polygenically controlled physiological process used to maintain the stability of tissues and organs throughout life [29]. Excessive cell proliferation and multiple tumors occur when regulation of apoptosis is imbalanced [30]. Hence, we detected the effect of compound **8** on the apoptosis of MDA-MB-231 cells, and found that compound **8** could induce apoptosis of MDA-MB-231 cells, and the number of apoptotic cells increased with the increase of drug concentration.

PI3K-Akt plays a key role in inhibiting apoptosis and promoting proliferation in cells, which is closely related to the occurrence and development of various human tumors [31]. Additionlly, Bcl-2 and Bax are the most representative apoptosis-inhibiting and apoptosis-promoting genes in the Bcl-2 family, which play an important regulatory role in tumor cell apoptosis [32]. Therefore, the effect of compound **8** on the expression of Bax, Bcl-2 and Akt was detected by Western blotting. It was found that compound **8** could up-regulate Bax and down-regulate Bcl-2 and Akt protein expression. In conclusion, compound **8** can induce apoptosis of MDA-MB-231 cells, and the occurrence of this programmed cell death is closely related to the up-regulation of Bax protein expression and the down-regulation of Bcl-2 and Akt protein expression.

In view of the good biological activity of Xn, the structural modification of Xn has achieved initial success. Anioł [18] found that 4,4'-OH substituted by monoacyl and diacyl will reduce the cytotoxic activity. Fang's team [19] found that α, β-unsaturated carbonyl is an active group, C3' position introduces electron-withdrawing groups have better cytotoxic activity relative to the C2', C4' position. Furthermore, Heilmann's [20, 21] research demonstrate that the introduction of hydroxy at the C3' position will have better cytotoxic activity, and the methylation and acetylation at the C4' position will be reduced cytotoxic activity. However, these studies are limited to a few modifiable sites and cannot fully elucidate the SAR of Xn. Therefore, in this study, we modified multiple sites of Xn. The Xn derivatives with phenolic hydroxyl methylation and the modification of α, β-unsaturated carbonyl groups were investigated by MTT assay, showed that its cytotoxicity was greatly reduced. The introduction of substituents at the C5 position was found to be effective in improving activity. For example, the cytotoxic activity of Mannich bases (compound **4**) and Schiff base derivatives (compound **8**) was significantly increased. Therefore, phenolic hydroxyl groups and α, β-unsaturated carbonyl may be the antitumor active pharmacophore of Xn. The introduction of trimethoxybenzyl and morpholine at the C5 position has a certain effect on the activity improvement, and the introduction of guanidino at the C5 position to generate Schiff base has a good effect on anti-cell proliferation, inhibition of invasion and migration, and induction of apoptosis.

3. Conclusions

In summary, eight Xn derivatives were designed and synthesized through modified at 4,4'-OH, α, β-unsaturated ketone and C5, 5' position, and their biological activities were evaluated. Compound **8** had

good cytotoxic activity and could inhibit the migration and invasion of MDA-MB-231 cells and down-regulate the invasion and migration related proteins HIF-1α, MMP-2 and MMP-9. In addition, flow cytometry experiments showed that compound **8** could induce apoptosis in MDA-MB-231 cells, and Western Blotting experiments showed that it induced apoptosis by up-regulating Bax and down-regulating the expression of Bcl-2 and Akt proteins. Hence, our study shows that compound **8** can be considered as a potential antitumor lead compound due to its good equilibrium solubility, lipid-water partition coefficient, cytotoxic activity, migration and invasion inhibition, and induction of apoptosis.

4. Materials And Methods

4.1 General

Unless otherwise noted, all solvents were obtained from commercial sources and used without further purifications. Xanthohumol was purchased from Chengdu Must Bio-Technology Co., Ltd (Sichuan, China). Flash column chromatography was performed using 200–300 mesh silica gel using petroleum ether/ethyl acetate or dichloromethane/methanol as eluents. Reactions were monitored by TLC plates purchased from Qingdao Haiyang Chemical Co., Ltd (Shandong, China), and a fluorescent indicator visualizable at 254 and 365 nm. ^{1}H-NMR and ^{13}C-NMR spectra were recorded on a Avance II 600 MHz spectrometer (Bruker, Billerica, MA, USA) using DMSO-d_6 as the solvent, respectively, using TMS as the internal standard (chemical shifts are given in δ values, J is given in Hz). High-resolution electrospray ionization mass spectrometry (HR-ESI-MS) spectra were obtained using a Thermo Scientific LTQ Orbitrap XL mass spectrometer (Bruker, Bremerhaven, Germany) with electrospray ionization. Apoptotic data was recorded on a FACSVerse flow cytometer (BD, New York, NY, USA). Anti-HIF-1α antibodies were purchased from Abcam (Cambridge, UK). Anti-MMP-9, MMP-2, Bax, Bcl-2 and Akt antibodies were purchased from Proteintech Group (Chicago, IL, USA).

4.2 Semi-synthesis and preparation of compounds

Compounds **1–2**: To a solution of Xn (130 mg, 0.37 mM) in *N, N*-dimethylformamide (DMF, 4 mL) was added sodium carbonate solution (10%, 1.5 mL) and CH_3I (35 μL, 0.56 mM) at 60℃ and stirred for 10 h. The reaction was quenched by addition of double distilled water (DDW), and adjusted with dilute hydrochloric acid pH to acidity, and extracted with ethyl acetate (EtOAc) three times. The combined organic extracts were concentrated under reduced pressure, dried over anhydrous Na_2SO_4, which was purified by chromatography on silica gel (petroleum ether/EtOAc = 30/1 to 10/1) to afford the compounds **1–2**.

(*E*)-1-(2,4-dihydroxy-6-methoxy-3-(3-methylbut-2-en-1-yl)phenyl)-3-(4-methoxyphenyl)prop-2-en-1-one (**1**): 37.5 mg, yield: 27.8%, yellow powder, ^{1}H-NMR (DMSO-d_6, 300 MHz) δ 14.57 (1H, s, 2-OH), 10.60 (1H, s, 4-OH), 7.84 (1H, d, J = 15.6 Hz, H-β), 7.72 (1H, d, J = 15.6 Hz, H-α), 7.70 (2H, d, J = 8.7 Hz, H-2', H-6'), 7.03 (2H, d, J = 8.6 Hz, H-3', H-5'), 6.09 (1H, s, H-5), 5.14 (1H, m, H-2"), 3.87 (3H, s, H-7), 3.81 (3H, s, H-7'), 3.15 (2H, J = 6.8 Hz, H-1"), 1.70 (3H, s, H-5"), 1.61 (3H, s, H-4"); ^{13}C NMR (DMSO-d_6, 75 MHz) δ 191.7 (C = O), 164.6 (C-

4), 162.5 (C-6), 161.1 (C-2), 160.5 (C-4'), 141.9 (C-*β*), 130.3 (C-2', C-6'), 129.9 (C-3"), 127.6 (C-*α*), 124.9 (C-1'), 123.0 (C-2"), 114.6 (C-3', C-5'), 107.3 (C-3), 104.6 (C-1), 91.0 (C-5), 55.8 (C-7), 55.4 (C-7'), 25.5 (C-5"), 21.0 (C-1"), 17.7 (C-4"); HR-ESI-MS: calcd. for $C_{22}H_{25}O_5^+$ $[M+H]^+$ 369.1697, found 369.1697.

(*E*)-1-(2-hydroxy-4,6-dimethoxy-3-(3-methylbut-2-en-1-yl)phenyl)-3-(4-methoxyphenyl)prop-2-en-1-one (**2**): 17.9 mg, yield: 12.8%, yellow powder, ^{1}H-NMR (DMSO-d_6, 500 MHz) δ 14.12 (1H, s, 2-OH), 7.81 (1H, d, *J* = 15.6 Hz, H-*β*), 7.73 (1H, d, *J* = 15.2 Hz, H-*α*), 7.71 (2H, d, *J* = 8.8 Hz, H-2', H-6'), 7.03 (2H, d, *J* = 8.8 Hz, H-3', H-5'), 6.27 (1H, s, H-5), 5.10 (1H, m, H-2"), 3.98 (3H, s, H-7), 3.91 (3H, s, H-8), 3.82 (3H, s, H-7'), 3.17 (2H, d, *J* = 5.7 Hz, H-1"), 1.70 (3H, s, H-5"), 1.60 (3H, s, H-4"); ^{13}C NMR (DMSO-d_6, 125 MHz) δ 192.4 (C = O), 163.3 (C-4), 162.8 (C-6), 161.3 (C-2), 161.2 (C-4'), 142.3 (C-*β*), 130.4 (C-3"), 130.3 (C-2', C-6'), 127.5 (C-*α*), 124.8 (C-1'), 122.7 (C-2"), 114.6 (C-3', C-5'), 108.4 (C-3), 105.6 (C-1), 87.7 (C-2"), 56.2 (C-8), 55.9 (C-7), 55.4 (C-7'), 25.5 (C-5"), 21.0 (C-1"), 17.6 (C-4"); HR-ESI-MS: calcd. for $C_{23}H_{27}O_5^+$ $[M+H]^+$ 383.1853, found 383.1854.

Compound **3**: To a solution of Xn (80 mg, 0.23 mM) in absolute ethyl alcohol was added 30 mg NaOH at 65℃ and stirred for 12 h. The reaction was quenched by addition of DDW, and adjusted with dilute hydrochloric acid pH to neutral, and extracted with EtOAc three times. The combined organic extracts were concentrated under reduced pressure, dried over anhydrous Na_2SO_4, which was purified by chromatography on silica gel (petroleum ether/EtOAc = 1/1 to 1/2) to afford the compound **3**.

7-hydroxy-2-(4-hydroxyphenyl)-5-methoxy-8-(3-methylbut-2-en-1-yl)chroman-4-one (**3**): 16.7 mg, yield: 19.4%, white powder, ^{1}H-NMR (DMSO-d_6, 300 MHz) δ 10.42 (1H, s, 4-OH), 9.53 (1H, s, 4'-OH), 7.30 (2H, d, *J* = 8.5 Hz, H-2', H-6'), 6.79 (2H, d, *J* = 8.5 Hz, H-3', H-5'), 6.14 (1H, s, H-5), 5.34 (1H, dd, *J* = 3.0 Hz, *J* = 12.4 Hz, H-*β*), 5.10 (1H, m, H-2"), 3.70 (3H, s, H-7), 3.13 (2H, *J* = 6.9 Hz, H-1"), 2.94 (2H, m, H-*α*), 1.59 (3H, s, H-5"), 1.54 (3H, s, H-4"); ^{13}C NMR (DMSO-d_6, 75 MHz) δ 188.3 (C = O), 161.6 (C-4), 161.4 (C-6), 159.6 (C-2), 157.4 (C-4'), 130.3 (C-3"), 129.6 (C-1'), 127.8 (C-2', C-6'), 122.9 (C-2"), 115.1 (C-3', C-5'), 107.5 (C-3), 104.6 (C-1), 92.7 (C-5), 77.9 (C-*β*), 55.4 (C-7), 44.7 (C-*α*), 25.5 (C-5"), 21.5 (C-1"), 17.6 (C-4"); HR-ESI-MS: calcd. for $C_{21}H_{23}O_5^+$ $[M+H]^+$ 354.1467, found 355.1539.

Compounds **4–5**: To a solution of Xn (300 mg, 0.85 mM) in acetonitrile (5 mL) was added morpholine (1.70 mM) at 100℃ and stirred for 3 h. The combined organic extracts were concentrated under reduced pressure, dried over anhydrous Na_2SO_4, which was purified by chromatography on silica gel (petroleum ether/EtOAc = 20/1 to 3/1) to afford the compounds **4–5**.

(*E*)-1-(3-((4-bromophenyl)(morpholino)methyl)-4,6-dihydroxy-2-methoxy-5-(3-methylbut-2-en-1-yl)phenyl)-3-(4-hydroxyphenyl)prop-2-en-1-one (**4**): 19.1 mg, yield: 3.7%, yellow powder, ^{1}H-NMR (DMSO-d_6, 300 MHz) δ 14.30 (1H, s, 2-OH), 13.69 (1H, s, 4'-OH), 10.17 (1H, s, 4-OH), 7.78 (1H, d, *J* = 15.5 Hz, H-*β*), 7.60 (2H, d, *J* = 8.4 Hz, H-2', H-6'), 7.58 (3H, d, *J* = 15.2 Hz, *J* = 7.3 Hz, H-*α*, H-4"', H-6"'), 7.44 (2H, d, *J* = 8.2 Hz, H-3"', H-7"'), 6.85 (2H, d, *J* = 8.3 Hz, H-3', H-5'), 5.21 (1H, m, H-2"), 4.99 (1H, s, H-1"'), 3.66 (4H, t, *J* = 7.1 Hz, H-9"', H-10"'), 3.42 (3H, s, H-7), 3.25 (2H, d, *J* = 4.7 Hz, H-1"), 2.40 (4H, t, *J* = 8.5 Hz, H-8"', H-11"'), 1.73 (3H, s, H-5"), 1.63 (3H, s, H-4"); ^{13}C NMR (DMSO-d_6, 75 MHz) δ 191.8 (C = O), 162.5 (C-4), 162.2 (C-6),

160.3 (C-4'), 158.8 (C-2), 144.2 (C-*β*), 138.4 (C-2‴), 131.9 (C-3", C-4‴, C-6‴), 130.7 (C-2', C-6'), 125.7 (C-3‴, C-7‴), 122.5 (C-1', C-*α*), 122.0 (C-2"), 121.4 (C-5‴), 116.1 (C-3', C-5'), 112.0 (C-3), 110.6 (C-1), 107.5 (C-5), 67.7 (C-1‴), 65.8 (C-7), 63.2 (C-9‴, C-10‴), 48.6 (C-8‴, C-11‴), 25.5 (C-5"), 24.1 (C-1"), 17.8 (C-4"); HR-ESI-MS: calcd. for $C_{32}H_{35}BrNO_5^+$ $[M + H]^+$ 608.1647, found 608.1642.

(*E*)-1-(2,4-dihydroxy-6-methoxy-3-(3-methylbut-2-en-1-yl)-5-(morpholino(3,4,5-trimethoxyphenyl)methyl)phenyl)-3-(4-hydroxyphenyl)prop-2-en-1-one (**5**): 25.2 mg, yield: 4.8%, yellow powder, ^{1}H-NMR (DMSO-d_6, 300 MHz) δ 14.38 (1H, s, 2-OH), 13.74 (1H, s, 4'-OH), 10.16 (1H, s, 4-OH), 7.79 (1H, d, *J* = 15.6 Hz, H-*β*), 7.63 (1H, d, *J* = 15.8 Hz, H-*α*), 7.57 (2H, d, *J* = 8.6 Hz, H-2', H-6'), 6.86 (2H, d, *J* = 8.1 Hz, H-3', H-5'), 6.83 (2H, d, *J* = 8.1 Hz, H-3‴, H-7‴), 5.26 (1H, m, H-2"), 4.98 (1H, s, H-1‴), 3.97 (3H, s, H-7), 3.73 (9H, s, H-12‴, H-13‴, H-14‴), 3.62 (4H, t, *J* = 9.1 Hz, H-9‴, H-10‴), 3.26 (2H, d, *J* = 5.7 Hz, H-1"), 2.42 (4H, t, *J* = 8.6 Hz, H-8‴, H-11‴), 1.74 (3H, s, H-5"), 1.60 (3H, s, H-4"); ^{13}C NMR (DMSO-d_6, 75 MHz) δ 191.7 (C = O), 162.2 (C-4), 160.3 (C-6), 158.7 (C-4'), 152.8 (C-2), 144.0 (C-4‴, C-6‴), 137.1 (C-*β*), 130.6 (C-2', C-6', C-3"), 130.5 (C-2‴, C-5‴), 125.7 (C-1'), 122.4 (C-*α*), 122.0 (C-2"), 116.1 (C-3', C-5'), 112.0 (C-3‴, C-7‴), 111.1 (C-1, C-3), 107.4 (C-5), 68.5 (C-1‴), 65.8 (C-7, C-13‴), 63.3 (C-9‴, C-10‴), 60.0 (C-12‴, C-14‴), 55.6 (C-8‴, C-11‴), 31.5 (C-5"), 25.5 (C-1"), 17.7 (C-4"); HR-ESI-MS: calcd. for $C_{35}H_{41}NO_9Na^+$ $[M + Na]^+$ 642.2674, found 642.2674.

Compounds **6–7**: To a solution of Xn (240 mg, 0.68 mM) in trichloromethane (8 mL) was added NaOH (25%, 4 mL) at 30°C and stirred for 9 h. The reaction was quenched by addition of double distilled water (DDW), and adjusted with dilute hydrochloric acid pH to acidity, and extracted with ethyl acetate (EtOAc) three times. The combined organic extracts were concentrated under reduced pressure, dried over anhydrous Na_2SO_4, which was purified by chromatography on silica gel (dichloromethane/methanol = 200/1 to 30/1) to afford the compounds **6–7**.

(*E*)-5-(3-(2,4-dihydroxy-6-methoxy-3-(3-methylbut-2-en-1-yl)phenyl)-3-oxoprop-1-en-1-yl)-2-hydroxybenzaldehyde (**6**): 7.2 mg, yield: 2.8%, yellow powder, ^{1}H-NMR (DMSO-d_6, 300 MHz) δ 10.28 (1H, s, H-7'), 7.77 (1H, d, *J* = 2.3 Hz, H-6'), 7.65 (1H, dd, *J* = 2.4 Hz, *J* = 8.6 Hz, H-2'), 7.05 (1H, d, *J* = 8.6 Hz, H-3'), 6.15 (1H, s, H-5), 5.43 (1H, dd, *J* = 3.1 Hz, *J* = 12.5 Hz, H-*β*), 5.10 (1H, m, H-2"), 3.70 (3H, s, H-7), 3.39 (4H, m, H-1", H-*α*), 1.58 (3H, s, H-4"), 1.52 (3H, s, H-5"); ^{13}C NMR (DMSO-d_6, 75 MHz) δ 191.0 (C = O), 187.9 (C-7'), 161.7 (C-4), 161.2 (C-6), 160.7 (C-4'), 159.7 (C-2), 134.5 (C-2'), 130.6 (C-3"), 126.8 (C-5'), 122.8 (C-6'), 122.1 (C-2"), 117.5 (C-3'), 107.6 (C-3), 104.6 (C-1), 92.8 (C-5), 77.3 (C-*β*), 55.4 (C-7), 44.6 (C-*α*), 25.5 (C-5"), 21.6 (C-1"), 17.6 (C-4"); HR-ESI-MS: calcd. for $C_{22}H_{23}O_6^+$ $[M + H]^+$ 383.1416, found 383.1490.

(*E*)-2,4-dihydroxy-5-(3-(4-hydroxyphenyl)acryloyl)-6-methoxy-3-(3-methylbut-2-en-1-yl)benzaldehyde (**7**): 21.5 mg, yield: 8.3%, yellow powder, ^{1}H-NMR (DMSO-d_6, 300 MHz) δ 10.23 (1H, s, 4'-OH), 10.06 (1H, d, *J* = 1.1 Hz, H-8), 7.83 (1H, d, *J* = 15.6 Hz, H-*β*), 7.64 (2H, d, *J* = 8.3 Hz, H-2', H-6'), 7.59 (1H, d, H-*α*), 6.87 (2H, d, *J* = 8.4 Hz, H-3', H-5'), 5.40 (1H, m, H-2"), 3.87 (3H, s, H-7), 3.20 (2H, d, *J* = 7.2 Hz, H-1"), 1.71 (3H, s, H-4"), 1.62 (3H, s, H-5"); ^{13}C NMR (DMSO-d_6, 75 MHz) δ 193.5 (C = O), 192.4 (C-8), 167.2 (C-4), 166.1 (C-2), 164.2 (C-6), 160.7 (C-4'), 145.8 (C-*β*), 131.4 (C-3"), 131.1 (C-2', C-6'), 125.4 (C-1'), 122.0 (C-*α*), 121.3 (C-2"), 116.1

(C-3', C-5'), 109.2 (C-5), 108.3 (C-1), 66.0 (C-7), 25.4 (C-5"), 20.5 (C-1"), 17.7 (C-4"); HR-ESI-MS: calcd. for $C_{22}H_{23}O_6^+$ $[M+H]^+$ 383.1416, found 383.1489.

Compound **8**: To a solution of compound **7** (20 mg, 0.05 mM) and Aminoguanidine bicarbonate (15 mg, 0.11 mM) in 3 mL absolute ethyl alcohol was added 500 μL aceticacid at 50℃ and stirred for 5 h. The combined organic extracts were concentrated under reduced pressure, dried over anhydrous Na_2SO_4, which was purified by chromatography on silica gel (dichloromethane/methanol = 50/1 to 20/1) to afford the compound **8**.

2-((*E*)-2,4-dihydroxy-5-((*E*)-3-(4-hydroxyphenyl)acryloyl)-6-methoxy-3-(3-methylbut-2-en-1-yl)benzylidene)hydrazine-1-carboximidamide (**8**): 5.1 mg, yield: 22.85%, yellow powder, ^{1}H-NMR (DMSO-d_6, 300 MHz) δ 13.68 (1H, s, 2-OH), 12.03 (1H, s, -NH), 10.57 (1H, s, 4-OH), 10.27 (1H, s, 4'-OH), 8.54 (1H, s, H-8), 7.90 (3H, s, -NH_2, =NH), 7.84 (1H, d, *J* = 15.5 Hz, H-*β*), 7.64 (1H, d, *J* = 14.2 Hz, H-*α*), 7.64 (2H, d, *J* = 9.2 Hz, H-2', H-6'), 6.88 (2H, d, *J* = 8.3 Hz, H-3', H-5'), 5.17 (1H, m, H-2"), 3.74 (3H, s, H-7), 3.29 (2H, d, *J* = 6.5 Hz, H-1"), 1.74 (3H, s, H-4"), 1.63 (3H, s, H-5"); ^{13}C NMR (DMSO-d_6, 75 MHz) δ 192.4 (C = O), 163.8 (C-4), 161.4 (C-2), 160.6 (C-6), 160.2 (C = NH), 154.4 (C-4'), 146.6 (C-*β*), 145.3 (C-8), 131.2 (C-3"), 131.0 (C-2', C-6'), 125.6 (C-1'), 122.1 (C-*α*), 121.9 (C-2"), 116.1 (C-3', C-5'), 112.0 (C-3), 109.0 (C-5), 104.6 (C-1), 64.8 (C-7), 25.5 (C-5"), 20.8 (C-1"), 17.8 (C-4"); HR-ESI-MS: calcd. for $C_{23}H_{26}N_4O_5^+$ $[M+H]^+$ 439.1903, found 439.1979.

4.3 MTT assay

Human breast cancer (MDA-MB-231 and MCF-7), human nasopharyngeal carcinoma (CNE-2Z), human hepatoma (SMMC-7721) and human lung adenocarcinoma (H1975) cell lines were cultured in RPMI 1640 or DMEM medium containing 10% fetal bovine serum and 1% penicillin/streptomycin. All the cell lines were cultured at 37°C in 5% CO_2 humidified air. Cells were seeded at the density of 5000 cells/well in 96-well plates and cultured for 72 h after treated with various concentrations (0, 2, 4, 8, 16 μg/mL) of the derivatives and shikonin was used as a positive control. The absorbance value of 490 nm was detected with the use of a microplate reader.

4.4 Wound-healing assay

The MDA-MB-231 cells were seeded at the density of 6×10^5 cells/well in 6-well plates. The wound was scratched perpendicular to the monolayered cells by 200 μL pipette tips and the suspension cells were washed with PBS. Then the cells were cultured in RPMI 1640 medium with compound **8** (0, 2, 4, 8 μM) for 24 h. The wound assay values were obtained under the light microscope from three randomly selected fields.

4.5 Cell migration assay

The MDA-MB-231 cells were prepared for RPMI 1640 medium without fetal bovine serum, which containing various concentrations (0, 2, 4, 8 μM) of compound **8**. It was added to the upper chambers at 3.0×10^5 cells/well, and RPMI 1640 medium containing fetal bovine serum was added to the lower

chambers. Then the 24-well plates were placed at 37°C in 5% CO_2 humidified air for 24 h. The unpassed cells in the upper chambers were removed, and cells of lower chambers were fixed with paraformaldehyde, stained with crystal violet for 15 min. The migratory cells was recorded under a light microscope (200 ×).

4.6 Cell invasion assay

The upper chambers were added 50 μL matrigel, and MDA-MB-231 cells were added at 3.0 ×10^5 cells/well in upper chambers with 8.0 μm pore membrane. Then various concentrations (0, 2, 4 and 8 μM) of compound **8** was added for 24 h. The remaining assays were the same as Section **3.5**.

4.7 Flow cytometry analysis of apoptosis

The MDA-MB-231 cells were seeded at the density of 1.5 × 10^5 cells/well in 12-well plates, and were treated by various concentrations (0, 5, 10 and 20 μM) of compound **8** for 48 h. Cells were collected and centrifuged at 1500 rpm for 10 min and wished with PBS, then Annexin V binding solution was added and mixed on ice. Annexin V-FITC and PI staining solution was added in the dark, respectively. The cells were filtered into the flow tube for detection by flow cytometry.

4.8 Western blotting

The MDA-MB-231 cells were seeded at the density of 3.0 × 10^5 cells/well in 6-well plates, and various concentrations (0, 5, 10 and 20 μM) of compound **8** were added for 48 h. Cells were harvested and homogenized in RIPA lysis buffer for 30 min on ice, and the lysates were centrifuged at 12,000 rpm for 30 min at 4°C. After quantification, the protein was separated by SDS-PAGE and PVDF membrane. The PVDF membrane was blocked with 5% skim milk and incubated with primary antibodies overnight at 4°C. Then incubated with the corresponding secondary antibodies at room temperature for 2 hours. Protein bands were observed using a chemiluminescence kit at a gel imaging system (Bio-Rad, Hercules, CA, USA).

4.9 Statistical analysis

Statistical analysis was performed with two samples using SPSS 16.0 software (Armonk, NY, USA), and * $p < 0.05$ or ** $p < 0.01$ were considered statistically significant differences.

Declarations

Supplementary Materials: The following are available online, Table S1 and Figures S0-S24.

Author contributions: Conceptualization, C.-Z.W. and Y.-X.Z.; methodology, X.-L.S., J.C.; software, F.D. and L.Z.; investigation, J.C. and X.-S. W.; formal analysis, L.Z.; data curation, F.D.; writing—original draft preparation, X.-L.S. and H.-M.L.; writing—review and editing, X.-S. W. and H.-M.L.; supervision, C.-Z.W., and Y.-X.Z.; project administration, C.-Z.W. and X.-L.S.; funding acquisition, C.-Z.W. All authors have read and agreed to the published version of the manuscript.

Conflict of interest: The authors declare no competing interests.

Acknowledgements: This work was financially supported by the Anhui Provincial Natural Science Foundation (2008085MH284); 512 Talent Cultivation Plan of Bengbu Medical College (By51202202); Anhui Engineering Technology Research Center of Biochemical Pharmaceutical Foundation (2022SYKFZ03).

References

1. Siegel RL, Miller KD, Fuchs HE, Jemal A (2022) Cancer statistics, 2022. CA Cancer J Clin 72(1):7–33. doi: 10.3322/caac.21708
2. Sung H, Ferlay J, Siegel RL, Laversanne M, Soerjomataram I, Jemal A, Bray F (2021) Global Cancer Statistics 2020: GLOBOCAN Estimates of Incidence and Mortality Worldwide for 36 Cancers in 185 Countries. CA Cancer J Clin 71(3):209–249. doi: 10.3322/caac.21660
3. Derakhshan F, Reis-Filho JS (2022) Pathogenesis of Triple-Negative Breast Cancer. Annu Rev Pathol 17:181–204. doi: 10.1146/annurev-pathol-042420-093238
4. Giaquinto AN, Sung H, Miller KD, Kramer JL, Newman LA, Minihan A, Jemal A, Siegel RL (2022) Breast Cancer Statistics, 2022. CA Cancer J Clin 72(6):524–541. doi: 10.3322/caac.21754
5. Zhu Y, Hu Y, Tang C, Guan X, Zhang W (2022) Platinum-based systematic therapy in triple-negative breast cancer. Biochim Biophys Acta Rev Cancer 1877(1):188678. doi: 10.1016/j.bbcan.2022.188678
6. Huppert LA, Gumusay O, Rugo HS (2022) Emerging treatment strategies for metastatic triple-negative breast cancer. Ther Adv Med Oncol 14:17588359221086916. doi: 10.1177/17588359221086916
7. Newman DJ, Cragg GM (2020) Natural Products as Sources of New Drugs over the Nearly Four Decades from 01/1981 to 09/2019. J Nat Prod 83(3):770–803. doi: 10.1021/acs.jnatprod.9b01285
8. Fabiani R (2020) Antitumoral Properties of Natural Products. Molecules 25(3):650. doi: 10.3390/molecules25030650
9. Singla RK, Wang X, Gundamaraju R, Joon S, Tsagkaris C, Behzad S, Khan J, Gautam R, Goyal R, Rakmai J, Dubey AK, Simal-Gandara J, Shen B (2022) Natural products derived from medicinal plants and microbes might act as a game-changer in breast cancer: a comprehensive review of preclinical and clinical studies. Crit Rev Food Sci Nutr [published online ahead of print]. doi: 10.1080/10408398.2022.2097196
10. Coley HM (2008) Mechanisms and strategies to overcome chemotherapy resistance in metastatic breast cancer. Cancer Treat Rev 34(4):378–90. doi: 10.1016/j.ctrv.2008.01.007
11. Guo D, Zhang B, Liu S, Jin M (2018) Xanthohumol induces apoptosis via caspase activation, regulation of Bcl-2, and inhibition of PI3K/Akt/mTOR-kinase in human gastric cancer cells. Biomed Pharmacother 106:1300–1306. doi: 10.1016/j.biopha.2018.06.166
12. Zhang XL, Zhang YD, Wang T, Guo HY, Liu QM, Su HX (2015) Evaluation on Antioxidant Effect of Xanthohumol by Different Antioxidant Capacity Analytical Methods. Journal of Chemistry 2014:1–6. doi: 10.1155/2014/249485

13. Cermak P, Olsovska J, Mikyska A, Dusek M, Kadleckova Z, Vanicek J, Nyc O, Sigler K, Bostikova V, Bostik P (2017) Strong antimicrobial activity of xanthohumol and other derivatives from hops (Humulus lupulus L.) on gut anaerobic bacteria. APMIS 125(11):1033–1038. doi: 10.1111/apm.12747
14. Yoshimaru T, Komatsu M, Tashiro E, Imoto M, Osada H, Miyoshi Y, Honda J, Sasa M, Katagiri T (2014) Xanthohumol suppresses oestrogen-signalling in breast cancer through the inhibition of BIG3-PHB2 interactions. Sci Rep 4:7355. doi: 10.1038/srep07355
15. Vene R, Benelli R, Minghelli S, Astigiano S, Tosetti F, Ferrari N (2012) Xanthohumol impairs human prostate cancer cell growth and invasion and diminishes the incidence and progression of advanced tumors in TRAMP mice. Mol Med 18:1292–302. doi: 10.2119/molmed.2012.00174
16. Wang CM, Chen J, Zhao J, Hu SS, Zhang SQ, Mi XQ, Shi X, Cao XH, Li Z (2021) Xanthohumol Induces ROS through NADPH Oxidase, Causes Cell Cycle Arrest and Apoptosis. Oxid Med Cell Longev 2021:9877170. doi: 10.1155/2021/9877170
17. Monteiro R, Becker H, Azevedo I, Calhau C (2006) Effect of hop (Humulus lupulus L.) flavonoids on aromatase (estrogen synthase) activity. J Agric Food Chem 54(8):2938–43. doi: 10.1021/jf053162t
18. Żołnierczyk AK, Baczyńska D, Potaniec B, Kozłowska J, Grabarczyk M, Woźniak E, Anioł M (2017) Antiproliferative and antioxidant activity of xanthohumol acyl derivatives. Medicinal Chemistry Research 26(8):1764–1771. doi: 10.1007/s00044-017-1887-9
19. Zhang B, Duan D, Ge C, Yao J, Liu Y, Li X, Fang J (2015) Synthesis of xanthohumol analogues and discovery of potent thioredoxin reductase inhibitor as potential anticancer agent. J Med Chem 58(4):1795–805. doi: 10.1021/jm5016507
20. Vogel S, Barbic M, Jurgenliemk G, Heilmann J (2010) Synthesis, cytotoxicity, anti-oxidative and anti-inflammatory activity of chalcones and influence of A-ring modifications on the pharmacological effect. Eur J Med Chem 45(6):2206–13. doi: 10.1016/j.ejmech.2010.01.060
21. Vogel S, Ohmayer S, Brunner G, Heilmann J (2008) Natural and non-natural prenylated chalcones: synthesis, cytotoxicity and anti-oxidative activity. Bioorg Med Chem 16(8):4286–93. doi: 10.1016/j.bmc.2008.02.079
22. Harish V, Haque E, Smiech M, Taniguchi H, Jamieson S, Tewari D, Bishayee A (2021) Xanthohumol for Human Malignancies: Chemistry, Pharmacokinetics and Molecular Targets. International Journal of Molecular Sciences 22(9):4478. doi: 10.3390/ijms22094478
23. Legette L, Ma L, Reed RL, Miranda CL, Christensen JM, Rodriguez-Proteau R, Stevens JF (2012) Pharmacokinetics of xanthohumol and metabolites in rats after oral and intravenous administration. Mol Nutr Food Res 56(3):466–74. doi: 10.1002/mnfr.201100554
24. Nookandeh A, Frank N, Steiner F, Ellinger R, Schneider B, Gerhauser C, Becker H (2004) Xanthohumol metabolites in faeces of rats. Phytochemistry 65(5):561–70. doi: 10.1016/j.phytochem.2003.11.016
25. Avula B, Ganzera M, Warnick JE, Feltenstein MW, Sufka KJ, Khan IA (2004) High-performance liquid chromatographic determination of xanthohumol in rat plasma, urine, and fecal samples. J Chromatogr Sci 42(7):378–82. doi: 10.1093/chromsci/42.7.378

26. Ottewell PD, O'Donnell L, Holen I (2015) Molecular alterations that drive breast cancer metastasis to bone. Bonekey Rep 4:643. doi: 10.1038/bonekey.2015.10
27. Ou M, Sun X, Liang J, Liu F, Wang L, Wu X, Tu J (2017) A polysaccharide from Sargassum thunbergii inhibits angiogenesis via downregulating MMP-2 activity and VEGF/HIF-1alpha signaling. Int J Biol Macromol 94:451–458. doi: 10.1016/j.ijbiomac.2016.10.046
28. Choi JY, Jang YS, Min SY, Song JY (2011) Overexpression of MMP-9 and HIF-1alpha in Breast Cancer Cells under Hypoxic Conditions. J Breast Cancer 14(2):88–95. doi: 10.4048/jbc.2011.14.2.88
29. Hengartner MO (2000) The biochemistry of apoptosis. Nature 407(6805):770–6. doi: 10.1038/35037710
30. Yuan L, Cai Y, Zhang L, Liu S, Li P, Li X (2021) Promoting Apoptosis, a Promising Way to Treat Breast Cancer With Natural Products: A Comprehensive Review. Front Pharmacol 12:801662. doi: 10.3389/fphar.2021.801662
31. Osaki M, Oshimura M, Ito H (2004) PI3K-Akt pathway: its functions and alterations in human cancer. Apoptosis 9(6):667–76. doi: 10.1023/B:APPT.0000045801.15585.dd
32. Nimmanapalli R, O'Bryan E, Kuhn D, Yamaguchi H, Wang HG, Bhalla KN (2003) Regulation of 17-AAG-induced apoptosis: role of Bcl-2, Bcl-XL, and Bax downstream of 17-AAG-mediated down-regulation of Akt, Raf-1, and Src kinases. Blood 102(1):269–75. doi: 10.1182/blood-2002-12-3718

Scheme

Scheme 1 is available in the Supplementary Files section

Figures

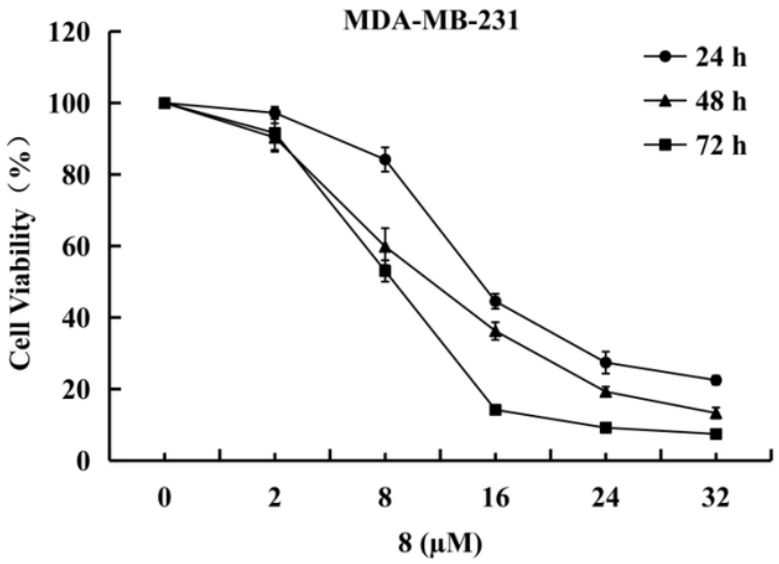

Figure 1

Anti-proliferative activity of compound **8** on MDA-MB-231 cells.

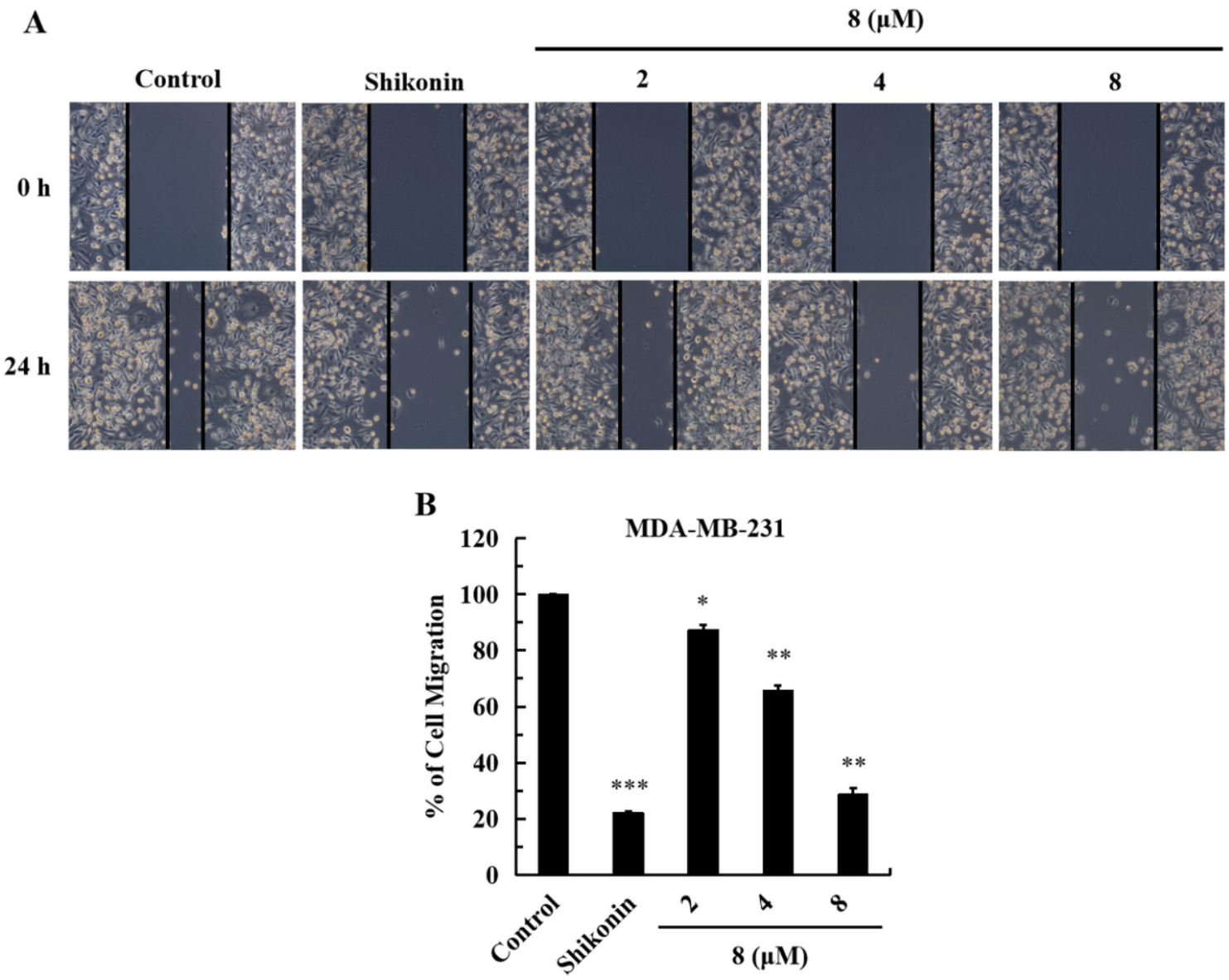

Figure 2

Effects of the compound **8** on the migration ability of MDA-MB-231 cells. (A) The migration effect of MDA-MB-231 cells treated with compound **8** (0, 2, 4 and 8 μM) for 24 h by wound-healing assay. (B) Relative migration of the treated MDA-MB-231 cells was analyzed. Data presented as mean ± SD. $^{***}p < 0.001$, $^{**}p < 0.01$, $^{*}p < 0.05$ ($n = 3$).

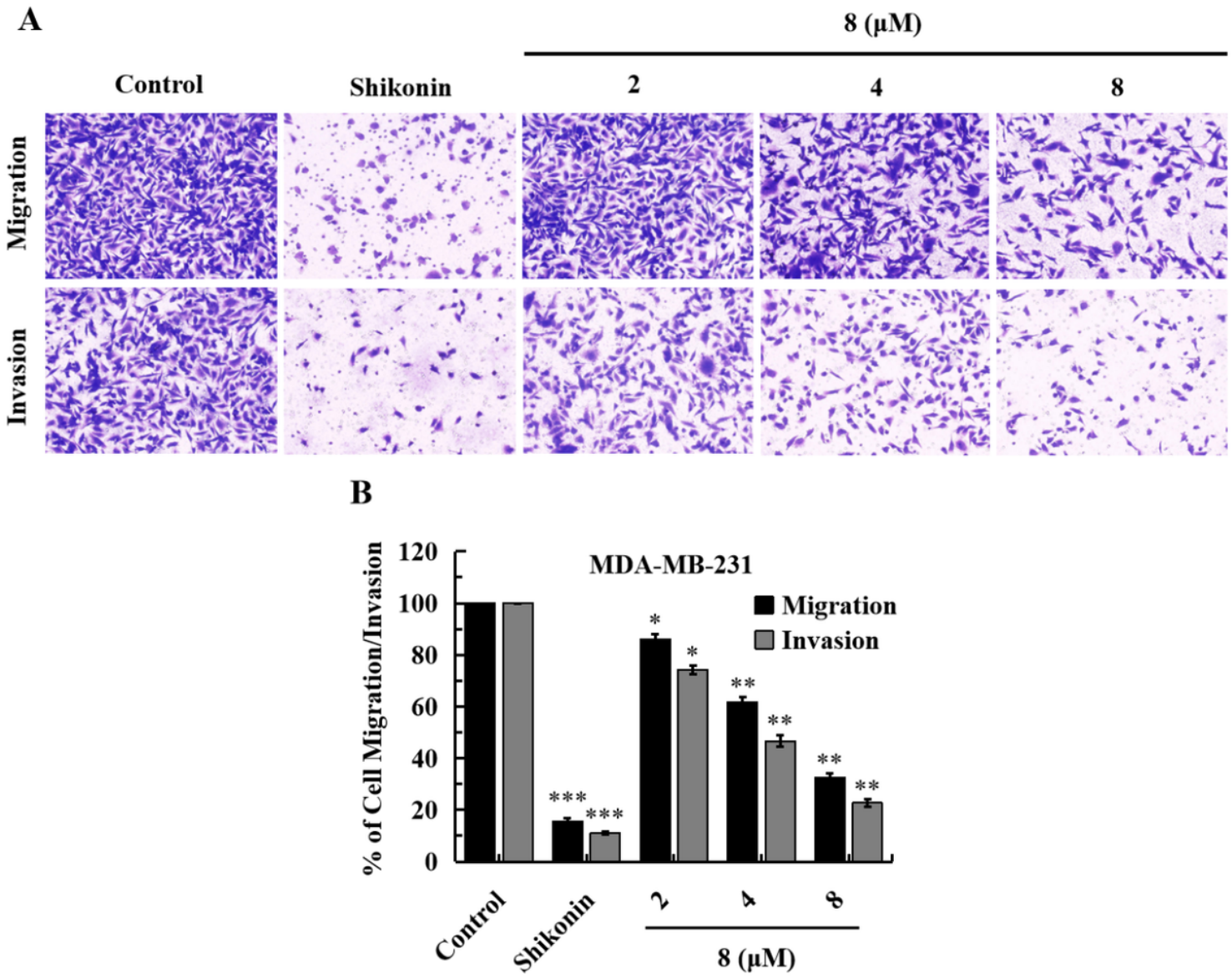

Figure 3

Effects of the compound **8** on the migration and invasion ability of MDA-MB-231 cells. (A) MDA-MB-231 cells were exposed to compound **8** (0, 2, 4, and 8 μM) for 24 h to evaluate the migration and invasion activity by transwell assay; (original magnification 200×). (B) Relative migration and invasion of the treated MDA-MB-231 cells was analyzed. Data presented as mean ± SD. $^{***}p < 0.001$, $^{**}p < 0.01$, $^{*}p < 0.05$ ($n = 3$).

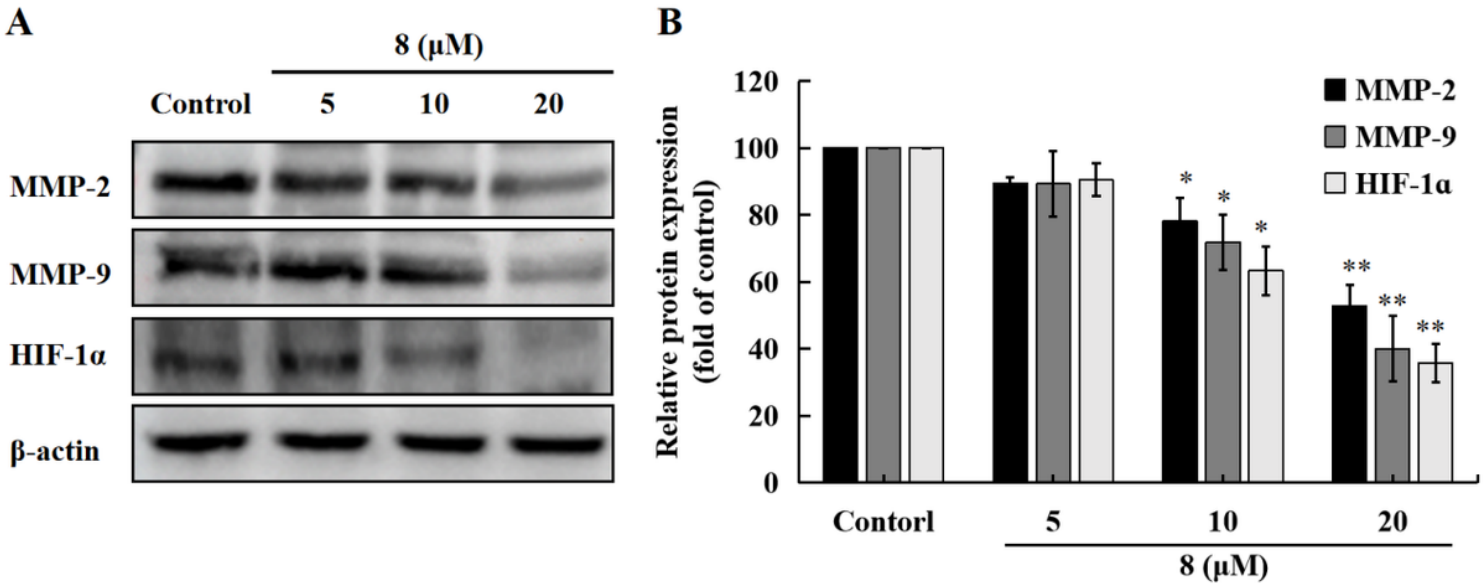

Figure 4

Effects of the compound **8** on HIF-1α, MMP-2 and MMP-9 protein of MDA-MB-231 cells. (A) HIF-1α, MMP-2 and MMP-9 protein levels were determined by western blotting following compound **8** (0, 5, 10 and 20 μM) treatment for 48 h. β-actin expression was included as an internal control. (B) Relative HIF-1α, MMP-2 and MMP-9 protein levels of the treated MDA-MB-231 cells was analyzed. Data presented as mean ± SD. $^{**}p < 0.01$, $^{*}p < 0.05$ vs. control.

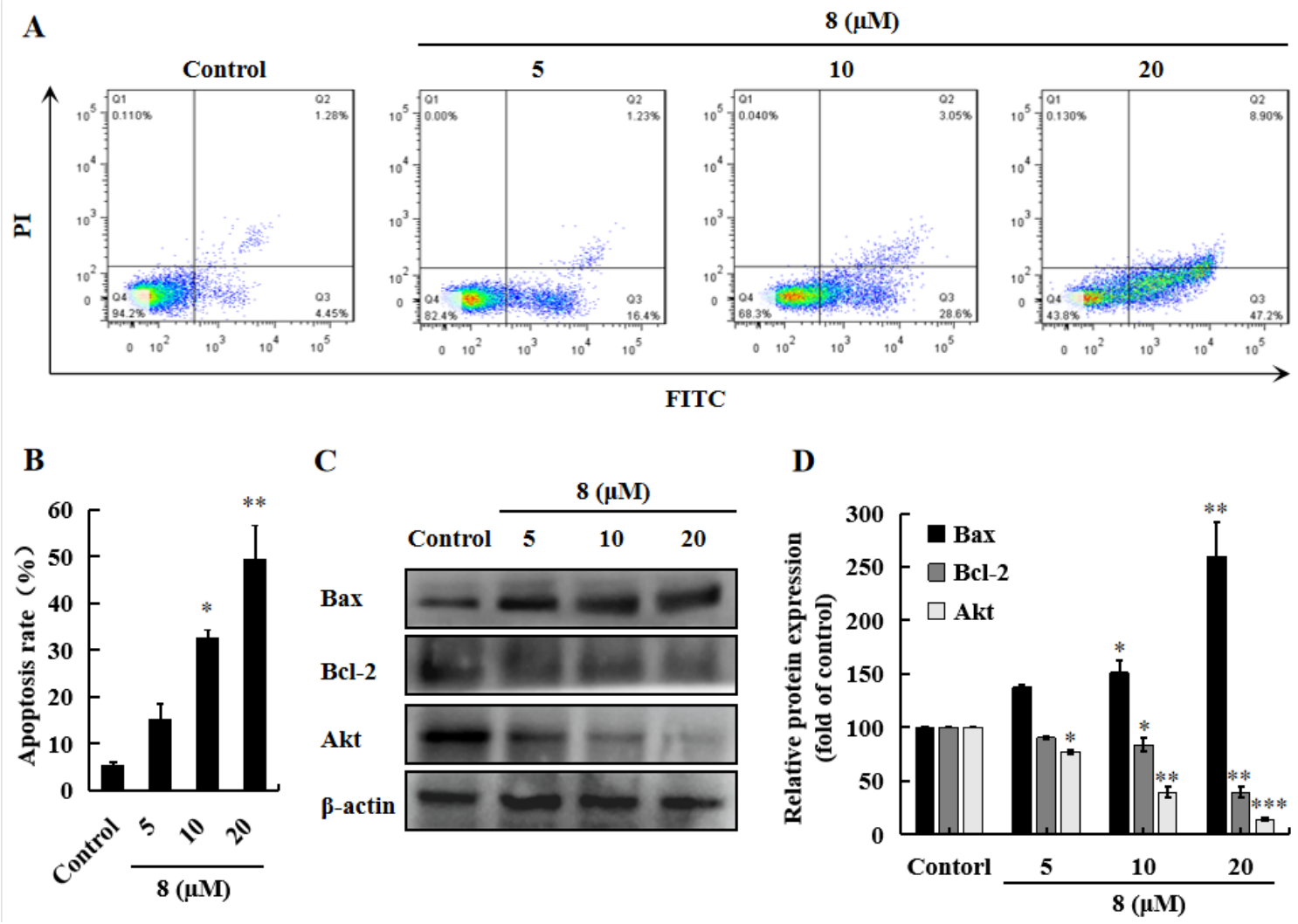

Figure 5

Effects of compound **8** on apoptosis of MDA-MB-231 cells. (A) MDA-MB-231 cells were exposed to compound **8** (0, 5, 10, and 20 μM) for 48 h to measure apoptosis by flow cytometry with Annexin V/PI staining. (B) Relative apoptosis of the treated MDA-MB-231 cells was analyzed. Data presented as mean ± SD. $^{**}p < 0.01$, $^{*}p < 0.05$ vs. control. (C) Bax, Bcl-2 and Akt protein levels were determined by western blotting following compound **8** (0, 5, 10 and 20 μM) treatment for 48 h. β-actin expression was included as an internal control. (D) Relative Bax, Bcl-2 and Akt protein levels of the treated MDA-MB-231 cells was analyzed. Data presented as mean ± SD. $^{***}p < 0.001$, $^{**}p < 0.01$, $^{*}p < 0.05$ vs. control.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- Supplementarymaterial.docx
- Onlinefloatimage1.png