

Synthesis of Novel Xanthone Analogues and Their Growth Inhibitory Activity Against Human Lung Cancer A549 Cells

This article was published in the following Dove Press journal:
Drug Design, Development and Therapy

Junqi Wu,^{1,2,*} Jinwei Dai,^{1,3,*}
Yuyang Zhang,^{1,3} Jing Wang,¹
Lei Huang,¹ Hongmei Ding,¹
Tiejun Li,^{1,4} Yuefan Zhang,^{1,5}
Junqin Mao,⁶ Shichong Yu¹

¹College of Pharmacy, Naval Medical University, Shanghai 200433, People's Republic of China; ²Department of Thoracic Surgery, Shanghai Pulmonary Hospital, Tongji University School of Medicine, Shanghai 200433, People's Republic of China; ³Department of Pharmacology, School of Life Science and Biopharmaceutics, Shenyang Pharmaceutical University, Shenyang 110016, People's Republic of China; ⁴Department of Pharmacy, Punan Hospital, Shanghai 200125, People's Republic of China; ⁵Biomedical Innovation R&D Center, School of Medicine, Shanghai University, Shanghai, 20444, People's Republic of China; ⁶Department of Pharmacy, Shanghai Pudong New Area People's Hospital, Shanghai 201299, People's Republic of China

*These authors contributed equally to this work

Purpose: Xanthones demonstrated an array of pharmacological activities via non-covalent DNA interaction and have been widely utilized in new drug research. The introduction of the polar 1,2,3-triazole ring located at the C3-position of xanthone has not been reported thus far.

Methods: In the present study, a series of xanthone derivatives were designed, synthesized, and characterized through ¹H NMR, ¹³C NMR, and MS. The methyl thiazolyl tetrazolium method was used to evaluate the cytotoxic activity of compounds. Furthermore, the structure–activity relationship and the potential mechanism of target compounds were investigated.

Results: The IC₅₀ showed that the inhibitory activity of 18 target compounds was higher than that of the original xanthone intermediate 4. In particular, compound 1j was the most active agent against A549 cancer cells (IC₅₀ = 32.4 ± 2.2 μM). Moreover, apoptosis analysis indicated different contributions of early/late apoptosis to cell death for compounds 1h and 1j. The results of Western blotting analysis showed that compound 1j significantly increased the expression of caspase 3, Bax, and c-Jun N-terminal kinase, and regulated p53 to a better healthy state in cancer cells.

Conclusion: We synthesized several derivatives of xanthone and evaluated their cytotoxicity. The evidence suggested that compound 1j possessed greater anticancer potential for further evaluations.

Keywords: synthesis, xanthone, derivatives, lung cancer cell, apoptosis

Introduction

Lung cancer is currently the most common type of cancer affecting both men and women, owing to the high prevalence of smoking worldwide.^{1,2} According to the American Cancer Society, lung cancer was the leading cause of cancer-related mortality in 2016 in the United States of America, with estimated 158,080 deaths (~27%).³ Conventional treatments against cancer, such as surgery and radiation therapy, are not feasible in advanced lung cancer mainly owing to the location of the cancer cells in the body.⁴ Chemotherapy is an effective systemic treatment option; chemotherapeutic drugs can disrupt the cell cycle/division and angiogenesis or induce apoptosis of cancer cells through several signaling pathways.⁵ Nevertheless, owing to the high rate of cancer-related mortality, development of resistance, and occurrence of serious adverse effects, continuous efforts are exerted by scientists to develop new drugs for the treatment of cancer.⁶

Xanthones are bioactive substances isolated and extracted from plants and microorganisms.⁷ Their basic skeleton had been confirmed as a biphenyl pyranone

Correspondence: Junqin Mao
Email maojq204@163.com

Shichong Yu
Tel/Fax +86-21-81871228
Email yuscc1008@163.com

with a planar three-ring structure (Figure 1A). Notably, the derivatives of xanthone possess an array of pharmacological activities (e.g., antitumor, antibacterial, antioxidant, hypolipidemic, etc.).^{8,9} Based on their planar structure, they act as efficient DNA intercalators and show anticancer activity via non-covalent DNA interaction.¹⁰ Previous studies have reported structures of natural xanthenes, such as 5,6-dimethylxanthone-4-acetic acid (DMXAA; IC₅₀ = 48.4 μM for MDA-MB-231 cells) (Figure 1) and globosuxanthone A, which showed outstanding anti-proliferative activity.^{11,12} In particular, DMXAA can interact with various biological targets via diverse actions. DMXAA is currently entering the phase III clinical trial stage; consequently, the synthesis of xanthone derivatives with excellent anticancer activity has attracted considerable attention. A series of xanthone analogues were reported and demonstrated improved in vitro antitumor activity versus the parent xanthone and drug-like properties.^{13,14} For example, a caged xanthone (Figure 1B; IC₅₀ = 3.60 μM for A549 cells) was synthesized using the prenyl moiety of its parent xanthone,^{15,16} while 3-phenylxanthone (Figure 1C; IC₅₀ = 6.27 μM for QGY-7703 cells) was identified as a potent and promising antitumor agent.¹⁷

In the side chain, 1,2,3-triazole could serve as a privileged building block for the synthesis of bioconjugates owing to its high stability, selectivity, and fewer adverse reactions.¹⁸ It exhibited formidable stability under basic and acid hydrolysis, including oxidative and reductive reactions. Moreover, this heterocycle was the bioisostere of amide and interacted with biomolecular targets through hydrogen-bonding.¹⁹ This attractive chromophore showed diverse activities (e.g., antibacterial, antiallergic, antiviral, antimalarial, antifungal, and anticancer).^{20–24} In addition, it interacted with DNA and acted as a supporting motif for

DNA targeting compounds, such as xanthenes.^{25,26} Cu(I)-catalyzed azide-alkyne cycloaddition (Click Reaction) rapidly yields bioactive molecules linked through 1,2,3-triazole with high atom economy, and have been widely applied to combinatorial synthesis and bio-conjugate chemistry.²⁷

However, to the best of our knowledge, the incorporation of the polar 1,2,3-triazole ring located at the C3-position of xanthone has not been reported in prior studies. Therefore, 22 heterocyclic xanthone derivatives were designed and synthesized to explore the antiproliferative effect associated with the 1,2,3-triazole and substituent benzyls of the xanthone framework (1a–v, Figure 1). The antiproliferative effects of all derivatives were evaluated in vitro using the MTT colorimetric method against human lung cancer cell line A549.^{28,29} Furthermore, we carefully selected potential compounds to examine the main mechanism involved in cancer cell death.

Materials and Methods

Chemistry

Target compounds 1a–v were synthesized on the basis of a reasonable synthetic route strategy (Scheme 1). In this method, synthesis of compound 4 was achieved by applying Eaton's reagents starting from 2-hydroxybenzoic acid 2 and 1, 3, 5-trihydroxybenzene 3. Compound 5 was synthesized through the *O*-alkylation reaction, in the presence of K₂CO₃, KI, and propargyl bromide in acetonitrile at room temperature. The target compounds 1a–v were obtained by performing a click reaction with variously substituted benzyl azides. All compounds were

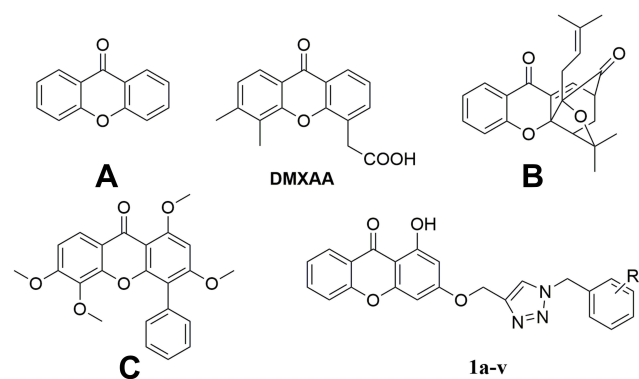
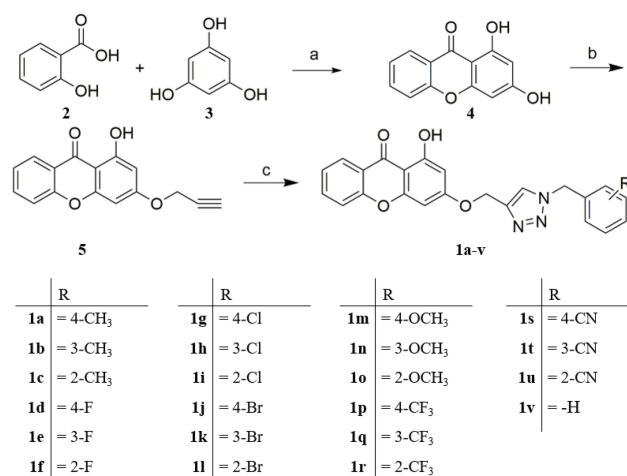


Figure 1 Chemical structures of xanthenes (A) Basic structure of xanthone, (B, C) Derivatives of xanthone.



Scheme 1 Synthesis of compounds 1a–v. Reagents and conditions: (A) P₂O₅/CH₃SO₃H, 85°C, 2.5 h; (B) Propargyl bromide, K₂CO₃, KI, acetonitrile, 6 h; (C) Corresponding benzyl azide, DMSO, CuSO₄·5H₂O, Vit-Na, r.t., 2 h.

characterized through ^1H NMR, ^{13}C NMR, and MS. The details are provided in the [Supplementary Information](#) of this article.

Biological Assays

We used the methyl thiazolyl tetrazolium (MTT) method to measure the cytotoxic activity of the target compounds against human lung cancer A549 cells. The results of this experiment are summarized in [Table 1](#).^{28,29} The A549 cells were obtained from the Cell Bank of the Chinese Academy of Sciences (Shanghai, China). Cells were seeded at a density of 5×10^4 cells/mL, and the compounds were added to the supernatant at different concentrations (i.e., 50 $\mu\text{g/mL}$, 30 $\mu\text{g/mL}$, 10 $\mu\text{g/mL}$, 2 $\mu\text{g/mL}$, 0.4 $\mu\text{g/mL}$). Cells not treated with compounds served as negative control, while those treated with pirarubicin hydrochloride served as positive control. Twelve hours later, MTT (20 μL , 5 mg/mL) was added to the cells, and the cells were incubated at 37°C for 4 h in the dark. Subsequently, dimethyl sulfoxide was added and the mixture was incubated for 10 min. All compounds

were incubated with A549 cells for 12 h. The absorbance was measured at 490 nm using a microplate reader (Multiskan MK3, Thermo Scientific, USA). Cell survival in the control group was considered 100% and that of all other groups was normalized to this value. These procedures were performed in triplicate.

Western blotting was performed to separate the proteins. For the extraction of total protein, 5×10^5 A549 cells were lysed in Protein Extraction Regent (Pierce, Rockford, USA) supplemented with a protease inhibitor cocktail and centrifuged (17,709 g) for 15 min at 4°C . Protein concentrations of the extracts were measured using the BCA assay (Pierce, Rockford, IL, USA) and equalized with the extraction reagent. Proteins were separated through sodium dodecyl sulfate-polyacrylamide gel electrophoresis and transferred to nitrocellulose membranes (Millipore, MA, USA). After the transfer, the proteins were immunoblotted with the corresponding primary antibody against caspase 3, p53, Bax, and c-Jun N-terminal kinase (JNK) (Cell Signaling Technology, Danvers, MA, USA) (dilution ratio: 1:5000). The blots were incubated with the corresponding horseradish peroxidase-conjugated secondary antibody (Kangchen, Shanghai, China) for 1 h at room temperature (dilution ratio: 1:5000). The intensity of each protein band was measured using Quantity One.

A549 cells (1×10^5 cells/mL) were seeded in six-well plates and treated with either vehicle or the indicated concentrations of 1j and 1h for 48 hr. The cells were collected through centrifugation at 4°C and washed twice with ice-cold phosphate-buffered saline. Subsequently, the cells were stained with Annexin-V-FITC/PI (KeyGEN; Nanjing, China) and analyzed via flow cytometry (BD FACS Calibur, Franklin Lakes, CA, USA).

Statistical Analysis

Data are presented as means \pm standard deviation. One-way analysis of variance was used to calculate statistical differences. All statistical analyses in this study were conducted using SPSS for Windows, version 22.0 (IBM, Armonk, NY, USA). A $p < 0.05$ denoted statistical significance.

Results and Discussion

As shown in [Table 1](#), 22 target compounds were synthesized after the introduction of 1,2,3-triazole and the heterocycle group. Of those, 18 compounds showed higher activity compared with intermediate 4 ($\text{IC}_{50} = 340.7 \pm 4.8 \mu\text{M}$) and 5 ($\text{IC}_{50} = 268.4 \pm 3.5 \mu\text{M}$). The extensional structure of various substituted groups resulted in a significantly

Table 1 Anticancer Activities of the Target Compounds in vitro (IC_{50} , μM)

Compounds	R	$\text{IC}_{50}(\mu\text{M})$ A549
1a	4- CH_3	113.8 \pm 6.2
1b	3- CH_3	92.5 \pm 3.9
1c	2- CH_3	67.3 \pm 3.3
1d	4-F	102.3 \pm 4.1
1e	3-F	292.4 \pm 6.0
1f	2-F	96.8 \pm 5.5
1g	4-Cl	51.5 \pm 2.6
1h	3-Cl	40.2 \pm 3.8
1i	2-Cl	596.6 \pm 9.8
1j	4-Br	32.4 \pm 2.2
1k	3-Br	71.5 \pm 6.3
1l	2-Br	96.4 \pm 5.8
1m	4- OCH_3	264.1 \pm 6.2
1n	3- OCH_3	56.0 \pm 2.8
1o	2- OCH_3	104.8 \pm 4.7
1p	4- CF_3	133.2 \pm 5.4
1q	3- CF_3	74.2 \pm 4.3
1r	2- CF_3	97.1 \pm 3.8
1s	4-CN	59.2 \pm 3.6
1t	3-CN	76.4 \pm 5.4
1u	2-CN	97.7 \pm 3.4
1v	H	133.2 \pm 4.2
4	-	340.7 \pm 4.8
5	-	268.4 \pm 3.5
Pirarubicin Hydrochloride ^a	-	5.8 \pm 0.3

Note: ^aPirarubicin Hydrochloride was used as a positive control.

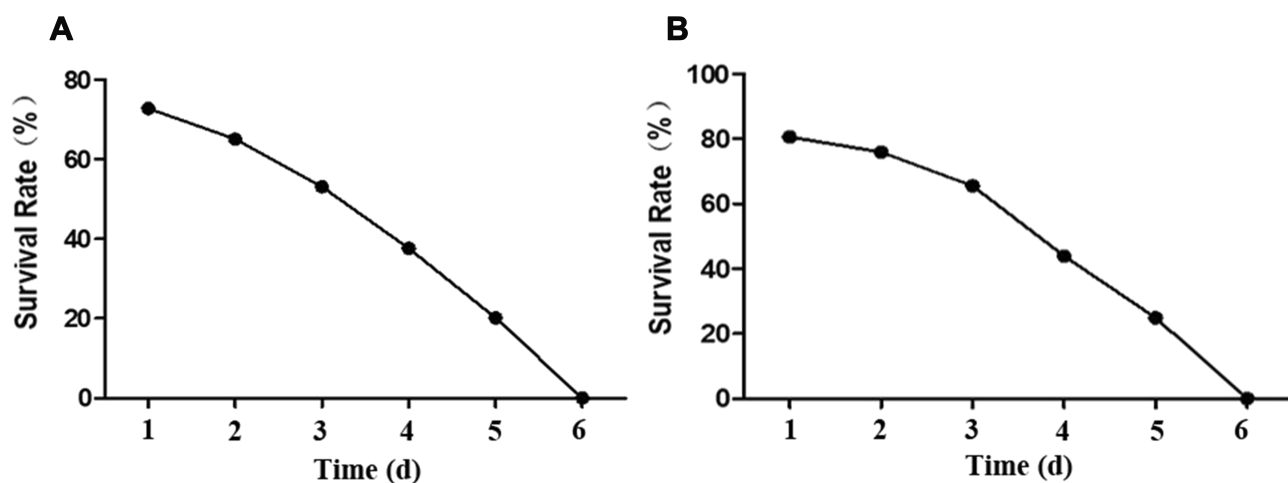


Figure 2 1h (A) (concentration: 46 μM) and 1j (B) (concentration: 46 μM) induced cell death in human umbilical vein endothelial cells in vitro.

increased anticancer effect versus compound 1v with the benzyl group ($\text{IC}_{50} = 133.2 \pm 4.2 \mu\text{M}$). In detail, the compounds with substituted deactivating groups (1d–k, 1p–u)

possessed higher activity versus those with electron-donating groups (1a–c, 1m–o). Of note, the compounds with weak electron-withdrawing groups (1g, 1h, 1j, 1k)

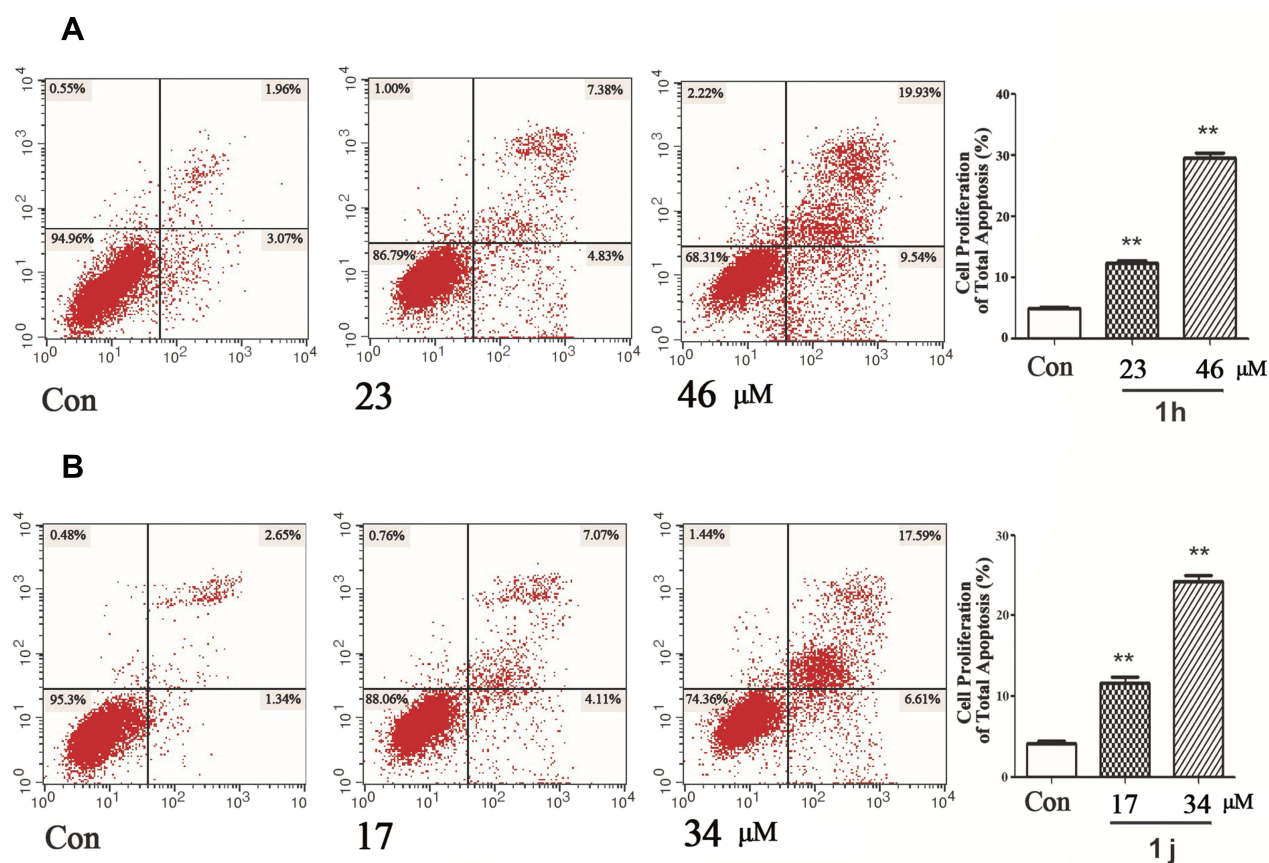


Figure 3 1h and 1j induced apoptosis in cancer A549 cells. Representative scatter diagrams. The A549 cells were pre-treated with (A) 1h at a dose of 0, 23, and 46 μM (0, 10, and 20 $\mu\text{g}/\text{mL}$), (B) 1j at a dose of 0, 17, and 34 μM (0, 8 and 16 $\mu\text{g}/\text{mL}$), respectively, for 48 h. Apoptotic cells were stained with Annexin V-FITC and PI, and measured using flow cytometry. ** $p < 0.01$ vs Con.

demonstrated the highest activity in this series. Among compounds with substituted halogen, the compound with replaced para bromide exhibited the highest anticancer activity ($IC_{50} = 32.4 \pm 2.2 \mu M$). However, the IC_{50} values of compounds 1a–v were lower than that of pirarubicin hydrochloride, an effective anticancer drug used in clinical practice. Two representative compounds, namely 1h ($IC_{50} = 40.2 \pm 3.8 \mu M$) and 1j ($IC_{50} = 32.4 \pm 2.2 \mu M$), presented strong activity against lung cancer A549 cells. In addition, these compounds obviously decreased the survival rate of human umbilical vein endothelial cells, and resulted in toxic effects on normal cells at concentrations of $46 \mu M$ and $34 \mu M$, respectively (Figure 2).

Owing to their higher activity against cancer cells, compounds 1h (low dose: $10 \mu g/mL$, $23 \mu M$, high dose:

$20 \mu g/mL$, $46 \mu M$) and 1j (low dose: $8 \mu g/mL$, $17 \mu M$; high dose: $16 \mu g/mL$, $34 \mu M$) were selected for further evaluation. Firstly, their effect of these compounds on apoptosis was investigated. Two doses (low dose, high dose) were used in the experiment and the results are presented in Figure 3. At the low dose, the rate of total apoptosis induced by 1h and 1j was only 12.21% and 11.18%, respectively. Notably, at the high dose, 1j resulted in 24.20% and 17.59% total and later apoptosis, respectively. The effect of 1h on later apoptosis also markedly contributed to cell death (19.93%). There was no significant difference between the low and high dose groups in terms of early apoptosis. Furthermore, A549 cells were incubated with 1h ($46 \mu M$) and 1j ($34 \mu M$) for 72 h. The rate of total apoptosis increased with time (Figure 4).

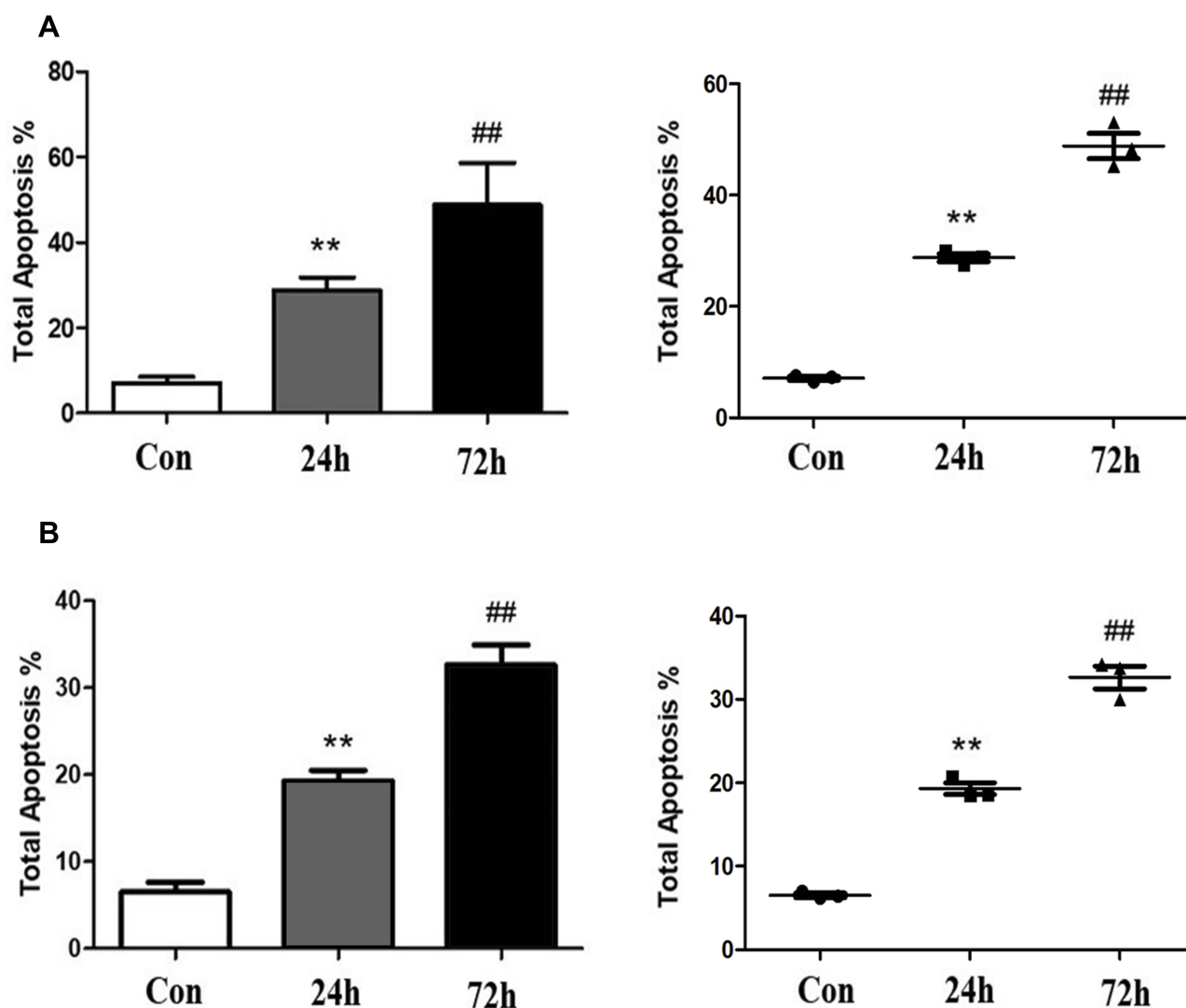


Figure 4 1h and 1j induced apoptosis in cancer A549 cells at 24 and 72 hrs. ** $p < 0.01$ vs Con. ## $p < 0.01$ vs 24hrs.

We used Western blotting to examine the expression of caspase 3, p53, Bax, and JNK in A549 cells after incubation with 1h and 1j for 12 h to disclose the mechanism through which compounds 1h and 1j prevented cancer cell growth. Caspase-3 was involved in the apoptotic process (i.e., chromatin condensation and DNA fragmentation). JNK played key roles in regulating cell proliferation, apoptosis, and differentiation. As shown in Figure 5, we found that both compounds 1j and 1h increased the expression of caspase-3 and JNK in A549 cells. Interestingly, these two compounds also enhanced the expression of p53. p53 is a tumor suppressor playing numerous roles in inducing DNA repair, senescence, cell cycle arrest, and apoptosis. This result was consistent with those of previous studies investigating other series of xanthone.^{30,31} Noticeably, only compound 1j

significantly up-regulated the expression of Bax protein. Bax, belonging to the Bcl-2 gene family, was central to the regulation of mitochondrial apoptosis. Collectively, these findings suggest that the high expression of apoptosis-related factors and p53 was the main mechanism of compound 1j.

Conclusion

Most of the target compounds exerted a marked inhibitory effect on cancer cells. The activity of 18 compounds was higher than that of the intermediates 4 and 5. Moreover, the extensional structure of the 1,2,3-triazole ring and heterocycle groups was beneficial to anticancer activity. Compounds 1h and 1j demonstrated the most potent inhibitory activity against A549 cells. The effects of compounds 1h and 1j on early apoptosis, necrosis, and late apoptosis

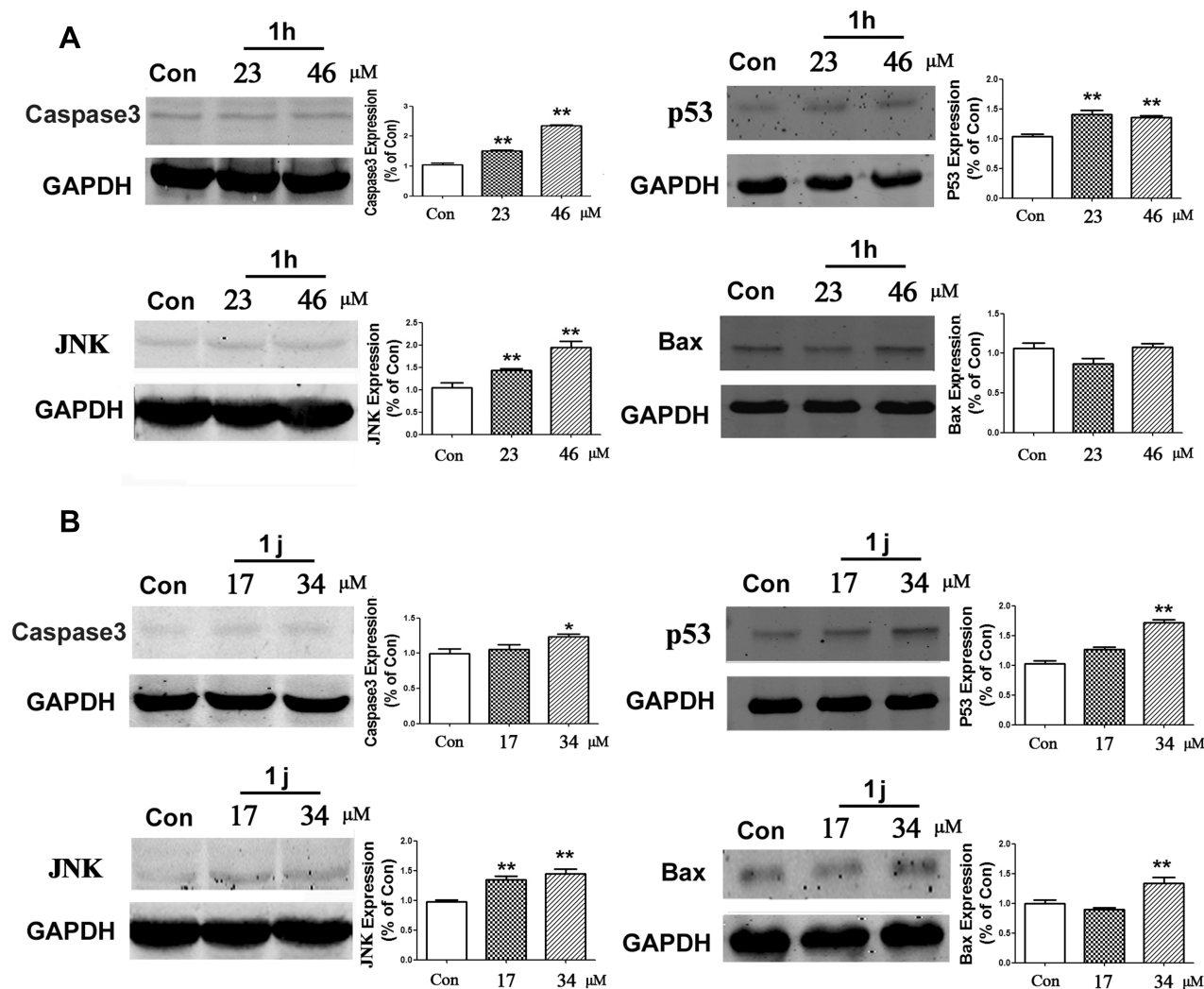


Figure 5 Effect of 1h (A) and 1j (B) on proteins related to cell death. A549 cells were treated with 1h (at a dose of 23 and 46 μM) and 1j (at a dose of 17 and 34 μM) for 12 hrs. The expression levels of proteins were measured using Western blotting. All data are presented as mean ± SE; n=3, *p<0.05 vs Con; **p<0.01 vs Con.

were similar. The results of the Western blotting analysis indicated that compound 1j increased the expression of caspase 3, JNK, p53, and particularly Bax, providing a novel viewpoint of the effects of xanthone derivatives.

Acknowledgments

This work was supported by the National Natural Science Foundation of China (No. 21202200) and by the Project of Shanghai Science and Technology Transformation and Industrialization (15431908500).

Disclosure

The authors declare no conflict of interests in this work.

References

1. Molina JR, Yang P, Cassivi SD, Schild SE, Adjei AA. Non-small cell lung cancer: epidemiology, risk factors, treatment, and survivorship. *Mayo Clin Proc.* 2008;83(5):584–594.
2. Godugu C, Patel AR, Doddapaneni R, Marepally S, Jackson T, Singh M. Inhalation delivery of Telmisartan enhances intratumoral distribution of nanoparticles in lung cancer models. *J Control Release.* 2013;172(1):86–95. doi:10.1016/j.jconrel.2013.06.036
3. Siegel RL, Miller KD, Jemal A. Cancer statistics, 2016. *CA Cancer J Clin.* 2016;66(1):7–30. doi:10.3322/caac.21332
4. Smith RA, Andrews KS, Brooks D, et al. Cancer screening in the United States, 2018: A review of current American Cancer Society guidelines and current issues in cancer screening. *CA Cancer J Clin.* 2018;68(4):297–316. doi:10.3322/caac.21446
5. Kumar CP, Reddy TS, Mainkar PS, et al. Synthesis and biological evaluation of 5, 10-dihydro-11H-dibenzo [b, e][1, 4] diazepam-11-one structural derivatives as anti-cancer and apoptosis inducing agents. *Eur J Med Chem.* 2016;108:674–686. doi:10.1016/j.ejmech.2015.12.007
6. Rao PV, Nallappan D, Madhavi K, Rahman S, Jun Wei L, Gan SH. Phytochemicals and biogenic metallic nanoparticles as anticancer agents. *Oxid Med Cell Longev.* 2016;2016.
7. El-Seedi HR, El-Barbary MA, El-Ghorab DM, et al. Recent insights into the biosynthesis and biological activities of natural xanthenes. *Curr Med Chem.* 2010;17(9):854–901. doi:10.2174/092986710790712147
8. Tarushi A, Raptopoulou CP, Psycharis V, et al. Copper (II) Inverse-[9-Metallacrown-3] compounds accommodating nitrate or diclofenac ligands: structure, magnetism, and biological activity. *Eur J Inorg Chem.* 2016;2016(2):219–231. doi:10.1002/ejic.201500769
9. Na Y. Recent cancer drug development with xanthone structures. *J Pharm Pharmacol.* 2009;61(6):707–712. doi:10.1211/jpp.61.06.0002
10. Tarushi A, Psomas G, Raptopoulou CP, Kessissoglou DP. Zinc complexes of the antibacterial drug oxolinic acid: structure and DNA-binding properties. *J Inorg Biochem.* 2009;103(6):898–905. doi:10.1016/j.jinorgbio.2009.03.007
11. Wijeratne EM, Turbyville TJ, Fritz A, Whitesell L, Gunatilaka AA. A new dihydroxanthone from a plant-associated strain of the fungus *Chaetomium globosum* demonstrates anticancer activity. *Bioorg Med Chem.* 2006;14(23):7917–7923. doi:10.1016/j.bmc.2006.07.048
12. Murata R, Horsman MR. Tumour-specific enhancement of thermo-radiotherapy at mild temperatures by the vascular targeting agent 5,6-dimethylxanthenone-4-acetic acid. *Int J Hyperther.* 2004;20(4):393–404. doi:10.1080/02656730310001619370
13. Shagufta AI. Recent insight into the biological activities of synthetic xanthone derivatives. *Eur J Med Chem.* 2016;116:267–280.
14. Castanheiro RA, Pinto MM, Silva AM, et al. Dihydroxyxanthenes prenylated derivatives: synthesis, structure elucidation, and growth inhibitory activity on human tumor cell lines with improvement of selectivity for MCF-7. *Bioorg Med Chem.* 2007;15(18):6080–6088. doi:10.1016/j.bmc.2007.06.037
15. Wang X, Lu N, Yang Q, et al. Studies on chemical modification and biology of a natural product, gambogic acid (III): determination of the essential pharmacophore for biological activity. *Eur J Med Chem.* 2011;46(4):1280–1290. doi:10.1016/j.ejmech.2011.01.051
16. Zhang X, Li X, Sun H, et al. Garcinia xanthenes as orally active antitumor agents. *J Med Chem.* 2013;56(1):276–292. doi:10.1021/jm301593r
17. Dai M, Yuan X, Kang J, et al. Synthesis and biological evaluation of phenyl substituted polyoxygenated xanthone derivatives as anti-hepatoma agents. *Eur J Med Chem.* 2013;69:159–166. doi:10.1016/j.ejmech.2013.08.020
18. Yan SJ, Liu YJ, Chen YL, Liu L, Lin J. An efficient one-pot synthesis of heterocycle-fused 1,2,3-triazole derivatives as anti-cancer agents. *Bioorg Med Chem Lett.* 2010;20(17):5225–5228. doi:10.1016/j.bmcl.2010.06.141
19. Wang XL, Wan K, Zhou CH. Synthesis of novel sulfanilamide-derived 1,2,3-triazoles and their evaluation for antibacterial and antifungal activities. *Eur J Med Chem.* 2010;45(10):4631–4639. doi:10.1016/j.ejmech.2010.07.031
20. Singh P, Sharma P, Anand A, et al. Azide-alkyne cycloaddition en route to novel 1H-1,2,3-triazole tethered isatin conjugates with in vitro cytotoxic evaluation. *Eur J Med Chem.* 2012;55:455–461. doi:10.1016/j.ejmech.2012.06.057
21. He R, Chen Y, Chen Y, et al. Synthesis and biological evaluation of triazol-4-ylphenyl-bearing histone deacetylase inhibitors as anticancer agents. *J Med Chem.* 2010;53(3):1347–1356. doi:10.1021/jm901667k
22. Kamal A, Prabhakar S, Janaki Ramaiah M, et al. Synthesis and anticancer activity of chalcone-pyrrollobenzodiazepine conjugates linked via 1,2,3-triazole ring side-armed with alkane spacers. *Eur J Med Chem.* 2011;46(9):3820–3831. doi:10.1016/j.ejmech.2011.05.050
23. Thomas KD, Adhikari AV, Chowdhury IH, Sumesh E, Pal NK. New quinolin-4-yl-1,2,3-triazoles carrying amides, sulphonamides and amidopiperazines as potential antitubercular agents. *Eur J Med Chem.* 2011;46(6):2503–2512. doi:10.1016/j.ejmech.2011.03.039
24. Wu J, Ni T, Chai X, et al. Molecular docking, design, synthesis and antifungal activity study of novel triazole derivatives. *Eur J Med Chem.* 2018;143:1840–1846.
25. Kamal A, Shankaraiah N, Devaiah V, et al. Synthesis of 1,2,3-triazole-linked pyrrollobenzodiazepine conjugates employing ‘click’ chemistry: DNA-binding affinity and anticancer activity. *Bioorg Med Chem Lett.* 2008;18(4):1468–1473. doi:10.1016/j.bmcl.2007.12.063
26. Isobe H, Fujino T, Yamazaki N, Guillot-Nieckowski M, Nakamura E. Triazole-linked analogue of deoxyribonucleic acid ((TL)DNA): design, synthesis, and double-strand formation with natural DNA. *Org Lett.* 2008;10(17):3729–3732. doi:10.1021/ol801230k
27. Lee LV, Mitchell ML, Huang SJ, Fokin VV, Sharpless KB, Wong CH. A potent and highly selective inhibitor of human alpha-1,3-fucosyltransferase via click chemistry. *J Am Chem Soc.* 2003;125(32):9588–9589. doi:10.1021/ja0302836
28. Qiu H-Y, Wang P-F, Li Z, et al. Synthesis of dihydropyrazole sulphonamide derivatives that act as anti-cancer agents through COX-2 inhibition. *Pharmacol Res.* 2016;104:86–96. doi:10.1016/j.phrs.2015.12.025
29. Zhu Q, Zhang Y, Liu Y, et al. MLIF alleviates SH-SY5Y neuroblastoma injury induced by oxygen-glucose deprivation by targeting eukaryotic translation elongation factor 1A2. *PLoS ONE.* 2016;11(2):e0149965. doi:10.1371/journal.pone.0149965

30. Lauria A, Tutone M, Ippolito M, Pantano L, Almerico AM. Molecular modeling approaches in the discovery of new drugs for anti-cancer therapy: the investigation of p53-MDM2 interaction and its inhibition by small molecules. *Curr Med Chem.* 2010;17(28):3142–3154. doi:10.2174/092986710792232021
31. Liu J, Zhang J, Wang H, et al. Synthesis of xanthone derivatives and studies on the inhibition against cancer cells growth and synergistic combinations of them. *Eur J Med Chem.* 2017;133:50–61. doi:10.1016/j.ejmech.2017.03.068

Drug Design, Development and Therapy

Dovepress

Publish your work in this journal

Drug Design, Development and Therapy is an international, peer-reviewed open-access journal that spans the spectrum of drug design and development through to clinical applications. Clinical outcomes, patient safety, and programs for the development and effective, safe, and sustained use of medicines are a feature of the journal, which has also

been accepted for indexing on PubMed Central. The manuscript management system is completely online and includes a very quick and fair peer-review system, which is all easy to use. Visit <http://www.dovepress.com/testimonials.php> to read real quotes from published authors.

Submit your manuscript here: <https://www.dovepress.com/drug-design-development-and-therapy-journal>