Study on Antitumor Activity of Piperidine Derivatives

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Abstract [**Objectives**] This study was conducted to investigate the effects of piperidine derivatives on the proliferation and apoptosis of tumor cells (Hele). [**Methods**] The target end product (piperidine derivative) was synthesized through a series of organic reactions. The MTT assay was adopted to detect the effect of piperidine derivative on the proliferation activity of Hele cells. The ROS fluorescence probe method was used to detect the changes of reactive oxygen species. The JC-1 method was applied to detect the changes of MMP in Hele cells. Flow cytometry was adopted to detect the apoptosis of Hele cells. [**Results**] The cell survival rates were 70.84%, 65.46% and 54.48% when the drug concentration was 100, 110 and 120 μmol/L, respectively. When the drug concentration increased to 120 μmol/L, the cell survival rate decreased by nearly half. The fluorescence intensity of active oxygen in the control group was 1, and when the drug concentrations were 100, 110 and 120 μmol/L, the fluorescence intensity of active oxygen was, respectively, 1.315, 1.478 and 1.677, which were higher than that in the control group. The red/green fluorescence intensity of the MMP control group was 1.819, and that of drug groups was, respectively, 1.643, 1.164 and 0.665, which were lower than that of the control group. The apoptosis rates were 10.79%, 22.91% and 38.54% at the drug concentrations of 100, 110 and 120 μmol/L, respectively, showing a concentration dependent effect. The results showed that the piperidine derivative could inhibit the proliferation of Hele cells and induce apoptosis, which was positively correlated with the concentration. [**Conclusions**] This study provides theoretical basis and reference for the anti-tumor research of piperidine.

Key words Piperidine derivative; Hele cell; Anti-cancer activity

DOI:10.19759/j.cnki.2164-4993.2024.03.019

Uterine myoma is a common tumor in female genital diseases. In China, about 40% of women suffer from uterine myoma, mostly in women of childbearing age. There are many reasons for the pathogenesis. Some hormone-regulating drugs have shown certain effects in the treatment of uterine myoma. Once the drugs are stopped, there is a risk of recurrence, and hormone-regulating drugs have certain adverse drug reactions[1-2]. According to epidemiological survey data, there is a certain correlation between the incidence of uterine fibroids and age, and the incidence of menopausal women is as high as 50%. Its five-year survival rate is less than 20%, so it is urgent to seek new treatment methods[3-4].

Piperidine is a kind of azacycloalkane, which is ubiquitous in natural products and has remarkable biological activity. With the deepening of research, it has been found that piperidine can also be used as the precursor of some new compounds to modify or transform the chemical structure, thus obtaining more efficient anti-tumor and antibacterial[5], antiviral, antimalarial and general anesthesia[6], anti-hyperlipidemia, anti-oxidation, anti-inflammatory and other pharmacological effects[7-11]. Like many N-containing groups, piperidine can form additional interaction with the target and easily cross the cell membrane, thus solving the drug resistance problem, improving the water solubility of drug molecules, adjusting the pH value, and improving the absorption and metabolism of drug molecules *in vivo*[12], which makes piperidine a favored dominant skeleton in drug synthesis, and molecular blocks containing piperidine are also frequently used in the synthesis of small drug molecules.

Chrysotin has a wide range of physiological activities, such as anti-oxidation and anti-inflammation[13], antibacterial and anticancer[14]. It is widely distributed in plants, mainly in passion fruit and propolis[15], with low toxicity, and it is a very important resource in the development and research of new drugs. In this study, piperidine was used as the skeleton, and chrysin was introduced to synthesize a piperidine derivative, which inhibited the proliferation of Hele cells and affected their apoptosis. This study provides theoretical basis and reference for the anti-tumor research of piperidine.

Materials and Methods

Experimental instruments, materials and medicines

Enzyme marker (Multiskan GO, Thermo Company, USA); inverted fluorescence microscope (Olympus IX71, Nanjing Eruoda Instrument Equipment Co., Ltd.); flow cytometer (Accurico6, Shanghai Shiwei Co., Ltd.); carbon dioxide incubator (BPN-80CRH, Shanghai Yiheng Scientific Instrument Co., Ltd.); automatic cell counter (IC1000, Yarong Instrument Co., Ltd.); Hele cell line, purchased from Shanghai Institute of Cell Biology, Chinese Academy of Sciences.

Pancreatic cell digestive juice (Solarbio Biotechnology Co., Ltd.); fetal bovine serum (Zhejiang Tianhang Biotechnology Co., Ltd.); thiazole blue (Qiyi Biotechnology (Shanghai) Co., Ltd.); DMEM high sugar medium (Wuhan Amyjet Scientific Inc); mitochondrial membrane potential detection kit (JC-1, Beyotime Biotechnology); Annexin V-FITC/PI apoptosis detection kit (Pricella).

Synthesis of target compound

Accurately, 1 mmol of piperidine and 1 mmol of bromoacetic acid were weighed into a 50 ml reaction bottle, and 35 ml of

Received: March 20, 2024 Accepted: May 28, 2024

Supported by Undergraduate Innovation and Entrepreneurship Training Program of Suzhou University in 2024 (Synthesis and Application of Novel Thiazole Fluorescent Probes); Key Project of Natural Science Research in Colleges and Universities of Department of Education of Anhui Province (KJ2021A1109).

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dichloromethanewas added as solvent, and 0.04 g of HOBT and 1.28 g of EDC · HCl were added as catalysts. The system reacted with reflux at 50 ℃ for 12 h, and TLC tracking was performed. After the reaction, filtration was performed, and the reaction product was extracted with dichloromethane for three times. An appropriate amount of anhydrous sodium sulfate was added, and after standing for 2 h, and rotary evaporation was performed to obtain an intermediate product I. The intermediate product I reacted with an appropriate amount of chrysin at 60 ℃ for 6 h, with anhydrous potassium carbonate as catalyst and acetone as solvent, and TLC tracking was performed. The final product was obtained by column chromatography.

Activity test of target compound

Test of cell activity One bottle of Hele cells and one bottle of 293T cells in which cells had grown to a layer were treated to obtain a 6 ml of cell suspension, respectively. The suspension was mixed with 20 μl of trypan blue, and then dyed for 3 min. The number of living cells (the number of dead cells was less than 5%) was counted with a cell counter, and then the concentration was adjusted to 1×10^5 cells/ml with cell culture fluid. Next, 100 μl of PBS buffer solution was added to each well in the outermost circle of a sterile 96-well plate, and the same amount of cell culture liquid was added to each well in the second column as a blank group, and the same amount of cell suspension was added to remaining wells. Finally, the plate was put into a cell incubator for culture at 37 ℃ with 5% carbon dioxide and saturated humidity.

Addition of drug: After the cells adhered to the wall, the cell culture plate was taken out, and the original culture liquid was removed. Next, 100 μl of new cell culture liquid was added to each well in the second column, and 100 μl of solutions containing samples with different concentrations were added to remaining wells, so that the final concentrations of the drug were 10, 20, 40, 60, 80, 100, 110 and 120 μmol/L, each of which was repeated for 6 times. In addition, six wells of cells added with the same amount of culture liquid were set as a control group. Finally, culture was continued.

Addition of MTT: After 24 h, the cell culture plate was taken out, and MTT solution with a concentration of 5 mg/ml was added with a volume of 15 μl/well in a weak light environment. After mixing well, the plate was wrapped with aluminum foil, and culture and incubation were continued.

Addition of DMSO: After 4 h, the cell culture plate was taken out, and the original cell culture liquid was discarded. Next, 150 μl of dimethyl sulfoxide was added to each well, and the plate was shaken in a horizontal shaking table for 8 – 10 min to dissolve the purple-blue precipitate.

Measurement of OD value: The absorbance (OD_{570}) of each well was measured at 570 nm by a microplate reader. The cell survival rate was calculated according to the formula.

Cell survival rate (%) = [Drug group A – Blank group A]/[Control Group A – Blank group A] × 100

Detection of ROS in cells by DCFH-DA Hele cells in the logarithmic growth phase were added into 6-well plates, and the cell quantity was adjusted to 1.0×10^5 cells/ml. They were incubated in a cell incubator for 24 h, with 5% carbon dioxide and saturated humidity at 37 ℃. After the culture was terminated, the original culture liquid was discarded, and 2 ml of solutions containing different concentrations of tested drug were added to various wells, so that the final concentrations were 100, 110 and 120 μmol/L, each of which was repeated three times. Three other wells were set up without tested drug as a control group. Next, culture was continued for 24 h. In a dark environment, the liquids in wells were mixed evenly with serum-free DMEM according to 1 DCFH-DA: 1000 serum-free DMEM, and the final concentration was 10 μmol/L. After the culture was terminated, the original culture liquid was discarded, and 1.5 ml of diluted DCFH-DA was added to each well. Finally, the cells were incubated in an incubator at 37 ℃ for 20 min.

After incubation, 1 ml of serum-free DMEM was added to each well, and after shaking gently, it was discarded, and each well was washed for three times. Next, 1 ml was added, and observation and photographing were performed with an inverted fluorescence microscope.

Semi-quantitative analysis was carried out by Image J software to explore changes of ROS in cells.

Determination of MMP in cells by JC-1 method Hele cells in the logarithmic growth phase were inoculated into 6-well plates, and the number of cells was adjusted to 1.0×10^5 cells/ml. They were cultured in an incubator for 24 h with 5% carbon dioxide and saturated humidity at 37 ℃. After discarding the original culture liquid, 1 ml of solutions containing different concentrations of tested drug were added to various wells, so that the final concentrations were 100, 110 and 120 μmol/L, each of which was repeated three times. Three other wells were set up as a control group. Next, culture was continued for 24 h. In a weak light condition, JC-1 (200X) was diluted according to the ratio of adding 8 ml of ultrapure water to every 50 μl of JC-1(200X), and 2 ml of JC-1 dyeing buffer (5X) was added after uniform mixing, and a JC-1 working solution was obtained after uniform mixing. Next, the 6-well plates were taken out, and the original culture liquid was discarded, and 1 ml of PBS was added to each well and then sucked out after shaking slowly. Subsequently, 1 ml of cell culture liquid and JC-1 working solution were added to each well, and the cells were incubated at 37 ℃ for 20 min, during which a cleaning solution was prepared. In specific, the JC-1 dyeing buffer (5X) was diluted with distilled water at a ratio of 1: 4 to obtain the cleaning solution, which was mixed evenly and put into a refrigerator at –20 ℃. After the culture was finished, the supernatant was discarded, and 1 ml of cleaning solution was added to each well, and then gently sucked out after slow shaking. The washing operation was performed three times. Next, 2 ml of cell culture liquid was added to each well after washing, and observation and photographing were performed with an inverted fluorescence microscope. Semi-quantitative analysis was carried out by Image J software,

and changes of mitochondrial membrane potential was explored with the relative red/green fluorescence intensity ratio as the index.

Detection of apoptosis rate by Annexin V-FITC/PI double staining Hele cells in the logarithmic growth phase were added into 6-well plates, which were divided into experimental groups and a control group. The cell density was 1.0×10^5 cells/ml, and they were cultured in a cell incubator with 5% carbon dioxide and saturated humidity at 37 ℃. After the cells adhered to the wall, the original culture liquid was discarded, and the drug was added to final concentrations of 100, 110 and 120 μmol/L, each of which was repeated three times. Meanwhile, the control group was added with the same amount of cell culture liquid, and culture was continued. After 24 h, the culture was terminated, and the original culture liquid was transferred to a 2 ml EP tube, and the cells were washed with PBS once. Next, 750 μl of trypsin was added to each well for digestion. When the adherent cells moved at room temperature, the original culture liquid collected before was added to stop digestion, and the cells were slowly blown down with a pipette and transferred to a centrifuge tube to perform centrifugation at 3 500 r/min for 5 min. After centrifugation, the supernatant was discarded, and the cells were collected and re-suspended with 1 ml of PBS to obtain a suspension, which was mixed slowly. Next, centrifugation was continued at 3 500 r/min for 5 min. After repeating the previous step, the cells were re-suspended with 200 μl of Annexin V-FITC binding solution. Subsequently, 5 μl of Annexin V-FITC and 10 μl of propidium iodide staining solution were added in sequence, and then, the cells were cultured at room temperature in the dark for 10 – 15 min. Finally, flow cytometry was used for detection within 1 h.

Results and Analysis

Characterization of drug

Name of target product: 5-Hydroxy-7-(2-oxo-2-(piperidin-1-yl) ethoxy)-2-phenyl-4H-chromen-4-one(4t) (hereinafter collectively referred to as piperidine derivative), light yellow solid powder, yield: 65.67%, melting point: 173.5 – 175.8 ℃. 1HNMR (DMSO-d6, 400 MHz) δ: 12.82 (s, 1H), 8.10 (d, J = 6.8 Hz, 2H), 7.59 – 7.63 (m, 3H), 7.06 (s, 1H), 6.81 (d, J = 2.2 Hz, 1H), 6.41 (d, J = 4.0 Hz, 1H), 5.00 (s, 2H), 3.40 – 3.46 (m, 4H), 1.60 (d, J = 9.4 Hz, 4H), 1.45 (s, 2H).

Effect of piperidine derivative on the survival rate of Hele cells

It can be seen from Table 3 that the cells in the control group grew normally, and the cell survival rates were 93.76%, 90.75%, 85.34%, 80.67%, 75.43% and 70.84% when the drug concentration was 10, 20, 40, 60, 80, 100, 110 and 120 μmol/L, respectively. Compared with the control group, the growth of cells in the drug group was obviously affected and showed a downward trend, and the higher the concentration, the lower the cell survival rate. When the drug concentration was 100 μmol/L, the cell survival rate was obviously affected, and when the drug concentration was 120 μmol/L, the cell survival rate was nearly half. With the concentration of the drug in the range of 10 – 120 μmol/L, there was hardly any harm to 293T normal cells, and various concentrations caused statistical significance compared with the control group ($P < 0.05$).

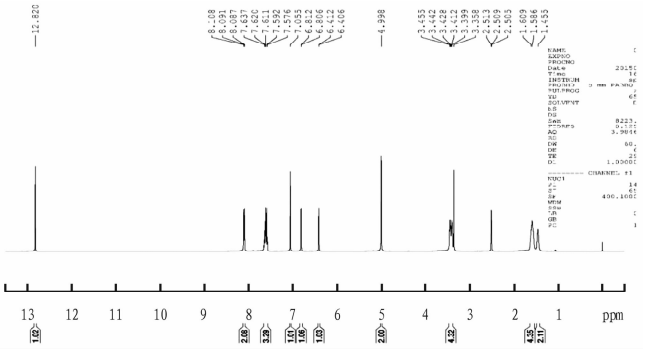


Fig. 1 ^{1}HNMR spectrum of piperidine derivative

Table 1 Effect of piperidine derivative on the survival rate of 293T/Hele cells %

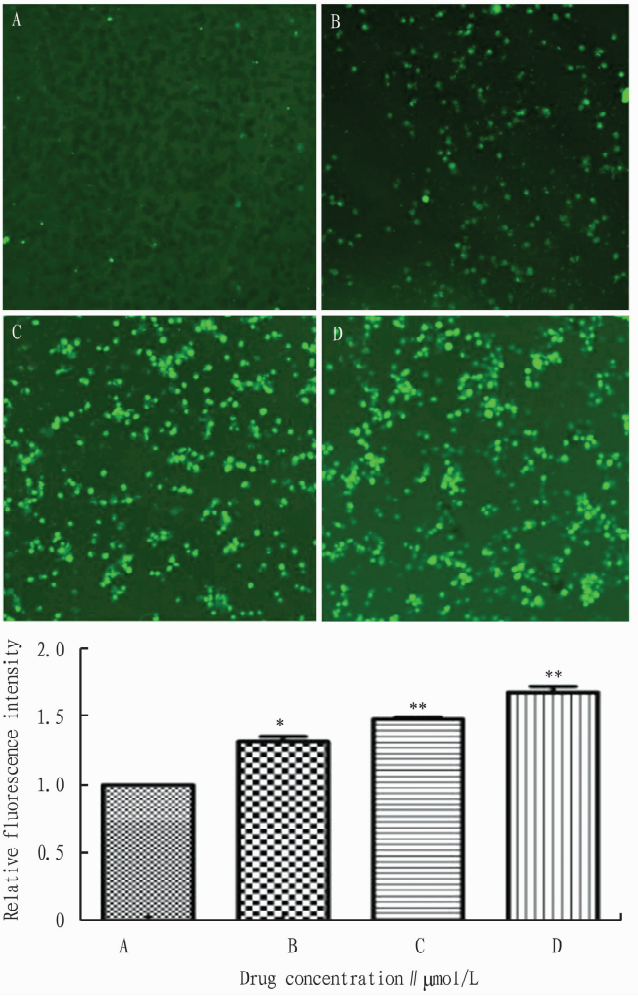
Drug concentration μmol/L	Survival rate of 293T cells	Survival rate of Hele cells
0	99.12 ± 0.57	99.87 ± 1.43
10	98.79 ± 0.77	93.76 ± 1.02
20	98.58 ± 0.87	90.75 ± 1.23
40	98.63 ± 1.13	85.34 ± 0.68
60	98.58 ± 0.72	80.67 ± 0.86
80	98.47 ± 0.75	75.43 ± 0.74
100	97.87 ± 0.56	70.84 ± 0.87
110	97.47 ± 1.62	65.46 ± 1.23
120	97.58 ± 1.74	54.48 ± 0.73

Effect of piperidine derivative on reactive oxygen species content in Hele cells

The level of reactive oxygen species in cells tends to be stable in the normal range, but when the level of reactive oxygen species increases, it will lead to apoptosis. In biological systems, using fluorescence technique for monitoring is a relatively effective method. DCFH-DA does not show fluorescence, but it can freely cross the cell membrane and be hydrolyzed by esterase in cells, thus generating fluorescent DCF. Since DCF cannot cross the cell membrane, the probe can be easily labeled in cells. The fluorescence intensity can reflect the level of reactive oxygen species in cells by converting non-fluorescent DCFH into fluorescent DCF.

As shown in Fig. 2, after cells were treated with the piperidine derivative with concentrations of 100, 110 and 120 μmol/L, the fluorescence intensity of reactive oxygen species was 1.32, 1.48 and 1.68 higher than that of the control group, respectively. When the drug concentration was 105 μmol/L, the fluorescence intensity of cells was the highest, about 1.7 times that of the control group, corresponding to the highest content of reactive oxygen species and the largest number of apoptosis. The cell fluorescence intensity was statistically significant different between the control group and various drug groups ($P < 0.05$). With the increase of drug concentration, the fluorescence intensity in cells also

increased, indicating that the level of reactive oxygen species in cells was on the rise.



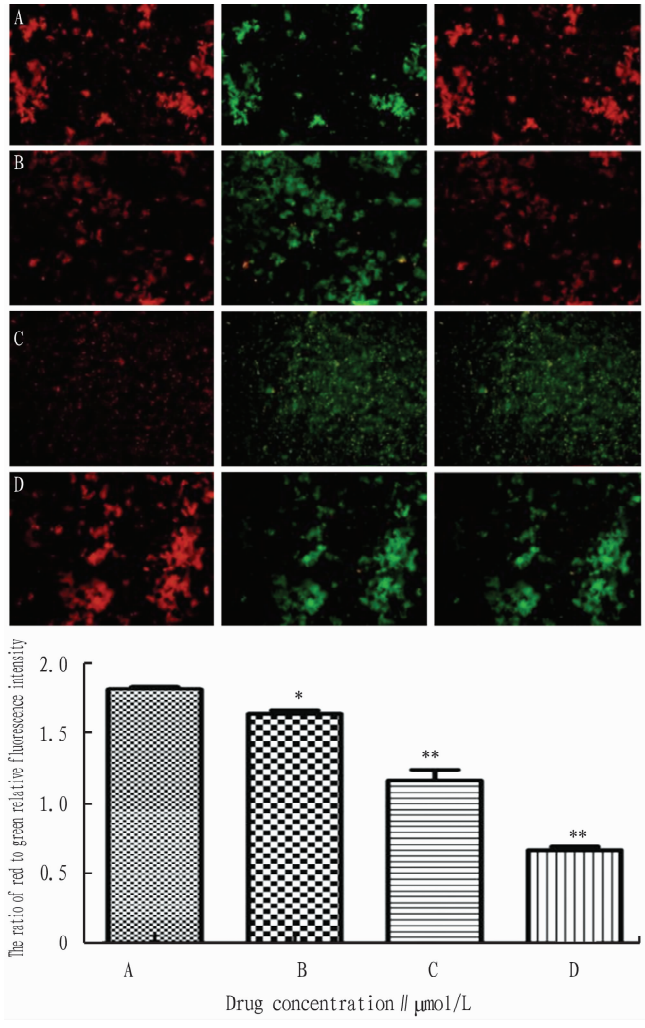
A. Control group; B. 100 μmol/L; C. 110 μmol/L; D. 120 μmol/L. Compared with the control group, $^{*}P<0.05$, $^{**}P<0.01$.

Fig. 2 Effect of piperidine derivative on reactive oxygen species content in Hele cells

Effect of piperidine derivative on MMP in Hele cells

In the process of apoptosis, with the polarization of MMP, JC-1 will enter mitochondria through the polarity of mitochondrial membrane, and with the increase of its concentration, the polymer will produce red fluorescence. When the transmembrane potential of apoptotic cells is depolarized, JC-1 will be released from mitochondria, and the concentration will gradually decrease, and finally it will be reversed to green fluorescence in monomer form. Therefore, the changes of MMP in cells can be judged by fluorescence intensity.

As shown in Fig. 3, the relative red/green fluorescence intensity of Hele cells treated with the piperidine derivative with concentrations of 100, 110 and 120 μmol/L was all lower than that of the control cells, and the red/green fluorescence intensity of the control cells was 1.11, and that of the drug group was 0.90, 0.63 and 0.41, respectively. When the drug concentration was 80 μmol/L, the cell mitochondrial membrane potential decreased obviously, and was 2.4 times different from that of the control group. The relative red/green fluorescence intensity was statistically significant different between the control group and the drug groups ($P<0.05$), indicating that the piperidine derivative could reduce the MMP of Hele cells.



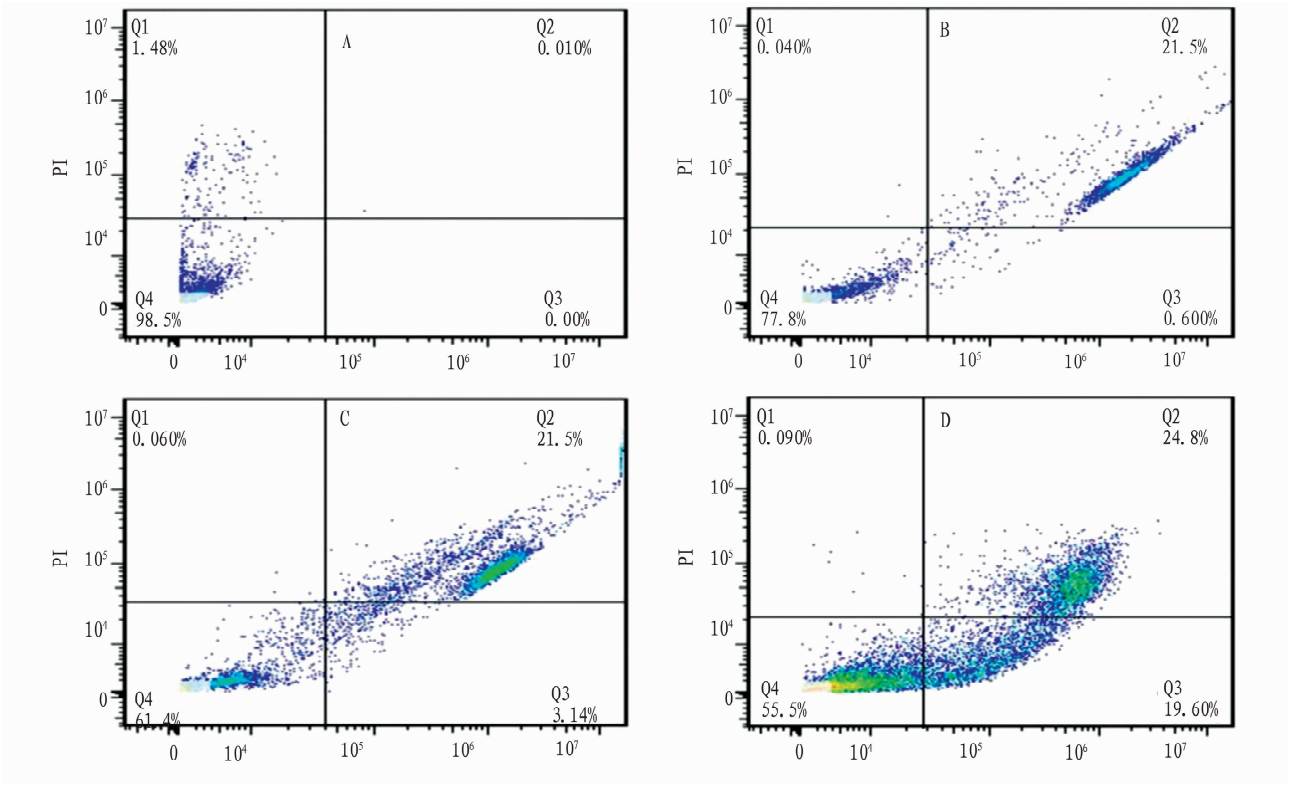
A. Control group; B. 100 μmol/L; C. 110 μmol/L; D. 120 μmol/L. Compared with the control group, $^{*}P<0.05$, $^{**}P<0.01$.

Fig. 3 Effect of piperidine derivative on mitochondrial membrane potential in Hele cells

Effect of piperidine derivative on apoptosis rate of Hele cells

The apoptosis of Hele cells was detected by Annexin V-FITC/PI double staining method. From Fig. 4, it can be seen that the cells in the control group, which were not treated with the piperidine derivative, showed a very small number of apoptotic cells, and the growth situation was good. When the drug concentration was 100 μmol/L, the total apoptosis rate was 10.79%, which was lower than that in the control group. When the drug concentration was 110 μmol/L, the total apoptosis rate was 22.91%, which was more obvious than that of the control group, and the number of apoptotic cells increased compared with that of the 100 μmol/L drug group. When the drug concentration was 120 μmol/L, the total apoptosis rate was 38.54%, which was obvious

compared with the control group, and the number of apoptotic cells increased compared with the first two drug groups, indicating that the apoptosis rate increased with the increase of piperidine derivative concentration.



A. Control group; B. 100 μmol/L; C. 110 μmol/L; D. 120 μmol/L. Compared with the control group, $^{*}P<0.05$, $^{**}P<0.01$.

Fig. 4 Effect of piperidine derivative on apoptosis rate of Hele cells

Conclusions and Discussion

In this study, piperidine was modified to explore its effects on the proliferation and apoptosis of tumor cells. Firstly, a piperidine derivative was synthesized through a series of organic reactions, and the target compound was determined by 1HNMR. With hepatoma cells, Hele cells as the research object, the piperidine derivative was exposed to different concentrations for 24 h, and the effects of the piperidine derivative on the proliferation of hepatoma cells were detected by MTT assay. When the drug concentration was 110 μmol/L, the cell survival rate decreased to 65.46%, which indicated that the piperidine derivative could inhibit cell proliferation, and the cell survival rate gradually decreased with the drug concentration increasing. The DCFH-DA method was adopted to detect the changes of reactive oxygen species (ROS) content in cells. The fluorescence intensity of cells in the drug groups was significantly higher than that in the control group, and with the drug concentration increasing, the fluorescence intensity gradually increased, and the content of ROS in cells increased. Compared with cells in the control group, the fluorescence intensity increased by 1.47 and 1.677 times when the drug concentration was 110 and 120 μmol/L, respectively. Cell MMP was detected by the JC-1 method. The red/green fluorescence intensity of the control group was 1.819, and that of the drug groups was 1.643, 1.164 and 0.665, respectively, so the fluorescence intensity was negatively correlated with the drug concentration. Compared with the control group, the cell fluorescence intensity of the 100 μmol/L drug group was significantly reduced by 1.107 times. The apoptosis rates of the drug groups were 10.79%, 22.91% and 38.54% by the AV/PI double staining method. When the concentration increased from 100 to 120 μmol/L, the apoptosis rate increased by 3.57 times, and there was a positive correlation between the apoptosis rate and the drug concentration.

In this study, when the concentration of the piperidine derivative was 80 μmol/L, the inhibitory effect on cell proliferation was obvious, and the piperidine derivative showed the characteristics of inducing cell apoptosis, and had a significant inhibitory effect on the growth of hepatoma cells.

References

[1] LIANG XL, ZHENG YD. Plumbagin induces autophagy and promotes apoptosis of uterine leiomyoma cells by down regulating the expression of miR-21[J]. The Chinese Journal of Clinical Pharmacology, 2023, 39(6): 814-818. (in Chinese).

[2] LI L. Effect of vitamin D3 on inflammatory uterine leiomyoma cells based on IL-6/STAT3 signaling pathway [J]. Chinese Journal of Immunology, 2023(39): 956-960. (in Chinese).

(Continued on page 89)

biology teaching mode based on research frontiers and hotspots [J]. Chinese Journal of Biotechnology, 2022, 38(7): 2639 – 2648. (in Chinese).
[2] LIU GY, ZHANG J, CHEN YH, *et al.* Research on course reform and teaching of synthetic biology based on OBE concept[J]. Guangxi Journal of Light Industry, 2022, 38(6): 178 – 180. (in Chinese).
[3] XU GQ, YU SQ, ZHOU JW. Teaching reform of graduate students in synthetic biology with the characteristics of fermentation engineering [J/OL]. Chinese Journal of Biotechnology, 1 – 9[2024-04-24]. (in Chinese).
[4] HUANG Y, PENG G, LI X, *et al.* Teaching research and practice of food engineering principle course under the background of "New Engineering"[J]. Yunnan Chemical Technology, 2023, 50(12): 161 – 163. (in Chinese).
[5] LIU SH, WU B, YANG SG, *et al.* Practice and research on microlecture-assisted classroom teaching[C]//Collection of Scientific Research Achievements of Research on Development of Teachers' Teaching Ability (Volume 16), 2018: 1255 – 1258. (in Chinese).
[6] ZHANG MJ. Reform and practice of practical teaching system construction under the background of new engineering[C]//Proceedings of the 2023 Symposium of Guangdong Higher Education Society. Software Engineering Institute of Guangzhou, 2023. (in Chinese).
[7] ZHANG L, MENG XH, DUAN LH. Practice and exploration of ideological and political education in colleges and universities under the background of new engineering: A case study of Industrial Catalysis Course [J]. Journal of Higher Education, 2024, 10(10): 168 – 172. (in Chinese).
[8] WAN YX, ZHANG J. Teaching reform of "food safety and hygiene experiment" based on OBE education concept under the background of new engineering[J]. Technology Wind, 2024(9): 16 – 18. (in Chinese).
[9] LI RY, ZHANG C, ZHANG K, *et al.* Research on the system composition and performance of college students' innovation and entrepreneurship ability[J/OL]. Theory and Practice of Education, 1 – 6[2024-04-25]. (in Chinese).
[10] WANG HQ, ZHANG WM. Exploration and practice of cultivating innovative talents relying on synthetic biology competition[J/OL]. Journal of Microbiology, 1 – 6[2024-04-25]. (in Chinese).
[11] LIU HC, XIA JL, NIE ZY. Exploration on teaching reform of synthetic biology under the background of strategic emerging industry development [J]. The Science Education Article Collects, 2023(8): 84 – 86. (in Chinese).
[12] SHI XC, LU ZQ, WANG ZH. Research and practice on the teaching reform of "PAD class" in synthetic biology[J]. Guangxi Journal of Light Industry, 2020, 36(4): 154 – 155, 182.
[13] JIN YZ. Global synthetic biology market exceeds $17 billion, Shanghai builds innovation center and develops three platforms[N]. China Business News, 2024-04-16(A02).
[14] WANG Z, SUN FF, ZHANG Y. Research progress of chemical synthetic biology[J/OL]. Chinese Bulletin of Life Sciences, 1 – 28[2024-04-26].
[15] HU YC, HUANG YQ, YUAN YM. Research on the teaching of integrating the internet and artificial intelligence under the background of "new engineering": Taking "introduction to automation" as an example[J]. Technology Wind, 2024(2): 105 – 108.

Editor: Yingzhi GUANG Proofreader: Xinxiu ZHU

(Continued from page 85)

[3] TANG J, HUANG BL, GAO LL. Effect of vitamin D3 on inflammatory uterine leiomyoma cells based on IL-6/STAT3 signaling pathway[J]. Hebei medical journal, 2022, 44(15): 2272 – 2275. (in Chinese).
[4] YANG Z, WU WP, MOURBOUL ABLISE, *et al.* Design, synthesis, anti-cervical cancer and reversal of cisplatin resistance activity of novel piperidine-chalcone derivatives[J]. Chemistry Bulletin, 2023, 86(9): 1126 – 1136. (in Chinese).
[5] QUAN YN. Design, synthesis and antiviral activity of 2-(4-substituted piperazine-1-yl) methylbenzonitrile compound[D]. Beijing: Peking Union Medical College, 2022. (in Chinese).
[6] ZHOU SY, YANG SB. Synthesis and biological activity of new type of local anesthetic drug 4-methoxy-N-2-(4-piperidine) ethyl benzamide compounds[J]. Journal of Guizhou Normal University: Natural Sciences, 2017, 35(6): 93 – 98. (in Chinese).
[7] LI HX, QIAN YM, XU LS. Design, synthesis and anticancer activity evaluation of novel quinazoline derivatives as EFGR inhibitors[J]. Chinese Journal of Structural Chemistry, 2021, 40(7): 933 – 941.
[8] NG JM, CURRAN T. The hedgehog's tale: Developing strategies for targeting cancer[J]. Nat Rev Cancer, 2011, 11(7): 493 – 501.
[9] HUANG HL, ZHOU SL, WU ZD. Comparison of therapeutic effects of sodium valproate and haloperidol on Tourette's syndrome in children [J]. Chinese Journal of Clinical Rational Drug Use, 2023, 16(9): 152 – 155. (in Chinese).
[10] WANG GQ, DUAN NN, CAO TY, *et al.* Antitumor fluoroquinolone C3-isostere derivatives (I)-synthesis and activity of bis-oxadiazole methyl-sulfide derivatives[J]. Chinese J Appl Chem, 2012, 29(7): 769 – 774.
[11] MA H, LU W, SUN Z, *et al.* Design, synthesis, and structure-activity-relationship of tetrahydrothiazolopyridine derivatives as potent smoothened antagonists[J]. Eur J Med Chem, 2015(89): 721 – 732.
[12] LI YL, ZONG W, CHANG SE, *et al.* Experimental study of miRNA-195 regulating apoptosis of hepatoma Hep3B cells by targeting BIRC5 [J]. Shaanxi Medical Journal, 2022, 51(3): 270 – 273. (in Chinese).
[13] BRAY F, FERLAY J, SOERJOMATARAM I, *et al.* Global cancer statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries[J]. CA Cancer J Clin, 2018, 68(6): 394 – 424.
[14] ZHAO YF. Single molecule fluorescence resonance energy transfer technique[J]. Biology, 2011, 23(11): 1140 – 1144. (in Chinese).
[15] YANG Z, WU WP, MOURBOUL ABLISE, *et al.* Design, synthesis anti-cervical cancer and reversal of cisplatin resistance activity of novel pipevidine-chalcone derivatives[J]. Chemistry Bulletin, 2023, 86(9): 1126 – 1136. (in Chinese).

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