Heliyon 10 (2024) e32241

Contents lists available at ScienceDirect

Heliyon

journal homepage: www.cell.com/heliyon

Research article

Exploring the potential of thiophene derivatives as dual inhibitors of β-tubulin and Wnt/β-catenin pathways for gastrointestinal cancers in vitro

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ARTICLE INFO

Keywords:
Thiophene derivative 1312
Gastrointestinal cancer
Wnt/β-catenin
Fluorouracil
Medicine resistance

ABSTRACT

Background: Gastrointestinal cancer poses a considerable global health risk, encompassing a heterogeneous spectrum of malignancies that afflict the gastrointestinal tract. It is significant to develop efficacious therapeutic agents, as they are indispensable for both the treatment and prevention of this formidable disease.

Methods: In this study, we synthesized a novel thiophene derivative, designated as compound 1312. An assessment was performed to investigate its anti-proliferative activity in several cancer cell lines (GES-1, EC9706, SGC7901, and HT-29). Furthermore, we performed molecular biology techniques to investigate the inhibitory impact of compound 1312 on gastrointestinal cell lines SGC-7901 and HT-29.

Results: Our findings reveal that compound 1312 exhibits significant efficacy in suppressing colony formation of cancer cells. Notably, it triggers cell cycle arrest at the G2/M phase in gastrointestinal cell lines SGC7901 and HT-29. Compound 1312 was confirmed to exert inhibitory effects on cell migration and invasion in SGC7901. Additionally, the compound elicits apoptotic cell death through the activation of the DNA repair enzyme poly (ADP-ribose) polymerase (PARP) and the caspase signaling cascade. Furthermore, *in vitro* experiments revealed that compound 1312 effectively suppresses both the β-tubulin cytoskeletal network and the Wnt/β-catenin signaling pathway. These multifaceted anti-cancer activities highlight the potential of compound 1312 as a promising therapeutic agent for the treatment of gastrointestinal malignancies.

Conclusion: This study indicates the promising potential of compound 1312 as a prospective candidate agent for gastrointestinal cancer treatment. Further comprehensive investigations are needed to explore its therapeutic efficacy in greater detail.

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https://doi.org/10.1016/j.heliyon.2024.e32241
Received 4 January 2024; Received in revised form 29 May 2024; Accepted 30 May 2024
Available online 31 May 2024

1. Introduction

Gastrointestinal malignancies, encompassing neoplasms arising from the stomach and colorectal regions, represent one of the most prevalent forms of cancer globally [1,2]. Common risk factors for gastrointestinal cancer are dietary patterns, *Helicobacter pylori* infection, medication use, and demographic factors [3,4]. Gastrointestinal cancer accounts for approximately one-third of global cancer-related deaths because of the limits of targeted therapies and late diagnoses [5]. Although treatment options such as chemotherapy, immunotherapy, radiation, and surgery have improved overall survival rates for patients, these approaches highly likely lead to off-target or side effects, reducing treating efficacy and patients' quality of life [6]. Despite advancements in diagnosis and therapy, the incidence of gastrointestinal cancer and associated mortality remain high each year, particularly in certain regions [7]. In parallel, the tumor micro-environment is complex and the heterogeneity from individuals poses challenges to gastrointestinal cancer treatment and diagnosis. These scenes strongly limit the efficiency of gastrointestinal cancer therapeutics [8,9]. Drug resistance has also been increasingly reported across various anti-cancer drugs, which undermined their effectiveness [10–12]. Therefore, finding novel, highly potent agents against cancer with reduced adverse effects holds great value in advancing more powerful and cost-effective approaches for cancer treatment.

Since the last century, significant emphasis has been placed on the development of new anticancer drugs, including natural products [13–15]. Anticancer drugs work based on various mechanisms, including cell cycle arrest, apoptosis, and inhibition of microtubule formation [16–19]. Tubulin inhibitors, such as Vinca alkaloids, taxanes, and eribulin, are widely used as anticancer agents [20,21]. Notably, Ginsenoside Rb1 (Rb1), derived from Ginseng, has showed promising antitumor and anti-inflammatory effects in various types of tumors [22–24]. However, the efficacy of most anticancer drugs varies across different cancer types due to diverse micro-environments and individual heterogeneity [25,26]. For instance, curcumin exhibits high efficiency against multiple tumors, but its application in gastrointestinal cancer treatment is limited because of its poor water solubility [27,28]. Thus, the development of anti-tumor agents for certain type of cancer would be benefit for precision medicine in cancer treatments. The Wnt/β-catenin signaling pathways have been identified to be essential in tumorigenesis and have usually been targeted for the development of antitumor drugs [29,30]. The Wnt signaling pathway is triggered by the binding of Wnt ligand proteins to specific cell surface receptor complexes. These receptor complexes include the Frizzled (FZD) family of receptors as well as the low-density lipoprotein receptor-related protein (LRP) co-receptors. The engagement of Wnt ligands with this receptor system triggers the downstream activation of the Wnt/β-catenin signaling cascade [31]. These binding trigger a cascade of intracellular events that result in β-catenin stabilization in the cell protoplasm and nuclear translocation [32]. In the cellular nucleus, the accumulated β-catenin protein interacts with transcription factor complexes belonging to the T-cell factor/lymphoid enhancer factor (TCF/LEF) family. This interaction between β-catenin and the TCF/LEF transcription factors leads to the transcriptional activation of Wnt target genes, which are involved in regulating key cellular processes such as cell survival and cell proliferation [33]. Therefore, the aberrant, dysregulated activation of the Wnt/β-catenin signaling pathway is a characteristic feature observed in a wide variety of cancer types [34]. Genetic aberrations impacting key components of the Wnt/β-catenin signaling cascade, including stimulating mutations in the CTNNB1 gene encoding β-catenin as well as suppressing mutations in the APC (adenomatous polyposis coli) gene, are frequently observed across a spectrum of cancer cell types [35].

Recent studies have shown that traditional Chinese medicines are useful for treating gastrointestinal cancers, such as berberine [36] and lycopene [37]. Although its clinic application can be limited by the poor solubility and absorption of berberine [38], while lycopene is constrained due to its high instability and limited oral bioavailability [39]. Moreover, traditional Chinese medicines and targeted small molecules have been explored as potential therapeutic options for colorectal cancer by modulating this pathway [40]. Thiophene derivatives, known for their diverse biological activities, including antibacterial and anti-allergic properties, have also shown promising inhibitory effects against various cancers [41–45]. Based on our prior studies, a series of thiophene derivatives were built and their anti-proliferative efficacy was evaluated. Particularly, their growth-inhibiting activities against three gastrointestinal cancer cells (SGC-7901, HT-29, and EC9706) were determined. Among them, Compound 1312 demonstrated the highest level of activity, exhibiting an IC50 of 340 nM against the SGC-7901 cell line. Therefore, developing an anticancer agent based on compound 1312 can be essential for designing safe and effective broad-spectrum antitumor drugs.

This study synthesized a novel thiophene derivative called compound 1312 and evaluated its ability to suppress tumor cells proliferation. The anti-proliferative effects of compound 1312, as well as the standard chemotherapy drug 5-fluorouracil (5-FU), were investigated on several gastrointestinal cancer cell lines, including GES-1, EC9706, SGC7901, and HT-29. Moreover, we conducted tumor biology experiments to assess the impact of compound 1312 on the occurrence and development of SGC7901 and HT-29 tumor cell lines. In parallel, the effect of compound 1312 on β-tubulin and Wnt/β-catenin signaling pathways was investigated. The present work aims to evaluate the efficacy of compound 1312 in inhibiting gastrointestinal cancer tumorigenesis *in vitro* and further investigate the underlying mechanisms behind its anti-tumor activity.

2. Materials and methods

2.1. Cell maintenance

The human gastric mucosa epithelial cells GES-1, human esophageal carcinoma cells EC-9706, human gastric adenocarcinoma cells SGC-7901, and human colon cancer cells HT-29 used in this work were acquired from the Cell Bank of the Chinese Academy of Sciences (Shanghai City, China). Cells EC-9706 were cultured in Dulbecco's Modified Eagle Medium (DMEM) [46]. Cells GES-1 and SGC-7901 were cultured in Roswell Park Memorial Institute (RPMI) 1640 medium [47,48]. HT-29 cells were maintained in McCoy's 5 A medium

[49]. To prepare the cell culture medium, a basal medium was mixed with 10 % (v/v) of fetal bovine serum and 1 % of penicillin-streptomycin.

2.2. Cell viability assay

The anti-proliferative activity of compound 1312 was assessed using the CCK-8 assay [50]. Briefly, GES-1, EC9706, SGC7901, and HT-29 cell lines were seeded in 96-well plates for 24 h. Then, cells were incubated with compound 1312 and the anti-tumor drug 5-FU under concentrations 0 nM, 10 nM, 100 nM, 500 nM, 1000 nM, 5000 nM, 10,000 nM and 20,000 nM. The treated cells were kept in a CO_2 incubator at 37 °C for 24, 48, and 72 h. After 24, 48, and 72 h, CCK-8 (10 μL) solution was added to each well and the plates were incubated for 1.5 h. The absorbance of the solution was measured at 450 nm by a multifunctional microplate reader (Bio-Rad, iMark, USA). Nonlinear regression analysis was undertaken in GraphPad Prism 8.0 (GraphPad Software, Inc., San Diego, CA, USA) [51] and IC50 values of each cell line under different treatments were calculated.

2.3. Wound healing test

Cell lines SGC-7901 and HT-29 were seeded and incubated to 90 % confluence. Cells were then scratched with a pipette tip and cultured with different concentrations (0 nM, 100 nM, 200 nM, 400 nM) of compound 1312 in the medium. Observation of snapshots was taken at different time points, including 0 h, 24 h, and 48 h with a Zeiss microscope (Zeiss, AxioObserVer, GER). Image J [52] software was performed to measure the scratch area and calculate the wound healing percentage.

2.4. β-tubulin immunofluorescence

SGC-7901 and HT-29 cell cultures were established on tissue slices. The cells were seeded onto the slices and treated with a basal culture medium containing varying concentrations (0 nM, 100 nM, 200 nM, and 400 nM) of compound 1312. After incubation, the cell-containing sections were washed and fixed with 4 % paraformaldehyde solution for 15 min. To permeabilize the cell membranes, the samples were then incubated with 0.5 % Triton-X-100 for 9 min. Nonspecific binding was blocked by incubating the samples with 5 % Bovine Serum Albumin (BSA) for 1 h. To detect β-tubulin expression, the cells were stained with a fluorescently-conjugated β-tubulin monoclonal antibody (dissolved in 2.5 % BSA at a 1:100 dilution, CL488-66240 Proteintech, China). The stained samples were incubated overnight in the dark at 4 °C. The following day, the cells were flushed with Phosphate Buffered Saline (PBS) and counterstained with DAPI for 3 min. Imaging and further analysis of the β-tubulin expression in the SGC-7901 and HT-29 cells treated with different concentrations of compound 1312 were performed using a laser scanning confocal microscope (ZEISS, LSM800, GER). This methodology provided insights into the impact of the compound on the β-tubulin pathway.

2.5. DAPI staining

Cells from the SGC-7901 and HT-29 cell lines were seeded in 24-well plates containing glass coverslips and incubated overnight. The cells were then cultured in basal medium added with varying concentrations of compound 1312 (0 nM, 100 nM, 200 nM, and 400 nM). After a 48-h incubation period, the cell-containing slices were fixed with 4 % paraformaldehyde solution for 15 min and washed three times using PBS. The fixed cells were then stained with the nuclear dye 4′,6-diamidino-2-phenylindole (DAPI) in the dark for 5 min. Fluorescence imaging of the stained samples was performed using a laser scanning confocal microscope (ZEISS, LSM800, GER).

2.6. Cell migration assay

SGC-7901 and HT-29 cells were seeded in 24-well plates containing Transwell chambers. A serum-free cell suspension, with a density of 2×10^5 cells, was added to the upper compartment of the Transwell insert. The lower chamber was filled with serum-free medium supplemented with varying concentrations of compound 1312 (0 nM, 100 nM, 200 nM, and 400 nM). After a 48-h incubation period, the medium was aspirated, and the non-migrated cells on the upper surface of the Transwell membrane were taken away using a cotton swab gently. The membrane was then rinsed three times with PBS buffer. The migrated cells adhering to the underside of the membrane were fixed with 4 % paraformaldehyde at room temperature for 25 min. The fixed cells were then stained with 1 mL of 1 % crystal violet solution for 30 min. Imaging and quantitative analysis of the stained, migrated cells were conducted using ImageJ software [52].

2.7. Cell invasion assay

Matrigel was first thawed at 4 °C and then diluted with ice-cold complete cell culture medium [53]. Prior to seeding the cells, diluted Matrigel (40 μL) was added to the upper chamber of the Transwell insert and allowed to solidify for 30 min. Then, a serum-free cell suspension containing 2×10^5 SGC-7901 or HT-29 cells was added to the upper chamber. Basal medium or serum-free medium containing varying concentrations of compound 1312 (0 nM, 100 nM, 200 nM, and 400 nM) was added to the lower chamber. The cells were then incubated for 48 h. After the incubation period, the medium was aspirated, and the Matrigel and non-migrated cells in the upper chamber were taken away with a cotton swab gently. The cells were then rinsed twice with PBS buffer. The migrated cells adhering to the underside of the Transwell membrane were fixed with 4 % paraformaldehyde for 25 min and stained with 0.1 % crystal

violet for 30 min. After staining, the membranes were washed with PBS and air-dried at room temperature. On the following day, five random fields of view were selected under a high-power microscope (ZEISS, AxioLAB A1, GER) to observe and quantify the migrated cells.

2.8. Colony formation experiment

Cells were seeded in 6-well plates for 3000 cells/well and cultured in basal medium containing compound 1312 at different concentrations (0 nM, 100 nM, 200 nM, and 400 nM). After 7–10 days of culture, cell colonies can be observed under the microscope (ZEISS, AxioLABA1, GER). Samples were fixed with 4 % of paraformaldehyde, then stained with 0.1 % of crystal violet, and counted by taking pictures with Image J software [54].

2.9. Western blot analysis

Cells of SGC-7901 and HT-29 were seeded in 6-well plates and cultured in a medium for 48 h with different concentrations (0 nM, 100 nM, 200 nM, and 400 nM) of compound 1312. The cell pellets were collected. After the invasion assay, the cell pellets were lysed on ice using a buffer containing protease inhibitors. This lysis step helped to extract the cellular proteins for further analysis. The protein lysates were then resolved by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) [55] and transferred to nitrocellulose membranes. The membranes were then blocked at room temperature with 5 % nonfat dry milk for 1 h, and primary antibodies were diluted with 5 % BSA. The primary antibodies are as follows: β-actin (ab8226, Abcam, UK 1:2000), GAPDH (ET1601-4, Huabio, China 1:10000), CDK1 (ET1607-51, Huabio, China 1:2000), Cyclin B1 (ab32053, Abcam, UK 1:5000), Cleaved PARP (ET1608-10, Huabio, China 1:500), Cleaved Caspase 9 (ab2324, Abcam, UK 1:1000), Axin2 (ab109307, Abcam, UK 1:2000) and β-catenin (ab32572, Abcam, UK 1:7500), incubated at 4 °C overnight. On the next day, the secondary antibody (goat-*anti*-rabbit: ZB-2301, ZSGB-bio, China 1:10000 and goat-*anti*-mouse: ZB-2305, ZSGB-bio, China 1:10000) was diluted with 5 % BSA, and finally exposed using Image lab.

2.10. Cell cycle experiments

Cells from the SGC-7901 and HT-29 cell lines were seeded in 6-well plates and cultured for 48 h in culture medium containing different concentrations of compound 1312 (0 nM, 100 nM, 200 nM, and 400 nM). After the treatment period, the cells were washed once with pre-cooled PBS buffer and then harvested by trypsinization to collect the cell pellets. The cell pellets were then fixed in pre-cooled 75 % ethanol. The fixed cells were washed once with pre-cooled PBS buffer. They were then incubated with 2 μg/mL of bovine pancreatic RNase for 30 min in a water bath to remove any contaminating RNA. Next, the cells were stained with propidium iodide (PI, 10 μg/mL) and incubated in the dark for 30 min at 4 °C. Propidium iodide is a fluorescent dye that binds to cellular DNA, allowing for the detection and quantification of DNA content. Using a flow cytometer, the red fluorescence emitted by PI-stained cells was detected at an excitation wavelength of 488 nm. The cells were acquired at a low speed for the analysis of their cellular DNA content [56].

2.11. Statistical analysis

Three replicates were performed in this study and statistical analysis was conducted in SPSS20.0 [57] (IBM Corp., Armonk, N·Y., USA). We normalized our treated cells by comparing them with the statues of cells in each control groups. Dunnett-t test was applied to assess the differences between the treatment groups and the control group. Data were displayed as mean ± standard deviation (SD). $P < 0.05$ was considered statistically significant.

3. Results

3.1. Chemical structure

A novel thiophene derivative, named 1312 (Fig. 1), was a synthetic compound, which identified by 1H and 13C nuclear magnetic resonance spectroscopy. The spectrums of 1H NMR and 13C NMR were listed in supplementary information file 1 (Fig. S1 and Fig. S2).

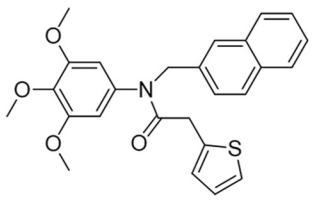

Fig. 1. Chemical structure of compound 1312.

3.2. Anti-proliferative activity

In the study, the anti-proliferative activity of compound 1312 was evaluated on cell lines GES-1, SGC-7901, EC9706, and HT-29. These cells underwent treatment with varying concentrations of compound 1312, and a positive control was treated by various concentrations of the drug 5-FU (Fig. 2A–H). The drug concentration ranged from 0 nM to 20,000 nM, with intermediate concentrations at 10 nM, 100 nM, 500 nM, 1000 nM, 5000 nM, and 10,000 nM. After a 48 h drug intervention, a significant decrease was observed in the proliferation of these cancer cells ($P < 0.05$). Based on these findings, the drug intervention time for subsequent experiments was set at 48 h.

After a 48-h drug intervention, we observed that the proliferation ability of HT-29 cells and SGC-7901 cells with 500 nM of

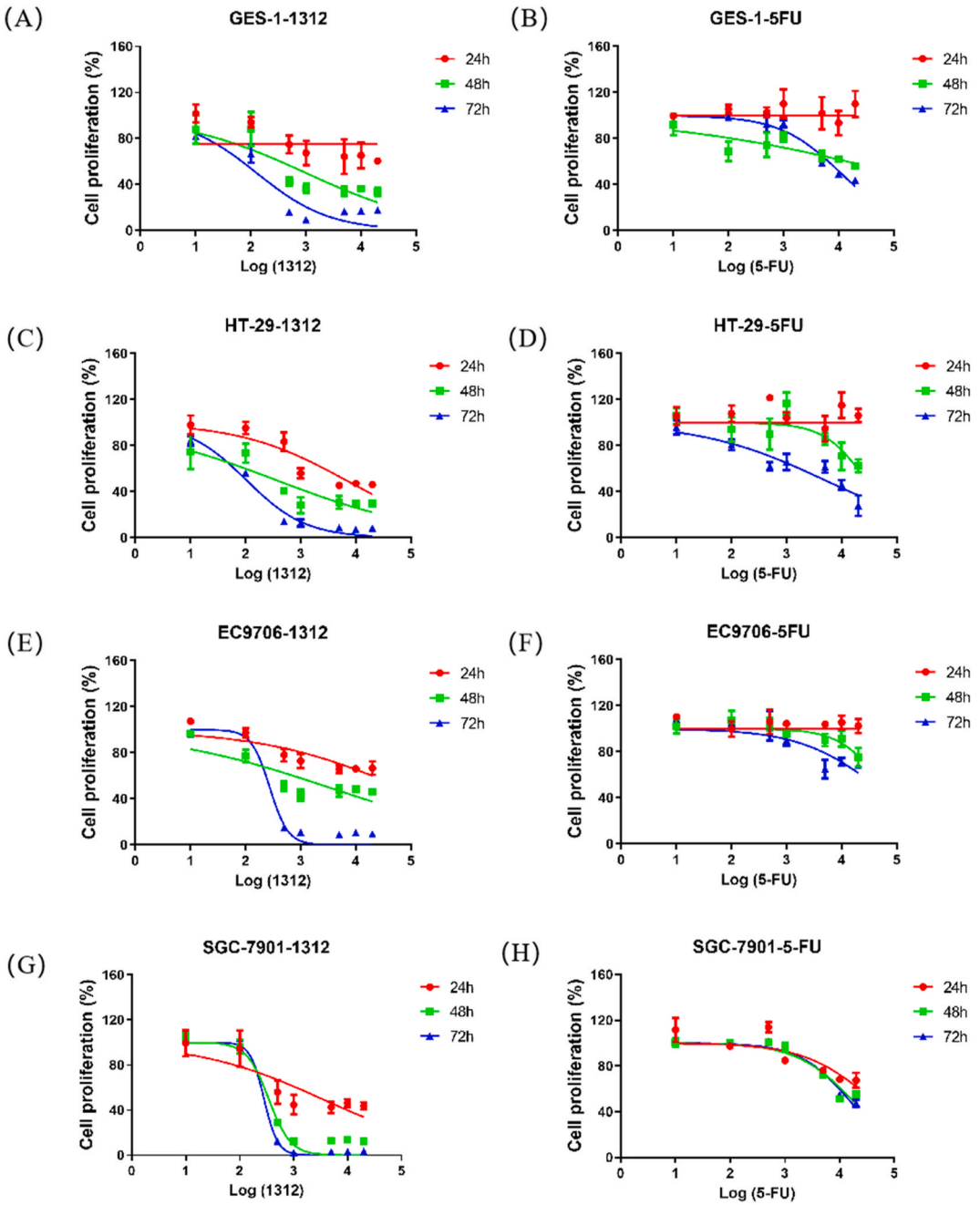

Fig. 2. The proliferation curves of different cell lines GES-1, HT-29, EC9706, and SGC-7901 under the treatment of compound 1312 and 5-FU at different times and drug concentrations (A–H).

compound 1312 was decreased by about 60 % and 70 %, respectively (Fig. 2C and G). On the contrary, the proliferative capacity of EC9706 cells was reduced by 50 % (Fig. 2E). We also found that the suppressive effect of 5-FU on these cancer cells showed lower than that of compound 1312 significantly ($P < 0.05$). Accordingly, we set the concentration gradient to 0 nM, 100 nM, 200 nM, and 400 nM for further studies in cell lines SGC-7901 and HT-29 (Fig. 2, Table S1, Table S2 and Table S3).

The CCK-8 assay was conducted to detect the inhibitory effect of compound 1312 on the proliferation of SGC-7901, HT-29, and EC-9706. For the treatment of 48 h, compound 1312 showed the inhibitory effect against SGC-7901, HT-29, and EC-9706 cell line with an IC50 value of 340 nM, 360 nM and 3170 nM, respectively (~50-fold, 38-fold, 18-fold more potent than 5-FU). It indicated that compound 1312 performed higher anti-proliferative than 5-FU, the anti-proliferation ability of compound 1312 on SGC-7901 and HT-29 was higher than that on EC-9706 cells. Based on IC50 results, the concentrations of 0 nM, 100 nM, 200 nM, and 400 nM were optimized for a follow-up study on SGC-7901 and HT-29 (Fig. 2).

3.3. Compound 1312 inhibited colony formation

We further verified the anti-proliferation ability of compound 1312 on tumor cells by plate cloning. We conducted combination 1312 at different concentrations to intervene in cell lines SGC-7901 and HT-29. Compared with the control, the above two cell lines showed fewer and smaller colonies along with the increase in the concentration of compound 1312. As the concentration of compound 1312 reached 50 nM, the capacity for proliferation of cell lines SGC-7901 and HT-29 was found to be inhibited (Fig. 3A–D, $P < 0.05$).

3.4. Effects of compound 1312 on SGC-7901 cell migration and invasion

SGC-7901 and HT-29 cell lines were intervened for 48 h with various concentrations of compound 1312 (0 nM, 100 nM, 200 nM,

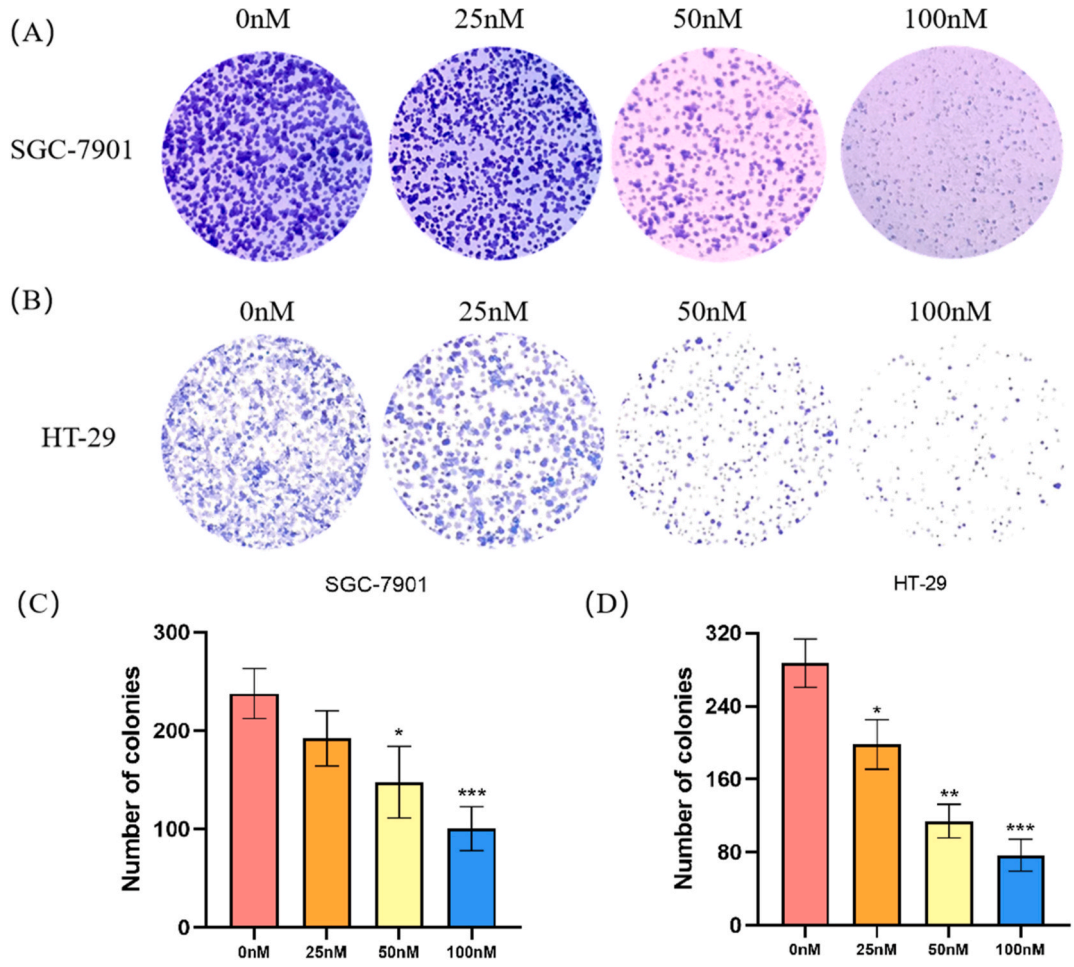

Fig. 3. Effect of compound 1312 on colony formation in gastrointestinal cancer cell lines SGC-7901 (A) and HT-29 (B). Statistical analysis of colony numbers of SGC-7901 and HT-29 cell lines under different concentrations of 1312 (C and D). The compound 1312 inhibits colony formation of SGC-7901 and HT-29 compared to their matched control cells.

and 400 nM) for Transwell and cell scratch assay. The results indicated that with the increase of the compound 1312 concentration, the wound healing ability of SGC-7901 was decreased, while the migration and invasion ability of SGC-7901 showed significantly inhibited (Fig. 4A–E, $P < 0.05$).

3.5. Effects of compound 1312 on cell cycle

To investigate the impact of compound 1312 on the modulation of the cell cycle and its anti-tumor effects, an assay was performed. The cells (SGC-7901 and HT-29) were cultured with compound 1312 of various concentrations (0 nM, 100 nM, 200 nM, and 400 nM) for 48 h. Flow cytometry was used to analyze the cell cycle distribution. Compared with the control, which shows a typical cell cycle, with the gradual increase of the concentration of compound 1312, the cell cycle of SGC-7901 was inhibited in the G2/M phase (Fig. 5C)

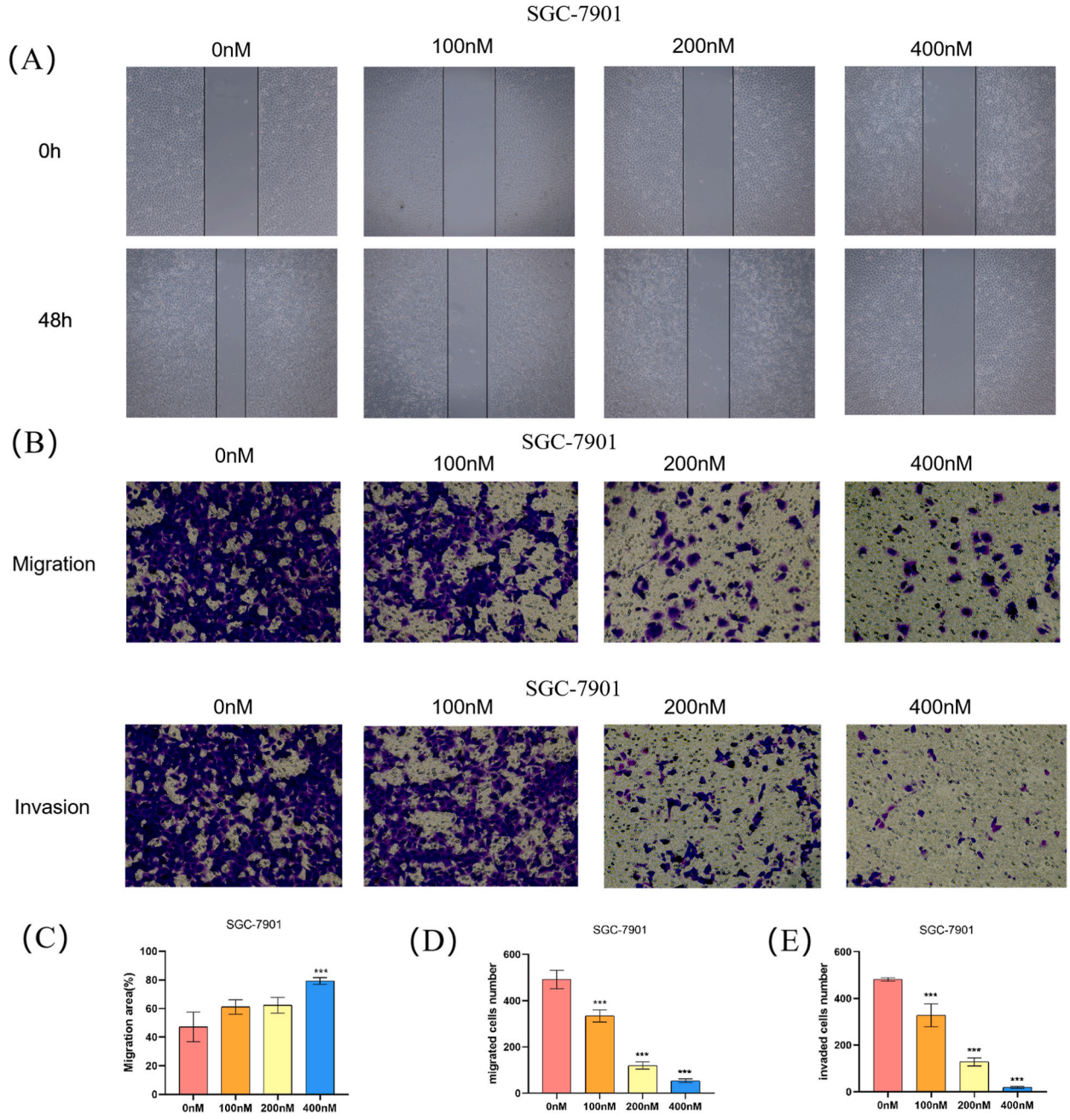

Fig. 4. Effects of compound 1312 on migration and invasion of SGC-7901. Compound 1312 inhibits migration and invasion ability of SGC-7901 cell lines, using wound healing test (A, C) and Boyden chambers (B, D, E). The data were presented as the mean ± SD (n = 3); $^{**}P < 0.01$, $^{***}P < 0.001$.

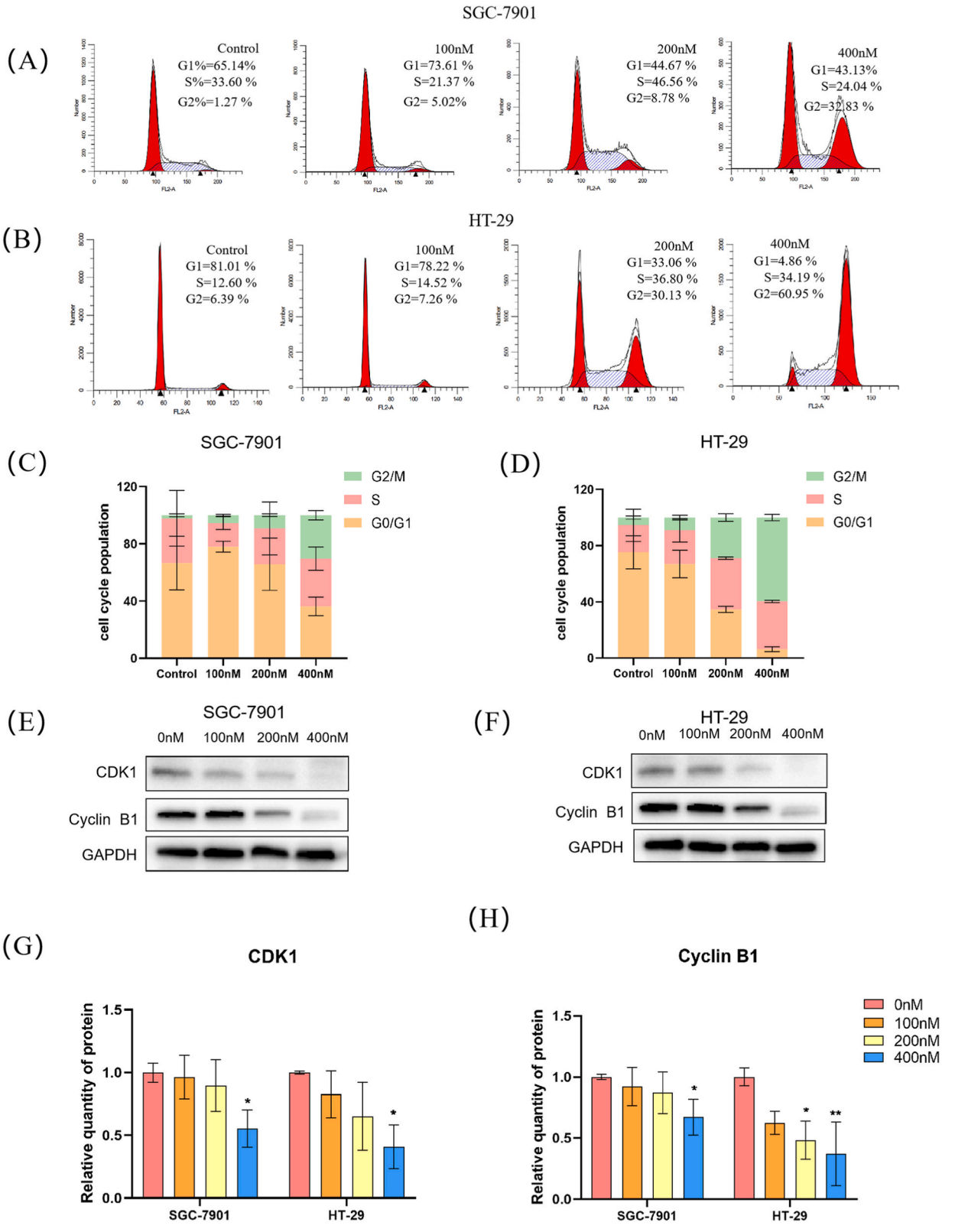

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Fig. 5. (A, B) Cell cycle flow cytometry analysis of gastrointestinal cancer cell lines, SGC-7901 (A) and HT-29 (B). Cells were incubated with 0, 100, 200, and 400 nM of compound 1312 for 48 h, followed by staining cells with Propidium Iodide (PI). Compared to controls, a representative DNA content histogram showing the phases of G0, G0/G1, S, and G2/M on tested cell lines upon treatment with compound 1312. (C, D) The cell cycle histogram results reveal that compound 1312 at high concentrations (400 nM) can significantly disrupt SGC-7901 cell mitosis by arresting cells in the G2/M phase, while HT-29 cell mitosis is blocked in a steady concentration-dependent manner. (E–H) Western Blot analyzed the expression level of Cyclin B1 and CDK1 in SGC-7901 cells and HT-29 cells. (The original blot is provided in the Supplementary file Fig. S3). The data were presented as the mean ± SD (n = 3); $^{*}P < 0.05$, $^{**}P < 0.01$.

while HT-29 cells were blocked at the G2/M phase (Fig. 5D). Furthermore, the percentage of SGC-7901 and HT-29 cells at G2 phase for the high concentration group (400 nM) were 32.83 % and 60.95 % (Fig. 5A and B), which were about 30 % and 50 % higher as compared with that of the control group, respectively. We verified the changes of cell cycle-related proteins and found a high concentration (400 nM) of compound 1312 down regulated significantly the expression of G2/M-phase related proteins CyclinB1 and CDK1 ($P < 0.05$) (Fig. 5E–H) and arrest the cell cycle (Fig. 5).

3.6. The compound 1312 on the polymerization ability of cancer cell tubulin

The immunofluorescence staining results revealed the organization of tubulins in the control group, where they were regularly arranged around the nucleus. As the concentration of compound 1312 increased, there was a decrease in the aggregation capacity of the tubulin pathways. Notably, when the concentration of compound 1312 reached 400 nM, the polymerization ability of β-tubulin in the treated group was inhibited (Fig. 6A and B). These findings suggest that compound 1312 showed a concentration-dependent suppressive effect in SGC-7901 and HT-29 cells on tubulin polymerization. By inhibiting tubulin polymerization, compound 1312 may disrupt cell division and intracellular transport, potentially leading to the prohibition of tumor cell growth and proliferation.

3.7. Effects of compound 1312 on tumor cell morphology and apoptosis-related proteins

As the increase of the concentration of compound 1312, we found that the cells SGC-7901 and HT-29 performed rounding and shrinkage as well as the number decreased significantly ($P < 0.05$) (Fig. 7A and B). Cells SGC-7901 showed increased nuclear pyknosis and fragmentation in morphology, while HT-29 cells showed no apparent alternation (Fig. 7C and D). We also investigated the changes of apoptosis-related proteins and found a high concentration (400 nM) of compound 1312 enhanced the expression of Cleaved PARP and Cleaved caspase 9 significantly (Fig. 7E–H).

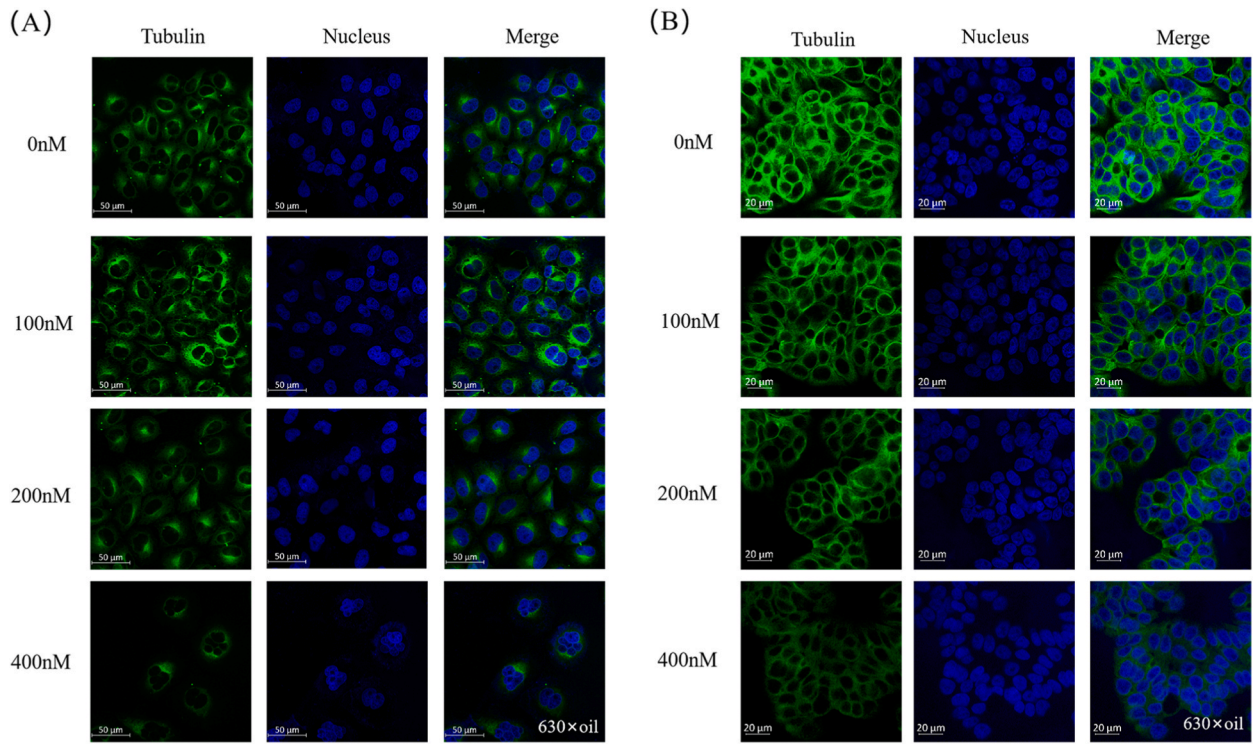

Fig. 6. Compound 1312 showed strong depolymerizing effects on the interphase microtubule network. SGC-7901 (A) and HT-29 (B) were incubated with 0, 100, 200, and 400 nM of compound 1312 for 48 h, respectively.

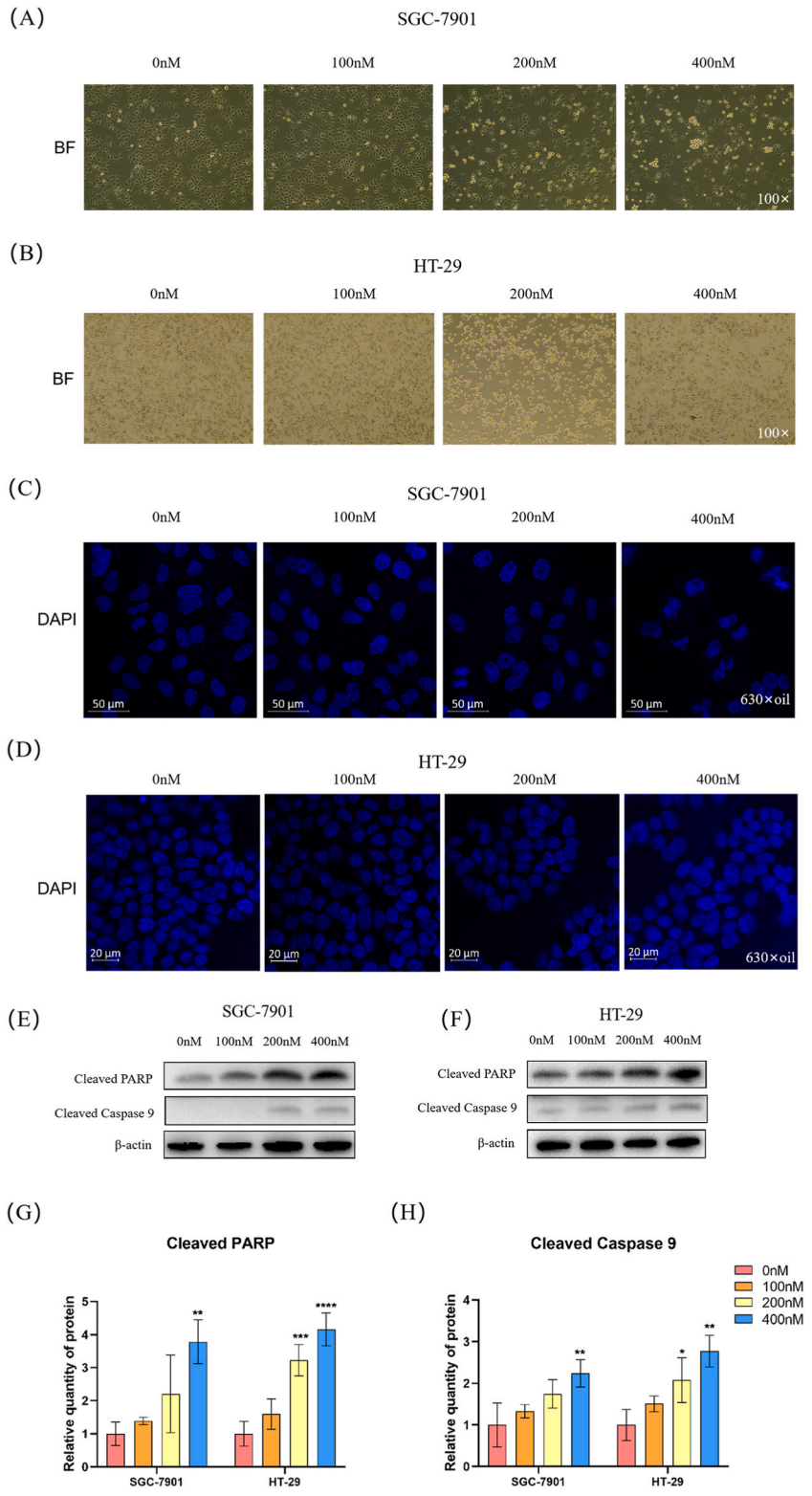

Fig. 7. Morphological changes analysis with phase-contrast microscopy and DAPI staining after 48 h of compound 1312 in SGC-7901 (A, C) and HT-29 cells (B, D). Western Blot analyzed the expression level of Cleaved PARP and Cleaved caspase 9 in SGC-7901 and HT-29 cells (E–H). (The original blot is provided in the Supplementary file Fig. S4). The data were presented as the mean ± SD (n = 3); $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$.

3.8. Effects of compound 1312 on proteins related to Wnt/β-catenin signaling pathway

The Western blot results showed that, with the increase of compound 1312 concentration, the protein expression level of β-catenin performed decreased significantly, while the expression of Axin2 increased significantly (Fig. 8A–D, $P < 0.05$).

4. Discussion

We investigated the effects of compound 1312, a thiophene derivative, on multiple cellular processes, for instance apoptosis, cell cycle, cellular migration, and microtubules *in vitro*. Our findings indicated that compound 1312 has potential anticancer properties by inducing apoptosis, inhibiting cellular proliferation, and impairing the migratory and invasive capabilities of gastrointestinal cancer cells, as thiophene derivatives that have been reported in the previous studies [8,58,59].

Thiophene, a five-membered ring structure containing a sulfur heteroatom, has been synthesized as a key scaffold in medicinal chemistry due to its diverse documented biological activities, including anticancer, antimicrobial, anti-inflammatory, and analgesic effects [41–45]. Thiophene derivatives, have been reported as anticancer agents via different mechanisms. Saad et al. synthesized thiophenes derivatives containing 1,2,4-triazino[3,4–b], including mono-substituted, di-substituted, or tri-substituted, and [1,3,4] thiadiazinones [60]. All of them showed significant cytotoxicity against cancer cells, such as cell lines HCT-116, MCF-7 and Hep-G2 [60]. AbdElhameid et al. found that thiophene carboxamides derivatives 5 and 21 exhibited cytotoxicity against two gastrointestinal solid cancer cells, HepG-2 and HCT-116 cell, via inhibiting VEGFR-2 and β-tubulin polymerization [61]. Amawi et al. suggested that thieno[3,2-*d*]pyrimidine-based compounds-010 showed anticancer efficacy against PC-3 and DU145 prostate cancer cells through Wnt/β-catenin signaling pathway *in vitro* [62]. Rogaratinib, a thiophene-containing derivative compound, has demonstrated good tolerability and clinical activity against a wide range of cancer types, including colorectal cancer, urothelial cancer, and cholangiocarcinoma. This thiophene-based drug candidate is currently being evaluated in several ongoing clinical trials, both as a monotherapy and in combination with immune checkpoint inhibitors or other targeted cancer therapies [63]. A significant increased was found in apoptosis from gastrointestinal cancer cells treated with compound 1312, which was supported by the activation of PARP and caspases. Additionally, compound 1312 caused a decrease in gastrointestinal cancer cellular proliferation, migration, colony formation, and impaired wound healing ability. These effects align with the mechanisms commonly associated with anticancer agents and support the potential of compound 1312 as an anticancer therapeutic.

Furthermore, treatment with compound 1312 resulted in G2/M phase cell cycle arrest, indicating it can be an anticancer agent potentially. Previous research has explored the anticancer activity of novel derivatives in multiple cancer cell lines, for instance, hepatocellular carcinoma, prostate cancer, and colorectal cancer [64,65]. Studies have also investigated the effects of benzo-N-heterocycles transition metal complexes on human esophageal cancer cell lines, and compounds containing carbazole and pyrazole on cancer cells [66]. Tubulin polymerization, which plays a crucial role in tumor formation [66], was also found to be significantly affected by compound 1312 [61,67]. The inhibition of β-tubulin and Wnt/β-catenin signaling pathways may contribute to the observed G2/M phase arrest and increased apoptosis in response to compound 1312. It is important to note that while compound 1312 showed higher efficacy than drug 5-FU in gastrointestinal cancer cell lines, our work has limitations, such as the lack of comparison between the efficacy of compound 1312 and 5-FU in animal models. Safety assessment in animal experiments is also necessary, in parallel, *in vivo* studies are needed to explore the bioavailability and effects of 1312 on animals. Briefly, further studies are needed to assess the adverse reactions associated with compound 1312 and to evaluate its long-term effects *in vivo*. Studying the adverse reactions associated with compound 1312 for gastrointestinal treatments is challenging. Therefore, long-term *in vivo* studies must record all benefits and adverse events related to compound 1312.

5. Conclusions

This study synthesized and assessed the anticancer effects of a novel compound, 1312, on different gastrointestinal cancer cell lines. The results demonstrated that compound 1312 exerted significant inhibitory effects on cellular proliferation by inducing apoptosis and cell cycle arrest. Additionally, compound 1312 effectively targeted tubulin polymerization through acting on β-tubulin and the Wnt/β-catenin signaling pathways *in vitro*. This mechanistic action contributes to the anticancer potential of compound 1312 against gastrointestinal cancer. These findings highlight the promising anticancer properties of compound 1312 and its potential as an effective therapeutic agent for gastrointestinal cancer treatment. However, further studies are warranted, including *in vivo* investigations and comprehensive safety assessments, to determine the efficacy and safety profile of compound 1312 as an anticancer treatment. Additionally, conducting clinical trials would provide valuable insights into the efficacy, adverse effects, and individual variations associated with thiophene derivatives as potential anticancer agents.

Funding

The accomplishment of this work was funded by the support of Scientific Research Project for Colleges and Universities of Henan Province (No. 22B350003), Key Discipline Project of Henan Province (No. 02031727), Preferential Scientific Research Funds for Overseas Chinese Students in 2020 and Natural ScienceFoundation of Henan province (No. 232300420235).

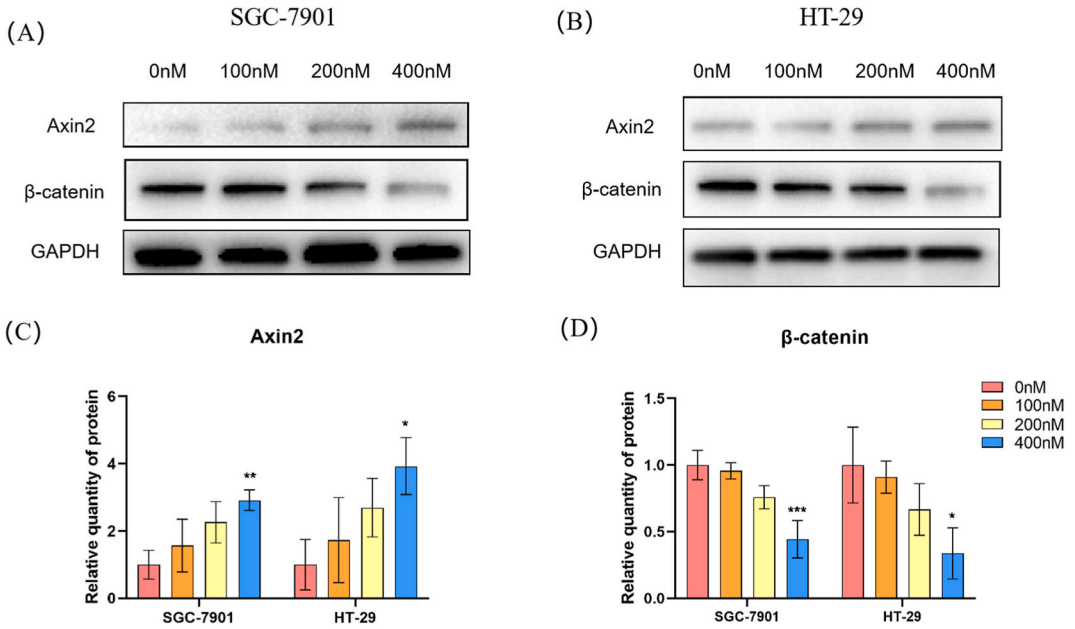

Fig. 8. The expression of the proteins Wnt/β-catenin pathway were determined in SGC-7901 cells and HT-29 cells with the treatment of compound 1312 (A–D). (The original blot is provided in the Supplementary file Fig. S3 and Fig. S5). The data were presented as the mean ± SD (n = 3); $*P < 0.05$, $**P < 0.01$, $***P < 0.001$.

Declarations

Consent for publication

No consent for publication is required for this manuscript.

Data availability

Data included in this manuscript could be accessed by contacting the corresponding author via email.

CRediT authorship contribution statement

Lina Fu: Writing – review & editing. **Fuhao Li:** Data curation. **Xia Xue:** Writing – review & editing. **Huayuan Xi:** Writing – review & editing, Data curation. **Xiangdong Sun:** Writing – review & editing. **Ruoyu Hu:** Writing – review & editing. **Huijuan Wen:** Writing – review & editing, Data curation. **Simeng Liu:** Writing – review & editing, Writing – original draft, Data curation.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgements

No specific acknowledgements are applicable to this work.

List of abbreviations

5-FU 5-Fluorouracil
PARP Poly ADP Ribose Polymerase
FZD Frizzled
LRP Lipoprotein Receptor-related Protein
TCF/LEF T-cell Factor/Lymphoid Enhancer Factor
Rb1 Ginsenoside Rb1

DMEM Dulbecco's Modified Eagle Medium
RPMI Roswell Park Memorial Institute
BSA Bovine Serum Albumin
PBS Phosphate Buffered Saline
DAPI 4′,6-diamidino-2-phenylindole

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.heliyon.2024.e32241.

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