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Synthesis of substituted aromatic heterocyclic sulfonyl hydrazone compounds and *in vitro* anti-hepatoma activity: preliminary results

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Abstract. – OBJECTIVE: Hydrazone compounds and their derivatives are a kind of special Schiff bases. The multiple hydrazone compounds and their derivatives have a variety of biological activities. This study aims to report the synthesis of diverse hydrazone derivatives and to explore the potent antitumor activities.

MATERIALS AND METHODS: A series of aromatic heterocyclic sulfonyl hydrazones W1-W15 synthesized from hydrazine or acylhydrazine and aldehydes or ketones were estimated for their *in vitro* antitumor activities against human cancers. Through the spectral (FT-IR, ^{1}H-NMR, MS) methods, the structure of the compounds was determined. Using MTT (3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-2-H-tetrazolium bromide) method, the effects of different concentrations of compounds on growth inhibition and viability of HepG-2 cells were detected.

RESULTS: Compound W9 exhibited anti-proliferation activity with IC_{50} values of 63.91 μmol/L in HepG-2 cell line. In addition, mechanism studies indicated that compound W9 could distinctly prohibit the propagation of HepG-2 cells by arresting the cell cycle at G_2/M and inducing apoptosis. Furthermore, we investigated the effectiveness of drug combination treatment with W_9 and cis-platinum (cis-DDP) or 5-fluorouracil (5-FU) on HepG-2 cell line.

CONCLUSIONS: Our results indicated that W9 with synergic treatment of 5-FU or cis-DDP shows better inhibitory cell growth. The combination of the two drugs blocks HepG-2 cells in the G_2/M phase. The inhibitory effect of W9 on cell apoptosis was decreased with the increase of drug concentration.

Key Words
Schiff base, Sulfonyl hydrazones, Antitumor, MTT, Cell cycle arrest, Apoptosis.

Introduction

Human liver cancer is the third leading cause of tumor death worldwide[1]. Although progresses have been made in the development of prevention and treatment of human liver cancer, there remains a big challenge in the successful treatment of human liver cancer. Potent treatments for liver cancer contain hepatic transplantation, surgical resection, and target-guided tumor ablation[2]. Although there are several treatments and the availability options for liver tumors, recurrence after treatment remains common[3]. As many solid tumors have been reported, systemic chemotherapy has marginal benefits for the therapy of liver cancer[4].

The hydrazone is a component of a lot of biologically active compounds stand for a category of Schiff base with significant pharmacological effects, including anti-bacterial, antiviral, anti-HIV, especially anticancer[5]. For instance, Mohareb et al[6] claimed that Pregnenolone structure of cyanide acetyl hydrazone derivatives (Figure 1A) was authenticated as a new and patent small molecule scaffolds for potential anticancer agents. On the other hand, the quinolone class containing fluorine is one of the most widely used antibacterial drugs. Quinolone selectively inhibits topoisomerase and DNA spiral enzyme. In particular, many applications of quinolone in the therapy of cancer have been developed. Among them, ciprofloxacin acyl hydrazone (Figure 1B), was found to exhibit extensive antitumor activities in liver cancer cells (SMMC-7221), leukemia cells (L1210) and human promyelocytic leukemia cells[7]. Furthermore, compounds containing furan group have been a

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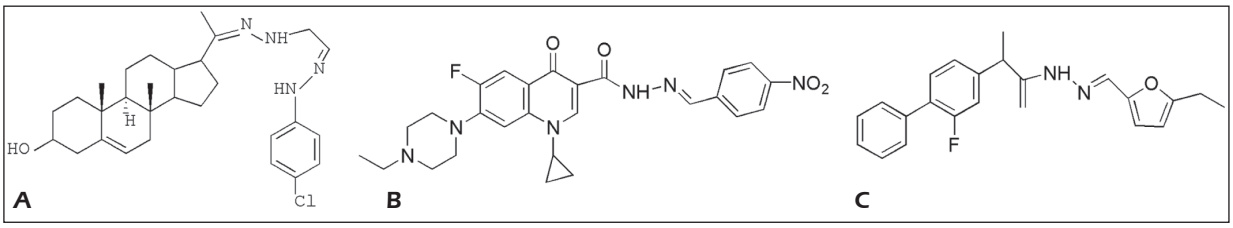

Figure 1. Chemical structures of the representative compounds as anticancer agents.

productive source of afflatus for medicinal chemists for many years due to their diverse inhibitory activities[8]. A recent research of flubiprofen ester hydrazide derivatives became much more attractive and promising for the devise of anticancer agents. Furan derivatives were reported to exhibit potential anticancer activity conjugated with a wide range of groups (Figure 1C)[9,10]. For instance, the compound flubiprofen ester hydrazide, inhibited the proliferation and metastasis of ovarian carcinoma and leukemia cells[11].

In the same field, recent results about the synthesis and anti-proliferative, anti-HIV and antibacterial properties of heterocyclic hydrazones have been reported[12]. In addition, Mansour et al[13] have identified hydrazone compounds containing sulfonic group presenting significance bioactivities as inhibitors of human breast cancer line (MCF-7)[14]. Hydrazide-hydrazones, due to their several biological and clinical applications, are one of the most popular intermediates in the synthesis of biological active derivatives[15]. Activities of hydrazones derivatives are considered to be associated with the positive pharmacophoric group. According to this knowledge, 15 compounds, which possess hydrazide-hydrazone, have been synthesized as main structure in this study starting from substituted hydrazine or acylhydrazine.

While continuing to our work on biological activities hydrazide-hydrazone derivatives, we indicated the synthesis of diverse hydrazone derivatives and their potential anticancer properties. What's more, compounds W_9 were evaluated for their growth inhibition and apoptotic activities against HepG-2 (liver hepatocellular carcinoma) tumor cells.

Materials and Methods

General Synthesis of Target Hydrazone Compounds

The general methods for synthesis of target hydrazone compounds and their derivatives were depicted in Figure 2[16]. For the sake of obtaining sulfonyl-hydrazone heterocycles in good yield (80%), nucleophilic addition-elimination reaction was applied, using hydrazide and aldehydes (ketones). The key of the synthesis was to improve the reaction temperature. The reaction rate depends on the nucleophilic reagent itself and the structure of the carbonyl compounds. Due to the carbonyl C atom connection with electronic group (alkyl or aryl), it increased the C atomic charge density, reduced nucleophilic reagent's ability to attack it and slowed down the reaction rate (Figure 2A). Through the recrystallization,

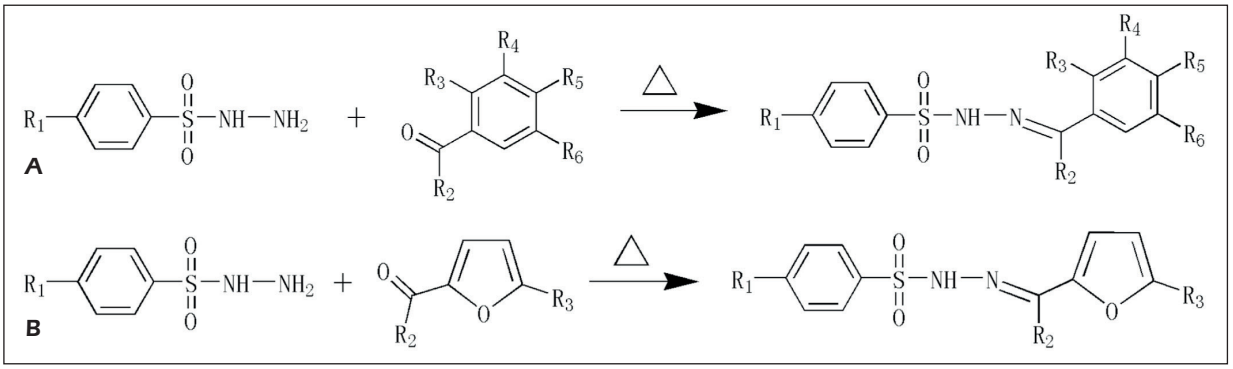

Figure 2. A, Synthesis of substituted aromatic sulfonyl hydrazone compounds. Reagents conditions and yields: ethanol or methanol, reflux 2 h-26 h, yield 20-90%. **B**, Synthesis of substituted aromatic heterocyclic sulfonyl hydrazine compounds. Reagents conditions and yields: ethanol or methanol, reflux 1 h-5 h, yield 30-90%.

Table I. The characteristics of the synthesized target compounds.

Compound	R_1	R_2	R_3	R_4	R_5	R_6	R_7	Formula	Yield (%)
W_1	H	H	H	OCH_3	OH	OCH_3	H	$C_{15}H_{16}N_2O_5S$	69.2
W_2	CH_3	CH_3	H	H	NO_2	H	H	$C_{15}H_{15}N_3O_4S$	62.9
W_3	CH_3	CH_3	NO_2	H	H	H	H	$C_{15}H_{15}N_3O_4S$	41.1
W_4	H	H	H	OCH_3	OCH_3	H	OCH_3	$C_{16}H_{18}N_2O_5S$	86.6
W_5	CH_3	CH_3	H	NO_2	H	H	H	$C_{15}H_{15}N_3O_4S$	43.6
W_6	H	CH_3	NO_2	H	H	H	H	$C_{14}H_{13}N_3O_4S$	26.4
W_7	CH_3	CH_3	H	H	NH_2	H	H	$C_{14}H_{15}N_3O_2S$	29.8

all synthesized molecules were purified. Purity was identified by TLC (Thin-Layer Chromatography) under UV (Ultra Violet) light exposure. Mass spectrometry (ESI-MS) verified molecular mass of the tested compounds. The ^{1}H-NMR spectra displayed three characteristic signals for the acylhydrazone, demonstrating the synthesis about the compounds. In the ^{1}H-NMR of W_1-W_{15}, NH proton signal shift ranged from 11.50 ppm in acylhydrazone to 10.08-12.25 ppm region. In addition, signals of NH2 protons were singlet, which shifted from 3.7 ppm to 4.7 ppm region. The benzene ring of NH2 moved to low field about 5.45 ppm. The spectra showed the characteristic signal for N=CH proton, it was a single peak at σ 8.0 ppm and signals of compounds W_2, W_3, W_5, W_6, W_7 and W_{12} disappeared (Figure 2B).

Evaluation of Drugs Interactions and Combination Index Analysis

HepG-2 cells were conducted with various concentrations of compounds at a constant combination proportion of the approximate IC_{50} (W_9/5-FU, W_9/cis-DDP) for 24 h, 48 h and 72 h to calculate a new IC_{50}. According to Chou's combination index (CI) model, all chemical solvents and reagents were commercially obtained from Energy Chemical without purification unless specific marked. The thin layer chromatography (TLC) was carried out on silicone gel GF254 plates with imagery through iodine vapors ultraviolet light. Melting points of the compounds were performed in open glass capillaries using a WSR-1B digital melting point device and were uncorrected. The ^{1}H-NMR spectra of DMSO-d_6 were recorded on Bruker AVANCE II 400 spectrometers and solutions tetramethylsilan (Me_4Si) were used as its internal standard. The analysis of mass spectra (ESI-MS) was recorded on an API 4000 spectrometer. The IR spectra were performed on a Nicolet iS5 FTIR spectrometer.

General Procedure for Synthesis of Compounds W_1-W_7

Sulfonyl hydrazide 0.945 g (5.5 mmol) or p-toluenesulfonyl hydrazide 1.13 g (6.1 mmol) was dissolved in ethanol (20 mL). Excessive secondary aromatic aldehyde (30 mL) was added, and twenty drips of glacial acetic acid were used as catalyst. The mixture was refluxed for 2-26 h and cooled down into ice water. The precipitate was produced, filtered from the solution, and washed with water. White to yellow solid was obtained after air dry. The crude compound was purified by recrystallization with ethanol or acetone to obtain the compounds W_1-W_7. In Table I, the characteristics of the synthesis target compounds were shown.

General Procedure for Preparation of Compounds W_8-W_{15}

Sulfonyl hydrazide 1.22 g (7.1 mmol) or p-Toluenesulfonyl hydrazide 1.94 g (10.4 mmol) was dissolved in ethanol (20 mL), excessive secondary furan aldehyde (30 mL) was added, and twenty drips of glacial acetic acid were used as catalyst. The mixture solution was refluxed for 2-5 h and poured into ice water. The producing precipitate was filtered, washed with water, and chemically dried to get a white to yellow solid. The crude compound was purified by recrystallization with ethanol or acetone to afford the compounds W_8-W_{15}. In Table II, the characteristics of the synthesis target compounds were shown.

In vitro Cytotoxicity and Antiproliferative Assay

Fifteen synthesis compounds were assessed for their *in vitro* anti-proliferative activities in HepG-2 (human hepatocellular carcinoma cell line) by cis-dichlorodiamine platinum (II) (cis-DDP), an effective and known anticancer agent used as the positive reference drug. The values of IC_{50}, an effective concentration in which 50%

Table II. The characteristics of the synthesized target compounds.

Compound	R_1	R_2	R_3	Formula	Yield (%)
W_8	CH_3	H	H	$C_{12}H_{12}N_2O_3S$	82.5
W_9	H	H	NO_2	$C_{11}H_9N_3O_5S$	69.9
W_{10}	CH_3	H	CH_3	$C_{13}H_{14}N_2O_3S$	31.6
W_{11}	H	H	CH_3	$C_{12}H_{12}N_2O_3S$	51.0
W_{12}	H	CH_3	H	$C_{12}H_{12}N_2O_3S$	70.4
W_{13}	H	H	CH_2OH	$C_{12}H_{12}N_2O_4S$	59.6
W_{14}	H	H	Br	$C_{11}H_9BrN_2O_3S$	91.0

of the tumor cells were inhibited[17-19], were calculated to evaluate the antiproliferative activities. The lower IC_{50} values manifested greater anticancer activity. Cell growth and proliferation were conducted by the MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide) experiment[20]. HepG-2 cells were cultured in Roswell Park Memorial Institute-1640 (RPMI-1640) medium (Gibco, Rockville, MD, USA) added with 15% fetal bovine serum (FBS) (Gibco, Rockville, MD, USA), then were planted into 96-well plates in 0.1 mL medium at a density of 5×10^3 cells/well. After 24 h, the cells were conducted with compounds (at concentration of 1,25,50,75,100 μmol/L) to produce a dose-dependent and time-dependent curves. After incubation for 24, 48, 72 h at 37°C, the drug containing medium was removed out and taken by 0.1 mL fresh complete medium. 0.5 mg/mL MTT was added to each well and cells were incubated for further 4 h at 37°C in 5% CO_2. The medium was removed and 150 μL dimethylsulfoxide (DMSO) (Sigma-Aldrich, St. Louis, MO, USA) were added to solubilize producing formazan. The optical density (OD) was performed at 595 nm using a FilterMax F5 MultiMode Microplate Reader (Multiskan spectrum, FilterMax F5 Molecular Devices, San Jose, CA, USA). The inhibition rate was calculated as (OD control-OD experiment)/OD control ×100%[15]. The value of the half maximal inhibitory concentration (IC_{50}) was then computed using Statistical Product and Service Solutions (SPSS) 17.0 software (SPSS Inc., Chicago, IL, USA).

Drug Combination of Compound W_9 cis-DDP and 5-FU

From Figure 3, it can be found that some of the synthesized compounds (W_2, W_3, W_5, W_9 and W_{11}) have certain inhibitory activity on HepG-2 cells than other compounds from the MTT experiments. We have provided the MTT results in the Tables III-V. Among them, the activity of the compound W_9 was the best, with the IC_{50} of 72.95 μmol/L, 69.56 μmol/L and 50.62 μmol/L for 24 h, 48 h and 72 h, respectively. Therefore, W_9 was needed to combine with other compounds (cis-DDP and 5-FU).

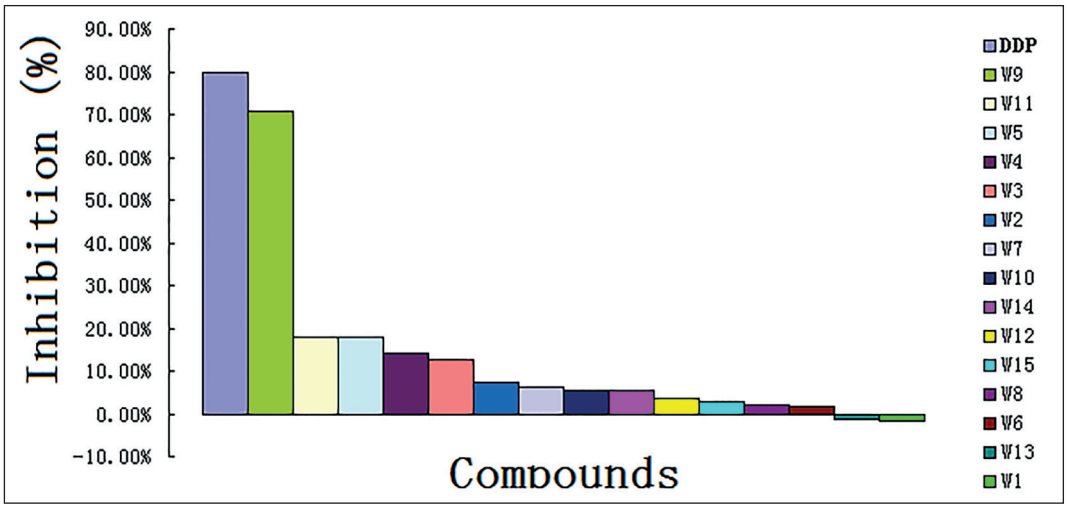

Figure 3. Cell proliferation inhibition rate of the compounds at the concentration of 100 μmol/L.

Table III. Inhibitory rate of compound on $HepG_2$ cells at different concentrations after 24 h.

Compounds	Concentrations (μmol/L)					IC_{50}
	1	25	50	75	100	
cis-DDP	1.34±1.79	70.68±0.75	80.24±0.65	82.46±0.50	82.83±0.92	16.23
W_2	0.46±1.87	2.75±2.60	3.39±2.50	4.48±1.77	5.63±1.41	>500
W_3	0.29±1.07	2.18±0.74	3.91±0.98	6.06±1.93	8.01±2.13	>500
W_5	0.13±1.94	4.86±2.28	5.88±1.95	6.91±4.04	9.91±0.58	>500
W_9	1.31±2.62	11.19±0.90	26.86±1.55	63.15±0.81	67.44±2.66	68.50
W_{11}	2.86±1.95	3.13±2.20	4.40±2.30	6.34±2.76	12.71±1.37	>500

Table IV. Inhibitory rate of compound on $HepG_2$ cells at different concentrations after 48 h.

Compounds	Concentrations (μmol/L)					IC_{50}
	1	25	50	75	100	
cis-DDP	10.69±1.12	83.72±1.05	84.30±0.54	86.32±0.50	86.67±0.70	7.81
W_2	1.74±1.40	3.72±1.78	5.07±1.50	5.56±2.19	7.71±1.60	>500
W_3	2.96±3.26	6.29±0.98	8.53±2.39	12.15±1.26	14.02±1.99	>500
W_5	0.56±1.39	5.22±1.02	13.57±2.13	15.99±2.58	17.86±2.09	>500
W_9	3.63±2.37	13.12±1.23	28.57±0.68	65.21±3.55	72.62±1.96	63.91
W_{11}	4.85±0.71	7.19±2.72	8.13±3.13	10.80±4.36	14.40±4.34	>500

Table V. Inhibitory rate of compound on HepG2 cells at different concentrations after 72 h.

Compounds	Concentrations (μmol/L)					IC_{50}
	1	25	50	75	100	
cis-DDP	29.08±2.43	85.49±3.69	88.20±2.57	89.52±3.36	90.18±2.44	3.91
W_2	2.95±2.54	4.26±1.22	5.65±1.58	6.50±1.84	11.38±2.21	>500
W_3	1.81±3.58	8.04±1.22	14.26±0.75	21.45±1.44	26.04±4.17	460.32
W_5	7.67±1.66	10.16±3.44	14.36±2.34	21.79±3.07	28.56±1.33	>500
W_9	11.39±0.77	26.88±1.19	47.80±0.64	80.85±1.50	82.19±1.72	31.90
W_{11}	9.072±1.83	11.57±3.02	13.51±4.50	28.13±3.70	31.00±4.71	>500

HepG-2 cells were conducted with disparate concentrations of compounds at an unaltered combination rate of the approximate IC_{50} (W_9/cis-DDP or W_9/5-FU) for 24, 48 and 72 h to calculate a new IC_{50}[21]. Cells were prepared into suspension and planted in 96-well plants yielding the densities of 5×10^3 cells/well. After treatment with compounds for 24 h at 37°C, cells were treated with 2 μmol/L cis- DDP and W_9 (at different concentrations of 0, 25, 50, 75 and 100 μmol/L) or 15 μmol/L 5-FU and W_9 (at different concentrations of 0, 25, 50, 75 and 100 μmol/L) or vehicle alone (control). Compounds were deliquescent in DMSO and prepared into different concentrations previously. Next, cells were treated and further incubated for 24, 48 and 72 h, 20 μL. MTT (5 mg/mL) were added to each well and incubated for further 4 h at 37°C. Using a FilterMax F5 MultiMode Microplate Reader, the optical density (OD) was measured at 595 nm. All the experiments were performed repeatedly at least three times for the independent tests.

Cell Cycle Arrest

The flow cytometry about the cell cycle experiment was conducted as depicted previously[22]. To the cell cycle analysis, HepG-2 cells (2×10^5 cells/well) were planted into 6-well plates, and then incubated for another 24 h. Cells were then treated with disparate concentrations (0, 25 and 75 μmol/L) of compound W_9 adding 2 μmol/L cis-DDP at 37°C for 48 h. After 48 h treatment, cells were harvested, then fixed with 75% ethanol for 24 h. The cells were centrifuged, washed and treated with RNase A, sequentially, and stained with PI[23]. At last, the total number of 1×10^4 cells

per sample was detected for cell cycle arresting on a BD Accuri C6 Cell Cytometer (BD Biosciences, Franklin Lakes, NJ, USA). All experimental data were quantitatively analyzed by Cell Quest software (BD Biosciences, Franklin Lakes, NJ, USA).

Cell Apoptosis Analysis

HepG-2 cells were planted into 6-well plates and treated with disparate concentrations (0, 25 and 75 μmol/L) of W_9 for 24 h. After that, collected cells and 5 μL Annexin V-FITC buffer and 5 μL Propidium Iodide (PI) double staining were used to detect apoptotic cells in a flow cytometer (FACScalibur BD Accuri™ C6) to analyze apoptosis on the basis of the manufacturer's instructions[24].

Statistical Analysis

The experimental values are presented as Mean±SD, and statistical analysis were conducted by Statistical Product and Service Solutions (SPSS) 17.0 (SPSS Inc., Chicago, IL, USA) through one-way ANOVA detected by SNK-LSD test as the post-hoc test. $p<0.05$ was considered statistically significant.

Results

Cytotoxicity Assay results

Figure 4 showed MTT results of HepG-2 cells incubated with disparate concentrations and disparate times (24, 48 and 72 h) of compounds W_2, W_3, W_5, W_9, W_{11} and cis-DDP, respectively. Values of proliferation inhibition ratio (%) were calculated as (OD control-OD experiment)/OD control ×100% (Figure 4). It can be found from the experiment that the synthesized compounds have certain inhibitory activity on HepG-2 cells. Among them, the activity of the compound W_9 was the best, with the IC_{50} of 72.95 μmol/L, 69.56 μmol/L and 50.62 μmol/L of 24 h, 48 h and 72 h, respectively. Results showed that the inhibitory activity of all the compounds measured on HepG-2 cells increased with the increasing of concentration. Besides, the inhibitory activity curve became slower when the concentration increased to a certain value. Figure 4D showed that the inhibitory activity of compound W_9 on HepG-2 cells increases with the increase of administration time. The longer the dosage time was, the more significant the inhibition was.

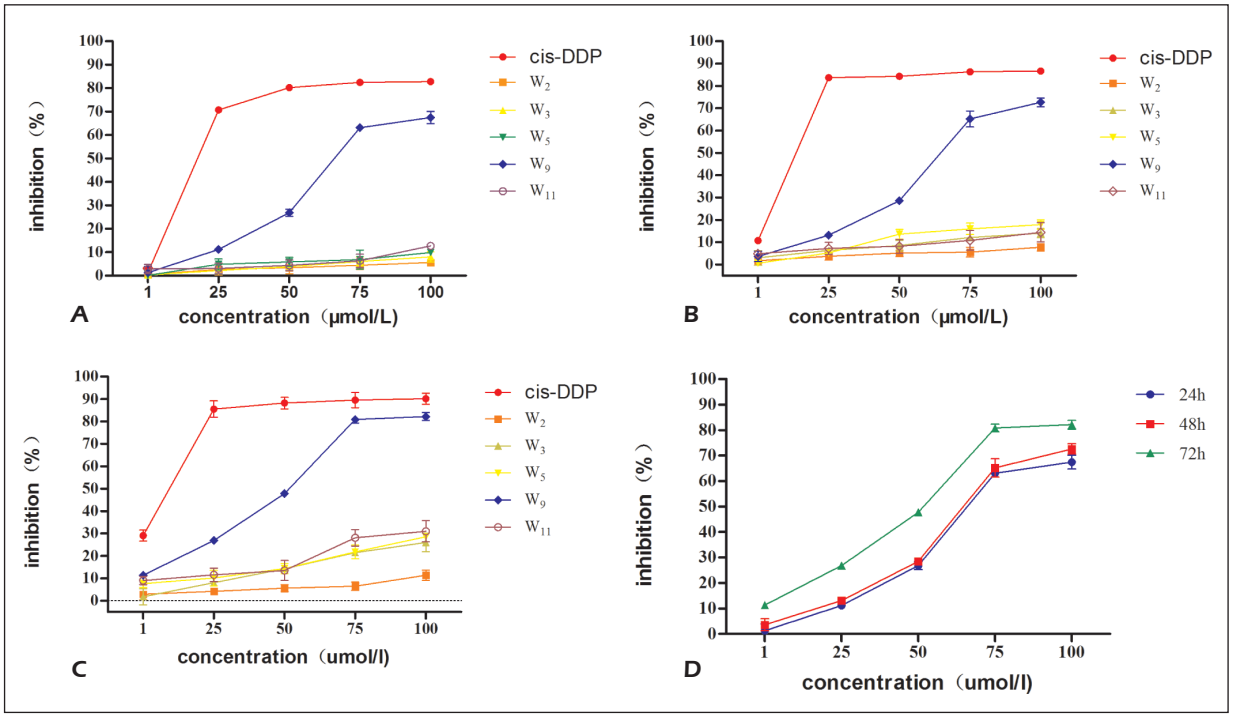

Figure 4. Represents the MTT assay results of HepG-2 cells incubated with disparate concentrations and different times of compounds W_2, W_3, W_5, W_9, W_{11} and cis-DDP. Respectively, values of growth inhibition ratio (%) represented the mean ±standard deviation (SD) of four wells. Three independent experiments were performed. Significant differences were indicated as $p<0.05$ compared with vehicle control group.

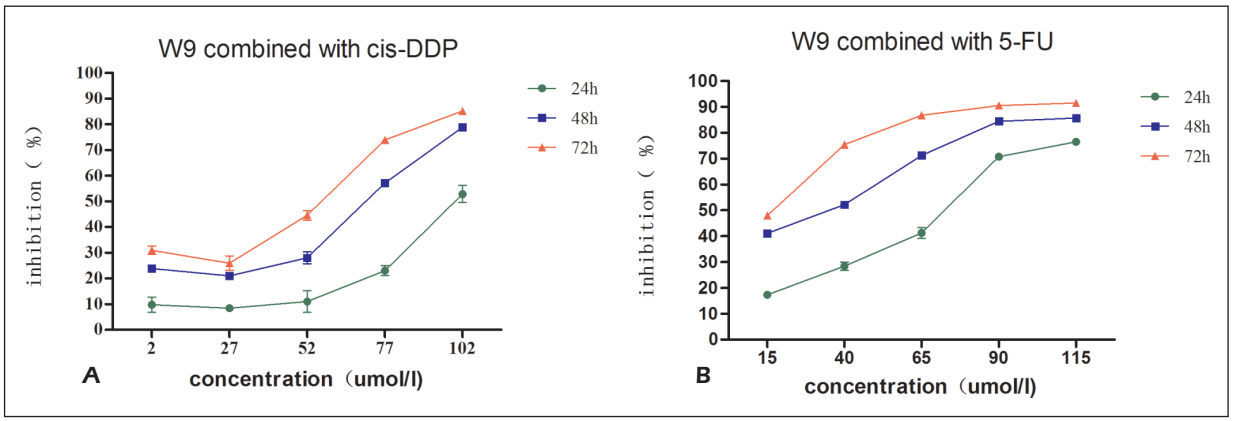

Figure 5. Represents the inhibition curve of HepG-2 cells at 24 h, 48 h, 72 h of W9 combined with cis-DDP (at different concentrations of 2,27,52,77,102 μmol/L), or 5-FU (at different concentration of 15, 40, 65, 90, 115 μmol/L). Significant differences were indicated as $p<0.05$ compared with vehicle control group.

Drugs Interaction Results

The evaluation of two drugs interaction index (IR) indicated the sharp reduction in the dosage of either combination treatment of compounds compared with the dosage of simplex compound at some extent effective level. After 24 h, the IC_{50} of W_9, which was combined with cis-DDP, was 102.85 μmol/L, that was regarded as 2 μmol/L cis-DDP+100.85 μmol/L W_9. After 48 h, the IC50 of W_9, which was combined with cis-DDP, was 69.52 μmol/L, that was regarded as 2 μmol/L cis-DDP+67.52 μmol/L W_9. After 72 h, the IC50 of W_9, which was combined with cis-DDP, was 64.66 μmol/L, that was regarded as 2 μmol/L cis-DDP+62.66 μmol/L W_9. From the experiment, we could conclude that the IR values of 2 μmol/L cis-DDP in HepG-2 cells were 9.82% for 24 h, 23.84% for 48 h, and 30.96% for 72 h, respectively. The IC_{50} values in HepG-2 cells were 1.59 for 24 h, 1.31 for 48 h, and 2.47 for 72 h, respectively (Figure 5). After 24 h, the IC_{50} of W_9, which was combined with 5-FU, was 60.49 μmol/L, that was regarded as 15 μmol/L 5-FU+45.94 μmol/L W_9. After 48 h, the IC_{50} of W_9, which was combined with 5-FU, was 25.45 μmol/L, that was regarded as 15 μmol/L 5-FU +20.45 μmol/L W_9. After 72 h, the IC_{50} of W_9, which was combined with 5-FU, was 16.01 μmol/L, that was regarded as 15 μmol/L cis-DDP+1.01 μmol/L W_9. From the experiment, we could conclude that the IR values of 15 μmol/L 5-FU in HepG-2 cells were 17.37% for 24 h, 41.15% for 48 h, and 47.94% for 72 h, respectively. The IC_{50} values in HepG-2 cells were 0.841 for 24 h, 0.964 for 48 h, and 0.794 for 72 h, respectively (Figure 5).

Influence of W_9 on the HepG-2 Cells Cycle and the Combination of cis-DDP and W_9 on the HepG-2 Cells Cycle

Arresting cell cycle was a significant marker for inhibiting cell proliferation and series of events that occurred in cells resulting in its replication and division[20]. As the inhibitory effect of W_9 and the combination of cis-DDP and W_9 cell multiplication were observed, we evaluated the influence on the cell cycle arresting and distribution analysis of HepG-2 cells through flow cytometry (Figure 6). From the cell cycle experiments, HepG-2 cells were treated with W_9 at 0, 25 μmol/L, 75 μmol/L, respectively. It can be found that the cell number arrested in G2/M phase was increased in a dose-dependent manner, which led to a significant augmentation in the cell percentage in G2/M phase from 18.67% to 19.66%, 37.30% of vehicle group. Moreover, cis-DDP 2 μmol/L+0 μmol/L W_9, cis-DDP 2 μmol/L+25 μmol/L W_9, cis-DDP 2 μmol/L+75 μmol/L W_9 combination led to cell percentage in G0/G1 phase increased from 14.40% to 15.57% and 22.77% ($p<0,01$). Cell percentage in G2/M phase was decreased from 63.30% to 61.70% and 44.97% ($p<0.01$), respectively. Compound W_9 could affect HepG-2 cell cycle progression in a dosage reliability manner at low micromolar concentrations. The combination of cis-DDP and W_9 had a more powerful inhibiting influence on cell growth than compound W_9 drug alone. However, their combination provokes the G2/M arresting ability, which had been weakened with the increasing of the concentration of W_9.

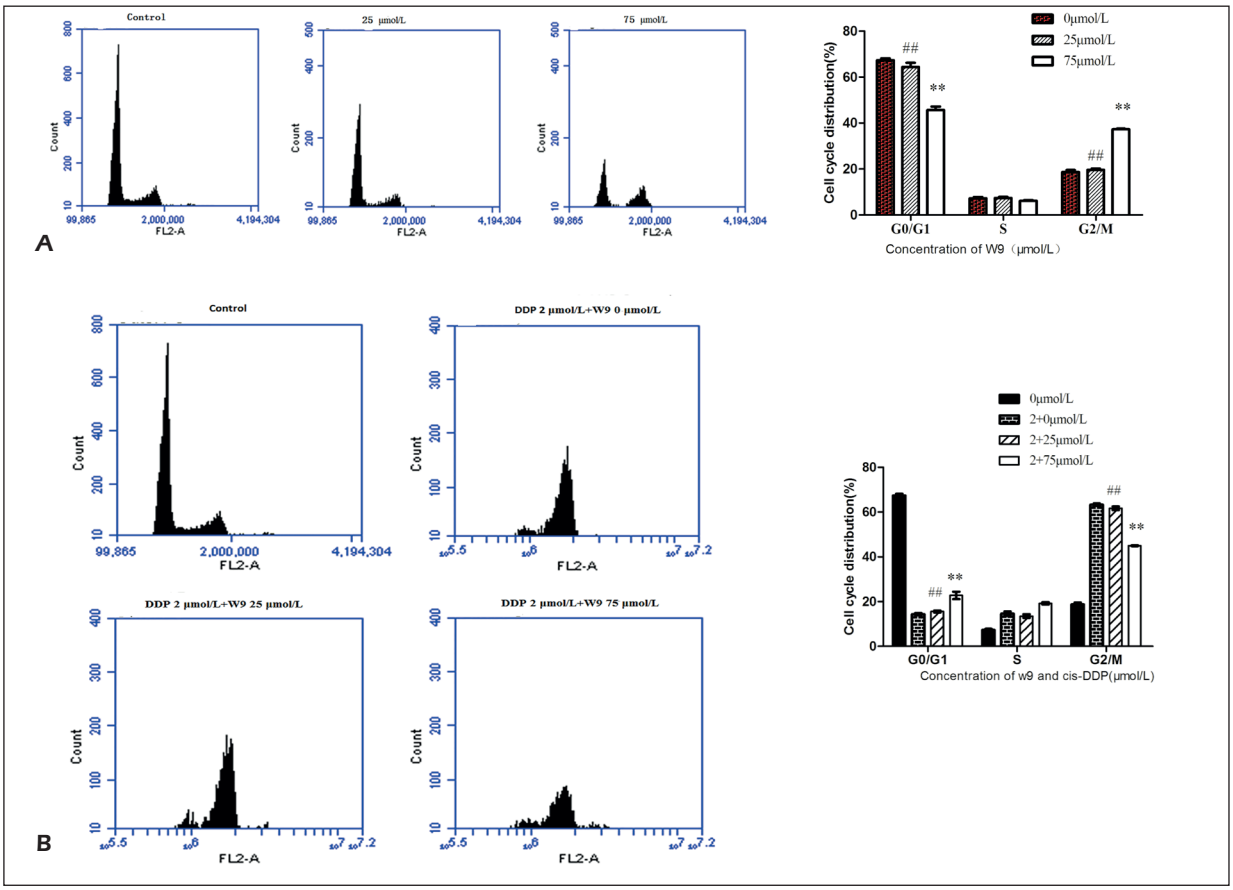

Figure 6. Effects compound W9 and W9 combined with DDP on cell cycle. A, Proportion of DNA content in the cell cycle at different concentrations of the compound W9. B, Proportion of DNA content in the cell cycle at DDP combined with different concentrations of the W9. Cell cycle distribution of HepG-2 cells. Compared with relevant control group, $^{**}p<0.01$, $^{\#\#}p<0.05$,(n=3).

Effects of Compound w_9 on Cell Apoptosis

To confirm whether the arresting of cell proliferation by compound W_9 was related to the induction of cell apoptosis, apoptosis analysis was performed after double staining cells with Propidium Iodide (PI) and Annexin V-FITC. As indicated in Figure 7, compound W_9 was remarkably induction of cell apoptosis in a dosage reliability manner in HepG-2 cells. Compound W_9 induced significant apoptosis at 25 and 75 μmol/L concentration after 24 h treatment (5.30±0.379% for control, 23.10±0.100% for 25 μmol/L, and 37.37±0.491% for 75 μmol/L). This result collectively indicated that the target compound W_9 might suppress the growth of HepG-2 cells through arresting cell cycle and apoptosis induction.

Discussion

Two series of aromatic heterocyclic sulfonyl-hydrazone compounds, W_1-W_7 and W_8-W_{15}, were synthesized by the reaction of nucleophilic addition elimination and the condensation of the target compounds. The target drugs were filtrated for their anti-proliferative activities in HepG-2 cell lines. Compound W_9 showed high activity against the cancer cells, which were considered as promising lead compounds for future optimization. The combinations of W_9 and cis-DDP or W_9 and 5-FU had a more powerful inhibition influence on cell growth than individual administration. Furthermore, the DRI values of the two compounds in the combination model were greater than either drug alone[5]. 5-nitro-2-furancarboxaldehyde salicylyl benzoyl hydrazone exhibited anti-cancer activity in a time-dependent manner and dosage reliability manner.

HepG-2 cells treated with W_9 induced a stronger augmentation in the proportion of cells in G2/M phase along with a rapid reducing the number of HepG-2 cells in G0/G1 phase. Co-administration of two drugs of W_9 and cis-DDP resulted in a remarkably higher percentage of cells in

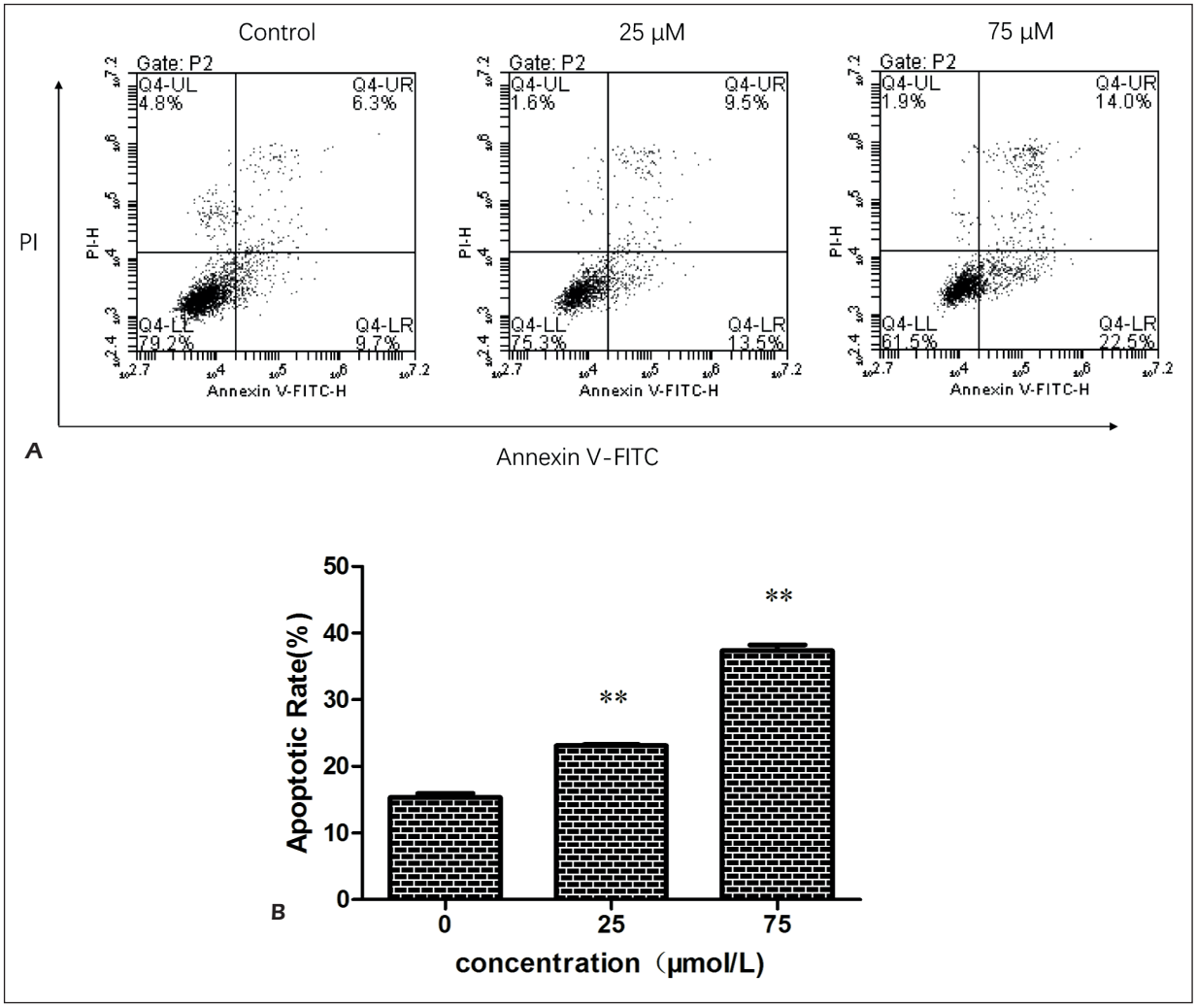

Figure 7. A, Effects of different concentrations of compound W9 on cell apoptosis. B, Percentage of apoptotic cells at different concentrations of the compound W9. Compared with relevant control group, $^{**}p<0.01$, (n=3).

G2/M phase compared with compound W_9d rug alone to some extent. However, with the increase of W_9 concentration, it caused a reduction in the percentage of cells in G2/M phase, some way to justifying the combination of W_9 and cisplatin presented antagonism phenomenon. It may be explained that the metal platinum forms a chelate with the acylhydrazone compound. Owning to 5-FU blocking uracil into thymidine nucleoside deoxyuridine interfere with DNA synthesis, it remarkably arrested the proportion of cells in S stage compared with the vehicle control. However, W_9 arrested cell cycle at G2/M phase, which may be explained for the synergistic effect of W_9 combined with 5-FU. Combination of the two drugs led to a remarkably inhibit cell growth compared with individual administration of each drug alone. According to the above, compound W_9, bearing furan hydrazone functionality, induced HepG-2 cells apoptosis.

Conclusions

We suggest that the hydrazones bearing furan structure may be considered for further research and scaffold optimization in designing anticancer drugs.

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Conflict of interest

The authors declared no conflict of interest.

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