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Original article

# Synthesis and anticancer activity of some novel 2-phenazinamine derivatives

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ABSTRACT

In this study, we report the synthesis and spectral characterization of a novel series of 2-phenazinamine derivatives. *In vitro* evaluation for their anticancer activity toward cultured K562 (human chronic myelogenous leukemia), HepG2 (human hepatocellular carcinoma), MGC803 (human gastric carcinoma), HCT116 (human colorectal carcinoma), MCF7 (human breast adenocarcinoma) cell lines, as well as 293T (epithelial cells from human embryo kidney) non-cancer cell was carried out. The compounds **4**, **7**, **16** and **19** showed good positive anticancer activity *in vitro*. In particular, compound **4**, 2-chloro-*N*-(phenazin-2-yl)benzamide, possessed a potent anticancer effect comparable to cisplatin against both K562 and HepG2 cancer cells but was very low or had no effect against 293T non-cancer cell. Preliminary anticancer mechanism of **4** was investigated by cell apoptosis assays compared with cisplatin using flow cytometry.
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## 1. Introduction

Cancer is now considered as one of the most serious health problems all over the world and also one of the leading causes of death [1–3]. Thus, in the past for several decades researchers have been struggling to find effective clinical approaches for the treatment of cancer and search for novel anticancer agents. Apart from the utility of surgical operations and irradiation in past years, chemotherapy still remains an important option for the management of cancer in the clinical settings [4].

Natural and synthetic phenazines have attracted considerable attention because they have shown interesting biological activities [5], including broad-spectrum antibiotics [6], antimalarial [7–9], trypanocidal [10], antihepatitis C viral replication activities [11], antitumor activity in leukemia and solid tumor [12], anti-carcinomatous activity [13–15], neuroprotective properties [16–19], antifungus [20], dual inhibitors of topoisomerase I and II [21]. Phenazines have been associated with anticancer activities since 1959 and they are small molecules that can permeate the tissues and organs easily [22,23]. Molecules of phenazine class have been regarded as secondary metabolites from *Streptomyces*, *Pseudomonas*, and other marine microorganisms [5].

Recently, our research group has reported a novel phenazine derivative *N*-(2-hydroxyphenyl)-2-phenazinamine (Fig. 1), isolated from a marine actinomycete BM-17, which showed high cancer cell cytotoxicity against several cancer cells [24]. This finding prompted us to further explore it as new potential anticancer agents. Thus, we describe herein, the synthesis and anticancer activity of a novel series of 2-phenazinamine derivatives.

## 2. Results and discussion

### 2.1. Chemistry

In this work, the synthesis of a series of 2-phenazinamine derivatives (**1**–**21**) was carried out according to the steps showed in Scheme 1. In the initial step, $N^1$-phenylbenzene-1,2,4-triamine **A** was synthesized via the reduction reaction of the commercially available *N*-(2,4-dinitrophenyl)benzeneamine in the presence of a 5% Pd/C catalyst under hydrogen atmosphere. The yield was high and without further purification, the ring closure reaction of the amine (**A**) with magnesium sulfate in nitrobenzene afforded the corresponding phenazin-2-amine (**B**) [25].

The treatment of phenazin-2-amine (**B**) with acyl chlorides in the presence of anhydrous pyridine by stirring under 15 °C for 0.5 h using dry dichloromethane as solvent of reaction gave the target

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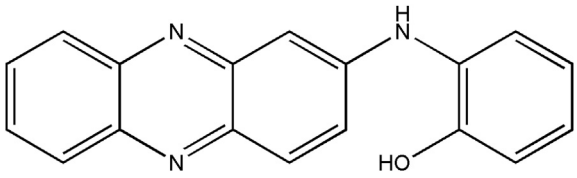


**Fig. 1.** Natural product.

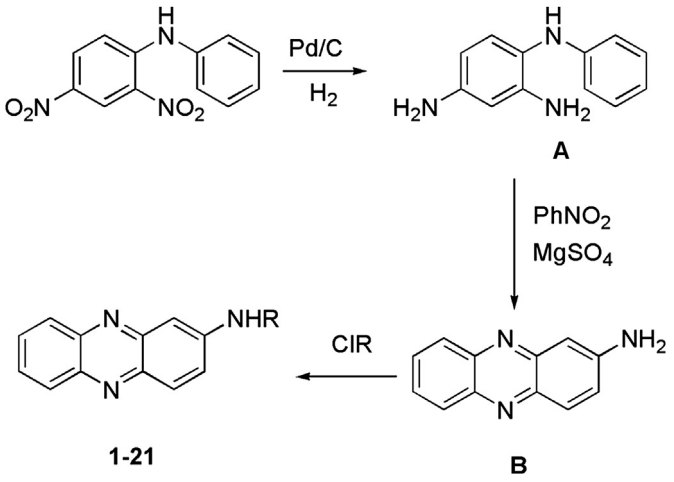
compounds (**1**–**19**). Similarly, phenazin-2-amine (**B**) and benzenesulfonyl chloride in the presence of anhydrous pyridine by refluxing for 1 h using dry dichloromethane as solvent of reaction afforded *N*-(2-phenazinyl)sulfon-amide (**20**) [26], while phenazin-2-amine (**B**) and dimethylcarbamyl chloride in the presence of triethylamine by refluxing for 2.5 h at 110 °C using *N*,*N*-dimethylformamide (DMF) as solvent of reaction gave 1,1-dimethyl-3-(phenazin-2-yl)urea (**21**).

However, it should be noted that many attempts having been made to synthesize compound **21** with a *N*,*N*-dimethyl substituent at C═O under various conditions failed, for example, phenazin-2-amine (**B**) and dimethylcarbamyl chloride in the presence of triethylamine by refluxing for 5 days at 77 °C using ethyl acetate as solvent of reaction afforded the same final product (**16**) as phenazin-2-amine (**B**) and ethyl carbonochloridate under the same procedure above, instead of target compound **21**. This showed that the solvent ethyl acetate participated in the reaction while the reactant dimethylcarbamyl chloride could work as a promoter [27,28].

The whole procedure was rather tedious, and the total yield was modest and significant amounts of byproducts under the harsh reaction conditions. To date, the synthesis yield of phenazines has been low since one of the first methods for preparation of phenazines was reported in 1901 [5,26].

The purity of compounds was checked by single-spot TLC using petroleum ether/ethyl acetate (1:1) solvent systems spots located under UV light. The final products were purified by flash chromatography column (petroleum ether/ethyl acetate gradient, 1:1–1:0) and recrystallized with ethyl acetate/petroleum ether or ethyl acetate, methanol, trichloromethane/petroleum ether, dichloromethane/petroleum ether, acetone/petroleum ether.

The structures of the newly synthesized compounds were appropriately characterized by spectral data. The $^1$H NMR spectrum of compound **B** showed that the presence of the two active proton and a broad singlet at $\delta$ 6.47 corresponded to an $NH_2$ group, one




**Scheme 1.** The synthesis of the compounds (**1**–**21**).

singlet at $\delta$ 6.91 indicated the $NH_2$ group at position 2 of phenazine core, four doublets and three triplets of the aromatic protons at $\delta$ 7.44–8.06, more downfield than normal aromatic protons due to the effect of two nitrogen heteroatoms, further confirmed the structure of phenazin-2-amine (**B**). Moreover, the $^{13}$C NMR data clearly showed the presence of the five aromatic quarter carbon atoms and seven aromatic carbon atoms containing hydrogen atoms at the appropriate chemical shift values. IR spectrum of compound **B** showed the presence of N–H symmetrical stretching vibration and asymmetrical stretching vibration of the amino group at 3194 and 3309 cm$^{-1}$, respectively, and N–H bending vibration at 1640 cm$^{-1}$.

Owing to high conjugation of phenazine core, its protons displayed a very low activity and difficult substitution [5], and the active protons of amino group were also poorly active and the nucleophilicity of phenazin-2-amine (**B**) was very weak. The nucleophilicity of the amines are key in Ullmann-type C–N bond formation coupling reaction [28,29]. Thus, many attempts of the reactions of phenazin-2-amine (**B**) with some chemical reagents under various conditions failed, for example, with other many aldehydes targeting Schiff bases were even not successful. In this paper, we selected strong electrophilic acyl chlorides as chemical reagents in mild conditions to successfully synthesize target compounds **1**–**21**.

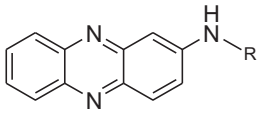
The structural assignments of a series of 2-phenazinamine derivatives (**1**–**21**) were based on a full characterization by IR, $^1$H NMR, $^{13}$C NMR, ESI-MS spectra, all target compounds were in accordance with the proposed structures, with spectral data reported in experimental section of this paper. Yield, melting point, molecular formula, molecular weight and recrystallization solvent of the newly synthesized compounds were provided in Table 1.

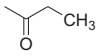
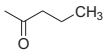
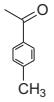
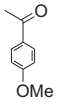
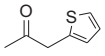
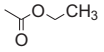
### 2.2. In vitro anticancer screening

Twenty-one of the newly synthesized compounds were subjected to *in vitro* cytotoxicity evaluation against K562 (human chronic myelogenous leukemia), HepG2 (human hepatocellular carcinoma), MGC803 (human gastric carcinoma), HCT116 (human colorectal carcinoma), MCF7 (human breast adenocarcinoma) cell lines, as well as 293T (epithelial cells from human embryo kidney) non-cancer cell by MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) reduction assay, respectively, using cisplatin as the reference drug [30,31]. Since the tested compounds showed poor aqueous solubility, they were all dissolved in DMSO and then diluted in culture medium so that the effective DMSO concentration did not exceed 0.2%. The percent inhibition of viability for each concentration of the compounds was calculated with respect to the control and $IC_{50}$ values were estimated with the software SPSS version 16.0 for Windows. Each experiment was repeated three times and the results were summarized in Table 2. Blank wells of all agents, containing the same concentrations of test compounds, but without MTT, had very low absorbance (data not shown). Therefore, interference of the color of compounds in the assay seemed unlikely. The response parameter calculated was $IC_{50}$ value (Table 2), which corresponded to the compound concentration causing 50% mortality in net cells.

Compound **4** showed 1.7-fold more potent against the human chronic myelogenous leukemia K562 cell line and 1.1-fold more potent against the human hepatocellular carcinoma HepG2 cell line than cisplatin in terms of $IC_{50}$ values. However it illustrated less potent against the human colorectal carcinoma HCT116 cell line than the positive controls in terms of $IC_{50}$ values and poor or no activity against other cell lines, while it was very low or no effect on the epithelial cells from human embryo kidney 293T non-cancer cell (Fig. 2 and Table 2).

**Table 1**
Some properties of the compounds (**1**–**21**).



| Compd. no. | R | Yield (%) | Mp (°C) | Mol. formula | Mol. weight | Recrystallization solvent |
|---|---|---|---|---|---|---|
| **1** |  | 32 | 200–202 | $C_{15}H_{13}N_3O$ | 251 | Ethyl acetate/petroleum ether |
| **2** |  | 25 | 184–186 | $C_{16}H_{15}N_3O$ | 265 | Ethyl acetate/petroleum ether |
| **3** |  | 34 | 183–186 | $C_{20}H_{15}N_3O$ | 313 | Ethyl acetate/petroleum ether |
| **4** |  | 28 | 187–189 | $C_{19}H_{12}ClN_3O$ | 333 | Ethyl acetate/petroleum ether |
| **5** |  | 34 | 227–229 | $C_{19}H_{12}ClN_3O$ | 333 | Ethyl acetate |
| **6** |  | 81 | 211–213 | $C_{20}H_{15}N_3O$ | 313 | Ethyl acetate/petroleum ether |
| **7** |  | 35 | 113–116 | $C_{20}H_{15}N_3O$ | 313 | Methanol |
| **8** |  | 11 | 105–108 | $C_{20}H_{15}N_3O$ | 313 | Trichloromethane/petroleum ether |
| **9** |  | 15 | 113–116 | $C_{20}H_{15}N_3O_2$ | 329 | Ethyl acetate/petroleum ether |
| **10** |  | 18 | 206–209 | $C_{19}H_{19}N_3O$ | 305 | Ethyl acetate/petroleum ether |
| **11** |  | 42 | 183–186 | $C_{20}H_{21}N_3O$ | 319 | Ethyl acetate/petroleum ether |
| **12** |  | 69 | 230–233 | $C_{23}H_{15}N_3O$ | 349 | Ethyl acetate/petroleum ether |
| **13** |  | 17 | 178–180 | $C_{17}H_{11}N_3O$ | 289 | Acetone/petroleum ether |
| **14** |  | 43 | 170–173 | $C_{17}H_{11}N_3OS$ | 305 | Dichloromethane/petroleum ether |
| **15** |  | 44 | 130–133 | $C_{18}H_{13}N_3OS$ | 319 | Ethyl acetate/petroleum ether |
| **16** |  | 42 | 199–200 | $C_{15}H_{13}N_2O_2$ | 267 | Ethyl acetate |
| **17** |  | 69 | 190–192 | $C_{15}H_{12}ClN_3O$ | 285 | Acetone |
| **18** |  | 15 | 188–190 | $C_{19}H_{13}N_3O$ | 299 | Ethyl acetate/petroleum ether |
| **19** |  | 23 | 234–236 | $C_{14}H_{11}N_3O$ | 237 | Ethyl acetate/petroleum ether |

(*continued on next page*)

**Table 1** (*continued*)

| Compd. no. | R | Yield (%) | Mp (°C) | Mol. formula | Mol. weight | Recrystallization solvent |
|---|---|---|---|---|---|---|
| **20** |  | 23 | 229–231 | $C_{18}H_{13}N_3O_2S$ | 335 | Ethyl acetate/petroleum ether |
| **21** |  | 13 | 113–115 | $C_{15}H_{14}N_3O$ | 266 | Ethyl acetate/petroleum ether |

Compound **7** exhibited the highest growth inhibitory activity against the human breast adenocarcinoma MCF7 cell line with $IC_{50}$ values of 11.63 μM, which was 2.4-fold more potent than positive control cisplatin with $IC_{50}$ values of 28.03 μM. However, it was less potent against the human gastric carcinoma MGC803 cell line and no activity against other cell lines. Compound **13** was close to cisplatin and compounds **12**, **17**, **21** showed significant activity against the MCF7 cell line. Although compound **8** was less potent against MGC803 cell line, it was 1.1-fold more potent against HepG2 cell line than cisplatin. Meantime, it had effect on the 293T non-cancer cell.

Compounds **16** and **19** indicated 1.2-fold more potent against the human hepatocellular carcinoma HepG2 cell line and 1.1-fold more potent against the human chronic myelogenous leukemia K562 cell line, respectively, while they both were less potent against the human breast adenocarcinoma MCF7 cell line than positive control cisplatin in terms of $IC_{50}$ values. All of the synthesized compounds were less sensitive and less potent against the human colorectal carcinoma HCT116 cell line than cisplatin excepted for compound **1**, which was close to positive control cisplatin in terms of $IC_{50}$ values.

In order to assess the effect of the newly synthesized compounds on non-cancer cells, the epithelial cells from human embryo kidney 293T non-cancer cell (Table 2) were used. Results showed that compounds **8** and **2** had effect on non-cancer cells, the rest of the compounds generally showed low or no effect. It should be noted that the standard reference anticancer drug cisplatin displayed magnificent effect on the normal cells, a phenomenon that has been reported in other literature [32–34].

As it could be seen in Tables 1 and 2, although the newly synthesized 2-phenazinamine derivatives, including aliphatic **1**, **2**, **17**, **19**, aromatic **3**–**9**, **12**, **18**, aliphatic cyclic **10**, **11**, heterocyclic **13**–**15**, ester **16**, sulfuryl **20** and ureal **21**, showed chemical structural diversity with this phenazine-containing structures, it was extremely difficult to derive a uniform structure–activity relationship due to the derivatization of the phenazine core with diverse pendant functionality [35].

**Table 2**
*In vitro* cytotoxicity ($IC_{50}$, μM)[a] of the compounds against human tumor cell lines.

| Compd. no. | $IC_{50}$ (μM) | | | | | |
|---|---|---|---|---|---|---|
| | K562[b] | HepG2[c] | MGC803[d] | HCT116[e] | MCF7[f] | 293T[g] |
| **1** | 57.67 | >160 | 63.93 | 18.94 | 40.73 | >160 |
| **2** | >160 | >160 | 43.47 | 62.33 | 68.82 | 109.63 |
| **3** | >160 | >160 | >160 | >160 | >160 | >160 |
| **4** | 33.43 | 16.46 | >160 | 91.93 | >160 | >160 |
| **5** | >160 | >160 | >160 | >160 | >160 | >160 |
| **6** | >160 | >160 | >160 | >160 | >160 | >160 |
| **7** | >160 | >160 | 27.22 | >160 | 11.63 | >160 |
| **8** | >160 | 16.74 | 89.01 | >160 | >160 | 56.16 |
| **9** | >160 | >160 | 50.06 | >160 | >160 | >160 |
| **10** | 78.85 | >160 | 67.09 | >160 | 136.71 | >160 |
| **11** | 157.32 | >160 | 122.33 | >160 | >160 | >160 |
| **12** | 61.22 | 44.22 | 87.86 | 130.6 | 30.09 | >160 |
| **13** | n.d.[h] | 52.25 | n.d. | 86.56 | 28.82 | >160 |
| **14** | >160 | >160 | >160 | 68.14 | >160 | >160 |
| **15** | n.d. | >160 | n.d. | 95.57 | >160 | >160 |
| **16** | >160 | 15.21 | >160 | >160 | 46.09 | >160 |
| **17** | 74.46 | >160 | 59.46 | 141.47 | 37.61 | >160 |
| **18** | >160 | >160 | >160 | 28.21 | >160 | >160 |
| **19** | 49.20 | >160 | 93.07 | >160 | 82.62 | >160 |
| **20** | >160 | >160 | >160 | >160 | >160 | >160 |
| **21** | n.d. | >160 | n.d. | 143.88 | 35.07 | >160 |
| Cisplatin | 56.04 | 18.44 | 9.51 | 16.26 | 28.03 | 13.77 |

[a] $IC_{50}$ is the drug concentration effective in inhibiting 50% of the cell growth measured by the MTT assay after 48 h drug exposure.
[b] Human chronic myelogenous leukemia cell line.
[c] Human hepatocellular carcinoma cell line.
[d] Human gastric carcinoma cell line.
[e] Human colorectal carcinoma cell line.
[f] Human breast adenocarcinoma cell line.
[g] Epithelial cells from human embryo kidney.
[h] n.d. = not determined.

### 2.3. Apoptosis assay for compound *4* by flow cytometry

In order to investigate whether the compounds exhibited its anti-proliferative effect on HepG2 cancer cell through induction of apoptosis, studies of flow cytometry were undertaken on compound **4** and cisplatin [36,37]. These compounds were incubated for 24 h at a concentration of 50 mM and the results were shown in Figs. 3 and 4. Four areas in the diagrams stand for necrotic cells (Q1, low Annexin V-FITC and high PI signal, left square on the top), late apoptosis or necrosis cells (Q2, high Annexin V-FITC and high PI signal, right square on the top), live cells (Q3, low Annexin V-FITC and low PI signal, left square at the bottom), apoptosis cells (Q4, high Annexin V-FITC and low PI signal, right square at the bottom), respectively. As it could be seen in Figs. 3 and 4, compound **4** showed a high population of apoptotic cells (56.6%) and 1.7-fold higher than cisplatin (33.5%) at the same concentration. The results demonstrated that the newly synthesized compounds could induce apoptosis of HepG2 cancer cells. But the proapoptotic property needs further investigations to better understand the precise mechanism of action of the compounds.

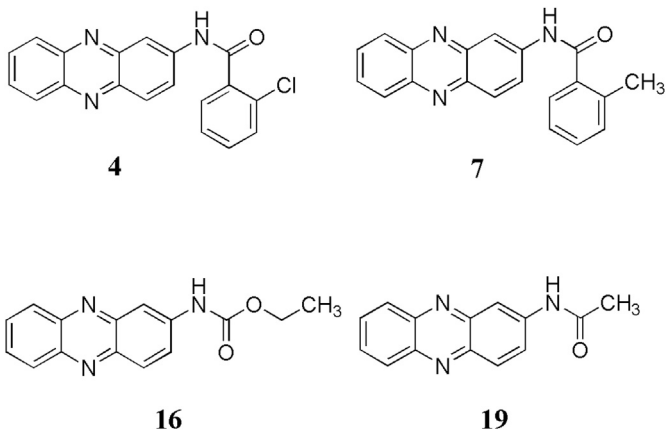



**Fig. 2.** Structures of highly active anticancer of newly synthesized compounds.

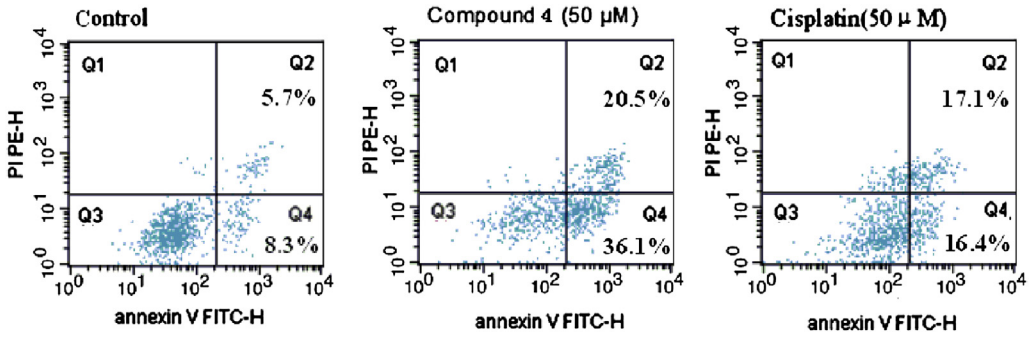



**Fig. 3.** Flow cytometric results after the exposure of HepG2 cell to the active compound **4** and cisplatin (50 μM). Four areas in the diagrams represent four different cell states: necrotic cells (Q1), late apoptotic or necrotic cells (Q2), living cells (Q3) and apoptotic cells (Q4).

## 3. Conclusion

In summary, a novel series of 2-phenazinamine derivatives were synthesized and characterized. The biological activities of all compounds were examined against five cancer cell lines and one non-cancer cell line. The results of *in vitro* anticancer activity indicated that compounds **4**, **7**, **16** and **19** were more active than cisplatin against both the K562 cell line and the HepG2 cell line, the MCF7 cell line, the HepG2 cell line, and K562 cell line, respectively. It was noted that the highly active anticancer compounds were no effect on the epithelial cells from the 293T non-cancer cell while the positive control cisplatin was very effective on the normal cells. Moreover, flow cytometry analysis of compound **4** indicated that it inhibited tumor proliferation by inducing apoptosis mechanism similar to cisplatin effect. In conclusion, it is worth studying compound **4** further as new potential anticancer agent for the treatment of human cancers.

## 4. Experimental section

### 4.1. Materials and instruments

All commercially available chemicals and solvents were of analytical reagent grade and were used without further purification unless otherwise specified. Column chromatography was carried out on silica gel (100–300 mesh). TLC was conducted on silica gel 250 micron, GF254 plates with short-wavelength UV light for visualization. Melting points were measured on a SGWX-4 hot stage apparatus. Infrared spectra were measured on KBr pellets on a Nicolet IR200 FT-IR spectrometer in the range of 4000–400 $cm^{-1}$. $^1H$ NMR and $^{13}C$ NMR spectroscopic measurements were performed on a Bruker AV-500 NMR spectrometer, using TSP and TMS as internal references at 298 K, respectively. Electro-spray ionization (ESI) mass spectra were recorded on a Finnigan MAT SSQ 710 mass spectrometer in a scan range of 100–1200 amu. K562 (human chronic myelogenous leukemia), HepG2 (human hepatocellular carcinoma), MGC803 (human gastric carcinoma), HCT116 (human colorectal carcinoma), MCF7 (human breast adenocarcinoma) cell lines, as well as 293T (epithelial cells from human embryo kidney) non-cancer cell were purchased from American type Cell Culture (ATCC, Shanghai, China) and maintained in Dulbecco's modified Eagle's medium (DMEM) with 10% fetal bovine serum and antibiotics.

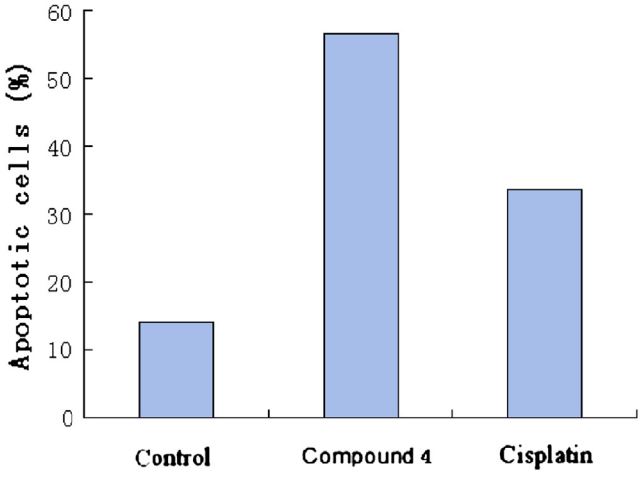



**Fig. 4.** Percentage of apoptotic HepG2 cell in total cells following treatment with compound **4** at 50 μM compared with cisplatin (50 μM).

### 4.2. Chemistry: general methods

#### 4.2.1. General procedure for the synthesis of intermediates *A* and *B*

*N*-(2,4-Dinitrophenyl)benzeneamine (5.0 g, 19.3 mmol) was dissolved in ethyl acetate (300 mL), then catalytically reduced in a Parr apparatus for 24 h using a 5% Pd/C catalyst under hydrogen atmosphere. After reduction, the reaction mixture was filtered into ethyl acetate. The solvent was removed in vacuo, giving the desired $N^1$-phenylbenzene-1,2,4-triamine **A** (3.76 g, 18.9 mmol, 98%), which was used without further purification. Compound **A** was immediately refluxed for 24 h in nitrobenzene (100 mL) containing $MgSO_4$ in a flask. After nitrobenzene removal in vacuo, and the residue was quenched with water, extracted with dichloromethane and dried over anhydrous $Na_2SO_4$. The solvent was removed under vacuum and the residue was purified by silica gel column chromatography, using petroleum ether/ethyl acetate, 3:1–1:1. The product phenazin-2-amine (**B**) was obtained as a red solid (1.11 g, 30%): mp 285–286 °C (lit. mp 280 °C) [25]. IR (KBr, $cm^{-1}$): 3309, 3194, 2918, 1640, 1597, 1514, 1476, 1453, 1335, 1232, 1132, 827, 755. $^1H$ NMR (DMSO-$d_6$, 500 MHz) $\delta$: 8.06–8.04 (d, $J$ = 8.5 Hz, 1H, Ar–H), 7.99–7.97 (d, $J$ = 8.5 Hz, 1H, Ar–H), 7.91–7.89 (d, $J$ = 9.2 Hz, 1H, Ar–H), 7.78–7.75 (t, $J$ = 7.5 Hz, 1H, Ar–H), 7.67–7.64 (t, $J$ = 7.5 Hz, 1H, Ar–H), 7.46–7.44 (d, $J$ = 9.2 Hz, 1H, Ar–H), 6.91 (s, 1H, Ar–H), 6.47 (s, 2H, $NH_2$). $^{13}C$ NMR (DMSO-$d_6$, 125 MHz) $\delta$: 151.0 (Ar–C), 145.8 (Ar–C), 143.2 (Ar–C), 139.7 (Ar–C), 139.3 (Ar–C), 130.1 (Ar–C), 130.0 (Ar–C), 129.2 (Ar–C), 128.0 (Ar–C), 127.0 (Ar–C), 126.7 (Ar–C), 101.2 (Ar–C). HRMS (ESI): $m/z$ $[M + H]^+$ calcd for $C_{12}H_9N_3$: 196.08747, found: 196.08707.

#### 4.2.2. General procedure for the synthesis of final target compounds (*1*–*21*)

Phenazin-2-amine (**B**) (195 mg, 1 mmol) was dissolved in the solution of dry $CH_2Cl_2$ (20 mL) and anhydrous pyridine (1 mL). Acyl

chlorides (14 mmol) diluted in $CH_2Cl_2$ (30 mL) then added dropwise to the above solution. The mixture was stirred under 15 °C for 0.5 h, and then 100 mL of $CH_2Cl_2$ was added, followed by washing with dilute 2 N hydrochloric acid, saturated sodium bicarbonate and water, respectively. Separating the organic layer, the solvent was removed by distillation. The residue was purified by flash chromatography on silica gel, using petroleum ether/ethyl acetate, 1:1–1:0, and then recrystallized with ethyl acetate/petroleum ether or ethyl acetate, methanol, trichloromethane/petroleum ether, dichloromethane/petroleum ether and acetone/petroleum ether to give the corresponding 2-phenazinamine derivatives (**1**–**19**), respectively. Compound **20** was prepared as follows: Phenazin-2-amine (**B**) (195 mg, 1 mmol) and benzenesulfonyl chloride (14 mmol) were dissolved in the solution of dry $CH_2Cl_2$ (50 mL) anhydrous pyridine (1 mL). The mixture was refluxed for 1 h and the other procedures were the same as the preparation of compounds (**1**–**19**). Compound **21** was prepared as follows: Phenazin-2-amine (**B**) (195 mg, 1 mmol) was dissolved in the solution of *N*,*N*-dimethylformamide (DMF, 20 mL) and triethylamine (3 mL). Dimethylcarbamyl chloride (14 mmol) was added to the above solution. The mixture was refluxed under 100 °C for 2.5 h and the other procedures were similar to the preparation of compounds (**1**–**19**). All the substituents at the phenazin-2-amine were listed in Scheme 1 and Table 1.

*N*-(Phenazin-2-yl)propionamide (**1**): bright yellow solid. IR (KBr, $cm^{-1}$): 3278, 3066, 2974, 1667, 1571, 1547, 1506, 1482, 1460, 1439, 1361, 1231, 1190, 1125, 829, 757. $^1$H NMR (DMSO-$d_6$, 500 MHz) $\delta$: 10.49 (s, 1H, NH), 8.70 (d, $J$ = 2.0 Hz, 1H, Ar–H), 8.22–8.18 (m, 3H, Ar–H), 8.00–7.97 (dd, $J$ = 9.5, 2.0 Hz, 1H, Ar–H), 7.94–7.87 (m, 2H, Ar–H), 2.51–2.47 (q, $J$ = 7.5, 2H, $CH_2$), 1.18–1.15 (t, $J$ = 7.5, 3H, $CH_3$). $^{13}$C NMR (DMSO-$d_6$, 125 MHz) $\delta$: 173.1 (CO), 143.9 (Ar–C), 143.1 (Ar–C), 141.7 (Ar–C), 140.8 (Ar–C), 140.4 (Ar–C), 130.8 (Ar–C), 129.8 (Ar–C), 129.7 (Ar–C), 129.3 (Ar–C), 128.9 (Ar–C), 126.4 (Ar–C), 113.3 (Ar–C), 29.7 ($CH_2$), 9.4 ($CH_3$). HRMS (ESI): $m/z$ [M + H]$^+$ calcd for: 252.11369, found: 252.11337.

*N*-(Phenazin-2-yl)butyramide (**2**): bright yellow solid. IR (KBr, $cm^{-1}$): 3164, 3060, 2961, 2870, 1665, 1571, 1549, 1482, 1460, 1437, 1219, 1197, 1121, 841, 755. $^1$H NMR (DMSO-$d_6$, 500 MHz) $\delta$: 10.49 (s, 1H, NH), 8.70 (d, $J$ = 2.0 Hz, 1H, Ar–H), 8.22–8.18 (m, 3H, Ar–H), 8.00–7.97 (dd, $J$ = 9.5, 2.0 Hz, 1H, Ar–H), 7.94–7.87 (m, 2H, Ar–H), 2.46–2.43 (t, $J$ = 7.5, 2H, $CH_2$), 1.72–1.68 (m, $J$ = 7.5, 2H, $CH_2$), 0.99–0.96 (t, $J$ = 7.5, 3H, $CH_3$). $^{13}$C NMR (DMSO-$d_6$, 125 MHz) $\delta$: 172.3 (CO), 143.9 (Ar–C), 143.1 (Ar–C), 141.7 (Ar–C), 140.8 (Ar–C), 140.4 (Ar–C), 130.8 (Ar–C), 129.8 (Ar–C), 129.7 (Ar–C), 129.3 (Ar–C), 128.9 (Ar–C), 126.4 (Ar–C), 113.4 (Ar–C), 38.5 ($CH_2$), 18.4 ($CH_2$), 13.6 ($CH_3$). HRMS (ESI): $m/z$ [M + H]$^+$ calcd for: 266.12934, found: 266.12769.

*N*-(Phenazin-2-yl)-2-phenylacetamide (**3**): yellow solid. IR (KBr, $cm^{-1}$): 3377, 3240, 3050, 1666, 1575, 1545, 1488, 1461, 1442, 1358, 1183, 1133, 835, 765, 717, 692. $^1$H NMR (DMSO-$d_6$, 500 MHz) $\delta$: 10.78 (s, 1H, NH), 8.69 (d, $J$ = 1.5 Hz, 1H, Ar–H), 8.22–8.18 (m, 3H, Ar–H), 8.01–7.99 (dd, $J$ = 9.5, 2.0 Hz, 1H, Ar–H), 7.94–7.87 (m, 2H, Ar–H), 7.42–7.35 (m, 4H, Ar–H), 7.30–7.26 (t, $J$ = 7.5, 1H, Ar–H). 3.81 (s, 2H, $CH_2$). $^{13}$C NMR (DMSO-$d_6$, 125 MHz) $\delta$: 170.2 (CO), 143.8 (Ar–C), 143.1 (Ar–C), 141.8 (Ar–C), 140.7 (Ar–C), 140.4 (Ar–C), 135.4 (Ar–C), 130.8 (Ar–C), 129.9 (Ar–C), 129.8 (Ar–C), 129.3 (Ar–C), 129.2 (2× Ar–C), 128.9 (Ar–C), 128.3 (2× Ar–C), 126.6 (Ar–C), 126.3 (Ar–C), 113.7 (Ar–C), 43.4 ($CH_2$). HRMS (ESI): $m/z$ [M + H]$^+$ calcd for: 314.12934, found: 314.12721.

2-Chloro-*N*-(phenazin-2-yl)benzamide (**4**): bright yellow solid. IR (KBr, $cm^{-1}$): 3316, 3263, 3058, 1661, 1572, 1547, 1484, 1459, 1437, 1355, 1292, 1190, 1118, 751. $^1$H NMR (DMSO-$d_6$, 500 MHz) $\delta$: 11.15 (s, 1H, NH), 8.83 (s, 1H, Ar–H), 8.27–8.22 (m, 3H, Ar–H), 8.13–8.11 (dd, $J$ = 9.5, 2.0 Hz, 1H, Ar–H), 7.97–7.91 (m, 2H, Ar–H), 7.73–7.72 (dd, $J$ = 7.5, 1.5 Hz, 1H, Ar–H), 7.65–7.63 (d, $J$ = 8.0 Hz, 1H, Ar–H), 7.60–7.57 (td, $J$ = 7.5, 1.5 Hz, 1H, Ar–H), 7.55–7.51 (t, $J$ = 7.5 Hz, 1H, Ar–H). $^{13}$C NMR (DMSO-$d_6$, 125 MHz) $\delta$: 165.8 (CO), 143.7 (Ar–C), 143.2 (Ar–C), 142.0 (Ar–C), 140.6 (Ar–C), 140.4 (Ar–C), 136.4 (Ar–C), 131.5 (Ar–C), 130.9 (Ar–C), 130.1 (Ar–C), 130.0 (Ar–C), 129.9 (Ar–C), 129.7 (Ar–C), 129.3 (Ar–C), 129.1 (Ar–C), 129.0 (Ar–C), 127.3 (Ar–C), 126.3 (Ar–C), 114.6 (Ar–C). HRMS (ESI): $m/z$ [M + H]$^+$ calcd for: 334.07471, found: 334.07432.

3-Chloro-*N*-(phenazin-2-yl)benzamide (**5**): bright yellow solid. IR (KBr, $cm^{-1}$): 3377, 3240, 3058, 1686, 1617, 1545, 1487, 1473, 1450, 1257, 1215, 1192, 1143, 758, 741. $^1$H NMR (DMSO-$d_6$, 500 MHz) $\delta$: 10.99 (s, 1H, NH), 8.88 (s, 1H, Ar–H), 8.30–8.24 (m, 4H, Ar–H), 8.13 (s, 1H, Ar–H), 8.04–8.03 (d, $J$ = 7.0 Hz, 1H, Ar–H), 7.96–7.92 (t, $J$ = 9.5 Hz, 2H, Ar–H), 7.74–7.73 (d, $J$ = 7.0 Hz, 1H, Ar–H), 7.65–7.62 (t, $J$ = 7.0 Hz, 1H, Ar–H). $^{13}$C NMR (DMSO-$d_6$, 125 MHz) $\delta$: 165.0 (CO), 143.5 (Ar–C), 142.9 (Ar–C), 141.9 (Ar–C), 140.7 (Ar–C), 140.6 (Ar–C), 136.3 (Ar–C), 133.2 (Ar–C), 131.7 (Ar–C), 130.9 (Ar–C), 130.4 (Ar–C), 130.0 (Ar–C), 129.6 (Ar–C), 129.2 (Ar–C), 128.8 (Ar–C), 127.6 (Ar–C), 127.0 (Ar–C), 126.7 (Ar–C), 115.0 (Ar–C). HRMS (ESI): $m/z$ [M + H]$^+$ calcd for: 334.07471, found: 334.07432.

4-Methyl-*N*-(phenazin-2-yl)benzamide (**6**): bright yellow solid. IR (KBr, $cm^{-1}$): 3471, 3255, 1652, 1573, 1546, 1505, 1486, 1460, 1438, 1354, 1312, 1279, 1190, 1118, 831, 759, 747. $^1$H NMR (DMSO-$d_6$, 500 MHz) $\delta$: 10.74 (s, 1H, NH), 8.86 (d, $J$ = 2.0 Hz, 1H, Ar–H), 8.29–8.22 (m, 4H, Ar–H), 7.99–7.97 (d, $J$ = 8.0 Hz, 2H, Ar–H), 7.96–7.89 (m, 2H, Ar–H), 7.41–7.40 (d, $J$ = 8.5 Hz, 2H, Ar–H), 2.43 (s, 3H, $CH_3$). $^{13}$C NMR (DMSO-$d_6$, 125 MHz) $\delta$: 166.3 (CO), 143.8 (Ar–C), 143.1 (Ar–C), 142.1 (Ar–C), 142.0 (Ar–C), 141.0 (Ar–C), 140.5 (Ar–C), 131.6 (Ar–C), 130.8 (Ar–C), 129.9 (Ar–C), 129.5 (Ar–C), 129.3 (Ar–C), 129.0 (2× Ar–C), 128.9 (Ar–C), 127.9 (2× Ar–C), 127.1 (Ar–C), 114.8 (Ar–C), 21.0 ($CH_3$). HRMS (ESI): $m/z$ [M + H]$^+$ calcd for: 314.12934, found: 314.12905.

2-Methyl-*N*-(phenazin-2-yl)benzamide (**7**): bright yellow solid. IR (KBr, $cm^{-1}$): 3253, 3059, 1668, 1569, 1540, 1499, 1482, 1453, 1432, 1355, 1307, 1265, 1188, 1125, 823, 756, 736. $^1$H NMR (DMSO-$d_6$, 500 MHz) $\delta$: 10.93 (s, 1H, NH), 8.85 (d, $J$ = 1.5 Hz, 1H, Ar–H), 8.25–8.21 (m, 3H, Ar–H), 8.17–8.15 (dd, $J$ = 9.5, 2.5 Hz, 1H, A-H), 7.96–7.89 (m, 2H, Ar–H), 7.60–7.59 (d, $J$ = 7.0 Hz, 1H, Ar–H), 7.48–7.46 (t, $J$ = 7.5 Hz, 1H, Ar–H), 7.38–7.35 (t, $J$ = 7.5 Hz, 2H, Ar–H), 2.46 (s, 3H, $CH_3$). $^{13}$C NMR (DMSO-$d_6$, 125 MHz) $\delta$: 168.7 (CO), 143.8 (Ar–C), 143.2 (Ar–C), 141.9 (Ar–C), 140.8 (Ar–C), 140.6 (Ar–C), 136.6 (Ar–C), 135.5 (Ar–C), 130.9 (Ar–C), 130.6 (Ar–C), 130.0 (Ar–C), 129.9 (Ar–C), 129.8 (Ar–C), 129.3 (Ar–C), 128.9 (Ar–C), 127.4 (Ar–C), 127.6 (Ar–C), 125.7 (Ar–C), 114.3 (Ar–C), 19.3 ($CH_3$). HRMS (ESI): $m/z$ [M + H]$^+$ calcd for: 314.12934, found: 314.12871.

3-Methyl-*N*-(phenazin-2-yl)benzamide (**8**): bright yellow solid. IR (KBr, $cm^{-1}$): 3346, 3324, 1651, 1541, 1507, 1487, 1458, 1439, 1356, 1309, 1279, 1224, 1190, 758, 739. $^1$H NMR (DMSO-$d_6$, 500 MHz) $\delta$: 10.80 (s, 1H, NH), 8.87 (s, 1H, Ar–H), 8.29–8.22 (m, 4H, Ar–H), 7.96–7.93 (m, 2H, Ar–H), 7.91–7.84 (m, 2H, Ar–H), 7.49–7.46 (m, 2H, Ar–H), 2.45 (s, 3H, $CH_3$). $^{13}$C NMR (DMSO-$d_6$, 125 MHz) $\delta$: 166.6 (CO), 143.8 (Ar–C), 143.2 (Ar–C), 141.9 (Ar–C), 140.9 (Ar–C), 140.6 (Ar–C), 137.9 (Ar–C), 134.5 (Ar–C), 132.6 (Ar–C), 130.8 (Ar–C), 130.0 (Ar–C), 129.6 (Ar–C), 129.3 (Ar–C), 128.9 (Ar–C), 128.4 (Ar–C), 128.3 (Ar–C), 127.1 (Ar–C), 125.0 (Ar–C), 114.9 (Ar–C), 20.9 ($CH_3$). HRMS (ESI): $m/z$ [M + H]$^+$ calcd for: 314.12934, found: 314.12893.

4-Methoxy-*N*-(phenazin-2-yl)benzamide (**9**): bright yellow solid. IR (KBr, $cm^{-1}$): 3422, 1651, 1601, 1537, 1502, 1457, 1312, 1255, 1193, 1177, 1022, 840, 758. $^1$H NMR (DMSO-$d_6$, 500 MHz) $\delta$: 10.68 (s, 1H, NH), 8.85 (d, $J$ = 2.2 Hz, 1H, Ar–H), 8.29–8.26 (m, 1H, Ar–H), 8.25–8.21 (m, 3H, Ar–H), 8.08–8.04 (m, 2H, Ar–H), 7.95–7.88 (m, 2H, Ar–H), 7.14–7.10 (m, 2H, Ar–H), 3.87 (s, 3H, $OCH_3$). $^{13}$C NMR (DMSO-$d_6$, 125 MHz) $\delta$: 165.8 (CO), 162.3 (Ar–C), 143.8 (Ar–C), 143.1 (Ar–C), 141.9 (Ar–C), 141.1 (Ar–C), 140.5 (Ar–C), 130.8 (Ar–C), 129.9 (2× Ar–C), 129.8 (Ar–C), 129.5 (Ar–C), 129.3 (Ar–C), 128.9 (Ar–C),

127.2 (Ar–C), 126.4 (Ar–C), 114.6 (Ar–C), 113.7 (2× Ar–C), 55.5 ($OCH_3$). HRMS (ESI): $m/z$ $[M + H]^+$ calcd for: 330.12425, found: 330.12676.

*N*-(Phenaazin-2-yl)cyclohexanecarboxamide (**10**): yellow solid. IR (KBr, $cm^{-1}$): 3435, 3255, 3042, 2932, 2853, 1670, 1573, 1548, 1512, 1486, 1461, 1441, 1250, 1204, 833, 759. $^1H$ NMR (DMSO-$d_6$, 500 MHz) $\delta$: 10.43 (s, 1H, NH), 8.71 (d, $J = 2.0$ Hz, 1H, Ar–H), 8.22–8.17 (m, 3H, Ar–H), 8.00–7.99 (dd, $J = 9.0$, 2.0 Hz, 1H, Ar–H), 7.94–7.90 (m, 1H, Ar–H), 7.89–7.86 (m, 1H, Ar–H), 2.49–2.44 (m, 1H, cyclohexyl-CH), 1.91–1.89 (m, 2H, cyclohexyl-$CH_2$), 1.81–1.79 (m, 2H, cyclohexyl-$CH_2$), 1.70–1.67 (d, $J = 12.0$ Hz, 1H, cyclohexyl-$CH_2$), 1.53–1.45 (m, 2H, cyclohexyl-$CH_2$), 1.36–1.27 (m, 2H, cyclohexyl-$CH_2$), 1.26–1.19 (m, 1H, cyclohexyl-$CH_2$). $^{13}C$ NMR (DMSO-$d_6$, 125 MHz) $\delta$: 175.4 (CO), 144.0 (Ar–C), 143.2 (Ar–C), 141.8 (Ar–C), 141.0 (Ar–C), 140.0 (Ar–C), 130.8 (Ar–C), 129.8 (2× Ar–C), 129.3 (Ar–C), 128.9 (Ar–C), 126.5 (Ar–C), 113.4 (Ar–C), 45.1 (cyclohexyl-CH), 29.1 (2× cyclohexyl-$CH_2$), 25.4 (cyclohexyl-$CH_2$), 25.2 (2× cyclohexyl-$CH_2$). HRMS (ESI): $m/z$ $[M + H]^+$ calcd for: 306.16064, found: 306.16178.

3-Cyclopentyl-*N*-(phenazin-2-yl)propanamide (**11**): yellow solid. IR (KBr, $cm^{-1}$): 3430, 3256, 3050, 2947, 2864, 1670, 1647, 1571, 1544, 1508, 1480, 1440, 1358, 1203, 832, 759. $^1H$ NMR (DMSO-$d_6$, 500 MHz) $\delta$: 10.49 (s, 1H, NH), 8.70 (d, $J = 2.0$ Hz, 1H, Ar–H), 8.21–8.17 (m, 3H, Ar–H), 7.98–7.96 (dd, $J = 9.0$, 2.0 Hz, 1H, Ar–H), 7.93–7.90 (m, 1H, Ar–H), 7.89–7.86 (m, 1H, Ar–H), 2.48–2.45 (m, 2H, $COCH_2$), 1.83–1.76 (m, 3H, $CH_2$ and cyclopentyl-CH), 1.71–1.66 (m, 2H, cyclopentyl-$CH_2$), 1.61–1.57 (m, 2H, cyclopentyl-$CH_2$), 1.54–1.45 (m, 2H, cyclopentyl-$CH_2$), 1.16–1.12 (m, 2H, cyclopentyl-$CH_2$). $^{13}C$ NMR (DMSO-$d_6$, 125 MHz) $\delta$: 172.4 (CO), 143.9 (Ar–C), 143.1 (Ar–C), 141.7 (Ar–C), 140.8 (Ar–C), 140.3 (Ar–C), 130.8 (Ar–C), 129.8 (Ar–C), 129.7 (Ar–C), 129.2 (Ar–C), 128.8 (Ar–C), 126.3 (Ar–C), 113.4 (Ar–C), 39.2 (cyclopentyl-CH), 36.0 ($CH_2$), 32.0 (2× cyclohexyl-$CH_2$), 31.1 (cyclohexyl-$CH_2$), 24.7 (2× cyclohexyl-$CH_2$). HRMS (ESI): $m/z$ $[M + H]^+$ calcd for: 320.17629, found: 320.17682.

*N*-(Phenazin-2-yl)-1-naphthamide (**12**): yellow solid. IR (KBr, $cm^{-1}$): 3430, 3278, 3050, 1649, 1560, 1537, 1509, 1485, 1459, 1430, 1287, 1252, 1194, 1126, 1053, 962, 781, 755. $^1H$ NMR (DMSO-$d_6$, 500 MHz) $\delta$: 11.22 (s, 1H, NH), 8.96 (d, $J = 2$ Hz, 1H, Ar–H), 8.29–8.20 (m, 4H, Ar–H), 8.17–8.15 (m, 2H, Ar–H), 8.08–8.06 (m, 1H, Ar–H), 7.98–7.96 (m, 1H, Ar–H), 7.95–7.90 (m, 2H, Ar–H), 7.70–7.67 (m, 1H, Ar–H), 7.66–7.63 (m, 2H, Ar–H). $^{13}C$ NMR (DMSO-$d_6$, 125 MHz) $\delta$: 168.1 (CO), 143.8 (Ar–C), 143.2 (Ar–C), 142.0 (Ar–C), 140.9 (Ar–C), 140.6 (Ar–C), 134.0 (Ar–C), 133.2 (Ar–C), 130.9 (Ar–C), 130.6 (Ar–C), 130.0 (Ar–C), 129.8 (Ar–C), 129.6 (Ar–C), 129.3 (Ar–C), 129.0 (Ar–C), 128.4 (Ar–C), 127.2 (Ar–C), 126.7 (Ar–C), 126.5 (Ar–C), 125.9 (Ar–C), 125.0 (Ar–C), 124.9 (Ar–C), 114.6 (Ar–C). HRMS (ESI): $m/z$ $[M + H]^+$ calcd for: 350.12934, found: 350.13123.

*N*-(Phenazin-2-yl)furan-2-carboxamide (**13**): yellow solid. IR (KBr, $cm^{-1}$): 3385, 3264, 3125, 1680, 1587, 1544, 1461, 1439, 1354, 1305, 1281, 1213, 1195, 1123, 1013, 755. $^1H$ NMR (DMSO-$d_6$, 500 MHz) $\delta$: 10.78 (s, 1H, NH), 8.82 (d, $J = 2.0$, 1H, Ar–H), 8.30–8.21 (m, 4H, Ar–H), 7.96–7.89 (m, 3H, 2× Ar–H and furan–CH), 7.49–7.46 (m, 1H, furan–H), 6.79–6.78 (m, 1H, furan–H). $^{13}C$ NMR (DMSO-$d_6$, 125 MHz) $\delta$: 156.8 (CO), 146.3 (furan–C), 140.4 (furan–C), 130.9 (Ar–C), 130.7 (Ar–C), 130.0 (Ar–C), 129.6 (Ar–C), 129.3 (Ar–C), 129.2 (Ar–C), 128.9 (Ar–C), 126.9 (Ar–C), 122.4 (Ar–C), 116.0 (Ar–C), 115.8 (Ar–C), 115.0 (furan–C), 112.5 (Ar–C), 112.4 (furan–C). HRMS (ESI): $m/z$ $[M + H]^+$ calcd for: 290.09295, found: 290.11491.

*N*-(Phenazin-2-yl)thiophene-2-carboxamide (**14**): yellow solid. IR (KBr, $cm^{-1}$): 3316, 3088, 1663, 1653, 1573, 1550, 1522, 1508, 1414, 1360, 1274, 1190, 758, 729. $^1H$ NMR (DMSO-$d_6$, 500 MHz) $\delta$: 10.79 (s, 1H, NH), 8.80 (d, $J = 0.5$ Hz, 1H, Ar–H), 8.25–8.22 (m, 4H, Ar–H), 8.17–8.16 (dd, $J = 4.0$, 1.0 Hz, 1H, thiophenyl-H), 7.97–7.96 (dd, $J = 5.0$, 1.0 Hz, 1H, thiophenyl-H), 7.95–7.92 (m, 1H, Ar–H), 7.91–7.88 (m, 1H, Ar–H), 7.32–7.30 (q, $J = 5.0$, 4.0 Hz, 1H, thiophenyl-H). $^{13}C$ NMR (DMSO-$d_6$, 125 MHz) $\delta$: 160.7 (CO), 143.7 (Ar–C), 143.2 (Ar–C), 142.0 (Ar–C), 140.6 (Ar–C), 140.5 (thiophenyl-C), 132.8 (thiophenyl-C), 130.9 (thiophenyl-C), 130.0 (2× Ar–C), 129.7 (Ar–C), 129.3 (Ar–C), 129.0 (Ar–C), 128.2 (Ar–C), 128.1 (Ar–C), 126.9 (thiophenyl-C), 115.0 (Ar–C). HRMS (ESI): $m/z$ $[M + H]^+$ calcd for: 306.07011, found: 306.07194.

*N*-(Phenazin-2-yl)-2-(thiophen-2-yl)acetamide (**15**): deep yellow solid. IR (KBr, $cm^{-1}$): 3408, 3256, 3163, 3052, 1670, 1576, 1551, 1487, 1460, 1439, 1354, 1312, 1245, 1182, 1121, 827, 764, 690. $^1H$ NMR (DMSO-$d_6$, 500 MHz) $\delta$: 10.84 (s, 1H, NH), 8.69 (d, $J = 2.5$ Hz, 1H, Ar–H), 8.22–8.19 (m, 3H, Ar–H), 8.00–7.98 (dd, $J = 9.5$, 2.5 Hz, 1H, Ar–H), 7.94–7.91 (m, 1H, Ar–H), 7.90–7.87 (m, 1H, Ar–H), 7.44–7.43 (dd, $J = 5.5$, 1.0 Hz, 1H, thiophenyl-H), 7.07–7.06 (dd, $J = 5.5$, 1.0 Hz, 1H, thiophenyl-H), 7.02–7.01 (q, $J = 5.5$ Hz, 1H, thiophenyl-H), 4.04 (s, 2H, $CH_2$). $^{13}C$ NMR (DMSO-$d_6$, 125 MHz) $\delta$: 169.1 (CO), 143.8 (Ar–C), 143.1 (Ar–C), 141.9 (Ar–C), 140.5 (Ar–C), 140.4 (thiophenyl-C), 136.5 (Ar–C), 130.9 (Ar–C), 130.0 (Ar–C), 129.9 (Ar–C), 129.3 (Ar–C), 128.9 (Ar–C), 126.7 (Ar–C), 126.6 (thiophenyl-C), 126.2 (thiophenyl-C), 125.2 (thiophenyl-C), 113.8 (Ar–C), 37.6 ($CH_2$). HRMS (ESI): $m/z$ $[M + H]^+$ calcd for: 320.08576, found: 320.09090.

Ethyl phenazin-2-ylcarbamate (**16**): yellow solid. IR (KBr, $cm^{-1}$): 3217, 3058, 2974, 2921, 1738, 1636, 1608, 1502, 1448, 1445, 1313, 1236, 1202, 1133, 1060, 851, 833, 756. $^1H$ NMR (DMSO-$d_6$, 500 MHz) $\delta$: 10.33 (s, 1H, NH), 8.40 (d, $J = 2.0$ Hz, 1H, Ar–H), 8.20–8.16 (m, 3H, Ar–H), 7.97–7.95 (dd, $J = 9.5$, 2.0 Hz, 1H, Ar–H), 7.92–7.85 (m, 2H, Ar–H), 4.26–4.22 (q, $J = 7.1$, 2H, $CH_2$), 1.33–1.30 (t, $J = 7.1$, 3H, $CH_3$). $^{13}C$ NMR (DMSO-$d_6$, 125 MHz) $\delta$: 153.5 (CO), 143.9 (Ar–C), 143.1 (Ar–C), 141.6 (Ar–C), 141.1 (Ar–C), 140.2 (Ar–C), 130.8 (Ar–C), 129.9 (Ar–C), 129.6 (Ar–C), 129.2 (Ar–C), 128.8 (Ar–C), 125.8 (Ar–C), 111.8 (Ar–C), 60.8 ($CH_2$), 14.4 ($CH_3$). HRMS (ESI): $m/z$ $[M + H]^+$ calcd for: 268.10860, found: 268.10704.

*N*-(Phenazin-2-yl)propanamide (**17**): black solid. IR (KBr, $cm^{-1}$): 3432, 1697, 1632, 1616, 1557, 1518, 1473, 1455, 1358, 1194, 1138, 756. $^1H$ NMR (DMSO-$d_6$, 500 MHz) $\delta$: 10.97 (s, 1H, NH), 8.74 (d, $J = 1.5$ Hz, 1H, Ar–H), 8.23–8.20 (m, 3H, Ar–H), 8.07–8.05 (dd, $J = 9.5$, 2.0 Hz, 1H, Ar–H), 7.96–7.89 (m, 2H, Ar–H), 3.98–3.95 (t, $J = 7.5$, 6.0 Hz, 2H, $CH_2$), 3.03–3.00 (t, $J = 7.5$, 6.0 Hz, 2H, $CH_2$). $^{13}C$ NMR (DMSO-$d_6$, 125 MHz) $\delta$: 169.3 (CO), 143.7 (Ar–C), 143.0 (Ar–C), 141.8 (Ar–C), 140.7 (Ar–C), 140.5 (Ar–C), 131.0 (Ar–C), 130.0 (Ar–C), 129.9 (Ar–C), 129.3 (Ar–C), 128.8 (Ar–C), 126.3 (Ar–C), 113.6 (Ar–C), 40.5 ($CH_2$), 39.5 ($CH_2$). HRMS (ESI): $m/z$ $[M + H]^+$ calcd for: 286.07471, found: 286.07370.

*N*-(Phenazin-2-yl)benzamide (**18**): yellow solid. IR (KBr, $cm^{-1}$): 3469, 3225, 3060, 1649, 1566, 1547, 1483, 1461, 1439, 1358, 1310, 1286, 1190, 1126, 837, 800, 760, 707. $^1H$ NMR (DMSO-$d_6$, 500 MHz) $\delta$: 10.83 (s, 1H, NH), 8.87 (s, 1H, Ar–H), 8.29–8.22 (m, 4H, Ar–H), 8.07–8.05 (d, 2H, Ar–H), 7.96–7.90 (m, 2H, Ar–H), 7.78–7.65 (t, $J = 7.5$ Hz, 1H, Ar–H), 7.62–7.59 (t, $J = 7.5$ Hz, 2H, Ar–H). $^{13}C$ NMR (DMSO-$d_6$, 125 MHz) $\delta$: 166.5 (CO), 143.7 (Ar–C), 143.2 (Ar–C), 142.0 (Ar–C), 140.9 (Ar–C), 140.6 (Ar–C), 134.5 (Ar–C), 132.0 (Ar–C), 130.9 (Ar–C), 130.0 (Ar–C), 129.6 (Ar–C), 129.3 (Ar–C), 129.0 (Ar–C), 128.5 (2× Ar–C), 127.9 (2× Ar–C), 127.1 (Ar–C), 114.9 (Ar–C). HRMS (ESI): $m/z$ $[M + H]^+$ calcd for: 300.11369, found: 300.11211.

*N*-(Phenazin-2-yl)acetamide (**19**): bright yellow solid. IR (KBr, $cm^{-1}$): 3490, 3258, 3158, 3046, 1675, 1574, 1550, 1461, 1441, 1378, 1356, 1292, 1122, 838, 765. $^1H$ NMR (DMSO-$d_6$, 500 MHz) $\delta$: 10.55 (s, 1H, NH), 8.68 (d, $J = 2.2$ Hz, 1H, Ar–H), 8.22–8.18 (m, 3H, Ar–H), 7.97–7.87 (m, 3H, Ar–H), 2.20 (s, 3H, $CH_3$). $^{13}C$ NMR (DMSO-$d_6$, 125 MHz) $\delta$: 169.4 (CO), 143.9 (Ar–C), 143.1 (Ar–C), 141.8 (Ar–C), 140.8 (Ar–C), 140.4 (Ar–C), 130.8 (Ar–C), 129.8 (Ar–C), 129.7 (Ar–C), 129.3 (Ar–C), 128.9 (Ar–C), 126.3 (Ar–C), 113.4 (Ar–C), 24.3 ($CH_3$). HRMS (ESI): $m/z$ $[M + H]^+$ calcd for: 238.09804, found: 238.09638.

*N*-(Phenazin-2-yl)benzenesulfonamide (**20**): red solid. IR (KBr, $cm^{-1}$): 3223, 3060, 1638, 1601, 1519, 1483, 1453, 1362, 1330, 1156, 1094, 922, 906, 832, 748. $^1H$ NMR (DMSO-$d_6$, 500 MHz) $\delta$: 11.26 (s,

1H, NH), 8.18–8.15 (m, 3H, Ar–H), 7.97–7.96 (d, $J$ = 7.2 Hz, 2H, Ar–H), 7.93–7.87 (m, 2H, Ar–H), 7.80–7.75 (td, $J$ = 10.2, 2.3 Hz, 2H, Ar–H), 7.64–7.59 (m, 3H, Ar–H). $^{13}$C NMR (DMSO-$d_6$, 125 MHz) $\delta$: 143.2 (Ar–C), 143.1 (Ar–C), 141.9 (Ar–C), 140.2 (Ar–C), 139.8 (Ar–C), 139.1 (Ar–C), 133.4 (Ar–C), 131.1 (Ar–C), 130.8 (Ar–C), 130.1 (Ar–C), 129.5 (2× Ar–C), 129.3 (Ar–C), 128.8 (Ar–C), 126.7 (2× Ar–C), 125.5 (Ar–C), 112.5 (Ar–C). HRMS (ESI): $m/z$ [M + H]$^+$ calcd for: 336.08067, found: 336.07862.

1,1-Dimethyl-3-(phenazin-2-yl)urea (**21**): brown solid. IR (KBr, cm$^{-1}$): 3380, 3047, 2915, 1636, 1617, 1582, 1476, 1449, 1396, 1360, 1099, 832, 771, 756. $^1$H NMR (DMSO-$d_6$, 500 MHz) $\delta$: 8.18–8.16 (d, $J$ = 7.5 Hz, 1H, Ar–H), 8.16 (s, 1H, Ar–H), 8.13–8.12 (d, $J$ = 8.5 Hz, 1H, Ar–H), 8.07–8.05 (d, $J$ = 9.5 Hz, 1H, Ar–H), 7.89–7.86 (m, 1H, Ar–H), 7.83–7.80 (m, 1H, Ar–H), 7.70–7.69 (d, $J$ = 9.0 Hz, 1H, Ar–H), 7.51 (s, 1H, NH), 3.14 (s, 3H, $CH_3$), 3.06 (s, 3H, $CH_3$). $^{13}$C NMR (DMSO-$d_6$, 125 MHz) $\delta$: 155.1 (2× C, CO and Ar–C), 154.6 (Ar–C), 144.8 (Ar–C), 142.9 (Ar–C), 141.0 (Ar–C), 131.4 (Ar–C), 130.3 (Ar–C), 129.3 (Ar–C), 129.2 (Ar–C), 128.8 (Ar–C), 128.6 (Ar–C), 112.4 (Ar–C), 39.7 ($CH_3$), 34.2 ($CH_3$). HRMS (ESI): $m/z$ [M + H]$^+$ calcd for $C_{15}H_{14}N_4O$: 267.12459, found: 267.12463.

### 4.3. In vitro anticancer screening

#### 4.3.1. Cell culture

All adherent cell lines including K562 (human chronic myelogenous leukemia), HepG2 (human hepatocellular carcinoma), MGC803 (human gastric carcinoma), HCT116 (human colorectal carcinoma), MCF7 (human breast adenocarcinoma), as well as 293T (epithelial cells from human embryo kidney) non-cancer cell cultured in a humidified, 5% $CO_2$ atmosphere at 37 °C, and maintained in monolayer culture in RPMI-1640 medium supplemented with 10% fetal bovine serum (FBS), 100 mg/mL of streptomycin and 100 mg/mL of penicillin.

#### 4.3.2. MTT assay

The *in vitro* cytotoxicity of all newly synthesized target compounds (**1**–**21**) against K562 (human chronic myelogenous leukemia), HepG2 (human hepatocellular carcinoma), MGC803 (human gastric carcinoma), HCT116 (human colorectal carcinoma), MCF7 (human breast adenocarcinoma) cell lines, as well as 293T (epithelial cells from human embryo kidney) non-cancer cell, was measured by the MTT assays [30,31]. This assay was based on the cleavage of the yellow tetrazolium salt (3-(4,5-dimethyl-thiazol-2-yl)-2,5-diphenyl-tetrazolium bromide, MTT, Sigma) forming purple formazan crystals by viable cells. The cells were plated in 96-well culture plates at a density of 5000 cells per well and incubated for 24 h at 37 °C in a 5% $CO_2$ incubator. The compounds were dissolved in DMSO and diluted with culture medium. The compounds were added to the wells and the final concentrations made reaching to 0.16, 0.63, 2.5, 10, 40 and 160 μM, before incubating the cells at 37 °C in a 5% $CO_2$ incubator for 48 h. After that, the cells were treated with 10% (v/v) MTT dye solution (5 mg/ml) for 4 h cultivation. The media with MTT solution were replaced with DMSO solution (150 μL). The absorbance was measured at 490 nm, using an Absorbance Plate Reader (Bio-Rad). The $IC_{50}$ value was determined from the chart of cell viability (%) against dose of compounds added (μM).

#### 4.3.3. Induction of cell apoptosis

The selected cell line HepG2 (human hepatocellular carcinoma) was grown in culture as described in the Experimental section 4.3.1. Briefly, cells were washed with PBS solution and digested by trypsin solution. A cell suspension was made with culture medium, and concentration adjusted to 1 × 10$^5$ cells/mL. Cells were plated into 6-well culture plates (2 mL/well) and incubated at 37 in 5% $CO_2$ overnight. A series of indicated doses of the selected compound were added into each well and incubated with cells for 24 h at 37 °C in 5% $CO_2$. Cisplatin was used as positive control and 10% CS was used as a negative control for HepG2. Cell apoptosis assays were performed by Flow cytometry using an Annexin V-FITC Apoptosis Detection Kit (Biouniquer, Nanjing, China) according to the manufacturer's instructions. Cells were harvested and washed twice with cold PBS, then collected by centrifugation for 5 min and stained with annexin V-FITC (100 ng/mL) and propidium iodide (2 μg/mL) in annexin-binding buffer (10 mM HEPES, 140 mM NaCl, 2.5 mM $CaCl_2$, pH 7.4). After 15 min incubation at room temperature, the fluorescence of cells was measured using a flow cytometer (FAC Scan, Becton Dickenson, USA) in FL1 and FL2 channel, respectively. The results were obtained by using FCSExpress software and represented as percentage of normal and apoptotic cells.

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## Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.ejmech.2013.07.017.

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