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Journal Name

ARTICLE

Synthesis and biological evaluation of 3-(1,3,4-oxadiazol-2-yl)-1,8-naphthyridin-4(1H)-ones as cisplatin sensitizers

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A series of novel 3-(1,3,4-oxadiazol-2-yl)-1,8-naphthyridin-4(1H)-one derivatives were synthesized and their anti-cancer as well as cisplatin sensitization activities were evaluated. Among them, compound **6e** and **6h** exhibited the significantly cisplatin sensitization activity against HCT116. Hoechst staining and annexin V-FITC/PI dual-labeling studies demonstrated that the combination of **6e/6h** and cisplatin can induce tumour cells apoptosis. Western blot showed the expression of ATR downstream protein, CHK1, decreased in **6e** + cisplatin and **6h** + cisplatin groups, compared with that in test compound and cisplatin group. Furthermore, docking of **6e/6h** into the ATR structure active site revealed that the N1 and N8 atoms in the naphthyridine ring and the hybrid atom in the oxadiazol ring are involved in hydrogen bonding with Val170, Glu168 and Tyr155. Additionally, the naphthyridine ring is also involved in π - π stack with Trp169. Accordingly, compounds **6e** and **6h** can be expected to be the potential cisplatin sensitizers to participate in HCT116 cancer therapy.

Introduction

1,8-naphthyridinone moiety is a structural feature of many drug molecules and pharmaceutical agents and lots of compounds bearing 1,8-naphthyridinone exhibit a variety of biological properties, such as anti-tumour,¹⁻³ anti-bacterial,⁴⁻⁵ anti-inflammatory,⁶ CB2 selective agonist activities,⁷⁻¹⁰ etc. As far as anti-cancer was concerned, Ke Chen and his co-workers discovered a series of 2-aryl-1,8-naphthyridin-4(1H)-ones and 2-phenyl-1,8-naphthyridin-4(1H)-ones as anti-tumour agents through inhibiting tubulin polymerization.²⁻³ Indeed, HKL-1 (2-(3-Methoxyphenyl)-5-methyl-1,8-naphthyridin-4(1H)-one) was an antimitotic agent that can induce G2/M arrest and mitotic catastrophe in human leukemia HL-60 cells.¹ Furthermore, 1,3,4-oxadiazole is a commonly used moiety for pharmacophore development, which has been thoroughly investigated because of its hydrogen bonding ability within the receptor site and good metabolic profile.¹¹ The azole group ($-N=C-O$) of 1,3,4-oxadiazole can increase the lipophilicity of drugs, promote drug's transportation through cell membranes to reach the target site, and facilitate drug's various biological activities.¹² In fact, a lot of researches showed that the synthetic compounds bear 1,3,4-oxadiazole moiety have excellent anticancer activities both *in vitro* and *in vivo*.¹³⁻¹⁹ Although various substituent 1,8-naphthyridinones and 1,3,4-oxadiazole have been developed, there are still a lot of analogues have not been designed and synthesized. Therefore, a novel molecular skeleton, 3-(1,3,4-oxadiazol-2-yl)-1,8-naphthyridin-4(1H)-one (Fig. 2), was designed and synthesized, which was seen as the core

structure of target compounds in this study.

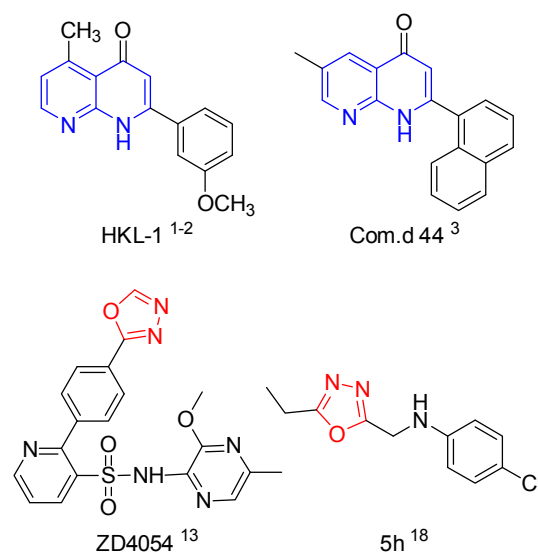


Fig. 1. Chemical structures of small molecule compounds bearing 1,8-naphthyridin-4(1H)-one or oxadiazole with anti-cancer activities.

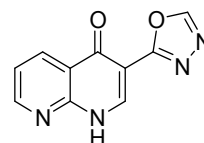


Fig. 2. Chemical structure of 3-(1,3,4-oxadiazol-2-yl)-1,8-naphthyridin-4(1H)-one.

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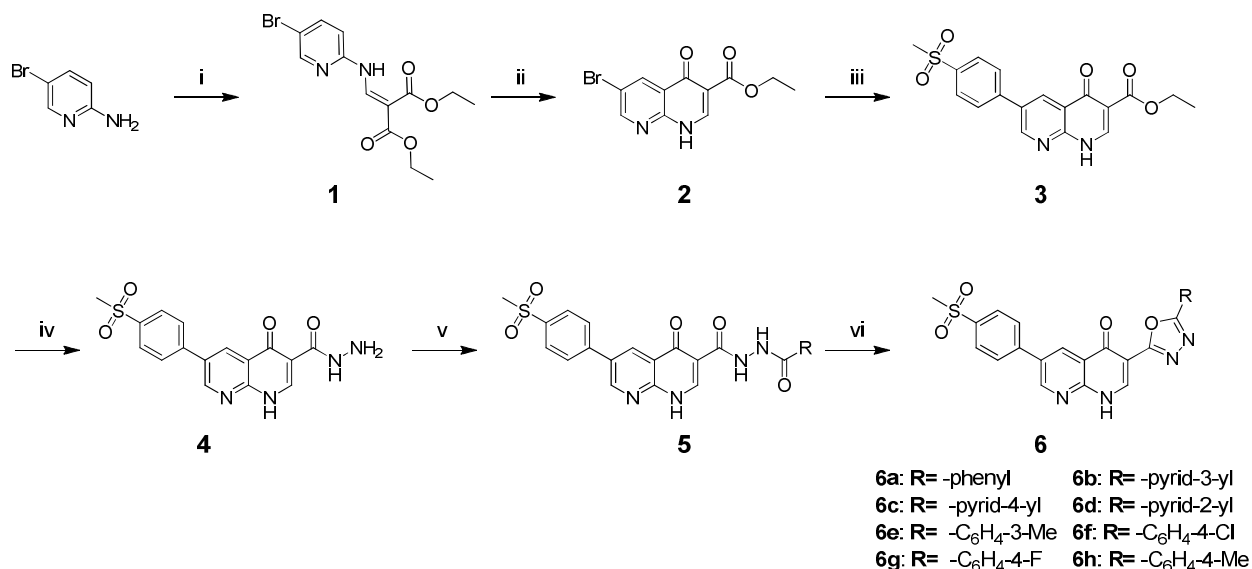
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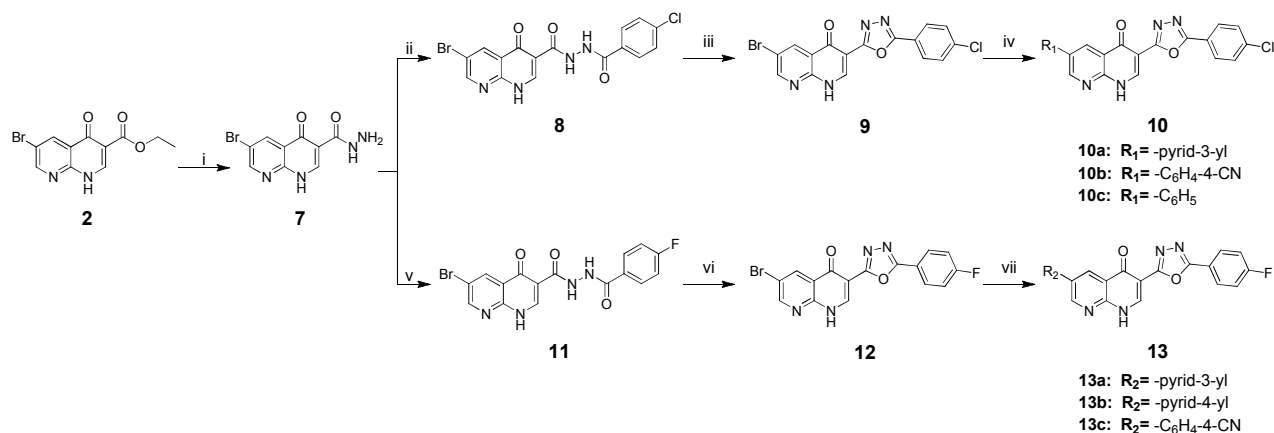
Cisplatin is a DNA-damaging agent that commonly used in the treatment of solid tumours, which are highly effective against certain cancers like metastatic testicular cancer.²⁰⁻²³ But for the majority of solid tumours such as ovarian, lung, and prostatic tumours, cisplatin only has modest benefit.²⁴ Additionally, the drug resistance, nephrotoxicity, ototoxicity and neurotoxicity of cisplatin also limit the scope of its application.¹⁶ Recent researches demonstrate that the poor response to those cancers and drug resistance are closely related to the DNA damage response (DDR).²⁶⁻³¹ DDR network is an elaborate transduction system which can sense different types of damage and coordinate a series of response, including activation of apoptosis, cell cycle control, transcription, senescence, and DNA repair processes.³²⁻³³ One critical signalling pathway in DDR network is the

ATR/CHK1 pathway, which activated by single-stranded DNA arising from stalled replication forks caused by replication stress or as an intermediated of DNA repair processes.³⁴⁻³⁵ Many studies showed that the cisplatin sensitivity activities can increase by the inhibition of ATR and its downstream protein CHK1.³⁶⁻³⁸

In this study, a series of novel 3-(1,3,4-oxadiazol-2-yl)-1,8-naphthyridin-4(1H)-one derivatives were designed and synthesized (Scheme 1 and 2), and their biological activities were evaluated as anti-cancer and cisplatin sensitizer agents. Among of these compounds, **6e** and **6h** were discovered the cisplatin sensitization effects. Hoechst staining, annexin V-FITC/PI dual-labelling, western blot and molecular docking experiments were employed to investigate the possible mechanism.



Scheme 1. Reagents and conditions: (i) diethyl 2-(ethoxymethylene)malonate, 130 °C, 2 h; (ii) Ph₂O, 250 °C, 30-40 min; (iii) (4-(methylsulfonyl)phenyl)boronic acid, Pd Cat., 60 °C, 8 h; (iv) H₂NNH₂, 50 °C, 3 h; (v) RCOCl, THF, DIEA, overnight; (vi) pyridine, SOCl₂, 3 h.



Scheme 2. Reagents and conditions: (i) H₂NNH₂, 50 °C, 3 h; (ii) R₁COCl, THF, DIEA, overnight; (iii) pyridine, SOCl₂, 3 h; (iv) aryl boronic acid or aryl borate, Pd Cat., 50 °C, 6 h; (v) R₂COCl, THF, DIEA, overnight; (vi) pyridine, SOCl₂, 3 h; (vii) aryl boronic acid or aryl borate, Pd Cat., 50 °C, 6 h.

Results and discussion

Chemistry

In this study, a new series of 3-(1,3,4-oxadiazol-2-yl)-1,8-naphthyridin-4(1H)-one derivatives were synthesized. The general procedures for the preparation of the 3-(1,3,4-oxadiazol-2-yl)-1,8-naphthyridin-4(1H)-one derivatives **6a-6h** were efficiently synthesized according to the protocol outlined in Scheme 1. Condensation of 5-bromopyridin-2-amine with diethyl ethoxymethylenemalonate were heated under 130 °C for 2 h to provide the intermediate **1**. Phenyl ether was heated under stirring at 250 °C, and then **1** was added slowly. The resulting mixture was refluxed for 4 h to get the intermediate **2**. Intermediate **3** was prepared from **2** by Suzuki coupling with 4-(methylsulfonyl)phenylboronic acid and dichlorobis(triphenylphosphine)palladium(II). Intermediate **4** was obtained by treating **3** with hydrazine hydrate in the presence of methanol at room temperature. A series of aromatic acid derivatives were reacted with SOCl₂ and DMF as catalyst refluxed for 1 h to afford acyl chloride derivatives, which were converted to amide intermediates **5a-5h** by the treatment with **4** in the presence of DIEA. Finally, the compounds **6a-6h** were accomplished by refluxing **5a-5h** with SOCl₂ in pyridine for 3 h.

As outlined in Scheme 2, compounds **10a-10c** and **13a-13c** were achieved starting from intermediate **2** and the synthesis began with hydrazine hydrate to prepare intermediate **7**. **8** and **11** were detected by the reaction of the corresponding aromatic acid derivatives with **7**. Refluxing **8** and **11** with SOCl₂ in pyridine for 3 h can provide the intermediate **9** and **12**. Bis(triphenylphosphine)palladium(II) chloride catalyzed coupling of **9** with a series of boronic acid derivatives in the presence of Na₂CO₃ to yield the compounds **10a-10c**. In addition, compounds **13a-13c** were obtained by the similar synthetic route with intermediate **12** and different kinds of boronic acid analogues. All synthetic compounds gave satisfactory analytical and spectroscopic data, which were consistent with their depicted structures.

In vitro inhibition evaluation of compounds

The proliferation inhibitory effects of compounds **6a-6h**, **10a-10c** and **13a-13c** in several tumour cell lines were evaluated primarily by MTT. It was shown that the cancer cells, including human non-small cell lung cancer A549, hepatocellular carcinoma HepG2, oral epidermoid carcinoma KB, breast cancer MCF-7, cervical carcinoma Hela and colorectal carcinoma HCT116, were inhibited by the treatment of each compound with different concentrations (Table 1 and 2). However, compared with that of cisplatin, the inhibitory effects of compounds were slight. Comfortingly, all compounds have lower cytotoxicity to LO2 normal cells (Table S1 of the ESI).

In vitro inhibition evaluation of compounds in combination with cisplatin

To investigate whether compounds **6a-6h**, **10a-10c** and **13a-13c** have activities in tumour therapy, the combination effects of each compound and cisplatin against HCT116 cells were evaluated in this study. As shown in Table 3, the inhibitory rate of cisplatin (2.5 μM) was 26.98%, while the inhibition rate of each compound (2.5 μM) was low. It is worthy to note that the compounds, especially **6e** and **6h**, can enhance the sensitivity of tumour cells to cisplatin ($p < 0.01$ or $p < 0.001$). The inhibition rate of cisplatin increased from 26.98% to 50.81% and 54.78%, after combining with **6e** and

6h, respectively. However, the cytotoxicity against LO2 normal cells did not significantly increase in **6e/6h** + cisplatin group (Table S2 of the ESI).

Morphological analysis by Hoechst staining

We next explored the morphology of cancer cell nuclei by Hoechst 33258 staining. Hoechst 33258 is a membrane-permeable fluorescent dye that can stain the chromatin of cells. In cells with normal morphology, the nuclei stained by Hoechst 33258 were round and light-blue coloured, and the chromatin was stained blue. While the apoptotic cells have condensed chromatins, bright blue nuclei, and nuclear fragmentation.⁴⁰⁻⁴¹ In this study, the treatment of cisplatin, **6e** or **6h** alone can induce morphological changes of HCT116 nuclei. In fact, the morphology of cancer cell nuclei could undergo further changes such as remarkable chromatin condensation, cell shrinkage, and evident reduction in the number of adherent cells (Fig. 3a). The number of nuclei were quantified and analyzed that shown in Fig. 3b, which was consistent with MTT assay.

Apoptosis analysis by FCM

To further understand the morphological changes of cancer cells, Annexin V-FITC/PI dual-labelling was performed by FCM. As shown in Fig. 4a, cisplatin, **6e** and **6h** at different concentrations can induce HCT116 cell apoptosis at different degrees. Remarkably, the percentage of Annexin V-positive cells significantly increase in the **6e** + cisplatin group, which indicated that cisplatin and **6e** could produce synergistic apoptosis in the appropriate concentration (Fig. 4b). At the same time, the percentage of PI-positive cells was enhanced remarkably ($p < 0.001$) compared with that with cisplatin or **6e** alone (Fig. 4c). Similar results were also observed in **6h** + cisplatin group. Together with Hoechst 33258 staining experiment, we propose that apoptosis may be one signalling pathway for the enhanced cisplatin sensitization effects of compounds **6e** and **6h**.

Western blot analysis

The cisplatin sensitization mechanism initiated by **6e/6h** in combination with cisplatin was explored by western blot. The ATR/CHK1 signalling pathway can regulate many common proteins, such as p53, CDC25 phosphatases, and Wee1 kinase, which further regulated G1, S and G2/M checkpoints. Besides, suppresses of CHK1 signalling pathway will lead to DDR damage and then may trigger DNA damage-induced apoptosis or senescence.⁴² It has been reported that some small molecule compounds, such as SCH900776 (MK-8776)³⁶ and CBP-93872³⁹, can enhance the cisplatin sensitivity by inhibiting phosphorylation of CHK1, and induce cell death.

Therefore, the expression of CHK1 in HCT116 cancer cells was examined in this study. The results showed that phosphorylated CHK1 was upregulated by cisplatin treatment (Fig. 5), which may be attributed to the DNA-damage agent cisplatin, leading to the DDR in cancer cells. However, the CHK1 expression was decreased in **6e** + cisplatin and **6h** + cisplatin groups with a concentration-dependent manner, indicating that **6e** and **6h** can inactivate the CHK1 induced by cisplatin treatment and then achieve the synergistic anti-tumour effects. The expression of CHK1 can recover to normal in **6e** (2.5 μM)

+ cisplatin and **6h** (5 μM) + cisplatin groups.

Table 1. In vitro cancer cell growth inhibiting rate of compound (10 μ M) alone.

Compound (10 μ M)	Inhibition rate (% of control)					
	A549	HepG2	KB	MCF-7	Hela	HCT116
6a	9.51 \pm 1.38	6.98 \pm 1.67	5.20 \pm 1.80	2.83 \pm 1.11	17.53 \pm 0.25	15.71 \pm 1.13
6b	-	-	-	-	-	14.73 \pm 0.59
6c	12.11 \pm 0.50	6.65 \pm 2.10	3.02 \pm 0.21	4.27 \pm 0.44	18.36 \pm 1.02	7.83 \pm 2.58
6d	-	-	-	-	-	16.22 \pm 1.46
6e	8.95 \pm 1.95	13.34 \pm 2.12	7.50 \pm 2.19	7.76 \pm 0.67	2.57 \pm 0.38	25.19 \pm 0.65
6f	26.76 \pm 0.90	11.61 \pm 2.75	9.30 \pm 0.66	13.07 \pm 0.38	1.64 \pm 0.76	17.00 \pm 2.69
6g	3.59 \pm 0.61	11.09 \pm 2.52	7.58 \pm 2.62	8.10 \pm 0.97	15.68 \pm 0.10	18.65 \pm 1.19
6h	11.22 \pm 0.95	8.94 \pm 1.91	3.19 \pm 1.41	10.58 \pm 1.44	18.48 \pm 1.25	24.44 \pm 0.69
10a	-	-	-	-	-	11.83 \pm 2.71
10b	12.51 \pm 0.78	12.67 \pm 1.84	9.73 \pm 3.29	19.69 \pm 0.56	13.65 \pm 0.43	18.30 \pm 2.66
10c	9.77 \pm 1.63	14.93 \pm 2.31	19.49 \pm 2.88	26.90 \pm 0.30	31.29 \pm 0.18	21.63 \pm 2.40
13a	-	-	-	-	-	19.68 \pm 1.54
13b	-	-	-	-	-	20.53 \pm 2.07
13c	-	-	-	-	-	18.89 \pm 0.77
Cisplatin	68.52 \pm 0.53	76.16 \pm 3.94	42.43 \pm 3.58	27.37 \pm 0.56	44.16 \pm 0.56	57.76 \pm 1.09

Table 2. In vitro cancer cell growth inhibiting rate of compound (5 μ M) alone.

Compound (5 μ M)	Inhibition rate (% of control)					
	A549	HepG2	KB	MCF-7	Hela	HCT116
6a	8.17 \pm 0.50	5.46 \pm 0.22	4.38 \pm 2.73	1.38 \pm 0.65	8.58 \pm 0.34	8.60 \pm 2.16
6b	-	-	-	-	-	9.26 \pm 1.42
6c	6.76 \pm 2.01	3.49 \pm 0.30	2.16 \pm 0.81	2.35 \pm 0.35	7.08 \pm 0.04	5.61 \pm 1.32
6d	-	-	-	-	-	11.34 \pm 3.27
6e	7.92 \pm 2.15	13.20 \pm 1.76	3.70 \pm 0.81	1.86 \pm 0.36	1.49 \pm 0.48	20.35 \pm 1.25
6f	23.77 \pm 0.21	11.25 \pm 3.26	2.15 \pm 2.10	0.86 \pm 0.10	1.07 \pm 0.57	16.38 \pm 1.25
6g	3.11 \pm 0.15	9.45 \pm 2.84	2.55 \pm 3.38	3.78 \pm 0.50	8.47 \pm 1.71	14.86 \pm 2.89
6h	6.69 \pm 0.67	6.90 \pm 1.65	0.43 \pm 0.60	6.26 \pm 0.30	10.45 \pm 0.34	20.44 \pm 1.46
10a	-	-	-	-	-	15.86 \pm 1.02
10b	7.60 \pm 0.99	11.51 \pm 4.45	7.35 \pm 0.81	4.65 \pm 3.23	4.80 \pm 0.58	17.86 \pm 2.56
10c	1.44 \pm 0.85	5.10 \pm 1.20	2.15 \pm 1.06	11.95 \pm 0.42	3.55 \pm 1.58	12.29 \pm 2.97
13a	-	-	-	-	-	11.09 \pm 0.24
13b	-	-	-	-	-	10.93 \pm 2.17
13c	-	-	-	-	-	12.56 \pm 1.84
Cisplatin	39.77 \pm 1.01	55.09 \pm 3.21	24.30 \pm 3.29	7.76 \pm 1.35	34.99 \pm 0.16	41.12 \pm 0.39

Table 3. In vitro cancer cell growth inhibiting rate of compound combined with cisplatin for HCT116 cells.

	Inhibition rate (% of control)		
	Cisplatin (2.5 μ M)	Compound (2.5 μ M)	Cisplatin (2.5 μ M) + Compound (2.5 μ M)
	26.98 \pm 2.84		
6a		0.53 \pm 2.17	27.19 \pm 2.16
6b		4.32 \pm 0.88	28.59 \pm 1.63
6c		2.64 \pm 1.71	29.16 \pm 1.88
6d		5.37 \pm 1.71	34.28 \pm 2.56
6e		8.13 \pm 3.63	50.81 \pm 1.35 ^{***###}
6f		2.06 \pm 3.38	24.91 \pm 2.06
6g		10.71 \pm 1.29	39.46 \pm 0.75
6h		7.47 \pm 0.73	54.78 \pm 2.70 ^{***###}
10a		10.42 \pm 2.08	20.13 \pm 1.37
10b		13.73 \pm 3.16	35.43 \pm 1.99
10c		9.81 \pm 5.15	29.83 \pm 0.36
13a		9.87 \pm 2.03	20.06 \pm 2.12
13b		7.54 \pm 1.26	19.57 \pm 0.29
13c		10.15 \pm 0.27	25.64 \pm 1.82

* p <0.05, ** p <0.01 and *** p <0.001 vs. cisplatin group; # p <0.05, ### p <0.01 and #### p <0.001 vs. compound group.

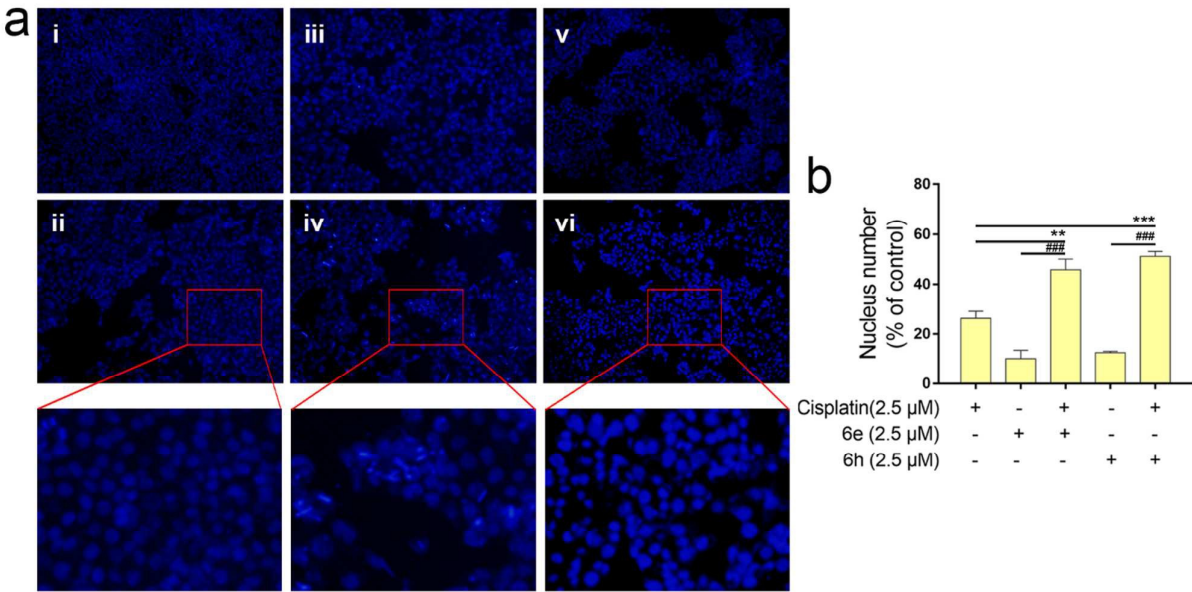


Fig. 3. Fluorescence microscopic appearance of Hoechst 33258-stained nuclei of HCT116 cells with the treatment of compounds **6e** and **6h** in combination with cisplatin. HCT116 cells were treated with **6e** or **6h** alone for 24 h before combination with cisplatin (2.5 μ M) for 48 h. (a) i, control; ii, cisplatin (2.5 μ M); iii, **6e** (2.5 μ M); iv, **6e** (2.5 μ M) + cisplatin (2.5 μ M); v, **6h** (2.5 μ M); vi, **6h** (2.5 μ M) + cisplatin (2.5 μ M). (b) The blue fluorescence was quantified and analyzed by *T* test. All data were from three independent experiments. * p <0.05, ** p <0.01 and *** p <0.001 vs. cisplatin group; # p <0.05, ### p <0.01 and #### p <0.001 vs. compound group.

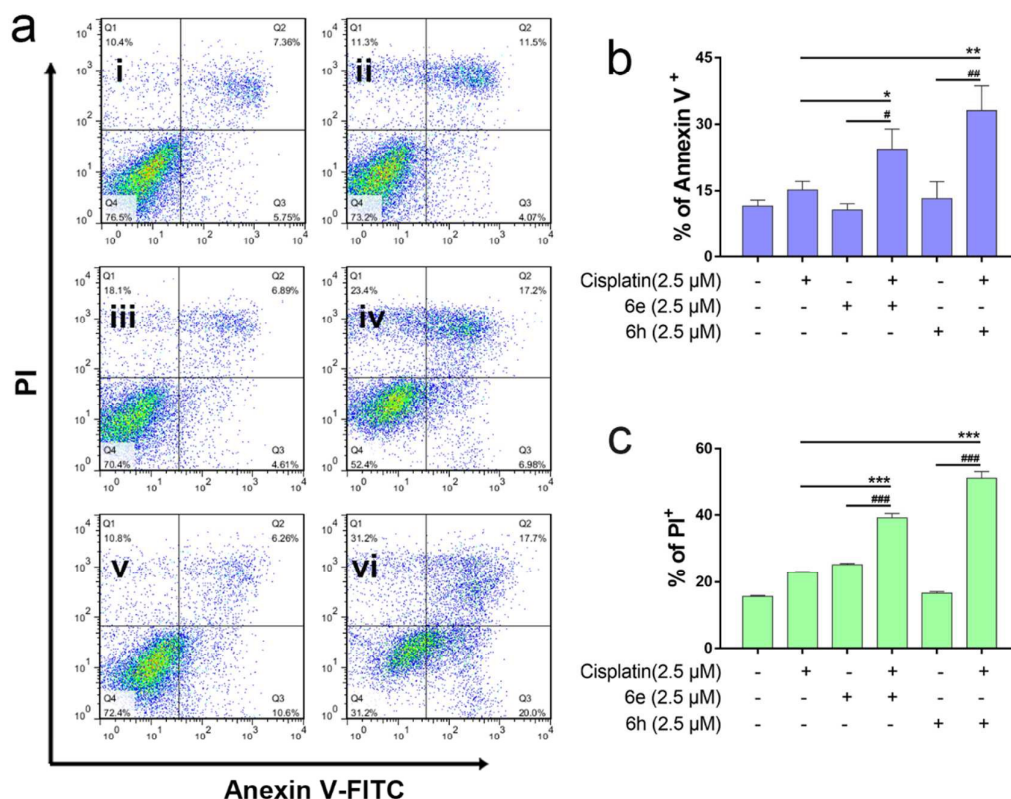


Fig. 4. Apoptosis analysis of compounds **6e** and **6h** in combination with cisplatin. HCT116 cells were treated with **6e/6h** alone for 24 h before combination with cisplatin for 48 h. Cell apoptosis were evaluated by flow cytometry. (a) i, control; ii, cisplatin (2.5 μ M); iii, **6e** (2.5 μ M); iv, **6e** (2.5 μ M) + cisplatin (2.5 μ M); v, **6h** (2.5 μ M); vi, **6h** (2.5 μ M) + cisplatin (2.5 μ M). (b and c) Percentage of Annexin V⁺ and PI⁺ cells analyzed of **6e** and **6h**. All data were from three independent experiments. * p <0.05, ** p <0.01 and *** p <0.001 vs. cisplatin group; # p <0.05, ## p <0.01 and ### p <0.001 vs. compound group.

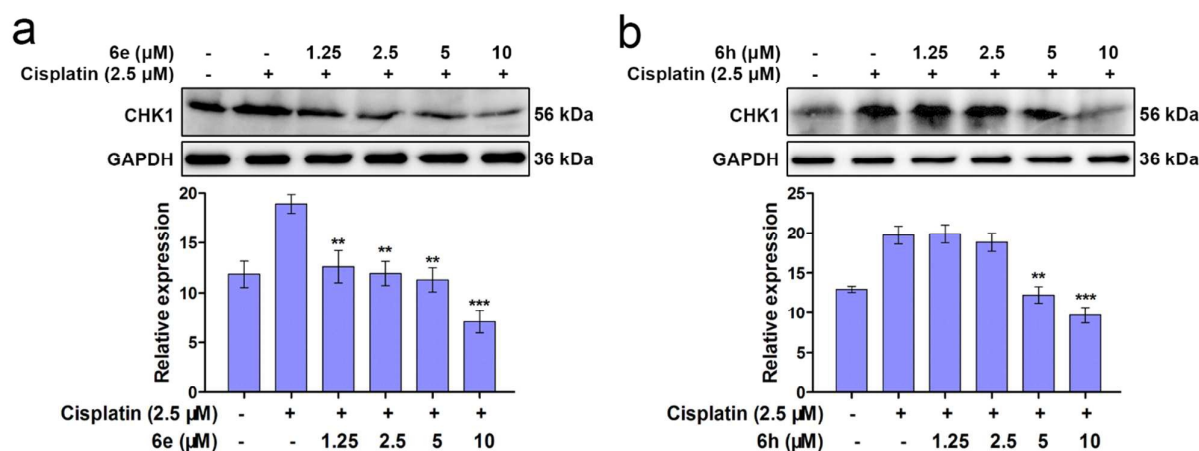


Fig. 5. Compounds **6e** (a) and **6h** (b) in combination with cisplatin inhibited Chk1 expression. HCT116 cells were treated with **6e** or **6h** alone for 24 h before combination with cisplatin (2.5 μ M) for 48 h. Chk1 expression was quantified by the densitometry analysis using ImageJ. * p <0.05, ** p <0.01 and *** p <0.001 vs. cisplatin group.

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Binding mode of **6e** and **6h** into ATR

Based on the above western blot result, we suspected that the **6e** and **6h** might interact with ATR signaling pathway. Therefore, in an attempt to explain the cisplatin sensitization effects of **6e** and **6h**, the molecular docking to ATR⁴³ was performed in our research and the results showed that the N1 and N8 in the naphthyridine ring and the hybrid atoms in the oxadiazol ring are involved in H-bond interactions with Val170, Glu168 and Tyr155 (Figure 6). Additionally, the naphthyridine ring is also involved in π - π stack with Trp169. Besides the key binding of the skeleton mentioned above, the sulfone group in **R**₂ also forms the hydrogen bond with Gly175. Moreover, the *p*-methylphenyl ring in **R**₁ is involved in hydrophobic interaction with Lys117, Leu122 and Pro159.

Biological activities of the tested compounds could be correlated to structure variations. In view of **6a-6h**, it was found that the replacement of phenyl with pyridine group resulted in the decrease in the sensitization activity, which can be ascribed to the decline of hydrophobic interaction with Lys117, Leu122 and Pro159 of ATR kinase pocket. Furthermore, exploration of the impact of the substitution on the different position of the phenyl group demonstrated that 4-modification is more beneficial than 3-position (**6e** and **6h**). With regard to 4-substituted compound counterparts in **6f**, **6g**, and **6h**, the sensitivity activities were decreased in the order of -CH₃ > -Cl > -F, hinting that the appropriate electron density is significant for the activity. Additionally, 4-(methylsulfonyl)benzene were converted to two different substitutions to explore the influence of the substituted group at 6-position on activity. However, the sensitivities against HCT116 was almost lost, which can be attributed to the absent of H-bond interaction between the sulfone group and Gly175.

Conclusions

In summary, a series of 3-(1,3,4-oxadiazol-2-yl)-1,8-naphthyridin-4(1*H*)-one derivatives were synthesized and their anti-tumour activities in vitro against A549, HepG2, KB, MCF-7, Hela, and HCT116 cells were tested in this research. Regrettably, all compounds have only slight activities. However, the anti-tumour activities initiated by compounds in combination with cisplatin were measured and the results showed that compound **6e** and **6h** bearing the good cisplatin sensitization. Hoechst staining and annexin V-FITC/PI dual-labeling studies demonstrated that the combination of **6e/6h** (2.5 μ M) and cisplatin (2.5 μ M) can significantly induce apoptosis of HCT116 cancer cells.

ATR is the most important protein in DDR network and western blot displayed that cisplatin treatment lead to the significant upregulating of CHK1 expression in HCT116 cancer cells, which indicated that the DNA-damage agent cisplatin treatment can cause HCT116 cells to initiate DDR, produce drug resistance and ultimately induce parts of tumour cell survival. While the CHK1 expression was decreased in **6e** + cisplatin and

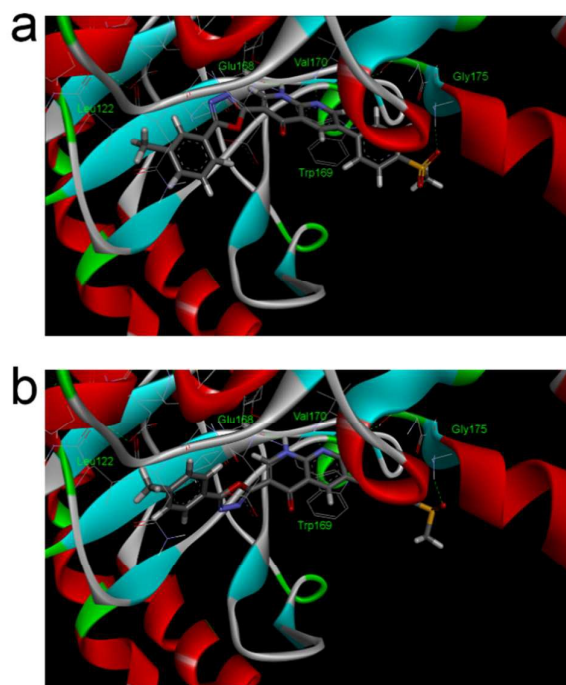


Fig. 6. Theoretical binding mode of **6e** (a) and **6h** (b) to ATR active binding pocket.

6h + cisplatin groups, suggesting that **6e** and **6h** can inhibit the DDR response, and increase the sensitivity of tumour cells to cisplatin. Furthermore, molecular docking was employed in our study and docking of **6e/6h** into the ATR structure active site revealed that the N1 and N8 atoms in the naphthyridine ring and the hybrid atom in the oxadiazol ring are involved in hydrogen bonding with Val170, Glu168 and Tyr155. Additionally, the naphthyridine ring is also involved in π - π stack with Trp169. Taken together, 3-(1,3,4-oxadiazol-2-yl)-1,8-naphthyridin-4(1*H*)-one derivatives **6e** and **6h** were discovered and deserved further investigation as potential cisplatin sensitizing agents for HCT116 cancer cells.

Conflicts of interest

The authors declare no conflict of interest.

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Experimental

Materials and instruments

Unless specified otherwise, all chemicals and reagents used in this study were of analytical grade and used without any purification. The reactions were monitored by thin layer chromatography (TLC) on pre-coated silica GF254 plates (Qingdao Ocean Chemical Factory, China). Melting points (uncorrected) were determined with a SGW X-4 melting points apparatus (Shanghai precision & scientific instrument Co., Ltd, China). Magnetic resonance spectra (NMR) were detected on a Bruker AC-E400 spectrometer

under 130 °C in an open system for 2 h. Then cooled down to room temperature followed by recrystallization in ethanol to get yellow solid **1** (2.6 g). Yield: 75.7%. ¹H NMR (400 MHz, CDCl₃) δ = 11.12 (d, *J* = 12.5 Hz, 1H), 9.07 (d, *J* = 12.7 Hz, 1H), 8.39 (d, *J* = 2.2 Hz, 1H), 7.75 (dd, *J* = 8.6 Hz, *J* = 2.4 Hz, 1H), 6.78 (d, *J* = 8.6 Hz, 1H), 4.50-4.12 (m, 4H), 1.36 dt, *J* = 15.9 Hz, *J* = 7.1 Hz, 6H).

Synthesis of ethyl 6-bromo-4-oxo-1,4-dihydro-1,8-naphthyridine-3-carboxylate (**2**)

Phenyl ether (30 mL) was heated under stirring at 250 °C. Diethyl 2-(((5-bromopyridin-2-yl)amino)methylene)malonate (10g, 29.1 mmol) was slowly added, and the resulting mixture was refluxed for 30-40 min. After the mixture was cooled at room temperature, the resulting precipitate was collected by filtration, washed with petroleum ether, and recrystallized from DMF to get the white solid **2** (7.8 g). Yield: 90.0%. ¹H NMR (400 MHz, CDCl₃) δ = 9.38 (d, *J* = 1.4 Hz, 1H), 9.04 (s, 1H), 7.97 (dd, *J* = 9.3 Hz, *J* = 2.0 Hz, 1H), 7.67 (d, *J* = 9.3 Hz, 1H), 4.43 (q, *J* = 7.1 Hz, 2H), 1.42 (t, *J* = 7.1 Hz, 3H).

Synthesis of ethyl 6-(4-(methylsulfonyl)phenyl)-4-oxo-1,4-dihydro-1,8-naphthyridine-3-carboxylate (**3**)

4-(Methylsulfonyl)phenylboronic acid (2.53 g, 12.65 mmol) was added to a solution of intermediate **2** (10.54 mmol) in 1,4-dioxane/H₂O mixture (5:1, 20 mL), followed by Na₂CO₃ (3.32 g, 31.62 mmol), Dichlorobis(triphenylphosphine)palladium(II) (220 mg, 0.32 mmol). The mixture was then heated and stirred at 60 °C under nitrogen atmosphere for 8 h. After being cooled to room temperature, the mixture was diluted with DCM (120 mL), washed with H₂O (3×40 mL), and dried (Na₂SO₄). The solvent was then evaporated under reduced pressure, and the residue was purified by chromatography to afford white solid **3** (3.7 g). Yield: 94.1%, mp, 233.5-234.5 °C. ¹H NMR (400 MHz, CDCl₃) δ = 9.52 (s, 1H), 9.08 (s, 1H), 8.21 (d, *J* = 9.0 Hz, 1H), 8.13 (d, *J* = 8.2 Hz, 2H), 7.92 (d, *J* = 9.1 Hz, 1H), 7.88 (d, *J* = 8.3 Hz, 2H), 4.44 (q, *J* = 7.1 Hz, 2H), 3.13 (s, 3H), 1.44 (t, *J* = 7.1 Hz, 3H). ESI-QTOF-MS: 395.0766 (C₂₃H₁₆N₄O₄SN_a, M+Na⁺), Anal. Calcd for C₁₈H₁₆N₂O₅S: 372.0780.

Synthesis of 6-(4-(methylsulfonyl)phenyl)-4-oxo-1,4-dihydro-1,8-naphthyridine-3-carbohydrazide (**4**)

3 (2.68 mmol) obtained by a known method was suspended in methanol (15 mL) and to this mixture, hydrazine monohydrate (1 mL) was added dropwise at room temperature for 3 h. The precipitate was separated from the reaction mixture by means of suction filtration to give the **4** (600 mg). Yield: 62.3%. ¹H NMR (400 MHz, DMSO-*d*₆) δ = 9.77 (s, 1H), 9.42 (s, 1H), 9.05

operating at a field strength of 400 MHz at room temperature with TMS and solvent signals allotted as internal standards. Chemical shifts are reported in ppm (δ) with references. Standard and peak multiplicities are designated as follows: s, singlet; d, doublet; dd, double doublets; t, triplet; q, quartet; brs, broad singlet; and m; multiplet. Mass spectrometry (MS) data were obtained by ESI-QTOF spectrometer. Purity of the all compounds was evaluated by a HPLC system (Dionex Ultimate 3000, USA) was found to be higher than 98%.

Synthesis of diethyl 2-(((5-bromopyridin-2-yl)amino)methylene)malonate (**1**)

Condensation of 5-bromopyridin-2-amine (1.73 g, 10 mmol) with diethyl ethoxymethylene-malonate (2.16 g, 10 mmol) were heated (s, 1H), 8.61 (dd, *J* = 9.1 Hz, *J* = 1.7 Hz, 1H), 8.14 (q, *J* = 8.4 Hz, 4H), 8.03 (d, *J* = 9.1 Hz, 1H), 4.71 (s, 2H), 3.31 (s, 3H).

Synthesis of compounds **5a-5h**

Appropriate aromatic acid (0.63 mmol) was reacted with SOCl₂ (4 mL) and DMF as catalyst refluxed for 1 h to prepare acid chlorides. The product was used as this in the next step. A solution of **4** (150 mg, 0.42 mmol) and DIEA (162 mg, 1.26 mmol) in THF (3 mL) was added to a stirring solution of acid chlorides in 30 mL of THF at 0 °C. The mixture was stirred at room temperature overnight. The white precipitate was recovered by filtration, washed three times with 5 mL portions of THF and dried overnight under vacuum.

General procedure for the synthesis of compounds **6a-6h**

To a mixture of the appropriate intermediate (**5a-5h**, 0.32 mmol) in SOCl₂ (5 mL), pyridine (50 mg, 0.64 mmol) was added dropwise. The reaction mixture was then gradually heated to the reflux temperature and maintained there for 3 h. The remaining SOCl₂ was removed by rotary evaporation and poured into saturated NaHCO₃ solution to acidify to pH 7. Then the aqueous layer was extracted with CH₂Cl₂, and the combined organic layers were dried over Na₂SO₄, filtered and concentrated. The products (**6a-6h**) were purified by column chromatography.

6-(4-(methylsulfonyl)phenyl)-3-(5-phenyl-1,3,4-oxadiazol-2-yl)-1,8-naphthyridin-4(1H)-one (6a). Yellow solid: yield 53.0%, mp 301.1-302.0 °C. ¹H NMR (400 MHz, DMSO-*d*₆) δ = 9.49 (s, 1H), 9.16 (s, 1H), 8.65 (d, *J* = 9.0 Hz, 1H), 8.19 (d, *J* = 8.1 Hz, 2H), 8.13 (d, *J* = 7.6 Hz, 4H), 8.07 (d, *J* = 9.1 Hz, 1H), 7.67 (s, 3H), 3.32 (s, 3H). ESI-QTOF-MS: 445.0891 (C₂₃H₁₇N₄O₄S, M+H⁺), Anal. Calcd for C₂₃H₁₆N₄O₄S: 444.0892.

6-(4-(methylsulfonyl)phenyl)-3-(5-(pyridin-3-yl)-1,3,4-oxadiazol-2-yl)-1,8-naphthyridin-4(1H)-one (6b). Yellow solid: yield 49.1%, mp 297.7-299.2 °C. ¹H NMR (400 MHz, DMSO-*d*₆) δ = 9.49 (s, 1H), 9.30 (s, 1H), 9.19 (s, 1H), 8.84 (s, 1H), 8.65 (d, *J* = 8.5 Hz, 1H), 8.49 (d, *J* = 6.8 Hz, 1H), 8.16 (d, *J* = 14.9 Hz, 4H), 8.07 (d, *J* = 8.1 Hz, 1H), 7.70 (s, 1H), 3.32 (s, 3H). ESI-QTOF-MS: 446.0847 (C₂₂H₁₆N₅O₄S, M+H⁺), Anal. Calcd for C₂₂H₁₅N₅O₄S, 445.0845.

6-(4-(methylsulfonyl)phenyl)-3-(5-(pyridin-4-yl)-1,3,4-oxadiazol-2-yl)-1,8-naphthyridin-4(1H)-one (6c). Yellow solid: yield 60.3%, mp 320.9-321.6 °C. ¹H NMR (400 MHz, DMSO-*d*₆) δ = 9.51 (s, 1H), 9.20 (s, 1H), 8.89 (d, *J* = 5.3 Hz, 2H), 8.67 (d, *J* = 9.5 Hz, 1H), 8.19 (d, *J* = 8.4 Hz, 2H), 8.14 (d, *J* = 8.3 Hz, 2H), 8.09 (d, *J* = 9.1 Hz, 1H), 8.05 (d, *J* = 5.4 Hz, 2H), 3.32 (s, 3H). ESI-QTOF-MS: 446.0848 (C₂₂H₁₆N₅O₄S, M+H⁺), Anal. Calcd for C₂₂H₁₅N₅O₄S, 445.0845.

6-(4-(methylsulfonyl)phenyl)-3-(5-(pyridin-2-yl)-1,3,4-oxadiazol-2-yl)-1,8-naphthyridin-4(1H)-one (6d). Yellow solid: yield 42.2%, mp 291.2–292.3 °C. ¹H NMR (400 MHz, DMSO-*d*₆): δ = 9.48 (d, *J* = 2.0 Hz, 1H), 9.11 (s, 1H), 8.83 (d, *J* = 4.8 Hz, 1H), 8.67 (d, *J* = 2.1 Hz, 1H), 8.64 (d, *J* = 2.2 Hz, 1H), 8.27 (d, *J* = 7.8 Hz, 1H), 8.18 (d, *J* = 8.5 Hz, 4H), 8.09 (dd, *J* = 9.5 Hz, *J* = 5.3 Hz, 2H), 7.67 (dd, *J* = 7.5 Hz, *J* = 5.0 Hz, 1H), 3.32 (s, 3H). ESI-QTOF-MS: 446.0847 (C₂₂H₁₆N₅O₄S, M+H⁺). Anal. Calcd for C₂₂H₁₅N₅O₄S, 445.0845.

6-(4-(methylsulfonyl)phenyl)-3-(5-(*m*-tolyl)-1,3,4-oxadiazol-2-yl)-1,8-naphthyridin-4(1H)-one (6e). Yellow solid: yield 47.4%, mp 311.2–312.9 °C. ¹H NMR (400 MHz, DMSO-*d*₆): δ = 9.49 (d, *J* = 1.9 Hz, 1H), 9.15 (s, 1H), 8.64 (dd, *J* = 9.1 Hz, *J* = 2.1 Hz, 1H), 8.19 (d, *J* = 8.5 Hz, 2H), 8.13 (d, *J* = 8.4 Hz, 2H), 8.06 (d, *J* = 9.1 Hz, 1H), 7.94 (s, 1H), 7.92 (d, *J* = 7.8 Hz, 1H), 7.54 (t, *J* = 7.6 Hz, 1H), 7.48 (d, *J* = 7.5 Hz, 1H), 3.32 (s, 3H), 2.45 (s, 3H). ESI-QTOF-MS: 459.1051 (C₂₄H₁₉N₄O₄S, M+H⁺). Anal. Calcd for C₂₄H₁₈N₄O₄S, 458.1049.

3-(5-(4-chlorophenyl)-1,3,4-oxadiazol-2-yl)-6-(4-(methylsulfonyl)phenyl)-1,8-naphthyridin-4(1H)-one (6f). Yellow solid: yield 55.0%, mp 320.0–320.9 °C. ¹H NMR (400 MHz, DMSO-*d*₆): δ = 9.49 (s, 1H), 9.16 (s, 1H), 8.65 (dd, *J* = 9.1 Hz, *J* = 1.9 Hz, 1H), 8.18 (d, *J* = 8.3 Hz, 2H), 8.13 (d, *J* = 7.6 Hz, 4H), 8.07 (d, *J* = 9.1 Hz, 1H), 7.74 (d, *J* = 8.5 Hz, 2H), 3.31 (s, 3H). ESI-QTOF-MS: 479.0505 (C₂₃H₁₆ClN₄O₄S, M+H⁺). Anal. Calcd for C₂₃H₁₅ClN₄O₄S, 478.0503.

3-(5-(4-fluorophenyl)-1,3,4-oxadiazol-2-yl)-6-(4-(methylsulfonyl)phenyl)-1,8-naphthyridin-4(1H)-one (6g). Yellow solid: yield 50.4%, mp 307.3–308.2 °C. ¹H NMR (400 MHz, DMSO-*d*₆): δ = 9.49 (d, *J* = 2.0 Hz, 1H), 9.16 (s, 1H), 8.65 (dd, *J* = 9.1 Hz, *J* = 2.2 Hz, 1H), 8.21–8.19 (m, 1H), 8.18 (d, *J* = 2.2 Hz, 2H), 8.13 (d, *J* = 8.5 Hz, 2H), 8.07 (d, *J* = 9.1 Hz, 1H), 7.51 (t, *J* = 8.9 Hz, 2H), 3.32 (s, 3H). ESI-QTOF-MS: 463.0800 (C₂₃H₁₆FN₄O₄S, M+H⁺). Anal. Calcd for C₂₃H₁₅FN₄O₄S, 462.0798.

6-(4-(methylsulfonyl)phenyl)-3-(5-(*p*-tolyl)-1,3,4-oxadiazol-2-yl)-1,8-naphthyridin-4(1H)-one (6h). Yellow solid: yield 59.2%, mp 300.0–301.2 °C. ¹H NMR (400 MHz, DMSO-*d*₆): δ = 9.48 (d, *J* = 1.7 Hz, 1H), 9.13 (s, 1H), 8.64 (dd, *J* = 9.1 Hz, *J* = 2.0 Hz, 1H), 8.18 (d, *J* = 8.4 Hz, 2H), 8.13 (d, *J* = 8.4 Hz, 2H), 8.05 (d, *J* = 9.1 Hz, 1H), 8.00 (d, *J* = 8.0 Hz, 2H), 7.45 (d, *J* = 8.0 Hz, 2H), 3.32 (s, 3H), 2.42 (s, 3H). ESI-QTOF-MS: 459.1051 (C₂₄H₁₉N₄O₄S, M+H⁺). Anal. Calcd for C₂₄H₁₈N₄O₄S, 458.1049.

Synthesis of 6-bromo-4-oxo-1,4-dihydro-1,8-naphthyridine-3-carbohydrazide (7)

Using a method similar to that of **4**, compound **7** was synthesized as a pink solid **7** (340 mg). Yield: 23.6%. ¹H NMR (400 MHz, DMSO-*d*₆): δ = 9.73 (s, 1H), 9.23 (d, *J* = 1.8 Hz, 1H), 9.02 (s, 1H), 8.32 (dd, *J* = 9.3 Hz, *J* = 2.1 Hz, 1H), 7.84 (d, *J* = 9.3 Hz, 1H), 4.79 (s, 2H).

Synthesis of compounds 8 and 11

Using a method similar to that of **5a**, compound **8** and **11** was synthesized as a brown solid. Yield: 76.1% and 90.0%, respectively.

Synthesis of 6-bromo-3-(5-(pyridin-3-yl)-1,3,4-oxadiazol-2-yl)-1,8-naphthyridin-4(1H)-one (9)

To a mixture of **8** (0.94 mmol) in SOCl₂ (5 mL), pyridine (160 mg, 2 mmol) was added dropwise. The reaction mixture was then gradually heated to the reflux temperature and maintained there for 3 h. The remaining SOCl₂ was removed by rotary evaporation and poured into saturated NaHCO₃ solution to acidify to pH 7. Then the aqueous layer was extracted with CH₂Cl₂, and the combined organic layers were dried over Na₂SO₄, filtered, and concentrated. The product **9a** was purified by column chromatography as a

yellow solid (250 mg, yield 65.9%). ¹H NMR (400 MHz, DMSO-*d*₆): δ = 9.27 (d, *J* = 2.2 Hz, 1H), 9.14 (s, 1H), 8.35 (dd, *J* = 9.3 Hz, *J* = 2.2 Hz, 1H), 8.13 (d, *J* = 8.6 Hz, 2H), 7.88 (d, *J* = 9.3 Hz, 1H), 7.73 (d, *J* = 8.6 Hz, 2H).

Synthesis of 6-bromo-3-(5-(4-chlorophenyl)-1, 3, 4-oxadiazol-2-yl)-1,8-naphthyridin-4(1H)-one (12)

Using a method similar to that of **9a**, compound **9b** was synthesized as a yellow solid (280 mg, yield 40.8%). ¹H NMR (400 MHz, DMSO-*d*₆): δ = 9.26 (d, *J* = 1.8 Hz, 1H), 9.12 (s, 1H), 8.34 (dd, *J* = 9.3 Hz, *J* = 2.2 Hz, 1H), 8.17 (dd, *J* = 8.8 Hz, *J* = 5.4 Hz, 2H), 7.87 (d, *J* = 9.3 Hz, 1H), 7.50 (t, *J* = 8.9 Hz, 2H).

General procedure for the synthesis of compounds 10a–10c and 13a–13c

Appropriate boronic acid or boronic acid ester (0.089 mmol) was added to a solution of intermediate **9a–9b** (0.074 mmol) in 1,4-dioxane/H₂O mixture (5:1, 5 mL), followed by Na₂CO₃ (23 g, 0.223 mmol), dichlorobis(triphenylphosphine)palladium(II) (3 mg, 0.004 mmol). The mixture was then heated and stirred at 60 °C under nitrogen atmosphere for 8 h. After being cooled to room temperature, the mixture was diluted with DCM, washed with H₂O, and dried (Na₂SO₄). The solvent was then evaporated under reduced pressure, and the residue was purified by chromatography to afford compounds **10a–10c**. Compounds **13a–13c** were synthesized by the similar method.

3-(5-(4-chlorophenyl)-1,3,4-oxadiazol-2-yl)-4-oxo-6-(pyridin-3-yl)-1,4-dihydro-1,8-naphthyridin-1-ium (10a). Yellow powder: yield 80.4%, mp 305.5–306.5 °C. ¹H NMR (400 MHz, DMSO-*d*₆): δ = 9.47 (s, 1H), 9.16 (s, 1H), 9.10 (s, 1H), 8.73 (d, *J* = 4.8 Hz, 1H), 8.65 (d, *J* = 8.9 Hz, 1H), 8.33 (d, *J* = 8.0 Hz, 1H), 8.13 (d, *J* = 8.5 Hz, 2H), 8.06 (d, *J* = 9.4 Hz, 1H), 7.74 (d, *J* = 8.5 Hz, 2H), 7.67–7.56 (m, 2H). ESI-QTOF-MS: 402.0682 (C₂₁H₁₃ClN₅O₂, M+H⁺). Anal. Calcd for C₂₁H₁₂ClN₅O₂, 401.0680.

4-(6-(5-(4-chlorophenyl)-1,3,4-oxadiazol-2-yl)-5-oxo-5,8-dihydro-1,8-naphthyridin-3-yl)benzonitrile (10b). Yellow powder: yield 80.1%, mp 292.5–293.5 °C. ¹H NMR (400 MHz, DMSO-*d*₆): δ = 9.48 (s, 1H), 9.16 (s, 1H), 8.64 (d, *J* = 8.9 Hz, 1H), 8.16–8.10 (m, 2H), 8.08 (s, 1H), 8.07–8.04 (m, 1H), 7.74 (d, *J* = 8.4 Hz, 2H). ESI-QTOF-MS: 426.0683 (C₂₃H₁₃ClN₅O₂, M+H⁺). Anal. Calcd for C₂₃H₁₂ClN₅O₂, 425.0680.

3-(5-(4-chlorophenyl)-1,3,4-oxadiazol-2-yl)-6-phenyl-1,8-naphthyridin-4(1H)-one (10c). Yellow powder: yield 81.3%, mp 238.9–239.2 °C. ¹H NMR (400 MHz, DMSO-*d*₆): δ = 9.39 (d, *J* = 1.9 Hz, 1H), 9.13 (s, 1H), 8.60 (dd, *J* = 9.1 Hz, *J* = 2.1 Hz, 1H), 8.12 (d, *J* = 8.5 Hz, 2H), 8.02 (d, *J* = 9.1 Hz, 1H), 7.88 (t, *J* = 8.0 Hz, 2H), 7.73 (d, *J* = 8.5 Hz, 2H), 7.61 (t, *J* = 7.4 Hz, 2H), 7.53 (t, *J* = 7.3 Hz, 1H). ESI-QTOF-MS: 401.0730 (C₂₂H₁₄ClN₄O₂, M+H⁺). Anal. Calcd for C₂₂H₁₃ClN₄O₂, 400.0727.

3-(5-(4-fluorophenyl)-1,3,4-oxadiazol-2-yl)-6-(pyridin-3-yl)-1,8-naphthyridin-4(1H)-one (13a). Yellow powder: yield 82.4%, mp 306.2–307.1 °C. ¹H NMR (400 MHz, DMSO-*d*₆): δ = 9.47 (s, 1H), 9.15 (s, 1H), 9.10 (s, 1H), 8.73 (s, 1H), 8.65 (d, *J* = 8.9 Hz, 1H), 8.33 (d, *J* = 7.3 Hz, 1H), 8.19 (s, 1H), 8.06 (d, *J* = 9.1 Hz, 1H), 7.62 (s, 1H), 7.51 (t, *J* = 8.6 Hz, 2H). ESI-QTOF-MS: 386.0978 (C₂₁H₁₃FN₅O₂, M+H⁺). Anal. Calcd for C₂₁H₁₂FN₅O₂, 385.0975.

3-(5-(4-fluorophenyl)-1,3,4-oxadiazol-2-yl)-6-(pyridin-4-yl)-1,8-naphthyridin-4(1H)-one (13b). Yellow powder: yield 76.2%, mp 260.1–262.1 °C. ¹H NMR (400 MHz, DMSO-*d*₆): δ = 9.53 (d, *J* = 1.8 Hz, 1H), 9.15 (s, 1H), 8.78 (d, *J* = 5.1 Hz, 2H), 8.65 (dd, *J* = 9.1 Hz, 2.0, 1H), 8.18 (dd, *J* = 8.7 Hz, *J* = 5.4 Hz, 2H), 8.06 (d, *J* = 9.1 Hz, 1H), 7.94 (d, *J* = 5.8 Hz, 2H), 7.50 (t, *J* = 8.8 Hz, 2H). ESI-QTOF-MS: 386.0978 (C₂₁H₁₃FN₅O₂, M+H⁺). Anal. Calcd for C₂₁H₁₂FN₅O₂, 385.0975.

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4-(6-(5-(4-fluorophenyl)-1,3,4-oxadiazol-2-yl)-5-oxo-5,8-dihydro-1,8-naphthyridin-3-yl)benzonitrile (13c). Yellow powder: yield 86.1%, mp 339.0–340.5 °C. ¹H NMR (400 MHz, DMSO-*d*₆): δ = 9.48 (s, 1H), 9.16 (s, 1H), 9.03 (s, 1H), 8.63 (d, *J* = 8.9 Hz, 1H), 8.22–8.15 (m, 2H), 8.12 (d, *J* = 8.4 Hz, 2H), 8.08 (s, 1H), 8.05 (d, *J* = 8.9 Hz, 1H). ESI-QTOF-MS: 410.1047 (C₂₁H₁₃FN₅O₂, M+H⁺). Anal. Calcd for C₂₁H₁₂FN₅O₂, 409.0975.

Cell lines and culture

A549, HepG2, KB, MCF-7, Hela, and HCT116 cancer cells were maintained in Dulbecco's minimum essential medium (DMEM, Gibco, USA) or Roswell Park Memorial Institute-1640 (RPMI-1640, Gibco, USA), supplemented with 10% fetal bovine serum (FBS; Gibco, Auckland, N.Z.), 100 U/mL penicillin and 100 U/mL streptomycin in a humidified atmosphere containing 5% CO₂ at 37 °C. In addition, the human normal hepatocyte cell line LO2 was introduced in this study to evaluated the cytotoxicity of 3-(1,3,4-oxadiazol-2-yl)-1,8-naphthyridin-4(1H)-one derivatives.

Cell viability assay by MTT

Cells were seeded into 96-well plate 24 h before experimental manipulation. Then, cells were treated with the compounds. Cells were treated with compound alone for 24 h before combination with cisplatin (2.5 μM) for 48 h. After incubation, 20 μL of a 5 mg/mL 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) solution was added, and the plates were incubated for an additional 2–4 h at 37 °C. The medium was subsequently removed, and DMSO was then added. The OD₅₇₀ was measured using a Spectra MAX M5 microplate spectrophotometer (Molecular Devices, CA, USA). For combination treatment, cells were treated with compounds alone for 24 h before combination with cisplatin (2.5 μM) for 48 h.

Morphological analysis by Hoechst staining

The morphological changes were evaluated by Hoechst 33258 staining. HCT116 cells were treated with 6e/6h alone for 24 h before combination with cisplatin (2.5 μM) for 48 h. Then, cells were fixed with 4% paraformaldehyde and stained with a solution of Hoechst 33258 dye (5 μg/mL). Next, the nuclear morphology of cells was observed by fluorescence microscopy (Zeiss, Axiovert 200, Germany), and the number of nuclei were satisfied and analyzed.

Apoptosis analysis by FCM

To detect the apoptosis effect of 6e/6h combined with cisplatin, the Annexin V-FITC/PI apoptosis detection kit was used in this section. Briefly, HCT116 cells were treated with 6e/6h alone for 24 h before combination with cisplatin (2.5 μM) for 48 h. The cells were then harvested and washed with cold PBS. After centrifugation, the cells were stained with Annexin V-FITC and PI and analyzed by flow cytometry (Becton-Dickinson, USA).

Western blot analysis

HCT116 cells were collected by scraping and lysis in RIPA buffer (50 mM Tris HCL, 1% NP-40, 0.1% SDS, 150 mM NaCl, 1 mM EDTA and 0.5% sodium deoxycholate) containing protease and phosphatase inhibitors. The total protein concentrations were measured by the enhanced BCA Protein Assay Kit (Beyotime[®] Biotechnology, China). Equal amounts of protein were loaded onto 12% SDS-PAGE gels (Willget Biotech, Shanghai, China) and were electro-transferred to Millipore 0.2 μm PVDF membranes. After washing, the membranes were blocked with 5% skimmed milk and incubated with the primary antibody (anti-CHK1 mouse mAb) at 4 °C overnight. Antibodies were detected with horseradish peroxidase (HRP)-conjugated secondary antibody and developed with an enhanced chemiluminescence detection kit

(Luminata Crescendo Western HRP Substrate or Immobilon Western Chemiluminescent HRP Substrate, Millipore Corporation, Billerica, MA, USA). The membranes were probed for GAPDH to confirm equal loading. In this study, the anti-CHK1 primary antibody (220064) was a product of Zen Bioscience Co., Ltd. (Chengdu) and GAPDH primary antibody (AF5718) was purchased from R&D Systems, Inc. (Minneapolis, MN).

Molecular docking

Structure based docking studies were carried out by using GOLD V3.0.1. 3D conformations of the 2 molecules were generated and minimized using molecular mechanics (MM2) method and hamiltonian approximations Austin model 1 (AM1) method available in the MOPAC2009. The RESP charges were assigned to the molecules by 3 steps: at first, ESP charges of the molecule were calculated (HF/6-31G* OPT ESP) based on the structure using Gaussian 03; then the restrained ESP fitting was conducted using Antechamber program in the AMBER; at last, the obtained RESP charges were assigned to the Sybyl mol2 files of each molecule. The kinase domain of ATR was built by homology modelling with PI3K γ as template. A sphere of 10 Å around the binding site in the ATR kinase domain was defined as the docking site. RMSD of early termination was set to 1.5 Å. GA parameters was set to GOLD default and for each molecule, a number of 30 dockings were performed.

References

- M.H. Hsu, C.Y. Liu, C.M. Lin, Y.J. Chen, C.J. Chen, Y.F. Lin, L.J. Huang, K.H. Lee, S.C. *Toxicol Appl Pharmacol.* **2011**, 259, 219–226.
- K. Chen, S.C. Kuo, M.C. Hsieh, A. Mauger, C.M. Lin, E. Hamel, K.H. Lee. *J Med Chem.* **1997**, 40, 2266–2275.
- K.Chen, S.C. Kuo, M.C. Hsieh, A.Mauger, C.M. Lin, E. Hamel, K.H. Lee. *J.Med.Chem.* **1997**, 40, 3049–3056.
- H.K. Gençer, S. Levent, U. Acar Çevik, Y. Özkay, S. Ilgin. *Bioorg Med Chem Lett.* **2017**, 27, 1162–1168.
- C. M. Bébéar, O. Grau, A. Charron, H. Renaudin, D. Gruson, and C. Bébéar. *Antimicrob Agents Chemother.* **2000**, 44, 2719–2727.
- T. Kuroda, F. Suzuki, T. Tamura, K. Ohmori, H. Hosoe. *J Med Chem.* **1992**, 35, 1130–1136.
- V. Lucchesi, D.P. Hurst, D.M. Shore, S. Bertini, B.M. Ehrmann, M. Allarà, L. Lawrence, A. Ligresti, F. Minutolo, G. Saccomanni, H. Sharir, M. acchia, V. Di Marzo, M.E. Abood, P.H. Reggio, C. Manera. *J Med Chem.* **2014**, 57, 8777–8791.
- G. Saccomanni, G.P. ascali, S.D. Carlo, D. Panetta, M. De Simone, S. Bertini, S. Burchielli, M. Digiacomio, M. Macchia, C. Manera, P.A. Salvadori. *Bioorg Med Chem Lett.* **2015**, 25, 2532–2535.
- A.M. Malfitano, C. Laezza, A. D'Alessandro, C. Procaccini, G. Saccomanni, T. Tuccinardi, C. Manera, M. Macchia, G. Matarese, P. Gazzerro, M. Bifulco. *PLoS One.* **2013**, 8, e62511.
- C. Manera, V. Benetti, M.P. Castelli, T. Cavallini, S. Lazzarotti, F. Pibiri, G. Saccomanni, T. Tuccinardi, A. Vannacci, A. Martinelli, P.L. Ferrarini. *J Med Chem.* **2006**, 49, 5947–5957.
- N. Yadav, P. Kumar, A. Chhikara, M. Chopra. *Biomed Pharmacother.* **2017**, 95, 721–730.
- A. Andreani, M. Granaola, A. Leoni, A. Locatelli, R. Morigi, M. Rambaldi, *Eur J Med Chem.* **2001**, 36, 743–746.
- C.D. Morris, A. Rose, J. Curwen, A.M. Hughes, D.J. Wilson, D.J. Webb. *Br J Cancer.* **2005**, 92, 2148–2152.
- J. Sławiński, K. Szafranski, A. Pogorzelska, B. Żołnowska, A. Kawiak, K. Macur, M. Belka, T. Bączek. *Eur J Med Chem.* **2017**, 132, 236–248.
- F.A.F. Ragab, S.M. Abou-Seri, S.A. Abdel-Aziz, A.M. Alfayomy, M. Aboelmagd. *Eur J Med Chem.* **2017**, 138, 140–151.
- M.D. Altıntop, B. Sever, G. Akalın Çiftçi, G. Turan-Zitouni, Z.A. Kaplançıklı, A. Özdemir. *Eur J Med Chem.* **2018**, 155, 905–924.

- 17 X. He, X.Y. Li, J.W. Liang, C. Cao, S. Li, T.J. Zhang, F.H. Meng. *Bioorg Med Chem Lett.* **2018**, 28, 847-852.
- 18 J. Sun, S.Z. Ren, X.Y. Lu, J.J. Li, F.Q. Shen, C. Xu, H.L. Zhu. *Bioorg Med Chem.* **2017**, 25, 2593-2600.
- 19 M.J. Ahsan, A. Choupra, R.K. Sharma, S.S. Jadav, P. Padmaja, M.Z. Hassan, A.B.S. Al-Tamimi, M.H. Geesi, M.A. Bakht. *Anticancer Agents Med Chem.* **2018**, 18, 121-128.
- 20 R. Trummer, W. Rangsimawong, W. Sajomsang, M. Kumpugdee-Vollrath, P. Opanasopit, P. Tonglairoum. *MedChemComm.* **2018**, 8, 22967-22973.
- 21 L.I. Toledo, M. Murga, O. Fernandez-Capetillo. *Mol Oncol.* **2011**, 5, 368-373.
- 21 J.M. Furgason, M. Bahassi el. Targeting DNA repair mechanisms in cancer. *Pharmacol Ther.* **2013**, 137, 298-308.
- 22 L.H. Einhorn. *Proc Natl Acad Sci U S A.* **2002**, 99, 4592-4595.
- 23 G.J. Peters, C.L. van der Wilt, C.J. van Moorsel, J.R. Kroep, A.M. Bergman, S.P. Ackland. *Pharmacol Ther.* **2000**, 87, 227-253.
- 24 A. Wilmes, C. Bielow, C. Ranninger, P. Bellwon, L. Aschauer, A. Limonciel, H. Chassaigne, T. Kristl, S. Aiche, C. G. Huber, C. Guillou, P. Hewitt, M. O. Leonard, W. Dekant, F. Bois, P. Jennings. *Toxicol. In Vitro.* **2015**, 30, 117-127.
- 25 S.P. Jackson, J. Bartek. *Nature.* **2009**, 461, 1071-1078.
- 26 V.G. Gorgoulis, L.V. Vassiliou, P. Karakaidos, P. Zacharatos, A. Kotsinas, T. Liloglou, M. Venere, R.A. Jr. Dittullo, N.G. Kastrinakis, B. Levy, D. Kletsas, A. Yoneta, M. Herlyn, C. Kittas, T.D. Halazonetis. *Nature.* **2005**, 434, 907-913.
- 27 J. Bartkova, Z. Horejsi, K. Koed, A. Krämer, F. Tort, K. Zieger, P. Guldberg, M. Sehested, J.M. Nesland, C. Lukas, T. Ørntoft, J. Lukas, J. Bartek. *Nature.* **2005**, 434, 864-870.
- 28 T.G. Oliver, K.L. Mercer, L.C. Sayles, J.R. Burke, D. Mendus, K.S. Lovejoy, M.H. Cheng, A. Subramanian, D. Mu, S. Powers, D. Crowley, R.T. Bronson, C.A. Whittaker, A. Bhutkar, S.J. Lippard, T. Golub, J. Thomale, T. Jacks, E.A. Sweet-Cordero. *Genes Dev.* **2010**, 24, 837-852.
- 29 P. Borst, S. Rottenberg, J. Jonkers. *Cell Cycle.* **2008**, 7, 1353-1359.
- 30 D.B. Longley, P.G. Johnston. *J. Pathol.* **2005**, 205, 275-292.
- 31 J.W. Harper, S.J. Elledge. *Mol Cell.* **2007**, 28, 739-745.
- 32 B.B. Zhou, S.J. Elledge. *Nature.* **2000**, 408, 433-439.
- 33 K. Krüger, K. Geist, F. Stuhldreier, L. Schumacher, L. Blümel, M. Remke, S. Wesselborg, B. Stork, N. Klöcker, S. Bormann, W.P. Roos, S. Honnen, G. Fritz. *Cancer Lett.* **2018**, 430, 34-46.
- 34 L. Duan, R.Z. Perez, M. Hansen, S. Gitelis, C.G. Maki. *Cancer Biol Ther.* **2014**, 15, 1600-1612.
- 35 J. Herúdková, K. Paruch, P. Khirsariya, K. Souček, M. Krkoška, O. Vondálová Blanářová, P. Sova, A. Kozubík, A. Hyršlová Vaculová. *Neoplasia.* **2017**, 19, 830-841.
- 36 T. Iwata, T. Uchino, A. Koyama, Y. Johmura, K. Koyama, T. Saito, S. Ishiguro, T. Arikawa, S. Komatsu, M. Miyachi, T. Sano, M. Nakanishi, M. Shimada. *PLoS One.* **2017**, 12-24.
- 37 A. Peralta-Sastre, C. Manguan-Garcia, A. de Luis, C. Belda-Iniesta, S. Moreno, R. Perona, I. Sanchez-Perez. *Int J Biochem Cell Biol.* **2010**, 42, 318-328.
- 38 L.M. Pouliot, Y.C. Chen, J. Bai, R. Guha, S.E. Martin, M.M. Gottesman, M.D. Hall. *Cancer Res.* **2012**, 72, 5945-5955.
- 39 X. Hou, C. Yang, L. Zhang, T. Hu, D. Sun, H. Cao, F. Yang, G. Guo, C. Gong, X. Zhang, A. Tong, R. Li, Y. Zheng. *Biomaterials.* **2017**, 124, 195-210.
- 40 J. Tang, J.F. He, C.L. Yang, Y. Mao, T.T. Hu, L.J. Zhang, H. Cao, A.P. Tong, X.R. Song, G.G. He, G. Guo, Y.F. Luo, X.N. Zhang, Y.M. Xie, Y. Zheng. *J. Nanoparticle Res.* **2014**, 16, 2682-2699.
- 41 B. Fang. *J Med Chem.* **2014**, 57, 7859-7873.
- 42 Q. Liu, S. Guntuku, X.S. Cui, S. Matsuoka, D. Cortez, K. Tamai, G. Luo, S. Carattini-Rivera, F. DeMayo, A. Bradley, L.A. Donehower, S.J. Elledge. *Genes Dev.* **2000**, 14, 1448-1459.
- 43 L. Hao, J.Y. Shi, L.F. Lu, F. Wu, M. Zhou, X.Y. Hou, W.J. Zhang, Z.Y. Ding, R. Li. *Med Chem Res.* **2014**, 23: 747-758.