

# A novel anti-lung cancer agent inhibits proliferation and epithelial–mesenchymal transition

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## Abstract

**Objective:** To synthesize a novel chalcone-1,3,4-thiadiazole hybrid and investigate its anticancer effects against NCI-H460 cells.

**Methods:** (E)-3-(4-bromophenyl)-1-(4-hydroxyphenyl)prop-2-en-1-one, 1,3-dibromopropane and 1,3,4-thiadiazole-2-thiol were used as chemical materials to synthesize compound ZW97. The NCI-H460 lung cancer cell line was selected to explore the antitumor effects of compound ZW97 *in vitro* and *in vivo*.

**Results:** Compound ZW97 selectively inhibited cell proliferation against lung cancer cell lines NCI-H460, HCC-44 and NCI-H3122 with IC<sub>50</sub> values of 0.15 μM, 2.06 μM and 1.17 μM, respectively. ZW97 suppressed migration and the epithelial–mesenchymal transition process in NCI-H460 cells in a concentration-dependent manner. Based on the kinase activity results and docking analysis, compound ZW97 is a novel tyrosine-protein kinase Met (c-Met kinase) inhibitor. It also inhibited NCI-H460 cell growth in xenograft models without obvious toxicity to normal tissues.

**Conclusions:** Compound ZW97 is a potential c-Met inhibitor that might be a promising agent to treat lung cancer by inhibiting the epithelial–mesenchymal transition process.

## Keywords

Chalcone-1,3,4-thiadiazole, synthesize, NCI-H460 cells, epithelial–mesenchymal transition, c-Met inhibitor

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## Introduction

Chalcones, as one of the most widely investigated active compounds, have been reported to possess antitumour activity against many cancer cell lines *in vitro* and *in vivo*.<sup>1,2</sup> Lonchocarpin (1, Figure 1) significantly reduces cell proliferation and inhibits tumour growth in S180-bearing mice.<sup>3</sup> Chalcone 2 is a novel colchicine binding site inhibitor on  $\beta$ -tubulin to induce mitotic arrest and cell death.<sup>4</sup> Chalcone 3 induces apoptosis and inhibits migration in liver cancer and lung cancer cells.<sup>5</sup> Chalcone 4 exhibits an excellent cytotoxicity and inhibits the microtubule dynamics in NCI-60 cells.<sup>6</sup> Based on these findings, chalcone scaffold was used as an antitumor unit to design novel antiproliferative agents.

The 1,3,4-thiadiazole nucleus constitutes a significant class of compounds with potentially anticancer activity for new drug development.<sup>7</sup> The imidazo [2,1-*b*] [1,3,4] thiadiazole derivative 5 (Figure 2) displays remarkable antiproliferative activity and inhibits epithelial-to-mesenchymal transition of pancreatic ductal adenocarcinoma cell lines.<sup>8</sup> Fluorinated 3,6-diaryl-[1,2,4]triazolo[3,4-*b*][1,3,4]thiadiazole 6 shows antiproliferative potency against MCF7,

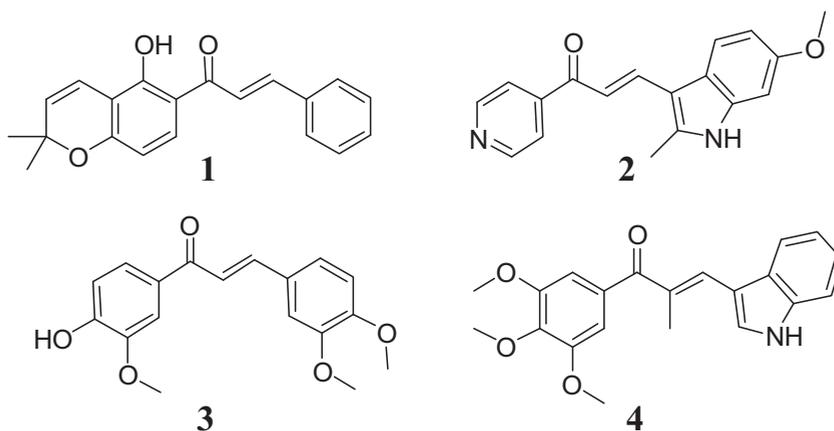
SaOS-2 and K562 cell lines.<sup>9</sup> The 2-arylamino-5-(indolyl)-1,3,4-thiadiazole 7 exhibits potent cytotoxicity with  $IC_{50}$  values of 0.91  $\mu$ M, 0.15  $\mu$ M and 0.44  $\mu$ M against MCF-7, LnCap and MDA-MB-231 cells, respectively.<sup>10</sup> The 5-aryl-2-(3-thienylamino)-1,3,4-thiadiazole 8 is synthesized in a good yield and has very interesting results with  $IC_{50} < 10 \mu$ M against H683 and B16F10 cells.<sup>11</sup>

Due to the frequent presence of the 1,3,4-thiadiazole nucleus and chalcone fragment in anticancer agents, this study hypothesized that a chalcone derivative with a 1,3,4-thiadiazole group might have excellent pharmacological activity. A chalcone-1,3,4-thiadiazole hybrid (ZW97) was designed and synthesized and its anticancer effects were evaluated in lung cancer cells.

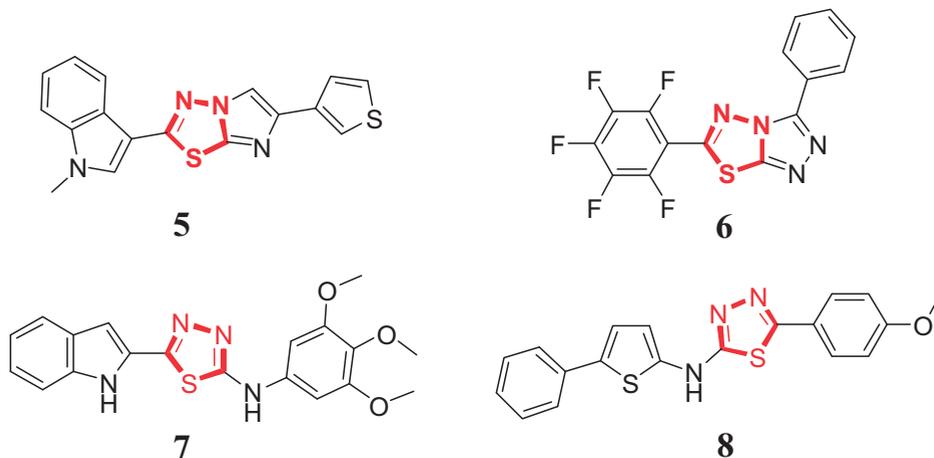
## Materials and methods

### General procedure of chalcone-1,3,4-thiadiazole hybrid ZW97

To a solution of (*E*)-3-(4-bromophenyl)-1-(4-hydroxyphenyl)prop-2-en-1-one (5 mmol) in acetone (20 ml), 1,3-dibromopropane (6 mmol) and potassium carbonate (5 mmol) were added carefully and the



**Figure 1.** Chalcone derivatives as anticancer agents.



**Figure 2.** The 1,3,4-thiadiazole derivatives as anticancer agents. The colour version of this figure is available at: <http://imr.sagepub.com>.

reaction mixture was stirred for 6 h at room temperature. Then, 1,3,4-thiadiazole-2-thiol (6 mmol) was added in the system and stirred for 3 h at room temperature. Upon completion, the residues were purified with column chromatography on silica gel (hexane/EtOAc = 10/1).

**(E)-1-(4-(3-((1,3,4-thiadiazol-2-yl)thio)propoxy)phenyl)-3-(4-bromophenyl)prop-2-en-1-one (ZW97)**

The structural information of the novel compound was as follows: white solid, yield: 81%; m.p.: 105–107°C;  $^1\text{H}$  NMR (400 MHz,  $\text{CDCl}_3$ )  $\delta$  9.02 (s, 1H), 8.03 (d,  $J = 8.8$  Hz, 2H), 7.73 (d,  $J = 15.6$  Hz, 1H), 7.64 – 7.41 (m, 5H), 6.98 (d,  $J = 8.8$  Hz, 2H), 4.21 (t,  $J = 5.8$  Hz, 2H), 3.60 (t,  $J = 7.0$  Hz, 2H), 2.53 – 2.21 (m, 2H).  $^{13}\text{C}$  NMR (100 MHz,  $\text{CDCl}_3$ )  $\delta$  188.36, 162.63, 151.48, 151.45, 142.58, 133.99, 132.17, 131.08, 130.87, 129.73, 124.56, 122.35, 114.36, 66.07, 30.73, 28.75. HRMS (ESI) calcd. for  $\text{C}_{20}\text{H}_{18}\text{BrN}_2\text{O}_2\text{S}_2$   $[\text{M} + \text{H}]^+$ : 460.9993, found: 460.9998.

**Cell viability using the MTT assay**

Cancer cell lines (TE-11, SK-BR-3, HO-8910, QGY-7701, SHG-44, HCC-44, COLO-320, SH-SY5Y, SW579, NCI-H460 and NCI-H3122) and normal cells (BEAS2B) were obtained from the Shanghai Institute of Biochemistry and Cell Biology (Shanghai, China). Cells were cultured in RPMI-1640 medium supplemented with 10% fetal bovine serum (RE-STEM BIOTECH, JiangShu, China) and penicillin-streptomycin (Aladdin, Shanghai, China).

Cells were seeded on a 24-well plate (SKILLBIO, Beijing, China) and incubated for 24 h at 37°C in a humidified atmosphere containing 5%  $\text{CO}_2$ . Compound ZW97 was added at different concentrations. Then, the medium was removed and the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) reagent (YuanYe BIO, Shanghai, China) was added to the cells and incubated for 2 h at 37°C. Cells were dissolved in dimethyl sulphoxide (DMSO) (YuanYe BIO) and the plate was shaken for 30 min. The absorbance was measured

on a DNM-9602G enzyme-labelled instrument (Beijing Planck New Technology, Beijing, China).

### *Wound healing*

NCI-H460 cells were seeded in a 24-well plate (SKILLBIO) and incubated for 24 h at 37°C in a humidified atmosphere containing 5% CO<sub>2</sub>. Then, the cell surface was scratched using a 2 µl pipette tip (SKILLBIO). NCI-H460 cells were cultured with compound ZW97 (0, 75 nM, 150 nM and 300 nM) for 48 h at 37°C in a humidified atmosphere containing 5% CO<sub>2</sub>. The plate was photographed on an inverted microscope (EVOS M5000; RE-STEM BIOTECH, Jiangsu, China).

### *Migration assay*

NCI-H460 cells were seeded in the top chamber of a 24-well transwell plate (SKILLBIO) in RPMI-1640 medium without fetal bovine serum. RPMI-1640 medium containing 10% fetal bovine serum was added to the lower chamber. After incubation for 48 h at 37°C in a humidified atmosphere containing 5% CO<sub>2</sub>, the NCI-H460 cells were fixed and stained with haematoxylin (YuanYe BIO). The extent of migration across the transwell was assessed by counting the number of cells that had migrated.

### *Western blotting*

NCI-H460 cells were seeded in 6-well plates (SKILLBIO) and treated with compound ZW97 for 48 h at 37°C in a humidified atmosphere containing 5% CO<sub>2</sub>. Then, 5 × 10<sup>6</sup> cells were collected and lysed using RIPA buffer (SKILLBIO). 10 µl of protein sample was loaded into each gel well. 20 µl proteins were separated by sodium dodecyl sulphate–polyacrylamide gel electrophoresis and transferred to polyvinylidene fluoride membranes (YuanYe BIO, Shanghai,

China). 5% defatted milk solution (YuanYe BIO, Shanghai, China) was used to block the membranes before adding the primary antibody for 2 h at 25°C. The primary antibodies were against E-cadherin and N-cadherin (both from Beyotime Biotechnology, Shanghai, China; dilution: 1:10000). The membranes were incubated with primary antibodies at 4°C for 12 h. After incubation with the primary antibody, 1 mM phosphate-buffered saline (PBS; pH 7.5; YuanYe BIO, Shanghai, China) was used to wash the membranes three times. Glyceraldehyde 3-phosphate dehydrogenase (GAPDH) was used the internal control using primary antibody diluted 1:50000 (YuanYe BIO, Shanghai, China). The membranes were incubated with anti-GAPDH antibodies at 25°C for 2 h. After incubation with the primary antibody, 1 mM PBS (pH 7.5) was used to wash the membranes three times. Finally, the bands were detected in using a FDbio-Pico enhanced chemiluminescence kit (YuanYe BIO, Shanghai, China). The protein levels were quantified in the experimental groups by comparing with the protein levels of the control group. The equipment for visualizing the immunoreactive signal was BIO-RADXR (Shanghai Shiwei Instrument Technology, Shanghai, China). The protein levels were quantified by the ImageJ software (National Institutes of Health, Maryland, America).

### *ELISA kinase assay*

The inhibitory effects of compound ZW97 against various tyrosine kinases (anaplastic lymphoma kinase [ALK], fibroblast growth factor receptor 1 [FGFR1], epidermal growth factor receptor [EGFR], tyrosine kinase A [TRKA], platelet-derived growth factor receptor α [PDGFRα]) were determined using enzyme-linked immunosorbent assays (ELISAs). Adenosine triphosphate solution diluted in kinase reaction buffer

(Cell Signaling Technology, Beverly, USA) was added to each well and compound ZW97 diluted in DMSO was added to each reaction well. DMSO was used as the negative control. The kinase reaction was initiated by the addition of purified tyrosine kinase proteins (Cell Signaling Technology®, Danvers, MA, USA). After incubation for 1 h at 37°C, the plate was washed three times with 1 mM PBS (pH 7.5) and horse radish peroxidase-conjugated goat anti-mouse IgG (Cell Signaling Technology®) was added. The plate was then incubated at 37°C for 30 min and washed three times with 1 mM PBS (pH 7.5) at 37°C for 10 min each time. Upon completion, the plate was analysed using a spectrophotometer (SKILLBIO). The minimum detectable concentration was 1 pg/ml for the targeted compound. Intra- and interassay coefficients of variation for all ELISAs were <0.05% and <0.05%, respectively.

### *Molecular docking study*

The crystal structure of tyrosine-protein kinase Met (c-Met kinase; code ID: 5ya5) was downloaded from the Protein Data Bank (<https://www.rcsb.org>). Firstly, waters and ligands were deleted. Hydrogen atoms were added to the protein using PyMOL software (<https://pymol.org/2/#products>). The 3D structures of compound ZW97 were built and minimized using molecular mechanics. Energy minimization and docking analysis were carried out using AutoDock version 4.2 software (The Scripps Research Institute, San Diego, USA).

### *Xenograft study*

BALB/c nude mice (20 mice; aged 4 weeks; 18–21 g) were purchased (Slack Laboratory Animals, Hunan, China) and xenograft models were established using NCI-H460 cells. The mice were housed under sterile conditions at 26–28°C and 40–60% relative

humidity in a 13-h light/11-h dark cycle with free access to food and water. When the tumour volumes had reached 100 mm<sup>3</sup>, tumour-bearing mice were randomly assigned to two groups (water vehicle or 50 mg/kg of compound ZW97 administered once every 3 days) with 5 mice per group mice. Mice in the treatment group received compound ZW97 by intragastric administration for 16 days. All mice were euthanized. The tumours were excised and weighed. The major organs, including heart, liver, spleen, lung and kidney were also collected from the mice. Haematoxylin and eosin staining was performed according to previous reports.<sup>12,13</sup>

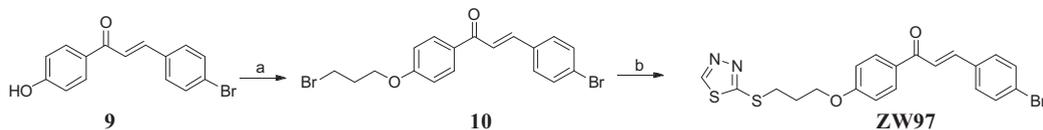
Experimentation on animals was undertaken according to the protocols from the Ethics Committee of Shanghai Jiao Tong University (no. 2019-ZW-012). The adequate care of the animals followed the ‘Guide for the Care and Use of Laboratory Animals, 8th Edition’. Efforts were made to minimize the number of animals utilized and to decrease their suffering.

### *Statistical analyses*

All statistical analyses were performed using the SPSS® statistical package, version 11.0 (SPSS Inc., Chicago, IL, USA) for Windows®. Data are presented as mean ± SEM and compared using factorial analysis of variance, Friedman test or Student’s *t*-test as appropriate. A *P*-value ≤0.05 was considered statistically significant.

## **Results**

The novel chalcone-1,3,4-thiadiazole hybrid ZW97 was synthesized as shown in Figure 3. Chalcone 9 was reacted with 1,3-dibromopropane in the presence of potassium carbonate to obtain chalcone 10. Chalcone-1,3,4-thiadiazole hybrid ZW97 was readily synthesized from chalcone 10 and 1,3,4-thiadiazole-2-thiol.



**Figure 3.** Reagents and conditions for the synthesis of the novel chalcone-1,3,4-thiadiazole hybrid ZW97. a) 1,3-dibromopropane in the presence of potassium carbonate and acetone; b) addition of 1,3,4-thiadiazole-2-thiol in the presence of acetone.

The NMR data of the compound are as follows:  $^1\text{H}$  NMR (400 MHz,  $\text{CDCl}_3$ )  $\delta$  9.02 (s, 1H), 8.03 (d,  $J=8.8$  Hz, 2H), 7.73 (d,  $J=15.6$  Hz, 1H), 7.64–7.41 (m, 5H), 6.98 (d,  $J=8.8$  Hz, 2H), 4.21 (t,  $J=5.8$  Hz, 2H), 3.60 (t,  $J=7.0$  Hz, 2H), 2.53 – 2.21 (m, 2H).  $^{13}\text{C}$  NMR (100 MHz,  $\text{CDCl}_3$ )  $\delta$  188.36, 162.63, 151.48, 151.45, 142.58, 133.99, 132.17, 131.08, 130.87, 129.73, 124.56, 122.35, 114.36, 66.07, 30.73, 28.75. HRMS (ESI) calcd. for  $\text{C}_{20}\text{H}_{18}\text{BrN}_2\text{O}_2\text{S}_2$   $[\text{M} + \text{H}]^+$ : 460.9993, found: 460.9998.

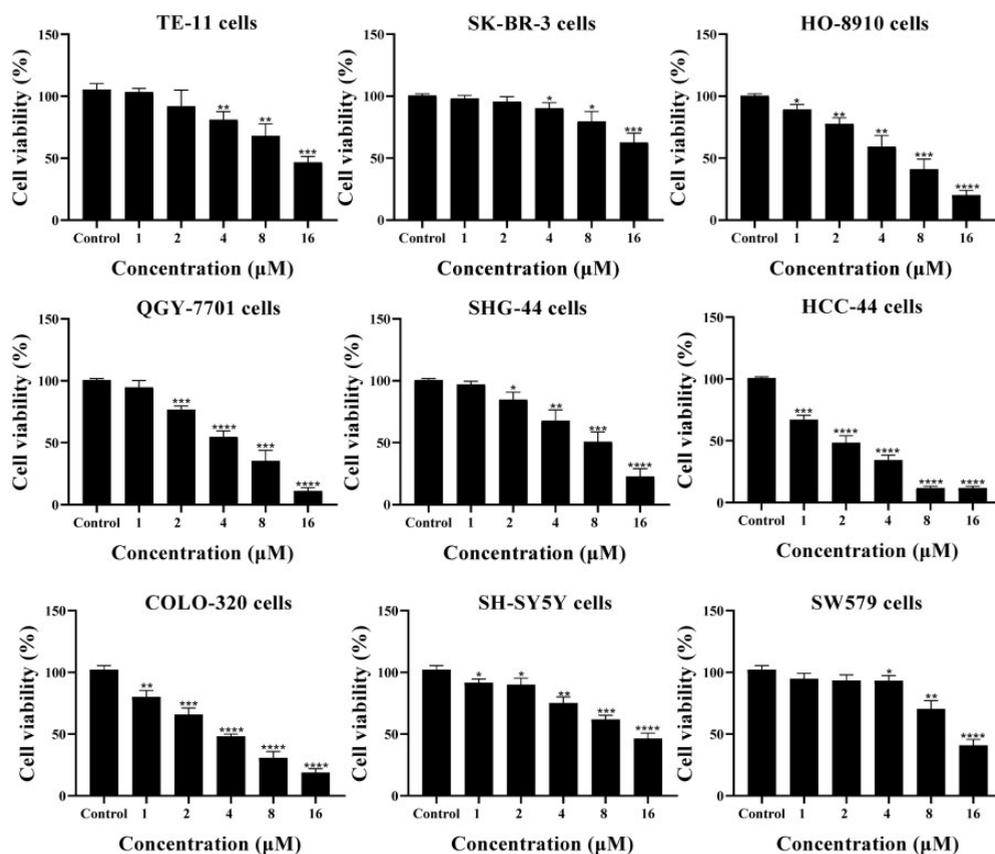
The MTT assay was used to measure cell viability in response to treatment with ZW97 for 48 h in the following nine cancer cell lines: TE-11 (oesophageal cancer cell line), SK-BR-3 (breast cancer cell line), HO-8910 (ovarian cancer cell line), QGY-7701 (liver cancer cell line), SHG-44 (glioma cell line), HCC-44 (lung cancer cell line), COLO-320 (colon cancer cell line), SH-SY5Y (neuroblastoma cell line) and SW579 (thyroid cancer cell line). As shown in Figure 4, compound ZW97 demonstrated antiproliferative activity against all nine cancer cell lines, indicating that it is a broad spectrum antiproliferative agent. Among the nine cell lines, chalcone-1,3,4-thiadiazole hybrid ZW97 showed the most potent antiproliferative efficacy of approximately 65.7% and 88.3% at  $4\ \mu\text{M}$  and  $8\ \mu\text{M}$ , respectively, against lung cancer HCC-44 cells.

As a consequence of the potent antiproliferative effects on lung cancer HCC-44 cells, different concentrations and durations

of treatment were chosen to test the anti-proliferative effects of compound ZW97 on the cell viability of three lung cancer cell lines (NCI-H460, NCI-H3122 and HCC-44). As shown in Figure 5, the  $\text{IC}_{50}$  values of compound ZW97 against NCI-H460, NCI-H3122 and HCC-44 cells were  $0.15\ \mu\text{M}$ ,  $1.17\ \mu\text{M}$ , and  $2.06\ \mu\text{M}$ , respectively. Compound ZW97 was further examined for possible cytotoxicity against BEAS2B (normal human epithelium cells), but it exhibited no cytotoxicity against these cells ( $\text{IC}_{50} > 64\ \mu\text{M}$ ). The results indicated that compound ZW97 had good selectivity between the selected lung cancer cells and normal cells.

From the results shown in Figure 6, chalcone-1,3,4-thiadiazole hybrid ZW97 inhibited the wound healing in a concentration dependent manner. The transwell assay demonstrated that compound ZW97 hindered NCI-H460 cell migration through the biological membrane. The levels of typical proteins involved in the epithelial–mesenchymal transition (EMT) process were measured using Western blot analysis.<sup>14–16</sup> Compound ZW97 upregulated the levels of E-cadherin, while N-cadherin, the mesenchymal cell biomarker, was downregulated. All these results indicated that chalcone ZW97 suppressed migration and inhibited epithelial–mesenchymal transition process in NCI-H460 cells.

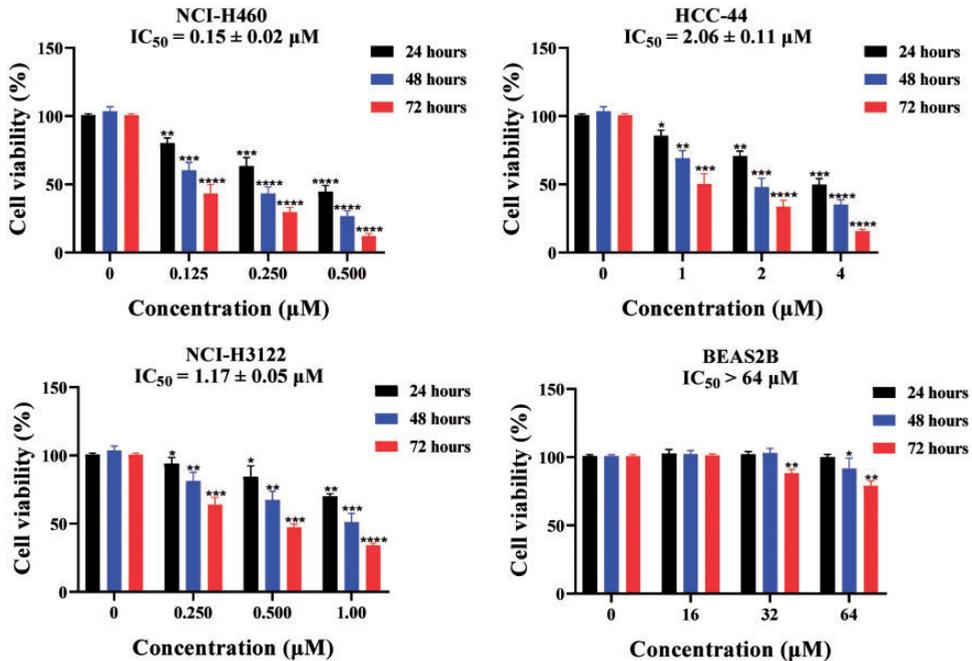
To examine the inhibitory effects on c-Met kinase and selectivity against a panel of tyrosine kinases (including ALK, FGFR1, EGFR, TRKA and PDGFR $\alpha$ ),



**Figure 4.** Cell viability of nine cancer cell lines after 48 h of treatment with a range of concentrations of the novel chalcone-1,3,4-thiadiazole hybrid ZW97. The data are presented as the mean  $\pm$  SEM. \* $P < 0.05$ , \*\* $P < 0.01$ , \*\*\* $P < 0.001$  and \*\*\*\* $P < 0.0001$  compared with the control group; Friedman test.

chalcone ZW97 was screened and the kinase inhibitory results were shown in Table 1. Compound ZW97 potently inhibited c-Met kinase with an  $IC_{50}$  value of 27.96 nM. However, compound ZW97 showed weak inhibitory activities against ALK, FGFR1, EGFR, TRKA and PDGFR $\alpha$  with  $IC_{50}$  values of  $>1500$  nM. In addition, the inhibitory activity of PHA-665752 as the reported c-Met inhibitor was also screened by this method. PHA-665752 displayed excellent inhibitory effects with an  $IC_{50}$  value of 16.32 nM. These data suggest that compound ZW97 might be a selective c-Met inhibitor.

To gain a better understanding of the binding processes of compound ZW97 with c-Met kinase, computational docking into the active sites of c-Met kinase to elucidate the structural basis was undertaken using AutoDock version 4.2 software. As shown in Figure 7A, chalcone-1,3,4-thiadiazole hybrid ZW97 was nicely bound to the active binding site and filled the cavity. The chalcone skeleton of compound ZW97 formed two hydrogen bonds with the residues of Tyr1230 and Asp1231 (Figure 7B). The 1,3,4-thiadiazole-2-thiol of compound ZW97 formed two hydrogen bonds with the residues of Leu1157 and



**Figure 5.** Cell viability of three lung cancer cell lines and normal BEAS2B cells after treatment with a range of concentrations of the novel chalcone-1,3,4-thiadiazole hybrid ZW97 for three treatment durations. The data are presented as the mean ± SEM. \* $P < 0.05$ , \*\* $P < 0.01$ , \*\*\* $P < 0.001$  and \*\*\*\* $P < 0.0001$  compared with the control group; Friedman test. The colour version of this figure is available at: <http://imr.sagepub.com>.

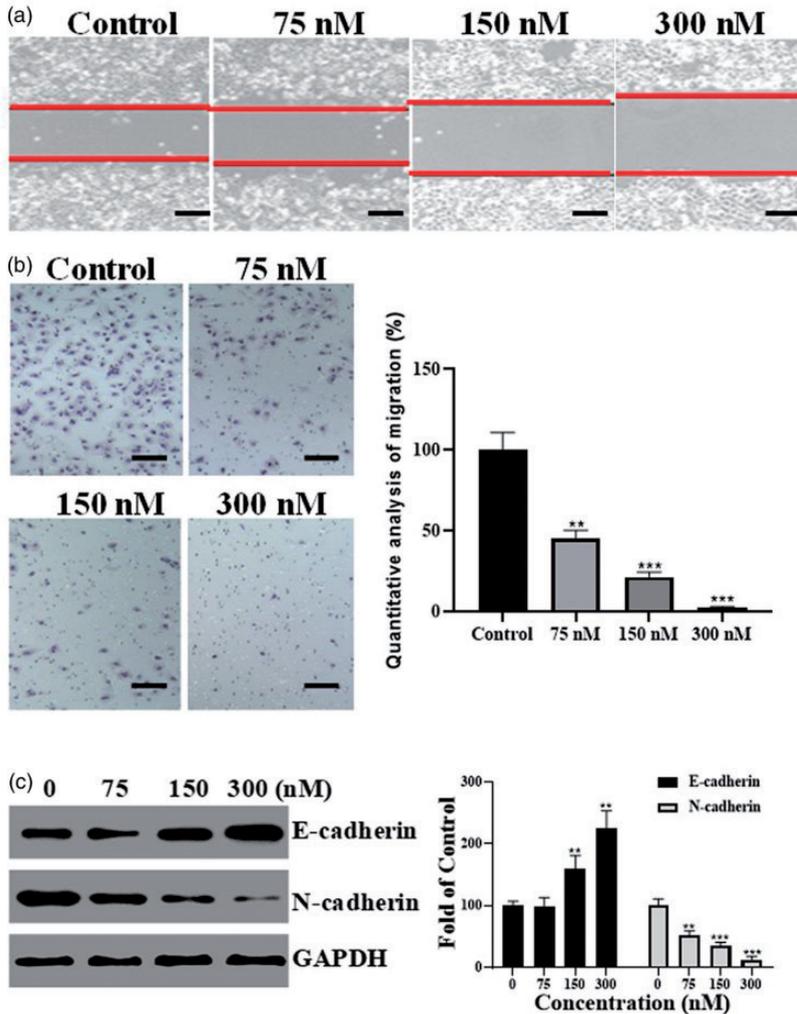
Met1160. The linker between the chalcone unit and the 1,3,4-thiadiazole unit formed a hydrogen bond with the residue of Asp1222. These molecular docking results also suggested that compound ZW97 might be a potential inhibitor of c-Met kinase.

To elucidate the antitumour effects of chalcone-1,3,4-thiadiazole hybrid ZW97, a xenograft model was established with NCI-H460 cells. Treated mice received intragastric administration of ZW97 for 16 days. As shown in Figure 8, chalcone-1,3,4-thiadiazole hybrid ZW97 suppressed NCI-H460 subcutaneous tumour growth. The mean ± SEM tumour weights of the vehicle group and ZW97 group were  $1.205 \pm 0.350$  g and  $0.463 \pm 0.125$  g (inhibitory rate: 61.58%) on day 16, respectively. Compound ZW97 did not significantly

decrease the mouse body weight compared with that of the control. In addition, haematoxylin and eosin staining of tumour sections suggested that chalcone-1,3,4-thiadiazole hybrid ZW97 displayed no obvious cytotoxicity on the major organs, including heart, liver, spleen, lung and kidney.

## Discussion

Lung cancer is a severe public health problem all around the world.<sup>17</sup> Chemotherapy using a range of chemical compounds is one of the most common cancer therapies, which is used to inhibit tumour cell division and induce cell death.<sup>18</sup> It is becoming increasingly important to design new anti-tumour drugs with potent biological activity against lung cancer cells.<sup>19</sup> In this current



**Figure 6.** Results of wound healing (a) and migration experiments (b) undertaken in NCI-H460 cells treated with three concentrations of the novel chalcone-1,3,4-thiadiazole hybrid ZW97 for 48 h; A, scale bar 100 μm; B, scale bar 100 μm. The levels of E-cadherin and N-cadherin protein in NCI-H460 cells after 48 h of treatment with three concentrations of ZW97 as measured using Western blot analysis (c). The internal control was glyceraldehyde 3-phosphate dehydrogenase (GAPDH). The data are presented as the mean ± SEM. \*\* $P < 0.01$  and \*\*\* $P < 0.001$  compared with the control group; Friedman test. The colour version of this figure is available at: <http://imr.sagepub.com>.

study, a novel chalcone-1,3,4-thiadiazole hybrid ZW97 was designed and synthesized based on the anticancer chalcone and 1,3,4-thiadiazole scaffolds. Chalcone-1,3,4-thiadiazole hybrid ZW97 as a broad spectrum antiproliferative agent potently

inhibited cell proliferation in lung cancer cell lines in a concentration-dependent and time-dependent manner. Its  $IC_{50}$  values against NCI-H460, NCI-H3122 and HCC-44 cells were 0.15 μM, 1.17 μM and 2.06 μM, respectively. Meanwhile,

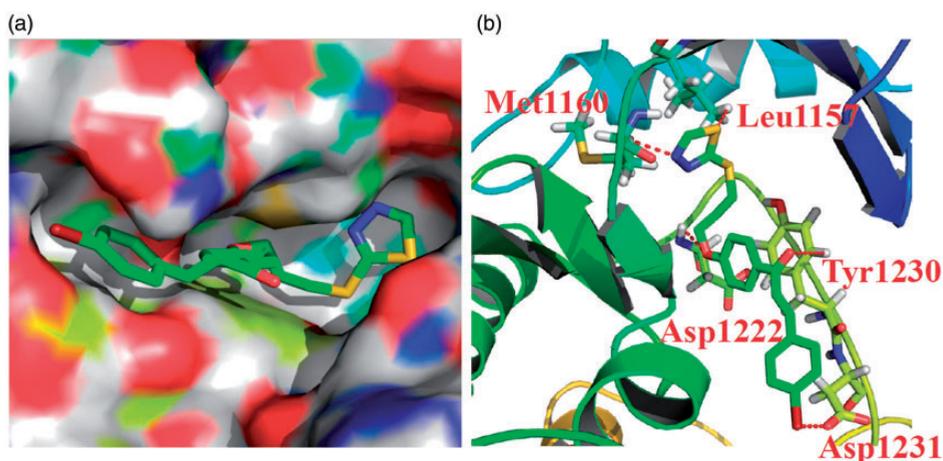
compound ZW97 exhibited no cytotoxicity against BEAS2B cells with an  $IC_{50}$  value of  $>64 \mu M$ , indicating that it had good selectivity between lung cancer cells and normal cells. In *in vivo* xenograft experiments, compound ZW97 also suppressed NCI-H460 cell growth. Due to the potent anticancer activity of compound ZW97, the design and synthesis of more analogues based on ZW97 as anticancer agents is planned for the future.

**Table 1.** Kinase inhibitory activity of the novel chalcone-1,3,4-thiadiazole hybrid ZW97.

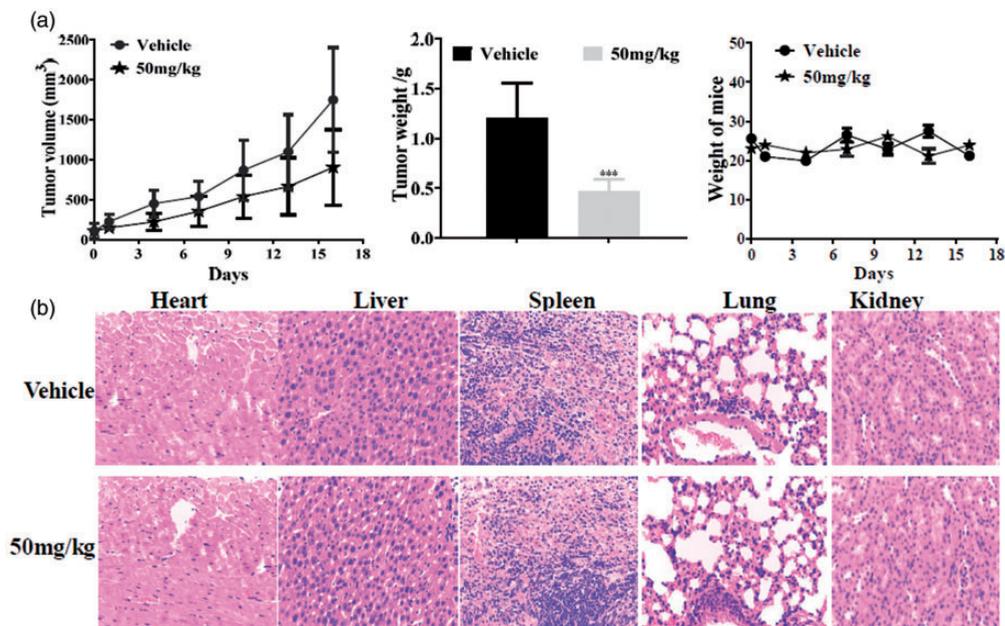
Kinase	Enzyme $IC_{50}$ , nM
c-Met	$27.96 \pm 0.14$
ALK	$>1500$
FGFR1	$>1500$
EGFR	$>1500$
TRKA	$>1500$
PDGFR $\alpha$	$>1500$

c-Met, tyrosine-protein kinase Met; ALK, anaplastic lymphoma kinase; FGFR1, fibroblast growth factor receptor 1; EGFR, epidermal growth factor receptor; TRKA, tyrosine kinase A; PDGFR $\alpha$ , platelet-derived growth factor receptor  $\alpha$ .

Tyrosine-protein kinase Met is a structurally distinct member of a heterodimeric transmembrane receptor tyrosine kinase family, which has an extracellular  $\alpha$  chain and a membrane spanning  $\beta$  chain.<sup>20</sup> Overexpression or abnormal activation of c-Met kinase has been reported to be associated with the formation and development of multiple cancers in liver, lung, prostate, breast, lymph, gastric and renal cancers.<sup>21</sup> Therefore, c-Met kinase has emerged as an attractive target for the development of antitumour agents. For example, among the reported c-Met inhibitors, crizotinib and cabozantinib are approved for the treatment of nonsmall-cell lung cancer and medullary thyroid cancer.<sup>22–29</sup> To date, no selective c-Met kinase inhibitors have been approved for clinical use. Based on the inhibitory results from the ELISA kinase assay, chalcone-1,3,4-thiadiazole hybrid ZW97 selectively and potently inhibited c-Met kinase with an  $IC_{50}$  of 27.96 nM. In addition, chalcone-1,3,4-thiadiazole hybrid ZW97 as a potent c-Met kinase inhibitor



**Figure 7.** Results of computational docking into the active sites of tyrosine-protein kinase Met (c-Met kinase) undertaken using AutoDock version 4.2 software demonstrated that the novel chalcone-1,3,4-thiadiazole hybrid ZW97 filled the cavity of c-Met kinase (a). The binding mode of ZW97 (green) at the c-Met kinase domain (b). The formed hydrogen bonds were marked in red. The colour version of this figure is available at: <http://imr.sagepub.com>.



**Figure 8.** Xenograft experiments established in BALB/c nude mice using NCI-H460 cells treated with the novel chalcone-1,3,4-thiadiazole hybrid ZW97: (a) Tumour volume, tumour weight (day 16) and weight of mice over the treatment period; (b) Haematoxylin and eosin staining of heart, liver, spleen, lung and kidney from mice treated with or without ZW97; scale bar 100 μm. The data were presented as the mean ± SEM. \*\*\* $P < 0.001$  compared with the control group; Friedman test. The colour version of this figure is available at: <http://imr.sagepub.com>.

also inhibited the epithelial–mesenchymal transition process in NCI-H460 cells. Thus, compound ZW97 might be a promising anticancer agent with potential clinical applications to treat lung cancer.

### Author contributions

All authors performed all experiments and analysed the experimental data. All authors approved the final manuscript.

### Declaration of conflicting interest

The authors declare that there are no conflicts of interest.

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