

Full Length Research Paper

Antitumor effect of N-(4-chlorophenyl)-5,6,7-trimethoxyquinazolin-4-amine dihydrochloride on tumor cells *in vitro*

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Accepted 9 May, 2012

Previous evidence demonstrated the antitumor ability of quinazolines and its derivatives. In this study, the anticancer activities of N-(4-chlorophenyl)-5,6,7-trimethoxyquinazolin-4-amine dihydrochloride against four kinds of cell lines were evaluated by 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) assay. The bioassay results indicated that title compound possessed wide spectrum of anticancer activity. Androgen-independent prostate cancer (PC-3) cells were employed and microscopic observation, lactate dehydrogenase (LDH) release assay, and Western blot were performed to study the antitumor mechanism of N-(4-chlorophenyl)-5,6,7-trimethoxyquinazolin-4-amine dihydrochloride against tumor cells. It was found that title compound executed anticancer activity in a dose-dependent manner with 24.46 μM IC_{50} of 72 h treatment. Microscopic observation and LDH release assay showed that the compound exerted such an effect through antiproliferation pathway rather than cytotoxicity. Furthermore, Western blot analysis revealed that treatment of cells with N-(4-chlorophenyl)-5,6,7-trimethoxyquinazolin-4-amine dihydrochloride (at more than 10 μM for 30 min) resulted in an almost complete inhibition of epidermal growth factor (EGF) induced phosphorylation of extracellular signal-regulated protein kinase1/2 (ERK1/2) which suggests that its antiproliferation effect is linked to its inhibition of ERK1/2 activation. These data imply that N-(4-chlorophenyl)-5,6,7-trimethoxyquinazolin-4-amine dihydrochloride is a potential anticancer agent capable of antiproliferation activity.

Key words: N-(4-chlorophenyl)-5,6,7-trimethoxyquinazolin-4-amine dihydrochloride, prostate cancer (PC-3) cell line, antiproliferation, extracellular signal-regulated protein kinase1/2 (ERK1/2).

INTRODUCTION

Heterocyclic compounds quinazolines and its derivatives are of important biological activity, including anticancer (Garrison et al., 2007; Harris et al., 2007; Martin et al., 2008; Yin et al., 2010; Assouline and Lipton, 2011), antiviral (el-Sherbeny et al., 2003; Kumar et al., 2010),

antimalarial (Nzila et al., 2010; Zhu et al., 2010), anti-inflammatory (Brullo et al., 2012) and antimicrobial activities (Ouyang et al., 2006; Yang et al., 2007; German et al., 2008; Rohini et al., 2009; Chevalier et al., 2010). Imatinib, Gefitinib, Erlotinib, and Vandetanib are a series of quinazoline anticancer drugs and have been commercialized and widely used in pharmaceuticals as cancer therapy agents. In recent years, some quinazoline anticancer drugs show selective quality, which are becoming research focus of molecular-targeted anticancer drug discovery. Consequently, significant emphasis

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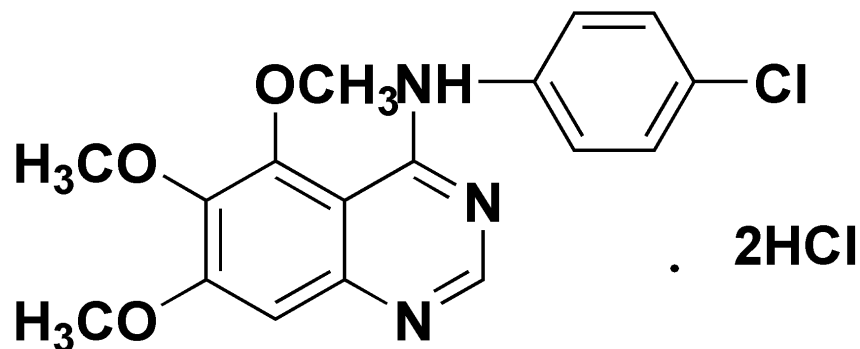


Figure 1. Structure of N-(4-chlorophenyl)-5,6,7-trimethoxyquinazolin-4-amine dihydrochloride.

emphasis is being placed on its antitumor activity (Burriss et al., 2009; Chen et al., 2011; Kubo et al., 2011; Addeo and Caraglia, 2011).

In our recent anticancer drug discovery screen study, we found that a quinazoline derivative N-(4-chlorophenyl)-5,6,7-trimethoxyquinazolin-4-amine dihydrochloride (1106) (Figure 1) showed a potential anticancer activity against four kinds of cell lines, namely prostate cancer (PC-3), human gastric cancer (BGC-823), human breast cancer (Bcap-37), and mouse embryonic (NIH 3T3). In order to investigate the anticancer mechanisms of quinazolines and its derivatives for the purpose of gaining useful knowledge for further design and synthesis of new compounds with novel biological activity, anticancer bioactivities and action mechanism of compound 1106 were investigated in PC-3 cells. In this study, we found out that the title compound exerted anticancer activity through antiproliferation pathway rather than cytotoxicity. Furthermore, the antiproliferation effect is largely associated with its inhibition of extracellular signal-regulated protein kinase1/2 (ERK1/2) activation. A further investigation on PC-3 cells will be conducted to observe if the inhibitory effect of compound 1106 on ERK1/2 activation is a direct effect or is due to impairment of upstream signaling involving both receptor tyrosine kinases and cytosolic signaling.

MATERIALS AND METHODS

Cell lines and other reagents

Androgen-independent prostate cancer cell line PC-3, human gastric cancer cell line BGC-823, human breast cancer cell line Bcap-37, and mouse embryonic cell line NIH 3T3 were obtained from cell bank of Chinese Academy of Sciences (Shanghai, China). Fetal bovine serum was purchased from Hangzhou Sijiqing Biological Engineering Materials Co., Ltd (Hangzhou, China). Roswell Park Memorial Institute (RPMI) 1640 and Dulbecco's Modified Eagle's Medium (DMEM) were from GIBICO. 3-(4,5-Dimethyl-2-thiazolyl)-2,5-diphenyltetrazolium bromide (MTT), Tween-20, dimethylsulfoxide (DMSO), trypsin, tetramethylethylene-

diamine (TEMED) were from Sigma. Lactate dehydrogenase (LDH) cytotoxicity measuring assay test kit was from Roche Applied Science. Rabbit antihuman phospho-ERK1/2 and actin antibodies and U0126 (ERK1/2 inhibitor) were from CST (Shanghai) Biological Reagents Company Limited (Shanghai, China). Goat antirabbit immunoglobulin-horseradish peroxidase-conjugated secondary antibodies were purchased from KPL Company. Enhanced chemiluminescence (ECL) was from Pierce. Adriamycin (ADM) was from Zhejiang Hisun Pharmaceutical Co., Ltd. (Taizhou, China). N-(4-chlorophenyl)-5,6,7-trimethoxyquinazolin-4-amine dihydrochloride was synthesized in the Center for Research and Development of Fine Chemicals of Guizhou University (Guiyang, China). N-(4-chlorophenyl)-5,6,7-trimethoxyquinazolin-4-amine dihydrochloride: yellow solid, yield: 65.0%, mp, 161 to 163°C. ¹H NMR (CDCl₃, 500 MHz) δ: 10.51 (s, 1H, NH), 8.59 (s, 1H, quinazoline H-2), 7.73 (s, 1H, quinazoline H-8), 7.67 to 7.65 (d, J=10.0 Hz, 2H, Ph-3, 5-H), 7.44 to 7.43 (d, J=5.0 Hz, 2H, Ph-2, 6-H), 4.28 to 3.97 (t, 9H, 3OCH₃); ¹³C NMR (CDCl₃, 500 MHz) δ: 160.8 (quinazoline C-4), 158.5 (quinazoline C-7), 149.8 (quinazoline C-2), 149.1 (Ph-1-C), 141.9 (quinazoline C-9), 138.8 (Ph-4-C), 134.9 (quinazoline C-6), 132.0 (quinazoline C-5), 129.5 (Ph-3,5-C), 124.3 (Ph-2,6-C), 102.3 (quinazoline C-10), 98.8 (quinazoline C-8), 54.3, 63.5, 62.9 (3C, quinazoline 5,6,7-site OCH₃); IR (KBr) ν: 3416 to 3271 (N-H), 3012.8 (Ar-H), 2987.7 (CH₃), 2893.2 (CH₃), 1616.6 to 1490.9 (quinazoline skeleton vibration), 1294.2 (Ar-O-C), 1155.4 (Ar-O-C), 809.8 (Ar-H) cm⁻¹; analytical calculation for C₁₇H₁₈Cl₃N₃O₃: C 48.77, H 4.33, N 10.04; found C 48.81, H 4.80, N 9.65. All the other reagents were analytical reagents without any modification.

Cell culture and drug treatments

Bcap-37, BGC-823 or PC-3 cells were respectively maintained in RPMI 1640 medium supplemented with 10% fetal bovine serum, at 37°C in a 5% CO₂ atmosphere; NIH 3T3 cells were maintained in DMEM medium supplemented with 10% fetal bovine serum, at 37°C in a 5% CO₂ atmosphere. The cells were passaged and harvested for the experiments by detachment with 0.05% trypsin and 0.5 mM ethylenediaminetetraacetic acid (EDTA) in phosphate-buffered saline (PBS). Compounds were dissolved in DMSO at a concentration of 20 mM as a stock solution. The stock was diluted to the required concentration directly in the medium before use. Negative control cultures received an equivalent amount of DMSO only and the final concentration of DMSO in culture medium during compound treatment did not exceed 0.1% (v/v).

MTT assay

Inhibition rates of title compound on tested cell lines were determined through MTT assay. The cells were plated into 96 well tissue culture plates in a range of 2 to 3 × 10³ cells per well in a final volume of 100 µl. After 24 h, the cells were treated with either 0.1% (v/v) DMSO as negative control or varying concentrations of tested compounds including ADM as positive control for 72 h. Subsequently, media containing compound were carefully removed and 100 µl of PBS with 0.5 mg/ml MTT was added to each well. The plate was incubated at 37°C for 4 h. Then, 100 µl of 10% sodium dodecyl sulfate (SDS) (w/v) solution was added to each well. The plate was maintained for 10 h at 37°C. The absorbance was subsequently measured at 595 nm using an enzyme-linked immunosorbent assay (ELISA) plate reader (Bio-Rad). At least, three independent experiments were performed. As a result, the inhibition activity of tested compound on tested cells was determined using the following formula.

$$\text{Inhibition rate (\%)} = \left(1 - \frac{\text{experiment value}}{\text{negative control value}}\right) \times 100\%$$

Morphological observation of PC-3 cells

PC-3 cells were plated into 96 well plates as discussed earlier. After 24 h, the cells were treated with ADM as positive control, 0.1% (v/v) DMSO as negative control or varying concentrations of tested compound for 24, 48 or 72 h. Subsequently, morphological changes of PC-3 cells were observed and pictures were taken using inverted microscope at 100 magnification ("MTT assay" and "Morphological observation of PC-3 cells" are two synchronization experiments).

LDH release assay

4.8 × 10⁴ cells per well (total volume 1.6 ml) were grown in a 6 well plate and was incubated for 24 h at 37°C in 5% CO₂. Cytotoxicity was evaluated based on measuring the release of LDH after 24 h compound treatment. Prior to each assay, the cells were lysed with 2% (v/v) Triton X-100 in culture media for 10 min at 37°C to obtain a representative maximal LDH release as the positive control with 100% toxicity, and cells in culture media alone were the low control. The amounts of LDH in the supernatant were determined and calculated as per kit instructions. All tests were performed in triplicate and assay was repeated three times independently with similar results. As a consequence, the percentage of cytotoxicity was determined using the following formula.

$$\text{Cytotoxicity (\%)} = \frac{\text{experiment value} - \text{low control}}{\text{positive control} - \text{low control}} \times 100\%$$

Western blot analysis

PC-3 cells were plated into 6-well plates, as detailed earlier and at 80% confluency they were treated with either varying concentrations of compounds or 0.1% (v/v) DMSO for 30 min, and were stimulated by 40 ng/ml epidermal growth factor (EGF), except DMSO control for 10 min. After treatment, the medium was aspirated, cells were washed two times with cold PBS and were directly dissolved in SDS sample buffer, and proteins were separated using 10% (w/v) sodium dodecyl sulfate-poly acrylamide gel electrophoresis (SDS-PAGE). The separated proteins were transferred onto polyvinylidene difluoride (PVDF) membrane.

Immunoblot was blocked with blocking buffer (5% nonfat milk, 10 mM Tris, pH 7.5, 100 mM NaCl, and 0.1% (v/v) Tween-20) at room temperature for 1 h. Protein was detected by 1:5000 (v/v) primary antibody (anti-phospho-ERK1/2) solution and was incubated overnight at 4°C. Then, the membrane was washed three times with TBS-T (1 × TBS, 1% (v/v) Tween 20) for 5 min. The second antibody solution at 1:1000 (v/v) dilution was incubated at room temperature for 1 h. The blots were developed using an ECL detection system. In order to study the expression of different proteins, membranes were sequentially stripped and were re probed with other antibodies (anti-actin).

Statistical analysis

All the statistical analyses were done under Statistical Package for Social Sciences (SPSS) Version 11.5 using personal computer. P value, standard deviation and IC₅₀ were tested using one-way analysis of variance (ANOVA), means and probit analysis, respectively. Each experiment had three replicates and all experiments were run three times with similar results. Measurements from all the replicates were combined and treatment effects were analyzed.

RESULTS

Antitumor activity

PC-3, BGC-823, Bcap-37 and NIH 3T3 cell lines were employed in MTT assay for anticancer drug discovery screen. As indicated in Table 1, compound 1106 at 10 µM showed considerable inhibition effects on the tested cell lines. The inhibition rates of title compound at 10 µM against Bcap-37, PC-3, BGC-823 and NIH 3T3 *in vitro* were 25.23 ± 11.69, 31.3 ± 7.1, 43 ± 2.74 and 15.7 ± 5.8%, respectively. The results of preliminary bioassays were compared with that of a commercial anticancer drug, ADM. Although, the inhibition effects of compound 1106 on tested cells were weaker than ADM treatment group at corresponding concentration, when compared with DMSO control, compound 1106 treatment at 10 µM, even 1 µM for 72 h exhibited remarkable antitumor activities against the tested cells (P < 0.01) (Table 1). The result implied that title compound 1106 may be a potential antitumor agent.

The anticancer activity of compound 1106 was further evaluated by additional dose test and microscopic observation in PC-3 cells. It was found that compound 1106 executed antitumor activity in a dose dependent pattern and its IC₅₀ of 72 h for PC-3 *in vitro* was 24.46 µM. The inhibition activity increased with increase in concentration (Table 2 and Figure 2). Compared with ADM treatment group, compound 1106 exhibited relatively low inhibition effect on PC-3 cells at corresponding concentration (data not shown). For example, even ADM at 5 µM dose resulted in 96.3 ± 3.5% inhibition of PC-3 cell growth after 72 h treatment. Further morphological observation revealed that ADM-treated cells became larger, some of them broke down eventually. Compound 1106 treatment did not show any morphological effect on cells. However, a proliferation inhibition was observed in

Table 1. Inhibition rates of compound 1106 and ADM at 1 and 10 μM against four kinds of cell lines *in vitro*.

Compound	Cell line	Inhibition rate (%)	
		1 μM	10 μM
ADM	Bcap-37	93.67 \pm 6.91**	97 \pm 5.63**
	PC-3	95.6 \pm 0.9**	97.6 \pm 0.5**
	BGC-823	96.87 \pm 1.02**	97.39 \pm 0.38**
	NIH 3T3	95.7 \pm 1.1**	98.3 \pm 1.0**
1106	Bcap-37	19.64 \pm 16.08*	25.23 \pm 11.69**
	PC-3	6.3 \pm 10.2*	31.3 \pm 7.1**
	BGC-823	15.52 \pm 3.91**	43 \pm 2.74**
	NIH 3T3	0.5 \pm 4.0*	15.7 \pm 5.8**

*, Compared with DMSO control, compound 1106 or ADM treatment of 72 h showed no statistically significant inhibition ($P > 0.05$) against cell growth.**, Compared with DMSO control, compound 1106 or ADM treatment of 72 h showed statistically significant inhibition ($P < 0.01$) against cell growth.

Table 2. Inhibition rates of compound 1106 against PC-3 cells *in vitro* and IC_{50} .

Concentration (μM)	1	5	8	10	20	40
Inhibition rate (%)	8.28 \pm 9.33*	10.96 \pm 8.44*	21.30 \pm 8.99**	22.52 \pm 8.52**	42.34 \pm 4.14**	75.25 \pm 3.67**
IC_{50} (μM)						24.46

*, Compared with DMSO control, after 72 h of compound 1106 treatment, 1 and 5 μM dose were not effective and showed no statistically significant inhibition ($P > 0.05$) against PC-3 growth. **, Compared with DMSO control, after 72 h of compound 1106 treatment, these doses showed statistically significant inhibition ($P < 0.05$) against PC-3 growth.

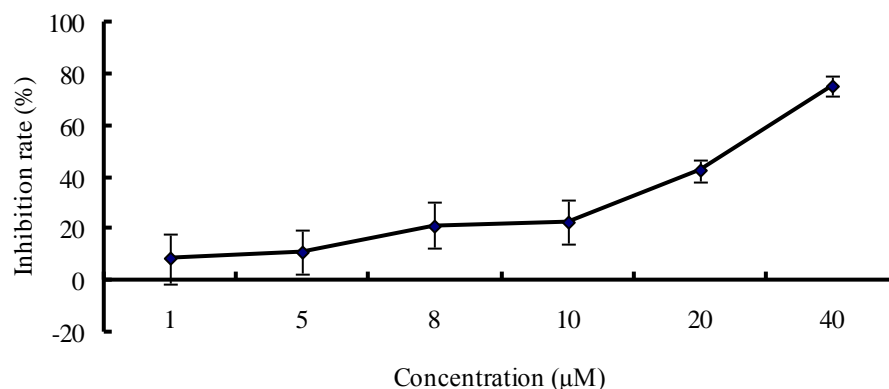


Figure 2. Dose-dependent manner of anticancer activity of compound 1106 in PC-3 cells. Compound 1106 was dissolved in DMSO at a concentration of 20 mM as a stock solution. The stock was diluted to 1, 5, 8, 10, 20, and 40 μM directly in the medium before use. Dose test was performed through MTT assay in PC-3 cells. The data shown are representative of three independent experiments.

compound 1106 treated cells in a concentration dependent manner (Figure 3). Together, these studies suggested that compound 1106 could execute antitumor activity through antiproliferation pathway rather than cytotoxicity which was confirmed by an additional LDH release assay.

Compound 1106-treated cells showed no cytotoxicity

LDH release assay was performed in PC-3 cells to assess the cytotoxicity of compound 1106. This assay quantifies cell death and lysis based on measuring the release of LDH which is present within the supernatant

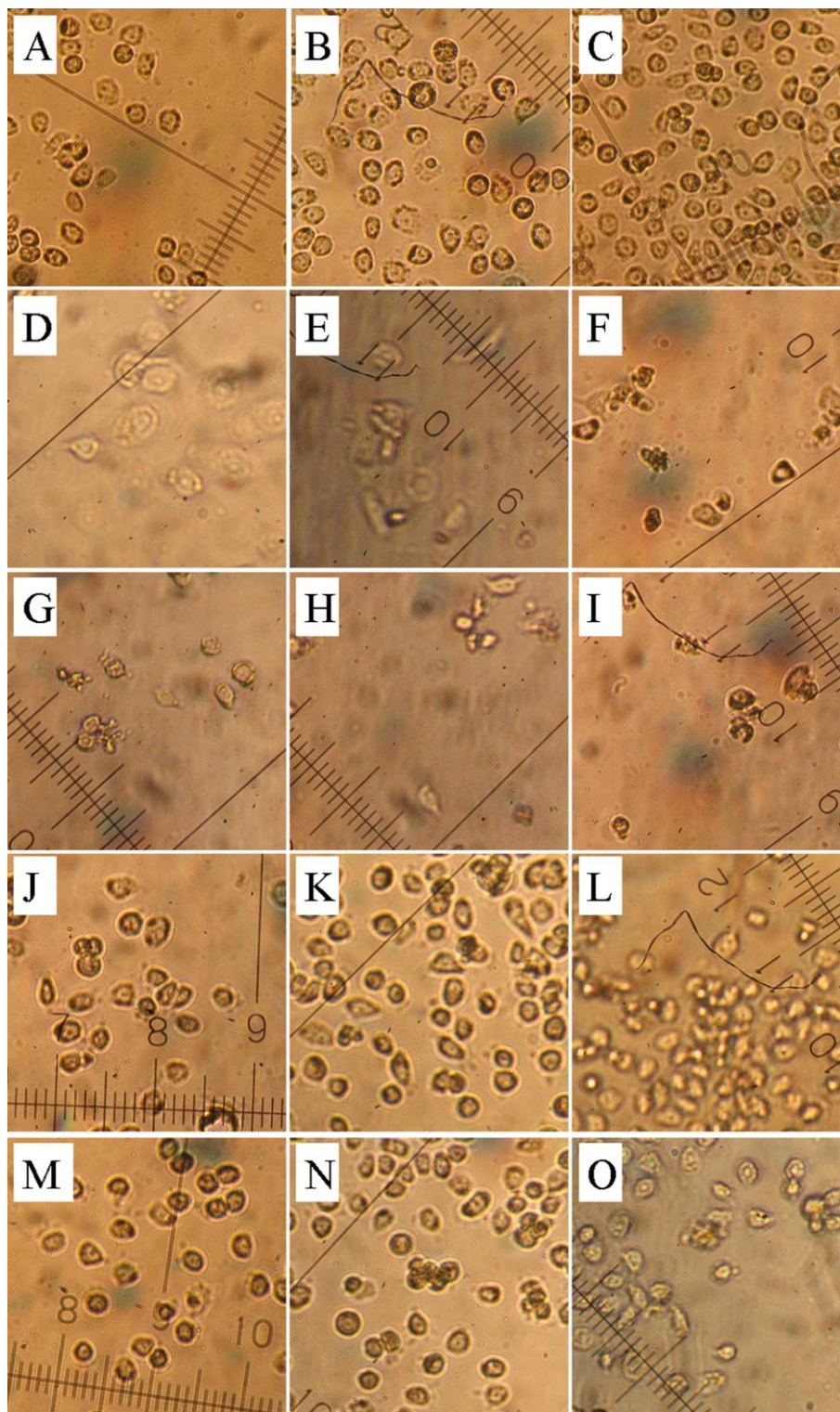


Figure 3. Effects of indicated concentrations of DMSO, ADM and compound 1106 treatment on PC-3 cell proliferation. Cells were plated into 96 well plates. After 24 h, the cells were treated either with 0.1% (v/v) DMSO, 1 and 10 μ M concentrations of compound 1106 or 1 and 10 μ M concentration of ADM for 24, 48, and 72 h. Representative images from 3 experiments are shown. A, DMSO (0.1% (v/v), 24 h). B, DMSO (0.1% (v/v), 48 h). C, DMSO (0.1% (v/v), 72 h). D, ADM (1 μ M, 24 h). E, ADM (1 μ M, 48 h). F, ADM (1 μ M, 72 h). G, ADM (10 μ M, 24 h). H, ADM (10 μ M, 48 h). I, ADM (10 μ M, 72 h). J, 1106 (1 μ M, 24 h). K, 1106 (1 μ M, 48 h). L, 1106 (1 μ M, 72 h). M, 1106 (10 μ M, 24 h). N, 1106 (10 μ M, 48 h). O, 1106 (10 μ M, 72 h).

Table 3. Cell cytotoxicity of PC-3 cells treated with compound 1106 or ADM for 24 h.

Concentration (μM)		0.1	0.5	1	5	10	spontaneous release well	Positive control
1106	Cytotoxicity (%)	34.47 \pm 2.2	35.62 \pm 2.98	35.58 \pm 2.30	34.43 \pm 1.08	35.58 \pm 2.3	34.47 \pm 2.2	100
	P ^a	1	1	1	1	1		0.002
	P ^b	0.002	0.002	0.004	0.002	0	0.002	
ADM	Cytotoxicity (%)	34.5 \pm 4.79	37.59 \pm 4.30	45.68 \pm 4.09	60.7 \pm 3.25	65.43 \pm 3.12	34.47 \pm 2.2	100
	P ^a	1	1	0.323	0.012	0.006		0.005
	P ^b	0.015	0.013	0.016	0.019	0.023	0.005	
P ^c		1	1	0.301	0.029	0.018		

^a, Compared with negative control (spontaneous release well), there is no difference ($P > 0.05$) in cell cytotoxicity, there is significant difference in cell cytotoxicity ($P < 0.05$). ^b, Compared with positive control (complete release well), there is no difference ($P > 0.05$) in cell cytotoxicity, there is significant difference in cell cytotoxicity ($P < 0.05$). ^c, When ADM treatment group was compared with 1106 treatment group at corresponding concentration, there is no difference ($P > 0.05$) in cell cytotoxicity, there is significant difference in cell cytotoxicity ($P < 0.05$).

following the loss of membrane integrity. Compared with negative control (spontaneous release well), 24 h of compound 1106 treatment at 0.1, 0.5, 1, 5, and 10 μM did not cause any cytotoxicity effect in PC-3 cells ($P > 0.05$). On the contrary, a significant increase in cytotoxicity was observed in positive control ($P < 0.05$) (Table 3). At the same time, cytotoxicity of ADM was evaluated in PC-3 cells by LDH release assay. As shown in Table 3, compared with positive control, although, the cytotoxicity of ADM for 24 h at varying concentrations are statistically low ($P < 0.05$), compared with negative control or compound 1106 treatment group at corresponding concentration, ADM treatment at 5 and 10 μM showed significant cytotoxicity ($P < 0.05$, even $P < 0.01$) (Table 3).

Compound 1106 inhibited activation of ERK1/2 in PC-3 Cells

On the basis of the data shown in Tables 2 and 3

and Figure 3, we further examined whether the observed inhibitory effect of compound 1106 on PC-3 cells was due to its antiproliferation activity. As shown in Figure 4, compared with loading control, treatment of PC-3 cells with compound 1106 resulted in almost complete inhibition of EGF induced phosphorylation of ERK1/2 at more than 10 μM for 30 min. Compared with DMSO control, the qualitative densitometric analysis indicated that EGF treatment alone stimulated phosphorylation of ERK1/2 remarkably. Compared with DMSO control and EGF treatment alone, after 30 min of compound 1106 treatment, the group of less than 5 μM dose were not effective, but the group of more than 10 μM dose showed almost complete inhibition of EGF stimulated phosphorylation of ERK1/2 (Figure 4).

DISCUSSION

The results of antitumor bioactivity preliminary screen and dose test indicate that title compound

possesses potential anticancer activity and executes the anticancer activity in a dose-dependent manner. Additional morphological observation and LDH release assay revealed that compound 1106 could execute antitumor activity through antiproliferation pathway rather than cytotoxicity which was further confirmed by the anticancer mechanism study in PC-3 cells. It has been documented that quinazolines are synthesized and used as Raf kinase inhibitors (Ioannou et al., 2011; Ramurthy et al., 2012). So, our attention was firstly focused on the effect of compound 1106 on activation of mitogen-activated protein kinase (MAPK), especially MAPK/ERK1/2, because the MAPK family composed ERKs, JNK (stress-activated protein kinase), and p38, which are functional units involved in three distinct signaling pathways (Pearson et al., 2001; Yu et al., 2010). There are studies which indicate that ERKs and p38 signaling correlate with proliferation and differentiation, whereas the JNK pathway is associated with apoptosis (Seger and Krebs, 1995; Cobb and Goldsmith, 1995; Chen et

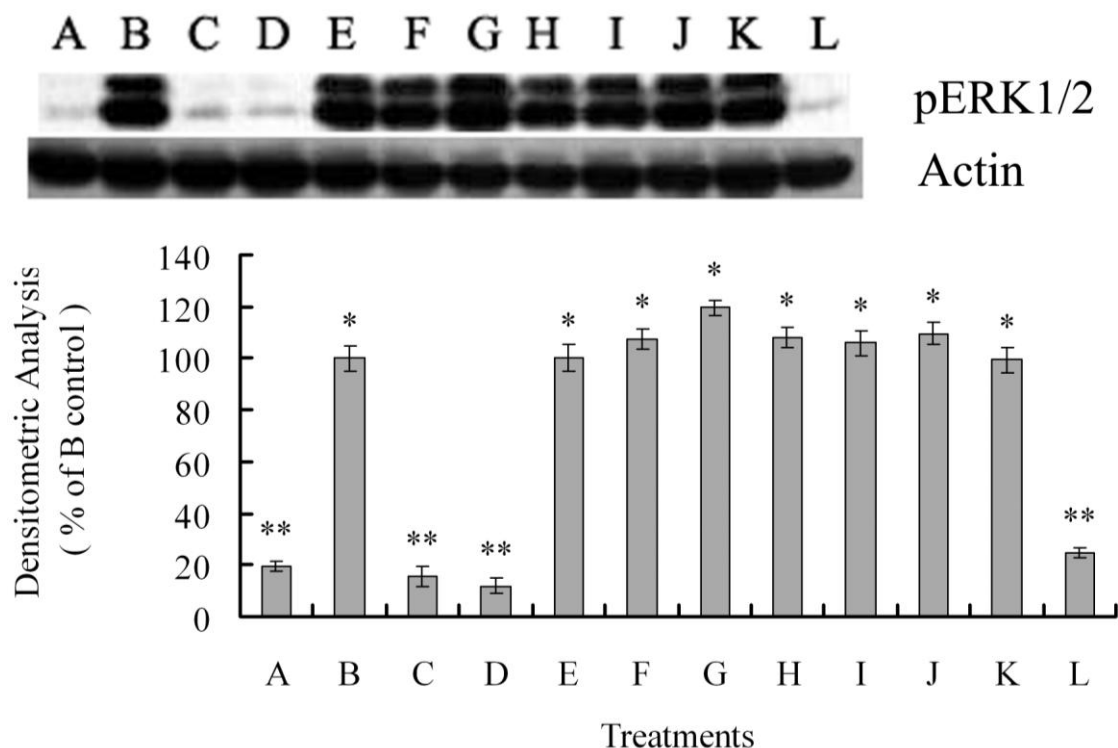


Figure 4. Inhibitory effect of compound 1106 on ERK1/2 phosphorylation in PC3 cells induced by EGF. PC-3 cells were cultured in RPMI 1640 with 10% serum and at 80% confluency were treated either with 0.1% (v/v) DMSO or 0.1, 0.2, 0.5, 1, 2, 5, 10, and 20 μM concentrations of N-(4-chlorophenyl)-5,6,7-trimethoxyquinazolin-4-amine dihydrochloride, 20 μM concentration of U0126 for 30 min and stimulated by 40 ng/ml EGF for 10 min except DMSO control. U0126 treatment was used as positive control. Cell lysates were prepared and subjected to SDS-PAGE, followed by Western blotting. The membranes were probed with antiphospho-ERK1/2, antiactin antibodies and then peroxidase-conjugated appropriate secondary antibody. Visualization of proteins was done using ECL detection system. Actin was used as a loading control. The data shown are representative of three independent experiments. A, DMSO control; B, DMSO+EGF; C, 20 μM compound 1106+EGF; D, 10 μM compound 1106+EGF; E, 5 μM compound 1106+EGF; F, 4 μM compound 1106+EGF; G, 2 μM compound 1106+EGF; H, 1 μM compound 1106+EGF; I, 0.5 μM compound 1106+EGF; J, 0.2 μM compound 1106+EGF; K, 0.1 μM compound 1106+EGF; L, U0126+EGF. *, Compared with B control (DMSO+EGF), E-K treatments showed no statistically significant difference in ERK1/2 phosphorylation ($P > 0.05$). **, Compared with B control (DMSO+EGF), A, C, D, and L treatments showed statistically significant decreasing in ERK1/2 phosphorylation ($P < 0.05$).

al., 1996; Pearson et al., 2001; Yu et al., 2010). The effect of compound 1106 on ERK1/2 in PC-3 cells was assessed by Western immunoblotting. It was found out that compound 1106 at more than 10 μM concentration for 30 min demonstrated almost complete inhibition action of EGF stimulated phosphorylation of ERK1/2.

To the best of our knowledge, quinazoline derivatives as anticancer drugs mostly target epidermal growth factor receptor (EGFR) (Bos et al., 1997; Kroep et al., 2010; Bouchalova et al., 2010; Dragowska et al., 2011; Yeh et al., 2011). Several studies have demonstrated that EGFR over expression correlates with human cancer progression (Resnik et al., 1998; Wang et al., 2010; Kim et al., 2011). EGFR mediated signaling pathways can ultimately activate MAPKs that then translocates to the nucleus and activate transcription factors for cell growth, differentiation, and proliferation (Burgering et al., 1993;

Matsuda et al., 1994; Krysan et al., 2005; Roberts and Der, 2007). So, ERK1/2 is a promising target for developing novel anticancer drugs. It should be noted that this study has examined inhibition effect of compound 1106 on ERK1/2 activation in PC-3 cells only. A further investigation will be conducted to observe if inhibitory effect of compound 1106 on ERK1/2 activation is a direct effect or due to impairment of upstream signaling involving both receptor tyrosine kinases and cytosolic signaling.

Conclusion

Conclusively, these findings suggest that compound 1106 possesses broad spectrum of anticancer activity and can execute the antitumor activity through antiproliferation

pathway rather than cytotoxicity. Furthermore, the inhibition effect of compound 1106 on PC-3 cells is largely associated with its inhibition of ERK1/2 phosphorylation.

ACKNOWLEDGEMENTS

We are grateful for the financial support from the National Key Project for Basic Research (2003CB114404), Guidance Project of Bengbu City for Science and Technology Research, Scientific Research Projects of Colleges and Universities in Anhui province, China (KJ2011B096).

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