

## Original Research Article

# Inhibitory effect of platinum (II) complex of curcumin on non-small cell lung cancer via the PI3K-AKT pathway

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### Abstract

**Purpose:** To study the effect of platinum (II) complex of curcumin (PCC) on non-small cell lung cancer (NSCLC) using human NSCLC cell line A549.

**Methods:** The anti-proliferative and apoptotic effects of PCC against NSCLC were investigated using 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT), Annexin V-FITC/propidium iodide double staining, quantitative real-time polymerase chain reaction (qRT-PCR), and western blot assays.

**Results:** First, MTT assay and Annexin V-FITC/propidium iodide double staining results revealed that PCC possesses strong anti-cancer potency and high safety, when compared with cisplatin. Western blotting and qRT-PCR results showed that PCC inhibited the viability of NSCLC cells, decreased the expressions of PI3K, VEGF, AKT and Bcl-2, and upregulated the expression of PTEN.

**Conclusion:** These results indicate that PCC promotes apoptosis in A549 cells via PTEN/PI3K/AKT signaling pathways.

**Keywords:** Platinum-curcumin complex, Non-small cell lung cancer, Apoptosis, PI3K-AKT

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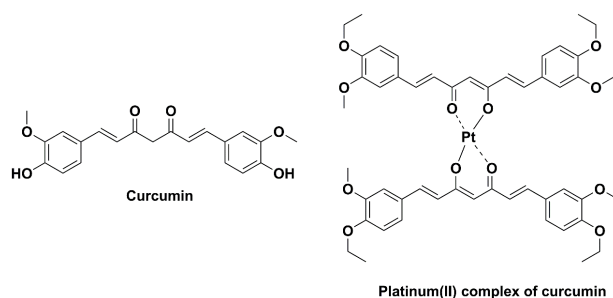
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## INTRODUCTION

Most lung cancer incidents (about 85 %) are due to NSCLC, while squamous cell carcinoma, large cell carcinoma, and adenocarcinoma occur less frequently [1-3]. Currently, surgery, chemotherapy, radiation and immunotherapy are the main courses of treatment for these cancers [4]. Among these, chemotherapy is the most widely used, but its effect is frequently unsatisfactory because of problems such as limited effectiveness, severe toxicity and multidrug resistance [5].

Consequently, natural products have attracted increasing attention in cancer therapy [6]. Curcumin (Cur) is a natural polyphenol (Figure 1) which plays role in inducing tumor differentiation and apoptosis, inhibiting blood vessel formation, and regulating the expressions of tumor suppressor genes and oncogene proteins [7,8]. The expectation is that this is a new type of natural antitumor drug with high efficiency and low toxicity. However the use of Cur has limitations because of its weak anti-tumor effect, low bioavailability and poor water solubility [9]. The gold standard primary therapy for NSCLC patients involves the application of platinum-

linked treatment [10,11]. However, the lack of selectivity between normal cells and cancer cells creates more side effects [12,13]. Therefore, in the present study, platinum (II) complex of curcumin was used (PCC, Figure 1) to investigate if the anti-cancer effects of curcumin could be improved, and also to see if the toxicity of cisplatin could be relieved. The specific mechanism of this complex was also investigated.



**Figure 1:** Structures of curcumin and platinum (II) complex of curcumin

## EXPERIMENTAL

### Materials and reagents

Professor Shuangsheng Zhou of the Anhui College of Traditional Chinese Medicine designed and provided the platinum (II) complex of curcumin. Curcumin and cisplatin (Cis) were products of Chengdu Herbpurify Co., LTD (Chengdu, China) and Sigma Chemical Co. (St. Louis, USA) respectively. They were dissolved in DMSO as stock solution. Sigma Chemical Co (St. Louis, USA) was the source of 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT).

### Cell lines and cell culture

Human non-small cell lung cancer cell lines (A549 and SPC-A1), and normal lung cell line HBE were bought from KeyGEN BioTECH (Nanjing, China). These cell lines were cultured in Roswell Park Memorial Institute (RPMI)-1640 medium containing 10 % heat-inactivated fetal bovine serum (FBS, Hyclone Laboratories) and 1 % penicillin–streptomycin antibiotic mixture (Gibco BRL) at 37 °C with 5 % CO<sub>2</sub>. The cells were used at the exponential growth phase.

### Cell viability assay

Each cell culture was collected and transfected for 24 h before digestion with 0.25 % trypsin. After re-suspending in complete medium, the cells were separately plated in 100- $\mu$ L culture medium in 96-well plates at density of  $1 \times 10^4$

cells/well, and incubated with various concentrations of the different drugs for 48 hours. Four hours before the end of the incubation, MTT dye (10  $\mu$ L of 2.5 mg/mL in PBS) was added to the wells, followed by further 4 hour-incubation at 37 °C in the 5 % CO<sub>2</sub> atmosphere.

Next, each plate was centrifuged for 15 min at 1500 rpm before discarding the supernatant without disturbing the well cells and the formazan crystals. The formazan crystals were solubilized in DMSO (150  $\mu$ L) before using a plate shaker to agitate the plates for 5 min. A microplate reader (Thermo, USA) was employed to read the optical density (OD) at 490 nm, and the IC<sub>50</sub> values were calculated using GraphPad Prism 6.0. All assays were carried out in triplicate.

### Determination of cell apoptosis

The A549 cells were seeded at  $1 \times 10^5$  cells per well in a 6-well microtiter plate. After exposure to PCC (0, 10, and 30  $\mu$ M) for 24 h, they were trypsinized before washing twice with chilled PBS and then re-suspending in a binding buffer. Next, each cell was subjected to Annexin V-FITC/PI double staining using apoptosis detection kit (Nanjing Jiancheng Bioengineering Institute) based on the manufacturers protocol. Flow cytometry (BD FACS Calibur, USA) was then used to analyse cellular fluorescence. Each experiment was carried out in triplicate.

### PCR analysis

Total RNA was extracted from the cells treated with 0, 10, and 30  $\mu$ M PCC, and also from cells treated 10  $\mu$ M Cis in different groups. To determine the mRNA expressions of VEGF, PTEN and P13K, the extracted total RNA was reverse-transcribed to cDNA using kits from First strand cDNA Synthesis (TransGen Biotech) in line with the kit manual protocol. The qRT-PCR was done using Top Green qPCR SuperMix (TransGen Biotech) coupled to a 96-well Piko Thermal Cycler system, with  $\beta$ -actin mRNA as internal control. Quantifications of mRNA expressions were carried out with comparative Ct procedure.

### Western blot

Total protein was extracted from each cell line by grinding in RIPA buffer containing 1 mM PMSF (Cell-Signalling Tech., US). The protein levels were quantified with BCA protein assay kit (Thermo Fisher Scientific, MA, USA) according to the kit protocol. Primary antibodies for PTEN, P13K and  $\beta$ -actin were bought from Cell Signalling Technology (Beverly, MA, USA), while

the primary antibodies for VEGF, AKT, Bcl-2 were obtained from Abcam (Cambridge, UK). The internal reference used was  $\beta$ -actin. Chemiluminescence was measured with an ECL kit (Pierce Biotechnology) and a Bio-Rad molecular imager was used to visualise and capture the images of the resultant immunoreactive protein bands. The intensity of each band was calculated with ImageJ, and the results were normalised to that of  $\beta$ -actin.

### Statistical analysis

Data are presented as mean  $\pm$  standard deviation (SD). Statistical analysis was done with one-way analysis of variance (ANOVA), followed by Tukey's multiple comparison. All analysis was done with GraphPad Prism 6. Statistical significance was set at  $p < 0.05$ .

## RESULTS

### *In vitro* cytotoxicity of PCC

The  $IC_{50}$  values depicted in Figure 2 reveal that the inhibitory effects of PCC on lung adenocarcinoma cells was comparable to that of the positive control Cis, but superior to that of Cur. Aside from that, in normal HBE cells, PCC

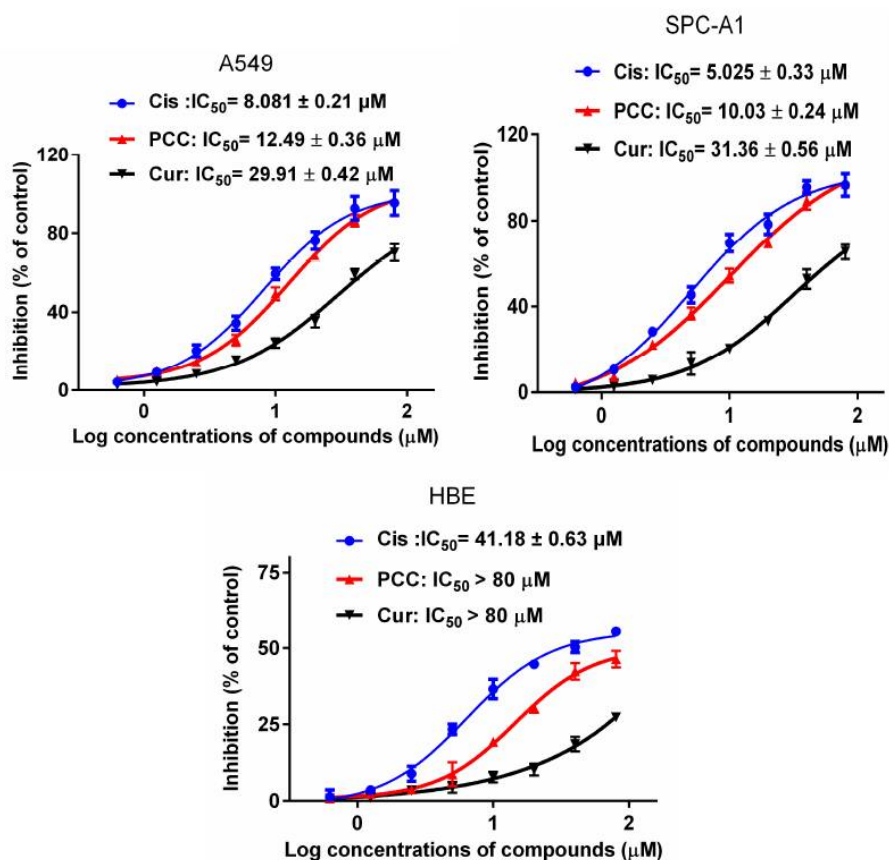
almost exhibited no toxic effects, while Cis showed obvious inhibitory effect on the growth of HBE cells, with  $IC_{50}$  of 41.18  $\mu$ M. This suggests that the complex sustained the relative safety of Cur and the potent activity of Cis.

### Results of flow cytometry for apoptosis

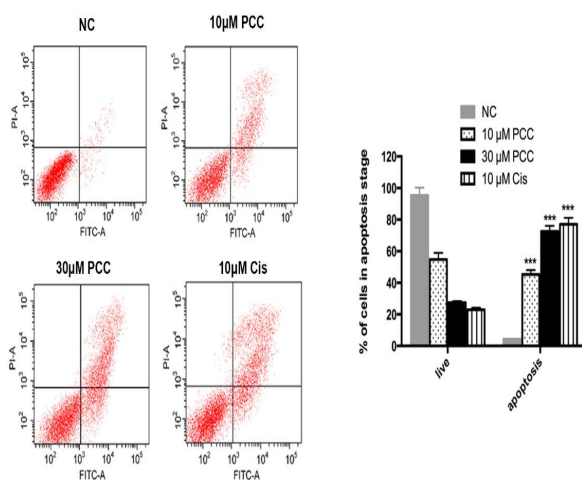
Results from cytometry demonstrated that the pro-apoptotic effect of PCC on A549 cells was dose-dependent. Apoptosis in the low-dose PCC group ( $45.3 \pm 2.8$  %) and the high-dose PCC group ( $72.6 \pm 3.5$  %) were significantly higher than that in the normal control group ( $p < 0.001$ ), indicating that the pro-apoptotic effect of PCC was comparable to that of Cis.

### Effect of PCC on the expressions of P13K, VEGF, and PI3K mRNAs

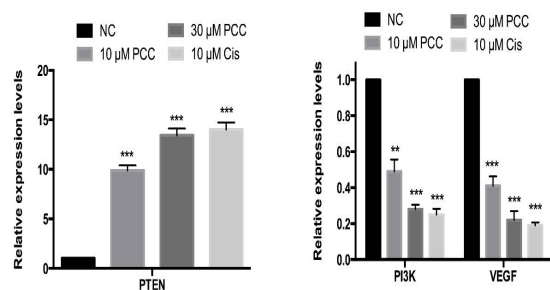
As shown in Figure 4, data obtained from qRT-PCR revealed that the expression of PTEN mRNA was significantly upregulated in the PCC and Cis groups, when compared to the normal control group, while the expressions of VEGF and PI3K mRNAs in the drug-treated groups were significantly lower ( $p < 0.05$ ).



**Figure 2:**  $IC_{50}$  for the potency of PCC on A549, SPC-A1 and HBE cell lines, as determined using MTT assay. Data are presented as mean  $\pm$  SD ( $n = 3$ )



**Figure 3:** Pro-apoptotic effects of PCC on A549 cells, as determined with Annexin V-FITC/PI staining and flow cytometry. Data are presented as mean ± SD (n = 3)



**Figure 4:** Effect of PCC on mRNA expressions of PTEN, PI3K and VEGF in A549 cells; \**p* < 0.05, compared with the control group

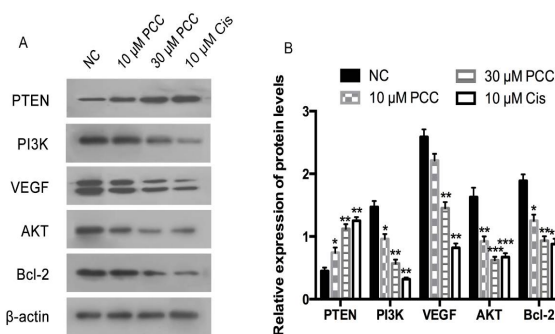
**Effect of PCC on the expressions of VEGF and AKT/PI3K pathway-related proteins in A549 cells**

Western blotting assay results revealed that in the drug-treated groups, PTEN expression was significantly increased while the PI3K, AKT, VEGF, and Bcl-2 expressions were depressed, relative to corresponding expressions in the normal control group (Figure 5). These results suggest that PCC exerts anti-lung cancer effect via regulation of the PTEN/PI3K/AKT signaling pathway.

**DISCUSSION**

Although anti-cancer therapies such as radiotherapy, chemotherapy, and targeted therapies have witnessed some advances, the five years survival remains low (< 15 %) [14]. Curcumin occupies a vital position as a paramount source of anti-cancer drugs, but its use is limited because of a weak anti-tumor effect, low bioavailability and poor water solubility [15]. The gold standard therapy for NSCLC is platinum-based chemotherapy, which also has

severe side effects [16]. In the present study, the anticancer effect of a combination of the two drugs was studied. First, the cytotoxicity of PCC on human NSCLC cell lines A549 and SPC-A1, as well as normal human bronchial epithelial cell line HBE was determined. Conclusions were easily drawn that PCC had potent ability to inhibit NSCLC cancer cell growth at a level comparable to that of cisplatin, and superior to that of curcumin. In addition, it had no effect on normal cells, indicating it was safer than cisplatin. Double staining with Annexin V-FITC/PI produced consistent results, showing the ability of PCC to induce significant cell apoptosis in a dose-dependent manner.



**Figure 5:** Effect of PCC on expressions of PTEN/PI3K/AKT signaling pathway-associated proteins. Data are expressed as mean ± SD (n = 3); \**p* < 0.05, \*\**p* < 0.01, \*\*\**p* < 0.001, compared with the normal control group

Researchers have found multiple signaling pathways which are linked to lung cancer. These include the PI3K-AKT [17 - 19], VEGF [20-22], and P53 [23-25] signaling pathways. There is agreement with the fact that PTEN is a dual protein/lipid phosphatase. The central substrate is a product of P13K, i.e. phosphatidylinositol 3,4,5 triphosphate (PIP3) [26]. Cyclins and Bcl-2 proteins correlate closely with cell cycles and apoptosis, respectively [27].

It is known that VEGF mediates the migration and proliferation of endothelial cells, as well as angiogenesis and survival [28-30]. Thus, in order to determine the effect of PCC on the related proteins, the relative expression levels of VEGF, PI3K and PTEN; and the relative protein expression levels of VEGF, PI3K, PTEN, AKT and Bcl-2 were determined using qPCR and western blot, respectively. The results showed that PCC downregulated VEGF and PI3K mRNAs, but upregulated PTEN mRNA. The changes in protein expressions were in agreement with the pattern of expressions of mRNAs.

## CONCLUSION

These results suggest that treatment of PCC prevents A549 cell proliferation via regulation of the PI3K/AKT signaling pathway (34-35). PCC exerts anticancer effect on NSCLC, most likely via regulation of PTEN side of PI3K/AKT signaling pathway rather than through PI3K axis. Therefore, PCC is a potential novel anti-lung cancer drug candidate.

## DECLARATIONS

### Conflict of Interest

No conflict of interest associated with this work.

### Contribution of Authors

This work was done by the authors named in this article and the authors accept all liability resulting from claims which relate to this article and its contents. The study was conceived and designed by Xingxiang Xu; Junjun Yang, Lingfeng Min, Zhenghua Jiang, Wenjing Xu and Hanqing Yao collected and analysed the data, while Yanming Geng and Jiarong Bian wrote the manuscript. All authors read and approved the manuscript prior to publication.

## REFERENCES

1. Ferlay J, Soerjomataram I, Dikshit R, Eser S, Mathers C, Rebelo M, Parkin DM, Forman D, Bray F. Cancer incidence and mortality worldwide: sources, methods and major patterns in GLOBOCAN 2012. *Int J Cancer* 2015; 136: 359-386.
2. Pikor LA, Ramnarine VR, Lam S, Lam WL. Genetic alterations defining NSCLC subtypes and their therapeutic implications. *Lung Cancer* 2013; 82: 179-189.
3. Lindsay CR, Shaw E, Popat S, Blyth KG, Walker I, Chaturvedi A, Dick C, Rassi D, Tanriere P, Johnson PWM, et al. 13 – KRAS Subtypes in non-small cell lung cancer (NSCLC) within Phase I of the Cancer Research UK Stratified Medicine Programme (SMP1). *Lung Cancer* 2018; 115.
4. Alongi F, Arcangeli S, Ramella S, Giaj-Levra N, Borghetti P, D'angelillo R, Ricchetti F, Maddalo M, Mazzola R, Trovò M, et al. From chemotherapy to target therapies associated with radiation in the treatment of NSCLC: a durable marriage? *Expert Rev Anticancer Ther* 2017; 17: 157.
5. Cascella M. Chemotherapy-induced peripheral neuropathy: limitations in current prophylactic strategies and directions for future research. *Curr Med Res Opin* 2017: 1-7.
6. Greenlee H. Natural products for cancer prevention. *Semin Oncol Nurs* 2016; 32: 215.
7. Nelson KM, Dahlin JL, Bisson J, Graham J, Pauli GF, Walters MA. The Essential Medicinal Chemistry of Curcumin. *J Med Chem* 2017; 60: 1620-1637.
8. Xu X, Zhu Y. Curcumin inhibits human non-small cell lung cancer xenografts by targeting STAT3 pathway. *Am J Transl Res* 2017; 9: 3633.
9. Maria DN, Mishra SR, Wang L, Abd-Elgawad AH, Soliman OA, El-Dahan MS, Jablonski MM. Water-soluble Complex Of Curcumin With Cyclodextrins: Enhanced Physical Properties For Ocular Drug Delivery. *Curr Drug Deliv* 2016; 13: 1-1.
10. Yin JY, Li X, Zhou HH, Liu ZQ. Pharmacogenomics of platinum-based chemotherapy sensitivity in NSCLC: toward precision medicine. *Pharmacogenomics* 2016; 00: 1365-1378.
11. Rossi A, Di MM. Platinum-based chemotherapy in advanced non-small-cell lung cancer: optimal number of treatment cycles. *Expert Rev Anticancer Ther* 2016.
12. Oun R, Moussa YE, Wheate NJ. The side effects of platinum-based chemotherapy drugs: a review for chemists. *Dalton Trans* 2018.
13. Rajeswaran A, Trojan A, Burnand B, Giannelli M. Efficacy and side effects of cisplatin- and carboplatin-based doublet chemotherapeutic regimens versus non-platinum-based doublet chemotherapeutic regimens as first line treatment of metastatic non-small cell lung carcinoma: a systematic review of randomiz. *Lung Cancer* 2008; 59: 1-11.
14. Leon G, Macdonagh L, Finn SP, Cuffe S, Barr MP. Cancer stem cells in drug resistant lung cancer: Targeting cell surface markers and signaling pathways ☆. *Pharmacol Ther* 2016; 158: 71-90.
15. Ji JL, Huang XF, Zhu HL. Curcumin and its formulations: potential anti-cancer agents. *Anticancer Agents Med Chem* 2012;12: 210-218.
16. Kosmidis P. Chemotherapy in NSCLC: historical review. *Lung Cancer* 2002; 38: 19-22.
17. Liu Y, Ma T. Metabolic regulation of mesenchymal stem cell in expansion and therapeutic application. *Biotechnol Prog* 2015; 31: 468-481.
18. Venkannagari S, Fiskus W, Peth K, Atadja P, Hidalgo M, Maitra A, Bhalla KN. Superior efficacy of co-treatment with dual PI3K/mTOR inhibitor NVP-BE2235 and pan-histone deacetylase inhibitor against human pancreatic cancer. *Oncotarget* 2012; 3: 1416.
19. Zhu H, Jiang XX, Guo ZK, Li H, Su YF, Yao YH, Wang XY, Li XS, Wu Y, Liu YL, et al. Tumor necrosis factor- $\alpha$  alters the modulatory effects of mesenchymal stem cells on osteoclast formation and function. *Stem Cells Dev* 2009; 18: 1473.
20. Peng N, Gao S, Guo X, Wang G, Cheng C, Li M, Liu K. Silencing of VEGF inhibits human osteosarcoma angiogenesis and promotes cell apoptosis via VEGF/PI3K/AKT signaling pathway. *Am J Transl Res* 2016; 8: 1005-1015.

21. Zhiping HE, Chen AY, Rojanasakul Y, Rankin GO, CHEN YI. Gallic acid, a phenolic compound, exerts anti-angiogenic effects via the PTEN/AKT/HIF-1 $\alpha$ /VEGF signaling pathway in ovarian cancer cells. *Oncol Rep* 2016; 35: 291-297.
22. Chen H, Cong Q, Du Z, Liao W, Zhang L, Yao Y, Ding K. Sulfated fucoidan FP08S2 inhibits lung cancer cell growth in vivo by disrupting angiogenesis via targeting VEGFR2/VEGF and blocking VEGFR2/Erk/VEGF signaling. *Cancer Lett* 2016; 382: 44-52.
23. Tao Z, Chen S, Mao G, Xia H, Huang H, Ma H. The PDRG1 is an oncogene in lung cancer cells, promoting radioresistance via the ATM-P53 signaling pathway. *Biomed Pharmacother* 2016; 83: 1471-1477.
24. Lin Y, Xu J, Liao H, Li L, Pan L. Piperine induces apoptosis of lung cancer A549 cells via p53-dependent mitochondrial signaling pathway. *Tumour Biol* 2014; 35: 3305-3310.
25. Vaughan CA, Singh S, Grossman SR, Windle B, Deb SP, Deb S. Gain-of-function p53 activates multiple signaling pathways to induce oncogenicity in lung cancer cells. *Mol Oncol* 2017; 11.
26. Buckles TC, Ziemba BP, Masson GR, Williams RL, Falke JJ. Single-Molecule Study Reveals How Receptor and Ras Synergistically Activate PI3K $\alpha$  and PIP3 Signaling. *Biophys J* 2017; 113: 2396-2405.
27. Harvey KJ, Blomquist JF, Ucker DS. Commitment and effector phases of the physiological cell death pathway elucidated with respect to Bcl-2 caspase, and cyclin-dependent kinase activities. *Mol Cell Biol* 1998; 18: 2912-2922.
28. Moffat BA, Chen M, Kariaapper MST, Hamstra DA, Hall DE, Stojanovska J, Johnson TD, Blaivas M, Chenevert TL, et al. Inhibition of vascular endothelial growth factor (VEGF)-A causes a paradoxical increase in tumor blood flow and up-regulation of VEGF-D. *Clin Cancer Res* 2006; 12: 1525-1532.
29. Fan J, Bao Y, Meng X, Wang S, Li T, Chang X, Yang G, Bo T. Mechanism of modulation through PI3K-AKT pathway about *Nepeta cataria* L.'s extract in non-small cell lung cancer. *Oncotarget* 2017; 8(19): 31395-31405.
30. Qiu Q, Liu B, Cui J, Li Z, Deng X, Qiang H, Li J, Liao C, Zhang B, Shi W, et al. Design, Synthesis, and Pharmacological Characterization of N-(4-(2 (6,7-Dimethoxy-3,4-dihydroisoquinolin-2(1H)yl)ethyl)phenyl)quinazolin-4-amine Derivatives: Novel Inhibitors Reversing P-Glycoprotein-Mediated Multidrug Resistance. *J Med Chem* 2017; 60(8): 3289-3302.