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Imidazo [4,5*f*][1,10] phenanthroline derivatives as inhibitor of c-myc gene expression in A549 cells via NF-κB pathway

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ABSTRACT

1,10-Phenanthroline has been shown to exhibit anticancer activity. Here, a series of imidazo [4,5*f*][1,10] phenanthroline derivatives 1−10 were synthesized and their biological activities were further elucidated. We found that 2-(4-Brominephenyl)-imidazo [4,5*f*][1,10] phenanthroline (compound **3**) possessed potent antiproliferation activities again a variety of tumor cell lines using 3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl tetrazolium bromide (MTT) assay. Flow cytometric analysis revealed that compound **3** induced both through apoptosis and necrosis in human lung adenocarcinoma cell line, A549. Moreover, compound **3** treatment led to up-regulation of IκBα and down-regulation of p65 and c-myc in A549 cells. Taken together, these results suggested that compound **3** inhibited cell proliferation by suppression of NF-κB activity and down-regulation of c-myc gene expression and may be a candidate for further evaluation as a chemopreventive and chemotherapeutic agent for human cancers, especially for lung cancer.

Lung cancer is one of the leading causes of cancer death in the world. Cancer death is mainly caused by metastasis and an increased resistance to chemotherapy. However, lung cancer is often presented at stages beyond surgical respectability. Novel therapies are necessary to reduce the increasing incidence in pulmonary neoplasm because current treatment modalities are inadequate. Classic chelates based on 1,10-phenanthroline and a number of its derivatives have attracted particular attention in the past few decades because of their unique properties as chelating agents. 1,10-Phenanthroline and substituted derivatives play a key role in the performance of a wide variety of biological systems in transition metal complexes, relying on the availability of multifunctional ligands.[1–4] Most of the investigations of their applications have been reported in chemistry, physics, and material, as well as biological, sciences.[5,6] Several mechanisms have been described to elucidate the anticancer activities of 1,10-phenanthroline based on metal-chelating, such as the inhibition of DNA synthesis[7,8] and mitochondria-mediated apoptosis.[9] However, the underlying mechanism of the action of 1,10-phenanthroline derivatives in lung cancer cells has remained largely unknown.

Most of the apoptosis-related genes are involved in two signaling pathways leading to apoptosis, such as the nuclear factor κB (NF-κB) or the mitochondrial-mediated signaling pathway. The NF-κB family of transcriptional factors play an important role in cancer, immunology, and inflammation,[10,11] and regulate the transcription of many genes that are involved in tumor promotion, angiogenesis, metastasis, and increased cell survival.[12–14] It has five cellular members: p105/p50 (NF-κB1), p100/p52 (NF-κB2), p65 (RelA), RelB, and c-Rel. In almost all cell types, NF-κB complexes are typically localized in the cytoplasm, where they bind to IκB inhibitory proteins, including IκBα, IκBβ, and IκBε. Upon stimulation, IκB proteins are rapidly phosphorylated by I-κB kinases α and β (IKKα and−β) and degraded via the ubiquitin–proteasome pathway. Most important, the activation of NF-κB is linked to increased resistance of tumors to chemotherapeutic drugs and radiation therapy.[15] Many classes of compounds have been reported to inhibit NF-κB activation, such as phenyl-piperazine analogs,[16] glucocorticoids,[17] and nonsteroidal anti-inflammatory agents.[18,19] Thus, NF-κB inhibition is a viable strategy for both chemotherapy and chemoprevention. However, the mechanisms of 1,10-phenanthroline derivatives to inhibit NF-κB activation are not described. In the present study, the antiproliferative activity of 1,10-phenanthroline derivatives was determined, and the effect of these compounds on apoptosis in the A549 human lung cancer cell line was examined. Furthermore, to establish the anticancer mechanism of 1,10-phenanthroline derivatives, the levels of I-κB-α, NF-κB P65, and c-myc, which are strongly associated with the

Abbreviations: NF-κB, nuclear factor-kappa B; MTT, 3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl tetrazolium bromide; Compound **3**, 2-(4-Brominephenyl)-imidazo [4,5*f*][1,10] phenanthroline; A549, human lung adenocarcinoma.

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signal transduction pathway of apoptosis and known to affect the chemosensitivity of tumor cells to anticancer agents, were assayed.

The cytotoxic and antitumor effects of compounds **1**–**10** were studied using MTT assay, and the inhibitory activities (IC_{50}) of these complexes are listed in Table S3. These compounds exhibited high activity against human lung adenocarcinoma (A549) cell line, with inhibitory activities IC_{50} ranging from 0.85 to 35.5 μM. The high cytotoxic activities of a few compounds were even beyond that of cisplatin (15.94 μM). The IC_{50} value for compound **3** was (0.85 ± 0.06) μM, confirming that this compound is more effective in inhibiting A549 cell growth. This result suggests that compounds that contain a pendant halogen functional group might be advantageous for inhibitory activities.

The following observations can be derived from Table S3: (1) Although belonging to the same family of halogen substituted compounds, the complexes might show some differences in activity. Cytotoxic activities increase as electronegativity decreases; (2) The same positions related to the electron-withdrawing group were fluorine, chlorine, and bromine, whereas those related to electron donor were methoxy, ethoxy, and propoxy. The latter also showed higher activities; and (3) The difference in results might involve the steric hindrance of substituents, and small group substitutes in *para*-positions could possess appreciable alteration in their properties.

According to the results of the MTT assay (Table S3), compound **3** is more active than the other compounds in inhibiting A549 cell growth. Thus, this compound was used to investigate further the underlying mechanisms. The IC_{50} values for compound **3** against human melanoma cells A375, human hepatoma cells HepG2, human ovarian carcinoma cells HO8910, human hepatoma cells Bel-7402, nasopharyngeal carcinoma cell CNE-1 were 0.98, 4.3, 1.56, 5.8 and 1.2 μM, respectively, (Table 1). These data indicate the high activity of compound **3** against these cell lines, not only against the A549 cell line. Compound **3** also exhibited higher activity compared with cisplatin.

Apoptosis is a physiological process that functions as an essential mechanism of tissue homeostasis and is regarded as the preferred way to eliminate unwanted cells. Most of the available cytotoxic anticancer drugs mediate their effects via induction of apoptosis in cancer cells.[20] Given that compound **3** had the lowest IC_{50} value of 0.85 μM (Table S3), this compound was used to substantiate further the mechanistic role of inhibition of A549 cancer cell proliferation. The flow cytometric (FCM) analysis (determined 24 h post drug treatment) of compound **3** at 1, 2.5, and 5 μM showed a significant increase in the cell apoptosis at 24 h. The ratio of cell apoptosis reached 50.94% for 5 μM compound **3** (Fig. 1). The result of control (10.81%) was approximately fivefold lower than that of compound **3**, indicating that the latter induced A549 cell apoptosis.

In view of the important role of NF-κB signal pathway in the cell proliferation, the effect of compound **3** upon the protein levels of p65, IκBα and c-myc, a well-documented downstream target gene

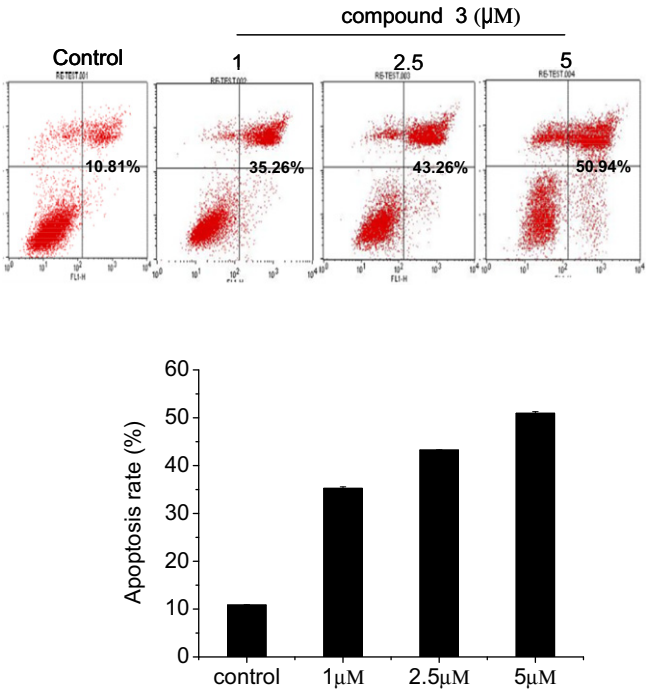
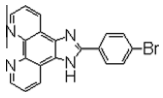


Figure 1. FCM scatter of the apoptosis of A549 cell treated with compound **3** after 24 h. The effect of 1, 2.5 and 5 μM of compound **3** on A549 cell apoptosis determined by flow cytometry. Untreated (control) cells or cells treated for 24 h were harvested, fixed, stained with Annexin V/PI, and assessed for cell apoptosis distribution by flow cytometric analysis. Percentages of apoptotic cells were measured by the annexin V/PI flow cytometry analysis, as described in Section 2. The *y*-axis plots the sum of early and late apoptotic cells as the mean ± SD of three independent experiments.

of NF-κB signal pathway, were assayed by Western blot. As shown in Figure 2A, compound **3** treatment of A549 cells for 24 h evidently induced IκBα protein expression in a dose-dependent manner. Meanwhile, the protein levels of c-myc and p65 were decreased after compound **3** exposure in a dose—and/or time-dependent manner (Fig. 2B and C). These data suggest that compound **3** inhibited NF-κB signal pathway by upregulation of IκBα and downregulation of p65, thus suppressing of c-myc expression and in turn inhibiting proliferation and inducing apoptosis in A549 cell.

The $2^{-\triangle\triangle ct}$ method was used to calculate relative changes in gene expression that were determined from real-time quantitative PCR experiments.[21] To investigate whether the induction of apoptosis by compound **3** is due to the downregulation of the *c-myc* gene, quantitative real-time PCR was conducted in compound **3**-treated A549 lung cancer cells. As shown in Figure 3, compound **3** had a markedly suppressing effect on the *c-myc* expression level in a dose-dependent manner. Therefore, compound **3** might be a potent agent for the treatment of A549 human lung cancer.

Table 1
Chemical structure of compound **3** and inhibitory activity (IC_{50} values) of compound **3** against human cancer cells[a]



Compd	IC_{50}(μM)					
	A549	A375	Hep G2	Ho8910	Bel-7402	CNE-1
3	0.85 ± 0.06	0.98 ± 0.07	4.3 ± 0.21	1.56 ± 0.09	5.8 ± 0.26	1.2 ± 0.08
DDP	15.94 ± 1.02	7.3 ± 0.34	8.3 ± 0.37	4.4 ± 0.22	15.3 ± 1.05	6.5 ± 0.05

[a] IC_{50} values are given in μM. Compd, compound, DDP, cisplatin. The values are expressed as the mean ± SD (triplicates).

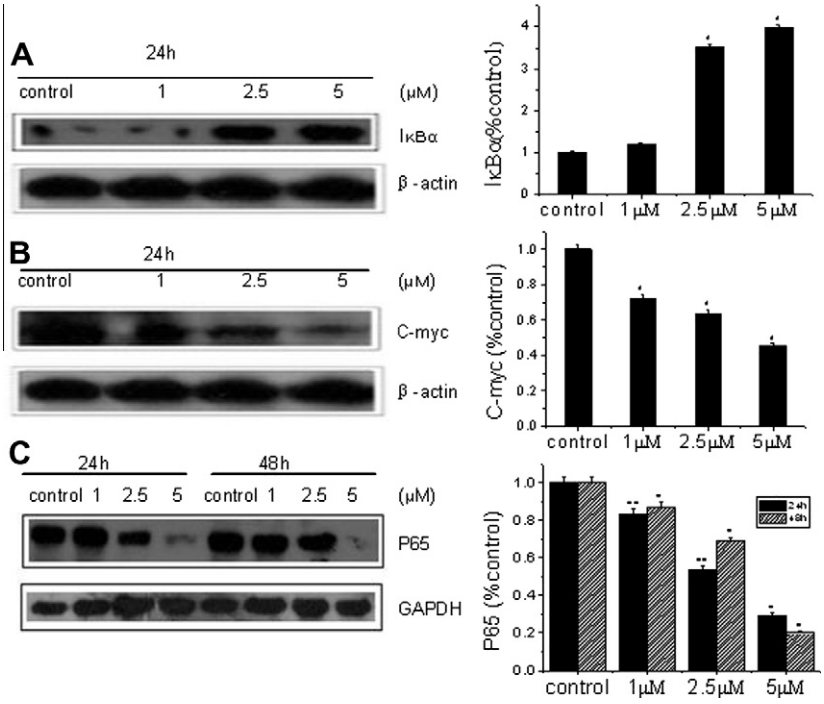


Figure 2. Effect of compound **3** on apoptosis-related proteins. Total cell lysates from cells treated for various concentrations with 1, 2.5 and 5 μM of compound **3** were separated electrophoretically on 10% polyacrylamide gels and immunoblotted with an antibody against each protein, β-actin and GAPDH as an internal control, respectively. (A) Effect of compound **3** on the expression of IκBα in A549 after 24 h; (B) Effect of compound **3** on the expression of c-myc in A549 after 24 h and 48 h, respectively; (C) Effect of compound **3** on the expression of p65 in A549 after 24 h. The values are expressed as the mean ± SD (triplicates), and statistical significance is indicated by $^{*}P$ <0.05, $^{**}P$ <0.01.

In conclusion, a novel class of potent NF-κB signaling inhibitors was discovered through the present study. Further studies by Western blot and PCR assay have shown that compound **3** significantly inhibited the expression levels of NF-κB proteins in the A549 cell line in a time-dependent manner. The results show that compound **3** impaired the activity of NF-κB to inhibit A549 cell proliferation by suppressing the c-myc expression. This class of compounds can be beneficial in the treatment of lung cancer, autoimmune diseases, anti-viral therapy, and interferon co-therapy. These compounds could lead to a novel concept of host cell pathway modulation toward an anticancer therapy. Further studies must be conducted to gain an in-depth comprehension of these preliminary findings on the activity of these promising wide-spectrum anticancer agents.

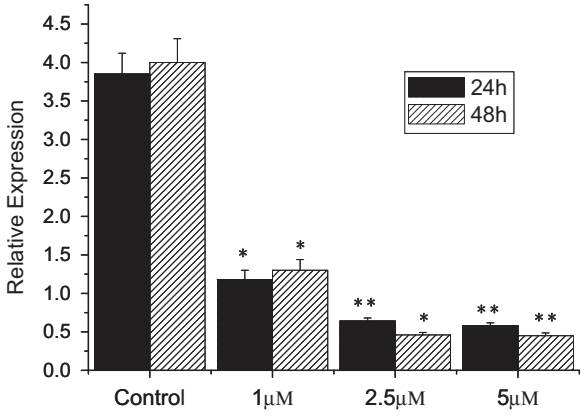


Figure 3. Quantitative PCR expression analysis of *c-myc* treated with compound **3**. Sample spreadsheet of data analysis using the $2^{-\triangle\triangle ct}$ method, where △△ct = (CT, Target—C, Actin)Time x—(CT, Target—C, Actin)Time 0. Black and gray bars represented the ratios of c-myc gene relative expression in the absence and presence of compound **3** treated for 24 and 48 h with 1, 2.5 and 5 μM, and fold changes in gene expression with respect to control cells were determined by quantitative real time reverse transcription-PCR. The values are expressed as the mean ± SD (triplicates), and statistical significance is indicated by $^{*}P$ <0.05, $^{**}P$ <0.01.

Acknowledgements

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Supplementary data

Supplementary data (biological methods, experimental procedures and the complete characterization data of the synthesized compounds are provided.) associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2011.11.063.

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