

## Original Article

# *In vitro* anticancer property of a novel thalidomide analogue through inhibition of NF- $\kappa$ B activation in HL-60 cells

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**Aim:** To investigate the anticancer property and possible mechanism of action of a novel sugar-substituted thalidomide derivative (STA-35) on HL-60 cells *in vitro*.

**Methods:** TNF- $\alpha$ -induced NF- $\kappa$ B activation was determined using a reporter gene assay. The MTT assay was used to measure cytotoxicity of the compound. The appearance of apoptotic Sub-G1 cells was detected by flow cytometry analysis. PARP cleavage and protein expression of NF- $\kappa$ B p65 and its inhibitor I $\kappa$ B were viewed by Western blotting.

**Results:** STA-35 (1–20  $\mu$ mol/L) suppressed TNF- $\alpha$ -induced NF- $\kappa$ B activation in transfected cells (HEK293/pNiFty-SEAP) in a dose- (1–20  $\mu$ mol/L) and time-dependent (0–48 h) manner. It was also shown that STA-35 exerted a dose-dependent inhibitory effect on HL-60 cell proliferation with an IC<sub>50</sub> value of 9.05  $\mu$ mol/L. In addition, STA-35 induced apoptosis in HL-60 cells, as indicated by the appearance of a Sub-G1 peak in the cell cycle distribution, as well as poly ADP-ribose polymerase (PARP) cleavage. Subsequently, both NF- $\kappa$ B p65 and its inhibitor I $\kappa$ B gradually accumulated in cytoplasmic extracts in a dose- and time-dependent manner, indicating the blockage of NF- $\kappa$ B translocation induced by TNF- $\alpha$  from the cytoplasm to the nucleus.

**Conclusion:** A novel sugar-substituted thalidomide derivative, STA-35, is potent toward HL-60 cells *in vitro* and induces apoptosis by the suppression of NF- $\kappa$ B activation.

**Keywords:** anticancer; HL-60; thalidomide; NF- $\kappa$ B; apoptosis

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## Introduction

Since the discovery of the NF- $\kappa$ B transcription factor in 1986<sup>[1]</sup>, increasing experimental evidence has demonstrated that this factor plays a major role in the development and progression of various human cancers<sup>[2]</sup>. NF- $\kappa$ B exists as a homodimer or heterodimer composed of Rel family member proteins p50/105, p52/p100, p65, RelB, and c-Rel, with p65/p50 heterodimer as the main member of cytokine-induced NF- $\kappa$ B activation. NF- $\kappa$ B is located in the cytoplasm and bound by inhibitory proteins such as I $\kappa$ B $\alpha$  or other I $\kappa$ B-like proteins. Upon stimulation by upstream inducers, a phosphorylation cascade results in dissociation of the inhibitory proteins from NF- $\kappa$ B, freeing the latter to activate the expression of genes involved in cell growth, suppression of apoptosis, metastasis, and immune and inflammatory

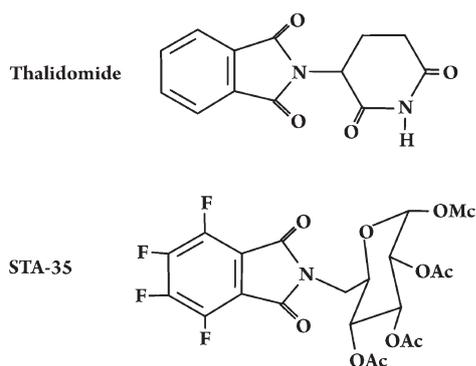
responses<sup>[3]</sup>. Therefore, the inhibition of NF- $\kappa$ B appears to be a promising strategy for cancer therapy<sup>[4]</sup>. Accordingly, a series of NF- $\kappa$ B inhibitors based on the mechanism of NF- $\kappa$ B antagonism have been identified<sup>[5]</sup>. Thalidomide is one of the drugs that can inhibit NF- $\kappa$ B activation.

Thalidomide ( $\alpha$ -N-phthalimideglutarimide, Figure 1) is a sedative/hypnotic drug that was withdrawn from the market in the 1960s because of its severe teratogenicity<sup>[6,7]</sup>. However, it is now being re-evaluated for its antiangiogenic effect and potential use in the treatment of various diseases including AIDS and cancer<sup>[8,9]</sup>. Recently, thalidomide has received great attention in structural development studies aimed at discovering new compounds with greater efficacy, less toxicity, and better bioavailability<sup>[8]</sup>. In the course of those studies, several thalidomide analogues were obtained, such as TNF- $\alpha$  production regulators<sup>[10,11]</sup>,  $\alpha$ -glucosidase inhibitors<sup>[12,13]</sup>, cyclooxygenase (COX) inhibitors<sup>[14,15]</sup>, nitric oxide synthase (NOS) inhibitors<sup>[16,17]</sup>, tubulin polymerization inhibitor<sup>[18]</sup>, and immunomodulatory analogues

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**Figure 1.** Chemical structure of thalidomide and STA-35.

(IMiDs)<sup>[19]</sup>. More recently, thalidomide and its analogues have shown promising immunomodulatory, antiangiogenic, antiproliferative, and pro-apoptotic properties, and were found to mediate antitumor responses in many kinds of human cancer. For example, the thalidomide analogue, CC-4047, displays profound cytostatic effects in stroma-supported human acute lymphoblastic leukemia (ALL) cells both *in vitro* and *in vivo*<sup>[20]</sup>. Interestingly, substitution of the four aromatic hydrogen atoms of phthalimide analogues of thalidomide with fluorine leads to more potent angiogenic and prostate cancer cell inhibitory activities compared to thalidomide<sup>[21]</sup>.

Phthalimide is a useful pharmacophore, as well as a common protecting group used in carbohydrate chemistry. Moreover, the carbohydrate moiety might play an important role in carrying the phthalimide to the specific site for its pharmacological activity<sup>[22, 23]</sup>. Thus, it would be of value to develop sugar-substituted phthalimide as a novel thalidomide analogue with better pharmacological profiles.

In the present study, a novel sugar-substituted phthalimide derivative, namely, 1-*O*-methyl-2,3,4-tri-acetyl-6-tetrafluorophthalimido-6-deoxy- $\alpha$ -*D*-glucopyranose (STA-35), was synthesized and investigated for its effect on NF- $\kappa$ B activation, as well as its anticancer property on HL-60 cells *in vitro*. We observed that STA-35 suppresses TNF- $\alpha$ -induced NF- $\kappa$ B activation and exerted an inhibitory effect accompanied by apoptosis induction in HL-60 cells *in vitro*.

## Materials and methods

**Cells and cell culture** A stably transfected cell line (HEK293/pNiFty-SEAP, provided by Dr DAVRICHE, INSERM 563, CHU Purpan, BP 3028, 31054 Toulouse Cédex, France), was grown in Dulbecco's modified Eagle's medium (GIBCO/BRL, Germany) supplemented with

10% heat-inactivated fetal bovine serum and antibiotics (100 U/mL penicillin, 100  $\mu$ g/mL streptomycin, and 200  $\mu$ g/mL Zeocin). Cell cultures were maintained in the same condition as other cell lines. The human promyeloleukemia cell line (HL-60) was maintained in RPMI-1640 medium (GIBCO/BRL, Germany) supplemented with 10% heat-inactivated fetal calf serum and antibiotics (100 U/mL penicillin and 100  $\mu$ g/mL streptomycin) in a 95% air, 5% CO<sub>2</sub> humidified atmosphere at 37 °C.

**Drugs and reagents** A novel sugar-substituted thalidomide analogue, 1-*O*-methyl-2,3,4-tri-acetyl-6-tetrafluorophthalimido-6-deoxy- $\alpha$ -*D*-glucopyranose, named STA-35, was synthesized by condensation of tetrafluorophthalic anhydride with related 6-aminoglucose according to the literature<sup>[24]</sup>. Its structure was determined by <sup>1</sup>H, <sup>13</sup>C NMR, and mass spectrum and elementary analyses (C<sub>21</sub>H<sub>19</sub>O<sub>10</sub>NF<sub>4</sub>, 521.38, Figure 1). It was purified to a higher than 98% purity by silica gel column chromatography. The compound is a white solid, mp 181–182 °C, with good stability at room temperature and solubility in organic solvents such as dimethyl sulfoxide (DMSO), CHCl<sub>3</sub>, acetone, and ethyl acetate, but less solubility in water. It was dissolved in 10% DMSO in PBS at a concentration of 10<sup>-2</sup> mol/L as a stock solution, and further diluted in PBS before use. The final concentration of DMSO in the solution was less than 0.1%.

TNF- $\alpha$  was purchased from R&D Company (Carlsbad, CA, USA). Thalidomide, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT), anti-actin antibody, and secondary antibodies against mouse or rabbit IgG were purchased from Sigma-Aldrich (Sheboygan, Wisconsin, USA). Monoclonal antibody against NF- $\kappa$ B p65 and polyclonal antibody against I $\kappa$ B $\alpha$  were obtained from Santa Cruz Biotechnology, Inc, USA.

**Gene reporter assay for NF- $\kappa$ B activity** NF- $\kappa$ B activities were measured by the secreted alkaline phosphatase (SEAP) reporter gene assay<sup>[25]</sup>. The inhibitory effect of STA-35 on NF- $\kappa$ B activation was analyzed by comparing absorbance values with those of the controls. To assess effects of STA-35 on NF- $\kappa$ B, a control of TNF- $\alpha$  plus 0.1% DMSO was used at each indicated time. The experiments were run in triplicate.

**Measurement of cytotoxicity by the MTT assay** The effects of STA-35 and thalidomide on cell proliferation were determined using a standard MTT-based colorimetric assay as described previously<sup>[26]</sup>. The experiments were run in triplicate.

**Flow cytometry** HL-60 cells were plated at a density of 2 $\times$ 10<sup>5</sup> cells/mL. On the following day, different concentrations of STA-35 (0.5–20  $\mu$ mol/L) were added. Control cells

were treated with 0.1% DMSO. At the times indicated, cells were harvested by centrifugation at 1000×g, washed in PBS, and fixed overnight in 70% ethanol at -20 °C. After addition of 0.1 mg/mL RNase for 30 min at 37 °C, PBS was used again to wash and resuspend the cells. When it was time for the assay, the cells were stained with 0.5 mg/mL of propidium iodide. Pro-apoptotic effects (Sub-G1 population of the cells) were determined with analytical flow cytometry using a BD-LSR flow cytometer (USA) with an excitation/emission wavelength of 488/525 nm. Experiments were performed in triplicate and yielded similar results.

**Western blotting** To determine the pro-apoptotic effect of STA-35, the PARP cleavage of HL-60 cells was determined using Western blotting. Cell lysates in lysis buffer (150 mmol/L NaCl, 1% NP40, 0.5% deoxycholate, 0.1% SDS, 50 mmol/L Tris) were homogenized with a pipette and centrifuged for 15 min at 10 000 r/min. Supernatants were collected and total proteins were quantified using a protein assay kit (Pierce, USA). Proteins 30 µg was boiled for 5 min in 5% β-mercaptoethanol reducing Laemli sample buffer, and samples were separated by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) in 10% denaturing polyacrylamide gels and transferred to nitrocellulose membranes (Hybond C, Amersham). Immunoblots were stained with ponceau red to visualize total proteins contained in each slot and probed alternatively with the following mouse monoclonal antibodies: anti-human PARP (clone 70, 1:500 dilution, Transduction Laboratories) and anti-human β-actin (clone AC-15, 1:2000 dilution, SIGMA). Blots were visualized using a peroxidase-conjugated polyclonal anti-mouse antibody (1:10,000 dilution, SIGMA) and an ECL detection kit (Amersham).

To analyze the expression of NF-κB p65 and IκBα proteins, the HL-60 cells were seeded into incubation flasks at 5×10<sup>5</sup> cells/mL. Following incubation for 24 h, the culture medium was replaced by fresh medium, and STA-35 (1–40 µmol/L) or solvent (0.1% DMSO) was added into the medium for 6 h, followed by TNF-α (50 ng/mL) for another 30 min. DMSO (0.1%) alone was added to the medium as a solvent control. Cytoplasmic extracts were obtained as previously described<sup>[27]</sup>. Protein quantification and Western blot analysis were performed as for viewing PARP cleavage, but probed alternatively with the mouse monoclonal antibody prepared against p65 (1:500) and polyclonal antibody prepared against IκBα (1:250). Blots were visualized using peroxidase-conjugated polyclonal anti-mouse or anti-rabbit antibodies (1:5000) and an ECL detection kit (Amersham). The images were scanned using Imagine J software to semi-quantify the expression of the proteins.

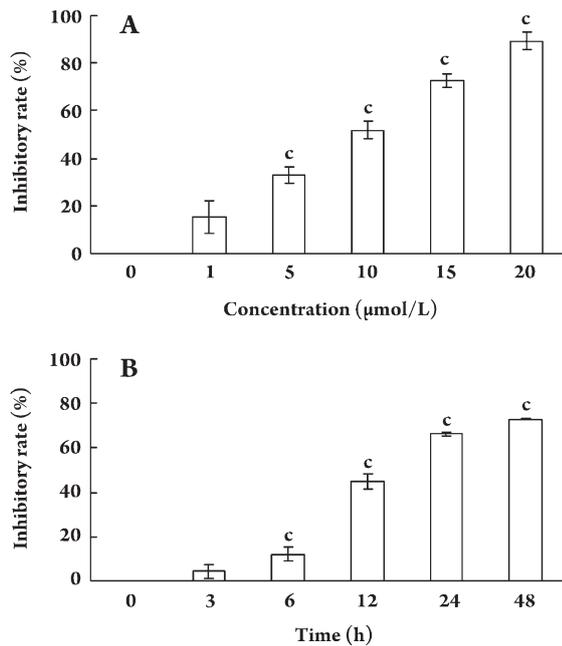
**Statistical analysis** The statistical analysis was performed with SPSS 13.0 software, and one way ANOVA was used to determine the significant difference between the various samples.

## Results

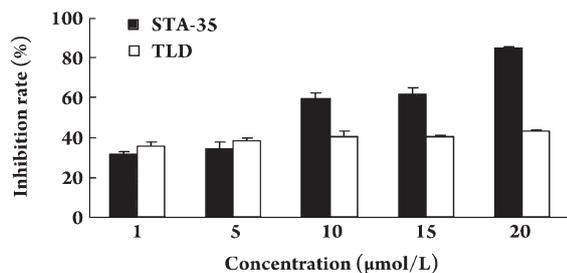
**STA-35 suppressed TNF-α-induced NF-κB activation by a reporter gene assay** A series of synthesized novel N-substituted phthalimide derivatives were analyzed by a random screen with a reporter gene assay. An N-sugar-substituted phthalimide, namely, 1-O-methyl-2,3,4-tri-acetyl-6-tetrafluorophthalimido-6-deoxy-α-D-glucopyranose (STA-35), showed inhibitory effects on TNF-α-induced NF-κB activation. As the dosages of STA-35 (1–20 µmol/L, increased, NF-κB activity induced by TNF-α decreased gradually, and 10 µmol/L STA-35 significantly decreased the SEAP activity. A maximal inhibitory effect was achieved with 20 µmol/L STA-35 (Figure 2A). In parallel, the inhibitory effect of 20 µmol/L STA-35 on NF-κB activity increased upon the increase of incubation time (Figure 2B). To rule out the possibility that the reduction of NF-κB activity is due to cell death, we investigated the cytotoxicity of compound STA-35 plus TNF-α on HEK293/pNiFty-SEAP cells. The result showed that the combination of TNF-α (0.2 ng/mL) and different concentrations (1–20 µmol/L) of compound STA-35 did not result in observable cell death at 24 h (data not shown). Therefore, it is most likely that the observed reduction in NF-κB in Figure 2 is not due to cell death.

**Cytotoxic effect of STA-35 on tumor cell proliferation** To investigate potential inhibition of the compound on tumor cell proliferation, the cytotoxicity of STA-35 and thalidomide on HL-60 cells after a 48-h treatment was evaluated using the MTT assay. As shown in Figure 3, STA-35 inhibited cell proliferation markedly in a dose-dependent manner. The IC<sub>50</sub> value of STA-35 was determined to be 9.05 µmol/L in HL-60 cells. In contrast, thalidomide gave a much lower inhibition rate on HL-60 cell proliferation than STA-35, indicating that the N-sugar substitution of thalidomide provides its biological activity.

**STA-35 induces appearance of Sub-G1 cells and PARP cleavage in HL-60 cells** Apoptosis is known to be a very important mechanism in the anticancer effects induced by chemopreventive and chemotherapeutic agents<sup>[28]</sup>. To assess whether STA-35 induced apoptosis, we first applied flow cytometry analysis to check for the appearance of a specific apoptotic fraction (Sub-G1 population). As illustrated in Figure 4, exposure to a gradient concentration of STA-35 from 1 to 20 µmol/L increased the number of apoptotic cells

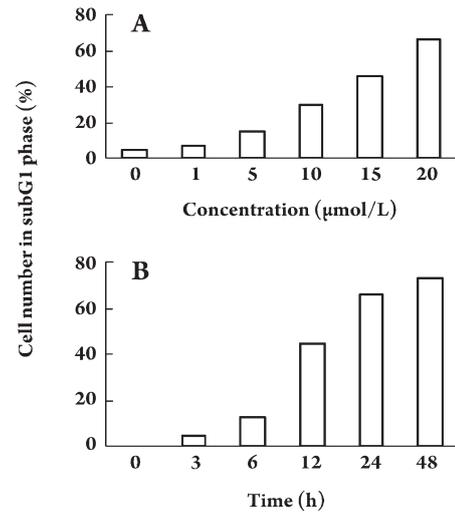


**Figure 2.** Inhibitory effect of STA-35 on TNF- $\alpha$ -induced NF- $\kappa$ B activation. The inhibitory effect of STA-35 on NF- $\kappa$ B activation induced by TNF- $\alpha$  (0.2 ng/mL) was determined by the SEAP reporter gene assay. Absorbance was measured at 405 nm. Inhibitory rates of STA-35 on NF- $\kappa$ B at each time point were calculated as follows: [the absorbance values at 405 nm (A values) of positive control (TNF- $\alpha$  plus 0.1% DMSO) minus the A values of sample treated with 20  $\mu$ mol/L STA-35 plus TNF- $\alpha$ ]/A values of positive control (TNF- $\alpha$  plus 0.1% DMSO) $\times$ 100%. (A) Dose-effect results of HEK293/pNiFty-SEAP cells exposed for 24 h to varying doses of STA-35 (1–20  $\mu$ mol/L). (B) Time-course results of HEK293/pNiFty-SEAP cells treated with 10  $\mu$ mol/L of STA-35 for varying times (0–48 h). The experiments were run in triplicate.  $n=3$ . Mean $\pm$ SD. \* $P<0.01$ .



**Figure 3.** STA-35 inhibits proliferation of HL-60 cells. HL-60 cells were exposed to increasing concentrations of STA-35 and thalidomide (TLD), respectively, for 48 h. The absorbance values were determined by the MTT assay. 0.1% DMSO was used as a solvent control. The inhibition rate is expressed as a percentage of solvent control. Each value represents the mean $\pm$ SD of data in triplicate for proliferation inhibition.

from 6.85% to 65.86% versus 4.16% in the control, indicating

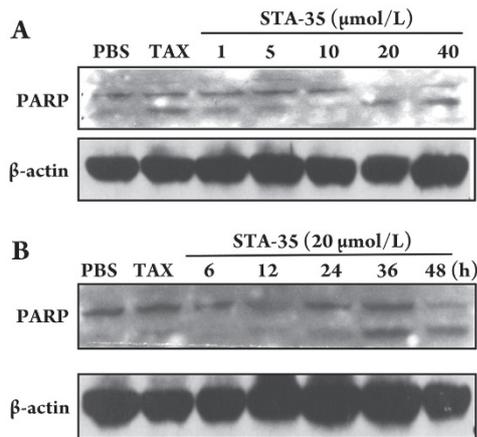


**Figure 4.** Pro-apoptosis effect of STA-35 on HL-60 cells. HL-60 cells were exposed to different concentrations of STA-35 for 24 h (A) or 20  $\mu$ mol/L of STA-35 for various time points (B). 0.1% DMSO was used as a control. Apoptotic fraction was determined using flow cytometry.

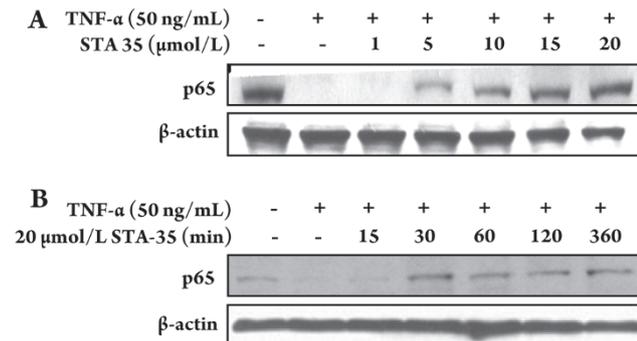
that the apoptotic induction by STA-35 is obviously dose-dependent after treatment for 12 h. In parallel, there is a linear increase of apoptotic cells during the incubation times from 6 h to 48 h in the presence of 20  $\mu$ mol/L STA-35.

The PARP protein, a substrate of caspase-3, is an early hallmark of apoptosis. To further address the pro-apoptotic effect of STA-35 on HL-60 cells, we analyzed PARP cleavage by Western blotting using a PARP antibody that recognizes native and fragmented PARP. Incubation of HL-60 cells with STA-35 (1–40  $\mu$ mol/L for 24 h or 20  $\mu$ mol/L for 6–48 h) induced a cleavage pattern of PARP characterized by the appearance of an 85 kDa fragment, which was similar to that obtained with paclitaxel treatment. Increasing both the dose and the incubation time of STA-35 enhanced the amount of the cleaved PARP (Figure 5).

**Determination of NF- $\kappa$ B protein expression by Western blotting** NF- $\kappa$ B is a transcription factor that plays a critical role in the inappropriate survival of various types of malignant cells<sup>[29]</sup>. To analyze the anticancer molecular mechanism of STA-35, as well as to validate the results of the reporter gene assay (Figure 2), we investigated the effect of STA-35 on NF- $\kappa$ B protein translocation using Western blotting. The cells treated with TNF- $\alpha$  alone showed a decreased expression of p65 and I $\kappa$ B $\alpha$  proteins (Figures 6, 7) in cytoplasmic extracts, when compared with those treated with PBS. However, both NF- $\kappa$ B p65 and its inhibitor I $\kappa$ B proteins accumulated gradually in the cytoplasmic extracts in a dose- and time-dependent manner, indicating blockage of

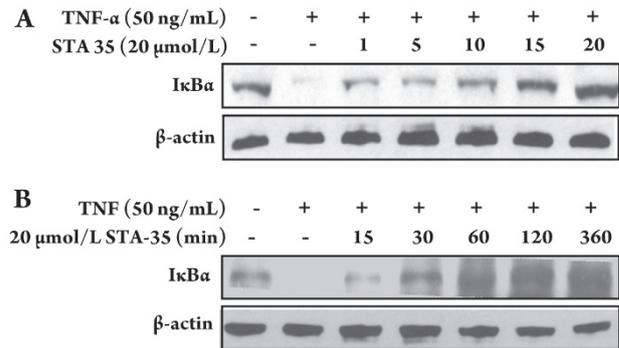


**Figure 5.** Effect of STA-35 on PARP cleavage in HL-60 cells. Total protein was extracted from HL-60 cells treated with 1–40 μmol/L STA-35 for 24 h (A) or 20 μmol/L STA-35 for 6–48 h (B). 0.1% DMSO and 10 μmol/L paclitaxel (TAX) were used as negative and positive controls, respectively. Protein samples were separated by 10% SDS-PAGE and electrophoretically transferred to nitrocellulose membranes. Immunoblots were probed alternatively with the following mouse monoclonal antibodies against PARP (1:500) and β-actin (1:2000), then visualized with peroxidase-conjugated monoclonal anti-mouse (1:8000) antibody and an ECL detection kit. Cytoplasmic β-actin protein was used as a control for equal loading.



**Figure 6.** Effect of STA-35 on nuclear translocation of NF-κB. Cytoplasmic proteins were extracted from HL-60 cells treated with different concentrations of STA-35 for 6 h (A) or 20 μmol/L STA-35 for varying time points (B) followed by the addition of TNF-α (50 ng/mL) into the culture medium for another 30 min. 0.1% DMSO and TNF-α (50 ng/mL) alone were used as controls. Protein samples were separated by 10% SDS-PAGE and electrophoretically transferred to nitrocellulose membranes. Immunoblots were probed alternatively with the following mouse monoclonal antibodies against p65 (1:500) and β-actin (1:2000), then visualized with peroxidase-conjugated monoclonal anti-mouse (1:8000) antibody and an ECL detection kit. Cytoplasmic β-actin protein was used as a control for equal loading.

TNF-α-induced NF-κB translocation from the cytoplasm to the nucleus. A semi-quantitative analysis (data not shown)



**Figure 7.** Effect of STA-35 on IκBα degradation. Cytoplasmic proteins were extracted and Western blot analysis was performed as indicated in Figure 6. Rabbit polyclonal antibody against IκBα was used to determine the expression of IκBα. β-actin was used as a control for equal loading.

revealed that the inhibition on p65 increased as the concentration (from 1 μmol/L to 20 μmol/L) and treatment time (from 60 to 360 min) of STA-35 increased. Similar results were obtained for IκBα expression when STA-35 concentration (from 5 to 20 μmol/L) and incubation time (from 15 min to 360 min) were increased. The effect of STA-35 on NF-κB protein expression suggests that STA-35 blocks NF-κB translocation, as well as inhibits NF-κB activity in a time- and dose-dependent manner.

## Discussion

Several lines of evidence suggest that the molecular mechanisms of the multiple pharmacological actions elicited by thalidomide and its analogues are related to the NF-κB signaling pathway<sup>[30,31]</sup>. For example, thalidomide was demonstrated to block NF-κB activation through suppression of IκB kinase activity<sup>[3]</sup>. In our research, we designed and synthesized an N-sugar-substituted thalidomide analogue named STA-35, and then explored its anticancer and pro-apoptotic properties, as well as its effects on the NF-κB signaling pathway.

Thalidomide can inhibit tumor growth in a concentration-dependent manner in HL-60 cells with an IC<sub>50</sub> of 22.14 μmol/L, *via* COX-2 degradation independent of antiangiogenesis<sup>[32]</sup>. Inatsuki *et al* reported that thalidomide analogues suppressed HL-60 cell proliferation by inhibition of tubulin polymerization, in which one of the compounds showed the most potent activity with an IC<sub>50</sub> of approximately 5 μmol/L<sup>[18]</sup>. We showed that STA-35 inhibited HL-60 cell proliferation at a much higher rate than thalidomide; STA-35 inhibited HL-60 cell growth with an IC<sub>50</sub> of

9.05  $\mu\text{mol/L}$ . The higher growth-inhibition rate of this novel N-sugar-substituted phthalimide indicates that the N-sugar substitution of thalidomide provides its biological activity. It was also shown that STA-35 inhibited cell proliferation by suppressing NF- $\kappa$ B activation, which prompted us to investigate the link between inhibition of cell-proliferation and suppression of NF- $\kappa$ B activation.

Suppression of the nuclear factor- $\kappa$ B (NF- $\kappa$ B)/inhibitor of nuclear factor- $\kappa$ B (I $\kappa$ B) signaling pathway is a potential property of thalidomide<sup>[33]</sup>. Moreover, the ability of NF- $\kappa$ B to inhibit apoptosis, as well as to promote cell proliferation, makes it an attractive target for cancer therapy<sup>[34]</sup>. Hideshima *et al* first demonstrated that thalidomide and its analogues induced tumor cell apoptosis, evidenced by increased Sub-G1 cells or induction of p21 and related G1 growth arrest<sup>[35]</sup>. In addition, thalidomide has been shown to directly affect multiple myeloma cells through the induction of apoptosis and growth arrest<sup>[36]</sup>. Our compound STA-35 not only induces the appearance of Sub-G1 cells but also causes PARP cleavage in HL-60 cells in a time- and dose-dependent manner, which is in agreement with its cytotoxic property.

The mammalian NF- $\kappa$ B family of proteins contains the K $\epsilon$ A/p65, NF- $\kappa$ B1, NF- $\kappa$ B2, C-Rel, and RelB subunits, which can form a variety of heterodimers and homodimers to differentially control downstream gene expression of signals elicited by cytokines, bacterial products, viral expression, growth factors, and stress stimuli. I $\kappa$ B is a very important regulator in the activation process of NF- $\kappa$ B. Therefore, we utilized Western blot analysis to evaluate the effect of STA-35 on NF- $\kappa$ B p65 and I $\kappa$ B in cytoplasmic proteins extracted from HL-60 cells. We demonstrated that STA-35 restored the expression of p65 and inhibited the degradation of cytoplasmic I $\kappa$ B in a dose- and time-dependent manner, consistent with the functional suppression of the NF- $\kappa$ B pathway by a reporter gene assay. It can be deduced that inactivation of NF- $\kappa$ B resulted from STA-35 treatment might be due to inhibition of I $\kappa$ B degradation. Since the NF- $\kappa$ B signaling pathway can be inhibited at multiple levels, the exact mechanism underlying STA-35 regulation of NF- $\kappa$ B activity needs to be elucidated for a better understanding of the anticancer and/or anti-inflammatory properties of STA-35.

In conclusion, a novel N-sugar-substituted phthalimide exhibited cytotoxic and pro-apoptotic activities in HL-60 cells, which may be mediated by the suppression of NF- $\kappa$ B activation. These results highlight the importance of further study of N-sugar substitution of thalidomide in terms of the chemical modification and pharmacological effect.

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## Author contribution

Min LI designed research; Min LI, Wan SUN, Ya-ping YANG, Bo XU, Wen-yuan YI, Yan-xia MA performed research; Bo XU, Zhong-jun LI contributed new analytical tools and reagents; Min LI and Wan SUN analyzed data; Min LI and Jing-rong CUI wrote the paper.

## Abbreviation

DMSO, dimethyl sulfoxide; NF- $\kappa$ B, nuclear factor- $\kappa$ B; I $\kappa$ B, Inhibitor of  $\kappa$ B; MTT, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide; TNF- $\alpha$ , tumor necrosis factor- $\alpha$ .

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