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Discovery of Novel Imidazothiazole Derivatives as NF-κB Inhibitors

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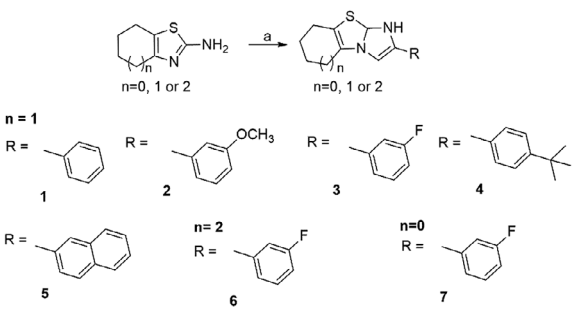
Nuclear factor kappa-B (NF-κB), a transcription factor that exists as a heterodimer or homodimer, is a key signaling molecule in innate and adaptive immune responses.[1] NF-κB is activated by pro-inflammatory cytokines like tumor necrosis factor α (TNF-α) and interleukin-1β (IL-1β), or pathogens like lipopolysaccharides (LPS), which results in nuclear translocation of NF-κB. Constitutive activation of NF-κB has been observed in the pathogenesis of a variety of diseases.[2] In many cancers, NF-κB promotes proliferation and survival, and regulates angiogenesis by inducing vascular endothelial growth factor expression.[3] NF-κB is also implicated in tumor metastasis and in remodeling tumor metabolism.[3] Inhibition of NF-κB could overcome tumor resistance and protect normal tissues from toxicities caused by radiation or chemotherapy.[4] Further, NF-κB is chronically and aberrantly activated in autoimmune diseases including multiple sclerosis lesions, Crohn's disease, inflammatory bowel disease and arthritis.[5,6] NF-κB is considered a promising therapeutic target in cancers and inflammatory diseases. Great efforts to identify NF-κB signaling inhibitors have afforded numerous small molecule inhibitors including natural products.[7,8] Several IκB kinase inhibitors have been developed, but to date, none are clinically useful despite promising preclinical results.[7] Further discovery of novel NF-κB inhibitors is still required and a pool of chemical regulators could provide a great opportunity to identify clinically useful agents.[8] Herein, we describe the discovery of small molecule regulators through phenotype-based assays from a chemical library. Chemicals were screened in LPS-stimulated RAW 264.7 cells and their inhibitory activity for nitric oxide (NO) production were determined by measuring nitrite concentration. Cells were stimulated with LPS (0.1 μg/mL) for 24 h after chemical treatment for 2 h. Nitrite levels in culture media were measured using the Griess reaction and cell viability was established with an MTT assay.

A series of tetrahydobenzoimidazothiazole derivatives were found to have inhibitory activity for nitric oxide production. The primary hits were re-synthesized and their synthetic scheme is outlined in Scheme 1. Coupling of 2-aminothiazoles with various α-bromo-acetophenones provided the desired imidazothiazole derivatives.

To investigate whether the imidazothiazoles inhibit LPS-induced inflammatory responses, NO production was measured in RAW264.7. The cells were treated with compounds and analyzed after 24 h incubation with or without LPS. Compounds **1–5** exhibited a considerable reduction of nitrite concentration at 10 and 30 μM (Figure 1). Compound **3** gave the most potent activity. A slight cytotoxicity was observed in cells treated with compounds, except for **2**, at high concentrations, compared to cells treated with LPS only. The reduction of nitrite concentrations was much greater than the decrease of the viable cells, indicating that activity of compounds has little to do with cytotoxicity. To understand whether imidazothiazoles inhibit NF-κB mediated signaling, we carried out an image-based assay visualizing translocation of NF-κB by using an immunofluorescent staining method.[9] Immunostaining was performed after the short-time exposure of chemicals. As shown in Figure 2, NF-κB was principally localized in the cytoplasm in the resting state. The treatment with LPS (1 μg/mL) led to the translocation of NF-κB into the nucleus. The translocation of NF-κB was inhibited by the pretreatment of the representative compound **3**. Image analysis indicated that compound **3** was strongly active for preventing translocation of NF-κB, supporting that imidazothiazole activity for NO production may result from blocking translocation of NF-κB. Next, to explore further structural activity relationships (SAR), we synthesized two derivatives that have different ring sizes. Ring expanded **6** inhibited NO production by more than 50%



Scheme 1. Reagents and condition: (a) EtOH, 2-bromo-1-arylethanone, 150 °C, microwave, 20 min

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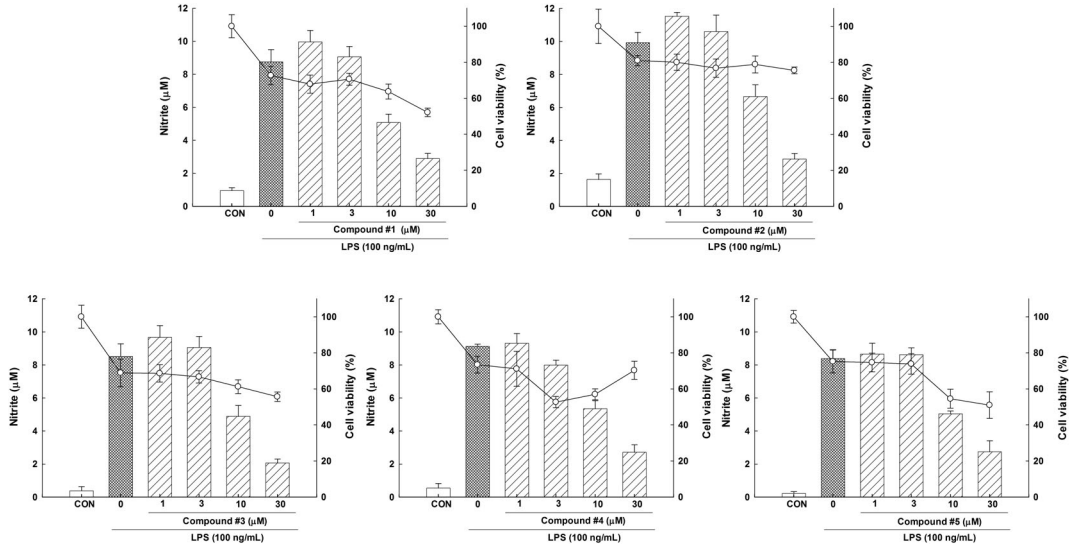

Figure 1. Inhibitory effects of the described compounds (1–5) on NO production in LPS-stimulated RAW 293.7 cells. Data present mean ± S.D. in triplicate. $^{*}p < 0.05$ compared to control, $^{\#}p < 0.05$ compared to LPS-treated group (LPS).

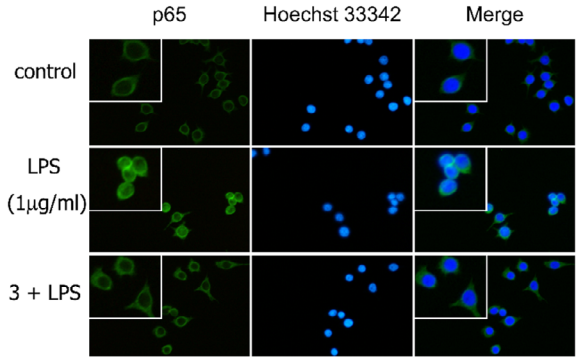

Figure 2. Immunocytochemistry for NF-κB translocation. Cells were pretreated with compound **3** at 30 μM for 2 h, and then stimulated with LPS (1 μg/mL) for 1 h. cells were stained with anti-NF-κB p65 (green) and Hoechst 33342 for nuclei (blue).

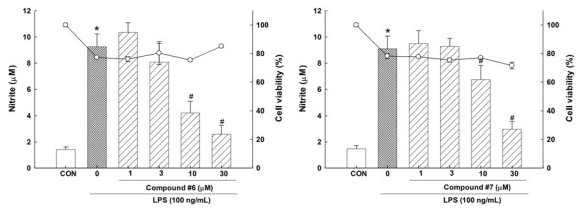

Figure 3. Inhibitory activity of **6** and **7** for NO production. Data present mean ± S.D. in triplicate. $^{*}p < 0.05$ compared to control, $^{\#}p < 0.05$ compared to LPS-treated group (LPS).

without cytotoxicity at 10 μM and ring contracted **7** showed similar activity to **2**. Therefore, cycloheptaimidazothialzole **6** would be a promising hit compound in locating candidates for NF-κB inhibitors (Figure 3).

In summary, we found novel NF-κB inhibitors through a phenotype-based assay and immunocytochemistry for translocation of NF-kB. Imidazothiazole derivative **6** showed promising anti-inflammatory activity without cytotoxicity. Further investigation for the mechanism of action and SAR of the described molecules will be reported in due course.

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9. For immunofluorescent staining, RAW 264.7 cells were plated on chamber slide (Thermofisher, Rochester, NY, USA) at a density of 2 × 104 cells/mL. After preincubation with or without compounds (30 μM) for 2 h cells were stimulated with LPS (1 μg/mL) for 1h, fixed with ice-cold acetone for 10 min, and then blocked with 1% BSA for 60 min. The cells were probed with rabbit anti-p65 NF-κB antibody (Cell Signaling Technology Inc. Danvers, MA, USA, diluted 1: 100) overnight at 4 °C, followed by Alexa Fluor 488-conjugated goat anti-rabbit IgG antibody (Invitrogen, diluted 1:100) 2 h at room temperature in the dark, washed with PBS three times, and then stained with Hoechst dye for 1 min. Finally, slides were mounted in an antifade reagent (Invitrogen) and analyzed under a fluorescent light microscope (Nikon, Tokyo, Japan) connected to a DS-R1 digital camera (Nikon).

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