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Synthesis and biological evaluation on novel analogs of 9-methylstreptimidone, an inhibitor of NF-κB

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ABSTRACT

Synthesis of 9-methylstreptimidone analogs and their inhibitory activities against NF-κB (nuclear factor-κB) are reported. Among several active derivatives synthesized in this study, **8** with a relatively simple structure, exhibited inhibitory activity against LPS-induced NO production comparable to that of 9-methylstreptimidone.

NF-κB (nuclear factor-κB) was first discovered in 1986 as a factor that bound to the enhancer of immunoglobulin-κ light-chain of B lymphocytes.[1] This transcription factor promotes expressions of immune, growth, and inflammation genes, such as IL-1,[2] IL-2, IL-6, IL-8, TNF-α;[3] cell adhesion molecules, such as E-selectin, intercellular adhesion molecule 1 (ICAM-1), and vascular cell adhesion protein (VCAM-1); anti-apoptotic proteins, such as inhibitors of apoptotic proteins (IAPs)[4] and Bcl-XL;[5] COX-2; and iNOS.[6–8] These transcriptional products are involved in cell survival, proliferation, angiogenesis, inflammation, invasion, and metastasis. NF-κB is often activated in cancer, leukemia cells and inflammatory cells, such as macrophages. Therefore, low molecular weight substances inhibiting NF-κB should be useful as anti-inflammatory and anticancer agents.

9-Methylstreptimidone (**1**), which was first isolated as an antibiotic,[9] was rediscovered from the culture filtrate of *Streptomyces* species by Umezawa and co-workers in 2006 as a novel inhibitor of NF-κB (Fig. 1).[10] 9-Methylstreptimidone (**1**) inhibited NO production and iNOS expression in LPS-stimulated RAW264.7 cells, and induced apoptosis in Jurkat cells and adult T-cell leukemia cells, similar to other NF-κB inhibitors. Therefore, **1** and/or its analogs would be new leads of anti-inflammatory or anticancer agents. However, only small supply from the culture broth disturbs further in vivo-scale assessment of **1**. We planned to synthesize simplified analogs of **1**. As shown in Figure 1, 9-methylstreptimidone (**1**) consisted of the glutarimide residue and hydrophobic alkyl chain. Thus, as part of our ongoing plan, we initially fixed the glutarimide structure and designed analogs possessing linear alkyl chains.

Analogs of **1** were synthesized according to the plan mentioned above (Scheme 1). Compound **2**,[11] readily available by coupling of diethyl 1,3-acetonedicarboxylate and cyanoacetic acid, was connected to appropriate alcohols, amines, or thiols to afford the analogs **3**–**9**.[12,13] Next, unsaturated ketone **11** and ester **12** were synthesized (Scheme 2). According to the Fukuyama protocol,[14] reduction of thioester **8** provided aldehyde **10**, which on the corresponding Wittig reagents gave **11** and **12**, respectively.

The synthetic analogs **3**–**9** and **11**–**12** were subjected to the assay for LPS-induced NO production. NO is produced by inducible NO synthase (iNOS), expression of which is mediated by NF-κB. Table 1 showed the results, along with the cytotoxicity, which was not observed in **2**–**4**, **6**, **7**, **9**, and **12**. Carboxylic acid **2**, as well as its ester and amide derivatives **3**–**7**, did not inhibit NO production in RAW264.3 cells. On the other hand, the thioester derivative **8** exhibited inhibitory activity comparable to that of 9-methylstreptimidone (**1**: IC_{50} 1 μg/mL; **8**: IC_{50} 1 μg/mL). The analog **9** derived from **2** and *n*-hexanethiol, also inhibited NO production, while the activity was diminished to 1/15 that of **8**. Ketone **11** exhibited

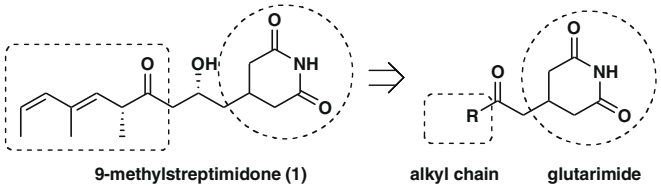

Figure 1. 9-Methylstreptimidone (**1**) and its analog.

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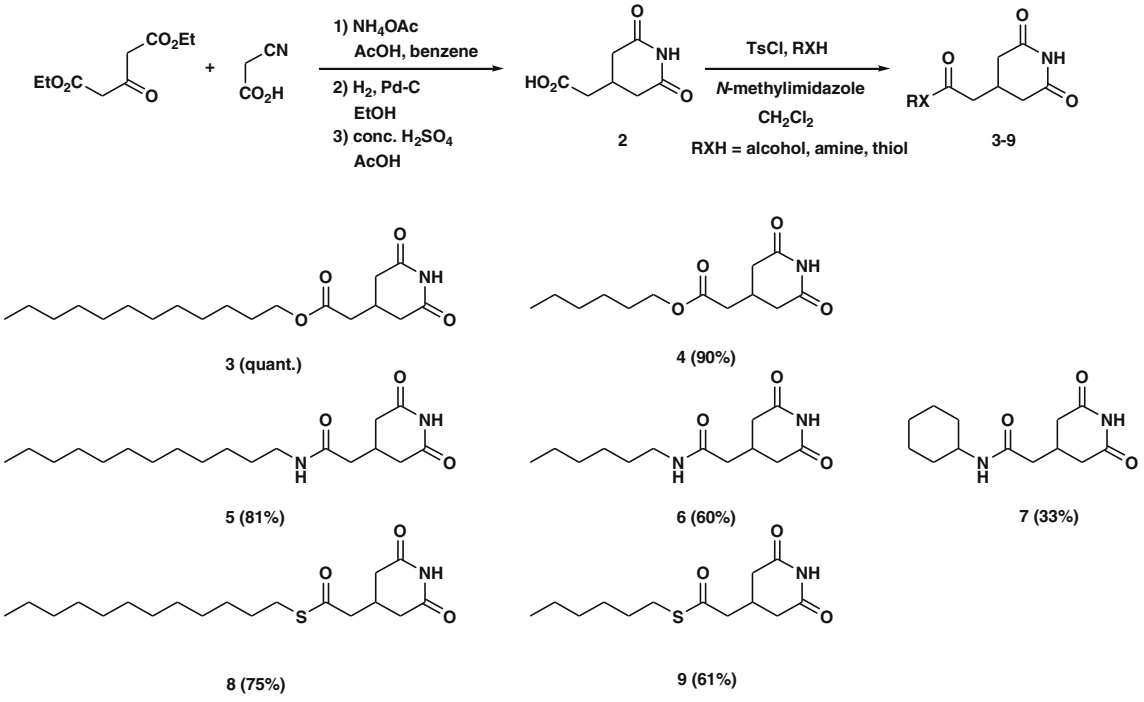
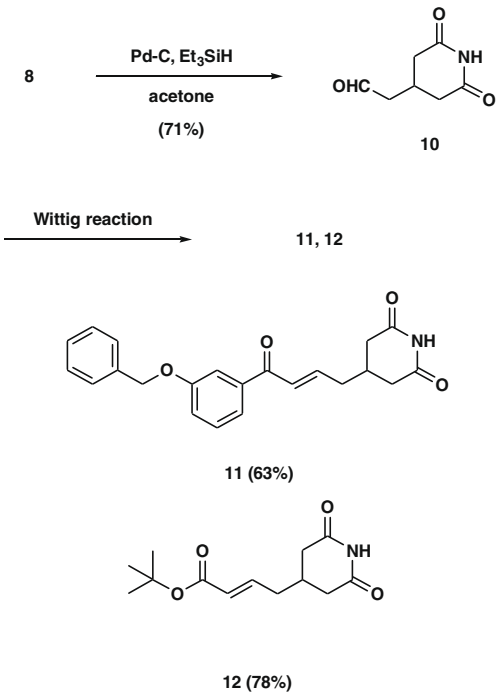


Scheme 1. Synthesis of analogs **2**–**9**.



Scheme 2. Synthesis of analogs **11** and **12**.

a good activity (IC_{50} 3 μg/mL), but ester **12** showed weak inhibition (IC_{50} 30 μg/mL).

To investigate the influence of the glutarimide structure, several derivatives were prepared from **8** (Scheme 3). Alkylation of **8** afforded *N*-alkyl derivatives **13**–**14** in good yields, which also exhibited good inhibitory activities.

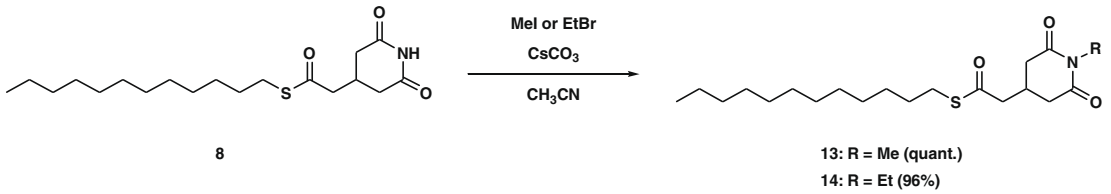
Table 1
Inhibitory activity (IC_{50}) for LPS-induced NO production and cytotoxicites (ED_{50}) of **2**–**9**, **11**, **12**, **13** and **14**[15]

Compound	IC_{50} (μg/mL)	ED_{50} (μg/mL)
2	>30	>30
3	>30	>30
4	>30	>30
5	>30	25
6	>30	>30
7	>30	>30
8	1	15
9	15	>30
11	3	8
12	30	>30
13	2	8
14	2	20

The cytotoxicity values were obtained in the presence of LPS.

These results suggested that the glutarimide and the thio ester structures might play important roles in the inhibitory activity of NO production. The most potent **8** can be synthesized in 4 steps. This will prompt further biological investigation and development as an effective chemotherapeutic agent. Mechanistic studies of the inhibition will be performed in due course.

In conclusion, we synthesized analogs of 9-methylstreptimidone, an inhibitor of NF-κB, and evaluated their activities. The analog **8** was shown to exhibit inhibitory activity against LPS-induced NO production comparable to that of 9-methylstreptimidone.



Scheme 3. Synthesis of analogs **13** and **14**.

Acknowledgment

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13. *Representative procedure for synthesis of 9-methylstreptimidone analogs (procedure for **8**)*: To a solution of acid **2** (0.10 g, 0.58 mmol) and *N*-methylimidazole (0.14 mL, 1.8 mmol) in CH_3CN (1 mL) was added a solution of TsCl (0.14 g, 0.73 mmol) in CH_3CN (1 mL) at 0 °C. After 40 min, to the solution was added a solution of 1-dodecanthiol (0.14 mL, 0.58 mmol). The mixture was stirred at the same temperature for 20 min, and then 2 N HCl (5 mL) was added. The resulted mixture was extracted with EtOAc. The combined organic phases were washed with satd aq $NaHCO_3$ and brine, dried over anhydrous Na_2SO_4, and concentrated in vacuo. The crude product was recrystallized from *n*-hexane and EtOAc to afford **8** (0.16 g, 75 %) as colorless needles: mp 93.2–94.0 °C; IR (KBr) υ 3188, 3084, 2922, 2850, 1702, 1670, 1271 cm^{-1}; ^1H NMR (270 MHz, $CDCl_3$) δ 0.88 (3H, t, J = 7.0 Hz), 1.27 (18H, complex), 1.55 (2H, dd, J = 6.5, 13.2 Hz), 2.36 (2H, complex), 2.71 (5H, complex), 2.93 (2H, t, J = 7.0 Hz); ^{13}C NMR (67.8 MHz, $CDCl_3$) δ14.2, 22.7, 27.7, 28.9, 29.1, 29.3, 29.4, 29.5, 29.6, 29.7, 32.0, 37.2, 47.8, 166.8, 171.0. HRMS (FAB) calcd for $C_{19}H_{34}NO_3S$ $[M+H]^+$: 356.2259. Found: *m/z* 356.2261.
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15. The assay to determine inhibition of LPS-induced NO production was performed as described below. Aliquots of the RAW264.7 cell suspension (1×10^5 cells/mL, 200 μL) were seeded into 96-well plates, and test compounds were added to the plates. After 24 h, the cells were stimulated with LPS at 3 μg/mL and incubated for 20 h. Then, 100 μL of Griess reagent was added to each plate. The concentration of NO was determined by measuring the absorbance at 570 nm. Cytotoxicity was determined using a 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) colorimetric assay. Aliquots of the RAW264.7 cell suspension (1×10^5 cells/mL, 200 μL) were seeded into 96-well plates, and test compounds were added to the plates. After 4 h, the cells were stimulated with LPS at 3 μg/mL and incubated for 20 h. Then, 20 μL of MTT solution (5 mg/mL) was added to each plate. After 4 h, the culture supernatant was replaced with 100 μL DMSO to dissolve formazan crystal. The optical density of the formazan solution was measured with microplate reader at 570 nm.