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Design and synthesis of pregnenolone/2-cyanoacryloyl conjugates with dual NF-κB inhibitory and anti-proliferative activities

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ABSTRACT

Twenty-five novel pregnenolone/2-cyanoacryloyl conjugates (**6**–**30**) were designed and prepared, with the aim of developing novel anticancer drugs with dual NF-κB inhibitory and anti-proliferative activities. Compounds **22** and **27**–**30** showed inhibition against TNF-α-induced NF-κB activation in luciferase assay, which was confirmed by Western blotting. Among them, compound **30** showed potent NF-κB inhibitory activity (IC_{50} = 2.5 μM) and anti-proliferative against MCF-7, A549, H157, and HL-60 cell lines (IC_{50} = 6.5–36.2 μM). The present study indicated that pregnenolone/2-cyanoacryloyl conjugate I can server as a novel scaffold for developing NF-κB inhibitors and anti-proliferative agents in cancer chemotherapy.

Cancer is a group of diseases characterized by uncontrolled cell growth, which has became a major public health concern over the last several decades.[1] The number of new diagnosed cancer cases reached nearly 14.5 million in 2014 and is expected to rise by about 30% in the next decade.[2] The abnormal cell growth can be a result of gene mutations induced by DNA damage or aberrant activation of cell signaling pathways, such as hormones cytokines and chemokines.[3–5] Among the factors involved in cancer, the nuclear factor-κB (NF-κB) as a ubiquitous eukaryotic transcription factor plays an important role in regulating the expression of more than 150 genes associated with inflammation, immunity, and cell growth.[6] In particular, the aberrant activation of NF-κB has been frequently observed in various types of human cancers, and suppression of NF-κB can limit the proliferation of cancer cells.[7,8] Therefore, NF-κB has been pointed as a therapeutical target in cancer, and inhibitors of NF-κB function could be developed into new anticancer drugs or leads.[9–11]

It is generally believed that the Michael acceptors in bioactive compounds can form adducts with reactive thiol groups of proteins to induce protein modification and misfolding, which might be responsible for their various biological effects, such as anti-proliferative activity.[12–14] As a Michael acceptor, 2-cyanoacryloyl moiety has been extensively applied in the design of anticancer drugs. For example, tyrphostin AG490 (**1a**, Fig. 1) is the first small molecular Jak2 inhibitor that is clinically used as an anticancer agent and is also effective in various models of inflammatory and autoimmune diseases[15]; indole/2-cyanoacryloyl hybrid **1b** was reported to show anti-proliferative activity against a range of cancer cells[16]; CDDO-Me (**1c**) is a semi-synthetic triterpenoid acting as an inhibitor of NF-κB pathway, which was shown to be a drug candidate for treating cancer, chronic kidney disease, and other diseases.[17]

Pregnenolone (**2a**, Fig. 1) is an important naturally occurring endogenous steroid and known as a precursor to most of steroid hormones like estrogen, progesterone, testosterone, and glucocorticoids.[18] The unique structural features and the broad-spectrum bioactivities of **2a** make it to be a promising drug leads, attracting numerous interest in structural modification from the field of medicinal chemistry.[19–21] As part of our project to develop new

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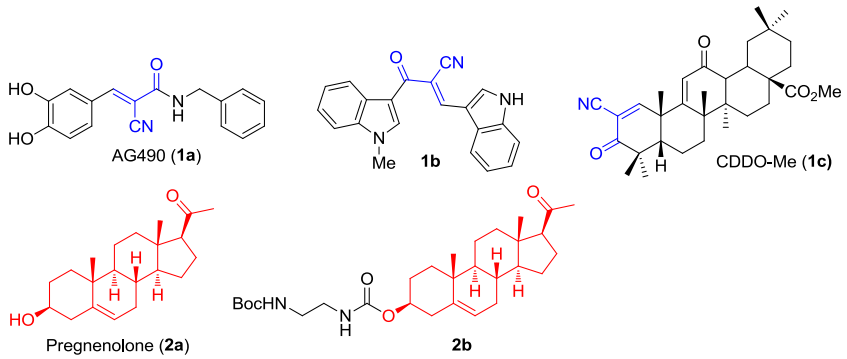


Fig. 1. 2-Cyanoacryloyl derivatives **1a**–**1c** and steroids **2a** and **2b**.

drugs derived from pregnenolone,[22,23] a new NF-κB inhibitor, derivative **2b** (Fig. 1) with an IC_{50} value of 12.2 μM, was recently screened out from our in-house compound library using NF-κB pathway luciferase assay. In anti-proliferation assay, **2b** did not show significant activity against A549 and MCF7 cancer cell lines.

In our continuous effort to discover anticancer drugs with dual NF-κB inhibitory and anti-proliferative activities, a privileged fragment combination (PFC) strategy was recently employed to further modify the structure of **2b**. Since pregnenolone is a privileged molecular skeletion and 2-cyanoacryloyl moiety as Michael acceptor is a very useful moiety in the design of anticancer drugs, both pharmacophores was combined to generate conjugates **I**, as shown in Fig. 2. In addition, the ethanediamine linker was preserved since numerous bioactive compounds or drugs contain the *N*-CH_2-CH_2-*N* moiety.[24] Herein, we reported the synthesis of pregnenolone/2-cyanoacryloyl conjugate **I**, and the biological evaluation for their dual NF-κB inhibitory and anti-proliferative activities.

The synthesis for target compounds **6**–**30** is depicted in Scheme 1. Briefly, pregnenolone (**1a**) was reacted with *p*-nitrophenyl chloroformate in dichloromethane using pyridine as the base to obtain the intermediate **3**, which was then reacted with *N*-Boc-ethylenediamine in the presence of Et_3N to yield compound **4**.[25] *N*-Boc protection in **4** was removed by treating with TFA followed by coupling with cyanoacetic acid in the presence of EDCI and HOBT to give amide **5**.[26,27] Knoevenagel condensation reaction of **5** with various substituted benzaldehydes catalyzed by piperidine[28] finally afforded pregnenolone/2-cyanoacryloyl conjugates **6**–**30**. The general procedures and spectroscopic data of all synthetic compounds were described in Supplementary data.

The NF-κB inhibitory activity of target compounds **6**–**30** and their synthetic intermediates **4** and **5** was evaluated in TNF-α-stimulated HEK293/NF-κB cells, according to a previously reported luciferase assay.[29] TNF-α (tumor necrosis factor alpha) is a known inducer of NF-κB activity,[30] and PS-341 (bortezomib) as known NF-κB inhibitor[31] was used as reference compound. In brief, The HEK293/NF-κB cells were inoculated into 96-well plates. After 24 h, the cells were treated with TNF-α and then incubated with tested compounds. Signal strength of luciferase was detected by EnVision. The results for these tests were summarized as percentage of inhibition or IC_{50} values in Table 1.

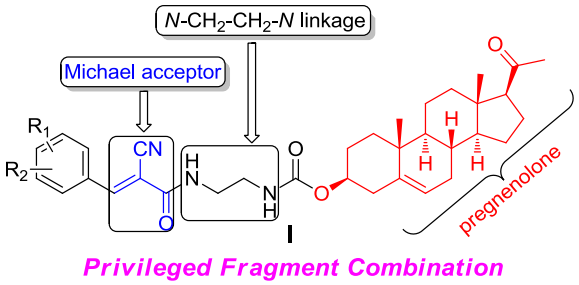
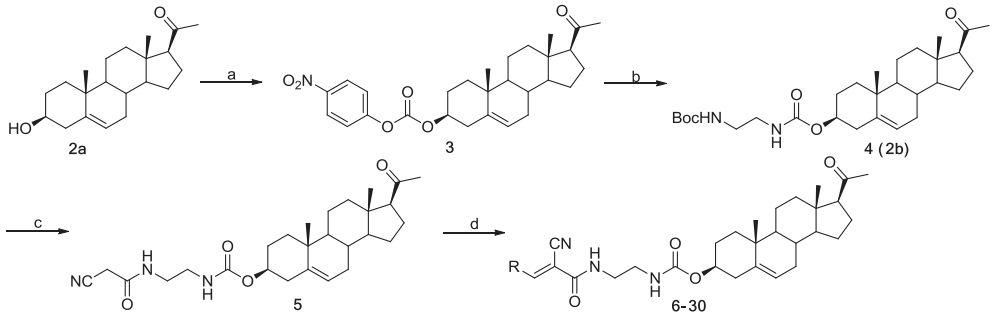


Fig. 2. Proposed novel pregnenolone/2-cyanoacryloyl conjugate **I**.

At first, the inhibitory activity of compounds against NF-κB was tested at the concentration of 20 μg/mL (equal to 42–31 μM for each different compound). The percentage inhibition of synthetic intermediates **4** and **5** was 76.6% and 74.4%. For most target compounds, especially those (**6**–**20**) with electron-donating substituents in benzene moiety, showed very weak activity (<50%) against NF-κB at the concentration of 20 μg/mL, while **22** and **27**–**30** possessing electron-withdrawing groups (such as F, CN, and NO_2) displayed significant inhibitory effect on NF-κB at the same tested concentration. These results indicated that electron-withdrawing effect of substituent in benzene ring plays an important role in their bioactivity. The cytotoxicity of selected compounds including **4**, **5**, **22** and **27**–**30** was evaluated at 20 μg/mL. From the results, these compounds did not show significant



Scheme 1. Synthesis of **6**–**30**. Reagents and conditions: (a) *p*-nitrophenyl chloroformate, pyridine, CH_2Cl_2, rt, overnight, 79%; (b) *N*-Boc-ethylenediamine, Et_3N, CH_2Cl_2, rt, overnight, 84%; (c) i. trifluoroacetic acid, CH_2Cl_2, rt, overnight; ii. cyanoacetic acid, EDCI, HOBT, DIEPA, CH_2Cl_2, 36 h, 84%; (d) substituted benzaldehyde, piperidine, EtOH, 19%–76%.