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Design, synthesis and molecular docking studies of sinomenine derivatives

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ABSTRACT

In order to search for drugs with excellent anti-inflammatory activities, a series of novel sinomenine derivatives were designed, synthesized, and evaluated for their inhibition activities against NF-κB activation induced by lipopolysaccharide (LPS). Compared with the natural parent sinomenine, compounds **2a–w** showed higher activity, while compounds **1a–o** showed similar activity against NF-κB. Moreover, a molecular model for the binding between compound **2v** and the active site of p50 was provided on the basis of the computational docking results.

Rheumatoid arthritis (RA) is a systemic inflammatory disease characterized by chronic and erosive synovitis that involves peripheral joints.[1] Three major classes of drugs used for RA are currently in clinical use: nonsteroidal anti-inflammatory drugs (NSAIDs) (such as indomethacin), antirheumatic drugs (such as methotrexate), and corticosteroids (such as dexamethasone) (Fig. 1). However, the long-term use of anti-inflammatory drugs has led to serious health hazards, such as interstitial pneumonitis, pulmonary fibrosis, nephritis, and neurocognitive impairment.[2] Thus, these have emphasized the urgent need for new, effective, and safe anti-inflammatory agents.

For many decades, numerous bioactive products isolated from various natural resources have influenced modern drug discovery across the therapeutic spectrum.[3] Therefore, great efforts have been made to discover lead compound from natural product in an attempt to obtain new anti-inflammatory drugs.

Sinomenine (7, 8-didehydro-4-hydroxy-3, 7-dimethoxy-17-methylmorphinan-6-one) (Fig. 2), a naturally abundant alkaloid isolated from the traditional chinese medicinal plant Sinomenium acutum, has been demonstrated to have variety of pharmacological effects, including anti-inflammation, immunosuppression, and anti-angiogenesis.[4–6] Pharmacology reveals that sinomenine decrease the mRNA expression of TNF-α by inhibiting the Nuclear Factor-κB (NF-κB) binding activity.[4] NF-kB has been found to play an active role in inflammatory responses. The family of NF-κB transcription factors is intimately involved in the regulation of expression of numerous genes in the setting of the inflammatory response. In mammals, the NF-κB family consists of five members: RelA (p65), RelB, c-Rel, NF-κB1 (p50 and its precursor p105), and NF-κB2 (p52 and its precursor p100). They form a variety of homodimers and heterodimers. The most prevalent activated form is the heterodimer p65–p50. Normally, NF-κB is present in the cytoplasm of almost all mammalians cells in an inactive form (IκBa-p50-p65) associated with the inhibitory κB proteins (IκB).[7–9]

However, few applications have been developed due to sinomenine's unsatisfactory immunomodulating activity and short biological half-life.[4,10,11] After comprehensive analysis of the natural structure of sinomenine and its previous modifications, we introduced nitrogen-containing heterocycles to sinomenine, which are frequently employed in modern drug design. 1, 2, 3-Triazole possess favorable properties for medicinal chemistry such as a moderate dipole character, hydrogen bonding capability, rigidity and stability under in vivo conditions.[12] A series of sinomenine derivatives (Fig. 3) have been designed and synthesized by introducing substituted 1, 2, 3-triazole group (**1a–o**) or substituted benzyl group (**2a–w**) into skeleton of sinomenine to test their in vitro inhibition activities against NF-κB activation induced by LPS.

The synthetic route of the target compounds **1a–o** and **2a–w** was outlined in Scheme 1. Compound **4** was synthesized by the reaction of sinomenine (**3**) with sodium hydroxide in CH_3CN at room temperature. The target compounds **1a–o** were synthesized in good yields by reacting compound **4** with substituted benzyl bromide in CH_3CN at room temperature. Compound **5** was synthe-

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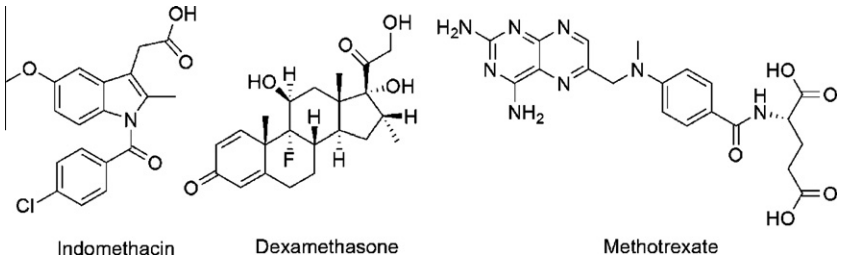

Figure 1. Agents used for RA in clinical therapy

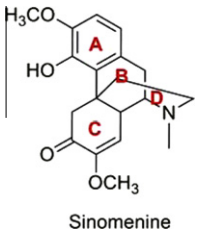

Figure 2. Structure of the sinomenine

sized from sinomenine (**3**) via reacting with propargyl bromide in CH_3CN in the presence of potassium tert-butyl alcohol. And the title compounds (**2a–w**) were accomplished in the presence of NaN_3 and substituted benzyl bromide, using $CuSO_4{\cdot}5H_2O$ and ascorbate sodium as catalyst in DMF, at room temperature. All the new compounds (**1a–o, 2a–w**) described above were characterized by NMR, LC-MS and Elemental analyses.[13]

The mis-regulation of the NF-κB signal pathway is involved in a variety of inflammatory diseases that leads to the production of inflammatory mediators.[14] We assessed the effects of sinomenine and sinomenine derivatives on NF-κB transcriptional activity in LPS-stimulated RAW264.7 cells using a reporter gene assay.[15] RAW264.7 cells (2×10^5) co-transfected for 24 h with the mixture of pGL3.5× κB-luciferase, and pRL-TK-Renilla-luciferase were pretreated with sinomenine or sinomenine derivatives (100 μM) for 30 min and then stimulated with LPS (1 μg/mL) for 6 h. NF-κB luciferase activities were measured using the Dual-Luciferase Reporter Assay System (Promega) according to the manufacturer's instructions. Data are normalized for transfection efficiency by dividing firefly luciferase activity with that of Renilla luciferase.

Thirty-eight sinomenine derivatives (**1a–o, 2a–w**) were evaluated for their inhibition activities against NF-κB activation induced by LPS. The results of assays are summarized in Table 1.

The control group was measured without stimulated by LPS (with the relative NF-κB activity (NF-κB/TK, fold): 1.18 ± 0.22). The LPS group was defined as the stimulated group (with the relative NF-κB activity (NF-κB/TK, fold): 5.18 ± 0.98). The inhibition values indicate that almost all the sinomenine derivatives could more effectively inhibit the NF-κB activation induced by LPS. Especially, compound **2c, 2m**, and **2v** exhibited excellent activities (respectively with the relative NF-κB activity (NF-κB/TK, fold): 1.98 ± 0.12, 1.96 ± 0.58 and 1.80 ± 0.17). Compared with the natural parent sinomenine, compounds **2a–w** showed higher activities while compounds **1a–o** showed similar activity against NF-κB. The high activity data of compounds **2a–w** suggests that the strategy of embedding a rigid nitrogen-containing heterocyclic moiety into the A-ring of sinomenine generally improves the inhibition activity against NF-κB. The introduction of 1, 2, 3-triazole between phenolic hydroxyl group and benzyl group is significant. It may act as H-bond acceptors.

To explain the results, we proposed a likely binding mode for **2v** to the active site of p50 based on computational docking results (Fig. 4). From Figure 4, we observed that the skeleton of sinomenine could be placed into the hydrophobic pocket formed by Tyr57, Lys59, Glu60, His141, Ser240 and Lys241. The substituted 1, 2, 3-triazole was oriented into a hydrophobic pocket formed by Lys144, Asp206, Leu207, Ser208, Ala242, Pro243 and Asn244. Besides, the N-2 of 1, 2, 3-triazole heterocyclic moiety formed hydrogen bond with His141. The oxygen atom of the carbonyl could also form hydrogen bond with the amino group of Lys272. In addition, heterocyclic moiety itself could generate indirect non-bonding interactions with the amino residues.

A series of novel sinomenine derivatives were successfully designed and synthesized. And their inhibition activities against NF-κB activation induced by LPS were evaluated. Compared with sinomenine, some of these derivatives showed improved activities, while others exhibited similar activity against NF-κB. Our present results clearly showed that the introduction of substituted 1, 2, 3-

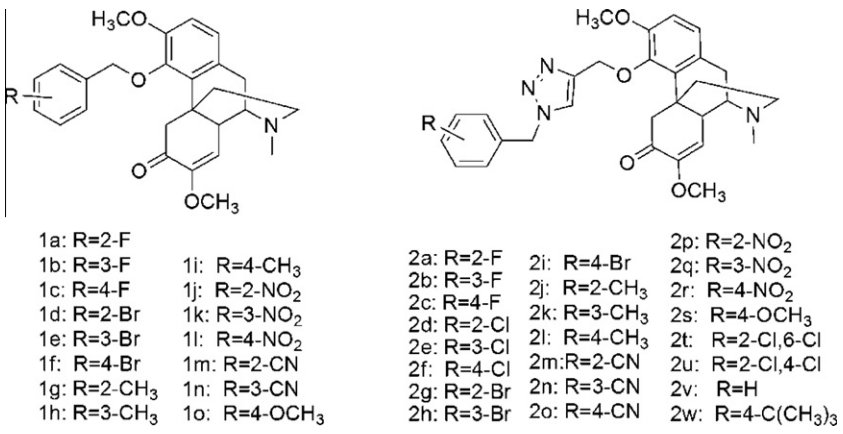

Figure 3. All the compounds we designed and synthesized