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NOVEL CYTOKINE RELEASE INHIBITORS. PART I: TRITERPENES

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Abstract: Tripterine and closely related triterpenoid derivatives as IL-1β release inhibitors are discussed.

Extracts of *Tripterygium wilfordii Hook F* (TWH), a Chinese medicinal herb, have been used in China for the treatment of autoimmune diseases such as rheumatoid arthritis and systemic lupus erythematosus with positive results.[1] The apparent clinical efficacy of TWH in autoimmune diseases prompted us to study this herbal medicine. Recently, there have been a number of published studies focusing on the possible bioactive components and the biological mechanism of action of TWH.[2–5] Results from these studies were not conclusive and some produced conflicting results. From our studies, we identified tripterine or celastrol[6–9] (**1**), one of the pentacyclic triterpenes from TWH, as a potent IL-1β release inhibitor. We report here the biological activity of **1** and several closely related analogs. A possible mechanism of action of **1** is also presented.

Tripterine **1** was isolated from TWH. Pristimerin **2**[10–14] and tingenone **3**[13–16] were isolated from Catha Cassinoides (*Mayytenus canariensis*). Reduction of **2** in ethanol with $NaBH_4$ gave pristimerol (**4**),[17] which was acetylated with acetic acid anhydride and pyridine at room temperature to give diacetyldihydropristimerine (**5**).[17] Methylation of **4** with Me_2SO_4 and K_2CO_3 in methanol gave the dimethyl ether **5**.[18]

LPS-stimulated IL-1β production by human monocytes was used to assess the inhibitory activity of these compounds on IL-1β release.[19] In this assay, tripterine **1** inhibited the production of IL-1β with an IC_{50} of 40 nM. As shown in Table 1, closely related analogs **2** and **3** also inhibited IL-1β production with similar potency. These compounds appear to be among the most potent IL-1β release inhibitors reported other than glucocorticoids. Compounds **4** and **5**, although slightly less potent than **2**, were still effective inhibitors of IL-1β release. In contrast, the dimethyl ether derivative **6** displayed no activity in this assay. Since it is expected that **4** can be slowly oxidized in the air to **2**,[20] it is conceivable that **4** and **5** serve as precursors for **2**. However, it remains to be established whether **2**, **4**, and **5** inhibit IL-1β production by the same mechanism. The relative

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insensitivity of the inhibitory activity to the change in different functional groups on ring E (e.g., **2** vs. **3**), together with the fact that modifications of the A/B rings affect the inhibitory activity, seem to suggest that the primary pharmacophore resides on the A/B rings for this class of inhibitors. Although the molecular target(s) in cells of compound **1** is unknown, it is conceivable that compound **1** could act as a Michael acceptor or an electron transfer agent due to its unique quinone methide structure (Figure 1). These prototypes, with proper tagging or labeling, could be used to identify novel molecular targets for potential therapeutic intervention.

Table 1. Inhibition of IL-1β release

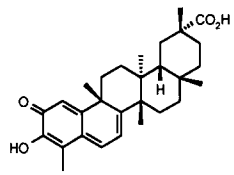
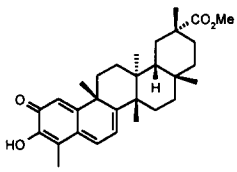
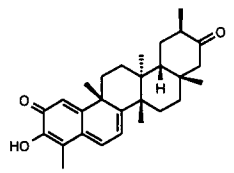
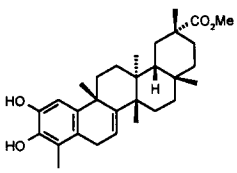
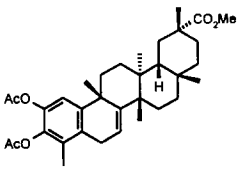
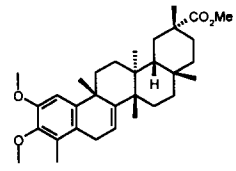
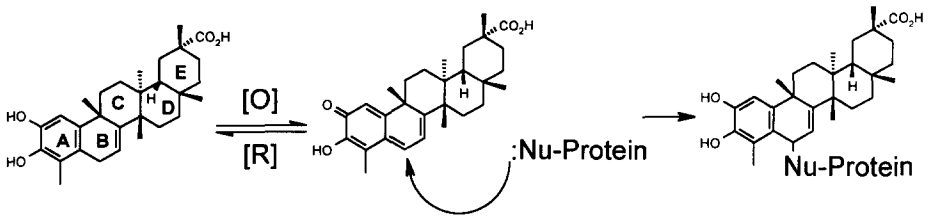
Compound	Structure	IC_{50}, nM (N)
1		40 ± 4 (8)
2		56 ± 4 (17)
3		58 ± 7 (11)
4		260 ± 38 (5)
5		79 ± 11 (5)
6		8% @ 1000

Figure 1.



In an attempt to establish the mechanism of action of **1**, the effect of **1** on IL-1β mRNA level and post-translational processing of IL-1β were examined. Preliminary data shows that **1** has no effect on the steady state level of LPS-stimulated IL-1β mRNA over the concentration range (25–100 nM) in which the production of IL-1β was progressively inhibited. These results are in contrast to glucocorticoids, which appear to inhibit the induction of IL-1β gene transcription.[19] Further study by [^{35}S]-pulse-chase experiments, as monitored by [^{35}S]-cysteine and methionine labeling, indicated that **1** did not affect the synthesis of the IL-1β 31 kd precursor. In the same experiment, the appearance of [^{35}S]-labeled 17 kd mature IL-1β was completely suppressed. Taken together, these results show that the inhibitory action of **1** on LPS-stimulated IL-1β production by cultured human monocytes are primarily directed at the post-translational processing/secretion steps.

Recent studies suggested that synthesis, processing and secretion of IL-1β may be specific to monocytes. A cysteine protease, an interleukin-1β converting enzyme (ICE), is involved in the processing of the pro-IL-1β 31 kd protein to the mature 17 kd IL-1β in monocytes.[21] It was reported that *p*-benzoquinone inhibited CPP32 (an ICE-like enzyme involved in apoptosis) with an IC_{50} of 3 μM.[22] Preliminary experiments using a partially purified ICE enzyme preparation[23] indicates that **1** is not an ICE inhibitor despite its quinone methide structure. This result does not rule out the possibility that **1** might inhibit the activation of ICE. We also examined whether **1** is involved in inhibiting the secretion mechanism. It has been suggested that IL-1β secretion involves a protein kinase C mediated mechanism.[24] No activity of **1** was detected in a PKC assay. Further studies are needed to delineate the exact mechanism of action of this class of molecules.

A streptococcal cell wall (SCW) induced arthritis model in rats[25] was selected to examine the in vivo activity of **1**. In this model about 50–60% of the inflammatory response was inhibited by treatment with IL-1ra for 4 days, indicating a prominent, although not exclusive, role of IL-1β in this model. Oral administration of **1** suppressed the SCW-induced recurrence in swelling of previously sensitized rat joints, with an ED_{50} of about 6 mg/kg/day (po). It is noteworthy that **1** also reduced joint damage in addition to the reduction in joint swelling by histological evaluation. In contrast, indomethacin at a dose of 0.67 mg/kg potently reduced the SCW-induced swelling by 70%, but failed to reduce joint damage. These results suggest that **1**, a potent IL-1β release inhibitor, has disease-modifying efficacy in the SCW-induced arthritis model.

Interleukin-1β is a major mediator in the pathogenesis of chronic and acute inflammatory diseases.[26] Therefore, the search for small molecules that are potent IL-1β inhibitors represents an attractive therapeutic approach. Several compounds have been reported to inhibit IL-1β production with inhibitory potency in the micromolar range.[5,27] The pentacyclic triterpenes described here represent a novel class of potent of IL-1β release inhibitors. Although the exact mechanism(s) of action is unknown, these compounds could be useful tools to elucidate the mechanism of IL-1β production. Finally, it remains to be established whether the inhibition of IL-1β release by compound **1** contributes significantly to the clinical efficacy of TWH in rheumatoid arthritis.

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