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NOVEL CYTOKINE RELEASE INHIBITORS. PART III: TRUNCATED ANALOGS OF TRIPTERINE

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Abstract: Truncated analogs of tripterine as cytokine (IL-1α, IL-1β, TNF-α, IL-6, and IL-8) release inhibitors are discussed.

Pro-inflammatory cytokines like Interleukin-1β (IL-1β) and Tumor Necrosis Factor-α (TNF-α) are important molecular mediators of immune and inflammatory responses.[3] Excessive production of IL-1β and TNF-α is believed to contribute to the onset and progression of a number of inflammatory and autoimmune pathologies, such as rheumatoid arthritis and septic shock. IL-1β receptor antagonists and monoclonal antibodies to TNF-α were shown to be efficacious in animal models of inflammatory diseases, with the latter being demonstrated to be effective clinical trials in humans.[4] Therefore, the inhibition of the production of both cytokines represents a good strategy for therapeutic intervention of immune and inflammatory diseases.

Recently, we disclosed the potent inhibitory activity of tripterine **1** and its closely related triterpenoid derivatives on IL-1β release with IC_{50}s approximating 50 nM.[5,6] This class of compounds does not inhibit IL-1β release the same way as glucocorticoids do. The exact mechanism of action of **1** on IL-1β release has not been fully elucidated, however, **1** has shown interesting disease-modifying activity in a streptococcus cell wall (SCW)-induced model of arthritis in rats.

For **1** and its analogs, the postulated primary pharmacophore seems to reside in the A/B rings—a unique quinone methide structure.[5] The role of C-, D-, and E- rings remains unclear, whether they interact with enzymes or merely stabilize the quinone methide A/B rings. More importantly, short supply of this natural product (0.0003% yield) and its complex structure (five-quaternary and six-stereogenic centers) have hindered the research of **1** and related compounds. Despite of its isolation in 1936 and its biological activities, the total synthesis of tripterine has not yet been reported. Our objectives, in addition to the investigations of the total synthesis of **1**, were to synthesize truncated analogs of **1** to explore SAR and to identify structurally simplified inhibitors.

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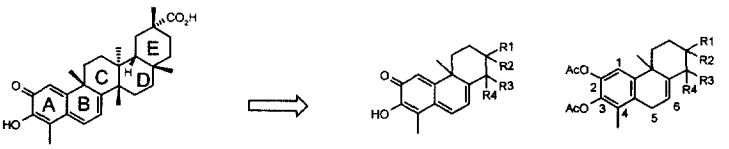
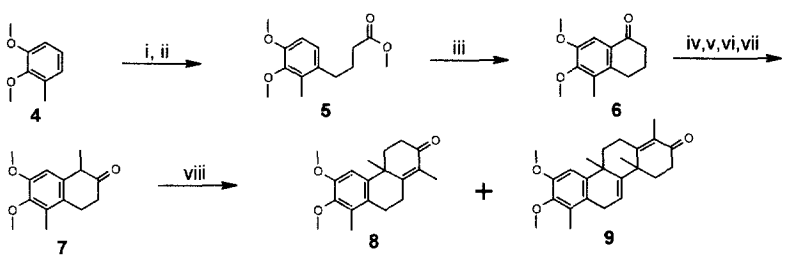


Tripterine 1 Truncated analogs **2** and **3**

The synthesis of potential IL-1β inhibitors with truncated structures of **1** is summarized in Schemes 1 and 2. Commercially available 2,3-dimethoxy toluene **4** was subjected to Lewis acid-catalyzed Friedel–Crafts acylation, followed by hydrogenation. Ester **5** (60% yield) was separated from its isomer by chromatography. Cyclization of **5** was achieved with PPA to give α-tetralone **6** (85% yield). Conversion of **6** to β-tetralone **7** was accomplished with an overall yield of 70% in four steps (Grignard addition, dehydration, epoxidation with MMPP[7] and rearrangement). Robinson annulation of the β-tetralone with ethyl vinyl ketone delivered tricyclic and tetracyclic compounds (60% and 15% yield, respectively) as the major products. With **8** and **9** as key substrates, a variety of analogs were synthesized as described in Scheme 2. Demethylation and acetylation of compound **8** gave diacetate **10**. Methylation of compound **8** produced ketone **13**, which was then demethylated, subsequently reduced and acetylated to afford triacetate **20**. Catechol **15**, derived from demethylation of compound **13**, is air-sensitive and can be oxidized with silver carbonate to give the unstable quinone methide **17**. Compound **18** was obtained similarly via the enantioselective Robinson annulation[8] of **7**. All other compounds were synthesized by methods similar to the chemistry described above.

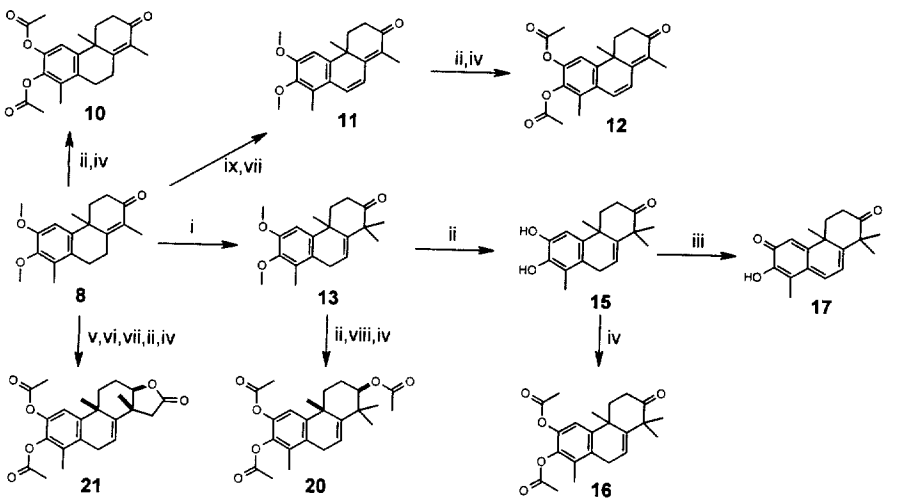
Scheme 1.



i. $MeO_2CCH_2CH_2COCl/TiCl_4/CH_2Cl_2$; ii. $H_2/Pd/C/AcOH$, 60% yield for two-steps; iii. PPA, 85% yield; iv. MeMgCl/THF; v. $TsOH/C_6H_6$; vi. MMPP/MeOH; vii. BF_3OEt_2/C_6H_6/heat, 70% yield for four-steps; viii. $EtCOCHCH_2$/KOH/MeOH/H_2O.

LPS-stimulated IL-1β production by human monocytes was used to assess the inhibitory activity of these compounds on IL-1β release.[9] Some of the representative truncated inhibitors were listed in Tables 1 and 2. Catechol diacetate **10** was a poor inhibitor of IL-1β release with an IC_{50} of 2400 nM while **1** and **2** were shown to be potent inhibitors in the same assay with IC_{50}s of 40 and 80 nM, respectively. The introduction of a carbon–carbon double bond between C5 and C6, which is conjugated to the aromatic ring and the enone

Scheme 2.

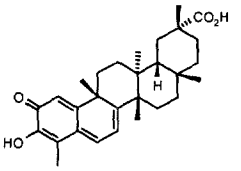
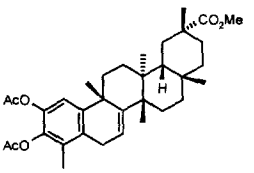
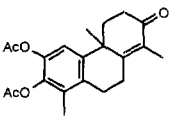
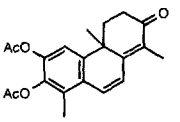
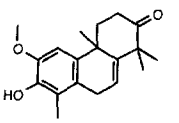
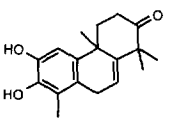
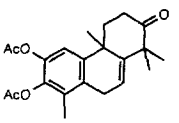
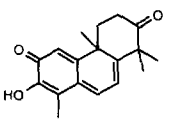
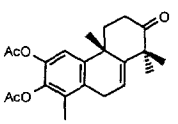


i. $^tBuOK/C_6H_6$/MeI, 70% yield; ii. BBr_3/CH_2Cl_2, 80% yield; iii, Ag_2CO_3; iv. AcCl/py/CH_2Cl_2, 90-95% yield; v. NaH/THF/$BrCH_2CO_2Me$, 71% yield; vi. $NaBH_4$/THF; vii. TsOH/C_6H_6, 80% yield for two steps; viii. LAH/THF, 85% yield; ix. $^tBuOK/C_6H_6/O_2$, 76% yield.

group, improved potency by threefold. However, more than a tenfold potency improvement was achieved with the introduction of a carbon–carbon double bond between C6 and C6a, which is not conjugated to the aromatic ring, in the B-ring (**16** vs **10**). Compounds with such a double bond between C6 and C6a in the B-ring are potent inhibitors of IL-1β release with IC_{50}s of 100–200 nM (e.g., **16**, **18**, **19**, **20**, **21**, **22**, and **23**). The potency improvement might result from the easier oxidation of catechols with a double bond in the B-ring to the corresponding quinone methides relative to catechols without such a double bond. As mentioned above, quinone methide **17** was not very stable. The solution of freshly prepared **17** was shown to inhibit IL-1β release with an IC_{50} of 80 nM. However, the inhibitory activity was gradually lost probably due to the decomposition of quinone methide **17**. Consequently, catechols were converted into diacetates, in an effort to increase their stability. These results indicate that C-, D-, E-rings of tripterine are not critical for potency, but perhaps play a very important role in the stabilization of the quinone methide pharmacophore of tripterine. Also, the methyl substitution of the aromatic ring does not have a major impact on potency, but may affect the stability of compounds. Compound **19** was marginally more potent than compound **24**. No dramatic change of activities was seen for compounds with several functional groups attached to the C-ring (**16**, **19**, **20**, **21**, **23**). Chirality does not play a role in potency because racemic compound **16** and its chiral analog **18** were equi-potent.

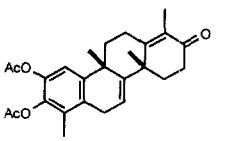
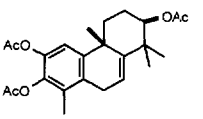
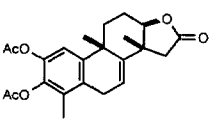
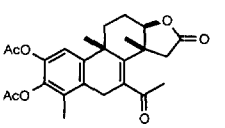
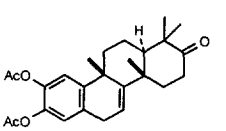
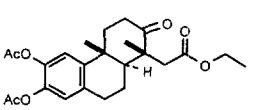
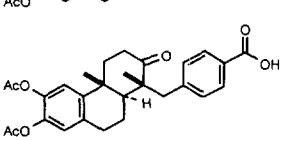
As reported previously, only catechol or diacetate derivatives of tripterine are active on IL-1β release.[5] Substitution of diacetoxy groups of compound **2** with dimethoxy groups resulted in a complete loss of activity.[5] It is conceivable that catechols (or diacetates) and tripterine **1** can participate in the same redox or Michael-addition cycles within cells.

Table 1. Inhibition of IL-1β Release

Compound[a]	Structure	IC_{50} or % inhibition (nM)[b]
1		40
2		80
10		2400
12		700
14		700
15		300
16		200
17		80-700
18		200

[a]Compounds were racemic except **1**, **2**, **18**. [b]IC_{50} values were determined from concentration-response curves (N = 3) in which concentrations ranged from 1 nM to 10 μM. Errors were within ±20%.

Table 2. Inhibition of IL-1β Release

Compound[a]	Structure	IC_{50} or % inhibition (nM)[b]
19		80
20		100
21		200
22		900
23		140
24		2200
25		16%@ 3000

[a]Compounds were racemic. [b]IC_{50} values were determined from concentration-response curves (N = 3) in which concentrations ranged from 1 nM to 10 μM. Errors were within ±20%.

Several potent IL-1β release inhibitors, tripterine and its truncated analogs, were selected for further evaluations such as the mechanism of action and the release inhibition of other cytokines and mediators (IL-1α, TNF-α, IL-6, IL-8, and PGE2). The molecular target of these inhibitors has not been identified. The inhibitory activity is not due to non-specific lysis of cells. LC_{50}s were larger than 1 and 10 μM for tripterine and its truncated analogs, respectively, 20–100 times greater than their IC_{50}s of inhibiting IL-1β release. A mechanism of action might involve in situ oxidation to reactive quinone methide intermediates and in situ reduction of such intermediates. These intermediates might also cross-link with protein nucleophiles via Michael additions.[10] Therefore, these inhibitors might be useful to identify novel molecular targets. The inhibitory effects of these

Table 3. Selectivity Profiles of Cytokine Release Inhibitors[a]

Compound	IL-1β	IL-1α	TNF-α	IL-6	IL-8	PGE2
1	0.04	0.04	0.21	0.08	0.21	0.11
7	0.19	0.24	0.46	0.43	1.0	0.52
10	0.08	0.10	0.19	0.25	0.30	0.20
11	0.10	0.13	0.19	0.34	0.48	0.45
12	0.18	0.22	0.5	0.40	0.93	0.60

[a]IC_{50} (μM) values were determined from concentration-response curves (N = 3) in which concentrations ranged from 1 nM to 10 μM. Errors were within ±20%.

compounds on the release of various cytokines and mediators are summarized in Table 3. Tripterine and its truncated analogs are more potent on IL-1α and IL-1β release than on TNF-α release. They are also active inhibiting the release of IL-6, IL-8, and PGE2. It is not surprising that this class of inhibitors has such broad activities on the release of the cytokines measured, since within the cytokine cascade the synthesis of one cytokine often induces the production of other cytokines. For example, both IL-1 and TNF-α induce the synthesis and release of IL-6 and IL-8.[11] In addition, the potential reactive intermediates might interfere with the common pathways of the release of cytokines. Further studies are needed to address these issues. The fact that only catechols and phenols are active against cytokine release may limit the therapeutic applications of this class of inhibitors to specific indications such as the topical treatment of inflammatory disorders psoriasis.

In summary, we have explored the inhibition of IL-1β release by the truncated analogs of tripterine. It is clearly demonstrated that the small synthetic compounds also have good inhibitory effects on IL-1β and TNF-α release, although less potent than tripterine. The results of the small molecules suggest that the primary pharmacophore resides on the A/B rings, and the C-, D-, and E- rings of tripterine do not contribute to potency but perhaps stabilize the quinone methide. The small molecules might be useful in the study of mechanism of action and the identification of novel targets. Optimization of these prototype compounds may lead to the discovery of novel antiinflammatory drugs.

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