Pergamon

Bioorganic & Medicinal Chemistry Letters 8 (1998) 2825–2828

BIOORGANIC & MEDICINAL CHEMISTRY LETTERS

NOVEL CYTOKINE RELEASE INHIBITORS. PART II: STEROIDS

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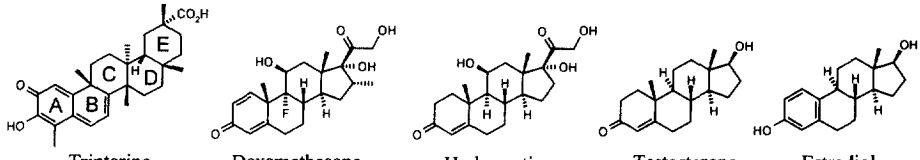
Received 5 June 1998; accepted 31 August 1998

Abstract: Steroidal derivatives as IL-1β release inhibitors are discussed.

In a preceding paper[1] we have disclosed potent inhibitory activities of tripterine and its closely related triterpenoid derivatives against IL-1β release with IC_{50}s approximating 50 nM. This class of compounds does not inhibit IL-1β release the same way glucocorticoids do. In addition, tripterine **1** has shown disease-modifying activity in the SCW-induced arthritis model. Glucocorticoids[2,3] such as dexamethasone and hydrocortisone inhibited IL-1β release in a dose-dependent manner with IC_{50}s ranging from 50–500 nM through inhibition of transcription of the IL-1β gene and decreasing the stability of IL-1β mRNA. However, preincubation of cells with compounds (5–16 h) prior to LPS- stimulation was needed to achieve the inhibition of IL-1β release for glucocorticoids. Nonglucocorticord steroids such as testosterone and β-estradiol, which lack antiinflammatory effects, were reported to have no effect on IL-1β release at 1 uM.[2]

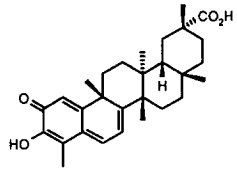
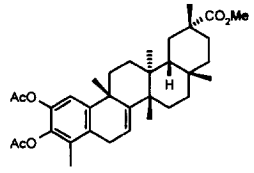
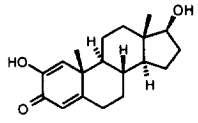
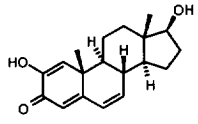
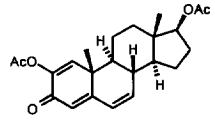
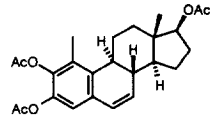
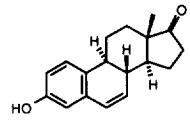
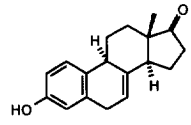
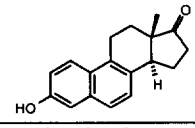
Figure 1



The postulated primary pharmacophore resides on the A/B rings, a unique quinone methide structure, for tripterine and its analogs.[1] The role of C-, D-, and E- rings is not very clear. More importantly, limited supply of this natural product and its complicated structure (five-quarternary and six-stereogenic centers) have hindered the research of tripterine. Despite of its isolation in 1936 and its biological activities, the total synthesis of tripterine has not been reported. Taking advantage of the SAR developed from tripterine and its analogs, we have hypothesized that manipulation of readily available steroids would generate structurally diverse potent IL-1β release inhibitors and might also provide a better understanding of SAR for further exploration. Here we report the inhibition of IL-1β release in human monocytes stimulated with LPS by compounds derived from testosterone.

0960-894X/98/$ - see front matter
PII: S0960-894X(98)00521-6

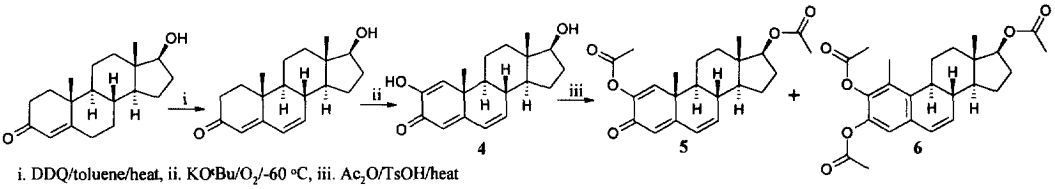
Table 1. Inhibition of IL-1β Release

Compound[a]	Structure	IC_{50} or % inhibition (nM)[b]
1		40
2		80
3		36% @ 3000
4		3000
5		3000
6		150
7		1% @1000
8		-11% @ 1000
9		27% @ 1000

[a]Compounds **1–2** were obtained as described previously. Compounds **7–9** were commercially available.
[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%.

Commercially available testosterone was converted into the corresponding 4,6-dien-3-one[4] with a yield of 44% according to a known procedure with minor modifications.[5] Oxidation of the 4,6-dien-3-one was achieved with KO^{t}Bu/O_2[6] to give 2-hydroxy-1,4,6-trien-3-one **4** (70% yield). The dienone-phenol rearrangement of compound **4** with acetic anhydride in the presence of *p*-toluenesulfonic acid[7] afforded a mixture of compounds **5** and **6** (59% and 39%, respectively) which are easily separated by chromatography.[8] To our knowledge, compounds **4**, **5**, and **6** are new and have never been disclosed before.

Scheme I



i. DDQ/toluene/heat, ii. KO^{t}Bu/O_2/-60 °C, iii. Ac_2O/TsOH/heat

Table 1 summarized the LPS-stimulated IL-1β production by human monocyte where no preincubation of cells with compounds was applied. Only 36% of inhibition of IL-1β release was observed for 2-hydroxy-1,4-dien-3-one **3**[5]at 3 uM. Introduction of a carbon-carbon double bond in the B-ring did not improve the potency as one might anticipate. 2-Hydroxy-1,4,6-trien-3-one **4** only inhibited the production of IL-1β with an IC_{50} of 3000 nM despite its structural feature that was similar to tripterine **1**. For comparison, tripterine **1** inhibited IL-1β release with an IC_{50} of 40 nM in the same assay. However, potency improvement was achieved with the product of the dienone phenol rearrangement of compound **4**. 1-Methyl-2,3,17β-triacetoxy-$\Delta^{1,3,5(10),6}$-estratetraene **6** was a potent inhibitor with an IC_{50} of 150 nM of against the release of IL-1β. In order to access the origin of 20–fold increase of activity, diacetate **5** was also tested in the same assay and was shown to have the same potency as the parent compound **4**. Therefore, the potency improvement does not result from acetylation. Triacetate **6**, although less potent than tripterine itself, has very similar activity against IL-1β release as diacetate **2** (a prodrug of tripterine). Furthermore, compound **6** is more potent than most of known IL-1β inhibitors which have been reported to inhibit IL-1β production with potencies in the micromolar range.[9,10]

In addition to the structural similarities (catechol derivatives, unsaturated bonds in B-rings), compound **6** is quite different from compound **2**. Compound **6** possesses only four rings and does not contain any quaternary center in the B-ring, along with the carbon-carbon double bond conjugated to the aromatic A-ring. The activity against IL-1β release of compound **6** indicates that the role of C-, D-, and E-rings of tripterine is not critical. The inhibitory potency of compound **6**, under conditions where no preincubation was needed, also seems to suggest that compound **6** inhibits IL-1β release via a different pathway from those of glucocorticord steroids. The exact mechanism of action of compound **6** against IL-1β release has not been elucidated, neither has that of tripterine **1**.

Structural similarities prompted us to examine estrogen derivatives such as **7** (6-dehydroestrone), **8** (7-dehydroestrone or equilin), and **9** [1,3,5(10),6,8-estrapentaen-3-ol-17-one or (+)-equilenine]. Those estrogen metabolites/derivatives were not as effective IL-1β release inhibitors as compound **6** despite structural similarities (Table 1). The apparent, important role of substituents at the C2 position of compound **6** remains to be explored.

Due to structural resemblance to estrogen and the important role of estrogen and its metabolites, compound **6** might have other biological activities and warrants further investigation. For example, closely related 2-methoxyestradiol, an anti-angiogenic agent, has been reported to inhibit the growth of lung cancer cells.[11]

In summary, we have explored the inhibition of IL-1β release by tripterine and expanded SAR into steroidal derivatives. A 20-fold potency improvement has been achieved with compound **6**, compared with the corresponding parent steroids. Simple structural manipulation of nonglucocorticord steroids, which lack antiinflammatory effects, has resulted in significant improvement of antiinflammatory activities as measured by the inhibition of IL-1β release. These interesting findings might ultimately lead to the discovery of novel antiinflammatory compounds.

References

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8. Compound **6**: a white solid, mp 178–179 °C, elemental analysis for $C_{25}H_{30}O_5$ calcd C 70.40 H 7.09, found C 70.14, H 6.74. ^{1}H NMR (300 MHz, $CDCl_3$) δ 6.74 (s, 1H), 6.33 (dd, 1H), 5.89 (dd, 1H), 4.67 (t, 1H), 2.62 (m, 2H), 2.31 (s, 6H), 2.27 (s, 3H), 2.23 (m, 2H), 2.06 (s, 3H), 2.04–1.85 (m, 3H), 1.56–1.25 (m, 4H), 0.84 (s, 3H).
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