Pergamon

Bioorganic & Medicinal Chemistry Letters 9 (1999) 469–474

BIOORGANIC & MEDICINAL CHEMISTRY LETTERS

NOVEL CYTOKINE RELEASE INHIBITORS. PART IV: ANALOGS OF PODOCARPIC ACID

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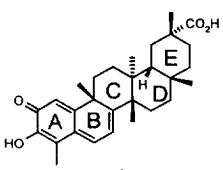
Received 9 November 1998; accepted 16 December 1998

Abstract: Podocarpic acid derivatives as cytokine (IL-1β) release inhibitors are discussed.

Recently, we have reported on the potent inhibitory activities of tripterine (**1**) and its analogs.[4-6] This class of compounds does not inhibit IL-1β release the same way as glucocorticoids do. For **1** and its analogs, the postulated primary pharmacophore appears to reside in the A/B rings—a unique quinone methide structure. In addition, **1** has shown interesting disease-modifying activity in the streptococcus cell wall (SCW)-induced arthritis model in rats.[4] However, short supply of this natural product and its complicated structure (five-quaternary and six-stereogenic centers) have hindered the research of **1** and related compounds. Although the truncated analogs **3** were also shown to be good inhibitors of cytokine release, the synthesis of these inhibitors has its limitations. For example, only catechols or catechol diesters can be prepared and it is difficult to selectively substitute the A-ring.[6]



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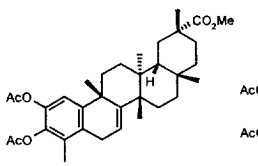

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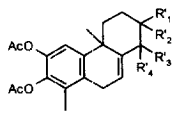

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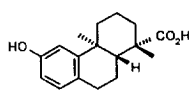

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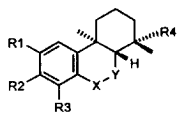

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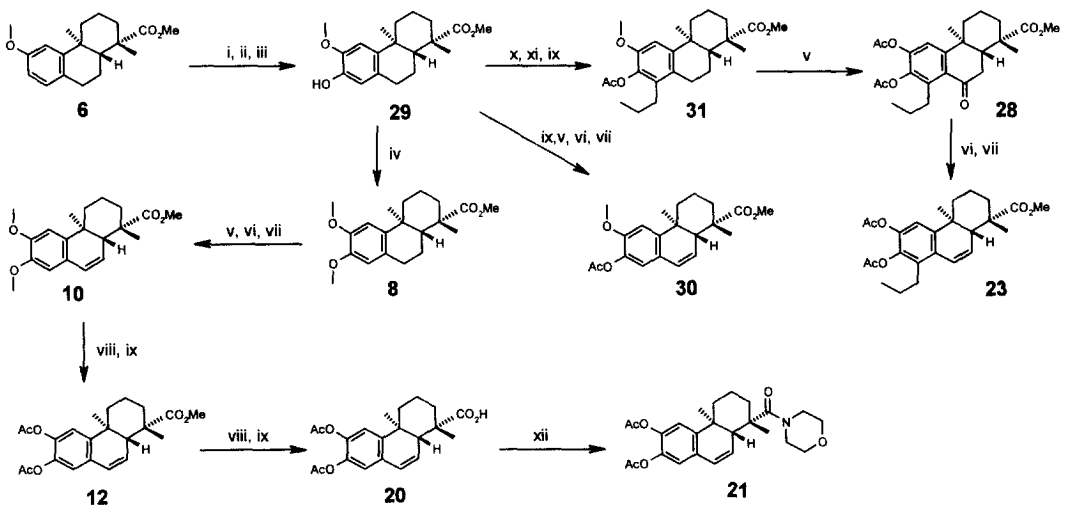
In a continued effort of to explore the SAR of tripterine-type cytokine-release inhibitors, podocarpic acid **4** and its ester derivatives seemed to be ideal starting materials for the preparation of structurally simplified inhibitors. In addition to being a readily available chiral material, **4** can be manipulated chemically without difficulty to provide various target inhibitors. For example, different R1, R2, and R3 groups can be easily installed as shown in compound **5**. Furthermore, noncatechol inhibitors could also be synthesized. Here we report the inhibition of IL-1β release by human monocytes stimulated with LPS[7] by compounds derived from **4**.

0960-894X/99/$ - see front matter
PII: S0960-894X(99)00023-2

The synthesis of potential IL-1β release inhibitors was outlined in Scheme 1 from commercially available podocarpic acid **4** or ester **6**. A few of such compounds, as cited below and in the tables, have previously been prepared via different methodologies as synthetic intermediates.[8–12] However, their biological activities such as the inhibition of cytokine release have never been explored. Acylation of ester **6** with HMTA,[13] followed by oxidation with MCPBA and hydrolysis with KOH afforded phenol **29**[8] (quantitative yield), which was a key intermediate for the synthesis of a variety of analogs. Oxidation of ester **8**,[9] and subsequent reduction and elimination produced **10** (40% overall yield). Demethylation of **10** with BBr_3 gave catechol **11**,[10] which is air-sensitive and gradually decomposes on standing. Consequently, catechol **11** was converted into the more stable diacetate **12**[10] in order to facilitate biological evaluation. Acid **20** was used as the precursor for the preparation of analogs **21** and **22**. Claisen rearrangement of allyl ether of phenol **29** generated the corresponding allyl-substituted phenol which was then hydrogenated and acetylated to give compound **31** (66% overall yield). Oxidation of ester **31** with BPPC[14] delivered ketone **28** which was then reduced with $NaBH_4$/methanol. Elimination of the corresponding alcohols gave ester **23** (30% overall yield). Oxidation of catechol **11** under various conditions failed to afford the corresponding quinone methide—a truncated analog of tripterine. All other compounds were synthesized by methods similar to the chemistry described above.

Scheme 1



i. $(CH_2)_6N_4$/TFA; ii. MCPBA; iii. NaOH; iv. K_2CO_3/MeI; v. BPPC; vi. $NaBH_4$; vii. TsOH/C_6H_6; viii. BBr_3; ix. AcCl/py; x. allyl bromide/K_2CO_3; xi. heat; xii. DCC/$HN(CH_2CH_2)_2O$.

As shown in Table 1, methyl podocarpate **7** only inhibited 15% of IL-1β release at a concentration of 1 μM while tripterine **1** and its derivative **2** were shown to be potent inhibitors in the same assay with IC_{50}s of 40 and 80 nM, respectively. Substitution of **7** with a hydroxyl group afforded catechol **9**[9] which was marginally

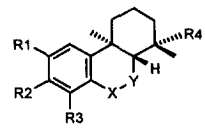
more potent than phenol **7**. A more than tenfold potency improvement is achieved with the introduction of a carbon–carbon double bond conjugated to the aromatic ring (**11** vs **9**, **32** vs **29**). Such potency improvement might result from easier in situ oxidation of catechols with a double bond in the B-ring to the corresponding quinone methides than that of catechols without such a double bond. The inhibitory activity of catechol **11** and diacetate **12** is close to that of tripterine **1** and its analog **2**. These results seem to indicate that C-, D-, E- rings of tripterine are not critical in terms of potency but perhaps play a role in the stabilization of the quinone methide pharmacophore of tripterine. The fact that the quinone methide analog of catechol **11** was not isolated under various conditions might be related to the chemical instability of such quinone methide. Although diacetates **2** and **12** have similar potencies, there are several structural differences. In addition to different ring systems and R3 substitutions, the carbon–carbon double bond in **2** is not conjugated to the aromatic ring as it is in **12**. Diacetates **2** and **12** are conceivable as prodrugs which could be hydrolyzed in cells to form **1** and **11**, respectively. Compounds (**12**, **15**) with ester bonds easier to be hydrolyzed are more potent than pivalate **14**. A tenfold loss of activity was observed for pivalate **14** while carbamide **16** was inactive, probably due to the difficult hydrolysis of the carbamide bond.

The R4 group seems not to contribute to the potency since compounds **12**, **20**, **21**, **22**, and **24** were all equipotent inhibitors of IL-1β release. The R3 substituent does not have a major impact on the inhibitory activity of this class of compounds either. A potency difference of threefold or less was observed for compounds **18**, **19**, **25**, and **26** (R3 = allyl, methylcarbonyl, bromo, and propyl). Similar results were seen for other subsets of compounds (**12–23**, and **30–31**).

It is clear that the R2 substituent can influence the inhibition of IL-1β release dramatically. When the hydroxyl group of catechol **11** was substituted with a methoxy, amino, and sulfonamide group, little change of activity was seen (**34**, **41**, and **42** vs **11**). It is interesting to note that these non-catechols derivatives are as active as catechols. It has not been determined that whether these compounds inhibit IL-1β release by the same mechanism. Substantial loss of activity was observed for compounds **38** and **39** when the hydroxyl group was replaced by an isopropyl or nitro groups, respectively.

The combination of R1 and R2 substitutions also has a major impact on the inhibitory activity of this class of compounds. The dimethyl ethers **8** and **10** were inactive on IL-1β release while the corresponding catechols and diacetates such as **11** and **12** were good inhibitors of IL-1β release. Interestingly, mono-methoxy phenols and mono-acetates (**30**, **32**, **33**, **34**, **35**) are as potent as catechol **11** and diacetate **12**. Furthermore, the position of the hydroxyl group at R1 or R2 seemed not to be important because phenols **32** and **35** were equipotent. It has not been established whether compounds **32** and **35** are converted into the same common intermediate in cells. Nevertheless, these mono-methyl ether derivatives might be more interesting to explore further because of potential stability advantages.

Table 1. Inhibition of IL-1β Release



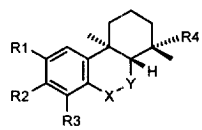
Compound	R1	R2	R3	R4	X	Y	IC_{50} (nM)[a]
1[4]							40
2[4]							80
7	HO	H	H	CO_2Me	CH_2	CH_2	15% @ 1 μM
9[9]	HO	HO	H	CO_2Me	CH_2	CH_2	42% @ 1μM
11[10]	HO	HO	H	CO_2Me	CH	CH	100
12[10]	$MeCO_2$	$MeCO_2$	H	CO_2Me	CH	CH	100
13	$MeCO_2$	$MeCO_2$	Me	CO_2Me	CH	CH	140
14	Me_3CCO_2	Me_3CCO_2	H	CO_2Me	CH	CH	540
15	Me_2CHCO_2	Me_2CHCO_2	H	CO_2Me	CH	CH	110
16	Me_2NCO_2	Me_2NCO_2	H	CO_2Me	CH	CH	> 3 μM
17	$MeCO_2$	$MeCO_2$	Me	CO_2Me	MeC	CH	200
18	$MeCO_2$	$MeCO_2$	CH_2CHCH_2	CO_2Me	CH_2	CH_2	200
19	$MeCO_2$	$MeCO_2$	MeCO	CO_2Me	CH_2	CH_2	600
20	$MeCO_2$	$MeCO_2$	H	CO_2H	CH	CH	110
21	$MeCO_2$	$MeCO_2$	H	$CON(CH_2CH_2)_2O$	CH	CH	110
22	$MeCO_2$	$MeCO_2$	H	$CONH(CH_2)_3CO_2Et$	CH	CH	150
23	$MeCO_2$	$MeCO_2$	$CH_3CH_2CH_2$	CO_2Me	CH	CH	110
24	$MeCO_2$	$MeCO_2$	H	$MeCO_2CH_2$	CH	CH	160
25	$MeCO_2$	$MeCO_2$	Br	CO_2Me	CH_2	CH_2	400
26	$MeCO_2$	$MeCO_2$	$CH_3CH_2CH_2$	CO_2Me	CH_2	CH_2	300
27[10]	$MeCO_2$	$MeCO_2$	H	CO_2Me	CO	CH_2	1000
28	$MeCO_2$	$MeCO_2$	$CH_3CH_2CH_2$	CO_2Me	CO	CH_2	800

[a] 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 podocarpic acid analogs were selected for further evaluations. They were also found to be active inhibiting the release of other cytokines and mediators (IL-1α, TNF-α, IL-6, IL-8, and PGE2) with selectivity profiles (data not shown) similar to those of tripterine and its truncated analogs disclosed in the previous paper.[6] In addition, the inhibitory activity is not due to non-specific lysis of cells. LC_{50}s of the potent podocarpic acid derivatives were larger than 10 μM, much greater than their IC_{50}s of inhibiting IL-1β release. It

is also true that the molecular targets of these inhibitors have not been identified. A possible mechanism of action might involve in situ oxidation to reactive quinone methide intermediates and in situ reduction of such intermediates. These intermediates may subsequently cross-link with protein nucleophiles via Michael additions.[15] The inhibitory potency of podocarpic acid analogs, and the fact simple phenols are not very active on cytokine release, seem to indicate that this series of inhibitors might be different from the typical protein-cross-link agents which are simple phenol derivatives and only exhibit activity around 50 μM.[15] Thus, podocarpic acid analogs could be useful to identify novel proteins that regulate cytokine release.

Table 2. Inhibition of IL-1β Release



Compound	R1	R2	R3	R4	X	Y	IC_{50} (nM)[a]
29[8]	MeO	HO	H	CO_2Me	CH_2	CH_2	1400
30	MeO	$MeCO_2$	H	CO_2Me	CH	CH	160
31	MeO	$MeCO_2$	$CH_3CH_2CH_2$	CO_2Me	CH	CH	300
32	MeO	HO	H	CO_2Me	CH	CH	100
33	MeO	HO	Me	CO_2H	CH	CH	100
34	$MeCO_2$	MeO	H	CO_2Me	CH	CH	100
35	HO	MeO	H	CO_2Me	CH	CH	100
36	MeO	$MeCO_2$	H	CO_2Me	CH_2	CO	260
37	$MeCO_2$	Br	H	CO_2Me	CH	CH	800
38[12]	HO	Me_2CH	H	CO_2Me	CH	CH	38% @ 1 μM
39	HO	O_2N	H	CO_2Me	CH	CH	5% @ 1 μM
40	HO	H_2N	H	CO_2Me	CH	CH	150
41	$MeCO_2$	CH_3SO_2NH	H	CO_2Me	CH	CH	100
42	$MeCO_2$	$MeCO_2CH_2$	H	CO_2Me	CH	CH	400

[a]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%.

The fact that only catechols and phenols are active on cytokine release may pose a great challenge to develop this class of inhibitors for oral administration. In addition to selectivity issues due to reactive intermediates, such prototype compounds might also encounter pharmacokinetic/pharmacodynamic problems. However, such compounds might be useful in the topical treatment of inflammatory disorders where cytokines have been implicated pathophysiologically.

In summary, we have explored the inhibition of IL-1β release by podocarpic acid derivatives which are structurally more diverse than compounds explored before.[4-6] It is clearly demonstrated that these compounds also have good inhibitory effects on IL-1β release, although somewhat less potent than tripterine itself. Our results here seem to further support 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. More importantly, several non-catechols derivatives have been shown to be active inhibiting IL-1β release. The analogs of podocarpic acid can be readily synthesized and might be useful in the study of mechanism of action of cytokine release and the identification of novel targets. Optimization of these prototype compounds may lead to the discovery of novel antiinflammatory drugs.

References

1. Current address: Bristol-Myers Squibb, Princeton, New Jersey.
2. Current address: Boehringer Ingelheim, Ridgefield, Connecticut.
3. Current address: Abbott Laboratories, Abbott Park, Illinois.
4. Huang, F.-C.; Chan, W. K.; Moriarty, K. J.; Zhang, D. C.; Chang, M. N.; He, W.; Yu, K. T.; Zilberstein, A. *Bioorg. Med. Chem. Lett.* **1998**, *8*, 1883.
5. He, W.; Huang, F.-C.; Morytko, M.; Jariwala, N.; Yu, K. T. *Bioorg. Med. Chem. Lett.* **1998**, *8*, 2825.
6. He, W.; Huang, F.-C.; Gavai, A.; Chan, W. K.; Amato, G.; Yu, K. T.; Zilberstein, A. *Bioorg. Med. Chem. Lett.* **1998**, *8*, 3659.
7. Lew, W.; Oppenheim, J. J.; Matsushima, K. J. *Immunol.* **1988**, *140*, 1895.
8. Cambie, R. C.; Rutledge, P. S.; Woodgate, P. D. *Aust. J. Chem.* **1993**, *46*, 1447.
9. Hill, J. A.; Johnson, A. W.; King, T. J. *J. Chem. Soc.* **1961**, 4430.
10. Hill, J. A.; Johnson, A. W.; King, T. J.; Natori, S.; Tam, S. W. *J. Chem. Soc.* **1965**, 361.
11. Cambie, R. C.; Grimsdale, A. C.; Rutledge, P. S.; Walker, M. F.; Woodgate, P. D. *Aust. J. Chem.* **1991**, *44*, 1553.
12. Matsumoto, T.; Imai, S.; Ondo, K.; Takeyama, N.; Kataoka, H.; Yamamoto, Y.; Fukui, K. *Bull. Chem. Soc. Jpn.* **1982**, *55*, 891.
13. Blazevic, N.; Kolbah, D.; Belin, B.; Sunjic, V.; Kajfez, F. *Synth.* **1979**, 161.
14. Guziec Jr., F. S.; Luzzio, F.A. *Synth.* **1980**, 691.
15. Stanwell, C.; Ye, B.; Yuspa, S. H.; Burke Jr., T. R. *Biochem. Pharm.* **1996**, *52*, 475.