Pergamon

Bioorganic & Medicinal Chemistry Letters 11 (2001) 559–562

BIOORGANIC & MEDICINAL CHEMISTRY LETTERS

Pimarane Cyclooxygenase 2 (COX-2) Inhibitor and its Structure–Activity Relationship

Young-Ger Suh,[a,*] Young-Ho Kim,[b] Mi-Hyoun Park,[b] Young-Hoon Choi,[a] Hye-Kyung Lee,[a] Ju-Yeon Moon,[a] Kyung-Hoon Min,[a] Dong-Yun Shin,[a] Jae-Kyung Jung,[a] Ok-Hui Park,[a] Ra-Ok Jeon,[d] Hyung-Sup Park[c] and Soon-Ah Kang[c]

[a]*College of Pharmacy, Seoul National University, San 56-1 Shinrim-Dong, Kwanak-Gu, Seoul 151-742, South Korea*
[b]*College of Pharmacy, Chungnam University, Taejon 305-764, South Korea*
[c]*College of Medicine, Ulsan University, Seoul 138-040, South Korea*
[d]*College of Pharmacy, Sookmyung Women's University, Seoul 140-742, South Korea*

Received 19 September 2000; accepted 12 December 2000

Abstract—The structure–activity relationship and molecular modelings of a novel pimarane COX-2 inhibitor are reported. Particularly, a series of linker extended analogues designed on the basis of these studies exhibited significantly enhanced COX-2 inhibitory activities and selectivities.

The cyclooxygenase enzymes catalyze the oxidative conversion of arachidonic acid into prostaglandin H_2 which mediates both beneficial and pathodological effects.[1] The COX-1, which is constitutively expressed in most tissues[2] and in blood platelets,[3] is responsible for the physiological production of prostaglandins whereas the expression of COX-2 isoform is induced in response to inflammatory stimuli such as cytokines.[4] Thus, the identification of a novel COX-2 selective inhibitor should offer an excellent anti-inflammatory activity with minimal side effects including gastrointestinal toxicity.[5] Recently, we have reported the isolation of a novel pimarane diterpene, acanthoic acid,[6] from the Korean medicinal plant which has been traditionally used for treating rheumatism. In particular, acanthoic acid turned out to be biologically attractive because it has been shown to exhibit an excellent suppression of interleukin-1 (IL-1) and tumor necrosis factor-α (TNF-α)[7] at 0.1–10 μg/mL level which are major proinflammatory cytokines. More recently, the COX-2 inhibitory activities of acanthoic acid have also been investigated by us as an extension of the studies on its anti-inflammatory effects as well as therapeutic utilities. We herein report acanthoic acid as a novel COX-2 inhibitor and its SAR. In addition, the interaction mode of acanthoic acid with the COX-2 active site and the highly bioactivity-enhanced acanthoic acid analogues are reported (Fig. 1).

The initial in vitro purified enzyme assay[8] of acanthoic acid showed the anticipated COX-2 inhibitory activities, although the inhibitory potency was not satisfactory. Our docking study[9] revealed that the COX-2 inhibitory activity of acanthoic acid is attributed to the interaction between the carboxyl group of acanthoic acid and Arg120 and Tyr355 of COX-2 active site[10] as shown in Figure 2. The hydrophobic part of acanthoic acid also seems to fit well the hydrophobic channel of COX-2 active site.

On the basis of the docking study, we expected that the introduction of an appropriate functionality at C16,

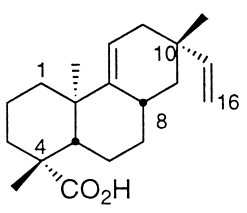


Figure 1. Structure of acanthoic acid.

*Corresponding author. Tel.: +82-02-880-7875; fax: +82-02-888-0649; e-mail: ygsuh@plaza.snu.ac.kr

0960-894X/01/$ - see front matter
PII: S0960-894X(01)00004-X

optimization of the linker length between C4 and the carboxyl group or modification of the carboxyl group would provide an enforced interaction of acanthoic acid with the active site. The syntheses of the requisite analogues are outlined in Schemes 1 and 2.

The derivatives **2**, **3**, **22** and **26** were prepared by conventional epoxidation, hydrogenation, esterification or reduction. Hydroboration of ester **5** of acanthoic acid and then *O*-alkylation or acylation followed by deprotection of the trimethylsilylethyl ester afforded the C16-analogues **7**, **8** and **9**. Direct fluorination[11] of the alcohol **6** followed by ester deprotection provided the fluorinated analogue **10** or **11** while oxidation of the alcohol **6** followed by fluorination gave the C16-difluorinated analogue **12**. The acid derivatives **23**–**25** were prepared by reaction of acid chloride **4** with the corresponding amines.

The synthetic variation of the linker between C4 and the carboxyl group is outlined in Scheme 2. The one carbon extended analogue **15** was prepared by the sequential olefination of the aldehyde **13**, hydrolysis of the resulting enol ether and then oxidation of the aldehyde **14**.

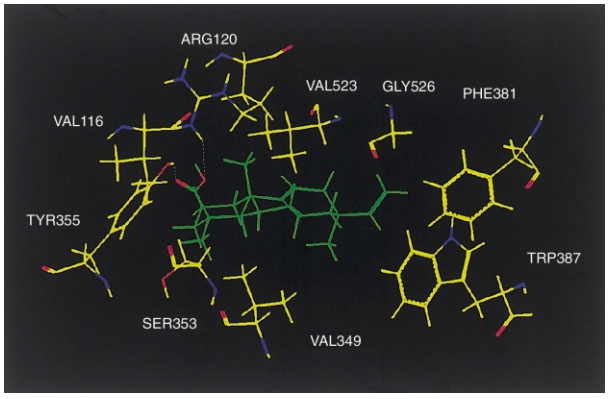
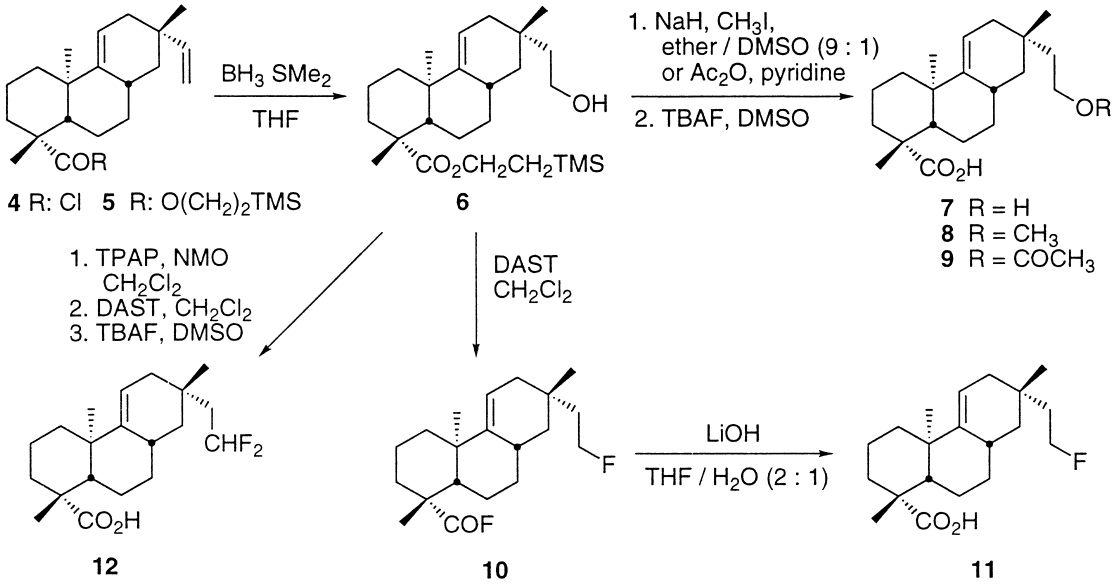


Figure 2. Binding of acanthoic acid in the COX-2 acitve site.

The two and three carbon extended analogues **17** and **19** were prepared from the aldehydes **13** and **14** by olefination, chemoselective olefin reduction[12] of the resulting α,β-unsaturated ester and ester hydrolysis. The four and five carbon extended analogues **20**[13] and **21**[14] were prepared from the esters **16** and **18** by the same procedure. The COX-2 and COX-1 inhibitory activities[15] of the synthesized analogues are summarized in Table 1.

Generally, C16-analogues **10**, **11** and **12** showed the potent inhibitory activities only against COX-1 isoform while other C16-analogues showed poor inhibitory activities against both COX-2 and COX-1. However, variation of the linker between C4 and the carboxyl group (analogues **15**, **17**, **19**, **20** and **21**) provided significant enhancement in inhibition of both COX-2 and COX-1. In particular, the four and five carbon extended analogues **20** and **21** showed 20 and 60 fold potencies in COX-2 inhibitory activities compared to that of acanthoic acid. Amidation of the carboxyl group of acanthoic acid (analogues **23** and **25**) reduced COX-2 inhibitory activities although the hydrazide **24** showed the enhanced inhibitory activities against both COX-2 and COX-1.

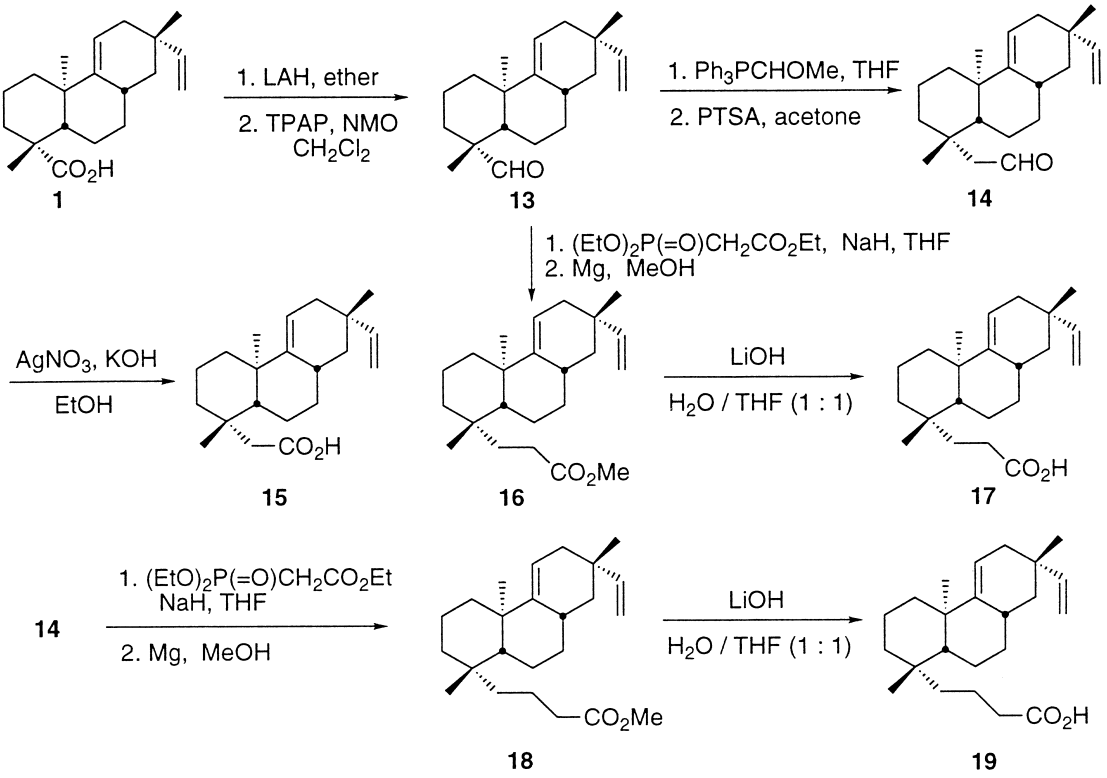
The structure–activity studies reveal that incorporation of an adequate functionality at C16 for additional hydrogen bondings in the active site generally increases COX-1 inhibitory activity while it decreases COX-2 inhibitory activity. Regarding modification of the carboxyl group, the functional group possessing both hydrogen bonding donor and acceptor such as carboxyl or hydrazido group seems to be crucial for the COX inhibitory activities. The poor or no inhibitory activities of the ester **22** and the alcohol **26** partly support this trend. The significant improvement in both inhibitory potency and selectivity by the linker extension is likely due to the enforced ligand–active site interaction by an additional hydrogen bonding. The docking study of the analogue **21** in COX-2 active site (Fig. 3) exhibits three hydrogen bondings of the carboxyl group of the analogue



Scheme 1. Syntheses of C16-analogues.

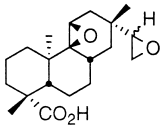
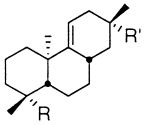
21 with Arg120, Ser119 and Tyr115. By contrast, the docking study of acanthoic acid shows only two hydrogen bondings as shown in Figure 2. The improved COX-2 selectivity can also be rationalized by the relatively weaker interaction of the analogue **21** in COX-1 isoform (two hydrogen bondings) compared to that in COX-2 isoform.[16]

In conclusion, a natural pimarane diterpene and a series of its analogues have been discovered as novel COX-2 inhibitors. Additionally, the structure–activity studies of acanthoic acid have revealed that modification of the carboxyl group or extension of the linker between C4 and the carboxyl group significantly enhances COX-2 inhibitory activity as well as selectivity. In particular,



Scheme 2. Syntheses of the linker-extended analogues.

Table 1. In vitro COX inhibitory activities of acanthoic acid and its analogues

Compound		R	R′	COX-2 IC_{50} (μM)	COX-1 IC_{50} (μM)
1	Acanthoic acid			790.4	116.4
2				>1000	1000
3		CO_2H	CH_2CH_3	425.0	229.0
7		CO_2H	CH_2CH_2OH	>1000	>1000
8		CO_2H	$CH_2CH_2OCH_3$	>1000	84.6
9		CO_2H	$CH_2CH_2OCOCH_3$	>1000	265.7
10		COF	CH_2CH_2F	193.7	18.1
11		CO_2H	CH_2CH_2f	>1000	32.4
12		CO_2H	CH_2CH_2f	796.0	56.0
15		CH_2CO_2H	$CHCH_2$	82.3	28.1
17		$CH_2CH_2CO_2H$	$CHCH_2$	105.0	21.1
19		$CH_2(CH_2)_2CO_2H$	$CHCH_2$	49.4	34.3
20		$CH_2(CH_2)_3CO_2H$	$CHCH_2$	38.7	52.6
21[a]		$CH_2(CH_2)_2CHCHCO_2H$	$CHCH_2$	13.2	32.7
22		CO_2CH_3	$CHCH_2$	>1000	>1000
23		$CONH_2$	$CHCH_2$	>1000	147.5
24		$CONHNH_2$	$CHCH_2$	41.9	18.4
25		CONHOH	$CHCH_2$	818.9	45.6
26		CH_2OH	$CHCH_2$	>1000	791.1

[a]The side chain (R) of the compound **21** consists of only *E*-olefin.

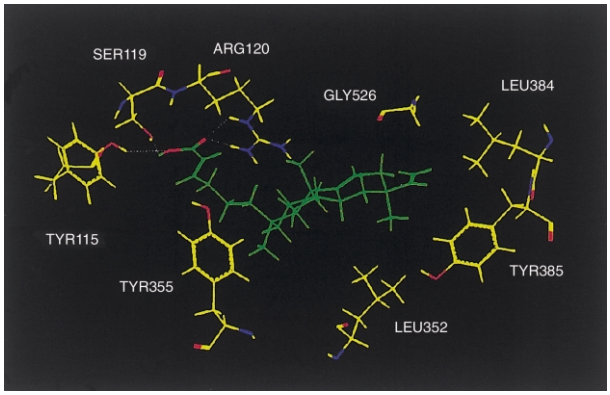


Figure 3. Binding of analogue **21** in the COX-2 acitve site.

our SAR studies and molecular modelings on these novel COX-2 inhibitors would provide quite useful information for the development of a new class of anti-inflammatory agents. More detailed SAR results and in vivo activities of the advanced analogues will be reported in the near future.

Acknowledgements

This work was supported by grant 1999-21500-001-2 from KOSEF and in part by 2000 BK21 project for Medicine, Dentistry and Pharmacy.

References and Notes

1. Vane, J. R.; Botting, R. M. *Inflamm. Res.* **1995**, *44*, 1.
2. Simmons, D. L.; Xie, W.; Chipman, J. G.; Evett, G. E. In *Prostaglandins, Leukotriens, Lipoxins, and PAF*; Bailey, J. M., Ed.; Plenum Press: New York, 1991; pp 67–78.
3. Funk, C. D.; Funk, L. B.; Kennedy, M. E.; Pong, A. S.; Fitzgerald, G. A. *FASEB. J.* **1991**, *5*, 2304.
4. Goppelt-Struebe, M. *Prostagl. Leukotr. Essen. Fatty Acids* **1995**, *52*, 213.
5. Laneuville, O.; Breuer, D. K.; Dewitt, D. L.; Hla, T.; Funk, C. D.; Smith, W. L. *J. Pharmacol. Exp. Ther.* **1994**, *271*, 927. For a recent review of COX-2 inhibitors, see: Marnett, L. J.; Kalgutkar, A. S. *Trends Pharmacol. Sci.* **1999**, *20*, 465.
6. Kim, Y. H.; Chung, B. S.; Sankawa, U. *J. Nat. Prod.* **1988**, *51*, 1080.
7. Kang, H. S.; Kim, Y. H.; Lee, J. S.; Lee, J. J.; Choi, I.; Pyun, K. H. *Cell. Immunol.* **1996**, *170*, 212.
8. The purified COX-2 enzyme assay was performed according to Bohlin protocol with a slight modification. Noreen, Y.; Ringbom, T.; Perera, P.; Danielson, H.; Bohlin, L. *J. Nat. Prod.* **1998**, *61*, 2. The purified COX-2 (prostaglandin endoperoxide H synthetase-2) from sheep placental cotyledons was purchased from Cayman Chemical Co., Ann Arbor, MI, USA.
9. Calculations were performed with SYBYL 6.5 program (Tripos Inc.) utilizing the Tripos force field and Gasteiger-Huckel charges.
10. The COX-2 structure was obtained from the Brookhaven Protein Data Bank, 1cx2.
11. Middleton, W. J. *J. Org. Chem.* **1975**, *40*, 574.
12. Hudlicky, T.; Sinai-Zingde, G.; Natchus, M. G. *Tetrahedron Lett.* **1987**, *28*, 5287.
13. Spectral data for compound **20**: $[\alpha]_D^{21}$ −13.6° (*c* 0.19, $CHCl_3$); IR (neat): 1714 cm^{-1}; ^1H NMR ($CDCl_3$, 300 MHz) δ 5.75 (dd, 1H, *J* = 17.5, 10.5 Hz), 5.27 (m, 1H), 4.86 (dd, 1H, *J* = 17.5, 1.5 Hz), 4.79 (dd, 1H, *J* = 10.5, 1.5 Hz), 2.30–0.74 (m, 25H), 1.01 (s, 3H), 0.90 (s, 3H), 0.76 (s, 3H); HRMS (EI) *m*/*z* calcd for $C_{24}H_{38}O_2$ 358.2872 (M^+), found 358.2871.
14. Spectral data for compound **21**: $[\alpha]_D^{21}$ −13.7° (*c* 0.21, $CHCl_3$); IR (neat): 1650 cm^{-1}; ^1H NMR ($CDCl_3$, 300 MHz) δ 7.01 (ddt, 1H, *J* = 15.7, 6.9, 2.9 Hz), 5.70–5.80 (m, 2H), 5.27 (m, 1H), 4.86 (dd, 1H, *J* = 17.5, 1.5 Hz), 4.79 (dd, 1H, *J* = 10.5, 1.5 Hz), 0.76–2.23 (m, 23H), 1.01 (s, 3H), 0.90 (s, 3H), 0.78 (s, 3H); HRMS (EI) *m*/*z* calcd for $C_{25}H_{38}O_2$ 370.2872 (M^+), found 370.2893.
15. The COX-1 inhibitory activity was measured according to the modified Sankawa protocol with the rabbit renal microsomes as the enzyme source. Sankawa, U.; Shibuya, M.; Ebizuka, Y.; Noguchi, H.; Kinoshita, T.; Iitaka, Y. *Prostaglandins* **1982**, *24*, 21.
16. The docking study of the analogue **21** in COX-1 isoform is not presented in this paper. However, the increased volume by linker extension seems to be an additional benefit for the COX-2 selectivity.