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Synthesis and Structure−Activity Relationship Studies of Anti-Inflammatory Epoxyisoprostane Analogues

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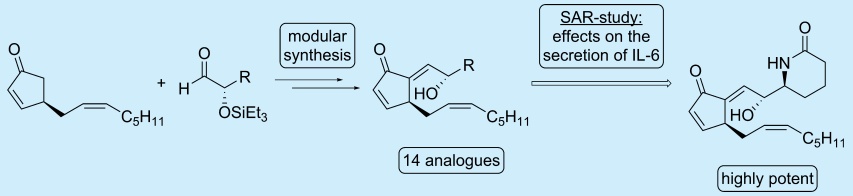
Supporting Information



ABSTRACT: The lactone derivative of the epoxyisoprostane EC is a highly effective inhibitor of the secretion of the proinflammatory cytokine IL-6. Herein, a modular synthesis of analogues is described, allowing flexible variations of the cyclic side chain of the parent lactone. A structure−activity relationship study identified a lactam analogue that retains the high activity. Furthermore, the exocyclic allylic alcohol was shown to be crucial for the observed effect.

Oxidized phospholipids (OxPLs) constitute a class of biomolecules generated upon exposure of biological membranes to reactive oxygen species (ROS).[1] These natural products have been recognized as active compounds in inflammation. Intriguingly, while a majority of investigations suggest proinflammatory effects of OxPLs via interaction with pattern recognition receptors of the innate immune system,[2] anti-inflammatory properties have also been observed.[3] Recently, we have shown that epoxyisoprostane EC (**1**, Figure 1), as well as to a lesser extent its phosphatidylcholine

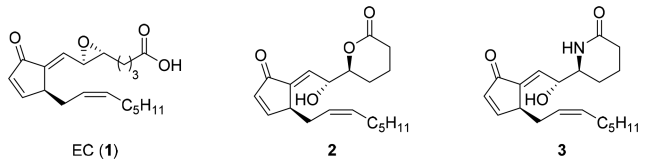


Figure 1. Structures of EC (**1**), lactone **2**, and lactam **3**.

derivative PECPC, act in an anti-inflammatory fashion.[4] This was demonstrated by the observed reduced secretion of the proinflammatory cytokines IL-6 and IL-12 by bone-marrow-derived dendritic cells (BMDCs). Furthermore, during studies aimed at understanding the underlying mechanisms for the action of these epoxyisoprostanes, we discovered lactone **2** derived from EC (**1**) as the most potent anti-inflammatory compound in the series.[5] Herein, we report a structure−activity relationship study that further investigates the effect of the cyclic side chain in **2**.

The promising activity of lactone **2** suggests this class of epoxycyclopentenone derivatives as a potential scaffold for future anti-inflammatory therapeutics. While our previous studies showed that the endo- and the exocyclic enone as well as the allylic epoxide in EC (**1**) are crucial for the observed high activity, the role of the side chain in lactone **2** vis-à-vis its anti-inflammatory effects has not been investigated. Therefore, a structure−activity relationship study was initiated. In this regard, the presence of a lactone in **2** creates ambiguity with respect to the generation of seco-acid; therefore, key structures targeted for synthesis include replacements for the lactone, such as lactams (**3**, Figure 1), sultams, and other rings.[6]

The general strategy to access the targeted analogues involved late-stage coupling of previously described cyclopentenone **4** with various side chains as the corresponding aldehydes **5** via aldol condensation reactions to afford dienones **6** (Scheme 1, I). This approach allowed for a modular synthesis of a variety of different target structures. The synthesis of lactam **3** commenced with sulfinimine **7** (Scheme 1, II).[7] Grignard addition proceeded with dr = 10:1, and the desired sulfinamide **8** was isolated in 69% yield.[8] Oxidative cleavage of the alkene in **8** led to the corresponding methyl ester, which following I_2-mediated auxiliary removal with concomitant amide bond formation furnished lactam **9**.[9] Aldehyde **11** was subsequently prepared in four steps and appended to **4** as described above to give lactam **3**. All other analogues described in this work were accessed employing closely related approaches (see the Supporting Information).

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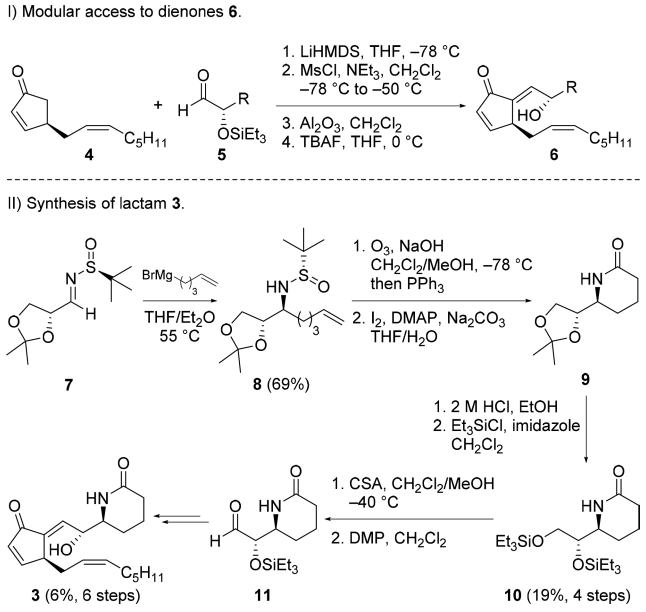
Scheme 1. Access to Dienones 6 and Synthesis of Lactam 3



Lactam **3** was tested together with its diastereomer **12** along with EC (**1**) and **2** for comparative purposes for their ability to inhibit IL-6 secretion by BDMCs (Figure 2). We were

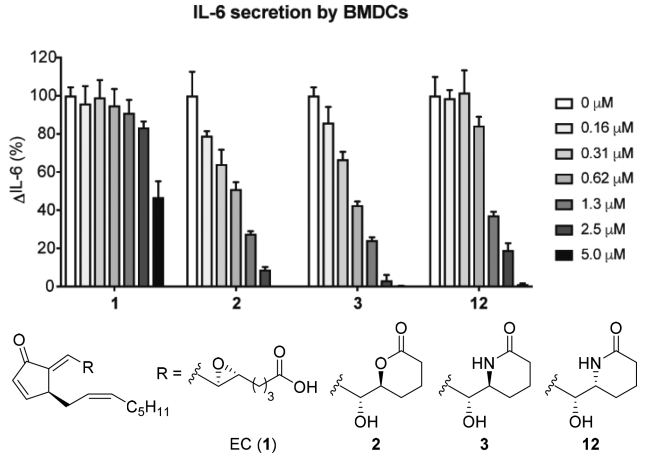


Figure 2. Comparison of the activity of EC (**1**), lactone **2**, and lactams **3** and **12** in inhibiting secretion of the proinflammatory cytokine IL-6.

delighted to observe that the substitution of the lactone for a lactam resulted in a slightly more active epoxyisoprostane analogue. Also, comparison with diastereomeric lactam **12** indicated that the stereogenic center in the six-membered ring only had a minor impact on the activity.

With these results in hand, ester and amide surrogates were further evaluated.[10] To this end, diastereomeric sultams **13** and **14** as well as ketone **15** (all as single diastereomers; Figure 3) were examined.[11] When subjected to the IL-6 secretion assay, sultam **13** displayed activity comparable to lactone **2**; however, cytotoxicity was observed at concentrations >1 μM. In contrast, sultam **14** was slightly less active but not cytotoxic in the observed concentration range. Interestingly, ketone **15** also exhibited potent activity, whereby the observed cytotoxic effect was observed at >2 μM.

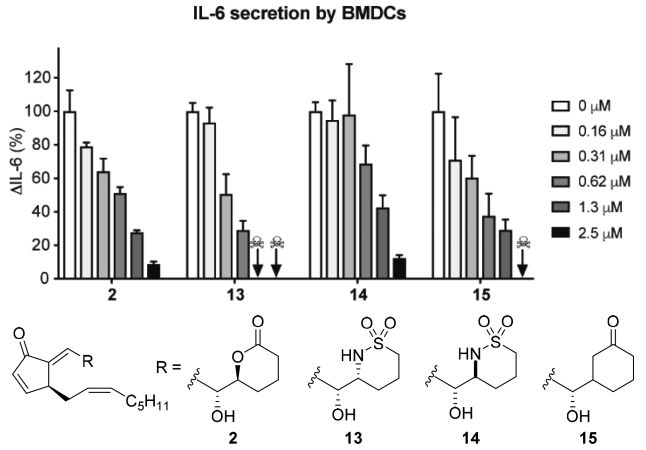


Figure 3. Comparison of the activity of lactone **2**, sultams **13** and **14**, and ketone **15** in inhibiting secretion of the proinflammatory cytokine IL-6.

In follow-up studies, structures incorporating simpler side-chain surrogates of the lactone were examined. Additionally, the significance of the allylic alcohol for activity was investigated. As described in the Supporting Information, two deoxy derivatives lactone **16** and lactam **17** were synthesized and tested for their ability to inhibit IL-6 secretion (Figure 4).

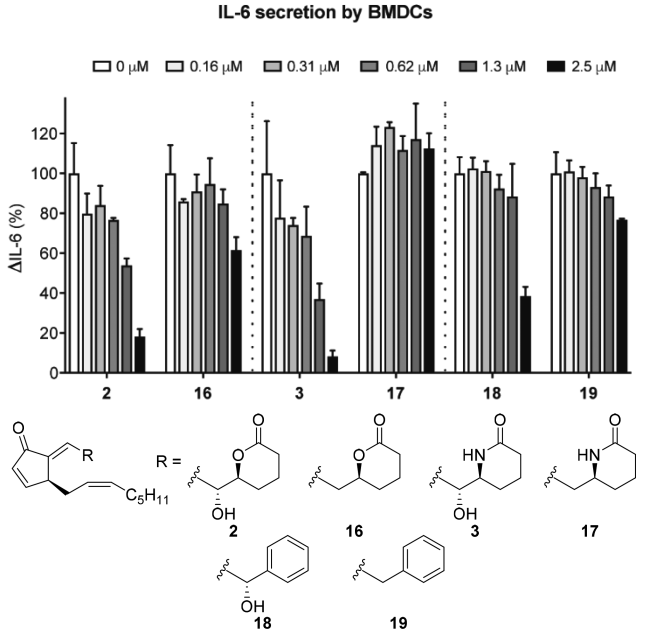


Figure 4. Comparison of the activity of **2**, **3**, and **18** and their deoxy analogues **16**, **17**, and **19** in inhibiting secretion of the proinflammatory cytokine IL-6.

Analysis of the data for **2** and **16** as well as for **3** and **17** in Figure 4 clearly showed that the presence of the allylic alcohol is crucial for potent activity. In order to further validate this finding, the phenyl analogue **18** was tested along with its deoxy derivative **19**. Although allylic alcohol **18** exhibited noticeable activity, **19** was virtually inactive.

Attention was next turned to the investigation of homologated and truncated analogues (Figure 5). Both *N*-substituted lactam **20** and γ-lactam **21** exhibited cytotoxicity at >2 μM, but while the former was significantly less active, the

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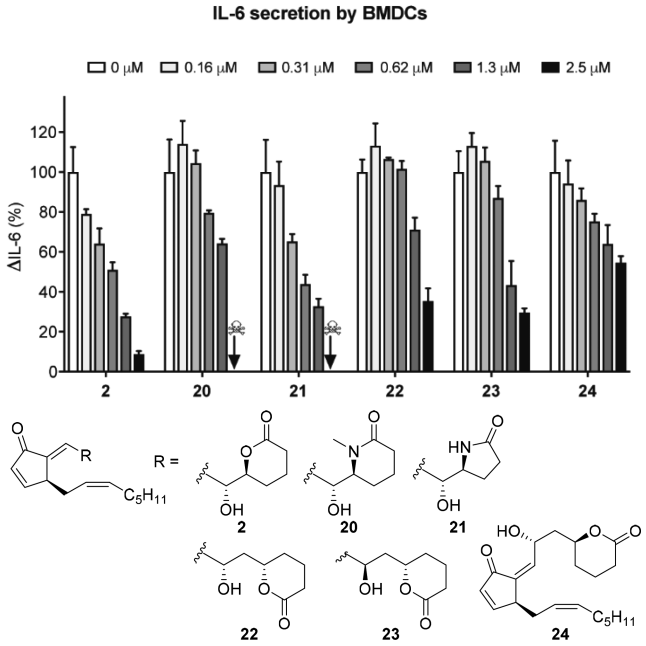

Figure 5. Comparison of the activity of **2**, **20**, **21**, **22**, **23**, and **24** in inhibiting secretion of the proinflammatory cytokine IL-6.

latter displayed similar activity to the parent lactone **2**. Homologated lactones **22** and **23** were less active than their C_{20} analogue **2**, and the olefin diastereomer of the α,β-unsaturated exocyclic enone (**24**) displayed decrease in potency. In addition to the experiments described above, we also investigated exocyclic enaminones as analogues. However, no perceivable anti-inflammatory activity was observed (see the Supporting Information).

In summary, we have described the convergent synthesis of anti-inflammatory epoxyisoprostane analogues derived from lactone **2** and analyzed their effect on the secretion of the proinflammatory cytokine IL-6. We demonstrated that lactam **3** retained the high anti-inflammatory activity, and diastereomeric lactam **12**, sultam **13**, ketone **15**, as well as γ-lactam **21** displayed comparable activity. The study also highlighted the necessity of the side-chain allylic alcohol for activity. Further modifications and study of this intriguing class of biomolecules are the subject of ongoing research in our laboratory and will be reported as they become available.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.orglett.8b01042.

Experimental procedures and characterization data for all new compounds (PDF)

Accession Codes

CCDC 1825895−1825896 contain the supplementary crystallographic data for this paper. These data can be obtained free of charge via www.ccdc.cam.ac.uk/data_request/cif, or by emailing data_request@ccdc.cam.ac.uk, or by contacting The Cambridge Crystallographic Data Centre, 12 Union Road, Cambridge CB2 1EZ, UK; fax: +44 1223 336033.

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Notes

The authors declare no competing financial interest.

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■ REFERENCES

(1) (a) Jahn, U.; Galano, J.-M.; Durand, T. *Angew. Chem., Int. Ed.* **2008**, *47*, 5894. (b) Bochkov, V. N.; Oskolkova, O. V.; Birukov, K. G.; Levonen, A.-L.; Binder, C. J.; Stöckl, J. *Antioxid. Redox Signaling* **2010**, *12*, 1009.

(2) (a) Imai, Y.; et al. *Cell* **2008**, *133*, 235. (b) Stewart, C. R.; et al. *Nat. Immunol.* **2010**, *11*, 155. (c) Seimon, T. A.; et al. *Cell Metab.* **2010**, *12*, 467.

(3) (a) Leitinger, N.; et al. *Proc. Natl. Acad. Sci. U. S. A.* **1999**, *96*, 12010. (b) Bochkov, V. N.; Kadl, A.; Huber, J.; Gruber, F.; Binder, B. R.; Leitinger, N. *Nature* **2002**, *419*, 77. (c) Blüml, S.; et al. *J. Immunol.* **2005**, *175*, 501. (d) Knapp, S.; Matt, U.; Leitinger, N.; van der Poll, T. *J. Immunol.* **2007**, *178*, 993.

(4) (a) Egger, J.; Bretscher, P.; Freigang, S.; Kopf, M.; Carreira, E. M. *Angew. Chem., Int. Ed.* **2013**, *52*, 5382. (b) PECPC = 1-palmitoyl-2-(5,6-epoxycyclopentenone)-*sn*-glycero-3-phosphorylcholine.

(5) (a) Egger, J.; Bretscher, P.; Freigang, S.; Kopf, M.; Carreira, E. M. *J. Am. Chem. Soc.* **2014**, *136*, 17382. (b) Bretscher, P.; Egger, J.; Shamshiev, A.; Trötzmüller, M.; Köfeler, H.; Carreira, E. M.; Kopf, M.; Freigang, S. *EMBO Mol. Med.* **2015**, *7*, 593.

(6) Testa, B.; Mayer, J. M. *Hydrolysis in Drug and Prodrug Metabolism: Chemistry, Biochemistry and Enzymology*; Verlag Helvetica Chimica Acta: Zürich, 2003; pp 163−164.

(7) Buesking, W. A.; Baguley, T. D.; Ellman, J. A. *Org. Lett.* **2011**, *13*, 964.

(8) The diastereomeric ratio was determined by ^{1}H NMR spectroscopy of the unpurified reaction mixture.

(9) Chen, W.; Ren, J.; Wang, M.; Dang, L.; Shen, X.; Yang, X.; Zhang, H. *Chem. Commun.* **2014**, *50*, 6259.

(10) Meanwell, N. A. *J. Med. Chem.* **2011**, *54*, 2529.

(11) The relative configuration of the amide and alcohol stereogenic centers in **14** is anti; however, we have not been able to establish their relationship to the γ-stereogenic center in the cyclopentenone. This also holds for the stereogenic center in cyclohexanone **15**.

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