Bioorganic & Medicinal Chemistry 23 (2015) 944–952

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry

journal homepage: www.elsevier.com/locate/bmc

Rat hormone sensitive lipase inhibition by cyclipostins and their analogs

Elena Vasilieva, Supratik Dutta, Raj K. Malla, Benjamin P. Martin, Christopher D. Spilling, Cynthia M. Dupureur *

Department of Chemistry & Biochemistry and the Center for Nanoscience, University of Missouri St. Louis, St. Louis, MO 63121, United States

ARTICLE INFO

Article history:
Received 19 November 2014
Revised 5 January 2015
Accepted 15 January 2015
Available online 22 January 2015

Keywords:
Lipase
Inhibition
Kinetics
Natural products

ABSTRACT

Cyclipostins are bicyclic lipophilic phosphate natural products. We report here that synthesized individual diastereomers of cyclipostins P and R have nanomolar IC_{50}s toward hormone sensitive lipase (HSL). The less potent diastereomers of these compounds have 10-fold weaker IC_{50}s. The monocyclic phosphate analog of cyclipostin P is nearly as potent as the bicyclic natural product. Bicyclic phosphonate analogs of both cyclipostins exhibit IC_{50}s similar to those of the weaker diastereomer phosphates (about 400 nM). The monocyclic phosphonate analog of cyclipostin P has similar potency. A series of monocyclic phosphonate analogs in which a hydrophobic tail extends from the lactone side of the ring are considerably poorer inhibitors, with IC_{50}s around 50 μM. Finally cyclophostin, a related natural product inhibitor of acetylcholinesterase (AChE) that lacks the hydrocarbon tail of cyclipostins, is not active against HSL. These results indicate a critical SAR for these compounds, the hydrophobic tail. The smaller lactone ring is not critical to activity, a similarity shared with cyclophostin and AChE. The HSL kinetics of inhibition for the cyclipostin P *trans* diastereomer were examined in detail. The reaction is irreversible with a K_I of 40 nM and a rate constant for inactivation of 0.2 min^{-1}. These results are similar to those observed for cyclophostin and AChE.

1. Introduction

The serine hydrolases constitute one of the largest classes of enzymes in biochemistry.[1] These biological catalysts share the classic α/β hydrolase fold structure and the well known catalytic triad composed of an acidic residue, His, and Ser. This last residue serves as the nucleophile in the attack on ester or amide substrates.[2] Among the serine hydrolases of therapeutic interest are acetylcholinesterase (AChE[†]), butyrylcholinesterase, and a number of lipases. For this reason, extensive literature has evolved from inhibition studies.[3–5]

An interesting class of esterase inhibitors has emerged from the natural product literature. The basic structure is represented by cyclophostin **1a** (Scheme 1), a bicyclic phosphate isolated from *Streptomyces* species strain DSM 13381.[6] As we have recently characterized, this compound is a potent (nanomolar) irreversible inhibitor of AChE.[7,8] Much like the chemical warfare agents Sarin and V/X,[9] it targets the conserved Ser residue in the catalytic triad of this enzyme through phosphorylation. We recently demonstrated that bicyclic phosphonate analogs (**2a**, **2b**) of cyclophostin are less potent AChE inhibitors than the natural product.[8] Monocyclic phosphonate analogs (large phosphate ring) are as potent as the corresponding bicyclic phosphonate.

Close structural relatives are the cyclipostins, long chain derivatives of cyclophostin (**3a**, **3b**, **4a**, **4b**). These compounds isolated from bacteria were shown to be strong inhibitors of hormone sensitive lipase (HSL).[10] This enzyme is able to catalyze the hydrolysis of mono-, di, and triacylglycerides to liberate fatty acids (Scheme 2).[11,12] Through insulin signaling, HSL becomes phosphorylated and translocates to lipid droplets in adipocytes.[13]

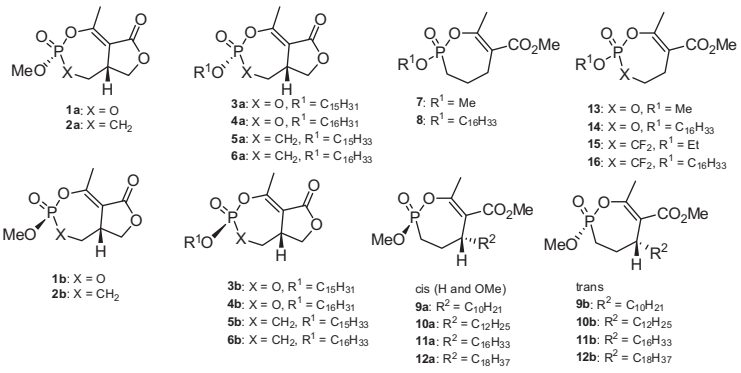
Lipases are of wide interest due to their relevance to obesity and diabetes, the latter of which exhibits disturbances in both sugar and fat metabolism. There are only a few obesity drugs on the market. The most well known is Orlistat (Fig. 1), obtained from the catalytic hydrogenation of the natural product lipstatin, which targets the digestive gastric and pancreatic lipases.[14] Many more are/have been in clinical trials, and similarly a couple target lipases.[3]

A number of low nanomolar HSL synthetic inhibitors have been reported (Fig. 1;[15–24]). Very few detailed kinetic and mechanistic studies of HSL inhibitors exist.[25]

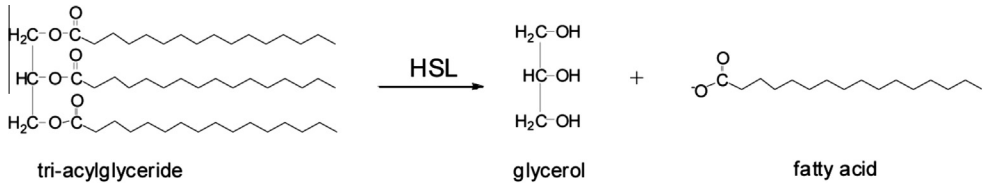
Cyclipostins represent a unique niche among HSL inhibitors because they are natural products and, by virtue of being both lipophilic and electrophilic, are substrate mimic-mechanism-based

* Corresponding author. Tel.: +1 314 516 4392; fax: +1 314 516 5342.
E-mail address: cdup@umsl.edu (C.M. Dupureur).
† AChE, acetylcholinesterase; HSL, hormone sensitive lipase.

http://dx.doi.org/10.1016/j.bmc.2015.01.028
0968-0896/



Scheme 1.



Scheme 2.

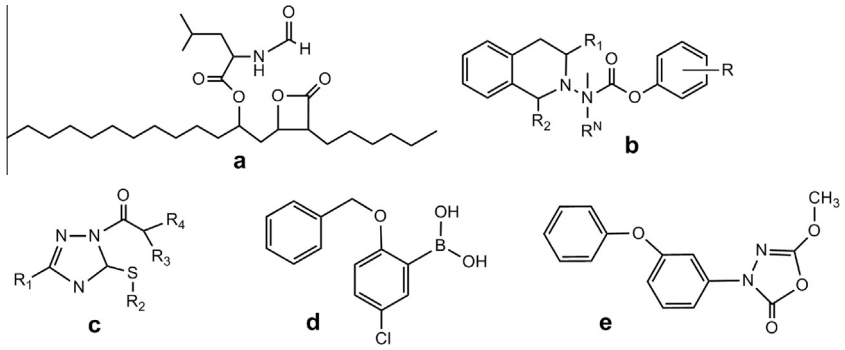


Figure 1. Other representative lipase inhibitors. (a) Pancreatic lipase inhibitor Orlistat;[42] HSL inhibitors (b) carbazates;[18] (c) carbamoyl triazole;[17] (d) boronic acids;[19] (e) 3-phenyl-5-alkoxy-1,3,4-oxadiazol-2-one.[24,25]

inhibitors. The long hydrocarbon tails suggest a substrate-type binding mode. Cyclophostins share the cyclic phosphate electrophilic center of cyclipostins, which have already been shown to irreversibly modify the active site Ser of AChE[8] and microbial lipases.[26] We recently reported the synthesis of cyclipostin P (**4a** and **4b**).[27] Herein we characterize the activities for two cyclipostins and their analogs against hormone sensitive lipase. To our knowledge, this represents the most detailed study of HSL inhibitors reported to date.

2. Results and discussion

2.1. Synthesis of inhibitors

Cyclipostin R (**3a** and **3b**) and the analogs used in this study were synthesized using our one-pot ester exchange process from (±)-cyclophostin **1a** and its diastereomer **1b**,[27] the phosphonate analogs thereof **2a** and **2b**,[7] and three monocyclic compounds **7**, **13** and **15** (Scheme 3). Characterization data for the monocyclic compounds **13** and **15** appear in Supplemental material. The syntheses these compounds will be reported in another article. Methyl esters of phosphates undergo transesterification cleanly in the presence of 5 mol % tetra-*n*-butylammonium iodide, but phosphonates and the α,α-difluorophosphonate ethyl ester **16** require 10 mol % for optimal conversion. Additional information can be found in Section 3 and Supplementary material.

Cyclophostin **1a** **1b**, cyclipostin P **4a**, **4b** and the phosphonate analogs of cyclophostin (**5a**, **5b**, **6a**, **6b**) were prepared following previously published procedures.[7,27] The synthesis of the monocyclic phosphonates (**7**, **8**, **9a**, **10a**, **10b**, **11a**, **11b**, **12a**, **12b**) also followed published procedures.[8,26]

2.2. Inhibition studies with cyclophostin and cyclipostin

Table 1 summarizes all of the cyclophostin and cyclipostin analogs that were tested against recombinant rat HSL. The natural product and AChE inhibitor cyclophostin **1b** is not potent against HSL ($IC_{50} \geqslant 100$ μM). The same is true of the synthetic isomer **1a** and phosphonate isomer **2a**. However, the cyclipostins with the same core cyclophostin structure with long carbon tails extending from the phosphate moiety (**3**–**6**) are more potent against HSL; a couple have IC_{50}'s near 50 nM. This indicates that the long hydrocarbon tail of the phosphate moiety is a critical part of the inhibitor