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PAPER

Synthesis and biological investigation of the β-thiolactone and β-lactam analogs of tetrahydrolipstatin†

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The synthesis of β-thiolactone and β-lactam analogs of tetrahydrolipstatin is described from a common late-stage β-lactone derivative. These analogs, and a *cis*-disubstituted β-lactone analog of tetrahydrolipstatin, were screened for activity against porcine pancreatic lipase and for inhibition of cell growth of a panel of four human cancer lines.

Introduction

Over the past few decades naturally occurring β-lactones (2-oxetanones) and their synthetic congeners have been recognized by the scientific community as promising drug candidates for several human disease states.[1] Tetrahydrolipstatin (Orlistat), a saturated analog of the natural lipstatin isolated from *Streptomyces toxytricini* in 1987,[2] is one such molecule that is currently marketed as Xenical® for the treatment of obesity (Fig. 1).[3] The biological target for tetrahydrolipstatin is the active site serine of the pancreatic and gastric lipases with which it forms an irreversible ester bond by ring opening of the *trans*-fused β-lactone,[4] resulting in a decrease of the rate of triglyceride hydrolysis and adsorption of dietary fat by the small intestine.[5] More recently, Orlistat and some of its analogs have been found to inhibit the thioesterase domain of fatty acid synthase, an essential enzymatic process involved in the growth and survival of tumor cells and a validated drug target for the discovery of new anti-tumor antibiotics.[6]

Unlike the β-lactones[1,7] and β-lactams,[8] which have received enormous attention from the synthetic and medicinal chemistry communities, the β-thiolactones have been essentially ignored and thus represent an untapped potential for drug discovery. The β-thiolactones present a unique reactivity profile toward active site nucleophiles, including both acylating and alkylating capabilities, which differ from those of the corresponding β-lactones. This is because of the different physicochemical properties of β-thiolactones arising from the longer C–S bond and the smaller C–S–C═O angle as compared to their oxygen analogs, which offer the potential of a useful compromise between stability and reactivity.[9]

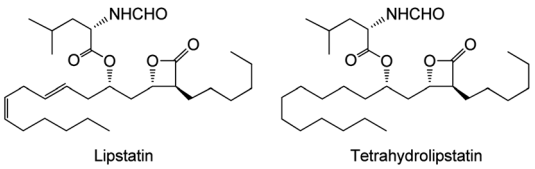

Fig. 1 Lipstatin and tetrahydrolipstatin.

As a part of a program to uncover the potential of the β-thiolactones in medicinal chemistry,[10] we report here on the synthesis of sulfur analogs (β-thiolactones) of tetrahydrolipstatin and, for the purposes of comparison of the corresponding nitrogen analogs (β-lactams), with the two series of compounds obtained efficiently from a common late-stage β-lactone intermediate. The β-thiolactones and β-lactams prepared in this manner were evaluated for activity against porcine pancreatic lipase and for the inhibition of four human cancer cell lines.

Synthesis

Selective ring opening of (*S*)-(−)-epichlorohydrin with $C_{10}H_{21}MgBr$ in presence of CuI in THF at −45 °C gave the chlorohydrin **1** in 87% yield (Scheme 1) that was converted to the epoxide **2** by the action of KOH in Et_2O in 81% yield.[11] Subsequent ring opening with vinylmagnesium bromide, again in the presence of CuI resulted in the formation of the homoallylic alcohol **3** in 92% yield, which was protected as PMB ether **4** in 92% yield. Oxidative cleavage of the alkene by a one-pot procedure[12] consisting of dihydroxylation in the presence of OsO_4 and NMO followed by diol cleavage with $PhI(OAc)_2$, afforded the corresponding aldehyde **5** in 88% yield. Subsequent reaction with the boron enolate derived from oxazolidinone

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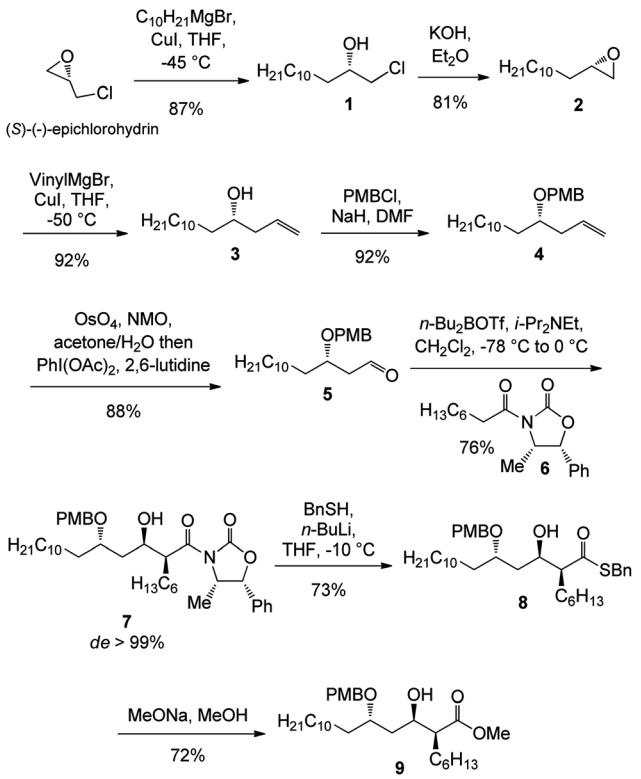
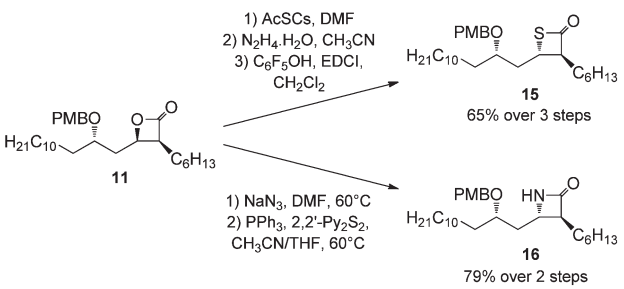
6 delivered the aldol **7** in 76% yield and a diastereomeric excess of >99% (Scheme 1).[13] Removal of the oxazolidinone group from **7** was accomplished by nucleophilic displacement with the lithium salt of BnSH at −10 °C in THF, furnishing the benzyl thioester **8** in 73% yield. Subsequent methanolysis of **8** with MeONa in MeOH then gave rise to the methyl ester **9** in 72% yield.

Attempted conversion of the alcohol **9** to the corresponding configurationally inverted thiol by Mitsunobu reaction with thioacetic acid, or by displacement of the derived mesylate or triflate esters was unsuccessful. Consequently, it was envisaged that the desired substitution might be achieved *via* the β-lactone. Accordingly, hydrolysis of methyl ester **9** was conducted to afford the corresponding acid **10** in quantitative yield, which was cyclized using $PhSO_2Cl$ in pyridine to give the β-lactone **11** in 67% yield (Scheme 2). With **11** in hand, PMB removal with TFA in CH_2Cl_2 gave the secondary alcohol **12** in 94% yield, which, on esterification with Boc-Leu-OH in the presence of EDCI and DMAP, provided the *N*-protected amino ester **13** in 86% yield. Subsequent removal of the Boc group with TFA was followed by formylation with formic acetic anhydride to afford the *cis*-isomer of tetrahydrolipstatin **14** in 86% yield (Scheme 2).[14]

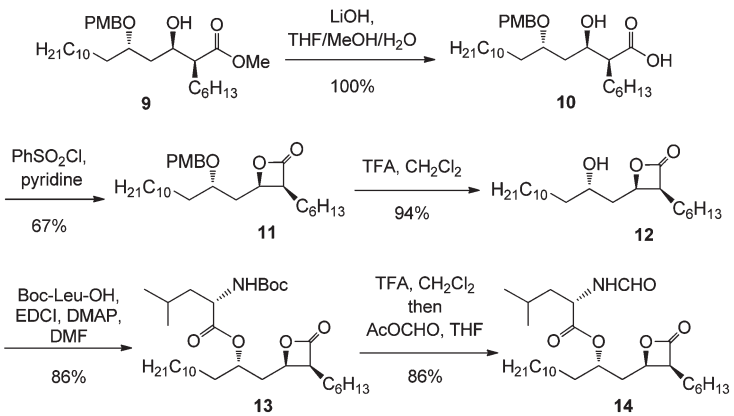
Returning to the formation of the β-thiolactone, and taking into consideration previous reports on the alkylation of soft nucleophiles including thiols by oxetanones,[15] β-lactone **11** was subjected to BnSLi, but only *O*-acyl fission of β-lactone **11** affording benzyl thioester **8** was observed. The use of NaSH as nucleophile also proved to be fruitless due to competitive intermolecular reaction after the initial S_N2 cleavage of **11**. Fortunately, the use of cesium thioacetate as nucleophile led to opening of the β-lactone **11** in the desired manner and gave rise to the corresponding thioacetate-substituted carboxylic acid intermediate (Scheme 3). Cleavage of acetate group from this intermediate was then accomplished by the action of hydrazine hydrate, and this was followed by cyclization with EDCI and C_6F_5OH in CH_2Cl_2, to cleanly afford the β-thiolactone **15** in 65% yield over three steps (Scheme 3). With this strategy for the preparation of β-thiolactone **15** in hand, the use of β-lactone **11** as a synthon for the β-lactam analog was considered. To this end, ring opening of **11** was achieved with NaN_3 in DMF at 60 °C and furnished the desired azido carboxylic acid as



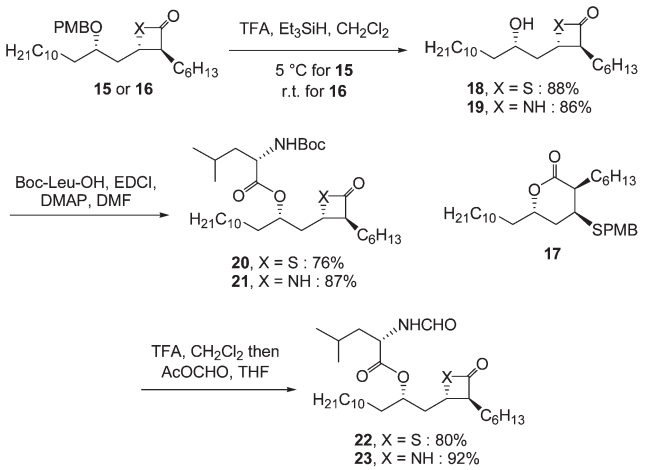
Scheme 1 Synthesis of methyl ester **9**.



Scheme 3 Synthesis of the β-thiolactone and β-lactam precursors.



Scheme 2 Synthesis of the β-lactone analog **14** of tetrahydrolipstatin.



Scheme 4 Completion of the synthesis of the β-thiolactone and β-lactam analogs of tetrahydrolipstatin.

demonstrated by mass spectrometric analysis using ESI in the negative ion mode (Scheme 3).[15c,16] In what might be dubbed an intramolecular Staudinger–Vilarrasa β-lactamization,[17] this intermediate was then directly engaged in a one-pot procedure consisting first of azide reduction and subsequent cyclization to the β-lactam **16** through the aegis of triphenylphosphine and 2,2′-dipyridyl disulfide in CH_3CN–THF at 60 °C in 79% over two steps. Related lactam-forming reactions from β-amino acids using Ohno's protocol have previously been reported by several groups (Scheme 3).[18]

Removal of the PMB group from **15** with TFA in CH_2Cl_2 proved to be more difficult than expected due the concomitant formation of a six-membered ring δ-lactone **17**, which was accompanied by the migration of the PMB group to the sulfur atom (Scheme 4). To avoid this issue, the reaction was conducted at 5 °C and, critically, in the presence of Et_3SiH as a scavenger when **18** was obtained in 88% yield. In the case of β-lactam **16**, acidic cleavage of the PMB ether gave the secondary alcohol **19** in 86% yield. Subsequent derivatization of **18** and **19**, by esterification of the secondary alcohol function was accomplished as described above for the lactone, and gave the corresponding *N*-protected amino ester **20** and **21** in 76 and 87% yield, respectively (Scheme 4). Finally, removal of the Boc group with TFA, followed by formylation with formic acetic anhydride led to the β-thiolactone **22** and β-lactam **23** in 80% and 92% yields, respectively (Scheme 4).

Biology

With compounds **14**, **22** and **23** in hand, their capacity to inhibit porcine pancreatic lipase was first assessed with the aid of the 6′-methylresorufin ester of 1,2-di-*O*-lauryl-*rac*-glycero-3-glutaric acid as substrate,[19] and Orlistat as control.[19] Orlistat proved to be the best inhibitor of porcine pancreatic lipase with an IC_{50} of 7.5 nM,[20] but its *cis*-isomer **14**, which retained the β-lactone functionality, showed only a two-fold loss of activity (IC_{50} = 15 nM) (Table 1, entries 1 and 2).[14] This observation potentially opens the way to more extensive studies of Orlistat analogs bearing a *cis*- rather than a *trans*-disubstituted β-lactone as potential inhibitors of fatty acid synthase.[21] In contrast to Orlistat and **14**, neither the β-thiolactone **22** nor the β-lactam **23** inhibited the lipase to any significant extent (Table 1, entries 3 and 4), thereby revealing the importance of the reactivity of the β-lactone for lipase inhibition. In addition, none of the synthetic intermediates (**11–13**, **15–16**, **18–21**) prepared, revealed any inhibition of the pancreatic lipase.[22]

Table 1 IC_{50} values of Orlistat, β-lactone **14**, β-thiolactone **22**, and β-lactam **23** for the inhibition of porcine pancreatic lipase

Entry	Compound	IC_{50}[a] (nM)
1	Orlistat	7.5
2	**14**	15.0
3	**22**	>100
4	**23**	>100

[a] Average of duplicate measures.

Table 2 IC_{50} (μM) values of Orlistat, β-lactone **14**, β-thiolactone **22**, and β-lactam **23** for the inhibition of KB, HCT116, PC3 and MDA231 human cancer cell line proliferation *in vitro*

Entry	Compound	KB[a]	PC3[a]	HCT116[a]	MDA231[a]
1	Orlistat	50.6	57.2	32.0	13.0
2	**14**	29.7	43.2	54.6	28.2
3	**22**	54.4	36.9	15.4	40.7
4	**23**	13.6	10.2	11.5	12.4

[a] Average of duplicate measures.

With respect to the inhibition of cancer cell proliferation, IC_{50} values were determined for compounds **14**, **22**, **23** and Orlistat against a range of four human cancer cell lines (KB nasopharynx human carcinoma, PC3 prostate carcinoma, MDA231 human breast adenocarcinoma, and HCT116 colorectal carcinoma). For both the KB and PC3 cell lines β-lactam **23** (Table 2, entry 4) was found to be between two to four-fold more cytotoxic in the 10 μM range than Orlistat, **14** and **22** (Table 2). Concerning the HCT116 cell line, β-thiolactone **22** and β-lactam **23** (Table 2, entries 3 and 4) exhibited similar cytotoxicity and were more potent than the β-lactone analogs (Table 2, entries 1 and 2). This observation may be linked to the inhibition of reactive cysteine residues, that might provide a different mechanism of action towards β-thiolactone or β-lactam.[23]

Finally, Orlistat and β-lactam **23** showed similar inhibitory activity against the MDA231 cancer cell line (Table 2, entries 1 and 4) and showed a similar cytotoxic profile, while the β-thiolactone **22** was the least cytotoxic of the four compounds surveyed (Table 2, entry 3). Overall, in this brief screen of cytotoxicity the β-lactam congener **23** of Orlistat was discovered to be generally more cytotoxic (IC_{50} = 10.2–13.6 μM) than either Orlistat itself, the *cis*-analog of Orlistat **14** and the β-thiolactone analog **22**, perhaps suggesting a parallel with earlier observations on the inhibition of the thioesterase domain of fatty acid synthase by β-lactam congeners of Orlistat.[6,8d]

Conclusions

To conclude, an efficient synthesis of the β-thiolactone and β-lactam analogs of tetrahydrolipstatin has been developed that

takes advantage of the S_N2 mode of ring opening of β-lactones by soft nucleophiles. A lipase inhibition assay revealed that the stereochemistry of the β-lactone moiety at the β-position has only a minor effect on pancreatic lipase activity of tetrahydrolipstatin, but that neither the β-thiolactone nor the β-lactam analogs show significant lipase inhibitory activity. Among the four compounds screened the β-lactam **23** was uniformly the most cytotoxic against four human cancer cell lines.

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