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β-Lactam congeners of orlistat as inhibitors of fatty acid synthase

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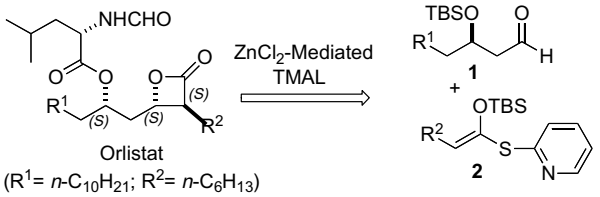
Abstract—β-Lactam derivatives of orlistat were prepared and their inhibitory activities toward the thioesterase domain of fatty acid synthase (FAS-TE) were evaluated using a recombinant form of the enzyme. While in general these derivatives showed lower potency compared to β-lactones, a reasonably potent, lead compound (−)-**9** (IC_{50} = 8.6 μM) was discovered that suggests that this class of compounds should be evaluated further.

As the sole complex machine responsible for cellular synthesis of palmitate, human fatty acid synthase[1] (FAS) has recently attracted attention as a drug target in oncology for its well-documented up-regulation in cancer cells,[2] including most carcinomas such as those of the breast,[3] prostate,[4] and ovaries.[5] The pharmacological inhibition of FAS has also been shown to enhance the effectiveness of current antineoplastic therapies such as paclitaxel[6] and trastuzumab.[7] Despite these promising results, a suitable FAS inhibitor for clinical use has not emerged.[8] Recently, the first FDA-approved over-the-counter weight-loss medication, tetrahydrolipstatin (orlistat), a pancreatic lipase inhibitor and a reduced form of the natural product lipstatin, was discovered to also be a potent inhibitor of the thioesterase domain of fatty acid synthase (FAS-TE).[9,10] This finding led to a renaissance in the synthesis of orlistat and congeners,[11,12] as it is an important lead compound for further structure–activity relationship (SAR) studies to identify FAS inhibitors as potential therapeutics.

In previous synthetics studies toward FAS inhibitors, a variety of orlistat congeners were prepared using our $ZnCl_2$-mediated tandem Mukaiyama aldol-lactonization (TMAL) process as a key step (Scheme 1).[12] In addition, the structural requirements for inhibition of FAS-TE and factors for improving solubility, potency, and selectivity were delineated.[13]

As part of ongoing efforts to develop new β-lactone-based transformations, we previously reported a mild, efficient two-step, one-pot method for conversion of β-lactones to β-lactams based on the method of Miller.[14] In conjunction with our ongoing SAR studies of orlistat targeting FAS, we envisioned that conversion of the β-lactone core to a β-lactam,[15] which of course has a long history as an effective pharmacophore,[16] might impart greater stability and lead to a new class of FAS inhibitors. Herein, we report the first synthesis of β-lactam derivatives of orlistat that exhibit inhibition of the recombinant form of FAS-TE.

Our strategy for the synthesis of orlistat β-lactams utilized our stereocomplementary $SnCl_4$-promoted TMAL process.[17] This provides the required *cis*-β-lactones since



Scheme 1. Key disconnection for the synthesis of orlistat and congeners employing the TMAL process.

Keywords: β-Lactone; Thioesterase domain; Anticancer; Tandem Mukaiayama aldol-lactonization.
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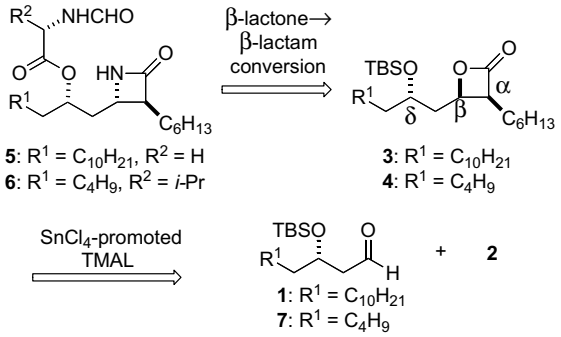
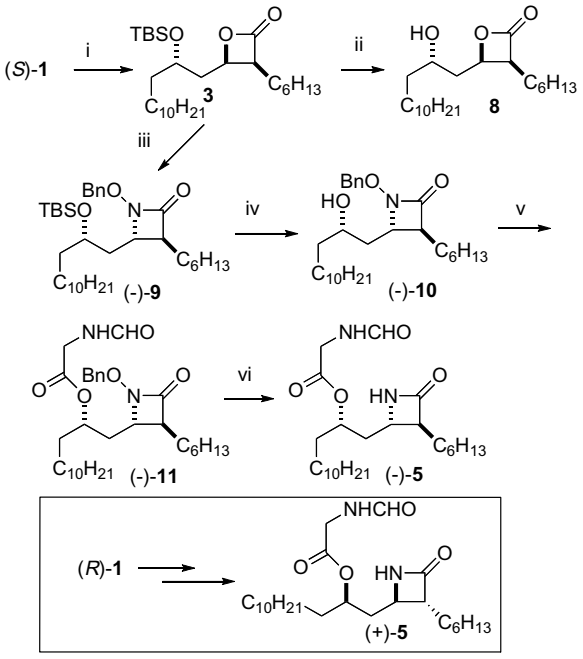
the conversion to β-lactams leads to inversion at the β-stereocenter of the β-lactone (Scheme 2). We initially targeted the synthesis of β-lactam (−)-**5**, which directly mimics the stereochemistry and substitution pattern of orlistat. The synthesis commenced with the $SnCl_4$-promoted TMAL reaction with known aldehyde (*S*)-**1**[12] and thiopyridyl ketene acetal **2**[18] to efficiently deliver the desired β-lactone **3** as a 15:1 mixture of *anti*/*syn* diastereomers (Scheme 3). Complete *cis*-selectivity of the β-lactone core was verified by analysis of coupling constants ($J_{H\alpha,H\beta} = 6.5$ Hz) as previously described,[18] whereas the relative stereochemistry with respect to the δ-center was confirmed by comparison with the known alcohol **8**[19] after desilylation. The sterochemical outcome is consistent with Evans' model for additions to β-silyloxy aldehydes[20] as previously observed for similar TMAL reactions.[18b] Next, application of the one-pot conversion of β-lactones to β-lactams generated the *N*-benzyloxy-β-lactam (−)-**9**.[14] Subsequent desilylation afforded the δ-hydroxy-β-lactam (−)-**10**, which following acylation with *N*-formyl glycine generated ester (−)-**11**. Finally, employing SmI_2-promoted reductive N–O bond cleavage of the benyloxy-β-lactam provided the orlistat-type β-lactam (−)-**5**.[14] In addition, the enantiomeric series was also prepared for comparison providing β-lactam (+)-**5** in comparable yields (not shown)[21] starting from aldehyde (*R*)-**1**.

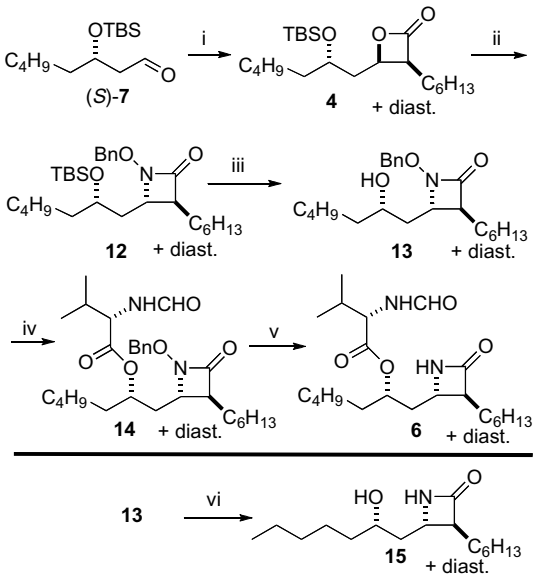
Based on our previous SAR studies with orlistat-derivatives,[13] compounds containing shorter side chains at the β-position often exhibited superior inhibitory activity. In efforts to determine if this finding translated to β-lactam inhibitors, we targeted the synthesis of β-lactam **6** using a similar strategy (Scheme 4). $SnCl_4$-promoted TMAL reaction of aldehyde (*S*)-**7** and thiopyridyl ketene acetal **2** afforded the *cis*-β-lactone **4** along with a minor diastereomer (not shown, dr 6:1). As with aldehyde (*S*)-**7**, both diastereomers possessed *cis*-β-lactones with differing relative stereochemistry at the δ-silyloxy stereocenter. Instead of doing a tedious separation, the mixture of diastereomers was carried forward for preliminary studies by conversion of the mixture to β-lactam **12** and diastereomer (not shown) via the single-pot protocol.[14] Subsequent desilylation afforded alcohol **13** and following acylation with *N*-formyl-L-valine this provided ester **14**. Reductive N–O cleavage using SmI_2 completed the synthesis of β-lactam **6** (dr 6:1). In addition,



Scheme 2. Strategy for the synthesis of orlistat-type β-lactam derivatives.



Scheme 3. Synthesis of β-lactams (−) and (+)-**5**. Reagents and conditions: (i) **2**, $SnCl_4$, −78 °C, CH_2Cl_2, 1.5 h, 60%, dr 15:1; (ii) HF, CH_3CN, 0 °C, 78%; (iii) $BnONH_2$; DIAD, Ph_3P, 56% over two steps; (iv) HF, CH_3CN, 0 °C, 75%; (v) DMAP, EDCI, *N*-formylglycine, 80%; (vi) SmI_2, THF/H_2O, 0 °C, 82%. DIAD: diisopropyl azodicarboxylate; EDCI: 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide); DMAP: 4-dimethylaminopyridine.



Scheme 4. Synthesis of β-lactam derivatives **6** and **15**. Reagents and conditions: (i) **2**, $SnCl_4$, −78 °C, CH_2Cl_2, 49%, dr 6:1; (ii) $BnONH_2$; DIAD, Ph_3P, 44% over two steps; (iii) HF, CH_3CN, 68%; (iv) DMAP, EDCI, *N*-formylvaline, 80%; (v) SmI_2, THF/H_2O, 66%; (vi) SmI_2, THF/H_2O, 35%.

β-lactam **15** was also obtained (dr 6:1) by reductive removal of the benzyloxy group from β-lactam **13** using SmI_2.

The inhibitory activities of the synthesized β-lactam derivatives were determined in a biochemical fluorogenic

Table 1. Inhibitory properties of β-lactam derivatives of orlistat to recombinant FAS-TE

Compound	Complement inhibition IC_{50} (μM)	Compound	Complement inhibition IC_{50} (μM)
(−)-**5**	No inhibition	(+)-**10**	61.2
(+)-**5**	No inhibition	(−)-**11**	68.9
6[a]	—[b]	(+)-**11**	39.2
(−)-**9**	8.6	**13**[a]	50.5
(+)-**9**	97.4	**14**[a]	58.2
(−)-**10**	86.8	**15**[a]	67.6

[a] dr = 6:1.
[b] Inconclusive (see text).

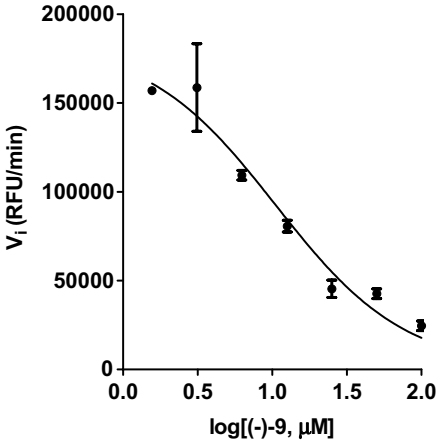


Figure 1. A representative dose–response curve illustrating the inhibition of FAS-TE by β-lactam (−)-**9** in the fluorogenic assay.

assay using recombinant FAS-TE applying a protocol previously described (Table 1).[12] Not surprisingly, in general the β-lactam derivatives showed reduced inhibitory activity compared to orlistat. Most of the β-lactam derivatives retaining the *N*-benzyloxy group including β-lactams **10**–**11** and **13**–**14** exhibited only modest activity with IC_{50}'s ranging from ~30 to 100 μM. A clear and surprising exception was β-lactam (−)-**9**, which retains the *N*-benzyloxy group, a silyl ether and also shares the same absolute stereochemistry with orlistat. This derivative showed the highest activity (IC_{50} = 8.6 μm) among all the β-lactams studied (Fig. 1). It is also noteworthy that a shorter chain at the β-position of the β-lactam, relative to the β-lactone of orlistat, did not show obvious improvement in inhibition activity as in the case of β-lactone inhibitors. Surprisingly, neither β-lactam (+)-**5** nor its enantiomer, which is structurally most similar to orlistat, showed inhibitory activity, however, this may be due to reasons of solubility as is the case with orlistat itself. Unfortunately, inhibitory studies of β-lactam **6** were inconclusive as it failed to exhibit a regular dose–response in the fluorogenic assay.

In summary, the first β-lactam derivatives of orlistat were synthesized and their inhibitory activities toward FAS-TE were evaluated against a recombinant form of FAS-TE. While in general these derivatives showed lower potency compared to β-lactones, one β-lactam (−)-**9** possessing a *N*-benzyloxy-β-lactam was discovered to have a good potency (IC_{50} = 8.6 μM) providing evidence that this class of compounds should be evaluated further as potential inhibitors of FAS.

Acknowledgments

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2008.02.043.

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