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Discovery of halogenated eurypamide B analogues as inhibitors of lipid droplet accumulation in macrophages

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Abstract—Halogenated cyclic isodityrosine–tripeptides were synthesized as analogues of a marine natural product, eurypamide B. Although the original eurypamides showed no inhibitory activity, the new analogues were found to inhibit lipid droplet accumulation in macrophages with a low micromolar IC_{50} value.

1. Introduction

Marine natural product eurypamides[1] represent a class of isodityrosine-derived 17-membered cyclic peptides including aminopeptidase B inhibitor OF4949I-IV,[2] angiotensin-I converting enzyme inhibitor K-13,[3] and renieramide[4] recently reported. The common scaffold for OF4949 and K-13 has been taken as a new lead for protease inhibitors (Fig. 1).[5] More recently, the design and synthesis of macrocyclic structures having a diaryl ether moiety or the related cyclic compounds (Fig. 1) were also reported for drug candidates, such as protease inhibitors and farnesyltransferase inhibitors.[6] We have recently completed a total synthesis of eurypamides A, B, and D using thallium(III) trinitrate (TTN)-oxidative coupling of an *o*, *o*′-dihalogenated tyrosine derivative for cyclization.[7] Although isodityrosine-derived 17-membered cyclic peptides showed a variety of biological activities, interestingly, eurypamides showed no antimicrobial, anti-inflammatory, or cytotoxic activities.[1] Among the molecular modeling study of eurypamide derivatives including synthetic intermediates, flexibility of the diaryl ether linkage and peptide chain in the halogenated analogue **1** were different from that of halogen-free structure **2** (Fig. 2).[8] Based on these findings, several halogenated eurypamide B derivatives were synthesized to examine the biological activity.

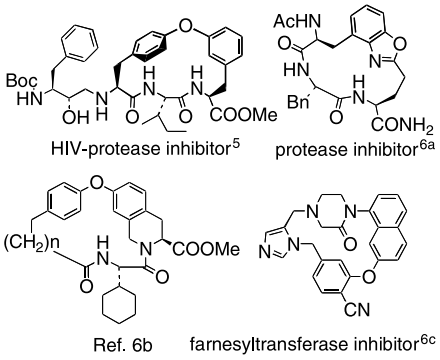

Figure 1. Reported cyclic compounds.

2. Synthesis of *o*,*o*′-halogenated eurypamide analogues

Halogenated eurypamide B derivatives (**5a–c**, **6a–c**) were prepared in good yield by following our total synthetic route[7] (Scheme 1). Coupling reaction of dibrominated tyrosine derivative **3**[7] with commercially available *N*-Boc-threonine or *N*-Boc-*O*-benzyl-threonine, under the conditions using BOP, provided the dipeptides. Subsequent deprotection of the *N*-Boc group and further condensation with a diiodinated tyrosine derivative,[9] readily

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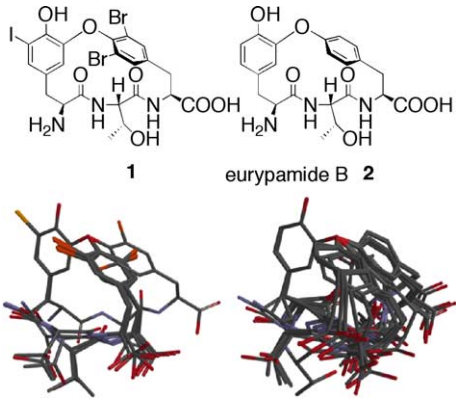

Figure 2. Model structures for conformational study. Lower left: overlapped minimum conformers of compound **1**. Lower right: overlapped minimum conformers of compound **2**.

prepared from tyrosine, yielded the linear tripeptides **4**. Key ring closure of **4–5** via intramolecular phenolic oxidation with TTN smoothly proceeded at 0 °C with yield of 89% at best. Deprotection of the *N*-Boc group of **5b** and **5c** gave **6a** and **6b**, respectively, and hydrolysis of **5c** gave **6c**. The NMR data of **5** and **6** are shown in Supplement data.

3. Biological activities of eurypamides and their analogues

The obtained analogues **5a**, **5b**, **6a–6c**, and parent eurypamides A (3″*R*,4″*S*), A′ (3″*S*,4″*R*), B, and D[7] were screened for their biological effects. Among biological assessments, a cell-based assay of lipid droplet synthesis using mouse peritoneal macrophages as a model of macrophage-derived foam-cell formation[10] showed positive results.[11] As shown in Table 1, compounds **5a** and **5b** inhibited accumulation of lipid droplets with IC_{50} values of 3.1 and 3.2 μM, respectively: these compounds reduce the size and number of the cytosolic lipid droplets in macrophages. Moreover, no morphological changes or cytotoxicity were observed at concentrations up to 10 μM. These indicated that **5a** and **5b** possessed no effect against cell viability, but specific inhibitory activity against accumulation of lipid droplets. The other three analogues **6a–c** and parent eurypamides A–D showed no inhibitory activity and cytotoxicity up to 12 μM. From these results of **5a**, **5b**, and **6a–c**, more hydrophobic characters, for example, carrying protecting groups and halogen atoms than that of parent eurypamide, are required to exhibit the biological activity for this assay.

Table 1. Inhibition of lipid droplet accumulation in macrophages

Compounds	Inhibitory activity (IC_{50}, μM)[a]
Eurypamide A[1]	>18.8
Eurypamide A′[7]	>18.8
Eurypamide B[7]	>22.6
Eurypamide D[7]	>22.6
5a	3.1
5b	3.2
5c	nt
6a	>12.1
6b	>13.5
6c	>12.1

[a] Values are the mean of three experiments (nt = not tested).

In conclusion, we have developed low cytotoxic and biologically active eurypamide analogues by simple modification. Further investigation in these areas including additional analogue synthesis with structure–activity relationship study, and other biological assays will be reported in due course.

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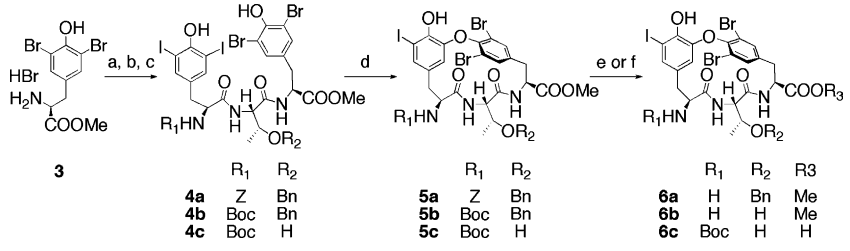
8. Calculations were done using SPRTAN'02 for Macintosh (Wavefunction Inc., CA, USA) on a Power Mac G5



Scheme 1. Reagents and conditions: (a) *N*-Boc-*O*-benzyl-Thr, BOP, Et_3N/MeCN 94%; (b) TFA/CH_2Cl_2; (c) *N*-*Z*-*o*,*o*′-diiodo-Tyr, BOP, Et_3N/MeCN **4a** 99% in two steps, *N*-Boc-*o*,*o*′-diiodo-Tyr, BOP, Et_3N/MeCN–dioxane **4b** 94% in two steps, **4c**;[7] (d) TTN, MeOH–THF (1:4), 0 °C, 40–60 min, **5a** 89%, **5b** 69%, **5c**;[7] (e) TFA/CH_2Cl_2, **5b** to **6a** 78%, **5c** to **6b** quant.; (f) 1 N-NaOH/MeOH, **5c** to **6c** 95%.

(Apple Computer Inc., CA, USA) computer. Halogenated **1** and halogen-free **2** model structures of the tripeptide bonds were generated as β-sheet conformation, then the tyrosine moiety was cyclized to construct an isodityrosine bond, and the models were pre-minimized. The stable local minimum conformers were distributed based on molecular mechanics calculations with MMFF94 force field. The first 20 stable minimum conformers were aligned at the residue of the DOPA moiety as shown in Figure 1. Decrease of conformational heterogeneity of the diaryl ether moiety and peptide chain linkage was observed in **1** compared to **2**.

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11. None of the tested compounds showed antimicrobial activity. Antimicrobial activity was tested against *Bacillus subtilis*, *Escherichia coli*, *Candida albicans*, *Staphylococcus aureus*, *Micrococcus luteus*, *Bacteroides fragilis*, *Acholeplasma laidlawii*, *Pyricularia oryzae*, *Aspergillus niger*, *Mucor racemosus*, *Saccharomyces cerevisiae*, and *Pseudomonas aeruginosa* at 10 μg/6 mm diameter paper disks.