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Synthesis and Biological Evaluation of Aspergillide A/Neopeltolide Chimeras

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In this study, stereoisomeric aspergillide A/neopeltolide chimeras were synthesized in a parallel manner, and their antiproliferative activity was evaluated to demonstrate the potential utility of the 14-membered macrolactone structure embedded with a tetrahydropyran substructure as a template for developing natural product-like antiproliferative agents.

Because of their unique molecular architecture and potent biological activities, there is an emerging interest in marine natural products as a source of novel therapeutics.[1] A number of 14-membered macrolide natural products containing a tetrahydropyran ring have been isolated from various marine organisms. Examples of such secondary metabolites include aspergillides A and B (**1** and **2**, respectively),[2] auriside A (**3**),[3] lyngbyaloside B (**4**),[4] and neopeltolide (**5**),[5] as shown in Figure 1. These naturally occurring macrolides have attracted considerable attention from the organic and medicinal chemistry communities owing to their synthetically intriguing structure and antiproliferative and/or cytotoxic activity;[6–9] e.g., **1** and **2** showed cytotoxicity against mouse lymphocytic leukemia cells (L1210) with LD_{50} values of 2.1 and 71 μg mL^{-1}, respectively.[2a] The mechanisms of action of these natural products have not been fully elucidated so far.[10] However, we envision that the tetrahydropyran-containing 14-membered macrolactone structure, commonly found in this class of natural products, might be a potentially useful template for developing natural product-like antiproliferative agents. In this context, structural hybridization of natural products would be an attractive means of generating natural product-like compounds.[11] Herein, we report the synthesis and biological evaluation of aspergillide A/neopeltolide stereoisomeric chimeras.

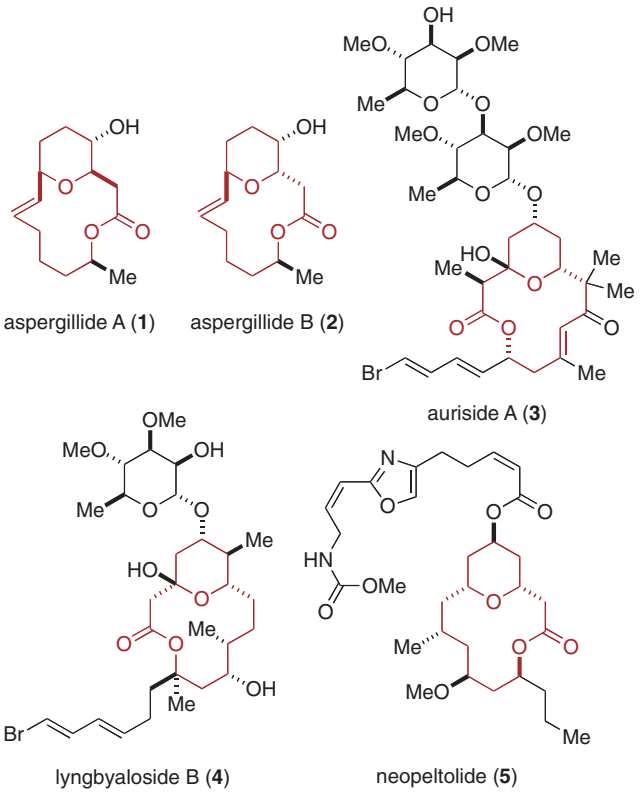


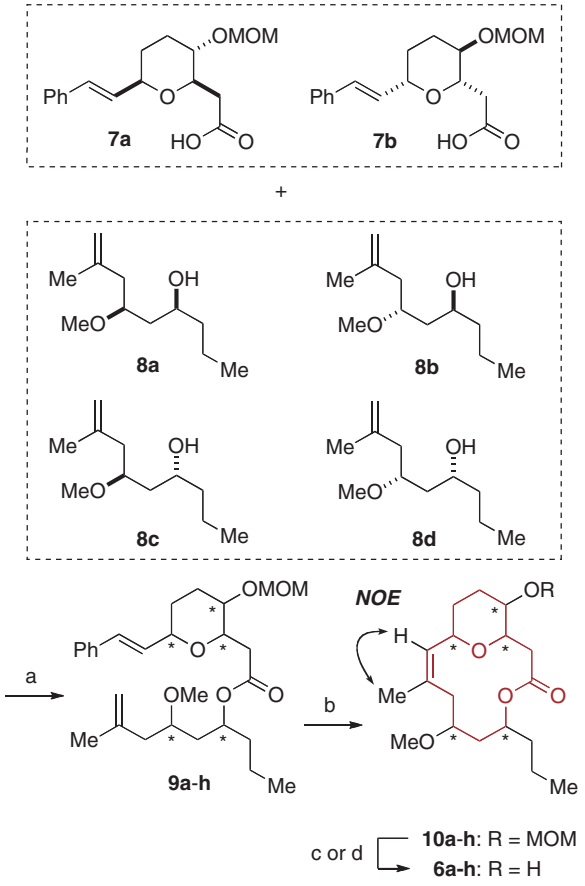
Figure 1. Structures of aspergillides A and B (**1** and **2**, respectively), auriside A (**3**), lyngbyaloside B (**4**), and neopeltolide (**5**).

We designed the aspergillide A/neopeltolide chimera **6a** and its stereoisomers **6b**–**6h** as the target of this study because of the structural simplicity of the macrolactone backbone of **1** and **5** (Scheme 1 and Table 1). Thus, structural hybridization of the upper-half domain of **1** and the lower-half domain of **5** would generate stereoisomeric chimeras **6a**–**6h**. We have previously reported the total syntheses of **1**,[12] **2**,[12] and **5**,[13] and learned that the 14-membered macrolactone backbone structure of **5** could be efficiently constructed via an esterification–ring-closing metathesis (RCM)[14] sequence. Accordingly, we planned to synthesize **6a**–**6h** from the carboxylic acids **7a**,[12] **7b**[15] and the alcohols **8a**–**8d**[13a] in a parallel manner.

The synthesis of **6a**–**6h** commenced with esterification of **7a** and **7b** with **8a**–**8d** under Yamaguchi conditions[17] to give the esters **9a**–**9h** in excellent yields (Scheme 1). RCM of **9a**–**9h** proceeded efficiently under the influence of the second-generation Grubbs catalyst ($(H_2IMes)(PCy_3)Ru(=CHPh)Cl_2$, H_2IMes: 1,3-dimesityl-4,5-dihydroimidazol-2-ylidene)[18] and 1,4-benzoquinone[19] in toluene at 100 °C to afford the macrolactone **10a**–**10h** in moderate to excellent yields. The stereochemistry of the newly generated double bond was determined to be *Z* by an NOE experiment. Finally, the MOM group of **10a**–**10h** was removed under acidic conditions (HCl, MeOH or $LiBF_4$, aqueous CH_3CN[20]) to furnish **6a**–**6h**.[21] The stereochemistry of **6a**–**6h** is summarized in Table 1.

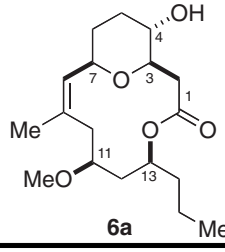
The antiproliferative activity of **6a**–**6h** was evaluated against mouse leukemia P388 cells by the WST-8 assay,[22] and the results are summarized in Table 2. A pair of enantiomers **6b** and **6g** showed significant activity among **6a**–**6h**. The IC_{50} values for **6b** and **6g** were determined to be 23 and 28 μg mL^{-1}, respectively. These results indicate the importance of the stereoisomerism of the macrocyclic backbone structure. Meanwhile, compounds **6a**–**6h** were found to be inactive against HL-60 human promyelocytic leukemia cells and HT1080 human fibrosarcoma cells at 75 μg mL^{-1}.[23,24]

Notably, reflecting the backbone chirality, each of the stereoisomers **6a**–**6d** showed distinct ^{1}H and ^{13}C NMR spectra, indicating that each stereoisomer has its unique conformational profile.[25] The solution structures of **6a**–**6d**, deduced on the basis of NMR-based conformational analysis and molecular model-



Scheme 1. Reagents and conditions: (a) 2,4,6-$Cl_3C_6H_2COCl$, Et_3N, THF, 0 °C to rt; then DMAP, toluene, rt, 92%–quant; (b) $(H_2IMes)(PCy_3)Ru(=CHPh)Cl_2$, 1,4-benzoquinone, toluene, 100 °C, 56–93%; (c) HCl, MeOH, rt, 98% for **6d**, 92% for **6e**; (d) $LiBF_4$, CH_3CN(aq), 70 °C, 79%–quant for **6a**–**6c** and **6f**–**6h**.

Table 1. Stereochemistry of **6a**–**6h**



6a

Compound	Stereochemistry
6a	3*R*,4*S*,7*R*,11*S*,13*S*
6b	3*R*,4*S*,7*R*,11*R*,13*S*
6c	3*R*,4*S*,7*R*,11*S*,13*R*
6d	3*R*,4*S*,7*R*,11*R*,13*R*
6e (*ent*-**6d**)	3*S*,4*R*,7*S*,11*S*,13*S*
6f (*ent*-**6c**)	3*S*,4*R*,7*S*,11*R*,13*S*
6g (*ent*-**6b**)	3*S*,4*R*,7*S*,11*S*,13*R*
6h (*ent*-**6a**)	3*S*,4*R*,7*S*,11*R*,13*R*

Table 2. Antiproliferative activity of **6a**–**6h**[a]

Compound	IC_{50}/μg mL^{-1}	Compound	IC_{50}/μg mL^{-1}
6a	39	**6e** (*ent*-**6d**)	75
6b	23	**6f** (*ent*-**6c**)	>75 (43%)[b]
6c	>75 (46%)[b]	**6g** (*ent*-**6b**)	28
6d	43	**6h** (*ent*-**6a**)	58

[a]Cell viability was determined after 48-h exposure of P388 cells to compounds using the WST-8 assay ($n = 3$). For details of the assay procedure, see ref 13c. [b]Percentage inhibition value at 75 μg mL^{-1}.

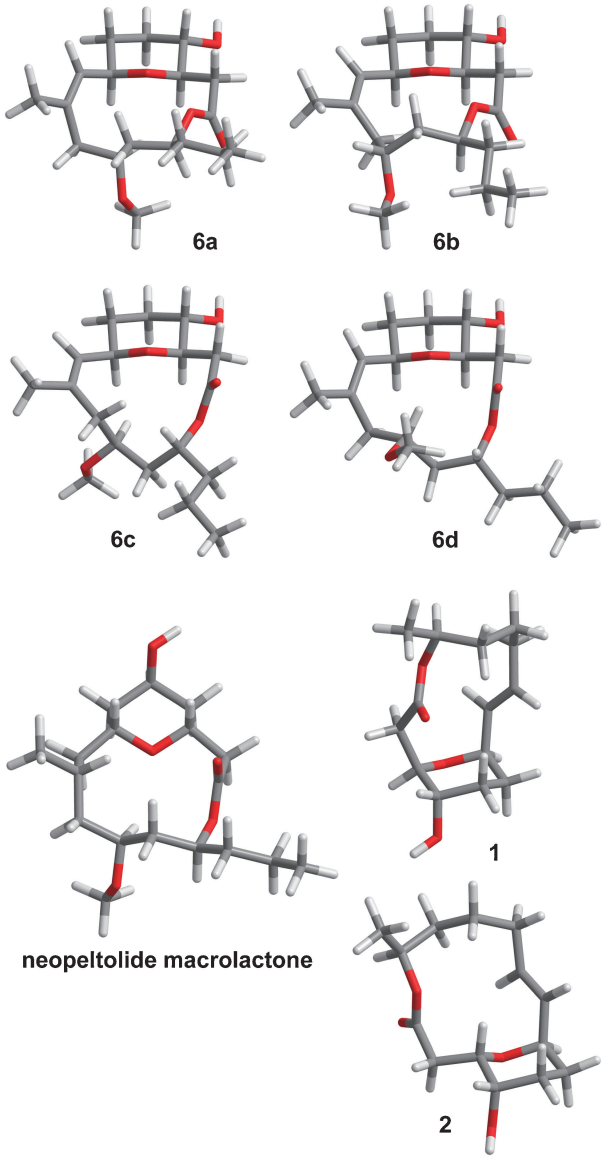


Figure 2. Energy-minimized ground state conformers of **1**, **2**, **6a**–**6d**, and neopeltolide macrolactone, generated by conformational searches using MMFFs94 followed by geometry optimization using ab initio calculations at HF/6-31G* level of theory.

ing, also supported the distinct conformational properties of these stereoisomers (Figure 2).[26] However, it is likely that in solution, **6a**–**6h** actually exist as mixtures of rapidly interconverting conformers because of their backbone flexibility. Because the time-averaged NMR-deduced structures of **6a**–**6h** do not necessarily represent their "bioactive" conformers, they

may not be helpful for rigorous analysis of the structure–activity relationship. Nevertheless, it can be roughly estimated that the molecular shape of **6a**–**6h** resembles that of the macrolactone domain of **5** ("flat"), but is considerably different from that of **1** and its stereoisomer **2** ("L-shaped").[12a,27] Importantly, **6a**–**6h** do not have the oxazole-containing side chain of **5**, which is indispensable for the potent antiproliferative activity of **5**.[13c]

In conclusion, we have synthesized stereoisomeric aspergillide A/neopeltolide chimeras **6a**–**6h** in a parallel manner by exploiting an esterification–RCM sequence, and evaluated their antiproliferative activity against mouse leukemia P388 cells. Among the eight stereoisomers synthesized, compounds **6b** and **6g** inhibited the proliferation of P388 cells with moderate potencies. Our conformational analysis indicated that the overall molecular shape of **6a**–**6h** is distinct from that of the parental natural products, i.e., **1** and **5**. Taken together, this study demonstrates the versatility of the tetrahydropyran-containing 14-membered macrolactone as a template for the development of natural product-like antiproliferative agents. Further studies along this line are currently ongoing and will be reported shortly.

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25 Copies of ^1H and ^{13}C NMR spectra of **6a**–**6h** are provided in the Supporting Information.[16]

26 For details on the NMR-based conformational analysis of **6a**–**6d**, see the Supporting Information.[16]

27 The X-ray crystallographic structures of *m*-bromobenzoates of **1** and **2** have been reported. See ref 2b.

