Studies toward the Synthesis of Biologically Important, Natural and Artificial Small Molecules

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Studies toward the Synthesis of Biologically Important, Natural and Artificial Small Molecules

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Summary

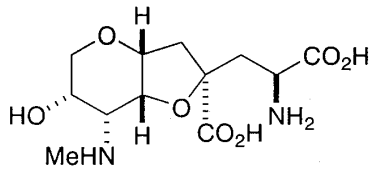
Shown in this article are our current research topics based on the organic syntheses of biologically important molecules. The synthetic studies on marine natural products, dysiherbaines, azaspiracids, and goniodomin are described. Another research aiming to discover biologically functional small molecules from artificial synthetic libraries is also described.

Key words: dysiherbaine, azaspiracid, goniodomin A, diversity-oriented organic synthesis, small molecule microarray

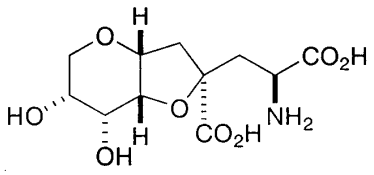
Synthetic organic chemistry is now recognized to play a key role in the research field of life sciences, since biologically functional organic small molecules has been realized useful for understanding the events caused by biologically important biomolecules such as proteins, RNA, and even DNA by modulating their functions. Chemical studies on these organic molecules found from natural sources and artificial libraries are underway in our laboratories as follows.

1. Dysiherbaine and Neodysiherbaine

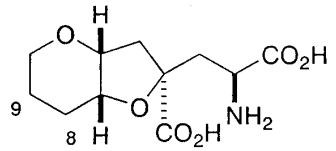
Dysiherbaine **(1)** (Sakai *et al.*, 1997) and neodysiherbaine A **(2)** (Sakai *et al.*, 2001) are excitatory amino acids isolated by Sakai *et al.* (Kitasato University, Japan) from Micronesian marine sponge *Dysidea herbacea*. These amino acids show potent and selective affinity to non-NMDA-type glutamate receptors (GluRs), especially GluR5 and GluR6 kainate receptor subunits (Sanders *et al.*, 2005). Toward the development of more subunit-selective ligands for GluRs, we initiated structure-activity relationship (SAR) studies of dysiherbaines. We first synthesized natural-type dysiherbaine **(1)** (Sasaki *et al.*, 2000) and neodysiherbaine A **(2)** (Sakai *et al.*, 2001) successfully. During the course of these studies,



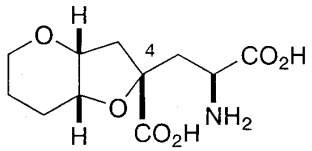
dysiherbaine (**1**)



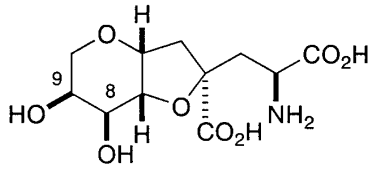
neodysiherbaine A (**2**)



analogue **3**



analogue **4**



8,9-*epi*-neodysiherbaine A (**5**)

FIG. 1.

simple analogues **3** and **4** that lack functional groups at the C_8 and C_9 positions were synthesized in 1999, and **3** was found to induce convulsant behaviors in mice upon intracerebroventricular injections (Sasaki *et al.*, 1999). Another C_4-*epi*-analogue **4** was inactive to mice, suggesting that the stereochemistry at the C_4 quaternary carbon is important for this activity. More recently, the third synthetic analogue, 8, 9-*epi*-neodysiherbaine A **(5)** was synthesized by a newly developed, efficient synthetic pathway in 2005, and **5** was determined to be inactive from the preliminary biological studies with mice (Shoji *et al.*, 2005). Encouraged by these findings, we are now working on the concise, diverted total syntheses of dysiherbaine analogues for more detailed SAR studies.

Another chemical approach to analyze and elucidate the mechanism for the biological function of GluRs using dysiherbaines is also underway in our laboratory. The basic strategy toward this is the use of functional, chemical probes of dysiherbaines, e.g. fluorescent or photoaffinity probes in combination with affinity probes such as biotin. Since the chemical probes of dysiherbaines are not easily prepared from natural specimens, we have initiated the program directed toward the chemical synthesis of these functional probes. We are currently working on the synthesis of structurally modified dysiherbaine analogues to explore the possible positions for structural modification to introduce the labeling fun-

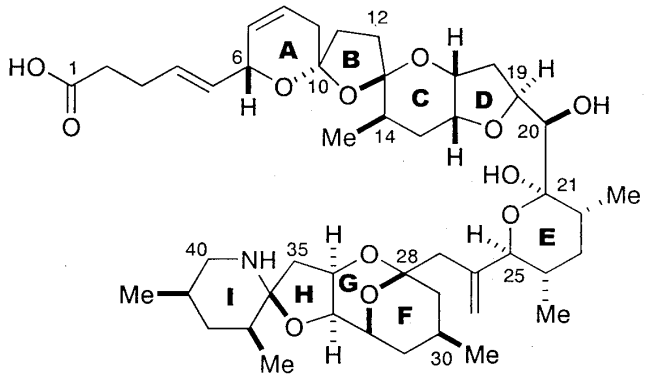
ctionalities.

2. Azaspiracids

Azaspiracids are a family of polyether natural products first reported in 1998 by a group of Satake and Yasumoto of Tohoku University as the causative agents for a new type of shellfish poisoning occurred in Europe since 1995 (Satake *et al.*, 1998 ; Ofuji *et al.*, 1999 ; Ofuji *et al.*, 2001). The toxic syndrome, which features nausea, vomiting, severe diarrhea, and stomach cramps, was named azaspiracid poisoning. Although the toxins were first recognized in Irish mussels in November of 1995, they are now detected widely from the coastal region of western Ireland to Spain at certain times of the year (Magdalena *et al.*, 2003). The characteristic feature of the structure for azaspiracid-1 **(6)**, finally established by a total synthesis by Nicolaou *et al.* at Scripps Research Institute (U.S.A.) in 2004, includes a bisspiro assembly, an unusual azaspiro ring structure fused with a 2, 9-dioxabicyclo[3.3.1]nonane ring, and a carboxylic acid (Nicolaou *et al.*, 2004b ; Nicolaou *et al.*, 2004a).

We have been working on the total synthesis of azaspiracid-1 **(6)** to elucidate its biological function, which causes poisoning by yet unknown molecular mechanism. Our synthetic strategy includes the final coupling between the ABCD- and EFGHI-ring fragments. We have been so far successful in the synthesis of the FGHI-ring domain employing dithiane anion-epoxide coupling for convergent fragment assembly between the C_{28}-C_{35} and C_{36}-C_{40} fragments (Sasaki *et al.*, 2003). The stereoselective synthesis of the E-ring domain and its coupling with the FGHI-ring domain so far obtained are under progress toward the EFGHI-ring domain, which corresponds to a lower half of azaspiracid-1 **(6)**.

The Ramsdell group recently reported azaspiracid-1 **(6)** is a potent teratogen



azaspiracid-1 (**6**)

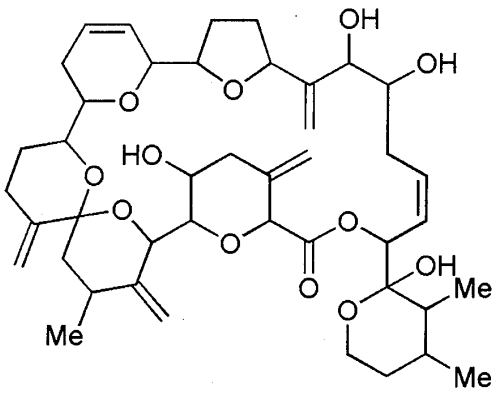
FIG. 2.

to finfish (Colman *et al.*, 2005). On the other hand, Nicolaou *et al.* have reported a preliminary study on the structure-activity relationships by evaluating the biological effects of the synthetic intermediates against mice (Ito *et al.*, 2006). They concluded that the entire or at least a major part of the structure of **6** is required for the biological activity. Our future SAR study will be focused on the phenotypic assay using fish embryos and the cell-based assay for the toxicity evaluation.

3. Goniodomin A

Goniodomin was first isolated as an antifungal compound from the dinoflagellate *Goniodoma* sp. collected in the bay of Puerto Rico (Chile) (Sharma *et al.*, 1968). Twenty years later in 1988, Murakami *et al.* (The University of Tokyo) isolated a closely related, antifungal agent from the dinoflagellate *Alexandrium hiranoi*, named goniodomin A, and determined the planar structure as a polyether macrolide **7** (Murakami *et al.*, 1988). More important biological activity of goniodomin A **(7)** was found after 1993 by a group led by Ohizumi of Tohoku University ; **7** affects actin directly to modulate the interaction between actin-myosin, activates the actomyosin ATPase, and finally affects the cytoskeletal reorganization (Furukawa *et al.*, 1993 ; Mizuno *et al.*, 1998 ; Yasuda *et al.*, 1998 ; Matsunaga *et al.*, 1999 ; Abe *et al.*, 2002).

To establish the molecular basis of goniodomin A **(7)**, we started a program directed toward the study related to, 1) the three-dimensional structural analysis, and 2) the total synthesis applicable to the divergent preparation of structural analogues used for the SAR study. We are currently working on the detailed NMR analyses and degradation experiments toward the determination of the absolute structure of goniodomin A. A synthetic approach to the degraded fragments to confirm both the relative and absolute three-dimensional structures



goniodomin A **(7)**

FIG. 3.

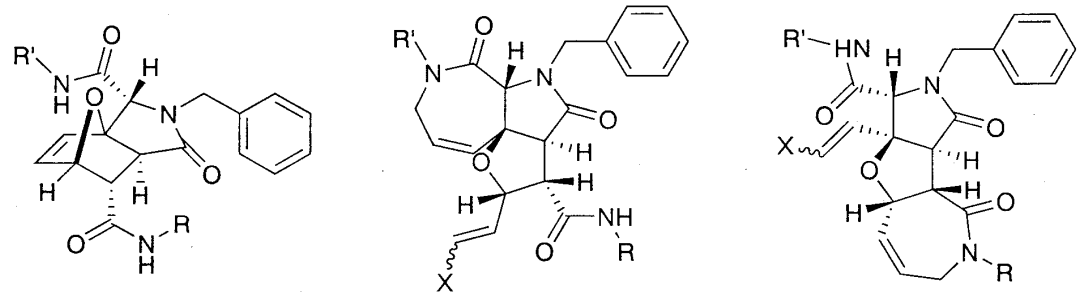
is also undertaken in a parallel manner.

4. Artificial Small Molecules for Biological Study

To understand the biological events at a molecular level, much attention has been recently paid to the biological study by using small molecules, which interact with the target biomolecules of interest to modulate their functions (Schreiber, 2003). Small molecules, which play a central role in this research called chemical genetics or chemical biology, are generally found in natural resources and/or artificial, synthetic small molecules library. Combinatorial chemistry has been developed for more than ten years to construct synthetic small molecules library used to discover biologically interesting, or even drug-like small molecules efficiently. In this chemical research area, efforts have been recently made to construct libraries not only with appendage-based diversity but also with skeletal diversity (Burke and Schreiber, 2004). Our current interest lies on the realization of this type of diverse small molecules library in an efficient way based on the strategy called "diversity-oriented organic synthesis (DOS)".

At first, up to 200 compounds library **8** was constructed by tandem Ugi/Diels-Alder reaction between furfural, amines, isocyanides, and fumaric acid derivatives by liquid phase technology using soluble polymer, poly(ethylene glycol) monomethyl ether (Oikawa *et al.*, 2005a). The oxanorbornene library **8** with appendage-based diversity thus constructed was further transformed into a library composed of diverse skeletons such as **9** and **10** by ring-opening/ring-closing/cross metathesis (Oikawa *et al.*, 2005b). This is the first demonstration of the DOS approach, which allows acquiring both the skeletal and appendage-based diversities simultaneously. Based on these strategies, we are currently working on the construction of highly functionalized amino acid library with skeletal diversity.

Biologically functional peptide mimetics with β-turn structural motif can be also synthesized by our DOS strategy starting from the Ugi four-component



8 **9** **10**

FIG. 4.

coupling reaction products. Works are also in progress to construct the β-turn mimetics library using newly developed soluble polymer platform.

5. Small Molecules Microarray for High-throughput Screening

To discover biologically interesting, drug-like compounds from naturally or synthetically derived small molecules libraries efficiently, it is essential to establish a simple but reliable method for biological screening to detect interactions between small molecules and proteins in a high-throughput manner. In this study, we are working on small molecules microarrays on glass plates via either tri(ethylene glycol) or tetra(ethylene glycol) synthetic linker by using photoaffinity groups (Kanoh *et al.*, 2003). The photoaffinity groups studied were aryl azide and aryl diazirine, and the latter was found to show better efficiency in immobilization of small molecules with diverse chemical structure.

Biological screenings of our diverse collection of small molecules are currently underway to discover potent agents, which interact with biologically important proteins such as kinases, proteases, and lectins.

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