Introduction and Objectives

- Living organisms synthesize a variety of organic compounds, collectively termed natural products. Increasingly, these natural products are being recognized as important sources of materials for use in such areas as medicine, pharmaceuticals, food processing, and biomaterials. The diversity of chemicals synthesized by marine organisms is enormous, and a number of these marine natural products (MNPs) have been found to possess pharmacological activity.
- Many MNPs contain halogens, especially bromine and chlorine. A recent survey identified more than 2,000 naturally occurring halogenated chemicals, most of them produced by marine organisms. Some of the halogenated MNPs have structures that resemble those of the halogenated dioxins, furans, and biphenyls, highly toxic contaminants of marine, estuarine, and freshwater environments.
- Halogenated dioxins (e.g., 2,3,7,8-tetrachlorodibenzo-p-dioxin or TCDD) and biphenyls (e.g., PCBs) cause toxicity through activation of an intracellular protein known as the aryl hydrocarbon receptor (Ah receptor). The ligand-activated Ah receptor is a transcription factor that causes increased expression (“induction”) of specific genes. One such gene is cytochrome P450 1A1 (CYP1A1), an enzyme that metabolizes (degrades) certain chemical contaminants. Induction of CYP1A1 is a widely used biomarker of marine animal exposure to toxic dioxins and PCBs.
- The objective of these studies was to determine if there are marine natural products that can bind to and activate the Ah receptor, causing dioxin-like effects such as induction of CYP1A1. We describe here an initial screening of 160 compounds, using three different bioassays representing three species (two mammals and a fish).

Marine Natural Products

Selected examples from the more than 160 compounds and extracts tested.

Compounds on the left were from a variety of sites and species and supplied by Brad Carté, formerly at SmithKline Beecham.

Right panel shows eight brominated indoles isolated from acorn worms (hemichordata) and provided by T. Higa.

Known Ah receptor ligands
(TCDD and a PCB)

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Bioassays

PLHC-1 Bioassay. The endogenous reporter CYP1A was measured in the PLHC-1 fish hepatoma (liver tumor) cells exposed to MNPs.
CALUX® Bioassay. (chemical-activated luciferase expression). The artificial reporter luciferase was measured in the Hepa-1 mouse hepatoma (liver tumor) cells exposed to MNPs. Hepa-1 cells were transfected with a reporter construct containing a luciferase gene under control of a mouse dioxin-responsive enhancer (DRE) sequence.

EMSA Bioassay. (electrophoretic mobility shift assay) This assay measures the ability of chemicals to bind to and activate the Ah receptor in guinea pig liver. Activated Ah receptor is measured by a gel retardation assay using oligonucleotides containing a mouse DRE sequence.

Results

- Seven brominated indoles were active in one or more of the assays, with efficacies of up to 90% as compared to 2,3,7,8-TCDD. Ascididemin, neopimaridione, kabiramides, manoalide, and bromotopsentin were also active. (Table and Figure).
- There were significant differences in bioavailability or metabolic fate of the MNPs in the three bioassay systems.
- There were differences in bioavailability or metabolic fate of the MNPs in the three bioassay systems.
- There were significant differences in the sensitivity and specificity of the fish, mouse, and guinea pig bioassays, possibly reflecting distinct Ah receptor binding characteristics in these species and/or differences in bioavailability or metabolic fate of the MNPs in the three bioassay systems.

Conclusions

- Compounds capable of activating the Ah receptor occur naturally in the marine environment.
- Studies with these and additional natural products with diverse structures may help to define the ligand specificity and binding site topology of vertebrate Ah receptors, as well as to identify novel compounds with potent Ah receptor agonist (stimulator) or antagonist (blocker) activity.