Dynamic Adsorbents

Isolation and Purification of Marine Organisms

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Isolating and purifying new therapeutic agents from plants and marine sources is essential to the continued success of the pharmaceutical industry. One out of four prescription drugs sold in the United States contains active ingredients extracted or derived from plants. 50% of the top selling drug products are derived from natural products. More than 40 different plant species are used, and yet less than 1% of the world's flora has been screened for biochemical activity, with even less of the marine species explored.

In the pharmaceutical industry the value of an organism, whether of plant or marine origin lies in its potential to contain a unique biologically active compound that can be fully synthesized in the laboratory. Signs of biochemical activity in a natural product extract must be followed by a long period of testing to isolate and characterize the active compound. The dominant strategy in the pharmaceutical industry is to maximize the number of diverse compounds screened in order to maximize the probability of discovering novel compounds for drug development. Chemical libraries, which are collections of chemical structures that have either been synthesized in the laboratory or isolated from natural sources are assembled for creating new lead drug compounds. Natural products chemistry encompasses isolation of a desired compound, use of purification technologies to get as pure a product as possible from the initial sample and subsequent analytical and structural analysis of the compound using spectroscopic and nuclear magnetic resonance techniques. The key role for the separation scientist is to isolate novel structures from natural products which can then serve as blueprints, or starting material for the biochemical synthesis of new drugs. Bioactive natural products serve as lead structures which are then optimized through classical medicinal chemistry techniques or combinatorial synthesis methods to yield new drug compounds having either superior activity or less toxicity.

Drug discovery offers a bright future for marine biotechnology research. Biochemicals produced by marine invertebrates, algae and bacteria are different than those from terrestrial organisms and offers the potential for new classes of medicines. Many of the marine derived natural products which are being isolated exhibit exceptional levels of biological activity, combined with unique modes of action. The oceans cover more than 70% of the earth's surface and in places are more than 5 miles deep. There are over 200,000 invertebrate and algal species, with nearly 150,000 species of algae (sea weed) alone. Other types of marine organisms include sponges (Porifera) cnidarians or coelenterates (corals, octocorals (including sea fans), hydroids and sea anemones), nemerteans (worms), ascidians (including sea squirts), mollusks (sea snails and sea slugs) and echinoderms (brittlestars, sea urchins, starfish ands sea cucumbers). Sponges are very simple animals that live permanently attached to a location in the water and they are sessile, being permanently attached to an underlying substrate and unable to move on their own. Presently, more than 35% of useful medical compounds from the sea are isolated from sponges. Important compounds for drug discovery come from soft bodied or slow moving marine creatures which do not have protective structures such as spines or protective shells and which produce toxic natural products as their means to fight off potential predators. The harsh marine environment which they inhabit, together with their lack of physical defenses has required these organisms to develop chemical deterrents in order to survive.

In addition to new medicines, other uses for marine derived compounds include cosmetics (from algae, crustacean and sea fan compounds), nutritional supplements (algae and fish compounds), artificial bone (corals), and industrial compounds (including fluorescent compounds from jellyfish, novel glues from mussels, and heat resistant enzymes from deep sea bacteria).

Marine organisms have been used for the development of pharmaceutical compounds for more than 50 years. Natural products tend to have higher molecular weight than synthetic counterparts, contain more rings and are sterically complex. This makes synthesis of these compounds challenging to medicinal chemists who attempt to synthesize the active agents. The first drugs were developed for the treatment of viral infections and cancer. The Caribbean sponge Cryptotethya crypta yielded arabinose nucleosides which were the lead compounds for the synthesis of analogs ara A (vidarabin) and ara C (cystosine arabinoside). The fungus Cephalospirium was isolated from sea water collected from Cagliari, Italy which led to the isolation of cephalosporins, which remain a most useful class of antibiotic agents. Large quantities of prostaglandins found in the gorgonian Plexaura homomalla has become the take off point for a systemic investigation of marine environments as sources of novel biologically active compounds.

New drug agents are being isolated from marine organisms and finding their way into clinical practice. Ziconotide (Prialt) is a novel non-opioid, non local anesthetic, developed for the treatment of severe chronic pain. Ziconotide is the synthetic form of a 25 amino acid peptide isolated from the venum of the marine snail Conus magus. The drug works through a unique mechanism of action, in that it binds to N type calcium channels in the spinal cord and blocks the ability to transmit pain signals to the brain. Its action is not blocked by opioid antagonists. In December 2004 the Food and Drug Administration approved ziconotide for use as an infusion into the cerebrospinal fluid using an intrathecal pump system. Trabectedin is an anti-tumor drug which was isolated from an extract from the sea squirt Ecteinascidia turbinata.. Sea squirts are so named as many species expel streams of water through a siphon. It is sold by Johnson and Johnson and Zeltia Yondelis and is currently approved for use in Europe, Russia and South Korea for the treatment of advanced soft tissue sarcoma.. It is also undergoing clinical trials for the treatment of breast, prostate, and pediatric m.

The European Commission and the US Food & Drug Administration have granted orphan drug status to trabectedin for soft tissue sarcomas and ovarian cancer. The mechanism of action for the drug is believed to involve the production of superoxide near the DNA strand, resulting in DNA backbone cleavage and cell apoptosis. It is being used for patients who have failed treatment with anthracycline drugs and ifosphamide, the most commonly used agents for the treatment of sarcomas.

Many compounds isolated from the marine environment display anti-cancer activity. Some appear to work as microtubule stabilizing agents, disrupting the formation and maintenance of microtubules in cells thus suppressing cell division. Much interest has been generated in examining the marine environment for such compounds because of the clinical success in the isolation of taxol from the plant species Taxus from the Pacific Yew tree. Taxol is the most powerful drug in the treatment of breast cancer, has significant activity in the treatment of ovarian tumors as well as having significant activity in the treatment of other squamous cancers and adenocarcinomas. Furthermore, it is used as an agent coating vascular stents to inhibit the regrowth of endothelial cells and is coated onto stents inserted during angioplasty procedures.

The marine natural product laulimalide has a similar mechanism of action as that of taxol. Although laulimalide is only 1/5th as potent as taxol in drug sensitivity laboratory cell lines, it is as much as 100x more potent than taxol in multi drug resistant cell lines. This compound was isolated from the Okinowan Ocean sponge Cacospongia mycofijensis and has proven effective in the treatment of breast and ovarian cancers which become resistant to taxol. Furthermore, the drug has now been completely synthetically synthesized. Multiple analogs of laulimalide have been isolated which initiate an increased density of interphase microtubules, aberrant mitotic spindles, and ultimately apoptosis or programmed cell death, which is the method of destruction of cells. Taxol and laulimalide bind at different sites on tubulin polymer and appear to have synergistic cytotoxic activity.

There are difficulties working with marine organisms as a natural source for drug development. Marine invertebrates cannot be easily cultured, cannot be collected on a large scale and compounds from marine compounds often cannot be synthesized. Preclinical and clinical developments are often hampered by the limited supply from the natural source. To that end, the goal of medicinal chemists is total synthesis of materials derived from the sea. Total synthesis allows for the ready production of synthetic analogs and for the elucidation of structure activity relationships and the design of more active or less toxic molecules. Analog design for synthetic compounds is based upon the assumption that only certain structural features are involved in discrete interactions with the biological target.

Marine sampling starts with freeze drying of the material until taken back to the laboratory. The samples are frozen at – 20 C until they are ready to be analyzed. Next the sample extract is fractionated by liquid-liquid partitioning followed by the separation and isolation of the individual components using chromatographic separation techniques including thin layer chromatography (TLC), vacuum liquid chromatography, column chromatography and preparative high performance reversed phase liquid chromatography. Isolation of bioactive secondary metabolites is usually monitored by bioactive and cytotoxicity assays. Structural elucidation of desired active compounds is studied using spectroscopic techniques especially 2D nuclear magnetic resonance and mass spectrometry.

Extracts from marine organisms may possess antibacterial, antifungal and cytotoxic activities. Bioassays rely on determining the biological activity of crude extracts for numerous target specific assays such as enzyme assays and receptor binding assays. Antimicrobial activity is analyzed by testing the crude extracts against the standard strains of gram positive Bacillus subtilis, gram negative Escherichia coli, the yeast Saccaromyces cerevisiae and the fungal strains Cladosporium herarum and Cladosporium cucmerinum.

Protein kinase screening assays are also performed to determine inhibition of the serine/threonine kinases, which control the transmission between the successive stages of the cell cycle. These are high volume, automated screening processes which may allow thousands of samples to be studied per week in larger pharmaceutical houses.

Complimenting these bioassays the separation techniques of TLC, column chromatography, UV and MS are used to isolate the chemically most interesting substances. In general, the more hydrophilic metabolites may best be isolated using ion exchange chromatography, reversed phase silica gel chromatography or size exclusion chromatography on polysaccharide resins. The more lipophilic metabolites can be further purified by chromatography on normal phase silica gel, Florisil, alumina or lipophilic size exclusion resins such as Sephadex LH-20. Due to its amphoteric properties alumina appears to be the superior method for clean up purification.

Further separation techniques are beyond the scope of this paper. However the benefits of using alumina for column chromatographic analysis of marine organisms appears to parallel the benefits of its use in the isolation and purification of plant alkaloids.

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