

Marine natural Products with a Special Reference to Aplidine as a New Bioactive Compound in Anti-Cancer Class

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ABSTRACT

The marine environment has proven to be a very rich source of extremely potent compounds that have demonstrated significant activities in antitumor, anti-parasitic, anti-microbial, anti-coagulants, anti-viral, cytotoxic compounds, anti-inflammatory, proteins. Aplidine is a promising anti-tumor agent that is derived from the Mediterranean tunicate *Aplidium albicans*. We have found that aplidine at nm concentrations (10-100 nm) induced apoptosis in human leukemic cell lines and in primary leukemic cell culture from leukemic patients. Aplidine induced a rapid and sustained c jun NH₂ –terminal kinase activation and apoptosis. Thus, Aplidine is an extremely potent and rapid apoptic inducer on leukemic cells that triggers Fas/CD 95- and mitochondrial mediated apoptotic singling routes, and shows a rather selective apoptotic action on cancer cells and activated T- cells.

Keywords: Anti-parasitic, anti-microbial, anti-inflammatory activity; anticoagulant, Aplidine.

INTRODUCTION

In companion to land plants and animals, the use of marine natural products is restricted. Several marine products are still are used as cod liver oil, spermaceti, protamine sulphate and some polysaccharide as agar, carrageenan and alginic acid.

1. Cod liver oil

Cod liver oil is obtained from fresh liver of the cod i.e *Gadus collorias*, family *Gadidae* with enriched “vitamin A & D”. Two types of oil are found that are -

- i. Type A (Prone to oxidation)
- ii. Type B (Commerical product)¹

2. Spermaceti

Spermaceti are obtained from the sperms of whole *Physeter macrocephalus* Linn², family *Physeteridae*. Spermaceti is oil contains-

- -Cetyl palmitate
- -Free cetyl alcohol
- -Ester of lauric acid
- Myristic acid
-

3. Protamine sulphate

Protamine sulphate is obtained from the sperms of fish belonging to family *Salmonidae*. It contains various proteins and is used as a heparin antagonist³.

4. Agar

Agar is a dried gelatinous substance obtained from *Gelidium amansii*, belonging to family *Geladiaceae*. Also some species of *Gracilaria* (*Gracilariaceae*) are a source of agar, which contains –Agarose and agarpectin

Agarose is responsible for gel strength and composed of D-galactose and 3,6 anhydrogalactose unit.

Agarpectin is responsible for viscosity of agar solution and is used as gelling agent and in formulations etc⁴.

5. Carrageenan

It is obtained from sea weeds called carrageenan or Irishmoss i.e red algae- *Chondrus crispus*, family-*Rhodophyceae*. It is used as emulsifying agent, stabilizing agent, viscosity builder etc.

In tooth paste, cream, lotions and other products.

Constituents- Depending upon position of sulphate and presence or absence of anhydrogalactose, two types are there-

i. Kappa (k)

ii. Lambda (α)

K-carrageenan contains D-galactose, 3,6 anhydrous D-galactose and ester sulphate group.

α-carrageenan contains D-galactose and mono, disulphate ester⁵.

6. Algenic acid

Algenic acid⁶ is composed of reduced manuronic and glucuronic acid which are obtained from algal growth species- *Laminaria degitata*, *Laminaria hyperborean*.

It is soluble in water forming viscous colloidal solution, insoluble in alcohol and ethers etc.

7. Occurrence of red tide caused by *Karenia mikimotoi* (toxic dinoflagellate) in the Southwest coast of India

Red tides are common especially in the coastal waters that threaten marine living resources' and local economy. At times, red tides are dominated by harmful algal bloom⁷ (HAB), species that adversely affect aquatic ecosystem by releasing toxic substances. So far several phytoplankton blooms were frequently reported from the west coast of India. Among them, blooms of *Nitzschia miliaris* (dinoflagellate) and *Trichodesmium erythraeum* (blue green algae) are frequently occurring red tides. Even through these two blooms are recurrent and sometimes cause fish kill⁸ due to oxygen depletion, both of them are not accounted to be toxic yet. Few years back (September-October 2004) an unusual stench event observed in the coastal waters off Trivandrum and Kollam had created some health problems (nausea, chest pain and breathlessness) to the local people in the region⁹.

8. Juvenile abundance and post-larval incursion of mud crabs (*Scylla* spp.) in Chilika lagoon

The mud crabs *Scylla* spp., represents a valuable component of small-scale coastal fisheries in many countries of tropical and subtropical Asia and African coast. Through the mud crabs are marine dwellers, they immigrate into brackish water system during their post-larval stages, grow fast attain maturity and form lucrative fishery in estuaries, backwaters and lagoons. With great demand for live export and increased price, fishery and aquaculture of mud crabs have gained importance in India and abroad. The two mud crab species (*S. serrata* and *S. tranquebarica*) are coexisting in the lagoon¹⁰. The lagoon was in a degraded state during the last few decades tending towards a freshwater ecosystem due to the natural changes coupled with anthropogenic pressure^{11, 12}.

9. Coral disease prevalence in the Palk Bay, South-eastern India –with special emphasis to black band¹³

Present study consists of the details which is related to the nature of coral disease in nine locations from Vethalai to Rameswaram north in the Palk Bay. Among the overall corals 21% were affected by diseases. Six disease types were till now recorded. Black Band Disease (BBD) is high with 9.8% followed by white band (5.5%), white spot (2.2%), pink spot (1.9%), white plague (1.15%) and yellow band (0.6%). Eight coral genera were found to be affected, wherein *Acropora* and *Porites* showed severe damage and the high prevalence of diseases. The coral genus, *Porites* was found to be affected by four different types of diseases. BBD affected colonies were tagged and photographed at regular intervals to quantify the progression rate in two coral genera, *Acropora* and *Platygyra* and the disease progression rate was 3 cm per month. White band disease was widespread and was found to affect exclusively *Acropora* sp. Corals like *Symphylia* sp. and *Cyphastrea* sp. in the Palk Bay were comparatively not affected by diseases.

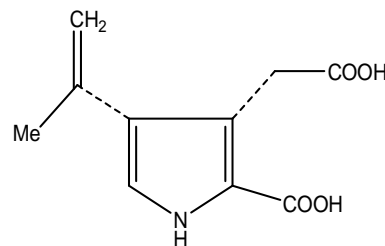
Classes of active compounds:

1. Anti-parasitic compounds

i. Red algae, *Digenia simplex*¹⁴ has been used as vermifuge since hundreds of years.

Many companies are marketing it as broad spectrum antihelmintic.

Effective against round worm, tape worm and in which kainic acid¹⁵ is the active constituent.

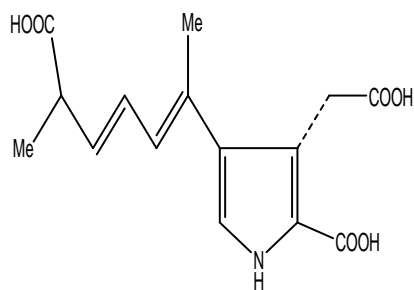


Kainic acid

ii. Red algae

Chondria armata and *Alsidium corallium* also used as antihelmintic properties.

Constitute- Domoic acid¹⁶

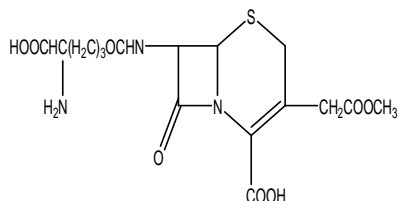


Domoic acid

2. Anti-microbial agent

Generally marine micro-organisms are isolated and characterized posse's anti-microbial activity.

e.g.fungus- cephalosporium acremonium, from which cephalosporin C¹⁷ is isolated – i.e. semi synthetic derivatives as cephalosporium sodium.



Cephalosporin c

Other marine anti-microbial compounds isolated from marine micro-organism are istamycins-A and B^{18, 19} produced by fermentation of marine streptomycete, Streptomyces tenyimariens. It is effective against gram (+) and gram (-).

3. Anti-coagulants

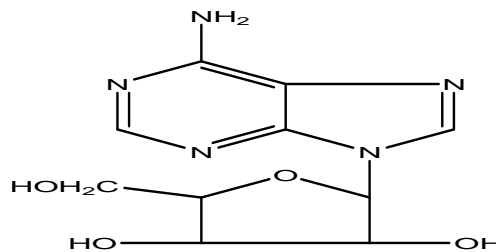
Generally polysaccharides collected from marine algae are considered as good source of anti-coagulants.

Sulphated galactum of Iridaea laminarioides¹⁴ is responsible for anti-coagulant effect.

Carrageenans isolated from Chondrus crispus, Euchema spinosum, Polyides rotundus are known for anti-coagulant activity and were reported to cause inactivation of thrombin.

4. Anti-viral

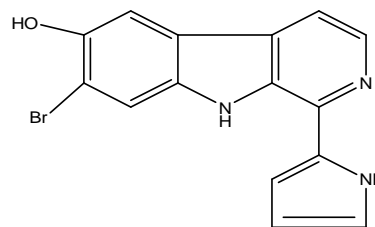
Anti-viral agents which are against human virus are in great demand because of AIDS epidemic. Ara-A²⁰ is semi synthetic substance (arabinosyl nucleosides) that is isolated from marine sponge-Tethya crypta.



Ara-A

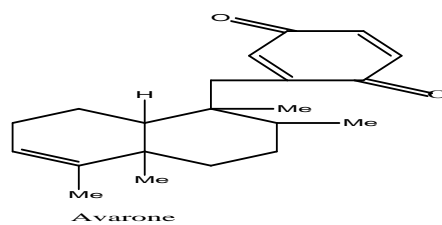
Several compounds which are isolated from the marine are reported for its anti-viral action invitro but didemnins (cyclic depsipeptide) shows an anti-viral activity – in vivo as well as anti-tumour activity.

Eudistomins (β -carboline) which are isolated from the Udistoma olivaceum²¹ (family polycytoridae) are known for its potent anti-viral activity.

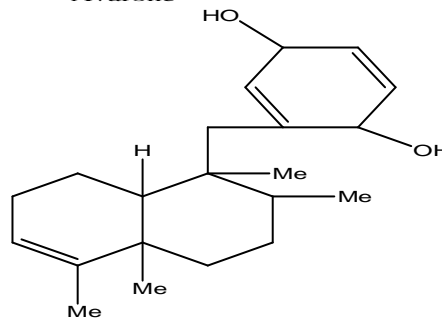


Eudistomin A

Avarol and Avarone which are extracted from sponge Disidea avara²² are known to inhibit immunodeficiency virus and cross bbb. Thus used as a potent anti-viral agent for AIDS treatment.



Avarone



Avarol

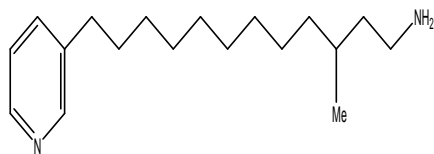
5. Cytotoxic Compounds

Cytotoxic compounds are a group of macrolides known as bryostatins isolated from bryozoon – *Bugula neritina*.²³ Some macrolides were also collected from sponges and tunicates.

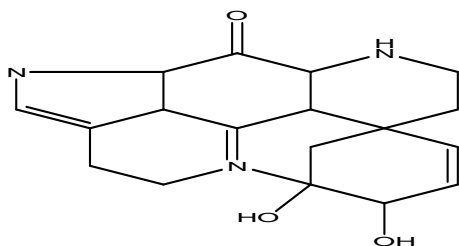
Bryostatins also stimulates human haemopoietic cells and are thus useful for treating neoplastic bone marrow failure.

Bryostatins activates and differentiate peripheral blood cells of lymphocytic leukemia patients and also activates protein kinase and arachidonic acid metabolite release. Bryostatin-1 is under phase-2 clinical trial.

Alkaloid-niphatesine is isolated from *Niphates* species,²⁴ and epinardins is isolated from *Stelletta globostellata* are cytotoxic agents from marine sponges.

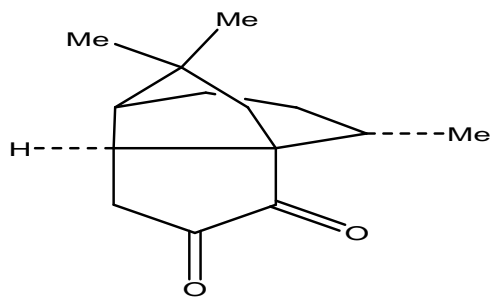


Niphatesine

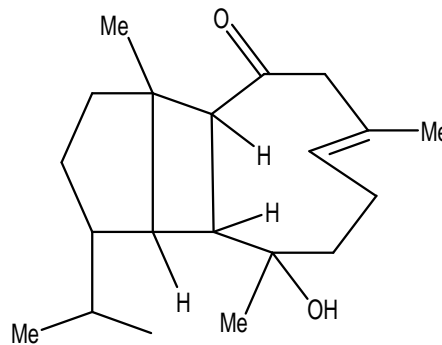


Epinardin A

Sesquiterpenes- Suberosenone extracted from *Subergorgia suberosa*,^{25,26} Diterpene sarcoglane from sarcophyton glaucum are examples of cytotoxic agent from coelenterates.

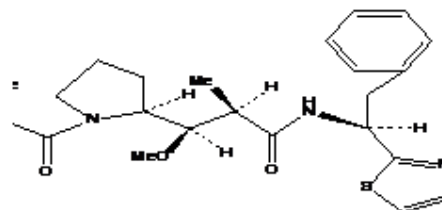


Suberosenone



Sarcoglane

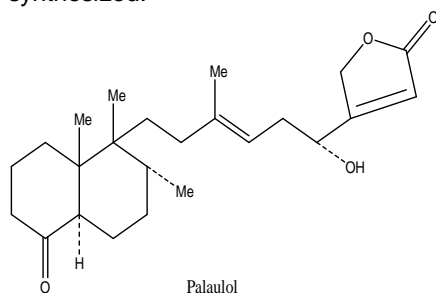
Dolastatins²⁷ are peptides derived from *Dolabella aericularia*, a mollusc found in the Indian ocean. Dolastatin 10 and the dolastatin 15 are included in dolastatin family. Structurally dolastatin is a pentapeptide with four of the residues being unique (dolavaline, dolaisoleucine, dolaproline and dolaphenine, in addition to valine). Dolastatin 10 has phase 1 clinical trials as anticancer agent and it is for used for the treatment of breast and liver cancers, solid tumours and leukaemia. Dolastatin 10 and 15 are small peptides. It blocks microtubule assembly, there by causing cells to accumulate in metaphase and is extremely potent in vitro. The side effects of dolastatin 10 are bone marrow toxicity in initial clinical trials, as well as local irritation at the injection site and mild peripheral neuropathy. Dolastatin 15, a seven subunit depsipeptide and it is derived from *Dolabella auricularia*, a potent antimitotic agent structurally related to the antitubulin agent dolastatin 10, a five subunit peptide isolated from extracts of the Indian ocean sea hare *D. Auricularia*. Indeed, numerous dolastatin 15- related peptides have been isolated from diverse marine cyanobacteria. Its linear depsipeptide sequence is composed of seven amino acid or hydroxyl acid residues²⁸.



Dolastatin

6. Anti-inflammatory compounds

Sesquiterpene 'Palaulol' from sponge *Fascaplysinopsis* sps²⁹ and Sesquiterpene furan³⁰ from coelenterates- *Sinularia* species are synthesized.



7. Proteins

The group of proteins known as Lectins^{31, 32} has received a good idea of attention in recent years. Lectins are carbohydrates-binding proteins and are found in a wide variety of life forms, including marine organisms. Probably the best known Lectins from a marine source are the potent haemagglutinins obtained from the haemolymph of *L. polyphemus*. One of the lectins, limulin, has that been isolated and shown to be a large, 18-subunit protein with a molecular mass of 350 kDa. Limulin shows specificity for binding to sialic acid, although other structures are recognized. Another lectin that shows specificity to sialic acid is that obtained from the haemolymph of the lobster and *Homarus americanus*.

8. Argochemical usage

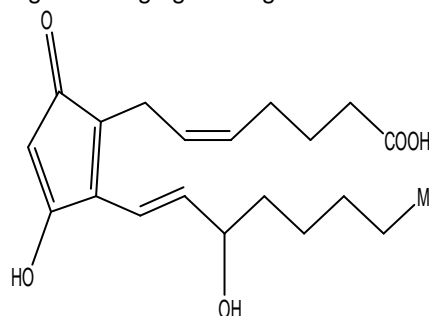
The marine annelid, *Lumbriconeris heteropoda*,¹⁴ has long been known to be toxic to insects. This activity was shown to be due to nereistoxin, which has rapid anaesthetic properties on insects and is toxic to fish and mammals, affecting the nervous system and heart. Studies on this compound led to the introduction of the synthetic pesticide Padan. the most widely used species in the preparation of the product are *Ascophyllum nodosum*, *Ecklonia maxima* and *Durvillaea patatorum*.

12. Prostaglandins

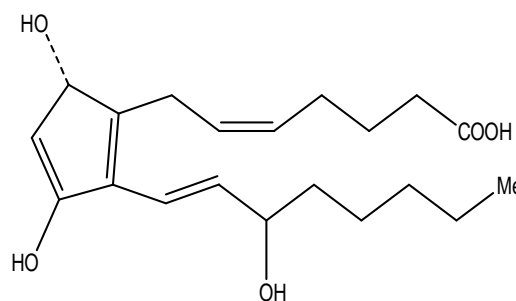
Prostaglandins³³ constitute a group of biologically potent substances of wide spectrum activity. Although first recognized in the 1930s, they remained of little interest until the structures of two of them were determined in 1962. Structurally, all the compounds are based on prostanic acid, which is inactive. Six series (A-E) arise by modification of the

cyclopentane ring and these are now available as synthetic products. However, prior to synthetic compounds being available, research on prostaglandins was restricted by inadequate supplies of the two active compounds, PGE₂ and PGF_{2α}. This discovery that the soft coral, *Plexaura homomalla*, was a rich source of 15-epi-PGA₂ and its acetate, methyl ester derivative, was a major breakthrough as the Upjohn Company developed a synthetic pathway to convert these inactive compounds into the required active ones.

Prostaglandins have also been isolated from red algae belonging to the genus *Gracilaria*.



Prostaglandins E₂

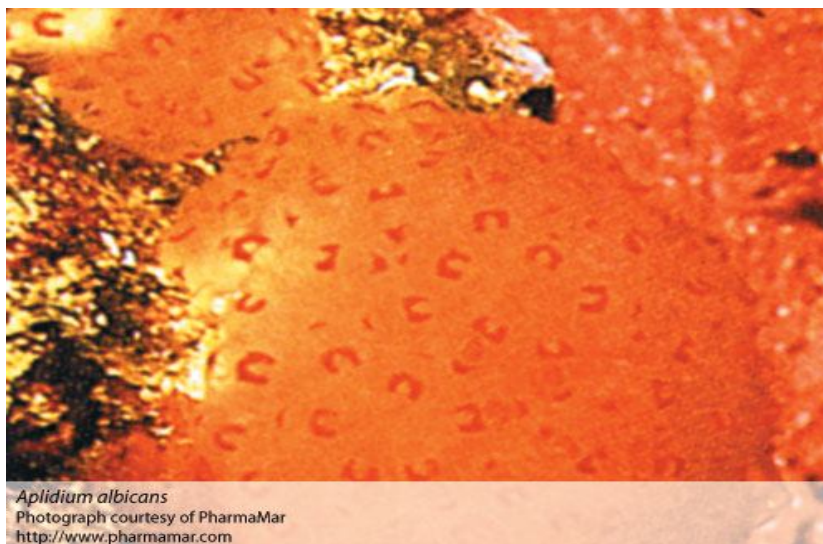
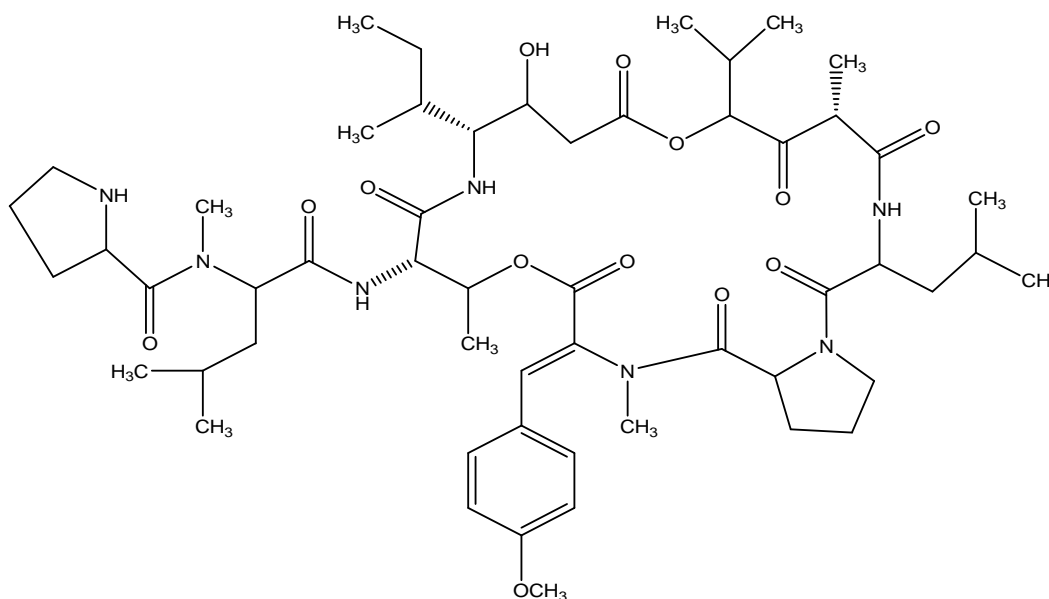


Prostaglandin F_{2α}

13. Aplidine

Its others name: Aplidin, Dihydrodidemnin B, plitidepsin; source: The tinucate *Aplidium albicans* (CHORDTA)^{34, 35}. Activity: Anti-cancer agent via apoptosis; status: Phase II clinical trials

Properties	
Molecular formula	C ₅₇ H ₈₇ N ₇ O ₁₅
Molar mass	1110.34 g mol ⁻¹

**Aplidium albicans****Chemical structure of aplidine**

Aplidine is a new marine natural products isolated from *Aplidium albicans*. It is a member of the class of compounds known as didemnins. It is a cyclic depsipeptide in which there is one or more ester bond in place of one or more of a peptide bond. It shows strong anti-tumour activity against different human cancer cells growing in vitro and in vivo³⁴⁻³⁵. It was first reported in a 1991 patent applications. The chemical structure of aplidine is very close to didemnin B, the only difference being that the lactate residue in didemnin B is present in the oxidized pyruvate

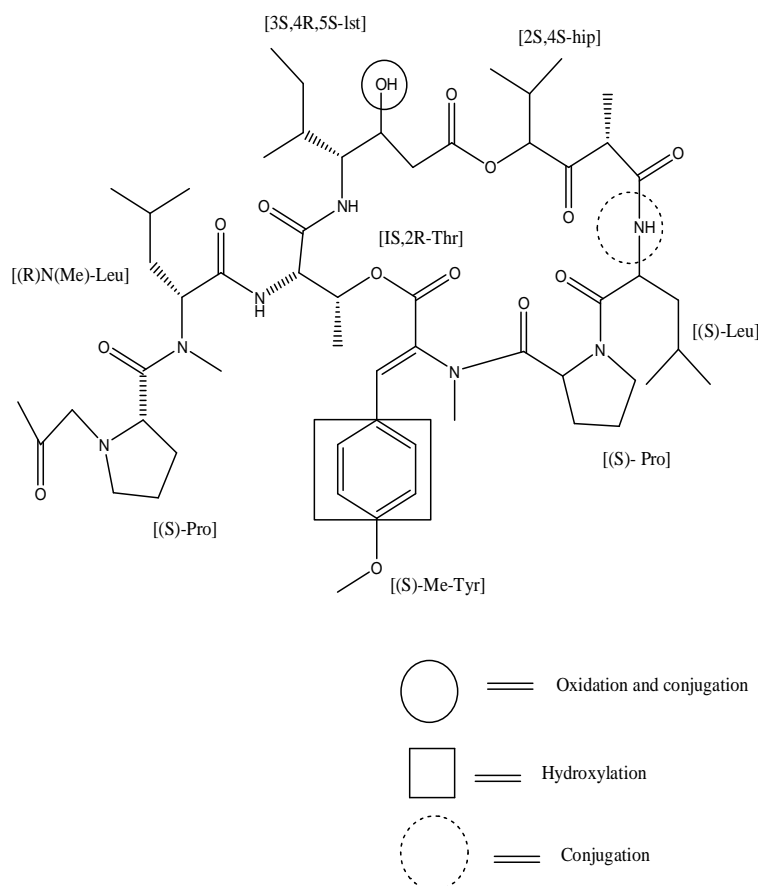
version. It has completed phase 1 trials and is under phase 2 development.

The exact mechanism of action of aplidine is still unknown. It induces cell growth inhibition and apoptosis in the human leukemia cell line MOLT-4 at G-1 phase in the cell cycle. This cell line has the ability to secrete VEGF (vascular endothelial growth factor) which is present on the surface of VEGF receptor -1 (VEGFR-1). A change in gene expression induced by aplidine in MOLT-4 cells is also seen and it also decreases the expression of VEGFR-1 which is bind to the angiogenic factor VEGF³⁶. Increasing suggests, however,

that certain cancer cells of different origin, including leukemic cells, also express VEGF receptors on their surface. On the other hand, VEGF is secreted by virtually all cancer cells as a homodimeric glycoprotein which is able to bind VEGFR-1 and other components of the VEGF receptors family with high affinity³⁷. The simultaneous presence of a receptor and the ability to secrete the ligand for it, suggests the possible presence of an autocrine loop important for the growth of cancer cells expressing these receptors. Aplidine has the ability to block the VEGF/VEGFR-1 loop thereby decreasing the production of VEGF and VEGFR-1. This reviews gives the evidence that aplidine has strong effect on the VEGF/VEGFR-1 autocrine loop regulating the growth of the human leukemic cell line MOLT-4³⁸.

In vitro characterization of Aplidine biotransformation-

The *in vitro* biotransformation of aplidine was characterized by using incubations with liver preparations, human plasma, cytochrome P450 (CYP) and uridine diphosphoglucuronosyl transferase (UGT) supersomes in combination with HPLC analysis and cytotoxicity assays with cell lines. Carboxyl esterases is an enzyme which metabolised aplidine in human plasma. It was shown that aplidine was metabolised mainly by CYP3A4 and also by CYP2A6, 2E1 and 4A11. Aplidine formed four metabolites after incubation with human liver microsomes, one formed by CYP2A6 (C-demethylation) and three by CYP3A4 (hydroxylation and/or C-dealkylation). This metabolites were further conjugated by the phase II enzymes UGT, GST and SULT. The human cancer cell lines Hep G2 and IGROV-1 were used to study the cytotoxicity of aplidine and its metabolites. The enzyme CYP3A4 has a major role in metabolising aplidine *in vitro* with additional involvement of CYP2A6, 2E1, and 4A11^{39,40}.



Chemical structure of aplidine. The different squares and ovals indicate potential sites for biotransformation. In addition all the ester and amide bonds are potential sites for hydrolysis. Hip-hiphydroxyisovalerylpropionyl, Ist-isostatine, Leu-leucine, Me-methyl, Pro-proline, pyr-pyruvoyl, Thr-threonine, Tyr-tyrosine.

CONCLUSION

It has been demonstrated in this review; the potential for marine natural products as sources and/or leads to drugs that cover a very wide range of pharmacological effects (i.e., cancer, anti-infective, analgesia, Alzheimer's disease, inflammation, immunomodulation) is only now being realized. It is probable that within the next two years at least one marine-derived novel agent will enter commerce as an anticancer or analgesia drug following governmental approval. Perhaps the most important current discovery, however, is the proof with Aplidine that, as suspected by many investigators over the years. The effects of aplidine were evaluated in experimental models. At the time of submission of the application for orphan designation, no clinical trials in patients with acute lymphoblastic leukaemia were initiated. Aplidine was not marketed anywhere worldwide for acute lymphoblastic leukaemia or designated as orphan medicinal product elsewhere for this condition, at the time of submission.

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