## L-12
## Recently Isolated Bioactive Compounds from Korean Marine Sponges

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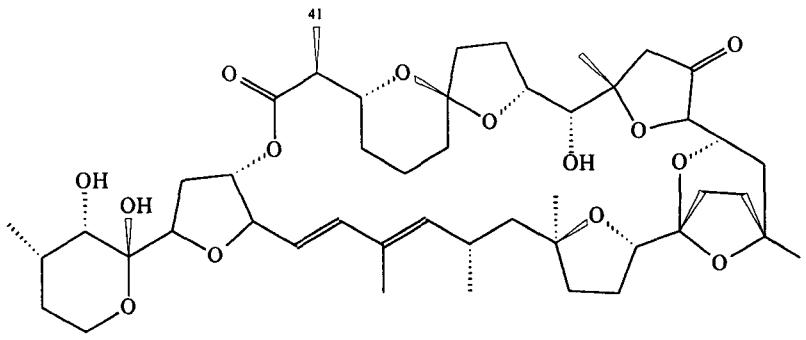
Marine sponges are recognized as a plentiful source of diverse biologically active secondary metabolites. Recently, we have initiated a research to discover antitumor constituents from the marine sponges collected from Korean Waters. Marine sponges collected from the South Sea of Korea were screened for several biological activities including such as brine shrimp lethality and cytotoxicity. Significant brine shrimp lethality was detected in the crude extract of a two-sponge association of *Poecillastra* sp. and *Jaspis* sp. A cross-section of this sample showed two layers of morphologically distinct sponges. The thin and dirty yellow outer layer was identified as *Poecillastra* sp. (Pachastrellidae), the surface of which was very rough. The light-grey inner layer was identified as *Jaspis* sp. (Jaspidae), the surface of which was smooth. This two-sponge association appears to be consistent as these sponges were always found in associated form regardless of collection site or collection period. Investigation of the bioactive constituents monitored by brine shrimp lethality assay led to the isolation of pectenotoxin II (PTX2) and psammaplin A as causative compounds for the brine shrimp lethality. $^{1}H$- and $^{13}C$-nmr signals of PTX2 was fully assigned utilizing TOCSY, HETCOR, Long-range HETCOR, and Homonuclear J-resolved 2D experiments. PTX2 displayed very potent and selective cytotoxicities in the 60 cell line panel antitumor assay at the NCI. PTX2 has progressed to acute toxicity determination and *in vivo* antitumor assay at the NCI (Table 1). However, significant *in vitro* antitumor activity of PTX2 can not be affirmed in the *in vivo* assay.




PTX 2

Table 1. *In vivo* Antitumor Assay on PTX2 (NCI, USA)

| tumor type | dose/unit (mg/kg/dose) | implant site | schedule | No. of mice | drug death | % T/C (day) |
|---|---|---|---|---|---|---|
| COLO205 (human colon cancer) | 0.45 | s.c. | IP Q4D x 3, day 10 | 6 | 6 | toxic |
| | 0.30 | | | 6 | 5 | toxic |
| | 0.20 | | | 6 | 3 | toxic |
| CAKI-1 (human renal cancer) | 0.27 | s.c. | IP QD x 5, day 18 | 6 | 6 | toxic |
| | 0.18 | | | 6 | 3 | toxic |
| | 0.12 | | | 6 | 0 | 100 |
| RXF393 (human renal cancer) | 0.27 | s.c. | IP QD x 5, day 7 | 6 | 6 | toxic |
| | 0.18 | | | 6 | 6 | toxic |
| | 0.12 | | | 6 | 1 | 86 |
| SNB-75 (human CNS cancer) | 0.27 | s.c. | IP QD x 5, day 10 | 6 | 6 | toxic |
| | 0.18 | | | 6 | 5 | toxic |
| | 0.12 | | | 6 | 0 | 74 |
| P388 (murine leukemia) | 0.15 | i.p. | IP QD x 5, day 1 | 6 | 0 | 14* |
| | 0.10 | | | 6 | 0 | 14* |
| | 0.067 | | | 6 | 0 | 9* |
| OVCAR (human ovarian cancer) | 0.45 | s.c. | IP Q4D x 3, day 7 | 8 | 2 | 9* |
| | 0.30 | | | 8 | 0 | 29* |
| | 0.20 | | | 8 | 0 | 27* |
| MDA-MB-231 (human breast cancer) | 0.30 | s.c. | IP QD x 5, day 13 | 6 | 0 | 112(30) |
| | 0.20 | | | 6 | 0 | 92(30) |
| | 0.13 | | | 6 | 0 | 78(26) |
| MDA-MB-435 | 0.30 | s.c. | IP QD x 5, day 15 | 6 | 0 | 65(26) |
| | 0.20 | | | 6 | 0 | 78(26) |
| | 0.13 | | | 6 | 0 | 101(26) |
| | 0.67 | s.c. | IP QD x 5, day 16 | 6 | 0 | 86(30) |
| | 0.45 | | | 6 | 0 | 95(23) |
| | 0.30 | | | 6 | 0 | 100(23) |
| MAXF401 (human breast cancer) | 0.30 | s.c. | IP QD x 5, day 15 | 6 | 0 | 108(26) |
| | 0.20 | | | 6 | 0 | 97(26) |
| | 0.13 | | | 6 | 0 | 74(22) |
| | 0.67 | s.c. | IP QD x 5, day 15 | 6 | 0 | 87(29) |
| | 0.45 | | | 6 | 0 | 86(29) |
| | 0.30 | | | 6 | 0 | 75(29) |
| A498 (human renal cancer) | 0.45 | s.c. | IP QD x 5, day 17 | 6 | 6 | toxic |
| | 0.30 | | | 6 | 4 | toxic |
| | 0.20 | | | 6 | 5 | toxic |

*% ILS (increase of life span)

A significant activity in the brine shrimp larvae lethality bioassay ($LD_{50}$ = 30 ppm) was detected in the methanol extract of the marine sponge *Petrosia* sp. Guided by the brine shrimp lethality, the methanol extract was further fractionated between water and $CH_2Cl_2$, followed by partitioning of the $CH_2Cl_2$ solubles between 90% methanol and *n*-hexane. The 90% MeOH fraction was then partitioned again between water and $CH_2Cl_2$ to afford the $CH_2Cl_2$ layer which was subjected to reverse phase flash column chromatography and HPLC to yield eight new polyacetylene alcohols, **3-10**, together with two known compounds. These compounds showed moderate to significant cytotoxicities against human tumor cells (Table 2, 3). The cytotoxicities of the compound **3**, **5**, and **6** were further determined in NCI 60 cell panel to show significant selectivity and potency. Compound **6** is now progressed to *in vivo* testing at NCI.

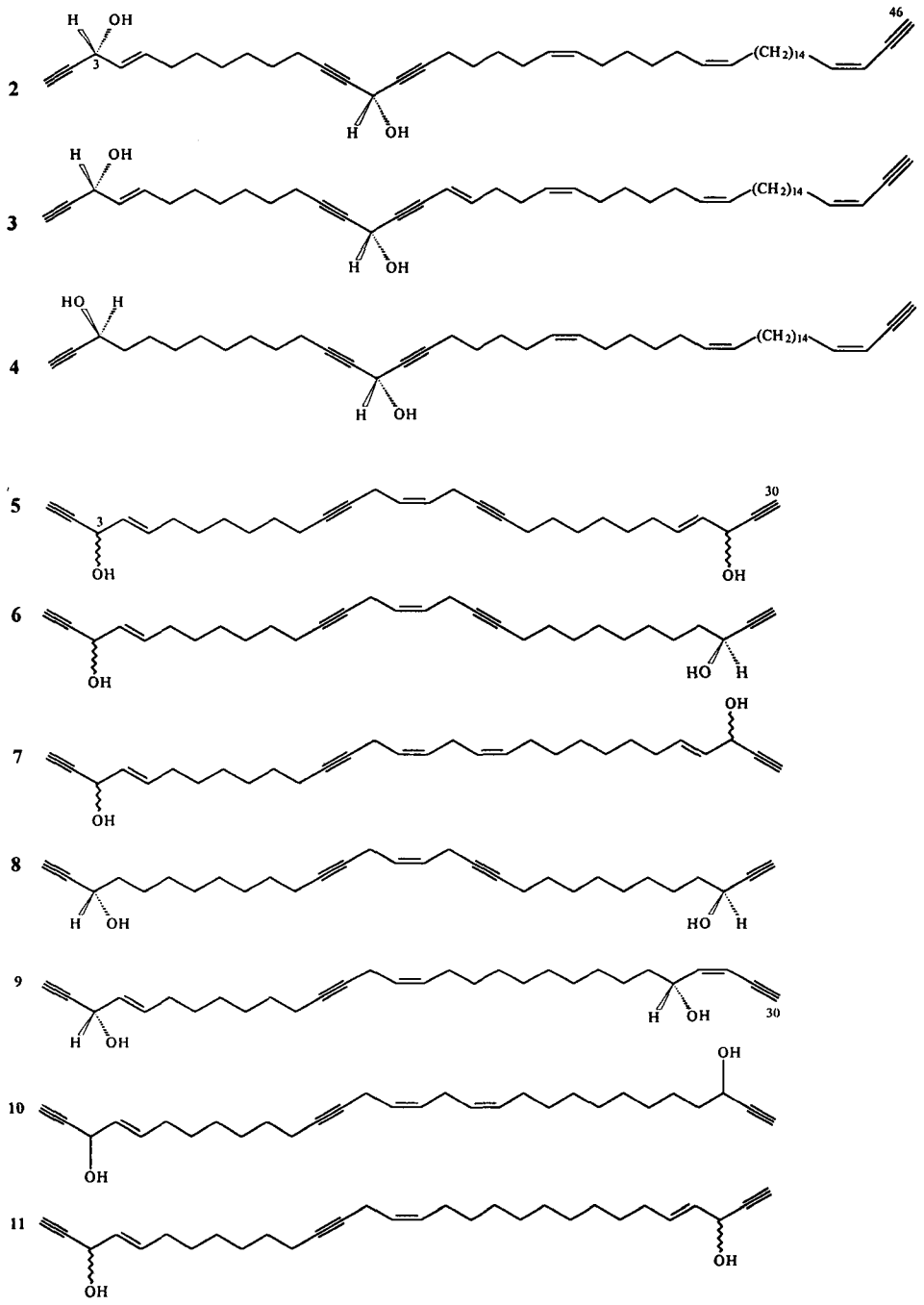


Table 2. *In vitro* Cytotoxicity Data of **5-8** Against a Small Panel of Human Solid Tumor Cell lines*

| compound | A549 | SK-OV-3 | SK-MEL-2 | XF498 | HCT15 |
|---|---|---|---|---|---|
| **5** | 1.43 | 0.02 | 0.01 | 0.16 | 0.17 |
| **6** | 1.98 | 0.21 | 0.11 | 1.83 | 1.56 |
| **7** | 12.41 | 1.83 | 1.27 | 1.83 | 1.87 |
| **8** | 5.78 | 0.02 | 0.02 | 3.02 | 1.94 |
| doxorubicin | 0.09 | 0.16 | 0.11 | 0.13 | 1.02 |

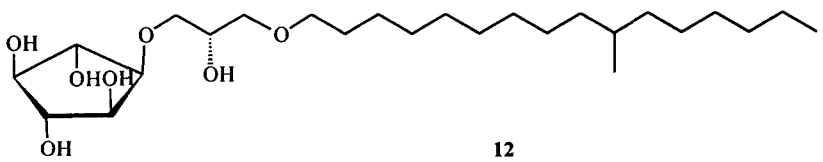
*Data are expressed as $ED_{50}$ values ($\mu$g/ml). Key to cell lines: A549, human lung carcinoma; SK-OV-3, human ovarian cancer; SK-MEL-2, human skin cancer; XF498, human CNS cancer; HCT15, human colon cancer.

Table 3. *In vitro* Cytotoxicity Data of **2-4** and **9-11** Against a Small Panel of Human Solid Tumor Cell lines*

| compound | A549 | SK-OV-3 | SK-MEL-2 | XF498 | HCT15 |
|---|---|---|---|---|---|
| **2** | 1.08 | 0.55 | 1.12 | 1.68 | 0.97 |
| **3** | 1.63 | 0.47 | 0.85 | 1.71 | 1.04 |
| **4** | 1.74 | 2.16 | 1.92 | >3.0 | 3.68 |
| **9** | 1.26 | 0.12 | 0.14 | 0.57 | 0.75 |
| **10** | >3.0 | >3.0 | >3.0 | >3.0 | >3.0 |
| **11** | 1.39 | 0.14 | 0.18 | 1.20 | 1.22 |
| doxorubicin | 0.10 | 0.18 | 0.18 | 0.20 | 0.94 |

*Data are expressed as $ED_{50}$ values ($\mu$g/ml). Key to cell lines: A549, human lung carcinoma; SK-OV-3, human ovarian cancer; SK-MEL-2, human skin cancer; XF498, human CNS cancer; HCT15, human colon cancer.

From the same sponge material, a cyclitol derivative (**12**) was isolated as a DNA replication inhibitor. Compound **12** displayed selective cytotoxicity on the ovarian cancer (SK-OV-3), CNS cancer (XF498), and colon cancer (HCT15) in a small panel of human tumor cell lines. Compared to cisplatin, **12** showed less potent but substantial activity. The effects of **12** on DNA replication were examined using SV40 DNA replication system *in vitro*. Addition of increasing amounts of **12** quantitatively inhibited SV40 DNA replication with HeLa cytosolic extracts (Figure 1). In the presence of 800 mM of **12**, more than 90% of the replication activity was inhibited. It is therefore plausible that **12** predominantly inhibits the initiation stage of DNA replication, and their inhibitory effects may be related to the interaction between **12** and protein(s) required to establish replication forks during the initiation process. To address this possibility, we examined whether **12** inhibited the activity of topoisomerase I (topo I), which is now considered to be an important target for cancer chemotherapeutics. The effects of **12** on the catalytic activity of topo I are shown in Figure 2. The plasmid DNA was in the superhelical form (Figure 2, lane 1), and topo I relaxed the supercoiled DNA (Figure 2, lane 2). Compound **12** did not show any inhibitory effect on topo I activity at all concentrations tested. It will be the subject of future study to address whether **12** inhibits DNA replication either by (a) interaction with DNA-protein complex or (b) inhibition of replication protein(s) or (c) intercalation with DNA.




**12**

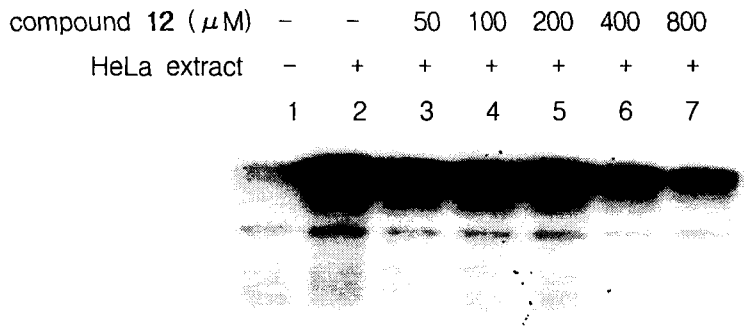



**Figure 1.** The effect of compound 12 on SV40 DNA replication *in vitro*. Replication reaction comprised SV40 origin-containing DNA (pUC), SV40 T-Ag, HeLa cytosolic extract (100 μg), [$^3$H]dTTP, and the indicated amounts of compound 12. Reaction mixtures were incubated for 2 h at 37℃, and the replication products were isolated and separated by 1% agarose gel electrophoresis (Tris-borate-EDTA buffer).

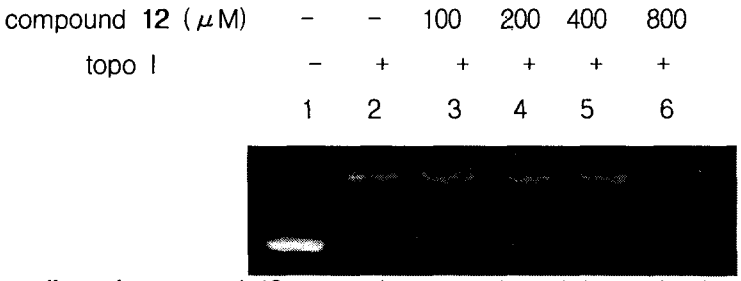
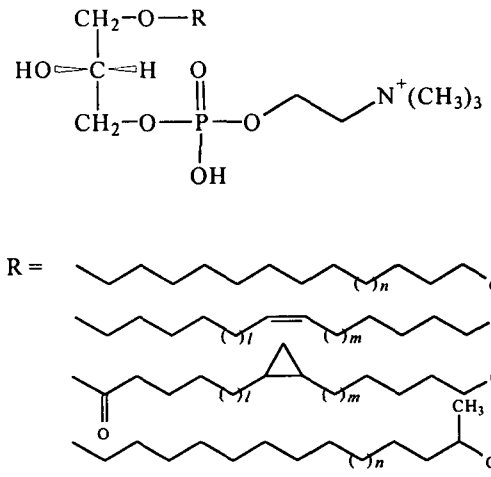



**Figure 2.** The effect of compound 12 on topoisomerase I catalytic activity. Topoisomerase was measured by the relaxation of superhelical plasmid DNA. The assay mixture (20 l) contained pSA (20 μg/ml), topoisomerase I, and various amounts of compound 12. After 30 min at 30℃, the reactions were stopped by the addition of 5 l of stop solution. The samples were then loaded onto the agarose gel (0.8%) for electrophoresis and photography was done.

From the marine sponge, *Spirastrella abata*, a series of lysophosphatidylcholines were isolated as the inhibitors of cholesterol biosynthesis. These lysophosphatidylcholins were found to be either 1-alkyl ether or ester form, with cyclopropyl moiety or methyl branching, which are not ordinary members of phospholipids. These compound inhibited the cholesterol biosynthesis at the midstream of the pathway unlike commercially available hypocholesteremic drugs which suppress synthesis at the level of upstream or downstream.



## References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2. Jung S. Kim, Kwang S. Kim, Jee H. Jung, Young-L. Kim, Jinwoong Kim, Chung J. Shim, and Chong-O. Lee, New Bioactive Polyacetylenes from the Marine Sponge *Petrosia* sp., *Tetrahedron*, 1998, **54**, 3151-3158.
3. Jung Sun Kim, Young Ja Lim, Kwang Sik Im, Jee H. Jung, Chung J. Shim, Chong-O. Lee, Jongki Hong, and Hongkum Lee, Cytotoxic Polyacetylenes from the Marine Sponge *Petrosia* sp., *J. Nat. Prod.*, 1998, accepted.
4. Dong-Kyoo Kim, Young Ja Lim, Jung Sun Kim, Jong Hee Park, Nam Deuk Kim, Kwang Sik Im, Jongki Hong, and Jee H. Jung, A Cyclitol Derivative as a Replication Inhibitor from the Marine Sponge *Petrosia* sp., *J. Nat. Prod.*, 1988, submitted.