CHAPTER V

CONCLUSION

Three new stabilized renieramycin-type including jorunnamycins A-C were isolated from the mantles, the visceral organs, and the egg ribbons of the Thai nudibranch *Jorunna funebris* which was collected from Si Chang Island, in the Gulf of Thailand along with the five known compounds, renieramycins M, N, O, and Q and mimosamycin. Renieramycin M was the major components in the mantles and the egg ribbons. Jorunnamycin A was absent in the visceral organs. Jorunnamycin B was only present in the visceral organs as the main components. Jorunnamycin C was only found in the egg ribbons as major components.

Large-scale preparation of the stabilized renieramycin M from the Thai blue sponge, *Xestospongia* sp. by pretreatment the sponge with potassium cyanide before extraction process was accomplished. The availability of renieramycin M in high yield has led us to prepare twenty-one acyl renieramycin analogs **60-80** including acyclic, alicyclic and aromatic acyl derivatives from the key intermediate deangeloylrenieramycin M. Two step transformations of renieramycin M into deangeloylrenieramycin M in 43.4% yield via the leuco compound, bisdihydroquinonerenieramycin M were accomplished through by hydrogenation over 20% $Pd(OH)_2/C$ and subsequent reduction with AlH_3 in THF and air oxidation. Acylations of deangeloylrenieramycin M into acyl renieramycin analogs were performed by treatment with the corresponding acid anhydrides or acid chlorides to give twenty-one acyl renieramycin analogs **60-78** about 25-85% yield. Moreover, conversions of 21-CN of **67** and **69** into 21-OH of **79** and **80** in 96% yield were easily carried out by treatment with silver nitrate in aqueous acetonitrile.

In vitro cytotoxicity of jorunnamycins A-C was assayed against human carcinoma cell lines including human colon carcinoma (HCT116), human lung carcinoma (QG56), and human prostate carcinoma (DU145). Among the three compounds, the cytotoxicity of jorunnamycin C containing 22-*O*-propionyl side chain was 10-100 times higher than that of jorunnamycin A containing 22-OH at nanomolar concentration. Jorunnamycin B, which contains a dihydroquinone at ring E, 22-OH,

and C-14 carbonyl, showed the least cytotoxicity at about 300-1500 times lower concentration than jorunnamycin C. Evaluation of 50% inhibited cell growth of twenty-one acyl renieramycin analogs was performed with a cell counting kit (DOJINDO, Osaka, Japan) against HCT116, QG56 and DU145, and MTT colorimetric assay against HCT116 and MDA-MB-435 (breast). Most analogs showed much less cytotoxicity than the parent compound renieramycin M exept acetate or propionate acyl renieramycin derivatives exhibited higher cytotoxicity than renieramycin M. Prostate and breast cancer cells were the most sensitive to acyl renieramycin analogs while lung cancer cell was the least sensitive. Free OH and bulky aliphatic acyl at C-22 dramatically reduced cytotoxic activity. The acyl part containing carbon atoms not more than 5 atoms showed high cytotoxicity, similar to renieramycin M. The alicyclic five-membered ring acyl derivatives have more cytotoxicity than the six-membered ring. Para substituted benzoyl derivatives and 4-quinolinyl derivative exhibited equal potency to renieramycin M.

The microarray analysis described in this thesis has made it possible to profile renieramycin M and jorunnamycin C on the basis of compound-induced changes in gene expression (transcriptional fingerprint patterns). The array data allowed us to identify PTPRK gene as a candidate biomarker potentially relevant to the anticancer effect of the tetrahydroisoquinoline class of anticancer agents. The finding that renieramycin M is more potent against cancer cell growth than jorunnamycin C was also confirmed by the gene expression analysis, suggesting that the side chain structure at the C-22 ester part should have a critical impact on the antiproliferative activity of this class of unique anticancer natural products.