学位論文内容の要旨

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学 位 論 文 題 目

Chemical Studies on β-Glucuronidase Inhibition of Compounds Derived from Marine Algae

（海藻由来化合物のβ - グルクロニダーゼ阻害に関する化学的研究）

β-Glucuronidase (EC 3.2.1.31) is an inducible enzyme commonly found in anaerobic *Escherichia*, *Bacteroides*, *Clostridia*, *Peptostreptococcus* genera and responsible to catalyze the cleavage of β-glucuronosyl-*O*-bond. This enzyme activity is related on capability of eliminating and excreting large number of xenobiotics from body as glucuronides via glucuronidation reaction. Inhibition of the enzyme is led to accelerate excretion of xenobiotics. Therefore, β-glucuronidase inhibitors were screened and isolated from algal extracts.

In Chapter 1, 151 algal samples (7 species of green algae, 22 species of brown algae, and 30 species of red algae) were screened in order to isolate the potential β-glucuronidase inhibitors. Based on the assay data, most of the collected red and brown algae showed strong inhibitory activity against *E. coli* β-glucuronidase, especially species from orders Ceramiales, Gigartinales of red algae and Fucales, Laminariales of brown algae.

In Chapter 2, β-glucuronidase inhibitors were isolated from marine red algae and determined structures. From *Neorhodomela aculeata* (Rhodomelaceae, Ceramiales) , three bromophenols were obtained as 2,3-dibromo-4,5-dihydroxybenzyl ether (**F1**), 3-bromo-2-(2,3-dibromo-4,5-dihydroxybenzyl)-4,5-dihydroxybenzyl methyl ether (**F2**) and 3-bromo-2-(2,3-dibromo-4,5-dihydroxybenzyl)-4,5-dihyroxybenzyl alcohol (**F3**). From *Neodilsea yendoana* (Dumontiaceae, Gigartinales), one novel compound (**A6**), 2-hydroxymethyl-2-methoxyoxolan-3-one named as isogloiosiphone B, was purified along with phytal (**A1**), a mixture of phytol (**A2**) and cholesta-4,22-diene-3,6-dione (**A3**), cholesterol (**A4**) and 22-dehydrocholesterol (**A5**).

In Chapter 3, β-glucuronidase inhibitors were isolated from marine brown algae.

From *Sargassum confusum*, *S. thunbergii* (Sargassaceae, Fucales) and *Agarum clathratum* (Agaraceae, Laminariales), three hydrophobic compounds were isolated and identified as the hydrophobic inhibitors (6*Z*,9*Z*,12*Z*,15*Z*)-1,6,9,12,15-henicosapentaene (**J1**), (6*Z*,9*Z*,12*Z*,15*Z*,18*Z*)-1,6,9,12,15,18-henicosahexanene (**U2**) and squalene (**C3**), and phloroglucinol (**G2**).

In Chapter 4, the isolated compounds were compared *in vitro* inhibitory activity and *in silico* accessibility to the active site of the enzyme. With the comparison of inhibitory activity of all the purified compounds against *Escherichia coli* β-glucuronidase (3LPG) were investigated by inhibition kinetic study and molecular docking simulations. A good correlation was observed between IC_{50} values and the molecular binding docking affinities. Hydrogen bonds, van der Waals forces, hydrophobic interactions and electrostatic forces showed important roles in the binding affinity. Bromophenol inhibitors **F2** and **F3** might access to the active site of *E. coli* β-glucuronidase through hydrogen bonding and hydrophobic interactions with residues Pro48, Arg302, Phe306 and Asn308, and Gly17, Ala46, Pro48, Arg302, Phe306, Asp307, Asn308 and Val309, respectively. Hydrophobic inhibitors **J1**, **U2** and **C3** might approach to the active site of *E. coli* β-glucuronidase via the hydrophobic interactions with residues Leu15, Gly17, Ala46, Pro48, Arg302, Phe306, Asp307, Asn308 and Val309.

In conclusion, marine red and brown algae may be the exceptional sources for the isolation of *E. coli* β-glucuronidase inhibitory compounds. Although further investigation is also warranted, the encouraging correlation between *in vitro* inhibition analysis and *in silico* molecular docking simulations may provide the guidance for the exploration of effective *E. coli* β-glucuronidase inhibitors.