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学 位 論 文 要 旨
Dissertation Abstract

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学位論文題目：
Title of Dissertation

Prediction of Binding Mode of Aplysiatoxin with Protein Kinase C and Development of a Synthetically-accessible Aplysiatoxin Analog
（アプリシアトキシンとプロテインキナーゼCとの結合様式の予測と合成が容易なアプリシアトキシンアナログの開発）

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Aplysiatoxin (ATX) and debromoaplysiatoxin (DAT), naturally-occurring tumor-promoters isolated from sea hare, are potent activators of protein kinase C (PKC). ATX and DAT bind to tandem C1 domains (C1A and C1B) in regulatory region of eight isozymes of conventional PKC (α, βI, βII, γ) and novel PKC (δ, ε, η, θ) to activate them. ATX and DAT show both anti-proliferative and tumor-promoting activities *in vitro* and *in vivo*, respectively. On the other hand, their simplified analogs, 10-Me-aplog-1 and 10,12-diMe-aplog-1, show significant anti-proliferative activity against several human cancer cell lines comparable to DAT, but exhibit little tumor-promoting activity. Although these analogs are promising as a potential chemotherapeutic agent, a more synthetically-accessible ATX analog is needed because synthesis of 10-Me-aplog-1 and 10,12-diMe-aplog-1 still requires at least 23 steps in a longest linear sequence. Therefore, the author attempted to develop a synthetically-accessible ATX analog with further simplification.

To rationally design ATX analogs, the author first predicted binding mode of ATX with PKCδ C1B domain (δ-C1B). The molecular docking simulation of ATX with δ-C1B and molecular dynamics simulation of ATX/δ-C1B complex in phospholipid membrane environment suggests that the phenolic hydroxy group at position 20, the carbonyl group at position 27, and the hydroxy group at position 30 of ATX are involved in the intermolecular hydrogen bondings and that the ester groups at position 1 might contribute to generate shape molecular electrostatic potential complimentary to that of the receptor rather than hydrogen bonding. The predicted binding mode of ATX with δ-C1B was consistent with structure–activity studies on aplysiatoxins reported previously and simple acyclic analogs of ATX (**1**–**3**) in this study, and provided a deeper understanding of receptor-recognition by ATX.

This binding model shows that a spiroketal moiety composed by two

tetrahydropyrane rings (A-ring and B-ring) in a conformation-controlling unit (CCU) of ATX is not involved in hydrogen bondings with the PKC C1 domain, which led the author to surmise that the B-ring is more important for PKC binding than the A-ring. Thus, the author designed and synthesized des-A-ring analog (**13**) of 18-deoxy-aplog-1 as a synthetically-accessible analog. Synthesis of **13** was achieved in a longest linear sequence of 11 steps, which is approximately half of that of 18-deoxy-aplog-1. Conformational analysis of the des-A-ring analog **13** revealed that the conformation of the PKC-recognition part of aplogs was hardly affected by the removal of the A-ring, but the conformation of CCU in **13** is slightly different from that of 18-deoxy-aplog-1 with the spiro-ring. This structural change selectively decreased the affinity for novel PKCs, which is somewhat surprising because nearly all structural modifications of aplogs in the previous studies increased the isozyme selectivity toward novel PKCs. Although the reason why **13** retained affinity for C1A domains of conventional PKCs is still not clear, the conformation of positions 2–7 in aplogs might be important in the recognition of C1B domains of novel PKCs. In addition, the spatial orientation of a dimethyl group at position 6 and/or hydrophobicity at positions 4–5 might be also responsible for this phenomenon. Because **13** showed selective anti-proliferative activity against NCI-H460 (lung) and MKN45 (stomach) human cancer cell lines and the installation of a methyl group at positions 10 and/or 12 would be promising to enhance biological activities of **13** as exemplified by 10-Me-aplog-1 and 10,12-diMe-aplog-1, **13** could serve as a synthetically-accessible lead compound for the development of selective chemotherapeutic agents for such types of cancer.

In conclusion, this study revealed that a modification of the spiroketal scaffold in the ATX analogs dose not affect so much the intermolecular hydrogen bondings and that such a modification is effective not only to achieve further simplification and shortening of the synthetic steps but also to provide different selectivity among target proteins and different biological activity from the original compound. These findings also suggest that a modification of spiroketal moiety of natural products in which the spiro ring plays a role in controlling the conformation, like ATX, could be an effective strategy to achieve simplification and change of selectivity between multiple targets of the natural products.