様式８

論　文　内　容　要　旨

報告番号	乙　先　第　34　号	氏　名	大倉　一人
学位論文題目	Exploring the interactive TX-molecules: Applicable 4-cyclopentene-1,3-dione and 2-nitroimidazole structure for drug design （相互作用性TX-分子の探索：4-サイクロペンテン-1,3-ジオンおよび2-ニトロイミダゾール構造の薬剤設計へ応用）		

内容要旨

In interactive analysis between protein and ligand, structural feature of ligand bindable pockets in protein molecule should be considered. The ligand binding pattern can be verified by simulating the ligand invasion mode into the cavity of protein. The compounds used in the present study include five-membered ring components such as 1,3-cyclopentenedione and 2-nitroimidazole. These ring structure can serve as a place for transferring electrons, molecular features can be changed by introducing various functional groups, and as a result, various functions are easily provided. Molecular features for designed compounds has been discussed as follows: 1) Molecular features of 2-hydroxyarylidene-4-cyclopentene-1,3-dione scaffold TX-1123 derivatives. 2) Molecular property of glyco-conjugated TX-1877 derivatives. 3) Molecular chirality and interactive ability of TX-2036 derivatives.

1) Molecular features of 2-hydroxyarylidene-4-cyclopentene-1,3-dione scaffold TX-1123 derivatives. TX-1123 exhibited potent inhibition of Src kinase, and IC_{50} was 2.2 μM. Src kinase inhibition was suppressed by addition of a methoxy group to TX-1123, and IC_{50} of TX-1925 was 3.1 μM. Replacement of two sterically-bulky *tert*-butyl groups in TX-1123 by two methyl groups reduced its inhibitory activity, with an IC_{50} of TX-1918 was 4.4 μM. Interaction was observed between phenolic hydroxyl group of TX-1123 and phenolic hydroxyl group of Tyr^{416} of Src kinase. Methylation of TX-1123 phenolic hydroxy group (*e.g.* TX-1925) also inhibited Src kinase activity. In TX-1918 without bulky *tert*-butyl groups of TX-1123, mobility of phenolic hydroxy group increased, and indicating reaction features different from those of TX-1123 by an interaction with Phe^{405} in Src kinase. Flexibility of TX-1926 was altered by six-membered ring addition to 1,3-cyclopentenedion site, and anti-kinase activity decreased. Reactivity of TX-1927 with Src kinase was not affected by methylation of phenolic hydroxy group.

COX2-inhibitory ($IC_{50} = 1.16\times10^{-6}$ M) effect of TX-1123 was higher than that of COX1-inhibitory ($IC_{50} = 1.57\times10^{-5}$ M) effect. TX-1123 bound at COX2, and oxygen

atom of 4-cyclopentene-1,3-dione region of TX-1123 interacted with Cys^{26} (nitrogen atom) and Gln^{447} (nitrogen atom of amide group). TX-1123 bound COX1, and oxygen atom of 4-cyclopentene-1,3-dione group of TX-1123 interacted with Cys^{41} (nitrogen atom) and Gln^{461} (nitrogen atom of amide group). The oxygen atom of TX-1123 phenolic group interacted with COX1 Arg^{469} (nitrogen atom of side chain).

2) Molecular property of glyco-conjugated TX-1877 derivatives. Heat formation energy of monosaccharide-conjugated TX-1877 derivatives (β-glucose: TX-2141, β-galactose: TX-2218, α-mannose: TX-2217) were greater than that of parent TX-1877. The energies of tetra-*O*-acetylated compounds (TX-2244, TX-2245, TX-2246) were lower than those of monosaccharide-conjugated compounds, and these molecules were stabilized by *O*-acetylation. In monosaccharide- or tetra-*O*-acetyl-conjugated TX compounds, stereo-hydrophobicity (dGW) increased with a decrease in stability. Conformations and hydrophobicities of designed compounds seem to be controlled by addition of monosaccharide- or tetra-*O*-acetyl-conjugated sugar to TX-1877. The tetra-*O*-acetylation of β-glucose moiety of parent TX-2141 significantly improved radiosensitizing efficacy (TX-2244). Acetylation of hydroxyl group in glucose moiety appears to be advantageous for radiosensitization. Regarding TX-2244, the balance between molecular stability and hydrophobicity was thought to work advantageously for radiosensitization ability.

3) Molecular chirality and interactive ability of TX-2036 derivatives. Synthesized TX-2036 *S*-derivatives (TX-2044, TX-2031, and TX-2037) had stronger radiosensitizing activity than the corresponding *R*-derivatives (TX-2043, TX-2030, and TX-2036). Conformation profile and stereo-hydrophobicity analysis did not reveal a difference between *S*- and *R*-configured TX-2036 derivatives. In ESP field analysis, small minus ESP fields were observed only in *R*-configurations in cyclopentene-1,3-dione region. These small minus fields influenced radiosensitizing activity. Radiosensitizing activity of TX-2046 (*S*-configuration) with a small ESP field was lower than that of TX-2045 (*R*-configuration), which suggests that the small ESP fields affect their radiosensitizing activity.

Restrained effect of *R*-configured TX-2036 derivatives on EGFR-tyk activity was higher than *S*-configured derivatives. EGFR-tyk inhibition was 10 times different between *R*-derivatives and *S*-derivatives, and IC_{50} of *R*- and *S*-enantiomers was 1.8 – 21.3 μM and 18.4 – 213.0 μM, respectively. Spatial configuration of TX-2036 derivatives seemed to affect the EGFR-tyk function. All *R*-configured derivatives (TX-2043, TX-2030, TX-2036) interacted with Lys^{721} and Thr^{766} of EGFR-tyk domain. Interacting amino acid residues of EGFR-tyk domain were different for each *S*-derivative as follows; TX-2044 (Ile^{765} and Thr^{766}), TX-2031 (Ser^{696}, Thr^{766}, Thr^{830}), TX-2037 (Gly^{772}, Cys^{773}, Thr^{830}).