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Topological analysis of drug-binding site on α1-acid glycoprotein

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7-hydroxystaurosporine (UCN-01) is a protein kinase inhibitor anticancer drug currently undergoing phase II clinical trial. The low distribution volumes and systemic clearance of UCN-01 in the human patients were found to be partly due to extraordinarily high affinity binding to human alpha1-acid glycoprotein (hAGP). In this study, we photolabeled hAGP with [^{3}H]UCN-01 without further chemical modification. The photolabeling specificity of [^{3}H]UCN-01 was confirmed with the inhibition of covalent bond formation by other hAGP binding ligands. The amino acid sequence of the photolabeled peptide was concluded to be SDVVYTDXK, corresponding to Ser153~Lys161 of hAGP. Since no PTH derivatives could be detected at the 8th cycle, which corresponded to the 160th Trp residue, it was suggested that Trp160 was photolabeled by [^{3}H]UCN-01. Three mutants, namely W25A, W122A and W160A as well as wild type hAGP were photolabeled by [^{3}H]UCN-01. In the experiments using Trp mutants (W25A, W122A and W160A), only W160A showed a marked decrease in the extent of photoincorporation. These results strongly supported that Trp160 play a prominent role in the high affinity binding of [^{3}H]UCN-01 to hAGP. A docking model of UCN-01 and hAGP around Trp160 provided further details on the binding site topology.