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Identification of Potent Inhibitors against Human Peptide Deformylase as Anticancer Agents

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Peptide deformylase (PDF) catalyzes the removal of a formyl group from the N-terminal methionine residue of newly synthesized polypeptides in order to yield mature proteins. This deformylation step is an essential process in bacteria.[1,2] For this reason, PDF has been proposed as an attractive antibacterial target.[3,4] While it has been thought that only prokaryote PDFs are functional, recent studies have shown that eukaryotic PDFs, including human mitochondrial PDF, are also active *in vitro* and *in vivo*.[5] The functionality of human PDF in human mitochondria strongly raises the possibility as a new cancer drug target. Moreover, the PDF inhibitors have been reported that they stimulate cell death or proliferation arrest in a variety of cancer cell lines.[5,6,7] Furthermore, inhibitors against PDF showed low toxicity to human and other animal cells.[8,9] Interestingly, it has been reported that the representative PDF inhibitor including actinonin has a considerable influence on innate immune reactions in human.[10,11] Such consequences can be beneficial for human and also serve to resist cancer.[11]

Several inhibitory mechanism of PDF inhibitors against cancers has been suggested.[12] The PDF inhibitors may induce a tumor-specific mitochondrial membrane depolarization and ATP depletion, which promote cell death or arrest proliferation in a wide variety of cancer cell lines.[5-7] However, the exact mechanism of anticancer effect of PDF inhibitors is still unclear.

In the present study, we aim to identify the new potent human PDF inhibitors for the development of anticancer reagents focused on breast cancer. These inhibitors were previously reported to show strong inhibitory activities against pathogenic bacteria.[13]

For the study, we tested two classes of PDF inhibitors, which consist of the hydroxamate/peptidomimetic [PMT387 (7a) and PMT497] and the reverse hydroxamate/nonpeptide scaffold inhibitors [PMT1039 (15e) and PMT1067] (Figure 1). Most known PDF inhibitors were developed based on the naturally occurring inhibitor, actinonin, which is composed of hydroxamate moiety and peptide backbone.

The compound possessing hydroxamte moiety such as actinonin is an effective inhibitor, however, it shows poor selectivity by chelating the metals of other metalloproteins. This kind of compound can be cytotoxic to mammalian cells and presents poor oral bioavailability related to characteristic rapid metabolization.[14-16] Therefore, our newly developed reverse hydroxamate/nonpeptide scaffold can aid in the design of new potential inhibitors against human PDF.

In a biochemical enzyme assay, the Ki values for the four compounds [PMT387 (7a), PMT497, PMT1039 (15e), and PMT1067] inhibiting the human PDF were measured as 243.5(± 3.2), 91.3(± 29.5), 360.3(± 54.5), and 62.9(± 8.2)

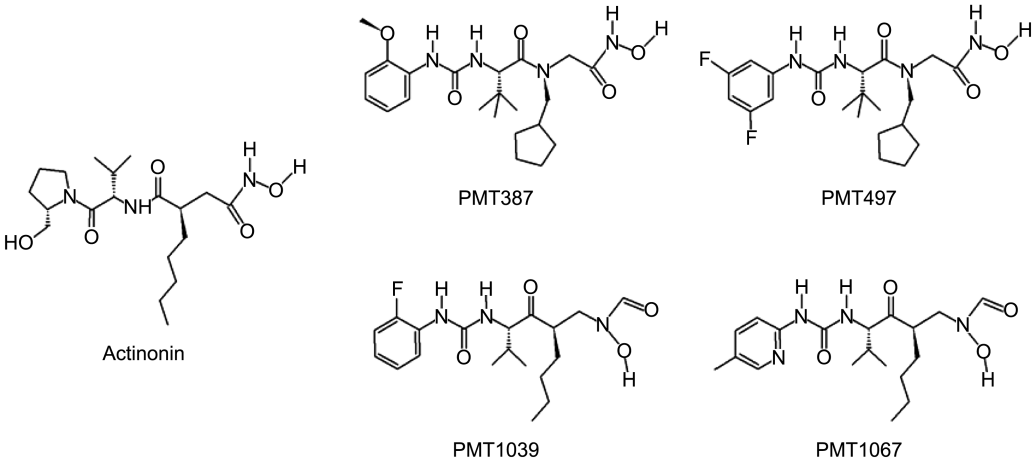

Figure 1. Chemical structures of the peptide-scaffold hydroxamate inhibitors [PMT387 (7a) and PMT497] and non-peptide scaffold reverse hydroxamate inhibitors [PMT1039 (15e) and PMT1067].

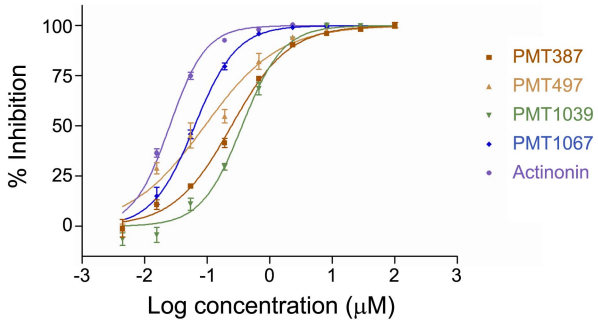

Figure 2. Biochemical PDF assay.

nM, respectively (Figure 2). IC_{50} value of actinonin was 25.1(± 4.4) nM.

We also performed cytotoxicity test for our four chemicals using two kinds of breast cancer cell lines (MDA-MB231 and MDA-MB468). These cell lines were obtained commercially from ATCC. The cancer cells were exposed to four PDF inhibitors ranging from 3 μM to 200 μM and the viability was measured using Sulforhodamine B (SRB) assay.[17] We found that our inhibitors effectively inhibit cell proliferation similar to actinonin (Figure 3). The strong anti-proliferative activities of hydroxamate/peptidomimetic [PMT387 (7a) and PMT497] and the reverse hydroxamate/nonpeptide scaffold inhibitors [PMT1039 (15e) and PMT1067] were detected with IC_{50} values of 28.8 (± 9.8), 20.9(± 5.3), 55.4 (± 5.6), and 54.9(± 10.7) μM, respectively (against MDA-MB231) and 70.6(± 31.9), 21.2(± 6.4), 69.1(± 10.1), and 37.6(± 5.4) μM, respectively (against MDA-MB468) (Figure 3). IC_{50} values of actinonin against MDA-MB231 and MDA-MB468 were 28.1(± 10.3) and 49.4(± 19.5) μM, respectively.

To understand the molecular interactions between human PDF and our two kinds of inhibitors, we have predicted the binding of two kinds of inhibitors to the active site in human PDF by molecular docking study. Human PDF has an approximate sequence identity of 28-34% and shares similar three-dimensional structures with bacterial PDFs.[18] When we superimposed the structure of human PDF to that of *S. aureus* PDF, the overall folds including the active site are highly similar to each other and had r.m.s. deviations

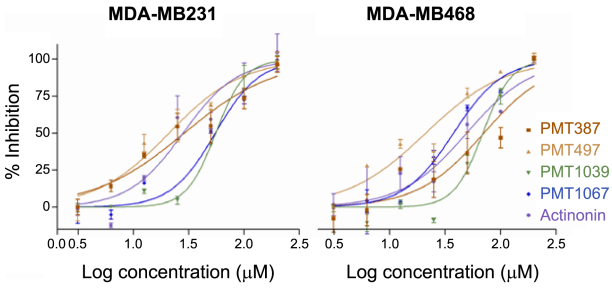

Figure 3. Sulforhodamine B proliferation assay. Dose-response curves of the peptide-scaffold hydroxamate inhibitors [PMT387 (7a) and PMT497] and non-peptide scaffold reverse hydroxamate inhibitors [PMT1039 (15e) and PMT1067] for the inhibition of breast cancer cell lines (MDA-MB213 and MDA-MB468) in the presence of variable concentrations of inhibitors.

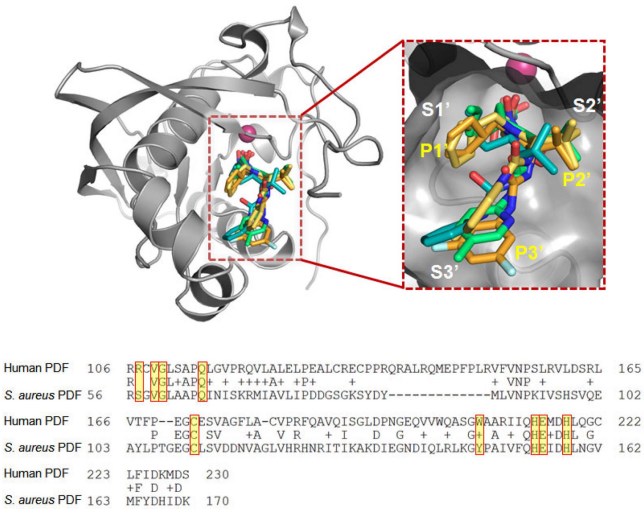

Figure 4. (a) The predicted models of the four inhibitors bound to human PDF (PDB code 3G5P), and (a) sequence alignment of human PDF and *S. aureus* PDF. The P1', P2', and P3' moieties of the inhibitors and the corresponding binding regions in the active site (S1', S2', and S3') are depicted. The one cobalt ion is shown as a sphere. Inhibitors-interacting residues in *S. aureus* PDF are highlighted with a yellow background, which are highly similar to those in human PDF. The figure of sequence alignment was generated using NCBI Blast (http://blast.ncbi.nlm.nih.gov/Blast.cgi).

(r.m.s.d) of only 0.6-0.7 Å for 96 of the Cα atom pairs. The ligand-binding sites which consisted of three major interacting regions (P1', P2', and P'3) as well as a metal are highly conserved in human PDF.

The presence of a cobalt ion at human PDF active site was important in the mode of action of inhibitors. The predicted binding mode of two kinds of PDF inhibitors were shown in Figure 4.

The docking data show good agreement with our previous crystallographic data of four inhibitors-bound *S. aureus* PDF structures.[13] The docking data also suggest that metal (cobalt ion) binder group and active site-interacting groups (S1, S2, and S3) are quite important to form the inhibitory conformation in the binding pocket. Especially, the hydroxamate moieties of PMT387 (7a) and PMT497 and the reverse hydroxamate moieties of PMT1039 (15e) and PMT1067 are expected to form the selective interactions with the cobalt ion. In addition, three ligand-interacting regions (P1', P2', and P3') in PDF also contribute the extra stabilization and selection on the enzyme surface to the corresponding interactive moieties of four ligands (S1, S2, and S3).

In summary, our four PDF inhibitors, which were designed to be developed as antibiotics, were shown to also inhibit human PDF enzyme. The inhibitors could effectively inhibit the proliferations of two representative cancer cell lines. We also predicted the binding modes of four inhibitors, which were very similar to those of our previously reported data.

Experimental Methods

Chemistry and Measurement of PDF Activity. The

inhibitors hydroxamate/peptidomimetic [PMT387 (7a) and PMT497] and the reverse hydroxamate/nonpeptide scaffold inhibitors [PMT1039 (15e) and PMT1067] were synthesized and supplied by ProMediTech (Figure 1).[19,20] Human PDF were purified according to previously reported method[18] and biochemical PDF assays were performed closely followed by previous studies.[13] The IC_{50} value was calculated using nonlinear regression and GraphPad Prism 5.

***In vitro* Cell-based Chemosensitivity Assay.** Cancer cells were cultured in RPMI 1640 containing 10% fetal calf serum, 100 U/mL penicillin, 100 μg/mL streptomycin, and 2 mM L-glutamine (complete RPMI medium). All cancer cells were plated at 1×10^4 cells per well in a 96 well plate. Drugs of different concentrations were then added. The sulforhodamine B assay is used for cell density determination, based on the measurement of cellular protein content.[17]

Molecular Docking Study. The binding modes of four inhibitors to human PDF (3G5P)[18] were predicted using AutoDokc-Vina.[21] One cobalt ion and the flexibility of side chains of the amino acids in the active site were considered.

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