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Synthesis and PDF Inhibitory Activities of Novel Benzothiazolylidenehydroxamic Acid Derivatives

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Abstract—A novel series of benzothiazolylidenehydroxamic acid derivatives has been designed and synthesized as PDF inhibitors. Some of this novel class of PDF inhibitors exhibited micromolar order enzyme inhibitory activity and antibacterial activity.

In recent years, many new antibiotic-resistant bacteria have been appeared throughout the world, and the increasing incidence emphasizes the need to discover and develop novel antibacterial drugs with new modes of action. So far, many successful antibacterial drugs are targeted to inhibit the protein synthesis of bacteria. Prokaryotic protein synthesis begins with formylmethionine-tRNA resulting in the synthesis of N-terminally formylated polypeptides, and the formyl group must be subsequently removed for the proteins to function. Peptide deformylase (PDF), a unique subclass of metalloenzymes, catalyzes the removal of the formyl group at the N-terminus of bacterial proteins.[1] Deformylation of nascent peptides is a unique feature of bacterial cells and is essential for their survival,[2] whereas the peptide deformylase is absent in mammalian cells.[3] Therefore, inhibition of PDF represents an attractive target for the discovery of novel antibiotics. Recent studies from several research groups have shown that PDF inhibitors act as broad-spectrum antibacterial agents.[4]

Based on the mechanism of deformylation of peptides[1] and the pharmacophore requirements for PDF inhibitor,[5] a novel series of benzothiazolylidenehydroxamic acid derivatives was designed and synthesized (Fig. 1). They were subsequently evaluated for their in vitro ability to inhibit PDF and antibacterial activity, and the observed structure–activity relationships were analyzed using computational docking simulation.

The general route for the synthesis of the benzothiazolylidenehydroxamic acid derivatives is outlined in Scheme 1. Benzothiazolylacetic acid ethyl esters **2**(**a**–**d**) were prepared following a published method.[6] Oxidation of **2**(**a**–**d**) with equimolar amount of mCPBA in CH_2Cl_2 afforded olefin esters **3**(**a**–**d**). The resulting esters **3**(**a**–**d**) were hydrolyzed with 4 N NaOH in ethanol to give the corresponding carboxylic acids **4**(**a**–**d**). To convert the carboxyl groups to hydroxamic function moieties, **4**(**a**–**d**) were treated with hydroxylamine hydrochloride in the presence of 1-hydroxybenzotriazole (HOBt), 1-ethyl-3-(3-dimethylaminopropyl)-carbodiimide hydrochloride (EDC) and Et_3N in DMF to afford **5**(**a**–**d**). Oxidation of **2c** with three equivalents of mCPBA gave a sulfonyl compound **6c**, which was then converted to **8c** by using a similar method to the preparation of **5c**. The structures of novel compounds were assigned based on NMR spectra and elemental analyses.[7] The stereochemistry of the double bonds of conjugated carbonyl compounds (**3**–**8**) was determined based on the result of nuclear Overhauser effect (NOE) experiment. For example, irradiation at a frequency corresponding to the methylene protons at $\delta = 3.46$ (2H, q, $J = 7.2$ Hz) of **5a** revealed a clear signal enhancement of proton signal ($\delta = 6.96$) of the double bond of conjugated carbonyl group. Thus, the double bond was assigned to *Z*.

The newly synthesized compounds were evaluated for their in vitro inhibitory effects on *Escherichia coli* Ni-PDF using a published method,[8] and their in vitro antibacterial activities were tested following a guideline of The Chemotherapy Society of Japan.[9] The activities

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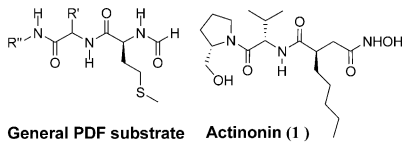


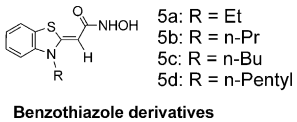
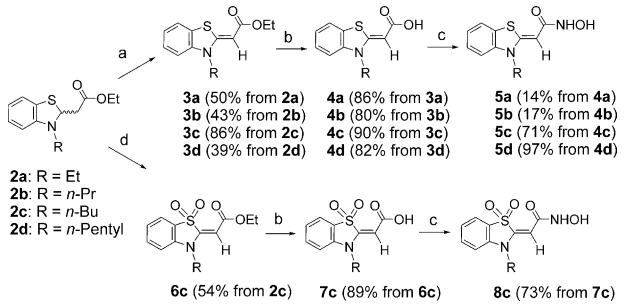


Figure 1. Chemical structures of general PDF substrate, actinonin and the novel benzothiazolylidenehydroxamic acid.



Scheme 1. Reagents and conditions: (a) 1 equiv mCPBA, CH_2Cl_2, rt, overnight; (b) 4 N KOH, EtOH, reflux, 5 h; (c) HOBt, EDC, $NH_2OH{\cdot}HCl$, Et_3N, rt overnight; (d) 3 equiv mCPBA, CH_2Cl_2, rt, overnight.

of these compounds compared to actinonin[8b] are summarized in Table 1.

The hydroxamic acid function group, a bidentate metal chelating moiety, is critical for the enzyme inhibitory activity (**5b**, **5c**, and **5d**). The compound with a carboxylic acid as a metal chelating group (**4c**) is devoid of PDF inhibitory activity. It is not sufficient, however, to only have hydroxamic acid functionality, because the PDF inhibitory activity of these inhibitors is strongly influenced by the nature of the *N*-alkyl substituent R. The activity enhanced as the length of the *N*-alkyl chain increased from ethyl (**5a**, $IC_{50} > 100$ μM), to *n*-propyl (**5b**, $IC_{50} = 8.39$ μM), and *n*-butyl (**5c**, $IC_{50} = 1.04$ μM). When the *N*-butyl group was replaced with *n*-pentyl, however, the activity decreased (**5d**, $IC_{50} = 9.91$ μM).

Table 1. In vitro PDF inhibitory and antibacterial activities of benzothiazolylidenehydroxamic acids

Compd	IC_{50} (μM)[a]	MIC (μg/mL)[b]		
		S. aureus[c]	*E. coli*[d]	*P. aeruginosa*[e]
1[f]	1.50×10^{-3}	3	50	100
4c	>100	>100	>100	>100
5a	>100	>100	>100	>100
5b	8.39	10	>100	100
5c	1.04	30	>100	100
5d	9.91	100	>100	100
8c	>100	>100	>100	>100

[a]The inhibitor concentration that can inhibit 50% of enzyme activity.
[b]Minimal inhibitory concentration.
[c]*Staphylococcus aureus* (ATCC 10390).
[d]*Escherichia coli* (ATCC 25922).
[e]*Pseudomonas aeruginosa* (ATCC 9027).
[f]$K_i = 0.3$ nM (lit.[8b]).

Oxidation of sulfur of **5c** to sulfone (**8c**) resulted in complete loss of PDF inhibitory activity. The PDF inhibitors, **5b**, **5c** and **5d**, showed antibacterial activity against *S. aureus* and *P. aeruginosa*, but they were inactive against *E. coli* (Table 1). There was not a good correlation between the IC_{50} values and the MICs. The precise reason remains to be investigated, but we can speculate that there might be several possible factors, such as poor penetration of the bacterial cell membrane, or recognition by an efficient efflux system of the bacteria.[8b]

Computational docking simulation provided a good explanation for the observed structure–activity relationship in enzyme inhibitory activity. A docking model was prepared by using a crystal structure of *E. coli* Ni-PDF with a ligand MAS (Met-Ala-Ser) reported by Becker et al.[10] and deposited in the Protein Data Bank (code 1BS6). In order to conduct the docking simulations by using the Tripos docking program FlexX™,[11] the Ni atom was displaced with a Zn atom. Minimization of the molecules was conducted by using the Tripos force field (Trips 60) with the Powell method,[12] and the enzyme–inhibitor interactions in FlexX include both polar (hydrogen bond and charge–charge) and nonpolar (hydrophobic) terms. Many of these interactions are geometrically quite restrictive, which allows FlexX to accurately place inhibitors and their fragments. Directly docking a potential inhibitor into the active site of the enzyme covers all possible docking combinations of inhibitor conformations and binding modes. Finally, the docking results were minimized again with the Tripos force field, defining monomers outside the 5-Å area around the inhibitor as an aggregate.

The docking result of actinonin is shown in Figure 2. The actinonin bounds in a similar fashion to that of the crystal structure of actinonin-PDF (PDB code 1G2A),[13] which suggests the reliability of this docking model. The

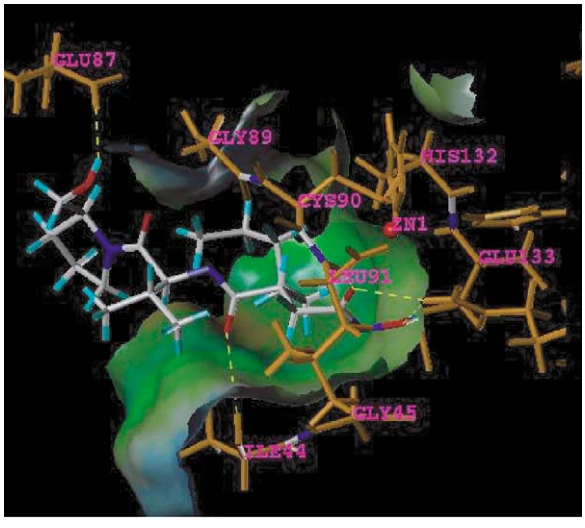


Figure 2. Binding mode of the most stable docking model for actinonin within the substrate pocket of PDF. Hydrogen bonds (distances less than 2.8 Å between the hydrogen bonded to an H-bond donor and an acceptor) are shown in dotted yellow lines. Carbon atoms are shown in white, hydrogens are cyan, nitrogens are blue, and oxygens are red, except those of the amino acids (orange). Zn atom is shown as a red ball.

binding mode of **5c** (Fig. 3), the most potent PDF inhibitor of this study, suggests that the inhibitor lie in a cleft on the enzyme surface and within the active site. The hydroxamic acid moiety was positioned favorably for chelating the Zn atom in the active site of the PDF. The distances from the metal to nitrogen-bound oxygen and carbonyl oxygen of **5c** are 1.70 and 2.65 Å, respectively, while in the case of actinonin the distances are 2.34 and 3.22 Å, respectively. Some hydrogen bonds were also made between carbonyl oxygen of **5c** and Leu 91, between nitrogen-bound oxygen of **5c** and Gln 50, and between NH of **5c** and His 136 of the enzyme. The *N*-*n*-butyl chain of **5c** sat in the hydrophobic S1′ pocket, which was generated by the residues Gly 43, Gly 45, Glu-88, Ile 128, Cys-129, and Glu-133 of the enzyme. The *n*-butyl group made a better fit than the other *N*-alkyl groups, such as ethyl (**5a**), *n*-propyl (**5b**), and *n*-pentyl (**5d**) groups. Since the ethyl group is not long enough to fit into the S1′ pocket of the enzyme, and the *n*-pentyl group is slightly long for the pocket, resulting in an eclipsing interaction at its terminus. While the *n*-propyl group is slightly shorter than that of *n*-butyl group for the S1′ pocket of the enzyme, which accounts for the less potent PDF inhibitory activity of **5b** comparing to **5c**. The benzothiazole ring of the inhibitor is positioned in the S2′ binding pocket of the enzyme to keep hydrophobic interaction with side chains of Ile 44 and Gly 89 of the enzyme. In addition, the FlexX energy of **5c** (−5.08 kcal/mol) was lower than that of **5b** (−5.04 kcal/mol) and **5d** (−4.95 kcal/mol). In the case of actinonin in this study, the FlexX energy was −5.36 kcal/mol. There is a trend of increased energy of interaction in FlexX resulting in decreased activity of enzyme inhibition. The sulfonyl compound **8c** could not fit into the active site of present docking model, which resulted in its lack of PDF inhibitory activity.

In summary, we have discovered benzothiazolylidene-hydroxamic acid as novel class of PDF inhibitors. Some of these inhibitors showed moderate antibacterial activity. In addition, the computational docking simulation study helps rationalize the structure–activity relationship and will assist further lead optimization studies.

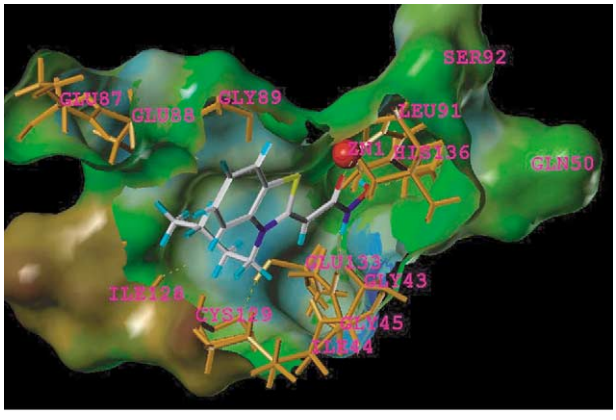


Figure 3. Binding mode of the most stable docking model for **5c** within the substrate pocket of PDF. Hydrogen bonds (distances less than 2.8 Å between the hydrogen bonded to an H-Bond donor and an acceptor) are shown in dotted yellow lines. Carbon atoms are shown in white, hydrogens are cyan, nitrogens are blue, oxygens are red, and sulfur is yellow, except those of the amino acids (orange). Zn atom is shown as a red ball.

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7. **5a:** Orange crystals, mp 63.7–65.9 °C. ^{1}H NMR (DMSO-d_6): δ 1.16 (3H, t, J = 7.2 Hz), 3.46 (2H, q, J = 7.2 Hz), 6.69 (1H, m), 6.78–6.79 (2H, m), 6.96 (1H, s), 6.98 (1H, m), 8.70 (1H, br s), 10.34 (1H, s). **5b:** Orange crystals, mp 118.6–119.4 °C. ^{1}H NMR (DMSO-d_6): δ 0.92 (3H, t, J = 7.2 Hz), 1.52–1.65 (2H, m), 3.36 (2H, t, J = 7.2 Hz), 6.68 (1H, m), 6.77–6.79 (2H, m), 6.96 (1H, s), 6.98 (1H, m), 8.69 (1H, s), 10.33 (1H, s). **5c:** Orange crystals, mp 126.5–126.9 °C. ^{1}H NMR (DMSO-d_6): δ 0.91 (3H, t, J = 7.2 Hz), 1.30–1.42 (2H, m), 1.49–1.59 (2H, m), 3.40 (2H, t, J = 7.2 Hz), 6.68 (1H, m), 6.76–6.79 (2H, m), 6.95 (1H, s), 6.98 (1H, m), 8.70 (1H, s), 10.34 (1H, s). **5d:** Orange crystals, mp 108.1–108.3 °C. ^{1}H NMR (DMSO-d_6): δ 0.87 (3H, d, J = 7.2 Hz), 1.32–1.33 (4H, m), 1.54–1.56 (2H, m), 3.39 (2H, t, J = 7.2 Hz), 6.67 (1H, m), 6.78–6.79 (2H, m), 6.95 (1H, s), 6.98 (1H, m), 8.67 (1H, s), 10.31 (1H, s). **8c:** Colorless crystals, mp 179.2–189.9 °C. ^{1}H NMR (DMSO-d_6): δ 0.89 (3H, t, J = 7.2 Hz), 1.24–1.37 (2H, m), 1.60–1.70 (2H, m), 4.14 (2H, t, J = 7.2 Hz), 7.45 (1H, m), 7.60 (1H, m), 7.74 (1H, m), 7.97 (1H, s), 7.99 (1H, m), 9.19 (1H, s), 10.20 (1H, s).
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