Bioorganic Chemistry **27,** 35–40 (1999)
Article ID bioo.1998.1111, available online at http://www.idealibrary.com on IDEAL®

Hydroxamate Inhibitors of *Aeromonas hydrophila* AE036 Metallo-β-lactamase

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Received August 3, 1998

Amino acid derived hydroxamates were synthesized and tested for inhibitory activity against different metallo-β-lactamases. Several compounds inhibited the clinically relevant enzyme from *Aeromonas hydrophila* AE036.

INTRODUCTION

Enzyme-mediated resistance to β-lactam antibiotics poses a major challenge for the efficient treatment of bacterial infections. Metallo-β-lactamases (class B) form a group of enzymes which contain zinc ions in their active site and whose relevance for the development of bacterial resistance has only recently been widely recognized (*1–3*). Previously known and clinically used inhibitors of "serine" β-lactamases, such as clavulanic acid, are ineffective against the class B metalloenzymes and few effective inhibitors of metallo-β-lactamases have been reported (*4,5*). Recently, we have demonstrated the activity of amino acid-derived trifluoromethyl alcohols and ketones as the first synthetic inhibitors of different metallo-β-lactamases (*6,7*). Herein, we describe the synthesis and the inhibitory activity of amino acid-derived hydroxamates against the metalloenzyme of *Aeromonas hydrophila* AE036. This pathogen is of particular interest as it causes a variety of infections in humans.

EXPERIMENTAL METHODS

Kinetic Essays

The enzymes from *A. hydrophila* and *Bacillus cereus* were prepared according to literature procedures (*12,13*). Analyses were performed at 25°C in 15 mM sodium

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0045-2068/99 $30.00

cacodylate buffer at pH 6.5, using 200 μM Imipenem as reporter substrate for the enzyme from *A. hydrophila* and with 150 μM nitrocefin as reporter substrate for the *B. cereus* enzyme. The pseudo-first-order rate constants were determined by the reporter substrate method with 200 μM imipenem (*14*). The hydrolysis of the antibiotics was monitored by following the absorbance variation resulting from the opening of the β-lactam ring, using an Uvikon 860 spectrophotometer equipped with thermostatically controlled cells and connected to a Copam PC88C microcomputer *via* a RS232C serial interface.

Synthesis

General Procedure 1: Preparation of O-benzyl hydroxamates. To an ice-cooled solution of *O*-benzylhydroxylamine hydrochloride salt (238 mg, 15.0 mmol) in dry dichloromethane (25 ml) was added triethylamine (0.21 ml, 15.0 mmol). After stirring for 5–10 min the required *N*-hydroxysuccinimide ester (10.0 mmol) was added and stirring continued for 4–5 h. The organic layer was washed with aqueous hydrochloric acid (25 ml), water (50 ml), and brine (50 ml). After drying over magnesium sulphate the solvents were evaporated *in vacuo* and the products crystallised from suitable solvents.

General Procedure 2: Preparation of hydroxamic acids. To a solution of the required *O*-benzyl-hydroxamate (2.00 mmol) in methanol (15 ml) was added palladium acetate (200 mg). The flask was placed under a balloon of hydrogen and stirred until (usually 1–2 h) t.l.c. analysis indicated completion of the reaction. The product mixture was diluted with methanol, filtered through a plug of Celite, and the solvents removed *in vacuo*. The products were obtained by crystallization from suitable solvents.

(*RS*)-*O*-Benzyl-*N*-phenoxyacetylalanine hydroxamate 4 was prepared from *N*-phenoxyacetylalanine-*N*-hydroxysuccinimide (3.20 g, 10.0 mmol) following **General Procedure 1** and obtained as fine white needles after crystallization from ethyl acetate/diethyl ether (2.00 g, 61%); mp 95–96°C ν_{max} (KBr): 3165s, 3100-2900br, 1650s, 1600m, 1565m, 1495m, 1450m, 1375m, 1175m cm^{-1}; δ_H (200 MHz, $CDCl_3$): 1.40 (3H, d, *J* 7Hz, CH_3), 4.37–4.48 (3H, m, 2-H and $PhOCH_2$), 4.92 (2H, s, $PhCH_2O$), 6.89–7.37 (11H, m, 10H of aromatic CH and amide NH), 9.30 (1H, s, hydroxamate NH); δ_C (50.3 MHz, $CDCl_3$): 18.3 (CH_3), 46.2 (C-2), 66.9 ($PhOCH_2$), 78.2 ($PhCH_2O$), 114.9, 122.1, 128.7, 128.9, 129.5, 130.0 (aryl CH), 135.5, 157.3 (*ipso* aryl), 168.7, 169.6 (C=O); *m/z* (Scan AP^+): 351 (M+Na, 10%), 268 (5%), 178 (100%), 149 (15%), 107 (35%). Required for $C_{18}H_{20}N_2O_4$: C, 65.84; H, 6.14; N, 8.53%. Found: C, 65.93; H, 6.10; N, 8.56%.

(*RS*)-*O*-Benzyl-*N*-phenoxyacetylphenylalanine hydroxamate 5 was prepared from *N*-phenoxyacetylphenylalanine-*N*-hydroxysuccinimide (3.96 g, 10.0 mmol) following **General Procedure 1** and obtained as fine white plates after crystallization from diethyl ether (3.59 g, 89%); mp 109–110°C ν_{max} (KBr): 3240s, 1660s, 1600m, 1550m, 1495s, 1390s, 1260m cm^{-1}; δ_H (200 MHz, $CDCl_3$): 3.04–3.11 (2H, m, $PhCH_2$), 4.17 (2H, s, $PhOCH_2$), 4.65–4.83 (3H, m, $PhCH_2O$, and 2-H), 6.84 (1H, br, d, *J* 8Hz, amide NH), 6.86–7.36 (15H, m, aromatic CH), 9.02 (1H, s, hydroxamate NH); δ_C (50.3 MHz, $CDCl_3$): 38.3 ($PhCH_2$), 51.6 (C-2), 66.8 ($PhOCH_2$), 78.2 ($PhCH_2O$), 114.7, 122.2, 127.1, 128.5, 128.7, 129.3, 129.4, 129.8 (aryl CH), 135.1,

135.9, 156.9 (*ipso* aryl), 167.8, 168.6 (C=O); *m/z* (NH_3): 405 (MH^+, 75%), 387 (5%), 299 (70%), 282 (90%), 254 (95%), 151 (35%), 120 (100%), 91 (65%). Required for $C_{24}H_{24}N_2O_4$: C, 71.27; H, 5.98; N, 6.93%. Found: C, 71.39; H, 5.90; N, 6.90%.

(*RS*)-*N*-Benzoyl-*O*-benzylalanine hydroxamate 6 was prepared from *N*-benzoyl-alanine-*N*-hydroxysuccinimide (2.90 g, 10.0 mmol) following **General Procedure 1** and obtained as fine white needles after crystallization from diethyl ether (2.83, 95%); mp 147–149°C ν_{max} (KBr): 3285s, 3180m, 3060m, 1690m, 1630s, 1575m, 1490m, 1375m, 1245m cm^{-1}; δ_H (200 MHz, $CDCl_3$): 1.43 (3H, d, *J* 7Hz, CH_3), 4.66 (1H, qui, *J* 7Hz, 2-H), 4.85 (2H, s, $PhCH_2O$), 7.10–7.78 (11H, m, 10H of aromatic CH, and amide NH), 10.02 (1H, s, br, hydroxamate NH); δ_C (50.3 MHz, $CDCl_3$): 18.1 (CH_3), 46.8 (C-2), 78.2 ($PhCH_2O$), 127.5, 127.7, 127.8, 129.1, 129.4, 132.2 (aryl CH), 133.5, 135.3 (*ipso* aryl), 168.7, 170.3 (C=O); *m/z* (Scan AP^+): 299 (MH^+, 5%), 225 (100%), 176 (80%), 148 (35%), 105 (25%). Required for $C_{17}H_{18}N_2O_3$: C, 68.44; H, 6.08; N, 9.39%. Found: C, 68.60; H, 6.14; N, 9.44%.

(*RS*)-*N*-Phenoxyacetylalanine hydroxamate 7 was prepared from **4** (656 mg, 2.00 mmol) following **General Procedure 2** and obtained as fine white needles after crystallization from methanol/diethyl ether (286 mg, 60%); mp 141–143°C; ν_{max} (KBr): 3340s, 3195s, 3400–2800br, 1650s, 1600m, 1540s, 1495m, 1460m, 1375m, 1290m cm^{-1}; δ_H (200 MHz, $(CD_3)_2SO$): 1.22 (3H, d, *J* 7Hz, CH_3), 4.26 (1H, qui, *J* 7Hz, 2-H), 4.50 (2H, s, $PhOCH_2$), 6.91–7.37 (5H, m, aromatic CH), 8.15 (1H, d, *J* 8Hz, NH), 8.90 (1H, br); δ_C (50.3 MHz, $(CD_3)_2SO$): 18.9 (CH_3), 46.2 (C-2), 67.0 ($PhOCH_2$), 115.2, 121.8, 130.2 (aryl CH), 158.5 (*ipso* aryl), 168.0, 169.5 (2×C=O); *m/z* (Scan AP^+): 239 (MH^+, 5%), 206 (25%), 178 (100%), 150 (15%), 111 (10%). HRMS: Calcd for $C_{11}H_{15}N_2O_4$ (MH^+): 239.1032. Found: 239.1032.

(*RS*)-*N*-Phenoxyacetylphenylalanine hydroxamate 8 was prepared from **5** (808 mg, 2.00 mmol) following **General Procedure 2** and obtained as fine white needles (354 mg, 56%) after crystallization from methanol/petroleum ether (30–40); mp 128–130°C; ν_{max} (KBr): 3400–3000br, 3215s, 1665s, 1645s, 1600m, 1540m, 1495s, 1375s, 1225m cm^{-1}; δ_H (200 MHz, $(CD_3)_2SO$): 3.81–3.00 (2H, m, $PhCH_2$), 3.28–3.38 (3H, m, $PhOCH_2$, and 2-H), 6.75–7.27 (11H, m, incl. 10H of aromatic CH), 8.24 (1H, d, *J* 7Hz, NH), 8.91 (1H, br, NHO*H*); δ_C (50.3 MHz, $(CD_3)_2SO$): 37.9 ($PhCH_2$), 51.7 (C-2), 66.6 ($PhOCH_2$), 114.7, 121.2, 126.5, 128.3, 129.3, 129.6 (aryl CH), 137.7, 157.9 (*ipso* aryl), 167.5, 167.6 (C=O); *m/z* (Scan AP^-): 314 (M^+, 20%), 313 (M^+_H, 100%). Required for $C_{17}H_{18}N_2O_4$: C, 64.96; H, 5.77; N, 8.91. Found: C, 64.83; H, 5.42; N, 8.64%.

(*RS*)-*N*-Benzoylalanine hydroxamate 9 was prepared from **6** (596 mg, 2.00 mol) following **General Procedure 2** and obtained as fine white needles (187 mg, 45%) after crystallization from methanol/petroleum ether (30–40); mp 144–146°C ν_{max} (KBr): 3400–2800br, 1665m, 1630s, 1605m, 1560s, 1490m, 1455m, 1320s, 1245m cm^{-1}; δ_H (200 MHz, $(CD_3)_2SO$): 1.32 (3H, d, *J* 7Hz, CH_3), 4.40 (1H, qui, *J* 7Hz, 2-H), 7.41–7.91 (5H, m, aromatic CH), 8.51 (1H, d, *J* 7Hz, NH), 8.85 (1H, br), 10.69 (1H, br); δ_C (50.3 MHz, $(CD_3)_2SO$): 18.1 (CH_3), 47.0 (C-2), 127.6, 128.2, 131.3 (aryl CH), 134.1 (*ipso* aryl), 166.1, 169.4 (C=O); *m/z* (Scan AP^+): 225 ($M-H+NH_4^+$, 100%), 209 (MH^+, 20%), 176 (60%), 148 (50%), 105 (30%). HRMS: Calcd for $C_{10}H_{13}N_2O_3$ (MH^+): 209.0927. Found: 209.0938.