Articles

2-(2-Oxo-1,4-dihydro-2*H*-quinazolin-3-yl)- and 2-(2,2-Dioxo-1,4-dihydro-2*H*-2λ^6-benzo[1,2,6]thiadiazin-3-yl)-*N*-hydroxy-acetamides as Potent and Selective Peptide Deformylase Inhibitors

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Received August 14, 2000

Potent, selective, and structurally new inhibitors of the Fe(II) enzyme *Escherichia coli* peptide deformylase (PDF) were obtained by rational optimization of the weakly binding screening hit (5-chloro-2-oxo-1,4-dihydro-2*H*-quinazolin-3-yl)-acetic acid hydrazide (**1**). Three-dimensional structural information, gathered from Ni-PDF complexed with **1**, suggested the preparation of two series of related hydroxamic acid analogues, 2-(2-oxo-1,4-dihydro-2*H*-quinazolin-3-yl)-*N*-hydroxy-acetamides (**A**) and 2-(2,2-dioxo-1,4-dihydro-2*H*-2λ^6-benzo[1,2,6]thiadiazin-3-yl)-*N*-hydroxy-acetamides **(B)**, among which potent PDF inhibitors (**37**, **42**, and **48**) were identified. Moreover, two selected compounds, one from each series, **36** and **41**, showed good selectivity for PDF over several endoproteases including matrix metalloproteases. However, these compounds showed only weak antibacterial activity.

Introduction

Peptide deformylase (PDF, EC 3.5.1.27) is believed to be an essential enzyme in both Gram-positive and Gram-negative bacteria.[1−3] In eukaryotes, gene sequences similar to *def* (PDF gene) have been identified, but their functions have not yet been rigorously established.[4,5] PDF catalyzes the removal of a formyl group attached to the N-terminus of the leading methionine from nascent polypeptide chains. Initial studies with PDF were hampered by the fact that PDF was isolated accidentally as an almost inactive but thermodynamically stable zinc complex.[6] The native enzyme, however, turned out to be the oxidatively very labile Fe(II) complex.[7,8] The mechanism of action of PDF has been extensively studied.[9,10] The search for selective inhibitors of PDF has relied on modifying the thermolysin inhibitor thiorphane,[11−13] the calpain inhibitor calpeptin,[14] the naturally occurring antibacterial hydroxamate actinonin,[15−18] the angiotensin II receptor antagonists from the biphenyl tetrazole class,[19] and the anticholesteremic thyropropic acid.[20] Recently, the orally bioavailable *N*-formyl hydroxylamine BB-3497 has given momentum to the validity of PDF as a novel antibacterial target.[21]

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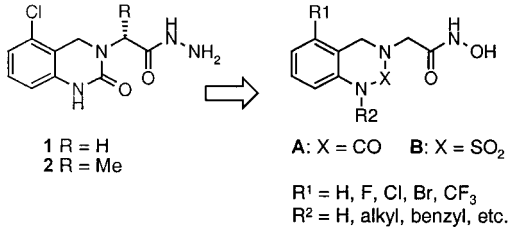
Screening, X-ray, and Modeling Results

High-throughput screening of our compound repository in an *Escherichia coli* PDF based assay afforded the weakly binding ($IC_{50} = 27\mu M$) hydrazide **1** (Scheme 1), which was successfully cocrystallized with *E.coli* Ni-PDF (Figure 1): The hydrazide group of **1** was observed to chelate the Ni^{2+} cation bidentally. The 5-chloro substituent of hydrazide **1** was localized in the hydrophobic P_1' pocket which accommodates the side chain of methionine in the natural enzyme/substrate complex. The urea carbonyl group of **1** did not undergo a hydrogen-bonding interaction with the enzyme. Compound **2** (Scheme 1), the α-methyl analogue of hydrazide **1**, was devoid of PDF inhibitory activity, indicating an unfavorable steric interaction with the protein in the vicinity of the hydrazide function. From our X-ray structure of PDF complexed with actinonin,[18] we knew that the backbone NH of Ile44 forms an H-bond with the substrate-like inhibitor actinonin. We superimposed the two structures of PDF complexed with actinonin and hydrazide **1**, respectively, and speculated that a tetrahedral SO_2 group instead of the in-plane carbonyl group of **1** might undergo a similar hydrogen-bonding interaction with the backbone (Figure 2).

On the basis of our X-ray and modeling studies, we proposed the general structures **A** and **B** (Scheme 1), wherein the hydrazide group of **1** was replaced by a hydroxamic acid function resulting in stronger metal coordination. Substituent R^1 should be a small lipophilic substituent (F, Cl, Br, or CF_3), filling the hydrophobic methionine pocket optimally. Substituent R^2, which points toward the surface of the enzyme, would probably allow broader structural variation.

10.1021/jm000352g CCC: $20.00
Published on Web 05/05/2001

Scheme 1



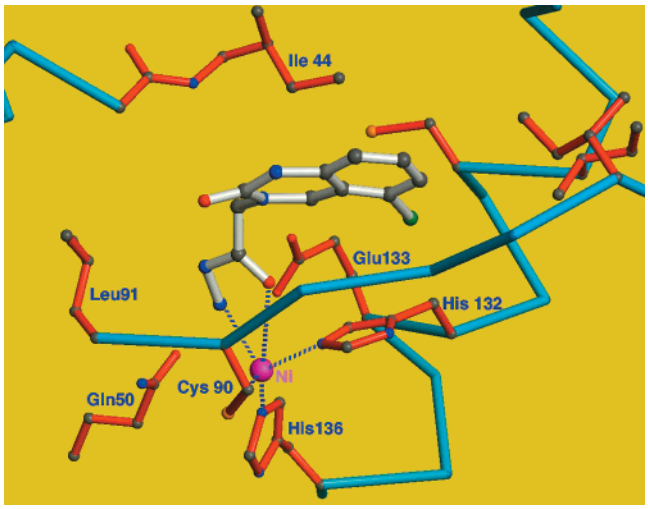


Figure 1. X-ray crystal structure of hydrazide **1** bound to Ni-PDF. The inhibitor is shown as ball-and-stick in white. The protein Cα trace is in cyan, with selected active site residues in red. The Ni^{2+} ion is shown as a magenta ball, with blue dashed bonds to its three protein ligands and to the hydrazide oxygen and nitrogen.

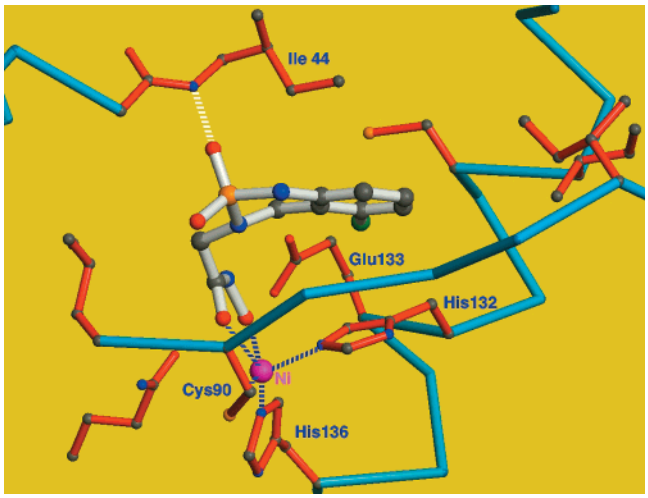


Figure 2. Model predicting the binding mode of hydroxamate **41**. Color scheme is as in Figure 1. The predicted hydrogen-bonding interaction with Ile44 is indicated with a white dashed line.

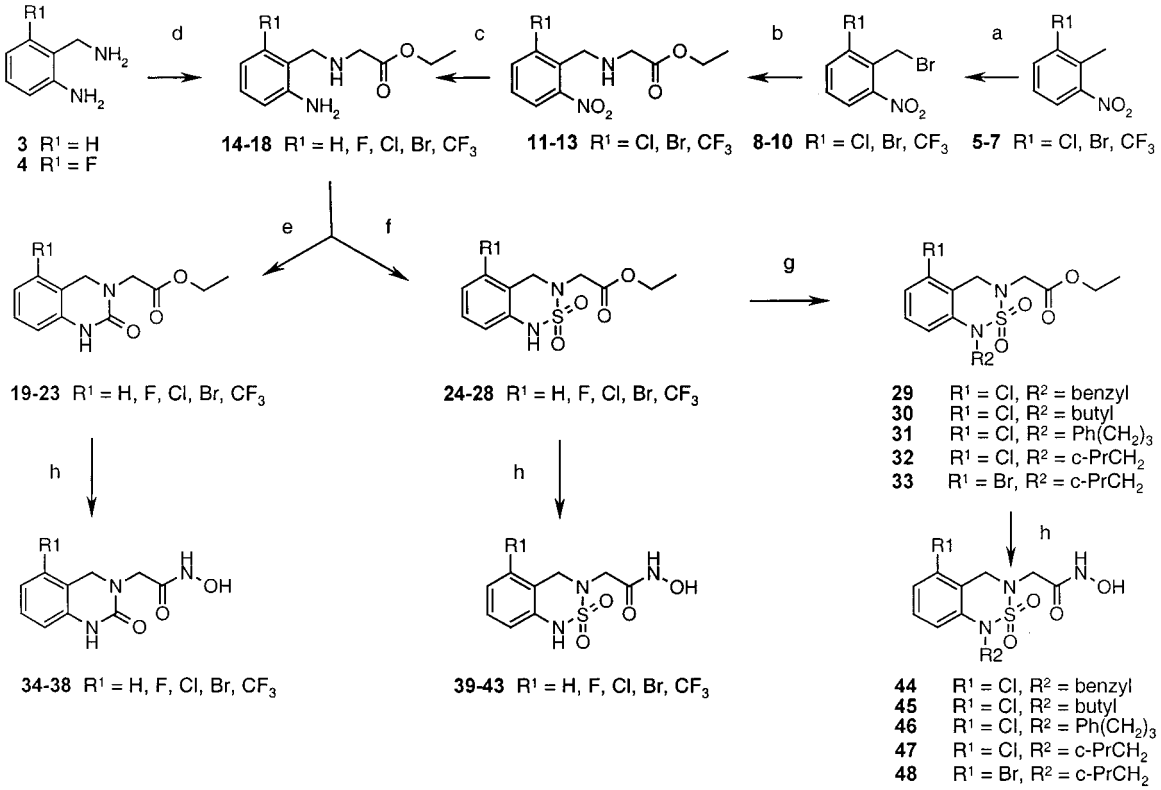
Chemistry

The choice of our synthetic routes (Scheme 2) was guided by the availability of suitable starting materials already carrying the substituent R^1. The 2-amino-benzylamines **3** (R^1 = H) and **4** (R^1 = F) were monoalkylated with ethyl bromoacetate in dioxane at reflux (method D).[22] The 2-nitrotoluenes **5**−**7** (R^1 = Cl, Br, CF_3) were readily photobrominated (method A) despite the presence of strongly deactivating substituents in the ortho position (**10**, R^1 = CF_3).[23] The resulting benzyl bromides **8**−**10** were then used to monoalkylate glycine ethyl ester with NaH in DMF (method B). Bisalkylation was not observed since the benzyl bromides **8**−**10** are deactivated by the bulk and the electron-withdrawing nature of the two ortho substituents (NO_2 and R^1 = Cl, Br, CF_3). The nitro groups of **11** and **12** were then reduced chemoselectively by catalytic hydrogenation over Raney nickel which left the sensitive substituents (R^1 = Cl, Br) untouched (method C-1).[24] The nitro group of **13** was reduced simply by catalytic hydrogenation over Pd/C in THF (method C-2). Ring closure of **14**−**18** was achieved with either bis(trichloromethyl)carbonate in dioxane (method E)[25] or with excess sulfamide in refluxing pyridine (method F).[26] The resulting compounds **19**−**28** were directly transformed into the corresponding hydroxamic acids **34**−**43** with an excess of potassium salt of hydroxylamine in THF/water at 0 °C (method H).[27] This transformation had to be controlled carefully because under the basic reaction conditions partial hydrolysis to the corresponding carboxylic acid can occur. The target compounds **34**−**43** (R^2 = H) were quite insoluble and precipitated either directly from the reaction medium or from the organic layer after extraction. The more lipophilic target compounds **44**−**48** (R^2 ≠ H) could be extracted and purified by silica gel chromatography, thus removing traces of the corresponding carboxylic acid. The ^{1}H NMR spectra of compounds **34**−**48** revealed that they exist in solution as mixture of *E*/*Z*-rotamers leading to doubling of most signals. A more laborious route to the hydroxamic acids **34**−**48** involved 1,1′-carbonyldiimidazole coupling of the acids derived from esters **21**−**33** with O-protected hydroxylamines (Bn, THP, MOM, TBDMS), followed by deprotection. While the coupling was straightforward, clean deprotection was difficult to reproduce, and we therefore abandoned this route. The library of final compounds was extended by introducing lipophilic substituents R^2 (benzyl, *n*-butyl, 3-phenylpropyl, and 1-cyclopropylmethyl) into compound **26** or **27** via standard alkylation with the corresponding alkyl bromides (method G), affording the intermediates **29**−**33**, which were transfomed into the corresponding hydroxamic acids **44**−**48** by method H.

Biological Results and Discussion

1. Inhibition of Native *E. coli* PDF. The proposed structural modifications of the initial screening hit **1** (Scheme 1) led to the discovery of potent inhibitors of the native *E. coli* PDF (Table 1). Compound **36**, the hydroxamate analogue of the original hydrazide **1**, was 90 times more potent. Compound **41**, the SO_2 analogue of **36**, was another 3 times more active, less than one would have expected for an additional strong H-bond but still beneficial. The small lipophilic substituent R^1 (F, Cl, Br, CF_3) was crucial for activity. The bromo substituent was optimum in size, as demonstrated with the most potent inhibitor **37**, which was 40 times more potent than its nonsubstituted analogue **34**. In series **B**, this phenomenon was less pronounced than in series **A**, where the three compounds **41** (R^1 = Cl), **42** (R^1 = Br), and **43** (R^1 = CF_3) were almost equally potent. Attachment of a lipophilic substituent R^2 to compound **41** did not improve the potency as demonstrated with derivatives **44**−**47** (R^2 = benzyl, *n*-butyl, 3-phenylpropyl,

Scheme 2[a]



[a] Reagents: (a) NBS, CCl_4, cat. BPO, light, reflux (method A); (b) ethyl glycinate, NaH, DMF, rt (method B); (c) RaNi, H_2, TEA, THF (method C-1); or Pd/C, H_2, THF (method C-2); (d) $BrCH_2CO_2Et$, TEA/dioxane, reflux (method D); (e) bis(trichloromethyl)carbonate, dioxane, rt (method E); (f) sulfamide, pyridine, reflux (method F); (g) R^2Br, NaH, DMF (method G); (h) 5 equiv NH_2OK, THF/H_2O, rt (method H).

Table 1. Preparation, Analytical Data, Inhibition of Native PDF, and in Vitro Antibacterial Activities against Selected Strains

compd	R^1	R^2	X	method[a]	yield[b] (%)	mp (°C)	elem. analysis[c]	MS (*m/z*)	IC_{50} (μM) PDF Fe[f]	MIC (μg/mL) *E.c.*[g]	*H.i.*[h]	*M.c.*[i]
1	Cl	H	CO	nd	nd	>300	$C_{10}H_{11}ClN_4O_2$	255[d]	27	50	nd	nd
34	H	H	CO	D, E, H	15	190	$C_{10}H_{11}N_3O_3$	220[e]	1.900	>64	>64	>64
35	F	H	CO	D, E, H	56	215	$C_{10}H_{10}FN_3O_3$	238[e]	0.870	>64	64	16
36	Cl	H	CO	A−C, E, H	58	209	$C_{10}H_{10}ClN_3O_3$	254[e]	0.310	16	8	2
37	Br	H	CO	A−C, E, H	62	208	$C_{10}H_{10}BrN_3O_3$	300[e]	0.049	32	16	4
38	CF_3	H	CO	A−C, E, H	45	198	$C_{11}H_{10}F_3N_3O_3$	288[e]	0.230	64	64	8
39	H	H	SO_2	D, F, H	63	foam	$C_9H_{11}N_3O_4S$	256[e]	0.760	>64	>64	>64
40	F	H	SO_2	D, F, H	55	144	$C_9H_{10}FN_3O_4S$	274[e]	0.870	>64	>64	64
41	Cl	H	SO_2	A−C, F, H	52	foam	$C_9H_{10}ClN_3O_4S$	290[e]	0.120	>64	16	8
42	Br	H	SO_2	A−C, F, H	50	85	$C_9H_{10}BrN_3O_4S$	336[e]	0.098	32	>64	4
43	CF_3	H	SO_2	A−C, F, H	35	145	$C_{10}H_{10}F_3N_3O_4S$	324[e]	0.130	>64	32	8
44	Cl	benzyl-	SO_2	A−C, F−H	55	foam	$C_{16}H_{16}ClN_3O_4S$	382[e]	2.000	64	32	8
45	Cl	*n*-butyl	SO_2	A−C, F−H	37	143	$C_{13}H_{18}ClN_3O_4S$	346[e]	0.590	>64	64	2
46	Cl	$Ph(CH_2)_3$-	SO_2	A−C, F−H	13	125	$C_{18}H_{20}ClN_3O_4S$	408[e]	0.290	>64	32	8
47	Cl	c-$PrCH_2$-	SO_2	A−C, F−H	80	145	$C_{13}H_{16}ClN_3O_4S$	344[e]	0.110	>64	64	4
48	Br	c-$PrCH_2$-	SO_2	A−C, F−H	60	foam	$C_{13}H_{16}BrN_3O_4S$	388[e]	0.069	64	64	1

[a] Methods A−H refer to the general procedures described in the Experimental Section. [b] For the final step. [c] C, H, N. [d] ISP $(M + H)^+$. [e] ISN $(M - H)^-$. [f] Inhibition of the isolated *E. coli* enzyme demonstrated to contain mainly iron. [g] *E. coli* DC2. [h] *H. influenzae* 11. [i] *M. catarrhalis* RA21.

1-cyclopropylmethyl). In contrast to our modeling expectations, the *N*-benzyl derivative **44** was almost 20 times less active, and this was the case for a whole series of benzyl analogues substituted on the benzene ring (data not shown). The best substituent R^2 was 1-cyclopropylmethyl (compound **47**), which brought the activity back to the level of **41**. A combination of the best substituents R^1 (Br) and R^2 (1-cyclopropylmethyl) afforded the second most potent compound **48** of the whole series.

2. Inhibition of Endoproteases. The hydroxamic acid derivatives **36** and **41** were selective for PDF over representative endopeptidases found in the cardiovascular system (Table 2). Moreover, these compounds showed selectivity for PDF over human macrophage

Table 2. Inhibition of Several Endopeptidases and MMPs by the PDF Inhibitors

compd	IC_{50} (μM)[a] PDF-Fe	ACE	ECE	NEP	COL-3	HME
36	0.31	90	>10	>100	0.46	>100
41	0.12	>100	>10	>100	>0.5	>100

[a] ACE, angiotensin-converting enzyme; ECE, endothelin-converting enzyme; NEP, neutral endopeptidase; COL-3, collagenase-3 (MMP-13); HME, human macrophage elastase (MMP-12).

elastase (MMP-12) and/or collagenase-3 (MMP-13), a goal we had missed by our first approach.[18]

3. Antibacterial in Vitro Activity. The PDF inhibitors were routinely tested for Gram-negative antibacterial activity with the permeable outer membrane mutant *E. coli* DC2, *Haemophilus influenzae*, and *Moraxella catarrhalis* RA21 (Table 1). However, despite potent enzyme inhibition, the hydroxamic acids **36**−**38, 41**−**43**, and **46**−**48** were only weakly active in the first two strains. The results of pertinent uptake studies will be published elsewhere.[28] In the strain *M. catarrhalis*, substantial antibacterial activity was observed.

Conclusions

Starting from a weakly binding screening hit, whose binding mode could be elucidated by X-ray analysis, we have rationally designed and synthesized highly potent inhibitors of PDF, some of which bound 500 times better than the initial screening hit. Upon broader testing, the inhibitors were found to exhibit selectivity for PDF over endoproteases, including matrix metalloproteinases (MMPs). The antibacterial activity was reasonable in *M. catarrhalis*, but only marginal in other Gram-negative strains such as *H. influenzae* and *E. coli* DC2. Our approach demonstrates how new structural scaffolds can be identified and optimized by applying a combination of screening, X-ray, and medicinal chemistry.

Experimental Section

General Procedures. The following reagents and intermediates were commercially available or prepared by literature procedures: 2-chloro-6-nitrobenzyl bromide **8** (commercial), 2-bromo-6-nitrobenzyl bromide **9** (method A),[29] *N*-(2-chloro-6-nitrobenzyl)glycine ethyl ester **11** (method B),[24] (2-bromo-6-nitro-benzylamino)-acetic acid ethyl ester **12** (method B),[24] and (2-amino-benzylamino)-acetic acid ethyl ester **14** (method D).[30] The benzyl bromides **8**−**10** are strong lacrimators and should be manipulated in a ventilated hood. All organic extracts were dried over Na_2SO_4, filtered, and evaporated under vacuum. All chromatographic separations were performed on prepacked Flash cartridges of KP-Sil silica, 32−63 μm, 6 nm (12, 40, or 90 g), unless otherwise stated. ^{1}H NMR spectra (400 MHz) were measured in $CDCl_3$ unless otherwise stated. Chemical shifts (δ) are reported in ppm relative to Me_4-Si as internal standard. Coupling constants (J) are given in hertz. Ion spray and EI mass spectra were measured on a Finningan MAT SSQ 7000 and Perkin-Elmer Siex API III recorder, respectively.

Method A: 2-Nitro-6-trifluoromethyl-benzyl Bromide (10). 2-Nitro-6-(trifluoromethyl)toluene (5.00 g, 24 mmol), *N*-bromosuccinimide (4.34 g, 24 mmol), and benzoyl peroxide (0.1 g, 0.4 mmol) were dissolved in CCl_4 (50 mL), heated at reflux, and irradiated with light (500 W bulb) for 16 h. The reaction mixture was filtered and concentrated. Chromatography: hexane/AcOEt 20:1. A yellow oil was obtained: 5 g (72%). ^{1}H NMR: δ 4.93 (s, 2H), 7.62 (t, J = 8, 1H), 7.95 (d, J = 8, 1H), 8.05 (d, J = 8, 1H).

Method B: (2-Nitro-6-trifluoromethyl-benzylamino)-acetic Acid Ethyl Ester (13). Glycine ethyl ester hydrochloride (2.0 g, 19 mmol) and sodium hydride (0.93 g, 39 mmol) were stirred under argon in DMF (30 mL) for 0.5 h. Then **10** (5.0 g, 18 mmol) in DMF (20 mL) was added dropwise over 15 min (slightly exothermic). Stirring at room temperature was continued for 5 h. Extraction: AcOEt, H_2O. Chromatography: hexane/AcOEt 5:1. A yellow oil was obtained: 3.20 g (60%). ^{1}H NMR: δ 1.28 (t, J = 8, 3H), 2.11 (s, 1H), 3.35 (s, 2H), 4.19 (q, J = 7, 4H), 7.54 (t, J = 8, 1H), 7.87 (t, J = 8, 2H).

Method C-1: *N*-(2-Amino-6-chlorobenzyl)glycine Ethyl Ester (16). Compound **11** (4.7 g, 18 mmol) and TEA (2.5 mL) were dissolved in THF (50 mL) and hydrogenated (1 bar H_2) at room temperature over fresh Raney nickel (ca. 2 g suspension in water) until 3 equiv of H_2 was absorbed. The mixture was filtered through Celite and evaporated. Chromatography: hexane/EtOAc 2:1. Yellow oil: 3 g (68%). ^{1}H NMR: δ 1.27 (t, 3H), 1.70 (br, 1H), 3.41 (s, 2H), 3.99 (s, 2H), 4.18 (q, 2H), 4.77 (br, 2H), 6.55 (d, 1H), 6.73 (d, 1H), 6.97 (t, 1H).

Method C-2: (2-Amino-6-trifluoromethyl-benzylamino)-acetic Acid Ethyl Ester (18). Compound **13** (2.0 g, 7 mmol) was dissolved in THF (5 mL) and hydrogenated (1 bar H_2) over 10% palladium on charcoal (0.5 g). The mixture was filtered through a pad of dicalite (5 g). The yellow filtrate was evaporated. A yellow oil was obtained: 1.5 g (83%). ^{1}H NMR: δ 1.29 (t, J = 8, 3H), 1.6 (br, 1H), 3.43 (s, 2H), 3.88 (s, 2H), 4.22 (q, J = 8, 2H), 5.00 (br, 2H), 6.83 (d, J = 8, 1H), 7.00 (d, J = 8, 1H), 7.14 (t, J = 8, 1H).

Method D: (2-Amino-6-fluoro-benzylamino)-acetic Acid Ethyl Ester (15). 2-Amino-6-fluoro-benzylamine (1.54 g, 10.1 mmol) and TEA (3.06 mL, 20.2 mmol) were refluxed in dioxane. Then a solution of ethyl bromoacetate (1.12 mL, 10.1 mmol) in dioxane (5 mL) was added slowly over 1 h. A precipitate formed. Stirring at reflux was continued for 2 h. Extraction: 2 × AcOEt, 2 × H_2O. Chromatography: CH_2Cl_2/MeOH 40:1. Yellow oil: 1.7 g (74%). ^{1}H NMR: δ 1.28 (t, 3 H), 1.65 (br, 1H), 3.40 (s, 2H), 3.84 (s, 2H), 4.23 (q, 2H), 4.75 (br, 2H), 6.42 (m, 2H), 7.00 (dd, J = 6, 1H).

Method E: (2-Oxo-5-trifluoromethyl-1,4-dihydro-2*H*-quinazolin-3-yl)-acetic Acid Ethyl Ester (23). Compound **18** (0.50 g, 2 mmol) and bis(trichloromethyl)carbonate (0.54 g, 2 mmol) were dissolved in dioxane (5 mL) and stirred at room temperature for 24 h. Extraction: AcOEt, H_2O. Chromatography: CH_2Cl_2/*t*-BuOMe 10:1. A colorless oil, which crystallized spontaneously, was obtained: 0.10 g (46%); mp 161 °C. ^{1}H NMR: δ 1.30 (t, J = 8, 3H), 4.19 (s, 2H), 4.25 (q, J = 8, 2H), 4.68 (s, 2H), 6.90 (t, J = 4, 1H), 7.26 (t, J = 4, 2H), 8.11 (s, 1H).

Method F: (5-Chloro-2,2-dioxo-1,4-dihydro-2*H*-2λ^6-benzo[1,2,6]thiadiazin-3-yl)-acetic Acid Ethyl Ester (26). Compound **16** (2.6 g, 11 mmol) and sulfamide (3.1 g, 32 mmol) were refluxed in pyridine (20 mL) for 24 h. Extraction: 2 × 3 N HCl, 2 × 50% saturated NaCl. Chromatography: CH_2Cl_2. The product crystallized spontaneously: 2.6 g (79%); mp 108 °C. ^{1}H NMR: δ 1.27 (t, J = 7, 3H), 3.84 (s, 2H), 4.22 (q, J = 7, 2H), 4.85 (s, 2H), 6.65 (s, 1H), 6.67 (s, 1H), 7.10 (d, J = 8, 1H), 7.17 (t, J = 8, 1H).

Method G: (1-Benzyl-5-chloro-2,2-dioxo-1,4-dihydro-2*H*-2λ^6-benzo[1,2,6]thiadiazin-3-yl)-acetic Acid Ethyl Ester (29). NaH (55% in oil, 78 mg, 1.8 mmol) was dissolved in DMF (5 mL) under argon, then **26** (0.5 g, 1.64 mmol) was added carefully, and stirring continued for 5 min. Benzylbromide (0.21 mL, 1.8 mmol) and catalytic Bu_4NI (61 mg, 0.18 mmol) were added, and the solution was heated at 80 °C for 2 h. Extraction: 2 × AcOEt, 2 × H_2O. Chromatography: hexane/CH_2Cl_2 1:1, then CH_2Cl_2. A white solid was obtained: 437 mg (67%); mp 63 °C. ^{1}H NMR: δ 1.30 (t, J = 7, 3H), 3.88 (s, 2H), 4.24 (q, J = 7, 2H), 4.89 (s, 2H), 5.00 (s, 2H), 6.68 (d, 1H), 7.07 (m, 2H), 7.28 (m, 1H), 7.28 (m, 4H).

Method H: *N*-Hydroxy-2-(2-oxo-1,4-dihydro-2*H*-quinazolin-3-yl)-acetamide (34). Hydroxylamine hydrochloride (552 mg, 7.9 mmol) was dissolved in ice-cooled 4 N KOH (3.9 mL) and stirred for 5 min. A solution of **19** (350 mg, 1.6 mmol) in THF (4 mL; *for compounds* **36**, **38**, *and* **43**: *40 mL because of low solubility!*) was added, and stirring continued for 2 h at 0 °C. The resulting emulsion was neutralized with 4 N HCl (1.95 mL) and stirred for 15 min. Extraction: AcOEt, H_2O. The organic layer was not dried, but it was concentrated until the product precipitated. The product was filtered off and dried at 80 °C under vacuum. White crystals were obtained: 51 mg, 15%; mp 190 °C dec. ^{1}H NMR (DMSO-d_6) (5:1 mixture of rotamers): δ 3.85 and 4.16 (2s, 2H), 4.47 (s, 2H), 6.76 (d, J = 8, 1H), 6.86 (t, J = 8, 1H), 7.05 (d, J = 8, 1H), 7.12 (t, J = 8, 1H), 8.82 and 9.16 (2s, 1H), 9.20 and 9.26 (2s, 1H), 10.1 and 10.6 (2s, 1H).

Enzyme Inhibition. *E. coli* PDF was isolated, as recom-

binant enzyme from *E. coli*, in its native iron-containing form and purified according to a published procedure.[8] PDF activity was measured at room temperature in a microtiter plate format with 5 mM formyl-methionine-alanine-serine (f-MAS) at pH 7.2 (100 mM MOPS/KOH, 250 mM KCl, 0.4% BSA, 100 μg/mL catalase) in a total volume of 50 μL. The reaction was started by the addition of PDF and Fe-PDF with approximately 1100 U/mg (final concentration: 5.7 nM), and it was stopped after 5 min with 200 μL of a 1.54 mM solution of fluorescamine in a 20:1 mixture of 2-methoxy-ethanol and 0.2 M sodium borate buffer (pH 8.5). After 30 min at room temperature, the optical density was measured at 380 nm and the amount of hydrolyzed substrate determined against a standard curve of MAS which was run in parallel. Inhibitors were tested at a final concentration of 1% DMSO.

Inhibition of ACE, ECE, NEP, and several MMPs was determined according to known literature procedures.[31−35]

In Vitro Antibacterial Activity. Bacterial strains used in this study were from our own strain collection. They have been obtained from the American Type Culture Collection (ATCC) and from various hospitals and kept at −80 °C. The minimal inhibitory concentrations (MICs) of the test compounds were determined by microdilution following the guidelines of the National Committee for Clinical Laboratory Standards.[36] The MIC of a compound was defined as the lowest concentration that prevents visible growth of bacteria after incubation at 37 °C for 18 to 24 h. Iso-Sensitest broth (Oxoid) was used as the test medium.

Structure Determination. For crystallization and X-ray structure determination, *E. coli* PDF containing nickel was used.[8] Crystals of space group *C*2 were obtained under conditions similar to those used by these authors and were soaked with a 1 mM solution of hydrazide **1** in 54% ammonium sulfate, 100 mM tris pH 8.5. Diffraction data were collected to 2.25 Å on an Enraf Nonius FR591 generator equipped with Supper mirrors and a MAR 30 cm image plate detector. Data were processed with the program XDS[37] and reduced with the CCP4 package.[38] The unit cell parameters were $a = 143.55$ Å, $b = 64.06$ Å, $c = 84.60$ Å, $\beta = 123.5°$. The inhibitor density was clear from a difference map computed against a previously determined model of Ni-PDF. The inhibitor complex was refined with the program CNS[39] to a final *R*-factor of 21.7% (*R*-free 26.6%) with tight geometrical constraints (rms bond errors 0.010 Å, rms angle errors 1.54°). Modeling and figure generation were performed with the in-house program Moloc.[40]

Acknowledgment. We are grateful to our colleagues from the central units for performing the spectroscopic measurements and for the determination of the microanalysis and from NRRC for running the enzyme inhibition screen. We also thank Dr. T. Hartung and P. Dreier for the synthesis of the radiolabeled compounds **36** and **41**, and E. Bald, N. Nock, E. Philipp, B. Prud'hon, C. Lacoste, B. Rutten, and O. Partouche for their excellent technical assistance. For enzyme preparation, we thank Dr. B. Takács, R. Remy, and B. Gsell. For crystal preparation, we thank M. Stihle and A. D'Arcy. We thank also D. Blum and Dr. B. Löffler for assays with ACE, ECE, and NEP, and R. T. MacKenzie for determination of metalloprotease inhibition. We thank Dr. V. Wagner, University of Heidelberg, Germany, for valuable advice on enzyme preparation and properties.

Supporting Information Available: Additional experimental data (^{1}H NMR, MS, yields, etc.) of all compounds not listed in the Experimental Section. This material is available free of charge via the Internet at http://pubs.acs.org.

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JM000352G