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Identification of novel potent bicyclic peptide deformylase inhibitors

Valentina Molteni,[a,*] Xiaohui He,[a] Juliet Nabakka,[a] Kunyong Yang,[a] Andreas Kreusch,[a] Perry Gordon,[a] Badry Bursulaya,[a] Ian Warner,[a] Tanya Shin,[a] Tanya Biorac,[a] Neil S. Ryder,[b] Ron Goldberg,[c] John Doughty[c] and Yun He[a]

[a]*Genomics Institute of the Novartis Research Foundation, 10675 John Jay Hopkins Drive, San Diego, CA 92121, USA*
[b]*Novartis Institutes for Biomedical Research, 100 Technology Square, Cambridge, MA 02139, USA*
[c]*Novartis Institute for Biomedical Research, One Health Plaza, East Hanover, NJ 07936, USA*

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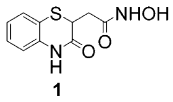
Abstract—Screening of our compound collection using *Staphylococcus aureus* Ni–Peptide deformylase (PDF) afforded a very potent PDF inhibitor with an IC_{50} in the low nanomolar range but with poor antibacterial activity (MIC). Three-dimensional structural information obtained from *Pseudomonas aeruginosa* Ni–PDF complexed with the inhibitor suggested the synthesis of a variety of analogues that would maintain high binding affinity while attempting to improve antibacterial activity. Many of the compounds synthesized proved to be excellent PDF–Ni inhibitors and some showed increased antibacterial activity in selected strains.

Bacterial resistance to many of the existing antibiotics is a growing health concern.[1] Therefore there is an urgent need to identify new antibiotics with unexploited modes of action. Peptide Deformylase (PDF) is an essential bacterial metalloenzyme responsible for the removal of the N-terminal formyl group from methionine residues following protein synthesis.[2] PDF has been considered an attractive target for antibacterial chemotherapy.[3] Several classes of inhibitors have already been investigated to date,[3e,g,4–9] and some compounds with pseudopeptidic backbone are now in development.[7,8,10] With concerns about possible low specificity for MMPs and poor metabolic stability associated with pseudopeptidic inhibitors,[11,12] there is still a need for the identification of novel and structurally diverse non-peptidic PDF inhibitors.

Screening of our compound collection for inhibitory activity against *Staphylococcus aureus* Ni–PDF led to the discovery of a very potent inhibitor **1** with an IC_{50} of less than 5 nM (Fig. 1). Compound **1**, with a metal chelating hydroxamic acid attached to a benzothiazinone core, showed good selectivity for PDF over representative mammalian metalloenzymes (MMP-2 and MMP-13) and also showed a desirable solubility and metabolic stability, as measured in rat liver microsomes. Despite its potency against the PDF enzyme, this compound lacked antibacterial activity (MIC >128 μg/mL) against *Escherichia coli*, *Klebsiella pneumoniae*, *S. aureus*, *Enterococcus faecalis*, and two efflux-deficient *E. coli* mutants.[13] The compound, however, displayed some activity against two selected strains of *Moraxella catarrhalis* and *Haemophilus influenzae* (Table 2).[13] The selected *H. influenzae* and *M. catarrhalis* strains were specifically selected from our collection on the basis of their hyper susceptibility to known proprietary PDF inhibitors currently under investigation (N.S. Ryder, unpublished data).

Compound **1** was co-crystallized with *Pseudomonas aeruginosa*-Ni–PDF (Fig. 2). The X-ray structure showed that compound **1** chelates to the nickel center



1

Ni-PDF IC_{50} < 5 nM
Solubility: > 500 μM at pH 6.8
361 μM at pH 1
Metabolic Stability (RLM): 99%
MMP 2 = 11% inhibition at 10 μM
MMP13 = 7% inhibition at 10 μM

Figure 1. Initial hit **1** from HTS.

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* Corresponding author. Tel.: +1-858-332-4736; fax: +1-858-812-1648; e-mail: vmolteni@gnf.org

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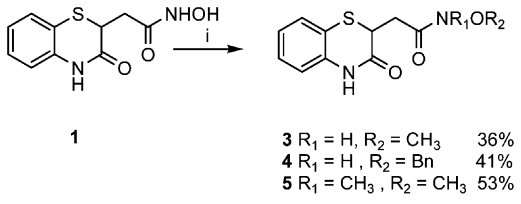
through the hydroxamic acid moiety. In particular, the carbonyl and hydroxyl oxygen atom of the hydroxamate group act as a bidentate ligand for the Ni^{2+} while the nitrogen atom forms a strong H-bond with the carboxylate group of Glu135. The benzothiazinone ring occupies the hydrophobic S1′ pocket, which usually binds the methionine side chain of the substrate and the carbonyl oxygen on the benzothiazinone ring forms a favorable hydrogen bond to Ile46-N. This particular H-bond is conserved in all available PDF inhibitor structures.

The interactions observed in the crystal structure in combination with molecular modeling suggested that substitution at N-4 on the benzothiazinone ring and small modifications of the heterocycle could be performed to improve the antibacterial activity without interfering with the key interactions responsible for the binding. Here we describe various modifications at N-4 and on the heterocyclic core and their effects on antibacterial activity. In addition, the replacement of the hydroxamic acid for other chelating moieties is described, since hydroxamic acids have been associated with poor bacterial penetration.[4]

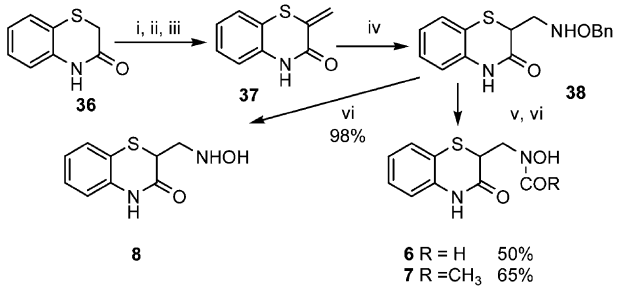
The synthesis of compounds **2**–**35** will be described, followed by a discussion of their biological activity.

The bidentate analogues (**3**, **4** and **5**) were synthesized using standard alkylation conditions (Scheme 1). In the case of compound **5**, 2.0 equivalents of K_2CO_3 were used to ensure alkylation of both NH and OH.

The reverse hydroxamate **6** was synthesized as shown in Scheme 2. The 4*H*-benzo[1,4]thiazin-3-one **36** was easily chlorinated to afford the 2-chloro derivative that was converted to the corresponding phosphonate by treatment with triethylphosphite (Scheme 2). Horner–



Scheme 1. (i) K_2CO_3, RX, DMF.



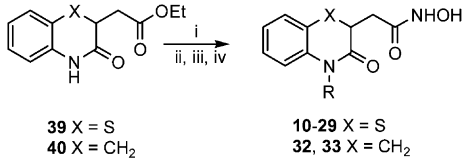
Scheme 2. (i) SO_2Cl_2, CH_2Cl_2 (99%); (ii) $P(OEt)_3$, 100 °C (79%); (iii) NaOMe, HCHO (72%); (iv) NH_2OBn, HCl, DMF, reflux; (76%); (v) Ac_2O, HCOOH for **6** (65%) Ac_2O for **7** (60%); (vi) 10% Pd/C, 1,4-cyclohexadiene, EtOH.

Emmons condensation with formaldehyde in the presence of sodium methoxide afforded the alkene **37**. Compound **37** underwent Michael addition with *O*-benzyl hydroxylamine hydrochloride under refluxing conditions in DMF to give **38**. Compound **38** was *N*-formylated or *N*-acetylated by treatment with formic acid/acetic anhydride or acetic anhydride respectively. Removal of the benzyl group by hydrogenolysis afforded the reverse hydroxamates, *N*-formyl hydroxamine **6** and the *N*-acetyl hydroxylamine **7**, respectively. Compound **38** was also directly converted into compound **8** by hydrogenolysis.

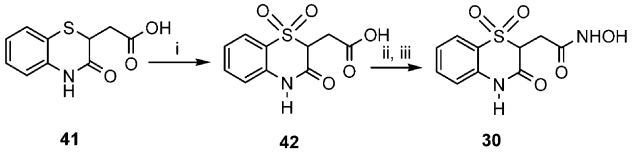
The N-substituted analogues of compound **1** and of 3,4-dihydro-1*H*-quinolin-2-one **31** were synthesized according to the sequence shown in Scheme 3. Esters **39** and **40** were *N*-alkylated by treatment with NaH in DMF followed by the appropriate alkylating agent. The esters were then hydrolyzed by LiOH in THF/water solution. The acids were then converted to the final hydroxamic acids, following the procedure of Reddy.[15]

Sulfone **30** was prepared by oxidation of acid **41** with *m*-CPBA to give **42** that was transformed to the corresponding hydroxamic acid under the same conditions described above.[15] Any attempt to directly oxidize compound **1** gave an inseparable mixture (Scheme 4).

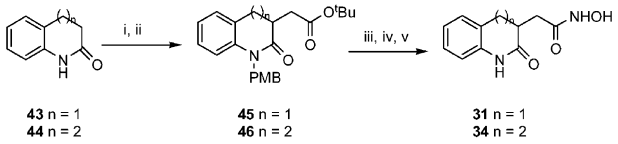
The carbon isosteres of **1**, **31** and **34** were synthesized according to Scheme 5. Protection of compounds **43** and **44** followed by α-alkylation with *tert*-butylbromoacetate afforded compounds **45** and **46**, respectively. Hydrolysis of the ester and deprotection with TFA



Scheme 3. (i) NaH, DMF, RX; (ii) LiOH 3 M, THF–H_2O (1:1); (iii) ethyl chloroformate, *N*-methylmorpholine, THF; (iv) NH_2OH (1 M in MeOH) (10–80% for 4 steps).



Scheme 4. (i) *m*CPBA, CH_2Cl_2 (27%); (ii) ethyl chloroformate, *N*-methylmorpholine, THF; (iii) NH_2OH (1M in MeOH) (38% for 2 steps).



Scheme 5. (i) NaH, DMF, pMBCl (90–95%); (ii) LDA, THF, $BrCH_2COO$*t*-Bu (30–53%); (iii) TFA, reflux (71–93%); (iv) ethyl chloroformate, *N*-methylphoporline, THF; (iv) NH_2OH (1 M in MeOH) (30–90% for 2 steps).

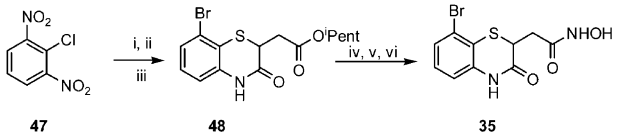
followed by conversion to the hydroxamic acid gave the target compounds.

The 8-bromo substituted compound **35** was prepared as shown in Scheme 6. Aromatic nucleophilic substitution of 2,6-dinitrochlorobenzene **47** with 2-mercapto succinic isopentylester followed by reduction of nitro groups with Fe/HCl afforded directly the cyclized compound. The second amino group was then converted into a diazonium salt which was displaced in situ with $CuBr_2$ to give **48**. The ester **48** was then converted to compound **35** with standard procedures.

Table 1 gives the *S. aureus* Ni–PDF IC_{50} for compounds **1**–**35**.

It has been proposed that potent hydroxamic acid containing PDF inhibitors may be inadequate antibacterial agents due to poor penetration and the action of efflux pumps.[4] As a consequence, one possible way to achieve antibacterial activity for compounds of type **1**, would be through replacement of the hydroxamic acid functionality, assuming that enzyme inhibitory activity could be retained. Several groups have previously described the replacement of the hydroxamic acid functionality with alternative chelating moieties.[6,10,16] For the few structural classes described the hydroxamic acid and the *N*-formyl hydroxylamine have proven to be the only chelating functionalities which would guarantee PDF enzyme inhibition.[6,10] However, it was shown that the hydroxamic acid motif alone is not sufficient for PDF inhibition and the nature of the backbone contribute greatly to the extent of inhibition.[6] Examples of PDF inhibitors that do not contain hydroxamic acid but other chelating moieties such as thiols have been also described.[16c,d] On this basis we decided that it was worthwhile to evaluate a series of analogues in which the hydroxamic acid in **1** was replaced with other related mono- or bidentate chelating moieties (Table 1).

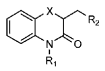
The bidentate hydrazide **2**[17] and the methyl and benzyl hydroxamate **3** and **4** were still PDF inhibitors but less potent than **1**. The decreased potency of **4** relative to **3** is due to increased steric hindrance, as observed from modeling. The *N*-methyl methyl hydroxamate **5** was inactive maybe also because of steric reasons. It may be also possible that the N–H is involved in a hydrogen bond. The crystal structure of compound **1** (Fig. 2) indeed showed a hydrogen bond interaction with the side chain of Glu 135. The reverse hydroxamate, *N*-formyl hydroxamine **6**, was equipotent to **1**, while the *N*-acetyl hydroxylamine **7**, was 40-fold less potent, once again due to steric hindrance. The monodentate hydroxylamine **8** and acid **9**[17] were inactive.

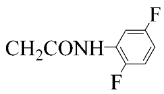
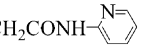


Scheme 6. (i) 2-Mercapto-succinic acid diisopentyl ester, TEA, DMF, 0 °C (45%); (ii) Fe, HCl, Water/EtOH (80%); (iii) *t*-BuONO, $CuBr_2$, CH_3CN (25%); (iv) NaOH, H_2O/EtOH (99%); (v) ethyl chloroformate, *N*-methylphoporline, THF; (vi) NH_2OH (1 M in MeOH) (17% for 2 steps).

The crystal structure of the *P. aeruginosa*-Ni–PDF-**1** complex (Fig. 2) clearly indicated that substituents at N-4 should not affect PDF activity as they are located outside the active site. Under the hypothesis that lack of antibacterial activity is due to poor penetration, substitution at the N-4 position could be used to improve the pharmacological properties of the compounds, which in turn should result in improved antibacterial

Table 1. PDF–Ni IC_{50} for analogues of **1**



	X	R_1	R_2	IC_{50}[a,b]
	Actinonin			<5
1	S	H	CONHOH	<5
2	S	H	$CONHNH_2$	330
3	S	H	$CONHOCH_3$	74
4	S	H	CONHOBn	230
5	S	H	$CONCH_3OCH_3$	>2000
6	S	H	N(CHO)OH	10
7	S	H	$N(COCH_3)OH$	427
8	S	H	NHOH	>2000
9	S	H	COOH	>2000
10	S	Methyl	CONHOH	37
11	S	Isopentyl	CONHOH	<5
12	S	Pentyl	CONHOH	<5
13	S	Isohexyl	CONHOH	<5
14	S	Bn	CONHOH	12
15	S	*c*-Propylmethyl	CONHOH	29
16	S	*c*-Butylmethyl	CONHOH	41
17	S	*c*-Hexylmethyl	CONHOH	302
18	S		CONHOH	18
19	S	2-*c*-Hexylethyl	CONHOH	57
20	S	Phenethyl	CONHOH	<5
21	S		CONHOH	<5
22	S	2-Methoxyethyl	CONHOH	<5
23	S	CH_2CN	CONHOH	515
24	S	CH_2CO*t*Bu	CONHOH	50
25	S	CH_2CONH_2	CONHOH	168
26	S	$CH(CH_3)CONH_2$	CONHOH	44
27	S		CONHOH	58
28	S		CONHOH	397
29	S		CONHOH	343
30	SO_2	H	CONHOH	>2000
31	CH_2	H	CONHOH	73
32	CH_2	Isopentyl	CONHOH	63
33	CH_2	*c*-Propylmethyl	CONHOH	175
34	C=C	H	CONHOH	169
35[c]	S	H	CONHOH	74

[a] IC_{50} (nM).

[b] Ni–PDF assays with the *S. aureus* enzyme were performed in duplicate according to Clements et al.[3e] using f-MAS as substrate and detecting the free amino group of the PDF product MAS with fluorescamine. The detection limit of the assay was 5 nM.

[c] 8-Bromo substituted analogue.

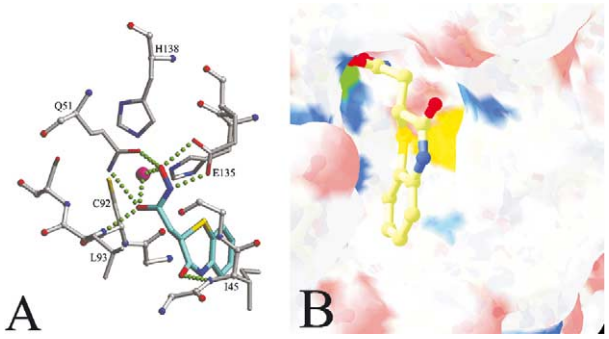


Figure 2. X-ray crystal structure of (R)-**1** bound to Ni–PDF from *P. aeruginosa*. **A**: Polar interactions between **1** (light blue carbon atoms) and PDF from *P. aeruginosa* (grey carbon atoms) are shown in green. The Ni^{2+} ion is colored in magenta, nitrogen atoms in blue, oxygen atoms in red, and sulfur atoms in yellow. **B**: The compound is shown as yellow ball and stick model, the protein is shown as a surface with the Ni^{2+} ion in green, carbon atoms in white, nitrogen atoms in blue, oxygen atoms in red, and sulfur atoms in dark yellow. X-ray analysis revealed that it is the (*R*)-enantiomer that strongly binds to the active site. This stereochemistry, was not confirmed by enzyme assay because the separated enantiomers were not available, but the crystallographic observation is in line with the stereochemistry observed for previously described PDF inhibitors.[4,10,14]

activity. Several N-4 substituted derivatives of the hydroxamic acid **1** and the related *N*-formyl hydroxylamine **6** (data not shown) were synthesized and tested. The nature of some of the substitutions performed were suggested by published data[5] and Novartis unpublished data on existing PDF inhibitors. As expected the substitutions were generally tolerated (Table 1). Very potent hydroxamic acid derivatives were obtained with both long linear and branched alkyl chains (**11**–**13**) and with phenethyl and 2-ethyl [1,3] dioxolane substituents (**20** and **21**) whereas cycloalkyl and benzyl substituents afforded compounds with lower activity (**15**–**19**). This was also the case for benzyl analogues substituted on the benzene ring (data not shown). These results are in contrast to modeling expectations but are in line with what has been observed by Apfel et al.[5] on a class of related PDF inhibitors. Activity was retained with more polar substitution (**23**–**26**) and in substituted acetamides (**27**–**29**) but the compounds showed a decrease in activity with respect to the parent molecule **1**.

In order to address the possibility that the lack of antimicrobial activity of **1** was due to intracellular metabolic oxidation, we prepared the corresponding sulfone **30** which was inactive against PDF–Ni (Table 1). This result prompted us to prepare a series of carbon isosteres. The carbon analogue **31** and the corresponding seven member ring analogue **34** were both PDF inhibitors, even if less potent than **1**, suggesting that sulfur oxidation is not the cause of lack of antibacterial activity for compound **1**. Derivatives of the carbon analogues were also prepared. They followed the same SAR trend as the sulfur analogue but with lower potency (**32** versus **11**, **33** versus **15**).

Compound **35** was synthesized to establish a comparison with the bicyclic urea described by Apfel et al.[5] For that core the 8-bromo substitution gave a 40-fold improvement in PDF activity. In our series the same substitution afforded a decrease of activity of 15-fold ($IC_{50} = 74$ nM).

Table 2. In vitro antibacterial activity against selected strains

Compd	MIC μg/mL *M. catarrhalis* NB50012	MIC μg/mL *M. catarrhalis* NB01050	MIC μg/mL *H. influenzae* NB65007
1	8	64	128
6	8	128	128
12	2	8	32
15	8	64	128
31	4	32	128
35	1	8	32

Compounds **2**–**35** were all tested for antibacterial activity on the standard panel of four bacterial strains (*E. coli*, *K. pneumonia, S. aureus* and *E. faecalis*). No significant improvement of antibacterial activity was obtained against these strains. Selected compounds were also tested against the three hyper susceptible strains of *M. catarrhalis* and *H. influenzae* (Table 2).

The *N*-pentyl substituted compound **12** showed improved activity with respect to **1**. The tetrahydroquinolone **31**, despite the fact that it is a much less potent PDF inhibitor than **1** (73 nM versus <5 nM) showed a similar MIC profile. Compound **35** showed the best improvement of antibacterial activity against these selected strains. In addition, it had a MIC of 64 μg/mL against *E. coli*, despite its lower PDF activity relative to **1** (74 nM versus <5 nM). These results reinforce the observation of Clements et al.,[3e] that variations of antibacterial activity cannot necessarily be attributed to a corresponding variation in enzyme inhibition.

Many factors that affect drug distribution, such as bacterial membrane permeability and efflux pumps contribute to antibacterial activity. In order to try to explain the poor antibacterial activity for the described inhibitors we decided to study the intracellular uptake of **1** in *E. coli* and *S. aureus*.[18] It was found that compound **1** was unable to permeate the *E. coli* cell membrane, explaining the lack of activity in this particular strain. When the experiment was performed with *S. aureus*, it appeared that the compound entered the cell at a concentration that was expected to be sufficient for PDF inhibition. It should be noted that compound molecules bound to the outer cell membrane and subsequently released during cell analysis could contribute to the measured intracellular compound concentration. It is likely, even if not proven, that this is what happened in the case of *S. aureus*.

This letter describes preliminary efforts to convert potent PDF inhibitors into antibacterial agents. We demonstrated that the PDF activity of **1** might be maintained and improved with appropriate structural modifications. In all cases the PDF enzyme inhibitory activity did not translate to antibacterial potency.

Nevertheless, two compounds showed a clear improved antibacterial activity on selected strains with respect to **1**. Further investigation of the reasons underlying the poor antibacterial activity of these potent PDF inhibitors may be the topic of future reports.

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Atomic coordinates have been deposited with the RCSB Protein Data Bank with access code 1S17.

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17. Compound commercially available.
18. *E. coli* and *S. aureus* were grown in LB medium to OD600 = 0.6. Compound **1** was added to the *E. coli* cells to a final concentration of 25 μM, 50 μM, 75 μM, respectively, and a final concentration of 100 μM and 300 μM for *S. aureus*. Samples were taken after 30 min, 1.5 h, and 3.0 h, respectively, and the cell pellet washed several times with LB media before stored at −30 °C overnight. Untreated *E. coli* and *S. aureus* samples served as controls. Thawed *E. coli* cells were resuspended in 200 μL lysis buffer (20 mM Tris pH 7.8, 150 mM NaCl), and lysed by sonication. For *S. aureus*, the lysis buffer contained an additional 0.5 mg/mL Lysostaphin. After cell lysis and pelleting the cell debris, the supernatant was filtered through a 3K centricon spin concentrator and 100 μL of the filtrate was analyzed by mass spectrometry.