Pergamon

Tetrahedron Letters 42 (2001) 3549–3551

TETRAHEDRON LETTERS

Isolation and structure elucidation of two novel deformylase inhibitors produced by *Streptomyces* sp.

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Received 19 March 2001; revised 22 March 2001; accepted 28 March 2001

Abstract—Sch 382582 (**1**) and Sch 382583 (**2**), two novel pseudopeptides, were isolated from fermentation broth of *Streptomyces* sp. as bacteria peptide deformylase inhibitors. Structure elucidation of **1** and **2** was accomplished by extensive 2D NMR spectroscopic studies including NOESY, HMQC-TOCSY and HMBC experiments, and the relative stereochemistry was determined by X-ray crystallography. Both compounds displayed potent inhibitory activity against *E. coli* deformylase.

Bacterial infections due to antibiotic resistant strains have emerged rapidly in recent years, and have become a serious threat to public health worldwide.[1,2] Especially in hospital-acquired infections, many pathogenic bacteria from *Enterococcus faecalis* to *Staphylococcus aureus* have been reported to be resistant to all available antibiotic treatments including, vancomycin, the glycopeptide that is considered as the antibiotic of last resort.[3] Therefore, the issue of antibiotic resistance has brought a new sense of urgency to the discovery and development of antibacterial drugs which differ from vancomycin in mode of action. A genomic-based antibacterial research program was launched to identify new targets for intervention. Bacterial peptide deformylase (PDF) belongs to a new subfamily of metalloproteases, which catalyzes the removal of the N-terminal formyl group from newly synthesized proteins.[4,5] PDF is essential in prokaryotes, is conserved throughout the eubacteria, and is absent in mammalian cells.[6,7] It is therefore an attractive target for developing new antibacterial agents. In the course of searching for deformylase inhibitors as potential leads of mechanism-based antibacterial agents, two novel metabolites Sch 382582 (**1**) and Sch 382583 (**2**) (Fig. 1), were discovered from the fermentation broth of *Streptomyces* sp. (culture 95-02600). Herewith, we report the isolation, structure determination and biological activity of **1** and **2**.

The fermentation whole broth (25 L) was filtered by paper filtration. The active filtrate was absorbed on Amberchrom CG-161 md resin (TosoHaas). The resin was washed with water, and then eluted with the solvent mixture of MeOH:EtOAc:acetone:CH_3CN (1:1:1:1). The crude eluate was partitioned by the modified Kupchan method.[8] The eluate residue was first dissolved in MeOH–H_2O (9:1) and partitioned with an equal volume of hexane. After layer separation, the aqueous MeOH lower phase was adjusted to 20% of water and partitioned with equal volume of CCl_4.[9] The upper MeOH layer was separated and adjusted to 40% of water, and then partitioned with an equal volume of CH_2Cl_2. The CH_2Cl_2 layer was found to be active in the deformylase assay, and chromatographed by reversed-phase HPLC (YMC-ODS semi-preparative column

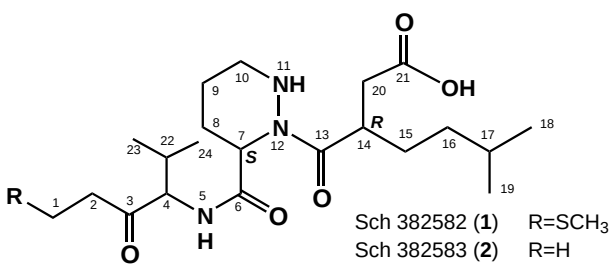

Figure 1.

Keywords: novel pseudopeptides; purification; structure elucidation; ^1H and ^{13}C NMR data.

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0040-4039/01/$ - see front matter
PII: S0040-4039(01)00525-1

Table 1. NMR spectral data of Sch 382582 (**1**)[a]

No.	^{1}H (δ)	^{13}C (δ)	No.	^{1}H (δ)	^{13}C (δ)
1	2.61 t, 6.6[b]	39.28 t[c]	14	3.66 m	35.50 d
2	2.77 m	26.94 t	15	1.33 m	29.55 t
3	–	207.53 s	16	1.09 m	35.08 t
4	4.23 dd, 6.2, 6.8	62.92 d	17	1.41 m	27.38 d
5	8.20 d, 6.8	–	18	0.78 d, 6.0	22.35 q
6	–	172.21 s	19	0.78 d, 6.0	22.30 q
7	5.12 m	49.52 d	20	2.22, 2.46 m, m	36.12 t
8	1.76, 2.02 m, m	26.40 t	21	11.92 br.s, OH	173.42 s
9	1.49 m	20.88 t	22	2.16 m	28.37 d
10	2.72, 2.95 m, m	46.38 t	23	0.87 d, 6.5	19.39 q
11	4.92 d, 8.0	–	24	0.82 d, 6.5	17.60 q
13	–	175.80 s	SCH_3	2.03 s	14.77 q

[a] Recorded on 400 MHz (^{1}H) and 100 MHz (^{13}C) at 25°C in DMSO-d_6 (GE Omega), respectively.
[b] Coupling constant in Hz.
[c] Multiplicity was determined by DEPT data.

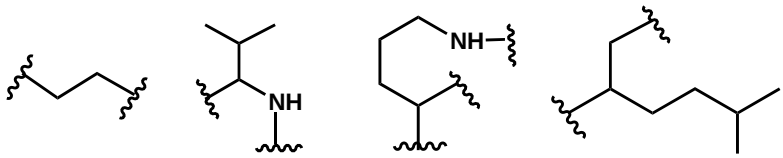

Figure 2. HMQC-TOCSY data of Sch 382582 (**1**).

250×20 mm, S-5, with guard 50×20 mm, 5–50% aqueous acetonitrile gradient in 25 min followed by 50–100% acetonitrile gradient in 12 min, 12 mL/min, UV = 220 nm). The enriched active fraction was further purified by HPLC with the same size of preparative column eluting with aqueous MeOH gradient of 60–80% in 15 min and 80–100% in 5 min to obtain pure **1** (10 mg) and **2** (20 mg) with the yield of ~0.02%.

The molecular weight of **1** was determined to be 457 Da based on LC/MS (ESI+) data that indicated the protonated molecular ion at m/z 458 $(M+H)^+$. The molecular formula was established as $C_{22}H_{39}N_3O_5S$ by high-resolution FAB-MS (calcd for $C_{22}H_{40}N_3O_5S$: 458.2689. Found: 458.2685). Elemental analysis of **1** further confirmed the molecular formula and the presence of sulfur (calcd: C, 57.77; H, 8.53; N, 9.19; S, 7.00%. Measured: C, 57.68; H, 8.54; N, 9.24; S, 6.19%). The UV spectrum of **1** showed end absorption only, which indicated the absence of conjugated unsaturation in the molecule. As shown in Table 1, the ^{1}H NMR spectral data indicated two exchangeable NH doublets at δ 4.92 and 8.20, and one carboxylic OH broad singlet at δ 11.92. The ^{13}C NMR data (Table 1) confirmed the carbon number in the molecular formula established by MS experiments. In the ^{13}C NMR spectrum, a carbonyl at δ 207.53 and three amide or carboxylic carbons at δ 172.21, 173.42 and 175.80 were observed, consistent with the ^{1}H NMR data. Extensive 2D NMR experiments were carried out for the purpose of structure elucidation. Four partial structures of **1** were determined based on HMQC-TOCSY experiments (Fig. 2). The connectivity of these fragments was assigned by the analysis of long-range ^{1}H and ^{13}C correlation data from the HMBC experiments, which are summarized in Fig. 3. The structure was confirmed by X-ray crystallographic analysis of the substrate **1**/deformylase complex at 1.5 Å resolution.[10]

The molecular weight of **2** was determined to be 411 Da based on LC/MS data that indicated the protonated molecular ion at m/z 412 $(M+H)^+$. The exact mass of $(M+H)^+$ was measured by high-resolution FAB-MS as m/z 412.2803 (calcd for $C_{21}H_{38}N_3O_5$: m/z 412.2811) indicating the molecular formula as $C_{21}H_{37}N_3O_5$. The end absorption was observed in the UV spectrum of **2**, which strongly indicated that **2** was an analog of **1**. As shown in Table 2, both ^{1}H and ^{13}C NMR spectral data demonstrated the close similarities between compounds **1** and **2**. The combined data revealed the absence of a SCH_3 group at C-1 in **2** with the remaining part of the molecule the same as **1**. The protons and carbons of **2** were further assigned based on the analysis of HMQC-TOCSY and HMBC data.

The stereochemistries of **1** at the chiral centers C-7 and C-14 were established as *S* and *R*, respectively by X-ray macromolecular crystallography. The chiral center C-4 was considered to have an *S* configuration in the crystal structure. However, this assignment remained uncertain

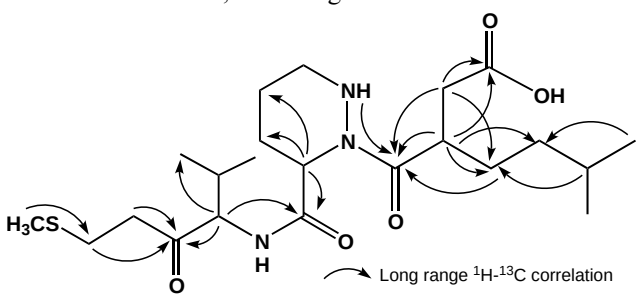

Figure 3.

Table 2. NMR spectral data of Sch 382583 (**2**)[a]

No.	^{1}H (δ)	^{13}C (δ)	No.	^{1}H (δ)	^{13}C (δ)
1	0.93 t, 6.6[b]	7.44 q[c]	13	–	175.78 s
2	2.47 m	32.45 t	14	3.67 m	35.49 d
3	–	209.53 s	15	1.33 m	29.55 t
4	4.22 dd, 6.2, 6.8	62.83 d	16	1.08 m	35.07 t
5	8.17 d, 6.8	–	17	1.41 m	27.39 d
6	–	172.15 s	18	0.79 d, 6.0	22.35 q
7	5.11 m	49.53 d	19	0.79 d, 6.0	22.30 q
8	1.75, 2.01 m, m	26.42 t	20	2.23, 2.46 m, m	36.06 t
9	1.48 m	20.87 t	21	11.86 s, OH	173.40 s
10	2.70, 2.95 m, m	46.39 t	22	2.15 m	28.53 d
11	4.92 d, 8.0	–	23	0.86 d, 6.2	19.42 q
12	–	–	24	0.80 d, 6.2	17.64 q

[a] Recorded on 400 MHz (^{1}H) and 100 MHz (^{13}C) at 25°C in DMSO-d_6 (GE Omega), respectively.
[b] Coupling constant in Hz.
[c] Multiplicity was determined by DEPT data.

because the C-4 moiety was away from the binding sites, which caused poor resolution. The stereochemistry of **2** was proposed as the same as **1** since they are analogs.

Compounds **1** and **2** exhibited DPF inhibitory activity with equal potency of $K_i^* = 60$ nM. Compounds **1** and **2** displayed much weaker activity against aeromonas amino-peptidase (both $K_i^* > 30$ μM) and amino-peptidase M (both $K_i^* = 33$ μM), indicating a selectivity greater than 500-fold. The MIC (minimum inhibition concentration) of both compounds was 32 ug/mL against a super sensitive *E. coli* strain. The previously reported natural product inhibitor of PDF, actinonin,[4] contained a hydroxamic acid functional group. Both compounds were possibly derived from the microorganism through the non-ribosomal biosynthetic pathway. To the best of our knowledge, **1** and **2** are the first examples of non-hydroxamic acid containing natural product inhibitors of PDF.

Acknowledgements

The authors would like to thank Mrs. D. Scott for the preparation of this manuscript.

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9. The method was modified by using CH_2Cl_2 to replace CCl_4 due to the environmental concern. Most of the PDF activity was found in the second CH_2Cl_2 partition.
10. Details of the X-ray crystallographic data of the substrate–enzyme complex will be published elsewhere.