Available online at www.sciencedirect.com
ScienceDirect
Tetrahedron Letters 49 (2008) 3067–3070
Tetrahedron Letters

WSS2220, a novel cyclic tetrapeptide with a new sulfonoamino acid, exhibits potent and selective inhibitory activity against GlyT1

Yuichi Terui [a,*], Chu Yi-wen [b], Li Jun-ying [b], Osamu Nozawa [a], Tsutomu Ando [a], Takuya Fukunaga [a], Takeshi Aoki [a], Yoshihisa Toda [a], Akira Kawashima [a]

[a] *Medicinal Chemistry Laboratories, Taisho Pharmaceutical Co., Ltd, 1-403, Yoshino-cho, Kita-ku, Saitama-shi, Saitama 331-9530, Japan*
[b] *Sichuan Industrial Institute of Antibiotics, 9 Shabanqiao Road, Chengdu, Sichuan 610051, China*

Received 7 February 2008; revised 10 March 2008; accepted 11 March 2008
Available online 14 March 2008

Abstract

In the course of our screening program for novel glycine transporter inhibitors, we obtained a novel cyclic tetrapeptide (WSS2220) from the culture broth of *Nonomuraea* sp. The structure of WSS2220 was elucidated based on physicochemical data. WSS2220 contained a novel amino acid (4-oxo-3′-sulfoisoleucine), the absolute stereochemistry of which was determined by chemical modifications and the modified Mosher's method. WSS2220 selectively inhibited glycine transporter type1 with an IC_{50} of 20 nM.

N-Methyl-D-aspartate (NMDA) receptor blockers, such as phencyclidine (PCP), are known to induce schizophrenic-like symptoms (both positive and negative symptoms) in humans. Thus, a dysfunction in glutamatergic transmission may play an important role in the pathophysiology of schizophrenia (hypoglutamatergic hypothesis). Glycine is known to act as a requisite co-agonist of glutamate for the activation of NMDA receptors, and its levels at synaptic clefts are controlled by glycine transporter type1 (GlyT1) on glial cells. Therefore, the blockers of GlyT1 are expected to function as antipsychotics. Several GlyT1 inhibitors have been reported, and some of these agents are now being investigated in clinical trials.[1]

To identify new inhibitors of GlyT1 from natural origins, we tested the inhibitory activities of thousands of fermentation extracts on [^{3}H]-glycine uptake in glial cells. During this screening program, we obtained a novel cyclic tetrapeptide, named WSS2220 (**1**), from a fermentation broth of *Nonomuraea* sp. strain TA-0426.[2] Compound **1** inhibited [^{3}H]-glycine uptake in a human glial cell line (T98G) with an IC_{50} of 12.5 nM. In this Letter, we report the isolation, structure determination, and biological activity of **1** (Fig. 1).

The WSS2220-producing organism, TA-0426, was isolated from a soil sample collected in Henan Province, China. The strain was taxonomically identified as genus *Nonomuraea* sp. To produce **1**, TA-0426 was cultured in a seed medium for 3 days at 28 °C on a rotary shaker at 200 rpm.[3] This seed medium was then inoculated into the production medium and cultured for 7 days at 28 °C on a rotary shaker at 200 rpm.[4] The culture broth (10 L) was extracted with *n*-BuOH and concentrated in vacuo to give an oily residue (14.9 g). This *n*-BuOH extract was suspended in distilled water (1 L) and extracted with EtOAc.

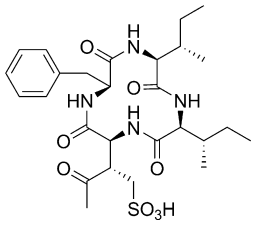


Fig. 1. Structure of WSS2220 (**1**).

* Corresponding author. Tel.: +81 48 669 3107; fax: +81 48 652 7254.
E-mail address: yuichi.terui@po.rd.taisho.co.jp (Y. Terui).

0040-4039/$ - see front matter © 2008 Elsevier Ltd. All rights reserved.
doi:10.1016/j.tetlet.2008.03.054

The resulting aqueous layer was passed through a column of Diaion HP-20. After being washed with 20% aqueous acetone (1 L), the materials containing **1** were eluted with 40% and 60% acetone. These fractions were combined and concentrated in vacuo. The resulting residue was then purified by silica gel column chromatography, eluting with $CHCl_3$–MeOH 9:1 to give WSS2220 (**1**, 363.9 mg) as a colorless powder.

HRTOF-MS of **1** showed a quasi-molecular ion peak at 625.2302 $[M+2Na]^+$, corresponding to the quasi-molecular formula $C_{27}H_{39}N_4O_8SNa_2$ (calcd for $C_{27}H_{39}N_4O_8SNa_2$: 625.2284). IR absorptions at 1693 and 1533 cm^{-1} suggested the presence of amide functions. Absorptions at 1221, 1172, and 1044 cm^{-1}, together with the high water-solubility of **1**, suggested the presence of a sulfonate group. The ^{13}C and ^1H NMR spectral data of **1** are shown in Table 1. In the ^{13}C NMR spectrum of **1**, the signals of four carbonyl carbons (δ 169.7, 171.0, 171.5, and 172.0) and four methines (δ 56.1, 57.0, 60.5, and 60.5) were observed. These data suggested that **1** was a tetrapeptide compound. Another carbonyl signal at δ 207.0 was assigned as a ketone. Signals of sp^2 carbon [δ 126.6 (d), 128.4 (d) (2C), 128.8 (d) (2C), and 136.8 (s)] and a methylene carbon (δ 35.8) suggested the presence of a phenylalanine residue (Phe), and five pairs of signals at 60.5 and 60.5 ppm (d), 33.3 and 34.0 ppm (d), 24.9 and 25.4 ppm (t), 15.3 and 15.4 ppm (q), and 9.8 and 10.0 ppm (q) suggested the presence of two isoleucine residues (Ile). The presence of these amino acid residues was also supported by a detailed analysis of 2D NMR data (Fig. 2) and the advanced Marfey's method (vide infra). The structure of the remaining amino acid residue was also determined by an analysis of the 2D NMR data. Analysis of the COSY spectrum indicated connectivity between the NH on C-2″ to CH_2-6″. The HMBC correlation between H-2″ and C-1″ indicated the connection of C-1″/C-2″. The HMBC correlations observed from H-5″ [δ 1.82 (3H, s)] to C-3″ and C-4″ (δ 207.0) allowed the connection of an acetyl group at the C-3″ position. The ^{13}C chemical shift value of the methylene carbon at position 6″ (δ 51.1) suggested that a sulfonate group was attached at this position. Thus, this amino acid residue was confirmed to be 4-oxo-3′-sulfoisoleucine (Osi), which is a novel amino acid.

Table 1
^1H NMR (500 MHz) and ^{13}C NMR (125 MHz) data for **1** in DMSO-d_6

Position		^{13}C	^1H
Ile-1	1	171.5	
	2	60.5	3.82 (dd, J = 11.0, 11.0)
	3	33.3	2.14 (m)
	4	24.9	1.46 (m), 1.08 (m)
	5	9.8	0.79[a]
	6	15.3	0.79[a]
	2-NH		7.05 (br)
Ile-2	1′	172.0	
	2′	60.5	3.82 (dd, J = 11.0, 11.0)
	3′	34.0	1.75 (m)
	4′	25.4	1.46 (m), 1.08 (m)
	5′	10.0	0.86 (d, J = 6.7 Hz)
	6′	15.4	0.79[a]
	2′-NH		7.72 (br)
Osi	1″	169.7	
	2″	56.1	4.18 (dd, J = 11.6, 11.6 Hz)
	3″	49.0	3.50 (m)
	4″	207.0	
	5″	30.6	1.82 (s)
	6″	51.1	2.96 (m), 2.81 (br)
	2″-NH		7.80 (br)
Phe	1‴	171.0	
	2‴	57.0	4.35 (m)
	3‴	35.8	3.11 (dd, J = 7.3, 14.0)
			2.99 (dd, J = 10.4, 14.0)
	4‴	136.8	
	5‴, 9‴	128.4	7.20–7.32[a]
	6‴, 8‴	128.8	7.20–7.32[a]
	7‴	126.6	7.16 (t, J = 7.3 Hz)
	2‴-NH		7.92 (br)

[a] Not assigned precisely because of signal overlapping.

Based on the degree of unsaturation, **1** was deduced to be a cyclic tetrapeptide. The HMBC correlation observed from the Phe α-proton (H-2‴) to the Osi carbonyl carbon (C-1″) established the two amino acid-sequence of **1**, as shown in Figure 2. The HMBC correlations of NH-2/C-1‴, NH-2′/C-1, NH-2″/C-1′, and NH-2‴/C-1″ and the NOESY correlations of NH-2/H-3‴, NH-2′/H-3, NH-2″/H-3′, and NH-2‴/H-3″ established all of the amino acid sequences of **1**. The configurations of Phe and Ile were determined to be L-Phe and L-Ile by acid hydrolysis and application of the advanced Marfey's method using standard amino acids with known configurations.[5] Since the FDLA (1-fluoro-2,4-dinitrophenyl-5-leucineamide) derivative of Osi was not retained on an ODS column, its absolute configuration at the α-position could not be determined by this method.

To determine the stereochemistry of Osi, **1** was chemically degraded as shown in Scheme 1. Compound **1** was reduced by $NaBH_4$ to give **2** as a diastereomeric mixture (3:1). Acid hydrolysis of **2** yielded L-Phe, L-Ile, and cyclo-4-hydroxy-3′-sulfoisoleucine (**3**). Compound **3** was purified using a strong cation exchange (SCX) resin. Compound **3** was a single isomer, and its diastereomer was not obtained. The relative stereochemistry of **3** was determined by analyzing the ^1H NMR spectrum in detail.[6] The coupling constants between 2-H/3-H and 3-H/4-H were 11.0 Hz

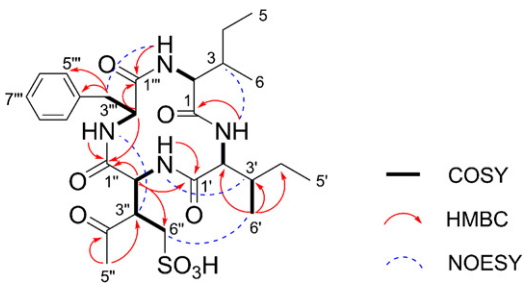
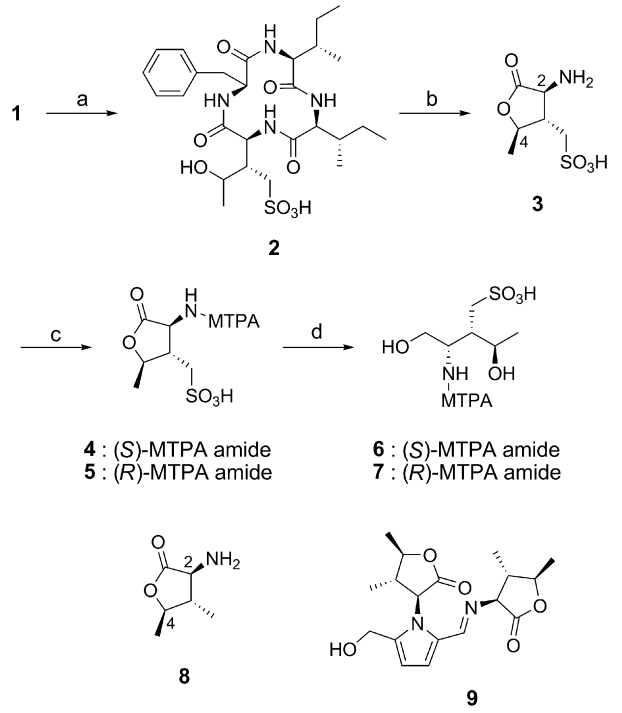


Fig. 2. 2D NMR analysis of **1**.



Scheme 1. Reagents and conditions: (a) $NaBH_4$, MeOH, rt, 24 h; (b) 6 M HCl, 110 °C, 12 h; (c) (*R*)-(−)- or (*S*)-(+)-MTPACl, CH_2Cl_2, pyridine, rt, 4 h; (d) $LiBH_4$, THF, rt, 6 h.

and 9.6 Hz, respectively, which reflected an anti/anti-configuration of 2-H/3-H/4-H. Thus, the relative configuration of **3** was determined to be 2*S**, 3*S**, and 4*R**. This configuration was also supported by a comparison of the ^{1}H NMR data between **3** and (2*S*,3*S*,4*R*)-cyclo-4-hydroxyisoleucine (**8**), which is contained in funebrine (**9**) isolated from *Quararibea funebris*.[7] The coupling constants between 2-H/3-H and 3-H/4-H of **8** were reported to be 11.6 Hz and 9.6 Hz, respectively, which agrees well with those of **3**.

To elucidate the absolute stereochemistry of **3**, **3** was converted to (*S*)- and (*R*)-MTPA amides (**4**, **5**).[8] The modified Mosher's method was applied to **4** and **5**, but no systematic $\Delta\delta$ shift values ($\delta_4 - \delta_5$) were observed. The MTPA groups seemed to assume a conformation different from the ideal one. Therefore, **4** and **5** were converted to linear alcohols **6** and **7** by reduction with $LiBH_4$. After reduction, the $\Delta\delta$ shift values ($\delta_6 - \delta_7$) indicated a 2*S* configuration (Fig. 3). Thus, the absolute configuration of Osi was established to be 2*S*, 3*S*, as shown in Figure 1.

To measure the inhibitory activity toward glycine uptake, T98G cells were cultured in Dulbecco's Modified

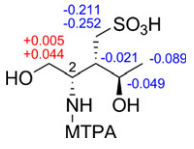


Fig. 3. $\Delta\delta$ shift values (ppm, 500 MHz, CD_3OD) obtained for (*S*)- and (*R*)-MTPA amides (**6**, **7**).

Table 2
GlyT-inhibitory activities of **1** (nM)

Compound	T98G	rGlyT1	rGlyT2
1	12.5	20.4	2261
ALX5407	4.8	3.0	NT
ALX1393	NT	NT	21.1

ALX5407 (Tocris): selective inhibitor of GlyT1.
ALX1393 (Sigma–Aldrich): selective inhibitor of GlyT2.

Eagle Medium with 10% fetal bovine serum. 2×10^4 cells/well were plated in a 96-well plate and incubated at 37 °C in a humidified incubator (95% air, 5% CO_2). After 24 h, the cells were washed and incubated at room temperature with 250 nM [^{3}H]-glycine and **1** in an assay buffer.[9] After 15 min, the assay buffer was removed, and the cells were washed twice with the assay buffer. The cells were then lysed with 0.5 M NaOH, a scintillation cocktail was added, and the cell lysates were counted using a scintillation counter. Compound **1** inhibited glycine uptake in T98G with an IC_{50} of 12.5 nM. ALX5407, a selective inhibitor of GlyT1, showed inhibitory activity in this assay system with an IC_{50} of 4.8 nM.[10]

Next, we evaluated the selectivity of **1** against GlyT1 and GlyT2 subtypes. The selectivity of GlyT inhibitory activity was determined by the ability of **1** to inhibit the uptake of [^{3}H]-glycine in COS7 cells that had been transfected with rat GlyT1 or GlyT2. Compound **1** inhibited glycine uptake in these cells with IC_{50} of 20.4 nM and 2261 nM, respectively. ALX1393, a selective inhibitor of GlyT2, showed inhibitory activity in this assay system with an IC_{50} of 21.1 nM.[11] These data revealed that **1** was a selective inhibitor of GlyT1 (Table 2).

In summary, we have isolated a novel cyclic tetrapeptide with selective GlyT1-inhibitory activity. Compound **1** contained a novel amino acid, 4-oxo-3′-sulfoisoleucine (Osi). While alkyl sulfonate groups have been found in some natural products,[12] a highly functionalized sulfonoamino acid such as Osi has not been reported previously.

Acknowledgments

We thank Dr. Yuriko Nozawa for performing the LC/MS analyses, Dr. Atsushi Okada for performing the NMR measurements, and Mr. Haruaki Yamamoto for fermenting TA-0426.

References and notes

1. (a) Depoortère, R.; Dargazanli, G.; Estenne-Bouhtou, G.; Coste, A.; Lanneau, C.; Desvignes, C.; Poncelet, M.; Heaulme, M.; Santucci, V.; Decobert, M.; Cudennec, A.; Voltz, C.; Boulay, D.; Terranova, J. P.; Stemmelin, J.; Roger, P.; Marabout, B.; Sevrin, M.; Vigé, X.; Biton, B.; Steinberg, R.; Françon, D.; Alonso, R.; Avenet, P.; Oury-Donat, F.; Perrault, G.; Griebel, G.; George, P.; Soubrié, P.; Scatton, B. *Neuropsychopharmacology* **2005**, *30*, 1963–1985; (b) Lowe, J. A., III. *Expert Opin. Ther. Patents.* **2005**, *15*, 1657–1662.
2. (a) Toda, Y.; Chu, Y.; Li, J.; Terui, Y., Fukunaga, T. WO2003104265; (b) Chu, Y.; Li, J.; Toda, Y. CN1535980.

3. The seed medium was composed of soluble starch 2.5%, glucose 1.0%, fish meal 0.5%, pharma media 0.3%, NZ case 0.3%, yeast extract 0.2% and $CaCO_3$ 0.2%.
4. The production medium was composed of corn starch 2.0%, glycerol 1.0%, glucose 0.5%, pharma media 0.5%, cottonseed flour 0.5%, malt extract 0.5%, polypepton 0.3%, Mg_2SO_4 0.05% and $CaCO_3$ 0.3%. The pH of the medium was adjusted to 7.0 before sterilization.
5. (a) Fujii, K.; Ikai, Y.; Oka, H.; Suzuki, M.; Harada, K. *Anal. Chem.* **1997**, *69*, 5146–5151; (b) FDLA-derivatives were analyzed using a YMC-Pack Pro C18 (100 × 4.6 mm; i.d., 3 μm; YMC) column at 40 °C. Acetonitrile–water containing 5 mM of ammonium formate and 5 mM of formic acid was used as the mobile phase under a linear-gradient elution mode (acetonitrile, 15–85%, 20 min) at a flow rate of 1.0 mL/min with UV detection at 215 nm. L- and D-FDLA derivatives of Phe from **1** were detected at 9.11 min and 14.93 min, respectively. L- and D-FDLA derivatives of Ile from **1** were detected at 10.60 min and 14.93 min, respectively. L-FDLA derivatives of standard L-Ile and L-*allo*-Ile were detected at 10.62 min and 10.36 min, respectively.
6. *NMR data for* **3**: ^{1}H NMR (500 MHz, D_2O): δ 1.54 (3H, d, $J = 6.3$ Hz), 2.78 (1H, m), 3.24 (1H, dd, $J = 10.2$, 15.0 Hz), 3.33 (1H, dd, $J = 4.1$, 15.0 Hz), 4.53 (1H, d, $J = 11.0$ Hz), 4.59 (1H, dq, $J = 9.6$, 6.3 Hz). ^{13}C NMR (125 MHz, D_2O): δ 20.6 (q), 46.7 (d), 53.0 (t), 57.5 (d), 82.4 (d), 175.3 (s).
7. (a) Raffauf, R. F.; Zennie, T. M.; Onan, K. D.; Le Quesne, P. W. *J. Org. Chem.* **1984**, *49*, 2714–2718; (b) Tamura, O.; Iyama, N.; Ishibashi, H. *J. Org. Chem.* **2004**, *69*, 1475–1480.
8. Ohtani, I.; Kusumi, T.; Kashman, Y.; Kakisawa, H. *J. Am. Chem. Soc.* **1991**, *113*, 4092–4096.
9. The assay buffer was composed of 10 mM HEPES (pH 7.4), 150 mM NaCl, 1 mM KCl, 1 mM $CaCl_2$, 1 mM $MgCl_2$ and 10 mM glucose.
10. Atkinson, B. N.; Bell, S. C.; De Vivo, M.; Kowalski, L. R.; Lechner, S. M.; Ognyanov, V. I.; Tham, C.-S.; Tsai, C.; Jia, J.; Ashton, D.; Klitenick, M. A. *Mol. Pharmacol.* **2001**, *60*, 1414–1420.
11. Xu, T. X.; Gong, N.; Xu, T. L. *Neuroreport* **2005**, *16*, 1227–1231.
12. (a) Matsunaga, S.; Fusetani, S.; Konosu, S. *J. Nat. Prod.* **1985**, *48*, 236–241; (b) Rinken, M.; Lehmann, W. D.; König, W. A. *Liebigs Ann. Chem.* **1984**, *10*, 1672–1684; (c) Kamiyama, T.; Umino, T.; Itezono, Y.; Nakamura, Y.; Sato, T.; Yokose, K. *J. Antibiot.* **1995**, *48*, 929–936; (d) Shinagawa, S.; Kasahara, S.; Wada, Y.; Harada, S.; Asai, M. *Tetrahedron* **1984**, *40*, 3465–3470; (e) Ito, T.; Ezaki, N.; Ohba, K. *J. Antibiot.* **1982**, *35*, 533–535; (f) Reshef, V.; Mizrachi, E.; Maretzki, T.; Silberstein, C.; Loya, S.; Hizi, A.; Carmeli, S. *J. Nat. Prod.* **1997**, *60*, 1251–1260.