Facile Total Synthesis of (+)-Spinoxazine B

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Herein we describe a short total synthesis of (+)-spinoxazine B, which inhibits nitric oxide (NO) production in BV-2 microglial cells. Spinoxazine B is the first example of a natural alkaloid containing an oxazinone-pyrrolidone nucleus, and it is expected to serve as a novel drug lead compound as well as a drug discovery scaffold.

Key words total synthesis; spinoxazine B; salinazinone B; 1,3-oxazine-6-one

The development of purification and structural analysis techniques in recent years has facilitated the isolation and structure determination of a wide variety of novel natural products. Natural products are potential candidates for novel leads in drug discovery, but their biological activity has not been sufficiently evaluated because of the limited amount of isolated compounds. Therefore, establishing easily accessible synthesis pathways for these natural products will increase their possible utility as novel drug discovery leads and contribute to the development of novel medicines.

Spinoxazine B (1) is a natural product that was independently isolated by the MacMillan group1) and the Kwon group2) (The Kwon group named it salinazinone B.) (Fig. 1). This natural product has an unprecedented skeleton with a 1,3-oxazin-6-one bearing oxopyrrolidine ring. From the perspective of medicinal chemistry, ring structures play a very important role in drug discovery; in other words, ring structures determine their affinity to the target protein, metabolic stability and pharmacokinetic properties, as well as the physicochemical properties of the compounds. Therefore, new ring system is expected to be a new resource for drug discovery.

The proposed synthesis of 1 involves the construction of the 1,3-oxazin-6-one ring via thermolysis of N-acylaminoethylidene-1,3-dioxane-2,4-one 7 (Chart 1). This type of thermolysis reaction was reported for simple substrates,3,4) but functionalized substrates have yet to be tested. At the outset, ketene dithioacetal 35) was reacted with amide 4 in the presence of NaH to afford ketene-N,S-acetal 5 in 50% yield. Next, the second substitution reaction was conducted with the enantioenriched amino ester 66) in AcOH. Although this reaction was rather slow, the yield of the desired ketene aminal 7 reached 76% after 2 d. Thermolysis of 7 in dimethyl sulfoxide (DMSO) at 190°C furnished the expected 1,3-oxazin-6-one derivative 9 in 66% yield. The thermolysis is believed to proceed via iminoketene intermediate 8.3,4)

The remaining task was the construction of the γ-lactam ring, but this was problematic. For example, no lactam formation was observed upon the treatment of 9 with acid or base, most likely due to the low nucleophilicity of the amino group. In addition, when reduction or hydrolysis of the ethoxycarbonyl group was attempted to improve the electrophilicity of the carbonyl group, decomposition of the 1,3-oxazin-6-one ring occurred preferentially.

With these results in hand, we decided to seek a new synthetic route (Chart 2). First, ketene-N,S-acetal 5 was coupled with amino acid 10 to generate ketene aminal 11 in 79% yield, envisioning that the carboxyl group in 11 could

Fig. 1. Structures of Spinoxazines (1, 2)

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be transformed into an activated ester to promote the final lactamization. To our surprise, thermolysis of 11 at 200°C directly afforded spinoxazine B (1) in 48% yield, implying that oxazinone ring formation and lactamization proceeded in one pot. At this stage, it is unclear why lactamization proceeded from 11 and not from 9; this will be a subject of our future research.

In conclusion, a concise total synthesis of spinoxazine B (1) in three steps from ketene dithioacetal 3 (prepared using Meldrum’s acid in one step) and amino acid 10 was accomplished by two addition-eliminations and an intramolecular double cyclization. To the best of our knowledge, the total synthesis of spinoxazine B (1) has not been reported so far. Additionally, there is no report on a skeleton bearing a 1,3-oxazin-6-one-pyrrolidione system other than the existing literature on spinoxazines.\(^{1,2}\) The development of this synthetic method is expected to open new avenues for drug discovery.

**Experimental**

All reactions were carried out in a round-bottom flask or a test tube fitted with a 3-way glass stopcock under Ar atmosphere unless otherwise stated. Flash chromatography was performed using silica gel 60N (particle size: 40–50 \(\mu\)m) purchased from Kanto Chemical unless otherwise stated. All work-up and purification procedures were carried out with reagent-grade solvents under ambient atmosphere. Compounds 3\(^{9}\) and 6\(^{10}\) were synthesized as reported in the literature. Other reagents were purchased from commercial suppliers and used as received. NMR spectra were recorded on JEOL ECA-600 or Bruker AVIII 400 spectrometers. Chemical shifts and coupling constant. High-resolution (HR) MS (electrospray ionization-time-of-flight (ESI-TOF)) (+) were measured on JEOL JMS-T100LP. Optical rotation was measured on JASCO 1D-2200.

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**Ethyl (S)-(2-(2-Methylbutyl-1-2-ethyl)-6-oxo-6H-1,3-oxazin-4-ylamino)pentanoate (9)** A solution of 7 (6.8 mg, 0.017 mmol) in DMSO (0.3 mL) was stirred at 190°C for 1.5 h. After cooling to r.t., water was added and the mixture was extracted with AcOEt. The combined organic layer was washed with water and brine and dried over Na\(_2\)SO\(_4\). After filtration and concentration under reduced pressure, the residue was purified by silica gel column chromatography (19:1 to 1:4) to afford 9 (3.3 mg, 0.011 mmol, 66%) as a yellow foam.

**Ethyl (S)-4-(((2,2-Dimethyl-4,6-dioxo-1,3-dioxan-5-ylidene)-(3-methylbut-2-enamido)methyl)amino)pentanoate (10)** A solution of 8 (25.1 mg, 0.0839 mmol) and NaOAc (206 mg, 2.51 mmol) in AcOH (0.50 mL) was added amino acid hydrochloride 10 (27.5 mg, 0.47 mmol) at r.t. After stirring at 50°C for 2 h, the reaction mixture was concentrated under reduced pressure. The resulting white solid (91.1 mg, quant.) was used in the next reaction without further purification.

**Ethyl (S)-4-(((2,2-Dimethyl-4,6-dioxo-1,3-dioxan-5-ylidene)-(3-methylbut-2-enamido)methyl)amino)pentanoate (11)** To a solution of 5 (25.1 mg, 0.0839 mmol) and NaOAc (206 mg, 2.51 mmol) in AcOH (0.50 mL) was added amino acid hydrochloride 10 (27.5 mg, 0.47 mmol) at r.t. After stirring at 70°C for 45 h, 0.1 M aqueous HCl was added and the mixture was extracted with AcOEt. The combined organic layer was washed with brine and dried over Na\(_2\)SO\(_4\). After filtration and concentration under reduced pressure, the residue was purified by silica gel column chromatography (1st column: CHCl\(_3\)-MeOH=1:0 to 10:1; 2nd column: MeOH–AcOEt=2:1 to 1:5) to afford 11 (24.4 mg, 0.0662 mmol, 79%) as a yellow solid.

**Ethyl (S)-4-(((2,2-Dimethyl-4,6-dioxo-1,3-dioxan-5-ylidene)-(3-methylbut-2-enamido)methyl)amino)pentanoate (12)** To a solution of 5 (66.8 mg, 0.223 mmol) and NaOAc (568 mg, 6.92 mmol) in AcOH (1.5 mL) was added amine hydrochloride 6 (240 mg, 1.32 mmol) at r.t. After stirring at 70°C for 2 d, 1 M aqueous HCl was added, and the mixture was extracted with AcOEt. The combined organic layer was washed with brine and dried over Na\(_2\)SO\(_4\). After filtration and concentration under reduced pressure, the residue was purified by silica gel column chromatography (n-hexane–AcOEt=19:1 to 2:1) to afford 7 (66.2 mg, 0.167 mmol, 76%) as a yellow oil.

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Spinoxazine B (1) A solution of 11 (4.9 mg, 0.013 mmol) in Ph$_2$O (0.27 mL) was stirred at 200°C for 14 h. After cooling to r.t., the mixture was purified by silica gel column chromatography (CHCl$_3$–MeOH=1:0 to 99:1; 2nd column: n-hexane–AcOEt=1:0 to 19:1) to afford 1 (1.6 mg, 0.064 mmol, 48%) as a white solid.

$^1$H-NMR (400 MHz, CD$_3$CN) $\delta$: 1.31 (3H, d, $J$=7.2 Hz), 1.70–1.77 (1H, m), 2.02 (3H, d, $J$=1.2 Hz), 2.25–2.29 (4H, m), 2.39–2.46 (1H, m), 2.70–2.79 (1H, m), 4.67–4.74 (1H, m), 5.92–5.93 (1H, m), 6.83 (1H, s). $^{13}$C-NMR (151 MHz, CD$_3$CN) $\delta$: 20.5, 21.5, 25.8, 28.5, 32.4, 55.5, 89.9, 117.3, 157.0, 159.4, 161.6, 165.1, 177.1. $[^{25}]$D 10.2 (c=0.08, MeOH). HR-MS (ESI$^+$) Calcd for C$_{13}$H$_{16}$N$_2$O$_3$Na$^+$ m/z: 271.1053 (M+Na)$^+$. Found 271.1031.

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Conflict of Interest The authors declare no conflict of interest.

Supplementary Materials The online version of this article contains supplementary materials. $^1$H- and $^{13}$C-NMR spectra of synthesized compounds.

References