Synthesis of New β-Lactam Analogs and Evaluation of Their Histone Deacetylase (HDAC) Activity

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A simple synthesis of the β-lactams 11–13 and 16–17 as novel histone deacetylase (HDAC) inhibitors is described. The key synthetic strategies involved the O-alkylation of 6-APA and the coupling reactions of freshly prepared N-carbobenzyloxy-L-prolines 5 and 6 and 6-aminopenicillanates 8–10 and 15 in high yields. It was found that all compounds show potent growth inhibitory activity on human tumor cell lines, the most potent compound 16 exhibiting an IC\textsubscript{50} = 2.1 µM \textit{in vitro}.

Key words: β-Lactams, Histone Deacetylase, Coupling Reaction, Anticancer, Synthesis

Introduction

Since the introduction of β-lactam antibiotics, β-lactam substrates have stimulated significant interest due to their wide range of intriguing biological activities [1] such as antibiotic [2], antioxidant [3], antiviral [4], and anticancer [5] properties. The variety of the pharmacological activities of these β-lactams and their unique structural features, including azetidin-2-one rings, generated a great deal of interest among synthetic chemists and biologists. Recently, β-lactam substrates with significant biological activities such as serine-dependent enzyme inhibitors [6], matrix-metalloprotease inhibitors [7], cysteine protease inhibitors [8], and apoptosis inducers [9] were reported in the literature. Furthermore, they have served as synths in the preparation of various heterocyclic compounds and potent anticancer agents such as paclitaxel, epothilones, and their analogs [10]. Current research priorities for the β-lactam moieties are focused on providing better antibacterial efficacy and on biochemical features as enzyme inhibitors including apoptosis-inducing properties. In addition, efforts to develop optimal β-lactams as anticancer agents are underway. More recently, histone acetylation was reasonably accepted as a mechanism of chromatin remodeling. It is highly governed by the antagonistic activity of histone acetyltransferases (HAT) and histone deacetylase (HDAC) as well as its acetylase inhibitors [11]. The small lactam (β and γ) derivatives are privileged structures for enzyme inhibition, mainly due to their ability to trap serine or cysteine residues in the DNA binding domain.

In a continuation of our medicinal chemistry program connected with the synthesis of new β-lactam moieties and evaluation of their biological properties, we required Cbz-protected L-prolines 5–6 and 6-aminopenicillanates 8–10, 15 as important fragments in order to generate novel histone acetylase inhibitors. We wish to report herein a simple synthesis and the evaluation of the anticancer activity of the 6-acylamopenicillanates 11–13 and 16–17, starting from 6-aminopenicillanic acid (6-APA, 1) via O-alkylation and coupling reactions.

Results and Discussion

To generate the Cbz-protected L-prolines 5 and 6, which are well known as a pharmacophore for HDAC inhibitors, the commercially available L-proline (1) and trans-4-hydroxy-L-proline (2) were treated with chlorotrimethylsilane (Me\textsubscript{3}SiCl) in the presence of diisopropylethylamine (DIEA) in dichloromethane to yield the silyl-protected L-prolines 3 and 4, which were used in the next step without purification. Intermediates 3 and 4 were subsequently treated with benzoylcarbonyl chloride (Cbz-Cl) to give the Cbz-protected...
l-prolines 5 and 6 in 97 and 95% two-step yields, respectively (Scheme 1).

The β-lactam moieties 11–13 were prepared from readily generated 6-aminopenicillanates 8–10 [12] with N-carbobenzyloxy-L-proline (5) and N-carbobenzyloxy-L-4-hydroxyproline (6) via common condensation reactions. 6-Aminopenicillanic acid (6-APA, 7) was treated with several alkylating reagents such as bromoacetonitrile, allyl bromide, and benzyl bromide to give esters 8–10. At this stage, we tried to generate the various esters using several alkylating reagents such as bromochloromethane, chloromethyl methyl ether, 4-methoxybenzyl bromide, and benzyl chloromethyl ether under SN2-type reaction conditions including K2CO3/DMF [13], DBU/CH3CN [14], and NaH/THF [15], but these reactions were all unsatisfactory, and for the most part the starting material was recovered. Compounds 8–10 were readily coupled with 6 in the presence of 2-(7-aza-1H-benzotriazol-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate (HATU) and DIPEA in dichloromethane to afford 11–13 in good yields (Scheme 2).

In addition, 6-APA (7) was treated with freshly prepared diazomethane [16] in dichloromethane to afford ester 15, in high yield. On the other hand, in
an effort to prepare the sulfoxide 14, 6-APA (7) was smoothly treated with 3,3-dimethyldioxirane (DMDO) [17] in dichloromethane to generate a sulfoxide, which was then readily treated with diazomethane [18]. Unfortunately, these reactions failed to afford sulfoxide 14, leaving only starting material and/or decomposed products. Compound 15 was condensed with acids 5 (Z-Hyp) or 6 (Z-Hyp-OH) using ethyl(dimethylaminopropyl)carbodiimide (EDCI) and 1-hydroybenzotriazole (HOBt) to generate 16, 17 in high yields [13]. At this stage, coupling of 15 with 5, 6 was also accomplished by dicyclohexylcarbodiimide (DCC)/CH2Cl2, HATU/CH2Cl2, and bis(2-oxo-3-oxazolidinyl)phosphinic chloride (BOP-Cl) in CH2Cl2. Although the latter conditions were more convenient, the HOB/EDCI method afforded a superior yield.

**In vitro inhibition of histone deacetylase**

A histone deacetylase fraction was prepared as described by Yoshida et al. [19]. Human leukemia K562 (2.5 × 10⁸) cells were disrupted in buffer-A [15 mM of potassium phosphate buffer (pH 7.5) containing 5% glycerol and 0.2 mM EDTA (15 mL)]. The nuclei were collected by centrifugation (35000 g, 10 min) and resuspended with buffer-A (15 mL) containing 1 M (NH4)2SO4. After sonication, the supernatant was collected by centrifugation, and ammonium sulfate was added to make the final concentration 3.5 M. After stirring for 1 h at 0 °C, the precipitate was collected by centrifugation, dissolved with buffer-A (4 mL), and dialyzed against buffer-A (2000 mL). The dialysate was collected by centrifugation, dissolved with buffer-A (4 mL), and dialyzed against buffer-A, and ammonium sulfate was added to make the final concentration 3.5 M. After stirring for 1 h at 0 °C, the precipitate was collected by centrifugation, dissolved with buffer-A (4 mL), and dialyzed against buffer-A (2000 mL). The dialysate was loaded onto a mono Q HR 5/5 column (Pharmacia) equilibrated against buffer-A (2000 mL). The dialysate was loaded onto a mono Q HR 5/5 column (Pharmacia) equilibrated with buffer-A, and eluted with a linear gradient of 0–1 M NaCl in buffer-A (30 mL). A single peak of histone deacetylase activity was eluted around 0.4 M NaCl, and the fraction was stored at −80 °C until use. Inhibition of histone deacetylase was estimated as described by Yoshida et al. with slight modifications. ³H-labeled histone was prepared as reported. K562 cells (10⁸ cells) were incubated in growth medium (25 mL) containing 0.5 Mci ml⁻¹ [³H]sodium acetate (152.8 GBq mmol⁻¹; NEN) and 5 mM sodium butyrate at 37 °C [18]. Histone deacetylase inhibitory activity of the test compound was measured as follows: The mixture (total volume 50 µL) containing the above histone deacetylase fraction (2 µL), ³H labeled histone (100 µg ml⁻¹), and the test compound (5 µL) was incubated for 10 min at 37 °C. [³H]Acetic acid, which was liberated from ³H-labeled histone, was extracted with ethyl acetate, and radioactivity was measured by a liquid scintillation counter.

The *in vitro* anticancer activity of β-lactam moieties 11–13 and 16, 17 were evaluated in human tumor cell lines, and the results are summarized in Table 1. It was found that all compounds showed potent growth inhibitory activity on human tumor cell lines with the most potent compound 16 exhibiting IC₅₀ = 2.1 µM. In addition, the methoxy esters 16, 17 (Table 1, entries 4, 5) or benzyl ester 13 (Table 1, entry 3) exhibited higher *in vitro* growth inhibitory activity when compared to cyanomethyl or allyl esters 11, 12. In addition, the novel β-lactam moieties 11–13 and 16, 17 showed better HDAC activity than sodium butyrate. However, all prepared sulfonamides exhibited less HDAC activity than trichostatin A.

In conclusion, a simple preparation of new histone deacetylase (HDAC) inhibitors has been described. Compound 16 exhibited the most potent anticancer activity among these analogs. We expect that simple synthesis of new β-lactam moieties and key fragments are useful for the modification of histone acetylase inhibitors.

**Experimental Section**

Reactions requiring anhydrous conditions were performed with the usual precautions for rigorous exclusion of air and moisture. Tetrahydrofuran was distilled from sodium benzenophenone ketyl prior to use. Thin layer chromatography (TLC) was performed on precoated silica gel G and GP umlates from Analtech and visualized with 254 nm UV light. Flash chromatography was carried out on silica gel 60 (Scientific Adsorbs Incorporated (SAI), particle size 32–63 µm, pore size 60 Å). ¹H NMR, ¹³C NMR, and 2D NMR spectra were recorded on Bruker DPX 500 instruments at 500 MHz (¹H) and 125 MHz (¹³C). The chemical shifts are reported in parts per million (ppm) downfield from tetramethylsilane. Infrared (IR) spectra were obtained on an ATI Mattson FT/IR spectrometer. Mass spectra were recorded with a Waters Micromass ZQ LC-Mass system and

<table>
<thead>
<tr>
<th>Compound</th>
<th>IC₅₀ enzyme (µM)</th>
<th>IC₅₀ cells (µM)</th>
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<tbody>
<tr>
<td>11</td>
<td>44.0</td>
<td>68.4</td>
</tr>
<tr>
<td>12</td>
<td>40.0</td>
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<td>12.8</td>
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<tr>
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<td>6.3</td>
<td>2.1</td>
</tr>
<tr>
<td>17</td>
<td>11.8</td>
<td>5.5</td>
</tr>
<tr>
<td>Sodium butyrate &amp; Trichostatin A</td>
<td>–</td>
<td>0.0046</td>
</tr>
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*HDAC enzyme assay; the values are means of three experiments [20]; materials for comparison.*
high-resolution mass spectra (HRMS) were measured with a Bruker BioApex FTMS system by direct injection using an electrospray interface (ESI). When necessary, chemicals were purified according to the reported procedures [21].

(4R,2S)-4-Hydroxyprolylaline-1,2-dicarboxylic acid 1-benzyl ester (6) [22]

To a stirred solution of 4 (7.5 g, 57.2 mmol) in dry CH2Cl2 (120 mL) was added DIPEA (24.3 g, 188.7 mmol) under argon atmosphere at r.t., followed by addition of Me3SiCl (27.9 g, 257.2 mmol). The mixture was refluxed for 2 h, and then the reaction mixture was cooled to 0 °C using an ice-salt bath. Cbz-Cl (9.3 g, 54.4 mmol) was added dropwise to the mixture and the resulting mixture was stirred at r.t. for 16 h. The reaction mixture was concentrated under reduced pressure, and the residue was dissolved in 3% aqueous sodium bicarbonate solution (120 mL). The aqueous layer was separated and acidified to pH = 2 by 5% aqueous HCl solution. The aqueous phase was extracted with ethyl acetate (100 mL) and washed with saturated aqueous NH4Cl solution (200 mL), and then the reaction mixture was cooled to 0°C, filtered, and concentrated under reduced pressure. The residue was purified by flash chromatography (silica gel, 70 : 25 : 5; hexane : ethyl acetate : methanol, v/v) to give pure carboxamides 11-13 as a beige oil. Rf = 0.3 (hexane ; ethyl acetate : methanol = 20 : 75 : 5, v/v) to give pure carboxamides 11-13.  

General procedure for the preparation of compounds 11-13 via condensation reaction of acid 6 and amino esters 8-10

To a stirred suspension of acid 6 (0.45 mmol), DIPEA (0.50 mmol) and HATU (0.54 mmol) in dry dichloromethane (8.0 mL) were added amino esters 8-10 (0.45 mmol) at 5°C. The mixture was stirred at r.t. for 16 h. The reaction mixture was diluted with dichloromethane (10 mL) and washed with sat. aqueous NaHCO3 solution (10 mL) and brine (12 mL). The organic layer was separated, dried over anhydrous MgSO4, filtered, and concentrated under reduced pressure. The residue was purified by flash column chromatography (silica gel, ethyl acetate : methanol = 20 : 75 : 5, v/v) to give pure carboxamides 11-13.
etate : methanol = 65 : 30 : 5, v/v). – IR (neat, NaCl): ν = 3345, 3013, 2956, 1785, 1751, 1698, 1586, 1531, 1416, 1357, 1301, 1123, 1089, 754 cm⁻¹. – 1HN M R( CDCl₃, 500.14 MHz): δ = 7.66 (br s, 2H), 7.41 – 7.16 (m, 10H), 6.91 (br s, 1/3H), 5.56 (d, J = 10.8 Hz, 2H), 4.41 (d, J = 10.8 Hz, 2H), 3.74 (s, 3H), 3.61 – 3.34 (m, 3H), 2.45 – 2.31 (m, 1H), 2.20 – 2.06 (m, 1H), 1.91 (s, 2H), 1.57 (s, 3H), 1.43 (s, 3H). – 13CN M R (CDCl₃, 125.67 MHz): δ = 173.5, 168.2, 156.7, 136.3, 128.6, 128.1, 127.9, 70.5, 67.9, 67.6, 65.8, 64.8, 58.2, 52.4, 39.7, 38.6, 31.6, 26.9. – HRMS: m/z = 462.1717 (calcd. 462.1699 for C₂₂H₂₈N₃O₇S, [M+H⁺]).

Yield: 78 %. White solid. Rf = 0.4 (n-hexane : ethyl acetate : methanol = 65 : 30 : 5, v/v). – 13C NMR (CDCl₃, 125.67 MHz): δ = 173.5, 167.1, 156.7, 136.2, 128.6, 128.1, 127.8, 70.5, 67.9, 67.6, 65.8, 64.8, 58.2, 52.4, 39.7, 38.6, 31.6, 26.9. – HRMS: m/z = 548.1767 (calcd. 548.1684 for C₂₂H₂₈N₃O₇S, [M+H⁺]).

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[20] All the analogs were evaluated at least at six different concentrations, each in duplicate. The mean values were used to generate the growth inhibition curves and determination of IC50 values.