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Bicyclic tetrapeptide histone deacetylase inhibitors with methoxymethyl ketone and boronic acid zinc-binding groups

Md. Nurul Islam [a,b,*], Md. Shahidul Islam [a,b], Md. Ashraful Hoque [a,c], Tamaki Kato [a], Norikazu Nishino [a], Akihiro Ito [d], Minoru Yoshida [d]

[a] Graduate School of Life Science and Systems Engineering, Kyushu Institute of Technology, Kitakyushu 808-0196, Japan
[b] Department of Chemistry, Faculty of Science, University of Rajshahi, Rajshahi 6205, Bangladesh
[c] Department of Biochemistry and Molecular Biology, Faculty of Science, University of Rajshahi, Rajshahi 6205, Bangladesh
[d] Chemical Genetics Laboratory/Chemical Genomics Research Group, RIKEN Advanced Science Institute, Saitama 351-0198, Japan

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ABSTRACT

Histone deacetylase (HDAC) inhibitors are a class of potential therapeutics for the treatment of cancer. Bicyclic tetrapeptides equipped with methoxymethyl ketone and boronic acid as zinc-binding group were designed and synthesized. The inhibitory activities of these compounds were evaluated against HDAC enzymes. The cell-free and cell-based assay data showed that both potency and selectivity changed with the change in zinc-binding group. Boronic acid-based compound showed poor activity whereas methoxymethyl ketone-based compound displayed impressive activity in both cell-free and cell-based conditions.

1. Introduction

Histone deacetylase (HDAC) inhibitors, belonging to an emerging class of therapeutics with potential as anticancer drugs, not only cause growth arrest, differentiation, and apoptosis of tumor cells but also have shown promise as anti-parasitic, anti-neurodegenerative, anti-rheumatologic, and immunosuppressant agents [1,2]. As HDAC inhibitors display their biological effects across multiple pathways within the malignant cell, including extrinsic and intrinsic apoptosis, autophagy, inhibiting proliferation, migration, and tumor angiogenesis and effects in the immune response [1], design and synthesis of HDAC inhibitors has become an attracting field for research. The United States Food and Drug Administration (FDA) has approved two HDAC inhibitors, vorinostat and romidepsin, for the treatment of cutaneous T-cell lymphoma (CTCL) which are now available in the market [3]. This approval has added a new dimension in this research field.

So far, several structurally distinct HDAC inhibitors including hydroxamates, benzamides, short-chain fatty acids, electrophilic ketones, and macrocyclic peptides have been reported [2]. However, most of the reported HDAC inhibitors including trichostatin A (TSA) and SAHA are regarded as broad spectrum with wide range of side effects [4]. Therefore, research in this field is now going on to develop isoform selective HDAC inhibitors. The main focus of these researches is on the optimization of cap groups and/or zinc-binding groups, as the spacer groups have been optimized to six methylene units [5]. Komatsu and co-workers reported a series of cyclic tetrapeptides hydroxamic acid [6]. Our group reported a number of cyclic tetrapeptides HDAC inhibitors containing a variety of functional groups such as retrohydroxamate [7], SS-hybrid [8], trifluoromethyl, pentafluoroethyl ketones [9], carbonyl group [10], acryloyl chloride, and chloroacetic acid [11]. To increase the size of macrocyclic cap group, Nishino and co-workers synthesized bicyclic peptide disulfide hybrids which showed good activity in both cell-free and cell-based conditions in nanomolar range [12]. As a continuation, we reported bicyclic terapeptides hydroxamic acid as potent HDAC inhibitors in which the aliphatic loop length was optimized to eleven methylene units (compound **2**, Fig. 1) [13]. Comparative study of bicyclic tetrapeptide HDAC inhibitors containing disulfide hybrid and hydroxamic acid showed a significant change in both activity and selectivity as the zinc-binding group (ZBG) was changed from SS-hybrid to hydroxamic acid [12,13]. Inspired by these facts we designed two more bicyclic tetrapeptide HDAC inhibitors to explore the effect of other ZBG on the activity of bicyclic tetrapeptides. The designing was originated from our report of boronic acid and methoxymethyl ketone-based cyclic tetrapeptide HDAC inhibitors (compound **3** and **4**, Fig. 1) [11].

* Corresponding author at: Department of Chemistry, Faculty of Science, University of Rajshahi, Rajshahi 6205, Bangladesh.
E-mail address: mnurulchem@gmail.com (M.N. Islam).

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In this paper, we describe the account on synthesis and a brief description of interesting biological results of methoxymethyl ketone and boronic acid-based bicyclic tetrapeptide HDAC inhibitors having optimized loop length (Fig. 2).

2. Results and discussion

2.1. Chemistry

Bicyclic tetrapeptide methoxymethyl ketone (**6**) was synthesized according to Scheme 1 by the conventional solution phase method. The synthesis was started by coupling H-D-Pro-O^{t}Bu (**7**) with Boc-L-2-amino-8-nonenoic acid (Boc-L-Ae9-OH) using DCC/HOBt to obtain protected dipeptide (**8**). Boc protection was selectively removed by 4 M HCl/dioxane, and the free amine was condensed with Boc-D-2-amino-7-octenoic acid (Boc-D-Ae8-OH) by the same DCC/HOBt method to obtain linear tripeptide (**9**). The linear tripeptide with fused side ring (**10**) was synthesized by ring-closing metathesis (RCM) between D-Ae8 and L-Ae9 using Grubb's first generation catalyst in dichloromethane (DCM), followed by catalytic hydrogenation in presence of Pd–C. After selective deprotection, Boc-L-Ae9-OH was incorporated to prepare the linear tetrapeptide (**11**). After removal of both side protections by treating with trifluoroacetic acid (TFA), cyclization reaction was carried out by the aid of HATU in DMF under high dilution conditions with minimum amount of DIEA (2.5 equiv) to yield bicyclic tetrapeptide with terminal alkene in the side chain (**12**). The side chain terminal alkene was modified to epoxide (**13**) by the aid of 3-Chloroperbenzoic acid (m-CPBA) in DCM. Opening of the epoxide ring by the use of NaOMe/MeOH and subsequent oxidation of alcoholic group ((**14**) to keto group using Dess–Martin periodinane (DMP) yielded the bicyclic tetrapeptide methoxymethyl ketone (**6**).

Bicyclic tetrapeptide boronic acid (**5**) was synthesized according to Scheme 2. In this case, the same linear tripeptide with fused ring (**10**) was used as starting material. After selective deprotection, Boc-L-2-amino-6-heptenoic acid (Boc-L-Ae7-OH) was incorporated to prepare the linear tetrapeptide (**15**). After removal of both the side protections by treating with trifluoroacetic acid, cyclization reaction was carried out by the aid of HATU in DMF (0.2 mM) with minimum amount of DIEA (2.5 equiv) to yield bicyclic tetrapeptide with terminal alkene in the side chain (**16**). Pinacole borane was incorporated to the side chain (**17**) by treating with pinacole borane in presence of $[Ir(cod)Cl]_2$ and bis(diphenylphosphino)methane (dppm) in DCM. Finally, pinacole protection was removed by treating with $NaIO_4$ and NH_4OAc to yield bicyclic tetrapeptide boronic acid (**5**).

All the synthesized compounds were characterized by ^{1}H NMR and high resolution FAB–MS. The purity of the compounds was determined by HPLC analysis all the synthesized cyclic/bicyclic tetrapeptides showed purity above 95%.

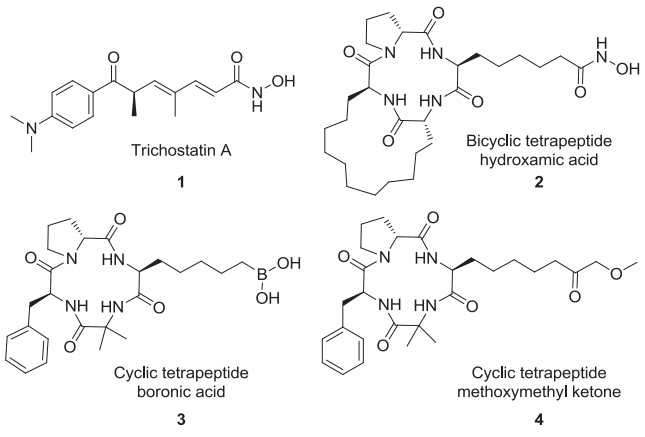

Fig. 1. Some reported HDAC inhibitors.

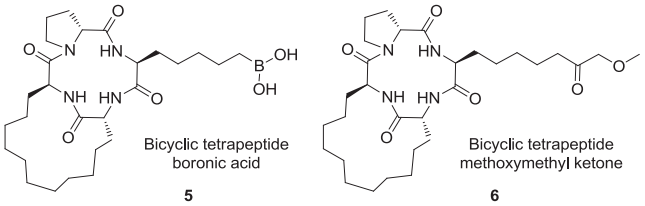

Fig. 2. Synthesized bicyclic tetrapeptide methoxymethyl ketone and boronic acid.

2.2. Enzyme inhibition and biological activity

The synthesized bicyclic tetrapeptide boronic acid and methoxymethyl ketone were assayed for HDAC inhibitory activity using HDAC1, HDAC4, and HDAC6 enzymes prepared from 293T cells [14]. Additionally, to know the inhibitory activity of these compounds in cell-based condition; we carried out p21 promoter assay according to the literature [10]. The detail experimental procedures are described in the experimental section of this paper. The results of the HDAC inhibitory activity and the p21 promoter assay of the compounds are shown in Table 1.

To evaluate the potency and selectivity of synthesized compounds **5** and **6** as HDAC inhibitors, their activity on distinct isoforms (HDAC1, 4 and 6) was compared with that of compounds **2**, **3** and **4** (Table 1). Compounds **2**, **5** and **6** contain the same scaffold with different ZBG, hydroxamic acid, boronic acid and methoxymethyl ketone respectively. Among them, boronic acid showed poor activity with micromolar range in both cell-free and cell-based conditions. Suzuki and co-workers reported a series of boronic acid-based HDAC inhibitors, which also showed inhibitory activity in micromolar range [15]. Our reported cyclic tetrapeptide boronic acid (**3**) also exhibited HDAC inhibitory activity in micromolar range.

Therefore, boronic acid seems to be not promising as zinc-binding group. It may be due to the poor zinc-binding affinity for this group in the mechanism of HDAC inhibition. On the other hand, bicyclic tetrapeptide methoxymethyl ketone **6** displayed impressive inhibitory activity against HDAC1, 4, and 6. This reveals that the extent of zinc-binding ability of methoxymethyl ketone in the mechanism of HDAC inhibition is highly effective. It also showed better activity than monocyclic tetrapeptide methoxymethyl ketone, **4**. It implies that bicyclic tetrapeptide scaffold has better interaction with surface region of the HDAC enzymes. This observation supports the fact that complex cap-groups interact with HDAC enzyme's outer rim and demonstrate improved HDAC inhibition [2]. The nanomolar inhibitory activity of compound **6** is similar to our formerly reported bicyclic tetrapeptide hydroxamic acid **2**. Moreover, compound **6** showed much more selectivity towards HDAC 1 and 4 over HDAC 6 compared to that of compound **2**. As hydroxamic acid-based HDAC inhibitors suffer from some limitations [16], methoxymethyl ketone-based HDAC inhibitor might be an alternative in the field of HDAC inhibitors.

3. Conclusion

To explore the effect of zinc-binding groups on activity and selectivity, bicyclic tetrapeptide HDAC inhibitors bearing methoxymethyl ketone and boronic acid were synthesized and their inhibitory activity were evaluated. The cell-free and cell-based assay