Pergamon

Bioorganic & Medicinal Chemistry Letters 12 (2002) 3089–3092

BIOORGANIC & MEDICINAL CHEMISTRY LETTERS

Hydroxamate Based Inhibitors of Adenylyl Cyclase. Part 2: The Effect of Cyclic Linkers on P-Site Binding

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Received 20 May 2002; accepted 1 August 2002

Abstract—The adenylyl cyclases (ACs) are a family of enzymes that are key elements of signal transduction by virtue of their ability to convert ATP to cAMP. The catalytic mechanism of this transformation proceeds through initial binding of ATP to the purine binding site (P-site) followed by metal mediated cyclization with loss of pyrophosphate. Previous work in our group identified novel inhibitors which possess an adenine ring joined to a metal-coordinating hydroxamic acid through flexible linkers. Considering the spatial positioning of the metals with respect to the adenine binding site coupled with potentially favorable entropic factors, conformational restriction of the tether through a stereochemistry based SAR employing a rigid cyclic scaffold was explored.

β-Adrenergic signaling is a key process in CV,[1] CNS[2] and metabolic regulation.[3] Unfortunately, the prolonged use of β-agonists/antagonists is plagued by poor tissue selectivity, sensitization/desensitization following therapy, and dynamic changes to the β-adrenergic receptors that are inconsistent among disease states.[4] Since the use of β-blockers in clinical settings is intended to regulate cAMP production, direct regulation of AC activity may afford a novel pharmacological approach to β-adrenergic signaling.

Previous studies in our labs demonstrated that AC P-site antagonists may be obtained by linking adenine to a metal chelating hydroxamic acid via flexible linkers. Figure 1 illustrates several representative inhibitors derived from this approach.[5] As shown in our initial study, inhibitory activity was dependent on both the length and heteroatom content of the linkers.

Previously reported crystallographic studies[6,7] of adenylyl cyclase/Gsα complexes with bound P-site substrates confirmed our hypothesis regarding the importance of metal chelating groups in enzyme catalysis. Figure 2, based on these studies, illustrates expected binding interactions between one of our designed inhibitors and the AC active site residues. Realizing the energy required to order flexible linkers within the active site has potentially negative effects on inhibitory activity, inhibitors bearing conformationally restricted linkers were proposed. As shown in Figure 3, linker rigidity was designed utilizing cyclopentene and cyclopentane rings. Because this modification results in the introduction of two stereogenic centers, all stereoisomeric hydroxamic acids were prepared and compared to their corresponding carboxylic acid and ester analogues.

As shown in Scheme 1, the commercially available (1*R*,3*S*)-cyclopentene, **1**, was alkylated with ethyl diazoacetate utilizing rhodium catalysis.[8] Subsequent cleavage of the acetate group, with simultaneous conversion of the ethyl ester to a methyl ester, gave the alcohol, **2**. Alkylation of adenine at N9 with alcohol, **2**, was achieved via Mitsunobu methodology[9,10] and gave the desired compound, **3a**, with inversion.

Conversion of **3a** to its corresponding cyclopentene and cyclopentane based carboxylic and hydroxamic acids is shown in Scheme 2. Although Scheme 2 is drawn with no inferred stereochemistry there was no compromise to the configurational purity of the starting ester during any of the illustrated steps. As shown, the ester was hydrolyzed to its carboxylic acid, **4a**, on treatment with sodium hydroxide in aqueous methanol. Alternately, the ester group was converted to its hydroxamic acid, **5a**, on treatment with hydroxylamine and potassium hydroxide in methanol.[11] Following hydrogenation of

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PII: S0960-894X(02)00654-6

the cyclopentene ester, **3a**, to its corresponding cyclopentane derivative, **6a**, the analogous carboxylic acid and hydroxamic acid analogues (compounds **7a** and **8a**, respectively) were prepared.

The cyclopentene products prepared according to Scheme 1 possess the (1*S*, 3*S*) configuration as defined by the numbering in Figure 3. Similarly, the cyclopentane analogues possess the (1*R*, 3*R*) configuration. As this study focused not only on the rigidity of the tether, but also on the stereochemistry of the substituents around the tether, preparation of all possible stereoisomers was of interest. Therefore, the next goal was to synthesize the enantiomer of compound **3a** (Scheme 1) and all relevant derivatives. As shown in Scheme 3, this goal was realized beginning with the same cyclopentene starting material, **1**. Initial protection of the alcohol as its TBDMS ether followed by cleavage of the acetate group and alkylation of the resulting hydroxyl group with ethyl diazoacetate gave compound **9**. Cleavage of the silyl group followed by coupling with adenine under Mitsunobu conditions gave the desired enantiomer, **3b**.

Utilizing the chemistry described in Scheme 2, compound **3b** was converted to its saturated ester and corresponding cyclopentene and cyclopentane linked carboxylic and hydroxamic acids (compounds **4b**–**8b**). In this series, the (1*R*, 3*R*) cyclopentene and (1*S*, 3*S*) cyclopentane configurations were realized.

Having prepared two series bearing enantiomeric linkers, attention was directed towards the synthesis of the series containing the corresponding diastereomeric linkers. As illustrated in Scheme 4, protecting group manipulation gave the intermediate alcohol, **10**. Subsequent inversion

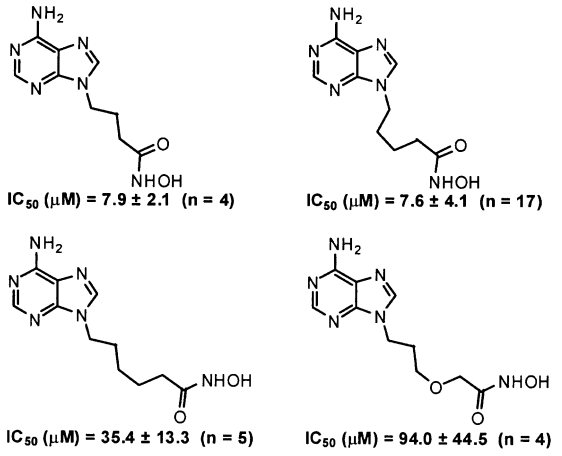


Figure 1. Representative P-site inhibitors with flexible linkers.

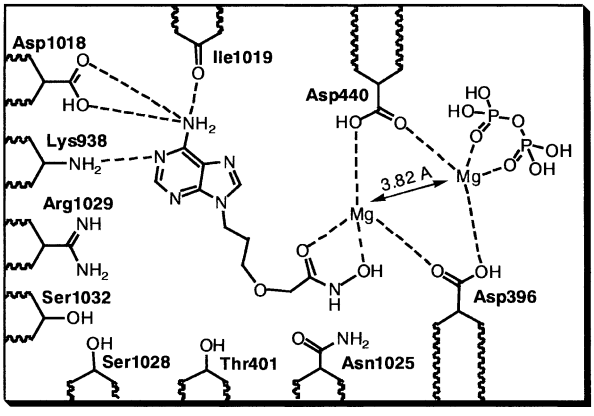


Figure 2. Proposed interactions between ATP binding site and flexible P-site inhibitor.

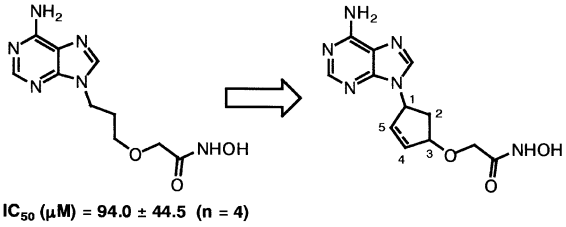
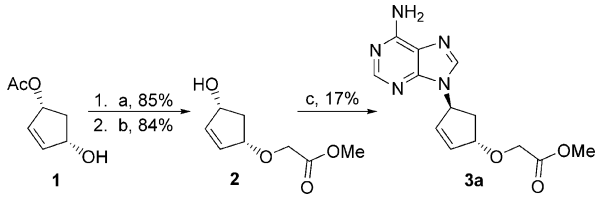
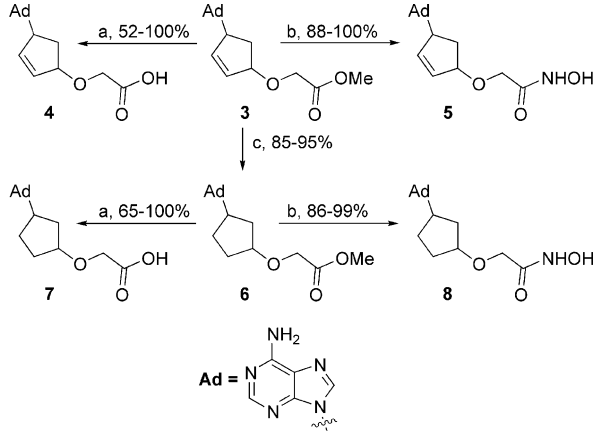


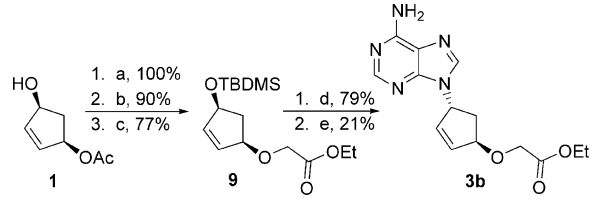
Figure 3. Design of cyclic tethers from acyclic analogues.



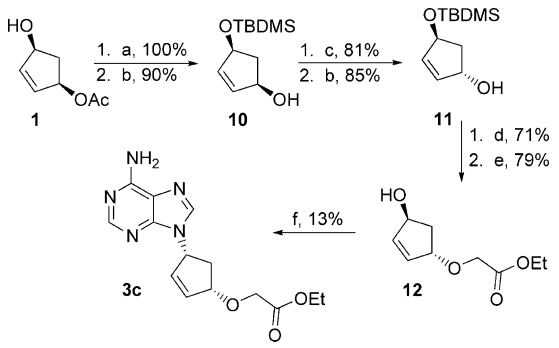
Scheme 1. Reagents and conditions: (a) ethyl diazoacetate, $[Rh(OAc)_2]_2$, CH_2Cl_2; (b) NaOMe, MeOH, rt; (c) adenine, DEAD, PPh_3, THF.



Scheme 2. Reagents and conditions: (a) NaOH, MeOH, H_2O, rt; (b) $HONH_2$•HCl, KOH, MeOH, rt; (c) H_2, 10% Pd/C, MeOH.



Scheme 3. Reagents and conditions: (a) TBDMS-Cl, imidazole, THF, rt; (b) NaOMe, MeOH, rt; (c) ethyl diazoacetate, $[Rh(OAc)_2]_2$, CH_2Cl_2; (d) AcOH, THF, H_2O, rt; (e) adenine, DEAD, PPh_3, THF.



Scheme 4. Reagents and conditions: (a) TBDMS-Cl, imidazole, THF, rt; (b) NaOMe, MeOH, rt; (c) *p*-nitrobenzoic acid, DEAD, PPh_3, THF; (d) ethyl diazoacetate, $[Rh(OAc)_2]_2$, CH_2Cl_2; (e) TBAF, THF, rt; (f) adenine, DEAD, PPh_3, THF.

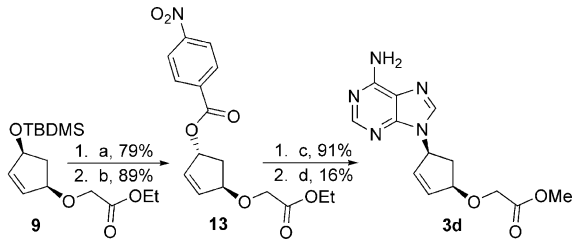
of the hydroxyl stereochemistry was achieved utilizing the Mitsunobu reaction with *p*-nitrobenzoic acid followed by cleavage of the resulting *p*-nitrobenzoate. The resulting alcohol, **11**, was alkylated with ethyl diazoacetate and the silyl group was cleaved. Treatment of compound **12** with adenine under Mitsunobu conditions gave the ester, **3c**, bearing the desired diastereomeric linker.

Applying the chemistry described in Scheme 2, compound **3c** was converted into its saturated ester and corresponding cyclopentene and cyclopentane linked carboxylic and hydroxamic acids (compounds **4c**–**8c**). The (1*R*, 3*S*) cyclopentene and (1*S*, 3*R*) cyclopentane configurations were realized in this scheme.

In pursuit of the final family of diastereomers, compound **9** (Scheme 3) was utilized as illustrated in Scheme 5. As shown, initial cleavage of the silyl group followed by inversion of the hydroxyl stereochemistry under Mitsunobu conditions gave the desired *p*-nitrobenzoate, **13**. Subsequent cleavage of the *p*-nitrobenzoate and coupling with adenine under Mitsunobu conditions gave the desired ester, **3d**.

Using previously described chemistry, compound **3d** was converted its saturated ester and corresponding cyclopentene and cyclopentane linked carboxylic and hydroxamic acids (compounds **4d**–**8d**). The (1*S*, 3*R*) cyclopentene and (1*R*, 3*S*) cyclopentane configurations were realized using this scheme.

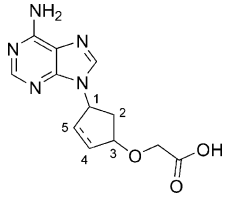
Following isolation, all adenine based esters, carboxylic and hydroxamic acids were evaluated against type V AC expressed in HEK 293 cells.[12]



Scheme 5. Reagents and conditions: (a) AcOH, THF, H_2O, rt; (b) *p*-nitrobenzoic acid, DEAD, PPh_3, THF; (c) NaOMe, MeOH, rt; (d) Adenine, DEAD, PPh_3, THF.

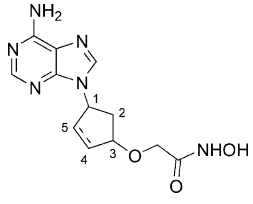
In agreement with our previously reported data,[5] all ester analogues, regardless of stereochemistry, failed to demonstrate any measure of inhibitory activity against type V AC (data not shown). However, unlike the carboxylic acids presented in previous reports,[5] some analogues presented herein did exhibit a weak level of inhibitory activity (Table 1). Due to the low level of inhibition, it is difficult to assess whether this activity relies on the stereochemistry around the cyclic tether. Perhaps the most striking results noted relate to the hydroxamic acid analogues (Table 2). While all of these compounds demonstrated some level of inhibitory activity, two compounds stood out, analogues **5b** and **8b**. These compounds (Fig. 4), were found to inhibit type V AC with approximately an eight-fold increase in activity over the predecessor acyclic analogue. While the activity did not appear to be dependent upon the presence of an endocyclic double bond in the linker, it was highly dependent upon the stereochemistry around the cyclic tether with a preferred trans relationship between the adenine and hydroxamate groups.

Table 1. IC_{50} values of carboxylic acids against type V AC



Compd	Stereochemistry	IC_{50} (μM)
4a	1*S*, 3*S*	98 ± 61 (n = 2)
7a	1*R*, 3*R*	> 200
4b	1*R*, 3*R*	78 ± 33 (n = 4)
7b	1*S*, 3*S*	79 ± 48 (n = 3)
4c	1*R*, 3*S*	72.0 (n = 1)
7c	1*S*, 3*R*	> 200
4d	1*S*, 3*R*	> 200
7d	1*R*, 3*S*	> 200

Table 2. IC_{50} values of hydroxamic acids against type V AC



Compd	Stereochemistry	IC_{50} (μM)
8b	1*S*, 3*S*	10.8 ± 3.6 (n = 13)
5b	1*R*, 3*R*	13.6 ± 4.0 (n = 12)
5d	1*S*, 3*R*	96 ± 78 (n = 2)
5a	1*S*, 3*S*	102 ± 13 (n = 2)
8d	1*R*, 3*S*	109 ± 46 (n = 3)
8a	1*R*, 3*R*	110 ± 12 (n = 2)
5c	1*R*, 3*S*	116 ± 35 (n = 3)
8c	1*S*, 3*R*	> 200

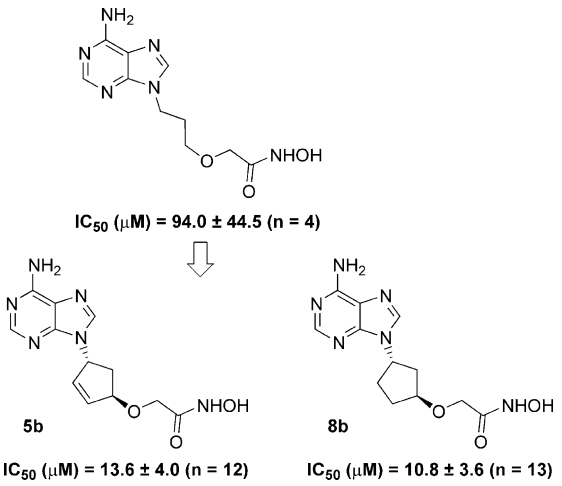


Figure 4. Most potent cyclic analogues derived from acyclic lead.

In conclusion, rationally designed inhibitors of type V AC were prepared and tested. Two hydroxamic acid based inhibitors with cyclic tethers showed significantly enhanced activity over the corresponding acyclic analogue. In addition, the level of activity was highly dependent upon the stereochemistry within the cyclic tether. While the most potent analogues possessed strongly chelating hydroxamic acid groups, the weaker chelating carboxylic acids showed some activity presumably due to favorable positioning of the adenine and chelator by the rigid linker. These results validate our hypothesis that more potent inhibitors could be obtained by introducing conformational restriction into the tether of the designed adenine-linked hydroxamic acids. Further attempts to improve the activity of these type V AC inhibitors will be the subject of future communications from this laboratory.

References and Notes

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12. Measurement of AC activities was achieved utilizing human type V recombinant AC expressed in HEK293 cells. Isolated membranes (140 ng/mL) were used in the presence of 60 mM HEPES, pH 8.0, 0.6 mM EDTA, 0.01% (w/v) Bovine serum albumin, 25 nM activated recombinant Gsα, 1 mM ATP, 2 mM isobutyl methyl xanthine and 2 mM $MgCl_2$. Compounds were added to the mixture and the reaction was run for 30 min at 30 °C. Terminated reactions were evaluated for the enzymatic product, cAMP, using a commercially available New England Nuclear flash plate system. The degree of inhibition was determined by comparing the measured cAMP concentrations to those measured in control reactions containing no compound.