Metal Coordination-Based Inhibitors of Adenylyl Cyclase: Novel Potent P-Site Antagonists

Daniel E. Levy,*,† Ming Bao, Diana B. Cherbavaz, James E. Tomlinson, David M. Sedlock, Charles J. Homcy, and Robert M. Scarborough*

Departments of Medicinal Chemistry and Biology, Millennium Pharmaceuticals, Inc., 256 East Grand Avenue, South San Francisco, California 94080

Received December 13, 2002

The adenylyl cyclases (ACs) are a family of intracellular enzymes associated with 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 so-called purine binding site (P-site) of the enzyme followed by metal-mediated cyclization with loss of pyrophosphate. Crystallographic analysis of ACs with known inhibitors reveals the presence of two metals in the active site. Presently, nine isoforms of adenylyl cyclase are known, and unique isoform combinations are expressed in a tissue-specific manner. The development of isoform-specific inhibitors of adenylyl cyclase may prove to be a useful strategy toward the design of unique signal transduction inhibitors. To develop novel AC inhibitors, we have chosen an approach to inhibitor design utilizing an adenine ring system joined to a metal-coordinating hydroxamic acid via various linkers. Previous work in our group has validated this approach and identified novel inhibitors that possess an adenine ring joined to a metal-coordinating hydroxamic acid through flexible acyclic linkers (Levy, D. E., et al. *Bioorg. Med. Chem. Lett.* **2002**, *12*, 3085−3088). Subsequent studies have focused on the introduction of conformational restrictions into the tether of the inhibitors with the goal of increasing potency (Levy, D. E., et al. *Bioorg. Med. Chem. Lett.* **2002**, *12*, 3089−3092). Building upon the favorable spatial positioning of the adenine and hydroxamate groups coupled with potentially favorable entropic factors, the unit joining the carbocycle to the hydroxamate was explored further and a stereochemical-based SAR was elucidated, leading to a new series of highly potent AC inhibitors.

Introduction

β-Adrenergic signaling is a key process in cardiovascular,[1] CNS,[2] and metabolic regulation.[3] Figure 1[4] illustrates the regulation of cAMP production through the β-adrenergic receptor. As shown, heterotrimeric G-protein complexes interact with the β-adrenergic receptor and release activated Gsα when β-adrenergic ligands are bound.[5] Following dissociation, activated Gsα binds to and stabilizes adenylyl cyclase (AC),[6,7] which catalyzes the conversion of ATP into cAMP.[8] One strategy for regulating β-adrenergic signaling that has been pursued is prolonged use of β-agonists/antagonists. This approach is unfortunately plagued by poor tissue selectivity, sensitization/desensitization following therapy, and dynamic changes to the β-adrenergic receptors that are inconsistent among disease states.[9]

Previous studies in our labs have demonstrated that AC P-site antagonists may be obtained by linking the adenine ring system to a metal chelating hydroxamic acid via flexible linkers.[10] Subsequent studies demonstrated the importance of stereochemistry and conformational rigidity in the tether.[11] Figure 2 illustrates several representative inhibitors derived from this approach.

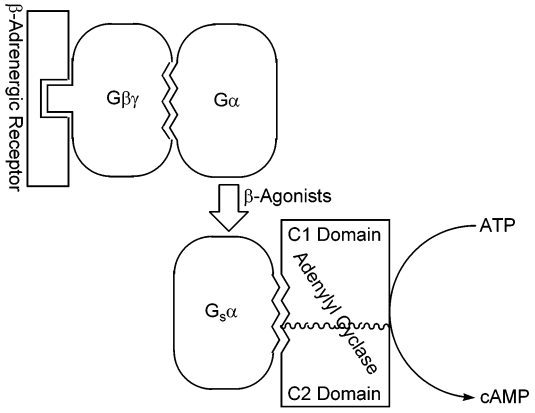


Figure 1. Interaction cascade leading to conversion of ATP to cAMP.

Successful crystallographic studies with known AC P-site inhibitors were reported utilizing a heterodimeric crystal comprised of the C1 domain from type V AC and the C2 domain from type II AC. As noted previously, these reported crystallographic studies[12,13] of adenylyl cyclase/Gsα complexes with bound P-site substrates confirmed our original inhibitor design hypothesis regarding the importance of metal chelating groups in enzyme catalysis. On the basis of these studies, Figure 3 illustrates the expected binding interactions between one of our designed inhibitors and the AC active site residues. Subsequently, having established the importance of stereochemistry and structural rigidity within

* Millennium Pharmaceuticals, Inc. 256 East Grand Avenue, South San Francisco, CA 94080. Tel: (650) 244-6822. Fax: (650) 244-9287. E-mail: bob.scarborough@mpi.com.

† Current address: Scios, Inc. 820 West Maude Avenue, Sunnyvale, CA 94085. Tel: (408) 616−8391. Fax: (408) 616−8317. E-mail: levyd@sciosinc.com.

10.1021/jm0205604 CCC: $25.00
Published on Web 04/19/2003

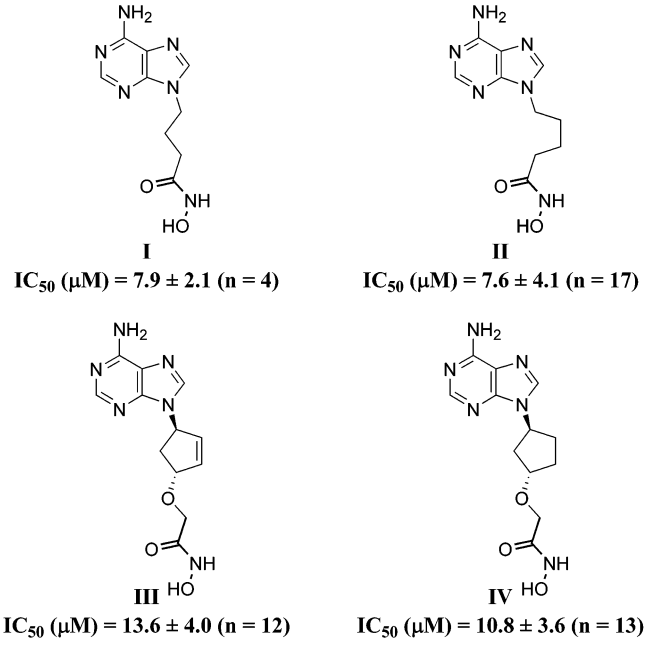


Figure 2. Representative synthetic P-site inhibitors.

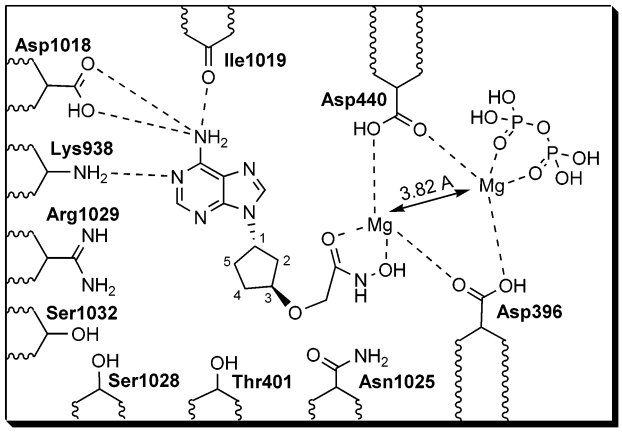


Figure 3. Proposed interactions between ATP binding site and a synthetic P-site inhibitor.

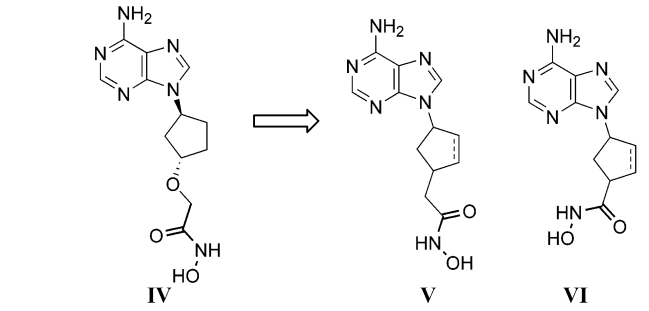
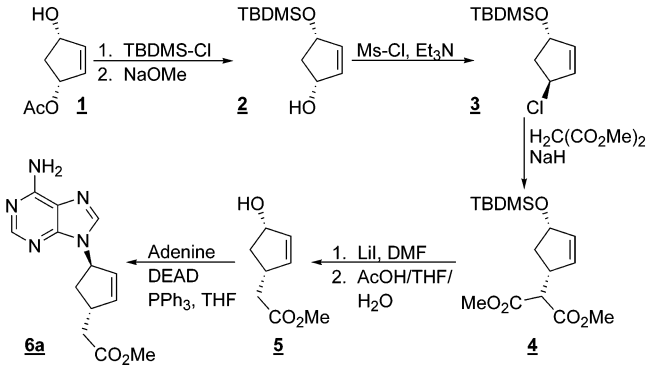


Figure 4. Modification of unit joining hydroxamate to cyclic tether.

the inhibitor tethers, the present study was initiated to probe the spatial requirements of the P-site through further modification of the unit joining the adenine ring and the metal-chelating hydroxamic acid (Figure 4). As noted in previous studies, there is a dependence upon the stereochemical configuration around the cyclopentene/cyclopentane ring tether which we continued to probe through the synthesis of all possible stereoisomeric hydroxamic acids. The inhibitory activities of the hydroxamic acids prepared in this study were compared to the activities of the analogous carboxylic acids and esters to confirm the importance of the metal-chelating functionality in enzyme inhibition.

Scheme 1

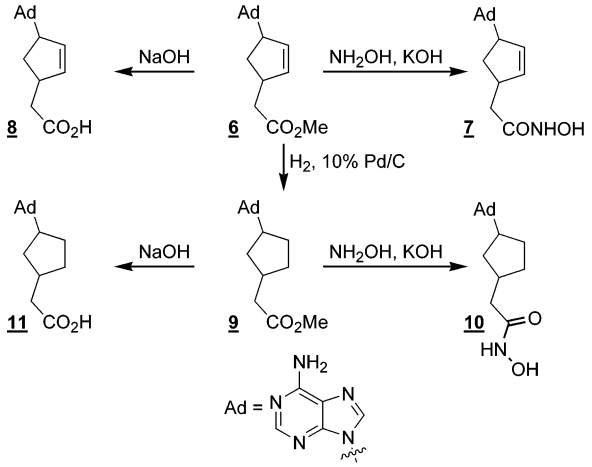


Chemistry. The compounds of the present study were prepared in two groups. The first group possessed the metal chelating functionality separated from the cyclopentene/cyclopentane ring by a methylene unit. This involved removal of the oxygen atom of our previous inhibitor **IV** affording the cyclopentene/cyclopentane acetic acid derived compounds **V**. The second group consisted of compounds analogous to the first with the methylene unit removed affording the cyclopentene/cyclopentane carboxylic acid-derived compounds **VI**. Beginning with the compounds of the first group, the starting material for all structures was commercially available (1*R*,3*S*)-4-cyclopentene-1,3-diol-1-acetate, **1**. As shown in Scheme 1, the hydroxyl group of compound **1** was converted into its corresponding silyl ether, and the acetate group was subsequently cleaved giving the allylic alcohol, **2**. The allylic alcohol was converted to the corresponding allylic chloride **3**, on treatment with mesyl chloride, with stereochemical inversion. Compound **3** was then used to alkylate dimethylmalonate, again with inversion of the stereochemistry at the chloride center. The resulting diester **4** was monodecarboxylated on treatment with lithium iodide,[14,15] and the silyl ether was subsequently cleaved under acidic conditions affording the allylic alcohol, **5**. Finally, utilizing Mitsunobu conditions,[16,17] compound **5** was coupled with adenine with stereochemical inversion at the hydroxide center giving the cyclopenteneacetic acid ester **6a**.

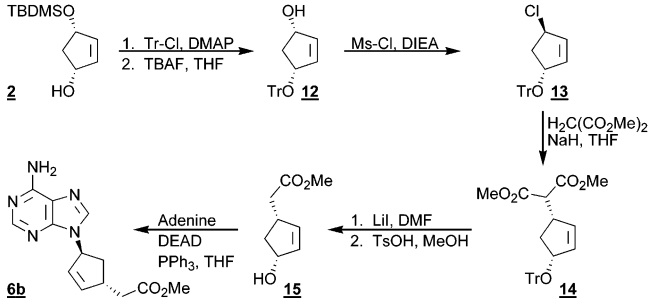
Conversion of **6a** to its corresponding cyclopentene- and cyclopentane-based carboxylic and hydroxamic acids is shown in Scheme 2. Although Scheme 2 is drawn with no inferred stereochemistry, there was no compromise to the configurational purity of the starting ester during any of the illustrated steps. As shown, the ester was converted into the hydroxamic acid, **7a**, on treatment with hydroxylamine and potassium hydroxide in methanol.[18] Alternately, the ester group was hydrolyzed to the carboxylic acid, **8a**, on treatment with sodium hydroxide in aqueous methanol. Following hydrogenation of the cyclopentene ester, **6a**, into the corresponding cyclopentane derivative **9a**, the analogous hydroxamic acid and carboxylic acid analogues (compounds **10a** and **11a**, respectively) were prepared.

The cyclopentene products prepared according to Scheme 1 possess the (1*R*, 3*S*) configuration as defined

Scheme 2



Scheme 3

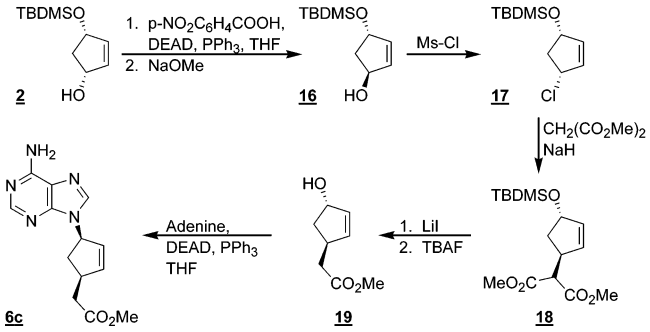


by the numbering in Figure 3. Similarly, the cyclopentane analogues possess the (1*S*, 3*S*) configuration. As this study focused not only on the rigidity of the tether, but also on the stereochemistry of the substituents within the tether, preparation of all possible stereoisomers was of interest. Therefore, the next goal was to synthesize the enantiomer of compound **6a** and all relevant derivatives. As shown in Scheme 3, this goal was realized beginning with the previously described intermediate **2**. Initial protection of the allylic alcohol as a trityl ether followed by cleavage of the silyl ether with TBAF gave the allylic alcohol, **12**. The allylic alcohol was converted to the corresponding allyl chloride, **13**, on treatment with mesyl chloride, with stereochemical inversion. Compound **13** was then used to alkylate dimethylmalonate with inversion of the stereochemistry at the chloride center. The resulting compound **14** was decarboxylated on treatment with lithium iodide and the trityl ether was subsequently cleaved under acidic conditions giving the allylic alcohol **15**. Finally, utilizing Mitsunobu conditions, compound **15** and adenine were coupled with stereochemical inversion at the hydroxyl center giving compound **6b**.

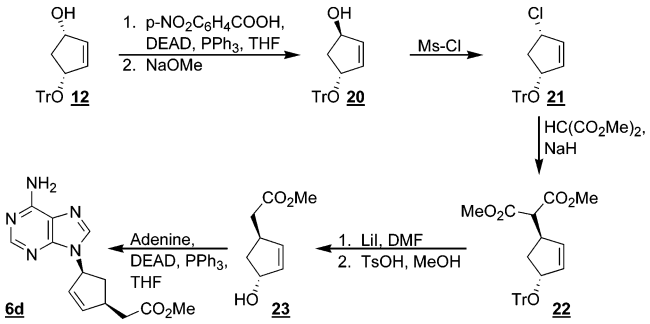
Utilizing the chemistry described in Scheme 2, compound **6b** was converted into the saturated ester and corresponding cyclopentene- and cyclopentane-linked carboxylic and hydroxamic acids (compounds **7b**−**1b**). In this series, the (1*S*, 3*R*) cyclopentene and (1*R*, 3*R*) cyclopentane configurations were obtained.

Having prepared two series of derivatives bearing enantiomeric linkers, attention was directed toward the synthesis of the series containing the corresponding diastereomeric linkers. As illustrated in Scheme 4, the key step in the preparation of the first set of diastereomers was inversion of the hydroxyl group stereocenter of compound **2**. This was accomplished by utilizing the Mitsunobu coupling of compound **2** with *p*-nitrobenzoic acid[19] followed by cleavage of the *p*-nitrobenzoate with sodium methoxide. The resulting allylic alcohol, **16**, was converted to the corresponding allyl chloride, **17**, on treatment with mesyl chloride, with stereochemical inversion. Compound **17** was then used to alkylate dimethylmalonate with inversion of the stereochemistry at the chloride center. The resulting compound **18** was decarboxylated on treatment with lithium iodide and the silyl ether was subsequently cleaved with tetrabutylammonium fluoride giving the allylic alcohol **19**. Finally, utilizing Mitsunobu conditions, compound **19** and adenine were coupled with stereochemical inversion at the hydroxide center giving compound **6c**.

Scheme 4



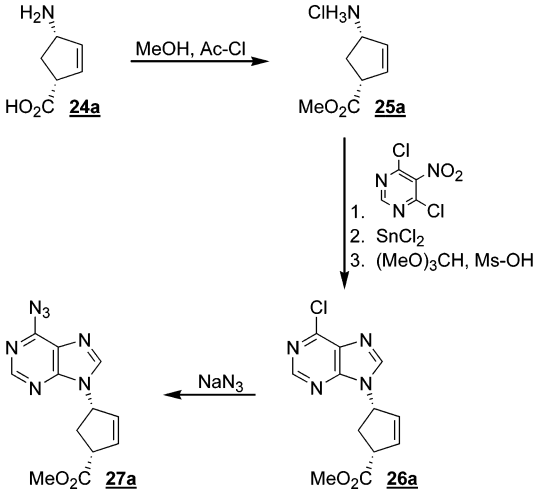
Scheme 5



Applying the chemistry described in Scheme 2, compound **6c** was converted into the saturated ester and corresponding cyclopentene- and cyclopentane-linked carboxylic and hydroxamic acids (compounds **7c**−**11c**). However, unlike previous examples, the carboxylic and hydroxamic acids for the cyclopentene and cyclopentane-based linkers were isolated from the hydroxamic acid synthesis. Once formed, these compounds were easily separated utilizing reversed-phase HPLC. The compounds isolated from this scheme possessed the (1*R*, 3*R*) cyclopentene and (1*S*, 3*S*) cyclopentane configurations.

In pursuit of the final family of diastereomers, compound **12** (Scheme 3) was utilized as illustrated in Scheme 5. Applying the Mitsunobu coupling of compound **12** with *p*-nitrobenzoic acid followed by cleavage of the *p*-nitrobenzoate with sodium methoxide, the stereochemistry of the hydroxyl group was inverted giving compound **20**. Conversion of **20** to the corresponding allyl chloride **21** was achieved on treatment with mesyl chloride accompanied by stereochemical inversion. Compound **21** was then used to alkylate dimethylmalonate with inversion of the stereochemistry

Scheme 6



at the chloride center. The resulting compound **22** was decarboxylated on treatment with lithium iodide and the trityl ether was subsequently cleaved under acidic conditions giving the allylic alcohol, **23**. Finally, utilizing Mitsunobu conditions, compound **23** and adenine were coupled with stereochemical inversion at the hydroxide center giving compound **6d**.

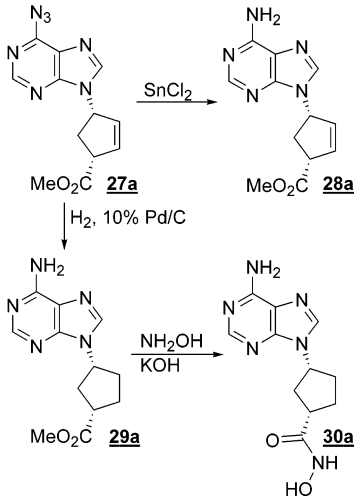
Using the previously described chemistry, compound **6d** was converted to its saturated ester and corresponding cyclopentene- and cyclopentane-linked carboxylic and hydroxamic acids (compounds **7d**−**11d**). The (1*S*, 3*S*) cyclopentene and (1*R*, 3*S*) cyclopentane configurations were obtained using this scheme.

Having completed the cyclopentene/cyclopentane acetic acid derived group, attention was focused on the preparation of compounds with a direct linkage between the cyclopentene/cyclopentane ring and the metal chelator. To accomplish this goal, commercially available (1*R*,4*S*)-4-amino-cyclopent-2-ene carboxylic acid, **24a**, was used. As shown in Scheme 6, compound **24a** was converted to its methyl ester, **25a**, in acidic methanol. The amine was then coupled with 4,6-dichloro-5-nitropyrimidine.[20] Following reduction of the nitro group with tin chloride,[21] cyclization to the chloropurine, **26a**, was achieved on treatment with trimethylorthoformate and catalytic methanesulfonic acid.[22] Subsequent conversion to the azidopurine, **27a**, was achieved on treatment with sodium azide.

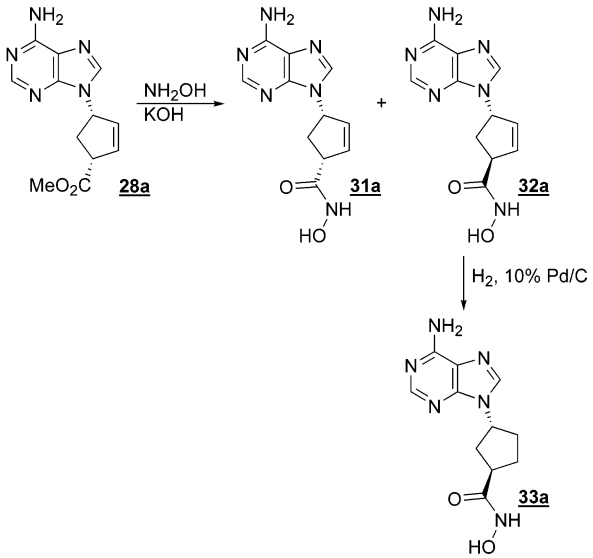
Continuing as illustrated in Scheme 7, the azidopurine, **27a**, was converted to its corresponding substituted adenine, **28a**, utilizing tin chloride. Alternately, the azide and the olefin were simultaneously reduced via catalytic hydrogenation giving compound **29a**. On treatment with hydroxylamine and potassium hydroxide, compound **29a** was converted to the corresponding hydroxamic acid, **30a**.

Completion of this family of compounds proceeded as shown in Scheme 8. As illustrated, compound **28a** was treated with hydroxylamine and potassium hydroxide forming a mixture of diastereomeric hydroxamic acids **31a** and **32a**, in a ratio of approximately 2:1 favoring compound **31a**. Following separation of these isomers by preparative reversed-phase HPLC, compound **32a** was subjected to catalytic hydrogenation giving compound **33a**. It should be noted that the enantiomers of

Scheme 7



Scheme 8

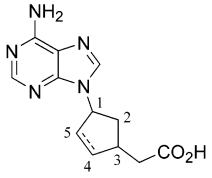


all compounds prepared in Schemes 6−8 (compounds **25b**−**33b**) were prepared under identical conditions beginning with commercially available (1*S*,4*R*)-4-amino-cyclopent-2-ene carboxylic acid, **24b**.

SAR Analysis. The compounds of the present study were assayed using recombinant human type V AC expressed in HEK293 cells. Membranes isolated from these cells demonstrated a 40−50-fold stimulation by recombinant activated Gsα when compared to empty vector (pcDNA3) control populations. Stimulation with activated Gsα demonstrates that 90−98% of the cAMP generated in the human AC V populations is due to expression of the recombinant adenylyl cyclase.

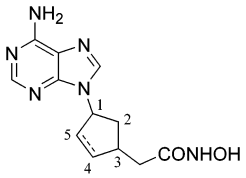
In agreement with our previously reported data,[10,11] all ester analogues, regardless of stereochemistry, failed to demonstrate any measurable inhibitory activity against type V AC (data not shown). With respect to the carboxylic acids prepared in this study (Table 1), some of the acids prepared did show moderate to weak inhibitory activity (compounds **8b**, **11b**, and **8d**). This activity was highly dependent upon the stereochemical configuration around the cyclopentene/cyclopentane ring and directly correlated with the activity of the most potent hydroxamic acids (Table 2) in this series (compounds **7b**, **10b**, and **7d**). With respect to the cyclopen-

Table 1. Cyclopentene/Cyclopentane Acetic Acids



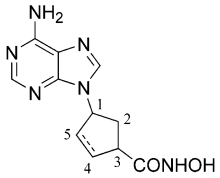
compds	stereochemistry	IC_{50} (μM)
8a	1*R*, 3*S*	>200
11a	1*S*, 3*S*	>100
8b	1*S*, 3*R*	8.2 ± 5.2 (n = 3)
11b	1*R*, 3*R*	28.8 ± 13.4 (n = 2)
8c	1*R*, 3*R*	>200
11c	1*S*, 3*R*	>200
8d	1*S*, 3*S*	73.2 (n = 1)
11d	1*R*, 3*S*	>200

Table 2. Cyclopentene/Cyclopentane Acetic Acid Hydroxamates



compds	stereochemistry	IC_{50} (μM)
7a	1*R*, 3*S*	4.3 ± 1.8 (n = 5)
10a	1*S*, 3*S*	9.4 ± 3.1 (n = 5)
7b	1*S*, 3*R*	0.4 ± 0.1 (n = 3)
10b	1*R*, 3*R*	0.6 ± 0.2 (n = 3)
7c	1*R*, 3*R*	16.3 ± 1.4 (n = 2)
10c	1*S*, 3*R*	55.0 ± 4.6 (n = 2)
7d	1*S*, 3*S*	2.6 ± 0.9 (n = 3)
10d	1*R*, 3*S*	25.1 ± 27.6 (n = 3)

Table 3. Cyclopentene/Cyclopentane Hydroxamic Acids



compds	stereochemistry	IC_{50} (μM)
30a	1*R*, 3*S*	4.6 ± 2.2 (n = 4)
30b	1*S*, 3*R*	> 70
31a	1*S*, 3*R*	188 ± 68 (n = 2)
31b	1*R*, 3*S*	> 200
32a	1*S*, 3*S*	4.9 ± 1.5 (n = 2)
32b	1*R*, 3*R*	100 ± 82 (n = 2)
33a	1*R*, 3*R*	0.2 ± 0.2 (n = 3)
33b	1*S*, 3*S*	78 ± 68 (n = 2)

tene/cyclopentane carboxylic acid-derived series (Table 3), compound **33a** was found to have exceptional inhibitory activity. As noted in the previous series, this activity was highly dependent upon stereochemistry and was only noted for the cyclopentane-containing analogue. The most potent hydroxamic acids prepared in this study, illustrated in Figure 5, inhibited type V AC with 13−89-fold increases in activity over the predecessor acyclic analogues. While the activity of compounds **7b** and **10b** 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. By

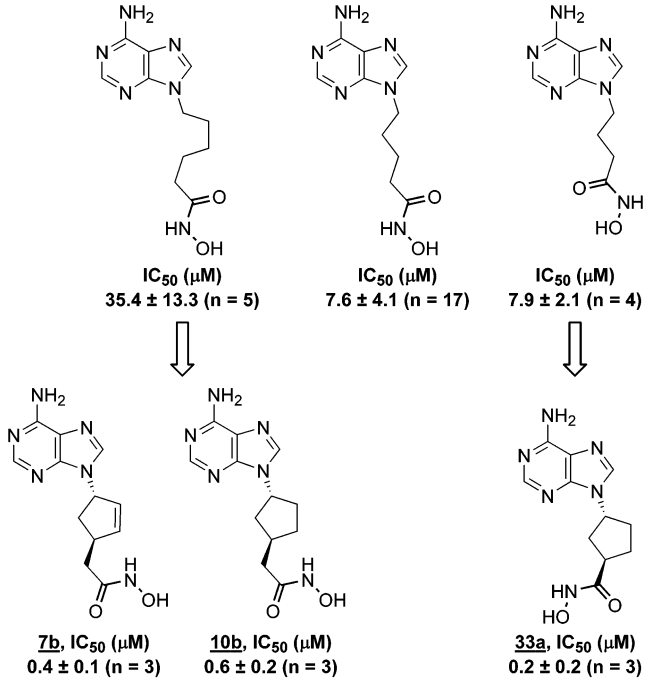


Figure 5. Evolution of potent hydroxamic acid-based AC inhibitors.

contrast, the activity of compound **33a** appeared to be dependent upon both the stereochemistry and the absence of an endocyclic double bond in the cyclic tether. The stereochemical relationship between the adenine and the hydroxamate were identical among all three compounds.

Conclusions

Rationally designed inhibitors of type V AC were prepared and tested in this study. Three hydroxamic acid-based inhibitors with cyclic tethers showed significantly enhanced activity over the corresponding acyclic analogues previously prepared. In addition, the level of activity was highly dependent upon the stereochemistry within the cyclic tether and, in one case, upon the absence of the endocyclic double bond within the tether. While the most potent analogues possessed strongly chelating hydroxamic acid functionality, some carboxylic acids showed activity presumably due to favorable positioning of the adenine and the weaker 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.

While the introduction of conformational restriction into the linker did enhance the potency of the inhibitors as measured against type V AC, it is currently unclear if these restrictions altered the expected mode of binding illustrated in Figure 3. However, the illustrated active site amino acid residues Arg1029, Ser1032, Ser1028, Thr401, and Asn1025, shown to interact with 2-deoxy-3′-AMP in crystallographic studies,[10,12,13] are not expected to interact with the reported synthetic inhibitors due to the absence of hydrogen bond donors and acceptors within the tether region. Although it is anticipated that the model reflects the probable binding mode, its relevance to type V AC is limited because of the inclusion of the C2 domain from type II AC. Neverthe-

less, crystallographic studies placing the inhibitors of this report into the active site of the type V/type II AC heterodimer are currently underway.

Experimental Section

General Procedure. ^{1}H Nuclear magnetic resonance (NMR) spectra were recorded on a Varian Unity +400 spectrometer. Low-resolution mass spectra were recorded with a HP 1100-MSD LC-MS spectrometer. High-resolution mass spectra (HRMS) were recorded with a VG ZAB2-EQ high-resolution mass spectrometer and performed at the University of California, Berkeley. Final compounds were purified by reversed phase high-performance liquid chromatography (HPLC) using a Waters 4000Prep, Waters 490E multiwavelength detector and Vydac 218TP1022 column (10 μM, C_{18}, 22 × 250 mm). Purity of the compounds was confirmed by two diverse HPLC systems using Waters 600 controller, Waters 996 photodiode array detector, and a Keystone Beta Basic column (C_{18}, 4.6 × 50 mm). HPLC method A (isocratic) − 2% CH_3CN/10 min HPLC method B (gradient) − 5−85% CH_3CN/9 min. The new compounds synthesized were characterized by mass spectrometry (MS) and NMR, and purity was determined by HRMS and two HPLC systems. We did not obtain melting points, elemental analyses, or optical rotations of these compounds as most of the derivatives were amorphous, hygroscopic, and prepared in small quantities. Commercially available reagents and starting materials were obtained from Aldrich and Chirotech and were used with no additional purification. Chromatography generally refers to flash silica gel chromatography (J. T. Baker, 40 μM, flash chromatography silica gel). The typical experimental procedures used in this study are described as follows.

General Procedure A − Hydroxamic Acids. KOH (3.8 M in MeOH, 0.45 mL) was added to $HONH_2$·HCl (1.6 M in MeOH, 0.67 mL) and cooled to 0 °C for 2 h. A methyl or ethyl ester (0.15 mmols) was dissolved in MeOH (0.31 mL) and the $HONH_2$ solution was added by filtration. After the sample was stirred for 45 min at room temperature, the reaction was concentrated to dryness and the residue was purified by reverse phase preparative HPLC (0−10% CH_3CN/30 min). The isolated product was desalted with MP-carbonate resin (Argonaut) in MeOH, filtered, and concentrated to dryness giving the desired hydroxamic acid.

General Procedure B − Carboxylic Acids. A methyl or ethyl ester (0.53 mmols) was dissolved in MeOH (2.40 mL) and NaOH (2.00 M in H_2O, 1.60 mmols) was added. The reaction was stirred at room temperature for 2.5 h after which it was acidified to pH = 2 with DOWEX acid resin (50WX2-100, MeOH washed). The reaction was filtered and concentrated to dryness giving the desired carboxylic acid.

General Procedure C − Acetate/*p*-Nitrobenzoate Cleavage (NaOMe). An acetate or *p*-nitrobenzoate (5.18 mmol) was dissolved in anhydrous MeOH (15 mL), and catalytic NaOMe (solution in MeOH) was added. The reaction was stirred at room temperature for 24 h after which it was quenched with H_2O (1.0 mL) and concentrated to dryness. The product was purified on silica gel (50% EtOAc/hexane).

General Procedure D − Adenine Mitsunobu. An allylic alcohol (10.93 mmol), triphenylphosphine (10.93 mmol), and adenine (10.93 mmol) were dissolved in THF (40 mL) and cooled to 0 °C. Diethyl azodicarboxylate (10.93 mmol) was added dropwise and the reaction was stirred at room temperature for 18 h. After heating the reaction to 40 °C for an additional 4 h, the mixture was cooled to room temperature and the solids were removed by filtration. The filtrate was concentrated to dryness and the residue was purified on silica gel (EtOAc then 5% MeOH/$CHCl_3$).

General Procedure E − Olefin Hydrogenation (Also Azide Reduction). An olefin (100 mg) and 10% Pd/C (25 mg) were placed under argon and MeOH (10 mL) was added. The mixture was degassed under vacuum and stirred under H_2 (1 atm) for 20 h. The reaction was filtered and concentrated giving the desired product.

General Procedure F − TBDMS Protection. An alcohol (72.24 mmol) was dissolved in THF (200 mL) and imidazole (108.36 mmol) was added followed by TBDMS-Cl (90.30 mmol). The reaction was stirred at room temperature for 24 h after which the solids were removed by filtration and the filtrate was concentrated to dryness. The residue was dissolved in EtOAc (300 mL) and washed with HCl (1 N, 3 × 50 mL), saturated $NaHCO_3$ (3 × 50 mL), and brine (50 mL). The organic phase was dried over anhydrous $MgSO_4$, filtered, and concentrated, and the residue was used with no further purification.

General Procedure G − TBDMS Cleavage (AcOH/THF/Water). A TBDMS ether (4.52 mmol) was combined with THF (1 mL), H_2O (1 mL), and acetic acid (3 mL). The reaction was stirred at room temperature for 6 h after which it was azeotroped with benzene (3 × 15 mL). The residue was dried under vacuum and purified on silica gel (25% EtOAc/hexane).

General Procedure H − *p*-Nitrobenzoic Acid Mitsunobu. An allylic alcohol (60.70 mmol), *p*-nitrobenzoic acid (242.81 mmol), and triphenylphospine (242.81 mmol) were combined with THF (200 mL) and cooled to 0 °C under argon. Diethylazodicarboxylate (242.81 mmol) was added dropwise and the reaction was stirred at room temperature for 15 h and 40 °C for an additional 3 h. After the sample was cooled to room temperature, the reaction was concentrated to dryness and the residue was diluted with EtOAc (200 mL). The resulting solution was washed with HCl (1 N, 3 × 50 mL), brine (50 mL), saturated $NaHOC_3$ (3 × 50 mL), and brine (50 mL). After drying of the sample over anhydrous $MgSO_4$, the organics were filtered, concentrated, and stirred with Et_2O (150 mL) for 18 h. The resulting solids were removed by filtration and the filtrate was concentrated to dryness. The isolated residue was used without further purification.

General Procedure I − TBDMS Cleavage (TBAF). A TBDMS ether (69.58 mmol) was dissolved in THF (500 mL) and tetrabutylammonium fluoride (1 M in THF, 104 mL) was added. The reaction was stirred at room temperature for 2 h and concentrated to dryness. The residue was filtered through silica gel (EtOAc) and again concentrated to dryness. Final purification was achieved on silica gel (10% then 25% then 50% EtOAc/hexane).

General Procedure J − Allyl Chloride. An allylic alcohol (46.17 mmol) was dissolved in CH_2Cl_2 and diisopropylethylamine (69.25 mmol) was added. The resulting solution was cooled to 0 °C under argon and methanesulfonyl chloride (57.71 mmol) was added. After stirring at 0 °C for 3 h, the reaction was diluted with EtOAc (600 mL). The mixture was then washed with HCl (1 N, 3 × 50 mL), saturated $NaHCO_3$ (3 × 50 mL), and brine (50 mL). The organics were dried over anhydrous $MgSO_4$, filtered, and concentrated, and the residue was purified on silica gel (5% EtOAc/hexane).

General Procedure K − Malonate Coupling. NaH (60%, 149.03 mmol) was suspended in anhydrous THF (400 mL) and cooled to 0 °C under argon. Dimethylmalonate (149.03 mmol) was added dropwise over 30 min and the reaction was allowed to warm to room temperature. An allyl chloride (29.81 mmols) was dissolved in anhydrous THF (100 mL) and added to the malonate solution via cannula. After heating of the sample to 75 °C for 19 h, the reaction was cooled, concentrated to a volume of 150 mL, and diluted with 50% EtOAc/hexane (300 mL). The resulting solution was washed with saturated NH_4Cl (3 × 50 mL) and brine (2 × 50 mL). Following concentration, the organics were partitioned between hexane (150 mL) and H_2O (150 mL). The hexane layer was further washed with H_2O (2 × 50 mL), dried over anhydrous $MgSO_4$, filtered, and concentrated. The residue was purified on silica gel (5% EtOAc/hexane).

General Procedure L − Decarboxylation (LiI). A substituted malonate (31.93 mmol) was combined with LiI (191.58 mmol) and dissolved in DMF (260 mL). The mixture was degassed under vacuum, placed under argon, and heated to 130 °C for 17 h. After the sample was cooled to room temperature, the reaction was diluted with 25% EtOAc/hexane (1500 mL) and washed with H_2O (3 × 300 mL) and brine (100

mL). The organic phase was dried over anhydrous $MgSO_4$, filtered, and concentrated. The resulting residue was purified on silica gel (5% EtOAc/hexane).

General Procedure M − Tritylation. An allylic alcohol (9.39 mmol), trityl chloride (46.96 mmol), and DMAP (56.36 mmol) were combined and dissolved in DMF (30 mL). After heating of the sample to 100 °C for 20 h, the reaction was cooled to room temperature and diluted with H_2O (200 mL). The aqueous mixture was washed with 50% EtOAc/hexane (200 mL), and the organics were sequentially washed with HCl (1 N, 3 × 25 mL), saturated $NaHCO_3$ (3 × 25 mL), and brine (25 mL). The organics were dried over $MgSO_4$, filtered, concentrated to dryness, and used without further purification.

General Procedure N − Trityl Cleavage (TsOH). A trityl ether (15.75 mmol) was dissolved in MeOH (100 mL) and *p*-toluenesulfonic acid (0.79 mmol) was added. After stirring of the sample at room temperature for 1.25 h, the reaction was quenched with saturated $NaHCO_3$ (100 mL). The resulting mixture was washed with EtOAc (3 × 100 mL), and the combined organic extracts were washed with brine (50 mL). After drying of the sample over anhydrous $MgSO_4$, the product was purified on silica gel (25% then 50% EtOAc/hexane).

General Procedure O − Methyl Ester Formation (MeOH, Ac−Cl). Acetyl chloride (9.00 mmol) was slowly added to MeOH (35.00 mL) and cooled to 0 °C. A carboxylic acid (7.87 mmol) was added and the resulting mixture was stirred at room temperature for 4 h. Concentration of the reaction mixture provided the desired product requiring no further purification.

General Procedure P − Coupling with Pyrimidine. An amine hydrochloride (7.97 mmol) was combined with dichloronitropyrimidine (11.95 mmol) and EtOH (80 mL). Triethylamine (23.90 mmol) was added and the reaction was stirred at room temperature for 3.5 h. Following dilution with EtOAc (320 mL), the mixture was sequentially washed with HCl (1 N, 3 × 50 mL), saturated $NaHCO_3$ (3 × 30 mL), and brine (30 mL). The organics were dried over anhydrous $MgSO_4$, filtered, and concentrated. The isolated residue was used with no further purification.

General Procedure Q − Nitro Group Reduction ($SnCl_2$). A nitropyrimidine (9.36 mmol) was dissolved in EtOH (75 mL), and $SnCl_2$ (28.09 mmol) was added. The reaction was heated to reflux for 50 min and cooled to room temperature. Following quenching with saturated $NaHCO_3$ (300 mL), the reaction was washed with EtOAc (3 × 75 mL). The organic extracts were washed with brine (2 × 75 mL), dried over anhydrous $MgSO_4$, filtered, and concentrated. No further purification was required.

General Procedure R − Purine Formation (Orthoformate, Ms-OH). A diaminopyrimidine (9.36 mmol) was dissolved in trimethylorthoformate (25 mL) and methanesulfonic acid (0.22 mL) was added. The reaction was stirred at room temperature for 4.5 h and diluted with EtOAc (150 mL). The resulting mixture was washed with saturated $NaHCO_3$ (3 × 25 mL) and brine (25 mL). The organic phase was dried over anhydrous $MgSO_4$, filtered, and concentrated. The product was purified on silica gel (50% EtOAc/hexane).

General Procedure S − Azidopurine. A chloropurine (3.02 mmol), sodium azide (9.06 mmol), EtOH (13 mL), and H_2O (6.5 mL) were combined and heated to 50 °C for 20 h. After stirring of the sample for an additional 17 h at room temperature, the reaction was concentrated to dryness. The residue was diluted with H_2O (20 mL) and the resulting solids were filtered, washed with H_2O, and dried in a desiccator. No further purification was required.

General Procedure T − Azide Reduction ($SnCl_2$). An azidopurine (0.42 mmol) was dissolved in EtOH (3.25 mL) and $SnCl_2$ (1.27 mmol) was added. The reaction was heated to reflux for 20 min and cooled to room temperature. Following quenching of the sample with saturated $NaHCO_3$ (15 mL), the reaction was washed with EtOAc (3 × 15 mL). The organic extracts were dried over anhydrous $MgSO_4$, filtered, and concentrated. No further purification was required.

(1*R*,3*S*)-1-Hydroxy-3-(*tert*-Butyl-dimethylsiloxy)-4-cyclopentene (2). (1*R*,3*S*)-1-Acetoxy-3-hydroxy-4-cyclopentene, **1**, was subjected to general procedure F. Yield = 100%. TLC: R_f = 0.48 (10% EtOAc/hexane). Subsequent subjection of the product to general procedure C gave compound **2**. Yield = 90%. TLC: R_f = 0.43 (25% EtOAc/hexane).

(1*R*,3*R*)-1-(*tert*-Butyl-dimethylsiloxy)-3-hydroxy-4-cyclopentene (16). Compound **2** was subjected to general procedure H. Yield = 81%. TLC: R_f = 0.50 (10% EtOAc/hexane). Subsequent subjection of the product to general procedure C gave compound **16**. Yield = 85%. TLC: R_f = 0.33 (25% EtOAc/hexane).

(1*S*,3*R*)-1-Hydroxy-3-triphenylmethoxy-4-cyclopentene (12). Compound **2** was subjected to general procedure M. TLC: R_f = 0.27 (hexane). Subsequent subjection of the product to general procedure I gave compound **12**. Yield = 87% (2 steps). TLC: R_f = 0.32 (25% EtOAc/hexane).

(1*R*,3*R*)-1-Chloro-3-triphenylmethoxy-4-cyclopentene (13). Compound **13** was prepared by subjecting compound **12** to general procedure J. Yield = 84%. TLC: R_f = 0.61 (10% EtOAc/hexane).

(1*S*,3*R*)-1-(2-Dimethylmalonyl)-3-triphenylmethoxy-4-cyclopentene (14). Compound **14** was prepared by subjecting compound **13** to general procedure K. Yield = 72%. TLC: R_f = 0.27 (10% EtOAc/hexane).

(1*R*,3*R*)-1-Hydroxy-3-(methyl-carboxymethyl)-4-cyclopentene (15). Compound **14** was subjected to general procedure L. Yield = 80%. TLC: R_f = 0.45 (10% EtOAc/hexane). Subsequent subjection of the product to general procedure N gave compound **15**. Yield = 39%. TLC: R_f = 0.38 (50% EtOAc/hexane).

(1*S*,3*R*)-1-(9-Adenenyl)-3-(methyl-carboxymethyl)-4-cyclopentene (6b). Compound **6b** was prepared by subjecting compound **15** to general procedure D. Yield = 9%. TLC: R_f = 0.22 (5% MeOH/$CHCl_3$). Purity: >85% (HPLC method B).

(1*S*,3*R*)-1-(9-Adenenyl)-3-(*N*-hydroxycarbamoylmethyl)-4-cyclopentene (7b). Compound **7b** was prepared by subjecting compound **6b** to general procedure A. Yield = 37%. TLC: R_f = 0.40 ($CHCl_3$/MeOH/H_2O 150/45/5). Purity: >97% (HPLC method A); >97% (HPLC method B).

(1*S*,3*R*)-1-(9-Adenenyl)-3-carboxymethyl-4-cyclopentene (8b). Compound **8b** was prepared by subjecting compound **6b** to general procedure B. Yield = 97%. TLC: R_f = 0.42 ($CHCl_3$/MeOH/H_2O 150/45/5). Purity: >86% (HPLC method B).

(1*R*,3*R*)-1-(9-Adenenyl)-3-(methyl-carboxymethyl)cyclopentane (9b). Compound **9b** was prepared by subjecting compound **6b** to general procedure E. Yield = 95%. TLC: R_f = 0.21 ($CHCl_3$/MeOH/H_2O 150/45/5). Purity: >86% (HPLC method B).

(1*R*,3*R*)-1-(9-Adenenyl)-3-(*N*-hydroxycarbamoylmethyl)cyclopentane (10b). Compound **10b** was prepared by subjecting compound **9b** to general procedure A. Yield = 39%. TLC: R_f = 0.34 ($CHCl_3$/MeOH/H_2O 150/45/5). Purity: >97% (HPLC method A); >99% (HPLC method B).

(1*R*,3*R*)-1-(9-Adenenyl)-3-carboxymethylcyclopentane (11b). Compound **11b** was prepared by subjecting compound **9b** to general procedure B. Yield = 83%. TLC: R_f = 0.38 ($CHCl_3$/MeOH/H_2O 150/45/5). Purity: >80% (HPLC method B).

(1*S*,3*S*)-1-Chloro-3-(*tert*-Butyl-dimethylsiloxy)-4-cyclopentene (3). Compound **3** was prepared by subjecting compound **2** to general procedure J. Yield = 79%. TLC: R_f = 0.80 (10% EtOAc/hexane).

(1*R*,3*S*)-1-(2-Dimethylmalonyl)-3-(*tert*-Butyl-dimethylsiloxy)-4-cyclopentene (4). Compound **4** was prepared by subjecting compound **3** to general procedure K. Yield = 74%. TLC: R_f = 0.32 (10% EtOAc/hexane).

(1*S*,3*S*)-1-Hydroxy-3-(methyl-carboxymethyl)-4-cyclopentene (5). Compound **4** was subjected to general procedure L. Yield = 75%. TLC: R_f = 0.57 (10% EtOAc/hexane). Subsequent subjection of the product to general procedure G gave compound **5**. Yield = 61%. TLC: R_f = 0.39 (50% EtOAc/hexane).

(1*R*,3*S*)-1-(9-Adenenyl)-3-(methyl-carboxymethyl)-4-cyclopentene (6a). Compound **6a** was prepared by subjecting compound **5** to general procedure D. Yield = 10%. TLC: R_f = 0.14 (5% MeOH/$CHCl_3$). Purity: >85% (HPLC method B).

(1*R*,3*S*)-1-(9-Adenenyl)-3-(*N*-hydroxycarbamoylmethyl)-4-cyclopentene (7a). Compound **7a** was prepared by subjecting compound **6a** to general procedure A. Yield = 63%. TLC: R_f = 0.40 ($CHCl_3$/MeOH/H_2O 150/45/5). Purity: >84% (HPLC method A); >90% (HPLC method B).

(1*R*,3*S*)-1-(9-Adenenyl)-3-carboxymethyl-4-cyclopentene (8a). Compound **8a** was prepared by subjecting compound **6a** to general procedure B. Yield = 100%. TLC: R_f = 0.31 ($CHCl_3$/MeOH/H_2O 150/45/5). Purity: >85% (HPLC method B).

(1*S*,3*S*)-1-(9-Adenenyl)-3-(methyl-carboxymethyl)cyclopentane (9a). Compound **9a** was prepared by subjecting compound **6a** to general procedure E. Yield = 93%. TLC: R_f = 0.22 ($CHCl_3$/MeOH/H_2O 150/45/5). Purity: >93% (HPLC method B).

(1*S*,3*S*)-1-(9-Adenenyl)-3-(*N*-hydroxycarbamoylmethyl)cyclopentane (10a). Compound **10a** was prepared by subjecting compound **9a** to general procedure A. Yield = 34%. TLC: R_f = 0.39 ($CHCl_3$/MeOH/H_2O 150/45/5). Purity: >98% (HPLC method A); >85% (HPLC method B).

(1*S*,3*S*)-1-(9-Adenenyl)-3-carboxymethylcyclopentane (11a). Compound **11a** was prepared by subjecting compound **9a** to general procedure B. Yield = 83%. TLC: R_f = 0.33 ($CHCl_3$/MeOH/H_2O 150/45/5). Purity: >80% (HPLC method B).

(1*R*,3*S*)-1-Chloro-3-(*tert*-Butyl-dimethylsiloxy)-4-cyclopentene (17). Compound **17** was prepared by subjecting compound **16** to general procedure J. Yield = 99%. TLC: R_f = 0.86 (10% EtOAc/hexane).

(1*S*,3*S*)-1-(2-Dimethylmalonyl)-3-(*tert*-Butyl-dimethylsiloxy)-4-cyclopentene (18). Compound **18** was prepared by subjecting compound **17** to general procedure K. Yield = 75%. TLC: R_f = 0.36 (10% EtOAc/hexane).

(1*S*,3*R*)-1-Hydroxy-3-(methyl-carboxymethyl)-4-cyclopentene (19). Compound **18** was subjected to general procedure L. Yield = 74%. TLC: R_f = 0.58 (10% EtOAc/hexane). Subsequent subjection of the product to general procedure I gave compound **19**. Yield = 59%. TLC: R_f = 0.40 (50% EtOAc/hexane).

(1*R*,3*R*)-1-(9-Adenenyl)-3-(methyl-carboxymethyl)-4-cyclopentene (6c). Compound **6c** was prepared by subjecting compound **19** to general procedure D. Yield = 4.3%. TLC: R_f = 0.17 (5% MeOH/$CHCl_3$). Purity: >81% (HPLC method B).

(1*R*,3*R*)-1-(9-Adenenyl)-3-(*N*-hydroxycarbamoylmethyl)-4-cyclopentene (7c) and (1*R*,3*R*)-1-(9-Adenenyl)-3-carboxymethyl-4-cyclopentene (8c). Compound **6c** was subjected to general procedure A, and the products were separated by preparative TLC ($CHCl_3$/MeOH/H_2O 150/45/5). Hydroxamic acid **(7c)**: Yield = 21%. TLC: R_f = 0.29 ($CHCl_3$/MeOH/H_2O 150/45/5). Purity: >89% (HPLC method A); >90% (HPLC method B). HRMS: calcd ($C_{12}H_{14}N_6O_2$) *m*/*z* 275.1257 (M^+ + 1), found 275.1257 (M^+ + 1). Carboxylic acid **(8c)**: Yield = 31%. TLC: R_f = 0.25 ($CHCl_3$/MeOH/H_2O 150/45/5). Purity: >99% (HPLC method B).

(1*S*,3*R*)-1-(9-Adenenyl)-3-(methyl-carboxymethyl)cyclopentane (9c). Compound **9c** was prepared by subjecting compound **6c** to general procedure E. Yield = 85%. TLC: R_f = 0.27 (5% MeOH/$CHCl_3$).

(1*S*,3*R*)-1-(9-Adenenyl)-3-(*N*-hydroxycarbamoylmethyl)cyclopentane (10c) and (1*S*,3*R*)-1-(9-Adenenyl)-3-carboxymethylcyclopentane (11c). Compound **9c** was subjected to general procedure A, and the products were separated by preparative HPLC and isolated as TFA salts. Hydroxamic acid **(10c)**: Yield = 32%. TLC: R_f = 0.33 ($CHCl_3$/MeOH/H_2O 150/45/5). Purity: >95% (HPLC method A); >90% (HPLC method B). HRMS: calcd ($C_{12}H_{16}N_6O_2$) *m*/*z* 277.1413 (M^+ + 1), found 277.1421 (M^+ + 1). Carboxylic acid **(11c)**: Yield = 11%. TLC: R_f = 0.33 ($CHCl_3$/MeOH/H_2O 150/45/5). Purity: >95% (HPLC method B).

(1*R*,3*R*)-1-Hydroxy-3-triphenylmethoxy-4-cyclopentene (20). Compound **12** was subjected to general procedure H. TLC: R_f = 0.36 (10% EtOAc/hexane). Subsequent subjection of the product to general procedure C gave compound **20**. Yield = 77% (two steps). TLC: R_f = 0.32 (25% EtOAc/hexane).

(1*S*,3*R*)-1-Chloro-3-triphenylmethoxy-4-cyclopentene (21). Compound **21** was prepared by subjecting compound **20** to general procedure J. Yield = 93%. TLC: R_f = 0.58 (10% EtOAc/hexane).

(1*R*,3*R*)-1-(2-Dimethylmalonyl)-3-triphenylmethoxy-4-cyclopentene (22). Compound **22** was prepared by subjecting compound **21** to general procedure K. Yield = 74%. TLC: R_f = 0.22 (10% EtOAc/hexane).

(1*R*,3*S*)-1-Hydroxy-3-(methyl-carboxymethyl)-4-cyclopentene (23). Compound **22** was subjected to general procedure L. Yield = 63%. TLC: R_f = 0.45 (10% EtOAc/hexane). Subsequent subjection of the product to general procedure N gave compound **23**. Yield = 54%. TLC: R_f = 0.35 (50% EtOAc/hexane).

(1*S*,3*S*)-1-(9-Adenenyl)-3-(methyl-carboxymethyl)-4-cyclopentene (6d). Compound **6d** was prepared by subjecting compound **23** to general procedure D. Yield = 16%. TLC: R_f = 0.17 (5% MeOH/$CHCl_3$). Purity: >87% (HPLC method B).

(1*S*,3*S*)-1-(9-Adenenyl)-3-(*N*-hydroxycarbamoylmethyl)-4-cyclopentene (7d). Compound **7d** was prepared by subjecting compound **6d** to general procedure A. Yield = 49%. TLC: R_f = 0.36 ($CHCl_3$/MeOH/H_2O 150/45/5). Purity: >88% (HPLC method A); >89% (HPLC method B).

(1*S*,3*S*)-1-(9-Adenenyl)-3-carboxymethyl-4-cyclopentene (8d). Compound **8d** was prepared by subjecting compound **6d** to general procedure B. Yield = 75%. TLC: R_f = 0.34 ($CHCl_3$/MeOH/H_2O 150/45/5). Purity: >84% (HPLC method B).

(1*R*,3*S*)-1-(9-Adenenyl)-3-(methyl-carboxymethyl)cyclopentane (9d). Compound **9d** was prepared by subjecting compound **6d** to general procedure E. Yield = 92%. TLC: R_f = 0.27 (5% MeOH/$CHCl_3$). Purity: >87% (HPLC method B).

(1*R*,3*S*)-1-(9-Adenenyl)-3-(*N*-hydroxycarbamoylmethyl)cyclopentane (10d). Compound **10d** was prepared by subjecting compound **9d** to general procedure A. Yield = 56%. TLC: R_f = 0.42 ($CHCl_3$/MeOH/H_2O 150/45/5). Purity: >96% (HPLC method A); >94% (HPLC method B).

(1*R*,3*S*)-1-(9-Adenenyl)-3-carboxymethylcyclopentane (11d). Compound **11d** was prepared by subjecting compound **9d** to general procedure B. Yield = 99%. TLC: R_f = 0.42 ($CHCl_3$/MeOH/H_2O 150/45/5). Purity: >82% (HPLC method B).

(1*S*,3*R*)-Methyl-1-aminocyclopent-4-ene-3-carboxylate hydrochloride (25a). Compound **25a** was prepared by subjecting (1*R*,4*S*)-4-amino-cyclopent-2-ene carboxylic acid to general procedure O. Yield = 100%.

(1*R*,3*S*)-Methyl-1-aminocyclopent-4-ene-3-carboxylate hydrochloride (25b). Compound **25b** was prepared by subjecting (1*S*,4*R*)-4-amino-cyclopent-2-ene carboxylic acid to general procedure O. Yield = 100%.

(1*S*,3*R*)-1-[9-(1-Chloroadenenyl)]-3-methylcarboxy-4-cyclopentene (26a). Compound **25a** was subjected to general procedure P. Subsequent subjection of the crude product to general procedure Q yielded the desired crude aminopyrimidine. Without purification, the crude aminopyrimidine was subjected to general procedure R giving compound **26a**. Yield = 40% (3 steps). TLC: R_f = 0.50% (EtOAc/hexane). Purity: >90% (HPLC method B).

(1*R*,3*S*)-1-[9-(1-Chloroadenenyl)]-3-methylcarboxy-4-cyclopentene (26b). Compound **25b** was subjected to general procedure P. Subsequent subjection of the crude product to general procedure Q yielded the desired crude aminopyrimidine. Without purification, the crude aminopyrimidine was subjected to general procedure R giving compound **26b**. Yield = 38% (three steps). TLC: R_f = 0.50% (EtOAc/Hexane). Purity: >90% (HPLC method B).

(1*S*,3*R*)-1-[9-(1-Azidoadenenyl)]-3-methylcarboxy-4-cyclopentene (27a). Compound **27a** was prepared by subjecting

compound **26a** to general procedure S. Yield = 52%. Purity: >99% (HPLC method B).

(1*R*,3*S*)-1-[9-(1-Azidoadenenyl)]-3-methylcarboxy-4-cyclopentene (27b). Compound **27b** was prepared by subjecting compound **26b** to general procedure S. Yield = 60%. Purity: >99% (HPLC method B).

(1*R*,3*S*)-1-(9-Adenenyl)-3-methylcarboxycyclopentane (29a). Compound **29a** was prepared by subjecting compound **27a** to general procedure E. Yield = 99%. Purity: >99% (HPLC method B).

(1*S*,3*R*)-1-(9-Adenenyl)-3-methylcarboxycyclopentane (29b). Compound **29b** was prepared by subjecting compound **27b** to general procedure E. Yield = 96%. Purity: >99% (HPLC method B).

(1*R*,3*S*)-1-(9-Adenenyl)-3-(*N*-hydroxycarbamoyl)cyclopentane (30a). Compound **30a** was prepared by subjecting compound **29a** to general procedure A. Yield = 53%. Purity: >91% (HPLC method A); >95% (HPLC method B).

(1*S*,3*R*)-1-(9-Adenenyl)-3-(*N*-hydroxycarbamoyl)cyclopentane (30b). Compound **30b** was prepared by subjecting compound **29b** to general procedure A. Yield = 53%. Purity: >98% (HPLC method A); >95% (HPLC method B).

(1*S*,3*R*)-1-(9-Adenenyl)-3-methylcarboxy-4-cyclopentene (28a). Compound **28a** was prepared by subjecting compound **27a** to general procedure T. Yield = 98%. Purity: >99% (HPLC method B).

(1*R*,3*S*)-1-(9-Adenenyl)-3-methylcarboxy-4-cyclopentene (28b). Compound **28b** was prepared by subjecting compound **27b** to general procedure T. Yield = 97%. Purity: >99% (HPLC method B).

(1*S*,3*R*)-1-(9-Adenenyl)-3-(*N*-hydroxycarbamoyl)-4-cyclopentene (31a) and (1*S*,3*S*)-1-(9-Adenenyl)-3-(*N*-hydroxycarbamoyl)-4-cyclopentene (32a). Compound **28a** was subjected to general procedure A, and the products were separated by preparative HPLC as described. The isolated TFA salts were converted to free bases utilizing MP-carbonate resin (Argonaut) in MeOH. Compound **31a**: Yield = 44%. TLC: R_f = 0.34 ($CHCl_3$/MeOH/H_2O 150/45/5). Purity: >96% (HPLC method A); >99% (HPLC method B). Compound **32a**: Yield = 26%. TLC: R_f = 0.29 ($CHCl_3$/MeOH/H_2O 150/45/5). Purity: >99% (HPLC method A); >99% (HPLC method B).

(1*R*,3*S*)-1-(9-Adenenyl)-3-(*N*-hydroxycarbamoyl)-4-cyclopentene (31b) and (1*R*,3*R*)-1-(9-Adenenyl)-3-(*N*-hydroxycarbamoyl)-4-cyclopentene (32b). Compound **28b** was subjected to general procedure A and the products were separated by preparative HPLC as described. The isolated TFA salts were converted to free bases utilizing MP-carbonate resin (Argonaut) in MeOH. Compound **31b**: Yield = 44%. TLC: R_f = 0.32 ($CHCl_3$/MeOH/H_2O 150/45/5). Purity: >99% (HPLC method A); >99% (HPLC method B). Compound **32b**: Yield = 24%. TLC: R_f = 0.26 ($CHCl_3$/MeOH/H_2O 150/45/5). Purity: >99% (HPLC method A); >99% (HPLC method B).

(1*R*,3*R*)-1-(9-Adenenyl)-3-(*N*-hydroxycarbamoyl)cyclopentane (33a). Compound **33a** was prepared by subjecting compound **32a** to general procedure E where 10% Pd/C was replaced with 20% $Pd(OH)_2$/C. Yield = 99%. TLC: R_f = 0.27 ($CHCl_3$/MeOH/H_2O 150/45/5). Purity: >99% (HPLC method A); >97% (HPLC method B).

(1*S*,3*S*)-1-(9-Adenenyl)-3-(*N*-hydroxycarbamoyl)cyclopentane (33b). Compound **33b** was prepared by subjecting compound **32b** to general procedure E where 10% Pd/C was replaced with 20% $Pd(OH)_2$/C. Yield = 95%. TLC: R_f = 0.27 ($CHCl_3$/MeOH/H_2O 150/45/5). Purity: >98% (HPLC method A); >97% (HPLC method B).

HEK293 Membrane Preparations. Cell membranes enriched with human adenylyl cyclase isozyme type-5 (hAC5) were generated by splicing hAC5 into pcDNA3 plasmid DNA and expressed in human embrionic kidney cells (HEK293). Cells were grown under nonadherent conditions in F-12 nutrient mixture (HAM) supplemented with 10% fetal bovine serum (Gemini Bio-Products), 450 μg/mL Geneticin (Gobco BRL), 1% penicillin−streptomycin, 2 mM final l-glutamine, 0.1% pluronic F-68, 10 units/mL heparin, and buffered with 10 mM HEPES. Cells were grown in T-150 tissue culture flasks for 3 days at 37 °C under 5% CO_2. Cells were washed with sterile phosphate buffered saline (PBS), trypsinized, concentrated, and seeded in 500 mL Bell-Co spinner flasks at a density of 1×10^6/mL and grown at 37 °C at medium speed on a Cellgro stirrer. After 5 days, cells were collected in 500 mL centrifuge bottles and pelleted at 3000 rpm for 10 min. Pellets were resuspended in tissue culture PBS and harvested at 1200 rpm for 10 min. Supernatants were discarded and pellets were frozen at −20 °C.

Adenylyl cyclase membranes were prepared after thawing cell pellets on ice. Thawed pellets were washed with 15 mL PBS and centrifugation at 3000 rpm for 10 min at 4 °C in a Beckman J2−21 centrifuge. Washed cells were resuspended in 20 mL of 20 mM Tris-HCl, 5 mM EDTA buffer freshly supplemented with 2 mM DTT and 1:25 dilution of complete protease inhibitor and dounced in a tissue grinder 10 times to homogenization. The suspension was sonicated for 18 cycles and centrifuged at 12000 rpm for 30 min in a JA-17 rotor. Pellets were resuspended in 300 mL of 25 mM Tris-HCl, 20 mM $MgCl_2$, and 10% sucrose freshly supplemented with DTT and Complete as described above to a final concentration of 10 mg/mL protein. Supernatants were then triturated seven times through a 22 $^1/_2$ gauge needle on a 5 mL syringe. Final protein concentration was determined and protein was aliquoted and stored at −80 °C.

Type V AC Assay. Type V AC activity was evaluated with or without added inhibitors. Membranes from HEK293 cells expressing recombinant human type V AC (1.4 μg/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. Degree of inhibition was determined by comparing to control reactions which did not contain compound.

Supporting Information Available: All ^{1}H NMR and HRMS data. This material is available free of charge via the Internet at http://pubs.acs.org.

References

(1) Toya, Y.; Schwencke, C.; Ishikawa, Y. Forskolin derivatives with increased selectivity for cardiac adenylyl cyclase. *J. Mol. Cell. Cardiol.* **1998**, *30*, 97−108.

(2) Hellevuo, K.; Hoffman, P. L.; Tabakoff, B. Adenylyl cyclases: mRNA and characteristics of enzyme activity in three areas of brain. *J. Neurochem.* **1996**, *67*, 177−185.

(3) Goldstein, J.; Silberstein, C.; Ibarra, C. Adenylyl cyclase types I and VI but not II and V are selectively inhibited by nitric oxide. *Braz. J. Med. Biol. Res.* **2002**, *35*, 145−151.

(4) Reprinted from *Bioorg. Med. Chem. Lett.*, Vol. 12, Levy, D. E.; Marlowe, C.; Kane-Maguire, K.; Bao, M.; Cherbavaz, D. B.; Tomlinson, J. E.; Sedlock, D. M.; Scarborough, R. M. Hydroxamate based inhibitors of adenylyl cyclase. Part 1: The effect of acyclic linkers on P-site binding, pp 3085−3088, Copyright 2002, with permission from Elsevier Science.

(5) Hamm, H. E. The many faces of G protein signaling. *J. Biol. Chem.* **1998**, *273*, 669−672.

(6) Taussig, R.; Gilman, A. G. Mammalian membrane-bound adenylyl cyclases. *J. Biol. Chem.* **1995**, *270*, 1−4.

(7) Ishikawa, Y.; Homcy, C. J. The adenylyl cyclases as integrators of transmembrane signal transduction. *Circ. Res.* **1997**, *80*, 297−304.

(8) Susanni, E. E.; Vatner, D. E.; Homcy, C. J. Beta-adrenergic receptor/adenylyl cyclase system. In *The Heart and Cardiovascular System*, 2nd ed.; Fozzard, H. A., Ed.; Raven Press: Ltd., New York, 1992.

(9) Braunwald, E.; Zipes, D. P.; Libby, P. Treatment of heart failure: Pharmacological methods. In *Heart Disease*, 6th ed.; W. B. Saunders Company: Philadelphia, 1997.

(10) Levy, D. E.; Marlowe, C.; Kane-Maguire, K.; Bao, M.; Cherbavaz, D. B.; Tomlinson, J. E.; Sedlock, D. M.; Scarborough, R. M. Hydroxamate based inhibitors of adenylyl cyclase. Part 1: The effect of acyclic linkers on P-site binding. *Bioorg. Med. Chem. Lett.* **2002**, *12*, 3085−3088.

(11) Levy, D. E.; Bao, M.; Tomlinson, J. E.; Scarborough, R. M. Hydroxamate based inhibitors of adenylyl cyclase. Part 2: The effect of cyclic linkers on P-site binding. *Bioorg. Med. Chem. Lett.* **2002**, *12*, 3089−3092.

(12) Dessauer, C. W.; Tesmer, J. J. G.; Sprang, S. R.; Gilman, A. G. The interactions of adenylate cyclases with P-site inhibitors. *Trends Pharmacol. Sci.* **1999**, *20*, 205−210.
(13) Tesmer, J. J. G.; Sunahara, R. K.; Johnson, R. A.; Gosselin, G.; Gilman, A. G.; Sprang, S. R. Two-metal-ion catalysis in adenylyl cyclase. *Science* **1999**, *285*, 756−760.
(14) Texier, F.; Carrie, F. 1,3-Dipolar cycloadditions to compounds with activated unsaturated bonds. X. Addition of azidobenzenes to α-cyanoacrylic acid esters, α-cyanocinnamonitrile, α-cyanocinnamamide, and benzylidene malonic acid esters. Production of pyrrolidines. *Bull. Soc. Chim. Fr.* **1972**, 258−263.
(15) Trost, B. M.; Dietsche, T. J. New synthetic reactions. Asymmetric induction in allylic alkylations. *J. Am. Chem. Soc.* **1973**, *95*, 8200−8201.
(16) Wu, Y.; Xu, J.-C.; Liu, J.; Jin, Y.-X. Synthesis of N-Boc and N-Fmoc dipeptoids with nucleobase residues as peptoid nucleic acid monomers. *Tetrahedron* **2001**, *57*, 3373−3381.
(17) Toyota, A.; Katagiri, N.; Kaneko, C. Mitsunobu reactions for the synthesis of carbocyclic analogues of nucleosides: examination of the regioselectivity. *Synthetic Commun.* 1993, *23*, 1295−1305.
(18) Levy, D. E.; Lapierre, F.; Liang, W.; Ye, W.; Lange, C. W.; Li, X.; Grobelny, D.; Casabonne, M.; Tyrrell, D.; Holme, K.; Nadzan, A.; Galardy, R. E. Matrix metalloproteinase inhibitors: A structure activity study. *J. Med. Chem.* **1998**, *41*, 199−223.
(19) Mitsunobu, O. The use of diethyl azodicarboxylate and triphenylphosphine in synthesis and transformation of natural products. *Synthesis* **1981**, *1*, 1−28.
(20) Kelley, J. L.; McLean, E. W.; Linn, J. A.; Krochmal, M. P.; Ferris, R. M.; Howard, J. L. Benzodiazepine receptor binding activity of 8-substituted-9-(3-substituted-benzyl)-6-(dimethylamino)-9H-purines. *J. Med. Chem.* **1990**, *33*, 196−202.
(21) Bellamy, F. D.; Ou, K. Selective reduction of aromatic nitro compounds with stannous chloride in nonacidic and nonaqueous medium. *Tetrahedron Lett.* 1984, *25*, 839−842.
(22) Thompson, R. D.; Secunda, S.; Daly, J. W.; Olsson, R. A. *N*,[6] 9-Disubstituted adenines: Potent, selective antagonists at the A_1 receptor. *J. Med. Chem.* **1991**, *34*, 2877−2882.

JM0205604