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Hydroxamate Based Inhibitors of Adenylyl Cyclase. Part 1: The Effect of Acyclic Linkers on P-Site Binding

Daniel E. Levy,* Charles Marlowe, Kim Kane-Maguire, Ming Bao, Diana B. Cherbavaz, James E. Tomlinson, David M. Sedlock and Robert M. Scarborough

Millennium Pharmaceuticals, Inc., 256 East Grand Avenue, South San Francisco, CA 94080, USA

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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. 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 novel therapeutic agents. In order to develop novel AC inhibitors, we have chosen a design approach utilizing molecules with the adenine ring system joined to a metal-coordinating hydroxamic acid via flexible acyclic linkers. The designed inhibitors were assayed against type V AC with the size and heteroatom content of the linkers varied to probe the interaction of the nucleotide and metal binding sites within the enzyme.

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]

Figure 1 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 in the presence of β-agonists.[5] Following dissociation, activated Gsα binds to and stabilizes adenylyl cyclase.[6,7] Adenylyl cyclase then converts ATP into cAMP.[8]

The dependence of metals on both AC catalytic activity and P-site mediated inhibition is known.[9] Drawing from this knowledge coupled with recently described PDE4 inhibitors where metal atom coordinating functionalities were found useful for designing potent inhibitors of this adenine binding enzyme, hydroxamic acid based inhibitors were proposed as phosphate surrogates in potential P-site inhibitors.[10] Subsequent publication of AC crystal structures with bound inhibitors has confirmed the importance of chelating groups.[11,12]

Analysis of the crystallographic data, illustrated in Figure 2, revealed a number of interesting interactions. These include hydrogen bonds between the adenine ring and residues Lys938, Asp1018 and Ile1019. Of particular interest is confirmation of the presence of two metal ions in the active site. These ions, required for the conversion of ATP to cAMP are shown coordinated to pyrophosphate and the phosphate of the bound inhibitor.

Considering the number of atoms separating the adenine unit from the α phosphate in ATP and 2′-deoxy-3′-AMP (Fig. 3), inhibitors were proposed where an adenine unit would be joined to a hydroxamic acid via various linkers. Based on the criterion derived from Figure 3, initial alkyl chain lengths of 4–6 carbon atoms were chosen. These parameters were later expanded to explore the upper and lower chain length limits required for observable inhibitory activity. For control purposes, the weakly coordinating carboxylic acids and non-coordinating ester analogues were also prepared. Initially, the linkers were designed to be flexible acyclic alkyl tethers.

*Corresponding author. Tel.: +1-650-246-7073; fax: +1-650-244-9287; e-mail: dan.levy@mpi.com

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As shown in Scheme 1, adenine, **1**, was alkylated at N9 on heating with haloesters in the presence of potassium carbonate in DMF.[13,14] The ester groups of the resulting functionalized adenines, **2**, were then hydrolyzed to carboxylic acids, **4**, on treatment with sodium hydroxide in aqueous methanol. Alternately, the ester groups were converted to hydroxamic acids, **3**, on treatment with hydroxylamine and potassium hydroxide in methanol.[15]

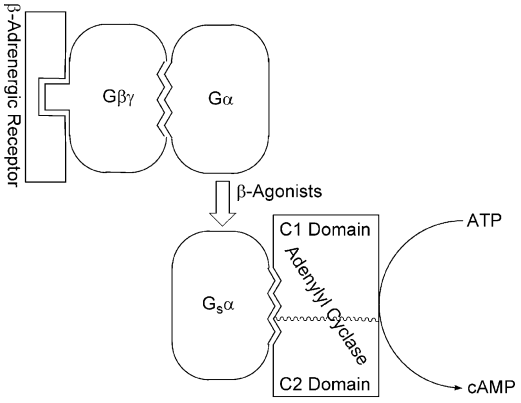


Figure 1. Interaction cascade leading to conversion of ATP to cAMP.

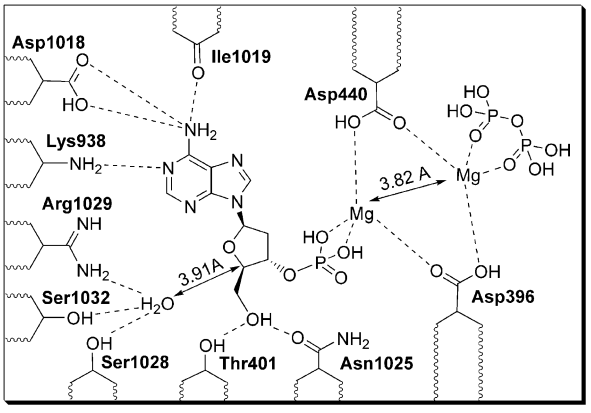


Figure 2. ATP binding site with bound P-site inhibitor and pyrophosphate.

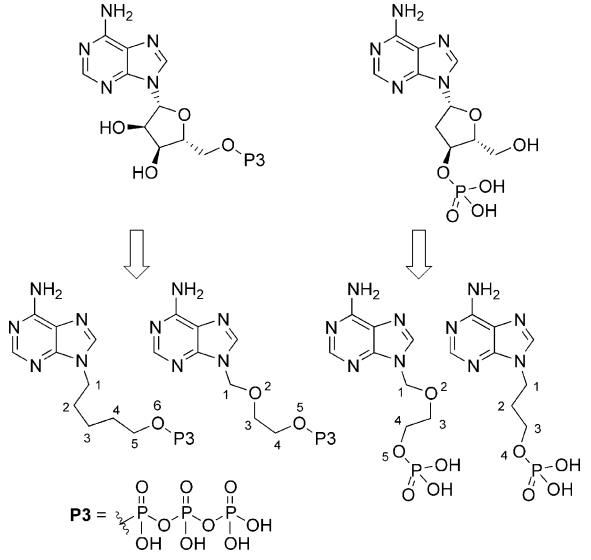
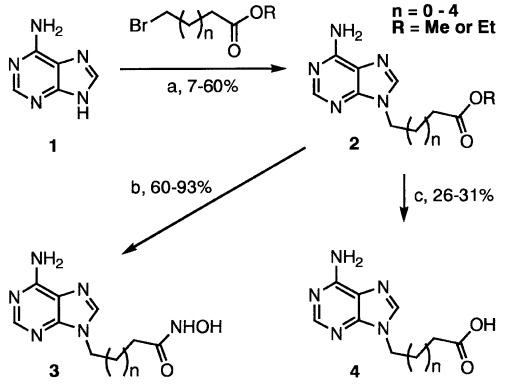


Figure 3. Linear atom count in ATP and 2-deoxy-3′-AMP.

Due to the presence of oxygen atoms in the tethers represented in Figure 3, expansion of the initial strategy to include heteroatom substitutions in the tethers became of interest. As shown in Scheme 2, ether-based tethers were created beginning with 3-benzyloxy-1-propanol, **5**. As illustrated, the alcohol was alkylated with ethyl diazoacetate utilizing rhodium catalysis.[16] Subsequent cleavage of the benzyl group was achieved by hydrogenation. The resulting alcohol, **6**, was used to alkylate the N9 position of adenine via Mitsunobu methodology[17,18] giving compound **7**. Final conversion of the ester group to its corresponding carboxylic and hydroxamic acids (compounds **8** and **9**, respectively) was achieved utilizing the conditions described in Scheme 1.

In order to expand the heteroatom substitutions to include nitrogen, the chemistry in Scheme 3 was utilized. As illustrated, ethanolamine, **10a**, was alkylated with chloroacetic acid and propanolamine, **10b**, was reductively alkylated with glyoxylic acid and sodium cyanoborohydride. Subsequent protection of the nitrogen as its BOC derivative followed by treatment with TMS-diazomethane gave the desired esters, **11**. The intermediate hydroxyl compound was then treated with methanesulfonyl chloride and the resulting mesylate was utilized to alkylate the N9 position of adenine[19] giving adducts **12**. Unlike other analogues presented herein, treatment of compounds bearing nitrogen components in the tether with hydroxylamine and potassium hydroxide resulted in the formation of a mixture of carboxylic and hydroxamic acids (compounds **13** and **14**, respectively). These compounds were subsequently separated by preparative HPLC. In order to expand the parameters of the SAR study, the BOC groups were removed on treatment with TFA converting carbamates **12**–**14** to amines **15**–**17**.

As illustrated in Scheme 4, compound **15a** ($n=0$) was acetylated on treatment with acetic anhydride giving compound **18**. The resulting acetamide was treated with hydroxylamine and potassium hydroxide giving a mixture of carboxylic and hydroxamic acids (compounds **19** and

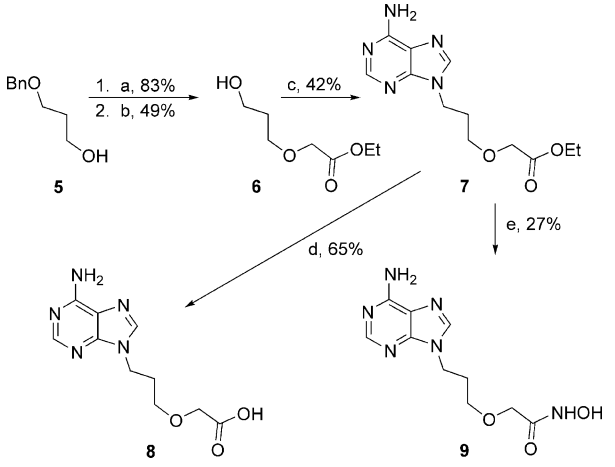


Scheme 1. Reagents and conditions: (a) K_2CO_3, DMF, 60 °C; (b) $HONH_2{\bullet}HCl$, KOH, MeOH, rt; (c) NaOH, MeOH, H_2O, rt.

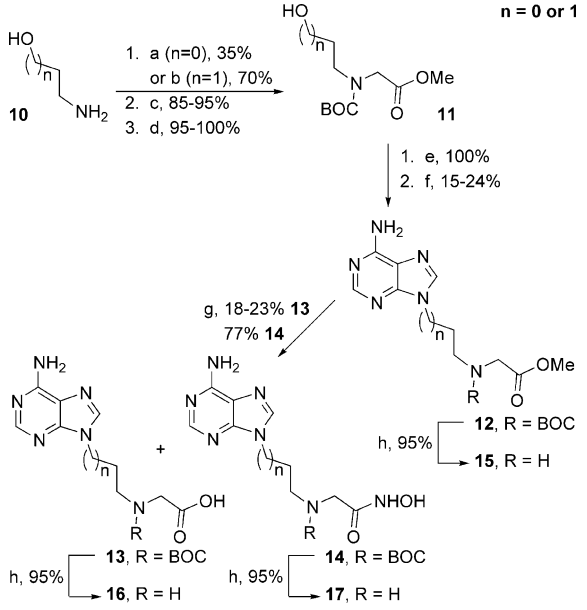
20, respectively) which were separated by preparative HPLC. Alternatively, compound **15a** was reductively methylated on treatment with formaldehyde and sodium cyanoborohydride giving compound **21**. Subsequent treatment with hydroxylamine and potassium hydroxide gave the hydroxamic acid, **22**, as the only product.

Following isolation, all adenine based esters, carboxylic and hydroxamic acids were evaluated against type V AC expressed in HEK 293 cells.[20]

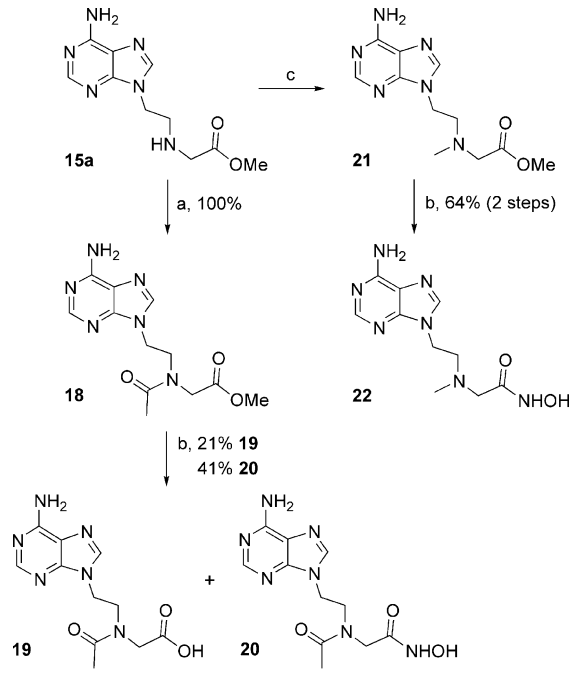
The data for all compounds prepared in this series are summarized in Table 1. As illustrated, among the alkyl tethered compounds, only linkers containing 3, 4 and 5 carbon atoms between N9 of the adenine moiety and



Scheme 2. Reagents and conditions: (a) Ethyl diazoacetate, $[Rh(OAc)_2]_2$, CH_2Cl_2; (b) H_2, 10% Pd/C, MeOH; (c) Adenine, DEAD, PPh_3, THF; (d) NaOH, MeOH, H_2O, rt; (e) $HONH_2{\bullet}HCl$, KOH, MeOH, rt.

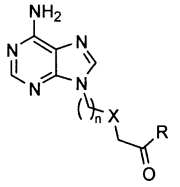


Scheme 3. Reagents and conditions: (a) Chloroacetic acid, H_2O, rt; (b) Glyoxylic acid$\bullet H_2O$, $NaCNBH_3$, AcOH, rt; (c) $(BOC)_2O$, NaOH, *t*BuOH, rt; (d) $TMSCHN_2$, Et_2O, 0 °C; (e) MsCl, DIEA, CH_2Cl_2,– 10 °C; (f) Adenine, Cs_2CO_3, DMF, 45 °C; (g) $HONH_2{\bullet}HCl$, KOH, MeOH, rt; (h) TFA, 0 °C.



Scheme 4. Reagents and conditions: (a) Ac_2O, AcOH, rt; (b) $HONH_2{\bullet}HCl$, KOH, MeOH, rt; (c) aq H_2CO, $NaCNBH_3$, AcOH, rt.

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



Compd	*n*	X	R	IC_{50} (μM)
2c	2	CH_2	OMe	>200
2d	3	CH_2	OEt	>200
2e	4	CH_2	OEt	>200
3a	0	CH_2	NHOH	>200
3b	1	CH_2	NHOH	7.9±2.1 (*n*=4)
3c	2	CH_2	NHOH	7.6±4.1 (*n*=17)
3d	3	CH_2	NHOH	35.4±13.3 (*n*=5)
3e	4	CH_2	NHOH	>200
4c	2	CH_2	OH	>200
4d	3	CH_2	OH	>200
4e	4	CH_2	OH	>200
7	3	O	OEt	>200
8	3	O	OH	140.5±49.1 (*n*=2)
9	3	O	NHOH	94.0±44.5 (*n*=4)
12a	2	N-BOC	OMe	>200
13a	2	N-BOC	OH	>200
13b	3	N-BOC	OH	>200
14a	2	N-BOC	NHOH	>200
14b	3	N-BOC	NHOH	>200
15a	2	NH	OMe	>200
15b	3	NH	OMe	>200
16a	2	NH	OH	>200
16b	3	NH	OH	>200
17a	2	NH	NHOH	>200
17b	3	NH	NHOH	>200
18	2	Nac	OMe	>200
19	2	Nac	OH	>200
20	2	Nac	NHOH	>200
21	2	Nme	OMe	>200
22	2	Nme	NHOH	15.3±7.6 (*n*=4)

the hydroxamic acid showed measurable activity. No inhibitory activity was noted for the corresponding esters or carboxylic acids. With respect to an oxygen substitution in the 5 atom tether (compound **9**), a marked decrease in activity was noted. Surprisingly, although compounds containing analogous tethers bearing substituted or unsubstituted nitrogen atoms demonstrated no measurable activity, a hydroxamate analogue bearing a methylated nitrogen in the tether (compound **22**) showed activity comparable to the native 4 carbon linked hydroxamate (compound **3c**).

In summary, mechanism based design of inhibitors of type V AC were prepared and tested. Only hydroxamic acid based inhibitors showed any measurable inhibitory activity presumably due to their ability to coordinate to magnesium atoms bound in the catalytic site. Among the hydroxamic acids, only 3, 4 and 5 atom tethers showed activity. These tethers appear to tolerate limited heteroatom substitutions with measurable losses in potency compared to the native carbon-based analogues. Based on these results, we would speculate that further conformational restriction of the tether may afford increased potencies of these novel inhibitors of type V AC.

References and Notes

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20. 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.