Bioorganic & Medicinal Chemistry 17 (2009) 6707–6714

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry

journal homepage: www.elsevier.com/locate/bmc

A new structural class of *S*-adenosylhomocysteine hydrolase inhibitors

Byung Gyu Kim [a], Tae Gyu Chun [b], Hee-Yoon Lee [b], Marc L. Snapper [a,*]

[a] *Merkert Chemistry Center, Department of Chemistry, Boston College, Chestnut Hill, MA 02467, USA*
[b] *Center for Molecular Design & Synthesis, Department of Chemistry, Korea Advanced Institute of Science & Technology, Daejon 305-701, Republic of Korea*

ARTICLE INFO

Article history:
Received 17 July 2008
Revised 21 July 2009
Accepted 23 July 2009
Available online 28 July 2009

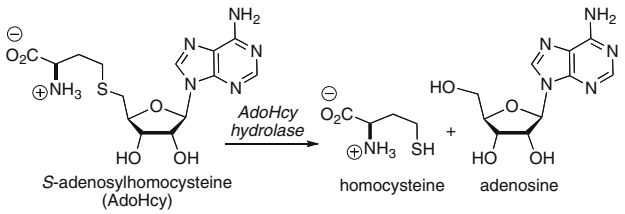
Keywords:
Ilimaquinone
S-Adenosylhomocysteine hydrolase
Inhibitor
Pharmacophore modeling

ABSTRACT

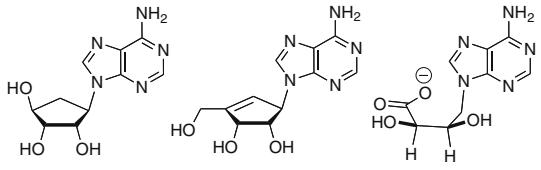
Effective inhibitors of *S*-adenosylhomocysteine hydrolase hold promise towards becoming useful therapeutic agents. Since most efforts have focused on the development of nucleoside analog inhibitors, issues regarding bioavailability and selectivity have been major challenges. Considering the marine sponge metabolite ilimaquinone was found to be a competitive inhibitor of *S*-adenosylhomocysteine hydrolase, new opportunities for developing selective new inhibitors of this enzyme have become available. Based on the activities of various hybrid analogs, SAR studies, pharmacophore modeling, and computer docking studies have lead to a predictive understanding of ilimaquinone's *S*-adenosylhomocysteine hydrolase inhibitory activities. These studies have allowed for the design and preparation of simplified structural variants possessing new furanoside bioisosteres with 100-fold greater inhibitory activities than that of the natural product.

1. Introduction

S-Adenosylhomocysteine hydrolase (AdoHcy hydrolase; EC 3.3.1.1),[1] which reversibly hydrolyzes *S*-adenosylhomocysteine (AdoHcy) to homocysteine and adenosine (Eq. 1),[2] has been considered an attractive target for the design of antiviral, antiparasitic, antiarthritic, and immunosuppressive agents.[3] Recent elucidation of the crystal structure of AdoHcy hydrolase isoforms from human and rat has contributed to our understanding of this enzyme.[4] Inhibition of AdoHcy hydrolase results in the accumulation of AdoHcy, which leads to feedback inhibition of *S*-adenosylmethionine (AdoMet) dependent methylations, a process that can impede viral replication,[5] and can lower serum cholesterol levels.[6] Furthermore, the corresponding reduction of homocysteine levels is believed to reduce the risk of developing coronary artery disease.[5]



Numerous inhibitors of AdoHcy hydrolase have been identified from natural and synthetic sources, but many function in an irreversible fashion, or are insufficiently selective, either of which can lead to undesired consequences and represent drawbacks for their development into clinically useful drugs.[7] Moreover, amongst the common nucleoside analogs, in addition to issues regarding potency and selectivity, there are also concerns of bioavailability. Eritadenine, for example, is a potent inhibitor of AdoHcy hydrolase, however, it is rapidly cleared by the liver. As a result, there is need for reversible selective inhibitors of AdoHcy hydrolase with improved bioavailability.

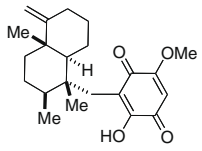


Our interest in this enzyme arose from the intriguing biological activities of (−)-ilimaquinone,[8] a natural product that was first isolated in 1979 from the Red Sea sponge *Smenospongia* sp. and over the years has been reported to possess antiviral, antimitotic, antimicrobial, and anti-HIV activities.[9] In addition, this marine sponge metabolite has also been shown to vesiculate the Golgi apparatus,[10] as well as to protect cells against the toxic effects of ricin and diphtheria.[11] In light of these myriad biological effects, we

* Corresponding author. Tel.: +1 617 552 8096; fax: +1 617 552 1442.
E-mail address: marc.snapper@BC.edu (M.L. Snapper).

0968-0896/$ - see front matter © 2009 Published by Elsevier Ltd.
doi:10.1016/j.bmc.2009.07.061

sought to use the natural product and structural variants to better understand the molecular basis of its activities.



(-)-ilimaquinone

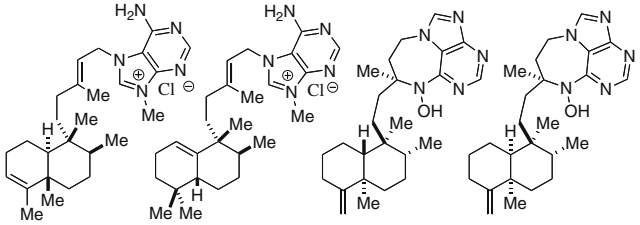
IC_{50} (40 μM)

Modifications to the total synthesis of (−)-ilimaquinone[12] allowed for the preparation, screening, and identification of active variants that could be used to report on the cellular interactions of the natural product.[13] Whole cell and cytosolic photoaffinity labeling and affinity chromatography studies with these reagents converged on AdoHcy hydrolase as a cellular target of the natural product.[14] Subsequent studies with isolated enzyme indicated that ilimaquinone is a competitive inhibitor of AdoHcy hydrolase. Moreover, new methylations, particularly amongst membrane-bound targets, were shown to be significantly reduced upon cellular incubation with ilimaquinone.[14a] This effect is consistent with inhibition of AdoHcy hydrolase elevating the concentration of AdoHcy, which leads to feedback inhibition of methyltransferases. Likewise, it was shown that the addition of AdoMet rescues the cell from the effects of ilimaquinone. In this case, the exogenous AdoMet serves to overcome AdoHcy's inhibition of the methyltransferases.[15]

Given these observations, ilimaquinone's inhibition of AdoHcy hydrolase and indirect inhibition of cellular methylations appears to account for many of ilimaquinone's biological activities. Moreover, from a structural perspective, (−)-ilimaquinone represents a unique inhibitor of AdoHcy hydrolase. Considering the biomedical potential for novel, non-toxic inhibitors of AdoHcy hydrolase in antiviral, antiparasitic, and antiarthritic therapies, a better understanding of (−)-ilimaquinone's inhibition of AdoHcy hydrolase could facilitate the development of new and non-toxic inhibitors of this enzyme. Our preliminary efforts toward this objective are described herein.

2. Results and discussion

Given ilimaquinone is a competitive inhibitor of AdoHcy hydrolase, and therefore competes with the nucleoside derived AdoHcy for binding to the enzyme's active site, perhaps the natural product is also functioning in a non-obvious manner as a nucleoside analog. Along these lines, we imagined several possibilities.[16] One scenario is the quinone moiety of the natural product, which is likely deprotonated under physiological conditions and therefore rendered non-electrophilic, is substituting for the adenine region of AdoHcy. This idea stemmed from the existence of natural products that possess adenine-like groups attached to a decalin ring system. Natural products such as kolavenol,[17] C-4–14,[18] asmarine A, and asmarine B,[19] all possess adenine-functionality in place of ilimaquinone's quinone group. An overlay of these two ring systems is shown in Figure 1 (Model 1). Unfortunately, our early evaluation of C-4–14 in a cellular secretion assay did not show much promise for these naturally occurring structural variants.[13]



Alternatively, it is also possible, especially considering the oxidized intermediates formed in AdoHcy hydrolase's enzymatic cycle, that the quinone group could serve as a replacement for the

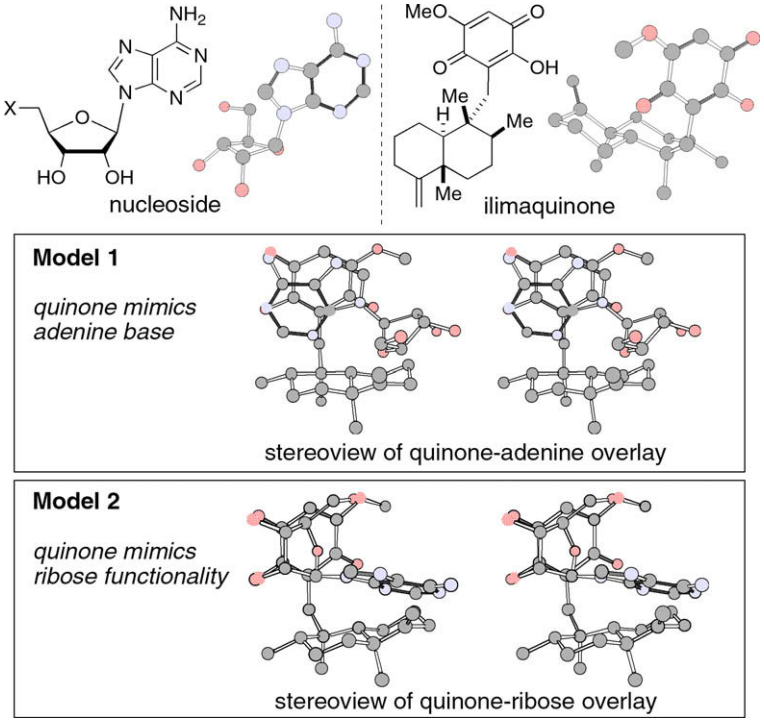
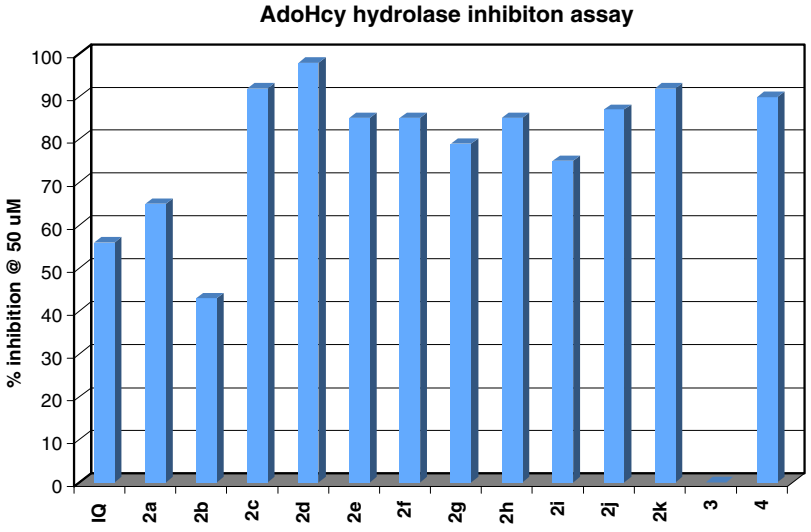


Figure 1. Pharmacophore modeling of (−)-ilimaquinone.



Graph 1. Comparison (−)-ilimaquinone (IQ) and analogs (50 μM) in a AdoHcy hydrolase inhibition assay.

ribose portion of AdoHcy. A similar situation was reported for the inhibition of Hsp90 by geldanamycin. Geldanamycin possesses a quinone moiety that binds to the active site of Hsp90 where the ribose portion of ATP binds.[20] This possibility for ilimaquinone is illustrated in Model 2. In either case, both pharmacophore models serve as inspiration for the design of new AdoHcy hydrolase inhibitors.

To understand ilimaquinone's interaction with AdoHcy hydrolase while identifying structural variants with improved inhibitory activities, we introduced simple modifications to the quinone moiety as suggested by the adenine-functionality in Model 1. We hoped that the inclusion of nitrogens (and other heteroatom-containing substituents) on the quinone would favorably influence binding with AdoHcy hydrolase. Synthesis of these variants started from the known chloroquinone **1**.[12,13] Replacement of chloride with fluoride and ethanethiol gave thioquinone **2a** and fluoroquinone **2b**, respectively. Treatment of **1** with methylamine and sodium bicarbonate afforded **2c** under short reaction times, but produced diaminated compound **2d** with longer reactions at elevated temperatures. Similarly, ethylamine, allylamine, *i*-propylamine, *n*-butylamine, and *t*-butylamine were installed on the quinone to provide analogs **2f–2k**.

The effects of adding other heteroatom substituents on the quinone moiety of ilimaquinone in an AdoHcy hydrolase inhibition assay were promising. As illustrated in Graph 1, with the exception of fluoroquinone **2b**, all other quinone variants (**2a**, **2c**–**2k**) displayed increased AdoHcy hydrolase inhibitory activities relative to ilimaquinone. While encouraged by these favorable results, their ability to provide insight into active site interactions and differentiate between binding models was limited.

A more telling approach, we believed, would be to build hybrid structures that paired portions of ilimaquinone with components of typical adenosyl inhibitors.[21] Four such compounds (**3**–**6**) are illustrated in Figure 2. Given our pharmacophore modeling, we were particularly interested in evaluating the relative activities of hybrids **3** and **4**. Coupling of alternative fragments such as in **5** and **6** were also of relevance. For example, hybrid analog **5** is reminiscent of the known adenosinyl containing natural products mentioned above.

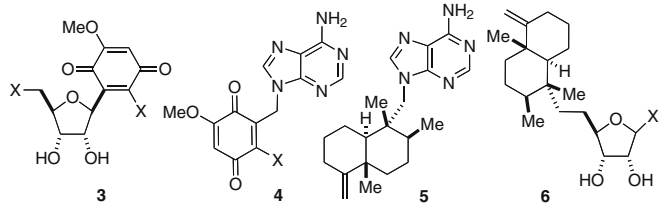
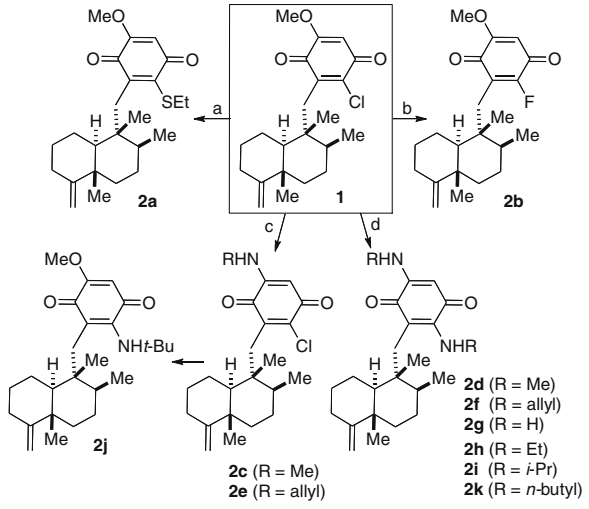


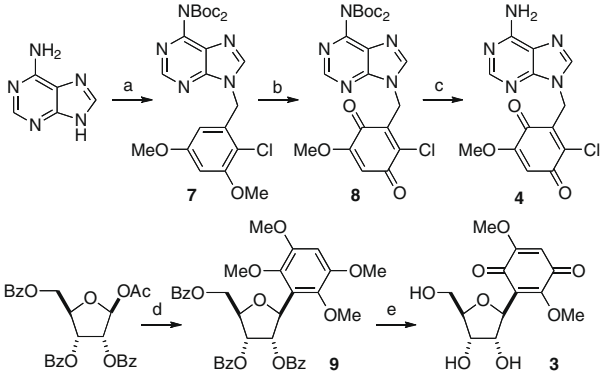
Figure 2. (−)-Ilimaquinone/adenosine hybrid analogs and natural products.

In any case, we prepared the ilimaquinone/adenosine hybrid structures **3** and **4** where the quinone moiety was substituted for either the adenine or the ribose portion of adenosine. As summarized in Scheme 2, coupling of adenine with 2-chloro-3,5-dimethoxybenzyl bromide followed by Boc protection afforded **7** in 48% yield, and subsequent CAN oxidation gave the quinone **8** in 79% (57% conversion). Deprotection of **8** using formic acid provided hybrid **4** in 83% yield. Synthesis of compound **3**, the quinone/ribose hybrid started from β-ribose-furanose 1-acetate 2,3,5-tribenzoate, which in the presence of a Lewis acid was coupled with 1,2,4,5-tetramethoxybenzene to give intermediate **9**. Deprotection of **9** with sodium methoxide and subsequent CAN oxidation afforded **3** in 25% yield for these final two steps.

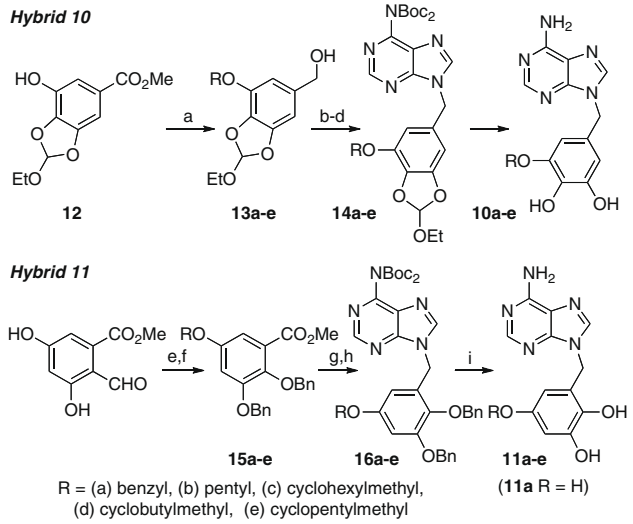
As before, compounds **3** and **4** were evaluated at 50 μM for inhibition of AdoHcy hydrolase. Their performance in this assay is also



Scheme 1. Preparation of quinone analogs of (−)-ilimaquinone. (a) EtSH, $NaHCO_3$, MeOH, 84%; (b) KF (excess), Bu_4NBr (cat.), 47%; (c) RNH_2, $NaHCO_3$, MeOH, rt, 10 min, 87%; (d) RNH_2, $NaHCO_3$, MeOH, 12 h, 40 °C, 81%.



Scheme 2. Preparation of hybrids **3** and **4**. (a) NaH, DMF, then 2-chloro-3,5-dimethoxybenzyl bromide; Boc_2O, DMAP, 48% (two steps); (b) CAN, CH_3CN/H_2O, 79% (57% conv.); (c) formic acid, 83%; (d) $AlCl_3$, 1,2,4,5-tetramethoxybenzene, 30%; (e) NaOMe, MeOH; CAN, CH_3CN/H_2O, 25% (two steps).



Scheme 3. Synthesis simplified aromatic hybrid analogs. (a) RBr, K_2CO_3, acetone; LAH, THF; (b) $SOCl_2$, pyridine, benzene, 70–80%; (c) bis-Boc-adenine, Bu_4NI, K_2CO_3, DMF; (d) HCO_2H; (e) RX, Cs_2CO_3, acetone; (f) H_2O_2, NaOH, 1,4-dioxane; BnBr, Cs_2CO_3, Bu_4NI, acetone; (g) LAH, THF; (h) $SOCl_2$, pyridine, benzene; bis-Boc-adenine, K_2CO_3, DMF, Bu_4NI; (i) HCO_2H; H_2, Pd/C (10% on carbon), EtOAc/MeOH, 1 atm.

summarized in Graph 1. Interestingly, hybrid **3** showed little inhibitory activity against the enzyme, whereas compound **4** displayed high activity at this concentration. Further evaluation indicated that hybrid **4** possesses an IC_{50} of 4.3 μM (±1.2 μM) against AdoHcy hydrolase, a 10-fold improvement over (−)-ilimaquinone's activity. These results provide support for binding Model 2 (Fig. 1), where ilimaquinone's quinone moiety may interact with AdoHcy hydrolase in a fashion similar to the ribose 2,3-dihydroxyl group of AdoHcy.[22]

Based on this binding model, we wondered whether the quinone ring could be simplified even further. For example, perhaps the quinone could be replaced by an aromatic ring. This system should still provide the same key hydrogen-bonding interactions with the enzyme target, while also interfering with the crucial redox chemistry that is indispensable for AdoHcy hydrolase activity. Along these lines, two simplified adenine/polyhydroxylated arene hybrids **10** and **11** were designed and prepared (Fig. 3).

As described in Scheme 3, the synthesis of hybrid **10** commenced with orthoformate protection of commercially available methyl 3,4,5-trihydroxybenzoate to afford compound **12** in 83% yield.[23] Coupling of **12** with various aliphatic R groups produced ethers, which were converted to the corresponding benzyl alcohols 13a–**e** by reduction with LAH in 85–90% yield (two steps). Attempts to prepare the corresponding benzyl bromides resulted in unsatisfactory yields (ca. 30%), presumably due to instability of these reactive alkylating agents. Reaction of **13a–e** with thionyl chloride and pyridine in dichloromethane gave the requisite benzyl chlorides in highly improved yields, which were directly coupled with bis-Boc-adenine[21] to provide analogs **14a–e** in 71–80% yield for these two steps. Deprotection of **14a–e** with formic acid furnished the desired hybrids **10a–e** in greater than 90% yields.

The preparation of the hybrid **11** analogs is also described in Scheme 3. Selective alkylation of methyl 3,5-dihydroxy-2-formylbenzoate[24] with various alkyl halides under the basic condition was followed by a Bayer–Villager reaction[25] and protection of the resulting diols with benzyl groups to afford compounds **15a–e** in approx. 60–65% yield (three steps). Reduction of ester **15a–e** with LAH and subsequent chlorination of the resulting benzyl alcohol using thionyl chloride and pyridine provided benzyl chloride, which was directly coupled with bis-Boc-adenine to provide

Table 1
Evaluation of compounds **10a–e** as AdoHcy hydrolase inhibitors

	% Inhibition					
	100 μM	50 μM	10 μM	2 μM	1 μM	0.1 μM
10a	93 (±7)	70 (±2)	53 (±2)	26 (±5)	27 (±2)	nd
10b	72 (±4)	47 (±8)	26 (±1)	12 (±2)	nd	nd
10c	100 (±1)	74 (±4)	64 (±6)	17 (±1)	15 (±4)	nd
10d	92 (±1)	nd	72 (±1)	nd	29 (±4)	7 (±1)
10e	89 (±2)	nd	62 (±2)	nd	31 (±1)	6 (±1)

Table 2
Evaluation of compounds **11a–e** as AdoHcy hydrolase inhibitors

	% Inhibition				
	100 μM	10 μM	1 μM	0.1 μM	0.01 μM
11a	89 (±3)	85 (±3)	67 (±14)	31 (±12)	10 (±4)
11b	95 (±2)	78 (±4)	48 (±11)	32 (±13)	13 (±10)
11c	98 (±2)	91 (±2)	69 (±4)	37 (±15)	4 (±6)
11d	95 (±1)	86 (±2)	62 (±12)	22 (±2)	6 (±1)
11e	104 (±4)	97 (±1)	74 (±3)	32 (±1)	20 (±1)

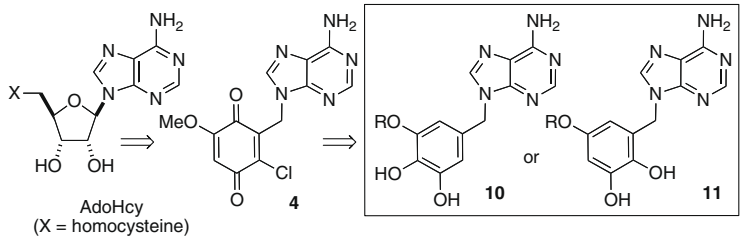


Figure 3. Simplified (−)-ilimaquinone/adenosine hybrids.

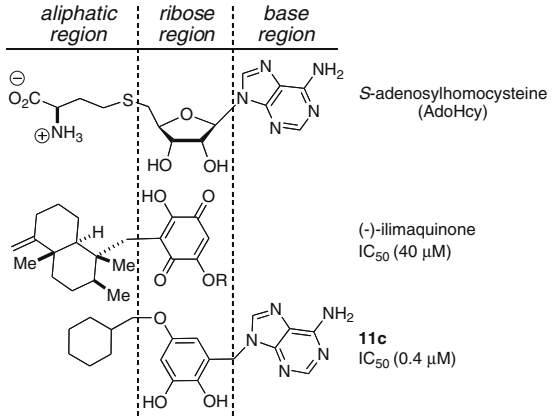


Figure 4. Refinement of the pharmocophore model.

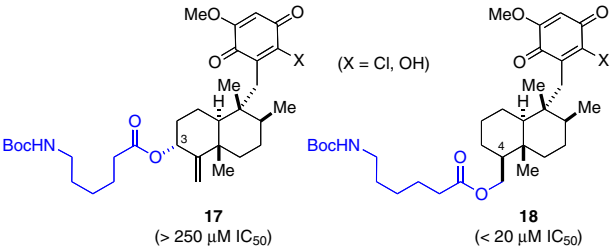
16a–e in 50–60% yield for the three steps. Successive Boc deprotection of **16a–e** with formic acid and hydrogenolysis of the resulting products furnished simplified adenine hybrids **11a–e** in approximately 50% yield.

The inhibitory activities of compounds **10a–e** and **11a–e** against AdoHcy hydrolase were investigated and an approximate IC_{50} value for each compound was obtained. Gratifyingly, as shown in Tables 1 and 2, most of these simplified analogs displayed at low μM range IC_{50}. In general, the hybrid **11** series were more potent inhibitors of AdoHcy hydrolase than the **10** series. Moreover, some of the hybrid **11** compounds possessed submicromolar IC_{50} activities, levels indicating 100-fold more potent inhibitory activities than that of (−)-ilimaquinone.

These favorable results encouraged further refinement of our pharmacophore model. Figure 4 illustrates the three key substrate/enzyme interacting regions and how they may correlate to ilimaquinone and simplified variants. Based on this model and crystallographic information of known inhibitors bound to AdoHcy hydrolase, computational studies of our simplified inhibitors docked with the enzyme were pursued. First, ilimaquinone was docked using the Discovery/Insight II into the catalytic domain of the enzyme with the quinone moiety positioned where the ribose unit of AdoHcy was located in the X-ray structure.[26] The hydrogens on the enzyme's residues and inhibitors were allowed to relax during energy minimization steps. As implied in Figure 4, and shown in Figure 5a and b, a binding comparison of ilimaquinone and AdoHcy indicates that the decalin portion of ilimaquinone can replace the homocysteine region of AdoHcy. Moreover, the ketomethoxy functionality of the quinone can effectively replace the ribose unit of AdoHcy with good overlap of the hydroxyl groups of the ribose.

Of particular note is the ability of this binding model to account for the inhibitory activities of previously examined ilimaquinone variants. For example, the functionalized reagents **17** and **18** were prepared and screened in a secretory assay designed to probe Golgi function.[13] Interestingly, analog **17**, which was generated from the natural product, showed no antisecretory activities at concentrations tested, while the synthetic analog **18** displayed activities comparable to the natural product. This notable difference in activity indicated that functional affinity and fluorescent probe reagents should be prepared from analog **18**. Now our AdoHcy hydrolase binding model can account for the activity differences in these two ilimaquinone-derived reagents.

As illustrated in Figure 5c, there is a narrow channel that extends from the enzyme's active site that can accommodate the C4 side chain found in ilimaquinone analog **18**. When the side chain emanates from C3 of the decalin ring system, however, this active site tunnel is not accessible to the substrate.



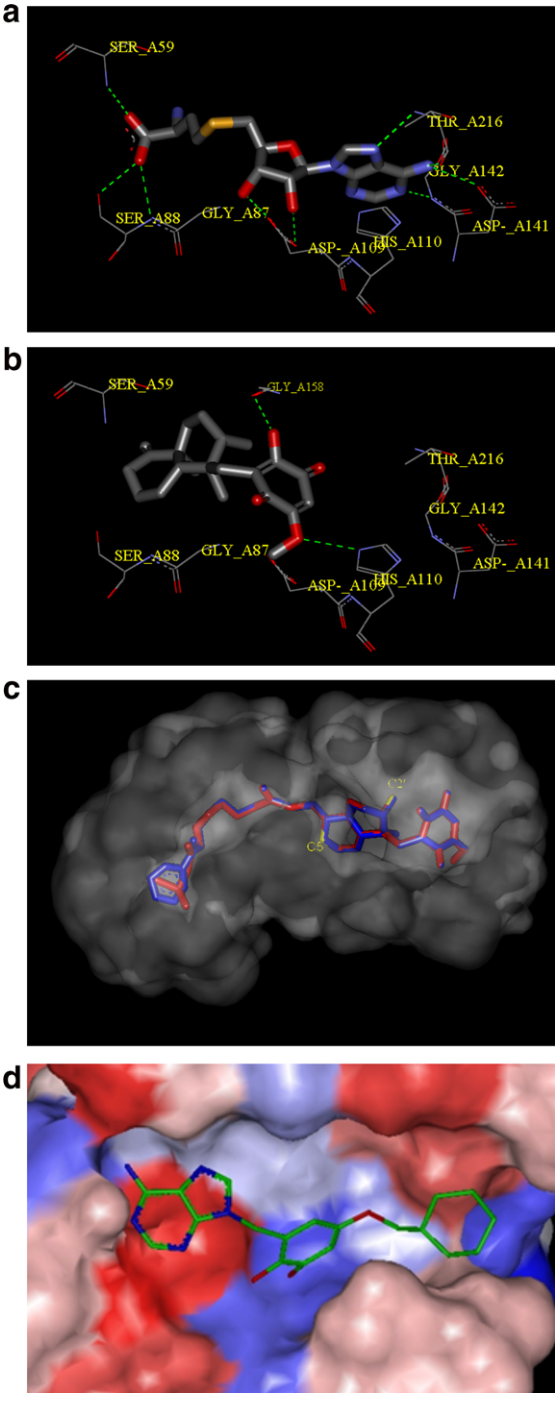
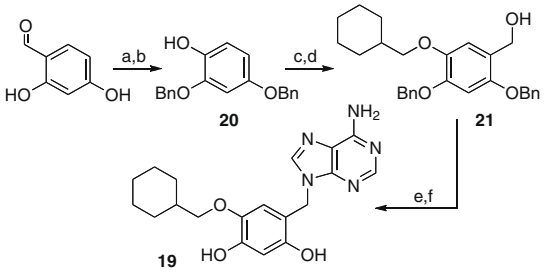


Figure 5. Comparison of binding of (a) AdoHcy and (b) ilimaquinone in AdoHcy hydrolase (c) modeling of ilimaquinone analog **18** made for affinity studies, and (d) the bonding mode of **11c**.

In a likewise gratifyingly manner, the binding model is also consistent with structure–activity relationships for compounds noted in Scheme 1. The methoxy group on the quinone, which functions as a hydrogen-bond acceptor, is replaceable by substituted nitrogens (**2c**, **2e**). The quinone hydroxyl group can also be exchanged with substituted nitrogens to provide improved activity (**2d**-**2k**) as the additional alkyl chain provides favorable hydrophobic interactions with the enzyme. The molecular modeling also can account for subtle differences in the inhibitory activities for the analogs **10a–e** and **11a–e**. Modeling of analogs **11a–e** show that the aromatic dihydroxyl groups, as well as the adenine group, are well positioned in the hydrophilic region typically occupied by the ribose and adenine groups of known inhibitors, while the pendent aliphatic functionality provides additional hydrophobic stabilization to the enzyme/ligand complex (Figs. 5d and 6). When compared with the analogs **11a–e**, the aromatic hydroxyl groups in analogs **10a–e**, on the other hand, have differing hydrogen-bonding interactions with the enzyme. Analogs **11a–e** hydrogen bond with Asp-109, while analogs **10a–e** have hydrogen-bonding interactions with Thr-57. This, in part, may account for the 10-fold greater activities of the **11a–e** analog series. More importantly, the molecular model provides guidance for the design of new ilimaquinone-based inhibitors.

With a predictive binding model in place, our strategy for optimizing interactions with the enzyme was to reexamine the three regions for optimal cooperative enzyme binding. In that regard, systematic changes to the aliphatic, ribose, and base regions were planned. As indicated in Figure 6, initial optimization studies focused on the placement of hydroxyl groups on the aromatic ring. Specifically, an alternative to the unattractive 1,2-catechol functionality would be preferred for further development of this series. Accordingly, 1,3-dihydroxyarenes were investigated as this functionality benefits from both a hydrogen bond with Asp-109, as in the **11** analog series, and a favorable interaction with Thr-57 as enjoyed by the **10** analog series (Fig. 6).

Toward this end, 1,3-dihydroxy analog **19** was prepared from commercially available 2,4-dihydroxybenzaldehyde, which was converted to phenol **20** through dibenzylation followed by basic Bayer–Villager reaction in 87% yield in two steps (Scheme 4). Reaction of phenol **20** with cyclohexylmethyl bromide and subsequent Vilsmeier formylation afforded the corresponding benzaldehyde, which was reduced to the benzyl alcohol **21**. Successive Mitsunobu



Scheme 4. Synthesis of analog **19**. (a) BnBr, K_2CO_3, acetone, 95%; (b) *m*-CPBA, CH_2Cl_2, then K_2CO_3, MeOH, 92%; (c) cyclohexylmethyl bromide, Cs_2CO_3, Bu_4NI, acetone, 85%; (d) DMF, $POCl_3$; $NaBH_4$, MeOH, 93%; (e) PPh_3, bis-Boc-adenine, DEAD, THF/benzene (1:1), 27%; (f) HCO_2H, 96%; Pd/C (10%), H_2, MeOH/EtOAc, 88%.

reaction, Boc deprotection, and hydrogenolysis furnished compound **19** in 23% overall yield for three steps.

Likewise, an adenine surrogate study was also pursued. It was envisioned that a late stage coupling of adenine variants with benzyl chloride **25** would be an efficient entry into these compounds (Scheme 5). Reduction of **22** with LAH and subsequent TBS protection of the resulting benzyl alcohol afforded compound **23**. Lithium anion of **23** generated by metal halogen exchange with *n*-BuLi, was reacted with triisopropyl borate followed by oxidative workup to provide a phenol, which was then reacted with bromomethyl cyclohexane to afford **24** in three steps. Successive TBS deprotection and chlorination of **24** then furnished the desired benzyl chloride **25**. With compound **25** in hand, we substituted adenine for various secondary amines. The coupling of benzyl chloride **25** and different amines were straightforward and gave us 6 analogs of the chimeric hybrid **26a**–**f** (Fig. 7).

In a similar fashion, we prepared an ester analog of inhibitor **11c**; also using a late stage coupling strategy (**29**, Scheme 5). The synthesis started from 5-bromobenzyl alcohol, which was converted to nitrile **27**. Hydrolysis of **27** followed by benzylation of the resulting acid afforded an ester, which was transformed to the corresponding benzyl chloride **28**. Coupling of **28** with bis-Boc-adenine and subsequent hydrogenolysis, EDC coupling, and deprotection of the resulting adduct furnished **29** in four steps.

Preliminary screening of inhibitory activity of compounds **26a–f** against AdoHcy hydrolase was carried out and percent inhibition

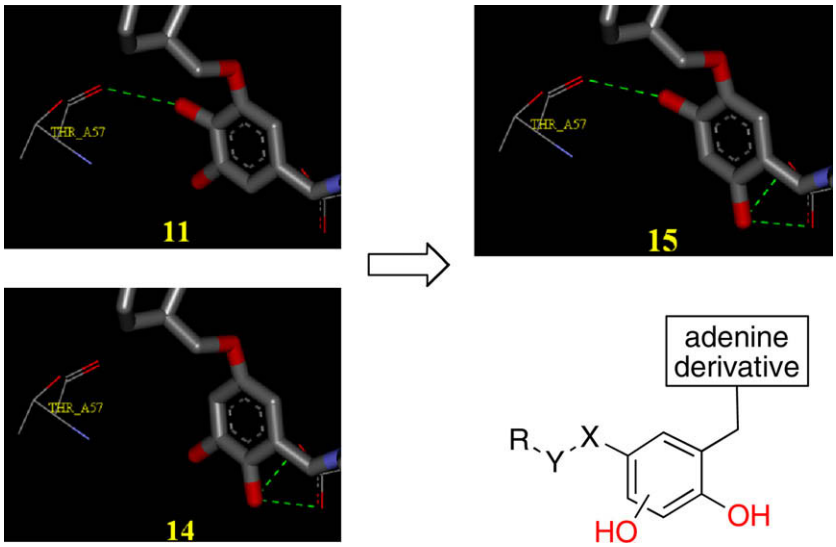
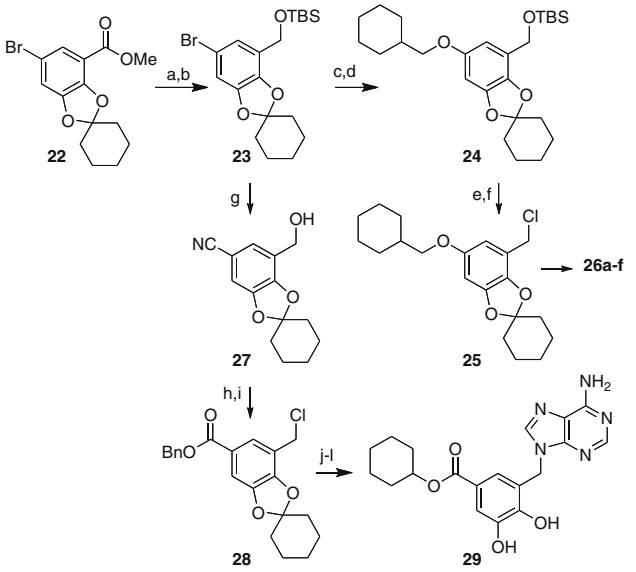


Figure 6. Design for new AdoHcy hydrolase inhibitors.



Scheme 5. Adenine and linker alternatives in hybrid **11** series. (a) LAH, THF, 99%; (b) TBSCl, Et_3N, CH_2Cl_2, 93%; (c) *n*-BuLi; $B(O^iPr)_3$; H_2O_2, NaOH, 86%; (d) Cs_2CO_3, cyclohexylmethyl bromide, Bu_4NI, DMF, 82%; (e) TBAF, THF, quant.; (f) $SOCl_2$, pyr, CH_2Cl_2, −50 °C, 82%; (g) CuCN, DMF, 150 °C, 61%; (h) LiOH, MeOH/H_2O, 70 °C; BnBr, K_2CO_3, DMF, 86%; (i) $SOCl_2$, CH_2Cl_2, pyr, 79%; (j) K_2CO_3, bis-Boc-adenine, K_2CO_3, DMF, Bu_4NI, rt, 72%; (k) Pd/C (10% on carbon), MeOH, 93%; (l) EDC, DMAP, cyclohexyl alcohol, CH_2Cl_2; formic acid 88%.

of AdoHcy hydrolase at 50 μM concentration of inhibitor is summarized in Graph 2. At 50 μM, none of these compounds showed improved inhibition of AdoHcy hydrolase over analog **11c**. Molecular modeling suggests that the adenine amine enjoys hydrogen-bonding interactions with Asp-141 and Thr-216 of the enzyme. While compounds **26a** and **26f** are capable of maintaining this hydrogen bonding interaction with AdoHcy hydrolase, the other analogs are devoid of such functional groups. This difference may be responsible for the inhibitory trends observed for compounds **26b–d**.

The inhibitory activity of compounds **19** and **29** were also investigated against AdoHcy hydrolase. As predicted from the molecular modeling study, ester **29** possesses an inhibitory activity similar to that of hybrid analog **11c** (IC_{50} = 0.4 μM ± 0.2 μM), and the *meta*-substituted dihydroxyl analog **19** also shows comparable inhibitory activities in the nanomolar range.

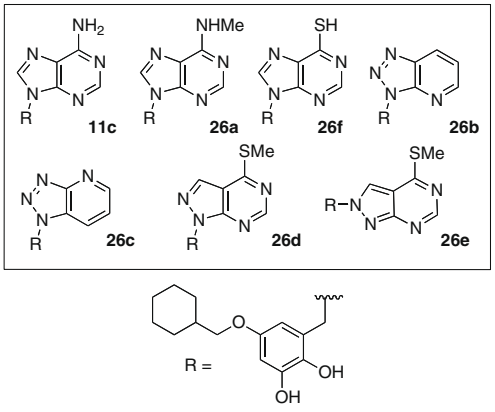
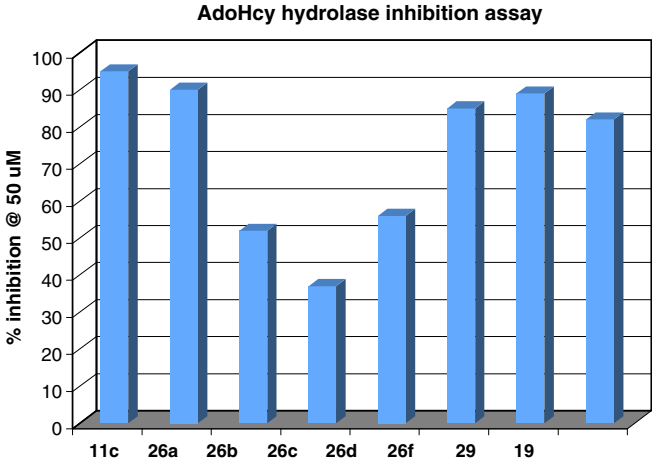


Figure 7. Adenine variants **11c** and **26a–f**.



Graph 2. Relative inhibition of AdoHcy hydrolase at 50 μM.

3. Conclusions

Several new ilimaquinone analogs were prepared and screened for their ability to inhibit AdoHcy hydrolase. The design of these inhibitors were based on the assumption that ilimaquinone is functioning as a nucleoside analog. These assumptions combined with molecular modeling have lead to the preparation of simplified analogs with improved inhibitory activities over that of ilimaquinone. Specifically, the preparation and evaluation of ilimaquinone/adenosine hybrids indicated that, when interacting with the enzyme, the quinone moiety of ilimaquinone is serving as a ribose mimic. These insights may lead to a new class of AdoHcy hydrolase inhibitors with improved specificity, activity and bioavailability.

4. Experimental section

4.1. *S*-Adenosylhomocysteine hydrolase assay

The ilimaquinone structural analogs were added to assay at final concentrations ranging between 0.01 and 100 μM. [8-^{14}C]-*S*-Adenosylhomocysteine (9.4 μg, 25.4 nM, 21.8 μCi) was incubated in the *S*-Adenosylhomocysteine hydrolase (0.35 μg) at 37 °C for 10 min in a total reaction volume of 250 μL. The incubation buffer was 15 mM HEPES, 5 mM $Mg(OAc)_2$, 150 mM KCl, 2 mM 2-mercaptoethanol, 0.25% bovine serum albumin, and pH 7.0 including adenosine deaminase (50 units/mL). The incubations were stopped by the addition of formic acid (5 N, 50 mL) and 250 mL from the solution was poured onto a SP-Sephadex-C25 column (10 mm × 30 mm) pre-equilibrated with 0.1 N formic acid. [^{14}C]-Inosine was eluted with formic acid (0.1 N, 6 mL) and collected in a scintillation vial with scintillation fluid (5 mL).

Acknowledgments

We thank the National Institutes of Health (CA-66617) for financial support. TGC and HYL acknowledge support by a grant from Marine Biotechnology Program funded by Ministry of Land, Transport and Maritime Affairs, Republic of Korea. We also thank Professor Mark P. Wentland for his critical reading and comments.

Supplementary data

Supplementary data (experimental procedures, as well as analytical and spectral characterization data for new compounds) associated with this article can be found, in the online version, at doi:10.1016/j.bmc.2009.07.061.

References and notes

1. Ueland, P. M. *Pharmacol. Rev.* **1982**, *34*, 223.
2. For initial mechanistic insight into AdoHcy, see: Palmer, J. L.; Abeles, R. H. *J. Biol. Chem.* **1979**, *254*, 1217.
3. (a) Yuan, C. S.; Saso, Y.; Lazarides, E.; Borchardt, R. T.; Robins, M. J. *Exp. Opin. Therap. Pat.* **1999**, *9*, 1197; Yuan, C. S. et al *Adv. Antiviral Drug Design* **1996**, *2*, 41; (b) Chiang, P. K. *Pharmacol. Ther.* **1998**, *77*, 115; (c) Daelemans, D.; Este, J. A.; Witvrouw, M.; Pannecouque, C.; Jonckheere, H.; Aquaro, S.; Perno, C.-F.; Clercq, E. D.; Vandamme, A.-M. *Pharmacology* **1997**, *52*, 1157; (d) De Clercq, E. *Nucleosides, Nucleotides, Nucleic Acids* **2005**, *24*, 1395.
4. (a) Turner, M. A.; Yuan, C.-S.; Borchardt, R. T.; Hershfield, M. S.; Smith, G. D.; Howell, P. L. *Nat. Struct. Biol.* **1998**, *5*, 369; (b) Hu, Y.; Komoto, J.; Huang, Y.; Gomi, T.; Ogawa, H.; Takata, Y.; Fujioka, M.; Takusagawa, F. *Biochemistry* **1999**, *38*, 8323.
5. (a) Wnuk, S. F. *Mini-Rev. Med. Chem.* **2001**, *1*, 307; (b) De Clercq, E.; Montgomery, J. A. *Antiviral Res.* **1983**, *3*, 17; (c) De Clercq, E. *Nucleosides, Nucleotides* **1998**, *17*, 625.
6. Yamada, T.; Komoto, J.; Lou, K.; Ueki, A.; Hua, D. H.; Sugiyama, K.; Takata, Y.; Ogawa, H.; Takusagawa, F. *Biochem. Pharmacol.* **2007**, *73*, 981.
7. (a) Wolfe, M. S.; Borchardt, R. T. *J. Med. Chem.* **1991**, *34*, 1521; For representative examples, also see: (b) Jarvi, E. T.; McCarthy, J. R.; Mehdi, S.; Matthews, D. P.; Edwards, M. L.; Prakash, N. J.; Bowlin, T. L.; Sunkara, P. S.; Bey, P. *J. Med. Chem.* **1991**, *34*, 647. and references cited therein; (c) Wnuk, S. F.; Mao, Y.; Yuan, C.-S.; Borchardt, R. T.; Andrei, G.; Balzarini, J.; De Clercq, E.; Robins, M. J. *J. Med. Chem.* **1998**, *41*, 3078; (d) Parry, R. J.; Muscate, A.; Askonas, L. J. *Biochemistry* **1991**, *30*, 9988; (e) Guillerm, G.; Muzard, M.; Glapski, C.; Pilard, S.; De Clercq, E. *J. Med. Chem.* **2006**, *49*, 1223; (f) Glazer, R. I.; Knode, M. C. *J. Biol. Chem.* **1984**, *259*, 12964.
8. (a) Luibrand, R. T.; Erdman, T. R.; Vollmer, J. J.; Scheuer, P. J.; Clardy, J. C. *Tetrahedron* **1979**, *35*, 609; (b) Capon, R. J.; MacLeod, J. K. *J. Org. Chem.* **1987**, *52*, 5059.
9. (a) Loya, S.; Tal, R.; Kashman, Y.; Hizi, A. *Antimicrob. Agents Chemother.* **1990**, *34*, 2009; (b) Kushlan, D. M.; Faulkner, D. J.; Parkanyi, L.; Clardy, J. C. *Tetrahedron* **1989**, *45*, 3307; (c) Bourguet-Kondracki, M.-L.; Longeon, A.; Morel, E.; Guyot, M. *Int. J. Immunopharm.* **1991**, *13*, 393; (d) Schroder, H. C.; Wenger, R.; Gerner, H.; Reuter, P.; Kuchino, Y.; Sladic, D.; Muller, W. E. G. *Cancer Res.* **1989**, *49*, 2069; (e) Sarin, P. S.; Sun, D.; Thornton, A.; Muller, W. E. G. *J. Natl. Cancer. Inst.* **1987**, *78*, 663; (f) Belisario, M. A.; Maturo, M.; Pecce, R.; De, R. S.; Villani, G. R. D. *Toxicology* **1992**, *72*, 221; (g) Muller, W. E. G.; Dogovic, N.; Zahn, R. K.; Maidhof, A.; Diehl, S. B.; Bedker, C.; Sachsse, W.; Gasic, M. J.; Schroeder, H. C. *Basic Appl. Histochem.* **1985**, *29*, 321; (h) Rangel, H. R.; Dagger, F.; Compagnone, R. S. *Cell Biol. Int.* **1997**, *21*, 337.
10. (a) Talizawa, P. A.; Yucel, J. K.; Veit, B.; Faulkner, D. J.; Deerinck, T.; Soto, G.; Ellisman, M.; Malhotra, V. *Cell* **1993**, *73*, 1079; (b) Veit, B.; Yucel, J. K.; Malhotra, V. *J. Biol. Chem.* **1993**, *122*, 11976; (c) Jamora, C.; Takizawa, P. A.; Zaarour, R. F.; Denesvre, C.; Faulkner, D. J.; Malhotra, V. *Cell* **1997**, *91*, 617.
11. Nambiar, M. P.; Wu, H. C. *Exp. Cell Res.* **1995**, *219*, 671.
12. Bruner, S. D.; Radeke, H. S.; Tallarico, J. A.; Snapper, M. L. *J. Org. Chem.* **1995**, *60*, 1114.
13. Radeke, H. S.; Digits, C. A.; Bruner, S. D.; Snapper, M. L. *J. Org. Chem.* **1997**, *62*, 2823.
14. (a) Radeke, H. S.; Digits, C. A.; Casaubon, R. L.; Snapper, M. L. *Chem. Biol.* **1999**, *6*, 639; (b) Casaubon, R. L.; Snapper, M. L. *Bioorg. Med. Chem. Lett.* **2001**, *14*, 133.
15. Cools, M.; De Clercq, E. *Biochem. Pharmacol.* **1990**, *40*, 2259.
16. (a) Romines, K. R.; Chrusciel, R. A. *Curr. Med. Chem.* **1995**, *2*, 825; (b) Wender, P. A.; Cribbs, C. M. *Adv. Med. Chem.* **1992**, *1*, 1; (c) Wender, P. A.; Cribbs, C. M.; Koehler, K. F. *Proc. Natl. Acad. Sci. U.S.A.* **1988**, *85*, 7197.
17. Total syntheses of the diterpenoids (−)-kolavenol and (−)-agelasine B: Piers, E.; Roberge, J. Y. *Tetrahedron Lett.* **1992**, *33*, 6923.
18. A sample was generously provided by Professor Sidney M. Hecht, University of Virginia.
19. Asmarines A–C; three novel cytotoxic metabolites from the marine sponge *Raspailia* sp.: Yosief, T.; Rudi, A.; Stein, Z.; Goldberg, I.; Gravalos, G. M. D.; Schleyer, M.; Kashman, Y. *Tetrahedron Lett.* **1998**, *39*, 3323.
20. (a) Stebbins, C. E.; Russo, A. A.; Schneider, C.; Rosen, N.; Hartl, F. U.; Pavletich, N. P. *Cell* **1997**, *89*, 239; (b) Prodromou, C.; Roe, S. M.; O'Brien, R.; Ladbury, J. E.; Piper, P. W.; Pearl, L. H. *Cell* **1997**, *90*, 65.
21. For recent AdoHcy hydrolase inhibitors, see: (a) Yin, X. Q.; Schneller, S. W. *Tetrahedron* **2005**, *61*, 1839; (b) Yang, M.; Schneller, S. W. *Bioorg. Med. Chem. Lett.* **2005**, *15*, 149; (c) Guillerm, G.; Muzard, M.; Glapski, C.; Pilard, S. *Bioorg. Med. Chem. Lett.* **2004**, *14*, 5803; (d) Guillerm, G.; Muzard, M.; Glapski, C. *Bioorg. Med. Chem. Lett.* **2004**, *14*, 5799; (e) Wnuk, S. F.; Lewandowska, E.; Sacasa, P. R.; Crain, L. N.; Zhang, J.; Borchardt, R. T.; De Clercq, E. *J. Med. Chem.* **2004**, *47*, 5251; (f) Yin, X.; Schneller, S. W. *Nucleosides, Nucleotides, Nucleic Acids* **2004**, *23*, 67; (g) Kumamoto, H.; Onuma, S.; Tanaka, H. *J. Org. Chem.* **2004**, *69*, 72; (h) Moon, H. R.; Lee, K. M.; Lee, H. J.; Lee, S. K.; Park, S. B.; Chun, M. W.; Jeong, L. S. *Nucleosides, Nucleotides, Nucleic Acids* **2005**, *24*, 707; (i) Kitade, Y.; Ando, T.; Yamaguchi, T.; Hori, A.; Nakanishi, M.; Ueno, Y. *Bioorg. Med. Chem.* **2006**, *14*, 5578.
22. This binding model implies that these compounds should function as competitive inhibitors of AdoHcy, however, we have not yet experimentally confirmed this prediction.
23. Alam, A.; Takaguchi, Y.; Ito, H.; Yoshida, T.; Tsuboi, S. *Tetrahedron* **2005**, *61*, 1909.
24. Dey, S.; Garner, P. *J. Org. Chem.* **2000**, *65*, 7697.
25. Witiak, D. T.; Patel, D. B.; Lin, Y. *J. Am. Chem. Soc.* **1967**, *89*, 1908.
26. Hagler, A. T.; Lifson, S.; Dauber, P. *J. Am. Chem. Soc.* **1979**, *101*, 5122.