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Bioorganic & Medicinal Chemistry Letters 17 (2007) 4456–4459
Bioorganic & Medicinal Chemistry Letters

Design, synthesis, and molecular modeling studies of 5′-deoxy-5′-ureidoadenosine: 5′-ureido group as multiple hydrogen bonding donor in the active site of *S*-adenosylhomocysteine hydrolase

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Received 16 April 2007; revised 19 May 2007; accepted 2 June 2007
Available online 8 June 2007

Abstract—5′-Deoxy-5′-ureidoadenosine was designed and synthesized as a potent inhibitor of *S*-adenosylhomocysteine hydrolase (SAH), in which 5′-ureido group acted as multiple hydrogen bonding donor in binding with active site residues of SAH in the molecular modeling study.

S-Adenosylhomocysteine hydrolase (SAH) is an enzyme catalyzing interconversion of *S*-adenosylhomocysteine into adenosine and L-homocysteine.[1–3] Inhibition of this enzyme accumulates *S*-adenosylhomocysteine intracellularly, resulting in feed-back inhibition of transmethylation by *S*-adenosylmethionine-dependent methyltransferase.[1–3] Since transmethylation is essential for *m*RNA capping of most animal infecting viruses, SAH has been a good therapeutic target for the development of broad-spectrum antiviral agents.[4,5]

Recently, we have reported the 5′-amino derivative **1** of fluoro-neplanocin A as a potent inhibitor of SAH, in which 5′-amino group acted as a good hydrogen bonding donor in the active site of SAH.[6] Thus, on the basis of these findings, we first designed the 5′-amino-5′-deoxyadenosine (**2**) and compared its SAH inhibitory activity with that of **1**. Then, in order to confirm the role of 5′-substituent as a hydrogen bonding donor in the active site of SAH, we designed the 5′-ureido derivative **3** to be capable of forming multiple hydrogen bonding. Herein, we report the synthesis, enzyme inhibitory activity, and molecular modeling studies of 5′-deoxy-5′-ureidoadenosine (**3**) (Fig. 1).

The key strategy to the synthesis of the desired nucleosides **2** and **3** was the conversion of the 5′-azido derivative to the 5′-amino- and 5′-ureido derivatives. Thus, we first synthesized the 5′-azido derivative, as shown in Scheme 1.

Synthesis began with commercially available N^6-benzoyladenosine (**4**). Treatment of **4** with 2,2-dimethoxypropane and acetone in the presence of catalytic amounts of sulfuric acid gave 2,3-isopropylidene derivative **5**. Compound **5** was treated with mesyl chloride followed by heating the resulting mesylate **6** with sodium azide in DMF yielded the 5′-azido derivative **7** in good yield. Removal of the isopropylidene group of **7** with aqueous trifluoroacetic acid gave diol **8**, which was treated with sodium methoxide to give 5′-azido-5′-deoxyadenosine (**9**).[7] Reduction of 5′-azido derivative **9** with Lindlar's catalyst afforded the 5′-amino derivative **2**.[7,8]

Conversion of the 5′-azido group to the 5′-ureido group is illustrated in Scheme 2. Catalytic hydrogenation of

Keywords: *S*-Adenosylhomocysteine hydrolase; 5′-Ureidoadenosine; Multiple hydrogen bonding donor.
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0960-894X/$ - see front matter © 2007 Published by Elsevier Ltd.
doi:10.1016/j.bmcl.2007.06.013

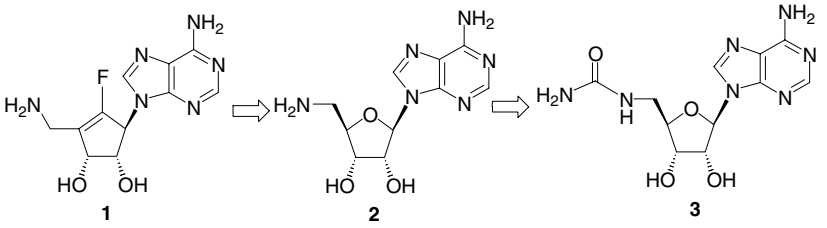
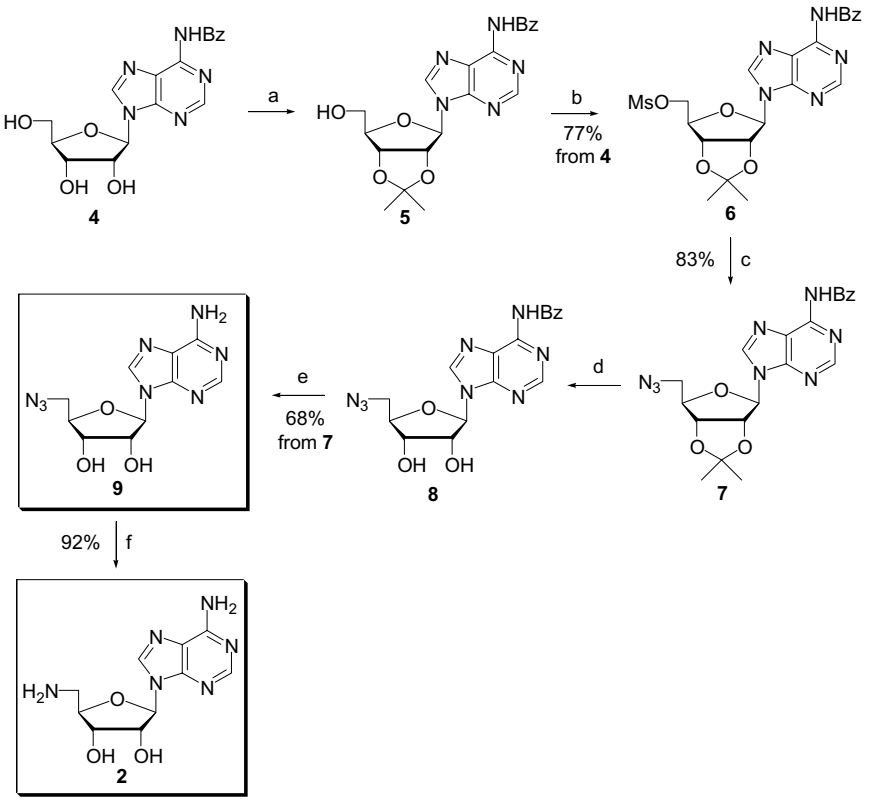
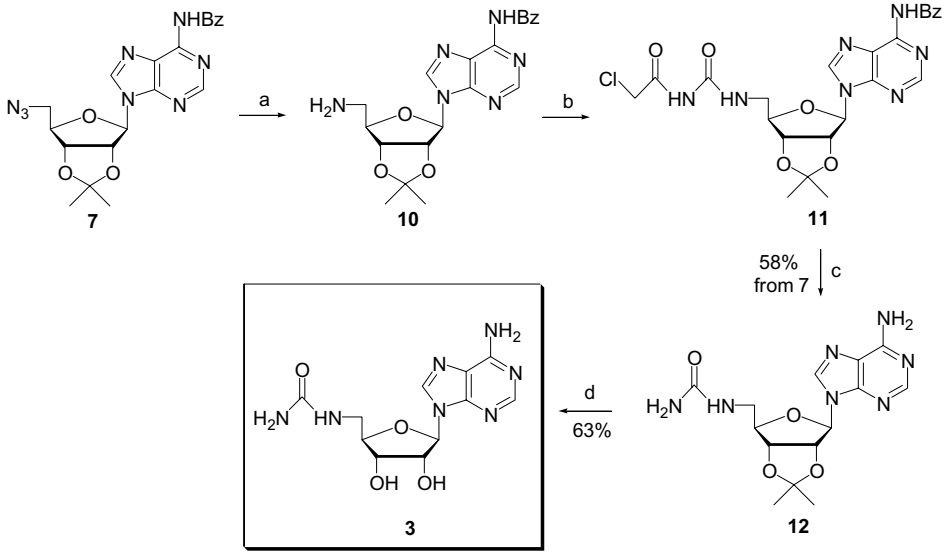


Figure 1. The rationale for the design of 5′-deoxy-5′-ureidoadenosine (**3**).

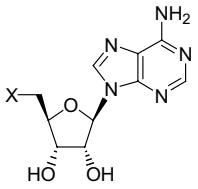


Scheme 1. Reagents and conditions: (a) 2,2-dimethoxypropane, acetone, H_2SO_4, rt, 30 min; (b) MsCl, pyridine, rt, 30 min; (c) NaN_3, DMF, 60 °C, 3 h; (d) 50% aq CF_3CO_2H, rt, 1 h; (e) NaOMe, MeOH, rt, 24 h; (f) Lindlar's catalyst, rt, 15 h.



Scheme 2. Reagents and conditions: (a) H_2, Pd/C, MeOH, rt, 24 h; (b) chloroacetyl isocyanate, DMF, 0 °C, 4 h; (c) NaOMe, MeOH, rt, overnight; (d) 50% aq CF_3CO_2H, rt, 1 h.

Table 1. SAH inhibitory activity of the final nucleosides **2**, **3**, and **9**



Compound	IC_{50}[a] (μM)
1	12.68[b]
2 (X = NH_2)	75.02
3 (X = $NHCONH_2$)	7.53
9 (X = N_3)	28.94

[a] Determined using pure recombinant human placental SAH obtained from E. coli JM109 containing the plasmid pPROKcd20.
[b] Data from Ref. 6.

the 5′-azido derivative **7** yielded 5′-amino derivative **10**, but it was gradually degraded. Thus, compound **10** was immediately treated with chloroacetyl isocyanate to give chloroacetyl urea derivative **11**. Treatment of **11** with sodium methoxide provided the 5′-ureido derivative **12** with concomitant removal of the benzoyl group.[9,10] Removal of the isopropylidene group in **12** with aqueous trifluoroacetic acid afforded the final nucleoside **3**.[8,11]

The SAH inhibitory activity of 5′-azido-, 5′-amino-, and 5′-ureido derivatives were measured using pure recombinant human placental SAH obtained from E. coli JM109 containing the plasmid pPROKcd20, as shown in Table 1.[12]

Compounds **2**, **3**, and **9** were preincubated with the enzyme SAH at various concentrations for 5 min at 37 °C and the residual activity of the enzyme was measured in the synthetic direction toward *S*-adenosylhomocysteine using adenosine and L-homocysteine. As shown in Table 1, 5′-amino-5′-deoxyadenosine (**2**) exhibited weak enzyme inhibitory activity (IC_{50} = 75.02 μM), indicating that the 5′-amino group is not enough to form strong hydrogen bonding in the active site of SAH. However, 5′-deoxy-5′-ureidoadenosine (**3**) showed more potent enzyme inhibitory activity (IC_{50} = 7.53 μM) than the 5′-amino derivative **1** (IC_{50} = 12.68 μM) or **2** (IC_{50} = 75.02 μM), indicating that the 5′-ureido moiety acted as more powerful and multiple hydrogen bonding donor than the 5′-amino moiety. Interestingly, the 5′-azido derivative **9** showed significant enzyme inhibitory activity (IC_{50} = 28.94 μM) and better inhibition than the 5′-amino derivative **2**, although 5′-azido group is not a hydrogen bonding donor, indicating that 5′-azido group might form significant binding in the active site due to its hydrogen bonding acceptor capability. These findings agreed with the previous report,[13] in which the 5′-azido derivative **9** showed better inhibition of SAH purified from hamster liver than the 5′-amino derivative **2**.

In order to examine the binding interactions of the synthesized compounds in the active site, flexible docking studies were performed with Surflex-Dock in SYBYL 7.3.3 (Tripos, Inc.) using a reported X-ray crystal structure of human SAH (i.e., 1LI4.pdb[14]).

Since X-ray co-crystal structure of human SAH with its ligand, neplanocin A, showed that neplanocin A was in its oxidized form at C3′ position which was enzymatically oxidized by the cofactor NAD^+, the synthesized ligands were modified to their oxidized forms and docked into SAH with the cofactor in its reduced, form NADH. The 5′-hydroxyl group of the original co-crystallized ligand, neplanocin A, showed a hydrogen bonding with the active site His301. Surflex-Dock well reproduced the active conformation of the crystal ligand. The 5′-amino group of compounds **1** and **2** was protonated and used for docking study to simulate its state in physiological conditions. The protonated 5′-amino group of docked compound **1** appeared to play as hydrogen bonding donors to active site residues such as His301, His55, and Asp131. In case of 5′-amino-5′-deoxyadenosine (**2**), the docking study indicated that its protonated 5′-amino group seemed to make smaller number of hydrogen bonds than **1**.

Surflex-Dock of 5′-deoxy-5′-ureidoadenosine (**3**) confirmed that its 5′-ureido group indeed played a role of multiple hydrogen bonding donor in its binding with active site residues of SAH as shown in Figure 2. The multiple hydrogen bondings of the 5′-ureido group with His301 and His55 would contribute to its strong binding to SAH, and that might cause its potent inhibitory activity. Compound **9**, although its 5′-azido group does not have a hydrogen bonding donor, showed a substantial level of inhibitory activity. According to the docking study, it appears to be because an azide nitrogen could

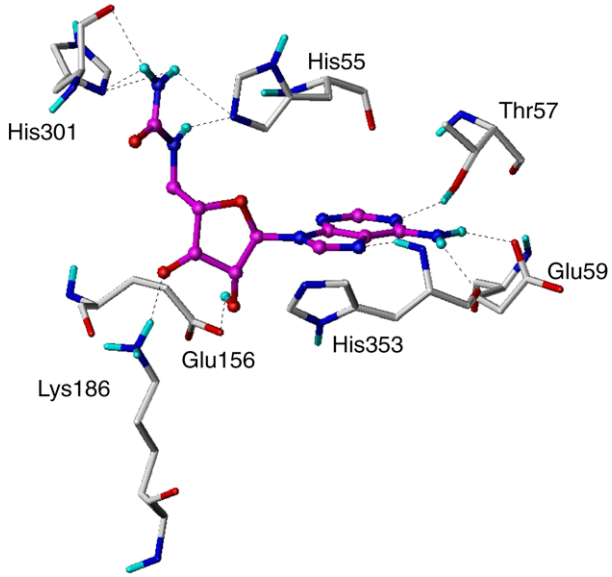


Figure 2. Interactions of 5′-deoxy-5′-ureidoadenosine (**3**) in the active site of SAH based on flexible docking. The Surflex-Docked ligand **3** is displayed in ball-and-stick whose carbon atoms are shown in magenta. The active site residues are displayed in capped-stick whose carbon atoms are in white. The non-polar hydrogen atoms are not displayed for clarity.

play a hydrogen bonding acceptor with the side-chain hydroxyl group of Thr157.

In conclusion, we have accomplished the synthesis of 5′-deoxy-5′-ureidoadenosine (**3**) as a potent SAH inhibitor. From this study, 5′-ureido moiety was discovered as effective multiple hydrogen bonding donor in the active site of SAH. We are sure that 5′-ureido moiety will be extensively utilized in designing enzyme inhibitors containing a hydrogen bonding donor as a pharmacophore feature.

Acknowledgments

This work was supported by grants from the Korea Research Foundation (KRF-2005-005-J01502) and the National Core Research Center (NCRC) program (No. R15-2006-020) of the Ministry of Science and Technology (MOST) and the Korea Science and Engineering Foundation (KOSEF) through the Center for Cell Signaling & Drug Discovery Research at Ewha Womans University.

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10. To a solution of compound **10** (0.24 g, 0.59 mmol) in anhydrous DMF (6 mL) was added chloroacetyl isocyanate (125.5 mL, 1.47 mmol) at 0 °C. After being stirred for 4 h at 0 °C, the reaction mixture was evaporated to give the crude product **11** (0.53 g) as a yellow syrup, which was directly used for next step. To a solution of **11** (0.53 g, 1.0 mmol) in methanol (10 mL) was added NaOMe (0.43 g, 8.01 mmol) at room temperature and stirred for overnight. The solvent was evaporated and the residue was purified by flash silica gel column chromatography (CH_2Cl_2/MeOH = 9:1) to give **12** (0.12 g, 58%, three steps) as a white solid: mp 199.2–202.8 °C; UV (MeOH) λ_{max} 258.5 nm; $[\alpha]_D^{18}$ −103.8; (*c* 0.50, MeOH); IR (KBr) 3433, 1637 cm^{-1}; ^1H NMR (400 MHz, CD_3OD) δ 8.26 (d, 2H, J = 6.0 Hz), 6.14 (d, 1H, J = 2.8 Hz), 5.43–5.45 (dd, 1H, J = 3.2 Hz, 6.4 Hz), 4.98–5.0 (dd, 1H, J = 3.2 Hz, J = 6.4 Hz), 4.27–4.30 (m, 1H), 3.40–3.43 (m, 2H), 1.59 (s, 3H), 1.37 (s, 3H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 158.5, 156.1, 152.7, 148.8, 139.8, 119.1, 113.4, 88.8, 84.9, 82.9, 81.6, 41.3, 26.9, 25.2.
11. A solution of compound **12** (0.12 g, 0.34 mmol) in 50% aqueous trifluoroacetic acid was stirred at room temperature for 1 h. After completion of reaction, the reaction mixture was evaporated under reduced pressure and co-evaporated with toluene. The residue was purified on Dowex 50WX8-200 (H^+) resin column to give the final nucleoside **3** (0.066 g, 63%), which was recrystallized from methonal: mp 251.3–252.2°C; UV (MeOH) λ_{max} 285.5 nm; $[\alpha]_D^{18}$ −27.2 (*c* 0.18, DMSO); IR (KBr): 3444, 1637 cm^{-1}; ^1H NMR (400 MHz, DMSO-d_6) δ 8.34 (s, 1H), 8.18 (s, 1H), 7.29 (s, 2H), 6.23 (t, 1H, J = 5.6 Hz,), 5.85 (d, 1H, J = 6.4 Hz), 5.49 (s, 2H), 5.42 (d, 1H, J = 6.4 Hz), 5.22 (d, 1H, J = 4.4 Hz), 4.63–4.68 (dd, 1H, J = 6.4 Hz, 11.6 Hz), 4.04–4.09 (m, 1H), 3.87–3.91 (m, 1H), 3.35–3.39 (m, 1H), 3.19–3.26 (m, 1H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 159.3, 156.1, 153.1, 149.7, 140.4, 119.4, 87.6, 84.4, 73.0, 71.3, 41.8. Anal. Calcd for $C_{11}H_{15}N_7O_4$: C, 42.72; H, 4.89; N, 31.70. Found: C, 42.70; H, 4.49; N, 31.41.
12. The enzyme (SAH) was incubated with 0.2 mM of adenosine and 5 mM of L-homocysteine in 50 mM potassium phosphate buffer (pH 7.2) (500 μL) containing 1 mM EDTA at 37 °C for 5 min. The reaction was terminated by the addition of 25 μL of 5 N $HClO_4$. The terminated reaction mixture was kept in ice for 5 min and microcentrifuged. The supernatant was analyzed for *S*-adenosylhomocysteine by HPLC equipped with C-18 reversed-phase column (Econosphere C18, 5 μm, 250 × 4.6 mm, Alltech, Deerfield, IL). The elution was carried out at a flow rate of 1 mL/min in two sequential linear gradients: 6–15% A over B for 15 min, 15–50% A over B for later 5 min, where mobile phase A was acetonitrile and B was 50 mM sodium phosphate buffer (NaH_2PO_4/H_3PO_4), pH 3.2 (HPLC buffer B). The peak of *S*-adenosylhomocysteine was monitored at 258 nm. The concentration was determined by the peak area of *S*-adenosylhomocysteine (Jeong, L. S.; Yoo, S. J.; Lee, K. M.; Koo, M. J.; Choi, W. J.; Kim, H. O.; Moon, H. R.; Lee, M. Y.; Park, J. G.; Lee, S. K.; Chun, M. W. *J. Med. Chem.* **2003**, *46*, 201).
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