Design and Synthesis of Amide Derivatives as S-Adenosyl-l-Homocysteine Hydrolase Inhibitors

Xiangduan Tan, Siyun Nian, and Guoping Wang

In this study, a series of amide derivatives were synthesized and evaluated for their S-adenosyl-l-homocysteine hydrolase (SAHase) inhibitory activities. The results demonstrated that most of compounds displayed potent SAHase inhibitory activities. Interestingly, compounds 11 and 14 exhibited more potent inhibitory effects than the aristeromycin, one of the most potent SAHase inhibitors known so far. Compounds 12, 13, 15 and 17 exhibited a moderate effect (IC_{50}<10.0 \mu M). The structure–activity relationship found that compounds with substituted indazole-5-yl group at Ar position and ethylamino group at the side chain showed better SAHase inhibitory activities.

Key words S-adenosyl-l-homocysteine hydrolase; S-adenosylhomocysteine; SAHase inhibitor; homocysteine; amide derivative

S-Adenosyl-l-homocysteine hydrolase (SAHase) can catalyze the reversible hydrolysis of S-adenosylhomocysteine (SAH) to adenosine (ADO) and l-homocysteine (Hcy). Transmethylation reactions are involved in various biological phenomena related to the development of pathological processes. S-Adenosyl-l-methionine (AdoMet) seems to be the most versatile methyl donor in mammalian systems. The formation of SAH from AdoMet, and the inhibition of cellular SAHase results in an intracellular accumulation of SAH, which leads to feedback inhibition of AdoMet dependent methylations.

In recent years, SAHase has become an attractive target for drug design, SAHase inhibitors have been shown to exhibit antiviral, antiparasitic, anti-cancer, and immunosuppressive effect. SAHase inhibitors have also been shown to exhibit plasma homocysteine-lowering effect. Numerous SAHase inhibitors have been extensively reported in literature, and all the existing SAHase inhibitors can be divided into three types according to the mechanisms of enzyme inhibition. Most of SAHase inhibitors were type I or type II inhibitor which were irreversible inhibitors, including aristeromycin, neplanocin A and 3-deazaadenosine (3-DZA) (Fig. 1). Type III inhibitors can reversibly bind to the open form of the enzyme, maintaining a similar potency with much reduced toxicity, such as DZ2002 (Fig. 1). However, most of the existing SAHase inhibitors are adenosine analogues, and most of the work previously reported on inhibitors of this enzyme has focused on systematically altering the structure of adenosine and evaluating the derivatives. There have been very few studies on new structure as SAHase inhibitors.

Recent studies found the N-(carbamoylmethyl)glycinamide derivatives exhibited SAHase inhibitory activities. In terms of the structure of these compounds, we chose the different substituted phenylazanediyl diacetic acid derivatives as the core scaffold, which firstly reacted with N-methyisoindolin-2-amino hydrochloride to obtain monoacid derivatives, and then acylation reaction with ethylenediamine derivatives to configure the side chain of this moiety. Accordingly, a novel class of amide derivatives (11–25) was synthesized and their SAHase inhibitory activity were estimated. These compounds were also designed to examine the role of different substitutes at aryl (Ar) position of phenylazanediyl diacetic acid and different substitutes in the side chain. It is hoped that continued research will lead to the development of new lead compounds from N-(carbamoylmethyl)glycinamide derivatives as effective SAHase inhibitors.

Results and Discussion

Chemistry The general method for the synthesis of the amide derivatives 11–25 was described in Chart 1. Bromination of the compound 1 with N-bromosuccinimide (NBS) gave intermediate 2 in 80% yield. Treatment of 2 with N-tert-butoxycarbonyl-N-methylhydrazine to afford compound 3 in 75% yield. Finally, the deprotection of tert-butoxycarbonyl (Boc) group on amino group by hydrolysis with concentrated

Fig. 1. Chemical Structures of Some of the SAHase Inhibitors

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* To whom correspondence should be addressed. e-mail: wgpsipich@163.com © 2014 The Pharmaceutical Society of Japan
hydrochloric acid was carried out at room temperature then transformed to compound 4 as hydrochloride salt in 70% yield. The corresponding aromatic amines 5a–e were firstly alkylated with ethyl bromoacetate under the basic condition to give compounds 6a–e, which were hydrolyzed with potassium hydroxide in ethanol afforded the desired substituted phenylazanediyl diacetic acids 7a–e. The intermediates 8a–e were obtained by dehydration of the substituted phenylazanediyl diacetic acids 7a–e in the presence of acetic anhydride at 90°C. Treatment of 8a–e with 4 under the basic condition to give monacid derivatives 9a–e. The amide derivatives 11–25 were prepared by condensation of monacid derivatives 9a–e and various ethylenediamine derivatives 10 with 1-ethyl-(3-(3-dimethylamino)propyl)-carboximide hydrochloride (EDCI) as condensation agent and 1-hydroxybenzotriazole (HOBt) as catalyst in N,N-dimethylformamide (DMF) in good yield. The chemical structures of all the synthesized compounds were characterized by 1H-NMR, MS and elemental analysis (C, H, N).

**Biological Activity** All the newly synthesized compounds, along with the reference compounds aristeromycin and 3-DZA, were screened for their SAHase inhibitory activities using the method which described in literature. The results, expressed as inhibition ration and IC₅₀ values, were summarized in Table 1.

As shown in Table 1, most of the compounds displayed potent SAHase inhibitory activities. Five compounds (11, 12, 14, 15, 17) displayed more potent SAHase inhibitory activities than the reference inhibitor 3-DZA (IC₅₀ = 5.52 µM). The most promising compound 11 (IC₅₀ = 0.48 µM) and 14 (IC₅₀ = 0.44 µM) exhibited more potent SAHase inhibition effects than the reference inhibitor aristeromycin (IC₅₀ = 0.49 µM), one of the most potent SAHase inhibitors known so far. Meanwhile, compounds 13, 18 and 19 also exhibited SAHase inhibitory activities in some extent.

**Chart 1. Synthetic Protocol of the Amide Derivatives 11–25**

Reagents and conditions: (a) NBS, benzoyl peroxide, CHCl₃, reflux, 5h, 80%; (b) NH₂–N(CH₃)Boc, Et₃N, DMF, rt, 75%; (c) conc. HCl, 70%; (d) BrCH₂CO₂Et, Na₂HPO₄, NaI, CH₃CN, reflux, 12h; (e) KOH, EtOH then 2N HCl, 35–45% in 2 steps; (f) Ac₂O, 90°C, 3h; (g) Et₃N, THF, rt; (h) EDCI, HOBt, DMF, Et₃N, rt, 34–47% in 3 steps.
Table 1. The SAHase Inhibitory Activity of 11–25

| Compound | IC_{50} (µM) | 0.5 µM | 2.5 µM | Inhibition ration (%) |
|----------|-------------|--------|--------|----------------------|
| 11       | 0.48        | 62.31±1.48 | 85.72±1.69 |
| 12       | 2.03        | 24.24±1.78 | 54.24±1.12 |
| 13       | 7.61        | 9.53±1.70 | 30.19±3.02 |
| 14       | 0.44        | 69.58±0.72 | 86.56±1.10 |
| 15       | 2.13        | 17.24±3.30 | 38.24±1.46 |
| 16       | 25.34       | 15.97±2.35 | 27.15±1.24 |
| 17       | 0.73        | 49.72±0.20 | 79.46±2.83 |
| 18       | 12.12       | 10.92±0.91 | 21.46±3.38 |
| 19       | 14.08       | 6.41±0.23  | 20.16±0.84 |
| 20       | 78.82       | 4.23±0.14  | 8.23±1.02  |
| 21       | >500        | 0±0.32     | 2.63±0.41  |
| 22       | >500        | 0±0.96     | 4.56±0.77  |
| 23       | 15.60       | 8.23±0.92  | 19.23±1.35 |
| 24       | 101.9       | 4.56±0.45  | 6.97±0.87  |
| 25       | 214         | 0±0.43     | 4.89±0.45  |
| Aristeromycin | 0.49^{a} | 83.93±0.90 | 98.35±0.16 |
| 3-DZA    | 5.52^{a}    | 2.16±0.47  | 35.37±3.32 |

\(^{a)} Values were determined from logarithmic concentration–inhibition curves and are given as means of three experiments. \(^{b)} Values in the literature is 0.2 µM, the SAHase from Mycobacterium tuberculosis. \(^{c)} Values in the literature is 20 µM, the SAHase from Mycobacterium tuberculosis.

SAHase inhibition potency was observed. However, when the 2-methyl-2H-indazole-5-yl group of compound 14 was replaced with 4-methylphenyl group as in compound 23, a significant decrease in SAHase inhibition potency was observed. Unfortunately replacement of 2-methyl-2H-indazole-5-yl group of compound 14 with 4-fluorophenyl to afford corresponding compounds 20–22 resulted in a loss of activity, and 4-methylphenyl derivatives 24 and 25 were also devoid of SAHase inhibitory activities. These facts imply that the introduction of substituted indazole-5-yl group on the Ar region obviously affected the SAHase inhibitory activity, and in the present investigation, 4-(1H-pyrrol-1-yl) phenyl group facilitated their inhibitory activities, while 4-fluorophenyl group and 4-methylphenyl group were unfavorable.

Substituted ethylenediamine structure at the side chain also affects the SAHase inhibitory activity. Compounds 11, 14, 17, 20 and 23 bearing ethylamino group at side chain exhibited highest influence on SAHase inhibitory activity, followed by the corresponding compounds with piperidinyl group at side chain (12, 15, 18, 21, 24). However, the corresponding compounds with diethylamino group at side chain (13, 16, 19, 22, 25) displayed lower SAHase inhibition potency. These results indicate that the ethylenyloxy group is considered to be the best substituent at side chain showing potent SAHase inhibitory activity.

Conclusion

In summary, a novel class of amide derivatives were designed, synthesized and evaluated as SAHase inhibitors. The results demonstrated that most of target compounds displayed potent SAHase inhibitory activities. Particularly, compounds 11 and 14 exhibited more potent inhibitory effects than the aristomycin and 3-DZA. The compounds with 1-methyl-1H-indazole-5-yl group or 2-methyl-2H-indazole-5-yl group at Ar position and ethylamino group at side chain showed better SAHase inhibitory activities. These results showed above are very encouraging, and further investigation of this kind of compounds may be of interest.

Experimental

Chemistry Melting points were determined on WRS-21 melting point apparatus and were uncorrected. \(^{1}H\)-NMR spectra were recorded on INOVA 400 (400 MHz) spectrometer with tetramethylsilane (TMS) as an internal standard. Chemical shifts (δ) are in ppm relative to TMS, and coupling constants (J) are expressed in hertz (Hz). Electron-spray ionization mass spectra (ESI-MS) in positive mode were recorded on a HP5989A mass spectrometer. Column chromatography was performed on 200–300 mesh silica gel. Analytical thin layer chromatography (TLC) was performed on precoated silica gel 60 F254 plates and visualization on TLC was achieved by UV light (254, 354 nm). Unless otherwise stated, all commercial reagents and solvents were used without additional purification.

1,2-Bis(bromomethyl)benzene (2) A mixture of compound 1 (40 g, 0.38 mol), NBS (140.8 g, 0.79 mol), benzoyl peroxide (0.91 g, 3.8 mmol) in CHCl\(_3\) (400 mL) was heated for 5 h under reflux. The reaction mixture was cooled to room temperature and CH\(_2\)Cl\(_2\) (400 mL) was added to the mixture. The organic layer was washed with water (2×200 mL) and dried over Na\(_2\)SO\(_4\) filtered, and the solvent were removed in vacuo. The residue was recrystallized from n-hexane–ethanol (30:1, 620 mL) to give compound 2 (79.0 g, 80%) as white solid, mp 92–93°C. \(^{1}H\)-NMR (400 MHz, CDCl\(_3\)) δ: 4.66 (4H, 4H), 7.29–7.38 (4H, m).

tert-Butyl Isoindolin-2-yl(methyl) Carbamate (3) A mixture of compound 2 (59.7 g, 0.22 mol), tert-butyl methylcarbamate (34.7 g, 0.23 mol) and triethylamine (44.5 g, 0.44 mol) in DMF (300 mL) was heated for 2 h at 65°C. The reaction mixture was cooled to room temperature and water (400 mL) was added to the mixture, the aqueous phase was extracted with ethyl acetate (3×300 mL). The combined organic layer was washed with brine and dried over Na\(_2\)SO\(_4\) filtered, and the solvent were removed in vacuo to get compound 3 (35.5 g, 65.0%) as white solid, mp 62–64°C. \(^{1}H\)-NMR (400 MHz, DMSO-d\(_6\)) δ: 1.35 (9H, s), 2.99 (3H, s), 4.33 (4H, s), 7.20–7.22 (4H, m). ESI-MS m/z: 249.16 [M+H]\(^+\)².

N-Methylisoindolin-2-amine Hydrochloride (4) Compound 3 (27.5 g, 0.11 mol) was dissolved in concentrated hydrochloric acid (83 mL), and stirred at room temperature for 12 h. The reaction mixture was evaporated under reduced pressure and co-evaporated with ethanol (1000 mL). The residue was recrystallized from ethanol (100 mL) to give compound 4 (14.2 g, 70%) as white solid, mp 160–162°C. \(^{1}H\)-NMR (400 MHz, DMSO-d\(_6\)) δ: 2.88 (3H, s), 4.59 (4H, s), 7.41–7.42 (4H, m). ESI-MS m/z: 149.11 [M+H]\(^+\)².

General Procedure for the Synthesis of Substituted Phenylazanediyl Diacetic Acids (7a–e) A mixture of aromatic amines 5a–e (0.1 mol), ethyl 2-bromoacetate (0.21 mol), Na\(_2\)HPO\(_4\) (0.25 mol) and NaI (0.05 mol) in CH\(_2\)CN (250 mL) was heated for 12 h under reflux. The reaction mixture was cooled to room temperature and concentrated in vacuo, and the water was added to the residue, the aqueous phase was extracted with ethyl acetate. The combined organic layer was washed with brine and dried over Na\(_2\)SO\(_4\) filtered, and the solvent were removed in vacuo to obtain 6a–e. The solution of KOH (0.25 mol) in ethanol (500 mL) was added to the so-
Yield 35% (2 steps), white solid, mp 156–158°C. 1H-NMR (400 MHz, DMSO-\text{d}_6): δ: 3.95 (3H, s), 4.14 (4H, s), 6.87–6.90 (1H, m), 7.47 (1H, d, J=9.2 Hz), 7.81 (1H, s). ESI-MS \text{m/z:} 264.12 [M+H]^+.

2.2′-(1-Methyl-1H-indazol-5-yl)azanediydiacetic Acid (7a): Yield 40% (2 steps), white solid, mp 172–174°C. 1H-NMR (400 MHz, DMSO-\text{d}_6): δ: 3.91 (3H, s), 4.12 (4H, s), 6.70 (1H, s), 6.85–6.88 (1H, m), 7.42–7.45 (1H, d, J=9.2 Hz), 7.79 (1H, s). ESI-MS \text{m/z:} 264.16 [M+H]^+.

2.2′-(1-(4H-Pyrrol-1-yl)phenyl)azanediydiacetic Acid (7c): Yield 30% (2 steps), brown solid, mp 150–152°C. 1H-NMR (400 MHz DMSO-\text{d}_6+D_2O) δ: 4.13 (4H, s), 6.17–6.20 (2H, m), 6.59–6.66 (2H, m), 7.10–7.26 (2H, m), 7.33 (2H, d, J=8.8 Hz). ESI-MS \text{m/z:} 275.12 [M+H]^+.

2.2′-(4-Fluorophenyl)azanediydiacetic Acid (7d): Yield 42% (2 steps), white solid, mp 192–194°C. 1H-NMR (400 MHz, CDCl_3) δ: 4.12 (4H, s), 6.55–6.57 (2H, m), 6.96–7.01 (2H, m). ESI-MS \text{m/z:} 228.06 [M+H]^+.

2.2′-(p-Tolylazanediydiacetic Acid (7e): Yield 45% (2 steps), white solid, mp 202–204°C. 1H-NMR (400 MHz, DMSO-\text{d}_6) δ: 2.16 (3H, s), 3.99 (4H, s), 6.37 (2H, d, J=8.8 Hz), 6.97 (2H, d, J=8.8 Hz). ESI-MS \text{m/z:} 224.10 [M+H]^+.

General Procedure for the Synthesis of Amidic Deriva-
tives (11–25) A mixture of substituted phenylazenediy-
diacetic acids 7a-e (3.8 mmol) and acetic anhydride (20 mL) was heated to 90°C for 3h, and the reaction mixture was concentrated in vacuo to get intermediates 8a-e. A solution of triethylamine (9.5 mmol) in tetrahydrofuran (THF) (20 mL) was added to the corresponding intermediates 8a-e, the mixture was stirred at room temperature for 10 min. Compound 4 (3.8 mmol) was added in three portions and the resulting mixture was stirred at room temperature for 12h. The solution was concentrated and water was added to the residue. The mixture was extracted with ethyl acetate-THF (10:1) and the combined organic layer was dried over Na_2SO_4, filtered, then evaporated to remove solvent to obtain corresponding monacid derivatives 9a-e. A stirred solution of 9a-e in DMF (20 mL) was added triethylamine (9.5 mmol), EDCl (7.6 mmol) and HOBr (7.6 mmol). After the addition was completed, the reaction mixture was stirred for 10 min at room temperature. A solution of corresponding ethylenediamine derivatives 10 (4.56 mmol) was added to the reaction mixture, and the resulting mixture was stirred at room temperature for 12h. The reaction mixture was poured into water. The pH of the sus-

2.2′-(1-(4H-Pyrrol-1-yl)phenyl)ethyl)amino)-2-oxoethyl)

Yield 40% (3 steps), white solid, mp 155–157°C. 1H-NMR (400 MHz, DMSO-\text{d}_6) δ: 1.25–1.37 (6H, m), 2.41–2.51 (6H, m), 2.98 (3H, s), 3.25 (2H, t, J=6.4 Hz), 3.95–3.98 (5H, m), 4.33 (4H, s), 4.61 (2H, s), 6.61 (1H, s), 6.73–6.76 (1H, m), 7.24–7.45 (5H, m), 7.81 (1H, s), 8.85 (1H, brs). ESI-MS \text{m/z:} 464.30 [M+H]^+. Anal. Calcd for C_{24}H_{23}N_{7}O_{2}: C, 64.77; H, 7.24; N, 19.75. Found: C, 64.69; H, 7.50; N, 19.18.

2-(2-(Diethylamino)ethyl)amino)-2-oxoethyl)-

Yield 35% (3 steps), white solid, mp 131–132°C. 1H-NMR (400 MHz, DMSO-\text{d}_6) δ: 0.97 (3H, t, J=7.2 Hz), 2.68–2.78 (4H, m), 2.90 (3H, s), 3.27–3.29 (2H, m), 3.94 (3H, s), 4.00 (2H, s), 4.29 (4H, s), 4.58 (2H, s), 6.61 (1H, s), 6.74–6.77 (1H, m), 7.22–7.43 (5H, m), 7.78 (1H, s), 8.98 (1H, brs). ESI-MS \text{m/z:} 492.32 [M+H]^+. Anal. Calcd for C_{27}H_{32}N_{7}O_{2}: C, 65.96; H, 7.59; N, 19.94. Found: C, 66.04; H, 7.50; N, 19.87.

2-(2-(Diethylamino)ethyl)amino)-2-oxoethyl)-

Yield 40% (3 steps), white solid, mp 155–157°C. 1H-NMR (400 MHz, DMSO-\text{d}_6) δ: 1.25–1.37 (6H, m), 2.41–2.51 (6H, m), 2.98 (3H, s), 3.25 (2H, t, J=6.4 Hz), 3.95–3.98 (5H, m), 4.33 (4H, s), 4.61 (2H, s), 6.61 (1H, s), 6.73–6.76 (1H, m), 7.24–7.45 (5H, m), 7.81 (1H, s), 8.85 (1H, brs). ESI-MS \text{m/z:} 464.30 [M+H]^+. Anal. Calcd for C_{24}H_{23}N_{7}O_{2}: C, 64.77; H, 7.24; N, 19.75. Found: C, 64.69; H, 7.50; N, 19.18.

N-(Isoindolin-2-yl)-N-methyl-2-((1-methyl-1H-indazol-5-

Yield 35% (3 steps), white solid, mp 133–135°C. 1H-NMR (400 MHz, DMSO-\text{d}_6) δ: 1.09 (3H, t, J=7.2 Hz), 2.79–2.87 (4H, m), 2.95 (3H, s), 3.20 (2H, t, J=6.4 Hz), 3.99 (2H, s), 4.34 (4H, s), 4.63 (2H, s), 6.18–6.19 (2H, m), 6.50 (2H, d, J=9.2 Hz), 7.11–7.13 (2H, m), 7.25–7.39
124–126°C. 1H-NMR (400 MHz, DMSO-\(d_6\)): \(\delta\) = 1.40–1.45 (6H, m), 2.49–2.51 (6H, m), 2.98 (3H, s), 3.98 (2H, s), 4.36 (4H, s), 4.65 (2H, s), 6.15–6.18 (2H, m), 6.48–6.51 (3 steps), white solid, mp 162–163°C. 1H-NMR (400 MHz, DMSO-\(d_6\)): \(\delta\) = 1.33–1.46 (3H, m), 2.42–2.50 (6H, m), 2.95 (3H, s), 3.20–3.28 (2H, m), 3.94 (2H, s), 4.33 (4H, s), 4.57 (2H, s), 6.38–6.42 (2H, m), 6.97–6.99 (2H, m), 7.25–7.30 (4H, m), 8.90 (1H, brs). ESI-MS m/z: 503.32 [M+H]+. Anal. Calcd for C27H34N6O2: C, 69.29; H, 7.62; N, 16.72. Found: C, 69.34; H, 7.66; N, 16.63.

2-((4-(1H-Pyrrol-1-yl)phenyl)(2-oxo-2-((2-(piperidin-1-yl)ethyl)amino)ethyl)-amino)-N-(isoindolin-2-yl)-N-methylacetamide (19): Yield 44% (3 steps), white solid, mp 70–72°C. 1H-NMR (400 MHz, DMSO-\(d_6\)): \(\delta\) = 1.07 (3H, t, \(J = 7.2\) Hz), 2.70–2.77 (4H, m), 2.95 (3H, s), 3.27–3.30 (2H, m), 3.94 (2H, s), 4.34 (4H, s), 4.61 (2H, s), 6.18–6.19 (2H, m), 6.48–6.51 (2H, m), 7.12–7.14 (2H, m), 7.28–7.32 (6H, m), 8.98 (1H, brs). ESI-MS m/z: 503.32 [M+H]+. Anal. Calcd for C27H34N6O2: C, 66.72; H, 7.62; N, 16.63.
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