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Discovery and structural analyses of *S*-adenosyl-L-homocysteine hydrolase inhibitors based on non-adenosine analogs

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ABSTRACT

Optimization of a new series of *S*-adenosyl-L-homocysteine hydrolase (AdoHcyase) inhibitors based on non-adenosine analogs led to very potent compounds **14n**, **18a**, and **18b** with IC_{50} values of 13 ± 3, 5.0 ± 2.0, and 8.5 ± 3.1 nM, respectively. An X-ray crystal structure of AdoHcyase with NAD^+ and **18a** showed a novel open form co-crystal structure. **18a** in the co-crystals formed intramolecular eight membered ring hydrogen bond formations. A single crystal X-ray structure of **14n** also showed an intramolecular eight-membered ring hydrogen bond interaction.

1. Introduction

Homocysteine (Hcy) is a sulfur-containing amino acid, which is an intermediate metabolite of an essential amino acid, methionine (Met). Met is condensed with ATP to form *S*-adenosyl-L-methionine (AdoMet), and AdoMet utilized as a methyl donor for methylation reactions is converted to *S*-adenosyl-L-homocysteine (AdoHcy). AdoHcy is hydrolyzed to Hcy and adenosine. There are two metabolic pathways of Hcy: (i) remethylation through methionine synthase or betaine–homocysteine methyltransferase (BHMT) into Met, and (ii) degradation to cysteine thorough cystathionine beta-synthase (CBS) and cystathionine γ-lyase (CTH).[1] Intracellular Hcy is highly regulated at low levels and redundant Hcy is released into the blood (see Fig. 1).

In the late 1960s, McCully reported that Hcy caused vascular pathology such as arteriosclerosis and myocardial infarction.[2] A meta-analysis verified a 25% lower usual Hcy level (3 μM in absolute level) was associated with about an 11% lower ischemic heart disease and about 19% lower stroke risk.[3] Elevated plasma Hcy levels are now recognized as independent risk factors for atherothrombotic diseases such as stroke. In addition, there is evidence that elevated plasma Hcy levels associate with other neuropsychiatric disorders.[4–6] Lowering Hcy to normal levels therefore might be one of the possible approaches to prevent and treat diseases such as coronary artery disease, ischemic stroke, and neuropsychiatric diseases.

One of the strategies to lower the Hcy levels is the inhibition of the Hcy synthetic enzyme, *S*-adenosyl-L-homocysteine hydrolase (AdoHcyase; EC 3.3.1.1.).[7] Almost all of the known AdoHcyase inhibitors are adenosine analogs.[8–10] Some of them inhibit the enzyme irreversibly, and many of them lack selectivity against related enzymes for producing adenosine, suggesting that there could remain concerns about adverse side effects.[8] A reversible inhibitor **4** has only a weak potency for the enzyme (K_i ~10^{-6} M) (see Fig. 2).[8]

We hypothesize that reversible, competitive AdoHcyase inhibitors based on non-adenosine analogs can provide some distinct advantages in terms of selectivity and toxicity. In our previous paper, we reported that a high throughput screening using Automated Ligand Identification System (ALIS) resulted in discovery of a new series of *S*-adenosyl-L-homocysteine hydrolase inhibitors based on non-adenosine analogs.[11,12] This paper describes detailed optimization strategies for lead discovery reported in the previous paper and an SAR study with X-ray crystal structural analyses, which elucidate a novel open form co-crystal structure and intramolecular eight-membered ring hydrogen bond formations.

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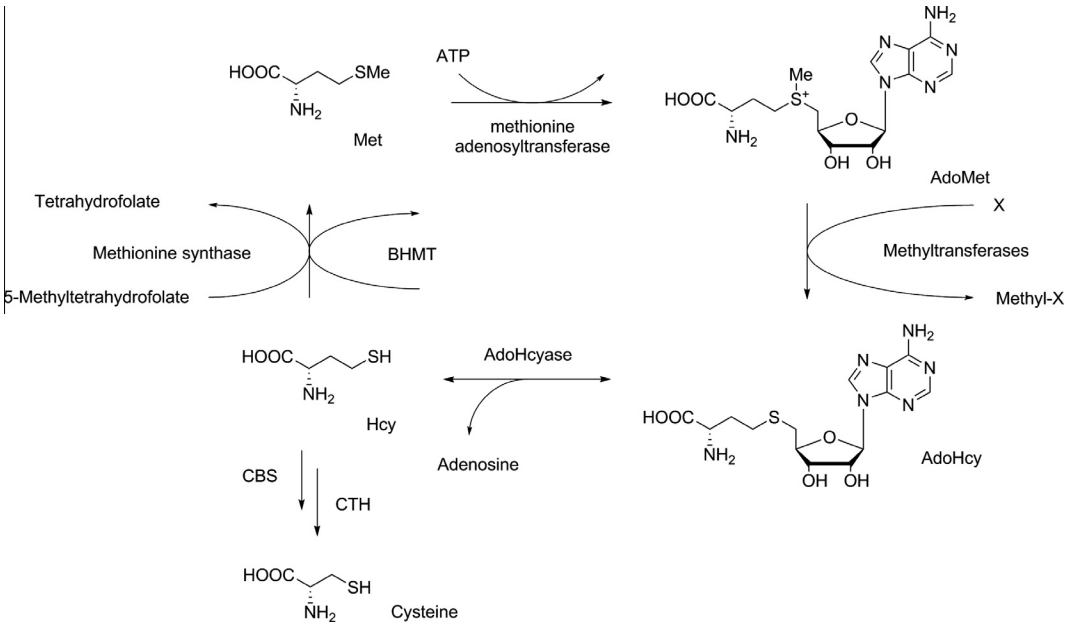


Figure 1. Homocysteine metabolism.

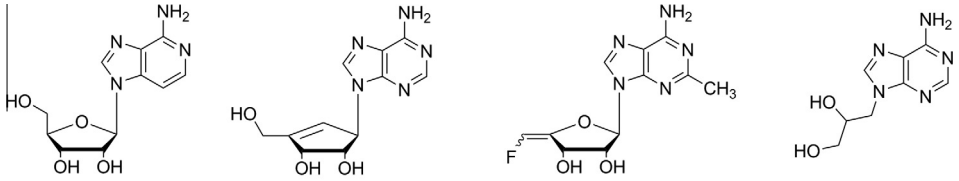


Figure 2. Known AdoHcyase inhibitors based on adenosine analogs.

The high throughput screening using ALIS led to a series of several lead candidate molecules (**5a**–**f**) illustrated in Figure 3. The structural features of the compounds are that they contain two amides: a hydrophobic amide and a hydrophilic amide with an amine group. **5a**, **5b**, and **5c** contained a methyl substituent at the hydrophobic amide nitrogen, and they were more potent than **5e** and **5f**, which did not have *N*-methyl groups in the hydrophobic amide parts.

In general, secondary amides present planar *trans*-peptide formations rather than *cis*-peptide formations, and the isomerization is restricted because of their partial double bond character.[13] Once N-alkylations of the secondary amide nitrogen atoms occurred, an energy barrier between *cis* and *trans* configurations is lowered and the resulting tertiary amides can rotate to form less sterically-hindered or electrochemically stable structures.[14] In the case of compound **5f**, its hydrophobic amide is presumed to form *trans*-amide conformation. We hypothesized that active conformations of **5** with AdoHcyase are *cis*-amide forms, and N-alkylation of less potent compound **5f** could easily access to *cis*-amide structure, resulting in stronger inhibitory activity against AdoHcyase (see Fig. 4). At the beginning of the optimization campaign, we addressed the replacement of the hydrophobic amide in **5** with various *N*,*N*′-substituted amides, including the amide of compound **14e**.

2. Chemistry

The synthesis of intermediate **10** was shown in Scheme 1. Reaction of 1,4-dichloro-2-nitrobenzene (**6**) with 4-chlorophenol in the presence of NaH in DMF provided biphenylether **7**. Reduction of the nitro group of **7** was performed by using hydrazine hydrate as hydrogen sources with a catalytic amount of $FeCl_3$ and charcoal activated in MeOH under reflux conditions. Dialkylation of **8** with ethyl bromoacetate in *N*,*N*-diisopropylethylamine gave **9**, and hydrolysis of **9** with aqueous NaOH in MeOH and THF produced **10**.

Compounds **14a**–**i**, and **14n**–**p** were synthesized from **10** in one-pot reactions described in Scheme 2. Treatment of **10** with EDC in DMF followed by addition of various amines **12a**–**i** and **12n**–**p**, and subsequent addition of 2-(1-pyrrolidinyl)ethylamine, HOBt, and EDC gave **14a**–**i**, and **14n**–**p**, respectively. Deprotection of *N*-Boc in **14i** led to **14j**, and acylation with acetic anhydride, sulfonylation with methylsulfonyl chloride, and carbamation with methyl chloroformate resulted in **14k**, **14l**, and **14m**, respectively. Treatment of **14p** with *p*-toluenethiol in the present of Cs_2CO_3 in MeCN gave **14q**, which was converted into **14r** and **14s** in a similar way to that used in the synthesis of **14l** and **14m**, respectively.

Compounds **16a**–**d** and **16m**–**t** were synthesized from **10** through the intermediate **13e** described in Scheme 3. Treatment of **10** with EDC in DMF followed by addition of **12a** with DIPEA gave 1**3e** as a colorless solid. Reaction of **13e** with **15a**–**l** gave **16a**–**l**, respectively. Deprotection of *N*-Boc in **16e**–**l** led to **16m**–**t**, respectively.

Compounds **18a** and **18b** were synthesized as described in Scheme 4. Treatment of **10** with EDC in DMF followed by addition of **12n** with DIPEA, and subsequent addition of **15e** and **15f** gave **17a** and **17b**, respectively. Deprotection of *N*-Boc in **17a** and **17b**, followed by their salt formation gave **18a** and **18b**, respectively.

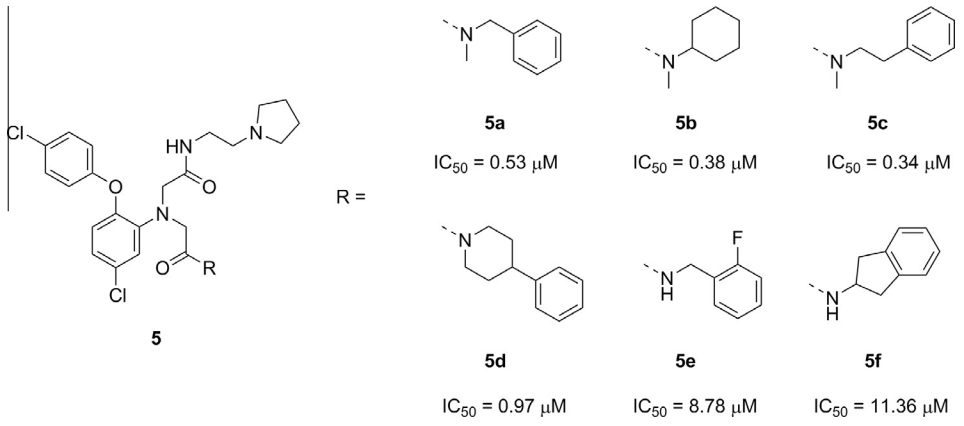


Figure 3. Representative compounds from ALIS screening and their IC_{50} values for AdoHcyase inhibition.

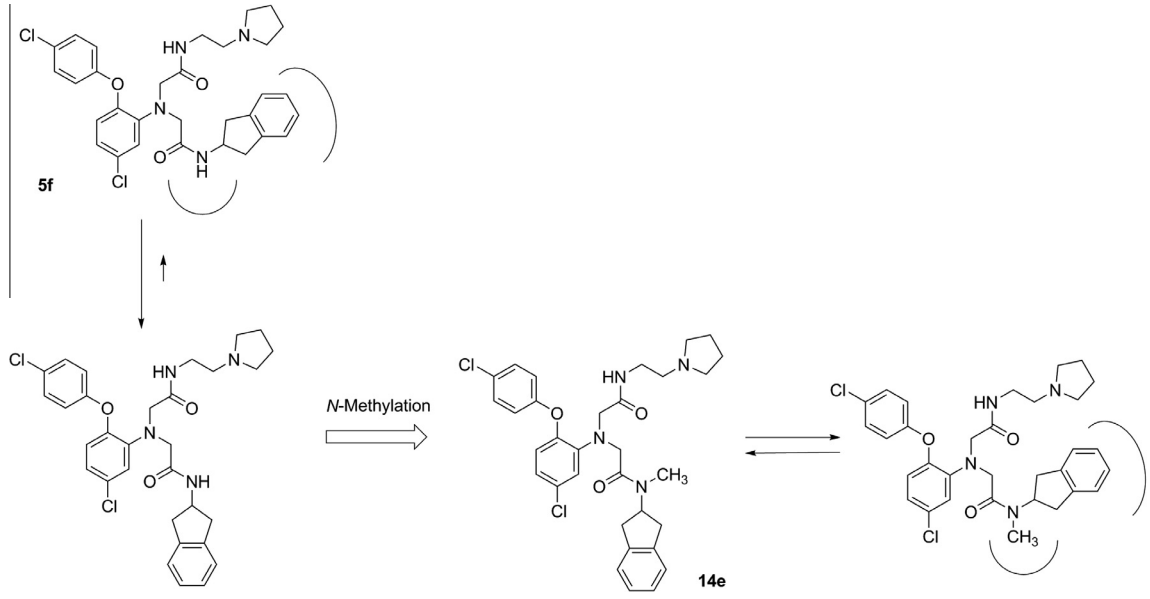
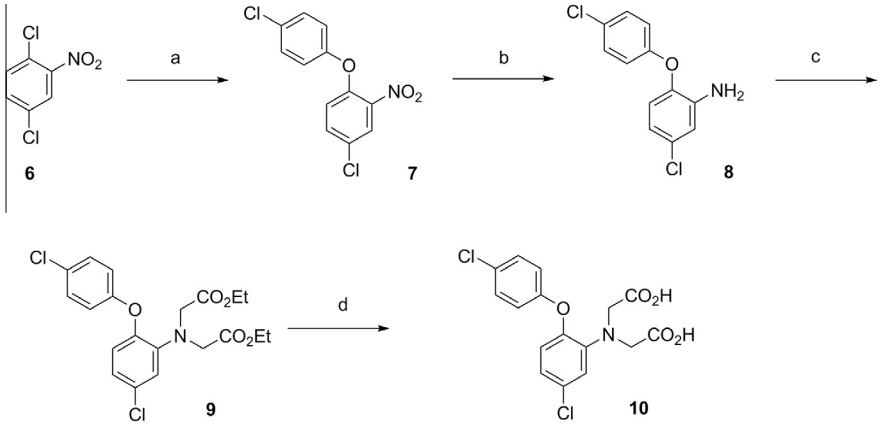
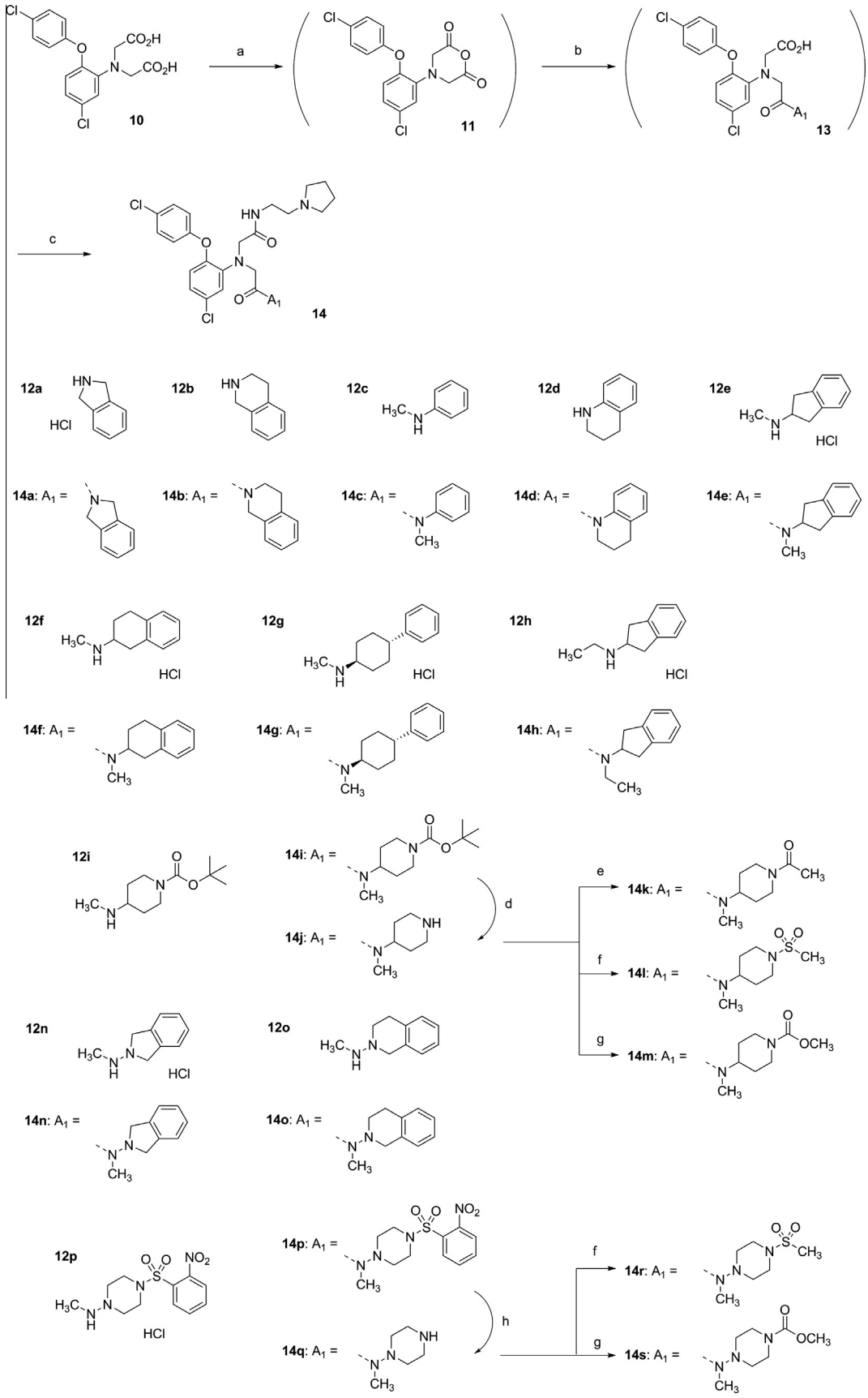


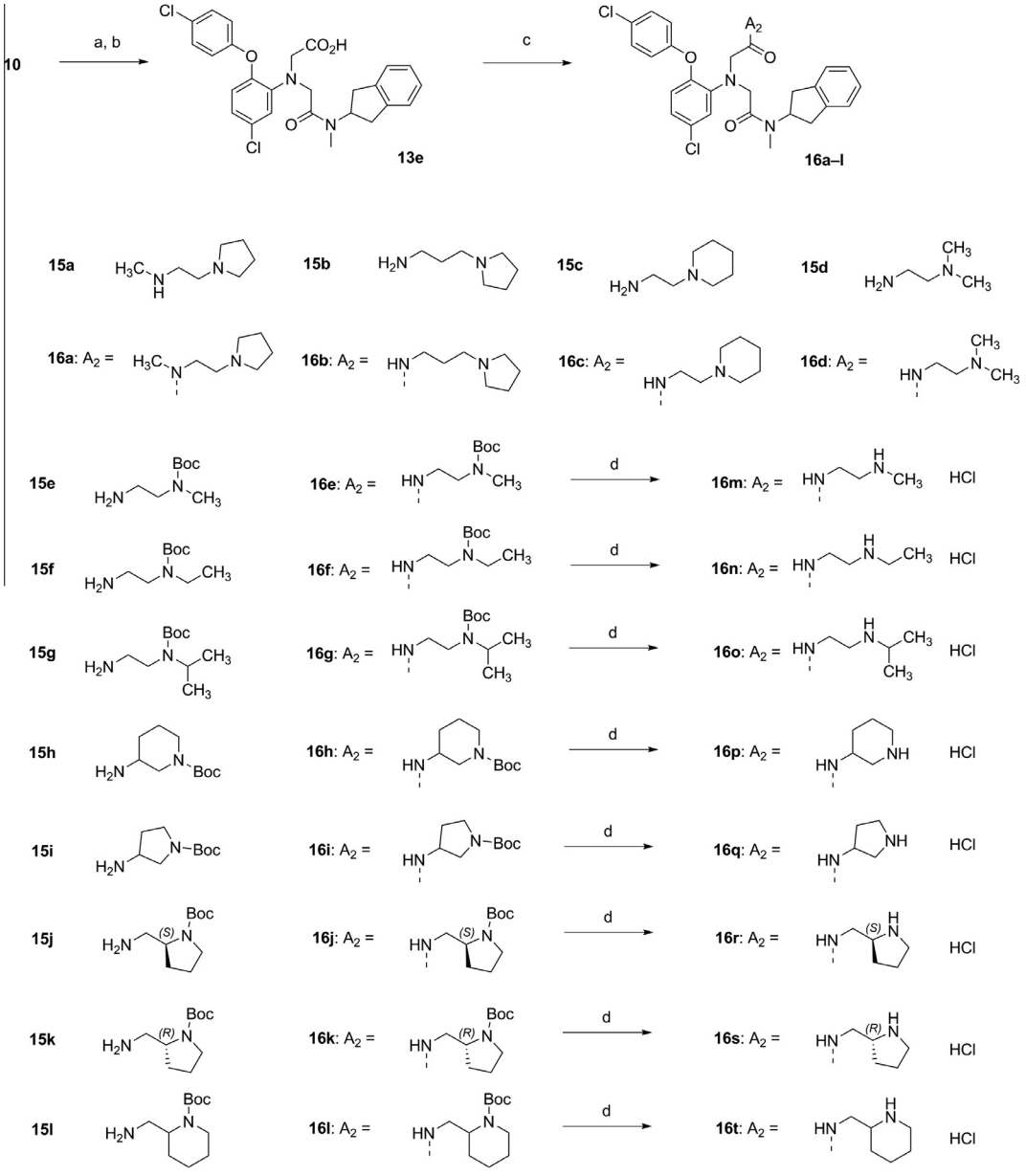
Figure 4. Proposed conformational changes by N-methylation of **5f** to **14e**.



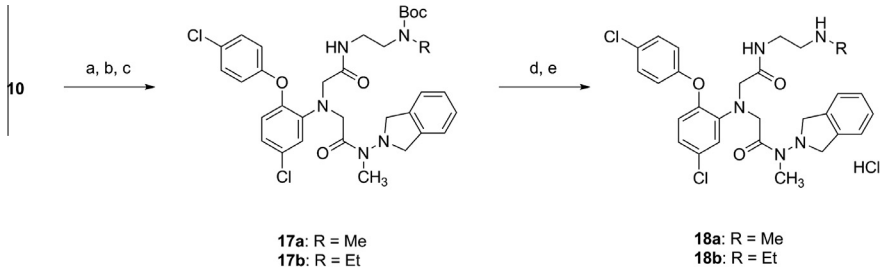
Scheme 1. Reagents and conditions: (a) 4-chlorophenol, NaH, DMF rt–80 °C; (b) $FeCl_3$, charcoal activated, $NH_2NH_2{\cdot}H_2O$, MeOH, 50 °C to reflux; (c) ethyl bromoacetate, *N,N*-diisopropylethylamine, 140 °C; (d) 1 mol/L aq NaOH, MeOH, THF, rt.



Scheme 2. Reagents and conditions: (a) EDC, DMF rt; (b) **12**, or **12**, DIPEA, 0 °C; (c) 2-(1-pyrrolidinyl)ethylamine, HOBt, EDC, 0 °C–rt; (d) 4 mol/L HCl in dioxane, CH_2Cl_2, rt; (e) Ac_2O, TEA, CH_2Cl_2, 0 °C–rt; (f) CH_3SO_2Cl, TEA, CH_2Cl_2, 0 °C–rt; (g) methyl chloroformate, TEA, CH_2Cl_2, 0 °C–rt; (h) *p*-toluenethiol, Cs_2CO_3, MeCN, rt.

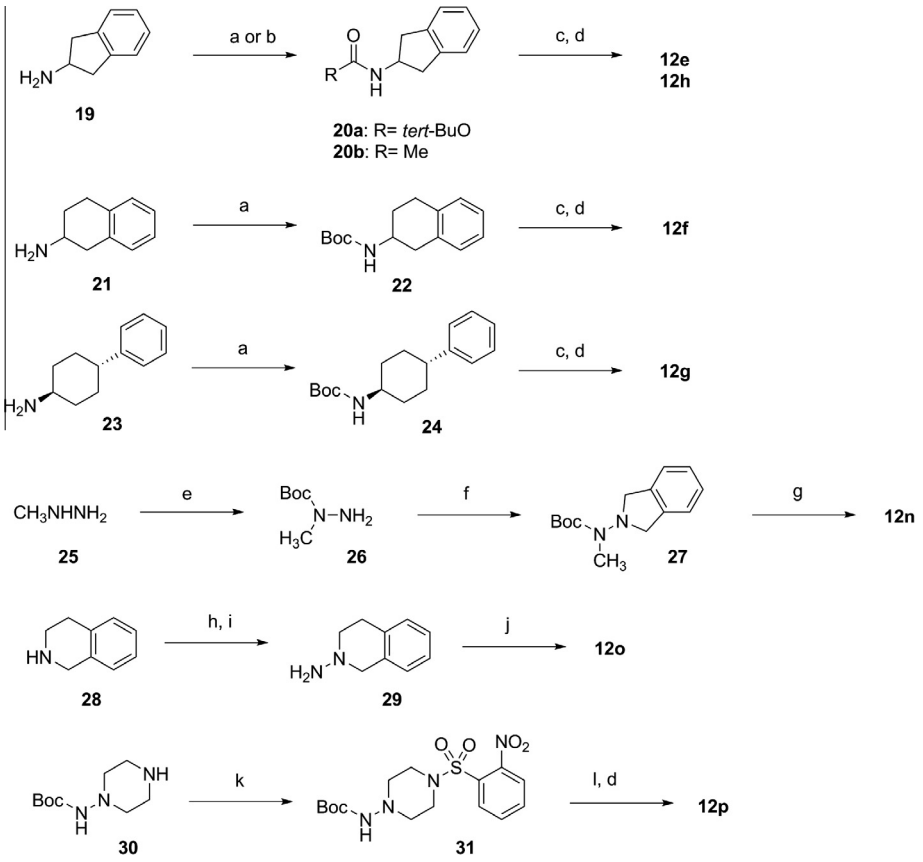


Scheme 3. Reagents and conditions: (a) EDC, DMF rt; (b) **12e**, DIPEA, 0 °C; (c) **15a–l**, HOBt, EDC, DMF, 0 °C–rt; (d) 4 mol/L HCl in dioxane, CH_2Cl_2, rt.



Scheme 4. Reagents and conditions: (a) EDC, DMF rt; (b) **12n**, DIPEA, 0 °C; (c) **15e–f**, HOBt, EDC, 0 °C–rt; (d) TFA, CH_2Cl_2, rt; (e) 4 mol/L HCl in dioxane, Et_2O, rt.

Compounds **12e–h** and **12n–p** were prepared shown in Scheme 5. Treatment of 2-aminoindane (**19**) with di-*tert*-butyl dicarbonate and acetic anhydride gave **20a** and **20b**, respectively. Reduction of **20a** and **20b** with $LiAlH_4$, followed by their salt formation afforded **12e** and **12h**, respectively. **12f** and **12g** were synthesized by a similar method that used in the synthesis of **20a**. Boc protection of methylhydrazine (**25**) with di-*tert*-butyl dicarbonate, followed by cyclization with *o*-xylylene dibromide gave **27**. Deprotection of *N*-Boc in **27** afforded **12n**. Treatment of 1,2,3,4-tetrahydroisoquinoline (**28**) with sodium nitrite, followed



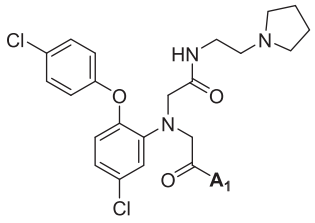
Scheme 5. Reagents and conditions: (a) Boc_2O, TEA, CH_2Cl_2, rt; (b) Ac_2O, TEA, CH_2Cl_2, 0 °C; (c) $LiAlH_4$, THF, 0 °C to reflux; (d) 4 mol/L HCl in dioxane, Et_2O, rt; (e) Boc_2O, EtOH, CH_2Cl_2, rt; (f) *o*-xylylene dibromide, TEA, NMP, 50 °C–rt; (g) 4 mol/L HCl in dioxane, CH_2Cl_2, rt; (h) $NaNO_2$, H_2O, AcOH, 5 °C–rt; (i) Zn, AcOH, MeOH, 0 °C–rt; (j) 37% formalin, AcOH, H_2O, 0 °C; $LiAlH_4$, THF, 0 °C to reflux; (k) *o*-nitrobenzenesulfonyl chloride, TEA, CH_2Cl_2, 0 °C–rt; (l) MeI, *t*-BuOK, THF, 0 °C.

by reduction with Zn and acetic acid in MeOH gave **29**. Treatment of **29** with formalin followed by reduction with $LiAlH_4$ afforded **12o**. Protection of **30** with *o*-nitrobenzenesulfonyl chloride gave **31**. Methylation of **31** with iodomethane, followed by removal of Boc provided **12p**.

3. Results and discussion

The compounds synthesized above were tested in an enzyme inhibition assay using human recombinant *S*-adenosyl-L-homocysteine hydrolase (Diazyme Laboratories) by measuring the hydrolysis activity of AdoHcy. In an effort to address the enhancement of the enzyme inhibition activity, we firstly investigated the replacement of the hydrophobic amide parts. Results are described in Table 1. Among compounds **5a**–**f**, **5d** only had a cyclic amine substituent in the hydrophobic amide parts. In order to make sure whether tertiary amides are simply required in the hydrophobic amide parts, we examined the effect of cyclic tertiary amides. Compounds **14a**, and **14b** decreased potency compared to **5a**, and **5c**. 1,2,3,4-Tetrahydroquinoline derivative **14d** had similar inhibitory activity to **14c**. These results suggested that the tertiary amide bond in the hydrophobic parts play an important role in directions and locations of hydrophobic substituents. Compound **14e**, designed for the conformational change of **5f**, significantly increased the inhibitory activity against AdoHcyase (>200-fold). Replacement of the indane in **14e** with 1,2,3,4-tetrahydronaphtalene maintained similar strong inhibitory activity (**14f**). On the

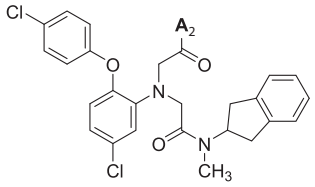
Table 1
AdoHcyase inhibition assay data of the conversion of tertiary amides



Compound	IC_{50}* (μM)
14a	>100
14b	17 ± 2
14c	2.7 ± 0.9
14d	2.3 ± 0.2
14e	0.052 ± 0.010
14f	0.081 ± 0.017
14g	0.53 ± 0.08
14h	0.64 ± 0.12
14j	>100
14k	4.6 ± 0.5
14l	0.23 ± 0.05
14m	0.89 ± 0.18
14n	0.013 ± 0.003
14o	0.20 ± 0.02
14r	0.049 ± 0.008
14s	0.044 ± 0.006

* Each datum represents the mean ± standard error of triplicate determinations.

Table 2
The conversion of hydrophilic amines



	IC_{50}* (μM)
14e	0.052 ± 0.010
16a	>100
16b	0.33 ± 0.05
16c	0.32 ± 0.05
16d	0.13 ± 0.03
16e	0.070 ± 0.012
16f	0.060 ± 0.006
16g	0.049 ± 0.005
16h	1.2 ± 0.1
16i	1.5 ± 0.2
16j	0.11 ± 0.01
16k	0.60 ± 0.07
16l	0.15 ± 0.02

* Each datum represents the mean ± standard error of triplicate determinations.

Table 3
Combination of the SAR on the two amide parts

	IC_{50}* (μM)
18a	0.0050 ± 0.0020
18b	0.0085 ± 0.0031

* Each datum represents the mean ± standard error of triplicate determinations.

other hand, replacement of the *N*-methyl group in **14e** with an *N*-ethyl group resulted in a 12-fold decrease in potency (**14h**). Introduction of a phenyl group into the 4-position of the cyclohexyl group in **5b** slightly decreased its potency (**14g**). Replacement of the cyclohexyl group in **5b** with piperidine (**14j**) led to a significant decrease in potency, but introduction of acetyl (**14k**), methanesulfonyl (**14l**), and methoxycarbonyl (**14m**) groups in **14j** regained the inhibitory activity. ^{1}H NMR spectra of the

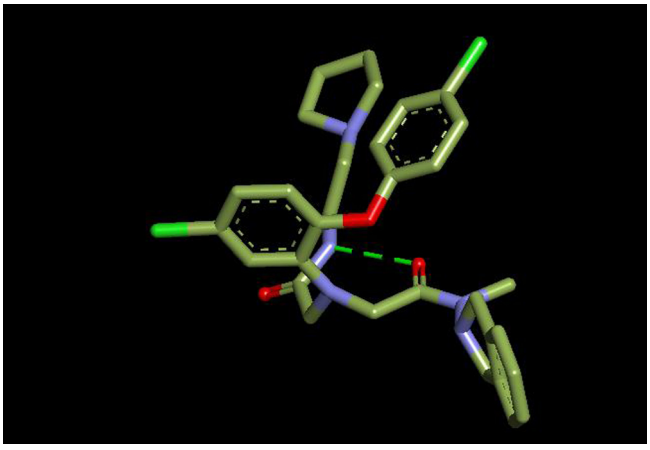


Figure 6. X-ray single crystal structure of **14n**.

compounds having a methine proton next to a tertiary amide nitrogen, such as **14e**, **14f**, **14l**, and **14m** showed two distinct peaks of the methine proton, suggesting the existence of rotational isomers. In the case of **14e**, for example, the spectrum of the methine proton exhibited two multiplets of 2:3 intensities at 4.47–4.57 ppm and 5.37–5.47 ppm, respectively. The rotationally restricted isomers might have a disadvantageous effect on the interaction with the target enzyme. This result encouraged us to perform the modification of *N*-methyl amine derivatives to methylhydrazine analogs. Replacement of the indane in (**14e**) with an isoindoline (**14n**) led to a 4-fold increase in potency, and conversion of piperidine moieties (**14l**, **14m**) into piperazines (**14r**, **14s**) also improved the inhibitory activities. On the other hand, replacement of the tetrahydronaphthalene (**14f**) with a tetrahydroisoquinoline (**14o**) gave a 2.5-fold loss in potency.

Next, we conducted the conversion of hydrophilic amines with the *N*-methylindan-2-amine on the hydrophobic parts (Table 2). N-methylation of the secondary amide in **14e** showed a marked decrease in potency (**16a**). Elongation of the methylene linker to give **16b** led to a 6-fold loss in potency. Replacement of the pyrrolidine with a piperidine (**16c**) and a dimethylamine (**16d**) showed 6 and 3 times losses in potency, respectively. The introductions of

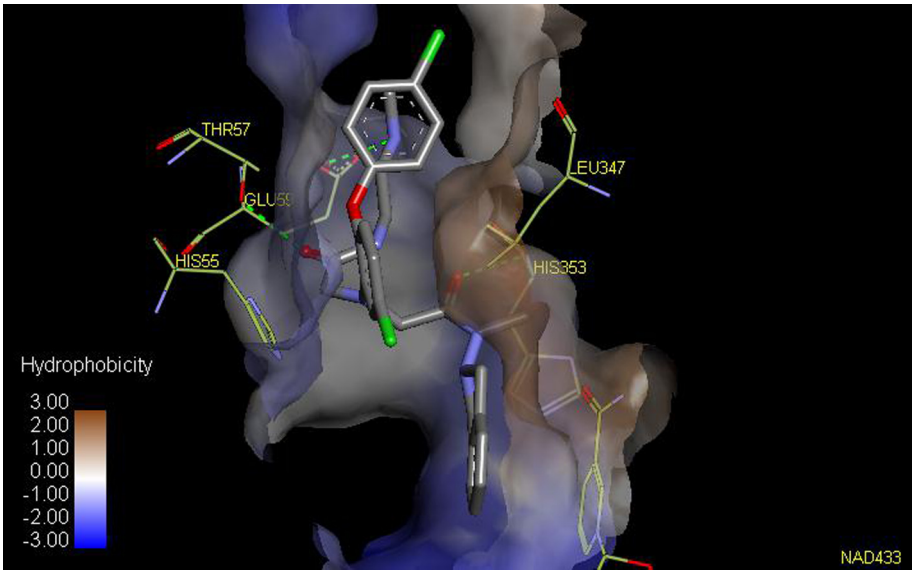


Figure 5. X-ray co-crystal structure of AdoHcyase, NAD^+, and **18a**.

N-monoalkylethylenediamines such as **16m**, **16n**, and **16o** showed comparable potency for AdoHcyase. The cyclic secondary amine series compounds **16p–t** showed weaker inhibitory activities than **14e**. Compounds **16j**, and **16l** were more potent than **16h**, **16i**, and **16k**.

Combined with the information of SAR on the two amides parts, we synthesized compounds **18a** and **18b**, which had the strongest inhibitory activities against AdoHcyase, with IC_{50} values of 0.0050 ± 0.0020, and 0.0085 ± 0.0031 μM, respectively (Table 3).[15]

To elucidate the active conformation of our inhibitors with AdoHcyase, X-ray co-crystal structure analysis was employed. We were successful in obtaining plate-like co-crystals of the AdoHcyase complexed with the NAD^+ coenzyme and compound **18a**, which diffracted X-rays to 2.7 Å resolutions (see Fig. 5). The complex showed tetrameric structures, and each subunit contained one NAD^+ molecule and one molecule of **18a**. The AdoHcyase in the complexes has an open conformation similar to the apo enzyme structure and different from the reported X-ray co-structures.[9b,16] **18a** was sandwiched between the catalytic domain and the NAD^+ binding domain. The benzene ring of the isoindoline in **18a** and the nicotinamide ring of the NAD^+ were located in a parallel displaced fashion with a separation of 3.9 Å, suggesting that there should be a cation–π stacking interaction.[17] 2-(4-Chlorophenoxyl)-5-chloroaniline part in **18a** was positioned between the two domains, indicating hydrophobic interactions with Leu347 and π–π stacking with an imidazole substituent in His55 (3.7 Å).[18] As expected, an active conformation of **18a** with AdoHcyase was a *cis*-amide form. There were four hydrogen bond interactions: (i) between the carbonyl oxygen of the hydrophobic amide part in **18a** and the amide of His353 in the NAD^+ binding domain (2.8 Å), (ii) between the carbonyl oxygen of the hydrophilic amide part in **18a** and the hydroxyl group of the side chain of Thr57 in the catalytic domain (2.5 Å), (iii) between the *N*-methylamine of the hydrophilic amide part in **18a** and the carboxyl group of the side chain of Glu59 in the catalytic domain (2.8 Å), and (iv) between the carbonyl oxygen of the hydrophobic amide part and the NH hydrogen of the hydrophilic amide part in **18a** (2.9 Å) as an unexpected intramolecular eight-membered ring hydrogen bond interaction.[19] The loss of activity observed for compound **16a** was consistent with the destruction of the intramolecular hydrogen bond interaction by N-methylation of **14e**. The reduced activity observed for **14h** was explained by the limited size of a hydrophobic pocket around the *N*-methyl amide region in **14e**. The increased activity observed for **14n** was thought to be due to the enhancement of a cation–π interaction by a small angle change from the indane in **14e** to the isoindoline in **14n**.

We speculated that the intramolecular eight-membered ring hydrogen bond formation in **18a** was induced only by the interaction of compound **18a** with the AdoHcyase and the NAD^+. To solve the molecular conformation of **18a** itself, we made an effort to obtain a single crystal of **18a** or other derivatives. As a result, a single crystal of **18a** was not obtained, however a single crystal of compound **14n** having a very similar structure to **18a** was fortunately obtained as a colorless prismatic crystal from diethyl ether. The single X-ray crystal structure revealed that **14n** formed not only a *cis*-amide formation, but also an intramolecular eight-membered ring hydrogen bond interaction (2.9 Å) similar to **18a** in the X-ray co-crystal structure (see Fig. 6), suggesting that the intramolecular eight-membered ring hydrogen bond interaction would be formed at least under hydrophobic environmental conditions such as binding pockets in enzymes or nonpolar solvents (see Fig. 6).[20]

4. Conclusions

In conclusion, we identified a novel series of *S*-adenosyl-L-homocysteine hydrolase inhibitors based on non-adenosine analogs through ALIS screening. During the optimization campaign, we hypothesized that active conformations of the inhibitors with AdoHcyase were *cis*-amide forms. Our effort led to very potent compounds **18a** and **18b** with IC_{50} values of 5.0 ± 2.0, and 8.5 ± 3.1 nM, respectively. A novel open form X-ray co-structure of AdoHcyase, **18a**, and NAD^+ revealed that the active conformation of compound **18a** was a *cis*-amide form, and there were three intermolecular and one intramolecular hydrogen bond interactions. A single X-ray crystal structure of compound **14n** showed an intramolecular eight-membered ring hydrogen bond interaction seen in **18a**. Compound **18a** and **18b** could be a promising lead compound for research to reduce elevated homocysteine levels, and the information of the X-ray crystal structures will help our further lead optimization.

5. Experimentals

5.1. Chemistry

5.1.1. General

All melting points were obtained on a Büchi 535 melting point apparatus and are uncorrected. Silica gel column chromatography was performed on a SHOKO Scientific Purif-α2 Flash Chromatography System using Purif-Pack silica gel columns or Yamazen Hi-Flash columns, and the described solvents as eluent under gradient condition. ^1H and ^{13}C NMR spectra were recorded on a Bruker ARX300 spectrometer (300 MHz) or a Bruker AVANCE400 (400 MHz) spectrometer. Chemical shifts are expressed in parts per million (ppm, δ units) relative to tetramethylsilane (TMS) as an internal standard, and the following abbreviations are used: s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet, dd = double doublet, dt = double triplet, br s = broad singlet. Mass spectra were measured in a combination with a Waters Acquity UPLC system (Acquity BEH C18 2.0 × 50 mm, mobile phase: A: H_2O + 0.05% HCOOH; B: CH_3CN + 0.05% HCOOH, a gradient of 5–98% B over 1 min, flow rate 0.6 mL/min, column temperature 40 °C) and a Micromass ZQ mass spectrometer in electrospray ionization (ESI) positive mode. Elemental analyses were performed on a Perkin–Elmer 2400 II CHN Elemental Analyzer. High resolution mass spectroscopy (HRMS) was measured in a combination with a Dionex UltiMate 3000 HPLC system (YMC Hydrosphere C18 (3 μm) 2.0 × 75 mm, mobile phase: A: H_2O + 0.1% HCOOH; B: CH_3CN, A/B = 50/50 over 3 min, flow rate 0.2 mL/min, column temperature 40 °C) and a Thermo Fisher Scientific LTQ Orbitrap Velos Pro mass spectrometer in ESI positive mode. Purity was determined by HPLC measured by an Agilent 1100 system (Sumipax ODS D-210SLP (3 μm) 4.6 × 50 mm, mobile phase: A: H_2O + 0.05% TFA; B: CH_3CN + 0.05% TFA, A/B = 40/60, 45/55, 50/50, 55/45, 60/40, or 70/30 over 12 min, flow rate 1.0 mL/min, column temperature 40 °C), and was >95% for all tested compounds. All chemicals and solvents were of reagent grade unless otherwise specified. The following abbreviations are used: DMF, *N*,*N*-dimethylformamide; DMSO, dimethyl sulfoxide; TEA, triethylamine; THF, tetrahydrofuran; EDC, 1-[3-(dimethylamino)propyl]-3-ethylcarbodiimide hydrochloride; HOBt, 3-hydroxybenztriazole.

5.1.2. 4-Chloro-1-(4-chlorophenoxy)-2-nitrobenzene (7)[21]

To a solution of 4-chlorophenol (10.6 g, 82.5 mmol) in DMF (100 mL) was added NaH (3.32 g, 60% in mineral oil, 83.0 mmol), and the resulting mixture was stirred at room temperature for 40 min. To the mixture was added 1,4-dichloro-2-nitrobenzene (**6**) (14.5 g, 75.5 mmol), and the mixture was stirred at 80 °C for 75 min. The reaction mixture was cooled down, and poured into ice water (400 mL). The precipitated solid was filtered, washed with H_2O (100 mL), 1 mol/L aqueous NaOH (100 mL × 2), and H_2O (100 mL), and dried under reduced pressure to give **7**

(21.8 g, 93%) as a yellow solid. Mp 74–76 °C. ^{1}H NMR (400 MHz, $CDCl_3$): δ (ppm) 6.98 (d, J = 8.7, 2H), 6.99 (d, J = 9.3, 1H), 7.35 (d, J = 8.7, 2H), 7.49 (dd, J = 2.6, 9.3, 1H), 7.96 (d, J = 2.6, 1H). ^{13}C NMR (100 MHz, $CDCl_3$): δ (ppm) 120.30, 121.81, 125.76, 128.85, 130.15, 130.23, 134.24, 141.50, 148.92, 154.22. MS (APCI) m/z 284, 286 $[M+H]^+$.

5.1.3. 5-Chloro-2-(4-chlorophenoxy)aniline (8)[22]

A solution of **7** (12.0 g, 42.2 mmol), $FeCl_3$ (702 mg, 4.33 mmol), and activated charcoal (3.70 g) in MeOH (360 mL) was stirred at 50 °C, and to the mixture was added hydrazine monohydrate (7.00 mL, 144 mmol) over 10 min, and then stirred under reflux for 1 h. The reaction mixture was cooled down, filtered through Celite, washed with MeOH, and the resulting solution was concentrated under reduced pressure. The residue oil was diluted with EtOAc, and the organic layer was washed with saturated aqueous $NaHCO_3$, brine, and dried over Na_2SO_4. The insoluble material was filtered off, and the solution was concentrated under reduced pressure to give **8** (10.6 g, 99%) as a colorless solid. Mp 59–61 °C. ^{1}H NMR (400 MHz, $CDCl_3$): δ (ppm) 3.85 (br s, 2H), 6.67 (dd, J = 2.6, 8.2, 1H), 6.76 (d, J = 8.2, 1H), 6.80 (d, J = 2.6, 1H), 6.89 (d, J = 8.7, 2H), 7.26 (d, J = 8.7, 2H). ^{13}C NMR (100 MHz, $CDCl_3$): δ (ppm) 116.07, 118.33, 118.42, 121.04, 127.98, 129.76, 130.17, 139.75, 141.32, 155.81. LC–MS (ESI) m/z 254, 256 $[M+H]^+$.

5.1.4. *N*-[5-Chloro-2-(4-chlorophenoxy)phenyl]iminodiacetic acid diethyl ester (9)

A solution of **8** (2.56 g, 10.1 mmol), ethyl bromoacetate (7.0 mL, 63 mmol), and *N*,*N*-diisopropylethylamine (7.0 mL, 41 mmol) was stirred at 140 °C for 5 h. The reaction mixture was cooled down, and diluted with EtOAc (100 mL). The organic layer was washed with 10% aqueous citric acid (100 mL × 2), saturated aqueous $NaHCO_3$, and brine, and dried over $MgSO_4$. The insoluble material was filtered off, and the solution was concentrated under reduced pressure. The residue oil was purified by silica gel column chromatography (hexane/EtOAc = 95:5 to 80:20) to afford **9** (3.68 g, 85%) as a pale yellow oil. ^{1}H NMR (400 MHz, $CDCl_3$): δ (ppm) 1.20 (t, J = 7.2, 6H), 4.10 (s, 4H), 4.11 (q, J = 7.2, 4H), 6.76 (d, J = 8.7, 1H), 6.81–6.87 (m, 4H), 7.24 (d, J = 8.7, 2H). ^{13}C NMR (100 MHz, $CDCl_3$): δ (ppm) 14.15, 54.04, 60.95, 118.72, 119.42, 121.65, 122.31, 127.95, 129.56, 129.94, 142.40, 145.35, 155.99, 170.52. LC–MS (ESI) m/z 426, 428 $[M+H]^+$.

5.1.5. *N*-[5-Chloro-2-(4-chlorophenoxy)phenyl]iminodiacetic acid (10)

To a solution of **9** (3.68 g, 8.63 mmol) in MeOH (20 mL) and THF (10 mL) was added 1 mol/L aqueous NaOH (30 mL, 30 mmol), and the resulting mixture was stirred at room temperature for 2 h. To the reaction mixture was added 1 mol/L aqueous HCl (40 mL, 40 mmol), and the precipitated solid was filtered, washed with H_2O, and dried under reduced pressure to give **10** (2.90 g, 91%) as a colorless solid. Mp 192–194 °C. ^{1}H NMR (400 MHz, DMSO-d_6): δ (ppm) 4.05 (s, 4H), 6.82–6.88 (m, 3H), 6.91 (d, J = 8.7, 2H), 7.36 (d, J = 8.7, 2H), 12.54 (br s, 2H). ^{13}C NMR (100 MHz, DMSO-d_6): δ (ppm) 53.50, 118.10, 118.73, 120.01, 122.99, 126.42, 128.73, 129.41, 142.55, 143.94, 155.93, 171.47. LC–MS (ESI) m/z 370, 372 $[M+H]^+$. Anal. Calculated for $C_{16}H_{13}Cl_2NO_5$: C, 51.91; H, 3.54; N, 3.78; Cl, 19.15. Found: C, 51.99; H, 3.49; N, 3.75; Cl, 19.07.

5.1.6. N^2-[5-Chloro-2-(4-chlorophenoxy)phenyl]-N^2-{2-[1,3-dihydro-2*H*-isoindol-2-yl(methyl)amino]-2-oxoethyl}-N^1-(2-pyrrolidin-1-ylethyl)glycinamide (14n)

To a solution of **10** (371 mg, 1.00 mmol) in DMF (5 mL) was added EDC (195 mg, 1.02 mmol), and the resulting mixture was stirred at room temperature for 1 h. Then, to the reaction mixture stirred under ice-cooling was added **12n** (192 mg, 1.04 mmol) and *N*,*N*-diisopropylethylamine (0.180 mL, 1.06 mmol), and the mixture was stirred at 0 °C for 1 h. To the reaction mixture was added 1-(2-aminoethyl)pyrrolidine (0.140 mL, 1.12 mmol), HOBt (174 mg, 1.14 mmol), and EDC (220 mg, 1.15 mmol), and the resulting mixture was stirred at room temperature for 6 h. The reaction mixture was diluted with EtOAc, and the organic layer was washed with H_2O, saturated aqueous $NaHCO_3$, and brine, and dried over Na_2SO_4. The insoluble material was filtered off, and the solution was concentrated under reduced pressure. The residue oil was purified by NH silica gel column chromatography (hexane/EtOAc = 50:50 to 0:100) to afford **14n** (497 mg, 83%) as a colorless solid. Mp 96–100 °C. ^{1}H NMR (400 MHz, $CDCl_3$): δ (ppm) 1.63–1.74 (m, 4H), 2.34–2.48 (m, 6H), 2.88 (s, 3H), 3.25 (q, J = 6.4, 2H), 3.95 (s, 2H), 4.07 (d, J = 11.3, 2H), 4.20 (d, J = 11.3, 2H), 4.40 (s, 2H), 6.77 (d, J = 8.2, 1H), 6.79–6.87 (m, 3H), 6.98 (d, J = 2.1, 1H), 7.18–7.30 (m, 6H), 8.06 (t, J = 5.4, 1H). ^{13}C NMR (100 MHz, $CDCl_3$): δ (ppm) 23.50, 24.70, 38.36, 53.98, 54.76, 54.92, 55.45, 58.66, 118.49, 118.82, 121.23, 122.61, 122.89, 127.59, 127.85, 129.69, 130.39, 136.97, 142.28, 144.75, 156.65, 170.08, 172.75. LC–MS (ESI) m/z 596, 598 $[M+H]^+$. HPLC purity: 98.87%. HRMS (ESI) m/z calculated for $C_{31}H_{36}Cl_2N_5O_3$ $[M+H]^+$ 596.21897, found 596.21902.

5.1.7. N^2-[5-Chloro-2-(4-chlorophenoxy)phenyl]-N^2-[2-(1,3-dihydro-2*H*-isoindol-2-yl)-2-oxoethyl]-N^1-(2-pyrrolidin-1-ylethyl)glycinamide (14a)

The title compound was synthesized according to the method described for the synthesis of **14n** to afford **14a** (350 mg, 72%) as a colorless oil. ^{1}H NMR (400 MHz, $CDCl_3$): δ (ppm) 1.73–1.94 (m, 4H), 2.40–2.95 (m, 6H), 3.35 (q, J = 6.2, 2H), 4.07 (s, 2H), 4.37 (br s, 2H), 4.64 (s, 2H), 4.66 (s, 2H), 6.73–6.82 (m, 4H), 6.86–6.92 (m, 1H), 7.14 (d, J = 9.3, 2H), 7.18–7.25 (m, 1H), 7.26–7.35 (m, 3H), 8.75 (broad, 1H). ^{13}C NMR (100 MHz, $CDCl_3$): δ (ppm) 23.42, 37.27, 51.51, 52.38, 53.96, 54.66, 56.10, 59.26, 118.07, 120.97, 122.66, 122.94, 123.24, 127.75, 127.79, 128.02, 129.61, 130.66, 135.58, 141.59, 143.79, 156.56, 169.09, 170.59. LC–MS (ESI) m/z 567, 569 $[M+H]^+$. HPLC purity 96.98%. HRMS (ESI) m/z calculated for $C_{30}H_{33}Cl_2N_4O_3$ $[M+H]^+$ 567.19242, found 567.19306.

5.1.8. N^2-[5-Chloro-2-(4-chlorophenoxy)phenyl]-N^2-{2-[3,4-dihydroisoquinolin-2(1*H*)-yl]-2-oxoethyl}-N^1-(2-pyrrolidin-1-ylethyl)glycinamide (14b)

The title compound was synthesized according to the method described for the synthesis of **14n** to afford **14b** (548 mg, 81%) as a colorless oil. ^{1}H NMR (400 MHz, $CDCl_3$): δ (ppm) 1.65–1.77 (m, 4H), 2.36–2.59 (m, 6H), 2.76, 2.79 (2t, J = 6.2, 2H), 3.32 (q, J = 6.2, 2H), 3.46 (t, J = 6.2, 1.2H), 3.67 (t, J = 6.2, 0.8H), 3.98 (s, 2H), 4.28, 4.29 (2s, 2H), 4.40 (s, 0.8H), 4.55 (s, 1.2H), 6.68–6.75 (m, 3H), 6.76–6.82 (m, 1H), 6.88–6.95 (m, 1H), 6.97–7.25 (m, 6H), 8.39 (br s, 1H). ^{13}C NMR (100 MHz, $CDCl_3$): δ (ppm) 23.53, 28.23, 28.97, 38.27, 40.11, 42.24, 44.26, 46.16, 54.00, 54.83, 55.92, 58.97, 118.32, 118.65, 121.12, 122.76, 122.81, 126.01, 126.57, 126.60, 126.81, 126.93, 127.26, 127.91, 128.17, 128.99, 129.62, 129.67, 130.51, 131.58, 132.96, 133.75, 134.79, 141.58, 141.63, 144.26, 144.33, 156.28, 156.33, 168.59, 168.70, 169.86. LC–MS (ESI) m/z 581, 583 $[M+H]^+$. HPLC purity: 96.39%. HRMS (ESI) m/z calculated for $C_{31}H_{35}Cl_2N_4O_3$ $[M+H]^+$ 581.20807, found 581.20863.

5.1.9. N^2-[5-Chloro-2-(4-chlorophenoxy)phenyl]-N^2-{2-[methy-(phenyl)amino]-2-oxoethyl}-N^1-(2-pyrrolidin-1-ylethyl)glycinamide (14c)

The title compound was synthesized according to the method described for the synthesis of **14n** to afford **14c** (548 mg, 86%) as a colorless oil. ^{1}H NMR (400 MHz, $CDCl_3$): δ (ppm) 1.65–1.81 (m, 4H), 2.31–2.59 (m, 6H), 3.21 (s, 3H), 3.23 (q, J = 6.2, 2H), 3.82 (s, 2H), 3.88 (s, 2H), 6.73–6.84 (m, 5H), 7.13 (d, J = 7.7, 2H),

7.22–7.28 (m, 2H), 7.35–7.49 (m, 3H), 8.21 (br s, 1H). ^{13}C NMR (100 MHz, $CDCl_3$): δ (ppm) 23.51, 37.57, 38.12, 53.98, 54.70, 55.49, 58.87, 118.59, 118.79, 121.52, 122.80, 124.34, 127.00, 127.95, 128.58, 129.70, 130.21, 130.27, 141.75, 142.28, 145.05, 156.50, 169.49, 170.11. LC–MS (ESI) *m*/*z* 555, 557 $[M+H]^+$. HPLC purity: 99.50%. HRMS (ESI) *m*/*z* calculated for $C_{29}H_{33}Cl_2N_4O_3$ $[M+H]^+$ 555.19242, found 555.19251.

5.1.10. N^2-[5-Chloro-2-(4-chlorophenoxy)phenyl]-N^2-{2-[3,4-dihydroquinolin-1(2*H*)-yl]-2-oxoethyl}-N^1-(2-pyrrolidin-1-ylethyl)glycinamide (14d)

The title compound was synthesized according to the method described for the synthesis of **14n** to afford **14d** (970 mg, 77%) as a colorless oil. ^{1}H NMR (400 MHz, $CDCl_3$): δ (ppm) 1.63–1.85 (m, 4H), 1.87–1.98 (m, 2H), 2.33–2.69 (m, 6H), 2.76 (t, *J* = 6.7, 2H), 3.23 (d, *J* = 6.2, 2H), 3.74 (br s, 2H), 3.96 (s, 2H), 4.30 (br s, 2H), 6.48–6.76 (m, 3H), 6.75 (d, *J* = 8.7, 1H), 6.81 (dd, *J* = 2.6, 8.7, 1H), 6.92–7.18 (broad, 1H), 7.15–7.31 (m, 5H), 8.30 (br s, 1H). ^{13}C NMR (100 MHz, $CDCl_3$): δ (ppm) 23.50, 23.79, 26.66, 37.93, 43.19, 54.01, 54.71, 55.11, 59.02, 118.24, 121.48, 123.16, 124.34, 126.41, 127.81, 129.02, 129.66, 130.38, 137.74, 141.80, 144.69, 156.52, 169.20, 170.24. LC–MS (ESI) *m*/*z* 581, 583 $[M+H]^+$. HPLC purity: 99.42%. HRMS (ESI) *m*/*z* calculated for $C_{31}H_{35}Cl_2N_4O_3$ $[M+H]^+$ 581.20807, found 581.20873.

5.1.11. N^2-[5-Chloro-2-(4-chlorophenoxy)phenyl]-N^2-{2-[2,3-dihydro-1*H*-inden-2-yl(methyl)amino]-2-oxoethyl}-N^1-(2-pyrrolidin-1-ylethyl)glycinamide (14e)

The title compound was synthesized according to the method described for the synthesis of **14n** to afford **14e** (502 mg, 67%) as a colorless oil. ^{1}H NMR (400 MHz, $CDCl_3$): δ (ppm) 1.65–1.81 (m, 4H), 2.37–2.59 (m, 6H), 2.66 (s, 1.8H), 2.70 (s, 1.2H), 2.78 (dd, *J* = 6.2, 16.4, 1.2H), 2.95 (dd, *J* = 6.2, 16.4, 0.8H), 3.04 (dd, *J* = 8.2, 16.4, 0.8H), 3.10 (dd, *J* = 8.2, 16.4, 1.2H), 3.32 (q, *J* = 6.2, 2H), 3.98 (s, 2H), 4.19 (s, 1.2H), 4.31 (s, 0.8H), 4.47–4.57 (m, 0.4H), 5.37–5.47 (m, 0.6H), 6.75 (d, *J* = 8.2, 2H), 6.78–6.98 (m, 3H), 7.14–7.23 (m, 4H), 7.28 (d, *J* = 8.2, 2H), 8.25, 8.36 (2 broad, 1H). ^{13}C NMR (100 MHz, $CDCl_3$): δ (ppm) 23.52, 27.92, 28.98, 36.11, 36.35, 38.23, 53.15, 54.00, 54.80, 55.64, 55.93, 56.12, 58.75, 59.00, 118.53, 118.60, 119.03, 121.07, 121.39, 122.68, 122.81, 124.44, 124.49, 126.85, 127.16, 128.06, 129.78, 129.81, 130.44, 130.50, 139.98, 140.91, 141.64, 141.79, 144.36, 144.75, 156.42, 156.48, 169.21, 169.57, 169.98. LC–MS (ESI) *m*/*z* 595, 597 $[M+H]^+$. HPLC purity: 97.75%. HRMS (ESI) *m*/*z* calculated for $C_{32}H_{37}Cl_2N_4O_3$ $[M+H]^+$ 595.22372, found 595.22444.

5.1.12. N^2-[5-Chloro-2-(4-chlorophenoxy)phenyl]-N^2-{2-[methyl(1,2,3,4-tetrahydronaphtalen-2-yl)amino]-2-oxoethyl}-N^1-(2-pyrrolidin-1-ylethyl)glycinamide (14f)

The title compound was synthesized according to the method described for the synthesis of **14n** to afford **14f** (565 mg, 67%) as a colorless oil. ^{1}H NMR (400 MHz, $CDCl_3$): δ (ppm) 1.64–2.00 (m, 6H), 2.36–2.76 (m, 8H), 2.78, 2.80 (2s, 3H), 2.84–3.00 (m, 2H), 3.24–3.37 (m, 2H), 3.74–3.85 (m, 0.4H), 3.98, 4.01 (2s, 2H), 4.30 (br s, 2H), 4.65–4.76 (m, 0.6H), 6.74 (d, *J* = 8.7, 1H), 6.76–6.95 (m, 4H), 6.98–7.17 (m, 4H), 7.23–7.30 (m, 2H), 8.1–8.9 (broad, 1H). ^{13}C NMR (100 MHz, $CDCl_3$): δ (ppm) 23.51, 26.75, 27.24, 27.85, 28.47, 29.18, 29.26, 31.76, 32.94, 37.94, 50.19, 52.74, 54.01, 54.77, 55.67, 56.24, 58.89, 59.25, 118.19, 118.74, 118.77, 120.71, 121.27, 122.58, 122.80, 125.90, 126.07, 126.20, 126.50, 128.07, 128.12, 128.67, 128.78, 129.27, 129.78, 130.38, 130.45, 133.97, 134.76, 134.82, 135.33, 141.49, 141.75, 144.31, 144.81, 156.41, 156.58, 169.41, 169.72, 170.16. LC–MS (ESI) *m*/*z* 609, 611 $[M+H]^+$. HPLC purity: 98.34%. HRMS (ESI) *m*/*z* calculated for $C_{33}H_{39}Cl_2N_4O_3$ $[M+H]^+$ 609.23937, found 609.24009.

5.1.13. N^2-[5-Chloro-2-(4-chlorophenoxy)phenyl]-N^2-{2-[methyl(*trans*-4-phenylcyclohexyl)amino]-2-oxoethyl}-N^1-(2-pyrrolidin-1-ylethyl)glycinamide (14g)

The title compound was synthesized according to the method described for the synthesis of **14n** to afford **14g** (366 mg, 87%) as a colorless solid. Mp 151–153 °C. ^{1}H NMR (400 MHz, $CDCl_3$): δ (ppm) 1.44–1.84 (m, 10H), 1.91–2.06 (m, 2H), 2.36–2.67 (m, 7H), 2.75, 2.77 (2s, 3H), 3.31 (q, *J* = 6.2, 2H), 3.37–3.48 (m, 0.35H), 3.99 (s, 2H), 4.21 (s, 1.3H), 4.29 (s, 0.7H), 4.35–4.44 (m, 0.65H), 6.75 (d, *J* = 8.7, 1H), 6.77–6.95 (m, 4H), 7.15–7.35 (m, 7H), 8.42 (broad, 1H). ^{13}C NMR (100 MHz, $CDCl_3$): δ (ppm) 23.50, 27.38, 28.51, 29.58, 30.63, 33.07, 33.20, 37.96, 43.19, 43.55, 52.72, 53.99, 54.74, 54.79, 55.46, 55.71, 55.90, 58.88, 59.11, 118.48, 118.58, 120.92, 121.22, 122.80, 122.91, 126.22, 126.39, 126.64, 126.73, 127.97, 128.45, 128.53, 129.68, 129.75, 130.42, 130.49, 141.66, 141.89, 144.22, 144.72, 145.77, 146.32, 156.52, 156.56, 169.14, 169.30, 170.17. LC–MS (ESI) *m*/*z* 637, 639 $[M+H]^+$. HPLC purity: 99.71%. HRMS (ESI) *m*/*z* calculated for $C_{35}H_{43}Cl_2N_4O_3$ $[M+H]^+$ 637.27067, found 637.27143.

5.1.14. N^2-[5-Chloro-2-(4-chlorophenoxy)phenyl]-N^2-{2-[2,3-dihydro-1*H*-inden-2-yl(ethyl)amino]-2-oxoethyl}-N^1-(2-pyrrolidin-1-ylethyl)glycinamide (14h)

The title compound was synthesized according to the method described for the synthesis of **14n** to afford **14h** (351 mg, 46%) as a colorless amorphous solid. ^{1}H NMR (400 MHz, $CDCl_3$): δ (ppm) 1.08 (t, *J* = 7.2, 1.5H), 1.14 (t, *J* = 7.2, 1.5H), 1.64–1.76 (m, 4H), 2.10–2.31 (m, 2H), 2.37–2.53 (m, 4H), 2.87–3.11 (m, 4H), 3.14–3.39 (m, 4H), 3.98 (s, 2H), 4.21 (s, 1H), 4.26 (s, 1H), 4.41–4.56 (m, 0.5H), 4.94–5.06 (m, 0.5H), 6.72–6.98 (m, 3H), 6.85 (d, *J* = 9.3, 2H), 7.11–7.24 (m, 4H), 7.27 (d, *J* = 9.3, 2H), 8.16–8.26 (m, 0.5H), 8.27–8.36 (m, 0.5H). ^{13}C NMR (100 MHz, $CDCl_3$): δ (ppm) 14.33, 15.85, 23.51, 36.47, 36.91, 37.62, 38.21, 39.02, 53.99, 54.82, 55.71, 55.83, 56.07, 56.83, 58.76, 59.02, 118.72, 118.83, 119.07, 121.14, 121.34, 122.64, 122.74, 124.43, 124.53, 126.73, 127.17, 128.10, 129.76, 129.80, 130.39, 139.89, 140.89, 141.75, 141.85, 144.80, 156.43, 156.52, 168.96, 169.62, 170.04. LC–MS (ESI) *m*/*z* 609, 611 $[M+H]^+$. HPLC purity: 98.56%. HRMS (ESI) *m*/*z* calculated for $C_{33}H_{39}Cl_2N_4O_3$ $[M+H]^+$ 609.23937, found 609.23984.

5.1.15. N^2-{2-{[1-(*tert*-Butoxycarbonyl)piperidin-4-yl](methyl)-amino}-2-oxoethyl}-N^2-[5-chloro-2-(4-chlorophenoxy)phenyl]-N^1-(2-pyrrolidin-1-ylethyl)glycinamide (14i)

The title compound was synthesized according to the method described for the synthesis of **14n** to afford **14i** (351 mg, 94%) as a colorless amorphous solid. ^{1}H NMR (400 MHz, $CDCl_3$): δ (ppm) 1.30–1.60 (m, 2H), 1.44 (s, 9H), 1.60–1.80 (m, 4H), 1.80–1.90 (m, 2H), 2.30–2.50 (m, 6H), 2.50–2.80 (m, 5H), 3.28 (q, *J* = 6.6, 2H), 3.35–3.50 (m, 0.2H), 3.96 (s, 2H), 4.05–4.30 (m, 4H), 4.30–4.50 (m, 0.8H), 6.65–6.90 (m, 5H), 7.25 (d, *J* = 8.7, 2H), 8.15 (broad t, 1H).

LC–MS (ESI) *m*/*z* 662, 664 $[M+H]^+$.

5.1.16. N^2-[5-Chloro-2-(4-chlorophenoxy)phenyl]-N^2-{2-[methyl(piperidin-4-yl)amino]-2-oxoethyl}-N^1-(2-pyrrolidin-1-ylethyl)glycinamide (14j)

To a solution of **14i** (650 mg, 0.980 mmol) in $CHCl_3$ (20 mL) was added 4 mol/L HCl in dioxane (2.0 mL, 8.0 mmol), and the resulting mixture was stirred at room temperature for 6 h. The reaction mixture was poured into diluted aqueous NaOH, and extracted with $CHCl_3$. The organic layer was washed with brine, dried over $MgSO_4$. The insoluble material was filtered off, and the solution was concentrated under reduced pressure to afford **14j** (387 mg, 70%) as a pale yellow solid. Mp 125–127 °C. ^{1}H NMR (400 MHz, $CDCl_3$): δ (ppm) 1.40–1.60 (m, 4H), 1.63–1.73 (m, 4H), 1.73–2.12 (broad, 1H), 2.37–2.53 (m, 6H), 2.54–2.72 (m, 2H), 2.72, 2.74 (2s, 3H), 3.06–3.20 (m, 2H), 3.28 (q, *J* = 6.6, 2H), 3.32–3.43 (m, 0.3H),

3.96 (s, 2H), 4.15 (s, 1.4H), 4.21 (s, 0.6H), 4.33–4.45 (m, 0.7H), 6.74 (d, J = 8.7, 1H), 6.77–6.95 (m, 4H), 7.25 (d, J = 8.7, 2H), 8.24 (broad t, 1H). ^{13}C NMR (100 MHz, $CDCl_3$): δ (ppm) 23.53, 27.50, 28.52, 29.90, 31.05, 38.34, 45.93, 46.07, 51.67, 54.04, 54.60, 54.87, 55.62, 55.73, 58.78, 58.96, 118.48, 118.52, 118.82, 119.00, 121.16, 121.38, 122.81, 128.01, 129.69, 129.78, 130.48, 141.72, 141.92, 144.44, 144.80, 156.49, 168.93, 169.08, 169.91. LC–MS (ESI) m/z 562, 564 $[M+H]^+$. HPLC purity: 98.94%. HRMS (ESI) m/z calculated for $C_{28}H_{38}Cl_2N_5O_3$ $[M+H]^+$ 562.23462, found 562.23404.

5.1.17. N^2-{2-[(1-Acetylpiperidin-4-yl)(methyl)amino]-2-oxoethyl}-N^2-[5-chloro-2-(4-chlorophenoxy)phenyl]-N^1-(2-pyrrolidin-1-ylethyl)glycinamide (14k)

To a solution of **14j** (102 mg, 0.131 mmol) and TEA (0.060 mL, 0.43 mmol) in CH_2Cl_2 (3 mL) was added Ac_2O (0.030 mL, 0.32 mmol), and the resulting mixture was stirred at room temperature for 5 h. The reaction mixture was diluted with EtOAc, and the organic layer was washed with saturated aqueous $NaHCO_3$ and brine, and dried over $MgSO_4$. The insoluble material was filtered off, and the solution was concentrated under reduced pressure. The residue oil was purified by NH silica gel column chromatography (EtOAc/MeOH = 100:0 to 95:5) to afford **14k** (118 mg, quant.) as a colorless oil. ^{1}H NMR (400 MHz, $CDCl_3$): δ (ppm) 1.40–1.60 (m, 4H), 1.64–1.76 (m, 4H), 2.10 (s, 3H), 2.37–2.48 (m, 6H), 2.49–2.59 (m, 1H), 2.68 (s, 3H), 2.96–3.17 (m, 1H), 3.21–3.35 (m, 2H), 3.43–3.60 (m, 0.2H), 3.80–3.90 (m, 1H), 3.96 (s, 2H), 4.15 (s, 2H), 4.46–4.59 (m, 0.8H), 4.68–4.80 (m, 1H), 6.75 (d, J = 8.7, 1H), 6.79–6.91 (m, 4H), 7.26 (d, J = 8.7, 2H), 8.04–8.15 (m, 1H). ^{13}C NMR (100 MHz, $CDCl_3$): δ (ppm) 21.42, 23.52, 28.36, 28.53, 29.24, 38.39, 40.87, 45.68, 51.22, 54.03, 54.88, 55.61, 58.61, 58.77, 118.48, 118.57, 119.00, 121.39, 121.60, 122.78, 128.12, 129.81, 130.50, 141.64, 141.79, 144.56, 144.93, 156.42, 168.84, 169.33, 169.78. LC–MS (ESI) m/z 604, 606 $[M+H]^+$. HPLC purity: 98.24%. HRMS (ESI) m/z calculated for $C_{30}H_{40}Cl_2N_5O_4$ $[M+H]^+$ 604.24519, found 604.24597.

5.1.18. N^2-[5-Chloro-2-(4-chlorophenoxy)phenyl]-N^2-{2-{methyl-[1-(methylsulfonyl)piperidin-4-yl]amino}-2-oxoethyl}-N^1-(2-pyrrolidin-1-ylethyl)glycinamide (14l)

To a solution of **14j** (350 mg, 0.620 mmol) and TEA (0.130 mL, 0.933 mmol) in CH_2Cl_2 (20 mL) was added methanesulfonyl chloride (0.060 mL, 0.775 mmol), and the resulting mixture was stirred overnight at room temperature. To the reaction mixture was added H_2O (100 mL), and extracted with $CHCl_3$ (100 mL). The organic layer was washed with brine, and dried over $MgSO_4$. The insoluble material was filtered off, and the solution was concentrated under reduced pressure. The residue oil was purified by silica gel column chromatography ($CHCl_3$/MeOH = 95:5 to 90:10) to afford **14l** (397 mg, 100%) as a colorless oil. ^{1}H NMR (400 MHz, $CDCl_3$): δ (ppm) 1.52–1.61 (m, 2H), 1.65–1.77 (m, 2H), 1.79–2.00 (m, 2H), 2.50–3.04 (m, 8H), 2.74 (s, 3H), 2.79 (s, 3H), 3.29 (q, J = 6.6, 2H), 3.27–3.44 (m, 2H), 3.56–3.64 (m, 0.2H), 3.82–3.95 (m, 2H), 4.01 (s, 1.6H), 4.07 (s, 0.4H), 4.30 (s, 1.6H), 4.35–4.46 (m, 0.8H), 4.59 (br s, 0.4H), 6.73 (d, J = 8.2, 1H), 6.75–6.87 (m, 2H), 6.82 (d, J = 9.0, 2H), 7.26 (d, J = 9.0, 2H), 8.60 (broad, 1H). ^{13}C NMR (100 MHz, $CDCl_3$): δ (ppm) 23.38, 23.45, 27.57, 28.36, 28.67, 29.38, 29.70, 35.16, 35.31, 37.05, 45.54, 50.73, 53.46, 53.92, 53.98, 54.60, 55.98, 56.10, 59.02, 118.19, 118.67, 118.74, 120.90, 122.90, 127.99, 128.15, 129.69, 129.81, 130.46, 141.42, 141.75, 144.25, 156.51, 156.61, 170.00, 170.59. LC–MS (ESI) m/z 640, 642 $[M+H]^+$. HPLC purity: 99.20%. HRMS (ESI) m/z calculated for $C_{29}H_{40}Cl_2N_5O_5S$ $[M+H]^+$ 640.21217, found 640.21258.

5.1.19. N^2-[5-Chloro-2-(4-chlorophenoxy)phenyl]-N^2-{2-{[1-(methoxycarbonyl)piperidin-4-yl](methyl)amino}-2-oxoethyl}-N^1-(2-pyrrolidin-1-ylethyl)glycinamide (14m)

To a solution of **14j** (101 mg, 0.130 mmol) and TEA (0.060 ml, 0.43 mmol) in CH_2Cl_2 (3 mL) was added methyl chloroformate (0.030 ml, 0.39 mmol), and the resulting mixture was stirred at room temperature for 5 h. The reaction mixture was diluted with EtOAc, and the organic layer was washed with saturated aqueous $NaHCO_3$ and brine, and dried over $MgSO_4$. The insoluble material was filtered off, and the solution was concentrated under reduced pressure. The residue oil was purified by NH silica gel column chromatography (EtOAc/MeOH = 100:0 to 95:5) to afford **14m** (105 mg, 94%) as a colorless oil. ^{1}H NMR (400 MHz, $CDCl_3$): δ (ppm) 1.41–1.58 (m, 4H), 1.65–1.78 (m, 4H), 2.37–2.48 (m, 6H), 2.69, 2.70 (2s, 3H), 2.72–2.89 (m, 2H), 3.28 (q, J = 6.6, 2H), 3.40–3.54 (m, 0.2H), 3.70, 3.71 (2s, 3H), 3.95 (s, 2H), 4.14, 4.21 (2s, 2H), 4.10–4.37 (m, 2H), 4.41–4.53 (m, 0.8H), 6.74 (d, J = 8.2, 1H), 6.78–6.96 (m, 4H), 7.25 (d, J = 9.3, 2H), 8.10 (broad t, J = 5.1, 1H). ^{13}C NMR (100 MHz, $CDCl_3$): δ (ppm) 23.55, 27.44, 28.52, 28.62, 29.68, 38.39, 43.33, 51.25, 52.70, 52.84, 54.03, 54.15, 54.89, 55.67, 58.67, 58.84, 118.52, 118.61, 119.02, 119.20, 121.37, 121.58, 122.77, 128.14, 129.81, 130.51, 141.69, 144.60, 144.98, 155.77, 156.45, 168.97, 169.28, 169.79. LC–MS (ESI) m/z 620, 622 $[M+H]^+$. HPLC purity: 98.23%. HRMS (ESI) m/z calculated for $C_{30}H_{40}Cl_2N_5O_5$ $[M+H]^+$ 620.24010, found 620.24091.

5.1.20. N^2-[5-Chloro-2-(4-chlorophenoxy)phenyl]-N^2-{2-[3,4-dihydroisoquinolin-2(1*H*)-yl(methyl)amino]-2-oxoethyl}-N^1-(2-pyrrolidin-1-ylethyl)glycinamide (14o)

The title compound was synthesized according to the method described for the synthesis of **14n** to afford **14o** (1.20 g, 97%) as a colorless amorphous oil. ^{1}H NMR (400 MHz, $CDCl_3$): δ (ppm) 1.64–1.80 (m, 4H), 2.32–2.64 (m, 6H), 2.77–3.07 (m, 3H), 2.95 (s, 3H), 3.10–3.22 (m, 1H), 3.26 (q, J = 6.6, 2H), 3.62 (d, J = 13.8, 1H), 3.94 (s, 2H), 4.07 (d, J = 13.8, 1H), 4.34 (d, J = 18.2, 1H), 4.45 (d, J = 18.2, 1H), 6.74 (d, J = 8.2, 1H), 6.79 (dd, J = 2.6, 8.2, 1H), 6.82 (d, J = 9.2, 2H), 6.92 (d, J = 2.6, 1H), 6.97–7.03 (m, 1H), 7.10–7.22 (m, 3H), 7.25 (d, J = 9.2, 2H), 8.29 (broad, 1H). ^{13}C NMR (100 MHz, $CDCl_3$): δ (ppm) 23.48, 23.81, 29.90, 38.00, 49.90, 52.57, 53.96, 54.67, 55.46, 58.77, 118.56, 121.04, 122.83, 126.14, 126.73, 126.81, 127.86, 128.65, 129.72, 130.32, 132.71, 132.87, 142.12, 144.69, 156.60, 170.31, 172.26. LC–MS (ESI) m/z 610, 612 $[M+H]^+$. HPLC purity: 99.31%. HRMS (ESI) m/z calculated for $C_{32}H_{38}Cl_2N_5O_3$ $[M+H]^+$ 610.23462, found 610.23456.

5.1.21. N^2-[5-Chloro-2-(4-chlorophenoxy)phenyl]-N^2-{2-[methyl-{4-[(2-nitrophenyl)sulfonyl]piperazin-1-yl}amino]-2-oxoethyl}-N^1-(2-pyrrolidin-1-ylethyl)glycinamide (14p)

The title compound was synthesized according to the method described for the synthesis of **14n** to afford **14p** (725 mg, 97%) as a pale yellow oil. ^{1}H NMR (400 MHz, $CDCl_3$): δ (ppm) 1.62–1.75 (m, 4H), 2.34–2.46 (m, 6H), 2.62 (d, J = 9.3, 2H), 2.84 (s, 3H), 2.87–3.03 (m, 4H), 3.24 (q, J = 6.5, 2H), 3.82 (d, J = 10.3, 2H), 3.90 (s, 2H), 4.25 (s, 2H), 6.74 (d, J = 8.7, 1H), 6.77–6.83 (m, 3H), 6.91 (d, J = 2.1, 1H), 7.24 (d, J = 8.7, 2H), 7.64–7.69 (m, 1H), 7.71–7.80 (m, 2H), 7.96–8.04 (m, 2H). ^{13}C NMR (100 MHz, $CDCl_3$): δ (ppm) 23.51, 24.14, 38.39, 45.66, 50.84, 53.98, 54.77, 55.52, 58.51, 118.45, 118.80, 121.44, 122.87, 124.35, 128.02, 129.75, 130.39, 131.09, 131.11, 131.81, 134.16, 142.07, 144.90, 148.31, 156.48, 169.86, 171.57. LC–MS (ESI) m/z 748, 750 $[M+H]^+$.

5.1.22. N^2-[5-Chloro-2-(4-chlorophenoxy)phenyl]-N^2-{2-[methyl-(piperazin-1-yl)amino]-2-oxoethyl}-N^1-(2-pyrrolidin-1-ylethyl)-glycinamide (14q)

To a solution of **14p** (711 mg, 0.950 mmol) in CH_3CN (8 mL) were added Cs_2CO_3 (747 mg, 2.29 mmol) and 4-ethylthiophenol

(0.200 mL, 1.48 mmol), the resulting mixture was stirred at room temperature for 2 h. The reaction mixture was diluted with H_2O, and extracted with $CHCl_3$ (50 mL × 3). The organic layers were combined, and dried over Na_2SO_4. The insoluble material was filtered off, and the solution was concentrated under reduced pressure. The residue oil was purified by NH silica gel column chromatography (EtOAc/MeOH = 100:0 to 90:10) to afford **14q** (491 mg, 92%) as a colorless oil. ^{1}H NMR (400 MHz, $CDCl_3$): δ (ppm) 1.61–1.79 (m, 5H), 2.37 (t, J = 6.9, 2H), 2.39–2.46 (m, 4H), 2.55 (d, J = 9.8, 2H), 2.72–2.93 (m, 4H), 2.86 (s, 3H), 3.02 (d, J = 11.3, 2H), 3.23 (q, J = 6.5, 2H), 3.94 (s, 2H), 4.32 (s, 2H), 6.76 (d, J = 8.2, 1H), 6.79–6.89 (m, 3H), 6.94 (d, J = 2.6, 1H), 7.25 (d, J = 8.7, 2H), 8.11 (t, J = 5.4, 1H). ^{13}C NMR (100 MHz, $CDCl_3$): δ (ppm) 23.52, 23.74, 38.39, 45.91, 52.39, 54.01, 54.80, 55.36, 58.70, 118.40, 118.75, 121.26, 122.97, 127.83, 129.68, 130.38, 142.28, 144.82, 156.64, 170.09, 171.71. LC–MS (ESI) m/z 563, 565 $[M+H]^+$.

5.1.23. N^2-[5-Chloro-2-(4-chlorophenoxy)phenyl]-N^2-{2-{methyl-[4-(methylsulfonyl)piperadin-1-yl]amino}-2-oxoethyl}-N^1-(2-pyrrolidin-1-ylethyl)glycinamide (14r)

The title compound was synthesized from **14q** according to the method described for the synthesis of **14l** to afford **14r** (235 mg, 93%) as a colorless solid. Mp 144–147 °C. ^{1}H NMR (400 MHz, $CDCl_3$): δ (ppm) 1.63–1.76 (m, 4H), 2.39 (t, J = 6.7, 2H), 2.39–2.47 (m, 4H), 2.65 (d, J = 10.3, 2H), 2.81–3.00 (m, 4H), 2.82 (s, 3H), 2.86 (s, 3H), 3.26 (q, J = 6.5, 2H), 3.78 (d, J = 10.8, 2H), 3.93 (s, 2H), 4.30 (s, 2H), 6.76 (d, J = 8.7, 1H), 6.79–6.86 (m, 3H), 6.93 (d, J = 2.1, 1H), 7.25 (d, J = 9.3, 2H), 8.04 (t, J = 5.1, 1H). ^{13}C NMR (100 MHz, $CDCl_3$): δ (ppm) 23.54, 24.17, 35.40, 38.42, 45.59, 50.78, 54.01, 54.81, 55.58, 58.63, 118.49, 118.85, 121.50, 122.88, 128.07, 129.75, 130.40, 142.14, 144.98, 156.52, 169.88, 171.57. LC–MS (ESI) m/z 641, 643 $[M+H]^+$. HPLC purity: 98.96%. HRMS (ESI) m/z calculated for $C_{23}H_{39}Cl_2N_6O_5S$ $[M+H]^+$ 641.20742, found 641.20796.

5.1.24. N^2-[5-Chloro-2-(4-chlorophenoxy)phenyl]-N^2-{2-{[4-(methoxycarbonyl)piperidin-1-yl](methyl)amino}-2-oxoethyl}-N^1-(2-pyrrolidin-1-ylethyl)glycinamide (14s)

The title compound was synthesized from **14q** according to the method described for the synthesis of **14m** to afford **14s** (273 mg, 97%) as a colorless oil. ^{1}H NMR (400 MHz, $CDCl_3$): δ (ppm) 1.64–1.77 (m, 4H), 2.38 (t, J = 6.9, 2H), 2.39–2.47 (m, 4H), 2.55 (d, J = 10.8, 2H), 2.75 (dt, J = 3.1, 11.3, 2H), 2.83 (s, 3H), 2.96 (t, J = 11.8, 2H), 3.25 (q, J = 6.3, 2H), 3.72 (s, 3H), 3.94 (s, 2H), 4.01–4.26 (broad, 2H), 4.32 (s, 2H), 6.76 (d, J = 8.2, 1H), 6.79–6.86 (m, 3H), 6.94 (d, J = 2.6, 1H), 7.25 (d, J = 9.3, 2H), 8.06 (t, J = 5.1, 1H). ^{13}C NMR (100 MHz, $CDCl_3$): δ (ppm) 23.53, 23.87, 38.40, 43.48, 50.98, 52.94, 54.01, 54.80, 55.45, 58.64, 118.45, 118.85, 121.40, 122.88, 127.99, 129.74, 130.40, 142.15, 144.90, 155.54, 156.54, 169.97, 171.67. LC–MS (ESI) m/z 621, 623 $[M+H]^+$. HPLC purity: 99.35%. HRMS (ESI) m/z calculated for $C_{29}H_{39}Cl_2N_6O_5$ $[M+H]^+$ 621.23535, found 621.23537.

5.1.25. N^2-[5-Chloro-2-(4-chlorophenoxy)phenyl]-N^2-{2-[1,3-dihydro-2*H*-isoindol-2-yl(methyl)amino]-2-oxoethyl}-N^1-[2-(methylamino)ethyl]glycinamide hydrochloride (18a)

To a solution of **10** (276 mg, 0.746 mmol) in DMF (4 mL) was added EDC (146 mg, 0.762 mmol), and the resulting mixture was stirred at room temperature for 1 h. To the reaction mixture stirred under ice-cooling were added **12n** (142 mg, 0.769 mmol) and *N*,*N*-diisopropylethylamine (0.140 mL, 0.823 mmol), and the mixture was stirred at 0 °C for 40 min. To the reaction mixture was added *tert*-butyl (2-aminoethyl)methylcarbamate (160 mg, 0.918 mmol), $HOBt{\cdot}H_2O$ (145 mg, 0.947 mmol), and EDC (180 mg, 0.939 mmol), and the resulting mixture was stirred at room temperature for 2 h. The reaction mixture was diluted with EtOAc, and the organic layer was washed with H_2O, diluted aqueous NaOH, and brine, and dried over $MgSO_4$. The insoluble material was filtered off, and the solution was concentrated under reduced pressure. The residue oil was purified by silica gel column chromatography (hexane/EtOAc = 70:30 to 0:100) to afford 338 mg of **17a** as a pale yellow oil. To the obtained oil in CH_2Cl_2 (2 mL) was added TFA (2 mL), and the resulting mixture was stirred at room temperature for 2 h. A diluted aqueous NaOH solution was added to the reaction mixture to give an alkaline solution with a pH greater than 10. The solution was extracted with CH_2Cl_2 (50 mL × 2), and the organic layers were combined, and dried over Na_2SO_4. The insoluble material was filtered off, and the solution was concentrated under reduced pressure. The residue oil was purified by NH silica gel column chromatography (EtOAc/MeOH = 100:0 to 90:10) to afford a colorless oil (227 mg). To the obtained oil dissolved with diethyl ether (2 mL) was added 4 mol/L HCl in dioxane (0.105 ml, 0.420 mmol), and the resulting mixture was stirred at room temperature for 20 min. The precipitated solid was filtered, washed with diethyl ether, and dried under reduced pressure to give **18a** (175 mg, 40%) as a colorless solid. Mp 90–100 °C. ^{1}H NMR (400 MHz, DMSO-d_6): δ (ppm) 2.49 (s, 3H), 2.78–2.87 (m, 2H), 2.83 (s, 3H), 3.25 (d, J = 6.2, 2H), 3.94 (s, 2H), 4.02 (d, J = 11.8, 2H), 4.21 (d, J = 11.8, 2H), 4.51 (s, 2H), 6.75–6.85 (m, 3H), 6.88 (d, J = 8.7, 2H), 7.25 (br s, 4H), 7.40 (d, J = 8.7, 2H), 8.50 (t, J = 5.7, 1H), 8.73 (br s, 2H). ^{13}C NMR (100 MHz, DMSO-d_6): δ (ppm) 24.21, 32.23, 34.64, 47.45, 53.85, 54.77, 56.98, 117.05, 118.68, 119.14, 122.40, 123.31, 126.31, 127.02, 128.85, 129.46, 137.34, 142.90, 143.21, 156.52, 170.49, 172.13. LC–MS (ESI) m/z 556, 558 $[M+H]^+$. HPLC purity: 97.77%. HRMS (ESI) m/z calculated for $C_{28}H_{32}Cl_2N_5O_3$ $[M+H]^+$ 556.18767, found 556.18816.

5.1.26. N^2-[5-Chloro-2-(4-chlorophenoxy)phenyl]-N^2-{2-[1,3-dihydro-2*H*-isoindol-2-yl(methyl)amino]-2-oxoethyl}-N^1-[2-(ethylamino)ethyl]glycinamide hydrochloride (18b)

The title compound was synthesized according to the method described for the synthesis of **18a** to afford **18b** (350 mg, 73%) as a colorless solid. Mp 98–108 °C. ^{1}H NMR (400 MHz, DMSO-d_6): δ (ppm) 1.15 (t, J = 7.2, 3H), 2.79–2.95 (m, 4H), 2.83 (s, 3H), 3.25 (q, J = 5.8, 2H), 3.94 (s, 2H), 4.02 (d, J = 11.3, 2H), 4.21 (d, J = 11.3, 2H), 4.51 (s, 2H), 6.75–6.85 (m, 3H), 6.88 (d, J = 9.3, 2H), 7.25 (s, 4H), 7.40 (d, J = 9.3, 2H), 8.52 (t, J = 5.7, 1H), 8.74 (broad, 2H). ^{13}C NMR (100 MHz, DMSO-d_6): δ (ppm) 10.79, 24.19, 34.75, 41.64, 45.32, 53.82, 54.77, 56.99, 117.01, 118.68, 119.11, 122.39, 123.31, 126.30, 127.01, 128.85, 129.46, 137.34, 142.90, 143.18, 156.54, 170.42, 172.15. LC–MS (ESI) m/z 570, 572 $[M+H]^+$. HPLC purity: 97.22%. HRMS (ESI) m/z calculated for $C_{29}H_{34}Cl_2N_5O_3$ $[M+H]^+$ 570.20332, found 570.20323.

5.1.27. *N*-[5-Chloro-2-(4-chlorophenoxy)phenyl]-*N*-{2-[2,3-dihydro-1*H*-inden-2-yl(methyl)amino]-2-oxoethyl}glycine (13e)

To a solution of **10** (556 mg, 1.50 mmol) in DMF (6 mL) was added EDC (294 mg, 1.53 mmol), and the resulting mixture was stirred at room temperature for 1 h. To the reaction mixture stirred under ice-cooling were added **12e** (284 mg, 1.55 mmol) and *N*,*N*-diisopropylethylamine (0.280 mL, 1.65 mmol), and the mixture was stirred at 0 °C for 1 h. To the reaction mixture were added H_2O (6 mL), 1 mol/L aqueous HCl (6 mL), and the precipitated solid was filtered, washed with H_2O, dried under reduced pressure, and washed with diethyl ether to give **13e** (684 mg, 91%) as a colorless solid. Mp 171–172 °C. ^{1}H NMR (400 MHz, DMSO-d_6): δ (ppm) 2.61 (s, 3H), 2.75–3.07 (m, 4H), 4.08 (s, 2H), 4.25 (s, 1.2H), 4.42 (s, 0.8H), 4.60–4.76 (m, 0.4H), 5.12–5.27 (m, 0.6H), 6.85 (br s, 3H), 6.91 (d, J = 8.7, 2H), 7.08–7.26 (m, 4H), 7.39 (d, J = 8.7, 2H), 12.91 (br s, 1H). ^{13}C NMR (100 MHz, DMSO-d_6): δ (ppm) 27.35, 28.78, 35.19,

35.43, 52.96, 54.48, 55.58, 117.89, 118.19, 118.65, 118.79, 119.54, 119.83, 122.88, 123.14, 124.14, 126.37, 126.44, 128.70, 128.84, 129.50, 140.58, 140.98, 142.48, 143.42, 143.81, 156.07, 156.16, 169.19, 169.37, 171.53, 171.59. LC–MS (ESI) *m*/*z* 499, 501 $[M+H]^+$. Anal. Calculated for $C_{26}H_{24}Cl_2N_2O_4 \cdot 1/3H_2O$: C, 61.79; H, 4.92; N, 5.54; Cl, 14.03. Found: C, 61.56; H, 4.63; N, 5.51; Cl, 13.96.

5.1.28. N^2-[5-Chloro-2-(4-chlorophenoxy)phenyl]-N^2-{2-[2,3-dihydro-1*H*-inden-2-yl(methyl)amino]-2-oxoethyl}-N^1-methyl (2-pyrrolidin-1-ylethyl)glycinamide (16a)

To a solution of **13e** (64 mg, 0.13 mmol) and 1-(2-methylaminoethyl)pyrrolidine (28 mg, 0.22 mmol) in DMF (2 mL) under ice-cooling were added HOBt·H_2O (39 mg, 0.25 mmol), and EDC (48 mg, 0.25 mmol), and the resulting mixture was stirred overnight at room temperature. The reaction mixture was diluted with EtOAc, and the organic layer was washed with H_2O, diluted aqueous NaOH, and brine, and dried over Na_2SO_4. The insoluble material was filtered off, and the solution was concentrated under reduced pressure. The residue oil was purified by NH silica gel column chromatography (hexane/EtOAc = 80:20 to 0:100) to afford **16a** (49 mg, 62%) as a colorless oil. ^{1}H NMR (400 MHz, $CDCl_3$): δ (ppm) 1.72–1.85 (m, 2H), 2.02–2.18 (m, 2H), 2.47–2.65 (m, 5H), 2.58, 2.61 (2s, 3H), 2.70–3.17 (m, 8H), 3.23–3.32, 3.45–3.52 (2 m, 2H), 4.17–4.36 (m, 4H), 4.53–4.64 (m, 0.4H), 5.46–5.58 (m, 0.6H), 6.67–6.93 (m, 5H), 7.13–7.29 (m, 6H). ^{13}C NMR (100 MHz, DMSO-d_6): δ (ppm) 23.52, 23.58, 27.61, 28.95, 33.77, 34.80, 36.04, 36.08, 36.32, 36.38, 47.03, 48.35, 52.77, 53.02, 53.33, 53.72, 53.80, 53.95, 54.15, 54.24, 54.40, 54.54, 56.02, 118.64, 118.70, 118.75, 118.99, 119.16, 119.45, 120.32, 120.60, 122.02, 122.15, 122.24, 124.42, 126.74, 126.97, 127.87, 129.65, 129.68, 130.05, 140.27, 141.11, 143.68, 143.82, 144.48, 144.68, 156.40, 169.26, 169.38, 169.51, 169.64, 169.73. LC–MS (ESI) *m*/*z* 609, 611 $[M+H]^+$. HPLC purity: 97.84%. HRMS (ESI) *m*/*z* calculated for $C_{33}H_{39}Cl_2N_4O_3$ $[M+H]^+$ 609.23937, found 609.23969.

5.1.29. N^2-[5-Chloro-2-(4-chlorophenoxy)phenyl]-N^2-{2-[2,3-dihydro-1*H*-inden-2-yl(methyl)amino]-2-oxoethyl}-N^1-(3-pyrrolidin-1-ylpropyl)glycinamide (16b)

The title compound was synthesized according to the method described for the synthesis of **16a** to afford **16b** (508 mg, 83%) as a colorless amorphous solid. ^{1}H NMR (400 MHz, $CDCl_3$): δ (ppm) 1.64–1.78 (m, 2H), 1.78–1.89 (m, 4H), 2.42–2.70 (m, 6H), 2.64 (s, 1.8H), 2.69 (s, 1.2H), 2.77 (dd, *J* = 6.1, 16.4, 1.2H), 2.94 (dd, *J* = 6.1, 16.4, 0.8H), 3.03 (dd, *J* = 8.7, 16.4, 0.8H), 3.09 (dd, *J* = 8.7, 16.4, 1.2H), 3.23 (q, *J* = 6.1, 2H), 3.97 (s, 2H), 4.20 (s, 1.2H), 4.32 (s, 0.8H), 4.43–4.58 (m, 0.4H), 5.34–5.44 (m, 0.6H), 6.73–6.92 (m, 5H), 7.12–7.23 (m, 4H), 7.29 (d, *J* = 8.7, 2H), 8.51, 8.61 (2 broad, 1H). ^{13}C NMR (100 MHz, $CDCl_3$): δ (ppm) 23.41, 27.84, 28.04, 29.04, 36.08, 36.36, 37.29, 53.33, 53.68, 53.83, 56.10, 56.21, 56.47, 58.95, 59.17, 117.89, 118.22, 118.59, 118.66, 120.69, 121.00, 122.85, 122.94, 124.43, 124.49, 126.88, 127.19, 128.20, 129.83, 129.88, 130.39, 130.44, 139.91, 140.83, 141.34, 141.51, 144.04, 144.42, 156.39, 156.43, 169.41, 169.78, 169.97. LC–MS (ESI) *m*/*z* 609, 611 $[M+H]^+$. HPLC purity: 98.19%. HRMS (ESI) *m*/*z* calculated for $C_{33}H_{39}Cl_2N_4O_3$ $[M+H]^+$ 609.23937, found 609.24003.

5.1.30. N^2-[5-Chloro-2-(4-chlorophenoxy)phenyl]-N^2-{2-[2,3-dihydro-1*H*-inden-2-yl(methyl)amino]-2-oxoethyl}-N^1-(2-piperidin-1-ylethyl)glycinamide (16c)

The title compound was synthesized according to the method described for the synthesis of **16a** to afford **16c** (554 mg, 91%) as a colorless amorphous solid. ^{1}H NMR (400 MHz, $CDCl_3$): δ (ppm) 1.31–1.44 (m, 2H), 1.45–1.59 (m, 4H), 2.20–2.45 (m, 6H), 2.65 (s, 1.8H), 2.70 (s, 1.2H), 2.78 (dd, *J* = 6.2, 16.4, 1.2H), 2.95 (dd, *J* = 6.2, 16.4, 0.8H), 3.03 (dd, *J* = 8.7, 16.4, 0.8H), 3.10 (dd, *J* = 8.7, 16.4, 1.2H), 3.28 (q, *J* = 6.2, 2H), 3.98 (s, 2H), 4.17 (s, 1.2H), 4.30 (s, 0.8H), 4.46–4.58 (m, 0.4H), 5.37–5.48 (m, 0.6H), 6.76 (d, *J* = 8.7, 1H), 6.78–6.97 (m, 2H), 6.86 (d, *J* = 9.3, 2H), 7.13–7.24 (m, 4H), 7.28 (d, *J* = 9.3, 2H), 8.14, 8.25 (2 broad, 1H). ^{13}C NMR (100 MHz, $CDCl_3$): δ (ppm) 24.22, 25.83, 27.90, 28.98, 36.11, 36.34, 36.46, 53.13, 54.34, 55.49, 55.78, 56.13, 57.52, 58.68, 58.92, 118.50, 118.58, 118.72, 119.10, 121.13, 121.43, 122.70, 122.82, 124.44, 124.48, 126.84, 127.16, 128.04, 129.78, 129.81, 130.44, 130.49, 139.97, 140.92, 141.72, 141.87, 144.37, 144.74, 156.42, 156.48, 169.05, 169.39, 169.83. LC–MS (ESI) *m*/*z* 609, 611 $[M+H]^+$. HPLC purity: 99.92%. HRMS (ESI) *m*/*z* calculated for $C_{33}H_{39}Cl_2N_4O_3$ $[M+H]^+$ 609.23937, found 609.24023.

5.1.31. N^2-[5-Chloro-2-(4-chlorophenoxy)phenyl]-N^2-{2-[2,3-dihydro-1*H*-inden-2-yl(methyl)amino]-2-oxoethyl}-N^1-[2-(dimethylamino)ethyl]glycinamide (16d)

The title compound was synthesized according to the method described for the synthesis of **16a** to afford **16d** (494 mg, 87%) as a colorless amorphous solid. ^{1}H NMR (400 MHz, $CDCl_3$); δ (ppm) 2.15, 2.16 (2s, 6H), 2.23–2.29 (m, 2H), 2.66 (s, 1.8H), 2.70 (s, 1.2H), 2.78 (dd, *J* = 6.2, 16.4, 1.2H), 2.96 (dd, *J* = 6.2, 16.4, 0.8H), 3.05 (dd, *J* = 8.7, 16.4, 0.8H), 3.10 (dd, *J* = 8.7, 16.4, 1.2H), 3.25 (q, *J* = 6.6, 2H), 3.98 (s, 2H), 4.17 (s, 1.2H), 4.30 (s, 0.8H), 4.48–4.59 (m, 0.4H), 5.37–5.58 (m, 0.6H), 6.762 (d, *J* = 8.7, 1H), 6.78–6.99 (m, 2H), 6.87 (d, *J* = 9.3, 2H), 7.13–7.22 (m, 4H), 7.28 (d, *J* = 9.3, 2H), 8.12 (t, *J* = 5.1, 0.4H), 8.25 (t, *J* = 5.1, 0.6H). ^{13}C NMR (100 MHz, $CDCl_3$): δ (ppm) 27.91, 28.98, 36.09, 36.35, 37.16, 45.38, 53.13, 55.50, 55.81, 56.11, 58.16, 58.69, 58.94, 118.59, 118.64, 118.89, 119.31, 121.21, 121.54, 122.62, 122.74, 124.44, 124.50, 126.85, 127.17, 128.07, 129.76, 129.81, 130.43, 130.48, 139.98, 140.90, 141.66, 141.82, 144.54, 144.93, 156.40, 156.47, 169.14, 169.50, 169.90. LC–MS (ESI) *m*/*z* 569, 571 $[M+H]^+$. HPLC purity: 99.13%. HRMS (ESI) *m*/*z* calculated for $C_{30}H_{35}Cl_2N_4O_3$ $[M+H]^+$ 569.20807, found 569.20824.

5.1.32. N^2-[5-Chloro-2-(4-chlorophenoxy)phenyl]-N^2-{2-[2,3-dihydro-1*H*-inden-2-yl(methyl)amino]-2-oxoethyl}-N^1-[2-(methylamino)ethyl]glycinamide hydrochloride (16m)

To a solution of **13e** (371 mg, 1.00 mmol) and *tert*-butyl (2-aminoethyl)methylcarbamate (160 mg, 0.918 mmol) in DMF (3 mL) under ice-cooling were added HOBt·H_2O (100 mg, 0.653 mmol), and EDC (119 mg, 0.621 mmol), and the resulting mixture was stirred overnight at room temperature. The reaction mixture was diluted with EtOAc, and the organic layer was washed with H_2O, diluted aqueous NaOH, and brine, and dried over $MgSO_4$. The insoluble material was filtered off, and the solution was concentrated under reduced pressure. The residue oil was purified by silica gel column chromatography (hexane/EtOAc = 70:30 to 0:100) to afford 233 mg of **16e** as a colorless oil. To the obtained oil in CH_2Cl_2 (1 mL) was added 4 mol/L HCl in dioxane (1.0 mL, 4.0 mmol), and the resulting mixture was stirred at room temperature for 1 h. To the reaction mixture was diluted with diethyl ether (20 mL) to allow precipitation of a solid. This solid was filtered, washed with diethyl ether, and dried under reduced pressure to give **16m** (166 mg, 56%) as a colorless solid. Mp 98–109 °C. ^{1}H NMR (400 MHz, DMSO-d_6): δ (ppm) 2.47–2.53 (m, 3H), 2.61 (s, 1.2H), 2.66 (s, 1.8H), 2.78–3.07 (m, 6H), 3.25 (d, *J* = 6.0, 2H), 3.94, 3.95 (2s, 2H), 4.35 (s, 1.2H), 4.51 (s, 0.8H), 4.61–4.72 (m, 0.4H), 5.16–5.27 (m, 0.6H), 6.77–6.93 (m, 5H), 7.12–7.24 (m, 4H), 7.41 (d, *J* = 9.2, 2H), 8.68 (t, *J* = 5.6, 1H), 8.73 (br s, 2H). ^{13}C NMR (100 MHz, DMSO-d_6): δ (ppm) 27.39, 28.76, 32.23, 34.61, 35.17, 35.44, 47.44, 52.93, 54.75, 54.89, 55.54, 57.14, 57.32, 117.12, 117.36, 118.61, 118.75, 119.06, 119.33, 123.05, 123.18, 124.16, 126.39, 126.49, 128.76, 128.87, 129.51, 140.56, 140.98, 142.53, 142.61, 142.95, 143.39, 156.38, 169.22, 169.49, 170.37. LC–MS (ESI) *m*/*z* 555, 557 $[M+H]^+$. HPLC purity: 98.38%. HRMS (ESI) *m*/*z* calculated for $C_{29}H_{33}Cl_2N_4O_3$ $[M+H]^+$ 555.19242, found 555.19282.

5.1.33. N^2-[5-Chloro-2-(4-chlorophenoxy)phenyl]-N^2-{2-[2,3-dihydro-1*H*-inden-2-yl(methyl)amino]-2-oxoethyl}-N^1-[2-(ethylamino)ethyl]glycinamide hydrochloride (16n)

The title compound was synthesized according to the method described for the synthesis of **16m** to afford **16n** (252 mg, 83%) as a colorless solid. Mp 97–108 °C. ^{1}H NMR (400 MHz, DMSO-d_6): δ (ppm) 1.11–1.22 (m, 3H), 2.62 (s, 1.2H), 2.66 (s, 1.8H), 2.76–3.09 (m, 8H), 3.26 (d, J = 6.0, 2H), 3.94, 3.95 (2s, 2H), 4.35 (s, 1.2H), 4.51 (s, 0.8H), 4.61–4.73 (m, 0.4H), 5.15–5.28 (m, 0.6H), 6.76–6.96 (m, 5H), 7.10–7.26 (m, 4H), 7.41 (d, J = 8.7, 2H), 8.64–8.75 (m, 1H), 8.77 (br s, 2H). ^{13}C NMR (100 MHz, DMSO-d_6): δ (ppm) 10.82, 27.39, 28.76, 34.75, 35.18, 35.43, 41.68, 45.34, 52.96, 54.80, 54.91, 55.56, 57.14, 57.29, 117.16, 117.37, 118.63, 118.75, 119.10, 119.37, 123.05, 123.16, 124.15, 126.40, 126.49, 128.76, 128.86, 129.51, 140.54, 140.97, 142.53, 142.59, 143.01, 143.42, 156.38, 169.26, 169.48, 170.35. LC–MS (ESI) m/z 569, 571 [M+H]$^+$. HPLC purity: 98.92%. HRMS (ESI) m/z calculated for $C_{30}H_{35}Cl_2N_4O_3$ [M+H]$^+$ 569.20807, found 569.20839.

5.1.34. N^2-[5-Chloro-2-(4-chlorophenoxy)phenyl]-N^2-{2-[2,3-dihydro-1*H*-inden-2-yl(methyl)amino]-2-oxoethyl}-N^1-[2-(isopropylamino)ethyl]glycinamide hydrochloride (16o)

The title compound was synthesized according to the method described for the synthesis of **16m** to afford **16o** (96 mg, 61%) as a colorless solid. Mp 93–101 °C. ^{1}H NMR (400 MHz, DMSO-d_6): δ (ppm) 1.19 (d, J = 6.2, 2.4H), 1.21 (d, J = 6.2, 3.6H), 2.62 (s, 1.2H), 2.66 (s, 1.8H), 2.72–3.07 (m, 6H), 3.19–3.34 (m, 3H), 3.94 (s, 1.2H), 3.95 (s, 0.8H), 4.34 (s, 1.2H), 4.51 (s, 0.8H), 4.61–4.72 (m, 0.4H), 5.16–5.27 (m, 0.6H), 6.78–6.86 (m, 3H), 6.87–6.95 (m, 2H), 7.11–7.25 (m, 4H), 7.41 (d, J = 9.3, 2H), 8.65–8.74 (m, 1H), 8.81 (br s, 2H). ^{13}C NMR (100 MHz, DMSO-d_6): δ (ppm) 18.33, 27.38, 28.77, 34.92, 35.20, 35.42, 42.85, 49.19, 52.97, 54.73, 54.83, 55.59, 57.12, 57.25, 117.23, 117.42, 118.69, 118.81, 119.13, 119.39, 122.97, 123.07, 124.15, 126.41, 126.49, 128.72, 128.81, 129.48, 129.51, 140.54, 140.97, 142.55, 142.60, 143.12, 143.51, 156.32, 156.35, 169.19, 169.39, 170.32. LC–MS (ESI) m/z 583, 585 [M+H]$^+$. HPLC purity: 97.96%. HRMS (ESI) m/z calculated for $C_{31}H_{37}Cl_2N_4O_3$ [M+H]$^+$ 583.22372, found 583.22415.

5.1.35. N^2-[5-Chloro-2-(4-chlorophenoxy)phenyl]-N^2-{2-[2,3-dihydro-1*H*-inden-2-yl(methyl)amino]-2-oxoethyl}-N^1-(piperidin-3-yl)glycinamide hydrochloride (16p)

The title compound was synthesized according to the method described for the synthesis of **16m** to afford **16p** (131 mg, 73%) as a colorless solid. Mp 124–136 °C. ^{1}H NMR (400 MHz, DMSO-d_6): δ (ppm) 1.22–1.37 (m, 1H), 1.55–1.86 (m, 3H), 2.50–2.60 (m, 1H), 2.60 (s, 1.2H), 2.67 (s, 1.8H), 2.72–3.16 (m, 7H), 3.77–3.90 (m, 1H), 3.93 (s, 2H), 4.34 (d, J = 18.0, 0.6H), 4.39 (d, J = 18.0, 0.6H), 4.48 (d, J = 18.0, 0.4H), 4.54 (d, J = 18.0, 0.4H), 4.61–4.72 (m, 0.4H), 5.14–5.26 (m, 0.6H), 6.77–6.94 (m, 5H), 7.11–7.25 (m, 4H), 7.42 (d, J = 9.3, 2H), 8.78 (d, J = 7.2, 1H), 8.96 (br s, 1H), 9.12 (br s, 1H). ^{13}C NMR (100 MHz, DMSO-d_6): δ (ppm) 20.27, 27.42, 27.67, 28.74, 35.16, 35.46, 42.49, 42.72, 45.80, 53.00, 54.98, 55.10, 55.49, 57.29, 57.52, 116.95, 117.16, 118.67, 118.79, 119.07, 119.31, 123.09, 123.17, 124.16, 126.39, 126.45, 126.50, 128.76, 128.86, 129.50, 129.56, 140.55, 140.94, 140.96, 142.18, 142.29, 143.03, 143.40, 156.37, 169.31, 169.44, 169.75. LC–MS (ESI) m/z 581, 583 [M+H]$^+$. HPLC purity: 95.88%. HRMS (ESI) m/z calculated for $C_{31}H_{35}Cl_2N_4O_3$ [M+H]$^+$ 581.20807, found 581.20836.

5.1.36. N^2-[5-Chloro-2-(4-chlorophenoxy)phenyl]-N^2-{2-[2,3-dihydro-1*H*-inden-2-yl(methyl)amino]-2-oxoethyl}-N^1-(pyrrolidin-3-yl)glycinamide hydrochloride (16q)

The title compound was synthesized according to the method described for the synthesis of **16m** to afford **16q** (106 mg, 68%) as a colorless solid. Mp 136–146 °C. ^{1}H NMR (400 MHz, DMSO-d_6): δ (ppm) 1.56–1.70 (m, 1H), 1.92–2.07 (m, 1H), 2.62 (s, 1.2H), 2.69 (s, 1.8H), 2.77–3.30 (m, 8H), 3.84–4.00 (m, 2H), 4.06–4.18 (m, 1H), 4.31 (d, J = 17.9, 0.6H), 4.44 (d, J = 17.9, 0.6H), 4.47 (d, J = 17.4, 0.4H), 4.59 (d, J = 17.4, 0.4H), 4.65–4.75 (m, 0.4H), 5.18–5.29 (m, 0.6H), 6.77–6.93 (m, 5H), 7.11–7.25 (m, 4H), 7.41 (d, J = 9.2, 2H), 8.88 (d, J = 6.1, 0.6H), 8.91 (d, J = 6.1, 0.4H), 9.14 (br s, 1H), 9.36 (br s, 1H). ^{13}C NMR (100 MHz, DMSO-d_6): δ (ppm) 27.44, 28.77, 29.58, 35.19, 35.50, 43.13, 43.20, 47.98, 48.04, 48.63, 52.91, 54.73, 54.90, 55.53, 57.35, 57.63, 116.96, 117.18, 118.59, 118.75, 119.12, 119.34, 123.13, 123.24, 124.16, 126.39, 126.49, 128.78, 128.89, 129.47, 129.51, 140.55, 140.96, 140.99, 142.34, 142.42, 142.99, 143.39, 156.40, 156.45, 169.41, 169.73, 169.89. LC–MS (ESI) m/z 567, 569 [M+H]$^+$. HPLC purity: 99.30%. HRMS (ESI) m/z calculated for $C_{30}H_{33}Cl_2N_4O_3$ [M+H]$^+$ 567.19242, found 567.19287.

5.1.37. N^2-[5-Chloro-2-(4-chlorophenoxy)phenyl]-N^2-{2-[2,3-dihydro-1*H*-inden-2-yl(methyl)amino]-2-oxoethyl}-N^1-[(2*S*)-pyrrolidin-2-ylmethyl]glycinamide hydrochloride (16r)

The title compound was synthesized according to the method described for the synthesis of **16m** to afford **16r** (117 mg, 75%) as a colorless solid. Mp 113–125 °C. ^{1}H NMR (400 MHz, DMSO-d_6): δ (ppm) 1.43–1.59 (m, 1H), 1.71–1.93 (m, 3H), 2.61 (s, 1.2H), 2.65 (s, 1.8H), 2.82 (dd, J = 6.7, 16.4, 1.2H), 2.90 (dd, J = 6.7, 16.4, 0.8H), 2.95 (dd, J = 8.7, 16.4, 1.2H), 3.00 (dd, J = 8.7, 16.4, 0.8H), 3.05–3.19 (m, 2H), 3.20–3.51 (m, 3H), 3.96, 3.97 (2s, 2H), 4.36 (s, 1.2H), 4.52 (s, 0.8H), 4.59–4.71 (m, 0.4H), 5.15–5.26 (0.6H), 6.76–6.94 (m, 5H), 7.11–7.24 (m, 4H), 7.42 (d, J = 8.7, 2H), 8.69 (br s, 1H), 8.85 (t, J = 5.4, 1H), 9.44 (br s, 1H). ^{13}C NMR (100 MHz, DMSO-d_6): δ (ppm) 22.70, 26.95, 27.37, 28.75, 35.16, 35.42, 44.37, 52.95, 54.90, 55.03, 55.52, 57.16, 57.35, 58.70, 66.25, 117.08, 117.33, 118.68, 118.81, 119.07, 119.33, 123.00, 123.11, 124.15, 126.40, 126.45, 126.49, 128.74, 128.84, 129.51, 129.55, 140.54, 140.96, 142.28, 142.39, 143.00, 143.42, 156.28, 156.31, 169.25, 169.53, 170.53. LC–MS (ESI) m/z 581, 583 [M+H]$^+$. HPLC purity: 99.80%. HRMS (ESI) m/z calculated for $C_{31}H_{35}Cl_2N_4O_3$ [M+H]$^+$ 581.20807, found 581.20828.

5.1.38. N^2-[5-Chloro-2-(4-chlorophenoxy)phenyl]-N^2-{2-[2,3-dihydro-1*H*-inden-2-yl(methyl)amino]-2-oxoethyl}-N^1-[(2*R*)-pyrrolidin-2-ylmethyl]glycinamide hydrochloride (16s)

The title compound was synthesized according to the method described for the synthesis of **16m** to afford **16s** (387 mg, 76%) as a colorless solid. Mp 110–118 °C. ^{1}H NMR (400 MHz, DMSO-d_6): δ (ppm) 1.44–1.59 (m, 1H), 1.73–1.92 (m, 3H), 2.61 (s, 1.2H), 2.65 (s, 1.8H), 2.82 (dd, J = 6.7, 16.4, 1.2H), 2.90 (dd, J = 6.7, 16.4, 0.8H), 2.95 (dd, J = 8.7, 16.4, 1.2H), 3.00 (dd, J = 8.7, 16.4, 0.8H), 3.05–3.17 (m, 2H), 3.18–3.49 (m, 3H), 3.97 (s, 2H), 4.34 (s, 1.2H), 4.50 (s, 0.8H), 4.59–4.70 (m, 0.4H), 5.14–5.26 (0.6H), 6.76–6.94 (m, 5H), 7.11–7.24 (m, 4H), 7.42 (d, J = 8.7, 2H), 8.52 (br s, 1H), 8.87 (t, J = 5.1, 1H), 9.19 (br s, 1H). ^{13}C NMR (100 MHz, DMSO-d_6): δ (ppm) 22.71, 26.96, 27.36, 28.75, 35.16, 35.41, 44.34, 52.94, 54.89, 55.02, 55.52, 57.16, 57.35, 58.69, 117.08, 117.32, 118.67, 118.82, 119.07, 119.32, 122.99, 123.12, 124.15, 126.39, 126.44, 126.49, 128.73, 128.84, 129.50, 129.55, 140.54, 140.96, 142.29, 142.39, 142.99, 143.41, 156.31, 169.25, 169.52, 170.51. LC–MS (ESI) m/z 581, 583 [M+H]$^+$. HPLC purity: 98.85%. HRMS (ESI) m/z calculated for $C_{31}H_{35}Cl_2N_4O_3$ [M+H]$^+$ 581.20807, found 581.20824.

5.1.39. N^2-[5-Chloro-2-(4-chlorophenoxy)phenyl]-N^2-{2-[2,3-dihydro-1*H*-inden-2-yl(methyl)amino]-2-oxoethyl}-N^1-(piperidin-2-ylmethyl)glycinamide hydrochloride (16t)

The title compound was synthesized according to the method described for the synthesis of **16m** to afford **16t** (369 mg, 84%) as a colorless solid. Mp 124–134 °C. ^{1}H NMR (400 MHz, DMSO-d_6): δ (ppm) 1.20–1.44 (m, 2H), 1.46–1.75 (m, 4H), 2.60 (s, 1.2H), 2.65

(s, 1.8H), 2.73–3.06 (m, 6H), 3.09–3.35 (m, 3H), 3.98 (s, 2H), 4.38 (s, 1.2H), 4.53 (s, 0.8H), 4.59–4.70 (m, 0.4H), 5.13–5.25 (m, 0.6H), 6.77–6.94 (m, 5H), 7.10–7.24 (m, 4H), 7.38–7.45 (m, 2H), 8.3–9.0 (broad, 2H), 8.77 (t, J = 6.2, 0.4H), 8.82 (t, J = 6.2, 0.6H). ^{13}C NMR (100 MHz, DMSO-d_6): δ (ppm) 21.27, 21.53, 25.60, 27.38, 28.77, 35.16, 35.40, 40.33, 43.81, 52.99, 54.90, 55.11, 55.27, 55.54, 57.26, 57.47, 117.02, 117.36, 118.65, 118.76, 118.98, 119.30, 123.01, 123.13, 124.15, 126.41, 126.44, 126.50, 128.78, 128.88, 129.50, 129.55, 140.54, 140.96, 142.22, 142.41, 142.94, 143.40, 156.28, 156.32, 169.43, 169.71, 170.36. LC–MS (ESI) m/z 595, 597 $[M+H]^+$. HPLC purity: 98.37%. HRMS (ESI) m/z calculated for $C_{32}H_{37}Cl_2N_4O_3$ $[M+H]^+$ 595.22372, found 595.22394.

5.1.40. *N*-Methyl-1,3-dihydro-2*H*-isoindol-2-amine hydrochloride (12n)[23]

Xylylene dibromide (160 g, 606 mmol) and *tert*-butyl 1-methylhydrazinecarboxylate (**26**) (88.5 g, 606 mmol) obtained according to the reported method[24] were dissolved in *N*-methylpyrrolidone (550 mL). To the stirred reaction mixture at 50–60 °C, TEA (190 mL, 1.36 mol) was gradually added dropwise. After the addition was completed, the reaction mixture was stood overnight at room temperature. To the reaction mixture was added 5% aqueous citric acid (700 mL), and the precipitate solid was collected by filtration, washed with H_2O, and dried under reduced pressure to give *tert*-butyl 1,3-dihydro-2*H*-isoindol-2-yl(methyl)carbamate (**27**) (126 g, 84%) as a pale pink solid. This compound was used in the next reaction without further purification. ^{1}H NMR (300 MHz, $CDCl_3$): δ (ppm) 1.41 (s, 9H), 3.09 (s, 3H), 4.44 (s, 4H), 7.1–7.2 (m, 4H). To a solution of **27** (126 g, 507 mmol) in CH_2Cl_2 (150 mL) and EtOH (150 mL) was added 4 mol/L HCl in dioxane (500 mL), and the reaction mixture was stirred at room temperature for 5 h. The reaction mixture was diluted with H_2O and CH_2Cl_2 and the aqueous layer was extracted. An ice-cooled aqueous NaOH solution was added to the ice-cooled aqueous layer to give a strongly-alkaline solution. The aqueous layer was extracted with CH_2Cl_2. The organic layer was dried over K_2CO_3, and the insoluble material was filtered off. The solution was concentrated under reduced pressure. The residue oil was dissolved in diethyl ether (500 mL), and 4 mol/L HCl in dioxane (140 mL) was added with stirring under ice-cooling to allow precipitation of a solid. This solid was filtered, washed with diethyl ether, and dried under reduced pressure to give **12n** (75.9 g, 81%) as a gray solid. Mp 159–165 °C. ^{1}H NMR (400 MHz, DMSO-d_6): δ (ppm) 2.77 (s, 3H), 4.42 (br s, 4H), 7.23–7.43 (m, 4H), 10.97 (br s, 2H). ^{13}C NMR (100 MHz, DMSO-d_6): δ (ppm) 32.81, 56.66, 122.67, 127.63, 136.10. LC–MS (ESI) m/z 149 $[M+H]^+$. Anal. Calculated for $C_9H_{13}ClN_2$: C, 58.54; H, 7.10; N, 15.17; Cl, 19.20. Found: C, 58.31; H, 7.08; N, 14.87; Cl, 18.84.

5.1.41. 3,4-Dihydroisoquinolin-2(1*H*)-amine (29)[25]

To a stirred solution of 1,2,3,4-tetrahydroisoquinoline (**28**) (2.00 g, 15.0 mmol) and sodium nitrite (2.07 g, 30.0 mmol) in H_2O (30 mL) was gradually added AcOH (1.30 mL, 22.7 mmol) at under 5 °C, and the mixture was stirred at room temperature for 90 min. The reaction mixture was diluted with EtOAc, and the organic layer was washed with H_2O, saturated aqueous $NaHCO_3$ and brine and dried over $MgSO_4$. The insoluble material was filtered off, and the solution was concentrated under reduced pressure. The residue oil was purified by silica gel column chromatography (hexane/EtOAc = 90:10 to 50:50) to afford 2.03 g of a purple oil. This oil was dissolved with MeOH (15 mL), and to the mixture was added Zn (4.00 g, 61.2 mmol). To the mixture was gradually added AcOH (15.0 mL) under ice-cooling, and the mixture was stirred at room temperature for 80 min. The insoluble material in the reaction mixture was filtered off through Celite, and washed with MeOH and $CHCl_3$. The solution was neutralized with saturated aqueous $NaHCO_3$ and the solution was extracted with $CHCl_3$ (200 mL × 3). The organic layers were combined, and dried over Na_2SO_4. The insoluble material was filtered, and the solution was concentrated under reduced pressure. The residue oil was purified by silica gel column chromatography (EtOAc/MeOH = 100:0 to 80:20) to afford **29** (1.50 g, 67%) as a pale yellow oil. ^{1}H NMR (400 MHz, $CDCl_3$): δ (ppm) 2.5–3.5 (broad, 2H), 2.94 (t, J = 5.7, 2H), 3.00 (t, J = 5.7, 2H), 3.82 (s, 2H), 7.00–7.05 (m, 1H), 7.09-7.19 (m, 3H). ^{13}C NMR (100 MHz, $CDCl_3$): δ (ppm) 29.27, 57.23, 62.01, 125.82, 126.46, 126.67, 128.37, 133.19, 134.00. LC–MS (ESI) m/z 149 $[M+H]^+$.

5.1.42. *N*-Methyl-3,4-dihydroisoquinolin-2(1*H*)-amine (12o)[26]

To a stirred solution of **29** (1.50 g, 10.1 mmol) in H_2O (15 mL) and AcOH (0.700 mL, 12.2 mmol) was added 37% aqueous formalin (0.950 mL, 12.6 mmol), and the resulting mixture was stirred at 0 °C for 10 min. The reaction mixture was diluted with diluted aqueous NaOH, and the solution was extracted with diethyl ether (100 mL). The organic layer was washed with brine, and dried over $MgSO_4$. The insoluble material was filtered off, and the solution was concentrated under reduced pressure. The residue oil was purified by silica gel column chromatography (hexane/EtOAc = 100:0 to 80:20) to afford 1.34 g of a colorless oil. The obtained oil in THF (10 mL) was added to a stirred suspension of $LiAlH_4$ (460 mg, 12.1 mmol) in diethyl ether (50 mL), the resulting mixture was stirred under reflux for 40 min. The reaction mixture was ice-cooled, and H_2O (0.460 mL), 15% aqueous NaOH (0.460 mL), H_2O (1.38 mL) and Na_2SO_4 were successively added with stirring. The insoluble material was filtered off through Celite, and the solution was concentrated under reduced pressure. The residue oil was purified by NH silica gel column chromatography (hexane/EtOAc = 100:0 to 50:50) to afford **12o** (1.30 g, 79%) as a colorless oil. ^{1}H NMR (400 MHz, $CDCl_3$): δ (ppm) 2.69 (s, 3H), 2.99 (s, 4H), 3.88 (s, 2H), 7.01–7.18 (m, 4H). ^{13}C NMR (100 MHz, $CDCl_3$): δ (ppm) 28.92, 35.48, 52.73, 57.96, 125.82, 126.44, 126.95, 128.46, 133.71, 133.99. LC–MS (ESI) m/z 163 $[M+H]^+$.

5.1.43. *tert*-Butyl 4-[(2-nitrophenyl)sulfonylpiperazin-1-yl]carbamate (31)

To an ice-cooled solution of *tert*-butyl piperazin-1-ylcarbamate (**30**) (820 mg, 4.07 mmol, Bepharma. Ltd) and TEA (0.850 ml, 6.10 mmol) in CH_2Cl_2 (10 mL) was added 2-nitrobenzenesulfonyl chloride (947 mg, 4.27 mmol), and the resulting mixture was stirred overnight at room temperature. The reaction mixture was diluted with EtOAc, and the organic layer was washed with 10% aqueous citric acid and brine, and dried over $MgSO_4$. The insoluble material was filtered off, and the solution was concentrated under reduced pressure. The residue oil was purified by silica gel column chromatography (hexane/EtOAc = 90:10 to 0:100) to afford **31** (1.39 g, 86%) as a pale blue solid. Mp 183–184 °C. ^{1}H NMR (400 MHz, DMSO-d_6): δ (ppm) 1.44 (s, 9H), 2.89 (t, J = 4.9, 4H), 3.43 (t, J = 4.9, 4H), 5.54 (s, 1H), 7.62 (dd, J = 1.5, 7.7, 1H), 7.66–7.77 (m, 2H), 7.95 (dd, J = 1.5, 7.2, 1H). ^{13}C NMR (100 MHz, DMSO-d_6): δ (ppm) 28.29, 45.74, 54.95, 80.75, 124.17, 130.81, 130.86, 131.52, 133.90, 148.48, 154.19. LC–MS (ESI) m/z 331 $[M+H]^+$. Anal. Calculated for $C_{15}H_{22}N_4O_6S$: C, 46.62; H, 5.74; N, 14.50. Found: C, 46.64; H, 5.58; N, 14.44.

5.1.44. *N*-Methyl-4-(2-nitrophenyl)sulfonyl-piperazin-1-amine hydrochloride (12p)

To a stirred solution of **31** (775 mg. 2.01 mmol) in THF (8 mL) was added potassium *tert*-butoxide (331 mg, 2.95 mmol), the resulting mixture was stirred at 0 °C for 5 min. Then, to the reaction mixture was added iodomethane (0.300 ml, 4.82 mmol), and the mixture was stirred at 0 °C for 30 min. The reaction mixture was diluted with EtOAc, and the organic layer was washed with H_2O and brine, and dried over $MgSO_4$. The insoluble material was

filtered off, and the solution was concentrated under reduced pressure. The residue oil was purified by silica gel column chromatography (hexane/EtOAc = 90:10 to 35:65) to afford 543 mg of a colorless oil. The obtained oil was dissolved with CH_2Cl_2 (2 mL), and to the solution was added 4 mol/L HCl in dioxane (2.0 mL, 8.0 mmol), and the resulting mixture was stirred at room temperature for 4 h. The reaction mixture was diluted with diethyl ether (8 mL). The precipitated solid was filtered, washed with diethyl ether and dried under reduced pressure to give **12p** (3.53 g, 83%) as a colorless solid. Mp 224–226 °C. ^{1}H NMR (400 MHz, DMSO-d_6): δ (ppm) 2.64 (br s, 3H), 3.09 (br s, 4H), 3.36 (br s, 4H), 7.86–7.92 (m, 1H), 7.95 (t, J = 7.7, 1H), 8.01–8.06 (m, 2H), 10.85, 10.98 (2 br s, 2H). ^{13}C NMR (100 MHz, DMSO-d_6): δ (ppm) 30.22, 44.65, 49.91, 124.25, 129.00, 130.32, 132.43, 135.02, 147.68. LC–MS (ESI) m/z 301 $[M+H]^+$. Anal. Calculated for $C_{11}H_{17}ClN_4O_4S{\cdot}1/5H_2O$: C, 38.81; H, 5.15; N, 16.46; Cl, 10.42; S, 9.42. Found: C, 38.99; H, 5.00; N, 16.09; Cl, 10.53; S, 9.23.

5.1.45. *N*-Methylindan-2-amine hydrochloride (12e)[27]

2-Aminoindane (**19**) (3.10 g, 23.3 mmol) was dissolved in CH_2Cl_2 (50 mL), Boc_2O (5.20 g, 23.8 mmol) and TEA (5 mL) were added under ice-cooling, and the mixture was stirred at room temperature for 2 h. The reaction mixture was diluted with a 1:1 EtOAc/hexane (100 mL) solution, and the organic layer was washed with 10% aqueous citric acid solution, saturated aqueous $NaHCO_3$ and brine and dried over $MgSO_4$. The insoluble material was filtered off, and the solution was concentrated under reduced pressure to give 4.68 g of **20a** as a colorless oil. This was added to a suspension of $LiAlH_4$ (2.56 g, 67.5 mmol) in THF (100 mL), and the mixture was heated under reflux conditions for 3 h. The reaction mixture was ice-cooled, and H_2O (2.56 mL), 15% aqueous NaOH (2.56 mL), H_2O (7.68 mL) and $MgSO_4$ were successively added with stirring. The insoluble material was filtered off through Celite, nd the solution was concentrated under reduced pressure to give an oil (3.15 g). This was dissolved in diethyl ether (50 mL), 4 mol/L HCl in dioxane (6 mL) was added, and the precipitated solid was filtered, washed with diethyl ether and dried under reduced pressure to give **12e** (3.53 g, 83%) as a colorless solid. Mp 233–236 °C.[20] ^{1}H NMR (400 MHz, DMSO-d_6): δ (ppm) 2.57 (s, 3H), 3.11 (dd, J = 6.7, 16.4, 2H), 3.27 (dd, J = 8.0, 16.4, 2H), 3.87–4.00 (m, 1H), 7.16–7.23 (m, 2H), 7.23–7.30 (m, 2H), 9.39 (br s, 2H). ^{13}C NMR (100 MHz, DMSO-d_6): δ (ppm) 30.76, 35.29, 58.17, 124.44, 126.89, 139.36. LC–MS (ESI) m/z 148 $[M+H]^+$.

5.1.46. *N*-Ethylindan-2-amine hydrochloride (12h)[27]

The title compound was synthesized according to the method described for the synthesis of **12e** from 2-aminoindane (**19**) (1.07 g, 8.03 mmol) and acetic anhydride (0.76 mL, 8.04 mmol) to afford **12h** (1.01 g, 94%) as a colorless solid. Mp 168–176 °C. ^{1}H NMR (400 MHz, DMSO-d_6): δ (ppm) 1.25 (t, J = 7.2, 3H), 2.92–3.06 (m, 2H), 3.08–3.19 (m, 2H), 3.22–3.33 (m, 2H), 3.90–4.07 (m, 1H), 7.15–7.31 (m, 4H), 9.32 (br s, 2H). ^{13}C NMR (100 MHz, DMSO-d_6): δ (ppm) 11.06, 35.47, 40.60, 56.66, 124.40, 126.87, 139.38. LC–MS (ESI) m/z 162 $[M+H]^+$.

5.1.47. *N*-Methyl-1,2,3,4-tetrahydronaphthalen-2-amine hydrochloride (12f)[27]

The title compound was synthesized according to the method described for the synthesis of **12e** from 1,2,3,4-tetrahydronaphthalen-2-amine (**21**) (2.50 g, 13.6 mmol) to afford **12f** (1.66 g, 62%) as a colorless solid. Mp 185–186 °C. ^{1}H NMR (400 MHz, DMSO-d_6): δ (ppm) 1.71–1.85 (m, 1H), 2.20–2.28 (m, 1H), 2.60 (s, 3H), 2.74–2.93 (m, 3H), 3.13–3.23 (m, 1H), 3.31–3.42 (m, 1H), 7.08–7.17 (m, 4H), 9.24 (br s, 2H). ^{13}C NMR (100 MHz, DMSO-d_6): δ (ppm) 24.85, 26.78, 29.37, 30.93, 53.97, 125.90, 126.15, 128.42, 128.93, 132.50, 134.86. LC–MS (ESI) m/z 162 $[M+H]^+$.

5.1.48. *trans*-*N*-Methyl-4-phenylcyclohexanamine hydrochloride (12g)

The title compound was synthesized according to the method described for the synthesis of **12e** from *trans*-4-phenylcyclohexanamine (**23**) (3.42 g, 16.2 mmol) to afford **12g** (3.11 g, 88%) as a colorless solid. Mp 269–272 °C. ^{1}H NMR (400 MHz, DMSO-d_6): δ (ppm) 1.38–1.58 (m, 4H), 1.81–1.95 (m, 2H), 2.08–2.22 (m, 2H), 2.44-2.60 (m, 4H), 2.91–3.09 (m, 1H), 7.15–7.33 (m, 5H), 8.88 (br s, 2H). ^{13}C NMR (100 MHz, DMSO-d_6): δ (ppm) 28.28, 31.37, 42.27, 56.23, 126.01, 126.56, 128.23, 145.88. LC–MS (ESI) m/z 190 $[M+H]^+$. Anal. Calculated for $C_{13}H_{20}ClN{\cdot}1/10H_2O$: C, 68.61; H, 8.95; N, 6.16; Cl, 15.58. Found: C, 68.77; H, 8.78; N, 6.16; Cl, 15.38.

5.2. X-ray crystallography

5.2.1. Crystallization, data, and structural refinement of human recombinant AdoHcyase, NAD^+, and 18a

The enzyme of human recombinant AdoHcyase purchased from Diazyme Laboratory was purified according to the method reported by Yuan et al.[28] The protein–inhibitor complex was produced by sitting-drop vapor diffusion method using a reservoir solution composed of 100 mM HEPES (pH 7.5), 13% (w/v) PEG 4000, 10% (v/v) 2-PrOH, and 0.5% (v/v) EtOAc. A plate-shape crystal suitable for X-ray diffraction having dimensions 0.1 mm × 0.05 mm × 0.005 mm in a drop was dipped into a cryoprotectant solution containing 100 mM HEPES (pH 7.5), 25% (w/v) PEG 4000, and 15% (v/v) glycerol for 2 h before it was frozen in liquid nitrogen. X-ray diffraction data were collected at SPring-8 BL24XU beam line. The diffraction data were measured up to 2.7 Å resolutions at −180 °C. The data were processed with the program HKL2000. The structure of AdoHcyase and inhibitor complex was solved by molecular replacement with the program AMoRe, utilizing the previously determined coordinates of AdoHcyase with Protein Data Bank accession code 1B3R. The structure was refined against all available data to 2.7 Å using Maximum likelihood (Refmac) to a crystallographic R-factor of 0.199 and free R-factor of 0.240. Data collection and model refinement statistics are summarized in Table 4. The crystallographic refinement parameters, final (2Fo Fc) maps, and conformational analysis by PROCHECK indicate that the crystal structure has been determined with acceptable statistics. Coordinates have been deposited with the Protein Data Bank, (Entry name: 4YVF).

5.2.2. X-ray crystal structure determination of 14n

A colorless prismatic crystal of compound **14n** suitable for X-ray single crystal analysis was obtained at room temperature by partial evaporation from diethyl ether. X-ray data (see Table 5) were collected at a temperature of 25 ± 1 °C on a Rigaku AFC7R diffractometer equipped with a graphite monochromated Cu-Kα radiation (λ = 1.5418 Å) and a rotating anode generator. The crystal structure was solved by direct methods.[29] The non-hydrogen atoms were refined anisotropically by full-matrix least squares refinement on F^2. The H atoms were refined using the riding model. All calculations were performed using the CrystalStructure crystallographic software package.[30] CCDC 1049632 contains the supplementary crystallographic data for this paper. These data can be obtained free of charge from The Cambridge Crystallographic Data Centre via www.ccdc.cam.ac.uk/data_request/cif.

5.3. In vitro AdoHcyase inhibition assays

The enzyme inhibitory activity was measured using the hydrolysis activity of AdoHcy as an index. The measurement method was modification of the reported method.[31] AdoHcy (10 μM) and adenosine deaminase (Roche) (4 units) were added to 50 mM phosphate buffer (pH 7.2, containing 1 mM EDTA) with the total

Table 4

Resolution (Å)	47.81–2.7	
No. of all reflections	31,525	(Highest range[a]: 4826)
Completeness (working + test) (%)	99.5	(Highest range[a]: 98.5)
R[b](working)	0.199	(Highest range[a]: 0.229)
Free R	0.240	(Highest range[a]: 0.282)
Free R test set size (%)	5.0	(Highest range[a]: 5.5)
No. of reflections used for free R	1565	(Highest range[a]: 280)
No. of non-hydrogen atoms used in refinement		
AdoHcyase	6644	
NAD^+	88	
18a	76	
Water	244	
B values		
From Wilson plot (Å^2)	22.1	
Mean B value all (Å^2)	23.9	
AdoHcyase	24.1	
NAD^+	18.6	
18a	20.4	
Water	21.5	
Estimated coordinate error		
ESD from Luzzati plot (Å)	0.25	
ESD from SIGMAA (Å)	0.19	
Low resolution cutoff (Å)	5.00	
RMS deviations from ideal values		
Bond length (Å)	0.006	
Bond angles (°)	1.2	
Dihedral angles (°)	22.0	
Improper angles (°)	0.73	

[a] 2.87–2.70 Å resolution.
[b] $R = \Sigma|Fo - Fc|/\Sigma|Fo|$.

Table 5
Crystal data and structure refinement of **14n**

Compound	**14n**
Empirical formula	$C_{31}H_{35}Cl_2N_5O_3$
Formula weight	596.56
Recryst. solvent	Diethyl ether
Crystal system	Monoclinic
Unit cell dimensions	a = 18.9093(17) Å, α = 90°
	b = 16.1873(9) Å, β = 131.002(5)°
	c = 13.2852(9) Å, γ = 90°
Volume (Å^3)	3068.9(5)
Space group	*Cc*
Z value	4
Density (calculated) (g/cm^3)	1.291
Absorption coefficient (cm^{-1})	22.252
Crystal size (mm^3)	0.300 × 0.200 × 0.100
Temperature (K)	298
2θ max (°)	135.8
Reflections collected	2876
Independent reflections	2790 [R(int) = 0.0347]
Data/restraints/parameters	2344/0/406
Goodness-of-fit on F^2	1.003
Residuals: R, Rw	0.0438, 0.1257
CCDC reference number	1049632

amount being 200 μL, and to the solution were added a test substance and then human recombinant *S*-adenosyl-L-homocysteine hydrolase (50 ng, Diazyme Laboratories) to start the reaction, and the mixture was incubated at 37 °C for 8 min. The reaction was quenched by the addition of 1 mol/L aqueous perchloric solution (20 μl), and the mixture was centrifuged under the conditions of 10,000 rpm, 5 min, 4 °C. The supernatant was collected, and the amount of AdoHcy after the reaction was quantified by HPLC. The inhibitory rate was determined with the amount of decrease in AdoHcy before and after the reaction without using the test substance as 100%. Inhibitory rate (%) = [(amount of decrease of AdoHcy in the presence of test substance)/(amount of decrease of AdoHcy in the absence of test substance)] × 100.

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