博士論文

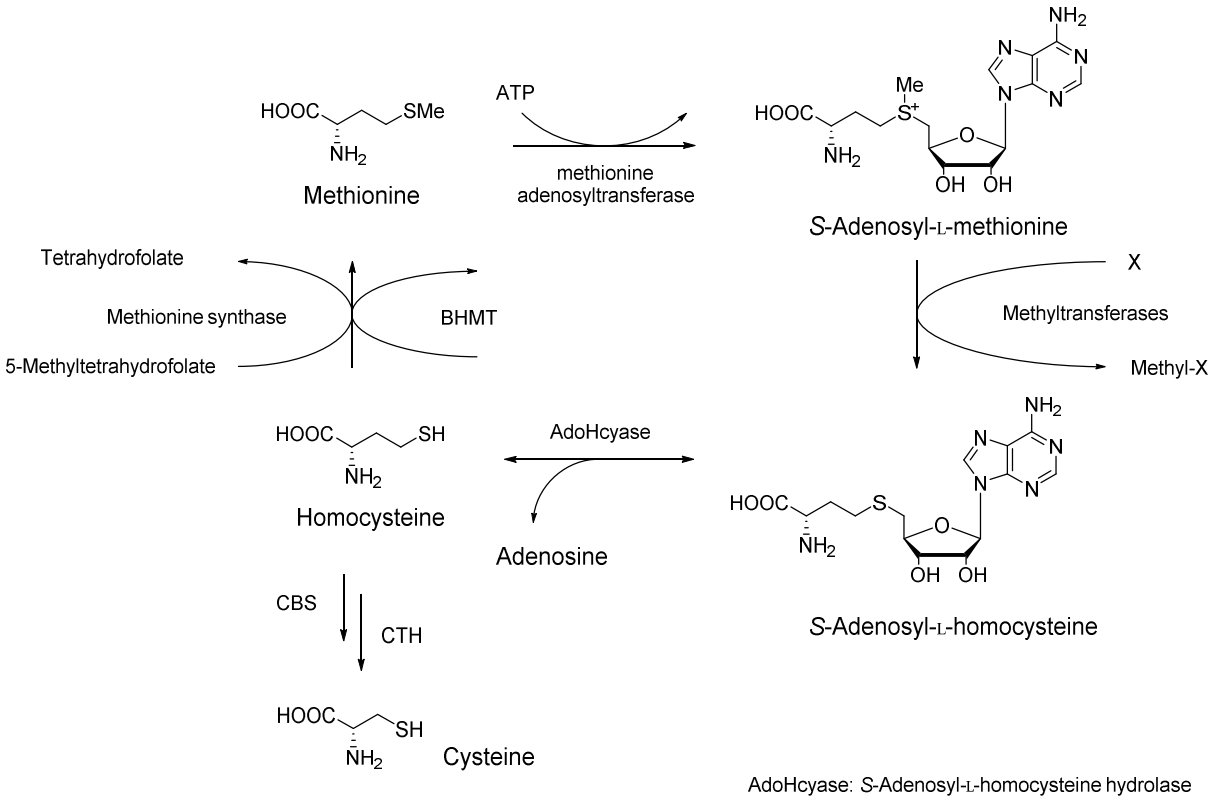
非アデノシン骨格を有する新規ホモシステイン合成酵素阻害剤の発見と X 線複合体解析研究

中尾　朗

目　次

序論

ホモシステインは含硫アミノ酸の１つであり、同じく含硫必須アミノ酸であるメチオニンの代謝産物の１つである。メチオニンは、メチオニンアデノシルトランスフェラーゼによってアデノシンと結合することにより *S* - アデノシル - L - メチオニンへと変換される。メチル化反応の供与体である *S* - アデノシル - L - メチオニンはメチルトランスフェラーゼによってメチル基を供与し *S* - アデノシル - L - ホモシステインへと変換される。ホモシステインはこの *S* - アデノシル - L - ホモシステインが *S* - アデノシル - L - ホモシステイン加水分解酵素によって加水分解することによって、アデノシンとともに合成される。ホモシステインは２つの代謝経路、すなわち、葉酸、ビタミン B12 等の関与によってホモシステインがメチオニンへ戻る経路とビタミン B6 が関与する含硫基移動によってシステインへと変換される経路がある [1]。



ホモシステイン代謝

１９６９年、McCully は、ホモシステインが動脈硬化、心筋梗塞といった血管性病態の原因になりうることを報告した [2]。それ以降、それを検証するため多くの臨床試験が行われてきた [3]。Perry らは、5,661 人を対象にしたコホート研究を行い、血漿総ホモシステイン値が高い人ほど脳卒中を発症する危険度が高く、相対危険率は 正常値 10.3 μ mol/L 以下の場合を１としたとき、15.4 μ mol/L 以上で 4.7 倍になることを報告した [4]。２００２年、Clarke らは、虚血性心疾患や脳梗塞とホモシステイン濃度との関連研究に対するメタアナリシスを実施し、ホモシステイン値を約

２５％低下させると脳梗塞のリスクが１９％低下することを報告した[5]。このような研究結果から、今日では高ホモシステイン状態は心筋梗塞や脳梗塞の独立したリスクファクターであると言われている。また近年では、アルツハイマー病、うつ病、統合失調症といった精神神経疾患への関与も示唆されており、コホート研究の結果から高ホモシステイン状態はアルツハイマー病のリスクが約２．５倍上昇することが報告されている[6]。一方、動物を用いた実験では、葉酸欠乏食で３ケ月飼育した高ホモシステインマウスを、中大脳動脈虚血再灌流モデルによって脳梗塞を起こすことにより、梗塞体積の増加や神経症状の悪化が見られることが報告されている[7]。apoE 欠損マウスをアテローム生成食で 12 週、および 24 週間飼育した際、ホモシステイン合成酵素阻害剤であるデアザアデノシンを一日一回投与することにより血中ホモシステイン値が低下することが報告されている[8]。これら既存の情報から、我々はホモシステイン合成酵素である *S* - アデノシル - L - ホモシステイン加水分解酵素（*S*-adenosyl-L-homocysteine hydrolase; AdoHcyase; EC 3.3.1.1.）を阻害し、高ホモシステイン状態を正常化させることにより脳梗塞、心筋梗塞等の冠動脈疾患の予防、治療が期待できるのではないかと考えた。

第１章　ハイスループットスクリーニングの実施

第１節　自動リガンド識別システムによるハイスループットスクリーニングの実施

研究開始当初から今日に至るまで AdoHcyase 阻害剤として知られているものはアデノシン骨格変換体のみであり、その多くが不可逆阻害剤であった[9,10]。不可逆阻害剤による毒性、アデノシン誘導体の非酵素選択性といった課題を解決するため、我々は非アデノシン骨格を有する新規リード化合物の探索を開始した。

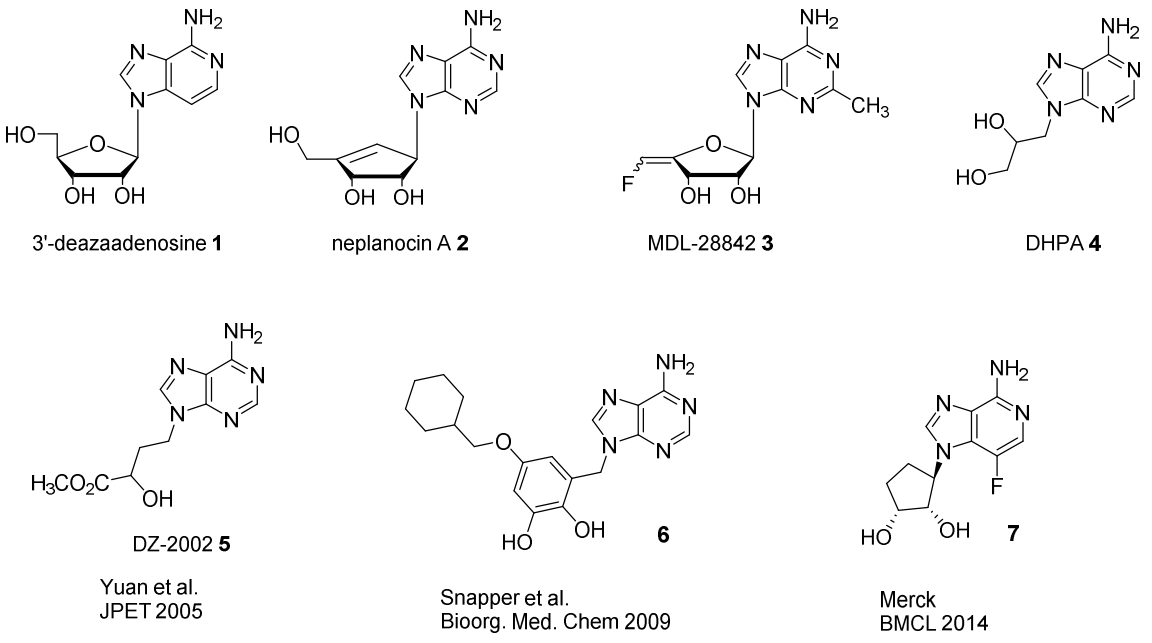
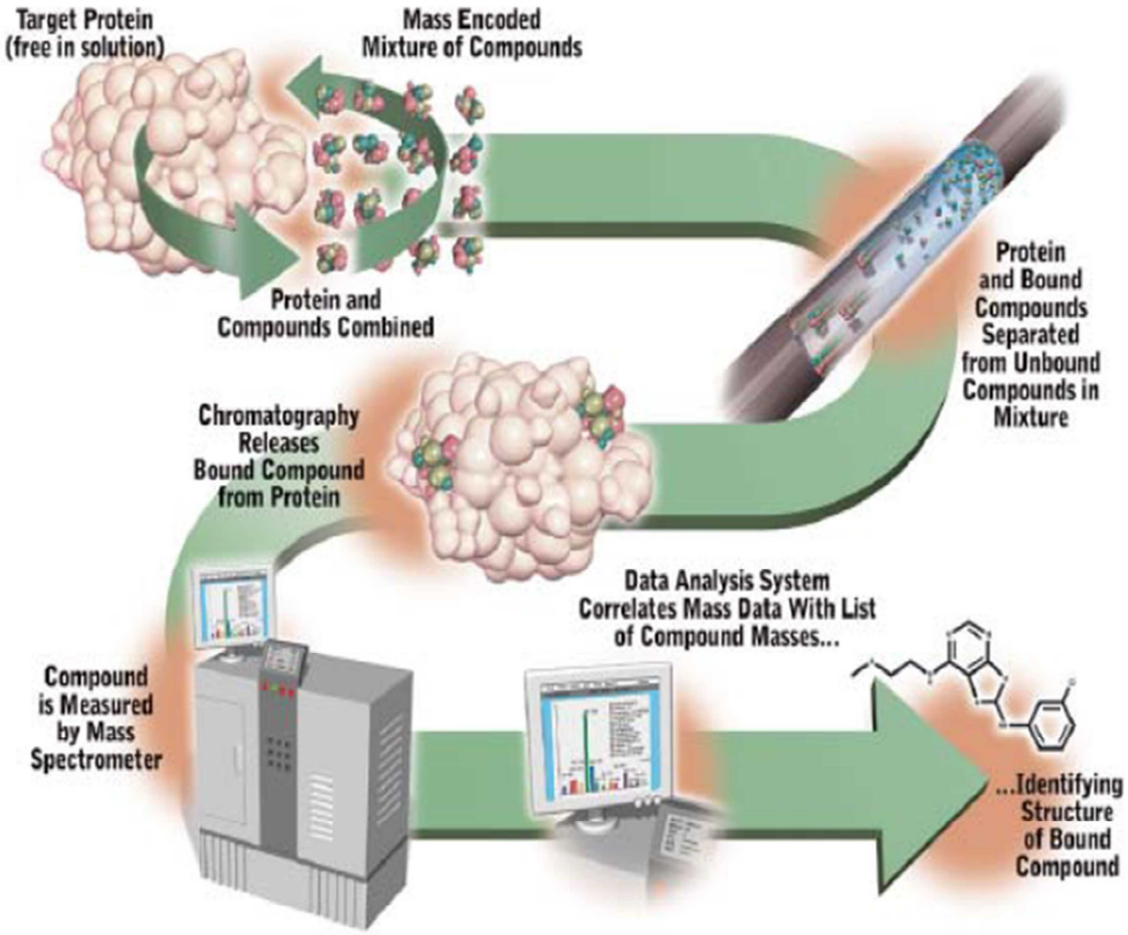


図１－１　既知の AdoHcyase 阻害剤

分子ふるいを応用した自動リガンド識別システム（ALIS）という、可逆阻害剤の取得を志向したハイスループットスクリーニング（HTS）技術[11]を用いて HTS を実施した結果、１系統の化合物群を HTS ヒット化合物として見出した。



Reprinted from Current Opinion in Chemical Biology 2007, 11, 518–526, Annis, D. Allen et al, Affinity selection-mass spectrometry screening techniques for small molecule drug discovery, with permission from Elsevier.

図１－２　自動リガンド識別システム概要

第２節　化合物最適化に向けた作業仮説

得られた HTS ヒット化合物は２つのアミド構造、すなわち１つは脂溶性アミド、もう一方は末端にアミノ基を有する鎖状アミドを有していた。

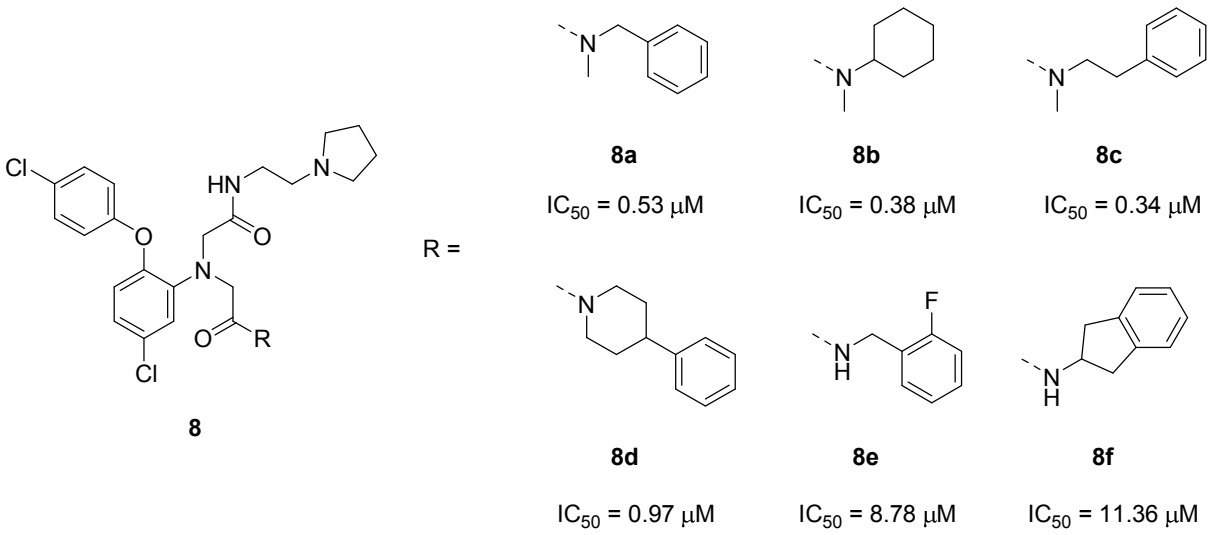


図１－３　ハイスループットスクリーニングで得られたＨＴＳヒット化合物

これら化合物のうち３化合物（化合物 **8a－c**）は脂溶性アミド部位に *N*- メチル基を有する３級アミド構造を有しており、他の化合物よりも酵素阻害活性が強いことがわかる。一般に２級アミドは平面構造をしており、シス体よりもトランス体として存在し、その二重結合性によりほぼ異性化は起こらないとされている[12]。しかし２級アミドのＮＨをアルキル化した３級アミドでは、トランス - シス異性化のエネルギー障壁が下がり、より立体障害の少ない構造、あるいは電気化学的に安定な構造をとるようになる[13]。首藤らは、アニリドのＮＨをメチル化するとコンフォメーション変化によりシス型アミドとなること X 線結晶解析、およびＮＭＲスペクトル解析から明らかにしている[14]。HTS ヒット化合物の中で化合物 **8f** は２級アミドを含む構造を有しているが、*N*- メチル基を有する化合物 **8a－c** と比較し、その活性が弱いことに着目した。すなわち、「化合物 **8a－c** が酵素タンパクである *S*- アデノシル - L - ホモシステイン加水分解酵素と相互作用する際、脂溶性アミド部位はシス型をとっている。一方化合物 **8f** はトランス - シス異性化が制限され、シス型をとりにくいため活性が弱い。**8f** のアミドをメチル化すればトランスーシス変換により活性コンフォメーションを取ることが容易となり、酵素阻害活性が大幅に向上する。」という仮説を立て合成展開を開始した。

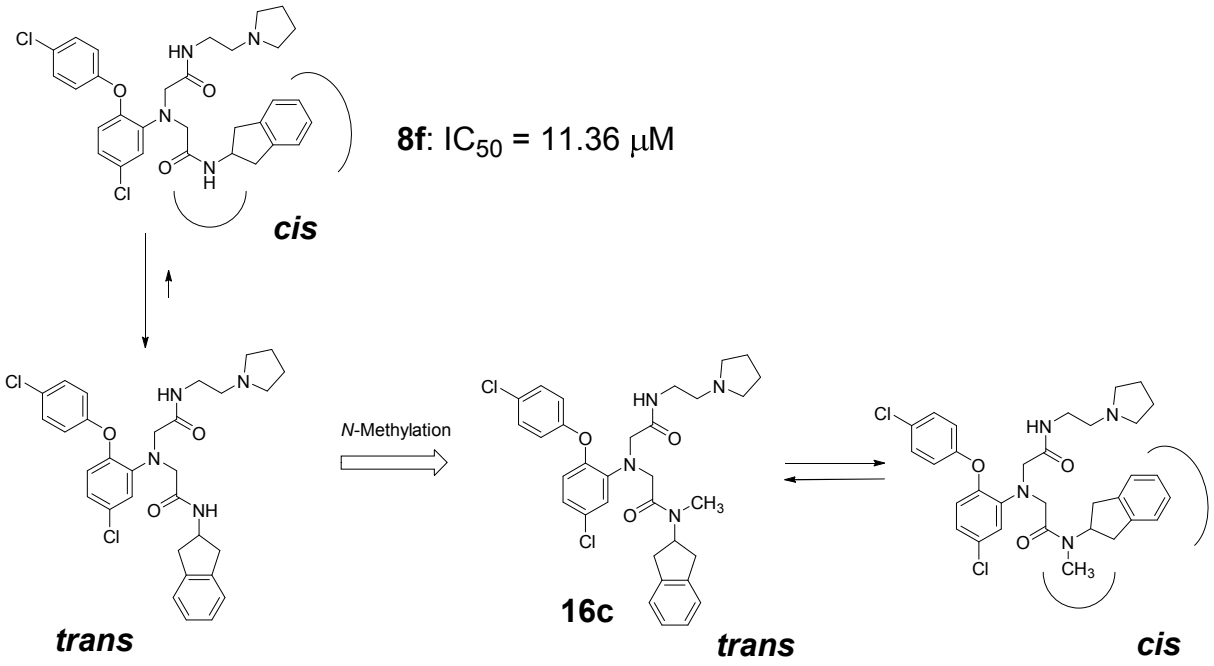


図１－４　Ｎ－メチル化によるコンフォメーション変化仮説

第２章　評価化合物の合成

阻害剤の最適化合成研究を行うに当たり、合成展開箇所を１）脂溶性アミド部位、および２）水溶性アミド部位、に定め種々化合物の合成を行った。

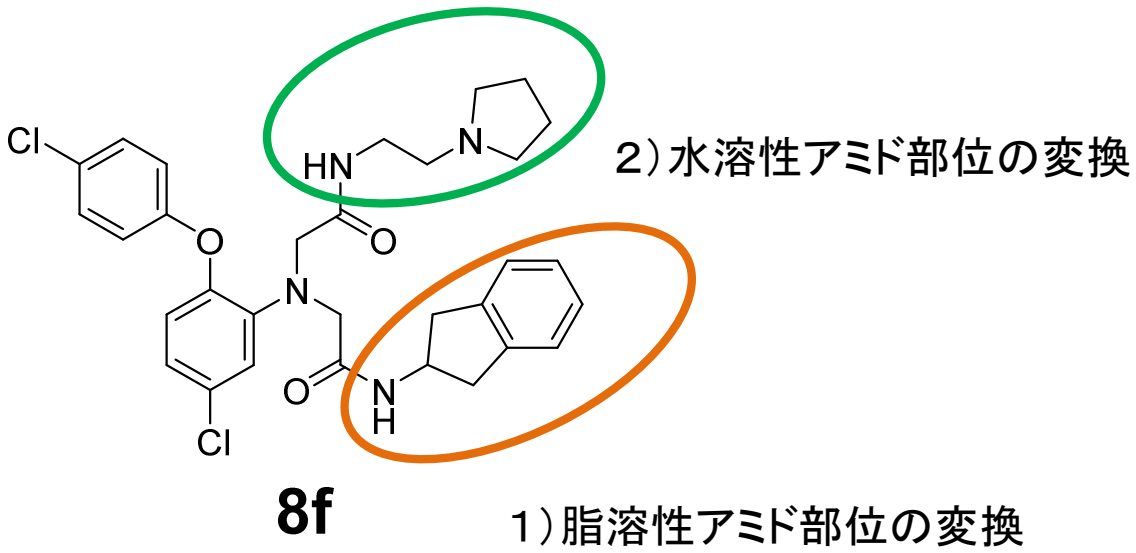


図２－１　評価化合物の合成展開箇所

第１節　鍵中間体の合成

評価化合物を合成するために必要な鍵中間体 **13** の合成を以下に示す方法で行った。４ - クロロフ

ェノール（**9**）を水素化ナトリウムで脱水素化した後、１，４‐ジクロロ‐２‐ニトロベンゼンと反応させ化合物 **10** を得た。**10** のニトロ基を塩化鉄、ヒドラジン条件により還元し化合物 **11** を得た。化合物 **11**、ブロモ酢酸エチル、およびジイソプロピルエチルアミン（DIPEA）を混合し、無溶媒下 140℃で反応させることによりジエステル体 **12** へと変換した。**12** のジエステルを水酸化ナトリウムでアルカリ加水分解することによりジカルボン酸 **13** を得た。

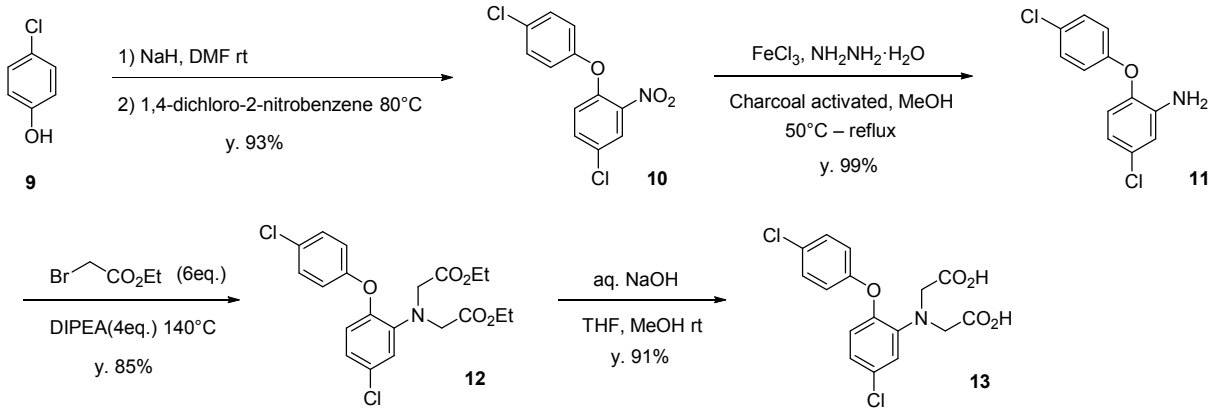
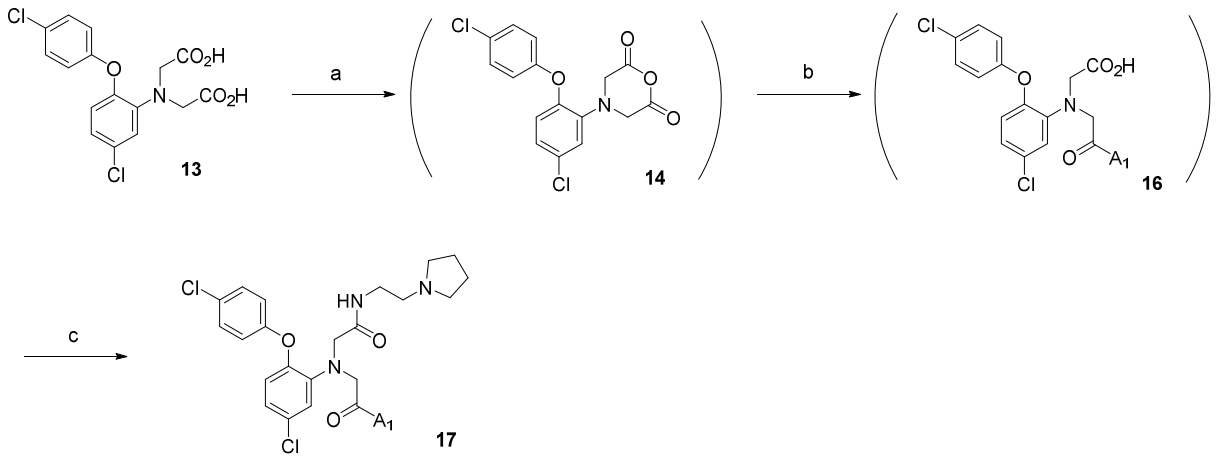


図２－２　鍵中間体 **13** の合成

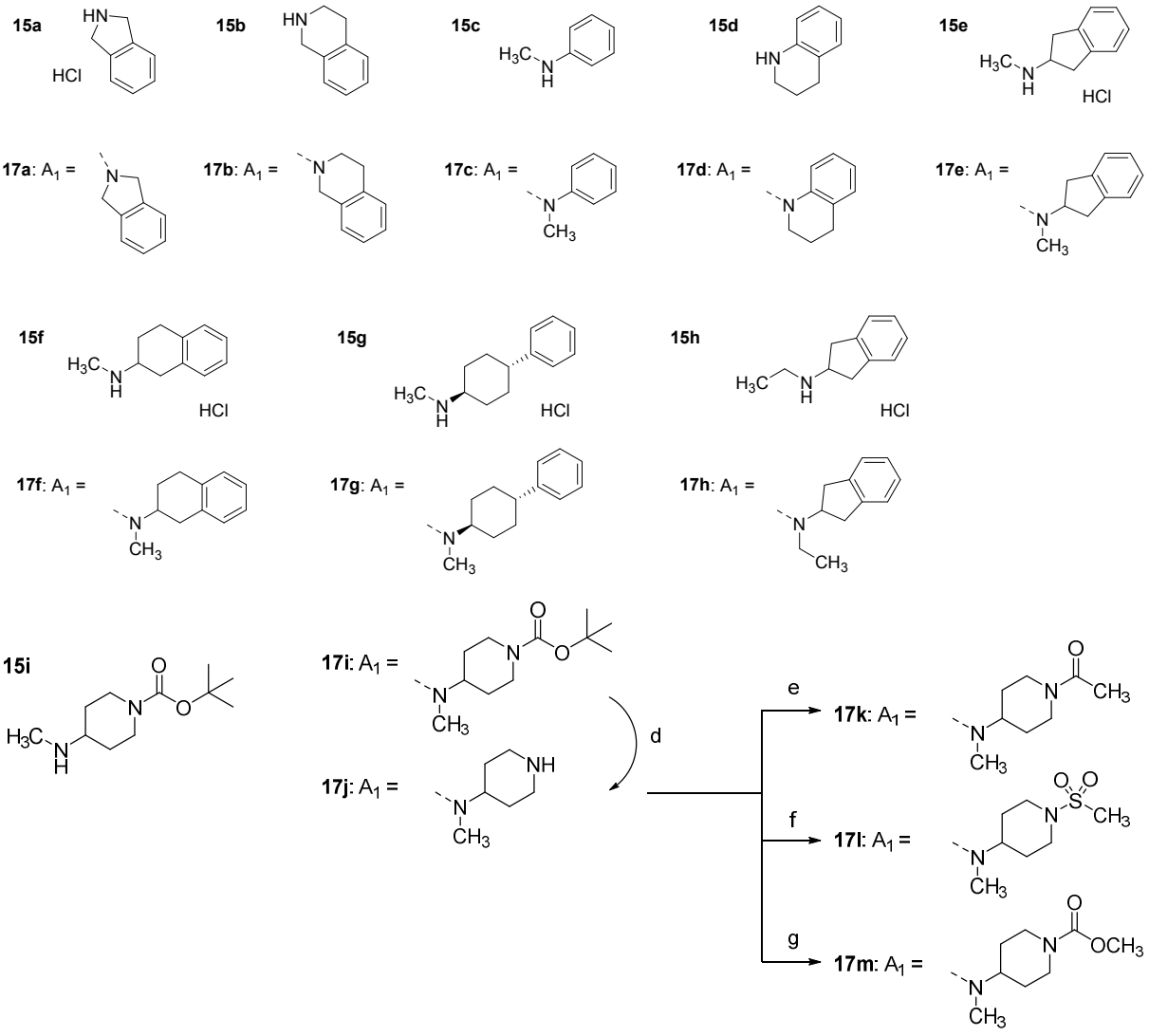
第２節　脂溶性アミド部位の変換

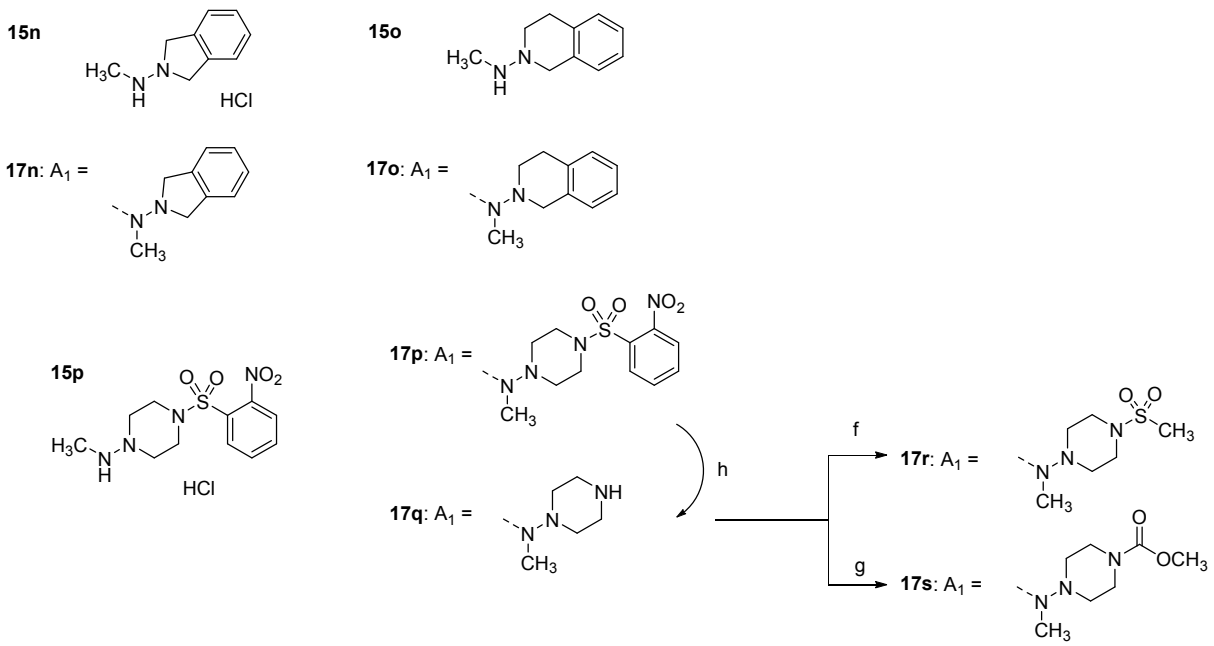
化合物 **13** を用いて、化合物 **17a-i** および化合物 **17n-p** の合成を行った。化合物 **13** を*N, N*‐ジメチルホルムアミド（DMF）溶媒下、１当量の１‐エチル‐３‐（３‐ジメチルアミノプロピル）カルボジイミド塩酸塩（EDC）と反応させ、反応系中で酸無水物 **14** を生成させた後、脂溶性アミド部位に相当するアミン **15a-i** および **15n-p** を加えアミド化した。次に反応系中に、２－（１－ピロリジニル）エチルアミン、１‐ヒドロキシベンゾトリアゾール（HOBt）、EDC を加え２つ目のアミド化を行い、それぞれ化合物 **17a-i** および **17n-p** を得た。



スキーム１　試薬と条件：(a) EDC, DMF rt; (b) **15**, or **15**, DIPEA, 0 °C; (c) 2-(1-pyrrolidinyl)ethylamine, HOBt, EDC, 0 °C to rt.

化合物 **17i** をジクロロメタン中 4mol/L 塩酸 - ジオキサン溶液で脱 Boc 化することにより化合物 **17j** を得た。**17j** をジクロロメタン溶媒下、トリエチルアミン（TEA）を塩基として加え、無水酢酸と反応させることにより化合物 **17k** を得た。同様にメタンスルホニルクロリドと反応させることにより化合物 **17l**、クロロギ酸メチルと反応させることにより化合物 **17m** を得た。化合物 **17p** をアセトニトリル中、塩基として炭酸セシウムを加え、パラ - トルエンチオールと反応させることにより化合物 **17q** を得た。**17q** をジクロロメタン溶媒下、TEA を塩基として加え、メタンスルホニルクロリドと反応させることにより化合物 **17r**、クロロギ酸メチルと反応させることにより化合物 **17s** を得た。

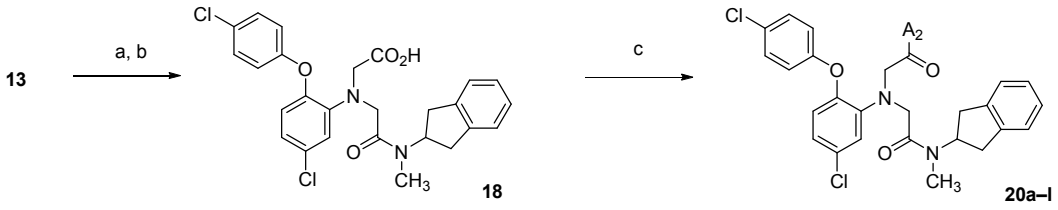


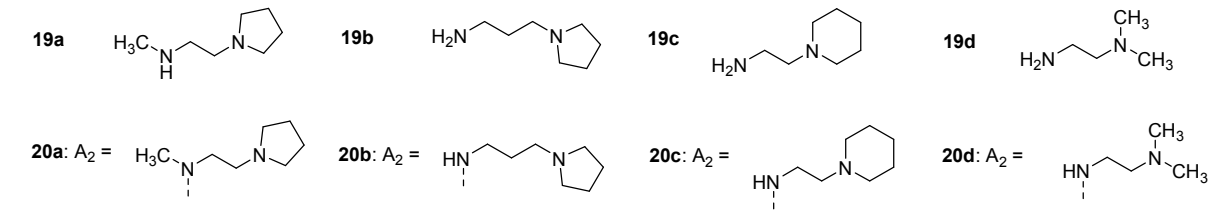


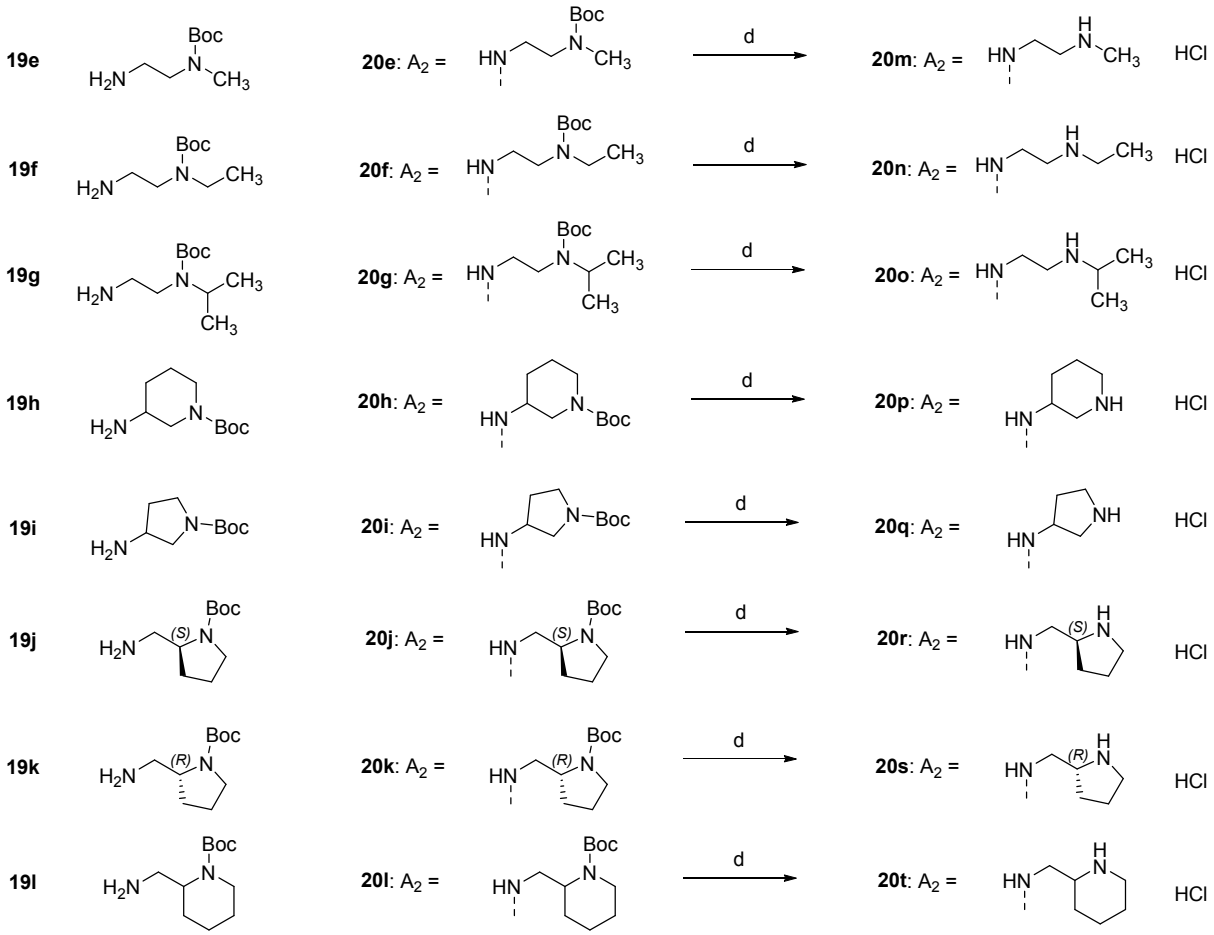
スキーム２　試薬と条件：(d) 4 mol/L HCl in dioxane, CH_2Cl_2, rt; (e) Ac_2O, TEA, CH_2Cl_2, 0 °C to rt; (f) CH_3SO_2Cl, TEA, CH_2Cl_2, 0 °C to rt; (g) Methyl chloroformate, TEA, CH_2Cl_2, 0 °C to rt; (h) *p*-Toluenethiol, Cs_2CO_3, CH_3CN, rt.

第３節　水溶性アミド部位の変換

化合物 **13** を用いて、化合物 **20a-d** および化合物 **20m-t** の合成を行った。化合物 **13** を DMF 溶媒下、１当量の EDC と反応させ酸無水物を生成させた後、化合物 **15e** および DIPEA を加え反応させることにより化合物 **18** を無色固体として単離した。**18** に対し、水溶性アミド部位に相当するアミンである化合物 **19a-l** を反応させることにより化合物 **20a-l** を得た。**20e-l** の N-Boc 基を塩酸で脱保護することにより化合物 **20m-t** を合成した。



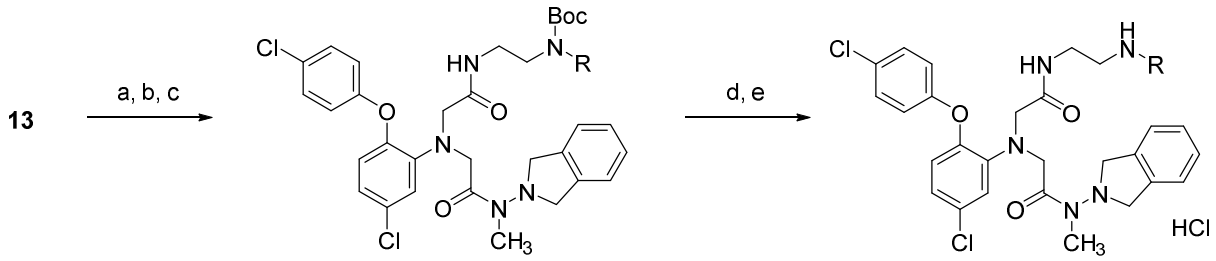




スキーム３　試薬と条件：(a) EDC, DMF, rt; (b) **15e**, DIPEA, 0 °C; (c) **19a-l**, HOBt, EDC, DMF, 0 °C to rt; (d) 4 mol/L HCl in dioxane, CH_2Cl_2, rt.

第４節　脂溶性アミド部位の変換と水溶性アミド部位の変換のコンビネーション

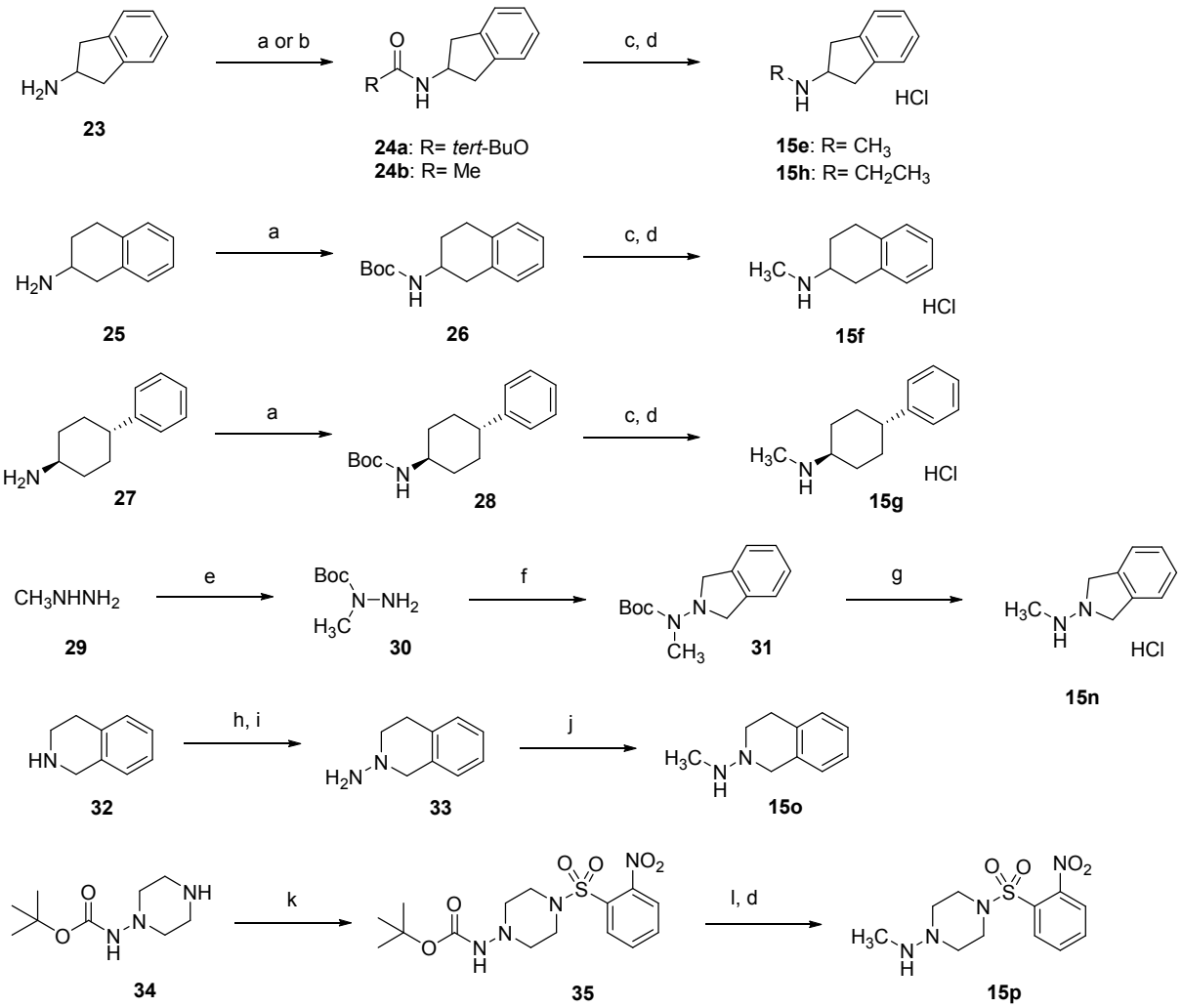
化合物 **13** を用いて化合物 **22a** および化合物 **22b** の合成を行った。化合物 **13** を DMF 溶媒下、1 当量の EDC と反応させ、酸無水物を生成させた後、化合物 **15n** および DIPEA を加え１つ目のアミド化を行った後、反応系中に **19e**、または **19f**、および HOBt、EDC を加え２つ目のアミド化を行い、それぞれ化合物 **21a**、**21b** を得た。化合物 **21a**、**21b** をジクロロメタン中、トリフルオロ酢酸（TFA）で脱 Boc 化した後、塩酸塩とすることで、それぞれ化合物 **22a**、**22b** を得た。



スキーム４　試薬と条件 : (a) EDC, DMF rt; (b) **15n**, DIPEA, 0 °C; (c) **19e-f**, HOBt, EDC, 0 °C to rt; (d) TFA, CH_2Cl_2, rt; (e) 4 mol/L HCl in dioxane, Et_2O, rt.

第５節　脂溶性アミド部位のアミン中間体の合成

脂溶性アミド部位のアミンに相当する化合物 **15e-h**、および化合物 **15n-p** の合成を以下に示す方法で実施した。２‐アミノインダン（**23**）を *tert*－ブトキシカルボニルジカーバメート（Boc_2O）で Boc 化、および無水酢酸でアセチル化することにより、それぞれ化合物 **24a**、**24b** を得た後、水素化リチウムアルミニウムで還元、塩酸塩化することにより、化合物 **15e**、**15h** を合成した。２‐アミノ‐１，２，３，４－テトラヒドロナフタレン（**25**）およびトランス－４－フェニルシクロヘキサン‐アミン（**27**）を Boc_2O で Boc 化、水素化リチウムアルミニウムで還元、塩酸塩化することにより、化合物 **15f**、**15g** を合成した。メチルヒドラジン（**29**）を Boc_2O で Boc 化した後、オルト‐キシリレンジブロミドと反応させ環化し化合物 **31** を得た後、脱保護により化合物 **15n** を合成した。１，２，３，４－テトラヒドロイソキノリン（**32**）を亜硝酸ナトリウムでニトロソ体とした後、亜鉛で還元することにより化合物 **33** を得た。**33** のアミノ基をホルマリンでイミンとした後、水素化リチウムアルミニウムで還元することにより化合物 **15o** を合成した。化合物 **34** をノシル化し、化合物 **35** を得た後、ヨウ化メチルで *N*－メチル化、脱 Boc 化により化合物 **15p** を合成した。



スキーム５　試薬と条件: (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, *N*-methylpyrrolidone, 50 °C to r.t; (g) 4 mol/L HCl in dioxane, CH_2Cl_2, rt; (h) $NaNO_2$, H_2O, AcOH, 5 °C to rt; (i) Zn, AcOH, MeOH, 0 °C to 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 to rt; (l) CH_3I, *t*-BuOK, THF, 0 °C.

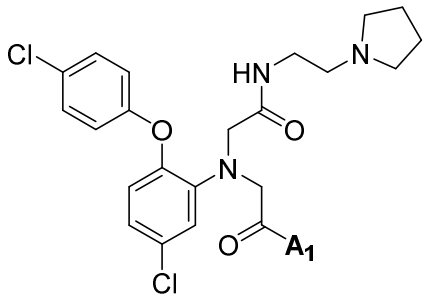
第３章　合成化合物の酵素阻害活性と考察

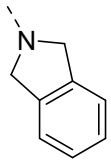
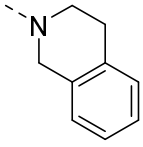
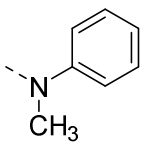
酵素阻害活性の測定は市販のヒト由来*S*-アデノシル-L-ホモシステイン加水分解酵素を用いて行った。本酵素反応は可逆反応であるので、酵素溶液中に、基質、阻害剤に加えアデノシンデアミナーゼを添加し、酵素反応によって精製したアデノシンをイノシンへと変換させることにより逆向きの反応が起きないようにし、残存した基質の量をＨＰＬＣで測定することにより実施した。

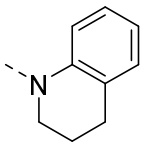
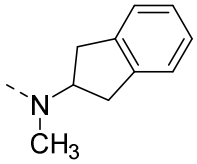
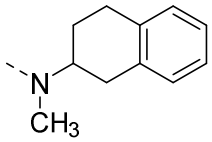
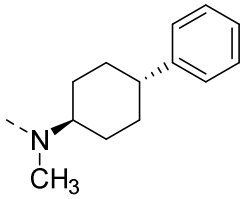
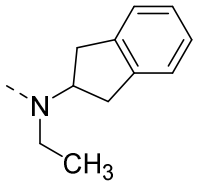
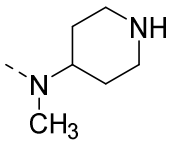
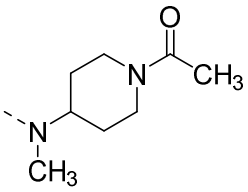
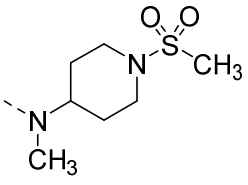
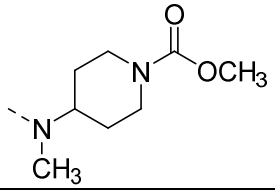
第１節　脂溶性アミド部位の変換結果

脂溶性アミド部位の変換の結果を以下に示す。HTS ヒット化合物 **8a-f** の中で、**8d** のみが脂溶性アミド部位のアミン置換基として環構造を有していた。脂溶性アミド部位が、環状、非環状に関係なく３級アミドであることが強い酵素阻害活性発現に重要なのかどうかを確認するため、化合物 **17a**、**17b** を合成、評価した。しかしながら、**17a**、**17b** の酵素阻害活性は大幅に減弱した。脂溶性アミド部位が３級アミドであることが強い酵素阻害活性を示すのに重要なのではなく、アミドの脂溶性置換基が適切な位置に配置されていることが重要であることが示唆された。シスアミド仮説を検証するため、HTS ヒット化合物 **8f** の２級アミドを *N*- メチル化した化合物 **17e** を合成、評価した。その結果、**17e** の酵素阻害活性は IC_{50} = 0.052±0.010 μM を示し **8f** と比較し約２００倍近い活性の向上が見られた。**17e** のインダン部位をテトラヒドロナフタレンに変換した化合物 **17f** にも同様に強い活性が見られたが、**17e** のメチルアミドをエチルアミドに変換した化合物 **17h** は活性が約１０倍低下した。化合物 **8b** のシクロヘキサン部位の変換においては、シクロヘキサン部位をベンゼン環に変換した化合物 **17c** は活性が低下する結果となった。**17c** のメチルフェニルアミンを１，２，３，４－テトラヒドロキノリンに変換した化合物 **17d** は **17c** とほぼ同様の酵素阻害活性を示した。シクロヘキサンの４位にベンゼン環を置換した化合物 **17g** は **8b** と比べやや活性が低下した。シクロヘキサンを４－ピペリジニル基へ変換した化合物 **17j** は酵素阻害活性が消失したが、ピペリジンのアミノ基にアセチル基（**17k**）、メタンスルホニル基（**17l**）、メトキシカルボニル基（**17m**）などの置換基を導入すると、阻害活性が復活した。

表３－１　脂溶性アミド部位の変換１



化合物番号	A_1	IC_{50} (μM)*
17a		>100
17b		17 ± 2
17c		2.7 ± 0.9

17d		2.3 ± 0.2
17e		0.052 ± 0.010
17f		0.081 ± 0.017
17g		0.53 ± 0.08
17h		0.64 ± 0.12
17j		>100
17k		4.6 ± 0.5
17l		0.23 ± 0.05
17m		0.89 ± 0.18

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

第２節　^{1}H-NMR スペクトルからの立体構造予測と次なる化合物デザイン

酵素阻害活性が向上した化合物 **17e** の ^{1}H-NMR を精査したところ、２－インダニルメチルアミド付近のメチンプロトン、メチル基、およびカルボニル基のα位のメチレンのプロトンピークがいずれも２：３の比率で別々に観測され、シス－トランス異性体の存在が示唆された。このような脂溶性アミド付近のプロトンピークが別々に観測される現象は、分割比率には差はあったが化合物 **17f**、**17h**、**17i-m** 等でも確認された。

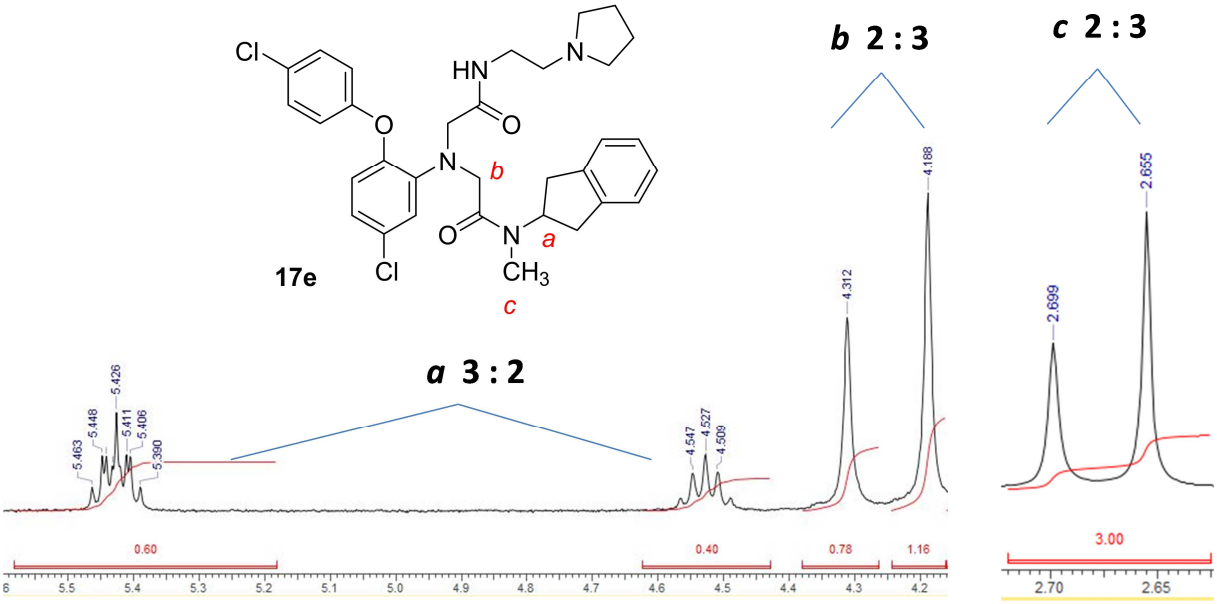


図３－１　化合物 **17e** の ^{1}H-NMR spectra

17e のその他の異性体としてインダニル基の回転に伴う異性体も考えられ、本化合物は多様なコンフォメーションを取りうると考えられた。

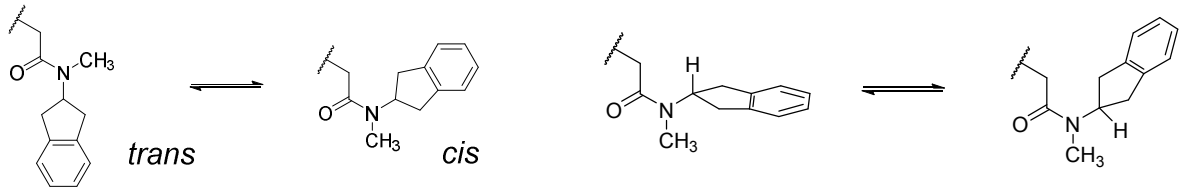
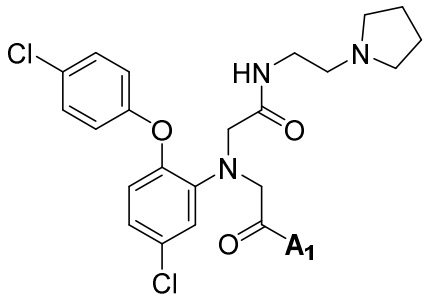


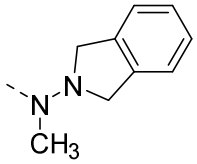
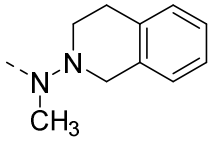
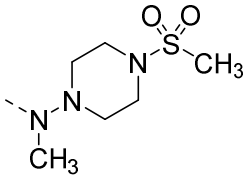
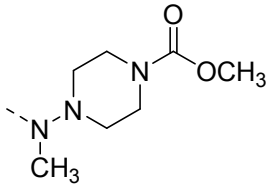
図３－２　化合物 **17e** の脂溶性アミド付近の幾何異性体模式図．左はトランスーシス異性、右はインダニル基の回転異性．

多様な異性体の存在は、化合物が酵素タンパクと相互作用する際に不利に働くと考えられる。２－アミノインダン基のメチン炭素部位を窒素に変換することで、少なくともインダニル基の回転異性の可能性は排除できると考え、化合物 **17e** のインダン基のイソインドリン基への変換を行った。その結果、約４倍の酵素阻害活性の向上が確認された。一方、化合物 **17f** のテトラヒドロナフタレン部位をイソインドリンへ変換した化合物 **17o** は活性が低下する結果となった。化合物 **17l** および **17m** のピペリジンをピペラジンへ変換した化合物 **17r** および **17s** はそれぞれ約５倍、２０

倍の活性の向上が見られた。

表３－２　脂溶性アミド部位の変換２



化合物番号	A_1	IC_{50} (μM)*
17n		0.013 ± 0.003
17o		0.20 ± 0.02
17r		0.049 ± 0.008
17s		0.044 ± 0.006

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

化合物 **17n** の ^{1}H-NMR スペクトルデータから、イソインドリンの２つのメチレン基、メチル基、カルボニルα位のメチレン基のピークから異性体の存在は確認されず、シス体またはトランス体どちらか単一の状態、あるいはシストランス相互変換が非常に速い状態として存在していることが示唆された。同様に化合物 **17o**、**17r**、**17s** の ^{1}H-NMR スペクトルデータについても異性体の存在を示唆するピークは確認されなかった。

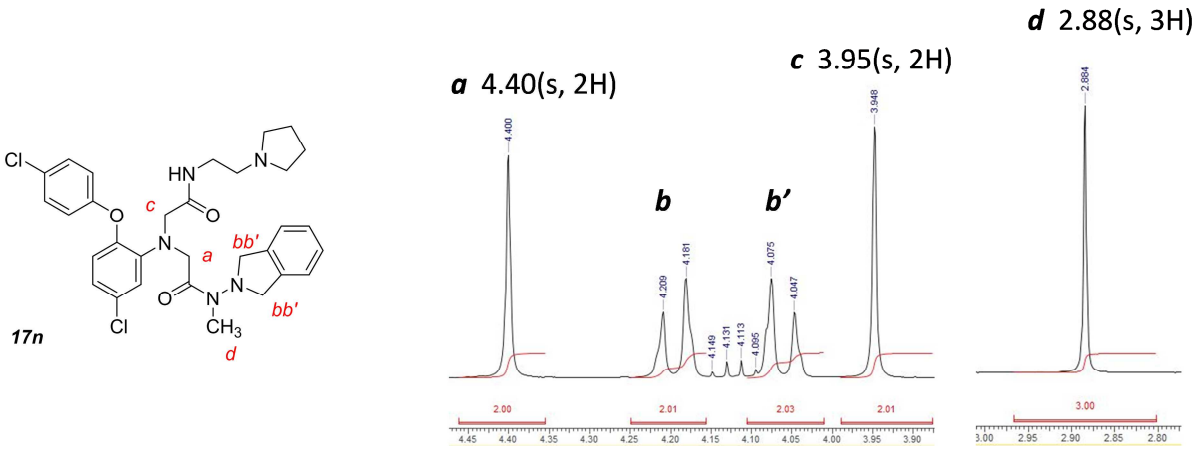
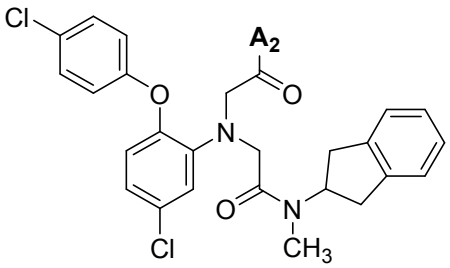


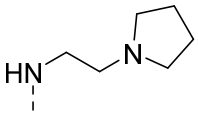
図３－３　化合物 **17n** の ^{1}H-NMR spectra

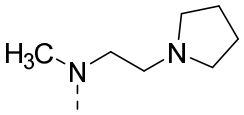
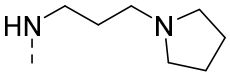
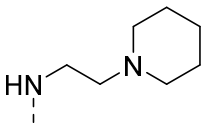
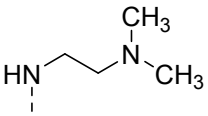
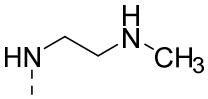
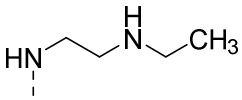
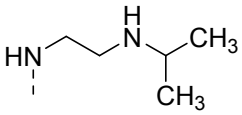
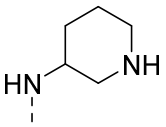
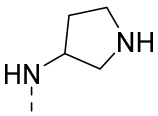
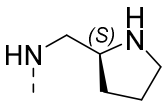
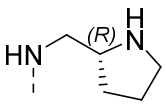
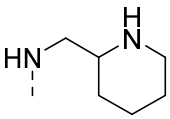
第３節　水溶性アミド部位の変換結果

水溶性アミド部位の変換の結果を以下に示す。化合物 **17e** の２－ピロリジルエチルアミドのアミド NH をメチル化した化合物 **20a** は活性が消失した。エチルアミドからプロピルアミドへの変換（化合物 **20b**）、ピロリジンからピペリジンへの変換（化合物 **20c**）、ジメチルアミンへの変換（化合物 **20d**）はともに活性が低下する結果となった。３級アミンから２級アミンへの変換、すなわちピロリジン部位のメチルアミン（化合物 **20m**）、エチルアミン（化合物 **20n**）、イソプロピルアミン（化合物 **20o**）への変換は活性を損なうことなく同等の酵素阻害活性を示すことが確認された。２つの窒素原子の距離が２炭素分である環状タイプの２級アミドへの変換は、環化パターンによって活性が大きく変動し、アミドの隣接炭素で環化した化合物 **20p** および **20q** では活性の大幅な低下が見られたが、アミドから２炭素分離れた位置で環化した化合物のうち、化合物 **20r**、**20t** は活性がやや低下する程度であった。

表３－３　水溶性アミド部位の変換



化合物番号	A_2	塩	IC_{50} (μM)*
17e		-	0.052 ± 0.010

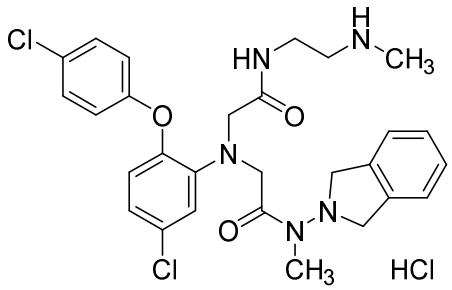
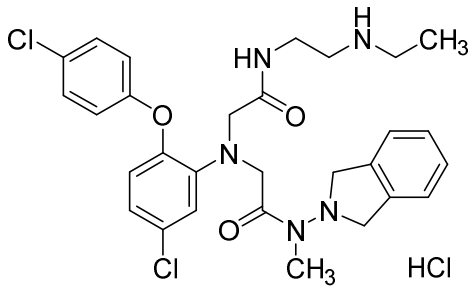
20a		-	>100
20b		-	0.33 ± 0.05
20c		-	0.32 ± 0.05
20d		-	0.13 ± 0.03
20m		HCl	0.070 ± 0.012
20n		HCl	0.060 ± 0.006
20o		HCl	0.049 ± 0.005
20p		HCl	1.2 ± 0.1
20q		HCl	1.5 ± 0.2
20r		HCl	0.11 ± 0.01
20s		HCl	0.60 ± 0.07
20t		HCl	0.15 ± 0.02

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

第４節　最適パーツの組み合わせ結果

脂溶性アミド部位の変換から見出されたイソインドリン構造と水溶性アミド部位の変換から見出された*N* - メチルアミン、*N* - エチルアミン構造を組み合わせた化合物 **22a**、および **22b** を合成、評価したところ、シングルナノモルオーダーの非常に強い酵素阻害活性を示すことが確認された。

表３－４　脂溶性アミド部位、水溶性アミド部位の最適パーツの組み合わせ

化合物番号	化学構造	IC_{50} (μM)*
22a		0.0050 ± 0.0020
22b		0.0085 ± 0.0031

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

第５節　阻害剤の酵素阻害様式の確認

化合物 **22a** を用いて、*S* - アデノシル - L - ホモシステイン加水分解酵素の酵素反応の阻害様式の検討を行った。基質である *S* - アデノシル - L - ホモシステインの濃度を 2.5, 5.0, 7.5, 10, 20 nmol/L と変化させ、0, 1, 3, および 6 nmol/L の濃度での化合物 **22a** の酵素反応阻害率を算出した。Lineweaver-Burk プロットによる解析の結果、**22a** の酵素阻害様式は拮抗阻害であり、*Ki* = 1.5 nmol/L であると計算された。

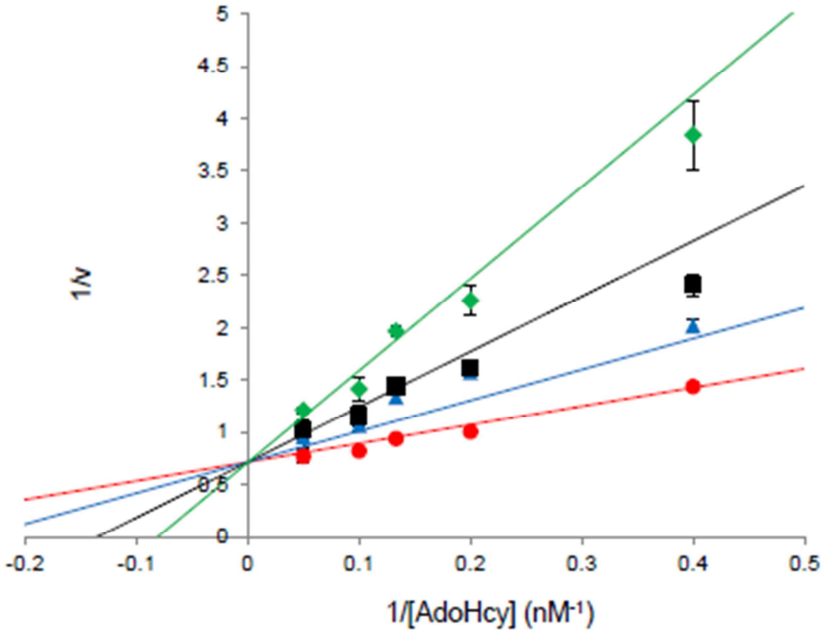


図３－４　化合物 **22a** による *S*-アデノシル-L-ホモシステイン加水分解酵素阻害の Lineweaver-Burk プロット：Assays were conducted by incubation of various concentrations of the substrate (AdoHcy) with AdoHcyase in the absence (red circles●) and presence of different concentrations [1 nM (blue triangles▲), 3 nM (black squares■), and 6 nM (green diamonds◆)] of **22a**. SAS software, version 9.2 (SAS Institute Inc.) was used for statistical analyses.

第４章　阻害剤と酵素タンパクとの X 線複合体解析

第１節　単結晶の取得と回折強度測定

酵素中での阻害剤の活性コンフォメーションを確認するため、X 線共結晶構造解析を行った。市販のヒト由来 *S*-アデノシル-L-ホモシステイン加水分解酵素を Yuan, C.-S. らの方法[15]を参考にタンパク精製し、5 mM **22a**, 40 %(v/v) 2-PrOH, 20 mM Tris-HCl(pH7.2), 0.1 M NaCl, 1 mM EDTA, 1 mM DTT 溶液を AdoHcyase 試料に 1/10 量添加した 7.7 mg/mL AdoHcyase の溶液 1 μL とリザーバー1 μL を混合してドロップとし、100 mM HEPES(pH 7.5), 13 %(w/v) PEG4K, 10 %(v/v) 2-PrOH, 0.5 %(v/v) Ethyl Acetate のリザーバーを用いて、シッティングドロップ蒸気拡散法により X 線結晶構造解析が可能な単結晶を調整した。100 mM HEPES(pH7.5), 25 %(w/v) PEG4K, 15 %(v/v) Glycerol 溶液に 2 時間浸漬した板状晶（大きさ 0.1×0.05×0.005 mm）を放射光施設 SPring-8 のビームライン BL24XU、結晶の回折強度測定を理学電機（株）の放射光用 X 線検出装置 R-AXIS V、データ処理を HKL Research Inc. の回折強度処理プログラム HKL2000 を使用して実施した。その結果、実効分解能 2.7 Å のデータセットを得た。

表４－１　測定条件

Beamline	BL24XU
Wave length （Å）	0.82565
Oscillation angle (°)	1.0
Measurement range (°)	-90 ～ 90
No. of images	180
Temperature (℃)	-180

表４－２　結晶学的データ

Resolution (Å)	50.0-2.7	
No. of observed reflections	195263	
No. of unique reflections	31605	
Completeness (%)	99.6	(highest range*2: 98.3)
Average I/σ(I)	25.4	(highest range*2: 10.1)
Rmerge*1	0.092	(highest range*2: 0.235)
Crystal system	*Orthorhombic*	
Space group	$C222_1$	
Cell constants (Å)	a = 91.53	
	b = 134.2	
	c = 185.2	

*1: Rmerge = Σ (|(I - <I>)|) / Σ (I)

*2: 2.80-2.70 Å resolution

第２節　結晶構造解析

英国 CCLRC の CCP4 プログラムスイートに含まれる AMoRe を用いて分子置換法による結晶構造解析を行った。プロテインデータバンク（PDB）にはヒトおよびその他の種の AdoHcyase 単体、および阻害剤との複合体が登録されている。AdoHcyase は４量体として存在し、それぞれの単量体には触媒ドメインと NAD 結合ドメインが存在し、その間に基質の結合部位が存在する。基質や阻害剤を含まない AdoHcyase はおもに open form として存在し、基質や阻害剤を含む複合体は closed form として存在することが報告されている[10b,16]。今回、ヒト closed 型 AdoHcyase（PDB ID:1A7A）、ラット closed 型 AdoHcyase（1K0U）、ラット open 型 AdoHcyase（1B3R）の４量体の半分（２分子）をそれぞれ探索分子モデルに選択し、分解能 15–4.0 Åの回折データを用いて交叉回転関数の計算を実施した。全ての探索分子モデルから解が得られたが、ラット open 型 AdoHcyase を探索分子モデルとした場合に最も高い相関係数を示した。回転関数で求めた回転行列を探索分子モデルに適用した後、分解能 15–4.0 Åの回折データを用いて並進関数を計算し、非対称単位に含まれる AdoHcyase 分子の位置を決定した。その結果、非対称単位には４

量体の半分（２分子）が存在することが明らかとなった。そこで、ラット open 型 AdoHcyase の２分子を解の位置に移動し、アミノ酸残基をヒト AdoHcyase に置換して NAD^+を除いた蛋白質部分を構造精密化の初期分子モデルとした。

表４－３　ラット open 型 AdoHcyase（２分子）を探索分子モデルとした分子置換法の解

交叉回転関数の解

No. of Solution	α	β	γ	T_x	T_y	T_z	CC_F*1	RF_F*2
1	85.30	4.50	95.33	0.0000	0.0000	0.0000	22.3	56.2
2	360.68	0.00	0.00	0.0000	0.0000	0.0000	12.1	59.0
3	180.50	0.00	0.00	0.0000	0.0000	0.0000	12.0	59.0
4	0.50	0.00	0.00	0.0000	0.0000	0.0000	12.0	59.0

並進関数の解

No. of Solution	α	β	γ	T_x	T_y	T_z	CC_F*1	RF_F*2
1	85.30	4.50	95.33	0.2817	0.9994	0.1051	58.2	45.8
2	85.30	4.50	95.33	0.2178	0.0003	0.1053	56.0	46.9
3	85.30	4.50	95.33	0.2817	0.9993	0.3942	42.5	52.6
4	85.30	4.50	95.33	0.2819	0.9993	0.0790	42.5	52.7
5	85.30	4.50	95.33	0.2178	0.0005	0.3933	42.2	52.6

*1: Correlation Coefficient

*2: R factor

第３節　構造精密化

Accelrys Inc.の結晶構造解析用プログラム CNX で結晶構造の精密化を実施した。データセットに含まれる回折点の中から無作為に抽出した５％の回折点を精密化計算から除外し、その５％のデータの R 因子（free R）を残りのデータの R 因子と比較し、精密化計算の妥当性評価に使用した。

まず、初期分子モデルを open 型と closed 型で位置が移動する４つのドメイン（非対称単位中の２分子の触媒ドメインと NAD 結合ドメイン）に分け、それぞれを構成する計８つのセグメントのグループを作り（アミノ酸残基番号 5–197、198–350、351–386、387–432）、分解能 6.0–3.0 Åのデータを用いてグループ内を剛体近似し、位置の精密化を行った。次に、仮想温度 2500Kから 300Kまでの Simulated Annealing を実施した後、３次元分子グラフィックスプログラム Quanta を用いた電子密度図に対するモデルフィッティングと、CNX による原子位置・温度因子の精密化を交互に行った。蛋白質部分の構造がほぼ決定された後、Fo-Fc を係数とする差フーリエ合成により得られた電子密度図に合わせて、NAD^+、水分子、化合物 **22a** と残りの水分子の順に構造を決定しながら、構造精密化を進めた。構造精密化に必要な各分子のパラメータファイルは蛋白質、水分子については CNX 内のものを、NAD^+及び **22a** についてはウプサラ大学のプログラム xplo2d によって作製したものを使用した。最終的に 50.0–2.7 Å分解能の回折データにおいて Bulk Solvent 領域の実空間での電子密度補正を適応し、AdoHcyase ２分子、NAD^+ ２分子、

22a ２分子、水 244 分子に対して、R=0.199、free R=0.240 (R = Σ | Fo-Fc | / Σ | Fo |)を得た。最終座標の蛋白質部分についてプログラム PROCHECK にて各種統計量が当該実効分解能での許容範囲に収まっていることを確認した。

表４－４　構造精密化統計値

Resolution (Å)	47.81-2.7	
No. of all reflections	31525	(highest range*1: 4826)
Completeness (working + test) (%)	99.5	(highest range*1: 98.5)
R*2(working)	0.199	(highest range*1: 0.229)
free R	0.240	(highest range*1: 0.282)
free R test set size (%)	5.0	(highest range*1: 5.5)
No. of reflections used for free R	1565	(highest range*1: 280)
No. of non-hydrogen atoms used in refinement		
AdoHcyase	6644	
NAD^+	88	
22a	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	
22a	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	

*1: 2.87–2.70 Å resolution

*2: R = Σ | Fo-Fc | /Σ | Fo |

第４節　化合物 **22a** と AdoHcyase、NAD^+の立体構造

上記の剛体近似を用いた精密化後の Fo-Fc 電子密度図において化合物 **22a** と予想される電子密度が観測された。AdoHcyase 、NAD^+及び水分子を含めた構造精密化後の Fo-Fc 電子密度図では更に明確な **22a** の電子密度が現れた。その電子密度の形状は **22a** の立体的特徴を反映しており、容易に **22a** の構造を電子密度に当てはめることができた。

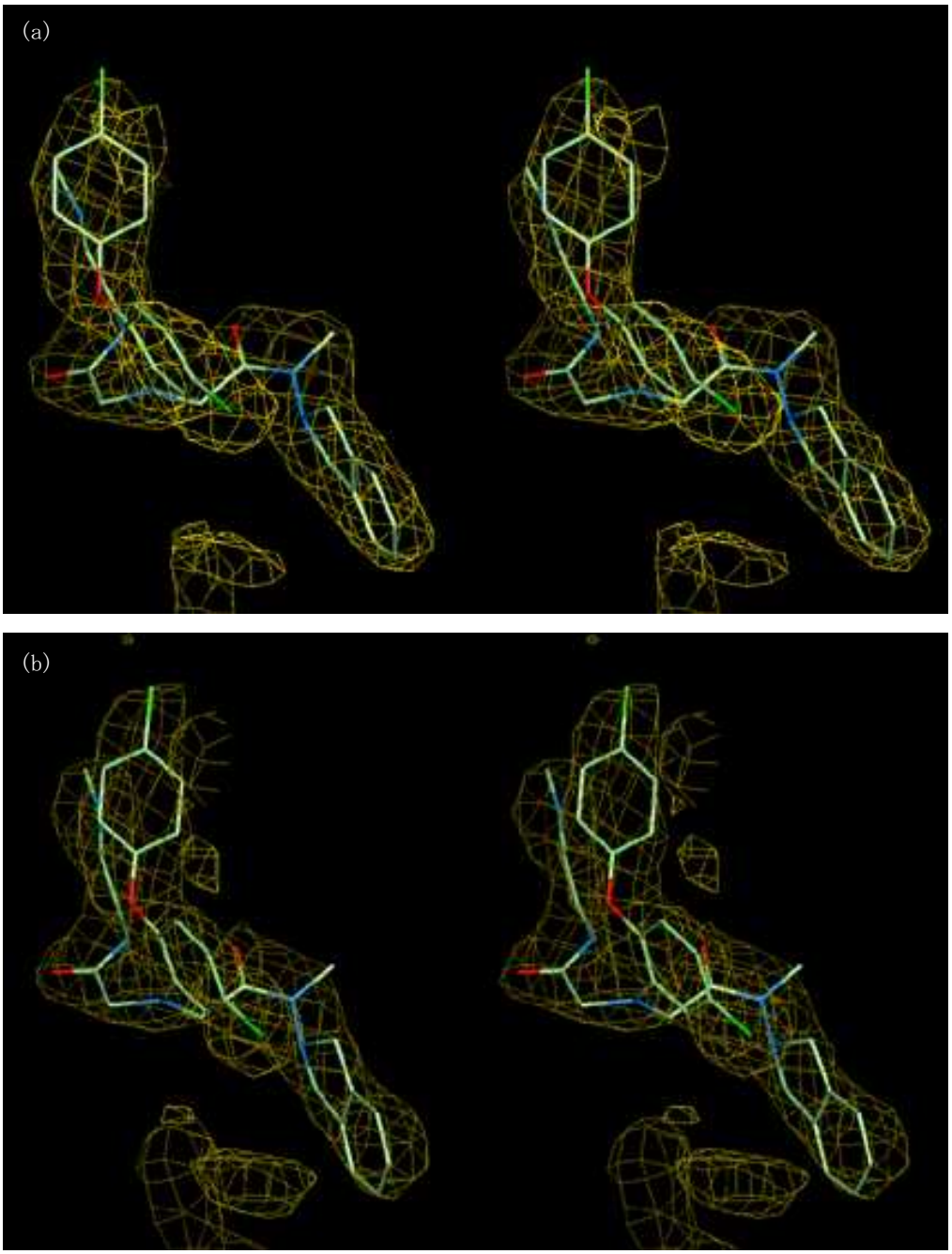


図４－１　**22a** の電子密度図（Fo-Fc map, 3σカット, ステレオ図）(a), (b):非対称単位中の２分子　黄色：電子密度図、白色：炭素原子、赤色：酸素原子、青色：窒素原子、緑色：塩素原子

AdoHcyase 単量体について、それぞれ NAD^+、**22a** が１分子ずつ結合していた。**22a** のイソインドリン部位は、既に構造解析されている closed 型を誘導する阻害剤（PDB ID:1A7A）と類似の位置に存在するが、その他の部分は AdoHcyase 単量体の２つのドメイン間に挟まれており、AdoHcyase 単量体は open 型に近いドメインの配置を取っていた（PDB ID:4YVF)。

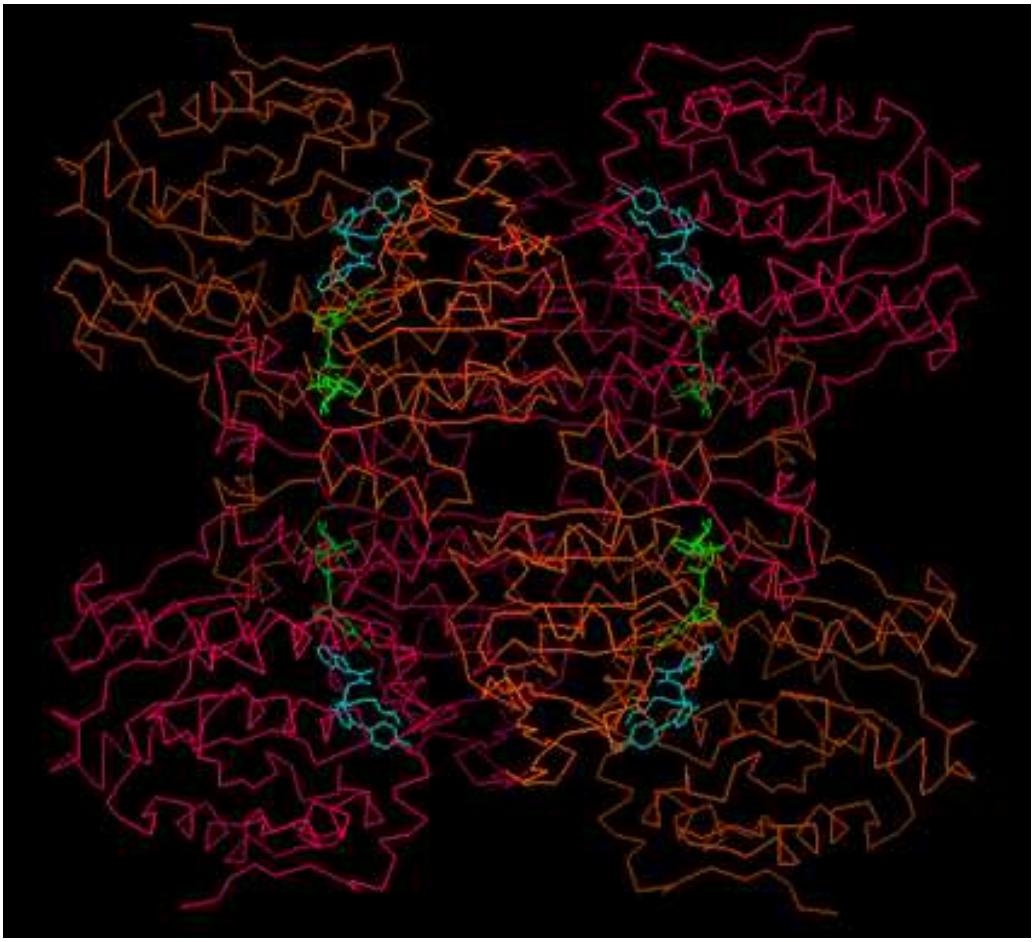


図４－２　AdoHcyase ４量体とそれに結合している NAD^+及び **22a**
紫色、橙色：AdoHcyase（Cα トレース、非対称単位２分子)、緑色：NAD^+、水色：**22a**

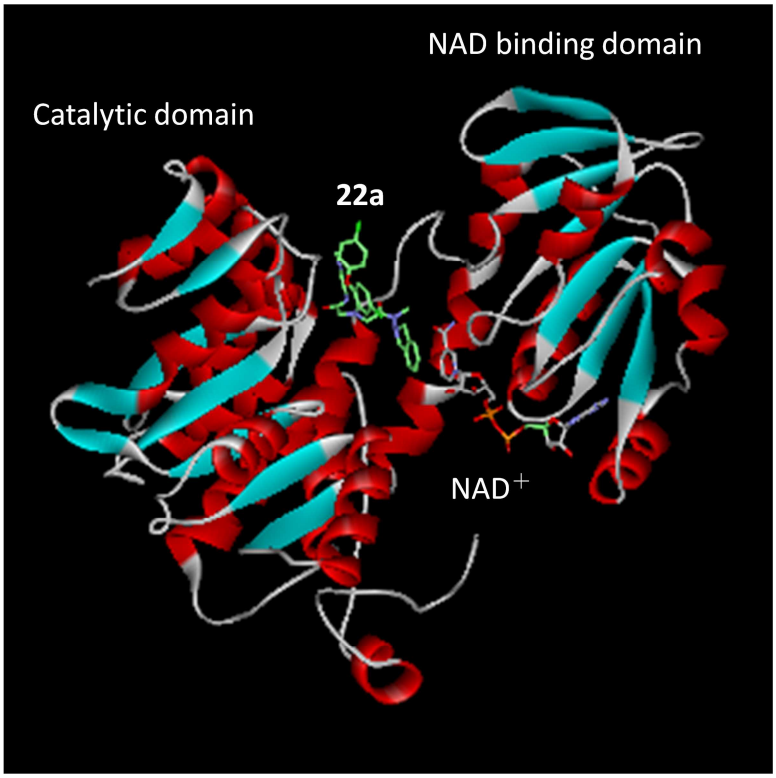


図４－３　AdoHcyase のドメイン（リボンモデル）と **22a**
赤：α ヘリックス、水色：β シート

化合物 **22a** のイソインドリン部位は NAD^+のニコチンアミド芳香環と 3.9~4.0Åの距離で平行に位置し、カチオン - π スタッキング相互作用[17]をしていることが示唆された。置換アニリン部分の芳香環は２つのドメインに挟まれた状態で位置し、NAD 結合ドメイン中の ロイシン 347 との疎水性相互作用、触媒ドメインのヒスチジン 55 とπ - π スタッキング相互作用[18]をしていることが示唆された。化合物 **22a** には４つの水素結合の存在が示唆された。すなわち、水溶性アミド部位のカルボニル酸素と触媒ドメイン中のスレオニン 57 の側鎖水酸基との水素結合、メチルアミノ基と触媒ドメインのグルタミン酸 59 の側鎖カルボキシル基との水素結合、脂溶性アミド部位のカルボニル酸素と NAD 結合ドメイン中のヒスチジン 353 主鎖アミドとの水素結合、および **22a** の水溶性アミド部位の NH と脂溶性アミド部位の酸素との分子内８員環水素結合[19,20]が示唆された。また、脂溶性アミド部位の３級アミド部位はカルボニル酸素に対し、*N*- メチル基がシスに位置していることが判明し、化合物最適化戦略時に立てた『シスアミド仮説』を支持する結果となった。

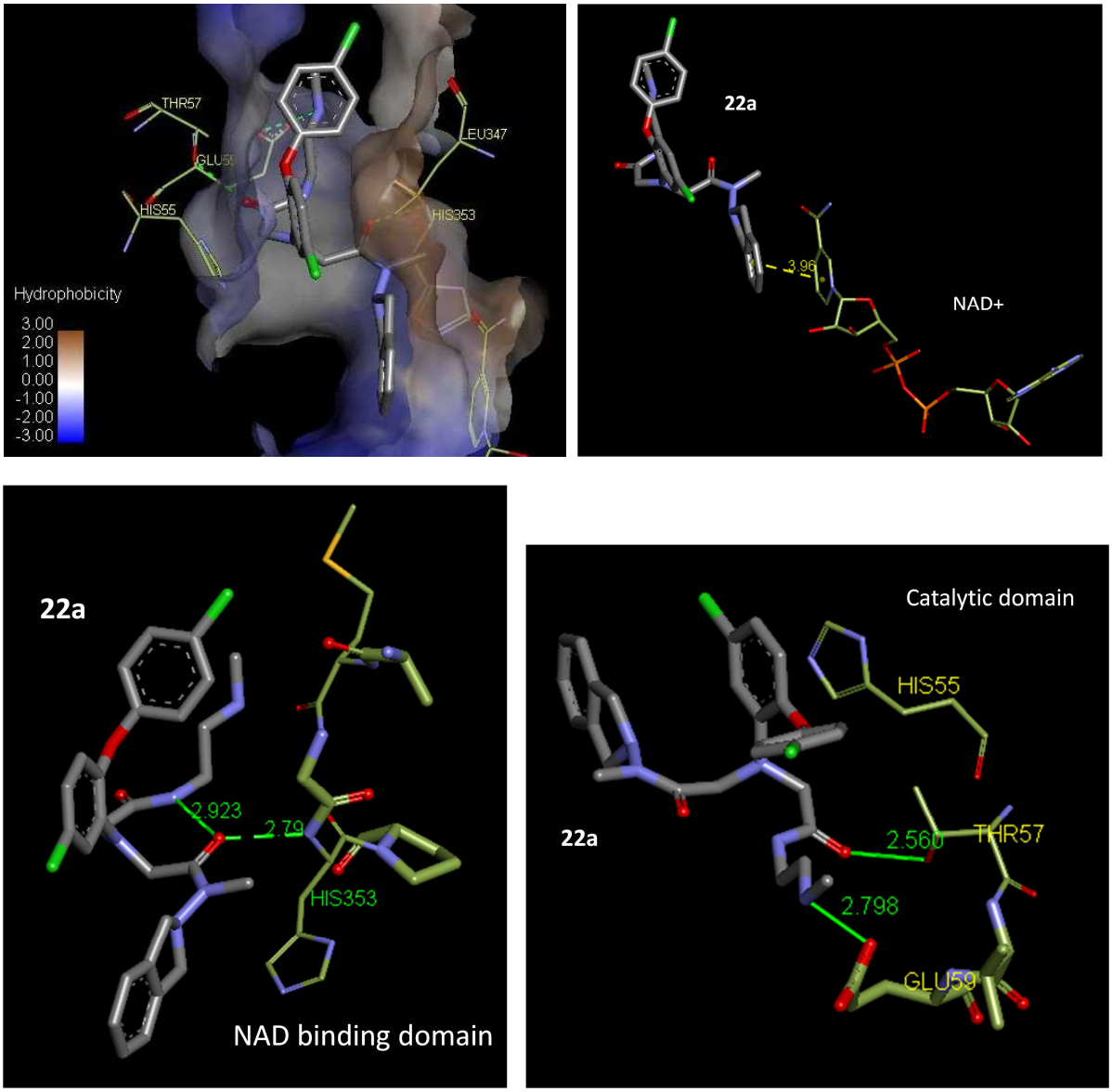


図４－４　Discovery Studio 4.1 による AdoHcyase、NAD+、化合物 **22n** の立体構造図（左上）：**22a** が触媒ドメインと NAD 結合ドメインに挟まれている図。（右上）：**22a** と NAD+のニコチンアミド芳香環とのカチオン - πスタッキング相互作用。（左下）：**22a** の脂溶性アミド部位カルボニル酸素と HIS353 主鎖アミドとの水素結合、および **22a** の分子内８員環水素結合。（右下）：**22a** の芳香族アニリン部位と HIS55 のイミダゾール環とのπ－πスタッキング相互作用、水溶性アミド部位カルボニル酸素と THR57 側鎖水酸基との水素結合、および水溶性アミド部位アミノ基と GLU59 側鎖カルボキシル基との水素結合。図中の数値は原子間距離（Å）を表す。

第５章　阻害剤単体の X 線構造解析

X 線複合体解析の結果、脂溶性アミド部位の３級アミドがシス型をとり、さらに分子内８員環水素結合をしていることが示唆されたが、これは化合物 **22a** が AdoHcysase および NAD+と相互作用することによって生じた結果であると考えられた。これを検証するため、阻害剤単体の立体構

造をX線構造解析により明らかにしようと考えた。残念ながら、化合物 **22a** の単結晶を取得することはできなかったが、幸運にも化合物 **22a** と非常に近い構造、すなわち末端のアミノ基がメチルアミノ基からピロリジンになった化合物 **17n** の結晶化に成功した。**17n** をジエチルエーテルから再結晶化することにより単結晶を作成しX線構造解析を実施した。

表５－１　化合物 **17n** の結晶データと構造精密化

Compound	**17n**
Empirical formula	$C_{31}H_{35}Cl_2N_5O_3$
Formula weight	596.56
Recryst. Solvent	Diethyl ether
Crystal Color, Habit	colorless, prismatic
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	3068.9(5) Å^3
Space group	*Cc*
Z value	4
Density (calculated)	1.291 g/cm^3
Absorption coefficient	22.252 cm^{-1}
Crystal size	0.300 x 0.200 x 0.100 mm^3
Temperature	298 K
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
Max Shift/Error in Final Cycle	0.000
Maximum peak in Final Diff. Map	0.27 e$^-$/Å^3
Minimum peak in Final Diff. Map	-0.25 e$^-$/Å^3
CCDC reference number	1049632

化合物 **17n** は化合物 **22a** と同様に、脂溶性アミド部位の３級アミドがシス型を取っており、さらに水溶性アミド部位の窒素原子（図５－１の N2）と脂溶性アミド部位の酸素原子（図５－１の O3）との原子間距離が 2.918 Å、水素原子を挟む角度が 166° であることから、分子内８員環水素結合をしていることが確認された[21)]。

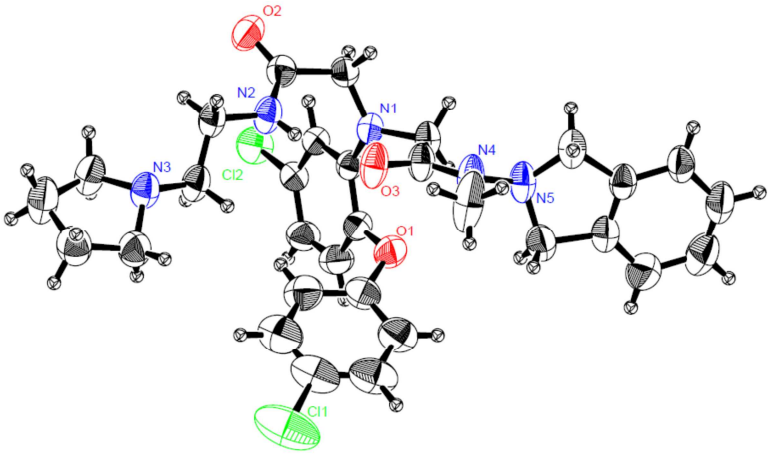


図５－１　ORTEP drawings of **17n** with 50% probability displacement ellipsoids

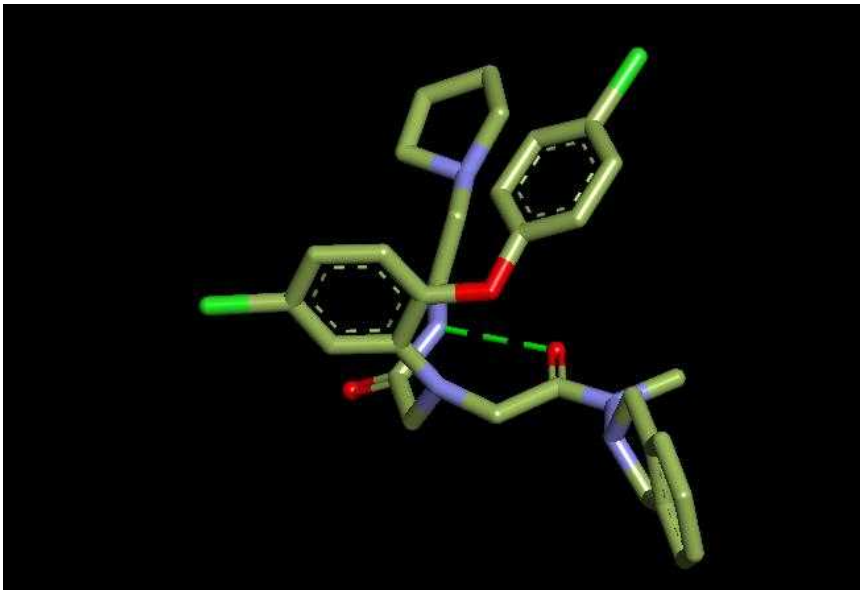


図５－２　Discovery Studio 3.5 による化合物 **17n** の立体構造図

表５－２　化合物 **17n** の分子内水素結合

A…H－D	A…D（Å）	∠A…H－D（°）
O3(A) …H－N2(D)	2.918(9)	166.16

化合物 **17n** の重クロロホルム中でのプロトン NMR 解析の結果、X 線単結晶構造解析の結果、および化合物 **22a** の X 線複合体解析の結果から、*N*-（イソインドリン-２－イル）-*N*-メチルアミド構造を有する化合物群は、少なくとも酵素タンパクとの結合部位や非極性有機溶媒中といった疎水性環境下において、脂溶性アミド部位の３級アミドはシス型となり、さらに分子内８員環水素結合を形成していることが示唆された。

第６章　総括

高ホモシステイン状態を正常化させることにより脳梗塞等の疾患の予防、治療ができるのではないかと考え、その合成酵素である *S*-アデノシル-L-ホモシステイン加水分解酵素の阻害剤の化合物最適化研究を実施した。阻害剤が酵素と相互作用する際の立体構造を予測し、３級アミド部位がシス型をとることが活性発現に重要であると考えた。化合物最適化研究によって得られた化合物 **22a** と酵素との X 線複合体解析の結果から、複合体中の酵素は open form として存在していることが確認された。また酵素中で阻害剤の３級アミド部位はシス型をとっていることが示され、分子内で８員環水素結合をとっていることが示唆された。X 線単結晶構造解析の結果、化合物 **17n** は分子内で８員環水素結合を形成するとともに、その３級アミド部位はシス型をとっていることが確認された。化合物 **22a** の酵素阻害様式が拮抗阻害であることを示し、*Ki* 値は *Ki* = 1.5 nmol/L であった。これまでにない非アデノシン骨格を有する高活性な化合物 **22a,b** は高ホモシステイン状態を改善する薬剤の研究におけるリード化合物となり得るだけでなく、その構造活性相関情報、および X 線複合体解析情報は我々の今後のさらなる最適化研究の一助となると考えている。

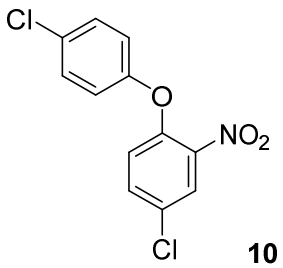
第 7 章　実験項

第 1 節　化合物合成

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. ^{1}H 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, brs =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 (3μm) 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 (3μm) 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.

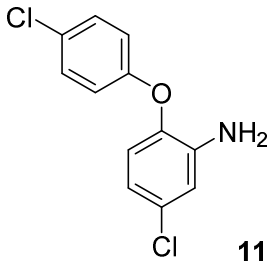
4-Chloro-1-(4-chlorophenoxy)-2-nitrobenzene (10)[22]



10

To a solution of 4-chlorophenol (10.6 g, 82.5 mmol) in DMF (100mL) 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 (**9**) (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 (400mL). The precipitated solid was filtered, washed with H_2O (100 mL), 1 mol/L aqueous NaOH (100 mL x2), and H_2O (100 mL), and dried under reduced pressure to give **10** (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 $[M+H]^+$.

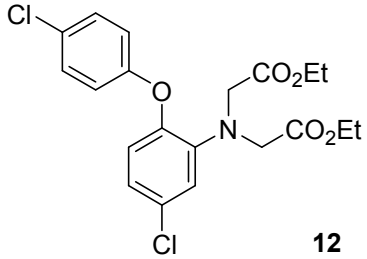
5-Chloro-2-(4-chlorophenoxy)aniline (11)[23]



11

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 **11** (10.6 g, 99%) as a colorless solid. Mp 59–61 °C. ^{1}H NMR (400 MHz, $CDCl_3$): δ (ppm) 3.85 (brs, 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 $[M+H]^+$.

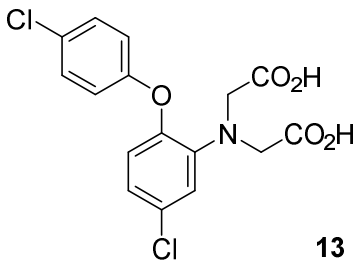
***N*-[5-Chloro-2-(4-chlorophenoxy)phenyl]iminodiacetic acid diethyl ester (12)**



12

A solution of **11** (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 (100mL x2), 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 **12** (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 $[M+H]^+$.

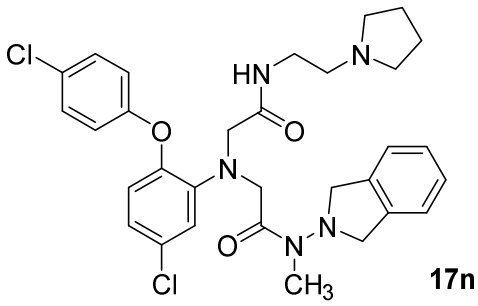
_N_-[5-Chloro-2-(4-chlorophenoxy)phenyl]iminodiacetic acid (13)



13

To a solution of **12** (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 **13** (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 (brs, 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.

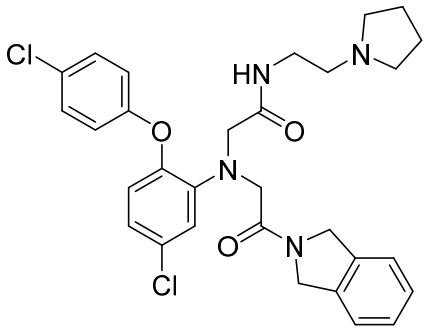
N^2-[5-Chloro-2-(4-chlorophenoxy)phenyl]-N^2-{2-[1,3-dihydro-2*H*-isoindol-2-yl(methyl)amino]-2-oxoethyl}-N^1-(2-pyrrolidin-1-ylethyl)glycinamide (17n)



17n

To a solution of **13** (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 **15n** (192 mg, 1.04 mmol) and *N,N*-diisopropylethylamine (0.180 mL, 1.06 mmol), and the mixture was stirred at 0 °C for 1h. 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 **17n** (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.

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 (17a)

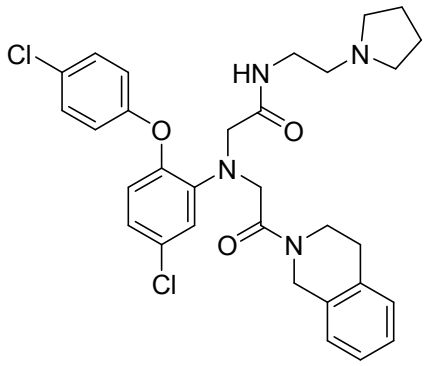


17a

The title compound was synthesized according to the method described for the synthesis of **17n** to afford **17a** (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 (brs, 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.

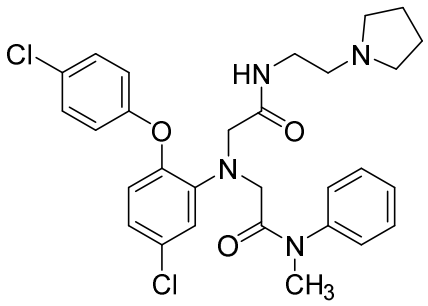
N^2-[5-Chloro-2-(4-chlorophenoxy)phenyl]-N^2-{2-[3,4-dihydroisoquinolin-2(1*H*)-yl]-2-oxoethyl}-N^1-(2-pyrrolidin-1-ylethyl)glycinamide (17b)



17b

The title compound was synthesized according to the method described for the synthesis of **17n** to afford **17b** (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 (brs, 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.

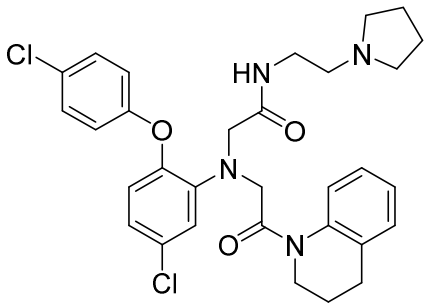
N^2-[5-Chloro-2-(4-chlorophenoxy)phenyl]-N^2-{2-[methy(phenyl)amino]-2-oxoethyl}-N^1-(2-pyrrolidin-1-ylethyl)glycinamide (17c)



17c

The title compound was synthesized according to the method described for the synthesis of **17n** to afford **17c** (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 (brs, 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.

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 (17d)

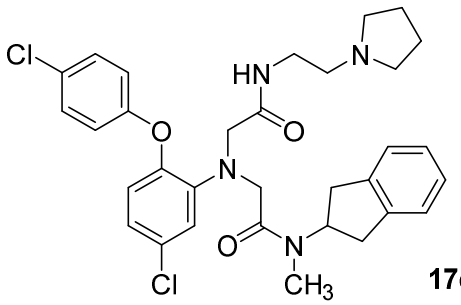


17d

The title compound was synthesized according to the method described for the synthesis of **17n** to afford **17d** (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 (brs, 2H), 3.96 (s, 2H), 4.30 (brs, 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 (brs, 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.

N^2-[5-Chloro-2-(4-chlorophenoxy)phenyl]-N^2-{2-[2,3-dihydro-1*H*-inden-2-yl(methyl)amino]-2-oxoeth

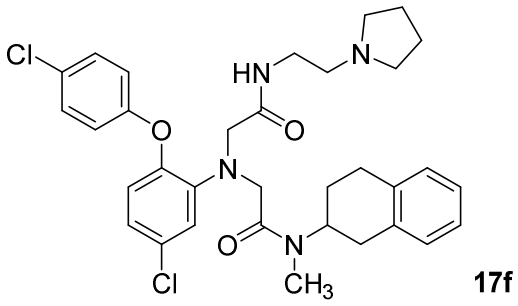
yl}-N^1-(2-pyrrolidin-1-ylethyl)glycinamide (17e)



17e

The title compound was synthesized according to the method described for the synthesis of **17n** to afford **17e** (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 (2broad, 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.

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 (17f)

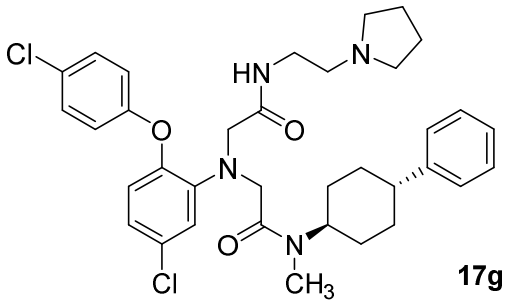


17f

The title compound was synthesized according to the method described for the synthesis of **17n** to afford **17f** (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 (brs, 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.

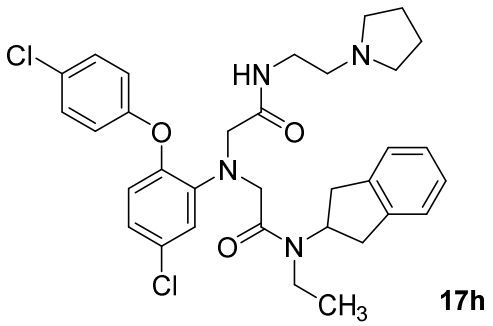
N^2-[5-Chloro-2-(4-chlorophenoxy)phenyl]-N^2-{2-[methyl(*trans*-4-phenylcyclohexyl)amino]-2-oxoethyl}-N^1-(2-pyrrolidin-1-ylethyl)glycinamide (17g)



17g

The title compound was synthesized according to the method described for the synthesis of **17n** to afford **17g** (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.

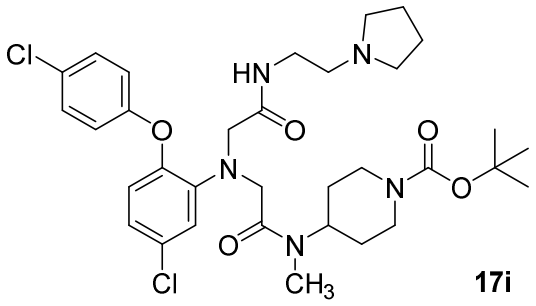
N^2-[5-Chloro-2-(4-chlorophenoxy)phenyl]-N^2-{2-[2,3-dihydro-1*H*-inden-2-yl(ethyl)amino]-2-oxoethyl}-N^1-(2-pyrrolidin-1-ylethyl)glycinamide (17h)



17h

The title compound was synthesized according to the method described for the synthesis of **17n** to afford **17h** (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.

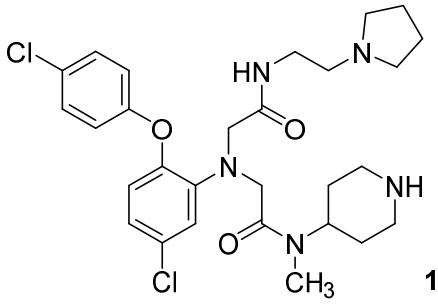
N^2-{2-{[1-(*tert*-Butoxycarbonyl)piperidin-4-yl](methyl)amino}-2-oxoethyl}-N^2-[5-chloro-2-(4-chlorophenoxy)phenyl] -N^1-(2-pyrrolidin-1-ylethyl)glycinamide (17i)



17i

The title compound was synthesized according to the method described for the synthesis of **17n** to afford **17i** (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 $[M+H]^+$.

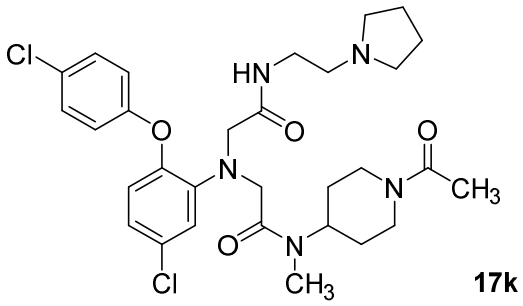
N^2-[5-Chloro-2-(4-chlorophenoxy)phenyl]-N^2-{2-[methyl(piperidin-4-yl)amino]-2-oxoethyl}-N^1-(2-pyrrolidin-1-ylethyl)glycinamide (17j)



17j

To a solution of **17i** (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 **17j** (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.

***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 (17k)**

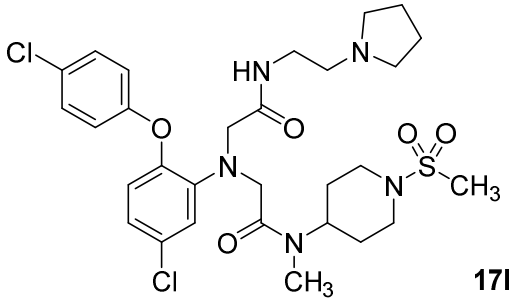


17k

To a solution of **17j** (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 **17k** (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.

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 (17l)

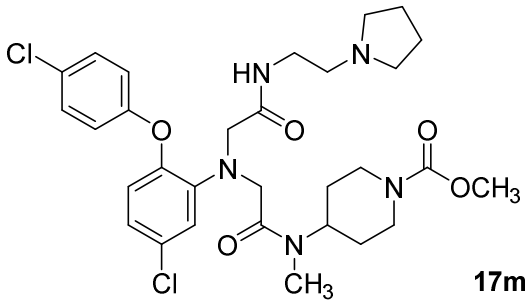


17l

To a solution of **17j** (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 **17l** (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 (brs, 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.

N^2-[5-Chloro-2-(4-chlorophenoxy)phenyl]-N^2-{2-{[1-(methoxycarbonyl)piperidin-4-yl](methyl)amino

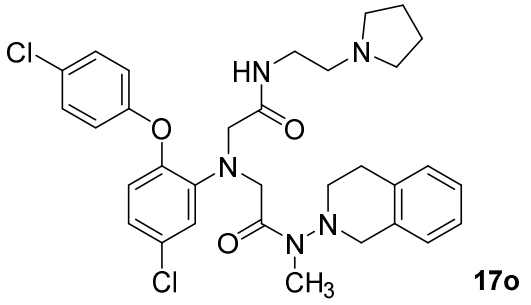
}-2-oxoethyl}-*N*^{1}-(2-pyrrolidin-1-ylethyl)glycinamide (17m)



17m

To a solution of **17j** (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 **17m** (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.

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 (17o)

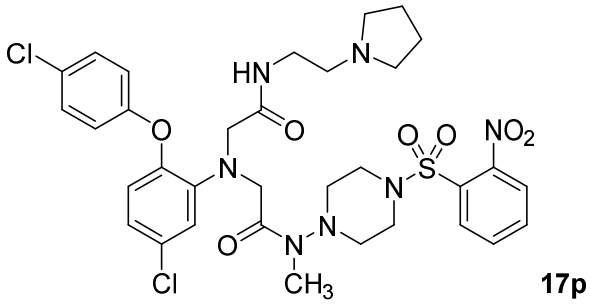


17o

The title compound was synthesized according to the method described for the synthesis of **17n** to afford **17o** (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.

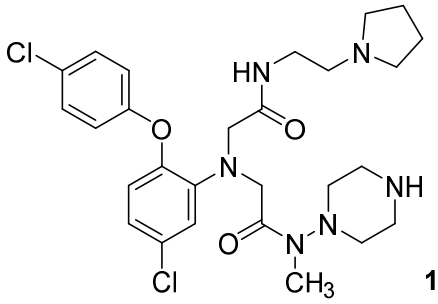
N^2-[5-Chloro-2-(4-chlorophenoxy)phenyl]-N^2-{2-[methyl{4-[(2-nitrophenyl)sulfonyl]piperazin-1-yl}amino]-2-oxoethyl}-N^1-(2-pyrrolidin-1-ylethyl)glycinamide (17p)



17p

The title compound was synthesized according to the method described for the synthesis of **17n** to afford **17p** (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 $[M+H]^+$.

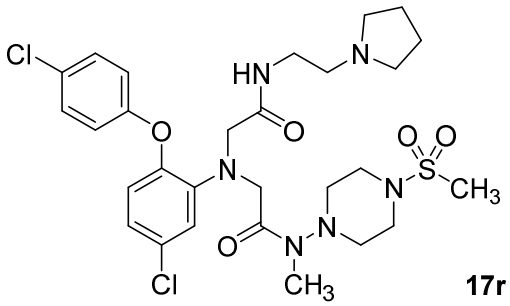
N^2-[5-Chloro-2-(4-chlorophenoxy)phenyl]-N^2-{2-[methyl(piperazin-1-yl)amino]-2-oxoethyl}-N^1-(2-pyrrolidin-1-ylethyl)glycinamide (17q)



17q

To a solution of **17p** (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 x3). 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 **17q** (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 $[M+H]^+$.

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 (17r)

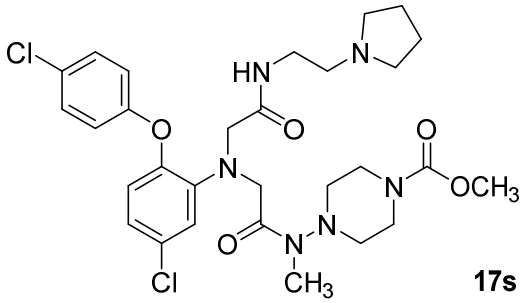


17r

The title compound was synthesized from **17q** according to the method described for the synthesis of **17l** to afford **17r** (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.

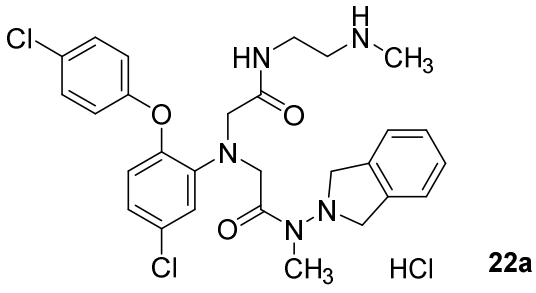
N^2-[5-Chloro-2-(4-chlorophenoxy)phenyl]-N^2-{2-{[4-(methoxycarbonyl)piperidin-1-yl](methyl)amino}-2-oxoethyl}-N^1-(2-pyrrolidin-1-ylethyl)glycinamide (17s)



17s

The title compound was synthesized from **17q** according to the method described for the synthesis of **17m** to afford **17s** (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.

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 (22a)

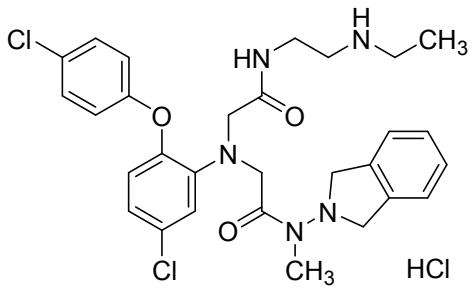


22a

To a solution of **13** (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 **17n** (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·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 **21a** 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 x2), 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 **22a** (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 (brs, 4H), 7.40 (d, J = 8.7, 2H), 8.50 (t, J = 5.7, 1H), 8.73 (brs, 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.

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 (22b)

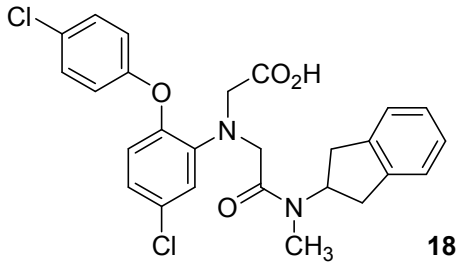


22b

The title compound was synthesized according to the method described for the synthesis of **22a** to afford **22b** (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.

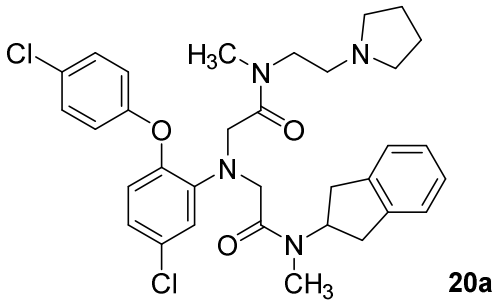
_N_-[5-Chloro-2-(4-chlorophenoxy)phenyl]-_N_-{2-[2,3-dihydro-1_H_-inden-2-yl(methyl)amino]-2-oxoethyl }glycine (18)



18

To a solution of **13** (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 **15e** (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 **18** (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 (brs, 3H), 6.91 (d, *J* = 8.7, 2H), 7.08–7.26 (m, 4H), 7.39 (d, *J* = 8.7, 2H), 12.91 (brs, 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.

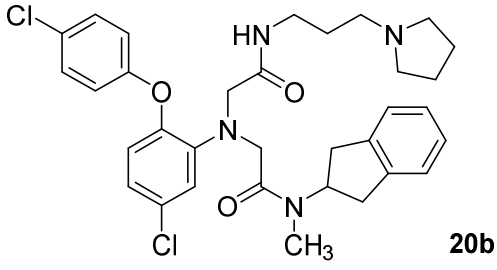
N^2-[5-Chloro-2-(4-chlorophenoxy)phenyl]-N^2-{2-[2,3-dihydro-1_H_-inden-2-yl(methyl)amino]-2-oxoethyl}-N^1-methyl(2-pyrrolidin-1-ylethyl)glycinamide (20a)



20a

To a solution of **18** (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 **20a** (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 (2m, 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.

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 (20b)

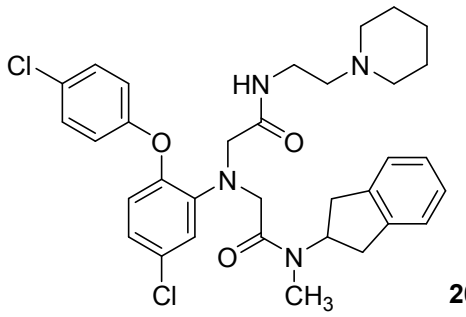


20b

The title compound was synthesized according to the method described for the synthesis of **20a** to afford **20b** (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 (2broad, 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.

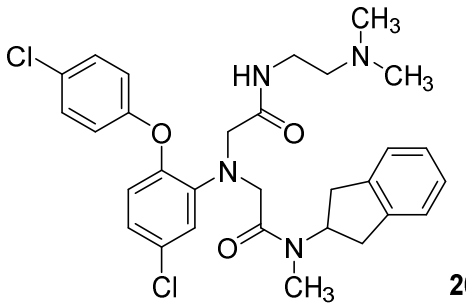
N^2-[5-Chloro-2-(4-chlorophenoxy)phenyl]-N^2-{2-[2,3-dihydro-1*H*-inden-2-yl(methyl)amino]-2-oxoeth yl}-N^1- (2-piperidin-1-ylethyl)glycinamide (20c)



20c

The title compound was synthesized according to the method described for the synthesis of **20a** to afford **20c** (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 (2broad, 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.

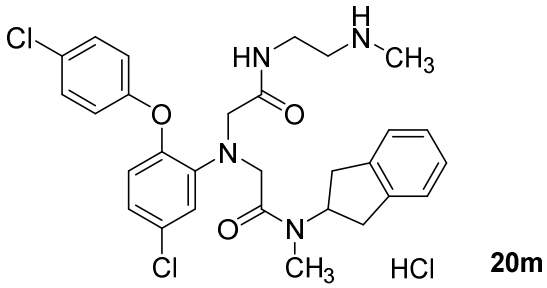
N^2-[5-Chloro-2-(4-chlorophenoxy)phenyl]-N^2-{2-[2,3-dihydro-1*H*-inden-2-yl(methyl)amino]-2-oxoeth yl}-N^1- [2-(dimethylamino)ethyl]glycinamide (20d)



20d

The title compound was synthesized according to the method described for the synthesis of **20a** to afford **20d** (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.

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 (20m)

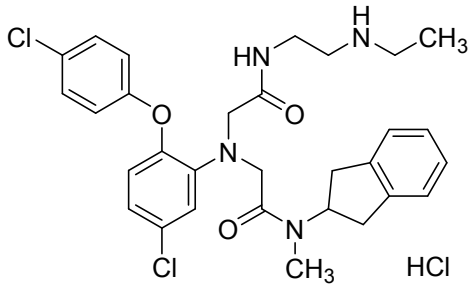


20m

To a solution of **18** (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 **20e** 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 **20m** (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 (brs, 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.

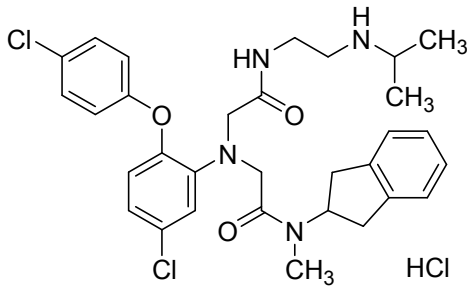
N^2-[5-Chloro-2-(4-chlorophenoxy)phenyl]-N^2-{2-[2,3-dihydro-1*H*-inden-2-yl(methyl)amino]-2-oxoeth yl}-N^1- [2-(ethylamino)ethyl]glycinamide hydrochloride (20n)



20n

The title compound was synthesized according to the method described for the synthesis of **20m** to afford **20n** (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 (brs, 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.

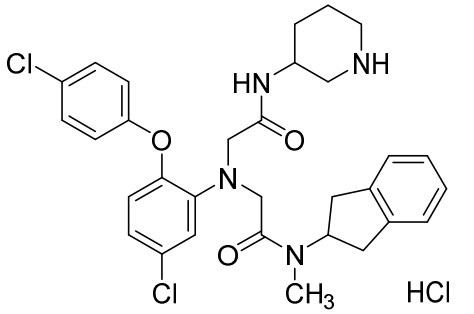
N^2-[5-Chloro-2-(4-chlorophenoxy)phenyl]-N^2-{2-[2,3-dihydro-1*H*-inden-2-yl(methyl)amino]-2-oxoeth yl}-N^1- [2-(isopropylamino)ethyl]glycinamide hydrochloride (20o)



20o

The title compound was synthesized according to the method described for the synthesis of **20m** to afford **20o** (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 (brs, 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.

N^2-[5-Chloro-2-(4-chlorophenoxy)phenyl]-N^2-{2-[2,3-dihydro-1*H*-inden-2-yl(methyl)amino]-2-oxoeth yl}-N^1- (piperidin-3-yl)glycinamide hydrochloride (20p)

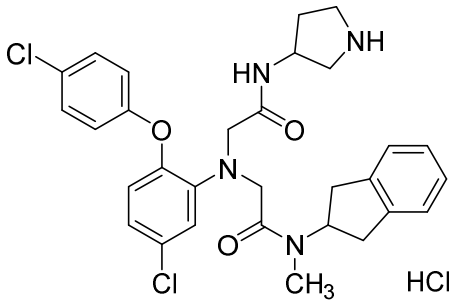


20p

The title compound was synthesized according to the method described for the synthesis of **20m** to afford **20p** (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 (brs, 1H), 9.12 (brs, 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.

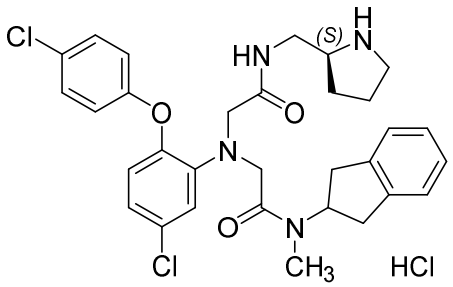
N^2-[5-Chloro-2-(4-chlorophenoxy)phenyl]-N^2-{2-[2,3-dihydro-1*H*-inden-2-yl(methyl)amino]-2-oxoeth yl}-N^1- (pyrrolidin-3-yl)glycinamide hydrochloride (20q)



20q

The title compound was synthesized according to the method described for the synthesis of **20m** to afford **20q** (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 (brs, 1H), 9.36 (brs, 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.

N^2-[5-Chloro-2-(4-chlorophenoxy)phenyl]-N^2-{2-[2,3-dihydro-1*H*-inden-2-yl(methyl)amino]-2-oxoeth yl}-N^1-[(2*S*)-pyrrolidin-2-ylmethyl]glycinamide hydrochloride (20r)

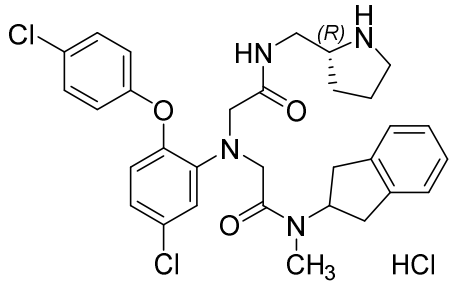


20r

The title compound was synthesized according to the method described for the synthesis of **20m** to afford

20r (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 (brs, 1H), 8.85 (t, J = 5.4, 1H), 9.44 (brs, 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.

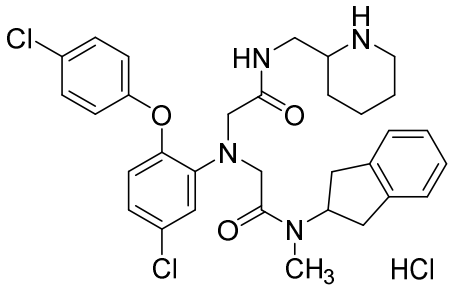
N^2-[5-Chloro-2-(4-chlorophenoxy)phenyl]-N^2-{2-[2,3-dihydro-1*H*-inden-2-yl(methyl)amino]-2-oxoethyl}-N^1-[(2*R*)-pyrrolidin-2-ylmethyl]glycinamide hydrochloride (20s)



20s

The title compound was synthesized according to the method described for the synthesis of **20m** to afford **20s** (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 (brs, 1H), 8.87 (t, J = 5.1, 1H), 9.19 (brs, 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.

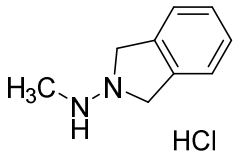
N^2-[5-Chloro-2-(4-chlorophenoxy)phenyl]-N^2-{2-[2,3-dihydro-1*H*-inden-2-yl(methyl)amino]-2-oxoethyl}-N^1-(piperidin-2-ylmethyl)glycinamide hydrochloride (20t)



20t

The title compound was synthesized according to the method described for the synthesis of **20m** to afford **20t** (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.

_N_-Methyl-1,3-dihydro-2_H_-isoindol-2-amine hydrochloride (15n)[24]

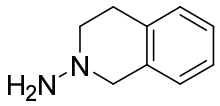


15n

Xylylene dibromide (160 g, 606 mmol) and *tert*-butyl 1-methylhydrazinecarboxylate (**30**) (88.5 g, 606 mmol) obtained according to the reported method[25] 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 (**31**) (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 **15n** (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 (brs, 4H), 7.23–7.43 (m, 4H), 10.97 (brs, 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.

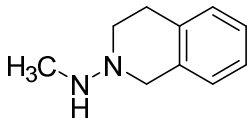
3,4-Dihydroisoquinolin-2(1*H*)-amine (33)[26]



33

To a stirred solution of 1,2,3,4-tetrahydroisoquinoline (**32**) (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 x3). 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 **33** (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 $[M+H]^+$.

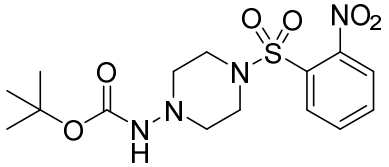
***N*-Methyl-3,4-dihydroisoquinolin-2(1*H*)-amine (15o)**[27]



15o

To a stirred solution of **33** (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 $[M+H]^+$.

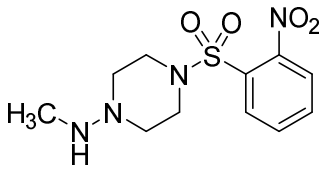
***tert*-Butyl 4-[(2-nitrophenyl)sulfonylpiperazin-1-yl]carbamate (35)**



35

To an ice-cooled solution of *tert*-Butyl piperazin-1-ylcarbamate (**34**) (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.

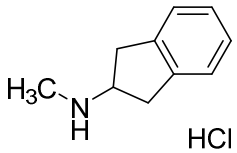
***N*-Methyl-4-(2-nitrophenyl)sulfonyl-piperazin-1-amine hydrochloride (15p)**



15p

To a stirred solution of **35** (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 **15p** (3.53 g, 83%) as a colorless solid. Mp 224–226 °C. ^{1}H NMR (400 MHz, DMSO-d_6): δ (ppm) 2.64 (brs, 3H), 3.09 (brs, 4H), 3.36 (brs, 4H), 7.86–7.92(m, 1H), 7.95(t, J = 7.7, 1H), 8.01–8.06(m, 2H), 10.85 10.98 (2brs, 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.

***N*-Methylindan-2-amine hydrochloride (15e)**[28]

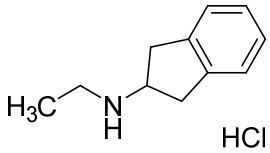


15e

2-Aminoindane (**23**) (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 **24a** 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, and 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 **15e** (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 (brs, 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 $[M+H]^+$.

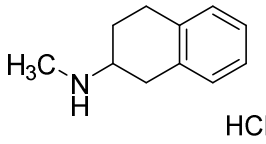
***N*-Ethylindan-2-amine hydrochloride (15h)**[28]



15h

The title compound was synthesized according to the method described for the synthesis of **15e** from 2-aminoindane (**23**) (1.07 g, 8.03 mmol) and acetic anhydride(0.76 mL, 8.04 mmol) to afford **15h** (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 (brs, 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 $[M+H]^+$.

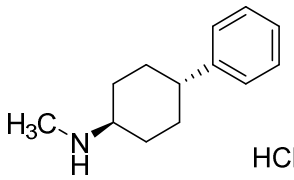
***N*-Methyl-1,2,3,4-tetrahydronaphthalen-2-amine hydrochloride (15f)**[28]



15f

The title compound was synthesized according to the method described for the synthesis of **15e** from 1,2,3,4-tetrahydronaphthalen-2-amine (**25**) (2.50g, 13.6 mmol) to afford **15f** (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 (brs, 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 $[M+H]^+$.

***trans*-*N*-Methyl-4-phenylcyclohexanamine hydrochloride (15g)**



HCl **15g**

The title compound was synthesized according to the method described for the synthesis of **15e** from *trans*-4-phenylcyclohexanamine (**27**) (3.42 g, 16.2 mmol) to afford **15g** (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 (brs, 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.

第２節　X 線結晶構造解析

Crystallization, data, and structural refinement of human recombinant AdoHcyase, NAD^+, and 22a

The enzyme of human recombinant AdoHcyase purchased from Diazyme Laboratory was purified according to the method reported by Yuan C.-S. et al.[15] 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 x 0.05 mm x 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 hours 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 4-4. The crystallographic refinement parameters, final (Fo-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, (PDB ID: 4YVF).

X-ray crystal structure determination of 17n

A colorless prismatic crystal of compound **17n** suitable for X-ray single crystal analysis was obtained at

room temperature by partial evaporation from diethyl ether. X-ray data 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.

第３節　in vitro 酵素阻害活性測定

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 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 10000 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.

参考文献

1. Tehlivets, O.; Malanovic, N.; Visram, M.; Pavkov-Keller, T.; Keller, W. *Biochim. Biophys. Acta* **2013**, *1832*, 204–215.
2. McCully, K. S. *Am. J. Pathol.* **1969**, 56, 111–128.
3. (a) Brattstrom, L. E.; Hardebo, J. E.; Hultberg, B. L. *Stroke* **1984**, *15*, 1012–1016. (b) Coull, B. M.; Malinow, M. R.; Beamer, N.; Sexton, G.; Nordt, F.; Garmo, P. *Stroke* **1990**, *21*, 572–576. (c) Brattstrom, L.; Lindgren, A.; Israelsson, B.; Malinow, M. R.; Norrving, B.; Upson, B.; Hamfelt, A. *Eur. J. Clin. Invest.*, **1992**, *22*, 214–221. (d) Nygård, O.; Vollset, S. E.; Refsum, H.; Stensvold, I.; Tverdal, A.; Nordrehaug, J. E.; Ueland, M.; Kvåle, G. *JAMA* **1995**, *274*, 1526–1533. Bots, M. L.; Launer, L. J.; Lindemans, J.; Hoffman, A.; Grobbee, P. E. *J. Intern. Med.* **1997**, *242*, 339–347. (e) The European Concerted Action Project, *JAMA* **1997**, *277*, 1775–1781. (f) Yoo, J-H.; Shung, C-H.; Kang, S-S. *Stroke* **1998**, *29*, 2478–2483.
4. Perry, I. J.; Morris, R. W.; Ebrahim, S. B.; Shaper, A. G.; Refsum, H.; Ueland, P. M. *Lancet* **1995**, *346*, 1395–1398.
5. Homocysteine and Risk of Ischemic Heart Disease and Stroke: A Meta-analysis, *JAMA* **2002**, *288*, 2015–2022.
6. (a) Seshadri, S.; Beiser, A.; Selhub, J.; Jacques, P. F.; Rosenberg, I. H.; D'Agostino, R. B.; Wilson, P. W.; Wolf, P. A. *N. Eng. J. Med.* **2002**, *346*, 476–483. (b) Van Dam, F.; Van Gool, W. A. *Arch. Gerontol. Geriatr.* **2009**, *48*, 425–430. (c) Stanger, O.; Fowler, B.; Pietrzik, K.; Huemer, M.; Haschke-Becher, E.; Semmler, A.; Lorenzl, S.; Linnebank, M. *Expert Rev. Neurother.* **2009**, *9*, 1393–1412.
7. Endres,M.; Ahmadi, M.; Kruman, I.; Biniszkiewicz, D.; Meisel, A.; Gertz, K. *Stroke* **2005**, *36*, 321–325.
8. Langheinrich, A. C.; Braun-Dullaeus, R. C.; Walker, G.; Jeide, I.; Schiling, R.; Tammoscheit, K.; Dreyer, T.; Fink, L.; Bohle, R. M.; Haberbosch, W. *Atherosclerosis* **2003**, *171*, 181–192.
9. Yuan, C.-S.; Saso, Y.; Lazarides, E.; Borchardt, R. T.; Robins, M. J. *Exp. Opin. Ther. Patents* **1999**, *9*, 1197–1206.
10. (a) Wu, Q.-L.; Fu, Y.-F.; Zhou, W.-L.; Wang, J.-X.; Feng, Y.-H.; Liu, J.; Xu, J.-Y.; He, P.-L.; Zhou, R.; Tang, W.; Wang, G.-F.; Zhou, Y.; Yang, Y.-F.; Ding, J.; Li, X.-Y.; Chen, X.-R.; Yuan, C.; Lawson, B. R.; Zuo, J.-P. *J. Pharmacol. Exp. Ther.* **2005**, *313*, 705–711. (b) Yamada, T.; Komoto, J.; Lou, K.; Ueki, A.; Hua, D. H.; Sugiyama, K.; Takata, Y.; Ogawa, H.; Takusagawa, F. *Biochem. Pharmacol.* **2007**, *73*, 981–989. (c) Zhang, Y.-M.; Ding, Y.; Tang, W.; Luo, W.; Gu, M.; Lu, W.; Tang, J.; Zuo, J.-P.; Nan, F.-J. *Bioorg. Med. Chem.* **2008**, *16*, 9212–9216. (d) Kim, B. G.; Chun, T. G.; Lee, H.- Y.; Snapper, M. L. *Bioorg. Med. Chem.* **2009**, *17*, 6707–6714. (e) Converso, A.; Hartingh, T.; Fraley, M. E.; Garbaccio, R. M.; Hartman, G. D.; Huang, S. Y.; Majercak, J. M.; McCampbell, A.; Na, S. J.; Ray, W. J.;

Savage, M. J.; Wolffe, C.; Yeh, S.; Yu, Y.; White, R.; Zhang, R. *Bioorg. Med. Chem. Lett.* **2014**, *24*, 2737–2740.

11. (a) Annis D. A.; Athanasopoulos, J.; Curran, P. J.; Felsch, J. S.; Kalghatgi, K.; Lee, W. H.; Nash, H. M.; Orminati, J-P. A.; Rosner, K. E.; Shipps, J. G. W.; Thaddupathy, G. R. A.; Tyler, A. N.; Vilenchik, L.; Wagner, C. R.; Wintner, E. A. *Int. J. Mass Spectrom.* **2004**, *238*, 77–83. (b) Annis, D. A.; Nickbarg, E.; Yang, X.; Ziebell, M. R.; Whitehurst, C. E. *Curr. Opin. Chem. Biol.* **2007**, *11*, 518–526.
12. Schulz, G. E.; Schirmer, R. H. *Principles of Protein Structure*; Springer: New York, 1979.
13. Laursen, J. S.; Engel-Andreasen, J.; Fristrup, P.; Harris, P.; Olsen, C. A. *J. Am. Chem. Soc.* **2013**, *135*, 2835–2844.
14. (a) Itai, A.; Toriumi, Y.; Saito, S.; Hiroyuki, Kagechika; Shudo, K. *J. Am. Chem. Soc.* **1992**, *114*, 10649–10650.
15. Yuan, C.-S.; Yeh, J.; Liu, S.; Borchardt, R. T. *J. Biol. Chem.* **1993**, *268*, 17030–17037.
16. (a) Hu, Y.; Komoto, J.; Huang Y.; Gomi, T.; Ogawa, H.; Takata, Y.; Fujioka, M.; Takusagawa, F. *Biochemistry* **1999**, *38*, 8323–8333. (b) Turner, M. A.; Yuan, C. S.; Borchardt, R. T.; Hershfield, M. S.; Smith, G. D.; Howell, P. L. *Nature Struct. Biol.* **1998**, *5*, 369–376. (c) Komoto, J.; Huang, Y.; Gomi, Y.; Ogawa, H.; Takata, Y.; Fujioka, M.; Takusagawa, F. *J. Biol. Chem.* **2000**, *275*, 32147–32156. (d) Huang, Y.; Komoto, J.; Takata, Y.; Powell, D. R.; Gomi, T.; Ogawa, H.; Fujioka, M.; Takusagawa, F. *J. Biol. Chem.* **2002**, *277*, 7477–7482. (e) Takata, Y.; Yamada, T.; Huang, Y.; Komoto, J.; Gomi, T.; Ogawa, H.; Fujioka, M.; Takusagawa, F. *J. Biol. Chem.* **2002**, *277*, 22670–22676. (f) Yang, X.; Hu, Y.; Yin, D. H.; Turner, M. A.; Wang, M.; Borchardt, R. T.; Howell, P. L.; Kuczera, K.; Schowen, R. L. *Biochemistry* **2003**, *42*, 1900–1909. (g) Yamada, T.; Takata, Y.; Komoto, J.; Gomi, T.; Ogawa, H.; Fujioka, M.; Takusagawa, F. *Int. J. Biochem. Cell Biol.* **2005**, *37*, 2417–2435. (h) Yamada, T.; Komoto, J.; Lou,K.; Ueki, A.; Hua, D. H.; Sugiyama, K.; Takata, Y.; Ogawa, H.; Takusagawa, F. *Biochem. Pharmacol.* **2007**, *37*, 981–989. (i) Lee, K. M.; Choi, W. J.; Lee, Y.; Lee, H. J.; Zhao, L. X.; Lee, H. W.; Park, J. G.; Kim, H. O.; Hwang, K. Y.; Heo, Y. S.; Choi, S.; Jeong, L. S. *J. Med. Chem.* **2011**, *54*, 930–938.
17. (a) Ma, J. C.; Dougherty, D. A. *Chem. Rev.* **1997**, *97*, 1303–1324. (b) Gallivan, J. P.; Dougherty, D. A. *Proc. Natl. Acad. Sci. USA* **1999**, *96*, 9459–9464. (c) Meyer, E. A.; Castellano, R. K.; Diederich, F. *Angew. Chem. Int. Ed.* **2003**, *42*, 1210–1250. (d) Salonen, L. M.; Ellermann, M.; Diederich, F. *Angew. Chem. Int. Ed.* **2011**, *50*, 4808–4842.
18. (a) Hunter, C. A.; Sanders, J. K. M. *J. Am. Chem. Soc.* **1990**, *112*, 5525–5534. (b) McGaughey, G. B.; Gagne, M.; and Rappe, A. K. *J. Biol. Chem.* **1998**, *273*, 15458–15463.
19. Kuhn, B.; Mohr, P.; Stahl, M. *J. Med. Chem.* **2010**, *53*, 2601–2611.
20. (a) Bates, R. B.; Hruby, V. J.; Kriek, G. R. *Acta Cryst.* **1979**, *B35*, 188–191. (b) Gellman, S. H.; Dado, G. P.; Liang, G.-B.; Adams, B. R. *J. Am. Chem. Soc.* **1991**, *113*, 1164–1173. (c) Benjamin, W.-G.; Zhaohai, Z. *Tetrahedron Lett.* **1996**, *37*, 2189–2192. (d) Yang, D.; Ng,

F.-F.; Li, Z.-J.; Wu, Y.-D.; Chan, K. W. K.; Wang, D.-P. *J. Am. Chem. Soc.* **1996**, *118*, 9794–9795. (e) Abele, S.; Seiler, P.; Seebach, D. *Eur. J. Org. Chem.* **2000**, *20*, 1–15.

21. Stout, G. H.; Jensen, L. H. *X-ray Structure Determination: A Practical Guide, 2nd Edition*; Wiley: New York, 1989.
22. Raiford, L. C.; Colbert, J. C. *J. Am. Chem. Soc.* **1926**, *48*, 2654–2662.
23. McCombie, H.; Macmillan, W. G.; Scarborough, H. A. *J. Chem. Soc.* **1931**, 529–537.
24. Gibbs, C. G. U.S. Patent 4 272 284, **1981**.
25. Metz, H. J.; Neunhoeffer, H. *Chem. Ber.* **1982**, 115, 2807–2818.
26. Johannes, C. J.; Lotte, G. S.; Hans, T.; Poul, J. *Bioorg. Med. Chem. Lett.* **2004**, *14*, 1741–1744.
27. Biel, J. H.; Drukker, A. E.; Mitchell, T. F., Jr. *J. Am.Chem. Soc.* **1960**, *82*, 2204–2208.
28. Cannon, J. G.; Perez, J. A.; Pease, J. P.; Long, J. P.; Flynn, J. R.; Rusterholz, D. B.; Dryer, S. E. *J. Med. Chem.* **1980**, *23*, 745–749.
29. SIR92: Altomare, A.; Cascarano, G.; Giacovazzo, C.; Guagliardi, A.; Burla, M.; Polidori, G.; Camalli, M. *J. Appl. Cryst.* **1994**, *27*, 435–436.
30. (a) CrystalStructure 4.0: Crystal Structure Analysis Package, Rigaku Corporation (2000–2010). Tokyo 196-8666, Japan. (b) CRYSTALS Issue 11: Carruthers, J. R., Rollett, J. S., Betteridge, P. W., Kinna, D., Pearce, L., Larsen, A., and Gabe, E. Chemical Crystallography Laboratory, Oxford, UK. (1999).
31. Richards, H. H.; Chiang, P. K.; Cantoni, G. L. *J. Biol. Chem.* **1978**, *253*, 4476–4480.

公表論文

Nakao, A., Suzuki, H., Ueno, H., Iwasaki, H. and Setsuta, T. Discovery of *S*-adenosyl-L-homocysteine hydrolase inhibitors based on non-adenosine analogs. Bioorganic & Medicinal Chemistry Letters 2014, 24, 4336–4340.
http://dx.doi.org/10.1016/j.bmcl.2014.06.008

Nakao, A., Suzuki, H., Ueno, H., Iwasaki, H., Setsuta, T. Kashima, A. and Sunada, S. Discovery and structural analyses of *S*-adenosyl-L-homocysteine hydrolase inhibitors based on non-adenosine analogs. Bioorganic & Medicinal Chemistry 2015, 23, 4952–4969.
http://dx.doi.org/10.1016/j.bmc.2015.05.018

謝辞

本研究の発表にあたり、ご指導ご鞭撻を賜りました東京大学大学院薬学系研究科　金井　求教授に謹んで感謝の意を表します。

本論文を執筆するにあたり、有益なご助言とご校閲をいただきました東京大学大学院薬学系研究科　清水　敏之教授、石川　稔准教授、尾谷　優子准教授、大戸　梅治講師に厚く御礼申し上げます。

本研究を開始するにあたり、創薬化学のプロジェクトリーダーに任命していただき、直接の上司としてご指導ご鞭撻を賜りました田辺三菱製薬株式会社　上野　裕明氏に深く感謝致します。また、本研究の共同研究者として、多大なるご協力を頂きました田辺三菱製薬株式会社　鈴木　裕子氏、岩﨑　博氏、説田　智文氏、鹿島　亜希子氏、砂田　真志氏に厚く御礼申し上げます。

その他、分析研究、構造解析研究を始めとする多くの方々が本研究にご協力下さいました。ここに厚く御礼申し上げます。

論文博士取得に際しご理解いただき、学術論文、博士論文執筆に至るまで、ご支援、ご指導ご鞭撻を賜りました田辺三菱製薬株式会社　大菊　剛氏、中村　恵宣氏、安達　邦知氏、渡邉　和俊氏に深く感謝の意を表します。

研究者としての研究への取り組み方、進め方についてご指導いただきました　故　古賀　憲司教授に心より感謝致します。

最後に、筆者の研究活動、学術論文、博士論文執筆に理解を示し、協力してくれた妻　知子と　長女　真菜に感謝致します。