新規糖尿病性黄斑浮腫治療剤の合成と構造活性相関に関する研究

２０１３年

井上 隆幸

目次

略語表

本論文において以下に示す略語及び略号を用いた。

Ac	acetyl
AcOEt	ethyl acetate
AcOH	ethyl acetate
AO	amine oxidase
aq	aqueous
AUC	area under the concentration-time curve
BA	bioavailability
Bn	benzyl
Boc	*tert*-butoxycarbonyl
c	concentration
CHO	chinese hamster ovary
DIBAL	diisobutylaluminum hydride
DAO	diamine oxidase
DBU	1,8-diazabicyclo[5.4.0]undec-7-ene
N.D.	not detected
DMAP	*N*, *N*-dimethyl-4-aminopyridine
DME	dimethoxyethane
DMF	*N*, *N*-dimethylformamide
DMSO	dimethyl sulfoxide
dppf	1,1’-bis(diphenylphosphino)ferrocene
EDC	1-[3-(dimethylamino)propyl]-3-ethylcarbodiimide
eq	equivalent
Et	ethyl
h	hour(s)
HOBT	1-hydroxybenzotriazole

HRMS	high resolution mass spectra
HTS	high-throughput screening
IC_{50}	half inhibition concentration
IPE	diisopropylether
IR	infrared
i.v.	intravenous
LAH	lithium aluminum hydride
MAO	monoamine oxidase
max	maximum
Me	methyl
MeI	iodomethane
min	minute(s)
NIS	*N*-iodosuccinimide
Mp	melting point
MS	mass spectra
NMR	nuclear magnetic resonance
*n*Hex	normal hexane
PD	pharmacodynamics
PK	pharmacokinetics
Ph	phenyl
*i*Pro	iso propyl
p.o.	per os
rt	room temperature
PDB	protein data bank
POC	proof of concept
SAR	structure-activity relationship
s.c.	subcutaneous

SSAO	semicarbazide-sensitive amine oxidase
STZ	streptozotocin
T	time
TFA	trifluoroacetic acid
THF	tetrahydrofuran
TMS	trimethylsilyl
TPQ	topaquinone (trihydroxyphenylalanine quinone)
VAP	vascular adhesion protein

序論

我々の眼は、瞳から入ってきた光を眼底の網膜で感じ取っている。網膜は、１億数千万個の、光の情報を感知するための視細胞と、その情報を脳へ送る神経細胞、それらの細胞に血液を送る血管などで構成されている。視細胞には、杆体細胞と錐体細胞の２種類が存在する。杆体細胞は、光の明暗を感じ取る役割をしている。一方、錐体細胞は、明るい所で良く働き、色の識別や細かい物を区別する機能を有し、視力を得るために重要な役割を担っている。

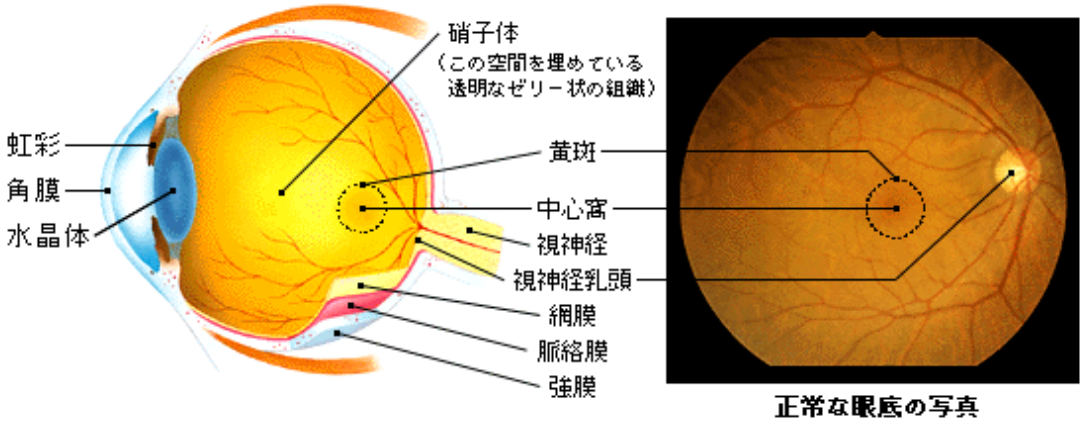


Figure 1. Ocular structure

眼の構造を **Figure 1** に示した。[1)] 網膜は、眼球の内側全体に広がっており、一点を見つめている場合でも上下左右広範を見る事が出来るが、一点を凝視しようとする時には、首や眼球を動かし、見たい所を視野の中央で捉える必要がある。網膜の中で最も視力が鋭い黄斑に、焦点を合わせる必要があるためである。黄斑とは、眼底のほぼ中央に位置する黄褐色の部分で、錐体細胞が密集している。そして、錐体細胞以外の組織は極端に少なく、特に黄斑の中心 0.4mm の中心窩には、血管さえ存在していない。黄斑には光を遮る物が殆ど無い事を意味し、この特殊な構造が、錐体細胞が密集している事と相まって、高い視力を作り出している。以上の事から黄斑は、網膜の中で視力に対して最も重要な役割を担っている。

黄斑症とは、黄斑が損傷し視力が低下する疾病である。黄斑症の原因には、加齢や高度

の近視、遺伝要因等の他、糖尿病も大きな原因に挙げられる。糖尿病の眼の合併症としては、糖尿病性網膜症が良く知られている。網膜症と黄斑症の違いは、症状が自覚できるかどうかである。網膜症の自覚症状は、硝子体出血や網膜剥離が起きた時に急に現れ、それまで患者は全く気付かない。一方で黄斑症の場合、黄斑以外の網膜が正常だとしても視力が著しく低下し、自覚症状として捉えられやすい。糖尿病患者の１１％に合併症として網膜症が起きていると言われている。黄斑は網膜上に存在し、網膜症の患者ほど黄斑症が起きる確率も高くなり、実際に増殖網膜症まで進行している患者の黄斑症発生率は７１％となっている（**Figure 2**）。[1)]

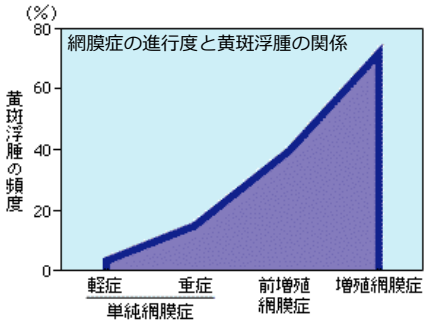


Figure 2. Frequency of macular edema in retinopathies

糖尿病性黄斑症（黄斑浮腫）の原因には、大きく次の三つに分類される（**Figure 3**）。[1)]

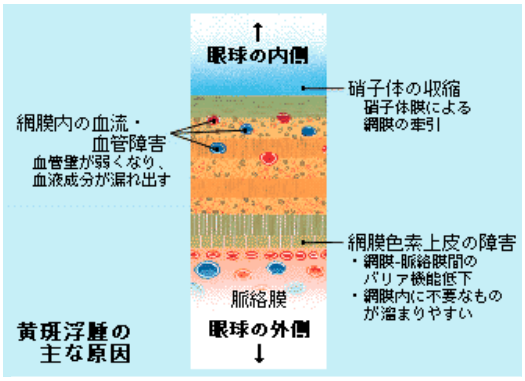


Figure 3. Major causes for diabetic macular edema

① 黄斑部の網膜・脈絡膜の血流・血管障害：眼球内血管は大変細いため、高血糖の影響が出やすく、多くの眼の合併症が現れる。網膜内の血流・血管障害が起こると、血管から血液中の成分の漏出や、血管の一部が瘤の様に膨れる毛細血管瘤が形成されて浮腫を起こす。黄斑以外の浮腫であれば自覚症状は無いが、黄斑浮腫の場合、視力低下・変視症等の自覚症状が出てくる。また、黄斑の中心窩には血管が存在しないため、酸素や栄養は周辺の血管より得ている。よって、血流障害が起きると、他の網膜より影響が出やすく、視細胞の機能低下に至る。

② 網膜色素上皮機能の障害：網膜色素上皮とは、網膜の一番外側にある、脈絡膜との境目の層で、両膜間では酸素・タンパク質等の様々な成分が必要量をコントロールしながら往来している。また、脈絡膜から網膜へ不要な成分が入り込むのを防いだり、網膜内に存在する不要物を脈絡膜へ戻したりする役割もある。糖尿病で高血糖状態が続くと、この網膜色素上皮がうまく機能しなくなり、網膜内に不要物が溜まり黄斑に浮腫が起きる。

③ 後部硝子体膜による黄斑部網膜の牽引：硝子体は網膜の内側に存在し、眼球の大部分を

占めるゼリー状の組織である。硝子体の表面を硝子体膜が覆い、この膜は通常は網膜と付着している。硝子体は、加齢や高血糖によるタンパク質の糖化等から徐々に収縮する事があり、黄斑付近の網膜が硝子体膜を介して牽引され、その結果黄斑に浮腫が発生する。

現在、糖尿病性黄斑症（黄斑浮腫）の治療に、次の四つの方法が用いられている。[1)]

① レーザー光凝固治療：毛細血管から水分が滲み出している場所（毛細血管瘤）がはっきりしている場合、その部分にレーザー光を当てて凝固させる方法である（レーザーは可能な限り弱く当てるので痛みを感じることはほとんどない)。しかし、この方法は、黄斑浮腫の進行を促進する可能性やレーザー光が硝子体を刺激して硝子体収縮の一因となるなど治癒率が低い。

② 硝子体手術：硝子体を手術で取り除く方法であり、黄斑部を眼球の内側へ向けて引っ張る力を解除したり、毛細血管の透過性を亢進させる物質を除去したり、あるいは網膜への酸素の供給を増やしたりすることが期待される。短所としては、手術の合併症（細菌感染、眼内の大規模出血）や再発が挙げられる。

③ ステロイドの眼球内注射：血管からの液体成分の滲み出しを減らし、黄斑浮腫を減らす効果があることが知られている。ステロイドは糖尿病を悪化させるため、全身投与（内服あるいは静脈注射）はできず、黄斑だけに効果を集中させるために、作用が長期間続くステロイド剤を眼球に直接注射する。短所には、白内障の進行が報告されている。

④ 抗 VEGF 抗体の硝子体内注射：眼の中に VEGF（血管内皮細胞成長因子）という物質がたまることが糖尿病性黄斑浮腫の原因であることがわかっており、その活動性を失わせる抗体を眼球内に注射する。短所としては抗体を使用するため高額であり、また継続投与する必要があることが挙げられる。

以上より、糖尿病性黄斑浮腫の治療には、未だ確実な方法は存在していない。また、どの方法も網膜症初期、特に視力低下の比較的軽微な時期にはこれらの治療が適用される事は無く、視力低下がありながら治療を受けることが出来ない現状を踏まえると、硝子体手術等の導入時期の延期などが期待できる黄斑浮腫での薬物治療のニーズは高いと考えられる。

Amine oxidases (AOs)は、活性中心のコファクターによって次の二つのタイプに分けられる。第一グループは、コファクターに flavin adenine dinucleotide (FAD)を含むタイプで、monoamine oxidase (MAO)-A/B や polyamine oxidase (PAO)を代表とする。第二グループは、コファクターに銅を含むタイプで、diamine oxidase (DAO)、lysyl oxidase (LO)や 遊離/膜結合型 semicarbazide-sensitive amine oxidase (SSAO)を代表とする。Vascular adhesion protein-1（VAP-1）は SSAO に属し、90kDa のホモダイマー構造をとっている。

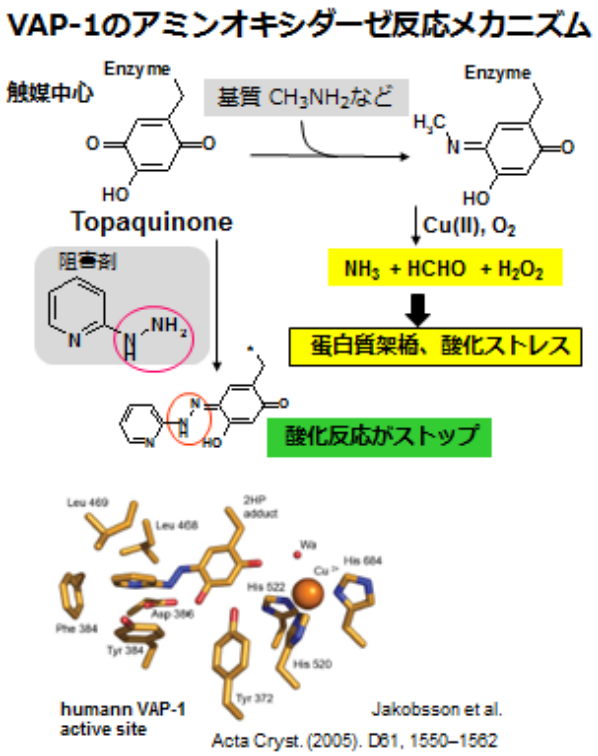


Figure 4. Mechanism of amineoxidase (VAP-1)

VAP-1 には、血管内皮に存在する膜結合型と血清中に存在する遊離型の二つのタイプが存在し、生体内でそれぞれ違った役割を担っている。また、その発現は炎症部位の血管内皮細胞に顕著である。先の膜結合型は、炎症部位[2)]に白血球やリンパ球を集積させるための接着分子として働き、後者の遊離型は、有している amine oxidase 活性によって生体内１級アミン（methylamine や aminoacetone 等）[3,4)]の解毒を行っている。その１級アミン類の代謝では、酵素部位の中心に存在する trihydroxyphenylalanine quinone（TPQ：topaquinone）を利用し、酸化的脱アミノ化を触媒し、相当するアルデヒドと共にアンモニアや過酸化水素を生成する（**eq. 1**、**Figure 4**）。[5)]

$RCH_2NH_2 + H_2O + O_2 \rightarrow RCHO + NH_3 + H_2O_2$ **(eq. 1)**

その反応産物は細胞毒性を有していることで知られ、多くの血管障害[6,7)]に関与していると考えられる。事実として、脂肪細胞や血管組織由来の遊離・膜結合型 VAP-1 の増加は、多くの炎症に関連した疾患で観察されている（I 型/II 型糖尿病[8-10)]、関節リウマチ[2,11)]、炎症性大腸炎、アテローム性動脈硬化、慢性心不全[12)]、アルツハイマー病[13)]）。

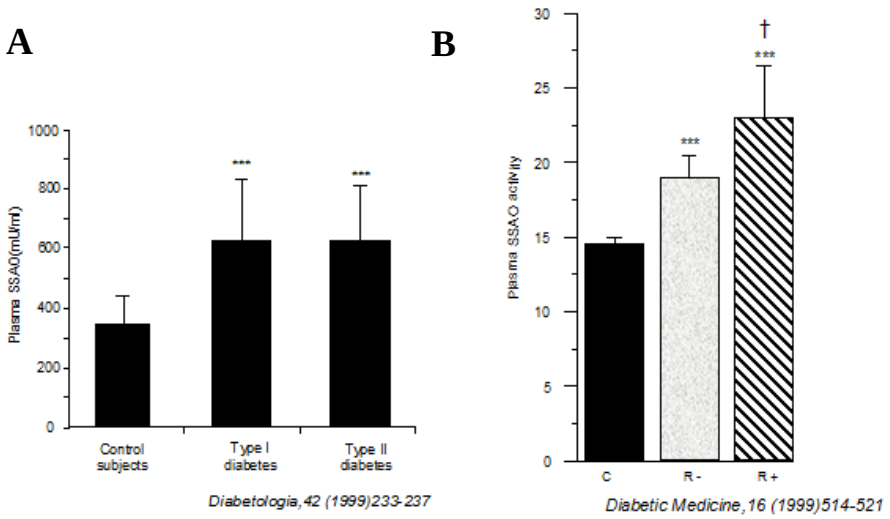


Figure 5. VAP-1 (SSAO) enzyme activity in diabetic patients

最近、糖尿病患者においてI型/II型を問わず血漿中VAP-1酵素活性が増加しており（**Figure 5A**）、同時に合併症の中でも特に網膜症を合併した糖尿病患者でさらに顕著であることが複数報告された（**Figure 5B**）。これはVAP-1と黄斑浮腫との関係を示唆する重要な報告である。すなわち網膜症は合併症の中でも初期に症状が出現する病態であり、黄斑浮腫は網膜症に共通する細小血管障害の初期変化を示唆する病変であることから、VAP-1は合併症の初期病変、特に血管のバリアー機能の破綻を起こすような細小血管障害と密に関係していると考えられる。以上より糖尿病に於いて、血糖および脂質代謝異常により起こる血漿中および組織レベルでのVAP-1酵素活性および酵素基質の増加は、酸化ストレス、グリケーション、さらにリンパ球接着の活性化現象の一因として働き、その後に起こる血管透過性の亢進（浮腫）、血流低下および血栓形成（虚血）などの原因になることが考えられる（**Figure 6**）。

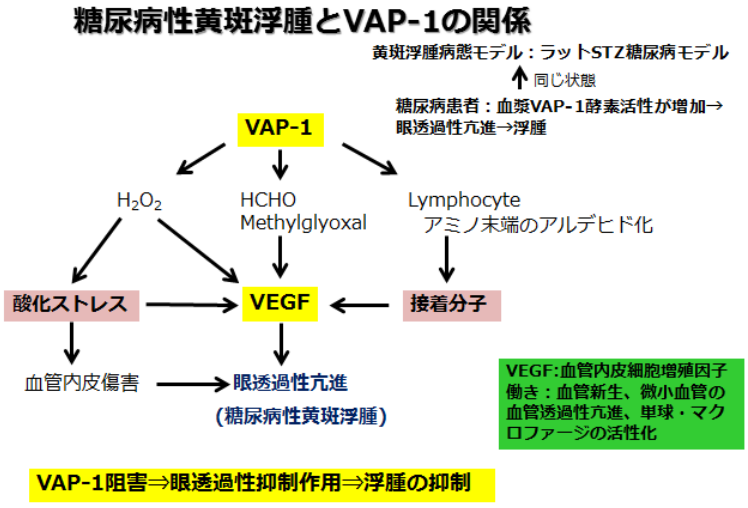


Figure 6. The hypothetical role of VAP-1 in diabetic macular edema

よって、VAP-1がこれら疾患の治療ターゲットとして有

望であることは明確である。さらに、VAP-1 が網膜血管内皮に発現し、網膜血管でのリンパ球集積に重要な役割を担う事が最近報告された。[14] この事象は、糖尿病性網膜症でみられる浮腫を導く blood–retinal barrier の破綻を引き起こすと考えられる。これらの事から、有害物の産生やリンパ球の接着活性は、糖尿病性網膜症、特に糖尿病性黄斑浮腫で見られる血管損傷で起きている病理的進行の原因である可能性が高い。

糖尿病性網膜症は、ヒトの失明原因として未だ存在し、糖尿病性黄斑浮腫はひどい視力障害をもたらす。黄斑部は網膜で視覚の中心的な役割を担う最も重要な領域で、一度そこで浮腫が起きると、たとえ小さな障害であっても重度の視力障害となる。さらに、未治療の浮腫は黄斑部での不可逆的変化を引き起こし、網膜症を悪化させ失明に至る。よって、それらの医療ニーズにより、VAP-1 阻害による黄斑浮腫治療剤として複数の化合物が現在開発下にある（**Figure 7**）。[15] いずれの化合物も前臨床段階であるが、Biotie Therapies 社（Roche 社との共同開発）の VAP-1 抗体（BTT-1023）は、慢性炎症性疾患に対して臨床開発[16]を行っており、乾癬や関節リウマチ患者に対する反復投与群に於いて重度の副作用がなく良好な有効性を示す事が報告され、[17]本創薬ターゲットに於けるヒトでの概念実証（POC）を証明した。

ヒトVAP-1のX線結晶解析の結果が論文で報告されて以来、低分子阻害剤の開発が一段と活発化している。2-hydrazinopyridineが阻害剤として結合している３次元VAP-1結晶構造（PDB code 2C11[18]）を用いたドッキング解析をヒントに、さまざまな構造の化合物が他社で合成されているが薬として不適な構造が多く（**Figure 7**）、それらは薬剤としての開発に困難を伴うことが想像できる。そこで著者は、未だ満たされていないこの疾患ターゲットに着目し、早期に開発可能なドラッグライクな構造を有する低分子薬の創製を目的として、本研究に着手した。

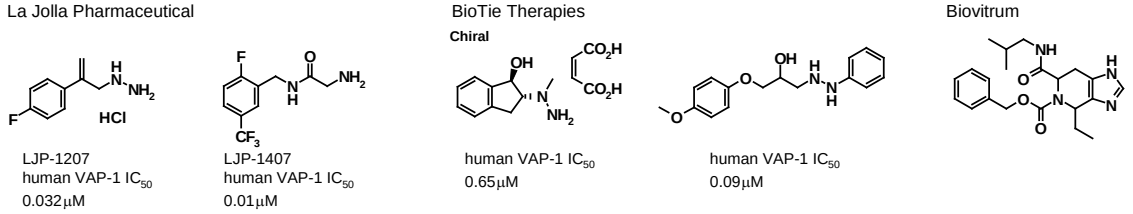


Figure 7. Structure of Competitors' Drug Candidates

本論

第１章　　新規VAP-1阻害剤の合成と構造活性相関に関する研究

第１節　　シード化合物の探索と生物活性

新規な化学構造を有するVAP-1阻害剤の探索を目的に、自社化合物ライブラリーを用いてハイスループットスクリーニング（HTS）を行った。その結果、チアゾール誘導体**1**（human VAP-1 : IC_{50} = 3.5 μM, rat : IC_{50} = 0.34 μM）とヒト活性特異的なピラゾール誘導体**2**（human : IC_{50} = 2.1 μM, rat : IC_{50} = >100 μM）を見出した（**Figure 8**）。本研究を開始するに当たり、ラットの病態モデルで有効性を確認し、糖尿病性黄斑浮腫におけるVAP-1阻害剤の概念実証（POC）を取得する事が優先された為、誘導体研究のシードとして化合物**1**を選択した。

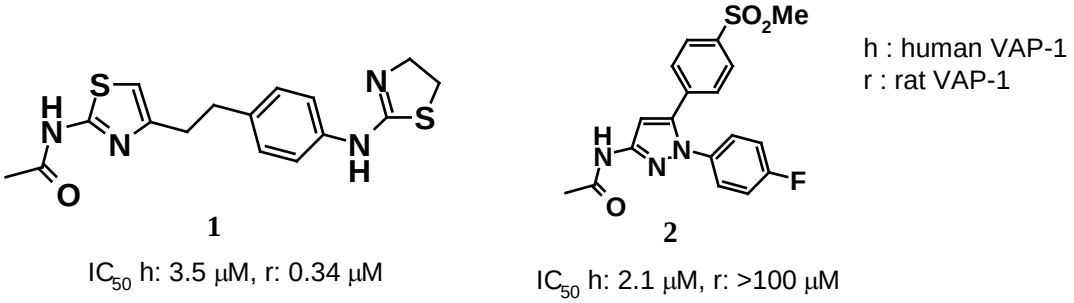


Figure 8.　HTS hit compounds

化合物の*in vitro*評価試験に於いて、ヒトとラットのVAP-1阻害活性を人工基質である^{14}C-benzylamineを用いたradiochemical-enzyme assay法によって測定した。使用したVAP-1酵素は、ヒトやラットのVAP-1酵素を安定的に発現させたCHO細胞から準備した。

第２節　　誘導体の合成方針

誘導体合成の方針は、本化合物の基本SARの取得を目的とし、化合物全体を四つのパートに分け、それぞれ誘導体合成を行った（**Figure 9**）。

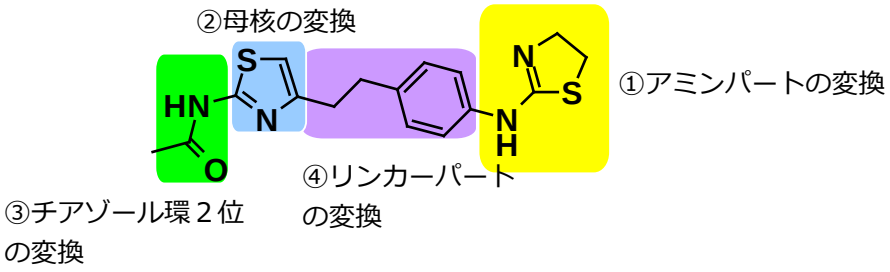
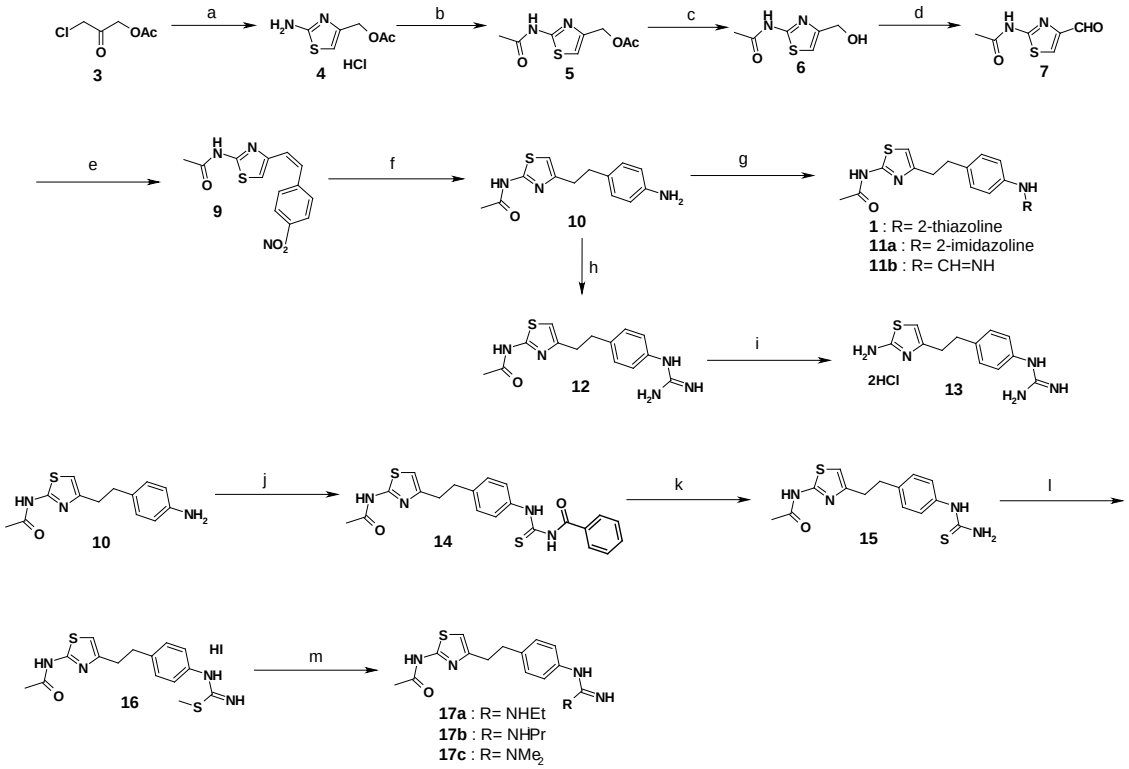


Figure 9. Structural modification of HTS hit compound **1**

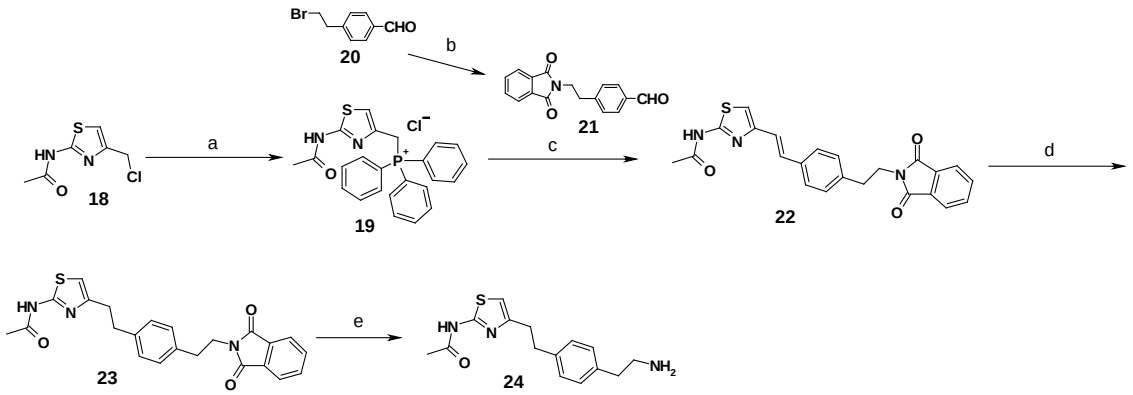
第３節　シード化合物１誘導体の合成

第１項　アミンパートの変換



Scheme 1. Reagents and conditions: (a) thiourea, EtOH, reflux, 51%; (b) AcCl, pyridine, CH_2Cl_2, 82%; (c) K_2CO_3, MeOH, 95%; (d) MnO_2, MeOH, $CHCl_3$, 73%; (e) 1) 4-nitrobenzyl bromide (**8**), PPh_3, DMF, 2) ^tBuOK, 62%; (f) H_2 (4 atm), 10% Pd-C, AcOH, MeOH, THF, 30%; (g) **1**: 2-(methylsulfanyl)-4,5-dihydro-1,3-thiazole, c.HCl, 2-methoxyethanol, 120 °C, 40%, **11a**: ethyl 2-(methylsulfanyl)-4,5-dihydro-1H-imidazole-1-carboxylate, AcOH, EtOH, reflux, 49%, **11b**: isopropyl formimidate hydrochloride, MeOH, 10%; (h) cyanamide, 4 M HCl-AcOEt, EtOH, reflux, 60%; (i) 6 M HCl, 90 °C, 70%; (j) benzoyl isothiocyanate, acetone, reflux, 74%; (k) 6 M NaOH, EtOH, 60 °C, 49%; (l) MeI, MeOH, reflux, 83%; (m) amines, EtOH, 42-59%.

N-(4-phenylethyl-1,3-thiazol-2-yl)acetamide 誘導体 **1, 11a,b, 12, 13, 17a–c** は **Scheme 1** に示す方法で合成した。出発原料の 3-chloro-2-oxopropyl acetate (**3**) と thiourea を加熱還流下で反応させることによりチアゾール環を構築し、(2-amino-1,3-thiazol-4-yl)methyl acetate hydrochloride (**4**)へと導いた。次に acetyl chloride を用いてアミノ基をアシル化し(**5**)、炭酸カリウム/メタノールでアセチル基を脱保護し、ヒドロキシメチル誘導体 **6** を得た。次に、ヒドロキシメチル基を manganese (IV) oxide でアルデヒド基へと酸化した。アルデヒド誘導体 **7** はさらに、4-nitrobenzyl bromide (**8**)とトリフェニルホスフィンから系内で合成した (4-nitrobenzyl)triphenylphosphonium bromide と Wittig 反応を行い、引き続き 10%パラジウム炭素触媒下、中圧接触還元を行い共通中間体であるアニリン誘導体 **10** へと導いた。化合物 (**1, 11a,b**) は、それぞれ対応する求電子剤と置換反応を行い合成した。グアニジン誘導体 **12** は、加熱還流下、シアナミド/塩酸を用いて置換反応により合成した。アニリン誘導体 **13** は加熱下、6M 塩酸を用いてアセチル基を脱保護して合成した。アルキル置換グアニジン誘導体(**17a–c**)は、化合物 **10** より benzoyl isothiocyanate とカップリング後、加熱下、6M 水酸化ナトリウムを用いてベンゾイル基を脱保護し、チオウレア体 **15** へと導き、ヨウ化メチルを用いて、チオウレア基の硫黄原子のメチル化を行い、対応するアミンを用いた置換反応を行うことによりそれぞれ合成した。

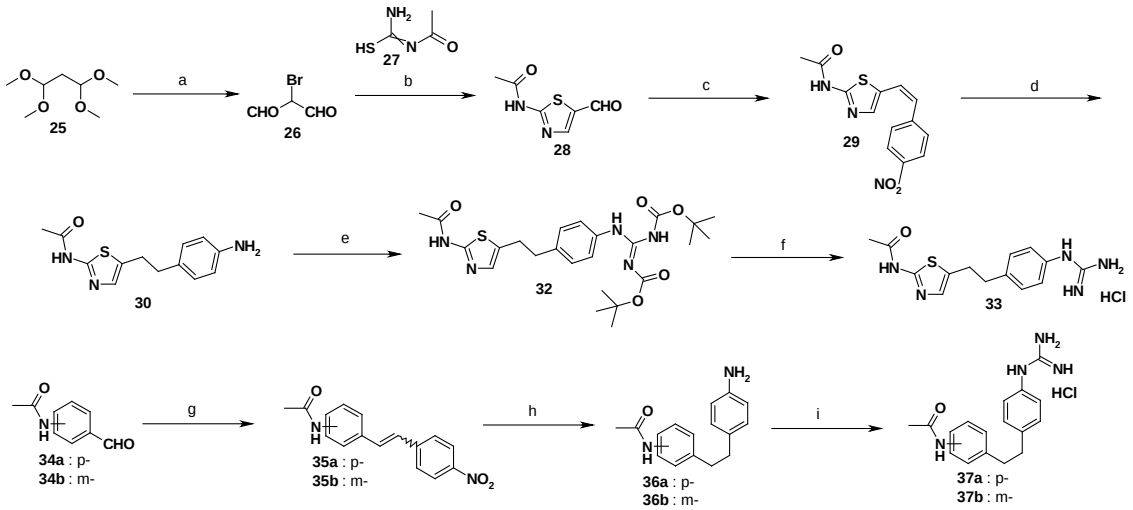


Scheme 2. Reagents and conditions: (a) PPh_3, CH_3CN, toluene, reflux, 64%; (b) phthalimide potassium salt, DMF, 60 °C, 37%; (c) ^tBuOK, DMF, 86%; (d) H_2 (4 atm), 10% Pd-C, AcOH, MeOH, DMF, 71%; (e) hydrazine hydrate, CH_3CN, 50 °C, 80%.

フェネチルアミン誘導体 **24** の合成を **Scheme 2** に示す。市販の化合物 **18** は、トリフェニルホスフィンを用いてホスホニウム塩 **19** へと変換し、化合物 **20** から得られたアルデヒド誘導体 **21** と Wittig 反応を行い、引き続き接触還元に付し、フタルイミド誘導体 **23** へと導

いた。最後に、ヒドラジン一水和物を用いて、フタルイミド基の脱保護を行い、フェネチルアミン誘導体 **24** を合成した。

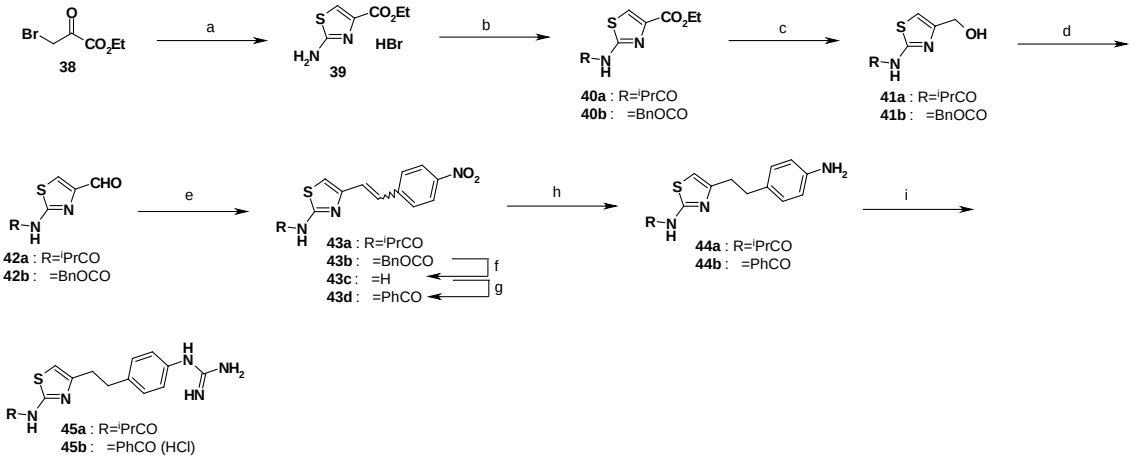
第２項　　母核の変換



Scheme 3. Reagents and conditions: (a) Br_2, c.HCl, H_2O, 39%; (b) **27**, acetone, reflux, 31%; (c) 1) **8**, PPh_3, DMF, 2) ^{t}BuOK, 24%; (d) H_2 (4 atm), 10% Pd-C, AcOEt, DMF, 27%; (e) *N*,*N*'-bis(*tert*-butoxycarbonyl)-1H-pyrazole-1-carboxamidine (**31**), THF, DMF, 50 °C, 49%; (f) 4 M HCl-dioxane, 103%; (g) 1) **8**, PPh_3, DMF, 2) ^{t}BuOK, 50-74%; (h) H_2 (3 atm), 10% Pd-C, MeOH, THF, 49-93%; (i) 1) **31**, THF, 2) 4 M HCl-dioxane, 45-48%.

チアゾール５位置換体 **33** とフェニルアセタミド誘導体 **37a–b** は **Scheme 3** に示す方法により合成した。市販の 1,1,3,3-tetramethoxypropane (**25**)を臭素化し 2-bromomalonaldehyde (**26**) を合成し、さらに *N'*-acetylcarbamimidothioic acid (**27**)を用いてアセトン中で加熱環流させ、チアゾール環を構築してアルデヒド誘導体 **28** へと導いた。化合物 **30** は化合物 **28** より、化合物 **10** と同様の方法にて合成した。次に、アニリン誘導体 (**30**) は *N*,*N'*-bis(*tert*-butoxycarbonyl)-1H-pyrazole-1-carboxamidine (**31**)を用いた置換反応により化合物 **32** とし、引き続き脱 Boc 化によりグアニジン誘導体 **33** へと変換した。グアニジン誘導体 **37a–b** は、 化合物 **33** と同様の方法を用いて、市販化合物 **34a–b** より合成した。

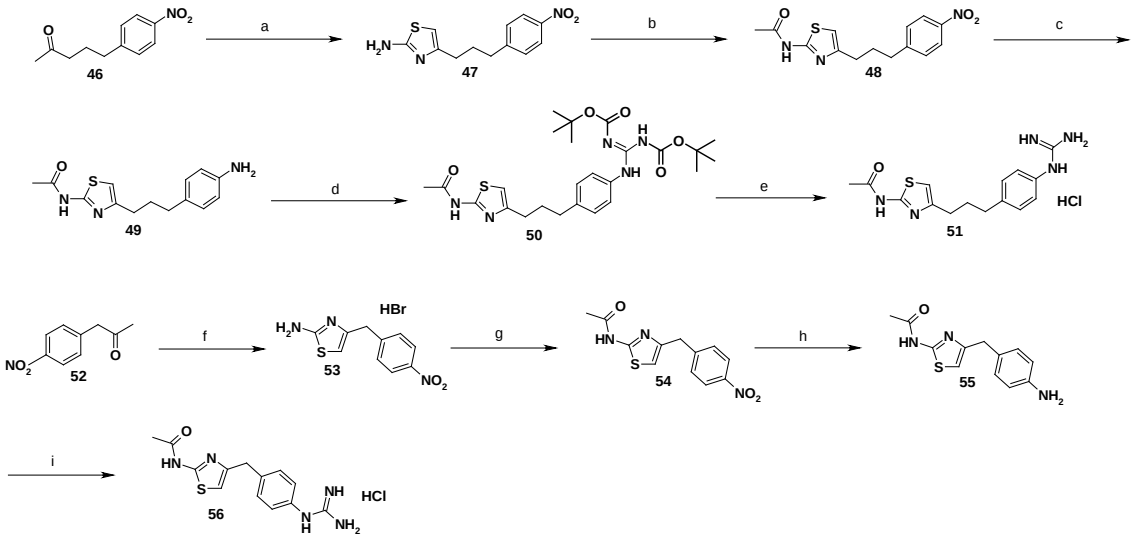
第３項　　チアゾール環２位の変換



Scheme 4. Reagents and conditions: (a) thiourea, EtOH, reflux, 131%; (b) R-Cl, pyridine, CH_2Cl_2, 70-84%; (c) $LiBH_4$, THF, reflux, 86-93%; (d) MnO_2, MeOH-$CHCl_3$, 71-73%; (e) 1) **8**, PPh_3, DMF, 2) ^{t}BuOK, 64-76%; (f) 6 M HCl, reflux, 77%; (g) benzoyl chloride, DMA, 110 °C, 70%; (h) H_2 (4 atm), 10% Pd-C, AcOH, MeOH, THF, 86-91%; (i) cyanamide, 4 M HCl-AcOEt, EtOH, reflux, 33-74%.

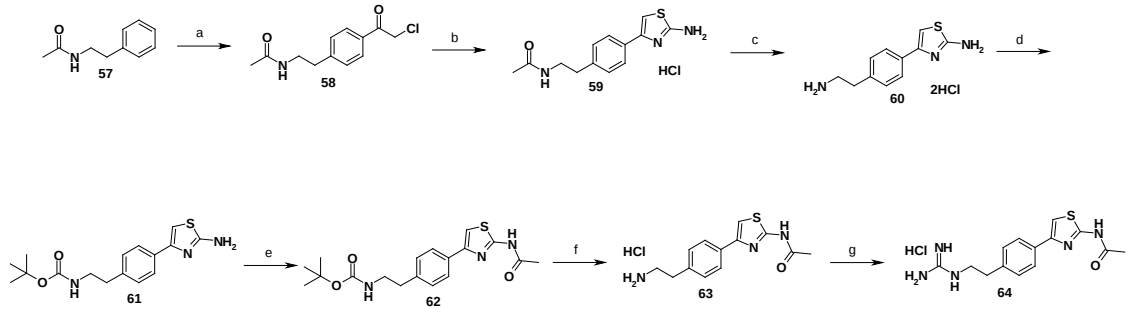
グアニジン化合物 **45a–b** の合成を **Scheme 4** に示す。市販のケトエステル体 **38** を原料とし、エタノール中加熱還流下、チオウレアと反応させてチアゾール環を構築し、続いて対応する酸クロライドでアシル化する事によりアミド誘導体 **40a–b** へと導いた。次に、化合物 **40a–b** をそれぞれ lithium borohydride でヒドロキシメチル体 **41a–b** へと還元後、manganese (IV) oxide で酸化してアルデヒド体 **42a–b** へと誘導した。このアルデヒド体と (4-nitrobenzyl)triphenylphosphonium bromide を用いて Wittig 反応を行い、オレフィン誘導体 **43a–b** を合成した。この内、化合物 **43b** は、６M 塩酸で加熱還流する事により、Cbz 基を脱保護し、化合物 **43c** へと変換し、benzoyl chloride を用いて再度アシル化を行った(**43d**)。その後、化合物 **12** の合成法を用いて、化合物 **43a, d** を化合物 **45a–b** へと変換した。

第４項　　リンカーパートの変換



Scheme 5. Reagents and conditions: (a) 1) Br_2, MeOH, 2) thiourea, K_2CO_3, 50 °C, 17%; (b) AcCl, pyridine, CH_2Cl_2, 72%; (c) H_2 (3 atm), 10% Pd-C, MeOH, THF, DMF, 105%; (d) **31**, THF, DMF, 50 °C, 84%; (e) 4 M HCl-dioxane, 85%; (f) 1) Br_2, HBr, AcOH, 2) acetone, 3) thiourea, EtOH, reflux, 72%; (g) AcCl, pyridine, CH_2Cl_2, 54%; (h) H_2 (3 atm), 10% Pd-C, MeOH, DMF, 90%; (i) cyanamide, 4 M HCl-AcOEt, EtOH, reflux, 77%.

Scheme 5 に、化合物 **51** と **56** の合成法を示す。2-Aminothiazole 誘導体 **47** は市販の 5-(4-nitrophenyl)pentan-2-one (**46**)を出発原料とし、臭素化後チオウレアを用いた環化により合成され、次にアセチル化とニトロ基の還元を行いアニリン誘導体 **49** へと導いた。更に、化合物 **33** の合成法を用いてグアニジン誘導体 **51** を合成した。化合物 **56** は、市販原料 **52** より Br_2/HBr/AcOH を用いた臭素化後、チオウレアによる環化反応により化合物 **53** を合成し、続いて、アシル化、ニトロ基の還元、シアナミドを用いたグアニジル基への変換を行うことにより、目的化合物 **56** を合成した。



Scheme 6. Reagents and conditions: (a) Chloroacetylchloride, $AlCl_3$, 1,2-dichloroethane, 80%; (b) thiourea, EtOH, reflux, 90%; (c) c.HCl, EtOH, reflux, 85%; (d) *tert*-butyl dicarbonate, 1 M NaOH, dioxane, H_2O, 61%; (e) Ac_2O, pyridine, DMAP, CH_2Cl_2, 85%; (f) 4 M HCl-AcOEt, 106%; (g) 1) **31**, THF, 2) 4 M HCl-dioxane, 35%.

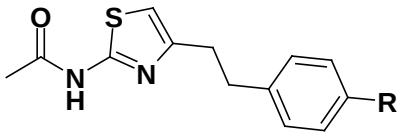
N-(4-Phenyl-1,3-thiazol-2-yl)acetamide 誘導体 **64** は、**Scheme 6** に示されたルートに従って合成した。原料 *N*-(2-phenylethyl)acetamide (**57**) に対して、Friedel-Crafts 反応によりクロロアセチル基を導入し(**58**)、引き続きチオウレアを用いて環化を行い 2-アミノチアゾール誘導体 **59** を合成した。次に、脱 Ac 化、Boc 化、Ac 化を順次行い化合物 **62** へと変換し、4M 塩酸で Boc 基を脱保護する事によりフェネチルアミン誘導体 **63** へと導いた。続いて、化合物 **33** と同様の方法にて目的化合物 **64** を合成した。

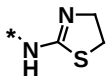
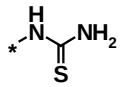
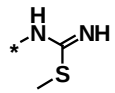
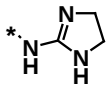
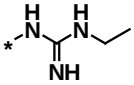
第４節　　シード化合物１誘導体の構造活性相関データの取得

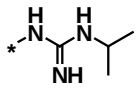
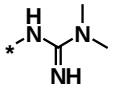
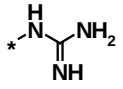
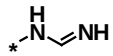
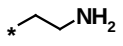
第１項　　アミンパートの変換

Table 1.

VAP-1 inhibitory activity of N-(4-phenylethyl-1,3-thiazol-2-yl)acetamide derivatives.



Compd	R	VAP-1 human IC_{50}(μM)
10	-NH_2	>100
1		3.5
15		>10
16[a]		1.1
11a		3.0
17a		0.68

Compd	R	VAP-1 human IC_{50}(μM)
17b		1.1
17c		>100
12		0.23
11b		0.73
24		5.0

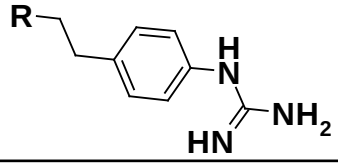
[a] Hydroiodide salt

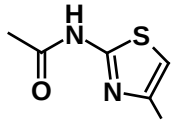
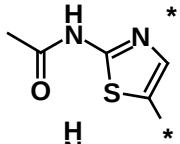
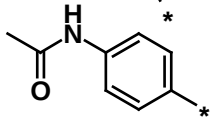
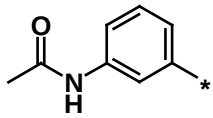
化合物 **1** でのアミンパート（チアゾリン部分）変換のヒト VAP-1 阻害活性を **Table 1** に示す。チアゾリン環を削除したアニリン体 **10** は阻害活性を全く示さなかった。これにより、チアゾリン環は阻害活性に何らかの役割を担っている事が推測できる。チアゾリン環を開裂したチオウレア体 **15** は全く阻害活性を示さなかったが、一方で S-メチルチオウレア体 **16** では、**1** と比較して 3 倍の活性改善を示した。このことより、アミノ基の塩基性が重要であると考えられる。チアゾリン環の硫黄原子を窒素原子に変換したイミダゾリン化合物 **11a** は、**1** と同等の活性を示した。又、イミダゾリン環を開裂させたエチルグアニジン誘導体 **17a** は、環化体 **11a** と比較して、約 4 倍ヒト VAP-1 阻害活性が増強した。**17a** のエチル基をさらに嵩高いイソプロピル基 **17b** に変換したところ、僅かであるが活性の減少がみられた。一方、ジメチルグアニジン **17c** では、活性が劇的に消失した。全ての化合物中、最も活性の強い化合物は、無置換グアニジン体 **12** で、**1** に比べ約 15 倍活性が改善された（IC_{50}=0.23μM）。無置換アミジン **11b** やエチルアミン **24** にグアニジン部分を変換したところ、グアニジン **12** より活性が低下する結果となった。これらの結果は、グアニジン部分の立体許容性は狭く、更にグアニジンが VAP-1 阻害活性に対して重要なパーツである事を示している。他の報告にも有る様に [19,20]、著者は **12** のグアニジン部分が VAP-1 酵素の活性中心にあるトパキノンと、基質と同様に Schiff base 複合体を形成していると推測しており、グアニジン窒素官能基の求核性は必須と考えている（**Figure 4**）。

第２項　　母核の変換

Table 2.

VAP-1 inhibitory activity of (*p*-guanidino)phenethyl derivatives.



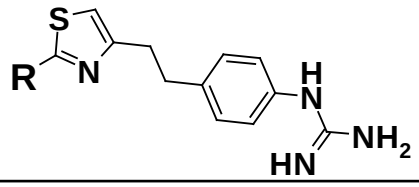
Compd	R	VAP-1 human IC_{50}(μM)
12		0.23
33[a]		9.6
37a[a]		18
37b[a]		15

[a] Hydrochloride salt

母核パート（チアゾール部分）の変換での酵素阻害活性を **Table 2** に示す。チアゾール環の位置異性体 **33** は、**12** に比べ活性が約 40 倍減弱した。又、チアゾール環をベンゼン環に変換した **37a** と **37b** でも同様の活性低下がみられ、これらの結果より、チアゾール環上のヘテロ原子が VAP-1 酵素と相互作用をしていると推測できる。

第３項　　チアゾール環２位の変換

Table 3.
VAP-1 inhibitory activity of 4-[(*p*-guanidino)phenethyl]-
1,3-thiazole derivatives.



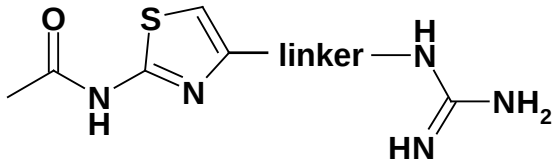
Compd	R	VAP-1 human IC_{50}(μM)
12	CH_3CONH-	0.23
45a	$^{i}PrCONH$-	0.52
45b[a]	PhCONH-	11
13[b]	NH_2-	22

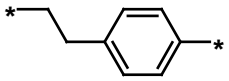
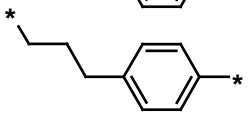
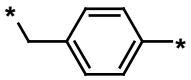
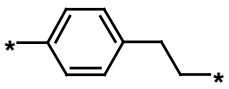
[a] Hydrochloride salt
[b] Dihydrochloride salt

チアゾール環２位に於ける置換基変換での酵素阻害活性を**Table 3**に示す。アセチル基**(12)**をイソプロピオニル基**(45a)**に変換しても、活性はほとんど変わらなかった。しかし、ベンゾイル基**(45b)**の様な更に嵩高い官能基に変換すると、活性は劇的に低下した。脱アセチル化したアミン体**(13)**でも、同様の活性低下がみられた。以上の事から、チアゾール環２位に於いて、アミド基は活性発現に必要であり、立体的な許容性は高く無い事が判明した。

第４項　　リンカーパートの変換

Table 4.
VAP-1 inhibitory activity of the compounds in various linkers.

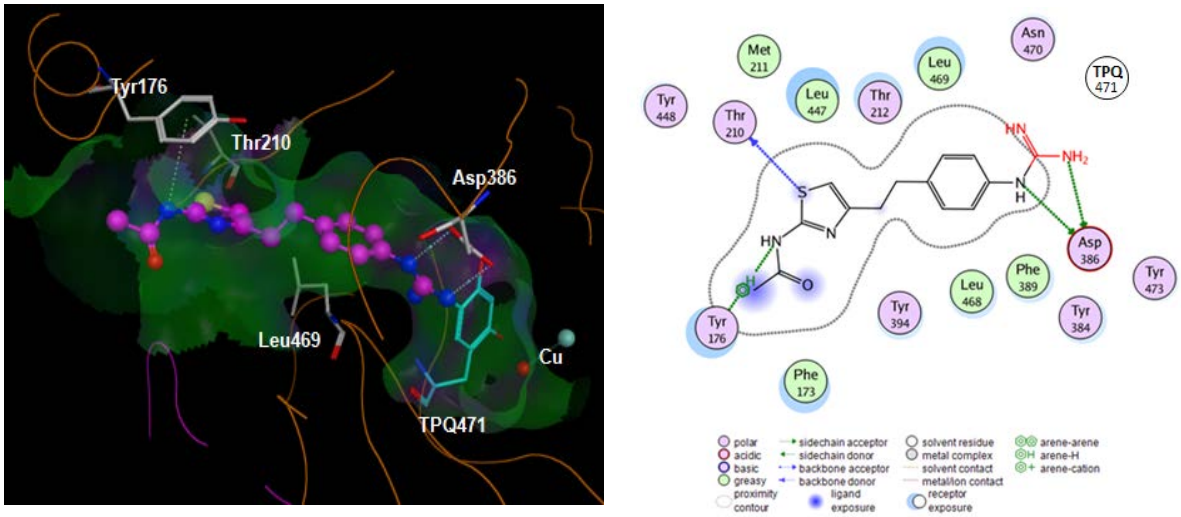


Compd	linker	VAP-1 human IC_{50}(μM)
12		0.23
51[a]		8.8
56[a]		42
64[a]		0.33

[a] Hydrochloride salt

チアゾール環とグアニジン部分を繋ぐリンカーユニットの変換結果を **Table 4** に示す。初めに、チアゾール環とベンゼン環の間の距離を変えた。エチレン(**12**)をプロピレン(**51**)やメチレン(**56**)に変換した所、活性は減弱した。一方、**12** のフェネチル部分を逆に接続した **64** は、僅かな活性の減弱に留まった。これらのデータは、チアゾール環とグアニジン部分の間の距離が重要である事を示し、フェネチルユニットが最適で、ベンゼン環の位置は活性に影響しない結果を示した。以上より、この部分がリンカーとして働いている事が確認できた。

第５節　　ヒトVAP-1ドッキングモデルの作成、及びリード化合物**12**のドッキング解析



A　　**B**

Figure 10. Computational human VAP-1 docking results for **12**.
The two-dimensional diagram was prepared using the ligand interactions application in MOE. (A) Best docking solution (lowest binding energy) calculated by GOLD *ver.5.0.* for **12** (stick representation; compound is colored blue for nitrogen, red for oxygen, yellow for sulfur and pink for carbon) surrounded by human VAP-1 active site residues (represented in surface). (B) Best docking solutions for **12** on the active site of human VAP-1 (represented in residues). Arrows indicate interaction.

ヒトVAP-1はホモダイマー構造をとっており、タンパクの内側の深く埋没した所にあるダイマー接合部位近傍に活性中心が存在し、そこにはコファクターであるtrihydroxyphenylalanine quinone (TPQ)が存在している。[18,21,22] その活性中心へのアプローチは、Leu469によって制限されており、基質のリガンド結合部位への流入をブロックしている。イメージとして、活性中心は狭く細長い筒状を成しており、Leu469が蓋の役割をしている。ヒトVAP-1の結晶構造は、PDB(code 2C11[18])から2-hydrazinopyridineとの共結晶として入手可能であり、ヒドラジンの１級アミノ基はコファクターであるTPQ と共有結合（Schiff base）している。加えて、2-hydrazinopyridine より大きな阻害剤の場合、Leu469の側鎖が回転し、ligand-binding cavity をオープンにし、リガンドが溶媒側からリガンド結合ポケットへ接近する事を可能にすると報告されている。[19,20] この情報を基に、著者はLeu469の側鎖を回転させたヒトVAP-1モデルを構築した。化合物**12**は、GOLD *version 5.0*を用いて、その

モデルにドッキングさせた（**Figure 10**）。**12**のドッキング開始時、グアニジンの１級アミノ基はTPQとシッフベースを形成させた。このモデルに於ける**12**のドッキング解析の結果、グアニジン部分は、TPQ 471との共有結合以外に、Asp386と水素結合ネットワークを形成しており、この事は活性に対するグアニジンの重要性を示唆している。さらに、チアゾール環の硫黄原子は、Thr210の主鎖カルボニル酸素原子とS-O相互作用[23)]を形成し、アミド基のNHはTyr176とproton-π相互作用を形成していた。これらの結果は、グアニジン、チアゾール環、及びアミド基が、VAP-1の阻害活性に対して全て重要な部分であるというSARからの知見と一致していた。よって、このヒトVAP-1ドッキングモデルを利用したドラッグデザインは、本研究に於いて非常に有用なツールになることが判明した。

第６節　　リード化合物**12**の生物活性

第１項 リード化合物 **12** の阻害様式検討

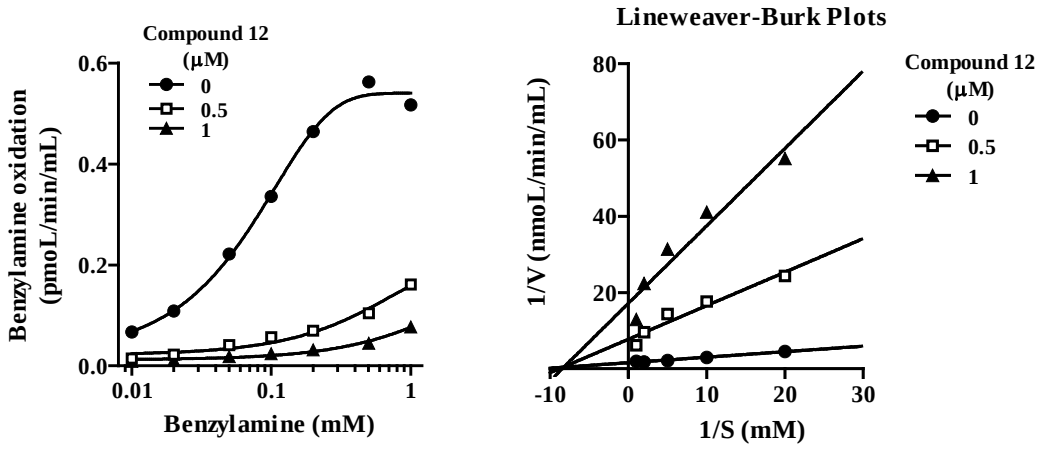


Figure 11.　Kinetic analysis of human VAP-1 inhibitory activity of **12**

化合物 **12** のヒト VAP-1 に対する阻害様式を、逆数プロット法（Lineweaver-Burk の式）により求めた。その結果、**12** の存在下、ヒト VAP-1 活性は、選択的基質である benzylamine の濃度に依存せず、また Lineweaver-Burk プロットは横軸で交わることから、**12** はヒト VAP-1 に対して非競合的阻害剤であることが判明した（**Figure 11**）。この阻害様式の結果は、一度阻害剤が活性中心に作用すると、作用持続が期待できることを示唆した。

第２項 リード化合物 **12** に於ける他のアミンオキシダーゼに対する選択性

リード化合物 **12** のラット VAP-1 活性（種差）、及びヒト DAO や MAO-B 等他のアミンオキシダーゼ活性（選択性）を、radiochemical-enzyme assay 法を用いて評価した（**Table 5**）。ヒト VAP-1 活性と比較して、**12** はラット VAP-1 活性が 16 倍強く、他のアミンオキシダーゼに対しても 435 倍以上の高い選択性を有していた。

Table 5.

Selectivity of **12** for various amine oxidases in human and rat.

Compd	VAP-1 human IC_{50}(μM)	VAP-1 rat IC_{50}(μM)	DAO[a] human IC_{50}(μM)	MAO-B[b] human IC_{50}(μM)
12	0.23	0.014	>100	>100

[a] DAO; diamine oxidase

[b] MAO-0B; monoamine oxidase-B

第３項 リード化合物 **12** の *in vivo* 薬効効果

リード化合物 **12** を用いてラット病態モデルで有効性を確認し、糖尿病性黄斑浮腫に於ける VAP-1 阻害剤の概念実証（POC）を取得する為に *in vivo* 評価を行った。初めに血中濃度推移（PK）[24]を確認する為に、Wistar 雄ラット (8-12 week-old)を用いて、**12** を 0.1 から 10 mg/kg を皮下投与した時のプラズマ中濃度を測定した（**Figure 12A**）。その結果、ラットのプラズマ中濃度は投与量依存的であった。次に、病態モデルの STZ ラットでのプラズマ VAP-1 活性に対する阻害効果を評価した。この pharmacodynamics（PD）[24]評価の結果、投与量 0.1 mg/kg 以上で有意な阻害効果がみられた（**Figure 12B**）。この PK/PD データより、**12** の血中濃度が、有効性を示す最低用量の 0.1 mg/kg で 21.8 ng/mL であり、この濃度は **12** の IC_{50} 値の 5. 1 倍に相当するので、PD 評価系での有効性は十分合理的であると考えている。また、血中濃度での持続以上に PD 評価系で有効性が持続しているのは、**12** の阻害様式が非競合的阻害であるためと考察している。最後に、黄斑浮腫の病態モデルである STZ ラット眼透過性試

験を実施した（**Figure 12C**）。**12** の眼透過性阻害効果は、皮下投与に於いて 1, 10 mg/kg で有意に観察された。またこの反復投与時のラットの一般状態は良好であった。これらの実験結果より、著者は黄斑浮腫の治療に対する VAP-1 阻害剤の概念実証取得に成功した。

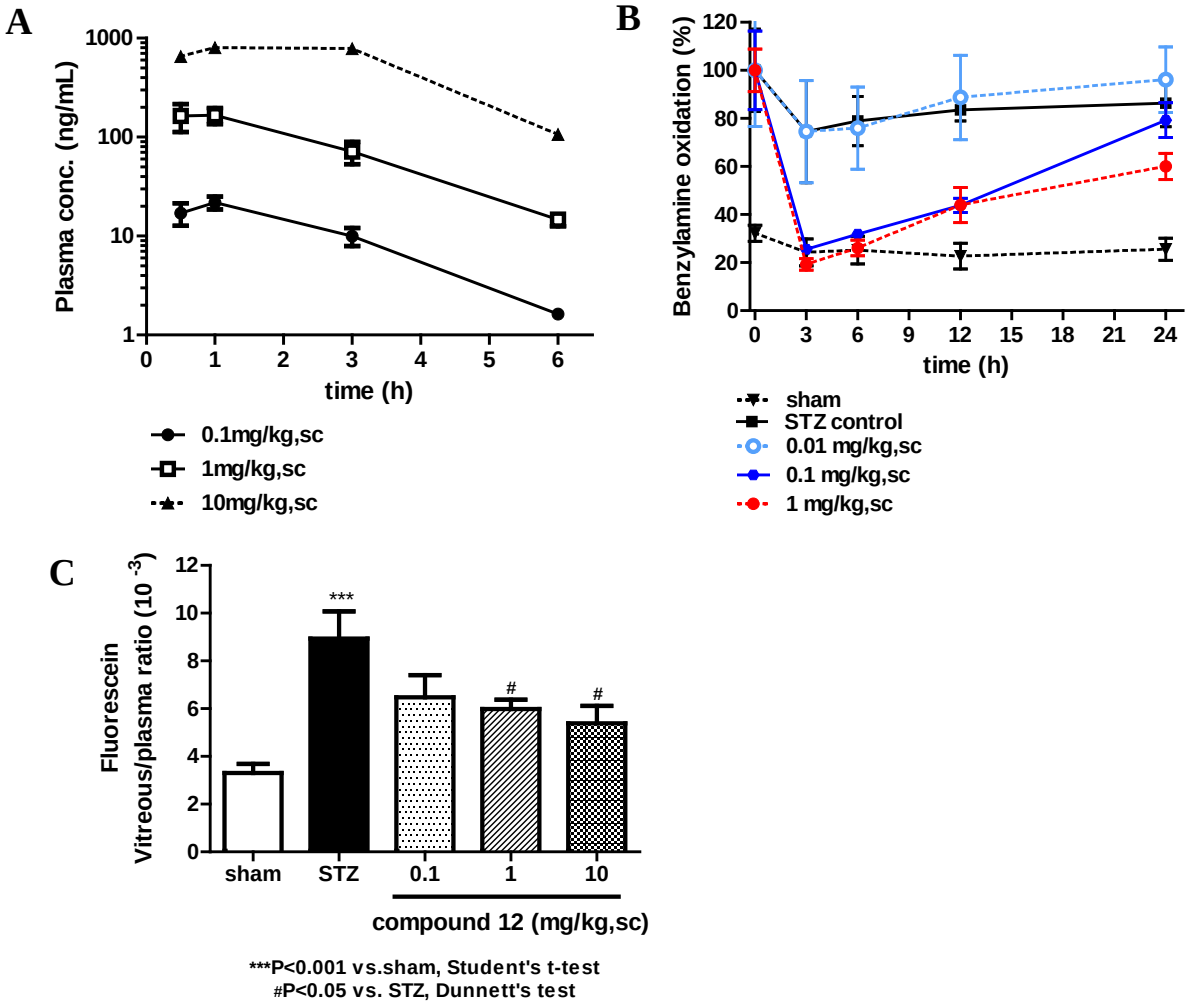


Figure 12. Profile of **12** on pharmacokinetics, pharmacodynamics and pharmacology in rats.
(A) Plasma concentration profile of **12** in normal rats (n=4, 0.1–10 mg/kg, s.c.). (B) Inhibitory effect on plasma VAP-1 activity in STZ-induced diabetic rats (n=4). The plasma VAP-1 activity was measured with radio enzyme assay using ^{14}C- benzylamine as the substrate. Values are % of VAP-1 activity at time 0 h. (C) Effect of **12** (n=10, 0.1–10 mg/kg, s.c.) on ocular permeability in STZ-induced diabetic rats. ***P<0.001, Student's t-test vs. sham. #P<0.05 vs. STZ control group, Dunnett's multiple comparison test.

第 7 節　　小括

著者は、VAP-1 阻害作用を有するチアゾール誘導体、及びその類縁体の SAR 情報を取得した。HTS ヒットである化合物 **1** に基づいた SAR 研究で、鍵ファーマコフォアを同定し、立体的にコンパクトな塩基性部分が、VAP-1 の強い阻害活性に対して重要であることを示した。**1** の SAR 研究の過程で、リード化合物 **12** でのグアニジンパートの重要性を確認した。この **12** を用いたヒト VAP-1 モデルでのドッキング結果より、グアニジンパートが VAP-1 酵素としっかりとした水素結合ネットワークを形成し、チアゾール環の硫黄原子は S-O 相互作用、アミドパートは proton-π 相互作用を形成している事を明らかにした。これらの結果は SAR データと近似していたため、本モデルはドラッグデザインに対して有用なツールになると考えられた。また、グアニジンパート以外にチアゾールアミド部分も、阻害活性に重要なファーマコフォアである事が明らかとなった。そして、糖尿病性黄斑浮腫病態モデルに於いて **12** が有効であったことから、VAP-1 阻害剤が黄斑浮腫の治療に対して有用である事を明確にした。

第２章　新規 VAP-1 阻害剤 **12** の改良研究１

第１節　リード化合物の課題とその改良方針

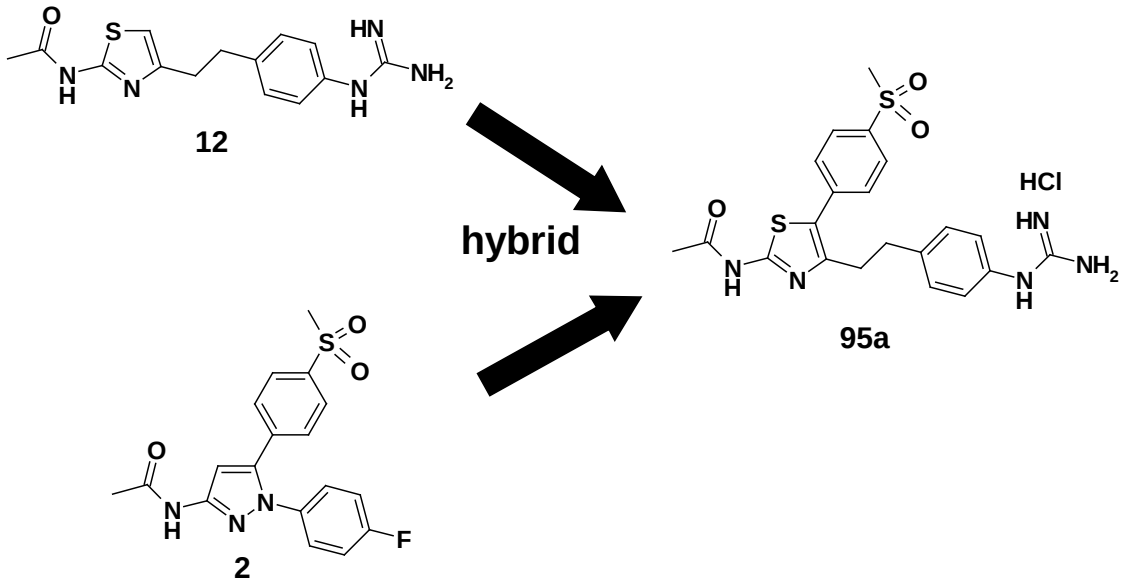


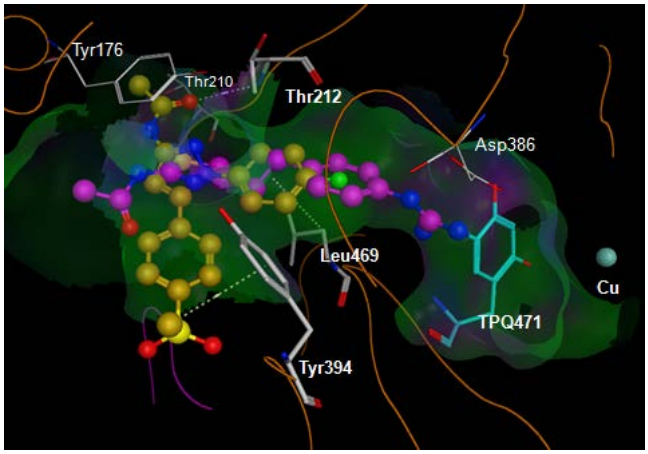
Figure 13. Hybrid design to improve the species difference in VAP-1 inhibitory activity of **12**.

第１章で見出されたリード化合物**12**は、課題として種差を有し、ラットよりヒトのVAP-1阻害活性が約20倍弱い(human IC_{50} of 230 nM; rat IC_{50} of 14 nM)。著者はこの種差改善を目的とし、ヒトの活性を特異的に有しているHTSヒット化合物**2**に着目した(human IC_{50} of 2.1 μM; rat IC_{50} of >100μM)。**2**は、VAP-1阻害に重要な役割を果たすグアニジル基を有していない事から、**2**をヒトVAP-1モデルにドッキングさせて解析する事により、ヒトVAP-1阻害に対する新たなファーマコフォア部位の発見を期待した。更には、**12**と**2**のそれぞれのファーマコフォアを利用したハイブリッド化合物をデザインする事を計画した（**Figure 13**）。

最初に、第１章で報告したヒトVAP-1モデルに対して、**2**をドッキングさせて結合モードの解析を行った（**Figure 14**）。その結果、アミド基の酸素原子がThr212主鎖のアミドの水素原子と水素結合を形成し、メタンスルホニル基はTyr394とproton-π相互作用、4-fluoro benzene部分はLeu469とπ-proton相互作用していることが判明した。これらの知見は、**2**のドッキングモードが**12**のものと顕著に違うことを示した。したがって、ヒト阻害活性の改善のために、**2**で見出された新たなファーマコフォア部位を**12**に導入する検討を、ドッキングモデル中で**12**

と**2**を重ね合わせる事により行った（**Figure 14A**）。その結果、**12**のチアゾール環５位の位置に、**2**のメタンスルホニルフェニル基が位置する事が判明したため、それをハイブリット化合物として合成する事とした。

A



B

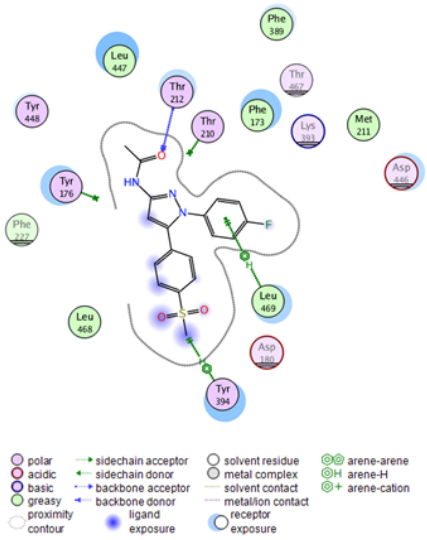
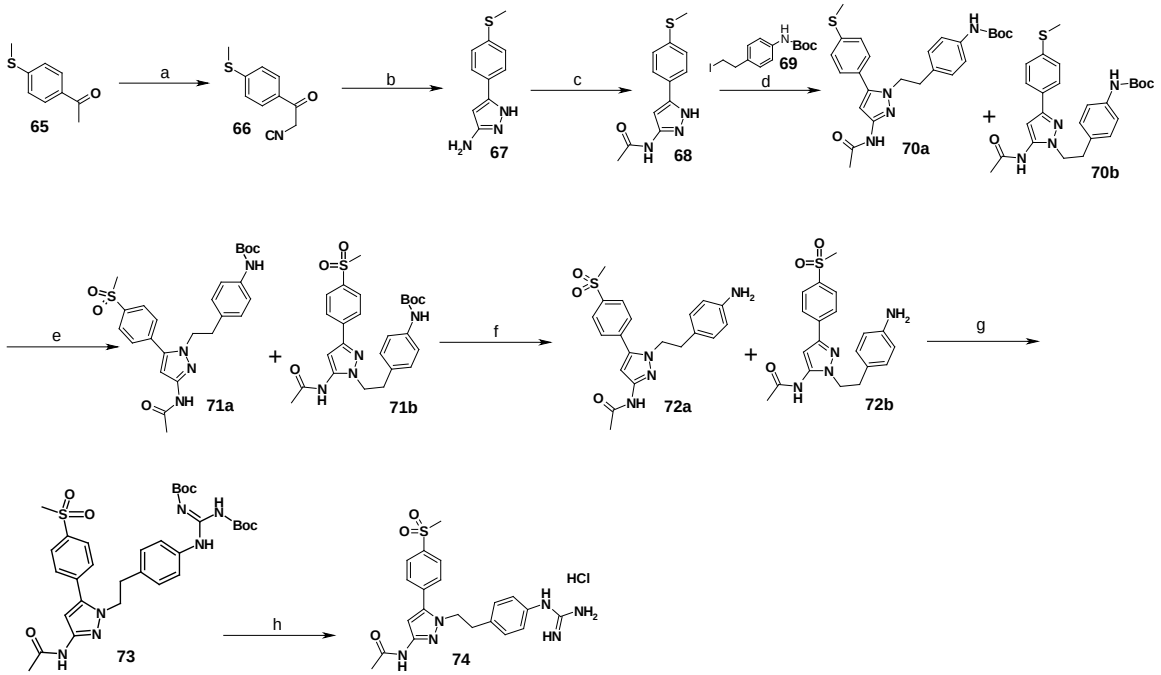


Figure 14. Computational human VAP-1 docking results for **12** and **2**.
A two-dimensional diagram was prepared using the ligand interactions application in MOE. (A) Best docking solution for **2** on the active site of human VAP-1 (represented in residues). Arrows reflect the interaction. (B) Best docking solution (lowest binding energy) calculated by GOLD *ver.5.0.* for **12** (pink) and **2** (yellow) when surrounded by human VAP-1 active site residues (represented in surface).

第２節　　ハイブリッド化合物の合成



Scheme 7. Reagents and conditions: (a) 1) pyridinium tribromide, HBr/AcOH, 2) NaCN, EtOH/H_2O, 43%; (b) $H_2NNH_2 \cdot H_2O$, c.HCl, EtOH/H_2O, reflux, 77%; (c) 1) Ac_2O, pyridine, 2) 1 M NaOH, EtOH, 73%; (d) **69**, K_2CO_3, DMF, 55 °C, 53%; (e) Oxone®, THF/H_2O, 82%; (f) 1) TFA, CH_2Cl_2, 2) separation (silicagel column), 29%; (g) *N*,*N*'-bis(*tert*-butoxycarbonyl)-1*H*-pyrazole-1-carboxamidine (**31**), DMF/THF, 50 °C, 36%; (h) 4 M HCl/dioxane, 98%.

ピラゾール誘導体 **74** の合成を **Scheme 7** に示す。3-amino-1*H*-pyrazole 誘導体 **67** は、市販のケトン体 **65** より、臭素化、sodium cyanide を用いた置換反応、ヒドラジンを用いた環化反応により合成された。次に、**67** のアセチル化後、ピラゾール環のアルキル化は、化合物 **70a** と **70b** の位置異性体混合物を生じさせた（**70a**:**70b** = 2:3）。このアルキル化の反応条件を更に検討したが（**Table 6**）、収率及び選択性は 50-60%；**70a**:**70b** = 1:1 から向上しなかった。この結果は、ピラゾール環の反応性が二つの置換基の立体的な要因に大きく影響し、選択性が出なかったと考察している（3-acetamido-*1H*-pyrazole の場合は、１位のアルキル体が優先する）。この混合物は、シリカゲルカラムによる分離が困難のため、分離可能な化合物まで混合物で進めることとした。混合物 **70a**, **b** はスルフィド基を Oxone®でスルホンに酸化し、TFA により Boc 基を脱保護した（**72a**, **b**）。この時点で位置異性体混合物のカラムクロマトグラフィーでの分離が可能となったため、望む **72a** を単離した。**72a** と **72b** の構造決定は、NMR 解析（^{1}H, ^{13}C, COSY, HSQC, HMBC）の結果より決定した（**Figure 15**）。**72a** は、**33** と

同様の方法で **74** へと導いた。

Table 6. Optimization of the reaction condition for **70a**.

Base	Solvent	Temp. (°C)	Time (h)	Yield
K_2CO_3	DMF	55°C	18	53%; **70a**:**70b** = 2:3
^tBuOK	DMF	55°C	18	59%; **70a**:**70b** =1:1
Et_3N	DMF	55°C	18	no reaction
NaH	DMF	rt	3	57%; **70a**:**70b** =1:1
NaH	DMSO	rt	3	53%; **70a**:**70b** =1:1

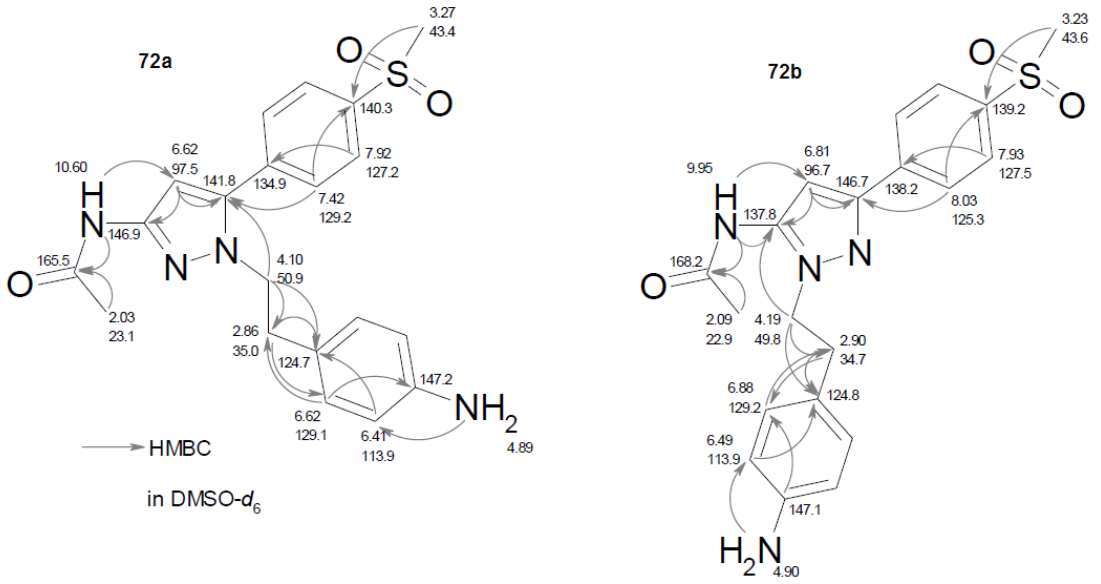
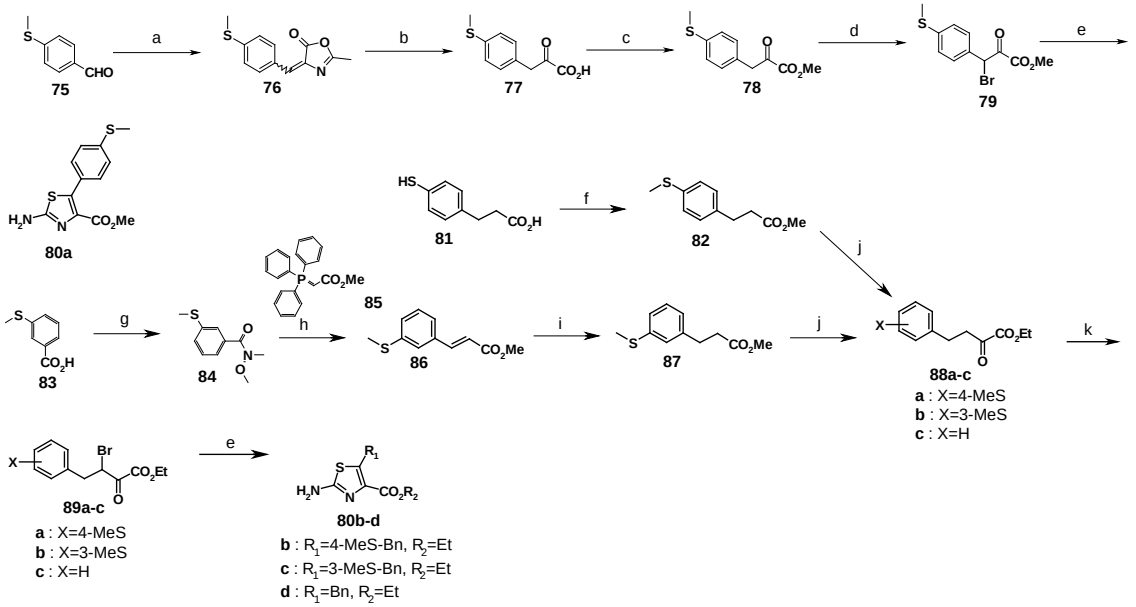
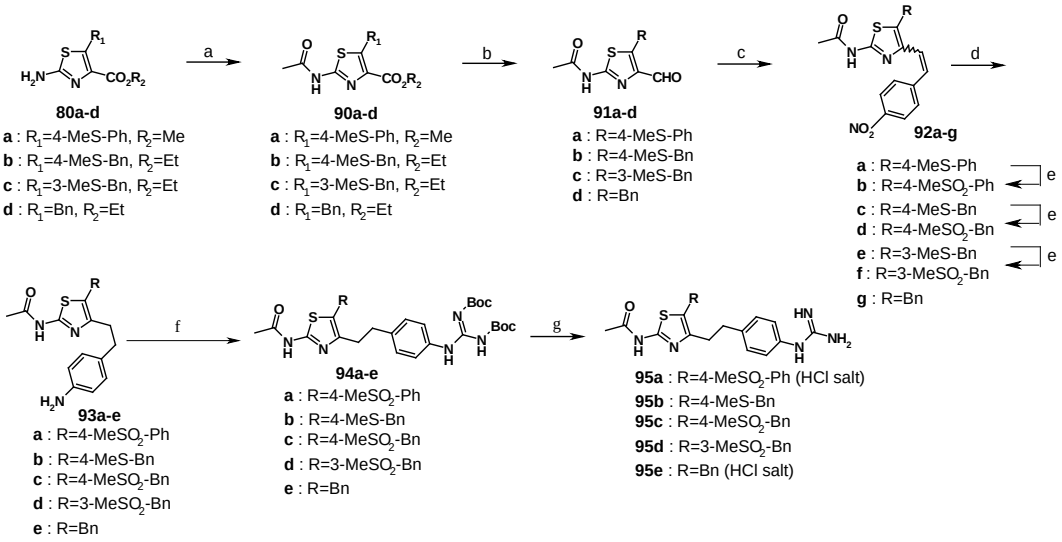


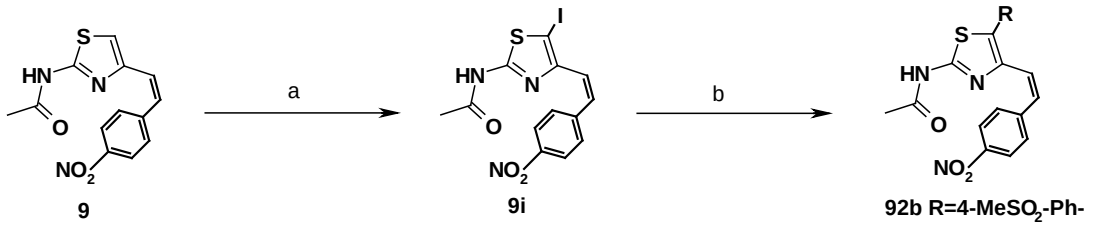
Figure 15. Structural determination of **72a** and **72b** by NMR.



Scheme 8. Reagents and conditions: (a) (acetylamino)acetic acid, Ac_2O, AcONa, reflux, 37%; (b) 4 M HCl/dioxane, reflux, 104%; (c) MeI, DBU, DMF, 59%; (d) pyridinium tribromide, AcOH, CH_2Cl_2, 0 °C, 105%; (e) thiourea, EtOH, reflux, 75-90%; (f) MeI, K_2CO_3, DMF, 73%; (g) *N*,*O*-dimethylhydroxylamine hydrochloride, EDC, HOBt, DMF, 97%; (h) 1) diisobutylaluminum hydride, THF, -78 °C–rt, 2) **85**, THF, reflux, 81%; (i) H_2 (4 atm), 10% Pd-C, AcOH, MeOH, 88%; (j) 1) diethyl oxalate, MeONa, MeOH, 15% aq.H_2SO_4, reflux, 2) c.H_2SO_4, EtOH, reflux, 49-51%; (k) $CuBr_2$, AcOEt/$CHCl_3$, reflux, 84-97%.



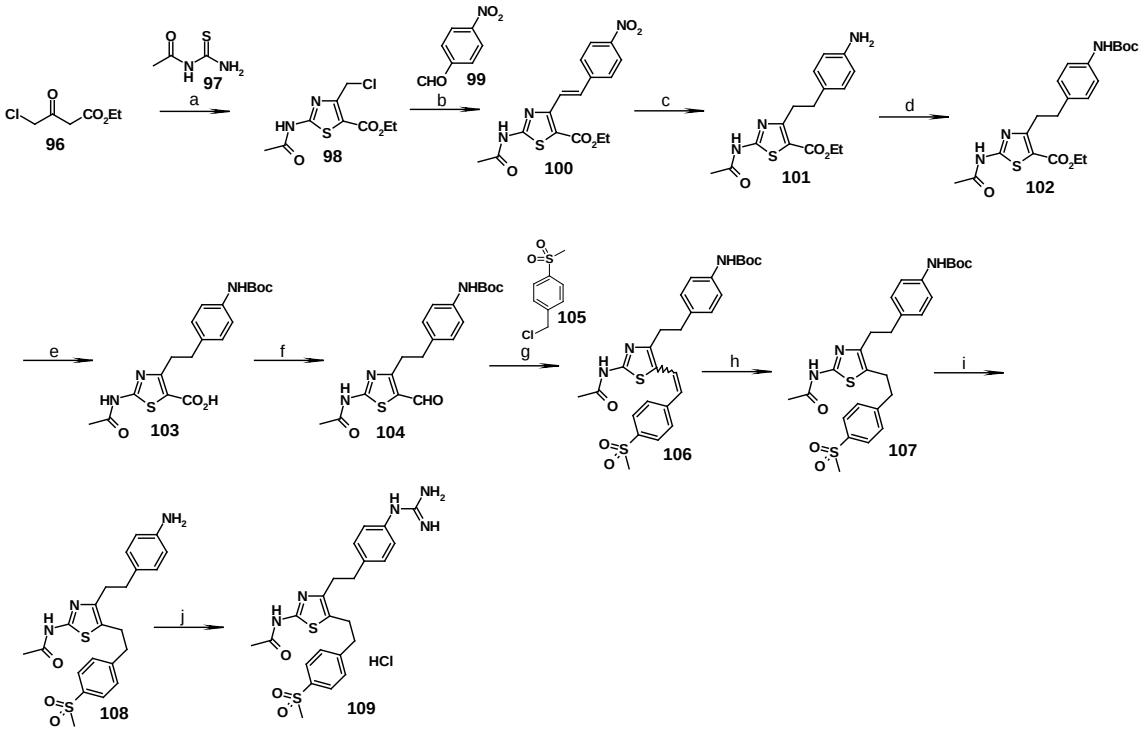
Scheme 9. Reagents and conditions: (a) AcCl, pyridine, 50 °C, 60-93%; (b) 1) $LiAlH_4$, THF, 2) MnO_2, MeOH/$CHCl_3$, 67-85%; (c) 1) 4-nitrobenzyl bromide (**8**), PPh_3, DMF, 2) ^{t}BuOK, 87-107%; (d) H_2 (3 atm), 10% Pd-C, AcOH, MeOH, THF, 51-90%; (e) Oxone®, THF/H_2O, 42-100%; (f) **31**, THF, 68-85%; (g) 4 M HCl/dioxane, 23-100%.



Scheme 10. Alternative synthetic route for 5-substituted thiazole derivatives.
Reagents and conditions: (a) NIS, THF/MeOH, rt, 94%; (b) 4-$MeSO_2PhB(OH)_2$, $Pd(PPh_3)_4$, Na_2CO_3, DME-H_2O, 80 ℃, 97%.

グアニジン誘導体 **95a–e** の合成を **Schemes 8, 9** に示す。原料 4-(methylsulfanyl)benzaldehyde（**75**）より、(acetylamino)acetic acid と無水酢酸を用いて sodium acetate 存在下、加熱還流する事により、2-methyl-4-[4-(methylsulfanyl)benzylidene]-1,3-oxazol-5(4*H*)-one（**76**）を合成した。ケトエステル誘導体 **78** は、4M 塩酸を用いた加水分解後、DBU 存在下、MeI を用いてエステル化を行い合成された。化合物 **78** は、臭素化後、チオウレアを用いて環化を行い、**80a** へと導いた。市販原料 **81** のエステル化により準備された化合物 **82** は、メタノール中 65℃で、diethyl oxalate と sodium methoxide を用いて反応を行い、続いて、15%硫酸と加熱還流下で反応させ 2−ケトカルボン酸誘導体を合成し、更にエタノール加熱還流下で濃硫酸と反応させ、2−ケトエステル誘導体 **88a** に変換した。**88b** は、市販のカルボン酸体 **83** を原料とし、*N*,*O*-ジメチルヒドロキシアミン塩酸塩を用いたワインレブアミド化（**84**）後、DIBAL を用いてアルデヒドへと還元し、methyl (triphenylphosphoranylidene)acetate（**85**）を用いて Wittig 反応を行い（**86**）、更に中圧接触還元によりオレフィンを還元し（**87**）、最後に **88a** と同様の合成方法を用いて 2−ケトエステル部分を構築する事により合成した。**88c** は市販品を使用し、**88a–c** の臭素化は臭化銅(II)を用いて AcOEt/$CHCl_3$ 加熱還流下行い、引き続き **80a** と同様の方法で環化体 **80b–d** へと導いた。これらアミノチアゾール誘導体 **80a–d** は、アセチル化後、還元剤として LAH を用いてエステル基をヒドロキシメチル基へと還元後、二酸化マンガンによりアルデヒド体 **91a–d** へと酸化した。続いて、系内で合成した (4-nitrobenzyl)triphenylphosphonium bromide を用いて Wittig 反応を行いオレフィン体 **92a,c,e,g** へと変換した。更に、**92a,c,e** は、Oxone®を用いてスルフィドをスルホン体 **92b,d,f** へと変換した。これらのオレフィン体 **92b,c,d,f,g** を中圧接触還元によりアニリン誘導体 **93a–e** へと導

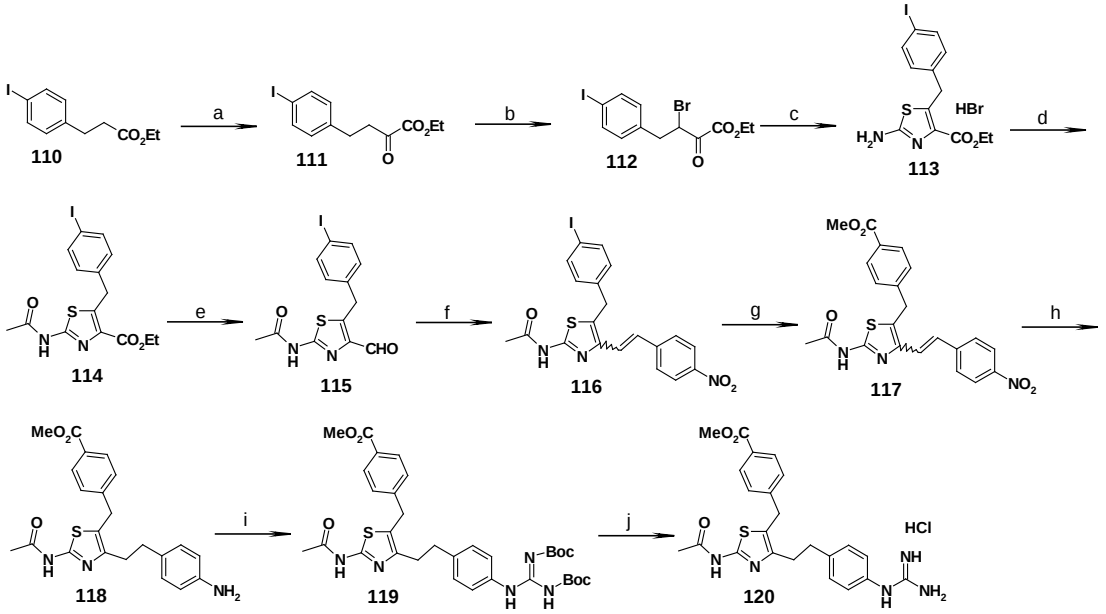
いた後、**74** と同様の方法で目的とする化合物 **95a–e** を合成した。更に、合成ルートの検討により、チアゾール環５位での置換基変換を容易にできるクロスカップリング反応（鈴木カップリング）を利用したルートを見出した(**Scheme 10**)。その結果、５位フェニル誘導体は、市販の各種フェニルボロン酸を使用して合成可能となった。



Scheme 11. Reagents and conditions: (a) 1) SO_2Cl_2, CH_2Cl_2, 2) **97**, acetone, reflux, 38%; (b) 1) PPh_3, DMF, 65 °C, 2) **99**, ^{t}BuOK, 73%; (c) H_2 (4 atm), 10% Pd-C, EtOH, THF, 73%; (d) $(Boc)_2O$, THF, reflux, 92%; (e) 1) 1 M NaOH, EtOH, reflux, 2) AcCl, pyridine, 93%; (f) 1) *N*,*O*-dimethylhydroxylamine hydrochloride, EDC, HOBt, DMF, 2) $LiAlH_4$, THF, 0 °C, 59%; (g) 1) **105**, PPh_3, DMF, 2) ^{t}BuOK, 125%; (h) H_2 (3 atm), 10%Pd-C, EtOH, 73%; (i) 4 M HCl/dioxane, 54%; (j) 1) **31**, THF, 2) 4 M HCl/dioxane, 105%.

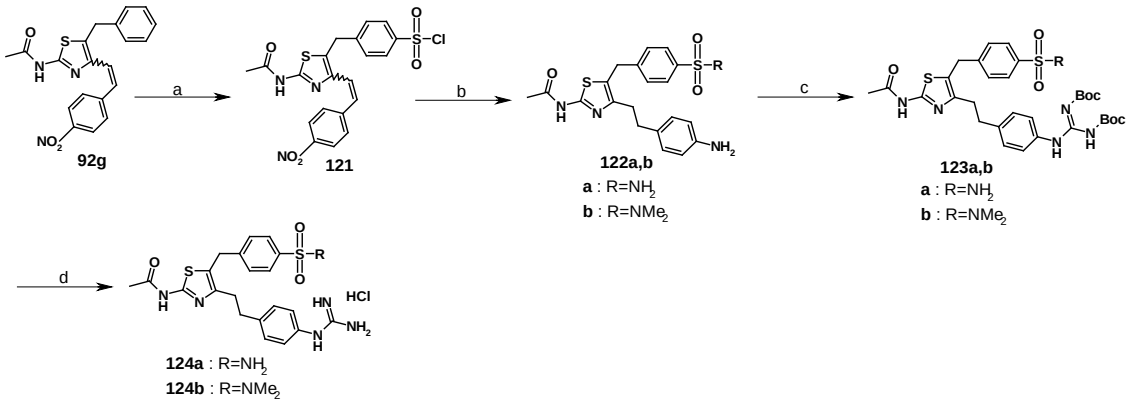
化合物 **109** は、**Scheme 11** に示したルートに従って合成した。市販原料 ethyl 4-chloro-3-oxobutanoate (**96**)に対して SO_2Cl_2 で塩素化し、引き続きアセチルチオウレア(**97**)を用いてチアゾール環を構築し、クロロメチル化合物 **98** を合成した。次に、triphenylphosphine と反応させ、反応系内でホスホニウム塩に変換した後、市販アルデヒド体 **99** と Wittig 反応を行い、更に中圧接触還元を行う事により、アニリン誘導体 **101** を合成した。次に、アミノ基を Boc 基で保護し(**102**)、エステルを加水分解後(**103**)、ワインレブアミドを経由し LAH で還元する事によりアルデヒド **104** へと変換した。このアルデヒド誘導体 **104** より、反応系内で合成した[4-(methylsulfonyl)benzyl]triphenylphosphonium chloride と Wittig 反応を行い、

続いて中圧接触還元を行う事により化合物 **107** へと導いた。更に、Boc 基を脱保護し、**74** と同様の方法で目的物 **109** へと導いた。



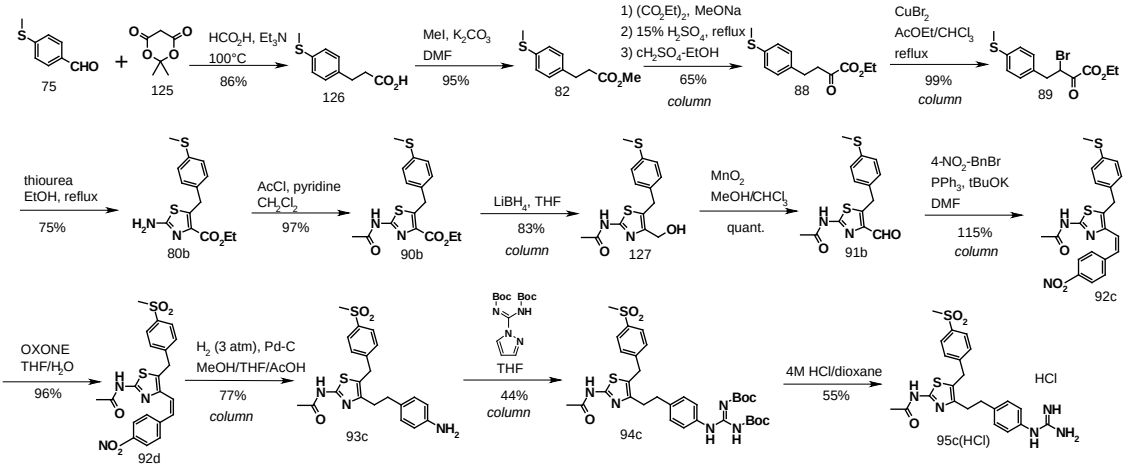
Scheme 12. Reagents and conditions: (a) 1) diethyl oxalate, MeONa, MeOH, 15% aq.H_2SO_4, reflux, 2) c.H_2SO_4, EtOH, reflux, 47%.; (b) $CuBr_2$, AcOEt/$CHCl_3$, reflux, 108%; (c) thiourea, EtOH, reflux, 94%; (d) AcCl, pyridine, CH_2Cl_2, 79%; (e) 1) $LiAlH_4$, THF, 2) MnO_2, MeOH/$CHCl_3$, 100%; (f) 1) **8**, PPh_3, DMF, 2) ^{t}BuOK, 70%; (g) $Pd(OAc)_2$, 1,3-bis(diphenylphosphino)propane, CO, Et_3N, MeOH/DMF, 65 °C, 77%; (h) H_2 (1.5 atm), 10% Pd-C, MeOH, THF, 93%; (i) **31**, THF, 83%; (j) 4 M HCl/dioxane, 68%.

化合物 **120** の合成を **Scheme 12** に示す。その中間体 **116** は、市販のエチルエステル体 **110** を原料とし、**92c** と同様の方法で合成した。次に、**116** のヨード基に対して、一酸化炭素雰囲気下、MeOH/DMF 混合溶媒中、1,3-bis(diphenylphosphino)propane と palladium (II) acetate を用いる事により、メトキシカルボニル基へと変換した。このメチルエステル体 **117** に対して中圧接触還元後、**74** と同様の方法で目的とする化合物 **120** を合成した。



Scheme 13. Reagents and conditions: (a) chlorosulfonic acid, $CHCl_3$, 43%; (b) 1) amines, THF, 2) H_2 (3 atm), 10% Pd-C, MeOH, DMF, AcOH, 22-48%; (c) **31**, ^{i}Pr$_2$NHEt, CH_2Cl_2, 88-100%; (d) 4 M HCl/dioxane, 76-82%.

スルホンアミド誘導体 **124a,b** の合成は、**Scheme 13** に示すルートで実施した。**92g** をクロロスルホニル化する事により **121** へと変換した。次に、それぞれ対応するアミンを用いてスルホンアミド体とし、続いて中圧接触還元を行い **122a,b** へと変換後、**74** と同様の方法で目的物 **124a,b** を合成した。



Scheme 14.　Large scale synthesis of **95c**.

著者は、**95c** のスケールアップ合成を行うために合成検討を行った（**Scheme 14**）。その結果、以下の三点を改良し、**75**（250g）を出発原料に 13 工程で **95c**（塩酸塩）を 58g 合成する事に成功した。

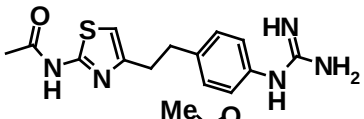
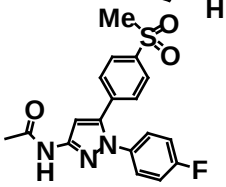
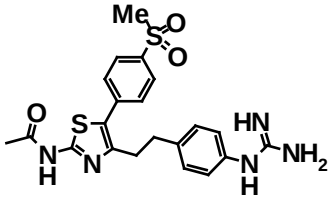
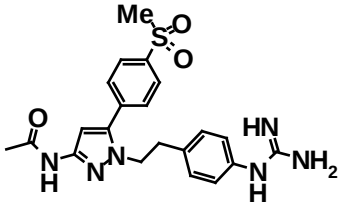
① 当初の出発原料である 3-(4-Mercaptophenyl)propionic acid (**81**) (TCI、5G：58,200 円)の高コストを、メルドラム酸と 4-(Methylthio)benzaldehyde との縮合反応を利用したルートに変更する事により改善した。

② 第 9 工程の Wittig 反応では、生成物のシス/トランス比が使用溶媒により異なった(THF : cis/trans=1:1, DMF：cis/trans=2:1)。トランス体はシス体に比べ難溶性で有り、第１１工程の接触還元に於いて反応の進行が非常に遅く、更に未還元のトランス体が残存していると精製で分離することが難しく、最終体まで残存して化合物純度の問題となる。よってトランス体を減少させる事が、最終物の純度確保に重要である事が判明した。

③ 第 13 工程（最終物）は、当初グアニジンのフリー体で合成していた。フリー体は有機溶媒に難溶の物性を有していたため、カラム精製や再結晶ができず、有機溶媒による洗浄で精製した。そのため最終品には総量 1.64%（8 個の類縁体を含有）の類縁物質を含み、純度の向上が難しかった。その後、塩酸塩での結晶化に成功し（一塩酸塩）、純度も向上した（不純物：一つの類縁体/0.13%）。塩酸塩に含有する唯一の不純物は、先に述べた接触還元での未還元トランスオレフィン体のグアニジン化合物と推測している（LC-MS より）。

第３節　　ハイブリッド化合物の構造活性相関

Table 7.
VAP-1 inhibitory activity of hybrid designs from compounds of **12** and **2**.

Compd		VAP-1 IC_{50}(μM) human	rat
12		0.23	0.014
2		2.1	>100
95a[a]		0.034	3.5
74[a]		0.39	9.2

[a] Hydrochloride salt

ハイブリッド型化合物**95a**と**74**の酵素阻害活性を**Table 7**に示す。**95a**は**12**と比較して、ヒトのVAP-1阻害活性は7倍改善したが、ラットの活性が250倍低下した。**74**は、**95a**に比べると、ヒトとラット共にVAP-1阻害活性が低下した。この事は、チアゾール環の硫黄原子がVAP-1酵素に対しS-O相互作用[23]で寄与しているので、結果としてチアゾール環はピラゾール環より優れたVAP-1阻害活性を示したと考察している。以上より、化合物**12**と**2**をハイブリッドした**95a**は、期待通りヒトの活性は向上したが、一方でラットの活性が減弱する結果となった。ラット病態モデルの有効性よりヒトでの臨床開発可能性を判断するため、開発化合物ではラットの阻害活性も有する事が必要である。よって、この種差の解決が次の課

題となった。

第４節　　ハイブリッド化合物**95a**の種差の回避方針

著者は、化合物**95a**のラットVAP-1阻害活性の低下理由を、VAP-1のヒトとラットのシークエンス情報を利用して分析した。ヒトとラットVAP-1酵素間のシークエンスホモロジーを調査したところ、リガンド結合ポケット周辺で2残基の違いが見られた（human/rat：F173/T, L447/F）。**95a**とヒトVAP-1のドッキングモードから、**95a**のメタンスルホニルフェニル部分がLeu447残基の近傍に存在する事が明らかとなった（**Figure 16**）。ラットでLeu447に相当する残基はPhe447となり、それはロイシンより立体的に嵩高い。その結果、**95a**のメタンスルホニルフェニル部分がPhe447と大きな立体的反発を起こし、ラットで最適な位置に結合する事が出来ないと推測した。この立体的反発は、ヒトでは比較的小さいため、活性の低下がみられないと考察している。そこで、著者はメタンスルホニルフェニル部分の自由度を高める事により、ラットで立体的な反発を下げるデザインを考えた。

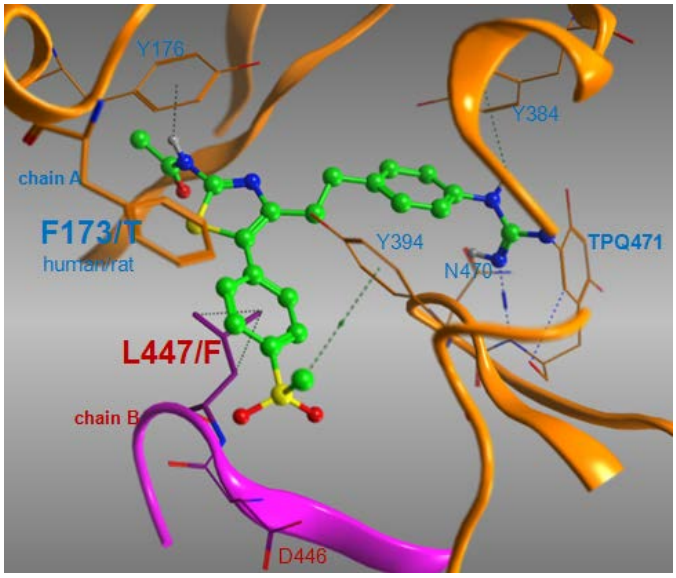
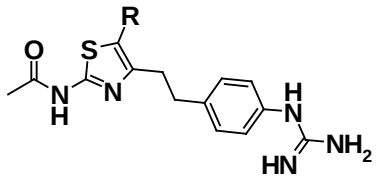


Figure 16. Computational human VAP-1 docking results for **95a** (as free base).
A two-dimensional diagram was prepared using the ligand interactions application in MOE. We compared sequence homology between human and rat VAP-1. Two residue differences (F173/T, L447/F) in the ligand binding pocket of human VAP-1 model were found. Best docking solution (lowest binding energy) calculated by GOLD *ver.5.0.* for **95a** (stick representation; compound is colored blue for nitrogen, red for oxygen, yellow for sulfur, and green for carbon) surrounded by human VAP-1 active site residues.

第５節　　チアゾール環５位への置換基導入と構造活性相関の取得

Table 8.
VAP-1 inhibitory activity of different substituents at 5-position of the thiazole ring.



Compd	R	VAP-1 IC_{50}(μM) human	rat
95a[a]	4-$MeSO_2$-Ph-	0.034	3.5
95b	4-MeS-Bn-	1.1	0.85
95c	4-$MeSO_2$-Bn-	0.020	0.072
95d	3-$MeSO_2$-Bn-	0.036	0.018
95e[a]	Bn-	0.21	0.30
109[a]	4-$MeSO_2$-Phenethyl-	0.16	0.092
120[a]	4-MeOCO-Bn-	0.32	0.45
124a[a]	4-NH_2SO_2-Bn-	0.053	0.12
124b[a]	4-NMe_2SO_2-Bn-	0.15	0.050

[a] Hydrochloride salt

ハイブリッド体**95a**誘導体の酵素阻害活性を**Table 8**に示す。前節の仮説に基づき、チアゾール環と５位フェニル基間にメチレンを挿入し自由度を持たせたベンジル基に変換した所（**95c**）、ラットのVAP-1阻害活性が50倍増加した。加えて、ヒトのVAP-1阻害活性も約2倍改善された。これらの結果は、立体障害に関する著者の仮説を強く支持しており、ヒトとラットに対するシークエンス情報を用いたこのアプローチの有用性を裏付けている。無置換

ベンジル体**95e**は、**95c**に比べてヒトとラット共にVAP-1阻害活性が明らかに減弱した。よって、メタンスルホニル基の重要性を明確にする事が出来た。更に、リンカーをメチレンからエチレンへと増炭したフェネチル体**109**では、ヒトの活性が1/8に低下した事から、ベンジルパーツが最適なユニットである事が判明した。メタンスルホニル基の置換位置を３位に変えた**95d**は、４位の**95c**に比べヒトで1/2の活性低下がみられた。ヒトの阻害活性は、スルホンをスルフィド(**95b**)に変換する事によって1/50に低下し、スルホンをメチルエステル(**120**)に変換しても1/16に低下した。スルホニル基のbioisostereであるスルホンアミド体**124a**,**b**でも、**95c**に比べてVAP-1阻害活性は改善しなかった。これらの結果より、４位のメタンスルホニル基はベンゼン環上での最適な置換基であり、VAP-1の阻害に対して重要な役割を果たしている事が明らかとなった。

第６節　　新規チアゾール誘導体**95c**のプロファイル検証

第１項　　ヒトVAP-1ドッキングモデルに於ける解析

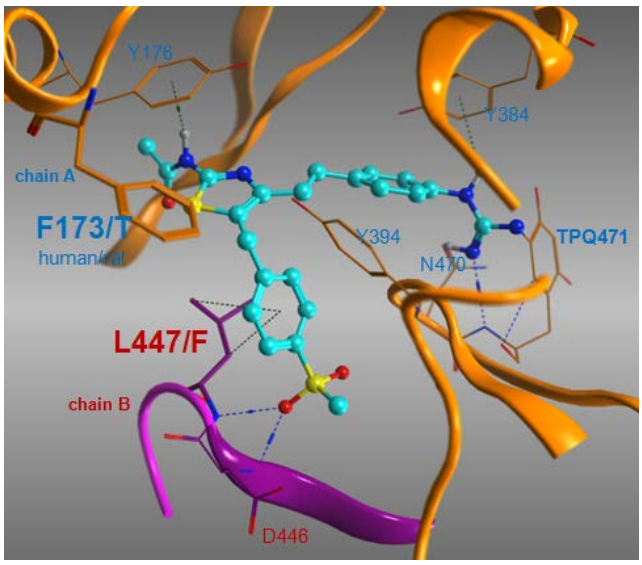


Figure 17. Computational human VAP-1 docking results for **95c**.
A two-dimensional diagram was prepared using the ligand interactions application in MOE. We compared the sequence homology between human and rat VAP-1. Two residue differences (F173/T, L447/F) in the ligand binding pocket of the human VAP-1 model were found. Best docking solution (lowest binding energy) calculated by GOLD *ver.5.0.* for **95c** (stick representation; compound is colored blue for nitrogen, red for oxygen, yellow for sulfur and light blue for carbon) surrounded by human VAP-1 active site residues.

ヒトVAP-1モデルで化合物**95c**のドッキング解析を行った(**Figure 17**)。**95c**のヒトVAP-1

に対するドッキングモードは、メチレンリンカーが挿入されているが **95a** と近似していた。グアニジン部分のリジッドな相互作用に加え、**95c** のスルホニル部分は Leu447 や Asp446 と水素結合を形成し、スルホニルフェニル部分は Leu447 と π-proton 相互作用、アミド基の NH は Tyr176 と proton-π 相互作用を形成していた。化合物 **12** に比べて **95c** のヒト VAP-1 阻害活性が改善された理由は、メタンスルホニルフェニル部分と酵素間の２つの水素結合と２つの π-proton 相互作用にあると、これらの結果は示唆している。

第２項　　他のアミンオキシダーゼに対する選択性

Table 9. Selectivity of **12** and **95c** for various amine oxidases in human and rat.

Compd	VAP-1 human $IC_{50}(\mu M)$[c]	VAP-1 rat $IC_{50}(\mu M)$[c]	DAO[a] human $IC_{50}(\mu M)$[c]	DAO[a] rat $IC_{50}(\mu M)$[c]	MAO-B[b] human $IC_{50}(\mu M)$[c]
12	0.23	0.014	>100	75	>100
95c	0.020	0.072	>100[d]	>100[d]	>100[d]

[a] DAO, diamine oxidase
[b] MAO-B, monoamine oxidase-B
[c] IC_{50} data for various amine oxidase in μM
[d] Hydrochloride salt's data

化合物 **95c** と **12** の各種 *in vitro* プロファイルを **Table 9** に示す。アミンオキシダーゼ内で大別される二つのグループの代表として、ヒト／ラットのジアミンオキシダーゼ(DAO)とヒトのモノアミンオキシダーゼ(MAO)-B を選択し、radiochemical-enzyme assay を用いて評価した。その結果、**95c** は他のアミンオキシダーゼに対して、1400 倍以上の優れた選択性を有している事が判明した。

第３項　　*in vivo* 薬効効果

Table 10.　Pharmacokinetic profile of **95c** in rats.

Route	Dose (mg/kg)	Cmax (ng/mL)	tmax (h)	AUC_{0-t} (ng・h/mL)	t1/2 (h)
p.o. (n=3)	1	N.D. [a]	N.D.[a]	N.D.[a]	N.D.[a]
s.c. (n=3)	1	139.7[b] ±9.3[c]	0.25[b] ±0.00[c]	205.8[b] ±8.4[c]	2.5[b] ±0.2[c]

[a] Not detected
[b] Mean
[c] S.E.

化合物 **95c** を用いて PK と PD の相関を確認するために、p.o.（経口）と s.c.（皮下）投与で PK 評価を行った（**Table 10**）。その結果、本化合物は p.o 投与で、全く血中に暴露されない事が明らかとなった。s.c.投与では **12** とほぼ同等の血中濃度が確保されており、**95c** は **12** と比較してラットの活性が 1/5 である事から、**12** の PD 評価系での最小有効用量 0.1mg/kg から類推すると、**95c** の PD 評価系の最小有効用量は 0.5 mg/kg 付近と推測できる。

次に、ストレプトゾトシン(STZ)誘導糖尿病ラットのプラズマ VAP-1 活性に対する、**95c** の阻害効果（PD 評価）を評価した(**Figure 18**)。0.32 から 3.2 mg/kg を s.c.投与した所、0.32mg/kg で若干 efficacy が弱いが全ての用量で阻害効果が観察された。この結果は、上記で予測した **95c** の最小有効用量とほぼ一致しており、PK と PD の相関を確認する事ができた。以上の結果より、**95c** は経口での黄斑浮腫治療剤の開発は難しいが、他の投与経路での開発可能性は十分有していると考えられる。

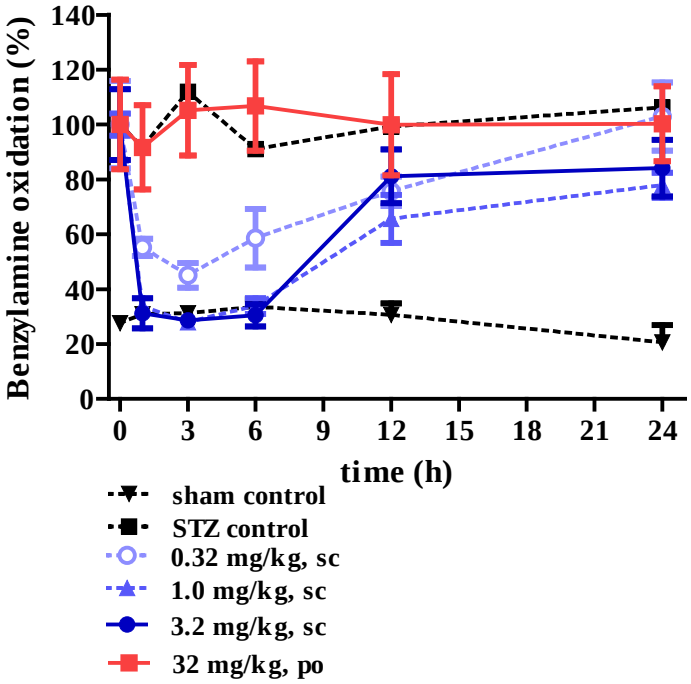


Figure 18. Profile of **95c** pharmacodynamics in rat (PD study).
Inhibitory effect on plasma VAP-1 activity in STZ-induced diabetic rats (n=4–5, 0.32–3.2 mg/kg, s.c.).
The plasma VAP-1 activity was measured with a radioenzyme assay using ^{14}C- benzylamine as the substrate. Values are % of VAP-1 activity at time 0 h.

第 7 節　小括

リード化合物 **12** が課題として有している種差の改善を目的とし、**12** のヒト VAP-1 阻害活性を向上させるために、ヒトの活性を特異的に有している HTS ヒット **2** を利用して、ヒト VAP-1 ドッキングモデル上で **12** と **2** のハイブリッドデザインを考えた。そのハイブリッド化合物 **95a** は、**12** と比較して期待通りにヒト阻害活性が 7 倍向上した。しかし、ラットの活性は減弱する結果となった。そこで、**95a** のラット阻害活性を向上させるために、ヒトとラットの VAP-1 シークエンス情報に基づいたドラッグデザインを考えた。その結果、化合物 **95c**(human IC_{50} of 20 nM; rat IC_{50} of 72 nM)を見出すことに成功し、その化合物は、高活性、高選択性、そして s.c.投与ではあるが PD 試験に於いて有効性を示した。更に、**95c** を用いて PK と PD に相関がある事を確認した。

第１節　リード化合物の課題とその改良方針

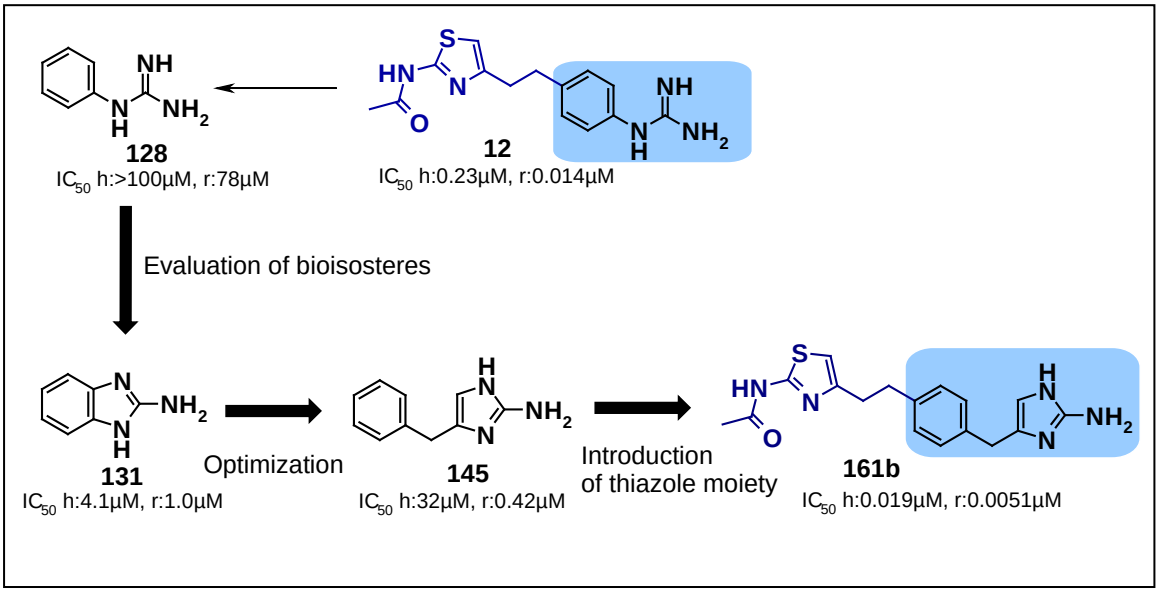


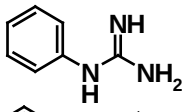
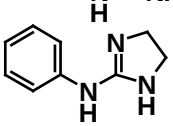
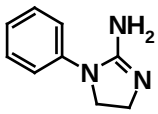
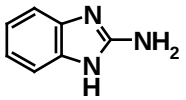
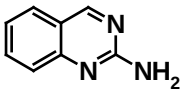
Figure 19.　Summary of the discovery of **161b** with bioisosteric conversion.

リード化合物**12**の種差の改善を、第２章とは異なる方法で実施した。リード化合物**12**(human IC_{50} of 0.23 μM; rat IC_{50} of 0.014 μM)に於けるヒトVAP-1阻害活性の向上を目的に、**12**とヒトVAP-1のドッキング研究結果よりヒト阻害活性に対して重要なアンカーパートと考察しているフェニルグアニジン部分の更なる変換を実施した。フェニルグアニジン部分の生物学的等価体探索のアウトラインを**Figure 19**に示す。最初に、フェニルグアニジン(**128**)の生物学的等価体探索をフラグメント化合物で実施し、続いて、最適化されたフラグメント化合物を**12**のフェニルグアニジン部分と置き換える方策をとった。

第２節　フェニルグアニジン生物学的等価体の探索

第１項　　構造活性相関の取得

Table 11.
VAP-1 inhibitory activities of phenylguanidine bioisosteres.

Compd	Structure	VAP-1 IC_{50}(μM) human	rat
128[c]		>100	78
129[a,c]		>100	>100
130[b,c]		>100	>100
131[c]		4.1	1.0
132[c]		>100	>100

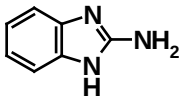
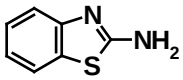
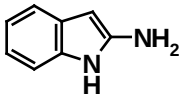
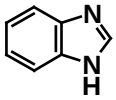
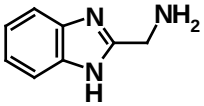
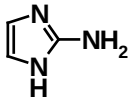
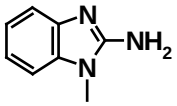
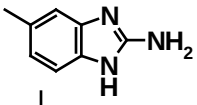
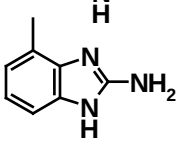
[a] Hydrochloride salt
[b] Hydrobromide salt
[c] Commercially available

第１章中でグアニジンパートは、TPQ471と共有結合する以外にAsp386と水素結合ネットワークを形成し、ヒトのVAP-1活性に対してとても重要である事を示した。よってこの情報を基に、フェニルグアニジンに非常に近い構造にフォーカスしてフラグメント探索を行った。初期デザインとしてグアニジン構造を維持し、窒素原子同士をリンカーで結び環を構築した、imidazoline誘導体（**129**, **130**）、1*H*-benzimidazol-2-amine（**131**）、quinazolin-2-amine（**132**）を評価した。化合物**129–132**のVAP-1酵素阻害活性を**Table 11**に示す。imidazoline誘導体**(129, 130)**、quinazolin-2-amine **(132)**は、阻害活性を全く示さなかった。これらの結果は、新たに構築した環構造の立体障害によりTPQとシッフベースを形成できなかったからと推測

している。一方、1*H*-benzimidazol-2-amine (**131**)は強い阻害活性を有し、**128**に比べてヒトとラット共に阻害活性が大幅に増強した。この化合物には種差がほとんどなく、アンカーパートとして良好な活性を持ち合わせていた。

Table 12.

VAP-1 inhibitory activities of *1H*-benzimidazol-2-amine derivatives.

Compd	Structure	VAP-1 IC_{50}(μM) human	rat
131[d]		4.1	1.0
133[d]		>100	>100
134[a,d]		74	63
135[d]		>100	>100
136[b,d]		>100	>100
137[c,d]		>100	90
138[d]		>100	80
139[d]		>100	9.3
140[d]		>100	1.0

[a] Hydrochloride salt
[b] Dihydrochloride salts
[c] Hemisulfate salt
[d] Commercially available

次に、化合物 **131** の周辺 SAR を検討し、活性向上、及びチアゾールパートの結合位置探

索を行った（**Table 12**）。ベンゾチアゾール体 **133** では、VAP-1 阻害活性が完全に消失した。イミダゾール部分の NH が活性には重要と推測し、NH を残したインドール体 **134** を評価したが、阻害活性は劇的に低下した。これらの結果は、グアニジン構造が阻害活性に重要な役割を果たしている事を示唆している。**131** の脱アミン体 **135** は、活性が完全に消失した。この結果は、シッフベースを形成する末端の１級アミンが阻害活性に対して必須である事と一致している。ベンゼン環を削除した **137** では、活性の大幅な減弱が生じたため、ベンゼン環も高活性には必須と考えている。アミノメチル体 **136** の阻害活性が完全に消失した事は、グアニジン部分に対してスペーサーを挿入できる空間的な余裕が無い事を示唆している。以上の結果より、最も良好な阻害活性を示したのは 1*H*-benzimidazol-2-amine (**131**)であった。続いて、化合物 **12** のファーマコフォアであるチアゾールパートを付加するための部位を探索するため、1*H*-benzimidazole-2-amine の 1、４、５位にメチル基を導入した。しかし、全ての化合物(**138–140**)でヒト VAP-1 阻害活性が消失し、置換基導入の可能性が否定された。

第２項 1*H*-Benzimidazole-2-amine (**131**)のドッキング解析

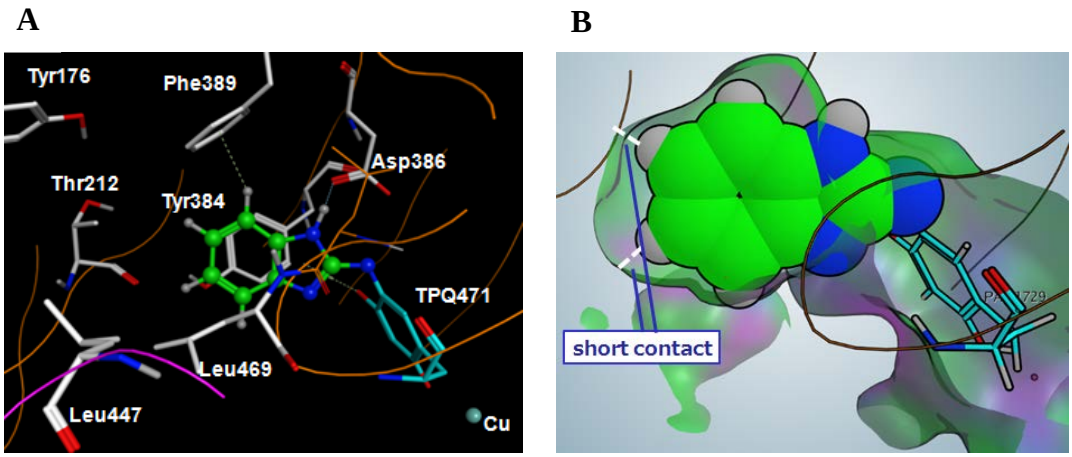
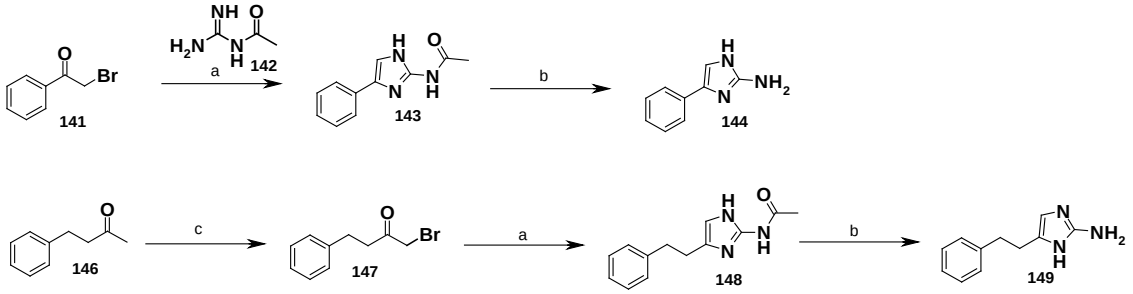


Figure 20. Computational docking results for **131** with human VAP-1.
(A) Best docking solution (lowest binding energy) calculated by GOLD *ver.5.1.* for **131** (ball and stick representation; compound is colored blue for nitrogen and green for carbon) surrounded by the human VAP-1 active site. Key receptor residues are indicated. (B) Results of the docking analysis represented as an Active LP image (green, hydrophobic regions of the surface; blue, mildly polar regions; and purple, hydrogen-bonding regions).

ヒト VAP-1 モデルを用いて、前項の結果を考察した。化合物 **131** は GOLD version 5.1 を

用いて、ヒト VAP-1 モデルにドッキングした(**Figure 20**)。初期構造として、**131** の２位アミノ基は、TPQ471 とシッフベースを形成させた。結果として、イミダゾール環内の NH は Asp386 と水素結合を形成し、イミダゾール環は TPQ471(-OH)と π-proton 相互作用、ベンゼン環は Tyr384 と π-π 相互作用、そしてベンゼン環の CH は Phe389 と proton-π 相互作用を形成していた。**131** のような低分子でも、これら５つの相互作用を有することで強い阻害活性を示したと推察している。加えて、**131** は活性中心のポケットに隙間なく結合している為、置換基を導入する空間は見られなかった。この結果は、SAR 研究で見出された知見と一致した。以上より、著者は **131** にチアゾールパートを導入する事は難しいと考え、引き続き、置換基導入可能な 1*H*-benzimidazol-2-amine の周辺誘導体を探索した。

第３項 1*H*-Benzimidazole-2-amine (**131**)の生物学的等価体探索

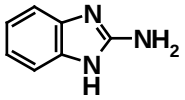
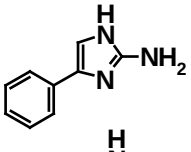
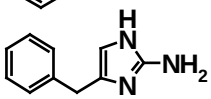
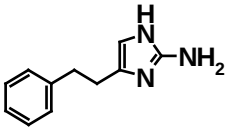
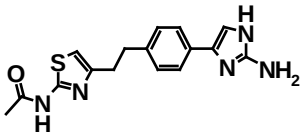
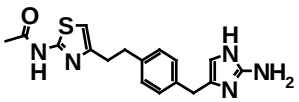
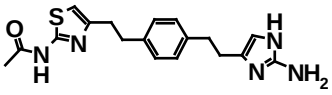


Scheme 15. Reagents and conditions: (a) **142**, DMF, 10-54%; (b) 6 M HCl, MeOH, reflux, 33-93%; (c) 1) Br_2, HBr, AcOH, 2) acetone, 34%.

1*H*-imidazol-2-amine 誘導体 **144** と **149** の合成を **Scheme 15** に示す。市販の 2-bromo-1-phenylethanone (**141**)を原料とし、*N*-carbamimidoylacetamide (**142**)を用いて環化させ *N*-(4-phenyl-1*H*-imidazol-2-yl)acetamide (**143**)へと変換し、更にアセチル基を脱保護し、4-phenyl-1*H*-imidazol-2-amine (**144**)へと導いた。市販の 4-phenylbutan-2-one (**146**)は、臭素体(**147**)へと変換後、**144** と同様の合成法によって 4-(2-phenylethyl)-1*H*-imidazol-2-amine (**149**)を合成した。

Table 13.

VAP-1 inhibitory activities of 1*H*-imidazol-2-amine derivatives.

Compd	Structure	VAP-1 IC_{50}(μM) human	rat
131[b]		4.1	1.0
144		>100	44
145[a,b]		32	0.42
149		>100	4.8
161a[a]		13	0.15
161b[a]		0.019	0.0051
161c[a]		2.2	0.018

[a] Hydrochloride salt

[b] Commercially available

化合物 **131** のフェニル環とイミダゾール環の間に自由度を持たせる事により、チアゾールパートの導入可能な空間を生じさせるデザインを考えた（**Table 13**）。リンカーの長さを調節してフェニル環とイミダゾール環の距離の最適化を実施した。その結果、メチレンを挿入した **145** が最も高活性を示した。直結の **144** やエチレンリンカーの **149** は、ヒト阻害活性がラットに比べて劇的に減少した。

第４項 4-Benzyl-1*H*-imidazol-2-amine (**145**)のドッキング解析

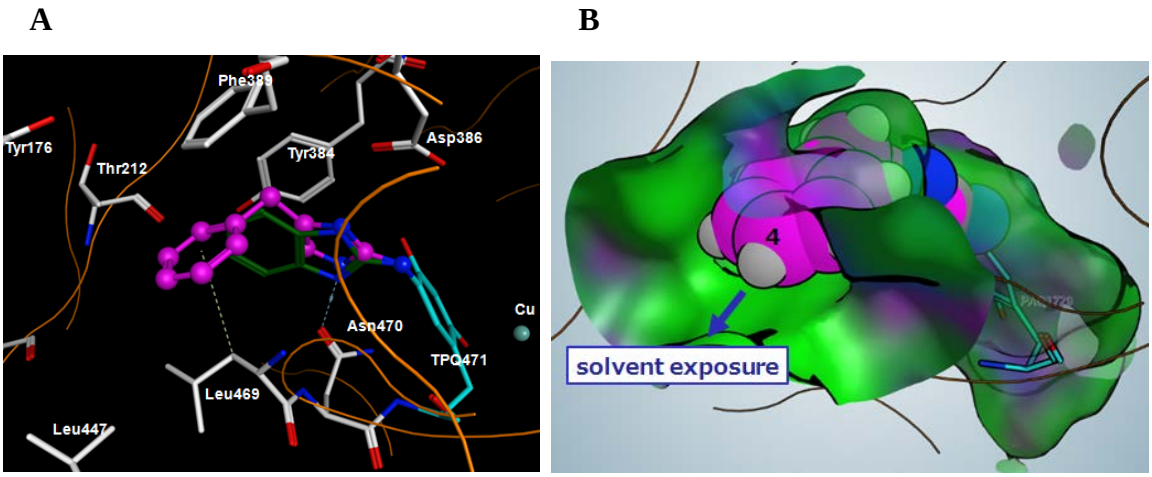
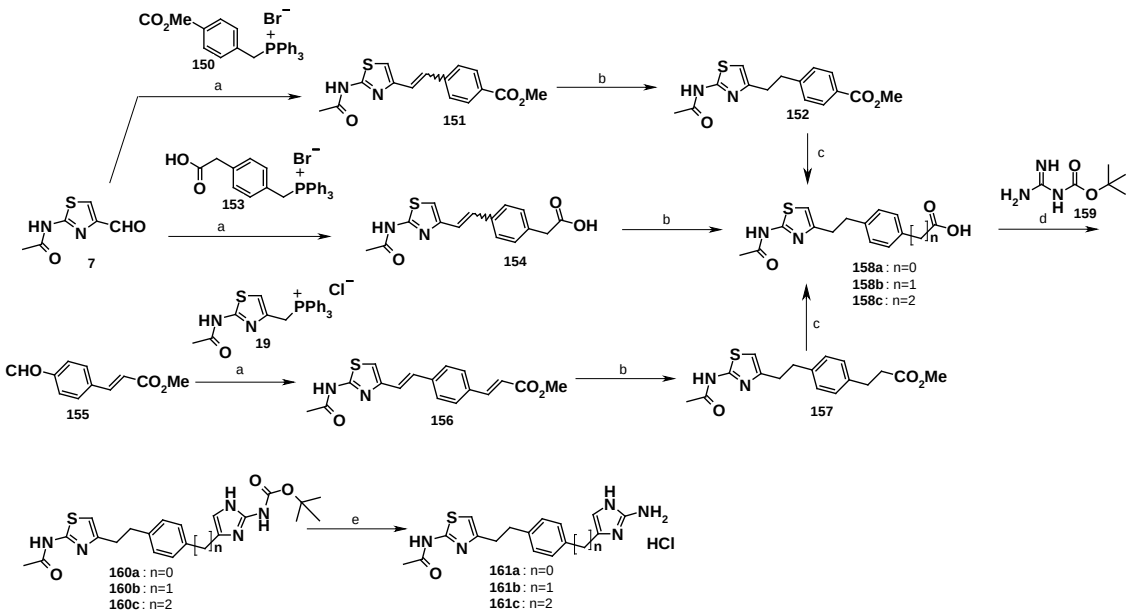


Figure 21. Computational docking results for **131** and **145** with human VAP-1.
(A) Best docking solution (lowest binding energy) calculated by GOLD *ver.5.1*. for **131** (stick representation; compound is colored blue for nitrogen and green for carbon) and **145** (ball and stick representation; compound is colored blue for nitrogen and pink for carbon) surrounded by the human VAP-1 active site. Key receptor residues are indicated. (B) Results of the docking analysis represented as an Active LP image (green, hydrophobic surface regions; blue, mildly polar regions; and purple, hydrogen-bonding regions).

化合物 **145** とヒト VAP-1 モデルとのドッキングからドッキングモードを解析した(**Figure 21**)。イミダゾール環の NH は Asn470 と水素結合を形成し、イミダゾール環は Tyr384 と π-π 相互作用、ベンゼン環は Leu469 と π-proton 相互作用を形成していた。**145** は **131** と比較して、約 8 倍ヒト VAP-1 阻害活性の低下を示した。これは、2つの環の間にメチレンリンカーを挿入する事によって、**145** のベンゼン環がポケットから僅かに露出し、その結果として親和性の低下が起きたと推測している。一方、ベンゼン環の4位が溶媒側に向いている為、この位置に置換基(チアゾールパート)を導入する事は可能と考えられた。また、**145** は初期化合物 **128** と比較すると、ヒトの阻害活性で 3 倍以上、ラットで 186 倍改善した。

第５項 低分子 1*H*-imidazol-2-amine 誘導体へのチアゾールパート導入



Scheme 16. Reagents and conditions: (a) **150** or **153** or **19**, ^{t}BuOK, DMF, 70-94%; (b) H_2 (4 atm), 10% Pd-C, AcOH, MeOH, THF, 29-76%; (c) 1 M NaOH, EtOH, reflux, 69-88%; (d) 1) $(COCl)_2$, CH_2Cl_2, DMF, 2) $TMSCH_2N_2$, CH_2Cl_2, 3) 4 M HCl/AcOEt, CH_2Cl_2/DMF, 4) **159**, DMF, 22-26%; (e) 4 M HCl/AcOEt, MeOH, 74-96%.

化合物 **161a-c** の合成ルートを **Scheme 16** に示す。アルデヒド体 **7** や **155** に対し、それらに対応するホスホニウム塩 **150**、**153**、**19** を用いた Wittig 反応を行い、引き続き接触還元を行い、中間体 **152**、**157**、**158b** を合成した。そのうちエステル体 **152** と **157** は加水分解により、カルボン酸誘導体 **158a, c** へと導いた。**158a–c** は oxalyl chloride を用いて酸クロライド体とし、(trimethylsilyl)diazomethane で処理しケトン体へと変換後、トリメチルシリル基を 4 M HCl/AcOEt によりクロロ基に変換し、*tert*-butyl carbamimidoylcarbamate (**159**)を用いて環化反応を行い、化合物 **160a–c** へとそれぞれ導いた。最後に、**160a–c** の Boc 基を脱保護し、目的物 **161a–c** を合成した。

著者は、前項でのドッキング結果に基づき、化合物 **145** のベンゼン環４位にチアゾールパートを導入した（**Table 13**）。その結果、目的化合物 **161b** は、ヒトとラット共に良好な活性を示し（human IC_{50} of 19 nM; rat IC_{50} of 5.1 nM）、**145** に比べてチアゾールパートを導入した事により、ヒトの活性が約 1700 倍向上した（ラットの活性は約 80 倍向上）。また、**161b** はヒトの VAP-1 阻害活性が **12** より 12 倍向上した。化合物 **144** と **149** に関してはヒトの活

性を有していないが、確認のために対応するチアゾール誘導体 **161a**, **c** を評価した。それらの結果は、**161b** に比べて非常に弱いものであった。

以上の結果より、フェニルグアニジンの生物学的等価体をフラグメント化合物で探索し、続いて最適化されたフラグメント化合物を **12** のフェニルグアニジン部分と置き換えるこの手法により、ヒト及びラット両方の阻害活性が良好な化合物 **161b** を見出すことに成功した。

第３節　新規1*H*-imidazol-2-amine誘導体**161b**のプロファイル検証

第１項　ヒトVAP-1ドッキングモデルに於ける解析

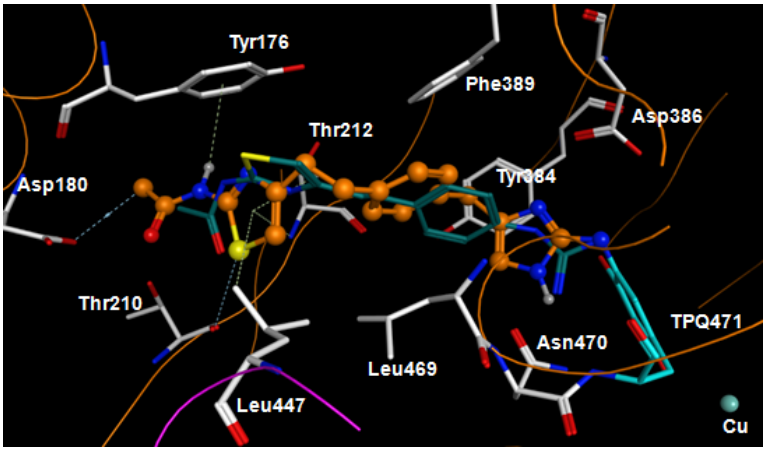


Figure 22.　Computational docking results for **12** and **161b** with human VAP-1.
Best docking solution (lowest binding energy) calculated by GOLD *ver.5.1*. for **12** (stick representation; compound is colored blue for nitrogen, red for oxygen, yellow for sulfur, and cyan for carbon) and **161b** (ball and stick representation; compound is colored blue for nitrogen, red for oxygen, yellow for sulfur, and orange for carbon) surrounded by the human VAP-1 active site. Key receptor residues are indicated.

化合物 **161b** のヒト VAP-1 モデルとのドッキング研究結果を **Figure 22** に示した。予想した通り最後に導入したチアゾールパートは、ヒト VAP-1 と複数の相互作用を示し活性向上に寄与した。チアゾール環は Thr212、Leu447 と π-proton 相互作用を形成、チアゾール環の硫黄原子は Thr210 の主鎖のカルボニル酸素と S-O 相互作用[23]を形成、アミドの NH は Tyr176 と proton-π 相互作用を形成、アセチル部分は Asp180 と CH-O 相互作用を形成していた。著者は、**12** より **161b** が高活性の理由として、次の２つを考えている：①**161b** のアンカーパートである 4-benzyl-1*H*-imidazol-2-amine は、**12** のアンカーパートである phenylguanidine よ

り高活性である事、②**161b** のチアゾールパートは、VAP-1 酵素の最適な場所に結合し、**12** より強固な相互作用ネットワークを形成している事。

第２項　他のアミンオキシダーゼに対する選択性

Table 14. Selectivity of **12** and **161b** for VAP-1 and MAO-A,B.

Compound	VAP-1 Human IC_{50} (μM)	VAP-1 Rat IC_{50} (μM)	MAO[a]-A Human IC_{50} (μM)	MAO[a]-B Human IC_{50} (μM)
12	0.23	0.014	>100	60
161b	0.019	0.0051	>100	11

[a] MAO, monoamine oxidase

化合物 **161b** は、他のアミンオキシダーゼに対する選択性として MAO-A/B を fluorometric-enzymatic assay 法を用いて評価した（**Table 14**）。**161b** は、MAO-A/B に対して約 580 倍の優れた選択性を有していた。

第３項　*in vivo* 薬効効果

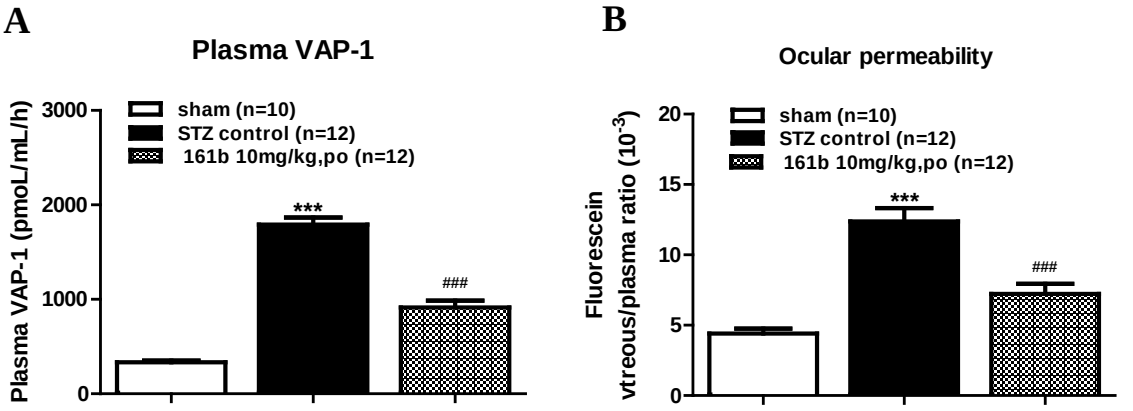


Figure 23.　Pharmacodynamic profile and pharmacology of **161b** in rats.
(A) Inhibitory effect on plasma VAP-1 activity in streptozotocin (STZ)-induced diabetic rats (n=10–12, 10 mg/kg, p.o.) at 2 weeks after treatment with **161b**. The plasma VAP-1 activity was measured using a radiolabeled enzyme assay with ^{14}C- benzylamine as the substrate. (B) Effect of **161b** (n=10–12, 10 mg/kg, p.o.) on ocular permeability in STZ-induced diabetic rats. ***P<0.001 and $^{###}$P<0.001, Student's t-test vs. sham and STZ control groups, respectively. Error bars indicate standard error.

STZ 糖尿病ラットでの、プラズマ VAP-1 活性に於ける化合物 **161b** の阻害効果を評価した(**Figure 23A**)。**161b** を２週間経口投与後、10mg/kg の用量に於いて有意な阻害効果を示した。次に、黄斑浮腫の病態モデルである STZ 糖尿病ラットでの眼透過性試験を行った(**Figure 23B**)。眼透過性の阻害効果は、**161b** を２週間経口投与後 PD 試験で有効であった 10mg/kg の用量に於いて、有意に観察された。この結果は、**161b** が糖尿病性黄斑浮腫の治療に対し、経口投与で有望であることを示唆している。

第４節　小括

第３章では、acetamidothiazoleを有する1*H*-imidazol-2-amine誘導体である新規の経口で有効なVAP-1阻害剤を見出した。リード化合物**12**のヒトVAP-1阻害活性を向上させ種差を改善するために、ヒトVAP-1阻害作用に対して重要なアンカーパートと判明しているフェニルグアニジンパートの更なる変換から始めた。最初に、フェニルグアニジン(**128**)の生物学的等価体探索をフラグメント化合物で実施し、引き続き最適化されたフラグメント化合物を**12**のフェニルグアニジン部分と置き換えた。その結果、化合物**161b** (human IC_{50} of 19 nM; rat IC_{50} of 5.1 nM)を見出した。その化合物は、高活性、高選択性であり、経口投与で糖尿病性黄斑浮腫病態モデルに於いて有効性を確認する事ができた。

結論

以上詳述したように、著者は、新規メカニズムによる糖尿病性黄斑浮腫治療薬の創製を目指し、VAP-1 阻害剤の開発研究を行った。以下にその研究成果について記す。

第 1 章：VAP-1 阻害作用を有するチアゾール誘導体、及びその類縁体の SAR 情報を取得した。HTS ヒットである化合物 **1** に基づいた SAR 研究で、鍵ファーマコフォアを同定し、立体的にコンパクトな塩基性部分が VAP-1 の阻害活性に対して重要であることを示した。**1** の SAR 研究の過程で、リード化合物 **12** でのグアニジンパートの重要性を確認した。ヒト VAP-1 のドッキングモデルを作成し **12** のドッキング解析を行い、グアニジンパートが強固な水素結合ネットワークを形成し、チアゾール環の硫黄原子は S-O 相互作用、アミドパートは proton-π 相互作用を形成している事を明らかにした。これらの結果が SAR データを支持していたため、本モデルはドラッグデザインに対して有用なツールになると考えられた。また、グアニジンパート以外にチアゾールアミド部分も、阻害活性に重要なファーマコフォアである事を明らかにした。更に、リード化合物 **12** を用いて皮下投与で *in vivo* 評価を行い、PK-PD の相関性を明らかにし、糖尿病性黄斑浮腫病態モデルに於いて **12** が有効性を示した事から、VAP-1 阻害剤が黄斑浮腫の治療に対して有用である概念実証の取得に成功した。

第 2 章: リード化合物 **12** の課題である種差の改善を目的に誘導体合成を行った。最初に、**12** のヒト VAP-1 阻害活性を向上させるために、ヒトの活性を特異的に有している HTS ヒット化合物 **2** を利用し、**12** とヒト VAP-1 ドッキング結果に基づいたハイブリッドデザインを考えた。そのハイブリッド化合物 **95a** は、**12** と比較してヒトの活性が 7 倍向上したが、一方でラットの活性は減弱した。ラットでの活性低下の原因を、ヒトとラットの VAP-1 シークエンス情報に基づいて考察し、その結果をドラッグデザインに反映させた。その結果、ヒトとラット共に阻害活性が良好な化合物 **95c**(human IC_{50} of 20 nM; rat IC_{50} of 72 nM)を見出すことに成功した。よって、ドッキングモデルから導いたファーマコフォアのハイブリッドの手法、及びヒトとラット間の VAP-1 シークエンス情報に基づいた考察の有用性を示す

ことができた。更に、**95c** を用いて、PK と PD の相関関係を確認する事ができた。本化合物は、糖尿病性黄斑浮腫の治療に対する経口剤としての開発は難しいが、他の投与経路での開発可能性を十分有しており現在探索中である。

第３章：リード化合物 **12** の課題である種差の改善を第２章とは別の手法を用いて行い、acetamidothiazole を有する 1*H*-imidazol-2-amine 誘導体である新規の経口で有効な VAP-1 阻害剤を見出した。リード化合物 **12** のヒト VAP-1 阻害活性を向上させ種差を改善するために、ヒト VAP-1 阻害作用に対して重要なアンカーパートと判明しているフェニルグアニジン部分の更なる変換を行った。最初に、フェニルグアニジン(**128**)の生物学的等価体探索をフラグメント化合物で実施し、引き続き最適化されたフラグメント化合物を **12** のフェニルグアニジン部分と置き換えた。その結果、化合物 **161b** (human IC_{50} of 19 nM; rat IC_{50} of 5.1 nM)を見出すことに成功した。本化合物は、高活性、高選択性であり、経口投与で糖尿病性黄斑浮腫病態モデルに於いて有効性を確認する事ができた。以上の結果より、このフラグメント化合物を用いた最適化法は、効率良く目的の化合物を取得できる有用な方法として実証する事ができた。

以上により、HTS ヒット化合物であるチアゾール誘導体 **1** から、計算化学の手法も取り入れ多面的な方法により、強力な阻害活性を有し他のアミンオキシダーゼに対して高い選択性を有する新規 VAP-1 阻害剤の創製に成功した。本研究により見出された成果は、今後の新たな糖尿病性黄斑浮腫治療薬の開発に有用な知見になると期待される。

謝辞

本論文の作成並びに発表にあたり、御懇切なる御指導を賜り、論文の御校閲を頂きました千葉大学大学院薬学研究院教授　西田篤司博士に謹んで感謝致します。

また、本論文に関して審査および貴重な御教示と御鞭撻を賜りました千葉大学大学院薬学研究院教授　石川 勉博士、濱田康正博士、高山廣光博士、並びに石橋正巳博士に厚く御礼申し上げます。

本研究の機会を与えて頂き、また過分の御便宜と御配慮を賜りましたアステラス製薬株式会社研究本部長　塚本紳一博士、化学研究所所長　渡辺俊博博士，化学研究所室長　冨島昌紀博士に深謝致します。

本研究を遂行するにあたり、終始暖かい御指導、御鞭撻を賜りましたアステラスリサーチテクノロジー株式会社社長　三宅 宏博士、同企画総務部長　大久保 充博士に厚く感謝いたします。

合成研究に於いて御協力頂きましたアステラス製薬株式会社化学研究所　東條 隆博士、森田真正博士、吉原耕生氏に謹んで感謝致します。

薬理評価を行っていただきました同薬理研究所 永島 亮博士に厚く感謝致します。

計算化学で貴重な御助言・御協力を頂きました同化学研究所 森友紋子氏、アステラスビジネスサービス株式会社研究支援センター長　今井啓祐博士に深く感謝致します。

論文作成時に有益な御助言を頂きました、アステラス製薬株式会社化学研究所　永嶋信也博士、川上晋平博士に厚く感謝致します。さらに、各種データを測定してくださいましたアステラス製薬の方々に心より感謝致します。

最後に、私の研究生活を激励し、常に支えてくれました妻、そして娘に深く感謝致します。

実験の部

化合物の合成法

^{1}H NMR spectra were measured with a JEOL EX400 or GX500 spectrometer; chemical shifts are expressed in δ units using tetramethylsilane as the standard (in NMR description: s, singlet; d, doublet; t, triplet; q, quartet, m, multiplet, and br, broad peak). Mass spectra were recorded with a Hitachi M-80 or JEOL JMS-DX300 spectrometer. IR spectra were measured in a HORIBA FT-720 spectrophotometer; the frequencies in the IR spectra are indicated in cm^{-1}. The elemental analyses were performed with a Yanaco MT-5 microanalyzer (C, H, N) and Yokogawa IC-7000S ion chromatographic analyzer (S and halogens) and were within ±0.4% of theoretical values. Melting points were determined with a Büchi B-545 melting point apparatus and left uncorrected. Silica gel column chromatography was performed using Wakogel C-200 or Merck Silica Gel 60. Unless otherwise noted, all commercial reagents and solvents were used without further purification.

第 1 章

(2-Amino-1,3-thiazol-4-yl)methyl acetate hydrochloride (4).

A mixture of 3-chloro-2-oxopropyl acetate (**3**) (5.0 g) and thiourea (2.5 g) in EtOH (25 mL) was refluxed for 4 h. The reaction mixture was cooled to room temperature and the resulting crystalline precipitate was collected by filtration and washed with EtOH (20 mL) to give **4** (3.5 g, 51%) as a white solid. ^{1}H NMR (DMSO-d_6) δ 2.07 (3H, s), 4.92 (2H, s), 6.87 (1H, s); FAB MS m/e $(M+H)^+$ 173.

[2-(Acetylamino)-1,3-thiazol-4-yl]methyl acetate (5).

To a mixture of **4** (56 g) and pyridine (45 g) in CH_2Cl_2 (560 mL) was added acetyl chloride (23 g) over a period of 30 min at 5 ˚C, and the reaction mixture was stirred for 10 min at the same temperature. The reaction mixture was poured into H_2O (500 mL) and extracted with $CHCl_3$ (1 L).

The organic layer was dried over anhydrous Na_2SO_4 and concentrated *in vacuo*. The residual solid was collected by filtration with $^iPr_2O$ to give **5** (47 g, 82%) as a white solid. ^{1}H NMR (DMSO-d_6) δ 2.12 (3H, s), 2.29 (3H, s), 5.08 (2H, s), 6.93 (1H, s); FAB MS m/e $(M+H)^+$ 215.

***N*-[4-(Hydroxymethyl)-1,3-thiazol-2-yl]acetamide (6).**

A mixture of **5** (46 g) and K_2CO_3 (30 g) in MeOH (640 mL) was stirred for 3 h at room temperature. The reaction mixture was concentrated *in vacuo*. The residue was diluted with $CHCl_3$, and the insoluble material was filtered off. The resulting solution was purified by flash column chromatography on silica-gel with MeOH-$CHCl_3$ (1:99) as an eluent and triturated with $^iPr_2O$ to give **6** (35 g, 95%) as a white solid. ^{1}H NMR (DMSO-d_6) δ 2.12 (3H, s), 4.44 (2H, d, J = 5.0 Hz), 5.20 (1H, t, J = 5.0 Hz), 6.88 (1H, s), 12.02 (1H, br s); FAB MS m/e $(M+H)^+$ 173.

***N*-(4-Formyl-1,3-thiazol-2-yl)acetamide (7).**

Compound **6** (2.8 g) was dissolved in MeOH (10 mL) and $CHCl_3$ (200 mL). Then manganese (IV) oxide (28.3 g) was added to the solution under a nitrogen atmosphere. The reaction mixture was stirred at room temperature for 7 h and filtered through a calcite® pad. The filtrate was concentrated *in vacuo*. The resulting solid was washed with Et_2O to give **7** (2.0 g, 73%) as an off-white solid. Mp 195.5–199 ˚C; ^{1}H NMR (DMSO-d_6) δ 2.17 (3H, s), 8.28 (1H, s), 9.79 (1H, s), 12.47 (1H, br s).

***N*-{4-[(Z)-2-(4-Nitrophenyl)ethenyl]-1,3-thiazol-2-yl}acetamide (9).**

To a mixture of 4-nitrobenzyl bromide (**8**) (1.9 g) in DMF (20 mL) was added triphenylphosphine (2.3 g) at 0 ˚C under a nitrogen atmosphere, and the reaction mixture was stirred at room temperature for 2.5 h. Then potassium *tert*-butoxide (1.2 g) and **7** (1.5 g) were added at 0 ˚C, and the mixture was stirred at room temperature for 14 h. The reaction mixture was poured into ice-H_2O and extracted with AcOEt. The organic layer was washed with 1 M HCl, H_2O and brine, dried over anhydrous $MgSO_4$, and concentrated *in vacuo*. The residue was purified by flash column chromatography over silica gel with Hexane-AcOEt (1:1–1:2) as an eluent and triturated with Et_2O

to give **9** (1.6 g, 62%) as a yellow solid. Mp 155–157 ˚C; ^{1}H NMR (DMSO-d_6) δ 2.13 (3H, s), 6.64 (1H, d, J = 12.5 Hz), 6.71 (1H, d, J = 12.5 Hz), 7.18 (1H, s), 7.79 (2H, d, J = 9.0 Hz), 8.17 (2H, d, J = 9.0 Hz), 12.02 (1H, br s); FAB MS m/e $(M+H)^+$ 290.

***N*-(4-(2-(4-Aminophenyl)ethyl)-1,3-thiazol-2-yl)acetamide (10).**

A mixture of **9** (2.0 g) and 10% palladium on carbon (400 mg) in MeOH (25 mL), THF (25 mL) and AcOH (18 mL) was stirred under hydrogen atmosphere (4 atm) at room temperature for 5 h. The reaction mixture was filtered through a celite® pad, and the filtrate was concentrated *in vacuo*. The residue was dissolved in AcOEt. The organic solution was washed with saturated aqueous $NaHCO_3$ and brine, dried over anhydrous $MgSO_4$, and concentrated *in vacuo*. The residue was purified by flash column chromatography over silica gel with Hexane-AcOEt (1:2–0:1) as an eluent and triturated with EtOH-Et_2O to give **10** (540 mg, 30%) as an off-white solid. Mp 102.5–104 ˚C; ^{1}H NMR (DMSO-d_6) δ 2.11 (3H, s), 2.75 (4H, br s), 4.82 (2H, s), 6.46 (2H, d, J = 8.5 Hz), 6.69 (1H, s), 6.83 (2H, d, J = 8.5 Hz), 12.07 (1H, br s); IR (KBr) cm^{-1}: 3438, 3357, 2914, 1658, 1630, 1560, 1516, 1452, 1375, 1333, 1300, 1178, 1001; FAB MS m/e $(M+H)^+$ 262; HRMS (ESI) Calcd for $C_{13}H_{16}N_3OS$ $(M+H)^+$: 262.1014, found: 262.1019.

***N*-(4-(2-(4-(4,5-Dihydro-1,3-thiazol-2-ylamino)phenyl)ethyl)-1,3-thiazol-2-yl)acetamide (1).**

A mixture of **10** (1.8 g), 2-(methylsulfanyl)-4,5-dihydro-1,3-thiazole (918 mg), concentrated HCl (0.57 mL) and 2-methoxyethanol (28 mL) was stirred at 120 ˚C for 10 h. After cooling to room temperature, the reaction mixture was concentrated *in vacuo*. The residue was dissolved in THF-H_2O and made basic with aqueous K_2CO_3. The mixture was extracted with AcOEt. The organic layer was dried over anhydrous $MgSO_4$, and evaporated *in vacuo*. The residue was purified by flash column chromatography over silica gel with $CHCl_3$-MeOH (30:1–20:1) as an eluent and triturated with AcOEt to give **1** (485 mg, 40%) as an off-white solid. Mp 218–219.5 ˚C; ^{1}H NMR (DMSO-d_6) δ 2.11 (3H, s), 2.84 (4H, s), 3.26 (2H, t, J = 7.5 Hz), 3.35 (2H, t, J = 7.5 Hz), 4.02 (1H, br s), 6.71 (1H, br s), 7.05 (2H, d, J = 8.5 Hz), 7.51 (1H, br s), 9.25 (1H, br s), 12.10 (1H, br s); FAB MS m/e

$(M+H)^+$ 347; HRMS (ESI) Calcd for $C_{16}H_{19}N_4OS_2$ $(M+H)^+$: 347.1000, found: 347.1003; Anal. Calcd for $C_{16}H_{18}N_4OS_2$: C, 55.47; H, 5.24; N, 16.17; S, 18.51. Found: C, 55.47; H, 5.28; N, 15.89; S, 18.03.

***N*-(4-{2-[4-(4,5-Dihydro-1H-imidazol-2-ylamino)phenyl]-ethyl}-1,3-thiazol-2-yl)acetamide (11a).**

A mixture of **10** (65 mg), ethyl 2-(methylsulfanyl)-4,5-dihydro-1H-imidazole-1-carboxylate (56 mg), AcOH (0.1 mL) and EtOH (0.9 mL) was stirred at 65 ˚C for 6 h and then refluxed for 5 h. The reaction mixture was poured into AcOEt (5 mL) and saturated aqueous $NaHCO_3$. The precipitated solid was filtered, and the solid was dissolved in 50% MeOH-$CHCl_3$. Insoluble materials were filtered, and the filtrate was concentrated *in vacuo*. The resulting solid was collected and washed with AcOEt to give **11a** (40 mg, 49%) as an off-white solid. ^{1}H NMR (DMSO-d_6) δ 2.11 (3H, s), 2.72 (4H, s), 3.33 (4H, s), 6.73 (1H, s), 6.85–7.08 (4H, m); IR (KBr) cm^{-1}: 1662, 1608, 1541, 1510, 1435, 1392, 1321, 1282, 1227, 1200, 1132, 1095, 1022; FAB MS m/e $(M+H)^+$ 330; HRMS (ESI) Calcd for $C_{16}H_{20}N_5OS$ $(M+H)^+$: 330.1389, found: 330.1390.

***N*-[4-(2-{4-[(Iminomethyl)amino]phenyl}ethyl)-1,3-thiazol-2-yl]acetamide (11b).**

Compound **10** (150 mg) was dissolved in MeOH (3 mL) under a nitrogen atmosphere. Then isopropyl formimidate hydrochloride (567 mg) was added to the solution at room temperature, and the reaction mixture was stirred at room temperature for 66 h. The precipitate was filtered off, and the filtrate was concentrated *in vacuo*. The residue was purified by preparative thin-layer chromatography (NH silicagel, Fuji Silysia Chemical Ltd.) with $CHCl_3$-MeOH (20:1) as an eluent to give **11b** (17 mg, 10%) as an off-white solid. Mp 225–227 ˚C; ^{1}H NMR (DMSO-d_6) δ 2.11 (3H, s), 2.86 (4H, s), 6.71 (1H, s), 7.10 (1H, d, J = 2.0 Hz), 7.13 (2H, d, J = 8.5 Hz), 7.47 (2H, d, J = 8.5 Hz), 8.23 (1H, d, J = 2.0 Hz), 10.09 (1H, br s), 12.07 (1H, br s); FAB MS m/e $(M+H)^+$ 289; HRMS (ESI) Calcd for $C_{14}H_{17}N_4OS$ $(M+H)^+$: 289.1123, found: 289.1105.

***N*-{4-[2-(4-{[Amino(imino)methyl]-amino}phenyl)ethyl]-1,3-thiazol-2-yl}acetamide (12).**

To a suspension of **10** (26.0 g) in EtOH (500 mL) was added 4 M HCl-AcOEt (25 mL) and cyanamide (6.3 g). The mixture was refluxed for 26 h. The reaction mixture was cooled to room temperature and poured into a mixture of AcOEt (500 mL) and saturated aqueous $NaHCO_3$ (500 mL). The resulting precipitate was collected by filtration and washed with H_2O (300 mL) and EtOH (300 mL) to give **12** (18.0 g, 60%) as a white solid. ^{1}H NMR (DMSO-d_6) δ 2.10 (3H, s), 2.85 (4H, s), 6.79 (1H, s), 6.83 (2H, d, J = 7 Hz), 7.10 (2H, d, J = 7 Hz); ^{13}C NMR (DMSO-d_6) δ 22.6, 32.4, 34.0, 107.7, 124.5, 129.6, 132.9, 133.0, 139.8, 149.8, 157.6, 168.5; IR (KBr) cm^{-1}: 3394, 1666, 1635, 1597, 1545, 1510, 1444, 1375, 1294, 1219, 1200, 1132, 1105, 1039, 1011; FAB MS m/e $(M+H)^+$ 304; HRMS (ESI) Calcd for $C_{14}H_{18}N_5OS$ $(M+H)^+$: 304.1232, found: 304.1230.

1-{4-[2-(2-Amino-1,3-thiazol-4-yl)ethyl]phenyl}guanidine dihydrochloride (13).

A mixture of **12** (200 mg) and 6 M HCl (2.2 mL) was heated at 90 ˚C for 6 h. After cooling to room temperature, the mixture was concentrated *in vacuo*. The residual solid was washed with MeCN to give **13** (153 mg, 70%) as an off-white solid. ^{1}H NMR (DMSO-d_6) δ 2.79–2.97 (4H, m), 6.51 (1H, s), 7.16 (2H, d, J = 8.5 Hz), 7.32 (2H, d, J = 8.5 Hz), 7.53 (3H, s), 9.23 (2H, s), 10.09 (1H, s); IR (KBr) cm^{-1}: 3479, 1660, 1628, 1604, 1579, 1516, 1442, 1404, 1252, 1215, 1140, 1026; FAB MS m/e $(M+H)^+$ 262; HRMS (ESI) Calcd for $C_{12}H_{16}N_5S$ $(M+H)^+$: 262.1126, found: 262.1128; Anal. Calcd for $C_{12}H_{15}N_5S\cdot 2HCl$: C, 43.12; H, 5.13; N, 20.95; S, 9.59; Cl, 21.21. Found: C, 42.60; H, 5.15; N, 20.56; S, 9.33; Cl, 21.39.

***N*-{4-[2-(4-{[(Benzoylamino)carbonothioyl]amino}phenyl)ethyl]-1,3-thiazol-2-yl}acetamide (14).**

To a mixture of **10** (300 mg) and acetone (5 mL) was added benzoyl isothiocyanate (187 mg) in an ice bath, and the mixture was refluxed for 2 h and cooled to 0 ˚C. The precipitated solid was filtered and washed with ice-cold acetone to give **14** (359 mg, 74%) as a yellow solid. ^{1}H NMR ($CDCl_3$) δ 2.25 (3H, s), 2.90–3.05 (4H, m), 6.51 (1H, s), 7.21 (2H, d, J = 7 Hz), 7.50–7.70 (5H, m), 7.89 (2H, d,

J = 7 Hz), 9.03 (1H, s), 9.12 (1H, s); FAB MS m/e $(M+H)^+$ 425.

_N_-[4-(2-{4-[(Aminocarbonothioyl)amino]phenyl}ethyl)-1,3-thiazol-2-yl]acetamide (15).

A mixture of **14** (200 mg), 6 M NaOH (0.19 mL) and EtOH (2 mL) was stirred at 60 ˚C for 2 h. The reaction mixture was cooled to room temperature and neutralized with 1 M HCl (1.2 mL). The precipitated solid was filtered and washed with water to give **15** (120 mg, 49%) as an off-white solid. Mp 191.5–192.5 ˚C; ^{1}H NMR (DMSO-d_6) δ 2.11 (3H, s), 2.88 (4H, s), 6.75 (1H, s), 7.15 (2H, d, *J* = 7 Hz), 7.27 (2H, d, *J* = 7 Hz), 9.60 (1H, s); IR (KBr) cm^{-1}: 3384, 3303, 1685, 1635, 1545, 1508, 1473, 1363, 1292; FAB MS m/e $(M+H)^+$ 321; HRMS (ESI) Calcd for $C_{14}H_{17}N_4OS_2$ $(M+H)^+$: 321.0844, found: 321.0841.

Methyl _N_-{4-[2-(2-acetamido-1,3-thiazol-4-yl)ethyl]phenyl}carbamimidothioate hydroiodide (16).

A mixture of **15** (123 mg), MeI (0.029 mL) and MeOH (2.5 mL) was refluxed for 3 h. The reaction mixture was concentrated *in vacuo*. The residue was diluted with AcOEt and stirred for 30 min. The precipitated solid was filtered and washed with AcOEt to give **16** (148 mg, 83%) as a pale yellow solid. ^{1}H NMR (DMSO-d_6) δ 2.13 (3H, s), 2.68 (3H, s), 2.87–3.05 (4H, m), 6.75 (1H, s), 7.24 (2H, d, *J* = 7 Hz), 7.35 (2H, d, *J* = 7 Hz); IR (KBr) cm^{-1}: 2958, 1668, 1645, 1593, 1556, 1539, 1512, 1456, 1417, 1375, 1329, 1286, 1225, 1136; FAB MS m/e $(M+H)^+$ 335; HRMS (ESI) Calcd for $C_{15}H_{19}N_4OS_2$ $(M+H)^+$: 335.1000, found: 335.1001; Anal. Calcd for $C_{15}H_{18}N_4OS_2 \cdot HI$: C, 38.96; H, 4.14; N, 12.12; S, 13.87; I, 27.45. Found: C, 38.72; H, 4.22; N, 11.84; S, 13.81; I, 26.85.

_N_-{4-[2-(4-{[(Ethylamino)(imino)methyl]amino}phenyl)ethyl]-1,3-thiazol-2-yl}acetamide (17a).

A mixture of **16** (50 mg), ethylamine (0.056 mL) and EtOH (1 mL) was stirred at room temperature for 20 h. The precipitated solid was filtered and washed with EtOH to give **17a** (15 mg, 42%) as an off-white solid. ^{1}H NMR (DMSO-d_6) δ 1.13 (3H, t, *J* = 6 Hz), 2.11(3H, s), 2.70–3.00 (6H, m), 6.70 (1H, s), 6.77 (2H, d, *J* = 7 Hz), 7.17 (2H, d, *J* = 7 Hz); FAB MS m/e $(M+H)^+$ 332.

***N*-(4-{2-[4-(*N'*-Isopropylcarbamimidamido)phenyl]ethyl}-1,3-thiazol-2-yl)acetamide (17b).**

A mixture of compound **16** (47 mg), EtOH (0.93 mL) and 2-aminopropane (119 mg) was refluxed for 16 h under a nitrogen atmosphere. After cooling to room temperature, the mixture was concentrated *in vacuo*. The crude oil was purified by flash column chromatography over NH_2-silica gel (Fuji Silysia Chemical Ltd.) with $CHCl_3$-MeOH (50:1-30:1) as an eluent. The yellow oil was solidified with MeCN to give **17b** (15 mg, 42%) as an off-white solid. ^{1}H NMR (DMSO-d_6) δ 1.08 (3H, s), 1.10 (3H, s), 2.11 (3H, s), 2.83 (4H, s), 3.77–3.88 (1H, m), 6.65 (2H, d, J = 8.5 Hz), 6.72 (1H, s), 7.00 (2H, d, J = 8.5 Hz); FAB MS m/e $(M+H)^+$ 346; HRMS (ESI) Calcd for $C_{17}H_{24}N_5OS$ $(M+H)^+$: 346.1702, found: 346.1701.

***N*-(4-{2-[4-(*N*,*N*-Dimethylcarbamimidamido)phenyl]ethyl}-1,3-thiazol-2-yl)acetamide (17c).**

Compound **17c** was prepared from **16** and 2 M dimethylamine in THF solution according to the same procedure as that of compound **17b**. Compound **17c** was obtained as an off-white solid (59% yield). ^{1}H NMR (DMSO-d_6) δ 2.11 (3H, s), 2.84 (4H, s), 2.85 (6H, s), 6.61 (2H, d, J = 8.5 Hz), 6.72 (1H, s), 7.02 (2H, d, J = 8.5 Hz); IR (KBr) cm^{-1}: 3465, 3371, 3111, 1670, 1628, 1576, 1560, 1498, 1431, 1406, 1371, 1302, 1284, 1136, 1014; FAB MS m/e $(M+H)^+$ 332; HRMS (ESI) Calcd for $C_{16}H_{22}N_5OS$ $(M+H)^+$: 332.1545, found: 332.1544.

{[2-(Acetylamino)-1,3-thiazol-4-yl]methyl}(triphenyl)phosphonium chloride (19).

To a solution of N-[4-(chloromethyl)-1,3-thiazol-2-yl]acetamide (**18**) (23.6 g) in toluene (200 mL) and MeCN (80 mL) was added triphenylphosphine (35.7 g) at 25 ˚C. The mixture was stirred at 130 ˚C for 12 h. The resulting precipitate was collected by filtration and washed with ^{i}Pr$_2$O to give **19** (35.7 g, 64%) as a colorless powder. ^{1}H NMR (DMSO-d_6) δ 2.11 (3H, s), 5.25 (2H, d, J = 15.3 Hz), 6.86 (1H, d, J = 3.8 Hz), 7.68-7.92 (15H, m), 12.06 (1H, s).

4-[2-(1,3-Dioxo-1,3-dihydro-2H-isoindol-2-yl)ethyl]benzaldehyde (21).

To a solution of phthalimide potassium salt (46.2 g) in DMF (300 mL) was added 4-(2-bromoethyl)benzaldehyde (**20**) (40.9 g) in DMF (50 mL) dropwise at 60 ˚C, and the mixture was stirred for 2 h. The reaction mixture was cooled to 20 ˚C and then poured into H_2O (1.5 L). The resulting precipitate was collected by filtration to give a yellow solid. The solid was dissolved in $CHCl_3$ (250 mL) and insoluble material was removed by filtration. The filtrate was concentrated *in vacuo*. The residue was washed with Et_2O and collected by filtration to give **21** (19.7 g, 37%) as an off-white solid. ^{1}H NMR (DMSO-d_6) δ 3.04 (2H, t, J = 7Hz), 3.88 (2H, t, J = 7 Hz), 7.44 (2H, d, J = 8.5 Hz), 7.75–7.89 (6H, m), 9.94 (1H, s); FAB MS m/e $(M+H)^+$ 280.

***N*-[4-((E)-2-{4-[2-(1,3-Dioxo-1,3-dihydro-2H-isoindol-2-yl)ethyl]phenyl}vinyl)-1,3-thiazol-2-yl]acetamide (22).**

Potassium *tert*-butoxide (12.8 g) was added to a mixture of compound **19** (46.9 g) and DMF (190 mL) at 0 ˚C under a nitrogen atmosphere. The reaction mixture was stirred at 0 ˚C for 15 min, and **21** (19.28 g) was added to the mixture at 0 ˚C. The reaction mixture was stirred at room temperature for 2 h and poured into H_2O. The resulting precipitate was collected by filtration to give a crude brown solid. The brown solid was washed with ^{i}Pr$_2$O-MeCN (1:1) and then MeCN to give **22** (24.9 g, 86%) as a beige solid. ^{1}H NMR (DMSO-d_6) δ 2.15 (3H, s), 2.94 (2H, t, J = 7.1 Hz), 3.83 (2H, t, J = 7.1 Hz), 7.12 (1H, d, J = 15.8 Hz), 7.14 (1H, d, J = 15.8 Hz), 7.16 (1H, s), 7.19 (2H, d, J = 8 Hz), 7.44 (2H, d, J = 8.4 Hz), 7.8–7.88 (4H, m), 12.22 (1H, s); FAB MS m/e $(M+H)^+$ 418.

***N*-[4-(2-{4-[2-(1,3-Dioxo-1,3-dihydro-2H-isoindol-2-yl)ethyl]phenyl}ethyl)-1,3-thiazol-2-yl]acetamide (23).**

A mixture of **22** (24.9 g) and 10% palladium on carbon (50% wet) (24.4 g) in MeOH (80 mL), DMF (800 mL) and AcOH (8 mL) was stirred under hydrogen atmosphere (4 atm) at room temperature for 16 h. The reaction mixture was filtered through a celite® pad, and the filtrate was concentrated *in vacuo*. The residue was washed with ^{i}Pr$_2$O (200 mL) and purified by flash column chromatography over silica gel with $CHCl_3$-AcOEt (1:1) as an eluent, and triturated with ^{i}Pr$_2$O (200 mL) to give **23**

(17.9 g, 71%) as an off-white solid. ^{1}H NMR (DMSO-d_6) δ 2.11 (3H, s), 2.78–2.92 (6H, m), 3.79 (2H, t, J = 7.3 Hz), 6.66 (1H, s), 7.08 (2H, d, J = 8.9 Hz), 7.1 (2H, d, J = 8.8 Hz), 7.79–7.89 (4H, m), 12.08 (1H, s); FAB MS m/e $(M+H)^+$ 420, $(M+Na)^+$ 442.

***N*-(4-{2-[4-(2-Aminoethyl)phenyl]ethyl}-1,3-thiazol-2-yl)acetamide (24)**

To a solution of **23** (2.1 g) in MeCN (20 mL) was added hydrazine monohydrate (2.4 mL), and the mixture was stirred at 50 ˚C for 2 h. Volatiles were evaporated. To the mixture was added $CHCl_3$ (10 mL), and an insoluble material was removed by filtration. The residue was purified by flash column chromatography over NH_2-silica gel with $CHCl_3$-MeOH (10:0–10:2) as an eluent to give **24** (1.1 g, 80%) as a pale yellow solid. ^{1}H NMR (DMSO-d_6) δ 2.11 (3H, s), 2.58 (2H, t, J = 7.3 Hz), 2.72 (2H, t, J = 7.1 Hz), 2.81–2.94 (4H, m), 6.73 (1H, s), 7.08 (2H, d, J = 8.4 Hz), 7.11 (2H, d, J = 8.4 Hz); FAB MS m/e $(M+H)^+$ 290.

2-Bromomalonaldehyde (26).

A solution of 1,1,3,3-tetramethoxypropane (**25**) (10 g) and concentrated HCl (0.43 mL) in H_2O (11 mL) was stirred at room temperature for 10 min. Br_2 (3.1 mL) was added dropwise to the solution at room temperature for more than 50 min. The reaction mixture was stirred at room temperature for 20 min and concentrated *in vacuo*. The residual solid was washed with H_2O to give **26** (3.6 g, 39%) as a yellow solid. Mp 147–148 ˚C; ^{1}H NMR ($CDCl_3$) δ 4.73–4.80 (1H, m), 8.47 (2H, br s); FAB MS m/e $(M-H)^-$ 149.

***N*-(5-Formyl-1,3-thiazol-2-yl)acetamide (28).**

To a solution of *N'*-acetylcarbamimidothioic acid (**27**) (2.7 g) in acetone (20 mL) was added **26** (3.5 g) under reflux. The reaction mixture was refluxed for an hour under a nitrogen atmosphere and cooled to room temperature. The precipitate was collected *in vacuo*. The solid was washed with H_2O and acetone, and purified by flash column chromatography over silica gel with $CHCl_3$-MeOH (20:1) as an eluent to give **28** (1.2 g, 31%) as an off-white solid. Mp 235–235.5 ˚C; ^{1}H NMR (DMSO-d_6) δ

2.21 (3H, s), 8.41 (1H, s), 9.95 (1H, s), 12.71 (1H, br s); FAB MS m/e $(M-H)^-$ 169.

***N*-{5-[(*Z*)-2-(4-Nitrophenyl)ethenyl]-1,3-thiazol-2-yl}acetamide (29).**

Compound **29** was prepared from **28** and 4-nitrobenzyl bromide (**8**) according to the same procedure as that of compound **9**. Compound **29** was obtained as a yellow solid (24% yield). Mp 221–223 ˚C; ^{1}H NMR (DMSO-d_6) δ 2.07 (3H, s), 6.63 (1H, d, J = 12.0 Hz), 6.92 (1H, d, J = 12.0 Hz), 7.55 (1H, s), 7.62 (2H, d, J = 9.0 Hz), 8.24 (2H, d, J = 9.0 Hz), 12.16 (1H, br s); FAB MS m/e $(M+H)^+$ 290.

***N*-{5-[2-(4-Aminophenyl)ethyl]-1,3-thiazol-2-yl}acetamide (30).**

A mixture of **29** (1.0 g) and 10% palladium on carbon (1.0 g) in AcOEt (100 mL) and DMF (20 mL) was stirred under hydrogen atmosphere (4 atm) at room temperature for 4 h. The reaction mixture was filtered through a celite® pad, and the filtrate was concentrated *in vacuo*. The residue was purified by flash column chromatography over silica gel with $CHCl_3$-MeOH (30:1–20:1) as an eluent and triturated with Et_2O to give **30** (241 mg, 27%) as an off-white solid. Mp 218–219.5 ˚C; ^{1}H NMR (DMSO-d_6) δ 2.09 (3H, s), 2.70 (2H, t, J = 7.5 Hz), 2.92 (2H, t, J = 7.5 Hz), 4.85 (2H, s), 6.47 (2H, d, J = 8.5 Hz), 6.86 (2H, d, J = 8.5 Hz), 7.08 (1H, s), 11.86 (1H, br s); FAB MS m/e $(M+H)^+$ 262.

Di-*tert*-butyl {[(4-{2-[2-(Acetylamino)-1,3-thiazol-5-yl]ethyl}phenyl)amino]-methylidene}-biscarbamate (32).

To a solution of **30** (100 mg) in DMF (1 mL) and THF (2 mL) was added *N*,*N*'-bis(*tert*-butoxycarbonyl)-1H-pyrazole-1-carboxamidine (**31**) (119 mg) under a nitrogen atmosphere, and the mixture was stirred at 50 ˚C for 5.5 h. After cooling to room temperature, the reaction mixture was concentrated *in vacuo*. The residue was purified by preparative silica gel column chromatography with hexane-AcOEt (1:2) as an eluent to give **32** (94 mg, 49%) as a colorless solid. Mp 203–205 ˚C; ^{1}H NMR (DMSO-d_6) δ 1.40 (9H, s), 1.51 (9H, s), 2.10 (3H, s), 2.87 (2H, t, J = 7.5 Hz), 3.02 (2H, t, J = 7.5 Hz), 7.11 (1H, s), 7.21 (2H, d, J = 8.5 Hz), 7.45 (2H, d, J =

8.5 Hz), 9.96 (1H, br s), 11.43 (1H, br s), 11.88 (1H, br s); FAB MS m/e $(M+H)^+$ 504.

_N_-{5-[2-(4-{[Amino(imino)methyl]amino}phenyl)ethyl]-1,3-thiazol-2-yl}acetamide hydrochloride (33).

A mixture of **32** (86 mg) and 4 M HCl-dioxane (2 mL) was stirred at room temperature for 8 h under a nitrogen atmosphere. The solvent was removed *in vacuo*. The residue was washed with AcOEt to give **33** (60 mg, 103%) as a colorless solid. Mp 105–107 ˚C; ^{1}H NMR (DMSO-d_6) δ 2.11 (3H, s), 2.91 (2H, t, J = 7.5 Hz), 3.04 (2H, t, J = 7.5 Hz), 7.14 (1H, s), 7.14 (2H, d, J = 8.5 Hz), 7.32 (2H, d, J = 8.5 Hz), 7.46 (3H, br s), 9.89 (1H, s), 11.95 (1H, br s); IR (KBr) cm^{-1}: 3411, 3257, 3186, 3086, 1709, 1670, 1631, 1608, 1577, 1558, 1514, 1439, 1369, 1338, 1300, 1275, 1257, 1223; FAB MS m/e $(M+H)^+$ 304; HRMS (ESI) Calcd for $C_{14}H_{18}N_5OS$ $(M+H)^+$: 304.1232, found: 304.1233.

A mixture of _N_-{4-[(_E_)-2-(4-Nitrophenyl)vinyl]phenyl}acetamide and _N_-{4-[(_Z_)-2-(4-Nitrophenyl)vinyl]phenyl}acetamide (35a).

Compound **35a** was prepared from **34a** and 4-nitrobenzyl bromide (**8**) according to the same procedure as that of compound **9**. Compound **35a** was obtained as a pale brown wax (50% yield, E : Z =2 : 5). ^{1}H NMR (DMSO-d_6) δ 2.03 (3Hx5/7, s), 2.07 (3Hx2/7, s), 6.65 (1Hx5/7, d, J = 12.5 Hz), 6.79 (1Hx5/7, d, J = 12.5 Hz), 7.16 (2Hx5/7, d, J = 9.0 Hz), 7.30 (1Hx2/7, d, J = 16.5 Hz), 7.47 (1Hx2/7, d, J = 16.5 Hz), 7.49 (2Hx5/7, d, J = 9.0 Hz), 7.61 (2Hx2/7, d, J = 9.0 Hz), 7.64 (2Hx2/7, d, J = 9.0 Hz), 7.83 (2Hx2/7, d, J = 9.0 Hz), 7.85 (2Hx5/7, d, J = 9.0 Hz), 8.13 (2Hx5/7, d, J = 9.0 Hz), 8.22 (2Hx2/7, d, J = 9.0 Hz); FAB MS m/e $(M+H)^+$ 283.

A mixture of _N_-{3-[(_E_)-2-(4-Nitrophenyl)vinyl]phenyl}acetamide and _N_-{3-[(_Z_)-2-(4-Nitrophenyl)vinyl]phenyl}acetamide (35b).

Compound **35b** was prepared from **34b** and 4-nitrobenzyl bromide (**8**) according to the same procedure as that of compound **9**. Compound **35b** was obtained as a yellow oil (74% yield, E : Z =2 : 3). ^{1}H NMR (DMSO-d_6) δ 1.99 (3Hx3/5, s), 2.07 (3Hx2/5, s), 6.74 (1Hx3/5, d, J = 12.5 Hz), 6.85

(1Hx3/5, d, *J* = 12.5 Hz), 6.86 (1Hx3/5, d, *J* = 7.5 Hz), 7.21 (1Hx3/5, t, *J* = 7.5 Hz), 7.27 (1Hx2/5, d, *J* = 16.5 Hz), 7.34 (1Hx2/5, t, *J* = 7.5 Hz), 7.37 (1Hx2/5, t, *J* = 7.5 Hz), 7.47 (2Hx3/5, d, *J* = 8.5 Hz), 7.47–7.90 (2H, m), 7.51 (1Hx2/5, d, *J* = 16.5 Hz), 7.90 (2Hx2/5, d, *J* = 8.5 Hz), 8.12 (2Hx3/5, d, *J* = 8.5 Hz), 8.23 (2Hx2/5, d, *J* = 8.5 Hz), 9.87 (1Hx3/5, s), 10.01 (1Hx2/5, s); FAB MS m/e $(M+H)^+$ 283.

***N*-{4-[2-(4-Aminophenyl)ethyl]phenyl}acetamide (36a).**

A mixture of **35a** (1.7 g) and 10% palladium on carbon (641 mg) in MeOH (34 mL) and THF (17 mL) was stirred at room temperature for 4 h under hydrogen atmosphere (3 atm). The mixture was filtered through a celite® pad, and the filtrate was concentrated *in vacuo*. The residual solid was washed with MeCN to give **36a** (743 mg, 49%) as a brown solid. ^{1}H NMR (DMSO-d_6) δ 2.01 (3H, s), 2.75 (4H, s), 6.80 (2H, d, *J* = 8.5 Hz), 7.03 (2H, d, *J* = 8.5 Hz), 7.09 (2H, d, *J* = 8.5 Hz), 7.46 (2H, d, *J* = 8.5 Hz), 7.53 (2H, brs), 9.88 (1H, s); FAB MS m/e $(M+H)^+$ 255.

***N*-{3-[2-(4-Aminophenyl)ethyl]phenyl}acetamide (36b).**

Compound **36b** was prepared from **35b** according to the same procedure as that of compound **36a**. Compound **36b** was obtained as an off-white amorphous (93% yield). ^{1}H NMR (DMSO-d_6) δ 2.02 (3H, s), 2.65–2.78 (4H, m), 5.38 (2H, br s), 6.5 3(2H, d, *J* = 8.5 Hz), 6.86 (1H, d, *J* = 7.5 Hz), 6.89 (2H, d, *J* = 8.5 Hz), 7.16 (1H, t, *J* = 7.5 Hz), 7.38–7.44 (2H, m), 9.85 (1H, s); IR (KBr) cm^{-1}: 3369, 3294, 2922, 1678, 1610, 1556, 1518, 1487, 1450, 1417, 1371, 1319, 1282, 1257; FAB MS m/e $(M+H)^+$ 255.

***N*-{4-[2-(4-Carbamimidamidophenyl)ethyl]phenyl}acetamide hydrochloride (37a).**

To a solution of **36a** (200 mg) in DMF (2 mL) and THF (4 mL) was added *N*,*N*'-bis(*tert*-butoxycarbonyl)-1H-pyrazole-1-carboxamidine (**31**) (366 mg) under a nitrogen atmosphere, and the mixture was stirred at 60 ˚C for 7 h. After cooling to room temperature, the mixture was concentrated *in vacuo*. The residue was purified by flash column chromatography over

silica gel with $CHCl_3$-MeOH (40:1–20:1) as an eluent to give *tert*-butyl {(*Z*)-({4-[2-(4-acetamidophenyl)ethyl]phenyl}amino)[(*tert*-butoxycarbonyl)amino]methylene}carba mate (276 mg) as a colorless amorphous. A mixture of di-Boc compound (276 mg) and 4 M HCl-dioxane (2.8 mL) was stirred at room temperature for 14 h under a nitrogen atmosphere. The solvent was removed *in vacuo*. The residue was washed with MeCN to give **37a** (82 mg, 45%) as a colorless solid. ^{1}H NMR (DMSO-d_6) δ 2.02 (3H, s), 2.78–2.91 (4H, m), 7.13 (2H, d, J = 8.5 Hz), 7.14 (2H, d, J = 8.5 Hz), 7.29 (2H, d, J = 8.5 Hz), 7.41 (3H, br s), 7.48 (2H, d, J = 8.5 Hz), 9.82 (1H, s), 9.90 (1H, s); IR (KBr) cm^{-1}: 2935, 2918, 2854, 1668, 1626, 1599, 1572, 1537, 1514, 1454, 1410, 1371, 1319, 1261, 1140; FAB MS m/e $(M+H)^+$ 297; HRMS (ESI) Calcd for $C_{17}H_{21}N_4O$ $(M+H)^+$: 297.1715, found: 297.1714; Anal. Calcd for $C_{17}H_{20}N_4O\cdot1.3HCl\cdot H_2O\cdot0.8C_2H_3N$: C, 56.61; H, 6.56; N, 17.04; Cl, 11.68. Found: C, 56.61; H, 6.37; N, 17.37; Cl, 11.75.

***N*-{3-[2-(4-carbamimidamidophenyl)ethyl]phenyl}acetamide hydrochloride (37b).**

Compound **37b** was prepared from **36b** and *N*,*N*'-bis(*tert*-butoxycarbonyl)-1H-pyrazole-1 -carboxamidine (**31**) according to the same procedure as that of compound **37a**. Compound **37b** was obtained as a colorless solid (48% yield). ^{1}H NMR (DMSO-d_6) δ 2.02 (3H, s), 2.79–2.93 (4H, m), 6.92 (1H, d, J = 7.5 Hz), 7.14 (2H, d, J = 8.5 Hz), 7.18 (1H, t, J = 7.5 Hz), 7.30 (2H, d, J = 8.5 Hz), 7.36 (1H, d, J = 7.5 Hz), 4.42 (3H, br s), 7.47 (1H, s), 9.82 (1H, s), 9.92 (1H, s); IR (KBr) cm^{-1}: 3411, 2962, 1670, 1635, 1608, 1595, 1552, 1510, 1496, 1441, 1419, 1375, 1319, 1257, 1092; FAB MS m/e $(M+H)^+$ 297; HRMS (ESI) Calcd for $C_{17}H_{21}N_4O$ $(M+H)^+$: 297.1715, found: 297.1711; Anal. Calcd for $C_{17}H_{20}N_4O\cdot1.1HCl\cdot0.4H_2O$: C, 59.41; H, 6.42; N, 16.30; Cl, 11.35. Found: C, 59.57; H, 6.40; N, 16.67; Cl, 11.04.

Ethyl 2-amino-1,3-thiazole-4-carboxylate hydrobromide (39).

A mixture of **38** (100 g), thiourea (39 g) and EtOH (500 mL) was refluxed for 2 h. The reaction mixture was concentrated *in vacuo*. The crystalline residue was collected and washed with AcOEt to give **39** (116 g, 131%) as a pale yellow solid. ^{1}H NMR (DMSO-d_6) δ 1.28 (3H, t, J = 7 Hz), 4.26 (2H,

q, *J* = 7 Hz), 7.60 (1H, s).

Ethyl 2-(*iso*-butyrylamino)-1,3-thiazole-4-carboxylate (40a).

To a mixture of **39** (2.0 g), pyridine (1.3 mL) and CH_2Cl_2 (20 mL) was added *i*-butyryl chloride (0.91 mL) in an ice-water bath, and the mixture was stirred for 30 min. To the mixture was then added saturated aqueous $NaHCO_3$ (30 mL) and the organic layer was separated, dried over Na_2SO_4 and concentrated *in vacuo*. The crystalline residue was collected and washed with AcOEt to give **40a** (1.3 g, 70%) as an off-white solid. ^{1}H NMR ($CDCl_3$) δ 1.30 (6H, d, *J* = 7 Hz), 1.40 (3H, t, *J* = 7 Hz), 2.57–2.73 (1H, m), 4.41 (2H, q, *J* = 7 Hz), 7.83 (1H, s), 8.98 (1H, s); FAB MS m/e $(M+H)^+$ 243.

Ethyl 2-{[(benzyloxy)carbonyl]amino}-1,3-thiazole-4-carboxylate (40b).

To a mixture of **39** (5.0 g), pyridine (3.4 mL) and CH_2Cl_2 (50 mL) was added benzyloxycarbonyl chloride (3.1 mL) in an ice-water bath, and the reaction mixture was stirred at room temperature for an hour. The mixture was washed with saturated aqueous $NaHCO_3$ (30 mL), dried over anhydrous Na_2SO_4 and concentrated *in vacuo*. The crystalline residue was collected and washed with $^iPr_2O$ to give **40b** (5.1 g, 84%) as an off-white solid. ^{1}H NMR ($CDCl_3$) δ 1.48 (3H, t, *J* = 7 Hz), 4.38 (2H, q, *J* = 7 Hz), 5.27 (2H, s), 7.36–7.44 (5H, m), 7.82 (1H, s); FAB MS m/e $(M+H)^+$ 307.

***N*-[4-(Hydroxymethyl)-1,3-thiazol-2-yl]-2-methylpropanamide (41a).**

To a mixture of **40a** (1.4 g) and THF (28 mL) was added lithium borohydride (252 mg) portionwise, and the mixture was refluxed for 6 h. The reaction mixture was cooled to 0 ˚C, quenched with MeOH (5 mL) and concentrated *in vacuo*. The residue was suspended with 10% MeOH-$CHCl_3$ (100 mL), and the insoluble materials were filtered. The filtrate was purified by flash column chromatography on silica-gel with 5% MeOH-$CHCl_3$ as an eluent. The crystalline residue was collected and washed with $^iPr_2O$ to give **41a** (1.0 g, 86%) as an off-white solid. ^{1}H NMR ($CDCl_3$) δ 1.32(6H, d, *J* = 5 Hz), 2.58–2.73 (1H, m), 4.68 (2H, s), 6.82 (1H, s); FAB MS m/e $(M+H)^+$ 200.

Benzyl 4-(hydroxymethyl)-1,3-thiazol-2-ylcarbamate (41b).

Compound **41b** was prepared from **40b** and lithium borohydride according to the same procedure as that of compound **41a**. Compound **41b** was obtained as an off-white solid (93% yield). ^{1}H NMR ($CDCl_3$) δ 4.56 (2H, s), 5.27 (2H, s), 6.80 (1H, s), 7.30–7.46 (5H, m); FAB MS m/e $(M+H)^+$ 265.

***N*-(4-Formyl-1,3-thiazol-2-yl)-2-methylpropanamide (42a).**

A mixture of **41a** (520 mg), manganese (IV) oxide (2.26 g), MeOH (0.5 mL) and $CHCl_3$ (5 mL) was stirred at room temperature for 18 h. The reaction mixture was filtered through a celite® pad, and the filtrate was concentrated *in vacuo*. The crystalline residue was collected and washed with $^iPr_2O$ to give **42a** (365 mg, 71%) as a yellow solid. ^{1}H NMR ($CDCl_3$) δ 1.13 (6H, d, J = 5 Hz), 2.60–2.77 (1H, m), 7.86 (1H, s); FAB MS m/e $(M+H)^+$ 199.

Benzyl 4-formyl-1,3-thiazol-2-ylcarbamate (42b).

Compound **42b** was prepared from **41b** and manganese (IV) oxide according to the same procedure as that of compound **42a** and obtained as an off-white solid (73% yield). ^{1}H NMR ($CDCl_3$) δ 5.29 (2H, s), 7.35–7.45 (5H, m), 7.81 (1H, s), 9.80 (1H, s); FAB MS m/e $(M+H)^+$ 263.

A mixture of 2-methyl-*N*-{4-[(*E*)-2-(4-nitrophenyl)ethenyl]-1,3-thiazol-2-yl}propanamide and 2-methyl-*N*-{4-[(*Z*)-2-(4-nitrophenyl)ethenyl]-1,3-thiazol-2-yl}propanamide (43a).

Compound **43a** was prepared from **42a** and 4-nitrobenzyl bromide (**8**) according to the same procedure as that of compound **9** and obtained as a yellow solid (64% yield, E : Z =1 : 2). ^{1}H NMR ($CDCl_3$) δ (ppm): 1.25 (6x2/3H, d, J = 5 Hz), 1.30 (6x1/3H, d, J = 5 Hz), 2.50–5.70 (1H, m), 6.63 (2Hx2/3H, s), 6.79 (1Hx2/3H, s), 6.97 (1Hx1/3H, s), 7.14 (1x1/3H, d, J = 15 Hz), 7.33 (1x1/3H, d, J = 15 Hz), 7.53 (2x2/3H, d, J = 7 Hz), 7.62 (2x1/3H, d, J = 7 Hz), 8.13 (2x2/3H, d, J = 7 Hz), 8.22 (2x1/3H, d, J = 7 Hz); FAB MS m/e $(M+H)^+$ 318.

Benzyl 4-[(*E*)-2-(4-nitrophenyl)ethenyl]-1,3-thiazol-2-ylcarbamate (43b)

Compound **43b** was prepared from **42b** and 4-nitrobenzyl bromide (**8**) according to the same procedure as that of compound **9** and obtained as a yellow solid (76% yield). FAB MS m/e $(M+H)^+$ 382.

4-[(*E*)-2-(4-Nitrophenyl)ethenyl]-1,3-thiazol-2-amine (43c).

A mixture of **43b** (2.7 g) and 6 M HCl (50 mL) was refluxed for 3 h. After cooling to room temperature, the mixture was concentrated *in vacuo*. To the residue were added AcOEt and saturated aqueous $NaHCO_3$. The precipitated solid was filtered and washed with AcOEt and H_2O to give **43c** (1.3 g, 77%) as a yellow solid. Mp 278–278.5 ˚C; ^{1}H NMR (DMSO-d_6) δ 7.02 (1H, s), 7.33 (2H, s), 7.77 (2H, d, J = 8.5 Hz), 8.25 (2H, d, J = 8.5 Hz); FAB MS m/e $(M+H)^+$ 248.

***N*-{4-[(*E*)-2-(4-Nitrophenyl)ethenyl]-1,3-thiazol-2-yl}benzamide (43d).**

To a mixture of **43c** (300 mg) and *N*,*N*-dimethylaniline (4 mL) was added benzoyl chloride (0.31 mL) dropwise under a nitrogen atmosphere, and the reaction mixture was stirred at 110 ˚C for 2 h. After cooling to room temperature, the mixture was diluted with AcOEt. The organic solution was washed with 1 M HCl, H_2O, saturated aqueous $NaHCO_3$ and brine, dried over anhydrous $MgSO_4$, and concentrated *in vacuo*. The residual solid was washed with Et_2O to give **43d** (299 mg, 70%) as a yellow solid. Mp 224.5-225 ˚C; ^{1}H NMR (DMSO-d_6) δ 7.40 (1H, d, J = 16.0 Hz), 7.45 (1H, s), 7.53 (1H, d, J = 16.0 Hz), 7.56 (2H, t, J = 7.0 Hz), 7.66 (1H, t, J = 7.0 Hz), 7.84 (2H, d, J = 8.5 Hz), 8.13 (2H, d, J = 7.0 Hz), 8.23 (2H, d, J = 8.5 Hz), 12.80 (1H, br s); FAB MS m/e $(M+H)^+$ 352.

***N*-{4-[2-(4-Aminophenyl)ethyl]-1,3-thiazol-2-yl}-2-methylpropanamide (44a).**

A mixture of **43a** (333 mg), 10% palladium on carbon (33 mg), AcOH (1 mL), MeOH (2 mL) and THF (2 mL) was stirred under hydrogen atmosphere (4 atm) at room temperature for 5 h. The catalyst was filtered, and the filtrate was concentrated *in vacuo*. The residue was purified by flash column chromatography on silica gel with 5% MeOH-AcOEt as an eluent. The residual solid was collected and washed with ^{i}Pr$_2$O to give **44a** (260 mg, 86%) as an off-white amorphous. ^{1}H NMR

($CDCl_3$) δ 1.38 (6H, d, J = 5 Hz), 2.57–2.73 (1H, m), 2.39–2.43 (4H, m), 6.45 (1H, s), 6.62 (2H, d, J = 7 Hz), 6.97 (2H, d, J = 7 Hz); FAB MS m/e $(M+H)^+$ 290.

***N*-{4-[2-(4-Aminophenyl)ethyl]-1,3-thiazol-2-yl}benzamide (44b).**

A mixture of **43d** (285 mg), 10% palladium on carbon (162 mg), AcOH (1 mL), MeOH (3 mL) and THF (3 mL) was stirred under hydrogen atmosphere (4 atm) at room temperature for 4 h. The reaction mixture was filtered through a celite® pad, and the filtrate was concentrated *in vacuo*. A mixture of the residual oil, 10% palladium on carbon (162 mg), AcOH (1 mL), MeOH (3 mL) and THF (3 mL) was stirred under hydrogen atmosphere (4 atm) at room temperature for 3 h. The reaction mixture was filtered through a celite® pad, and the filtrate was concentrated *in vacuo*. The residue was dissolved in AcOEt. The organic solution was washed with saturated aqueous $NaHCO_3$ and brine, dried over anhydrous $MgSO_4$ and concentrated *in vacuo* to give **44b** (238 mg, 91%) as an off-white amorphous. ^{1}H NMR ($CDCl_3$) δ 2.82 (4H, s), 3.57 (2H, br s), 6.53 (1H, s), 6.61 (2H, d, J = 8.0 Hz), 6.92 (2H, d, J = 8.0 Hz), 7.50 (2H, t, J = 7.0 Hz), 7.60 (1H, t, J = 7.0 Hz), 7.93 (2H, d, J = 7.0 Hz), 10.15 (1H, br s); FAB MS m/e $(M+H)^+$ 324.

***N*-{4-[2-(4-{[Amino(imino)methyl]amino}phenyl)ethyl]-1,3-thiazol-2-yl}-2-methylpropanamide (45a).**

A mixture of **44a** (125 mg), cyanamide (26.1 mg), 4 M HCl-AcOEt (0.1 mL) and EtOH (2 mL) was stirred at 100 ˚C for 72 h. The reaction mixture was concentrated *in vacuo*. To the residue were added AcOEt and saturated aqueous $NaHCO_3$. The precipitated solid was filtered and washed with AcOEt and H_2O to give **45a** (45 mg, 33%) as an off-white solid. ^{1}H NMR (DMSO-d_6) δ 1.01 (6H, d, J = 5 Hz), 2.62–2.78 (1H, m), 2.83 (4H, s), 6.72 (2H, d, J = 7 Hz), 6.75 (1H, s), 7.04 (2H, d, J = 7 Hz); FAB MS m/e $(M+H)^+$ 332.

***N*-{4-[2-(4-{[Amino(imino)methyl]amino}phenyl)ethyl]-1,3-thiazol-2-yl}benzamide hydrochloride (45b).**

A mixture of **44b** (180 mg), cyanamide (23.4 mg), 4 M HCl-AcOEt (0.14 mL) and EtOH (4 mL) was stirred at 100 ˚C for 72 h. The reaction mixture was concentrated *in vacuo*. To the residue were added AcOEt and saturated aqueous $NaHCO_3$. The precipitated solid was filtered and washed with AcOEt and H_2O. A mixture of the solid and 4 M HCl-dioxane (2 mL) was concentrated *in vacuo*. The residue was washed with AcOEt to give **45b** (165 mg, 74%) as an off-white solid. Mp 229–232 ˚C; ^{1}H NMR (DMSO-d_6) δ 2.91–3.05 (4H, m), 6.88 (1H, s), 7.15 (2H, d, J = 8.5 Hz), 7.32 (2H, d, J = 8.5 Hz), 7.44 (3H, br s), 7.54 (2H, t, J = 7.5 Hz), 7.64 (1H, t, J = 7.5 Hz), 8.10 (2H, d, J = 7.5 Hz), 9.88 (1H, s); IR (KBr) cm^{-1}: 3143, 1687, 1658, 1631, 1595, 1566, 1529, 1514, 1493, 1448, 1379, 1329 1308, 1265, 1240, 1192, 1093; FAB MS m/e $(M+H)^+$ 366; HRMS (ESI) Calcd for $C_{19}H_{20}N_5OS$ $(M+H)^+$: 366.1389, found: 366.1382.

4-[3-(4-Nitrophenyl)propyl]-1,3-thiazol-2-amine (47).

To a solution of 5-(4-nitrophenyl)pentan-2-one (**46**) (4.0 g) in MeOH (35 mL) was added Br_2 (1.0 mL), and the mixture was stirred at room temperature for 2 h. Then to the reaction mixture were added thiourea (1.5 g) and K_2CO_3 (6.7 g), and the mixture was stirred at 50 ˚C for 2 h. Volatiles were concentrated *in vacuo*. The residue was dissolved in H_2O (50 mL) and THF (15 mL), extracted with AcOEt (50 mL, 3 times), washed with brine (50 mL), dried over anhydrous $MgSO_4$ and evaporated *in vacuo*. The residue was purified by flash column chromatography over silica gel with CH_2Cl_2-MeOH (100:0–100:1.5) as an eluent to give **47** (855 mg, 17%) as a pale brown solid. ^{1}H NMR (DMSO-d_6) δ 1.78–2.01 (2H, m), 2.41 (2H, t, J = 7.4 Hz), 2.73 (2H, t, J = 7.6 Hz), 6.13 (1H, s), 6.81 (2H, s), 7.49 (2H, d, J = 8.7 Hz), 8.15 (2H, d, J = 8.8 Hz); FAB MS m/e $(M+H)^+$ 264.

***N*-{4-[3-(4-Nitrophenyl)propyl]-1,3-thiazol-2-yl}acetamide (48).**

Compound **47** (847 mg) was dissolved in CH_2Cl_2 (8.5 mL) under a nitrogen atmosphere. Then pyridine (0.83 mL) and acetyl chloride (0.37 mL) were added dropwise to the solution at 0 ˚C. The reaction mixture was stirred at room temperature for 30 min. The organic solution was washed with 1 M HCl, H_2O and brine, dried over anhydrous $MgSO_4$, and concentrated *in vacuo*. The solid was

washed with AcOEt-Et_2O (1:1) to give **48** (704 mg, 72%) as a pale yellow solid. ^{1}H NMR (DMSO-d_6) δ 1.87–2.06 (2H, m), 2.61 (2H, t, J = 7.4 Hz), 2.75 (2H, t, J = 7.6 Hz), 6.77 (1H, s), 7.5 (2H, d, J = 8.6 Hz), 8.16 (2H, d, J = 8.7 Hz), 12.03 (1H, s); FAB MS m/e $(M+H)^+$ 306.

.

***N*-{4-[3-(4-Aminophenyl)propyl]-1,3-thiazol-2-yl}acetamide (49).**

Compound **48** (100 mg) and 10% palladium on carbon (50% wet) (98 mg) in MeOH (2 mL), THF (2 mL) and AcOH (0.3 mL) were stirred under hydrogen atmosphere (3 atm) at room temperature for 5 h. The reaction mixture was filtered through a celite® pad, and the filtrate was concentrated *in vacuo*. The residue was dissolved in AcOEt. The organic solution was washed with saturated aqueous $NaHCO_3$ and brine, dried over anhydrous $MgSO_4$, and concentrated *in vacuo* to give **49** (94 mg, 105%) as pale yellow oil. FAB MS m/e $(M+H)^+$ 276.

Di-*tert*-butyl {(*Z*)-[(4-{3-[2-(acetylamino)-1,3-thiazol-4-yl]propyl}phenyl)amino]methylidene}-biscarbamate (50).

To a solution of **49** (90 mg) in THF (1 mL) was added **31** (152 mg), and the mixture was stirred at room temperature for 14 h. Volatiles were evaporated *in vacuo*, and the residue was purified by preparative TLC (0.5 mm×2, $CHCl_3$-MeOH (15:1), then 0.5 mm×1, AcOEt) to give **50** (142 mg, 84%) as an off-white amorphous. ^{1}H NMR ($CDCl_3$) δ 1.50 (9H, s), 1.53 (9H, s), 1.86–2.07 (2H, m), 2.22 (3H, s), 2.62 (2H, t, J = 8 Hz), 2.66 (2H, t, J = 8 Hz), 6.53 (1H, s), 7.12 (2H, d, J = 8.4 Hz), 7.48 (2H, d, J = 8.4 Hz), 10.26 (1H, s), 11.64 (1H, s); FAB MS m/e $(M+H)^+$ 518, $(M+Na)^+$ 540.

***N*-{4-[3-(4-{[Amino(imino)methyl]amino}phenyl)propyl]-1,3-thiazol-2-yl}acetamide hydrochloride (51).**

A mixture of **50** (140 mg) and 4 M HCl-dioxane (2 mL) was stirred at room temperature for 38 h under a nitrogen atmosphere. The solvent was removed *in vacuo* to give **51** (81 mg, 85%) as a pale yellow solid. ^{1}H NMR (DMSO-d_6) δ 1.86–1.98 (2H, m), 2.11 (3H, s), 2.57–2.65 (4H, m), 6.75 (1H, s), 7.15 (2H, d, J = 8.3 Hz), 7.27 (2H, d, J = 8.3 Hz), 7.41 (4H, s), 9.82 (1H, s), 12.03 (1H, s); IR

(KBr) cm^{-1}: 3492, 3361, 3165, 3089, 1716, 1662, 1622, 1606, 1577, 1550, 1514, 1458, 1431, 1371, 1298, 1255, 1228; FAB MS m/e $(M+H)^+$ 318; HRMS (ESI) Calcd for $C_{15}H_{20}N_5OS$ $(M+H)^+$: 318.1389, found: 318.1387.

4-(4-Nitrobenzyl)-1,3-thiazol-2-amine hydrobromide (53).

To a solution of 4-nitrophenylacetone (**52**) (5.0 g) in AcOH (10 mL) and 48% aqueous HBr (5 mL) was added a solution of Br_2 (9.0 g) in AcOH (8 mL) at 0 ˚C. The reaction mixture was stirred at room temperature for 4 h. Then acetone (50 mL) was added to the solution, and the reaction mixture was stirred at room temperature for 14 h. The mixture was concentrated *in vacuo*, diluted with brine, and extracted with CH_2Cl_2 (twice). Anhydrous $MgSO_4$ and SiO_2 were added to the combined CH_2Cl_2 extracts, which were then filtered *in vacuo*. The filtrate was concentrated *in vacuo* to give 1-bromo-3-(4-nitrophenyl)acetone (11.6 g) as a brown solid. To a solution of 1-bromo-3-(4-nitrophenyl)acetone (11.6 g) in EtOH (232 mL) was added thiourea (3.4 g). The reaction mixture was refluxed for 3 h. After cooling to room temperature, the mixture was concentrated *in vacuo*. The residual solid was washed with MeCN to give **53** (6.3 g, 2 steps yield:72%) as an off-white solid. ^{1}H NMR (DMSO-d_6) δ 4.07 (2H,s), 6.53 (1H, s), 7.56 (2H, d, J = 9.0 Hz), 8.22 (2H, d, J = 9.0 Hz), 9.04 (2H, br s); FAB MS m/e $(M+H)^+$ 236.

***N*-[4-(4-Nitrobenzyl)-1,3-thiazol-2-yl]acetamide (54).**

Compound **53** (2.0 g) was suspended in CH_2Cl_2 (20 mL) under a nitrogen atmosphere. Then pyridine (2.3 mL) and acetyl chloride (0.68 mL) were added dropwise to the solution at 0 ˚C. The reaction mixture was stirred at room temperature for 18 h. The organic solution was washed with 1 M HCl, H_2O, saturated aqueous $NaHCO_3$ and brine, dried over anhydrous $MgSO_4$, and concentrated *in vacuo*. The residual solid was washed with MeCN to give **54** (949 mg, 54%) as an off-white solid. ^{1}H NMR (DMSO-d_6) δ 2.08 (3H,s), 4.10 (2H,s), 6.91 (1H, s), 7.50 (2H, d, J = 9.0 Hz), 8.17 (2H, d, J = 9.0 Hz), 12.06 (1H, s); IR (KBr) cm^{-1}: 3172, 3116, 3059, 1647, 1608, 1577, 1523, 1489, 1456, 1429, 1346, 1306, 1242, 1176, 1161, 1107, 1003; FAB MS m/e $(M+H)^+$ 278.

***N*-[4-(4-Aminobenzyl)-1,3-thiazol-2-yl]acetamide (55).**

Compound **54** (500 mg) was suspended in MeOH (10 mL) and DMF (2.5 mL). Then 10% palladium on carbon (360 mg) was added to the solution under a nitrogen atmosphere. The reaction mixture was stirred at room temperature for 2 h under hydrogen atmosphere (3 atm) and filtered through a celite® pad. The filtrate was concentrated *in vacuo*. The residue was solidified with MeCN-Et_2O to give **55** (400 mg, 90%) as an off-white solid. ^{1}H NMR (DMSO-d_6) δ 2.08 (3H,s), 3.73 (2H,s), 5.15 (2H, brs), 6.51 (2H, d, J = 8.5 Hz), 6.65 (1H, s), 6.89 (2H, d, J = 8.5 Hz), 12.00 (1H, s); FAB MS m/e $(M+H)^+$ 248.

***N*-[4-(4-Carbamimidamidobenzyl)-1,3-thiazol-2-yl]acetamide (56).**

Compound **56** was prepared from **55** and cyanamide according to the same procedure as that of compound **45a**. Compound **56** was obtained as an off-white solid (77% yield). ^{1}H NMR (DMSO-d_6) δ 2.08 (3H,s), 3.83 (2H,s), 6.73 (1H, s), 6.77 (2H, d, J = 8.0 Hz), 7.09 (2H, d, J = 8.0 Hz); IR (KBr) cm^{-1}: 1670, 1635, 1577, 1543, 1516, 1458, 1417, 1373, 1340, 1309, 1242, 1134; FAB MS m/e $(M+H)^+$ 290; HRMS (ESI) Calcd for $C_{13}H_{16}N_5OS$ $(M+H)^+$: 290.1076, found: 290.1075.

***N*-{2-[4-(2-Chloroacetyl)phenyl]ethyl}-acetamide (58).**

Aluminium chloride (1.6 g) was dissolved in 1,2-dichloroethane (15 mL). Chloroacetylchloride (0.73 mL) was added to the mixture at 0 ˚C, and the mixture was stirred additionally for 20 min followed by the dropwise addition of *N*-(2-phenylethyl)acetamide (**57**) (1.0 g) in 1,2-dichloroethane (5 mL). The mixture was stirred at room temperature for an hour and then poured into ice-H_2O. It was then extracted with $CHCl_3$, washed with H_2O and brine, dried over anhydrous Na_2SO_4 and concentrated *in vacuo*. The solid was washed with AcOEt and Et_2O, and dried *in vacuo* to give **58** (1.2 g, 80%) as a white powder. ^{1}H NMR (DMSO-d_6) δ 1.98 (3H, s), 2.90–2.94 (2H, m), 3.55–3.60 (2H, m), 4.70 (2H, s), 5.66 (1H, br), 7.34 (2H, d, J = 6 Hz), 7.92 (2H, d, J = 6 Hz).

***N*-{2-[4-(2-Amino-1,3-thiazol-4-yl)phenyl]ethyl}acetamide hydrochloride (59).**

Compound **58** (1.1 g) and thiourea (505 mg) were dissolved in EtOH (20 mL). The mixture was refluxed for an hour and cooling to room temperature. The white solid was collected with filtration and washed with EtOH to give **59** (1.2 g, 90%) as a white solid. ^{1}H NMR (DMSO-d_6) δ 1.78 (3H, s), 2.70–2.76 (2H, m), 3.23–3.30 (2H, m), 7.16 (1H, s), 7.30 (2H, d, J = 6 Hz), 7.69 (2H, d, J = 6 Hz), 7.93–7.96 (2H, m); FAB MS m/e $(M+H)^+$ 262.

4-[4-(2-Aminoethyl)phenyl]-1,3-thiazol-2-amine dihydrochloride (60).

Compound **59** (0.6 g) was dissolved in EtOH (10 mL) and concentrated HCl (10 mL). The mixture was refluxed for 5 h. The solvent was evaporated *in vacuo*. The residue was washed with Et_2O to give **60** (0.5 g, 85%) as a white solid. ^{1}H NMR (DMSO-d_6) δ 2.90–2.98 (2H, m), 3.03–3.10 (2H, m), 7.24 (1H, s), 7.39 (2H, d, J = 6 Hz), 7.78 (2H, d, J = 6 Hz), 8.15 (3H, br s); FAB MS m/e $(M+H)^+$ 220.

***tert*-Butyl {2-[4-(2-amino-1,3-thiazol-4-yl)phenyl]ethyl}-carbamate (61).**

Compound **60** (450 mg) was dissolved in dioxane (10 mL), H_2O (3 mL) and 1 M NaOH (3.1 mL). Di-*tert*-butyl dicarbonate (336 mg) was added at 0 ˚C. The reaction mixture was stirred at room temperature overnight, then extracted with AcOEt, washed with H_2O and brine, dried over anhydrous Na_2SO_4 and concentrated *in vacuo*. The solid was washed with Et_2O to give **61** (311 mg, 63%) as a white solid. ^{1}H NMR (DMSO-d_6) δ 1.37 (9H, s), 2.65–2.72 (2H, m), 3.10–3.27 (2H, m), 7.02 (2H, brs), 7.18 (2H, d, J = 6 Hz), 7.69 (1H, s), 7.69 (2H, d, J = 6 Hz); FAB MS m/e $(M+H)^+$ 320.

***tert*-Butyl (2-{4-[2-(acetylamino)-1,3-thiazol-4-yl]phenyl}ethyl)carbamate (62).**

Compound **61** (290 mg) was dissolved in CH_2Cl_2 (5 mL), then Ac_2O (0.10 mL), 4-dimethylaminopyridine (10 mg) and pyridine (0.15 mL) were added. The reaction mixture was stirred at room temperature overnight. The mixture was extracted with $CHCl_3$, washed with H_2O and

brine, dried over anhydrous Na_2SO_4 and concentrated *in vacuo*. The solid was washed with Et_2O to give **62** (280 mg, 85%) as a white solid. ^{1}H NMR (DMSO-d_6) δ 1.37 (9H, s), 2.16 (3H, s), 2.16–2.63 (2H, m), 3.12–3.18 (2H, m), 6.90 (1H, m), 7.24 (2H, d, J = 6 Hz), 7.53 (1H, s), 7.80 (2H, d, J = 6 Hz); FAB MS m/e $(M+H)^+$ 362.

***N*-{4-[4-(2-Aminoethyl)phenyl]-1,3-thiazol-2-yl}acetamide hydrochloride (63).**

Compound **62** (250 mg) was dissolved in AcOEt (4 mL) and 4 M HCl-AcOEt (2 mL). The solvent was evaporated *in vacuo*. The solid was washed with AcOEt and Et_2O to give **63** (220 mg, 106%) as a white solid. ^{1}H NMR (DMSO-d_6) δ 2.16 (3H, s), 2.88–2.94 (2H, m), 3.12–3.18 (2H, m), 7.32 (2H, d, J = 6 Hz), 7.58 (1H, s), 7.85 (2H, d, J = 6 Hz), 8.05 (3H, br s); FAB MS m/e $(M+H)^+$ 262.

***N*-{4-[4-(2-{[Amino(imino)methyl]amino}ethyl)phenyl]-1,3-thiazol-2-yl}acetamide hydrochloride (64).**

Compound **63** (200 mg), *N*,*N'*-bis(*tert*-butoxycarbonyl)-1H-pyrazole-1-carboxamidine (**31**) (208 mg) and diisopropylethylamine (0.18 mL) were dissolved in THF (5 mL). The reaction mixture was stirred at room temperature overnight, and evaporated *in vacuo*. The residue was purified by flash column chromatography on silica-gel with 5% MeOH-$CHCl_3$ as an eluent to give di-*tert*-butyl {[(2-{4-[2-(acetylamino)-1,3-thiazol-4-yl]phenyl}ethyl)amino]methylidene}-biscarbamate (268 mg, 79%).

Di-*tert*-butyl {[(2-{4-[2-(acetylamino)-1,3-thiazol-4-yl]phenyl}ethyl)amino]methylidene}-biscarbamate (170 mg) was dissolved in 4 M HCl-dioxane (5 mL). The mixture was stirred at room temperature for 2 days and then evaporated *in vacuo*. The residue was washed with Et_2O, dried *in vacuo* to give **64** (50 mg, 44%) as a white solid. ^{1}H NMR (DMSO-d_6) δ 2.16 (3H, s), 2.78–2.85 (2H, m), 3.37–3.45 (2H, m), 7.34 (2H, d, J = 8 Hz), 7.56 (1H, s), 7.62–7.66 (1H, m), 7.83 (2H, d, J = 8 Hz); FAB MS m/e $(M+H)^+$ 304; HRMS (ESI) Calcd for $C_{14}H_{18}N_5OS$ $(M+H)^+$: 304.1232, found: 304.1226; Anal. Calcd for $C_{14}H_{17}N_5OS \cdot 2HCl \cdot 0.5H_2O$: C, 43.64; H, 5.23; N, 18.18; S, 8.32; Cl, 18.40. Found: C, 43.58; H, 5.25; N, 18.15; S, 8.32; Cl, 18.36.

3-[4-(Methylsulfanyl)phenyl]-3-oxopropanenitrile (66).

Compound **65** (5.2 g) was dissolved in AcOH (52 mL), and then 90% pyridinium bromide perbromide (11.1 g) and 30% HBr/AcOH (5.2 mL) were added to the solution at 0 ˚C. The reaction mixture was stirred at room temperature for an hour and then poured into H_2O and extracted with AcOEt. The organic layer was washed with saturated aqueous $NaHCO_3$ and brine, dried over anhydrous $MgSO_4$, and concentrated in vacuo.

A solution of sodium cyanide (4.6 g) in H_2O (39 mL) was added dropwise to the suspension of the residual yellow solid (8.2 g) in EtOH (52 mL) at 0 ˚C over 10 min. The reaction mixture was stirred at room temperature for 4 h and then poured into H_2O. The mixture was neutralized with 1 M HCl and extracted with AcOEt (twice). The combined organic layer was washed with brine, dried over anhydrous $MgSO_4$, and concentrated in vacuo. The residual solid was recrystallized from AcOEt/$^{i}Pr_2O$ to give **66** (2.6 g, 43%) as a brown solid. Mp 97–97.5 ˚C; ^{1}H NMR (DMSO-d_6) δ 2.55 (3H, s), 4.71 (2H, s), 7.40 (2H, d, J = 8.5 Hz), 7.85 (2H, d, J = 8.5 Hz); FAB MS m/e $(M-H)^-$ 190.

3-[4-(Methylsulfanyl)phenyl]-1*H*-pyrazol-5-amine (67)

To a solution of **66** (2.0 g) in EtOH (10 mL) and H_2O (10 mL) were added hydrazine hydrate (0.66 mL) and concentrated HCl (0.2 mL), and the reaction mixture was refluxed for 2.5 h. After cooling to room temperature, the mixture was poured into AcOEt/H_2O. The mixture was made basic with saturated aqueous $NaHCO_3$ and extracted. The organic layer was dried over anhydrous $MgSO_4$ and concentrated in vacuo. The resulting solid was washed with EtOH/Et_2O to give **67** (1.7 g, 77%) as a pale brown solid. Mp 164–165 ˚C; ^{1}H NMR (DMSO-d_6) δ 2.48 (3H, s), 5.73 (1H, br s), 7.25 (2H, d, J = 8.5 Hz), 7.58 (2H, d, J = 8.5 Hz); FAB MS m/e $(M+H)^+$ 206.

***N*-{3-[4-(Methylsulfanyl)phenyl]-1*H*-pyrazol-5-yl}acetamide (68).**

Compound **67** (1.6 g) was dissolved in pyridine (16 mL) under a nitrogen atmosphere, and then

Ac_2O (1.5 mL) was added to the solution at 0 ˚C. The reaction mixture was stirred at 0 ˚C for 30 min and concentrated in vacuo. The residue was suspended in EtOH (30 mL), and then 1 M NaOH (7.8 mL) was added. The mixture was stirred at room temperature for an hour, and the precipitate was collected. The solid was washed with H_2O and EtOH to give **68** (1.4 g, 73%) as an off-white solid. Mp 231.5–232 ˚C; ^{1}H NMR (DMSO-d_6) δ 2.02 (3H, s), 2.50 (3H, s), 6.84 (1H, br s), 7.31 (2H, d, J = 8.5 Hz), 7.65 (2H, d, J = 8.5 Hz), 10.40 (1H, br s), 12.75 (1H, br s); FAB MS *m*/*e* $(M+H)^+$ 248.

A mixture of *tert*-butyl [4-(2-{3-acetamido-5-[4-(methylsulfanyl)phenyl]-1*H*-pyrazol-1-yl}-ethyl)phenyl]carbamate (70a) and *tert*-butyl [4-(2-{5-acetamido-3-[4-(methylsulfanyl)phenyl]-1*H*-pyrazol-1-yl}ethyl)phenyl]carbamate (70b).

Compound **68** (700 mg) was dissolved in DMF (14 mL) under a nitrogen atmosphere. K_2CO_3 (469 mg) and *tert*-butyl [4-(2-iodoethyl)phenyl]carbamate (**69**) (1.1 g) were then added to the solution at 0 ˚C. The reaction mixture was stirred at 55 ˚C for 18 h. After cooling to room temperature, the mixture was poured into H_2O and extracted with AcOEt (twice). The combined organic layer was washed with H_2O and brine, dried over anhydrous $MgSO_4$, and concentrated in vacuo. The residual solid was washed with MeOH/$CHCl_3$, and the filtrate was concentrated in vacuo. The residue was purified by flash column chromatography over silica gel with hexane/AcOEt (3:1) as an eluent to give a mixture of **70a** and **70b** (696 mg, 53%; 70a:70b=2:3) as a brown oil. ^{1}H NMR (DMSO-d_6) δ 1.49 (9H, s), 2.01 (3H×2/5, s), 2.07 (3H×3/5, s), 2.50 (3H, s), 2.91–3.03 (2H, m), 4.07–4.23 (2H, m), 6.49 (1H×2/5, s), 6.60 (1H×3/5, s), 6.86 (2H×2/5, d, J=8.5Hz), 7.06–7.14 (2H, m), 7.23–7.29 (2H×8/5, m), 7.36 (2H×2/5, d, J = 8.5 Hz), 7.70 (2H×3/5, d, J = 8.5 Hz), 9.24 (1H×2/5, br s), 9.26 (1H×3/5, br s), 9.89 (1H×3/5, br s), 10.52 (1H×2/5, br s); FAB MS *m*/*e* $(M+H)^+$ 467.

A mixture of *tert*-butyl [4-(2-{3-acetamido-5-[4-(methylsulfonyl)phenyl]-1*H*-pyrazol-1-yl}-ethyl)phenyl]carbamate (71a) and *tert*-butyl [4-(2-{5-acetamido-3-[4-(methylsulfonyl)phenyl]-1*H*-pyrazol-1-yl}ethyl)phenyl]carbamate (71b).

Potassium peroxymonosulfate (Oxone®) (1.1 g) was suspended in H_2O (3 mL) and THF (14 mL),

and then a mixture of **70a** and **70b** (652 mg) was added portionwise to the suspension at 0 ˚C. The reaction mixture was stirred at room temperature for 1.5 h, and then H_2O was added to the suspension. The solution was made basic with saturated aqueous $NaHCO_3$ and extracted with $CHCl_3$ (twice). The combined organic layer was washed with brine, dried over anhydrous $MgSO_4$, and concentrated in vacuo to give a mixture of **71a** and **71b** (570 mg, 82%) as pale brown amorphous. FAB MS *m*/*e* $(M+H)^+$ 499.

***N*-{1-[2-(4-Aminophenyl)ethyl]-5-[4-(methylsulfonyl)phenyl]-1*H*-pyrazol-3-yl}acetamide (72a) and *N*-{1-[2-(4-aminophenyl)ethyl]-3-[4-(methylsulfonyl)phenyl]-1*H*-pyrazol-5-yl}acetamide (72b).**

A mixture of **71a** and **71b** (550 mg) was dissolved in CH_2Cl_2 (2 mL), and then TFA (2 mL) was added to the solution at 0 ˚C. The reaction mixture was stirred at room temperature for an hour, and concentrated in vacuo. The residue was dissolved in $CHCl_3$, washed with 1 M NaOH, H_2O and brine, dried over anhydrous $MgSO_4$, and concentrated in vacuo. The residue was purified by preparative silica gel column chromatography with $CHCl_3$/MeOH (10:1) as an eluent to give **72a** (126 mg, 29%) as an off-white solid and **72b** (170 mg, 39%) as an off-white solid.

72a : Mp 203–204 ˚C; ^{1}H NMR (DMSO-d_6) δ 2.03 (3H, s), 2.86 (2H, t, *J* = 7.0 Hz), 3.27 (3H, s), 4.10 (2H, t, *J* = 7.0 Hz), 4.89 (2H, s), 6.41 (2H, d, *J* = 8.5 Hz), 6.62 (2H, d, *J* = 8.5 Hz), 6.62 (1H, s), 7.42 (2H, d, *J* = 8.5 Hz), 7.92 (2H, d, *J* = 8.5 Hz), 10.60 (1H, br s); ^{13}C NMR (DMSO-d_6) δ 23.1, 35.0, 43.4, 50.9, 97.5, 113.9, 124.7, 127.2, 129.1, 129.2, 134.9, 140.3, 141.8, 146.9, 147.2, 165.5; FAB MS *m*/*e* $(M+H)^+$ 399.

72b : Mp 188–189°C; ^{1}H NMR (DMSO-d_6) δ 2.09 (3H, s), 2.90 (2H, t, *J* = 7.5 Hz), 3.23 (3H, s), 4.19 (2H, t, *J* = 7.5 Hz), 4.90 (2H, s), 6.49 (2H, d, *J* = 8.5 Hz), 6.81 (1H, s), 6.88 (2H, d, *J* = 8.5 Hz), 7.93 (2H, d, *J* = 8.5 Hz), 8.03 (2H, d, *J* = 8.5 Hz), 9.95 (1H, br s); ^{13}C NMR (DMSO-d_6) δ 22.9, 34.7, 43.6, 49.8, 96.7, 113.9, 124.8, 125.3, 127.5, 129.2, 137.8, 138.2, 139.2, 146.7, 147.1, 168.2; FAB MS *m*/*e* $(M+H)^+$399.

***tert*-Butyl {(*Z*)-{[4-(2-{3-acetamido-5-[4-(methylsulfonyl)phenyl]-1*H*-pyrazol-1-yl}ethyl)-phenyl]amino}[(*tert*-butoxycarbonyl)amino]methylene}carbamate (73).**

A mixture of **72a** (105 mg), *N*,*N'*-bis(*tert*-butoxycarbonyl)-1*H*-pyrazole-1-carboxamidine (**11**) (82 mg), DMF (0.5 mL) and THF (2 mL) was stirred at 50 ˚C for 10 h and at room temperature for 75 h under a nitrogen atmosphere. The solvent was removed *in vacuo*, and the residue was purified by preparative silica gel column chromatography with $CHCl_3$/MeOH (20:1) as an eluent to give **73** (62 mg, 36%) as an off-white wax. ^{1}H NMR (DMSO-d_6) δ 1.38 (9H, s), 1.51 (9H, s), 2.02 (3H, s), 3.00 (2H, t, *J* = 7.0 Hz), 3.25 (3H, s), 4.24 (2H, t, *J* = 7.0 Hz), 6.60 (1H, s), 6.90 (2H, d, *J* = 8.5 Hz), 7.32 (2H, d, *J* = 8.5 Hz), 7.48 (2H, d, *J* = 8.5 Hz), 7.92 (2H, d, *J* = 8.5 Hz), 9.90 (1H, br s), 10.61 (1H, br s), 11.43 (1H, br s); FAB MS *m*/*e* $(M+H)^+$ 641.

***N*-{1-[2-(4-Carbamimidamidophenyl)ethyl]-5-[4-(methylsulfonyl)phenyl]-1*H*-pyrazol-3-yl}acetamide hydrochloride (74).**

Compound **73** (50 mg) was dissolved in 4 M HCl/dioxane (4 mL). The mixture was stirred at room temperature for 7 h and then evaporated in vacuo. The residual solid was washed with Et_2O to give **74** (37 mg, 98%) as an off-white solid. Mp 155–156 ˚C; ^{1}H NMR (DMSO-d_6) δ 2.03 (3H, s), 3.05 (2H, t, *J* = 7.5 Hz), 3.28 (3H, s), 4.25 (2H, t, *J* = 7.5 Hz), 6.64 (1H, s), 7.03 (2H, d, *J* = 8.5 Hz), 7.07 (2H, d, *J* = 8.5 Hz), 7.37 (4H, br s), 7.50 (2H, d, *J* = 8.5 Hz), 7.97 (2H, d, *J* = 8.5 Hz), 9.77 (1H, br s), 10.61 (1H, br s); FAB MS *m*/*e* $(M+H)^+$ 441.

2-Methyl-4-[4-(methylsulfanyl)benzylidene]-1,3-oxazol-5(4*H*)-one (76).

To a mixture of 4-(methylsulfanyl)benzaldehyde (**75**) (31.8 g), (acetylamino)acetic acid (24.5 g) and Ac_2O (35 mL) was added sodium acetate (8.6 g) at room temperature under a nitrogen atmosphere. The reaction mixture was refluxed for 3.5 h. After cooling to room temperature, the mixture was poured into ice-H_2O and AcOEt with stirring, and filtered in vacuo. The filtrate was separated, and the organic layer was washed with brine, dried over anhydrous $MgSO_4$, and concentrated in vacuo. The residue and the previously obtained solid were combined, and the mixture was purified by flash

column chromatography over silica gel with $CHCl_3$-AcOEt (30:1) as an eluent and triturated with $^{i}Pr_2O$ to give **76** (17.8 g, 37%) as a brown solid. Mp 154–155 ˚C; ^{1}H NMR (DMSO-d_6) δ 2.38 (3H, s), 2.53 (3H, s), 7.19 (1H, s), 7.36 (2H, d, J = 8.5 Hz), 8.12 (2H, d, J = 8.5 Hz).

3-(4-(Methylsulfanyl)phenyl)-2-oxopropanoic acid (77).

To a solution of **76** (17.5 g) in dioxane (100 mL) was added 4 M HCl (27 mL), and the reaction mixture was refluxed for 3 h. After cooling to room temperature, the mixture was concentrated in vacuo. AcOEt and H_2O were added to the residue, and the precipitate was filtered in vacuo to give **77** (16.4g, 104%) as a pale brown solid. Mp 165–167 ˚C; ^{1}H NMR (DMSO-d_6) δ 2.48 (3H, s), 6.37 (1H, s), 7.23 (2H, d, J = 8.5 Hz), 7.70 (2H, d, J = 8.5 Hz), 9.44 (1H, s); FAB MS m/e $(M-H)^-$ 209.

Methyl 3-(4-(methylsulfanyl)phenyl)-2-oxopropanoate (78).

To a solution of **77** (16.2 g) in DMF (81 mL) was added 1,8-diazabicyclo[5.4.0]undec-7-ene (11.5 mL) at 0 ˚C under a nitrogen atmosphere. The mixture was stirred at the same temperature for an hour, and then MeI (9.6 mL) was added to the solution at the same temperature. The reaction mixture was stirred at room temperature for 4 h, poured into 1 M HCl, and extracted with AcOEt (twice). The combined organic layer was washed with brine, dried over anhydrous $MgSO_4$, and concentrated in vacuo. The residue was purified by flash column chromatography over silica gel with $CHCl_3$/AcOEt (30:1) as an eluent, and triturated with hexane/$^{i}Pr_2O$ to give **78** (10.2 g, 59%) as a dark yellow solid. Mp 112–113 ˚C; ^{1}H NMR (DMSO-d_6) δ 2.48 (3H, s), 3.79 (3H, s), 6.41 (1H, s), 7.24 (2H, d, J = 8.5 Hz), 7.72 (2H, d, J = 8.5 Hz), 9.52 (1H, br s); FAB MS m/e $(M-H)^-$ 223.

Methyl 3-bromo-3-[4-(methylsulfanyl)phenyl]-2-oxopropanoate (79).

To a solution of **78** (2.8 g) in CH_2Cl_2 (140 mL) and AcOH (0.5 mL) was added pyridinium tribromide (5.0 g) at 0 ˚C under a nitrogen atmosphere. The reaction mixture was stirred at the same temperature for 2 h, and poured into H_2O. The mixture was extracted with AcOEt (twice). The combined organic layer was dried over anhydrous $MgSO_4$ and concentrated in vacuo to give **79** (4.1

g, 105%) as a brown oil.

Methyl 3-[4-(methylthio)phenyl]propanoate (82).

To a mixture of 3-(4-mercaptophenyl)propanoic acid (**81**) (5.0 g) and K_2CO_3 (11.4 g) in DMF (30 mL) was added MeI (5.1 mL) at 0 ˚C under a nitrogen atmosphere. The reaction mixture was stirred at room temperature for 13 h and poured into ice-H_2O. The mixture was extracted with AcOEt. The organic layer was washed with H_2O (twice) and brine, dried over anhydrous $MgSO_4$, and concentrated in vacuo to give **82** (4.2 g, 73%) as a pale yellow oil. ^{1}H NMR ($CDCl_3$) δ 2.47 (3H, s), 2.61 (2H, t, J = 8.0 Hz), 2.91 (2H, t, J = 8.0 Hz), 3.67 (3H, s), 7.12 (2H, d, J = 8.5 Hz), 7.20 (2H, d, J = 8.5 Hz).

***N*-Methoxy-*N*-methyl-3-(methylsulfanyl)benzamide (84).**

1-Hydroxybenzotriazole (HOBt) (3.7 g) and 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide (EDC) (4.1 g) were added to a solution of 3-(methylsulfanyl)benzoic acid (**83**) (15.0 g) in DMF (150 mL) at 0 ˚C. After stirring for 30 min at room temperature, *N,O*-dimethylhydroxylamine hydrochloride (8.7 g) was added at 0 ˚C. The reaction mixture was stirred at room temperature for 13 h and poured into saturated aqueous $NaHCO_3$ at 0 ˚C. The mixture was extracted with AcOEt (twice). The combined organic layer was washed with brine, dried over anhydrous $MgSO_4$, and concentrated in vacuo to give **84** (18.3 g, 97%) as a yellow oil. ^{1}H NMR ($CDCl_3$) δ 2.50 (3H, s), 3.36 (3H, s), 3.56 (3H, s), 7.28–7.45 (3H, m), 7.54 (1H, s); FAB MS *m*/*e* $(M+H)^+$ 212.

Methyl (2*E*)-3-[3-(methylsulfanyl)phenyl]acrylate (86).

To a stirred solution of **84** (18.0 g) in THF (360 mL) was added diisobutylaluminum hydride (170 mL) dropwise at -78 ˚C over 40 min under a nitrogen atmosphere. The reaction mixture was let warm to room temperature over 2.5 h and then quenched with MeOH. 1 M HCl was added and the mixture was extracted with AcOEt. The organic layer was washed with brine, dried over anhydrous $MgSO_4$, and concentrated in vacuo. A mixture of the residual yellow oil (12.9 g), methyl

(triphenylphosphoranylidene)acetate (**85**) (28.5 g) and THF (260 mL) was refluxed for 2 h. The solvent was removed in vacuo, and the residue was suspended in AcOEt. The solid was filtered off, and the filtrate was concentrated in vacuo. The residue was purified by flash column chromatography over silica gel with hexane/AcOEt (3:1) as an eluent to give **86** (14.4 g, 81%) as a colorless wax. ^{1}H NMR (DMSO-d_6) δ 2.51 (3H, s), 3.81 (3H, s), 6.44 (1H, d, J = 16.0 Hz), 7.24–7.32 (3H, m), 7.38 (1H, m), 7.65 (1H, d, J = 16.0 Hz).

Methyl 3-[3-(methylthio)phenyl]propanoate (87).

A mixture of **86** (14.0 g) and 10% palladium on carbon (6.7 g) in MeOH (140 mL) and AcOH (70 mL) was stirred at room temperature for 9 h under a hydrogen atmosphere (4 atm) and filtered through a celite® pad. The filtrate was concentrated in vacuo. The residue was purified by flash column chromatography over silica gel with hexane/AcOEt (3:1) as an eluent to give **87** (12.5 g, 88%) as a colorless oil. ^{1}H NMR (DMSO-d_6) δ 2.48 (3H, s), 2.62 (2H, t, J = 8.0 Hz), 2.92 (2H, t, J = 8.0 Hz), 3.68 (3H, s), 6.94–7.00 (1H, m), 7.07–7.14 (2H, m), 7.15–7.24 (1H, m).

Ethyl 4-[4-(methylthio)phenyl]-2-oxobutanoate (88a).

Sodium methoxide, 28% solution in MeOH (3.7 mL), was added dropwise to a mixture of **82** (4.0 g) and diethyl oxalate (5.2 mL) at 0 ˚C with stirring. The reaction mixture was stirred at 65 ˚C for 30 min under reduced pressure. 15% aqueous H_2SO_4 (35 mL) was added to the mixture, and the mixture was refluxed for 15 h. After cooling to room temperature, the mixture was extracted with AcOEt. The organic layer was washed with H_2O and brine, dried over anhydrous $MgSO_4$, and concentrated in vacuo. The residual oil was dissolved in EtOH (20 mL), and concentrated H_2SO_4 (0.4 mL) was added dropwise to the solution. The reaction mixture was refluxed for 2 h. After cooling to room temperature, EtOH was removed in vacuo. AcOEt and H_2O were added to the residue, and extracted. The organic layer was washed with H_2O and brine, dried over anhydrous $MgSO_4$, and concentrated in vacuo. The residue was purified by flash column chromatography over silica gel with hexane/AcOEt (6:1) as an eluent to give **88a** (2.4 g, 51%) as a yellow oil. ^{1}H NMR ($CDCl_3$) δ 1.35

(3H, t, *J* = 7.0 Hz), 2.46 (3H, s), 2.92 (2H, t, *J* = 7.0 Hz), 3.16 (2H, t, *J* = 7.0 Hz), 4.31 (2H, q, *J* = 7.0 Hz), 7.13 (2H, d, *J* = 8.5 Hz), 7.20 (2H, d, *J* = 8.5 Hz).

Ethyl 4-[3-(methylthio)phenyl]-2-oxobutanoate (88b).

Compound **88b** was prepared from **87** according to the same procedure as that of compound **88a**. Compound **88b** was obtained as a pale yellow oil (49% yield). ^{1}H NMR ($CDCl_3$) δ 1.36 (3H, t, *J* = 7.5 Hz), 2.48 (3H, s), 2.92 (2H, t, *J* = 7.0 Hz), 3.17 (2H, t, *J* = 7.0 Hz), 4.32 (2H, q, *J* = 7.5 Hz), 6.94–7.01 (1H, m), 7.05–7.13 (2H, m), 7.17–7.26 (1H, m).

Ethyl 3-bromo-4-[4-(methylthio)phenyl]-2-oxobutanoate (89a).

To a suspension of copper (II) bromide (212 g) in AcOEt (1600 mL) was added a solution of **88a** (80 g) in 800 mL of $CHCl_3$. The reaction mixture was refluxed for 13 h, cooled to room temperature, and filtered in vacuo. The filtrate was concentrated in vacuo, and the residue was purified by flash column chromatography over silica gel with hexane/AcOEt (1:1) as an eluent to give **89a** (102 g, 97%) as a yellow oil. ^{1}H NMR ($CDCl_3$) δ 1.37 (3H, t, *J* = 7.0 Hz), 2.47 (3H, s), 3.20 (1H, dd, *J* = 14.5, 7.5 Hz), 3.49 (1H, dd, *J* = 14.5, 7.5 Hz), 4.35 (2H, q, *J* = 7.0 Hz), 5.22 (1H, d, *J* = 7.5 Hz), 7.17 (2H, d, *J* = 8.5 Hz), 7.20 (2H, d, *J* = 8.5 Hz).

Ethyl 3-bromo-4-[3-(methylthio)phenyl]-2-oxobutanoate (89b).

Compound **89b** was prepared from **88b** and copper (II) bromide according to the same procedure as that of compound **89a**. Compound **89b** was obtained as a pale brown oil (96% yield). ^{1}H NMR ($CDCl_3$) δ 1.38 (3H, t, *J* = 7.5 Hz), 2.47 (3H, s), 3.21 (1H, dd, *J* = 14.5, 7.5 Hz), 3.50 (1H, dd, *J* = 14.5, 7.5 Hz), 4.36 (2H, q, *J* = 7.5 Hz), 5.21 (1H, t, *J* = 7.5 Hz), 6.98–7.05 (1H, m), 7.11–7.29 (3H, m).

Ethyl 3-bromo-2-oxo-4-phenylbutanoate (89c).

Compound **89c** was prepared from **88c** and copper (II) bromide according to the same procedure as

that of compound **89a.** Compound **89c** was obtained as a yellow oil (84% yield). ^{1}H NMR ($CDCl_3$) δ 1.37 (3H, t, J = 7.0 Hz), 3.25 (1H, dd, J = 14.5, 7.5 Hz), 3.54 (1H, dd, J = 14.5, 7.5 Hz), 4.35 (2H, q, J = 7.0 Hz), 5.27 (1H, d, J = 7.5 Hz), 7.18–7.41 (5H, m).

Methyl 2-amino-5-[4-(methylthio)phenyl]-1,3-thiazole-4-carboxylate (80a).

Compound **79** (4.1 g) was dissolved in EtOH (55 mL), and then thiourea (1.3 g) was added to the solution. The reaction mixture was refluxed for an hour under a nitrogen atmosphere. The cooled reaction mixture was evaporated in vacuo. The residual solid was suspended in saturated aqueous $NaHCO_3$ and H_2O. The solid was collected by filtration, and purified by flash column chromatography over silica gel with $CHCl_3$/MeOH (10:1) as an eluent to give **80a** (2.7 g, 75%) as a brown solid. Mp 184–185 ˚C; ^{1}H NMR (DMSO-d_6) δ 2.50 (3H, s), 3.64 (3H, s), 7.25 (2H, d, J = 8.5 Hz), 7.34 (2H, d, J = 8.5 Hz); FAB MS m/e $(M+H)^+$ 281.

Ethyl 2-amino-5-[4-(methylthio)benzyl]-1,3-thiazole-4-carboxylate (80b).

Compound **89a** (39.0 g) was dissolved in EtOH (600 mL), and then thiourea (17.9 g) was added to the solution. The reaction mixture was refluxed for 2 h under a nitrogen atmosphere. The cooled reaction mixture was evaporated in vacuo. Saturated aqueous $NaHCO_3$ and H_2O were added to the residue, and the mixture was extracted with AcOEt. The organic layer was washed with H_2O and brine, dried over anhydrous $MgSO_4$, and concentrated in vacuo. The residue was purified by flash column chromatography over silica gel with $CHCl_3$/MeOH (40:1–10:1) as an eluent to give **80b** (30.9 g, 85%) as a yellow solid. Mp 111–112.5 ˚C; ^{1}H NMR (DMSO-d_6) δ 1.25 (3H, t, J = 7.0 Hz), 2.44 (3H, s), 4.20 (2H, q, J = 7.0 Hz), 4.28 (2H, s), 7.02 (2H, s), 7.19 (4H, s); IR (KBr) cm^{-1}: 3465, 3429, 3253, 1718, 1703, 1620, 1597, 1545, 1489, 1437, 1371, 1323, 1232, 1215, 1194, 1130, 1109, 1093, 1034; FAB MS m/e $(M+H)^+$ 309; HRMS (ESI) Calcd for $C_{14}H_{17}N_2O_2S_2$ $(M+H)^+$: 309.0731, found: 309.0738.

Ethyl 2-amino-5-[3-(methylthio)benzyl]-1,3-thiazole-4-carboxylate (80c).

Compound **80c** was prepared from **89b** and thiourea according to the same procedure as that of compound **80b**. Compound **80c** was obtained as a yellow amorphous (90% yield). ^{1}H NMR (DMSO-d_6) δ 1.25 (3H, t, J = 7.0 Hz), 2.45 (3H, s), 4.21 (2H, q, J = 7.0 Hz), 4.30 (2H, s), 6.96-7.29 (4H, m); FAB MS *m*/*e* $(M+H)^+$ 309.

Ethyl 2-amino-5-benzyl-1,3-thiazole-4-carboxylate (80d).

Compound **80d** was prepared from **89c** and thiourea according to the same procedure as that of compound **80b**. Compound **80d** was obtained as an off-white solid (75% yield). Mp 86–88 ˚C; ^{1}H NMR (DMSO-d_6) δ 1.25 (3H, t, *J* = 7.0 Hz), 4.21 (2H, q, *J* = 7.0 Hz), 4.33 (2H, s), 7.02 (2H, s), 7.11–7.39 (5H, m); FAB MS *m*/*e* $(M+H)^+$ 263.

Methyl 2-(acetylamino)-5-[4-(methylthio)phenyl]-1,3-thiazole-4-carboxylate (90a).

Compound **80a** (8.8 g) was dissolved in pyridine (88 mL), and then acetyl chloride (6.7 mL) was added dropwise to the solution at 0 ˚C under a nitrogen atmosphere. The reaction mixture was stirred at room temperature for 30 min and at 50 ˚C for 2 h. After cooling to 0 ˚C, H_2O was added to the solution. The precipitate was filtered in vacuo, and the solid was washed with Et_2O to give **90a** (9.3 g, 92%) as an off-white solid. Mp 253–254.5 ˚C; ^{1}H NMR (DMSO-d_6) δ 2.16 (3H, s), 2.52 (3H, s), 3.70 (3H, s), 7.30 (2H, d, *J* = 8.5 Hz), 7.44 (2H, d, *J* = 8.5 Hz); FAB MS *m*/*e* $(M+H)^+$ 323.

Ethyl 2-(acetylamino)-5-[4-(methylthio)benzyl]-1,3-thiazole-4-carboxylate (90b).

Compound **90b** was prepared from **80b** and acetyl chloride according to the same procedure as that of compound **90a**. Compound **90b** was obtained as an off-white solid (93% yield). Mp 205–206 ˚C; ^{1}H NMR (DMSO-d_6) δ 1.28 (3H, t, *J* = 7.0 Hz), 2.09 (3H, s), 2.45 (3H, s), 4.27 (2H, q, *J* = 7.0 Hz), 4.43 (2H, s), 7.22 (4H, s), 12.41 (1H, s); IR (KBr) cm^{-1}: 3271, 2991, 1720, 1691, 1537, 1493, 1373, 1338, 1294, 1261, 1232, 1198, 1149, 1111, 1092, 1024; FAB MS *m*/*e* $(M+H)^+$ 351; HRMS (ESI) Calcd for $C_{16}H_{19}N_2O_3S_2$ $(M+H)^+$: 351.0837, found: 351.0851; Anal. Calcd for $C_{16}H_{18}N_2O_3S_2 \cdot 0.1H_2O$: C, 54.55; H, 5.21; N, 7.95; S, 18.21. Found: C, 54.24; H, 5.19; N, 8.04; S,

18.37.

Ethyl 2-(acetylamino)-5-[3-(methylthio)benzyl]-1,3-thiazole-4-carboxylate (90c).

Compound **90c** was prepared from **80c** and acetyl chloride according to the same procedure as that of compound **90a**. Compound **90c** was obtained as a colorless solid (60% yield). Mp 187.5–188.5 ˚C; ^{1}H NMR (DMSO-d_6) δ 1.28 (3H, t, J = 7.0 Hz), 2.09 (3H, s), 2.45 (3H, s), 4.28 (2H, q, J = 7.0 Hz), 4.45 (2H, s), 7.00–7.23 (3H, m), 7.26 (1H, t, J = 7.5 Hz), 12.43 (1H, s); FAB MS *m*/*e* $(M+H)^+$ 351.

Ethyl 2-(acetylamino)-5-benzyl-1,3-thiazole-4-carboxylate (90d).

Compound **90d** was prepared from **80d** and acetyl chloride according to the same procedure as that of compound **90a**. Compound **90d** was obtained as a brown solid (79% yield). Mp 178–180 ˚C; ^{1}H NMR (DMSO-d_6) δ 1.28 (3H, t, J = 7.0 Hz), 2.09 (3H, s), 4.28 (2H, q, J = 7.0 Hz), 4.48 (2H, s), 7.19–7.39 (5H, m), 12.41 (1H, s); FAB MS *m*/*e* $(M+H)^+$ 305.

***N*-{4-Formyl-5-[4-(methylsulfanyl)phenyl]-1,3-thiazol-2-yl}acetamide (91a).**

Compound **90a** (200 mg) was dissolved in THF (2 mL), and then lithium aluminum hydride (35 mg) was added portionwise to the solution at 0 ˚C under a nitrogen atmosphere. The reaction mixture was stirred at 0 ˚C for 30 min, at room temperature for 30 min, and then quenched with MeOH. 1 M HCl was added and the mixture was extracted with AcOEt. The aqueous layer was extracted with AcOEt (twice). The combined organic layer was washed with brine, dried over anhydrous $MgSO_4$, and concentrated in vacuo. The residual solid was dissolved in MeOH (0.4 mL) and $CHCl_3$ (7 mL). Manganese (IV) oxide (1.1 g) was then added to the solution under a nitrogen atmosphere. The reaction mixture was stirred at room temperature for 13 h and filtered through a celite® pad. The filtrate was concentrated in vacuo. The residue was purified by flash column chromatography over silica gel with $CHCl_3$/MeOH (20:1) as an eluent to give **91a** (154 mg, 85%) as a pale brown amorphous. ^{1}H NMR (DMSO-d_6) δ 2.18 (3H, s), 2.54 (3H, s), 7.38 (2H, d, J = 8.5 Hz), 7.58 (2H, d,

J = 8.5 Hz), 9.77 (1H, s), 12.59 (1H, br s); FAB MS m/e $(M+H)^+$ 293.

N-{4-Formyl-5-[4-(methylthio)benzyl]-1,3-thiazol-2-yl}acetamide (91b).

Compound **91b** was prepared from **90b** according to the same procedure as that of compound **91a**. Compound **91b** was obtained as a yellow wax (71% yield). ^{1}H NMR (DMSO-d_6) δ 2.12 (3H, s), 2.45 (3H, s), 4.48 (2H, s), 7.23 (4H, s), 10.03 (1H, s), 12.33 (1H, s); FAB MS m/e $(M+H)^+$ 307.

N-{4-Formyl-5-[3-(methylthio)benzyl]-1,3-thiazol-2-yl}acetamide (91c).

Compound **91c** was prepared from **90c** according to the same procedure as that of compound **91a**. Compound **91c** was obtained as an off-white solid (71% yield). Mp 148–149.5 ˚C; ^{1}H NMR (DMSO-d_6) δ 2.12 (3H, s), 2.45 (3H, s), 4.50 (2H, s), 6.99–7.25 (3H, m), 7.27 (1H, t, J = 7.5 Hz), 10.04 (1H, s), 12.35 (1H, br s); FAB MS m/e $(M+H)^+$ 307.

N-(5-benzyl-4-formyl-1,3-thiazol-2-yl)acetamide (91d).

Compound **91d** was prepared from **90d** according to the same procedure as that of compound **91a**. Compound **91d** was obtained as a pale yellow solid (67% yield). Mp 191–192.5 ˚C; ^{1}H NMR (DMSO-d_6) δ 2.12 (3H, s), 4.53 (2H, s), 7.19–7.40 (5H, m), 10.04 (1H, s), 12.34 (1H, s); FAB MS m/e $(M+H)^+$ 261.

A mixture of N-{5-[4-(methylthio)phenyl]-4-[(*E*)-2-(4-nitrophenyl)ethenyl]-1,3-thiazol-2-yl}-acetamide and N-{5-[4-(methylthio)phenyl]-4-[(*Z*)-2-(4-nitrophenyl)ethenyl]-1,3-thiazol-2-yl}-acetamide (92a).

To a mixture of 4-nitrobenzyl bromide (**8**) (182 mg) in DMF (2 mL) was added triphenylphosphine (221 mg) at 0 ˚C under a nitrogen atmosphere, and the reaction mixture was stirred at room temperature for 3 h.

Potassium *tert*-butoxide (111 mg) and **91a** (145mg) were then added to the mixture at 0 ˚C, which was stirred at room temperature for 15 h. The reaction mixture was poured into ice-H_2O and

extracted with AcOEt. The organic layer was washed with 1 M HCl, H_2O and brine, dried over anhydrous $MgSO_4$, and concentrated in vacuo. The residue was purified by flash column chromatography over silica gel with $CHCl_3$/MeOH (40:1) as an eluent to give **92a** (E : Z = 2 : 1) (177 mg, 87%) as a brown wax. FAB MS *m*/*e* $(M+H)^+$ 412.

A mixture of *N*-{5-[4-(methylsulfonyl)phenyl]-4-[(*E*)-2-(4-nitrophenyl)ethenyl]-1,3-thiazol-2-yl}acetamide and *N*-{5-[4-(methylsulfonyl)phenyl]-4-[(*Z*)-2-(4-nitrophenyl)ethenyl]-1,3-thiazol-2-yl}acetamide (92b).

Oxone® (408 mg) was suspended in H_2O (1 mL) and THF (1 mL), and then **92a** (182 mg) in THF (3 mL) was added dropwise to the suspension at 0 ˚C. The reaction mixture was stirred at room temperature for 2 h, and then H_2O was added to the suspension. The precipitate was filtered in vacuo. The solid was washed with H_2O and AcOEt to give **92b** (83 mg, 42%, E : Z = 2 : 1) as a yellow solid. Mp 294–295 ˚C; FAB MS *m*/*e* $(M+H)^+$ 444.

A mixture of *N*-{5-[4-(methylthio)benzyl]-4-[(*E*)-2-(4-nitrophenyl)vinyl]-1,3-thiazol-2-yl}-acetamide and *N*-{5-[4-(methylthio)benzyl]-4-[(*Z*)-2-(4-nitrophenyl)vinyl]-1,3-thiazol-2-yl}-acetamide (92c).

Compound **92c** was prepared from **91b** according to the same procedure as that of compound **92a**. Compound **92c** was obtained as a yellow amorphous (97% yield, E : Z = 1 : 2). FAB MS *m*/*e* $(M+H)^+$ 426.

A mixture of *N*-{5-[4-(methylsulfonyl)benzyl]-4-[(*E*)-2-(4-nitrophenyl)vinyl]-1,3-thiazol-2-yl}-acetamide and *N*-{5-[4-(methylsulfonyl)benzyl]-4-[(*Z*)-2-(4-nitrophenyl)vinyl]-1,3-thiazol-2-yl}-acetamide (92d).

Compound **92d** was prepared from **92c** according to the same procedure as that of compound **92b**. Compound **92d** was obtained as a yellow solid (99% yield, E : Z = 1 : 2). FAB MS *m*/*e* $(M+H)^+$ 458.

A mixture of *N*-{5-[3-(methylthio)benzyl]-4-[(*E*)-2-(4-nitrophenyl)vinyl]-1,3-thiazol-2-yl}-acetamide and *N*-{5-[3-(methylthio)benzyl]-4-[(*Z*)-2-(4-nitrophenyl)vinyl]-1,3-thiazol-2-yl}-acetamide (92e).

Compound **92e** was prepared from **91c** according to the same procedure as that of compound **92a**. Compound **92e** was obtained as a yellow amorphous (97% yield, E : Z = 1 : 2). FAB MS *m*/*e* $(M+H)^+$ 426.

A mixture of *N*-{5-[3-(methylsulfonyl)benzyl]-4-[(*E*)-2-(4-nitrophenyl)vinyl]-1,3-thiazol-2-yl}-acetamide and *N*-{5-[3-(methylsulfonyl)benzyl]-4-[(*Z*)-2-(4-nitrophenyl)vinyl]-1,3-thiazol-2-yl}-acetamide (92f).

Compound **92f** was prepared from **92e** according to the same procedure as that of compound **92b**. Compound **92f** was obtained as a brown amorphous (100% yield, E : Z = 1 : 2). FAB MS *m*/*e* $(M+H)^+$ 458.

A mixture of *N*-{5-benzyl-4-[(*E*)-2-(4-nitrophenyl)vinyl]-1,3-thiazol-2-yl}acetamide and *N*-{5-benzyl-4-[(*Z*)-2-(4-nitrophenyl)vinyl]-1,3-thiazol-2-yl}acetamide (92g).

Compound **92g** was prepared from **91d** according to the same procedure as that of compound **92a**. Compound **92g** was obtained as a yellow amorphous (107% yield, E : Z = 1 : 2). FAB MS *m*/*e* $(M+H)^+$ 380.

***N*-{4-[2-(4-Aminophenyl)ethyl]-5-[4-(methylsulfonyl)phenyl]-1,3-thiazol-2-yl}acetamide (93a).**

A mixture of **92b** (600 mg), 10% palladium on carbon (906 mg), MeOH (6 mL), THF (6 mL) and AcOH (1 mL) was stirred at room temperature for 3 h under a hydrogen atmosphere (3 atm), and filtered through a celite® pad. 1 M NaOH was added to the residue, and the mixture was extracted with AcOEt. The organic layer was washed with H_2O and brine, dried over anhydrous $MgSO_4$, and concentrated in vacuo. The residual solid was washed with Et_2O to give **93a** (366 mg, 65%) as a pale

yellow solid. Mp 202–204 ˚C; ^{1}H NMR (DMSO-d_6) δ 2.17 (3H, s), 2.77–2.88 (4H, m), 3.24 (3H, s), 6.84 (2H, br s), 6.45 (2H, d, J = 8.5 Hz), 6.77 (2H, d, J = 8.5 Hz), 7.49 (2H, d, J = 8.5 Hz), 7.91 (2H, d, J = 8.5 Hz), 12.34 (1H, br s); FAB MS m/e $(M+H)^+$ 416.

***N*-{4-[2-(4-Aminophenyl)ethyl]-5-[4-(methylsulfanyl)benzyl]-1,3-thiazol-2-yl}acetamide (93b).**

Compound **93b** was prepared from **92c** according to the same procedure as that of compound **93a**. Compound **93b** was obtained as a yellow solid (51% yield). ^{1}H NMR (DMSO-d_6) δ 2.07 (3H, s), 2.43 (3H, s), 2.64–2.79 (4H, m), 3.80 (2H, s), 4.83 (2H, s), 6.46 (2H, d, J = 8.5 Hz), 6.77 (2H, d, J = 8.5 Hz), 6.97 (2H, d, J = 8.5 Hz), 7.16 (2H, d, J = 8.5 Hz), 11.94 (1H, br s); IR (KBr) cm^{-1}: 3392, 3319, 1684, 1606, 1558, 1518, 1491, 1456, 1437, 1369, 1281, 1180, 1090; FAB MS m/e $(M+H)^+$ 398; HRMS (ESI) calcd for $C_{21}H_{24}N_3OS_2$ $(M+H)^+$: 398.1361, found: 398.1359.

***N*-{4-[2-(4-Aminophenyl)ethyl]-5-[4-(methylsulfonyl)benzyl]-1,3-thiazol-2-yl}acetamide (93c).**

Compound **93c** was prepared from **92d** according to the same procedure as that of compound **93a**. Compound **93c** was obtained as a brown oil (90% yield). ^{1}H NMR (DMSO-d_6) δ 2.08 (3H, s), 2.58–2.87 (4H, m), 3.18 (3H, s), 3.98 (2H, s), 4.85 (2H, s), 6.46 (2H, d, J = 8.5 Hz), 6.77 (2H, d, J = 8.5 Hz), 7.27 (2H, d, J = 8.5 Hz), 7.82 (2H, d, J = 8.5 Hz), 12.02 (1H, s); FAB MS m/e $(M+H)^+$ 430.

***N*-{4-[2-(4-Aminophenyl)ethyl]-5-[3-(methylsulfonyl)benzyl]-1,3-thiazol-2-yl}acetamide (93d).**

Compound **93d** was prepared from **92f** according to the same procedure as that of compound **93a**. Compound **93d** was obtained as a yellow amorphous (51% yield). ^{1}H NMR (DMSO-d_6) δ 2.08 (3H, s), 2.59–2.86 (4H, m), 3.18 (3H, s), 4.02 (2H, s), 4.84 (2H, br s), 6.46 (2H, d, J = 8.5 Hz), 6.78 (2H, d, J = 8.5 Hz), 7.25–7.88 (4H, m), 12.03 (1H, s); FAB MS m/e $(M+H)^+$ 430.

***N*-{4-[2-(4-Aminophenyl)ethyl]-5-benzyl-1,3-thiazol-2-yl}acetamide (93e).**

Compound **93e** was prepared from **92g** according to the same procedure as that of compound **93a**. Compound **93e** was obtained as an off-white amorphous (61% yield). ^{1}H NMR (DMSO-d_6) δ 2.07

(3H, s), 2.59–2.85 (4H, m), 3.85 (2H, s), 4.84 (2H, s), 6.46 (2H, d, *J* = 8.5 Hz), 6.78 (2H, d, *J* = 8.5 Hz), 7.07 (2H, d, *J* = 8.0 Hz), 7.16–7.31 (3H, m), 11.96 (1H, s); FAB MS *m*/*e* $(M+H)^+$ 352.

Di-*tert*-butyl {[(4-{2-[2-(acetylamino)-5-(4-(methylsulfonyl)phenyl)-1,3-thiazol-4-yl]ethyl}-phenyl)amino]methylidene}biscarbamate (94a).

A mixture of **93a** (360 mg), *N*,*N'*-bis(*tert*-butoxycarbonyl)-1*H*-pyrazole-1-carboxamidine (**31**) (296 mg) and THF (7 mL) was stirred at room temperature for 12 h, and then concentrated in vacuo. The residue was purified by flash column chromatography over silica gel with $CHCl_3$/AcOEt (1:1) as an eluent to give **94a** (451 mg, 79%) as a colorless amorphous. ^{1}H NMR (DMSO-d_6) δ 1.39 (9H, s), 1.51 (9H, s), 2.17 (3H, s), 2.97 (4H, s), 3.24 (3H, s), 7.11 (2H, d, *J* = 8.5 Hz), 7.38 (2H, d, *J* = 8.5 Hz), 7.56 (2H, d, *J* = 8.5 Hz), 7.92 (2H, d, *J* = 8.5 Hz), 9.92 (1H, s), 11.43 (1H, br s), 12.34 (1H, br s); FAB MS *m*/*e* $(M+H)^+$ 658.

***tert*-Butyl {(*Z*)-{[4-(2-{2-acetamido-5-[4-(methylsulfanyl)benzyl]-1,3-thiazol-4-yl}ethyl)phenyl]-amino}[(*tert*-butoxycarbonyl)amino]methylene}carbamate (94b).**

Compound **94b** was prepared from **93b** and **31** according to the same procedure as that of compound **94a**. Compound **94b** was obtained as a pale yellow oil (73% yield). ^{1}H NMR (DMSO-d_6) δ 1.39 (9H, s), 1.51 (9H, s), 2.08 (3H, s), 2.42 (3H, s), 2.85 (4H, s), 3.83 (2H, s), 6.99 (2H, d, J = 8.5 Hz), 7.12 (2H, d, J = 8.5 Hz), 7.14 (2H, d, J = 8.5 Hz), 7.43 (2H, d, J = 8.5 Hz), 9.69 (1H, s), 11.44 (1H, s), 11.97 (1H, s); FAB MS *m*/*e* $(M+H)^+$ 640.

Di-*tert*-butyl ((*Z*)-{[4-(2-{2-(acetylamino)-5-[4-(methylsulfonyl)benzyl]-1,3-thiazol-4-yl}ethyl)-phenyl]amino}methylidene)biscarbamate (94c).

Compound **94c** was prepared from **93c** and **31** according to the same procedure as that of compound **94a**. Compound **94c** was obtained as an off-white amorphous (73% yield). ^{1}H NMR (DMSO-d_6) δ 1.39 (9H, s), 1.51 (9H, s), 2.08 (3H, s), 2.86 (4H, s), 3.16 (3H, s), 4.03 (2H, s), 7.13 (2H, d, *J* = 8.5 Hz), 7.33 (2H, d, *J* = 8.5 Hz), 7.43 (2H, d, *J* = 8.5 Hz), 7.81 (2H, d, *J* = 8.5 Hz), 9.97 (1H, s), 11.45

(1H, s), 12.05 (1H, s); FAB MS *m*/*e* $(M+H)^+$ 672.

Di-*tert*-butyl ({[4-(2-{2-(acetylamino)-5-[3-(methylsulfonyl)benzyl]-1,3-thiazol-4-yl}ethyl)-phenyl]amino}methylidene)biscarbamate (94d).

Compound **94d** was prepared from **93d** and **31** according to the same procedure as that of compound **94a**. Compound **94d** was obtained as a yellow amorphous (68% yield). ^{1}H NMR (DMSO-d_6) δ 1.39 (9H, s), 1.51 (9H, s), 2.09 (3H, s), 2.85 (4H, s), 3.18 (3H, s), 4.06 (2H, s), 7.13 (2H, d, J = 8.0 Hz), 7.37–7.45 (1H, m), 7.41 (2H, d, J = 8.0 Hz), 7.56 (1H, t, J = 8.0 Hz), 7.74–7.80 (2H, m), 9.94 (1H, s), 11.44 (1H, s), 12.05 (1H, s); FAB MS *m*/*e* $(M+H)^+$ 672.

Di-*tert*-butyl {(*Z*)-[(4-{2-[2-(acetylamino)-5-benzyl-1,3-thiazol-4-yl]ethyl}phenyl)amino]-methylidene}biscarbamate (94e).

Compound **94e** was prepared from **93e** and **31** according to the same procedure as that of compound **94a**. Compound **94e** was obtained as an off-white amorphous (85% yield). ^{1}H NMR (DMSO-d_6) δ 1.39 (9H, s), 1.51 (9H, s), 2.07 (3H, s), 2.85 (4H, s), 3.89 (2H, s), 7.05–7.33 (7H, m), 7.42 (2H, d, J = 8.5 Hz), 9.95 (1H, s), 11.44 (1H, s), 11.99 (1H, s); FAB MS *m*/*e* $(M+H)^+$ 594.

***N*-{4-[2-(4-{[Amino(imino)methyl]amino}phenyl)ethyl]-5-[4-(methylsulfonyl)phenyl]-1,3-thiazol-2-yl}acetamide hydrochloride (95a).**

A mixture of **94a** (130 mg) and 4 M HCl/dioxane (2 mL) was stirred at room temperature for 13 h under a nitrogen atmosphere. The solvent was removed in vacuo. The residue was solidified with AcOEt to give **95a** (83 mg, 85%) as an off-white solid. Mp 228–229.5 ˚C; ^{1}H NMR (DMSO-d_6) δ 2.18 (3H, s), 2.99 (4H, br s), 3.25 (3H, s), 7.11 (2H, d, J = 8.0 Hz), 7.22 (2H, d, J = 8.0 Hz), 7.38 (3H, br s), 7.57 (2H, d, J = 8.0 Hz), 7.94 (2H, d, J = 8.0 Hz), 9.79 (1H, s), 12.36 (1H, br s); FAB MS *m*/*e* $(M+H)^+$ 458.

***N*-{4-[2-(4-{[Amino(imino)methyl]amino}phenyl)ethyl]-5-[4-(methylsulfanyl)benzyl]-1,3-thiazo**

l-2-yl}acetamide (95b).

A mixture of **94b** (200 mg) and 4 M HCl/dioxane (8 mL) was stirred at room temperature for 2 h under a nitrogen atmosphere. The solvent was removed in vacuo, and the residue was dissolved in H_2O and AcOEt. The solution was made basic (pH=8) by saturated aqueous $NaHCO_3$. The precipitate was filtered in vacuo to give **95b** (31 mg, 23%) as a colorless solid. ^{1}H NMR (DMSO-d_6) δ 2.07 (3H, s), 2.44 (3H, s), 2.72–2.84 (4H, m), 3.85 (2H, s), 6.73 (2H, d, J = 8.5 Hz), 6.99 (2H, d, J = 8.5 Hz), 7.05 (2H, d, J = 8.5 Hz), 7.17 (2H, d, J = 8.5 Hz); IR (KBr) cm^{-1}: 2922, 1682, 1670, 1576, 1558, 1543, 1514, 1493, 1456, 1404, 1369, 1338, 1319, 1292, 1093; FAB MS *m*/*e* $(M+H)^+$ 440; HRMS (ESI). calcd for $C_{22}H_{26}N_5OS_2$ $(M+H)^+$: 440.1579, found: 440.1581.

***N*-{4-[2-(4-{[Amino(imino)methyl]amino}phenyl)ethyl]-5-[4-(methylsulfonyl)benzyl]-1,3-thiazo l-2-yl}acetamide (95c).**

Compound **95c** was prepared from **94c** according to the same procedure as that of compound **95b**. Compound **95c** was obtained as a pale yellow solid (100% yield). Mp 228–229.5 ˚C; ^{1}H NMR (DMSO-d_6) δ 2.08 (3H, s), 2.79 (4H, m), 3.18 (3H, s), 4.05 (2H, s), 6.72 (2H, d, *J* = 8.0 Hz), 6.99 (2H, d, *J* = 8.0 Hz), 7.37 (2H, d, *J* = 8.5 Hz), 7.84 (2H, d, *J* = 8.5 Hz); ^{13}C NMR (DMSO-d_6) δ 23.1, 30.7, 30.9, 34.8, 43.8, 122.0, 123.1, 127.5, 129.0, 129.3, 133.7, 139.0, 146.1, 146.9, 147.1, 152.8, 156.6, 168.8; IR (KBr) cm^{-1}: 3429, 2927, 1682, 1668, 1624, 1597, 1514, 1444, 1398, 1304, 1271, 1205, 1184, 1147, 1090; FAB MS *m*/*e* $(M+H)^+$ 472; HRMS (ESI) calcd for $C_{22}H_{26}N_5O_3S_2$ $(M+H)^+$: 472.1477, found: 472.1471; Anal. Calcd for $C_{22}H_{25}N_5O_3S_2 \cdot 0.4H_2O$: C, 55.19; H, 5.43; N, 14.63; S, 13.39. Found: C, 55.19; H, 5.49; N, 14.71; S, 13.34.

***N*-{4-[2-(4-{[Amino(imino)methyl]amino}phenyl)ethyl]-5-[3-(methylsulfonyl)benzyl]-1,3-thiazo l-2-yl}acetamide (95d).**

Compound **95d** was prepared from **94d** according to the same procedure as that of compound **95b**. Compound **95d** was obtained as an off-white solid (47% yield). Mp 212–213.5 ˚C; ^{1}H NMR (DMSO-d_6) δ 2.08 (3H, s), 2.67–2.91 (4H, m), 3.19 (3H, s), 4.08 (2H, s), 6.80 (2H, d, *J* = 8.0 Hz),

7.04 (2H, d, *J* = 8.0 Hz), 7.42 (1H, d, *J* = 8.0 Hz), 7.57 (1H, t, *J* = 8.0 Hz), 7.77 (1H, s), 7.79 (1H, d, *J* = 8.0 Hz); IR (KBr) cm^{-1}: 3425, 1697, 1685, 1635, 1576, 1560, 1516, 1456, 1367, 1348, 1296, 1257, 1207, 1138, 1084; FAB MS *m*/*e* $(M+H)^+$ 472; HRMS (ESI) calcd for $C_{22}H_{26}N_5O_3S_2$ $(M+H)^+$: 472.1477, found: 472.1481.

***N*-{4-[2-(4-{[Amino(imino)methyl]amino}phenyl)ethyl]-5-benzyl-1,3-thiazol-2-yl}acetamide hydrochloride (95e).**

Compound **95e** was prepared from **94e** according to the same procedure as that of compound **95a**. Compound **95e** was obtained as a pale green solid (96% yield). Mp 97–99 ˚C; ^{1}H NMR (DMSO-d_6) δ 2.09 (3H, s), 2.86 (4H, s), 3.93 (2H, s), 7.05–7.37 (9H, m), 7.47 (3H, s), 9.98 (1H, s), 12.01 (1H, br s); FAB MS *m*/*e* $(M+H)^+$ 394.

N-{5-Iodo-4-[(Z)-2-(4-nitrophenyl)ethenyl]-1,3-thiazol-2-yl}acetamide (9i).

To a solution of **9** (400 mg) in MeOH (8 mL) and THF (4 mL) was added NIS (470 mg) at 0 ˚C under a nitrogen atmosphere, and the reaction mixture was stirred at room temperature for 12 h. The mixture was concentrated in vacuo. The residue was dissolved in $CHCl_3$. The organic solution was washed with saturated aqueous $NaHCO_3$, H_2O and brine, dried over anhydrous $MgSO_4$, and concentrated in vacuo. The residue was purified by flash column chromatography over silica gel with $CHCl_3$/MeOH (50:1–20:1) as an eluent to give **9i** (542 mg, 94%) as a yellow solid. ^{1}H NMR (DMSO-d_6) δ 2.12 (3H, s), 6.61 (1H, d, *J* = 12.5 Hz), 6.80 (1H, d, *J* = 12.5 Hz), 7.64 (2H, d, *J* = 8.5 Hz), 8.14 (2H, d, *J* = 8.5 Hz), 12.19 (1H, br s); FAB MS m/e $(M+H)^+$ 416.

N-{5-[4-(Methylsulfonyl)phenyl]-4-[(Z)-2-(4-nitrophenyl)ethenyl]-1,3-thiazol-2-yl}acetamide (92b:*Z isomer*).

A mixture of **9i** (80 mg) and $Pd(PPh_3)_4$ (97 mg) in DME (4 mL) was stirred at room temperature for 15 min. [4-(methylsulfonyl)phenyl]boronic acid (160 mg), Na_2CO_3 (85 mg) and H_2O (0.8 mL) were added under a nitrogen atmosphere and the mixture heated at 80 ˚C for 5 h. The solvents were then

removed in vacuo and the residue partitioned between H_2O and AcOEt. The organic layer was dried over anhydrous $MgSO_4$, and concentrated in vacuo. The residue was purified by flash column chromatography over silica gel with $CHCl_3$/MeOH (20:1–10:1) as an eluent to give **92b** (83 mg, 97%) as a yellow amorphous. ^{1}H NMR(DMSO-d_6) δ 2.15 (3H, s), 3.24 (3H, s), 6.72 (1H, d, J = 12.5 Hz), 6.85 (1H, d, J = 12.5 Hz), 7.53 (2H, d, J = 8.5 Hz), 7.71 (2H, d, J = 8.5 Hz), 7.94 (2H, d, J = 8.5 Hz), 8.09 (2H, d, J = 8.5 Hz), 12.21 (1H, br s); FAB MS m/e $(M+H)^+$ 444.

Ethyl 2-(acetylamino)-4-(chloromethyl)-1,3-thiazole-5-carboxylate (98).

Ethyl 4-chloro-3-oxobutanoate (**96**) (35.0 g) was dissolved in CH_2Cl_2 (70 mL), and then sulfuryl chloride (17.1 mL) in CH_2Cl_2 (20 mL) was added dropwise to the solution at 0 ˚C over 15 min under a nitrogen atmosphere. The reaction mixture was stirred at room temperature for 3 h and concentrated in vacuo. A mixture of the residual oil, *N*'-((*E*)-ethanoyl)carbamimidothioic acid (**97**) (25.1 g) and acetone (600 mL) was refluxed for 2.5 h. After cooling to room temperature, the reaction mixture was concentrated in vacuo. The residual solid was washed with H_2O and $^iPr_2O$ to give **98** (21.2 g, 38%) as a pale yellow solid. Mp 164–165 ˚C; ^{1}H NMR (DMSO-d_6) δ 1.30 (3H, t, J = 7.0 Hz), 2.19 (3H, s), 4.29 (2H, q, J = 7.0 Hz), 5.00 (2H, s), 12.72 (1H, s); FAB MS *m*/*e* $(M+H)^+$ 263.

Ethyl 2-(acetylamino)-4-[(*E*)-2-(4-nitrophenyl)ethenyl]-1,3-thiazole-5-carboxylate (100).

To a stirring solution of **98** (1.0 g) in DMF (20 mL) was added triphenylphosphine (1.2 g) at room temperature. The resultant mixture was stirred at 65 ˚C for 5 h. To the mixture was added potassium *tert*-butoxide (555 mg) at 0 ˚C, and the resultant mixture was stirred at 0 ˚C for 30 min. *p*-Nitrobenzaldehyde (**99**) (805 mg) was added at 0 ˚C. After stirring for an hour at room temperature, the reaction mixture was quenched with H_2O, and the precipitate was collected to give **100** (1.0 g, 73%) as a yellow solid. ^{1}H-NMR ($CDCl_3$), δ (ppm): 1.40 (3H, t, J = 7.2 Hz), 2.33 (3H, s), 4.38 (2H, q, J = 7.2 Hz), 7.59 (1H, d, J = 16.0 Hz), 7.70 (2H, d, J = 8.8 Hz), 8.18 (1H, d, J = 16.0 Hz), 8.22 (2H, d, J = 8.8 Hz), 8.90 (1H, m).

Ethyl 2-(acetylamino)-4-[2-(4-aminophenyl)ethyl]-1,3-thiazole-5-carboxylate (101).

Compound **100** (394 mg) was suspended in EtOH (4 mL) and THF (4 mL), and then 10% palladium on carbon (305 mg) was added to the mixture under a nitrogen atmosphere. The reaction mixture was stirred at room temperature for 3 h under a hydrogen atmosphere (4 atm), filtered through a celite® pad, and the filtrate was concentrated in vacuo. The residual amorphous was crystallized from Et_2O to give **101** (266 mg, 73%) as an off-white solid. Mp 184–185 ˚C; ^{1}H NMR (DMSO-d_6) δ 1.27 (3H, t, J = 7.0 Hz), 2.17 (3H, s), 2.73 (2H, t, J = 7.5 Hz), 3.17 (2H, t, J = 7.5 Hz), 4.23 (2H, q, J = 7.0 Hz), 4.84 (2H, br s), 6.47 (2H, d, J = 8.5 Hz), 6.82 (2H, d, J = 8.5 Hz), 12.51 (1H, br s); FAB MS *m*/*e* $(M+H)^+$ 334.

Ethyl 2-(acetylamino)-4-(2-{4-[(*tert*-butoxycarbonyl)amino]phenyl}-ethyl)-1,3-thiazole-5-carboxylate (102).

Compound **101** (310 mg) was dissolved in THF (6 mL) under a nitrogen atmosphere. bis(*tert*-Butyl)dicarbonate (223 mg) in THF (1 mL) was then added to the solution at room temperature. The reaction mixture was refluxed for 2 h. After cooling to room temperature, the mixture was concentrated in vacuo. The residual solid was washed with Et_2O to give **102** (371 mg, 92%) as an off-white solid. Mp 213–214 ˚C; ^{1}H NMR (DMSO-d_6) δ 1.26 (3H, t, J = 7.0 Hz), 1.46 (9H, s), 2.17 (3H, s), 2.85 (2H, t, J = 7.5 Hz), 3.23 (2H, t, J = 7.5 Hz), 4.22 (2H, q, J = 7.0 Hz), 7.04 (2H, d, J = 8.5 Hz), 7.33 (2H, d, J = 8.5 Hz), 9.23 (1H, br s), 12.55 (1H, br s); FAB MS *m*/*e* $(M+H)^+$ 434.

2-(Acetylamino)-4-(2-{4-[(*tert*-butoxycarbonyl)-amino]phenyl}ethyl)-1,3-thiazole-5-carboxylic acid (103)

1 M NaOH (17.5 mL) was added to a solution of **102** (3.0 g) in EtOH (30 mL) and the mixture was refluxed for 5 h. After cooling to room temperature, the organic solvent was removed in vacuo. The aqueous solution was acidified (pH=4) with 1 M HCl and extracted with AcOEt (twice). The

combined organic layer was dried over anhydrous $MgSO_4$ and concentrated in vacuo. The residual solid (2.4 g) was dissolved in pyridine (45 mL), and then acetyl chloride (1.5 mL) was added dropwise to the solution at 0 ˚C under a nitrogen atmosphere. The reaction mixture was stirred at room temperature for 13 h, and pyridine was removed in vacuo. H_2O was added to the residue, and acidified with 1 M HCl. The precipitate was collected in vacuo. The solid was washed with H_2O and Et_2O to give **103** (2.7 g, 93%) as an off-white solid. Mp 237–238 ˚C; ^{1}H NMR (DMSO-d_6) δ 1.46 (9H, s), 2.16 (3H, s), 2.85 (2H, m), 3.23 (2H, m), 7.04 (2H, d, J = 8.5 Hz), 7.33 (2H, d, J = 8.5 Hz), 9.24 (1H, s), 12.46 (1H, s); FAB MS *m*/*e* $(M-H)^-$ 404.

***tert*-Butyl (4-{2-[2-(acetylamino)-5-formyl-1,3-thiazol-4-yl]ethyl}phenyl)carbamate (104).**

To a solution of **103** (8.3 g) in 44 mL of CH_2Cl_2 and 44 mL of DMF was added *N*,*O*-dimethylhdroxylamine hydrochloride (2.6 g), EDC (9.5 g) and HOBt (4.1 g). The solution was then stirred at room temperature for 3 h under a nitrogen atmosphere. The reaction mixture was diluted with 400 mL of AcOEt and washed with 100 mL×3 of H_2O. The organic layer was dried over anhydrous $MgSO_4$ and evaporated in vacuo. The residual solid was triturated with $^iPr_2O$ and collected by filtration to give *tert*-butyl {4-[2-(2-(acetylamino)-5-{[methoxy(methyl)amino]-carbonyl}-1,3-thiazol-4-yl)ethyl]phenyl}carbamate (7.0 g, 76%) as a pale yellow solid.

Lithium aluminum hydride (499 mg) was slowly added (over 15 min) at 0 °C under a nitrogen atmosphere to a solution of the compound obtained in Step 1 (3.9 g) in THF (80 mL). The mixture was stirred at 0 ˚C for an hour. 1 M aqueous potassium sodium tartrate solution (30 mL) was added slowly under ice-cooled conditions, and then the mixture was stirred at room temperature for another 30 min. The mixture was extracted with AcOEt, and the organic layer was dried over anhydrous $MgSO_4$, and then concentrated in vacuo. This pale yellow oil was triturated with $^iPr_2O$/AcOEt to give **104** (2.7 g, 78%, 2 steps yield:59%) as a pale yellow powder. ^{1}H NMR (DMSO-d_6) δ 1.46 (9H, s), 2.19 (3H, s), 2.90 (2H, t, J = 7.3 Hz), 3.22 (2H, t, J = 7.3 Hz), 7.01(2H, d, J = 8.5 Hz), 7.32 (2H, d, J = 8.5 Hz), 9.22 (1H, s), 9.77 (1H, s), 12.68 (1H, s); FAB MS *m*/*e* $(M+H)^+$ 390.

A mixture of *tert*-butyl {4-[2-(2-acetamido-5-{(*E*)-2-[4-(methylsulfonyl)phenyl]vinyl}-1,3-thiazol-4-yl)ethyl]phenyl}carbamate and *tert*-butyl {4-[2-(2-acetamido-5-{(*Z*)-2-[4-(methylsulfonyl)phenyl]vinyl}-1,3-thiazol-4-yl)ethyl]phenyl}carbamate (106).

To a mixture of 1-(chloromethyl)-4-(methylsulfonyl)benzene (**105**) (768 mg) in DMF (10 mL) was added triphenylphosphine (943 mg) at 0 ˚C under a nitrogen atmosphere, and the reaction mixture was stirred at 65 ˚C for 2 h. Potassium *tert*-butoxide (461 mg) and **104** (400 mg) were then added to the solution at 0 ˚C, and the mixture was stirred at room temperature for 30 h. The reaction mixture was poured into 1 M HCl under ice-cooled conditions and extracted with AcOEt. The organic layer was washed with H_2O and brine, dried over anhydrous $MgSO_4$, and concentrated in vacuo. The residue was purified by flash column chromatography over silica gel with hexane/AcOEt (1:1–1:2) as an eluent to give **106** (697 mg, 125%) as a pale brown oil. FAB MS *m*/*e* $(M+H)^+$ 542.

***tert*-Butyl *N*-{4-[2-(2-(acetylamino)-5-{2-[4-(methylsulfonyl)phenyl]ethyl}-1,3-thiazol-4-yl)-ethyl]phenyl}carbamate (107).**

A mixture of **106** (697 mg), 10% palladium on carbon (150 mg) and MeOH (10 mL) was stirred at room temperature for 6 h under a hydrogen atmosphere (3 atm). The reaction mixture was filtered through a celite® pad, and the filtrate was concentrated in vacuo. The residue was purified by flash column chromatography over silica gel with toluene/AcOEt (1:1) as an eluent to give **107** (508 mg, 73%) as a pale brown oil. FAB MS *m*/*e* $(M+H)^+$ 544.

***N*-(4-[2-(4-Aminophenyl)ethyl]-5-{2-[4-(methylsulfonyl)phenyl]ethyl}-1,3-thiazol-2-yl)-acetamide (108).**

A mixture of **107** (500 mg) and 4 M HCl/dioxane (10 mL) was stirred at room temperature for 5 h under a nitrogen atmosphere. The solvent was removed in vacuo. The residue was dissolved in H_2O. The solution was neutralized with 1 M NaOH and extracted with AcOEt (twice). The combined organic layer was dried over anhydrous $MgSO_4$ and concentrated in vacuo to give **108** (220 mg, 54%) as a white powder. ^{1}H NMR ($CDCl_3$) δ 2.23 (3H, s), 2.61 (4H, s), 2.78 (4H, s), 2.98 (3H, s),

3.55 (2H, br s), 6.57 (2H, d, *J* = 8.5 Hz), 6.81 (2H, d, *J* = 8.5 Hz), 7.25 (2H, d, *J* = 8.5 Hz), 7.82 (2H, d, *J* = 8.5 Hz), 8.80 (1H, s); FAB MS *m*/*e* $(M+H)^+$ 444.

_N_-(4-[2-(4-{[Amino(imino)methyl]amino}phenyl)ethyl]-5-{2-[4-(methylsulfonyl)phenyl]ethyl}-1,3-thiazol-2-yl)acetamide hydrochloride (109).

Compound **108** (200 mg), *N*,*N'*-bis(*tert*-butoxycarbonyl)-1*H*-pyrazole-1-carboxamidine (**31**) (420 mg) and diisopropylethylamine (0.12 mL) were dissolved in THF (4 mL). The mixture was stirred at room temperature for 12 h and then evaporated in vacuo. The residue was purified with silica gel chromatography (5% $CHCl_3$/MeOH) to give *tert*-butyl {(*E*)-({4-[2-(2-acetamido-5-{2-[4-(methylsulfonyl)phenyl]ethyl}-1,3-thiazol-4-yl)ethyl]phenyl}amino)[(*tert*-butoxycarbonyl)amino]methylene}carbamate (307 mg, 99%).

tert-Butyl {(*E*)-({4-[2-(2-acetamido-5-{2-[4-(methylsulfonyl)phenyl]ethyl}-1,3-thiazol-4-yl)ethyl]phenyl}amino)[(*tert*-butoxycarbonyl)amino]methylene}carbamate (228 mg) was dissolved in 4 M HCl/dioxane (5 mL). The mixture was stirred at room temperature overnight and then evaporated in vacuo. The residue was triturated with AcOEt to give **109** (183 mg, 106%) as a white solid. ^{1}H NMR (DMSO-d_6) δ 2.16 (3H, s), 2.67 (4H, br s), 2.82–2.94 (4H, m), 3.14 (3H, s), 7.12 (2H, d, *J* = 8.4 Hz), 7.20 (2H, d, *J* = 8.4 Hz), 7.43 (2H, d, *J* = 8.4 Hz), 7.82 (2H, d, *J* = 8.4 Hz), 9.87 (1H, s), 11.97 (1H, s); FAB MS *m*/*e* $(M+H)^+$ 486; HRMS calcd for $C_{23}H_{28}N_5O_3S_2$ $(M+H)^+$: 486.1634, found: 486.1633.

Ethyl 4-(4-iodophenyl)-2-oxobutanoate (111).

Sodium methoxide, 28% solution in MeOH (4.2 mL), was added dropwise to a mixture of **110** (6.6 g) and diethyl oxalate (5.9 mL) at 0 ˚C with stirring. The reaction mixture was stirred at 65 ˚C for 30 min under reduced pressure. 15% aqueous H_2SO_4 (44 mL) was added to the mixture, and the mixture was refluxed for 12 h. After cooling to room temperature, the mixture was extracted with AcOEt. The organic layer was washed with H_2O and brine, dried over anhydrous $MgSO_4$, and concentrated in vacuo. The residual oil (7.7 g) was dissolved in EtOH (25 mL), and concentrated H_2SO_4 (0.5 mL)

was added dropwise to the solution. The reaction mixture was refluxed for 3 h. After cooling to room temperature, EtOH was removed in vacuo. AcOEt and H_2O were added to the residue, and extracted. The organic layer was washed with H_2O and brine, dried over anhydrous $MgSO_4$, and concentrated in vacuo. The residue was purified by flash column chromatography over silica gel with hexane/AcOEt (15:1–9:1) as an eluent to give **111** (3.4 g, 47%) as a pale yellow oil. ^{1}H NMR ($CDCl_3$) δ 1.35 (3H, t, J = 7.0 Hz), 2.90 (2H, t, J = 7.5 Hz), 3.15 (2H, t, J = 7.5 Hz), 4.31 (2H, q, J = 7.0 Hz), 6.96 (2H, d, J = 8.0 Hz), 7.61 (2H, d, J = 8.0 Hz); FAB MS m/e $(M-H)^-$ 331.

Ethyl 3-bromo-4-(4-iodophenyl)-2-oxobutanoate (112).

To a suspension of copper(II) bromide (2.8 g) in AcOEt (50 mL) was added a solution of **111** (1.0 g) in 25 mL of $CHCl_3$. The mixture was refluxed for 13 h. After cooling to room temperature, the suspension was filtered in vacuo. The filtrate was concentrated in vacuo. The residue was purified by flash column chromatography over silica gel with hexane/AcOEt (1:1) as an eluent to give **112** (1.3 g, 108%) as a pale yellow oil. ^{1}H NMR ($CDCl_3$) δ 1.38 (3H, t, J = 7.0 Hz), 3.19 (1H, dd, J = 7.5, 14.6 Hz), 3.47 (1H, dd, J = 7.5, 14.6 Hz), 4.36 (2H, q, J = 7.0 Hz), 5.21 (1H, dd, J = 7.5, 7.5 Hz), 7.00 (2H, d, J = 8.5 Hz), 7.65 (2H, d, J = 8.5 Hz).

Ethyl 2-amino-5-(4-iodobenzyl)-1,3-thiazole-4-carboxylate hydrobromide (113).

Compound **112** (1.3 g) was dissolved in EtOH (26 mL), and then thiourea (244 mg) was added to the solution. The reaction mixture was refluxed for an hour under a nitrogen atmosphere. The cooled reaction mixture was evaporated in vacuo. The crude material was triturated with Et_2O to give **113** (1.4 g, 94%) as a pale yellow solid. ^{1}H NMR (DMSO-d_6) δ 1.27 (3H, t, J = 7.0 Hz), 4.28(2H, q, J = 7.0 Hz), 4.31 (2H, s), 7.10 (2H, d, J = 8.5 Hz), 7.69 (2H, d, J = 8.5 Hz); FAB MS m/e $(M+H)^+$ 389.

Ethyl 2-(acetylamino)-5-(4-iodobenzyl)-1,3-thiazole-4-carboxylate (114).

Compound **113** (1.4 g) was dissolved in CH_2Cl_2 (14 mL) under a nitrogen atmosphere. Pyridine (0.77 mL) and acetyl chloride (0.34 mL) were then added dropwise to the solution at 0 ˚C. The

reaction mixture was stirred at room temperature for an hour. The organic solution was washed with 1 M HCl, H_2O and brine, dried over anhydrous $MgSO_4$, and concentrated in vacuo. The residual solid was washed with $^{i}Pr_2O$ to give **114** (1.0 g, 79%) as a white solid. ^{1}H NMR (DMSO-d_6) δ 1.27 (3H, t, J = 7.0 Hz), 2.09 (3H, s), 4.26 (2H, q, J = 7.0 Hz), 4.43 (2H, s), 7.10 (2H, d, J = 8.0 Hz), 7.67 (2H, d, J = 8.0 Hz), 12.44 (1H, s); FAB MS *m*/*e* $(M+H)^+$ 431.

***N*-[4-Formyl-5-(4-iodobenzyl)-1,3-thiazol-2-yl]acetamide (115).**

Compound **114** (978 mg) was dissolved in THF (20 mL), and then lithium borohydride (150 mg) was added portionwise to the solution at 0 ˚C under a nitrogen atmosphere. The reaction mixture was refluxed for 4 h and quenched with MeOH and 1 M HCl at 0 ˚C. Anhydrous $MgSO_4$ was added to the mixture, and stirred at room temperature for an hour. The suspension was filtered in vacuo, and the filtrate was concentrated in vacuo to give a hydroxymethyl compound (1.3 g, 143%) as a white solid. The solid was dissolved in MeOH (2 mL) and $CHCl_3$ (20 mL). Manganese (IV) oxide (4 g) was then added to the solution under a nitrogen atmosphere. The reaction mixture was stirred at room temperature for 34 h and filtered through a celite® pad. The filtrate was concentrated in vacuo. The residual solid was washed with $^{i}Pr_2O$ to give **115** (873 mg, 100%) as a pale yellow solid. ^{1}H NMR (DMSO-d_6) δ 2.11 (3H, s), 4.48 (2H, s), 7.11 (2H, d, J = 8.5 Hz), 7.68 (2H, d, J = 8.5 Hz), 10.00 (1H, s); FAB MS *m*/*e* $(M+Na)^+$ 409.

A mixture of *N*-{5-(4-iodobenzyl)-4-[(*Z*)-2-(4-nitrophenyl)vinyl]-1,3-thiazol-2-yl}acetamide and *N*-{5-(4-iodobenzyl)-4-[(*E*)-2-(4-nitrophenyl)vinyl]-1,3-thiazol-2-yl}acetamide (116).

To a mixture of 4-nitrobenzyl bromide (**8**) (482 mg) in DMF (8.6 mL) was added triphenylphosphine (585 mg) at 0 ˚C under a nitrogen atmosphere, and the reaction mixture was stirred at room temperature for 4 h. Potassium *tert*-butoxide (300 mg) and **115** (861 mg) were then added to the mixture at 0 ˚C, and the mixture was stirred at room temperature for 2.5 h. The reaction mixture was poured into ice-H_2O and extracted with AcOEt. The organic layer was washed with 1 M HCl, H_2O and brine, dried over anhydrous $MgSO_4$, and concentrated in vacuo. The residue was purified by

flash column chromatography over silica gel with hexane/AcOEt (2:1–3:2) as an eluent to give **116** (Z: E = 2 : 1) (897 mg, 70%) as an orange amorphous. ^{1}H NMR ($CDCl_3$) δ 2.07 (3Hx2/3, s), 2.15 (3Hx1/3, s), 3.96 (2Hx2/3, s), 4.12 (2Hx1/3, s), 6.63 (1Hx2/3, d, J = 12.6 Hz), 6.70 (1Hx2/3, d, J = 12.6 Hz), 6.94 (2Hx2/3, d, J = 8.0 Hz), 6.99 (2Hx1/3, d, J = 8.0 Hz), 7.12 (1Hx1/3, d, J = 15.6 Hz), 7.25 (1Hx1/3, d, J = 15.6 Hz), 7.39 (2Hx2/3, d, J = 9.0 Hz), 7.56 (2Hx1/3, d, J = 8.5 Hz), 7.62 (2Hx2/3, d, J = 8.0 Hz), 7.65 (2Hx1/3, d, J = 8.5 Hz), 8.00 (2Hx2/3, d, J = 8.5 Hz), 8.22 (2Hx1/3, d, J = 8.5 Hz), 9.85 (1Hx1/3, s), 10.18 (1Hx2/3, s); FAB MS m/e $(M+H)^+$ 528.

A mixture of *N*-{5-(4-(methoxycarbonyl)benzyl)-4-[(*Z*)-2-(4-nitrophenyl)vinyl]-1,3-thiazol-2-yl}acetamide and *N*-{5-(4-(methoxycarbonyl)benzyl)-4-[(*E*)-2-(4-nitrophenyl)vinyl]-1,3-thiazol-2-yl}acetamide (117).

To a solution of **116** (Z:E=2:1) (558 mg) in MeOH (2.8 mL) and DMF (5.5 mL) were added palladium(II) acetate (50 mg), 1,3-bis(diphenylphosphino)propane (109 mg) and Et_3N (0.3 mL). Carbon monooxide gas was bubbled through the solution at room temperature for 30 min. The reaction mixture was then stirred at 70 ˚C for 6 h under a carbon monooxide atmosphere. The reaction mixture was cooled to room temperature, diluted with AcOEt, washed with brine, dried over anhydrous $MgSO_4$, and concentrated in vacuo. The residue was purified by flash column chromatography over silica gel with toluene/AcOEt (2:1–3:2) as an eluent, and triturated with Et_2O to give **117** (Z:E=2:3) (371 mg, 77%) as a yellow solid. ^{1}H NMR ($CDCl_3$) δ 2.09 (3Hx2/5, s), 2.20 (3Hx3/5, s), 3.91 (3H, s), 4.10 (2Hx2/5, s), 4.25 (2Hx3/5, s), 7.27 (2Hx2/5, s), 7.14 (1Hx3/5, d, J = 15.6 Hz), 7.25 (2Hx2/5, d, J = 9.0 Hz), 7.29 (1Hx3/5, d, J = 15.6 Hz), 7.31 (2Hx3/5, d, J = 8.5 Hz), 7.38 (2Hx2/5, d, J = 9.0 Hz), 7.57 (2Hx3/5, d, J = 8.5 Hz), 7.97 (2Hx2/5, d, J = 8.5 Hz), 7.99 (2Hx2/5, d, J = 9.0 Hz), 8.00 (2Hx3/5, d, J = 8.5 Hz), 8.20 (2Hx3/5, d, J = 9.0 Hz), 9.55 (1Hx3/5, br s), 10.11 (1Hx2/5, br s); FAB MS m/e $(M+Na)^+$ 460.

Methyl 4-({2-(acetylamino)-4-[2-(4-aminophenyl)ethyl]-1,3-thiazol-5-yl}methyl)benzoate (118).

A mixture of **117** (290 mg), 10% palladium on carbon (597 mg), MeOH (15 mL) and THF (15 mL)

was stirred at room temperature for an hour under a hydrogen atmosphere (1.5 atm). The reaction mixture was filtered through a celite® pad. The filtrate was concentrated in vacuo to give **118** (251 mg, 93%) as a pale yellow amorphous. ^{1}H NMR ($CDCl_3$) δ 2.20 (3H, s), 2.80 (4H, s), 3.40–3.67 (2H, m), 3.83 (2H, s), 3.90 (3H, s), 6.57 (2H, d, *J* = 8.5 Hz), 6.84 (2H, d, *J* = 8.5 Hz), 7.09 (2H, d, *J* = 8.0 Hz), 7.91 (2H, d, *J* = 8.5 Hz), 8.96 (1H, br s); FAB MS *m*/*e* $(M+H)^+$ 410.

Methyl 4-[(2-(acetylamino)-4-{2-[4-({(*Z*)-[(*tert*-butoxycarbonyl)amino][(*tert*-butoxycarbonyl)-imino]methyl}amino)phenyl]ethyl}-1,3-thiazol-5-yl)methyl]benzoate (119).

A mixture of **118** (85 mg), *N*,*N'*-bis(*tert*-butoxycarbonyl)-1*H*-pyrazole-1-carboxamidine (**31**) (83 mg) and THF (1 mL) was stirred at room temperature for 17 h under a nitrogen atmosphere. The solvent was removed in vacuo. The residue was purified by flash column chromatography over silica gel with $CHCl_3$/MeOH (30:1) as an eluent to give **119** (40 mg, 83%) as a colorless amorphous. ^{1}H NMR ($CDCl_3$) δ 1.49 (9H, s), 1.54 (9H, s), 2.20 (3H, s), 2.83 (4H, s), 3.88 (2H, s), 3.89 (3H, s), 7.03 (2H, d, *J* = 8.5 Hz), 7.17 (2H, d, *J* = 8.0 Hz), 7.44 (2H, d, *J* = 8.0 Hz), 7.93 (2H, d, *J* = 8.5 Hz), 9.09 (1H, br s), 10.24 (1H, s), 11.64 (1H, s); FAB MS *m*/*e* $(M+H)^+$ 652.

Methyl 4-({2-(acetylamino)-4-[2-(4-{[amino(imino)methyl]amino}phenyl)ethyl]-1,3-thiazol-5-yl}methyl)benzoate hydrochloride (120).

A mixture of **119** (40 mg) and 4 M HCl/dioxane (1 mL) was stirred at room temperature overnight under a nitrogen atmosphere. The solvent was removed in vacuo. The residual solid was washed with AcOEt to give **120** (20 mg, 68%) as a white solid. ^{1}H NMR (DMSO-d_6) δ 2.09 (3H, s), 2.86 (4H, s), 3.83 (3H, s), 3.96–4.10 (2H, m), 7.13 (2H, d, *J* = 8.5 Hz), 7.24 (2H, d, *J* = 9.0 Hz), 7.28 (2H, d, *J* = 8.5 Hz), 7.35 (3H, s), 7.89 (2H, d, *J* = 8.0 Hz), 9.71 (1H, s), 12.01 (1H, s); FAB MS *m*/*e* $(M+H)^+$ 452.

A mixture of 4-({2-(acetylamino)-4-[(*E*)-2-(4-nitrophenyl)vinyl]-1,3-thiazol-5-yl}methyl)-benzenesulfonyl chloride and 4-({2-(acetylamino)-4-[(*Z*)-2-(4-nitrophenyl)vinyl]-1,3-thiazol-

5-yl}methyl)benzenesulfonyl chloride (121).

To the solution of **92g** (346 mg) in $CHCl_3$ (7 mL) was added chlorosulfonic acid (0.30 mL) dropwise under ice-cooling. The reaction mixture was stirred at room temperature for 15 h and then evaporated to a reduced volume. To the solution was added saturated aqueous $NaHCO_3$. The mixture was extracted with THF. The organic layer was dried over anhydrous $MgSO_4$ and concentrated in vacuo to give **121** (185 mg, 43%) as an off-white solid. FAB MS *m*/*e* $(M+H)^+$ 478.

***N*-{4-[2-(4-Aminophenyl)ethyl]-5-[4-(aminosulfonyl)benzyl]-1,3-thiazol-2-yl}acetamide (122a).**

To a solution of **121** (170 mg) in THF (5 mL) was added 28% ammonium hydroxide (1.5 mL) at 5 ˚C. The reaction mixture was stirred at room temperature for 12 h and quenched with saturated aqueous NH_4Cl. The mixture was extracted with AcOEt, dried over anhydrous $MgSO_4$, and concentrated in vacuo. The resulting orange powder was dissolved in MeOH (6 mL) and AcOH (1 mL). To the solution was added 10 % palladium on carbon (170 mg). The reaction mixture was stirred at room temperature for 15 h under a hydrogen atmosphere (3 atm) and filtered through a celite® pad. To the filtrate was added 0.1 M NaOH. The mixture was extracted with AcOEt. The organic layer was dried over anhydrous $MgSO_4$ and concentrated in vacuo. The residue was purified by flash column chromatography over silica gel with $CHCl_3$/MeOH (20:1) as an eluent to give **122a** (33 mg, 22%) as a white powder. ^{1}H NMR (DMSO-d_6) δ 1.88 (3H, s), 3.65–2.76 (4H, m), 3.93 (2H, s), 4.84 (2H, s), 6.46 (2H, d, J = 8.3 Hz), 6.78 (2H, d, J = 8.3 Hz), 7.22 (2H, d, J = 8.3 Hz), 7.28 (2H, s), 7.72 (2H, d, J = 8.3 Hz), 12.00 (1H, s); FAB MS *m*/*e* $(M+H)^+$ 431.

***N*-(4-[2-(4-Aminophenyl)ethyl]-5-{4-[(dimethylamino)sulfonyl]benzyl}-1,3-thiazol-2-yl)-acetamide (122b).**

Compound **122b** was prepared from **121** according to the same procedure as that of compound **122a**. Compound **122b** was obtained as a white powder (48% yield). ^{1}H NMR (DMSO-d_6) δ 2.08 (3H, s), 2.56 (4H, s), 3.36 (6H, s), 3.99 (2H, s), 4.84 (2H, s), 6.45 (2H, d, J = 8.5 Hz), 6.76 (2H, d, J = 8.5 Hz), 7.27 (2H, d, J = 8.5 Hz), 7.65 (2H, d, J = 8.5 Hz), 12.03 (1H, s); FAB MS *m*/*e* $(M+H)^+$ 459.

Di-*tert*-butyl ((*Z*)-{[4-(2-{2-(acetylamino)-5-[4-(aminosulfonyl)benzyl]-1,3-thiazol-4-yl}ethyl)phenyl]amino}methylidene)biscarbamate (123a).

A mixture of **122a** (30 mg), *N*,*N'*-bis(*tert*-butoxycarbonyl)-1*H*-pyrazole-1-carboxamidine (**31**) (48 mg), diisopropylethylamine (0.02 mL) and CH_2Cl_2 (0.6 mL) was stirred at room temperature for 12 h under a nitrogen atmosphere. The solvent was removed in vacuo. The residue was purified by flash column chromatography over silica gel with $CHCl_3$/MeOH (10:1) as an eluent to give **123a** (47 mg, 100%) as a colorless powder. ^{1}H NMR (DMSO-d_6) δ 1.39 (9H, s), 1.50 (9H, s), 2.08 (3H, s), 2.85 (4H, br s), 4.00 (2H, s), 7.15 (2H, d, J = 8.5 Hz), 7.31 (2H, d, J = 8.5 Hz), 7.43 (2H, d), 7.73 (2H, d, J = 8.5 Hz), 9.95 (1H, s), 11.44 (1H, s), 12.03 (1H, s); FAB MS *m*/*e* $(M+H)^+$ 673.

Di-*tert*-butyl [(*Z*)-({4-[2-(2-(acetylamino)-5-{4-[(dimethylamino)sulfonyl]benzyl}-1,3-thiazol-4-yl)ethyl]phenyl}amino)methylidene]biscarbamate (123b).

Compound **123b** was prepared from **122b** according to the same procedure as that of compound **123a**. Compound **123b** was obtained as a white powder (88% yield). ^{1}H NMR (DMSO-d_6) δ 1.38 (9H, s), 1.50 (9H, s), 2.08 (3H, s), 2.54 (6H, s), 2.84 (4H, s), 4.02 (2H, s), 7.10 (2H, d, J = 8.4 Hz), 7.27 (2H, d, J = 8.4 Hz), 7.43 (2H, d, J = 8.4 Hz), 7.61 (2H, d, J = 8.4 Hz), 9.99 (1H, s), 11.45 (1H, s), 12.03 (1H, s).

***N*-{4-[2-(4-{[Amino(imino)methyl]amino}phenyl)ethyl]-5-[4-(aminosulfonyl)benzyl]-1,3-thiazol-2-yl}acetamide hydrochloride (124a).**

A mixture of **123a** (74 mg) and 4 M HCl/dioxane (3 mL) was stirred at room temperature for 12 h under a nitrogen atmosphere. The solvent was removed in vacuo. The residue was solidified with AcOEt to give **124a** (42 mg, 76%) as a white powder. ^{1}H NMR (DMSO-d_6) δ 1.95 (3H, s), 2.86 (4H, s), 3.99 (2H, s), 7.14 (2H, d, J = 8.5 Hz), 7.25 (2H, d, J = 8.5 Hz), 7.27–7.35 (7H, m), 7.74 (2H, d, J = 8.5 Hz), 10.30 (1H, s), 12.03 (1H, s); FAB MS *m*/*e* $(M+H)^+$ 473.

***N*-(4-[2-(4-{[Amino(imino)methyl]amino}phenyl)ethyl]-5-{4-[(dimethylamino)sulfonyl]benzyl}-1,3-thiazol-2-yl)acetamide hydrochloride (124b).**

Compound **124b** was prepared from **123b** according to the same procedure as that of compound **124a**. Compound **124b** was obtained as a white powder (82% yield). ^{1}H NMR (DMSO-d_6) δ 2.09 (3H, s), 2.57 (6H, s), 2.84 (4H, s), 4.08 (2H, s), 7.12 (2H, d, J = 8.4 Hz), 7.23 (2H, d, J = 8.4 Hz), 7.40 (2H, d, J = 8.0 Hz), 7.42 (3H, br s), 8.02 (2H, d, J = 8.0 Hz), 9.86 (1H, s), 12.05 (1H, s); FAB MS m/e $(M+H)^+$ 501.

3-[4-(Methylsulfanyl)phenyl]propanoic acid (126).

To HCO_2H (368 mL) was added dropwise Et_3N (546 mL) at 0 ˚C under a nitrogen atmosphere. Then to the solution was added dropwise 4-(methylsulfanyl)benzaldehyde (250 g) and 2,2-dimethyl-1,3-dioxane-4,6-dione (237 g) at 0 ˚C under a nitrogen atmosphere. The reaction mixture was heated at 100 ˚C for 2 h, and poured into ice-H_2O (3 L). The aqueous solution was adjusted to pH=1 by 2M HCl. The precipitate was collected in vacuo and washed with $^iPr_2O$ to give **126** (276 g, 86%) as a pale yellow solid. Mp 79–80.5 ˚C; ^{1}H NMR ($CDCl_3$) δ 2.47 (3H, s), 2.66 (2H, t, J = 8.0 Hz), 2.92 (2H, t, J = 8.0 Hz), 7.13 (2H, d, J = 8.5 Hz), 7.21 (2H, d, J = 8.5 Hz).

Methyl 3-[4-(methylsulfanyl)phenyl]propanoate (82).

To a mixture of **126** (85 g) and K_2CO_3 (72 g) in DMF (850 mL) was added dropwise MeI (32 mL) at 0 ˚C under a nitrogen atmosphere. The reaction mixture was stirred at room temperature for 16 h and poured into ice-H_2O. The mixture was extracted with AcOEt. The organic layer was washed with H_2O (twice) and brine, dried over anhydrous $MgSO_4$, and concentrated in vacuo to give **82** (87 g, 95%) as a pale yellow oil.

N-{4-[2-(4-Carbamimidamidophenyl)ethyl]-5-[4-(methylsulfonyl)benzyl]-1,3-thiazol-2-yl}acetamide <u>hydrochloride</u> (95c:<u>HCl salt</u>).

A mixture of **94c** (140 g), dioxane (700 mL) and 4 M HCl/dioxane (700 mL) was stirred at room

temperature for 19 h under a nitrogen atmosphere. Then MeOH (200 mL) was added to the mixture, and stirred at room temperature for 6 h. The solvent was removed in vacuo, and the residue was dissolved in H_2O. The solution was filtered in vacuo. The filtrate was washed with AcOEt. The aqueous layer and AcOEt were combined, and the mixture was made neutral (pH=7) by saturated aqueous $NaHCO_3$ with stirring. The precipitate was filtered in vacuo to give crude **95c** (100 g) as a hydrochloride salt. The solid was suspended in CH_3CN and refluxed for 1.5 h. The precipitate was filtered in vacuo, and recrystallized from H_2O (1 L) to give **95c** (58 g, 55% / HCl salt) as an off-white solid. Mp 195–196 ˚C; ^{1}H NMR (DMSO-d_6) δ 2.08 (3H, s), 2.79 (4H, m), 3.18 (3H, s), 4.05 (2H, s), 6.73 (2H, d, J = 8.0 Hz), 6.99 (2H, d, J = 8.0 Hz), 7.37 (2H, d, J = 8.5 Hz), 7.84 (2H, d, J = 8.5 Hz); FAB MS m/e $(M+H)^+$ 472; Anal. Calcd for $C_{22}H_{25}N_5O_3S_2 \cdot HCl \cdot H_2O$: C, 50.23; H, 5.36; N, 13.31; S, 12.19; Cl, 6.74. Found: C, 50.56; H, 5.31; N, 13.44; S, 12.22; Cl, 6.81.

第３章

The following compounds were purchased from commercial sources and used without further purification; **128** (2002-16-6), **129** (1848-75-5), **130** (41213-54-1), **131** (934-32-7), **132** (1687-51-0), **133** (136-95-8), **134** (27878-37-1), **135** (51-17-2), **136** (5993-91-9), **137** (1450-93-7), **138** (1622-57-7), **139** (6285-68-3), **140** (171082-91-0), **145** (862281-44-5).

_N_-(4-Phenyl-1_H_-imidazol-2-yl)acetamide (143).

A solution of 2-bromo-1-phenylethanone (**141**) (1.0 g) in DMF (20 mL) was added to a solution of *N*-carbamimidoylacetamide (**142**) (1.0 g) at 0 ˚C. The reaction mixture was stirred at room temperature for 15 h, and concentrated in vacuo. The solid was washed with CH_3CN to give **143** (546 mg, 54%) as an off-white solid. ^{1}H NMR (DMSO-d_6) δ 2.07 (3H, s), 7.16 (1H, t, J = 7.5 Hz), 7.25 (1H, s), 7.32 (2H, t, J = 7.5 Hz), 7.71 (2H, d, J = 7.5 Hz), 11.22 (1H, s), 11.61(1H, br s); IR (KBr) cm^{-1}: 3371, 1687, 1622, 1599, 1576, 1556, 1531, 1487, 1456, 1419, 1371, 1335, 1277, 1211, 1167, 1041; FAB MS *m*/*e* $(M+H)^+$ 202; HRMS (ESI) Calcd for $C_{11}H_{12}N_3O$ $(M+H)^+$: 202.0980, found: 202.0986.

4-Phenyl-1*H*-imidazol-2-amine (144).

A mixture of **143** (500 mg), 6 M HCl (2.2 mL) and MeOH (2.5 mL) was refluxed for 3 h. After cooling to room temperature, the precipitate was filtered off. The filtrate was concentrated in vacuo. The residue was dissolved in H_2O. The solution was neutralized with 1 M NaOH. The precipitate was collected in vacuo. The solid was washed with H_2O and Et_2O to give **144** (131 mg, 33%) as a pale brown solid. ^{1}H NMR (DMSO-d_6) δ 5.25 (2H, s), 6.96 (1H, s), 7.07 (1H, t, J = 7.5 Hz), 7.26 (2H, t, J = 7.5 Hz), 7.57 (2H, d, J = 7.5 Hz), 10.53 (1H, br s); IR (KBr) cm^{-1}: 3413, 3383, 1635, 1577, 1558, 1508, 1489, 1473, 1178; FAB MS *m*/*e* $(M+H)^+$ 160; HRMS (ESI) Calcd for $C_9H_{10}N_3$ $(M+H)^+$: 160.0875, found: 160.0871; Anal. Calcd for $C_9H_9N_3 \cdot 0.3H_2O$: C, 65.68; H, 5.88; N, 25.53. Found: C, 65.81; H, 5.49; N, 25.19.

1-Bromo-4-phenylbutan-2-one (147).

To a solution of 4-phenylbutan-2-one (**146**) (3.0 g) in AcOH (7 mL) and 48% aqueous HBr (3 mL) was added a solution of Br_2 (6.5 g) in AcOH (5 mL) at 0 ˚C. The reaction mixture was stirred at room temperature for 4 h. Then acetone (30 mL) was added to the solution, and the reaction mixture was stirred at room temperature for 14 h. The mixture was concentrated in vacuo, diluted with brine, and extracted with CH_2Cl_2 (twice). The combined organic layer was dried over anhydrous $MgSO_4$, and evaporated in vacuo. The residue was purified by flash column chromatography over silica gel with hexane/AcOEt (40:1–10:1) as an eluent to give **147** (1.5 g, 34%) as a pale brown oil. CI MS *m*/*e* $(M)^+$ 227.

***N*-[4-(2-Phenylethyl)-1*H*-imidazol-2-yl]acetamide (148).**

Compound **148** was prepared from **147** and *N*-carbamimidoylacetamide (**142**) according to the same procedure as that of compound **143** and obtained as an off-white solid (10% yield). ^{1}H NMR (DMSO-d_6) δ 2.02 (3H, s), 2.67–2.74 (2H, m), 2.81–2.88 (2H, m), 6.39 (1H, s), 7.13–7.29 (5H, m), 10.97 (2H, br s); IR (KBr) cm^{-1}: 3330, 1674, 1622, 1577, 1520, 1456, 1367, 1292, 1255, 1157, 1078,

1045; FAB MS *m*/*e* $(M+H)^+$ 230; HRMS (ESI) Calcd for $C_{13}H_{16}N_3O$ $(M+H)^+$: 230.1293, found: 230.1293.

4-(2-Phenylethyl)-1*H*-imidazol-2-amine (149).

Compound **149** was prepared from **148** according to the same procedure as that of compound **144** and obtained as a pale yellow solid (93% yield). ^{1}H NMR (DMSO-d_6) δ 2.55–2.61 (2H, m), 2.77–2.83 (2H, m), 5.05 (2H, br s), 6.12 (1H, s), 7.13–7.29(5H, m), 10.11 (1H, br s); IR (KBr) cm^{-1}: 3028, 1716, 1697, 1684, 1653, 1637, 1560, 1541, 1489, 1456, 1313; FAB MS *m*/*e* $(M+H)^+$ 188; HRMS (ESI) Calcd for $C_{11}H_{14}N_3$ $(M+H)^+$: 188.1188, found: 188.1190.

A mixture of methyl 4-[(*Z*)-2-(2-acetamido-1,3-thiazol-4-yl)vinyl]benzoate and methyl 4-[(*E*)-2-(2-acetamido-1,3-thiazol-4-yl)vinyl]benzoate (151).

Potassium *tert*-butoxide (3.9 g) was added to a mixture of [4-(methoxycarbonyl)benzyl](triphenyl)-phosphonium bromide (**150**) (14.3 g) and DMF (75 mL) at 0 ˚C under a nitrogen atmosphere. The reaction mixture was stirred at 0 ˚C for 15 min, and *N*-(4-formylthiazol-2-yl)acetamide (**7**)[9] (5.0 g) was added to the mixture at 0 ˚C. The reaction mixture was stirred at room temperature for 13 h, and poured into ice-H_2O. The precipitate was collected in vacuo and washed with Et_2O to give **151** (Z:E = 2:1) (7.8 g, 88%) as an off-white solid. Mp 175–177 ˚C; ^{1}H NMR (DMSO-d_6) δ 2.13 (3H×2/3, s), 2.16 (3H×1/3, s), 3.85 (3H, s), 6.61 (2H×2/3, s), 7.05 (1H×2/3, s), 7.26 (1H×1/3, d, J = 15.5 Hz), 7.26 (1H×1/3, s), 7.37 (1H×1/3, d, J = 15.5 Hz), 7.64 (2H×2/3, d, J = 8.5 Hz), 7.69 (2H×1/3, d, J = 8.5 Hz), 7.90 (2H×2/3, d, J = 8.5 Hz), 7.94 (2H×1/3, d, J = 8.5 Hz), 12.05 (1H, br s); FAB MS *m*/*e* $(M+H)^+$ 303.

Methyl 4-[2-(2-acetamido-1,3-thiazol-4-yl)ethyl]benzoate (152).

A mixture of **151** (119 g), 10% palladium on carbon (118 g), AcOH (300 mL) and DMF (2000 mL) was stirred under a hydrogen atmosphere (3 atm) at room temperature for 6 h, and filtered through a celite® pad. The filtrate was concentrated in vacuo. The residual solid was suspended in AcOEt,

and filtered in vacuo. The filtrate was concentrated in vacuo. The residue was purified by flash column chromatography over silica gel with hexane/AcOEt (1:2) to $CHCl_3$/MeOH (10:1) as an eluent and triturated with Et_2O to give **152** (69 g, 57%) as a pale yellow solid. Mp 170–171 ˚C; ^{1}H NMR (DMSO-d_6) δ 2.11 (3H, s), 2.86–2.95 (2H, m), 2.97–3.05 (2H, m), 3.83 (3H, s), 6.72 (1H, s), 7.35 (2H, d, J = 8.5 Hz), 7.87 (2H, d, J = 8.5 Hz), 12.08 (1H, br s); IR (KBr) cm^{-1}: 3176, 3064, 1736, 1716, 1647, 1608, 1577, 1558, 1543, 1508, 1456, 1431, 1308, 1277, 1178, 1101; FAB MS *m*/*e* $(M+H)^+$ 305; HRMS (ESI) Calcd for $C_{15}H_{17}N_2O_3S$ $(M+H)^+$: 305.0960, found: 305.0966.

A mixture of {4-[(*Z*)-2-(2-acetamido-1,3-thiazol-4-yl)vinyl]phenyl}acetic acid and {4-[(*E*)-2-(2-acetamido-1,3-thiazol-4-yl)vinyl]phenyl}acetic acid (154).

Compound **154** was prepared from **7** and [4-(carboxymethyl)benzyl](triphenyl) phosphonium bromide (**153**) according to the same procedure as that of compound **151** and obtained as a white powder (Z:E = 5:1) (94% yield). ^{1}H NMR (DMSO-d_6) δ 2.12 (3H×5/6, s), 2.15 (3H×1/6, s), 3.52 (2H×5/6, s), 3.54 (2H×1/6, s), 6.46 (1H×5/6, d, J = 12.5 Hz), 6.54 (1H×5/6, d, J = 12.5 Hz), 6.95 (1H, s), 7.11–7.49 (6H×1/6, m), 7.20 (2H×5/6, d, J = 8.0 Hz), 7.41 (2H×5/6, d, J = 8.0 Hz), 12.10 (1H, br s), 12.42 (1H, br s); FAB MS *m*/*e* $(M+H)^+$ 303.

Methyl (2*E*)-3-{4-[(*E*)-2-(2-acetamido-1,3-thiazol-4-yl)vinyl]phenyl}acrylate (156).

Compound **156** was prepared from methyl (2*E*)-3-(4-formylphenyl)acrylate (**155)** and [(2-acetamido-1,3-thiazol-4-yl)methyl](triphenyl)phosphonium chloride (**19**)[9] according to the same procedure as that of compound **151** and obtained as a pale yellow wax (70% yield). ^{1}H NMR (DMSO-d_6) δ 2.15 (3H, s), 3.73 (3H, s), 6.66 (1H, d, J = 16.0 Hz), 7.24 (1H, d, J = 14.5 Hz), 7.55–7.78 (2H, m), 7.95 (4H, s), 12.20 (1H, br s); FAB MS *m*/*e* $(M+H)^+$ 329; HRMS (ESI) Calcd for $C_{17}H_{17}N_2O_3S$ $(M+H)^+$: 329.0960, found: 329.0960.

Methyl 3-{4-[2-(2-acetamido-1,3-thiazol-4-yl)ethyl]phenyl}propanoate (157).

Compound **157** was prepared from **156** according to the same procedure as that of compound **152**

and obtained as a colorless wax (76% yield). ^{1}H NMR (DMSO-d_6) δ 2.11 (3H, s), 2.60 (2H, t, J = 7.5 Hz), 2.80 (2H, t, J = 7.5 Hz), 2.89 (4H, m), 3.57 (3H, s), 6.72 (1H, s), 7.11 (4H, s), 12.06 (1H, br s); FAB MS *m*/*e* $(M+H)^+$ 333; HRMS (ESI) Calcd for $C_{17}H_{21}N_2O_3S$ $(M+H)^+$: 333.1273, found: 333.1274.

4-[2-(2-Acetamido-1,3-thiazol-4-yl)ethyl]benzoic acid (158a).

To a solution of **152** (330 mg) in dioxane (3.3 mL) was added 1 M NaOH (0.43 mL) at 0 ˚C, and then the mixture was refluxed for 2 h. Volatiles were evaporated in vacuo. The residue was dissolved in H_2O and washed with AcOEt. The aqueous layer was adjusted to pH=2, and the resulting precipitate was collected by filtration to give **158a** (218 mg, 69%) as a colorless solid. ^{1}H NMR (DMSO-d_6) δ 2.11 (3H, s), 2.87–3.05 (4H, m), 6.73 (1H, s), 7.32 (2H, d, J = 8.0 Hz), 7.84 (2H, d, J = 8.0 Hz), 12.06 (1H, br s), 12.78 (1H, br s); IR (KBr) cm^{-1}: 3186, 1693, 1610, 1570, 1435, 1417, 1371, 1311, 1296, 1236, 1176, 1153, 1126, 1101; FAB MS *m*/*e* $(M+H)^+$ 291; HRMS (ESI) Calcd for $C_{14}H_{15}N_2O_3S$ $(M+H)^+$: 291.0803, found: 291.0808; Anal. Calcd for $C_{14}H_{14}N_2O_3S \cdot 0.08C_2H_4O_2$: C, 57.62; H, 4.89; N, 9.49; S, 10.86. Found: C, 58.00; H, 4.80; N, 9.23; S, 10.38.

{4-[2-(2-Acetamido-1,3-thiazol-4-yl)ethyl]phenyl}acetic acid (158b).

Compound **158b** was prepared from **154** according to the same procedure as that of compound **152** and obtained as an off-white solid (29% yield). ^{1}H NMR (DMSO-d_6) δ 2.11 (3H, s), 2.86–2.93 (4H, m), 3.50 (2H, s), 6.74 (1H, s), 7.14 (4H, s), 12.07 (1H, s), 12.24 (1H, br s); IR (KBr) cm^{-1}: 3107, 2362, 1695, 1576, 1541, 1516, 1456, 1375, 1331, 1298, 1240, 1161, 1003; FAB MS *m*/*e* $(M+H)^+$ 305; HRMS (ESI) Calcd for $C_{15}H_{17}N_2O_3S$ $(M+H)^+$: 305.0960, found: 305.0962.

3-{4-[2-(2-Acetamido-1,3-thiazol-4-yl)ethyl]phenyl}propanoic acid (158c).

Compound **158c** was prepared from **157** according to the same procedure as that of compound **158a** and obtained as a colorless solid (88% yield). ^{1}H NMR (DMSO-d_6) δ 2.11 (3H, s), 2.52 (2H, t, J = 7.5 Hz), 2.77 (2H, t, J = 7.5 Hz), 2.89 (4H, m), 6.73 (1H, s), 7.11 (4H, s), 12.10 (1H, br s); FAB MS

m/*e* $(M+H)^+$ 319; HRMS (ESI) Calcd for $C_{16}H_{19}N_2O_3S$ $(M+H)^+$: 319.1116, found: 319.1122.

***tert*-Butyl (4-{4-[2-(2-acetamido-1,3-thiazol-4-yl)ethyl]phenyl}-1*H*-imidazol-2-yl)carbamate (160a).**

To a solution of **158a** (300 mg) in CH_2Cl_2 (4.5 mL) was added dropwise oxalyl chloride (0.16 mL) at 0 ˚C under a nitrogen atmosphere. After stirring for 5 min, 2 drops of DMF were added. The reaction mixture was stirred under ice-cooling for 1 h. Then the solvent was evaporated to give darkish yellow powder. This acid chloride was dissolved in CH_2Cl_2 (4.5 mL) and the solution was cooled in an ice-bath. To the solution was added dropwise 2 M (trimethylsilyl)diazomethane/hexane (1.0 mL) at 0 ˚C under a nitrogen atmosphere. The reaction mixture was stirred at room temperature for 2 h. To the solution was added 4M HCl/AcOEt (1.0 mL) and DMF (2 mL) at 0 ˚C under a nitrogen atmosphere. After stirring at room temperature for 12 h, the organic solvent was removed in vacuo. This halo-ketone was dissolved in DMF (6 mL). To the solution was added *tert*-butyl carbamimidoylcarbamate (**35**) (493 mg) at 0 ˚C under a nitrogen atmosphere. The reaction mixture was stirred at 65 ˚C for 7h, and concentrated in vacuo. The residue was purified by flash column chromatography over silica gel with $CHCl_3$/MeOH (50:1–20:1) as an eluent to give **160a** (101 mg, 23%) as an off-white solid. ^{1}H NMR (DMSO-d_6) δ 1.58 (9H, s), 2.12 (3H, s), 2.85–2.98 (4H, m), 6.55 (1H, s), 6.72 (1H, s), 7.16 (2H, d, J = 8.0 Hz), 7.62 (2H, d, J = 8.0 Hz), 8.25 (1H, br s), 12.08 (1H, br s); FAB MS *m*/*e* $(M+H)^+$ 428; HRMS (ESI) Calcd for $C_{21}H_{26}N_5O_3S$ $(M+H)^+$: 428.1756, found: 428.1760.

***tert*-Butyl (4-{4-[2-(2-acetamido-1,3-thiazol-4-yl)ethyl]benzyl}-1*H*-imidazol-2-yl)carbamate (160b).**

Compound **160b** was prepared from **158b** and **159** according to the same procedure as that of compound **160a** and obtained as a yellow amorphous (22% yield). ^{1}H NMR (DMSO-d_6) δ 1.52 (9H, s), 2.11 (3H, s), 2.79–2.99 (4H, m), 3.52–3.64 (2H, m), 5.15 (1H, br s), 6.36 (1H, s), 6.49–6.59 (1H, m), 6.72 (1H, s), 7.06–7.18 (4H, m), 12.05 (1H, br s); FAB MS *m*/*e* $(M+H)^+$ 442; HRMS (ESI)

Calcd for $C_{22}H_{28}N_5O_3S$ $(M+H)^+$: 442.1913, found: 442.1913.

***tert*-Butyl (4-{4-[2-(2-acetamido-1,3-thiazol-4-yl)ethyl]phenethyl}-1*H*-imidazol-2-yl)carbamate (160c).**

Compound **160c** was prepared from **158c** and **159** according to the same procedure as that of compound **160a** and obtained as an off-white amorphous (26% yield). ^{1}H NMR (DMSO-d_6) δ 1.53 (9H, s), 2.11 (3H, s), 2.70–2.93 (8H, m), 6.38 (1H, s), 6.72 (1H, s), 7.10 (4H, s), 12.06 (1H, br s); IR (KBr) cm^{-1}: 1734, 1684, 1558, 1541, 1508, 1473, 1458, 1371, 1340, 1288, 1254, 1149, 1122; FAB MS *m*/*e* $(M+H)^+$ 456; HRMS (ESI) Calcd for $C_{23}H_{30}N_5O_3S$ $(M+H)^+$: 456.2069, found: 456.2074.

***N*-(4-{2-[4-(2-Amino-1*H*-imidazol-4-yl)phenyl]ethyl}thiazol-2-yl)acetamide hydrochloride (161a).**

A mixture of **160a** (32 mg), 4 M HCl/AcOEt (0.4 mL) and MeOH (1 mL) was stirred at room temperature for 10 h under a nitrogen atmosphere. The solvent was removed in vacuo. The residual amorphous was solidified with AcOEt to give **161a** (21 mg, 76%) as an off-white solid. ^{1}H NMR (DMSO-d_6) δ 2.11 (3H, s), 2.88–2.96 (4H, m), 6.50 (1H, s), 6.71 (1H, s), 7.25 (2H, d, J = 8.0 Hz), 7.55 (2H, d, J = 8.0 Hz), 9.14 (1H, br s), 12.01 (1H, br s); FAB MS *m*/*e* $(M+H)^+$ 328; HRMS (ESI) Calcd for $C_{16}H_{18}N_5OS$ $(M+H)^+$: 328.1232, found: 328.1231.

***N*-[4-(2-{4-[(2-Amino-1*H*-imidazol-4-yl)methyl]phenyl}ethyl)thiazol-2-yl]acetamide hydrochloride (161b).**

Compound **161b** was prepared from **160b** according to the same procedure as that of compound **161a** and obtained as a pale yellow solid (74% yield). ^{1}H NMR (DMSO-d_6) δ 2.12 (3H, s), 2.77–2.96 (4H, m), 3.71–3.85 (2H, m), 6.57 (1H, s), 6.72 (1H, s), 7.078–7.21 (4H, m), 11.64(1H, s), 12.05 (1H, s); *free form*_^{13}C NMR (DMSO-d_6) δ 22.3, 32.9, 34.1, 39.9, 79.1, 107.2, 127.8, 128.4, 138.4, 138.5, 149.3, 150.3, 157.3, 159.4, 168.1; *free form*_IR (KBr) cm^{-1}: 2927, 1716, 1682, 1668, 1558, 1543, 1508, 1489, 1456, 1373, 1296; FAB MS *m*/*e* $(M+H)^+$ 342; HRMS (ESI) Calcd for

$C_{17}H_{20}N_5OS$ $(M+H)^+$: 342.1389, found: 342.1388.

***N*-[4-(2-{4-[(2-Amino-1*H*-imidazol-4-yl)ethyl]phenyl}ethyl)thiazol-2-yl]acetamide hydrochloride (161c).**

Compound **161c** was prepared from **160c** according to the same procedure as that of compound **161a** and obtained as an off-white solid (96% yield). ^{1}H NMR (DMSO-d_6) δ 2.12 (3H, s), 2.65–2.95 (8H, m), 6.51 (1H, br s), 6.71 (1H, s), 7.12 (4H, s), 11.58 (1H, br s), 12.07 (1H, br s); FAB MS *m*/*e* $(M+H)^+$ 356; HRMS (ESI) Calcd for $C_{18}H_{22}N_5OS$ $(M+H)^+$: 356.1545, found: 356.1540.

化合物の modeling 法

Human VAP-1 model

A side-chain conformational search and minimization for Leu469 of the three-dimensional (3D) structure of VAP-1 with TPQ in an active conformation (PDB-code: 2C11,[18] resolution: 2.90 Å) was performed using the LowMode MD[25] function in the Molecular Operating Environment (MOE) program[26] with the MMFF94x forcefield. The bound 2-hydrazinopyridine ligand was then deleted.

Docking Study

Compounds were docked to the human VAP-1 model using the docking program GOLD (version 5.0. / 5.1.).[27] The ligand-binding pocket was defined using $C^{\beta}H$ from Leu468 as the central atom with a radius of 20 Å. The ligand was docked covalently to nitrogen atom N1 of PAQ1729 (PAQ is used PDB entry 2C11[18]) to assign TPQ and the ligand. Each ligand was docked 10 times.

Sequence Homology between human and rat

The homology between human and rat VAP-1 sequences was evaluated using MOE.[26]

化合物の評価法

Inhibitory effect on human and rat VAP-1 enzyme activities

Human and rat VAP-1 enzyme activities were measured using a radiochemistry enzymatic assay with ^{14}C-benzylamine (American Radiolabeled Chemicals, St. Louis, MO, USA) as a substrate.[28] An enzyme suspension prepared from Chinese Hamster Ovary (CHO) cells stably expressing human or rat VAP-1 enzyme was preincubated with the test compound in a 96-well microplate at room temperature for 30 min. The enzyme suspension was then incubated with ^{14}C-benzylamine (final concentration of 1×10^{-5} mol/L) in a final volume of 50 μL at 37 °C for 1 h. The enzymatic reaction was stopped by the addition of 2 mol/L (50 μL) citric acid. The oxidation products were extracted directly with a toluene scintillator (200 μL), and the radioactivity was measured with a scintillation spectrometer.

Inhibitory effect on MAO-B and DAO activities by a radiochemical-enzyme assay

MAO-B and DAO enzyme activities were measured by a radiochemical-enzyme assay using ^{14}C-phenethylamine and ^{14}C-putrescine as the enzyme substrate, respectively. The enzyme suspension prepared from CHO cells transiently expressing each enzyme was used in the human MAO-B and DAO assays, and rat DAO enzyme suspension was prepared from rat colon.

Compounds **12** and **95c**, and a substrate mixture were reacted at 37 °C for 1 h with the enzyme suspension. The enzymatic reaction of MAO-B and DAO was stopped by the addition of 2 mol/L citric acid and 0.6 mol/L $NaHCO_3$, respectively. The oxidation products were extracted with toluene scintillator, and the radioactivity was measured with a scintillation spectrometer.

Inhibitory effect on MAO-A and MAO-B activities by a fluorometric enzymatic assay

MAO-A and MAO-B enzyme activities were measured using a fluorometric enzymatic assay with 5-hydroxytriptamine and benzylamine as enzyme substrates, respectively. An enzyme suspension prepared with recombinant monoamine oxidase (MAO)-A and B (Supersomes MAO A/B®, BD

Gentest, MA, USA) was used in the human MAO-A and MAO-B assays. The enzymatic reaction was performed using the Amplex® Red Oxidase Assay kit (Molecular Probes). Briefly, compounds **12** and **161b,** and a substrate mixture containing Amplex® Red reagent were reacted at 37 °C for 1 h with the MAO-A and MAO-B enzyme suspensions. The fluorescence of the resulting mixture was measured using a fluorescent plate reader (SPECTRA Max® M2; Molecular Devices, Osaka, Japan).

Measurement of the Plasma Concentration (pharmacokinetics)

Wistar male rats (8-12 week-old) were fasted for 20 h and subcutaneously administered with compound **12**. Heparin blood collection from the tail vein was performed at 0.5 h, 1 h, 3 h and 6 h after administration in the pharmacokinetic study.

Measurement of the effect on plasma VAP-1 activity (pharmacodynamics)

In an *ex vivo* study, STZ rats at 2 weeks after the induction of diabetes were subcutaneously administered compounds **12**, **95c**, or orally administered compound **161b** and heparin blood collection from the tail vein was performed immediately before and 1, 3, 6, 12 and 24 h after administration of compounds. The resulting plasma was obtained by centrifugation at 14,000 rpm for 5 min, and stored at -80 °C until measurement. The plasma VAP-1 activity was measured by a radioenzyme assay with ^{14}C-benzylamine as the substrate.[28] Specific VAP-1 activity was measured in a reaction mixture of 100 µL plasma and ^{14}C-benzylamine (10 µmol/L) at 37 °C for 1 h with and without compounds. Radioactivity of a reacted metabolite (^{14}C-benzaldehyde) was measured using a liquid scintillation counter (TRI-CARB 2100TR, Perkin Elmer Japan, Osaka, Japan). The effect of the test drug was calculated from the ratio (%) of the VAP-1 activity after the administration of compounds relative to the VAP-1 activity in the plasma immediately before administration (100%).

Measurement of ocular permeability in the diabetic rats.

Male Sprague Dawley (SD) rats (6 weeks old) were purchased from Japan Charles River Laboratories (Yokohama, Japan). Following 1 week of acclimation, one group of animals was

weighed and injected intraperitoneally with 65 mg/kg STZ (Sigma-Aldrich, St. Louis, MO, USA) in citrate-buffered saline. A second group underwent sham treatments with citrate-buffered saline. Two weeks after STZ injection, plasma glucose was measured from collected plasma samples. The blood glucose value was measured using a colorimetric method, and rats that showed 350 mg/dL blood glucose levels 2 weeks after STZ treatment were diagnosed with diabetes.

Compounds **12** and **161b** were given daily for 2 weeks starting 2 weeks after the completion of STZ treatment. Ocular vascular permeability was examined 24 h after the date of the final administration. Ocular permeability was evaluated based on fluorescein leakage into the vitreous 30 min after tail vein administration of 40 mg/mL/kg sodium fluorescein solution. Permeability was expressed as the intravitreal concentration/plasma concentration ratio of fluorescein. Measurement of the fluorescein was performed using a SpectraMax® fluorescent plate reader (Molecular Devices, Osaka, Japan).

参考文献およびノート

1) 目と健康シリーズNo3, 特集：糖尿病黄斑症, 北野滋彦. Available from: http://www.skk-health.net/me/03/ [Last accessed Oct. 2013]

2) Marttila-Ichihara, F.; Smith, D. J.; Stolen, C.; Yegutkin, G. G.; Elima, K.; Mercier, N.; Kiviranta, R.; Pihlavisto, M.; Alaranta, S.; Pentikäinen, U.; Pentikäinen, O.; Fülöp, F.; Jalkanen, S.; Salmi, M.,
Arthritis Rheum., **2006**, *54*, 2852.

3) Smith, D.J.; Salmi, M.; Bono, P.; Hellman, J.; Leu, T.; Jalkanen, S., *J. Exp. Med.*, **1998**, *188*, 17.

4) Yu, P. H.; Wright, S.; Fan, E. H.; Lun, Z. R.; Gubisne-Harberle, D., *Biochim. Biophys. Acta*, **2003**, *1647*, 193.

5) Buffoni, F.; Ignesti, G.; *Mol. Genet. Metab.*, **2000**, *71*, 559.

6) Yu, P. H.; Lu, L. X.; Fan, H.; Kazachkov, M.; Jiang, Z. J.; Jalkanen, S.; Stolen, C., *Am. J. Pathol.*, **2006**, *168*, 718.

7) Yu, P. H.; Deng, Y. L., *Atherosclerosis,* **1998**, *140*, 357.

8) Boomsma, F.; Derkx, F. H.; van den Meiracker, A. H.; Man in't Veld, A. J.; Schalekamp, M. A.,
Clin. Sci. (London), **1995**, *88*, 675.

9) Boomsma, F.; Bhaggoe, U. M.; van der Houwen, A. M.; van den Meiracker, A. H., *Biochim. Biophys. Acta*, **2003**, *1647*,48.

10) Garpenstrand, H.; Ekblom, J.; Backlund, L. B.; Oreland, L.; Rosenqvist, U., *Diabet. Med.*, **1999**, *16*, 514.

11) Akin, E.; Aversa, J.; Steere, A. C. *Infect. Immun.,* **2001**, *69*, 1774.

12) Boomsma, F.; van Veldhuisen, D. J.; de Kam, P. J.; Man in't Veld, A. J.; Mosterd, A.; Lie, K. I.; Schalekamp, M. A., *Cardiovasc. Res.*, **1997**, *33*, 387.

13) Yu, P., *Med. Hypotheses*, **2001**, *57*, 175.

14) Almulki, L.; Noda, K.; Nakao, S.; Hisatomi, T.; Thomas, KL.; Hafezi-Moghadam, A., *Exp Eye Res.*, **2010**, *90(1)*, 26.

15) Petra, D.; Balázs, B.; Rita M.; Elias, M.; Klára, G.; Péter M., *Expert Opin. Ther. Patents,* **2011**, *21(9)*, 1453.

16) Smith, D.; Vainio, P.; Mikkola, J.; Vuorio, P.; Vainio, J., WO 2008129124, **2008**.

17) VAP-1 antibody product profile., Biotie 2011. Available from: http://www.biotie.com/en/product_and_development/inflammation/vap1_antibody [Last accessed Oct. 2012]

18) Emma, J.; Joakim, N.; Derek, O.; Gerard, J. K., *Acta Crystallogr., Set. D: Biol. Crystallogr.*, **2005**, *61*, 1550.

19) Elisa, M. N.; Marjo, P.; László, L.; Zsolt, S.; Ulla, P.; Ferenc, F.; Olli, T. P., *J. Med. Chem.*, **2010**, *53*, 6301.

20) Elisa, M. N.; Marjo, P.; László, L.; Ulla, P.; Ferenc, F.; Olli, T. P., *J. Med. Chem.*, **2011**, *54*, 2143.

21) Emma, J.; Joakim, N.; Ulla, K.; Derek, O.; Gerard, J. K., *Acta Crystallogr., Set. F: Struc.Biol. Cryst. Commun.*, **2005**, *61*, 274.

22) Tomi, T. A.; Yvonne, N.; Heidi, K.; David, J. S.; Marjo, P.; Marko, S.; Sirpa, J.; Mark, S.J.; Tiina, A. S., *Protein Sci.*, **2005**, *14*, 1964.

23) Caterina, B.; Bernd, K.; Martin, S., *J. Med. Chem.*, **2010**, *53*, 5061.

24) PK (Pharmacokinetics) は、生体に投与した薬物の体内動態過程を明らかにするもの。この論文では、薬剤をラットに投与（経口、または皮下）した時の血中濃度測定試験を PK と記載している。PD（Pharmacodynamics）は、生体に対する薬物の薬理学的効果についての試験で、用量や薬物濃度と効果との関連を調べることを目的とする。この論文では、薬剤をラットに投与（経口、または皮下）した時の血中 VAP-1 阻害活性測定試験を PD と記載している。

25) Paul, L., *J. Chem. Inf. Model.*, **2010**, *50*, 792.

26) MOE2010.10, Chemical Computing Group, Montreal, QC, Canada

27) GOLD5.0. / 5.1., The Cambridge Crystallographic Data Centre, Cambridge, UK

28) Hayes, B. E.; Clarke, D. E., Res Commun Chem Pathology and Pharmacology, **1990**, 69, 71.

主論文目録

本学位論文内容は下記の発表論文による。

1. Inoue, T.; Morita, M.; Tojo, T.; Yoshihara, K.; Nagashima, A.; Moritomo, A.; Ohkubo, M.; Miyake, H.: Synthesis and SAR study of new thiazole derivatives as vascular adhesion protein-1 (VAP-1) inhibitors for the treatment of diabetic macular edema. *Bioorg. Med. Chem.*, **2013**, *21*, 1219-1233.

2. Inoue, T.; Morita, M.; Tojo, T.; Nagashima, A.; Moritomo, A.; Imai, K. ; Miyake, H.: Synthesis and SAR study of new thiazole derivatives as vascular adhesion protein-1 (VAP-1) inhibitors for the treatment of diabetic macular edema: Part 2. *Bioorg. Med. Chem.*, **2013**, *21*, 2478-2494.

3. Inoue, T.; Morita, M.; Tojo, T.; Nagashima, A.; Moritomo, A.; Miyake, H.: Novel 1*H*-imidazol-2-amine derivatives as potent and orally active vascular adhesion protein-1 (VAP-1) inhibitors for diabetic macular edema treatment. *Bioorg. Med. Chem.*, **2013**, *21*, 3873-3881.

参考関連特許

1) Inoue, T.; Tojo, T.; Morita, M.; Ohkubo, M.; Yoshihara, K.; Nagashima, A., Thiazole derivatives and their use as VAP-1 inhibitors, WO2004/067521 (2004).
2) Ueno, R.; Nagashima, A; Inoue, T.; Ohkubo, M.; Yoshihara, K., Method for treating vascular hyperpermeable disease, WO2004/087138 (2004).
3) Inoue, T.; Tojo, T.; Morita, M., Thiazole derivatives having VAP-1 inhibitory activity, WO2006/011631 (2006).
4) Inoue, T.; Tojo, T.; Morita, M., Thiazole derivatives having VAP-1 inhibitory activity, WO2006/028269 (2006).

主査および副査名

本学位論文の審査は千葉大学大学院薬学研究院で指名された下記の審査委員により行われた（敬称略）。

主査　千葉大学教授　（薬学研究院）薬学博士　西田 篤司

副査　千葉大学教授　（薬学研究院）薬学博士　石川　勉

副査　千葉大学教授　（薬学研究院）薬学博士　濱田 康正

副査　千葉大学教授　（薬学研究院）薬学博士　高山 廣光

副査　千葉大学教授　（薬学研究院）理学博士　石橋 正己