博士論文

基質結合様式に基づくプロテアーゼ阻害薬基本骨格の分子設計

—ジペプチジルペプチダーゼ４による検証—

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略号表

本論文中において下記の略号を使用した。

Ac_2O	acetic anhydride
ACE	angiotensin I-converting enzyme
BINAP	(±)-2,2'-bis(diphenylphosphino)-1,1'-binaphthalene
Bn	benzyl
Boc	*tert*-butoxycarbonyl
Boc_2O	di-*tert*-butyl dicarbonate
Bu	butyl
Cbz	(benzyloxy)carbonyl
CDI	1,1'-carbonyldiimidazole
CHAPS	3-((3-cholamidopropyl) dimethylammonio)-1-propanesulfonate
DCM	dichloromethane
DIBALH	diisobutylaluminum hydride
DMAP	4-dimethylaminopyridine
DME	1,2-dimethoxyethane
DMF	*N*,*N*-dimethylformamide
DMSO	dimethylsulfoxide
DPP2	dipeptidyl peptidase-2
DPP4	dipeptidyl peptidase-4
DPP8	dipeptidyl peptidase-8
DPP9	dipeptidyl peptidase-9
dppf	1,1'-bis(diphenylphosphino)- ferrocene
EDC	1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride
ESI	electrospray ionization
Et	ethyl
Ext_HB	protein-ligand hydrogen bond energy
Ext_vdW	protein-ligand van der Waals energy
GHRH	growth hormone releasing hormone
GIP	glucose-dependent insulinotropic polypeptide (or gastric inhibitory polypeptide)
GLP-1	glucagon-like peptide-1

HOBt	1-hydroxy-1*H*-benzotriazole
HTS	high-throughput screening
i-Bu	isobutyl
IC_{50}	half maximal (50%) inhibitory concentration
Int_Torsion	ligand torsional strain energy
Int_vdW	ligand internal van der Waals energy
IRI	immunoreactive insulin
LC–MS	low-resolution liquid chromatography mass spectra
Me	methyl
MM/PBSA	molecular mechanics Poisson–Boltzmann surface area
mp	melting point
MsCl	methanesulfonyl chloride
MsOH	methanesulfonic acid
neo-Pen	neopentyl
NMP	1-methyl-2-pyrrolidinone
NMR	nuclear magnetic resonance
NPY	neuropeptide Y
OAc	acetate
Ph	phenyl
*p*NA	*p*-nitoraniline
prep HPLC	preparative high performance liquid chromatography
RIA	radioimmunoassay
SAR	structure–activity relationship
TBS	*tert*-butyl(dimethyl)silyl
Tf	trifluoromethanesulfonyl
TFA	trifluoroacetic acid
THF	tetrahydrofuran
TLC	thin layer chromatography
Tris	tris(hydroxymethyl)aminomethane

第 1 章　緒言

1-1　本研究の目的

1-1-1　プロテアーゼと阻害薬

プロテアーゼ（protease, EC 3.4）とは、ペプチドやタンパク質を基質としてそのアミド結合の加水分解による基質分解を触媒する酵素の総称である。プロテアーゼにはペプシン（pepsin A, EC 3.4.23.1）やトリプシン（trypsin, EC 3.4.21.4）などに代表される基質認識能が比較的低いグループと、アンジオテンシン変換酵素（angiotensin I-converting enzyme, ACE, EC 3.4.15.1）やレニン（renin, EC 3.4.23.15）、ジペプチジルペプチダーゼ 4（dipeptidyl peptidase-4, DPP4, EC3.4.14.5）などに代表される基質認識能の高いグループがあり、基質認識能の高いグループの中には、生理学的意義の大きい特定の生体分子を分解あるいは生成することにより、生体の恒常性に深く関わるものが多い。Kinase に対する phosphatase、acyltransferase に対する deacylase など、生物学的意義の大きい酵素には生成物を基質に戻す逆反応系が存在することが多いが、プロテアーゼには逆反応を触媒する酵素が存在せず、一旦作用すると、基質の再生にはコードする遺伝子 DNA の転写・翻訳からの生合成過程を経る必要があり一定時間を要することになる。このように基質分解という不可逆反応を触媒するという機序から、プロテアーゼは生理作用発現の鍵因子となることが多く、その活性は生体内で精緻に制御されている。従って、多くのプロテアーゼの機能異常は、恒常性の破綻を招来しうることから、幅広い疾患領域において治療薬研究の重要な標的として注目され続けてきた。

プロテアーゼは、触媒活性中心が基質の特定のアミド結合のカルボニル基を活性化ならびに求核攻撃あるいは水分子の求核攻撃を支援することによりアミド結合を切断する。触媒活性中心の違いにより、セリン、システイン、スレオニン、アスパラギン酸、金属プロテアーゼなどのタイプに分類され

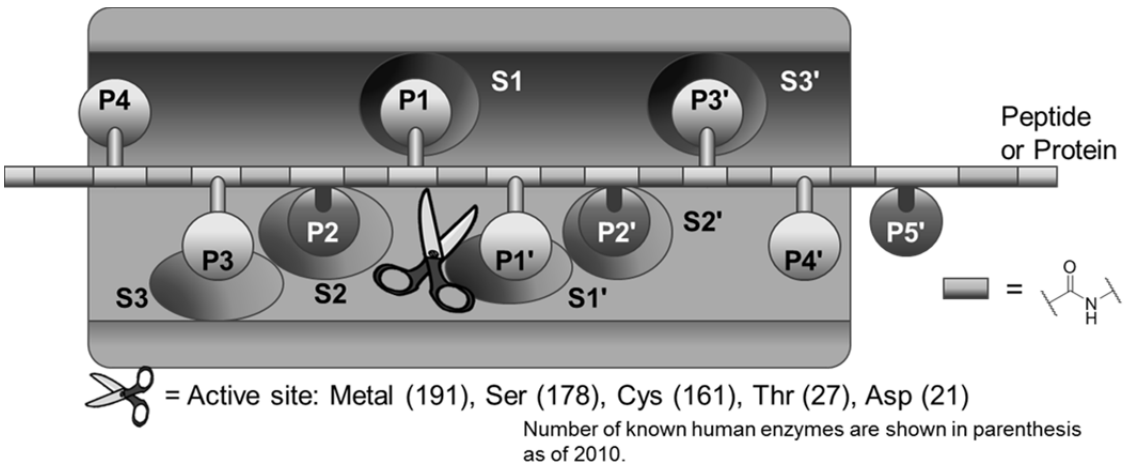


Figure 1-1.　Protease cleaves a specific amide bond of its substrate.

るが、いずれのタイプにおいても、その基質認識能は Figure 1-1 に示すように活性中心の比較的近傍にある複数の結合ポケット（S2', S1', S1, S2, S3 など）と対応する基質アミノ酸残基（P2', P1', P1, P2, P3 など）が相互作用することにより生み出されている。例えば、Figure 1-1 (A) に示した ACE は、基質である angiotensin I の Phe8（P1）のカルボニル基を活性中心の亜鉛イオンが活性化し、Glu384 の支援による水分子の求核攻撃を引き起こして His9（P1'）との間のアミド結合を切断する。その際、C 末端 Leu10（P2'）のカルボキシ基を S2'ポケットの Lys511 と Tyr520 で、また Leu10（P2'）のイソブチル基、His9 の側鎖と主鎖カルボニル基、Phe8（P1）のベンジル基、ならびに Pro7（P2）のピロリジン環を、それぞれ S2'（Phe457 など）、S1'（Gln162、Val380 など）、S1（Phe512、Val518 など）、S2 ポケット（Phe391 など）のアミノ酸残基による相互作用を利用して認識し、アミド結合切断のための最適な反応場を構築していると考えられている[1-5]。その他、Figure 1-2 に示した結合様式のように、プロテアーゼは、複数の結合ポケットで結合できる基質のみを選択し、標的となるアミド結合を活性中心の反応場に引き寄せて切断していると考えられる[6, 7]。

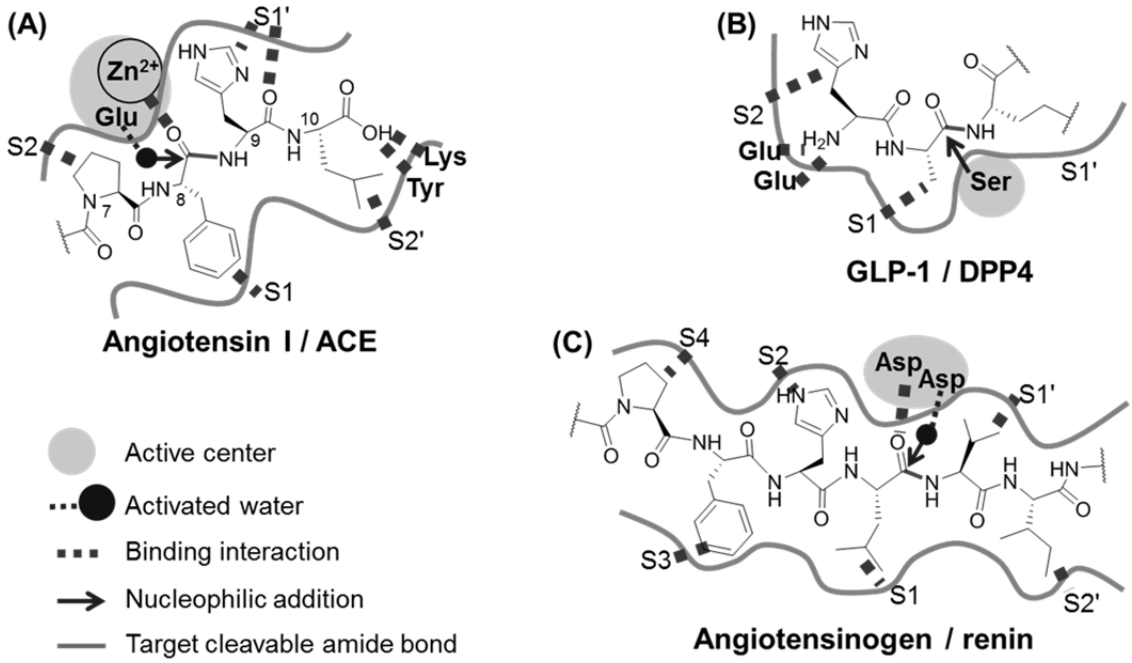


Figure 1-2. Putative 2D diagrams of catalytic domain in protease with a substrate. (A) ACE and angiotensin I; (B) DPP4 and glucagon-like peptide-1 (GLP-1); (C) Renin and angiotensinogen.

そのような基質認識の本質である結合ポケットとの相互作用を利用して多くの阻害薬が創出され、さらには疾患治療薬として上市されてきたが、低分子リードとの共結晶構造が公知となっていた特定の酵素を除き、ほとんどのプロテアーゼ阻害薬が基質ペプチドの模倣から始められたペプチドミメティクスに分類され、一般的な有機合成低分子の阻害薬は比較的数が少ない（Chart 1-1）[8-14]。プロテア

ーゼの基質認識能が高ければ高いほど、基質はより多くの結合ポケットによって認識されていることになるため、プロテアーゼー基質間の相互作用は、近年新たな創薬標的として注目を集めているタンパクータンパク間相互作用の要素が増すと考えられる。プロテアーゼ阻害薬はこの相互作用を断つ必要があることから、より多くの結合ポケットと相互作用しうるペプチドやペプチドミメティクスの方が阻害活性発現のためには有利であると考えられる。このような基質認識の機序ゆえか、一般に低分子化合物ライブラリーからのプロテアーゼ阻害薬のハイスループットスクリーニング（high-throughput screening, HTS）の成功確率は低いことが知られており、低分子ヒット化合物に高い阻害活性を期待することは現実的ではない[15, 16]。また、阻害活性の低いヒット化合物では酵素との結合部位が明確にならない場合が多く、具体的な阻害薬デザインに利用するまでには多くの仮説検証の繰り返しが必要となる。一方、プロテアーゼとの強い相互作用を得やすいと考えられるペプチドおよびペプチドミメティクスにおいては、溶解性などの物理化学的性質や代謝安定性、膜透過性といった薬物動態に関する特性などのドラッグライクネスの面、さらには製造、製剤化などのプロセスの面での課題が多く、リード発見から治療薬候補となるまでに結局長期の研究開発期間を要することも稀ではない[17, 18]。これらペプチドおよびペプチドミメティクスにおける課題は、有機合成低分子においても同様に生じることはあるが、その課題解決のための方法論やノウハウが創薬化学の分野に数多く集積されているため、リード発見から治療薬候補となるまでの研究開発がペプチドやペプチドミメティクスに比べて短期間となることが期待できる。いわば創薬化学者の得意分野とも言えるこの強みをプロテアーゼ阻害薬開発で生かすためには、効率良くリード化合物を創出できる何らかの方法論が必要であると考えられる。

そこで、プロテアーゼ阻害薬の新たな低分子リード創出戦略を確立すべく研究を開始した。

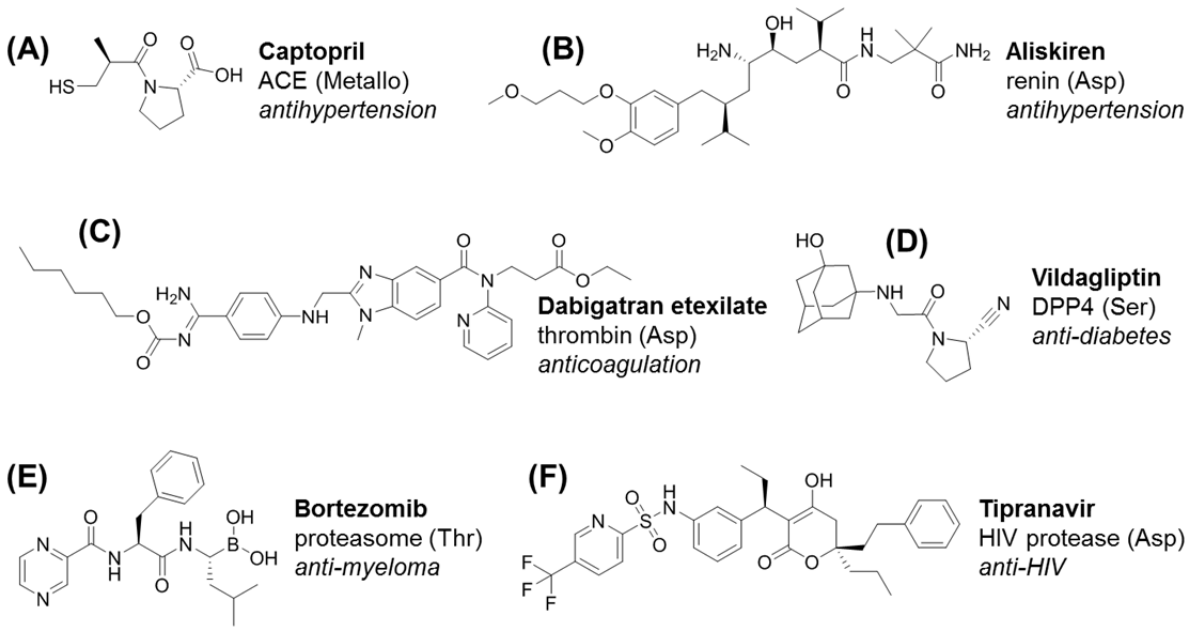


Chart 1-1. Representative protease inhibitors.

1-1-2　スキャフォールド戦略の着想

これまでに創製された代表的な基質認識能の高いプロテアーゼ阻害薬の結合様式[19]を酵素との共結晶の X 線構造から考察したところ、ペプチドミメティクスに代表されるプロテアーゼ阻害薬は、概して酵素活性中心のアミノ酸残基に加えその近傍の 2 つ以上の結合ポケットとの相互作用を利用しているが（Figure 1-3 (A) ~ (D)）、実例は少ないものの一部の低分子阻害薬は、活性中心とは相互作用せずその近傍の 3 つ以上の結合ポケットとの相互作用を利用していると考えられた（Figure 1-3 (E)）。

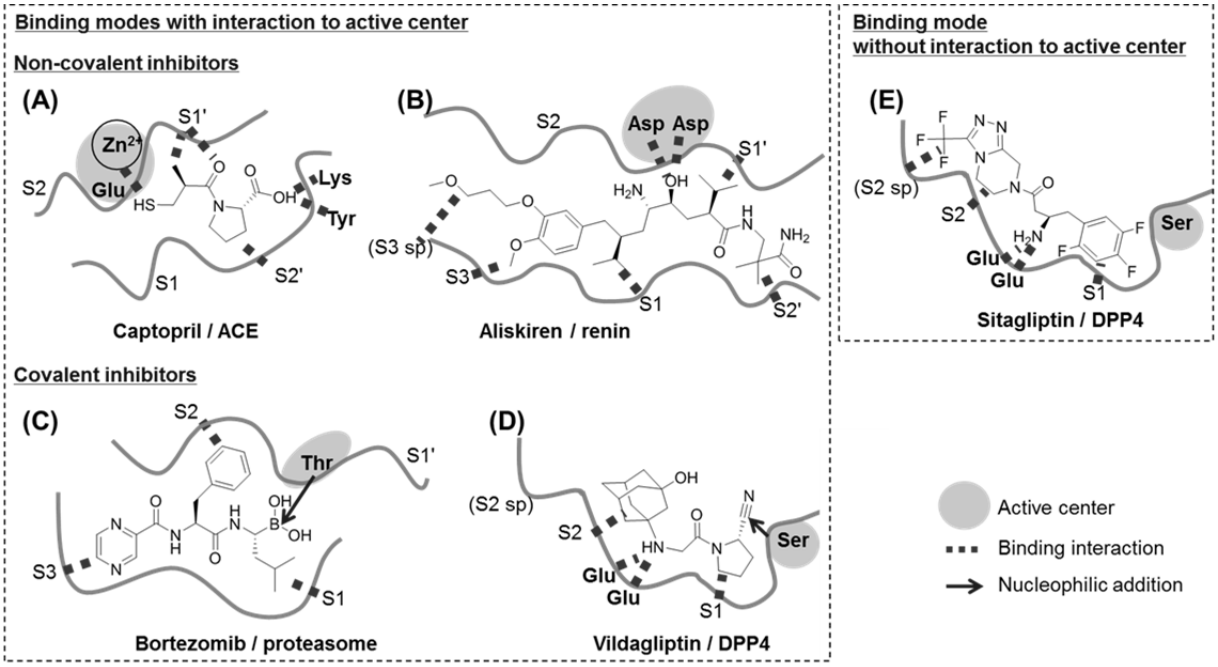


Figure 1-3. Binding modes of representative protease inhibitors with interaction to active center (Left, (A) ACE and captopril; (B) Renin and aliskiren; (C) Proteasome and bortezomib; (D) DPP4 and vildagliptin) and that without interaction to active center (Right, (E) DPP4 and sitagliptin).

例えば、Figure 1-3 (A) に示す ACE 阻害薬の captopril は、その共結晶構造から、メルカプト基が活性中心の亜鉛イオンに配位し、ピロリジン環とカルボキシ基が S2'ポケットと、またアミド結合のカルボニル基とα 位メチル基が S1' ポケットとそれぞれ相互作用していることが知られている。すなわち、ACE 阻害薬のうち最も単純な構造を有する captopril でさえも、活性中心を含め酵素の 3 つの認識部位を利用して阻害活性を発揮していることになる[1, 2]。Renin 阻害薬の aliskiren（Figure 1-3 (B)）においては、基質分解の遷移状態を模倣した 4 位水酸基が、活性中心の Asp32 および Asp215 と水素結合を形成し、その他の側鎖が活性中心近傍の S2'、 S1'、 S1、 S3 ポケットおよび S3 サブポケットとそれぞれ結合しており、酵素の 6 つの認識部位を利用していることが知られている[9, 17]。共有結合型で最も単

純な構造を有する DPP4 阻害薬の一つである vildagliptin（Figure 1-3 (D)）は、シアノ基が活性中心の Ser630 と共有結合を形成し、ピロリジン環が S1 ポケットと、二級アミノ基および 3-ヒドロキシ-1-アダマンチル基が S2 ポケットとそれぞれ相互作用することで 3 つの認識部位を利用することにより酵素を阻害している [11, 20]。これに対して、非共有結合型の DPP4 阻害薬の一つである sitagliptin（Figure 1-3 (E)）は、S1、S2 ポケットとは同様に相互作用するが、活性中心とは相互作用せず、代わりに S2 ポケットの延長上の S2 サブポケットとの相互作用を利用して 3 点認識を維持している [20, 21]。このように、活性中心との相互作用の有無や共有結合性の有無に関わらず、創薬リードレベルのプロテアーゼ阻害薬には合計 3 つ以上の相互作用（3 点認識）が必要であると考えられた。

上述の考察に基づき、基質認識能の高いプロテアーゼ阻害薬のリード創出の戦略として、基質ペプチドをリードとしてペプチドミメティクスの創出を目指すものではなく、基質ペプチドの結合様式を基に有機合成低分子阻害薬のリードを創出するための「スキャフォールド戦略」を着想した。

この戦略は以下に示す 2 つの段階を経て高活性の創薬リードの創出を目指すものである。

1) 酵素の活性中心およびその近傍の結合ポケットのうち、連続する 2 つの認識部位に対する相互作用点を導入したヘテロ環中心骨格（スキャフォールド）の構築（Figure 1-4 の Step 1）
2) スキャフォールドへの酵素の第 3 の認識部位との相互作用点の追加による活性向上（Figure 1-4 の Step 2）

また、スキャフォールド戦略を用いた場合、次のような可能性が期待できる。

1) リード最適化や経口吸収性、製造、製剤化などでの課題解決において、ペプチドミメティクスよりも優れた低分子創薬の特長を利用できる可能性
2) 中心骨格が基質ペプチドの主鎖とは立体的に異なる空間を占有する可能性が高く、これにより新たな結合部位を見出せる可能性
3) 得られたスキャフォールドを、置換基の変換などの構造修飾により他のプロテアーゼ阻害薬に転用できる可能性

本戦略により創出される低分子プロテアーゼ阻害薬には、標的とする認識部位の組み合わせにより 2 つのタイプが想定される。一方は、Figure 1-4 の Type A の方法により創出される、酵素活性中心に加えその近傍の 2 つの結合ポケットとの合計 3 つの相互作用部位を利用する阻害薬、他方は、Figure 1-4 の Type B の方法により創出される、酵素活性中心近傍の 3 つの結合ポケットと相互作用し、活性中心とは相互作用しない阻害薬である。その実例が少ないことを鑑みると、後者のような活性中心と相互作用しないプロテアーゼ阻害薬のリード創出は一般に困難と考えられ、その創出戦略（Type B）を確立することの創薬化学的意義は大きいと考えられた。

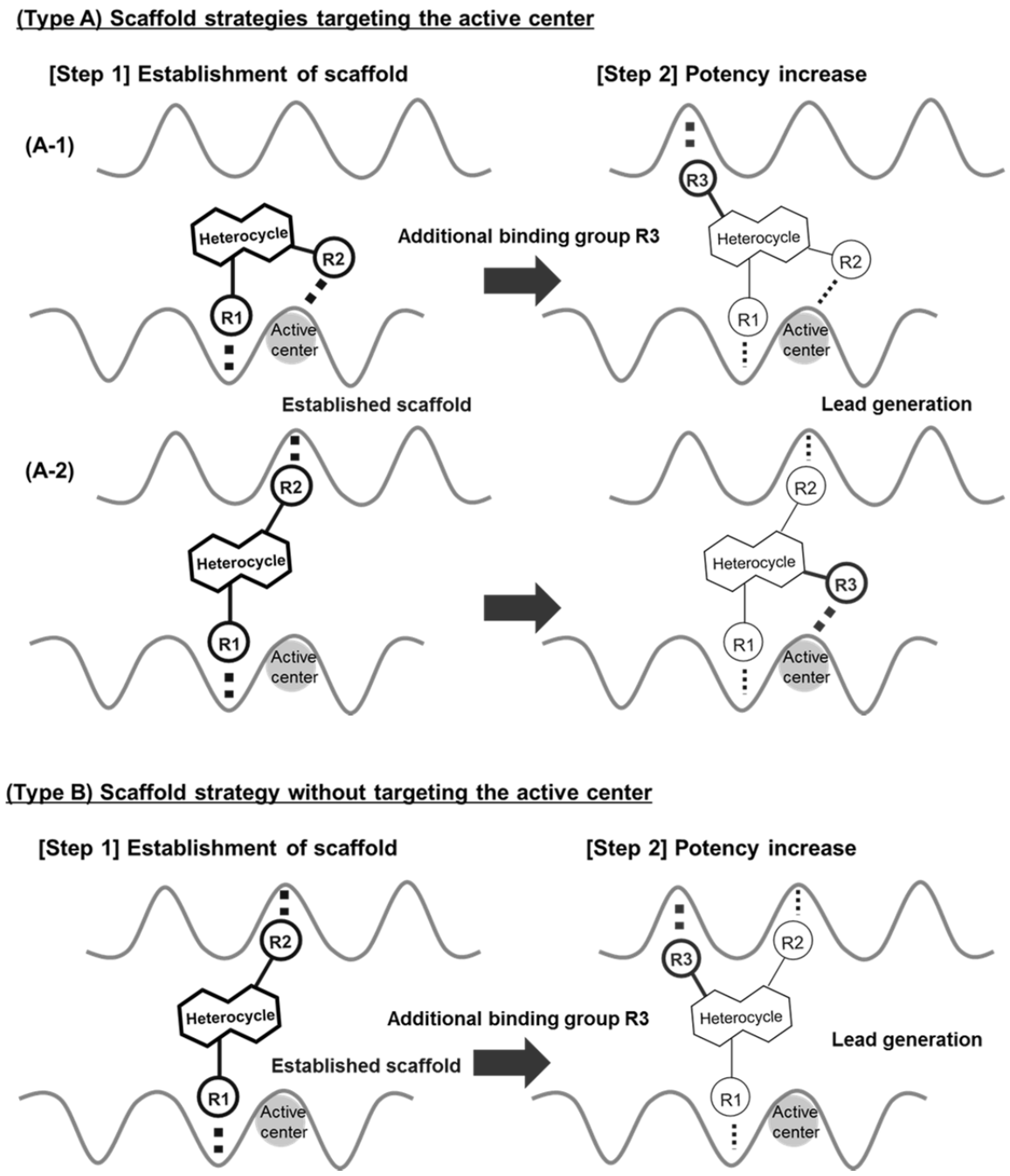


Figure 1-4. Outlines of scaffold strategy. Step 1, establishment of a heterocyclic core bearing substituents (R1 and R2) targeting two binding sites; Step 2, generation of highly potent lead compound by introducing an additional binding group (R3); Types A, strategies for inhibitors that interact to the active center; Type B, strategy for inhibitors that do not interact to the active center.

1-1-3　本研究の目的

以上の点を踏まえて、本研究の目的を、基質認識能の高いプロテアーゼの阻害薬に対する創薬リード創出の方法論として、着想したスキャフォールド戦略が有効か否かを確認することに設定し、その検証の題材には、近年糖尿病治療薬として重要性を増しているジペプチジルペプチダーゼ 4（DPP4）を選択した。

現在では、Chart 1-2 に示すように数多くの DPP4 阻害薬が糖尿病治療薬として上市されている。特に vildagliptin（Chart 1-1 の D、Chart 1-2 の A）など、2 位に求電子性置換基を導入したピロリジン環を有する阻害薬に代表されるような触媒活性中心と直接共有結合するタイプの阻害薬は、そのデザインが十分に研究されてきており、一つの化合物クラスを形成している[22]。これに対し、sitagliptin（Chart 1-2 の C）などの活性中心と直接相互作用しないタイプには、いずれも独自性の高い多様な化合物クラスの阻害薬が含まれている。

そこで本研究では、活性中心と直接相互作用しないタイプの阻害薬を目標とするスキャフォールド戦略（Figure 1-4 の Type B）を選択し、酵素活性中心近傍の 3 つの結合ポケットとの相互作用を指向することによる創薬リードレベルの低分子 DPP4 阻害薬創出の可否、ならびに創出されるリードの独自性によりその有効性を確認することで本戦略の完成を目指すことにした。

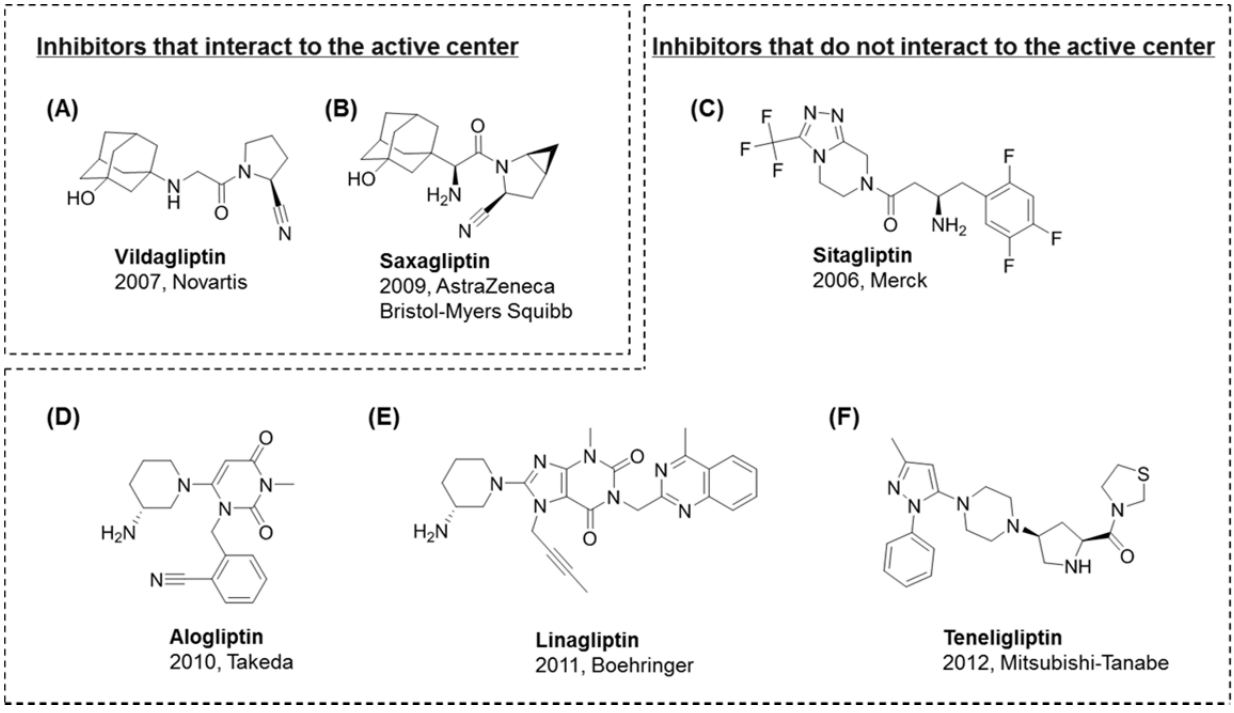


Chart 1-2.　Representatives of launched DPP4 inhibitors.

1-2　ジペプチジルペプチダーゼ４とその基質認識

DPP4 は N 末端から 2 番目と 3 番目のアミノ酸残基（P1 および P1'）の間のアミド結合を切断するジペプチジルペプチダーゼ（dipeptidyl peptidase, EC3.4.14）の一種である。Table 1-1 に例示したようにその基質には、血糖濃度依存性のインスリン分泌促進作用や膵保護作用などの様々な抗糖尿病作用を有する glucagon-like peptide-1 (GLP-1) や glucose-dependent insulinotropic polypeptide (GIP) といった、インクレチンホルモンに代表される重要な生理活性ペプチドが数多く含まれており、DPP4 はこれらを分解し不活性化することでその生理作用を調節しているものと考えられる。一方で、血糖調節機能が破綻した糖尿病患者においては、GLP-1 の極めて短い血中半減期（$t_{1/2}$ < 2 min）をその主要な原因である分解酵素 DPP4 の阻害により延長させる治療は、現在最も有用な糖尿病治療法の一つとなっている [23-25]。さらに近年では、DPP4 と脳卒中 [26] や心不全 [27] との関連を示唆する報告もなされており、DPP4 阻害薬は今後幅広い疾患の治療薬になりうることが期待されている。

DPP4 の触媒活性中心は Ser630 であり、Ser630 側鎖の水酸基が標的アミド結合のカルボニル基を求核攻撃することで加水分解を引き起こし結合を切断する。DPP4 は基質として P1 が Ala あるいは Pro 残基であるペプチドを選択することが知られている（Table 1-1）。その基質認識能は、P1 の側鎖を疎水性アミノ酸残基で構成される S1 ポケット（Val656、Trp659、Tyr662、Tyr666、Val711 など）で、さらに N 末端のアミノ酸残基（P2）の遊離アミノ基を S2 ポケット内の Glu motif（Glu205、Glu206）に代表される酸性アミノ酸残基が多く集まる領域で、同じく P2 の側鎖を S2 ポケット（Arg125、Val207、Ser209、Phe357、Arg358 など）で、それぞれ認識することにより達成されると考えられる [28, 29]。

Table 1-1. Representative Substrate for DPP4 and N-Terminal Amino Acid Sequence[a]

Substrate	P2–**P1**–P1'–P2'–
Glucagon-like peptide-1 (GLP-1) [7-36]	His–**Ala**–Glu–Gly–
Glucose-dependent insulinotropic polypeptide (GIP) [1-42]	Tyr–**Ala**–Glu–Gly–
Neuropeptide Y (NPY) [1-36]	Tyr–**Pro**–Ser–Lys–
Growth hormone releasing hormone (GHRH) [1-44]	Tyr–**Ala**–Asp–Ala–
Substance P	Arg–**Pro**–Lys–Pro–

[a] DPP4 degrades substrates with Ala or Pro at the penultimate N-terminal position as P1.

1-3　ジペプチジルペプチダーゼ４阻害薬創出のためのスキャフォールド戦略

1-3-1　スキャフォールド戦略のジペプチジルペプチダーゼ４への適用

DPP4 は第 1-2 節に記したように、基質の P1 および N 末端の P2 のアミノ基を強く認識していると考えられる。そこで、DPP4 の連続する重要な基質認識部位である S1 および S2 ポケットを Step 1 での標的相互作用部位に設定し、それらへの相互作用点となる置換基をヘテロ環中心骨格に導入することで DPP4 に対する新たな阻害薬のスキャフォールドを構築しうると考えた（Figure 1-5, Step 1）。具体的には、S1 ポケットに結合する基質の P1 アミノ酸残基（Ala/Pro）の側鎖に対応して芳香環などの脂溶性基を、S2 ポケットの Glu motif に結合する基質の N 末端アミノ基に対応して遊離または *N*-置換のアミノアルキル基を、また S2 ポケットに結合する P2 アミノ酸残基の側鎖に対応して脂溶性基をそれぞれ適切に配置したヘテロ環中心骨格の構築を検討することにした。この構築したスキャフォールドに Step 2 で導入する活性向上のための第 3 の相互作用点の標的部位としては、S1 ポケットに隣接する S1' ポケットが分子設計上容易であると考えられた（Figure 1-5, Step 2）。そこで、S2、S1、および S1' ポケットとの相互作用を強化して DPP4 阻害薬の新たなリード創出を試みる方針で、実際に創薬研究を開始した。

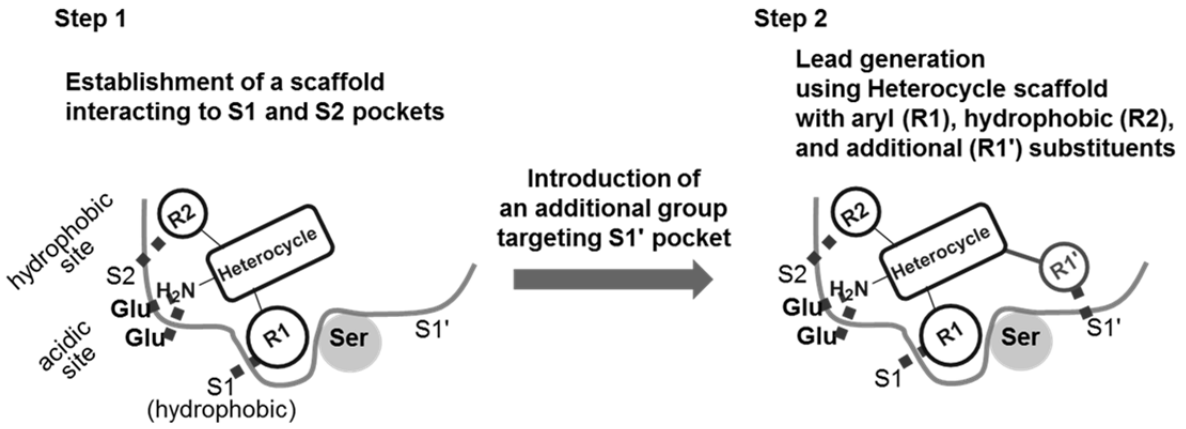


Figure 1-5. Outline of scaffold strategy (Type B) for lead generation of DPP4 inhibitors. Step 1) establishment of a heterocyclic core bearing substituents (R1 and R2) targeting S1 and S2 pockets, respectively; Step 2) generation of highly potent lead compound by introducing an additional binding group (R1') targeting S1' pocket.

1-3-2　イソキノロン系スキャフォールドでの予備検討

DPP4 阻害薬のリード創出に対してスキャフォールド戦略を適用するにあたり、社内化合物ライブラリーからの HTS ヒット化合物を用いた予備検討を実施した結果を著者らは既に報告している[30]。この検討では、HTS ヒット化合物であるイソキノロン系化合物 **1a** に基づいて、化合物 **1e** の構造で表されるイソキノロン系スキャフォールドを構築し、構造修飾により化合物 **2a** にまで導いた（Figure 1-6）。より詳細には、まずスクリーニングチームが実施した HTS により得られた 10^{-5}~10^{-4} M オーダーの低い阻害活性を有する複数のヒット化合物の中からイソキノロン系化合物 **1a** を選択した。その際、この化合物の 4 位フェニル基が DPP4 の基質の P1 プロリンの 5 員環が結合する S1 ポケットと、3 位置換基上のアミノ基および 2 位置換基が P2 アミノ酸残基の末端アミノ基および側鎖が結合する S2 ポケットと、それぞれ相互作用して DPP4 阻害のための足場となることで、本戦略のコンセプトに合致しうるとの仮説を立てた。この仮説に基づいて 2 位、3 位、および 4 位置換基の構造活性相関を調べることで化合物 **1e** を得て、活性発現のための基本構造であるイソキノロン系スキャフォールドを構築した。次いで、活性向上のための新たな相互作用部位に DPP4 の S1' ポケットを設定し、イソキノロン系スキャフォールドに導入する相互作用点として 6 位の置換基が適することを確認した。この 6 位置換基最適化の結果、DPP4 阻害活性がヒット化合物 **1a** から約 80 倍向上した化合物 **2a** を見出すことに成功した。

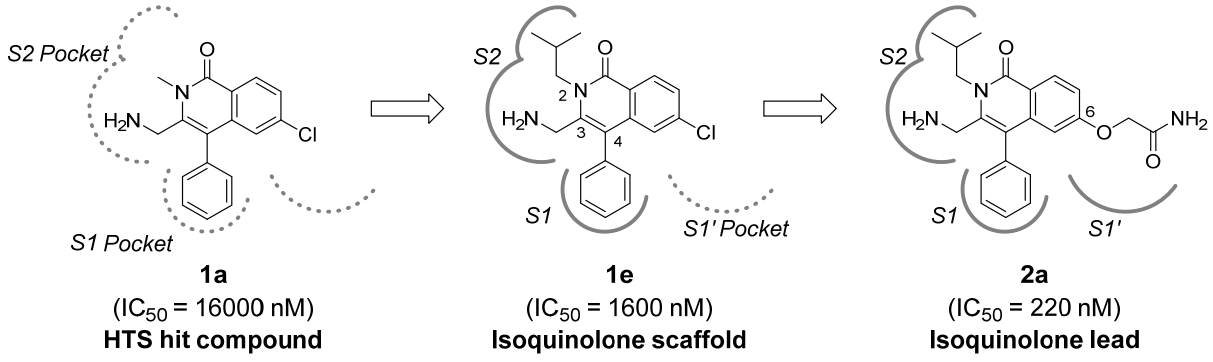
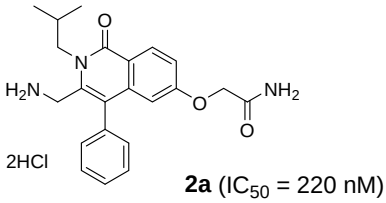


Figure 1-6. Identification of isoquinolone-based DPP4 inhibitor **2a** via scaffold **2e** derived from HTS hit compound **1a**.

構造生物学チームにより取得された、見出した化合物 **2a** と DPP4 との共結晶構造を Figure 1-7 に示した。この結果から、4 位置換基が S1 ポケットに結合し、3 位アミノメチル基が S2 ポケットの酸性アミノ酸残基（Glu205、Glu206）と塩橋を形成、さらに 2 位イソブチル基は S2 ポケット側面の Phe357 と CH–π 相互作用により結合しており、スキャフォールド部分がほぼ仮説通りの結合様式を取ることが明らかとなった。また、追加導入した相互作用点である 6 位置換基は、末端のカルボニル基が Lys554

と水素結合を形成しており、アミドの NH_2 と S1' ポケット底面の Trp629 の主鎖カルボニル基との水素結合も認められた。さらに、Glide[31] を用いた結合エネルギー計算ではその寄与は S1' のアミノ酸残基との全水素結合エネルギーの 5%未満と小さいことが示唆されたが、6 位エーテル結合で水分子を介して活性中心の Ser630 および His740 と水素結合していることも確認できた。



2a (IC_{50} = 220 nM)

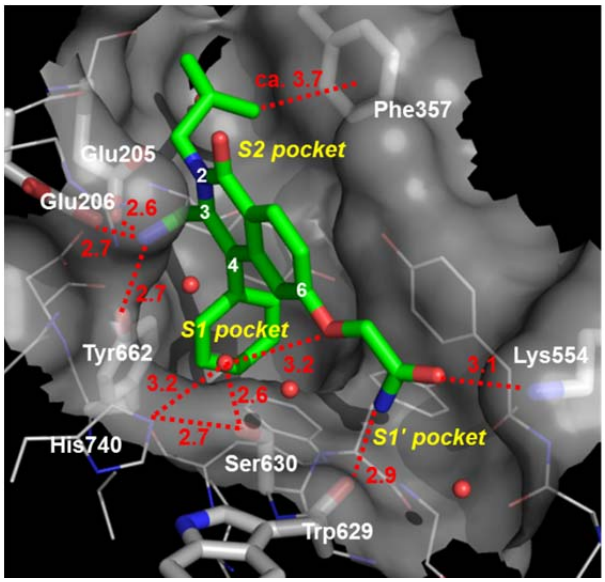


Figure 1-7. X-ray structure of compound **2a** in complex with human DPP4 (Protein Data Bank Code: 3OPM).[30] Representative amino acid residues (grey carbons, white texts) and waters (red oxygens) in binding sites of **2a** (green carbons) are indicated. Values listed in red are distances between heavy atoms in angstroms.

予備検討で見出した **2a** は阻害活性がやや低く、創薬リードとするには更なる活性向上が必要であると考えられたが、この予備検討の結果によりスキャフォールド戦略によるリード創出の手応えを得ることができた。そこで、**2a** の構造に基づいてより高い阻害活性を発揮しうる新たなスキャフォールドを設計し、リード創出につなげることでスキャフォールド戦略の完成を目指すことにした。

なお以降の本研究において、特筆する場合を除き化合物のデザインおよび合成は合成チームで実施し、得られた阻害薬の酵素阻害活性の評価は、Gly–Pro–*p*-nitoraniline (*p*NA, for DPP4, DPP8, and DPP9) および H–Lys–Ala–*p*NA（for DPP2）を基質に用いてスクリーニングチームが実施した。またドッキングスタディーは、構造生物学チームにより解かれた X 線共結晶構造に基づき計算化学チームが実施した。

第 2 章　新規キノリン系ジペプチジルペプチダーゼ 4 阻害薬の創出

2-1　キノリン系ジペプチジルペプチダーゼ 4 阻害薬の分子設計

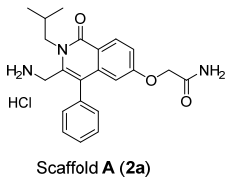
2-1-1　新たな中心骨格の in silico 探索

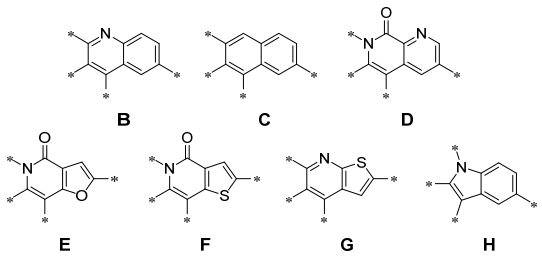
新たなスキャフォールドとして、イソキノロン系と同じ空間配置に相互作用点を保持でき、かつ追加導入する相互作用点の構造変換が容易なものが望ましいと考え、誘導化の容易さも考慮してイソキノロン環に代わる新たな中心骨格を探索することにした。

新たな中心骨格の in silico 探索は、計算化学チームの主導によりドッキングプログラム GOLD[32] を利用して実施した。まず、イソキノロン系スキャフォールドが有する DPP4 の S1 および S2 ポケットに対する相互作用点（**2a** の 2 位、3 位、4 位置換基）および S1' ポケットとの相互作用点（**2a** の 6 位置換基）を、**2a** と対応する置換位置に導入した縮合芳香環化合物 B–H を設計した。次いで、これらデザイン化合物と DPP4 とのドッキングモデルを、化合物 **2a** と DPP4 との共結晶構造に基づき GOLD を用いて作成し、その親和性を GoldScore で評価した（Table 2-1）。

評価した化合物のうち、最も高い GoldScore を示す化合物の一つであり、かつ合成の容易なキノリン骨格を有する B に注目した。GOLD による評価では、キノリン誘導体 B はイソキノロン誘導体 A に比べてリガンド分子内歪みに関するスコアが良好で、構造上無理なく酵素と結合できる点で有利である可能性が示唆された。

Table 2-1. In Silico Screening for Alternative Core Structure Using GOLD





Scaffold	GoldScore[a]	Ext_HB	Ext_vdW	Int_Torsion	Int_vdW
A (**2a**)	73.8	24.3	64.6	–11.8	–3.3
B (**9d**)	78.4	24.6	62.1	–8.0	–0.3
C	79.7	21.7	64.4	–5.3	–1.1
D	76.6	23.0	61.7	–6.4	–1.7
E	69.7	23.5	62.9	–12.6	–4.1
F	69.0	24.2	58.5	–10.1	–3.7
G	70.1	21.6	60.5	–9.4	–2.5
H	68.4	22.4	56.5	–10.1	–0.5

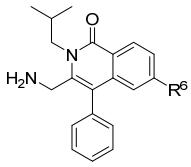
[a] GoldScore consists of protein–ligand hydrogen bond energy (Ext_HB), protein–ligand van der Waals energy (Ext_vdW), ligand torsional strain energy (Int_Torsion) and ligand internal van der Waals energy (Int_vdW).

2-1-2　In silico 探索結果の検証

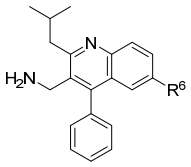
GOLD による評価結果の妥当性を、実際に誘導体を合成し阻害活性を評価することにより検証した。具体的には、イソキノロン環（A）とキノリン環（B）に加えて、代表的な中心骨格として化合物 D の 7,8-ジヒドロ-1,7-ナフチリジン-8-オン環を有する化合物を合成してその DPP4 阻害活性を調べた（Table 2-2）。その結果、阻害活性の序列は GoldScore の序列とほぼ同様であり、三者の中でキノリン誘導体は最も高いレベルにあって、in silico での探索結果およびキノリン環の選択の妥当性が実測結果により支持された。

以上の検討により中心骨格選択の妥当性がある程度支持されたことから、これ以降はキノリン系スキャフォールドに的を絞って検証を進めることにした。

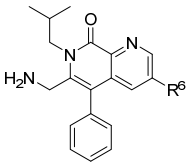
Table 2-2. DPP4 Inhibitory Activity of Representative Designed Compounds



A



B



D

R^2	human DPP4 IC_{50} (nM) [a]		
	A	B	D
Br	1200 (**2b**)	630 (**43a**)	1200 (**40a**)
$CONH_2$	360 (**2c**) [b]	170 (**43b**)	470 (**40b**)
OCH_2CONH_2	220 (**2a**) [b]	270 (**9d**)	not tested

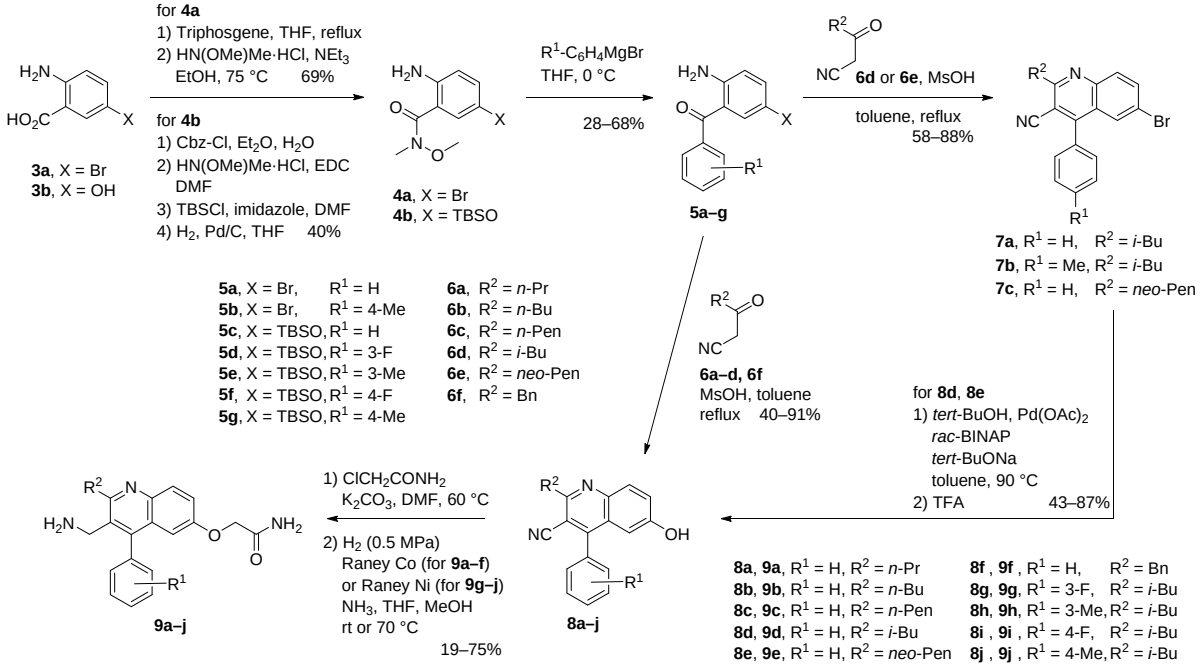
[a] 50% inhibitory concentration against human DPP4 using Lys–Ala–*p*-nitro-aniline (*p*NA) as a substrate.

[b] Banno, Y., et al. *Bioog. Med. Chem.* **2011**, *19*, 4953–4970.

2-2　キノリン系ジペプチジルペプチダーゼ 4 阻害薬の合成

キノリン誘導体および関連誘導体の合成は Scheme 2-1–2-6 に示す方法により行った。Scheme 2-1、Scheme 2-2 には、主に 6 位鎖状置換基を有する誘導体の合成法を示した。まず Scheme 2-1 に示すように、市販の置換アントラニル酸 **3a,b** から導いた Weinreb アミド体 **4a,b** に [33]、対応する置換フェニルマグネシウム試薬を反応させて 2-アミノベンゾフェノン誘導体 **5a–g** へと変換した。これら **5a–g** と 2-アシルアセトニトリル **6a–f** とを、Friedlander のキノリン合成法の改良法 [34, 35] を用いてメタンスルホン酸存在下で縮合させ、キノリン環を構築することで 6-ブロモ-3-シアノキノリン体 **7a–c** および 3-シアノ-6-ヒドロキシキノリン体 **8a–c**、**8f–j** へと導いた。このうち 6-ブロモ-3-シアノキノリン体 **7a** および **7c** は、Buchwald の方法 [36] で *tert*-ブチルエーテル化した後、酸処理して **8d** および **8e** へと変換した。得られた **8a–j** の 6 位水酸基を 2-クロロアセトアミドを用いてアルキル化することで 6 位側鎖を構築した後、3 位シアノ基を展開ニッケルまたはコバルト触媒により接触還元して 3-(アミノメチル)キノリン誘導体 **9a–j** へと導いた。

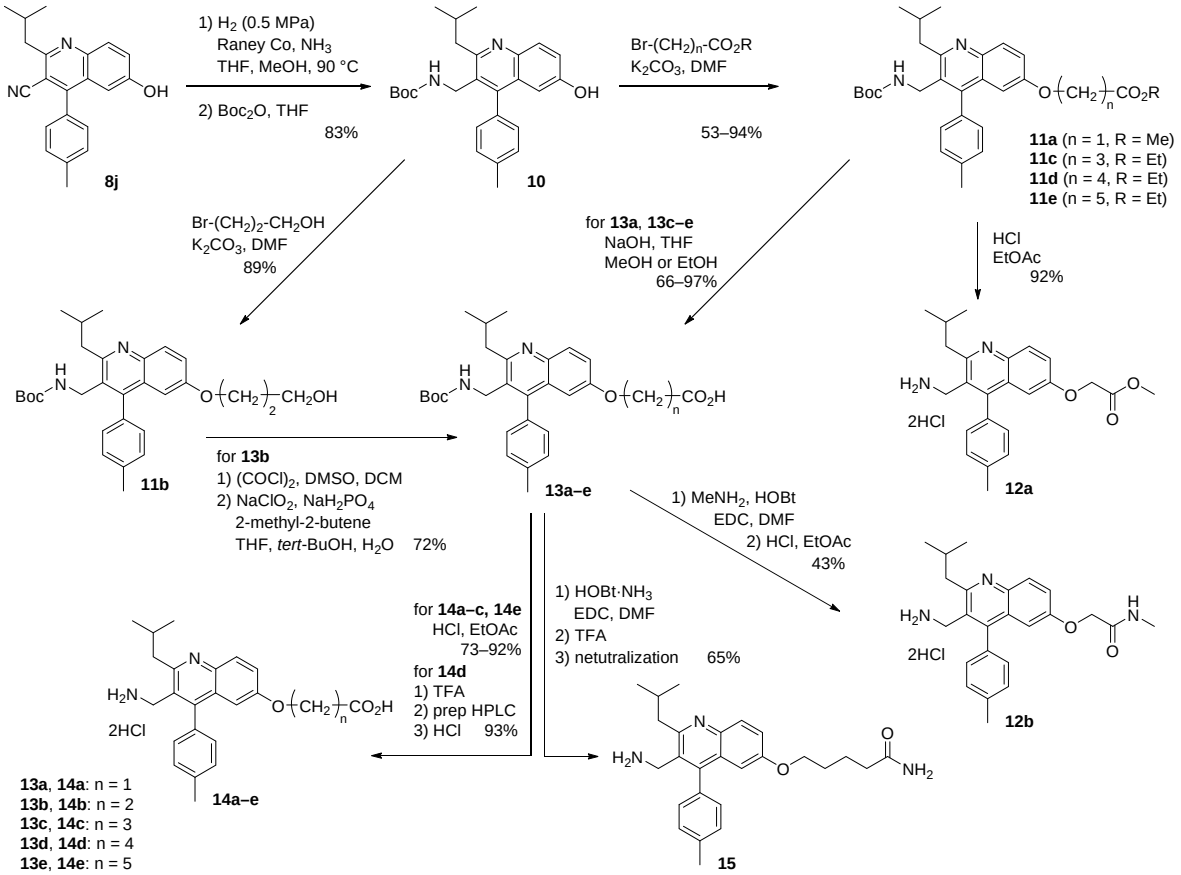
Scheme 2-1.



また Scheme 2-2 に示すように、中間体 **8j** の 3 位シアノ基を接触還元した後、N-Boc 保護することで共通中間体 **10** を合成した。この中間体 **10** の 6 位水酸基を各種アルキル化して **11a–e** へと変換し、これらのうち **11a** を脱 N-Boc 化することで **12a** を得た。また、**11a** および **11c–e** の 6 位置換基末端のエステルを加水分解、あるいは **11b** の 6 位置換基末端のアルコール部分を酸化することでカルボン酸中

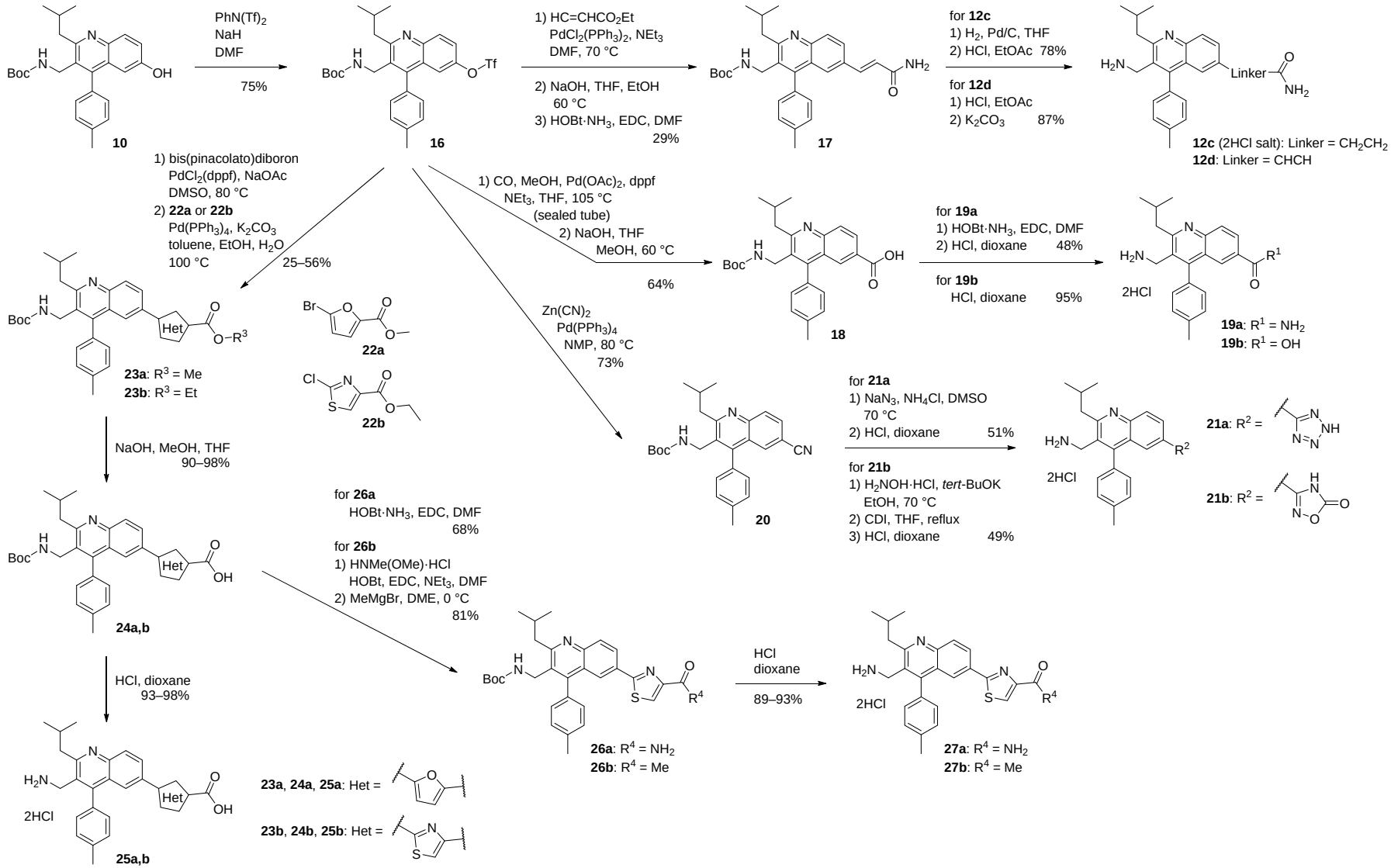
間体 **13a–e** へと変換し、これらの 3 位 *N*-Boc 基を脱保護することで、リンカー長の異なるカルボン酸誘導体 **14a–e** を合成した。さらに中間体 **13a** および **13d** のカルボキシ基をアミド化し、脱 N-Boc 化することで、**12b** および **15** へと導いた。

Scheme 2-2.



Scheme 2-3 には、主にパラジウム触媒を用いたクロスカップリング反応による 6 位置換基の変換方法を示した。まず、中間体 **10** の 6 位水酸基をトリフルオロスルホニル化して共通中間体 **16** を得た。この 6 位に Heck 反応を利用してアクリル酸エチル部分構造を導入した後 [37]、末端エステル部分を加水分解、次いで酸アミド化して **17** へと変換した。得られたアクリルアミド誘導体 **17** を、パラジウム触媒下で接触還元した後、脱 N-Boc 化してプロピオンアミド誘導体 **12c** へと導いた。また **17** をそのまま脱 N-Boc 化したアクリルアミド誘導体 **12d** も合成した。同様にパラジウム触媒下で、**10** を原料に一酸化炭素を用いカルボニル挿入してエステル体とした後 [38]、エステル部分をアルカリ加水分解してカルボン酸誘導体 **18** へと変換した。得られたカルボン酸誘導体 **18** を酸アミドに変換した後、脱 N-Boc 化して酸アミド誘導体 **19a** へと導いた。また、**18** をそのまま脱 N-Boc 化したカルボン酸誘導体 **19b** も合成した。さらに、パラジウム触媒下で **10** の 6 位にシアノ基を挿入した中間体 **20** を用いて [39]、6 位シ

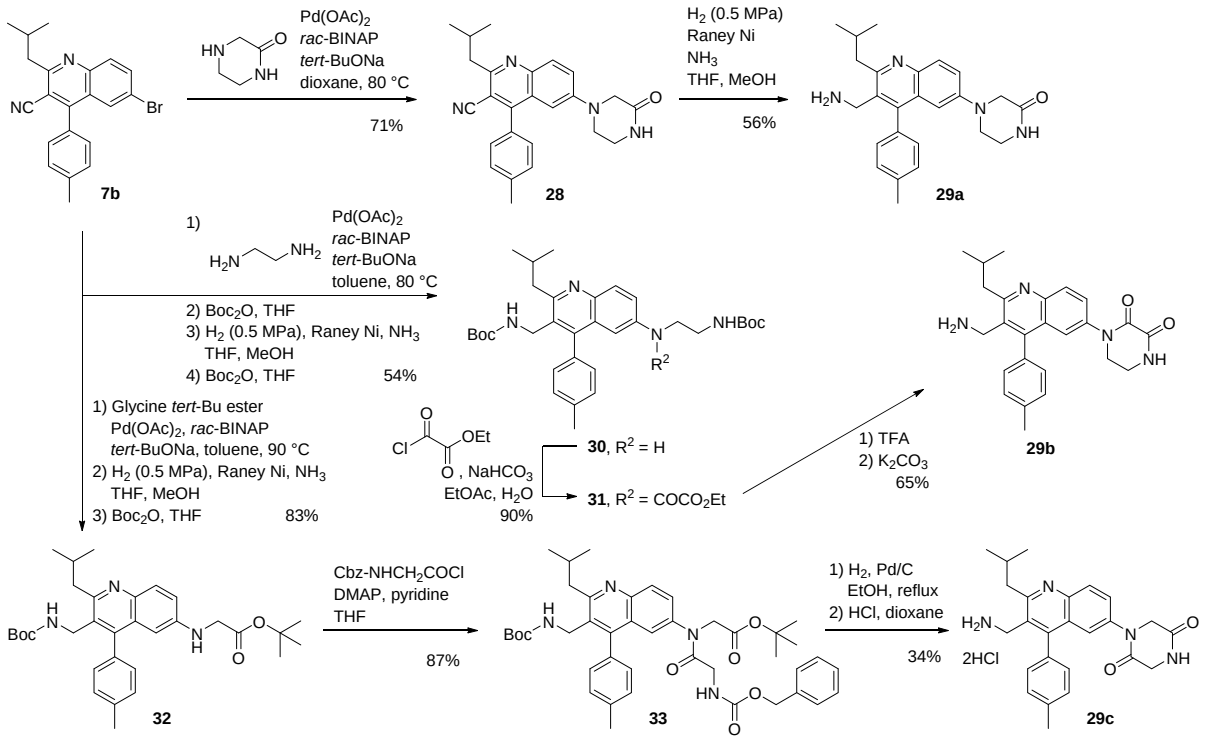
Scheme 2-3.


PhN(Tf)2
NaH
DMF
75%
1) HC=CHCO2Et
PdCl2(PPh3)2, NEt3
DMF, 70 °C
2) NaOH, THF, EtOH
60 °C
3) HOBt·NH3, EDC, DMF
29%
for 12c
1) H2, Pd/C, THF
2) HCl, EtOAc 78%
for 12d
1) HCl, EtOAc
2) K2CO3 87%
12c (2HCl salt): Linker = CH2CH2
12d: Linker = CHCH
1) bis(pinacolato)diboron
PdCl2(dppf), NaOAc
DMSO, 80 °C
2) 22a or 22b
Pd(PPh3)4, K2CO3
toluene, EtOH, H2O
100 °C
25–56%
1) CO, MeOH, Pd(OAc)2, dppf
NEt3, THF, 105 °C
(sealed tube)
2) NaOH, THF
MeOH, 60 °C
64%
for 19a
1) HOBt·NH3, EDC, DMF
2) HCl, dioxane 48%
for 19b
HCl, dioxane 95%
19a: R1 = NH2
19b: R1 = OH
Zn(CN)2
Pd(PPh3)4
NMP, 80 °C
73%
for 21a
1) NaN3, NH4Cl, DMSO
70 °C
2) HCl, dioxane 51%
for 21b
1) H2NOH·HCl, tert-BuOK
EtOH, 70 °C
2) CDI, THF, reflux
3) HCl, dioxane 49%
21a: R2 =
21b: R2 =
NaOH, MeOH, THF
90–98%
HCl, dioxane
93–98%
for 26a
HOBt·NH3, EDC, DMF
68%
for 26b
1) HNMe(OMe)·HCl
HOBt, EDC, NEt3, DMF
2) MeMgBr, DME, 0 °C
81%
HCl
dioxane
89–93%
23a: R3 = Me
23b: R3 = Et
26a: R4 = NH2
26b: R4 = Me
27a: R4 = NH2
27b: R4 = Me
23a, 24a, 25a: Het =
23b, 24b, 25b: Het =

アノ基をアジ化ナトリウムと反応させてテトラゾール環に変換した後[40]、脱 N-Boc 化して **21a** へと導いた。同様に、**20** のシアノ基をヒドロキシルアミンと反応させて得られたアミドキシム誘導体を 1,1'-カルボニルジイミダゾールと反応させて 1,2,4-オキサジアゾール環に変換し[41]、脱 N-Boc 化することで **21b** も合成した。また中間体 **16** は、ピナコールジボランとのクロスカップリングによりホウ酸エステル誘導体とすることで、種々の鈴木アリールカップリングの中間体として利用し[42]、これを用いて複素環ハライド **22a** あるいは **22b** とのカップリングにより複素環置換基を導入したエステル誘導体 **23a** および **23b** を合成した。得られたエステル誘導体 **23a** および **23b** をアルカリ加水分解してカルボン酸誘導体 **24a** および **24b** とした後、脱 N-Boc 化して **25a** および **25b** へと導いた。また、カルボン酸 **24b** は酸アミド **26a** に変換、あるいは Weinreb アミドに変換した後に、メチルマグネシウム試薬と反応させてアセチル体 **26b** に変換し、それぞれ脱 N-Boc 化して **27a** および **27b** へと導いた。

Scheme 2-4 には、Buchwald のアミノ化反応[43]を利用した 6 位環状置換基を有する誘導体の合成法を示した。Buchwald タイプの反応は 6-ブロモ-3-シアノキノリン誘導体 **7b** を原料として行った。この **7b** の 6 位を 2-オキソピペラジンでアミノ化した後（**28**）、3 位シアノ基を接触還元してアミノメチル基へと変換して **29a** を合成した。また **7b** の 6 位を 1,2-エチレンジアミンでアミノ化してその末端アミノ基を選択的に N-Boc 保護した後、3 位シアノ基を接触還元した。得られた 3-アミノメチル体を二炭酸ジ-*tert*-ブチルと反応させることで、3 位アミノ基が N-Boc 保護された中間体 **30** へと導いた。次いで、中間体 **30** の 6 位の二級アミノ基をアシル化してシュウ酸アミド体 **31** とし、酸存在下で 2 つの *N*-Boc アミノ基を脱保護

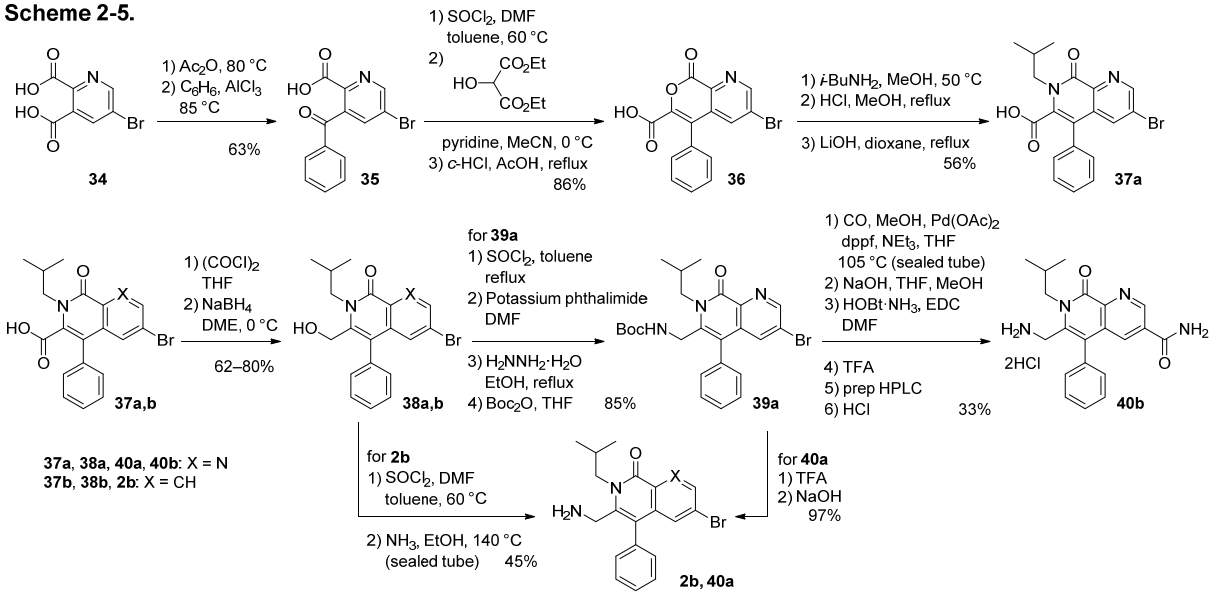
Scheme 2-4.



したところ、6 位置換基の環化反応も進行して 2,3-ジオキソピペラジン環を有する **29b** へと変換できた。同様に **7b** の 6 位をグリシンエステルでアミノ化した後、3 位シアノ基を *N*-Boc アミノメチル基へと変換して **32** とした。次いで 6 位の二級アミノ基を *N*-Cbz グリシンの酸クロリドと反応させてアシル化し（**33**）た後、加水素分解で脱 N-Cbz 化したところ、6 位置換基の環化反応も進行して 2,5-ジオキソピペラジン環を有する中間体が生成した。この中間体の 3 位 *N*-Boc アミノメチル基を酸存在下で脱保護して、目的の **29c** を合成した。

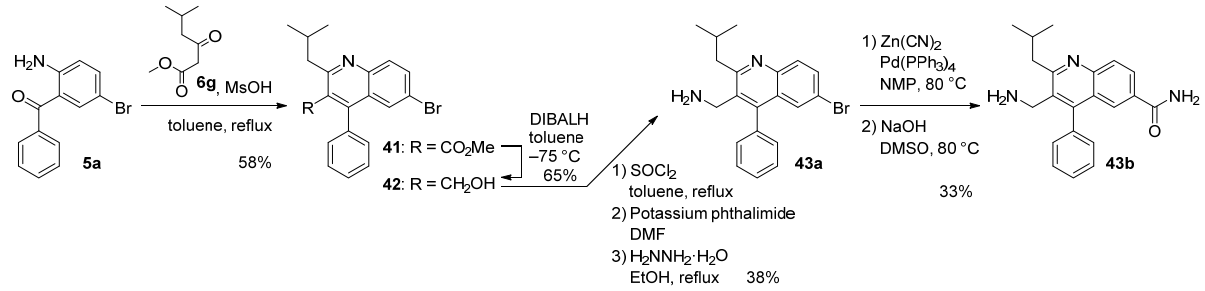
関連する 1,7-ナフチリジン-8-オン誘導体 **40a,b** およびイソキノロン誘導体 **2b** は、既存の合成法[30]と同様の方法で合成した（Scheme 2-5）。まず、ピリジン-2,3-ジカルボン酸誘導体 **34** から変換した酸無水物を用いた Friedel–Crafts 反応によりベンゼンをアシル化して、3-ベンゾイルピリジン-2-カルボン酸 **35** を合成した。このカルボキシ基を酸クロリドに変換し、次いで 2-ヒドロキシマロン酸エステルと反応させてトリエステルとして、これを塩酸存在下で加熱還流することで環化とエステル加水分解が進行したピラノピリジン誘導体 **36** を得た。次に **36** にブチルアミンを反応させることで 1,7-ナフチリジン-8-オン環を構築した（**37a**）。得られた **37a** の 6 位カルボキシ基を酸クロリドに変換した後、水素化ホウ素ナトリウムでヒドリド還元して 6-ヒドロキシメチル体 **38a** に導いた。文献既知のイソキノロン誘導体 **37b**[30]についても同様の方法で **38b** に導いた。中間体 **38a** の水酸基を塩素化し、Gabriel 合成法によりフタルイミド体を経て 3-アミノメチル体に導き、N-Boc 化して中間体 **39a** を合成した。得られた中間体 **39a** を酸存在下で脱 N-Boc 化して **40a** を合成した。また、中間体 **39a** をパラジウム触媒下、一酸化炭素を用いて 3 位にカルボニルを挿入してエステル体とし、エステル加水分解、酸アミド化を行った後に脱 N-Boc 化することで **40b** へと変換した。中間体 **38b** については水酸基を塩素化した後、アンモニアを反応させて 3-アミノメチル体 **2b** に変換した。

Scheme 2-5.



キノリン誘導体 **43a,b** については、Scheme 2-6 に示すように **5a** と β-ケトエステル **6g** とを縮合して得られる 3 位にエステルを有するキノリン体 **41** を経て合成した。中間体 **41** のエステル部分のヒドリド還元、塩素化、Gabriel 合成により 3-アミノメチル体 **43a** へと導き、その 6 位へのシアノ基挿入で得られたニトリル体のアルカリ加水分解により **43b** を合成した。

Scheme 2-6.



2-3　キノリン系ジペプチジルペプチダーゼ 4 阻害薬の構造活性相関

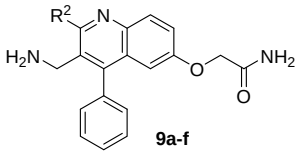
2-3-1　キノリン系スキャフォールドの構築

まず、選択したキノリン環を中心骨格とするスキャフォールドの構築を検討すべく、2 位および 4 位置換基の最適化を行った。

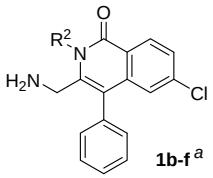
このうち 2 位置換基（R^2）の構造活性相関を、イソキノロン誘導体の構造活性相関とともに Table 2-3 に示した。R^2 は炭素鎖長が 3 が好ましく（**9a** > **9b**, **9c**）、その末端に分枝のあるイソブチル基（**9d**）やネオペンチル基（**9e**）の時に比較的高い阻害活性を示し、この傾向はイソキノロン誘導体の時と同様であった（**1e** および **1f**）。一方、S2 ポケットの Phe357 との疎水性相互作用の増強を期待してベンジル基を導入した場合は逆に阻害活性は減弱した（**9f**）。このことから、**9f** ではベンジル基のかさ高さが Phe357 周辺の空間には許容されなかったものと考えられる。

以上の結果から、キノリン系スキャフォールドの 2 位置換基としてイソキノロン誘導体の場合と同様にイソブチル基を選択した。

Table 2-3. Structure–Activity Relationship of 2-Substituent (R^2) of Quinoline Derivatives



9a-f

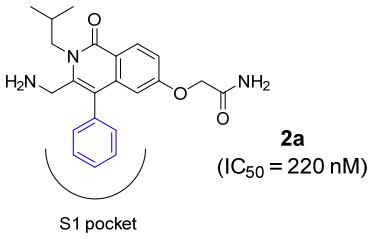


1b-f [a]

Compound	R^2	IC_{50} (nM) [b]
9a	*n*-Pr	510
9b	*n*-Bu	620
9c	*n*-Pen	540
9d	*i*-Bu	270
9e	*neo*-Pen	250
9f	Bn	720
1b	*n*-Pr	2400
1c	*n*-Bu	2400
1d	*n*-Pen	3800
1e	*i*-Bu	1600
1f	*neo*-Pen	1900

[a] Banno, Y., et al. *Bioog. Med. Chem.* **2011**, *19*, 4953-4970.
[b] Inhibitory activity for human DPP4.

次に、4 位置換基の最適化を行った。まず最適化に先立って、第 1 章第 1-3-2 節で述べたイソキノロン **2a** の DPP4 との共結晶構造について S1 ポケット周辺の結合様式を解析した（Figure 2-1）。その結果、**2a** の 4 位フェニル基と最も近接する S1 ポケット壁面の脂溶性残基（Tyr631、Val656、Val711 など）との炭素間距離は 3.9~4.4 Å であり、4 位フェニル基のメタ位からパラ位付近には S1 ポケット壁面との間に 1 炭素分程度の空間があることが確認できた。このことから、4 位フェニル基のメタ位あるいはパラ位への小さな脂溶性置換基の導入が阻害活性を向上させる可能性が示唆された。



2a
(IC_{50} = 220 nM)

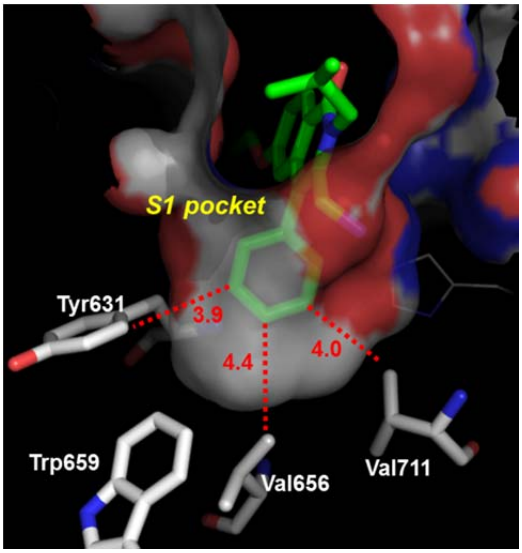
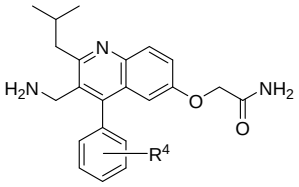


Figure 2-1. X-ray structure of compound **2a** in complex with human DPP4 (Protein Data Bank Code: 3OPM).[30] Representative amino acid residues (grey carbons, white texts) in S1 binding sites of **2a** (green carbons) are indicated in cross-sectional view. Values listed are distances between carbon atoms in angstroms.

そこでこの考察に基づいて、実際に 4 位フェニル基上に小さな置換基を導入し阻害活性への効果を調べた（Table 2-4)。その結果、4 位フェニル基のメタ位への置換基導入は阻害活性を若干減弱させる結果となり、導入による活性向上効果は認められなかった。一方、パラ位への導入は阻害活性を高め、中でもメチル基の導入は阻害活性を約 13 倍向上させた（**9j**)。

以上の結果から、キノリン環 4 位の置換基として *p*-トリル基を選択し、上述の 2 位置換基の結果も合わせることで、DPP4 の S1 および S2 ポケットを標的とするキノリン系スキャフォールドとしての、3-(アミノメチル)-2-イソブチル-4-(4-メチルフェニル)キノリン構造が構築できた。

Table 2-4. Effect of Substitution on the 4-Phenyl Group on DPP4 Inhibition



Compound	R^4	IC_{50} (nM) [a]
9g	3-F	350
9h	3-Me	340
9i	4-F	150
9j	4-Me	20
9d	H	270

[a] Inhibitory activity for human DPP4.

2-3-2　キノリン系リード化合物の創出

次に、得られたキノリン系スキャフォールドへの新たな相互作用点導入による活性向上とリード化合物の創出を検討した。新たな相互作用点の標的とした S1' ポケットは、活性中心の Ser630 に隣接し Tyr547 などの脂溶性アミノ酸残基により形成される結合ポケットであり、その最深部に Lys554 が位置している（Figure 2-2）。

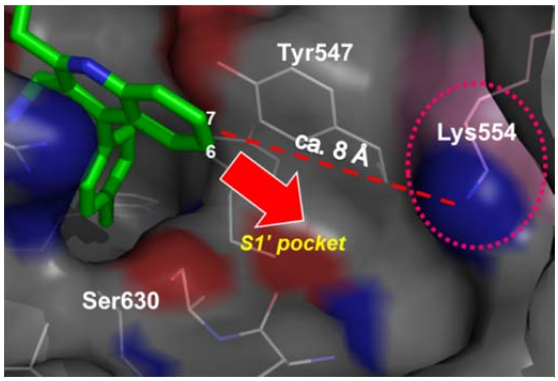


Figure 2-2. Docking model of quinoline-based scaffold (green carbons) into DPP4 binding site. S1' pocket and representative amino acid residues are depicted.

この Lys 残基を標的とする DPP4 阻害薬のデザインは当初報告されていなかったこともあり、独自性の高い結合様式の阻害薬を目指して、Lys554 との相互作用獲得を以下の 2 通りの方針により検討した。

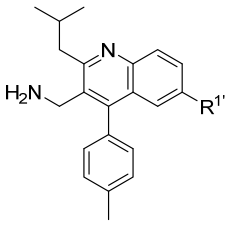
方針 1：Lys554 との水素結合獲得（6 位置換基への水素結合性置換基導入）

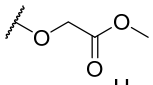
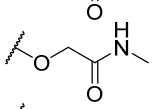
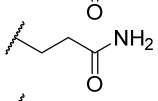
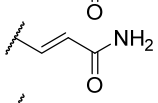
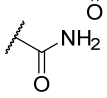
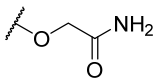
方針 2：Lys554 との塩橋形成（6 位置換基へのカルボキシ基導入）

2-3-2-1　方針 1 に基づく Lys554 との水素結合可能な 6 位置換基の検討

Table 2-5 に示すように、6 位の鎖状置換基末端に種々の水素結合性置換基を導入したところ、エステル体（**12a**）や置換アミド体（**12b**）では、無置換アミド体（**9j**）に比べて活性が減弱した。無置換アミドとのリンカー部分については、**9j** のエーテル結合をメチレン鎖に変換した場合（**12c**）は **9j** と同等の阻害活性を、リンカー部分をエチレン鎖に変換した場合（**12d**）には 3 倍高い阻害活性を示した。前章 Figure 1-7 に示した **2a** の共結晶構造と同様に、**9j** もエーテル部分で水分子を介して Ser630 と間接的に相互作用していることが想定されたが、その相互作用よりも、**12d** の 6 位エチレン鎖に想定される S1' ポケット内脂溶性アミノ酸残基との疎水性相互作用のほうが、阻害活性への寄与が大きいことが示唆された。一方、6 位直結のアミド体 **19a** では阻害活性が **9j** に比べ約 10 倍減弱しており、Lys554 との距離および脂溶性アミノ酸残基との位置関係ともに不利に働いたものと考えられた。

Table 2-5. Introduction of Polar Moiety onto 6-Substituent of Quinoline Derivatives

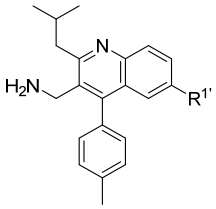


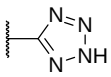
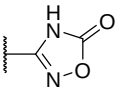
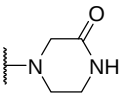
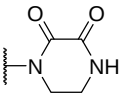
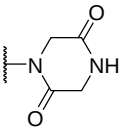
Compound	$R^{1'}$	IC_{50} (nM) [a]
12a		330
12b		270
12c		25
12d		6.1
19a		210
9j		20

[a] Inhibitory activity for human DPP4.

ところで、上述の **19a** の類縁体であるカルボン酸 **19b** に対し、その生物学的等価体であるテトラゾール誘導体 **21a** は 14 倍高い阻害活性を示すことが分かった。このことから 6 位置換基として環状置換基が好ましい可能性が考えられたため、6 位への環状置換基の導入を検討した（Table 2-6）。その結果、極性基を有する環状置換基が高い阻害活性を示すことが分かったが、特に 3-オキソピペラジン構造を有する **29a–c** の阻害活性が高かった。とりわけ **29c** は、IC_{50} 値が 10^{-9} M オーダーの非常に高い阻害活性を示した。

Table 2-6. Introduction of Cyclic Group at the 6-Position of Quinoline Derivatives



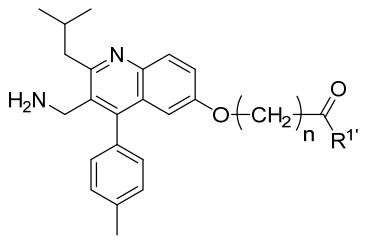
Compound	$R^{1'}$	IC_{50} (nM) [a]
19a	$CONH_2$	210
19b	CO_2H	330
21a		23
21b		19
29a		11
29b		12
29c		4.9

[a] Inhibitory activity for human DPP4.

2-3-2-2　方針 2 に基づく Lys554 との塩橋形成可能な 6 位置換基の検討

S1' ポケットの Lys554 との塩橋形成を指向し、末端にカルボキシ基を有する 6 位置換基のキノリン系スキャフォールドへの導入を検討した。まず、鎖状置換基の検討としてカルボキシ基とのリンカーの炭素鎖長による阻害活性への影響を調べた（Table 2-7）。第 2-3-2 節の Figure 2-2 に示したように、標的とする Lys554 とキノリン環 6 位との距離は約 8 Å と考えられ、6 位置換基が伸長した場合、Table 2-2 における至適炭素鎖長は **14a,b** の n = 1~2 であると予想された。しかしながら、実際に最も高い阻害活性を示したのは炭素鎖長が n = 4（**14d**）の場合であり、この時の母核とカルボキシ基との間のリンカー長は、明らかに Lys554 との距離よりも長かった。一方、共結晶構造は明らかにはなっていないものの、カルボン酸体 **14d** に対応するアミド体 **15** の阻害活性が 3 倍以上低下することから、**14d** は Lys554 との塩橋を形成して阻害活性を高めていることが示唆される。したがって、**14d** はカルボキシ基を Lys554 との塩橋形成に適する位置に配置するため、その 6 位リンカーの炭素鎖を折り畳んで S1' ポケットに納めているとともに、折り畳んだ炭素鎖により S1' の脂溶性アミノ酸残基との間の疎水性相互作用を増強しているものと考えられた。この結合様式は、結果は示さないが **14d** を用いたドッキングモデルからも支持された。また、第 2-3-2-1 節 Table 2-5 においてリンカーに π 電子を有する **12d** が高い阻害活性を示したことや、同 Table 2-6 においてカルボン酸 **19b** よりも環構造を有する生物学的等価体 **21a** の方が高い阻害活性を示したことを考慮すると、折り畳まれた 6 位の炭素鎖が、S1' ポケット壁面にキノリン環と平行に配置された Tyr547 と相互作用している可能性が示唆された。

Table 2-7. Effect of 6-Linker Length of Quinoline-based Carboxylic Acids

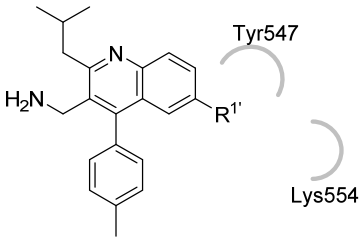


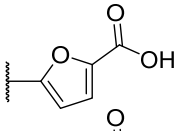
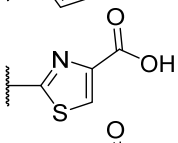
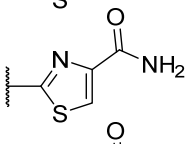
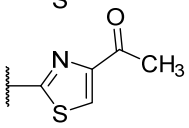
Compound	n	$R^{1'}$	IC_{50} (nM) [a]
14a	1	OH	140
14b	2	OH	29
14c	3	OH	17
14d	4	OH	4.6
14e	5	OH	6.8
15	4	NH_2	13

[a] Inhibitory activity for human DPP4.

そこで次に、Tyr547 との疎水性相互作用獲得を検討し、6 位置換基のカルボキシ基との間のリンカーとして、Tyr547 のベンゼン環と π–π スタッキングしうる芳香環をデザインしその導入を行った。その結果、Table 2-8 に示す 5 員複素環で高い阻害活性が得られ、とりわけチアゾール環を有する **25b** では IC_{50} 値 0.38 nM と、過去に報告のある DPP4 阻害薬の中でも最高レベルの DPP4 阻害活性を示した。カルボン酸 **25b** に対して類縁体であるアミド体（**27a**）およびケトン体（**27b**）では阻害活性が 5 倍および 20 倍以上低下しており、**25b** のカルボキシ基による塩橋形成が阻害活性に重要であることが示唆された。

Table 2-8. DPP4 Inhibitory Activity of Quinoline Derivatives with Heterocyclic Linker

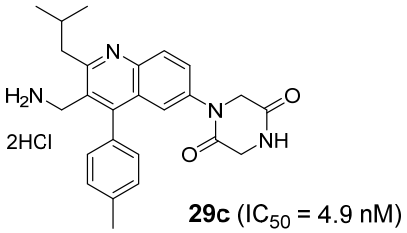


Compound	$R^{1'}$	IC_{50} (nM) [a]
25a		28
25b		0.38
27a		2.1
27b		8.7

[a] Inhibitory activity for human DPP4.

2-3-3　ドッキングモデルを用いた考察

スキャフォールド戦略を用いて見出したキノリン系DPP4阻害薬のDPP4とのドッキングモデルを作成し、その結合様式を考察した。Figure 2-3 に示すモデルでは、Lys554 との水素結合形成を指向して合成した **29c** は、イソキノロン系と同様に 2 位イソブチル基が Phe357 と CH–π 相互作用可能な位置に、また 3 位アミノメチル基が Glu205、Glu206、Tyr662 と塩橋形成可能な位置にあり、4 位 *p*-トリル基は S1 ポケットと結合して、スキャフォールド部分に想定された S1 および S2 ポケットとの結合が可能であることが示唆された。追加の相互作用部位に設定した S1' ポケットとの相互作用では、6 位置換基であるピペラジン環の 3 位オキソ基が Lys554 との水素結合形成可能な位置にあり、6 位オキソ基は Tyr631 の主鎖 NH と水素結合可能な位置にあった。さらにピペラジン環は Tyr547 に対して、炭素鎖が CH–π あるいはカルボニルが π–π スタッキングすることで、S1' での疎水性相互作用を高めているものと考えられた。



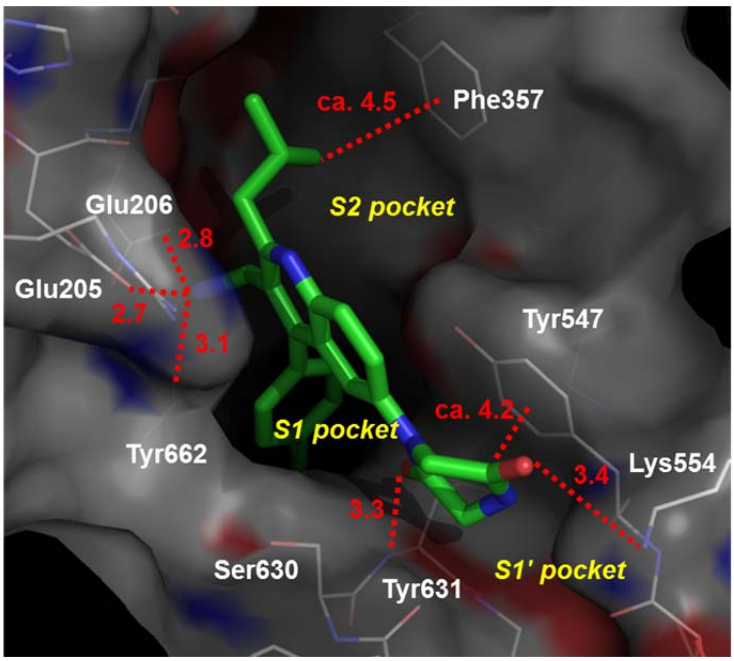
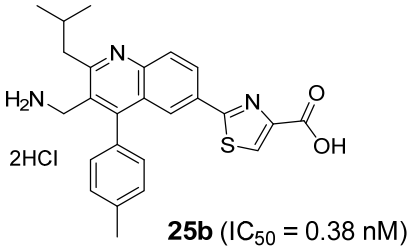


Figure 2-3.　Docking model of **29c** in the catalytic domain of DPP4.

一方、Lys554 との塩橋形成を指向して合成した **25b** のドッキングモデルは、Figure 2-4 に示すように、キノリンスキャフォールド部分が **29c** の場合と同様の結合様式となっていることを示唆している。S1' ポケットとの相互作用では、**25b** のカルボキシ基は Lys554 の側鎖末端アミノ基と近く、塩橋形成しうることが示唆された。また、チアゾール環は Tyr554 のベンゼン環と約 4 Å の距離をおいてほぼ平行に配置しており、良好な π–π スタッキングをしていることが示唆された。この **25b** も、Figure 1-7 に示したイソキノロン誘導体 **2a** の共結晶構造と同様に水分子を介して Ser630 と間接的に相互作用している可能性は否定できないが、キノリン環の 6 位周辺の極性が高い **29c** に比べてその寄与はより小さく、またイソキノロン **2a** の場合と同様に S1' との相互作用においてもエネルギー的には大きな寄与にはなっていないと考えられる。



25b (IC_{50} = 0.38 nM)

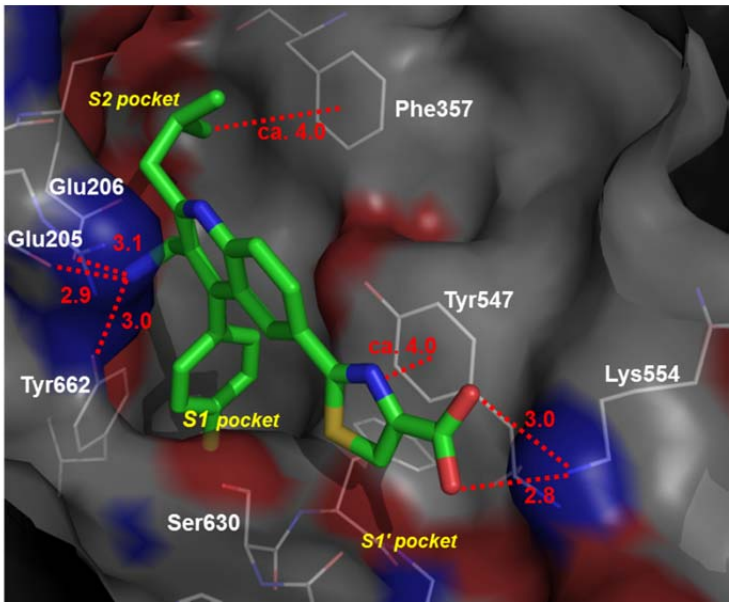


Figure 2-4. Docking model of **25b** in the catalytic domain of DPP4.

2-4　結論

スキャフォールド戦略の Step 1 として、イソキノロン環に代わる中心骨格の in silico 探索および置換基最適化により、新たなスキャフォールドである 3-(アミノメチル)-2-イソブチル-4-(4-メチルフェニル)キノリン構造を構築した。次いで Step 2 として、このスキャフォールドに新たな相互作用点を導入すべく、DPP4 の S1' ポケットの Lys554 との水素結合形成および塩橋形成を目的とした 6 位置換基探索を実施した。その結果、IC_{50} 値 0.38 nM の非常に高い阻害活性を有するカルボン酸誘導体 **25b** を始め、高い阻害活性を有する **12d** や **29c**、**14d** を見出すことに成功した。ドッキングモデルの解析などから、これら高活性の阻害薬がスキャフォールド部分で S1 および S2 ポケットと良好な相互作用を形成し、S1' ポケットにおいては Lys554 と相互作用するだけでなく、Tyr547 と疎水性相互作用することにより阻害活性を高めている可能性が示唆され、一方で活性中心である Ser630 との相互作用の寄与は小さいと考えられた。

第 3 章　新規ピリジン系ジペプチジルペプチダーゼ 4 阻害薬の創出

3-1　ピリジン系ジペプチジルペプチダーゼ 4 阻害薬の分子設計

DPP4 阻害薬創出のためのスキャフォールド戦略の新たな応用例として、前章で述べたキノリン系スキャフォールドの中心骨格を単純化し、ピリジン系スキャフォールドをデザインしてリード創出を検討した。

第 2 章第 2-2-3 節の Figure 2-3 および Figure 2-4 に示したドッキングモデルでは、キノリン環のベンゼン環部分は DPP4 のどのアミノ酸残基とも明確な相互作用をしていないと考えられた。このことから、このベンゼン環部分の役割が置換基の方向性を規定することのみであると考察し、キノリン環からベンゼン環部分を除去したピリジン環を中心骨格とするスキャフォールドが構築でき、スキャフォールド戦略を適用できるものと仮定した。

キノリン誘導体の結果から、S1' ポケットの 2 つの重要な相互作用（Tyr547 との疎水性相互作用および Lys554 との塩橋形成による相互作用）を利用する阻害薬のファーマコフォアーは Figure 3-1 のように表現でき、これら相互作用を維持できれば、ピリジン系スキャフォールドにおいても高活性の阻害薬を創出できると考えた。

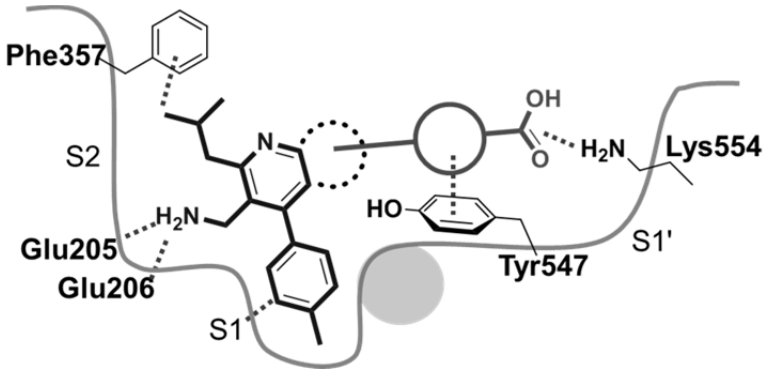
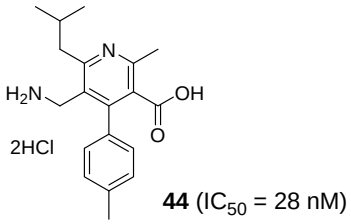


Figure 3-1.　Pharmacophore model for a potent DPP4 inhibitor employing interactions with Lys554 and Tyr547 in S1' pocket.

既に著者らは、3-アミノメチル-2-イソブチル-4-(*p*-トリル)ピリジン構造が、DPP4 阻害活性発現の最小構造単位であることを確認済みである [44]。例えば、この構造を基本構造として有する最小構造単位として合成したニコチン酸誘導体 **44** は、IC_{50} 値 28 nM の DPP4 阻害活性を示した。Figure 3-2 に示す DPP4 との共結晶構造から、**44** は 5 位カルボキシ基で Tyr547 の水酸基や活性中心の Ser630 の水酸基と水素結合しており、S1' ポケット全体を利用できてはいない。しかしながら、その基本構造部分は、2 位イソブチル基と 3 位アミノメチル基とで S2 ポケットと、4 位 *p*-トリル基で S1 ポケットとそれぞれ良好な結合を形成していることから、**44** の基本構造部分は新たな相互作用点を付与するためのスキャフォールドとして好適であることが期待できる。

そこで、この基本部分構造をピリジン系スキャフォールドとして採用し、その 5 位に S1' ポケットとの相互作用を指向した置換基を導入することにした。



44 (IC_{50} = 28 nM)

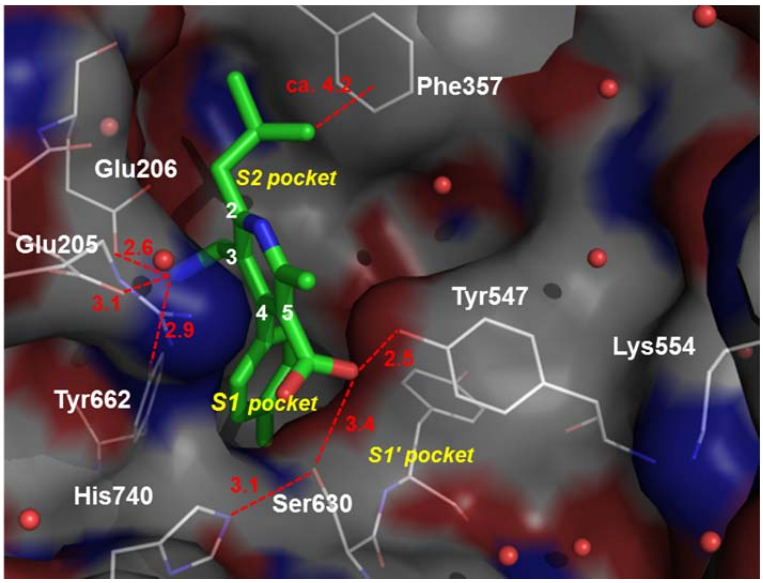
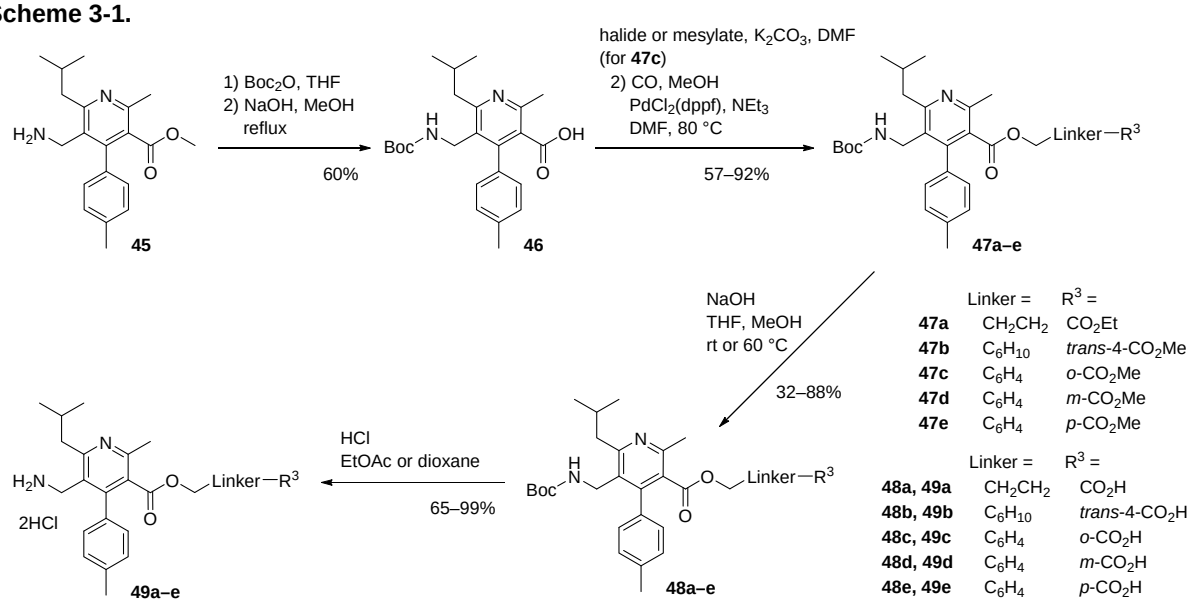


Figure 3-2. X-ray structure of compound **44** in complex with human DPP4 (Protein Data Bank Code: 3O9V).[44] Amino acids (light grey carbons, white texts) and waters (red oxygens) in the binding sites of **44** (green carbons) are indicated. Major interactions are depicted as red dotted lines. Values listed are heavy atom distances in angstroms.

3-2　ピリジン系ジペプチジルペプチダーゼ４阻害薬の合成

ピリジン誘導体は、既に報告済みのニコチン酸誘導体 **45**[44] を出発原料として、Scheme 3-1 に示す方法により合成した。まず、5 位にエステルリンカーを有する誘導体 **49a–e** は、**45** を N-Boc 保護した後、アルカリ条件下で加熱還流することによりエステル加水分解したカルボン酸 **46** を中間体として、アルキル化により対応するエステル体 **47a–e** へと導いた。なお **47c** に関しては *o*-ブロモベンジルエステル体を経て、パラジウム触媒を用いた一酸化炭素挿入反応により **47c** へと導いた。得られたエステル体を室温下でアルカリ加水分解することで、末端のエステルのみが加水分解された **48a–e** が選択的に得られ、次いで脱 N-Boc 化することにより目的のカルボン酸 **48a–e** を合成した。

Scheme 3-1.

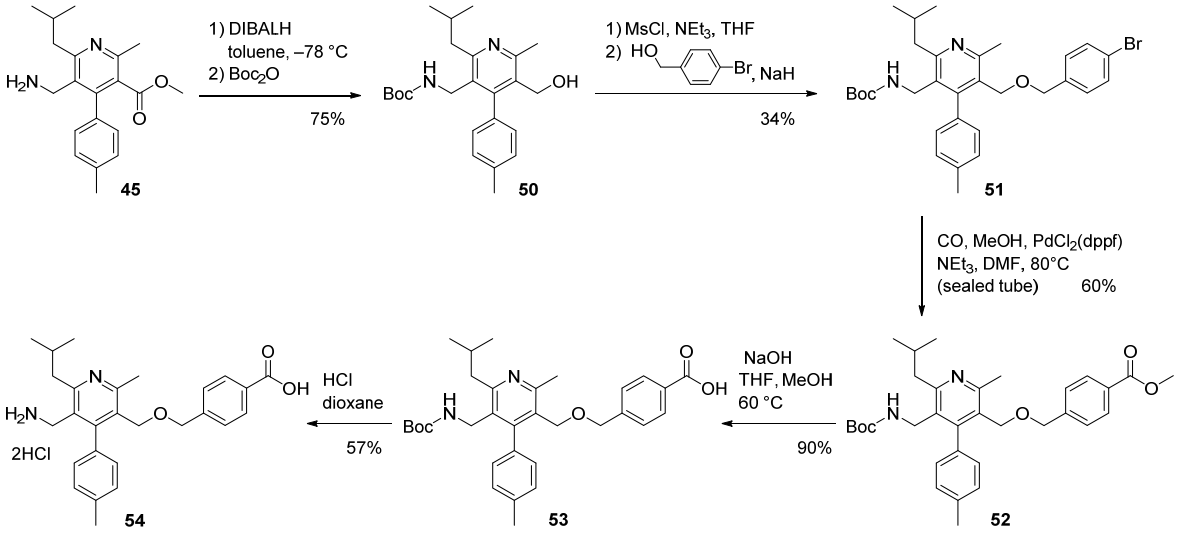


	Linker =	R^3 =
47a	CH_2CH_2	CO_2Et
47b	C_6H_{10}	*trans*-4-CO_2Me
47c	C_6H_4	*o*-CO_2Me
47d	C_6H_4	*m*-CO_2Me
47e	C_6H_4	*p*-CO_2Me

	Linker =	R^3 =
48a, 49a	CH_2CH_2	CO_2H
48b, 49b	C_6H_{10}	*trans*-4-CO_2H
48c, 49c	C_6H_4	*o*-CO_2H
48d, 49d	C_6H_4	*m*-CO_2H
48e, 49e	C_6H_4	*p*-CO_2H

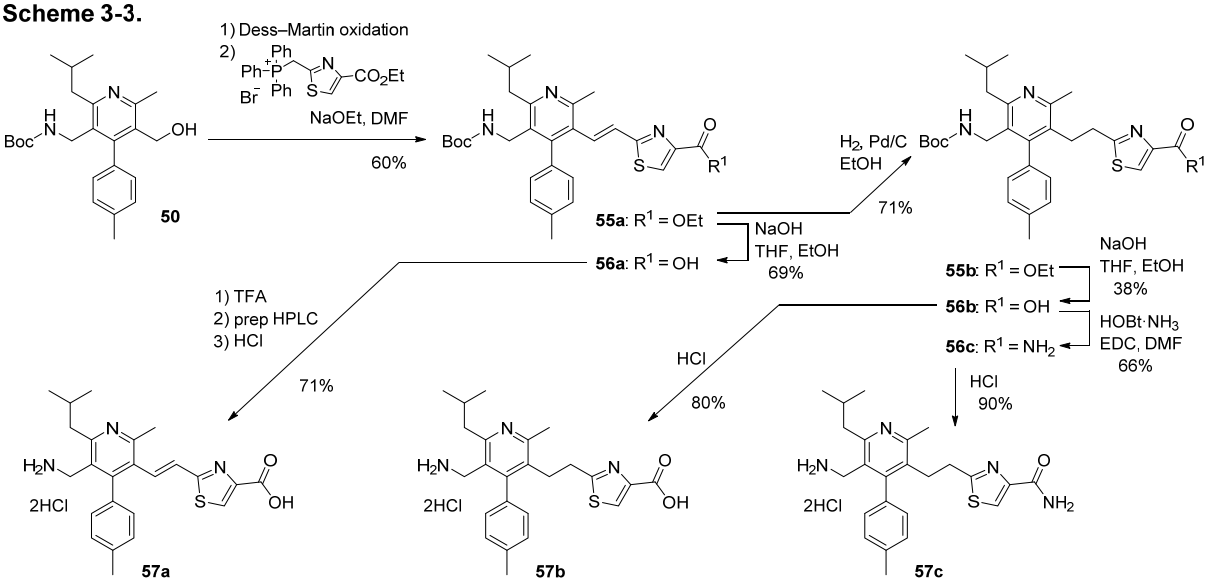
一方、**45** の 5 位エステルをヒドリド還元によりヒドロキシメチル基へと変換し、N-Boc 化してアルコール体 **50** を合成した。このアルコール体の水酸基をメシラートにして *p*-ブロモベンジルアルコールと反応させることでエーテルリンカーを有する **51** へと変換し、次いでパラジウム触媒を用いた一酸化炭素挿入反応によりエステル体 **52** へと変換した。このエステルを加水分解し（**53**）、脱 N-Boc 化することで、**54** を合成した（Scheme 3-2）。

Scheme 3-2.



また、中間体 **50** を Dess–Martin 酸化によりアルデヒド体とし、チアゾールエステルを有するリン試薬を用いた Wittig 反応により **55a** へと変換した。得られた **55a** のエステル加水分解（**56a**）と脱 N-Boc 化により目的のカルボン酸 **57a** を合成した他、**55a** の 5 位オレフィンを接触還元してエチレンリンカーへと変換した後（**55b**）、エステル加水分解（**56b**）と脱 N-Boc 化により目的のカルボン酸 **57b** を合成した。さらに、カルボン酸 **56b** を常法により酸アミド化（**56c**）した後に脱 N-Boc 化することで **57b** に対応する酸アミド体 **57c** を合成した（Scheme 3-3）。

Scheme 3-3.



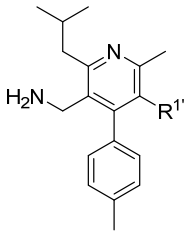
3-3　ピリジン系ジペプチジルペプチダーゼ４阻害薬の構造活性相関

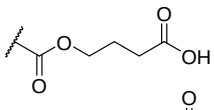
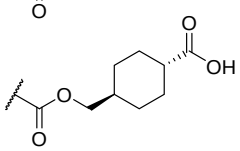
3-3-1　ピリジン系リード化合物の創出

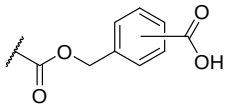
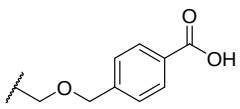
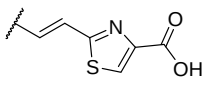
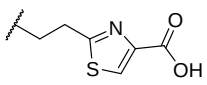
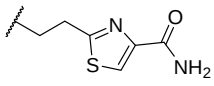
合成したピリジン誘導体の DPP4 阻害活性を評価した（Table 3-1）。まず、炭素鎖リンカーを持つ **49a** の阻害活性は 10^{-7} M レベルで **44** に比べて 15 倍減弱しており、**49a** では **44** ほど強く Ser630 と結合できなくなっていることが推測された。この炭素鎖リンカーをシクロヘキシル環に置換すると阻害活性は 5 倍向上し（**49b**）、更にベンゼン環に置換した場合は、阻害活性はカルボキシ基の位置がメタ（**49d**）＜オルト（**49c**）＜パラ（**49e**）の順に活性が向上し、**49e** では **49a** に比べて 25 倍の向上が認められた。さらに **49e** のリンカー部分を、エステルから自由度の高いエーテルに変換することで活性は向上し、誘導体 **54** は 10^{-9} M レベルの高い阻害活性を示した。次に、リンカー部分を、非常に高い活性を示したキノリン **25b** のベンゼン環を開環した構造に変換した **57a** および **57b** の阻害活性を調べたところ、いずれも 10^{-9} M レベルの高い阻害活性を示し、**57a** に対してより自由度の高いエチレンリンカーを有する **57b** は IC_{50} 値 2.6 nM の非常に高い阻害活性を示した。一方、カルボン酸 **57b** を対応するカルボキサミド（**57c**）に変換すると阻害活性は減弱し、この傾向は他のカルボン酸誘導体の場合でも一貫していた。このことから、これらカルボキシ基が Lys554 と塩橋を形成していることが強く示唆された。

以上の結果から、ピリジン誘導体においても、5 位側鎖に環リンカーを介したカルボキシ基、特に芳香環リンカーを介したカルボキシ基の導入によって Lys554 と Tyr547 の両方との相互作用を獲得することで、高活性の阻害薬を創出しうることが示された。

Table 3-1. Inhibitory Activity of Pyridine-based Carboxylic Acids and Carboxamide against Human DPP4[a]



Compound	$R^{1'}$	IC_{50} (nM)
49a		470
49b		80
44		28

Compound	$R^{1'}$		IC_{50} (nM)
49c		*ortho*	29
49d		*meta*	87
49e		*para*	17
54			7.2
57a			5.5
57b			2.6
57c			8.5

[a] Inhibitory activity against human DPP4 was assayed using Gly–Pro–*p*NA as a substrate.

3-3-2　ドッキングモデルを用いた考察

活性の高かった **57b** について DPP4 との結合様式をドッキングモデルを用いて考察した（Figure 3-3）。ピリジン誘導体 **57b** は、キノリン誘導体（Figure 2-3 および 2-4）と同様に 2 位イソブチル基、3 位アミノメチル基で S2 ポケットと、4 位 *p*-トリル基で S1 ポケットと相互作用しうることが示唆され、スキャフォールドとしての機能を維持できていることが示唆された。追加の相互作用点として導入した 5 位置換基についても、末端のカルボキシ基が S1' ポケットの Lys554 と塩橋形成可能な位置に、またチアゾール環が Tyr547 と π–π スタッキング可能な位置にあることが示唆された。

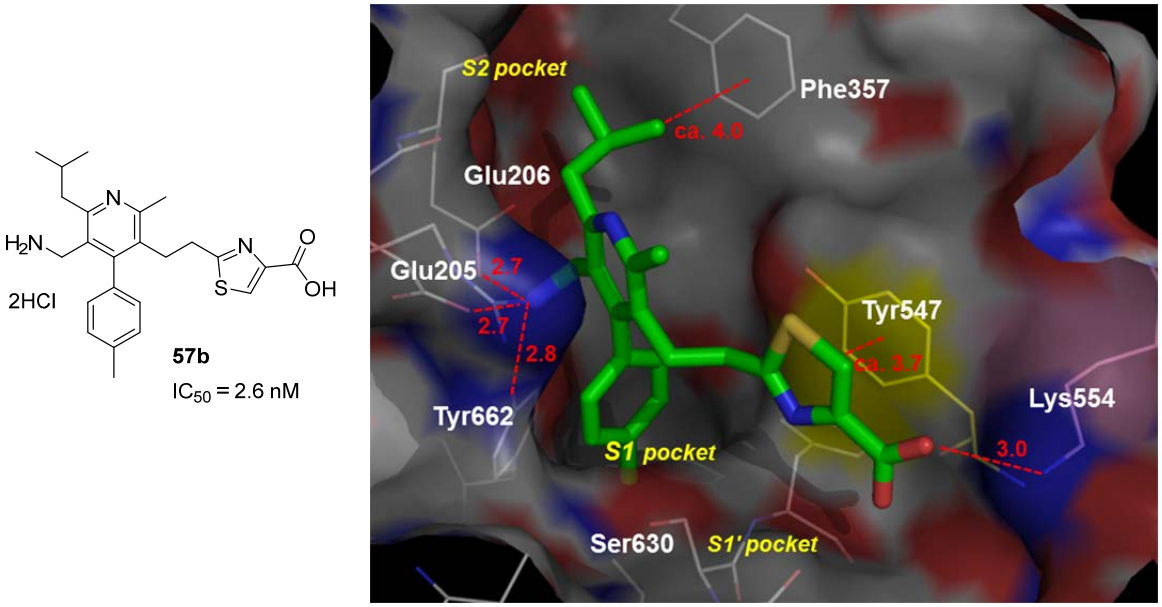


Figure 3-3.　Docking model between DPP4 protein and compound **57b**. Values in red are heavy atom distances in angstrom.

3-4　結論

第 2 章で見出したキノリン系スキャフォールドの中心ヘテロ環を単純化し、ピリジン系スキャフォールドをデザインしてスキャフォールド戦略を適用したところ、S1' ポケットとの相互作用点として導入した 5 位置換基の末端に芳香環を介してカルボキシ基を有する誘導体が 10^{-9} M レベルの高い阻害活性を示した。ドッキングモデルは、デザインしたピリジン系スキャフォールドが目的の機能を有していることを示唆した。さらに SAR 情報も合わせて考察すると、芳香環と Tyr547 との π–π スタッキングならびにカルボキシ基と Lys554 との塩橋形成の両方の相互作用をデザインに取り入れることで、ピリジン系スキャフォールドからも高活性の DPP4 阻害薬を創出しうることが強く示唆された。

第 4 章　新規ジペプチジルペプチダーゼ 4 阻害薬の生物学的特性

4-1　実験動物における抗糖尿病作用

スキャフォールド戦略を用いて見出した高活性の DPP4 阻害薬が、創薬リードレベルの化合物であることを確認する目的で、薬効である抗糖尿病作用を評価した。

抗糖尿病作用の評価は薬理チームが実施した。まず経口投与後の体内での DPP4 阻害活性の強度および持続性を調べるために、血中 DPP 活性を測定する ex vivo 試験を実施し、次いで食餌による血糖上昇の抑制能を調べるために、病態モデル動物を用いた経口糖負荷試験を実施して耐糖能を調べた。

4-1-1　ラット血中の酵素阻害活性（ex vivo 試験）

第 2 章および第 3 章で得られた DPP4 阻害薬の生体内での阻害活性を評価するため、化合物を経口投与した後の Sprague-Dawley ラットの血漿中 DPP 酵素活性の推移を調べた。キノリン誘導体 **25b** の結果を Figure 4-1 に例示する。化合物 **25b** は、1 mg/kg の経口投与でラット血漿中の DPP4 を阻害し、かつ投与後 1 時間から 6 時間の測定期間を通して約 60%以上の阻害を維持した。陽性対照として用いたイソキノロン **2a** に比べ、**25b** は ex vivo において明確な活性向上を示した。また同じく陽性対照として用いた活性中心と共有結合するタイプの市販薬である vildagliptin と同等以上の阻害活性を示すことが明らかになった。

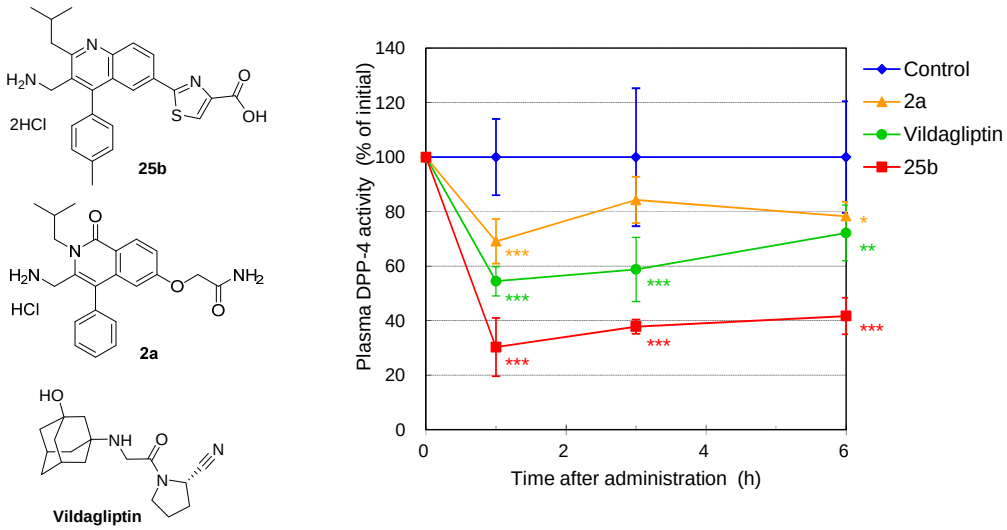


Figure 4-1. Effect of oral administration (1 mg/kg) of compound **25b** on plasma dipeptidyl peptidase activity of Sprague-Dawley rats. Compound **2a** and **Vildagliptin** were used as positive controls. Data are indicated by mean ± standard deviation ($n = 5$).
*, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$ vs control by Dannett test.

4-1-2　ラット経口糖負荷試験（in vivo 薬効試験）

経口糖負荷試験は食餌による血糖上昇の抑制能を測定する薬効モデル試験の一つであり、特に雌性 Wistar fatty ラット [45] においては耐糖能が低下した病態での評価モデルとなる。Figure 4-2 (A) および (B) に示したように、**25b** は 1 mg/kg の経口投与後にグルコース投与による雌性 Wistar fatty ラットの血糖上昇を有意に抑制し、インスリン分泌促進により血漿中の免疫反応性インスリン（IRI）レベルを有意に増大させた。その作用強度は、市販薬である vildagliptin とほぼ同等であった。

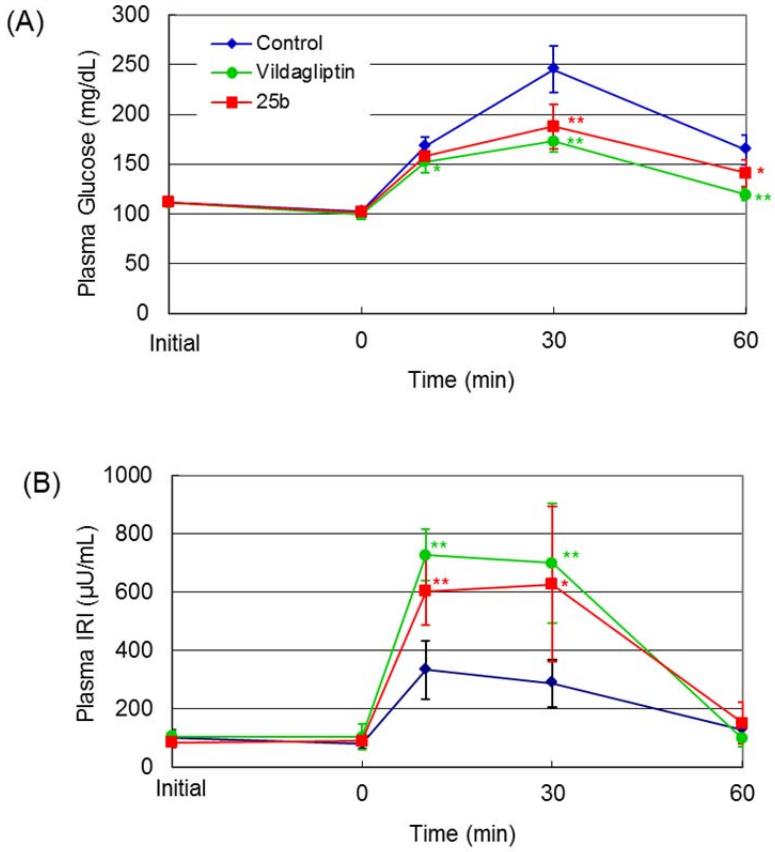
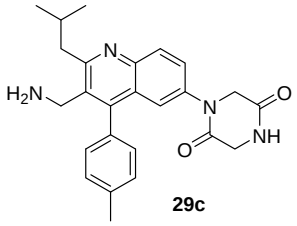


Figure 4-2. Oral glucose tolerance test in female Wistar fatty rats. Plasma glucose (A) and plasma IRI levels (B) of female Wistar fatty rats administered with compounds (1 mg/kg, p.o.) or 0.1% methylcellulose 1 h before oral glucose load (1 g/kg at 0 min). **Vildagliptin** was used as a positive control. Data are indicated by mean ± standard deviation ($n = 6$). *, $p < 0.05$; **, $p < 0.01$ vs control by Dannett test.

4-2　他のジペプチジルペプチダーゼとのアイソザイム選択性

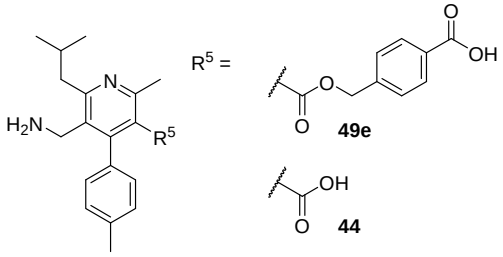
最後に、スキャフォールド戦略を用いて見出した高活性の DPP4 阻害薬のアイソザイム選択性を確認した（Table 4-1）。キノリン **29c** の DPP2（EC3.4.14.2）および DPP8[46]、DPP9[47] に対する阻害活性は非常に低く、それぞれに対する DPP4 選択性は 4,000 倍以上および 20,000 倍以上、12,000 倍以上であった。ピリジン系阻害薬も高い DPP8 阻害活性を示し、例えば **49e** およびニコチン酸誘導体 **44** は、それぞれ DPP8 に対して 5,800 倍以上および 390 倍の DPP4 選択性を示した。スキャフォールドの構造に近く S1' ポケットと相互作用していないと考えられる **44** でも高い選択性を獲得できていることから、今回見出された阻害薬の DPP8 に対する選択性はスキャフォールド構造に由来すると考えられる。一方、**44** に S1' ポケットとの相互作用点を導入した **49e** では、選択性は更に 14 倍以上向上しており、S1' ポケットとの相互作用点にも DPP8 に対する選択性を増す構造的要素が含まれるものと考えられる。

Table 4-1.　Selectivity for DPP4 against Other Dipeptidyl Peptidases[a]



29c

	IC_{50} (nM)			
	DPP4	DPP2	DPP8	DPP9
29c	4.9	20000	>100000	>60000



49e　　**44**

	IC_{50} (nM)	
	DPP4	DPP8
49e	17	>100000
44	28	11000

[a] Inhibitory activity against human DPP4, DPP8, and DPP9 were evaluated using Gly–Pro–*p*NA as a substrate, and that against rat DPP2 were done using H–Lys–Ala–*p*NA as a substrate.

DPP8 の結晶構造は未だ報告されていないが、Rummey らの報告[48]によると、DPP8 の S2 および S1' ポケットでは、DPP4 の Phe357 および Lys554 に相当する位置にそれぞれ His および Ile が配置している。今回見出した阻害薬では、2 位イソブチル基が DPP4 の Phe357 と相互作用するが、DPP8 では対応する位置にそのような疎水性の領域が存在しないため、2 位イソブチル基との結合が弱いと考えられる。また、S1' ポケットの Lys554 との結合を指向してアミドやカルボン酸などの極性基を導入したため、DPP8 では

対応する位置にある脂溶性残基 Ile が、阻害薬との相互作用を著しく減弱させたと考えられる。以上の 2 点のアミノ酸配列による相互作用部位の物性の相違が、今回得られた DPP4 阻害薬の DPP8 に対する非常に高い選択性の主な要因であると考えられる。

4-3　結論

スキャフォールド戦略を用いて見出されたDPP4阻害薬の中には、**25b**などのように、実験動物において経口投与により市販されている DPP4 阻害薬とほぼ同等の抗糖尿病作用を示すものもあった。この結果から、本戦略により創薬リードレベルの薬効を示す低分子阻害薬リード候補を創出しうることが実証された。また、見出された阻害薬はDPP8を始めとするアイソザイムに対するDPP4選択性が高く、阻害薬がアイソザイム間でのアミノ酸配列の違いによる基質認識部位の物性の差異を利用して選択性を引き出していると考えられる。

結語

本研究は、有機合成低分子での新規なプロテアーゼ阻害薬リード創出法の開発を目的とした、基質結合様式に基づくプロテアーゼ阻害薬基本骨格の分子設計に関するものである。

1) 有機合成低分子プロテアーゼ阻害薬のリード創出戦略としてスキャフォールド戦略を着想した。本戦略は、①酵素活性中心近傍の 2 つの相互作用部位と相互作用するスキャフォールドを構築し、次いで②新たな相互作用部位との相互作用点を導入することにより、リード創出を行う戦略である。

2) 本研究の目的を、この着想した戦略のうち、相互作用部位として酵素活性中心ではなく結合ポケットを標的とするスキャフォールド戦略 Type B を完成し、酵素活性中心と相互作用しないタイプの低分子プロテアーゼ阻害薬リード創出における有効性を確認することと定め、DPP4 阻害薬を題材として、創薬リードレベルで独自性のあるリード化合物候補が創出可能かどうかを検証した。

3) 上記の戦略に従い、DPP4 の S1 および S2 ポケットと相互作用する 3 置換キノリンならびに 3 置換ピリジンを DPP4 阻害薬スキャフォールドとして見出し、これに新たな結合部位として S1' ポケットの Lys554 および Tyr547 との相互作用点を導入することにより、両スキャフォールドで、高活性、高選択的で、かつ経口投与で薬効を示すリード化合物の創出に成功した。

4) 本戦略で得られたリード化合物は、化合物の構造上の独自性だけでなく、酵素活性中心との強い直接結合なしに 10^{-9} M レベル以上の非常に高い阻害活性を示しており、本戦略の利用によって、一般的なペプチドミメティクス阻害薬にはない独自性を有する低分子阻害薬リードを創出することに成功した。

5) 以上の結果から、本研究は、スキャフォールド戦略 Type B が独自性の高い低分子プロテアーゼ阻害薬のリード創出の方法論として有用であることを示した。また、一般的に活性中心と相互作用しないプロテアーゼ阻害薬の創製は困難と考えられ、このような難易度の高い低分子プロテアーゼ阻害薬創出においても有効な方法論を提案した。

General information. The proton nuclear magnetic resonance (^{1}H NMR) spectra were measured on a Bruker AVANCE 300 (300 MHz), Varian Ultra-300 (300 MHz), or Varian Gemini-200 (200 MHz) spectrometer. Chemical shifts are given in δ values (ppm) using tetramethylsilane as the internal standard. All *J* values are given in hertz. The following abbreviations are used: s, singlet; d, doublet; t, triplet; q, quartet; quin, quintet; spt, septet; m, multiplet; dd, doublet of doublets; br, broad. Low-resolution liquid chromatography mass spectra (LC–MS) were recorded on an Agilent 1200 LC/MS system, a Waters ZQ LC/MS system, or a Shimadzu LCMS-2020 UFLC/MS system in an electrospray ionization (ESI) mode. Melting points (mp) were determined on a Yanagimoto melting point apparatus, a Büchi melting point apparatus B-545, or an OptiMelt melting point apparatus MPA100 and were uncorrected. Elemental analyses (C, H, N) were within ±0.4% of theoretical values. For thin layer chromatography (TLC) analysis throughout this work, Merck precoated TLC plates (silica gel 60 F_{254}) and basic TLC plates (NH silica gel, Fuji Silysia Chemical Ltd.) were used. The products were purified on silica gel 60 (0.040–0.063 or 0.063–0.200 mm, E. Merck), basic silica gel (Chromatorex NH, 100–200 mesh, Fuji Silysia Chemical Ltd.), or Purif-Pack (Si or NH, Shoko scientific Co., Ltd.). Preparative high performance liquid chromatography (HPLC) was performed on a Gilson preparative HPLC system (CombiPrep ODS-A (50 × 20 mm I.D., YMC) with UV detection at 220 nm) or a Waters 2525 system (L-column2 ODS (20 × 150 mm I.D., CERI) with MS (ESI) detection using Waters ZQ2000) eluting with a gradient of acetonitrile–distilled water containing 0.1% trifluoroacetic acid. Reagents and solvents were obtained from commercial sources and used without further purification. Amounts of Raney cobalt and Raney nickel are theoretical values calculated from their weights in water. Yields are unoptimized.

3-(Aminomethyl)-6-bromo-2-isobutyl-4-phenylisoquinolin-1(2*H*)-one (2b).

i) 6-Bromo-3-(chloromethyl)-2-isobutyl-4-phenylisoquinolin-1(2*H*)-one (2ba). To a solution of **38b** (2.2 g, 5.7 mmol) in tetrahydrofuran (30 mL) was added $SOCl_2$ (0.90 mL, 12 mmol) at room temperature. After stirring for 15 h, the resulting mixture was concentrated in vacuo. The residual oil was crystallized from hexanes to afford the title compound **2ba** (1.6 g, 69%) as colorless crystals. ^{1}H NMR (300 MHz, DMSO-d_6): δ 0.93 (d, *J* = 6.69 Hz, 6H), 2.20 (spt, *J* = 7.50 Hz, 1H), 4.07 (d, *J* = 7.24 Hz, 2H), 4.50 (s, 2H), 6.99 (s, 1H), 7.38 (d, *J* = 7.06 Hz, 2H), 7.52–7.65 (m, 3H), 7.74 (dd, *J* = 8.49, 1.60 Hz, 1H), 8.24 (d, *J* = 8.61 Hz, 1H). LC–MS (ESI) *m*/*z*: 404 $(M + H)^+$.

ii) 3-(Aminomethyl)-6-bromo-2-isobutyl-4-phenylisoquinolin-1(2*H*)-one (2b). A mixture of **2ba** (0.80 g, 2.0 mmol) in tetrahydrofuran (10 mL) and 2 M NH_3 in ethanol (35 mL, 70 mmol) was stirred in a sealed tube at 140 °C for 5 h. After evaporation of the solvent, the residue was diluted with ethyl acetate, washed with saturated $NaHCO_3$ and brine, dried over Na_2SO_4, and concentrated in vacuo. The residue was purified by column chromatography (silica gel, eluting with ethyl acetate) to afford the title compound **2b** (0.50 g, 65%) as colorless crystals. ^{1}H NMR

(300 MHz, DMSO-d_6): δ 0.91 (d, J = 6.69 Hz, 6H), 1.70 (br s, 2H), 2.15 (spt, J = 6.80 Hz, 1H), 3.48 (s, 2H), 4.20 (d, J = 7.43 Hz, 2H), 6.94 (s, 1H), 7.39 (d, J = 7.34 Hz, 2H), 7.46–7.58 (m, 3H), 7.63 (dd, J = 8.53, 1.60 Hz, 1H), 8.20 (d, J = 8.51 Hz, 1H). LC–MS (ESI) m/z: 385 $(M + H)^+$. Anal. Calcd for $C_{20}H_{21}BrN_2O$: C, 62.35; H, 5.49; N, 7.27. Found: C, 62.34; H, 5.49; N, 7.02.

2-Amino-5-bromo-*N*-methoxy-*N*-methylbenzamide (4a).

i) 6-Bromo-2*H*-3,1-benzoxazine-2,4(1*H*)-dione (4aa). To a solution of 2-amino-5-bromobenzoic acid (**3a**, 25 g, 0.12 mol) in tetrahydrofuran (350 mL) was added bis(trichloromethyl) carbonate (24.4 g, 0.082 mol) was added, and the resulting suspension was stirred at reflux for 4 h. The reaction mixture was poured onto ice, and the precipitate was collected by filtration, washed with methanol, and dried to afford the title compound **4aa** (27.1 g, 96%) as a white powder. ^{1}H NMR (200 MHz, $CDCl_3$) δ: 7.19 (d, J = 8.4 Hz, 1H), 7.96–8.02 (m, 1H), 8.09 (d, J = 2.6 Hz, 1H).

ii) 2-Amino-5-bromo-*N*-methoxy-*N*-methylbenzamide (4a). A mixture of *N*,*O*-dimethylhydroxylamine hydrochloride (16.4 g, 168 mmol) and triethylamine (13.4 mL, 168 mmol) in ethanol (70 mL) was stirred at room temperature for 30 min. To the resulting suspension was added **4aa** (27.1 g, 112 mmol), and the resulting mixture was stirred at 75 °C for 17 h. The reaction mixture was cooled and filtered. The filtrate was concentrated in vacuo, and the residue was neutralized with saturated $NaHCO_3$ and extracted with ethyl acetate. The extract was washed sequentially with saturated $NaHCO_3$ and brine, dried over $MgSO_4$, and concentrated in vacuo. Purification by column chromatography (silica gel, eluting with a gradient of 50–80% ethyl acetate in hexanes) was followed by recrystallization from diisopropyl ether–hexanes to afford the title compound **4a** (21.8 g, 72%) as pale orange crystals; mp 78–81 °C. ^{1}H NMR (200 MHz, $CDCl_3$) δ: 3.34 (s, 3H), 3.59 (s, 3H), 4.67 (br s, 2H), 6.64 (d, J = 8.4 Hz, 1H), 7.27 (dd, J = 8.4, 2.4 Hz, 1H), 7.50 (d, J = 2.4 Hz, 1H). LC–MS (ESI) m/z: 198 $(M - N(Me)OMe)^+$.

2-Amino-5-((*tert*-butyl(dimethyl)silyl)oxy)-*N*-methoxy-*N*-methylbenzamide (4b).

i) 2-(((Benzyloxy)carbonyl)amino)-5-hydroxybenzoic acid (4ba). A solution of benzyl chloroformate (52 mL, 0.36 mmol) in diethyl ether (200 mL) was added dropwise with a vigorous stirring to a mixture of 2-amino-5-hydroxybenzoic acid (**3b**, 50 g, 0.33 mmol), $NaHCO_3$ (109 g, 1.3 mol), diethyl ether (200 mL), and water (200 mL) at room temperature. The resulting mixture was stirred at room temperature for 30 min, and then neutralized with concd HCl. The reaction mixture was extracted with ethyl acetate, and the extract was washed with brine, dried over $MgSO_4$, and concentrated in vacuo. The residue was crystallized from diisopropyl ether–hexanes to afford the title compound **4ba** (92 g, 98%) as a white powder. ^{1}H NMR (200 MHz, $CDCl_3$) δ: 5.13 (s, 2H), 7.00 (dd, J = 9.0, 3.0 Hz, 1H), 7.33–7.41 (m, 6H), 8.00 (d, J = 9.0 Hz, 1H), 9.50 (br s, 1H), 10.32 (s, 1H).

ii) Benzyl (4-((*tert*-butyl(dimethyl)silyl)oxy)-2-(methoxy(methyl)carbamoyl)phenyl)carbamate (4bb). To a mixture of **4ba** (91 g, 0.32 mol) and *N*,*O*-dimethyl hydroxylamine hydrochloride (36 g, 0.36 mol) of in *N*,*N*-dimethylformamide (500 mL) were sequentially added triethylamine (55 mL, 0.36 mol) and 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (70 g, 0.36 mol) at room temperature, and the resulting mixture was stirred at room temperature for 2.5 h. After quenching with 1 M hydrochloric acid, the mixture was extracted with ethyl acetate. The extract was washed with brine, dried over $MgSO_4$, and concentrated in vacuo. Purification by column chromatography (silica gel, eluting with a gradient of 25–100% ethyl acetate in hexanes) gave a brown oil. A mixture of the obtained brown oil, *tert*-butyl(chloro)dimethylsilane (48 g, 0.32 mol), and imidazole (22 g, 0.32 mol) in *N*,*N*-dimethylformamide (500 mL) was stirred at room temperature for 14 h. The reaction mixture was partitioned between ethyl acetate and water. The extract was washed sequentially with 1 M HCl and brine, dried over $MgSO_4$, and concentrated in vacuo. Purification by column chromatography (silica gel, eluting with 25% ethyl acetate in hexanes) gave the tile compound **4bb** (85 g, 59%) as a white powder. ^{1}H NMR (200 MHz, $CDCl_3$) δ: 0.28 (s, 6H), 1.08 (s, 9H), 3.45 (s, 3H), 3.62 (s, 3H), 5.28 (s, 2H), 7.02 (dd, *J* = 8.8, 2.6 Hz, 1H), 7.06 (d, *J* = 2.6 Hz, 1H), 7.44–7.51 (m, 5H), 8.04 (d, *J* = 8.8 Hz, 1H), 8.45 (br s, 1H).

iii) 2-Amino-5-((*tert*-butyl(dimethyl)silyl)oxy)-*N*-methoxy-*N*-methylbenzamide (4b). A mixture of **4bb** (85 g, 0.19 mol), 10% palladium on charcoal (2.5 g), ethanol (250 mL), and tetrahydrofuran (250 mL) was stirred at room temperature for 20 h under a hydrogen atmosphere. The reaction mixture was filtered, and the filtrate was concentrated in vacuo to afford the title compound **4b** (40 g, 69%) as a white powder. ^{1}H NMR (200 MHz, $CDCl_3$) δ: 0.25 (s, 6H), 1.07 (s, 9H), 3.45 (s, 3H), 3.68 (s, 3H), 6.70 (d, *J* = 8.8 Hz, 1H), 6.84 (dd, *J* = 8.8, 3.0 Hz, 1H), 6.97 (d, *J* = 3.0 Hz, 1H).

(2-Amino-5-bromophenyl)(phenyl)methanone (5a). To a solution of **4a** (5.0 g, 19 mmol) in diethyl ether (50 mL) was added 2 M phenylmagnesium bromide in tetrahydrofuran (24 mL, 48 mmol) dropwise at 0 °C. The resulting mixture was stirred at 0 °C for 15 min. After quenching with saturated NH_4Cl, the reaction mixture was extracted with ethyl acetate. The extract was washed with brine, dried over dried over $MgSO_4$, and concentrated in vacuo. Purification by column chromatography (silica gel, eluting with a gradient of 5–25% ethyl acetate in hexanes) afforded title compound **5a** (3.21 g, 60%) as yellow crystals. ^{1}H NMR (200 MHz, $CDCl_3$) δ: 6.08 (br s, 2H), 6.65 (d, *J* = 8.8 Hz, 1H), 7.26–7.66 (m, 7H).

(2-Amino-5-bromophenyl)(4-methylphenyl)methanone (5b). By a procedure similar to that described for the synthesis of compound **5a** using *p*-tolylmagnesium bromide, the title compound **5b** was obtained as yellow crystals (3.5 g, 63%). ^{1}H NMR (300 MHz, $CDCl_3$) δ: 2.44 (s, 3H), 5.98 (br s, 2H), 6.64 (d, *J* = 8.9 Hz, 1H), 7.28 (d, *J* = 7.9 Hz, 2H), 7.35 (dd, *J* = 8.9, 2.3 Hz, 1H), 7.55–7.60 (m, 3H). LC–MS (ESI) *m*/*z*: 290 $(M + H)^+$.

(2-Amino-5-((*tert*-butyl(dimethyl)silyl)oxy)phenyl)(phenyl)methanone (5c). To a solution of **4b** (5.0 g, 16 mmol) in tetrahydrofuran (100 mL) was added 2 M phenylmagnesium bromide in tetrahydrofuran (20 mL, 40 mmol) at −78 °C. The resulting mixture was stirred for 30 min and allowed to warm to room temperature. The reaction was quenched with saturated NH_4Cl and extracted with ethyl acetate. The extract was washed with brine, dried over $MgSO_4$, and concentrated in vacuo. The residue was purified by column chromatography (silica gel, eluting with 17% ethyl acetate in hexanes) to afford the title compound **5c** (3.4 g, 65%) as an orange syrup. ^{1}H NMR (300 MHz, $CDCl_3$): δ 0.09 (s, 6H), 0.91 (s, 9H), 5.72 (br s, 2H), 6.60–6.67 (m, 1H), 6.80–6.90 (m, 2H), 7.40–7.55 (m, 3H), 7.60–7.70 (m, 2H). LC–MS (ESI) *m*/*z*: 328 $(M + H)^+$.

(2-Amino-5-((*tert*-butyl(dimethyl)silyl)oxy)phenyl)(3-fluorophenyl)methanone (5d*).* By a procedure similar to that described for the synthesis of compound **5c** using 3-fluorophenylmagnesium bromide, the title compound **5d** was obtained as a yellow syrup (1.2 g, 43%). ^{1}H NMR (200 MHz, $CDCl_3$) δ: 0.09 (s, 6H), 0.92 (s, 9H), 5.75 (br s, 2H), 6.66 (d, *J* = 8.4 Hz, 1H), 6.80–6.95 (m, 2H), 7.15–7.25 (m, 1H), 7.30–7.50 (m, 3H). LC–MS (ESI) *m*/*z*: 346 $(M + H)^+$.

(2-Amino-5-((*tert*-butyl(dimethyl)silyl)oxy)phenyl)(3-methylphenyl)methanone (5e). By a procedure similar to that described for the synthesis of compound **5c** using *m*-tolylmagnesium bromide, the title compound **5e** was obtained as a crude brown syrup (1.5 g, 68%). LC–MS (ESI) *m*/*z*: 342 $(M + H)^+$.

(2-Amino-5-((*tert*-butyl(dimethyl)silyl)oxy)phenyl)(4-fluorophenyl)methanone (5f). By a procedure similar to that described for the synthesis of compound **5c** using 4-fluorophenylmagnesium bromide prepared from *p*-bromofluorobenzene, the title compound **5f** was obtained as an orange syrup (1.3 g, 47%). ^{1}H NMR (200 MHz, $CDCl_3$) δ: 0.10 (s, 6H), 0.93 (s, 9H), 5.63 (br s, 2H), 6.65 (d, *J* = 9.2 Hz, 1H), 6.80–6.95 (m, 2H), 7.05–7.20 (m, 2H), 7.60–7.75 (m, 2H).

(2-Amino-5-((*tert*-butyl(dimethyl)silyl)oxy)phenyl)(4-methylphenyl)methanone (5g). By a procedure similar to that described for the synthesis of compound **5c** using *p*-tolylmagnesium bromide prepared from *p*-bromofluorobenzene, the title compound **5g** was obtained as a yellow syrup (1.4 g, 28%). ^{1}H NMR (200 MHz, $CDCl_3$) δ: 0.10 (s, 6H), 0.93 (s, 9H), 2.43 (s, 3H), 5.58 (br s, 2H), 6.64 (d, *J* = 8.8 Hz, 1H), 6.70–6.75 (m, 2H), 7.00–7.10 (m, 2H), 7.25 (d, *J* = 8.8 Hz, 2H), 7.55–7.65 (m, 2H). LC–MS (ESI) *m*/*z*: 342 $(M + H)^+$.

6-Bromo-2-isobutyl-4-phenylquinoline-3-carbonitrile (7a). A mixture of **5a** (4.2 g, 15 mmol), **6d** (2.28 g, 18.2 mmol), methanesulfonic acid (1.46 g, 15.2 mmol), and toluene (200 mL) was heated to reflux for 6 h using a Dean-Stark trap. The reaction mixture was allowed to cool to room temperature, washed sequentially with saturated $NaHCO_3$ and brine, dried over $MgSO_4$, and concentrated in vacuo. Purification by column chromatography (silica gel, eluting with a gradient of 5–20% ethyl acetate in hexanes) was followed by recrystallization from ethyl

acetate–hexanes to afford the title compound **7a** (4.85 g, 88%) as colorless crystals; mp 136–137 °C. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 1.06 (d, *J* = 6.6 Hz, 6H), 2.25–2.55 (m, 1H), 3.11 (d, *J* = 7.3 Hz, 2H), 7.40–7.50 (m, 2H), 7.55–7.65 (m, 3H), 7.79 (d, *J* = 2.2 Hz, 1H), 7.87 (dd, *J* = 8.4, 2.2 Hz, 1H), 8.80 (d, *J* = 8.4 Hz, 1H). LC–MS (ESI) *m*/*z*: 365 $(M + H)^+$.

6-Bromo-2-isobutyl-4-(4-methylphenyl)quinoline-3-carbonitrile (7b). By a procedure similar to that described for the synthesis of compound **7a**, the title compound **7b** was obtained from **5b** as pale orange crystals (6.6 g, 58%); mp 168–169 °C. ^{1}H NMR (200 MHz, $CDCl_3$) δ: 1.05 (6H, d, *J* = 6.8 Hz), 2.30–2.50 (1H, m), 2.50 (3H, s), 3.10 (2H, d, *J* = 7.4 Hz), 7.34 (2H, d, *J* = 8.2 Hz), 7.42 (2H, d, *J* = 8.2 Hz), 7.80–7.90 (2H, m), 7.99 (1H, d, *J* = 9.0 Hz). LC–MS (ESI) *m*/*z*: 379 $(M + H)^+$.

6-Bromo-2-(2,2-dimethylpropyl)-4-phenylquinoline-3-carbonitrile (7c). By a procedure similar to that described for the synthesis of compound **7a** using 5,5-dimethyl-3-oxohexanenitrile (**6e**), the title compound **7c** was obtained as a white powder (1.99 g, 66%); mp 163–164 °C. ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.11 (s, 9H), 3.18 (s, 2H), 7.42–7.47 (m, 2H), 7.58–7.65 (m, 3H), 7.78 (d, *J* = 2.2 Hz, 1H), 7.87 (dd, *J* = 8.6, 2.2 Hz, 1H), 8.01 (d, *J* = 8.6 Hz, 1H). LC–MS (ESI) *m*/*z*: 379 $(M + H)^+$.

6-Hydroxy-4-phenyl-2-propylquinoline-3-carbonitrile (8a). A mixture of **5c** (2.0 g, 6.1 mmol), 3-oxohexanenitrile (**6a**, 0.811 g, 7.3 mmol), and methanesulfonic acid (0.586 g, 0.1 mmol) in toluene (50 mL) was stirred at reflux for 15 h with azeotropic removal of water. The reaction mixture was partitioned between ethyl acetate and water, and the organic layer was washed with brine, dried over $MgSO_4$, and concentrated in vacuo. The residue was triturated with diisopropyl ether to afford the title compound **8a** (0.980 g, 56%) as an orange powder. ^{1}H NMR (300 MHz, DMSO-d_6): δ 1.00 (t, *J* = 7.2 Hz, 3H), 1.80–1.89 (m, 2H), 3.01–3.07 (m, 2H), 6.78 (d, *J* = 1.8 Hz, 1H), 7.42 (dd, *J* = 9.3, 1.8 Hz, 1H), 7.49–7.52 (m, 2H), 7.60–7.65 (m, 3H), 7.95 (d, *J* = 9.3 Hz, 1H), 10.19 (s, 1H). LC–MS (ESI) *m*/*z*: 289 $(M + H)^+$.

2-Butyl-6-hydroxy-4-phenylquinoline-3-carbonitrile (8b). By a procedure similar to that described for the synthesis of compound **8a** using 3-oxoheptanenitrile (**6b**), the title compound **8b** was obtained as a brown powder (0.75 g, 65%). ^{1}H NMR (300 MHz, $CDCl_3$): δ 0.98 (t, *J* = 7.2 Hz, 3H), 1.46–1.54 (m, 2H), 1.81–1.92 (m, 2H), 3.15–3.21 (m, 2H), 6.91 (d, *J* = 2.7 Hz, 1H), 7.39–7.42 (m, 3H), 7.51–7.56 (m, 3H), 8.01 (d, *J* = 9.3 Hz, 1H). LC–MS (ESI) *m*/*z*: 303 $(M + H)^+$.

6-Hydroxy-2-pentyl-4-phenylquinoline-3-carbonitrile (8c). By a procedure similar to that described for the synthesis of compound **8a** using 3-oxooctanenitrile (**6c**), the title compound **8c** was obtained as a white powder (0.96 g, 55%). ^{1}H NMR (300 MHz, $CDCl_3$): δ 0.89 (t, *J* = 7.2 Hz, 3H), 1.27–1.49 (m, 4H), 1.82–1.91 (m, 2H), 3.16

(t, *J* = 7.2 Hz, 2H), 6.66 (br s, 1H), 6.56–6.74 (m, 1H), 6.92 (d, *J* = 2.7 Hz, 1H), 7.39–7.43 (m, 3H), 7.52–7.54 (m, 3H), 8.00 (d, *J* = 9.3 Hz, 1H). LC–MS (ESI) *m*/*z*: 317 $(M + H)^+$.

6-Hydroxy-2-isobutyl-4-phenylquinoline-3-carbonitrile (8d).

i) 6-*tert*-Butoxy-2-isobutyl-4-phenylquinoline-3-carbonitrile (8da). A mixture of palladium(II) acetate (0.062 g, 0.27 mmol) and (±)-2,2'-bis(diphenylphosphino)-1,1'-binaphthalene (0.098 g, 0.15 mmol) in toluene (20 mL) was stirred at 40 °C for 1 h under a nitrogen atmosphere. To the mixture were added **7a** (1.89 g, 4.98 mmol), sodium *tert*-butoxide (0.79 g, 8.2 mmol), and *tert*-butanol (1.1 mL, 11 mmol), and the resulting mixture was stirred at 90 °C for 1 h. The reaction mixture was cooled to room temperature and filtered through Celite. The filtrate was washed with brine, dried over $MgSO_4$, and concentrated in vacuo. The residue was purified by column chromatography (silica gel, eluting with a gradient of 5–20% ethyl acetate in hexanes) to afford the title compound **8da** (1.8 g, 92%) as a white powder. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 1.06 (d, *J* = 6.6 Hz, 6H), 1.33 (s, 9H), 2.30–2.50 (m, 1H), 3.10 (d, *J* = 7.2 Hz, 2H), 7.14 (d, *J* = 2.5 Hz, 1H), 7.40–7.65 (m, 6H), 8.03 (d, *J* = 9.1 Hz, 1H). LC–MS (ESI) *m*/*z*: 369 $(M + H)^+$.

ii) 6-Hydroxy-2-isobutyl-4-phenylquinoline-3-carbonitrile (8d). A mixture of **8da** (1.0 g, 2.8 mmol), trifluoroacetic acid (10 mL) in tetrahydrofuran (2 mL) was stirred at room temperature for 1 h. The reaction mixture was poured into water and extracted with ethyl acetate. The extract was washed with brine, dried over $MgSO_4$, and concentrated in vacuo. The residue was crystallized from diisopropyl ether –hexanes to afford the title compound **8d** (0.81 g, 95%) as a pale yellow powder. ^{1}H NMR (200 MHz, $CDCl_3$) δ: 1.10 (d, *J* = 6.6 Hz, 6H), 2.28–2.43 (m, 1H), 3.29 (d, *J* = 7.5 Hz, 2H), 7.13 (d, *J* = 2.4 Hz, 1H), 7.45–7.51 (m, 2H), 7.62–7.70 (m, 3H), 7.73 (dd, *J* = 9.3, 2.4 Hz, 1H), 8.32 (br s, 1H), 8.57 (d, *J* = 9.3 Hz, 1H).

2-(2,2-Dimethylpropyl)-6-hydroxy-4-phenylquinoline-3-carbonitrile (8e).

i) 6-*tert*-Butoxy-2-(2,2-dimethylpropyl)-4-phenylquinoline-3-carbonitrile (8ea). By a procedure similar to that described for the synthesis of compound **8da**, the title compound **8ea** was obtained from **7c** as a white powder (0.81 g, 44%); mp 129–130 °C. ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.12 (s, 9H), 1.33 (s, 9H), 3.17 (s, 2H), 7.13 (d, *J* = 2.4 Hz, 1H), 7.42–7.62 (m, 6H), 8.03 (d, *J* = 9.2 Hz, 1H). LC–MS (ESI) *m*/*z*: 373 $(M + H)^+$.

ii) 2-(2,2-Dimethylpropyl)-6-hydroxy-4-phenylquinoline-3-carbonitrile (8e). By a procedure similar to that described for the synthesis of compound **8d**, the title compound **8e** was obtained from **8ea** as a pale yellow powder (0.62 g, 98%); mp 181–182 °C. ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.16 (s, 9H), 3.39 (s, 2H), 7.15 (d, *J* = 2.6 Hz, 1H), 7.45–7.50 (m, 2H), 7.61–7.73 (m, 4H), 8.58 (d, *J* = 9.2 Hz, 1H), 9.67 (br s, 1H). LC–MS (ESI) *m*/*z*: 317 $(M + H)^+$.

2-Benzyl-6-hydroxy-4-phenylquinoline-3-carbonitrile (8f). By a procedure similar to that described for the synthesis of compound **8a** using 3-oxo-4-phenylbutanenitrile (**6f**), the title compound **8f** was obtained as a pale yellow powder (2.21 g, 83%); mp 235–236 °C. ^{1}H NMR (300 MHz, $CDCl_3$): δ 4.50 (s, 2H), 6.91 (d, *J* = 2.6 Hz, 1H), 7.13–7.50 (m, 12H), 8.02 (d, *J* = 8.8 Hz, 1H). LC–MS (ESI) *m*/*z*: 337 $(M + H)^+$.

4-(3-Fluorophenyl)-6-hydroxy-2-isobutylquinoline-3-carbonitrile (8g). By a procedure similar to that described for the synthesis of compound **8a** using 5-methyl-3-oxohexanenitrile (**6d**), the title compound **8g** was obtained from **5d** as an off-white powder (0.83 g, 75%); mp 273–276 °C. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 1.05 (d, *J* = 6.6 Hz, 6H), 2.25–2.45 (m, 1H), 3.08 (d, *J* = 7.4 Hz, 2H), 5.46 (br s, 1H), 6.68 (d, *J* = 2.8 Hz, 1H), 7.10–7.35 (m, 3H), 7.48 (dd, *J* = 9.2, 2.8 Hz, 1H), 7.50–7.65 (m, 1H), 8.05 (d, *J* = 9.2 Hz, 1H). LC–MS (ESI) *m*/*z*: 321 $(M + H)^+$.

6-Hydroxy-2-isobutyl-4-(3-methylphenyl)quinoline-3-carbonitrile (8h). By a procedure similar to that described for the synthesis of compound **8a** using **6d**, the title compound **8h** was obtained from **5e** as an off-white powder (0.050 g, 40%); mp 275 °C (decomp). ^{1}H NMR (200 MHz, $CDCl_3$) δ: 0.99 (d, *J* = 6.6 Hz, 6H), 2.20–2.35 (m, 1H), 2.43 (s, 3H), 2.96 (d, *J* = 7.3 Hz, 2H), 6.81 (d, *J* = 2.6 Hz, 1H), 7.25–7.60 (m, 5H), 7.97 (d, *J* = 9.2 Hz, 1H), 10.20 (s, 1H).

4-(4-Fluorophenyl)-6-hydroxy-2-isobutylquinoline-3-carbonitrile (8i). By a procedure similar to that described for the synthesis of compound **8a** using **6d**, the title compound **8i** was obtained from **5f** as an off-white powder (0.88 g, 68%); mp 247–249 °C. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 1.04 (d, *J* = 6.6 Hz, 6H), 2.15–2.45 (m, 1H), 3.07 (d, *J* = 7.2 Hz, 2H), 5.87 (br s, 1H), 6.89 (d, *J* = 2.6 Hz, 1H), 7.10–7.50 (m, 5H), 8.04 (d, *J* = 9.2 Hz, 1H). LC–MS (ESI) *m*/*z*: 321 $(M + H)^+$.

6-Hydroxy-2-isobutyl-4-(4-methylphenyl)quinoline-3-carbonitrile (8j). By a procedure similar to that described for the synthesis of compound **8a** using **6d**, the title compound **8j** was obtained from **5g** as pale yellow crystals (7.2 g, 91%); mp 247 °C (decomp). ^{1}H NMR (200 MHz, $CDCl_3$) δ: 1.03 (d, *J* = 6.6 Hz, 6H), 2.20–2.45 (m, 1H), 2.46 (s, 3H), 3.06 (d, *J* = 7.3 Hz, 2H), 6.28 (d, *J* = 2.9 Hz, 1H), 7.25–7.40 (m, 4H), 7.41 (dd, *J* = 9.2, 2.9 Hz, 1H), 8.02 (d, *J* = 9.2 Hz, 1H). LC–MS (ESI) *m*/*z*: 317 $(M + H)^+$

2-((3-(Aminomethyl)-4-phenyl-2-propylquinolin-6-yl)oxy)acetamide (9a).

i) 2-((3-Cyano-4-phenyl-2-propylquinolin-6-yl)oxy)acetamide (9aa). A mixture of **8a** (0.98 g, 3.4 mmol), 2-chloroacetamide (0.355 g, 3.8 mmol), and K_2CO_3 (0.553 g, 4.0 mmol) in *N*,*N*-dimethylformamide (10 mL) was stirred at 60 °C for 12 h. The reaction mixture was partitioned between ethyl acetate and water. The organic layer was washed with brine, dried over $MgSO_4$, and concentrated in vacuo. The residue was purified by column chromatography (silica gel, eluting with a gradient of 50–100% ethyl acetate in hexanes) to afford the title

compound **9aa** (1.03 g, 88%) as a pale yellow powder. ^{1}H NMR (300 MHz, DMSO-d_6): δ 1.00 (t, J = 7.5 Hz, 3H), 1.81–1.91 (m, 2H), 3.08 (t, J = 7.5 Hz, 2H), 4.39 (s, 2H), 6.86 (d, J = 2.7 Hz, 1H), 7.36 (br s, 1H), 7.51–7.60 (m, 3H), 7.60–7.66 (m, 4H), 8.06 (d, J = 9.3 Hz, 1H). LC–MS (ESI) m/z: 346 $(M + H)^+$.

ii) 2-((3-(Aminomethyl)-4-phenyl-2-propylquinolin-6-yl)oxy)acetamide (9a). A mixture of **9aa** (1.0 g, 2.9 mmol), Raney cobalt (5 mL), 28% NH_3 (5 mL), tetrahydrofuran (2.5 mL), and methanol (2.5 mL) was stirred at 70 °C for 6 h in a sealed tube under a hydrogen atmosphere (0.5 MPa). The reaction mixture was filtered, and the filtrate was partitioned between ethyl acetate and water. The organic layer was washed with brine, dried over $MgSO_4$, and concentrated in vacuo. The residue was purified by column chromatography (silica gel, eluting with a gradient of 0–25% methanol in ethyl acetate) to afford the title compound **9a** (0.62 g, 62%) as a pale yellow powder. ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.10 (t, J = 7.2 Hz, 3H), 1.85–1.96 (m, 2H), 3.01–3.11 (m, 2H), 3.77 (s, 2H), 4.33 (s, 2H), 5.75 (br s, 1H), 6.54 (d, J = 1.8 Hz, 1H), 6.55 (br s, 1H), 7.25–7.33 (m, 3H), 7.52–7.58 (m, 3H), 8.02 (d, J = 9.0 Hz, 1H). LC–MS (ESI) m/z: 350 $(M + H)^+$. Anal. Calcd for $C_{21}H_{23}N_3O_2$: C, 72.18; H, 6.63; N, 12.03. Found: C, 71.49; H, 6.88; N 11.71.

2-((3-(Aminomethyl)-2-butyl-4-phenylquinolin-6-yl)oxy)acetamide (9b).

i) 2-((2-Butyl-3-cyano-4-phenylquinolin-6-yl)oxy)acetamide (9ba). By a procedure similar to that described for the synthesis of compound **9aa**, the title compound **9ba** was obtained from **8b** as a brown powder (0.71 g, 82%). ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.00 (t, J = 7.5 Hz, 3H), 1.48–1.60 (m, 2H), 1.84–1.95 (m, 2H), 3.19–3.24 (m, 2H), 4.41 (s, 2H), 5.65 (br s, 1H), 6.52 (br s, 1H), 6.92 (d, J = 2.7 Hz, 1H), 7.42–7.47 (m, 2H), 7.50 (dd, J = 8.7, 2.7 Hz, 1H), 7.58–7.63 (m, 3H), 8.08 (d, J = 8.7 Hz, 1H). LC–MS (ESI) m/z: 360 $(M + H)^+$.

ii) 2-((3-(Aminomethyl)-2-butyl-4-phenylquinolin-6-yl)oxy)acetamide (9b). By a procedure similar to that described for the synthesis of compound **9a**, the title compound **9b** was obtained from **9ba** as a pale yellow powder (0.56 g, 82%). ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.00 (t, J = 7.4 Hz, 3H), 1.44–1.62 (m, 2H), 1.84–1.90 (m, 2H), 3.07–3.15 (m, 2H), 3.77 (s, 2H), 4.33 (s, 2H), 5.68 (br s, 1H), 6.55 (d, J = 3.0 Hz, 1H), 6.45–6.60 (m, 1H), 7.24–7.33 (m, 3H), 7.52–7.57 (m, 3H), 8.03 (d, J = 9.2 Hz, 1H). LC–MS (ESI) m/z: 364 $(M + H)^+$. Anal. Calcd for $C_{22}H_{25}N_3O_2 \cdot 0.75H_2O$: C, 70.10; H, 7.09; N, 11.15. Found: C, 69.90; H, 6.89; N 11.09.

2-((3-(Aminomethyl)-2-pentyl-4-phenylquinolin-6-yl)oxy)acetamide (9c).

i) 2-((3-Cyano-2-pentyl-4-phenylquinolin-6-yl)oxy)acetamide (9ca). By a procedure similar to that described for the synthesis of compound **9aa**, the title compound **9ca** was obtained from **8c** as a pale yellow powder (1.09 g, 98%). ^{1}H NMR (300 MHz, DMSO-d_6): δ 0.99 (t, J = 6.6 Hz, 3H), 1.45–1.55 (m, 4H), 1.86–2.00 (m, 2H), 3.16–

3.27 (m, 2H), 4.50 (s, 2H), 6.96 (d, *J* = 2.6 Hz, 1H), 7.47 (br s, 1H), 7.61–7.79 (m, 6H), 7.95–8.11 (m, 1H), 8.18 (d, *J* = 9.0 Hz, 1H). LC–MS (ESI) *m*/*z*: 374 $(M + H)^+$.

ii) 2-((3-(Aminomethyl)-2-pentyl-4-phenylquinolin-6-yl)oxy)acetamide (9c). By a procedure similar to that described for the synthesis of compound **9a**, the title compound **9c** was obtained from **9ca** as a white powder (0.72 g, 71%). ^{1}H NMR (300 MHz, $CDCl_3$): δ 0.93 (t, *J* = 6.9 Hz, 3H), 1.39–1.54 (m, 4H), 1.82–1.95 (m, 2H), 3.07–3.13 (m, 2H), 3.77 (s, 2H), 4.33 (s, 2H), 5.66 (br s, 1H), 6.55 (d, *J* = 3.0 Hz, 1H), 6.45–6.60 (m, 1H), 7.25–7.29 (m, 2H), 7.31 (dd, *J* = 9.6, 3.0 Hz, 1H), 7.51–7.50 (m, 3H), 8.01 (d, *J* = 9.6 Hz, 1H). LC–MS (ESI) *m*/*z*: 378 $(M + H)^+$. Anal. Calcd for $C_{23}H_{27}N_3O_2 \cdot H_2O$: C, 69.85; H, 7.39; N, 10.62. Found: C, 69.86; H, 7.40; N 10.55.

2-((3-(Aminomethyl)-2-isobutyl-4-phenylquinolin-6-yl)oxy)acetamide (9d).

i) 2-((3-Cyano-2-isobutyl-4-phenylquinolin-6-yl)oxy)acetamide (9da). By a procedure similar to that described for the synthesis of compound **9aa**, the title compound **9da** was obtained from **8d** as a white powder (0.71 g, 79%). ^{1}H NMR (200 MHz, $CDCl_3$) δ: 1.05 (d, *J* = 6.6 Hz, 6H), 2.32–2.43 (m, 1H), 3.09 (d, *J* = 7.2 Hz, 2H), 4.40 (s, 2H), 5.75 (br s, 1H), 6.51 (br s, 1H), 6.92 (d, *J* = 3.0 Hz, 1H), 7.41–7.48 (m, 2H), 7.49 (dd, *J* = 9.0, 3.0 Hz, 1H), 7.57–7.64 (m, 3H), 8.09 (d, *J* = 9.0 Hz, 1H).

ii) 2-((3-(Aminomethyl)-2-isobutyl-4-phenylquinolin-6-yl)oxy)acetamide (9d). By a procedure similar to that described for the synthesis of compound **9a**, the title compound **9d** was obtained from **9da** as a white powder (0.43 g, 66%); mp 139–141 °C. ^{1}H NMR (200 MHz, $CDCl_3$) δ: 1.04 (d, *J* = 6.6 Hz, 6H), 1.24 (br s, 2H), 2.32–2.44 (m, 1H), 3.00 (d, *J* = 7.2 Hz, 2H), 3.78 (s, 2H), 4.33 (s, 2H), 5.88 (br s, 1H), 5.55 (br s, 1H), 6.56 (d, *J* = 2.7 Hz, 1H), 7.25–7.28 (m, 2H), 7.30 (dd, *J* = 9.3, 2.7 Hz, 1H), 7.49–7.58 (m, 3H), 8.02 (d, *J* = 9.3 Hz, 1H). Anal. Calcd for $C_{22}H_{25}N_3O_2$: C, 72.70; H, 6.93; N, 11.56. Found: C, 72.35; H, 6.80; N 11.28. HPLC purity: 96.69% (λ 220 nm), 95.60% (λ 254 nm).

2-((3-(Aminomethyl)-2-(2,2-dimethylpropyl)-4-phenylquinolin-6-yl)oxy)acetamide (9e).

i) 2-((3-Cyano-2-(2,2-dimethylpropyl)-4-phenylquinolin-6-yl)oxy)acetamide (9ea). By a procedure similar to that described for the synthesis of compound **9aa**, the title compound **9ea** was obtained from **8e** as a pale yellow powder (0.41 g, 61%); mp 184–185 °C. ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.11 (s, 9H), 3.17 (s, 2H), 4.41(s, 2H), 5.69 (br s, 1H), 6.52 (br s, 1H), 6.92 (d, *J* = 2.8 Hz, 1H), 7.41–7.48 (m, 2H), 7.51 (dd, *J* = 9.0, 2.8 Hz, 1H), 7.57–7.64 (m, 3H), 8.11 (d, *J* = 9.0 Hz, 1H). LC–MS (ESI) *m*/*z*: 374 $(M + H)^+$.

ii) 2-((3-(Aminomethyl)-2-(2,2-dimethylpropyl)-4-phenylquinolin-6-yl)oxy)acetamide (9e). By a procedure similar to that described for the synthesis of compound **9a**, the title compound **9e** was obtained from **9ea** as a white

powder (0.15 g, 47%); mp 152–154 °C. ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.08 (s, 9H), 1.34 (br s, 2H), 3.07 (s, 2H), 3.84 (s, 2H), 4.33(s, 2H), 5.73 (br s, 1H), 6.54 (d, *J* = 2.7 Hz, 1H), 6.55 (br s, 1H), 7.25–7.30 (m, 2H), 7.31 (dd, *J* = 9.0, 2.7 Hz, 1H), 7.51–7.57 (m, 3H), 8.02 (d, *J* = 9.0 Hz, 1H). LC–MS (ESI) *m*/*z*: 378 $(M + H)^+$. Anal. Calcd for $C_{23}H_{27}N_3O_2$: C, 73.18; H, 7.21; N, 11.13. Found: C, 72.91; H, 7.24; N 10.83.

2-((3-(Aminomethyl)-2-benzyl-4-phenylquinolin-6-yl)oxy)acetamide (9f).

i) 2-((2-Benzyl-3-cyano-4-phenylquinolin-6-yl)oxy)acetamide (9fa). By a procedure similar to that described for the synthesis of compound **9aa**, the title compound **9fa** was obtained from **8f** as a white powder (0.40 g, 85%); mp 178–179 °C. ^{1}H NMR (300 MHz, $CDCl_3$): δ 4.40 (s, 2H), 4.56 (s, 2H), 5.63 (br s, 1H), 6.50 (br s, 1H), 6.91 (d, *J* = 3.0 Hz, 1H), 7.19–7.43 (m, 6H), 7.49–7.62 (m, 5H), 8.14 (d, *J* = 9.2 Hz, 1H). LC–MS (ESI) *m*/*z*: 394 $(M + H)^+$.

ii) 2-((3-(Aminomethyl)-2-benzyl-4-phenylquinolin-6-yl)oxy)acetamide (9f). By a procedure similar to that described for the synthesis of compound **9a**, the title compound **9f** was obtained from **9fa** as a white powder (0.29 g, 74%); mp 180–182 °C. ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.32 (br s, 2H), 3.68 (s, 2H), 4.33 (s, 2H), 4.56 (s, 2H), 5.83 (br s, 1H), 6.56 (d, *J* = 3.0 Hz, 1H), 6.58 (br s, 1H), 7.15–7.30 (m, 7H), 7.34 (dd, *J* = 9.0, 3.0 Hz, 1H), 7.45–7.54 (m, 3H), 8.08 (d, *J* = 9.0 Hz, 1H). LC–MS (ESI) *m*/*z*: 398 $(M + H)^+$. Anal. Calcd for $C_{25}H_{23}N_3O_2 \cdot 0.5H_2O$: C, 73.87; H, 5.95; N, 10.34. Found: C, 74.17; H, 5.95; N 10.04.

2-((3-(Aminomethyl)-4-(3-fluorophenyl)-2-isobutylquinolin-6-yl)oxy)acetamide (9g).

i) 2-((3-Cyano-4-(3-fluorophenyl)-2-isobutylquinolin-6-yl)oxy)acetamide (9ga). By a procedure similar to that described for the synthesis of compound **9aa**, the title compound **9ga** was obtained from **8g** as an off-white powder (0.38 g, 81%); mp 186–193 °C. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 1.06 (d, *J* = 6.6 Hz, 6H), 2.30–2.50 (m, 1H), 3.10 (d, *J* = 7.3 Hz, 2H), 4.43 (s, 2H), 5.74 (br s, 1H), 6.52 (br s, 1H), 6.88 (d, *J* = 2.8 Hz, 1H), 7.10–7.25 (m, 2H), 7.25–7.40 (m, 1H), 7.52 (dd, *J* = 9.2, 2.8 Hz, 1H), 7.55–7.65 (m, 1H), 8.11 (d, *J* = 9.2 Hz, 1H). LC–MS (ESI) *m*/*z*: 378 $(M + H)^+$.

ii) 2-((3-(Aminomethyl)-4-(3-fluorophenyl)-2-isobutylquinolin-6-yl)oxy)acetamide (9g). A mixture of **9ga** (0.35 g, 0.93 mmol), Raney nickel (ca. 4 g), 25% NH_3 (4 mL), tetrahydrofuran (50 mL), and methanol (50 mL) was stirred at room temperature for 9 h in a sealed tube under a hydrogen atmosphere (0.5 MPa). The reaction mixture was filtered, and the filtrate was concentrated in vacuo. The residue was purified by column chromatography (silica gel, eluting with a gradient of 0–20% methanol in ethyl acetate) followed by recrystallization from aqueous ethanol to afford the title compound **9g** (0.27 g, 76%) as colorless crystals; mp 106–108 °C. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 1.04 (d, *J* = 6.6 Hz, 6H), 1.51 (br s, 2H), 2.25–2.50 (m, 1H), 3.00 (d, *J* = 7.2 Hz, 2H), 3.78 (s, 2H), 4.36 (s, 2H), 5.62 (br s, 1H), 6.53 (d, *J* = 2.8 Hz, 1H), 6.55 (br s, 1H), 6.90–7.30 (m, 3H), 7.34 (dd, *J* = 9.2, 2.8 Hz, 1H), 7.45–

7.60 (m, 1H), 8.03 (d, *J* = 9.2 Hz, 1H). LC–MS (ESI) *m*/*z*: 382 $(M + H)^+$. Anal. Calcd for $C_{22}H_{24}FN_3O_2 \cdot H_2O$: C, 66.15; H, 6.56; N, 10.52. Found: C, 65.92; H, 6.43; N 10.31. HPLC purity: 98.31% (λ 220 nm), 97.68% (λ 254 nm).

2-((3-(Aminomethyl)-2-isobutyl-4-(3-methylphenyl)quinolin-6-yl)oxy)acetamide (9h).

i) 2-((3-Cyano-2-isobutyl-4-(3-methylphenyl)quinolin-6-yl)oxy)acetamide (9ha). By a procedure similar to that described for the synthesis of compound **9aa**, the title compound **9ha** was obtained from **8h** as an off-white powder (0.25 g, 71%); mp 123–125 °C. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 1.05 (d, *J* = 6.8 Hz, 6H), 2.25–2.45 (m, 1H), 2.48 (s, 3H), 3.10 (d, *J* = 7.2 Hz, 2H), 4.41 (s, 2H), 5.64 (br s, 1H), 6.53 (br s, 1H), 6.93 (d, *J* = 2.8 Hz, 1H), 7.15–7.30 (m, 2H), 7.30–7.55 (m, 3H), 8.09 (d, *J* = 9.2 Hz, 1H). LC–MS (ESI) *m*/*z*: 374 $(M + H)^+$.

ii) 2-((3-(Aminomethyl)-2-isobutyl-4-(3-methylphenyl)quinolin-6-yl)oxy)acetamide (9h). By a procedure similar to that described for the synthesis of compound **9g**, the title compound **9h** was obtained from **9ha** as a white powder (50 mg, 27%); mp 98–101 °C. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 1.04 (d, *J* = 6.6 Hz, 6H), 1.60 (br s, 2H), 2.30–2.45 (m, 1H), 2.45 (s, 3H), 2.99 (d, *J* = 7.4 Hz, 2H), 3.78 (br s, 2H), 4.34 (s, 2H), 5.63 (br s, 1H), 6.57 (d, *J* = 2.6 Hz, 1H), 6.60 (br s, 1H), 7.05 (d, *J* = 7.2 Hz, 1H), 7.25–7.40 (m, 1H), 7.32 (dd, *J* = 9.2, 2.6 Hz, 2H), 7.40–7.50 (m, 1H), 8.01 (d, *J* = 9.2 Hz, 1H). LC–MS (ESI) *m*/*z*: 378 $(M + H)^+$; HRMS (ESI) calcd for $C_{23}H_{27}N_3O_2$ $(M + H)^+$ *m*/*z* 378.2176, found *m*/*z* 378.2151. Anal. Calcd for $C_{23}H_{27}N_3O_2 \cdot 2H_2O$: C, 66.81; H, 7.56; N, 10.16. Found: C, 66.73; H, 7.05; N 9.93. HPLC purity: 95.87% (λ 220 nm), 95.11% (λ 254 nm).

2-((3-(Aminomethyl)-4-(4-fluorophenyl)-2-isobutylquinolin-6-yl)oxy)acetamide (9i).

i) 2-((3-Cyano-4-(4-fluorophenyl)-2-isobutylquinolin-6-yl)oxy)acetamide (9ia). By a procedure similar to that described for the synthesis of compound **9aa**, the title compound **9ia** was obtained from **8i** as pale yellow crystals (0.93 g, 94%); mp 153–154 °C. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 1.05 (d, *J* = 6.6 Hz, 6H), 2.30–2.50 (m, 1H), 3.10 (d, *J* = 7.4 Hz, 2H), 4.43 (s, 2H), 5.68 (br s, 1H), 6.51 (br s, 1H), 6.90 (d, *J* = 2.9 Hz, 1H), 7.25–7.40 (m, 2H), 7.40–7.50 (m, 2H), 7.52 (dd, *J* = 9.2, 2.9 Hz, 1H), 8.11 (d, *J* = 9.2 Hz, 1H). LC–MS (ESI) *m*/*z*: 378 $(M + H)^+$.

ii) 2-((3-(Aminomethyl)-4-(4-fluorophenyl)-2-isobutylquinolin-6-yl)oxy)acetamide (9i). By a procedure similar to that described for the synthesis of compound **9g**, the title compound **9i** was obtained from **9ia** as a white powder (0.42 g, 49%); mp 142 °C. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 1.04 (d, *J* = 6.6 Hz, 6H), 1.54 (br s, 2H), 2.25–2.50 (m, 1H), 2.99 (d, *J* = 7.2 Hz, 2H), 3.77 (s, 2H), 4.36 (s, 2H), 5.63 (br s, 1H), 6.54 (br s, 1H), 6.54 (d, *J* = 2.8 Hz, 1H), 7.20–7.30 (m, 4H), 7.33 (dd, *J* = 9.2, 2.8 Hz, 1H), 8.03 (d, *J* = 9.2 Hz, 1H). LC–MS (ESI) *m*/*z*: 378 $(M + H)^+$. Anal. Calcd for $C_{22}H_{24}FN_3O_2 \cdot 0.75H_2O$: C, 66.90; H, 6.51; N, 10.64. Found: C, 66.56; H, 6.61; N 10.43. HPLC purity: 99.54% (λ 220 nm), 99.39% (λ 254 nm).

2-((3-(Aminomethyl)-2-isobutyl-4-(4-methylphenyl)quinolin-6-yl)oxy)acetamide (9j).

i) 2-((3-Cyano-2-isobutyl-4-(4-methylphenyl)quinolin-6-yl)oxy)acetamide (9ja). By a procedure similar to that described for the synthesis of compound **9aa**, the title compound **9ja** was obtained from **8j** as a white powder (0.37 g, 78%); mp 184–186 °C. ^{1}H NMR (200 MHz, $CDCl_3$) δ: 1.05 (d, *J* = 6.6 Hz, 6H), 2.30–2.50 (m, 1H), 2.51 (s, 3H), 3.04 (d, *J* = 7.3 Hz, 2H), 4.42 (s, 2H), 5.71 (br s, 1H), 6.53 (br s, 1H), 6.96 (d, *J* = 2.9 Hz, 1H), 7.25–7.45 (m, 4H), 7.50 (dd, *J* = 9.2, 2.9 Hz, 1H), 8.08 (d, *J* = 9.2 Hz, 1H). LC–MS (ESI) *m*/*z*: 374 $(M + H)^+$.

ii) 2-((3-(Aminomethyl)-2-isobutyl-4-(4-methylphenyl)quinolin-6-yl)oxy)acetamide (9j). By a procedure similar to that described for the synthesis of compound **9g**, the title compound **9j** was obtained from **9ja** as a white powder (0.24 g, 72%); mp 175–177 °C. ^{1}H NMR (200 MHz, $CDCl_3$) δ: 1.04 (d, *J* = 6.6 Hz, 6H), 1.60 (br s, 2H), 2.25–2.45 (m, 1H), 2.50 (s, 3H), 2.99 (d, *J* = 7.3 Hz, 2H), 3.78 (br s, 2H), 4.34 (s, 2H), 5.68 (br s, 1H), 6.58 (br s, 1H), 6.59 (d, *J* = 2.9 Hz, 1H), 7.15 (d, *J* = 8.1 Hz, 2H), 7.25–7.40 (m, 3H), 8.01 (d, *J* = 9.2 Hz, 1H). LC–MS (ESI) *m*/z: 378 $(M + H)^+$. Anal. Calcd for $C_{23}H_{27}N_3O_2 \cdot 1.25H_2O$: C, 69.06; H, 7.43; N, 10.51. Found: C, 69.27; H, 7.50; N 10.11. HPLC purity: 99.10% (λ 220 nm), 98.84% (λ 254 nm).

***tert*-Butyl ((6-hydroxy-2-isobutyl-4-(4-methylphenyl)quinolin-3-yl)methyl)carbamate (10).** A mixture of **8j** (27.4 g, 86.6 mmol), Raney cobalt (ca. 101 g), 25% aqueous ammonia (50 mL), methanol (300 mL), and tetrahydrofuran (150 mL) was stirred at 90 °C for 8 h in a sealed tube under a hydrogen atmosphere (0.5 MPa). The reaction mixture was cooled to room temperature and filtered, and the filtrate was poured into brine and extracted with ethyl acetate. The extract was dried over $MgSO_4$ and concentrated in vacuo. The residue was dissolved in tetrahydrofuran (500 mL) and then di-*tert*-butyl dicarbonate (22.7 g, 104 mmol) was added. After stirring at room temperature for 96 h, the reaction mixture was concentrated in vacuo. The residual solid was triturated with diisopropyl ether to afford the title compound **10** (30.2 g, 83%) as an off-white powder. ^{1}H NMR (200 MHz, $CDCl_3$) δ: 0.97 (d, *J* = 6.6 Hz, 6H), 1.40 (s, 9H), 2.15–2.30 (m, 1H), 2.42 (s, 3H), 2.92 (d, *J* = 7.3 Hz, 2H), 4.25–4.35 (m, 3H), 6.67 (d, *J* = 2.5 Hz, 1H), 7.08 (d, *J* = 7.3 Hz, 2H), 7.20–7.30 (m, 3H), 7.88 (d, *J* = 8.9 Hz, 1H). LC–MS (ESI) *m*/*z*: 421 $(M + H)^+$.

Methyl ((3-(((*tert*-butoxycarbonyl)amino)methyl)-2-isobutyl-4-(4-methylphenyl)quinolin-6-yl)-oxy)acetate (11a). A mixture of **10** (1.50 g, 3.56 mmol), methyl bromoacetate (0.92 g, 6.0 mmol), and K_2CO_3 (0.55 g, 4.0 mmol) in *N*,*N*-dimethylformamide (10 mL) was stirred at room temperature for 2.5 h. The reaction mixture was partitioned between ethyl acetate and water. The organic layer was washed with brine, dried over $MgSO_4$, and concentrated in vacuo. The residual solid was triturated with diisopropyl ether to afford the title compound **11a** (1.66 g, 94%) as a white powder. ^{1}H NMR (300 MHz, DMSO-d_6): δ 0.99 (d, *J* = 6.6 Hz, 6H), 1.35 (s, 9H), 2.26–2.32 (m, 1H), 2.43 (s, 3H), 3.09–3.10 (m, 2H), 3.60 (s, 3H), 4.12 (s, 2H), 4.77 (s, 2H), 6.54 (s, 1H), 7.12 (s, 1H),

7.29 (d, *J* = 7.8 Hz, 2H), 7.40 (d, *J* = 7.8 Hz, 2H), 7.75–7.77 (m, 1H), 8.48–8.51 (m, 1H). LC–MS (ESI) *m*/*z*: 493 $(M + H)^+$.

***tert*-Butyl ((6-(3-hydroxypropoxy)-2-isobutyl-4-(4-methylphenyl)quinolin-3-yl)methyl)carbamate (11b).** By a procedure similar to that described for the synthesis of compound **11a** using 3-bromo-1-propanol, the title compound **11b** was obtained as a white powder (0.84 g, 89%). ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.12 (d, *J* = 6.6 Hz, 6H), 1.40 (s, 9H), 1.97–2.05 (m, 2H), 2.34–2.45 (m, 1H), 2.50 (s, 3H), 3.51 (d, *J* = 7.5 Hz, 2H), 3.83 (t, *J* = 6.0 Hz, 2H), 3.99 (t, *J* = 6.0 Hz, 2H), 4.38 (br s, 3H), 6.99 (d, *J* = 2.6 Hz, 1H), 7.16 (d, *J* = 8.0 Hz, 2H), 7.44 (d, *J* = 8.0 Hz, 2H), 7.54 (dd, *J* = 9.3, 2.6 Hz, 1H), 9.09 (d, *J* = 9.3 Hz, 1H). LC–MS (ESI) *m*/*z*: 479 $(M + H)^+$.

Ethyl 4-((3-(((*tert*-butoxycarbonyl)amino)methyl)-2-isobutyl-4-(4-methylphenyl)quinolin-6-yl)oxy)butanoate (11c). K_2CO_3 (73 mg, 0.53 mmol) was added to a mixture of compound **10** (201 mg, 0.48 mmol) and ethyl 4-bromobutyrate (105 mg, 0.54 mmol) in *N*,*N*-dimethylformamide (5 mL) at room temperature. The resulting mixture was stirred at room temperature for 12 h. The reaction mixture was partitioned between ethyl acetate and water. The organic layer was separated, washed with brine, dried over $MgSO_4$, and concentrated in vacuo. The residue was purified by column chromatography (silica gel, eluting with a gradient of 19–40% ethyl acetate in hexanes) to afford the title compound **11c** (175 mg, 69%) as a white powder. ^{1}H NMR (300 MHz, DMSO-d_6): δ 0.97 (d, *J* = 6.51 Hz, 6H), 1.14 (t, *J* = 7.11 Hz, 3H), 1.38 (s, 9H), 1.92 (quin, *J* = 6.70 Hz, 2H), 2.32 (spt, *J* = 6.80 Hz, 1H), 2.40 (t, *J* = 7.30 Hz, 2H), 2.42 (s, 3H), 2.77 (d, *J* = 6.79 Hz, 2H), 3.83 (t, *J* = 5.96 Hz, 2H), 3.97–4.03 (m, 2H), 4.02 (q, *J* = 7.20 Hz, 2H), 6.51 (d, *J* = 1.90 Hz, 1H), 7.01 (t, *J* = 3.90 Hz, 1H), 7.26–7.37 (m, 5H), 7.89 (d, *J* = 9.05 Hz, 1H). LC–MS (ESI) *m*/*z*: 535 $(M + H)^+$.

Ethyl 5-((3-(((*tert*-butoxycarbonyl)amino)methyl)-2-isobutyl-4-(4-methylphenyl)quinolin-6-yl)oxy)-pentanoate (11d). By a procedure similar to that described for the synthesis of compound **11c** using ethyl 5-bromovalerate, the title compound **11d** was obtained as a white powder (161 mg, 61%). ^{1}H NMR (300 MHz, DMSO-d_6): δ 0.97 (d, *J* = 6.3 Hz, 6H), 1.15 (t, *J* = 7.0 Hz, 3H), 1.38 (s, 9H), 1.54–1.73 (m, 4H), 2.30 (t, *J* = 6.8 Hz, 2H), 2.34 (quin, *J* = 6.5 Hz, 1H), 2.42 (s, 3H), 2.77 (d, *J* = 6.9 Hz, 2H), 3.81 (t, *J* = 5.4 Hz, 2H), 3.97–4.07 (m, 2H), 4.02 (q, *J* = 7.0 Hz, 2H), 6.52 (d, *J* = 1.9 Hz, 1H), 7.01 (t, *J* = 4.8 Hz, 1H), 7.26–7.38 (m, 5H), 7.89 (d, *J* = 9.2 Hz, 1H). LC–MS (ESI) *m*/*z*: 549 $(M + H)^+$.

Ethyl 6-((3-(((*tert*-butoxycarbonyl)amino)methyl)-2-isobutyl-4-(4-methylphenyl)quinolin-6-yl)oxy)hexanoate (11e). By a procedure similar to that described for the synthesis of compound **11c** using ethyl 6-bromohexanoate, the title compound **11e** was obtained from compound **10** as a white powder (0.90 g, 53%). ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.12 (d, *J* = 6.6 Hz, 6H), 1.25 (t, *J* = 7.2 Hz, 3H), 1.40 (s, 9H), 1.43–1.53 (m, 2H), 1.61–1.81 (m, 4H), 2.29–2.43 (m, 3H), 2.52 (s, 3H), 3.51 (d, *J* = 7.5 Hz, 2H), 3.83 (t, *J* = 6.0 Hz, 2H), 4.12 (q, *J* = 7.2 Hz, 2H), 4.37 (br s, 3H), 6.64 (d, *J* = 2.4 Hz, 1H), 7.16 (t, *J* = 7.6 Hz, 2H), 7.44 (t, *J* = 7.6 Hz, 2H), 7.53 (dd, *J* = 9.3, 2.4 Hz, 1H),

9.09 (d, *J* = 9.3 Hz, 1H).

Methyl ((3-(aminomethyl)-2-isobutyl-4-(4-methylphenyl)quinolin-6-yl)oxy)acetate dihydrochloride (12a). To a solution of **11a** (0.15 g, 0.3 mmol) in ethyl acetate (5 mL) was added 4 M HCl in ethyl acetate (5 mL) at room temperature. The resulting mixture was stirred at room temperature for 1.5 h and then concentrated in vacuo. The residue was crystallized from diisopropyl ether to afford the title compound **12a** (0.13 g, 92%) as a yellow powder. ^{1}H NMR (200 MHz, DMSO-d_6) δ: 1.00 (d, *J* = 7.2 Hz, 6H), 2.24–2.32 (m, 1H), 2.46 (s, 3H), 3.25 (s, 2H), 3.59 (s, 3H), 3.93–3.99 (m, 2H), 4.75 (s, 2H), 6.49 (s, 1H), 7.33 (d, *J* = 7.5 Hz, 2H), 7.45 (d, *J* = 7.5 Hz, 2H), 7.72–7.75 (m, 1H), 8.39–8.42 (m, 1H), 8.56 (br s, 3H). HRMS (ESI) calcd for $C_{24}H_{28}N_2O_3$ $(M + H)^+$ *m/z* 393.2173, found *m/z* 393.2155. HPLC purity: 98.26% (λ 220 nm), 98.12% (λ 254 nm).

2-((3-(Aminomethyl)-2-isobutyl-4-(4-methylphenyl)quinolin-6-yl)oxy)-*N*-methylacetamide dihydrochloride (12b).

i) *tert*-Butyl ((2-isobutyl-6-(2-(methylamino)-2-oxoethoxy)-4-(4-methylphenyl)quinolin-3-yl)methyl)-carbamate (12ba). To a mixture of **13a** (0.48 g, 1.0 mmol), 1 M methylamine in tetrahydrofuran (2 mL, 2 mmol) in *N*,*N*-dimethylformamide (3 mL) were added 1-hydroxy-1*H*-benzotriazole (0.16 g, 1.2 mmol) and 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (0.23 g, 1.2 mmol). The resulting mixture was stirred at room temperature for 15 h. The reaction mixture was diluted with ethyl acetate, washed sequentially with 1 M HCl and brine, dried over $MgSO_4$, and concentrated in vacuo. The residue was purified by column chromatography (silica gel, eluting with 50% ethyl acetate in hexanes) to give the title compound **12ba** (0.27 g, 55%) as a pale yellow solid. ^{1}H NMR (200 MHz, $CDCl_3$) δ: 1.00 (d, *J* = 6.6 Hz, 6H), 1.41 (s, 9H), 2.30–2.33 (m, 1H), 2.49 (s, 3H), 2.88–2.98 (m, 5H), 4.28–4.34 (m, 4H), 6.58–6.59 (m, 2H), 6.60 (br s, 1H), 7.07 (d, *J* = 8.1 Hz, 2H), 7.32 (d, *J* = 8.1 Hz, 2H), 7.33–7.34 (m, 1H), 8.02 (d, *J* = 9.0 Hz, 1H).

ii) 2-((3-(Aminomethyl)-2-isobutyl-4-(4-methylphenyl)quinolin-6-yl)oxy)-*N*-methylacetamide dihydro-chloride (12b). By a procedure similar to that described for the synthesis of compound **12a**, the title compound **12b** was obtained from **12ba** as a pale yellow powder (0.20 g, 79%). ^{1}H NMR (200 MHz, DMSO-d_6) δ: 0.99 (d, *J* = 6.6 Hz, 6H), 2.26–2.30 (m, 1H), 2.46 (s, 3H), 2.58 (s, 3H), 3.18 (s, 2H), 3.97 (s, 2H), 4.42 (s, 2H), 6.64 (s, 1H), 7.32 (d, *J* = 8.4 Hz, 2H), 7.44 (d, *J* = 8.4 Hz, 2H), 7.72–7.73 (m, 1H), 8.07–8.08 (m, 1H), 8.41 (br s, 3H). HRMS (ESI) calcd for $C_{24}H_{29}N_3O_2$ $(M + H)^+$ *m/z* 392.2333, found *m/z* 392.2316. HPLC purity: 99.08% (λ 220 nm), 99.05% (λ 254 nm).

3-(3-(Aminomethyl)-2-isobutyl-4-(4-methylphenyl)quinolin-6-yl)propanamide dihydrochloride (12c).

i) *tert*-butyl ((6-(3-amino-3-oxopropyl)-2-isobutyl-4-(4-methylphenyl)quinolin-3-yl)methyl)carbamate (12ca).

A mixture of **17** (0.25 g, 0.53 mmol), 10% palladium on charcoal (0.25 g), ethanol (5 mL), and tetrahydrofuran (5 mL) was stirred at room temperature for 3.5 h under a hydrogen atmosphere. The reaction mixture was filtered, and the filtrate was concentrated in vacuo. The residue was triturated with diisopropyl ether to afford the title compound **12ca** (0.20 g, 81%) as a white powder. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 1.03 (d, *J* = 6.6 Hz, 6H), 1.41 (s, 9H), 2.31–2.39 (m, 1H), 2.46 (t, *J* = 7.8 Hz, 2H), 2.49 (s, 3H), 3.00 (t, *J* = 7.8 Hz, 2H), 3.00–3.03 (m, 2H), 4.31 (s, 2H), 4.34 (br s, 1H), 5.28 (br s, 1H), 5.40 (br s, 1H), 7.12 (d, *J* = 8.1 Hz, 2H), 7.12–7.15 (m, 1H), 7.35 (d, *J* = 5.1 Hz, 2H), 7.55–7.61 (m, 1H), 8.17 (br s, 1H).

ii) 3-(3-(Aminomethyl)-2-isobutyl-4-(4-methylphenyl)quinolin-6-yl)propanamide dihydrochloride (12c). By a procedure similar to that described for the synthesis of compound **12a**, the title compound **12c** was obtained from **12ca** as a white powder (0.14 g, 96%) as a pale yellow powder. ^{1}H NMR (200 MHz, DMSO-d_6) δ: 0.84 (d, *J* = 6.3 Hz, 6H), 2.11–2.17 (m, 1H), 2.15 (t, *J* = 7.2 Hz, 2H), 2.36 (s, 3H), 2.71 (t, *J* = 7.2 Hz, 2H), 3.09 (s, 2H), 3.82–3.88 (m, 2H), 6.57 (br s, 1H), 7.03 (s, 1H), 7.09 (br s, 1H), 7.20 (d, *J* = 7.8 Hz, 2H), 7.29 (d, *J* = 7.8 Hz, 2H), 7.75–7.76 (m, 1H), 8.28–8.33 (m, 1H), 8.32 (br s, 3H). HRMS (ESI) calcd for $C_{24}H_{29}N_3O$ $(M + H)^+$ *m/z* 376.2383, found *m/z* 376.2366. HPLC purity: 99.10% (λ 220 nm), 99.50% (λ 254 nm).

(2*E*)-3-(3-(Aminomethyl)-2-isobutyl-4-(4-methylphenyl)quinolin-6-yl)acrylamide (12d).

i) (2*E*)-3-(3-(Aminomethyl)-2-isobutyl-4-(4-methylphenyl)quinolin-6-yl)acrylamide (12da). By a procedure similar to that described for the synthesis of compound **12a**, the title compound **12da** was obtained from **17** as a white powder (0.83 g, 98%). ^{1}H NMR (200 MHz, DMSO-d_6) δ: 0.99 (d, *J* = 6.6 Hz, 6H), 2.28–2.30 (m, 1H), 2.43 (s, 3H), 3.18–3.19 (m, 2H), 3.94–3.96 (m, 2H), 6.60 (d, *J* = 15.6 Hz, 1H), 7.11–7.12 (m, 1H), 7.35–7.45 (m, 5H), 7.56 (d, *J* = 15.6 Hz, 1H), 7.60–7.62 (m, 1H), 8.13–8.14 (m, 1H), 8.45 (br s, 3H).

ii) (2*E*)-3-(3-(Aminomethyl)-2-isobutyl-4-(4-methylphenyl)quinolin-6-yl)acrylamide (12d). A mixture of **12da** (0.22 g, 0.50 mmol) and 10% K_2CO_3 (100 mL) was stirred at room temperature for 5 min. The mixture was partitioned between ethyl acetate and water. The organic layer was washed with brine, dried over $MgSO_4$, and concentrated in vacuo. The residual solid was recrystallized from aqueous ethanol to afford the title compound **12d** (0.16 g, 89%) as a white powder. ^{1}H NMR (200 MHz, DMSO-d_6) δ: 1.04 (d, *J* = 6.6 Hz, 6H), 1.26 (br s, 2H), 2.33–2.44 (m, 1H), 2.50 (s, 3H), 3.02 (d, *J* = 6.9 Hz, 2H), 3.82 (s, 2H), 5.51 (br s, 2H), 6.42 (d, *J* = 15.6 Hz, 1H), 7.16 (d, *J* = 8.1 Hz, 2H), 7.34–7.36 (m, 3H), 7.59 (d, *J* = 8.1 Hz, 1H), 7.81 (dd, *J* = 8.7, 1.8 Hz, 1H), 8.03 (d, *J* = 8.7 Hz, 1H). LC–MS (ESI) *m*/z: 374 $(M + H)^+$. Anal. Calcd for $C_{24}H_{27}N_3O \cdot 2H_2O$: C, 70.39; H, 7.63; N, 10.26. Found: C, 70.36; H, 7.77; N, 10.25. HPLC purity: 96.28% (λ 220 nm), 95.83% (λ 254 nm).

((3-(((*tert*-Butoxycarbonyl)amino)methyl)-2-isobutyl-4-(4-methylphenyl)quinolin-6-yl)-oxy)acetic acid (13a). To a mixture of compound **11a** (1.60 g, 3.25 mmol), tetrahydrofuran (5 mL), and methanol (5 mL) was added 1 M

NaOH (10 mL) at room temperature. After stirring at room temperature for 1.5 h, the reaction mixture was acidified with 1 M HCl and extracted with ethyl acetate. The extract was washed with brine, dried over $MgSO_4$, and concentrated in vacuo. The residual solid was triturated with diisopropyl ether to afford the title compound **13a** (1.32 g, 85%) as a pale yellow powder. ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.05 (d, *J* = 6.6 Hz, 6H), 1.40 (s, 9H), 2.29–2.31 (m, 1H), 2.48 (s, 3H), 3.36 (s, 2H), 4.35 (s, 2H), 4.53 (s, 2H), 4.89 (br s, 1H), 6.71 (s, 1H), 7.12–7.13 (m, 2H), 7.27–7.40 (m, 3H), 8.57 (d, *J* = 8.1 Hz, 1H). LC–MS (ESI) *m*/*z*: 493 $(M + H)^+$.

3-((3-(((*tert*-Butoxycarbonyl)amino)methyl)-2-isobutyl-4-(4-methylphenyl)quinolin-6-yl)oxy)propanoic acid (13b).

i) *tert*-Butyl ((2-isobutyl-4-(4-methylphenyl)-6-(3-oxopropoxy)quinolin-3-yl)methyl)carbamate (13ba). To a solution of oxalyl chloride (0.20 mL, 2.3 mmol) was added dimethylsulfoxide (0.33 mL, 4.6 mmol) at −78 °C in a dropwise manner. After stirring at −78 °C for 15 min, a solution of compound **11b** (0.72 g, 1.5 mmol) in CH_2Cl_2 (15 mL) was added at the same temperature. The resulting mixture was stirred at −78 °C for 30 min. To the reaction mixture was added triethylamine (1.0 mL, 7.5 mmol), and the resulting mixture was stirred at 0 °C for 30 min. The mixture was poured into ice water and extracted with ethyl acetate. The extract was washed with brine, dried over $MgSO_4$, and concentrated in vacuo. The residue was crystallized from ethyl acetate–diisopropyl ether to afford the title compound **13ba** (0.56 g, 78%) as a white powder. ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.02 (d, *J* = 6.9 Hz, 6H), 1.42 (s, 9H), 2.29–2.38 (m, 1H), 2.48 (s, 3H), 2.83–2.87 (m, 2H), 2.93 (d, *J* = 7.5 Hz, 2H), 4.16 (d, *J* = 6.0 Hz, 2H), 4.27 (br s, 3H), 6.60 (d, *J* = 2.7 Hz, 1H), 7.12 (d, *J* = 7.8 Hz, 2H), 7.27–7.34 (m, 3H), 7.97 (d, *J* = 9.3 Hz, 1H), 9.83 (s, 1H).

ii) 3-((3-(((*tert*-Butoxycarbonyl)amino)methyl)-2-isobutyl-4-(4-methylphenyl)quinolin-6-yl)oxy)propanoic acid (13b). To a mixture of **13ba** (0.71 g, 1.5 mmol), NaH_2PO_4 (0.18 g, 1.5 mmol), and 2-methyl-2-butene (0.64 mL, 6.0 mmol) in tetrahydrofuran–water–*tert*-butanol (1:1:1, v/v, 30 mL) was added $NaClO_2$ (0.27 g, 3.0 mmol) at room temperature. After stirring at room temperature for 1 h, the reaction mixture was poured into water and extracted with ethyl acetate. The extract was washed with brine, dried over $MgSO_4$, and concentrated in vacuo. The residue was crystallized from ethyl acetate–diisopropyl ether to afford the title compound **13b** (0.69 g, 92%) as a white powder. ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.10 (d, *J* = 6.3 Hz, 6H), 1.37 (s, 9H), 2.24–2.35 (m, 1H), 2.45 (s, 3H), 2.69 (t, *J* = 6.0 Hz, 2H), 3.06 (br s, 2H), 4.06 (t, *J* = 6.0 Hz, 2H), 4.11 (s, 2H), 6.67 (d, *J* = 2.4 Hz, 1H), 7.13 (br s, 2H), 7.35 (d, *J* = 8.0 Hz, 2H), 7.43 (d, *J* = 8.0 Hz, 2H), 7.71 (br s, 1H), 8.36 (br s, 1H).

4-((3-(((*tert*-Butoxycarbonyl)amino)methyl)-2-isobutyl-4-(4-methylphenyl)quinolin-6-yl)-oxy)-butanoic acid (13c). By a procedure similar to that described for the synthesis of compound **13a**, the title compound **13c** was obtained from compound **11c** as a white powder (0.16 g, 97%). ^{1}H NMR (300 MHz, DMSO-d_6): δ 0.99 (d, *J* = 6.3 Hz, 6H), 1.37 (s, 9H), 1.89 (quin, *J* = 6.5 Hz, 2H), 2.26–2.36 (m, 1H), 2.34 (t, *J* = 7.2 Hz, 2H), 2.44 (s, 3H), 2.82–

3.10 (m, 2H), 3.86 (t, *J* = 5.5 Hz, 2H), 3.98–4.16 (m, 2H), 6.60 (br s, 1H), 7.09 (br s, 1H), 7.32 (d, *J* = 7.5 Hz, 2H), 7.39 (d, *J* = 8.5 Hz, 2H), 7.48–7.69 (m, 1H), 8.15 (br s, 1H), 12.15 (br s, 1H). LC–MS (ESI) *m*/*z*: 507 $(M + H)^+$.

5-((3-(((*tert*-Butoxycarbonyl)amino)methyl)-2-isobutyl-4-(4-methylphenyl)quinolin-6-yl)oxy)pentanoic acid (13d). By a procedure similar to that described for the synthesis of compound **13a**, the title compound **13d** was obtained from compound **11d** as a pale yellow powder (98 mg, 66%). ^{1}H NMR (300 MHz, DMSO-d_6): δ 0.97 (d, *J* = 6.4 Hz, 6H), 1.38 (s, 9H), 1.52–1.72 (m, 4H), 2.22 (t, *J* = 6.9 Hz, 2H), 2.34 (spt, *J* = 6.2 Hz, 1H), 2.42 (s, 3H), 2.77 (d, *J* = 6.8 Hz, 2H), 3.81 (t, *J* = 5.5 Hz, 2H), 4.01 (d, *J* = 3.6 Hz, 2H), 6.52 (d, *J* = 1.9 Hz, 1H), 7.03 (t, *J* = 4.7 Hz, 1H), 7.24–7.43 (m, 5H), 7.89 (d, *J* = 9.2 Hz, 1H), 12.02 (br s, 1H). LC–MS (ESI) *m*/*z*: 521 $(M + H)^+$.

6-((3-(((*tert*-Butoxycarbonyl)amino)methyl)-2-isobutyl-4-(4-methylphenyl)quinolin-6-yl)oxy)hexanoic acid (13e). By a procedure similar to that described for the synthesis of compound **13a**, the title compound **13e** was obtained from compound **11e** as a white powder (0.61 g, 95%). ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.07 (d, *J* = 6.6 Hz, 6H), 1.40 (s, 9H), 1.42–1.53 (m, 2H), 1.61–1.79 (m, 4H), 2.29–2.41 (m, 3H), 2.50 (s, 3H), 3.26 (br s, 2H), 3.82 (t, *J* = 6.2 Hz, 2H), 4.32 (d, *J* = 4.8 Hz, 2H), 4.41 (br s, 1H), 6.61 (d, *J* = 2.7 Hz, 1H), 7.15 (d, *J* = 8.0 Hz, 2H), 7.37–7.44 (m, 3H), 8.54–8.62 (m, 1H).

((3-(Aminomethyl)-2-isobutyl-4-(4-methylphenyl)quinolin-6-yl)oxy)acetic acid dihydrochloride (14a). By a procedure similar to that described for the synthesis of compound **12a**, the title compound **14a** was obtained from compound **13a** as a slightly brown powder (0.10 g, 73%). ^{1}H NMR (300 MHz, DMSO-d_6): δ 1.00 (d, *J* = 6.6 Hz, 6H), 2.20–2.31 (m, 1H), 2.45 (s, 3H), 3.20 (br s, 2H), 3.97 (s, 2H), 4.64 (s, 2H), 6.57 (d, *J* = 2.7 Hz, 1H), 7.32 (d, *J* = 8.1 Hz, 2H), 7.40 (d, *J* = 8.1 Hz, 2H), 7.72 (br s, 1H), 8.46 (br s, 4H). LC–MS (ESI) *m*/*z*: 379 $(M + H)^+$. Anal. Calcd for $C_{23}H_{26}N_2O_3 \cdot 2HCl \cdot 3H_2O$: C, 54.66; H, 6.78; N, 5.54. Found: C, 54.86; H, 6.69; N, 5.50.

3-((3-(aminomethyl)-2-isobutyl-4-(4-methylphenyl)quinolin-6-yl)oxy)propanoic acid dihydrochloride (14b). By a procedure similar to that described for the synthesis of compound **12a**, the title compound **14b** was obtained from compound **13b** as a white powder (0.12 g, 92%). ^{1}H NMR (300 MHz, DMSO-d_6): δ 1.02 (d, *J* = 6.6 Hz, 6H), 2.24–2.36 (m, 1H), 2.48 (s, 3H), 2.69 (d, *J* = 5.4 Hz, 2H), 3.20 (br s, 2H), 3.99–4.07 (m, 4H), 6.63 (br s, 1H), 7.38 (d, *J* = 7.5 Hz, 2H), 7.48 (d, *J* = 7.5 Hz, 2H), 7.70 (br s, 1H), 8.43 (br s, 4H). LC–MS (ESI) *m*/*z*: 393 $(M + H)^+$. Anal. Calcd for $C_{24}H_{28}N_2O_3 \cdot 2HCl \cdot 2H_2O$: C, 57.49; H, 6.83; N, 5.59. Found: C, 57.34; H, 6.58; N, 5.35.

4-((3-(Aminomethyl)-2-isobutyl-4-(4-methylphenyl)quinolin-6-yl)oxy)butanoic acid dihydrochloride (14c). By a procedure similar to that described for the synthesis of compound **12a**, the title compound **14c** was obtained from compound **13c** as a white powder (0.30 g, 79%). ^{1}H NMR (300 MHz, DMSO-d_6): δ 1.02 (d, *J* = 6.5 Hz, 6H), 1.90 (quin, *J* = 6.5 Hz, 2H), 2.23–2.37 (m, 1H), 2.35 (t, *J* = 7.2 Hz, 2H), 2.48 (s, 3H), 3.26 (br s, 2H), 3.90 (t, *J* = 5.7 Hz, 2H), 3.99 (d, *J* = 3.9 Hz, 2H), 6.62 (br s, 1H), 7.39 (d, *J* = 7.6 Hz, 2H), 7.48 (d, *J* = 7.6 Hz, 2H), 7.72 (br s,

1H), 8.32–8.64 (m, 4H). LC–MS (ESI) *m*/*z*: 507 $(M + H)^+$. Anal. Calcd for $C_{25}H_{30}N_2O_3 \cdot 2HCl \cdot 0.5H_2O$: C, 61.47; H, 6.81; N, 5.74. Found: C, 61.48; H, 6.53; N 5.63.

5-((3-(Aminomethyl)-2-isobutyl-4-(4-methylphenyl)quinolin-6-yl)oxy)pentanoic acid dihydrochloride (14d). Compound **13d** (65 mg, 0.12 mmol) was dissolved in trifluoroacetic acid (2 mL), and the resulting solution was stirred at room temperature for 1 h. The reaction mixture was concentrated in vacuo, and the residue was purified by preparative HPLC. The obtained solid was dissolved in 1 M HCl (1 mL) and concentrated in vacuo. The residue was solidified with diisopropyl ether to afford the title compound **14d** (57 mg, 93%) as a pale yellow powder. ^{1}H NMR (300 MHz, DMSO-d_6): δ 1.03 (d, *J* = 6.3 Hz, 6H), 1.51–1.78 (m, 4H), 2.18–2.35 (m, 3H), 2.48 (s, 3H), 3.36 (d, *J* = 6.5 Hz, 2H), 3.91 (t, *J* = 5.5 Hz, 2H), 4.02 (d, *J* = 4.2 Hz, 2H), 6.66 (s, 1H), 7.11–7.28 (m, 1H), 7.43 (d, *J* = 8.3 Hz, 2H), 7.49 (d, *J* = 8.2 Hz, 2H), 7.80 (d, *J* = 9.2 Hz, 1H), 8.59 (d, *J* = 8.7 Hz, 1H), 8.69 (br s, 3H). LC–MS (ESI) *m*/*z*: 421 $(M + H)^+$. Anal. Calcd for $C_{26}H_{32}N_2O_3 \cdot 2HCl \cdot H_2O$: C, 61.05; H, 7.09; N, 5.48. Found: C, 61.22; H, 6.77; N 5.34.

6-((3-(Aminomethyl)-2-isobutyl-4-(4-methylphenyl)quinolin-6-yl)oxy)hexanoic acid dihydrochloride (14e). By a procedure similar to that described for the synthesis of compound **12a**, the title compound **14e** was obtained from compound **13e** as a white powder (0.14 g, 93%). ^{1}H NMR (300 MHz, DMSO-d_6): δ 1.03 (d, *J* = 6.3 Hz, 6H), 1.30–1.40 (m, 2H), 1.45–1.55 (m, 2H), 1.63–1.72 (m, 2H), 2.20 (t, *J* = 7.2 Hz, 2H), 2.23–2.34 (m, 1H), 2.84 (s, 3H), 3.35 (d, *J* = 6.9 Hz, 2H), 3.89 (t, *J* = 6.3 Hz, 2H), 3.99 (br s, 2H), 6.65 (d, *J* = 2.4 Hz, 1H), 7.42 (d, *J* = 7.8 Hz, 2H), 7.49 (d, *J* = 7.8 Hz, 2H), 7.78 (d, *J* = 8.4 Hz, 1H), 8.56–8.65 (m, 4H). LC–MS (ESI) *m*/*z*: 435 $(M + H)^+$. Anal. Calcd for $C_{27}H_{34}N_2O_3 \cdot 2HCl \cdot H_2O$: C, 61.71; H, 7.29; N, 5.33. Found: C, 61.42; H, 7.18; N 5.27.

5-((3-(Aminomethyl)-2-isobutyl-4-(4-methylphenyl)quinolin-6-yl)oxy)pentanamide (15).

i) *tert*-Butyl ((6-((5-amino-5-oxopentyl)oxy)-2-isobutyl-4-(4-methylphenyl)quinolin-3-yl)methyl)carbamate (15a). A mixture of **13d** (0.80 g, 1.5 mmol), ammonium salt of 1-hydroxy-1*H*-benzotriazole (0.25 g, 1.6 mmol), and 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (0.38 g, 2.0 mmol) in *N*,*N*-dimethylformamide (10 mL) was stirred at room temperature for 17 h. The reaction mixture was partitioned between ethyl acetate and water. The organic layer was washed with 1 M HCl, saturated $NaHCO_3$, and brine, dried over $MgSO_4$, and concentrated in vacuo. The residue was crystallized from diisopropyl ether to afford the title compound **15a** (0.60 g, 77%) as a colorless powder. ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.02 (d, *J* = 6.6 Hz, 6H), 1.41 (s, 9H), 1.76–1.81 (m, 4H), 2.24–2.30 (m, 2H), 2.33 (spt, *J* = 6.9 Hz, 1H), 2.48 (s, 3H), 2.93 (d, *J* = 7.1 Hz, 2H), 3.79–3.85 (m, 2H), 4.27 (d, *J* = 4.6 Hz, 2H), 4.32 (br s, 1H), 5.30 (br s, 1H), 5.41 (br s, 1H), 6.56 (d, *J* = 2.4 Hz, 1H), 7.12 (d, *J* = 7.5 Hz, 2H), 7.29 (dd, *J* = 9.1, 2.4 Hz, 1H), 7.33 (d, *J* = 7.6 Hz, 2H), 7.96 (d, *J* = 9.1 Hz, 1H). LC–MS (ESI) *m*/*z*: 520 $(M + H)^+$.

ii) 5-((3-(Aminomethyl)-2-isobutyl-4-(4-methylphenyl)quinolin-6-yl)oxy)pentanamide (15). A mixture of compound **15a** (0.40 g, 0.77 mmol) and trifluoroacetic acid (10 mL) was stirred at 0 °C for 3 h. The reaction mixture was concentrated in vacuo, and the residue was dissolved in ethyl acetate. The solution was washed with saturated $NaHCO_3$ and brine, dried over $MgSO_4$, and concentrated in vacuo. The residue was crystallized from diethyl ether to afford the title compound **15** (0.27 g, 84%) as a pale yellow powder. ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.03 (d, *J* = 6.8 Hz, 6H), 1.74–1.82 (m, 4H), 2.23–2.30 (m, 2H), 2.37 (spt, *J* = 6.8 Hz, 1H), 2.48 (s, 3H), 2.97 (d, *J* = 7.2 Hz, 2H), 3.77 (s, 2H), 3.79–3.85 (m, 2H), 5.34 (br s, 1H), 5.42 (br s, 1H), 6.54 (d, *J* = 3.1 Hz, 1H), 7.16 (d, *J* = 7.9 Hz, 2H), 7.27 (dd, *J* = 9.1, 3.1 Hz, 3H), 7.33 (d, *J* = 7.9 Hz, 2H), 7.95 (d, *J* = 9.1 Hz, 1H). LC–MS (ESI) *m*/*z*: 420 $(M + H)^+$. Anal. Calcd for $C_{26}H_{33}N_3O_2 \cdot 1.2H_2O$: C, 70.78; H, 8.09; N, 9.52. Found: C, 70.96; H, 7.86; N 9.26.

3-(((*tert*-butoxycarbonyl)amino)methyl)-2-isobutyl-4-(4-methylphenyl)quinolin-6-yl trifluoromethanesulfonate (16). To a mixture of compound **10** (1.5 g, 3.6 mmol) and NaH (60% suspension in oil, 0.21 g, 5.4 mmol) in *N*,*N*-dimethylformamide (10 mL) was added *N*-phenyl-bis(trifluoromethanesulfonimide) (1.9 g, 5.4 mmol) at 0 °C. The resulting mixture was stirred at room temperature for 1 h. The reaction mixture was diluted with water and extracted with ethyl acetate. The extract was washed with brine, dried over $MgSO_4$, and concentrated in vacuo. The residue was triturated with diisopropyl ether–hexanes to afford the title compound **16** (1.5 g, 75%) as a white powder. ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.01 (d, *J* = 6.6 Hz, 6H), 1.41 (s, 9H), 2.33–2.42 (m, 1H), 2.49 (s, 3H), 2.97–3.01 (m, 2H), 4.33 (s, 2H), 7.12 (d, *J* = 8.0 Hz, 2H), 7.25 (d, *J* = 3.0 Hz, 1H), 7.36 (d, *J* = 8.0 Hz, 2H), 7.52 (dd, *J* = 7.4, 3.0 Hz, 1H), 8.13 (d, *J* = 7.4 Hz, 1H).

***tert*-Butyl**
((6-((1*E*)-3-amino-3-oxoprop-1-en-1-yl)-2-isobutyl-4-(4-methylphenyl)quinolin-3-yl)methyl)carbamate (17).

i) Ethyl (2*E*)-3-(3-(((*tert*-butoxycarbonyl)amino)methyl)-2-isobutyl-4-(4-methylphenyl)-quinolin-6-yl)acrylate (17a). A mixture of **16** (2.0 g, 3.6 mmol), ethyl acrylate (0.65 mL, 6.0 mmol), bis(triphenylphosphine)palladium(II) (dichloride complex, 0.021 mg, 0.03 mmol), and triethylamine (4.2 mL, 30 mmol) in *N*,*N*-dimethylformamide (10 mL) was stirred at 70 °C for 20 h under a nitrogen atmosphere. The reaction mixture was partitioned between ethyl acetate and water, and the organic layer was washed with brine, dried over $MgSO_4$, and concentrated in vacuo. Purification by column chromatography (silica gel, eluting with a gradient of 0–25% ethyl acetate in hexanes) afforded the title compound **17a** (0.89 g, 50%) as a white powder. ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.03 (d, *J* = 6.6 Hz, 6H), 1.32 (t, *J* = 7.2 Hz, 3H), 1.42 (s, 9H), 2.38 (spt, *J* = 7.2 Hz, 1H), 2.50 (s, 3H), 2.97 (d, *J* = 7.2 Hz, 2H), 4.25 (q, *J* = 7.2 Hz, 2H), 4.31 (br s, 3H), 6.40 (d, *J* = 15.6 Hz, 1H), 7.12 (d, *J* = 7.9 Hz, 2H), 7.36 (d, *J* = 7.9 Hz, 2H), 7.39 (d, *J* = 1.7 Hz, 1H), 7.64 (d, *J* = 16.1 Hz, 1H), 7.85 (dd, *J* = 8.8, 1.9 Hz, 1H), 8.04 (d, *J* = 8.7 Hz, 1H).

ii) (2*E*)-3-(3-(((*tert*-Butoxycarbonyl)amino)methyl)-2-isobutyl-4-(4-methylphenyl)quinolin-6-yl)acrylic acid (17b). To a mixture of **17a** (0.62 g, 1.2 mmol), ethanol (5 mL), and tetrahydrofuran (5 mL) was added 1 M NaOH

(10 mL) at room temperature. The resulting mixture was stirred at 60 °C for 4 h. The reaction mixture was cooled to room temperature, neutralized with 1 M HCl, and extracted with ethyl acetate. The extract was washed with brine, dried over $MgSO_4$, and concentrated in vacuo. The residual solid was triturated with diisopropyl ether to afford the title compound **17b** (0.41 g, 70%) as a white powder. ^{1}H NMR (200 MHz, $CDCl_3$) δ: 1.13 (d, *J* = 6.0 Hz, 6H), 1.41 (s, 9H), 2.36–2.39 (m, 1H), 2.52 (s, 3H), 3.15 (s, 2H), 4.35 (s, 2H), 4.35 (br s, 1H), 6.43 (d, *J* = 15.6 Hz, 1H), 7.14 (d, *J* = 8.1 Hz, 2H), 7.26 (s, 1H), 7.28 (d, *J* = 8.1 Hz, 2H), 7.45 (s, 1H), 7.70 (d, *J* = 15.6 Hz, 1H), 7.93 (s, 1H).

iii) *tert*-Butyl ((6-((1*E*)-3-amino-3-oxoprop-1-en-1-yl)-2-isobutyl-4-(4-methylphenyl)quinolin-3-yl)methyl)-carbamate (17). By a procedure similar to that described for the synthesis of compound **15a**, the title compound **17** was obtained from **17b** as a white powder (0.25 g, 84%). ^{1}H NMR (300 MHz, $CDCl_3$) δ: 1.03 (d, *J* = 6.6 Hz, 6H), 1.41 (s, 9H), 2.35–2.39 (m, 1H), 2.50 (s, 3H), 2.97 (d, *J* = 7.2 Hz, 2H), 4.32 (s, 3H), 5.49 (br, 2H), 6.42 (d, *J* = 15.6 Hz, 1H), 7.11 (d, *J* = 3.1 Hz, 2H), 7.33–7.37 (m, 3H), 7.58 (d, *J* = 15.6 Hz, 1H), 7.82 (dd, *J* = 8.7, 2.1 Hz, 1H), 8.04 (d, *J* = 8.7 Hz, 1H).

3-(((*tert*-Butoxycarbonyl)amino)methyl)-2-isobutyl-4-(4-methylphenyl)quinoline-6-carboxylic acid (18).

i) Methyl 3-(((*tert*-butoxycarbonyl)amino)methyl)-2-isobutyl-4-(4-methylphenyl)quinoline-6-carboxylate (18a). By a procedure similar to that described for the synthesis of compound **40ba**, the title compound **18a** was obtained from **16** as a white powder (6.8 g, 67%); mp 192–194 °C. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 1.04 (d, *J* = 6.3 Hz, 6H), 1.42 (s, 9H), 2.34–2.37 (m, 1H), 2.49 (s, 3H), 2.99 (d, *J* = 7.2 Hz, 2H), 3.87 (s, 3H), 4.32 (br s, 3H), 7.13 (d, *J* = 7.8 Hz, 2H), 7.35 (d, *J* = 7.8 Hz, 2H), 8.06–8.10 (m, 2H), 8.23 (dd, *J* = 8.7, 2.1 Hz, 1H). Anal. Calcd for $C_{28}H_{34}N_2O_4$: C, 72.70; H, 7.41; N, 6.06. Found: C, 72.69; H, 7.69; N 5.84.

ii) 3-(((*tert*-Butoxycarbonyl)amino)methyl)-2-isobutyl-4-(4-methylphenyl)quinoline-6-carboxylic acid (18). A mixture of **18a** (6.5 g, 14 mmol), 1 M NaOH (28 mL), tetrahydrofuran (20 mL), and methanol (20 mL) was stirred at 60 °C for 1 h. The reaction mixture was poured into water, acidified with 1 M hydrochloric acid, and extracted with ethyl acetate. The extract was washed with brine, dried over $MgSO_4$, and concentrated in vacuo. Recrystallization of the residual solid from tetrahydrofuran–diisopropyl ether afforded the title compound **18** (6.0 g, 95%) as a white powder; mp 276–277 °C. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 1.05 (d, *J* = 6.6 Hz, 6H), 1.41 (s, 9H), 2.30–2.49 (m, 4H), 3.18 (br s, 2H), 4.35 (d, *J* = 5.1 Hz, 2H), 4.49 (br s, 1H), 7.15 (d, *J* = 7.5 Hz, 2H), 7.33–7.38 (m, 3H), 8.19 (s, 1H), 8.29–8.41 (m, 2H). Anal. Calcd for $C_{27}H_{32}N_2O_4 \cdot 0.5H_2O$: C, 70.87; H, 7.27; N, 6.12. Found: C, 70.54; H, 7.00; N 5.94.

3-(Aminomethyl)-2-isobutyl-4-(4-methylphenyl)quinoline-6-carboxamide dihydrochloride (19a).

***tert*-Butyl ((6-carbamoyl-2-isobutyl-4-(4-methylphenyl)quinolin-3-yl)methyl)carbamate (19aa).** By a procedure similar to that described for the synthesis of compound **15a**, the title compound **19aa** was obtained from **18** as a white powder (0.12 g, 55%); mp 206–208 °C. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 1.02 (d, *J* = 6.3 Hz, 6H), 1.41 (s, 9H), 2.29–2.48 (m, 1H), 2.52 (s, 3H), 3.49 (d, *J* = 6.6 Hz, 2H), 4.37 (d, *J* = 5.1 Hz, 2H), 5.26 (br s, 1H), 5.84 (br s, 1H), 7.23 (d, *J* = 7.8 Hz, 2H), 7.39 (d, *J* = 7.8 Hz, 2H), 8.08 (br s, 2H), 8.55 (d, *J* = 9.0 Hz, 1H), 8.79 (d, *J* = 9.0 Hz, 1H).

ii) 3-(Aminomethyl)-2-isobutyl-4-(4-methylphenyl)quinoline-6-carboxamide dihydrochloride (19a). A mixture of **19aa** (90 mg, 0.2 mmol) and 4 M HCl in 1,4-dioxane (5 mL) was stirred at room temperature for 1 h. The reaction mixture was concentrated in vacuo, and the residual solid was triturated with ethyl acetate–diisopropyl ether to afford the title compound **19a** as a white powder (70 mg, 88%); mp 194–196 °C. ^{1}H NMR (300 MHz, DMSO-d_6) δ: 1.03 (d, *J* = 6.3 Hz, 6H), 2.32–2.49 (m, 4H), 3.17 (br s, 2H), 3.99 (d, *J* = 6.3 Hz, 2H), 7.36 (d, *J* = 7.8 Hz, 2H), 7.47 (d, *J* = 7.8 Hz, 2H), 7.59 (br s, 1H), 7.89 (s, 1H), 8.21–8.33 (m, 3H), 8.42 (br s, 3H). HRMS (ESI) calcd for $C_{22}H_{25}N_3O$ $(M + H)^+$ *m/z* 348.2070, found *m/z* 348.2044. HPLC purity: 98.61% (λ 220 nm), 98.20% (λ 254 nm).

3-(Aminomethyl)-2-isobutyl-4-(4-methylphenyl)quinoline-6-carboxylic acid dihydrochloride (19b). By a procedure similar to that described for the synthesis of compound **19a**, the title compound **19b** was obtained from **18** as a white powder (0.20 g, 95%); mp 284–286 °C. ^{1}H NMR (300 MHz, DMSO-d_6) δ: 1.04 (d, *J* = 6.3 Hz, 6H), 2.28–2.44 (m, 1H), 2.49 (s, 3H), 3.27 (d, *J* = 6.9 Hz, 2H), 4.02 (d, *J* = 5.4 Hz, 2H), 7.41 (d, *J* = 8.0 Hz, 2H), 7.49 (d, *J* = 8.0 Hz, 2H), 8.00 (d, *J* = 1.5 Hz, 1H), 8.34–8.45 (m, 2H), 8.62 (br s, 3H). HRMS (ESI) calcd for $C_{22}H_{24}N_2O_2$ $(M + H)^+$ *m/z* 349.1911, found *m/z* 349.1876; calcd for $C_{22}H_{24}N_2O_2$ $(M - H)^-$ *m/z* 347.1765, found *m/z* 347.1771. Anal. Calcd for $C_{22}H_{24}N_2O_2 \cdot 2HCl \cdot 0.45H_2O$: C, 61.53; H, 6.31; N, 6.52. Found: C, 61.89; H, 6.48; N 6.14. HPLC purity: 99.74% (λ 220 nm), 99.57% (λ 254 nm).

***tert*-Butyl ((6-cyano-2-isobutyl-4-(4-methylphenyl)quinolin-3-yl)methyl)carbamate (20).** By a procedure similar to that described for the synthesis of compound **43ba**, the title compound **20** was obtained from **16** as a white powder (2.18 g, 73%). ^{1}H NMR (300 MHz, $CDCl_3$) δ: 1.03 (d, *J* = 6.6 Hz, 6H), 1.42 (s, 9H), 2.34–2.47 (m, 1H), 2.50 (s, 3H), 3.00 (d, *J* = 7.2 Hz, 2H), 4.34 (br s, 3H), 7.11 (d, *J* = 7.8 Hz, 2H), 7.37 (d, *J* = 7.8 Hz, 2H), 7.72 (d, *J* = 1.8 Hz, 1H), 7.77 (dd, *J* = 8.7, 1.8 Hz, 1H), 8.12 (d, *J* = 8.7 Hz, 1H). Anal. Calcd for $C_{27}H_{31}N_3O_2 \cdot 0.1H_2O$: C, 75.18; H, 7.29; N, 9.74. Found: C, 74.96; H, 7.45; N 9.53.

1-(2-Isobutyl-4-(4-methylphenyl)-6-(2*H*-tetrazol-5-yl)quinolin-3-yl)methanamine dihydrochloride (21a).

i) *tert*-Butyl ((2-isobutyl-4-(4-methylphenyl)-6-(2*H*-tetrazol-5-yl)quinolin-3-yl)methyl)carbamate (21aa). A mixture of **20** (0.60 g, 1.4 mmol), NaN_3 (0.18 g, 2.8 mmol), and NH_4Cl (0.30 g, 5.6 mmol) in dimethylsulfoxide

(10 mL) was stirred at 70 °C for 48 h. The reaction mixture was partitioned between ethyl acetate and 0.1 M HCl. The organic layer was washed with brine, dried over $MgSO_4$, and concentrated in vacuo. The residue was crystallized from ethyl acetate–hexanes to afford the title compound **21aa** (0.38 g, 57%) as a white powder. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 1.00 (d, *J* = 6.4 Hz, 6H), 1.39 (s, 9H), 2.25–2.45 (m, 1H), 2.47 (s, 3H), 2.86 (d, *J* = 7.0 Hz, 2H), 4.07 (d, *J* = 4.3 Hz, 2H), 7.09 (t, *J* = 4.3 Hz, 1H), 7.35 (d, *J* = 8.1 Hz, 2H), 7.41 (d, *J* = 8.1 Hz, 2H), 8.05 (d, *J* = 1.8 Hz, 1H), 8.18 (d, *J* = 8.7 Hz, 1H), 8.28 (dd, *J* = 8.7, 1.8 Hz, 1H).

ii) 1-(2-Isobutyl-4-(4-methylphenyl)-6-(2*H*-tetrazol-5-yl)quinolin-3-yl)methanamine dihydrochloride (21a). By a procedure similar to that described for the synthesis of compound **19a**, the title compound **21a** was obtained from **21aa** as a pale yellow powder (0.14 g, 89%). ^{1}H NMR (300 MHz, DMSO-d_6) δ: 1.05 (d, *J* = 6.8 Hz, 6H), 2.30–2.50 (m, 1H), 2.49 (s, 3H), 3.08 (d, *J* = 7.0 Hz, 2H), 4.00 (d, *J* = 5.5 Hz, 2H), 7.38 (d, *J* = 7.8 Hz, 2H), 7.49 (d, *J* = 7.8 Hz, 2H), 8.08 (d, *J* = 1.5 Hz, 1H), 8.29 (d, *J* = 8.8 Hz, 1H), 8.33 (br s, 3H), 8.43 (dd, *J* = 8.8, 1.5 Hz, 1H). LC–MS (ESI) *m*/*z*: 373 $(M + H)^+$; HRMS (ESI) calcd for $C_{22}H_{24}N_6$ $(M + H)^+$ *m*/*z* 373.2135, found *m*/*z* 373.2115. HPLC purity: 99.44% (λ 220 nm), 99.42% (λ 254 nm).

3-(3-(Aminomethyl)-2-isobutyl-4-(4-methylphenyl)quinolin-6-yl)-1,2,4-oxadiazol-5(4*H*)-one dihydrochloride (21b).

i) *tert*-Butyl ((6-(*N*-hydroxycarbamimidoyl)-2-isobutyl-4-(4-methylphenyl)quinolin-3-yl)methyl)carbamate (21ba). To a suspension of **20** (3.0 g, 7.0 mmol) and hydroxylamine hydrochloride (0.73 g, 11 mmol) in ethanol (75 mL) was added potassium *tert*-butoxide (1.18 g, 11 mmol). The resulting mixture was stirred at 70 °C for 6 h, cooled to room temperature, and filtered. The filtrate was concentrated in vacuo, and the residue was partitioned between ethyl acetate and water. The organic layer was washed with brine, dried over $MgSO_4$, and concentrated in vacuo. The residual solid was triturated with dichloromethane–ethyl acetate to afford the title compound **21ba** (2.38 g, 74%) as an off-white powder; mp 224–225 °C. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 1.03 (d, *J* = 6.6 Hz, 6H), 1.43 (s, 9H), 2.25–2.45 (m, 1H), 2.47 (s, 3H), 2.96 (d, *J* = 7.2 Hz, 2H), 4.29 (d, *J* = 4.7 Hz, 2H), 4.49 (t, *J* = 4.7 Hz, 1H), 4.75 (br s, 2H), 7.12 (d, *J* = 7.5 Hz, 2H), 7.33 (d, *J* = 7.7 Hz, 2H), 7.40–7.50 (m, 1H), 7.80–7.95 (m, 1H), 8.01 (d, *J* = 8.7 Hz, 1H). LC–MS (ESI) *m*/*z*: 463 $(M + H)^+$.

ii) *tert*-Butyl ((2-isobutyl-4-(4-methylphenyl)-6-(5-oxo-4,5-dihydro-1,2,4-oxadiazol-3-yl)quinolin-3-yl)-methyl)carbamate (21bb). To a mixture of **21ba** (0.25 g, 0.54 mmol), tetrahydrofuran (10 mL), and ethyl acetate (10 mL) was added 1,1'-carbonyldiimidazole (0.26 g, 1.62 mmol), and the resulting mixture was stirred at reflux for 3 h. The reaction mixture was poured into 0.1 M aqueous citric acid solution and extracted with ethyl acetate–tetrahydrofuran (2:1, v/v). The extract was washed with brine, dried over $MgSO_4$, and concentrated in vacuo. The residual solid was crystallized from ethyl acetate–hexanes to afford the title compound **21bb** (0.24 g, 90%) as a white powder. ^{1}H NMR (300 MHz, DMSO-d_6) δ: 0.99 (d, *J* = 6.6 Hz, 6H), 1.38 (s, 9H), 2.30–2.50 (m, 1H), 2.45 (s,

3H), 2.85 (d, *J* = 6.8 Hz, 2H), 4.05 (d, *J* = 4.2 Hz, 2H), 7.08 (t, *J* = 4.2 Hz, 1H), 7.31 (d, *J* = 8.0 Hz, 2H), 7.38 (d, *J* = 8.0 Hz, 2H), 7.81 (d, *J* = 1.9 Hz, 1H), 8.03 (dd, *J* = 8.8, 1.9 Hz, 1H), 8.14 (d, *J* = 8.8 Hz, 1H), 13.21 (br s, 1H).

iii) 3-(3-(Aminomethyl)-2-isobutyl-4-(4-methylphenyl)quinolin-6-yl)-1,2,4-oxadiazol-5(4*H*)-one dihydrochloride (21b). By a procedure similar to that described for the synthesis of compound **19a**, the title compound **21b** was obtained from **21bb** as pale yellow crystals (0.14 g, 74%). ^{1}H NMR (300 MHz, DMSO-d_6) δ: 1.04 (d, *J* = 6.6 Hz, 6H), 2.30–2.45 (m, 1H), 2.49 (s, 3H), 3.08 (d, *J* = 7.2 Hz, 2H), 3.97 (d, *J* = 5.3 Hz, 2H), 7.34 (d, *J* = 7.9 Hz, 2H), 7.46 (d, *J* = 7.9 Hz, 2H), 7.83 (d, *J* = 1.8 Hz, 1H), 8.16 (dd, *J* = 8.9, 1.9 Hz, 1H), 8.25 (d, *J* = 8.9 Hz, 1H), 8.36 (br s, 3H), 13.33 (br s, 1H). HRMS (ESI) calcd for $C_{23}H_{24}N_4O_2$ $(M + H)^+$ *m/z* 389.1972, found *m/z* 389.1956. HPLC purity: 99.96% (λ 220 nm), 99.92% (λ 254 nm).

Methyl 5-(3-(((*tert*-butoxycarbonyl)amino)methyl)-2-isobutyl-4-(4-methylphenyl)quinolin-6-yl)-2-furoate (23a). A mixture of compound **16** (5.5 g, 10 mmol), bis(pinacolato)diboron (5.1 g, 20 mmol), (1,1′-Bis(diphenylphosphino)ferrocene)dichloropalladium(II) (dichloromethane complex, 0.22 g, 0.30 mmol), and potassium acetate (3.0 g, 30 mmol) in dimethylsulfoxide (50 mL) was stirred at 40 °C for 2 h under an argon atmosphere. The reaction mixture was cooled and partitioned between ethyl acetate and water. The organic layer was washed with brine, dried over $MgSO_4$, and concentrated in vacuo. The residue was purified by column chromatography (silica gel, eluting with a gradient of 5–25% ethyl acetate in hexanes) to afford 2.8 g of white solid, which was dissolved in toluene–methanol–water (8:1:1, v/v, 50 mL). To the solution were added tetrakis(triphenylphosphine)palladium(0) (0.62 g, 0.54 mmol), K_2CO_3 (4.1 g, 30 mmol), and methyl 5-bromo-2-furoate (**22a**, 3.3 g, 16 mmol). The resulting mixture was stirred at 100 °C for 17 h under an argon atmosphere. The reaction mixture was cooled and partitioned between water and ethyl acetate. The organic layer was washed with brine, dried over $MgSO_4$, and concentrated in vacuo. The residue was purified by column chromatography (silica gel, eluting with a gradient of 20–100% ethyl acetate in hexanes) followed by preparative HPLC to afford the title compound **23a** (1.4 g, 27%) as a white powder. ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.04 (d, *J* = 6.6 Hz, 6H), 1.42 (s, 9H), 2.36–2.39 (m, 1H), 2.50 (s, 3H), 3.00 (d, *J* = 6.9 Hz, 2H), 3.89 (s, 3H), 4.32 (s, 3H), 6.66 (d, *J* = 3.3 Hz, 1H), 7.16 (d, *J* = 7.5 Hz, 2H), 7.20 (d, *J* = 3.3 Hz, 1H), 7.36 (d, *J* = 7.5 Hz, 2H), 7.68 (s, 1H), 8.09 (d, *J* = 1.2 Hz, 2H). LC–MS (ESI) *m*/*z*: 529 $(M + H)^+$.

Ethyl 2-(3-(((*tert*-butoxycarbonyl)amino)methyl)-2-isobutyl-4-(4-methylphenyl)quinolin-6-yl)-1,3-thiazole-4-carboxylate (23b). A mixture of compound **16** (0.50 g, 0.90 mmol), bis(pinacolato)diboron (0.25 g, 1.0 mmol), and potassium acetate (0.27 g, 2.7 mmol) in dimethylsulfoxide (20 mL) was stirred at 80 °C for 10 min under an argon atmosphere. To the resulting solution was added (1,1′-Bis(diphenyl-phosphino)ferrocene)dichloropalladium(II) (dichloromethane complex, 20 mg, 0.027 mmol). After stirring at 100 °C for 15 min under an argon atmosphere, the reaction mixture was cooled and partitioned between ethyl acetate and water. The organic layer was washed with brine, dried over $MgSO_4$, and concentrated in vacuo. The residue was dissolved in toluene–methanol–water

(5:1:1, v/v, 28 mL). To the solution were added ethyl 2-chloro-1,3-thiazole-4-carboxylate (**22b**, 0.17 g, 0.90 mmol), K_2CO_3 (0.63 g, 4.5 mmol), and tetrakis(triphenylphosphine)palladium(0) (0.10 g, 0.090 mmol), and the resulting mixture was stirred at reflux for 17 h under an argon atmosphere. The reaction mixture was cooled and partitioned between water and ethyl acetate. The organic layer was washed with brine, dried over $MgSO_4$, and concentrated in vacuo. The residue was purified by column chromatography (silica gel, eluting with a gradient of 10–30% ethyl acetate in hexanes) followed by preparative HPLC to afford the title compound **23b** (0.28 g, 56%) as a white powder; mp 193–194 °C. ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.04 (d, *J* = 6.6 Hz, 6H), 1.42 (t, *J* = 7.0 Hz, 3H), 1.42 (s, 9H), 2.34–2.49 (m, 4H), 2.89 (d, *J* = 7.2 Hz, 2H), 4.28–4.35 (m, 3H), 4.42 (q, *J* = 7.0 Hz, 2H), 7.16 (d, *J* = 7.8 Hz, 2H), 7.35 (d, *J* = 7.8 Hz, 2H), 7.86 (d, *J* = 1.8 Hz, 1H), 8.11 (d, *J* = 9.3 Hz, 1H), 8.35 (dd, *J* = 9.3, 1.8 Hz, 1H). LC–MS (ESI) *m*/*z*: 560 $(M + H)^+$.

5-(3-(((*tert*-Butoxycarbonyl)amino)methyl)-2-isobutyl-4-(4-methylphenyl)quinolin-6-yl)-2-furoic acid (24a). A mixture of compound **23a** (1.2 g, 2.3 mmol), 1 M NaOH (10 mL), and methanol (10 mL) was stirred at room temperature for 22 h. The reaction mixture was acidified with 1 M HCl and extracted with ethyl acetate. The extract was washed with brine, dried over $MgSO_4$, and concentrated in vacuo. The residual solid was triturated with diisopropyl ether to afford the title compound **24a** (1.1 g, 98%) as a pale yellow powder. ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.09 (d, *J* = 6.3 Hz, 6H), 1.42 (s, 9H), 2.22–2.26 (m, 1H), 2.52 (s, 3H), 3.32 (s, 2H), 4.35 (s, 2H), 4.82 (br s, 1H), 6.68 (s, 1H), 7.20–7.23 (m, 3H), 7.41–7.43 (m, 2H), 7.67 (s, 1H), 8.12–8.13 (m, 1H), 8.55–8.58 (m, 1H). LC–MS (ESI) *m*/*z*: 515 $(M + H)^+$.

2-(3-(((*tert*-Butoxycarbonyl)amino)methyl)-2-isobutyl-4-(4-methylphenyl)quinolin-6-yl)-1,3-thiazole-4-carboxylic acid (24b). A mixture of compound **23b** (0.21 g, 0.36 mmol), 1 M NaOH (4 mL), tetrahydrofuran (8 mL), and methanol (8 mL) was stirred at room temperature for 15 min. The reaction mixture was acidified with 1 M HCl and extracted with ethyl acetate. The extract was washed with brine, dried over $MgSO_4$, and concentrated in vacuo. The residual solid was triturated with diisopropyl ether to afford the title compound **24b** (0.18 g, 90%) as a pale yellow solid. ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.05 (d, *J* = 6.6 Hz, 6H), 1.43 (s, 9H), 2.25–2.50 (m, 1H), 2.50 (s, 3H), 3.03 (d, *J* = 7.2 Hz, 2H), 4.33 (d, *J* = 4.4 Hz, 2H), 4.41 (br s, 1H), 7.18 (d, *J* = 7.7 Hz, 2H), 7.37 (d, *J* = 7.7 Hz, 2H), 7.80–7.95 (m, 1H), 8.10–8.25 (m, 1H), 8.20 (s, 1H), 8.32 (dd, *J* = 9.1, 2.2 Hz, 1H). LC–MS (ESI) *m*/*z*: 532 $(M + H)^+$.

5-(3-(Aminomethyl)-2-isobutyl-4-(4-methylphenyl)quinolin-6-yl)-2-furoic acid dihydrochloride (25a). By a procedure similar to that described for the synthesis of compound **19a**, the title compound **25a** was obtained from **24a** as a yellow powder (133 mg, 93%). ^{1}H NMR (300 MHz, CD_3OD): δ 1.16 (d, *J* = 6.5 Hz, 6H), 2.34 (spt, *J* = 6.5 Hz, 1H), 2.57 (s, 3H), 3.32 (d, *J* = 6.7 Hz, 2H), 4.34 (s, 2H), 7.14 (d, *J* = 3.6 Hz, 1H), 7.31 (d, *J* = 3.8 Hz, 1H), 7.44 (d, *J* = 7.4 Hz, 2H), 7.62 (d, *J* = 3.8 Hz, 2H), 7.97 (s, 1H), 8.41 (d, *J* = 9.0 Hz, 1H), 8.60 (d, *J* = 9.0 Hz, 1H). LC–MS (ESI) *m*/*z*: 415 $(M + H)^+$. Anal. Calcd for $C_{26}H_{26}N_2O_3 \cdot 2HCl \cdot 3.5H_2O$: C, 56.73; H, 6.41; N, 5.09. Found: C,

56.98; H, 6.22; N 4.98.

2-(3-(Aminomethyl)-2-isobutyl-4-(4-methylphenyl)quinolin-6-yl)-1,3-thiazole-4-carboxylic acid dihydrochloride (25b). By a procedure similar to that described for the synthesis of compound **19a**, the title compound **25b** was obtained from **24b** as a pale yellow powder (0.13 g, 98%); mp 266–269 °C. ^{1}H NMR (300 MHz, CD_3OD): δ 1.16 (d, *J* = 6.6 Hz, 6H), 2.25–2.42 (m, 1H), 2.56 (s, 3H), 3.28 (d, *J* = 7.0 Hz, 2H), 4.33 (s, 2H), 7.44 (d, *J* = 8.1 Hz, 2H), 7.60 (d, *J* = 8.1 Hz, 2H), 8.15 (d, *J* = 1.9 Hz, 1H), 8.41 (d, *J* = 8.9 Hz, 1H), 8.46 (s, 1H), 8.76 (dd, *J* = 8.9, 1.9 Hz, 1H). LC–MS (ESI) *m*/*z*: 432 $(M + H)^+$. Anal. Calcd for $C_{25}H_{25}N_3O_2S{\cdot}2HCl{\cdot}H_2O$: C, 57.47; H, 5.59; N, 8.04. Found: C, 57.36; H, 5.88; N 7.76.

***tert*-Butyl ((6-(4-carbamoyl-1,3-thiazol-2-yl)-2-isobutyl-4-(4-methylphenyl)quinolin-3-yl)-methyl)carbamate (26a).** A mixture of **24b** (0.20 g, 0.38 mmol), ammonium salt of 1-hydroxy-1*H*-benzotriazole (0.086 g, 0.56 mmol), and 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (0.11 g, 0.56 mmol) in *N*,*N*-dimethylformamide (5 mL) was stirred at room temperature for 3 h. The reaction mixture was partitioned between ethyl acetate and water. The organic layer was washed with brine, dried over $MgSO_4$, and concentrated in vacuo. The residue was purified by column chromatography (silica gel, eluting with a gradient of 50–80% ethyl acetate in hexanes) to afford the title compound **26a** (0.14 g, 68%) as colorless crystals; mp 256–258 °C. ^{1}H NMR (300 MHz, DMSO-d_6) δ 1.00 (d, *J* = 6.6 Hz, 6H), 1.39 (s, 9H), 2.30–2.55 (m, 1H), 2.46 (s, 3H), 2.85 (d, *J* = 6.6 Hz, 2H), 4.08 (d, *J* = 4.5 Hz, 2H), 7.17 (t, *J* = 4.5 Hz, 1H), 7.30–7.45 (m, 4H), 7.67 (br s, 1H), 7.76 (d, *J* = 2.2 Hz, 1H), 7.84 (br s, 1H), 8.12 (d, *J* = 8.8 Hz, 1H), 8.23 (s, 1H), 8.42 (dd, *J* = 8.8, 2.2 Hz, 1H). LC–MS (ESI) *m*/*z*: 531 $(M + H)^+$.

***tert*-Butyl ((6-(4-acetyl-1,3-thiazol-2-yl)-2-isobutyl-4-(4-methylphenyl)quinolin-3-yl)methyl)carbamate (26b).**

i) *tert*-Butyl ((2-isobutyl-6-(4-(methoxy(methyl)carbamoyl)-1,3-thiazol-2-yl)-4-(4-methylphenyl)quinolin-3-yl)methyl)carbamate (26ba). A mixture of **24b** (0.27 g, 0.51 mmol), *N*,*O*-dimethylhydroxylamine hydrochloride (0.060 g, 0.62 mmol), triethylamine (80 μL, 0.57 mmol), 1-hydroxy-1*H*-benzotriazole monohydrate (0.098 g, 0.59 mmol), and 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (0.12 g, 0.63 mmol) in *N*,*N*-dimethylformamide (10 mL) was stirred at room temperature for 2 h. The reaction mixture was poured into water and extracted with ethyl acetate. The extract was washed with brine, dried over $MgSO_4$, and concentrated in vacuo. The residue was crystallized from diisopropyl ether–ethyl acetate to afford the title compound **26ba** (0.24 g, 86%) as a white powder. ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.05 (d, *J* = 6.8 Hz, 6H), 1.42 (s, 9H), 2.41 (spt, *J* = 6.6 Hz, 1H), 2.49 (s, 3H), 2.99 (d, *J* = 7.2 Hz, 2H), 3.41 (s, 3H), 3.72 (s, 3H), 4.30–4.37 (m, 3H), 7.17 (d, *J* = 7.4 Hz, 2H), 7.34 (d, *J* = 7.5 Hz, 2H), 7.98 (d, *J* = 1.5 Hz, 1H), 8.00 (s, 1H), 8.12 (d, *J* = 9.0 Hz, 1H), 8.19 (dd, *J* = 9.0, 2.3 Hz, 1H). LC–MS (ESI) *m*/*z*: 575 $(M + H)^+$.

ii) *tert*-Butyl ((6-(4-acetyl-1,3-thiazol-2-yl)-2-isobutyl-4-(4-methylphenyl)quinolin-3-yl)methyl)carbamate

(26b). To a suspension of **26ba** (0.23 g, 0.40 mmol) in tetrahydrofuran (10 mL) was added 3 M methylmagnesium bromide in diethyl ether (400 μL, 1.2 mmol) at 0 °C. After stirring at 0 °C for 1 h, the reaction was quenched with saturated NH_4Cl. The mixture was partitioned between ethyl acetate and water. The organic layer was washed with brine, dried over $MgSO_4$, and concentrated in vacuo. The residue was purified by column chromatography (silica gel, eluting with a gradient of 5–25% ethyl acetate in hexanes) to afford the title compound **26b** (0.20 g, 95%) as a white powder. ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.05 (d, *J* = 6.6 Hz, 6H), 1.43 (s, 9H), 2.41 (spt, *J* = 6.7 Hz, 1H), 2.50 (s, 3H), 2.70 (s, 3H), 3.00 (d, *J* = 7.2 Hz, 2H), 4.30–4.38 (m, 3H), 7.18 (d, *J* = 8.1 Hz, 2H), 7.38 (d, *J* = 7.8 Hz, 2H), 7.86 (d, *J* = 1.9 Hz, 1H), 8.07 (s, 1H), 8.15 (d, *J* = 8.7 Hz, 1H), 8.34 (dd, *J* = 8.8, 1.9 Hz, 1H). Anal. Calcd for $C_{31}H_{35}N_3O_3S$: C, 70.29; H, 6.66; N, 7.93. Found: C, 70.05; H, 6.51; N, 8.03.

2-(3-(Aminomethyl)-2-isobutyl-4-(4-methylphenyl)quinolin-6-yl)-1,3-thiazole-4-carboxamide dihydrochloride (27a). By a procedure similar to that described for the synthesis of compound **19a**, the title compound **27a** was obtained from compound **26a** as pale yellow crystals (0.10 g, 89%); mp 212–215 °C. ^{1}H NMR (300 MHz, DMSO-d_6): δ 1.06 (d, *J* = 6.6 Hz, 6H), 2.30–2.40 (m, 1H), 2.43 (s, 3H), 3.04 (br s, 2H), 4.00 (d, *J* = 7.0 Hz, 2H), 7.35–7.45 (m, 2H), 7.49 (d, *J* = 8.2 Hz, 2H), 7.68 (br s, 1H), 7.75–7.80 (m, 1H), 7.85 (br s, 1H), 8.15–8.25 (m, 1H), 8.26 (s, 1H), 8.35 (br s, 3H), 8.45–8.60 (m, 1H). LC–MS (ESI) *m*/*z*: 431 $(M + H)^+$. Anal. Calcd for $C_{25}H_{26}N_4OS{\cdot}2HCl{\cdot}2H_2O$: C, 55.65; H, 5.98; N, 10.38. Found: C, 55.59; H, 6.17; N 10.20.

1-(2-(3-(Aminomethyl)-2-isobutyl-4-(4-methylphenyl)quinolin-6-yl)-1,3-thiazol-4-yl)ethanone dihydrochloride (27b). By a procedure similar to that described for the synthesis of compound **19a**, the title compound **27b** was obtained from compound **26b** as a pale yellow powder (0.14 g, 93%). ^{1}H NMR (300 MHz, DMSO-d_6) δ 1.05 (d, *J* = 6.6 Hz, 6H), 2.40 (spt, *J* = 6.8 Hz, 1H), 2.49 (br s, 3H), 2.57 (s, 3H), 3.15 (d, *J* = 6.4 Hz, 2H), 4.01 (d, *J* = 5.3 Hz, 2H), 7.42 (d, *J* = 8.1 Hz, 2H), 7.49 (d, *J* = 7.9 Hz, 2H), 7.89 (d, *J* = 1.7 Hz, 1H), 8.33 (d, *J* = 9.0 Hz, 1H), 8.45 (d, *J* = 9.4 Hz, 1H), 8.45–8.54 (m, 3H), 8.58 (s, 1H). LC–MS (ESI) *m*/*z*: 430 $(M + H)^+$. Anal. Calcd for $C_{26}H_{27}N_3OS{\cdot}2HCl{\cdot}0.5H_2O$: C, 61.05; H, 5.91; N, 8.21. Found: C, 60.92; H, 5.99; N, 7.83.

2-Isobutyl-4-(4-methylphenyl)-6-(3-oxopiperazin-1-yl)quinoline-3-carbonitrile (28). A mixture of palladium(II) acetate (0.014 g, 0.063 mmol) and (±)-2,2'-bis(diphenylphosphino)-1,1'-binaphthalene (0.12 g, 0.19 mmol) in 1,4-dioxane (30 mL) was stirred at 40 °C for 30 min under a nitrogen atmosphere. To the mixture were added **7b** (0.50 g, 1.25 mmol), piperazin-2-one (0.25 g, 2.5 mmol), and Cs_2CO_3 (0.57 g, 1.8 mmol), and the resulting mixture was stirred at 80 °C for 24 h under a nitrogen atmosphere. The reaction mixture was cooled to room temperature and filtered through Celite. The filtrate was washed sequentially with water and brine, dried over $MgSO_4$, and concentrated in vacuo. Purification by column chromatography (silica gel, eluting with a gradient of 0–5% methanol in ethyl acetate) afforded the title compound **28** (0.35 g, 71%) as a yellow powder; mp 237–238 °C. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 1.04 (d, *J* = 6.6 Hz, 6H), 2.25–2.45 (m, 1H), 2.50 (s, 3H), 3.07 (d, *J* = 7.4 Hz, 2H), 3.45–3.60 (m, 4H), 3.81 (s, 2H), 6.22 (s, 1H), 6.83 (d, *J* = 2.8 Hz, 1H), 7.30–7.45 (m, 4H), 7.53 (dd, *J* = 9.4, 2.7 Hz,

1H), 8.04 (d, *J* = 9.4 Hz, 1H). LC–MS (ESI) *m/z*: 399 $(M + H)^+$.

4-(3-(Aminomethyl)-2-isobutyl-4-(4-methylphenyl)quinolin-6-yl)piperazin-2-one (29a). By a procedure similar to that described for the synthesis of compound **9g**, the title compound **29a** was obtained from **28** as a pale yellow powder (0.18 g, 56%); mp 155–157 °C. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 1.03 (d, *J* = 6.6 Hz, 6H), 1.55 (br s, 2H), 2.25–2.40 (m, 1H), 2.49 (s, 3H), 2.97 (d, *J* = 7.2 Hz, 2H), 3.35–3.45 (m, 2H), 3.45–3.55 (m, 2H), 3.72 (s, 2H), 3.75 (s, 2H), 6.21 (d, *J* = 10.9 Hz, 1H), 6.50 (d, *J* = 2.8 Hz, 1H), 7.15 (d, *J* = 7.8 Hz, 2H), 7.33 (d, *J* = 7.8 Hz, 2H), 7.37 (dd, *J* = 9.2, 2.8 Hz, 1H), 7.99 (d, *J* = 9.2 Hz, 1H). LC–MS (ESI) *m/z*: 403 $(M + H)^+$; HRMS (ESI) calcd for $C_{25}H_{30}N_4O$ $(M + H)^+$ *m/z* 403.2492, found *m/z* 403.2478. HPLC purity: 97.72% (λ 220 nm), 95.11% (λ 254 nm).

1-(3-(Aminomethyl)-2-isobutyl-4-(4-methylphenyl)quinolin-6-yl)piperazine-2,3-dione (29b). A mixture of **31** (0.50 g, 0.75 mmol) and trifluoroacetic acid (3 mL) was stirred at room temperature for 30 min. The reaction mixture was concentrated in vacuo, and the residue was partitioned between ethyl acetate and 10% K_2CO_3. The organic layer was dried over anhydrous K_2CO_3, and concentrated in vacuo. Purification by column chromatography (silica gel, eluting with a gradient of 0–20% methanol in ethyl acetate) afforded the title compound **29b** (0.21 g, 65%) as a pale yellow powder; mp 229–232 °C. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 1.02 (d, *J* = 6.6 Hz, 6H), 1.66 (br s, 2H), 2.30–2.45 (m, 1H), 2.46 (s, 3H), 3.01 (d, *J* = 7.2 Hz, 2H), 3.55–3.65 (m, 2H), 3.80 (s, 2H), 3.85–3.90 (m, 2H), 7.16 (d, *J* = 8.0 Hz, 2H), 7.18 (d, *J* = 2.3 Hz, 1H), 7.32 (d, *J* = 8.0 Hz, 2H), 7.57 (dd, *J* = 9.0, 2.3 Hz, 1H), 8.06 (d, *J* = 9.0 Hz, 1H), 8.08 (br s, 1H). LC–MS (ESI) *m/z*: 417 $(M + H)^+$; HRMS (ESI) calcd for $C_{25}H_{28}N_4O_2$ $(M + H)^+$ *m/z* 417.2285, found *m/z* 417.2271. HPLC purity: 99.67% (λ 220 nm), 99.42% (λ 254 nm).

1-(3-(Aminomethyl)-2-isobutyl-4-(4-methylphenyl)quinolin-6-yl)piperazine-2,5-dione dihydrochloride (29c).

i) *tert*-Butyl ((6-(2,5-dioxopiperazin-1-yl)-2-isobutyl-4-(4-methylphenyl)quinolin-3-yl)methyl)carbamate (29ca). A mixture of **33** (0.45 g, 0.62 mmol), 10% palladium on charcoal (0.04 g), ethanol (100 mL) was stirred at room temperature for 24 h under a hydrogen atmosphere. The reaction mixture was filtered through Celite, and the filtrate was concentrated in vacuo. The residue was dissolved in ethanol (10 mL), and the resulting solution was stirred at reflux for 17 h. The reaction mixture was concentrated in vacuo, and the residue was purified by column chromatography (silica gel, eluting with a gradient of 0–10% methanol in ethyl acetate) followed by crystallization from ethyl acetate to afford the title compound **29ca** (0.16 g, 44%) as pale yellow crystals; mp 128–129 °C. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 1.02 (d, *J* = 6.6 Hz, 6H), 1.42 (s, 9H), 2.25–2.45 (m, 1H), 2.48 (s, 3H), 2.98 (d, *J* = 7.2 Hz, 2H), 4.16 (s, 2H), 4.28 (s, 2H), 4.30 (br s, 3H), 6.13 (s, 1H), 7.12 (d, *J* = 7.8 Hz, 2H), 7.14 (d, *J* = 2.3 Hz, 1H), 7.34 (d, *J* = 7.8 Hz, 2H), 7.62 (dd, *J* = 9.0, 2.3 Hz, 1H), 8.11 (d, *J* = 9.0 Hz, 1H). LC–MS (ESI) *m/z*: 517 $(M + H)^+$.

ii) 1-(3-(Aminomethyl)-2-isobutyl-4-(4-methylphenyl)quinolin-6-yl)piperazine-2,5-dione dihydrochloride (29c). By a procedure similar to that described for the synthesis of compound **19a**, the title compound **29c** was

obtained from **29ca** as a pale yellow powder (0.11 g, 83%); mp 250 °C (decomp). ^{1}H NMR (300 MHz, DMSO-d_6) δ: 1.03 (d, *J* = 6.6 Hz, 6H), 2.25–2.40 (m, 1H), 2.46 (s, 3H), 3.13 (d, *J* = 7.0 Hz, 2H), 3.92 (s, 2H), 3.98 (d, *J* = 5.5 Hz, 2H), 4.21 (s, 2H), 7.23 (d, *J* = 1.9 Hz, 1H), 7.34 (d, *J* = 7.9 Hz, 2H), 7.45 (d, *J* = 7.9 Hz, 2H), 7.88 (d, *J* = 8.8 Hz, 1H), 8.24 (dd, *J* = 8.8, 1.9 Hz, 1H), 8.30 (brs, 1H), 8.35 (brs, 3H). LC–MS (ESI) *m/z*: 417 $(M + H)^+$; HRMS (ESI) calcd for $C_{25}H_{28}N_4O_2$ $(M + H)^+$ *m/z* 417.2285, found *m/z* 417.2272. Anal. Calcd for $C_{25}H_{28}N_4O_2 \cdot 2HCl$: C, 61.35; H, 6.18; N, 11.45; Cl, 14.49. Found: C, 61.11; H, 6.22; N, 11.33; Cl, 14.44. HPLC purity: 99.90% (λ 220 nm), 99.93% (λ 254 nm).

***tert*-Butyl ((6-((2-((*tert*-butoxycarbonyl)amino)ethyl)amino)-2-isobutyl-4-(4-methyl-phenyl)quinolin-3-yl)-methyl)carbamate (30).**

i) *tert*-Butyl (2-((3-cyano-2-isobutyl-4-(4-methylphenyl)quinolin-6-yl)amino)ethyl)carbamate (30a). A mixture of palladium(II) acetate (0.030 g, 0.13 mmol) and (±)-2,2'-bis(diphenylphosphino)-1,1'-binaphthalene (0.246 g, 0.40 mmol) in toluene (20 mL) was stirred at 40 °C for 30 min under a nitrogen atmosphere. To the mixture were added **7b** (1.00 g, 2.64 mmol), sodium *tert*-butoxide (0.35 g, 3.7 mmol), and a solution of ethylenediamine (0.44 mL, 6.6 mmol) in toluene (2 mL). The resulting mixture was stirred at 80 °C for 2 h under a nitrogen atmosphere. The reaction mixture was cooled to room temperature and filtered through Celite. The filtrate was washed with saturated $NaHCO_3$ and brine, dried over $MgSO_4$, and concentrated in vacuo to afford crude amine (2.02 g) as a yellow syrup. The obtained crude amine (2.02 g) was dissolved in tetrahydrofuran (100 mL), and then di-*tert*-butyl dicarbonate (0.70 g, 3.2 mmol) was added. The resulting mixture was stirred at room temperature for 1 h and concentrated in vacuo. Purification by column chromatography (silica gel, eluting with a gradient of 20–40% ethyl acetate in hexanes) afforded the title compound **30a** (0.85 g, 70%) as a yellow powder. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 1.03 (d, *J* = 6.6 Hz, 6H), 1.43 (s, 9H), 2.25–2.40 (m, 1H), 2.48 (s, 3H), 3.03 (d, *J* = 7.4 Hz, 2H), 3.15 (q, *J* = 5.5 Hz, 2H), 3.36 (q, *J* = 5.5 Hz, 2H), 4.57 (br s, 1H), 4.74 (br s, 1H), 6.49 (d, *J* = 2.5 Hz, 1H), 7.18 (dd, *J* = 9.1, 2.5 Hz, 1H), 7.30–7.40 (m, 4H), 7.89 (d, *J* = 9.1 Hz, 1H). LC–MS (ESI) *m/z*: 459 $(M + H)^+$.

ii) *tert*-Butyl ((6-((2-((*tert*-butoxycarbonyl)amino)ethyl)amino)-2-isobutyl-4-(4-methylphenyl)quinolin-3-yl)-methyl)carbamate (30). A mixture of **30a** (0.80 g, 1.7 mmol), Raney nickel (ca. 2 g), 25% aqueous ammonia (5 mL), methanol (50 mL), and tetrahydrofuran (10 mL) was stirred at room temperature for 6 h in a sealed tube under a hydrogen atmosphere (0.5 MPa). The reaction mixture was filtered, and the filtrate was concentrated in vacuo. The residue was dissolved in tetrahydrofuran (50 mL), and then di-*tert*-butyl dicarbonate (0.57 g, 2.6 mmol) was added. The resulting mixture was stirred at room temperature for 30 min and concentrated in vacuo. Purification by column chromatography (silica gel, eluting with a gradient of 20–50% ethyl acetate in hexanes) afforded the title compound **30** (0.75 g, 77%) as a yellow powder; mp 57 °C. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 1.01 (d, *J* = 6.6 Hz, 6H), 1.41 (s, 9H), 1.43 (s, 9H), 2.20–2.40 (m, 1H), 2.46 (s, 3H), 2.89 (d, *J* = 7.2 Hz, 2H), 3.10 (q, *J* = 5.3 Hz, 2H), 3.31 (q, *J* = 5.3 Hz, 2H), 4.20–4.25 (m, 3H), 4.31 (t, *J* = 3.6 Hz, 1H), 4.72 (br s, 1H), 6.17 (d, *J* = 2.5 Hz, 1H), 7.05 (dd,

J = 9.0, 2.5 Hz, 1H), 7.11 (d, *J* = 7.8 Hz, 2H), 7.30 (d, *J* = 7.8 Hz, 2H), 7.85 (d, *J* = 9.0 Hz, 1H). LC–MS (ESI) *m*/*z*: 563 $(M + H)^+$.

Ethyl ((2-((*tert*-butoxycarbonyl)amino)ethyl)(3-(((*tert*-butoxycarbonyl)amino)methyl)-2-isobutyl-4-(4-methylphenyl)quinolin-6-yl)amino)(oxo)acetate (31). Ethyl chloro(oxo)acetate (0.30 g, 2.2 mmol) was added to a vigorously stirred mixture of **30** (0.50 g, 0.89 mmol), ethyl acetate (50 mL), and saturated $NaHCO_3$ (50 mL) at room temperature. After vigorous stirring for 30 min, the organic layer was separated, washed with brine, dried over $MgSO_4$, and concentrated in vacuo. Purification by column chromatography (silica gel, eluting with a gradient of 20–40% ethyl acetate in hexanes) afforded the title compound **31** (0.53 g, 90%) as a white powder; mp 110–114 °C. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 0.93 (t, *J* = 7.1 Hz, 3H), 1.03 (d, *J* = 6.6 Hz, 6H), 1.33 (s, 9H), 1.42 (s, 9H), 2.30–2.45 (m, 1H), 2.48 (s, 3H), 2.98 (d, *J* = 7.4 Hz, 2H), 3.31 (q, *J* = 6.0 Hz, 2H), 3.85–3.90 (m, 2H), 3.90 (q, *J* = 7.1 Hz, 2H), 4.30 (br s, 3H), 4.75 (t, *J* = 4.7 Hz, 1H), 7.08 (d, *J* = 7.9 Hz, 2H), 7.14 (d, *J* = 2.1 Hz, 1H), 7.34 (d, *J* = 7.9 Hz, 2H), 7.56 (dd, *J* = 8.9, 2.1 Hz, 1H), 8.09 (d, *J* = 8.9 Hz, 1H). LC–MS (ESI) *m*/*z*: 663 $(M + H)^+$.

***tert*-Butyl *N*-(3-(((*tert*-butoxycarbonyl)amino)methyl)-2-isobutyl-4-(4-methylphenyl)-quinolin-6-yl)glycinate (32).**

i) *tert*-Butyl *N*-(3-cyano-2-isobutyl-4-(4-methylphenyl)quinolin-6-yl)glycinate (32a). By a procedure similar to that described for the synthesis of compound **8da** using *tert*-butyl glycinate, the title compound **32a** was obtained from **7b** as yellow crystals (1.41 g, 83%); mp 128–129 °C. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 1.03 (d, *J* = 6.6 Hz, 6H), 1.44 (s, 9H), 2.25–2.40 (m, 1H), 2.49 (s, 3H), 3.04 (d, *J* = 7.2 Hz, 2H), 3.71 (d, *J* = 5.2 Hz, 2H), 4.81 (t, *J* = 5.2 Hz, 1H), 6.45 (d, *J* = 2.6 Hz, 1H), 7.22 (dd, *J* = 9.0, 2.6 Hz, 1H), 7.30–7.40 (m, 4H), 7.92 (d, *J* = 9.0 Hz, 1H). LC–MS (ESI) *m*/*z*: 430 $(M + H)^+$.

ii) *tert*-Butyl *N*-(3-(((*tert*-butoxycarbonyl)amino)methyl)-2-isobutyl-4-(4-methylphenyl)-quinolin-6-yl)-glycinate (32). By a procedure similar to that described for the synthesis of compound **30**, the title compound **32** was obtained from **32a** as pale yellow crystals (0.96 g, quantitative); mp 181–185 °C. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 1.01 (d, *J* = 6.6 Hz, 6H), 1.41 (s, 9H), 1.44 (s, 9H), 2.20–2.40 (m, 1H), 2.48 (s, 3H), 2.89 (d, *J* = 7.4 Hz, 2H), 3.65 (d, *J* = 5.3 Hz, 2H), 4.22 (d, *J* = 4.9 Hz, 2H), 4.30 (br, 1H), 4.42 (t, *J* = 4.9 Hz, 1H), 6.14 (d, *J* = 2.5 Hz, 1H), 7.08 (dd, *J* = 9.0, 2.5 Hz, 1H), 7.11 (d, *J* = 8.0 Hz, 2H), 7.31 (d, *J* = 8.0 Hz, 2H), 7.87 (d, *J* = 9.0 Hz, 1H). LC–MS (ESI) *m*/*z*: 534 $(M + H)^+$.

***tert*-Butyl *N*-((benzyloxy)carbonyl)glycyl-*N*-(3-(((*tert*-butoxycarbonyl)amino)methyl)-2-isobutyl-4-(4-methyl-phenyl)quinolin-6-yl)glycinate (33).** To an ice-cooled mixture of *N*-((benzyloxy)carbonyl)glycine (0.78 g, 3.75 mmol), *N*,*N*-dimethylformamide (0.1 mL), and tetrahydrofuran (20 mL) was added oxalyl chloride (0.33 mL, 3.7 mmol) in a dropwise manner. The resulting mixture was stirred at 0 °C for 1 h and added dropwise to a mixture of

32 (0.40 g, 0.75 mmol), pyridine (0.3 mL), and tetrahydrofuran (100 mL) at room temperature. After stirring at room temperature for 1 h, 4-dimethylaminopyridine (0.01 g, 0.08 mmol) was added, and the resulting mixture was stirred at room temperature for 60 h. The reaction mixture was partitioned between ethyl acetate and water. The organic layer was washed sequentially with water, saturated $NaHCO_3$, and brine, dried over $MgSO_4$, and concentrated in vacuo. Purification by column chromatography (silica gel, eluting with a gradient of 25–50% ethyl acetate in hexanes) afforded the title compound **33** (0.47 g, 87%) as a white powder; mp 81–83 °C. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 1.04 (d, *J* = 6.8 Hz, 6H), 1.37 (s, 9H), 1.42 (s, 9H), 2.25–2.45 (m, 1H), 2.46 (s, 3H), 3.00 (d, *J* = 7.4 Hz, 2H), 3.69 (d, *J* = 4.3 Hz, 2H), 4.22 (s, 2H), 4.32 (br s, 3H), 5.04 (s, 2H), 5.60 (t, *J* = 4.3 Hz, 1H), 7.11 (d, *J* = 7.9 Hz, 2H), 7.25–7.40 (m, 8H), 7.60 (dd, *J* = 8.9, 2.1 Hz, 1H), 8.11 (d, *J* = 8.9 Hz, 1H). LC–MS (ESI) *m*/*z*: 725 $(M + H)^+$.

3-Benzoyl-5-bromopyridine-2-carboxylic acid (35).

i) 3-Bromofuro[3,4-*b*]pyridine-5,7-dione (35a). A mixture of 5-bromopyridine-2,3-dicarboxylic acid (**34**, 10.0 g, 40.6 mmol) and acetic anhydride (20 mL, 200 mmol) was stirred at 80 °C for 2 h. The reaction mixture was concentrated in vacuo, and the residual solid was triturated with hexanes to afford the title compound **35a** (8.69 g, 94%) as a pale yellow powder; mp 142–144 °C. ^{1}H NMR (200 MHz, DMSO-d_6): δ 8.50 (d, *J* = 2.20 Hz, 1H), 9.22 (d, *J* = 2.20 Hz, 1H).

ii) 3-Benzoyl-5-bromopyridine-2-carboxylic acid (35). To an ice-cooled mixture of **35a** (14.5 g, 63.6 mmol) and benzene (17 mL, 190 mol) in nitrobenzene (29 mL) was added $AlCl_3$ (18.7 g, 140 mmol), and the resulting mixture was stirred at 85 °C for 17 h. The reaction mixture was diluted with nitrobenzene (50 mL), and crushed ice was carefully added. The mixture was extracted with ethyl acetate. The organic layer was washed with 1 M HCl and then extracted with 4 M NaOH. The aqueous layer was washed with diethyl ether, acidified to pH 2 with 6 M HCl, and extracted with ethyl acetate. The extract was washed with brine, dried over $MgSO_4$, and concentrated in vacuo. The residual solid was triturated with diisopropyl ether to afford the title compound **35** (13.0 g, 67%) as a pale yellow powder; mp 166–167 °C. ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.40–7.50 (m, 2H), 7.60 (tt, *J* = 7.5, 1.5 Hz, 1H), 7.70–7.75 (m, 2H), 7.97 (d, *J* = 2.1 Hz, 1H), 8.81 (d, *J* = 2.1 Hz, 1H). LC–MS (ESI) *m*/*z*: 306 $(M + H)^+$.

3-Bromo-8-oxo-5-phenyl-8*H*-pyrano[3,4-*b*]pyridine-6-carboxylic acid (36). To a mixture of **35** (8.00 g, 26.1 mmol) and *N*,*N*-dimethylformamide (0.5 mL) in toluene (50 mL) was added $SOCl_2$ (2.3 mL, 31 mmol) at room temperature. The resulting mixture was stirred at 60 °C for 2 h. After evaporation of the solvent, the residue was dissolved in acetonitrile (80 mL). To the solution was added a solution of diethyl 2-hydroxymalonate (5.85 g, 31.4 mmol) in acetonitrile (20 mL) and then pyridine (5.7 mL, 71 mmol) was added dropwise at 0 °C. After stirring at 0 °C for 2 h, the reaction mixture was partitioned between ethyl acetate and 4 M HCl. The organic layer was washed with brine, dried over $MgSO_4$, and concentrated in vacuo. The residue was dissolved in acetic acid (12 mL)

and concd HCl (12 mL) was added. The resulting mixture was stirred at reflux for 24 h and then cooled to 80 °C. To the resulting suspension water (25 mL) was added in a dropwise manner. The suspension was cooled to 0 °C, and the precipitated crystals were collected by filtration to afford the title compound **36** (7.72 g, 86%) as a pale yellow powder. ^{1}H NMR (200 MHz, $CDCl_3$): δ 7.22–7.32 (m, 2H), 7.50–7.60 (m, 3H), 7.62 (d, *J* = 2.2 Hz, 1H), 8.99 (d, *J* = 2.2 Hz, 1H).

3-Bromo-7-isobutyl-8-oxo-5-phenyl-7,8-dihydro-1,7-naphthyridine-6-carboxylic acid (37a). To a solution of **36** (3.50 g, 10.1 mmol) in methanol (100 mL) was added isobutylamine (3.0 mL, 30 mmol), and the resulting mixture was stirred at 50 °C for 3 h. The reaction mixture was concentrated in vacuo, and the residue was partitioned between ethyl acetate and 0.1 M HCl. The organic layer was washed with brine, dried over $MgSO_4$, and concentrated in vacuo. The residue was dissolved in 5% HCl in methanol (50 mL) and stirred at reflux for 2.5 days. The reaction mixture was concentrated in vacuo, and the residue was dissolved in 1,4-dioxane (20 mL). To the solution was added 4 M LiOH (20 mL), and the resulting mixture was stirred at reflux for 3 h. The reaction mixture was partitioned between ethyl acetate and 1 M HCl. The organic layer was washed with brine, dried over $MgSO_4$, and concentrated in vacuo. The residual solid was recrystallized from toluene to afford the title compound **37a** (2.25 g, 56%) as a pale yellow powder. ^{1}H NMR (200 MHz, $CDCl_3$): δ 0.95 (d, *J* = 6.6 Hz, 6H), 2.33 (spt, *J* = 6.6 Hz, 1H), 4.15 (d, *J* = 7.8 Hz, 2H), 7.35–7.42 (m, 2H), 7.45–7.53 (m, 3H), 7.72 (d, *J* = 2.2 Hz, 1H), 8.63 (d, *J* = 1.8 Hz, 1H). LC–MS (ESI) *m*/*z*: 401 $(M + H)^+$.

3-Bromo-6-(hydroxymethyl)-7-isobutyl-5-phenyl-1,7-naphthyridin-8(7*H*)-one (38a). By a procedure similar to that described for the synthesis of compound **38b**, the title compound **38a** was obtained from **37a** as a yellow powder (0.36 g, 62%). ^{1}H NMR (300 MHz, $CDCl_3$): δ 0.98 (d, *J* = 6.6 Hz, 6H), 2.20–2.35 (m, 1H), 2.61 (br s, 1H), 4.29 (d, *J* = 7.5 Hz, 2H), 4.46 (d, *J* = 6.0 Hz, 2H), 7.30–7.45 (m, 2H), 7.42 (d, *J* = 2.3 Hz, 1H), 7.50–7.60 (m, 3H), 8.70 (d, *J* = 2.3 Hz, 1H). LC–MS (ESI) *m*/*z*: 387 $(M + H)^+$.

6-Bromo-3-(hydroxymethyl)-2-isobutyl-4-phenylisoquinolin-1(2*H*)-one (38b). To a solution of 6-bromo-2-isobutyl-1-oxo-4-phenyl-1,2-dihydroisoquinoline-3-carboxylic acid[30] (**37b**, 4.0 g, 10 mmol) in tetrahydrofuran (30 mL) was added oxalyl chloride (1.7 mL, 20 mmol) and *N*,*N*-dimethylformamide (catalytic amount) at room temperature. After stirring at 2 h, the reaction mixture was concentrated in vacuo, and the residue was crystallized from hexanes to afford 3.8 g of white powder. The obtained powder was dissolved in tetrahydrofuran (30 mL) and added to an ice-cooled suspension of sodium borohydride (1.1 g, 29 mmol) in 1,2-dimethoxyethane (30 mL). The resulting mixture was stirred at 0 °C for 15 h. The reaction mixture was poured into ice-water with HCl and extracted with ethyl acetate. The extract was washed with saturated $NaHCO_3$ and brine, dried over Na_2SO_4, and concentrated in vacuo. The residual solid was crystallized from diisopropyl ether–ethyl acetate to afford the title compound **38b** (3.1 g, 80%) as colorless crystals. ^{1}H NMR (300 MHz, DMSO-d_6): δ 0.91 (d, *J* = 6.60 Hz, 6H), 2.21 (spt, *J* = 6.60 Hz, 1H), 4.15 (d, *J* = 7.24 Hz, 2H), 4.25 (d, *J* = 4.68 Hz, 2H), 5.35 (t, *J* = 4.72 Hz, 1H), 6.99–7.04 (m,

1H), 7.35 (d, *J* = 4.41 Hz, 2H), 7.46–7.62 (m, 3H), 7.68 (dd, *J* = 8.62, 1.65 Hz, 1H), 8.23 (d, *J* = 8.53 Hz, 1H). LC–MS (ESI) *m*/*z*: 386 $(M + H)^+$.

***tert*-Butyl ((3-bromo-7-isobutyl-8-oxo-5-phenyl-7,8-dihydro-1,7-naphthyridin-6-yl)methyl)carbamate (39a).** To a solution of **38a** (0.35 g, 0.90 mmol) in toluene (15 mL) was added $SOCl_2$ (0.13 mL, 1.8 mmol), and the resulting mixture was stirred at reflux for 2 h. The reaction mixture was concentrated in vacuo, and the residue was partitioned between ethyl acetate and saturated $NaHCO_3$. The organic layer was washed with brine, dried over $MgSO_4$, and concentrated in vacuo to afford crude chloride (0.41 g). The chloride was dissolved in *N*,*N*-dimethylformamide (5 mL) and potassium phthalimide (0.25 g, 1.4 mmol) was added. After stirring at room temperature for 12 h, the reaction mixture was partitioned between ethyl acetate and saturated $NaHCO_3$. The organic layer was washed with brine, dried over $MgSO_4$, and concentrated in vacuo to afford crude imidate (0.58 g). The imidate was dissolved in ethanol and hydrazine monohydrate (1.0 mL, 21 mmol) was added. The resulting mixture was stirred at reflux for 2h. The reaction mixture was partitioned between ethyl acetate and saturated $NaHCO_3$. The organic layer was washed with brine, dried over $MgSO_4$, and concentrated in vacuo to afford crude amine (0.36 g). The obtained amine was dissolved in tetrahydrofuran (10 mL), and di-*tert*-butyl dicarbonate (0.30 g, 1.4 mmol) was added at room temperature. After stirring for 1 h, the reaction mixture was concentrated in vacuo. The residue was purified by column chromatography (silica gel, eluting with a gradient of 33–60% ethyl acetate in hexanes) to afford the title compound **39a** (0.37 g, 85%) as a white solid. ^{1}H NMR (200 MHz, $CDCl_3$): δ 1.00 (d, *J* = 6.6 Hz, 6H), 1.44 (s, 9H), 2.15–2.40 (m, 1H), 4.12 (d, *J* = 7.2 Hz, 2H), 4.21 (d, *J* = 5.6 Hz, 2H), 4.79 (br s, 1H), 7.25–7.35 (m, 2H, overlapped), 7.42 (d, *J* = 2.0 Hz, 1H), 7.50–7.60 (m, 3H), 8.78 (d, *J* = 2.0 Hz, 1H). LC–MS (ESI) *m*/*z*: 486 $(M + H)^+$.

6-(Aminomethyl)-3-bromo-7-isobutyl-5-phenyl-1,7-naphthyridin-8(7*H*)-one (40a). A mixture of **39a** (0.35 g, 0.73 mmol) and trifluoroacetic acid (5 mL) was stirred at room temperature for 15 min. The reaction mixture was concentrated in vacuo, and the residue was partitioned between ethyl acetate and 1 M NaOH. The organic layer was washed with 1 M NaOH and brine, dried over $MgSO_4$, and concentrated in vacuo. The residue was crystallized from ethyl acetate to afford the title compound **40a** (0.27 g, 97%) as a slightly brown solid. ^{1}H NMR (200 MHz, $CDCl_3$): δ 1.00 (d, *J* = 6.6 Hz, 6H), 2.15–2.40 (m, 1H), 3.69 (s, 2H), 4.27 (d, *J* = 7.8 Hz, 2H), 7.20–7.30 (m, 2H, overlapped), 7.46 (d, *J* = 2.2 Hz, 1H), 7.50–7.60 (m, 3H), 8.82 (d, *J* = 2.2 Hz, 1H). LC–MS (ESI) *m*/*z*: 386 $(M + H)^+$.

6-(Aminomethyl)-7-isobutyl-8-oxo-5-phenyl-7,8-dihydro-1,7-naphthyridine-3-carboxamide dihydrochloride (40b).

i) Methyl 6-(((*tert*-butoxycarbonyl)amino)methyl)-7-isobutyl-8-oxo-5-phenyl-7,8-dihydro-1,7-naphthyridine-3-carboxylate (40ba). To a mixture of compound **39a** (0.25 g, 0.51 mmol), triethylamine (0.11 mL, 0.76 mmol),

methanol (25 mL), and tetrahydrofuran (25 mL) was added palladium(II) acetate (6 mg, 0.03 mmol) and 1,1'-ferrocenediyl-bis(diphenylphosphine) (14 mg, 0.03 mmol). The resulting mixture was stirred at 105 °C for 4 h under a CO atmosphere (0.5 MPa). The reaction mixture was partitioned between ethyl acetate and 0.1 M HCl. The insoluble was removed by filtration, and the organic layer was washed with brine, dried over $MgSO_4$, and concentrated in vacuo. The residue was purified by column chromatography (silica gel, eluting with a gradient of 60–80% ethyl acetate in hexanes) to afford the title compound **40ba** as a yellow powder (0.21 g, 88%). ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.01 (d, *J* = 6.6 Hz, 6H), 1.44 (s, 9H), 2.20–2.35 (m, 1H), 3.89 (s, 3H), 4.14 (d, *J* = 8.7 Hz, 2H), 4.23 (d, *J* = 6.0 Hz, 2H), 4.77 (br s, 1H), 7.25–7.30 (m, 2H, overlapped), 7.45–7.60 (m, 3H), 7.90 (d, *J* = 1.8 Hz, 1H), 9.27 (d, *J* = 1.8 Hz, 1H). LC–MS (ESI) *m*/*z*: 466 $(M + H)^+$.

ii) 6-(((*tert*-Butoxycarbonyl)amino)methyl)-7-isobutyl-8-oxo-5-phenyl-7,8-dihydro-1,7-naphthyridine-3-carboxylic acid (40bb). A mixture of **40ba** (0.20 g, 0.43 mmol), 1 M NaOH (2 mL), methanol (2 mL), and tetrahydrofuran (2 mL) was stirred at room temperature for 17 h. The reaction mixture was partitioned between ethyl acetate and 0.5 M HCl, and the organic layer was washed with brine, dried over $MgSO_4$, and concentrated in vacuo. The residual solid was triturated with diisopropyl ether to afford the title compound **40bb** (0.18 g, 93%) as a yellow solid. ^{1}H NMR (300 MHz, $CDCl_3$): δ 0.99 (d, *J* = 6.6 Hz, 6H), 1.49 (s, 9H), 2.15–2.30 (m, 1H), 4.00–4.20 (m, 4H), 6.18 (br s, 1H), 7.45–7.60 (m, 5H), 7.65 (br s, 1H), 8.99 (d, *J* = 1.2 Hz, 1H). LC–MS (ESI) *m*/*z*: 452 $(M + H)^+$.

iii) 6-(Aminomethyl)-7-isobutyl-8-oxo-5-phenyl-7,8-dihydro-1,7-naphthyridine-3-carboxamide dihydrochloride (40b). A mixture of **40bb** (0.17 g, 0.38 mmol), ammonium salt of 1-hydroxy-1*H*-benzotriazole (0.12 g, 0.75 mmol), and 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (0.15 g, 0.75 mmol) in *N*,*N*-dimethylformamide (5 mL) was stirred at room temperature for 4 h. The reaction mixture was partitioned between ethyl acetate and 0.1 M citric acid. The organic layer was washed with saturated $NaHCO_3$ and brine, dried over $MgSO_4$, and concentrated in vacuo. The residual solid was triturated with diisopropyl ether to afford crude amide (0.15 g) as a white solid. LC–MS (ESI) *m*/*z*: 451 $(M + H)^+$. The amide was dissolved in trifluoroacetic acid (3 mL), and the solution was stirred at room temperature for 30 min. The reaction mixture was concentrated in vacuo, and the residue was purified by preparative HPLC. The obtained solid was dissolved in 1 M HCl (1 mL) and concentrated in vacuo. The residue was solidified with diisopropyl ether to afford the title compound **40b** (66 mg, 40%) as a yellow powder. ^{1}H NMR (200 MHz, DMSO-d_6): δ 0.94 (d, *J* = 6.6 Hz, 6H), 2.00–2.25 (m, 1H), 3.90 (d, *J* = 4.6 Hz, 2H), 4.11 (d, *J* = 6.6 Hz, 2H), 7.35–7.45 (m, 2H), 7.55–7.65 (m, 3H), 7.74 (d, *J* = 2.0 Hz, 1H), 7.83 (br s, 1H), 8.43 (br s, 1H), 8.63 (br s, 3H), 9.24 (d, *J* = 2.0 Hz, 1H). LC–MS (ESI) *m*/*z*: 351 $(M + H)^+$. Anal. Calcd for $C_{20}H_{22}N_4O_2 \cdot 2HCl$: C, 54.43; H, 5.94; N, 12.69. Found: C, 54.18; H, 6.12; N 12.60.

Methyl 6-bromo-2-isobutyl-4-phenylquinoline-3-carboxylate (41). A mixture of **5a** (3.67 g, 13.3 mmol), methyl 5-methyl-3-oxohexanoate (**6g**, 2.18 g, 13.8 mmol), and methanesulfonic acid (1.28 g, 13.3 mmol) in toluene (5 mL)

was stirred at reflux for 14 h with azeotropic removal of water. The reaction mixture was partitioned between ethyl acetate and saturated $NaHCO_3$, and the organic layer was washed with brine, dried over $MgSO_4$, and concentrated in vacuo. The residue was purified by column chromatography (silica gel, eluting with a gradient of 5–15% ethyl acetate in hexanes) to afford the title compound **41** (3.08 g, 58%) as a pale yellow solid; mp 108–110 °C. ^{1}H NMR (300 MHz, $CDCl_3$): δ 0.98 (d, *J* = 6.6 Hz, 6H), 2.20–2.40 (m, 1H), 2.87 (d, *J* = 7.2 Hz, 2H), 3.55 (s, 3H), 7.35–7.40 (m, 2H), 7.45–7.55 (m, 3H), 7.71 (d, *J* = 2.4 Hz, 1H), 7.79 (d, *J* = 9.0 Hz, 1H), 7.98 (d, *J* = 9.0 Hz, 1H). LC–MS (ESI) *m*/*z*: 398 $(M + H)^+$.

(6-Bromo-2-isobutyl-4-phenylquinolin-3-yl)methanol (42). To a solution of **41** (3.00 g, 7.53 mmol) in toluene (100 mL) was added a solution of diisobutylaluminum hydride in toluene (1 M, 20 mL, 20 mmol) in a dropwise manner over 30 min at −75 °C. The resulting mixture was stirred at −75 °C for 1 h, and $Na_2SO_4{\cdot}10H_2O$ (2.67 g, 8.29 mmol) was added portionwise. The reaction mixture was allowed to warm to room temperature, stirred for 1 h, and then filtered. The filtrate was concentrated in vacuo, and the residual solid was recrystallized from toluene–hexanes to afford the title compound **42** (1.78 g, 64%) as colorless crystals; mp 98–100 °C. ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.04 (d, *J* = 6.6 Hz, 6H), 1.51 (t, *J* = 6.0 Hz, 1H), 2.30–2.50 (m, 1H), 3.05 (d, *J* = 7.2 Hz, 2H), 4.60 (d, *J* = 6.0 Hz, 2H), 7.10–7.60 (m, 6H, overlapped), 7.72 (dd, *J* = 9.0, 2.1 Hz, 1H), 7.94 (d, *J* = 9.0 Hz, 1H). LC–MS (ESI) *m*/*z*: 370 $(M + H)^+$.

1-(6-Bromo-2-isobutyl-4-phenylquinolin-3-yl)methanamine (43a).

i) 2-((6-Bromo-2-isobutyl-4-phenylquinolin-3-yl)methyl)-1*H*-isoindole-1,3(2*H*)-dione (43aa). To a solution of **42** (1.55 g, 4.19 mmol) in toluene (30 mL) was added $SOCl_2$ (0.61 mL, 8.4 mmol), and the resulting mixture was stirred at 55 °C for 30 min. The reaction mixture was concentrated in vacuo, and the residue was dissolved in *N*,*N*-dimethylformamide (20 mL). To the solution potassium phthalimide (1.16 g, 6.28 mmol) was added. After stirring at 55 °C for 1 h, the reaction mixture was partitioned between ethyl acetate and water. The organic layer was washed with saturated $NaHCO_3$ and brine, dried over $MgSO_4$, and concentrated in vacuo. The residue was purified by column chromatography (silica gel, eluting with a gradient of 10–20% ethyl acetate in hexanes) to afford the title compound **43aa** (1.02 g, 82%) as a pale yellow solid; mp 164–165 °C. ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.01 (d, *J* = 6.6 Hz, 6H), 2.20–2.45 (m, 1H), 3.00 (d, *J* = 7.2 Hz, 2H), 4.91 (s, 2H), 7.15–7.25 (m, 2H), 7.30–7.40 (m, 4H), 7.65–7.75 (m, 5H), 7.93 (d, *J* = 8.8 Hz, 1H). LC–MS (ESI) *m*/*z*: 499 $(M + H)^+$.

ii) 1-(6-Bromo-2-isobutyl-4-phenylquinolin-3-yl)methanamine (43a). A mixture of **43aa** (1.70 g, 3.40 mmol) and hydrazine monohydrate (5 mL, 103 mmol) in ethanol (150 mL) was stirred at reflux for 2 h. The reaction mixture was partitioned between ethyl acetate and saturated $NaHCO_3$. The organic layer was washed with saturated $NaHCO_3$ and brine, dried over $MgSO_4$, and concentrated in vacuo. The residue was purified by column chromatography (silica gel, eluting with a gradient of 0–10% methanol in ethyl acetate) followed by

recrystallization from diisopropyl ether–hexanes to afford the title compound **43a** (0.90 g, 72%) as colorless crystals; mp 129–130 °C. ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.04 (d, *J* = 6.6 Hz, 6H), 1.26 (br s, 2H), 2.30–2.50 (m, 1H), 3.01 (d, *J* = 7.2 Hz, 2H), 3.79 (s, 2H), 7.25–7.35 (m, 2H), 7.35–7.45 (m, 1H), 7.50–7.60 (m, 3H), 7.69 (dd, *J* = 9.0, 2.1 Hz, 1H), 7.95 (dd, *J* = 9.0, 0.3 Hz, 1H). LC–MS (ESI) *m*/*z*: 369 $(M + H)^+$. Anal. Calcd for $C_{20}H_{21}BrN_2$: C, 65.05; H, 5.73; N, 7.59. Found: C, 65.47; H, 5.79; N 7.90.

3-(Aminomethyl)-2-isobutyl-4-phenylquinoline-6-carboxamide (43b).

i) 3-(Aminomethyl)-2-isobutyl-4-phenylquinoline-6-carbonitrile (43ba). A mixture of **43a** (0.20 g, 0.54 mmol), $Zn(CN)_2$ (0.038 g, 0.32 mmol), and tetrakis(triphenylphosphine)palladium(0) (0.032 g, 0.027 mmol) in 1-methyl-2-pyrrolidinone (8 mL) was stirred at 80 °C for 1 h under a nitrogen atmosphere. The reaction mixture was partitioned between ethyl acetate and a mixture of 28% NH_3–saturated NH_4Cl–water (1:1:4, v/v). The organic layer was washed with 28% NH_3–saturated NH_4Cl–water (1:1:4, v/v) and brine, dried over $MgSO_4$, and concentrated in vacuo. The residue was purified by preparative HPLC to afford a colorless paste. The obtained paste was partitioned between ethyl acetate and saturated $NaHCO_3$. The organic layer was washed with brine, dried over $MgSO_4$, and concentrated in vacuo. The residue was crystallized from ethyl acetate–hexanes to afford the title compound **43ba** (0.153 g, 90%) as a white powder; mp 128–129 °C. ^{1}H NMR (200 MHz, $CDCl_3$): δ 1.06 (d, *J* = 6.6 Hz, 6H), 1.40 (br s, 2H), 2.30–2.55 (m, 1H), 3.06 (d, *J* = 7.4 Hz, 2H), 3.82 (br s, 2H), 7.20–7.35 (m, 2H), 7.50–7.60 (m, 3H), 7.67 (d, *J* = 1.4 Hz, 1H), 7.76 (dd, *J* = 8.8, 1.4 Hz, 1H), 8.12 (d, *J* = 8.8 Hz, 1H). LC–MS (ESI) *m*/*z*: 316 $(M + H)^+$.

ii) 3-(Aminomethyl)-2-isobutyl-4-phenylquinoline-6-carboxamide (43b). A mixture of **43ba** (0.111 g, 0.35 mmol), 1 M NaOH (2 mL), and dimethylsulfoxide (4 mL) was stirred at 80 °C for 3 h. The reaction mixture was extracted with ethyl acetate. The extract was washed with brine, dried over $MgSO_4$, and concentrated in vacuo. The residue was crystallized from ethyl acetate–diisopropyl ether to afford the title compound **43b** (0.043 g, 37%) as pale yellow crystals; mp 128–129 °C. ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.05 (d, *J* = 6.6 Hz, 6H), 2.35–2.50 (m, 1H), 3.05 (d, *J* = 7.1 Hz, 2H), 3.80 (s, 2H), 5.64 (br s, 1H), 5.94 (br s, 1H), 7.25–7.35 (m, 2H), 7.50–7.60 (m, 3H), 7.74 (d, *J* = 1.4 Hz, 1H), 8.01 (dd, *J* = 8.6, 2.0 Hz, 1H), 8.11 (d, *J* = 6.6 Hz, 1H). LC–MS (ESI) *m*/*z*: 334 $(M + H)^+$. Anal. Calcd for $C_{21}H_{23}N_3O$: C, 75.65; H, 6.95; N, 11.60. Found: C, 75.16; H, 7.16; N, 11.65.

5-(((*tert*-Butoxycarbonyl)amino)methyl)-6-isobutyl-2-methyl-4-(4-methylphenyl)nicotinic acid (46).

i) Methyl 5-(((*tert*-butoxycarbonyl)amino)methyl)-6-isobutyl-2-methyl-4-(4-methylphenyl)nicotinate (46a). Di-*tert*-butyl dicarbonate (0.72 g, 3.3 mmol) was added to a solution of compound **45** (0.90 g, 2.8 mmol) in tetrahydrofuran (25 mL), and the resulting mixture was stirred at room temperature for 12 h. The reaction mixture was concentrated in vacuo, and the residue was purified by column chromatography (silica gel, eluting with a

gradient of 5–15% ethyl acetate in hexanes) to afford the title compound **46a** (1.2 g, 98%) as a white powder; mp 139–142 °C. ^{1}H NMR (300 MHz, $CDCl_3$): δ 0.97 (d, *J* = 6.8 Hz, 6H), 1.39 (s, 9H), 2.10–2.30 (m, 1H), 2.39 (s, 3H), 2.54 (s, 3H), 2.78 (d, *J* = 7.2 Hz, 2H), 3.50 (s, 3H), 4.15 (d, *J* = 4.9 Hz, 2H), 4.24 (br s, 1H), 7.06 (d, *J* = 7.9 Hz, 2H), 7.20 (d, *J* = 7.9 Hz, 2H). LC–MS (ESI) *m*/*z*: 427 $(M + H)^+$.

ii) 5-(((*tert*-Butoxycarbonyl)amino)methyl)-6-isobutyl-2-methyl-4-(4-methylphenyl)nicotinic acid (46). A mixture of compound **46a** (1.0 g, 2.3 mmol) and 1 M NaOH (10 mL) in methanol (30 mL) was stirred at reflux for 3 days. The reaction mixture was acidified with 0.5 M HCl and extracted with ethyl acetate. The extract was washed with brine, dried over $MgSO_4$, and concentrated in vacuo. The residue was crystallized from methanol–water to afford the title compound **46** (0.58 g, 60%) as a white powder; mp 182–183 °C. ^{1}H NMR (300 MHz, $CDCl_3$): δ 0.87 (d, *J* = 6.4 Hz, 6H), 1.39 (s, 9H), 1.95–2.10 (m, 1H), 2.38 (s, 3H), 2.67 (s, 3H), 2.75 (d, *J* = 7.2 Hz, 2H), 4.13 (d, *J* = 4.7 Hz, 2H), 4.30 (t, *J* = 4.7 Hz, 1H), 7.15 (d, *J* = 7.9 Hz, 2H), 7.22 (d, *J* = 7.9 Hz, 2H). LC–MS (ESI) *m*/*z*: 413 $(M + H)^+$.

4-Ethoxy-4-oxobutyl 5-(((*tert*-butoxycarbonyl)amino)methyl)-6-isobutyl-2-methyl-4-(4-methylphenyl)-nicotinate (47a). A mixture of compound **46** (0.41 g, 1.0 mmol), ethyl 4-bromobutyrate (0.21 g, 1.1 mmol), and K_2CO_3 (0.15 g, 1.1 mmol) in *N*,*N*-dimethylformamide (20 mL) was stirred at room temperature for 1 h. The reaction mixture was partitioned between ethyl acetate and brine, and the organic layer was separated, dried over $MgSO_4$, and concentrated in vacuo. The residue was purified by column chromatography (silica gel, eluting with a gradient of 5–30% ethyl acetate in hexanes) to afford the title compound **47a** (0.45 g, 85%) as a colorless syrup. ^{1}H NMR (300 MHz, $CDCl_3$): δ 0.98 (d, *J* = 6.6 Hz, 6H), 1.25 (t, *J* = 7.2 Hz, 3H), 1.39 (s, 9H), 1.55–1.70 (m, 2H), 2.08 (t, *J* = 7.5 Hz, 2H), 2.15–2.30 (m, 1H), 2.38 (s, 3H), 2.54 (s, 3H), 2.78 (d, *J* = 7.3 Hz, 2H), 3.95 (t, *J* = 6.2 Hz, 2H), 4.11 (q, *J* = 7.2 Hz, 2H), 4.14 (d, *J* = 5.3 Hz, 2H), 4.21 (br s, 1H), 7.07 (d, *J* = 8.0 Hz, 2H), 7.21 (d, *J* = 8.0 Hz, 2H). LC–MS (ESI) *m*/*z*: 527 $(M + H)^+$.

(4-(Methoxycarbonyl)cyclohexyl)methyl 5-(((*tert*-butoxycarbonyl)amino)methyl)-6-isobutyl-2-methyl-4-(4-methylphenyl)nicotinate (47b). A solution of methanesulfonyl chloride (0.27 mL, 3.5 mmol) in tetrahydrofuran (2 mL) was added dropwise to a mixture of methyl *trans*-4-(hydroxymethyl)cyclohexanecarboxylate (0.40 g, 2.5 mmol) and triethylamine (0.65 mL, 4.6 mmol) in tetrahydrofuran (10 mL) at room temperature. The resulting mixture was stirred at room temperature. The reaction mixture was diluted with ethyl acetate, washed with brine, dried over $MgSO_4$, and concentrated in vacuo. The residue was dissolved in *N*,*N*-dimethylformamide (5 mL), and the solution was added in a dropwise manner to a mixture of compound **46** (0.96 g, 2.3 mmol) and K_2CO_3 (0.48 g, 3.5 mmol) in *N*,*N*-dimethylformamide (10 mL). The resulting mixture was stirred at 70 °C for 15 h. The reaction mixture was partitioned between ethyl acetate and water. The organic layer was washed with brine, dried over $MgSO_4$, and concentrated in vacuo. The residue was purified by column chromatography (silica gel, eluting with a gradient of 20–40% ethyl acetate in hexanes) to afford the title compound **47b** (0.75 g, 57%) as a colorless paste.

^{1}H NMR (300 MHz, $CDCl_3$): δ 0.97 (d, *J* = 6.6 Hz, 6H), 1.02–1.20 (m, 2H), 1.30–1.50 (m, 5H), 1.38 (s, 9H overlapped), 1.77–1.99 (m, 2H), 2.12–2.32 (m, 1H), 2.39 (s, 3H), 2.46–2.60 (m, 4H), 2.78 (d, *J* = 7.3 Hz, 2H), 3.67 (s, 3H), 3.78 (d, *J* = 6.8 Hz, 2H), 4.11–4.18 (m, 2H), 4.23 (s, 1H), 7.07 (d, *J* = 7.9 Hz, 2H), 7.20 (d, *J* = 7.7 Hz, 2H). LC–MS (ESI) *m*/*z*: 567 $(M + H)^+$.

2-(Methoxycarbonyl)benzyl 5-(((*tert*-butoxycarbonyl)amino)methyl)-6-isobutyl-2-methyl-4-(4-methylphenyl)nicotinate (47c).

i) 2-Bromobenzyl 5-(((*tert*-butoxycarbonyl)amino)methyl)-6-isobutyl-2-methyl-4-(4-methylphenyl)nicotinate (47ca). A mixture of compound **46** (1.1 g, 2.7 mmol), 2-bromobenzyl bromide (0.61 g, 2.4 mmol), and K_2CO_3 (0.50 g, 3.7 mmol) in *N*,*N*-dimethylformamide (10 mL) was stirred at room temperature for 15 min. The reaction mixture was partitioned between ethyl acetate and brine, and the organic layer was separated, dried over $MgSO_4$, and concentrated in vacuo. The residue was purified by column chromatography (silica gel, eluting with a gradient of 8–20% ethyl acetate in hexanes) to afford the title compound **47ca** (1.2 g, 79%) as a white powder. ^{1}H NMR (300 MHz, $CDCl_3$): δ 0.97 (d, *J* = 6.8 Hz, 6H), 1.38 (s, 9H), 2.14–2.25 (m, 1H), 2.35 (s, 3H), 2.56 (s, 3H), 2.78 (d, *J* = 7.2 Hz, 2H), 4.11–4.13 (m, 2H), 4.22 (br s, 1H), 5.05 (s, 2H), 7.02–7.05 (m, 3H), 7.11 (d, *J* = 7.9 Hz, 2H), 7.16–7.21 (m, 2H), 7.51–7.54 (m, 1H). LC–MS (ESI) *m*/*z*: 583 $(M + H)^+$.

ii) 2-(Methoxycarbonyl)benzyl 5-(((*tert*-butoxycarbonyl)amino)methyl)-6-isobutyl-2-methyl-4-(4-methylphenyl)nicotinate (47c). By a procedure similar to that described for the synthesis of compound **52**, the title compound **47c** was obtained from compound **47ca** as a white powder (0.88 g, 74%). ^{1}H NMR (300 MHz, $CDCl_3$): δ 0.97 (d, *J* = 6.6 Hz, 6H), 1.38 (s, 9H), 2.16–2.25 (m, 1H), 2.35 (s, 3H), 2.56 (s, 3H), 2.78 (d, *J* = 7.2 Hz, 2H), 3.87 (s, 3H), 4.11–4.16 (m, 2H), 4.21 (br s, 1H), 5.39 (s, 2H), 7.01–7.06 (m, 3H), 7.11 (d, *J* = 7.9 Hz, 2H), 7.32–7.42 (m, 2H), 7.93–7.96 (m, 1H). LC–MS (ESI) *m*/*z*: 561 $(M + H)^+$.

3-(Methoxycarbonyl)benzyl 5-(((*tert*-butoxycarbonyl)amino)methyl)-6-isobutyl-2-methyl-4-(4-methylphenyl)nicotinate (47d). By a procedure similar to that described for the synthesis of compound **47a** using methyl 3-(bromomethyl)benzoate, the title compound **47d** was obtained as a white powder (1.8 g, 78%). ^{1}H NMR (300 MHz, $CDCl_3$): δ 0.96 (d, *J* = 6.6 Hz, 6H), 1.38 (s, 9H), 2.16–2.25 (m, 1H), 2.33 (s, 3H), 2.53 (s, 3H), 2.77 (d, *J* = 7.4 Hz, 2H), 3.94 (s, 3H), 4.13 (br s, 2H), 4.20 (br s, 1H), 4.95 (s, 2H), 7.01 (d, *J* = 8.1 Hz, 2H), 7.09 (d, *J* = 7.9 Hz, 2H), 7.22 (d, *J* = 7.7 Hz, 1H), 7.35 (d, *J* = 7.7 Hz, 1H), 7.83 (s, 1H), 7.98 (d, *J* = 7.7 Hz, 1H). LC–MS (ESI) *m*/*z*: 561 $(M + H)^+$.

4-(Methoxycarbonyl)benzyl 5-(((*tert*-butoxycarbonyl)amino)methyl)-6-isobutyl-2-methyl-4-(4-methylphenyl)nicotinate (47e). By a procedure similar to that described for the synthesis of compound **47a** using methyl 4-(bromomethyl)benzoate, the title compound **47e** was obtained as a white powder (2.5 g, 92%). ^{1}H NMR (300

MHz, $CDCl_3$): δ 0.96 (d, *J* = 6.6 Hz, 6H), 1.38 (s, 9H), 2.14–2.25 (m, 1H), 2.35 (s, 3H), 2.54 (s, 3H), 2.78 (d, *J* = 7.2 Hz, 2H), 3.93 (s, 3H), 4.12 (d, *J* = 7.0 Hz, 2H), 4.21 (br s, 1H), 4.98 (s, 2H), 7.01 (d, *J* = 7.9 Hz, 2H), 7.07–7.12 (m, 4H), 7.93 (d, *J* = 8.3 Hz, 2H). LC–MS (ESI) *m*/*z*: 561 $(M + H)^+$.

4-(((5-(((*tert*-Butoxycarbonyl)amino)methyl)-6-isobutyl-2-methyl-4-(4-methylphenyl)pyridin-3-yl)carbonyl)-oxy)butanoic acid (48a). By a procedure similar to that described for the synthesis of compound **13a**, the title compound **48a** was obtained from compound **47a** as a white powder (0.23 g, 82%); mp 70–72 °C. ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.02 (d, *J* = 6.6 Hz, 6H), 1.39 (s, 9H), 1.55–1.70 (m, 2H), 2.12 (t, *J* = 7.1 Hz, 2H), 2.15–2.30 (m, 1H), 2.39 (s, 3H), 2.75 (br s, 3H), 2.85–3.20 (m, 2H), 4.00 (t, *J* = 6.2 Hz, 2H), 4.20 (d, *J* = 3.6 Hz, 2H), 4.37 (br s, 1H), 7.10 (d, *J* = 7.7 Hz, 2H), 7.26 (d, *J* = 7.7 Hz, 2H). LC–MS (ESI) *m*/*z*: 499 $(M + H)^+$.

4-((((5-(((*tert*-butoxycarbonyl)amino)methyl)-6-isobutyl-2-methyl-4-(4-methylphenyl)pyridin-3-yl)carbonyl)-oxy)methyl)cyclohexanecarboxylic acid (48b). By a procedure similar to that described for the synthesis of compound **53**, the title compound **48b** was obtained from compound **47b** as a white powder (0.55 g, 75%). ^{1}H NMR (300 MHz, $CDCl_3$): δ 0.97 (d, *J* = 6.6 Hz, 6H), 1.04–1.22 (m, 2H), 1.26–1.70 (m, 5H), 1.39 (s, 9H overlapped), 1.82–1.99 (m, 2H), 2.10–2.31 (m, 1H), 2.38 (s, 3H), 2.48–2.64 (m, 4H), 2.78 (s, 2H), 3.78 (d, *J* = 6.6 Hz, 2H), 4.08–4.19 (m, 2H), 4.24 (s, 1H), 7.07 (d, *J* = 7.9 Hz, 2H), 7.20 (d, *J* = 7.7 Hz, 2H). LC–MS (ESI) *m*/*z*: 553 $(M + H)^+$.

2-((((5-(((*tert*-Butoxycarbonyl)amino)methyl)-6-isobutyl-2-methyl-4-(4-methylphenyl)pyridin-3-yl)carbonyl)-oxy)methyl)benzoic acid (48c). By a procedure similar to that described for the synthesis of compound **53**, the title compound **48c** was obtained from compound **47c** as a white powder (0.75 g, 88%). ^{1}H NMR (300 MHz, $CDCl_3$): δ 0.96 (d, *J* = 6.6 Hz, 6H), 1.37 (s, 9H), 2.12–2.21 (m, 1H), 2.36 (s, 3H), 2.54 (s, 3H), 2.83 (d, *J* = 7.2 Hz, 2H), 4.13–4.18 (m, 2H), 4.25 (br s, 1H), 5.38 (s, 2H), 7.01–7.04 (m, 3H), 7.11 (d, *J* = 7.5 Hz, 2H), 7.38–7.46 (m, 2H), 8.06–8.09 (m, 1H). LC–MS (ESI) *m*/*z*: 547 $(M + H)^+$.

3-((((5-(((*tert*-Butoxycarbonyl)amino)methyl)-6-isobutyl-2-methyl-4-(4-methylphenyl)pyridin-3-yl)carbonyl)-oxy)methyl)benzoic acid (48d). By a procedure similar to that described for the synthesis of compound **53**, the title compound **48d** was obtained from compound **47d** as a white powder (1.4 g, 87%). ^{1}H NMR (300 MHz, $CDCl_3$): δ 0.96 (d, *J* = 6.6 Hz, 6H), 1.38 (s, 9H), 2.13–2.25 (m, 1H), 2.34 (s, 3H), 2.55 (s, 3H), 2.80 (d, *J* = 7.4 Hz, 2H), 4.11–4.16 (m, 2H), 4.22 (br s, 1H), 4.98 (s, 2H), 7.02 (d, *J* = 7.9 Hz, 2H), 7.11 (d, *J* = 7.7 Hz, 2H), 7.26–7.30 (m, 1H), 7.39 (d, *J* = 7.7 Hz, 1H), 7.89 (s, 1H), 8.04 (d, *J* = 7.5 Hz, 1H). LC–MS (ESI) *m*/*z*: 547 $(M + H)^+$.

4-((((5-(((*tert*-Butoxycarbonyl)amino)methyl)-6-isobutyl-2-methyl-4-(4-methylphenyl)pyridin-3-yl)carbonyl)-oxy)methyl)benzoic acid (48e). By a procedure similar to that described for the synthesis of compound **53**, the title compound **48e** was obtained from compound **47e** as a white powder (0.34 g, 32%). ^{1}H NMR (300 MHz, $CDCl_3$): δ

0.91 (d, *J* = 6.5 Hz, 6H), 1.35 (s, 9H), 2.21 (spt, *J* = 6.5 Hz, 1H), 2.31 (s, 3H), 2.43 (s, 3H), 2.62 (d, *J* = 7.1 Hz, 2H), 3.87 (d, *J* = 3.5 Hz, 2H), 5.04 (s, 2H), 6.99 (t, *J* = 4.0 Hz, 1H), 7.07–7.16 (m, 6H), 7.84 (d, *J* = 8.0 Hz, 2H), 13.00 (br s, 1H). LC–MS (ESI) *m*/*z*: 547 $(M + H)^+$.

4-(((5-(Aminomethyl)-6-isobutyl-2-methyl-4-(4-methylphenyl)pyridin-3-yl)carbonyl)oxy)butanoic acid dihydrochloride (49a). By a procedure similar to that described for the synthesis of compound **12a**, the title compound **49a** was obtained from compound **48a** as a white powder (0.20 g, 99%); mp 221–223 °C. ^{1}H NMR (300 MHz, DMSO-d_6): δ 0.97 (d, *J* = 6.6 Hz, 6H), 1.40–1.55 (m, 2H), 2.00 (t, *J* = 7.4 Hz, 2H), 2.15–2.30 (m, 1H), 2.36 (s, 3H), 2.52 (br s, 3H, overlapped), 2.80–2.95 (m, 2H), 3.83 (d, *J* = 4.3 Hz, 2H), 3.92 (t, *J* = 6.2 Hz, 2H), 7.20 (d, *J* = 7.7 Hz, 2H), 7.29 (d, *J* = 7.7 Hz, 2H), 8.29 (br s, 3H). LC–MS (ESI) *m*/*z*: 447 $(M + H)^+$. Anal. Calcd for $C_{27}H_{30}N_2O_4 \cdot 2HCl \cdot H_2O$: C, 57.49; H, 6.83; N, 5.59. Found: C, 57.69; H, 6.93; N 5.70.

4-((((5-(Aminomethyl)-6-isobutyl-2-methyl-4-(4-methylphenyl)pyridin-3-yl)carbonyl)oxy)methyl)cyclohexanecarboxylic acid dihydrochloride (49b). By a procedure similar to that described for the synthesis of compound **19a**, the title compound **49b** was obtained from compound **48b** as a white powder (0.25 g, 83%). ^{1}H NMR (300 MHz, DMSO-d_6): δ 0.97 (d, *J* = 6.6 Hz, 6H), 1.13–1.48 (m, 7H), 1.60–1.86 (m, 2H), 2.09–2.30 (m, 1H), 2.37 (s, 3H), 2.40–2.48 (m, 1H), 2.54 (s, 3H), 2.82–3.00 (m, 2H), 3.76 (d, *J* = 6.6 Hz, 2H), 3.83 (d, *J* = 4.7 Hz, 2H), 7.20 (d, *J* = 7.9 Hz, 2H), 7.30 (d, *J* = 8.1 Hz, 2H), 8.34 (br s, 3H). LC–MS (ESI) *m*/*z*: 453 $(M + H)^+$. Anal. Calcd for $C_{27}H_{36}N_2O_4 \cdot 2HCl \cdot 1.5H_2O$: C, 58.69; H, 7.48; N, 5.07. Found: C, 59.04; H, 7.29; N 4.93.

2-((((5-(Aminomethyl)-6-isobutyl-2-methyl-4-(4-methylphenyl)pyridin-3-yl)carbonyl)oxy)methyl)benzoic acid dihydrochloride (49c). By a procedure similar to that described for the synthesis of compound **19a**, the title compound **49c** was obtained from compound **48c** as a white powder (0.28 g, 65%). ^{1}H NMR (300 MHz, DMSO-d_6): δ 0.98 (d, *J* = 6.5 Hz, 6H), 2.22 (spt, *J* = 6.8 Hz, 1H), 2.36 (s, 3H), 2.62 (s, 3H), 2.99 (d, *J* = 6.1 Hz, 2H), 3.85 (d, *J* = 4.9 Hz, 2H), 5.34 (s, 2H), 7.00 (d, *J* = 7.1 Hz, 1H), 7.19–7.28 (m, 4H), 7.41–7.53 (m, 2H), 7.90 (d, *J* = 7.2 Hz, 1H), 8.44 (br s, 3H). LC–MS (ESI) *m*/*z*: 447 $(M + H)^+$. Anal. Calcd for $C_{27}H_{30}N_2O_4 \cdot 2HCl \cdot 1.5H_2O$: C, 59.34; H, 6.46; N, 5.13. Found: C, 59.15; H, 6.40; N, 4.97.

3-((((5-(Aminomethyl)-6-isobutyl-2-methyl-4-(4-methylphenyl)pyridin-3-yl)carbonyl)oxy)methyl)benzoic acid dihydrochloride (49d). By a procedure similar to that described for the synthesis of compound **19a**, the title compound **49d** was obtained from compound **48d** as a white powder (0.28 g, 65%). ^{1}H NMR (300 MHz, DMSO-d_6): δ 0.96 (d, *J* = 6.6 Hz, 6H), 2.16–2.25 (m, 1H), 2.32 (s, 3H), 2.54 (s, 3H), 2.90 (d, *J* = 6.6 Hz, 2H), 3.81 (d, *J* = 5.1 Hz, 2H), 5.04 (s, 2H), 7.13 (d, *J* = 8.5 Hz, 2H), 7.17 (d, *J* = 8.3 Hz, 2H), 7.26–7.30 (m, 1H), 7.44 (t, *J* = 7.6 Hz, 1H), 7.73–7.74 (m, 1H), 7.89–7.92 (m, 1H), 8.30 (br s, 3H). LC–MS (ESI) *m*/*z*: 447 $(M + H)^+$. Anal. Calcd for $C_{27}H_{30}N_2O_4 \cdot 2HCl \cdot 0.25H_2O$: C, 61.89; H, 6.25; N, 5.35. Found: C, 61.78; H, 6.27; N 5.02.

4-((((5-(Aminomethyl)-6-isobutyl-2-methyl-4-(4-methylphenyl)pyridin-3-yl)carbonyl)oxy)methyl)benzoic acid dihydrochloride (49e). By a procedure similar to that described for the synthesis of compound **19a**, the title compound **49e** was obtained from compound **48e** as a white powder (0.33 g, 94%). ^{1}H NMR (300 MHz, DMSO-d_6): δ 0.97 (d, J = 6.5 Hz, 6H), 2.21 (spt, J = 6.7 Hz, 1H), 2.34 (s, 3H), 2.56–2.68 (m, 3H), 3.02 (br s, 2H), 3.83 (br s, 2H), 5.08 (s, 2H), 7.13 (d, J = 7.9 Hz, 2H), 7.16–7.23 (m, 4H), 7.85 (d, J = 7.9 Hz, 2H), 8.48 (br s, 3H), 11.30 (br s, 1H). LC–MS (ESI) m/z: 447 $(M + H)^+$. Anal. Calcd for $C_{27}H_{30}N_2O_4 \cdot 2HCl \cdot 0.25H_2O$: C, 61.89; H, 6.25; N, 5.35. Found: C, 61.90; H, 6.23; N 5.29.

***tert*-Butyl ((5-(hydroxymethyl)-2-isobutyl-6-methyl-4-(4-methylphenyl)pyridin-3-yl)methyl)carbamate (50).** A solution of diisobutylaluminum hydride in toluene (1 M, 100 mL, 100 mmol) was added in a dropwise manner over 30 min to a stirred solution of methyl 5-(aminomethyl)-6-isobutyl-2-methyl-4-(4-methylphenyl)nicotinate[44] (**45**, 9.3 g, 29 mmol) in toluene (150 mL) at −78 °C. The reaction mixture was allowed to warm, and acetone (10 mL) and sodium sulfate decahydrate (40 g) were added at 0 °C. The resulting suspension was stirred at room temperature for 15 h, and insoluble materials were filtered off and washed with ethyl acetate. The filtrate and the wash were combined, and 1 M NaOH (30 mL) and di-*tert*-butyl dicarbonate (6.9 mL, 30 mmol) were added. The mixture was stirred at room temperature for 30 min. The reaction mixture was washed sequentially with water and brine, dried over anhydrous $MgSO_4$ and concentrated in vacuo. The residue was purified by column chromatography (silica gel, eluting with 50% ethyl acetate in hexanes) to afford the title compound **50** (8.5 g, 75%) as colorless crystals; mp 140–141 °C. ^{1}H NMR (300 MHz, $CDCl_3$): δ 0.97 (6H, d, J = 6.6 Hz), 1.32 (9H, s), 2.13–2.25 (1H, m), 2.42 (3H, s), 2.68 (3H, s), 2.75 (2H, d, J = 7.4 Hz), 4.05 (2H, d, J = 4.7 Hz), 4.19 (1H, br s), 4.36 (2H, d, J = 5.7 Hz), 7.05 (2H, d, J = 7.9 Hz), 7.24–7.26 (2H, m). LC–MS (ESI) m/z: 399 $(M + H)^+$.

***tert*-Butyl ((5-(((4-bromobenzyl)oxy)methyl)-2-isobutyl-6-methyl-4-(4-methylphenyl)pyridin-3-yl)methyl)-carbamate (51).** Methanesulfonyl chloride (0.15 mL, 1.9 mmol) was added to a mixture of compound **50** (0.50 g, 1.3 mmol) and triethylamine (0.35 mL, 2.5 mmol) in tetrahydrofuran (10 mL), and the resulting mixture was stirred at room temperature for 30 min. The reaction mixture was diluted with ethyl acetate, washed with saturated $NaHCO_3$ and brine, dried over $MgSO_4$, and concentrated in vacuo. The residual mesylate was dissolved in tetrahydrofuran (10 mL). NaH (60% suspension in oil, 60 mg, 1.5 mmol) was added to a solution of (4-bromophenyl)methanol (0.33 g, 1.8 mmol) in tetrahydrofuran (10 mL) at room temperature and stirred for 30 min. To the resulting mixture was added the solution of mesylate dropwise, and the mixture was stirred at reflux for 17 h. The reaction mixture was quenched with water, extracted with ethyl acetate. The extract was washed with brine, dried over $MgSO_4$, and concentrated in vacuo. The residue was purified by column chromatography (silica gel, eluting with a gradient of 20–40% ethyl acetate in hexanes) to afford the title compound **51** (0.24 g, 34%) as a white powder. ^{1}H NMR (300 MHz, $CDCl_3$): δ 0.95 (d, J = 6.8 Hz, 6H), 1.37 (s, 9H), 2.13–2.22 (m, 1H), 2.42 (s, 3H), 2.61 (s, 3H), 2.74 (d, J = 7.2 Hz, 2H), 4.05 (d, J = 4.9 Hz, 2H), 4.26 (s, 2H), 7.00 (d, J = 7.9 Hz, 2H), 7.06 (d, J = 8.3 Hz, 2H), 7.19 (d, J = 7.7 Hz, 2H), 7.39 (d, J = 8.3 Hz, 2H). LC–MS (ESI) m/z: 569 $(M + H)^+$.

Methyl 4-(((5-(((*tert*-butoxycarbonyl)amino)methyl)-6-isobutyl-2-methyl-4-(4-methylphenyl)-pyridin-3-yl)-methoxy)methyl)benzoate (52). To a mixture of compound **51** (1.7 g, 3.0 mmol), triethylamine (0.84 mL, 6.1 mmol), *N*,*N*-dimethylformamide (15 mL), and methanol (5 mL) was added (1,1′-Bis(diphenylphosphino)-ferrocene)dichloropalladium(II) (dichloromethane complex, 0.25 g, 0.30 mmol), and the resulting mixture was stirred at 80 °C for 14 h under a CO atmosphere. The reaction mixture was diluted with ethyl acetate, washed with brine, dried over $MgSO_4$, and concentrated in vacuo. The residue was purified by column chromatography (silica gel, eluting with a gradient of 15–40% ethyl acetate in hexanes) to afford the title compound **52** (1.0 g, 60%) as a white powder. ^{1}H NMR (300 MHz, $CDCl_3$): δ 0.96 (d, *J* = 6.6 Hz, 6H), 1.38 (s, 9H), 2.14–2.23 (m, 1H), 2.41 (s, 3H), 2.63 (s, 3H), 2.75 (d, *J* = 7.4 Hz, 2H), 3.92 (s, 3H), 4.06 (d, *J* = 4.7 Hz, 2H), 4.14 (s, 2H), 4.37 (s, 2H), 7.02 (d, *J* = 8.1 Hz, 2H), 7.20 (d, *J* = 7.7 Hz, 2H), 7.26 (d, *J* = 8.3 Hz, 2H), 7.95 (d, *J* = 8.3 Hz, 2H). LC–MS (ESI) *m*/*z*: 547 $(M + H)^+$.

4-(((5-(((*tert*-Butoxycarbonyl)amino)methyl)-6-isobutyl-2-methyl-4-(4-methylphenyl)pyridin-3-yl)methoxy)-methyl)benzoic acid (53). A mixture of compound **52** (0.91 g, 1.7 mmol), 1 M NaOH (5 mL), tetrahydrofuran (5 mL), and methanol (5 mL) was stirred at 60 °C for 1 h. The reaction mixture was acidified with 1 M HCl and extracted with ethyl acetate. The extract was washed with brine, dried over $MgSO_4$, and concentrated in vacuo. The residual solid was triturated with diisopropyl ether–hexanes to afford the title compound **53** (0.80 g, 90%) as a white powder. ^{1}H NMR (300 MHz, $CDCl_3$): δ 0.99 (d, *J* = 6.6 Hz, 6H), 1.38 (s, 9H), 2.12–2.28 (m, 1H), 2.42 (s, 3H), 2.77 (s, 3H), 2.93 (br s, 2H), 4.10 (d, *J* = 5.1 Hz, 2H), 4.16 (s, 2H), 4.25 (br s, 1H), 4.39 (s, 2H), 7.02 (d, *J* = 7.2 Hz, 2H), 7.21–7.28 (m, 4H), 8.01 (d, *J* = 7.9 Hz, 2H). LC–MS (ESI) *m*/*z*: 533 $(M + H)^+$.

4-(((5-(Aminomethyl)-6-isobutyl-2-methyl-4-(4-methylphenyl)pyridin-3-yl)methoxy)methyl)benzoic acid dihydrochloride (54). By a procedure similar to that described for the synthesis of compound **19a**, the title compound **54** was obtained from compound **53** as a white powder (0.22 g, 57%). ^{1}H NMR (300 MHz, DMSO-d_6): δ 0.98 (d, *J* = 6.2 Hz, 6H), 2.11–2.22 (m, 1H), 2.40 (s, 3H), 2.87 (br s, 3H), 3.14 (br s, 2H), 3.75–3.86 (m, 2H), 4.18 (s, 2H), 4.43 (s, 2H), 7.23 (d, *J* = 7.9 Hz, 2H), 7.28 (d, *J* = 8.3 Hz, 2H), 7.33 (d, *J* = 7.9 Hz, 2H), 7.87 (d, *J* = 8.1 Hz, 2H), 8.40 (br s, 3H). LC–MS (ESI) *m*/*z*: 433 $(M + H)^+$. Anal. Calcd for $C_{27}H_{32}N_2O_3 \cdot 2HCl \cdot 0.5H_2O$: C, 63.03; H, 6.86; N, 5.44. Found: C, 62.88; H, 6.74; N, 5.40.

Ethyl 2-((*E*)-2-(5-(((*tert*-butoxycarbonyl)amino)methyl)-6-isobutyl-2-methyl-4-(4-methylphenyl)pyridin-3-yl)vinyl)-1,3-thiazole-4-carboxylate (55a).

i) *tert*-Butyl ((5-formyl-2-isobutyl-6-methyl-4-(4-methylphenyl)pyridin-3-yl)methyl)carbamate (55aa). A mixture of compound **50** (3.4 g, 8.5 mmol) and Dess–Martin reagent (5.4 g, 13 mmol) in dichloromethane (50 mL)

was stirred at room temperature for 30 min. To the reaction mixture was added 10% $Na_2S_2O_3$ (50 mL) and saturated $NaHCO_3$ (50 mL). After vigorous stirring at room temperature for 1 h, the organic layer was separated, washed with brine, dried over $MgSO_4$, and concentrated in vacuo. The residue was purified by column chromatography (silica gel, eluting with 75% ethyl acetate in hexanes) to afford the title compound **55aa** (3.4 g, quantitative) as a white powder. ^{1}H NMR (300 MHz, DMSO-d_6) δ 0.93 (d, J = 6.6 Hz, 6H), 1.36 (s, 9H), 2.25 (spt, J = 6.5 Hz, 1H), 2.38 (s, 3H), 2.62–2.71 (m, 5H), 3.88 (d, J = 4.1 Hz, 2H), 6.98 (br t, J = 3.7 Hz, 1H), 7.25 (d, J = 7.7 Hz, 2H), 7.30 (d, J = 7.9 Hz, 2H), 9.59 (s, 1H). LC–MS (ESI) m/z: 397 $(M + H)^+$.

ii) Ethyl 2-((*E*)-2-(5-(((*tert*-butoxycarbonyl)amino)methyl)-6-isobutyl-2-methyl-4-(4-methylphenyl)pyridin-3-yl)vinyl)-1,3-thiazole-4-carboxylate (55a). To a solution of ethyl 2-(bromomethyl)-1,3-thiazole-4-carboxylate (2.4 g, 9.7 mmol) in toluene (20 mL) triphenylphosphine (2.7 g, 10 mmol) was added at room temperature, and the resulting mixture was stirred at 100 °C for 3 h. The reaction mixture was cooled to room temperature, and the precipitate was collected by filtration, washed with hexanes, and dried to afford ((4-(ethoxycarbonyl)-1,3-thiazol-2-yl)methyl)(triphenyl)phosphonium bromide (**55ab**, 3.7 g, 74%) as a brown powder, which was used without further purification. To a mixture of compound **55aa** (2.2 g, 5.5 mmol) and compound **55ab** (3.7 g, 7.2 mmol) in *N*,*N*-dimethylformamide (20 mL) was added sodium ethoxide (0.75 g, 11 mmol) at room temperature, and the resulting mixture was stirred at room temperature for 3 h. The reaction mixture was partitioned between ethyl acetate and water. The organic layer was separated, washed with brine, dried over $MgSO_4$, and concentrated in vacuo. The residue was purified by column chromatography (silica gel, eluting with a gradient of 10–50% ethyl acetate in hexanes) to afford the title compound **55a** (1.8 g, 60%) as a pale yellow powder. ^{1}H NMR (300 MHz, $CDCl_3$): δ 0.99 (d, J = 6.6 Hz, 6H), 1.36–1.44 (m, 3H), 1.39 (s, 9H), 2.24 (spt, J = 6.6 Hz, 1H), 2.39 (s, 3H), 2.68 (s, 3H), 2.78 (d, J = 7.3 Hz, 2H), 4.11 (d, J = 4.9 Hz, 2H), 4.18–4.29 (m, 1H), 4.36–4.48 (m, 2H), 6.76 (dd, J = 16.8, 0.8 Hz, 1H), 6.95 (s, 1H), 6.99 (d, J = 7.7 Hz, 2H), 7.23 (d, J = 7.7 Hz, 2H), 8.01 (dd, J = 17.3, 0.8 Hz, 1H). LC–MS (ESI) m/z: 550 $(M + H)^+$.

Ethyl 2-(2-(5-(((*tert*-butoxycarbonyl)amino)methyl)-6-isobutyl-2-methyl-4-(4-methylphenyl)pyridin-3-yl)-ethyl)-1,3-thiazole-4-carboxylate (55b). A mixture of compound **55a** (1.5 g, 2.7 mmol) and 10% palladium on charcoal (0.15 g) in ethanol (20 mL) was stirred at room temperature for 1 h in a sealed tube under a hydrogen atmosphere (0.5 MPa). The reaction mixture was filtered, and the filtrate was concentrated in vacuo. The residue was purified by column chromatography (silica gel, eluting with a gradient of 20–50% ethyl acetate in hexanes) to afford the title compound **55b** (1.1 g, 71%) as a white powder. ^{1}H NMR (300 MHz, $CDCl_3$): δ 0.97 (d, J = 6.6 Hz, 6H), 1.39 (t, J = 7.1 Hz, 3H), 1.38 (s, 9H), 2.19 (spt, J = 6.8 Hz, 1H), 2.41 (s, 3H), 2.55 (s, 3H), 2.74 (d, J = 7.3 Hz, 2H), 2.77–2.87 (m, 2H), 2.98–3.06 (m, 2H), 4.02 (d, J = 4.9 Hz, 2H), 4.20 (br s, 1H), 4.40 (q, J = 7.2 Hz, 2H), 6.98 (d, J = 7.9 Hz, 2H), 7.25 (d, J = 7.9 Hz, 2H, overlapped), 7.98 (s, 1H). LC–MS (ESI) m/z: 552 $(M + H)^+$.

2-((*E*)-2-(5-(((*tert*-Butoxycarbonyl)amino)methyl)-6-isobutyl-2-methyl-4-(4-methylphenyl)pyridin-3-yl)vinyl)-

1,3-thiazole-4-carboxylic acid (56a). A mixture of compound **55a** (0.15 g, 0.27 mmol), 1 M NaOH (1 mL), tetrahydrofuran (1 mL), and ethanol (1 mL) was stirred at room temperature for 12 h. The reaction mixture was diluted with water, acidified to pH 2 with 1 M HCl, and extracted with ethyl acetate. The extract was washed with brine, dried over $MgSO_4$, and concentrated in vacuo. The residual solid was triturated with diisopropyl ether to afford the title compound **56a** (95 mg, 69%) as a white powder. ^{1}H NMR (300 MHz, $CDCl_3$): δ 0.95 (d, *J* = 6.5 Hz, 6H), 1.36 (s, 9H), 2.22 (spt, *J* = 6.1 Hz, 1H), 2.35 (s, 3H), 2.58–2.75 (m, 5H), 3.86 (br s, 2H), 6.70 (d, *J* = 17.1 Hz, 1H), 6.94 (br s, 1H), 7.04 (d, *J* = 16.5 Hz, 1H), 7.18 (d, *J* = 8.3 Hz, 2H), 7.26 (d, *J* = 8.1 Hz, 2H), 8.32 (s, 1H), 13.04 (br s, 1H). LC–MS (ESI) *m*/*z*: 522 $(M + H)^+$.

2-(2-(5-(((*tert*-Butoxycarbonyl)amino)methyl)-6-isobutyl-2-methyl-4-(4-methylphenyl)pyridin-3-yl)ethyl)-1,3-thiazole-4-carboxylic acid (56b). By a procedure similar to that described for the synthesis of compound **56a**, the title compound **56b** was obtained from compound **55b** as a pale yellow powder (0.30 g, 38%). ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.06 (d, *J* = 6.2 Hz, 6H), 1.38 (s, 9H), 2.21–2.37 (m, 1H), 2.46 (s, 3H), 3.00 (s, 3H), 3.28–3.44 (m, 2H), 3.82–4.24 (m, 4H), 4.13 (d, *J* = 2.1 Hz, 2H), 4.52 (br s, 1H), 7.05 (d, *J* = 7.2 Hz, 2H), 7.36 (d, *J* = 7.0 Hz, 2H), 8.10 (s, 1H). LC–MS (ESI) *m*/*z*: 524 $(M + H)^+$.

***tert*-Butyl ((5-(2-(4-carbamoyl-1,3-thiazol-2-yl)ethyl)-2-isobutyl-6-methyl-4-(4-methylphenyl)pyridin-3-yl)-methyl)carbamate (56c).** A mixture of **56b** (0.17 g, 0.32 mmol), ammonium salt of 1-hydroxy-1*H*-benzotriazole (0.074 g, 0.49 mmol), and 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (0.094 g, 0.49 mmol) in *N*,*N*-dimethylformamide (6 mL) was stirred at room temperature for 5 days. The reaction mixture was partitioned between ethyl acetate and water. The organic layer was washed with brine, dried over $MgSO_4$, and concentrated in vacuo. The residue was purified by column chromatography (silica gel, eluting with a gradient of 50–100% ethyl acetate in hexanes) to afford the title compound **56c** (0.11 g, 66%) as a white powder; mp 174–176 °C. ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.97 (d, *J* = 6.6 Hz, 6H), 1.39 (s, 9H), 2.20 (spt, *J* = 7.3 Hz, 1H), 2.41 (s, 3H), 2.59 (s, 3H), 2.75 (d, *J* = 7.2 Hz, 2H), 2.77–3.00 (m, 4H), 4.03 (d, *J* = 5.1 Hz, 2H), 4.20 (br s, 1H), 5.60 (br s, 1H), 6.97 (d, *J* = 6.9 Hz, 2H), 7.03 (br s, 1H), 7.25 (d, *J* = 6.9 Hz, 2H), 7.94 (s, 1H). LC–MS (ESI) *m*/*z*: 523 $(M + H)^+$.

2-((*E*)-2-(5-(Aminomethyl)-6-isobutyl-2-methyl-4-(4-methylphenyl)pyridin-3-yl)vinyl)-1,3-thiazole-4-carboxylic acid dihydrochloride (57a). Compound **56a** (75 mg, 0.14 mmol) was dissolved in trifluoroacetic acid (2 mL), and the resulting solution was stirred at room temperature for 1 h. The reaction mixture was concentrated in vacuo, and the residue was purified by preparative HPLC. The obtained solid was dissolved in 1 M HCl (1 mL) and concentrated in vacuo. The residue was solidified with diisopropyl ether to afford the title compound **57a** (51 mg, 71%) as a pale yellow powder. ^{1}H NMR (300 MHz, CD_3OD): δ 1.13 (d, *J* = 6.4 Hz, 6H), 2.21 (spt, *J* = 6.5 Hz, 1H), 2.41 (s, 3H), 2.95 (s, 3H), 3.14 (d, *J* = 7.2 Hz, 2H), 4.20 (s, 2H), 6.88 (d, *J* = 16.9 Hz, 1H), 7.11 (d, *J* = 16.5 Hz, 1H), 7.29 (d, *J* = 7.8 Hz, 2H), 7.40 (d, *J* = 6.5 Hz, 2H), 8.32 (s, 1H). LC–MS (ESI) *m*/*z*: 422 $(M + H)^+$. Anal. Calcd for $C_{24}H_{27}N_3O_2S{\cdot}2HCl{\cdot}1.5H_2O$: C, 55.27; H, 6.18; N, 8.06. Found: C, 55.38; H, 6.32; N 8.02.

2-(2-(5-(Aminomethyl)-6-isobutyl-2-methyl-4-(4-methylphenyl)pyridin-3-yl)ethyl)-1,3-thiazole-4-carboxylic acid dihydrochloride (57b). A mixture of compound **56b** (0.20 g, 0.38 mmol) and 6 M HCl (5 mL) was stirred at room temperature for 30 min. The reaction mixture was concentrated in vacuo, and the residual solid was recrystallized from water–acetonitrile (1:100, v/v) to afford the title compound **57b** (0.15 g, 80%) as a white powder; mp 235–240 °C. ^{1}H NMR (300 MHz, DMSO-d_6): δ 0.98 (d, J = 6.6 Hz, 6H), 2.15 (spt, J = 6.6 Hz, 1H), 2.41 (s, 3H), 2.79–2.96 (m, 2H), 2.89 (s, 3H), 2.98–3.08 (m, 2H), 3.12–3.28 (m, 2H), 3.78 (d, J = 4.5 Hz, 2H), 7.28 (d, J = 7.7 Hz, 2H), 7.40 (d, J = 7.9 Hz, 2H), 8.29 (s, 1H), 8.38 (br s, 3H). LC–MS (ESI) m/z: 424 $(M + H)^+$. Anal. Calcd for $C_{24}H_{29}N_3O_2S{\cdot}2HCl{\cdot}3H_2O$: C, 52.36; H, 6.77; N, 7.63. Found: C, 52.11; H, 6.50; N 7.90.

2-(2-(5-(Aminomethyl)-6-isobutyl-2-methyl-4-(4-methylphenyl)pyridin-3-yl)ethyl)-1,3-thiazole-4-carboxamide dihydrochloride (57c). By a procedure similar to that described for the synthesis of compound **19a**, the title compound **57c** was obtained from compound **56c** as a pale yellow powder (0.94 g, 90%). ^{1}H NMR (300 MHz, DMSO-d_6): δ 0.98 (d, J = 6.3 Hz, 6H), 2.16 (spt, J = 6.1 Hz, 1H), 2.41 (s, 3H), 2.79–3.07 (m, 4H), 2.86 (br s, 3H), 3.16 (br d, J = 2.6 Hz, 2H), 3.78 (br d, J = 3.9 Hz, 2H), 7.28 (d, J = 7.5 Hz, 2H), 7.39 (d, J = 7.8 Hz, 2H), 7.45 (br s, 1H), 7.58 (br s, 1H), 8.08 (s, J = 3.0 Hz, 1H), 8.42 (br s, 3H). LC–MS (ESI) m/z: 423 $(M + H)^+$. Anal. Calcd for $C_{24}H_{30}N_4OS{\cdot}2HCl{\cdot}3H_2O$: C, 52.45; H, 6.97; N, 10.19. Found: C, 52.68; H, 6.96; N 9.98.

Docking study. Docking studies in Table 2-1 and Figures 2-3 and 2-4 were carried out with a docking software Glide[49] or with an automatic docking using GOLD[32] and the subsequent energy-minimization at the MMFF94s force field using MOE.[50] Docking study in Figure 3-3 was performed using Maestro.[31] The protein coordinates of the cocrystal structure of **2a** (Protein Data Bank Code: 3OPM) or **44** (Protein Data Bank Code: 3O9V) was used as a template for docking study. The binding free energies of each compound were calculated using molecular mechanics Poisson–Boltzmann surface area (MM/PBSA) method[51] or using Maestro.[31]

In vitro DPP4, DPP2, DPP8, and DPP9 enzyme assay. Human DPP4 was partially purified from Caco-2 cells (ATCC No. HTB-37). The compounds (1 μL in DMSO) at each concentration were added to 79 μL of assay buffer (0.25 M Tris–HCl pH 7.5, 0.25% bovine serum albumin, 0.125% CHAPS) and mixed with 20 μL of human DPP4 fraction. After the mixture was incubated at room temperature for 15 min, the reaction was initiated by adding 100 μL of 1 mM of Gly–Pro–*p*NA·*p*-tosylate in distilled water as a substrate and run for 60 min at 37 °C. Rat DPP2 was partially purified from rat kidney according to the method previously reported.[52] One microliter of compounds dissolved in DMSO was mixed with 29 μL of distilled water, 10 μL of 1 M 3,3-dimethylglutamic acid buffer (pH 5.5), and 10 μL of the DPP-2 fraction. After the mixture was incubated at room temperature for 20 min, the reaction was initiated by adding 50 μL of 1 mM of H–Lys–Ala–*p*NA·2HCl and run at 37 °C for 60 min. Human DPP8 and DPP9 were purified respectively by affinity chromatography from the 293-F cells expressing each FLAG®-tagged protein. One microliter of compounds dissolved in DMSO was mixed with 29 μL of distilled water, 10 μL of 1 M

Tris–HCl buffer (pH 7.5), and 10 μL of the enzyme fraction. After the mixture was incubated at room temperature for 20 min, the reaction was initiated by adding 50 μL of 2 mM of Gly–Pro–*p*NA·*p*-tosylate in distilled water for DPP8 or 4 mM of Gly–Pro–*p*NA·*p*-tosylate in distilled water for DPP9 and run at 37 °C for 90 min. Absorbance at 405 nm of each reaction mixture was measured using a microplate reader at the initial time and the end of the reaction. The well containing substrate alone was used as a basal control. The well containing the substrate and the enzyme without the compound was used as a total reaction.

Ex vivo plasma DPP4 enzyme assay. Male Sprague-Dawley rats were purchased from CLEA Japan, Inc. (Tokyo, Japan). Rats were orally administered vehicle (0.5% methylcellulose) or compounds at a dose of 1 mg/kg. Blood samples were collected from tail vein at 1, 3, and 6 hours after dosing. Plasma sample was prepared from each blood sample and the residual DPP4 activity was measured using the method employed in the in vitro DPP4 enzyme assay described above.

Effects of single administration of compounds on glucose tolerance in female Wistar fatty rats. Female Wistar fatty rats were obtained from Takeda Rabics, Ltd. At the age of 10 weeks, the rats were fasted overnight and divided into 4 groups based on plasma glucose levels and body weights (6 rats in each group). Each group was orally administered vehicle (0.5% methylcellulose) or compounds at a dose of 1 mg/kg. Four hours later, all animals were received an oral glucose load (1 g/kg). Blood samples were collected from tail vein at 0, 10, 30, and 60 minutes after the glucose load. Plasma glucose level was determined by an enzyme assay method (L-type Glucose 2; Wako Pure Chemical Ind., Ltd.). Plasma insulin level was determined as immunoreactive insulin (IRI) level in plasma by using a radioimmunoassay (RIA) kit (Shionogi, Japan).

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謝辞

本稿を取りまとめるにあたり、終始ご指導ご鞭撻を賜りました、東京大学大学院薬学系研究科薬化学教室教授　大和田智彦先生に深く感謝いたします。

本稿の構想段階から完成に至るまで、終始ご助言と温かいご支援を賜りました、武田薬品工業株式会社　長展生博士に深く感謝いたします。

本研究は武田薬品工業株式会社において実施したものであり、本研究実施の機会を与えて頂き、終始有用なご助言と励ましを頂きました、武田薬品工業株式会社　三宅昭夫博士、左右田隆博士、大川滋紀博士、加藤金芳博士、石原雄二博士、百瀬祐博士に感謝いたします。

本論文作成の機会を与えて頂き、温かい励ましを頂きました、武田薬品工業株式会社　内川治博士、一川隆史博士、前川毅志博士に感謝いたします。

本研究の共同研究者であり、武田薬品工業株式会社医薬研究本部 DPP-IV 阻害薬研究チームのメンバーである以下の皆様の多大なご支援と有益な議論に心から感謝いたします。共同研究者の皆様の優れた能力とチームワークなくして本研究は完成しえませんでした。

研究計画および化合物のデザインを議論し、質の高い合成研究を実施して本研究の推進に多大なる貢献を頂いた合成チームのメンバーで、本研究の出発点となったヒット化合物の選択と本研究全般の合成研究でご貢献頂いた大井悟氏、阪野義広氏、本研究全般の合成研究でご貢献頂いた宮本泰史博士、本論文第 3 章の合成研究でご貢献頂いた山下徹博士、ヒット化合物の選択とイソキノロン系の合成研究で本研究にご貢献頂いた佐々木満氏

薬理研究を実施し、合成化合物が創薬リードレベルの薬理作用を有することを証明して頂いた薬理チームの鈴木伸宏博士、武内浩二博士、片岡修氏、淺川智子博士、森藤雄亮博士

ヒット化合物の発見につながる HTS の実施と、合成化合物の阻害活性および選択性評価を実施して頂いたスクリーニングチームの谷昭義博士、小阪拓男博士、坪谷重利博士、池堂孝治氏、坂本潤一博士、佐々木雅子博士

本研究化合物の薬物動態評価を実施して頂いた動態チームの船見みゆき氏

ホモロジーモデルやドッキングモデルの構築と分析を実施して頂いた計算化学チームの山本善雄博士、多和田倫子氏

X 線共結晶構造解析を実施して頂いた構造生物学チームの Dr. Kathleen Aertgeerts、Dr. Jason Yano

また、化合物のバルク合成等を通じて合成研究に有用な情報提供や議論を頂いた武田薬品工業株式会社　多和田紘之博士、武藤英吾博士、水船秀哉博士、瀬良美佐代博士、梶野正博博士に感謝いたします。

本論文の執筆にあたり、有用な議論とご助言、励ましを頂きました武田薬品工業株式会社　奈良洋氏に感謝いたします。

同じく本論文の執筆にあたり、ドッキングモデルの分子化学計算でご助力頂いた武田薬品工業株式会社　奈良禎氏、生物試験結果の解析でご助力頂いた武田薬品工業株式会社　鎌田祐輔氏、薬理試験結果の解析でご助力頂いた武田薬品工業株式会社　服部ハル実氏に感謝いたします。

その他、本研究の完成および本論文の作成のために様々なご支援とご配慮を頂きました、武田薬品工業株式会社の皆様、ならびに私の現所属であります Axcelead Drug Discovery Partners 株式会社の皆様に心より感謝いたします。

最後に、私の研究生活を常に応援し支えてくれています家族に深く感謝いたします。

（注）皆様のご所属は研究当時のものを記載いたしました。