博士論文

X線共結晶解析に基づく高活性かつ高選択的な非亜鉛結合型MMP-13阻害薬の分子設計と合成

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本論文掲載出版社よりの学位論文への再利用の許諾に関して

目次

略号表

本論文中において下記の略号を使用した。

AIBN	2,2'-azobis(isobutyronitrile)
Bn	benzyl
Boc	*tert*-butoxycarbonyl
Boc_2O	di-*tert*-butyl dicarbonate
Bu	butyl
DABCO	1,4-diazabicyclo[2.2.2]octane
Dba	dibenzylideneacetone
DIEA	*N*,*N*-diisopropylethylamine
DMA	dimethylacetamide
DMF	*N*,*N*-dimethylformamide
DMAP	4-dimethylaminopyridine
DMSO	dimethylsulfoxide
EDC	1-ethyl-3-(3-dimethylaminopropyl)carbodiimide
LDA	lithium diisopropylamide
Mp	melting point
MsCl	methylsulfonyl chloride
NBS	*N*-bromosuccinimide
NMO	*N*-methylmorpholine *N*-oxide
NMR	nuclear magnetic resonance
MS4A	molecular sieves 4A
PTFE	polytetrafluoroethylene
RMS	root mean square
SD	standard deviation
SEM	2-(trimethylsilyl)ethoxymethyl
TFA	trifluoroacetic acid

THF	tetrahydrofuran
TPAP	tetrapropylammonium perruthenate
Tris	*tris*(hydroxymethyl)aminomethane
mCPBA	*m*-chloroperoxybenzoic acid
WSCD	water soluble carbodiimide

第1章　緒言

1-1　変形性関節症とは

変形性関節症（osteoarthritis: OA）は、非炎症性の関節変性疾患であり、関節軟骨の摩耗と破壊を主徴とする。日本におけるResearch on Osteoarthritis/osteoporosis Against Disability (ROAD) studyと呼ばれる大規模調査の結果、40歳以上の推定患者数が2,530万人と言われており、慢性関節リウマチの総患者数（約70～80万人）と比べても極めて罹患数が多い疾患と言え、40歳以上に腰や、膝に多く見られることが報告されている（**Figure 1-1**）[1]。

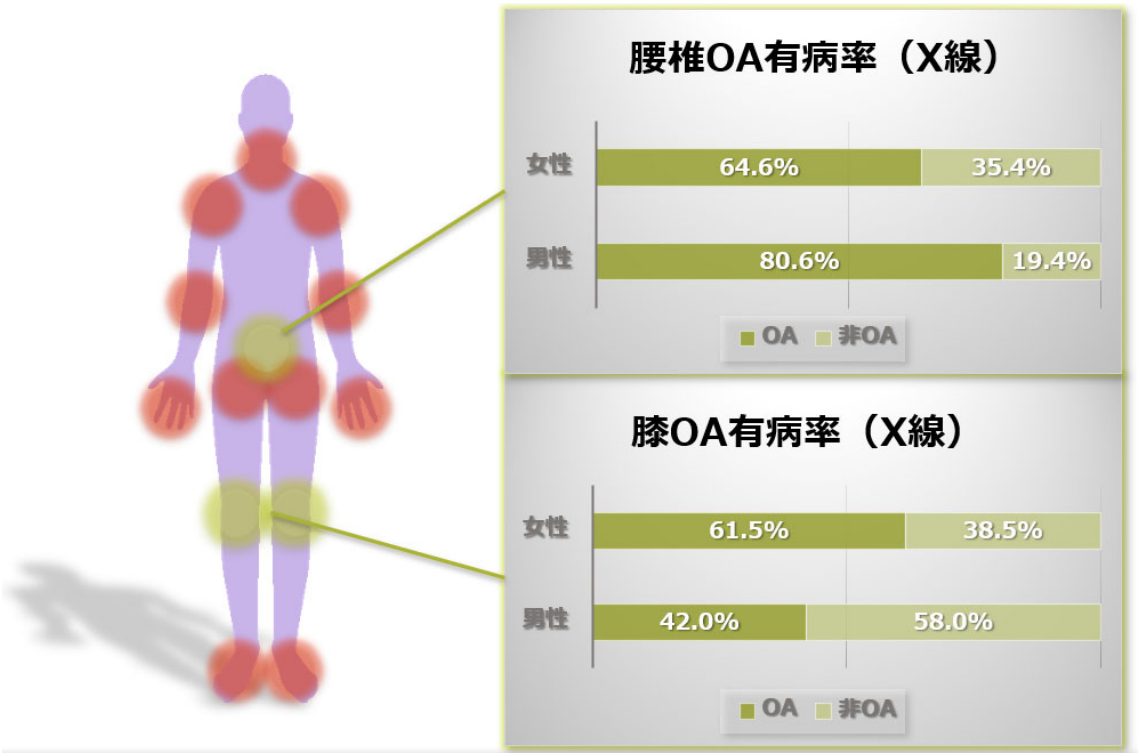


Figure 1-1. Epidemiology of Osteoarthritis (OA).

膝関節の模式図を**Figure 1-2**に示す。OAの関節は軟骨が摩耗し、これを補うために軟骨細胞が増加する。また、軟骨細胞の成熟が見られ、肥大した軟骨細胞から分泌されるプロテアーゼによって軟骨基質の変性がさらに進行する。軟骨の摩滅が進むと足がO脚やX脚になり関節が変形し、痛みを生じ、可動域が制限され、起立や歩行に大きな影響を与える。さらに進行すると軟骨下骨が露出し、骨そのものも変形しはじめ、とげのように飛び出した骨棘（こつきょく）と言われる骨変性を生じ、大きな痛みを伴う。現在の治療法は、非ステロイド性抗炎症薬（Non-Steroidal Anti-Inflammatory Drugs: NSAID）または選択的シクロオキシゲナーゼ-2阻害剤（COXIB）による症状の緩和、ヒアルロン酸の関節内注射、または外科的関節置換術に限定されている。中でもCOXIBは心臓発作や脳卒中などの心血管イベントの増加により、その使用に制限があるため[2, 3]、疾患の進行を変えることができ、安全性が高く有効な、疾患修飾性変形性関節症薬（disease modifying drugs for osteoarthritis: DMOAD）の開発が急務となっている[4]。

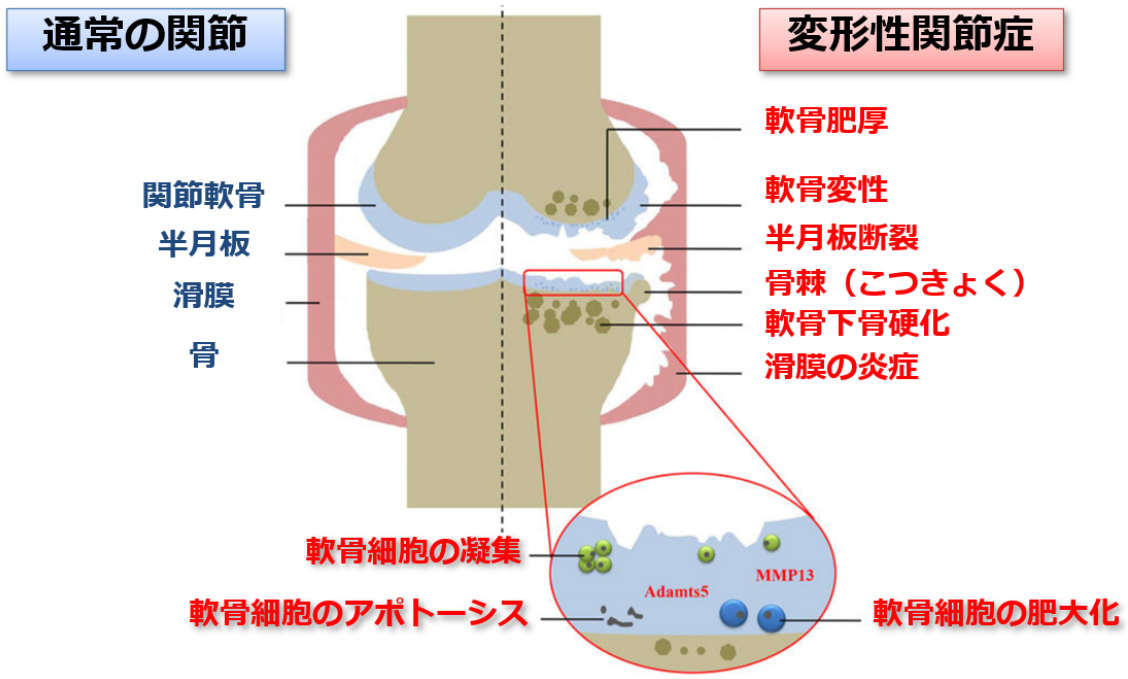


Figure 1-2. Comparison of normal knee joint and osteoarthritis. [4]

1-2　軟骨の構造

軟骨は骨の表面を覆うように存在しており、常に摩擦が発生する部位であるため、大変小さな摩擦係数をもつすぐれた力学特性を有している。また、軟骨は血管やリンパ管、神経の無い組織で、全体の7割程度を占める水を除いては、軟骨細胞と、コラーゲンやアグリカンからなる細胞外基質（extracellular matrix: ECM）により構成されている。ECMの主な成分はコラーゲンで、その多くの部分はタイプIIコラーゲンである。その他のタンパク質として大部分はアグリカンと言われるプロテオグリカンとなっている（**Figure 1-3**）[5]。OA患者の関節では、関節軟骨の機械的負荷によって軟骨の形成よりも分解が優位となり、ECMの減少をきたす。さらに軟骨欠損を補うために、増加し肥大した活性化軟骨細胞から分泌されるマトリックスメタロプロテアーゼ（matrix metalloproteinases: MMPs）やアグリカナーゼによりコラーゲンやアグリカンが分解されることによって軟骨基質の変性が進行する[6]。

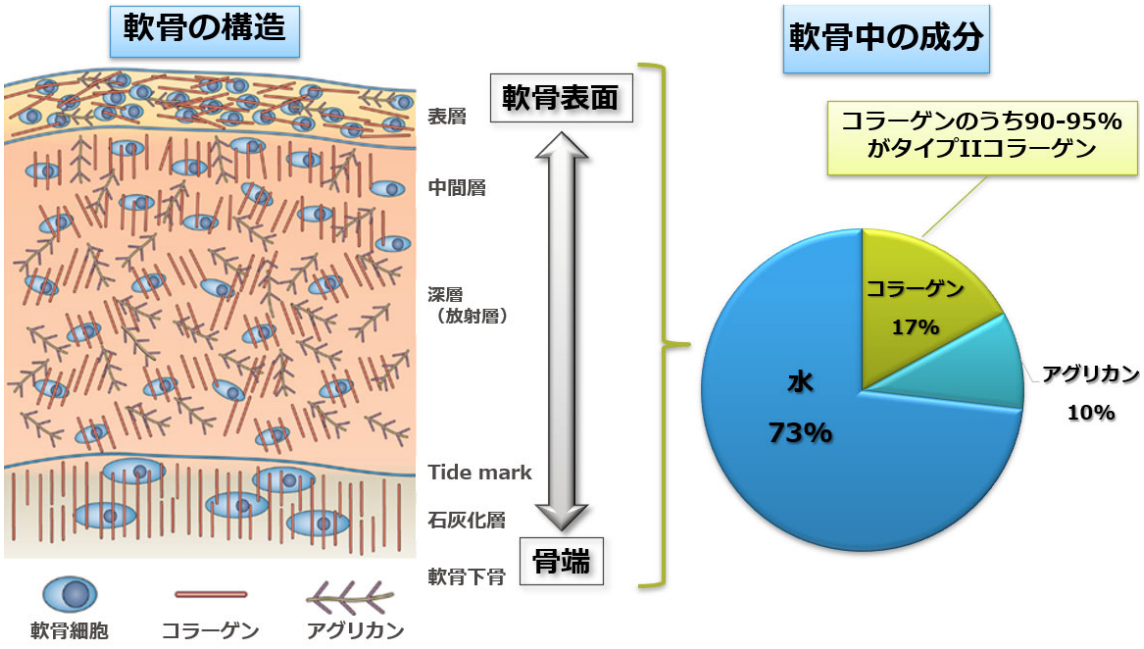


Figure 1-3. Cartilage structure and component distribution. [7]

1-3　MMPの種類

MMP の基質となる、コラーゲンは、かつては骨や腱など強固な組織に含まれる ECM 成分とともに活発には代謝されないと考えられていたが、MMP ファミリー[8,9]のうち、MMP-1（collagenase 1）は、オタマジャクシのカエルへの変態において、尾ひれが溶解することにコラーゲンのリモデリングが関与していることから 1962 年に、Jerome Gross と Charles Lapiere により最初に発見された[10]。また、ヒトでのコラゲナーゼの発見は、歯周炎患者からのものが最初であった[11]。その後、MMP は現在までに次々と非常に多くの種類が同定されており、ヒトでは 20 種以上が知られている。MMP の基質となるコラーゲンは生体組織の主要な構造タンパク質であり、約 30 年前から MMP が発生期および癌や関節炎において極めて重要な役割を果たすことが示されており、昨今では、変形性関節症（OA）、関節リウマチ、癌、炎症性腸疾患、歯周病、角膜潰瘍などのさまざまな疾患において MMP の関与が示唆されている[12]。MMP ファミリーは、共通のドメイン構造を有する 20 以上の亜鉛依存性酵素からなるが、これらの酵素群は、歴史的に基質に基づく命名および、発見の順序に基づくナンバリングからの命名がなされている（**Figure 1-4**）。なお、MMP-4, 5, 6 は既知の MMP と同じものであったため、欠番となっている[9]。MMP は基質特異性と相同性に基づいて、コラゲナーゼ、ゼラチナーゼ、ストロメライシン、マトリライシン、膜型 MMP の 5 つのグループに分類される。

	MMPs
コラゲナーゼ	MMP-1 (コラゲナーゼ1), MMP-8 (コラゲナーゼ2), **MMP-13 (コラゲナーゼ3),** MMP-18 (コラゲナーゼ4)
ゼラチナーゼ	MMP-2 (ゼラチナーゼA), MMP-9 (ゼラチナーゼB)
ストロメライシン	MMP-3 (ストロメライシン1), MMP-10 (ストロメライシン2), MMP-11 (ストロメライシン3)
マトリライシン	MMP-7, MMP-26
膜型MMPs	MMP-14 (MT1-MMP), MMP-15 (MT2-MMP), MMP-16 (MT3-MMP), MMP-17 (MT4-MMP), MMP-24 (MT5-MMP), MMP-25 (MT6-MMP)

MMP: matrix metalloproteinase; **MT**: membrane type

Figure 1-4. Types of MMPs and their classification.

1-4　MMP-13とOAの関係

MMPファミリーの中でも、ヒト乳がん細胞から最初にクローニングされたMMP-13は、

1）OA患者の軟骨に高発現している[13-16]

2）MMP酵素群の中でタイプIIコラーゲンに高い分解活性を示す[17, 18, 18]

3）MMP-13高発現マウスはOA様の病態の進行に関与している[19]

4）MMP-13ノックアウトマウスでOAモデルを作成しても軟骨変性・破壊が惹起されにくい[20]

等の報告から、OAにとって重要な役割を担うことが示唆されている。

MMPの基質となる、コラーゲンはグリシン-X-Yというアミノ酸配列の繰り返しの3重らせん構造からなっており、タイプIIコラーゲンはMMP-13によりGly975-Leu976が最初に切断され（**Figure 1-5**）、その後変性してゼラチンとなり繊維を形成することが出来なくなる[21]。また、軟骨の主成分のタイプIIコラーゲンはMMP-13により最も効率よく切断されることが報告されている[18, 22]。

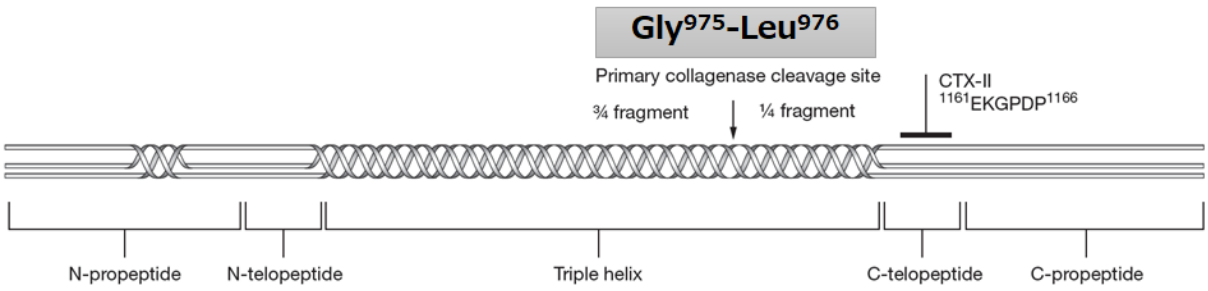


Figure 1-5. Collagen structure and its cleavage site.[23]

1-5　MMPの構造と触媒メカニズム

Figure 1-6 に示したように、MMP は亜鉛イオンに結合する 3 つのヒスチジン残基（H）とメチオニンターン”HEXXHXXGXXH…Met-turn”モチーフを構造上の特徴としている [24, 25]。

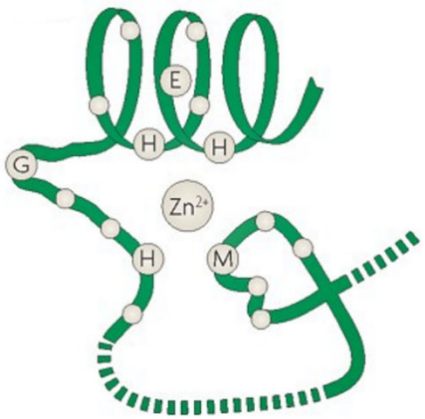


Figure 1-6. A structural feature of the active center of MMP.[26]

活性型 MMP は、活性中心の亜鉛および [27]、グルタミン酸側鎖 [28] により亜鉛の配位水が活性化されており（下図）(I)、ペプチド基質とミカエリス複合体（Michaelis complex）を形成した後 (II)、水分子がアミド結合のカルボニル基にアタックし、四面体反応中間体（III）を経て、ペプチド結合が切断された、生成物複合体（IV、V）を生成する（**Figure 1-7**）。

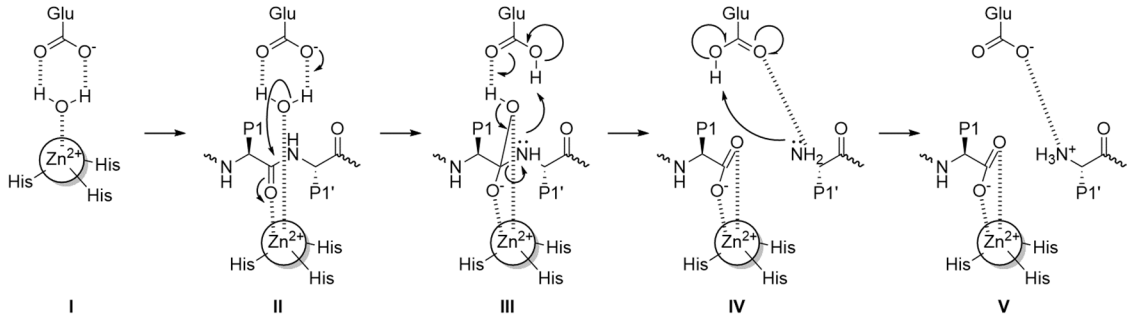


Figure 1-7. Catalytic mechanism of MMP.[29]

1-6　MMPの構造と亜鉛結合型非選択的阻害薬の歴史

MMP には触媒活性中心の亜鉛を中心として、近傍に unprimed pocket や left-handed サイドと呼ばれる S3、S2、および S1 ポケットと、primed pocket や right-handed サイドと呼ばれる S3’、S2’、および S1’ポケットが見られ、これらのポケットの中で、S1’ポケットは、最も深いポケットであり、また、それを構成するアミノ酸組成にも最も多様性が見られる。MMP はしばしばこの S1’ポケットの深さで分類され、深い（MMP-3, 11, 12, 13, 14）、中間（MMP-2, 8, 9）、浅い（MMP-1, 7）ポケットが知られており（**Figure 1-8**）、この S1’ポケットの特徴を利用して、種々の選択的 MMP 阻害薬の合成が試みられてきた [30, 31]。

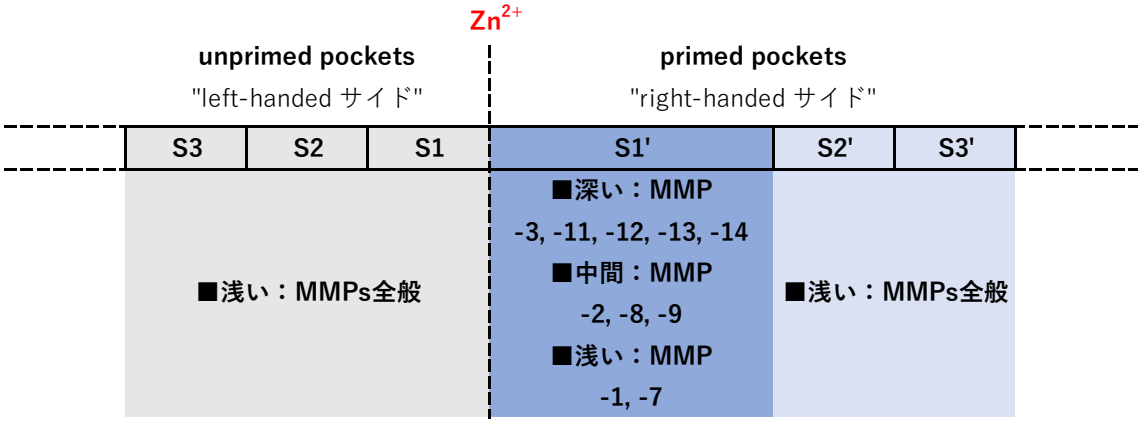


Figure 1-8. Structural features of the pocket of the catalytically active center of the MMP.

MMP-2、9など、特定のMMP類はがんに関与していることが知られており、がんを指向したMMP阻害薬の開発研究が盛んに行われたが[12, 32]、初期のMMP阻害薬のデザインは、酵素の活性中心に存在する亜鉛を補足するためのヒドロキサム酸などの亜鉛結合基（zinc binding group: ZBG）の近傍に、コラーゲン等の基質の被切断部位付近のアミノ酸側鎖を模倣した置換基（P1'）を結合した偽基質を合成するというアプローチ[10, 11]を基本としており（**Figure 1-9**）、これらの多くは関節痛や筋肉痛を代表とする筋骨格系症候群（musculoskeletal syndrome: MSS）と称される一連の副作用により臨床開発がことごとく中断された[33-35]。

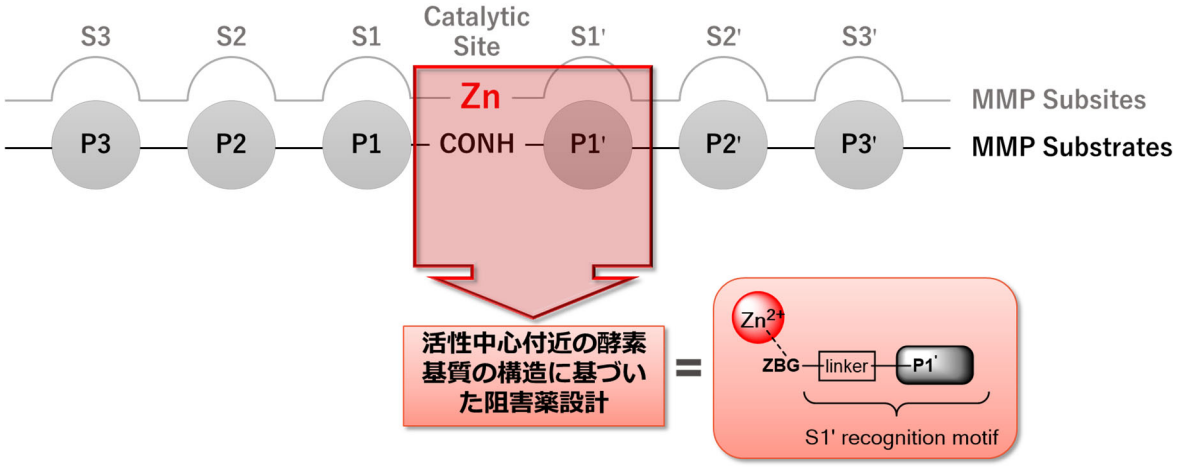


Figure 1-9. Traditional MMP inhibitor design.

具体的には、**Figure 1-10**に示した初期のMMP阻害薬は血管新生阻害や、転移抑制薬として、抗がん薬を志向したものがほとんどで、全てMMPの活性中心に存在する亜鉛を補足する亜鉛結合基を有するものであり、様々な癌に対して50種類以上のMMP阻害剤の臨床試験が行われてきたが、多くはMSSに代表される副作用や、薬効の不足により臨床試験は全て失敗に終わった[36]。これらの副作用は投薬を中断すると消失するものであったが、この副作用の理由としては、他の金属酵素や、MMPファミリーへの低い選択性に起因すると考えられた。

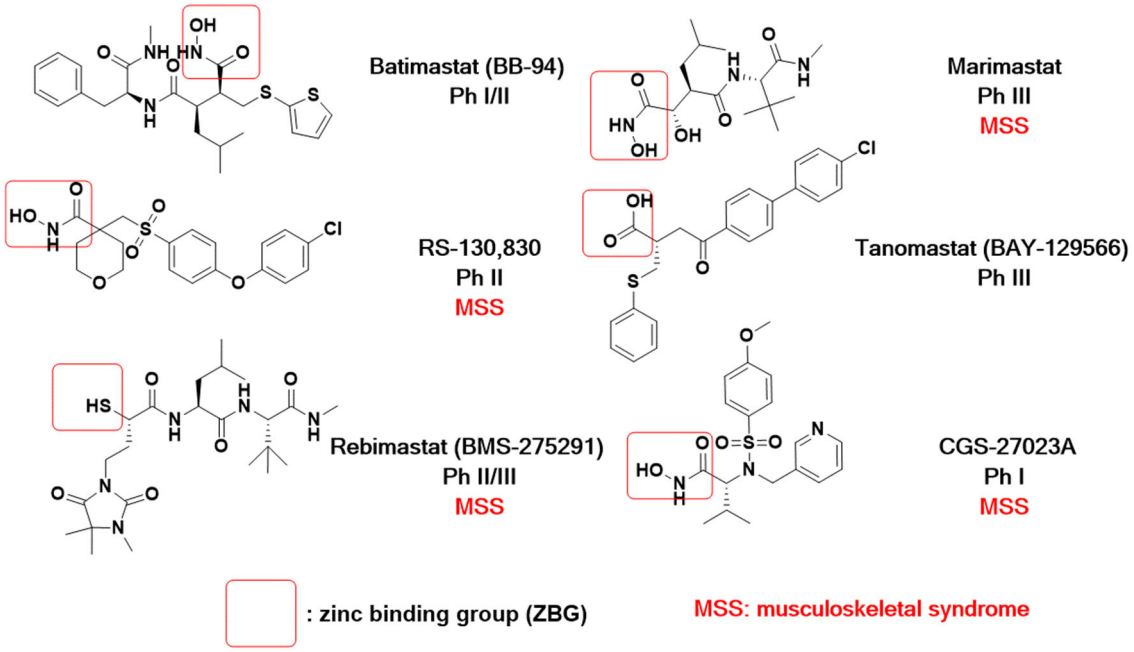


Figure 1-10. Clinically tested non-selective inhibitors with zinc binding groups.

そこで、軟骨の主成分であるタイプIIコラーゲンの分解活性の高いMMP-13に着目し、選択性の向上を達成するためにMMPサブファミリー間で高度に構造が保存されている活性中心から離れた部分構造を利用した選択的MMP-13阻害薬を創出する新たな戦略を立案した。具体的には、MMP-13との共結晶解析像に基づき、精密なStructure-Based Drug Design（SBDD）を行うことで、活性中心の亜鉛との相互作用が無くても、それを補完し得る新たな相互作用を探索し、デザイン・合成することとした。

1-7　他のプロテアーゼ阻害薬との比較

現在臨床で用いられているプロテアーゼ阻害薬と、我々が目指す MMP-13 阻害薬（一番上）のデザインの違いをまとめた（**Figure 1-11**）。プロテアーゼ阻害薬一般が活性中心の金属やアミノ酸をターゲットとし、基質認識サイトを広く使うのに対し、今回は活性中心との相互作用を用いず、S1'と、MMP-13 に特異的な S1"ポケットを活用するユニークなアプローチとなる。

酵素	臨床薬	適応	相互作用するサイト							
			S4	S3	S2	S1	活性中心	S1'	S2'	S3'
MMP-13	-	(変形性関節症)						S1'/S1''		
MMPs	-	癌					Zn			
アンジオテンシン変換酵素	カプトプリル	高血圧症					Zn			
HIVプロテアーゼ	リトナビル	HIV-1感染症					Asp			
レニン	アリスキレン	高血圧症		S3/S3sp			Asp			
NS3/4Aプロテアーゼ	シメプレビル	C型肝炎			S2/exS2		Ser			
ジペプチジルペプチダーゼ	ビルダグリプチン	2型糖尿病					Ser			
プロテアソーム	ボルテゾミブ	多発性骨髄腫					Thr			

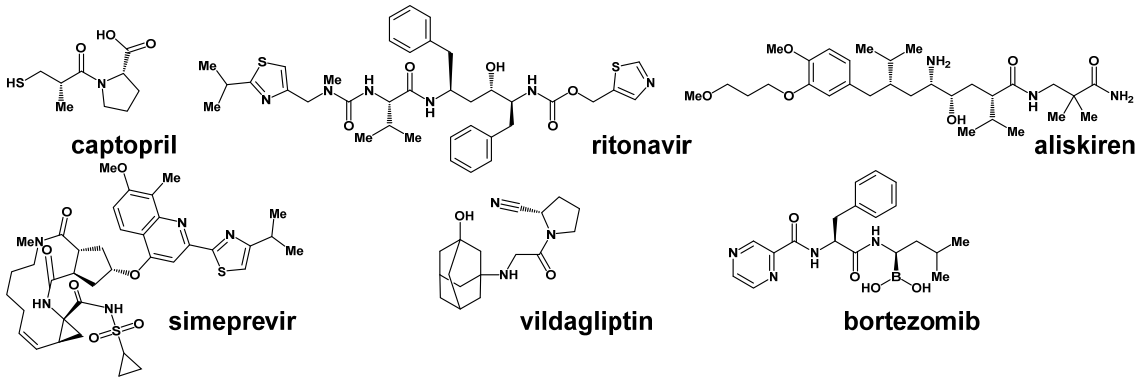


Figure 1-11. Comparison with other protease inhibitors.

2-1　非亜鉛結合型MMP-13選択的阻害薬のデザイン

武田薬品工業株式会社の化合物ライブラリーからMMP-13阻害活性を指標としたハイスループットスクリーニングにより、キナゾリン誘導体**1**が発見された。他の多くのヒットケモタイプとは異なり、キナゾリン誘導体**1**の構造には、ヒドロキサム酸や、チオール、カルボン酸などの典型的な亜鉛結合基が見られなかったため、キナゾリン誘導体**1**とMMP-13の触媒ドメインとの共結晶構造解析を行ったところ、MMP-13のアミノ酸残基245～253からなる柔軟で大きな疎水性S1'ポケット[37]に結合しており、MMP活性中心の亜鉛とは結合していないことが判明した。また、亜鉛とは離れた側のS1'ポケットの側面にそれまでは見られなかった、新たな脂溶性ポケット（S1"ポケット[38]）が認められた（**Figure 2-1**）。

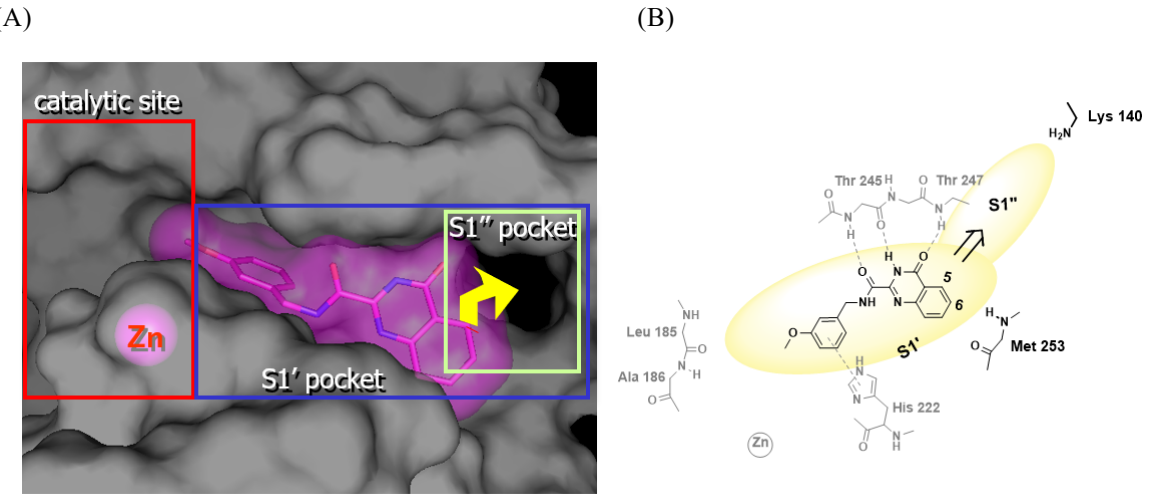


Figure 2-1. Crystal structure of the complex of hit quinazoline compound **1** and MMP-13 (PDB code: 3WV2). (A) Surface representation of MMP-13 illustrating the binding cavity. The inhibitor is buried deeply into the S1' pocket. (B) Schematic representation of the binding mode of compound **1** and MMP-13.

続いて、解析像を基に仮説を立て、以下の化合物デザイン（structure-based drug design: SBDD）を行うこととした[39-41]。即ち、

1. 縮合ピリミジン核と側鎖ベンゼン環を結ぶリンカー部分の修飾
2. S1'ポケットに収まる、P1'に相当するキナゾリンの部分構造であるA環の探索
3. 新たに認められた脂溶性S1"ポケットを志向した脂溶性P1"の導入および、S1"ポケットの奥に認められたLys残基との水素結合の獲得

を検討することにした（**Figure 2-2**）。

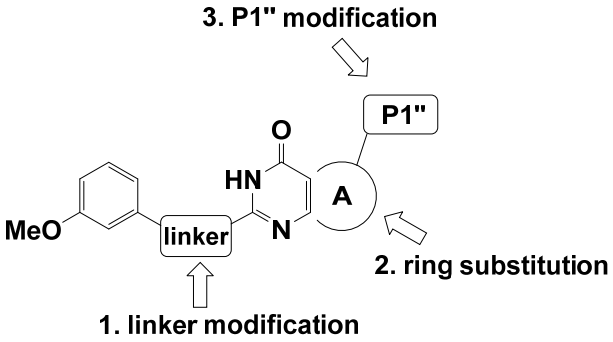


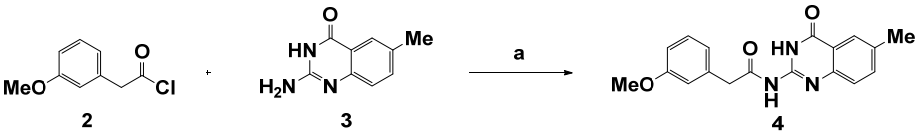
Figure 2-2. Structural Modification of Fused Pyrimidine MMP Inhibitors

リード化合物 **1** の左側に位置するベンジルアミド部分を効率的に最適化するために、ハイスループット合成により、類似誘導体からなる小ライブラリーを調製し予備検討を行った。結果、リード化合物 **1** の左側部分の置換ベンジル誘導体のフェニル基の 3、4 位が置換基の導入に有望であることがわかった。それらの中で、3-メトキシ基は、末端アリール基の最良の置換基の 1 つであることがわかった。従って、構造活性相関を検討するために、3-メトキシフェニル誘導体を用い、最適化を実行することとした。

2-2　キナゾリン誘導体の合成

リバースアミド誘導体 **4** は、トリエチルアミンの存在下で市販の **3** を 2-(3-メトキシフェニル)アセチルクロリド **2** と反応し、71%の収率で得た（**Scheme 2-1**）。

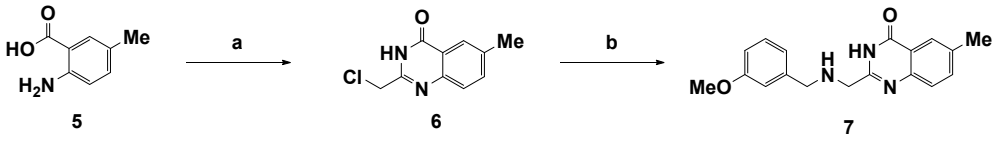
Scheme 2-1. Synthesis of Reverse Amide Derivative **4**[a]



[a]Reagents and conditions: (a) Et_3N, THF, DMA, 90 °C, 71%.

Scheme 2-2 に示すように、メタノール中、80 °C でナトリウムメトキシド存在下、メチルアントラニル酸 **5** とクロロアセトニトリルの反応により、2-（クロロメチル）キナゾリノン誘導体 **6** が 67%の収率で得られた。**6** の塩素原子を DMF 中、炭酸カリウム存在下、3-メトキシベンジルアミンで置換し、アミンリンカー誘導体 **7** を 41%の収率で得た。

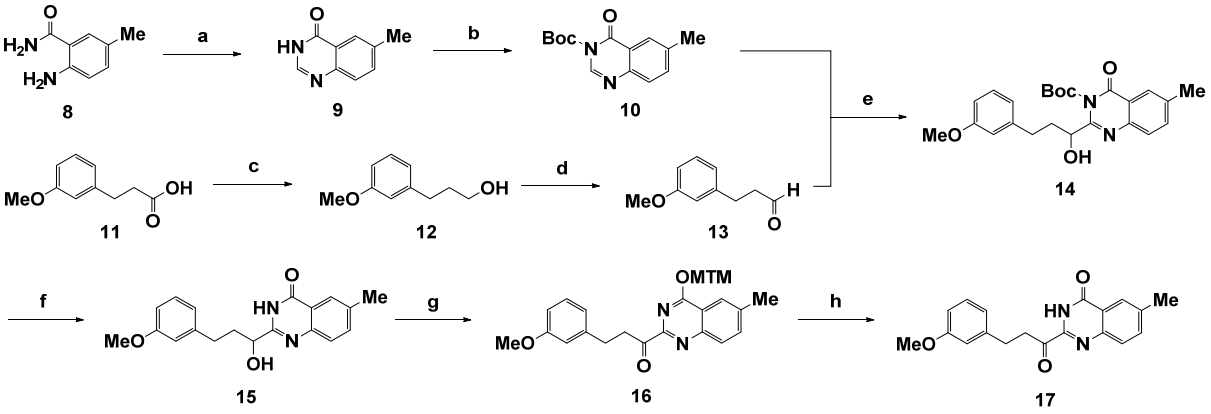
Scheme 2-2. Synthesis of Amine Derivative **7**[a]



[a]Reagents and conditions: (a) chloroacetonitrile, MeONa, MeOH, rt to 80 °C, 67%; (b) 3-methoxybenzylamine, K_2CO_3, THF, 40 °C, 41%.

カルボニルリンカーを有する **17** の合成経路を **Scheme 2-3** に示した。即ち、ベンズアミド誘導体 **8** とオルトギ酸トリメチルから、環化体である 6-メチル-3,4-ジヒドロキナゾリン-4-オン誘導体 **9** を得た後、化合物 **9** の 3 位窒素原子を Boc 基で保護した **10** を LDA で処理後、カルボン酸誘導体 **11** から２工程で合成したアルデヒド誘導体 **13** を反応してヒドロキシル誘導体 **14** を得た。14 の Boc 基を脱保護した後、**15** の２級水酸基を、塩化オキサリルとジメチルスルホキシド条件下 Swern 酸化に付し、同時にキナゾリン環の 4-カルボニルをメチルチオメチル（methylthiomethyl：MTM）エーテルとして保護して *O*- MTM 誘導体 **16** を得た後、 MTM 基の酸性条件下の加水分解により、標的化合物 **17** を得た。

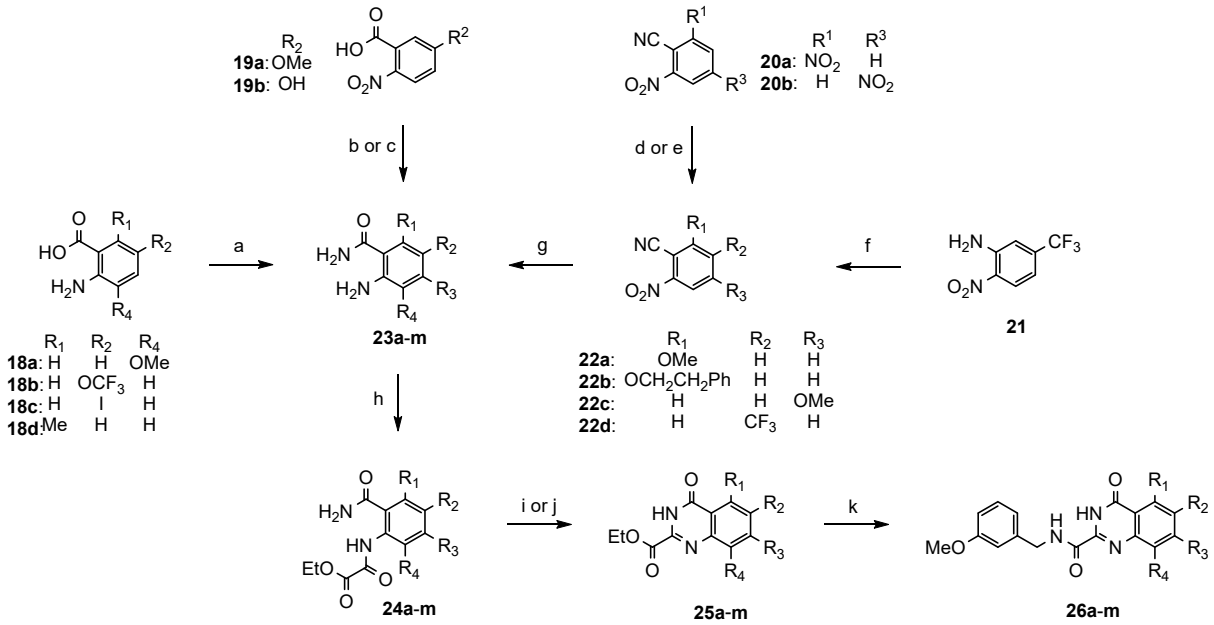
Scheme 2-3. Synthesis of Ketone Derivative **17**[a]



[a]Reagents and conditions: (a) $CH(OMe)_3$, conc. HCl, 0 °C to rt, 81%; (b) Boc_2O, NaH, THF, 0 °C to rt, 91%; (c) (1) oxalyl chloride, DMF, THF, rt; (2) $NaBH_4$, THF, reflux, 92%; (d) TPAP, NMO, MS4A, CH_2Cl_2, rt, 63%; (e) (1) **10**, LDA, THF, –78 °C; (2) **13**, –78 °C to rt, 44%; (f) TFA, CH_2Cl_2, rt, 45%; (g) DMSO, oxalyl chloride, Et_3N, –78 °C to rt, 41%; (h) TFA, H_2O, CH_2Cl_2, rt, 64%.

鍵中間体である 4-オキソ-3,4-ジヒドロキナゾリン-2-カルボン酸エチルエステル誘導体 **25** は、アントラニル酸アミド類 **23** からアシル化/環化の 2 段階の反応を経由して（method A、**Scheme 2-4**）、またはアントラニル酸エステル類 **32** の環化反応（method B、**Scheme 2-5**）により合成した。即ち、置換アントラニル酸誘導体 **18a–d** とトリホスゲンから調製したオキサジン誘導体をアンモニアでアミノリシスして、目的のアントラニル酸アミド誘導体 **23d**、**23h**、**23j**、および **23l** を得た。オルトニトロ安息香酸誘導体 **19a** を塩化オキサリルで塩化アシル誘導体に変換し、アンモニアと反応してカルボキサミド誘導体を得た後、ニトロベンズアミドのニトロ基の還元により、アントラニル酸アミド **23b** を得た。**23i** は、**19b** より、同様の方法論により得ることができた。ニトロベンゾニトリル誘導体 **22a–c** は、ジニトロベンゾニトリル類 **20a,b** を、対応するナトリウムアルコキシドと縮合させることにより合成した。 Raney ニッケル存在下でのニトロベンゾニトリル誘導体 **22a–c** とヒドラジンの反応により、ニトロ基をアミンに、シアノ基をカルボキサミドに同時に変換して、それぞれアントラニル酸アミド誘導体 **23a**、**23m**、および **23c** を合成した [42]。アントラニル酸アミド誘導体 **23g** は、2-ニトロ-5-トリフルオロメチルフェニルアミン（**21**）から Sandmeyer シアン化法によって得られたニトロベンゾニトリル誘導体 **22d** を使用して同様に合成した。アントラニル酸アミド誘導体 **23a–m** をクロログリオキシル酸エチルで *N*-アシル化した後、得た **24a–m** を、酸または塩基で処理して、4-オキソ-3,4-ジヒドロキナゾリン-2-カルボン酸エチルエステル誘導体 **25a–m** を得た。**25a–m** を DMF または EtOH 中で 3-メトキシベンジルアミン存在下、加熱し、アミノリシスすることにより対応するアミド誘導体 **26a–m** を得ることができた。**25a–m** の 2 位のエステル基は、第一級脂肪族アミンに対して反応性が高く、塩基非存在下でも反応が進行した。この 2 位エステルの高い反応性は、隣接するピリミジン 3 位 NH 基による分子内水素結合による隣接基関与によるものであると考えられる。

Scheme 2-4. Synthesis of 4-Oxo-3,4-dihydroquinazoline-2-carboxamide Derivatives **26a-m** (method A)[a]



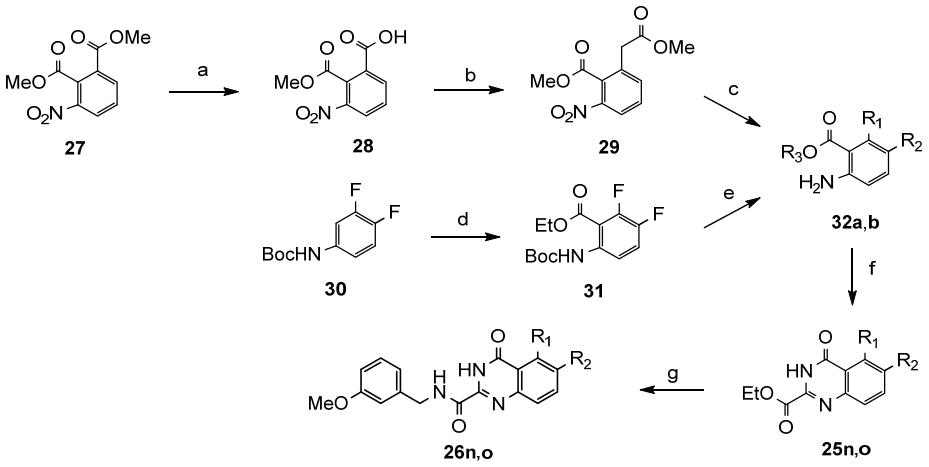
compound				R_1	R_2	R_3	R_4
23	**24**	**25**	**26**				
23a	**24a**	**25a**	**26a**	OMe	H	H	H
23b	**24b**	**25b**	**26b**	H	OMe	H	H
23c	**24c**	**25c**	**26c**	H	H	OMe	H
23d	**24d**	**25d**	**26d**	H	H	H	OMe
23e	**24e**	**25e**	**26e**	H	F	H	H
23f	**24f**	**25f**	**26f**	H	Me	H	H
23g	**24g**	**25g**	**26g**	H	CF_3	H	H
23h	**24h**	**25h**	**26h**	H	OCF_3	H	H
23i	**24i**	**25i**	**26i**	H	OBn	H	H
23j	**24j**	**25j**	**26j**	H	I	H	H
23k	**24k**	**25k**	**26k**	F	H	H	H
23l	**24l**	**25l**	**26l**	Me	H	H	H
23m	**24m**	**25m**	**26m**	OCH_2CH_2Ph	H	H	H

[a]Reagents and conditions: (a) (1) triphosgene, THF, 50 °C; (2) NH_3, H_2O, 50 °C, 13–75% over 2 steps; (b) (1) $(COCl)_2$, DMF, THF, 0 °C to rt; NH_3, H_2O, rt, 56%; (2) H_2, Pd on carbon, MeOH, rt, 99% (for **23b**); (c) (1) BnBr, K_2CO_3, rt, 98%; (2) KOH, MeOH, H_2O, reflux, 84%; (3) $(COCl)_2$, DMF, THF, 0 °C to rt; NH_3, H_2O, rt, 93%; (4) Fe powder, NH_4Cl, EtOH, H_2O, reflux, 100% (for **23i**); (d) MeONa, MeOH, reflux, 38–99% (for **22a**,**c**); (e) 2-phenylethanol, NaH, DMF, 90 °C, 32% (for **22b**); (f) $NaNO_2$, HCl, H_2O, 0 °C to rt; CuCN, NaCN, toluene, H_2O, 0 °C to reflux; (g) NH_2NH_2•H_2O, Raney Ni, EtOH, 40 °C to reflux, 40–88% (for **23a**, **23c**, and **23m**), 16% over 2 steps (for **23g**); (h) ethyl chloroglyoxylate, Et_3N, THF, 0 °C to rt, 79–100%; (i) NaOEt, EtOH, 0 °C to rt, 38–87% (for **25b** and **25d**–**m**); (j) *p*-TsOH, toluene, reflux, 22–27% (for **25a** and **25c**); (k) 3-methoxybenzylamine, DMF or EtOH, 80–90 °C, 28–92%.

鍵中間体 **25n,o** は、シアノギ酸エチルと 1 M HCl/AcOH 条件下の 1 工程（method B、**Scheme 2-5**）で容易に合成できるアントラニル酸エステル類 **32a,b** から合成した[43]。**32a,b** の合成は以下のように行った。即ち、ジエステル誘導体 **27** の水酸化リチウム水溶液による加水分解により、モノカルボン酸誘導体 **28** を得た後、Arndt–Eistert 反応によりホモロゲーションを行い、メチルエステル誘導体 **29** を得た[44]後、パラジウム炭素存在下、**29** のニトロ基を接触還元し、アントラニル酸エステル誘導体 **32a** を得た。一方、2 当量の *n*-ブチルリチウムを用いた **30** の 2 位の選択的リチウム化、続いてクロロ炭酸エチルへの求核付加反応により、6-アミノ-2,3-ジフルオロ安息香酸エチルエステル誘導体 **31** を得た[45]後、**31** の Boc 基を除去し、**32b** が得られた。

鍵中間体 **25n,o** を 3-メトキシベンジルアミンでアミノリシスし、**26n,o** を得た。

Scheme 2-5. Synthesis of 4-Oxo-3,4-dihydroquinazoline-2-carboxamide Derivatives **26n**–**o** (method B)[a]



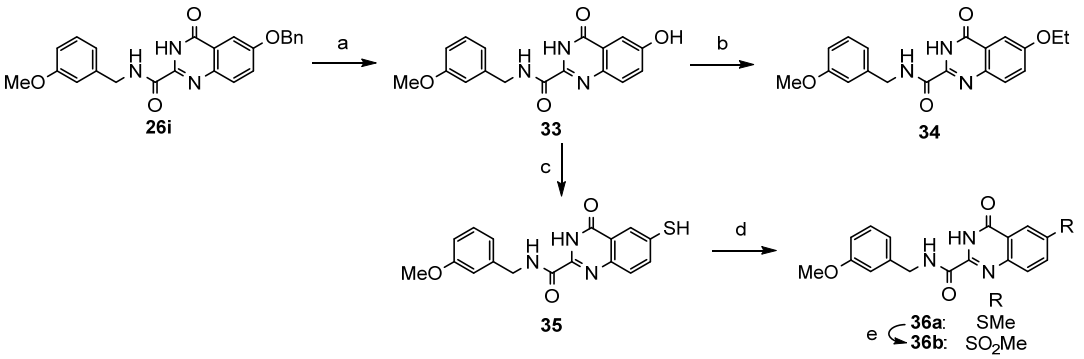
compound					
32	**25**	**26**	R_1	R_2	R_3 (for **32**)
32a	**25n**	**26n**	CH_2CO_2Me	H	Me
32b	**25o**	**26o**	F	F	Et

[a]Reagents and conditions: (a) LiOH, H_2O, THF, rt, 96%; (b) (1) $(COCl)_2$, DMF, THF, rt; (trimethylsilyl)diazomethane, TEA, Et_2O, THF, CH_3CN, 0 °C; (2) MeOH, AgOBz, TEA, THF, rt, 67% over 2 steps; (c) H_2, Pd on carbon, THF, MeOH, rt, 99%; (d) *n*-BuLi, $ClCO_2Et$, THF, –78 °C; (e) HCl, AcOEt, rt, 70% over 2 steps; (f) $NCCO_2Et$, HCl, AcOH, 80 °C, 76–82%; (g) 3-methoxybenzylamine, EtOH, 80 °C, 78–84%.

Scheme 2-6 に示すように、6-ベンジルオキシ誘導体 **26i** をパラジウム炭素存在下、水素化分解により脱ベンジル化してフェノール誘導体 **33** を得た後、ヨウ化エチルを用いてエチル化を行い、6-エトキシ誘導体 **34** に変換した。なお、このアルキル化の 21%という低収率は、ピリミジン環の競合するアルキル化によるものであった。

上記のフェノール誘導体 **33** を *O*-アリールチオカルバメートに変換し、これを Newman–Kwart 熱転位反応 [46] に付し、*S*-アリールチオカルバメートを得た。この反応中間体は容易に加水分解されてチオール誘導体 **35** が得られ、続いてヨウ化メチルでアルキル化し、6-メチルチオ誘導体 **36a** を得た。**36a** のスルフィドの酸化により、スルホン誘導体 **36b** を得た。

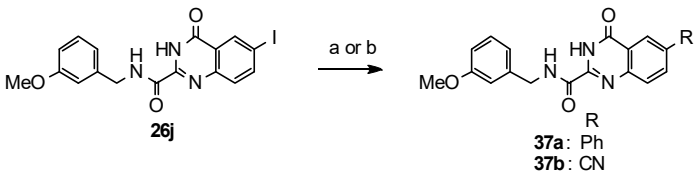
Scheme 2-6. Modification of Substituents at C-6 Position[a]



[a]Reagents and conditions: (a) H_2, Pd on carbon, THF, MeOH, rt, 100%; (b) EtI, Cs_2CO_3, DMF, THF, rt, 21%; (c) (1) *N*,*N*-dimethylthiocarbamoyl chloride, DABCO, DMF, rt, 96%; (2) *N*,*N*-diethylaniline, 210 °C, 82%; (3) KOH, MeOH, reflux, 95%; (d) MeI, Et_3N, rt, 85%; (e) mCPBA, $CHCl_3$, rt, 95%.

37a および **37b** の合成は、パラジウムカップリング反応で行った。化合物 **37a** および **37b** は、**Scheme 2-7** に示すように、共通の中間体 **26j** から合成した。

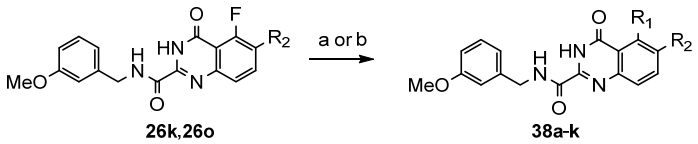
Scheme 2-7. Modification of Substituents at C-6 Position[a]



[a]Reagents and conditions: (a) $PhB(OH)_2$, $Pd(PPh_3)_4$, Na_2CO_3, EtOH, toluene, H_2O, reflux, 64% (for **37a**); (b) $Zn(CN)_2$, $Pd(PPh_3)_4$, DMF, 80 °C, 68% (for **37b**).

Scheme 2-8 に示すように、**26k** の 5-フルオロ基をさまざまな求核試薬（RONa、RSNa、RNH_2）との芳香族求核置換反応により置換し 5-置換誘導体 **38a–g** を合成した[47]。一方、5,6-ジフルオロ誘導体 **26o** への位置選択的な 5 位への反応は、より穏やかな条件下で行うことができ、フェネチルアルコール類による置換は、競合するスチレン誘導体への脱離反応を伴うものの、エーテル誘導体 **38h–k** を中程度の収率で得ることができた。

Scheme 2-8. Nucleophilic Substitution Reaction of 5-Fluoro Derivatives **26k** and **26o**[a]



26k,**26o** **38a-k**

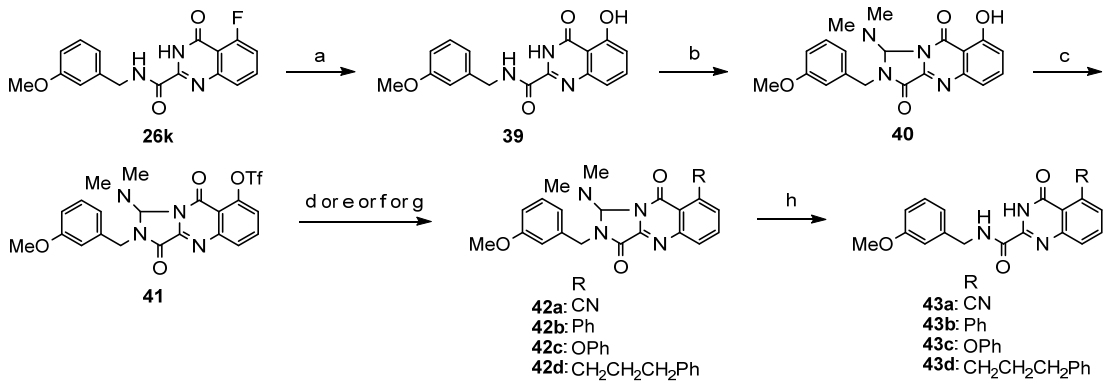
compound	R_1	R_2	yield (%)
38a	O Ph	H	81
38b	O Ph	H	44
38c	O O Ph	H	76
38d	HN Ph	H	51
38e	S Ph	H	55
38f	O	H	55
38g	O N	H	35
38h	O	F	35
38i	O F	F	32
38j	O NH_2	F	54
38k	O CO_2H	F	29

[a]Reagents and conditions: (a) ROH or RSH, NaH, DMA, rt–80 °C (for **38a**–**c** and **38e**–**k**); (b) RNH_2, DMA, 80–100 °C (for **38d**).

化合物 **43a–d** は、**Scheme 2-9** に示すように、フッ化物誘導体 **26k** から数工程で得られる共通中間体 **41** から合成した。即ち、**26k** の 5 位フッ素原子をフェネチルオキシ基で置換し、引き続くフェネチル基の β-脱離反応による除去をワンポット行い、**39** を得た。**39** のピリミジン環と、側鎖アミドは副生成物の生成につながる反応に関与することがわかったため、トリフレーションに先立ち保護を行った。即ち、新規に見出した、5 員環状 DMF-アミナールを保護基として利用して、イミダゾリジン環を形成することによりキナゾリン-4-オン-2-カルボキサミド誘導体 **39** を効率的に保護した。この保護基は、Vilsmeier 条件下で容易に導入でき、塩基や求核試薬に対して安定であり、一方、酢酸中、60°C で 2〜3 時間塩酸で処理することにより、効果的に除去できることがわかった。

保護されたフェノール誘導体 **40** は、トリフラート誘導体 **41** に変換し、種々の求核試薬とのパラジウム触媒カップリングにより、カップリング生成物 **42a–d** が得られた。最後に、新規保護基を酢酸中、塩酸で脱保護して、目的とした **43a–d** を得た。

Scheme 2-9. Synthesis of 5-Substituted Derivatives **43a**–**d**[a]

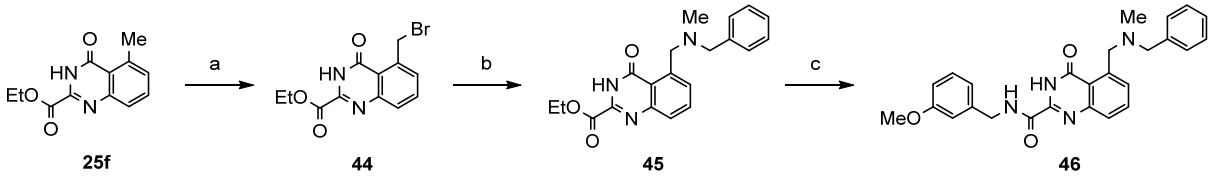


[a]Reagents and conditions: (a) 2-phenylethanol, NaH, THF, 90 °C, 97%; (b) (1) $(COCl)_2$, DMF, THF, 0 °C to rt; (2) NaOH, H_2O, THF, MeOH, rt, 90% over 2 steps; (c) Tf_2O, pyridine, CH_2Cl_2, 0 °C to rt, 97%; (d) $Zn(CN)_2$, $Pd(PPh_3)_4$, DMF, 150 °C under microwave, 82% (for **42a**); (e) $PhB(OH)_2$, $Pd(PPh_3)_4$, Na_2CO_3, EtOH, toluene, H_2O, reflux, 91% (for **42b**); (f) PhOH, $Pd_2(dba)_3$, 2-(di-*t*-butylphosphino)biphenyl, DIEA, toluene, 80 °C, 19% (for **42c**); (g) (1) 3-phenyl-1-propyne, $Pd(PPh_3)_4$, CuI, DIEA, DMF, rt; (2) H_2, Pd on carbon, THF, MeOH, rt, 21% over 2 steps (for **42d**); (h) HCl, H_2O, AcOH, 60 °C, 53–80%.

3 原子長のリンカー鎖を有する 5-置換 4-オキソ-3,4-ジヒドロキナゾリン（**46**、**51**、**53**）の合成は、対応する 5-ブロモメチル誘導体 **44**（**Scheme 2-10**）、**48**（**Scheme 2-11**）、または 5-カルボキシメチル誘導体 **52**（**Scheme 2-12**）を前駆体として合成した。

Scheme 2-10 に示すように、5-メチル-4-オキソ-3,4-ジヒドロキナゾリン **25f** のベンジル位の臭素化に続いて、二級アミンである、*N*-メチルベンジルアミンと反応して **45** を得、続いて一級アミンである、3-メトキシベンジルアミンと反応し、化合物 **46** を得た。

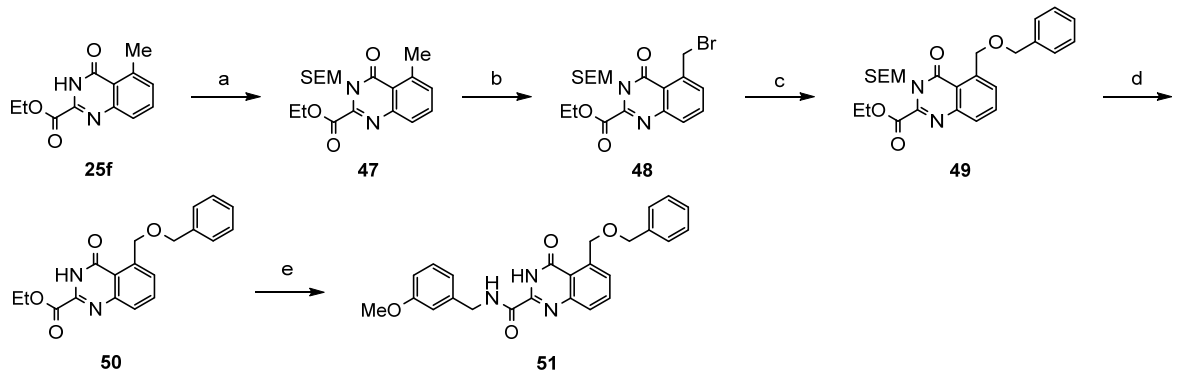
Scheme 2-10. Synthesis of Amino Linker Analogue **46**[a]



[a]Reagents and conditions: (a) NBS, AIBN, $CHCl_3$, reflux, 79%; (b) *N*-methylbenzylamine, pyridine, DMF, THF, 0 °C to rt, 59%; (c) 3-methoxybenzylamine, EtOH, 80 °C, 8%.

同様の方法で、エーテルリンカー類似体 **51** の合成を試みたが、構造不明の複数の副生成物が生成したため、**Scheme 2-11** に示したように、**25f** のピリミジン環を適切な保護基によって保護することとした[48]。即ち、SEM-Cl によるピリミジン環の 3 位の窒素の保護と、続く NBS によるベンジル位の臭素化により、臭化物 **48** を得た後、ベンジルアルコールと反応し、**49** を得た。酸性条件下で SEM 保護基を除去した後、**Scheme 2-10** に示した **46** の合成と同様の方法で、EtOH 中、3-メトキシベンジルアミンで処理することにより活性エステル（**50**）をアミノリシスし、化合物 **51** を得た。

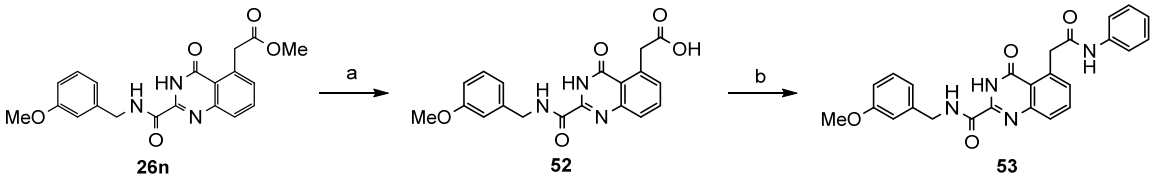
Scheme 2-11. Synthesis of Ether Linker Analogue **51**[a]



[a]Reagents and conditions: (a) SEMCl, NaH, DMF, rt, 77%; (b) NBS, AIBN, $CHCl_3$, reflux, 40%; (c) benzyl alcohol, NaH, THF, 0 °C to rt; (d) TFA, CH_2Cl_2, 0 °C to rt, 30% over 2 steps; (e) 3-methoxybenzylamine, EtOH, 80 °C, 51%.

また、**Scheme 2-12** に示すように、**26n** のメチルエステルの加水分解により、得たカルボン酸誘導体 **52** をアニリンと縮合して、P1"方向にアミドリンカーを有する誘導体 **53** を得た。

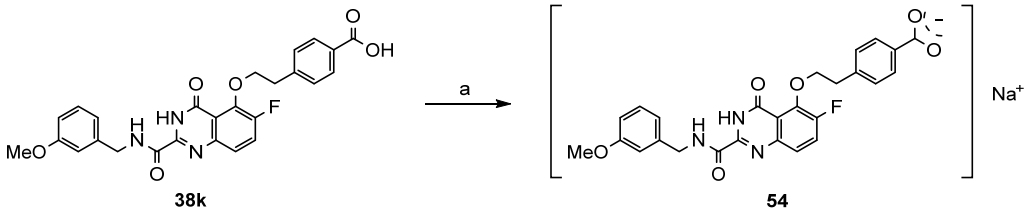
Scheme 2-12. Synthesis of Amide Linker Analogue **53**[a]



[a]Reagents and conditions: (a) NaOH, H_2O, THF, MeOH, 80 °C, 87%; (b) WSCD•HCl, HOBt, DMAP, DMF, 50 °C, 81%.

化合物 **38k** のナトリウム塩 **54** は、**Scheme 2-13** に示すように、含水溶媒中、1 または 2 当量の炭酸水素ナトリウムで **38k** を処理することによって調製した。小スケールのナトリウム塩の調製では、THF/MeOH/H_2O の混合溶媒系を採用したが、難溶性のカルボン酸誘導体 **38k** を溶解するために大量の溶媒が必要なため、**54**（**38k** の一ナトリウム塩）の数キログラムスケールの調製を行うために、溶媒を精査したところ、共溶媒として少量の DMSO を添加すると、**38k** を少量の溶媒に溶解するのに効果的であった。種々検討の結果、THF/MeOH/DMSO/H_2O（8 : 2 : 1 : 2 v/v）からなる混合溶媒系を用い、**54** のキログラムスケールでの調製を行うことが可能となった。

Scheme 2-13. Preparation of Salts of Compound **38k**[a]

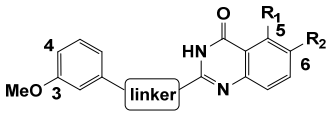


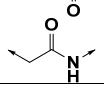
[a]Reagents and condition: (a) $NaHCO_3$ (1 equiv.), DMSO, THF, MeOH, H_2O, 80 °C; MeOH, reflux, 89%

2-3　キナゾリン誘導体の構造活性相関

キナゾリン環と 3-メトキシベンゼン環の間のリンカーの重要性を確認するために、リンカーの検討をまず行った。**Table 2-1** に、3 原子リンカー誘導体の構造活性相関を示した。リード化合物 **1** のアミドカルボニル基をメチレン（**7**）に置換すると、活性が大幅に低下し、逆アミドリンカー誘導体 **4** でも同様に活性が減弱することがわかった。対照的に、アミド窒素をメチレン基で置換するとある程度の活性が保持され(**17**)、リード化合物 **1** の X 線結晶解析から Tyr244 および Thr245 と相互作用すると想定されるカルボニル基の重要性が示された（**Table 2-1**）。また、**17** のカルボニル基をヒドロキシル基（**15**）に還元すると、活性が大幅に低下した。これらの結果より、オリジナルタイプのアミドリンカー（**1**、**26l**、および **26f**）が強力な酵素阻害活性を発揮するために重要であることが明らかとなった。一方、X 線結晶解析より示唆された、S1"ポケットを志向した置換基の導入は、キナゾリンの 5 位または 6 位（**26l** および **26f**）にメチル基を導入した化合物が高い阻害活性を保持したため、キナゾリンの 5 位または 6 位へのさらなる置換基導入が許容されることが示唆された。

Table 2-1. Effect of Linker Variation within 3,4-Dihydroquinazolin-4-one Series



compound	linker	R_1	R_2	IC_{50} (nM) [a]
1		H	H	12 ± 1.5
26l		Me	H	29 ± 3.4
26f		H	Me	26 ± 3.1
15 [b]		H	Me	>10000
7		H	Me	>10000
17		H	Me	220 ± 25
4		H	Me	>10000

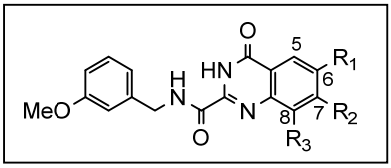
[a] IC_{50} (nM) represents the average IC_{50} value in nanomolars for the inhibition assay of the compound using the human MMP-13 catalytic domain enzyme. Data are expressed as mean IC_{50} ± SD, n = 3. [b] Tested as a racemic mixture.

ハイスループットスクリーニングにより新規 MMP-13 阻害薬として同定されたリード化合物 **1** と MMP-13 の X 線共結晶解析により、キナゾリン環が深く S1'ポケット結合していることが明らかになった。また、S1'ポケットのより深い領域に位置するサイドポケット（S1''）を、MMP-13 との選択的な相互作用を強化するための最初のターゲットとして選択した。さらに化合物 **1** のキナゾリン環の 5 位または 6 位は、置換基を導入するのに適した位置であると予測した。

実際に、キナゾリン環の 7 位または 8 位に小さなメトキシ基を導入すると、両化合物の活性は著しく低下した（**26c** および **26d**）ため（**Table 2-2**）、キナゾリン環の 7 位または 8 位の周辺の空間的余裕は無いと考え、構造活性相関の検討は、キナゾリン環の 5 位および 6 位の置換誘導体で行うこととした。

Table 2-2 に示すように、6 位のフッ素置換誘導体 **26e** は、6 位無置換のリード化合物 **1** と比較して MMP-13 阻害活性を維持した。一方、6 位メチル **26f** またはトリフルオロメチル **26g** 誘導体は、活性が低下した。対照的に、6 位をメトキシ基（**26b**）またはエトキシ基（**34**）で置換すると、MMP-13 阻害活性が 3〜5 倍向上し、トリフルオロメトキシ（**26h**）や、ベンジルオキシ（**26i**）基で置換すると、6 位メトキシ誘導体 **26b** と比較して活性は減弱した。メトキシ基のアイソスターであるメチルチオ誘導体 **36a** やシアノ誘導体 **37b** は 6 位メトキシ誘導体 **26b** と同等の活性を示したが、メチルスルホニル誘導体 **36b** は活性が低下した。以上のように 6 位への置換基の導入では、Met253 主鎖との水素結合が可能と考えられる、立体的に比較的小さな置換基（フルオロ、メトキシ、エトキシ、メチルチオ、およびシアノ基）が好ましいことがわかった（IC_{50} = 1.8–11 nM）。以上の知見を踏まえ、MMP-13 との効率的な相互作用が可能な置換基を 5 位に導入し、さらなる検討を行うこととした。

Table 2-2. Inhibitory Activities against MMP-13 of 6-, 7-, 8-Substitued Derivatives

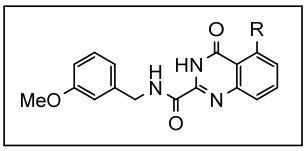


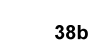
compound	R_1	R_2	R_3	IC_{50} (nM)[a]
1	H	H	H	12 ± 1.5
26e	F	H	H	11 ± 1.6
26f	Me	H	H	26 ± 3.1
26g	CF_3	H	H	97 ± 13
26b	OMe	H	H	4.0 ± 0.53
34	OEt	H	H	2.4 ± 0.16
26h	OCF_3	H	H	27 ± 3.7
26i	OBn	H	H	110 ± 17
36a	SMe	H	H	1.8 ± 0.19
36b	SO_2Me	H	H	23 ± 0.77
37a	Ph	H	H	9.8 ± 0.56
37b	CN	H	H	6.3 ± 0.53
26c	H	OMe	H	2200 ± 960
26d	H	H	OMe	>10000

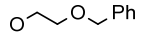
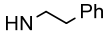
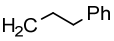
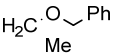
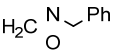
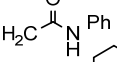
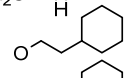
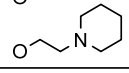
[a]IC_{50} against MMP-13. Each value is the mean ± SD from triplicate assay in a single experiment.

Table 2-3 に示すように、6 位置換誘導体（**Table 2-2**）とは異なり、5 位への立体的に小さい置換基の導入（**26k**、**26a**、および **43a**）では、大きな活性の向上は見られなかった。この結果により、5 位のフルオロ、メトキシ、およびシアノ基が S1'奥の、S1"ポケット [38] に到達するのに十分なサイズでは無いことが示唆された。従って、さらに強力な MMP-13 阻害剤を設計するために、適切なリンカーを介することによって S1"ポケットに結合するフラグメント（P1"）をキナゾリン骨格 5 位に導入する戦略をとることとした。 **Table 2-3** に 5 位へ P1"フラグメントを導入した化合物を示した。キナゾリン骨格と P1"フェニル基の間のリンカーを伸長すると、MMP-13 阻害活性が向上した（**43b**、**43c**、**38a**、**26m**、**38b**、および **38c**）。中でも、非置換のリード化合物 **1** よりも 17 倍強力な活性を示したフェネチルオキシ誘導体 **26m** において、化合物の物性や、合成の容易さを考慮し、さらなる精査を行うこととした。一方、フェネチルオキシ誘導体 **26m** のリンカーの酸素原子を他の原子（窒素、硫黄、炭素）で置き換えた、**38d**、**38e**、および **43d** は、MMP-13 阻害活性が 4〜14 倍低下した。同様に、3 原子からなるリンカー内のヘテロ原子の配置が異なる一連の化合物（**51**、**46**、および **53**）は、アニリンアミド誘導体 **53** を除いて、フェネチルオキシ誘導体 **26m** と比較して活性が低下した。 P1"に相当する、芳香族フェニル環をシクロヘキシル基で置換すると、阻害活性がわずかに低下し（**38f** 対 **26m**）、シクロヘキシル基を塩基性のピペリジニル基に変換すると活性が大きく減弱した（**38g** 対 **26m**）。

Table 2-3. Inhibitory Activities against MMP-13 of 5-Substitued Derivatives



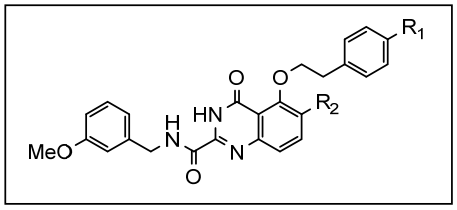
compound	R	IC_{50} (nM)[a]
1	H	12 ± 1.5
26k	F	5.0 ± 0.79
26l	Me	29 ± 3.4
26a	OMe	25 ± 4.6
43a	CN	8.6 ± 0.67
43b	Ph	2.2 ± 0.074
43c	OPh	0.53 ± 0.079
38a		0.31 ± 0.0091
26m		0.69 ± 0.058
38b		0.52 ± 0.052

compound	R	IC_{50} (nM)[a]
38c		1.6 ± 0.14
38d		4.3 ± 0.68
38e		9.7 ± 1.2
43d		2.9 ± 0.49
51		1.7 ± 0.2
46		16 ± 1.4
53		0.16 ± 0.017
38f		0.99 ± 0.1
38g		34 ± 7

[a]IC_{50} against MMP-13. Each value is the mean ± SD from triplicate assay in a single experiment.

Table 2-4 に示すように、フェネチルオキシ誘導体 **26m** のキナゾリン 6 位（R_2）にフルオロ基を導入すると、MMP-13 阻害活性が向上した（**38h**）ため、誘導体合成の容易等を勘案して、6 位フッ素体で、P1"末端フェニル環の修飾の検討を行った。末端ベンゼン環のパラ位は酸化的代謝を受けやすい経験則があること、また、S1"ポケットの終端に位置する Lys140 の ε-アミノ基への相互作用には最適な位置であることから水素結合供与/受容官能基の導入を行った。結果、無置換誘導体 **38h**（R_1 = H）と比較して、フルオロ置換誘導体 **38i**（R_1 = F）は、やや活性が減弱し、アミノ置換誘導体 **38j**（R_1 = NH_2）は活性を維持した。一方、カルボン酸誘導体 **38k** は 10 倍の活性の向上が見られ、非常に強力な MMP-13 阻害活性を示した（IC_{50} = 0.0039 nM）。

Table 2-4. Inhibitory Activities of 5-Phenethyloxy Derivatives against MMP-13

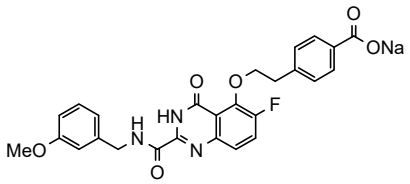


compound	R_1	R_2	IC_{50} (nM) [a]
26m	H	H	0.69 ± 0.058
38h	H	F	0.040 ± 0.013
38i	F	F	0.15 ± 0.013
38j	NH_2	F	0.030 ± 0.0018
38k	CO_2H	F	0.0039 ± 0.0011

[a] IC_{50} against MMP-13. Each value is the mean ± SD from triplicate assay in a single experiment.

一方、化合物 **38k** は、試験したすべての種（ラット、モルモット、ウサギ、ビーグル犬、カニクイザル、**Table 2-5**）において経口バイオアベイラビリティが十分とは言えないことがわかった（*F*% = 1.5-11）。肝ミクロソームでの高い代謝安定性（**Table 2-5**）と Caco-2 膜での高い透過性（見かけの透過係数（Papp）= 47.7 nm/s）から、この低いバイオアベイラビリティは投与液への低い溶解度が原因と考え、バイオアベイラビリティを改善するために **38k** の塩の調製の検討を行った。結果、一ナトリウム塩 **54** が安定で非吸湿性であるという好ましい特性を有し、ラット、モルモット、ウサギ、ビーグル犬、およびカニクイザルで経口吸収性を評価したところ、すべての試験種でフリー体と比較して良好な経口バイオアベイラビリティを示すことがわかった（それぞれ、*F*% = 23、74、35、58、および 45）。

Table 2-5. Pharmacokinetic Parameters of Sodium Salt **54**[a]



54

	Intravenous[b]			Oral[c]					
Species	Dose (mg/kg)	Vd,ss (mL/kg)	CLtotal (mL/h/kg)	Dose (mg/kg)	Tmax (h)	Cmax (ng/mL)	AUC (ng･h/mL)	*F*[d] (%)	Metabolic stability[e]
Rat	1.0	995	2077	10	0.25	439	1597	23 (4.9[f])	70
Guinea pig	1.0	5387	1615	3.0	0.50	439	1413	74 (6.3[f])	1.0
Rabbit	0.1	1142	222	10	1.8	2293	15677	35 (11[g])	3.0
Dog	1.0	731	185	10	1.7	3873	31810	58 (3.0[f])	ND[h]
Monkey	1.0	7025	1256	1.0	2.8	45	456	45 (1.5[f])	2.0
Human	NT	NT	NT	NT	NT	NT	NT	NT	3.0

[a]All experiments were performed using three male animals. NT = not tested. [b]Compounds were dosed in DMA/PEG400 or DMSO. [c]Compounds were dosed in 0.5% methyl cellulose. [d]Bioavailabilities of **38k** (free form) are given in parentheses. [e]Hepatic microsomal metabolic stability (μL/min/mg) of free acid of **54**. [f]Bioavailability at a dose of 3 mg/kg, po. [g]Bioavailability at a dose of 1 mg/kg, po. [h]No elimination of **54** was observed. ND = not determined.

2-4　キナゾリン誘導体**38k** のMMP-13との共結晶X線構造解析

合成した **38k** の MMP-13 との共結晶が得られ、**Figure 2-3** に示すように、**38k**–MMP-13 複合体の X 線解析像から、化合物 **38k** はリード化合物 **1** と同じ結合モードで MMP-13 に結合し、活性中心の亜鉛とは結合していないことが確認された。化合物 **38k** のキナゾリン環部分は、MMP-13 の特異性ポケットである深い S1'ポケットを充填しており、β シートタイプの以下の 3 箇所の水素結合によって安定化されていることが確認された: (a) キナゾリン環の 4 位カルボニル酸素と Thr247 の骨格アミド（b）キナゾリン環の 3 位アミド水素と Thr245 のカルボニル酸素、および（c）キナゾリン環の 2 位の環外カルボニル酸素と Thr245 の主鎖アミド、また、化合物 **38k** のフェネチルオキシ基は MMP-13 特異的な S1"ポケットに深く埋め込まれており、末端のフェニル環のカルボキシル基は、Lys140 の ε-アミノ基との塩橋を介してタンパク質へのアンカーとなっていることがわかった。化合物 **38k** の 6 位のフルオロ基は、やや長い結合距離（3.3Å）で Met253 の主鎖アミドと弱く相互作用していることが推測された。

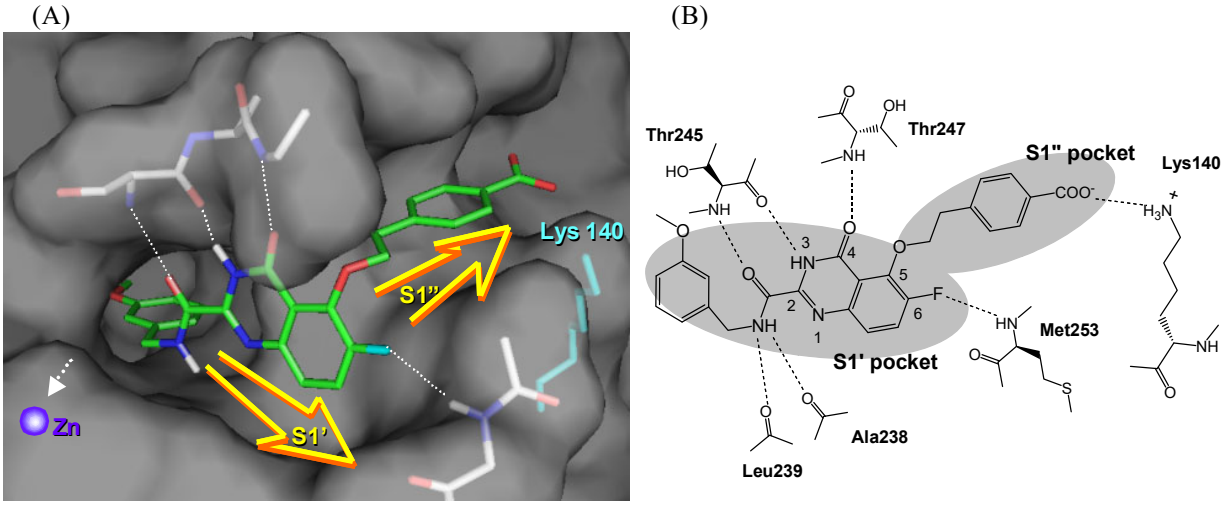


Figure 2-3. Crystal Structure of the Complex of Carboxylate **38k** and MMP-13. (A) Surface representation of MMP-13 illustrating the binding cavity. The inhibitor is buried deeply into the S1′ pocket and extends into an additional S1′ side pocket (S1″), which is unique to MMP-13. The figure was made with program PyMOL.[49] (B) The distances of hydrogen bonds and ionic interactions between **38k** and MMP-13 are depicted as dashed lines.

2-5　結論

キナゾリン-2-カルボキサミド誘導体を効率的に合成する方法を開発し、種々の新規 MMP-13 選択的阻害薬を合成した。リード化合物 **1** と MMP-13 の複合体の X 線結晶構造解析に基づいて、MMP-13 に特徴的な S1'ポケットのより深い領域（S1"ポケット）、Met253 の主鎖 NH、および S1"ポケットの終端に位置する Lys140 の ε-アミノ基との相互作用を獲得するために、一連の MMP-13 選択的阻害薬をデザイン、合成し、強力な阻害活性を有する化合物を見出した。中でも、カルボン酸誘導体 **38k** は、非常に強力な MMP-13 阻害活性（IC_{50} = 0.0039 nM）を示した。

合成した、**38k** と MMP-13 の複合体の X 線像解析により、S1 'ポケットにまたがる酵素の主鎖との間に β シート様の水素結合を形成することにより、**38k** が MMP-13 に固有の S1'ポケットおよび、S1'サイドポケット（S1'ポケット）に結合し、また、Met253 の主鎖 NH、および S1"ポケットの終端に位置する Lys140 の ε-アミノ基との相互作用により、強い MMP-13 阻害活性が発揮されていることが確認された。

カルボン酸 **38k** は水系溶媒に難溶性であったため、一ナトリウム塩 **54** を調製したころ、様々な動物種で、好ましい経口バイオアベイラビリティと薬物動態パラメータを示した。

3-1　縮合ピリミジン環を中心骨格とした非亜鉛結合型MMP-13選択的阻害薬のデザイン

前章で述べた、キナゾリン骨格を有する非亜鉛結合型 MMP-13 阻害薬で得られた知見を用い、キナゾリンのベンゼン環部分に相当する（i）A 環の変更が可能かどうか、また、（ii）A 環を変更した場合の P1"の最適化を行い、新たな骨格での選択的 MMP-13 阻害薬の合成を検討した。

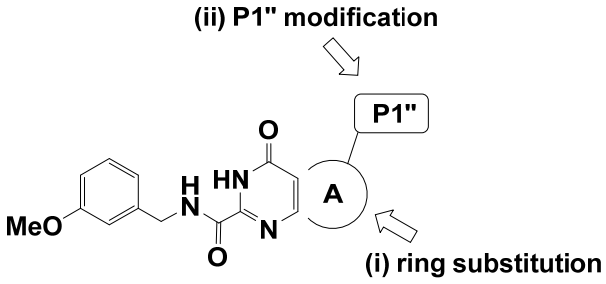
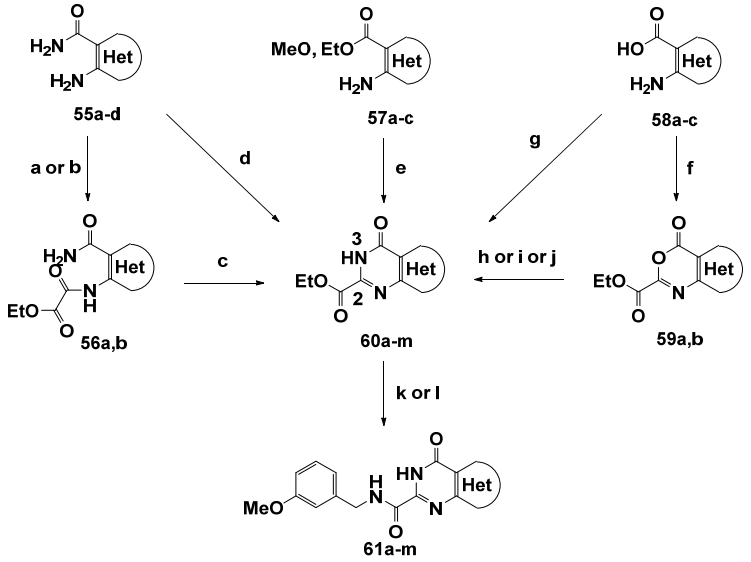


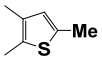
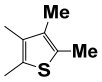
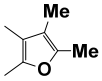
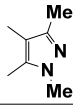
Figure 3-1. Structural Modification of Fused Pyrimidine MMP Inhibitors

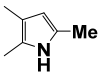
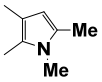
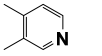
3-2　縮合ピリミジン環誘導体の合成

縮合ピリミジン-2-カルボン酸誘導体 **61a-m** は、2-アミノヘテロアリールカルボン酸誘導体 **55a–d**、**57a–c**、および **58a–c** を出発原料とし、共通中間体としてエステル **60a–m** から、1 または 2 ステップの工程で合成した（**Scheme 3-1**）。**60a–m** の 2 位エチルエステルは、アリファティック一級アミンと容易に反応し、対応するヘテロアリールアミド体 **61a–m** を得た。

Scheme 3-1. Synthesis of Fused Pyrimidine-2-carboxamide Derivatives **61a–m**[a]



compound					Het
55	56	57	60	61	
55a	56a	-	60a	61a	
-	-	-	60b	61b	
-	-	-	60c	61c	
-	-	-	60d	61d	
55b	56b	-	60e	61e	
55c	-	-	60f	61f	
55d	-	-	60g	61g	

compound					Het
57	58	59	60	61	
57a	-	-	60h	61h	
57b	-	-	60i	61i	
57c	-	-	60j	61j	
-	58a	59a	60k	61k	
-	58b	59b	60l	61l	
-	58c	-	60m	61m	

[a] Reagents and conditions: (a) ethyl chloroglyoxylate, Et_3N, THF, 0 °C to rt; (b) (1) diethyl oxalate, EtONa, EtOH, reflux; (2) oxalyl chloride, DMF, THF, rt; (3) EtOH, pyridine, THF, rt; (c) *p*-TsOH, toluene or xylene, reflux; (d) diethyl oxalate, EtONa, EtOH, reflux; (e) $CNCO_2Et$, HCl, AcOH, 80 °C; (f) ethyl chloroglyoxylate, pyridine, rt to 50 °C [for **58a**,**b**]; (g) (1) ethyl chloroglyoxylate, pyridine, rt; (2) oxalyl chloride, DMF, THF, 0 °C to rt [for **58c**]; (h) NH_4OAc, EtOH, reflux [for **59a**]; (i) NH_4OAc, AcOH, EtOH, reflux [for **59b**]; (j) (1) NH_3, EtOH, THF, 0 °C; (2) EtONa, EtOH, 0 °C to rt [for **59c**]; (k) 3-methoxybenzylamine, DMF or EtOH, 80–90 °C [for **60a–h**, **j–m**]; (l) 3-methoxybenzylamine, *N*-ethyldiisopropylamine, DMA, 80–90 °C [for **60i**].

チエノ[2,3-*d*]ピリミジンの 5 位に 3 原子長のスペーサーを介し、置換フェニル基を有する誘導体の合成を、**Scheme 3-2** に示した。市販のエステル **60b** の 5 位メチル基をクロロベンゼン中、*N*-ブロモスクシンイミド（NBS）によるラジカル臭素化により、モノブロモ体 **62** を得た。THF 中、ベンジルアルコールと NaH でエーテル化する際に、エステルの部分的加水分解が進行したため、生じたカルボン酸の再エステル化を行った。こうして、チエノピリミジン-2-カルボン酸エチルエステル **63a–d** を得た後、エステル部分を 3-メトキシベンジルミンでアミノリシスすることによりエーテルリンカー誘導体 **64a,b,d,e** を合成した。*N*-メチルまたはチオエーテルリンカー誘導体 **64f** および **64g** の合成も同様の方法で行った。

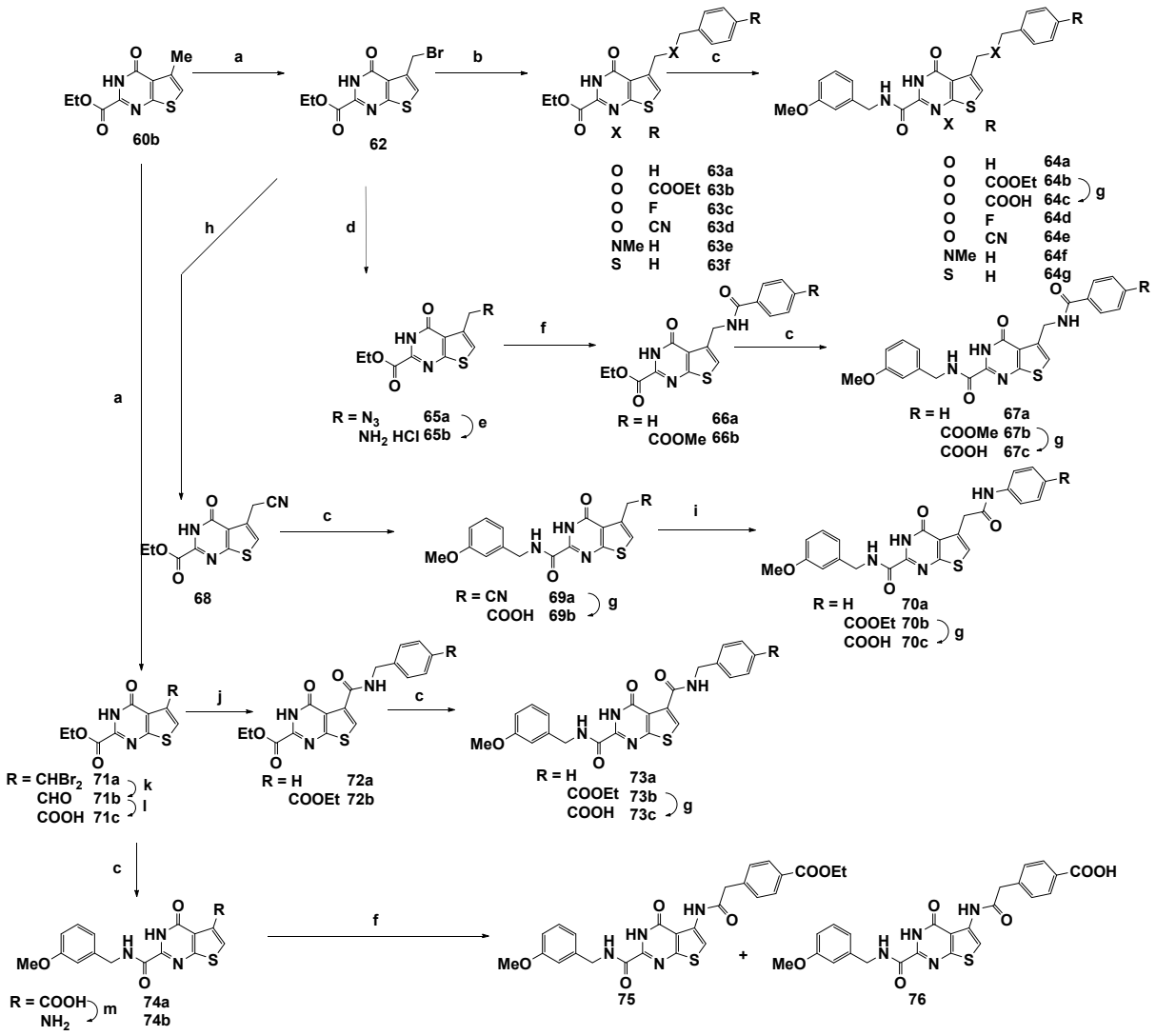
5-[（フェニルカルバモイル）メチル]誘導体 **67a–c** は、5-ブロモメチル中間体 **62** から合成した。即ち、臭素原子をアジ化ナトリウムで置換し、得られたアジド誘導体 **65a** を一級アミンに還元し（**65b**）、続いてアシル化することにより **66a,b** を得た後、エタノール中、3-メトキシベンジルアミンによる **66a,b** のエステル部のアミノリシスにより **67a,b** を得た。次に、**67b** のメチルエステルをアルカリ加水分解することにより、カルボン酸誘導体 **67c** に変換した。

また、ブロモメチル中間体 **62** をシアン化ナトリウムによってシアノメチル誘導体 **68** に変換し、次いでエチルエステルのアミノリシスを行い、シアノ誘導体 **69a** を得た。**69a** を水酸化ナトリウム水溶液で加水分解して、カルボン酸誘導体 **69b** を得た後、酸塩化物に変換し、アニリンと縮合し、**70a,b** を得た。エステル誘導体 **70b** は水酸化ナトリウムで加水分解し、カルボン酸誘導体 **70c** を得た。

60b を AIBN の存在下で 2.3 当量の NBS を用い臭素化し、*gem*-ジブロモ誘導体 **71a** を得た後、**71a** の加水分解により、アルデヒド誘導体 **71b** を得た。アセトニトリル水溶液中、亜塩素酸ナトリウムを用い、カルボン酸誘導体 **71c** へと酸化後、カルボン酸部分を酸塩化物に変換した後、ベンジルアミンとカップリングし、続いて 3-メトキシベンジルアミンでアミノリシスすることにより、**73a,b** を得た。**73b** のエチルエステルを加水分解して、対応するカルボン酸誘導体 **73c** を 94%の収率で得た。

5-置換 4-オキソ-3*H*,4*H*-チエノ[2,3-*d*]ピリミジン誘導体 **75** および **76** の合成は、エステル誘導体 **71c** と 3-メトキシベンジルアミンとの反応により得たアミド誘導体 **74a** をトルエン中でジフェニルホスホリルアジド（DPPA）およびトリエチルアミンと加熱することにより、系中で発生したイソシアナートを *tert*-ブタノールでトラップして、粗 *tert*-ブチルカルバマート体を得た後、HCl-EtOAc で処理して Boc 保護基を除去し、続いて水酸化ナトリウム水溶液で処理すること[50]でフリーのアミン誘導体 **74b** を得た。**74b** を 4-（2-クロロ-2-オキソエチル）安息香酸エチルでアシル化すると、エチルエステル誘導体 **75** と加水分解生成物 **76** を同時に得ることができた。

Scheme 3-2. Synthesis of Thieno[2,3-*d*]pyrimidines **64a–g**, **67a–c**, **70a–c**, **73a–c**, **75**, and **76**[a]

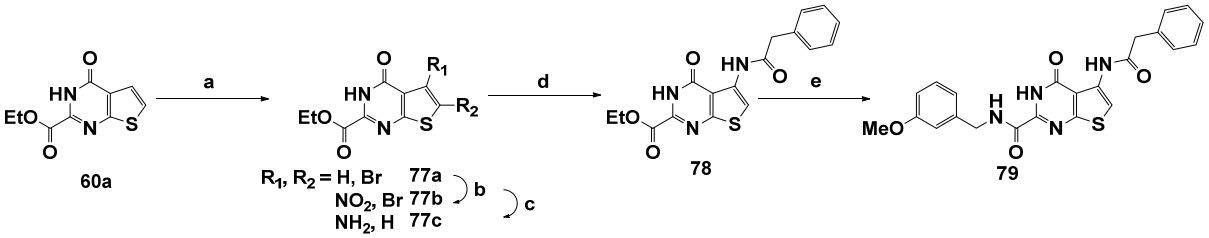


[a] Reagents and conditions: (a) 1.2 eq. NBS, AIBN, chlorobenzene, 80 °C, 54% [for **62**]; 2.3 eq. NBS, AIBN, carbon tetrachloride, 80 °C, 85% [for **71a**]; (b) (1) benzyl alcohol, NaH, THF; (2) oxalyl chloride, DMF, THF; (3) EtOH, THF, 16% [for **63a**]; (1) 4-hydroxymethylbenzoic acid ethyl ester, NaH, THF; (2) oxalyl chloride, DMF, THF; (3) EtOH, pyridine, THF, 75% [for **63b**]; (1) (4-fluorophenyl)methanol, THF, NaH; (2) EtOH, EDC HCl, DMAP, THF, 8% [for **63c**]; (1) 4-(hydroxymethyl)benzonitrile, THF, NaH; (2) EtOH, EDC HCl, DMAP, THF, 52% [for **63d**]; *N*-methyl-1-phenylmethanamine, Et_3N, THF, 52% [for **63e**]; phenylmethanethiol, Et_3N, DMA, 30% [for **63f**]; (c) 1-[3-(methyloxy)phenyl]methanamine, EtOH, 80–90 °C, 60% [for **64a**]; 90% [for **64b**]; 81% [for **64d**]; 79% [for **64e**]; 58% [for **64f**]; 67% [for **64d**]; 82% [for **67a**]; 89% [for **67b**]; 76% [for **69a**]; 85% [for **73a**]; 91% [for **73b**]; 1-[3-(methyloxy)phenyl]methanamine, ethyldiisopropylamine, EtOH, 90 °C, 67% [for **74a**]; (d) NaN_3, DMF, 76%; (e) H_2, HCl, Pd/C, EtOH–THF, 94%; (f) benzoyl chloride, Et_3N, THF, 72% [for **66a**]; 4-

chlorocarbonylbenzoicacidmethyl ester (prepared from monomethyl terephthalate, oxalyl chloride, DMF, THF), Et_3N, THF, 82% [for **66b**]; (1) ethyl 4-(2-chloro-2-oxoethyl)benzoate (prepared from 2-[4-(ethoxycarbonyl)phenyl]acetic acid, oxalyl chloride, DMF, THF), pyridine, THF; (2) aqueous NaOH solution, EtOH/THF, 6% [for **75**]; 17% [for **76**]; (g) aqueous NaOH solution, EtOH or THF, MeOH, 80–100 °C, quant. [for **64c**]; 98% [for **67c**]; 62% [for **69b**]; 86% [for **70c**]; 62% [for **69b**]; 86% [for **70c**]; 94% [for **73c**]; (h) NaCN, DMF/H_2O, 0 °C–rt, 69%; (i) (1) oxalyl chloride, THF; (2) aniline, pyridine, THF, 64% [for **70a**]; (1) oxalyl chloride, THF; (2) ethyl 4-aminobenzoate, pyridine, THF, 76% [for **70b**]; (j) (1) oxalyl chloride, DMF, THF (2) benzylamine, THF, 70% [for **72a**]; (1) oxalyl chloride, DMF, THF (2) ethyl 4-(aminomethyl)benzoate hydrochloride, THF, 80% [for **72b**]; (k) 1 N HCl, THF, MeOH, 60 °C, 69%; (l) $NaClO_2$, MeCN, H_2O, 57%; (m) (1) DPPA, Et_3N, toluene, 100 °C; (2) *tert*-butyl alcohol, 100 °C; (3) HCl, AcOEt; (4) aqueous NaOH, THF, MeOH, 80 °C, 26%.

Scheme 3-3 に示すように、**60a** の臭素化、続く硫酸中の硝酸カリウムを用いたニトロ化により、6-ブロモ-5-ニトロ-4-オキソ-3*H*,4*H*-チエノ[2,3-*d*]ピリミジン-2-カルボン酸エチル誘導体 **77b** を得た後、パラジウム触媒の存在下での水素化により、ニトロ基の還元とブロモ基の水素化分解が同時に進行し、**77c** を得た。**77c** をフェニルアセチルクロリドでアシル化し、続いて 3-メトキシベンジルアミンでアミノリシスすることで、ジアミド誘導体 **79** が得られた。

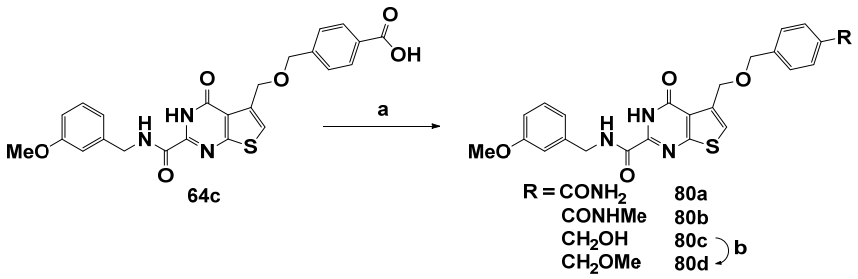
Scheme 3-3. Synthesis of 5-(2-Phenylacetamido)thieno[2,3-*d*]pyrimidine **79**[a]



[a]Reagents and conditions:(a) Br_2, AcOH, 93%; (b) conc. H_2SO_4, $NaNO_3$, 0 °C, 80%; (c) H_2, Pd/C, EtOH/THF, 36%; (d) phenylacetyl chloride, Et_3N, THF, 77%; (e) 3-methoxybenzylamine, EtOH, 90 °C, 93%.

Scheme 3-4 に示すように、カルボキサミド誘導体 **80a** はカルボン酸誘導体 **64c** から、塩化オキサリル、続いてアンモニア水溶液との反応により合成した。*N*-メチルアミド誘導体 **80b** は、DMAP の存在下でカップリング剤として 1-エチル-3- [3-（ジメチルアミノ）プロピル]カルボジイミド塩酸塩（EDC）を使用して、カルボン酸誘導体 **64c** をメチルアミン塩酸塩と縮合することにより合成した。アルコール誘導体 **80c** は、**64c** から調製した酸塩化物に、DMA 中、水素化ホウ素ナトリウムで還元することにより得た。アルコール誘導体 **80c** を THF 中、メタンスルホニルクロリドと反応し、得られたメシレートをナトリウムメトキシドで置換することにより、メチルエーテル誘導体 **80d** を合成した。

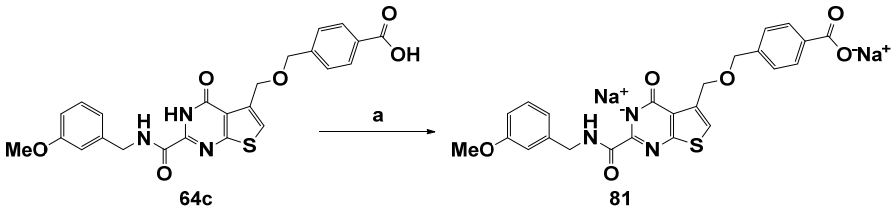
Scheme 3-4. Synthesis of Compounds **80a**, **80b**, **80c**, and **80d**[a]



[a]Reagents and conditions: (a) (1) oxalyl chloride, DMF, THF; (2) 28% aqueous ammonia solution, THF, 87% [for **80a**]; methylamine hydrochloride, EDC, DMAP, THF, 67% [for **80b**]; (1) oxalyl chloride, DMF, THF; (2) $NaBH_4$, DMA, 65% [for **80c**]; (b) (1) MsCl, Et_3N, THF; (2) sodium methoxide, MeOH/THF, 80 °C, 66% [for **80d**].

64c の二ナトリウム塩の調製を **Scheme 3-5** に示した。炭酸水素ナトリウム水溶液 2.0 当量を **64c** の THF-EtOH 溶液に加え、得た沈殿物を、濾過により溶液から分離した。続いて 90°C の EtOH で再沈殿を行い、二ナトリウム塩 **81** を得た。

Scheme 3-5. Synthesis of Disodium Salt **81**[a]

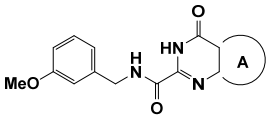


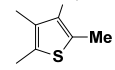
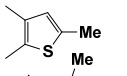
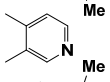
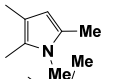
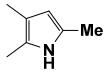
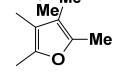
[a] Reagents and conditions: (a) (1) THF, EtOH, aqueous $NaHCO_3$; (2) EtOH, 90 °C, 91%.

3-3　縮合ピリミジン環誘導体の構造活性相関

キナゾリン骨格のベンゼン環を、生物学的等価体であるチオフェン環や、他のヘテロ環で置換可能かどうかの検討を行った（**Table 3-1**）。チオフェン環のメチル置換誘導体 **61b–c** は、リードキナゾリン **1** と比較して 4〜10 倍改善された活性を示した（**Table 2-1** を参照）。また、対応する 5-メチルキナゾリン類似体 **26l** と比較して（**Table 2-1** を参照）、5-メチルチエノピリミジン誘導体 **61b** は 20 倍以上の高活性を示し、チオフェン環の 5 位方向への置換が許容であることが示唆された。一方、チオフェン環をピロロ-、フロ-、ピラゾロ-、イソキサゾロ-、およびピリジン環などの他のヘテロ芳香族環で置換すると、活性が低下した。即ち、チオフェン環をフラン環に置換すると、活性は 1/10 の以下に減弱し（**61d** 対 **61e**）、チオフェン環をピリジン環などの 6 員ヘテロアリール環に置換すると 1/100 以下に減弱した（**61a** 対 **61l**）。以上の結果から、4-オキソ-3*H*,4*H*-チエノ[2,3-*d*]ピリミジン環を中心骨格として選択し、さらなる修飾を行うこととした。

Table 3-1. In Vitro Data for Ring A Substituted *N*-[(3-Methoxyphenyl)methyl]-6-oxo-1,6-dihydropyrimidine-2-carboxamide Derivatives



compound	A	IC_{50} (nM) [a]	compound	A	IC_{50} (nM) [a]
61b		1.1 ± 0.043	61k		29 ± 5.2
61d		1.2 ± 0.069	61g		65 ± 10
61c		3.1 ± 0.29	61m		200 ± 38
61h		12 ± 1.6	61f		760 ± 95
61j		14 ± 2.2	61i		1100 ± 220
61e		16 ± 2.1	61l		3200 ± 300
61a		24 ± 3.7			

[a] IC_{50} (nM) represents the average IC_{50} value in nanomolars for the inhibition assay of the compound using the human MMP-13 catalytic domain enzyme. Data are expressed as mean IC_{50} ± SD, n = 3.

Figure 3-2に示すように、X線共結晶構造解析により、チオフェン誘導体**61a**もリード化合物**1**と同様の結合モードをとっていることが確認され、また、S1"ポケットに結合するP1"置換基の導入は、構造活性相関の結果からも（**Table 3-1**）、4-オキソ-3*H*,4*H*-チエノ[2,3-*d*]ピリミジン骨格の5位が適切である可能性が示唆された。X線共結晶構造解析から、S1"ポケットが主に疎水性であり、3原子のリンカーを介してP1"に相当する置換フェニル基を収容する十分な空間があること、S1"ポケットの終端に位置するLys140残基とP1"置換基との相互作用を通じて、追加の水素結合またはイオン相互作用を形成することが可能であると考えられた。

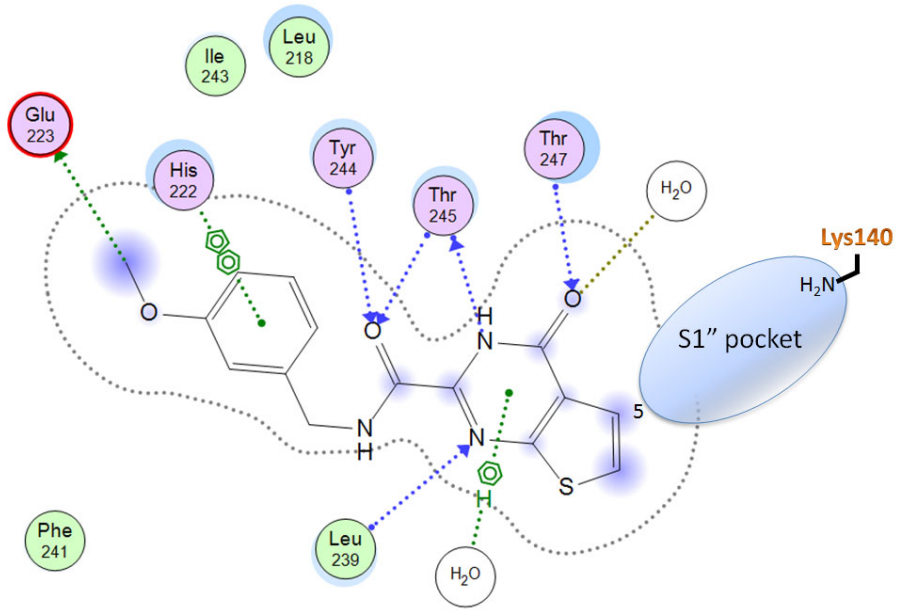
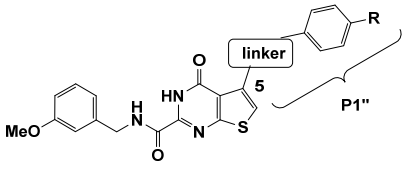
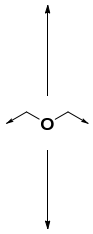


Figure 3-2. X-ray Co-crystal Structure of Prototype Compound **61a** in Complex with MMP-13 Catalytic Domain (PDB code: 3WV3) in a Schematic Representation. The ligand interacts in a similar fashion as **1** with MMP-13 residues of the specific S1′ pocket (dotted curved line). Molecular modeling suggested that attachment of the P1″ substituent via a linker to the thiophene 5-position of the 4-oxo-3*H*,4*H*-thieno[2,3-*d*]pyrimidine scaffold would afford a target molecule. An additional possible interaction of P1″ with Lys140 residue at the bottom of the S1″ pocket is present in the protein.

P1"を構成する、5 位の末端フェニル基の置換基 R および、チエノ[2,3-*d*]ピリミジンコアの間の 3 原子リンカーの MMP-13 阻害活性に与える影響を示した（**Table 3-2**）。ベンジルオキシメチル基を 5 位に導入したところ（**64a**）、5-メチル誘導体 **61b**（**Table 3-1**）と比較して約 6 倍の活性向上が見られ、サブ nM オーダーの活性を示した（IC_{50} = 0.19 nM）。**64a** の末端フェニル基のパラ位に水素結合が可能な種々の置換基を導入したところ（**64c**）、カルボキシル基では活性が劇的に向上し、IC_{50} 値が 0.0069 nM を示した。一方、フルオロ（**64d**）、ヒドロキシメチル（**80c**）、メトキシメチル（**80d**）、シアノ（**64e**）、カルボキサミド（**80a**）、および *N*-メチルカルボキサミド（**80b**）の、他の置換は、無置換化合物（**64a**）と比較して効力が増強されたものの、カルボキシ誘導体 **64c** よりも強力な阻害を示すには至らなかった。

Table 3-2. Inhibition of MMP-13 by Derivatives with Different Linkers



cpd	linker	R	IC_{50} (nM) [a]
64a		H	0.19 ± 0.021
64d		F	0.13 ± 0.016
80c		CH_2OH	0.032 ± 0.0031
80d		CH_2OMe	0.080 ± 0.0063
64e		CN	0.018 ± 0.0032
80a		$CONH_2$	0.015 ± 0.0013
80b		CONHMe	0.029 ± 0.0012
64c		COOH	0.0069 ± 0.00078
64g		H	0.66 ± 0.075
64f		H	39 ± 8.3

cpd	linker	R	IC_{50} (nM) [a]
70a		H	0.028 ± 0.0033
70c		COOH	0.005 ± 0.0006
67a		H	0.018 ± 0.0038
67c		COOH	0.0022 ± 0.00073
73a		H	0.026 ± 0.0022
73c		COOH	0.0077 ± 0.00095
79		H	0.053 ± 0.0075
76		COOH	0.021 ± 0.0018

[a] IC_{50} (nM) represents the average IC_{50} value in nanomolars for the inhibition assay of the compound using the human MMP-13 catalytic domain enzyme. Data are expressed as mean IC_{50} ± SE, n = 3.

P1"末端の R が水素である誘導体で比較したところ、エーテルリンカー（**64a**）をチオエーテル（**64g**）または第三級アミン（**64f**）リンカーで置換すると、活性が低下したが、アミドリンカー（**70a**、**67a**、**73a**、および **79**）を有する誘導体は、リンカーへのアミド構造の導入位置や、向きに関係無く 2 桁の pM オーダーの IC_{50} 値を示し、これらの化合物にカルボキシル基を導入すると、活性がさらに向上し、1 桁の pM オーダーの IC_{50} 値を示すことがわかった（**70c**、**67c**、および **73c**）。高い活性を示した、カルボン酸誘導体 **64c**、**70c**、**67c**、**73c**、および **76** のうち、阻害活性、化合物のハンドリングのしやすさ（物性）に基づいて、4-カルボキシベンジルオキシメチル誘導体 **64c** を選択し、高次の評価を行うことにした。

Table 3-3 に示すように、モルモットにフリーのカルボン酸誘導体 **64c**（10 mg/kg）の二ナトリウム塩 **81**（10.9 mg/kg：フリー体換算で 10 mg/kg）を経口投与すると、**64c** と比較して、AUC および Cmax 値が大きく向上した（それぞれ、8,357 ng•h/mL および 1,445 ng/mL）。 また、二ナトリウム塩 **81** は、10〜20 mg/kg の経口投与量で良く吸収され、ラット（11 mg/kg）、イヌ（10.9 mg/kg）とサル（20 mg/kg）の **81** の経口 AUC 値は、それぞれ 3,414 ng•h/mL、2,7136 ng•h/mL および、82,360 ng•h/mL であることがわかった。

Table 3-3. Pharmacokinetics of **64c** and Its Disodium Salt **81** in Guinea Pig, Dog, and Monkey

species	compound	C_{max} ng/mL (po)	T_{max} h (po)	AUC ng·h/mL (po)	Vd,ss[f] mL/kg (iv)	CL[g] mL/h/kg (iv)	% *F*
Rat[a]	81	335	2.8	3414	799	606	20
Guinea pig[b]	64c	911	0.83	6478	923	431	28
Guinea pig[c]	81	1445	0.67	8357	-	-	-
Dog[d]	81	2438	2.0	27136	395	111	29
Monkey[e]	81	6607	3.0	82360	-	-	-

[a] i.v. 1.1 mg/kg, p.o. 11 mg/kg male (n = 3). [b] i.v. 1 mg/kg, p.o. 10 mg/kg male (n = 3). [c] p.o. 10.9 mg/kg male (n = 3). [d] i.v. 1.09 mg/kg, p.o. 10.9 mg/kg male (n = 3). [e] p.o. 20 mg/kg male (n = 2) and female (n = 2). [f] Volume of distribution at steady state. [g]Total body clearance.

3-4　結論

キナゾリン以外の、中央の骨格の探索を行い、キナゾリンリード化合物 **1** および、チオフェン誘導体 **61a** との MMP-13 の X 線共結晶構造解析像をもとに、中央の骨格として、チエノ[2,3-*d*]ピリミジンを用い、MMP-13 に特徴的な S1"ポケットとの相互作用が可能な置換基（P1"）および、S1"ポケットの終端に位置する Lys140 残基との水素結合相互作用を形成することで、活性と選択性を改善可能であるという仮定に基づいて、種々のリンカーを介した P1"置換基を導入した MMP-13 選択的阻害薬をデザイン、合成した結果、非常に選択的で、MMP-13 に強力な阻害を示す化合物 **64c** を発見するに至った。

ピリミジンの 3、4 位と Thr245、247 残基は、S1'および S1"ポケットにまたがる酵素の主鎖との間に緊密な β シート様の相互作用を形成する。P1"と S1"ポケットの終端に位置する Lys140 残基との水素結合相互作用を形成することを目的としたリンカーを介した P1"置換基の結合により、IC_{50} = 0.0069 nM の非常に強力な MMP13 阻害薬 **64c** を見出した。

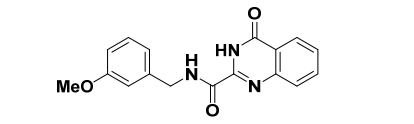
フリーのカルボン酸誘導体 **64c** を二ナトリウム塩 **81** に誘導することで、モルモットでは AUC および Cmax 値が大きく向上し、また、二ナトリウム塩 **81** はラット、イヌ、および、サルで、高い経口 AUC 値を示すことがわかった。

4-1　MMPアイソザイムおよびTACEに対する選択性

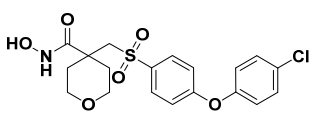
キナゾリン誘導体 **38k**、チエノ[2,3-*d*]ピリミジン誘導体 **64c** の MMP-1、2、3、7、8、9、10、14、および tumor necrosis factor-alpha converting enzyme（TACE）を含む、他のマトリックスメタロプロテイナーゼホモログに対する選択性プロファイルを精査した（**Table 4-1**）。非選択的阻害薬として知られている、ロシュバイオサイエンス社の研究グループが報告したヒドロキサム酸系 MMP 阻害薬 **82**（RS-130,830[51]）は他の金属酵素に対する MMP-13 の選択性が、3 倍未満と、十分な選択性を示さないのに対し、キナゾリン誘導体 **38k** は、他の測定した酵素と比較して、MMP-13 に対して>41,000 倍の選択性を示した。また、**38k** は、MMP-1、7、9、14、および TACE に対して優れた MMP-13 選択性（>1,000,000 倍）も示した。一方、チエノ[2,3-*d*]ピリミジン誘導体 **64c** は、他の測定した酵素と比較して、>2,600 倍の選択性を示し、キナゾリン誘導体と同様に、MMP-1、7、9、14、および TACE に対して優れた MMP-13 選択性（>1,000,000 倍）を示した。

リード化合物 **1** と比較して、キナゾリン誘導体 **38k** の MMP-13 阻害活性は約 3,000 倍向上し、選択性は約 1,600 倍の向上を示した。また、チエノ[2,3-*d*]ピリミジン誘導体 **64c** の MMP-13 阻害活性は 1,700 倍向上し、選択性は約 100 倍の向上を示した。

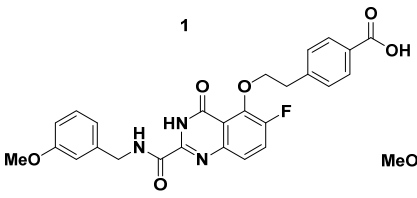
Table 4-1. IC_{50} Values for Inhibition of MMPs and TACE



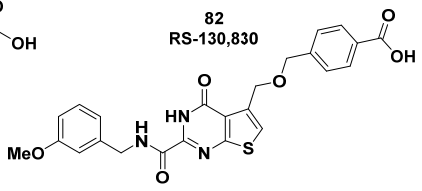
1



82
RS-130,830



38k



64c

	IC_{50} (nM)[a]									
Compound	MMP-13	MMP-1	MMP-2	MMP-3	MMP-7	MMP-8	MMP-9	MMP-10	MMP-14	TACE
1	12	>10000	300	>10000	>10000	1100	>10000	3400	>10000	>10000
38k	0.0039	>10000	5300	4000	>10000	720	>10000	160	>10000	>10000
64c	0.0069	>10000	18	600	>10000	780	>10000	160	>10000	>10000
82	0.01	34	0.029	0.3	210	0.097	0.11	0.54	1.1	14

[a] Values are shown as the mean IC_{50} ± SD of triplicates.

4-2　ex vivo ウシ鼻軟骨分解抑制試験

軟骨組織におけるキナゾリン誘導体 **38k** および、チエノピリミジン誘導体 **64c** の in vitro での有効性を評価するために、ウシ鼻中隔軟骨を用いた分解抑制活性の測定を行った [52, 53]。即ち、ウシ鼻中隔軟骨片を 2 種のサイトカインである、IL-1β とオンコスタチン M（oncostatin M: OSM）処理により、軟骨細胞から種々の MMP 類の産生を誘導し、組織培養液中に放出された軟骨片中の II 型コラーゲンの分解産物の測定を行った（コラーゲンに特異的なヒドロキシプロリンとして測定）。結果、被験化合物 1 μM 添加での軟骨分解抑制率は、非選択的 MMP 阻害薬 **82**（RS-130,830）が 100%、キナゾリン誘導体 **38k** が 73%、チエノピリミジン誘導体 **64c** が 100%となることがわかった。さらに低濃度でも **64c** は、コラーゲンの分解を有意に阻害し（0.1 μM で 87%阻害）、非選択的 MMP 阻害薬 **82**（RS-130,830）の分解阻害活性（0.1 μM で 76%の阻害）に迫る活性を示した（**Figure 4-1**）。一方、キナゾリン誘導体 **38k** が、その高い MMP-13 阻害活性に比較して強い分解抑制活性を示さなかったのは、軟骨培養溶液中に対して、十分な溶解性を示さなかったことが理由だと推測される。

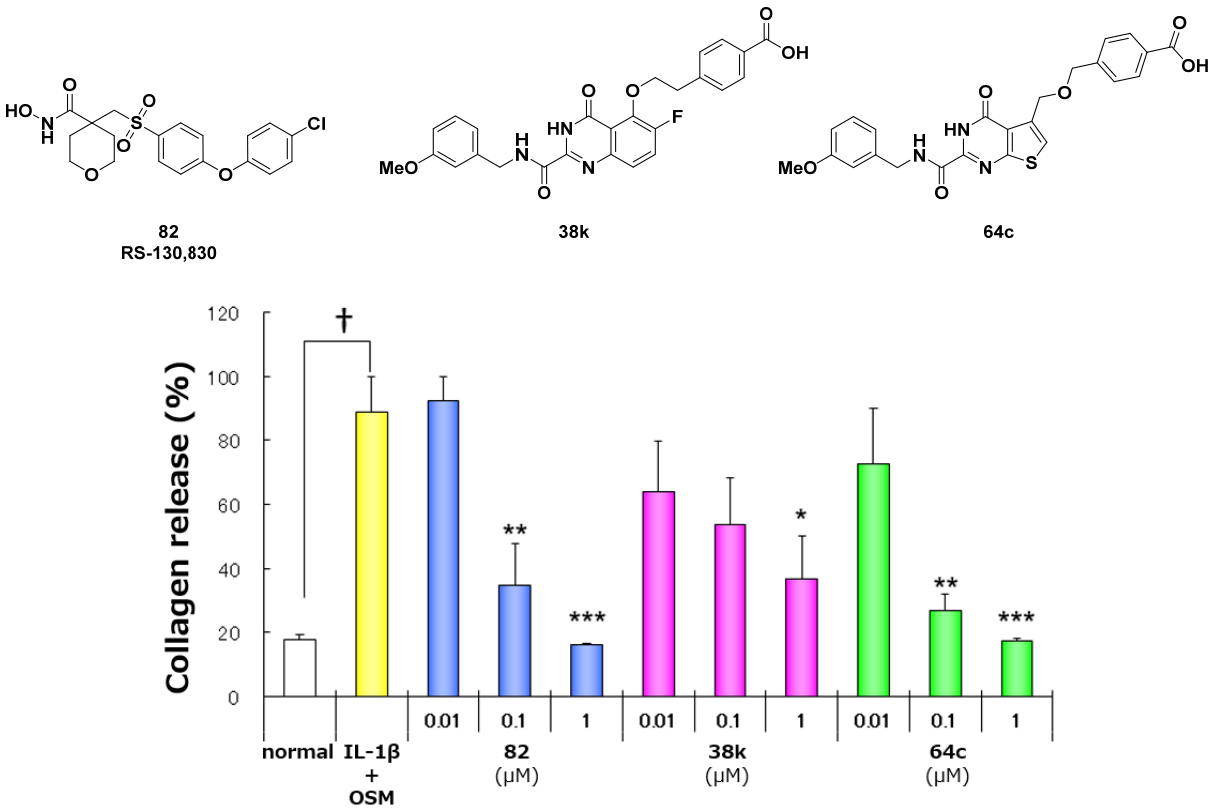
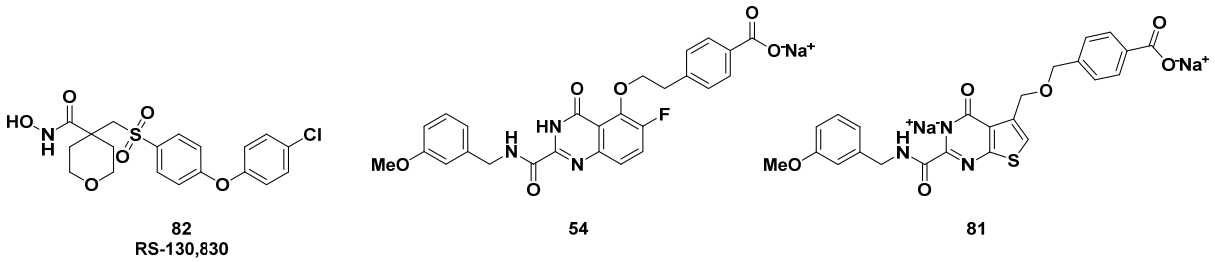


Figure 4-1. Inhibition Activity in Bovine Nasal Cartilage Assay. Data are represented as means ± SEM (n = 6). † indicates $P < 0.05$ by t-test compared to untreated group. *, **, and *** denote $P < 0.05$, $P < 0.01$, and $P < 0.001$, respectively by one-tailed Williams' test.

4-3　MIA による OA 様症状誘発ラットモデルでの化合物評価

モノヨード酢酸（monoiodoacetate: MIA）による OA 様症状誘発ラットモデルを使用して、軟骨分解に対する選択的 MMP-13 阻害薬の経口投与による分解抑制効果を評価した。ラットの膝関節腔内に MIA を注射すると、関節軟骨内の軟骨細胞が壊死し、種々の MMP 活性が上昇し[54-56]、MMP 類による軟骨コラーゲン分解によって II 型コラーゲンのマーカーである C-テロペプチド（C-terminal telopeptides of type II collagen: CTX-II）が生成する[57, 58]。関節腔内に MIA を注射後、7 日目に 10 mg/kg の用量で試験化合物を経口投与してから 4 時間後、関節からの滑液サンプルを採取し、CTX-II 量を測定したところ、MIA 注射後、vehicle 投与群で関節内 CTX-II 濃度の通常レベルの 18.6 倍の増加が観察されたが（**Figure 4-2**）、キナゾリン誘導体のナトリウム塩 **54** は CTX-II 量の有意な抑制を示した（69%）。一方、非選択的で広域スペクトルの MMP 阻害活性を持つ、ヒドロキサム酸型 MMP 阻害薬 **82**（RS-130,830）は 87%の抑制率を示した。一方、in vitro ウシ鼻軟骨分解抑制試験では良好な活性を示した、チエノピリミジン誘導体 **64c** の二ナトリウム塩 **81** は、本評価系では、抑制傾向（31%）を示すにとどまった。この動物モデルは MMP-13 以外の種々の MMP が亢進していると考えられるため、MMP-13 選択的阻害薬の薬効評価を行うには必ずしも最適な系では無い可能性があるが、キナゾリン誘導体 **54** で MMP-13 選択的阻害薬が、経口投与で、ラット MIA 誘発 OA モデルにおけるコラーゲン分解を抑制することを示した。



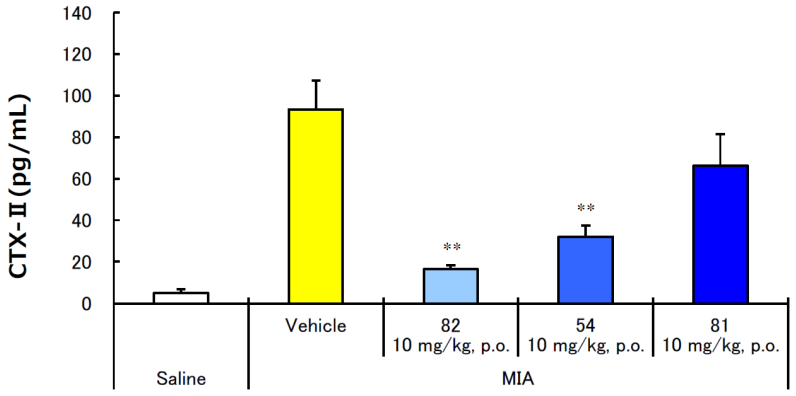


Figure 4-2. Protective Effect of MMPs Inhibitors on Cartilage Degradation in the Rat Model of monoiodoacetate (MIA)-induced OA. Synovial C-terminal telopeptide of type II collagen (CTX-II) levels were measured at 4 h after intraarticular injection of MIA. Data are expressed as means ± SEM (n = 6). ** denotes $P < 0.01$ versus vehicle group by two-way analysis of variance with Dunnett's test.

4-4　ラット毒性試験

キナゾリン誘導体 **38k** の一ナトリウム塩 **54** は、ラットを用いた経口投与での 2 週間の毒物試験では、200 mg/kg/日の用量まで、毒性所見は観察されなかった。また、チエノピリミジン誘導体 **64c** の二ナトリウム塩 **81** に関しても、ラットでの 2 週間の反復投与経口毒性試験を行い、60 mg/kg/日の無毒性量を確認した。

以上のことから、**54** および、**81** は MMP-13 関連疾患の治療に向けたさらなる前臨床評価の有望な候補となった。

4-5　結論

キナゾリン誘導体 **38k** は MMP-13 に対し IC_{50} = 0.0039 nM の非常に強力な阻害活性を示し、他の MMP ファミリーに対し、41,000 倍以上の極めて高い選択性を示した。また、チエノピリミジン誘導体 **64c** も MMP-13 に対し、IC_{50} = 0.0069 nM の活性及び 2,600 倍以上の高い選択性を示した。

ウシ鼻軟骨培養系を用いたコラーゲン分解抑制試験において 1 μM 添加でキナゾリン誘導体 **38k** は 73%、チエノピリミジン誘導体 **64c** は 100%の抑制活性を示した。

ラットを用いたモノヨード酢酸（MIA）誘発関節炎モデル（10 mg/kg）に経口投与し、タイプ II コラーゲンの分解抑制率を調べたところ、キナゾリン誘導体 **38k** の一ナトリウム塩 **54** は 69%の有意な抑制を、チエノピリミジン誘導体 **64c** の二ナトリウム塩 **81** は 31%の軟骨分解抑制傾向を示した。

ラットの 2 週間の経口毒性試験ではキナゾリン誘導体 **54** は 200 mg/kg/日、チエノピリミジン誘導体 **81** は 60 mg/kg/日まで、毒性所見は見られなかった。

結語

本研究は、副作用の無い変形性関節症（OA）治療薬の創製を目指し、従来の亜鉛結合型非選択的阻害薬とは異なり、活性中心の亜鉛と結合しないMMP-13選択的阻害薬の分子設計に関するものである。

1） 従来のMMP阻害薬が、MMPファミリー内で高度に保存された触媒活性中心付近の構造を利用したものであり、亜鉛結合基を有し、非選択的であったことに対し、X線共結晶解析像に基づき、活性中心から遠く離れたMMP-13に特有のS1"ポケットとの相互作用を活用して、触媒中心の亜鉛とは相互作用をしない、MMP-13に選択的な阻害薬を創出するという戦略をとった。

2） structure-based drug design (SBDD)により、合成を行った結果、強力なMMP-13阻害活性と極めて高い選択性を有する新規キナゾリン誘導体および、チエノ[2,3-*d*]ピリミジン誘導体の創出に成功し、立案したデザイン戦略の妥当性を示した。

3） 創出した高活性・高選択的MMP-13阻害薬の高次評価の結果、in vitro軟骨組織培養系およびin vivo OAモデルで軟骨分解抑制活性を確認した。

4） in vitro軟骨組織培養系活性およびin vivo OAモデルの活性と、酵素阻害活性との乖離が共に大きく、その有効性を十分に実証するには至らなかったが、これら高次評価系では、MMP-13選択的阻害薬のOA治療への適用可否判断は困難であると考えられた。より病態を反映した、適切なin vitro、in vivo評価系の開発は必須と考えられ、これらの研究の進展が待たれる。

5） 優れた活性・選択性・物性・経口吸収性と、ラットの2週反復投与毒性試験より確認された高い安全性を示すMMP-13高選択的阻害薬チエノピリミジン**81**は現在、The Structural Genomics Consortium（SGC）を通じ、創薬および機能解析のツールとして、全世界の研究者が入手し医薬品開発研究に活用出来るように公開・配布されている。

General. Melting points were determined in open capillary tubes on a Büchi melting point apparatus B545 and are uncorrected. ^{1}H NMR spectra were recorded on a Varian Gemini-200 (200 MHz) or Varian Gemini-300 (300 MHz) or Bruker DPX-300 (300 MHz) spectrometer and are reported in parts per million (δ) relative to tetramethylsilane (TMS: δ 0.0 ppm). Data are reported as follows: chemical shift, integration, multiplicity (s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet, dd = doublet of doublet, ddd = doublet of doublet of doublet, bs = broad singlet), and coupling constants (J, Hz). Unless otherwise specified, all solvents and reagents were obtained from commercial suppliers and used without further purification. Column chromatography was performed using Merck silica gel 60 (70–230 mesh). Thin-layer chromatography (TLC) was performed on Merck silica gel plates $60F_{254}$. LC-MS analysis was performed on a Shiseido CAPCELL PACK C-18 UG120 S-3 column (1.5 mmΦ × 35 mm) in a Waters Alliance 2795 or an Agilent 1100 LC system equipped with a Waters 2487 absorbance detector and a Micromass ZQ2000 mass spectrometer. Analytes were eluted using a linear gradient of water (0.05% TFA)/acetonitrile (0.04% TFA) from 90:10 to 0:100 over 4 min at a flow rate of 0.5 mL/min. UV detection was at 220 nm. Preparative HPLC was performed on a Shiseido CAPCELL PACK C-18 UG120 S-5 column (20 mmΦ × 50 mm), eluting at 25 mL/min with a gradient of water (0.1% TFA)/acetonitrile (0.1% TFA). UV detection was at 220 nm. Compound purity for all tested compounds was determined by elemental analysis or HPLC analysis. Experimentally determined hydrogen, carbon, and nitrogen composition by elemental analysis was within ± 0.4% of the expected value, implying a purity of ≥ 95%. Analytical HPLC was performed with Corona charged aerosol detector (CAD) on an L-column 2 ODS (30 mm × 2.0 mm I.D., CERI, Japan) operated at 50 °C, eluting at 0.5 mL/min using a linear gradient. Mobile phase was A, 50 mmol/L ammonium acetate, water, and acetonitrile (1:8:1, v/v/v); and B, 50 mmol/L ammonium acetate and acetonitrile (1:9, v/v). The ratio of mobile phase B was increased linearly from 5% to 95% over 3 min, 95% over the next 1 min. All experiments using animals were reviewed and approved by the Internal Animal Care and Use Committee of Takeda Pharmaceutical Research Division.

2-(3-Methoxyphenyl)-*N*-(6-methyl-4-oxo-3,4-dihydroquinazolin-2-yl)acetamide (**4**). A mixture of commercially available 2-amino-6-methyl-3,4-dihydroquinazolin-4-one (**3**, 150 mg, 0.856 mmol), compound **2** (380 mg, 2.06 mmol), triethylamine (260 mg, 2.56 mmol), THF (6 mL), and DMF (4 mL) was stirred at 90 °C for 4 h. The insoluble materials were filtered off and the filtrate was concentrated under reduced pressure. The residue was suspended with ethanol and the resulting precipitate was collected by filtration. The solid was washed with ethanol and dried to give **4** as a white powder (198 mg, 72%). mp 208–210 °C. ^{1}H NMR (200 MHz, DMSO-d_6) δ 2.41 (3H, s), 3.70–7.80 (5H, m), 6.82–6.94 (3H, m), 7.26 (1H, t, J = 8.0 Hz), 7.40 (1H, d, J = 8.4 Hz), 7.57–7.62 (1H, m), 7.85 (1H, s), 11.8 (1H, bs), 11.9 (1H, bs).

Anal. Calcd for $C_{18}H_{17}N_3O_3$•0.1H_2O: C, 66.49; H, 5.33; N, 12.92. Found: C, 66.26; H, 5.43; N, 13.14.

2-(Chloromethyl)-6-methylquinazolin-4(3*H*)-one (**6**)[59]. Sodium methylate (357 mg, 6.62 mmol) was added to a solution of chloroacetonitrile (2.75 g, 36.4 mmol) in methanol (75 mL) at 0 °C and the mixture was stirred at room temperature for 1 h. In another flask, commercially available 2-amino-5-methylbenzoic acid **5** (5.00 g, 33.1 mmol) was added to a solution of sodium methylate (179 mg, 3.31 mmol) in methanol (75 mL) and this solution was added to the above solution of chloroacetoimidate at room temperature. The reaction mixture was stirred at room temperature for 1 h and then heated at 80 °C for 2 h. After the mixture was cooled to room temperature, the precipitated solid was collected and washed with methanol to give **6** as a pale gray powder (4.60 g, 67%). ^{1}H NMR (300 MHz, DMSO-d_6) δ 2.45 (3H, s), 4.54 (2H, s), 7.58 (1H, d, J = 8.1 Hz), 7.66 (1H, dd, J = 8.1, 1.8 Hz), 7.92 (1H, d, J = 0.6 Hz), 12.5 (1H, bs).

2-({[(3-Methoxyphenyl)methyl]amino}methyl)-6-methyl-3,4-dihydroquinazolin-4-one (**7**).
A mixture of compound **6** (200 mg, 0.959 mmol), 3-methoxybenzylamine (263 mg, 1.92 mmol), and K_2CO_3 (132 mg, 0.959 mmol) in THF (6 mL) was stirred at 40 °C for 15 h. The reaction mixture was concentrated under reduced pressure. The residue was diluted with ethyl acetate, washed with brine, dried over anhydrous Na_2SO_4 and concentrated under reduced pressure. The crude product was purified by preparative HPLC and crystallized from diethyl ether to give **7** as a white powder (120 mg, 41%). mp 172–174 °C. ^{1}H NMR (300 MHz, DMSO-d_6) δ 2.43 (3H, s), 3.64 (2H, s), 3.71 (2H, s), 3.73 (3H, s), 6.76–6.80 (1H, m), 6.89–6.93 (2H, m), 7.21 (1H, t, J = 7.8 Hz), 7.53 (1H, d, J = 8.1 Hz), 7.61 (1H, dd, J = 8.4, 2.1 Hz), 7.89 (1H, d, J = 0.6 Hz). Anal. Calcd for $C_{18}H_{19}N_3O_2$•0.4H_2O: C, 68.29; H, 6.30; N, 13.27. Found: C, 68.23; H, 6.07; N, 13.23.

6-Methylquinazolin-4(3*H*)-one (**9**). Concentrated hydrochloric acid (4.16 mL, 49.9 mmol) was added to a mixture of commercially available 2-amino-5-methylbenzamide **8** (5.00 g, 33.3 mmol) and trimethyl orthoformate (51.0 mL, 466 mmol) at 0 °C and the mixture was stirred at room temperature for 2 h. After the mixture was concentrated under reduced pressure, the residue was diluted with water and neutralized with 2 M aqueous sodium hydroxide solution. The precipitated solid was collected and washed with H_2O, methanol and diethyl ether to give **9** as a white powder (4.33 g, 81%). ^{1}H NMR (300 MHz, DMSO-d_6) δ 2.44 (3H, s), 7.57 (1H, d, J = 8.4 Hz), 7.62–7.66 (1H, m), 7.91–7.93 (1H, m), 8.03 (1H, s), 12.2 (1H, bs).

***tert*-Butyl 6-methyl-4-oxo-3,4-dihydroquinazoline-3-carboxylate** (**10**). To a solution of compound **9** (3.00 g, 18.7 mmol) in THF (250 mL) was added sodium hydride (60% oil dispersion, 0.824 g, 20.6 mmol) at 0 °C and the mixture was stirred at room temperature for 3 h. A solution of di-*tert*-butyl dicarbonate (6.13 g, 28.1 mmol) in THF (50 mL) was added 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 washed with brine, dried over anhydrous Na_2SO_4 and concentrated under reduced pressure. The crude product was purified by silica gel column chromatography (25% ethyl acetate/hexane) to give **10** as a pale yellow oil (4.45 g, 91%). ^{1}H NMR (300 MHz, DMSO-d_6) δ 1.59 (9H, s), 2.46 (3H, s), 7.60 (1H, d, J = 8.4 Hz), 7.69–7.72 (1H, m), 7.97 (1H, bs), 8.45 (1H, s).

3-[3-(Methyloxy)phenyl]propan-1-ol (**12**). To a solution of commercially available 3-[3-(methyloxy)phenyl]propanoic acid (**11**, 3.00 g, 16.6 mmol) and DMF (0.10 mL) in THF (100 mL) was added dropwise oxalyl chloride (2.32 g, 18.3 mmol) at 0 °C, and the mixture was stirred at room temperature for 3 h. The reaction mixture was concentrated under reduced pressure and dissolved in THF (100 mL). Sodium borohydride (1.26 g, 33.2 mmol) was added to the solution at 0 °C and the resulting mixture was stirred at reflux for 2 h. The mixture was partitioned between ethyl acetate and H_2O. The organic layer was washed with brine and dried over anhydrous Na_2SO_4. The filtrate was concentrated under reduced pressure to give **12** as a pale yellow oil (2.53 g, 92%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.87–1.92 (2H, m), 2.69 (2H, t, J = 7.5 Hz), 3.68 (2H, t, J = 6.3 Hz), 3.80 (3H, s), 6.73–6.81 (3H, m), 7.20–7.26 (1H, m).

3-[3-(Methyloxy)phenyl]propanal (**13**). To a mixture of compound **12** (2.45 g, 14.7 mmol), *N*-methylmorpholine-*N*-oxide (2.58 g, 22.1 mmol) and molecular sieves 4A (500 mg) in CH_2Cl_2 (50 mL) was added tetrapropylammonium perruthenate (259 mg, 0.737 mmol), and the mixture was stirred at room temperature for 2 h. The reaction mixture was purified by silica gel column chromatography (60% ethyl acetate/hexane) to give **13** as a pale yellow oil (1.52 g, 63%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 2.75–2.81 (2H, m), 2.94 (2H, t, J = 7.5 Hz), 3.80 (3H, s), 6.74–6.80 (3H, m), 7.18–7.26 (1H, m), 9.83 (1H, t, J = 1.5 Hz).

***tert*-Butyl 2-[1-hydroxy-3-(3-methoxyphenyl)propyl]-6-methyl-4-oxo-3,4-dihydroquinazoline-3-carboxylate** (**14**). To a solution of compound **10** (500 mg, 1.92 mmol) in THF (20 mL) was added dropwise lithium diisopropylamide (1.8 M in a mixed solvent of heptane, THF and ethylbenzene, 1.28 mL, 2.30 mmol) at –78 °C, and the mixture was stirred at –78 °C for 10 min. A solution of compound **13** (631 mg, 3.84 mmol) in THF (5 mL) was added at –78 °C and the reaction mixture was allowed to warm to room temperature followed by stirring at room temperature for 1 h. The reaction was quenched 1 M hydrochloric acid and the resulting mixture was partitioned between ethyl acetate and H_2O. The organic layer was washed with brine, dried over anhydrous Na_2SO_4 and concentrated under reduced pressure. The crude product was purified by silica gel column chromatography (25–33% ethyl acetate/hexane) to give **14** as a yellow oil (815 mg, 44%). ^{1}H NMR (300 MHz, DMSO-d_6) δ 1.40 (9H, s), 2.14–2.27 (2H, m), 2.44 (3H, s), 2.64–2.76 (2H, s), 3.71 (3H, s), 5.17–5.22 (1H, m), 6.65–6.81 (3H, m), 7.14–7.21 (1H, m), 7.52–7.55 (1H, m), 7.61–7.65 (1H, m), 7.89 (1H, s).

2-[1-Hydroxy-3-(3-methoxyphenyl)propyl]-6-methyl-3,4-dihydroquinazolin-4-one (**15**). A mixture of compound **14** (360 mg, 0.848 mmol), trifluoroacetic acid (3 mL), and CH_2Cl_2 (6 mL) was stirred at room temperature for 1 h. The reaction mixture was concentrated under reduced pressure. The residue was purified by silica gel column chromatography (60% ethyl acetate/hexane) and crystallized from diethyl ether to give **15** as a white powder (125 mg, 0.385 mmol, 45%). mp 149–151 °C. ^{1}H NMR (300 MHz, DMSO-d_6) δ 2.00–2.09 (2H, m), 2.43 (3H, s), 2.57–2.72 (2H, m), 3.72 (3H, s), 4.38–4.44 (1H, m), 5.76 (1H, t, J = 4.8 Hz), 6.72–6.81 (3H, m), 7.18 (1H, t, J = 7.8 Hz), 7.54 (1H, d, J = 8.4 Hz), 7.62 (1H, d, J = 7.8 Hz), 7.90 (1H, s), 11.7 (1H, bs). Anal. Calcd for $C_{19}H_{20}N_2O_3$•0.1H_2O: C, 69.96; H, 6.24; N, 8.59. Found: C, 69.67; H, 6.18;

N, 8.51.

2-[3-(3-Methoxyphenyl)propanoyl]-6-methyl-3,4-dihydroquinazolin-4-one (**17**). Dimethylsufoxide (0.011 mL, 0.154 mmol) was added to a solution of oxalyl chloride (0.013 mL, 0.154 mmol) in CH_2Cl_2 (4 mL) at –78 °C under nitrogen atmosphere and the mixture was stirred at –78 °C for 2 min. A solution of compound **15** (25 mg, 0.077 mmol) and dimethylsulfoxide (0.020 mL) in CH_2Cl_2 (2 mL) was added and the mixture was stirred at –78 °C for 1 h. After triethylamine (0.107 mL, 0.771 mmol) was added, the resulting mixture was allowed to warm to room temperature and stirred at room temperature for 2 h. The reaction mixture was partitioned between ethyl acetate and water. The organic layer was washed with brine, dried over anhydrous Na_2SO_4 and concentrated under reduced pressure. The crude product was purified by silica gel column chromatography (25% ethyl acetate/hexane) to give compound **16** (1-(6-methyl-4-{[(methylthio)methyl]oxy}quinazolin-2-yl)-3-[3-(methyloxy)phenyl]propan-1-one) as a pale yellow oil (12 mg). To a solution of the pale yellow oil obtained above in CH_2Cl_2 (1 mL) was added 90% aqueous trifluoroacetic acid (0.5 mL), and the mixture was stirred at room temperature for 10 min. The reaction mixture was concentrated under reduced pressure and the residue was purified by preparative HPLC to give **17** as a white powder (6.5 mg, 26%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 2.53 (3H, s), 3.03–3.08 (2H, m), 3.54–3.59 (2H, m), 3.80 (3H, s), 6.73–6.86 (3H, m), 7.22 (1H, t, J = 8.1 Hz), 7.65 (1H, dd, J = 8.4, 2.1 Hz), 7.75 (1H, d, J = 8.4 Hz), 8.13–8.14 (1H, m), 10.1 (1H, bs).

Representative Procedure for the Synthesis of Compounds 22a and 22c: 2-(Methyloxy)-6-nitrobenzonitrile (**22a**). To a solution of 2,6-dinitrobenzonitrile (6.00 g, 31.1 mmol) in methanol (120 mL) was added dropwise a solution of sodium methoxide (1.68 g, 31.1 mmol) in methanol (30 mL) at room temperature and the mixture was refluxed for 3 h. The mixture was concentrated under reduced pressure and the residue was partitioned between ethyl acetate and H_2O. The organic layer was washed with brine, dried over Na_2SO_4 and concentrated under reduced pressure to give the title compound as a brown powder (5.50 g, 30.9 mmol, 99%). ^{1}H NMR (200 MHz, DMSO-d_6) δ 4.04 (3H, s), 7.70–7.78 (1H, m), 7.90–7.95 (2H, m).

4-(Methyloxy)-2-nitrobenzonitrile (**22c**). Compound **22c** was prepared from 2,4-dinitrobenzonitrile (pale yellow powder, 38%). ^{1}H NMR (200 MHz, $CDCl_3$) δ 3.98 (3H, s), 7.23–7.30 (1H, m), 7.73–7.83 (2H, m).

2-Nitro-6-[(2-phenylethyl)oxy]benzonitrile (**22b**). To a mixture of 2-phenylethanol (1.27 g, 10.4 mmol), DMF (20 mL) and THF (5 mL) was added sodium hydride (60% oil dispersion, 460 mg, 11.4 mmol) at 0 °C and the mixture was stirred at room temperature for 30 min. A solution of 2,6-dinitrobenzonitrile (2.00 g, 10.4 mmol) in THF (5 mL) was added to the mixture and the resultant mixture was heated at 90 °C for 3 h. The mixture was concentrated under reduced pressure and the residue was partitioned between ethyl acetate and H_2O. The organic layer was washed with brine and dried over Na_2SO_4. After removal of the solvent, the residue was triturated with ethanol to give the title compound as a brown powder (894 mg, 3.33 mmol,

32%). ^{1}H NMR (200 MHz, DMSO-d_6) δ 3.12 (2H, t, J = 6.6 Hz), 4.46 (2H, t, J = 6.8 Hz), 7.19–7.41 (5H, m), 7.74 (1H, dd, J = 6.6, 3.0 Hz), 7.79–7.93 (2H, m).

Representative Procedure for the Synthesis of Compounds 23a, 23c, and 23m: 2-Amino-6-(methyloxy) benzamide (**23a**). To a mixture of 2-(methyloxy)-6-nitrobenzonitrile (2.60 g, 14.6 mmol) and hydrazine monohydrate (1.53 g, 30.7 mmol) in ethanol (60 mL) was added portionwise Raney Ni (600 mg) and the mixture was stirred at 60 °C for 2 h. Additional Raney Ni (300 mg) was added to the reaction mixture and the resultant mixture was refluxed for 2 h. The insoluble materials were filtered off through a PTFE membrane filter and concentrated under reduced pressure. The residue was partitioned between ethyl acetate and 1 N hydrochloric acid. The aqueous layer was basified with 1 N aqueous sodium hydroxide solution and extracted with ethyl acetate. The organic layer was washed with brine, dried over Na_2SO_4 and concentrated under reduced pressure to give the title compound as a brown powder (980 mg, 5.90 mmol, 40%). ^{1}H NMR (200 MHz, DMSO-d_6) δ 3.76 (3H, s), 6.15–6.19 (1H, m), 6.28–6.35 (3H, m), 7.01 (1H, t, J = 8.0 Hz), 7.25 (1H, bs), 7.53 (1H, bs).

2-Amino-4-(methyloxy)benzamide (**23c**). Compound **23c** was prepared from compound **22c** (pale yellow powder, 88%). ^{1}H NMR (200 MHz, DMSO-d_6) δ 3.69 (3H, s), 6.06 (1H, dd, J = 8.8, 2.6 Hz), 6.19 (1H, d, J = 2.6 Hz), 6.73 (2H, bs), 7.48 (1H, d, J = 8.8 Hz).

2-Amino-6-[(2-phenylethyl)oxy]benzamide (**23m**). Compound **23m** was prepared from compound **22b** (brown powder, 44%). ^{1}H NMR (200 MHz, DMSO-d_6) δ 3.07 (2H, t, J = 6.6 Hz), 4.20 (2H, t, J = 6.6 Hz), 6.19 (1H, d, J = 8.2 Hz), 6.30 (1H, d, J = 7.6 Hz), 6.40 (2H, bs), 6.99 (1H, t, J = 8.2 Hz), 7.22–7.33 (7H, m).

2-Amino-5-(methyloxy)benzamide (**23b**). To a solution of 5-methoxy-2-nitrobenzoic acid (18.0 g, 91.3 mmol) and DMF (0.1 mL) in THF (150 mL) was added dropwise oxalyl chloride (12.7 g, 100 mmol) at 0 °C. After being stirred at room temperature for 3 h, the mixture was added to 7% aqueous ammonia solution (200 mL) at 0 °C. The mixture was concentrated under reduced pressure and the residue was suspended in H_2O. The precipitated solid was collected and washed with H_2O to give 5-methoxy- 2-nitrobenzamide as a pale yellow powder (10.0 g, 50.9 mmol, 56%). A mixture of 5-methoxy- 2-nitrobenzamide (9.70 g, 49.4 mmol) and 10% palladium on carbon (2.00 g) in methanol (250 mL) was stirred at room temperature for 6 h under hydrogen atmosphere (1 atm). After the insoluble materials were filtered off through a PTFE membrane filter, the filtrate was concentrated under reduced pressure to give the title compound as a pale yellow powder (8.20 g, 49.3 mmol, 99%). ^{1}H NMR (200 MHz, DMSO-d_6) δ 3.67 (3H, s), 6.11 (2H, bs), 6.63 (1H, d, J = 8.8 Hz), 6.83 (1H, dd, J = 8.8, 2.8 Hz), 7.09 (1H, bs), 7.10 (1H, d, J = 2.8 Hz), 7.75 (1H, bs).

Representative Procedure for the Synthesis of Compounds 23d, 23h, 23j, and 23l: 2-Amino-3-(methyloxy)benzamide (**23d**). To a solution of 3-methoxyanthranilic acid (4.90 g, 29.3 mmol) in THF (40 mL) was added triphosgene (2.90 g, 9.77 mmol) and the mixture was stirred at 60 °C for 15 h. The mixture

was concentrated under reduced pressure and 1 N aqueous ammonia solution (150 mL) was added to the residue. After being stirred at 60 °C for 2 h, the mixture was extracted with ethyl acetate. The organic layer was washed with brine, dried over Na_2SO_4 and filtered. The filtrate was concentrated under reduced pressure to give the title compound as a pale yellow powder (3.65 g, 22.0 mmol, 75%). ^{1}H NMR (300 MHz, DMSO-d_6) δ 3.79 (3H, s), 6.27 (2H, bs), 6.48 (1H, t, J = 8.1 Hz), 6.88 (1H, dd, J = 8.1, 1.2 Hz), 7.09 (1H, bs), 7.18 (1H, dd, J = 8.1, 1.2 Hz), 7.71 (1H, bs).

2-Amino-5-[(trifluoromethyl)oxy]benzamide (**23h**). Compound **23h** was prepared from 5-[(trifluoromethyl)oxy]anthranilic acid (yellow powder, 43%). ^{1}H NMR (200 MHz, DMSO-d_6) δ 6.74 (1H, d, J = 9.2 Hz), 6.78 (2H, bs), 7.12–7.25 (2H, m), 7.55 (1H, d, J = 2.8 Hz), 7.85 (1H, bs).

2-Amino-5-iodobenzamide (**23j**). Compound **23j** was prepared from 5-iodoanthranilic acid (white powder, 64%). ^{1}H NMR (200 MHz, DMSO-d_6) δ 6.54 (1H, d, J = 8.8 Hz), 6.69 (2H, bs), 7.14 (1H, bs), 7.37 (1H, dd, J = 8.8, 2.2 Hz), 7.81 (1H, d, J = 2.2 Hz), 7.80–7.83 (1H, m).

2-Amino-6-methylbenzamide (**23l**). Compound **23l** was prepared from 6-methylanthranilic acid (pale yellow powder, 13%). ^{1}H NMR (200 MHz, DMSO-d_6) δ 2.21 (3H, s), 4.89 (2H, bs), 6.38 (1H, d, J = 7.6 Hz), 6.50 (1H, d, J = 8.0 Hz), 6.91 (1H, t, J = 7.8 Hz), 7.4 (1H, bs), 7.61 (1H, bs).

2-Amino-5-(trifluoromethyl)benzamide (**23g**). To a solution of 2-nitro-5-(trifluoromethyl)aniline (5.00 g, 24.3 mmol) in concentrated hydrochloric acid (50 mL) was added dropwise a solution of sodium nitrite (1.84 g, 26.7 mmol) in H_2O (15 mL) at 0 °C and the mixture was stirred at room temperature for 1 h. After the insoluble materials were filtered off, to the filtrate was added dropwise a mixture of copper(I) cyanide (2.91 g, 29.2 mmol) and sodium cyanide (600 mg, 12.2 mmol) in H_2O (20 mL) followed by the addition of toluene (9 mL). After being stirred at room temperature for 15 h, the mixture was diluted with chloroform, washed with H_2O and brine, dried over Na_2SO_4 and concentrated under reduced pressure to give crude 2-nitro-5-(trifluoromethyl)benzonitrile (**22d**) as a brown oil. Compound **23g** was prepared from compound **22d** obtained above with the same procedure as described for **23a** (brown powder, 16% over 2 steps). ^{1}H NMR (200 MHz, DMSO-d_6) δ 6.81 (1H, d, J = 8.4 Hz), 7.20 (3H, m), 7.41 (1H, dd, J = 8.6, 2.0 Hz), 7.89 (1H, bs), 7.99 (1H, bs).

2-Amino-5-[(phenylmethyl)oxy]benzamide (**23i**). To a solution of 5-hydroxy-2-nitrobenzoic acid (10.0 g, 54.6 mmol) in DMF (200 mL) were added benzyl bromide (20.5 g, 120 mmol) and potassium carbonate (18.9 g, 137 mmol) and the mixture was stirred at room temperature for 15 h. The mixture was concentrated under reduced pressure and the residue was suspended in H_2O. The precipitated solid was collected and washed with H_2O to give benzyl 5-benzyloxy-2-nitrobenzoate as a yellow powder (19.4 g, 53.5 mmol, 98%). To a solution of benzyl 5-benzyloxy-2-nitrobenzoate (19.0 g, 52.2 mmol) in methanol (100 mL) was added a solution of potassium hydroxide (8.79 g, 157 mmol) in H_2O (50 mL) and the mixture was refluxed for 1 h. After acidification with 4 N hydrochloric acid, the mixture was concentrated under reduced pressure and the residue was partitioned between ethyl acetate and H_2O. Eighty mL of 1 N aqueous sodium hydroxide

solution was added to the organic layer and the precipitated solid was collected. The solid was dissolved with methanol and 1 N hydrochloric acid (60 mL) was added. The product was extracted with ethyl acetate, washed with brine, dried over Na_2SO_4 and concentrated under reduced pressure to give 5-benzyloxy-2-nitrobenzoic acid as a pale yellow powder (12.0 g, 44.1 mmol, 84%). To a solution of 5-benzyloxy-2-nitrobenzoic acid (11.9 g, 43.6 mmol) and DMF (0.12 mL) in THF (120 mL) was added dropwise oxalyl chloride (6.08 g, 47.9 mmol) at 0 °C. After being stirred at room temperature for 3 h, the mixture was added to 3% aqueous ammonia solution (270 mL) at 0 °C and stirred at room temperature for 1 h. The precipitated solid was collected and washed with H_2O and diethyl ether to give 5-benzyloxy-2-nitrobenzamide as a pale yellow powder (11.0 g, 40.6 mmol, 93%). A mixture of 5-benzyloxy-2-nitrobenzamide (10.5 g, 38.8 mmol), ammonium chloride (10.0 g), Fe powder (10.0 g), ethanol (100 mL) and H_2O (100 mL) was refluxed for 1.5 h. The insoluble materials were filtered off and concentrated under reduced pressure. The residue was partitioned between ethyl acetate and 1 N aqueous sodium hydroxide solution. The organic layer was washed with brine, dried over Na_2SO_4 and concentrated under reduced pressure to give the title compound as a yellow powder (9.42 g, 38.8 mmol, 100%). ^{1}H NMR (200 MHz, DMSO-d_6) δ 4.99 (2H, s), 6.15 (2H, bs), 6.63 (1H, d, J = 8.8 Hz), 6.91 (1H, dd, J = 8.8, 2.8 Hz), 7.07 (1H, bs), 7.24 (1H, d, J = 2.8 Hz), 7.28–7.45 (5H, m), 7.74 (1H, bs).

Representative Procedure for the Synthesis of Compounds 24a–m: Ethyl {[2-(Aminocarbonyl)-3-(methyloxy)phenyl]amino}(oxo)acetate (**24a**). To a solution of compound **23a** (960 mg, 5.78 mmol) and triethylamine (701 mg, 6.93 mmol) in THF (20 mL) was added dropwise ethyl chloroglyoxylate (868 mg, 6.36 mmol) at 0 °C and the mixture was stirred at room temperature for 2 h. The mixture was diluted with ethyl acetate, washed with H_2O and brine, dried over Na_2SO_4. After removal of the solvent, the residue was triturated with ethanol to give the title compound as a yellow powder (1.56 g, 5.85 mmol, 100%). ^{1}H NMR (200 MHz, DMSO-d_6) δ 1.31 (3H, t, J = 7.2 Hz), 3.86 (3H, s), 4.30 (2H, q, J = 7.2 Hz), 6.97 (1H, d, J = 8.4 Hz), 7.46 (1H, d, J = 8.4 Hz), 7.91–8.06 (2H, m), 8.02 (1H, d, J = 8.0 Hz), 12.27 (1H, bs).

Ethyl {[2-(Aminocarbonyl)-4-(methyloxy)phenyl]amino}(oxo)acetate (**24b**). Compound **24b** was prepared from compound **23b** (pale yellow powder, 100%). ^{1}H NMR (200 MHz, DMSO-d_6) δ 1.31 (3H, t, J = 7.2 Hz), 3.81 (3H, s), 4.30 (2H, q, J = 7.2 Hz), 7.18 (1H, dd, J = 9.0, 3.0 Hz), 7.43 (1H, d, J = 3.0 Hz), 7.86 (1H, bs), 8.39 (1H, bs), 8.47 (1H, d, J = 8.8 Hz).

Ethyl {[2-(Aminocarbonyl)-5-(methyloxy)phenyl]amino}(oxo)acetate (**24c**). Compound **24c** was prepared from compound **23c** (pale yellow powder, 100%). ^{1}H NMR (200 MHz, DMSO-d_6) δ 1.32 (3H, t, J = 7.2 Hz), 3.82 (3H, s), 4.30 (2H, q, J = 7.2 Hz), 6.80 (1H, dd, J = 8.8, 2.6 Hz), 7.64 (1H, bs), 7.88 (1H, d, J = 8.8 Hz), 8.18–8.21 (1H, m), 8.20 (1H, d, J = 2.6 Hz).

Ethyl {[2-(Aminocarbonyl)-6-(methyloxy)phenyl]amino}(oxo)acetate (**24d**). Compound **24d** was prepared from compound **23d** (white powder, 81%). ^{1}H NMR (200 MHz, DMSO-d_6) δ 1.30 (3H, t, J = 7.2 Hz), 3.79 (3H, s), 4.29 (2H, q, J = 7.2 Hz), 7.15 (1H, dd, J = 7.6, 1.4 Hz), 7.20 (1H, dd, J = 8.4, 1.4 Hz),

7.34 (1H, dd, *J* = 8.4, 7.6 Hz), 7.45 (1H, bs), 7.68 (1H, bs), 10.15 (1H, bs).

Ethyl {[2-(Aminocarbonyl)-4-fluorophenyl]amino}(oxo)acetate (**24e**). Compound **24e** was prepared from compound **23e** (white powder, 87%). ^{1}H NMR (200 MHz, DMSO-d_6) δ 1.36 (3H, t, *J* = 7.2 Hz), 4.31 (2H, q, *J* = 7.2 Hz), 7.42–7.53 (1H, m), 7.76 (1H, dd, *J* = 9.8, 2.8 Hz), 7.99 (1H, bs), 8.41 (1H, bs), 8.57 (1H, dd, *J* = 9.2, 5.2 Hz).

Ethyl {[2-(Aminocarbonyl)-4-methylphenyl]amino}(oxo)acetate (**24f**). Compound **24f** was prepared from compound **23f** (white powder, 100%). ^{1}H NMR (200 MHz, DMSO-d_6) δ 1.31 (3H, t, *J* = 7.2 Hz), 2.32 (3H, s), 4.29 (2H, q, *J* = 7.2 Hz), 7.38 (1H, dd, *J* = 8.4, 1.4 Hz), 7.71 (1H, d, *J* = 1.4 Hz), 7.79 (1H, bs), 8.29 (1H, bs), 8.42 (1H, d, *J* = 8.4 Hz).

Ethyl {[2-(Aminocarbonyl)-4-(trifluoromethyl)phenyl]amino}(oxo)acetate (**24g**). Compound **24g** was prepared from compound **23g** (brown powder, 99%). ^{1}H NMR (200 MHz, DMSO-d_6) δ 1.33 (3H, t, *J* = 7.2 Hz), 4.32 (2H, q, *J* = 7.2 Hz), 7.93–8.04 (2H, m), 8.23–8.28 (1H, m), 8.63 (1H, bs), 8.74 (1H, d, *J* = 8.8 Hz).

Ethyl ({2-(Aminocarbonyl)-4-[(trifluoromethyl)oxy]phenyl}amino)(oxo)acetate (**24h**). Compound **24i** was prepared from compound **23h** (yellow powder, 100%). ^{1}H NMR (200 MHz, DMSO-d_6) δ 1.32 (3H, t, *J* = 7.0 Hz), 4.31 (2H, q, *J* = 7.0 Hz), 7.62–7.67 (1H, m), 7.90 (1H, d, *J* = 3.2 Hz), 8.02 (1H, bs), 8.48 (1H, bs), 8.64 (1H, d, *J* = 9.6 Hz).

Ethyl ({2-(Aminocarbonyl)-4-[(phenylmethyl)oxy]phenyl}amino)(oxo)acetate (**24i**). Compound **24i** was prepared from compound **23i** (yellow powder, 100%). ^{1}H NMR (200 MHz, DMSO-d_6) δ 1.31 (3H, t, *J* = 7.2 Hz), 4.30 (2H, q, *J* = 7.2 Hz), 5.15 (2H, s), 7.26 (1H, dd, *J* = 9.2, 3.0 Hz), 7.30–7.50 (5H, m), 7.55 (1H, d, *J* = 2.6 Hz), 7.85 (1H, bs), 8.37 (1H, bs), 8.47 (1H, d, *J* = 9.0 Hz).

Ethyl {[2-(Aminocarbonyl)-4-iodophenyl]amino}(oxo)acetate (**24j**). Compound **24j** was prepared from compound **23j** (white powder, 96%). ^{1}H NMR (200 MHz, DMSO-d_6) δ 1.31 (3H, t, *J* = 7.0 Hz), 4.30 (2H, q, *J* = 7.0 Hz), 7.91 (1H, dd, *J* = 8.8, 2.2 Hz), 7.92 (1H, bs), 8.20 (1H, d, *J* = 2.2 Hz), 8.34 (1H, d, *J* = 8.8 Hz), 8.43 (1H, bs).

Ethyl {[2-(Aminocarbonyl)-3-fluorophenyl]amino}(oxo)acetate (**24k**). Compound **24k** was prepared from compound **23k** (white powder, 79%). ^{1}H NMR (200 MHz, DMSO-d_6) δ 1.31 (3H, t, *J* = 7.2 Hz), 4.3 (2H, q, *J* = 7.2 Hz), 7.14 (1H, dd, *J* = 10.4, 8.2 Hz), 7.55 (1H, dd, *J* = 14.8, 8.2 Hz), 8.12–8.19 (3H, m), 11.64 (1H, bs).

Ethyl {[2-(Aminocarbonyl)-3-methylphenyl]amino}(oxo)acetate (**24l**). Compound **24l** was prepared from compound **23l** (white powder, 87%). ^{1}H NMR (200 MHz, DMSO-d_6) δ 1.31 (3H, t, *J* = 7.0 Hz), 2.37 (3H, s), 4.30 (2H, q, *J* = 7.0 Hz), 7.10 (1H, d, *J* = 7.2 Hz), 7.34 (1H, t, *J* = 7.8 Hz), 7.88 (1H, d, *J* = 8.6 Hz), 7.94–7.98 (2H, m), 10.24 (1H, bs).

Ethyl ({2-(Aminocarbonyl)-3-[(2-phenylethyl)oxy]phenyl}amino)(oxo)acetate (**24m**). Compound **24m** was prepared from compound **23m** (white powder, 100%). ^{1}H NMR (200 MHz, DMSO-d_6) δ 1.31 (3H,

t, J = 7.2 Hz), 3.11 (2H, t, J = 6.6 Hz), 4.24–4.38 (4H, m), 6.98 (1H, d, J = 8.0 Hz), 7.20–7.34 (5H, m), 7.44 (1H, t, J = 8.4 Hz), 7.72 (1H, bs), 8.02 (1H, d, J = 8.4 Hz), 8.04 (1H, bs), 12.28 (1H, bs).

Representative Procedure for the Synthesis of Compounds 25a and 25c: Ethyl 5-(Methyloxy)-4-oxo-3,4-dihydroquinazoline-2-carboxylate (**25a**). A mixture of compound **24a** (1.50 g, 5.63 mmol) and *p*-toluenesulfonic acid monohydrate (535 mg, 2.81 mmol) in toluene (60 mL) was refluxed for 15 h. Additional *p*-toluenesulfonic acid monohydrate (535 mg, 2.81 mmol) was added and the mixture was refluxed for further 24 h. The mixture was diluted with ethyl acetate, washed with H_2O and brine, dried over Na_2SO_4. After removal of the solvent, the residue was triturated with ethanol to give the title compound as a pale yellow powder (304 mg, 1.22 mmol, 22%). ^{1}H NMR (200 MHz, DMSO-d_6) δ 1.34 (3H, t, J = 7.2 Hz), 3.89 (3H, s), 4.36 (2H, q, J = 7.2 Hz), 7.15 (1H, d, J = 8.0 Hz), 7.32 (1H, d, J = 7.6 Hz), 7.76 (1H, t, J = 8.2 Hz).

Ethyl 7-(Methyloxy)-4-oxo-3,4-dihydroquinazoline-2-carboxylate (**25c**). Compound **25c** was prepared from compound **24c** (brown powder, 27%). ^{1}H NMR (200 MHz, DMSO-d_6) δ 1.36 (3H, t, J = 7.2 Hz), 3.92 (3H, s), 4.38 (2H, q, J = 7.2 Hz), 7.21 (1H, dd, J = 8.8, 2.6 Hz), 7.30 (1H, d, J = 2.6 Hz), 8.07 (1H, d, J = 8.8 Hz).

Representative Procedure for the Synthesis of Compounds 25b and 25d-m: Ethyl 6-(Methyloxy)-4-oxo-3,4-dihydroquinazoline-2-carboxylate (**25b**). To a suspension of compound **24b** (1.50 g, 5.63 mmol) in ethanol (30 mL) was added dropwise sodium ethoxide (20% in ethanol, 2.30 g, 6.76 mmol) at 0 °C and the mixture was stirred at the same temperature for 2 h. The mixture was acidified with 1 N hydrochloric acid to pH 3–4. The resulting precipitate was collected, washed with H_2O and ethanol, and dried to give the title compound as a white powder (1.04 g, 4.19 mmol, 74%). ^{1}H NMR (200 MHz, DMSO-d_6) δ 1.35 (3H, t, J = 7.2 Hz), 3.91 (3H, s), 4.38 (2H, q, J = 7.2 Hz), 7.49 (1H, dd, J = 8.8, 3.0 Hz), 7.57 (1H, d, J = 3.0 Hz), 7.79 (1H, d, J = 8.8 Hz).

Ethyl 8-(Methyloxy)-4-oxo-3,4-dihydroquinazoline-2-carboxylate (**25d**). Compound **25d** was prepared from compound **24d** (yellow powder, 38%). ^{1}H NMR (200 MHz, DMSO-d_6) δ 1.36 (3H, t, J = 7.2 Hz), 3.94 (3H, s), 4.39 (2H, q, J = 7.2 Hz), 7.44 (1H, dd, J = 7.8, 1.4 Hz), 7.58 (1H, t, J = 7.8 Hz), 7.72 (1H, dd, J = 7.8, 1.4 Hz).

Ethyl 6-Fluoro-4-oxo-3,4-dihydroquinazoline-2-carboxylate (**25e**). Compound **25e** was prepared from compound **24e** (white powder, 75%). ^{1}H NMR (200 MHz, DMSO-d_6) δ 1.32 (3H, t, J = 7.2 Hz), 4.39 (2H, q, J = 7.2 Hz), 7.77–7.97 (3H, m).

Ethyl 6-Methyl-4-oxo-3,4-dihydroquinazoline-2-carboxylate (**25f**). Compound **25f** was prepared from compound **24f** (pale orange powder, 64%). ^{1}H NMR (200 MHz, DMSO-d_6) δ 1.36 (3H, t, J = 7.2 Hz), 2.48 (3H, s), 4.39 (2H, q, J = 7.2 Hz), 7.69–7.79 (2H, m), 7.98 (1H, s).

Ethyl 4-Oxo-6-(trifluoromethyl)-3,4-dihydroquinazoline-2-carboxylate (**25g**). Compound **25g** was prepared from compound **24g** (brown powder, 53%). ^{1}H NMR (200 MHz, DMSO-d_6) δ 1.37 (3H, t, J = 7.0 Hz), 4.41 (2H, q, J = 7.0 Hz), 8.03 (1H, d, J = 8.8 Hz), 8.20 (1H, dd, J = 8.8, 2.2 Hz), 8.38–8.42 (1H, m).

Ethyl 4-Oxo-6-[(trifluoromethyl)oxy]-3,4-dihydroquinazoline-2-carboxylate (**25h**). Compound **25h** was prepared from compound **24h** (beige powder, 70%). ^{1}H NMR (200 MHz, DMSO-d_6) δ 1.36 (3H, t, J = 7.2 Hz), 4.40 (2H, q, J = 7.2 Hz), 7.85–7.91 (1H, m), 7.96–8.02 (2H, m).

Ethyl 4-Oxo-6-[(phenylmethyl)oxy]-3,4-dihydroquinazoline-2-carboxylate (**25i**). Compound **25i** was prepared from compound **24i** (pale yellow powder, 87%). ^{1}H NMR (200 MHz, DMSO-d_6) δ 1.35 (3H, t, J = 7.0 Hz), 4.38 (2H, q, J = 7.0 Hz), 5.28 (2H, s), 7.34–7.59 (6H, m), 7.66 (1H, d, J = 3.0 Hz), 7.80 (1H, d, J = 8.8 Hz).

Ethyl 6-Iodo-4-oxo-3,4-dihydroquinazoline-2-carboxylate (**25j**). Compound **25j** was prepared from compound **24j** (pale pink powder, 86%). ^{1}H NMR (200 MHz, DMSO-d_6) δ 1.35 (3H, t, J = 7.0 Hz), 4.38 (2H, q, J = 7.0 Hz), 7.61 (1H, d, J = 8.5 Hz), 8.18 (1H, dd, J = 8.5, 2.0 Hz), 8.44 (1H, d, J = 2.0 Hz).

Ethyl 5-Fluoro-4-oxo-3,4-dihydroquinazoline-2-carboxylate (**25k**). Compound **25k** was prepared from compound **24k** (pale yellow powder, 57%). ^{1}H NMR (200 MHz, DMSO-d_6) δ 1.35 (3H, t, J = 7.2 Hz), 4.37 (2H, q, J = 7.2 Hz), 7.34–7.41 (1H, m), 7.62 (1H, d, J = 8.1 Hz), 7.81–7.89 (1H, m), 12.64 (1H, bs).

Ethyl 5-Methyl-4-oxo-3,4-dihydroquinazoline-2-carboxylate (**25l**). Compound **25l** was prepared from compound **24l** (white powder, 64%). ^{1}H NMR (200 MHz, DMSO-d_6) δ 1.36 (3H, t, J = 7.2 Hz), 2.79 (3H, s), 4.37 (2H, q, J = 7.2 Hz), 7.37 (1H, d, J = 7.5 Hz), 7.61 (1H, d, J = 7.5 Hz), 7.70 (1H, t, J = 7.5 Hz), 12.35 (1H, bs).

Ethyl 4-Oxo-5-[(2-phenylethyl)oxy]-3,4-dihydroquinazoline-2-carboxylate (**25m**). Compound **25m** was prepared from compound **24m** (pale yellow powder, 47%). ^{1}H NMR (200 MHz, DMSO-d_6) δ 1.35 (3H, t, J = 7.2 Hz), 3.10 (2H, t, J = 6.6 Hz), 4.23–4.32 (2H, m), 4.37 (2H, q, J = 7.2 Hz), 6.94–8.04 (8H, m), 12.20 (1H, bs).

Representative Procedure for the Synthesis of Compounds 25n and 25o: Ethyl 5,6-Difluoro-4-oxo-3,4-dihydroquinazoline-2-carboxylate (**25o**). A mixture of compound **32b** (2.50 g, 10.5 mmol), ethyl cyanoformate (1.14 g, 11.6 mmol) and 1 N HCl in acetic acid (50 mL) was stirred at 80 °C for 3 h. After removal of the solvent, the residue was suspended in ethanol. The resulting precipitate was collected and washed with ethanol and diethyl ether to give the title compound as a white powder (2.18 g, 8.58 mmol, 82%). ^{1}H NMR (300 MHz, DMSO-d_6) δ 1.35 (3H, t, J = 7.2 Hz), 4.38 (2H, q, J = 7.2 Hz), 7.70 (1H, ddd, J = 9.0, 7.5 Hz, 2.1 Hz), 7.92–8.02 (1H, m), 12.78 (1H, bs).

Ethyl 5-[2-(Methyloxy)-2-oxoethyl]-4-oxo-3,4-dihydroquinazoline-2-carboxylate (**25n**). Compound **25n** was prepared from compound **32a** (white powder, 76%). ^{1}H NMR (300 MHz, DMSO-d_6) δ 1.35 (3H, t, J = 7.2 Hz), 3.58 (3H, s), 4.22 (2H, s), 4.39 (2H, q, J = 7.2 Hz), 7.46 (1H, dd, J = 7.0, 1.7 Hz), 7.72–7.84 (2H, m), 12.52 (1H, s).

Representative Procedure for the Synthesis of Compounds 26a–o: 5-(Methyloxy)-*N*-{[3-(methyloxy) phenyl]methyl}-4-oxo-3,4-dihydroquinazoline-2-carboxamide (**26a**). A mixture of compound **25a** (150 mg, 0.604 mmol) and 3-methoxybenzylamine (166 mg, 1.21 mmol) in DMF (4 mL) was stirred at 80 °C for

15 h. The mixture was concentrated under reduced pressure and the residue was triturated with diisopropylether to give crude **26a**. The crude product was recrystallized from ethanol–diisopropylether to give the title compound as a pale yellow powder (117 mg, 0.345 mmol, 57%). mp 188–190 °C. ^{1}H NMR (300 MHz, DMSO-d_6) δ 3.73 (3H, s), 3.87 (3H, s), 4.42 (2H, d, J = 6.6 Hz), 6.79–6.83 (1H, m), 6.88–6.93 (2H, m), 7.10 (1H, d, J = 8.1 Hz), 7.23 (1H, t, J = 8.1 Hz), 7.28 (1H, d, J = 8.1 Hz), 7.74 (1H, t, J = 8.1 Hz), 9.45 (1H, t, J = 6.6 Hz), 11.75 (1H, bs). Anal. Calcd for $C_{18}H_{17}N_3O_4$•0.1H_2O: C, 63.37; H, 5.08; N, 12.32. Found: C, 63.38; H, 4.90; N, 12.21.

6-(Methyloxy)-*N*-{[3-(methyloxy)phenyl]methyl}-4-oxo-3,4-dihydroquinazoline-2-carboxamide (**26b**). Compound **26b** was prepared from compound **25b** (white powder, 28%). mp 174–176 °C. ^{1}H NMR (200 MHz, DMSO-d_6) δ 3.74 (3H, s), 3.91 (3H, s), 4.45 (2H, d, J = 6.6 Hz), 6.81–6.94 (3H, m), 7.25 (1H, t, J = 8.0 Hz), 7.49 (1H, dd, J = 8.8, 3.0 Hz), 7.56 (1H, d, J = 3.0 Hz), 7.74 (1H, d, J = 8.6 Hz), 9.42–9.52 (1H, m). Anal. Calcd for $C_{18}H_{17}N_3O_4$•0.2H_2O: C, 63.04; H, 5.11; N, 12.25. Found: C, 62.93; H, 5.11; N, 12.08.

7-(Methyloxy)-*N*-{[3-(methyloxy)phenyl]methyl}-4-oxo-3,4-dihydroquinazoline-2-carboxamide (**26c**). Compound **26c** was prepared from compound **25c** (white powder, 47%). mp 228–230 °C. ^{1}H NMR (300 MHz, DMSO-d_6) δ 3.74 (3H, s), 3.91 (3H, s), 4.45 (2H, d, J = 6.2 Hz), 6.81–6.94 (3H, m), 7.16–7.29 (3H, m), 8.05–8.10 (1H, m), 9.50 (1H, t, J = 6.2 Hz), 12.12 (1H, bs). Anal. Calcd for $C_{18}H_{17}N_3O_4$: C, 63.71; H, 5.05; N, 12.38. Found: C, 63.41; H, 5.02; N, 12.42.

8-(Methyloxy)-*N*-{[3-(methyloxy)phenyl]methyl}-4-oxo-3,4-dihydroquinazoline-2-carboxamide (**26d**). Compound **26d** was prepared from compound **25d** (white powder, 71%). mp 238–239 °C. ^{1}H NMR (200 MHz, DMSO-d_6) δ 3.74 (3H, s), 3.94 (3H, s), 4.47 (2H, d, J = 6.4 Hz), 6.81–6.99 (3H, m), 7.21–7.29 (1H, m), 7.41–7.58 (2H, m), 7.70–7.74 (1H, m), 9.21 (1H, t, J = 6.4 Hz), 12.26 (1H, bs). Anal. Calcd for $C_{18}H_{17}N_3O_4$: C, 63.71; H, 5.05; N, 12.38. Found: C, 63.50; H, 5.14; N, 12.29.

6-Fluoro-*N*-{[3-(methyloxy)phenyl]methyl}-4-oxo-3,4-dihydroquinazoline-2-carboxamide (**26e**). Compound **26e** was prepared from compound **25e** (pale yellow powder, 69%). mp 177–179 °C. ^{1}H NMR (200 MHz, DMSO-d_6) δ 3.74 (3H, s), 4.45 (2H, d, J = 6.4 Hz), 7.25 (1H, t, J = 8.0 Hz), 6.80–6.94 (3H, m), 7.74–7.89 (3H, m), 9.50–9.57 (1H, m). Anal. Calcd for $C_{17}H_{14}FN_3O_3$•0.2H_2O: C, 61.70; H, 4.39; N, 12.70. Found: C, 61.44; H, 4.27; N, 2.71.

6-Methyl-*N*-{[3-(methyloxy)phenyl]methyl}-4-oxo-3,4-dihydroquinazoline-2-carboxamide (**26f**). Compound **26f** was prepared from compound **25f** (white powder, 68%). mp 175–177 °C. ^{1}H NMR (200 MHz, DMSO-d_6) δ 2.48 (3H, s), 3.74 (3H, s), 4.45 (2H, d, J = 6.6 Hz), 6.80–6.94 (3H, m), 7.25 (1H, t, J = 8.0 Hz), 7.65–7.75 (2H, m), 7.98 (1H, s), 9.50 (1H, t, J = 6.4 Hz), 12.16 (1H, bs). Anal. Calcd for $C_{18}H_{17}N_3O_4$•0.2H_2O: C, 63.04; H, 5.11; N, 12.25. Found: C, 62.93; H, 5.11; N, 12.08.

***N*-{[3-(Methyloxy)phenyl]methyl}-4-oxo-6-(trifluoromethyl)-3,4-dihydroquinazoline-2-carboxamide** (**26g**). Compound **26g** was prepared from compound **25g** (pale yellow powder, 61%). mp

186–187 °C. ^{1}H NMR (300 MHz, DMSO-d_6) δ 3.73 (3H, s), 4.46 (2H, d, J = 6.3 Hz), 6.80–6.84 (1H, m), 6.90–6.93 (2H, m), 7.24 (1H, t, J = 8.1 Hz), 7.95 (1H, dd, J = 8.1, 0.6 Hz), 8.18 (1H, dd, J = 8.4, 2.1 Hz), 8.38 (1H, d, J = 0.9 Hz), 9.63 (1H, t, J = 6.3 Hz), 12.71 (1H, bs). Anal. Calcd for $C_{18}H_{14}F_3N_3O_3$: C, 57.30; H, 3.74; N, 11.14. Found: C, 57.22; H, 3.78; N, 11.22.

***N*-{[3-(Methyloxy)phenyl]methyl}-4-oxo-6-[(trifluoromethyl)oxy]-3,4-dihydroquinazoline-2-carboxamide** (**26h**). Compound **26h** was prepared from compound **25h** (white powder, 62%). mp 156–159 °C. ^{1}H NMR (300 MHz, DMSO-d_6) δ 3.74 (3H, s), 4.46 (2H, d, J = 6.3 Hz), 6.83 (1H, dd, J = 8.1, 2.4 Hz), 6.90–6.94 (2H, m), 7.25 (1H, t, J = 8.1 Hz), 7.86–7.93 (2H, m), 8.00 (1H, s), 9.58 (1H, t, J = 6.3 Hz), 12.59 (1H, bs). Anal. Calcd for $C_{18}H_{14}F_3N_3O_4$: C, 54.97; H, 3.59; N, 10.68. Found: C, 54.80; H, 3.53; N, 10.73.

***N*-{[3-(Methyloxy)phenyl]methyl}-4-oxo-6-[(phenylmethyl)oxy]-3,4-dihydroquinazoline-2-carboxamide** (**26i**). Compound **26i** was prepared from compound **25i** (white powder, 86%). mp 179–182 °C. ^{1}H NMR (300 MHz, DMSO-d_6) δ 3.73 (3H, s), 4.44 (2H, d, J = 6.6 Hz), 5.27 (2H, s), 6.82 (1H, dd, J = 8.1, 2.4 Hz), 6.90–6.94 (2H, m), 7.24 (1H, t, J = 7.6 Hz), 7.32–7.42 (3H, m), 7.43–7.51 (1H, m), 7.54–7.58 (1H, m), 7.66 (1H, d, J = 2.4 Hz), 7.75 (1H, d, J = 8.7 Hz), 9.48 (1H, t, J = 6.4 Hz), 11.80–11.90 (1H, m). Anal. Calcd for $C_{24}H_{21}N_3O_4$: C, 69.39; H, 5.10; N, 10.11. Found :C, 69.09; H, 5.07; N, 10.21. **6-Iodo-*N*-{[3-(methyloxy)phenyl]methyl}-4-oxo-3,4-dihydroquinazoline-2-carboxamide** (**26j**). Compound **26j** was prepared from compound **25j** (white powder, 92%). mp 219–220 °C. ^{1}H NMR (200 MHz, DMSO-d_6) δ 3.74 (3H, s), 4.45 (2H, d, J = 6.2 Hz), 6.81–6.93 (3H, m), 7.25 (1H, d, J = 8.0 Hz), 7.55 (1H, d, J = 8.8 Hz), 8.17 (1H, dd, J = 8.8, 1.8 Hz), 8.43 (1H, d, J = 1.8 Hz), 9.54 (1H, t, J = 6.2 Hz), 12.22 (1H, bs). Anal. Calcd for $C_{17}H_{14}IN_3O_3$: C, 46.92; H, 3.24; N, 9.66. Found: C, 46.83; H, 3.18; N, 9.82.

5-Fluoro-*N*-{[3-(methyloxy)phenyl]methyl}-4-oxo-3,4-dihydroquinazoline-2-carboxamide (**26k**). Compound **26k** was prepared from compound **25k** (pale yellow powder, 89%). mp 159–161 °C. ^{1}H NMR (300 MHz, DMSO-d_6) δ 3.74 (3H, s), 4.44 (2H, d, J = 6.3 Hz), 6.80–6.84 (1H, m), 6.89–6.92 (2H, m), 7.21–7.27 (1H, m), 7.31–7.38 (1H, m), 7.58 (1H, d, J = 8.1 Hz), 7.81–7.88 (1H, m), 9.54 (1H, t, J = 6.3 Hz), 12.28 (1H, bs). Anal. Calcd for $C_{17}H_{14}FN_3O_3$: C, 62.38; H, 4.31; N, 12.84. Found: C, 62.43; H, 4.38; N, 12.88.

5-Methyl-*N*-{[3-(methyloxy)phenyl]methyl}-4-oxo-3,4-dihydroquinazoline-2-carboxamide (**26l**). Compound **26l** was prepared from compound **25l** (white powder, 78%). mp 150–152 °C. ^{1}H NMR (300 MHz, DMSO-d_6) δ 2.79 (3H, s), 3.74 (3H, s), 4.44 (2H, d, J = 6.3 Hz), 6.82 (1H, dd, J = 8.1, 2.4 Hz), 6.89–6.93 (2H, m), 7.24 (1H, t, J = 8.1 Hz), 7.35 (1H, d, J = 7.5 Hz), 7.58 (1H, d, J = 7.8 Hz), 7.7 (1H, t, J = 7.8 Hz), 9.48 (1H, t, J = 6.3 Hz), 11.94 (1H, bs). Anal. Calcd for $C_{18}H_{17}N_3O_3$: C, 66.86; H, 5.30; N, 13.00. Found: C, 66.86; H, 5.37; N, 13.08.

***N*-{[3-(Methyloxy)phenyl]methyl}-4-oxo-5-[(2-phenylethyl)oxy]-3,4-dihydroquinazoline-2-carboxamide** (**26m**). Compound **26m** was prepared from compound **25m** (pale yellow powder, 61%). mp 150–151 °C. ^{1}H NMR (300 MHz, DMSO-d_6) δ 3.10 (2H, d, J = 6.6 Hz), 3.73 (3H, s), 4.25 (2H, t, J = 6.6

Hz), 4.43 (2H, d, *J* = 6.6 Hz), 6.82 (1H, dd, *J* = 8.1, 1.8 Hz), 6.89–6.92 (2H, m), 7.09 (1H, d, *J* = 8.1 Hz), 7.17–7.30 (5H, m), 7.46–7.49 (2H, m), 7.71 (1H, t, *J* = 8.1 Hz), 9.47 (1H, t, *J* = 6.6 Hz), 11.77 (1H, bs). Anal. Calcd for $C_{25}H_{23}N_3O_4$•0.1H_2O: C, 69.62; H, 5.42; N, 9.68. Found: C, 69.44; H, 5.40; N, 9.68.

Methyl {2-[({[3-(Methyloxy)phenyl]methyl}amino)carbonyl]-4-oxo-3,4-dihydroquinazolin-5-yl} acetate (**26n**). Compound **26n** was prepared from compound **25n** (white powder, 84%). mp 180–182 °C. ^{1}H NMR (300 MHz, DMSO-d_6) δ 3.58 (3H, s), 3.74 (3H, s), 4.21 (2H, s), 4.45 (2H, d, *J* = 6.4 Hz), 6.79–6.87 (1H, m), 6.88–6.96 (2H, m), 7.25 (1H, t, *J* = 8.1 Hz), 7.42 (1H, dd, *J* = 7.3, 1.2 Hz), 7.68–7.74 (1H, m), 7.75–7.83 (1H, m), 9.53 (1H, t, *J* = 6.3 Hz), 12.08 (1H, s). Anal. Calcd for $C_{20}H_{19}N_3O_5$•0.1H_2O: C, 62.69; H, 5.05; N, 10.97. Found: C, 62.65; H, 5.03; N, 11.08.

5,6-Difluoro-*N*-{[3-(methyloxy)phenyl]methyl}-4-oxo-3,4-dihydroquinazoline-2-carboxamide (**26o**). Compound **26o** was prepared from compound **25o** (pale yellow powder, 78%). mp 189–191 °C. ^{1}H NMR (300 MHz, DMSO-d_6) δ 3.73 (3H, s), 4.44 (2H, d, *J* = 6.3 Hz), 6.80–6.84 (1H, m), 6.88–6.91 (2H, m), 7.24 (1H, t, *J* = 8.1 Hz), 7.59–7.63 (1H, m), 7.90–7.99 (1H, m), 8.05 (1H, d, *J* = 7.8 Hz), 9.53 (1H, t, *J* = 6.3 Hz), 12.44 (1H, bs). Anal. Calcd for $C_{17}H_{13}F_2N_3O_3$: C, 59.13; H, 3.79; N, 12.17. Found: C, 58.97; H, 3.77; N, 12.25.

2-[(Methyloxy)carbonyl]-3-nitrobenzoic acid (**28**). A mixture of dimethyl 3-nitrobenzene-1,2-dicarboxylate (**27**, 25.7 g, 107 mmol) and 1 N aqueous lithium hydroxide solution (107 mL, 107 mmol) in THF (250 mL) was stirred at room temperature for 15 h. The mixture was acidified with 1 N hydrochloric acid (115 mL). The solvent was removed by evaporation under reduced pressure and the residue was suspended in H_2O. The resulting precipitate was collected, washed with H_2O and dried in a stream of air to give the title compound as a white powder (23.1 g, 103 mmol, 96%). ^{1}H NMR (200 MHz, DMSO-d_6) δ 3.85 (3H, s), 7.87 (1H, t, *J* = 8.2 Hz), 8.32 (1H, d, *J* = 8.0 Hz), 8.42 (1H, d, *J* = 8.4 Hz).

Methyl 2-[2-(Methyloxy)-2-oxoethyl]-6-nitrobenzoate (**29**). To a solution of compound **28** (5.00 g, 22.2 mmol) and DMF (0.0516 mL) in THF (50 mL) was added dropwise oxalyl chloride (2.32 mL, 26.6 mmol) at 0 °C and the mixture was stirred at room temperature for 3 h. After removal of the solvent, the residue was coevaporated with toluene and dissolved in THF (50 mL). The solution was added dropwise to a solution of (trimethylsilyl)diazomethane (2 M in diethyl ether, 12.2 mL, 24.4 mmol) and triethylamine (2.47 g, 24.4 mmol) in acetonitrile (50 mL) at 0 °C and the reaction mixture was stirred at 0 °C for 2 h. The mixture was concentrated under reduced pressure and the residue was suspended in diethyl ether. The precipitated solids were collected and washed with diethyl ether to give a brown powder (3.90 g). To a suspension of the brown powder (3.80 g) in methanol (70 mL) and THF (35 mL) was added dropwise a solution of silver benzoate (349 mg, 1.53 mmol) in triethylamine (7.4 mL) and the mixture was stirred at room temperature for 1 h. The reaction mixture was concentrated under reduced pressure, diluted with ethyl acetate, and washed with saturated aqueous $NaHCO_3$ solution, H_2O, 10% aqueous $KHSO_4$ solution and brine. The organic layer was dried over Na_2SO_4 and the solvent was removed by evaporation. The crude materials were purified by silica

gel column chromatography (50% ethyl acetate/hexane) to give the title compound as a brown oil (3.69 g, 14.6 mmol, 67% over 2 steps). ^{1}H NMR (300 MHz, DMSO-d_6) δ 3.62 (3H, s), 3.81 (3H, s), 3.88 (2H, s), 7.76 (1H, t, J = 7.9 Hz), 7.81–7.86 (1H, m), 8.10 (1H, dd, J = 8.0, 1.2 Hz).

Methyl 2-Amino-6-[2-(methyloxy)-2-oxoethyl]benzoate (**32a**). A mixture of compound **29** (3.50 g, 13.8 mmol), 10% palladium on carbon (1.17 g), THF (35 mL) and methanol (35 mL) was stirred at room temperature for 3 h under hydrogen atmosphere (1 atm). After the insoluble materials were filtered off through a PTFE membrane filter, the filtrate was concentrated under reduced pressure to give the title compound as a brown powder (3.06 g, 13.8 mmol, 99%). ^{1}H NMR (300 MHz, DMSO-d_6) δ 3.58 (3H, s), 3.70 (3H, s), 3.74 (2H, s), 6.17 (2H, s), 6.43 (1H, dd, J = 7.2, 1.1 Hz), 6.71 (1H, dd, J = 8.4, 1.2 Hz), 7.11 (1H, dd, J = 8.3, 7.3 Hz).

Ethyl 6-Amino-2,3-difluorobenzoate Hydrochloride (**32b**). To a solution of compound **30** (5.00 g, 21.8 mmol, synthesized by the method of Carretero et al.[45]) in THF (50 mL) was added dropwise *n*-butyl lithium (1.6 M in hexane, 30.0 mL, 48.0 mmol) at –78 °C and the mixture was stirred at the same temperature for 3 h. A solution of ethyl chlorocarbonate (2.60 g, 24.0 mmol) in THF (15 mL) was added followed by stirring at –78 °C for further 1 h. The reaction was quenched with saturated aqueous NH_4Cl solution (50 mL) and the resulting mixture was partitioned between ethyl acetate and H_2O. The organic layer was washed with brine, dried over Na_2SO_4 and concentrated under reduced pressure to give compound **31** as a yellow oil. To a solution of the yellow oil in ethyl acetate (10 mL) was added 4 N HCl in ethyl acetate (40 mL) at room temperature. The mixture was stirred at room temperature for 3 h and then diethyl ether (20 mL) was added. The resulting precipitate was collected and washed with diethyl ether to give the title compound as a white powder (2.91 g, 12.2 mmol, 70% over 2 steps). ^{1}H NMR (300 MHz, DMSO-d_6) δ 1.29 (3H, t, J = 7.2 Hz), 4.30 (2H, q, J = 7.2 Hz), 6.57 (1H, ddd, J = 9.3, 4.2, 2.1 Hz), 7.27–7.37 (1H, m).

6-Hydroxy-*N*-{[3-(methyloxy)phenyl]methyl}-4-oxo-3,4-dihydroquinazoline-2-carboxamide (**33**). A mixture of compound **26i** (2.00 g, 4.81 mmol), 10% palladium on carbon (500 mg), THF (50 mL) and methanol (20 mL) was stirred at room temperature for 3 h under hydrogen atmosphere (1 atm). After the insoluble materials were filtered off through a PTFE membrane filter, the filtrate was concentrated under reduced pressure to give the title compound as a pale yellow powder (1.60 g, 4.91 mmol, 100%). An analytical sample was obtained by recrystallization from ethanol. mp 268–271 °C. ^{1}H NMR (300 MHz, DMSO-d_6) δ 3.73 (3H, s), 4.44 (2H, d, J = 6.3 Hz), 6.82 (1H, dd, J = 8.4, 2.7 Hz), 6.89–6.92 (2H, m), 7.24 (1H, t, J = 8.1 Hz), 7.33 (1H, dd, J = 8.7, 3.0 Hz), 7.46 (1H, d, J = 3.0 Hz), 7.66 (1H, d, J = 8.7 Hz), 9.42 (1H, t, J = 6.4 Hz), 10.33 (1H, bs), 11.99 (1H, bs). Anal. Calcd for $C_{17}H_{15}N_3O_4$: C, 62.76; H, 4.65; N, 12.92. Found: C, 62.60; H, 4.82; N, 12.72.

6-(Ethyloxy)-*N*-{[3-(methyloxy)phenyl]methyl}-4-oxo-3,4-dihydroquinazoline-2-carboxamide (**34**). A mixture of compound **33** (120 mg, 0.368 mmol), cesium carbonate (240 mg, 0.736 mmol), iodoethane (0.087 mL, 1.10 mmol), THF (3 mL) and DMF (1 mL) was stirred at room temperature for 4 h. The mixture

was partitioned between ethyl acetate and H_2O. The organic layer was washed with brine, dried over Na_2SO_4 and concentrated under reduced pressure. The crude materials were purified by preparative HPLC and recrystallization from ethanol to give the title compound as a white powder (27.0 mg, 0.0764 mmol, 21%). mp 163–165 °C. ^{1}H NMR (300 MHz, DMSO-d_6) δ 1.38 (3H, t, J = 6.9 Hz), 3.74 (3H, s), 4.17 (2H, q, J = 6.9 Hz), 4.44 (2H, d, J = 6.3 Hz), 6.83 (1H, dd, J = 8.4, 2.4 Hz), 6.90–6.93 (2H, m), 7.24 (1H, t, J = 8.1 Hz), 7.47 (1H, dd, J = 9.0, 3.0 Hz), 7.54 (1H, d, J = 3.0 Hz), 7.73 (1H, d, J = 9.3 Hz), 9.47 (1H, t, J = 6.3 Hz), 12.18 (1H, bs). Anal. Calcd for $C_{19}H_{19}N_3O_4$: C, 64.58; H, 5.42; N, 11.89. Found: C, 64.55; H, 5.52; N, 11.89.

6-Mercapto-*N*-{[3-(methyloxy)phenyl]methyl}-4-oxo-3,4-dihydroquinazoline-2-carboxamide (**35**). To a solution of compound **33** (500 mg, 1.54 mmol) and *N*,*N*-dimethylthiocarbamoylchloride (380 mg, 3.08 mmol) in DMF (5 mL) was added 1,4-diazabicyclo[2.2.2]octane (345 mg, 3.08 mmol). The mixture was stirred at room temperature for 2h and poured into ice cold water. The precipitated solid was collected and washed with H_2O, ethanol and diethyl ether to give *O*-{2-[({[3-(methyloxy)phenyl]methyl}amino) carbonyl]-4-oxo-3,4-dihydroquinazolin-6-yl} dimethylthiocarbamate as a white powder (608 mg, 1.47 mmol, 96%). A mixture of the white powder obtained above (540 mg, 1.31 mmol) and *N*,*N*-diethylaniline (5 mL) was stirred at 210 °C for 7 h and then poured into 3 N hydrochloric acid (30 mL). The precipitated solid was collected and washed with H_2O, ethanol and diethyl ether to give *S*-{2-[({[3-(methyloxy)phenyl]-methyl}amino)carbonyl]-4-oxo-3,4-dihydroquinazolin-6-yl} dimethylthiocarbamate as a pale gray powder (440 mg, 1.07 mmol, 82%). A mixture of the pale gray powder obtained above (380 mg, 0.921 mmol) and potassium hydroxide (259 mg, 4.61 mmol) in methanol (10 mL) was refluxed for 2h. The mixture was acidified with 1 N hydrochloric acid to pH 2–3 and extracted with a mixed solvent of ethyl acetate and THF. The organic layer was washed with brine, dried over Na_2SO_4 and concentrated under reduced pressure to give crude **35**. The crude product was recrystallized from ethanol to give the title compound as a pale yellow powder (300 mg, 0.879 mmol, 95%). mp 170–172 °C. ^{1}H NMR (300 MHz, DMSO-d_6) δ 3.73 (3H, s), 4.44 (2H, d, J = 6.6 Hz), 6.17 (1H, bs), 6.81 (1H, dd, J = 8.1, 2.4 Hz), 6.88–6.91 (2H, m), 7.23 (1H, t, J = 8.1 Hz), 7.64 (1H, d, J = 8.4 Hz), 7.75 (1H, dd, J = 8.4, 2.1 Hz), 8.07 (1H, d, J = 2.1 Hz), 9.48 (1H, t, J = 6.3 Hz), 12.25 (1H, bs). Anal. Calcd for $C_{17}H_{15}N_3O_3S$•$0.1H_2O$: C, 59.50; H, 4.46; N, 12.24. Found: C, 59.44; H, 4.58; N, 12.24.

***N*-{[3-(Methyloxy)phenyl]methyl}-6-(methylthio)-4-oxo-3,4-dihydroquinazoline-2-carboxamide** (**36a**). A mixture of compound **35** (270 mg, 0.791 mmol), iodomethane (0.049 mL, 0.791 mmol), and triethylamine (0.110 mL, 0.791 mmol) in THF (5 mL) was stirred at room temperature for 1 h. The mixture was partitioned between ethyl acetate and H_2O. The organic layer was washed with brine, dried over Na_2SO_4 and concentrated under reduced pressure. The crude product was purified by recrystallization from ethanol to give the title compound as a pale yellow powder (240 mg, 0.675 mmol, 85%). mp 168–170 °C. ^{1}H NMR (200 MHz, DMSO-d_6) δ 2.60 (3H, s), 3.74 (3H, s), 4.45 (2H, d, J = 6.2 Hz), 6.81–6.94 (3H, m), 7.25 (1H,

t, *J* = 8.0 Hz), 7.70 (1H, d, *J* = 8.8 Hz), 7.77 (1H, dd, *J* = 8.8, 2.2 Hz), 7.89 (1H, d, *J* = 1.8 Hz), 9.50 (1H, t, *J* = 6.2 Hz), 12.28 (1H, bs). Anal. Calcd for $C_{18}H_{17}N_3O_3S$: C, 60.83; H, 4.82; N, 11.82. Found: C, 60.65; H, 4.76; N, 11.98.

***N*-{[3-(Methyloxy)phenyl]methyl}-6-(methylsulfonyl)-4-oxo-3,4-dihydroquinazoline-2-carboxamide** (**36b**). To a solution of compound **36a** (60.0 mg, 0.169 mmol) in chloroform (2 mL) was added 3-chloroperoxybenzoic acid (84.0 mg, 338 mmol) at 0 °C and the mixture was stirred at room temperature for 2 h. The mixture was partitioned between ethyl acetate and H_2O. The organic layer was washed with brine, dried over Na_2SO_4 and concentrated under reduced pressure. The crude product was purified by recrystallization from ethanol to give the title compound as a pale yellow powder (62.0 mg, 0.161 mmol, 95%). mp 186–188 °C. ^{1}H NMR (300 MHz, DMSO-d_6) δ 3.33 (3H, s), 3.74 (3H, s), 4.46 (2H, d, *J* = 6.6 Hz), 6.80–6.84 (1H, m), 6.90–6.93 (2H, m), 7.24 (1H, t, *J* = 8.1 Hz), 7.96 (1H, d, *J* = 8.4 Hz), 8.31 (1H, dd, *J* = 8.4, 2.1 Hz), 8.60 (1H, s), 9.63 (1H, t, *J* = 6.3 Hz), 12.77 (1H, bs). Anal. Calcd for $C_{18}H_{17}N_3O_5S$•$0.4H_2O$: C, 54.79; H, 4.55; N, 10.65. Found: C, 54.83; H, 4.36; N, 10.66.

***N*-{[3-(Methyloxy)phenyl]methyl}-4-oxo-6-phenyl-3,4-dihydroquinazoline-2-carboxamide** (**37a**). A mixture of compound **26j** (300 mg, 0.689 mmol), phenylboronic acid (167 mg, 1.37 mmol), tetrakis(triphenylphosphine)palladium(0) (32.0 mg, 0.0277 mmol) and 2 N aqueous Na_2CO_3 solution (1.03 mL, 2.06 mmol) in a mixed solvent of ethanol (2 mL) and toluene (6 mL) was refluxed for 18 h under nitrogen atmosphere. The reaction mixture was diluted with ethyl acetate, washed with H_2O and brine, dried over Na_2SO_4 and concentrated under reduced pressure. The crude product was purified by recrystallization from ethanol to give the title compound as a white powder (170 mg, 0.441 mmol, 64%). mp 202–204 °C. ^{1}H NMR (300 MHz, DMSO-d_6) δ 3.74 (3H, s), 4.46 (2H, d, *J* = 6.6 Hz), 6.81–6.85 (1H, m), 6.90–6.94 (2H, m), 7.25 (1H, t, *J* = 8.1 Hz), 7.40–7.45 (1H, m), 7.49–7.55 (2H, m), 7.78–7.88 (3H, m), 8.20 (1H, dd, *J* = 8.4, 2.4 Hz), 8.37 (1H, d, *J* = 2.1 Hz), 9.56 (1H, t, *J* = 6.3 Hz), 12.34 (1H, bs). Anal. Calcd for $C_{23}H_{19}N_3O_3$•$0.2H_2O$: C, 71.01; H, 5.03; N, 10.80. Found: C, 71.12; H, 5.00; N, 10.51.

6-Cyano-*N*-{[3-(methyloxy)phenyl]methyl}-4-oxo-3,4-dihydroquinazoline-2-carboxamide (**37b**). A mixture of compound **26j** (1.00 g, 2.30 mmol), zinc cyanide (148 mg, 1.26 mmol) and tetrakis(triphenylphosphine)palladium(0) (132 mg, 0.115 mmol) in DMF (10 mL) was stirred at 80 °C for 3 h under nitrogen atmosphere. The reaction mixture was concentrated under reduced pressure and the residue was suspended in ethyl acetate. The resulting precipitate was collected to give crude **37b** as a white powder (537 mg). Two hundred milligrams of the crude product was recrystallized from ethanol to give the title compound as a white powder (193 mg, 0.577 mmol, 68%). mp 206–208 °C. ^{1}H NMR (200 MHz, DMSO-d_6) δ 3.74 (3H, s), 4.46 (2H, d, *J* = 6.6 Hz), 6.81–6.95 (3H, m), 7.25 (1H, t, *J* = 8.0 Hz), 7.89 (1H, d, *J* = 8.4 Hz), 8.23 (1H, dd, *J* = 8.6, 2.0 Hz), 8.55 (1H, d, *J* = 2.0 Hz), 9.63 (1H, t, *J* = 6.6 Hz). Anal. Calcd for $C_{18}H_{14}N_4O_3$•$0.1H_2O$: C, 64.32; H, 4.26; N, 16.67. Found: C, 64.17; H, 4.23; N, 16.74.

Representative Procedure for the Synthesis of Compounds 38a–c, 38e–g, and 38k: *N*-{[3-

(Methyloxy)-phenyl]methyl}-4-oxo-5-[(phenylmethyl)oxy]-3,4-dihydroquinazoline-2-carboxamide (**38a**). To a solution of benzyl alcohol (99.0 mg, 0.917 mmol) in DMA (6 mL) was added sodium hydride (60% oil dispersion, 122 mg, 3.06 mmol) and the mixture was stirred at room temperature for 30 min. Compound **26k** (200 mg, 0.611 mmol) was added to the mixture and the resulting mixture was stirred at 80 °C for 1 h. The reaction mixture was acidified with 0.5 N hydrochloric acid to pH 3–4 and extracted with a mixed solvent of ethyl acetate and THF. The organic layer was washed with H_2O and brine, dried over Na_2SO_4 and concentrated under reduced pressure to give crude **38a**. The crude product was crystallized from ethanol to give the title compound as a pale yellow powder (207 mg, 0.498 mmol, 81%). mp 188–190 °C. ^{1}H NMR (300 MHz, DMSO-d_6) δ 3.74 (3H, s), 4.44 (2H, d, J = 6.6 Hz), 5.28 (2H, s), 6.80–6.84 (1H, m), 6.90–6.93 (2H, m), 7.18–7.33 (4H, m), 7.37–7.43 (2H, m), 7.75 (1H, t, J = 8.4 Hz), 7.61–7.63 (2H, m), 9.50 (1H, t, J = 6.3 Hz), 11.81 (1H, bs). Anal. Calcd for $C_{24}H_{21}N_3O_4$: C, 69.39; H, 5.10; N, 10.11. Found: C, 69.11; H, 4.97; N, 10.40.

***N*-{[3-(Methyloxy)phenyl]methyl}-4-oxo-5-[(3-phenylpropyl)oxy]-3,4-dihydroquinazoline-2-carboxamide** (**38b**). Compound **38b** was prepared from compound **26k** and 3-phenylpropan-1-ol (pale yellow powder, 44%). mp 165–167 °C. ^{1}H NMR (300 MHz, DMSO-d_6) δ 2.01–2.11 (2H, m), 2.89 (2H, t, J = 7.5 Hz), 3.74 (3H, s), 4.05 (2H, t, J = 6 Hz), 4.44 (2H, d, J = 6.3 Hz), 6.80–6.84 (1H, m), 6.90–6.92 (2H, m), 7.06 (1H, d, J = 8.4 Hz), 7.13–7.29 (7H, m), 7.71 (1H, t, J = 8.1 Hz), 9.48 (1H, t, J = 6.3 Hz), 11.76 (1H, bs). Anal. Calcd for $C_{26}H_{25}N_3O_4$•0.1H_2O: C, 70.13; H, 5.70; N, 9.44. Found: C, 69.91; H, 5.62; N, 9.69.

***N*-{[3-(Methyloxy)phenyl]methyl}-4-oxo-5-({2-[(phenylmethyl)oxy]ethyl}oxy)-3,4-dihydroquinazoline-2-carboxamide** (**38c**). Compound **38c** was prepared from compound **26k** and 2-[(phenylmethyl)oxy] ethanol (pale yellow powder, 76%). mp 136–138 °C. ^{1}H NMR (300 MHz, DMSO-d_6) δ 3.73 (3H, s), 3.84 (2H, t, J = 4.5 Hz), 4.26 (2H, t, J = 4.5 Hz), 4.43 (2H, d, J = 6.6 Hz), 4.69 (2H, s), 6.80–6.84 (1H, m), 6.89–6.92 (2H, m), 7.12 (1H, d, J = 8.4 Hz), 7.21–7.38 (7H, m), 7.72 (1H, t, J = 8.1 Hz), 9.47 (1H, t, J = 6.3 Hz), 11.76 (1H, bs). Anal. Calcd for $C_{26}H_{25}N_3O_5$: C, 67.96; H, 5.48; N, 9.14. Found: C, 67.76; H, 5.52; N, 9.22.

***N*-{[3-(Methyloxy)phenyl]methyl}-4-oxo-5-[(2-phenylethyl)amino]-3,4-dihydroquinazoline-2-carboxamide** (**38d**). A mixture of compound **26k** (100 mg, 0.309 mmol) and 2-phenylethanamine (280 mg, 2.32 mmol) in DMA (2 mL) was stirred at 80 °C for 72 h. The reaction mixture was concentrated under reduced pressure. The residue was diluted with ethyl acetate, washed with 0.1 N hydrochloric acid and brine, dried over Na_2SO_4 and concentrated under reduced pressure to give crude **38d**. The crude product was crystallized from ethanol to give the title compound as a yellow powder (68.0 mg, 159 mmol, 51%). mp 164–166 °C. ^{1}H NMR (300 MHz, DMSO-d_6) δ 2.94 (2H, t, J = 7.2 Hz), 3.39–3.47 (2H, m), 3.73 (3H, s), 4.42 (2H, d, J = 6.3 Hz), 6.67 (1H, d, J = 8.4 Hz), 6.81–6.92 (4H, m), 7.21–7.32 (6H, m), 7.55 (1H, t, J = 8.1 Hz), 8.66–8.70 (1H, m), 9.40–9.45 (1H, m), 11.89 (1H, bs). Anal. Calcd for $C_{25}H_{24}N_4O_3$•0.1H_2O: C, 69.78; H, 5.67; N, 13.02. Found: C, 69.55; H, 5.56; N, 13.19.

***N*-{[3-(Methyloxy)phenyl]methyl}-4-oxo-5-[(2-phenylethyl)thio]-3,4-dihydroquinazoline-2-carboxamide** (**38e**). Compound **38e** was prepared from compound **26k** and 2-phenylethanethiol (pale yellow powder, 55%). mp 178–180 °C. ^{1}H NMR (300 MHz, DMSO-d_6) δ 2.98 (2H, t, J = 7.5 Hz), 3.20 (2H, t, J = 7.5 Hz), 3.74 (3H, s), 4.44 (2H, d, J = 6.3 Hz), 6.81–6.84 (1H, m), 6.90–6.92 (2H, m), 7.22–7.34 (6H, m), 7.41–7.59 (2H, m), 7.75 (1H, t, J = 7.8 Hz), 9.50 (1H, t, J = 6.6 Hz), 12.17 (1H, bs). Anal. Calcd for $C_{25}H_{23}N_3O_3S$•0.4H_2O: C, 66.32; H, 5.30; N, 9.28. Found: C, 66.16; H, 5.10; N, 9.52.

5-[(2-Cyclohexylethyl)oxy]-*N*-{[3-(methyloxy)phenyl]methyl}-4-oxo-3,4-dihydroquinazoline-2-carboxamide (**38f**). Compound **38f** was prepared from compound **26k** and 2-cyclohexylethanol (white powder, 55%). mp 130–132 °C. ^{1}H NMR (300 MHz, DMSO-d_6) δ 0.90–1.05 (2H, m), 1.13–1.30 (3H, m), 1.60–1.78 (8H, m), 3.73 (3H, s), 4.10 (2H, t, J = 6.3 Hz), 4.43 (2H, d, J = 6.3 Hz), 6.80–6.83 (1H, m), 6.89–6.92 (2H, m), 7.10 (1H, d, J = 8.1 Hz), 7.21–7.27 (2H, m), 7.71 (1H, t, J = 8.1 Hz), 9.46 (1H, t, J = 6.3 Hz), 11.69 (1H, bs). Anal. Calcd for $C_{25}H_{29}N_3O_4$: C, 68.95; H, 6.71; N, 9.65. Found: C, 68.71; H, 6.71; N, 9.77.

***N*-{[3-(Methyloxy)phenyl]methyl}-4-oxo-5-[(2-piperidin-1-ylethyl)oxy]-3,4-dihydroquinazoline-2-carboxamide** (**38g**). Compound **38g** was prepared from compound **26k** and 2-piperidin-1-ylethanol (pale yellow powder, 35%). mp 225–227 °C. ^{1}H NMR (300 MHz, DMSO-d_6) δ 1.45–1.79 (2H, m), 1.76–1.83 (4H, m), 3.05–3.39 (2H, m), 3.48–3.75 (4H, m), 3.73 (3H, s), 4.43 (2H, d, J = 6.3 Hz), 4.49–4.55 (2H, m), 6.81–6.85 (1H, m), 6.88–6.92 (2H, m), 7.18–7.27 (2H, m), 7.39 (1H, d, J = 8.4 Hz), 7.81 (1H, t, J = 8.1 Hz), 9.52 (1H, t, J = 6.3 Hz), 11.90–12.10 (1H, m). Anal. Calcd for $C_{24}H_{28}N_4O_4$•1.5H_2O: C, 62.19; H, 6.74; N, 12.09. Found: C, 61.92; H, 6.45; N, 11.85.

Representative Procedure for the Synthesis of Compounds 38h-j: 6-Fluoro-*N*-{[3-(methyloxy) phenyl]methyl}-4-oxo-5-[(2-phenylethyl)oxy]-3,4-dihydroquinazoline-2-carboxamide (**38h**). To a solution of compound **26o** (100 mg, 0.290 mmol) in DMA (2 mL) was added sodium hydride (60% oil dispersion, 46.0 mg, 1.16 mmol). After the mixture was stirred at room temperature for 30 min, 2-phenylethanol (53.0 mg, 0.434 mmol) was added and the resulting mixture was stirred at room temperature for 3 h. The reaction mixture was acidified with 0.5 N hydrochloric acid to pH 3–4 and extracted with ethyl acetate. The organic layer was washed with H_2O and brine, dried over Na_2SO_4 and concentrated under reduced pressure. The crude product was purified by preparative HPLC and crystallization from ethanol/diethyl ether to give the title compound as a pale yellow powder (45.0 mg, 0.101 mmol, 35%). mp 134–135 °C. ^{1}H NMR (300 MHz, DMSO-d_6) δ 3.10 (2H, t, J = 7.2 Hz), 3.74 (3H, s), 4.28 (2H, t, J = 7.2 Hz), 4.44 (2H, d, J = 6.3 Hz), 6.81–6.84 (1H, m), 6.89–6.92 (2H, m), 7.20–7.33 (6H, m), 7.52 (1H, dd, J = 9.0, 4.5 Hz), 7.79 (1H, t, J = 9.6 Hz), 9.50 (1H, t, J = 5.7 Hz), 12.14 (1H, bs). Anal. Calcd for $C_{25}H_{22}FN_3O_4$•0.7H_2O: C, 65.27; H, 5.13; N, 9.13. Found: C, 65.04; H, 5.09; N, 8.84.

6-Fluoro-5-{[2-(4-fluorophenyl)ethyl]oxy}-*N*-{[3-(methyloxy)phenyl]methyl}-4-oxo-3,4-dihydroquinazoline-2-carboxamide (**38i**). Compound **38i** was prepared from compound **26o** and 2-(4-fluorophenyl)ethanol (pale yellow powder, 32%). mp 146–148 °C. ^{1}H NMR (300 MHz, DMSO-d_6) δ 3.09

(2H, t, *J* = 7.2 Hz), 3.73 (3H, s), 4.27 (2H, t, *J* = 7.2 Hz), 4.44 (2H, d, *J* = 6.3 Hz), 6.80–6.84 (1H, m), 6.88–6.92 (2H, m), 7.11 (2H, t, *J* = 8.7 Hz), 7.25 (1H, t, *J* = 8.1 Hz), 7.37 (2H, dd, *J* = 8.4, 5.7 Hz), 7.53 (1H, dd, *J* = 9.0, 4.8 Hz), 7.78 (1H, dd, *J* = 10.2, 9.0 Hz), 9.50 (1H, t, *J* = 6.3 Hz), 12.15 (1H, bs). Anal. Calcd for $C_{25}H_{21}F_2N_3O_4$•0.6H_2O: C, 63.05; H, 4.70; N, 8.82. Found: C, 62.85; H, 4.57; N, 8.96.

5-{[2-(4-Aminophenyl)ethyl]oxy}-6-fluoro-*N*-{[3-(methyloxy)phenyl]methyl}-4-oxo-3,4-dihydroquinazoline-2-carboxamide (**38j**). Compound **38j** was prepared from compound **26o** and 2-(4-aminophenyl)ethanol (beige powder, 54%). mp 129–131 °C. ^{1}H NMR (300 MHz, DMSO-d_6) δ 2.91 (2H, t, *J* = 7.5 Hz), 3.73 (3H, s), 4.14 (2H, t, *J* = 7.8 Hz), 4.44 (2H, d, *J* = 6.3 Hz), 4.90 (2H, bs), 6.48 (2H, d, *J* = 8.4 Hz), 6.80–6.84 (1H, m), 6.90–6.94 (4H, m), 7.24 (1H, t, *J* = 7.8 Hz), 7.53 (1H, dd, *J* = 9.0, 4.8 Hz), 7.76–7.83 (1H, m), 9.51 (1H, t, *J* = 6.6 Hz), 12.12 (1H, bs). Anal. Calcd for $C_{25}H_{23}FN_4O_4$•0.1H_2O: C, 64.67; H, 5.04; N, 12.07. Found: C, 64.61; H, 5.05; N, 12.20.

4-[2-({6-Fluoro-2-[({[3-(methyloxy)phenyl]methyl}amino)carbonyl]-4-oxo-3,4-dihydroquinazolin-5-yl}oxy)ethyl]benzoic Acid (**38k**). Compound **38k** was prepared from compound **26o** and 4-(2-hydroxyethyl)benzoic acid synthesized by the method of Gilman et al.[60] (pale yellow powder, 29%). mp 227–229 °C. ^{1}H NMR (300 MHz, DMSO-d_6) δ 3.18 (2H, t, *J* = 6.9 Hz), 3.73 (3H, s), 4.32 (2H, t, *J* = 6.9 Hz), 4.44 (2H, d, *J* = 6.0 Hz), 6.81–6.84 (1H, m), 6.89–6.92 (2H, m), 7.24 (1H, t, *J* = 8.1 Hz), 7.46 (2H, d, *J* = 8.4 Hz), 7.53 (1H, dd, *J* = 6.3, 4.8 Hz), 7.79 (1H, t, *J* = 9.6 Hz), 7.87 (2H, d, *J* = 8.1 Hz), 9.50 (1H, t, *J* = 6.3 Hz), 12.20 (1H, bs), 12.87 (1H, bs). Anal. Calcd for $C_{26}H_{22}FN_3O_6$•0.1H_2O: C, 63.31; H, 4.54; N, 8.52. Found: C, 63.21; H, 4.52; N, 8.36.

5-Hydroxy-*N*-{[3-(methyloxy)phenyl]methyl}-4-oxo-3,4-dihydroquinazoline-2-carboxamide (**39**). To a solution of compound **26k** (4.00 g, 12.2 mmol) and 2-phenylethanol (1.64 g, 13.4 mmol) in DMA (100 mL) was added sodium hydride (60% oil dispersion, 2.44 g, 61.1 mmol) and the mixture was stirred at 90 °C for 6 h. After the mixture was allowed to cool to room temperature, H_2O (100 mL) and 1 N hydrochloric acid (60 mL) was added. The resulting precipitate was collected and washed with H_2O and ethanol, and dried under reduced pressure to give the title compound as a pale yellow powder (3.84 g, 11.8 mmol, 97%). ^{1}H NMR (300 MHz, DMSO-d_6) δ 3.74 (3H, s), 4.45 (2H, d, *J* = 6.4 Hz), 6.79–6.86 (1H, m), 6.89–6.98 (3H, m), 7.20–7.30 (2H, m), 7.73 (1H, t, *J* = 8.2 Hz), 9.59 (1H, t, *J* = 5.7 Hz), 12.05 (1H, s), 12.89 (1H, s).

1-(Dimethylamino)-8-hydroxy-2-{[3-(methyloxy)phenyl]methyl}-1,2-dihydroimidazo[5,1-*b*]quinazoline-3,9-dione (**40**). To a mixture of compound **39** (1.00 g, 3.07 mmol), DMF (4.8 mL) and THF (10 mL) was added dropwise oxalyl chloride (1.17 g, 9.22 mmol) at 0 °C and the reaction mixture was stirred at room temperature for 2 h. The mixture was concentrated under reduced pressure and partitioned between ethyl acetate and aqueous $NaHCO_3$ solution. The organic layer was washed with brine, dried over Na_2SO_4 and concentrated under reduced pressure to give a yellow solid. The yellow solid obtained above was dissolved in a mixed solvent of THF (8 mL) and methanol (8 mL), and then 1 N aqueous sodium

hydroxide solution (3.07 mL, 3.07 mmol) was added. The mixture was stirred at room temperature for 4 h followed by neutralization with 1 N hydrochloric acid. The resulting mixture was concentrated under reduced pressure and the residue was suspended in H_2O. The precipitated solid was collected, washed with H_2O and dried to give the title compound as a white powder (1.05 g, 2.76 mmol, 90%). ^{1}H NMR (300 MHz, DMSO-d_6) δ 2.36 (6H, s), 3.75 (3H, s), 4.35 (1H, d, J = 15.1 Hz), 4.89 (1H, d, J = 14.8 Hz), 6.16 (1H, s), 6.84–6.98 (3H, m), 7.02 (1H, d, J = 8.3 Hz), 7.29 (1H, t, J = 8.0 Hz), 7.34 (1H, d, J = 7.2 Hz), 7.79 (1H, t, J = 8.1 Hz), 11.40 (1H, s).

1-(Dimethylamino)-2-{[3-(methyloxy)phenyl]methyl}-3,9-dioxo-1,2,3,9-tetrahydroimidazo[5,1-*b*] quinazolin-8-yl Trifluoromethanesulfonate (**41**). To a solution of compound **40** (3.53 g, 9.28 mmol) in dichloromethane (32 mL) were added pyridine (1.95 mL, 24.2 mmol) and trifluoromethanesulfonic anhydride (2.03 mL, 12.1 mmol) at 0 °C and the mixture was stirred at room temperature for 2 h. After the mixture was concentrated under reduced pressure, the residue was diluted with ethyl acetate and H_2O, and neutralized with 1 N hydrochloric acid (5 mL). The organic layer was separated, washed with brine and dried over Na_2SO_4. After removal of the solvent, the residue was triturated with diethyl ether to give the title compound as a pale yellow powder (4.63 g, 9.04 mmol, 97%). ^{1}H NMR (300 MHz, DMSO-d_6) δ 2.34 (6H, s), 3.75 (3H, s), 4.35 (1H, d, J = 15.1 Hz), 4.88 (1H, d, J = 15.1 Hz), 6.19 (1H, s), 6.84–7.00 (3H, m), 7.29 (1H, t, J = 7.8 Hz), 7.66 (1H, d, J = 7.6 Hz), 7.97–8.11 (2H, m).

1-(Dimethylamino)-2-{[3-(methyloxy)phenyl]methyl}-3,9-dioxo-1,2,3,9-tetrahydroimidazo[5,1-*b*] quinazoline-8-carbonitrile (**42a**). Compound **42a** was prepared from compound **41** with a similar procedure as described for **37b** (white powder, 82%). ^{1}H NMR (300 MHz, DMSO-d_6) δ 2.38 (6H, s), 3.75 (3H, s), 4.35 (1H, d, J = 14.8 Hz), 4.91 (1H, d, J = 15.1 Hz), 6.17 (1H, s), 6.85–6.98 (3H, m), 7.29 (1H, t, J = 7.8 Hz), 8.02–8.10 (1H, m), 8.14–8.21 (2H, m).

1-(Dimethylamino)-2-{[3-(methyloxy)phenyl]methyl}-8-phenyl-1,2-dihydroimidazo[5,1-*b*]quinazoline-3,9-dione (**42b**). Compound **42b** was prepared from compound **41** with a similar procedure as described for **37a** (pale yellow powder, 91%). ^{1}H NMR (300 MHz, DMSO-d_6) δ 2.24 (6H, s), 3.74 (3H, s), 4.31 (1H, d, J = 14.9 Hz), 4.87 (1H, d, J = 15.1 Hz), 6.05 (1H, s), 6.84–6.96 (3H, m), 7.23–7.42 (7H, m), 7.86–7.94 (2H, m).

1-(Dimethylamino)-2-{[3-(methyloxy)phenyl]methyl}-8-(phenyloxy)-1,2-dihydroimidazo[5,1-*b*] quinazoline-3,9-dione (**42c**). A mixture of compound **41** (300 mg, 0.585 mmol), phenol (66 mg, 0.700 mmol), tris(dibenzylideneacetone)dipalladium(0) (27 mg, 0.029 mmol), 2-(di-*t*-butylphosphine)biphenyl (17 mg, 0.059 mmol), and K_3PO_4 (0.352 mL, 1.23 mmol) in toluene (2 mL) was stirred at 80 °C for 72 h under nitrogen atmosphere. The reaction mixture was diluted with ethyl acetate, and washed with H_2O and brine. The organic layer was dried over Na_2SO_4 and concentrated under reduced pressure. The crude product was purified by silica gel column chromatography (30–50% ethyl acetate/hexane) to give the title compound as a pale yellow oil (50.1 mg, 0.110 mmol, 19%). ^{1}H NMR (300 MHz, DMSO-d_6) δ 2.27 (6H, bs), 3.74 (3H,

s), 4.31 (1H, d, *J* = 14.8 Hz), 4.87 (1H, d, *J* = 14.8 Hz), 6.08 (1H, s), 6.84–6.97 (5H, m), 7.05–7.15 (2H, m), 7.27 (1H, t, *J* = 8.0 Hz), 7.33–7.40 (2H, m), 7.66 (1H, dd, *J* = 8.3, 1.1 Hz), 7.85 (1H, t, *J* = 8.1 Hz).

1-(Dimethylamino)-2-{[3-(methyloxy)phenyl]methyl}-8-(3-phenylpropyl)-1,2-dihydroimidazo[5,1-*b*]quinazoline-3,9-dione (**42d**). To a mixture of compound **41** (200 mg, 0.390 mmol), *N*,*N*-diisopropyl-ethylamine (0.102 mL, 0.585 mmol), copper(I) iodide (22 mg, 0.120 mmol) and tetrakis(triphenylphosphine)palladium(0) (45 mg, 0.039 mmol) in DMF (4 mL) was added 3-phenyl-1-propyne (68 mg, 0.590 mmol) and the mixture was stirred at room temperature for 24 h. The reaction mixture was diluted with ethyl acetate and washed with aqueous NH_4Cl solution (twice) and brine. The organic layer was dried over Na_2SO_4 and concentrated under reduced pressure. The crude product was purified by silica gel column chromatography (40% ethyl acetate/hexane) and crystallized from diethyl ether to give 1-(dimethylamino)- 2-{[3-(methyloxy)phenyl]methyl}-8-(3-phenylprop-1-yn-1-yl)-1,2-dihydroimidazo[5,1-*b*]quinazoline-3,9- dione as a pale yellow power (85.0 mg). A mixture of the pale yellow powder obtained above (80 mg, 0.170 mmol) and 10% palladium on carbon (160 mg) in a mixed solvent of THF (10 mL) and methanol (1 mL) was stirred at room temperature for 2 h under hydrogen atmosphere (1 atm). After the insoluble materials were filtered off through a PTFE membrane filter, the filtrate was concentrated under reduced pressure to give the title compound as a colorless oil (36 mg, 0.075 mmol, 21% over 2 steps). ^{1}H NMR (300 MHz, DMSO-d_6) δ 1.80–1.97 (2H, m), 2.24–2.40 (6H, m), 2.65 (2H, t, *J* = 7.8 Hz), 3.25–3.39 (2H, m), 3.75 (3H, s), 4.33 (1H, d, *J* = 14.8 Hz), 4.88 (1H, d, *J* = 15.1 Hz), 6.15 (1H, s), 6.84–6.99 (3H, m), 7.10–7.32 (6H, m), 7.41 (1H, d, *J* = 6.1 Hz), 7.67–7.84 (2H, m).

Representative Procedure for the Synthesis of Compounds 43a-d: 5-Cyano-*N*-{[3-(methyloxy) phenyl]-methyl}-4-oxo-3,4-dihydroquinazoline-2-carboxamide (**43a**). A mixture of compound **42a** (29 mg, 0.074 mmol), 6 N hydrochloric acid (0.496 mL), and acetic acid (1 mL) was heated at 80 °C for 2 h. After completeness of reaction was checked by LC-MS, the reaction mixture was concentrated under reduced pressure and the residue was crystallized from ethanol to give the title compound as a white powder (20 mg, 0.060 mmol, 80%). mp 185–187 °C. ^{1}H NMR (300 MHz, DMSO-d_6) δ 3.74 (3H, s), 4.46 (2H, d, *J* = 6.4 Hz), 6.79–6.87 (1H, m), 6.88–6.97 (2H, m), 7.20–7.30 (1H, m), 7.92–8.13 (3H, m), 9.61 (1H, t, *J* = 6.1 Hz), 12.74 (1H, s). Anal. Calcd for $C_{18}H_{14}N_4O_3$: C, 64.66; H, 4.22; N, 16.76. Found: C, 64.39; H, 4.25; N, 17.04.

***N*-{[3-(Methyloxy)phenyl]methyl}-4-oxo-5-phenyl-3,4-dihydroquinazoline-2-carboxamide** (**43b**). Compound **43b** was prepared from compound **42b** (white powder, 53%). mp 196–198 °C. ^{1}H NMR (300 MHz, DMSO-d_6) δ 3.74 (3H, s), 4.46 (2H, d, *J* = 6.1 Hz), 6.80–6.87 (1H, m), 6.90–6.97 (2H, m), 7.21–7.40 (7H, m), 7.75–7.89 (2H, m), 9.55 (1H, t, *J* = 6.2 Hz), 12.00 (1H, bs). Anal. Calcd for $C_{23}H_{19}N_3O_3$: C, 71.67; H, 4.97; N, 10.90. Found: C, 71.38; H, 5.07; N, 10.72.

***N*-{[3-(Methyloxy)phenyl]methyl}-4-oxo-5-(phenyloxy)-3,4-dihydroquinazoline-2-carboxamide** (**43c**). Compound **43c** was prepared from compound **42c** (white powder, 55%). Chromatographic purity

(HPLC) 95.7%. mp 148-150 °C. ^{1}H NMR (300 MHz, DMSO-d_6) δ 3.74 (3H, s), 4.45 (2H, d, J = 6.4 Hz), 6.80–6.86 (1H, m), 6.89–6.96 (4H, m), 7.00–7.14 (2H, m), 7.25 (1H, t, J = 8.1 Hz), 7.31–7.40 (2H, m), 7.57 (1H, d, J = 8.0 Hz), 7.81 (1H, t, J = 8.1 Hz), 9.54 (1H, t, J = 6.2 Hz), 12.03 (1H, bs). Anal. Calcd for $C_{23}H_{19}N_3O_4$•0.25H_2O: C, 68.05; H, 4.84; N, 10.35. Found: C, 67.77; H, 4.70; N, 10.51.

***N*-{[3-(Methyloxy)phenyl]methyl}-4-oxo-5-(3-phenylpropyl)-3,4-dihydroquinazoline-2-carboxamide** (**43d**). Compound **43d** was prepared from compound **42d** (white powder, 56%). mp 132–134 °C. ^{1}H NMR (300 MHz, DMSO-d_6) δ 1.80–1.94 (2H, m), 2.63–2.71 (2H, m), 3.22–3.33 (2H, m), 3.73 (3H, s), 4.45 (2H, d, J = 6.4 Hz), 6.78–6.87 (1H, m), 6.88–6.95 (2H, m), 7.10–7.31 (6H, m), 7.34 (1H, d, J = 7.2 Hz), 7.57–7.66 (1H, m), 7.73 (1H, t, J = 7.8 Hz), 9.50 (1H, t, J = 6.1 Hz), 12.00 (1H, bs). Anal. Calcd for $C_{26}H_{25}N_3O_3$•0.1H_2O: C, 72.74; H, 5.92; N, 9.79. Found: C, 72.70; H, 5.85; N, 9.87.

Ethyl 5-(Bromomethyl)-4-oxo-3,4-dihydroquinazoline-2-carboxylate (**44**). A mixture of compound **25f** (3.00 g, 12.9 mmol), *N*-bromosuccinimide (2.53 g, 14.2 mmol) and 2,2'-azobis(isobutyronitrile) (106 mg, 0.645 mmol) in chloroform (60 mL) was refluxed for 1 h. The mixture was concentrated under reduced pressure and the residue was triturated with ethyl acetate to give the title compound as a white powder (3.17 g, 10.2 mmol, 79%). ^{1}H NMR (300 MHz, DMSO-d_6) δ 1.36 (3H, t, J = 6.9 Hz), 4.39 (2H, q, J = 6.9 Hz), 5.38 (2H, s), 7.67–7.69 (1H, m), 7.77–7.87 (2H, m), 12.66 (1H, bs).

Ethyl 5-{[Methyl(phenylmethyl)amino]methyl}-4-oxo-3,4-dihydroquinazoline-2-carboxylate (**45**). To a suspension of compound **44** (300 mg, 0.964 mmol) in a mixed solvent of THF (6 mL) and DMF (2 mL) were added pyridine (0.078 mL, 0.964 mmol) and *N*-methyl-1-phenylmethan- amine (117 mg, 0.964 mmol) at 0 °C and the mixture was stirred at room temperature for 2 h. Water and 1 N hydrochloric acid (2 mL) were added to the reaction mixture and washed with ethyl acetate. The aqueous layer was basified with 1 N aqueous sodium hydroxide solution and the product was extracted with ethyl acetate. The organic layer was washed with brine, dried over Na_2SO_4 and concentrated under reduced pressure. The residue was triturated with diethyl ether to give the title compound as a pale yellow powder (200 mg, 0.569 mmol, 59%). ^{1}H NMR (300 MHz, DMSO-d_6) δ 1.67 (3H, t, J = 7.2 Hz), 2.50 (3H, s), 3.99 (2H, bs), 4.58 (2H, s), 4.69 (2H, q, J = 6.9 Hz), 7.56–7.71 (5H, m), 8.02 (1H, d, J = 9.0 Hz), 8.13–8.22 (2H, m), 12.69 (1H, bs).

***N*-{[3-(Methyloxy)phenyl]methyl}-5-{[methyl(phenylmethyl)amino]methyl}-4-oxo-3,4-dihydro-quinazoline-2-carboxamide** (**46**). Compound **46** was prepared from compound **45** with the same procedure as described for **26a** (white powder, 8%). Chromatographic purity (HPLC) 93.4%. mp 127–128 °C. ^{1}H NMR (300 MHz, DMSO-d_6) δ 2.17 (3H, s), 3.64 (2H, s), 3.73 (3H, s), 4.24 (2H, s), 4.45 (2H, d, J = 5.7 Hz), 6.81–6.93 (3H, m), 7.21–7.38 (6H, m), 7.64–7.67 (1H, m), 7.82–7.90 (2H, m), 9.52 (1H, t, J = 5.7 Hz), 11.42 (1H, bs).

Ethyl 5-Methyl-4-oxo-3-({[2-(trimethylsilyl)ethyl]oxy}methyl)-3,4-dihydroquinazoline-2-carboxy-late (**47**). To a mixture of compound **25f** (1.50 g, 6.46 mmol) in DMF (30 mL) was added sodium hydride (60% oil dispersion, 284 mg, 7.10 mmol) at 0 °C and the mixture was stirred at room temperature for 30

min. The mixture was cooled to 0 °C again and {2-[(chloromethyl)oxy]ethyl}(trimethyl)silane (1.25 mL, 7.10 mmol) was added. After being stirred at room temperature for 12 h, the reaction mixture was diluted with ethyl acetate, washed with H_2O (twice) and brine, and dried over Na_2SO_4. After removal of the solvent, the residue was purified by silica gel column chromatography (17–50% ethyl acetate/hexane) to give the title compound as a pale yellow oil (1.80 g, 4.97 mmol, 77%). ^{1}H NMR (300 MHz, DMSO-d_6) δ –0.05 (9H, s), 0.83 (2H, t, J = 8.1 Hz), 1.34 (3H, t, J = 6.9 Hz), 2.79 (3H, s), 3.51 (2H, t, J = 8.1 Hz), 4.39 (2H, q, J = 6.9 Hz), 5.54 (2H, s), 7.42 (1H, d, J = 7.5 Hz), 7.56 (1H, d, J = 8.1 Hz), 7.74 (1H, t, J = 8.1 Hz).

Ethyl 5-(Bromomethyl)-4-oxo-3-({[2-(trimethylsilyl)ethyl]oxy}methyl)-3,4-dihydroquinazoline-2-carboxylate (**48**). Compound **48** was prepared from compound **47** with a similar procedure as described for **44** (pale yellow oil, 40%). ^{1}H NMR (300 MHz, DMSO-d_6) δ –0.06–0.00 (9H, m), 0.84 (2H, t, J = 8.1 Hz), 1.32–1.37 (3H, m), 3.48–3.56 (2H, m), 4.36–4.45 (2H, m), 5.34–5.59 (4H, m), 7.70–7.87 (3H, m).

Ethyl 4-Oxo-5-{[(phenylmethyl)oxy]methyl}-3,4-dihydroquinazoline-2-carboxylate (**50**). To a mixture of benzyl alcohol (108 mg, 0.996 mmol) in THF (2 mL) was added sodium hydride (60% oil dispersion, 36.0 mg, 0.906 mmol) at 0 °C and the mixture was stirred at room temperature for 30 min. The mixture was cooled to 0 °C again and a solution of compound **48** (200 mg, 0.453 mmol) in THF (3 mL) was added. After being stirred at room temperature for 3 h, the reaction mixture was quenched with saturated aqueous NH_4Cl solution and extracted with ethyl acetate. The organic layer was washed with H_2O and brine, dried over Na_2SO_4 and concentrated under reduced pressure to give ethyl 4-oxo-5-{[(phenylmethyl)oxy]methyl}-3-({[2-(trimethylsilyl)ethyl]oxy}methyl)-3,4-dihydroquinazoline-2-carboxylate (**49**) as a yellow oil (220 mg). To a solution of the yellow oil (200 mg) obtained above in dichloromethane (8 mL) was added trifluoroacetic acid (2 mL) and the mixture was stirred at room temperature for 2 h. After removal of the solvent, the residue was purified by silica gel column chromatography (25–100% ethyl acetate/hexane) to give the title compound as a brown powder (42 mg, 120 mmol, 30% over 2 steps). ^{1}H NMR (300 MHz, DMSO-d_6) δ 1.49 (3H, t, J = 7.2 Hz), 4.57 (2H, q, J = 7.2 Hz), 4.76 (2H, s), 5.32 (2H, s), 7.30–7.46 (5H, m), 7.78–7.86 (2H, m), 7.97–8.01 (1H, m), 9.92 (1H, bs).

***N*-{[3-(Methyloxy)phenyl]methyl}-4-oxo-5-{[(phenylmethyl)oxy]methyl}-3,4-dihydroquinazoline-2-carboxamide** (**51**). Compound **51** was prepared from compound **50** with the same procedure as described for **26a** (white powder, 51%). mp 160–161 °C. ^{1}H NMR (300 MHz, DMSO-d_6) δ 3.73 (3H, s), 4.44 (2H, d, J = 6.3 Hz), 4.71 (2H, s), 5.19 (2H, s), 6.81–6.93 (3H, m), 7.21–7.44 (6H, m), 7.68 (1H, d, J = 7.8 Hz), 7.79–7.89 (2H, m), 9.53 (1H, t, J = 6.3 Hz), 12.10 (1H, bs). Anal. Calcd for $C_{25}H_{23}N_3O_4$•0.1H_2O: C, 69.62; H, 5.42; N, 9.74. Found: C, 69.47; H, 5.39; N, 9.91.

{2-[({[3-(Methyloxy)phenyl]methyl}amino)carbonyl]-4-oxo-3,4-dihydroquinazolin-5-yl}acetic Acid (**52**). A mixture of compound **26n** (230 mg, 0.603 mmol), 4 N aqueous sodium hydroxide solution (0.528 mL, 2.11 mmol), THF (4 mL), methanol (4 mL) and H_2O (4 mL) was stirred at 80 °C for 10 h. After being allowed to cool to room temperature, the mixture was diluted with H_2O and acidified with 1 N hydrochloric

acid (3 mL). The resulting mixture was partitioned between ethyl acetate and H_2O, and the organic layer was washed with brine and dried over Na_2SO_4. After removal of the solvent, the residue was crystallized from ethyl acetate–diethyl ether to give the title compound as a white powder (192 mg, 0.523 mmol, 87%). mp 227–228 °C. ^{1}H NMR (300 MHz, DMSO-d_6) δ 3.74 (3H, s), 4.16 (2H, s), 4.45 (2H, d, J = 6.4 Hz), 6.79–6.87 (1H, m), 6.88–6.96 (2H, m), 7.20–7.30 (1H, m), 7.40 (1H, d, J = 6.8 Hz), 7.66–7.73 (1H, m), 7.77 (1H, t, J = 7.8 Hz), 9.53 (1H, t, J = 6.2 Hz), 12.05–12.20 (2H, m). Anal. Calcd for $C_{19}H_{17}N_3O_5$•0.1H_2O: C, 61.82; H, 4.70; N, 11.38. Found: C, 61.59; H, 4.69; N, 11.30.

***N*-{[3-(Methyloxy)phenyl]methyl}-4-oxo-5-[2-oxo-2-(phenylamino)ethyl]-3,4-dihydroquinazoline-2-carboxamide** (**53**). A mixture of compound **52** (120 mg, 0.327 mmol), aniline (0.0600 mL, 0.653 mmol), 1-[3-(dimethylamino)propyl]-3-ethylcarbodiimide hydrochloride (125 mg, 0.653 mmol), 1-hydroxybenzotriazole (88.0 mg, 0.653 mmol) and 4-dimethylaminopyridine (40.0 mg, 0.327 mmol) in DMF (3 mL) was stirred at 50 °C for 12 h. The reaction mixture was diluted with ethyl acetate, and washed with H_2O, 0.1 N hydrochloric acid, H_2O and brine. The organic layer was dried over Na_2SO_4 and concentrated under reduced pressure. The residue was triturated with ethanol to give the title compound as a pale yellow powder (117 mg, 0.264 mmol, 81%). mp 206–208 °C. ^{1}H NMR (300 MHz, DMSO-d_6) δ 3.74 (3H, s), 4.33 (2H, s), 4.45 (2H, d, J = 6.4 Hz), 6.79–6.86 (1H, m), 6.89–6.95 (2H, m), 6.99 (1H, t, J = 7.4 Hz), 7.20–7.31 (3H, m), 7.43 (1H, d, J = 7.2 Hz), 7.57 (2H, d, J = 7.6 Hz), 7.67–7.73 (1H, m), 7.79 (1H, t, J = 7.8 Hz), 9.53 (1H, t, J = 6.2 Hz), 10.08 (1H, bs), 12.10 (1H, bs). Anal. Calcd for $C_{25}H_{22}N_4O_4$•0.1H_2O: C, 67.59; H, 5.04; N, 12.61. Found: C, 67.54; H, 4.91; N, 12.82.

Sodium 4-[2-({6-Fluoro-2-[({[3-(methyloxy)phenyl]methyl}amino)carbonyl]-4-oxo-3,4-dihydroquinazolin-5-yl}oxy)ethyl]benzoate (**54**). To a solution of compound **38k** (1.50 g, 3.05 mmol) in a mixed solvent of THF (30 mL), methanol (7.5 mL), and DMSO (3.75 mL) was added a solution of sodium hydrogen carbonate (256 mg, 3.05 mmol) in H_2O (7.5 mL) at 80 °C, and the mixture was stirred at 80 °C for 30 min. After THF and methanol were removed by evaporation, methanol (30 mL) was added to the residual suspension. The resulting suspension was stirred at 80 °C for 30 min and allowed to cool to room temperature. The precipitated solid was collected and washed with methanol to give the title compound as a pale yellow powder (1.40 g, 2.73 mmol, 89%). mp 319–321 °C. ^{1}H NMR (300 MHz, DMSO-d_6) δ 3.10 (2H, t, J = 7.3 Hz), 3.73 (3H, s), 4.26 (2H, t, J = 7.3 Hz), 4.43 (2H, d, J = 6.2 Hz), 6.79–6.85 (1H, m), 6.88–6.93 (2H, m), 7.20–7.30 (3H, m), 7.45 (1H, dd, J = 9.0, 4.7 Hz), 7.63 (1H, t, J = 9.7 Hz), 7.79 (2H, d, J = 8.1 Hz), 9.33 (1H, t, J = 6.5 Hz), 12.51 (1H, s). Anal. Calcd for $C_{26}H_{21}N_3O_6FNa$: C, 60.82; H, 4.12; N, 8.18. Found: C, 60.62; H, 4.11; N, 8.38.

2-Amino-4,5-dimethylfuran-3-carboxamide (**55b**). A mixture of 2-amino-4,5-dimethylfuran-3-carbonitrile[61] (4.50 g, 33.1 mmol, synthesized by a method of Hayashi et al.) and concentrated sulfuric acid (20 mL) was stirred with heating at 60 °C for 30 min. After the reaction mixture was cooled to 0 °C, crushed ice (40 g) was added carefully, and 28% aqueous ammonia solution (45 mL) was added dropwise to adjust

pH to 9. The precipitated solid was collected by filtration, washed with water and ethanol and dried under reduced pressure to give **55b** as a brown powder (3.71 g, 73%). ^{1}H NMR (200 MHz, DMSO-d_6) δ 1.98 (3H, s), 2.03 (3H, s), 6.30 (2H, bs), 6.51 (2H, bs).

Ethyl [(3-carbamoylthiophen-2-yl)carbamoyl]formate (**56a**). To a solution of 2-amino-3-thiophenecarboxylic amide[62] (**55a**) (5.00 g, 35.2 mmol, synthesized by the method of Dumaitre and Dodic) and triethylamine (5.39 mL, 38.7 mmol) in THF (200 mL) was added dropwise ethyl chloroglyoxylate (4.81 g, 35.2 mmol) at 0 °C, and the mixture was stirred at room temperature for 1 h. The mixture was partitioned between ethyl acetate and water. The organic layer was washed with brine, dried over anhydrous Na_2SO_4 and concentrated under reduced pressure. The residue was triturated with ethanol to give **56a** as a pale yellow powder (8.32 g, 97%). ^{1}H NMR (200 MHz, DMSO-d_6) δ 1.32 (3H, t, J = 7.0 Hz), 4.33 (2H, q, J = 7.0 Hz), 7.15 (1H, d, J = 6.0 Hz), 7.50 (1H, d, J = 6.0 Hz), 7.67 (1H, bs), 8.03 (1H, bs).

Ethyl [(3-carbamoyl-4,5-dimethylfuran-2-yl)carbamoyl]formate (**56b**). Step 1. To a suspension of compound **55b** (1.50 g, 10.7 mmol) and diethyl oxalate (6.26 g, 42.8 mmol) in ethanol (70 mL) was added sodium ethylate (27.3 g, 80.2 mmol) at 0 °C, and the mixture was heated under reflux for 20 h. The reaction mixture was allowed to cool to room temperature, and poured into 1 M hydrochloric acid cooled to 0 °C. The mixture was concentrated under reduced pressure and the residue was suspended with water. The precipitated solid was collected by filtration, washed with water and dried to give 5,6-dimethyl-4-oxo-3*H*,4*H*-furo[2,3-*d*]pyrimidine-2-carboxylic acid as a brown powder (1.67 g, 75%). ^{1}H NMR (300 MHz, DMSO-d_6) δ 2.07 (3H, s), 2.15 (3H, s), 11.2 (1H, bs), 12.7 (1H, bs).

Step 2. To a suspension of 5,6-dimethyl-4-oxo-3*H*,4*H*-furo[2,3-*d*]pyrimidine-2-carboxylic acid (500 mg, 2.40 mmol) obtained above and DMF (0.1 mL) in THF (10 mL) was added dropwise oxalyl chloride (457 mg, 3.60 mmol) at 0 °C, and the mixture was stirred at room temperature for 3 h. The reaction mixture was concentrated under reduced pressure and ethanol (5 mL) and THF (5 mL) were added to the concentrated residue. Pyridine (0.291 mL, 3.60 mmol) was further added dropwise to the mixture. The resulting mixture was stirred at room temperature for 2 h and then with heating at 50 °C for 15 h. The reaction mixture was concentrated under reduced pressure and the residue was partitioned between ethyl acetate and water. The organic layer was washed with brine and dried over anhydrous Na_2SO_4. After removal of the solvent, the residue was triturated with diethyl ether–ethanol to give **56b** as a yellow powder (297 mg, 49%). ^{1}H NMR (300 MHz, DMSO-d_6) δ 1.29 (3H, t, J = 6.9 Hz), 2.02 (3H, s), 2.16 (3H, s), 4.28 (2H, q, J = 6.9 Hz), 6.85–7.38 (2H, m), 11.2 (1H, bs).

3-Aminothiophene-2-carboxylic Acid (**58a**). A mixture of commercially available methyl 3-aminothiophene-2-carboxylate (10.0 g, 63.6 mmol) and 1 M aqueous sodium hydroxide solution (70 mL, 70 mmol) was stirred at reflux for 1.5 h. The mixture was acidified with 2 M hydrochloric acid to pH 3–4 and extracted with a mixed solvent of ethyl acetate and THF. The organic layer was dried over anhydrous Na_2SO_4 and concentrated under reduced pressure to give **58a** as a beige powder (6.40 g, 70%). ^{1}H NMR

(200 MHz, DMSO-d_6) δ 6.58 (1H, d, J = 5.2 Hz), 7.44 (1H, d, J = 5.2 Hz).

Ethyl 4-oxo-4*H*-thieno[3,2-*d*][1,3]oxazine-2-carboxylate (**59a**). To a suspension of compound **58a** (3.84 g, 26.5 mmol) in pyridine (100 mL) was added dropwise ethyl chloroglyoxylate (7.69 g, 56.3 mmol) at 0 °C, and the mixture was stirred at room temperature for 2 h. The reaction mixture was concentrated under reduced pressure and the residue was suspended with water. The resulting precipitate was collected by filtration to give **59a** as a brown powder (4.86 g, 81%). ^{1}H NMR (200 MHz, DMSO-d_6) δ 1.35 (3H, t, J = 7.2 Hz), 4.39 (2H, q, J = 7.2 Hz), 7.64 (1H, d, J = 5.6 Hz), 8.49 (1H, d, J = 5.6 Hz).

Ethyl 4-oxo-4*H*-pyrido[2,3-*d*][1,3]oxazine-2-carboxylate (**59b**). To a suspension of commercially available 2-aminopyridine-3-carboxylic acid **58b** (2.50 g, 18.1 mmol) in pyridine (30 mL) was added dropwise ethyl chloroglyoxylate (4.94 g, 36.2 mmol) at 0 °C. The mixture was stirred at room temperature for 2 h and then at 50 °C for further 1 h. The reaction mixture was concentrated under reduced pressure and the residue was suspended with water. The resulting precipitate was collected by filtration to give **59b** as a pale yellow powder (2.21 g, 56%). ^{1}H NMR (200 MHz, $CDCl_3$) δ 1.47 (3H, t, J = 7.2 Hz), 4.52 (2H, q, J = 7.2 Hz), 7.64 (1H, dd, J = 8.0, 4.8 Hz), 8.61 (1H, dd, J = 8.0, 1.8 Hz), 9.11 (1H, dd, J = 4.8, 1.8 Hz).

Ethyl 4-oxo-3*H*,4*H*-thieno[2,3-*d*]pyrimidine-2-carboxylate (**60a**). A mixture of compound **56a** (2.00 g, 8.26 mmol) and *p*-toluenesulfonic acid monohydrate (514 mg, 2.70 mmol) in xylene (50 mL) was heated under reflux for 11 h. The reaction mixture was diluted with ethyl acetate, washed with water and brine, dried over anhydrous Na_2SO_4 and concentrated under reduced pressure. The residue was purified by silica gel column chromatography (67% ethyl acetate/hexane) to give **60a** as a pale yellow powder (531 mg, 29%). ^{1}H NMR (200 MHz, DMSO-d_6) δ 1.35 (3H, t, J = 7.2 Hz), 4.38 (2H, q, J = 7.2 Hz), 7.49 (1H, d, J = 5.6 Hz), 7.81 (1H, d, J = 5.6 Hz).

Ethyl 5,6-dimethyl-4-oxo-3*H*,4*H*-furo[2,3-*d*]pyrimidine-2-carboxylate (**60e**). A mixture of compound **56b** (280 mg, 1.10 mmol) and *p*-toluenesulfonic acid monohydrate (105 mg, 0.550 mmol) in toluene (20 mL) was heated under reflux for 4 h. The reaction mixture was partitioned between ethyl acetate and water. The organic layer was washed with brine, dried over anhydrous Na_2SO_4, and concentrated under reduced pressure. The residue was crystallized from ethanol to give **60e** as a pale yellow powder (150 mg, 0.635 mmol, 58%). ^{1}H NMR (300 MHz, DMSO-d_6) δ 1.33 (3H, t, J = 7.2 Hz), 2.19 (3H, s), 2.34 (3H, s), 4.35 (2H, q, J = 7.2 Hz), 12.8 (1H, bs).

Ethyl 1,3-dimethyl-4-oxo-1*H*,4*H*,5*H*-pyrazolo[3,4-*d*]pyrimidine-6-carboxylate (**60g**). To a solution of 5-amino-1,3-dimethyl-1*H*-pyrazole-4-carboxamide[63] (**55d**) (3.00 g, 19.5 mmol, synthesized by the method of Cheng et al.) and diethyl oxalate (11.4 g, 77.8 mmol) in ethanol (500 mL) was added sodium ethylate (33.1 g, 97.3 mmol) at 0 °C, and the mixture was heated under reflux for 18 h. The reaction mixture was allowed to cool to room temperature and poured into 1 M hydrochloric acid (100 mL). The mixture was concentrated under reduced pressure and the residue was suspended with water. The precipitated solid was collected by filtration and washed with water and ethanol to give **60g** as a pale yellow powder (2.52 g, 55%).

^{1}H NMR (300 MHz, DMSO-d_6) δ 1.35 (3H, t, J = 7.2 Hz), 2.44 (3H, s), 3.86 (3H, s), 4.38 (2H, q, J = 7.2 Hz), 12.4 (1H, bs).

Ethyl 5-methyl-4-oxo-3*H*,4*H*-thieno[3,4-*d*]pyrimidine-2-carboxylate (**60h**). A mixture of methyl 4-amino-2-methylthiophene-3-carboxylate hydrochloride[64] (**57a**) (1.90 g, 9.15 mmol, synthesized by the method of Barker et al.), ethyl cyanoformate (1.36 g, 13.7 mmol) and 1 M hydrochloric acid in acetic acid (40 mL) was stirred at 80 °C for 2 h. After removal of the solvent, the residue was suspended with water. The resulting precipitate was collected and washed with water and diethyl ether to give **60h** as a brown powder (1.48 g, 68%). ^{1}H NMR (300 MHz, DMSO-d_6) δ 1.33 (3H, d, J = 7.2 Hz), 2.88 (3H, s), 4.34 (2H, q, J = 7.2 Hz), 7.79 (1H, s), 11.7 (1H, bs).

Ethyl 6-methyl-4-oxo-3*H*,4*H*,7*H*-pyrrolo[2,3-*d*]pyrimidine-2-carboxylate (**60i**). Compound **60i** was prepared from ethyl 2-amino-5-methyl-1*H*-pyrrole-3-carboxylate[65] (**57b**) (synthesized by a method of Toja et al.) with a similar procedure as described for **60h** (white powder, 50%). ^{1}H NMR (300 MHz, DMSO-d_6) δ 1.33 (3H, t, J = 7.2 Hz), 2.31 (3H, s), 4.33 (2H, q, J = 7.2 Hz), 6.26 (1H, s), 12.0 (1H, bs), 12.1 (1H, bs).

Ethyl 6,7-dimethyl-4-oxo-4,7-dihydro-3*H*-pyrrolo[2,3-*d*]pyrimidine-2-carboxylate (**60j**). Compound **60j** was prepared from ethyl 2-amino-1,5-dimethyl-1*H*-pyrrole-3-carboxylate[65] (**57c**, synthesized by a method of Toja et al.) with a similar procedure as described for **60h** (white powder, 26%). ^{1}H NMR (300 MHz, DMSO-d_6) δ 1.34 (3H, t, J = 6.9 Hz), 2.36 (3H, s), 3.66 (3H, s), 4.36 (2H, q, J = 7.2 Hz), 6.37 (1H, s), 12.1 (1H, bs).

Ethyl 4-oxo-3*H*,4*H*-thieno[3,2-*d*]pyrimidine-2-carboxylate (**60k**). A mixture of compound **59a** (2.50 g, 11.1 mmol) and ammonium acetate (941 mg, 12.2 mmol) in ethanol (30 mL) was heated under reflux for 1 h. The reaction mixture was concentrated under reduced pressure. The residue was diluted with ethyl acetate, washed with water and brine, dried over anhydrous Na_2SO_4 and concentrated under reduced pressure. The crude product was purified by silica gel column chromatography (67–80% ethyl acetate/hexane) to give **60k** as a brown powder (478 mg, 19%). ^{1}H NMR (200 MHz, DMSO-d_6) δ 1.35 (3H, t, J = 7.2 Hz), 4.38 (2H, q, J = 7.2 Hz), 7.56 (1H, d, J = 5.6 Hz), 8.28 (1H, d, J = 5.6 Hz).

Ethyl 4-oxo-3*H*,4*H*-pyrido[2,3-*d*]pyrimidine-2-carboxylate (**60l**). A mixture of compound **59b** (2.20 g, 9.99 mmol), ammonium acetate (770 mg, 9.99 mmol) and acetic acid (240 mg, 4.00 mmol) in ethanol (30 mL) was heated under reflux for 1 h. After the reaction mixture was cooled to room temperature, the precipitated solid was collected by filtration and washed with ethanol to give **60l** as a pale yellow powder (1.11 g, 51%). ^{1}H NMR (200 MHz, DMSO-d_6) δ 1.38 (3H, t, J = 7.0 Hz), 4.41 (2H, q, J = 7.0 Hz), 7.65 (1H, dd, J = 7.8, 4.4 Hz), 8.56 (1H, dd, J = 7.8, 1.8 Hz), 9.04 (1H, dd, J = 4.4, 1.8 Hz).

Ethyl 4-oxo-3*H*,4*H*-pyrido[3,4-*d*]pyrimidine-2-carboxylate (**60m**).

Step 1. To a suspension of commercially available 3-aminopyridine-4-carboxylic acid (4.84 g, 35.1 mmol) in pyridine (60 mL) was added dropwise ethyl chloroglyoxylate (9.58 g, 70.2 mmol) at 0 °C, and the mixture was stirred at room temperature for 15 h. The reaction mixture was concentrated under reduced pressure

and the residue was suspended with water. The resulting precipitate was collected by filtration to give 3-(2-ethoxy-2-oxoacetamido)pyridine-4-carboxylic acid as a pale yellow powder (3.74 g, 45%). ^{1}H NMR (200 MHz, DMSO-d_6) δ 1.33 (3H, t, J = 7.0 Hz), 4.33 (2H, q, J = 7.0 Hz), 7.88 (1H, d, J = 5.0 Hz), 8.53 (1H, d, J = 5.0 Hz), 9.71 (1H, s), 12.10 (1H, s).

Step 2. To a solution of 3-(2-ethoxy-2-oxoacetamido)pyridine-4-carboxylic acid (100 mg, 0.420 mmol) and DMF (0.030 mL) in THF (3 mL) was added dropwise oxalyl chloride (0.040 mL, 0.460 mmol) at 0 °C, and the mixture was stirred at room temperature for 2 h. The reaction mixture was cooled to 0 °C followed by the addition of 2 M ammonia in ethanol (0.693 mL, 1.39 mmol). The mixture was stirred at 0 °C for 1 h and then partitioned between ethyl acetate and water. The organic layer was washed with brine and dried over anhydrous Na_2SO_4. The filtrate was concentrated under reduced pressure to give ethyl [(4-carbamoylpyridin-3-yl)carbamoyl]formate as a white powder (76 mg, 0.320 mmol, 76%). ^{1}H NMR (200 MHz, DMSO-d_6) δ 1.32 (3H, t, J = 7.0 Hz), 4.31 (2H, q, J = 7.4 Hz), 7.78 (1H, d, J = 5.2 Hz), 8.17 (1H, bs), 8.50 (1H, d, J = 5.2 Hz), 8.60 (1H, bs), 9.68 (1H, s).

Step 3. To a suspension of ethyl [(4-carbamoylpyridin-3-yl)carbamoyl]formate (76 mg, 0.320 mmol) in ethanol (4 mL) was added dropwise sodium ethylate (20% ethanol solution, 120 mg, 0.350 mmol) at 0 °C, and the mixture was stirred at room temperature for 1 h. The reaction was quenched with 1 M hydrochloric acid (0.5 mL) and the resulting mixture was neutralized with aqueous $NaHCO_3$ solution. The mixture was extracted with ethyl acetate, washed with brine, dried over anhydrous Na_2SO_4 and concentrated under reduced pressure to give **60m** as a white amorphous form (22 mg, 31%). The crude **60m** was used for the next reaction without purification.

***N*-[(3-Methoxyphenyl)methyl]-4-oxo-3*H*,4*H*-thieno[2,3-*d*]pyrimidine-2-carboxamide (61a).**

A mixture of compound **60a** (240 mg, 4.00 mmol) and 3-methoxybenzylamine (138 mg, 1.00 mmol) in DMF (3 mL) was heated at 90 °C for 5 h. The reaction mixture was concentrated under reduced pressure. The residue was diluted with ethyl acetate, washed with brine, dried over anhydrous Na_2SO_4 and concentrated under reduced pressure. The crude product was purified by preparative HPLC and crystallized from ethanol-diethyl ether to give **61a** as a beige powder (88.5 mg, 42%). mp 179–182 °C. ^{1}H NMR (200 MHz, DMSO-d_6) δ 3.73 (3H, s), 4.42 (2H, d, J = 6.6 Hz), 6.80–6.92 (3H, m), 7.24 (1H, t, J = 8.0 Hz), 7.43 (1H, d, J = 5.6 Hz), 7.66 (1H, d, J = 5.6 Hz), 9.56 (1H, m), 1H hidden. Anal. Calcd for $C_{15}H_{13}N_3O_3S$•0.2CF_3CO_2H·0.6H_2O: C, 53.00; H, 4.16; N, 12.04. Found: C, 53.05; H, 4.13; N, 11.85.

***N*-[(3-Methoxyphenyl)methyl]-5-methyl-4-oxo-3*H*,4*H*-thieno[2,3-*d*]pyrimidine-2-carboxamide (61b).** Compound **61b** was prepared from ethyl 5-methyl-4-oxo-3*H*,4*H*-thieno[2,3-*d*]pyrimidine-2-carboxylate[66] with a similar procedure as described for **61a** (white powder, 86%). mp 148–151 °C. ^{1}H NMR (300 MHz, $CDCl_3$) δ 2.60 (3H, s), 3.81 (3H, s), 4.60 (2H, d, J = 6.0 Hz), 6.83–6.92 (4H, m), 7.25–7.32 (1H, m), 7.91 (1H, br). Anal. Calcd for $C_{16}H_{15}N_3O_3S$•0.60H_2O: C, 56.49; H, 4.80; N, 12.35. Found: C, 56.47; H, 4.62; N 12.44.

***N*-[(3-Methoxyphenyl)methyl]-6-methyl-4-oxo-3*H*,4*H*-thieno[2,3-*d*]pyrimidine-2-carboxamide (61c).** Compound **61c** was prepared from ethyl 6-methyl-4-oxo-3*H*,4*H*-thieno[2,3-*d*]pyrimidine-2-carboxylate (**60c**)[67] with a similar procedure as described for **61a** (white powder, 84%). mp 187 °C. ^{1}H NMR (300 MHz, DMSO-d_6) δ 2.55 (3H, d, J = 1.1 Hz), 3.73 (3H, s), 4.41 (2H, d, J = 6.4 Hz), 6.78–6.94 (3H, m), 7.13–7.30 (2H, m), 9.63 (1H, t, J = 6.3 Hz), 12.4 (1H, s). Anal. Calcd for $C_{16}H_{15}N_3O_3S$: C, 58.34; H, 4.59; N, 12.76. Found: C, 58.14; H, 4.61; N, 12.73.

***N*-[(3-Methoxyphenyl)methyl]-5,6-dimethyl-4-oxo-3*H*,4*H*-thieno[2,3-*d*]pyrimidine-2-carboxamide (61d).** Compound **61d** was prepared from ethyl 5,6-dimethyl-4-oxo-3*H*,4*H*-thieno[2,3-*d*]pyrimidine-2-carboxylate (**60d**)[67] with a similar procedure as described for **61a** (white powder, 84%). mp 194 °C. ^{1}H NMR (300 MHz, DMSO-d_6) δ 2.40 (3H, s), 2.42 (3H, s), 3.73 (3H, s), 4.40 (2H, d, J = 6.4 Hz), 6.78–6.93 (3H, m), 7.18–7.29 (1H, m), 9.60 (1H, t, J = 6.3 Hz), 12.2 (1H, s). Anal. Calcd for $C_{17}H_{17}N_3O_3S$•0.25H_2O: C, 58.69; H, 5.07; N, 12.08. Found: C, 58.77; H, 4.93; N, 11.94.

***N*-[(3-Methoxyphenyl)methyl]-5,6-dimethyl-4-oxo-3*H*,4*H*-furo[2,3-*d*]pyrimidine-2-carboxamide (61e).** Compound **61e** was prepared from compound **60e** with a similar procedure as described for **61a** (white powder, 26%). mp 178–180 °C. ^{1}H NMR (300 MHz, DMSO-d_6) δ 2.18 (3H, s), 2.32 (3H, s), 3.73 (3H, s), 4.41 (2H, d, J = 6.3 Hz), 6.80–6.84 (1H, m), 6.88–6.92 (2H, m), 7.24 (1H, t, J = 8.1 Hz), 9.55 (1H, t, J = 6.0 Hz), 12.3 (1H, bs). Anal. Calcd for $C_{17}H_{17}N_3O_4$: C, 62.38; H, 5.23; N, 12.84. Found: C, 62.16; H, 5.29; N, 12.77.

***N*-[(3-Methoxyphenyl)methyl]-3-methyl-4-oxo-4*H*,5*H*-[1,2]oxazolo[5,4-*d*]pyrimidine-6-carboxamide (61f).** Compound **61f** was prepared from compound **60f**[68] with a similar procedure as described for **61a** (pale pink powder, 71%). mp 236–237 °C. ^{1}H NMR (300 MHz, DMSO-d_6) δ 2.49 (3H, m), 3.73 (3H, s), 4.42 (2H, d, J = 6.3 Hz), 6.80–6.84 (1H, m), 6.89–6.92 (2H, m), 7.24 (1H, t, J = 8.1 Hz), 9.80 (1H, t, J = 6.3 Hz), 13.0 (1H, bs). Anal. Calcd for $C_{15}H_{14}N_4O_4$: C, 57.32; H, 4.49; N, 17.83. Found: C, 57.35; H, 4.47; N, 17.81.

***N*-[(3-Methoxyphenyl)methyl]-1,3-dimethyl-4-oxo-1*H*,4*H*,5*H*-pyrazolo[3,4-*d*]pyrimidine-6-carboxamide (61g).** Compound **61g** was prepared from compound **60g** with a similar procedure as described for **61a** (white powder, 90%). mp 186–188 °C. ^{1}H NMR (300 MHz, DMSO-d_6) δ 2.43 (3H, s), 3.73 (3H, s), 3.90 (3H, s), 4.45 (2H, d, J = 6.6 Hz), 6.80–6.84 (1H, m), 6.88–6.92 (2H, m), 7.24 (1H, t, J = 8.1 Hz), 9.56 (1H, t, J = 6.6 Hz), 11.9 (1H, bs). Anal. Calcd for $C_{16}H_{17}N_5O_3$: C, 58.71; H, 5.23; N, 21.39. Found: C, 58.41; H, 5.20; N, 21.23.

***N*-[(3-Methoxyphenyl)methyl]-5-methyl-4-oxo-3*H*,4*H*-thieno[3,4-*d*]pyrimidine-2-carboxamide (61h).** Compound **61h** was prepared from compound **60h** with a similar procedure as described for **61a** (beige powder, 76%). mp 168–170 °C. ^{1}H NMR (300 MHz, DMSO-d_6) δ 2.88 (3H, s), 3.73 (3H, s), 4.41 (2H, d, J = 6.0 Hz), 6.81–6.91 (3H, m), 7.24 (1H, t, J = 8.1 Hz), 7.64 (1H, s), 9.43 (1H, t, J = 6.0 Hz), 11.3 (1H, bs). Anal. Calcd for $C_{16}H_{15}N_3O_3S$: C, 58.34; H, 4.59; N, 12.76. Found: C, 58.24; H, 4.69; N, 12.49.

***N*-[(3-Methoxyphenyl)methyl]-6-methyl-4-oxo-3*H*,4*H*,7*H*-pyrrolo[2,3-*d*]pyrimidine-2-carboxamide (61i).** Compound **61i** was prepared from compound **60i** with a similar procedure as described for **61a** (white powder, 86%). mp 255–256 °C. ^{1}H NMR (300 MHz, DMSO-d_6) δ 2.32 (3H, s), 3.73 (3H, s), 4.44 (2H, d, J = 6.3 Hz), 6.25 (1H, s), 6.81–6.91 (3H, m), 7.24 (1H, t, J = 8.1 Hz), 9.14 (1H, t, J = 6.6 Hz), 11.5 (1H, bs), 11.8 (1H, bs). Anal. Calcd for $C_{16}H_{16}N_4O_3$: C, 61.53; H, 5.16; N, 17.94. Found: C, 61.44; H, 5.16; N, 17.89.

***N*-[(3-Methoxyphenyl)methyl]-6,7-dimethyl-4-oxo-3*H*,4*H*,7*H*-pyrrolo[2,3-*d*]pyrimidine-2-carboxamide (61j).** Compound **61j** was prepared from compound **60j** with a similar procedure as described for **61a** (white powder, 31%). mp 205–207 °C. ^{1}H NMR (300 MHz, DMSO-d_6) δ 2.35 (3H, s), 3.72 (3H, s), 3.73 (3H, s), 4.46 (2H, d, J = 6.3 Hz), 6.33 (1H, s), 6.80–6.85 (1H, m), 6.88–6.92 (2H, m), 7.25 (1H, t, J = 8.1 Hz), 9.47 (1H, t, J = 6.3 Hz), 11.6 (1H, bs). Anal. Calcd for $C_{17}H_{18}N_4O_3 \cdot H_2O$: C, 59.45; H, 5.50; N, 16.30. Found: C, 59.29; H, 5.85; N, 16.27.

***N*-[(3-Methoxyphenyl)methyl]-4-oxo-3*H*,4*H*-thieno[3,2-*d*]pyrimidine-2-carboxamide (61k).** Compound **61k** was prepared from compound **60k** with a similar procedure as described for **61a** (pale yellow powder, 36%). mp 201–202 °C. ^{1}H NMR (200 MHz, DMSO-d_6) δ 3.73 (3H, s), 4.44 (2H, d, J = 6.6 Hz), 6.80–6.93 (3H, m), 7.24 (1H, t, J = 8.0 Hz), 7.48 (1H, d, J = 5.4 Hz), 8.27 (1H, d, J = 5.4 Hz), 9.57 (1H, t, J = 6.6 Hz), 1H hidden. Anal. Calcd for $C_{15}H_{13}N_3O_3S$: C, 57.13; H, 4.16; N, 13.33. Found: C, 56.95; H, 4.14; N, 13.08.

***N*-[(3-Methoxyphenyl)methyl]-4-oxo-3*H*,4*H*-pyrido[2,3-*d*]pyrimidine-2-carboxamide (61l).** Compound **61l** was prepared from compound **60l** with a similar procedure as described for **61a** (white powder, 76%). mp 181–183 °C. ^{1}H NMR (200 MHz, DMSO-d_6) δ 3.74 (3H, s), 4.46 (2H, d, J = 6.2 Hz), 6.80–6.95 (3H, m), 7.25 (1H, t, J = 8.0 Hz), 7.62 (1H, dd, J = 8.0, 4.6 Hz), 8.55 (1H, dd, J = 8.2, 2.2 Hz), 9.02 (1H, dd, J = 4.6, 2.0 Hz), 9.71 (1H, m), 1H hidden. Anal. Calcd for $C_{16}H_{14}N_4O_3 \bullet 0.1$AcOEt: C, 61.72; H, 4.67; N, 17.56. Found: C, 61.64; H, 4.56; N, 17.52.

***N*-[(3-Methoxyphenyl)methyl]-4-oxo-3*H*,4*H*-pyrido[3,4-*d*]pyrimidine-2-carboxamide (61m).** Compound **61m** was prepared from compound **60m** with a similar procedure as described for **61a** (white powder, 65%). mp 231–233 °C. ^{1}H NMR (300 MHz, DMSO-d_6) δ 3.73 (3H, s), 4.45 (2H, d, J = 6.3 Hz), 6.80–6.84 (1H, m), 6.90–6.93 (2H, m), 7.24 (1H, t, J = 8.1 Hz), 7.99 (1H, d, J = 5.1 Hz), 8.72 (1H, d, J = 5.4 Hz), 9.12 (1H, s), 9.63 (1H, t, J = 6.3 Hz), 12.7 (1H, bs). Anal. Calcd for $C_{16}H_{14}N_4O_3 \bullet 0.2H_2O$: C, 61.22; H, 4.62; N, 17.85. Found: C, 61.04; H, 4.42; N, 17.88.

Ethyl 5-(bromomethyl)-4-oxo-3*H*,4*H*-thieno[2,3-*d*]pyrimidine-2-carboxylate (62). A mixture of commercially available **60b** (50.0 g, 210 mmol), NBS (44.8 g, 252 mmol), and AIBN (3.45 g, 21.0 mmol) in chlorobenzene (1000 mL) was stirred at 80 °C for 1.5 h. The reaction mixture was concentrated in vacuo. The resulting semi-solid was suspended in diethyl ether (250 mL), filtered, and washed with diethyl ether (2×75 mL), H_2O (5×250 mL), diethyl ether (2×200 mL) and dried to provide **62** as a white powder (35.7 g, 54%). mp 176 °C. ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.49 (3H, t, J = 7.0 Hz), 4.57 (2H, q, J = 6.9 Hz), 4.92 (2H,

d, J = 0.8 Hz), 7.52 (1H, s), 10.1 (1H, s). Anal. Calcd for $C_{10}H_9BrN_2O_3S$: C, 37.87; H, 2.86; N, 8.83. Found: C, 37.78; H, 2.90; N, 8.99.

Ethyl 5-[(benzyloxy)methyl]-4-oxo-3*H*,4*H*-thieno[2,3-*d*]pyrimidine-2-carboxylate (**63a**). To a solution of benzyl alcohol (0.157 mL) in THF (10 mL) was gradually added 60% sodium hydride (116 mg, 3.03 mmol), and the mixture was stirred at room temperature for 10 min. Compound **62** (400 mg, 1.26 mmol) was added at once, and the mixture was stirred at room temperature for 1 h. The solvent was evaporated under reduced pressure and the residue was extracted with ethyl acetate. The mixture was washed with 1 M hydrochloric acid and a 1:1 mixture of 1 M hydrochloric acid-saturated brine, and after drying over anhydrous Na_2SO_4, the solvent was evaporated. The residue was suspended in diethyl ether, filtrated, dried and suspended in THF (5 mL). Oxalyl chloride (0.550 mL, 6.31 mmol) and DMF (one drop) were added, and the mixture was stirred at room temperature for 2.5 h. The solvent was evaporated under reduced pressure. The obtained residue was dissolved in EtOH–THF (1:1) solution, and the mixture was stirred at room temperature for 12 h. The solvent was evaporated under reduced pressure and the residue was extracted with ethyl acetate, washed with water and saturated brine, and dried over anhydrous Na_2SO_4. The solvent was evaporated under reduced pressure. The obtained residue was purified by silica gel column chromatography (20–40% ethyl acetate/hexane) to give **63a** (68.7 mg, 16%) as a colorless powder. mp 155–156 °C. ^{1}H NMR (300 MHz, DMSO-d_6) δ 1.34 (3H, t, J = 7.1 Hz), 4.36 (2H, q, J = 7.0 Hz), 4.66 (2H, s), 4.86 (2H, d, J = 0.9 Hz), 7.13–7.51 (5H, m), 7.66 (1H, s), 12.9 (1H, s). Anal. Calcd for $C_{17}H_{16}N_2O_4S$•0.25H_2O: C, 58.52; H, 4.77; N, 8.03. Found: C, 58.30; H, 4.53; N, 8.30.

Ethyl 5-({[4-(ethoxycarbonyl)phenyl]methoxy}methyl)-4-oxo-3*H*,4*H*-thieno[2,3-*d*]pyrimidine-2-carboxylate (**63b**). Compound **63b** was prepared from compound **62** and ethyl 4-(hydroxymethyl)benzoate with a similar procedure as described for **63a** (pale yellow powder, 75%). mp 181 °C. ^{1}H NMR (300 MHz, DMSO-d_6) δ 1.33 (6H, q, J = 7.0 Hz), 4.27–4.41 (4H, m), 4.75 (2H, s), 4.89 (2H, d, J = 1.1 Hz), 7.54 (2H, d, J = 8.5 Hz), 7.70 (1H, s), 7.91–7.99 (2H, m), 12.9 (1H, s). Anal. Calcd for $C_{20}H_{20}N_2O_6S$•0.05H_2O: C, 57.56; H, 4.85; N, 6.71. Found: C, 57.55; H, 4.82; N, 6.70.

Ethyl 5-{[(4-fluorophenyl)methoxy]methyl}-4-oxo-3*H*,4*H*-thieno[2,3-*d*]pyrimidine-2-carboxylate (63c). Compound **63c** was prepared from compound **62** and ethyl (4-fluorophenyl)methanol with a similar procedure as described for **63a** (pale yellow powder, 8%). mp 195–196 °C. ^{1}H NMR (300 MHz, DMSO-d_6) δ 1.34 (3H, t, J = 7.1 Hz), 4.37 (2H, q, J = 7.0 Hz), 4.64 (2H, s), 4.85 (2H, d, J = 1.3 Hz), 7.18 (2H, t, J = 9.0 Hz), 7.44 (2H, dd, J = 8.7, 5.7 Hz), 7.66 (1H, t, J = 1.2 Hz), 12.9 (1H, s). Anal. Calcd for $C_{17}H_{15}FN_2O_4S$•0.25H_2O: C, 55.65; H, 4.26; N, 7.64. Found: C, 55.51; H, 4.13; N, 7.84.

Ethyl 5-{[(4-cyanophenyl)methoxy]methyl}-4-oxo-3*H*,4*H*-thieno[2,3-*d*]pyrimidine-2-carboxylate (**63d**). Compound **63d** was prepared from compound **62** and 4-(hydroxymethyl)benzonitrile with a similar procedure as described for **63a** (pale yellow powder, 52%). mp 236–237 °C. ^{1}H NMR (300 MHz, DMSO-d_6) δ 1.34 (3H, t, J = 7.1 Hz), 4.37 (2H, q, J = 7.1 Hz), 4.76 (2H, s), 4.90 (2H, d, J = 1.1 Hz), 7.59 (2H, d,

J = 8.5 Hz), 7.71 (1H, t, *J* = 1.1 Hz), 7.77–7.89 (2H, m), 12.9 (1H, s). Anal. Calcd for $C_{18}H_{15}N_3O_4S$•$0.25H_2O$: C, 57.82; H, 4.18; N, 11.24. Found: C, 57.68; H, 4.09; N, 11.44.

Ethyl 5-{[benzyl(methyl)amino]methyl}-4-oxo-3*H*,4*H*-thieno[2,3-*d*]pyrimidine-2-carboxylate (63e). To a mixture of compound **62** (600 mg, 1.89 mmol) obtained and THF (12 mL) were added *N*-methyl-1-phenylmethanamine (0.269 mL, 2.08 mmol) and triethylamine (0.527 mL, 3.78 mmol) at room temperature and the mixture was stirred for 1 h. The reaction mixture was concentrated under reduced pressure and ethyl acetate was added to the residue. The mixture was washed with saturated brine, dried over anhydrous Na_2SO_4 and concentrated under reduced pressure. The concentrated residue was purified by silica gel column chromatography (1–8% methanol/ethyl acetate). The obtained crude crystals were recrystallized from ethyl acetate–hexane to give **63e** as a white powder (351 mg, 52%). mp 129 °C. ^{1}H NMR (300 MHz, DMSO-d_6) δ 1.33 (3H, t, *J* = 7.2 Hz), 2.32 (3H, s), 4.15 (4H, s), 4.32 (2H, q, *J* = 7.0 Hz), 7.28–7.51 (5H, m), 7.64 (1H, s). Anal. Calcd for $C_{18}H_{19}N_3O_3S$•$0.05H_2O$: C, 60.33; H, 5.37; N, 11.73. Found: C, 60.37; H, 5.28; N, 11.74.

Ethyl 5-[(benzylsulfanyl)methyl]-4-oxo-3*H*,4*H*-thieno[2,3-*d*]pyrimidine-2-carboxylate (63f). To a mixture of compound **62** (600 mg, 1.89 mmol) and DMA (12 mL) were added phenylmethanethiol (0.244 mL, 2.08 mmol) and triethylamine (0.527 mL, 3.78 mmol) at room temperature and the mixture was stirred for 1 h. The reaction mixture was concentrated under reduced pressure, and ethyl acetate was added to the obtained residue. The mixture was washed with 1 M hydrochloric acid and saturated brine, dried over anhydrous Na_2SO_4 and concentrated under reduced pressure. The concentrated residue was purified by silica gel column chromatography (5–50% ethyl acetate/hexane). The obtained crude crystals were recrystallized from ethyl acetate–hexane to give **63f** as a white powder (207 mg, 30%). mp 171 °C. ^{1}H NMR (300 MHz, DMSO-d_6) δ 1.35 (3H, t, *J* = 7.2 Hz), 3.73 (2H, s), 3.99 (2H, s), 4.37 (2H, q, *J* = 7.1 Hz), 7.15–7.34 (5H, m), 7.57 (1H, s), 12.8 (1H, s). Anal. Calcd for $C_{17}H_{16}N_2O_3S_2$•$0.20H_2O$: C, 56.09; H, 4.54; N, 7.69. Found: C, 55.93; H, 4.41; N, 7.96.

5-[(Benzyloxy)methyl]-*N*-[(3-methoxyphenyl)methyl]-4-oxo-3*H*,4*H*-thieno[2,3-*d*]pyrimidine-2-carboxamide (64a). Compound **64a** was prepared from compound **63a** with a similar procedure as described for **61a** (white powder, 60%). mp 145 °C. ^{1}H NMR (300 MHz, DMSO-d_6) δ 3.73 (3H, s), 4.41 (2H, d, *J* = 6.4 Hz), 4.66 (2H, s), 4.85 (2H, d, *J* = 0.8 Hz), 6.82 (1H, dd, *J* = 8.3, 2.3 Hz), 6.87–6.95 (2H, m), 7.18–7.45 (6H, m), 7.60 (1H, s), 9.66 (1H, t, *J* = 6.5 Hz), 12.4 (1H, s). Anal. Calcd for $C_{23}H_{21}N_3O_4S$•$0.25H_2O$: C, 62.78; H, 4.93; N, 9.55. Found: C, 62.54; H, 4.90; N, 9.88.

Ethyl 4-{[(2-{[(3-Methoxyphenyl)methyl]carbamoyl}-4-oxo-3*H*,4*H*-thieno[2,3-*d*]pyrimidin-5-yl)methoxy]methyl}benzoate (**64b**). Compound **64b** was prepared from compound **63b** with a similar procedure as described for **61a** (white powder, 90%). mp 173 °C. ^{1}H NMR (300 MHz, DMSO-d_6) δ 1.32 (3H, t, *J* = 7.1 Hz), 3.73 (3H, s), 4.31 (2H, q, *J* = 7.2 Hz), 4.42 (2H, d, *J* = 6.2 Hz), 4.75 (2H, s), 4.89 (2H, d, *J* = 0.9 Hz), 6.79–6.85 (1H, m), 6.87–6.93 (2H, m), 7.24 (1H, t, *J* = 8.1 Hz), 7.54 (2H, d, *J* = 8.5 Hz),

7.63 (1H, s), 7.88–8.03 (2H, m), 9.65 (1H, t, *J* = 6.5 Hz), 12.4 (1H, s). Anal. Calcd for $C_{26}H_{25}N_3O_6S$•0.10H_2O: C, 61.31; H, 4.99; N, 8.25. Found: C, 61.17; H, 4.94; N, 8.42.

4-{[(2-{[(3-Methoxyphenyl)methyl]carbamoyl}-4-oxo-3*H*,4*H*-thieno[2,3-*d*]pyrimidin-5-yl)methoxy]methyl}benzoic acid (**64c**). A mixture of **64b** (126 g, 249 mmol) and 8 M NaOH (77.7 mL, 622 mmol) in a mixture of MeOH (600 mL), THF (600 mL) and water (600 mL) was stirred at 90 °C for 1 h. The mixture was concentrated in vacuo. After acidification with 1 M hydrochloric acid (933 mL, 933 mmol), the crude materials were collected by filtration, washed with water (7×500 mL) and MeOH (6×500 mL) to give a white powder. The crude product was suspended in MeOH (2500 mL) at refluxed temperature for 1.5 h, cooled to room temperature, and collected by filtration, washed with MeOH and air dried to give **64c** as a white powder (122 g, quant.). mp 229 °C. ^{1}H NMR (300 MHz, DMSO-d_6) δ 3.73 (3H, s), 4.41 (2H, d, *J* = 6.0 Hz), 4.74 (2H, s), 4.89 (2H, s), 6.82 (1H, dd, *J* = 8.5, 1.9 Hz), 6.86–6.97 (2H, m), 7.24 (1H, t, *J* = 8.1 Hz), 7.51 (2H, d, *J* = 8.1 Hz), 7.63 (1H, s), 7.93 (2H, d, *J* = 8.3 Hz), 9.66 (1H, t, *J* = 6.4 Hz), 12.5 (1H, s), 12.9 (1H, s). Anal. Calcd for $C_{24}H_{21}N_3O_6S$: C, 60.12; H, 4.41; N, 8.76. Found: C, 60.30; H, 4.53; N, 8.61.

5-{[(4-Fluorophenyl)methoxy]methyl}-*N*-[(3-methoxyphenyl)methyl]-4-oxo-3*H*,4*H*-thieno[2,3-*d*]pyrimidine-2-carboxamide (**64d**). Compound **64d** was prepared from compound **63c** with a similar procedure as described for **61a** (white powder, 81%). mp 166 °C. ^{1}H NMR (300 MHz, DMSO-d_6) δ 3.73 (3H, s), 4.41 (2H, d, *J* = 6.0 Hz), 4.64 (2H, s), 4.85 (2H, d, *J* = 0.8 Hz), 6.74–6.94 (3H, m), 7.11–7.30 (3H, m), 7.38–7.49 (2H, m), 7.59 (1H, s), 9.64 (1H, s), 12.4 (1H, s). Anal. Calcd for $C_{23}H_{20}FN_3O_4S$•0.15H_2O: C, 60.56; H, 4.49; N, 9.21. Found: C, 60.48; H, 4.44; N, 9.27.

5-{[(4-Cyanophenyl)methoxy]methyl}-*N*-[(3-methoxyphenyl)methyl]-4-oxo-3*H*,4*H*-thieno[2,3-*d*]pyrimidine-2-carboxamide (64e). Compound **64e** was prepared from compound **63d** with a similar procedure as described for **61a** (white powder, 79%). mp 205 °C. ^{1}H NMR (300 MHz, DMSO-d_6) δ 3.73 (3H, s), 4.42 (2H, d, *J* = 6.4 Hz), 4.76 (2H, s), 4.89 (2H, d, *J* = 1.1 Hz), 6.79–6.93 (3H, m), 7.24 (1H, t, *J* = 8.1 Hz), 7.55–7.66 (3H, m), 7.77–7.90 (2H, m), 9.65 (1H, t, *J* = 6.4 Hz), 12.4 (1H, s). Anal. Calcd for $C_{24}H_{20}N_4O_4S$•0.25H_2O: C, 61.99; H, 4.44; N, 12.05. Found: C, 62.02; H, 4.36; N, 12.13.

5-{[Benzyl(methyl)amino]methyl}-*N*-[(3-methoxyphenyl)methyl]-4-oxo-3*H*,4*H*-thieno[2,3-*d*]pyrimidine-2-carboxamide (**64f**). Compound **64f** was prepared from compound **63e** with a similar procedure as described for **61a** (white powder, 58%). mp 127 °C. ^{1}H NMR (300 MHz, DMSO-d_6) δ 2.18 (3H, s), 3.60 (2H, s), 3.73 (3H, s), 3.94 (2H, s), 4.42 (2H, d, *J* = 6.4 Hz), 6.78–6.86 (1H, m), 6.87–6.93 (2H, m), 7.17–7.44 (6H, m), 7.59 (1H, s), 9.63 (1H, s), 12.3 (1H, s). Anal. Calcd for $C_{24}H_{24}N_4O_3S$: C, 64.27; H, 5.39; N, 12.49. Found: C, 64.02; H, 5.21; N, 12.39.

5-[(Benzylsulfanyl)methyl]-*N*-[(3-methoxyphenyl)methyl]-4-oxo-3*H*,4*H*-thieno[2,3-*d*]pyrimidine-2-carboxamide (64g). Compound **64g** was prepared from compound **63f** with a similar procedure as described for **61a** (white powder, 67%). mp 126 °C. ^{1}H NMR (300 MHz, DMSO-d_6) δ 3.73 (2H, s), 3.74 (3H, s), 3.99 (2H, s), 4.42 (2H, d, *J* = 6.4 Hz), 6.79–6.93 (3H, m), 7.19–7.32 (6H, m), 7.51 (1H, s), 9.64 (1H, t, *J* = 6.1

Hz), 12.4 (1H, s). Anal. Calcd for $C_{23}H_{21}N_3O_3S_2$•0.45H_2O: C, 60.10; H, 4.80; N, 9.14. Found: C, 59.95; H, 4.57; N, 9.38.

Ethyl 5-(azidomethyl)-4-oxo-3*H*,4*H*-thieno[2,3-*d*]pyrimidine-2-carboxylate (65a). To a mixture of ethyl compound **62** (2.00 g, 6.31 mmol) and DMF (20 mL) was added sodium azide (861 mg, 13.2 mmol), and the mixture was stirred at room temperature for 12 h. Hydrochloric acid (1 M) was added to the reaction mixture, and the mixture was extracted with ethyl acetate. The organic layer was washed 5 times with water and once with saturated brine, dried over anhydrous Na_2SO_4 and concentrated under reduced pressure. The concentrated residue was crystallized from ethyl acetate to give **65a** as a white powder (1.33 g, 76%). mp 192 °C. ^{1}H NMR (300 MHz, DMSO-d_6) δ 1.35 (3H, t, J = 7.2 Hz), 4.37 (2H, q, J = 7.2 Hz), 4.76 (2H, s), 7.81 (1H, s), 13.0 (1H, s). Anal. Calcd for $C_{10}H_9N_5O_3S$•0.05H_2O: C, 42.87; H, 3.27; N, 25.00. Found: C, 43.30; H, 3.26; N, 24.57.

Ethyl 5-(aminomethyl)-4-oxo-3*H*,4*H*-thieno[2,3-*d*]pyrimidine-2-carboxylate hydrochloride (**65b**). A mixture of compound **65a** (100 mg, 0.358 mmol), 2 M hydrochloric acid/EtOH (0.268 mL, 1.07 mmol), and 10% Pd/C (50% wet) (25.0 mg) in EtOH–THF (1.0 mL–1.0 mL) was vigorously stirred under H_2 atmosphere for 1 h at room temperature. After removal of the catalyst by filtration (washed with MeOH and H_2O) (Celite), the filtrate was concentrated in vacuo. The residue was crystallized from ethyl acetate to give **65b** (104 mg, 94%) as a white powder. mp 262–263 °C. ^{1}H NMR (300 MHz, DMSO-d_6) δ 1.35 (3H, t, J = 7.1 Hz), 4.05–4.64 (4H, m), 7.87 (1H, s), 8.34 (3H, s), 13.2 (1H, s). Anal. Calcd for $C_{10}H_{12}ClN_3O_3S$•0.30H_2O: C, 40.69; H, 4.30; N, 14.24. Found: C, 40.51; H, 4.12; N, 14.35.

Ethyl 5-[(benzoylamino)methyl]-4-oxo-3,4-dihydrothieno[2,3-*d*]pyrimidine-2-carboxylate (**66a**). To a mixture of compound **65b** (250 mg, 0.863 mmol) in THF (3.0 mL) were added benzoyl chloride (0.110 mL, 0.949 mmol) and triethylamine (0.253 mL, 1.81 mmol). After being stirred for 1 h at room temperature, the mixture was concentrated in vacuo. The residue was taken up in ethyl acetate, washed with saturated sodium hydrogen carbonate solution (twice), brine, 1 M hydrochloric acid, brine, dried over Na_2SO_4 and concentrated in vacuo. The residue was crystallized from ethyl acetate to give **66a** (223 mg, 72%) as a white powder. mp 204 °C. ^{1}H NMR (300 MHz, DMSO-d_6) δ 1.35 (3H, t, J = 7.1 Hz), 4.38 (2H, q, J = 7.0 Hz), 4.80 (2H, d, J = 4.9 Hz), 7.43–7.60 (4H, m), 7.86–7.94 (2H, m), 9.03 (1H, t, J = 5.8 Hz), 12.9 (1H, s). Anal. Calcd for $C_{17}H_{15}N_3O_4S$: C, 57.13; H, 4.23; N, 11.76. Found: C, 57.28; H, 4.27; N, 11.56.

Ethyl 5-({[4-(methoxycarbonyl)benzoyl]amino}methyl)-4-oxo-3,4-dihydrothieno[2,3-*d*]pyrimidine-2-carboxylate (**66b**). To a mixture of monomethyl terephthalate (373 mg, 2.07 mmol) in THF (5.0 mL) were added oxalyl chloride (0.450 mL, 5.18 mmol) and *N*,*N*-dimethylformamide (1 drop). After being stirred for 1 h at room temperature, the mixture was concentrated in vacuo. The residue was resusupended in THF (5.0 mL), and to the mixture were added **65b** (500 mg, 1.73 mmol) and triethylamine (0.960 mL, 6.90 mmol). After being stirred for 1 h at room temperature, the mixture was concentrated in vacuo. The residue was taken up in ethyl acetate, washed with saturated sodium hydrogen carbonate solution (twice), brine, 1 M

hydrochloric acid, brine, dried over Na_2SO_4 and concentrated in vacuo. The residue was crystallized from ethyl acetate to give **66b** (588 mg, 82%) as a white powder. mp 236 °C. ^{1}H NMR (300 MHz, DMSO-d_6) δ 1.35 (3H, t, J = 7.2 Hz), 3.89 (3H, s), 4.37 (2H, q, J = 7.2 Hz), 4.82 (2H, d, J = 5.5 Hz), 7.52 (1H, s), 7.77–8.22 (4H, m), 9.23 (1H, s), 12.9 (1H, s). Anal. Calcd for $C_{19}H_{17}N_3O_6S$: C, 54.93; H, 4.12; N, 10.12. Found: C, 54.65; H, 4.24; N, 9.86.

5-[(Benzoylamino)methyl]-*N*-(3-methoxybenzyl)-4-oxo-3,4-dihydrothieno[2,3-*d*]pyrimidine-2-carboxamide (**67a**). Compound **67a** was prepared from compound **66a** with a similar procedure as described for **61a** (white powder, 82%). mp 195–196 °C. ^{1}H NMR (300 MHz, DMSO-d_6) δ 3.73 (3H, s), 4.42 (2H, d, J = 6.2 Hz), 4.80 (2H, d, J = 5.1 Hz), 6.74–6.98 (3H, m), 7.24 (1H, t, J = 8.1 Hz), 7.38–7.62 (4H, m), 7.82–7.98 (2H, m), 9.02 (1H, s), 9.64 (1H, t, J = 6.3 Hz), 12.5 (1H, s). Anal. Calcd for $C_{23}H_{20}N_4O_4S$•0.15H_2O: C, 61.23; H, 4.53; N, 12.42. Found: C, 61.28; H, 4.47; N, 12.33.

Methyl 4-{[(2-{[(3-methoxyphenyl)methyl]carbamoyl}-4-oxo-3*H*,4*H*-thieno[2,3-*d*]pyrimidin-5-yl)methyl]carbamoyl}benzoate (67b). Compound **67b** was prepared from compound **66b** with a similar procedure as described for **61a** (white powder, 89%). mp 237 °C. ^{1}H NMR (300 MHz, DMSO-d_6) δ 3.73 (3H, s), 3.89 (3H, s), 4.42 (2H, d, J = 6.4 Hz), 4.80 (2H, d, J = 5.7 Hz), 6.80–6.93 (3H, m), 7.24 (1H, t, J = 8.1 Hz), 7.41–7.46 (1H, m), 8.00–8.08 (4H, m), 9.26 (1H, s), 9.59 (1H, s), 12.5 (1H, s). Anal. Calcd for $C_{25}H_{22}N_4O_6S$: C, 59.28; H, 4.38; N, 11.06. Found: C, 59.20; H, 4.41; N, 11.05.

4-{[(2-{[(3-Methoxyphenyl)methyl]carbamoyl}-4-oxo-3*H*,4*H*-thieno[2,3-*d*]pyrimidin-5-yl)methyl]carbamoyl}benzoic acid (67c). A mixture of compound **67b** (275 mg, 0.543 mmol) and 12 M NaOH (0.113 mL, 1.36 mmol) in THF–MeOH–H_2O (1:1:1) (6.0 mL) was stirred at 90 °C for 1 h. After evaporation in vacuo, the residue was dissolved in ethyl acetate–THF (1:1) (700 mL) and the organic layer was washed with 1 M hydrochloric acid, brine, dried over Na_2SO_4, and concentrated in vacuo. The residue was crystallized from ethyl acetate to give **67c** as a white powder (263 mg, 98%). mp 285–286 °C. ^{1}H NMR (300 MHz, DMSO-d_6) δ 3.74 (3H, s), 4.42 (2H, d, J = 6.2 Hz), 4.81 (2H, d, J = 5.3 Hz), 6.80–6.93 (3H, m), 7.21–7.27 (1H, m), 7.46 (1H, s), 7.98–8.06 (4H, m), 9.15 (1H, t, J = 5.8 Hz), 9.66 (1H, t, J = 6.3 Hz), 12.5 (1H, s), 13.1 (1H, s). Anal. Calcd for $C_{24}H_{20}N_4O_6S$•0.35H_2O: C, 57.79; H, 4.18; N, 11.23. Found: C, 57.86; H, 4.12; N, 11.14.

Ethyl 5-(cyanomethyl)-4-oxo-3*H*,4*H*-thieno[2,3-*d*]pyrimidine-2-carboxylate (68). Compound **62** (4.20 g, 13.2 mmol) in DMF (80 mL) was added dropwise to an ice-cooled solution of NaCN (162 mg, 33.1 mmol) in H_2O (20 mL)–DMF (20 mL). After being stirred for 2 h, to the mixture was added 0.1 M hydrochloric acid (pH 6). The mixture was extracted with ethyl acetate, washed with 0.1 M hydrochloric acid, water, dried over Na_2SO_4, and concentrated in vacuo. The residue was crystallized from ethyl acetate to give **68** (2.41 g, 69%) as a pale yellow powder. mp 183 °C. ^{1}H NMR (300 MHz, DMSO-d_6) δ 1.35 (3H, t, J = 7.2 Hz), 4.28 (2H, d, J = 1.1 Hz), 4.37 (2H, q, J = 7.2 Hz), 7.75 (1H, t, J = 1.0 Hz), 13.0 (1H, s). Anal. Calcd for $C_{11}H_9N_3O_3S$•0.10H_2O: C, 49.84; H, 3.50; N, 15.85. Found: C, 49.55; H, 3.41; N, 16.19.

5-(Cyanomethyl)-*N*-[(3-methoxyphenyl)methyl]-4-oxo-3*H*,4*H*-thieno[2,3-*d*]pyrimidine-2-carboxamide (69a). Compound **69a** was prepared from compound **68** with a similar procedure as described for **61a** (brown powder, 76%). mp 217 °C. ^{1}H NMR (300 MHz, DMSO-d_6) δ 3.73 (3H, s), 4.27 (2H, d, J = 0.9 Hz), 4.42 (2H, d, J = 6.4 Hz), 6.78–6.94 (3H, m), 7.24 (1H, t, J = 8.1 Hz), 7.69 (1H, t, J = 0.9 Hz), 9.66 (1H, t, J = 6.4 Hz), 12.6 (1H, s). Anal. Calcd for $C_{17}H_{14}N_4O_3S$•0.20H_2O: C, 57.04; H, 4.05; N, 15.65. Found: C, 57.23; H, 4.08; N, 15.44.

2-(2-{[(3-Methoxyphenyl)methyl]carbamoyl}-4-oxo-3*H*,4*H*-thieno[2,3-*d*]pyrimidin-5-yl)acetic acid (69b). A mixture of compound **68** (200 mg, 0.564 mmol), 2 M aqueous sodium hydroxide solution (4 mL, 8 mmol) and ethanol (2 mL) was stirred at 100 °C for 1 h. The reaction mixture was concentrated under reduced pressure, and ethyl acetate was added to the obtained residue. The organic layer was washed with 1 M hydrochloric acid and saturated brine, dried over anhydrous Na_2SO_4 and concentrated under reduced pressure. The concentrated residue was crystallized from ethyl acetate to give **69b** as a brown powder (131 mg, 62%). mp 228–229 °C. ^{1}H NMR (300 MHz, DMSO-d_6) δ 3.73 (3H, s), 3.91 (2H, s), 4.42 (2H, d, J = 6.2 Hz), 6.77–6.93 (3H, m), 7.24 (1H, t, J = 8.1 Hz), 7.49 (1H, s), 9.64 (1H, t, J = 6.3 Hz), 12.3 (1H, s), 12.4 (1H, s). Anal. Calcd for $C_{17}H_{15}N_3O_5S$•0.35H_2O: C, 53.78; H, 4.17; N, 11.07. Found: C, 53.62; H, 4.15; N, 11.32.

***N*-[(3-Methoxyphenyl)methyl]-4-oxo-5-[(phenylcarbamoyl)methyl]-3*H*,4*H*-thieno[2,3-*d*]pyrimidine-2-carboxamide (70a).** To a mixture of compound **69b** (200 mg, 0.536 mmol) in THF (3.0 mL) was added oxalyl chloride (0.140 mL, 1.61 mmol). After being stirred at room temperature for 15 h, the mixture was concentrated in vacuo, and the resulting residue was dissolved in THF (3.0 mL). To this solution was added aniline (0.146 mL, 1.61 mmol) and pyridine (0.217 mL, 2.68 mmol). After being stirred for 1 h at room temperature, the mixture was concentrated in vacuo, and the residue was taken up in ethyl acetate. The organic layer was washed with saturated $NaHCO_3$ (×3), 1 M hydrochloric acid (×2), and brine and dried over Na_2SO_4. The solvent was removed, and the residue was crystallized from ethyl acetate to give **70a** as a pale yellow powder (154 mg, 64%). mp 194–195 °C. ^{1}H NMR (300 MHz, DMSO-d_6) δ 3.73 (3H, s), 4.04 (2H, s), 4.42 (2H, d, J = 6.2 Hz), 6.78–6.93 (3H, m), 7.02 (1H, t, J = 7.3 Hz), 7.20–7.33 (3H, m), 7.52 (1H, s), 7.58 (2H, d, J = 7.5 Hz), 9.66 (1H, t, J = 6.5 Hz), 10.1 (1H, s), 12.4 (1H, s). Anal. Calcd for $C_{23}H_{20}N_4O_4S$: C, 61.59; H, 4.49; N, 12.49. Found: C, 61.29; H, 4.47; N, 12.47.

Ethyl 4-[2-(2-{[(3-methoxyphenyl)methyl]carbamoyl}-4-oxo-3*H*,4*H*-thieno[2,3-*d*]pyrimidin-5-yl)acetamido]benzoate (70b). To a mixture of compound **69b** (350 mg, 0.937 mmol) in THF (3.0 mL) was added oxalyl chloride (0.245 mL, 2.81 mmol). After being stirred at room temperature for 15 h, the mixture was concentrated in vacuo, and the resulting residue was dissolved in THF (3.0 mL). To this solution was added ethyl 4-aminobenzoate (465 mg, 2.81 mmol) and pyridine (0.379 mL, 4.69 mmol). After being stirred for 1 h at room temperature, the mixture was concentrated in vacuo, and the residue was taken up in ethyl acetate. The organic layer was washed with saturated $NaHCO_3$ (×3), 1 M hydrochloric acid (×4), and brine

and dried over Na_2SO_4. The solvent was removed, and the residue was crystallized from ethyl acetate to give **70b** as a pale yellow powder (370 mg, 76%). mp 222–223 °C. ^{1}H NMR (300 MHz, DMSO-d_6) δ 1.3 (3H, t, J = 7.2 Hz), 3.73 (3H, s), 4.07 (2H, s), 4.28 (2H, q, J = 7.1 Hz), 4.42 (2H, d, J = 6.2 Hz), 6.77–6.95 (3H, m), 7.24 (1H, t, J = 8.1 Hz), 7.52 (1H, s), 7.71 (2H, d, J = 8.9 Hz), 7.9 (2H, d, J = 8.7 Hz), 9.64 (1H, s), 10.5 (1H, s), 12.4 (1H, s). Anal. Calcd for $C_{26}H_{24}N_4O_6S$•0.15H_2O: C, 59.68; H, 4.68; N, 10.71. Found: C, 59.90; H, 4.69; N, 10.43.

4-[2-(2-{[(3-Methoxyphenyl)methyl]carbamoyl}-4-oxo-3*H*,4*H*-thieno[2,3-*d*]pyrimidin-5-yl)acetamido]benzoic acid (70c). A mixture of **70b** (230 mg, 0.442 mmol) and 12 M NaOH (0.092 mL, 1.1 mmol) in THF–MeOH–H_2O (1:1:1) (3.0 mL) was stirred at 80 °C for 1 h. After evaporation in vacuo, the residue was dissolved in ethyl acetate and the organic layer was washed with 1 M hydrochloric acid (×2), brine, dried over Na_2SO_4, and concentrated in vacuo. The residue was crystallized from ethyl acetate to give **70c** as a pale yellow powder (188 mg, 86%). mp 279 °C. ^{1}H NMR (300 MHz, DMSO-d_6) δ 3.73 (3H, s), 4.07 (2H, s), 4.42 (2H, d, J = 6.4 Hz), 6.73–6.96 (3H, m), 7.24 (1H, t, J = 8.1 Hz), 7.52 (1H, s), 7.69 (2H, d, J = 8.9 Hz), 7.87 (2H, d, J = 8.9 Hz), 9.61 (1H, s), 10.5 (1H, s), 12.5 (2H, s). Anal. Calcd for $C_{24}H_{20}N_4O_6S$•0.50H_2O: C, 57.48; H, 4.22; N, 11.17. Found: C, 57.57; H, 4.11; N, 10.96.

Ethyl 5-(dibromomethyl)-4-oxo-3*H*,4*H*-thieno[2,3-*d*]pyrimidine-2-carboxylate (71a). A mixture of compound **60b** (10.0 g, 42.0 mmol), NBS (17.2 g, 96.5 mmol), and AIBN (0.689 g, 4.20 mmol) in carbon tetrachloride (300 mL) was stirred at 80 °C for 1.5 h. The reaction mixture was concentrated in vacuo. The resulting solid was suspended in diethyl ether (100 mL), filtered, and washed with H_2O (5×100 mL), diethyl ether (4×10 mL) and dried to provide **71a** as an off-white powder (14.2 g, 85%). mp 214–215 °C. ^{1}H NMR (300 MHz, DMSO-d_6) δ 1.35 (3H, t, J = 7.1 Hz), 4.38 (2H, q, J = 7.0 Hz), 7.57 (1H, s), 8.26 (1H, s), 13.2 (1H, s). Anal. Calcd for $C_{10}H_8Br_2N_2O_3S$: C, 30.33; H, 2.04; N, 7.07. Found: C, 30.63; H, 2.12; N, 7.28.

Ethyl 5-formyl-4-oxo-3*H*,4*H*-thieno[2,3-*d*]pyrimidine-2-carboxylate (71b). A mixture of **71a** (13.5 g, 34.1 mmol) and 1 M hydrochloric acid (170 mL) in THF–MeOH (1:1) (280 mL) was stirred at 60 °C for 1.5 h. After removal of the solvent, the residue was taken up in ethyl acetate, washed with H_2O and brine, dried over Na_2SO_4, and concentrated in vacuo. The residue was crystallized from ethyl acetate to give **71b** as a white powder (5.93 g, 69). mp 213–214 °C. ^{1}H NMR (300 MHz, DMSO-d_6) δ 1.36 (3H, t, J = 7.1 Hz), 4.39 (2H, q, J = 7.0 Hz), 8.61 (1H, s), 10.51 (1H, s), 13.30 (1H, s). Anal. Calcd for $C_{10}H_8N_2O_4S$•0.25H_2O: C, 46.78; H, 3.34; N, 10.91. Found: C, 46.82; H, 3.18; N, 10.86.

2-(Ethoxycarbonyl)-4-oxo-3*H*,4*H*-thieno[2,3-*d*]pyrimidine-5-carboxylic acid (71c). To a mixture of **71b** (3.00 g, 11.9 mmol) in a mixture of MeCN (50 mL) and H_2O (50 mL) was added $NaClO_2$ (4.30 g, 45.6 mmol). The resulting mixture was stirred at room temperature for 12 h, and concentrated in vacuo. The residue was acidified with 1 M hydrochloric acid, and taken up in a mixture of ethyl acetate–THF, and washed with brine, dried over Na_2SO_4, and concentrated in vacuo. The residue was crystallized from ethyl acetate to give **71c** as a white powder (1.81 g, 57%). mp 258 °C. ^{1}H NMR (300 MHz, DMSO-d_6) δ 1.36 (3H,

t, J = 7.2 Hz), 4.41 (2H, q, J = 7.2 Hz), 8.69 (1H, s). Anal. Calcd for $C_{10}H_8N_2O_5S$•0.20H_2O: C, 44.18; H, 3.11; N, 10.30. Found: C, 44.11; H, 3.02; N, 10.49.

Ethyl 5-(benzylcarbamoyl)-4-oxo-3*H*,4*H*-thieno[2,3-*d*]pyrimidine-2-carboxylate (**72a**).
To a mixture of **71c** (400 mg, 1.49 mmol) in THF (10 mL) were added oxalyl chloride (0.390 mL, 4.47 mmol) and *N*,*N*-dimethylformamide (1 drop). After being stirred for 1h at room temperature, the mixture was concentrated in vacuo. The residue was resuspended in THF (10 mL), and to the mixture was added benzylamine (0.326 mL, 2.98 mmol). After being stirred for 12h at room temperature, the mixture was concentrated in vacuo. The residue was taken up in ethyl acetate, washed with 1 M hydrochloric acid (×2), saturated sodium hydrogen carbonate solution, 1 M hydrochloric acid, brine, dried over Na_2SO_4 and concentrated in vacuo. The residue was crystallized from ethyl acetate to give **72a** (373 mg, 70%) as a white powder. mp 215–216 °C. ^{1}H NMR (300 MHz, DMSO-d_6) δ 1.35 (3H, t, J = 7.2 Hz), 4.39 (2H, q, J = 7.1 Hz), 4.56 (2H, d, J = 5.5 Hz), 7.13–7.53 (5H, m), 8.56 (1H, s), 11.2 (1H, s), 13.5 (1H, s). Anal. Calcd for $C_{17}H_{15}N_3O_4S$: C, 57.13; H, 4.23; N, 11.76. Found: C, 57.08; H, 4.19; N, 11.83.

Ethyl 5-({[4-(ethoxycarbonyl)phenyl]methyl}carbamoyl)-4-oxo-3*H*,4*H*-thieno[2,3-*d*]pyrimidine-2-carboxylate (**72b**). To a mixture of compound **71c** (600 mg, 2.24 mmol) in THF (6.0 mL) were added oxalyl chloride (0.590 mL, 6.72 mmol) and *N*,*N*-dimethylformamide (1 drop). After being stirred for 1 h at room temperature, the mixture was concentrated in vacuo. The residue was resuspended in THF (6.0 mL), and to the mixture were added ethyl 4-(aminomethyl)benzoate hydrochloride (965 mg, 4.48 mmol) and triethylamine (1.20 mL, 8.96 mmol). After being stirred for 12 h at room temperature, the mixture was concentrated in vacuo. The residue was taken up in ethyl acetate, washed with 1 M hydrochloric acid (×2), saturated sodium hydrogen carbonate solution, 1 M hydrochloric acid, brine, dried over Na_2SO_4 and concentrated in vacuo. The residue was crystallized from ethyl acetate to give **72b** (771 mg, 80%) as a white powder. mp 227–228 °C. ^{1}H NMR (300 MHz, DMSO-d_6) δ 1.31 (3H, t, J = 6.2 Hz), 1.36 (3H, t, J = 6.2 Hz), 4.31 (2H, q, J = 7.1 Hz), 4.40 (2H, q, J = 7.1 Hz), 4.65 (2H, d, J = 5.5 Hz), 7.50 (2H, d, J = 8.5 Hz), 7.93 (2H, d, J = 8.5 Hz), 8.57 (1H, s), 11.3 (1H, s), 13.5 (1H, s). Anal. Calcd for $C_{20}H_{19}N_3O_6S$•0.20H_2O: C, 55.47; H, 4.52; N, 9.70. Found: C, 55.50; H, 4.39; N, 9.73.

***N*5-Benzyl-*N*2-[(3-methoxyphenyl)methyl]-4-oxo-3*H*,4*H*-thieno[2,3-*d*]pyrimidine-2,5-dicarboxamide (73a).** Compound **73a** was prepared from compound **72a** with a similar procedure as described for **61a** (white powder, 85%). mp 217 °C. ^{1}H NMR (300 MHz, DMSO-d_6) δ 3.73 (3H, s), 4.43 (2H, d, J = 6. 2 Hz), 4.56 (2H, d, J = 5.5 Hz), 6.80–6.85 (1H, m), 6.89–6.93 (2H, m), 7.21–7.29 (2H, m), 7.30–7.38 (4H, m), 8.52 (1H, s), 9.74 (1H, s), 11.3 (1H, s), 13.2 (1H, s). Anal. Calcd for $C_{23}H_{20}N_4O_4S$: C, 61.59; H, 4.49; N, 12.49. Found: C, 61.48; H, 4.48; N, 12.41.

Ethyl 4-{[({2-[(3-Methoxybenzyl)carbamoyl]-4-oxo-3,4-dihydrothieno[2,3-*d*]pyrimidin-5-yl}carbonyl)amino]methyl}benzoate (73b). Compound **73b** was prepared from compound **72b** with a similar procedure as described for **61a** (white powder, 91%). mp 229–230 °C. ^{1}H NMR (300 MHz, DMSO-

d_6) δ 1.31 (3H, t, J = 7.1 Hz), 3.73 (3H, s), 4.31 (2H, q, J = 7.1 Hz), 4.44 (2H, d, J = 6.2 Hz), 4.64 (2H, d, J = 5.3 Hz), 6.78–6.96 (3H, m), 7.24 (1H, t, J = 8.1 Hz), 7.49 (2H, d, J = 8.3 Hz), 7.88–7.97 (2H, m), 8.51 (1H, s), 9.73 (1H, t, J = 6.0 Hz), 11.4 (1H, s), 13.2 (1H, s). Anal. Calcd for $C_{26}H_{24}N_4O_6S$•0.20H_2O: C, 59.58; H, 4.69; N, 10.69. Found: C, 59.41; H, 4.46; N, 10.61.

4-{[({2-[(3-Methoxybenzyl)carbamoyl]-4-oxo-3,4-dihydrothieno[2,3-*d*]pyrimidin-5-yl}carbonyl)amino]methyl}benzoic acid (73c). A mixture of **73b** (330 mg, 0.634 mmol) and 12 M NaOH (0.132 mL, 1.59 mmol) in a mixture of THF–MeOH–H_2O (2.0 mL–2.0 mL–2.0 mL) was refluxed at 90 °C for 1 h. The mixture was concentrated in vacuo to give a residue, which was taken up in ethyl acetate–THF (1:1, ca 1L), washed with 1 M hydrochloric acid and brine, and dried over Na_2SO_4. The organic extract was concentrated in vacuo, and the residue was crystallized from ethyl acetate to give **73c** (294 mg, 94%) as a white powder. mp 254–255 °C. ^{1}H NMR (300 MHz, DMSO-d_6) δ 3.73 (3H, s), 4.44 (2H, d, J = 6.4 Hz), 4.64 (2H, d, J = 5.5 Hz), 6.74–6.97 (3H, m), 7.24 (1H, t, J = 8.1 Hz), 7.47 (2H, d, J = 8.3 Hz), 7.91 (2H, d, J = 8.3 Hz), 8.52 (1H, s), 9.76 (1H, t, J = 6.4 Hz), 11.3 (1H, t, J = 5.6 Hz), 13.2 (1H, s). Anal. Calcd for $C_{24}H_{20}N_4O_6S$•2.0H_2O: C, 54.54; H, 4.58; N, 10.60. Found: C, 54.19; H, 4.31; N, 10.45.

2-{[(3-Methoxyphenyl)methyl]carbamoyl}-4-oxo-3*H*,4*H*-thieno[2,3-*d*]pyrimidine-5-carboxylic acid (**74a**). Compound **74a** was prepared from compound **71c** with a similar procedure as described for **61a** (white powder, 81%). mp 236–237 °C. ^{1}H NMR (300 MHz, DMSO-d_6) δ 3.74 (3H, s), 4.45 (2H, d, J = 6.4 Hz), 6.76–6.96 (3H, m), 7.25 (1H, t, J = 8.1 Hz), 8.65 (1H, s), 9.82 (1H, t, J = 6.3 Hz), 13.9 (1H, s), 15.4 (1H, s). Anal. Calcd for $C_{16}H_{13}N_3O_5S$•0.30AcOEt: C, 53.55; H, 4.02; N, 10.89. Found: C, 53.86; H, 3.85; N, 11.07.

Ethyl 4-{[(2-{[(3-methoxyphenyl)methyl]carbamoyl}-4-oxo-3*H*,4*H*-thieno[2,3-*d*]pyrimidin-5-yl)carbamoyl]methyl}benzoate (75) and 4-{[(2-{[(3-Methoxyphenyl)methyl]carbamoyl}-4-oxo-3*H*,4*H*-thieno[2,3-*d*]pyrimidin-5-yl)carbamoyl]methyl}benzoic acid (76). To a suspension of **74a** (4.45 g, 12.4 mmol) in toluene (45 mL) were added triethylamine (10.0 mL, 74.3 mmol) and DPPA (6.90 mL, 32.2 mmol). The mixture was stirred for 2 min at 100 °C, followed by addition of *tert*-butyl alcohol (12.0 mL, 124 mmol), and heated at 100 °C for 12 h. After the removal of solvent, the residue was taken up in ethyl acetate. The organic layer was extracted with saturated $NaHCO_3$ (×2), and brine and dried over Na_2SO_4. The solvent was removed, and the residue was dried in vacuo to give a residue, which was used in the next step without further purification. A mixture of the above residue in 4 M hydrochloric acid/ethyl acetate (45 mL) was stirred overnight at room temperature. The resulting solid was filtered and washed with ethyl acetate and dried to provide a brown powder (3.14 g). A mixture of above powder and 12 M NaOH (4.10 mL, 49.5 mmol) in THF–MeOH–H_2O (1:1:1, 45 mL) was stirred at 80 °C for 2h. After neutralization with 1 M hydrochloric acid, the mixture was concentrated in vacuo. The residue was taken up in ethyl acetate, washed with water and brine and concentrated in vacuo to give a brown foam (1.18 g, 26%). The crude product **74b** was used without purification.

To a mixture of {4-[(ethyloxy)carbonyl]phenyl}acetic acid (215 mg, 1.033 mmol) and THF (3 mL) were added oxalyl chloride (0.183 mL, 2.10 mmol) and DMF (1 drop), and the mixture was stirred at room temperature for 2 h. The reaction mixture was concentrated under reduced pressure, THF (3 mL), **74b** (276 mg, 0.837 mmol) and pyridine (0.418 mL, 5.17 mmol) were added to the obtained residue, and the mixture was stirred at room temperature for 2 h. The reaction mixture was concentrated under reduced pressure and ethyl acetate was added to the obtained residue. The organic layer was washed with 1 M hydrochloric acid and saturated brine, dried over anhydrous Na_2SO_4 and concentrated under reduced pressure. Aqueous sodium hydroxide solution (1 M, 2 mL), ethanol (1 mL) and THF (1 mL) were added to the concentrated residue and the reaction mixture was stirred at room temperature for 12 h. The reaction mixture was concentrated under reduced pressure, and ethyl acetate was added to the obtained residue. The organic layer was washed with water and saturated brine, dried over anhydrous Na_2SO_4 and concentrated under reduced pressure. The concentrated residue was crystallized from ethyl acetate to give **75** as a brown powder (29.6 mg, 5.5%). mp 218–219 °C. ^{1}H NMR (300 MHz, DMSO-d_6) δ 1.32 (3H, t, J = 7.1 Hz), 3.73 (3H, s), 3.93 (2H, s), 4.31 (2H, q, J = 7.0 Hz), 4.41 (2H, d, J = 6.2 Hz), 6.77–6.95 (3H, m), 7.23 (1H, t, J = 8.1 Hz), 7.52 (2H, d, J = 8.3 Hz), 7.86 (1H, s), 7.95 (2H, d, J = 8.3 Hz), 9.67 (1H, t, J = 6.1 Hz), 9.95 (1H, s), 12.8 (1H, s).

The aqueous layer was acidified with 1 M hydrochloric acid. The mixture was extracted with ethyl acetate, and the organic layer was washed with 1 M hydrochloric acid and saturated brine, dried over anhydrous Na_2SO_4 and concentrated under reduced pressure. The concentrated residue was crystallized from ethyl acetate to give **76** as a brown powder (83.9 mg, 17%). mp 218–219 °C. ^{1}H NMR (300 MHz, DMSO-d_6) δ 3.73 (3H, s), 3.92 (2H, s), 4.40 (2H, d, J = 6.4 Hz), 6.73–6.96 (3H, m), 7.23 (1H, t, J = 8.1 Hz), 7.49 (2H, d, J = 8.3 Hz), 7.87 (1H, s), 7.93 (2H, d, J = 8.3 Hz), 9.67 (1H, t, J = 6.5 Hz), 9.94 (1H, s), 12.8 (1H, s), 12.9 (1H, s). Anal. Calcd for $C_{24}H_{20}N_4O_6S$•0.30H_2O: C, 57.89; H, 4.17; N, 11.25. Found: C, 57.81; H, 4.23; N, 11.52.

Ethyl 6-bromo-4-oxo-3*H*,4*H*-thieno[2,3-*d*]pyrimidine-2-carboxylate (77a). A mixture of **60a** (1000 mg, 4.46 mmol), bromine (1.37 mL, 26.8 mmol) and acetic acid (20 mL) was stirred at room temperature for 6 h. The reaction mixture was concentrated under reduced pressure, and ethyl acetate was added to the obtained residue. The organic layer was washed with water and saturated brine, dried over anhydrous Na_2SO_4 and concentrated under reduced pressure. The concentrated residue was crystallized from ethyl acetate to give **77a** as a pale gray powder (1.25 g, 93%). mp 252–253 °C. ^{1}H NMR (300 MHz, DMSO-d_6) δ 1.34 (3H, t, J = 7.2 Hz), 4.36 (2H, q, J = 7.0 Hz), 7.67 (1H, s), 13.1 (1H, s). Anal. Calcd for $C_9H_7BrN_2O_3S$: C, 35.66; H, 2.33; N, 9.24. Found: C, 35.75; H, 2.27; N, 9.09.

Ethyl 6-bromo-5-nitro-4-oxo-3*H*,4*H*-thieno[2,3-*d*]pyrimidine-2-carboxylate (77b). To a mixture of **77a** (800 mg, 2.64 mmol) and concentrated sulfuric acid (13 mL) was added sodium nitrate (269 mg, 3.17 mmol) at 0 °C. After stirring for 30 min, the reaction mixture was added to a mixed solution of ice water

(200 mL) and diethyl ether (100 mL) to remove the aqueous layer. The insoluble material in the upper layer was collected by filtration, washed 4 times with water and twice with diethyl ether, and dried to give **77b** as pale yellow crystal (734 mg, 80%). mp 284 °C. ^{1}H NMR (300 MHz, DMSO-d_6) δ 1.34 (3H, t, J = 7.1 Hz), 4.38 (2H, q, J = 7.0 Hz), 13.6 (1H, s).

Ethyl 5-amino-4-oxo-3*H*,4*H*-thieno[2,3-*d*]pyrimidine-2-carboxylate hydrobromide (77c). A mixture of **77b** (600 mg, 1.72 mmol), 10% palladium carbon (50% wet, 240 mg), ethanol (15 mL) and THF (15 mL) was stirred under a hydrogen atmosphere (1 atm) at room temperature for 12 h. The catalyst was filtered off, and the filtrate was concentrated under reduced pressure. The obtained concentrated residue was crystallized from ethanol. The obtained crude crystals were dissolved in methanol and an insoluble material was filtered off and the filtrate was concentrated under reduced pressure. The obtained concentrated residue was crystallized from ethanol to give **77c** as a brown powder (198 mg, 36%). mp 201 °C. ^{1}H NMR (300 MHz, DMSO-d_6) δ 1.34 (3H, t, J = 7.2 Hz), 4.36 (2H, q, J = 7.2 Hz), 6.67 (1H, s), 12.9 (1H, s). Anal. Calcd for $C_9H_{10}BrN_3O_3S$•1.0H_2O: C, 31.96; H, 3.58; N, 12.43. Found: C, 32.23; H, 3.52; N, 12.30.

Ethyl 4-oxo-5-(2-phenylacetamido)-3*H*,4*H*-thieno[2,3-*d*]pyrimidine-2-carboxylate (78). To a mixture of **77c** (160 mg, 0.500 mmol) and THF (2 mL) were added phenylacetyl chloride (0.0727 mL, 0.550 mmol) and triethylamine (0.146 mL, 1.05 mmol) at room temperature. The mixture was stirred for 1 h at room temperature and concentrated under reduced pressure. Ethyl acetate was added to the obtained residue, and the organic layer was washed with 1 M hydrochloric acid, water and saturated brine, dried over anhydrous Na_2SO_4 and concentrated under reduced pressure. The concentrated residue was crystallized from ethyl acetate to give **78** as a pale purple powder (138 mg, 77%). mp 237 °C. ^{1}H NMR (300 MHz, DMSO-d_6) δ 1.33 (3H, t, J = 7.1 Hz), 3.81 (2H, s), 4.36 (2H, q, J = 7.2 Hz), 7.22–7.41 (5H, m), 7.92 (1H, s), 9.89 (1H, s), 13.1 (1H, s). Anal. Calcd for $C_{17}H_{15}N_3O_4S$•0.25H_2O: C, 56.42; H, 4.32; N, 11.61. Found: C, 56.58; H, 4.30; N, 11.72.

***N*-[(3-Methoxyphenyl)methyl]-4-oxo-5-(2-phenylacetamido)-3*H*,4*H*-thieno[2,3-*d*]pyrimidine-2-carboxamide (79).** Compound **79** was prepared from compound **78** with a similar procedure as described for **61a** (white powder, 93%). mp 201 °C. ^{1}H NMR (300 MHz, DMSO-d_6) δ 3.73 (3H, s), 3.80 (2H, s), 4.41 (2H, d, J = 6.2 Hz), 6.77–6.94 (3H, m), 7.18–7.42 (6H, m), 7.86 (1H, s), 9.67 (1H, t, J = 6.4 Hz), 9.93 (1H, s), 12.8 (1H, s). Anal. Calcd for $C_{23}H_{20}N_4O_4S$•0.10AcOEt•0.25H_2O: C, 60.86; H, 4.65; N, 12.13. Found: C, 60.99; H, 4.62; N, 12.21.

5-{[(4-Carbamoylphenyl)methoxy]methyl}-*N*-[(3-methoxyphenyl)methyl]-4-oxo-3*H*,4*H*-thieno[2,3-*d*]pyrimidine-2-carboxamide (80a). To a mixture of **64c** (200 mg, 0.417 mmol) and THF (2 mL) were added oxalyl chloride (0.0500 mL, 0.573 mmol) and DMF (1 drop) and the mixture was stirred at room temperature for 1 h. The reaction mixture was concentrated under reduced pressure. To a suspension of the concentrated residue in THF (3 mL) was added 28% aqueous ammonia (2 mL), and the mixture was stirred at room temperature for 10 min. The reaction mixture was concentrated under reduced pressure, and ethyl

acetate and THF were added to the obtained residue. The organic layer was washed with 1 M hydrochloric acid and saturated brine, dried over anhydrous Na_2SO_4 and concentrated under reduced pressure. The concentrated residue was crystallized from ethyl acetate–THF to give **80a** as a white powder (173 mg, 87%). mp 224 °C. ^{1}H NMR (300 MHz, DMSO-d_6) δ 3.73 (3H, s), 4.42 (2H, d, J = 6.2 Hz), 4.71 (2H, s), 4.87 (2H, d, J = 1.1 Hz), 6.76–6.95 (3H, m), 7.18–7.28 (1H, m), 7.34 (1H, s), 7.47 (2H, t, J = 7.6 Hz), 7.62 (1H, s), 7.86 (2H, d, J = 8.3 Hz), 7.95 (1H, s), 9.64 (1H, t, J = 6.4 Hz), 12.4 (1H, s). Anal. Calcd for $C_{24}H_{22}N_4O_5S$•0.50H_2O: C, 59.13; H, 4.76; N, 11.49. Found: C, 59.06; H, 4.72; N, 11.22.

***N*-[(3-Methoxyphenyl)methyl]-5-({[4-(methylcarbamoyl)phenyl]methoxy}methyl)-4-oxo-3*H*,4*H*-thieno[2,3-*d*]pyrimidine-2-carboxamide (80b).** To a mixture of **64c** (110 mg, 0.229 mmol), 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride (65.9 mg, 0.344 mol), 4-dimethylaminopyridine (283 mg, 2.31 mol) and THF (5 mL) was added methylamine hydrochloride (155 mg, 2.29 mmol). The mixture was stirred at room temperature for 12 h. The reaction mixture was concentrated under reduced pressure, and ethyl acetate was added to the obtained residue. The organic layer was washed with 1 M hydrochloric acid and saturated brine, dried over anhydrous Na_2SO_4 and concentrated under reduced pressure. The concentrated residue was crystallized from ethyl acetate to give **80b** as a white powder (75.9 mg, 67%). mp 190–191 °C. ^{1}H NMR (300 MHz, DMSO-d_6) δ 2.78 (3H, d, J = 4.5 Hz), 3.73 (3H, s), 4.42 (2H, d, J = 6.4 Hz), 4.71 (2H, s), 4.87 (2H, d, J = 0.8 Hz), 6.82 (1H, dd, J = 9.0, 1.8 Hz), 6.86–6.97 (2H, m), 7.24 (1H, t, J = 8.1 Hz), 7.46 (2H, d, J = 8.1 Hz), 7.62 (1H, s), 7.82 (2H, d, J = 8.1 Hz), 8.42 (1H, d, J = 4.5 Hz), 9.65 (1H, t, J = 6.4 Hz), 12.4 (1H, s). Anal. Calcd for $C_{25}H_{24}N_4O_5S$•0.80H_2O: C, 59.23; H, 5.09; N, 11.05. Found: C, 59.33; H, 5.04; N, 11.00.

5-({[4-(Hydroxymethyl)phenyl]methoxy}methyl)-*N*-[(3-methoxyphenyl)methyl]-4-oxo-3*H*,4*H*-thieno[2,3-*d*]pyrimidine-2-carboxamide (**80c**). To a mixture of **64c** (1200 mg, 2.50 mmol) and THF (12 mL) were added oxalyl chloride (0.419 mL, 4.80 mmol) and DMF (1 drop), and the mixture was stirred at room temperature for 1.5 h. The reaction mixture was concentrated under reduced pressure. A Mixture of the concentrated residue and sodium borohydride (189 mg, 5.01 mmol) was stirred in DMA (15 mL) at room temperature for 5 min. The reaction mixture was stirred until generation of gas ceased and concentrated under reduced pressure. Ethyl acetate was added to the obtained residue. The organic layer was washed with water, 1 M hydrochloric acid, water and saturated brine, dried over anhydrous Na_2SO_4 and concentrated under reduced pressure. The concentrated residue was crystallized from ethyl acetate to give **80c** as a white powder (753 mg, 65%). mp 156–157 °C. ^{1}H NMR (300 MHz, DMSO-d_6) δ 3.73 (3H, s), 4.42 (2H, d, J = 6.4 Hz), 4.49 (2H, d, J = 5.7 Hz), 4.64 (2H, s), 4.83 (2H, d, J = 1.1 Hz), 5.16 (1H, t, J = 5.8 Hz), 6.75–6.95 (3H, m), 7.19–7.28 (1H, m), 7.28–7.39 (4H, m), 7.58 (1H, s), 9.64 (1H, t, J = 6.5 Hz), 12.4 (1H, s). Anal. Calcd for $C_{24}H_{23}N_3O_5S$•0.50H_2O: C, 60.75; H, 5.10; N, 8.86. Found: C, 60.72; H, 4.98; N, 8.89.

5-({[4-(Methoxymethyl)phenyl]methoxy}methyl)-*N*-[(3-methoxyphenyl)methyl]-4-oxo-3*H*,4*H*-thieno[2,3-*d*]pyrimidine-2-carboxamide (80d). To a mixture of **64c** (100 mg, 0.215 mmol) and THF (2

mL) were added methanesulfonyl chloride (0.0266 mL, 0.344 mmol) and triethylamine (0.0929 mL, 0.667 mmol), and the mixture was stirred at room temperature for 1 h. The reaction mixture was concentrated under reduced pressure, and ethyl acetate was added to the obtained residue. The organic layer was washed with water and saturated brine, dried over anhydrous Na_2SO_4 and concentrated under reduced pressure. To a mixture of the concentrated residue and sodium methylate (58.1 mg, 1.08 mmol) were added methanol (2 mL) and THF (2 mL) and the mixture was stirred at 80 °C for 1 h. The reaction mixture was concentrated under reduced pressure, and ethyl acetate was added to the obtained residue. The organic layer was washed with 1 M hydrochloric acid and saturated brine, dried over anhydrous Na_2SO_4 and concentrated under reduced pressure. The concentrated residue was crystallized from ethyl acetate to give **80d** as a white powder (68.5 mg, 66%). mp 156–157 °C. ^{1}H NMR (300 MHz, DMSO-d_6) δ 3.28 (3H, s), 3.73 (3H, s), 4.34–4.48 (4H, m), 4.65 (2H, s), 4.85 (2H, d, J = 0.9 Hz), 6.77–6.95 (3H, m), 7.18–7.40 (5H, m), 7.59 (1H, s), 9.64 (1H, t, J = 6.0 Hz), 12.4 (1H, s). Anal. Calcd for $C_{25}H_{25}N_3O_5S$•0.25H_2O: C, 62.03; H, 5.31; N, 8.68. Found: C, 61.98; H, 5.32; N, 8.63.

Disodium 4-[({2-[(3-Methoxybenzyl)carbamoyl]-4-oxo-4*H*-thieno[2,3-*d*]pyrimidin-3-id-5-yl}methoxy)methyl]benzoate (**81**). To a mixed solution of **64c** (150 mg, 0.313 mmol) in THF (24 mL) and ethanol (6 mL) was added an aqueous solution (3 mL) of sodium hydrogen carbonate (52.6 mg, 0.626 mmol) and the mixture was stirred for 30 min. The reaction mixture was concentrated under reduced pressure, ethanol (18 mL) was added to the obtained residue, and the ethanol suspension was stirred at 90 °C for 30 min. The mixture was allowed to cool to room temperature, and the precipitated solid was collected by filtration, washed with ethanol and dried to give **81** as a white powder (149 mg, 91%). mp >300 °C. ^{1}H NMR (300 MHz, DMSO-d_6) δ 3.73 (3H, s), 4.40 (2H, d, J = 6.4 Hz), 4.63 (2H, s), 4.88 (2H, d, J = 1.3 Hz), 6.74–6.94 (3H, m), 6.98 (1H, t, J = 1.3 Hz), 7.14–7.33 (3H, m), 7.84 (2H, d, J = 8.1 Hz), 9.03 (1H, t, J = 6.4 Hz). Anal. Calcd for $C_{24}H_{19}N_3Na_2O_6S$•1.0H_2O: C, 53.23; H, 3.91; N, 7.76. Found: C, 53.51; H, 4.09; N, 7.83.

MMPs and TACE enzyme inhibition assay. Human recombinant MMP precursors were purchased from Genzyme-Techne (MMP-1, 2, 7, 8, 9, 10, 13, and TACE) or Biogenesis (MMP-3). Human recombinant GST-MMP-14 was prepared as described by Sato et al.[69] The MMP assay buffer consisted of 50 mM Tris–HCl (pH 7.5), 10 mM $CaCl_2$, 150 mM NaCl, and 0.05% Brij-35. The pro-MMPs were activated by preincubation with 1 mM aminophenylmercuric acetate (APMA) in assay buffer at 37 °C for 2 h (MMP-1, 2, 7, 8, 10, and 13) or 18 h (MMP-3 and 9). The TACE assay buffer consisted of 25 mM Tris–HCl (pH 9.0), 2.5 μM $ZnCl_2$, and 0.005% Brij-35. The pro-MMPs were activated by preincubation with 1 mM aminophenylmercuric acetate (APMA) in assay buffer at 37 °C for 2 h (MMP-1, 2, 7, 8, 10, and 13) or 18 h (MMP-3 and 9). Enzyme inhibition assays were performed in an assay buffer containing enzymes and fluorescence peptide (Cy3-PLGLK(Cy5Q)AR-NH_2 for MMPs, Cy3-PLAQAV(Cy5Q-L-2,3-diaminopropionic acid)-RSSSR-NH_2 for TACE, Amersham Biosciences) in the presence of the various concentrations of inhibitors. Following

incubation at 37 °C for 40 min, the reaction was terminated by addition of EDTA (pH 8.0). The increase in fluorescence was measured by Farcyte spectrofluorimeter (Amersham Bioscience, λem 535 nm, λex 595 nm). Enzyme activity (%) was determined as following equation: Enzyme activity (%) = (X – C)/(T – C) ×100, where X = the fluorescence count with inhibitor, T = the fluorescence count without inhibitor and C = the fluorescence count with EDTA. IC_{50} values of inhibitors were obtained with iterative fitting package (GraphPad Prism software).

Crystallization and structure determination. The human MMP-13 catalytic domain was prepared as described previously. Crystals were grown in the hanging drop vapor diffusion method at 20 °C (the temperature was modified). Prior to crystallization, a solution containing 6–14 mg/mL MMP-13 catalytic domain, 5 μM $Zn(OAc)_2$, 5 mM $CaCl_2$, 50 mM NaCl, 20 mM Tris HCl buffer (pH 8.0), and 0.5 mM compound were prepared. Equal volumes (0.5 μL) of the protein solution and reservoir solution containing 8–16% w/v PEG8000, 1.0–1.5 M ammonium formate and 0.1 M Tris HCl (pH 8.5) buffer were mixed and equilibrated in the hanging drop against a reservoir solution. Crystals were dipped into a 1:1:1 mixture of protein solution, reservoir solution and glycerol, soaked for a few minutes and then treated by flash-cooling method. X-ray diffraction data were collected at SPring-8 BL32B2, and processed with the program CrystalClear™ (Rigaku/MSC, Inc.). Phase determination was solved by the molecular replacement method with the program MOLREP[70] using the structure with PDB accession number 830C.[37] This initial model was excluded solvents and inhibitors. The refinement program REFMAC[71] was used, and some rebuilding of parts of the molecule was performed with WinCoot. [72] X-ray coordinates have been deposited with the RCSB Protein Data Bank (PDB) for **1** in complex with MMP-13 (3WV2), **38k** in complex with MMP-13 (3WV1). The statistic data and the refinement statistics are shown in **Table E1**.

Table E1. X-ray Crystallographic Data Collection and Refinement Statistics for Complex of **1** and **38k** with MMP-13

	1 (3WV2)	**38k (3WV1)**
Data Collection		
X-ray source	SPring-8 BL32B2	SPring-8 BL32B2
Wavelength (Å)	1.0	1.0
Space group	*C*2	*C*2
Unit cell dimensions	a = 143.5 Å, b = 35.7 Å, c = 94.8 Å, α = 90.0°, β = 135.3°, γ = 90.0°	a = 134.4 Å, b = 36.1 Å, c = 95.3 Å, α = 90.0°, β = 130.9°, γ = 90.0°
Resolution (Å)	2.30	1.98
Unique reflections	14744	24131
Redundancy	4.0	3.69
Completeness (%)	95.4 (97.2)	98.0 (80.5)
$I/\sigma(I)$	9.0 (3.6)	9.3 (3.3)
R_{sym}[a]	0.077 (0.201)	0.085 (0.321)
Refinement		
Reflections used	14007	22911
RMS Bonds (Å)	0.009	0.009
RMS Angles (°)	1.212	1.318
Average B value (Å^2)	13.0	26.1
R-value[b]	0.174	0.177
R_{free}[b]	0.241	0.220

[a]Rsym = ΣhΣj |<I(h)> - I(h)j | / ΣhΣj <I(h)>, where <I(h)> is the mean intensity of symmetry-related reflections. [b]R-value = Σ | |Fobs| - |Fcalc| | / Σ |Fobs|. Rfree for 5% of reflections excluded from refinement. Values in parentheses are for the highest resolution shell.

The protein solution was incubated with 0.5 mM of the compounds for 1 h on ice, and then centrifuged to remove the precipitation. Equal volumes (0.5 μL) of the protein solution and reservoir solution containing 8–16% w/v PEG8000, 1.0–1.5 M ammonium formate and 0.1 M Tris HCl (pH 8.5) buffer were mixed and equilibrated in the hanging drop against a reservoir solution. Crystals were dipped into a 1:1:1 mixture of protein solution, reservoir solution and glycerol, soaked for a few minutes and then treated by flash-cooling method. The crystals were stored in liquid nitrogen until use. X-ray diffraction data were collected at the Advanced Light Source (ALS) beamline 5.0.3 (Berkeley, CA), and processed using the program HKL2000.[73] The crystal was diffracted to 1.60 Å, providing an unambiguous electron density for **61a**. The structure was determined by molecular replacement using MOLREP,[70] using the only protein structure of MMP-13 previously reported (PDB code: 830C).[37] Subsequently, structure refinement and model building were performed utilizing REFMAC.[71] The solved structure was modeled with WinCoot (version 0.3.3) .[72] Coordinates have been deposited in the PDB as entry 3WV3. The statistic data and the refinement statistics are shown in **Table E2**.

Table E2. X-ray Crystallographic Data Collection and Refinement Statistics for Complex of **61a** with MMP-13

Data Collection	
X-ray source	ALS BL5.0.3
Wavelength (Å)	0.976
Space group	*C*2
Unit cell dimensions	a = 135.6 Å, b = 36.4 Å, c = 95.9 Å, α = 90.0°, β = 130.6°, γ = 90.0°
Resolution (Å)	1.60
Unique reflections	46984
Redundancy	3.9
Completeness (%)	99.1 (87.2)
I/σ(I)	15.1 (1.6)
R_{sym}[a]	0.078 (0.667)
Refinement	
Reflections used	44606
RMS Bonds (Å)	0.010
RMS Angles (°)	1.248
Average B value (Å^2)	21.1
R-value[b]	0.166
R_{free}[b]	0.212

[a]Rsym = ΣhΣj |<I(h)> - I(h)j | / ΣhΣj <I(h)>, where <I(h)> is the mean intensity of symmetry-related reflections. [b]R-value = Σ | |Fobs| - |Fcalc| | / Σ |Fobs|. Rfree for 5% of reflections excluded from refinement. Values in parentheses are for the highest resolution shell.

Assay for inhibitory activity against collagen degradation. Bovine nasal septum cartilage was sliced, and the slices were maintained in the medium of a 1 : 1 (v/v) mixture of Dulbecco's modified Eagle's MEM and Ham's F-12 medium (DMEM/F-12) containing 10 % fetal calf serum overnight. After confirming that the slices were not contaminated, they were cultured in DMEM/F-12 medium containing 20 μg/mL gentamycin, 50 μg/mL streptomycin, and 50 U/mL penicillin (culture medium) for 2 days at 37 °C. The cartilage slices were cut into small cubes (ca. 1mm^3) and transferred individually into wells of a 96 well plate with 100 μL of culture medium. For the collagen degradation assay, the medium was supplemented with 10 ng/mL IL-1β and 50 ng/mL oncostatin M in the presence or absence of compounds. The cartilage

was incubated for 2 weeks. The supernatants were harvested and replaced with fresh medium containing identical test compounds every 7 days. Supernatants of day 7 and day 14 were collected and stored at −20 °C until assay. At the end of the culture, the remaining cartilage was completely digested with papain. Hydroxyproline release in the media from each explant was determined as a measure of collagen degradation by use of chloramine T and *p*-dimethylaminobenzaldehyde. The percentage of inhibitory activity against collagen degradation was calculated as follows: % of inhibition = [(% of collagen degradation with IL-1β and OSM) – (% of collagen degradation with IL-1β, OSM, and test sample)]/[(% of collagen degradation with IL-1β and OSM) – (% of collagen degradation without additives)] × 100.

Chemically induced OA.

Mono-iodoacetate (MIA, Wako Pure Chemical Industries LTD, Japan) was dissolved in saline at 20 mg/mL. Diethylether-anesthetized female Sprague–Dawley rats (12 weeks old, Charles River Japan) were injected with 25 μL of 20 mg/mL MIA in the right knee using a sterile syringe and 27-gauge needle. Control animals were injected with equivalent amounts of saline as the treatment group. On day 7, rats were administered 10 mg/kg **82** (**RS-130,830**) or **54** orally. Four hours after oral administration of MMP inhibitors, the rats were euthanized by CO_2, and the joints were lavaged with saline (50 μL) and analyzed for CTX-II in synovial fluid by the Serum Pre-Clinical CartiLaps ELISA (3CAL4000, Nordic Bioscience Diagnostics, Denmark).

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