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Article

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Abstract

On the basis of a superposition study of X-ray crystal structures of complexes of quinazoline derivative **1** and triazole derivative **2** with matrix metalloproteinase (MMP)-13 catalytic domain, a novel series of fused pyrimidine compounds which possess a 1,2,4-triazol-3-yl group as a zinc binding group (ZBG) was designed. Among the herein described and evaluated compounds, **31f** exhibited excellent potency for

MMP-13 (IC_{50} = 0.036 nM) and selectivities (greater than 1,500-fold) over other MMPs (MMP-1, 2, 3, 7, 8, 9, 10, and 14) and tumor necrosis factor-α converting enzyme (TACE). Furthermore, the inhibitor was shown to protect bovine nasal cartilage explants against degradation induced by interleukin-1 and oncostatin M. In this article, we report the discovery of extremely potent, highly selective, and orally bioavailable fused pyrimidine derivatives that possess a 1,2,4-triazol-3-yl group as a novel ZBG for selective MMP-13 inhibition.

INTRODUCTION

Osteoarthritis (OA) is one of the most common forms of arthritis affecting more than 30 million patients worldwide.[1] The principal morphological characteristic of OA is progressive cartilage damage that leads to pain and reduced mobility in affected joints. The recommended pharmaceutical therapies in the guidelines from the American College of Rheumatology are limited to alleviation of pain and inhibition of inflammation.[2-5] The therapies are oral treatment with acetaminophen, non-steroidal anti-inflammatory drugs (NSAIDs), or cyclooxygenase-2 (COX-2) inhibitors and intra-articular injections of hyaluronic acid or corticosteroids. In addition, because some COX-2 selective inhibitors (rofecoxib and valdecoxib) were withdrawn from the market in 2004 and 2005, [6, 7] there is a significant unmet medical need for safe oral disease-modifying osteoarthritis drugs that may be able to prevent, slow down, or reverse any advanced cartilage destruction.[8, 9]

The matrix metalloproteinases (MMPs) are a family of structurally related zinc-dependent endopeptidases that degrade varied extracellular matrix. Among MMPs, MMP-13 (collagenase-3) specifically catalyzes the hydrolysis of type II collagen,[10, 11]

the main structural component of the cartilage matrix, while the protein is resistant to most proteases. MMP-13 is expressed at higher levels by OA chondrocytes than by normal chondrocytes.[12] In addition, regulated expression of human MMP-13 in joint cartilages induces OA in genetically modified mice.[13] Further, an MMP inhibitor that preferentially inhibits MMP-13 has been shown to block the degradation of explanted human OA cartilage.[14] These findings suggest that MMP-13 plays a crucial role in the destruction of articular cartilage in OA.

In preclinical testing, MMP inhibitors have inhibited the destruction of cartilage in some animal models of OA.[15] However, most clinical trials of broad-spectrum MMP inhibitors have been discontinued due to concerns of dose-limiting toxicity (skin rash and musculoskeletal side effects (MSS) characterized by joint stiffness and pain). Although a number of hypotheses have been proposed for the cause of MSS, including a non-selective inhibition of other metalloproteinases or a combined inhibition of a series of critical MMPs, the pharmacological basis for such joint side effects remains unknown.[16-18] Therefore, considerable interest has been directed toward potent inhibitors of MMP-13 with a high degree of selectivity over other MMPs, which may avoid undesirable side effects. [19-29] Most MMP inhibitors generally incorporate a P1' fragment that can be accommodated in the S1' subsite of the enzyme active site and a

functional group capable of binding the catalytic zinc ion. While a number of P1' fragment allows modulation of potency and selectivity against various MMPs, only a small set of zinc binding groups (ZBGs) have been identified and employed in the design of selective MMP inhibitors.[27, 30-32]

High-throughput screening for MMP-13 inhibition led to a moderately potent (IC_{50} = 12 nM) quinazoline-2-carboxamide **1**[23, 25, 33] and a weakly potent (IC_{50} = 1,900 nM) triazole **2**, which were successfully co-crystallized with MMP-13 catalytic domain (**Figure 1**). These observations allowed the design of a hybrid molecule that combined the quinazoline **1** with the triazole ZBG **2** as shown in **Figure 2**.

We have previously used the fused pyrimidine-2-carboxamide-4-one scaffold represented by compound **3** for initial validation of a strategy to obtain inhibitory potency and selectivity for MMP-13 by utilizing (i) a hydrophobic interaction with the S1' and its side pocket (S1") as clearly revealed by X-ray analysis and (ii) a hydrogen bonding interaction with the ε-amino group of Lys140 at the bottom of the S1" pocket (**Figure 3**). [23, 25, 33, 34] Furthermore, the fused pyrimidine system has been successfully applied by several groups.[35-37] To further increase our repertoire of selective MMP-13 inhibitors, we next investigated the potential of substituents on the aromatic ring of the

N-benzyl group of **1**, thereby potentially addressing the catalytic zinc as shown in **Figure 2** (C). Beside our early patent application,[34] a similar combination approach was recently reported by Fischer et al.[38]

In this article, we report the design, synthesis, and biological activity of novel fused pyrimidine derivatives which possess a 1,2,4-triazol-3-yl group as a ZBG with potent MMP-13 inhibitory activities, excellent selectivities, and good oral bioavailabilities.

CHEMISTRY

Scheme 1 and **Scheme 2** depict the synthesis of benzylamine derivatives used in **Scheme 4** and **Scheme 5**. As shown in **Scheme 1**, benzylamine with an aliphatic side chain bearing a carboxylic ester functionality **6** was synthesized by catalytic hydrogenation of the cyano group of benzonitrile **5** prepared by alkylation of 3-cyanophenol **4** with ethyl 4-bromobutanoate. Another series of compounds that has a thioether linker was also synthesized. Benzylamines **9a–c** were obtained in the following manner. Alkylation of 3-cyanophenol (**4**) with the corresponding 1-bromoalkyl chloride afforded alkyl chlorides **7a–c**. Substitution reaction of the chlorides **7a–c** with triazolylthiols afforded **8a, 8b**, and **8d**. Benzonitrile **8a** underwent

Raney nickel catalyzed hydrogenation to give the benzylamine derivative **9a**. Triphenylmethyl protection followed by reduction with lithium aluminum hydride of each cyano group of benzonitriles **8b** and **8d** led to the benzylamines **9b** and **9c**, respectively. Benzylamine analogues **15a** and **15b** with diether linkers were synthesized by the following method. Commercially available nitrotriazole **10** was protected in a regioselective manner with the sterically demanding triphenylmethyl group to give 3-nitro-1-(triphenylmethyl)-1*H*-1,2,4-triazole (**11**). Alkylation of commercially available 3-cyanophenol (**4**) with ethyl bromoacetate gave ethyl ester **12**. Subsequent chemoselective reduction of the ester group with sodium borohydride afforded benzonitrile **13a**. One-carbon homologue of **13a**, benzonitrile **13b**, was obtained directly from 3-cyanophenol **4** by alkylation with 3-bromopropanol.[39] Precursors of benzylamines **15a** and **15b,** benzonitriles **14a** and **14b**, were synthesized by aromatic nucleophilic substitution reaction of nitrotriazole **11** with hydroxyalkyl substituted benzonitriles **13a** or **13b**, respectively. Lithium aluminum hydride reduction of the cyano group of benzonitriles **14a** and **14b** led to the desired benzylamine derivatives **15a** and **15b**.

Carboxylic acid derivatives **20a–c** were prepared as shown in **Scheme 3**. Thiosemicarbazide **16** was reacted with ethyl 3-chloro-3-oxopropanoate or ethyl

succinyl chloride followed by cyclization with sodium ethoxide to give triazole-3-thiol **18a** and **18b**, respectively. Desulfurization of **18a** using Raney Ni provided ester **19c** in a moderate yield (54%), while desulfurization of **18b** with sodium nitrite in nitric acid and concomitant hydrolysis of the ester group led to carboxylic acid **19d** in excellent yield (99%). Reesterification of **19d** and triphenylmethyl protection gave ester **19f**. Alkaline hydrolysis of esters **19b**, **19c**, and **19f** afforded the desired carboxylic acids **20a–c**, respectively.

The synthetic routes for 4-oxo-3,4-dihydroquinazoline-2-carboxamide derivatives **22a–c** and **23** via ethyl 4-oxo-3,4-dihydroquinazoline-2-carboxylate (**21**) are shown in **Scheme 4**. Taking advantage of the high reactivity of the ester group at the 2-position of the quinazoline **21**, amide formations at the 2-position of 4-oxo-3,4-dihydroquinazoline were achieved by aminolysis of the ethyl 4-oxo-3,4-dihydroquinazoline-2-carboxylate (**21**) with the corresponding benzylamines **6** and **9a** (see **Scheme 1** for preparation) to give the compounds **22a** and **23**, respectively.[23, 25, 40] The ethyl ester **22a** was hydrolyzed, and the resulting carboxylic acid **22b** was treated with oxalyl chloride followed by hydroxylamine to afford the desired hydroxamic acid **22c**.

The synthesis of 4-oxo-3,4-dihydrothienopyrimidine-2-carboxamide derivatives is

demonstrated in **Scheme 5**. The thienopyrimidine derivatives **25a–g** were synthesized in the same manner as described for the quinazoline derivatives in **Scheme 4**.[23] Accordingly, aminolysis of the commercially available ethyl 5-methyl-4-oxo-3,4-dihydrothieno[2,3-*d*]pyrimidine-2-carboxylate (**24**) with corresponding benzylamines, either commercially available or synthesized (**9a–c** and **15a**) as described in **Scheme 1** and **Scheme 2**, and if required, followed by the removal of the triphenylmethyl groups under an acidic condition gave the amide derivatives **26a**, **26b**, and **25a–d**. Deprotection of the Boc group of the amide **26b** afforded amine **26c**. Condensation of the resulting amines **26a** and **26c** with corresponding carboxylic acids **20c** and **20a** (see **Scheme 3** for preparation), followed by removal of the triphenylmethyl group afforded the amide-linked derivatives **25g** and **25e**, respectively. Similarly, condensation of **26c** with **20b** (see **Scheme 3** for preparation) led to **25f**. In the triphenylmethyl deprotection step for **25b–e** and **25g**, cation scavengers are essential for achieving consistently high yields of the triazoles, with triethylsilane proving to be superior to anisole in terms of reaction reproducibility.

The synthesis of 5-substituted 4-oxo-3,4-dihydrothienopyrimidine-2-carboxamide derivatives **31a–q** is described in **Scheme 6**. Ethyl 2-aminothiophenecarboxylates **29c**, **29e**, **29f**, **29h**, **29i**, **29k**, and **29l** were prepared from the corresponding ketones **27a–g** in

one-pot Gewald reaction.[41] Thus, Knoevenagel adducts of the ketones **27a–g** and ethyl cyanoacetate **28** were prepared and subsequently treated with elemental sulfur and morpholine in toluene.[42] Ester intermediates **30a–m** were obtained by reaction of thiophenes **29a–m** with ethyl cyanoformate in 1 M hydrogen chloride in acetic acid solution. In the same fashion as for the synthesis of 5-methylthienopyrimidine derivatives shown in **Scheme 5**, aminolysis of the reactive ester group at the 2-position of the key intermediates **30a–m** with benzylamines **15a** and **15b** (see **Scheme 2** for preparation) followed by deprotection of the triphenylmethyl group led to the corresponding amide derivatives **31a–q**.

RESULTS AND DISCUSSION

A lead quinazoline derivative **1**[23, 25] and a triazole derivative **2** were identified by high throughput screening as MMP-13 inhibitors (**Figure 1**). Interestingly, the quinazoline **1** did not possess an obvious ZBG, such as a hydroxamic acid or a carboxylic acid, and displayed only 25-fold selectivity over other MMP isoenzymes (**Table 4**).

An X-ray crystallographic study of the quinazoline **1** bound to MMP-13 revealed that no interaction with the catalytic zinc was observed and that the quinazoline ring

occupies the deep S1' subsite[17, 25](**Figure 4** (A) and **4** (B)). On the other hand, the 1,2,4-triazol-3-yl moiety of the triazole **2** coordinates in a monodentate fashion to the catalytic zinc (II) center. Furthermore, unlike the quinazoline **1**, the phenylaminothiazole moiety of **2** only partially fills the available space within the MMP-13 S1' pocket (**Figure 4** (C) and (D)). On the basis of a superposition of the crystal structures, the distance between the phenyl ring on the side chain and the ZBG was estimated to be about 4 Å (**Figure 5**). As shown in **Table 1**, analogues were designed and synthesized by connection of the methoxy moiety of the benzyl unit of the quinazoline **1** with various ZBGs by a simple linker element.

Compounds **22a** and **22b** having an ester or a carboxylic acid group via a *n*-propyleneoxy linker at the 3-position of the left-hand phenyl group showed a decrease in MMP-13 inhibitory activity in comparison to the original lead quinazoline **1**. Conversion of the carboxylic acid to hydroxamic acid, known as a potent ZBG, resulted in a slight increase in potency (**22c** vs **1**). On the other hand, quinazoline/triazole hybrid compound **23** that is linked to the triazolyl group through a four-atom linker showed a 28-fold increase in inhibitory activity against MMP-13 (**23** vs **1**).

We have previously shown that the fused pyrimidine core markedly influences the

MMP-13 inhibitory potency, and thus, the quinazoline scaffold was replaced with a previously identified thieno[2,3-*d*]pyrimidine core **25a–g** (**Table 2**). [23] The scaffold gave analogues that were potent compounds (**25a**, **25b**, and **25d**) in the MMP-13 inhibitory assay, however, an increased CYP3A4 inhibition was observed for the compounds with sulfur-containing linkers (**25a–c**). Investigation of the length of the linker between the benzylamide at the 2-position of the pyrimidine and the 1,2,4-triazol-3-yl group revealed that the compound with a five-atom linker exhibited comparable activity (**25b** vs **25a**) to that of a four-atom linker, but a six-atom linker derivative showed reduced activity (**25c** vs **25a**). Since the inhibition of the CYP3A4 enzyme is well known to potentially induce drug–drug interactions, elimination of such undesired property was clearly necessary. Fortuitously, the undesirable CYP3A4 inhibition was decreased in the oxygen linker analogue **25d**. A series of compounds with more rigid linkers (**25e–g**) showed decreased potency compared to that of compound **25a**. Subsequent rat pharmacokinetic studies revealed that thienopyrimidine **25d** with an ethylenedioxy linker was one of the most potent inhibitors, had the least risk of CYP3A4 inhibition among a series of fused pyrimidine derivatives, and showed high oral exposure (AUC = 10855 ng•h/mL) in rats (*F*% = 18). Further optimization to improve the pharmacokinetic profile and attenuation of off-target CYP3A4 inhibition

while maintaining high potency was attempted at 5-position of the thienopyrimidine core.

In the course of another study of non-zinc binding inhibitors having the thienopyrimidine core, it was found that the 5-position of the core can accommodate a wide range of substituents without loss of potency.[23] In **Table 3**, MMP-13 inhibitory activities, CYP3A4 inhibitory activities, and pharmacokinetic parameters of 5-substituted thienopyrimidine derivatives are summarized. Introduction of an isopropyl group at the 5-position maintained a potent MMP-13 inhibitory activity (**31a**), and substitution of the isopropyl group with a phenyl group showed a 3-fold increased MMP-13 inhibitory activity (**31d**). Replacement of the phenyl group with thiophenes maintained potent MMP-13 inhibitory activities (**31b** and **31c**), however, increased unwanted CYP3A4 inhibitory activities. Introduction of an additional substituent at the para position of the phenyl ring tended to reduce MMP-13 inhibitory activities (**31g**, **31j**, and **31m**). On the other hand, substitution at the meta position exhibited a tendency to retain potency even with rather bulky substituent (**31f**, **31i**, and **31l**). Substitution by a fluoro group (**31e**) or a chloro group (**31h**) at the ortho position of the phenyl ring also retained MMP-13 inhibitory activities. However, a methoxy group at the ortho-position of the benzene ring (**31k**) significantly reduced the activity. The reduction of the activity

observed for **31k** compared to **31l** could be attributed to a steric repulsion between the relatively large *o*-methoxy group of **31k** and the wall of the S1' subsite. Five-atom linker compounds, such as **31n**, **31o**, **31p**, and **31q** gave comparable MMP-13 inhibitory activities compared to those of the corresponding four-atom linker series **31e**, **31f**, **31h**, and **31i**, but the five-atom series showed potent CYP3A4 inhibition. It is noteworthy that in contrast to the triazole ZBG series exemplified by **31i** and **31q**, incorporation of the *m*-chloro-substituted 5-aryl group into other triazolone ZBG series was not proved to be well tolerated in terms of MMP-13 inhibitory activity.[19] Among the 5-substituted thienopyrimidine derivatives, *m*-fluorophenyl **31f** showed the best combination of CYP3A4 inhibition risk and oral exposure at a dose of 1 mg/kg in rats and mice (F% = 33 and 38, respectively). The overall properties of **31f** made it an attractive candidate for further preclinical evaluation towards the treatment of MMP-13 related diseases.

Compound **31f**, which exhibited a highly potent MMP-13 inhibitory activity and a promising DMPK profile, was assessed for its selectivity profile against other matrix metalloproteinase homologues including MMP-1, 2, 3, 7, 8, 9, 10, 14, and TACE as shown in **Table 4**. Compound **31f** exhibited 5,000-fold selectivity for MMP-13 over MMP-2, >1,500-fold selectivity over MMP-10, and >27,000-fold selectivity over MMP-1, 3, 7, 8, 9, 14, and TACE. Consequently, compound **31f** is one of the most

selective and potent MMP-13 inhibitors containing a ZBG.

As exemplified by the crystal structure of the triazole inhibitor **3** bound to MMP-13, obtained by soaking experiment, the 1,2,4-triazol-3-yl group of **23** coordinates to the catalytic zinc ion of MMP-13 shown in **Figure 6**. The quinazoline ring system of the hybrid inhibitor **23** fills the deep S1' pocket, the enzyme's specificity pocket, of MMP-13 consisting of Thr245 and Thr247 in the same fashion as the binding mode of the lead quinazoline **1** (**Figure 4** (B)). Although the four-atom linker between the triazole ring and the phenyl group has no obvious interaction with MMP-13, the linker should play an important role in the orientation of the triazolyl group to form the metal binding. The triazolyl group of **23** is coordinated to the zinc ion in a monodentate fashion as that of the lead triazole **2** (**Figure 4** (D)). The inhibitor **23** is stabilized at the S1' site of MMP-13 by three possible hydrogen bonds: (a) the O4 carbonyl oxygen of the quinazoline ring and the backbone amide of Thr247, (b) the N3 amide hydrogen of quinazoline ring and the carbonyl oxygen of Thr245, and (c) the exocyclic carbonyl oxygen at the 2-position of the quinazoline ring and the backbone amide of Thr245. The observed β-sheet type interaction confers the potent inhibitory activity of the quinazolin-4-one-2-carboxamide inhibitors.

Compound **31f** was further evaluated for its ability to block the release of collagen from cartilage. To examine the chondroprotective effect of the MMP-13 selective inhibitor, a bovine nasal cartilage (BNC) assay was performed. The BNC assay is an established and widely used procedure that allows evaluation of cartilage degradation by upregulating proteolytic enzymes such as the collagenases MMP-1 and MMP-13 in vitro.[43-49] The chondrocyte-mediated degradation of cartilage was studied using bovine nasal cartilage slices cultured for up to 14 days in the presence or absence of MMP inhibitors. Collagen release, measured quantitatively as hydroxyproline, was stimulated by pro-inflammatory cytokines interleukin-1 (IL-1) and oncostatin M (OSM).

As shown in **Table 5**, the protection of cartilage degradation by compound **31f** in this assay was concentration dependent and statistically significant at 1 μM (70.8% of inhibition), whereas at the same concentration of 1 μM, **32** (**RS-130,830**[50]), which inhibits MMPs broadly, showed complete inhibition. This difference of inhibitory activity profile between **31f** and **32** in this assay may be explained by high isoform selectivity of **31f** for MMP-13 over other collagenolytic enzymes in the chondroprotective efficacy and/or a difference in the degree of tissue permeability of the two inhibitors.

CONCLUSIONS

On the basis of the superposition of X-ray crystal structures of the complexes of quinazoline derivative **1** and triazole derivative **2** with MMP-13 catalytic domain, we have designed and identified a new class of MMP-13 selective inhibitors that possesses a 1,2,4-triazol-3-yl group as a ZBG, and developed an efficient synthetic method to prepare quinazoline-2-carboxamide and thienopyrimidine-2-carboxamide derivatives. Quinazoline and its isosteric thienopyrimidine derivatives that were linked via the 2-position with the 1,2,4-triazol-3-yl group by a 4-atom linker induced a considerable increase in potency. Particularly, diether linked 1,2,4-triazol-3-yl derivatives retained potent inhibition and reduced undesirable inhibition of CYP3A4. X-ray analysis of the complex, as exemplified by **23** with the MMP-13 catalytic domain, confirmed that the 1,2,4-triazol-3-yl group directly interacts with the catalytic zinc ion and that the quinazoline core is buried deeply into the S1' pocket by forming a β-sheet type interaction with hydrogen bonding to the enzyme's backbone spanning the S1' pocket. Among a series of quinazoline and thienopyrimidine derivatives investigated, 5-(3-fluorophenyl)-4-oxo-*N*-{3-[2-(1*H*-1,2,4-triazol-3-yl-oxy)ethoxy]benzyl}-3,4-dihyd

rothieno[2,3-*d*]pyrimidine-2-carboxamide (**31f**) exhibited excellent potency (IC_{50} = 0.036 nM) and selectivity (greater than 1,500-fold) over other MMPs (MMP-1, 2, 3, 7, 8, 9, 10, and 14) and TACE, and demonstrated favorable pharmacokinetic parameters, making it an attractive candidate for further studies. To evaluate compound **31f** for its potential utility in the treatment of collagenase related disease and disorders, BNC assay was employed. The selective MMP-13 inhibitor **31f** was effective at preventing the IL-1/OSM induced in vitro degradation of BNC (70.8% inhibition of cartilage degradation at 1 μM). Taken together with the fact that MMP-13 is indicated as the primary collagenase in the human OA cartilage, selective MMP-13 inhibitors may be a potential treatment of OA while avoiding the toxicity associated with inhibition of MMPs other than MMP-13.

EXPERIMENTAL SECTION

General Methods. 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 Bruker DPX-300 (300 MHz) or a Bruker Avance 400 (400 MHz) spectrometer and are reported in parts per million (δ) relative to tetramethylsilane (TMS: δ 0.00 ppm). Data

are reported as follows: chemical shift, integration, multiplicity (s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet, dd = doublet of doublet, dt = doublet of triplet, tt = triplet of triplet, 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 60F254. Preparative HPLC was performed on a Shiseido CAPCELL PACK C-18 UG120 S-5 column (20 mmΦ × 50 mm), eluting at a flow rate of 25 mL/min with a linear gradient of water (0.1% TFA)/acetonitrile (0.1% TFA) from 90:10 to 0:100 over 10 min. UV detection was at 220 nm.

The purity of all compounds used in biological studies was determined to be ≥ 95% by elemental analysis or HPLC analysis. Elemental analyses were performed by Takeda Analytical Research Laboratories, Ltd. Experimentally determined hydrogen, carbon, and nitrogen composition by elemental analysis was within ± 0.4% of the expected value, implying a purity of ≥ 95%. Liquid chromatography−mass spectrometry (LC/MS) analysis was performed using one of the following two conditions: (a) L-column 2 ODS (50 mm × 3.0 mm I.D., 3 μm particle size, CERI, Japan) in a Shimadzu LC-20AD equipped with a Shimadzu LCMS-2020 eluting with 5 mM

$AcONH_4$ in ultrapure water/acetonitrile = 90/10 (mobile phase A) and 5 mM $AcONH_4$ in ultrapure water/acetonitrile = 10/90 (mobile phase B), using the following elution gradient of 5% B to 90% B over 0.9 min followed by 90% B isocratic over 1.1 min at a flow rate of 1.5 mL/min (UV detection at 220 or 254 nm); (b) 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.

Ethyl 4-[(3-cyanophenyl)oxy]butanoate (**5**). A solution of 4-bromobutanoic acid (25.0 g, 150 mmol), DMF (5 drops), and oxalyl chloride (17.0 mL, 195 mmol) in dichloromethane (250 mL) was stirred at room temperature for 5 h. The reaction mixture was concentrated under reduced pressure, dichloromethane (200 mL) and ethanol (9.0 mL, 170 mmol) were added to the residue, and the mixture was stirred at room temperature for 15 h. The reaction mixture was washed with saturated aqueous $NaHCO_3$ solution and brine, and the organic layer was dried over Na_2SO_4, and concentrated. The residue was purified by silica gel column chromatography (0–5% ethyl acetate/hexane) to give ethyl 4-bromobutanoate as a colorless oil (24.5 g, 84%). A

suspension of 3-hydroxybenzonitrile (**4**) (5.00 g, 42.0 mmol) and 60% sodium hydride (oil dispersion, 2.01 g, 50.3 mmol) in DMF (200 mL) was stirred at room temperature for 30 min, and ethyl 4-bromobutanoate (9.82 g, 50.3 mmol) was added to the mixture. The mixture was stirred at room temperature for 15 h and concentrated under reduced pressure. The residue was extracted with ethyl acetate and saturated aqueous NH_4Cl solution. The organic layer was washed with saturated aqueous NH_4Cl solution and brine, dried over Na_2SO_4, and concentrated. The obtained residue was purified by silica gel column chromatography (0–10% ethyl acetate/hexane) to give the title compound as a colorless oil (10.2 g, quant.). ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.27 (3H, t, J = 7.2 Hz), 2.13 (2H, tt, J = 6.7, 6.7 Hz), 2.52 (2H, t, J = 7.2 Hz), 4.03 (2H, t, J = 6.1 Hz), 4.16 (2H, q, J = 7.2 Hz), 7.08–7.16 (2H, m), 7.21–7.26 (1H, m), 7.32–7.40 (1H, m).

Ethyl 4-{[3-(Aminomethyl)phenyl]oxy}butanoate Hydrochloride (**6**). A suspension of compound **5** (8.50 g, 36.4 mmol), 10% palladium on carbon (containing 50% water) (12.8 g), and formic acid (98 mL) in methanol (80 mL) was stirred at room temperature for 5 h under hydrogen atmosphere at 1 atm. The catalyst was filtered off through a Celite pad, and the filtrate was concentrated under reduced pressure. To the residue was added 4 N hydrogen chloride in ethyl acetate solution (15.0 mL, 60.0 mmol). The mixture was stirred at room temperature for 1 h and concentrated under reduced

pressure. The residue was crystallized from toluene, and the crude crystals were recrystallized from diethyl ether to give the title compound as a white powder (7.79 g, 78% for 2 steps). ^{1}H NMR (300 MHz, DMSO-d_6) δ 1.18 (3H, t, J = 7.2 Hz), 1.98 (2H, tt, J = 6.8, 6.8 Hz), 2.46 (2H, t, J = 7.3 Hz), 3.95–4.03 (4H, m), 4.07 (2H, q, J = 7.1 Hz), 6.92 (1H, dd, J = 8.2, 2.2 Hz), 7.04 (1H, d, J = 7.3 Hz), 7.12 (1H, s), 7.31 (1H, t, J = 7.9 Hz), 8.26 (3H, s).

3-[(2-Chloroethyl)oxy]benzonitrile (**7a**). A suspension of 3-hydroxybenzonitrile (**4**) (5.00 g, 42.0 mmol), 1-bromo-2-chloroethane (9.00 g, 62.8 mmol), and potassium hydroxide (2.50 g, 44.6 mmol) in ethanol (100 mL) was stirred at 90 °C for 24 h. After cooling to room temperature, the reaction mixture was concentrated under reduced pressure. To the residue were added diethyl ether and water. The organic layer was washed with brine, dried over Na_2SO_4, and concentrated. The residual oil was purified by silica gel column chromatography (2–50% ethyl acetate/hexane) to give the title compound as a colorless oil (1.39 g, 18%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 3.83 (2H, t, J = 5.7 Hz), 4.25 (2H, t, J = 5.7 Hz), 7.14–7.19 (2H, m), 7.29 (1H, dt, J = 7.6, 1.3 Hz), 7.37–7.43 (1H, m).

3-[(3-Chloropropyl)oxy]benzonitrile (**7b**). A suspension of 1-bromo-3-chloropropane

(13.9 g, 88.1 mmol) and 60% sodium hydride (oil dispersion, 3.02 g, 126 mmol) in ethanol (50 mL) was stirred at room temperature for 30 min. To the reaction mixture was added 3-hydroxybenzonitrile (**4**) (10.0 g, 83.9 mmol) at 0 °C, and the mixture was stirred at 60 °C for 15 h and concentrated under reduced pressure. To the residue was added ethyl acetate and water. The organic layer was washed with brine, dried over Na_2SO_4, and concentrated. The residual oil was purified by silica gel column chromatography (5–15% ethyl acetate/hexane) to give the title compound as a colorless oil (14.2 g, 86%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 2.26 (2H, tt, J = 6.0, 6.0 Hz), 3.75 (2H, t, J = 6.2 Hz), 4.14 (2H, t, J = 5.8 Hz), 7.11–7.18 (2H, m), 7.23–7.28 (1H, m), 7.34–7.41 (1H, m).

3-[(4-Chlorobutyl)oxy]benzonitrile (**7c**). Compound **7c** was prepared from 3-hydroxybenzonitrile (**4**) and 1-bromo-4-chlorobutane with a similar procedure as described for compound **7b** (colorless oil, 95%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.94–2.02 (4H, m), 3.60–3.66 (2H, m), 4.02 (2H, t, J = 5.4 Hz), 7.08–7.15 (2H, m), 7.21–7.27 (1H, m), 7.33–7.41 (1H, m).

3-{[2-(1*H*-1,2,4-Triazol-3-ylthio)ethyl]oxy}benzonitrile (**8a**). A solution of compound **7a** (11.3 g, 62.3 mmol), 1*H*-1,2,4-triazole-3-thiol (6.00 g, 59.3 mmol), and triethylamine

(8.40 mL, 62.3 mmol) in ethanol (50 mL) was stirred at 80 °C for 15 h. After cooling to room temperature, the reaction mixture was concentrated under reduced pressure. To the residue were added ethyl acetate and water. The organic layer was washed with brine, dried over Na_2SO_4, and concentrated to give the title compound as a white powder (14.4 g, 98%). ^{1}H NMR (300 MHz, DMSO-d_6) δ 3.48 (2H, t, J = 6.7 Hz), 4.32 (2H, t, J = 6.6 Hz), 7.29–7.36 (1H, m), 7.38–7.43 (1H, m), 7.44–7.54 (2H, m), 8.46 (1H, s), 14.09 (1H, s).

3-{[3-(1*H*-1,2,4-Triazol-3-ylthio)propyl]oxy}benzonitrile (**8b**). Compound **8b** was prepared from compound **7b** and 1*H*-1,2,4-triazole-3-thiol with a similar procedure as described for compound **8a** (white powder, quant.). ^{1}H NMR (300 MHz, DMSO-d_6) δ 2.11 (2H, tt, J = 6.8, 6.6 Hz), 3.22 (2H, t, J = 7.1 Hz), 4.14 (2H, t, J = 6.2 Hz), 7.26–7.53 (4H, m), 8.42 (1H, s), 14.03 (1H, s).

3-[(3-{[1-(Triphenylmethyl)-1*H*-1,2,4-triazol-3-yl]thio}propyl)oxy]benzonitrile (**8c**). A solution of compound **8b** (4.00 g, 15.4 mmol), triphenylmethyl chloride (6.43 g, 23.0 mmol), and triethylamine (2.33 g, 23.0 mmol) in THF (50 mL) was stirred at room temperature for 48 h and concentrated under reduced pressure. To the residue were added ethyl acetate and water. The organic layer was washed with brine, dried over

Na_2SO_4, and concentrated. The obtained oil was purified by silica gel column chromatography (5–40% ethyl acetate/hexane) to give the title compound as a white powder (3.52 g, 46%). ^{1}H NMR (300 MHz, DMSO-d_6) δ 2.00–2.09 (2H, m), 3.16 (2H, t, J = 7.1 Hz), 4.02–4.09 (2H, m), 7.01–7.11 (6H, m), 7.19–7.49 (13H, m), 8.13 (1H, s).

3-{[4-(1*H*-1,2,4-Triazol-3-ylthio)butyl]oxy}benzonitrile (**8d**). Compound **8d** was prepared from compound **7c** and 1*H*-1,2,4-triazole-3-thiol with a similar procedure as described for compound **8a** (white powder, quant.). ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.87–2.02 (5H, m), 3.20–3.31 (2H, m), 3.96–4.05 (2H, m), 7.06–7.16 (2H, m), 7.20–7.27 (1H, m), 7.32–7.40 (1H, m), 8.13 (1H, s).

3-[(4-{[1-(Triphenylmethyl)-1*H*-1,2,4-triazol-3-yl]thio}butyl)oxy]benzonitrile (**8e**). Compound **8e** was prepared from compound **8d** with a similar procedure as described for compound **8c** (white powder, 78%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.81–1.89 (4H, m), 3.07–3.16 (2H, m), 3.83–3.90 (2H, m), 7.01–7.06 (2H, m), 7.11–7.17 (6H, m), 7.19–7.24 (1H, m), 7.28–7.36 (10H, m), 7.87 (1H, s).

1-(3-{[2-(1*H*-1,2,4-Triazol-3-ylthio)ethyl]oxy}phenyl)methanamine (**9a**). A solution of compound **8a** (9.00 g, 36.5 mmol) and Raney-nickel (5.00 g) in 5 N ammonia in methanol solution (300 mL) was stirred at room temperature under hydrogen

atmosphere at 1 atm for 15 h. The catalyst was filtered off through a Celite pad, and the filtrate was concentrated. The obtained residue was crystallized from toluene to give the title compound as a pale blue powder (6.45 g, 71%). The compound was used without further purification.

1-{3-[(3-{[1-(Triphenylmethyl)-1*H*-1,2,4-triazol-3-yl]thio}propyl)oxy]phenyl}metha namine (**9b**). Compound **9b** was prepared from compound **8c** with a similar procedure as described for compound **15a** (pale yellow oil, 95%). ^{1}H NMR (300 MHz, DMSO-d_6) δ 1.94–2.08 (2H, m), 3.16 (2H, t, J = 7.1 Hz), 3.62–3.67 (2H, m), 3.97 (2H, t, J = 6.1 Hz), 6.60–6.74 (1H, m), 6.82–6.92 (2H, m), 6.98–7.11 (6H, m), 7.12–7.45 (12H, m), 8.14 (1H, s).

1-{3-[(4-{[1-(Triphenylmethyl)-1*H*-1,2,4-triazol-3-yl]thio}butyl)oxy]phenyl}methan amine (**9c**). Compound **9c** was prepared from compound **8e** with a similar procedure as described for compound **15a** (yellow oil, 86%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.79–1.89 (4H, m), 2.35 (2H, s), 3.09–3.16 (2H, m), 3.81 (2H, s), 3.85–3.93 (2H, m), 6.71 (1H, dd, J = 8.1, 2.3 Hz), 6.81 (1H, s), 6.87 (1H, d, J = 7.0 Hz), 7.08–7.37 (16H, m), 7.86 (1H, s).

3-Nitro-1-(triphenylmethyl)-1*H*-1,2,4-triazole (**11**). A solution of

3-nitro-1*H*-1,2,4-triazole (**10**) (1.00 g, 8.77 mmol), triphenylmethyl chloride (4.89 g, 17.5 mmol), and *N*,*N*-diisopropylethylamine (3.05 mL, 17.5 mmol) in THF (50 mL) was stirred at room temperature for 15 h. The mixture was concentrated under reduced pressure, and the residue was extracted with ethyl acetate and water. The organic layer was washed with brine, dried over Na_2SO_4, and concentrated. The residue was purified by silica gel column chromatography (15–30% ethyl acetate/hexane) to give the title compound as a white powder (2.90 g, 93%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.08–7.17 (6H, m), 7.33–7.47 (9H, m), 8.04 (1H, s).

Ethyl [(3-Cyanophenyl)oxy]acetate (**12**). Ethyl bromoacetate (14.7 g, 88.1 mmol) was added dropwise to a suspension of potassium carbonate (12.8 g, 92.3 mmol) and 3-hydroxybenzonitrile (**4**) (10.0 g, 83.9 mmol) in THF (50 mL) at room temperature, and the mixture was stirred at 50 °C for 24 h. The mixture was extracted with ethyl acetate and saturated aqueous NH_4Cl solution, and the organic layer was washed with brine, dried over Na_2SO_4, and concentrated to give the title compound as a pale yellow powder (17.5 g, 99%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.31 (3H, t, J = 7.2 Hz), 4.29 (2H, q, J = 7.1 Hz), 4.65 (2H, s), 7.13–7.18 (2H, m), 7.28–7.32 (1H, m), 7.36–7.44 (1H, m).

3-[(2-Hydroxyethyl)oxy]benzonitrile (**13a**). A suspension of compound **12** (8.00 g,

39.0 mmol) and sodium borohydride (1.47 g, 39.0 mmol) in ethanol (120 mL) was stirred at 50 °C for 15 h, and the mixture was concentrated under reduced pressure. The residue was extracted with ethyl acetate and water, and the organic layer was washed with brine, dried over Na_2SO_4, and concentrated to give the title compound as a white powder (5.85 g, 92%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.67 (1H, bs), 3.97–4.02 (2H, m), 4.08–4.13 (2H, m), 7.13–7.20 (2H, m), 7.24–7.30 (1H, m), 7.35–7.43 (1H, m).

3-[(2-{[1-(Triphenylmethyl)-1*H*-1,2,4-triazol-3-yl]oxy}ethyl)oxy]benzonitrile (**14a**). A solution of compound **13a** (19.2 g, 118 mmol) in THF (100 mL) was added dropwise to a suspension of compound **11** (40.0 g, 112 mmol) and 60% sodium hydride (oil dispersion, 6.06 g, 152 mmol) in THF (300 mL), and the mixture was stirred at room temperature for 12 h. To the mixture cooled to 0 °C were added water and ethyl acetate. The organic layer was washed with brine, dried over Na_2SO_4, and concentrated. The residue was purified by silica gel column chromatography (0–30% ethyl acetate/hexane) to give the title compound as a white powder (25.0 g, 46%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 4.23–4.29 (2H, m), 4.56–4.63 (2H, m), 7.07–7.41 (19H, m), 7.67 (1H, s).

3-[(3-{[1-(Triphenylmethyl)-1*H*-1,2,4-triazol-3-yl]oxy}propyl)oxy]benzonitrile (**14b**). A solution of compound **13b**[39] (4.97 g, 28.1 mmol) in THF (50 mL) was added

dropwise to a mixture of compound **11** (10.0 g, 28.1 mmol) and 60% sodium hydride (oil dispersion, 2.25 g, 56.1 mmol) in THF (150 mL) at room temperature and the resulting mixture was stirred at room temperature for 15 h. 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 to give the title compound as a pale yellow amorphous (14.0 g). The crude **14b** was used for the next reaction without further purification. ^{1}H NMR (300 MHz, DMSO-d_6) δ 2.08–2.19 (2H, m), 4.14 (2H, t, J = 6.2 Hz), 4.30 (2H, t, J = 6.2 Hz), 7.04–7.12 (6H, m), 7.24–7.30 (1H, m), 7.34–7.43 (11H, m), 7.47 (1H, t, J = 7.9 Hz), 7.82 (1H, s).

1-{3-[(2-{[1-(Triphenylmethyl)-1*H*-1,2,4-triazol-3-yl]oxy}ethyl)oxy]phenyl}methanamine (**15a**). To a suspension of lithium aluminum hydride (3.21 g, 84.6 mmol) in THF (30 mL) was added dropwise a solution of compound **14a** (4.00 g, 8.46 mmol) in THF (20 mL) at room temperature, and the mixture was stirred at room temperature for 4 h. To the mixture cooled to 0 °C was added Na_2SO_4•$10H_2O$ (10.9 g, 33.9 mmol). The mixture was stirred at room temperature for 30 min. The insoluble material was filtered off through a Celite pad. The filtrate was dried over Na_2SO_4 and concentrated. The residual solid was washed with diethylether and dried to give the title compound as a white powder (2.35 g, 58%). ^{1}H NMR (300 MHz, DMSO-d_6) δ 1.58–2.00 (2H, m), 3.66

(2H, s), 4.20–4.27 (2H, m), 4.45 (2H, dd, *J* = 5.5, 3.4 Hz), 6.75 (1H, dd, *J* = 8.1, 2.1 Hz), 6.86–6.96 (2H, m), 7.06–7.14 (6H, m), 7.19 (1H, t, *J* = 7.8 Hz), 7.34–7.44 (9H, m), 7.86 (1H, s).

1-{3-[(3-{[1-(Triphenylmethyl)-1*H*-1,2,4-triazol-3-yl]oxy}propyl)oxy]phenyl}metha namine (**15b**). Compound **15b** was prepared from compound **14b** with a similar procedure as described for compound **15a** (white powder, 77%). ^{1}H NMR (300 MHz, DMSO-d_6) δ 2.06–2.17 (2H, m), 3.65 (2H, s), 3.99–4.09 (2H, m), 4.29 (2H, t, *J* = 6.3 Hz), 6.70–6.76 (1H, m), 6.84–6.89 (1H, m), 6.90–6.93 (1H, m), 7.05–7.11 (6H, m), 7.12–7.32 (1H, m), 7.34–7.41 (9H, m), 7.82 (1H, s).

Ethyl 3-[2-(Aminocarbonothioyl)hydrazino]-3-oxopropanoate (**17a**). To a suspension of hydrazinecarbothioamide (**16**) (15.0 g, 165 mmol) in pyridine (100 mL) was added dropwise ethyl 3-chloro-3-oxopropanoate (24.8 g, 165 mmol) over 30 min at 0 °C, and the reaction mixture was stirred at room temperature for 2 days. The reaction mixture was concentrated under reduced pressure, and methanol was added to the residue. The resulting solid was filtered off, the filtrate was concentrated under reduced pressure, and to the residue were added ethyl acetate, THF, and saturated aqueous NH_4Cl solution. The organic layer was washed with saturated aqueous NH_4Cl solution,

dried over Na_2SO_4, and concentrated. The residue was crystallized from ethyl acetate and diisopropyl ether to give the title compound as a pale yellow powder (14.2 g, 42%). ^{1}H NMR (300 MHz, DMSO-d_6) δ 1.10–1.27 (3H, m), 3.28 (2H, s), 4.09 (2H, q, J = 7.2 Hz), 7.36 (1H, s), 7.98 (1H, s), 9.38 (1H, s), 10.02 (1H, s).

Ethyl 4-[2-(Aminocarbonothioyl)hydrazino]-4-oxobutanoate (**17b**). Ethyl 4-[2-(aminocarbonothioyl) hydrazino]-4-oxobutanoate (**17b**) was prepared from hydrazinecarbothioamide (**16**) and ethyl 4-chloro-4-oxobutanoate with a similar procedure as described for compound **17a** (white powder, 35%). ^{1}H NMR (400 MHz, DMSO-d_6) δ 1.18 (3H, t, J = 7.1 Hz), 2.33–2.47 (2H, m), 2.52–2.67 (2H, m), 4.04 (2H, q, J = 7.2 Hz), 7.30 (1H, bs), 7.89 (1H, bs), 9.22 (1H, s), 9.83 (s, 1H).

Ethyl (5-Mercapto-1*H*-1,2,4-triazol-3-yl)acetate (**18a**). A suspension of compound **17a** (14.0 g, 68.2 mmol) and sodium ethoxide (9.52 g, 140 mmol) in ethanol (200 mL) was stirred at 80 °C for 15 h. After cooling to room temperature, the mixture was concentrated under reduced pressure, and the residue was acidified with 1 N hydrochloric acid and extracted with ethyl acetate. The organic layer was washed with brine, dried over Na_2SO_4, and concentrated to give the title compound as an orange powder (12.2 g, 96%). ^{1}H NMR (300 MHz, DMSO-d_6) δ 1.20 (3H, t, J = 7.1 Hz), 3.74

(2H, s), 4.12 (2H, q, J = 7.0 Hz).

Ethyl 3-(5-Mercapto-1*H*-1,2,4-triazol-3-yl)propanoate (**18b**). Compound **18b** was prepared from compound **17b** with a similar procedure as described for compound **18a** (white powder, 91%). ^{1}H NMR (300 MHz, DMSO-d_6) δ 1.01–1.28 (3H, m), 2.64–2.83 (4H, m), 4.05 (2H, q, J = 7.2 Hz), 13.10 (1H, bs), 13.21 (1H, bs).

Methyl 1-(Triphenylmethyl)-1*H*-1,2,4-triazole-3-carboxylate (**19b**). Compound **19b** was prepared from commercially available methyl 1*H*-1,2,4-triazole-3-carboxylate (**19a**) with a similar procedure as described for compound **11** (TrCl, DIEA, 60 °C) (white powder, 59%). ^{1}H NMR (300 MHz, DMSO-d_6) δ 3.84 (3H, s), 7.07 (6H, dd, J = 6.8, 2.8 Hz), 7.34–7.46 (9H, m), 8.39 (1H, s).

Ethyl 1*H*-1,2,4-Triazol-3-ylacetate (**19c**). A suspension of compound **18a** (2.00 g, 10.7 mmol) and Raney-nickel (5.00 g) in ethanol (20 mL) was stirred at 80 °C for 15 h. The catalyst was filtered off through a Celite pad, and the filtrate was concentrated under reduced pressure. To a suspension of the residue in ethyl acetate was added activated carbon, and the mixture was filtered. The filtrate was concentrated under reduced pressure to give the title compound as a pale green powder (900 mg, 54%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.32 (3H, t, J = 6.8 Hz), 3.96 (2H, s), 4.26 (2H, q, J = 6.5 Hz),

8.03 (1H, s), 11.53 (1H, bs).

3-(1*H*-1,2,4-Triazol-3-yl)propanoic Acid (**19d**). Compound **18b** (650 mg, 3.23 mmol) was added slowly to a solution of sodium nitrite (8.91 mg, 0.129 mmol) and nitric acid (3 mL) in water (6 mL), maintaining the temperature below 45 °C. The solution was stirred at room temperature for 15 h, and neutralized with saturated aqueous $NaHCO_3$ solution. The mixture was concentrated under reduced pressure. The residue was triturated with THF, collected on a filter, and washed with THF. The collected solid was suspended in ethanol. The insoluble material was filtered off, and the filtrate was concentrated under reduced pressure to give the title compound as a pale yellow powder (454 mg, 99%). ^{1}H NMR (300 MHz, DMSO-d_6) δ 2.24 (2H, t, J = 7.5 Hz), 2.80 (2H, t, J = 7.5 Hz), 7.82 (1H, s).

Ethyl 3-(1*H*-1,2,4-Triazol-3-yl)propanoate (**19e**). To a suspension of compound **19d** (11.6 g, 3.54 mmol) in ethanol (100 mL) was added a 2 N hydrogen chloride in ethanol solution (100 mL) at room temperature. The mixture was stirred at 90 °C for 15 h. After cooling to room temperature, the mixture was filtered through a Celite pad, and the filtrate was concentrated under reduced pressure. To the residue was added ethyl acetate and water. The aqueous layer was neutralized with saturated aqueous $NaHCO_3$ solution

and extracted with ethyl acetate. The organic layer was washed with brine, dried over Na_2SO_4, and concentrated to give the title compound as a yellow oil (4.36 g, 33%). ^{1}H NMR (300 MHz, DMSO-d_6) δ 1.11–1.20 (3H, m), 2.72 (2H, d, J = 1.7 Hz), 2.92 (2H, s), 4.00–4.08 (2H, m), 7.79 (1H, s), 13.59 (1H, bs).

Ethyl 3-[1-(Triphenylmethyl)-1*H*-1,2,4-triazol-3-yl]propanoate (**19f**). Compound **19f** was prepared from compound **19e** with a similar procedure as described for compound **11** (TrCl, DIEA, rt) (pale yellow oil, 83%). ^{1}H NMR (300 MHz, DMSO-d_6) δ 1.10 (3H, t, J = 7.2 Hz), 2.67 (2H, t, J = 7.1 Hz), 2.90 (2H, t, J = 7.0 Hz), 3.98 (2H, q, J = 6.9 Hz), 6.99–7.08 (6H, m), 7.34–7.40 (9H, m), 7.95 (1H, s).

1-(Triphenylmethyl)-1*H*-1,2,4-triazole-3-carboxylic Acid (**20a**). Compound **20a** was prepared from compound **19b** with a similar procedure as described for compound **22b** (white powder, 98%). ^{1}H NMR (300 MHz, DMSO-d_6) δ 7.07 (6H, dd, J = 6.9, 2.9 Hz), 7.30–7.56 (9H, m), 8.31 (1H, s), 13.49 (1H, s).

(1*H*-1,2,4-Triazol-3-yl)acetic Acid (**20b**). Compound **20b** was prepared from compound **19c** with a similar procedure as described for compound **22b** (white powder, 50%). ^{1}H NMR (300 MHz, DMSO-d_6) δ 3.49 (2H, s), 7.92 (1H, s).

3-[1-(Triphenylmethyl)-1*H*-1,2,4-triazol-3-yl]propanoic Acid (**20c**). Compound **20c**

was prepared from compound **19f** with a similar procedure as described for compound **22b** (white powder, 85%). ^{1}H NMR (300 MHz, DMSO-d_6) δ 2.59 (2H, t, J = 7.3 Hz), 2.86 (2H, t, J = 7.3 Hz), 7.04 (6H, dd, J = 6.7, 2.9 Hz), 7.33–7.45 (9H, m), 7.93 (1H, s), 12.17 (1H, bs).

Ethyl 4-{[3-({[(4-Oxo-3,4-dihydroquinazolin-2-yl)carbonyl]amino}methyl)phenyl]oxy}butanoate (**22a**). A suspension of ethyl 4-oxo-3,4-dihydro-2-quinazolinecarboxylate (**21**) (500 mg, 2.29 mmol), ethyl 4-{[3-(aminomethyl)phenyl]oxy}butanoate hydrochloride (**6**) (878 mg, 3.21 mmol), and *N*,*N*-diisopropylethylamine (0.798 mL, 4.58 mmol) in THF (10 mL) was stirred at 80 °C for 15 h. The reaction mixture was evaporated under reduced pressure, and to the residue were added ethyl acetate and 0.1 N aqueous hydrochloric acid solution. The organic layer was washed with saturated aqueous NH_4Cl solution, dried over Na_2SO_4, and concentrated. The residual solid was recrystallized from ethyl acetate to give the title compound as a white powder (282 mg, 30%). Evaporation of the filtrate gave another crop of the title compound as a white powder (525 mg, 56%). mp 130–131 °C. ^{1}H NMR (300MHz, DMSO-d_6) δ 1.15 (3H, t, J = 7.1 Hz), 1.89–2.01 (2H, m), 2.44 (2H, t, J = 7.3 Hz), 3.96 (2H, t, J = 6.3 Hz), 4.04 (2H, q, J = 7.0 Hz), 4.45 (2H, d, J = 6.2 Hz), 6.77–6.84 (1H, m), 6.88–6.94 (2H, m),

7.19–7.27 (1H, m), 7.57–7.65 (1H, m), 7.75–7.81 (1H, m), 7.84–7.94 (1H, m), 8.17 (1H, dd, J = 7.9, 1.1 Hz), 9.54 (1H, t, J = 6.3 Hz), 12.29 (1H, bs). Anal. Calcd for $C_{22}H_{23}N_3O_5$: C, 64.54; H, 5.66; N, 10.26. Found: C, 64.30; H, 5.51; N, 10.22.

4-{[3-({[(4-Oxo-3,4-dihydroquinazolin-2-yl)carbonyl]amino}methyl)phenyl]oxy}butanoic Acid (**22b**). A mixture of compound **22a** (525 mg, 1.28 mmol), 4 N aqueous sodium hydroxide solution (1.6 mL) in THF (10 mL), methanol (10 mL), and water (10 mL) was stirred at 100 °C for 2 h. The mixture was allowed to cool to room temperature, and the solvent was evaporated under reduced pressure. Water and 1 N hydrochloric acid (6.41 mL) were added to the residue, and the resulting precipitate was collected by filtration, washed with water, and dried. The obtained crude crystals were recrystallized from ethanol to give the title compound as a white powder (339 mg, 69%). mp 189–190 °C. ^{1}H NMR (300MHz, DMSO-d_6) δ 1.85–1.99 (2H, m), 2.37 (2H, t, J = 7.3 Hz), 3.96 (2H, t, J = 6.4 Hz), 4.45 (2H, d, J = 6.4 Hz), 6.76–6.86 (1H, m), 6.88–6.96 (2H, m), 7.23 (1H, t, J = 8.1 Hz), 7.56–7.67 (1H, m), 7.75–7.82 (1H, m), 7.83–7.93 (1H, m), 8.18 (1H, dd, J = 7.9, 1.1 Hz), 9.55 (1H, t, J = 6.3 Hz), 12.21(2H, s). Anal. Calcd for $C_{20}H_{19}N_3O_5$: C, 62.99; H, 5.02; N, 11.02. Found: C, 62.74; H, 5.04; N, 10.98.

***N*-[(3-{[4-(Hydroxyamino)-4-oxobutyl]oxy}phenyl)methyl]-4-oxo-3,4-dihydroquina**

zoline-2-carboxamide (**22c**). To a solution of compound **22b** (90.0 mg, 0.236 mmol) in THF (2 mL) were added DMF (0.02 mL) and oxalyl chloride (59.9 mg, 0.472 mmol) at 0 °C, and the mixture was stirred at room temperature for 2 h. To the reaction mixture was added a mixed solution of 50% aqueous hydroxylamine solution (0.5 mL), *tert*-butanol (0.5 mL), and THF (0.5 mL) at 0 °C, and the mixture was stirred for 15 min. The mixture was concentrated under reduced pressure, and the residue was purified by preparative HPLC and recrystallization from ethyl acetate-hexane to give the title compound as a pale yellow powder (8.0 mg, 9%). mp 164–168 °C. ^{1}H NMR (300MHz, DMSO-d_6) δ 1.85–2.00 (2H, m), 2.07–2.16 (2H, m), 3.89–3.98 (2H, m), 4.44 (2H, d, J = 7.0 Hz), 6.77–6.95 (3H, m), 7.23 (1H, t, J = 7.9 Hz), 7.56 (1H, t, J = 7.8 Hz), 7.72–7.89 (2H, m), 8.15 (1H, d, J = 7.9 Hz), 9.48 (1H, bs), 10.41 (1H, bs), 12.22 (1H, s).

4-Oxo-*N*-[(3-{[2-(1*H*-1,2,4-triazol-3-ylthio)ethyl]oxy}phenyl)methyl]-3,4-dihydroquinazoline-2-carboxamide (**23**). A suspension of compound **21** (200 mg, 0.917 mmol), 1-(3-{[2-(1*H*-1,2,4-triazol-3-ylthio)ethyl]oxy}phenyl)methanamine (**9a**) (298 mg, 1.19 mmol), and *N*,*N*-diisopropylethylamine (237 mg, 1.83 mmol) in ethanol (10 mL) was stirred at 90 °C for 15 h. After cooling to room temperature, the mixture was evaporated under reduced pressure, and the residue was extracted with ethyl acetate and water. The organic layer was washed with brine, dried over Na_2SO_4, and concentrated. The residue

was purified by preparative HPLC and recrystallization from ethanol to give the title compound as a white powder (29.2 mg, 8%). mp 177–179 °C. ^{1}H NMR (300 MHz, DMSO-d_6) δ 3.45 (2H, t, J = 6.5 Hz), 4.23 (2H, t, J = 6.7 Hz), 4.45 (2H, d, J = 6.2 Hz), 6.85 (1H, dd, J = 8.6, 1.6 Hz), 6.90–6.98 (2H, m), 7.24 (1H, t, J = 7.9 Hz), 7.61 (1H, t, J = 7.0 Hz), 7.78 (1H, d, J = 7.7 Hz), 7.85–7.92 (1H, m), 8.17 (1H, d, J = 7.7 Hz), 9.50–9.57 (1H, m). Anal. Calcd for $C_{20}H_{18}N_6O_3S$: C, 56.86; H, 4.29; N, 19.89. Found: C, 56.77; H, 4.23; N, 19.65.

5-Methyl-4-oxo-*N*-[(3-{[2-(1*H*-1,2,4-triazol-3-ylthio)ethyl]oxy}phenyl)methyl]-3,4-dihydro-thieno[2,3-*d*]pyrimidine-2-carboxamide (**25a**). A suspension of ethyl 5-methyl-4-oxo-3,4-dihydrothieno[2,3-*d*]pyrimidine-2-carboxylate (**24**) (200 mg, 0.839 mmol) and 1-(3-{[2-(1*H*-1,2,4-triazol-3-ylthio)ethyl]oxy}phenyl)methanamine (**9a**) (305 mg, 1.22 mmol) in ethanol (10 mL) and DMA (2 mL) was stirred at 90 °C for 3 days. The reaction mixture was evaporated under reduced pressure, and to the residue were added ethyl acetate and 0.1 N aqueous hydrochloric acid solution. The organic layer was washed with saturated aqueous NH_4Cl solution, dried over Na_2SO_4, and concentrated. The residual solid was recrystallized from ethanol to give the title compound as a white powder (67.2 mg, 18%). mp 178–179 °C. ^{1}H NMR (300 MHz, DMSO-d_6) δ 3.45 (2H, t, J = 6.5 Hz), 3.56–3.64 (1H, m), 4.22 (2H, t, J = 6.4 Hz), 4.40

(2H, d, *J* = 6.2 Hz), 6.80–6.96 (3H, m), 7.17–7.30 (2H, m), 8.46 (1H, s), 9.56 (1H, s). Anal. Calcd for $C_{19}H_{18}N_6O_3S_2$: C, 51.57; H, 4.10; N, 18.99. Found: C, 51.32; H, 4.08; N, 18.74.

5-Methyl-4-oxo-*N*-[(3-{[3-(1*H*-1,2,4-triazol-3-ylthio)propyl]oxy}phenyl)methyl]-3,4-dihydro-thieno[2,3-*d*]pyrimidine-2-carboxamide (**25b**). A solution of ethyl 5-methyl-4-oxo-3,4-dihydrothieno[2,3-*d*]pyrimidine-2-carboxylate (**24**) (200 mg, 0.839 mmol) and 1-{3-[(3-{[1-(triphenylmethyl)-1*H*-1,2,4-triazol-3-yl]thio}propyl)oxy]phenyl}methanamine (**9b**) (723 mg, 1.43 mmol) in DMA (10 mL) was stirred at 100 °C for 12 h. After cooling to room temperature, the reaction mixture was evaporated under reduced pressure, and a solution of the residue in dichloromethane was treated with acidic resin (MP-TsOH: 100 mg) to give a brown oil. To the solution of the obtained oil in dichloromethane (10 mL) were added TFA (3 mL) and triethylsilane (0.141 mL, 0.881 mmol) at room temperature. The mixture was stirred for 1 h and evaporated under reduced pressure. The residue was purified by silica gel column chromatography (30–100% ethyl acetate/hexane) to give a solid. The suspension of the obtained solid in ethyl acetate was stirred under heating at 90 °C for 1 h to give the title compound as a white powder (171 mg, 45% for 2 steps). mp 178–179 °C. ^{1}H NMR (300 MHz, DMSO-d_6) δ

2.03–2.14 (2H, m), 3.21 (2H, t, J = 7.0 Hz), 4.04 (2H, t, J = 6.1 Hz), 4.41 (2H, d, J = 6.4 Hz), 6.80–6.84 (1H, m), 6.87–6.93 (2H, m), 7.23 (1H, t, J = 8.1 Hz), 7.32 (1H, d, J = 1.1 Hz), 8.52 (1H, bs), 9.62 (1H, t, J = 6.4 Hz), 12.28 (1H, bs), 14.04 (1H, bs). Anal. Calcd for $C_{20}H_{20}N_6O_3S_2$: C, 52.62; H, 4.42; N, 18.41. Found: C, 52.72; H, 4.56; N, 18.27.

5-Methyl-4-oxo-*N*-[(3-{[4-(1*H*-1,2,4-triazol-3-ylthio)butyl]oxy}phenyl)methyl]-3,4-dihydro-thieno[2,3-*d*]pyrimidine-2-carboxamide (**25c**). Compound **25c** was prepared from ethyl 5-methyl-4-oxo-3,4-dihydrothieno[2,3-*d*]pyrimidine-2-carboxylate (**24**) and 1-{3-[(4-{[1-(triphenyl-methyl)-1*H*-1,2,4-triazol-3-yl]thio}butyl)oxy]phenyl}methanamine (**9c**) with a similar procedure as described for compound **25b** (pale yellow powder, 48% for 2 steps). mp 161–163 °C. ^{1}H NMR (300 MHz, DMSO-d_6) δ 1.73–1.85 (4H, m), 2.46–2.52 (3H, m), 3.09–3.19 (2H, m), 3.90–4.01 (2H, m), 4.40 (2H, d, J = 6.4 Hz), 6.80 (1H, dd, J = 8.2, 1.6 Hz), 6.85–6.90 (2H, m), 7.22 (1H, t, J = 8.1 Hz), 7.31 (1H, d, J = 1.1 Hz), 8.40 (1H, bs), 9.59 (1H, t, J = 6.3 Hz), 12.30 (1H, bs), 14.00 (1H, bs). Anal. Calcd for $C_{21}H_{22}N_6O_3S_2$: C, 53.60; H, 4.71; N, 17.86. Found: C, 53.48; H, 4.72; N, 18.06.

5-Methyl-4-oxo-*N*-[(3-{[2-(1*H*-1,2,4-triazol-3-yloxy)ethyl]oxy}phenyl)methyl]-3,4-di

hydro-thieno[2,3-*d*]pyrimidine-2-carboxamide (**25d**). A suspension of ethyl 5-methyl-4-oxo-3,4-dihydro-thieno[2,3-*d*]pyrimidine-2-carboxylate (**24**) (200 mg, 0.839 mmol) and 1-{3-[(2-{[1-(triphenylmethyl)-1*H*-1,2,4-triazol-3-yl]oxy}ethyl)oxy]phenyl}methanamine (**15a**) (420 mg, 0.881 mmol) in DMA (3 mL) was stirred under microwave irradiation at 180 °C (150W, run time: 15 min, hold time: 15 min). After cooling to room temperature, the reaction mixture was evaporated under reduced pressure. To the residue were added ethyl acetate and water. The organic layer was washed with brine, dried over Na_2SO_4, and concentrated. The residue was purified by silica gel column chromatography (50–67% ethyl acetate/hexane) to give a white powder (283 mg). To a solution of the obtained powder (250 mg, 0.374 mmol) in dichloromethane (10 mL) were added TFA (3 mL) and triethylsilane (0.063 mL, 0.393 mmol) at room temperature. The mixture was stirred for 30 min and evaporated under reduced pressure. The residual solid was crystallized from ethanol and diethylether to give a white powder. The powder was recrystallized from ethanol to give the title compound as a white powder (126 mg, 35% for 2 steps). mp 225–227 °C. ^{1}H NMR (300 MHz, DMSO-d_6) δ 4.24–4.29 (2H, m), 4.41 (2H, d, J = 6.2 Hz), 4.45–4.50 (2H, m), 6.83–6.88 (1H, m), 6.89–6.97 (2H, m), 7.24 (1H, t, J = 7.9 Hz), 7.32 (1H, d, J = 1.1 Hz), 8.24 (1H, s), 9.62 (1H, t, J = 6.3 Hz),

12.27 (1H, bs), 13.33 (1H, bs). Anal. Calcd for $C_{19}H_{18}N_6O_4S$: C, 53.51; H, 4.25; N, 19.71. Found: C, 53.40; H, 4.26; N, 19.69.

5-Methyl-4-oxo-*N*-[(3-{[(1*H*-1,2,4-triazol-3-ylcarbonyl)amino]methyl}phenyl)methyl]-3,4-dihydrothieno[2,3-*d*]pyrimidine-2-carboxamide (**25e**). A solution of compound **26c** (365 mg, 1.00 mmol), 1-(triphenylmethyl)-1*H*-1,2,4-triazole-3-carboxylic acid (**20a**) (427 mg, 1.20 mmol), *N*,*N*-diisopropylethylamine (129 mg, 1.00 mmol), 1-[3-(dimethylamino)propyl]-3-ethylcarbodiimide hydrochloride (230 mg, 1.20 mmol), and 1-hydroxybenzotriazole (162 mg, 1.20 mmol) in DMF (10 mL) was stirred at 40 °C for 15 h. The reaction mixture was evaporated under reduced pressure. To the residue was added water. The resulting solid was collected on a filter, washed with water and diisopropyl ether, and dried to give a white solid. The obtained solid was washed with ethanol and diisopropyl ether to give a white powder (597 mg). A solution of the white powder (565 mg, 0.849 mmol) and triethylsilane (0.142 mL, 0.891 mmol) in dichloromethane (10 mL) and TFA (3 mL) was stirred at room temperature for 0.5 h. The reaction mixture was evaporated under reduced pressure. To the residue was added diisopropyl ether. The resulting solid was collected on a filter, washed with diisopropyl ether, and dried. The suspension of the obtained solid in ethanol was stirred under

heating at 90 °C for 2 h to give the title compound as a white powder (306 mg, 85% for 2 steps). mp 290–291 °C. ^{1}H NMR (300 MHz, DMSO-d_6) δ 4.42 (4H, d, J = 6.0 Hz), 7.16–7.34 (5H, m), 8.44 (1H, bs), 9.15 (1H, bs), 9.64 (1H, t, J = 6.4 Hz), 12.25 (1H, bs).

5-Methyl-4-oxo-*N*-[(3-{[(1*H*-1,2,4-triazol-3-ylacetyl)amino]methyl}phenyl)methyl]-3,4-dihydrothieno[2,3-*d*]pyrimidine-2-carboxamide (**25f**). A solution of compound **26c** (239 mg, 0.656 mmol), 1*H*-1,2,4-triazol-3-ylacetic acid (**20b**) (100 mg, 0.787 mmol), *N*,*N*-diisopropylethylamine (84.8 mg, 0.656 mmol), 1-[3-(dimethylamino)propyl]-3-ethylcarbodiimide hydrochloride (151 mg, 0.787 mmol), and 1-hydroxy-benzotriazole (106 mg, 0.787 mmol) in DMF (10 mL) was stirred for 15 h at 40 °C. The reaction mixture was concentrated under reduced pressure, and water was added to the residue. The resulting solid was collected on a filter, washed with water, ethanol and diisopropyl ether, and dried. The obtained solid was recrystallized from ethyl acetate–diisopropyl ether to give the title compound as a white powder (151 mg, 53%). mp 251–253 °C. ^{1}H NMR (300 MHz, DMSO-d_6) δ 3.61–3.68 (2H, m), 4.27 (2H, d, J = 5.7 Hz), 4.43 (2H, d, J = 6.4 Hz), 7.09–7.35 (5H, m), 8.50–8.68 (1H, m), 9.62 (1H, t, J = 6.3 Hz), 12.30 (1H, bs), 13.76 (1H, bs). Anal. Calcd for $C_{20}H_{19}N_7O_3S$•0.3H_2O: C, 54.24; H, 4.46; N, 22.14. Found: C, 54.52; H, 4.45; N, 21.81.

5-Methyl-4-oxo-*N*-[(3-{[3-(1*H*-1,2,4-triazol-3-yl)propanoyl]amino}phenyl)methyl]-3,4-dihydrothieno[2,3-*d*]pyrimidine-2-carboxamide (**25g**). Compound **25g** was prepared from compound **26a** and 3-[1-(triphenylmethyl)-1*H*-1,2,4-triazol-3-yl]propanoic acid (**20c**) with a similar procedure as described for compound **25e** (white powder, 45% for 2 steps). mp 248–250 °C. ^{1}H NMR (300 MHz, DMSO-d_6) δ 2.67–2.80 (2H, m), 2.96 (2H, bs), 4.40 (2H, d, J = 6.4 Hz), 7.00 (1H, d, J = 7.5 Hz), 7.18–7.27 (1H, m), 7.31 (1H, d, J = 1.1 Hz), 7.44–7.55 (2H, m), 9.63 (1H, t, J = 6.3 Hz), 9.99 (1H, bs), 12.29 (1H, bs), 13.64 (1H, bs). Anal. Calcd for $C_{20}H_{19}N_7O_3S$•0.3H_2O: C, 54.24; H, 4.46; N, 22.14. Found: C, 54.58; H, 4.45; N, 21.87.

***N*-[(3-Aminophenyl)methyl]-5-methyl-4-oxo-3,4-dihydrothieno[2,3-*d*]pyrimidine-2-carbox-amide** (**26a**). A suspension of ethyl 5-methyl-4-oxo-3,4-dihydrothieno[2,3-*d*]pyrimidine-2-carboxylate (**24**) (1.50 g, 6.30 mmol) and 3-(aminomethyl)aniline (0.923 g, 7.55 mmol) in THF (15 mL) was stirred at 80 °C for 15 h. To the reaction mixture cooled to room temperature was added diisopropyl ether, and the insoluble material was collected on a filter, washed with diisopropyl ether, and dried in vacuo to give the title compound as a yellow powder (1.92 mg, 97%). ^{1}H NMR (300 MHz, DMSO-d_6) δ 3.33 (3H, s), 4.30 (2H, d, J = 6.2

Hz), 5.03 (2H, s), 6.40–6.52 (3H, m), 6.94 (1H, t, J = 7.7 Hz), 7.31 (1H, d, J = 1.3 Hz), 9.48 (1H, t, J = 6.3 Hz), 12.20 (1H, s). Anal. Calcd for $C_{15}H_{14}N_4O_2S$: C, 57.31; H, 4.49; N, 17.82. Found: C, 57.11; H, 4.52; N, 17.65.

1,1-Dimethylethyl {[3-({[(5-methyl-4-oxo-3,4-dihydrothieno[2,3-*d*]pyrimidin-2-yl)carbonyl]amino}methyl)phenyl]methyl}carbamate (**26b**). Compound **26b** was prepared from ethyl 5-methyl-4-oxo-3,4-dihydrothieno[2,3-*d*]pyrimidine-2-carboxylate (**24**) and *tert*-butyl *N*-{[3-(aminomethyl)phenyl]methyl}carbamate with a similar procedure as described for compound **26a** (white powder, 82%). ^{1}H NMR (300 MHz, DMSO-d_6) δ 1.35 (9H, s), 2.47–2.53 (3H, m), 4.10 (2H, d, J = 6.2 Hz), 4.43 (2H, d, J = 6.4 Hz), 7.08–7.41 (6H, m), 9.62 (1H, t, J = 6.3 Hz), 12.25 (1H, s).

***N*-{[3-(Aminomethyl)phenyl]methyl}-5-methyl-4-oxo-3,4-dihydrothieno[2,3-*d*]pyrimidine-2-carboxamide Hydrochloride** (**26c**). To a suspension of compound **26b** (1.78 g, 4.15 mmol) in ethyl acetate (20 mL) was added 4 N hydrogen chloride in ethyl acetate solution (50 mL), and the mixture was stirred at room temperature for 15 h. The reaction mixture was concentrated under reduced pressure, and the residue was crystallized from toluene. The obtained crude crystals were suspended in ethyl acetate,

and the suspension was stirred under heating for 3 h to give the title compound as a white powder (1.39 g, 92%). ^{1}H NMR (300 MHz, DMSO-d_6) δ 3.33 (3H, s), 3.99 (2H, s), 4.46 (2H, d, J = 6.4 Hz), 7.25–7.52 (5H, m), 8.74–10.18 (4H, m).

Ethyl 5-Isopropyl-4-oxo-3,4-dihydrothieno[2,3-*d*]pyrimidine-2-carboxylate (**30a**). A suspension of commercially available ethyl 2-amino-4-isopropylthiophene-3-carboxylate (**29a**) (1.00 g, 4.69 mmol) and ethyl cyanoformate (511 mg, 5.16 mmol) in 1 N hydrochloride in acetic acid solution (10 mL) was stirred at 90 °C for 10 h. After cooling to room temperature the mixture was concentrated under reduced pressure. The residue was triturate with water, collected on a filter, washed with water and diethyl ether, and dried to give the title compound as a pale yellow powder (301 mg, 24%). ^{1}H NMR (300 MHz, DMSO-d_6) δ 1.23 (3H, s), 1.25 (3H, s), 1.34 (3H, t, J = 7.0 Hz), 3.59–3.70 (1H, m), 4.36 (2H, q, J = 7.2 Hz), 7.41 (1H, s), 12.72 (1H, bs).

Ethyl 4-Oxo-5-(2-thienyl)-3,4-dihydrothieno[2,3-*d*]pyrimidine-2-carboxylate (**30b**). Compound **30b** was prepared from commercially available ethyl 5'-amino-2,3'-bithiophene-4'-carboxylate (**29b**) with a similar procedure as described for compound **30a** (brown powder, 88%). ^{1}H NMR (300 MHz, DMSO-d_6) δ 1.36 (3H, t,

J = 7.1 Hz), 4.39 (2H, q, *J* = 7.2 Hz), 7.12 (1H, dd, *J* = 5.1, 3.6 Hz), 7.57 (1H, dd, *J* = 5.1, 1.3 Hz), 7.65 (1H, dd, *J* = 3.6, 1.3 Hz), 7.87 (1H, s), 12.89 (1H, bs).

Ethyl 4-Oxo-5-(3-thienyl)-3,4-dihydrothieno[2,3-*d*]pyrimidine-2-carboxylate (**30c**). A solution of 3-acetylthiophene (**27a**) (5.00 g, 39.6 mmol), ethyl cyanoacetate (**28**) (4.48 g, 39.6 mmol) and morpholine (3.45 g, 39.6 mmol) in toluene (40 mL) was stirred at 120 °C for 10 h. Sulfur (1.27 g, 39.6 mmol) and ethanol (40 mL) were added to the reaction mixture, and the mixture was stirred at 70 °C for 10 h. After cooling to room temperature the mixture was concentrated under reduced pressure. The residue was purified by silica gel column chromatography (9–20% ethyl acetate/hexane) to give ethyl 5-amino-3,3'-bithiophene-4-carboxylate (**29c**) as a yellow powder (3.30 g, 33%). Compound **30c** was prepared from compound **29c** with a similar procedure as described for compound **30a** (green powder, 56%). ^{1}H NMR (300 MHz, DMSO-d_6) δ 1.35 (3H, t, *J* = 7.0 Hz), 4.38 (2H, q, *J* = 6.9 Hz), 7.46–7.50 (1H, m), 7.56 (1H, dd, *J* = 4.9, 3.0 Hz), 7.86 (1H, s), 8.01 (1H, d, *J* = 1.9 Hz), 12.86 (1H, bs).

Ethyl 4-Oxo-5-phenyl-3,4-dihydrothieno[2,3-*d*]pyrimidine-2-carboxylate (**30d**). Compound **30d** was prepared from commercially available methyl 2-amino-4-phenylthiophene-3-carboxylate (**29d**) with a similar procedure as described

for compound **30a** (brown powder, 80%). ^{1}H NMR (300 MHz, DMSO-d_6) δ 1.36 (3H, t, J = 7.2 Hz), 4.39 (2H, q, J = 7.2 Hz), 7.32–7.45 (3H, m), 7.51–7.57 (2H, m), 7.74 (1H, s), 12.80 (1H, bs).

Ethyl 5-(2-Fluorophenyl)-4-oxo-3,4-dihydrothieno[2,3-*d*]pyrimidine-2-carboxylate (**30e**). Ethyl 2-amino-4-(2-fluorophenyl)thiophene-3-carboxylate (**29e**) was prepared from 2-fluoroaceto-phenone (**27b**) with a similar procedure as described for compound **29c** (yellow powder, 26%). Compound **30e** was prepared from compound **29e** with a similar procedure as described for compound **30a** (pale yellow powder, 60%). ^{1}H NMR (300 MHz, DMSO-d_6) δ 1.35 (3H, t, J = 7.2 Hz), 4.38 (2H, q, J = 7.0 Hz), 7.19–7.30 (2H, m), 7.38–7.50 (2H, m), 7.79 (1H, s), 12.83 (1H, bs).

Ethyl 5-(3-Fluorophenyl)-4-oxo-3,4-dihydrothieno[2,3-*d*]pyrimidine-2-carboxylate (**30f**). Ethyl 2-amino-4-(3-fluorophenyl)thiophene-3-carboxylate (**29f**) was prepared from 3-fluoroacetophenone (**27c**) with a similar procedure as described for compound **29c** (brown oil, 18%).

Compound **30f** was prepared from compound **29f** with a similar procedure as described for compound **30a** (brown powder, 38%). ^{1}H NMR (300 MHz, DMSO-d_6) δ 1.36 (3H, t, J = 7.2 Hz), 4.38 (2H, q, J = 7.1 Hz), 7.16–7.27 (1H, m), 7.36–7.51 (3H, m), 7.84 (1H,

s), 12.91 (1H, bs).

Ethyl 5-(4-Fluorophenyl)-4-oxo-3,4-dihydrothieno[2,3-*d*]pyrimidine-2-carboxylate (**30g**). Compound **30g** was prepared from commercially available ethyl 2-amino-4-(4-fluorophenyl)thiophene-3-carboxylate (**29g**) with a similar procedure as described for compound **30a** (brown powder, 78%). ^{1}H NMR (300 MHz, DMSO-d_6) δ 1.36 (3H, t, J = 7.2 Hz), 4.39 (2H, q, J = 7.1 Hz), 7.18–7.28 (2H, m), 7.52–7.63 (2H, m), 7.72–7.77 (1H, m), 12.81 (1H, bs).

Ethyl 5-(2-Chlorophenyl)-4-oxo-3,4-dihydrothieno[2,3-*d*]pyrimidine-2-carboxylate (**30h**). Ethyl 2-amino-4-(2-chlorophenyl)thiophene-3-carboxylate (**29h**) was prepared from 2-chloroacetophenone (**27d**) with a similar procedure as described for compound **29c** (brown powder, 27%).

Compound **30h** was prepared from compound **29h** with a similar procedure as described for compound **30a** (pale yellow powder, 5%). ^{1}H NMR (300 MHz, DMSO-d_6) δ 1.35 (3H, t, J = 7.1 Hz), 4.37 (2H, q, J = 7.0 Hz), 7.33–7.55 (4H, m), 7.70 (1H, s), 12.82 (1H, s).

Ethyl 5-(3-Chlorophenyl)-4-oxo-3,4-dihydrothieno[2,3-*d*]pyrimidine-2-carboxylate (**30i**). Ethyl 2-amino-4-(3-chlorophenyl)thiophene-3-carboxylate (**29i**) was prepared

from 3-chloroacetophenone (**27e**) with a similar procedure as described for compound **29c** (brown oil, 51%). Compound **30i** was prepared from compound **29i** with a similar procedure as described for compound **30a** (pale yellow powder, 36%). ^{1}H NMR (300 MHz, DMSO-d_6) δ 1.36 (3H, t, J = 7.2 Hz), 4.39 (2H, q, J = 7.2 Hz), 7.41–7.47 (2H, m), 7.48–7.54 (1H, m), 7.62 (1H, d, J = 0.8 Hz), 7.85 (1H, s), 12.88 (1H, s).

Ethyl 5-(4-Chlorophenyl)-4-oxo-3,4-dihydrothieno[2,3-*d*]pyrimidine-2-carboxylate (**30j**). Compound **30j** was prepared from commercially available ethyl 2-amino-4-(4-chlorophenyl)thiophene-3-carboxylate (**29j**) with a similar procedure as described for compound **30a** (pale yellow powder, 86%). ^{1}H NMR (300 MHz, DMSO-d_6) δ 1.35 (3H, t, J = 7.2 Hz), 4.38 (2H, q, J = 7.0 Hz), 7.42–7.51 (2H, m), 7.53–7.60 (2H, m), 7.78 (1H, s), 12.89 (1H, bs).

Ethyl 5-(2-Methoxyphenyl)-4-oxo-3,4-dihydrothieno[2,3-*d*]pyrimidine-2-carboxylate (30k). Ethyl 2-amino-4-(2-methoxyphenyl)thiophene-3-carboxylate (**29k**) was prepared from 2-methoxy-acetophenone (**27f**) with a similar procedure as described for compound **29c** (yellow oil, 36%). Compound **30k** was prepared from compound **29k** with a similar procedure as described for compound **30a** (brown powder, 5%). ^{1}H NMR

(300 MHz, DMSO-d_6) δ 1.36 (3H, t, J = 7.2 Hz), 3.97 (3H, s), 4.38 (2H, q, J = 7.1 Hz), 7.08 (1H, t, J = 7.4 Hz), 7.22 (1H, d, J = 8.0 Hz), 7.37–7.45 (1H, m), 7.89–7.97 (2H, m), 12.87 (1H, bs).

Ethyl

5-(3-Methoxyphenyl)-4-oxo-3,4-dihydrothieno[2,3-*d*]pyrimidine-2-carboxylate (**30l**). Ethyl 2-amino-4-(3-methoxyphenyl)thiophene-3-carboxylate (**29l**) was prepared from 3-methoxy-acetophenone (**27g**) with a similar procedure as described for compound **29c** (brown oil, 77%). Compound **30l** was prepared from compound **29l** with a similar procedure as described for compound **30a** (brown powder, 32%). ^{1}H NMR (300 MHz, DMSO-d_6) δ 1.36 (3H, t, J = 7.2 Hz), 3.78 (3H, s), 4.38 (2H, q, J = 7.2 Hz), 6.94 (1H, dd, J = 7.8, 2.1 Hz), 7.08–7.16 (2H, m), 7.31 (1H, t, J = 7.8 Hz), 7.76 (1H, s), 12.82 (1H, bs).

Ethyl

5-(4-Methoxyphenyl)-4-oxo-3,4-dihydrothieno[2,3-*d*]pyrimidine-2-carboxylate (**30m**). Compound **30m** was prepared from commercially available ethyl 2-amino-4-(4-methoxyphenyl)thiophene-3-carboxylate (**29m**) with a similar procedure as described for compound **30a** (pale yellow powder, 79%). ^{1}H NMR (300 MHz,

DMSO-d_6) δ 1.35 (3H, t, J = 7.1 Hz), 3.80 (3H, s), 4.38 (2H, q, J = 7.0 Hz), 6.96 (2H, d, J = 8.7 Hz), 7.49 (2H, d, J = 8.5 Hz), 7.65 (1H, s), 12.79 (1H, bs).

5-Isopropyl-4-oxo-*N*-{3-[2-(1*H*-1,2,4-triazol-3-yloxy)ethoxy]benzyl}-3,4-dihydrothieno[2,3-*d*]pyrimidine-2-carboxamide (**31a**). A suspension of compound **30a** (150 mg, 0.563 mmol) and 1-{3-[(2-{[1-(triphenylmethyl)-1*H*-1,2,4-triazol-3-yl]oxy}ethyl)oxy]phenyl}methanamine (**15a**) (322 mg, 0.676 mmol) in ethanol (10 mL) was stirred at 90 °C for 4 days. After cooling to room temperature the reaction mixture was evaporated under reduced pressure. To the residue were added ethyl acetate and water. The organic layer was washed with water, 1 N aqueous hydrochloric acid solution, and brine, dried over Na_2SO_4, and concentrated to give a yellow powder. The residue was purified by silica gel column chromatography (50% ethyl acetate/hexane) to give a white powder. To the solution of the white powder (380 mg) in CH_3CN (10 mL) were added TFA (1.01 mL) and triethylsilane (0.105 mL, 0.654 mmol) at 50 °C. The mixture was stirred at 50 °C for 3 h and evaporated under reduced pressure. The residual solid was crystallized from toluene, ethanol, and diethyl ether to give a powder. The powder was recrystallized from ethanol to give the title compound as a white powder (161 mg, 63% for 2 steps). mp 220–221 °C. ^{1}H NMR (300 MHz, DMSO-d_6) δ 1.24 (6H, d, J = 6.8 Hz), 3.58–3.71 (1H,

m), 4.27 (2H, dd, *J* = 5.3, 3.4 Hz), 4.42 (2H, d, *J* = 6.4 Hz), 4.47–4.53 (2H, m), 6.81–6.97 (3H, m), 7.24 (1H, t, *J* = 7.8 Hz), 7.34 (1H, s), 9.59 (1H, t, *J* = 5.9 Hz), 12.30 (1H, bs), 13.33 (1H, bs). Anal. Calcd for $C_{21}H_{22}N_6O_4S$: C, 55.49; H, 4.88; N, 18.49. Found: C, 55.42; H, 4.82; N, 18.58.

4-Oxo-5-(2-thienyl)-*N*-[(3-{[2-(1*H*-1,2,4-triazol-3-yloxy)ethyl]oxy}phenyl)methyl]-3,4-dihydrothieno[2,3-*d*]pyrimidine-2-carboxamide (**31b**). Compound **31b** was prepared from compound **30b** and compound **15a** with a similar procedure as described for compound **31a** (white powder, 46%). mp 162–164 °C. ^{1}H NMR (300 MHz, DMSO-d_6) δ 4.25–4.30 (2H, m), 4.40–4.53 (4H, m), 6.87 (1H, dd, *J* = 8.0, 2.4 Hz), 6.90–6.98 (2H, m), 7.11 (1H, dd, *J* = 5.1, 3.6 Hz), 7.25 (1H, t, *J* = 7.9 Hz), 7.56 (1H, dd, *J* = 5.1, 1.1 Hz), 7.65 (1H, dd, *J* = 3.6, 1.1 Hz), 7.79 (1H, s), 8.24 (1H, bs), 9.67 (1H, t, *J* = 6.0 Hz), 12.47 (1H, bs), 13.34 (1H, bs). Anal. Calcd for $C_{22}H_{18}N_6O_4S_2 \bullet H_2O$: C, 51.55; H, 3.93; N, 16.40. Found: C, 51.41; H, 3.55; N, 16.33.

4-Oxo-5-(3-thienyl)-*N*-{3-[2-(1*H*-1,2,4-triazol-3-yloxy)ethoxy]benzyl}-3,4-dihydrothieno[2,3-*d*]pyrimidine-2-carboxamide (**31c**). Compound **31c** was prepared from compound **30c** and compound **15a** with a similar procedure as described for compound **31a** (white powder, 34%). mp 186–189 °C. ^{1}H NMR (300 MHz, DMSO-d_6) δ 4.26–4.31

(2H, m), 4.43 (2H, d, J = 6.2 Hz), 4.51 (2H, bs), 6.80–7.02 (3H, m), 7.25 (1H, t, J = 7.8 Hz), 7.47–7.51 (1H, m), 7.54 (1H, dd, J = 4.9, 3.0 Hz), 7.77 (1H, s), 8.03 (1H, d, J = 3.0 Hz), 8.19 (1H, bs), 9.63 (1H, t, J = 6.2 Hz), 12.39 (1H, bs), 13.34 (1H, bs). Anal. Calcd for $C_{22}H_{18}N_6O_4S_2$•0.4H_2O: C, 52.66; H, 3.78; N, 16.75. Found: C, 52.92; H, 3.73; N, 16.76.

4-Oxo-5-phenyl-*N*-{3-[2-(1*H*-1,2,4-triazol-3-yloxy)ethoxy]benzyl}-3,4-dihydrothieno[2,3-*d*]pyrimidine-2-carboxamide (**31d**). Compound **31d** was prepared from compound **30d** and compound **15a** with a similar procedure as described for compound **31a** (white powder, 72%). mp 183–185 °C. ^{1}H NMR (300 MHz, DMSO-d_6) δ 4.28 (2H, dd, J = 5.3, 3.4 Hz), 4.44 (2H, d, J = 6.2 Hz), 4.50 (2H, s), 6.87 (1H, dd, J = 8.0, 2.0 Hz), 6.91–6.98 (2H, m), 7.25 (1H, t, J = 7.8 Hz), 7.32–7.43 (3H, m), 7.51–7.57 (2H, m), 7.67 (1H, s), 8.24 (1H, bs), 9.68 (1H, t, J = 6.3 Hz), 12.37 (1H, bs), 13.33 (1H, bs). Anal. Calcd for $C_{24}H_{20}N_6O_4S$: C, 59.01; H, 4.13; N, 17.20. Found: C, 58.74; H, 4.18; N, 17.08.

5-(2-Fluorophenyl)-4-oxo-*N*-{3-[2-(1*H*-1,2,4-triazol-3-yloxy)ethoxy]benzyl}-3,4-dihydro-thieno[2,3-*d*]pyrimidine-2-carboxamide (**31e**). Compound **31e** was prepared from compound **30e** and compound **15a** with a similar procedure as described for

compound **31a** (white powder, 46%). mp 178–179 °C. ^{1}H NMR (300 MHz, DMSO-d_6) δ 4.28 (2H, bs), 4.43 (2H, d, J = 6.0 Hz), 4.50 (2H, bs), 6.82–7.00 (3H, m), 7.18–7.30 (3H, m), 7.36–7.48 (2H, m), 7.67 (1H, bs), 8.23 (1H, bs), 9.63 (1H, bs), 12.30 (1H, s), 13.34 (1H, bs). Anal. Calcd for $C_{24}H_{19}FN_6O_4S$•0.5H_2O: C, 55.92; H, 3.91; N, 16.30. Found: C, 55.68; H, 3.81; N, 16.52.

5-(3-Fluorophenyl)-4-oxo-*N*-{3-[2-(1*H*-1,2,4-triazol-3-yloxy)ethoxy]benzyl}-3,4-dihydro-thieno[2,3-*d*]pyrimidine-2-carboxamide (**31f**). Compound **31f** was prepared from compound **30f** and compound **15a** with a similar procedure as described for compound **31a** (white powder, 55%). mp 158–161 °C. ^{1}H NMR (300 MHz, DMSO-d_6) δ 4.27 (2H, dd, J = 3.0, 2.4 Hz), 4.41–4.52 (4H, m), 6.87 (1H, dd, J = 8.2, 1.8 Hz), 6.91–6.98 (2H, m), 7.14–7.30 (2H, m), 7.35–7.50 (3H, m), 7.78 (1H, s), 8.24 (1H, s), 9.69 (1H, t, J = 6.3 Hz), 12.45 (1H, bs), 13.33 (1H, bs). Anal. Calcd for $C_{24}H_{19}FN_6O_4S$: C, 56.91; H, 3.78; N, 16.59. Found: C, 56.71; H, 3.72; N, 16.58.

5-(4-Fluorophenyl)-4-oxo-*N*-{3-[2-(1*H*-1,2,4-triazol-3-yloxy)ethoxy]benzyl}-3,4-dihydro-thieno[2,3-*d*]pyrimidine-2-carboxamide (**31g**). Compound **31g** was prepared from compound **30g** and compound **15a** with a similar procedure as described for compound **31a** (white powder, 69%). mp 188–190 °C. ^{1}H NMR (300 MHz, DMSO-d_6)

δ 4.25–4.30 (2H, m), 4.44 (2H, d, J = 6.2 Hz), 4.50 (2H, s), 6.87 (1H, dd, J = 8.1, 2.1 Hz), 6.90–6.98 (2H, m), 7.18–7.29 (3H, m), 7.53–7.62 (2H, m), 7.68 (1H, s), 8.24 (1H, bs), 9.68 (1H, t, J = 6.3 Hz), 12.40 (1H, bs), 13.33 (1H, bs). Anal. Calcd for $C_{24}H_{19}FN_6O_4S$: C, 56.91; H, 3.78; N, 16.59. Found: C, 56.77; H, 3.70; N, 16.53.

5-(2-Chlorophenyl)-4-oxo-*N*-{3-[2-(1*H*-1,2,4-triazol-3-yloxy)ethoxy]benzyl}-3,4-dihydro-thieno[2,3-*d*]pyrimidine-2-carboxamide (**31h**). Compound **31h** was prepared from compound **30h** and compound **15a** with a similar procedure as described for compound **31a** (white powder, 44%). mp 169–171 °C. ^{1}H NMR (300 MHz, DMSO-d_6) δ 4.26–4.30 (2H, m), 4.40–4.53 (4H, m), 6.83–6.98 (3H, m), 7.25 (1H, t, J = 7.9 Hz), 7.33–7.45 (3H, m), 7.48–7.53 (1H, m), 7.65 (1H, s), 8.24 (1H, bs), 9.67 (1H, t, J = 6.5 Hz), 12.40 (1H, bs), 13.34 (1H, bs). Anal. Calcd for $C_{24}H_{19}ClN_6O_4S$•0.2H_2O: C, 54.74; H, 3.71; N, 15.96. Found: C, 54.78; H, 3.61; N, 16.09.

5-(3-Chlorophenyl)-4-oxo-*N*-{3-[2-(1*H*-1,2,4-triazol-3-yloxy)ethoxy]benzyl}-3,4-dihydro-thieno[2,3-*d*]pyrimidine-2-carboxamide (**31i**). Compound **31i** was prepared from compound **30i** and compound **15a** with a similar procedure as described for compound **31a** (white powder, 45%). mp 153–154 °C. ^{1}H NMR (300 MHz, DMSO-d_6) δ 4.25–4.30 (2H, m), 4.40–4.52 (4H, m), 6.84–6.97 (3H, m), 7.25 (1H, t, J = 7.8 Hz),

7.41–7.45 (2H, m), 7.47–7.55 (1H, m), 7.62 (1H, t, *J* = 1.2 Hz), 7.79 (1H, s), 8.24 (1H, bs), 9.70 (1H, t, *J* = 6.3 Hz), 12.46 (1H, bs), 13.34 (1H, bs). Anal. Calcd for $C_{24}H_{19}ClN_6O_4S$: C, 55.12; H, 3.66; N, 16.07. Found: C, 54.90; H, 3.60; N, 16.16.

5-(4-Chlorophenyl)-4-oxo-*N*-{3-[2-(1*H*-1,2,4-triazol-3-yloxy)ethoxy]benzyl}-3,4-dihydro-thieno[2,3-*d*]pyrimidine-2-carboxamide (**31j**). Compound **31j** was prepared from compound **30j** and compound **15a** with a similar procedure as described for compound **31a** (white powder, 80%). mp 160–164 °C. ^{1}H NMR (300 MHz, DMSO-d_6) δ 4.28 (2H, dd, *J* = 5.3, 3.0 Hz), 4.43 (2H, d, *J* = 6.4 Hz), 4.47–4.53 (2H, m), 6.87 (1H, dd, *J* = 8.0, 1.9 Hz), 6.90–6.98 (2H, m), 7.25 (1H, t, *J* = 7.8 Hz), 7.42–7.49 (2H, m), 7.51–7.60 (2H, m), 7.71 (1H, s), 8.23 (1H, bs), 9.68 (1H, t, *J* = 6.1 Hz), 12.44 (1H, bs), 13.33 (1H, bs). Anal. Calcd for $C_{24}H_{19}ClN_6O_4S$: C, 55.12; H, 3.66; N, 16.07. Found: C, 55.05; H, 3.63; N, 16.01.

5-(2-Methoxyphenyl)-4-oxo-*N*-{3-[2-(1*H*-1,2,4-triazol-3-yloxy)ethoxy]benzyl}-3,4-dihydro-thieno[2,3-*d*]pyrimidine-2-carboxamide (**31k**). Compound **31k** was prepared from compound **30k** and compound **15a** with a similar procedure as described for compound **31a** (white powder, 49%). mp 211–213 °C. ^{1}H NMR (300 MHz, DMSO-d_6) δ 3.96 (3H, s), 4.25–4.31 (2H, m), 4.40–4.54 (4H, m), 6.83–6.98 (3H, m), 7.04–7.11

(1H, m), 7.18–7.29 (2H, m), 7.36–7.44 (1H, m), 7.88–7.92 (2H, m), 8.23 (1H, bs), 9.65 (1H, t, *J* = 6.3Hz), 12.45 (1H, bs), 13.33 (1H, bs). Anal. Calcd for $C_{25}H_{22}N_6O_5S$•1.2EtOH: C, 52.33; H, 3.86; N, 14.65. Found: C, 52.35; H, 3.94; N, 14.51.

5-(3-Methoxyphenyl)-4-oxo-*N*-{3-[2-(1*H*-1,2,4-triazol-3-yloxy)ethoxy]benzyl}-3,4-dihydro-thieno[2,3-*d*]pyrimidine-2-carboxamide (**31l**). Compound **31l** was prepared from compound **30l** and compound **15a** with a similar procedure as described for compound **31a** (white powder, 50%). mp 163–165 °C. ^{1}H NMR (300 MHz, DMSO-d_6) δ 3.78 (3H, s), 4.24–4.31 (2H, m), 4.40–4.53 (4H, m), 6.87 (1H, dd, *J* = 8.2, 2.0 Hz), 6.90–6.97 (3H, m), 7.08–7.15 (2H, m), 7.22–7.34 (2H, m), 7.69 (1H, s), 8.23 (1H, s), 9.66 (1H, t, *J* = 5.8 Hz), 12.35 (1H, s), 13.33 (1H, bs). Anal. Calcd for $C_{25}H_{22}N_6O_5S$•0.5H_2O: C, 56.92; H, 4.39; N, 15.93. Found: C, 56.93; H, 4.27; N, 16.06.

5-(4-Methoxyphenyl)-4-oxo-*N*-{3-[2-(1*H*-1,2,4-triazol-3-yloxy)ethoxy]benzyl}-3,4-dihydro-thieno[2,3-*d*]pyrimidine-2-carboxamide (**31m**). Compound **31m** was prepared from compound **30m** and compound **15a** with a similar procedure as described for compound **31a** (white powder, 50%). mp 93–98 °C. ^{1}H NMR (300 MHz, DMSO-d_6) δ 3.80 (3H, s), 4.25–4.30 (2H, m), 4.43 (2H, d, *J* = 5.7 Hz), 4.51 (2H, bs), 6.87 (1H, dd, *J*

= 7.6, 1.1 Hz), 6.90–6.99 (4H, m), 7.18–7.32 (2H, m), 7.45–7.51 (2H, m), 7.59 (1H, s), 9.69 (1H, t, *J* = 6.6 Hz), 12.35 (1H, s).

5-(2-Fluorophenyl)-4-oxo-*N*-[(3-{[3-(1*H*-1,2,4-triazol-3-yloxy)propyl]oxy}phenyl)methyl]-3,4-dihydrothieno[2,3-*d*]pyrimidine-2-carboxamide (**31n**). A mixture of compound **15b** (0.300 g, 0.611 mmol), ethyl 5-(2-fluorophenyl)-4-oxo-3,4-dihydrothieno[2,3-*d*]pyrimidine-2-carboxylate (**30e**, 0.195 g, 0.611 mmol), and *N*,*N*-diisopropylethylamine (0.213 mL, 1.22 mmol) in ethanol (4.5 mL) was heated under microwave for 3 h at 100 °C (50 W, run time: 5 min, hold time 1 h × 3 set). The reaction mixture was concentrated under reduced pressure and coevaporated with toluene (twice). The residue was purified by silica-gel column chromatography (33–40% ethyl acetate/hexane) to give a pale yellow amorphous (0.305 g). The amorphous product (0.280 g) was crystallized from CH_3CN to give a white powder (0.245 g, 0.321 mmol, 57%). To a suspension of the white powder (0.220 g, 0.288 mmol) in CH_3CN (4 mL) were added TFA (0.857 mL) and triethylsilane (0.055 mL, 0.346 mmol) at room temperature and the mixture was stirred at room temperature for 3 h. The mixture was concentrated under reduced pressure and the residue was crystallized from diethyl ether to give the title compound as a pale yellow powder (0.145 g, 97%) (55% for 2 steps). mp 159–161 °C. ^{1}H NMR (300 MHz, DMSO-d_6) δ

2.09–2.22 (2H, m), 4.08 (2H, t, *J* = 6.2 Hz), 4.27–4.38 (2H, m), 4.43 (2H, d, *J* = 6.2 Hz), 6.81–6.88 (1H, m), 6.88–6.97 (2H, m), 7.18–7.28 (3H, m), 7.38–7.50 (2H, m), 7.72 (1H, s), 8.21 (1H, bs), 9.67 (1H, t, *J* = 6.4 Hz), 12.39 (1H, bs), 13.28 (1H, bs). Anal. Calcd for $C_{25}H_{21}FN_6O_4S$: C, 57.68; H, 4.07; N, 16.14. Found: C, 57.48; H, 4.12; N, 16.01.

5-(3-Fluorophenyl)-4-oxo-*N*-[(3-{[3-(1*H*-1,2,4-triazol-3-yloxy)propyl]oxy}phenyl)methyl]-3,4-dihydrothieno[2,3-*d*]pyrimidine-2-carboxamide (**31o**). Compound **31o** was prepared from compound **30f** and compound **15b** with a similar procedure as described for compound **31n** (pale yellow powder, 59%). mp 130–132 °C. ^{1}H NMR (300 MHz, DMSO-d_6) δ 2.08–2.22 (2H, m), 4.08 (2H, t, *J* = 6.2 Hz), 4.25–4.38 (2H, m), 4.43 (2H, d, *J* = 6.4 Hz), 6.80–6.88 (1H, m), 6.88–6.98 (2H, m), 7.16–7.28 (2H, m), 7.35–7.49 (3H, m), 7.78 (1H, s), 8.21 (1H, bs), 9.69 (1H, t, *J* = 6.3 Hz), 12.45 (1H, bs), 13.28 (1H, bs). Anal. Calcd for $C_{25}H_{21}FN_6O_4S\bullet0.1H_2O$: C, 57.49; H, 4.09; N, 16.09. Found: C, 57.20; H, 4.12; N, 15.99.

5-(2-Chlorophenyl)-4-oxo-*N*-[(3-{[3-(1*H*-1,2,4-triazol-3-yloxy)propyl]oxy}phenyl)methyl]-3,4-dihydrothieno[2,3-*d*]pyrimidine-2-carboxamide (**31p**). Compound **31p** was prepared from compound **30h** and compound **15b** with a similar procedure as described for compound **31n** (pale yellow powder, 63%). mp 165–167 °C. ^{1}H NMR

(300 MHz, DMSO–d_6) δ 2.09–2.21 (2H, m), 4.08 (2H, t, J = 6.2 Hz), 4.28–4.39 (2H, m), 4.43 (2H, d, J = 6.2 Hz), 6.84 (1H, dd, J = 7.9, 1.9 Hz), 6.88–6.96 (2H, m), 7.24 (1H, t, J = 7.9 Hz), 7.32–7.46 (3H, m), 7.48–7.54 (1H, m), 7.65 (1H, s), 8.19 (1H, bs), 9.66 (1H, t, J = 6.2 Hz), 12.26 (1H, bs), 13.26 (1H, bs). Anal. Calcd for $C_{25}H_{21}ClN_6O_4S•0.2H_2O$: C, 55.54; H, 3.99; N, 15.55. Found: C, 55.33; H, 4.05; N, 15.54.

5-(3-Chlorophenyl)-4-oxo-*N*-[(3-{[3-(1*H*-1,2,4-triazol-3-yloxy)propyl]oxy}phenyl)methyl]-3,4-dihydrothieno[2,3-*d*]pyrimidine-2-carboxamide (**31q**). Compound **31q** was prepared from compound **30i** and compound **15b** with a similar procedure as described for compound **31n** (white powder, 67%). mp 167–169 °C. ^{1}H NMR (300 MHz, DMSO–d_6) δ 2.09–2.22 (2H, m), 4.08 (2H, t, J = 6.2 Hz), 4.26–4.39 (2H, m), 4.43 (2H, d, J = 6.2 Hz), 6.84 (1H, dd, J = 8.1, 1.9 Hz), 6.88–6.97 (2H, m), 7.24 (1H, t, J = 7.8 Hz), 7.39–7.47 (2H, m), 7.47–7.55 (1H, m), 7.62 (1H, s), 7.79 (1H, s), 8.21 (1H, bs), 9.69 (1H, t, J = 6.3 Hz), 12.45 (1H, bs), 13.28 (1H, bs). Anal. Calcd for $C_{25}H_{21}ClN_6O_4S•0.2H_2O$: C, 55.54; H, 3.99; N, 15.55. Found: C, 55.42; H, 3.97; N, 15.36.

Crystallization and Structure Determination. Crystals were grown by the hanging

drop vapor diffusion method at 20 ℃ (the temperature was modified). Prior to crystallization, a solution containing 6–14 mg/mL human MMP-13 catalytic domain, 5 μM $Zn(OAc)_2$, 5 mM $CaCl_2$, 50 mM NaCl, 20 mM Tris HCl buffer (pH 8.0) were prepared. 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 reservoir solution containing 25% ethylene glycol, 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.[51] The structure was determined by molecular replacement using MOLREP,[52] using the only protein structure of MMP-13 previously reported (PDB accession number 830C).[53] Subsequently, structure refinement and model building were performed utilizing REFMAC[54]. The solved structure was modeled with WinCoot (version 0.3.3)[55]. X-ray coordinates have been deposited at the Cambridge Crystallographic Data Centre; deposition numbers: 5B5O for **2** and 5B5P for **23**. The statistic data and the refinement statistics are shown in **Table 6**.

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.[56] 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. Enzyme inhibition assays were performed in assay buffer containing enzymes and fluorescence peptide (Cy3-PLGLK(Cy5Q)AR-NH_2 for MMPs and 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, λ_{em} = 535 nm; λ_{ex} = 595 nm). Enzyme activity (%) was determined according to the following equation: Enzyme activity (%) = $(X - C)/(T - C) \times 100$, where X is the fluorescence count with inhibitor, T is the fluorescence count without inhibitor, and C is the fluorescence count with EDTA. IC_{50} values of inhibitors

were obtained with iterative fitting package (GraphPad Prism software).

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 OSM 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 reagents 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 normalized degree of the collagen degradation was shown as % of collagen degradation, calculated by following formulation:

(hydroxyproline content in media)/[(hydroxyproline content in media) + (hydroxyproline content in remaining cartilage)] × 100. 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.

ASSOCIATED CONTENT

Accession Codes

PDB entries for **2** in complex with MMP-13 and for **23** in complex with MMP-13 are 5B5O and 5B5P, respectively. Authors will release the atomic coordinates and experimental data upon article publication.

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Notes

The authors declare no competing financial interest.

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ABBREVIATIONS USED

TEA, triethylamine; DIEA, *N*,*N*-diisopropylethylamine; WSC, water-soluble

carbodiimide; HOBt, 1-hydroxybenzotriazole; SEM, standard error of the mean

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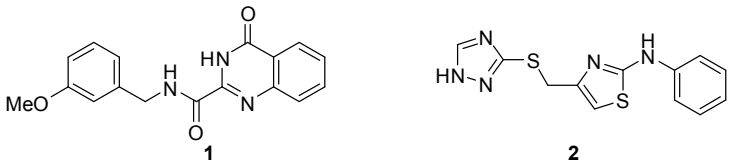
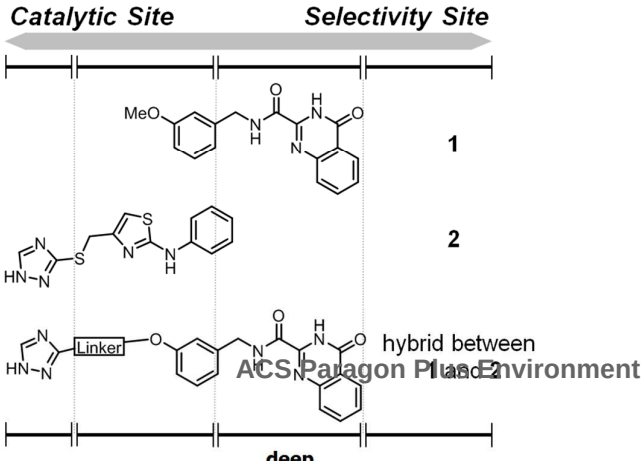


Figure 1. Structures of initial hit compounds.

(A)



(B)

(C)

Figure 2. Comparison of X-ray alignment of (A) quinazoline **1** and (B) triazole **2** with (C) hybrid structure incorporating key features of **1** and **2**.

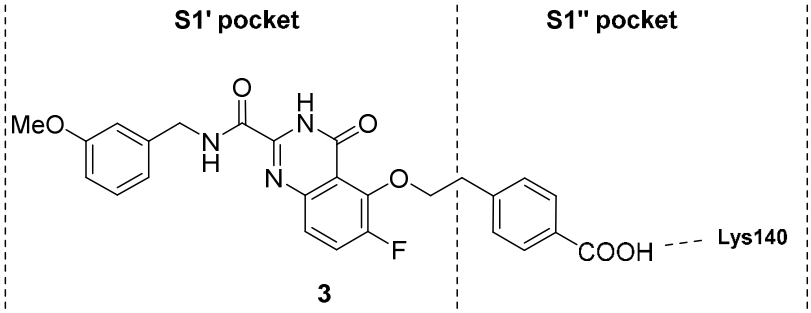
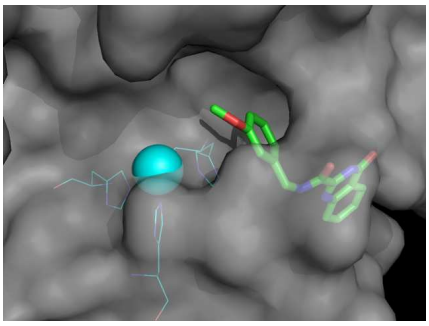
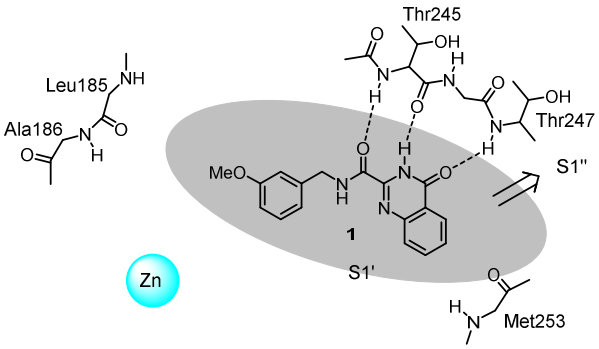


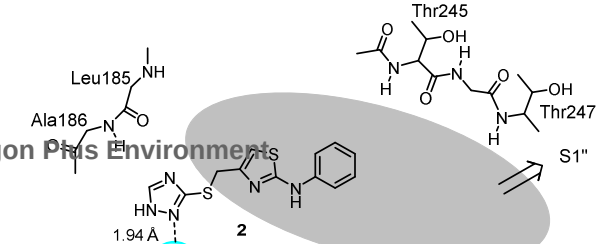
Figure 3. Chemical structure of **3**.

(A) (B)





(C) (D)



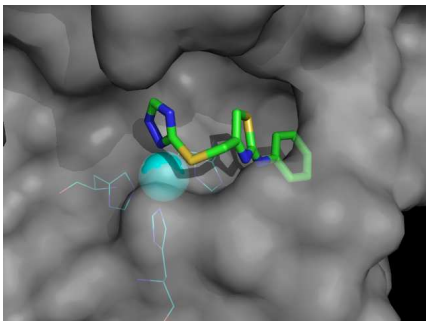


Figure 4. Crystal structures of complex of quinazoline **1** (PDB code 3WV2) and triazole **2** with human MMP-13 catalytic domain. (A) Surface representation of MMP-13 illustrating the binding cavity. Catalytic zinc ion is shown in blue sphere. The enzyme is in gray. Three residues His222, 226, and 232 in which imidazol-5-yl groups are binding to the zinc ion are represented in blue line model. Quinazoline **1** is buried deeply into the S1' pocket. The figure was made with program PyMOL 0.99.[57] (B) Schematic representation of the binding mode of quinazoline **1** and MMP-13. Hydrogen bonds are shown as dashed lines. (C) Molecular surface diagram of MMP-13 illustrating the binding cavity. Triazole **2** binds to the catalytic zinc ion and occupies the part of the S1' pocket. (D) Schematic representation of the binding mode of triazole **2** and MMP-13. Interaction between triazole **2** and the zinc ion is shown as a dashed line.

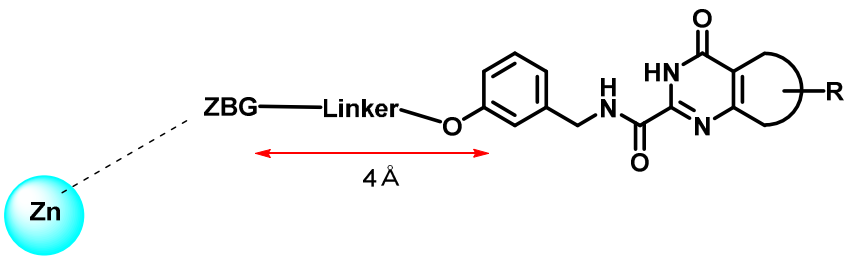


Figure 5. Design of quinazoline-based zinc binding MMP-13 selective inhibitors.

(A) (B)

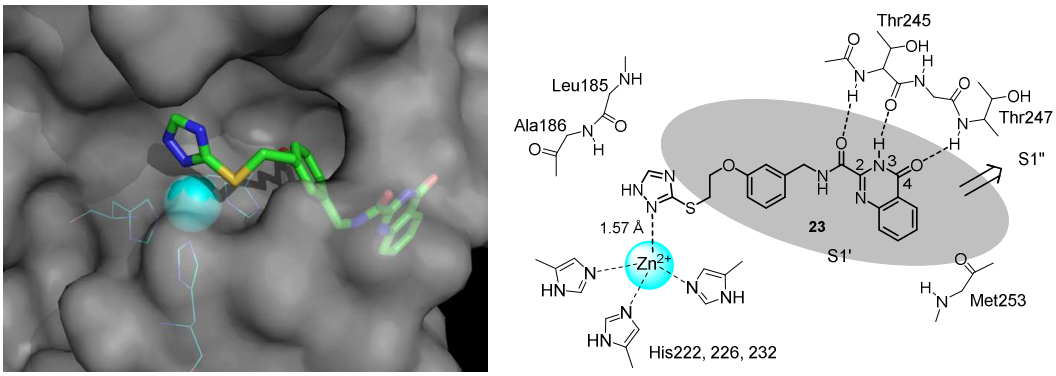
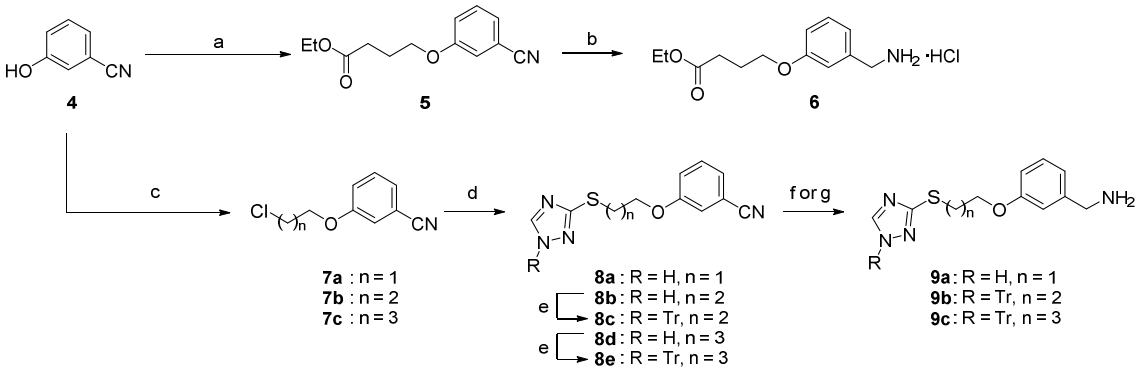


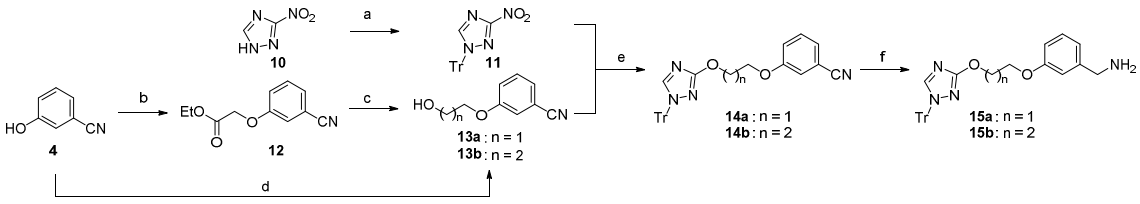
Figure 6. Crystal structure of the human MMP-13 catalytic domain complexed with a hybrid zinc binding inhibitor **23**. (A) Molecular surface diagram of MMP-13 illustrating the binding cavity. The catalytic zinc ion is shown in a blue sphere. The enzyme is in gray. Three residues His222, 226, and 232, whose imidazol-5-yl groups are coordinated to the zinc ion are represented in blue line model. Triazole **23** binds to the zinc ion and is buried deeply into the S1' pocket. The figure was made with program PyMOL.[57] (B) Schematic representation of the binding mode of triazole **23** and MMP-13. Hydrogen bonds and interaction between the triazole ring of **23** and the zinc ion are shown as dashed lines.

Scheme 1. Synthesis of Benzylamine Derivatives **6** and **9a–c**[a]



[a]Reagents and conditions: (a) ethyl 4-bromobutanoate, NaH, DMF, rt, quant. (for **5**); (b) (1) H_2 (1 atm), Pd/C, HCO_2H, MeOH, rt; (2) 4 N HCl/AcOEt, rt, 78% over 2 steps; (c) 1-bromoalkyl chloride, KOH or NaH, EtOH, 60 °C or 90 °C, 18–95%; (d) 1,2,4-triazole-3-thiol, TEA, EtOH, 80 °C, 98%–quant. (for **8a**, **8b**, and **8d**); (e) TrCl, TEA, THF, rt, 46 and 78% (for **8c** and **8e**, respectively); (f) H_2 (1 atm), Raney Ni, 5 N NH_3/MeOH, rt, 71% (for **9a**); (g) $LiAlH_4$, THF, rt, 95 and 86% (for **9b** and **9c**, respectively).

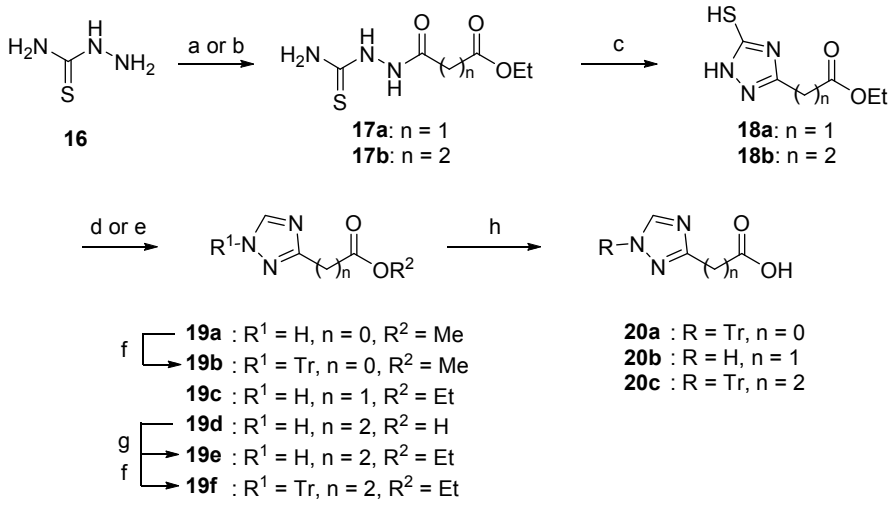
Scheme 2. Synthesis of Benzylamine Derivatives **15a** and **15b**[a]



[a]Reagents and conditions: (a) TrCl, DIEA, THF, rt, 93%; (b) ethyl bromoacetate, K_2CO_3, THF, 50 °C, 99%; (c) $NaBH_4$, EtOH, 50 °C, 92% (for **13a**) ; (d) 3-bromopropanol,

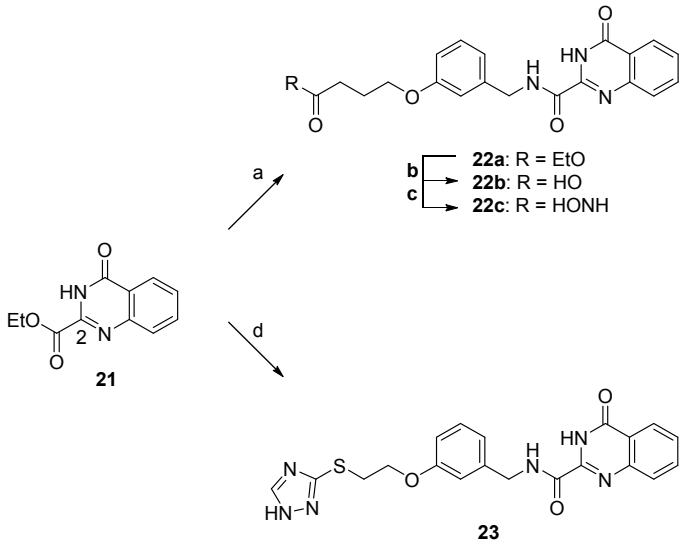
K_2CO_3, DMF, 80 °C, 53% (for **13b**); (e) NaH, THF, rt, 46% and quant. (crude) (for **14a** and **14b**, respectively); (f) $LiAlH_4$, THF, rt, 58 and 77% (for **15a** and **15b**, respectively).

Scheme 3. Synthesis of Carboxylic Acid Derivatives **20a–c**[a]



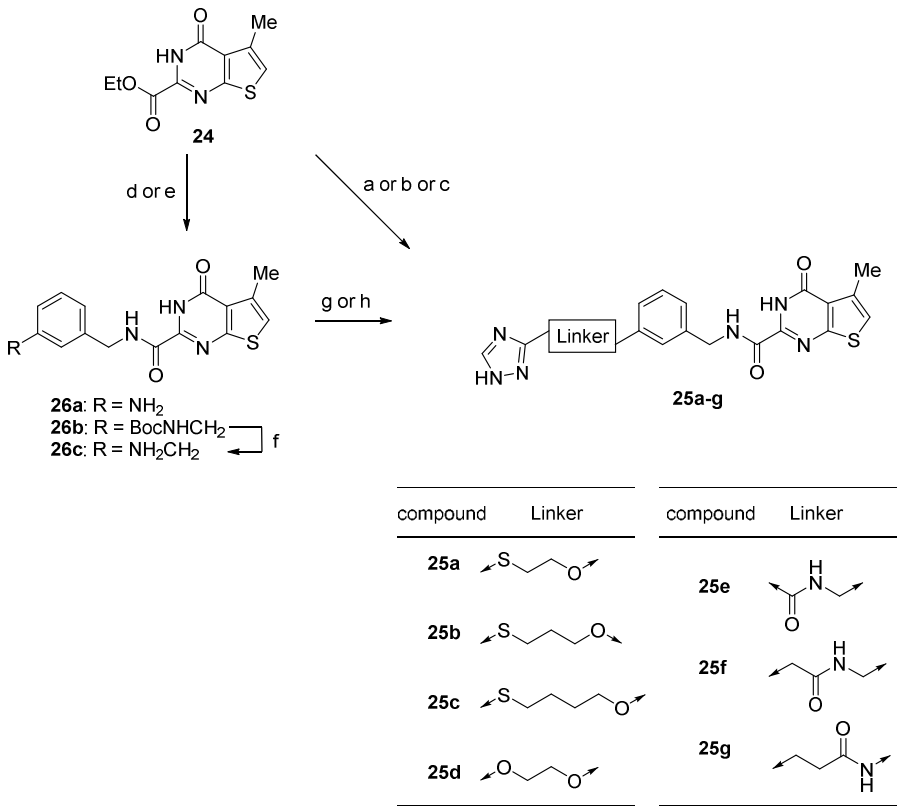
[a]Reagents and conditions: (a) ethyl 3-chloro-3-oxopropanoate, pyridine, 0 °C–rt, 42% (for **17a**); (b) ethyl succinyl chloride, pyridine, rt, 35% (for **17b**); (c) EtONa, EtOH, 80 °C, 96 and 91% (for **18a** and **18b**, respectively); (d) Raney Ni, EtOH, 80 °C, 54% (for **19c**); (e) $NaNO_2$, HNO_3, H_2O, rt, 99% (for **19d**); (f) TrCl, DIEA, THF, 60 °C or rt, 59 and 83% (for **19b** and **19f**, respectively); (g) 2 N HCl/EtOH, 90 °C, 33%; (h) 4 N NaOH, THF, MeOH, H_2O, 100 °C, 50–98% (for **20a–c**).

Scheme 4. Synthesis of 4-Oxo-3,4-dihydroquinazoline-2-carboxamide Derivatives **22a–c** and **23**[a]



[a]Reagents and conditions: (a) amine **6**, DIEA; THF, 80 °C, 86%; (b) 4 N aq. NaOH, THF–MeOH–H_2O, 100 °C, 69%; (c) (1) oxalyl chloride, cat. DMF, THF, 0 °C to rt; (2) 50% aq. NH_2OH, *t*-BuOH, THF, 0 °C–rt, 9% over 2 steps; (d) amine **9a**, DIEA, EtOH, 90 °C, 8%.

Scheme 5. Synthesis of 5-Methyl-4-oxo-3,4-dihydrothienopyrimidine-2-carboxamide Derivatives **25a–g**[a]

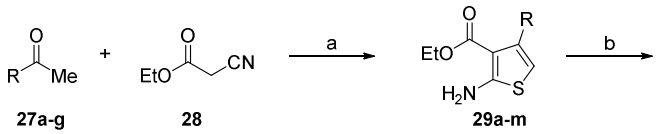


compound	Linker	compound	Linker
25a		25e	
25b		25f	
25c		25g	
25d			

[a]Reagents and conditions: (a) amine **9a**, DIEA, DMA–EtOH, 90 °C, 18% (for **25a**); (b) (1) amine **9b** or **9c**, DMA, 100 °C; (2) TFA, Et_3SiH, DCM, rt, 45 and 48% (for **25b** and **25c**, respectively, each over 2 steps); (c) (1) amine **15a**, DMA, 180 °C (microwave); (2) TFA, Et_3SiH, DCM, rt, 35% over 2 steps (for **25d**); (d) 3-(aminomethyl)aniline, THF, 80 °C, 97% (for **26a**); (e) *t*-butyl *N*-{[3-(aminomethyl)phenyl]methyl}carbamate, THF, 80 °C, 82% (for **26b**); (f) 4 N HCl/AcOEt, rt, 92% (for **26c**); (g) **26c**, carboxylic acid **20b**, WSC•HCl, HOBt, DIEA, DMF, 40 °C, 53% (for **25f**); (h) (1) **26c** or **26a**, carboxylic acid **20a** or **20c**, respectively, WSC•HCl, HOBt, DIEA, DMF, 40 °C; (2) TFA, Et_3SiH, DCM, rt, 85 and 45% (for **25e** and **25g**, respectively, each over 2 steps).

Scheme 6. Synthesis of 5-Substituted

4-Oxo-3,4-dihydrothienopyrimidine-2-carboxamide Derivatives **31a–q**[a]

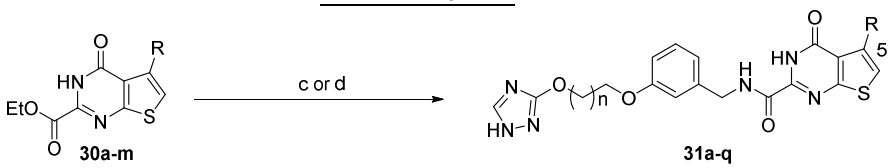


27a-g **28** **29a-m**

compound	R
27a	3-thiophenyl
27b	2-F-Ph
27c	3-F-Ph
27d	2-Cl-Ph
27e	3-Cl-Ph
27f	2-MeO-Ph
27g	3-MeO-Ph

compound	R
29a	*i*Pr
29b	2-thiophenyl
29c	3-thiophenyl
29d	phenyl
29e	2-F-Ph
29f	3-F-Ph
29g	4-F-Ph
29h	2-Cl-Ph

compound	R
29i	3-Cl-Ph
29j	4-Cl-Ph
29k	2-MeO-Ph
29l	3-MeO-Ph
29m	4-MeO-Ph



30a-m **31a-q**

compound	R
30a	*i*Pr
30b	2-thiophenyl
30c	3-thiophenyl
30d	phenyl
30e	2-F-Ph
30f	3-F-Ph
30g	4-F-Ph
30h	2-Cl-Ph

compound	R
30i	3-Cl-Ph
30j	4-Cl-Ph
30k	2-MeO-Ph
30l	3-MeO-Ph
30m	4-MeO-Ph

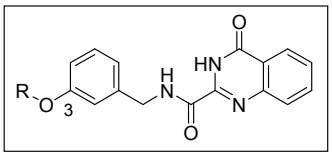
compound	n	R
31a	1	*i*Pr
31b	1	2-thiophenyl
31c	1	3-thiophenyl
31d	1	phenyl
31e	1	2-F-Ph
31f	1	3-F-Ph
31g	1	4-F-Ph
31h	1	2-Cl-Ph

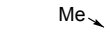
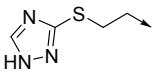
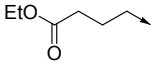
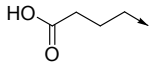
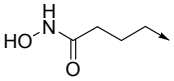
compound	n	R
31i	1	3-Cl-Ph
31j	1	4-Cl-Ph
31k	1	2-MeO-Ph
31l	1	3-MeO-Ph
31m	1	4-MeO-Ph
31n	2	2-F-Ph
31o	2	3-F-Ph
31p	2	2-Cl-Ph
31q	2	3-Cl-Ph

[a]Reagents and conditions: (a) (1) morpholine, toluene, 120 °C; (2) sulfur, toluene, EtOH, 70 °C, 18–77% (for **29c**, **29e**, **29f**, **29h**, **29i**, **29k**, and **29l** over 2steps); (b) ethyl cyanoformate, 1 N HCl/AcOH, 90 °C, 5–88% (for **30a–m**); (c) (1) **15a**, EtOH, 90 °C; (2) TFA, Et_3SiH, MeCN, 50 °C, 34–80% (for **31a–m** over 2 steps); (d) (1) **15b**, DIEA, EtOH, microwave, 100 °C; (2) TFA, Et_3SiH, MeCN, rt, 63–69% (for **31n–q** over 2

steps).

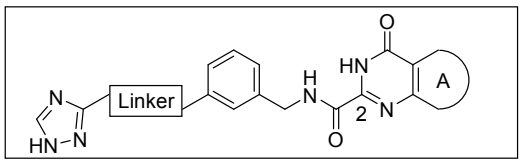
Table 1. Inhibitory Activities Against MMP-13 of Quinazoline Derivatives

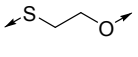
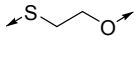
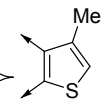
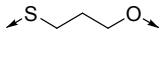
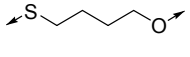
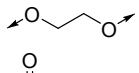
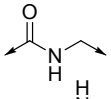
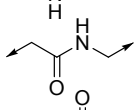
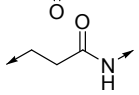


compound	R	IC_{50} (nM)[a]
1		12[b]
23		0.20[b]
22a		29[b]
22b		9.4[b]
22c		1.6[c]

[a]IC_{50} against MMP-13. [b]Each value is the mean from triplicate assay in a single experiment. [c]Values are reported as the mean of two experiments.

Table 2. MMP-13 Inhibitory Activities, Undesirable CYP3A4 Inhibitory Activities, and Oral AUC Values of Fused Pyrimidine Derivatives with 1,2,4-Triazol-3-yl ZBG

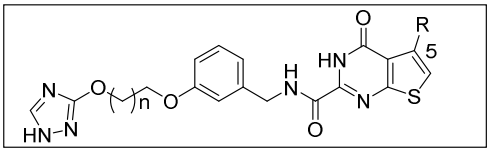


compound	Linker	A	IC_{50} (nM)[a] MMP-13	MMP-2	MMP-8	MMP-10	CYP3A4[b]	AUC[c]
23			0.20	83	140	140	49	8077
25a			0.074	ND[d]	ND[d]	ND[d]	67	23603
25b			0.033	62	21	140	60	22782
25c			2.6	230	510	>1000	74	5237
25d			0.064	14	11	30	20	10855
25e			1.5	660	110	460	ND[d]	19580
25f			4.9	>1000	480	>1000	15	1389
25g			0.29	670	76	270	8.8	1049

[a]IC_{50} against MMP-13. Each value is the mean from triplicate assay in a single experiment. [b]Inhibition (%) of CYP3A4 metabolic activity at 10 μM. [c]Rat AUC (ng•h/mL) following a single 1 mg/kg oral gavage dose in rats.

[d]Not determined.

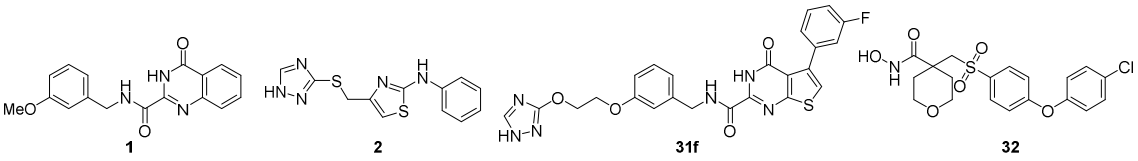
Table 3. MMP-13 Inhibitory Activities, Undesirable CYP3A4 Inhibitory Activities, and Rat Pharmacokinetic Parameters for 5-Substituted Thienopyrimidine Derivatives with 1,2,4-Triazol-3-yl ZBG



compound	n	R	IC_{50} (nM)[a]	CYP3A4[b]	AUC[c]
31a	1	*i*Pr	0.091	59	1322
31b	1	2-thiophenyl	0.027	57	2900
31c	1	3-thiophenyl	0.043	71	3194
31d	1	phenyl	0.034	45	469
31e	1	2-F-Ph	0.024	ND[d]	307
31f	1	3-F-Ph	0.036	35	2254
31g	1	4-F-Ph	0.093	60	299
31h	1	2-Cl-Ph	0.054	31	224
31i	1	3-Cl-Ph	0.057	41	266
31j	1	4-Cl-Ph	0.44	48	629
31k	1	2-MeO-Ph	0.29	30	271
31l	1	3-MeO-Ph	0.026	28	24
31m	1	4-MeO-Ph	0.10	25	109
31n	2	2-F-Ph	0.062	63	167
31o	2	3-F-Ph	0.14	62	1265
31p	2	2-Cl-Ph	0.059	52	44
31q	2	3-Cl-Ph	0.078	61	49

[a]IC_{50} against MMP-13. Each value is the mean from triplicate assay in a single experiment. [b]Inhibition (%) of CYP3A4 metabolic activity at 10 μM. [c]Rat AUC (ng•h/mL) following a single 1 mg/kg oral gavage dose in rats. [d]Not determined.

Table 4. Selectivity Profiles for **1**, **2**, **31f**, and **32**



1 **2** **31f** **32**

	IC_{50} (nM)									
compound	MMP-13	MMP-1	MMP-2	MMP-3	MMP-7	MMP-8	MMP-9	MMP-10	MMP-14	TACE
1	12[a]	> 10,000[a]	300[a]	> 10,000[a]	> 10,000[a]	1,100[a]	> 10,000[a]	3,400[a]	> 10,000[a]	> 10,000[a]
2	1,900[b]	> 10,000[b]	3,600[b]	> 10,000[b]	> 10,000[b]	9,300[b]	2,200[b]	83,000[b]	7,500[b]	ND[c]
31f	0.036[a]	> 10,000[a]	180[a]	1,100[a]	> 10,000[a]	> 10,000[a]	> 10,000[a]	55[a]	> 10,000[a]	> 10,000[a]
32	0.010[a]	34[a]	0.029[a]	0.30[a]	210[a]	0.097[a]	0.11[a]	0.54[a]	1.1[a]	14[a]

[a]Each value is the mean from triplicate assay in a single experiment without BSA (see

Experimental Section). [b]Values are means of two experiments in the presence of 0.5% BSA. [c]Not determined.

Table 5. Inhibition Activity in Bovine Nasal Cartilage Assay

compound	concentration (μM)	inhibition[a] (%)
32	0.01	-4.7 ± 10.8
	0.1	76.3 ± 18.5 *
	1	102.3 ± 0.6 *
31f	0.01	-17.6 ± 0.0
	0.1	48.4 ± 16.3
	1	70.8 ± 12.8 *

[a]Data are represented as means ± SEM (n = 6). * denotes $P < 0.025$ by one-tailed Williams' test.

Table 6. X-ray Crystallographic Data Collection and Refinement Statistics for Complex of **2** and **23** with MMP-13

	2 (5B5O)	**23** (5B5P)
Data Collection		
X-ray source	ALS BL5.0.3	ALS BL5.0.3

Wavelength (Å)	0.976	0.976
Space group	C2	C2
Unit cell dimensions	a = 136.4 Å, b = 36.1 Å, c = 95.9 Å, α = 90.0°, β = 131.1°, γ = 90.0°	a = 135.8 Å, b = 36.3 Å, c = 96.4 Å, α = 90.0°, β = 130.1°, γ = 90.0°
Resolution (Å)	1.20	1.60
Unique reflections	104586	46398
Redundancy	3.0	3.8
Completeness (%)	94.9 (60.4)	97.1 (68.5)
$I/\sigma(I)$	19.2 (2.3)	16.3 (2.3)
R_{sym}[a]	0.051 (0.363)	0.074 (0.443)
Refinement		
Reflections used	99347	44050
RMS Bonds (Å)	0.008	0.008

RMS Angles (°)	1.224	1.181
Average B value (Å^2)	15.2	23.1
R-value[b]	0.165	0.175
R $_{free}$[b]	0.185	0.208

[a]$R_{sym} = \Sigma h \Sigma j |<I(h)> - I(h)j| / \Sigma h \Sigma j <I(h)>$, where $<I(h)>$ is the mean intensity of symmetry-related reflections. [b]R-value $= \Sigma ||F_{obs}| - |F_{calc}|| / \Sigma |F_{obs}|$. R_{free} for 5% of reflections excluded from refinement. Values in parentheses are for the highest resolution shell.

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