Bioorganic & Medicinal Chemistry 22 (2014) 5487–5505

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry

journal homepage: www.elsevier.com/locate/bmc

Thieno[2,3-*d*]pyrimidine-2-carboxamides bearing a carboxybenzene group at 5-position: Highly potent, selective, and orally available MMP-13 inhibitors interacting with the S1″ binding site

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ARTICLE INFO

Article history:
Received 10 June 2014
Revised 15 July 2014
Accepted 16 July 2014
Available online 7 August 2014

Keywords:
Matrix metalloproteinase
MMP-13
Thieno[2,3-*d*]pyrimidin-4-one
Osteoarthritis
OA
X-ray crystallography
Structure-based drug design

ABSTRACT

On the basis of X-ray co-crystal structures of matrix metalloproteinase-13 (MMP-13) in complex with its inhibitors, our structure-based drug design (SBDD) strategy was directed to achieving high affinity through optimal protein–ligand interaction with the unique S1″ hydrophobic specificity pocket. This report details the optimization of lead compound **44** to highly potent and selective MMP-13 inhibitors based on fused pyrimidine scaffolds represented by the thienopyrimidin-4-one **26c**. Furthermore, we have examined the release of collagen fragments from bovine nasal cartilage in response to a combination of IL-1 and oncostatin M.

1. Introduction

Osteoarthritis is characterized by a loss of articular cartilage resulting in chronic pain and disability. Current treatments are limited to symptomatic relief with NSAIDs or COXIBs (selective COX-2 inhibitors), intra-articular injections of hyaluronic acid conjugates (Synvisc) or surgical joint replacement. Additionally, COXIBs' increased cardiovascular events such as heart attacks and stroke led to the recent withdrawal of the COX-2 inhibitor Vioxx from the market.[1,2]

The matrix metalloproteinases (MMPs) are a family of zinc-dependent enzymes involved in the degradation and remodeling of extra-cellular matrix.[3,4] The first matrix metalloproteinase (MMP-1) was a collagenase found in metamorphosing tadpole tail.[5] Since then, three members have been added to the collagenase family. These four collagenases MMP-1, MMP-8, MMP-13, and MMP-18 (a Xenopus collagenase-4) are capable of degrading interstitial collagens I, II, and III into two segments of 1/4 and 3/4 length.[4] MMPs are important therapeutic targets for various diseases[6] such as osteoarthritis (OA), rheumatoid arthritis, cancer, inflammatory bowel diseases, periodontal disease, and corneal ulceration. Among the MMP family, MMP-13 has been suggested to be intimately involved in the destruction of joint components in OA.[7,8] Therefore, MMP-13 inhibitors are expected to be disease-modifying drugs for OA.

In the past two decades, a large number of potent MMP inhibitors have been disclosed in the literature.[6,9] Earlier generation inhibitors designed based on the peptide fragment of substrates recognized by the MMP active site were found to fill the unprimed side of the active site by X-ray co-crystallographic studies. Most of the inhibitors have hydroxamic acid moiety as the zinc-binding group (ZBG).[10,11] Many of these small-molecule inhibitors showed a broad-spectrum inhibitory effect and were not selective between the MMP isoforms. Furthermore, a number of non-selective MMP inhibitors have been abandoned because of their musculoskeletal side effects (MSS) characterized by joint stiffness and pain.[12–14] Therefore, there is a great need for a new generation of inhibitors that can prevent degrading activities of specific MMPs with improved potency and reduced toxicity.

These findings prompted us to pursue selective MMP-13 inhibitors for the treatment of OA. Previously we have discovered a new class of human MMP-13 selective nonpeptide inhibitors based on

Abbreviations: MMP-13, matrix metalloproteinase 13; OA, osteoarthritis.

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http://dx.doi.org/10.1016/j.bmc.2014.07.025
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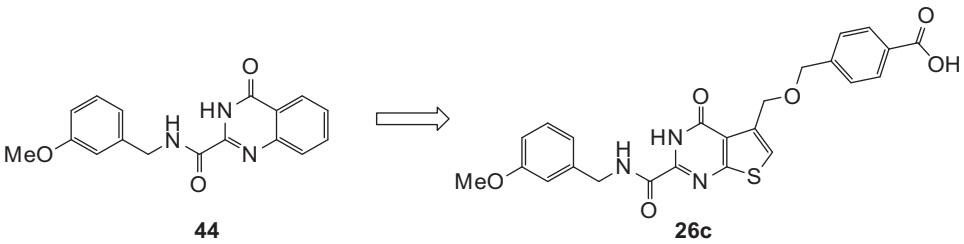


Figure 1. Chemical structures of **44** and **26c**.

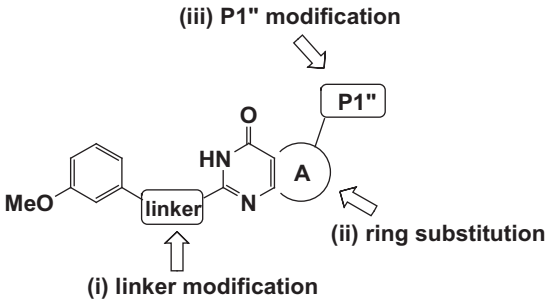


Figure 2. Structural modification of fused pyrimidine MMP inhibitors.

the lead compound **44**[15] (Fig. 1). In a parallel effort, a series of related fused pyrimidine analogues were also investigated.

Novel non-zinc chelating MMP-13 inhibitors were pursued with the goals of improving potency and selectivity for MMP-13 and physicochemical profile. As described in Figure 2, optimization studies were focused on (i) modification of the left-hand linker; (ii) modification/replacement of the ring A; (iii) modification of the right-hand P1″ group that could fit into the S1″ pocket.[16]

Optimization of this chemotype was conducted utilizing structure-based drug design (SBDD). The X-ray co-crystallographic analysis of **44** suggests that there is a unique and MMP-13-specific S1″ hydrophobic pocket[16] adjacent to the S1′ site accommodating the quinazolinone template (Fig. 3). To achieve an effective interaction with the S1″ binding site, the computer-aided analysis led to the design of inhibitors bearing a benzoic acid P1″ group, which can form a salt bridge with Lys140 in the S1″ site, tethered via a linker to the ring A, providing highly potent inhibitor thienopyrimidin-4-one **26c** (Fig. 1), which could potentially improve physicochemical properties such as water solubility and hence could improve the oral bioavailability. Furthermore, **26c** significantly inhibited degradation of explants of bovine nasal cartilage (BNC) induced by the treatment with a combination of interleukin-1 (IL-1) and oncostatin M (OSM).

In this report, we describe design, synthesis and SARs of a new generation of fused pyrimidine-based nonpeptide MMP-13 selective inhibitors.

2. Chemistry

Several routes were investigated for the synthesis of the fused pyrimidine-2-carboxylic acid derivatives, each of which generated the ester **6a**–**m** as a key intermediate for further functionalization. Starting from 2-aminoheteroaryl carboxylic acid derivatives **1a**–**d**, **3a**–**c**, and **4a**–**c**, the key intermediates **6a**–**m** were prepared by the standard one- or two-step procedure. The 2-ester groups of **6a**–**m** readily react with primary amines. Indeed, the compounds **6a**–**m** were easily converted to the corresponding amides **7a**–**m** by the general method described below in Scheme 1. Additionally, amide formation with a variety of substituted benzylamines in a parallel array led to a small library of the amides. The enhanced reactivity of the 2-ester group is probably attributed to anchimeric assistance by the neighboring 3-NH moiety. Activation of the 2-ester group through intramolecular hydrogen bonding with the 3-NH group readily accounts for the exquisite sensitivity of the 2-ester amidation. However, several analogous amidation could not be achieved. The ester is inert toward secondary amines in DMF at reflux temperature for 12 h or anilinic amines in DMF at reflux temperature over similar periods of time. Such a lack of reactivity in this aminolysis reaction is probably due to steric hindrance or low nucleophilicity.

Reaction of commercially available **9** with 2-(3-methoxyphenyl)acetyl chloride in the presence of triethylamine provided reverse amide-linked analogue **10** in 71% yield (Scheme 2).

As shown in Scheme 3, reaction of methylanthranilic acid **11** with chloroacetonitrile in methanol in the presence of sodium methoxide at 80 °C provided the 2-(chloromethyl)quinazolinone **12** in 67% yield. Replacement of the chlorine atom of **12** by

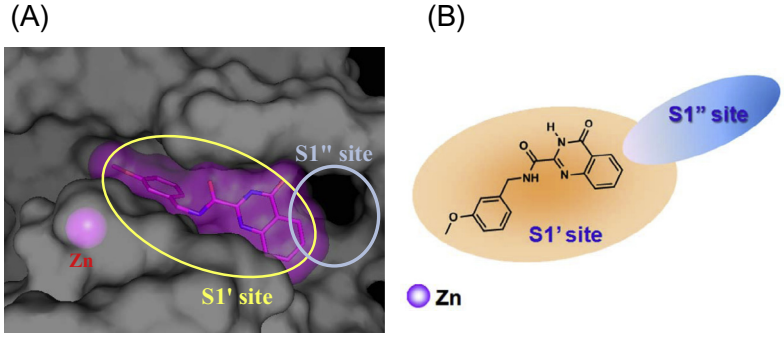
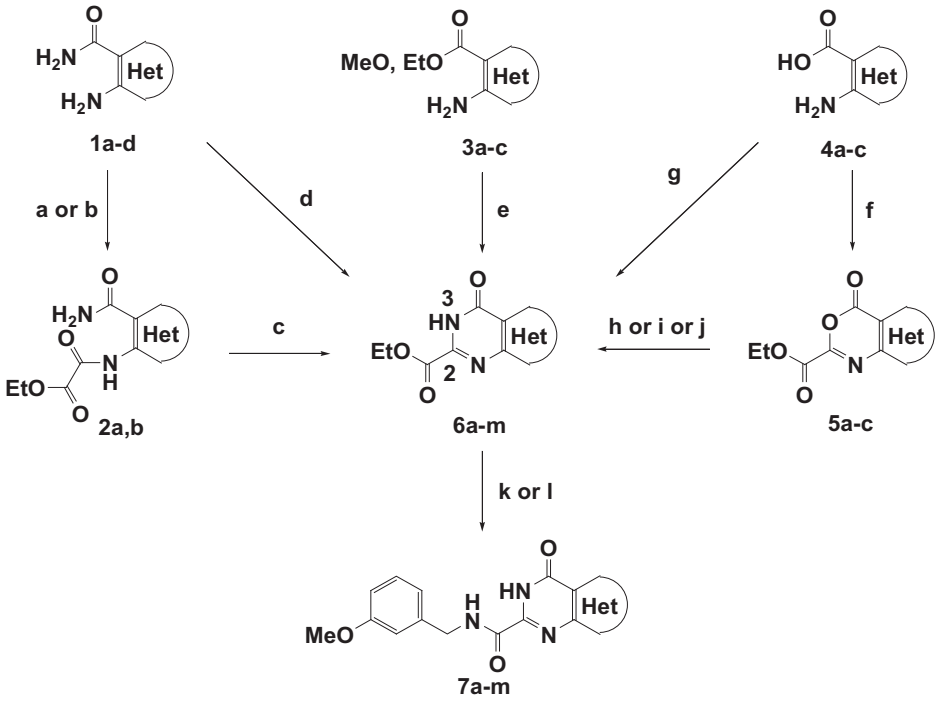


Figure 3. Crystal structure of the complex of **44** and MMP-13 (PDB code: 3WV2). (A) Surface representation of MMP-13 illustrating the binding cavity. The inhibitor is buried deeply into the S1′ pocket. (B) Schematic representation of the binding mode of **44** and MMP-13.



Compound					Het
1	2	3	6	7	
1a	2a	-	6a	7a	
-	-	-	6b	7b	
-	-	-	6c	7c	
-	-	-	6d	7d	
1b	2b	-	6e	7e	
1c	-	-	6f	7f	
1d	-	-	6g	7g	

Compound					Het
3	4	5	6	7	
3a	-	-	6h	7h	
3b	-	-	6i	7i	
3c	-	-	6j	7j	
-	4a	5a	6k	7k	
-	4b	5b	6l	7l	
-	4c	-	6m	7m	

Scheme 1. Synthesis of fused pyrimidine-2-carboxamide derivatives **7a**–**m**. Reagents and conditions: (a) ethyl chloroglyoxylate, Et_3N, THF, 0 °C to rt; (b) (1) diethyl oxalate, EtONa, EtOH, reflux, (2) oxalyl chloride, DMF, THF, rt, (3) EtOH, pyridine, THF, rt; (c) *p*-TsOH, toluene or xylene, reflux; (d) diethyl oxalate, EtONa, EtOH, reflux; (e) $CNCO_2Et$, HCl, AcOH, 80 °C; (f) ethyl chloroglyoxylate, pyridine, rt to 50 °C [for **4a**,**b**]; (g) (1) ethyl chloroglyoxylate, pyridine, rt, (2) oxalyl chloride, DMF, THF, 0 °C to rt [for **4c**]; (h) NH_4OAc, EtOH, reflux [for **5a**]; (i) NH_4OAc, AcOH, EtOH, reflux [for **5b**]; (j) (1) NH_3, EtOH, THF, 0 °C, (2) EtONa, EtOH, 0 °C to rt [for **5c**]; (k) 3-methoxybenzylamine, DMF or EtOH, 80–90 °C [for **6a**–**h**, **j**–**m**]; (l) 3-methoxybenzylamine, *N*-ethyldiisopropylamine, DMA, 80–90 °C [for **6i**].

3-methoxybenzylamine gave **13** in 41% yield by using potassium carbonate in DMF.

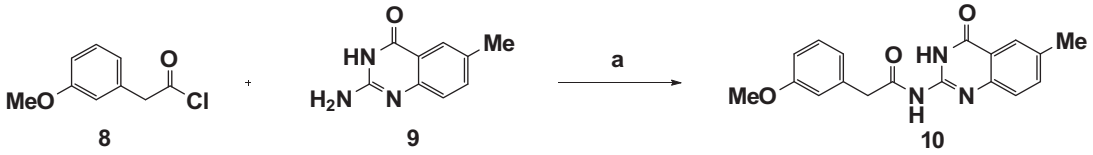
The general synthetic pathway to the 2-[3-(3-methoxyphenyl)propanoyl]-6-methyl-3,4-dihydroquinazolin-4-one **23** is shown in Scheme 4. The benzamide **14** was cyclized to the 6-methyl-3,4-dihydroquinazolin-4-one **15** by reaction with trimethyl orthoformate. Subsequently, the 3-nitrogen atom of compound **15** was easily protected by Boc group to give **16**, which upon treatment with LDA, was reacted with 3-(3-methoxyphenyl)propanal **19** prepared from carboxylic acid **17** to furnish hydroxyl derivative **20**. After deprotection of the Boc group of **20**, the alcohol function of **21** was then subjected to Swern oxidation using oxalyl chloride and dimethylsulfoxide with concomitant protection of the 4-carbonyl of the quinazoline ring as methylthiomethyl (MTM) ether to give the *O*-MTM compound **22**. Aqueous acidic hydrolysis of the MTM group afforded the target compound **23**.

The synthesis of the 3-methoxybenzylamide derivatives bearing para-substituted phenyl group via various 3-atom spacers linked to the 5-carbon of thieno[2,3-*d*]pyrimidine are described in Scheme 5.

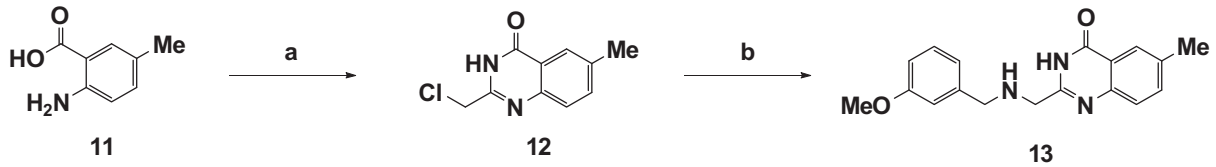
Starting with commercially available ester **6b**, the six thienopyrimidine-2-carboxylic acid ethyl esters bearing various substituents at the 5-position **25a–f** have been prepared by a modified Williamson procedure. Radical bromination of the C5-methyl of **6b** using *N*-bromosuccinimide (NBS) in chlorobenzene provided **24** (Scheme 5). The ether analogues **26a**,**b**,**d**,**e** derived from the bromide **24** were prepared by etherification with benzylalcohols and NaH in THF, followed by aminolysis of the resulting ester **25a–d** with 3-methoxybenzylmine. During the etherification step, partial hydrolysis of the ester occasionally occurred to give a carboxylic acid as a byproduct. The carboxylic acid was cleanly re-esterified under standard conditions to recover the ethyl ester, which upon treatment with 3-methoxybenzylamine was converted to the target amide. The synthesis of **26f** and **26g** containing the *N*-methyl or thioether spacers was achieved in a similar manner.

Alternately, the bromine atom of **24** can be displaced by nucleophiles such as sodium azide or cyanide to generate the corresponding azide analogue **27a** and cyano analogue **30**. The 5-[(phenylcarbamoyl)methyl] derivatives **29a–c** were prepared from the 5-bromomethyl intermediate **24** according to Scheme 5. The bromide **24** was converted by displacement with sodium azide, reduction of the resulting azide to the primary amine, and acylation to give **28a**,**b**. Aminolysis of the esters **28a**,**b** with 3-methoxybenzylamine in ethanol produced **29a**,**b**. The ester **29b** was next converted into the desired carboxylic acid **29c** by alkaline hydrolysis.

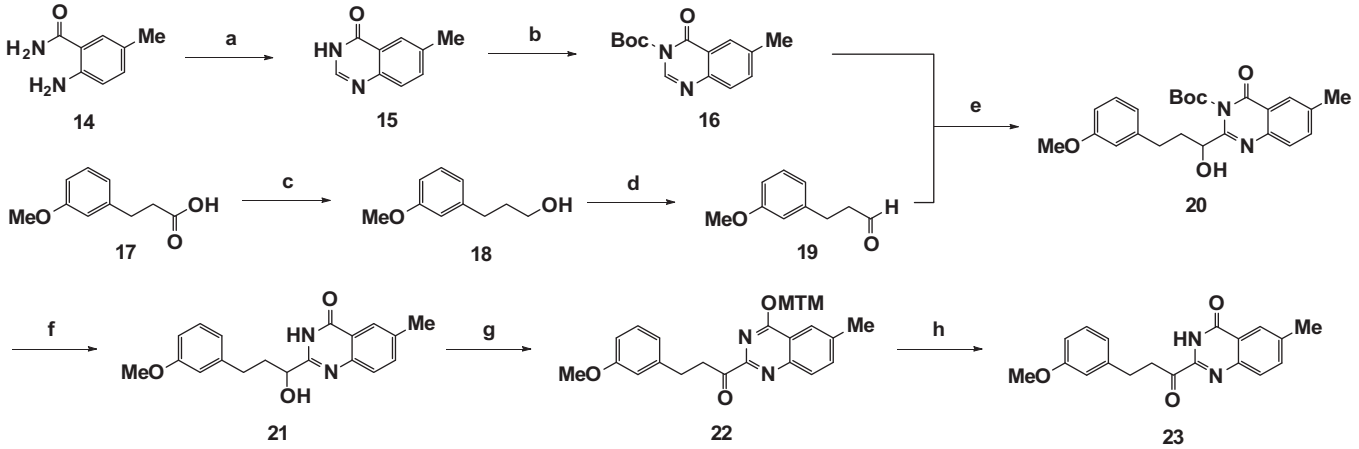
The bromomethyl intermediate **24** was converted by means of sodium cyanide to the cyanomethyl compound **30**, which was subjected to aminolysis of the ethyl ester to give **31a**. Cyano



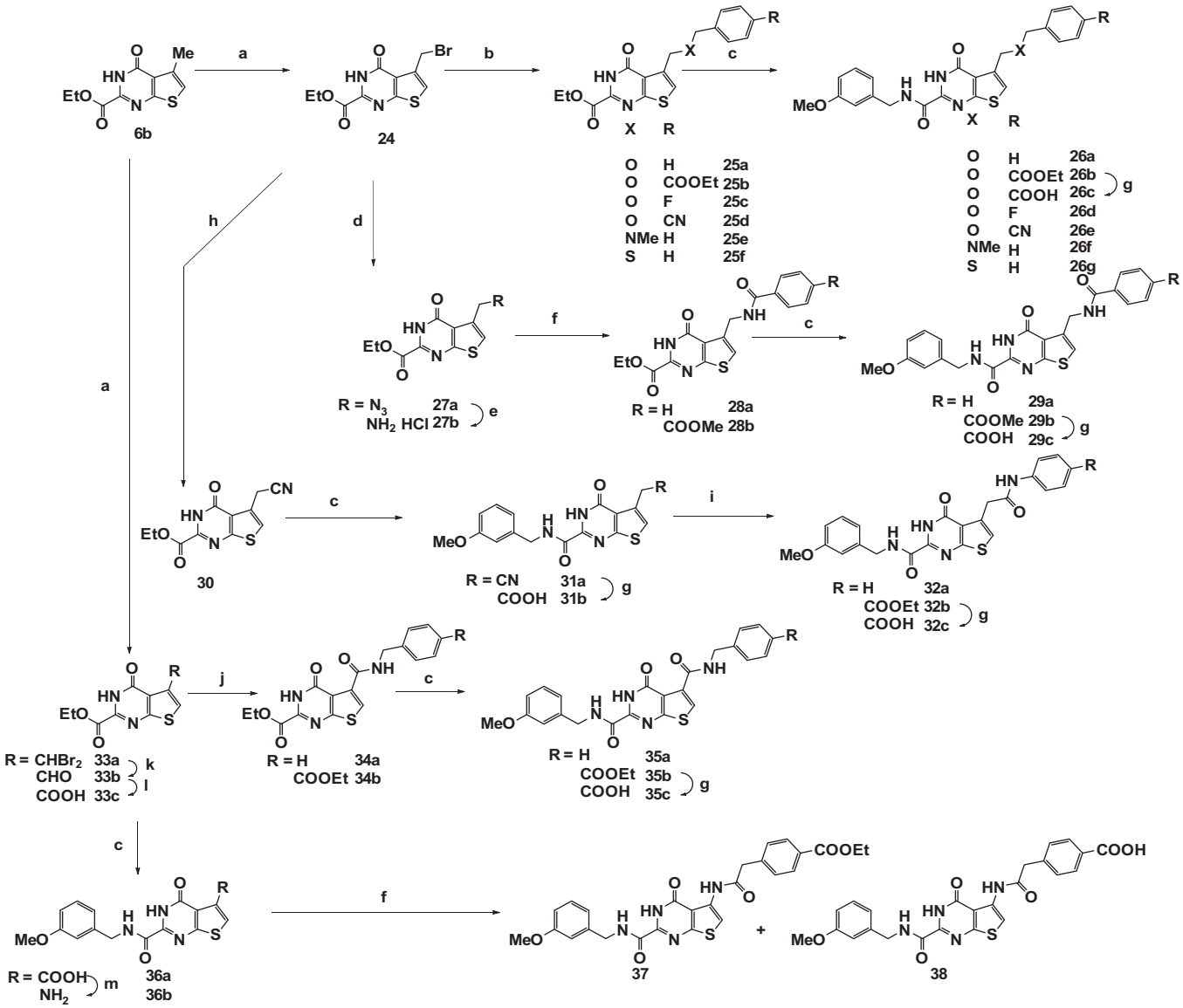
Scheme 2. Synthesis of reverse amide derivative **10**. Reagents and conditions: (a) TEA, THF, DMA, 90 °C, 71%.



Scheme 3. Synthesis of amine derivative **13**. Reagents and conditions: (a) chloroacetonitrile, MeONa, MeOH, rt to 80 °C, 67%; (b) 3-methoxybenzylamine, K_2CO_3, THF, 40 °C, 41%.



Scheme 4. Synthesis of ketone derivative **23**. Reagents and conditions: (a) $CH(OMe)_3$, concd HCl, 0 °C to rt, 81%; (b) Boc_2O, NaH, THF, 0 °C to rt, 91%; (c) (1) oxalyl chloride, DMF, THF, rt, (2) $NaBH_4$, THF, reflux, 92%; (d) TPAP, NMO, MS4A, CH_2Cl_2, rt, 63%; (e) (1) **16**, LDA, THF, −78 °C, (2) **19**, −78 °C to rt, 44%; (f) TFA, CH_2Cl_2, rt, 45%; (g) DMSO, oxalyl chloride, TEA, −78 °C to rt, 41%; (h) TFA, H_2O, CH_2Cl_2, rt, 64%.



Scheme 5. Synthesis of thieno[2,3-*d*]pyrimidines **26a**–**g**, **29a**–**c**, **32a**–**c**, **35a**–**c**, **37**, and **38**. Reagents and conditions: (a) 1.2 equiv NBS, AIBN, chlorobenzene, 80 °C, 54% [for **24**]; 2.3 equiv NBS, AIBN, carbon tetrachloride, 80 °C, 85% [for **33a**]; (b) (1) benzyl alcohol, NaH, THF, (2) oxalyl chloride, DMF, THF, (3) EtOH, THF, 16% [for **25a**], (1) 4-hydroxymethylbenzoic acid ethyl ester, NaH, THF, (2) oxalyl chloride, DMF, THF, (3) EtOH, pyridine, THF, 75% [for **25b**], (1) (4-fluorophenyl)methanol, THF, NaH, (2) EtOH, EDC HCl, DMAP, THF, 8% [for **25c**], (1) 4-(hydroxymethyl)benzonitrile, THF, NaH, (2) EtOH, EDC HCl, DMAP, THF, 52% [for **25d**]; *N*-methyl-1-phenylmethanamine, Et_3N, THF, 52% [for **25e**]; phenylmethanethiol, Et_3N, DMA, 30% [for **25f**]; (c) 1-[3-(methyloxy)phenyl]methanamine, EtOH, 80–90 °C, 60% [for **26a**]; 90% [for **26b**]; 81% [for **26d**]; 79% [for **26e**]; 58% [for **26f**]; 67% [for **26d**]; 82% [for **29a**]; 89% [for **29b**]; 76% [for **31a**]; 85% [for **35a**]; 91% [for **35b**]; 1-[3-(methyloxy)phenyl]methanamine, ethyldiisopropylamine, EtOH, 90 °C, 67% [for **36a**]; (d) NaN_3, DMF, 76%; (e) H_2, HCl, Pd/C, EtOH–THF, 94%; (f) benzoyl chloride, triethylamine, THF, 72% [for **28a**]; 4-chlorocarbonylbenzoicacidmethyl ester (prepared from monomethyl terephthalate, oxalyl chloride, DMF, THF), Et_3N, THF, 82% [for **28b**], (1) ethyl 4-(2-chloro-2-oxoethyl)benzoate (prepared from 2-[4-(ethoxycarbonyl)phenyl]acetic acid, oxalyl chloride, DMF, THF), pyridine, THF, (2) aqueous NaOH solution, EtOH/THF, 6% [for **37**]; 17% [for **38**]; (g) aqueous NaOH solution, EtOH or THF, MeOH, 80–100 °C, quant. [for **26c**]; 98% [for **29c**]; 62% [for **31b**]; 86% [for **32c**]; 62% [for **31b**]; 86% [for **32c**]; 94% [for **35c**]; (h) NaCN, DMF/H_2O, 0 °C to rt, 69%; (i) (1) oxalyl chloride, THF, (2) aniline, pyridine, THF, 64% [for **32a**], (1) oxalyl chloride, THF, (2) ethyl 4-aminobenzoate, pyridine, THF, 76% [for **32b**]; (j) (1) oxalyl chloride, DMF, THF (2) benzylamine, THF, 70% [for **34a**], (1) oxalyl chloride, DMF, THF (2) ethyl 4-(aminomethyl)benzoate hydrochloride, THF, 80% [for **34b**]; (k) 1 N HCl, THF, MeOH, 60 °C, 69%; (l) $NaClO_2$, MeCN, H_2O, 57%; (m) (1) DPPA, Et_3N, toluene, 100 °C, (2) *tert*-butyl alcohol, 100 °C, (3) HCl, AcOEt, (4) aqueous NaOH, THF, MeOH, 80 °C, 26%.

compound **31a** was hydrolyzed with aqueous sodium hydroxide to afford carboxylic acid **31b**. The carboxylic acid was converted to the acid chloride, which was then condensed with anilines to give **32a**,**b**. Ester **32b** was converted by the treatment of sodium hydroxide to give compound **32c**.

Bromination of **6b** with 2.3 equiv of NBS in the presence of AIBN afforded *gem*-dibrominated product **33a**. Hydrolysis of the *gem*-dibromo compound gave the aldehyde **33b**. Oxidation of the resulting aldehyde intermediate to the corresponding carboxylic acid **33c** was achieved using sodium chlorite in aqueous acetonitrile. Conversion of the carboxylic acid moiety to acid chloride followed by coupling with benzylamines and subsequent aminolysis by 3-methoxybenzylamine provided **35a**,**b**. The ethyl ester of **35b** was hydrolyzed to give the corresponding carboxylic acid **35c** in 94% yield.

The synthesis of 5-substituted 4-oxo-3*H*,4*H*-thieno[2,3-*d*]pyrimidine derivatives **37** and **38** was performed according to Scheme 5. Reaction of the ester **33c** with 3-methoxybenzylamine proceeded at the 2-position to provide the amide **36a**. Heating a solution of **36a** with diphenyl phosphoryl azide (DPPA) and triethylamine in toluene generated the isocyanate in situ, which could be trapped with *tert*-butanol to afford *tert*-butyl carbamate

accompanied by a side product, as identified by LCMS spectra[17] of the crude reaction mixture. This is interpreted as a result of intramolecular cyclization via O-4 attack on the isocyanate. The mixture was treated with HCl–EtOAc to remove the Boc protecting group and subsequently treated with aqueous sodium hydroxide to hydrolyze the cyclized byproduct[17]. Neutralization the reaction mixture afforded the free primary amine **36b**. Acylation of amine **36b** with ethyl 4-(2-chloro-2-oxoethyl)benzoate readily afforded the 5-substituted thienopyrimidine derivatives that was subjected to alkaline hydrolysis to provide a separable mixture of unreacted **37** and hydrolyzed product **38**.

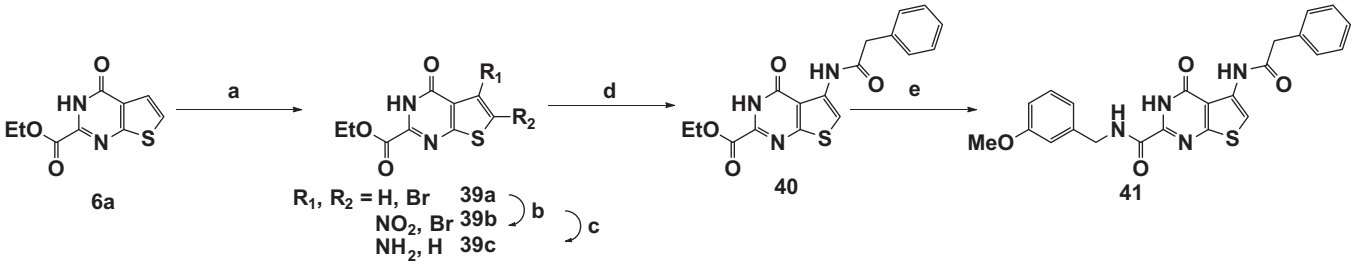
As shown in Scheme 6, bromination followed by nitration with potassium nitrate in sulfuric acid exclusively provided ethyl 6-bromo-5-nitro-4-oxo-3*H*,4*H*-thieno[2,3-*d*]pyrimidine-2-carboxylate **39b**. Hydrogenation in the presence of palladium catalyst produced a simultaneous reduction of the nitro group and hydrogenolysis of the bromo group to give **39c**. Acylation of **39c** with phenylacetyl chloride, followed by aminolysis with 3-methoxybenzylamine provided the diamide compound **41**.

The carboxylic acid **26c** was converted to carboxamide **42a** by reaction with oxalyl chloride followed by ammonia (Scheme 7). *N*-Methylamide **42b** was prepared by condensation of the carboxylic acid **26c** with methylamine hydrochloride using 1-ethyl-3-[3-(dimethylamino)propyl]carbodiimide hydrochloride (EDC) as a coupling agent in the presence of DMAP. The acid chloride prepared from **26c** was converted to the corresponding primary alcohol **42c** by reduction with sodium borohydride in DMA. The alcohol **42c** was reacted with methanesulfonyl chloride in THF and the resulting mesylate was then converted to the corresponding methyl ether **42d** by displacement with sodium methoxide.

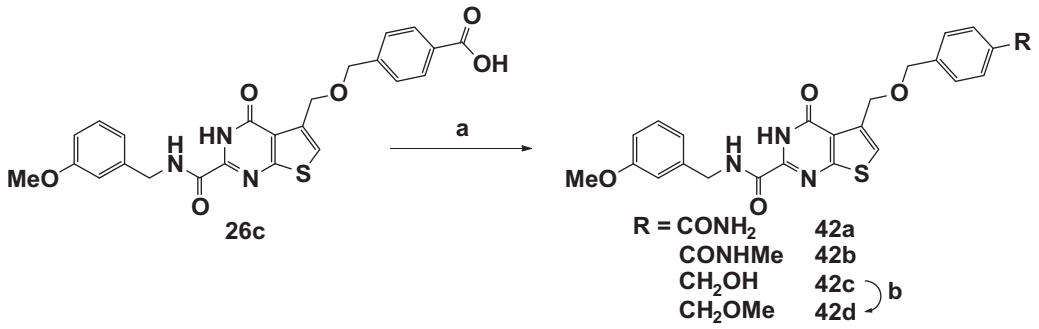
Disodium salt of **26c** was prepared as shown in Scheme 8. The precipitate, obtained by addition of 2.0 equivalent of aqueous sodium hydrogen carbonate to a solution of **26c** in THF–EtOH, was separated from the solution by filtration. Reprecipitation was carried out in EtOH at 90 °C to obtain the disodium salt **43**.

3. Results and discussion

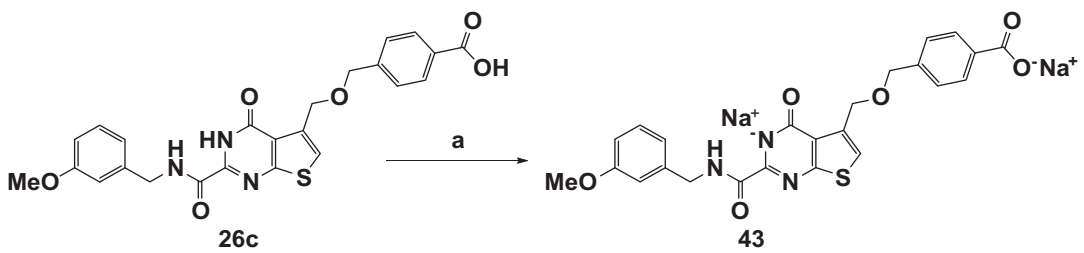
The high-throughput screening first identified compound **44** as a lead compound of MMP-13 inhibitor. The compound exhibited potent inhibitory activity with an IC_{50} value of 12 nM and selective properties by inhibiting MMP-13 showing weak inhibitory activity towards MMP-1, 3, 7, 9, 14, and TACE (see Table 4).



Scheme 6. Synthesis of 5-(2-phenylacetamido)thieno[2,3-*d*]pyrimidine **41**. Reagents and conditions: (a) Br_2, AcOH, 93%; (b) concd H_2SO_4, $NaNO_3$, 0 °C, 80%; (c) H_2, Pd/C, EtOH/THF, 36%; (d) phenylacetyl chloride, Et_3N, THF, 77%; (e) 3-methoxybenzylamine, EtOH, 90 °C, 93%.



Scheme 7. Synthesis of compounds **42a**, **42b**, **42c**, and **42d**. Reagents and conditions: (a) (1) oxalyl chloride, DMF, THF, (2) 28% aqueous ammonia solution, THF, 87% [for **42a**]; methylamine hydrochloride, EDC, DMAP, THF, 67% [for **42b**], (1) oxalyl chloride, DMF, THF, (2) $NaBH_4$, DMA, 65% [for **42c**]; (b) (1) MsCl, Et_3N, THF, (2) sodium methoxide, MeOH/THF, 80 °C, 66% [for **42d**].



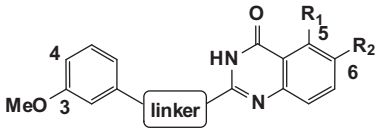
Scheme 8. Synthesis of disodium salt **43**. Reagents and conditions: (a) (1) THF, EtOH, aqueous $NaHCO_3$, (2) EtOH, 90 °C, 91%.

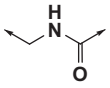
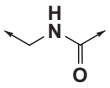
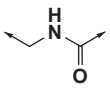
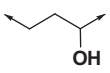
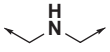
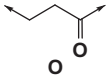
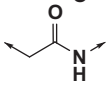
In an effort to optimize the left-hand portion of **44** in an efficient fashion, a small library of its analogues was synthesized by an automated high throughput approach. The biological activity of the first set of substituted benzyl derivatives on the terminal aryl ring in the left hand side portion of the molecule demonstrated that the C-3, 4 positions were promising for introduction of small substituents. Among them, 3-methoxy group was found to be one of the best substituent of the terminal aryl group. We therefore carried out further optimization of compound bearing 3-methoxy phenyl group to explore the SAR.

To confirm the pivotal importance of the linkers between the quinazoline and 3-methoxybenzene rings, compounds bearing modified linkers were initially investigated. Table 1 shows the SAR of the three-atom linker analogues. Replacement of the amide carbonyl with a methylene group (**13**) gave a large decrease in activity, and the same was observed in the reverse amide linker analogue **10**. In contrast, replacement of the amide nitrogen with a methylene group retained moderate activity (**23**), demonstrating the importance of the carbonyl group which is assumed to interact with Tyr244 and Thr245 from X-ray crystallographic analysis (Fig. 4). Also, reduction of the carbonyl group of **23** to a secondary hydroxyl group (**21**) resulted in a drastic drop in potency. These results revealed that the original amide linker (**44**, **45**, and **46**) is crucial for potent enzyme inhibition. The addition of a methyl group at the 5- or 6-position of the quinazoline (**45** and **46**) was found to be acceptable, since both amide analogues retained good inhibitory activity.

Replacement of the benzene ring of the core quinazoline with a thiophene ring, a known bioisostere, was studied (Table 2). Methyl-substituted compound **7b–c** exhibited approximately 4- to 10-fold improved activity compared to the original inhibitor **44** (see Table 1). It should be noted that the 5-methylthienopyrimidine

Table 1
Effect of linker variation within 3,4-dihydroquinazolin-4-one series



Compound	Linker	R_1	R_2	IC_{50} (nM)[a]
44		H	H	12 ± 1.5
45		Me	H	29 ± 3.4
46		H	Me	26 ± 3.1
21[b]		H	Me	>10,000
13		H	Me	>10,000
23		H	Me	220 ± 25
10		H	Me	>10,000

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

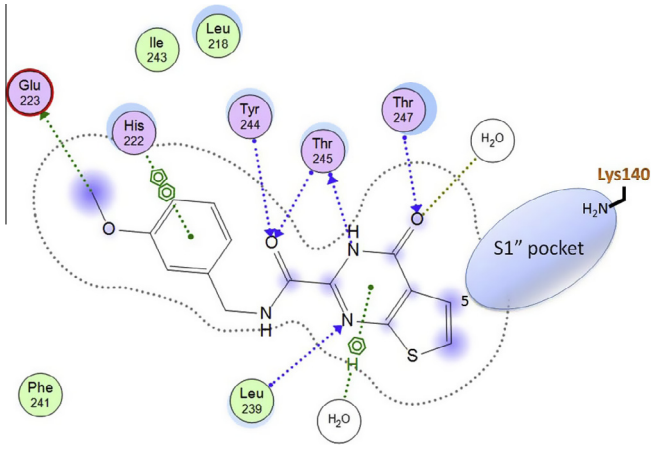


Figure 4. X-ray co-crystal structure of prototype compound **7a** in complex with MMP-13 catalytic domain (PDB code: 3WV3) in a schematic representation. The ligand interacts in a similar fashion as **44** with MMP-13 residues of the specific S1′ pocket (dotted curved line). Molecular modeling suggested that attachment of the P1″ substituent via a linker to the thiophene 5-position of the 4-oxo-3*H*,4*H*-thieno[2,3-*d*]pyrimidine scaffold would afford a target molecule. An additional possible interaction of P1″ with Lys140 residue at the bottom of the S1″ pocket is present in the protein.

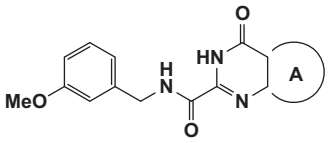
7b is more than 20-fold more potent than the corresponding 5-methylquinazoline analogue **45** (see Table 1). Replacement of the thiophene ring with the other heteroaromatic rings such as pyrrolo-, furo-, pyrazolo-, isoxazolo-, and pyridopyrimidines generally decreased activity. Specifically, even replacing the thiophene with a furan caused a more than 10-fold loss of potency (**7d** vs **7e**), and replacement of the thiophene with a six-membered heteroaryl group such as pyridine led to a more than 100-fold reduction in potency (**7a** vs **7l**). Because of its activity, 4-oxo-3*H*,4*H*-thieno[2,3-*d*]pyrimidine ring system was chosen for systematic exploration.

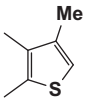
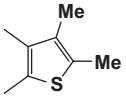
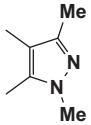
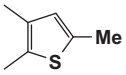
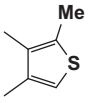
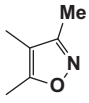
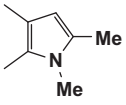
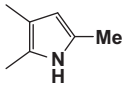
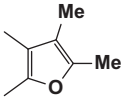
As mentioned earlier, the X-ray co-crystallographic analysis revealed that compound **44** does not interact with the catalytic zinc atom of MMP-13 and binds deeply in the S1′ pocket. The analysis showed an important finding that an MMP-13-specific hydrophobic pocket, which is referred to as the S1″ pocket, exists adjacent to the S1′ pocket (see Figs. 2 and 4). As shown in Figure 4, the thiophene congener **7a** proved to have a similar binding mode, suggesting that the 5-position of the 4-oxo-3*H*,4*H*-thieno[2,3-*d*]pyrimidine scaffold can be suitable for incorporation of a substituent binding to the S1″ pocket (P1″ substituent). The co-crystallographic analysis indicates that the S1″ pocket is mainly hydrophobic and provides sufficient binding space accommodating a substituted phenyl group via a linker length of three atoms. In addition, the structural studies have shown that an additional hydrogen bond or ionic interaction can be exploited through interaction of P1″ substituent with Lys140 residue at the bottom of the S1″ pocket present in the protein. Additionally, the phenyl group of the 3-methoxybenzyl amide of compound **7a** could participate in a nearly co-planar stacking interaction with His222.

To improve the overall profile such as MMP-13 inhibitory activity, aqueous solubility, and pharmacokinetic property, we utilized a strategy of incorporation of hydrogen bond donor/acceptor groups at the 4-position of the P1″ phenyl group. Molecular modeling suggested that attachment of the phenyl group via a three-atom linker to the 5-position of the thienopyrimidine core would afford a target molecule which could bind to MMP-13 with little conformational strain.

We therefore set out to explore favorable P1″ substituents. The effects of the substituent of the terminal phenyl group at the 5-position and the three-atom linker between the phenyl group

Table 2
In vitro data for ring A substituted *N*-[(3-methoxyphenyl)methyl]-6-oxo-1,6-dihydropyrimidine-2-carboxamide derivatives



Compound	A	IC_{50} (nM)[a]	Compound	A	IC_{50} (nM)[a]
7b		1.1 ± 0.043	**7k**		29 ± 5.2
7d		1.2 ± 0.069	**7g**		65 ± 10
7c		3.1 ± 0.29	**7m**		200 ± 38
7h		12 ± 1.6	**7f**		760 ± 95
7j		14 ± 2.2	**7j**		1,100 ± 220
7e		16 ± 2.1	**7l**		3,200 ± 300
7a		24 ± 3.7			

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

and thieno[2,3-*d*]pyrimidine core were investigated (Table 3). Considering synthetic feasibility, a benzyloxy group was initially incorporated into the 5-methyl group of **7b** (see Table 2), resulting in an about 6-fold increase in potency (**26a**). Indeed, the benzyloxymethyl analogue **26a** exhibited subnanomolar potency (IC_{50} = 0.19 - nM). The para position of the terminal phenyl group of **26a** was then substituted with hydrogen bonding groups. Incorporation of a carboxylic acid moiety to the para position dramatically enhanced the activity, affording the extremely potent inhibitor **26c** with an IC_{50} value of 6.9 pM. Other substitution such as fluoro, hydroxymethyl, methoxymethyl, cyano, carboxamide, and *N*-methylcarboxamide gave less potent inhibition than the carboxy substituent **26c** although the potencies were enhanced compared to the corresponding non-substituted compounds.

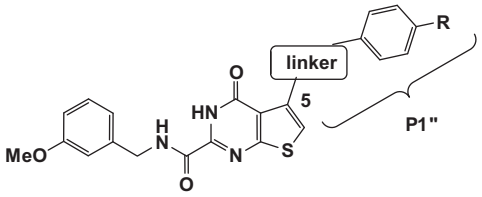
Modification of the linker with or without a carboxylic acid at the terminal phenyl group was next examined (Table 3). When compared with compounds possessing non-substituted phenyl group, replacement of the ether linker with a thioether or tertiary amine linker significantly decreased potency. In contrast, replacement of the ether linker with amide-containing linkers increased the potency by more than 3-fold. The compounds with amide linkers (**32a**, **29a**, **35a**, and **41**) displayed two-digit pM order potency, indicating that there is little preference of the positions of the carbonyl and NH groups. Incorporation of a carboxylic acid moiety into these compounds gave an additional boost in activity, affording picomolar inhibitors (**32c**, **29c**, and **35c**).

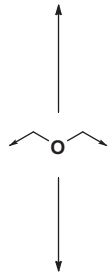
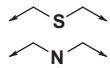
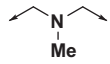
On the basis of the activities and pharmacokinetic properties of the carboxylic acid derivatives **26c**, **32c**, **29c**, **35c**, and **38**, the 4-carboxybenzyloxymethyl analogue **26c** was selected for further investigation.

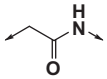
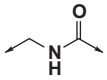
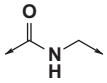
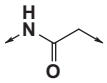
Compound **26c** that has the most favorable profile was subsequently evaluated for selectivity against MMP-1, -2, -3, -7, -8, -9, -10, -14, and tumor necrosis factor-alpha converting enzyme (TACE). As shown in Table 4, the hydroxamic acid-based MMP inhibitor **47** (**RS-130,830**[18]) reported by Roche Biosciences research group did not exhibit sufficient selectivity. For instance, its selectivity for MMP-13 over the other metalloenzymes tested proved to be less than 3-fold. On the other hand, the thieno[2,3-*d*]pyrimidine analogue **26c** showed improved selectivity over all other related metalloenzymes tested by approximately 100-fold relative to **44**. As a consequence, **26c** showed more than 2600-fold selectivity over the other related metalloenzymes. Furthermore, **26c** was ca. 1700-fold more potent than the lead compound **44**.

The pharmacokinetics of **26c** have been studied in guinea pigs, dogs, and monkeys. As shown in Table 5, oral administration of the disodium salt formulations of **26c** (**43**) to guinea pigs resulted in significant increases in AUC (8357 ng h/mL) and C_{max} (1445 ng/mL) compared with those of the free acid **26c** (AUC = 6478 ng h/mL and C_{max} = 911 ng/mL). The compound was well absorbed in all species at the oral dose of 10–20 mg/kg. The oral AUC values of **43** in dogs and monkeys were found to be 27136 and 82360 ng·h/mL, respectively.

Table 3
Inhibition of MMP-13 by derivatives with different linkers and their pharmacokinetic parameters in male rats



Cpd	Linker	R	IC_{50} (nM)[a]	AUC[b,c]	Vd,ss[b,d]	CL[b,e]
26a		H	0.19 ± 0.021	82	1061	2179
26d		F	0.13 ± 0.016	ND[f]	ND[f]	ND[f]
42c		CH_2OH	0.032 ± 0.0031	< 1	1285	5216
42d		CH_2OMe	0.080 ± 0.0063	< 1	960	1946
26e		CN	0.018 ± 0.0032	42	813	961
42a		$CONH_2$	0.015 ± 0.0013	6	874	2472
42b		CONHMe	0.029 ± 0.0012	47	780	2722
26c		COOH	0.0069 ± 0.00078	366	878	693
26d		H	0.66 ± 0.075	12	1175	1624
26f		H	39 ± 8.3	411	767	778

Cpd	Linker	R	IC_{50} (nM)[a]	AUC[b,c]	Vd,ss[b,d]	CL[b,e]
32a		H	0.028 ± 0.0033	311	867	1037
32c		COOH	0.005 ± 0.0006	201	1417	1362
29a		H	0.018 ± 0.0038	2289	314	214
29c		COOH	0.0022 ± 0.00073	22	871	1218
35a		H	0.026 ± 0.0022	1685	238	153
35c		COOH	0.0077 ± 0.00095	304	1072	865
41		H	0.053 ± 0.0075	2705	315	120
38		COOH	0.021 ± 0.0018	1044	773	501

[a] IC_{50} (nM) represents the average IC_{50} value in nanomolars for the inhibition assay of the compound using the human MMP-13 catalytic domain enzyme. Data are expressed as mean IC_{50} ± SE, n = 3.
[b] Data are given as mean values of triplicates.
[c] Rat AUC (ng h/mL) following a single 1 mg/kg oral cassette gavage dose in rats.
[d] Rat volume of distribution at steady state (Vd,ss) (mL/kg) at an intravenous dose of 0.1 mg/kg.
[e] Rat total body clearance (mL/h/kg) at an intravenous dose of 0.1 mg/kg.
[f] Not determined.

To assess the in vitro efficacy of **26c** in tissues, bovine nasal septum cartilage explants method was used.[19,20] The cartilage explants were treated with a combination of two cytokines, IL-1β and oncostatin M, to induce production of endogenous MMPs from chondrocytes, resulting in the degradation and the release of type II collagen fragments into the tissue culture fluid. Compound **26c** significantly inhibited the breakdown of collagen (87.4% inhibition at 0.1 μM) in IL-1β and oncostatin M stimulated cartilage (as measured by hydroxyproline release), which is comparable to broad spectrum MMP inhibitor **47** (**RS-130,830**) (76.3% inhibition at 0.1 μM) (Table 6).

Furthermore, compound **43** was evaluated for 2-week repeated dose oral toxicity study in rats and a no observed adverse effect level of 60 mg/kg/day was established.

4. Conclusion

On the basis of the X-ray co-crystal structure of MMP-13 in complex with **44**, we designed a new structural class of MMP-13 selective inhibitors employing a thieno[2,3-*d*]pyrimidine as the central scaffold. Our strategy to identify highly selective and potent inhibitors is based on the assumption that additional interaction with an unexplored selectivity binding site (S1″) of MMP-13 could improve the potency and selectivity.

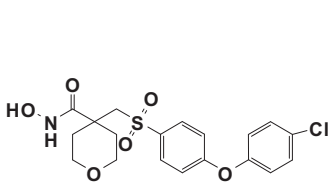
Using the X-ray structural analyses and computer modeling studies, we optimized the inhibitory activity and selectivity against MMP-13 of these compounds. The thienopyrimidine-based inhibitors that could bind to the unprimed side of MMP-13 form a tight β-sheet type interaction between the 3, 4 position of the pyrimidine and Thr245, 247 residues from the enzyme's backbone spanning the S1′ and S1″ pockets (see Fig. 4). Attachment of the P1″ substituent via a linker aimed to form hydrogen bond interactions of P1″ with Lys140 residue at the bottom of the S1″ pocket led to the discovery of the highly potent and selective MMP13 inhibitor **26c** with an IC_{50} value of 6.9 pM and more than 2600-fold selectivity over the other related metalloenzymes. Furthermore, the inhibitor was shown to be active in bovine nasal cartilage explants assay and possesses favorable ADME and safety profiles.

5. Experimental section

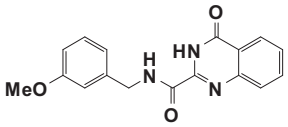
5.1. Chemistry

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) 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. LC-MS analysis was performed on a Shiseido CAPCELL PACK C-18 UG120 S-3 column (1.5 mm Φ × 35 mm) in a Waters Alliance 2795 or an Agilent 1100 LC system equipped with a Waters 2487 absorbance detector and a Micromass ZQ2000 mass spectrometer. Analytes were eluted using a linear gradient of water (0.05% TFA)/acetonitrile (0.04% TFA) from 90:10 to 0:100 over 4 min at a flow rate of 0.5 mL/min. UV detection was at 220 nm. Preparative HPLC was performed on a Shiseido CAPCELL PACK

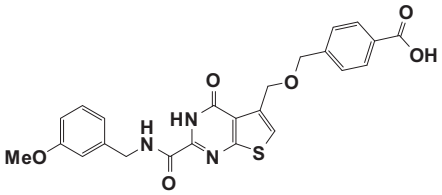
Table 4
IC_{50} values for inhibition of MMPs and TACE



47
RS-130,830



44



26c

Compound	IC_{50} (nM)[a] MMP-13	MMP-1	MMP-2	MMP-3	MMP-7
47	0.010 ± 0.0016	34 ± 16	0.029 ± 0.0036	0.30 ± 0.051	210 ± 15
44	12 ± 1.5	>10,000	300 ± 9.0	>10,000	>10,000
26c	0.0069 ± 0.00078	>10,000	18 ± 3.6	600 ± 220	>10,000

Compound	IC_{50} (nM)[a] MMP-8	MMP-9	MMP-10	MMP-14	TACE
47	0.097 ± 0.024	0.11 ± 0.0013	0.54 ± 0.076	1.1 ± 0.16	14 ± 1.3
44	1,100 ± 150	>10,000	3,400 ± 1,000	>10,000	>10,000
26c	780 ± 290	>10,000	160 ± 35	>10,000	>10,000

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

Table 5
Pharmacokinetics of **26c** and its disodium salt **43** in guinea pig, dog, and monkey

	Compound	C_{max} ng/mL(po)	T_{max} h (po)	AUC ng·h/mL (po)	Vd,ss[e] mL/kg (iv)	CL[f] mL/h/kg (iv)	% *F*
Guinea pig[a]	26c	911	0.83	6478	923	431	28
Guinea pig[b]	43	1445	0.67	8357	—	—	—
Dog[c]	43	2438	2.0	27136	395	111	29
Monkey[d]	43	6607	3.0	82360	—	—	—

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

Table 6
Inhibition activity in bovine nasal cartilage assay

Compound	Concentration (μM)	Inhibition[a] (%)
47 (**RS-130,830**)	0.01	−4.7 ± 10.83
	0.1	76.3 ± 18.54*
	1	102.3 ± 0.61*
26c	0.01	22.6 ± 24.18
	0.1	87.4 ± 7.64*
	1	100.3 ± 1.09*

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

C-18 UG120 S-5 column (20 mm $\Phi \times$ 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. Elemental analyses were performed by Takeda Analytical Research Laboratories, Ltd.

5.1.1. Ethyl 4-oxo-3*H*,4*H*-thieno[2,3-*d*]pyrimidine-2-carboxylate (6a)

A mixture of compound **2a** (2.00 g, 8.26 mmol) and *p*-toluenesulfonic acid monohydrate (514 mg, 2.70 mmol) in xylene (50 mL) was heated under reflux for 11 h. The reaction mixture

was diluted with ethyl acetate, washed with water and brine, dried over anhydrous Na_2SO_4 and concentrated under reduced pressure. The residue was purified by silica gel column chromatography (67% ethyl acetate/hexane) to give **6a** as a pale yellow powder (531 mg, 29%). ^{1}H NMR (200 MHz, DMSO-d_6) δ 1.35 (3H, t, J = 7.2 Hz), 4.38 (2H, q, J = 7.2 Hz), 7.49 (1H, d, J = 5.6 Hz), 7.81 (1H, d, J = 5.6 Hz).

5.1.2. Ethyl 5,6-dimethyl-4-oxo-3*H*,4*H*-furo[2,3-*d*]pyrimidine-2-carboxylate (6e)

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

5.1.3. Ethyl 1,3-dimethyl-4-oxo-1*H*,4*H*,5*H*-pyrazolo[3,4-*d*]-pyrimidine-6-carboxylate (6g)

To a solution of 5-amino-1,3-dimethyl-1*H*-pyrazole-4-carboxamide[21] (**1d**) (3.00 g, 19.5 mmol, synthesized by the method of Cheng et al.) and diethyl oxalate (11.4 g, 77.8 mmol) in ethanol (500 mL) was added sodium ethylate (33.1 g, 97.3 mmol) at 0 °C, and the mixture was heated under reflux for 18 h. The reaction mixture was allowed to cool to room temperature and poured into 1 M hydrochloric acid (100 mL). The mixture was concentrated under reduced pressure and the residue was suspended with water. The precipitated solid was collected by filtration and washed with water and ethanol to give **6g** as a pale yellow powder (2.52 g, 55%). ^{1}H NMR (300 MHz, DMSO-d_6) δ 1.35 (3H, t, J = 7.2 Hz), 2.44 (3H, s), 3.86 (3H, s), 4.38 (2H, q, J = 7.2 Hz), 12.4 (1H, bs).

5.1.4. Ethyl 5-methyl-4-oxo-3*H*,4*H*-thieno[3,4-*d*]pyrimidine-2-carboxylate (6h)

A mixture of methyl 4-amino-2-methylthiophene-3-carboxylate hydrochloride[22] (**3a**) (1.90 g, 9.15 mmol, synthesized by the method of Barker et al.), ethyl cyanoformate (1.36 g, 13.7 mmol) and 1 M hydrochloric acid in acetic acid (40 mL) was stirred at 80 °C for 2 h. After removal of the solvent, the residue was suspended with water. The resulting precipitate was collected and washed with water and diethyl ether to give **6h** as a brown powder (1.48 g, 68%). ^{1}H NMR (300 MHz, DMSO-d_6) δ 1.33 (3H, d, J = 7.2 Hz), 2.88 (3H, s), 4.34 (2H, q, J = 7.2 Hz), 7.79 (1H, s), 11.7 (1H, bs).

5.1.5. Ethyl 6-methyl-4-oxo-3*H*,4*H*,7*H*-pyrrolo[2,3-*d*]pyrimidine-2-carboxylate (6i)

Compound **6i** was prepared from ethyl 2-amino-5-methyl-1*H*-pyrrole-3-carboxylate[23] (**3b**) (synthesized by a method of Toja et al.) with a similar procedure as described for **6h** (white powder, 50%). ^{1}H NMR (300 MHz, DMSO-d_6) δ 1.33 (3H, t, J = 7.2 Hz), 2.31 (3H, s), 4.33 (2H, q, J = 7.2 Hz), 6.26 (1H, s), 12.0 (1H, bs), 12.1 (1H, bs).

5.1.6. Ethyl 6,7-dimethyl-4-oxo-4,7-dihydro-3*H*-pyrrolo[2,3-*d*]pyrimidine-2-carboxylate (6j)

Compound **6j** was prepared from ethyl 2-amino-1,5-dimethyl-1*H*-pyrrole-3-carboxylate[23] (**3c**, synthesized by a method of Toja et al.) with a similar procedure as described for **6h** (white powder, 26%). ^{1}H NMR (300 MHz, DMSO-d_6) δ 1.34 (3H, t, J = 6.9 Hz), 2.36 (3H, s), 3.66 (3H, s), 4.36 (2H, q, J = 7.2 Hz), 6.37 (1H, s), 12.1 (1H, bs).

5.1.7. Ethyl 4-oxo-3*H*,4*H*-thieno[3,2-*d*]pyrimidine-2-carboxylate (6k)

A mixture of compound **5a** (2.50 g, 11.1 mmol) and ammonium acetate (941 mg, 12.2 mmol) in ethanol (30 mL) was heated under reflux for 1 h. The reaction mixture was concentrated under reduced pressure. The residue was diluted with ethyl acetate, washed with water and brine, dried over anhydrous Na_2SO_4 and concentrated under reduced pressure. The crude product was purified by silica gel column chromatography (67–80% ethyl acetate/hexane) to give **6k** as a brown powder (478 mg, 19%). ^{1}H NMR (200 MHz, DMSO-d_6) δ 1.35 (3H, t, J = 7.2 Hz), 4.38 (2H, q, J = 7.2 Hz), 7.56 (1H, d, J = 5.6 Hz), 8.28 (1H, d, J = 5.6 Hz).

5.1.8. Ethyl 4-oxo-3*H*,4*H*-pyrido[2,3-*d*]pyrimidine-2-carboxylate (6l)

A mixture of compound **5b** (2.20 g, 9.99 mmol), ammonium acetate (770 mg, 9.99 mmol) and acetic acid (240 mg, 4.00 mmol) in ethanol (30 mL) was heated under reflux for 1 h. After the reaction mixture was cooled to room temperature, the precipitated solid was collected by filtration and washed with ethanol to give **6l** as a pale yellow powder (1.11 g, 51%). ^{1}H NMR (200 MHz, DMSO-d_6) δ 1.38 (3H, t, J = 7.0 Hz), 4.41 (2H, q, J = 7.0 Hz), 7.65 (1H, dd, J = 7.8, 4.4 Hz), 8.56 (1H, dd, J = 7.8, 1.8 Hz), 9.04 (1H, dd, J = 4.4, 1.8 Hz).

5.1.9. Ethyl 4-oxo-3*H*,4*H*-pyrido[3,4-*d*]pyrimidine-2-carboxylate (6m)

Step 1. To a suspension of commercially available 3-aminopyridine-4-carboxylic acid (4.84 g, 35.1 mmol) in pyridine (60 mL) was added dropwise ethyl chloroglyoxylate (9.58 g, 70.2 mmol) at 0 °C, and the mixture was stirred at room temperature for 15 h. The reaction mixture was concentrated under reduced pressure and the residue was suspended with water. The resulting precipitate was collected by filtration to give 3-(2-ethoxy-2-oxoacetamido)pyridine-4-carboxylic acid as a pale yellow powder (3.74 g, 45%). ^{1}H NMR (200 MHz, DMSO-d_6) δ 1.33 (3H, t, J = 7.0 Hz), 4.33 (2H, q, J = 7.0 Hz), 7.88 (1H, d, J = 5.0 Hz), 8.53 (1H, d, J = 5.0 Hz), 9.71 (1H, s), 12.10 (1H, s).

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

Step 3. To a suspension of ethyl [(4-carbamoylpyridin-3-yl)carbamoyl]formate (76 mg, 0.320 mmol) in ethanol (4 mL) was added dropwise sodium ethylate (20% ethanol solution, 120 mg, 0.350 mmol) at 0 °C, and the mixture was stirred at room temperature for 1 h. The reaction was quenched with 1 M hydrochloric acid (0.5 mL) and the resulting mixture was neutralized with aqueous $NaHCO_3$ solution. The mixture was extracted with ethyl acetate, washed with brine, dried over anhydrous Na_2SO_4 and concentrated under reduced pressure to give **6m** as a white amorphous form (22 mg, 31%). The crude **6m** was used for the next reaction without purification.

5.1.10. *N*-[(3-Methoxyphenyl)methyl]-4-oxo-3*H*,4*H*-thieno[2,3-*d*]pyrimidine-2-carboxamide (7a)

A mixture of compound **6a** (240 mg, 4.00 mmol) and 3-methoxybenzylamine (138 mg, 1.00 mmol) in DMF (3 mL) was heated at 90 °C for 5 h. The reaction mixture was concentrated under reduced pressure. The residue was diluted with ethyl acetate, washed with brine, dried over anhydrous Na_2SO_4 and concentrated under reduced pressure. The crude product was purified by preparative HPLC and crystallized from ethanol-diethyl ether to give **7a** as a beige powder (88.5 mg, 42%). mp 179–182 °C. ^{1}H NMR (200 MHz, DMSO-d_6) δ 3.73 (3H, s), 4.42 (2H, d, J = 6.6 Hz), 6.80–6.92 (3H, m), 7.24 (1H, t, J = 8.0 Hz), 7.43 (1H, d, J = 5.6 Hz), 7.66 (1H, d, J = 5.6 Hz), 9.56 (1H, m), 1H hidden. Anal. Calcd for $C_{15}H_{13}N_3O_3S{\cdot}0.2CF_3CO_2H{\cdot}0.6H_2O$: C, 53.00; H, 4.16; N, 12.04. Found: C, 53.05; H, 4.13; N, 11.85.

5.1.11. *N*-[(3-Methoxyphenyl)methyl]-5-methyl-4-oxo-3*H*,4*H*-thieno[2,3-*d*]pyrimidine-2-carboxamide (7b)

Compound **7b** was prepared from ethyl 5-methyl-4-oxo-3*H*,4*H*-thieno[2,3-*d*]pyrimidine-2-carboxylate[24] with a similar procedure as described for **7a** (white powder, 86%). mp 148–151 °C. ^{1}H NMR (300 MHz, $CDCl_3$) δ 2.60 (3H, s), 3.81 (3H, s), 4.60 (2H, d, J = 6.0 Hz), 6.83–6.92 (4H, m), 7.25–7.32 (1H, m), 7.91 (1H, br). Anal. Calcd for $C_{16}H_{15}N_3O_3S{\cdot}0.60H_2O$: C, 56.49; H, 4.80; N, 12.35. Found: C, 56.47; H, 4.62; N 12.44.

5.1.12. *N*-[(3-Methoxyphenyl)methyl]-6-methyl-4-oxo-3*H*,4*H*-thieno[2,3-*d*]pyrimidine-2-carboxamide (7c)

Compound **7c** was prepared from ethyl 6-methyl-4-oxo-3*H*,4*H*-thieno[2,3-*d*]pyrimidine-2-carboxylate[25] with a similar procedure as described for **7a** (white powder, 84%). mp 187 °C. ^{1}H NMR (300 MHz, DMSO-d_6) δ 2.55 (3H, d, J = 1.1 Hz), 3.73 (3H, s), 4.41 (2H, d, J = 6.4 Hz), 6.78–6.94 (3H, m), 7.13–7.30 (2H, m), 9.63 (1H, t, J = 6.3 Hz), 12.4 (1H, s). Anal. Calcd for $C_{16}H_{15}N_3O_3S$: C, 58.34; H, 4.59; N, 12.76. Found: C, 58.14; H, 4.61; N, 12.73.

5.1.13. *N*-[(3-Methoxyphenyl)methyl]-5,6-dimethyl-4-oxo-3*H*,4*H*-thieno[2,3-*d*]pyrimidine-2-carboxamide (7d)

Compound **7d** was prepared from ethyl 5,6-dimethyl-4-oxo-3*H*,4*H*-thieno[2,3-*d*]pyrimidine-2-carboxylate[25] with a similar procedure as described for **7a** (white powder, 84%). mp 194 °C. ^{1}H NMR (300 MHz, DMSO-d_6) δ 2.40 (3H, s), 2.42 (3H, s), 3.73 (3H, s), 4.40 (2H, d, J = 6.4 Hz), 6.78–6.93 (3H, m), 7.18–7.29 (1H, m), 9.60 (1H, t, J = 6.3 Hz), 12.2 (1H, s). Anal. Calcd for $C_{17}H_{17}N_3O_3S{\cdot}0.25H_2O$: C, 58.69; H, 5.07; N, 12.08. Found: C, 58.77; H, 4.93; N, 11.94.

5.1.14. *N*-[(3-Methoxyphenyl)methyl]-5,6-dimethyl-4-oxo-3*H*,4*H*-furo[2,3-*d*]pyrimidine-2-carboxamide (7e)

Compound **7e** was prepared from compound **6e** with a similar procedure as described for **7a** (white powder, 26%). mp 178–180 °C. ^{1}H NMR (300 MHz, DMSO-d_6) δ 2.18 (3H, s), 2.32 (3H, s), 3.73 (3H, s), 4.41 (2H, d, J = 6.3 Hz), 6.80–6.84 (1H, m), 6.88–6.92 (2H, m), 7.24 (1H, t, J = 8.1 Hz), 9.55 (1H, t, J = 6.0 Hz), 12.3 (1H, bs). Anal. Calcd for $C_{17}H_{17}N_3O_4$: C, 62.38; H, 5.23; N, 12.84. Found: C, 62.16; H, 5.29; N, 12.77.

5.1.15. *N*-[(3-Methoxyphenyl)methyl]-3-methyl-4-oxo-4*H*,5*H*-[1,2]oxazolo[5,4-*d*]pyrimidine-6-carboxamide (7f)

Compound **7f** was prepared from compound **6f**[26] with a similar procedure as described for **7a** (pale pink powder, 71%). mp 236–237 °C. ^{1}H NMR (300 MHz, DMSO-d_6) δ 2.49 (3H, m), 3.73 (3H, s), 4.42 (2H, d, J = 6.3 Hz), 6.80–6.84 (1H, m), 6.89–6.92 (2H, m), 7.24 (1H, t, J = 8.1 Hz), 9.80 (1H, t, J = 6.3 Hz), 13.0 (1H, bs). Anal. Calcd for $C_{15}H_{14}N_4O_4$: C, 57.32; H, 4.49; N, 17.83. Found: C, 57.35; H, 4.47; N, 17.81.

5.1.16. *N*-[(3-Methoxyphenyl)methyl]-1,3-dimethyl-4-oxo-1*H*,4*H*,5*H*-pyrazolo[3,4-*d*]pyrimidine-6-carboxamide (7g)

Compound **7g** was prepared from compound **6g** with a similar procedure as described for **7a** (white powder, 90%). mp 186–188 °C. ^{1}H NMR (300 MHz, DMSO-d_6) δ 2.43 (3H, s), 3.73 (3H, s), 3.90 (3H, s), 4.45 (2H, d, J = 6.6 Hz), 6.80–6.84 (1H, m), 6.88–6.92 (2H, m), 7.24 (1H, t, J = 8.1 Hz), 9.56 (1H, t, J = 6.6 Hz), 11.9 (1H, bs). Anal. Calcd for $C_{16}H_{17}N_5O_3$: C, 58.71; H, 5.23; N, 21.39. Found: C, 58.41; H, 5.20; N, 21.23.

5.1.17. *N*-[(3-Methoxyphenyl)methyl]-5-methyl-4-oxo-3*H*,4*H*-thieno[3,4-*d*]pyrimidine-2-carboxamide (7h)

Compound **7h** was prepared from compound **6h** with a similar procedure as described for **7a** (beige powder, 76%). mp 168–170 °C. ^{1}H NMR (300 MHz, DMSO-d_6) δ 2.88 (3H, s), 3.73 (3H, s), 4.41 (2H, d, J = 6.0 Hz), 6.81–6.91 (3H, m), 7.24 (1H, t, J = 8.1 Hz), 7.64 (1H, s), 9.43 (1H, t, J = 6.0 Hz), 11.3 (1H, bs). Anal. Calcd for $C_{16}H_{15}N_3O_3S$: C, 58.34; H, 4.59; N, 12.76. Found: C, 58.24; H, 4.69; N, 12.49.

5.1.18. *N*-[(3-Methoxyphenyl)methyl]-6-methyl-4-oxo-3*H*,4*H*,7*H*-pyrrolo[2,3-*d*]pyrimidine-2-carboxamide (7i)

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

5.1.19. *N*-[(3-Methoxyphenyl)methyl]-6,7-dimethyl-4-oxo-3*H*,4*H*,7*H*-pyrrolo[2,3-*d*]pyrimidine-2-carboxamide (7j)

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

5.1.20. *N*-[(3-Methoxyphenyl)methyl]-4-oxo-3*H*,4*H*-thieno[3,2-*d*]pyrimidine-2-carboxamide (7k)

Compound **7k** was prepared from compound **6k** with a similar procedure as described for **7a** (pale yellow powder, 36%). mp 201–202 °C. ^{1}H NMR (200 MHz, DMSO-d_6) δ 3.73 (3H, s), 4.44 (2H, d, J = 6.6 Hz), 6.80–6.93 (3H, m), 7.24 (1H, t, J = 8.0 Hz), 7.48 (1H, d, J = 5.4 Hz), 8.27 (1H, d, J = 5.4 Hz), 9.57 (1H, t, J = 6.6 Hz), 1H hidden. Anal. Calcd for $C_{15}H_{13}N_3O_3S$: C, 57.13; H, 4.16; N, 13.33. Found: C, 56.95; H, 4.14; N, 13.08.

5.1.21. *N*-[(3-Methoxyphenyl)methyl]-4-oxo-3*H*,4*H*-pyrido[2,3-*d*]pyrimidine-2-carboxamide (7l)

Compound **7l** was prepared from compound **6l** with a similar procedure as described for **7a** (white powder, 76%). mp 181–183 °C. ^{1}H NMR (200 MHz, DMSO-d_6) δ 3.74 (3H, s), 4.46 (2H, d, J = 6.2 Hz), 6.80–6.95 (3H, m), 7.25 (1H, t, J = 8.0 Hz), 7.62 (1H, dd, J = 8.0, 4.6 Hz), 8.55 (1H, dd, J = 8.2, 2.2 Hz), 9.02 (1H, dd, J = 4.6, 2.0 Hz), 9.71 (1H, m), 1H hidden. Anal. Calcd for $C_{16}H_{14}N_4O_3{\cdot}0.1$AcOEt: C, 61.72; H, 4.67; N, 17.56. Found: C, 61.64; H, 4.56; N, 17.52.

5.1.22. *N*-[(3-Methoxyphenyl)methyl]-4-oxo-3*H*,4*H*-pyrido[3,4-*d*]pyrimidine-2-carboxamide (7m)

Compound **7m** was prepared from compound **6m** with a similar procedure as described for **7a** (white powder, 65%). mp 231–233 °C. ^{1}H NMR (300 MHz, DMSO-d_6) δ 3.73 (3H, s), 4.45 (2H, d, J = 6.3 Hz), 6.80–6.84 (1H, m), 6.90–6.93 (2H, m), 7.24 (1H, t,

J = 8.1 Hz), 7.99 (1H, d, *J* = 5.1 Hz), 8.72 (1H, d, *J* = 5.4 Hz), 9.12 (1H, s), 9.63 (1H, t, *J* = 6.3 Hz), 12.7 (1H, bs). Anal. Calcd for $C_{16}H_{14}N_4O_3 \cdot 0.2H_2O$: C, 61.22; H, 4.62; N, 17.85. Found: C, 61.04; H, 4.42; N, 17.88.

5.1.23. 2-(3-Methoxyphenyl)-*N*-(6-methyl-4-oxo-3,4-dihydroquinazolin-2-yl)acetamide (10)

A mixture of commercially available 2-amino-6-methyl-3,4-dihydroquinazolin-4-one (**9**, 150 mg, 0.856 mmol), compound **8** (380 mg, 2.06 mmol), triethylamine (260 mg, 2.56 mmol), THF (6 mL), and DMF (4 mL) was stirred at 90 °C for 4 h. The insoluble materials were filtered off and the filtrate was concentrated under reduced pressure. The residue was suspended with ethanol and the resulting precipitate was collected by filtration. The solid was washed with ethanol and dried to give **10** as a white powder (198 mg, 72%). mp 208–210 °C. ^{1}H NMR (200 MHz, DMSO-d_6) δ 2.41 (3H, s), 3.70–7.80 (5H, m), 6.82–6.94 (3H, m), 7.26 (1H, t, *J* = 8.0 Hz), 7.40 (1H, d, *J* = 8.4 Hz), 7.57–7.62 (1H, m), 7.85 (1H, s), 11.8 (1H, bs), 11.9 (1H, bs). Anal. Calcd for $C_{18}H_{17}N_3O_3 \cdot 0.1H_2O$: C, 66.49; H, 5.33; N, 12.92. Found: C, 66.26; H, 5.43; N, 13.14.

5.1.24. 2-(Chloromethyl)-6-methylquinazolin-4(3*H*)-one (12)[27]

Sodium methylate (357 mg, 6.62 mmol) was added to a solution of chloroacetonitrile (2.75 g, 36.4 mmol) in methanol (75 mL) at 0 °C and the mixture was stirred at room temperature for 1 h. In another flask, commercially available 2-amino-5-methylbenzoic acid **11** (5.00 g, 33.1 mmol) was added to a solution of sodium methylate (179 mg, 3.31 mmol) in methanol (75 mL) and this solution was added to the above solution of chloroacetoimidate at room temperature. The reaction mixture was stirred at room temperature for 1 h and then heated at 80 °C for 2 h. After the mixture was cooled to room temperature, the precipitated solid was collected and washed with methanol to give **12** as a pale gray powder (4.60 g, 67%). ^{1}H NMR (300 MHz, DMSO-d_6) δ 2.45 (3H, s), 4.54 (2H, s), 7.58 (1H, d, *J* = 8.1 Hz), 7.66 (1H, dd, *J* = 8.1, 1.8 Hz), 7.92 (1H, d, *J* = 0.6 Hz), 12.5 (1H, bs).

5.1.25. 2-({[(3-Methoxyphenyl)methyl]amino}methyl)-6-methyl-3,4-dihydroquinazolin-4-one (13)

A mixture of compound **12** (200 mg, 0.959 mmol), 3-methoxybenzylamine (263 mg, 1.92 mmol), and K_2CO_3 (132 mg, 0.959 mmol) in THF (6 mL) was stirred at 40 °C for 15 h. The reaction mixture was concentrated under reduced pressure. The residue was diluted with ethyl acetate, washed with brine, dried over anhydrous Na_2SO_4 and concentrated under reduced pressure. The crude product was purified by preparative HPLC and crystallized from diethyl ether to give **13** as a white powder (120 mg, 41%). mp 172–174 °C. ^{1}H NMR (300 MHz, DMSO-d_6) δ 2.43 (3H, s), 3.64 (2H, s), 3.71 (2H, s), 3.73 (3H, s), 6.76–6.80 (1H, m), 6.89–6.93 (2H, m), 7.21 (1H, t, *J* = 7.8 Hz), 7.53 (1H, d, *J* = 8.1 Hz), 7.61 (1H, dd, *J* = 8.4, 2.1 Hz), 7.89 (1H, d, *J* = 0.6 Hz). Anal. Calcd for $C_{18}H_{19}N_3O_2 \cdot 0.4H_2O$: C, 68.29; H, 6.30; N, 13.27. Found: C, 68.23; H, 6.07; N, 13.23.

5.1.26. *tert*-Butyl 2-[1-hydroxy-3-(3-methoxyphenyl)propyl]-6-methyl-4-oxo-3,4-dihydroquinazoline-3-carboxylate (20)

To a solution of compound **16** (500 mg, 1.92 mmol) in THF (20 mL) was added dropwise lithium diisopropylamide (1.8 M in a mixed solvent of heptane, THF and ethylbenzene, 1.28 mL, 2.30 mmol) at −78 °C, and the mixture was stirred at −78 °C for 10 min. A solution of compound **19** (631 mg, 3.84 mmol) in THF (5 mL) was added at −78 °C and the reaction mixture was allowed to warm to room temperature followed by stirring at room temperature for 1 h. The reaction was quenched 1 M hydrochloric acid and the resulting mixture was partitioned between ethyl acetate and H_2O. The organic layer was washed with brine, dried over anhydrous Na_2SO_4 and concentrated under reduced pressure. The crude product was purified by silica gel column chromatography (25–33% ethyl acetate/hexane) to give **20** as a yellow oil (815 mg, 44%). ^{1}H NMR (300 MHz, DMSO-d_6) δ 1.40 (9H, s), 2.14–2.27 (2H, m), 2.44 (3H, s), 2.64–2.76 (2H, s), 3.71 (3H, s), 5.17–5.22 (1H, m), 6.65–6.81 (3H, m), 7.14–7.21 (1H, m), 7.52–7.55 (1H, m), 7.61–7.65 (1H, m), 7.89 (1H, s).

5.1.27. 2-[1-Hydroxy-3-(3-methoxyphenyl)propyl]-6-methyl-3,4-dihydroquinazolin-4-one (21)

A mixture of compound **20** (360 mg, 0.848 mmol), trifluoroacetic acid (3 mL), and CH_2Cl_2 (6 mL) was stirred at room temperature for 1 h. The reaction mixture was concentrated under reduced pressure. The residue was purified by silica gel column chromatography (60% ethyl acetate/hexane) and crystallized from diethyl ether to give **21** as a white powder (125 mg, 0.385 mmol, 45%). mp 149–151 °C. ^{1}H NMR (300 MHz, DMSO-d_6) δ 2.00–2.09 (2H, m), 2.43 (3H, s), 2.57–2.72 (2H, m), 3.72 (3H, s), 4.38–4.44 (1H, m), 5.76 (1H, t, *J* = 4.8 Hz), 6.72–6.81 (3H, m), 7.18 (1H, t, *J* = 7.8 Hz), 7.54 (1H, d, *J* = 8.4 Hz), 7.62 (1H, d, *J* = 7.8 Hz), 7.90 (1H, s), 11.7 (1H, bs). Anal. Calcd for $C_{19}H_{20}N_2O_3 \cdot 0.1H_2O$: C, 69.96; H, 6.24; N, 8.59. Found: C, 69.67; H, 6.18; N, 8.51.

5.1.28. 2-[3-(3-Methoxyphenyl)propanoyl]-6-methyl-3,4-dihydroquinazolin-4-one (23)

Dimethylsulfoxide (0.011 mL, 0.154 mmol) was added to a solution of oxalyl chloride (0.013 mL, 0.154 mmol) in CH_2Cl_2 (4 mL) at −78 °C under nitrogen atmosphere and the mixture was stirred at −78 °C for 2 min. A solution of compound **21** (25 mg, 0.077 mmol) and dimethylsulfoxide (0.020 mL) in CH_2Cl_2 (2 mL) was added and the mixture was stirred at −78 °C for 1 h. After triethylamine (0.107 mL, 0.771 mmol) was added, the resulting mixture was allowed to warm to room temperature and stirred at room temperature for 2 h. The reaction mixture was partitioned between ethyl acetate and water. The organic layer was washed with brine, dried over anhydrous Na_2SO_4 and concentrated under reduced pressure. The crude product was purified by silica gel column chromatography (25% ethyl acetate/hexane) to give compound **22** (1-(6-methyl-4-{[(methylthio)methyl]oxy}quinazolin-2-yl)-3-[3-(methyloxy)phenyl]propan-1-one) as a pale yellow oil (12 mg). To a solution of the pale yellow oil obtained above in CH_2Cl_2 (1 mL) was added 90% aqueous trifluoroacetic acid (0.5 mL), and the mixture was stirred at room temperature for 10 min. The reaction mixture was concentrated under reduced pressure and the residue was purified by preparative HPLC to give **23** as a white powder (6.5 mg, 26%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 2.53 (3H, s), 3.03–3.08 (2H, m), 3.54–3.59 (2H, m), 3.80 (3H, s), 6.73–6.86 (3H, m), 7.22 (1H, t, *J* = 8.1 Hz), 7.65 (1H, dd, *J* = 8.4, 2.1 Hz), 7.75 (1H, d, *J* = 8.4 Hz), 8.13–8.14 (1H, m), 10.1 (1H, bs).

5.1.29. Ethyl 5-[(benzyloxy)methyl]-4-oxo-3*H*,4*H*-thieno[2,3-*d*]pyrimidine-2-carboxylate (25a)

To a solution of benzyl alcohol (0.157 mL) in THF (10 mL) was gradually added 60% sodium hydride (116 mg, 3.03 mmol), and the mixture was stirred at room temperature for 10 min. Compound **24** (400 mg, 1.26 mmol) was added at once, and the mixture was stirred at room temperature for 1 h. The solvent was evaporated under reduced pressure and the residue was extracted with ethyl acetate. The mixture was washed with 1 M hydrochloric acid and a 1:1 mixture of 1 M hydrochloric acid-saturated brine, and after drying over anhydrous Na_2SO_4, the solvent was evaporated. The residue was suspended in diethyl ether, filtrated, dried and suspended in THF (5 mL). Oxalyl chloride (0.550 mL, 6.31 mmol) and DMF (one drop) were added, and the mixture was stirred

at room temperature for 2.5 h. The solvent was evaporated under reduced pressure. The obtained residue was dissolved in EtOH–THF (1:1) solution, and the mixture was stirred at room temperature for 12 h. The solvent was evaporated under reduced pressure and the residue was extracted with ethyl acetate, washed with water and saturated brine, and dried over anhydrous Na_2SO_4. The solvent was evaporated under reduced pressure. The obtained residue was purified by silica gel column chromatography (20–40% ethyl acetate/hexane) to give **25a** (68.7 mg, 16%) as a colorless powder. mp 155–156 °C. ^{1}H NMR (300 MHz, DMSO-d_6) δ 1.34 (3H, t, J = 7.1 Hz), 4.36 (2H, q, J = 7.0 Hz), 4.66 (2H, s), 4.86 (2H, d, J = 0.9 Hz), 7.13–7.51 (5H, m), 7.66 (1H, s), 12.9 (1H, s). Anal. Calcd for $C_{17}H_{16}N_2O_4S{\cdot}0.25H_2O$: C, 58.52; H, 4.77; N, 8.03. Found: C, 58.30; H, 4.53; N, 8.30.

5.1.30. Ethyl 5-({[4-(ethoxycarbonyl)phenyl]methoxy}methyl)-4-oxo-3*H*,4*H*-thieno[2,3-*d*]pyrimidine-2-carboxylate (25b)

Compound **25b** was prepared from compound **24** and ethyl 4-(hydroxymethyl)benzoate with a similar procedure as described for **25a** (pale yellow powder, 75%). mp 181 °C. ^{1}H NMR (300 MHz, DMSO-d_6) δ 1.33 (6H, q, J = 7.0 Hz), 4.27–4.41 (4H, m), 4.75 (2H, s), 4.89 (2H, d, J = 1.1 Hz), 7.54 (2H, d, J = 8.5 Hz), 7.70 (1H, s), 7.91–7.99 (2H, m), 12.9 (1H, s). Anal. Calcd for $C_{20}H_{20}N_2O_6S{\cdot}0.05H_2O$: C, 57.56; H, 4.85; N, 6.71. Found: C, 57.55; H, 4.82; N, 6.70.

5.1.31. Ethyl 5-{[(4-fluorophenyl)methoxy]methyl}-4-oxo-3*H*,4*H*-thieno[2,3-*d*]pyrimidine-2-carboxylate (25c)

Compound **25c** was prepared from compound **24** and ethyl (4-fluorophenyl)methanol with a similar procedure as described for **25a** (pale yellow powder, 8%). mp 195–196 °C. ^{1}H NMR (300 MHz, DMSO-d_6) δ 1.34 (3H, t, J = 7.1 Hz), 4.37 (2H, q, J = 7.0 Hz), 4.64 (2H, s), 4.85 (2H, d, J = 1.3 Hz), 7.18 (2H, t, J = 9.0 Hz), 7.44 (2H, dd, J = 8.7, 5.7 Hz), 7.66 (1H, t, J = 1.2 Hz), 12.9 (1H, s). Anal. Calcd for $C_{17}H_{15}FN_2O_4S{\cdot}0.25H_2O$: C, 55.65; H, 4.26; N, 7.64. Found: C, 55.51; H, 4.13; N, 7.84.

5.1.32. Ethyl 5-{[(4-cyanophenyl)methoxy]methyl}-4-oxo-3*H*,4*H*-thieno[2,3-*d*]pyrimidine-2-carboxylate (25d)

Compound **25d** was prepared from compound **24** and 4-(hydroxymethyl)benzonitrile with a similar procedure as described for **25a** (pale yellow powder, 52%). mp 236–237 °C. ^{1}H NMR (300 MHz, DMSO-d_6) δ 1.34 (3H, t, J = 7.1 Hz), 4.37 (2H, q, J = 7.1 Hz), 4.76 (2H, s), 4.90 (2H, d, J = 1.1 Hz), 7.59 (2H, d, J = 8.5 Hz), 7.71 (1H, t, J = 1.1 Hz), 7.77–7.89 (2H, m), 12.9 (1H, s). Anal. Calcd for $C_{18}H_{15}N_3O_4S{\cdot}0.25H_2O$: C, 57.82; H, 4.18; N, 11.24. Found: C, 57.68; H, 4.09; N, 11.44.

5.1.33. Ethyl 5-{[benzyl(methyl)amino]methyl}-4-oxo-3*H*,4*H*-thieno[2,3-*d*]pyrimidine-2-carboxylate (25e)

To a mixture of compound **24** (600 mg, 1.89 mmol) obtained and THF (12 mL) were added *N*-methyl-1-phenylmethanamine (0.269 mL, 2.08 mmol) and triethylamine (0.527 mL, 3.78 mmol) at room temperature and the mixture was stirred for 1 h. The reaction mixture was concentrated under reduced pressure and ethyl acetate was added to the residue. The mixture was washed with saturated brine, dried over anhydrous Na_2SO_4 and concentrated under reduced pressure. The concentrated residue was purified by silica gel column chromatography (1–8% methanol/ethyl acetate). The obtained crude crystals were recrystallized from ethyl acetate–hexane to give **25e** as a white powder (351 mg, 52%). mp 129 °C. ^{1}H NMR (300 MHz, DMSO-d_6) δ 1.33 (3H, t, J = 7.2 Hz), 2.32 (3H, s), 4.15 (4H, s), 4.32 (2H, q, J = 7.0 Hz), 7.28–7.51 (5H, m), 7.64 (1H, s). Anal. Calcd for $C_{18}H_{19}N_3O_3S{\cdot}0.05H_2O$: C, 60.33; H, 5.37; N, 11.73. Found: C, 60.37; H, 5.28; N, 11.74.

5.1.34. Ethyl 5-[(benzylsulfanyl)methyl]-4-oxo-3*H*,4*H*-thieno[2,3-*d*]pyrimidine-2-carboxylate (25f)

To a mixture of compound **24** (600 mg, 1.89 mmol) and DMA (12 mL) were added phenylmethanethiol (0.244 mL, 2.08 mmol) and triethylamine (0.527 mL, 3.78 mmol) at room temperature and the mixture was stirred for 1 h. The reaction mixture was concentrated under reduced pressure, and ethyl acetate was added to the obtained residue. The mixture was washed with 1 M hydrochloric acid and saturated brine, dried over anhydrous Na_2SO_4 and concentrated under reduced pressure. The concentrated residue was purified by silica gel column chromatography (5–50% ethyl acetate/hexane). The obtained crude crystals were recrystallized from ethyl acetate–hexane to give **25f** as a white powder (207 mg, 30%). mp 171 °C. ^{1}H NMR (300 MHz, DMSO-d_6) δ 1.35 (3H, t, J = 7.2 Hz), 3.73 (2H, s), 3.99 (2H, s), 4.37 (2H, q, J = 7.1 Hz), 7.15–7.34 (5H, m), 7.57 (1H, s), 12.8 (1H, s). Anal. Calcd for $C_{17}H_{16}N_2O_3S_2{\cdot}0.20H_2O$: C, 56.09; H, 4.54; N, 7.69. Found: C, 55.93; H, 4.41; N, 7.96.

5.1.35. 5-[(Benzyloxy)methyl]-*N*-[(3-methoxyphenyl)methyl]-4-oxo-3*H*,4*H*-thieno[2,3-*d*]pyrimidine-2-carboxamide (26a)

Compound **26a** was prepared from compound **25a** with a similar procedure as described for **7a** (white powder, 60%). mp 145 °C. ^{1}H NMR (300 MHz, DMSO-d_6) δ 3.73 (3H, s), 4.41 (2H, d, J = 6.4 Hz), 4.66 (2H, s), 4.85 (2H, d, J = 0.8 Hz), 6.82 (1H, dd, J = 8.3, 2.3 Hz), 6.87–6.95 (2H, m), 7.18–7.45 (6H, m), 7.60 (1H, s), 9.66 (1H, t, J = 6.5 Hz), 12.4 (1H, s). Anal. Calcd for $C_{23}H_{21}N_3O_4S{\cdot}0.25H_2O$: C, 62.78; H, 4.93; N, 9.55. Found: C, 62.54; H, 4.90; N, 9.88.

5.1.36. Ethyl 4-{[(2-{[(3-Methoxyphenyl)methyl]carbamoyl}-4-oxo-3*H*,4*H*-thieno[2,3-*d*]pyrimidin-5-yl)methoxy]methyl}-benzoate (26b)

Compound **26b** was prepared from compound **25b** with a similar procedure as described for **7a** (white powder, 90%). mp 173 °C. ^{1}H NMR (300 MHz, DMSO-d_6) δ 1.32 (3H, t, J = 7.1 Hz), 3.73 (3H, s), 4.31 (2H, q, J = 7.2 Hz), 4.42 (2H, d, J = 6.2 Hz), 4.75 (2H, s), 4.89 (2H, d, J = 0.9 Hz), 6.79–6.85 (1H, m), 6.87–6.93 (2H, m), 7.24 (1H, t, J = 8.1 Hz), 7.54 (2H, d, J = 8.5 Hz), 7.63 (1H, s), 7.88–8.03 (2H, m), 9.65 (1H, t, J = 6.5 Hz), 12.4 (1H, s). Anal. Calcd for $C_{26}H_{25}N_3O_6S{\cdot}0.10H_2O$: C, 61.31; H, 4.99; N, 8.25. Found: C, 61.17; H, 4.94; N, 8.42.

5.1.37. 4-{[(2-{[(3-Methoxyphenyl)methyl]carbamoyl}-4-oxo-3*H*,4*H*-thieno[2,3-*d*]pyrimidin-5-yl)methoxy]methyl}benzoic acid (26c)

A mixture of **26b** (126 g, 249 mmol) and 8 M NaOH (77.7 mL, 622 mmol) in a mixture of MeOH (600 mL), THF (600 mL) and water (600 mL) was stirred at 90 °C for 1 h. The mixture was concentrated in vacuo. After acidification with 1 M hydrochloric acid (933 mL, 933 mmol), the crude materials were collected by filtration, washed with water (7 × 500 mL) and MeOH (6×500 mL) to give a white powder. The crude product was suspended in MeOH (2500 mL) at refluxed temperature for 1.5 h, cooled to room temperature, and collected by filtration, washed with MeOH and air dried to give **26c** as a white powder (122 g, quant.). mp 229 °C. ^{1}H NMR (300 MHz, DMSO-d_6) δ 3.73 (3H, s), 4.41 (2H, d, J = 6.0 Hz), 4.74 (2H, s), 4.89 (2H, s), 6.82 (1H, dd, J = 8.5, 1.9 Hz), 6.86–6.97 (2H, m), 7.24 (1H, t, J = 8.1 Hz), 7.51 (2H, d, J = 8.1 Hz), 7.63 (1H, s), 7.93 (2H, d, J = 8.3 Hz), 9.66 (1H, t, J = 6.4 Hz), 12.5 (1H, s), 12.9 (1H, s). Anal. Calcd for $C_{24}H_{21}N_3O_6S$: C, 60.12; H, 4.41; N, 8.76. Found: C, 60.30; H, 4.53; N, 8.61.

5.1.38. 5-{[(4-Fluorophenyl)methoxy]methyl}-*N*-[(3-methoxyphenyl)methyl]-4-oxo-3*H*,4*H*-thieno[2,3-*d*]pyrimidine-2-carboxamide (26d)

Compound **26d** was prepared from compound **25c** with a similar procedure as described for **7a** (white powder, 81%). mp 166 °C.

^{1}H NMR (300 MHz, DMSO-d_6) δ 3.73 (3H, s), 4.41 (2H, d, J = 6.0 Hz), 4.64 (2H, s), 4.85 (2H, d, J = 0.8 Hz), 6.74–6.94 (3H, m), 7.11–7.30 (3H, m), 7.38–7.49 (2H, m), 7.59 (1H, s), 9.64 (1H, s), 12.4 (1H, s). Anal. Calcd for $C_{23}H_{20}FN_3O_4S{\cdot}0.15H_2O$: C, 60.56; H, 4.49; N, 9.21. Found: C, 60.48; H, 4.44; N, 9.27.

5.1.39. 5-{[(4-Cyanophenyl)methoxy]methyl}-*N*-[(3-methoxyphenyl)methyl]-4-oxo-3*H*,4*H*-thieno[2,3-*d*]pyrimidine-2-carboxamide (26e)

Compound **26e** was prepared from compound **25d** with a similar procedure as described for **7a** (white powder, 79%). mp 205 °C. ^{1}H NMR (300 MHz, DMSO-d_6) δ 3.73 (3H, s), 4.42 (2H, d, J = 6.4 Hz), 4.76 (2H, s), 4.89 (2H, d, J = 1.1 Hz), 6.79–6.93 (3H, m), 7.24 (1H, t, J = 8.1 Hz), 7.55–7.66 (3H, m), 7.77–7.90 (2H, m), 9.65 (1H, t, J = 6.4 Hz), 12.4 (1H, s). Anal. Calcd for $C_{24}H_{20}N_4O_4S{\cdot}0.25H_2O$: C, 61.99; H, 4.44; N, 12.05. Found: C, 62.02; H, 4.36; N, 12.13.

5.1.40. 5-{[Benzyl(methyl)amino]methyl}-*N*-[(3-methoxyphenyl)methyl]-4-oxo-3*H*,4*H*-thieno[2,3-*d*]pyrimidine-2-carboxamide (26f)

Compound **26f** was prepared from compound **25e** with a similar procedure as described for **7a** (white powder, 58%). mp 127 °C. ^{1}H NMR (300 MHz, DMSO-d_6) δ 2.18 (3H, s), 3.60 (2H, s), 3.73 (3H, s), 3.94 (2H, s), 4.42 (2H, d, J = 6.4 Hz), 6.78–6.86 (1H, m), 6.87–6.93 (2H, m), 7.17–7.44 (6H, m), 7.59 (1H, s), 9.63 (1H, s), 12.3 (1H, s). Anal. Calcd for $C_{24}H_{24}N_4O_3S$: C, 64.27; H, 5.39; N, 12.49. Found: C, 64.02; H, 5.21; N, 12.39.

5.1.41. 5-[(Benzylsulfanyl)methyl]-*N*-[(3-methoxyphenyl)-methyl]-4-oxo-3*H*,4*H*-thieno[2,3-*d*]pyrimidine-2-carboxamide (26g)

Compound **26g** was prepared from compound **25f** with a similar procedure as described for **7a** (white powder, 67%). mp 126 °C. ^{1}H NMR (300 MHz, DMSO-d_6) δ 3.73 (2H, s), 3.74 (3H, s), 3.99 (2H, s), 4.42 (2H, d, J = 6.4 Hz), 6.79–6.93 (3H, m), 7.19–7.32 (6H, m), 7.51 (1H, s), 9.64 (1H, t, J = 6.1 Hz), 12.4 (1H, s). Anal. Calcd for $C_{23}H_{21}N_3O_3S_2{\cdot}0.45H_2O$: C, 60.10; H, 4.80; N, 9.14. Found: C, 59.95; H, 4.57; N, 9.38.

5.1.42. Ethyl 5-[(benzoylamino)methyl]-4-oxo-3,4-dihydrothieno[2,3-*d*]pyrimidine-2-carboxylate (28a)

To a mixture of compound **27b** (250 mg, 0.863 mmol) in THF (3.0 mL) were added benzoyl chloride (0.110 mL, 0.949 mmol) and triethylamine (0.253 mL, 1.81 mmol). After being stirred for 1 h at room temperature, the mixture was concentrated in vacuo. The residue was taken up in ethyl acetate, washed with saturated sodium hydrogen carbonate solution (twice), brine, 1 M hydrochloric acid, brine, dried over Na_2SO_4 and concentrated in vacuo. The residue was crystallized from ethyl acetate to give **28a** (223 mg, 72%) as a white powder. mp 204 °C. ^{1}H NMR (300 MHz, DMSO-d_6) δ 1.35 (3H, t, J = 7.1 Hz), 4.38 (2H, q, J = 7.0 Hz), 4.80 (2H, d, J = 4.9 Hz), 7.43–7.60 (4H, m), 7.86–7.94 (2H, m), 9.03 (1H, t, J = 5.8 Hz), 12.9 (1H, s). Anal. Calcd for $C_{17}H_{15}N_3O_4S$: C, 57.13; H, 4.23; N, 11.76. Found: C, 57.28; H, 4.27; N, 11.56.

5.1.43. Ethyl 5-({[4-(methoxycarbonyl)benzoyl]amino}methyl)-4-oxo-3,4-dihydrothieno[2,3-*d*]pyrimidine-2-carboxylate (28b)

To a mixture of monomethyl terephthalate (373 mg, 2.07 mmol) in THF (5.0 mL) were added oxalyl chloride (0.450 mL, 5.18 mmol) and *N*,*N*-dimethylformamide (1 drop). After being stirred for 1 h at room temperature, the mixture was concentrated in vacuo. The residue was resuspended in THF (5.0 mL), and to the mixture were added **27b** (500 mg, 1.73 mmol) and triethylamine (0.960 mL, 6.90 mmol). After being stirred for 1 h at room temperature, the mixture was concentrated in vacuo. The residue was taken up in ethyl acetate, washed with saturated sodium hydrogen carbonate solution (twice), brine, 1 M hydrochloric acid, brine, dried over Na_2SO_4 and concentrated in vacuo. The residue was crystallized from ethyl acetate to give **28b** (588 mg, 82%) as a white powder. mp 236 °C. ^{1}H NMR (300 MHz, DMSO-d_6) δ 1.35 (3H, t, J = 7.2 Hz), 3.89 (3H, s), 4.37 (2H, q, J = 7.2 Hz), 4.82 (2H, d, J = 5.5 Hz), 7.52 (1H, s), 7.77–8.22 (4H, m), 9.23 (1H, s), 12.9 (1H, s). Anal. Calcd for $C_{19}H_{17}N_3O_6S$: C, 54.93; H, 4.12; N, 10.12. Found: C, 54.65; H, 4.24; N, 9.86.

5.1.44. 5-[(Benzoylamino)methyl]-*N*-(3-methoxybenzyl)-4-oxo-3,4-dihydrothieno[2,3-*d*]pyrimidine-2-carboxamide (29a)

Compound **29a** was prepared from compound **28a** with a similar procedure as described for **7a** (white powder, 82%). mp 195–196 °C. ^{1}H NMR (300 MHz, DMSO-d_6) δ 3.73 (3H, s), 4.42 (2H, d, J = 6.2 Hz), 4.80 (2H, d, J = 5.1 Hz), 6.74–6.98 (3H, m), 7.24 (1H, t, J = 8.1 Hz), 7.38–7.62 (4H, m), 7.82–7.98 (2H, m), 9.02 (1H, s), 9.64 (1H, t, J = 6.3 Hz), 12.5 (1H, s). Anal. Calcd for $C_{23}H_{20}N_4O_4S{\cdot}0.15H_2O$: C, 61.23; H, 4.53; N, 12.42. Found: C, 61.28; H, 4.47; N, 12.33.

5.1.45. Methyl 4-{[(2-{[(3-methoxyphenyl)methyl]carbamoyl}-4-oxo-3*H*,4*H*-thieno[2,3-*d*]pyrimidin-5-yl)methyl]carbamoyl}-benzoate (29b)

Compound **29b** was prepared from compound **28b** with a similar procedure as described for **7a** (white powder, 89%). mp 237 °C. ^{1}H NMR (300 MHz, DMSO-d_6) δ 3.73 (3H, s), 3.89 (3H, s), 4.42 (2H, d, J = 6.4 Hz), 4.80 (2H, d, J = 5.7 Hz), 6.80–6.93 (3H, m), 7.24 (1H, t, J = 8.1 Hz), 7.41–7.46 (1H, m), 8.00–8.08 (4H, m), 9.26 (1H, s), 9.59 (1H, s), 12.5 (1H, s). Anal. Calcd for $C_{25}H_{22}N_4O_6S$: C, 59.28; H, 4.38; N, 11.06. Found: C, 59.20; H, 4.41; N, 11.05.

5.1.46. 4-{[(2-{[(3-Methoxyphenyl)methyl]carbamoyl}-4-oxo-3*H*,4*H*-thieno[2,3-*d*]pyrimidin-5-yl)methyl]carbamoyl}benzoic acid (29c)

A mixture of compound **29b** (275 mg, 0.543 mmol) and 12 M NaOH (0.113 mL, 1.36 mmol) in THF–MeOH–H_2O (1:1:1) (6.0 mL) was stirred at 90 °C for 1 h. After evaporation in vacuo, the residue was dissolved in ethyl acetate–THF (1:1) (700 mL) and the organic layer was washed with 1 M hydrochloric acid, brine, dried over Na_2SO_4, and concentrated in vacuo. The residue was crystallized from ethyl acetate to give **29c** as a white powder (263 mg, 98%). mp 285–286 °C. ^{1}H NMR (300 MHz, DMSO-d_6) δ 3.74 (3H, s), 4.42 (2H, d, J = 6.2 Hz), 4.81 (2H, d, J = 5.3 Hz), 6.80–6.93 (3H, m), 7.21–7.27 (1H, m), 7.46 (1H, s), 7.98–8.06 (4H, m), 9.15 (1H, t, J = 5.8 Hz), 9.66 (1H, t, J = 6.3 Hz), 12.5 (1H, s), 13.1 (1H, s). Anal. Calcd for $C_{24}H_{20}N_4O_6S{\cdot}0.35H_2O$: C, 57.79; H, 4.18; N, 11.23. Found: C, 57.86; H, 4.12; N, 11.14.

5.1.47. 5-(Cyanomethyl)-*N*-[(3-methoxyphenyl)methyl]-4-oxo-3*H*,4*H*-thieno[2,3-*d*]pyrimidine-2-carboxamide (31a)

Compound **31a** was prepared from compound **30** with a similar procedure as described for **7a** (brown powder, 76%). mp 217 °C. ^{1}H NMR (300 MHz, DMSO-d_6) δ 3.73 (3H, s), 4.27 (2H, d, J = 0.9 Hz), 4.42 (2H, d, J = 6.4 Hz), 6.78–6.94 (3H, m), 7.24 (1H, t, J = 8.1 Hz), 7.69 (1H, t, J = 0.9 Hz), 9.66 (1H, t, J = 6.4 Hz), 12.6 (1H, s). Anal. Calcd for $C_{17}H_{14}N_4O_3S{\cdot}0.20H_2O$: C, 57.04; H, 4.05; N, 15.65. Found: C, 57.23; H, 4.08; N, 15.44.

5.1.48. 2-(2-{[(3-Methoxyphenyl)methyl]carbamoyl}-4-oxo-3*H*,4*H*-thieno[2,3-*d*]pyrimidin-5-yl)acetic acid (31b)

A mixture of compound **30** (200 mg, 0.564 mmol), 2 M aqueous sodium hydroxide solution (4 mL, 8 mmol) and ethanol (2 mL) was stirred at 100 °C for 1 h. The reaction mixture was concentrated under reduced pressure, and ethyl acetate was added to the obtained residue. The organic layer was washed with 1 M hydrochloric acid and saturated brine, dried over anhydrous Na_2SO_4

and concentrated under reduced pressure. The concentrated residue was crystallized from ethyl acetate to give **31b** as a brown powder (131 mg, 62%). mp 228–229 °C. ^{1}H NMR (300 MHz, DMSO-d_6) δ 3.73 (3H, s), 3.91 (2H, s), 4.42 (2H, d, J = 6.2 Hz), 6.77–6.93 (3H, m), 7.24 (1H, t, J = 8.1 Hz), 7.49 (1H, s), 9.64 (1H, t, J = 6.3 Hz), 12.3 (1H, s), 12.4 (1H, s). Anal. Calcd for $C_{17}H_{15}N_3O_5S{\cdot}0.35H_2O$: C, 53.78; H, 4.17; N, 11.07. Found: C, 53.62; H, 4.15; N, 11.32.

5.1.49. *N*-[(3-Methoxyphenyl)methyl]-4-oxo-5-[(phenylcarbamoyl)methyl]-3*H*,4*H*-thieno[2,3-*d*]pyrimidine-2-carboxamide (32a)

To a mixture of compound **31b** (200 mg, 0.536 mmol) in THF (3.0 mL) was added oxalyl chloride (0.140 mL, 1.61 mmol). After being stirred at room temperature for 15 h, the mixture was concentrated in vacuo, and the resulting residue was dissolved in THF (3.0 mL). To this solution was added aniline (0.146 mL, 1.61 mmol) and pyridine (0.217 mL, 2.68 mmol). After being stirred for 1 h at room temperature, the mixture was concentrated in vacuo, and the residue was taken up in ethyl acetate. The organic layer was washed with saturated $NaHCO_3$ (×3), 1 M hydrochloric acid (×2), and brine and dried over Na_2SO_4. The solvent was removed, and the residue was crystallized from ethyl acetate to give **32a** as a pale yellow powder (154 mg, 64%). mp 194–195 °C. ^{1}H NMR (300 MHz, DMSO-d_6) δ 3.73 (3H, s), 4.04 (2H, s), 4.42 (2H, d, J = 6.2 Hz), 6.78–6.93 (3H, m), 7.02 (1H, t, J = 7.3 Hz), 7.20–7.33 (3H, m), 7.52 (1H, s), 7.58 (2H, d, J = 7.5 Hz), 9.66 (1H, t, J = 6.5 Hz), 10.1 (1H, s), 12.4 (1H, s). Anal. Calcd for $C_{23}H_{20}N_4O_4S$: C, 61.59; H, 4.49; N, 12.49. Found: C, 61.29; H, 4.47; N, 12.47.

5.1.50. Ethyl 4-[2-(2-{[(3-methoxyphenyl)methyl]carbamoyl}-4-oxo-3*H*,4*H*-thieno[2,3-*d*]pyrimidin-5-yl)acetamido]benzoate (32b)

To a mixture of compound **31b** (350 mg, 0.937 mmol) in THF (3.0 mL) was added oxalyl chloride (0.245 mL, 2.81 mmol). After being stirred at room temperature for 15 h, the mixture was concentrated in vacuo, and the resulting residue was dissolved in THF (3.0 mL). To this solution was added ethyl 4-aminobenzoate (465 mg, 2.81 mmol) and pyridine (0.379 mL, 4.69 mmol). After being stirred for 1 h at room temperature, the mixture was concentrated in vacuo, and the residue was taken up in ethyl acetate. The organic layer was washed with saturated $NaHCO_3$ (×3), 1 M hydrochloric acid (×4), and brine and dried over Na_2SO_4. The solvent was removed, and the residue was crystallized from ethyl acetate to give **32b** as a pale yellow powder (370 mg, 76%). mp 222–223 °C. ^{1}H NMR (300 MHz, DMSO-d_6) δ 1.3 (3H, t, J = 7.2 Hz), 3.73 (3H, s), 4.07 (2H, s), 4.28 (2H, q, J = 7.1 Hz), 4.42 (2H, d, J = 6.2 Hz), 6.77–6.95 (3H, m), 7.24 (1H, t, J = 8.1 Hz), 7.52 (1H, s), 7.71 (2H, d, J = 8.9 Hz), 7.9 (2H, d, J = 8.7 Hz), 9.64 (1H, s), 10.5 (1H, s), 12.4 (1H, s). Anal. Calcd for $C_{26}H_{24}N_4O_6S{\cdot}0.15H_2O$: C, 59.68; H, 4.68; N, 10.71. Found: C, 59.90; H, 4.69; N, 10.43.

5.1.51. 4-[2-(2-{[(3-Methoxyphenyl)methyl]carbamoyl}-4-oxo-3*H*,4*H*-thieno[2,3-*d*]pyrimidin-5-yl)acetamido]benzoic acid (32c)

A mixture of **32b** (230 mg, 0.442 mmol) and 12 M NaOH (0.092 mL, 1.1 mmol) in THF–MeOH–H_2O (1:1:1) (3.0 mL) was stirred at 80 °C for 1 h. After evaporation in vacuo, the residue was dissolved in ethyl acetate and the organic layer was washed with 1 M hydrochloric acid (×2), brine, dried over Na_2SO_4, and concentrated in vacuo. The residue was crystallized from ethyl acetate to give **32c** as a pale yellow powder (188 mg, 86%). mp 279 °C. ^{1}H NMR (300 MHz, DMSO-d_6) δ 3.73 (3H, s), 4.07 (2H, s), 4.42 (2H, d, J = 6.4 Hz), 6.73–6.96 (3H, m), 7.24 (1H, t, J = 8.1 Hz), 7.52 (1H, s), 7.69 (2H, d, J = 8.9 Hz), 7.87 (2H, d, J = 8.9 Hz), 9.61 (1H, s), 10.5 (1H, s), 12.5 (2H, s). Anal. Calcd for $C_{24}H_{20}N_4O_6S{\cdot}0.50H_2O$: C, 57.48; H, 4.22; N, 11.17. Found: C, 57.57; H, 4.11; N, 10.96.

5.1.52. Ethyl 5-(benzylcarbamoyl)-4-oxo-3*H*,4*H*-thieno[2,3-*d*]pyrimidine-2-carboxylate (34a)

To a mixture of **33c** (400 mg, 1.49 mmol) in THF (10 mL) were added oxalyl chloride (0.390 mL, 4.47 mmol) and *N*,*N*-dimethylformamide (1 drop). After being stirred for 1 h at room temperature, the mixture was concentrated in vacuo. The residue was resuspended in THF (10 mL), and to the mixture was added benzylamine (0.326 mL, 2.98 mmol). After being stirred for 12 h at room temperature, the mixture was concentrated in vacuo. The residue was taken up in ethyl acetate, washed with 1 M hydrochloric acid (×2), saturated sodium hydrogen carbonate solution, 1 M hydrochloric acid, brine, dried over Na_2SO_4 and concentrated in vacuo. The residue was crystallized from ethyl acetate to give **34a** (373 mg, 70%) as a white powder. mp 215–216 °C. ^{1}H NMR (300 MHz, DMSO-d_6) δ 1.35 (3H, t, J = 7.2 Hz), 4.39 (2H, q, J = 7.1 Hz), 4.56 (2H, d, J = 5.5 Hz), 7.13–7.53 (5H, m), 8.56 (1H, s), 11.2 (1H, s), 13.5 (1H, s). Anal. Calcd for $C_{17}H_{15}N_3O_4S$: C, 57.13; H, 4.23; N, 11.76. Found: C, 57.08; H, 4.19; N, 11.83.

5.1.53. Ethyl 5-({[4-(ethoxycarbonyl)phenyl]methyl}carbamoyl)-4-oxo-3*H*,4*H*-thieno[2,3-*d*]pyrimidine-2-carboxylate (34b)

To a mixture of compound **33c** (600 mg, 2.24 mmol) in THF (6.0 mL) were added oxalyl chloride (0.590 mL, 6.72 mmol) and *N*,*N*-dimethylformamide (1 drop). After being stirred for 1 h at room temperature, the mixture was concentrated in vacuo. The residue was resuspended in THF (6.0 mL), and to the mixture were added ethyl 4-(aminomethyl)benzoate hydrochloride (965 mg, 4.48 mmol) and triethylamine (1.20 mL, 8.96 mmol). After being stirred for 12 h at room temperature, the mixture was concentrated in vacuo. The residue was taken up in ethyl acetate, washed with 1 M hydrochloric acid (×2), saturated sodium hydrogen carbonate solution, 1 M hydrochloric acid, brine, dried over Na_2SO_4 and concentrated in vacuo. The residue was crystallized from ethyl acetate to give **34b** (771 mg, 80%) as a white powder. mp 227–228 °C. ^{1}H NMR (300 MHz, DMSO-d_6) δ 1.31 (3H, t, J = 6.2 Hz), 1.36 (3H, t, J = 6.2 Hz), 4.31 (2H, q, J = 7.1 Hz), 4.40 (2H, q, J = 7.1 Hz), 4.65 (2H, d, J = 5.5 Hz), 7.50 (2H, d, J = 8.5 Hz), 7.93 (2H, d, J = 8.5 Hz), 8.57 (1H, s), 11.3 (1H, s), 13.5 (1H, s). Anal. Calcd for $C_{20}H_{19}N_3O_6S{\cdot}0.20H_2O$: C, 55.47; H, 4.52; N, 9.70. Found: C, 55.50; H, 4.39; N, 9.73.

5.1.54. *N*5-Benzyl-*N*2-[(3-methoxyphenyl)methyl]-4-oxo-3*H*,4*H*-thieno[2,3-*d*]pyrimidine-2,5-dicarboxamide (35a)

Compound **35a** was prepared from compound **34a** with a similar procedure as described for **7a** (white powder, 85%). mp 217 °C. ^{1}H NMR (300 MHz, DMSO-d_6) δ 3.73 (3H, s), 4.43 (2H, d, J = 6. 2 Hz), 4.56 (2H, d, J = 5.5 Hz), 6.80–6.85 (1H, m), 6.89–6.93 (2H, m), 7.21–7.29 (2H, m), 7.30–7.38 (4H, m), 8.52 (1H, s), 9.74 (1H, s), 11.3 (1H, s), 13.2 (1H, s). Anal. Calcd for $C_{23}H_{20}N_4O_4S$: C, 61.59; H, 4.49; N, 12.49. Found: C, 61.48; H, 4.48; N, 12.41.

5.1.55. Ethyl 4-{[({2-[(3-Methoxybenzyl)carbamoyl]-4-oxo-3,4-dihydrothieno[2,3-*d*]pyrimidin-5-yl}carbonyl)amino]methyl}-benzoate (35b)

Compound **35b** was prepared from compound **34b** with a similar procedure as described for **7a** (white powder, 91%). mp 229–230 °C. ^{1}H NMR (300 MHz, DMSO-d_6) δ 1.31 (3H, t, J = 7.1 Hz), 3.73 (3H, s), 4.31 (2H, q, J = 7.1 Hz), 4.44 (2H, d, J = 6.2 Hz), 4.64 (2H, d, J = 5.3 Hz), 6.78–6.96 (3H, m), 7.24 (1H, t, J = 8.1 Hz), 7.49 (2H, d, J = 8.3 Hz), 7.88–7.97 (2H, m), 8.51 (1H, s), 9.73 (1H, t, J = 6.0 Hz), 11.4 (1H, s), 13.2 (1H, s). Anal. Calcd for $C_{26}H_{24}N_4O_6$

S·0.20H_2O: C, 59.58; H, 4.69; N, 10.69. Found: C, 59.41; H, 4.46; N, 10.61.

5.1.56. 4-{[({2-[(3-Methoxybenzyl)carbamoyl]-4-oxo-3,4-dihydrothieno[2,3-*d*]pyrimidin-5-yl}carbonyl)amino]methyl}-benzoic acid (35c)

A mixture of **35b** (330 mg, 0.634 mmol) and 12 M NaOH (0.132 mL, 1.59 mmol) in a mixture of THF–MeOH–H_2O (2.0 mL–2.0 mL–2.0 mL) was refluxed at 90 °C for 1 h. The mixture was concentrated in vacuo to give a residue, which was taken up in ethyl acetate–THF (1:1, ca. 1L), washed with 1 M hydrochloric acid and brine, and dried over Na_2SO_4. The organic extract was concentrated in vacuo, and the residue was crystallized from ethyl acetate to give **35c** (294 mg, 94%) as a white powder. mp 254–255 °C. ^{1}H NMR (300 MHz, DMSO-d_6) δ 3.73 (3H, s), 4.44 (2H, d, J = 6.4 Hz), 4.64 (2H, d, J = 5.5 Hz), 6.74–6.97 (3H, m), 7.24 (1H, t, J = 8.1 Hz), 7.47 (2H, d, J = 8.3 Hz), 7.91 (2H, d, J = 8.3 Hz), 8.52 (1H, s), 9.76 (1H, t, J = 6.4 Hz), 11.3 (1H, t, J = 5.6 Hz), 13.2 (1H, s). Anal. Calcd for $C_{24}H_{20}N_4O_6S$·2.0H_2O: C, 54.54; H, 4.58; N, 10.60. Found: C, 54.19; H, 4.31; N, 10.45.

5.1.57. 2-{[(3-Methoxyphenyl)methyl]carbamoyl}-4-oxo-3*H*,4*H*-thieno[2,3-*d*]pyrimidine-5-carboxylic acid (36a)

Compound **36a** was prepared from compound **33c** with a similar procedure as described for **7a** (white powder, 81%). mp 236–237 °C. ^{1}H NMR (300 MHz, DMSO-d_6) δ 3.74 (3H, s), 4.45 (2H, d, J = 6.4 Hz), 6.76–6.96 (3H, m), 7.25 (1H, t, J = 8.1 Hz), 8.65 (1H, s), 9.82 (1H, t, J = 6.3 Hz), 13.9 (1H, s), 15.4 (1H, s). Anal. Calcd for $C_{16}H_{13}N_3O_5S$·0.30AcOEt: C, 53.55; H, 4.02; N, 10.89. Found: C, 53.86; H, 3.85; N, 11.07.

5.1.58. Ethyl 4-{[(2-{[(3-methoxyphenyl)methyl]carbamoyl}-4-oxo-3*H*,4*H*-thieno[2,3-*d*]pyrimidin-5-yl)carbamoyl]methyl}-benzoate (37) and 5.1.76. 4-{[(2-{[(3-Methoxyphenyl)methyl]-carbamoyl}-4-oxo-3*H*,4*H*-thieno[2,3-*d*]pyrimidin-5-yl)-carbamoyl]methyl}benzoic acid (38)

To a suspension of **36a** (4.45 g, 12.4 mmol) in toluene (45 mL) were added triethylamine (10.0 mL, 74.3 mmol) and DPPA (6.90 mL, 32.2 mmol). The mixture was stirred for 2 min at 100 °C, followed by addition of *tert*-butyl alcohol (12.0 mL, 124 mmol), and heated at 100 °C for 12 h. After the removal of solvent, the residue was taken up in ethyl acetate. The organic layer was extracted with saturated $NaHCO_3$ (×2), and brine and dried over Na_2SO_4. The solvent was removed, and the residue was dried in vacuo to give a residue, which was used in the next step without further purification. A mixture of the above residue in 4 M hydrochloric acid/ethyl acetate (45 mL) was stirred overnight at room temperature. The resulting solid was filtered and washed with ethyl acetate and dried to provide a brown powder (3.14 g). A mixture of above powder and 12 M NaOH (4.10 mL, 49.5 mmol) in THF–MeOH–H_2O (1:1:1, 45 mL) was stirred at 80 °C for 2 h. After neutralization with 1 M hydrochloric acid, the mixture was concentrated in vacuo. The residue was taken up in ethyl acetate, washed with water and brine and concentrated in vacuo to give a brown foam (1.18 g, 26%). The crude product **36b** was used without purification.

To a mixture of {4-[(ethyloxy)carbonyl]phenyl}acetic acid (215 mg, 1.033 mmol) and THF (3 mL) were added oxalyl chloride (0.183 mL, 2.10 mmol) and DMF (1 drop), and the mixture was stirred at room temperature for 2 h. The reaction mixture was concentrated under reduced pressure, THF (3 mL), **36b** (276 mg, 0.837 mmol) and pyridine (0.418 mL, 5.17 mmol) were added to the obtained residue, and the mixture was stirred at room temperature for 2 h. The reaction mixture was concentrated under reduced pressure and ethyl acetate was added to the obtained residue. The organic layer was washed with 1 M hydrochloric acid and saturated brine, dried over anhydrous Na_2SO_4 and concentrated under reduced pressure. Aqueous sodium hydroxide solution (1 M, 2 mL), ethanol (1 mL) and THF (1 mL) were added to the concentrated residue and the reaction mixture was stirred at room temperature for 12 h. The reaction mixture was concentrated under reduced pressure, and ethyl acetate was added to the obtained residue. The organic layer was washed with water and saturated brine, dried over anhydrous Na_2SO_4 and concentrated under reduced pressure. The concentrated residue was crystallized from ethyl acetate to give **37** as a brown powder (29.6 mg, 5.5%). mp 218–219 °C. ^{1}H NMR (300 MHz, DMSO-d_6) δ 1.32 (3H, t, J = 7.1 Hz), 3.73 (3H, s), 3.93 (2H, s), 4.31 (2H, q, J = 7.0 Hz), 4.41 (2H, d, J = 6.2 Hz), 6.77–6.95 (3H, m), 7.23 (1H, t, J = 8.1 Hz), 7.52 (2H, d, J = 8.3 Hz), 7.86 (1H, s), 7.95 (2H, d, J = 8.3 Hz), 9.67 (1H, t, J = 6.1 Hz), 9.95 (1H, s), 12.8 (1H, s).

The aqueous layer was acidified with 1 M hydrochloric acid. The mixture was extracted with ethyl acetate, and the organic layer was washed with 1 M hydrochloric acid and saturated brine, dried over anhydrous Na_2SO_4 and concentrated under reduced pressure. The concentrated residue was crystallized from ethyl acetate to give **38** as a brown powder (83.9 mg, 17%). mp 218–219 °C. ^{1}H NMR (300 MHz, DMSO-d_6) δ 3.73 (3H, s), 3.92 (2H, s), 4.40 (2H, d, J = 6.4 Hz), 6.73–6.96 (3H, m), 7.23 (1H, t, J = 8.1 Hz), 7.49 (2H, d, J = 8.3 Hz), 7.87 (1H, s), 7.93 (2H, d, J = 8.3 Hz), 9.67 (1H, t, J = 6.5 Hz), 9.94 (1H, s), 12.8 (1H, s), 12.9 (1H, s). Anal. Calcd for $C_{24}H_{20}N_4O_6S$·0.30H_2O: C, 57.89; H, 4.17; N, 11.25. Found: C, 57.81; H, 4.23; N, 11.52.

5.1.59. Ethyl 4-oxo-5-(2-phenylacetamido)-3*H*,4*H*-thieno[2,3-*d*]pyrimidine-2-carboxylate (40)

To a mixture of **39c** (160 mg, 0.500 mmol) and THF (2 mL) were added phenylacetyl chloride (0.0727 mL, 0.550 mmol) and triethylamine (0.146 mL, 1.05 mmol) at room temperature. The mixture was stirred for 1 h at room temperature and concentrated under reduced pressure. Ethyl acetate was added to the obtained residue, and the organic layer was washed with 1 M hydrochloric acid, water and saturated brine, dried over anhydrous Na_2SO_4 and concentrated under reduced pressure. The concentrated residue was crystallized from ethyl acetate to give **40** as a pale purple powder (138 mg, 77%). mp 237 °C. ^{1}H NMR (300 MHz, DMSO-d_6) δ 1.33 (3H, t, J = 7.1 Hz), 3.81 (2H, s), 4.36 (2H, q, J = 7.2 Hz), 7.22–7.41 (5H, m), 7.92 (1H, s), 9.89 (1H, s), 13.1 (1H, s). Anal. Calcd for $C_{17}H_{15}N_3O_4S$·0.25H_2O: C, 56.42; H, 4.32; N, 11.61. Found: C, 56.58; H, 4.30; N, 11.72.

5.1.60. *N*-[(3-Methoxyphenyl)methyl]-4-oxo-5-(2-phenylacetamido)-3*H*,4*H*-thieno[2,3-*d*]pyrimidine-2-carboxamide (41)

Compound **41** was prepared from compound **40** with a similar procedure as described for **7a** (white powder, 93%). mp 201 °C. ^{1}H NMR (300 MHz, DMSO-d_6) δ 3.73 (3H, s), 3.80 (2H, s), 4.41 (2H, d, J = 6.2 Hz), 6.77–6.94 (3H, m), 7.18–7.42 (6H, m), 7.86 (1H, s), 9.67 (1H, t, J = 6.4 Hz), 9.93 (1H, s), 12.8 (1H, s). Anal. Calcd for $C_{23}H_{20}N_4O_4S$·0.10AcOEt·0.25H_2O: C, 60.86; H, 4.65; N, 12.13. Found: C, 60.99; H, 4.62; N, 12.21.

5.1.61. 5-{[(4-Carbamoylphenyl)methoxy]methyl}-*N*-[(3-methoxyphenyl)methyl]-4-oxo-3*H*,4*H*-thieno[2,3-*d*]pyrimidine-2-carboxamide (42a)

To a mixture of **26c** (200 mg, 0.417 mmol) and THF (2 mL) were added oxalyl chloride (0.0500 mL, 0.573 mmol) and DMF (1 drop) and the mixture was stirred at room temperature for 1 h. The reaction mixture was concentrated under reduced pressure. To a suspension of the concentrated residue in THF (3 mL) was added 28% aqueous ammonia (2 mL), and the mixture was stirred at room temperature for 10 min. The reaction mixture was concentrated under reduced pressure, and ethyl acetate and THF were added to the obtained residue. The organic layer was washed with 1 M

hydrochloric acid and saturated brine, dried over anhydrous Na_2SO_4 and concentrated under reduced pressure. The concentrated residue was crystallized from ethyl acetate–THF to give **42a** as a white powder (173 mg, 87%). mp 224 °C. ^{1}H NMR (300 MHz, DMSO-d_6) δ 3.73 (3H, s), 4.42 (2H, d, J = 6.2 Hz), 4.71 (2H, s), 4.87 (2H, d, J = 1.1 Hz), 6.76–6.95 (3H, m), 7.18–7.28 (1H, m), 7.34 (1H, s), 7.47 (2H, t, J = 7.6 Hz), 7.62 (1H, s), 7.86 (2H, d, J = 8.3 Hz), 7.95 (1H, s), 9.64 (1H, t, J = 6.4 Hz), 12.4 (1H, s). Anal. Calcd for $C_{24}H_{22}N_4O_5S{\cdot}0.50H_2O$: C, 59.13; H, 4.76; N, 11.49. Found: C, 59.06; H, 4.72; N, 11.22.

5.1.62. *N*-[(3-Methoxyphenyl)methyl]-5-({[4-(methylcarbamoyl)phenyl]methoxy}methyl)-4-oxo-3*H*,4*H*-thieno[2,3-*d*]pyrimidine-2-carboxamide (42b)

To a mixture of **26c** (110 mg, 0.229 mmol), 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride (65.9 mg, 0.344 mol), 4-dimethylaminopyridine (283 mg, 2.31 mol) and THF (5 mL) was added methylamine hydrochloride (155 mg, 2.29 mmol). The mixture was stirred at room temperature for 12 h. The reaction mixture was concentrated under reduced pressure, and ethyl acetate was added to the obtained residue. The organic layer was washed with 1 M hydrochloric acid and saturated brine, dried over anhydrous Na_2SO_4 and concentrated under reduced pressure. The concentrated residue was crystallized from ethyl acetate to give **42b** as a white powder (75.9 mg, 67%). mp 190–191 °C. ^{1}H NMR (300 MHz, DMSO-d_6) δ 2.78 (3H, d, J = 4.5 Hz), 3.73 (3H, s), 4.42 (2H, d, J = 6.4 Hz), 4.71 (2H, s), 4.87 (2H, d, J = 0.8 Hz), 6.82 (1H, dd, J = 9.0, 1.8 Hz), 6.86–6.97 (2H, m), 7.24 (1H, t, J = 8.1 Hz), 7.46 (2H, d, J = 8.1 Hz), 7.62 (1H, s), 7.82 (2H, d, J = 8.1 Hz), 8.42 (1H, d, J = 4.5 Hz), 9.65 (1H, t, J = 6.4 Hz), 12.4 (1H, s). Anal. Calcd for $C_{25}H_{24}N_4O_5S{\cdot}0.80H_2O$: C, 59.23; H, 5.09; N, 11.05. Found: C, 59.33; H, 5.04; N, 11.00.

5.1.63. 5-({[4-(Hydroxymethyl)phenyl]methoxy}methyl)-*N*-[(3-methoxyphenyl)methyl]-4-oxo-3*H*,4*H*-thieno[2,3-*d*]pyrimidine-2-carboxamide (42c)

To a mixture of **26c** (1200 mg, 2.50 mmol) and THF (12 mL) were added oxalyl chloride (0.419 mL, 4.80 mmol) and DMF (1 drop), and the mixture was stirred at room temperature for 1.5 h. The reaction mixture was concentrated under reduced pressure. A Mixture of the concentrated residue and sodium borohydride (189 mg, 5.01 mmol) was stirred in DMA (15 mL) at room temperature for 5 min. The reaction mixture was stirred until generation of gas ceased and concentrated under reduced pressure. Ethyl acetate was added to the obtained residue. The organic layer was washed with water, 1 M hydrochloric acid, water and saturated brine, dried over anhydrous Na_2SO_4 and concentrated under reduced pressure. The concentrated residue was crystallized from ethyl acetate to give **42c** as a white powder (753 mg, 65%). mp 156–157 °C. ^{1}H NMR (300 MHz, DMSO-d_6) δ 3.73 (3H, s), 4.42 (2H, d, J = 6.4 Hz), 4.49 (2H, d, J = 5.7 Hz), 4.64 (2H, s), 4.83 (2H, d, J = 1.1 Hz), 5.16 (1H, t, J = 5.8 Hz), 6.75–6.95 (3H, m), 7.19–7.28 (1H, m), 7.28–7.39 (4H, m), 7.58 (1H, s), 9.64 (1H, t, J = 6.5 Hz), 12.4 (1H, s). Anal. Calcd for $C_{24}H_{23}N_3O_5S{\cdot}0.50H_2O$: C, 60.75; H, 5.10; N, 8.86. Found: C, 60.72; H, 4.98; N, 8.89.

5.1.64. 5-({[4-(Methoxymethyl)phenyl]methoxy}methyl)-*N*-[(3-methoxyphenyl)methyl]-4-oxo-3*H*,4*H*-thieno[2,3-*d*]pyrimidine-2-carboxamide (42d)

To a mixture of **26c** (100 mg, 0.215 mmol) and THF (2 mL) were added methanesulfonyl chloride (0.0266 mL, 0.344 mmol) and triethylamine (0.0929 mL, 0.667 mmol), and the mixture was stirred at room temperature for 1 h. The reaction mixture was concentrated under reduced pressure, and ethyl acetate was added to the obtained residue. The organic layer was washed with water and saturated brine, dried over anhydrous Na_2SO_4 and concentrated under reduced pressure. To a mixture of the concentrated residue and sodium methylate (58.1 mg, 1.08 mmol) were added methanol (2 mL) and THF (2 mL) and the mixture was stirred at 80 °C for 1 h. The reaction mixture was concentrated under reduced pressure, and ethyl acetate was added to the obtained residue. The organic layer was washed with 1 M hydrochloric acid and saturated brine, dried over anhydrous Na_2SO_4 and concentrated under reduced pressure. The concentrated residue was crystallized from ethyl acetate to give **42d** as a white powder (68.5 mg, 66%). mp 156–157 °C. ^{1}H NMR (300 MHz, DMSO-d_6) δ 3.28 (3H, s), 3.73 (3H, s), 4.34–4.48 (4H, m), 4.65 (2H, s), 4.85 (2H, d, J = 0.9 Hz), 6.77–6.95 (3H, m), 7.18–7.40 (5H, m), 7.59 (1H, s), 9.64 (1H, t, J = 6.0 Hz), 12.4 (1H, s). Anal. Calcd for $C_{25}H_{25}N_3O_5S{\cdot}0.25H_2O$: C, 62.03; H, 5.31; N, 8.68. Found: C, 61.98; H, 5.32; N, 8.63.

5.1.65. Disodium 4-[({2-[(3-Methoxybenzyl)carbamoyl]-4-oxo-4*H*-thieno[2,3-*d*]pyrimidin-3-id-5-yl}methoxy)methyl]-benzoate (43)

To a mixed solution of **26c** (150 mg, 0.313 mmol) in THF (24 mL) and ethanol (6 mL) was added an aqueous solution (3 mL) of sodium hydrogen carbonate (52.6 mg, 0.626 mmol) and the mixture was stirred for 30 min. The reaction mixture was concentrated under reduced pressure, ethanol (18 mL) was added to the obtained residue, and the ethanol suspension was stirred at 90 °C for 30 min. The mixture was allowed to cool to room temperature, and the precipitated solid was collected by filtration, washed with ethanol and dried to give **43** as a white powder (149 mg, 91%). mp >300 °C. ^{1}H NMR (300 MHz, DMSO-d_6) δ 3.73 (3H, s), 4.40 (2H, d, J = 6.4 Hz), 4.63 (2H, s), 4.88 (2H, d, J = 1.3 Hz), 6.74–6.94 (3H, m), 6.98 (1H, t, J = 1.3 Hz), 7.14–7.33 (3H, m), 7.84 (2H, d, J = 8.1 Hz), 9.03 (1H, t, J = 6.4 Hz). Anal. Calcd for $C_{24}H_{19}N_3Na_2O_6S{\cdot}1.0H_2O$: C, 53.23; H, 3.91; N, 7.76. Found: C, 53.51; H, 4.09; N, 7.83.

5.2. 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.[28] 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 mM $ZnCl_2$, and 0.005% Brij-35. The pro-MMPs were activated by preincubation with 1 mM aminophenylmercuric acetate (APMA) in assay buffer at 37 °C for 2 h (MMP-1, 2, 7, 8, 10, and 13) or 18 h (MMP-3 and 9). Enzyme inhibition assays were performed in an assay buffer containing enzymes and fluorescence peptide (Cy3-PLGLK(Cy5Q)AR-NH_2 for MMPs, Cy3-PLAQAV(Cy5Q-L-2,3-diaminopropionic acid)-RSSSR-NH_2 for TACE, Amersham Biosciences) in the presence of the various concentrations of inhibitors. Following incubation at 37 °C for 40 min, the reaction was terminated by addition of EDTA (pH 8.0). The increase in fluorescence was measured by Farcyte spectrofluorimeter (Amersham Bioscience, λ_{em} 535 nm, λ_{ex} 595 nm). Enzyme activity (%) was determined as following equation: Enzyme activity (%) = $(X - C)/(T - C) \times 100$, where X = the fluorescence count with inhibitor, T = the fluorescence count without inhibitor and C = the fluorescence count with EDTA. IC_{50} values of inhibitors were obtained with iterative fitting package (GraphPad Prism software).

5.3. Assay for inhibitory activity against collagen degradation

Bovine nasal septum cartilage was sliced, and the slices were maintained in the medium of a 1:1 (v/v) mixture of Dulbecco's

modified Eagle's MEM and Ham's F-12 medium (DMEM/F-12) containing 10 % fetal calf serum overnight. After confirming that the slices were not contaminated, they were cultured in DMEM/F-12 medium containing 20 μg/mL gentamycin, 50 μg/mL streptomycin, and 50 U/mL penicillin (culture medium) for 2 days at 37 °C. The cartilage slices were cut into small cubes (ca. 1mm^3) and transferred individually into wells of a 96 well plate with 100 μL of culture medium. For the collagen degradation assay, the medium was supplemented with 10 ng/mL IL-1β and 50 ng/mL oncostatin M in the presence or absence of compounds. The cartilage was incubated for 2 weeks. The supernatants were harvested and replaced with fresh medium containing identical test compounds every 7 days. Supernatants of day 7 and day 14 were collected and stored at −20 °C until assay. At the end of the culture, the remaining cartilage was completely digested with papain. Hydroxyproline release in the media from each explant was determined as a measure of collagen degradation by use of chloramine T and *p*-dimethylaminobenzaldehyde. The percentage of inhibitory activity against collagen degradation was calculated as follows: % of inhibition = [(% of collagen degradation with IL-1β and OSM) − (% of collagen degradation with IL-1β, OSM, and test sample)]/[(% of collagen degradation with IL-1β and OSM) − (% of collagen degradation without additives)] × 100.

Acknowledgements

We thank Mr. Hiroaki Omae and Ms. Yoko Tanaka for the preparation of the catalytic domain of human MMP-13 for X-ray structure analysis, Ms. Mika Murabayashi for hit identification by NMR measurements, and Mr. Akira Fujishima for supporting crystallographic study. The authors thank our supervisors: Dr. Shigenori Ohkawa, Dr. Yuji Ishihara, Dr. Yoshinori Ikeura, and Dr. Masayuki Takizawa, for valuable discussions and critical comments throughout this work. We thank Dr. Keiji Kamiyama and Dr. Nobuo Cho for careful reading of the manuscript. We gratefully acknowledge fruitful discussions with Mr. Akira Kaieda. We also thank the members of drug metabolism & pharmacokinetics research laboratories, biomolecular research laboratories, and analytical development laboratories for pharmacokinetic and physicochemical studies, the members of drug safety research laboratories for toxicokinetic and toxicological studies, and the members of the Takeda analytical research laboratories, Ltd. for elemental analyses.

Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bmc.2014.07.025.

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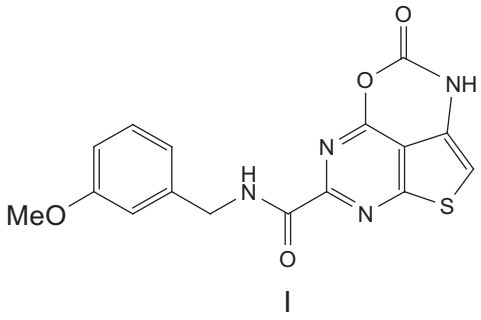

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