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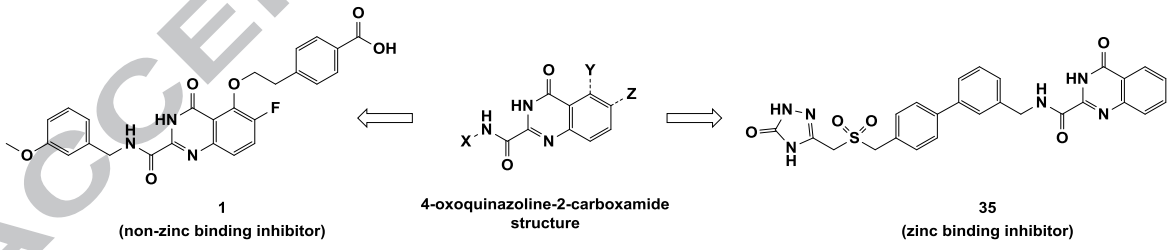
ABSTRACT

Matrix metalloproteinase-13 (MMP-13), a member of the collagenase family of enzymes, has been implicated to play a key role in the pathology of osteoarthritis. Recently, we have reported the discovery of a series of quinazoline-2-carboxamide based non-zinc-binding MMP-13 selective inhibitors, as exemplified by compound **1**. We then continued our research of a novel class of zinc-binding inhibitors to obtain follow-up compounds with different physicochemical, pharmacokinetic, and biological activity profiles. In order to design selective MMP-13 inhibitors, we adopted a strategy of connecting a zinc-binding group with the quinazoline-2-carboxamide system, a unique S1' binder, by an appropriate linker. Among synthesized compounds, a triazolone inhibitor **35** exhibited excellent potency (IC_{50} = 0.071 nM) and selectivity (greater than 170-fold) over other MMPs (MMP-1, 2, 3, 7, 8, 9, 10, 12, and 14) and tumor necrosis factor-α converting enzyme (TACE). In this article, the design, synthesis, and biological activity of novel zinc-binding MMP-13 inhibitors are described.



1. Introduction

Osteoarthritis (OA) is a chronic disease characterized by destruction of articular cartilage upon aging and progressive degradation due to mechanical stress. OA differs from systemic autoimmune diseases such as rheumatoid arthritis. The most common clinical signs are joint swelling, instability, and restricted range of motion. OA is at the top of arthralgia for elderly patients[1-3] and is a major health concern for an increasingly aging worldwide population.[4] The pathogenesis of OA is incompletely understood, but multiple genetic and environmental factors are thought to be involved in the development of OA. Genetic studies have identified multiple gene variations associated with an increased risk of OA.[5-8] Current OA treatments are limited to symptomatic relief with NSAIDs, intra-articular injections of hyaluronic acid conjugates, or surgical joint replacement. In addition, the COX-2 inhibitors cause an increase in the risk of cardiovascular side effects,[9] resulting in the withdrawal of Vioxx (rofecoxib) and Bextra (valdecoxib).

Thus, there is still an urgent unmet medical need to develop new and safer disease modifying osteoarthritis drugs ("DMOADs") that reduce or reverse the cartilage destruction associated with OA. Matrix metalloproteinases (MMPs) are a family of zinc-dependent, calcium-containing endopeptidases, many of which may play a role in OA. Preclinical testing of MMP inhibitors has shown that these inhibitors are able to prevent the destruction of cartilage in some OA animal models.[10] However, most clinical trials of broad spectrum MMP inhibitors have been halted by dose-limiting toxicity (skin rash and musculoskeletal side effects, which are characterized by joint stiffness, pain, inflammation, and tendinitis). This side effect has been postulated to result from the inhibition of MMP-1, or MMP-14, or sheddases, such as TACE.[11-13] As a result, interest in more selective MMP-13 inhibitors has increased. MMP-13 (collagenase-3) is the most efficient collagenase which cleaves type II collagen[14, 15] and is overexpressed in OA cartilage.[14, 16] Therefore, inhibition of this enzyme is one of the most promising approaches for the treatment of cartilage degradation in arthritis.[17-32]

Recently, we described highly selective MMP-13 inhibitors, which do not bind the catalytic zinc ion (**Figure 1**).[33-36] The inhibitors possess a pyrimidin-4-one-2-carboxamide core, which binds effectively to the primed regions (S1') of the catalytic active site[36, 37], and the fused pyrimidine carboxamide systems were successfully applied to other selective MMP-13 inhibitors.[38-40] Among the previously synthesized compounds in our laboratories, promising derivatives, quinazoline-2-carboxamide and thienopyrimidine-2-carboxamide based MMP-13 inhibitors, as exemplified by **1** and **2**, were identified, which exhibit potent inhibition of MMP-13, but weak inhibitory activity toward other members of the MMP family. This article describes the strategy employed to modify the previous series in order to obtain a follow-up compound with improved physicochemical and pharmacokinetic properties.

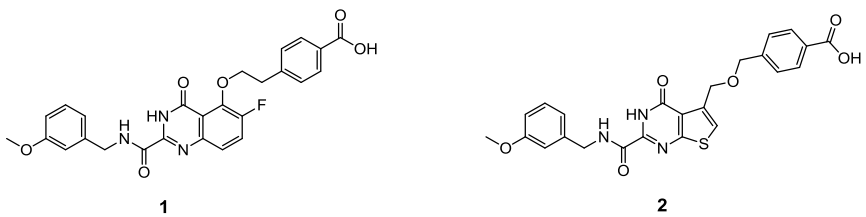


Figure 1. Structure of fused pyrimidine-derived MMP-13 selective inhibitors **1** and **2**.

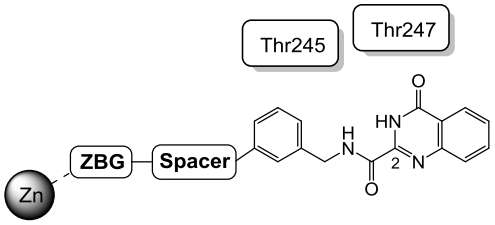


Figure 2. Schematic structure of quinazoline-2-carboxamide-based zinc-binding MMP-13 inhibitors.

There are three major components to most MMP inhibitors as follows: (1) the zinc binding group (ZBG); (2) side chains which could interact with amino acids around the catalytic zinc ion; (3) the pocket-occupying functionality referred to as the P1' group, which is bound in the S1' pocket.[41-47] Historically, most MMP inhibitors gained affinity through interacting with the catalytic zinc via a chelating moiety and by positioning hydrogen bonding groups such as backbone NH of Leu185, backbone carbonyl oxygen of Ala186, and carboxylate of the Glu202 residue near the catalytic zinc. However, one major concern with this approach is the issue of selectivity, since many of the important residues in the catalytic site are well-conserved among the MMP family. In contrast, conformational diversity is localized at the S1' loop region, which forms the bottom half of the S1' subsite. MMPs such as MMP-2, MMP-3, MMP-8, MMP-9, MMP-12, MMP-13, and MMP-14 have large S1' pockets and can accommodate more diverse large P1' groups. Thus, targeting the large S1' loop region assists in identifying MMP-13-specific inhibitors.[19-28, 48-52] As mentioned above, we described the discovery of potent MMP-13 inhibitors which form β-sheet type hydrogen bond interactions with both Thr245 and Thr247 of the S1' site. As a result, we designed MMP-13 inhibitors by incorporating a ZBG via appropriate spacers into the quinazoline-2-carboxamide scaffold that can be recognized by the enzyme and forms a complex with it (**Figure 2**). In addition, spacers were chosen that had a potential to form hydrogen bonds with the backbone amides near the catalytic zinc.

Drugs for the treatment of OA are required to reach the site of action by diffusing into the cartilage matrix. However, a carboxyl function may lead to poor penetration through the negatively charged cartilage matrix because the carboxyl acid would be ionized at physiological pH.[53-55] Hence, efforts were focused on incorporation of non-carboxylate moieties or intra-articular therapy.[56]

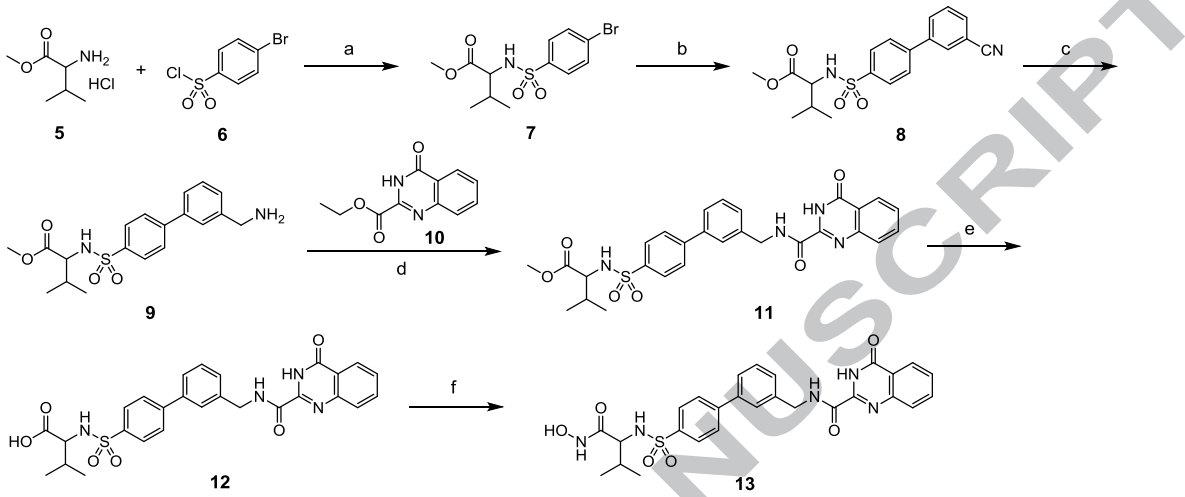
We now describe the design, synthesis, in vitro MMP inhibitory activity, and oral pharmacokinetic parameters of the MMP-13 inhibitors possessing a non-carboxylate zinc-binding function attached to the pyrimidin-4-one-2-carboxamide core, which binds effectively to the primed regions of the catalytic active site of the enzyme.

2. Chemistry

The synthesis of the biphenyl sulfonamides **12** and **13** is shown in **Scheme 1**. Benzylamine **9** was prepared by sulfonylation of *H*-(DL)-Val-OMe (**5**) with 4-bromophenylsulfonyl chloride (**6**), followed by Suzuki coupling with (3-cyanophenyl)boronic acid and cyano group reduction. Substituted-benzylamine **9** was condensed with quinazoline-2-carboxylic acid ethyl ester **10** by heating in the absence of base to give amide **11**. The pyrimidine NH of the quinazoline **10** accelerates rapid aminolysis of the ester through intramolecular acid catalysis.[33, 34] This neighboring group effect explains the high reactivity of the ester

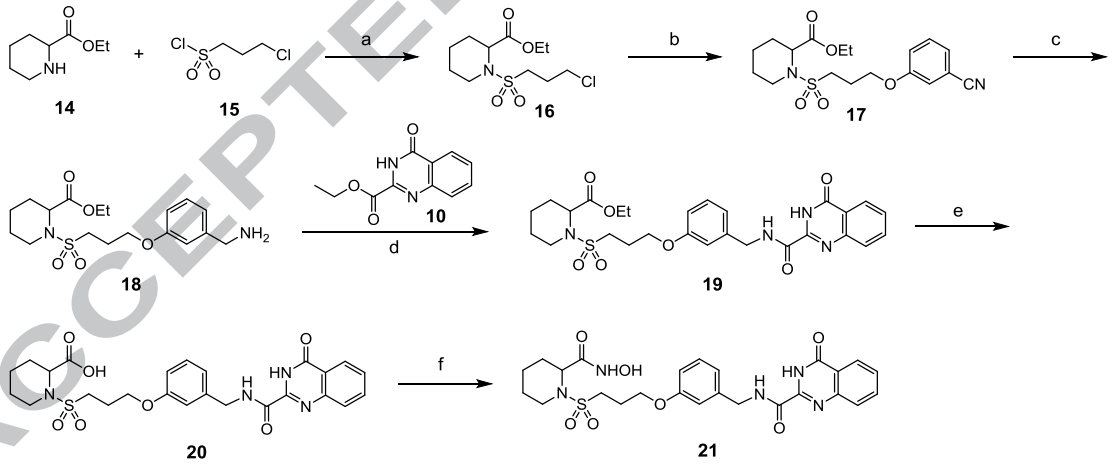
function. The fully functionalized biphenyl ester sulfonamide **11** was then hydrolyzed to the carboxylic acid **12**, and converted into the hydroxamic acid **13**.

Scheme 1. Synthesis of Biphenylsulfonamide Derivatives **12** and **13**[a]



[a] Reagents and conditions: (a) Na_2CO_3, acetone, H_2O, 0 °C–rt, 84%; (b) (3-cyanophenyl)boronic acid, $Pd(PPh_3)_4$, Na_2CO_3, EtOH, toluene, H_2O, reflux, 97%; (c) H_2, Raney Ni, NH_3, THF, MeOH, H_2O, rt, 76%; (d) **10**, EtOH, 90 °C, 86%; (e) NaOH, H_2O, THF, MeOH, 80 °C, 83%; (f) $TMSONH_2$, WSCD·HCl, HOBt, DMF, rt, 71%.

Scheme 2. Synthesis of 3-Phenoxypropane-1-sulfonamide Derivatives **20** and **21**[a]

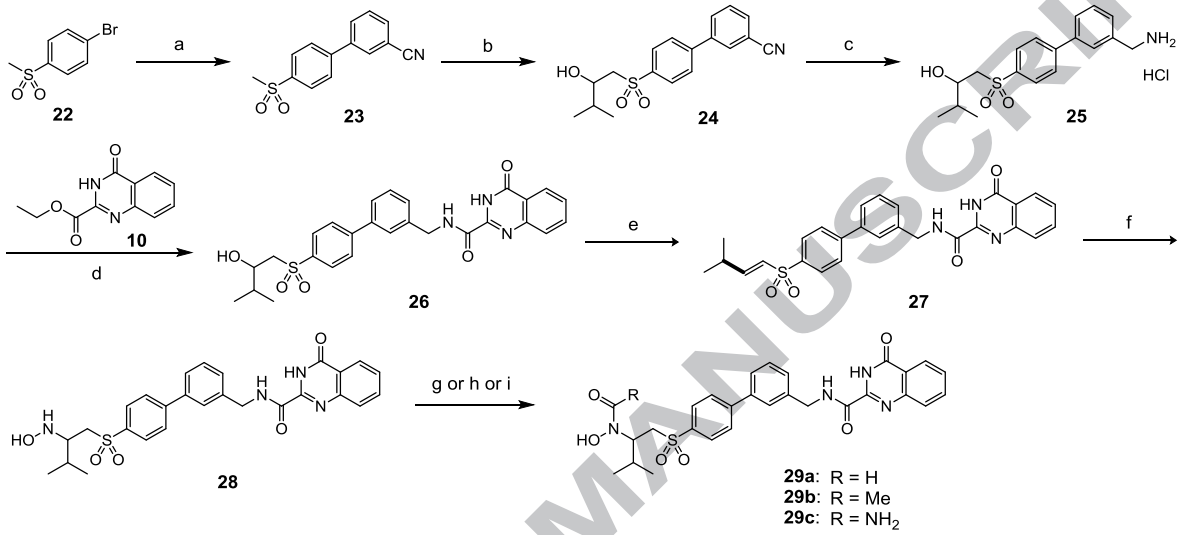


[a] Reagents and conditions: (a) TEA, THF, 0 °C–rt, 81%; (b) 3-hydroxybenzonitrile, K_2CO_3, NaI, DMF, 100 °C, 52%; (c) H_2, Raney Ni, NH_3, THF, MeOH, H_2O, rt, quant.; (d) **10**, TEA, EtOH, 80 °C, 59%; (e) NaOH, H_2O, THF, MeOH, 80 °C, 97%; (f) $TMSONH_2$, WSCD·HCl, HOBt, DMF, rt, 78%.

Synthetic pathway to 3-phenoxypropane-1-sulfonamides **20** and **21** is shown in **Scheme 2**. The 3-phenoxypropane-1-sulfonamides **20** and **21** were prepared from the commercially available ethyl pipecolate (**14**). Sulfonylation with 3-chloropropane-1-sulfonyl chloride (**15**) afforded sulfonamide **16**. Alkylation of 3-hydroxybenzonitrile with the chloride **16** yielded ether **17**, and the following nitrile reduction of **17** gave benzylamine **18**. Condensation of the resulting benzylamine **18** with

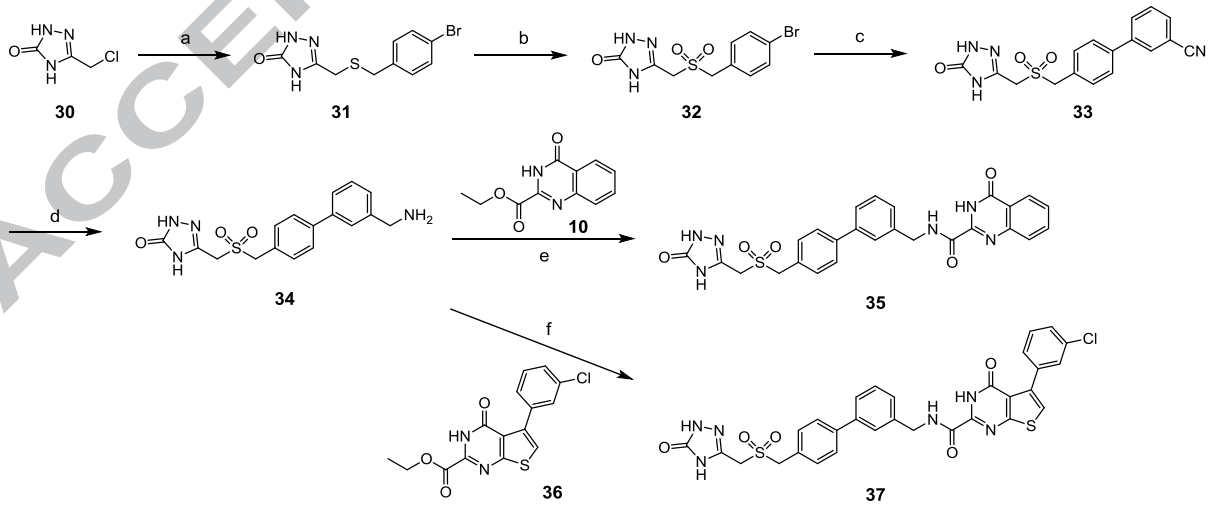
quinazoline-2-carboxylic acid ethyl ester **10**, accomplished in the same manner as **11** in **Scheme 1**, and basic hydrolysis of the ethyl ester **19** afforded the carboxylic acid **20**, which was then subsequently converted into the desired hydroxamic acid **21** by condensation with *O*-(trimethylsilyl)hydroxylamine as described in **Scheme 2**.

Scheme 3. Synthesis of Reverse Hydroxamate **29a** and Its Analogues **29b**–**c**[a]



[a] Reagents and conditions: (a) (3-cyanophenyl)boronic acid, $Pd(PPh_3)_4$, Na_2CO_3, EtOH, toluene, H_2O, reflux, 95%; (b) (1) *n*-BuLi, THF, –78 °C; (2) isobutyraldehyde, –78 °C, 65%; (c) (1) H_2, Raney Ni, NH_3, THF, MeOH, H_2O, rt; (2) HCl, AcOEt, MeOH, Et_2O, rt, 80%; (d) **10**, TEA, EtOH, 80 °C, 78%; (e) MsCl, TEA, THF, 0 °C–rt, 76%; (f) $TMSONH_2$, THF, rt, 65%; (g) HCO_2H, Ac_2O, THF, rt, 37%; (h) Ac_2O, AcOH, THF, rt, 79%; (i) (1) triphosgene, DIEA, THF, 0 °C–rt; (2) NH_3, H_2O, rt, 83%.

Scheme 4. Synthesis of Triazolone Derivatives **35** and **37**[a]



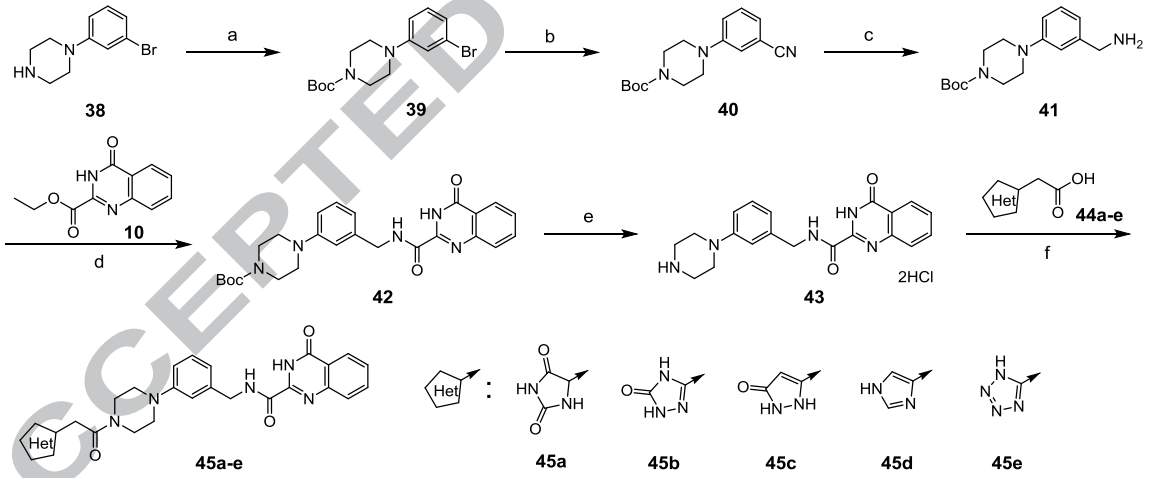
[a] Reagents and conditions: (a) (4-bromophenyl)methanethiol, K_2CO_3, DMF, rt, 88%; (b) *m*CPBA, DMF, rt, 92%; (c) (3-cyanophenyl)boronic acid, $Pd(PPh_3)_4$, Na_2CO_3, EtOH, toluene, H_2O, reflux, 60%; (d) H_2, Raney Ni, NH_3, THF, MeOH, H_2O, rt, 79%; (e) **10**, TEA, EtOH, DMA, 90 °C, 55%; (f) **36**[35], TEA, EtOH, DMA, 90 °C, 45%.

Reverse hydroxamates **29a–c** were prepared according to refs.[57, 58] as illustrated in **Scheme 3**. Biphenyl formation of **22** using Suzuki conditions, followed by treatment of a lithium anion of methyl sulfone **23** with isobutyraldehyde gave alcohol adduct **24**. Reduction of the cyano group of **24** by catalytic hydrogenation afforded aminomethyl derivative **25**, which yielded amide **26** on aminolysis of **10**. To prepare reverse hydroxamates **29a–c**, the hydroxy sulfone **26** was first converted to 1,2-unsaturated sulfone **27** by formation of mesylate of **26** followed by elimination. Treatment of the 1,2-unsaturated sulfone **27** with *O*-(trimethylsilyl)hydroxylamine in THF gave 1,4-Michael adduct **28**. Acylation or carbamoylation of **28** afforded *N*-formyl hydroxylamine **29a**, *N*-acetyl hydroxylamine **29b**, and *N*-carbamoyl hydroxylamine **29c**.

Conversion of chloromethyltriazolone **30**[59] to sulfide **31** followed by oxidation led to sulfone **32** as shown in **Scheme 4**. Triazolone derivatives **35** and **37** were prepared from the bromide **32** using the same synthetic sequence as described in **Schemes 1** and **3**.

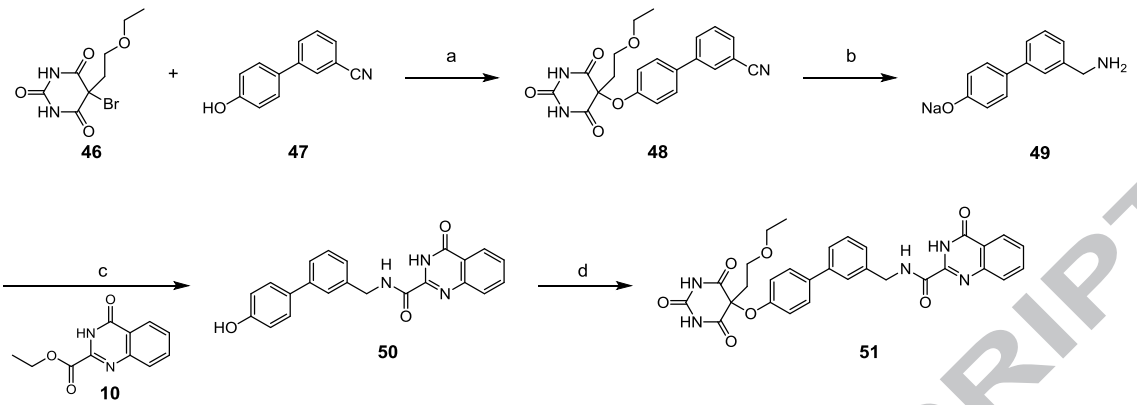
Installation of a variety of 5-membered heterocycles linked via a piperazine spacer (**45a-e**) was performed using divergent synthetic strategy whereby various potential ZBGs could be introduced on a common intermediate **43** at the last stage of the synthesis (**Scheme 5**). The common intermediate **43** was prepared from commercially available 1-(3-bromophenyl)piperazine (**38**) in five steps.

Scheme 5. Synthesis of Phenylpiperazine Derivatives **45a**–**e**[a]



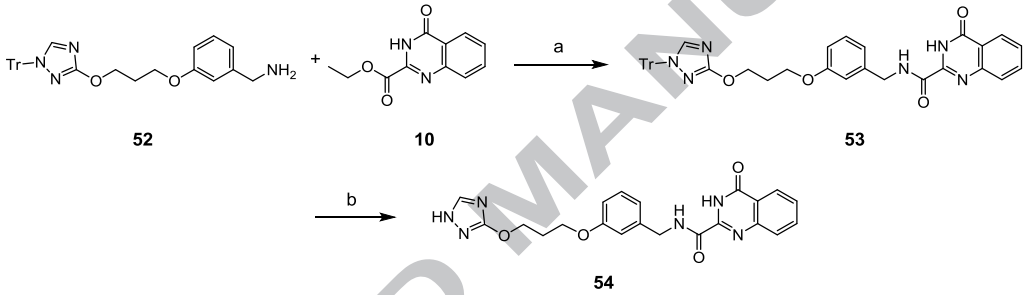
[a] Reagents and conditions: (a) Boc_2O, TEA, THF, rt, 64%; (b) $Zn(CN)_2$, $Pd(PPh_3)_4$, DMF, 80 °C, 80%; (c) H_2, Raney Ni, NH_3, THF, MeOH, H_2O, rt, 100%; (d) **10**, TEA, EtOH, 80 °C, 92%; (e) HCl, AcOEt, MeOH, THF, H_2O, rt–50 °C, 97%; (f) **44a**–**e**, WSCD·HCl, HOBt, TEA, DMF, rt, 32–79%.

Scheme 6. Synthesis of Pyrimidine-2,4,6-trione Derivative **51**[a]



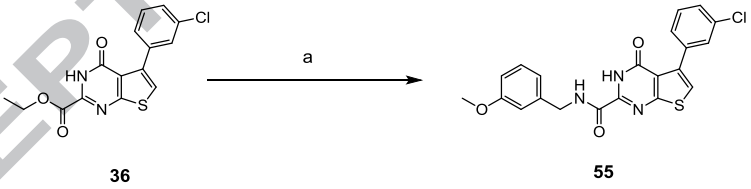
[a] Reagents and conditions: (a) K_2CO_3, DMF, rt, 69%; (b) (1) H_2, Raney Ni, NH_3, THF, MeOH, H_2O, rt; (2) NaOH, H_2O, 95%; (c) **10**, TEA, EtOH, 80 °C, 67%; (d) **46**, K_2CO_3, DMF, rt, 51%.

Scheme 7. Synthesis of 1,2,4-Triazole Derivative **54**[a]



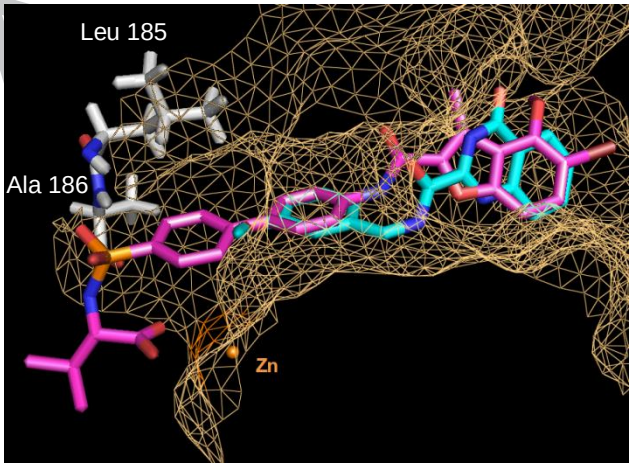
[a] Reagents and conditions: (a) DIEA, EtOH, microwave, 100 °C, 78%; (b) TFA, Et_3SiH, CH_3CN, rt, 96%.

Scheme 8. Synthesis of 3-Chlorophenyl Derivative **55**[a]



[a] Reagents and conditions: 3-methoxybenzylamine , EtOH, Et_3N, 80 °C, 62%.

(A) (B)



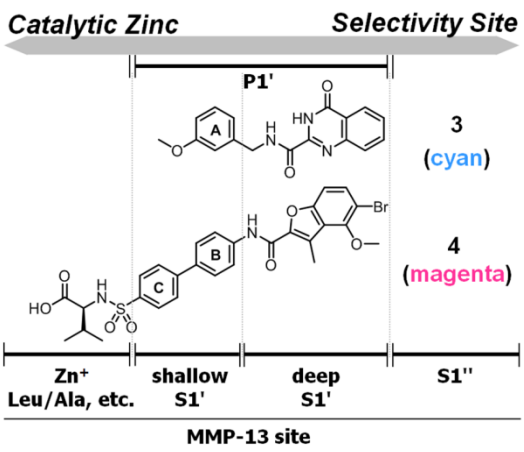


Figure 3. Superposition of the X-ray structures of MMP-13 selective inhibitors **3**[33-35] (cyan) and **4**[24, 27] (magenta). (A) Surface representation of MMP-13 illustrating the binding cavity. The figure was made with program PyMOL.[60] (B) Schematic representation of the binding site of MMP-13 and the inhibitors **3** and **4**.

An attempt to introduce a barbiturate functionality into the quinazoline-2-carboxamide via a biphenyl linker was performed using 3-(4-hydroxyphenyl)benzonitrile (**47**) as a starting material (**Scheme 6**). Alkylation of **47** with alkyl bromide **46** to ether **48** followed by reduction of the nitrile to primary amine with hydrogen gas in the presence of Raney nickel and ammonia proceeded with an unexpected elimination of the barbiturate functionality to afford 4-[3-(aminomethyl)phenyl]phenol (**49**) as a sodium salt. After condensation of **49** with **10**, the barbiturate functionality was reinstalled using **46** under basic condition to provide pyrimidine-2,4,6-trione derivative **51**.

As shown in **Scheme 7**, ester **10** was subjected to aminolysis by treatment with amine **52**[35] in EtOH to afford compound **53**, which was converted to 1,2,4-triazole **54** under acidic condition.

Reaction of ester **36**[35] and 3-methoxybenzylamine afforded 3-chlorophenyl derivative **55** (**Scheme 8**).

3. Results and Discussion

3-1. Drug Design Recently, we have described quinazoline-2-carboxamide potent inhibitors that occupy the S1' pocket of MMP-13 without interacting with the catalytic zinc and the residues near the zinc.[33] X-ray crystallography data have confirmed the unique binding mode that is characterized by forming a β-sheet type interaction with hydrogen bonding to the enzyme's backbone spanning the S1' pocket. In order to design potent MMP-13 inhibitors, we adopted a strategy of connecting ZBGs with our P1' quinazoline system by an appropriate linker (**Figure 2**). Molecule alignment study between our quinazoline-2-carboxamide **3** and biphenyl sulfonamide carboxylate MMP inhibitor **4**[24, 27] discovered at Wyeth was performed. For comparison of the ligands complexed in the binding sites of the crystal structures, the binding sites were superimposed using PyMOL. The structure diagrams of the inhibitors are shown in **Figure 3**. The biphenyl sulfonamide inhibitor **4** consists of an extra terminal valine moiety complexing the catalytic zinc and a benzofuran moiety extending deep into the S1' pocket. Two hydrogen bonds are formed between the inhibitor's sulfone oxygen and NH of Leu185 and Ala186. Alignment of compound **3** (cyan) with Wyeth's compound **4** (magenta) shows the proximity of the phenyl ring A of quinazoline-2-carboxamide **3** to the phenyl ring B of biphenyl sulfonamide inhibitor **4**. On the basis of the above observations, the substituent on the phenyl ring A of quinazoline-2-carboxamide **3** should be positioned in a meta-position to fill the hydrophobic S1' tunnel toward Leu185, Ala186, and the catalytic zinc. Hence, we newly designed quinazoline-2-carboxamide derivatives with various ZBGs via spacers (**Figure 4**). For a comprehensive schematic representation of the designed compounds to MMP-13, see **Figure 5**. Spacers were chosen that had the potential to form hydrogen bonds with the backbone amides of Leu185 and Ala186, as observed between these residues and the sulfone oxygen of the biphenyl sulfonamide carboxylate inhibitor **4** (**Figure 3**). Thus, ethers, amides, sulfones, and sulfonamides were selected as linker elements that contain at least one hydrogen bond acceptor atom. In addition, efforts were focused on inhibitors based on a variety of non-carboxylate ZBGs including heterocyclic ZBGs.

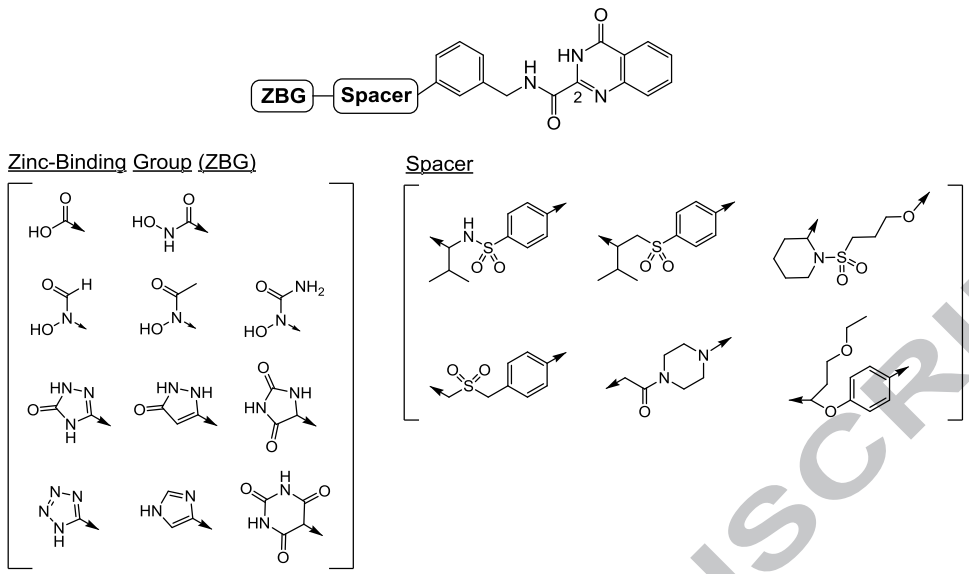


Figure 4. Quinazoline-2-carboxamide based zinc-binding MMP-13 inhibitors.

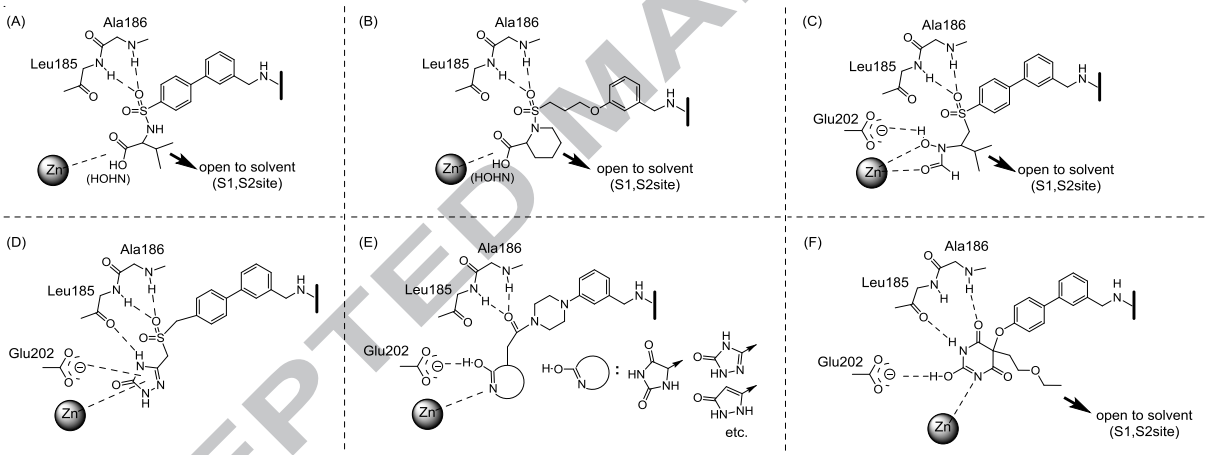


Figure 5. Schematic representation of the putative binding mode of the designed compounds to MMP-13. Hydrogen bonds are shown as dashed lines.

3-2. MMPs Activity IC_{50} values of quinazoline derivatives have been determined on ten recombinant MMP subtypes, namely MMP-1, MMP-2, MMP-3, MMP-7, MMP-8, MMP-9, MMP-10, MMP-12, MMP-13, MMP-14, and TACE. The IC_{50} values are displayed in **Table 1**. As expected, all compounds showed improved MMP-13 inhibitory activities in comparison with the lead compound **3**. Hydroxamic acid derivative **13** gave a > 1,000-fold increased potency in MMP-13 (IC_{50} = 3.2 pM) to **3**, but decreased selectivity over most of the MMPs showing at least nanomolar potency. Hydroxamate **21**, which has a methylene linker, showed a 100-fold increase in potency when compared to the lead **3** with subtype selectivity for MMP-1, 7, and 14, but the selectivity of this compound over MMP-2, 3, 8, 10, and 12 was relatively low.

Carboxylate series **12** and **20** showed improved potency compared to that of the lead **3** but had no improved MMP-13 inhibitory activity over the corresponding hydroxamic acid derivatives **13** and **21**,

respectively, resulting in sub-nanomolar inhibitory activity. However, in contrast to the biphenyl linker series **12**, the methylene linker series **20** showed greater MMP-13 selectivity than that of **12** against all enzymes tested.

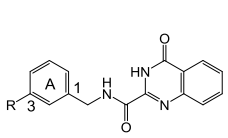
Additionally, substituents present on the nitrogen atom of reverse hydroxamates **29a-c** affected the potency to some extent. An *N*-formyl derivative **29a** was the most potent compound of the series, showing about 100-fold more potent inhibition of MMP-13 than that of the lead compound **3** accompanied by an increase of unintended activity toward other MMPs. The *N*-Acetyl derivative **29b** demonstrated a more attractive balance of potency and selectivity than that of the *N*-formyl derivative **29a** showing a somewhat (5- to 6-fold) less potent MMP-13 inhibitory activity than that of **29a**, and the selectivity against MMP-2, 8, and 10 was high (> 200-fold). Urea **29c** showed a lower degree of enzyme specificity toward subtypes of MMPs than that of *N*-acetyl derivative **29b**.

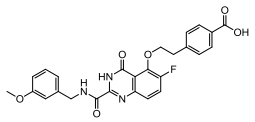
Incorporation of a triazolone ZBG, through a biphenylmethylsulfone linker group, to the pyrimidin-4-one-2-carboxamide core (**35**) resulted in about 100-fold improved inhibitory potency (IC_{50} = 71 pM) with substantial selectivity (> 170-fold) over all other enzymes tested.

On the contrary, incorporation of 5-membered heterocycles via a piperazine linker was not effective, giving relatively weak (IC_{50} > 100 pM) inhibitors **45a–e**. Introduction of a hydantoin (**45a**), a triazolone (**45b**), and a pyrazolone (**45c**), resulted in a 10-fold improved MMP-13 inhibitory activity in comparison to that of the other non-carbonyl analogues (imidazole **45d** and tetrazole **45e**). This result indicates that the carbonyl oxygen on the rings forms favorable hydrogen bonds with the MMP-13 backbone atoms close to the active site zinc or interacts with the zinc itself. Among the compounds **45a–c**, triazolone **45b** showed the highest potency but relatively low selectivity towards MMP-2, 3, 8, and 10 (MMP-8/MMP-13 ratio 35).

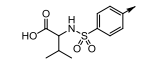
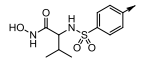
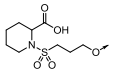
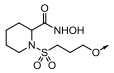
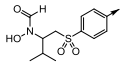
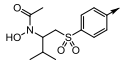
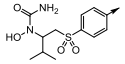
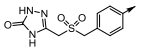
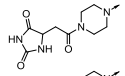
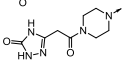
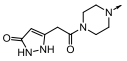
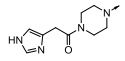
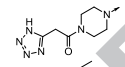
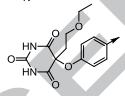
As shown in **Table 1**, an improvement in both potency and selectivity was observed for the biphenylmethylsulfone linker analogue **35**, which maintained the same triazolone ZBG, as compared to the piperazine linker analogue **45b**. This result indicates that an appropriate fine-tuning of the linker moiety would improve selectivity toward MMP-13.

Table 1. Inhibitory Activities Against MMPs of Quinazoline Derivatives: Effect of ZBG and Linker Attached to Phenyl Ring (A-Ring) at 3-Position





1

compound	R	IC$_{50}$ (nM) MMP-13[a]	MMP-1[b]	MMP-2	MMP-3[b]	MMP-7[b]	MMP-8	MMP-9[b]	MMP-10	MMP-12[b]	MMP-14[b]	TACE[b]
12		0.17 ± 0.026	3400	8.3[b]	30	1000	8.9[b]	36	2.4[b]	16	140	>10000
13		0.0032 ± 0.00026	11	1.9[b]	1.5	7.3	0.82[b]	4.7	0.30[b]	8.1	3.8	>10000
20		0.24 ± 0.02	>10000	14[b]	370	>10000	14[b]	>10000	240[b]	300	>10000	>10000
21		0.052[b]	>10000	0.57[b]	2.6	2000	1.2[b]	72	2.6[b]	1.8	550	510
29a		0.051 ± 0.013	18	3.0[b]	2.5	30	1.3[b]	5.3	0.35[b]	8.5	5.4	>10000
29b		0.30 ± 0.064	NT	65[c]	NT	NT	110[c]	NT	64[c]	NT	NT	NT
29c		0.26 ± 0.11	NT	<10[c]	NT	NT	19[c]	NT	<10[c]	NT	NT	NT
35		0.071 ± 0.013	>10000	23[b]	2400	>10000	12[b]	>10000	150[b]	9500	>10000	>10000
45a		0.19 ± 0.036	NT	<10[c]	NT	NT	<10[c]	NT	<10[c]	NT	NT	NT
45b		0.11 ± 0.02	>10000	10[b]	23	>10000	3.9[b]	2900	6.2[b]	260	8900	>10000
45c		0.36 ± 0.062	NT	<10[c]	NT	NT	<10[c]	NT	<10[c]	NT	NT	NT
45d		1.7 ± 0.17	NT	120[c]	NT	NT	45[c]	NT	460[c]	NT	NT	NT
45e		1.5 ± 0.067	NT	160[c]	NT	NT	32[c]	NT	640[c]	NT	NT	NT
51		0.23 ± 0.035	>10000	110[b]	3300	>10000	56[b]	1600	930[b]	540	3700	>10000
3		12 ± 1.5	>10000[c]	300[c]	>10000[c]	>10000[c]	1100[c]	>10000[c]	3400[c]	NT	>10000[c]	>10000[c]
1	—	0.0039 ± 0.0011	>10000[c]	5300[c]	4000[c]	>10000[c]	720[c]	>10000[c]	160[c]	NT	>10000[c]	>10000[c]

[a] IC$_{50}$ values were calculated as an average of three experiments and are represented as means ± standard deviations. [b] Results are means of two experiments. [c] IC$_{50}$ values were calculated as an average of three experiments.

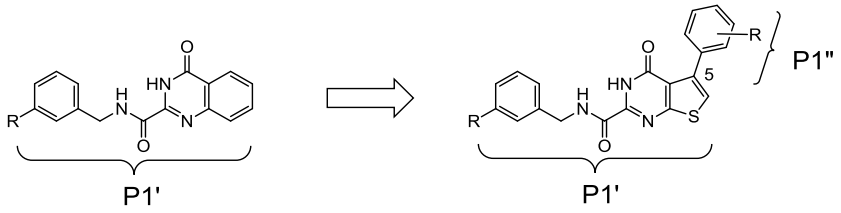


Figure 6. Introduction of P1" group: 5-aryl-thienopyrimidines from quinazolines.

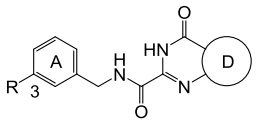
The MMP-13 inhibitory activity of the barbituric acid derivative **51** was not enough (IC$_{50}$ = 230 pM),

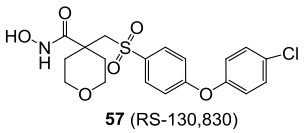
however, this compound showed at least 240-fold selectivity for MMP-13 over other MMPs.

Previously described non-zinc-binding MMP-13 inhibitors by our group such as compound **1** took advantage of both S1' and S1" site binding pockets to achieve potent inhibition and high selectivity.[33] The zinc-binding inhibitors with P1' in this report resulted in an increased MMP-13 potency but decreased selectivity over the other MMP subtypes. The use of strong chelators such as hydroxamates or reverse hydroxamates as ZBGs may preclude the development of selective MMP inhibitors. Accordingly, further attempts were made to improve both potency and selectivity by interacting with S1" side pocket, which is unique to MMP-13. In the course of the study of the MMP inhibitor without a ZBG aimed at the designing new P1" in our laboratory, we have found that introduction of a small aryl group into the 5-position of the thienopyrimidine-2-carboxamide structure resulted in the excellent potency/selectivity profile (**Figure 6**).[35] Thus, the 5-arylthienopyrimidine derivative **55** with a benzene ring directly attached to the thiophene ring not only enhanced the inhibitory activity but also improved the selectivity profile compared to the quinazoline **3** (**Table 2**, **3** vs **55**). Therefore, in a series of derivatives containing ZBGs, small aromatic rings were P1" substituents of choice for the inhibition of MMP-13 (**Table 2**, **37** and **56**). As a result, improvement of potency has been achieved by the combination of a simple linker with a triazole group as a ZBG (**Table 2**, **56**). [35] However, in contrast to the triazole ZBG series **56**, incorporation of the 5-arylthienopyrimidine-type P1" group into the triazolone **35** was not proved to be well tolerated (**Table 2**, **35** vs **37**). Probably, coupled with the size of the triazolone ZBG group, introduction of a rigid biphenyl P1' linker and a sulfonyl group resulted in a small shift of the pyrimidine-4-one-2-carboxamide core structure toward the primed side of the binding site, which would result in a significant loss in potency.

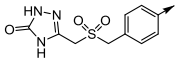
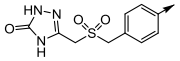
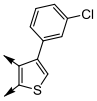
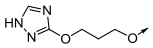
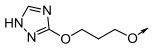
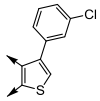
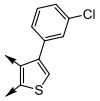
Further discussion on this issue, however, would best await X-ray crystal structures of the compounds **35** and **37** bound to MMP-13. On the other hand, it is presumed that a highly flexible alkylene linker of **54** and **56** that also have ZBG enabled 5-arylthienopyrimidine structure to interact effectively with the S1' and S1" pockets of MMP-13, which resulted in an improvement of potency and selectivity.

Table 2. Inhibitory Activities Against MMPs: Effect of Introduction of P1" Group





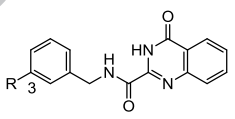
57 (RS-130,830)

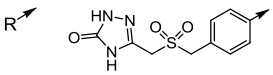
compound	R	D	IC_{50} (nM) MMP-13[a]	MMP-2[b]	MMP-8[b]	MMP-10[b]
35			0.071 ± 0.013	23[c]	12[c]	150[c]
37			0.38 ± 0.088	86	1000	34
54			0.59 ± 0.03	32	22	220
56			0.078 ± 0.015	510	>1000	820
3			5.6 ± 0.52	300	1100	3400
55			0.12 ± 0.017	2600	>10000	6300
57	RS-130,830[c]		0.010 ± 0.0016	0.029	0.097	0.54

[a] IC_{50} values were calculated as an average of three experiments and are represented as means ± standard deviations. [b] IC_{50} values were calculated as an average of three experiments. [c] Results are average of two experiments. [c] A sulfonehydroxamate MMP inhibitor RS-130,830[61] was used as a reference for comparison.

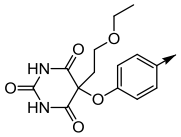
Table 3. DMPK/tox Profiles of Representative Compounds

compound	Solubility (μg/mL) pH6.8	PAMPA (nm/sec) pH7.4	Metabolic stability[a] (μL/min/mg) human	rat	Rat pharmacokinetic profiles[b] Vd (mL/kg)	CL (mL/h/kg)	*F* (%)	CYP3A4 inhibition (%inhibition at 10 μM)	Cytotoxicity[c] (% of control at 10μM)
35	0.48	7	7	13	320	323	1.3	29	98
51	9.1	95	12	-5	54	21	32	22	38
29b	0.93	452	250	250	< 1	< 1	< 1	99	29

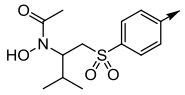




35



51



29b

[a] Human/rat liver microsomal clearance. [b] 0.1 mg/kg iv, 1 mg/kg po. [c] ATP was measured as an indicator of viable cells.

3-3. DMPK/tox Profile Table 3 shows DMPK/tox properties of the derivatives **35**, **51**, and **29b** with more than 100-fold selectivity against related MMPs. Despite possessing good properties (high stability in rat and human liver microsomes, low cytochrome P450 (CYP) 3A4 inhibition, and low toxicity by ATP measurement), an oral bioavailability of the triazolone compound **35** in rats was less than 5%, perhaps due

to low solubility and permeability (assessed by parallel artificial membrane permeability assay (PAMPA)) issues. Alternatively, the barbiturate derivative **51** demonstrated good PAMPA permeability, good stability against liver microsomes, and acceptable oral bioavailability ($F\% > 30$) in rats. However, **51** showed a very low volume of distribution, which may reflect high plasma protein binding. *N*-Acetylhydroxylamine derivative **29b** displayed poor in vitro metabolic stability and oral bioavailability in rat, and showed potent CYP3A4 inhibition and cytotoxic effect.

4. Conclusion

We have previously used a fused pyrimidine-2-carboxamide scaffold represented by compound **1** for an initial validation of two strategies to obtain potency and selectivity for MMP-13 by utilizing (i) the unique side pocket of S1' cavity, referred to as S1" pocket and (ii) interactions toward catalytic zinc through a linker.[35] With the goal of further expanding the chemical diversity, we next investigated the potential of ZBGs. In addition to accessing unique structure space, we expected that the inclusion of non-carboxylic acid-based ZBGs could impart useful biological properties such as improved pharmacokinetics or permeability into cartilage matrix. In conclusion, by applying a structure-based approach, we have discovered a new series of MMP-13 inhibitors represented by **35** with high potency against MMP-13 activity (IC_{50} = 0.071 nM) and high selectivity against other MMPs (> 170-fold over MMP-1, 2, 3, 7, 8, 9, 10, 12, 14, and TACE). The series is characterized by having a triazolone as the zinc binding moiety connected via a biphenyl spacer with a sulfonyl linkage to the P1' group, quinazoline-2-carboxamide. While analog **35** showed a slow rat liver microsomal turnover rate, its oral availability in rat PK studies was found to be much lower than expected. The discrepancy of these results may be due to solubility and permeability issues. By application of known SAR, P1" substituents could be placed on the quinazoline core with a view to improving the enzyme inhibition activity, selectivity, physicochemical, and PK properties of this class of compounds. In vivo animal efficacy will be the subject of a future publication.

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 Varian Gemini-200 (200 MHz), Varian Gemini-300 (300 MHz), or Bruker DPX-300 (300 MHz) spectrometer and are reported in parts per million (δ) relative to tetramethylsilane (TMS: δ 0.00 ppm). Data are reported as follows: chemical shift, integration, multiplicity (s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet, dd = doublet of doublet, ddd = doublet of doublet of doublet, bs = broad singlet), and coupling constants (*J*, Hz). Unless otherwise specified, all solvents and reagents were obtained from commercial suppliers and used without further purification. Column chromatography was performed using Merck silica gel 60 (70–230 mesh). Thin-layer chromatography (TLC) was performed on Merck silica gel plates $60F_{254}$. LC-MS analysis was performed on a Shiseido CAPCELL PACK C-18 UG120 S-3 column (1.5 mmϕ x 35 mm) in a Waters Alliance 2795 or an Agilent 1100 LC system equipped with a Waters 2487 absorbance detector and a Micromass ZQ2000 mass spectrometer. Analytes were eluted using a linear gradient of water (0.05% TFA)/acetonitrile (0.04%

TFA) from 90:10 to 0:100 over 4 min at a flow rate of 0.5 mL/min. UV detection was at 220 nm. Preparative HPLC was performed on a Shiseido CAPCELL PACK C-18 UG120 S-5 column (20 mmϕ x 50 mm), eluting at 25 mL/min with a gradient of water (0.1% TFA)/acetonitrile (0.1% TFA). UV detection was at 220 nm. Elemental analyses were performed by Takeda Analytical Research Laboratories, Ltd.

Methyl *N*-[(4-Bromophenyl)sulfonyl]valinate (**7**). To a mixture of methyl valinate hydrochloride (**5**, 10.0 g, 59.7 mmol) and Na_2CO_3 (15.8 g, 149 mmol) in a mixed solvent of acetone (200 mL) and H_2O (100 mL) was added 4-bromobenzenesulfonyl chloride (**6**, 12.7 g, 49.7 mmol) at 0 °C, and the mixture was stirred at room temperature for 12 h. The mixture was concentrated under reduced pressure, diluted with ethyl acetate, and successively washed with 0.1 N hydrochloric acid, H_2O and brine. The organic layer was dried over Na_2SO_4 and concentrated under reduced pressure. The residue was purified by crystallization from ethyl acetate–hexane to give the title compound as a white powder (14.7 g, 42.0 mmol, 84%). ^{1}H NMR (300 MHz, DMSO-d_6) δ 0.76–0.85 (6H, m), 1.84–1.98 (1H, m), 3.37 (3H, s), 3.57 (1H, d, J = 7.2 Hz), 7.63–7.71 (2H, m), 7.76–7.83 (2H, m), 8.39 (1H, s).

Methyl *N*-[(3'-Cyanobiphenyl-4-yl)sulfonyl]valinate (**8**). A mixture of compound **7** (5.00 g, 14.3 mmol), (3-cyanophenyl)boronic acid (2.52 g, 17.1 mmol), tetrakis(triphenylphosphine)palladium(0) (0.330 g, 0.286 mmol), 2 M aqueous Na_2CO_3 solution (8.57 mL, 17.1 mmol), ethanol (15 mL), and toluene (50 mL) was refluxed for 8 h. After completeness of reaction was checked by LC-MS, the reaction mixture was diluted with ethyl acetate and washed with H_2O and brine. The organic layer was dried over Na_2SO_4 and concentrated under reduced pressure. The residue was purified by crystallization from ethyl acetate–hexane to give the title compound as a yellow powder (5.18 g, 13.9 mmol, 97%). ^{1}H NMR (300 MHz, DMSO-d_6) δ 0.81 (3H, d, J = 6.8 Hz), 0.84 (3H, d, J = 6.8 Hz), 1.86–1.98 (1H, m), 3.34 (3H, s), 3.55–3.64 (1H, m), 7.72 (1H, t, J = 7.8 Hz), 7.81–7.86 (2H, m), 7.91 (1H, d, J = 7.6 Hz), 7.94–8.00 (2H, m), 8.10 (1H, d, J = 8.0 Hz), 8.27 (1H, s), 8.32–8.40 (1H, m).

Methyl *N*-{[3'-(Aminomethyl)biphenyl-4-yl]sulfonyl}valinate (**9**). A mixture of compound **8** (5.00 g, 13.4 mmol), Raney Ni (5.0 g), 28% aqueous NH_3 solution (50 mL), THF (50 mL), and methanol (50 mL) was stirred at room temperature for 15 h under hydrogen atmosphere (1 atm). The catalyst was removed by vacuum filtration through a PTFE membrane and the filtrate was concentrated under reduced pressure. The residue was diluted with a mixed solvent of ethyl acetate and hexane, and the insoluble materials were filtered off. The filtrate was washed with brine, and dried over Na_2SO_4. After removal of the solvent, the residue was triturated with diethyl ether to give the title compound as a white powder (3.84 g, 10.2 mmol, 76%). ^{1}H NMR (300 MHz, DMSO-d_6) δ 0.80 (3H, d, J = 6.8 Hz), 0.84 (3H, d, J = 6.8 Hz), 1.85–1.98 (1H, m), 3.34 (3H, s), 3.58 (1H, d, J = 7.2 Hz), 3.80 (2H, s), 7.35–7.48 (2H, m), 7.56 (1H, d, J = 7.2 Hz), 7.71 (1H, s), 7.78–7.84 (2H, m), 7.84–7.91 (2H, m).

Methyl *N*-{[3'-({[(4-Oxo-3,4-dihydroquinazolin-2-yl)carbonyl]amino}methyl)biphenyl-4-yl]sulfonyl}valinate (**11**). The mixture of compound **9** (0.300 g, 0.797 mmol) and ethyl 4-oxo-3,4-dihydroquinazoline-2-carboxylate[62] (**10**, 0.145 g, 0.664 mmol) in ethanol (9 mL) was stirred at 90 °C for 5 h. The mixture was concentrated under reduced pressure, diluted with ethyl acetate, washed with 0.1 N hydrochloric acid and brine, and dried over Na_2SO_4. The filtrate was concentrated under reduced pressure

to give the title compound as a colorless gum (0.375 g, 0.684 mmol, 86%). ^{1}H NMR (300 MHz, DMSO-d_6) δ 0.76–0.86 (6H, m), 1.84–1.97 (1H, m), 3.33 (3H, s), 3.57 (1H, d, J = 7.0 Hz), 4.58 (2H, d, J = 6.2 Hz), 7.38–7.52 (2H, m), 7.57–7.67 (2H, m), 7.71–7.94 (7H, m), 8.13–8.21 (1H, m), 8.32 (1H, s), 9.65 (1H, t, J = 6.2 Hz), 11.95 (1H, s).

***N*-{[3'-({[(4-Oxo-3,4-dihydroquinazolin-2-yl)carbonyl]amino}methyl)biphenyl-4-yl]sulfonyl}valine** (**12**). A mixture of compound **11** (250 mg, 0.456 mmol), 4 N aqueous sodium hydroxide solution (0.399 mL, 1.60 mmol), H_2O (3 mL), THF (3 mL) and methanol (3 mL) was stirred at 80 °C for 15 h. The mixture was concentrated under reduced pressure, diluted with H_2O, acidified with 1 N hydrochloric acid (2.4 mL), and partitioned between ethyl acetate and H_2O. The organic layer was washed with brine and dried over Na_2SO_4. After removal of the solvent, the residue was purified by crystallization from methanol to give the title compound as a white powder (202 mg, 0.456 mmol, 83%). mp 141–143 °C. ^{1}H NMR (300 MHz, DMSO-d_6) δ 0.75–0.88 (6H, m), 1.88–2.01 (1H, m), 3.54 (1H, dd, J = 8.6 Hz, 5.9 Hz), 4.57 (2H, d, J = 6.4 Hz), 7.40–7.53 (2H, m), 7.58–7.67 (2H, m), 7.72–7.94 (7H, m), 8.06 (1H, d, J = 8.9 Hz), 8.15–8.21 (1H, m), 9.65 (1H, t, J = 6.3 Hz), 12.27 (1H, s), 12.61 (1H, s). Anal. Calcd for $C_{27}H_{26}N_4O_6S$: C, 60.66; H, 4.90; N, 10.48. Found: C, 60.57; H, 5.11; N, 10.48.

***N*-({4'-[({1-[(Hydroxyamino)carbonyl]-2-methylpropyl}amino)sulfonyl]biphenyl-3-yl}methyl)-4-oxo-3,4-dihydroquinazoline-2-carboxamide** (**13**). The mixture of compound **12** (80 mg, 0.15 mmol), *O*-(trimethylsilyl)hydroxylamine (0.055 mL, 0.45 mmol), 1-[3-(dimethylamino)propyl]-3-ethylcarbodiimide hydrochloride (86 mg, 0.45 mmol), and 1-hydroxybenzotriazole (61 mg, 0.45 mmol) in DMF (5 mL) was stirred at room temperature for 12 h. The mixture was diluted with ethyl acetate, successively washed with 0.05 N hydrochloric acid, aqueous $NaHCO_3$ solution, H_2O and brine, and dried over Na_2SO_4. After removal of the solvent, the residue was purified by crystallization from methanol–diethyl ether to give the title compound as a white powder (58 mg, 0.11 mmol, 71%). mp 153–155 °C. ^{1}H NMR (300 MHz, DMSO-d_6) δ 0.75 (6H, dd, J = 6.1 Hz, 3.4 Hz), 1.70–1.84 (1H, m), 3.28–3.39 (1H, m), 4.57 (2H, d, J = 6.1 Hz), 7.37–7.52 (2H, m), 7.57–7.67 (2H, m), 7.71–7.93 (7H, m), 7.98 (1H, d, J = 9.1 Hz), 8.17 (1H, d, J = 8.0 Hz), 8.79 (1H, s), 9.63 (1H, t, J = 6.6 Hz), 10.52 (1H, s), 12.26 (1H, bs). Anal. Calcd for $C_{27}H_{27}N_5O_6S$: C, 59.00; H, 4.95; N, 12.74. Found: C, 58.72; H, 5.05; N, 12.56.

Ethyl 1-[(3-Chloropropyl)sulfonyl]piperidine-2-carboxylate (**16**). Ethyl piperidine-2-carboxylate (**14**, 4.19 g, 26.7 mmol) and triethylamine (3.72 mL, 26.7 mmol) were added to a mixture of 3-chloropropane-1-sulfonyl chloride (**15**, 4.72 g, 26.7 mmol) in THF (100 mL) at 0 °C, and the mixture was stirred for 12 h at room temperature. After the mixture was concentrated under reduced pressure, the residue was diluted with ethyl acetate, successively washed with H_2O, 0.1 N hydrochloric acid and brine, and dried over Na_2SO_4. The filtrate was concentrated under reduced pressure to give the title compound as a yellow oil (6.44 g, 21.6 mmol, 81%). ^{1}H NMR (300 MHz, DMSO-d_6) δ 1.13–1.27 (1H, m), 1.22 (3H, t, J = 7.0 Hz), 1.33–1.50 (1H, m), 1.59–1.72 (3H, m), 2.05–2.17 (3H, m), 3.06–3.27 (3H, m), 3.54–3.63 (1H, m), 3.70–3.77 (2H, m), 4.12–4.22 (2H, m), 4.52–4.59 (1H, m).

Ethyl 1-({3-[(3-Cyanophenyl)oxy]propyl}sulfonyl)piperidine-2-carboxylate (**17**). A mixture of compound **16** (2.00 g, 6.72 mmol), 3-hydroxybenzonitrile (800 mg, 6.72 mmol), K_2CO_3 (928 mg, 6.72 mmol), and sodium iodide (101 mg, 0.672 mmol) in DMF (20 mL) was stirred at 100 °C for 15 h. The

mixture was diluted with ethyl acetate, successively washed with H_2O, 0.5 N aqueous sodium hydroxide solution and brine, and dried over Na_2SO_4. After removal of the solvent, the residue was triturated with methanol to give the title compound as a pale yellow powder (1.34 g, 3.52 mmol, 52%). ^{1}H NMR (300 MHz, DMSO-d_6) δ 1.13–1.27 (1H, m), 1.21 (3H, t, J = 7.2 Hz), 1.30–1.47 (1H, m), 1.59–1.73 (3H, m), 2.04–2.16 (3H, m), 3.08–3.20 (1H, m), 3.21–3.31 (2H, m), 3.56–3.66 (1H, m), 4.10–4.21 (4H, m), 4.53–4.59 (1H, m), 7.27–7.33 (1H, m), 7.38–7.46 (2H, m), 7.50 (1H, t, J = 8.0 Hz).

Ethyl 1-[(3-{[3-(Aminomethyl)phenyl]oxy}propyl)sulfonyl]piperidine-2-carboxylate (**18**). A mixture of compound **17** (1.20 g, 3.15 mmol), Raney Ni (1.2 g), 28% aqueous NH_3 solution (12 mL), THF (24 mL), and methanol (12 mL) was stirred at room temperature for 12 h under hydrogen atmosphere (1 atm). The catalyst was removed by vacuum filtration through a PTFE membrane, and the filtrate was concentrated under reduced pressure to give the title compound as a green oil (1.21 g, 3.15 mmol, quant.). ^{1}H NMR (300 MHz, DMSO-d_6) δ 1.13–1.28 (4H, m), 1.31–1.48 (1H, m), 1.59–1.75 (3H, m), 2.03–2.15 (3H, m), 3.07–3.38 (5H, m), 3.61 (1H, d, J = 13.0 Hz), 3.98–4.09 (2H, m), 4.11–4.22 (2H, m), 4.51–4.63 (1H, m), 6.69–6.79 (1H, m), 6.80–6.96 (2H, m), 7.22 (1H, bs).

Ethyl 1-[(3-{[3-({[(4-Oxo-3,4-dihydroquinazolin-2-yl)carbonyl]amino}methyl)phenyl]oxy}propyl)-sulfonyl]piperidine-2-carboxylate (**19**). A mixture of compound **18** (600 mg, 1.56 mmol), compound **10**[62] (310 mg, 1.42 mmol), and triethylamine (0.297 mL, 2.13 mmol) in ethanol (20 mL) was stirred at 80 °C for 12 h. After the mixture was allowed to cool to room temperature, 2 N ethanolic HCl solution (1.5 mL) was added to the mixture. The precipitated solid was collected and washed with ethanol to give the title compound as a white powder (464 mg, 0.834 mmol, 59%). mp 84–86 °C. ^{1}H NMR (300 MHz, DMSO-d_6) δ 1.08–1.25 (1H, m), 1.19 (3H, t, J = 7.2 Hz), 1.27–1.45 (1H, m), 1.55–1.71 (3H, m), 2.00–2.15 (3H, m), 3.05–3.18 (1H, m), 3.19–3.29 (2H, m), 3.54–3.63 (1H, m), 4.05 (2H, t, J = 6.2 Hz), 4.09–4.18 (2H, m), 4.45 (2H, d, J = 6.4 Hz), 4.51–4.60 (1H, m), 6.83 (1H, d, J = 8.3 Hz), 6.90–6.97 (2H, m), 7.25 (1H, t, J = 8.0 Hz), 7.61 (1H, t, J = 7.6 Hz), 7.74–7.82 (1H, m), 7.84–7.93 (1H, m), 8.17 (1H, d, J = 8.0 Hz), 9.54 (1H, t, J = 6.2 Hz), 12.25 (1H, bs). Anal. Calcd for $C_{27}H_{32}N_4O_7S$: C, 58.26; H, 5.79; N, 10.07. Found: C, 57.97; H, 5.63; N, 10.04.

1-[(3-{[3-({[(4-Oxo-3,4-dihydroquinazolin-2-yl)carbonyl]amino}methyl)phenyl]oxy}propyl)sulfonyl]piperidine-2-carboxylic Acid (**20**). A mixture of compound **19** (360 mg, 0.647 mmol), 4 N aqueous sodium hydroxide solution (0.566 mL, 2.26 mmol), H_2O (4 mL), THF (4 mL), and methanol (4 mL) was stirred at 80 °C for 4 h. The mixture was diluted with H_2O, acidified with 1 N hydrochloric acid (3 mL), and partitioned between ethyl acetate and H_2O. The organic layer was washed with brine, and dried over Na_2SO_4. After removal of the solvent, the residue was purified by crystallization from ethyl acetate–diethyl ether to give the title compound as a white powder (332 mg, 0.628 mmol, 97%). mp 204–206 °C. ^{1}H NMR (300 MHz, DMSO-d_6) δ 1.06–1.45 (2H, m), 1.54–1.70 (3H, m), 2.02–2.17 (3H, m), 3.08–3.42 (3H, m), 3.49–3.63 (1H, m), 3.97–4.11 (2H, m), 4.36–4.51 (3H, m), 6.83 (1H, d, J = 8.0 Hz), 6.87–6.97 (2H, m), 7.25 (1H, t, J = 8.1 Hz), 7.62 (1H, t, J = 7.6 Hz), 7.74–7.83 (1H, m), 7.89 (1H, t, J = 7.6 Hz), 8.18 (1H, d, J = 8.0 Hz), 9.55 (1H, t, J = 5.7 Hz), 12.28 (1H, bs), 13.04 (1H, bs). Anal. Calcd for $C_{25}H_{28}N_4O_7S$: C, 56.81; H, 5.34; N, 10.60. Found: C, 56.82; H, 5.58; N, 10.33.

***N*-[(3-{[3-({2-[(Hydroxyamino)carbonyl]piperidin-1-yl}sulfonyl)propyl]oxy}phenyl)methyl]-4-oxo-**

3,4-dihydroquinazoline-2-carboxamide (**21**). The mixture of compound **20** (150 mg, 0.284 mmol), *O*-(trimethylsilyl)hydroxylamine (0.104 mL, 0.851 mmol), 1-[3-(dimethylamino)propyl]-3-ethylcarbodiimide hydrochloride (163 mg, 0.851 mmol), and 1-hydroxybenzotriazole (115 mg, 0.851 mmol) in DMF (5 mL) was stirred at room temperature for 12 h. The mixture was diluted with ethyl acetate, successively washed with 0.05 N hydrochloric acid, H_2O and brine, and dried over Na_2SO_4. After removal of the solvent, the residue was purified by crystallization from methanol–ethyl acetate–diethyl ether–hexane to give the title compound as a white powder (120 mg, 0.221 mmol, 78%). mp 106–108 °C. ^{1}H NMR (300 MHz, DMSO-d_6) δ 1.29–1.48 (2H, m), 1.53–1.72 (3H, m), 1.85–1.94 (1H, m), 2.04–2.17 (2H, m), 3.07–3.59 (4H, m), 4.00–4.08 (2H, m), 4.27 (1H, d), 4.45 (2H, d, *J* = 6.4 Hz), 6.79–6.86 (1H, m), 6.90–6.97 (2H, m), 7.25 (1H, t, *J* = 8.1 Hz), 7.61 (1H, t, *J* = 7.6 Hz), 7.75–7.81 (1H, m), 7.84–7.92 (1H, m), 8.17 (1H, d, *J* = 8.0 Hz), 8.91 (1H, bs), 9.54 (1H, t, *J* = 6.4 Hz), 10.66 (1H, bs), 12.22 (1H, bs). Anal. Calcd for $C_{25}H_{29}N_5O_7S\cdot 0.3H_2O$: C, 54.69; H, 5.43; N, 12.76. Found: C, 54.98; H, 5.44; N, 12.47.

4'-(Methylsulfonyl)biphenyl-3-carbonitrile (**23**). A mixture of 1-bromo-4-(methylsulfonyl)benzene (**22**, 10.0 g, 42.5 mmol), (3-cyanophenyl)boronic acid (7.50 g, 51.0 mmol), tetrakis(triphenylphosphine)-palladium(0) (983 mg, 0.851 mmol), 2 M aqueous Na_2CO_3 solution (25.5 mL, 51.0 mmol), ethanol (30 mL), and toluene (100 mL) was refluxed for 8 h. The reaction mixture was diluted with ethyl acetate and washed with H_2O and brine. The organic layer was dried over Na_2SO_4 and concentrated under reduced pressure. The residue was triturated with diisopropyl ether to give the title compound as a yellow powder (10.4 g, 40.4 mmol, 95%). ^{1}H NMR (300 MHz, DMSO-d_6) δ 3.28 (3H, s), 7.73 (1H, t, *J* = 8.0 Hz), 7.89–7.95 (1H, m), 7.99–8.06 (4H, m), 8.09–8.15 (1H, m), 8.29 (1H, t).

4'-[(2-Hydroxy-3-methylbutyl)sulfonyl]biphenyl-3-carbonitrile (**24**). To a solution of compound **23** (2.00 g, 7.77 mmol.) in THF (40 mL) was added dropwise *n*-butyl lithium (1.6 M in hexane, 5.34 mL, 8.55 mmol) at –78 °C. The mixture was stirred at –78 °C for 1 h followed by the addition of isobutyraldehyde (1.06 mL, 11.7 mmol). After the resulting mixture was stirred at –78 °C for further 3 h, the reaction was quenched with saturated aqueous NH_4Cl solution. The mixture was extracted with ethyl acetate, washed with brine, and dried over Na_2SO_4. After removal of the solvent, the residue was purified by silica gel column chromatography (25–50% ethyl acetate/hexane) to give the title compound as a pale yellow oil (1.66 g, 5.04 mmol, 65%). ^{1}H NMR (300 MHz, DMSO-d_6) δ 0.78 (3H, d, *J* = 6.8 Hz), 0.82 (3H, d, *J* = 6.8 Hz), 1.57–1.70 (1H, m), 3.39 (2H, d, *J* = 6.1 Hz), 3.74–3.82 (1H, m), 4.84 (1H, d, *J* = 5.7 Hz), 7.73 (1H, t, *J* = 8.0 Hz), 7.90–7.95 (1H, m), 7.98–8.05 (4H, m), 8.10–8.15 (1H, m), 8.29 (1H, t, *J* = 1.5 Hz).

1-{[3'-(Aminomethyl)biphenyl-4-yl]sulfonyl}-3-methylbutan-2-ol Hydrochloride (**25**). A mixture of compound **24** (1.60 g, 4.86 mmol), Raney Ni (1.6 g), 28% aqueous NH_3 solution (16 mL), THF (32 mL), and methanol (16 mL) was stirred at room temperature for 12 h under hydrogen atmosphere (1 atm). After the catalyst was removed by vacuum filtration through a PTFE membrane, the filtrate was concentrated under reduced pressure and the residual oil was dissolved in a mixed solvent of ethyl acetate, diethyl ether, and methanol. To the solution was added dropwise 4 N HCl in ethyl acetate (1.82 mL) at room temperature. The precipitated solid was collected and washed with diethyl ether to give the title compound as a white powder (1.44 g, 3.89 mmol, 80%). ^{1}H NMR (300 MHz, DMSO-d_6) δ 0.77 (3H, d, *J* = 6.8 Hz), 0.81 (3H, d, *J* = 6.8 Hz), 1.58–1.71 (1H, m), 3.35–3.40 (2H, m), 3.73–3.82 (1H, m), 4.12 (2H, bs), 4.84 (1H, d, *J* = 5.7

Hz), 7.53–7.60 (2H, m), 7.76–7.81 (1H, m), 7.92–8.04 (5H, m), 8.47 (3H, bs).

***N*-({4'-[(2-Hydroxy-3-methylbutyl)sulfonyl]biphenyl-3-yl}methyl)-4-oxo-3,4-dihydroquinazoline-2-carboxamide** (**26**). A mixture of compound **25** (800 mg, 2.16 mmol), compound **10**[62] (429 mg, 1.97 mmol) and triethylamine (0.411 mL, 2.95 mmol) in ethanol (21 mL) was stirred at 80 °C for 12 h. After the mixture was allowed to cool to room temperature, 2 N ethanolic HCl solution (2.2 mL) was added to the mixture. The precipitated solid was collected and washed with ethanol to give the title compound as a white powder (774 mg, 1.53 mmol, 78%). mp 169–171 °C. ^{1}H NMR (300 MHz, DMSO-d_6) δ 0.76 (3H, d, J = 6.8 Hz), 0.81 (3H, d, J = 6.8 Hz), 1.56–1.69 (1H, m), 3.34–3.38 (2H, m), 3.72–3.81 (1H, m), 4.58 (2H, d, J = 6.4 Hz), 4.81 (1H, d, J = 5.8 Hz), 7.42–7.53 (2H, m), 7.57–7.68 (2H, m), 7.74–7.81 (2H, m), 7.85–7.92 (3H, m), 7.95–8.01 (2H, m), 8.17 (1H, dd, J = 7.9, 1.3 Hz), 9.65 (1H, t, J = 6.4 Hz), 12.03 (1H, bs). Anal. Calcd for $C_{27}H_{27}N_3O_5S$: C, 64.14; H, 5.38; N, 8.31. Found: C, 63.99; H, 5.36; N, 8.22.

***N*-[(4'-{[(1*E*)-3-Methylbut-1-en-1-yl]sulfonyl}biphenyl-3-yl)methyl]-4-oxo-3,4-dihydroquinazoline-2-carboxamide** (**27**). To a mixture of compound **26** (300 mg, 0.593 mmol) and triethylamine (0.579 mL, 4.15 mmol) in THF (6 mL) was added methanesulfonyl chloride (0.083 mL, 1.1 mmol) at 0 °C, and the mixture was stirred at room temperature for 12 h. The reaction mixture was diluted with ethyl acetate, washed with 0.1 N hydrochloric acid and brine, and dried over Na_2SO_4. After removal of the solvent, the residue was purified by crystallization from ethyl acetate–diethyl ether to give the title compound as a pale yellow powder (220 mg, 0.593 mmol, 76%). mp 213–214 °C. ^{1}H NMR (300 MHz, DMSO-d_6) δ 1.02 (6H, d, J = 6.8 Hz), 2.44–2.59 (1H, m), 4.58 (2H, d, J = 6.1 Hz), 6.71–6.80 (1H, m), 6.86–6.96 (1H, m), 7.37–7.54 (2H, m), 7.55–7.68 (2H, m), 7.70–7.83 (2H, m), 7.82–7.98 (5H, m), 8.17 (1H, d, J = 7.6 Hz), 9.65 (1H, t, J = 5.9 Hz), 12.29 (1H, bs). Anal. Calcd for $C_{27}H_{25}N_3O_4S \cdot 0.2H_2O$: C, 66.02; H, 5.21; N, 8.50. Found: C, 65.85; H, 5.21; N, 8.44.

***N*-[(4'-{[2-(Hydroxyamino)-3-methylbutyl]sulfonyl}biphenyl-3-yl)methyl]-4-oxo-3,4-dihydroquinazoline-2-carboxamide** (**28**). To a solution of compound **27** (180 mg, 0.369 mmol) in THF (4 mL) was added *O*-(trimethylsilyl)hydroxylamine (0.271 mL, 2.22 mmol) at room temperature, and the mixture was stirred at room temperature for 12 h. The mixture was concentrated under reduced pressure and crystallized from ethyl acetate–diethyl ether to give the title compound as a white powder (125 mg, 0.240 mmol, 65%). mp 197–199 °C. ^{1}H NMR (300 MHz, DMSO-d_6) δ 0.76 (3H, d, J = 6.8 Hz), 0.80 (3H, d, J = 6.8 Hz), 1.87–2.03 (1H, m), 2.86–2.96 (1H, m), 3.25–3.43 (2H, m), 4.58 (2H, d, J = 6.2 Hz), 5.52 (1H, bs), 7.31 (1H, s), 7.41–7.55 (2H, m), 7.57–7.70 (2H, m), 7.74–7.83 (2H, m), 7.84–8.05 (5H, m), 8.14–8.21 (1H, m), 9.66 (1H, t, J = 6.4 Hz), 12.26 (1H, bs). Anal. Calcd for $C_{27}H_{28}N_4O_5S$: C, 62.29; H, 5.42; N, 10.76. Found: C, 62.04; H, 5.50; N, 10.49.

***N*-{[4'-({2-[Formyl(hydroxy)amino]-3-methylbutyl}sulfonyl)biphenyl-3-yl]methyl}-4-oxo-3,4-dihydroquinazoline-2-carboxamide** (**29a**). Acetic anhydride (0.054 mL, 0.58 mmol) was added to formic acid (0.217 mL) at room temperature and the mixture was stirred at room temperature for 2 h. The above solution was added to a mixture of compound **28** (200 mg, 0.384 mmol) and formic acid (2 mL) in THF (20 mL) at room temperature and the mixture was stirred at room temperature for 4 h. After the mixture was concentrated under reduced pressure, the residue was purified by preparative HPLC and crystallization from ethanol–ethyl acetate–diethyl ether to give the title compound as a pale yellow powder (77.0 mg,

0.140 mmol, 37%). mp 153–155 °C. ^{1}H NMR (300 MHz, DMSO-d_6) δ 0.72–0.84 (6H, m), 1.68–1.86 (1H, m), 3.46–3.57 (1H, m), 3.60–3.77 (1.6H, m), 4.24 (0.4H, t, J = 8.2 Hz), 4.59 (2H, d, J = 6.2 Hz), 7.42–7.55 (2H, m), 7.58–7.71 (2H, m), 7.74–7.83 (2H, m), 7.85–8.04 (5.6H, m), 8.12–8.22 (1.4H, m), 9.55 (0.6H, s), 9.66 (1H, t, J = 6.2 Hz), 9.89 (0.4H, s), 12.28 (1H, bs). Anal. Calcd for $C_{28}H_{28}N_4O_6S\cdot1.3H_2O$: C, 58.79; H, 5.39; N, 9.79. Found: C, 58.58; H, 5.10; N 9.64.

***N*-{[4'-({2-[Acetyl(hydroxy)amino]-3-methylbutyl}sulfonyl)biphenyl-3-yl]methyl}-4-oxo-3,4-dihydroquinazoline-2-carboxamide** (**29b**). Acetic anhydride (0.018 mL, 0.19 mmol) was added to a mixture of compound **28** (100 mg, 0.192 mmol) and acetic acid (2 mL) in THF (20 mL) at room temperature and the mixture was stirred at room temperature for 2 h. After the mixture was concentrated under reduced pressure, the residue was purified by crystallization from ethanol–ethyl acetate–diethyl ether–hexane to give the title compound as a white powder (85.0 mg, 0.152 mmol, 79%). mp 167–169 °C. ^{1}H NMR (300 MHz, DMSO-d_6) δ 0.78 (3H, d, J = 6.8 Hz), 0.84 (3H, d, J = 6.8 Hz), 1.87–2.00 (4H, m), 3.14–3.24 (1H, m), 3.31–3.42 (1H, m), 3.49–3.59 (1H, m), 4.58 (2H, d, J = 6.4 Hz), 7.42–7.54 (2H, m), 7.57–7.70 (2H, m), 7.74–7.82 (2H, m), 7.83–8.05 (6H, m), 8.18 (1H, d, J = 6.8 Hz), 9.65 (1H, t, J = 5.9 Hz), 12.27 (1H, bs). Anal. Calcd for $C_{29}H_{30}N_4O_6S$: C, 61.91; H, 5.37; N, 9.96. Found: C, 61.68; H, 5.29; N, 9.94.

***N*-{[4'-({2-[(Aminocarbonyl)(hydroxy)amino]-3-methylbutyl}sulfonyl)biphenyl-3-yl]methyl}-4-oxo-3,4-dihydroquinazoline-2-carboxamide** (**29c**). A suspension of compound **28** (50 mg, 0.096 mmol) and *N*,*N*-diethylisopropylamine (0.017 mL, 0.096 mmol) in THF (10 mL) was added to a mixture of triphosgene (9.4 mg, 0.032 mmol) in THF (2 mL) at 0 °C. After the mixture was stirred at room temperature for 2 h, 28% aqueous NH_3 solution (0.065 mL, 0.96 mmol) was added at room temperature and the resulting mixture was stirred at room temperature for 2 h. The mixture was concentrated under reduced pressure and the residue was triturated with ethanol–H_2O to give the title compound as a white powder (44.7 mg, 0.0793 mmol, 83%). mp 193–195 °C. ^{1}H NMR (300 MHz, DMSO-d_6) δ 0.69–0.80 (6H, m), 1.66–1.81 (1H, m), 3.29–3.39 (1H, m), 3.62 (1H, dd, J = 15.3, 8.3 Hz), 4.26–4.36 (1H, m), 4.58 (2H, d, J = 6.2 Hz), 6.10 (2H, bs), 7.40–7.54 (2H, m), 7.57–7.71 (2H, m), 7.73–7.82 (2H, m), 7.85–8.04 (5H, m), 8.18 (1H, d, J = 8.3 Hz), 9.07 (1H, s), 9.65 (1H, bs), 12.28 (1H, bs). Anal. Calcd for $C_{28}H_{29}N_5O_6S\cdot0.2H_2O$: C, 59.29; H, 5.22; N, 12.35. Found: C, 59.50; H, 5.10; N, 12.08.

5-({[(4-Bromophenyl)methyl]thio}methyl)-2,4-dihydro-3*H*-1,2,4-triazol-3-one (**31**). A mixture of 5-(chloromethyl)-2,4-dihydro-3*H*-1,2,4-triazol-3-one (**30**, 2.00 g, 15.0 mmol, synthesized by the method of Cowden et al.[59]), (4-bromophenyl)methanethiol (3.04 g, 15.0 mmol), and K_2CO_3 (2.48 g, 18.0 mmol) in DMF (40 mL) was stirred at room temperature for 12 h. After H_2O was added to the reaction mixture, the precipitated solid was collected and washed with H_2O and diethyl ether to give the title compound as a white powder (3.97 g, 13.2 mmol, 88%). ^{1}H NMR (300 MHz, DMSO-d_6) δ 3.36 (2H, s), 3.70 (2H, s), 7.28 (2H, d, J = 8.3 Hz), 7.52 (2H, d, J = 8.3 Hz), 11.29 (1H, bs), 11.39 (1H, bs).

5-({[(4-Bromophenyl)methyl]sulfonyl}methyl)-2,4-dihydro-3*H*-1,2,4-triazol-3-one (**32**). To a solution of compound **31** (500 mg, 1.67 mmol) in DMF (5 mL) was added 3-chloroperoxybenzoic acid (903 mg, 3.67 mmol) at 0 °C, and the mixture was stirred at room temperature for 12 h. After aqueous $NaHCO_3$ solution was added to the mixture, the precipitated solid was collected and washed with H_2O and diethyl ether to give the title compound as a white powder (508 mg, 1.53 mmol, 92%). ^{1}H NMR (300 MHz,

DMSO-d_6) δ 4.32 (2H, s), 4.60 (2H, s), 7.38 (2H, d, J = 8.3 Hz), 7.63 (2H, d, J = 8.3 Hz), 11.62 (1H, bs), 11.68 (1H, bs).

4'-({[(5-Oxo-4,5-dihydro-1*H*-1,2,4-triazol-3-yl)methyl]sulfonyl}methyl)biphenyl-3-carbonitrile (**33**). A mixture of compound **32** (480 mg, 1.44 mmol), (3-cyanophenyl)boronic acid (255 mg, 1.73 mmol), tetrakis(triphenylphosphine)palladium(0) (33 mg, 0.029 mmol), and 2 M aqueous Na_2CO_3 solution (1.73 mL, 3.47 mmol), ethanol (3 mL), and toluene (10 mL) was refluxed for 12 h. The mixture was diluted with H_2O and washed with ethyl acetate. The aqueous layer was acidified with 1 N hydrochloric acid and the precipitated solid was collected. The solid was washed with H_2O and diethyl ether to give the title compound as a pale yellow solid (308 mg, 0.869 mmol, 60%). ^{1}H NMR (300 MHz, DMSO-d_6) δ 4.36 (2H, s), 4.67 (2H, s), 7.56 (2H, d, J = 8.3 Hz), 7.69 (1H, t, J = 7.8 Hz), 7.79–7.89 (3H, m), 8.06 (1H, d, J = 8.0 Hz), 8.20 (1H, s), 11.65 (1H, bs), 11.70 (1H, bs).

5-[({[3'-(Aminomethyl)biphenyl-4-yl]methyl}sulfonyl)methyl]-2,4-dihydro-3*H*-1,2,4-triazol-3-one (**34**). A mixture of compound **33** (75 mg, 0.21 mmol), Raney Ni (200 mg), 28% aqueous NH_3 solution (2.5 mL), THF (5 mL), and methanol (2.5 mL) was stirred at room temperature for 2 h under hydrogen atmosphere (1 atm). The catalyst was removed by vacuum filtration through a PTFE membrane and the filtrate was concentrated under reduced pressure. The residue was triturated with diethyl ether to give the title compound as a pale blue powder (60 mg, 0.17 mmol, 79%). The pale blue powder was used for the next reaction without purification. MS *m/z* 359 $[M + H]^+$.

4-Oxo-*N*-{[4'-({[(5-oxo-4,5-dihydro-1*H*-1,2,4-triazol-3-yl)methyl]sulfonyl}methyl)biphenyl-3-yl]methyl}-3,4-dihydroquinazoline-2-carboxamide (**35**). A mixture of compound **34** (60 mg, 0.17 mmol), compound **10**[62] (24 mg, 0.11 mmol), triethylamine (0.389 mL, 1.12 mmol), ethanol (3 mL), and *N*,*N*-dimethylacetamide (3 mL) was stirred at 90 °C for 24 h. The mixture was diluted with ethyl acetate, washed with 0.1 N hydrochloric acid and brine, and dried over Na_2SO_4. After removal of the solvent, the residue was crystallized from ethanol–diethyl ether to give the title compound as a pale yellow powder (32 mg, 0.061 mmol, 55%). mp 245–247 °C. ^{1}H NMR (300 MHz, DMSO-d_6) δ 4.34 (2H, s), 4.57 (2H, d, J = 6.4 Hz), 4.64 (2H, s), 7.35–7.48 (2H, m), 7.52 (2H, d, J = 8.1 Hz), 7.56–7.74 (5H, m), 7.75–7.82 (1H, m), 7.89 (1H, t, J = 7.4 Hz), 8.17 (1H, d, J = 7.9 Hz), 9.62 (1H, t, J = 5.6 Hz), 11.52–11.78 (2H, m), 12.02–12.37 (1H, m). Anal. Calcd for $C_{26}H_{22}N_6O_5S\cdot0.5H_2O$: C, 57.88; H, 4.30; N, 15.58. Found: C, 57.61; H, 4.40; N, 15.43.

5-(3-Chlorophenyl)-4-oxo-*N*-{[4'-({[(5-oxo-4,5-dihydro-1*H*-1,2,4-triazol-3-yl)methyl]sulfonyl}methyl)biphenyl-3-yl]methyl}-3,4-dihydrothieno[2,3-*d*]pyrimidine-2-carboxamide (**37**). A mixture of compound **34** (0.100 g, 0.279 mmol), compound **36**[35] (0.078 g, 0.23 mmol), and triethylamine (0.162 ml, 1.16 mmol) in a mixed solvent of ethanol (5 mL) and *N*,*N*-dimethylacetamide (5 mL) was stirred at 90 °C for 24 h. After removal of the solvent, the residue was purified by preparative HPLC and crystallized from methanol to give the title compound as a pale yellow powder (0.0671 g, 0.104 mmol, 45%). mp 276–278 °C. ^{1}H NMR (300 MHz, DMSO-d_6) δ 4.34 (2H, s), 4.55 (2H, d, J = 6.1 Hz), 4.64 (2H, s), 7.33–7.90 (13H, m), 9.77 (1H, t, J = 5.9 Hz), 11.63 (1H, bs), 11.69 (1H, bs), 12.47 (1H, bs). Anal. Calcd for $C_{30}H_{23}ClN_6O_5S_2\cdot0.7H_2O$: C, 54.62; H, 3.73; N, 12.74. Found: C, 54.78; H, 3.76; N, 12.45.

1,1-Dimethylethyl 4-(3-Bromophenyl)piperazine-1-carboxylate (**39**). To a solution of 1-(3-bromo-

phenyl)piperazine (**38**, 6.00 g, 27.4 mmol) and triethylamine (3.82 mL, 27.4 mmol) in THF (60 mL) was added dropwise di-*t*-butyl dicarbonate (5.97 g, 27.4 mmol) at room temperature, and the mixture was stirred at room temperature for 3 h. After the mixture was concentrated under reduced pressure, the residue was diluted with ethyl acetate and H_2O, and neutralized with 1 N hydrochloric acid. The organic layer was separated, washed with brine, and dried over Na_2SO_4. After removal of the solvent, the residue was crystallized from hexane to give the title compound as a white powder (5.39 g, 15.8 mmol, 64%). ^{1}H NMR (300 MHz, DMSO-d_6) δ 1.42 (9H, s), 3.09–3.17 (4H, m), 3.39–3.47 (4H, m), 6.91–6.97 (2H, m), 7.09 (1H, t, J = 2.1 Hz), 7.12–7.19 (1H, m).

1,1-Dimethylethyl 4-(3-Cyanophenyl)piperazine-1-carboxylate (**40**). A mixture of compound **39** (3.10 g, 9.09 mmol), zinc cyanide (587 mg, 5.00 mmol), and tetrakis(triphenylphosphine)palladium(0) (525 mg, 0.454 mmol) in DMF (30 mL) was heated at 80 °C on an oil bath for 12 h under nitrogen atmosphere. The mixture was diluted with ethyl acetate, washed with H_2O and brine, dried over Na_2SO_4, and concentrated under reduced pressure. The residue was purified by silica gel column chromatography (25–40% ethyl acetate/hexane) and triturated with hexane to give the title compound as a white power (2.10 g, 7.31 mmol, 80%). ^{1}H NMR (300 MHz, DMSO-d_6) δ 1.42 (9H, s), 3.16–3.24 (4H, m), 3.40–3.48 (4H, m), 7.16–7.21 (1H, m), 7.25–7.31 (1H, m), 7.33–7.36 (1H, m), 7.36–7.43 (1H, m).

1,1-Dimethylethyl 4-[3-(Aminomethyl)phenyl]piperazine-1-carboxylate (**41**). A mixture of compound **40** (2.00 g, 6.96 mmol), Raney Ni (2.0 g), 28% aqueous NH_3 solution (20 mL), THF (20 mL) and methanol (20 mL) was stirred at room temperature for 4 h under hydrogen atmosphere (1 atm). The catalyst was removed by vacuum filtration through a PTFE membrane and the filtrate was concentrated under reduced pressure. The residue was dissolved in ethyl acetate, dried over Na_2SO_4, and concentrated under reduced pressure to give the title compound as a pale blue oil (2.03 g, 6.97 mmol, 100%). ^{1}H NMR (300 MHz, DMSO-d_6) δ 1.42 (9H, s), 3.04–3.13 (4H, m), 3.17–3.51 (6H, m), 3.50–3.82 (2H, m), 6.71–6.80 (2H, m), 6.92 (1H, bs), 7.16 (1H, t, J = 7.2 Hz).

1,1-Dimethylethyl 4-[3-({[(4-Oxo-3,4-dihydroquinazolin-2-yl)carbonyl]amino}methyl)phenyl]-piperazine-1-carboxylate (**42**). A mixture of compound **41** (2.00 g, 6.86 mmol), compound **10**[62] (1.25 g, 5.72 mmol), and triethylamine (0.797 mL, 5.72 mmol) in ethanol (30 mL) was stirred at 80 °C for 12 h. After the mixture was allowed to cool to room temperature, 1 N hydrochloric acid (5.7 mL) was added. The precipitated solid was collected and washed with ethanol to give the title compound as a white powder (2.44 g, 5.26 mmol, 92%). mp 184–185 °C. ^{1}H NMR (300 MHz, DMSO-d_6) δ 1.41 (9H, s), 3.03–3.14 (4H, m), 3.39–3.50 (4H, m), 4.43 (2H, d, J = 6.4 Hz), 6.76–6.88 (2H, m), 6.97 (1H, s), 7.18 (1H, t, J = 7.8 Hz), 7.56–7.66 (1H, m), 7.74–7.82 (1H, m), 7.84–7.94 (1H, m), 8.17 (1H, dd, J = 7.9, 1.1 Hz), 9.48 (1H, t, J = 6.4 Hz), 12.27 (1H, bs). Anal. Calcd for $C_{25}H_{29}N_5O_4$: C, 64.78; H, 6.31; N, 15.11. Found: C, 64.62; H, 6.30; N, 15.12.

4-Oxo-*N*-[(3-piperazin-1-ylphenyl)methyl]-3,4-dihydroquinazoline-2-carboxamide Dihydrochloride (**43**). To a mixture of compound **42** (2.40 g, 6.86 mmol), ethyl acetate (10 mL), THF (50 mL), and methanol (40 mL) was added 4 N HCl in ethyl acetate (10.4 mL) at room temperature. The mixture was stirred at room temperature for 4 h and then heated at 50 °C for 2 h. Diethyl ether (100 mL) was added to the mixture and the precipitated solid was collected to give the title compound as a white powder (2.18 g,

5.00 mmol, 97%). mp 243–245 °C. ^{1}H NMR (300 MHz, DMSO-d_6) δ 3.14–3.26 (4H, m), 3.31–3.42 (4H, m), 4.45 (2H, d, J = 6.4 Hz), 6.83–6.93 (2H, m), 7.00 (1H, bs), 7.22 (1H, t, J = 7.8 Hz), 7.62 (1H, t, J = 7.6 Hz), 7.75–7.83 (1H, m), 7.90 (1H, t, J = 7.6 Hz), 8.18 (1H, d, J = 8.0 Hz), 9.25–9.40 (2H, m), 9.52 (1H, t, J = 6.4 Hz). Anal. Calcd for $C_{20}H_{21}N_5O_2 \cdot 2HCl \cdot 0.2H_2O$: C, 51.52; H, 4.14; N, 7.51. Found: C, 51.42; H, 4.11; N, 7.51.

***N*-[(3-{4-[(2,5-Dioxoimidazolidin-4-yl)acetyl]piperazin-1-yl}phenyl)methyl]-4-oxo-3,4-dihydroquinazoline-2-carboxamide** (**45a**). A mixture of compound **43** (109 mg, 0.250 mmol), (2,5-dioxoimidazolidin-4-yl)acetic acid (47 mg, 0.30 mmol), 1-[3-(dimethylamino)propyl]-3-ethylcarbodiimide hydrochloride (96 mg, 0.50 mmol), and 1-hydroxybenzotriazole (68 mg, 0.50 mmol) in DMF (5 mL) was stirred at room temperature for 1 h, and triethylamine (0.139 mL, 1.00 mmol) was added. The reaction mixture was stirred at room temperature for further 12 h. After the reaction mixture was concentrated under reduced pressure, the residue was purified by preparative HPLC and crystallization from methanol–diethyl ether to give the title compound as a pale yellow powder (78 mg, 0.16 mmol, 62%). mp 280–282 °C. ^{1}H NMR (300 MHz, DMSO-d_6) δ 2.69–2.91 (2H, m), 3.04–3.21 (4H, m), 3.58 (4H, d, J = 3.8 Hz), 4.21–4.29 (1H, m), 4.43 (2H, d, J = 6.1 Hz), 6.76–6.90 (2H, m), 6.98 (1H, bs), 7.19 (1H, t, J = 8.0 Hz), 7.62 (1H, t, J = 7.4 Hz), 7.72 (1H, bs), 7.76–7.83 (1H, m), 7.89 (1H, t, J = 7.0 Hz), 8.18 (1H, d, J = 8.3 Hz), 9.49 (1H, t, J = 6.1 Hz), 10.56 (1H, bs), 12.26 (1H, bs). Anal. Calcd for $C_{25}H_{25}N_7O_5 \cdot 0.5H_2O$: C, 58.59; H, 5.11; N, 19.13. Found: C, 58.58; H, 5.17; N, 19.04.

4-Oxo-*N*-[(3-{4-[(5-oxo-4,5-dihydro-1*H*-1,2,4-triazol-3-yl)acetyl]piperazin-1-yl}phenyl)methyl]-3,4-dihydroquinazoline-2-carboxamide (**45b**). A mixture of compound **43** (109 mg, 0.250 mmol), (5-oxo-4,5-dihydro-1*H*-1,2,4-triazol-3-yl)acetic acid[63] (43 mg, 0.30 mmol), 1-[3-(dimethylamino)propyl]-3-ethylcarbodiimide hydrochloride (96 mg, 0.50 mmol), and 1-hydroxybenzotriazole (68 mg, 0.50 mmol) in DMF (5 mL) was stirred at room temperature for 1 h, and triethylamine (0.139 mL, 1.00 mmol) was added. The reaction mixture was stirred at room temperature for further 12 h. After the reaction mixture was concentrated under reduced pressure, the residue was purified by preparative HPLC, and crystallization from methanol–diethyl ether to give the title compound as a pale yellow powder (77 mg, 0.16 mmol, 63%). mp 311–313 °C. ^{1}H NMR (300 MHz, DMSO-d_6) δ 3.06–3.22 (4H, m), 3.55–3.69 (6H, m), 4.44 (2H, d, J = 6.4 Hz), 6.77–6.90 (2H, m), 6.98 (1H, bs), 7.19 (1H, t, J = 8.0 Hz), 7.62 (1H, t, J = 7.4 Hz), 7.75–7.82 (1H, m), 7.85–7.94 (1H, m), 8.18 (1H, d, J = 7.2 Hz), 9.49 (1H, t, J = 6.2 Hz), 11.14 (1H, bs), 11.18 (1H, bs), 12.27 (1H, bs). Anal. Calcd for $C_{24}H_{24}N_8O_4 \cdot 0.5H_2O$: C, 57.94; H, 5.06; N, 22.52. Found: C, 57.82; H, 5.01; N, 22.43.

4-Oxo-*N*-[(3-{4-[(5-oxo-2,5-dihydro-1*H*-pyrazol-3-yl)acetyl]piperazin-1-yl}phenyl)methyl]-3,4-dihydroquinazoline-2-carboxamide (**45c**). A mixture of compound **43** (109 mg, 0.250 mmol), compound **44c** (43 mg, 0.30 mmol), 1-[3-(dimethylamino)propyl]-3-ethylcarbodiimide hydrochloride (96 mg, 0.50 mmol), and 1-hydroxybenzotriazole (68 mg, 0.50 mmol) in DMF (5 mL) was stirred at room temperature for 1 h, and triethylamine (0.139 mL, 1.00 mmol) was added. The reaction mixture was stirred at room temperature for further 12 h. Water (5 mL) was added to the mixture and the resulting mixture was stirred at 50 °C for 4 h. After the reaction mixture was concentrated under reduced pressure, the residue was purified by preparative HPLC, and crystallization from methanol–diethyl ether to give the title compound as a pale

yellow powder (40 mg, 0.082 mmol, 33%). mp 183–185 °C. ^{1}H NMR (300 MHz, DMSO-d_6) δ 3.03–3.17 (4H, m), 3.53–3.72 (6H, m), 4.43 (2H, d, J = 6.2 Hz), 5.33 (1H, s), 6.77–6.90 (2H, m), 6.97 (1H, bs), 7.19 (1H, t, J = 7.9 Hz), 7.57–7.67 (1H, m), 7.74–7.82 (1H, m), 7.84–7.95 (1H, m), 8.18 (1H, dd, J = 7.9, 0.9 Hz), 9.49 (1H, t, J = 6.1 Hz), 12.26 (1H, bs). Anal. Calcd for $C_{25}H_{25}N_7O_4 \cdot 2H_2O$: C, 57.35; H, 5.58; N, 18.73. Found: C, 57.66; H, 5.27; N, 18.35.

***N*-({3-[4-(1*H*-Imidazol-4-ylacetyl)piperazin-1-yl]phenyl}methyl)-4-oxo-3,4-dihydroquinazoline-2-carboxamide Trifluoroacetate** (**45d**). A mixture of compound **43** (109 mg, 0.250 mmol), 1*H*-imidazol-4-ylacetic acid hydrochloride (49 mg, 0.30 mmol), 1-[3-(dimethylamino)propyl]-3-ethylcarbodiimide hydrochloride (96 mg, 0.50 mmol), and 1-hydroxybenzotriazole (68 mg, 0.50 mmol) in DMF (5 mL) was stirred at room temperature for 1 h, and triethylamine (0.174 mL, 1.25 mmol) was added. The reaction mixture was stirred at room temperature for further 12 h. After the reaction mixture was concentrated under reduced pressure, the residue was purified by preparative HPLC, and crystallization from methanol–diethyl ether to give the title compound as a pale yellow powder (93 mg, 0.196 mmol, 79%). mp 202–204 °C. ^{1}H NMR (300 MHz, DMSO-d_6) δ 3.08–3.24 (4H, m), 3.59–3.73 (4H, m), 3.96 (2H, s), 4.44 (2H, d, J = 6.4 Hz), 6.79–6.92 (2H, m), 6.99 (1H, bs), 7.21 (1H, t, J = 7.8 Hz), 7.46 (1H, s), 7.62 (1H, t, J = 7.4 Hz), 7.75–7.82 (1H, m), 7.90 (1H, t, J = 7.2 Hz), 8.18 (1H, d, J = 8.0 Hz), 9.00 (1H, s), 9.51 (1H, t, J = 5.9 Hz), 12.27 (1H, bs), 14.17 (2H, bs). Anal. Calcd for $C_{25}H_{25}N_7O_3 \cdot CF_3CO_2H \cdot H_2O$: C, 53.73; H, 4.68; N, 16.25. Found: C, 53.60; H, 4.74; N, 16.12.

4-Oxo-*N*-({3-[4-(1*H*-tetrazol-5-ylacetyl)piperazin-1-yl]phenyl}methyl)-3,4-dihydroquinazoline-2-carboxamide (**45e**). A mixture of compound **43** (109 mg, 0.250 mmol), 1*H*-tetrazol-5-ylacetic acid (38 mg, 0.30 mmol), 1-[3-(dimethylamino)propyl]-3-ethylcarbodiimide hydrochloride (96 mg, 0.50 mmol), and 1-hydroxybenzotriazole (68 mg, 0.50 mmol) in DMF (5 mL) was stirred at room temperature for 1 h, and triethylamine (0.139 mL, 1.00 mmol) was added. The reaction mixture was stirred at room temperature for further 12 h. After the reaction mixture was concentrated under reduced pressure, the residue was purified by preparative HPLC, and crystallization from methanol–diethyl ether to give the title compound as a pale yellow powder (38 mg, 0.080 mmol, 32%). mp 163–165 °C. ^{1}H NMR (300 MHz, DMSO-d_6) δ 3.07–3.24 (4H, m), 3.57–3.76 (4H, m), 4.27 (2H, s), 4.44 (2H, d, J = 6.4 Hz), 6.78–6.91 (2H, m), 6.99 (1H, bs), 7.20 (1H, t, J = 8.1 Hz), 7.62 (1H, t, J = 7.4 Hz), 7.75–7.83 (1H, m), 7.89 (1H, t, J = 7.8 Hz), 8.18 (1H, d, J = 7.6 Hz), 9.49 (1H, t, J = 5.7 Hz), 12.26 (1H, bs). Anal. Calcd for $C_{23}H_{23}N_9O_3 \cdot 1.2H_2O$: C, 55.80; H, 5.17; N, 25.46. Found: C, 56.10; H, 4.87; N, 25.17.

(5-Oxo-2,5-dihydro-1*H*-pyrazol-3-yl)acetic Acid (**44c**). Hydrazine (4.38 g, 129 mmol) was added to a solution of diethyl 3-oxopentanedioate (26.0 g, 129 mmol) in ethanol (250 mL) at room temperature and the mixture was stirred at 80 °C for 3 h. After the mixture was concentrated under reduced pressure, the residue was crystallized from diethyl ether–hexane to give ethyl (5-oxo-2,5-dihydro-1*H*-pyrazol-3-yl)acetate as a white powder (9.79 g, 57.5 mmol, 45%). ^{1}H NMR (300 MHz, DMSO-d_6) δ 1.19 (3H, t, J = 7.0 Hz), 3.53 (2H, s), 4.08 (2H, q, J = 6.9 Hz), 5.34 (1H, s), 9.53 (1H, bs), 11.39 (1H, bs).

A mixture of ethyl (5-oxo-2,5-dihydro-1*H*- pyrazol-3-yl)acetate (5.00 g, 29.4 mmol), 8 N aqueous sodium hydroxide solution (11.0 mL, 88.0 mmol), H_2O (20 mL), THF (20 mL), and methanol (20 mL) was

stirred at 80 °C for 3 h. After THF and methanol were removed by evaporation, the residue was acidified with 6 N hydrochloric acid (15 mL). The precipitated solid was collected, washed with H_2O, and dried in a stream of air to give the title compound as a white powder (3.43 g, 24.1 mmol, 82%). ^{1}H NMR (300 MHz, DMSO-d_6) δ 3.45 (2H, s), 5.33 (1H, s), 10.52–12.13 (3H, m).

4'-({5-[2-(Ethyloxy)ethyl]-2,4,6-trioxohexahydropyrimidin-5-yl}oxy)biphenyl-3-carbonitrile (**48**). A mixture of 5-bromo-5-[2-(ethyloxy)ethyl]pyrimidine-2,4,6(1*H*,3*H*,5*H*)-trione[20, 64] (**46**, 2.00 g, 7.17 mmol), 4'-hydroxybiphenyl-3-carbonitrile[65] (**47**, 1.40 g, 7.17 mmol), and K_2CO_3 (3.47 g, 25.1 mmol) in DMF (40 mL) was stirred at room temperature for 4 h. The reaction mixture was diluted with ethyl acetate, washed with H_2O and brine, and dried over Na_2SO_4. After removal of the solvent, the residue was purified by crystallization from methanol–diethyl ether to give the title compound as a white powder (1.95 g, 4.96 mmol, 69%). ^{1}H NMR (300 MHz, DMSO-d_6) δ 1.08 (3H, t, J = 7.0 Hz), 2.44 (2H, t, J = 5.9 Hz), 3.30–3.39 (2H, m), 3.52 (2H, t, J = 5.9 Hz), 6.78–6.84 (2H, m), 7.60–7.70 (3H, m), 7.76–7.81 (1H, m), 7.92–7.98 (1H, m), 8.06–8.10 (1H, m), 11.88 (2H, bs).

Sodium 3'-(Aminomethyl)biphenyl-4-olate (**49**). A mixture of compound **48** (1.50 g, 3.81 mmol), Raney Ni (1.5 g), 28% aqueous NH_3 solution (15 mL), THF (30 mL), and methanol (15 mL) was stirred at room temperature for 12 h under hydrogen atmosphere (1 atm). The catalyst was removed by vacuum filtration through a PTFE membrane and the filtrate was concentrated under reduced pressure. The residue was diluted with H_2O, and acidified with 1 N hydrochloric acid. The insoluble materials were filtered off through a pad of Celite and then the filtrate was basified with 1 N aqueous sodium hydroxide solution. The precipitated solid was collected and washed with H_2O and ethanol to give the title compound as a pale green powder (800 mg, 3.62 mmol, 95%). ^{1}H NMR (300 MHz, DMSO-d_6) δ 3.74 (2H, bs), 6.68–6.93 (2H, m), 7.05–7.68 (6H, m).

***N*-[(4'-Hydroxybiphenyl-3-yl)methyl]-4-oxo-3,4-dihydroquinazoline-2-carboxamide** (**50**). A mixture of compound **49** (200 mg, 0.904 mmol), compound **10**[62] (132 mg, 0.603 mmol), and triethylamine (0.336 mL, 2.41 mmol) in ethanol (10 mL) was stirred at 80 °C for 12 h. After the mixture was allowed to cool to room temperature, 2 N HCl in ethanol (2 mL) was added. The precipitated solid was collected and washed with ethanol to give the title compound as a white powder (149 mg, 0.401 mmol, 67%). mp 244–246 °C. ^{1}H NMR (300 MHz, DMSO-d_6) δ 4.53 (2H, d, J = 6.1 Hz), 6.84 (2H, d, J = 8.3 Hz), 7.23–7.30 (1H, m), 7.37 (1H, t, J = 7.6 Hz), 7.42–7.50 (3H, m), 7.55–7.64 (2H, m), 7.73–7.80 (1H, m), 7.87 (1H, t, J = 7.6 Hz), 8.16 (1H, d, J = 8.0 Hz), 9.51–9.64 (2H, m), 12.01 (1H, bs). Anal. Calcd for $C_{22}H_{17}N_3O_3 \cdot 0.3H_2O$: C, 70.13; H, 4.71; N, 11.15. Found: C, 69.96; H, 4.56; N, 11.24.

***N*-{[4'-({5-[2-(Ethyloxy)ethyl]-2,4,6-trioxohexahydropyrimidin-5-yl}oxy)biphenyl-3-yl]methyl}-4-oxo-3,4-dihydroquinazoline-2-carboxamide** (**51**). A mixture of compound **46** (75 mg, 0.27 mmol), compound **50** (100 mg, 0.269 mmol), and K_2CO_3 (186 mg, 1.34 mmol) in DMF (5 mL) was stirred at room temperature for 5 h. The reaction mixture was diluted with ethyl acetate, washed with H_2O and brine, and dried over Na_2SO_4. After removal of the solvent, the residue was purified by crystallization from ethyl acetate–diisopropyl ether to give the title compound as a white powder (78 mg, 0.137 mmol, 51%). mp 163–165 °C. ^{1}H NMR (300 MHz, DMSO-d_6) δ 1.01–1.08 (3H, m), 2.37–2.47 (2H, m), 3.29–3.39 (2H, m), 3.44–3.55 (2H, m), 4.53 (2H, d, J = 6.1 Hz), 6.73–6.82 (2H, m), 7.26–7.66 (7H, m), 7.73–7.94 (2H, m),

8.14–8.21 (1H, m), 9.60 (1H, t, *J* = 6.1 Hz), 11.73–12.01 (2H, m), 12.10–12.39 (1H, m). Anal. Calcd for $C_{30}H_{27}N_5O_7 \cdot 0.1^iPr_2O$: C, 63.39; H, 4.94; N, 12.08. Found: C, 63.16; H, 5.01; N, 11.79.

4-Oxo-*N*-(3-{3-[(1-trityl-1*H*-1,2,4-triazol-3-yl)oxy]propoxy}benzyl)-3,4-dihydroquinazoline-2-carboxamide (**53**). A mixture of compound **52**[35] (0.300 g, 0.611 mmol.), compound **10**[62] (0.133 g, 0.611 mmol), and DIEA (0.213 mL, 1.22 mmol) in ethanol (4.5 mL) was heated under microwave irradiation at 100 °C for 1.5 h. After the reaction mixture was cooled to room temperature, the precipitated solid was collected and washed with ethanol to give the title compound **53** as a white powder (0.315 g, 0.475 mmol, 78%). MS *m/z* 663.2 $[M + H]^+$. ^{1}H NMR (300 MHz, DMSO-d_6) δ 2.05–2.17 (2H, m), 4.06 (2H, t, *J* = 6.1 Hz), 4.29 (2H, t, *J* = 6.1 Hz), 4.44 (2H, d, *J* = 6.4 Hz), 6.78–6.85 (1H, m), 6.88–6.96 (2H, m), 7.02–7.11 (6H, m), 7.23 (1 H, t, J = 8.0 Hz), 7.30–7.42 (9H, m), 7.60 (1H, t, *J* = 7.6 Hz), 7.74–7.82 (2H, m), 7.87 (1H, t, *J* = 7.6 Hz), 8.17 (1H, d, *J* = 7.2 Hz), 9.51 (1H, t, *J* = 6.2 Hz), 11.86 (1H, bs).

4-Oxo-*N*-{3-[3-(1*H*-1,2,4-triazol-3-yloxy)propoxy]benzyl}-3,4-dihydroquinazoline-2-carboxamide (**54**). TFA (1.27 mL) and triethylsilane (0.082 mL, 0.52 mmol) were added to a mixture of compound **53** (0.285 g, 0.430 mmol) in CH_3CN (6 mL) at room temperature, and the mixture was stirred at room temperature for 12 h. The reaction mixture was concentrated in vacuo. The residue was crystallized from diethyl ether–ethanol to give the title compound as a white powder (0.173 g, 0.411 mmol, 96%). MS *m/z* 421.1 $[M + H]^+$. ^{1}H NMR (300 MHz, DMSO-d_6) δ 2.09–2.21 (2H, m), 4.08 (2H, t, *J* = 6.2 Hz), 4.29–4.40 (2H, m), 4.45 (2H, d, *J* = 6.4 Hz), 6.84 (1H, dd, *J* = 8.1, 2.1 Hz), 6.89–6.98 (2H, m), 7.24 (1H, t, *J* = 7.8 Hz), 7.61 (1H, t, *J* = 7.4 Hz), 7.74–7.82 (1H, m), 7.84–7.94 (1H, m), 8.12–8.26 (2H, m), 9.53 (1H, t, *J* = 6.1 Hz), 12.24 (1H, bs), 13.27 (1H, bs). mp 190–192 °C. Anal. Calcd for $C_{21}H_{20}N_6O_4 \cdot 0.2H_2O$: C, 59.48; H, 4.85; N, 19.82. Found: C, 59.40; H, 4.72; N, 19.80.

5-(3-Chlorophenyl)-*N*-(3-methoxybenzyl)-4-oxo-3,4-dihydrothieno[2,3-d]pyrimidine-2-carboxamide (**55**). A mixture of compound **36** (0.110 g, 0.346 mmol) and 3-methoxybenzylamine (0.066 mL, 0.518 mmol) , and triethylamine (0.096 mL, 0.691 mmol) in ethanol (2.0 mL) was stirred at 80 °C for 12 hr (clear solution). After the reaction mixture was allowed to cool to rt, the mixture was concentrated in vacuo. The residue was taken up in ethyl acetate, and washed with 1 N HCl twice and brine. The organic phase was dried over Na_2SO_4, and concentrated in vacuo to give a foam which was crystallized from ethyl acetate to give the title compound as a white powder (0.092 g, 0.216 mmol, 62%). mp 164 °C. ^{1}H NMR (300 MHz, DMSO-d_6) δ 3.74 (3H, s), 4.44 (2H, d, *J* = 6.4 Hz), 6.79–6.86 (1H, m), 6.88–6.95 (2H, m), 7.25 (1H, t, *J* = 8.1 Hz), 7.40–7.54 (3H, m), 7.61 (1H, q, *J* = 1.6 Hz), 7.8 (1H, s), 9.69 (1H, t, *J* = 6.4 Hz), 12.46 (1H, s). Anal. Calcd for $C_{21}H_{16}ClN_3O_3S$: C, 59.22; H, 3.79; N, 9.87. Found: C, 59.11; H, 3.87; N, 9.69.

5-2. MMPs and TACE enzyme inhibition assay. Human recombinant MMP precursors were purchased from Genzyme-Techne (MMP-1, 2, 7, 8, 9, 10, 12, 13, and TACE) or Biogenesis (MMP-3). Human recombinant GST-MMP-14 was prepared as described by Sato et al.[66] 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, 12, and 13) or 18 h (MMP-3 and 9). The TACE assay buffer consisted of 25 mM Tris–HCl (pH 9.0), 2.5 μM $ZnCl_2$, and 0.005% Brij-35. Enzyme inhibition assays were performed in assay buffer containing enzymes and fluorescence peptide (Cy3-PLGLK(Cy5Q)AR-NH_2 for MMPs and

Cy3-PLAQAV(Cy5Q-L-2,3-diaminopropionic acid)-RSSSR-NH_2 for TACE, Amersham Biosciences) in the presence of the various concentrations of inhibitors. Following incubation at 37 °C for 40 min, the reaction was terminated by addition of EDTA (pH 8.0). The increase in fluorescence was measured by Farcyte spectrofluorimeter (Amersham Bioscience, λ_{em} = 535 nm; λ_{ex} = 595 nm). Enzyme activity (%) was determined according to the following equation: Enzyme activity (%) = $(X - C)/(T - C) \times 100$, where X is the fluorescence count with inhibitor, T is the fluorescence count without inhibitor, and C is the fluorescence count with EDTA. IC_{50} values of inhibitors were obtained with iterative fitting package (GraphPad Prism software).

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7. References

(1) Badley, E. M.; Tennant, A. Disablement associated with rheumatic disorders in a British population: problems with activities of daily living and level of support. *Br. J. Rheumatol.* **1993**, 32, 601-608.

(2) Buckwalter, J. A.; Martin, J. A. Osteoarthritis. *Adv Drug Deliv Rev* **2006**, 58, 150-167.

(3) Helmick, C. G.; Felson, D. T.; Lawrence, R. C.; Gabriel, S.; Hirsch, R.; Kwoh, C. K.; Liang, M. H.; Kremers, H. M.; Mayes, M. D.; Merkel, P. A.; Pillemer, S. R.; Reveille, J. D.; Stone, J. H. Estimates of the prevalence of arthritis and other rheumatic conditions in the United States. Part I. *Arthritis Rheum.* **2008**, 58, 15-25.

(4) Miura, H.; Iwamoto, Y. Epidemiology of osteoarthritis. *The Bone* **2000**, 14, 287-291.

(5) Spector, T. D.; MacGregor, A. J. Risk factors for osteoarthritis: genetics. *Osteoarthritis Cartilage* **2004**, 12, 39-44.

(6) Ikegawa, S. New gene associations in osteoarthritis: what do they provide, and where are we going? *Curr. Opin. Rheumatol.* **2007**, 19, 429-434.

(7) Miyamoto, Y.; Shi, D.; Nakajima, M.; Ozaki, K.; Sudo, A.; Kotani, A.; Uchida, A.; Tanaka, T.; Fukui, N.; Tsunoda, T.; Takahashi, A.; Nakamura, Y.; Jiang, Q.; Ikegawa, S. Common variants in DVWA on chromosome 3p24.3 are associated with susceptibility to knee osteoarthritis. *Nat. Genet.* **2008**, 40, 994-998.

(8) Mototani, H.; Iida, A.; Nakajima, M.; Furuichi, T.; Miyamoto, Y.; Tsunoda, T.; Sudo, A.; Kotani, A.; Uchida, A.; Ozaki, K.; Tanaka, Y.; Nakamura, Y.; Tanaka, T.; Notoya, K.; Ikegawa, S. A functional SNP in EDG2 increases susceptibility to knee osteoarthritis in Japanese. *Hum. Mol. Genet.* **2008**, 17, 1790-1797.

(9) FitzGerald, G. A. Coxibs and Cardiovascular Disease. *N. Engl. J. Med.* **2004**, 351, 1709-1711.

(10) Sabatini, M.; Lesur, C.; Thomas, M.; Chomel, A.; Anract, P.; de Nanteuil, G.; Pastoureau, P.; Janusz, M. J.; Bendele, A. M.; Brown, K. K.; Taiwo, Y. O.; Hsieh, L.; Heitmeyer, S. A. Effect of inhibition of matrix metalloproteinases on cartilage loss in vitro and in a guinea pig model of osteoarthritis. *Arthritis Rheum.* **2005**,

52, 171-180.

(11) Hoekstra, R.; Eskens, F. A.; Verweij, J. Matrix metalloproteinase inhibitors: current developments and future perspectives. *Oncologist* **2001**, 6, 415-427.

(12) Peterson, J. T. The importance of estimating the therapeutic index in the development of matrix metalloproteinase inhibitors. *Cardiovasc. Res.* **2006**, 69, 677-687.

(13) Reiter, L. A.; Robinson, R. P.; McClure, K. F.; Jones, C. S.; Reese, M. R.; Mitchell, P. G.; Otterness, I. G.; Bliven, M. L.; Liras, J.; Cortina, S. R.; Donahue, K. M.; Eskra, J. D.; Griffiths, R. J.; Lame, M. E.; Lopez-Anaya, A.; Martinelli, G. J.; McGahee, S. M.; Yocum, S. A.; Lopresti-Morrow, L. L.; Tobiassen, L. M.; Vaughn-Bowser, M. L. Pyran-containing sulfonamide hydroxamic acids: potent MMP inhibitors that spare MMP-1. *Bioorg. Med. Chem. Lett.* **2004**, 14, 3389-3395.

(14) Mitchell, P. G.; Magna, H. A.; Reeves, L. M.; Lopresti-Morrow, L. L.; Yocum, S. A.; Rosner, P. J.; Geoghegan, K. F.; Hambor, J. E. Cloning, expression, and type II collagenolytic activity of matrix metalloproteinase-13 from human osteoarthritic cartilage. *J. Clin. Invest.* **1996**, 97, 761-768.

(15) Knäuper, V.; López-Otin, C.; Smith, B.; Knight, G.; Murphy, G. Biochemical Characterization of Human Collagenase-3. *J. Biol. Chem.* **1996**, 271, 1544-1550.

(16) Yocum, S. A.; Lopresti-Morrow, L. L.; Reeves, L. M.; Mitchell, P. G. MMP-13 and MMP-1 expression in tissues of normal articular joints. *Ann. N. Y. Acad. Sci.* **1999**, 878, 583-586.

(17) Engel, C. K.; Pirard, B.; Schimanski, S.; Kirsch, R.; Habermann, J.; Klingler, O.; Schlotte, V.; Weithmann, K. U.; Wendt, K. U. Structural Basis for the Highly Selective Inhibition of MMP-13. *Chem. Biol.* **2005**, 12, 181-189.

(18) Li, J. J.; Nahra, J.; Johnson, A. R.; Bunker, A.; O'Brien, P.; Yue, W.-S.; Ortwine, D. F.; Man, C.-F.; Baragi, V.; Kilgore, K.; Dyer, R. D.; Han, H.-K. Quinazolinones and Pyrido[3,4-*d*]pyrimidin-4-ones as Orally Active and Specific Matrix Metalloproteinase-13 Inhibitors for the Treatment of Osteoarthritis. *J. Med. Chem.* **2008**, 51, 835-841.

(19) Kim, S.-H.; Pudzianowski, A. T.; Leavitt, K. J.; Barbosa, J.; McDonnell, P. A.; Metzler, W. J.; Rankin, B. M.; Liu, R.; Vaccaro, W.; Pitts, W. Structure-based design of potent and selective inhibitors of collagenase-3 (MMP-13). *Bioorg. Med. Chem. Lett.* **2005**, 15, 1101-1106.

(20) Blagg, J. A.; Noe, M. C.; Wolf-Gouveia, L. A.; Reiter, L. A.; Laird, E. R.; Chang, S.-P. P.; Danley, D. E.; Downs, J. T.; Elliott, N. C.; Eskra, J. D.; Griffiths, R. J.; Hardink, J. R.; Haugeto, A. I.; Jones, C. S.; Liras, J. L.; Lopresti-Morrow, L. L.; Mitchell, P. G.; Pandit, J.; Robinson, R. P.; Subramanyam, C.; Vaughn-Bowser, M. L.; Yocum, S. A. Potent pyrimidinetrione-based inhibitors of MMP-13 with enhanced selectivity over MMP-14. *Bioorg. Med. Chem. Lett.* **2005**, 15, 1807-1810.

(21) Aranapakam, V.; Grosu, G. T.; Davis, J. M.; Hu, B.; Ellingboe, J.; Baker, J. L.; Skotnicki, J. S.; Zask, A.; DiJoseph, J. F.; Sung, A.; Sharr, M. A.; Killar, L. M.; Walter, T.; Jin, G.; Cowling, R. Synthesis and Structure-Activity Relationship of α-Sulfonylhydroxamic Acids as Novel, Orally Active Matrix Metalloproteinase Inhibitors for the Treatment of Osteoarthritis. *J. Med. Chem.* **2003**, 46, 2361-2375.

(22) Aranapakam, V.; Davis, J. M.; Grosu, G. T.; Baker; Ellingboe, J.; Zask, A.; Levin, J. I.; Sandanayaka, V. P.; Du, M.; Skotnicki, J. S.; DiJoseph, J. F.; Sung, A.; Sharr, M. A.; Killar, L. M.; Walter, T.; Jin, G.; Cowling, R.; Tillett, J.; Zhao, W.; McDevitt, J.; Xu, Z. B. Synthesis and Structure-Activity Relationship of N-Substituted

4-Arylsulfonylpiperidine-4-hydroxamic Acids as Novel, Orally Active Matrix Metalloproteinase Inhibitors for the Treatment of Osteoarthritis. *J. Med. Chem.* **2003**, 46, 2376-2396.

(23) Wu, J.; Rush, T. S., 3rd; Hotchandani, R.; Du, X.; Geck, M.; Collins, E.; Xu, Z. B.; Skotnicki, J.; Levin, J. I.; Lovering, F. E. Identification of potent and selective MMP-13 inhibitors. *Bioorg. Med. Chem. Lett.* **2005**, 15, 4105-4109.

(24) Li, J.; Rush III, T. S.; Li, W.; DeVincentis, D.; Du, X.; Hu, Y.; Thomason, J. R.; Xiang, J. S.; Skotnicki, J. S.; Tam, S.; Cunningham, K. M.; Chockalingam, P. S.; Morris, E. A.; Levin, J. I. Synthesis and SAR of highly selective MMP-13 inhibitors. *Bioorg. Med. Chem. Lett.* **2005**, 15, 4961-4966.

(25) Reiter, L. A.; Freeman-Cook, K. D.; Jones, C. S.; Martinelli, G. J.; Antipas, A. S.; Berliner, M. A.; Datta, K.; Downs, J. T.; Eskra, J. D.; Forman, M. D.; Greer, E. M.; Guzman, R.; Hardink, J. R.; Janat, F.; Keene, N. F.; Laird, E. R.; Liras, J. L.; Lopresti-Morrow, L. L.; Mitchell, P. G.; Pandit, J.; Robertson, D.; Sperger, D.; Vaughn-Bowser, M. L.; Waller, D. M.; Yocum, S. A. Potent, selective pyrimidinetrione-based inhibitors of MMP-13. *Bioorg. Med. Chem. Lett.* **2006**, 16, 5822-5826.

(26) Freeman-Cook, K. D.; Reiter, L. A.; Noe, M. C.; Antipas, A. S.; Danley, D. E.; Datta, K.; Downs, J. T.; Eisenbeis, S.; Eskra, J. D.; Garmene, D. J.; Greer, E. M.; Griffiths, R. J.; Guzman, R.; Hardink, J. R.; Janat, F.; Jones, C. S.; Martinelli, G. J.; Mitchell, P. G.; Laird, E. R.; Liras, J. L.; Lopresti-Morrow, L. L.; Pandit, J.; Reilly, U. D.; Robertson, D.; Vaughn-Bowser, M. L.; Wolf-Gouviea, L. A.; Yocum, S. A. Potent, selective spiropyrrolidine pyrimidinetrione inhibitors of MMP-13. *Bioorg. Med. Chem. Lett.* **2007**, 17, 6529-6534.

(27) Hu, Y.; Xiang, J. S.; DiGrandi, M. J.; Du, X.; Ipek, M.; Laakso, L. M.; Li, J.; Li, W.; Rush, T. S.; Schmid, J.; Skotnicki, J. S.; Tam, S.; Thomason, J. R.; Wang, Q.; Levin, J. I. Potent, selective, and orally bioavailable matrix metalloproteinase-13 inhibitors for the treatment of osteoarthritis. *Biorg. Med. Chem.* **2005**, 13, 6629-6644.

(28) Chen, J. M.; Nelson, F. C.; Levin, J. I.; Mobilio, D.; Moy, F. J.; Nilakantan, R.; Zask, A.; Powers, R. Structure-Based Design of a Novel, Potent, and Selective Inhibitor for MMP-13 Utilizing NMR Spectroscopy and Computer-Aided Molecular Design. *J. Am. Chem. Soc.* **2000**, 122, 9648-9654.

(29) Johnson, A. R.; Pavlovsky, A. G.; Ortwine, D. F.; Prior, F.; Man, C.-F.; Bornemeier, D. A.; Banotai, C. A.; Mueller, W. T.; McConnell, P.; Yan, C.; Baragi, V.; Lesch, C.; Roark, W. H.; Wilson, M.; Datta, K.; Guzman, R.; Han, H.-K.; Dyer, R. D. Discovery and Characterization of a Novel Inhibitor of Matrix Metalloprotease-13 That Reduces Cartilage Damage in Vivo without Joint Fibroplasia Side Effects. *J. Biol. Chem.* **2007**, 282, 27781-27791.

(30) Vandenbroucke, R. E.; Libert, C. Is there new hope for therapeutic matrix metalloproteinase inhibition? *Nat. Rev. Drug Discov.* **2014**, 13, 904-927.

(31) Cathcart, J. M.; Cao, J. MMP Inhibitors: Past, present and future. *Frontiers in bioscience (Landmark edition)* **2015**, 20, 1164-1178.

(32) Wang, K.; Xu, J.; Hunter, D. J.; Ding, C. Investigational drugs for the treatment of osteoarthritis. *Expert opinion on investigational drugs* **2015**, 24, 1539-1556.

(33) Nara, H.; Sato, K.; Naito, T.; Mototani, H.; Oki, H.; Yamamoto, Y.; Kuno, H.; Santou, T.; Kanzaki, N.; Terauchi, J.; Uchikawa, O.; Kori, M. Discovery of novel, highly potent, and selective quinazoline-2-carboxamide-based matrix metalloproteinase (MMP)-13 inhibitors without a zinc binding group

using a structure-based design approach. *J. Med. Chem.* **2014**, 57, 8886-8902.

(34) Nara, H.; Sato, K.; Naito, T.; Mototani, H.; Oki, H.; Yamamoto, Y.; Kuno, H.; Santou, T.; Kanzaki, N.; Terauchi, J.; Uchikawa, O.; Kori, M. 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. *Bioorg. Med. Chem.* **2014**, 22, 5487-5505.

(35) Nara, H.; Kaieda, A.; Sato, K.; Naito, T.; Mototani, H.; Oki, H.; Yamamoto, Y.; Kuno, H.; Santou, T.; Kanzaki, N.; Terauchi, J.; Uchikawa, O.; Kori, M. Discovery of Novel, Highly Potent, and Selective Matrix Metalloproteinase (MMP)-13 Inhibitors with a 1,2,4-Triazol-3-yl Moiety as a Zinc Binding Group Using a Structure-Based Design Approach. Submitted for publication.

(36) Terauchi, J.; Kuno, H.; Nara, H.; Oki, H.; Sato, K. Preparation of heterocyclic amides as MMP-13 inhibitors for treating osteoarthritis and rheumatoid arthritis. WO2005105760A1, 2005.

(37) Nara, H.; Kaieda, A.; Sato, K.; Terauchi, J. Preparation of heterocyclic amide compounds as matrix metalloproteinase inhibitors. WO2007049820A1, 2007.

(38) Schnute, M. E.; Ruminski, P. G.; Hanau, C. E.; Strohbach, J. W.; Carroll, J. N.; Sample, K. Hetero bicyclic carboxamide derivatives, processes for preparing them, pharmaceutical compositions containing them, and their uses as inhibitors of matrix metalloproteinase enzymes. WO2008149191A1, 2008.

(39) Pochetti, G.; Montanari, R.; Gege, C.; Chevrier, C.; Taveras, A. G.; Mazza, F. Extra binding region induced by non-zinc chelating inhibitors into the S1' subsite of matrix metalloproteinase 8 (MMP-8). *J. Med. Chem.* **2009**, 52, 1040-1049.

(40) Gege, C.; Chevrier, C.; Schneider, M.; Bluhm, H.; Hochguertel, M.; Deng, H.; Gallagher, B. M.; Sucholeiki, I.; Taveras, A. Preparation of heterotricyclic metalloprotease inhibitors. WO2008063667A1, 2008.

(41) Breuer, E.; Frant, J.; Reich, R. Recent non-hydroxamate matrix metalloproteinase inhibitors. *Expert Opin. Ther. Pat.* **2005**, 15, 253-269.

(42) Rao, B. G. Recent Developments in the Design of Specific Matrix Metalloproteinase Inhibitors aided by Structural and Computational Studies. *Curr. Pharm. Des.* **2005**, 11, 295-322.

(43) Skiles, J. W.; Gonnella, N. C.; Jeng, A. Y. The Design, Structure, and Therapeutic Application of Matrix Metalloproteinase Inhibitors. *Curr. Med. Chem.* **2001**, 8, 425-474.

(44) Puerta, D. T.; Cohen, S. M. A Bioinorganic Perspective on Matrix Metalloproteinase Inhibition. *Curr. Top. Med. Chem.* **2004**, 4, 1551-1573.

(45) Whittaker, M.; Floyd, C. D.; Brown, P.; Gearing, A. J. H. Design and Therapeutic Application of Matrix Metalloproteinase Inhibitors. *Chem. Rev.* **1999**, 99, 2735-2776.

(46) Skiles, J. W.; Gonnella, N. C.; Jeng, A. Y. The Design, Structure, and Clinical Update of Small Molecular Weight Matrix Metalloproteinase Inhibitors. *Curr. Med. Chem.* **2004**, 11, 2911-2977.

(47) Fischer, T.; Riedl, R. Molecular Recognition of the Catalytic Zinc(II) Ion in MMP-13: Structure-Based Evolution of an Allosteric Inhibitor to Dual Binding Mode Inhibitors with Improved Lipophilic Ligand Efficiencies. *International journal of molecular sciences* **2016**, 17, 314.

(48) O'Brien, P. M.; Ortwine, D. F.; Pavlovsky, A. G.; Picard, J. A.; Sliskovic, D. R.; Roth, B. D.; Dyer, R. D.; Johnson, L. L.; Man, C. F.; Hallak, H. Structure-Activity Relationships and Pharmacokinetic Analysis for a Series of Potent, Systemically Available Biphenylsulfonamide Matrix Metalloproteinase Inhibitors. *J. Med.*

Chem. **2000**, 43, 156-166.

(49) Natchus, M. G.; Bookland, R. G.; Laufersweiler, M. J.; Pikul, S.; Almstead, N. G.; De, B.; Janusz, M. J.; Hsieh, L. C.; Gu, F.; Pokross, M. E.; Patel, V. S.; Garver, S. M.; Peng, S. X.; Branch, T. M.; King, S. L.; Baker, T. R.; Foltz, D. J.; Mieling, G. E. Development of New Carboxylic Acid-Based MMP Inhibitors Derived from Functionalized Propargylglycines. *J. Med. Chem.* **2001**, 44, 1060-1071.

(50) Pikul, S.; Ohler, N. E.; Ciszewski, G.; Laufersweiler, M. C.; Almstead, N. G.; De, B.; Natchus, M. G.; Hsieh, L. C.; Janusz, M. J.; Peng, S. X.; Branch, T. M.; King, S. L.; Taiwo, Y. O.; Mieling, G. E. Potent and Selective Carboxylic Acid-Based Inhibitors of Matrix Metalloproteinases. *J. Med. Chem.* **2001**, 44, 2499-2502.

(51) Natchus, M. G.; Bookland, R. G.; De, B.; Almstead, N. G.; Pikul, S.; Janusz, M. J.; Heitmeyer, S. A.; Hookfin, E. B.; Hsieh, L. C.; Dowty, M. E.; Dietsch, C. R.; Patel, V. S.; Garver, S. M.; Gu, F.; Pokross, M. E.; Mieling, G. E.; Baker, T. R.; Foltz, D. J.; Peng, S. X.; Bornes, D. M.; Strojnowski, M. J.; Taiwo, Y. O. Development of New Hydroxamate Matrix Metalloproteinase Inhibitors Derived from Functionalized 4-Aminoprolines. *J. Med. Chem.* **2000**, 43, 4948-4963.

(52) Ruminski, P. G.; Massa, M.; Strohbach, J.; Hanau, C. E.; Schmidt, M.; Scholten, J. A.; Fletcher, T. R.; Hamper, B. C.; Carroll, J. N.; Shieh, H. S.; Caspers, N.; Collins, B.; Grapperhaus, M.; Palmquist, K. E.; Collins, J.; Baldus, J. E.; Hitchcock, J.; Kleine, H. P.; Rogers, M. D.; McDonald, J.; Munie, G. E.; Messing, D. M.; Portolan, S.; Whiteley, L. O.; Sunyer, T.; Schnute, M. E. Discovery of *N*-(4-Fluoro-3-methoxybenzyl)-6-(2-(((2*S*,5*R*)-5-(hydroxymethyl)-1,4-dioxan-2-yl)met hyl)-2*H*-tetrazol-5-yl)-2-methylpyrimidine-4-carboxamide. A Highly Selective and Orally Bioavailable Matrix Metalloproteinase-13 Inhibitor for the Potential Treatment of Osteoarthritis. *J. Med. Chem.* **2016**, 59, 313-327.

(53) Janusz, M.; Hookfin, E.; Brown, K.; Hsieh, L.; Heitmeyer, S.; Taiwo, Y.; Natchus, M.; Pikul, S.; Almstead, N.; Peng, B.; Baker, T.; Patel, V. Comparison of the pharmacology of hydroxamate- and carboxylate-based matrix metalloproteinase inhibitors (MMPIs) for the treatment of osteoarthritis. *Inflammation Res.* **2006**, 55, 60-65.

(54) Kreiselmeier, A.; Ulmer, W.; Stove, J.; Wieland, H. A.; Gerwin, N.; Bartnik, E.; Schudok, M.; Heute, S.; Breitenfelder, M.; Scharf, H.-P.; Schwarz, M. L. R. Validation of a diffusion chamber as in vitro system for the analysis of compound diffusibility through cartilage tissue. *Biomedecine & Pharmacotherapy* **2005**, 59, 395-401.

(55) Peng, S. X.; VonBargen, E. C.; Bornes, D. M.; Pikul, S. Permeability of Articular Cartilage to Matrix Metalloprotease Inhibitors. *Pharm. Res.* **1998**, 15, 1414-1418.

(56) Gege, C.; Bao, B.; Bluhm, H.; Boer, J.; Gallagher, B. M.; Korniski, B.; Powers, T. S.; Steeneck, C.; Taveras, A. G.; Baragi, V. M. Discovery and evaluation of a non-Zn chelating, selective matrix metalloproteinase 13 (MMP-13) inhibitor for potential intra-articular treatment of osteoarthritis. *J. Med. Chem.* **2012**, 55, 709-716.

(57) Wada, C. K.; Holms, J. H.; Curtin, M. L.; Dai, Y.; Florjancic, A. S.; Garland, R. B.; Guo, Y.; Heyman, H. R.; Stacey, J. R.; Steinman, D. H.; Albert, D. H.; Bouska, J. J.; Elmore, I. N.; Goodfellow, C. L.; Marcotte, P. A.; Tapang, P.; Morgan, D. W.; Michaelides, M. R.; Davidsen, S. K. Phenoxyphenyl Sulfone *N*-Formylhydroxylamines (Retrohydroxamates) as Potent, Selective, Orally Bioavailable Matrix Metalloproteinase Inhibitors. *J. Med. Chem.* **2002**, 45, 219-232.

(58) Wada, C. K. The Evolution of the Matrix Metalloproteinase Inhibitor Drug Discovery Program at

Abbott Laboratories. *Curr. Top. Med. Chem.* **2004**, 4, 1255-1267.

(59) Cowden, C. J.; Wilson, R. D.; Bishop, B. C.; Cottrell, I. F.; Davies, A. J.; Dolling, U.-H. A new synthesis of 1,2,4-triazolin-5-ones: application to the convergent synthesis of an NK1 antagonist. *Tetrahedron Lett.* **2000**, 41, 8661-8664.

(60) The PyMOL Molecular Graphics System. DeLano Scientific LLC, Palo Alto, CA.

(61) Abbenante, G.; Fairlie, D. P. Protease inhibitors in the clinic. *Med. Chem.* **2005**, 1, 71-104.

(62) Sugiyama, Y.; Sasaki, T.; Nagato, N. Acid-catalyzed reaction of ethyl cyanoformate with aromatic amines in acetic acid: facile synthesis of N-substituted amidinoformic acids and ethyl 4-quinazolone-2-carboxylate. *J. Org. Chem.* **1978**, 43, 4485-4487.

(63) Jacobsen, N. W.; McCarthy, B. L.; Smith, S. *N*-Aminopyrimidines: the Isomerization of 1-Aminobarbituric Acids to 2-(5-Oxo-4,5-dihydro-1*H*-1,2,4-triazol-3-yl) Aliphatic Acids. *Aust. J. Chem.* **1979**, 32, 161-165.

(64) Noe, M. C.; Reiter, L. A.; Wythes, M. J. Pyrimidine-2,4,6-trione metalloproteinase inhibitors. WO2002034726.

(65) Edsall, R. J.; Harris, H. A.; Manas, E. S.; Mewshaw, R. E. ER_β Ligands. Part 1: The discovery of ER_β selective ligands which embrace the 4-hydroxy-biphenyl template. *Biorg. Med. Chem.* **2003**, 11, 3457-3474.

(66) Sato, H.; Kinoshita, T.; Takino, T.; Nakayama, K.; Seiki, M. Activation of a recombinant membrane type 1-matrix metalloproteinase (MT1-MMP) by furin and its interaction with tissue inhibitor of metalloproteinases (TIMP)-2. *FEBS Lett.* **1996**, 393, 101-104.