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# 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

*Hiroshi Nara, \* Akira Kaieda, Kenjiro Sato, Takako Naito, Hideyuki Mototani, Hideyuki*

*Oki, Yoshio Yamamoto, Haruhiko Kuno, Takashi Santou, Naoyuki Kanzaki, Jun*

*Terauchi, Osamu Uchikawa, and Masakuni Kori*

Pharmaceutical Research Division, Takeda Pharmaceutical Company Limited, 26-1,

Muraoka-Higashi 2-chome, Fujisawa, Kanagawa 251-8555, Japan

## Abstract

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

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6 MMP-13 ( $IC_{50} = 0.036$  nM) and selectivities (greater than 1,500-fold) over other MMPs  
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9 (MMP-1, 2, 3, 7, 8, 9, 10, and 14) and tumor necrosis factor- $\alpha$  converting enzyme  
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11 (TACE). Furthermore, the inhibitor was shown to protect bovine nasal cartilage  
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13 explants against degradation induced by interleukin-1 and oncostatin M. In this article,  
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15 we report the discovery of extremely potent, highly selective, and orally bioavailable  
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18 fused pyrimidine derivatives that possess a 1,2,4-triazol-3-yl group as a novel ZBG for  
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21 selective MMP-13 inhibition.  
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6 INTRODUCTION  
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10 Osteoarthritis (OA) is one of the most common forms of arthritis affecting more than 30  
11 million patients worldwide.<sup>1</sup> The principal morphological characteristic of OA is  
12 progressive cartilage damage that leads to pain and reduced mobility in affected joints.  
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22 pharmaceutical therapies in the guidelines from the American College of Rheumatology  
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29 limited to alleviation of pain and inhibition of inflammation.<sup>2-5</sup> The therapies are oral  
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32 treatment with acetaminophen, non-steroidal anti-inflammatory drugs (NSAIDs), or  
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35 cyclooxygenase-2 (COX-2) inhibitors and intra-articular injections of hyaluronic acid or  
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38 corticosteroids. In addition, because some COX-2 selective inhibitors (rofecoxib and  
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40  
41 valdecoxib) were withdrawn from the market in 2004 and 2005,<sup>6,7</sup> there is a significant  
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44 unmet medical need for safe oral disease-modifying osteoarthritis drugs that may be  
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47 able to prevent, slow down, or reverse any advanced cartilage destruction.<sup>8,9</sup>  
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50 The matrix metalloproteinases (MMPs) are a family of structurally related  
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53 zinc-dependent endopeptidases that degrade varied extracellular matrix. Among MMPs,  
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56 MMP-13 (collagenase-3) specifically catalyzes the hydrolysis of type II collagen,<sup>10,11</sup>  
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6 the main structural component of the cartilage matrix, while the protein is resistant to  
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9 most proteases. MMP-13 is expressed at higher levels by OA chondrocytes than by  
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12 normal chondrocytes.<sup>12</sup> In addition, regulated expression of human MMP-13 in joint  
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15 cartilages induces OA in genetically modified mice.<sup>13</sup> Further, an MMP inhibitor that  
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18 preferentially inhibits MMP-13 has been shown to block the degradation of explanted  
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21 human OA cartilage.<sup>14</sup> These findings suggest that MMP-13 plays a crucial role in the  
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24 destruction of articular cartilage in OA.

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27 In preclinical testing, MMP inhibitors have inhibited the destruction of cartilage in some  
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30 animal models of OA.<sup>15</sup> However, most clinical trials of broad-spectrum MMP  
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33 inhibitors have been discontinued due to concerns of dose-limiting toxicity (skin rash  
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35  
36 and musculoskeletal side effects (MSS) characterized by joint stiffness and pain).

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39 Although a number of hypotheses have been proposed for the cause of MSS, including a  
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42 non-selective inhibition of other metalloproteinases or a combined inhibition of a series  
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45 of critical MMPs, the pharmacological basis for such joint side effects remains  
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47  
48 unknown.<sup>16-18</sup> Therefore, considerable interest has been directed toward potent  
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51 inhibitors of MMP-13 with a high degree of selectivity over other MMPs, which may  
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54 avoid undesirable side effects.<sup>19-29</sup> Most MMP inhibitors generally incorporate a P1'  
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57 fragment that can be accommodated in the S1' subsite of the enzyme active site and a  
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6 functional group capable of binding the catalytic zinc ion. While a number of P1'  
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9 fragment allows modulation of potency and selectivity against various MMPs, only a  
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11 small set of zinc binding groups (ZBGs) have been identified and employed in the  
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13 design of selective MMP inhibitors.<sup>27, 30-32</sup>  
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18 High-throughput screening for MMP-13 inhibition led to a moderately potent ( $IC_{50} = 12$   
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20 nM) quinazoline-2-carboxamide **1**<sup>23, 25, 33</sup> and a weakly potent ( $IC_{50} = 1,900$  nM)  
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22 triazole **2**, which were successfully co-crystallized with MMP-13 catalytic domain  
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27 (**Figure 1**). These observations allowed the design of a hybrid molecule that combined  
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29  
30 the quinazoline **1** with the triazole ZBG **2** as shown in **Figure 2**.  
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33  
34 We have previously used the fused pyrimidine-2-carboxamide-4-one scaffold  
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36 represented by compound **3** for initial validation of a strategy to obtain inhibitory  
37  
38 potency and selectivity for MMP-13 by utilizing (i) a hydrophobic interaction with the  
39  
40 S1' and its side pocket (S1'') as clearly revealed by X-ray analysis and (ii) a hydrogen  
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42 bonding interaction with the  $\epsilon$ -amino group of Lys140 at the bottom of the S1'' pocket  
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48 (**Figure 3**).<sup>23, 25, 33, 34</sup> Furthermore, the fused pyrimidine system has been successfully  
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50 applied by several groups.<sup>35-37</sup> To further increase our repertoire of selective MMP-13  
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52 inhibitors, we next investigated the potential of substituents on the aromatic ring of the  
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6 *N*-benzyl group of **1**, thereby potentially addressing the catalytic zinc as shown in

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9 **Figure 2 (C)**. Beside our early patent application,<sup>34</sup> a similar combination approach was  
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11 recently reported by Fischer et al.<sup>38</sup>  
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16 In this article, we report the design, synthesis, and biological activity of novel fused  
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18 pyrimidine derivatives which possess a 1,2,4-triazol-3-yl group as a ZBG with potent  
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20 MMP-13 inhibitory activities, excellent selectivities, and good oral bioavailabilities.  
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## 24 25 26 27 28 29 CHEMISTRY

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33 **Scheme 1** and **Scheme 2** depict the synthesis of benzylamine derivatives used in  
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36 **Scheme 4** and **Scheme 5**. As shown in **Scheme 1**, benzylamine with an aliphatic side  
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38 chain bearing a carboxylic ester functionality **6** was synthesized by catalytic  
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40 hydrogenation of the cyano group of benzonitrile **5** prepared by alkylation of  
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42 3-cyanophenol **4** with ethyl 4-bromobutanoate. Another series of compounds that has a  
43  
44 thioether linker was also synthesized. Benzylamines **9a–c** were obtained in the  
45  
46 following manner. Alkylation of 3-cyanophenol (**4**) with the corresponding  
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48 1-bromoalkyl chloride afforded alkyl chlorides **7a–c**. Substitution reaction of the  
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50 chlorides **7a–c** with triazolylthiols afforded **8a**, **8b**, and **8d**. Benzonitrile **8a** underwent  
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6 Raney nickel catalyzed hydrogenation to give the benzylamine derivative **9a**.  
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9 Triphenylmethyl protection followed by reduction with lithium aluminum hydride of  
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11 each cyano group of benzonitriles **8b** and **8d** led to the benzylamines **9b** and **9c**,  
12  
13 respectively. Benzylamine analogues **15a** and **15b** with diether linkers were synthesized  
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15 by the following method. Commercially available nitrotriazole **10** was protected in a  
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17 regioselective manner with the sterically demanding triphenylmethyl group to give  
18  
19 3-nitro-1-(triphenylmethyl)-1*H*-1,2,4-triazole (**11**). Alkylation of commercially  
20  
21 available 3-cyanophenol (**4**) with ethyl bromoacetate gave ethyl ester **12**. Subsequent  
22  
23 chemoselective reduction of the ester group with sodium borohydride afforded  
24  
25 benzonitrile **13a**. One-carbon homologue of **13a**, benzonitrile **13b**, was obtained  
26  
27 directly from 3-cyanophenol **4** by alkylation with 3-bromopropanol.<sup>39</sup> Precursors of  
28  
29 benzylamines **15a** and **15b**, benzonitriles **14a** and **14b**, were synthesized by aromatic  
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31 nucleophilic substitution reaction of nitrotriazole **11** with hydroxyalkyl substituted  
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33 benzonitriles **13a** or **13b**, respectively. Lithium aluminum hydride reduction of the  
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35 cyano group of benzonitriles **14a** and **14b** led to the desired benzylamine derivatives  
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49 **15a** and **15b**.  
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53 Carboxylic acid derivatives **20a–c** were prepared as shown in **Scheme 3**.  
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56 Thiosemicarbazide **16** was reacted with ethyl 3-chloro-3-oxopropanoate or ethyl  
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6 succinyl chloride followed by cyclization with sodium ethoxide to give triazole-3-thiol  
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9 **18a** and **18b**, respectively. Desulfurization of **18a** using Raney Ni provided ester **19c** in  
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11 a moderate yield (54%), while desulfurization of **18b** with sodium nitrite in nitric acid  
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13 and concomitant hydrolysis of the ester group led to carboxylic acid **19d** in excellent  
14  
15 yield (99%). Reesterification of **19d** and triphenylmethyl protection gave ester **19f**.  
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20 Alkaline hydrolysis of esters **19b**, **19c**, and **19f** afforded the desired carboxylic acids  
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23 **20a–c**, respectively.  
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27 The synthetic routes for 4-oxo-3,4-dihydroquinazoline-2-carboxamide derivatives **22a–**  
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29 **c** and **23** via ethyl 4-oxo-3,4-dihydroquinazoline-2-carboxylate (**21**) are shown in  
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33 **Scheme 4**. Taking advantage of the high reactivity of the ester group at the 2-position of  
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35 the quinazoline **21**, amide formations at the 2-position of 4-oxo-3,4-dihydroquinazoline  
36  
37 were achieved by aminolysis of the ethyl 4-oxo-3,4-dihydroquinazoline-2-carboxylate  
38  
39 (**21**) with the corresponding benzylamines **6** and **9a** (see **Scheme 1** for preparation) to  
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41  
42 give the compounds **22a** and **23**, respectively.<sup>23, 25, 40</sup> The ethyl ester **22a** was  
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45 hydrolyzed, and the resulting carboxylic acid **22b** was treated with oxalyl chloride  
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48 followed by hydroxylamine to afford the desired hydroxamic acid **22c**.  
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54 The synthesis of 4-oxo-3,4-dihydrothienopyrimidine-2-carboxamide derivatives is  
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6 demonstrated in **Scheme 5**. The thienopyrimidine derivatives **25a–g** were synthesized in  
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8 the same manner as described for the quinazoline derivatives in **Scheme 4**.<sup>23</sup>

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10 Accordingly, aminolysis of the commercially available ethyl  
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12 5-methyl-4-oxo-3,4-dihydrothieno[2,3-*d*]pyrimidine-2-carboxylate (**24**) with  
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14 corresponding benzylamines, either commercially available or synthesized (**9a–c** and  
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16 **15a**) as described in **Scheme 1** and **Scheme 2**, and if required, followed by the removal  
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18 of the triphenylmethyl groups under an acidic condition gave the amide derivatives **26a**,  
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20 **26b**, and **25a–d**. Deprotection of the Boc group of the amide **26b** afforded amine **26c**.

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Condensation of the resulting amines **26a** and **26c** with corresponding carboxylic acids  
20c and **20a** (see **Scheme 3** for preparation), followed by removal of the  
triphenylmethyl group afforded the amide-linked derivatives **25g** and **25e**, respectively.

Similarly, condensation of **26c** with **20b** (see **Scheme 3** for preparation) led to **25f**. In  
the triphenylmethyl deprotection step for **25b–e** and **25g**, cation scavengers are essential  
for achieving consistently high yields of the triazoles, with triethylsilane proving to be  
superior to anisole in terms of reaction reproducibility.

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

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6 one-pot Gewald reaction.<sup>41</sup> Thus, Knoevenagel adducts of the ketones **27a–g** and ethyl  
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8 cyanoacetate **28** were prepared and subsequently treated with elemental sulfur and  
9  
10 morpholine in toluene.<sup>42</sup> Ester intermediates **30a–m** were obtained by reaction of  
11  
12 thiophenes **29a–m** with ethyl cyanoformate in 1 M hydrogen chloride in acetic acid  
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14 solution. In the same fashion as for the synthesis of 5-methylthienopyrimidine  
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16 derivatives shown in **Scheme 5**, aminolysis of the reactive ester group at the 2-position  
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18 of the key intermediates **30a–m** with benzylamines **15a** and **15b** (see **Scheme 2** for  
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20 preparation) followed by deprotection of the triphenylmethyl group led to the  
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22 corresponding amide derivatives **31a–q**.  
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## 37 RESULTS AND DISCUSSION

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40 A lead quinazoline derivative **1**<sup>23, 25</sup> and a triazole derivative **2** were identified by high  
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42 throughput screening as MMP-13 inhibitors (**Figure 1**). Interestingly, the quinazoline **1**  
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44 did not possess an obvious ZBG, such as a hydroxamic acid or a carboxylic acid, and  
45  
46 displayed only 25-fold selectivity over other MMP isoenzymes (**Table 4**).  
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53 An X-ray crystallographic study of the quinazoline **1** bound to MMP-13 revealed that  
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55 no interaction with the catalytic zinc was observed and that the quinazoline ring  
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6 occupies the deep S1' subsite<sup>17, 25</sup>(**Figure 4** (A) and **4** (B)). On the other hand, the  
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9 1,2,4-triazol-3-yl moiety of the triazole **2** coordinates in a monodentate fashion to the  
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11  
12 catalytic zinc (II) center. Furthermore, unlike the quinazoline **1**, the  
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14 phenylaminothiazole moiety of **2** only partially fills the available space within the  
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17 MMP-13 S1' pocket (**Figure 4** (C) and (D)). On the basis of a superposition of the  
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20 crystal structures, the distance between the phenyl ring on the side chain and the ZBG  
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23 was estimated to be about 4 Å (**Figure 5**). As shown in **Table 1**, analogues were  
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26 designed and synthesized by connection of the methoxy moiety of the benzyl unit of the  
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29 quinazoline **1** with various ZBGs by a simple linker element.  
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33 Compounds **22a** and **22b** having an ester or a carboxylic acid group via a  
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36 *n*-propyleneoxy linker at the 3-position of the left-hand phenyl group showed a decrease  
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39 in MMP-13 inhibitory activity in comparison to the original lead quinazoline **1**.  
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42 Conversion of the carboxylic acid to hydroxamic acid, known as a potent ZBG, resulted  
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45 in a slight increase in potency (**22c** vs **1**). On the other hand, quinazoline/triazole hybrid  
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48 compound **23** that is linked to the triazolyl group through a four-atom linker showed a  
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51 28-fold increase in inhibitory activity against MMP-13 (**23** vs **1**).  
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54 We have previously shown that the fused pyrimidine core markedly influences the  
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6 MMP-13 inhibitory potency, and thus, the quinazoline scaffold was replaced with a  
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9 previously identified thieno[2,3-*d*]pyrimidine core **25a–g** (Table 2).<sup>23</sup> The scaffold  
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11  
12 gave analogues that were potent compounds (**25a**, **25b**, and **25d**) in the MMP-13  
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15 inhibitory assay, however, an increased CYP3A4 inhibition was observed for the  
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18 compounds with sulfur-containing linkers (**25a–c**). Investigation of the length of the  
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21 linker between the benzylamide at the 2-position of the pyrimidine and the  
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24 1,2,4-triazol-3-yl group revealed that the compound with a five-atom linker exhibited  
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27 comparable activity (**25b** vs **25a**) to that of a four-atom linker, but a six-atom linker  
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30 derivative showed reduced activity (**25c** vs **25a**). Since the inhibition of the CYP3A4  
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33 enzyme is well known to potentially induce drug–drug interactions, elimination of such  
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36 undesired property was clearly necessary. Fortuitously, the undesirable CYP3A4  
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39 inhibition was decreased in the oxygen linker analogue **25d**. A series of compounds  
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42 with more rigid linkers (**25e–g**) showed decreased potency compared to that of  
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45 compound **25a**. Subsequent rat pharmacokinetic studies revealed that thienopyrimidine  
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48 **25d** with an ethylenedioxy linker was one of the most potent inhibitors, had the least  
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51 risk of CYP3A4 inhibition among a series of fused pyrimidine derivatives, and showed  
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54 high oral exposure (AUC = 10855 ng•h/mL) in rats (*F*% = 18). Further optimization to  
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57 improve the pharmacokinetic profile and attenuation of off-target CYP3A4 inhibition  
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6 while maintaining high potency was attempted at 5-position of the thienopyrimidine  
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9 core.

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12 In the course of another study of non-zinc binding inhibitors having the  
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14 thienopyrimidine core, it was found that the 5-position of the core can accommodate a  
15  
16 wide range of substituents without loss of potency.<sup>23</sup> In **Table 3**, MMP-13 inhibitory  
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18 activities, CYP3A4 inhibitory activities, and pharmacokinetic parameters of  
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20 5-substituted thienopyrimidine derivatives are summarized. Introduction of an isopropyl  
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22 group at the 5-position maintained a potent MMP-13 inhibitory activity (**31a**), and  
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24 substitution of the isopropyl group with a phenyl group showed a 3-fold increased  
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26 MMP-13 inhibitory activity (**31d**). Replacement of the phenyl group with thiophenes  
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28 maintained potent MMP-13 inhibitory activities (**31b** and **31c**), however, increased  
29  
30 unwanted CYP3A4 inhibitory activities. Introduction of an additional substituent at the  
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32 para position of the phenyl ring tended to reduce MMP-13 inhibitory activities (**31g**, **31j**,  
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34 and **31m**). On the other hand, substitution at the meta position exhibited a tendency to  
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36 retain potency even with rather bulky substituent (**31f**, **31i**, and **31l**). Substitution by a  
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38 fluoro group (**31e**) or a chloro group (**31h**) at the ortho position of the phenyl ring also  
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40 retained MMP-13 inhibitory activities. However, a methoxy group at the ortho-position  
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42 of the benzene ring (**31k**) significantly reduced the activity. The reduction of the activity  
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6 observed for **31k** compared to **31l** could be attributed to a steric repulsion between the  
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8 relatively large *o*-methoxy group of **31k** and the wall of the S1' subsite. Five-atom  
9  
10 linker compounds, such as **31n**, **31o**, **31p**, and **31q** gave comparable MMP-13 inhibitory  
11  
12 activities compared to those of the corresponding four-atom linker series **31e**, **31f**, **31h**,  
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14 and **31i**, but the five-atom series showed potent CYP3A4 inhibition. It is noteworthy  
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16 that in contrast to the triazole ZBG series exemplified by **31i** and **31q**, incorporation of  
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18 the *m*-chloro-substituted 5-aryl group into other triazolone ZBG series was not proved  
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20 to be well tolerated in terms of MMP-13 inhibitory activity.<sup>19</sup> Among the 5-substituted  
21  
22 thienopyrimidine derivatives, *m*-fluorophenyl **31f** showed the best combination of  
23  
24 CYP3A4 inhibition risk and oral exposure at a dose of 1 mg/kg in rats and mice ( $F^0$  =  
25  
26 33 and 38, respectively). The overall properties of **31f** made it an attractive candidate  
27  
28 for further preclinical evaluation towards the treatment of MMP-13 related diseases.  
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30

31  
32 Compound **31f**, which exhibited a highly potent MMP-13 inhibitory activity and a  
33  
34 promising DMPK profile, was assessed for its selectivity profile against other matrix  
35  
36 metalloproteinase homologues including MMP-1, 2, 3, 7, 8, 9, 10, 14, and TACE as  
37  
38 shown in **Table 4**. Compound **31f** exhibited 5,000-fold selectivity for MMP-13 over  
39  
40 MMP-2, >1,500-fold selectivity over MMP-10, and >27,000-fold selectivity over  
41  
42 MMP-1, 3, 7, 8, 9, 14, and TACE. Consequently, compound **31f** is one of the most  
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6 selective and potent MMP-13 inhibitors containing a ZBG.  
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10 As exemplified by the crystal structure of the triazole inhibitor **3** bound to MMP-13,  
11  
12 obtained by soaking experiment, the 1,2,4-triazol-3-yl group of **23** coordinates to the  
13  
14 catalytic zinc ion of MMP-13 shown in **Figure 6**. The quinazoline ring system of the  
15  
16 hybrid inhibitor **23** fills the deep S1' pocket, the enzyme's specificity pocket, of  
17  
18 MMP-13 consisting of Thr245 and Thr247 in the same fashion as the binding mode of  
19  
20 the lead quinazoline **1** (**Figure 4 (B)**). Although the four-atom linker between the  
21  
22 triazole ring and the phenyl group has no obvious interaction with MMP-13, the linker  
23  
24 should play an important role in the orientation of the triazolyl group to form the metal  
25  
26 binding. The triazolyl group of **23** is coordinated to the zinc ion in a monodentate  
27  
28 fashion as that of the lead triazole **2** (**Figure 4 (D)**). The inhibitor **23** is stabilized at the  
29  
30 S1' site of MMP-13 by three possible hydrogen bonds: (a) the O4 carbonyl oxygen of  
31  
32 the quinazoline ring and the backbone amide of Thr247, (b) the N3 amide hydrogen of  
33  
34 quinazoline ring and the carbonyl oxygen of Thr245, and (c) the exocyclic carbonyl  
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36 oxygen at the 2-position of the quinazoline ring and the backbone amide of Thr245. The  
37  
38 observed  $\beta$ -sheet type interaction confers the potent inhibitory activity of the  
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quinazolin-4-one-2-carboxamide inhibitors.



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6 Compound **31f** was further evaluated for its ability to block the release of collagen from  
7  
8 cartilage. To examine the chondroprotective effect of the MMP-13 selective inhibitor, a  
9  
10 bovine nasal cartilage (BNC) assay was performed. The BNC assay is an established  
11  
12 and widely used procedure that allows evaluation of cartilage degradation by  
13  
14 upregulating proteolytic enzymes such as the collagenases MMP-1 and MMP-13 in  
15  
16 *vitro*.<sup>43-49</sup> The chondrocyte-mediated degradation of cartilage was studied using bovine  
17  
18 nasal cartilage slices cultured for up to 14 days in the presence or absence of MMP  
19  
20 inhibitors. Collagen release, measured quantitatively as hydroxyproline, was stimulated  
21  
22 by pro-inflammatory cytokines interleukin-1 (IL-1) and oncostatin M (OSM).  
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33 As shown in **Table 5**, the protection of cartilage degradation by compound **31f** in this  
34  
35 assay was concentration dependent and statistically significant at 1  $\mu$ M (70.8% of  
36  
37 inhibition), whereas at the same concentration of 1  $\mu$ M, **32 (RS-130,830<sup>50</sup>)**, which  
38  
39 inhibits MMPs broadly, showed complete inhibition. This difference of inhibitory  
40  
41 activity profile between **31f** and **32** in this assay may be explained by high isoform  
42  
43 selectivity of **31f** for MMP-13 over other collagenolytic enzymes in the  
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45 chondroprotective efficacy and/or a difference in the degree of tissue permeability of the  
46  
47 two inhibitors.  
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## CONCLUSIONS

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

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6 rothieno[2,3-*d*]pyrimidine-2-carboxamide (**31f**) exhibited excellent potency ( $IC_{50}$  =  
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8 0.036 nM) and selectivity (greater than 1,500-fold) over other MMPs (MMP-1, 2, 3, 7,  
9  
10 8, 9, 10, and 14) and TACE, and demonstrated favorable pharmacokinetic parameters,  
11  
12 making it an attractive candidate for further studies. To evaluate compound **31f** for its  
13  
14 potential utility in the treatment of collagenase related disease and disorders, BNC assay  
15  
16 was employed. The selective MMP-13 inhibitor **31f** was effective at preventing the  
17  
18 IL-1/OSM induced in vitro degradation of BNC (70.8% inhibition of cartilage  
19  
20 degradation at 1  $\mu$ M). Taken together with the fact that MMP-13 is indicated as the  
21  
22 primary collagenase in the human OA cartilage, selective MMP-13 inhibitors may be a  
23  
24 potential treatment of OA while avoiding the toxicity associated with inhibition of  
25  
26 MMPs other than MMP-13.  
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#### 43 EXPERIMENTAL SECTION

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46 **General Methods.** Melting points were determined in open capillary tubes on a Büchi  
47  
48 melting point apparatus B545 and are uncorrected.  $^1H$  NMR spectra were recorded on a  
49  
50 Bruker DPX-300 (300 MHz) or a Bruker Avance 400 (400 MHz) spectrometer and are  
51  
52 reported in parts per million ( $\delta$ ) relative to tetramethylsilane (TMS:  $\delta$ 0.00 ppm). Data  
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6 are reported as follows: chemical shift, integration, multiplicity (s = singlet, d = doublet,  
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8 t = triplet, q = quartet, m = multiplet, dd = doublet of doublet, dt = doublet of triplet, tt =  
9  
10 triplet of triplet, bs = broad singlet), and coupling constants ( $J$ , Hz). Unless otherwise  
11  
12 specified, all solvents and reagents were obtained from commercial suppliers and used  
13  
14 without further purification. Column chromatography was performed using Merck silica  
15  
16 gel 60 (70–230 mesh). Thin-layer chromatography (TLC) was performed on Merck  
17  
18 silica gel plates 60F254. Preparative HPLC was performed on a Shiseido CAPCELL  
19  
20 PACK C-18 UG120 S-5 column (20 mm $\Phi$   $\times$  50 mm), eluting at a flow rate of 25  
21  
22 mL/min with a linear gradient of water (0.1% TFA)/acetonitrile (0.1% TFA) from 90:10  
23  
24 to 0:100 over 10 min. UV detection was at 220 nm.  
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36 The purity of all compounds used in biological studies was determined to be  $\geq 95\%$  by  
37  
38 elemental analysis or HPLC analysis. Elemental analyses were performed by Takeda  
39  
40 Analytical Research Laboratories, Ltd. Experimentally determined hydrogen, carbon,  
41  
42 and nitrogen composition by elemental analysis was within  $\pm 0.4\%$  of the expected  
43  
44 value, implying a purity of  $\geq 95\%$ . Liquid chromatography–mass spectrometry  
45  
46 (LC/MS) analysis was performed using one of the following two conditions: (a)  
47  
48 L-column 2 ODS (50 mm  $\times$  3.0 mm I.D., 3  $\mu$ m particle size, CERI, Japan) in a  
49  
50 Shimadzu LC-20AD equipped with a Shimadzu LCMS-2020 eluting with 5 mM  
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6 AcONH<sub>4</sub> in ultrapure water/acetonitrile = 90/10 (mobile phase A) and 5 mM AcONH<sub>4</sub>  
7  
8  
9 in ultrapure water/acetonitrile = 10/90 (mobile phase B), using the following elution  
10  
11  
12 gradient of 5% B to 90% B over 0.9 min followed by 90% B isocratic over 1.1 min at a  
13  
14  
15 flow rate of 1.5 mL/min (UV detection at 220 or 254 nm); (b) Shiseido CAPCELL  
16  
17  
18 PACK C-18 UG120 S-3 column (1.5 mm $\Phi$   $\times$  35 mm) in a Waters Alliance 2795 or an  
19  
20  
21 Agilent 1100 LC system equipped with a Waters 2487 absorbance detector and a  
22  
23  
24 Micromass ZQ2000 mass spectrometer. Analytes were eluted using a linear gradient of  
25  
26  
27 water (0.05% TFA)/acetonitrile (0.04% TFA) from 90:10 to 0:100 over 4 min at a flow  
28  
29  
30 rate of 0.5 mL/min. UV detection was at 220 nm.

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32  
33 **Ethyl 4-[(3-cyanophenyl)oxy]butanoate (5)**. A solution of 4-bromobutanoic acid (25.0  
34  
35  
36 g, 150 mmol), DMF (5 drops), and oxalyl chloride (17.0 mL, 195 mmol) in  
37  
38  
39 dichloromethane (250 mL) was stirred at room temperature for 5 h. The reaction  
40  
41  
42 mixture was concentrated under reduced pressure, dichloromethane (200 mL) and  
43  
44  
45 ethanol (9.0 mL, 170 mmol) were added to the residue, and the mixture was stirred at  
46  
47  
48 room temperature for 15 h. The reaction mixture was washed with saturated aqueous  
49  
50  
51 NaHCO<sub>3</sub> solution and brine, and the organic layer was dried over Na<sub>2</sub>SO<sub>4</sub>, and  
52  
53  
54 concentrated. The residue was purified by silica gel column chromatography (0–5%  
55  
56  
57 ethyl acetate/hexane) to give ethyl 4-bromobutanoate as a colorless oil (24.5 g, 84%). A  
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6 suspension of 3-hydroxybenzonitrile (**4**) (5.00 g, 42.0 mmol) and 60% sodium hydride  
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8  
9 (oil dispersion, 2.01 g, 50.3 mmol) in DMF (200 mL) was stirred at room temperature  
10  
11  
12 for 30 min, and ethyl 4-bromobutanoate (9.82 g, 50.3 mmol) was added to the mixture.  
13  
14  
15 The mixture was stirred at room temperature for 15 h and concentrated under reduced  
16  
17  
18 pressure. The residue was extracted with ethyl acetate and saturated aqueous NH<sub>4</sub>Cl  
19  
20  
21 solution. The organic layer was washed with saturated aqueous NH<sub>4</sub>Cl solution and  
22  
23  
24 brine, dried over Na<sub>2</sub>SO<sub>4</sub>, and concentrated. The obtained residue was purified by silica  
25  
26  
27 gel column chromatography (0–10% ethyl acetate/hexane) to give the title compound as  
28  
29  
30 a colorless oil (10.2 g, quant.). <sup>1</sup>H NMR (300 MHz, CDCl<sub>3</sub>) δ 1.27 (3H, t, *J* = 7.2 Hz),  
31  
32 2.13 (2H, tt, *J* = 6.7, 6.7 Hz), 2.52 (2H, t, *J* = 7.2 Hz), 4.03 (2H, t, *J* = 6.1 Hz), 4.16 (2H,  
33  
34 q, *J* = 7.2 Hz), 7.08–7.16 (2H, m), 7.21–7.26 (1H, m), 7.32–7.40 (1H, m).  
35  
36  
37  
38

39 **Ethyl 4-{{3-(Aminomethyl)phenyl}oxy}butanoate Hydrochloride (6)**. A suspension  
40  
41  
42 of compound **5** (8.50 g, 36.4 mmol), 10% palladium on carbon (containing 50% water)  
43  
44  
45 (12.8 g), and formic acid (98 mL) in methanol (80 mL) was stirred at room temperature  
46  
47  
48 for 5 h under hydrogen atmosphere at 1 atm. The catalyst was filtered off through a  
49  
50  
51 Celite pad, and the filtrate was concentrated under reduced pressure. To the residue was  
52  
53  
54 added 4 N hydrogen chloride in ethyl acetate solution (15.0 mL, 60.0 mmol). The  
55  
56  
57 mixture was stirred at room temperature for 1 h and concentrated under reduced  
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6 pressure. The residue was crystallized from toluene, and the crude crystals were  
7  
8  
9 recrystallized from diethyl ether to give the title compound as a white powder (7.79 g,  
10  
11 78% for 2 steps).  $^1\text{H}$  NMR (300 MHz, DMSO- $d_6$ )  $\delta$  1.18 (3H, t,  $J = 7.2$  Hz), 1.98 (2H, tt,  
12  
13  $J = 6.8, 6.8$  Hz), 2.46 (2H, t,  $J = 7.3$  Hz), 3.95–4.03 (4H, m), 4.07 (2H, q,  $J = 7.1$  Hz),  
14  
15  
16  
17 6.92 (1H, dd,  $J = 8.2, 2.2$  Hz), 7.04 (1H, d,  $J = 7.3$  Hz), 7.12 (1H, s), 7.31 (1H, t,  $J = 7.9$   
18  
19 Hz), 8.26 (3H, s).  
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22  
23

24 **3-[(2-Chloroethyl)oxy]benzonitrile (7a)**. A suspension of 3-hydroxybenzonitrile (**4**)  
25  
26 (5.00 g, 42.0 mmol), 1-bromo-2-chloroethane (9.00 g, 62.8 mmol), and potassium  
27  
28 hydroxide (2.50 g, 44.6 mmol) in ethanol (100 mL) was stirred at 90 °C for 24 h. After  
29  
30 cooling to room temperature, the reaction mixture was concentrated under reduced  
31  
32 pressure. To the residue were added diethyl ether and water. The organic layer was  
33  
34 washed with brine, dried over  $\text{Na}_2\text{SO}_4$ , and concentrated. The residual oil was purified  
35  
36 by silica gel column chromatography (2–50% ethyl acetate/hexane) to give the title  
37  
38 compound as a colorless oil (1.39 g, 18%).  $^1\text{H}$  NMR (300 MHz,  $\text{CDCl}_3$ )  $\delta$  3.83 (2H, t,  $J$   
39  
40 = 5.7 Hz), 4.25 (2H, t,  $J = 5.7$  Hz), 7.14–7.19 (2H, m), 7.29 (1H, dt,  $J = 7.6, 1.3$  Hz),  
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50  
51 7.37–7.43 (1H, m).  
52  
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54 **3-[(3-Chloropropyl)oxy]benzonitrile (7b)**. A suspension of 1-bromo-3-chloropropane  
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6 (13.9 g, 88.1 mmol) and 60% sodium hydride (oil dispersion, 3.02 g, 126 mmol) in  
7  
8  
9 ethanol (50 mL) was stirred at room temperature for 30 min. To the reaction mixture  
10  
11  
12 was added 3-hydroxybenzotrile (**4**) (10.0 g, 83.9 mmol) at 0 °C, and the mixture was  
13  
14  
15 stirred at 60 °C for 15 h and concentrated under reduced pressure. To the residue was  
16  
17  
18 added ethyl acetate and water. The organic layer was washed with brine, dried over  
19  
20  
21 Na<sub>2</sub>SO<sub>4</sub>, and concentrated. The residual oil was purified by silica gel column  
22  
23  
24 chromatography (5–15% ethyl acetate/hexane) to give the title compound as a colorless  
25  
26  
27 oil (14.2 g, 86%). <sup>1</sup>H NMR (300 MHz, CDCl<sub>3</sub>) δ 2.26 (2H, tt, *J* = 6.0, 6.0 Hz), 3.75 (2H,  
28  
29 t, *J* = 6.2 Hz), 4.14 (2H, t, *J* = 5.8 Hz), 7.11–7.18 (2H, m), 7.23–7.28 (1H, m), 7.34–7.41  
30  
31  
32 (1H, m).

33  
34  
35  
36 **3-[(4-Chlorobutyl)oxy]benzotrile (7c)**. Compound **7c** was prepared from  
37  
38  
39 3-hydroxybenzotrile (**4**) and 1-bromo-4-chlorobutane with a similar procedure as  
40  
41  
42 described for compound **7b** (colorless oil, 95%). <sup>1</sup>H NMR (300 MHz, CDCl<sub>3</sub>) δ 1.94–  
43  
44  
45 2.02 (4H, m), 3.60–3.66 (2H, m), 4.02 (2H, t, *J* = 5.4 Hz), 7.08–7.15 (2H, m), 7.21–7.27  
46  
47  
48 (1H, m), 7.33–7.41 (1H, m).

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50  
51 **3-{[2-(1*H*-1,2,4-Triazol-3-ylthio)ethyl]oxy}benzotrile (8a)**. A solution of compound  
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54 **7a** (11.3 g, 62.3 mmol), 1*H*-1,2,4-triazole-3-thiol (6.00 g, 59.3 mmol), and triethylamine



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6 (8.40 mL, 62.3 mmol) in ethanol (50 mL) was stirred at 80 °C for 15 h. After cooling to  
7  
8  
9 room temperature, the reaction mixture was concentrated under reduced pressure. To the  
10  
11 residue were added ethyl acetate and water. The organic layer was washed with brine,  
12  
13 dried over Na<sub>2</sub>SO<sub>4</sub>, and concentrated to give the title compound as a white powder (14.4  
14  
15 g, 98%). <sup>1</sup>H NMR (300 MHz, DMSO-*d*<sub>6</sub>) δ 3.48 (2H, t, *J* = 6.7 Hz), 4.32 (2H, t, *J* = 6.6  
16  
17 Hz), 7.29–7.36 (1H, m), 7.38–7.43 (1H, m), 7.44–7.54 (2H, m), 8.46 (1H, s), 14.09 (1H,  
18  
19 s).  
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27 **3-{{3-(1*H*-1,2,4-Triazol-3-ylthio)propyl}oxy}benzotrile (8b)**. Compound **8b** was  
28  
29 prepared from compound **7b** and 1*H*-1,2,4-triazole-3-thiol with a similar procedure as  
30  
31 described for compound **8a** (white powder, quant.). <sup>1</sup>H NMR (300 MHz, DMSO-*d*<sub>6</sub>) δ  
32  
33 2.11 (2H, tt, *J* = 6.8, 6.6 Hz), 3.22 (2H, t, *J* = 7.1 Hz), 4.14 (2H, t, *J* = 6.2 Hz), 7.26–  
34  
35 7.53 (4H, m), 8.42 (1H, s), 14.03 (1H, s).  
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43 **3-[(3-{{1-(Triphenylmethyl)-1*H*-1,2,4-triazol-3-yl}thio}propyl)oxy]benzotrile (8c)**.

44  
45 A solution of compound **8b** (4.00 g, 15.4 mmol), triphenylmethyl chloride (6.43 g, 23.0  
46  
47 mmol), and triethylamine (2.33 g, 23.0 mmol) in THF (50 mL) was stirred at room  
48  
49 temperature for 48 h and concentrated under reduced pressure. To the residue were  
50  
51 added ethyl acetate and water. The organic layer was washed with brine, dried over  
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6 Na<sub>2</sub>SO<sub>4</sub>, and concentrated. The obtained oil was purified by silica gel column  
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8  
9 chromatography (5–40% ethyl acetate/hexane) to give the title compound as a white  
10  
11 powder (3.52 g, 46%). <sup>1</sup>H NMR (300 MHz, DMSO-*d*<sub>6</sub>) δ 2.00–2.09 (2H, m), 3.16 (2H, t,  
12  
13 *J* = 7.1 Hz), 4.02–4.09 (2H, m), 7.01–7.11 (6H, m), 7.19–7.49 (13H, m), 8.13 (1H, s).  
14  
15  
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17  
18 **3-{[4-(1*H*-1,2,4-Triazol-3-ylthio)butyl]oxy}benzonitrile (8d)**. Compound **8d** was  
19  
20 prepared from compound **7c** and 1*H*-1,2,4-triazole-3-thiol with a similar procedure as  
21  
22 described for compound **8a** (white powder, quant.). <sup>1</sup>H NMR (300 MHz, CDCl<sub>3</sub>) δ  
23  
24 1.87–2.02 (5H, m), 3.20–3.31 (2H, m), 3.96–4.05 (2H, m), 7.06–7.16 (2H, m), 7.20–  
25  
26 7.27 (1H, m), 7.32–7.40 (1H, m), 8.13 (1H, s).  
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34 **3-[(4-{[1-(Triphenylmethyl)-1*H*-1,2,4-triazol-3-yl]thio}butyl)oxy]benzonitrile (8e)**.  
35  
36 Compound **8e** was prepared from compound **8d** with a similar procedure as described  
37  
38 for compound **8c** (white powder, 78%). <sup>1</sup>H NMR (300 MHz, CDCl<sub>3</sub>) δ 1.81–1.89 (4H,  
39  
40 m), 3.07–3.16 (2H, m), 3.83–3.90 (2H, m), 7.01–7.06 (2H, m), 7.11–7.17 (6H, m),  
41  
42 7.19–7.24 (1H, m), 7.28–7.36 (10H, m), 7.87 (1H, s).  
43  
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49 **1-(3-{[2-(1*H*-1,2,4-Triazol-3-ylthio)ethyl]oxy}phenyl)methanamine (9a)**. A solution  
50  
51 of compound **8a** (9.00 g, 36.5 mmol) and Raney-nickel (5.00 g) in 5 N ammonia in  
52  
53 methanol solution (300 mL) was stirred at room temperature under hydrogen  
54  
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6 atmosphere at 1 atm for 15 h. The catalyst was filtered off through a Celite pad, and the  
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8  
9 filtrate was concentrated. The obtained residue was crystallized from toluene to give the  
10  
11  
12 title compound as a pale blue powder (6.45 g, 71%). The compound was used without  
13  
14  
15 further purification.

16  
17  
18 **1-{3-[(3-{[1-(Triphenylmethyl)-1*H*-1,2,4-triazol-3-yl]thio}propyl)oxy]phenyl}metha**  
19  
20  
21 **namine (9b)**. Compound **9b** was prepared from compound **8c** with a similar procedure  
22  
23 as described for compound **15a** (pale yellow oil, 95%). <sup>1</sup>H NMR (300 MHz, DMSO-*d*<sub>6</sub>)  
24  
25  $\delta$  1.94–2.08 (2H, m), 3.16 (2H, t, *J* = 7.1 Hz), 3.62–3.67 (2H, m), 3.97 (2H, t, *J* = 6.1  
26  
27 Hz), 6.60–6.74 (1H, m), 6.82–6.92 (2H, m), 6.98–7.11 (6H, m), 7.12–7.45 (12H, m),  
28  
29 8.14 (1H, s).

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36  
37 **1-{3-[(4-{[1-(Triphenylmethyl)-1*H*-1,2,4-triazol-3-yl]thio}butyl)oxy]phenyl}methan**  
38  
39  
40 **amine (9c)**. Compound **9c** was prepared from compound **8e** with a similar procedure as  
41  
42 described for compound **15a** (yellow oil, 86%). <sup>1</sup>H NMR (300 MHz, CDCl<sub>3</sub>)  $\delta$  1.79–  
43  
44 1.89 (4H, m), 2.35 (2H, s), 3.09–3.16 (2H, m), 3.81 (2H, s), 3.85–3.93 (2H, m), 6.71  
45  
46 (1H, dd, *J* = 8.1, 2.3 Hz), 6.81 (1H, s), 6.87 (1H, d, *J* = 7.0 Hz), 7.08–7.37 (16H, m),  
47  
48 7.86 (1H, s).

49  
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52  
53  
54  
55 **3-Nitro-1-(triphenylmethyl)-1*H*-1,2,4-triazole (11)**. A solution of

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6 3-nitro-1*H*-1,2,4-triazole (**10**) (1.00 g, 8.77 mmol), triphenylmethyl chloride (4.89 g,  
7  
8 17.5 mmol), and *N,N*-diisopropylethylamine (3.05 mL, 17.5 mmol) in THF (50 mL) was  
9  
10 stirred at room temperature for 15 h. The mixture was concentrated under reduced  
11  
12 pressure, and the residue was extracted with ethyl acetate and water. The organic layer  
13  
14 was washed with brine, dried over Na<sub>2</sub>SO<sub>4</sub>, and concentrated. The residue was purified  
15  
16 by silica gel column chromatography (15–30% ethyl acetate/hexane) to give the title  
17  
18 compound as a white powder (2.90 g, 93%). <sup>1</sup>H NMR (300 MHz, CDCl<sub>3</sub>) δ 7.08–7.17  
19  
20 (6H, m), 7.33–7.47 (9H, m), 8.04 (1H, s).  
21  
22  
23  
24  
25  
26  
27  
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29

30 **Ethyl [(3-Cyanophenyl)oxy]acetate (12)**. Ethyl bromoacetate (14.7 g, 88.1 mmol) was  
31  
32 added dropwise to a suspension of potassium carbonate (12.8 g, 92.3 mmol) and  
33  
34 3-hydroxybenzotrile (**4**) (10.0 g, 83.9 mmol) in THF (50 mL) at room temperature,  
35  
36 and the mixture was stirred at 50 °C for 24 h. The mixture was extracted with ethyl  
37  
38 acetate and saturated aqueous NH<sub>4</sub>Cl solution, and the organic layer was washed with  
39  
40 brine, dried over Na<sub>2</sub>SO<sub>4</sub>, and concentrated to give the title compound as a pale yellow  
41  
42 powder (17.5 g, 99%). <sup>1</sup>H NMR (300 MHz, CDCl<sub>3</sub>) δ 1.31 (3H, t, *J* = 7.2 Hz), 4.29 (2H,  
43  
44 q, *J* = 7.1 Hz), 4.65 (2H, s), 7.13–7.18 (2H, m), 7.28–7.32 (1H, m), 7.36–7.44 (1H, m).  
45  
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47  
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49  
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51  
52  
53

54 **3-[(2-Hydroxyethyl)oxy]benzotrile (13a)**. A suspension of compound **12** (8.00 g,  
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6 39.0 mmol) and sodium borohydride (1.47 g, 39.0 mmol) in ethanol (120 mL) was  
7  
8  
9 stirred at 50 °C for 15 h, and the mixture was concentrated under reduced pressure. The  
10  
11 residue was extracted with ethyl acetate and water, and the organic layer was washed  
12  
13 with brine, dried over Na<sub>2</sub>SO<sub>4</sub>, and concentrated to give the title compound as a white  
14  
15 powder (5.85 g, 92%). <sup>1</sup>H NMR (300 MHz, CDCl<sub>3</sub>) δ 1.67 (1H, bs), 3.97–4.02 (2H, m),  
16  
17 4.08–4.13 (2H, m), 7.13–7.20 (2H, m), 7.24–7.30 (1H, m), 7.35–7.43 (1H, m).  
18  
19  
20  
21  
22  
23

24 **3-[(2-{[1-(Triphenylmethyl)-1*H*-1,2,4-triazol-3-yl]oxy}ethyl)oxy]benzotrile (14a).**

25  
26  
27 A solution of compound **13a** (19.2 g, 118 mmol) in THF (100 mL) was added dropwise  
28  
29 to a suspension of compound **11** (40.0 g, 112 mmol) and 60% sodium hydride (oil  
30  
31 dispersion, 6.06 g, 152 mmol) in THF (300 mL), and the mixture was stirred at room  
32  
33 temperature for 12 h. To the mixture cooled to 0 °C were added water and ethyl acetate.  
34  
35  
36  
37 The organic layer was washed with brine, dried over Na<sub>2</sub>SO<sub>4</sub>, and concentrated. The  
38  
39 residue was purified by silica gel column chromatography (0–30% ethyl acetate/hexane)  
40  
41 to give the title compound as a white powder (25.0 g, 46%). <sup>1</sup>H NMR (300 MHz,  
42  
43 CDCl<sub>3</sub>) δ 4.23–4.29 (2H, m), 4.56–4.63 (2H, m), 7.07–7.41 (19H, m), 7.67 (1H, s).  
44  
45  
46  
47  
48  
49  
50

51 **3-[(3-{[1-(Triphenylmethyl)-1*H*-1,2,4-triazol-3-yl]oxy}propyl)oxy]benzotrile**

52 **(14b).** A solution of compound **13b**<sup>39</sup> (4.97 g, 28.1 mmol) in THF (50 mL) was added  
53  
54  
55  
56  
57  
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6 dropwise to a mixture of compound **11** (10.0 g, 28.1 mmol) and 60% sodium hydride  
7  
8  
9 (oil dispersion, 2.25 g, 56.1 mmol) in THF (150 mL) at room temperature and the  
10  
11  
12 resulting mixture was stirred at room temperature for 15 h. The reaction mixture was  
13  
14  
15 diluted with ethyl acetate and washed with H<sub>2</sub>O and brine. The organic layer was dried  
16  
17  
18 over Na<sub>2</sub>SO<sub>4</sub> and concentrated under reduced pressure to give the title compound as a  
19  
20  
21 pale yellow amorphous (14.0 g). The crude **14b** was used for the next reaction without  
22  
23  
24 further purification. <sup>1</sup>H NMR (300 MHz, DMSO-*d*<sub>6</sub>) δ 2.08–2.19 (2H, m), 4.14 (2H, t, *J*  
25  
26 = 6.2 Hz), 4.30 (2H, t, *J* = 6.2 Hz), 7.04–7.12 (6H, m), 7.24–7.30 (1H, m), 7.34–7.43  
27  
28  
29 (11H, m), 7.47 (1H, t, *J* = 7.9 Hz), 7.82 (1H, s).

30  
31  
32  
33 **1-{3-[(2-{{1-(Triphenylmethyl)-1*H*-1,2,4-triazol-3-yl}oxy}ethyl)oxy]phenyl}methan**  
34  
35  
36 **amine (15a)**. To a suspension of lithium aluminum hydride (3.21 g, 84.6 mmol) in THF  
37  
38  
39 (30 mL) was added dropwise a solution of compound **14a** (4.00 g, 8.46 mmol) in THF  
40  
41  
42 (20 mL) at room temperature, and the mixture was stirred at room temperature for 4 h.  
43  
44  
45 To the mixture cooled to 0 °C was added Na<sub>2</sub>SO<sub>4</sub>•10H<sub>2</sub>O (10.9 g, 33.9 mmol). The  
46  
47  
48 mixture was stirred at room temperature for 30 min. The insoluble material was filtered  
49  
50  
51 off through a Celite pad. The filtrate was dried over Na<sub>2</sub>SO<sub>4</sub> and concentrated. The  
52  
53  
54 residual solid was washed with diethylether and dried to give the title compound as a  
55  
56  
57 white powder (2.35 g, 58%). <sup>1</sup>H NMR (300 MHz, DMSO-*d*<sub>6</sub>) δ 1.58–2.00 (2H, m), 3.66  
58  
59  
60

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4  
5  
6 (2H, s), 4.20–4.27 (2H, m), 4.45 (2H, dd,  $J = 5.5, 3.4$  Hz), 6.75 (1H, dd,  $J = 8.1, 2.1$  Hz),  
7  
8  
9 6.86–6.96 (2H, m), 7.06–7.14 (6H, m), 7.19 (1H, t,  $J = 7.8$  Hz), 7.34–7.44 (9H, m), 7.86  
10  
11  
12 (1H, s).

13  
14  
15 **1-{3-[(3-{[1-(Triphenylmethyl)-1*H*-1,2,4-triazol-3-yl]oxy}propyl)oxy]phenyl}metha**

16 **namine (15b)**. Compound **15b** was prepared from compound **14b** with a similar

17  
18  
19 procedure as described for compound **15a** (white powder, 77%).  $^1\text{H}$  NMR (300 MHz,

20  
21  
22 DMSO- $d_6$ )  $\delta$  2.06–2.17 (2H, m), 3.65 (2H, s), 3.99–4.09 (2H, m), 4.29 (2H, t,  $J = 6.3$

23  
24  
25 Hz), 6.70–6.76 (1H, m), 6.84–6.89 (1H, m), 6.90–6.93 (1H, m), 7.05–7.11 (6H, m),

26  
27  
28  
29  
30 7.12–7.32 (1H, m), 7.34–7.41 (9H, m), 7.82 (1H, s).

31  
32  
33 **Ethyl 3-[2-(Aminocarbonothioyl)hydrazino]-3-oxopropanoate (17a)**. To a

34  
35  
36 suspension of hydrazinecarbothioamide (**16**) (15.0 g, 165 mmol) in pyridine (100 mL)

37  
38  
39 was added dropwise ethyl 3-chloro-3-oxopropanoate (24.8 g, 165 mmol) over 30 min at

40  
41  
42 0 °C, and the reaction mixture was stirred at room temperature for 2 days. The reaction

43  
44  
45 mixture was concentrated under reduced pressure, and methanol was added to the

46  
47  
48 residue. The resulting solid was filtered off, the filtrate was concentrated under reduced

49  
50  
51 pressure, and to the residue were added ethyl acetate, THF, and saturated aqueous

52  
53  
54  $\text{NH}_4\text{Cl}$  solution. The organic layer was washed with saturated aqueous  $\text{NH}_4\text{Cl}$  solution,

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2  
3  
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5  
6 dried over Na<sub>2</sub>SO<sub>4</sub>, and concentrated. The residue was crystallized from ethyl acetate  
7  
8  
9 and diisopropyl ether to give the title compound as a pale yellow powder (14.2 g, 42%).  
10  
11  
12 <sup>1</sup>H NMR (300 MHz, DMSO-*d*<sub>6</sub>) δ 1.10–1.27 (3H, m), 3.28 (2H, s), 4.09 (2H, q, *J* = 7.2  
13  
14 Hz), 7.36 (1H, s), 7.98 (1H, s), 9.38 (1H, s), 10.02 (1H, s).  
15  
16

17  
18 **Ethyl 4-[2-(Aminocarbonothioyl)hydrazino]-4-oxobutanoate (17b).** Ethyl  
19  
20  
21 4-[2-(aminocarbonothioyl) hydrazino]-4-oxobutanoate (**17b**) was prepared from  
22  
23  
24 hydrazinecarbothioamide (**16**) and ethyl 4-chloro-4-oxobutanoate with a similar  
25  
26  
27 procedure as described for compound **17a** (white powder, 35%). <sup>1</sup>H NMR (400 MHz,  
28  
29 DMSO-*d*<sub>6</sub>) δ 1.18 (3H, t, *J* = 7.1 Hz), 2.33–2.47 (2H, m), 2.52–2.67 (2H, m), 4.04 (2H,  
30  
31 q, *J* = 7.2 Hz), 7.30 (1H, bs), 7.89 (1H, bs), 9.22 (1H, s), 9.83 (s, 1H).  
32  
33  
34  
35  
36

37 **Ethyl (5-Mercapto-1*H*-1,2,4-triazol-3-yl)acetate (18a).** A suspension of compound  
38  
39 **17a** (14.0 g, 68.2 mmol) and sodium ethoxide (9.52 g, 140 mmol) in ethanol (200 mL)  
40  
41  
42 was stirred at 80 °C for 15 h. After cooling to room temperature, the mixture was  
43  
44  
45 concentrated under reduced pressure, and the residue was acidified with 1 N  
46  
47  
48 hydrochloric acid and extracted with ethyl acetate. The organic layer was washed with  
49  
50  
51 brine, dried over Na<sub>2</sub>SO<sub>4</sub>, and concentrated to give the title compound as an orange  
52  
53  
54 powder (12.2 g, 96%). <sup>1</sup>H NMR (300 MHz, DMSO-*d*<sub>6</sub>) δ 1.20 (3H, t, *J* = 7.1 Hz), 3.74  
55  
56  
57  
58  
59  
60



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5  
6 (2H, s), 4.12 (2H, q,  $J = 7.0$  Hz).  
7  
8

9  
10 **Ethyl 3-(5-Mercapto-1*H*-1,2,4-triazol-3-yl)propanoate (18b)**. Compound **18b** was  
11  
12 prepared from compound **17b** with a similar procedure as described for compound **18a**  
13  
14 (white powder, 91%).  $^1\text{H}$  NMR (300 MHz, DMSO- $d_6$ )  $\delta$  1.01–1.28 (3H, m), 2.64–2.83  
15  
16 (4H, m), 4.05 (2H, q,  $J = 7.2$  Hz), 13.10 (1H, bs), 13.21 (1H, bs).  
17  
18  
19

20  
21  
22 **Methyl 1-(Triphenylmethyl)-1*H*-1,2,4-triazole-3-carboxylate (19b)**. Compound **19b**  
23  
24 was prepared from commercially available methyl 1*H*-1,2,4-triazole-3-carboxylate  
25  
26 (**19a**) with a similar procedure as described for compound **11** (TrCl, DIEA, 60 °C)  
27  
28 (white powder, 59%).  $^1\text{H}$  NMR (300 MHz, DMSO- $d_6$ )  $\delta$  3.84 (3H, s), 7.07 (6H, dd,  $J =$   
29  
30 6.8, 2.8 Hz), 7.34–7.46 (9H, m), 8.39 (1H, s).  
31  
32  
33  
34  
35  
36

37  
38 **Ethyl 1*H*-1,2,4-Triazol-3-ylacetate (19c)**. A suspension of compound **18a** (2.00 g, 10.7  
39  
40 mmol) and Raney-nickel (5.00 g) in ethanol (20 mL) was stirred at 80 °C for 15 h. The  
41  
42 catalyst was filtered off through a Celite pad, and the filtrate was concentrated under  
43  
44 reduced pressure. To a suspension of the residue in ethyl acetate was added activated  
45  
46 carbon, and the mixture was filtered. The filtrate was concentrated under reduced  
47  
48 pressure to give the title compound as a pale green powder (900 mg, 54%).  $^1\text{H}$  NMR  
49  
50 (300 MHz, CDCl $_3$ )  $\delta$  1.32 (3H, t,  $J = 6.8$  Hz), 3.96 (2H, s), 4.26 (2H, q,  $J = 6.5$  Hz),  
51  
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6 8.03 (1H, s), 11.53 (1H, bs).  
7  
8  
9

10 **3-(1*H*-1,2,4-Triazol-3-yl)propanoic Acid (19d)**. Compound **18b** (650 mg, 3.23 mmol)  
11  
12 was added slowly to a solution of sodium nitrite (8.91 mg, 0.129 mmol) and nitric acid  
13  
14 (3 mL) in water (6 mL), maintaining the temperature below 45 °C. The solution was  
15  
16 stirred at room temperature for 15 h, and neutralized with saturated aqueous NaHCO<sub>3</sub>  
17  
18 solution. The mixture was concentrated under reduced pressure. The residue was  
19  
20 triturated with THF, collected on a filter, and washed with THF. The collected solid was  
21  
22 suspended in ethanol. The insoluble material was filtered off, and the filtrate was  
23  
24 concentrated under reduced pressure to give the title compound as a pale yellow powder  
25  
26 (454 mg, 99%). <sup>1</sup>H NMR (300 MHz, DMSO-*d*<sub>6</sub>) δ 2.24 (2H, t, *J* = 7.5 Hz), 2.80 (2H, t,  
27  
28 *J* = 7.5 Hz), 7.82 (1H, s).  
29  
30  
31  
32  
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38  
39

40 **Ethyl 3-(1*H*-1,2,4-Triazol-3-yl)propanoate (19e)**. To a suspension of compound **19d**  
41  
42 (11.6 g, 3.54 mmol) in ethanol (100 mL) was added a 2 N hydrogen chloride in ethanol  
43  
44 solution (100 mL) at room temperature. The mixture was stirred at 90 °C for 15 h. After  
45  
46 cooling to room temperature, the mixture was filtered through a Celite pad, and the  
47  
48 filtrate was concentrated under reduced pressure. To the residue was added ethyl acetate  
49  
50 and water. The aqueous layer was neutralized with saturated aqueous NaHCO<sub>3</sub> solution  
51  
52  
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54  
55  
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60

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5  
6 and extracted with ethyl acetate. The organic layer was washed with brine, dried over  
7  
8  
9 Na<sub>2</sub>SO<sub>4</sub>, and concentrated to give the title compound as a yellow oil (4.36 g, 33%). <sup>1</sup>H  
10  
11 NMR (300 MHz, DMSO-*d*<sub>6</sub>) δ 1.11–1.20 (3H, m), 2.72 (2H, d, *J* = 1.7 Hz), 2.92 (2H, s),  
12  
13 4.00–4.08 (2H, m), 7.79 (1H, s), 13.59 (1H, bs).  
14  
15  
16  
17

18 **Ethyl 3-[1-(Triphenylmethyl)-1*H*-1,2,4-triazol-3-yl]propanoate (19f)**. Compound **19f**

19  
20 was prepared from compound **19e** with a similar procedure as described for compound  
21  
22  
23  
24 **11** (TrCl, DIEA, rt) (pale yellow oil, 83%). <sup>1</sup>H NMR (300 MHz, DMSO-*d*<sub>6</sub>) δ 1.10 (3H,  
25  
26 t, *J* = 7.2 Hz), 2.67 (2H, t, *J* = 7.1 Hz), 2.90 (2H, t, *J* = 7.0 Hz), 3.98 (2H, q, *J* = 6.9 Hz),  
27  
28 6.99–7.08 (6H, m), 7.34–7.40 (9H, m), 7.95 (1H, s).  
29  
30  
31  
32  
33

34 **1-(Triphenylmethyl)-1*H*-1,2,4-triazole-3-carboxylic Acid (20a)**. Compound **20a** was

35  
36 prepared from compound **19b** with a similar procedure as described for compound **22b**  
37  
38  
39 (white powder, 98%). <sup>1</sup>H NMR (300 MHz, DMSO-*d*<sub>6</sub>) δ 7.07 (6H, dd, *J* = 6.9, 2.9 Hz),  
40  
41 7.30–7.56 (9H, m), 8.31 (1H, s), 13.49 (1H, s).  
42  
43  
44  
45

46 **(1*H*-1,2,4-Triazol-3-yl)acetic Acid (20b)**. Compound **20b** was prepared from

47  
48  
49 compound **19c** with a similar procedure as described for compound **22b** (white powder,  
50  
51 50%). <sup>1</sup>H NMR (300 MHz, DMSO-*d*<sub>6</sub>) δ 3.49 (2H, s), 7.92 (1H, s).  
52  
53  
54  
55

56 **3-[1-(Triphenylmethyl)-1*H*-1,2,4-triazol-3-yl]propanoic Acid (20c)**. Compound **20c**

1  
2  
3  
4  
5  
6 was prepared from compound **19f** with a similar procedure as described for compound  
7  
8  
9 **22b** (white powder, 85%). <sup>1</sup>H NMR (300 MHz, DMSO-*d*<sub>6</sub>)  $\delta$  2.59 (2H, t, *J* = 7.3 Hz),  
10  
11  
12 2.86 (2H, t, *J* = 7.3 Hz), 7.04 (6H, dd, *J* = 6.7, 2.9 Hz), 7.33–7.45 (9H, m), 7.93 (1H, s),  
13  
14  
15 12.17 (1H, bs).

### 16 17 18 19 Ethyl

20  
21  
22 **4-{{3-(((4-Oxo-3,4-dihydroquinazolin-2-yl)carbonyl)amino)methyl}phenyl}oxy}bu**  
23  
24  
25 **tanoate (22a)**. A suspension of ethyl 4-oxo-3,4-dihydro-2-quinazolinecarboxylate (**21**)  
26  
27 (500 mg, 2.29 mmol), ethyl 4-{{3-(aminomethyl)phenyl}oxy}butanoate hydrochloride  
28  
29 (**6**) (878 mg, 3.21 mmol), and *N,N*-diisopropylethylamine (0.798 mL, 4.58 mmol) in  
30  
31  
32 THF (10 mL) was stirred at 80 °C for 15 h. The reaction mixture was evaporated under  
33  
34  
35 reduced pressure, and to the residue were added ethyl acetate and 0.1 N aqueous  
36  
37  
38 hydrochloric acid solution. The organic layer was washed with saturated aqueous  
39  
40  
41 NH<sub>4</sub>Cl solution, dried over Na<sub>2</sub>SO<sub>4</sub>, and concentrated. The residual solid was  
42  
43  
44 recrystallized from ethyl acetate to give the title compound as a white powder (282 mg,  
45  
46  
47 30%). Evaporation of the filtrate gave another crop of the title compound as a white  
48  
49  
50 powder (525 mg, 56%). mp 130–131 °C. <sup>1</sup>H NMR (300MHz, DMSO-*d*<sub>6</sub>)  $\delta$  1.15 (3H, t,  
51  
52  
53 *J* = 7.1 Hz), 1.89–2.01 (2H, m), 2.44 (2H, t, *J* = 7.3 Hz), 3.96 (2H, t, *J* = 6.3 Hz), 4.04  
54  
55  
56 (2H, q, *J* = 7.0 Hz), 4.45 (2H, d, *J* = 6.2 Hz), 6.77–6.84 (1H, m), 6.88–6.94 (2H, m),  
57  
58  
59  
60

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2  
3  
4  
5  
6 7.19–7.27 (1H, m), 7.57–7.65 (1H, m), 7.75–7.81 (1H, m), 7.84–7.94 (1H, m), 8.17 (1H,  
7  
8  
9 dd,  $J = 7.9, 1.1$  Hz), 9.54 (1H, t,  $J = 6.3$  Hz), 12.29 (1H, bs). Anal. Calcd for  
10  
11  $C_{22}H_{23}N_3O_5$ : C, 64.54; H, 5.66; N, 10.26. Found: C, 64.30; H, 5.51; N, 10.22.  
12  
13

14  
15  
16 **4-{{3-((4-Oxo-3,4-dihydroquinazolin-2-yl)carbonyl)amino}methyl}phenyl}oxy}butanoic Acid (22b)**

17  
18 **tanoic Acid (22b)**. A mixture of compound **22a** (525 mg, 1.28 mmol), 4 N aqueous  
19  
20 sodium hydroxide solution (1.6 mL) in THF (10 mL), methanol (10 mL), and water (10  
21  
22 mL) was stirred at 100 °C for 2 h. The mixture was allowed to cool to room temperature,  
23  
24 mL) was stirred at 100 °C for 2 h. The mixture was allowed to cool to room temperature,  
25  
26 and the solvent was evaporated under reduced pressure. Water and 1 N hydrochloric  
27  
28 acid (6.41 mL) were added to the residue, and the resulting precipitate was collected by  
29  
30 filtration, washed with water, and dried. The obtained crude crystals were recrystallized  
31  
32 from ethanol to give the title compound as a white powder (339 mg, 69%). mp 189–  
33  
34 190 °C.  $^1\text{H NMR}$  (300MHz,  $\text{DMSO-}d_6$ )  $\delta$  1.85–1.99 (2H, m), 2.37 (2H, t,  $J = 7.3$  Hz),  
35  
36 3.96 (2H, t,  $J = 6.4$  Hz), 4.45 (2H, d,  $J = 6.4$  Hz), 6.76–6.86 (1H, m), 6.88–6.96 (2H, m),  
37  
38 7.23 (1H, t,  $J = 8.1$  Hz), 7.56–7.67 (1H, m), 7.75–7.82 (1H, m), 7.83–7.93 (1H, m), 8.18  
39  
40 (1H, dd,  $J = 7.9, 1.1$  Hz), 9.55 (1H, t,  $J = 6.3$  Hz), 12.21(2H, s). Anal. Calcd for  
41  
42  $C_{20}H_{19}N_3O_5$ : C, 62.99; H, 5.02; N, 11.02. Found: C, 62.74; H, 5.04; N, 10.98.  
43  
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53  
54 ***N*-[(3-{{4-(Hydroxyamino)-4-oxobutyl}oxy}phenyl)methyl]-4-oxo-3,4-dihydroquina**  
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6 **zoline-2-carboxamide (22c)**. To a solution of compound **22b** (90.0 mg, 0.236 mmol) in  
7  
8  
9 THF (2 mL) were added DMF (0.02 mL) and oxalyl chloride (59.9 mg, 0.472 mmol) at  
10  
11  
12 0 °C, and the mixture was stirred at room temperature for 2 h. To the reaction mixture  
13  
14  
15 was added a mixed solution of 50% aqueous hydroxylamine solution (0.5 mL),  
16  
17  
18 *tert*-butanol (0.5 mL), and THF (0.5 mL) at 0 °C, and the mixture was stirred for 15 min.  
19  
20  
21 The mixture was concentrated under reduced pressure, and the residue was purified by  
22  
23  
24 preparative HPLC and recrystallization from ethyl acetate-hexane to give the title  
25  
26  
27 compound as a pale yellow powder (8.0 mg, 9%). mp 164–168 °C. <sup>1</sup>H NMR (300MHz,  
28  
29 DMSO-*d*<sub>6</sub>) δ 1.85–2.00 (2H, m), 2.07–2.16 (2H, m), 3.89–3.98 (2H, m), 4.44 (2H, d, *J*  
30  
31 = 7.0 Hz), 6.77–6.95 (3H, m), 7.23 (1H, t, *J* = 7.9 Hz), 7.56 (1H, t, *J* = 7.8 Hz), 7.72–  
32  
33  
34 7.89 (2H, m), 8.15 (1H, d, *J* = 7.9 Hz), 9.48 (1H, bs), 10.41 (1H, bs), 12.22 (1H, s).  
35  
36  
37  
38

39 **4-Oxo-*N*-[(3-{[2-(1*H*-1,2,4-triazol-3-ylthio)ethyl]oxy}phenyl)methyl]-3,4-dihydroqu**  
40  
41  
42 **inazoline-2-carboxamide (23)**. A suspension of compound **21** (200 mg, 0.917 mmol),  
43  
44  
45 1-(3-{[2-(1*H*-1,2,4-triazol-3-ylthio)ethyl]oxy}phenyl)methanamine (**9a**) (298 mg, 1.19  
46  
47  
48 mmol), and *N,N*-diisopropylethylamine (237 mg, 1.83 mmol) in ethanol (10 mL) was  
49  
50  
51 stirred at 90 °C for 15 h. After cooling to room temperature, the mixture was evaporated  
52  
53  
54 under reduced pressure, and the residue was extracted with ethyl acetate and water. The  
55  
56  
57 organic layer was washed with brine, dried over Na<sub>2</sub>SO<sub>4</sub>, and concentrated. The residue  
58  
59  
60

1  
2  
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5  
6 was purified by preparative HPLC and recrystallization from ethanol to give the title  
7  
8  
9 compound as a white powder (29.2 mg, 8%). mp 177–179 °C. <sup>1</sup>H NMR (300 MHz,  
10  
11 DMSO-*d*<sub>6</sub>) δ 3.45 (2H, t, *J* = 6.5 Hz), 4.23 (2H, t, *J* = 6.7 Hz), 4.45 (2H, d, *J* = 6.2 Hz),  
12  
13 6.85 (1H, dd, *J* = 8.6, 1.6 Hz), 6.90–6.98 (2H, m), 7.24 (1H, t, *J* = 7.9 Hz), 7.61 (1H, t, *J*  
14  
15 = 7.0 Hz), 7.78 (1H, d, *J* = 7.7 Hz), 7.85–7.92 (1H, m), 8.17 (1H, d, *J* = 7.7 Hz), 9.50–  
16  
17 9.57 (1H, m). Anal. Calcd for C<sub>20</sub>H<sub>18</sub>N<sub>6</sub>O<sub>3</sub>S: C, 56.86; H, 4.29; N, 19.89. Found: C,  
18  
19 56.77; H, 4.23; N, 19.65.  
20  
21  
22  
23  
24  
25  
26

27 **5-Methyl-4-oxo-*N*-[3-{[2-(1*H*-1,2,4-triazol-3-ylthio)ethyl]oxy}phenyl)methyl]-3,4-*d***

28 **ihydro-thieno[2,3-*d*]pyrimidine-2-carboxamide (25a)**. A suspension of ethyl  
29  
30 **5-methyl-4-oxo-3,4-dihydrothieno[2,3-*d*]pyrimidine-2-carboxylate (24)** (200 mg, 0.839  
31  
32 mmol) and 1-(3-{[2-(1*H*-1,2,4-triazol-3-ylthio)ethyl]oxy}phenyl)methanamine (**9a**)  
33  
34 (305 mg, 1.22 mmol) in ethanol (10 mL) and DMA (2 mL) was stirred at 90 °C for 3  
35  
36 days. The reaction mixture was evaporated under reduced pressure, and to the residue  
37  
38 were added ethyl acetate and 0.1 N aqueous hydrochloric acid solution. The organic  
39  
40 layer was washed with saturated aqueous NH<sub>4</sub>Cl solution, dried over Na<sub>2</sub>SO<sub>4</sub>, and  
41  
42 concentrated. The residual solid was recrystallized from ethanol to give the title  
43  
44 compound as a white powder (67.2 mg, 18%). mp 178–179 °C. <sup>1</sup>H NMR (300 MHz,  
45  
46 DMSO-*d*<sub>6</sub>) δ 3.45 (2H, t, *J* = 6.5 Hz), 3.56–3.64 (1H, m), 4.22 (2H, t, *J* = 6.4 Hz), 4.40  
47  
48  
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(2H, d,  $J = 6.2$  Hz), 6.80–6.96 (3H, m), 7.17–7.30 (2H, m), 8.46 (1H, s), 9.56 (1H, s).

Anal. Calcd for  $C_{19}H_{18}N_6O_3S_2$ : C, 51.57; H, 4.10; N, 18.99. Found: C, 51.32; H, 4.08; N, 18.74.

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

**-dihydro-thieno[2,3-*d*]pyrimidine-2-carboxamide (25b).** A solution of ethyl

5-methyl-4-oxo-3,4-dihydrothieno[2,3-*d*]pyrimidine-2-carboxylate (**24**) (200 mg, 0.839

mmol) and

1-{3-[(3-{[1-(triphenylmethyl)-1*H*-1,2,4-triazol-3-yl]thio}propyl)oxy]phenyl}methana

mine (**9b**) (723 mg, 1.43 mmol) in DMA (10 mL) was stirred at 100 °C for 12 h. After

cooling to room temperature, the reaction mixture was evaporated under reduced

pressure, and a solution of the residue in dichloromethane was treated with acidic resin

(MP-TsOH: 100 mg) to give a brown oil. To the solution of the obtained oil in

dichloromethane (10 mL) were added TFA (3 mL) and triethylsilane (0.141 mL, 0.881

mmol) at room temperature. The mixture was stirred for 1 h and evaporated under

reduced pressure. The residue was purified by silica gel column chromatography (30–

100% ethyl acetate/hexane) to give a solid. The suspension of the obtained solid in ethyl

acetate was stirred under heating at 90 °C for 1 h to give the title compound as a white

powder (171 mg, 45% for 2 steps). mp 178–179 °C.  $^1\text{H}$  NMR (300 MHz,  $\text{DMSO-}d_6$ )  $\delta$



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3  
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5  
6 2.03–2.14 (2H, m), 3.21 (2H, t,  $J = 7.0$  Hz), 4.04 (2H, t,  $J = 6.1$  Hz), 4.41 (2H, d,  $J = 6.4$   
7  
8 Hz), 6.80–6.84 (1H, m), 6.87–6.93 (2H, m), 7.23 (1H, t,  $J = 8.1$  Hz), 7.32 (1H, d,  $J =$   
9  
10 1.1 Hz), 8.52 (1H, bs), 9.62 (1H, t,  $J = 6.4$  Hz), 12.28 (1H, bs), 14.04 (1H, bs). Anal.  
11  
12  
13 Calcd for  $C_{20}H_{20}N_6O_3S_2$ : C, 52.62; H, 4.42; N, 18.41. Found: C, 52.72; H, 4.56; N,  
14  
15 18.27.  
16  
17  
18  
19

20  
21  
22 **5-Methyl-4-oxo-*N*-[(3-{[4-(1*H*-1,2,4-triazol-3-ylthio)butyl]oxy}phenyl)methyl]-3,4-d**

23  
24 **ihydro-thieno[2,3-*d*]pyrimidine-2-carboxamide (25c).** Compound **25c** was prepared  
25  
26 from ethyl 5-methyl-4-oxo-3,4-dihydrothieno[2,3-*d*]pyrimidine-2-carboxylate (**24**) and  
27  
28 1-{3-[(4-{[1-(triphenyl-methyl)-1*H*-1,2,4-triazol-3-yl]thio}butyl)oxy]phenyl}methanam  
29  
30 ine (**9c**) with a similar procedure as described for compound **25b** (pale yellow powder,  
31  
32 48% for 2 steps). mp 161–163 °C.  $^1\text{H}$  NMR (300 MHz,  $\text{DMSO-}d_6$ )  $\delta$  1.73–1.85 (4H, m),  
33  
34 2.46–2.52 (3H, m), 3.09–3.19 (2H, m), 3.90–4.01 (2H, m), 4.40 (2H, d,  $J = 6.4$  Hz),  
35  
36 6.80 (1H, dd,  $J = 8.2, 1.6$  Hz), 6.85–6.90 (2H, m), 7.22 (1H, t,  $J = 8.1$  Hz), 7.31 (1H, d,  
37  
38  $J = 1.1$  Hz), 8.40 (1H, bs), 9.59 (1H, t,  $J = 6.3$  Hz), 12.30 (1H, bs), 14.00 (1H, bs). Anal.  
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60 **5-Methyl-4-oxo-*N*-[(3-{[2-(1*H*-1,2,4-triazol-3-yloxy)ethyl]oxy}phenyl)methyl]-3,4-di**

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5  
6 **hydro-thieno[2,3-*d*]pyrimidine-2-carboxamide (25d)**. A suspension of ethyl  
7  
8  
9 5-methyl-4-oxo-3,4-dihydro-thieno[2,3-*d*]pyrimidine-2-carboxylate (**24**) (200 mg, 0.839  
10  
11 mmol) and  
12  
13  
14 1-{3-[(2-{{1-(triphenylmethyl)-1*H*-1,2,4-triazol-3-yl}oxy)ethyl}oxy]phenyl}methanami  
15  
16 ne (**15a**) (420 mg, 0.881 mmol) in DMA (3 mL) was stirred under microwave  
17  
18 irradiation at 180 °C (150W, run time: 15 min, hold time: 15 min). After cooling to  
19  
20 room temperature, the reaction mixture was evaporated under reduced pressure. To the  
21  
22 residue were added ethyl acetate and water. The organic layer was washed with brine,  
23  
24 dried over Na<sub>2</sub>SO<sub>4</sub>, and concentrated. The residue was purified by silica gel column  
25  
26 chromatography (50–67% ethyl acetate/hexane) to give a white powder (283 mg). To a  
27  
28 solution of the obtained powder (250 mg, 0.374 mmol) in dichloromethane (10 mL)  
29  
30 were added TFA (3 mL) and triethylsilane (0.063 mL, 0.393 mmol) at room temperature.  
31  
32 The mixture was stirred for 30 min and evaporated under reduced pressure. The residual  
33  
34 solid was crystallized from ethanol and diethylether to give a white powder. The powder  
35  
36 was recrystallized from ethanol to give the title compound as a white powder (126 mg,  
37  
38 35% for 2 steps). mp 225–227 °C. <sup>1</sup>H NMR (300 MHz, DMSO-*d*<sub>6</sub>) δ 4.24–4.29 (2H, m),  
39  
40 4.41 (2H, d, *J* = 6.2 Hz), 4.45–4.50 (2H, m), 6.83–6.88 (1H, m), 6.89–6.97 (2H, m),  
41  
42 7.24 (1H, t, *J* = 7.9 Hz), 7.32 (1H, d, *J* = 1.1 Hz), 8.24 (1H, s), 9.62 (1H, t, *J* = 6.3 Hz),  
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6 12.27 (1H, bs), 13.33 (1H, bs). Anal. Calcd for C<sub>19</sub>H<sub>18</sub>N<sub>6</sub>O<sub>4</sub>S: C, 53.51; H, 4.25; N,  
7  
8 19.71. Found: C, 53.40; H, 4.26; N, 19.69.

9  
10  
11  
12 **5-Methyl-4-oxo-*N*-[(3-{{(1*H*-1,2,4-triazol-3-ylcarbonyl)amino}methyl}phenyl)meth**

13 **yl]-3,4-dihydrothieno[2,3-*d*]pyrimidine-2-carboxamide (25e).** A solution of

14  
15  
16 compound **26c** (365 mg, 1.00 mmol),

17  
18  
19 1-(triphenylmethyl)-1*H*-1,2,4-triazole-3-carboxylic acid (**20a**) (427 mg, 1.20 mmol),

20  
21  
22 *N,N*-diisopropylethylamine (129 mg, 1.00 mmol),

23  
24  
25 1-[3-(dimethylamino)propyl]-3-ethylcarbodiimide hydrochloride (230 mg, 1.20 mmol),

26  
27  
28 and 1-hydroxybenzotriazole (162 mg, 1.20 mmol) in DMF (10 mL) was stirred at 40 °C

29  
30  
31 for 15 h. The reaction mixture was evaporated under reduced pressure. To the residue

32  
33  
34 was added water. The resulting solid was collected on a filter, washed with water and

35  
36  
37 diisopropyl ether, and dried to give a white solid. The obtained solid was washed with

38  
39  
40 ethanol and diisopropyl ether to give a white powder (597 mg). A solution of the white

41  
42  
43 powder (565 mg, 0.849 mmol) and triethylsilane (0.142 mL, 0.891 mmol) in

44  
45  
46 dichloromethane (10 mL) and TFA (3 mL) was stirred at room temperature for 0.5 h.

47  
48  
49 The reaction mixture was evaporated under reduced pressure. To the residue was added

50  
51  
52 diisopropyl ether. The resulting solid was collected on a filter, washed with diisopropyl

53  
54  
55 ether, and dried. The suspension of the obtained solid in ethanol was stirred under

1  
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5  
6 heating at 90 °C for 2 h to give the title compound as a white powder (306 mg, 85% for  
7  
8  
9 2 steps). mp 290–291 °C. <sup>1</sup>H NMR (300 MHz, DMSO-*d*<sub>6</sub>) δ 4.42 (4H, d, *J* = 6.0 Hz),  
10  
11  
12 7.16–7.34 (5H, m), 8.44 (1H, bs), 9.15 (1H, bs), 9.64 (1H, t, *J* = 6.4 Hz), 12.25 (1H, bs).  
13

14  
15  
16 **5-Methyl-4-oxo-*N*-[(3-[(1*H*-1,2,4-triazol-3-ylacetyl)amino]methyl}phenyl)methyl]-**

17  
18  
19 **3,4-dihydrothieno[2,3-*d*]pyrimidine-2-carboxamide (25f)**. A solution of compound

20  
21  
22 **26c** (239 mg, 0.656 mmol), 1*H*-1,2,4-triazol-3-ylacetic acid (**20b**) (100 mg, 0.787

23  
24  
25 mmol), *N,N*-diisopropylethylamine (84.8 mg, 0.656 mmol),

26  
27  
28 1-[3-(dimethylamino)propyl]-3-ethylcarbodiimide hydrochloride (151 mg, 0.787 mmol),  
29

30  
31  
32 and 1-hydroxy-benzotriazole (106 mg, 0.787 mmol) in DMF (10 mL) was stirred for 15

33  
34  
35 h at 40 °C. The reaction mixture was concentrated under reduced pressure, and water

36  
37  
38 was added to the residue. The resulting solid was collected on a filter, washed with

39  
40  
41 water, ethanol and diisopropyl ether, and dried. The obtained solid was recrystallized

42  
43  
44 from ethyl acetate–diisopropyl ether to give the title compound as a white powder (151

45  
46  
47 mg, 53%). mp 251–253 °C. <sup>1</sup>H NMR (300 MHz, DMSO-*d*<sub>6</sub>) δ 3.61–3.68 (2H, m), 4.27

48  
49  
50 (2H, d, *J* = 5.7 Hz), 4.43 (2H, d, *J* = 6.4 Hz), 7.09–7.35 (5H, m), 8.50–8.68 (1H, m),

51  
52  
53 9.62 (1H, t, *J* = 6.3 Hz), 12.30 (1H, bs), 13.76 (1H, bs). Anal. Calcd for

54  
55  
56 C<sub>20</sub>H<sub>19</sub>N<sub>7</sub>O<sub>3</sub>S•0.3H<sub>2</sub>O: C, 54.24; H, 4.46; N, 22.14. Found: C, 54.52; H, 4.45; N, 21.81.  
57  
58  
59  
60

**5-Methyl-4-oxo-*N*-[(3-{[3-(1*H*-1,2,4-triazol-3-yl)propanoyl]amino}phenyl)methyl]-3**

**,4-dihydrothieno[2,3-*d*]pyrimidine-2-carboxamide (25g).** Compound **25g** was

prepared from compound **26a** and

3-[1-(triphenylmethyl)-1*H*-1,2,4-triazol-3-yl]propanoic acid (**20c**) with a similar

procedure as described for compound **25e** (white powder, 45% for 2 steps). mp 248–250

°C. <sup>1</sup>H NMR (300 MHz, DMSO-*d*<sub>6</sub>) δ 2.67–2.80 (2H, m), 2.96 (2H, bs), 4.40 (2H, d, *J*=

6.4 Hz), 7.00 (1H, d, *J* = 7.5 Hz), 7.18–7.27 (1H, m), 7.31 (1H, d, *J* = 1.1 Hz), 7.44–

7.55 (2H, m), 9.63 (1H, t, *J* = 6.3 Hz), 9.99 (1H, bs), 12.29 (1H, bs), 13.64 (1H, bs).

Anal. Calcd for C<sub>20</sub>H<sub>19</sub>N<sub>7</sub>O<sub>3</sub>S•0.3H<sub>2</sub>O: C, 54.24; H, 4.46; N, 22.14. Found: C, 54.58; H,

4.45; N, 21.87.

***N*-[(3-Aminophenyl)methyl]-5-methyl-4-oxo-3,4-dihydrothieno[2,3-*d*]pyrimidine-2-**

**carbox-amide (26a).** A suspension of ethyl

5-methyl-4-oxo-3,4-dihydrothieno[2,3-*d*]pyrimidine-2-carboxylate (**24**) (1.50 g, 6.30

mmol) and 3-(aminomethyl)aniline (0.923 g, 7.55 mmol) in THF (15 mL) was stirred at

80 °C for 15 h. To the reaction mixture cooled to room temperature was added

diisopropyl ether, and the insoluble material was collected on a filter, washed with

diisopropyl ether, and dried in vacuo to give the title compound as a yellow powder

(1.92 mg, 97%). <sup>1</sup>H NMR (300 MHz, DMSO-*d*<sub>6</sub>) δ 3.33 (3H, s), 4.30 (2H, d, *J* = 6.2

1  
2  
3  
4  
5  
6 Hz), 5.03 (2H, s), 6.40–6.52 (3H, m), 6.94 (1H, t,  $J = 7.7$  Hz), 7.31 (1H, d,  $J = 1.3$  Hz),  
7  
8  
9 9.48 (1H, t,  $J = 6.3$  Hz), 12.20 (1H, s). Anal. Calcd for  $C_{15}H_{14}N_4O_2S$ : C, 57.31; H, 4.49;  
10  
11  
12 N, 17.82. Found: C, 57.11; H, 4.52; N, 17.65.

### 1,1-Dimethylethyl

13  
14  
15  
16 **{[3-({[(5-methyl-4-oxo-3,4-dihydrothieno[2,3-*d*]pyrimidin-2-yl)carbonyl]amino}me**

17  
18  
19  
20  
21  
22 **thyl)phenyl]methyl}carbamate (26b)**. Compound **26b** was prepared from ethyl

23  
24  
25 5-methyl-4-oxo-3,4-dihydrothieno[2,3-*d*]pyrimidine-2-carboxylate (**24**) and *tert*-butyl

26  
27  
28 *N*-{[3-(aminomethyl)phenyl]methyl}carbamate with a similar procedure as described

29  
30 for compound **26a** (white powder, 82%).  $^1\text{H}$  NMR (300 MHz,  $\text{DMSO-}d_6$ )  $\delta$  1.35 (9H, s),

31  
32  
33 2.47–2.53 (3H, m), 4.10 (2H, d,  $J = 6.2$  Hz), 4.43 (2H, d,  $J = 6.4$  Hz), 7.08–7.41 (6H,

34  
35  
36 m), 9.62 (1H, t,  $J = 6.3$  Hz), 12.25 (1H, s).

37  
38  
39  
40 *N*-{[3-(Aminomethyl)phenyl]methyl}-5-methyl-4-oxo-3,4-dihydrothieno[2,3-*d*]pyri

41  
42  
43 **midine-2-carboxamide Hydrochloride (26c)**. To a suspension of compound **26b** (1.78

44  
45  
46 g, 4.15 mmol) in ethyl acetate (20 mL) was added 4 N hydrogen chloride in ethyl

47  
48  
49 acetate solution (50 mL), and the mixture was stirred at room temperature for 15 h. The

50  
51  
52 reaction mixture was concentrated under reduced pressure, and the residue was

53  
54  
55 crystallized from toluene. The obtained crude crystals were suspended in ethyl acetate,

1  
2  
3  
4  
5  
6 and the suspension was stirred under heating for 3 h to give the title compound as a  
7  
8  
9 white powder (1.39 g, 92%). <sup>1</sup>H NMR (300 MHz, DMSO-*d*<sub>6</sub>) δ 3.33 (3H, s), 3.99 (2H,  
10  
11 s), 4.46 (2H, d, *J* = 6.4 Hz), 7.25–7.52 (5H, m), 8.74–10.18 (4H, m).  
12  
13

14  
15  
16 **Ethyl 5-Isopropyl-4-oxo-3,4-dihydrothieno[2,3-*d*]pyrimidine-2-carboxylate (30a).** A

17  
18 suspension of commercially available ethyl  
19  
20  
21 2-amino-4-isopropylthiophene-3-carboxylate (**29a**) (1.00 g, 4.69 mmol) and ethyl  
22  
23  
24 cyanoformate (511 mg, 5.16 mmol) in 1 N hydrochloride in acetic acid solution (10 mL)  
25  
26  
27 was stirred at 90 °C for 10 h. After cooling to room temperature the mixture was  
28  
29  
30 concentrated under reduced pressure. The residue was triturate with water, collected on  
31  
32  
33 a filter, washed with water and diethyl ether, and dried to give the title compound as a  
34  
35  
36 pale yellow powder (301 mg, 24%). <sup>1</sup>H NMR (300 MHz, DMSO-*d*<sub>6</sub>) δ 1.23 (3H, s),  
37  
38  
39 1.25 (3H, s), 1.34 (3H, t, *J* = 7.0 Hz), 3.59–3.70 (1H, m), 4.36 (2H, q, *J* = 7.2 Hz), 7.41  
40  
41  
42 (1H, s), 12.72 (1H, bs).  
43  
44

45  
46 **Ethyl 4-Oxo-5-(2-thienyl)-3,4-dihydrothieno[2,3-*d*]pyrimidine-2-carboxylate (30b).**

47  
48 Compound **30b** was prepared from commercially available ethyl  
49  
50  
51 5'-amino-2,3'-bithiophene-4'-carboxylate (**29b**) with a similar procedure as described  
52  
53  
54 for compound **30a** (brown powder, 88%). <sup>1</sup>H NMR (300 MHz, DMSO-*d*<sub>6</sub>) δ 1.36 (3H, t,  
55  
56  
57  
58  
59  
60

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2  
3  
4  
5  
6  $J = 7.1$  Hz), 4.39 (2H, q,  $J = 7.2$  Hz), 7.12 (1H, dd,  $J = 5.1, 3.6$  Hz), 7.57 (1H, dd,  $J =$   
7  
8  
9 5.1, 1.3 Hz), 7.65 (1H, dd,  $J = 3.6, 1.3$  Hz), 7.87 (1H, s), 12.89 (1H, bs).

10  
11  
12  
13 **Ethyl 4-Oxo-5-(3-thienyl)-3,4-dihydrothieno[2,3-*d*]pyrimidine-2-carboxylate (30c).**

14  
15 A solution of 3-acetylthiophene (**27a**) (5.00 g, 39.6 mmol), ethyl cyanoacetate (**28**)  
16 (4.48 g, 39.6 mmol) and morpholine (3.45 g, 39.6 mmol) in toluene (40 mL) was stirred  
17  
18 (4.48 g, 39.6 mmol) and morpholine (3.45 g, 39.6 mmol) in toluene (40 mL) was stirred  
19  
20 at 120 °C for 10 h. Sulfur (1.27 g, 39.6 mmol) and ethanol (40 mL) were added to the  
21  
22 at 120 °C for 10 h. Sulfur (1.27 g, 39.6 mmol) and ethanol (40 mL) were added to the  
23  
24 reaction mixture, and the mixture was stirred at 70 °C for 10 h. After cooling to room  
25  
26 reaction mixture, and the mixture was stirred at 70 °C for 10 h. After cooling to room  
27  
28 temperature the mixture was concentrated under reduced pressure. The residue was  
29  
30 purified by silica gel column chromatography (9–20% ethyl acetate/hexane) to give  
31  
32 purified by silica gel column chromatography (9–20% ethyl acetate/hexane) to give  
33  
34 ethyl 5-amino-3,3'-bithiophene-4-carboxylate (**29c**) as a yellow powder (3.30 g, 33%).

35  
36 Compound **30c** was prepared from compound **29c** with a similar procedure as described  
37  
38 for compound **30a** (green powder, 56%). <sup>1</sup>H NMR (300 MHz, DMSO-*d*<sub>6</sub>)  $\delta$  1.35 (3H, t,  
39  
40  $J = 7.0$  Hz), 4.38 (2H, q,  $J = 6.9$  Hz), 7.46–7.50 (1H, m), 7.56 (1H, dd,  $J = 4.9, 3.0$  Hz),  
41  
42  $J = 7.0$  Hz), 4.38 (2H, q,  $J = 6.9$  Hz), 7.46–7.50 (1H, m), 7.56 (1H, dd,  $J = 4.9, 3.0$  Hz),  
43  
44 7.86 (1H, s), 8.01 (1H, d,  $J = 1.9$  Hz), 12.86 (1H, bs).

45  
46  
47  
48 **Ethyl 4-Oxo-5-phenyl-3,4-dihydrothieno[2,3-*d*]pyrimidine-2-carboxylate (30d).**

49  
50 Compound **30d** was prepared from commercially available methyl  
51  
52 2-amino-4-phenylthiophene-3-carboxylate (**29d**) with a similar procedure as described  
53  
54  
55  
56  
57  
58  
59  
60



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2  
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4  
5  
6 for compound **30a** (brown powder, 80%). <sup>1</sup>H NMR (300 MHz, DMSO-*d*<sub>6</sub>) δ 1.36 (3H, t,  
7  
8  
9 *J* = 7.2 Hz), 4.39 (2H, q, *J* = 7.2 Hz), 7.32–7.45 (3H, m), 7.51–7.57 (2H, m), 7.74 (1H,  
10  
11  
12 s), 12.80 (1H, bs).

13  
14  
15  
16 **Ethyl 5-(2-Fluorophenyl)-4-oxo-3,4-dihydrothieno[2,3-*d*]pyrimidine-2-carboxylate**

17  
18  
19 (**30e**). Ethyl 2-amino-4-(2-fluorophenyl)thiophene-3-carboxylate (**29e**) was prepared  
20  
21 from 2-fluoroaceto-phenone (**27b**) with a similar procedure as described for compound  
22  
23  
24 **29c** (yellow powder, 26%). Compound **30e** was prepared from compound **29e** with a  
25  
26 similar procedure as described for compound **30a** (pale yellow powder, 60%). <sup>1</sup>H NMR  
27  
28 (300 MHz, DMSO-*d*<sub>6</sub>) δ 1.35 (3H, t, *J* = 7.2 Hz), 4.38 (2H, q, *J* = 7.0 Hz), 7.19–7.30  
29  
30  
31 (2H, m), 7.38–7.50 (2H, m), 7.79 (1H, s), 12.83 (1H, bs).

32  
33  
34  
35  
36  
37 **Ethyl 5-(3-Fluorophenyl)-4-oxo-3,4-dihydrothieno[2,3-*d*]pyrimidine-2-carboxylate**

38  
39  
40 (**30f**). Ethyl 2-amino-4-(3-fluorophenyl)thiophene-3-carboxylate (**29f**) was prepared  
41  
42 from 3-fluoroacetophenone (**27c**) with a similar procedure as described for compound  
43  
44  
45 **29c** (brown oil, 18%).

46  
47  
48  
49 Compound **30f** was prepared from compound **29f** with a similar procedure as described  
50  
51 for compound **30a** (brown powder, 38%). <sup>1</sup>H NMR (300 MHz, DMSO-*d*<sub>6</sub>) δ 1.36 (3H, t,  
52  
53  
54  
55 *J* = 7.2 Hz), 4.38 (2H, q, *J* = 7.1 Hz), 7.16–7.27 (1H, m), 7.36–7.51 (3H, m), 7.84 (1H,  
56  
57  
58  
59  
60

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2  
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5  
6 s), 12.91 (1H, bs).  
7  
8

9  
10 **Ethyl 5-(4-Fluorophenyl)-4-oxo-3,4-dihydrothieno[2,3-*d*]pyrimidine-2-carboxylate**

11  
12 (30g). Compound 30g was prepared from commercially available ethyl  
13  
14 2-amino-4-(4-fluorophenyl)thiophene-3-carboxylate (29g) with a similar procedure as  
15  
16 described for compound 30a (brown powder, 78%). <sup>1</sup>H NMR (300 MHz, DMSO-*d*<sub>6</sub>) δ  
17  
18 1.36 (3H, t, *J* = 7.2 Hz), 4.39 (2H, q, *J* = 7.1 Hz), 7.18–7.28 (2H, m), 7.52–7.63 (2H, m),  
19  
20 7.72–7.77 (1H, m), 12.81 (1H, bs).  
21  
22  
23  
24  
25  
26

27  
28 **Ethyl 5-(2-Chlorophenyl)-4-oxo-3,4-dihydrothieno[2,3-*d*]pyrimidine-2-carboxylate**

29  
30 (30h). Ethyl 2-amino-4-(2-chlorophenyl)thiophene-3-carboxylate (29h) was prepared  
31  
32 from 2-chloroacetophenone (27d) with a similar procedure as described for compound  
33  
34 29c (brown powder, 27%).  
35  
36  
37  
38

39  
40 Compound 30h was prepared from compound 29h with a similar procedure as  
41  
42 described for compound 30a (pale yellow powder, 5%). <sup>1</sup>H NMR (300 MHz, DMSO-*d*<sub>6</sub>)  
43  
44 δ 1.35 (3H, t, *J* = 7.1 Hz), 4.37 (2H, q, *J* = 7.0 Hz), 7.33–7.55 (4H, m), 7.70 (1H, s),  
45  
46 12.82 (1H, s).  
47  
48  
49  
50

51  
52  
53 **Ethyl 5-(3-Chlorophenyl)-4-oxo-3,4-dihydrothieno[2,3-*d*]pyrimidine-2-carboxylate**

54  
55 (30i). Ethyl 2-amino-4-(3-chlorophenyl)thiophene-3-carboxylate (29i) was prepared  
56  
57  
58  
59  
60

1  
2  
3  
4  
5  
6 from 3-chloroacetophenone (**27e**) with a similar procedure as described for compound  
7  
8  
9 **29c** (brown oil, 51%). Compound **30i** was prepared from compound **29i** with a similar  
10  
11 procedure as described for compound **30a** (pale yellow powder, 36%). <sup>1</sup>H NMR (300  
12  
13 MHz, DMSO-*d*<sub>6</sub>)  $\delta$  1.36 (3H, t, *J* = 7.2 Hz), 4.39 (2H, q, *J* = 7.2 Hz), 7.41–7.47 (2H, m),  
14  
15 7.48–7.54 (1H, m), 7.62 (1H, d, *J* = 0.8 Hz), 7.85 (1H, s), 12.88 (1H, s).  
16  
17  
18  
19

20  
21 **Ethyl 5-(4-Chlorophenyl)-4-oxo-3,4-dihydrothieno[2,3-*d*]pyrimidine-2-carboxylate**  
22

23  
24 (**30j**). Compound **30j** was prepared from commercially available ethyl  
25  
26 2-amino-4-(4-chlorophenyl)thiophene-3-carboxylate (**29j**) with a similar procedure as  
27  
28 described for compound **30a** (pale yellow powder, 86%). <sup>1</sup>H NMR (300 MHz,  
29  
30 DMSO-*d*<sub>6</sub>)  $\delta$  1.35 (3H, t, *J* = 7.2 Hz), 4.38 (2H, q, *J* = 7.0 Hz), 7.42–7.51 (2H, m),  
31  
32 7.53–7.60 (2H, m), 7.78 (1H, s), 12.89 (1H, bs).  
33  
34  
35  
36  
37  
38  
39

40 **Ethyl**

41  
42 **5-(2-Methoxyphenyl)-4-oxo-3,4-dihydrothieno[2,3-*d*]pyrimidine-2-carboxylate**  
43

44  
45 (**30k**). Ethyl 2-amino-4-(2-methoxyphenyl)thiophene-3-carboxylate (**29k**) was prepared  
46  
47 from 2-methoxy-acetophenone (**27f**) with a similar procedure as described for  
48  
49 compound **29c** (yellow oil, 36%). Compound **30k** was prepared from compound **29k**  
50  
51 with a similar procedure as described for compound **30a** (brown powder, 5%). <sup>1</sup>H NMR  
52  
53  
54  
55  
56  
57  
58  
59  
60

(300 MHz, DMSO-*d*<sub>6</sub>)  $\delta$  1.36 (3H, t, *J* = 7.2 Hz), 3.97 (3H, s), 4.38 (2H, q, *J* = 7.1 Hz), 7.08 (1H, t, *J* = 7.4 Hz), 7.22 (1H, d, *J* = 8.0 Hz), 7.37–7.45 (1H, m), 7.89–7.97 (2H, m), 12.87 (1H, bs).

### Ethyl

#### 5-(3-Methoxyphenyl)-4-oxo-3,4-dihydrothieno[2,3-*d*]pyrimidine-2-carboxylate

(**30l**). Ethyl 2-amino-4-(3-methoxyphenyl)thiophene-3-carboxylate (**29l**) was prepared from 3-methoxy-acetophenone (**27g**) with a similar procedure as described for compound **29c** (brown oil, 77%). Compound **30l** was prepared from compound **29l** with a similar procedure as described for compound **30a** (brown powder, 32%). <sup>1</sup>H NMR (300 MHz, DMSO-*d*<sub>6</sub>)  $\delta$  1.36 (3H, t, *J* = 7.2 Hz), 3.78 (3H, s), 4.38 (2H, q, *J* = 7.2 Hz), 6.94 (1H, dd, *J* = 7.8, 2.1 Hz), 7.08–7.16 (2H, m), 7.31 (1H, t, *J* = 7.8 Hz), 7.76 (1H, s), 12.82 (1H, bs).

### Ethyl

#### 5-(4-Methoxyphenyl)-4-oxo-3,4-dihydrothieno[2,3-*d*]pyrimidine-2-carboxylate

(**30m**). Compound **30m** was prepared from commercially available ethyl 2-amino-4-(4-methoxyphenyl)thiophene-3-carboxylate (**29m**) with a similar procedure as described for compound **30a** (pale yellow powder, 79%). <sup>1</sup>H NMR (300 MHz,

1  
2  
3  
4  
5  
6 DMSO-*d*<sub>6</sub>)  $\delta$  1.35 (3H, t,  $J = 7.1$  Hz), 3.80 (3H, s), 4.38 (2H, q,  $J = 7.0$  Hz), 6.96 (2H, d,  
7  
8  
9  $J = 8.7$  Hz), 7.49 (2H, d,  $J = 8.5$  Hz), 7.65 (1H, s), 12.79 (1H, bs).

10  
11  
12 **5-Isopropyl-4-oxo-*N*-{3-[2-(1*H*-1,2,4-triazol-3-yloxy)ethoxy]benzyl}-3,4-dihydrothi**

13 **eno[2,3-*d*]pyrimidine-2-carboxamide (31a)**. A suspension of compound **30a** (150 mg,  
14  
15  
16 0.563 mmol) and

17  
18  
19  
20  
21 1-{3-[(2-{{1-(triphenylmethyl)-1*H*-1,2,4-triazol-3-yl}oxy)ethyl}oxy]phenyl}methanami  
22  
23  
24 ne (**15a**) (322 mg, 0.676 mmol) in ethanol (10 mL) was stirred at 90 °C for 4 days. After

25  
26  
27 cooling to room temperature the reaction mixture was evaporated under reduced

28  
29  
30 pressure. To the residue were added ethyl acetate and water. The organic layer was

31  
32  
33 washed with water, 1 N aqueous hydrochloric acid solution, and brine, dried over

34  
35  
36 Na<sub>2</sub>SO<sub>4</sub>, and concentrated to give a yellow powder. The residue was purified by silica

37  
38  
39 gel column chromatography (50% ethyl acetate/hexane) to give a white powder. To the

40  
41  
42 solution of the white powder (380 mg) in CH<sub>3</sub>CN (10 mL) were added TFA (1.01 mL)

43  
44  
45 and triethylsilane (0.105 mL, 0.654 mmol) at 50 °C. The mixture was stirred at 50 °C

46  
47  
48 for 3 h and evaporated under reduced pressure. The residual solid was crystallized from

49  
50  
51 toluene, ethanol, and diethyl ether to give a powder. The powder was recrystallized from

52  
53  
54 ethanol to give the title compound as a white powder (161 mg, 63% for 2 steps). mp

55  
56 220–221 °C. <sup>1</sup>H NMR (300 MHz, DMSO-*d*<sub>6</sub>)  $\delta$  1.24 (6H, d,  $J = 6.8$  Hz), 3.58–3.71 (1H,  
57  
58  
59  
60

1  
2  
3  
4  
5  
6 m), 4.27 (2H, dd,  $J = 5.3, 3.4$  Hz), 4.42 (2H, d,  $J = 6.4$  Hz), 4.47–4.53 (2H, m), 6.81–  
7  
8  
9 6.97 (3H, m), 7.24 (1H, t,  $J = 7.8$  Hz), 7.34 (1H, s), 9.59 (1H, t,  $J = 5.9$  Hz), 12.30 (1H,  
10  
11  
12 bs), 13.33 (1H, bs). Anal. Calcd for  $C_{21}H_{22}N_6O_4S$ : C, 55.49; H, 4.88; N, 18.49. Found:  
13  
14  
15 C, 55.42; H, 4.82; N, 18.58.

16  
17  
18  
19 **4-Oxo-5-(2-thienyl)-*N*-[(3-{[2-(1*H*-1,2,4-triazol-3-yloxy)ethyl]oxy}phenyl)methyl]-3,**

20  
21 **4-dihydrothieno[2,3-*d*]pyrimidine-2-carboxamide (31b).** Compound **31b** was

22  
23 prepared from compound **30b** and compound **15a** with a similar procedure as described

24  
25  
26 for compound **31a** (white powder, 46%). mp 162–164 °C.  $^1H$  NMR (300 MHz,

27  
28 DMSO- $d_6$ )  $\delta$  4.25–4.30 (2H, m), 4.40–4.53 (4H, m), 6.87 (1H, dd,  $J = 8.0, 2.4$  Hz),

29  
30  
31 6.90–6.98 (2H, m), 7.11 (1H, dd,  $J = 5.1, 3.6$  Hz), 7.25 (1H, t,  $J = 7.9$  Hz), 7.56 (1H, dd,

32  
33  
34  $J = 5.1, 1.1$  Hz), 7.65 (1H, dd,  $J = 3.6, 1.1$  Hz), 7.79 (1H, s), 8.24 (1H, bs), 9.67 (1H, t,  $J$

35  
36  
37 = 6.0 Hz), 12.47 (1H, bs), 13.34 (1H, bs). Anal. Calcd for  $C_{22}H_{18}N_6O_4S_2 \cdot H_2O$ : C, 51.55;

38  
39  
40  
41  
42 H, 3.93; N, 16.40. Found: C, 51.41; H, 3.55; N, 16.33.

43  
44  
45 **4-Oxo-5-(3-thienyl)-*N*-{3-[2-(1*H*-1,2,4-triazol-3-yloxy)ethoxy]benzyl}-3,4-dihydroth**

46  
47  
48 **ieno[2,3-*d*]pyrimidine-2-carboxamide (31c).** Compound **31c** was prepared from

49  
50  
51 compound **30c** and compound **15a** with a similar procedure as described for compound

52  
53  
54 **31a** (white powder, 34%). mp 186–189 °C.  $^1H$  NMR (300 MHz, DMSO- $d_6$ )  $\delta$  4.26–4.31

1  
2  
3  
4  
5  
6 (2H, m), 4.43 (2H, d,  $J = 6.2$  Hz), 4.51 (2H, bs), 6.80–7.02 (3H, m), 7.25 (1H, t,  $J = 7.8$   
7  
8 Hz), 7.47–7.51 (1H, m), 7.54 (1H, dd,  $J = 4.9, 3.0$  Hz), 7.77 (1H, s), 8.03 (1H, d,  $J = 3.0$   
9  
10 Hz), 8.19 (1H, bs), 9.63 (1H, t,  $J = 6.2$  Hz), 12.39 (1H, bs), 13.34 (1H, bs). Anal. Calcd  
11  
12 for  $C_{22}H_{18}N_6O_4S_2 \cdot 0.4H_2O$ : C, 52.66; H, 3.78; N, 16.75. Found: C, 52.92; H, 3.73; N,  
13  
14 16.76.  
15  
16  
17  
18  
19

20  
21  
22 **4-Oxo-5-phenyl-*N*-{3-[2-(1*H*-1,2,4-triazol-3-yloxy)ethoxy]benzyl}-3,4-dihydrothien**

23  
24 **o[2,3-*d*]pyrimidine-2-carboxamide (31d)**. Compound **31d** was prepared from

25  
26  
27 compound **30d** and compound **15a** with a similar procedure as described for compound

28  
29  
30 **31a** (white powder, 72%). mp 183–185 °C.  $^1H$  NMR (300 MHz, DMSO- $d_6$ )  $\delta$  4.28 (2H,  
31  
32 dd,  $J = 5.3, 3.4$  Hz), 4.44 (2H, d,  $J = 6.2$  Hz), 4.50 (2H, s), 6.87 (1H, dd,  $J = 8.0, 2.0$  Hz),  
33  
34 6.91–6.98 (2H, m), 7.25 (1H, t,  $J = 7.8$  Hz), 7.32–7.43 (3H, m), 7.51–7.57 (2H, m), 7.67  
35  
36 (1H, s), 8.24 (1H, bs), 9.68 (1H, t,  $J = 6.3$  Hz), 12.37 (1H, bs), 13.33 (1H, bs). Anal.  
37  
38  
39  
40

41  
42 Calcd for  $C_{24}H_{20}N_6O_4S$ : C, 59.01; H, 4.13; N, 17.20. Found: C, 58.74; H, 4.18; N,  
43  
44 17.08.  
45  
46  
47

48  
49 **5-(2-Fluorophenyl)-4-oxo-*N*-{3-[2-(1*H*-1,2,4-triazol-3-yloxy)ethoxy]benzyl}-3,4-dih**

50  
51 **ydro-thieno[2,3-*d*]pyrimidine-2-carboxamide (31e)**. Compound **31e** was prepared

52  
53  
54 from compound **30e** and compound **15a** with a similar procedure as described for  
55  
56  
57  
58  
59  
60

1  
2  
3  
4  
5  
6 compound **31a** (white powder, 46%). mp 178–179 °C. <sup>1</sup>H NMR (300 MHz, DMSO-*d*<sub>6</sub>)

7  
8  
9  $\delta$  4.28 (2H, bs), 4.43 (2H, d, *J* = 6.0 Hz), 4.50 (2H, bs), 6.82–7.00 (3H, m), 7.18–7.30

10  
11  
12 (3H, m), 7.36–7.48 (2H, m), 7.67 (1H, bs), 8.23 (1H, bs), 9.63 (1H, bs), 12.30 (1H, s),

13  
14  
15 13.34 (1H, bs). Anal. Calcd for C<sub>24</sub>H<sub>19</sub>FN<sub>6</sub>O<sub>4</sub>S•0.5H<sub>2</sub>O: C, 55.92; H, 3.91; N, 16.30.

16  
17  
18 Found: C, 55.68; H, 3.81; N, 16.52.

19  
20  
21  
22 **5-(3-Fluorophenyl)-4-oxo-*N*-{3-[2-(1*H*-1,2,4-triazol-3-yloxy)ethoxy]benzyl}-3,4-dih**

23  
24  
25 **ydro-thieno[2,3-*d*]pyrimidine-2-carboxamide (31f)**. Compound **31f** was prepared

26  
27  
28 from compound **30f** and compound **15a** with a similar procedure as described for

29  
30  
31 compound **31a** (white powder, 55%). mp 158–161 °C. <sup>1</sup>H NMR (300 MHz, DMSO-*d*<sub>6</sub>)

32  
33  
34  $\delta$  4.27 (2H, dd, *J* = 3.0, 2.4 Hz), 4.41–4.52 (4H, m), 6.87 (1H, dd, *J* = 8.2, 1.8 Hz),

35  
36  
37 6.91–6.98 (2H, m), 7.14–7.30 (2H, m), 7.35–7.50 (3H, m), 7.78 (1H, s), 8.24 (1H, s),

38  
39  
40 9.69 (1H, t, *J* = 6.3 Hz), 12.45 (1H, bs), 13.33 (1H, bs). Anal. Calcd for C<sub>24</sub>H<sub>19</sub>FN<sub>6</sub>O<sub>4</sub>S:

41  
42  
43 C, 56.91; H, 3.78; N, 16.59. Found: C, 56.71; H, 3.72; N, 16.58.

44  
45  
46 **5-(4-Fluorophenyl)-4-oxo-*N*-{3-[2-(1*H*-1,2,4-triazol-3-yloxy)ethoxy]benzyl}-3,4-dih**

47  
48  
49 **ydro-thieno[2,3-*d*]pyrimidine-2-carboxamide (31g)**. Compound **31g** was prepared

50  
51  
52 from compound **30g** and compound **15a** with a similar procedure as described for

53  
54  
55 compound **31a** (white powder, 69%). mp 188–190 °C. <sup>1</sup>H NMR (300 MHz, DMSO-*d*<sub>6</sub>)



1  
2  
3  
4  
5  
6  $\delta$  4.25–4.30 (2H, m), 4.44 (2H, d,  $J = 6.2$  Hz), 4.50 (2H, s), 6.87 (1H, dd,  $J = 8.1, 2.1$   
7  
8  
9 Hz), 6.90–6.98 (2H, m), 7.18–7.29 (3H, m), 7.53–7.62 (2H, m), 7.68 (1H, s), 8.24 (1H,  
10  
11  
12 bs), 9.68 (1H, t,  $J = 6.3$  Hz), 12.40 (1H, bs), 13.33 (1H, bs). Anal. Calcd for  
13  
14  $C_{24}H_{19}FN_6O_4S$ : C, 56.91; H, 3.78; N, 16.59. Found: C, 56.77; H, 3.70; N, 16.53.  
15  
16

17  
18 **5-(2-Chlorophenyl)-4-oxo-*N*-{3-[2-(1*H*-1,2,4-triazol-3-yloxy)ethoxy]benzyl}-3,4-dih**  
19  
20  
21 **ydro-thieno[2,3-*d*]pyrimidine-2-carboxamide (31h)**. Compound **31h** was prepared  
22  
23  
24 from compound **30h** and compound **15a** with a similar procedure as described for  
25  
26  
27 compound **31a** (white powder, 44%). mp 169–171 °C.  $^1H$  NMR (300 MHz, DMSO- $d_6$ )  
28  
29  
30  $\delta$  4.26–4.30 (2H, m), 4.40–4.53 (4H, m), 6.83–6.98 (3H, m), 7.25 (1H, t,  $J = 7.9$  Hz),  
31  
32  
33 7.33–7.45 (3H, m), 7.48–7.53 (1H, m), 7.65 (1H, s), 8.24 (1H, bs), 9.67 (1H, t,  $J = 6.5$   
34  
35 Hz), 12.40 (1H, bs), 13.34 (1H, bs). Anal. Calcd for  $C_{24}H_{19}ClN_6O_4S \cdot 0.2H_2O$ : C, 54.74;  
36  
37  
38 H, 3.71; N, 15.96. Found: C, 54.78; H, 3.61; N, 16.09.  
39  
40  
41

42  
43 **5-(3-Chlorophenyl)-4-oxo-*N*-{3-[2-(1*H*-1,2,4-triazol-3-yloxy)ethoxy]benzyl}-3,4-dih**  
44  
45  
46 **ydro-thieno[2,3-*d*]pyrimidine-2-carboxamide (31i)**. Compound **31i** was prepared  
47  
48  
49 from compound **30i** and compound **15a** with a similar procedure as described for  
50  
51  
52 compound **31a** (white powder, 45%). mp 153–154 °C.  $^1H$  NMR (300 MHz, DMSO- $d_6$ )  
53  
54  
55  $\delta$  4.25–4.30 (2H, m), 4.40–4.52 (4H, m), 6.84–6.97 (3H, m), 7.25 (1H, t,  $J = 7.8$  Hz),  
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5  
6 7.41–7.45 (2H, m), 7.47–7.55 (1H, m), 7.62 (1H, t,  $J = 1.2$  Hz), 7.79 (1H, s), 8.24 (1H,  
7  
8  
9 bs), 9.70 (1H, t,  $J = 6.3$  Hz), 12.46 (1H, bs), 13.34 (1H, bs). Anal. Calcd for  
10  
11  $C_{24}H_{19}ClN_6O_4S$ : C, 55.12; H, 3.66; N, 16.07. Found: C, 54.90; H, 3.60; N, 16.16.  
12  
13

14  
15 **5-(4-Chlorophenyl)-4-oxo-*N*-{3-[2-(1*H*-1,2,4-triazol-3-yloxy)ethoxy]benzyl}-3,4-dih**  
16  
17 **ydro-thieno[2,3-*d*]pyrimidine-2-carboxamide (31j).** Compound **31j** was prepared  
18  
19 from compound **30j** and compound **15a** with a similar procedure as described for  
20  
21 compound **31a** (white powder, 80%). mp 160–164 °C.  $^1H$  NMR (300 MHz, DMSO- $d_6$ )  
22  
23  $\delta$  4.28 (2H, dd,  $J = 5.3, 3.0$  Hz), 4.43 (2H, d,  $J = 6.4$  Hz), 4.47–4.53 (2H, m), 6.87 (1H,  
24  
25 dd,  $J = 8.0, 1.9$  Hz), 6.90–6.98 (2H, m), 7.25 (1H, t,  $J = 7.8$  Hz), 7.42–7.49 (2H, m),  
26  
27 7.51–7.60 (2H, m), 7.71 (1H, s), 8.23 (1H, bs), 9.68 (1H, t,  $J = 6.1$  Hz), 12.44 (1H, bs),  
28  
29 13.33 (1H, bs). Anal. Calcd for  $C_{24}H_{19}ClN_6O_4S$ : C, 55.12; H, 3.66; N, 16.07. Found: C,  
30  
31 55.05; H, 3.63; N, 16.01.  
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42  
43 **5-(2-Methoxyphenyl)-4-oxo-*N*-{3-[2-(1*H*-1,2,4-triazol-3-yloxy)ethoxy]benzyl}-3,4-di**  
44  
45 **hydro-thieno[2,3-*d*]pyrimidine-2-carboxamide (31k).** Compound **31k** was prepared  
46  
47 from compound **30k** and compound **15a** with a similar procedure as described for  
48  
49 compound **31a** (white powder, 49%). mp 211–213 °C.  $^1H$  NMR (300 MHz, DMSO- $d_6$ )  
50  
51  $\delta$  3.96 (3H, s), 4.25–4.31 (2H, m), 4.40–4.54 (4H, m), 6.83–6.98 (3H, m), 7.04–7.11  
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6 (1H, m), 7.18–7.29 (2H, m), 7.36–7.44 (1H, m), 7.88–7.92 (2H, m), 8.23 (1H, bs), 9.65

7  
8  
9 (1H, t,  $J = 6.3$  Hz), 12.45 (1H, bs), 13.33 (1H, bs). Anal. Calcd for

10  
11  
12  $C_{25}H_{22}N_6O_5S \cdot 1.2EtOH$ : C, 52.33; H, 3.86; N, 14.65. Found: C, 52.35; H, 3.94; N,

13  
14  
15 14.51.

16  
17  
18  
19 **5-(3-Methoxyphenyl)-4-oxo-*N*-{3-[2-(1*H*-1,2,4-triazol-3-yloxy)ethoxy]benzyl}-3,4-di**

20  
21 **hydro-thieno[2,3-*d*]pyrimidine-2-carboxamide (31l)**. Compound **31l** was prepared

22  
23  
24 from compound **30l** and compound **15a** with a similar procedure as described for

25  
26  
27 compound **31a** (white powder, 50%). mp 163–165 °C.  $^1H$  NMR (300 MHz, DMSO- $d_6$ )

28  
29  $\delta$  3.78 (3H, s), 4.24–4.31 (2H, m), 4.40–4.53 (4H, m), 6.87 (1H, dd,  $J = 8.2, 2.0$  Hz),

30  
31  
32 6.90–6.97 (3H, m), 7.08–7.15 (2H, m), 7.22–7.34 (2H, m), 7.69 (1H, s), 8.23 (1H, s),

33  
34  
35 9.66 (1H, t,  $J = 5.8$  Hz), 12.35 (1H, s), 13.33 (1H, bs). Anal. Calcd for

36  
37  
38  $C_{25}H_{22}N_6O_5S \cdot 0.5H_2O$ : C, 56.92; H, 4.39; N, 15.93. Found: C, 56.93; H, 4.27; N, 16.06.

39  
40  
41  
42 **5-(4-Methoxyphenyl)-4-oxo-*N*-{3-[2-(1*H*-1,2,4-triazol-3-yloxy)ethoxy]benzyl}-3,4-di**

43  
44 **hydro-thieno[2,3-*d*]pyrimidine-2-carboxamide (31m)**. Compound **31m** was prepared

45  
46  
47 from compound **30m** and compound **15a** with a similar procedure as described for

48  
49  
50 compound **31a** (white powder, 50%). mp 93–98 °C.  $^1H$  NMR (300 MHz, DMSO- $d_6$ )  $\delta$

51  
52  
53 3.80 (3H, s), 4.25–4.30 (2H, m), 4.43 (2H, d,  $J = 5.7$  Hz), 4.51 (2H, bs), 6.87 (1H, dd,  $J$

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5  
6 = 7.6, 1.1 Hz), 6.90–6.99 (4H, m), 7.18–7.32 (2H, m), 7.45–7.51 (2H, m), 7.59 (1H, s),  
7  
8  
9 9.69 (1H, t,  $J = 6.6$  Hz), 12.35 (1H, s).  
10

11  
12 **5-(2-Fluorophenyl)-4-oxo-*N*-[(3-{{3-(1*H*-1,2,4-triazol-3-yloxy)propyl}oxy}phenyl)m**

13 **ethyl]-3,4-dihydrothieno[2,3-*d*]pyrimidine-2-carboxamide (31n)**. A mixture of  
14

15  
16 compound **15b** (0.300 g, 0.611 mmol), ethyl  
17

18  
19 5-(2-fluorophenyl)-4-oxo-3,4-dihydrothieno[2,3-*d*]pyrimidine-2-carboxylate (**30e**,  
20

21  
22 0.195 g, 0.611 mmol), and *N,N*-diisopropylethylamine (0.213 mL, 1.22 mmol) in  
23

24  
25 ethanol (4.5 mL) was heated under microwave for 3 h at 100 °C (50 W, run time: 5 min,  
26

27  
28 hold time 1 h × 3 set). The reaction mixture was concentrated under reduced pressure  
29

30  
31 and coevaporated with toluene (twice). The residue was purified by silica-gel column  
32

33  
34 chromatography (33–40% ethyl acetate/hexane) to give a pale yellow amorphous (0.305  
35

36  
37 g). The amorphous product (0.280 g) was crystallized from CH<sub>3</sub>CN to give a white  
38

39  
40 powder (0.245 g, 0.321 mmol, 57%). To a suspension of the white powder (0.220 g,  
41

42  
43 0.288 mmol) in CH<sub>3</sub>CN (4 mL) were added TFA (0.857 mL) and triethylsilane (0.055  
44

45  
46 mL, 0.346 mmol) at room temperature and the mixture was stirred at room temperature  
47

48  
49 for 3 h. The mixture was concentrated under reduced pressure and the residue was  
50

51  
52 crystallized from diethyl ether to give the title compound as a pale yellow powder  
53

54  
55 (0.145 g, 97%) (55% for 2 steps). mp 159–161 °C. <sup>1</sup>H NMR (300 MHz, DMSO-*d*<sub>6</sub>)  $\delta$   
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6 2.09–2.22 (2H, m), 4.08 (2H, t,  $J = 6.2$  Hz), 4.27–4.38 (2H, m), 4.43 (2H, d,  $J = 6.2$  Hz),  
7  
8  
9 6.81–6.88 (1H, m), 6.88–6.97 (2H, m), 7.18–7.28 (3H, m), 7.38–7.50 (2H, m), 7.72 (1H,  
10  
11 s), 8.21 (1H, bs), 9.67 (1H, t,  $J = 6.4$  Hz), 12.39 (1H, bs), 13.28 (1H, bs). Anal. Calcd  
12  
13 for  $C_{25}H_{21}FN_6O_4S$ : C, 57.68; H, 4.07; N, 16.14. Found: C, 57.48; H, 4.12; N, 16.01.  
14  
15  
16  
17

18 **5-(3-Fluorophenyl)-4-oxo-*N*-[(3-{[3-(1*H*-1,2,4-triazol-3-yloxy)propyl]oxy}phenyl)m**  
19  
20  
21 **ethyl]-3,4-dihydrothieno[2,3-*d*]pyrimidine-2-carboxamide (31o)**. Compound **31o**  
22  
23

24 was prepared from compound **30f** and compound **15b** with a similar procedure as  
25  
26  
27 described for compound **31n** (pale yellow powder, 59%). mp 130–132 °C.  $^1H$  NMR  
28  
29 (300 MHz,  $DMSO-d_6$ )  $\delta$  2.08–2.22 (2H, m), 4.08 (2H, t,  $J = 6.2$  Hz), 4.25–4.38 (2H, m),  
30  
31 4.43 (2H, d,  $J = 6.4$  Hz), 6.80–6.88 (1H, m), 6.88–6.98 (2H, m), 7.16–7.28 (2H, m),  
32  
33 7.35–7.49 (3H, m), 7.78 (1H, s), 8.21 (1H, bs), 9.69 (1H, t,  $J = 6.3$  Hz), 12.45 (1H, bs),  
34  
35 13.28 (1H, bs). Anal. Calcd for  $C_{25}H_{21}FN_6O_4S \cdot 0.1H_2O$ : C, 57.49; H, 4.09; N, 16.09.  
36  
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38  
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41  
42 Found: C, 57.20; H, 4.12; N, 15.99.  
43  
44

45 **5-(2-Chlorophenyl)-4-oxo-*N*-[(3-{[3-(1*H*-1,2,4-triazol-3-yloxy)propyl]oxy}phenyl)m**  
46  
47  
48 **ethyl]-3,4-dihydrothieno[2,3-*d*]pyrimidine-2-carboxamide (31p)**. Compound **31p**  
49  
50

51 was prepared from compound **30h** and compound **15b** with a similar procedure as  
52  
53  
54 described for compound **31n** (pale yellow powder, 63%). mp 165–167 °C.  $^1H$  NMR  
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6 (300 MHz, DMSO-*d*<sub>6</sub>)  $\delta$  2.09–2.21 (2H, m), 4.08 (2H, t, *J* = 6.2 Hz), 4.28–4.39 (2H, m),  
7  
8  
9 4.43 (2H, d, *J* = 6.2 Hz), 6.84 (1H, dd, *J* = 7.9, 1.9 Hz), 6.88–6.96 (2H, m), 7.24 (1H, t,  
10  
11  
12 *J* = 7.9 Hz), 7.32–7.46 (3H, m), 7.48–7.54 (1H, m), 7.65 (1H, s), 8.19 (1H, bs), 9.66  
13  
14 (1H, t, *J* = 6.2 Hz), 12.26 (1H, bs), 13.26 (1H, bs). Anal. Calcd for  
15  
16 C<sub>25</sub>H<sub>21</sub>ClN<sub>6</sub>O<sub>4</sub>S•0.2H<sub>2</sub>O: C, 55.54; H, 3.99; N, 15.55. Found: C, 55.33; H, 4.05; N,  
17  
18 15.54.  
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24 **5-(3-Chlorophenyl)-4-oxo-*N*-[(3-{[3-(1*H*-1,2,4-triazol-3-yloxy)propyl]oxy}phenyl)m**  
25  
26  
27 **ethyl]-3,4-dihydrothieno[2,3-*d*]pyrimidine-2-carboxamide (31q).** Compound **31q**  
28

29  
30 was prepared from compound **30i** and compound **15b** with a similar procedure as  
31  
32 described for compound **31n** (white powder, 67%). mp 167–169 °C. <sup>1</sup>H NMR (300  
33  
34 MHz, DMSO-*d*<sub>6</sub>)  $\delta$  2.09–2.22 (2H, m), 4.08 (2H, t, *J* = 6.2 Hz), 4.26–4.39 (2H, m),  
35  
36 4.43 (2H, d, *J* = 6.2 Hz), 6.84 (1H, dd, *J* = 8.1, 1.9 Hz), 6.88–6.97 (2H, m), 7.24 (1H, t,  
37  
38  
39 *J* = 7.8 Hz), 7.39–7.47 (2H, m), 7.47–7.55 (1H, m), 7.62 (1H, s), 7.79 (1H, s), 8.21 (1H,  
40  
41  
42 bs), 9.69 (1H, t, *J* = 6.3 Hz), 12.45 (1H, bs), 13.28 (1H, bs). Anal. Calcd for  
43  
44  
45 C<sub>25</sub>H<sub>21</sub>ClN<sub>6</sub>O<sub>4</sub>S•0.2H<sub>2</sub>O: C, 55.54; H, 3.99; N, 15.55. Found: C, 55.42; H, 3.97; N,  
46  
47 15.36.  
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54 **Crystallization and Structure Determination.** Crystals were grown by the hanging  
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6 drop vapor diffusion method at 20 °C (the temperature was modified). Prior to  
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9 crystallization, a solution containing 6–14 mg/mL human MMP-13 catalytic domain, 5  
10  
11  $\mu\text{M}$   $\text{Zn}(\text{OAc})_2$ , 5 mM  $\text{CaCl}_2$ , 50 mM  $\text{NaCl}$ , 20 mM Tris HCl buffer (pH 8.0) were  
12  
13  
14 prepared. The protein solution was incubated with 0.5 mM of the compounds for 1 h on  
15  
16  
17 ice, and then centrifuged to remove the precipitation. Equal volumes (0.5  $\mu\text{L}$ ) of the  
18  
19  
20 protein solution and reservoir solution containing 8–16% w/v PEG8000, 1.0–1.5 M  
21  
22  
23 ammonium formate, and 0.1 M Tris HCl (pH 8.5) buffer were mixed, and equilibrated  
24  
25  
26 in the hanging drop against a reservoir solution. Crystals were dipped into a reservoir  
27  
28  
29 solution containing 25% ethylene glycol, and then treated by flash-cooling method. The  
30  
31  
32 crystals were stored in liquid nitrogen until use. X-ray diffraction data were collected at  
33  
34  
35 the Advanced Light Source (ALS) beamline 5.0.3 (Berkeley, CA), and processed using  
36  
37  
38 the program HKL2000.<sup>51</sup> The structure was determined by molecular replacement using  
39  
40  
41 MOLREP,<sup>52</sup> using the only protein structure of MMP-13 previously reported (PDB  
42  
43  
44 accession number 830C).<sup>53</sup> Subsequently, structure refinement and model building were  
45  
46  
47 performed utilizing REFMAC<sup>54</sup>. The solved structure was modeled with WinCoot  
48  
49  
50 (version 0.3.3)<sup>55</sup>. X-ray coordinates have been deposited at the Cambridge  
51  
52  
53 Crystallographic Data Centre; deposition numbers: 5B5O for **2** and 5B5P for **23**. The  
54  
55  
56 statistic data and the refinement statistics are shown in **Table 6**.  
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6 **MMPs and TACE Enzyme Inhibition Assay.** Human recombinant MMP precursors  
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8  
9 were purchased from Genzyme-Techne (MMP-1, 2, 7, 8, 9, 10, 13, and TACE) or  
10  
11 Biogenesis (MMP-3). Human recombinant GST-MMP-14 was prepared as described by  
12  
13 Sato et al.<sup>56</sup> The MMP assay buffer consisted of 50 mM Tris-HCl (pH 7.5), 10 mM  
14  
15 CaCl<sub>2</sub>, 150 mM NaCl, and 0.05% Brij-35. The pro-MMPs were activated by  
16  
17 preincubation with 1 mM aminophenylmercuric acetate (APMA) in assay buffer at  
18  
19 37 °C for 2 h (MMP-1, 2, 7, 8, 10, and 13) or 18 h (MMP-3 and 9). The TACE assay  
20  
21 buffer consisted of 25 mM Tris-HCl (pH 9.0), 2.5 μM ZnCl<sub>2</sub>, and 0.005% Brij-35.  
22  
23 Enzyme inhibition assays were performed in assay buffer containing enzymes and  
24  
25 fluorescence peptide (Cy3-PLGLK(Cy5Q)AR-NH<sub>2</sub> for MMPs and  
26  
27 Cy3-PLAQAV(Cy5Q-L-2,3-diaminopropionic acid)-RSSSR-NH<sub>2</sub> for TACE, Amersham  
28  
29 Biosciences) in the presence of the various concentrations of inhibitors. Following  
30  
31 incubation at 37 °C for 40 min, the reaction was terminated by addition of EDTA (pH  
32  
33 8.0). The increase in fluorescence was measured by Farcyte spectrofluorimeter  
34  
35 (Amersham Bioscience, λ<sub>em</sub> = 535 nm; λ<sub>ex</sub> = 595 nm). Enzyme activity (%) was  
36  
37 determined according to the following equation: Enzyme activity (%) =  $(X - C)/(T - C)$   
38  
39 × 100, where  $X$  is the fluorescence count with inhibitor,  $T$  is the fluorescence count  
40  
41 without inhibitor, and  $C$  is the fluorescence count with EDTA. IC<sub>50</sub> values of inhibitors  
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6 were obtained with iterative fitting package (GraphPad Prism software).  
7  
8

9  
10 **Assay for Inhibitory Activity Against Collagen Degradation.** Bovine nasal septum  
11  
12 cartilage was sliced, and the slices were maintained in the medium of a 1 : 1 (v/v)  
13  
14 mixture of Dulbecco's modified Eagle's MEM and Ham's F-12 medium (DMEM/F-12)  
15  
16 containing 10 % fetal calf serum overnight. After confirming that the slices were not  
17  
18 contaminated, they were cultured in DMEM/F-12 medium containing 20 µg/mL  
19  
20 gentamycin, 50 µg/mL streptomycin, and 50 U/mL penicillin (culture medium) for 2  
21  
22 days at 37 °C. The cartilage slices were cut into small cubes (ca. 1mm<sup>3</sup>) and transferred  
23  
24 individually into wells of a 96 well plate with 100 µL of culture medium. For the  
25  
26 collagen degradation assay, the medium was supplemented with 10 ng/mL IL-1β and 50  
27  
28 ng/mL OSM in the presence or absence of compounds. The cartilage was incubated for  
29  
30 2 weeks. The supernatants were harvested and replaced with fresh medium containing  
31  
32 identical test reagents every 7 days. Supernatants of day 7 and day 14 were collected  
33  
34 and stored at -20 °C until assay. At the end of the culture, the remaining cartilage was  
35  
36 completely digested with papain. Hydroxyproline release in the media from each  
37  
38 explant was determined as a measure of collagen degradation by use of chloramine T  
39  
40 and *p*-dimethylaminobenzaldehyde. The normalized degree of the collagen degradation  
41  
42 was shown as % of collagen degradation, calculated by following formulation:  
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6 (hydroxyproline content in media)/[(hydroxyproline content in media) +  
7  
8  
9 (hydroxyproline content in remaining cartilage)] × 100. The percentage of inhibitory  
10  
11 activity against collagen degradation was calculated as follows: % of inhibition = [(% of  
12  
13 collagen degradation with IL-1β and OSM) – (% of collagen degradation with IL-1β,  
14  
15 OSM, and test sample)]/[(% of collagen degradation with IL-1β and OSM) – (% of  
16  
17 collagen degradation without additives)] × 100.  
18  
19  
20  
21  
22  
23

#### 24 ASSOCIATED CONTENT

##### 25 26 27 28 Accession Codes

29  
30  
31  
32 PDB entries for **2** in complex with MMP-13 and for **23** in complex with MMP-13 are  
33  
34 5B5O and 5B5P, respectively. Authors will release the atomic coordinates and  
35  
36 experimental data upon article publication.  
37  
38  
39

#### 40 41 42 AUTHOR INFORMATION

##### 43 44 45 Corresponding Author

46  
47  
48  
49 \*E-mail: [hiroshi.nara@takeda.com](mailto:hiroshi.nara@takeda.com)

50  
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52  
53 Phone: +81-46-632-1230  
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6 Notes  
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10 The authors declare no competing financial interest.  
11

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15  
16  
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38  
39 analyses.  
40  
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## 51 52 ABBREVIATIONS USED 53

54 TEA, triethylamine; DIEA, *N,N*-diisopropylethylamine; WSC, water-soluble  
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6 carbodiimide; HOBt, 1-hydroxybenzotriazole; SEM, standard error of the mean  
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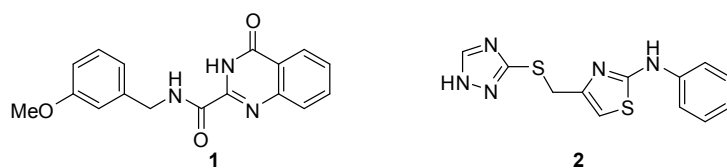
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recombinant membrane type 1-matrix metalloproteinase (MT1-MMP) by furin and its

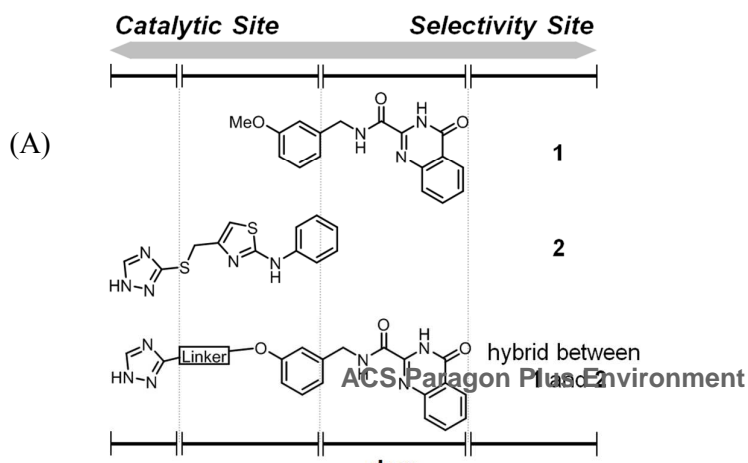
interaction with tissue inhibitor of metalloproteinases (TIMP)-2. *FEBS Lett.* **1996**, *393*,

101-104.

(57) *The PyMOL molecular graphics system.* In 2006.



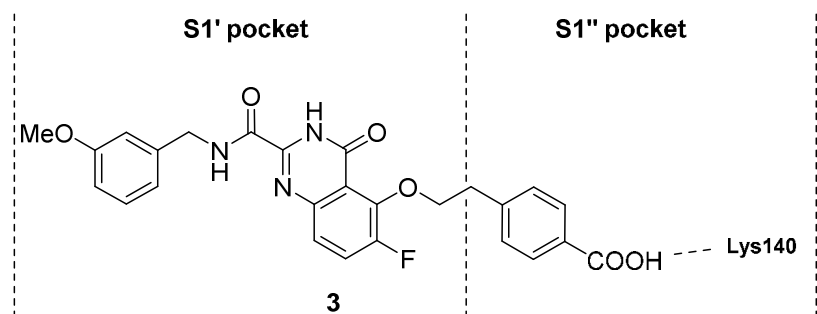
**Figure 1.** Structures of initial hit compounds.



(B)

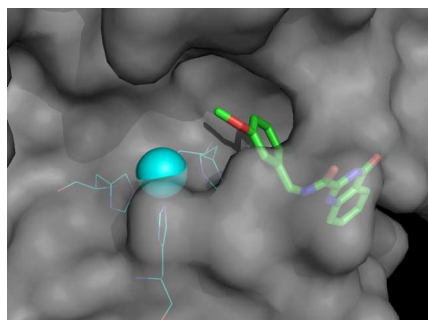
(C)

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

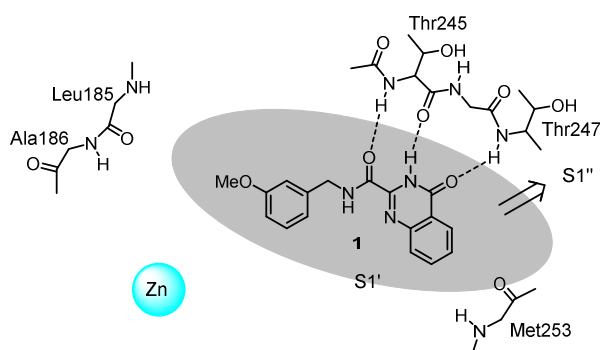


**Figure 3.** Chemical structure of **3**.

(A)

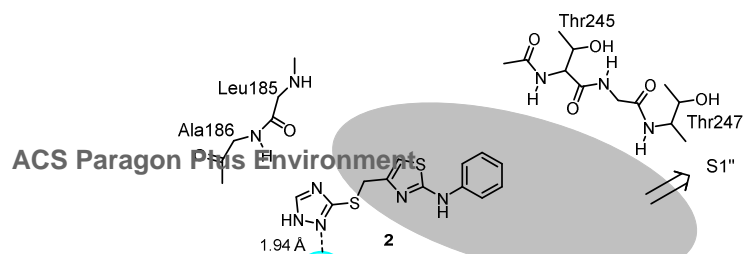


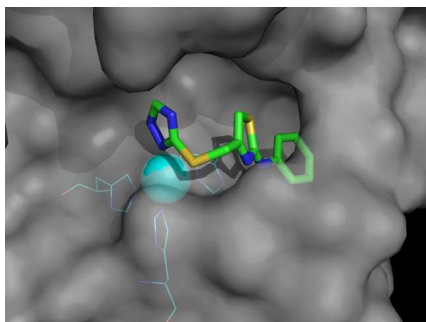
(B)



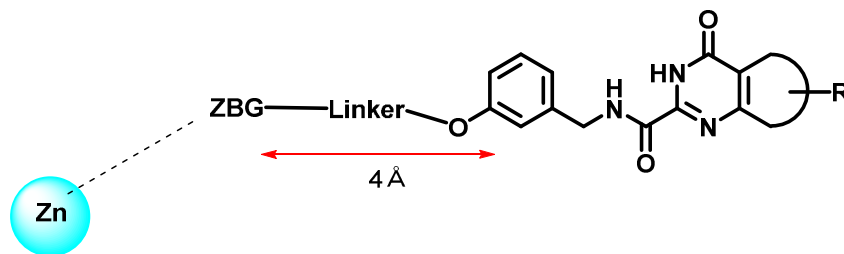
(C)

(D)



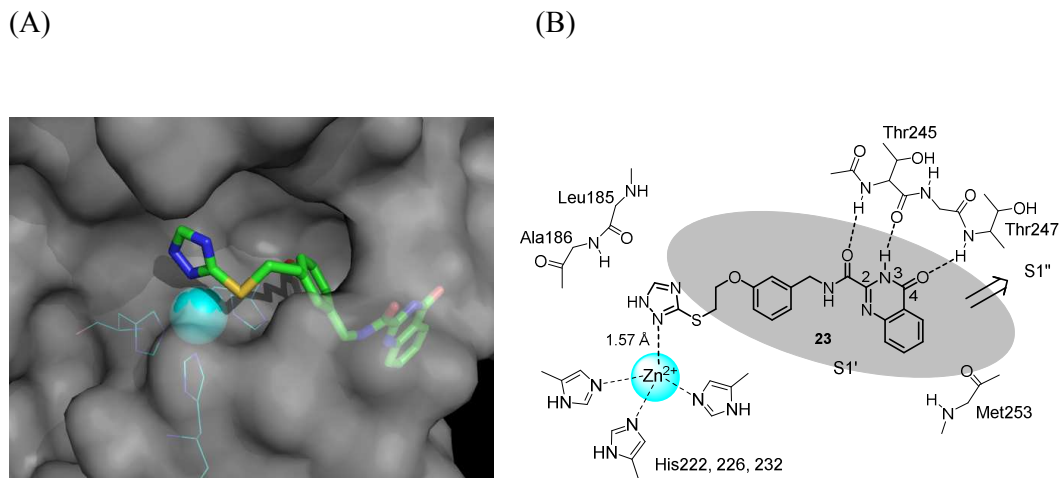


**Figure 4.** Crystal structures of complex of quinazoline **1** (PDB code 3WV2) and triazole **2** with human MMP-13 catalytic domain. (A) Surface representation of MMP-13 illustrating the binding cavity. Catalytic zinc ion is shown in blue sphere. The enzyme is in gray. Three residues His222, 226,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



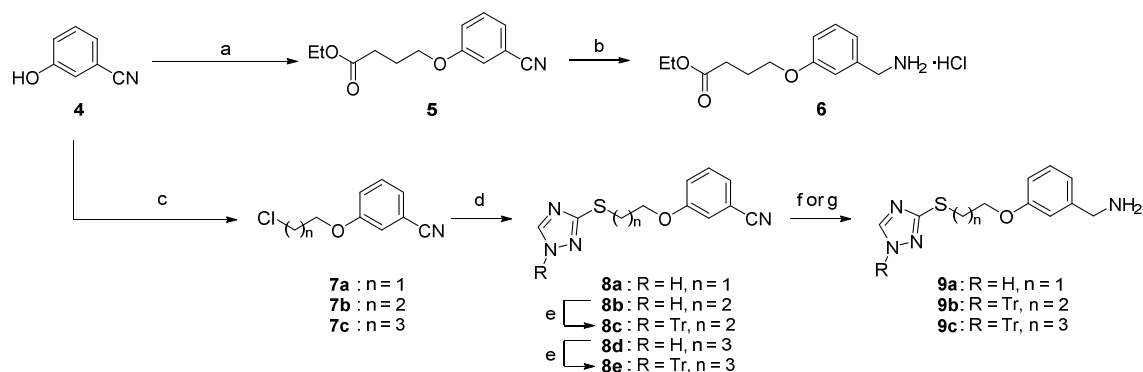


1  
2  
3  
4  
5  
6 **Figure 5.** Design of quinazoline-based zinc binding MMP-13 selective inhibitors.  
7  
8  
9



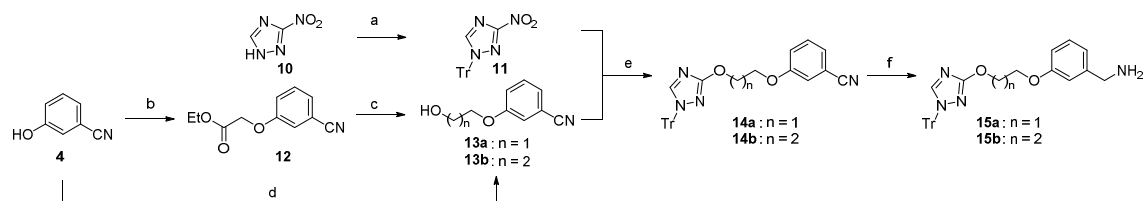
**Figure 6.** Crystal structure of the human MMP-13 catalytic domain complexed with a hybrid zinc binding inhibitor **23**. (A) Molecular surface diagram of MMP-13 illustrating the binding cavity. The catalytic zinc ion is shown in a blue sphere. The enzyme is in gray. Three residues His222, 226, and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

**Scheme 1.** Synthesis of Benzylamine Derivatives **6** and **9a-c**<sup>a</sup>



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

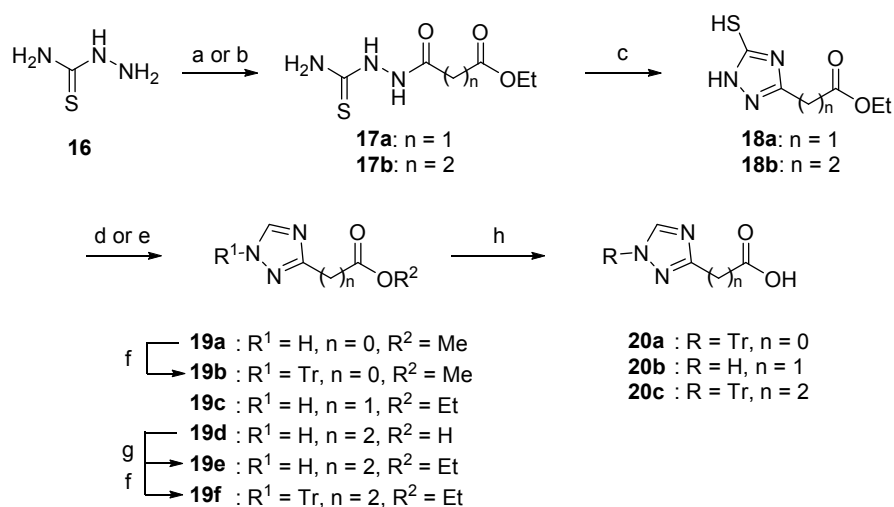
### Scheme 2. Synthesis of Benzylamine Derivatives **15a** and **15b**<sup>a</sup>



<sup>a</sup>Reagents and conditions: (a) TrCl, DIEA, THF, rt, 93%; (b) ethyl bromoacetate, K<sub>2</sub>CO<sub>3</sub>, THF, 50 °C, 99%; (c) NaBH<sub>4</sub>, EtOH, 50 °C, 92% (for **13a**); (d) 3-bromopropanol,

K<sub>2</sub>CO<sub>3</sub>, DMF, 80 °C, 53% (for **13b**); (e) NaH, THF, rt, 46% and quant. (crude) (for **14a** and **14b**, respectively); (f) LiAlH<sub>4</sub>, THF, rt, 58 and 77% (for **15a** and **15b**, respectively).

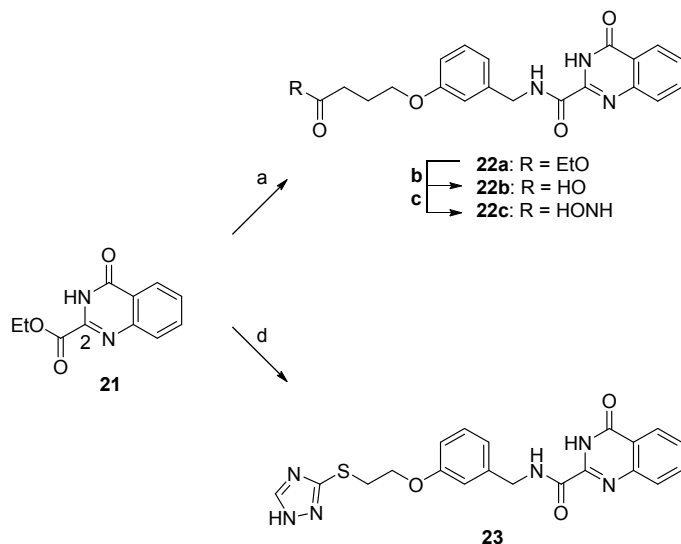
**Scheme 3.** Synthesis of Carboxylic Acid Derivatives **20a–c**<sup>a</sup>



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

**Scheme 4.** Synthesis of 4-Oxo-3,4-dihydroquinazoline-2-carboxamide Derivatives **22a–**

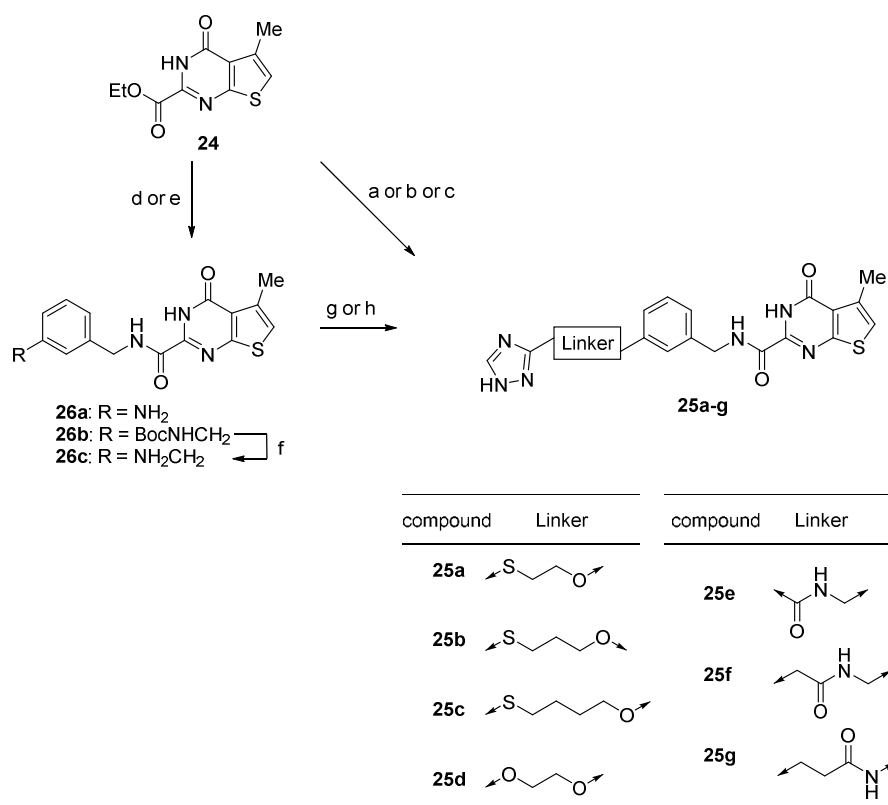
**c** and **23<sup>a</sup>**



<sup>a</sup>Reagents and conditions: (a) amine **6**, DIEA; THF, 80 °C, 86%; (b) 4 N aq. NaOH, THF–MeOH–H<sub>2</sub>O, 100 °C, 69%; (c) (1) oxalyl chloride, cat. DMF, THF, 0 °C to rt; (2) 50% aq. NH<sub>2</sub>OH, *t*-BuOH, THF, 0 °C–rt, 9% over 2 steps; (d) amine **9a**, DIEA, EtOH, 90 °C, 8%.

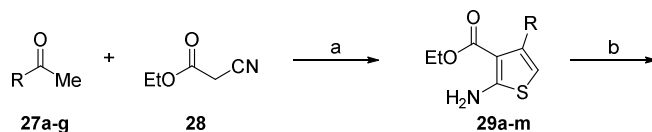
**Scheme 5.** Synthesis of 5-Methyl-4-oxo-3,4-dihydrothienopyrimidine-2-carboxamide

Derivatives **25a–g<sup>a</sup>**



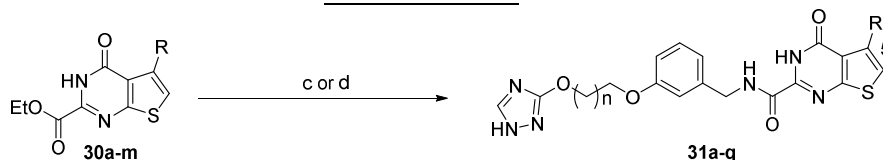
<sup>a</sup>Reagents and conditions: (a) amine **9a**, DIEA, DMA–EtOH, 90 °C, 18% (for **25a**); (b) (1) amine **9b** or **9c**, DMA, 100 °C; (2) TFA, Et<sub>3</sub>SiH, DCM, rt, 45 and 48% (for **25b** and **25c**, respectively, each over 2 steps); (c) (1) amine **15a**, DMA, 180 °C (microwave); (2)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

## Scheme 6. Synthesis of 5-Substituted

4-Oxo-3,4-dihydrothienopyrimidine-2-carboxamide Derivatives **31a–q**<sup>a</sup>

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

compound	R	compound	R
<b>29a</b>	<i>i</i> Pr	<b>29i</b>	3-Cl-Ph
<b>29b</b>	2-thiophenyl	<b>29j</b>	4-Cl-Ph
<b>29c</b>	3-thiophenyl	<b>29k</b>	2-MeO-Ph
<b>29d</b>	phenyl	<b>29l</b>	3-MeO-Ph
<b>29e</b>	2-F-Ph	<b>29m</b>	4-MeO-Ph
<b>29f</b>	3-F-Ph		
<b>29g</b>	4-F-Ph		
<b>29h</b>	2-Cl-Ph		



compound	R	compound	R
<b>30a</b>	<i>i</i> Pr	<b>30i</b>	3-Cl-Ph
<b>30b</b>	2-thiophenyl	<b>30j</b>	4-Cl-Ph
<b>30c</b>	3-thiophenyl	<b>30k</b>	2-MeO-Ph
<b>30d</b>	phenyl	<b>30l</b>	3-MeO-Ph
<b>30e</b>	2-F-Ph	<b>30m</b>	4-MeO-Ph
<b>30f</b>	3-F-Ph		
<b>30g</b>	4-F-Ph		
<b>30h</b>	2-Cl-Ph		

compound	n	R	compound	n	R
<b>31a</b>	1	<i>i</i> Pr	<b>31i</b>	1	3-Cl-Ph
<b>31b</b>	1	2-thiophenyl	<b>31j</b>	1	4-Cl-Ph
<b>31c</b>	1	3-thiophenyl	<b>31k</b>	1	2-MeO-Ph
<b>31d</b>	1	phenyl	<b>31l</b>	1	3-MeO-Ph
<b>31e</b>	1	2-F-Ph	<b>31m</b>	1	4-MeO-Ph
<b>31f</b>	1	3-F-Ph	<b>31n</b>	2	2-F-Ph
<b>31g</b>	1	4-F-Ph	<b>31o</b>	2	3-F-Ph
<b>31h</b>	1	2-Cl-Ph	<b>31p</b>	2	2-Cl-Ph
			<b>31q</b>	2	3-Cl-Ph

<sup>a</sup>Reagents and conditions: (a) (1) morpholine, toluene, 120 °C; (2) sulfur, toluene, EtOH,

70 °C, 18–77% (for **29c**, **29e**, **29f**, **29h**, **29i**, **29k**, and **29l** over 2steps); (b) ethyl

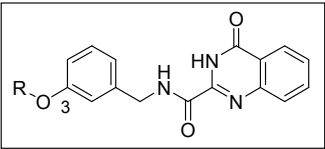
cyanofornate, 1 N HCl/AcOH, 90 °C, 5–88% (for **30a–m**); (c) (1) **15a**, EtOH, 90 °C;

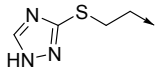
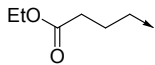
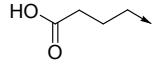
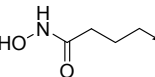
(2) TFA, Et<sub>3</sub>SiH, MeCN, 50 °C, 34–80% (for **31a–m** over 2 steps); (d) (1) **15b**, DIEA,

EtOH, microwave, 100 °C; (2) TFA, Et<sub>3</sub>SiH, MeCN, rt, 63–69% (for **31n–q** over 2

steps).

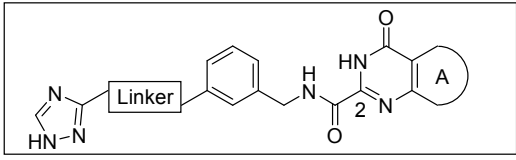
**Table 1.** Inhibitory Activities Against MMP-13 of Quinazoline Derivatives


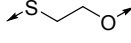

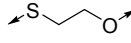
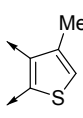
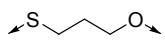
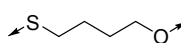
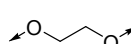
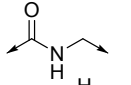
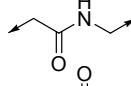
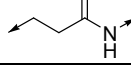


compound	R	IC <sub>50</sub> (nM) <sup>a</sup>
<b>1</b>	Me	12 <sup>b</sup>
<b>23</b>		0.20 <sup>b</sup>
<b>22a</b>		29 <sup>b</sup>
<b>22b</b>		9.4 <sup>b</sup>
<b>22c</b>		1.6 <sup>c</sup>

<sup>a</sup>IC<sub>50</sub> against MMP-13. <sup>b</sup>Each value is the mean from triplicate assay in a single experiment. <sup>c</sup>Values are reported as the mean of two experiments.

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



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

<sup>a</sup>IC<sub>50</sub> against MMP-13. Each value is the mean from triplicate assay in a single

experiment. <sup>b</sup>Inhibition (%) of CYP3A4 metabolic activity at 10 μM. <sup>c</sup>Rat AUC

(ng•h/mL) following a single 1 mg/kg oral gavage dose in rats.

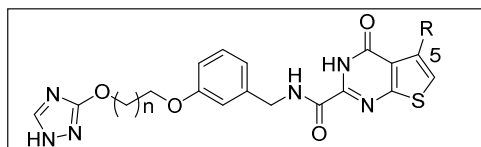
<sup>d</sup>Not determined.

**Table 3.** MMP-13 Inhibitory Activities, Undesirable CYP3A4 Inhibitory Activities, and

Rat Pharmacokinetic Parameters for 5-Substituted Thienopyrimidine Derivatives with

1,2,4-Triazol-3-yl ZBG





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

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

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

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

<sup>a</sup>Each value is the mean from triplicate assay in a single experiment without BSA (see

Experimental Section). <sup>b</sup>Values are means of two experiments in the presence of 0.5%

BSA. <sup>c</sup>Not determined.

**Table 5.** Inhibition Activity in Bovine Nasal Cartilage Assay

compound	concentration ( $\mu$ M)	inhibition <sup>a</sup> (%)
<b>32</b>	0.01	-4.7 $\pm$ 10.8
	0.1	76.3 $\pm$ 18.5 *
	1	102.3 $\pm$ 0.6 *
<b>31f</b>	0.01	-17.6 $\pm$ 0.0
	0.1	48.4 $\pm$ 16.3
	1	70.8 $\pm$ 12.8 *

<sup>a</sup>Data are represented as means  $\pm$  SEM (n = 6). \* denotes  $P < 0.025$  by one-tailed

Williams' test.

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

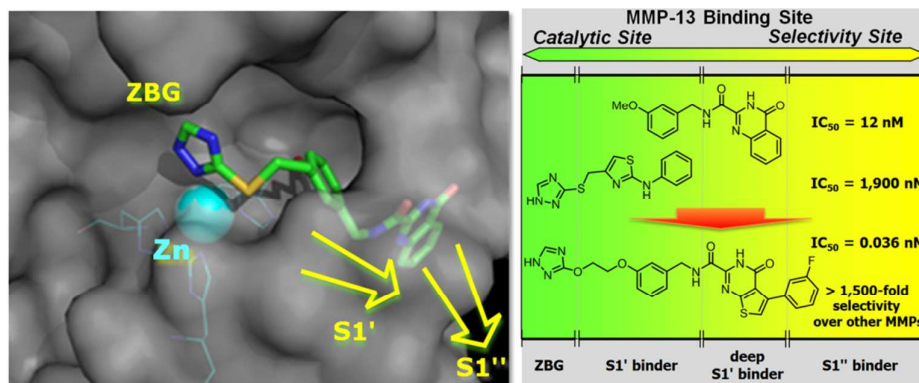
	<b>2</b> (5B5O)	<b>23</b> (5B5P)
<b>Data Collection</b>		
X-ray source	ALS BL5.0.3	ALS BL5.0.3

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

RMS Angles (°)	1.224	1.181
Average B value (Å <sup>2</sup> )	15.2	23.1
R-value <sup>b</sup>	0.165	0.175
R <sub>free</sub> <sup>b</sup>	0.185	0.208

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

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