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New chemotypes for cathepsin K inhibitors

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Abstract—Cyano pyrimidine acetylene and cyano pyrimidine *t*-amine, which belong to a new chemical class, were prepared and tested for inhibitory activities against cathepsin K and the highly homologous cathepsins L and S. The use of novel chemotypes in the development of cathepsin K inhibitors has been demonstrated by derivatives of compounds **1** and **8**.

Osteoporosis is a debilitating disease that is caused by an imbalance between bone matrix resorption and bone remodeling. Cathepsin K, which is selectively and highly expressed in osteoclasts, is a lysosomal cysteine protease of the papain superfamily that has high homology to cathepsins S and L.[1,2] Studies using cathepsin K antisense[3] and cathepsin K deficient mice[4] have shown that the proteinase is primarily involved in osteoclastic bone resorption. Cathepsin K inhibitors therefore are regarded as a potential therapy for the treatment of bone loss, such as osteoporosis.[5] One significant consideration in the design of cathepsin K inhibitors is selectivity for its highly homologous lysosomal cysteine proteases, cathepsins L and S. This has been shown to be possible in many inhibitors having new scaffolds that have been described in previous publications.[6–9]

As depicted in Figure 1, we have reported novel scaffolds, **A**,[6] **B**,[8] and **C**[9] for non-peptidic cathepsin K inhibitors with P2 (R2 group in Fig. 1) and P3 (R1 group in Fig. 1) moieties that make a significant contribution to

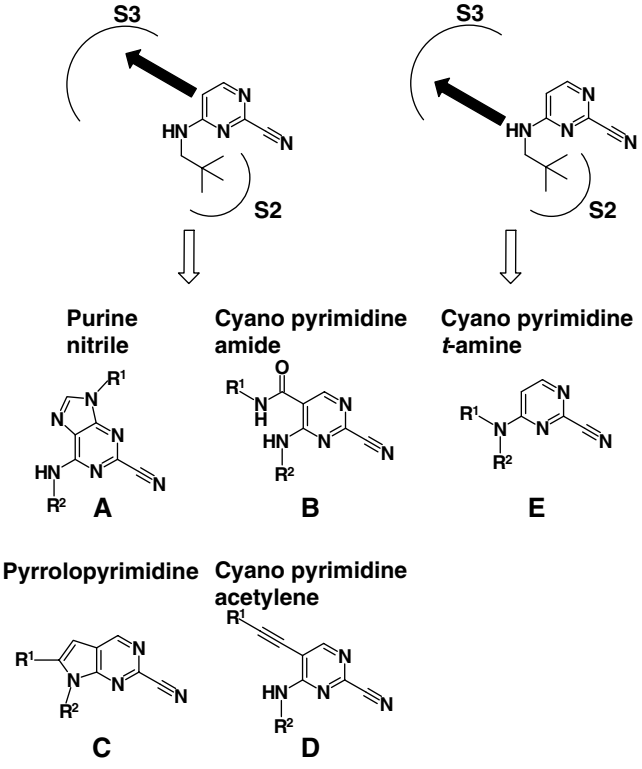

Figure 1. Structure-based design of diverse pyrimidine-based scaffold.

Keywords: Cathepsin K; Non-peptidic inhibitors; New chemotype; Cyano pyrimidine acetylene; Cyano pyrimidine *t*-amine.
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the interaction with S2 and S3 subsites. The derivatives based on the scaffolds of purine nitrile **A**[6] are potent inhibitors for cathepsin K but they exhibit only moderate specificity toward the highly homologous cathepsins L and S. Starting from the purine structure, cyano pyrimidine amide **B**[8] has been explored as a series of cathepsin K inhibitors. The novel scaffold **C**[9] derived from the pyrimidine derivatives contributed to the discovery of new and specific cathepsin K inhibitors. In this communication, we report two additional new chemotypes, cyano pyrimidine acetylene **D** and cyano pyrimidine *t*-amine **E** (Fig. 1).

In general, when designing selective inhibitors for cysteine proteases, it is crucial to create ideas to occupy the S2 and S3 subsites by appropriate parts extending from the core structure of the inhibitor. Of the various types of the P2 substituents investigated, a neopentyl as the P2 moiety of scaffolds **B** and **C** proved to be the most favorable for the S2 subsite.[8,9] Encouraged by this observation, the neopentyl moiety was chosen as the first candidate for the P2 moiety for the new scaffolds. The modeling suggested that the P3 moiety can reach to the S3 subsite if an appropriate rigid spacer could linearly extend from the 5-position of the pyrimidine toward the S3 subsite. The compound **1** with acetylenes as rigid linkers was designed as the first derivative for scaffold **D** (Fig. 2). To further pursue the structural diversity of the scaffold for cathepsin K inhibitor, we focused on the amino group at the 4-position of the pyrimidine. For scaffold **E**, the compound **8** was designed to elucidate whether the appropriate residue at the 4-position of the pyrimidine pointed toward the S3 subsite.

Unlike the peptidic inhibitors with rigorous hydrogen bonds between the peptide sequence and amino acids of the enzyme,[10] non-peptidic inhibitors **1** and **8** may have a flexible P2/S2 and P3/S3 interaction (molecular parts colored by beige and pink in Figures 3 and 4, respectively). The acetylene inhibitor **1** displayed an inhibitory activity for cathepsin K at single digit nM range with 100-fold selectivity against cathepsins L and S (Table 1). As indicated in Table 2, the parent compound **8** for scaffold **E** represented moderate inhibition for cathepsin K with selectivity against the highly homologous cathepsins L and S. Therefore, both the structures **1** and **8** were selected as a starting point for further optimization. We expected to achieve a further increase in the potency by extending the P3 moiety into the S3 subsite in cathepsin K, which is a crucial binding pocket comprising Asp61 and Tyr67. Crystal structures of cathepsins L[11] and S[12] indicate that the corresponding residues to the two amino acids in cathepsin K are Glu63 and Leu69 in cathepsin L, and Lys64 and Phe70 in cathepsin S. Since the comparison of the S3 subsites among human cathepsins K, L, and S revealed significant variations, higher selectivity over related proteases, cathepsins L and S, was anticipated.

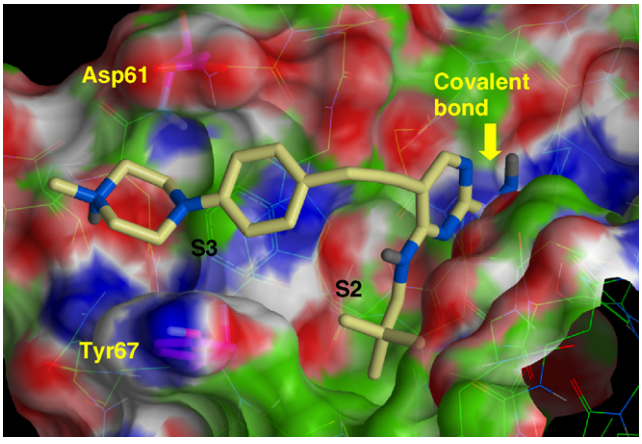


Figure 3. Compound **1** (beige) docked into the active site of human cathepsin K.

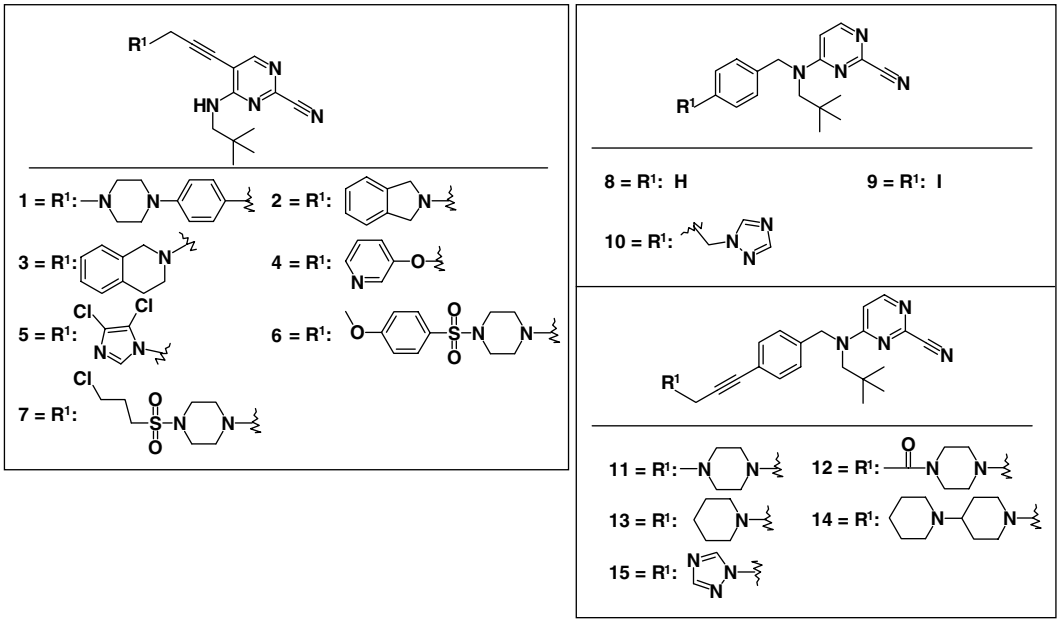


Figure 2. Structures of cathepsin K inhibitors with scaffold **D** or **E**.

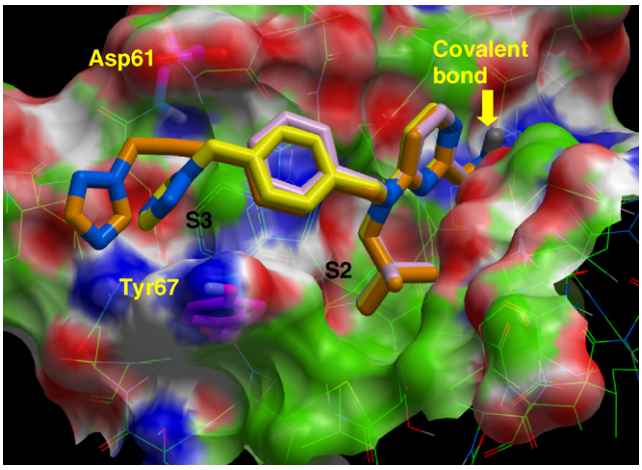


Figure 4. Compound **8** (pink), **10** (yellow), and **15** (orange) docked into the active site of human cathepsin K.

Table 1. Inhibition of human cathepsins K, L, and S by compounds **1**–**7**

Compound	IC_{50}[a] (nM)		
	Cat K	Cat L	Cat S
1	7.1	980	760
2	10	>1000	920
3	3.2	150	840
4	8.6	1000	460
5	7.2	190	68
6	7.1	>1000	>1000
7	3.7	>1000	800

[a] Inhibition of recombinant human cathepsins K, L, and S in a fluorescence assay.[6]

Table 2. Inhibition of human cathepsins K, L, and S by compounds **8**–**15**

Compound	IC_{50}[a] (nM)		
	Cat K	Cat L	Cat S
8	69	>1000	>1000
9	19	>1000	>1000
10	4.8	>1000	>1000
11	3.8	>1000	>1000
12	6.4	>1000	>1000
13	7.3	>1000	>1000
14	3.6	>1000	>1000
15	8.6	>1000	>1000

[a] Inhibition of recombinant human cathepsins K, L, and S in a fluorescence assay.[6]

The initial design of the P3 moiety (R1 in Fig. 1) in scaffold **D** or **E** aims to have electric and hydrophobic affinity with Asp61 and Tyr67, respectively, in the S3 subsite of cathepsin K. The designed derivatives having scaffold **D** or **E** are illustrated in Figure 2.

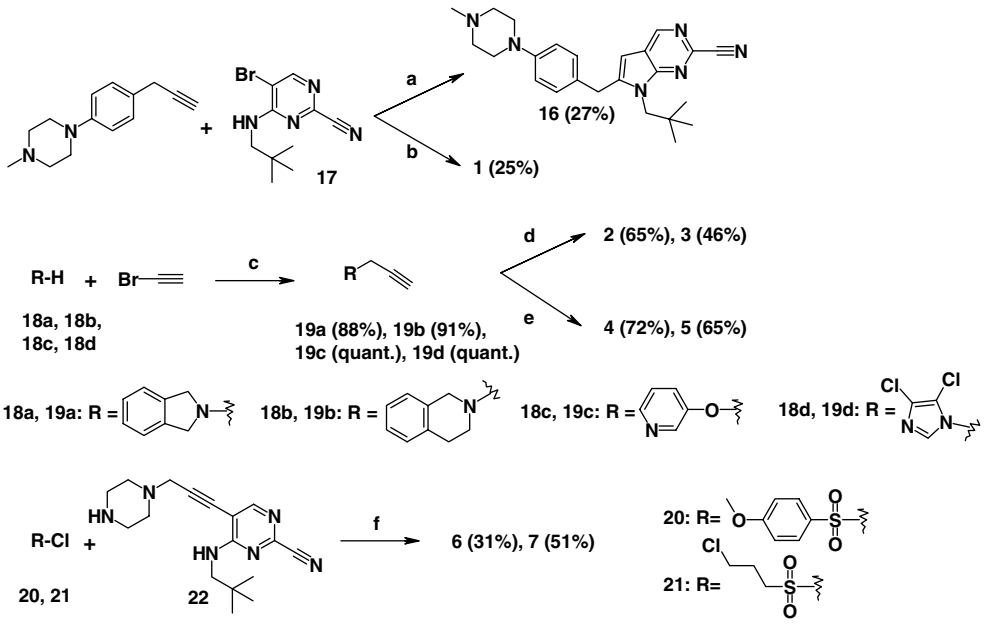
The IC_{50} values of derivatives of **1** with scaffold **D** are listed in Table 1. Acetylene inhibitors **2**–**5** having a heterocyclic or a heteroaromatic group as the P3 moiety inhibited cathepsin K equipotently to **1** but their selectivity against cathepsin L and S showed a declining trend. In particular, introduction of the imidazole derivative led to the poorly selective inhibitor **5** against cathepsins L and S. The sulfonamides **6** and **7** caused no substantial change in a potency for cathepsin K and the selectivity against cathepsins L and S. Although there is a possibility on the optimization of the P3 moiety to improve the selectivity against untargeted cathepsins, it turned out that **1**, **6**, and **7** are selective cathepsin K inhibitors.

The derivatives derived from **8** as representatives of scaffold **E** are shown in Table 2. As indicated above and in Figure 4, the structure of **8** implies that the *para*-substituents in the benzyl group of **8** contributed to the increase in inhibitory activity against cathepsin K while having selectivity against other cathepsins. Indeed, even synthetic intermediate **9** showed an about 3.5-fold-increase in the inhibitory activity for the target enzyme. In addition, **10**–**15** had substantial changes (8- to 18-fold-increase) in the target enzyme inhibition compared to **8**. As depicted in Figure 4, compounds **10** (yellow) and **15** (orange) occupied the critical subsites in the active site of cathepsin K. Note here that all of the inhibitors listed in Table 2 had no affinity to the highly homologous cathepsins L and S. From the examples prepared in this series, it is evident that the P3 moiety extended from scaffold **E** points toward the S3 subsite of cathepsin K.

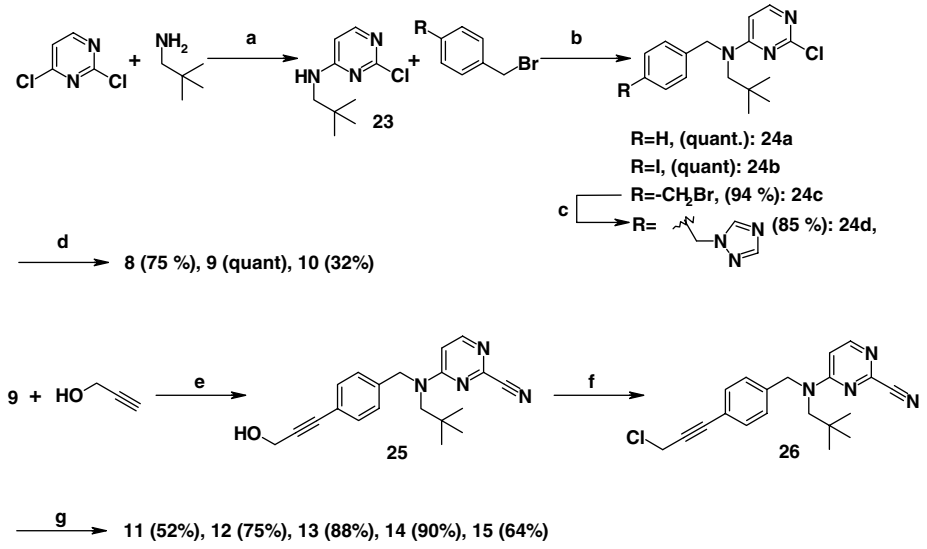
The synthesis of inhibitors discussed here is illustrated in Schemes 1 and 2. The cyclization[13,14] occurred predominantly to yield **16** (scaffold **C**) under the conditions, $Pd(PPh_3)_2Cl_2$, CuI, Et_3N, DMF, 80 °C.[9] Moreover, we found that a change of the solvent to THF or dioxane and/or reaction temperature (rt to 60 °C) in this reaction led to non-cyclized product **1** (scaffold **D**). Further investigation on the reaction condition using different acetylenes as a starting material was carried out in order to obtain non-cyclized derivatives as major products. The synthesis of **2**–**5** is outlined in Scheme 1, a coupling of **17**, and **19a** and **19b** (prepared from **18a** and **18b** and bromo-ethyne) carried out by using $Pd(PhCN)_2Cl_2$ and P(*t*-Bu)$_3$ as catalyst and ligand[15] gave **2** and **3**, respectively. The preparation of **4** and **5** was performed by using $Pd(PPh_3)_2Cl_2$, CuI, Et_3N, DMF, 60 °C. Sulfonyl chloride derivatives **20** and **21** were coupled with **22**[16] to afford sulfonamide **6** and **7**, respectively.

The 2-chloropyrimidin derivative **23** was prepared from 2,4-dichloropyrimidine and 2,2-dimethyl-propylamine, which was coupled with commercially available benzyl bromide derivatives to yield **24a**–**c** (Scheme 2). Amination of **24c** with 1H-[1,2,4]triazole in DMF gave **24d**. The intermediates, **24a**, **24b**, and **24d** were reacted with NaCN in DMSO-H_2O to yield **8**, **9**, and **10**, respectively. The intermediate **25** was prepared by Sonogashira coupling of **9** and prop-2-yn-1-ol in the presence of $Pd(PPh_3)_2Cl_2$ and CuI as catalysts. The hydroxyl group of **25** was converted to chloride by methanesulfonyl chloride to give **26**. Various amines or heteroaromatic derivatives were coupled with **26** to afford **11**–**15**.

In summary, the new inhibitors reported here are an important first step in tackling chemical modification. The chemical modification of **1** and **8** with the scaffolds **D** and **E** was focused on the P3 moiety which contrib-



Scheme 1. Reagents and conditions: (a) $Pd(PPh_3)_2Cl_2$, CuI, Et_3N, DMF, 80 °C, 2 h; (b) $Pd(PPh_3)_2Cl_2$, CuI, Et_3N, THF, 60 °C; (c) DMF, 60 °C, 5–7 h; (d) **17**, $Pd(PhCN)_2Cl_2$, CuI, P(*t*-Bu)$_3$, HN(*i*-Pr)$_2$, Dioxane, rt, 12 h; (e) **17**, $Pd(PPh_3)_2Cl_2$, CuI, Et_3N, DMF, 60 °C, 0.5 h; (f) Et_3N, CH_2Cl_2, 0 °C, stirred at rt for 12 h.



Scheme 2. Reagents and conditions: (a) K_2CO_3, THF, 80 °C, 8 h, 58%; (b) NaH, DMF, rt, 18 h; (c) DMF, rt, 18 h; (d) NaCN, DABCO, DMSO-H_2O, 75 °C, 24 h; (e) $Pd(PPh_3)_2Cl_2$, CuI, EtN(*i*-Pr)$_2$, DMF, rt, 1 h, rt, 82%; (f) methansulfonyl chloride, EtN(*i*-Pr)$_2$, CH_2Cl_2, rt, 15 h; (g) various amines or heteroaromatic rings (2.0 equiv), DMF, rt, 15 h.

uted to the selectivity against other cathepsins. The capability of the novel chemotypes in the development of cathepsin K inhibitors has been demonstrated by the derivatives represented by **1** and **8**, and appropriate moieties extending from each scaffold engaged with key pockets in the active site of cathepsin K.

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