Available online at www.sciencedirect.com
ScienceDirect
Bioorganic & Medicinal Chemistry Letters 17 (2007) 6096–6100
Bioorganic & Medicinal Chemistry Letters

Novel scaffold for cathepsin K inhibitors

Naoki Teno,[a,*] Takahiro Miyake,[a] Takeru Ehara,[a] Osamu Irie,[a] Junichi Sakaki,[a] Osamu Ohmori,[a] Hiroki Gunji,[a] Naoko Matsuura,[a] Keiichi Masuya,[a,†] Yuko Hitomi,[a] Kazuhiko Nonomura,[a] Miyuki Horiuchi,[a,‡] Keigo Gohda,[a,§] Atsuko Iwasaki,[a] Ichiro Umemura,[a] Sachiyo Tada,[a,¶] Motohiko Kometani,[a] Genji Iwasaki,[a] Sandra W. Cowan-Jacob,[b] Martin Missbach,[b] René Lattmann[b] and Claudia Betschart[a,†]

[a]*Novartis Institutes for BioMedical Research, Ohkubo 8, Tsukuba, Ibaraki 300-2611, Japan*
[b]*Novartis Institutes for BioMedical Research, CH-4002 Basel, Switzerland*

Received 20 August 2007; revised 10 September 2007; accepted 12 September 2007
Available online 15 September 2007

Abstract—Pyrrolopyrimidine, a novel scaffold, allows to adjust interactions within the S3 subsite of cathepsin K. The core intermediate **10** facilitated the P3 optimization and identified highly potent and selective cathepsin K inhibitors **11–20**.

A healthy bone metabolism depends on an appropriate balance between osteoclast-mediated bone resorption and osteoblast-mediated bone formation. If the bone resorption is exceeding the bone formation, bone mass is reduced and leads to disease conditions like post-menopausal osteoporosis and tumor-induced osteolysis. A reduction of the bone formation rate, which is observed in senile-osteoporosis, also reduces bone mass and finally induces bone fractures. In the 1980s bisphosphonates were found to act as bone resorption inhibitors (likely through inhibition of farnesyl diphosphate synthase in osteoclasts), and today, bisphosphonates are widely used for the treatment of osteoporosis and tumor-induced osteolysis.[1,2]

In the 1990s three independent groups have reported on cathepsin K, a cysteine protease predominantly expressed in osteoclasts.[3–5] In the meantime, the role of cathepsin K in degradation of bone matrix was established, and its inhibition is now perceived as a promising new principle for a treatment of the diseases caused by increased bone resorption.[6]

The sugar derivative **1** was identified in a high throughput screening as a potent inhibitor of cathepsin K [IC_{50} = 14 nM].[7] Surprisingly, removal of the hydrophilic sugar moiety and also simplification of the ring system to the pyrimidine **2** resulted with retained potency [IC_{50} = 170 nM].[8] A molecular modeling with the nitrile group covalently bound to the active site cysteine suggests that the cyclohexyl group occupies the S2 subsite while the S3 is unoccupied. Therefore, the small molecule **2** was a good starting point for further optimization. We expected to make further increase in the potency by extending into the S3. In addition, due to differences in the corresponding S3 subsites, higher selectivity over related proteases, such as cathepsin L and cathepsin S, was anticipated.

Three attachment sites for the P3 moiety were considered (**A**,[7] **B**[9], and **C** in Fig. 1). For scaffold **C**, the modeling suggested acetylenes as rigid linkers between the pyrimidine and S3 (scaffold **D** in Fig. 2). Indeed, the compounds with high potency and good selectivity against related proteases could be identified in this series as reported elsewhere.[9]

Depending on the structure of R^2 (in the case of R^2 with benzyl propargyl moiety) in Figure 2, the direct product

Keywords: Cathepsin K inhibitors; Pyrrolopyrimidine; Novel scaffold.
* Corresponding author. Tel.: +81 29 865 2360; fax: +81 29 865 2308; e-mail: naoki.teno@novartis.com
† Present address: Novartis Institutes for BioMedical Research, CH-4002 Basel, Switzerland.
‡ YASUTOMI & Associates, MT-2 BLDG, 5-36, Miyahara 3-chome, Yodogawa-ku, Osaka 532-0003 Japan.
§ Computer-Aided Molecular Modeling Research Center Kansai, 1-3-12, Honjyocho, Higashinada-ku, Kobe, Hyogo 658-0012, Japan.
¶ Central Research Laboratories, Sysmex Co., 4-4-4, Takatsuka-dai, Nishi-ku, Kobe, Hyogo 651-2271, Japan.

0960-894X/$ - see front matter © 2007 Elsevier Ltd. All rights reserved.
doi:10.1016/j.bmcl.2007.09.047

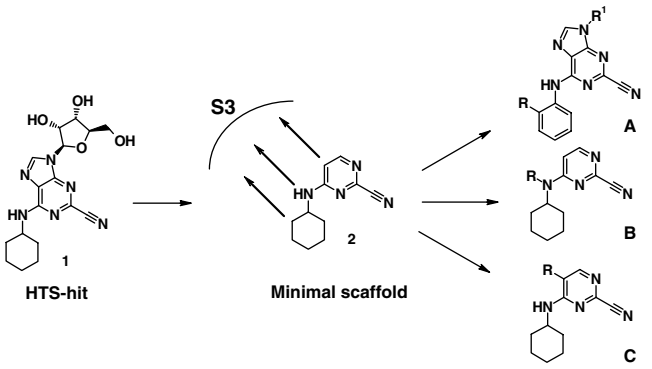


Figure 1. Design of new pyrimidine scaffolds, based on a HTS hit, reaching the S3.

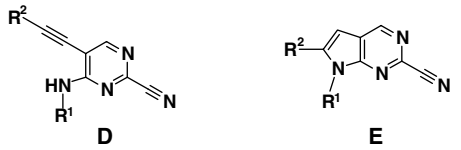
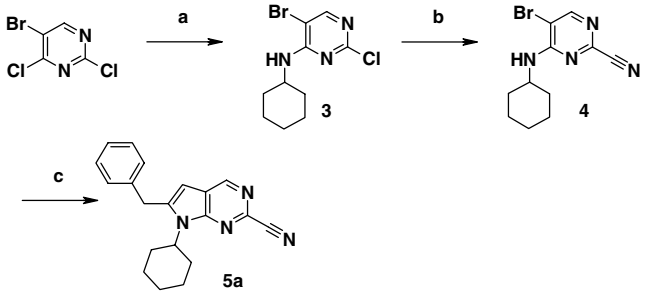


Figure 2. Design of new chemotypes based on the pyrimidine scaffold C.

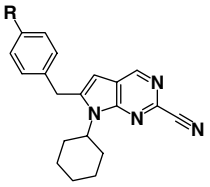
of the coupling could not be obtained but the product immediately rearranged to form a substituted pyrrolopyrimidine **E**. These pyrrolopyrimidine compounds also inhibited cathepsin K with comparable potency as to the acetylene derivatives. In this paper we report on the synthetic methods and the biological activity of cathepsin K inhibitors.

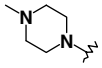
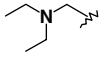
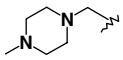
The synthesis of pyrrolopyrimidine **5a** is outlined in Scheme 1. Regioselective substitution of the chloride at the 4- position of 5-bromo-2,4-dichloropyrimidine gave the intermediate **3**. The second chloride was exchanged with cyanide to give the pyrimidine **4**. Castro-Stephens type cyclization[10,11] followed by immediate ring closure afforded the pyrrolopyrimidine **5a**. The new inhibitor **5a** exhibited an IC_{50} value of 15 nM against cathepsin K but with poor selectivity against cathepsins S and L (Table 1). Compounds **5b**–**5g** were prepared by an analogous procedure to **5a**, and their inhibitory activity against cathepsins



Scheme 1. Reagents and conditions: (a) Cyclohexylamine, $NaHCO_3$, MeOH, rt, 6 h, 54%; (b) KCN, 1,4-diazabicyclo[2.2.2]octane, DMSO-H_2O, 60 °C, 6 h, 84%; (c) prop-2-ynyl-benzene, $Pd(PPh_3)_2Cl_2$, CuI, Et_3N, DMF, 80 °C, 12 h, 15%.

Table 1. Inhibition of human cathepsins K, L, and S by compounds **5a–g**



Compound	R	IC_{50}[a] (nM)		
		Cat K	Cat L	Cat S
5a	H	15	120	390
5b	Cl	2.3	82	490
5c		47	>1000	>1000
5d		3.4	>1000	980
5e		<1	410	460
5f		4.7	260	>1000
5g		1.5	110	730

[a] Inhibition of recombinant human cathepsins K, L, and S in a fluorescence assay.[7]

was tested. The compounds **5f** and **5g** with a negative charge in the P3 moiety showed inhibitory activity for cathepsin K at single digit nM range, but not high selectivity against both cathepsins S and L simultaneously, while **5c** and **5d** revealed the countertrend. The methylpiperizine derivative **5e** was found to show sub nM potency and the selectivity against cathepsins L and S was obtained in more than 400-folds.

A co-crystal structure of the compound **5e** with cathepsin K showed that the benzyl group is pointing toward the S3 subsite (Fig. 3). The aromatic ring is involved in an edge-to-face interaction with the side chain of Tyr67. The co-crystal structure further indicated that the para-substituents on the compound **5a** are positioned between the Asp61 and Tyr67, as seen for **5e**. The cyclohexyl group of **5e** occupies the lipophilic S2 subsite.

On the basis of the results from a quenched fluorogenic substrate library for cathepsin K[12], the neopentyl moiety having a branched alkyl group was introduced instead of the cyclohexyl group of **5a**. Introduction of the neopentyl P2 group provided a potent cathepsin K inhibitor **5h** [IC_{50} (cathepsin K) = 1.2 nM] (Table 2), which was prepared in the same way as for **5a** shown in Scheme 1. The compound, however, still had moderate inhibitory activity toward cathepsins S and L. [IC_{50} (cathepsin S) = 100 nM, IC_{50} (cathepsin L) = 380 nM]. It should be noted that an increase in inhibitory activity for cathepsin K is caused by a suitable interaction between the

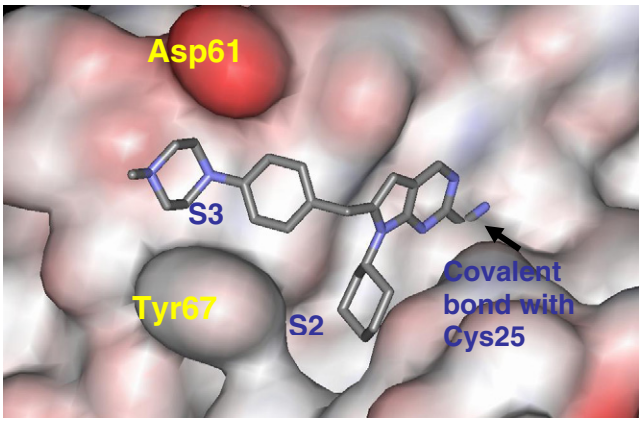


Figure 3. Co-crystal structure of compound **5e** with cathepsin K inhibitor is extending into S3 between Asp61 and Tyr67 (PDB code: 2R6N).

neopentyl group and the S2 subsite. The observation is consistent with that of the previous report.[8]

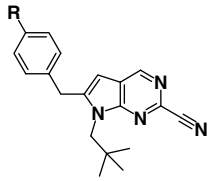
Another promising result was observed for compounds **5i–5w**.[13] As the preparation of **5q** depicted in Scheme 2, the P3 with benzyl propargyl group was coupled with 5-bromo-4-(2,2-dimethyl-propylamino)-pyrimidine-2-carbonitrile (**6**), which was prepared in the same way as for **4** shown in Scheme 1. As listed in Table 2, the compounds **5m**, **5o**, **5t**, and **5v** were considered satisfactory in terms of the potency for the target enzyme and the selectivity against other cysteine protease, cathepsins S and L.

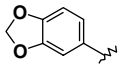
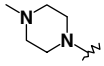
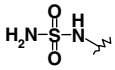
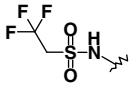
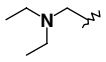
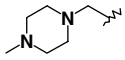
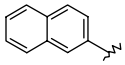
As shown in Scheme 3, we designed and prepared a useful intermediate **10** which can be used to introduce a wide variety of P3 moieties at the late stage of the compound preparation. Bromide **6** was coupled with 2-prop-2-ynyloxy-tetrahydro-pyran[14] by using $Pd(PPh_3)_2Cl_2$ and CuI as catalysts to afford **7**. Formation of the pyrrolopyrimidine ring was carried out by DBU[15], followed by tetrahydropyran removal using TsOH-H_2O. Alcohol **9** was converted to the bromine **10** and the P3 optimization using a parallel synthesis became feasible as depicted in Figure 4.

The parallel synthesis approach allowed us to access compounds with structural diversity by using an almost unlimited choice of commercially available nucleophiles with the functional groups (amine, hydroxyl, thiol). By this approach, structurally diverse P3 moieties can be introduced using the common intermediate **10**. In addition, we will be able to control the lipophilicity of the inhibitor by modifying P3 moiety, leading to improving physicochemical properties.

Various P3 parts (R in Table 3) were coupled with **10** in polar solvent (e.g., DMF, DMSO, or acetone) in the presence of a base (K_2CO_3, NaH, or diisopropylethylamine).[13,16] Representative inhibitors in this series are listed in Table 3. Unlike the purine derivatives[7] and 2-cyano-pyrimidine inhibitors,[8] the inhibitors **11–20** displayed highly potent cathepsin K inhibition combined with significant selectivity for cathepsin K over cathepsins L and S.

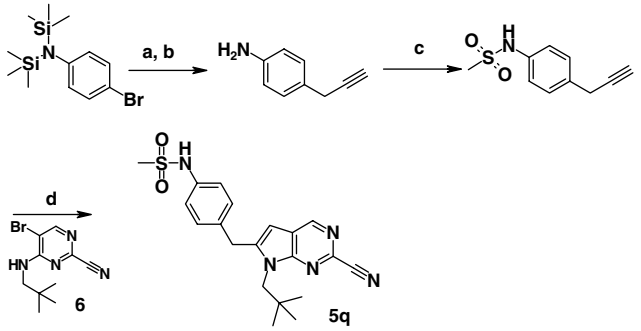
Table 2. Inhibition of human cathepsins K, L, and S by compounds **5h–w**



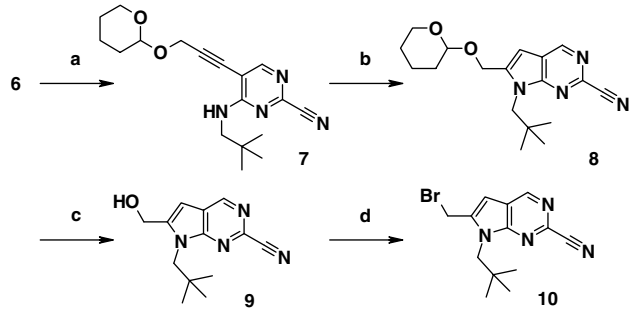
Compound	R	IC_{50}[a] (nM)		
		Cat K	Cat L	Cat S
5h	H	1.2	380	100
5i	CH_3	<1	170	930
5j	Cl	<1	96	460
5k		<1	540	820
5l		1.1	110	>1000
5m		<1	970	>1000
5n		<1	>1000	320
5o		<1	>1000	>1000
5p		<1	140	>1000
5q		<1	>1000	160
5r		<1	>1000	220
5s		1.1	>1000	440
5t		<1	>1000	>1000
5u		<1	430	>1000
5v		<1	>1000	>1000
5w		<1	520	>1000

[a] Inhibition of recombinant human cathepsins K, L, and S in a fluorescence assay.[7]

In summary, a novel pyrrolopyrimidine scaffold **E** derived from the pyrimidine derivative contributed to the discovery of new and specific cathepsin K inhibitors. The use of a key intermediate **10** has facilitated the P3 optimization and identified novel cathepsin K inhibitors



Scheme 2. Reagents and conditions: (a) Mg (powder), I_2, THF; (b) 1-methoxy-propa-1,2-diene, CuBr, THF, rt, 24 h, 61%; (c) MsCl, pyridine, CH_2Cl_2, rt, 1 h, quant.; (d) $Pd(PPh_3)_2Cl_2$, CuI, Et_3N, DMF, 70 °C, 2 h, 61%.



Scheme 3. Reagents and conditions: (a) 2-prop-2-ynyloxy-tetrahydropyran, $Pd(PPh_3)_2Cl_2$, CuI, Et_3N, DMF, 80 °C, 2 h, 92%; (b) DBU, DMF, 100 °C, 2 h, 93%; (c) $TsOH{\cdot}H_2O$, THF, MeOH, rt, 3 h, 87%; (d) CBr_4, Ph_3P, CH_2Cl_2, 0 °C, 0.5 h, rt, 3 h, 78%.

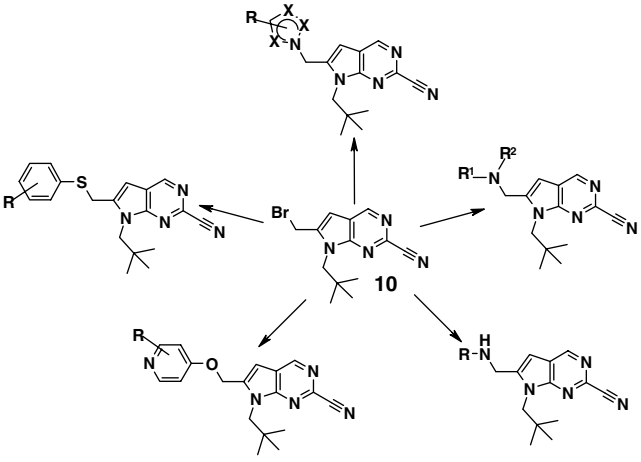


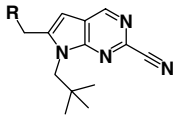
Figure 4. Exploring the P3 moiety by a parallel synthesis.

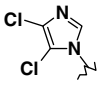
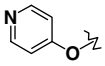
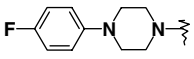
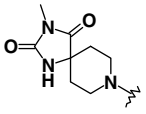
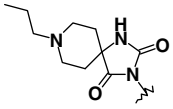
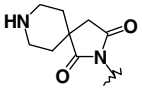
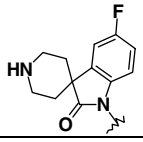
with nM potency with excellent selectivity against cathepsins L and S. The structure activity relationships and the pharmacokinetic properties of this series reported here will be covered in future publications.

Acknowledgments

We thank Mr. Junichi Yamanaka and Ms. Ibuki Misawa for excellent technical assistance. The authors are grateful to Dr. Robert Hof and Dr. Muneto Mogi for helpful critical reading of the manuscript.

Table 3. Inhibitory activity of new chemotype with azole, heterocyclic or spirohydantoin group against cathepsins K, L, and S



Compound	R	IC_{50}[a] (nM)		
		Cat K	Cat L	Cat S
11		6	>1000	>1000
12		4	>1000	>1000
13		5	>1000	>1000
14		3	>1000	>1000
15		4	>1000	>1000
16		1	>1000	>1000
17		<1	>1000	>1000
18		<1	770	>1000
19		<1	>1000	>1000
20		1.7	>1000	930

[a] Inhibition of recombinant human cathepsins K, L, and S in a fluorescence assay.[7]

References and notes

1. Van Beek, E.; Pieterman, E.; Cohen, L.; Lowik, C.; Papapoulos, S. *Biochme. Biophys. Res. Commun.* **1999**, *264*, 108.
2. Dunford, J. E.; Thompson, K.; Coxon, F. P.; Luckman, S. P.; Hahn, F. M.; Poulter, C. D.; Ebetino, F. H.; Rogers, M. J. *J. Pharmacol. Exp. Ther.* **2001**, *296*, 235.
3. Tezuka, K.; Tezuka, Y.; Maejima, A.; Sato, T.; Nemoto, K.; Kamioka, H.; Hakeda, Y.; Kumegawa, M. *J. Biol. Chem.* **1994**, *269*, 1106.
4. Brömme, D.; Okamoto, K.; Wang, B. B.; Biroc, S. *J. Biol. Chem.* **1996**, *271*, 2126.

5. Draket, F. H.; Dodds, R. A.; James, I. E.; Connor, J. R.; Debouck, C.; Richardson, S.; Lee-Rykaczewski, E.; Coleman, L.; Rieman, D.; Barthlow, R.; Hastings, G.; Gowen, M. *J. Biol. Chem.* **1996**, *271*, 12511.
6. Grabowska, U. B.; Chambers, T. J.; Shiroo, M. *Curr. Opin. Drug Dis. Dev.* **2005**, *8*, 619.
7. Altmann, E.; Cowan-Jacob, S. W.; Missbach, M. *J. Med. Chem.* **2004**, *47*, 5833.
8. Altmann, E.; Aichholz, R.; Betschart, C.; Buhl, T.; Green, J.; Irie, O.; Teno, N.; Lattmann, R.; Tintelnot-Blomley, M.; Missbach, M. *J. Med. Chem.* **2007**, *50*, 691.
9. Paper in preparation.
10. Stephens, R. D.; Castro, C. E. *J. Org. Chem.* **1963**, *28*, 3313.
11. Kondo, Y.; Watanabe, R.; Sakamoto, T.; Yamanaka, H. *Chem. Pharm. Bull.* **1989**, *37*, 2933.
12. Teno, N.; Kometani, M.; Betschart, C.; Toriyama, K.; Niwa, S.; Sakaki, J.; Altmann, E.; Buhl, T.; Gamse, R.; Gasser, J.A.; Lattmann, R.; Missbach, M. *Abstracts of Papers*, 12th Annual Meeting of Division of Medicinal Chemistry, Tsukuba, Japan, Nov 24–26, 2004.; The Pharmaceutical Society of Japan, 2004; P1-28.
13. Betschart, C.; Hayakawa, K.; Irie, O.; Sakaki, J.; Iwasaki, G.; Lattmann, R.; Missbach, M.; Teno, N. WO 2003020721, 2003.
14. Trost, B. M.; Jonasson, C.; Wuchrer, M. *J. Am. Chem. Soc.* **2001**, *123*, 12736.
15. Unlike the synthesis of **5a** (Scheme 1), the coupling of **6** and 2-prop-2-ynyloxy-tetrahydro-pyran did not give **8** directly. The intermediate **8** was prepared from **7** by using the reaction condition analogous to the synthesis of indoles Kondo, Y.; Kojima, S.; Sakamoto, T. *J. Org. Chem.* **1997**, *62*, 6507.
16. Irie, O.; Iwasaki, G.; Masuya, K.; Miyake, T.; Teno, N. WO 2004076455, 2004.