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4-Aminophenoxyacetic acids as a novel class of reversible cathepsin K inhibitors

Tsuyoshi Shinozuka,[a,*] Kousei Shimada,[a] Satoshi Matsui,[a] Takahiro Yamane,[a] Mayumi Ama,[a] Takeshi Fukuda,[a] Motohiko Taki[b] and Satoru Naito[a]

[a]*Medicinal Chemistry Research Laboratories, Sankyo Co., Ltd, 1-2-58 Hiromachi, Shinagawa-ku, Tokyo 140-8710, Japan*
[b]*Lead Discovery Research Laboratories, Sankyo Co., Ltd, 1-2-58 Hiromachi, Shinagawa-ku, Tokyo 140-8710, Japan*

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Abstract—We have designed and synthesized a novel series of 3-biphenylamino acid amides as cathepsin K inhibitors based on compound **I**. In these inhibitors, we have discovered 4-aminophenoxyacetic acids **43** and **47** with good IC_{50} values, although lipophilic groups are favorable for the hydrophobic S1′ pocket.

Cathepsin K[1] is a cysteine protease that is highly expressed in osteoclasts. The primary role of cathepsin K is the degradation of type I collagen, one of the main constituents of bone matrix. The inhibition of cathepsin K has been expected to provide a therapeutic means for the treatment of osteoporosis, osteoarthritis, rheumatoid arthritis, and Paget's disease. Derivatives of cyanamide,[2] nitrile,[3] ketone,[4] aldehyde,[5] and ketoamide[6] are known as cathepsin K inhibitors, which covalently interact with the thiol group of the cysteine residue at the active site of the enzyme. Besides those inhibitors, arylamine derivatives were reported by a Novartis group[7] and were shown to be non-covalent and reversible inhibitors. Herein, we report on arylamine-based inhibitors of cathepsin K whose P3 unit is replaced with a biphenyl group. We also report subsequent replacement of the methyl group in the P1′ unit with an acetate group led to a novel class of reversible cathepsin K inhibitors.

Our modification was started from Novartis compound **I**[7] which was chosen because non-covalent inhibitors were generally thought to be more advantageous in terms of toxicity compared to covalent inhibitors. Scrutiny of the X-ray crystallographic data of cathepsin K complexed with inhibitors bearing a benzyloxycarbonyl (Cbz) group[8] revealed that the carbonyl oxygen of the Cbz group did not interact with the enzyme. Therefore, we converted the Cbz group of compound **I** to a biphenyl group by tethering the carbonyl oxygen to the benzylic carbon as shown in Figure 1 for obtaining structurally novel inhibitors as such P3 sub-structures had not been reported when we started this project.[3]

Compounds **11**–**35** were prepared from the corresponding carboxylic acids and amines. As described in Scheme 1, aryloxycarboxylic acids **3** were prepared by etheration of phenols **1** with α-bromo esters **2**. The synthesis of *N*-aryl amino acids **6** was accomplished by coupling aryl

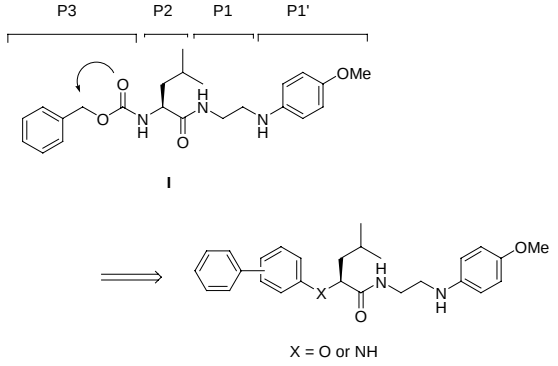

Figure 1. Schematic representation of the assumed binding mode of Novartis compound **I** and O- and NH-linked compound.

Keywords: Cathepsin K inhibitors; Osteoporosis; 3-Biphenylamino acid amides; 4-Aminophenoxyacetic acids.
* Corresponding author. Tel.: +81 3 3492 3131; fax: +81 3 5436 8563; e-mail: sinozu@sankyo.co.jp

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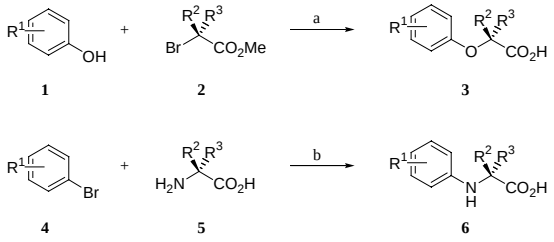


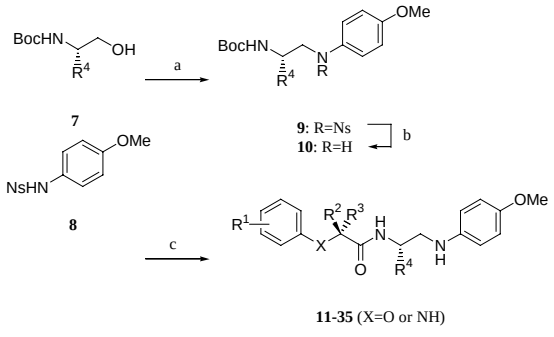
Scheme 1. Synthesis of carboxylic acids. Reagents and conditions: (a) KOH, *tert*-BuOH, reflux; (b) CuI, K_2CO_3, DMA, 90 °C.

bromides **4** with L-leucine or 1-aminocyclohexanecarboxylic acid (**5**) using a copper catalyst.[9]

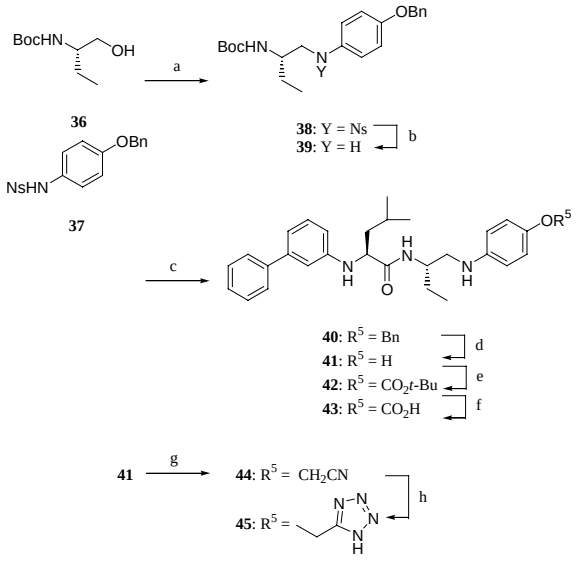
The preparation of compounds **11**–**35** is shown in Scheme 2. Mitsunobu reaction[10] of *N*-Boc aminoethanols **7**[11] with 2-nitrobenzenesulfonamide **8** afforded *N*-Boc ethylenediamines **9**, which were deprotected with PhSH to provide ethylenediamine derivatives **10**.[12] Subsequent coupling reaction of **10** with carboxylic acid **3** or **6** furnished compounds **11**–**35**.

The preparation of compounds **42**, **43**, and **45** is shown in Scheme 3. Mitsunobu reaction of *N*-Boc aminoethanol **36**[11] with 2-nitrobenzenesulfonamide **37** and subsequent deprotection provided **39**.[12] The coupling of **39** with carboxylic acid **6**, followed by cleavage of benzyl ether (H_2, Pd/C, EtOH), provided phenol **41**. Alkylation of **41** with *tert*-butyl bromoacetate afforded ester **42**. Saponification of ester **42** furnished carboxylic acid **43**. Tetrazole **45** was prepared from nitrile **44** under the usual reaction conditions. Ester **46** and carboxylic acid **47** were prepared in the same manner.

Purified procathepsin K is efficiently activated at lower pH, such as pH 4.0.[13] In addition, the activity of mature cathepsin K at pH 3.8 was around 40% when compared at pH 6.0 using Cbz-Phe-Arg-AMC as a substrate (data not shown). Therefore, we examined the inhibitory activity of O-linked compounds against recombinant human cathepsin K at pH 3.8 as shown in Table 1. Isobutyl and cyclohexyl groups were employed as the P2 unit, and isobutyl derivatives (compounds **11**–**16**) were prepared as racemic mixtures. Non-substituted phenoxy derivative **11** exhibited an IC_{50} value in the micromolar

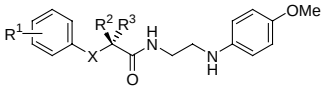


Scheme 2. Synthesis of compounds **11**–**35**. Reagents: (a) DEAD, PPh_3, THF; (b) PhSH, K_2CO_3, MeCN; (c) TFA, CH_2Cl_2, then **3** or **6**, WSC·HCl, HOBt, DMF.



Scheme 3. Synthesis of compounds **42**, **43**, and **45**. Reagents and conditions: (a) DEAD, PPh_3, THF; (b) PhSH, K_2CO_3, MeCN, 82% (2 steps); (c) TFA, CH_2Cl_2, then **6**, WSC·HCl, HOBt, DMF, 99%; (d) H_2, Pd/C, EtOH, 87%; (e) *tert*-butyl bromoacetate, NaH, DMF/THF, 81%; (f) HCl, 1,4-dioxane, 74%; (g) NaH, bromoacetonitrile, DMF/THF, 59%; (h) NaN_3, NH_4Cl, DMF, 100 °C, 69%.

Table 1. IC_{50} values of human cathepsin K inhibitors **11**–**28**



Compound	X	R^1	R^2	R^3	IC_{50}[a] (μM)
I					0.09
11[b]	O	H	*i*-Bu	H	3
12[b]	O	2-Ph	*i*-Bu	H	1.2
13[b]	O	3-Ph	*i*-Bu	H	0.090
14[b]	O	4-Ph	*i*-Bu	H	0.10
15[b]	O	3-PhO	*i*-Bu	H	0.069
16[b]	O	4-PhO	*i*-Bu	H	0.60
17	O	H	$-(CH_2)_5-$		3
18	O	3-Ph	$-(CH_2)_5-$		0.32
19	O	4-Ph	$-(CH_2)_5-$		0.19
20	O	4-Et	$-(CH_2)_5-$		0.13
21	O	4-*i*-Pr	$-(CH_2)_5-$		0.20
22	O	4-*c*-hex	$-(CH_2)_5-$		0.099
23	NH	H	$-(CH_2)_5-$		11
24	NH	2-Ph	$-(CH_2)_5-$		>20
25	NH	3-Ph	$-(CH_2)_5-$		0.083
26	NH	4-Ph	$-(CH_2)_5-$		0.10
27	NH	3-Ph	*i*-Bu	H	0.040
28	NH	3-*c*-hex	*i*-Bu	H	0.060

[a] Inhibition of recombinant human cathepsin K activity in a fluorescence assay using 8 μM Cbz-Phe-Arg-AMC as a substrate in 200 mM NaOAc, 10 mM DTT, 120 mM NaCl, and buffer (pH 3.8). The IC_{50} values represent the average of at least $n = 2$.

[b] Racemic compound.

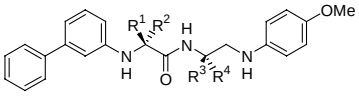
range, whereas compound **I** indicated good inhibition (IC_{50} = 0.09 μM).[14] In isobutyl derivatives, substitution of a phenyl group to a phenoxy group in compound **11** at the 3- or 4-position increased the inhibitory activity by 30 times (compounds **11**, **13**, and **14**), while

substitution at the 2-position (compound **12**) brought a little improvement in activity. The cyclohexyl derivatives **18** and **19** were less potent than the corresponding isobutyl derivatives **13** and **14**. 3-Phenoxy derivative **15** showed good inhibitory activity (IC_{50} = 0.069 μM), whereas 4-phenoxy derivative **16** showed 10 times reduced activity compared to **15**. Alkyl derivatives **20**–**22** retained good activity, and cyclohexyl derivative **22** was 2-fold more potent than 4-biphenyl ether **19**.

We then examined the inhibitory activity of NH-linked compounds as also summarized in Table 1. Biphenylamine derivatives **25** and **26** possessed increased activity compared to the corresponding biphenyl ethers **18** and **19**. The improvement in activity could be ascribed to a hydrogen bond between the amino group and the enzyme as mentioned in the literature.[3] In the arylamine series, 3-biphenylamine derivative **25** displayed better activity than 4-biphenylamine derivative **26**, and the 3-biphenyl-L-leucyl group was found to be a better P2 substituent than the 3-biphenylaminocyclohexylcarbonyl group (Compounds **25** and **27**). The terminal phenyl group of **27** was replaced with a cyclohexyl group; however, no enhancement in activity was attained. This is unlike the same displacement in aryl ether **19**, which resulted in more potent **22**.

It was expected that the introduction of an alkyl group extending into the S1 pocket would enhance the inhibitory activity, so derivatives with an alkyl side chain in the ethylenediamine moiety of compounds **25** and **27** were synthesized and the inhibitory activities were determined, as depicted in Table 2. First, both stereoisomers of methyl derivatives, **29** and **30**, were prepared, and (*S*)-isomer **29** was found to be more potent. Therefore, derivatives of various alkyl side chains with the (*S*)-configuration were examined, and the maximum activity was observed for the ethyl group (compound **31**). Similarly, good activity was displayed by the isobutyl derivative substituted with an ethyl group (compound **35**).

Table 2. IC_{50} values of human cathepsin K inhibitors **25**, **27**, and **29**–**35**



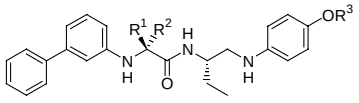
Compound	R^1	R^2	R^3	R^4	IC_{50}[a] (nM)
25	–$(CH_2)_5$–		H	H	83
29	–$(CH_2)_5$–		H	Me	10
30	–$(CH_2)_5$–		Me	H	82
31	–$(CH_2)_5$–		H	Et	2.7
32	–$(CH_2)_5$–		H	*n*-Pr	3.9
33	–$(CH_2)_5$–		H	*n*-Bu	5.6
27	*i*-Bu	H	H	H	40
34	*i*-Bu	H	H	Me	10
35	*i*-Bu	H	H	Et	3.8

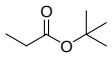
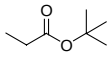
[a] Inhibition of recombinant human cathepsin K activity in a fluorescence assay using 8 μM Cbz-Phe-Arg-AMC as substrate in 200 mM NaOAc, 10 mM DTT, 120 mM NaCl, and buffer (pH 3.8). The IC_{50} values represent the average of at least $n = 2$.

Next the substituent on the benzene ring in the P1′ unit of ethyl derivatives **31** and **35** was examined. The result is summarized in Table 3. Incorporation of a tetrazole group in the P1′ unit led to moderate inhibitor **45**, which was only 3-fold less potent than **35**. This result encouraged us to synthesize carbonyl derivatives such as esters and carboxylic acids, although it has been shown that lipophilic groups are favorable for the hydrophobic S1′ pocket.[7b] *tert*-Butyl ester **42** showed a 2-fold increase in the inhibitory activity compared to tetrazole **45**. Interestingly, the IC_{50} value of carboxylic acid **43** was 4.5 nM, which was more potent than ester **42** and similar to that of methoxy derivative **35**. 3-Biphenylaminocyclohexyl analogues of the above derivatives were also prepared and evaluated for inhibitory activity. Compound **47**, a cyclohexane analogue of **43**, possessed good inhibitory activity with an IC_{50} of 4.8 nM. The reason for the good inhibitory activities of these carboxylic acid derivatives was obscure.

In summary, a 3-biphenylamino group was found to be an efficient P3 sub-structure for arylamine-based inhibitors of cathepsin K. Addition of an ethyl group in the (*S*)-configuration to the ethylenediamine moiety and a carboxyl group in the P1′ unit resulted in compounds **43** and **47** with a good IC_{50} value against cathepsin K. We expect the addition of the acidic group brings different features in enzyme selectivities and/or ADME properties from other cathepsin K inhibitors. These results will be reported in due course.

Table 3. IC_{50} values of human cathepsin K inhibitors **21** and **25**–**30**



Compound	R^1	R^2	R^3	IC_{50}[a] (nM)
31	–$(CH_2)_5$–		Me	2.7
35	*i*-Bu	H	Me	3.8
45	*i*-Bu	H		11
42	*i*-Bu	H		6.4
46	–$(CH_2)_5$–			4.8
43[b]	*i*-Bu	H		4.5
47[b]	–$(CH_2)_5$–			4.8

[a] Inhibition of recombinant human cathepsin K activity in a fluorescence assay using 8 μM Cbz-Phe-Arg-AMC as a substrate in 200 mM NaOAc, 10 mM DTT, 120 mM NaCl, and buffer (pH 3.8). The IC_{50} values represent the average of at least $n = 2$.

[b] 2 HCl salt.

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14. The IC_{50} value obtained in our assay was similar to the reported value (0.07 μM) see: Ref. 7.