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Structure-based Design of Non-peptide, Carbohydrazide-based Cathepsin K Inhibitors

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Abstract—Using binding models which were based on the X-ray crystal structure of an amino acid-based active site-spanning inhibitor complexed with cathepsin K, Cbz-leucine mimics have been developed, leading ultimately to the design of a potent cathepsin K inhibitor free of amino acid components. These mimics, which consist of α-substituted biphenylacetyl groups in place of Cbz-leucine moieties, effectively mimic all aspects of the Cbz-leucine moieties which are important for inhibitor binding. The predicted directions of binding for the inhibitors were confirmed by mass spectral analysis of their complexes with cathepsin K, which gave results consistent with acylation of the enzyme and loss of the acylhydrazine portion of the inhibitor which binds on the S′ side of the active site. The binding models were found to be very predictive of relative inhibitor potency as well as direction of inhibitor binding. These results strengthen the validity of a strategy involving iterative cycles of structure-based design and inhibitor synthesis and evaluation for the discovery of non-peptide inhibitors.

Introduction

Cysteine proteases have been broadly implicated as targets for therapeutic intervention (e.g. cancer, arthritis, viral and parasitic diseases).[1] Cathepsin K, a cysteine protease of the papain superfamily, has been implicated in the process of bone resorption.[2,3] Selective inhibitors of cathepsin K could therefore be promising therapeutic agents for the treatment of diseases characterized by excessive bone loss, such as osteoporosis.

The structure-based design and X-ray crystallographic analysis of a new class of active-site spanning cathepsin K inhibitors which contain a 1,5-diacylcarbohydrazide scaffold has recently been reported from these laboratories.[4] Compound **1** (Fig. 1), for example, is a potent, selective, kinetically irreversible inhibitor of cathepsin K but contains many groups, such as amino acid residues, with hydrogen-bonding potential thought to have a negative impact on oral bioavailability. Based on these considerations, a key aspect of the design of therapeutically useful enzyme inhibitors has been the replacement of amino acid groups by isosteric elements. The design of such elements has met with much success in the recent past, owing greatly to the availability of crystallographic data from enzyme:inhibitor complexes. We have recently reported the structure-based design and synthesis of compound **2** (Fig. 1), which contains a *meta*-benzyloxy substituted benzoyl group as a mimic for a benzyloxycarbonyl (Cbz) leucine moiety.[5] The design hypothesis was subsequently confirmed by X-ray crystallographic analysis of the complex of compound **2** and cathepsin K. However, the *meta*-benzyloxy substituted benzoyl group is an imperfect mimic of the Cbz-leucine moiety in that it does not mimic the γ-methyl groups of the leucine side chain, as is evidenced by an apparent second-order rate constant for inactivation of cathepsin K for compound **2** which is roughly 10-fold lower than that of compound **1**. The X-ray crystal structure of compound **2** in complex with cathepsin K also supports the lack of mimicry of the γ-methyl groups of the leucine side chain. The present report describes the structure-based design and synthesis of a 1,5-diacylcarbohydrazide-based inhibitor which contains

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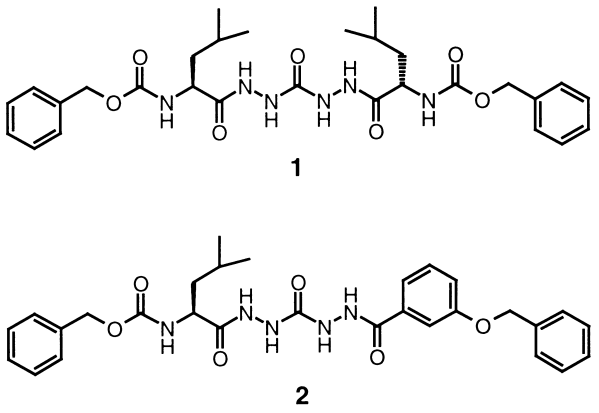


Figure 1. Structures of compounds **1** and **2**.

a substituted *para*-biphenylacetyl group in place of the benzyloxycarbonyl leucine moiety, and which retains the high potency of compound **1**, presumably by complete mimicry of the leucine side chain. We also report the structure-based design of a substituted *meta*-biphenylacetyl group as a replacement for the remaining Cbz-leucine moiety, and the use of this mimic in combination with the *meta*-benzyloxy substituted benzoyl group to construct a potent inhibitor which lacks any amino acid residues.

Results and Discussion

Inhibitor synthesis

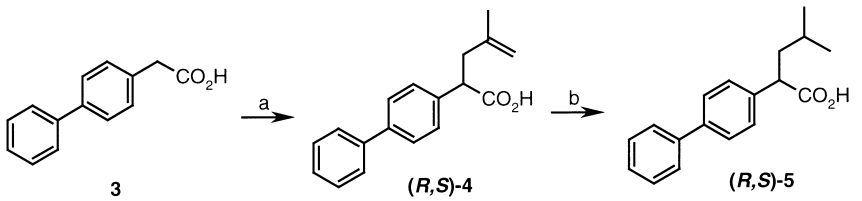
The 2-isobutyl-substituted biphenylacetic acid **5** used in the preparation of the inhibitors was prepared as outlined in Scheme 1. Sequential treatment of 4-biphenylacetic acid (**3**) with 2.2 equivalents of LDA and 3-bromo-2-methylpropene gave carboxylic acid **4**, which was subsequently hydrogenated over Pd/C to provide carboxylic acid **5**. The (*R*)-isobutyl-substituted 3-biphenylacetic acid ((*R*)-**6**) was prepared in six steps from 3-bromophenylacetic acid.[6]

The mono-acyl carbohydrazides **13** and **14** used in the preparation of the inhibitors were prepared as outlined in Scheme 2. Preparation of methyl ester **8** was accomplished by benzylation of methyl 3-hydroxybenzoate (**9**). Hydrazinolysis of methyl esters **7**[7] and **8**[8] provided acylhydrazines **9**[9] and **10**, respectively, which were subsequently treated with phosgene in refluxing toluene to provide 1,3,4-oxadiazol-2-ones **11** and **12**. Hydrazinolysis of **11** and **12** gave mono-acyl carbohydrazides **13** and **14**, respectively.

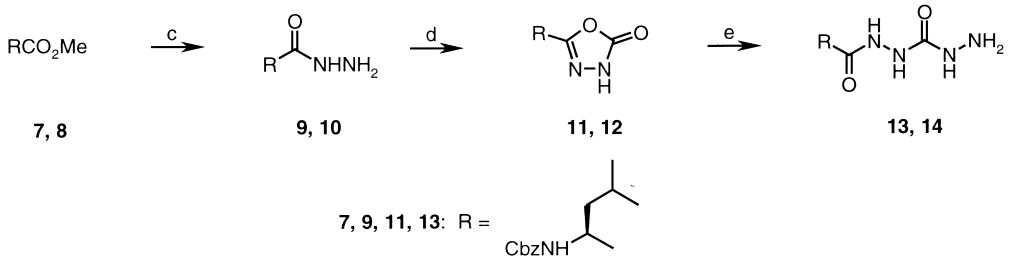
Compounds **15**–**18** were prepared as shown in Scheme 3. EDCl-promoted coupling of **13** with (*R*,*S*)-**5** provided compounds **15** and **16** as a 1:1 mixture which was separated by HPLC. Likewise, EDCl-promoted coupling of **14** with (*R*,*S*)-**5** gave (*R*,*S*)-**17**, and coupling of **14** with (*R*)-**6** provided (*R*)-**18**. The generally low yields obtained for compounds **15**–**18** are likely due to incomplete elution of the compound from the silica gel column as a result of their highly polar nature.

Inhibitor design and evaluation

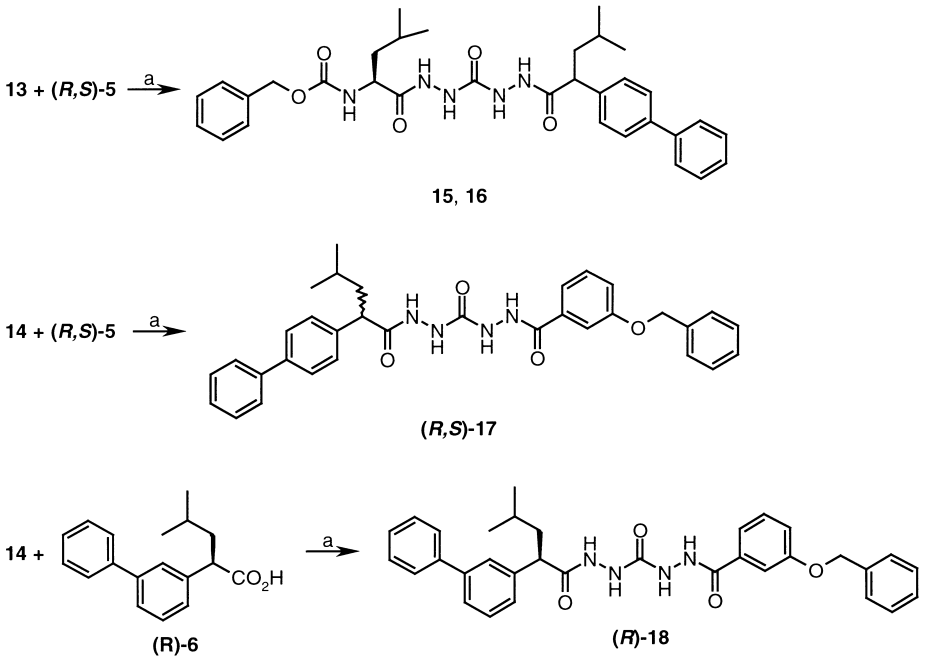
The failure of the *meta*-benzyloxybenzoyl group in compound **2** to mimic the γ-methyl groups of the S′ side leucine side chain (nomenclature of Schechter and Berger)[10] appears to be largely a consequence of the flat nature of the benzoyl group. To achieve the desired mimicry of the entire Cbz-leucine moiety, the use of a mimic that retains a stereocenter was explored. The position of the phenyl portion of the Cbz group relative to the leucine suggested a *para*-biphenylacetyl group wherein substitution of an isobutyl group at the α-position



Scheme 1. (a) i. 2.2 equiv. LDA (lithium diisopropylamide), THF; ii. 3-bromo-2-methylpropene, 72%; (b) H_2, Pd-C, EtOAc, 95%.



Scheme 2. (a) Benzyl chloroformate, Na_2CO_3, 1,4-dioxane/H_2O, 100%; (b) benzyl bromide, K_2CO_3, acetone, 98%; (c) $H_2NNH_2{\cdot}H_2O$, MeOH, **9**, 100%; **10**, 94%; (d) Cl_2CO, toluene, 110 °C; **11**, 96%; **12**, 96%; (e) $H_2NNH_2{\cdot}H_2O$, MeOH, **13**, 60%; **14**, 93%.



Scheme 3. (a) EDC·HCl, 1-HOBT, DMF, **15**, **16**, 42% combined; (***R***,***S***)-**17**, 53%; (***R***)-**18**, 36%.

would function to mimic the leucine side chain as in compounds **15** and **16** (Scheme 3). Thus, a binding model of this putative mimic was constructed using SYBYL version 6.3 and minimized in MAXMIN using the standard settings. The model was then fitted interactively into the cathepsin K active site by overlapping the terminal phenyl group, the carbon bearing the isobutyl group and the carbonyl of the model with the phenyl group, the α-carbon and the carbonyl, respectively, of the S′ side Cbz-leucine moiety of compound **1** from the X-ray crystal structure of compound **1** bound to cathepsin K.[4] Torsional angles were then manually adjusted to optimize the fit of the model. Indeed, the binding model (white) shows very good overlap with both the phenyl group and side chain of the S′ side Cbz-leucine moiety when superimposed with inhibitor **1** (Fig. 2). The internal phenyl group of the biphenyl system appears to function as a spacer to properly orient the terminal phenyl group for an aromatic–aromatic interaction with Trp-184, a key interaction between cathepsin K and this class of inhibitors, but also appears to be oriented to participate in a face–face aromatic–aromatic interaction with Trp-184. While the aromatic–aromatic interaction between the terminal phenyl group of the biphenyl system and Trp-184 suggested by the binding model is an edge–face interaction rather than the face–face interaction observed in the X-ray crystal structure of the cathepsin K/**1** complex, this type of interaction has been observed on the S′ side of the active site in X-ray crystal structures of other cathepsin K:inhibitor complexes.[4,5,11] Thus the diastereomeric mixture of compounds **15** and **16** was synthesized and subsequently separated into individual diastereomers. Although compounds **15** and **16** could be efficiently separated, they did, upon standing, undergo some degree of epimerization at the α-stereocenter of the substituted biphenylacetyl group (~10% after 12 h). The two "purified" diastereomers were therefore evaluated for cathepsin K inhibition as 90:10 mixtures. Compounds **15** and **16** were found to inhibit cathepsin

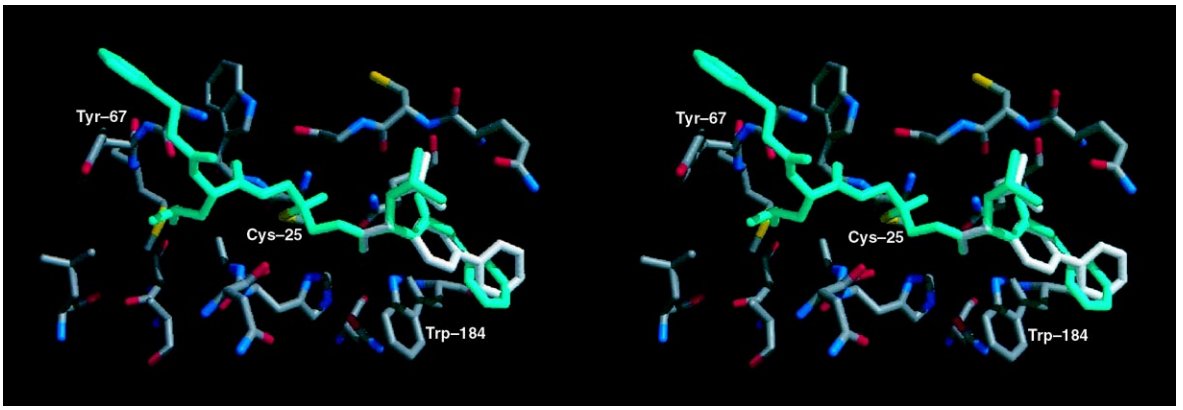
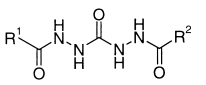
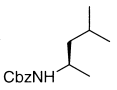
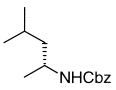
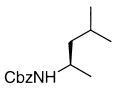
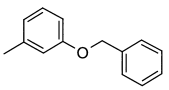
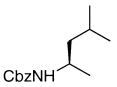
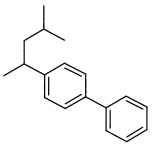
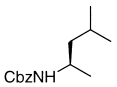
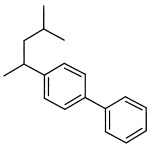
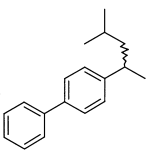
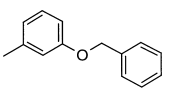
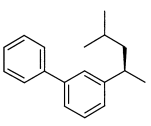
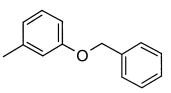


Figure 2. Stereoview of the binding model for the 2-isobutyl-4-biphenylacetyl peptidomimetic (white) superimposed with inhibitor **1** (cyan). Selected active site residues are colored by atom (C = grey, O = red, N = blue, S = yellow).

K in a time-dependent manner, exhibiting apparent second-order rate constants for inhibition of 2,900,000 and 650,000 $M^{-1} s^{-1}$, respectively (Table 1). The equivalent inhibition constants of compounds **1** and **15** indicate that the α-isobutyl-*para*-biphenylacetyl group functions very effectively as a mimic of all aspects of the S′ side Cbz-leucine moiety which are important for inhibitor binding. The absolute stereochemistry at the α-stereocenter is presumably *R*, corresponding to the natural amino acid stereochemistry, although this has not been determined experimentally. Compound **16** exhibits 5-fold lower potency than compound **15**. Although, since the sample of compound **16** contains about 10% of compound **15**, it is likely that about half of the inhibitory potency exhibited by the 90:10 mixture is due to the presence of compound **15**. A similar decrease in potency is observed for compound **2** relative to compound **1**. This result may mean that the isobutyl group in **16** is not effectively interacting with the P_2' binding site.

Table 1. Inhibition of cathepsin K by compounds **1**, **2** and **17**–**20**



Compound	R^1	R^2	$k_{obs}/[I]$ $(M^{-1} s^{-1})$[a]
1			3,100,000
2			224,000
15			2,900,000
16			650,000
17			6,300
18			240,000

[a]Assays were conducted as previously described.[12]

Having designed an effective mimic of all aspects of the S′ side Cbz-leucine moiety, which are important for inhibitor binding, attention was turned towards the design of a mimic of the remaining Cbz-leucine moiety. The slightly less effective *meta*-benzyloxybenzoyl group present in compound **2** rather than the α-isobutyl-*para*-biphenylacetyl group present in **15** was chosen as the S′ side Cbz-leucine mimic for evaluation of mimics of the S side Cbz-leucine since it has been shown to bind exclusively on the S′ side of the active site,[4,5] thereby maximizing the likelihood that the designed S side Cbz-leucine mimics will bind in the active site as proposed. Furthermore, the choice of this mimic alleviates concerns about the stereochemical lability of the α-stereocenter in the α-isobutyl-*para*-biphenylacetyl group. Although a binding model of the α-isobutyl-*para*-biphenylacetyl group (white), constructed as described above, suggests that it would fit into the active site in place of the S side Cbz-leucine of compound **1** and effectively mimic binding of the leucine side chain in the P_2 binding pocket, it does not place the terminal phenyl group properly to mimic the interaction of the phenyl portion of the Cbz-group with Tyr-67 (Fig. 3). Indeed, racemic compound **17** is about 35-fold less potent than its Cbz-leucine analogue **2**. Further examination of the binding model shown in Figure 3 suggested that a *meta*-biphenylacetyl system (magenta) might allow for the desired aromatic–aromatic interaction. This group has previously proven to be effective as a mimic of the S side Cbz-leucine in a structurally related class of 1,3-diaminoketone cathepsin K inhibitors.[6] Since the model also suggested that the *R* stereochemistry at the α-stereocenter was desirable, the target compound (**18**) was synthesized in enantiomerically pure *R* form. Unlike compounds **15** and **16**, compound **18** was not prone to racemization at the benzylic stereocenter under the conditions that promoted epimerization of the corresponding stereocenter in **15** and **16**. The greater stability of the benzylic stereocenter of compound **18** relative to that of compounds **15** and **16** is not fully understood at present, but may be a result of the greater ability of the *para* phenyl ring in **15** and **16** to stabilize a negative charge at the benzylic position through resonance. Nonetheless, compound **18** was found to be equivalent in potency to compound **2** (Table 1), thus demonstrating effective mimicry of both the side chain and aromatic components of the Cbz-leucine moiety.

Confirmation of the direction of binding of inhibitors

Compound **1** exhibits time-dependent kinetics consistent with an essentially irreversible mechanism of inhibition. Mass spectral and NMR analysis of the complex of **1** with cathepsin K give results consistent with formation of an acyl enzyme intermediate with cleavage of one of the acylhydrazine bonds and loss of Cbz-leucinylhydrazine as reported previously.[4] Mass spectral analysis of the complex of compound **2** with cathepsin K gave similar results with exclusive loss of *meta*-benzyloxybenzoylhydrazine, indicating a single direction of binding of the inhibitor.[4] Consistent with this hypothesis, X-ray crystallographic studies of the complex of **2** with cathepsin K show both halves of the

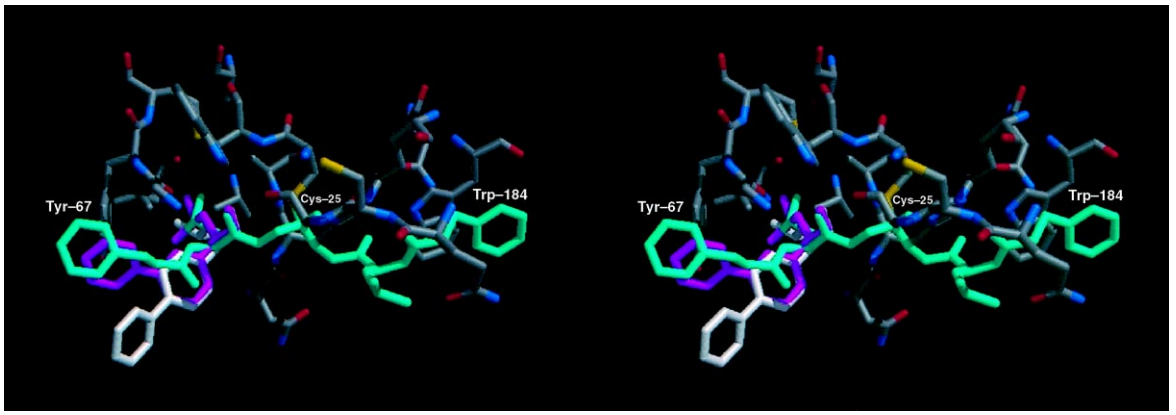


Figure 3. Stereoview of the binding models for the 2-isobutyl-4-biphenylacetyl peptidomimetic (white) and 2-isobutyl-3-biphenylacetyl peptidomimetic (magenta) superimposed with inhibitor **1** (cyan). Selected active site residues are colored by atom (C = grey, O = red, N = blue, S = yellow).

inhibitor present in the active site with the inhibitor bound across both S and S′ sides in a single orientation and the peptidomimetic portion bound on the S′ side of the active site. Thus, mass spectral analysis of complexes of these inhibitors with cathepsin K appears to be a useful analytical tool for prediction of the direction of inhibitor binding based on the exclusive loss of the acylhydrazine group that is bound on the S′ side of the active site. Mass spectral analysis of the complex(es) of cathepsin K with the mixture of inhibitors **15** and **16** showed exclusive formation of an acyl enzyme intermediate containing Cbz-leucine ($M_r = M + 306.0$) and exclusive loss of α-isobutyl-*para*-biphenylacetylhydrazine ($M_r = 283.2$ $[M + H]^+$) (Fig. 4). These results are consistent with the direction of binding predicted by the design hypothesis. Likewise, the proposed binding mode of compound **18** was confirmed by mass spectral analysis of its complex with cathepsin K which showed exclusive formation of an acyl enzyme intermediate containing the α-isobutyl-*meta*-phenylacetyl group ($M_r = M + 309.6$) and exclusive loss of *meta*-benzyloxybenzoylhydrazine ($M_r = 243.2[M + H]^+$) (Fig. 4). Thus, the binding models have proven to be very predictive of relative inhibitor potency as well as direction of inhibitor binding.

Conclusions

Through the use of binding models, we have utilized the crystal structure of a cathepsin K:inhibitor complex to design Cbz-leucine replacements which effectively mimic

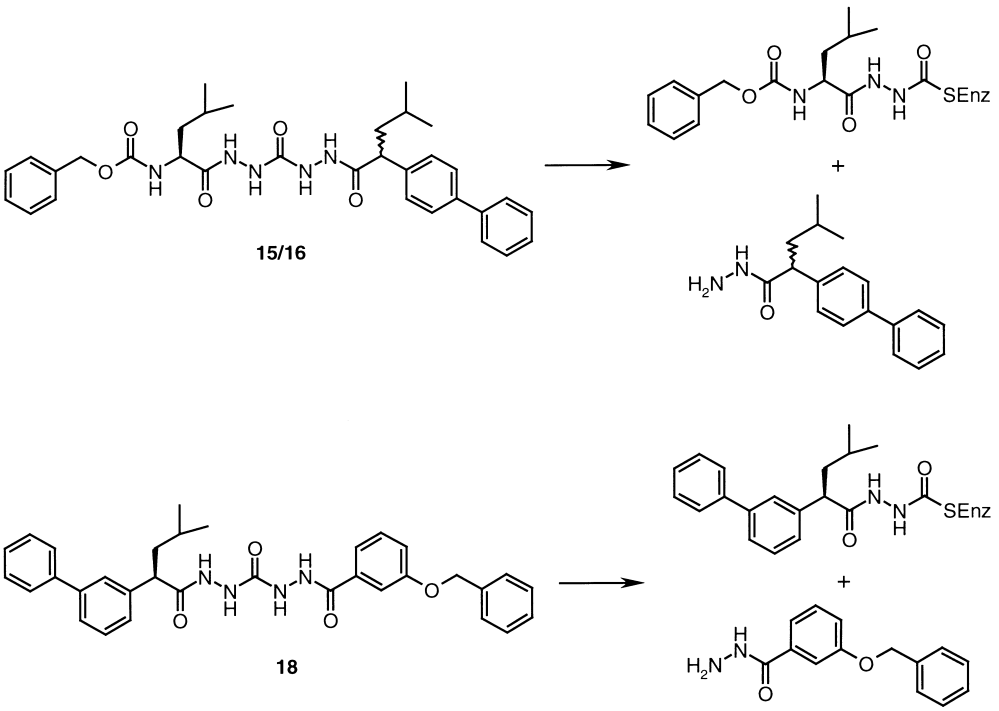


Figure 4. Products of the inhibition of cathepsin K by compounds **15**/**16** and compound **18**.

all aspects of both Cbz-leucine moieties in an amino acid-based inhibitor (**1**), ultimately leading to the design of a potent inhibitor free of amino acid components. The predicted directions of binding for the inhibitors were confirmed by mass spectral analysis of their complexes with cathepsin K, which gave results consistent with acylation of the enzyme and loss of the acylhydrazine portion of the inhibitor which binds on the S′ side of the active site. The binding models were found to be very predictive of relative inhibitor potency as well as direction of inhibitor binding. The strategy involving iterative cycles of structure-based design and inhibitor synthesis and evaluation has thus proven to be a viable approach to obtain potent cathepsin K inhibitors wherein amino acid elements have been replaced with non-amino acid surrogates.

Experimental

Unless otherwise noted, materials were obtained from commercial suppliers and were used without further purification. THF, 1,4-dioxane and DMF were anhydrous grade (Aldrich). All reactions involving organometallic reagents were conducted under an atmosphere of dry argon in oven-dried glassware. Electrospray ionization mass spectra (MS (ESI)) were obtained with a Sciex API-III triple quadrupole mass spectrometer. ^{1}H NMR spectra were obtained at 400 MHz. All extracts were dried over anhydrous magnesium sulfate, and solvents were removed with a Buchi rotary evaporator at aspirator pressure. Flash chromatography using Kieselgel 60 silica gel was performed as previously reported.

(*RS*)-2-(4-Biphenyl)-4-methyl-4-pentenoic acid ((*RS*)-4). To a stirring solution of *N*,*N*-diisopropylamine (537 mg, 5.31 mmol, 0.74 mL) in 5 mL of THF, cooled to 0 °C, was added dropwise 2.1 mL (5.22 mmol) of a 2.5 M solution of *n*-butyllithium in hexanes. After stirring for 15 min at 0 °C, the mixture was cooled to −78 °C and a solution of 4-biphenylacetic acid (**3**) (500 mg, 2.36 mmol) in 2 mL of THF was added dropwise. After warming to 0 °C and again cooling to −78 °C, 3-bromo-2-methylpropene (485 mg, 3.54 mmol, 0.36 mL) was added to the mixture in one portion. After stirring at −78 °C for 1 h, the reaction was quenched with 2 mL of water then concentrated. The residue was re-dissolved in water and extracted with ether (1×100 mL). The aqueous layer was acidified (3N HCl) and extracted with ether (3×100 mL). The organic layers were combined, dried ($MgSO_4$), filtered and concentrated to yield 449 mg (72%) of (***RS***)-**4** as a white solid: ^{1}H NMR ($CDCl_3$) δ 11.3 (bs, 1H), 7.69 (m, 4H), 7.55 (m, 4H), 7.46 (m, 1H), 4.89 (d, 2H), 4.01 (t, 1H), 3.01 (dd, 1H), 2.59 (dd, 1H), 1.84 (s, 3H).

(*RS*)-2-(4-Biphenyl)-4-methylpentanoic acid ((*RS*)-5). To a stirring solution of (***RS***)-**4** (449 mg, 1.69 mmol) in 25 mL of ethyl acetate was added 225 mg of 10% palladium on carbon. After stirring under a balloon of hydrogen for 16 h, the mixture was filtered through Celite. The filtrate was concentrated to yield 430 mg (95%) of (***RS***)-**5** as an off white solid: ^{1}H NMR ($CDCl_3$) δ 7.58 (m, 4H), 7.45 (m, 4H), 7.37 (m, 1H), 3.72 (d, 1H), 2.02 (m, 1H), 1.75 (m, 1H), 1.55 (m, 1H), 0.92 (d, 6H).

General procedure for the hydrazinolysis of methyl esters. To a stirring 0.1 M solution of methyl ester in methanol was added 10 equivalents of hydrazine hydrate. The solution was allowed to stir at room temperature for 16 h then concentrated to yield the desired hydrazides.

Spectral data for compounds 9 and 10. *N*-Benzyloxycarbonyl-L-leucinylhydrazide (9). ^{1}H NMR ($CDCl_3$) δ 8.32 (s b, 1H), 7.32 (m, 5H), 5.77 (d, 1H), 5.05 (q, 2H), 4.19 (m, 1H), 3.76 (s b, 2H), 1.56 (m, 3H), 0.89 (m, 6H).

3-Benzyloxybenzoylhydrazide (10). ^{1}H NMR ($CDCl_3$) δ 7.45–7.27 (m, 9H), 7.24 (m, 1H), 5.12 (s, 2H), 4.10 (s b, 2H).

General procedure for the conversion of hydrazides to 1,3,4-oxadiazol-2-ones. To a stirring 0.2 M solution of hydrazide in toluene was added 3 equivalents of phosgene as a 1.93 M solution in toluene. The solution was heated at reflux for 4 h then concentrated to yield the desired 1,3,4-oxadiazol-2-ones.

Spectral data for compounds 11 and 12. (1*S*)-1-Benzyloxycarbonylamino-3-methyl-1-(1,3,4-oxadiazol-2-on-5-yl)-butane (11). ^{1}H NMR ($CDCl_3$) δ 9.32 (s, 1H), 7.35 (m, 6H), 5.13 (m, 2H), 4.79 (m, 1H), 1.68 (m, 3H), 0.96 (m, 6H).

5-(3-Benzyloxyphenyl)-1,3,4-oxadiazol-2-one (12). ^{1}H NMR ($CDCl_3$) δ 7.41–7.28 (m, 9H), 7.06 (m, 1H), 5.06 (s, 2H).

General procedure for the conversion of 1,3,4-oxadiazol-2-ones to acyl carbohydrazides. To a stirring 0.25 M solution of 1,3,4-oxadiazol-2-one in methanol was added 10 equivalents of hydrazine hydrate. The solution was allowed to stir at room temperature for 24 h then concentrated and purified by column chromatography (silica gel, 8% methanol:dichloromethane) to yield the desired acyl carbohydrazides.

Spectral data for compounds 13 and 14. 2-[*N*-(*N*-Benzyloxycarbonyl-L-leucinyl)]carbohydrazide (13). ^{1}H NMR ($CDCl_3$) δ 7.30 (m, 5H), 6.29 (d, 1H), 5.10 (d, 1H), 4.95 (d, 1H), 4.36 (m, 1H), 1.58 (m, 3H), 0.87 (m, 6H).

2-[*N*-(3-Benzyloxybenzoyl)]carbohydrazide (14). ^{1}H NMR (CD_3OD) δ 7.54 (m, 1H), 7.47 (m, 3H), 7.38 (m, 3H), 7.31 (m, 1H), 7.20 (m, 1H), 5.14 (s, 2H).

General procedure for the EDC-promoted coupling of acyl carbohydrazides with carboxylic acids. To a stirring 0.15 M solution of acyl carbohydrazide in DMF was added 1.1 equivalents of carboxylic acid, 0.2 equivalents of 1-hydroxybenzotriazole and 1.1 equivalents of 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride. After stirring at room temperature for 16 h, the solution was diluted with ethyl acetate and washed successively with saturated aqueous sodium bicarbonate, water (2×), and brine. The organic layer was

dried, filtered and concentrated. The residue was purified by column chromatography (silica gel, 5% methanol: dichloromethane) to yield the desired diacyl carbohydrazides.

Spectral data for diacyl carbohydrazides. (2′*R*)-2-[*N*-(*N*-Benzyloxycarbonyl-L-leucinyl)]-2′-[*N*′-[2-(4-biphenyl)-4-methylpentanoyl)]]carbohydrazide and (2′*S*)-2-[*N*-(*N*-benzyloxycarbonyl-L-leucinyl)]-2′-[*N*′-[2-(4-biphenyl)-4-methylpentanoyl)]]carbohydrazide (15 and 16). ^{1}H NMR ($CDCl_3/CD_3OD$) δ 7.48 (m, 5H), 7.35 (m, 5H), 7.23 (m, 4H), 4.92 (m, 2H), 4.08 (m, 1H), 3.54 (m, 1H), 1.91 (m, 1H), 1.74–1.40 (m, 5H), 0.84 (m, 12H).

Compounds **15** and **16** were separated by HPLC (Sumipax OA-3100, 4.6×150 mm, 80:20 hexane:ethanol, 1.0 mL min^{-1}). Compound 15: retention time = 5.9 min; compound 16: retention time = 8.1 min.

(2′*RS*)-2-[*N*-(3-Benzyloxybenzoyl)]-2′-[*N*′-[2-(4-biphenyl)-4-methylpentanoyl)]]carbohydrazide ((*RS*)-17). ^{1}H NMR ($CDCl_3/CD_3OD$) δ 7.45 (m, 4H), 7.35 (m, 9H), 7.26 (m, 4H), 7.04 (dd, 1H), 4.98 (s, 2H), 3.59 (t, 1H), 1.92 (m, 1H), 1.71 (m, 1H), 1.44 (m, 1H), 0.85 (m, 6H).

(2′*R*)-2-[*N*-(3-Benzyloxybenzoyl)]-2′-[*N*′-[2-(3-biphenyl)-4-methylpentanoyl)]]carbohydrazide ((*R*)-18). ^{1}H NMR ($CDCl_3/CD_3OD$) δ 7.48 (m, 2H), 7.41–7.24 (m, 15H), 7.03 (m, 1H), 4.96 (s, 2H), 3.59 (t, 1H), 1.92 (m, 1H), 1.69 (m, 1H), 1.43 (m, 1H), 0.81 (m, 6H).

Mass spectral analysis of compounds 15/16 and compound 18

Cathepsin K (27 μM in MES buffer at pH 6.0) was activated as previously described[13] and stored at −70 to −40 °C. LC/MS analysis revealed the observed molecular weights of 23645.4 and 23700.3 for the N-terminal sequences beginning RAPD and GRAPD, in approximately a 5:4 ratio (calculated masses are 23645.6 and 23702.7 Da, respectively). Incubations were performed by adding 5 nmol compound in DMSO to 1 nmol protein and sufficient water so that the final protein concentration was 13.5 μM and the DMSO concentration was 5% (compounds **15** and **16**) or 10% (compound **18**). After vortexing, the solutions were allowed to stand at room temperature for 60 and 90 min, respectively. Then, they were loaded onto a peptide trap cartridge (Michrom), washed with 50 μL of 0.1% TFA/water (solvent A), and back-eluted onto a 1×50 mm Inertsil C18 reversed phase HPLC column with a gradient from 5% to 95% solvent B (0.1% TFA:90% MeCN:10% water) in 10 min. The mass spectrometer was scanned from m/z 200 to 1800 once every 3.9 s.

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References and Notes

1. McKerrow, J. H.; James, M. N. G., Eds.; *Perspectives in Drug Discovery and Design* **1997**, *6*, 1–125.
2. Drake, 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*, 12511–12516.
3. Bossard, M. J.; Tomaszek, T. A.; Thompson, S. K.; Amegadzie, B. Y.; Hanning, C. R.; Jones, C.; Kurdyla, J. T.; McNulty, D. E.; Drake, F. H.; Gowen, M.; Levy, M. A. *J. Biol. Chem.* **1996**, *271*, 12517–12524.
4. Thompson, S. K.; Halbert, S. M.; Bossard, M. J.; Tomaszek, T. A.; Levy, M. A.; Zhao, B.; Smith, W. W.; Abdel-Meguid, S. S.; Janson, C. A.; D'Alessio, K. J.; McQueney, M. S.; Amegadzie, B. Y.; Hanning, C. R.; DesJarlais, R. L.; Briand, J.; Sarkar, S. K.; Huddleston,M. J.; Ijames, C. F.; Carr, S. A.; Garnes, K. T.; Shu, A.; Heys, J. R.; Bradbeer, J.; Zembryki, D.; Lee-Rykaczewski, L.; James, I. E.; Lark, M. W.; Drake, F. H.; Gowen, M.; Gleason, J. G.; Veber, D. F. *Proc. Natl. Acad. Sci., USA* **1997**, *94*, 14249–14254.
5. Thompson, S. K.; Smith, W. W.; Zhao, B.; Halbert, S. M.; Tomaszek, T. A.; Tew, D. G.; Levy, M. A.; Janson, C. A.; D'Alessio, K. J.; McQueney, M. S.; Kurdyla, J.; Jones, C. S.; DesJarlais, R. L.; Abdel-Meguid, S. S.; Veber, D. F. *J. Med. Chem.* **1998**, *41*, 3923–3927.
6. DesJarlais, R. L.; Yamashita, D. S.; Oh, H.-J.; Uzinskas, I. N.; Erhard, K. F.; Allen, A. C.; Haltiwanger, R. C.; Zhao, B; Smith, W. W.; Abdel-Meguid, S. S.; D'Alessio, K. J.; Janson, C. A.; McQueney, M. S.; Tomaszek, T. A.; Levy, M. A.; Veber, D. F. *J. Am. Chem. Soc.* **1998**, *120*, 9114–9115.
7. Yamada, T.; Isono, N.; Inui, A.; Miyazawa, T.; Kuwata, S.; Watanabe, H. *Bull. Chem. Soc. Jpn.* **1978**, *51*, 1897–1898.
8. Subramanyam, C.; Bell, M. R.; Ferguson, E; Gordon, R. G.; Dunlap, R. P.; Franke, C.; Mura, A. J. *Bioorg. Med. Chem. Lett.* **1995**, *5*, 319–324.
9. Szarvasi, E.; Fontaine, L.; Betbeder-Matibet, A. *J. Med. Chem.* **1973**, *16*, 281–287.
10. Schechter, I.; Berger, A. *Biochem. Biophys. Res. Commun.* **1967**, *27*, 157–162.
11. Yamashita, D. S.; Smith, W. W.; Zhao, B.; Janson, C. A.; Tomaszek, T. A.; Bossard, M. J.; Levy, M. A.; Oh, H.-J.; Carr, T. J.; Thompson, S. K.; Ijames, C. F.; Carr, S. A.; McQueney, M. S.; D'Alessio, K. J.; Amegadzie, B. Y.; Hanning, C. R.; Abdel-Meguid, S. S.; DesJarlais, R. L.; Gleason, J. G., Veber, D. F. *J. Am. Chem. Soc.* **1997**, *119*, 11351.
12. Votta, B.; Levy, M. A.; Badger, A.; Bradbeer, J.; Dodds, R. A.; James, I. E.; Thompson, S.; Bossard, M. J.; Carr, T.; Connor, J. R.; Tomaszek, T. A.; Szewczuk, L.; Drake, F. H.; Veber, D. F.; Gowen, M. *J. Bone Miner. Res.* **1997**, *12*, 1396.
13. McQueney, M. S.; Amegadzie, B. Y.; D'Alessio, K. D.; Hanning, C. R.; McLaughlin, M. M.; McNulty, D.; Carr, S. A.; Ijames, C.; Kurdyla, J.; Jones, C. S. (1997) *J. Biol. Chem.* **272**, 13955–13960.