Novel, Nonpeptidic Cyanamides as Potent and Reversible Inhibitors of Human Cathepsins K and L

Jean-Pierre Falgueyret,[†] Renata M. Oballa,*[,‡] Osamu Okamoto,[‡] Gregg Wesolowski,[§] Yves Aubin,[‡] Robert M. Rydzewski,[∥] Peppi Prasit,[‡] Denis Riendeau,[†] Sevgi B. Rodan,[§] and M. David Percival[†]

Department of Biochemistry and Molecular Biology and Department of Chemistry, Merck Frosst Centre for Therapeutic Research, 16711 TransCanada Highway, Kirkland, Quebec H9H 3L1, Canada, Department of Bone Biology & Osteoporosis, Merck Research Laboratories, West Point, Pennsylvania, and Department of Medicinal Chemistry, Axys Pharmaceuticals, South San Francisco, California

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Compounds containing a 1-cyanopyrrolidinyl ring were identified as potent and reversible inhibitors of cathepsins K and L. The original lead compound **1** inhibits cathepsins K and L with IC_{50} values of 0.37 and 0.45 μM, respectively. Modification of compound **1** by replacement of the quinoline moiety led to the synthesis of *N*-(1-cyano-3-pyrrolidinyl)benzenesulfonamide (**2**). Compound **2** was found to be a potent inhibitor of cathepsins K and L with a K_i value of 50 nM for cathepsin K. Replacement of the 1-cyanopyrrolidine of compound **2** by a 1-cyanoazetidine increased the potency of the inhibitor by 10-fold. This increase in potency is probably due to an enhanced chemical reactivity of the compound toward the thiolate of the active site of the enzyme. This is demonstrated when the assay is performed in the presence of glutathione at pH 7.0 which favors the formation of a GSH thiolate anion. Under these assay conditions, there is a loss of potency in the 1-cyanoazetidine series due to the formation of an inactive complex between the GSH thiolate and the 1-cyanoazetidine inhibitors. 1-Cyanopyrrolidinyl inhibitors exhibited time-dependent inhibition which allowed us to determine the association and dissociation rate constants with human cathepsin K. The kinetic data obtained showed that the increase of potency observed between different 1-cyanopyrrolidinyl inhibitors is due to an increase of k_{on} values and that the association of the compound with the enzyme fits an apparent one-step mechanism. ^{13}C NMR experiments performed with the enzyme papain showed that compound **2** forms a covalent isothiourea ester adduct with the enzyme. As predicted by the kinetic analysis, the addition of the irreversible inhibitor E64 to the enzyme−cyanopyrrolidinyl complex totally abolished the signal of the isothiourea bond as observed by ^{13}C NMR, thereby demonstrating that the formation of the covalent bond with the active site cysteine residue is reversible. Finally, compound **2** inhibits bone resorption in an in vitro assay involving rabbit osteoclasts and bovine bone with an IC_{50} value of 0.7 μM. 1-Cyanopyrrolidine represents a new class of nonpeptidic compounds that inhibit cathepsin K and L activity and proteolysis of bone collagen.

Introduction

Cysteine proteases comprise a group of proteolytic enzymes involved in many physiological processes and which could potentially play important roles in a number of pathological situations. Cathepsins are defined as lysosomal proteases, and the majority are members of the papain-like cysteine protease family. Eleven different sequences of human cysteinyl cathepsins (B, H, L, S, C, K, O, F, V, X, and W) have been identified to date.[1] Cysteine proteases contain a cysteine and histidine pair at the active site which forms a stable thiolate−imidazolium ion pair and is required for enzyme activity.[2]

The physiological role of cathepsins is in intracellular protein degradation. In general, cathepsins have a broad tissue distribution, cathepsins L and B being highly expressed in lysosomes of many cells and secreted outside lysosomes in diseases such as cancer, muscular dystrophy, Alzheimer's disease, and multiple sclerosis.[3] Recently, two cathepsins, cathepsins K and S which are members of the cathepsin L subfamily, have been described as being more tissue-specific and were found to be involved in specialized cellular processes. Cathepsin S has a specific function in antigen processing[4] and represents a potential target for the treatment of autoimune diseases. Cathepsin K is almost exclusively expressed in bone-resorbing osteoclasts.[5] Thus, development of specific inhibitors for this enzyme could potentially be used as treatment for diseases involving excessive bone resorption. Moreover, recent work has demonstrated that nonselective inhibitors of cysteine proteases which are active against cathepsin K significantly reduce bone resorption in in vitro and in vivo models.[6]

* To whom correspondence should be addressed. Tel: (514) 428-3617. Fax: (514) 428-4900. E-mail: renata_oballa@merck.com.
[†] Department of Biochemistry and Molecular Biology, Merck Frosst Centre for Therapeutic Research.
[‡] Department of Chemistry, Merck Frosst Centre for Therapeutic Research.
[§] Merck Research Laboratories.
[∥] Axys Pharmaceuticals.

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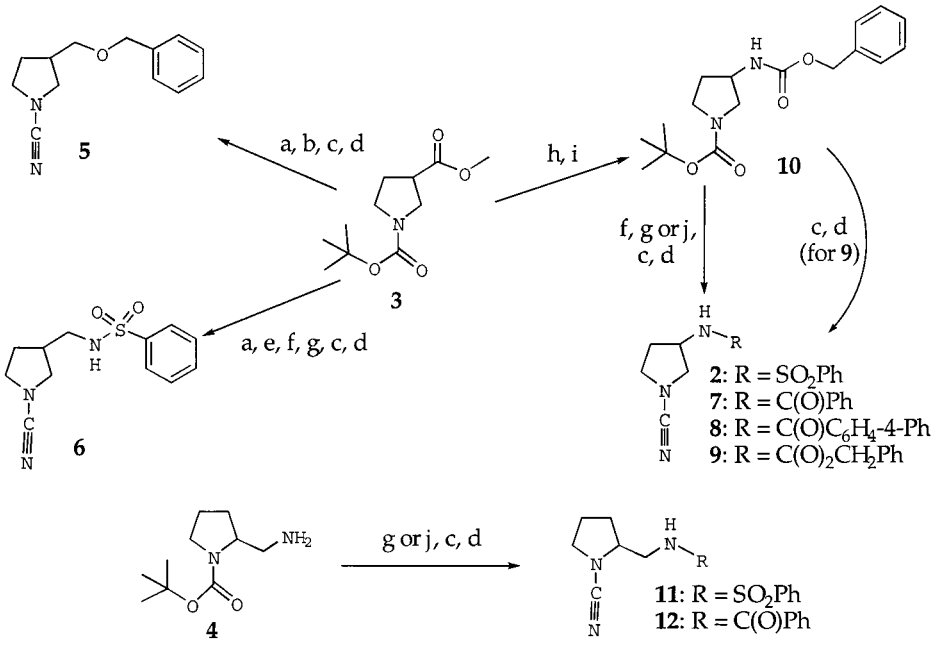
Scheme 1[a]



[a] Conditions: (a) $NaBH_4$, MeOH, THF; (b) NaH, DMF, $PhCH_2Cl$, 0 °C; (c) TFA, CH_2Cl_2; (d) BrCN, Et_3N, CH_2Cl_2; (e) MsCl, Et_3N, THF, 0 °C; NaN_3, DMSO, △; (f) H_2, Pd/C, MeOH; (g) $PhSO_2Cl$, Et_3N, CH_2Cl_2, 0 °C; (h) NaOH, MeOH; (i) DPPA, Et_3N, $PhCH_3$, △, $PhCH_2OH$; (j) RC(O)Cl, Et_3N, CH_2Cl_2.

Many chemotypes have been described as inhibitors of cysteine proteases.[7] The majority, if not all, are dependent for their activity on the presence of an electrophilic moiety which can form either a reversible or an irreversible covalent bond with the active site cysteine of the enzyme. Additionally, most of the compounds described so far for the inhibition of cathepsins are peptide-based molecules that suffer the liabilities of high molecular weight and the presence of a peptidic backbone. While these compounds are potent and selective inhibitors of the cathepsins, they generally possess poor pharmacokinetic properties.

In the present report, we describe a new class of nonpeptidic and low-molecular-weight compounds with some degree of selectivity for the inhibition of cathepsins K and L over cathepsin B. This new class of the cathepsin L subfamily inhibitors is based on a 1-cyanopyrrolidinyl ring, and they form a reversible isothiourea ester bond with the enzyme. Furthermore, these compounds were shown to be potent inhibitors of the degradation of denaturated collagen by cathepsin K and of bone resorption in an in vitro model.

Chemistry

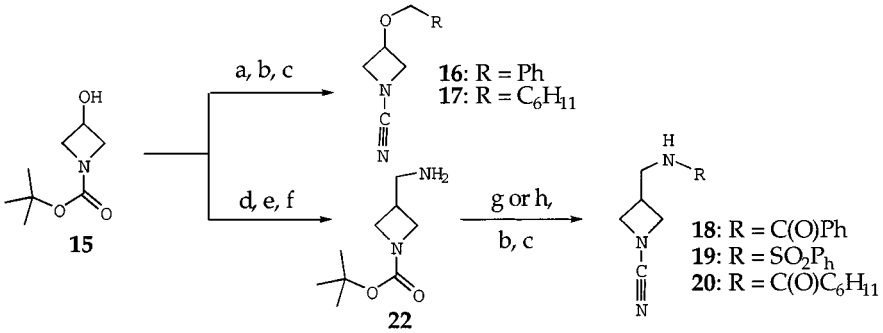
As described in Scheme 1, the synthesis of the 1-cyanopyrrolidines began with either 1-*tert*-butyl 3-methyl 1,3-pyrrolidinedicarboxylate (**3**)[11] or the commercially available *N*-Boc-2-aminomethylpyrrolidine (**4**). $NaBH_4$ reduction of **3** and benzylation of the resultant primary alcohol moiety followed by Boc-deprotection and treatment with BrCN led to the synthesis of cyanamide **5**. Cyanamide **6** was obtained by conversion of the primary alcohol to an amino group through mesylate displacement with NaN_3 and subsequent hydrogenolysis. The amino group was then converted to the benzenesulfonamide, and treatment with TFA followed by BrCN afforded the 3-substituted 1-cyanopyrrolidine **6**. The cyanamides **2** and **7**−**9** were synthesized from the carbamate intermediate **10** via (i) hydrogenolysis of the carbamate, (ii) amide or sulfonamide bond formation, and (iii) conversion of the N-Boc to the desired N-CN moiety. The carbamate intermediate **10** was, in turn, synthesized from the ester **3** via Curtius rearrangement using diphenyl phosphorazidate (DPPA) and trapping the resultant isocyanate with benzyl alcohol. Finally, the 2-substituted 1-cyanopyrrolidines **11** and **12** were obtained from **4** via amide or sulfonamide bond formation followed by conversion of the N-Boc to the cyanamide moiety.

The synthesis of the 1-cyanoazetidines is described in Scheme 2. Starting with *tert*-butyl 3-hydroxy-1-azetidinecarboxylate (**15**), the cyanamides **16** and **17** were synthesized by first forming the benzyl or cyclohexylmethyl ether linkage followed by deprotection of the Boc group and subsequent cyanamide formation with BrCN. In the case of the amide and sulfonamide analogues **18**−**20**, their synthesis originated from the primary amine **22** which, in turn, was derived by displacing the mesylate of **15** with NaCN followed by Raney nickel reduction. The amide or sulfonamide bond was then formed by reacting the amine **22** with RC(O)Cl or $PhSO_2Cl$ in the presence of Et_3N. Finally, deprotection of the Boc-amine and subsequent reaction with BrCN generated the desired azetidine cyanamides **18**−**20**.

Biochemistry

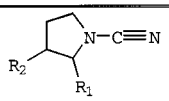
All compounds were tested against cathepsins K, L, and B. Purified recombinant cathepsins K and L were obtained from Axys Pharmaceuticals Inc., and purified human cathepsin B was obtained from Sigma. To determine inhibitory potency, compounds were preincubated 30 min with the enzyme, prior to the addition of the substrate Z-Phe-Arg-pNA (25 μM). All assays

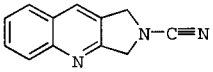
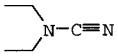
Scheme 2[a]



[a] Conditions: (a) NaH, DMF, RCH_2Br, 0 °C; (b) TFA, CH_2Cl_2; (c) BrCN, Et_3N, CH_2Cl_2; (d) MsCl, Et_3N, EtOAc, 0 °C; (e) NaCN, DMSO, 130 °C; (f) Raney Ni, NH_3, H_2, MeOH; (g) RC(O)Cl, Et_3N, CH_2Cl_2; (h) $PhSO_2Cl$, Et_3N, CH_2Cl_2, 0 °C.

Table 1. Inhibition of Human Cathepsins K, L, and B by 1-Cyanopyrrolidinyl Analogues



Compound	Molecular Formula	R_1	R_2	IC_{50} (μM) Cat K	Cat L	Cat B	Cat K Gelatinase Assay	Cat K Bone Resorption Assay
1	$C_{12}H_9N_3$			0.37	0.45	2.3	0.36	-
2	$C_{11}H_{13}N_3O_2S$	H	$NHSO_2Ph$	0.05	0.08	1.4	0.018	0.75
5	$C_{12}H_{15}N_3O_2S$	H	CH_2NHSO_2Ph	0.2	0.35	1.5	-	-
6	$C_{13}H_{16}N_2O$	H	CH_2OCH_2Ph	0.1	0.15	1.1	-	-
7	$C_{12}H_{13}N_3O$	H	NHCOPh	0.37	1.75	4.2	-	-
8	$C_{18}H_{17}N_3O$	H	$NHCOC_6H_4$-4-Ph	0.29	0.85	0.3	-	-
9	$C_{13}H_{15}N_3O_2$	H	$NHCO_2CH_2Ph$	0.04	0.054	0.2	0.020	1.41
11	$C_{12}H_{15}N_3O_2S$	CH_2NHSO_2Ph	H	12.1	11.5		-	-
12	$C_{13}H_{15}N_3O$	$CH_2NHCOPh$	H	13.7	23	75	-	-
13	$C_5H_8N_2$	H	H	1.0	4.0	15	-	-
14	$C_5H_{10}N_2$			> 100	> 100	> 100	-	-

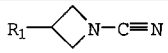
were performed in 96-wells plates at compound concentrations ranging from 100 μM to 1 nM. IC_{50} values were determined by fitting experimental values to a four-parameter logistic model. To determine pre-steady-state kinetic parameters, enzymatic activity was measured at room temperature in stirred cells using a fluorometer (PTI fluorescence system) and Z-Phe-Arg-AMC as substrate.

Results and Discussion

As shown in Table 1, the original lead compound **1**, identified through screening of the Merck sample collection, inhibits human cathepsins K and L with IC_{50} values of 0.37 and 0.45 μM, respectively. If the quinoline moiety is removed, the unsubstituted 1-cyanopyrrolidine **13** partially retains potency against cathepsin K. However, the acyclic cyanamide **14** loses all potency against cathepsins K, L, and B. Thus, the structure−activity relationship (SAR) of substituted 1-cyanopyrrolidines was examined. The addition of substituents at the 2-position (i.e. compounds **11** and **12**) resulted in loss of potency. However, substitution at the 3-position (i.e. compounds **2** and **5**−**9**) afforded potent inhibitors of cathepsins K and L. The amides **7** and **8**, in which the amide substituent is directly attached to the 3-position of the cyanopyrrolidine, afforded moderate inhibition of cathepsin K (0.37 and 0.29 μM, respectively). By converting the amide functionality to a benzenesulfonamide (compound **2**) or a benzyl carbamate (compound **9**), the inhibition of cathepsin K increased to 0.05 and 0.04 μM, respectively. The benzensulfonamide **2** proved to be the most potent and selective (cathepsins K and L versus B) compound in the 1-cyanopyrrolidine series. Moreover, compound **2** inhibits both rat and human cathepsin K with similar IC_{50} values.

The SAR in the 1-cyanoazetidine series is depicted in Table 2. The unsubstituted 1-cyanoazetidine **21** inhibits human cathepsins K and L with IC_{50} values of 0.1 and 0.43 μM, respectively. Compound **21** is 10-fold more active than the corresponding unsubstituted analogue **13** in the five-membered ring pyrrolidine series. This trend of an increase in potency in the four-

Table 2. Inhibition of Human Cathepsins K, L, and B by 1-Cyanoazetidinyl Analogues



Compound	Molecular Formula	R_1	IC$_{50}$ (μM) Cat K	Cat L	Cat B	Cat K Gelatinase Assay
16	$C_{11}H_{12}N_2O$	OCH_2Ph	0.04	0.1	0.5	-
17	$C_{11}H_{18}N_2O$	$OCH_2C_6H_{11}$	0.02	0.1	0.5	-
18	$C_{12}H_{13}N_3O$	$CH_2NHCOPh$	0.07	0.65	0.25	-
19	$C_{11}H_{13}N_3O_2S$	CH_2NHSO_2Ph	0.02	0.05	0.3	-
20	$C_{12}H_{19}N_3O$	$CH_2NHCOC_6H_{11}$	0.005	0.006	0.15	0.010
21	$C_4H_6N_2$	H	0.1	0.43	2.0	-

membered ring azetidine series when compared to the five-membered ring pyrrolidine series is seen across the board (i.e. compare compound **19** in Table 2 to compound **5** in Table 1). The most potent compound in the 1-cyanoazetidine series, compound **20**, possesses a cyclohexylamide substituent in the 3-position, and it inhibits cathepsins K and L with IC$_{50}$ values of 0.005 and 0.006 μM, respectively.

The 1-cyanopyrrolidines and 1-cyanoazetidines were also shown to be active in the gelatinase and bone-resorption assays (see Tables 1 and 2). For example, the lead compound **1** was equipotent in the gelatinase and human cathepsin K assays (0.36 and 0.37 μM, respectively). The more potent 3-substituted 1-cyanopyrrolidines **2** and **9** (0.05 and 0.04 μM versus cathepsin K, respectively) were also very potent in the gelatinase assay with IC$_{50}$ values of 0.018 and 0.02 μM, respectively. Likewise, in the 1-cyanoazetidine series, the most potent cathepsin K and L inhibitor **20** had an IC$_{50}$ value of 0.01 μM in the gelatinase assay. In the functional in vitro bone-resorption assay, compounds **2** and **9** were shown to inhibit bone resorption with IC$_{50}$ values of 0.75 and 1.41 μM, respectively. In the same assay, E64, a potent irreversible epoxide inhibitor of cysteine proteases, was found to inhibit bone resorption with an IC$_{50}$ value of 0.02 μM.

Kinetic Experiments. Inhibition of cathepsins K and L by 1-cyanopyrrolidinyl analogues was found to be time-dependent and fully reversible. Figure 1 shows the reaction progress curves for the onset of inhibition of human cathepsin K by compound **19** (Figure 1A) and compound **1** (Figure 1B). The curves show that for an equal final concentration of inhibitor, the same steady-state velocity is obtained either when the reaction is initiated by the addition of enzyme or when the enzyme and the inhibitor are preincubated and then diluted into the assay mixture containing substrate. These kinetics are consistent with a reversible time-dependent mechanism of inhibition. Generally, two possible mechanisms can describe reversible time-dependent inhibitors. One mechanism characterizes compounds where the initial velocity of the enzymatic reaction is independent of the inhibitor concentration and a linear correlation between the inhibitor concentration and the apparent first-order rate constant for the onset of inhibition (k_{obs}) is observed. This mechanism is often referred to as an apparent single step binding process[8] and is described by eq 1:

$$E + I \underset{k_{off}}{\overset{k_{on}}{\rightleftharpoons}} EI^* \quad (1)$$

The second mechanism involves a two-step process characterized by an initial rapidly reversible binding, followed by a second slow formation of a more tightly bound complex. In this two step process, binding in the initial complex is tight enough to result in an inhibition of the initial reaction velocity. This mechanism is characterized by curved plots of k_{obs} versus inhibitor concentration, where the asymptote approached at high inhibitor concentration represents the value of k_2 for the second equilibrium (eq 2):

$$E + I \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} EI \underset{k_{-2}}{\overset{k_2}{\rightleftharpoons}} EI^* \quad (2)$$

The progress curves for the onset of inhibition (at a range of inhibitor concentrations) were fitted to the eq 3 (see Experimental Section) describing slow binding inhibition. The obtained values of k_{obs} were then plotted versus inhibitor concentration. The results for both the 1-cyanopyrrolidine compounds **1**, **2**, and **9** and the 1-cyanoazetidine compound **19** showed that the rate of association of cathepsin K and the inhibitors is linearly dependent on inhibitor concentration. This is therefore consistent with an apparent one-step binding mechanism of the inhibitor to the enzyme. From the progress curves for the onset of inhibition, it is possible to determine the pre-steady-state kinetics parameters k_{on} and k_{off} (eq 3) as well as the dissociation constant K_i (see Experimental Section). The data presented in Table 3 shows that the increase in the potency of the inhibitors is mainly driven by an increase in the value of k_{on}, while the value of the k_{off} remains largely unchanged. Although the kinetic data obtained with the 1-cyanopyrrolidines fits a one-step mechanism, it is unlikely that the time dependency of these small molecules is due to a slow rate of diffusion into the enzyme active site. As hypothesized for other low-molecular-weight time-dependent inhibitors,[9] it is possible that one or more transient enzyme−inhibitor complexes occurs before the formation of the final EI complex. However, if the

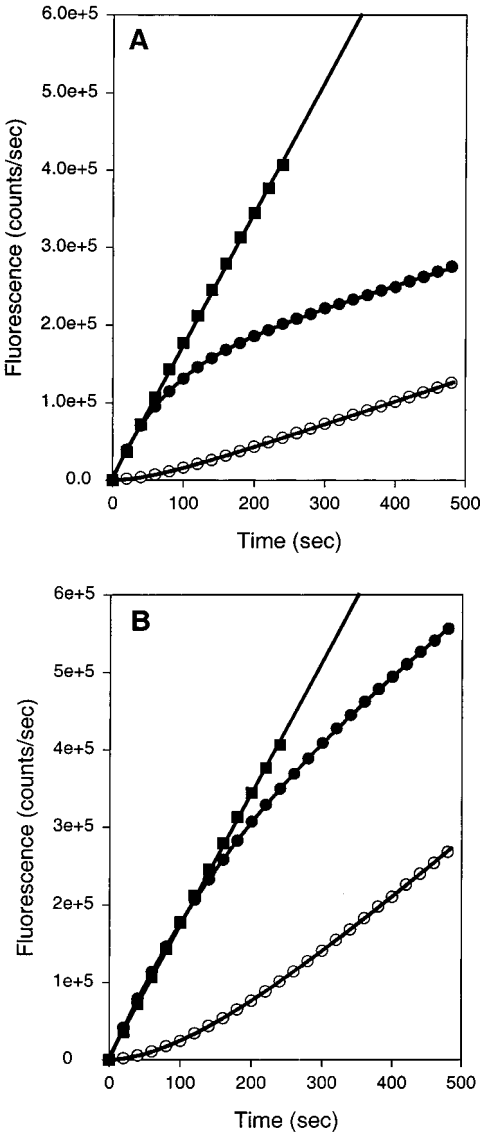


Figure 1. Time-dependent inhibition and reversibilty of 1-cyanoazetidine and 1-cyanopyrrolidinyl compounds. Progress curves were obtained when the reaction was initiated either by the addition of 0.5 nM cathepsin K (●) or when cathepsin K and the inhibitor were preincubated for 15 min and then diluted 100-fold prior to the initiation of the enzymatic reaction (○): (A) 130 nM compound **19**; (B) 500 nM compound **1**; control reaction (■) without inhibitor.

Table 3. Inhibition of Human Cathepsin K by 1-Cyanopyrrolidinyl and 1-Cyanoazetidinyl Analogues

compd	k_{off} (s^{-1}) ± SE	k_{on} (M^{-1} s^{-1}) ± SE	K_i (μM) ± SE	IC_{50} (μM)
1	0.0025 ± 0.0003	6594 ± 129	0.38 ± 0.084	0.37
2	0.0019 ± 0.001	19899 ± 1079	0.09 ± 0.001	0.081
9	0.0056 ± 0.0003	67303 ± 7800	0.14 ± 0.017	0.04
19	0.0028 ± 0.0002	140722 ± 24637	0.02 ± 0.0002	0.017

dissociation constant of this intermediate complex is much higher than the K_i of the final EI complex, its contribution will be kinetically insignificant and difficult to observe experimentally.

Reactivity of the Cyanamide with Thiols. To determine if the increase in potency observed between the 1-cyanopyrrolidine series (Table 1) and the 1-cyanoazetidine series (Table 2) is due to an increase in the reactivity of the cyanamide moiety toward the active site cysteine thiolate anion, IC_{50} values were determined in the presence and absence of glutathione (GSH) at pH 5.5 and 7.0. Increasing the pH to 7.0 will favor the formation of the GSH thiolate anion which is a better nucleophile and can react with the inhibitor during the preincubation step. Results shown in Table 4 show that when the assay was performed at acidic pH (pH 5.5, i.e. the optimal pH for cathepsins K and L), IC_{50} values were not significantly affected by the presence of GSH. However, at pH 7.0, compounds containing the 1-cyanoazetidinyl ring (i.e. compounds **20** and **21**) became completely inactive in the presence of GSH indicating that these four-membered ring cyanamides possess a higher reactivity than the five-membered ring cyanamides (i.e. compounds **1**, **9**, and **13**) toward the thiolate of the GSH. This increase in reactivity could in part explain the lower IC_{50} values observed with this class of compounds. Compounds containing a 1-cyanopyrrolidinyl ring were only slightly affected by the presence of GSH at either pH 5.5 or 7.0. In fact, compound **1** which has an IC_{50} value of 0.4 μM for cathepsin K is shifted by a factor of 30 in the presence of GSH at pH 7.0, while the IC_{50} value of compound **9** which is 10-fold more potent for cathepsin K than compound **1** is not significantly affected by GSH at pH 7.0. This observation suggests that the increase in potency obtained with compounds in the 1-cyanopyrrolidinyl series (Table 1) is probably not due to an increase in reactivity toward the thiolate anion of the enzyme but likely due to an increase in the affinity of the compound for the enzyme.

Table 4. Effect of GSH on the Inhibitory Activity of 1-Cyanopyrrolidinyl and 1-Cyanoazetidinyl Analogues at pH 5.5 and 7.0 toward Cathepsin K[a]

	no GSH		+10 mM GSH	
compd	pH 5.5	pH 7.0	pH 5.5	pH 7.0
1	0.34	0.28	0.36	11.4
9	0.06	0.05	0.09	0.15
13	1.63	2.3	1.5	3.4
20	0.016	0.038	0.027	>100
21	0.45	0.88	0.48	>100

[a] Compounds were preincubated 30 min with GSH prior to the addition of the enzyme.

NMR Experiment. Peptidic nitriles are known to be potent and reversible inhibitors of cysteine proteases of the papain family.[10] Unlike aldehydes, which are inhibitors of both serine and cysteine proteases, nitriles seem to be more specific for cysteine proteases.[10d] ^{13}C NMR studies with papain have demonstrated that peptidic nitriles form a reversible thioimidate ester adduct with papain.[10a,c,d] Because of the obvious similarity of the electrophile of the 1-cyanopyrrolidines and the peptidic nitriles, the ^{13}C analogue of compound **2** was synthesized and used to probe the formation of an isothiourea bond with the active site thiol of a cysteine protease as illustrated in Scheme 3. Although compound **2** is more potent for cathepsin K (Table 1), it is also relatively potent for the inhibition of papain with an IC_{50} value of 0.23 μM at pH 5.5. The results of the NMR studies are presented in Figure 2. Incubation of 0.5 equiv of glutathione and 1 equiv of compound **2** at pH 8.0 led to the formation of a new resonance peak that appeared at 161.7 ppm (Figure 2A). As previously described,[10d] this resonance peak is consistent with the

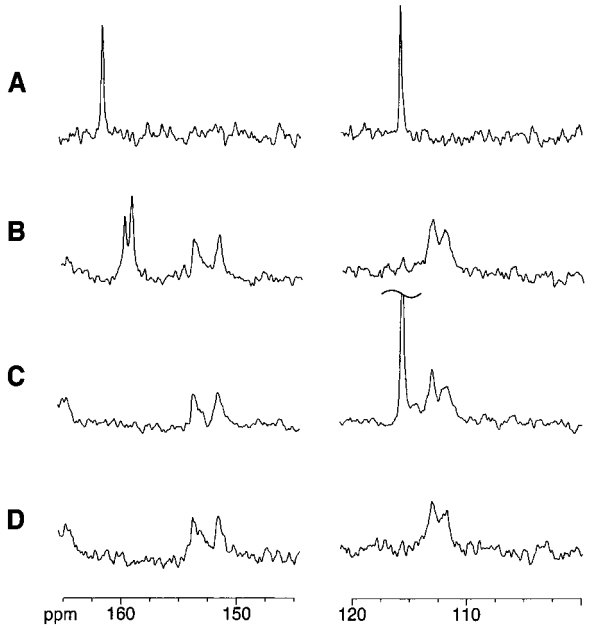
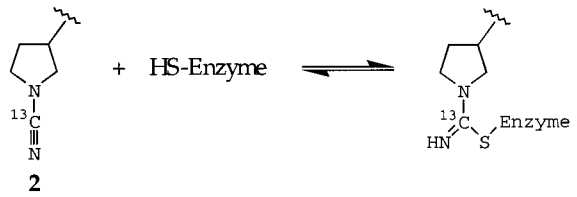


Figure 2. ^{13}C NMR spectrum of the papain−compound **2** complex: (A) NMR spectrum obtained when 0.5 equiv of GSH was incubated with 1 equiv of compound **2** (^{13}CN) at pH 8.0; (B) 1 equiv of compound **2** (^{13}CN) with 1 equiv of papain (2.0 mM); (C) 1 equiv of E64 (2.0 mM) was added to the mixture obtained in B; (D) 1 equiv of papain (2.0 mM) with no inhibitor present.

Scheme 3



formation of an isothiourea linkage since it falls between the range for a thioamide carbonyl (200−210 ppm) and an amide and peptide carbonyl (160−170 ppm). The chemical shift value was used as a reference when ^{13}C-**2** was incubated with papain. When 1.1 equiv of compound **2** was incubated with 1 equiv of papain, we observed the formation of two resonance peaks at 159.3 and 159.9 ppm and the disappearance of the nitrile peak at 115.7 ppm (Figure 2B). The existence of two resonance peaks for the isothiourea ester is most probably due to rotational isomers (i.e. restricted rotation about the isothiourea ester bond). Further addition of the irreversible inhibitor E64 to the EI complex totally abolished the resonance peaks observed at 159.3 and 159.9 ppm while the nitrile peak at 115.7 ppm reappeared (C, Figure 2). Consistent with the kinetic results, this observation demonstrates the reversibility of the covalent enzyme−inhibitor isothiourea ester bond.

Pharmacokinetic Profile of the Cyanamide 2. At neutral pH, 1-cyanopyrrolidinyl compounds might form stable isothiourea ester bonds with small thiol-containing molecules such as GSH or protein thiols. To determine if the reactivity of the cyanamide toward thiols could prevent this class of compounds from possessing a suitable pharmacokinetic profile, in vivo dosing studies were performed with compound **2**. The results of this study are presented in Figure 3. When compound **2** was

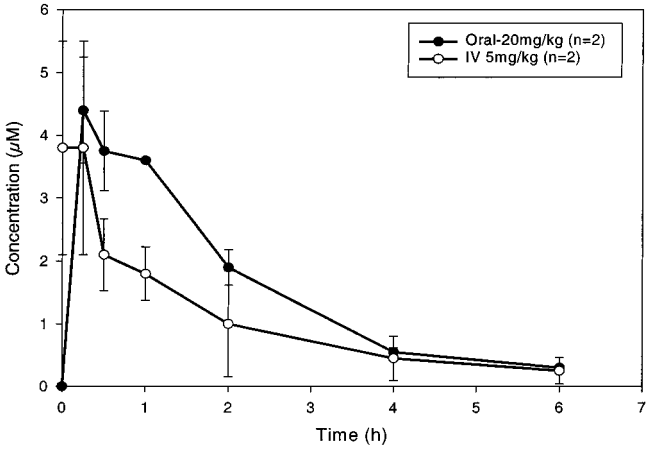


Figure 3. Mean plasma levels after intravenous administration of compound **2** in PEG-200/water (3:2) and after oral administration of **2** as a 1% methocel suspension in rats. Dosing volumes for intravenous and oral administration were 1 and 10 mL/kg, respectively.

administered orally in rats at a dose of 20 mg/kg in a 1% methocel suspension, the compound was well-absorbed with a bioavailability of 38%. The half-life of compound **2** was determined after intravenous dosing in PEG-200/water (3:2) and was found to be 2.0 h in rats. These results clearly demonstrate that compound **2** possesses good pharmacokinetic properties in rats and could be eventually used for in vivo studies.

Conclusion

In the present report we have shown that cathepsins K and L can be inhibited by compounds containing a 1-cyanopyrrolidine or 1-cyanoazetidine moiety. These cyanamide-based compounds represent a new class of nonpeptidic cysteine protease inhibitors. They inhibit cathepsins K and L in a time-dependent manner and form a reversible isothiourea ester link with the active site cysteine of the enzyme. The SARs with 1-cyanopyrrolidines showed that substitution at the 3-position is required to increase potency within this class of inhibitors. Addition of a benzenesulfonamide in the 3-position resulted in compound **2**, a potent inhibitor of cathepsins K and L which was also active in an in vitro model of bone resorption. Moreover, this inhibitor proved to have good pharmacokinetic properties in rats and thus would be suitable for animal studies where the roles of cathepsins K and L could be studied.

Experimental Section

General. Proton (^1H NMR) magnetic resonance spectra were recorded on a Bruker AM instrument operating at 300, 400 or 500 MHz. All spectra were recorded using residual solvent ($CHCl_3$ or acetone) as internal standard. Signal multiplicity was designated according to the following abbreviations: s = singlet, d = doublet, dd = doublet of doublets, t = triplet, m = multiplet, br s = broad singlet, br t = broad triplet. A Sciex API-100 mass spectrometer operating in positive ion APCI was used to obtain the $[M + H^+]^+$ ion of the target molecules. High-resolution mass spectrometry was carried out on a VG ZAB 2F mass spectrometer using positive ion FAB at 10000 resolution. Reactions were carried out with continuous stirring under a positive pressure of nitrogen except where noted. Flash chromatography was carried out with silica gel 60, 230−400 mesh. Compound **3** was prepared as previously described.[11] BrCN was prepared as described below. The 1-cyanoazetidine **21** was prepared by reacting the commer-

cially available azetidine with BrCN. Cyanamides **13** and **14** were purchased from Aldrich.

Cyanogen Bromide (BrCN). To a cold (−5 °C), stirred suspension of bromine (5.5 mL, 1 equiv) in H_2O (15 mL) was added, dropwise over 30 min, a solution of sodium cyanide (5.0 g, 1 equiv) in H_2O (15 mL). The temperature of the reaction mixture during the addition of sodium cyanide was maintained at −5 to 5 °C. The resultant suspension was stirred an additional 10 min and was then extracted with CH_2Cl_2 (3 × 33.3 mL). The resultant solution of BrCN (1 M in CH_2Cl_2) was stored over $CaCl_2$ at 4 °C.

General Procedure 1. To a stirred solution of the Boc-protected amine (1 equiv) in CH_2Cl_2 (∼1 mL/mmol of the amine) was added TFA (16 equiv). The solution was stirred at room temperature for 0.5 h and the CH_2Cl_2/TFA solvent was removed under reduced pressure. The residue was diluted with CH_2Cl_2 and the solvent was removed once again under reduced pressure (repeated 2×). The resultant amine was dissolved in CH_2Cl_2 (∼5 mL/mmol of amine) and cooled to 0 °C. To this cold solution was added Et_3N (1.5 equiv) followed by a solution of BrCN in CH_2Cl_2 (1 M, 1.1−5 equiv). The resultant reaction mixture was stirred at 0 °C for 1 h. The solution was warmed to room temperature, diluted with EtOAc and washed with H_2O and brine. The organic extract was dried ($MgSO_4$), concentrated under reduced pressure and the resultant residue was purified by flash chromatography to afford the desired cyanamide.

***N*-[(1-Cyano-3-pyrrolidinyl)methyl]benzenesulfonamide (5).** To a refluxing suspension of 1-*tert*-butyl 3-methyl 1,3-pyrrolidinedicarboxylate (**3**)[11] (2.29 g, 1 equiv) and $NaBH_4$ (757 mg, 2 equiv) in THF (10 mL) was added, dropwise, MeOH (2 mL). After the addition of MeOH, the resulting mixture was refluxed for 1 h. The mixture was then poured into 10% citric acid and extracted with EtOAc (3×). The combined organic extracts were washed with H_2O and brine, dried ($MgSO_4$) and concentrated under reduced pressure. The resultant oil was purified using flash chromatography (gradient elution: 50% EtOAc in hexane to 70% EtOAc in hexane) to afford the desired primary alcohol (1.54 g, 77% yield). To a cold (0 °C), stirred solution of this alcohol (250 mg, 1 equiv) in THF (25 mL) was added Et_3N (346 μL, 2 equiv) followed by MsCl (144 μL, 1.5 equiv). The resultant suspension was stirred at room temperature for 40 min. The suspension was then diluted with EtOAc and the mixture was washed with 10% citric acid, H_2O, saturated aqueous $NaHCO_3$ and brine. The combined aqueous washings were back extracted with EtOAc (3×) and the combined organic extracts were dried over $MgSO_4$ and concentrated under reduced pressure to afford the desired mesylate which was used immediately in the next reaction. This crude oil (mesylate) was dissolved in DMSO (3 mL) and NaN_3 (173 mg, 2 equiv) was added. The resultant reaction mixture was stirred at 100 °C for 5 h. The reaction mixture was then poured into H_2O and extracted with Et_2O (4×). The combined organic extracts were washed with H_2O and brine, dried over $MgSO_4$ and concentrated under reduced pressure to afford the crude azide (295 mg, 98% yield). To a stirred solution of this crude azide (295 mg, 1 equiv) in MeOH/$CHCl_3$ (2 mL each) was added 10% Pd on carbon (30 mg, 10%). The solution was evacuated, placed under a H_2 atmosphere (1 atm) and stirred at room temperature for 16 h. The suspension was then filtered through Celite and washed with MeOH. The filtrate was concentrated under reduced pressure to afford the desired primary amine (∼300 mg, 115% yield). To a stirred solution of this amine (109 mg, 1 equiv) and Et_3N (130 μL, 1.7 equiv) in CH_2Cl_2 (7 mL) was added benzenesulfonyl chloride (97 μL, 1.4 equiv). The resultant mixture was stirred at room temperature for 16 h. The mixture was then diluted with EtOAc and washed successively with 10% citric acid, H_2O and brine. The organic extract was dried ($MgSO_4$) and concentrated under reduced pressure. The residue was purified by flash chromatography (50% EtOAc in hexane) to afford the desired coupled product (123 mg, 66% yield). The N-Boc group of the coupled product was then converted to the cyanamide (N-CN) following general procedure 1 (i.e. successive treatment with TFA and BrCN). The crude material was purified by flash chromatography (50% EtOAc in hexane) to afford the desired cyanamide **5** (23 mg, 12% yield). ^{1}H NMR (400 MHz, acetone-d_6): δ 7.88 (m, 2H), 7.62 (m, 3H), 6.68 (br s, 1H), 3.38 (m, 3H), 3.12 (dd, 1H), 2.97 (t, 2H), 2.46 (m, 1H), 2.02 (m, 1H), 1.70 (m, 1H). *m*/*z* (+APCI): 266.0 $[M + H^+]^+$. HRMS (+FAB): calcd for $C_{13}H_{17}N_2O$ $[MH^+]$ 217.13411, found 217.13409. Anal. ($C_{13}H_{16}N_2O$) H, N; C: calcd, 72.19; found, 71.59.

3-[(Benzyloxy)methyl]-1-cyanopyrrolidine (6). To a refluxing suspension of 1-*tert*-butyl 3-methyl 1,3-pyrrolidinedicarboxylate (**3**)[11] (2.29 g, 1 equiv) and $NaBH_4$ (757 mg, 2 equiv) in THF (10 mL) was added, dropwise, MeOH (2 mL). After the addition of MeOH, the resulting mixture was refluxed for 1 h. The mixture was then poured into 10% citric acid and extracted with EtOAc (3×). The combined organic extracts were washed with H_2O and brine, dried ($MgSO_4$) and concentrated under reduced pressure. The resultant oil was purified using flash chromatography (gradient elution: 50% EtOAc in hexane to 70% EtOAc in hexane) to afford the desired primary alcohol (1.54 g, 77% yield). To a cold (0 °C), stirred solution of this alcohol (188 mg, 1 equiv) in DMF (9 mL) was added NaH (60% in oil, 56 mg, 1.5 equiv). The suspension was stirred at 0 °C for 40 min followed by the addition of the benzyl chloride (215 μL, 2 equiv). The suspension was warmed to room temperature and stirred for 16 h. MeOH was added followed by saturated aqueous NH_4Cl. The aqueous phase was extracted with EtOAc (3×) and the combined organic extracts were washed with brine, dried ($MgSO_4$) and concentrated under reduced pressure. The residue was purified by flash chromatography (20% EtOAc in hexane) to afford the desired benzyl ether (228 mg, 84% yield). The N-Boc group of this benzyl ether was then converted to the cyanamide (N-CN) following general procedure 1 (i.e. successive treatment with TFA and BrCN). The crude material was purified by flash chromatography (gradient elution: 30% EtOAc in hexane to 50% EtOAc in hexane) to afford the desired cyanamide **6** (165 mg, 98% yield). ^{1}H NMR (400 MHz, acetone-d_6): δ 7.30 (m, 5H), 4.53 (s, 2H), 3.43 (m, 5H), 3.18 (dd, 1H), 2.59 (m, 1H), 2.03 (m, 1H), 1.74 (m, 1H). *m*/*z* (+APCI): 217.1 $[M + H^+]^+$. HRMS (+FAB): calcd for $C_{12}H_{16}N_3O_2S$ $[MH^+]$ 266.09622, found 266.09632. Anal. ($C_{12}H_{15}N_2O_2S$) C, H, N.

***tert*-Butyl 3-{[(Benzyloxy)carbonyl]amino}-1-pyrrolidinecarboxylate (10).** To a solution of 1-*tert*-butyl 3-methyl 1,3-pyrrolidinedicarboxylate (**3**)[11] (2.29 g, 1 equiv) in MeOH (24 mL) was added 1 N NaOH (12 mL, 1.2 equiv). The mixture was stirred at room temperature for 3 days and then concentrated under reduced pressure. The residue was poured into a solution of 1 N HCl (10 mL) and 10% citric acid (20 mL). The mixture was extracted with EtOAc (3×) and the combined organic extracts were washed with H_2O and brine, dried ($MgSO_4$) and concentrated under reduced pressure to yield the corresponding acid (1.83 g, 85% yield). To a stirred solution of the acid (1.505 g, 1 equiv) and Et_3N (1.17 mL, 1.2 equiv) in toluene (20 mL) was added DPPA (1.66 mL, 1.1 equiv). After stirring at room temperature for 10 min, the mixture was refluxed for 1 h. To this refluxing mixture was added benzyl alcohol (1.45 mL, 2 equiv) and the refluxing was continued for 14 h. The resultant mixture was poured into 1 N aqueous NaOH and extracted with Et_2O (3×). The combined organic extracts were washed successively with H_2O, 10% citric acid, H_2O and brine. The organic extracts were dried ($MgSO_4$), concentrated under reduced pressure and the residue was purified by flash chromatography (gradient elution: 20% EtOAc in hexane to 67% EtOAc in hexane) to afford the benzyl carbamate **10** (2.06 g, 92% yield). ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.36 (m, 5H), 5.10 (s, 2H), 4.81 (m, 1H), 4.25 (m, 1H), 3.60 (dd, 1H), 3.42 (m, 2H), 3.19 (m, 1H), 2.15 (m, 1H), 1.83 (m, 1H), 1.45 (s, 9 H).

***N*-(1-Cyano-3-pyrrolidinyl)benzenesulfonamide (2).** To a stirred solution of the benzyl carbamate **10** (1.95 g, 1 equiv) in MeOH (20 mL) was added 10% palladium on carbon (200 mg, 10%). The solution was evacuated, placed under a H_2 atmosphere (35 atm) and shaken for 5 h. The suspension was then filtered through Celite and washed with MeOH. The

filtrate was concentrated under reduced pressure to afford the desired amine (1.358 g, 120% yield). To a stirred solution of this amine (100 mg, 1 equiv) and Et_3N (120 μL, 1.6 equiv) in CH_2Cl_2 (5 mL) was added benzenesulfonyl chloride (82 μL, 1.2 equiv). The resultant mixture was stirred at room temperature for 16 h. The mixture was then diluted with EtOAc and washed successively with 10% citric acid, H_2O and brine. The organic extract was dried ($MgSO_4$) and concentrated under reduced pressure. The residue was purified by flash chromatography (gradient elution: 10% EtOAc in hexane to 67% EtOAc in hexane) to afford the desired coupled product (135 mg, 77% yield). The N-Boc group of the coupled product was then converted to the cyanamide (N-CN) following general procedure 1 (i.e. successive treatment with TFA and BrCN). The crude material was purified by flash chromatography (gradient elution: 33% EtOAc in hexane to 70% EtOAc in hexane) to afford the desired cyanamide **2** (94 mg, 90% yield). ^{1}H NMR (500 MHz, $CDCl_3$): δ 7.78 (d, 2H), 7.53 (m, 1H), 7.43 (m, 2H), 4.90 (d, 1H), 3.79 (m, 1H), 3.32 (m, 3H), 3.03 (m, 1H), 1.95 (m, 1H), 1.73 (m, 1H). *m/z* (+APCI): 251.9 $[M + H^+]^+$. HRMS (+FAB): calcd for $C_{11}H_{14}N_3O_2S$ $[MH^+]$ 252.08068, found 252.08067. Anal. ($C_{11}H_{13}N_3O_2S$) H; C: calcd, 51.72; found, 52.57; N: calcd, 16.29; found, 16.72.

***N*-(1-Cyano-3-pyrrolidinyl)benzamide (7).** To a stirred solution of the benzyl carbamate **10** (1.95 g, 1 equiv) in MeOH (20 mL) was added palladium on carbon (200 mg, 10%). The solution was evacuated, placed under a H_2 atmosphere (35 atm) and shaken for 5 h. The suspension was then filtered through Celite and washed with MeOH. The filtrate was concentrated under reduced pressure to afford the desired amine (1.358 g, 120% yield). To a stirred solution of this amine (105 mg, 1 equiv) and Et_3N (160 μL, 2 equiv) in CH_2Cl_2 (5 mL) was added benzoyl bromide (90 μL, 1.3 equiv). The resultant mixture was stirred at room temperature for 10 min. The mixture was concentrated under reduced pressure and the residue was purified by flash chromatography (50% EtOAc in hexane) to afford the desired coupled product (134 mg, 83% yield). The N-Boc group of the coupled product was then converted to the cyanamide (N-CN) following general procedure 1 (i.e. successive treatment with TFA and BrCN). The crude material was purified by flash chromatography (gradient elution: 50% EtOAc in hexane to 67% EtOAc in hexane) to afford the desired cyanamide **7** (69 mg, 70% yield). ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.76 (d, 2H), 7.47 (m, 3H), 6.23 (m, 1H), 4.70 (m, 1H), 3.75 (dd, 1H), 3.57 (m, 2H), 3.39 (dd, 1H), 2.30 (m, 1H), 2.03 (m, 1H). *m/z* (+APCI): 216.1 $[M + H^+]^+$. HRMS (+FAB): calcd for $C_{12}H_{14}N_3O$ $[MH^+]$ 216.11358, found 216.11369. Anal. ($C_{12}H_{13}N_3O$) H; C: calcd, 66.96; found, 66.42; N: calcd, 19.52; found, 18.87.

***N*-(1-Cyano-3-pyrrolidinyl)[1,1′-biphenyl]-4-carboxamide (8).** To a stirred solution of the benzyl carbamate **10** (1.95 g, 1 equiv) in MeOH (20 mL) was added palladium on carbon (200 mg, 10%). The solution was evacuated, placed under a H_2 atmosphere (35 atm) and shaken for 5 h. The suspension was then filtered through Celite and washed with MeOH. The filtrate was concentrated under reduced pressure to afford the desired amine (1.358 g, 120% yield). To a stirred solution of this amine (100 mg, 1 equiv) and Et_3N (110 μL, 1.5 equiv) in CH_2Cl_2 (5 mL) was added 4-biphenylcarbonyl chloride (152 mg, 1.3 equiv). The resultant mixture was stirred at room temperature for 10 min. The mixture was concentrated under reduced pressure and the residue was purified by flash chromatography (50% EtOAc in hexane) to afford the desired coupled product (106 mg, 54% yield). The N-Boc group of the coupled product was then converted to the cyanamide (N-CN) following general procedure 1 (i.e. successive treatment with TFA and BrCN). The crude material was purified by flash chromatography (gradient elution: 50% EtOAc in hexane to 75% EtOAc in hexane) to afford the desired cyanamide **8** (73 mg, 87% yield). ^{1}H NMR (500 MHz, $CDCl_3$): δ 7.75 (d, 2H), 7.55 (m, 4H), 7.34 (m, 3H), 6.44 (d, 1H), 4.61 (m, 1H), 3.68 (m, 1H), 3.50 (m, 2H), 3.33 (m, 1H), 2.19 (m, 1H), 1.96 (m, 1H). HRMS (+FAB): calcd for $C_{18}H_{18}N_3O$ $[MH^+]$ 292.14505, found 292.14499. Anal. ($C_{18}H_{17}N_3O$) C, H, N.

Benzyl 1-Cyano-3-pyrrolidinylcarbamate (9). To a stirred solution of the benzyl carbamate **10** (100 mg, 1 equiv) in CH_2Cl_2 (1 mL) was added TFA (1 mL). The solution was stirred at room temperature for 0.5 h and the CH_2Cl_2/TFA solvent was removed under reduced pressure. The residue was diluted with CH_2Cl_2 and the solvent was removed once again under reduced pressure (repeated 2×). The resultant amine was dissolved in CH_2Cl_2 (2 mL) and cooled to 0 °C. To this cold solution was added Et_3N (0.22 mL, 5 equiv) followed by a solution of BrCN in CH_2Cl_2 (1M, 0.37 mL, 1.2 equiv). The resultant reaction mixture was stirred at 0 °C for 1 h. The solution was warmed to room temperature, diluted with EtOAc and washed with H_2O and brine. The organic extract was dried ($MgSO_4$), concentrated under reduced pressure and the resultant residue was purified by flash chromatography (50% EtOAc in hexane) to afford the desired cyanamide **9** (51 mg, 66% yield). ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.35 (m, 5H), 5.10 (s, 2H), 4.82 (m, 1H), 4.29 (m, 1H), 3.62 (dd, 1H), 3.50 (m, 2H), 3.28 (dd, 1H), 2.19 (m, 1H), 1.90 (m, 1H). *m/z* (+APCI): 246.0 $[M + H^+]^+$. HRMS (+FAB): calcd for $C_{13}H_{16}N_3O_2$ $[MH^+]$ 246.12419, found 246.12425. Anal. ($C_{13}H_{15}N_3O_2$) C, H, N.

***N*-[(1-Cyano-2-pyrrolidinyl)methyl]benzenesulfonamide (11).** To a stirred solution of *tert*-butyl 2-(aminomethyl)-1-pyrrolidinecarboxylate (**4**, commercially available) (200 mg, 1 equiv) and Et_3N (0.21 mL, 1.5 equiv) in CH_2Cl_2 (5 mL) was added benzenesulfonyl chloride (0.14 mL, 1.1 equiv). The resultant mixture was stirred at room temperature for 30 min. The mixture was then diluted with EtOAc and washed successively with 10% citric acid, H_2O and brine. The organic extract was dried ($MgSO_4$) and concentrated under reduced pressure. The residue was purified by flash chromatography (gradient elution: 10% EtOAc in hexane to 33% EtOAc in hexane) to afford the desired coupled product (350 mg, 103% yield). The N-Boc group of the coupled product was then converted to the cyanamide (N-CN) following general procedure 1 (i.e. successive treatment with TFA and BrCN). The crude material was purified by flash chromatography (gradient elution: 50% EtOAc in hexane to 67% EtOAc in hexane) to afford the desired cyanamide **11** (190 mg, 72% yield). ^{1}H NMR (500 MHz, $CDCl_3$): δ 7.76 (d, 2H), 7.48 (m, 3H), 4.70 (br t, 1H), 3.58 (m, 1H), 3.30 (m, 2H), 3.02 (m, 2H), 1.82 (m, 4H). HRMS (+FAB): calcd for $C_{12}H_{16}N_3O_2S$ $[MH^+]$ 266.09622, found 266.09632. Anal. ($C_{12}H_{15}N_3O_2S$) C, H, N.

***N*-[(1-Cyano-2-pyrrolidinyl)methyl]benzamide (12).** To a stirred solution of *tert*-butyl 2-(aminomethyl)-1-pyrrolidinecarboxylate (**4**, commercially available) (200 mg, 1 equiv) and Et_3N (0.21 mL, 1.5 equiv) in CH_2Cl_2 (5 mL) was added benzoyl bromide (0.13 mL, 1.1 equiv). The resultant mixture was stirred at room temperature for 30 min. The mixture was then diluted with EtOAc and washed successively with 10% citric acid, H_2O and brine. The organic extract was dried ($MgSO_4$) and concentrated under reduced pressure. The residue was purified by flash chromatography (gradient elution: 10% EtOAc in hexane to 33% EtOAc in hexane) to afford the desired coupled product (301 mg, 99% yield). The N-Boc group of the coupled product was then converted to the cyanamide (N-CN) following general procedure 1 (i.e. successive treatment with TFA and BrCN). The crude material was purified by flash chromatography (gradient elution: 50% EtOAc in hexane to 67% EtOAc in hexane) to afford the desired cyanamide **12** (173 mg, 76% yield). ^{1}H NMR (500 MHz, $CDCl_3$): δ 7.67 (d, 2H), 7.35 (m, 3H), 6.45 (br s, 1H), 3.72 (m, 2H), 3.37 (m, 3H), 1.96 (m, 1H), 1.70 (m, 2H), 1.60 (m, 1H). HRMS (+FAB): calcd for $C_{13}H_{16}N_3O$ $[MH^+]$ 230.12935, found 230.12934.

***tert*-Butyl 3-Hydroxy-1-azetidinecarboxylate (15).** To a stirred solution of 1-benzhydryl-3-azetidinol[12] (10.6 g, 1 equiv) in EtOH/H_2O (100 and 20 mL, respectively) was added 5% Pd on carbon (1 g, 10%). The solution was evacuated, placed under a H_2 atmosphere (60 atm) and stirred at room temperature for 16 h. The suspension was then filtered through Celite and washed with MeOH. The filtrate was concentrated under reduced pressure to afford the desired amine. To a cold (0 °C), stirred solution of the amine (2 g, 1 equiv) in EtOH (35 mL) was added Et_3N (7.5 mL, 2 equiv) followed by Boc_2O (6.47 g,

1.1 equiv). The resultant solution was stirred at room temperature for 30 min and then concentrated under reduced pressure. The residue was diluted with EtOAc, washed with 10% citric acid, H_2O and brine. The organic extract was dried over $MgSO_4$ and concentrated under reduced pressure to afford an oil which was purified by flash chromatography (gradient elution: 67% EtOAc in hexane to 100% EtOAc) to yield the Boc-protected azetidine **15** (2.71 g, 58% yield).

3-(Benzyloxy)-1-cyanoazetidine (16). To a cold (0 °C), stirred solution of *tert*-butyl 3-hydroxy-1-azetidinecarboxylate (**15**) (173 mg, 1 equiv) in DMF (2 mL) was added NaH (60% in oil, 60 mg, 1.5 equiv). The suspension was stirred at 0 °C for 30 min followed by the addition of the benzyl chloride (230 μL, 2 equiv). The resulting suspension was poured into H_2O and extracted with Et_2O (3 ×). The combined organic extracts were washed with H_2O and brine, dried ($MgSO_4$) and concentrated under reduced pressure. The residue was purified by flash chromatography (gradient elution: 10% EtOAc in hexane to 25% EtOAc in hexane) to afford the desired benzyl ether (273 mg, 104% yield). The N-Boc group of the benzyl ether was then converted to the cyanamide (N-CN) following general procedure 1 (i.e. successive treatment with TFA and BrCN). The crude material was purified by flash chromatography (gradient elution: 10% EtOAc in hexane to 33% EtOAc in hexane) to afford the desired cyanamide **16** (148 mg, 79% yield). ^{1}H NMR (500 MHz, $CDCl_3$): δ 7.22 (m, 5H), 4.34 (s, 2H), 4.25 (m, 1H), 4.10 (m, 2H), 3.97 (m, 2H). HRMS (+FAB): calcd for $C_{11}H_{13}N_2O$ [MH^+] 189.10276, found 189.10279. Anal. ($C_{11}H_{12}N_2O$) C, H, N.

1-Cyano-3-azetidinyl Cyclohexylmethyl Ether (17). To a cold (0 °C), stirred solution of *tert*-butyl 3-hydroxy-1-azetidinecarboxylate (**15**) (173 mg, 1 equiv) in DMF (2 mL) was added NaH (60% in oil, 60 mg, 1.5 equiv). The suspension was stirred at 0 °C for 30 min followed by the addition of the cyclohexylmethyl bromide (280 μL, 2 equiv). The resulting suspension was poured into H_2O and extracted with Et_2O (3×). The combined organic extracts were washed with H_2O and brine, dried ($MgSO_4$) and concentrated under reduced pressure. The residue was purified by flash chromatography (gradient elution: 5% EtOAc in hexane to 50% EtOAc in hexane) to afford the desired cyclohexylmethyl ether (215 mg, 80% yield). The N-Boc group of the cyclohexymethyl ether was then converted to the cyanamide (N-CN) following general procedure 1 (i.e. successive treatment with TFA and BrCN). The crude material was purified by flash chromatography (gradient elution: 10% EtOAc in hexane to 20% EtOAc in hexane) to afford the desired cyanamide **17** (82 mg, 53% yield). ^{1}H NMR (500 MHz, $CDCl_3$): δ 4.16 (m, 3H), 3.95 (m, 2H), 3.00 (m, 2H), 1.38−1.65 (m, 6H), 0.72−1.19 (m, 5H). *m/z* (+APCI): 195.2 [M + H^+]$^+$. HRMS (+FAB): calcd for $C_{11}H_{19}N_2O$ [MH^+] 195.14968, found 195.14974. Anal. ($C_{11}H_{18}N_2O$) N; C: calcd, 68.01; found, 67.25; H: calcd, 9.34; found, 8.60.

***tert*-Butyl 3-(Aminomethyl)-1-azetidinecarboxylate (22).** To a cold (0 °C), stirred solution of *tert*-butyl 3-hydroxy-1-azetidinecarboxylate (**15**) (1.23 g, 1 equiv) in EtOAc (10 mL) was added Et_3N (1.28 mL, 1.3 equiv) followed by MsCl (0.66 mL, 1.2 equiv). The resultant suspension was stirred at 0 °C for 1 h. The undissolved material (Et_3N·HCl) was filtered off and washed with EtOAc. The organic filtrate was concentrated under reduced pressure to afford an oil. This crude oil (mesylate) was dissolved in DMSO (5 mL) and NaCN (0.696 g, 2 equiv) was added. The resultant reaction mixture was stirred at 130 °C for 2 days. The reaction mixture was then poured into H_2O and extracted with Et_2O (2×). The combined organic extracts were washed with H_2O and brine, dried over $MgSO_4$ and concentrated under reduced pressure to afford a residue which was purified by flash chromatography (gradient elution: 25% EtOAc in hexane to 33% EtOAc in hexane) to yield the intermediate nitrile (735 mg, 57% yield). To a stirred solution of this nitrile (728 mg, 1 equiv) in MeOH (5 mL) was added NH_3 (2M in MeOH, 10 mL) and Raney nickel (1 mL). The solution was evacuated, placed under a H_2 atmosphere (40 atm) and stirred at room temperature for 3.5 h. The suspension was then filtered through Celite and washed with MeOH. The filtrate was concentrated under reduced pressure to afford the desired primary amine **22** (856 mg, 116% yield). ^{1}H NMR (500 MHz, $CDCl_3$): δ 3.90 (m, 2H), 3.50 (m, 2H), 2.80 (m, 2H), 2.43 (m, 1H), 1.32 (s, 9H).

***N*-[(1-Cyano-3-azetidinyl)methyl]benzamide (18).** To a stirred solution of the amine **22** (100 mg, 1 equiv) and Et_3N (0.11 mL, 1.5 equiv) in CH_2Cl_2 (5 mL) was added benzoyl bromide (0.07 mL, 1.1 equiv). The resultant mixture was stirred at room temperature for 10 min. The mixture was then diluted with EtOAc and washed successively with 10% citric acid, H_2O and brine. The organic extract was dried ($MgSO_4$) and concentrated under reduced pressure. The residue was purified by flash chromatography (gradient elution: 50% EtOAc in hexane to 70% EtOAc in hexane) to afford the desired coupled product (92 mg, 59% yield). The N-Boc group of the coupled product was then converted to the cyanamide (N-CN) following general procedure 1 (i.e. successive treatment with TFA and BrCN). The crude material was purified by flash chromatography (gradient elution: 50% EtOAc in hexane to 67% EtOAc in hexane) to afford the desired cyanamide **18** (21 mg, 30% yield). ^{1}H NMR (500 MHz, $CDCl_3$): δ 7.65 (m, 2H), 7.38 (m, 3H), 6.34 (br s, 1H), 4.17 (m, 2H), 3.84 (m, 2H), 3.59 (m, 2H), 2.93 (m, 1H). *m/z* (+APCI): 216.1 [M + H^+]$^+$. HRMS (+FAB): calcd for $C_{12}H_{14}N_3O$ [MH^+] 216.11369, found 216.11369. Anal. ($C_{12}H_{13}N_3O$) H; C: calcd, 66.96; found, 66.50; N: calcd, 19.52; found, 18.74.

***N*-[(1-Cyano-3-azetidinyl)methyl]benzenesulfonamide (19).** To a cold (0 °C), stirred solution of the amine **22** (100 mg, 1 equiv) and Et_3N (0.12 mL, 1.5 equiv) in CH_2Cl_2 (5 mL) was added benzenesulfonyl chloride (0.08 mL, 1.1 equiv). The resultant mixture was stirred at room temperature for 16 h. The mixture was then diluted with EtOAc and washed successively with 10% citric acid, H_2O and brine. The organic extract was dried ($MgSO_4$) and concentrated under reduced pressure. The residue was purified by flash chromatography (gradient elution: 30% EtOAc in hexane to 50% EtOAc in hexane) to afford the desired coupled product (121 mg, 69% yield). The N-Boc group of the coupled product was then converted to the cyanamide (N-CN) following general procedure 1 (i.e. successive treatment with TFA and BrCN). The crude material was purified by flash chromatography (gradient elution: 30% EtOAc in hexane to 67% EtOAc in hexane) to afford the desired cyanamide **19** (53 mg, 57% yield). ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.85 (m, 2H), 7.58 (m, 3H), 5.20 (br t, 1H), 4.16 (t, 2H), 3.81 (dd, 2H), 3.13 (t, 2H), 2.83 (m, 1H). *m/z* (+APCI): 251.9 [M + H^+]$^+$. HRMS (+FAB): calcd for $C_{11}H_{14}N_3O_2S$ [MH^+] 252.08068, found 252.080671.

***N*-[(1-Cyano-3-azetidinyl)methyl]cyclohexanecarboxamide (20).** To a cold (0 °C), stirred solution of the amine **22** (100 mg, 1 equiv) and Et_3N (0.15 mL, 2 equiv) in CH_2Cl_2 (5 mL) was added cyclohexanecarbonyl chloride (0.11 mL, 1.5 equiv). The resultant mixture was stirred at room temperature for 16 h. The mixture was then diluted with EtOAc and washed successively with 10% citric acid, H_2O and brine. The organic extract was dried ($MgSO_4$) and concentrated under reduced pressure. The residue was purified by flash chromatography (gradient elution: 50% EtOAc in hexane to 75% EtOAc in hexane) to afford the desired coupled product (96 mg, 60% yield). The N-Boc group of the coupled product was then converted to the cyanamide (N-CN) following general procedure 1 (i.e. successive treatment with TFA and BrCN). The crude material was purified by flash chromatography (gradient elution: 50% EtOAc in hexane to 85% EtOAc in hexane) to afford the desired cyanamide **20** (25 mg, 21% yield). ^{1}H NMR (500 MHz, $CDCl_3$): δ 5.5 (br s, 1H), 4.10 (m, 2H), 3.74 (m, 2H), 3.31 (m, 2H), 2.78 (m, 1H), 1.96 (m, 1H), 1.06−1.75 (m, 10 H). *m/z* (+APCI): 222.2 [M + H^+]$^+$. HRMS (+FAB): calcd for $C_{12}H_{20}N_3O$ [MH^+] 222.16064, found 222.16064.

Enzyme Inhibition. To measure enzyme activity, enzymatic assays were carried out in 50 mM MES pH 5.5 containing 2.5 mM DTT, 2.5 mM EDTA and 10% DMSO. IC_{50} values of compounds were determined for cathepsins K, L and

B using 25 μM of Z-Phe-Arg-pNA as substrate. Prior to the addition of substrate, different concentrations of the inhibitor ranging from 100 μM down to 2 nM were preincubated for 30 min with the enzyme (2−4 nM) to allow the establishment of the enzyme−inhibitor complex. Substrate was then added and the enzyme activity measured from the increase of OD at 405 nm. The final volume of the reaction was 300 μL. Assays were performed in 96-well plate format and the plates read using a Vmax (Molecular Devices) plate reader. The percent inhibition of the reaction was calculated from a control reaction containing only the compound vehicle. IC_{50} curves were generated by fitting percentage inhibition values to a four-parameter logistic model using a data analysis computer program (SOFTmax PRO, Molecular Devices). The inhibition of human cathepsin K gelatinase activity was measured using a fluorescent assay based on the release of quenched fluorescein from DQ gelatin (Molecular Probes). Assays were performed under the same conditions as for the colorimetric assay. The final concentration of DQ gelatin was 0.25 mg/mL and the reaction was measured using excitation and emission wavelengths of 495 and 515 nm, respectively.

Kinetic Analysis. The measurement of enzyme activity to determine kinetic parameters was performed in 50 mM MES pH 5.5 containing 2.5 mM DTT, 2.5 mM EDTA and 10% DMSO. Z-Phe-Arg-AMC was used as substrate and enzymatic activity was measured at room temperature in 1 mL stirred cells using a Quantamaster spectrofluorometer (Photon Technology International) with excitation and emission wavelengths of 355 nm of 460 nm, respectively. Product formation was measured at different inhibitor concentrations following initiation of the reaction by the addition of the enzyme (0.1 nM final concentration). The K_m value for Z-Phe-Arg-AMC was determined (30 μM) for cathepsin K, and inhibition assays were performed at a substrate concentration 10-fold below the K_m value. The formation of product (P) with time for an enzyme inhibited by a slow-binding inhibitor is described by the following equation:[13]

$$P = v_s t - (v_s - v_o)(1 - e^{-k_{obs}})/k_{obs} \quad (3)$$

where v_s is the rate of the reaction at steady-state, v_o is the initial velocity of the reaction, and k_{obs} is the apparent first-order rate constant characterizing the establishment of the steady-state velocity. Experimental values were fitted by nonlinear regression to eq 3 and fitted parameters v_s, v_o, and k_{obs} were used to estimate pre-steady-state kinetic parameters k_{on} and k_{off} (eq 1) and the dissociation constant K_i using the following relationships:[13]

$$K_i = [I]/((v_o/v_s) - 1) \quad (4)$$

$$k_{on} = k_{obs}/([I] + K_i) \quad (5)$$

$$k_{off} = k_{on}K_i \quad (6)$$

The value of K_i was calculated using eq 4 for each inhibitor concentration which gave a final steady-state velocity (v_s) of between 20% and 80% that of the initial velocity (v_o). The pre-steady-state constants k_{on} and k_{off} were calculated from the above values of k_{obs} and K_i using eqs 5 and 6. The values of K_i, k_{on}, and k_{off}, determined for at least three inhibitor concentrations, were averaged to give the values shown in Table 3.

Bone-Resorption Assay. The long bones were aseptically isolated from a 10-day-old New Zealand White hare (Covance Research Products, Denver, PA) and the soft tissue removed. The bones were minced into ~1-mm pieces with scissors in 10 mL of α-MEM (Gibco BRL, Gaithersburg, MD) containing penicillin/streptomycin, pH 7.1. The volume was brought to 25 mL and the tissue transferred to a 50-mL tube. The tube was rocked gently for 60 cycles, the tissue allowed to settle for 1 min and the supernatant withdrawn with a pipet and retained. 25 mL of medium was added back to the tissue and rocked again. The second supernatant was combined with the first. Cells were diluted 1:10 in 2% acetic acid in PBS, counted by hemacytometer and diluted to 5×10^6 cells/mL in α-MEM containing 2% FBS. 200-μL aliquots were plated onto 6-mm diameter × 200-μm bovine bone slices. After 2 h, test compounds were diluted in α-MEM containing 2% FBS, 10 nM $1,25(OH)_2D_3$, the plating medium removed and 200 μL of test media was added to triplicate wells. The cultures were incubated for 3 days at 37 °C in a 5% CO_2 atmosphere. The medium was then removed from the cultures and collagen fragments released into the medium measured by the CROSSLAPS elisa assay (Osteometer Biotech, Herlev, Denmark).

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