Synthesis, Calpain Inhibitory Activity, and Cytotoxicity of P_2-Substituted Proline and Thiaproline Peptidyl Aldehydes and Peptidyl α-Ketoamides

Rajani Korukonda,[†] Na Guan,[‡] James T. Dalton,[‡] Jiuyu Liu,[†] and Isaac O. Donkor[†,*]

Department of Pharmaceutical Sciences, The University of Tennessee Health Science Center, Memphis, Tennessee 38163 and Division of Pharmaceutics, The Ohio State University, Columbus, Ohio 43210

Received August 25, 2005

Calpain is a cytosolic cysteine endopeptidase that has been implicated in a number of disorders including cancer. We have synthesized and studied the μ-calpain inhibitory activity and cytotoxicity of peptidyl aldehydes and peptidyl α-ketoamides with N-substituted D-proline or L-thiaproline residues at the P_2-postion. The most potent and most selective members of the series were (*R*)-1-(4-nitrophenylsulfonyl)-*N*-((*R,S*)-1-oxo-3-phenylpropan-2-yl)pyrrolidine-2-carboxamide (**1j**) and (*R*)-1-(4-iodophenylsulfonyl)-*N*-((*R,S*)-1-oxo-3-phenylpropan-2-yl)pyrrolidine-2-carboxamide (**1n**). The compounds inhibited μ-calpain with K_i values of 0.02 μM and 0.03 μM, respectively, and displayed over 180-fold (**1j**) and 130-fold (**1n**) greater affinity for μ-calpain compared to cathepsin B. The cytotoxic effect of the compounds was evaluated in two leukemia cell lines (Daudi and Jurkat) and three solid tumor cell lines (DU-145, PC-3, and HeLa). Generally the compounds were modestly cytotoxic and displayed no correlation between the cytotoxic activity and μ-calpain inhibition.

Introduction

Calpains are unique among the cysteine protease family of enzymes because they require Ca^{2+} for activation.[1] Several calpain isoenzymes have been identified of which μ-calpain (calpain I) and m-calpain (calpain II) are the most widely distributed calpain isoenzymes in mammalian cells.[2] Calpains have been implicated in diverse range of cellular functions, including signal transduction, platelet activation, cell cycle progression, long-term potentiation, cell proliferation, membrane fusion, and apoptosis.[3−6] Calpain is considered an attractive therapeutic target because overactivation and/or improper regulation of the enzyme is associated with several diseases including neurological disorders and cancer.[3,7]

Calpain has been suggested as a potential anticancer target because of the cellular functions of some calpain substrates, including the c-Fos and c-Jun transcription factors,[8] the tumor suppressor protein p53,[9−11] the antiapoptotic protein Bcl-1, and the pro-apoptotic protein Bax.[12−16] All of these calpain substrates have been implicated in the pathogenesis of many human tumors, suggesting an important regulatory role of calpain in cancer. Furthermore, calpain has been shown to be involved in the regulation of cell proliferation and apoptosis in various types of cells.[4−7] Most data seem to indicate a level of collaboration between calpains and caspases in the induction of apoptosis.[10,17−19] However, other investigators have observed induction of apoptosis when cellular calpains were selectively inhibited.[20−23] For example, Zhu and Uckun[21] showed that calpain inhibitor II triggers rapid apoptosis in acute lymphoblastic leukemia (ALL) and non-Hodgkin's lymphoma (NHL) cells as well as some solid tumor cells. Witowski et al.[22] have also demonstrated that the activity and expression of μ-calpain is significantly higher in chronic lymphocytic leukemia (B-CLL) cells than in non-malignant cells. These observations implicate calpain as a new molecular target for the discovery of novel anticancer agents. We therefore synthesized and studied the cytotoxic activity of the compounds in Table 1. Tripathy et al.[24] demonstrated that calpain can accommodate D-proline and L-proline at the P_2-position of peptidyl aldehyde inhibitors. Following this report we studied the effect of incorporating ring-contracted and ring-expanded analogues of proline at the P_2-position on calpain inhibition.[25] In concert with the work of Tripathy et al.[24] compounds **1a** and **1b** (Table 1) with D-proline and L-thiaproline, respectively, at the P_2-position were the most potent calpain inhibitors of the series. In this report we present the synthesis, calpain inhibitory potency, and in vitro cytotoxic activity of a series of peptidyl aldehydes and peptidyl α-ketoamides related to compounds **1a** and **1b**. The compounds incorporate proline and thiaproline residues with various *N*-sulfonyl substituents at the P_2-position (Table 1).

Chemistry. The peptidyl aldehydes in Table 1 were synthesized following our previously reported procedure for the synthesis of compounds **1a** and **1b** and is summarized in Scheme 1.[25] The synthesis of the α-ketoamides (**2a**−**c**) commenced with the preparation of α-hydroxy-β-amino acid **6** as previously reported.[26,27] Compound **6** was Boc protected and coupled with phenylethylamine to give **8**, which was deprotected using TFA to give **9** (Scheme 2). Coupling of **9** with the appropriately sulfonylated D-proline afforded hydroxyl amide intermediates **11**−**13**, which were oxidized (Dess−Martin reagent) to give target compounds **2**. The target compounds were purified by flash chromatography over silical gel to give diastereomeric mixtures that are epimeric at the P_1 position. The diasteriomeric ratios of selected compounds that demonstrated potent calpain inhibition and/or significant cytotoxic activity were determined by HPLC using a C_{18} reversed phase column with 6.2% aqueous TFA and acetonitrile as the mobile phase (Table 2).

Results and Discussion

SAR Studies. The compounds were evaluated as inhibitors of μ-calpain from porcine erythrocytes and cathepsin B from human liver. The calpain assay was performed as previously reported[25] using two different concentrations (0.2 and 1.0 mM)

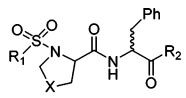
* Corresponding author: Isaac O. Donkor, Ph.D., Department of Pharmaceutical Sciences, Johnson Bldg. Room 327E, 847 Monroe Ave., Memphis, TN 38163. Phone: (901) 448-7736. Fax: (901) 448-6828. idonkor@utmem.edu

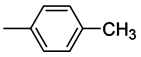
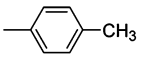
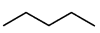
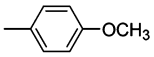
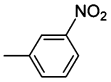
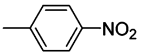
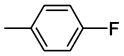
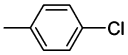
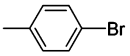
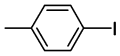
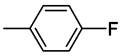
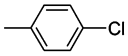
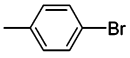
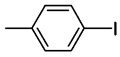
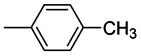
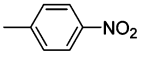
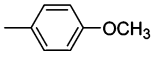
[†] University of Tennessee Health Science Center.

[‡] The Ohio State University.

10.1021/jm050849w CCC: $33.50
Published on Web 07/26/2006

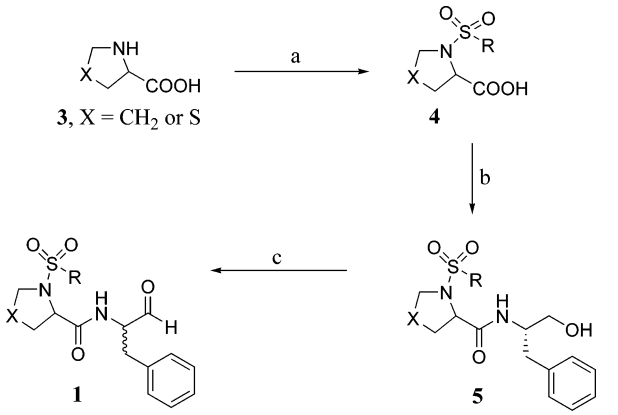
Table 1. μ-Calpain and Cathepsin B Inhibitory Activities of Peptidyl Aldehydes (**1a**−**r**) and α-Ketoamides (**2a**−**c**) Synthesized as Diastereomeric Mixtures[a]



#	P_2[b]	R_1	R_2	X	μ-Calpain[c] K_i(μM)	Cat. B[d] K_i(μM)	SR[e]
1a[f]	D		H	-CH_2-	0.081 ± 0.001	12.1 ± 0.74	151
1b[f]	L		H	-S-	0.07 ± 0.035	0.119 ± 0.01	1.70
1c	D		H	-CH_2-	0.313 ± 0.002	1.48 ± 0.018	4.72
1d	D		H	-CH_2-	0.836 ± 0.004	3.50 ± 0.07	1.19
1e	D		H	-CH_2-	0.191 ± 0.012	2.54 ± 0.05	13.0
1f	D		H	-CH_2-	0.196 ± 0.004	2.47 ± 0.07	13.0
1g	D		H	-CH_2-	0.037 ± 0.001	1.48 ± 0.02	40
1h	D		H	-CH_2-	0.424 ± 0.012	4.61 ± 0.09	10.9
1i	D		H	-CH_2-	0.256 ± 0.011	5.45 ± 0.11	21.3
1j	D		H	-CH_2-	0.020 ± 0.014	3.75 ± 0.09	188
1k	D		H	-CH_2-	0.386 ± 0.015	6.99 ± 0.17	18.1
1l	D		H	-CH_2-	0.141 ± 0.013	5.40 ± 0.12	38.2
1m	D		H	-CH_2-	0.128 ± 0.016	4.42 ± 0.08	34.5
1n	D		H	-CH_2-	0.033 ± 0.002	4.18 ± 0.14	131
1o	L		H	-S-	0.110 ± 0.016	0.34 ± 0.09	3.09
1p	L		H	-S-	0.090 ± 0.008	0.23 ± 0.01	2.50
1q	L		H	-S-	0.074 ± 0.011	0.09 ± 0.01	1.21
1r	L		H	-S-	0.042 ± 0.009	0.11 ± 0.02	2.61
2a	D		CONHEtPh	-CH_2-	0.850 ± 0.039	6.90 ± 0.17	8.12
2b	D		CONHEtPh	-CH_2-	0.280 ± 0.021	5.18 ± 0.13	18.1
2c	D		CONHEtPh	-CH_2-	0.450 ± 0.081	2.59 ± 0.11	5.70

[a] K_i values were determined by Dixon plots using the average of triplicate assays and plotting $1/v$ versus I to give intersecting lines with correlation coefficient ≥ 0.95. [b] P_2 = Configuration of the proline or thiaproline residue at the P_2-position of the compound. [c] Porcine erythrocyte μ-calpain (Calbiochem). [d] Cat. B = Human liver cathepsin B (Calbiochem). [e] SR = selectivity ratio, which was determined by dividing the K_i for cathepsin B inhibition by the K_i for μ-calpain inhibition. [f] The values for compounds **1a** and **1b** are from ref 25.

Scheme 1[a]



[a] Reagents: (a) aq NaOH/THF, R-SO_2Cl; (b) L-phenylalaninol hydrochloride, NMM, HOBT, EDC, CH_2Cl_2; (c) Dess−Martin reagent, CH_2Cl_2.

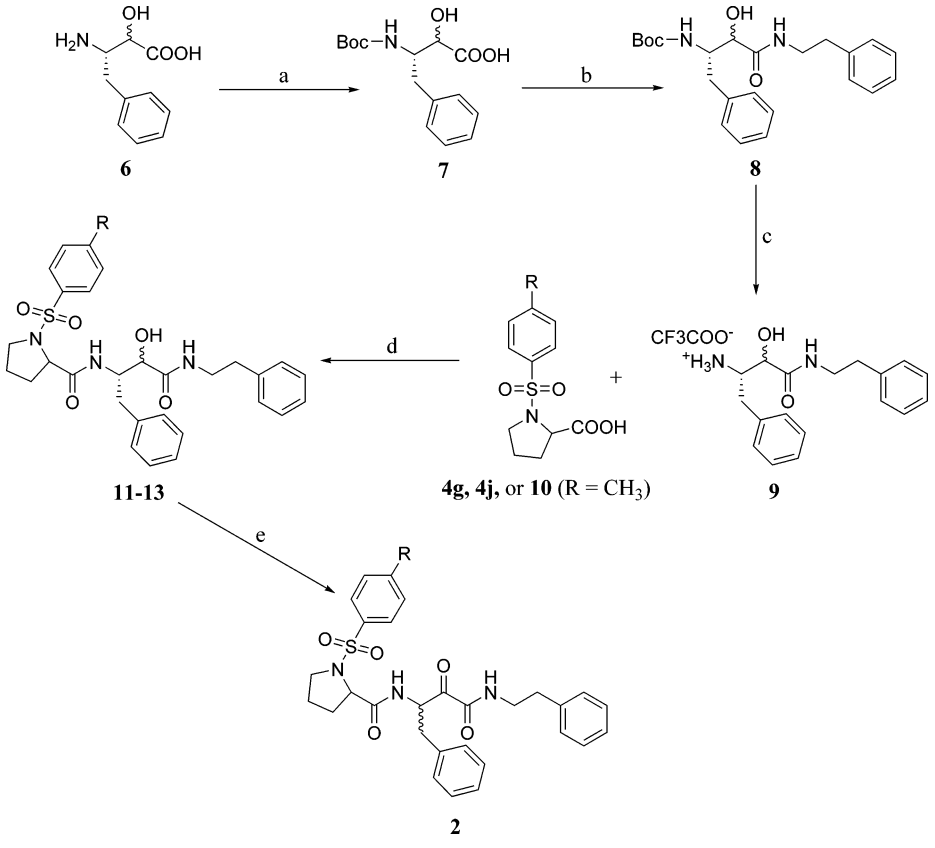
of Suc-Leu-Tyr-AMC as the fluorogenic substrate. The cathepsin B assay was performed in a similar manner using Z-Arg-Arg-AMC (50 μM and 250 μM) as the substrate. The compounds were tested against cathepsin B because several calpain inhibitors lack selectivity for calpain and are known to inhibit other cysteine proteases in particular the cathepsins.[28] The K_i values for inhibition of the enzymes were computed using Dixon plots.[29] Table 1 shows the results of the study. Compounds with *N*-arylsulfonyl substituents (**1e** and **1f**) were more potent than those with *N*-alkylsulfonyl groups (**1c** and **1d**). This is consistent with previous reports, which suggest that the S_3-subsite of calpain prefers bulky substitutes at the P_3-position of inhibitors.[30] Compound **1j**, with an electron-withdrawing *p*-nitro-*N*-phenylsulfonyl substituent was the most potent and the most selective calpain inhibitor of the series. It inhibited μ-calpain with a K_i value of 0.02 μM. The compound was over 180-fold selective for μ-calpain compared to cathepsin B. The position of the nitro group of the *N*-phenylsulfonyl substituent significantly affected the μ-calpain inhibitory potency of the compounds but not cathepsin B inhibition. Inhibition of μ-calpain followed the order *p*-nitro (**1j**) > *m*-nitro (**1i**) > *o*-nitro (**1h**). The μ-calpain inhibitory activity of **1g** and **1j** suggest that the difference in the electronic properties of the methoxy and nitro groups does not significantly impact μ-calpain inhibition. We also synthesized and studied the μ-calpain inhibitory activity of compounds **1k**−**n** with *p*-halo phenylsulfonyl substituents. In this series compound **1n** with K_i of 0.033 μM versus μ-calpain and 4.18 μM versus cathepsin B was the most potent and the most selective inhibitor of the halogenated derivatives. Generally, the inhibitory potency and selectivity of the halogenated compounds for calpain increased with an increase in the steric bulk of the halogen substituent. The halogenated thiaproline derivatives **1o**−**r** displayed a similar rank order of potency toward μ-calpain inhibition, which is consistent with previous reports that μ-calpain can accommodate proline and thiaproline equally well at P_2 position of inhibitors.[24, 25] However, the thiaproline derivatives were better inhibitors of cathepsin B compared to the proline

Table 2. Diastereomeric Ratios

compound	DR[a] DL:DD
1a	81:19[b]
1b	86:14[b]
1g	84:16
1j	88:12
1n	83:17
1o	87:13
1r	85:15

[a] DR is diastereomeric ratio determine by HPLC. [b] Values from ref 25.

Scheme 2[a]



[a] Reagents: (a) (*t*-ButOCO)$_2$, NaOH; (b) Ph$(CH_2)_2NH_2$, NMM, HOBT, EDC; (c) TFA; (d) NMM, HOBT, EDC; (e) Dess−Martin reagent, CH_2Cl_2.

Table 3. Cytotoxicity (GI_{50} in μM) of Selected Calpain Inhibitors against Leukemia and Solid Tumor Cell Lines[a]

no.	Daudi	Jurkat	DU-145	PC-3	HeLa	K_i (μM)[b]	CLogP[c]
1a	16.7 ± 1.85	17.6 ± 1.34	45.2 ± 3.10	40.1 ± 2.96	48.9 ± 3.05	0.081 ± 0.001	3.32
1b	2.17 ± 0.03	3.21 ± 0.08	6.00 ± 0.91	5.17 ± 0.79	4.37 ± 0.09	0.070 ± 0.035	3.47
1g	35.3 ± 2.71	25.4 ± 2.27	63.2 ± 3.92	19.7 ± 1.76	38.5 ± 2.13	0.037 ± 0.001	2.99
1j	32.5 ± 2.63	33.8 ± 2.51	42.4 ± 2.94	49.3 ± 3.01	40.4 ± 2.69	0.020 ± 0.014	2.57
1k	24.8 ± 2.12	24.2 ± 2.31	24.8 ± 1.98	27.1 ± 2.04	27.4 ± 2.55	0.386 ± 0.041	2.97
1o	3.54 ± 0.17	6.13 ± 0.96	6.22 ± 0.87	7.04 ± 0.92	7.12 ± 0.89	0.110 ± 0.001	3.11
1r	4.48 ± 0.06	5.97 ± 0.83	6.29 ± 0.75	6.38 ± 0.61	5.42 ± 0.47	0.042 ± 0.001	4.09
2a	19.6 ± 1.87	21.9 ± 1.65	22.6 ± 1.22	20.8 ± 1.09	22.9 ± 1.31	0.850 ± 0.035	5.23
2b	23.1 ± 2.10	21.3 ± 1.96	31.8 ± 2.03	27.8 ± 2.11	27.6 ± 2.25	0.280 ± 0.007	4.48
2c	22.4 ± 2.13	23.8 ± 2.17	30.0 ± 2.79	14.6 ± 1.52	31.4 ± 2.07	0.450 ± 0.003	4.90

[a] The cyctotoxicity of the compounds are reported as the concentration of the inhibitor required to inhibit growth of the cancer cell line by 50% (i.e., GI_{50}) in micromoles. The GI_{50} values were determined by nonlinear regression analysis using WinNonlin. [b] K_i values are those for μ-calpain inhibition and are the same as reported in Table 1. [c] CLog P is the calculated partition coefficients of the inhibitors and were determined with ChemDraw Ultra ver. 9.0.

derivatives and as such were not selective for μ-calpain. For example, compound **1n** with a P_2 proline residue was 130-fold selective for μ-calpain over cathepsin B while the corresponding thiaproline derivative **1r** was only about 3-fold selective for μ-calpain.

Peptidyl aldehydes are generally potent inhibitors of calpain but the compounds form hydrates and undergo tautomerization and rapid oxidation in vivo.[31,32] These factors limit their usefulness as tools for studying calpain function in vivo and as potential therapeutic agents. Unlike an aldehyde, the α-ketoamide functional group is stable to oxidation.[32,33] We therefore synthesized and studied the calpain inhibitory activity of peptidyl α-ketoamides **2a**−**c**. The calpain inhibitory activity of the compounds mirrored that of the aldehydes and displayed the following the rank order of potency: **2b** (with *p*-nitro) > **2c** (with *p*-methoxy) > **2a** (with *p*-methyl). The α-ketoamides were, however, about 10-fold less potent than the corresponding aldehydes.

Cytotoxicity Studies. Calpain inhibitors have been shown to prevent apoptosis.[17,34,35] In contrast to these reports Zhu et al.[23] demonstrated that calpain inhibitors such as calpain inhibitor I and Z-Leu-Leu-Tyr-diazomethyl ketone are cytotoxic to human prostate cell lines (LNCaP and PC-3). Other investigators[21,22] have also shown that calpain inhibitors trigger apoptosis in acute lymphoblastic leukemia (ALL) cells, non-Hodgkin's lymphoma (NHL) cells, and lymphocytic leukemia (B-CLL) cells. We therefore studied the relationship between μ-calpain inhibition and the antiproliferative activity (GI_{50}s) of our compounds in two leukemia cell lines (Jurkat and Daudi) and three solid tumor cell lines (DU-145, PC-3, and HeLa). Table 3 shows the results of the study. The compounds were more cytotoxic versus the leukemia cell lines compared to the solid tumor cell lines with GI_{50} values between 2.17 μM to 35.3 μM versus Daudi and Jurkat, and 4.37 μM to 63.2 μM versus the solid tumor cells. Compound **1b**, which was one of the most potent μ-calpain inhibitors of the series, was also the most effective cytotoxic agent versus all of the cell lines tested but overall no correlation was observed between calpain inhibition and cytotoxicity.

Compound **1j**, which was the most potent and the most selective μ-calpain inhibitor (K_i = 0.02 μM; SR = 188), was among the least effective antiproliferative agents (GI_{50} between 32.5 μM to 49.3 μM). We hypothesized that the poor cytotoxicity of **1j** despite its potent in vitro μ-calpain inhibitory activity compared to **1b** was due to limited cell penetration. To test this hypothesis we determined the abilities of the compounds to penetrate cells and inhibit intracellular calpain activity using a modification of the procedure reported by Chatterjee et al.[36] In this study Jurkat cells are treated with Ca^{2+} and Ionomycin (a calcium ionophore). This results in increased intracellular Ca^{2+} concentration, which in turn activates calpain. Activated calpain then degrades its cellular substrate protein, α-spectrin, and generates specific 145/150 KDa spectrin breakdown products (SBDP). Inhibition of formation of SBDP by a compound is used as a marker for the ability of the compound to enter cells and inhibit the proteolytic action of calpain.[37] In this assay **1b** (CLogP = 3.47) inhibited the production of SBDP by 82.4% while **1j** (CLogP = 2.57) effected only 22.8% inhibition of the production of SBDP at the same concentration, suggesting that poor cellular permeation may account for the poor cytotoxic activity of **1j** despite its potent in vitro μ-calpain inhibitory activity. The peptidyl α-ketoamides displayed similar cytotoxic effects as the corresponding peptidyl aldehydes despite the 10-fold greater μ-calpain inhibitory potency of the peptidyl aldehydes in the cell free assay. The difference in the cytotoxic effectiveness may be due in part to better cell penetration of the peptidyl α-ketoamides (due to greater lipophilicity) and/or better cellular stability compared to the peptidyl aldehydes.[31−33]

Conclusion

In this report we have described the synthesis, μ-calpain inhibitory potency, and cytotoxicity of a series of peptidyl aldehydes and peptidyl α-ketoamides incorporating D-proline and L-thiaproline residues with various *N*-sulfonyl substituents at the P_2-position of the inhibitors. The compounds displayed moderate cytotoxicity against the cell lines studied, but no correlation between calpain inhibition and cytotoxicity was observed. The peptidyl α-ketoamides were about 10-fold less potent but displayed similar SAR toward calpain inhibition and equivalent cytotoxic activity to their aldehyde counterparts.

Experimental Section

Chemistry. General Methods. All evaporations were carried out in vacuo with a rotary evaporator. Thin-layer chromatography (TLC) was performed on silica gel chromatogram plates purchased from Analtech, Inc. Spots were visualized by UV light (254 and 365 nm). Fischer silica gel S732−25 (100−400 mesh) was used for column chromatography. The amount (weight) of silica gel for column chromatography was in the range of 50−100 times the amount (weight) of the crude compounds being separated. Melting points (mp) were obtained utilizing a Fisher-Johns melting point apparatus and are uncorrected. Molecular masses were recorded on Bruker/Hewlett-Packard Esquire LC/MS. Proton nuclear magnetic resonance (^{1}H NMR) spectra were recorded on Bruker ARX 300 MHz and Varian Inova 500 MHz instruments. The chemical shift (δ) values are reported as parts per million (ppm) relative to tetramethylsilane as internal standard: s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet, br = broad singlet. All analytical samples were homogeneous on TLC in at least two different solvent systems. Analytical samples were dried in vacuo (0.2 mmHg) in a drying apparatus over P_2O_5. Elemental analyses

were performed by Atlantic Microlab, Inc., Norcross, GA. Elemental compositions of target compounds were within ±0.4% of the calculated values. Fractional moles of water found in analytical samples could not be removed even after 24 h of drying in vacuo and were confirmed, where possible, by their presence in the ^{1}H NMR spectrum. All reagents and solvents were purchased from Advanced ChemTech Inc., Bachem, Calbiochem, Fischer Scientific, and Sigma Aldrich and used without further purification.

General Procedure 1. Sulfonylation. Method A. D-Proline or L-thiaproline (1 equiv) was dissolved in a mixture of 10% K_2CO_3 (10 mL) and THF (10 mL). The appropriate phenylsulfonyl chloride (2 equiv) was added and stirred at 50 °C for 5 h followed by acidification with 3 N HCl, extraction with EtOAc (3 × 30 mL), drying (Na_2SO_4), and evaporation to afford the product.

Method B. D-Proline or L-thiaproline (1 equiv) was dissolved in a mixture of saturated NaOH/THF (50 mL) or saturated $NaHCO_3$/Et_2O (50 mL). *p*-Toluenesulfonyl chloride (1.5 equiv) was added and stirred overnight at RT, and then the mixture was acidified with 3 N HCl and extracted with Et_2O (3 × 30 mL). The organic extracts were dried (Na_2SO_4) and evaporated to give the product.

General Procedure 2. Coupling. A solution of the carboxylic acid (1 equiv) in CH_2Cl_2 was cooled in an ice-bath, EDC (1.05 equiv), HOBT (1.05 equiv), amine (1 equiv), and NMM (3 equiv) were added consecutively, and the mixture was stirred overnight at RT, H_2O (40 mL) was added, and the mixture was extracted with CH_2Cl_2 (3 × 30 mL). The combined organic extracts were washed with saturated $NaHCO_3$ solution (30 mL), 0.5 N HCl (30 mL), and H_2O (30 mL) successively and dried ($NaSO_4$). Evaporation of the solvent followed by column chromatographic purification over silica gel afforded the desired amide.

General Procedure 3. Dess−Martin Oxidation. Method A. Dess−Martin reagent (1.05 equiv) was added to a solution of the alcohol (1 equiv) in CH_2Cl_2 (20 mL), and the mixture was stirred at RT for 2 h. Sodium thiosulfate pentahydrate (14−28 equiv) in saturated $NaHCO_3$ solution was added and stirred for an additional 10 min, and the mixture was extracted with CH_2Cl_2 (3 × 30 mL). The combined extracts were washed with 0.5 N HCl (30 mL) and H_2O (30 mL) and dried (Na_2SO_4). The solvent was evaporated, and the crude product was purified by column chromatography over silica gel.

Method B. *t*-BuOH (1.05 equiv) was added to Dess−Martin reagent (1.05 equiv) in CH_2Cl_2. After 20 min, a solution of the alcohol (1 equiv) in CH_2Cl_2 (20 mL) was added and the mixture was stirred at RT for 2 h followed by workup as described in Method A.

Synthesis of Compounds 4c−r. Compounds **4c**−**e**, and **4g** were synthesized using sulfonylation Method A. Compounds **4f** and **4h**−**m** were synthesized using sulfonylation Method B with saturated NaOH/THF (50 mL) as the solvent. Compounds **4n**−**r** were synthesized using sulfonylation Method B with saturated $NaHCO_3$/Et_2O (50 mL) as the solvent.

(*R*)-1-(Butylsulfonyl)pyrrolidine-2-carboxylic Acid (4c). Compound **4c** was obtained as colorless viscous oil in 93.1% yield. $[\alpha]^{28}_D = +65.1°$ (*c* 1, $CHCl_3$). ^{1}H NMR ($CDCl_3$): δ 10.44 (br, 1H), 4.55 (m, 1H), 3.58 (m, 1H), 3.46 (m, 1H), 3.11 (m, 2H), 2.30 (m, 1H), 2.15 (m, 1H), 2.01 (m, 2H), 1.82 (m, 2H), 1.45 (m, 2H), 0.95 (t, 1H, J = 7.50 Hz).

(*R*)-1-(Isopropylsulfonyl)pyrrolidine-2-carboxylic Acid (4d). Compound **4d** was obtained as a white solid in 92.5% yield. mp 67−69 °C. $[\alpha]^{28}_D = +55.7°$ (*c* 1, $CHCl_3$). ^{1}H NMR ($CDCl_3$): δ 4.57 (m, 1H), 3.67 (m, 1H), 3.45 (m, 1H), 3.34 (m, 1H), 2.32 (m, 1H), 2.17 (m, 1H), 2.02 (m, 2H), 1.27 (d, J = 7.20 Hz, 6H).

(*R*)-1-(Naphthalen-1-ylsulfonyl)pyrrolidine-2-carboxylic Acid (4e). Compounds **4e** was obtained as a white solid in 96.2% yield. mp 111−113 °C. $[\alpha]^{21}_D = +61.5°$ (*c* 1, $CHCl_3$). ^{1}H NMR ($CDCl_3$): δ 10.78 (s, 1H), 8.47 (s, 1H), 7.92 (m, 6H), 4.41 (m, 1H), 3.56 (m, 1H), 3.36 (m, 1H), 2.05 (m, 1H), 1.98 (m, 2H), 1.79 (m, 1H).

(*R*)-1-(Phenylsulfonyl)pyrrolidine-2-carboxylic Acid (4f). Compound **4f** was obtained as a white solid in 90.4% yield. mp 81−83 °C. $[\alpha]^{24}_D = +102.1°$ (*c* 1, $CHCl_3$). ^{1}H NMR ($CDCl_3$): δ 8.01 (s, br. 1H), 7.90 (m, 2H), 7.58 (m, 3H), 4.32 (m, 1H), 3.54 (m, 1H), 3.31 (m, 1H), 2.01 (m, 3H), 1.99 (m, 1H).

(*R*)-1-(4-Methoxyphenylsulfonyl)pyrrolidine-2-carboxylic Acid (4g). Compound **4g** was obtained as yellow oil in 90.4% yield. $[\alpha]^{27}_D = +84.2°$ (*c* 1, $CHCl_3$). ^{1}H NMR ($CDCl_3$): δ 7.83 (m, 2H), 7.02 (m, 2H), 4.26 (m, 1H), 3.90 (s, 3H), 3.52 (m, 1H), 3.24 (m, 1H), 2.18 (m, 1H), 1.98 (m, 2H), 1.81 (m, 1H).

(*R*)-1-(2-Nitrophenylsulfonyl)pyrrolidine-2-carboxylic Acid (4h). Compound **4h** was obtained as a white solid in 77.5% yield. mp 83−84 °C. $[\alpha]^{28}_D = +111.5°$ (*c* 1, $CHCl_3$). ^{1}H NMR (DMSO-d_6): δ 8.08 (m, 1H), 7.98 (m, 1H), 7.87 (m, 2H), 4.38 (dd, J = 3.0, 3.0 Hz, 1H), 3.47 (m, 1H), 3.33 (m, 1H), 2.18 (m, 1H), 1.87 (m, 3H).

(*R*)-1-(3-Nitrophenylsulfonyl)pyrrolidine-2-carboxylic Acid (4i). Compound **4i** was obtained as a white solid in 81.6% yield. mp 147−149 °C. $[\alpha]^{26}_D = +74.2°$ (*c* 1, $CHCl_3$). ^{1}H NMR (DMSO-d_6): δ 8.53 (m, 1H), 8.27 (m, 1H), 7.92 (m, 1H), 7.61 (m, 1H), 4.25 (m, 1H), 3.38 (m, 2H), 1.87 (m, 4H).

(*R*)-1-(4-Nitrophenylsulfonyl)pyrrolidine-2-carboxylic Acid (4j). Compound **4j** was obtained as a white solid in 82% yield. mp 141−143 °C. $[\alpha]^{26}_D = +65.1°$ (*c* 1, $CHCl_3$). ^{1}H NMR (DMSO-d_6): δ 8.41 (m, 2H), 8.09 (m, 2H), 4.61 (m, 1H), 4.21 (m, 1H), 3.71 (m, 1H), 1.86 (m, 4H).

(*R*)-1-(4-Fluorophenylsulfonyl)pyrrolidine-2-carboxylic Acid (4k). Compound **4k** was obtained as a white solid in 87.3% yield. mp 110−112 °C. $[\alpha]^{27}_D = +86.8°$ (*c* 1, $CHCl_3$). ^{1}H NMR ($CDCl_3$): δ 9.97 (s, 1H), 7.92 (m, 2H), 7.23 (m, 2H), 4.34 (m, 1H), 3.50 (m, 1H), 3.32 (m, 1H), 2.13 (m, 2H), 1.99 (m, 1H), 1.84 (m, 1H).

(*R*)-1-(4-Chlorophenylsulfonyl)pyrrolidine-2-carboxylic Acid (4l). Compound **4l** was obtained as a white solid in 88% yield. mp 102−103 °C. $[\alpha]^{26}_D = +83.5°$ (*c* 1, $CHCl_3$). ^{1}H NMR ($CDCl_3$): δ 8.85 (s, br, 1H), 7.82 (m, 2H), 7.53 (m, 2H), 4.34 (m, 1H), 3.97 (m, 1H), 3.31 (m, 1H), 1.94 (m, 4H).

(*R*)-1-(4-Bromophenylsulfonyl)pyrrolidine-2-carboxylic Acid (4m). Compound **4m** was obtained as a white solid in 87.2% yield: mp 175−177 °C. $[\alpha]^{26}_D = +75.8°$ (*c* 1, $CHCl_3$). ^{1}H NMR ($CDCl_3$): δ 8.60 (s, br, 1H), 7.74 (m, 2H), 7.67 (m, 2H), 4.33 (m, 1H), 3.97 (m, 1H), 3.29 (m, 1H), 1.98 (m, 4H).

(*R*)-1-(4-Iodophenylsulfonyl)pyrrolidine-2-carboxylic Acid (4n). Compound **4n** was obtained as a white solid in 72.7% yield. mp 121−123 °C. $[\alpha]^{27}_D = +66.6°$ (*c* 1, $CHCl_3$). ^{1}H NMR ($CDCl_3$): δ 8.95 (s, br, 1H), 7.91 (m, 2H), 7.61 (m, 2H), 4.33 (m, 1H), 3.93 (m, 1H), 3.42 (m, 1H), 1.98 (m, 4H).

(*S*)-3-(4-Fluorophenylsulfonyl)thiazolidine-4-carboxylic Acid (4o). Compound **4o** was obtained as a white solid in 94.5% yield. mp 96−99 °C. $[\alpha]^{25}_D = -172.1°$ (*c* 1, $CHCl_3$). ^{1}H NMR ($CDCl_3$): δ 9.93 (s, br, 1H), 7.92 (m, 2H), 7.23 (m, 2H), 4.91 (m, 1H), 4.68 (d, J = 9.31 Hz, 1H), 4.42 (d, J = 9.31 Hz, 1H), 3.26 (m, 1H), 3.08 (m, 1H).

(*S*)-3-(4-Chlorophenylsulfonyl)thiazolidine-4-carboxylic Acid (4p). Compound **4p** was obtained as a white solid in 91.1% yield: mp 115−118 °C. $[\alpha]^{28}_D = -161.4°$ (*c* 1, $CHCl_3$). ^{1}H NMR ($CDCl_3$): δ 7.83 (m, 2H), 7.51 (m, 2H), 4.89 (m, 1H), 4.68 (d, J = 9.20 Hz, 1H), 4.42 (d, J = 9.30 Hz, 1H), 3.27 (m, 1H), 3.08 (m, 1H).

(*S*)-3-(4-Bromophenylsulfonyl)thiazolidine-4-carboxylic Acid (4q). Compound **4q** was obtained as a white solid in 80.6% yield. mp 127−130 °C. $[\alpha]^{23}_D = -141.4°$ (*c* 1, $CHCl_3$). ^{1}H NMR ($CDCl_3$): δ 7.74 (m, 4H), 4.89 (m, 1H), 4.67 (d, J = 9.30 Hz, 1H), 3.27 (m, 1H), 3.07 (m, 1H).

(*S*)-3-(4-Iodophenylsulfonyl)thiazolidine-4-carboxylic Acid (4r). Compound **4r** was obtained as a white solid in 86.4% yield: mp 149−152 °C. $[\alpha]^{28}_D = -124.5°$ (*c* 1, $CHCl_3$). ^{1}H NMR ($CDCl_3$): δ 7.91 (m, 2H), 7.61 (m, 2H), 4.90 (dd, J = 3.60, 3.30 Hz, 1H), 4.68 (d, J = 9.30 Hz, 1H), 4.44 (d, J = 9.30 Hz, 1H), 3.29 (dd, J = 3.60, 3.3 Hz, 1H), 3.08 (m, 1H).

Synthesis of compounds 5c−r. Compounds **5c**−**r** were synthesized using General Procedure 2.

(*R*)-1-(Butylsulfonyl)-*N*-((*S*)-1-hydroxy-3-phenylpropan-2-yl)-pyrrolidine-2-carboxamide (5c). Compound **5c** was obtained as

viscous oil in 79.2% yield. ^{1}H NMR (DMSO-d_6): δ 7.95 (s, 1H), 7.67 (d, J = 9.0 Hz, 1H), 7.22 (m, 5H), 4.81 (t, J = 5.4 Hz, 1H), 4.13 (dd, J = 3.6, 3.6 Hz, 1H), 3.88 (m, 1H), 3.06 (m, 2H), 2.85 (m, 1H), 2.60 (m, 2H), 2.50 (m, 2H), 1.96 (m, 1H), 1.68 (m, 2H), 1.63 (m, 3H), 1.38 (m, 2H), 0.88 (t, J = 7.2 Hz, 3H).

(*R*)-*N*-((*S*)-1-Hydroxy-3-phenylpropan-2-yl)-1-(isopropylsulfonyl)pyrrolidine-2-carboxamide (5d). Compound **5d** was obtained as viscous oil in 28% yield. ^{1}H NMR ($CDCl_3$): δ 7.27 (m, 5H), 6.59 (d, J = 7.8 Hz, 1H), 4.29 (m, 1H), 4.14 (m, 1H), 3.70 (m, 1H), 3.64 (m, 1H), 3.44 (m, 2H), 3.31 (m, 1H), 2.97 (s, 1H), 2.64 (t, J = 5.7 Hz, 1H), 2.16 (m, 2H), 1.93 (m, 2H), 1.37 (d, J = 6.9, 6H).

(*R*)-*N*-((*S*)-1-Hydroxy-3-phenylpropan-2-yl)-1-(naphthalen-1-ylsulfonyl)pyrrolidine-2-carboxamide (5e). Compound **5e** was obtained as a white crystalline solid in 68.4% yield: mp 120−122 °C; ^{1}H NMR ($CDCl_3$): δ 8.42 (s, 1H), 7.96 (m, 3H), 7.84 (m, 1H), 7.67 (m, 2H), 7.34 (m, 5H), 7.13 (d, J = 7.2 Hz, 1H), 4.16 (m, 1H), 3.70 (m, 2H), 3.53 (m, 1H), 3.23 (m, 1H), 2.96 (m, 3H), 2.10 (m, 1H), 1.65 (m, 3H). ESI MS: m/z 461 $(M + Na)^+$.

(*R*)-*N*-((*S*)-1-Hydroxy-3-phenylpropan-2-yl)-1-(phenylsulfonyl)-pyrrolidine-2-carboxamide (5f). Compound **5f** was obtained as a white solid in 84.6% yield: mp 98−100 °C; ^{1}H NMR ($CDCl_3$): δ 7.85 (m, 2H), 7.60 (m, 3H), 7.30 (m, 5H), 7.10 (d, J = 7.2 Hz, 1H), 4.07 (m, 2H), 3.67 (m, 2H), 3.50 (m, 1H), 3.17 (m, 1H), 3.03 (m, 2H), 2.78 (m, 1H), 2.11 (m, 1H), 1.59 (m, 2H).

(*R*)-*N*-((*S*)-1-Hydroxy-3-phenylpropan-2-yl)-1-(4-methoxyphenylsulfonyl)pyrrolidine-2-carboxamide (5g). Compound **5g** was obtained as a white solid in 73.8% yield: mp 146−148 °C; ^{1}H NMR ($CDCl_3$): δ 7.78 (m, 2H), 7.32 (m, 5H), 7.13 (d, J = 7.5 Hz, 1H), 7.02 (m, 2H), 4.11 (m, 1H), 4.00 (m, 1H), 3.90 (s, 3H), 3.72 (m, 2H), 3.49 (m, 1H), 3.14 (m, 1H), 2.97 (m, 2H), 2.71 (m, 1H), 2.10 (m, 1H), 1.60 (m, 2H).

(*R*)-*N*-((*S*)-1-Hydroxy-3-phenylpropan-2-yl)-1-(2-nitrophenylsulfonyl)pyrrolidine-2-carboxamide (5h). Compound **5h** was obtained as colorless oil in 49% yield. ^{1}H NMR ($CDCl_3$, 300 MHz): δ 8.08 (m, 1H), 7.72 (m, 3H), 7.26 (m, 5H), 6.79 (d, J = 8.1 Hz, 1H), 4.43 (dd, J = 4.0, 4.0 Hz, 5H), 4.09 (m, 1H), 3.66 (m, 1H), 3.54 (m, 3H), 2.74 (dd, J = 2.7, 2.7 Hz, 2H), 2.14 (m, 1H), 1.86 (m, 3H).

(*R*)-*N*-((*S*)-1-Hydroxy-3-phenylpropan-2-yl)-1-(3-nitrophenylsulfonyl)pyrrolidine-2-carboxamide (5i). Compound **5i** was obtained as a white solid in 76% yield: mp 119−121 °C; ^{1}H NMR ($CDCl_3$): δ 8.70 (m, 1H), 8.50 (m, 1H), 8.19 (m, 1H), 7.80 (t, J = 4.8 Hz, 1H), 7.24 (m, 5H), 6.89 (d, J = 6.3 Hz, 1H), 4.23 (m, 2H), 3.58 (m, 2H), 3.52 (m, 1H), 3.25 (m, 1H), 2.98 (m, 2H), 1.54 (m, 4H).

(*R*)-*N*-((*S*)-1-Hydroxy-3-phenylpropan-2-yl)-1-(4-nitrophenylsulfonyl)pyrrolidine-2-carboxamide (5j). Compound **5j** was obtained as a white solid in 73% yield: mp 184−186 °C; ^{1}H NMR ($CDCl_3$): δ 8.42 (d, J = 9.0 Hz, 2H), 8.06 (d, J = 9.0 Hz, 2H), 7.33 (m, 5H), 6.83 (d, J = 9.0 Hz, 1H), 4.17 (m, 1H), 4.08 (m, 2H), 3.68 (m, 2H), 3.53 (m, 1H), 3.19 (m, 1H), 2.97 (m, 2H), 2.43 (m, 1H), 2.14 (m, 1H), 1.73 (m, 2H).

(*R*)-1-(4-Fluorophenylsulfonyl)-*N*-((*S*)-1-hydroxy-3-phenylpropan-2-yl)pyrrolidine-2-carboxamide (5k). Compound **5k** was obtained as a white crystalline solid in 81.1% yield: mp 150−152 °C; ^{1}H NMR ($CDCl_3$): δ 7.86 (m, 2H), 7.28 (m, 7H), 7.03 (d, J = 7.20 Hz 1H), 4.13 (m, 1H), 4.01 (m, 1H), 3.68 (m, 2H), 3.48 (m, 1H), 3.06 (m, 3H), 2.63 (m, 1H), 2.12 (m, 1H), 1.64 (m, 3H). ESI MS: m/z 429 $(M + Na)^+$.

(*R*)-1-(4-Chlorophenylsulfonyl)-*N*-((*S*)-1-hydroxy-3-phenylpropan-2-yl)pyrrolidine-2-carboxamide (5l). Compound **5l** was obtained as a white crystalline solid in 52% yield: mp 100−102 °C; ^{1}H NMR ($CDCl_3$): δ 7.71 (m, 3H), 7.28 (m, 6H), 7.03 (d, J = 7.20 Hz, 1H), 4.13 (m, 1H), 4.01 (m, 1H), 3.68 (m, 2H), 3.48 (m, 1H), 3.06 (m, 3H), 2.63 (m, 1H), 2.12 (m, 1H), 1.64 (m, 2H). ESI MS: m/z 445.5 $(M + Na)^+$.

(*R*)-1-(4-Bromophenylsulfonyl)-*N*-((*S*)-1-hydroxy-3-phenylpropan-2-yl)pyrrolidine-2-carboxamide (5m). Compound **5m** was obtained as a white crystalline solid in 50.4% yield. ^{1}H NMR ($CDCl_3$): δ 7.48 (m, 4H), 7.29 (m, 5H), 7.03 (d, J = 7.50 Hz, 1H), 4.13 (m, 1H), 3.68 (m, 2H), 3.48 (m, 1H), 3.36 (m, 1H), 3.13 (m, 1H), 2.96 (m, 2H), 2.12 (m, 1H), 1.64 (m, 3H). ESI MS: m/z 489.6 $(M + Na)^+$.

(*R*)-*N*-((*S*)-1-Hydroxy-3-phenylpropan-2-yl)-1-(4-iodophenylsulfonyl)pyrrolidine-2-carboxamide (5n). Compound **5n** was obtained as a white crystalline solid in 30.8% yield: mp 178−180 °C; ^{1}H NMR ($CDCl_3$): δ 8.96 (s, br, 1H), 7.90 (m, 2H), 7.61 (m, 2H), 7.32 (d, J = 7.50 Hz, 1H), 4.32 (m, 1H), 3.90 (m, 1H), 3.49 (m, 1H), 3.30 (m, 1H), 2.01 (m, 6H). ESI MS: m/z 537.10 $(M + Na)^+$.

(*S*)-3-(4-Fluorophenylsulfonyl)-*N*-((*S*)-1-hydroxy-3-phenylpropan-2-yl)thiazolidine-4-carboxamide (5o). Compound **5o** was obtained as colorless viscous oil in 26.3% yield. ^{1}H NMR ($CDCl_3$, 500 MHz): δ 7.84 (m, 2H), 7.32 (m, 2H), 7.24 (m, 5H), 6.82 (d, J = 7.20 Hz, 1H), 4.46 (d, J = 6.91 Hz, 1H), 4.40 (m, 1H), 4.24 (m, 1H), 4.02 (d, J = 6.91 Hz, 1H), 3.78 (d, J = 9.10 Hz, 1H), 3.64 (m, 1H), 3.15 (dd, J = 5.80, 5.80 Hz, 1H), 3.02 (m, 1H), 2.84 (m, 1H), 2.65 (m, 1H), 2.58 (s, br, 1H). ESI MS: m/z 447.3 $(M + Na)^+$.

(*S*)-3-(4-Chlorophenylsulfonyl)-*N*-((*S*)-1-hydroxy-3-phenylpropan-2-yl)thiazolidine-4-carboxamide (5p). Compound **5p** was obtained as a yellow white solid in 54.3% yield: mp 102−104 °C; ^{1}H NMR ($CDCl_3$): δ 7.78 (m, 2H), 7.56 (m, 2H), 7.28 (m, 5H), 6.82 (d, J = 8.10 Hz, 1H), 4.48 (d, J = 10.50 Hz, 1H), 4.40 (m, 1H), 4.24 (m, 1H), 4.04 (d, J = 10.50 Hz, 1H), 3.80 (m, 1H), 3.65 (m, 1H), 3.18 (dd, J = 3.60, 3.60 Hz, 1H), 3.02 (m, 1H), 2.81 (m, 1H), 2.65 (m, 1H), 2.58 (s, br, 1H). ESI MS: m/z 463.10 $(M + Na)^+$.

(*S*)-3-(4-Bromophenylsulfonyl)-*N*-((*S*)-1-hydroxy-3-phenylpropan-2-yl)thiazolidine-4-carboxamide (5q). Compound **5q** was obtained as a yellow white solid in 38.7% yield: mp 113−115 °C; ^{1}H NMR ($CDCl_3$, 300 MHz): δ 7.69 (m, 4H), 7.25 (m, 5H), 6.79 (d, J = 8.1 Hz, 1H), 4.44 (m, 2H), 4.24 (m, 1H), 4.03 (d, J = 10.5 Hz, 1H), 3.78 (m, 1H), 3.63 (m, 1H), 3.15 (dd, J = 3.60, 3.60 Hz, 1H), 3.01 (m, 1H), 2.81 (m, 1H), 2.65 (m, 1H). ESI MS: m/z 509.10 $(M + Na)^+$.

(*S*)-*N*-((*S*)-1-Hydroxy-3-phenylpropan-2-yl)-3-(4-iodophenylsulfonyl)thiazolidine-4-carboxamide (5r). Compound **5r** was obtained as a yellow white solid in 40.3% yield: mp 100−102 °C; ^{1}H NMR ($CDCl_3$, 300 MHz): δ 7.94 (m, 2H), 7.54 (m, 2H), 7.27 (m, 5H), 6.79 (d, J = 7.8 Hz, 1H), 4.47 (d, J = 10.5 Hz, 1H), 4.39 (m, 1H), 4.23 (m, 1H), 4.04 (d, J = 10.5 Hz, 1H), 3.78 (m, 1H), 3.66 (m, 1H), 3.18 (dd, J = 3.60, 3.60 Hz, 1H), 3.02 (m, 1H), 2.82 (m, 1H), 2.65 (m, 1H), 2.53 (m, 1H). ESI MS: m/z 559.10 $(M + Na)^+$.

Synthesis of Compounds 1a−r. The synthesis of compounds **1a** and **1b** have been previously reported.[25] Compounds **1c−m** were synthesized using oxidation Method A while **1n−r** were synthesized using oxidation Method B.

(*R*)-1-(Butylsulfonyl)-*N*-((*R,S*)-1-oxo-3-phenylpropan-2-yl)-pyrrolidine-2-carboxamide (1c). Compound **1c** was obtained as colorless oil in 32% yield. ^{1}H NMR ($CDCl_3$): δ 9.62 (s, 1H), 7.27 (m, 6H), 4.69 (m, 1H), 4.30 (m, 1H), 3.40 (m, 2H), 3.19 (m, 2H), 2.95 (m, 2H), 2.15 (m, 2H), 1.93 (m, 2H), 1.79 (m, 2H), 1.43 (m, 2H), 0.97 (t, J = 7.2 Hz, 3H). Anal. ($C_{18}H_{26}N_2O_4S$), C, H, N. ESI MS: m/z 421 $(M + Na + CH_3OH)^+$.

(*R*)-1-(Isopropylsulfonyl)-*N*-((*R,S*)-1-oxo-3-phenylpropan-2-yl)pyrrolidine-2-carboxamide (1d). Compound **1d** was obtained as colorless oil in 68% yield. ^{1}H NMR ($CDCl_3$): δ 9.62 (s, 1H), 7.23 (m, 5H), 7.01 (m, 1H), 4.68 (q, J = 6.9, 6.9 Hz, 2H), 4.39 (m, 1H), 3.49 (m, 1H), 3.37 (m, 1H), 3.16 (m, 3H), 2.13 (m, 2H), 1.90 (m, 2H), 1.31 (m, 6H). Anal. ($C_{17}H_{24}N_2O_4S \cdot 0.6H_2O$), C, H, N. ESI MS: m/z 407 $(M + Na + CH_3OH)^+$.

(*R*)-1-(Naphthalen-1-ylsulfonyl)-*N*-((*R,S*)-1-oxo-3-phenylpropan-2-yl)pyrrolidine-2-carboxamide (1e). Compound **1e** was obtained as a white foamy solid in 75.8% yield: mp 60−62 °C; ^{1}H NMR ($CDCl_3$, 300 MHz): δ 9.65 (s, 1H), 8.42 (s, 1H), 7.98 (m, 3H), 7.82 (m, 1H), 7.67 (m, 2H), 7.31 (m, 6H), 4.71 (q, J = 7.5, 7.5 Hz, 1H), 4.25 (m, 1H), 3.56 (m, 1H), 3.22 (m, 3H), 2.11 (m, 1H), 1.74 (m, 1H), 1.58 (m, 2H). Anal. ($C_{24}H_{24}N_2O_4S \cdot 0.16H_2O$), C, H, N. ESI MS: m/z 491 $(M + Na + CH_3OH)^+$.

(*R*)-*N*-((*R,S*)-1-oxo-3-phenylpropan-2-yl)-1-(phenylsulfonyl)-pyrrolidine-2-carboxamide (1f). Compound **1f** was obtained as colorless oil in 43% yield. ^{1}H NMR ($CDCl_3$, 300 MHz): δ 9.64 (s, 1H), 7.93 (m, 2H), 7.60 (m, 4H), 7.37 (m, 5H), 4.68 (t, J = 6.90, 6.90 Hz, 1H), 3.50 (m, 1H), 3.24 (m, 4H), 1.63 (m, 4H). Anal. ($C_{20}H_{22}N_2O_4S \cdot 1.02H_2O$), C, H, N. ESI MS: m/z 441 (M + Na + CH_3OH)$^+$.

(*R*)-1-(4-Methoxyphenylsulfonyl)-*N*-((*R,S*)-1-oxo-3-phenylpropan-2-yl)pyrrolidine-2-carboxamide (1g). Compound **1g** was obtained as colorless oil in 96% yield. ^{1}H NMR ($CDCl_3$): δ 9.64 (s, 1H), 7.77 (d, J = 8.70, 2H), 7.30 (m, 6H), 7.02 (d, J = 9.00, 2H), 4.68 (t, J = 6.90, 6.90 Hz, 1H), 4.60 (m, 1H), 3.45 (m, 1H), 3.19 (m, 3H), 1.65 (m, 4H). Anal. ($C_{21}H_{24}N_2O_5S \cdot 1.0H_2O$), C, H, N. ESI MS: m/z 471 (M + Na + CH_3OH)$^+$.

(*R*)-1-(2-Nitrophenylsulfonyl)-*N*-((*R,S*)-1-oxo-3-phenylpropan-2-yl)pyrrolidine-2-carboxamide (1h). Compound **1h** was obtained as colorless oil in 48.4% yield. ^{1}H NMR ($CDCl_3$): δ 9.57 (s, 1H), 8.06 (m, 1H), 7.71 (m, 3H), 7.26 (m, 5H), 7.02 (d, J = 7.5 Hz, 1H), 4.61 (m, 5H), 4.49 (m, 1H), 3.48 (m, 2H), 3.10 (d, J = 6.9 Hz 2H), 2.24 (m, 1H), 1.93 (m, 3H). Anal. ($C_{20}H_{21}N_3O_6S \cdot 0.2H_2O$), C, H, N. ESI MS: m/z 486 (M + Na + CH_3OH)$^+$.

(*R*)-1-(3-Nitrophenylsulfonyl)-*N*-((*R,S*)-1-oxo-3-phenylpropan-2-yl)pyrrolidine-2-carboxamide (1i). Compound **1i** was obtained as colorless oil in 60% yield. ^{1}H NMR ($CDCl_3$): δ 9.67 (d, J = 6.0 Hz, 1H), 8.68 (m, 1H), 8.51 (m, 1H), 8.16 (m, 1H), 7.80 (t, J = 8.1 Hz, 1H), 7.32 (m, 5H), 7.08 (d, J = 6.9 Hz, 1H), 4.75 (m, 1H), 4.15 (m, 1H), 3.56 (m, 1H), 3.22 (m, 3H), 1.80 (m, 4H). Anal. ($C_{20}H_{21}N_3O_6S \cdot 0.2H_2O$), C, H, N. ESI MS: m/z 486 (M + Na + CH_3OH)$^+$.

(*R*)-1-(4-Nitrophenylsulfonyl)-*N*-((*R,S*)-1-oxo-3-phenylpropan-2-yl)pyrrolidine-2-carboxamide (1j). Compound **1j** was obtained as colorless oil in 77% yield. ^{1}H NMR ($CDCl_3$): δ 9.68 (s, 1H), 8.42 (d, J = 9.0 Hz, 2H), 8.02 (d, J = 9.0 Hz, 2H), 7.32 (m, 5H), 7.05 (d, J = 9.0 Hz, 1H), 4.74 (t, J = 6.0, 6.0 Hz, 1H), 4.16 (m, 1H), 3.53 (m, 1H), 3.24 (m, 3H), 2.16 (m, 1H), 1.85 (m, 1H), 1.70 (m, 2H). Anal. ($C_{20}H_{21}N_3O_6S \cdot 0.16H_2O$), C, H, N. ESI MS: m/z 486 (M + Na + CH_3OH)$^+$.

(*R*)-1-(4-Fluorophenylsulfonyl)-*N*-((*R,S*)-1-oxo-3-phenylpropan-2-yl)pyrrolidine-2-carboxamide (1k). Compound **1k** was obtained as a white foamy solid in 85.7% yield: mp 45−47 °C; ^{1}H NMR ($CDCl_3$): δ 9.62 (s, 1H), 7.84 (m, 2H), 7.26 (m, 8H), 4.68 (q, J = 6.90, 6.90 Hz, 1H), 4.11 (m, 1H), 3.48 (m, 1H), 3.13 (m, 3H), 2.11 (m, 1H), 1.66 (m, 3H). Anal. ($C_{20}H_{21}FN_2O_4S \cdot 0.4H_2O$), C, H, N. ESI MS: m/z 459 (M + Na + CH_3OH)$^+$.

(*R*)-1-(4-Chlorophenylsulfonyl)-*N*-((*R,S*)-1-oxo-3-phenylpropan-2-yl)pyrrolidine-2-carboxamide (1l). Compound **1l** was obtained as a white foamy solid in 56.9% yield: mp 42−44 °C; ^{1}H NMR ($CDCl_3$): δ 9.64 (s, 1H), 7.77 (m, 2H), 7.29 (m, 6H), 4.72 (q, J = 6.90, 7.20 Hz, 1H), 4.12 (m, 1H), 3.48 (m, 1H), 3.21 (m, 2H), 3.10 (m, 1H), 2.13 (m, 1H), 1.66 (m, 3H). Anal. ($C_{20}H_{21}ClN_2O_4S \cdot 0.4H_2O$), C, H, N. ESI MS: m/z 475 (M + Na + CH_3OH)$^+$.

(*R*)-1-(4-Bromophenylsulfonyl)-*N*-((*R,S*)-1-oxo-3-phenylpropan-2-yl)pyrrolidine-2-carboxamide (1m). Compound **1m** was obtained as a white foamy solid in 86.7% yield: mp 43−45 °C; ^{1}H NMR ($CDCl_3$): δ 9.61 (s, 1H), 7.68 (m, 4H), 7.26 (m, 6H), 4.69 (q, J = 6.90, 6.90 Hz, 1H), 4.10 (m, 1H), 3.48 (m, 1H), 3.19 (m, 3H), 2.10 (m, 1H), 1.63 (m, 3H). Anal. ($C_{20}H_{21}BrN_2O_4S$), C, H, N. ESI MS: m/z 520 (M + Na + CH_3OH)$^+$.

(*R*)-1-(4-Iodophenylsulfonyl)-*N*-((*R,S*)-1-oxo-3-phenylpropan-2-yl)pyrrolidine-2-carboxamide (1n). Compound **1n** was obtained as a white foamy solid in 69.4% yield: mp 50−52 °C; ^{1}H NMR ($CDCl_3$): δ 9.64 (s, 1H), 7.93 (m, 2H), 7.54 (m, 2H), 7.32 (m, 6H), 4.72 (q, J = 6.90, 6.90 Hz, 1H), 4.10 (m, 1H), 3.48 (m, 1H), 3.19 (m, 3H), 2.10 (m, 1H), 1.63 (m, 3H). Anal. ($C_{20}H_{21}IN_2O_4S$) C, H, N. ESI MS: m/z 511.10 (M − H)$^+$.

(*S*)-3-(4-Fluorophenylsulfonyl)-*N*-((*R,S*)-1-oxo-3-phenylpropan-2-yl)thiazolidine-4-carboxamide (1o). Compound **1o** was obtained as a white foamy solid in 55.7% yield: mp 36−38 °C; ^{1}H NMR ($CDCl_3$): δ 9.64 (s, 1H), 7.87 (m, 2H), 7.24 (m, 8H), 4.72 (m, 1H), 4.63 (m, 1H), 4.54 (d, J = 10.50 Hz, 1H), 4.03 (d, J = 10.50 Hz, 1H), 3.29 (m, 2H), 3.09 (m, 1H), 2.59 (m, 1H). Anal. ($C_{19}H_{19}FN_2O_4S_2 \cdot 0.4H_2O$), C, H, N. ESI MS: m/z 477.3 (M + Na + CH_3OH)$^+$.

(*S*)-3-(4-Chlorophenylsulfonyl)-*N*-((*R,S*)-1-oxo-3-phenylpropan-2-yl)thiazolidine-4-carboxamide (1p). Compound **1p** was obtained as a white foamy solid in 50.4% yield: mp 44−46 °C; ^{1}H NMR ($CDCl_3$): δ 9.62 (s, 1H), 7.78 (d, J = 8.40 Hz, 2H), 7.52 (d, J = 8.40 Hz, 2H), 7.23 (m, 6H), 4.63 (m, 3H), 4.04 (d, J = 10.20 Hz, 1H), 3.26 (m, 2H), 2.59 (m, 1H). Anal. ($C_{19}H_{19}ClN_2O_4S_2 \cdot 0.01H_2O$), C, H, N. ESI MS: m/z 493.3 (M + Na + CH_3OH)$^+$.

(*S*)-3-(4-Bromophenylsulfonyl)-*N*-((*R,S*)-1-oxo-3-phenylpropan-2-yl)thiazolidine-4-carboxamide (1q). Compound **1q** was obtained as a white foamy solid in 64.4% yield: mp 45−47 °C; ^{1}H NMR ($CDCl_3$, 300 MHz): δ 9.67 (s, 1H), 7.71 (m, 4H), 7.29 (m, 6H), 4.60 (m, 3H), 4.03 (d, J = 10.20 Hz, 1H), 3.27 (m, 3H), 2.63 (m, 1H). Anal. ($C_{19}H_{19}BrN_2O_4S_2$), C, H, N. ESI MS: m/z 537.2 (M + Na + CH_3OH)$^+$.

(*S*)-3-(4-Iodophenylsulfonyl)-*N*-((*R,S*)-1-oxo-3-phenylpropan-2-yl)thiazolidine-4-carboxamide (1r). Compound **1r** was obtained as a white foamy solid in 86.3% yield: mp 55−57 °C; ^{1}H NMR ($CDCl_3$): δ 9.66 (s, 1H), 7.91 (m, 2H), 7.53 (m, 2H), 7.29 (m, 6H), 4.67 (m, 1H), 4.58 (m, 1H), 4.50 (d, J = 10.2 Hz, 1H), 4.00 (d, J = 10.5 Hz, 1H), 3.30 (m, 2H), 3.13 (m, 1H), 2.61 (m, 1H). Anal. ($C_{19}H_{19}IN_2O_4S_2 \cdot 0.14$EtOAc), C, H, N. ESI MS: m/z 585.2 (M + Na + CH_3OH)$^+$.

Synthesis of α-Ketoamides 2a−c. 2(*R,S*)-Hydroxy-3(*S*)-amino-4-phenylbutanoic Acid (6). Compound **6** was synthesized as previously reported.[27]

2(*R,S*)-Hydroxy-3(*S*)-(*tert*-butoxycarbonylamino)-4-phenylbutanoic Acid (7). To **6** (0.4 g, 2.04 mmol) in 1N NaOH (5 mL), 2-methyl-2-propranol (5 mL) and di-*tert*-butyl carbonate (0.44 mg, 2.04 mmol) were added and stirred overnight at RT. H_2O (15 mL) was added and the mixture was extracted with hexanes (2 × 20 mL). The pH of the aqueous layer was acidified (pH = 1) and extracted with EtOAc (3 × 30 mL). The combined extracts were washed with H_2O (30 mL), brine (30 mL), dried (Na_2SO_4), and concentrated to give a white solid in 60% yield: mp 123−125 °C; ^{1}H NMR ($CDCl_3$): δ 7.27 (m, 5H), 4.26 (m, 1H), 2.93 (m, 2H), 1.38 (s, 9H).

***tert*-butyl-2(*S*)-3(*R,S*)-Hydroxy-4-oxo-4-(phenylethylamino)-1-phenylbutan-2-ylcarbamate (8).** Coupling of **7** with phenylethylamine afforded **8** as a white solid in 77.6% yield: mp 118−120 °C; ^{1}H NMR ($CDCl_3$): δ 7.25 (m, 10H), 5.45 (m, 1H), 5.05 (m, 1H), 4.07 (m, 1H), 3.98 (m, 1H), 3.52 (m, 2H), 3.01 (m, 2H), 2.81 (m, 2H), 1.35 (s, 9H).

3(*S*)-3-Amino-2(*R,S*)-hydroxy-*N*-phenethyl-4-phenylbutanamide (9). Compound **8** (0.129 g, 0.323 mmol) was dissolved in TFA (0.3 mL) and CH_2Cl_2 (0.5 mL) and the mixture was stirred for 4 h at RT. The solvent was removed and the residue was recrystallized from and CH_2Cl_2 /hexanes to afford **9** in 53% yield: mp 164−166 °C; ^{1}H NMR (MeOD): δ 7.29 (m, 10H), 3.77 (m, 1H), 3.58 (m, 1H), 3.41 (m, 1H), 3.33 (m, 1H), 3.02 (m, 1H), 2.92 (m, 1H), 2.83 (m, 2H).

2(*R*)-*N*-((2*S*)-3(*R,S*)-Hydroxy-4-oxo-4-(phenethylamino)-1-phenylbutan-2-yl)-1-tosyl-pyrrolidine-2-carboxamide (11). Coupling of **9** with 1-(toluene-4-sulfonyl)-pyrrolidine-2-carboxylic acid (**10**) gave **11** as a white solid in 93.1% yield: ^{1}H NMR ($CDCl_3$): δ 7.70 (d, J = 8.4 Hz, 2H), 7.25 (m, 12H), 7.01 (m, 1H), 6.89 (m, 1H), 4.37 (m, 1H), 4.16 (m, 1H), 3.97 (m, 1H), 3.65 (m, 1H), 3.55 (m, 1H), 3.45 (m, 1H), 3.24(m, 1H), 3.06 (m, 2H), 2.91 (m, 2H), 2.45 (s, 3H), 2.05 (m, 1H), 1.72 (m, 1H), 1.59 (m, 1H), 1.48 (m, 1H).

2(*R*)-*N*-((2*S*)-3(*R,S*)-Hydroxy-4-oxo-4-(phenethylamino)-1-phenylbutan-2-yl)-1-(4-nitro-phenylsulfonyl)pyrrolidine-2-carboxamide (12). Coupling of **9** with **4j** gave **12** as a white solid in 92.4% yield: mp 75−77 °C; ^{1}H NMR ($CDCl_3$): δ 8.38 (d, J = 8.2 Hz, 2H), 7.98 (t, J = 9 Hz, 2H), 7.26 (m, 10H), 7.04 (m, 1H), 6.83 (m, 1H), 5.02 (m, 1H), 4.36 (m, 1H), 4.16 (m, 1H), 3.97 (m, 1H), 3.52 (m, 3H), 3.21 (m, 1H), 3.08(m, 2H), 2.84 (m, 2H), 1.79 (m, 1H), 1.73 (m, 1H), 1.44 (m, 1H). ESI MS: m/z 603.65 (M + Na)$^+$.

2(*R*)-*N*-((2*S*)-3(*R,S*)-Hydroxy-4-oxo-4-(phenethylamino)-1-phenylbutan-2-yl)-1-(4-methoxy-phenylsulfonyl)pyrrolidine-2-carboxamide (13). Coupling of **9** with **4g** gave **13** as a white solid in 81.9% yield: mp 90−92 °C;^{1}H NMR ($CDCl_3$): δ 7.73 (m, 2H), 7.27 (m, 10H), 7.02 (m, 2H), 6.78 (m, 1H), 5.61 (m, 1H), 4.35 (m, 1H), 4.16 (m, 1H), 3.97 (m, 1H), 3.87 (s, 3H), 3.56 (m, 2H), 3.28 (m, 2H), 3.07 (m, 2H), 2.86 (m, 2H), 1.90 (m, 1H), 1.42 (m, 3H). ESI MS: *m*/*z* 573.6 $(M + Na)^+$.

(*R*)-*N*-((2*R,S*)-3,4-Dioxo-4-(phenethylamino)-1-phenylbutan-2-yl)-1-tosylpyrrolidine-2-carboxamide (2a). Oxidation (Method A) of **11** gave **2a** as a white solid in 62.3% yield: mp 118−120 °C; ^{1}H NMR ($CDCl_3$): δ 7.70 (d, J = 8.4 Hz, 2H), 7.25 (m, 12H), 6.98 (m, 1H), 5.58 (m, 1H), 4.08 (m, 1H), 3.61 (m, 2H), 3.44 (m, 1H), 3.31 (m, 2H), 3.08 (m, 2H), 2.88 (m, 2H), 2.44(s, 3H), 2.03 (m, 1H), 1.65 (m, 1H), 1.48 (m, 2H). Anal. ($C_{30}H_{33}N_3O_5S$), C, H, N. ESI MS: *m*/*z* 570 $(M + Na)^+$.

(*R*)-*N*-((2*R,S*)-3,4-Dioxo-4-(phenethylamino)-1-phenylbutan-2-yl)-1-(4-nitrophenyl-sulfonylpyrrolidine-2-carboxamide (2b). Oxidation (Method A) of **12** gave **2b** as a white solid 72.5% yield: mp 110−112 °C;^{1}H NMR ($CDCl_3$): δ 8.36 (m, 2H), 7.99 (m, 2H), 7.26 (m, 10H), 6.93 (m, 2H), 5.59 (m, 1H), 4.13 (m, 1H), 3.63 (m, 2H), 3.46 (m, 2H), 3.24 (m, 2H), 2.89 (m, 2H), 2.09 (m, 1H), 1.69 (m, 3H). Anal. ($C_{29}H_{30}N_4O_7S$), C, H, N. ESI MS: *m*/*z* 601.64 $(M + Na)^+$.

(*R*)-*N*-((2*R,S*)-3,4-Dioxo-4-(phenethylamino)-1-phenylbutan-2-yl)-1-(4-methoxyphenyl-sulfonylpyrrolidine-2-carboxamide (2c). Oxidation (Method A) of **13** gave **2c** as a white solid in 69.6% yield: mp 130−132 °C;^{1}H NMR ($CDCl_3$): δ 7.75 (m, 2H), 7.26 (m, 11H), 6.99 (m, 2H), 6.80 (m, 1H), 5.63 (m, 1H), 4.07 (m, 1H), 3.86 (s, 3H), 3.59 (m, 2H), 3.44(m, 1H), 3.28 (m, 2H), 3.09 (m, 1H), 2.87 (m, 2H), 2.04 (m, 1H), 1.58 (m, 3H). Anal. ($C_{30}H_{33}N_3O_6S$), C, H, N. ESI MS: *m*/*z* 586.6 $(M + Na)^+$.

Biology. Enzymological assays. The K_i values for inhibition of μ-calpain activity was monitored in a reaction mixture containing 50 mM Tris HCl (pH 7.4), 50 mM NaCl, 10 mM dithiothreitol, 1 mM EDTA, 1 mM EGTA, 0.2 mM or 1.0 mM Suc-Leu-Tyr-AMC (Calbiochem), 1 μg of porcine erythrocyte μ-calpain (Calbiochem), varying concentrations of inhibitor dissolved in DMSO (2% total concentration) and 5 mM $CaCl_2$ in a final volume of 250 μL in a polystyrene microtiter plate as previously reported.[25]

The K_i values for inhibition of human liver cathepsin B activity was determined using a reaction mixture containing 1 nM human liver cathepsin B (Calbiochem), 50 mM NaOAc (pH 6.0), 1 mM EDTA, 0.5 mM DTT, 50 μM or 250 μM of substrate (Z-Arg-Arg-AMC), and varying concentrations of inhibitor dissolved in DMSO (2% total concentration) in a final volume of 250 μL.

MTT Assay. Cells were distributed into 96-well plates at density of 2000 to 15000 cells/well and exposed to a range of drug concentrations (1 to 100 μM) for 96 h at 37 °C and 5% CO_2 atmosphere. Wells to which no drug was added were used as negative controls. At the end of treatment, an aliquot (25 μL) of MTT (3-(4,5-Dimethyl-2-thiazolyl)-2,5-diphenyl-2*H*-tetrazolium bromide) dye (5 mg/mL) was added to each well for the final 2−4 h of incubation. The plates were then centrifuged at 300*g* for 15 min. The supernatant media in each well was aspirated, and the formazen product was solubilized with 100 μL of DMSO. The absorbance values of wells were determined at 595 nm using a MRX microplate reader (DYNEX Technologies). Percentage cell survival was plotted against the drug concentration, and the GI_{50} (the concentration of drug required to reduce living cell number by 50% as compared to non-drug-treated wells) was determined by nonlinear regression analysis using WinNonlin (Pharsight Corporation).

Spectrin Breakdown Assay. Jurkat cell line, a unique cellular system, was used for evaluation of inhibitory activity for μ-calpain since it only expresses μ-calpain but not m-calpain.[38] Hence, the use of Jurkat cell line for evaluation of inhibitory activity for μ-calpain eliminates any proteolytic effects from m-calpain. Briefly, Jurkat cells were washed with HEPES-buffered saline (HBS) and preincubated with inhibitors for 10 min at 20 μM. Then, HBS containing ionomycin and Ca^{2+} was added to active intracellular calpain activity at a final concentration of 20 μM and 5 mM, respectively. After 60 min incubation at 37 °C, cells were collected and lysed in a lysis buffer. The supernatant was collected after centrifugation, and equal amounts of protein were loaded and resolved on SDS−PAGE gel and then transferred to nitrocellulose membranes. The blots were probed with monoclonal anti-α-spectrin (Chemicon) antibody and β-actin (Abcam). Appropriate conjugated secondary antibodies were used prior to detection by chemiluminescence (Amersham). The relative intensities of the calpain-specific 145/150-kDa spectrin fragment doublet, calpain-mediated spectrin breakdown products (SBDPs), were obtained by using Quantity One densitometry software (PDI Imageware Systems), corrected with the β-actin loading control.

Acknowledgment. The studies presented in this paper were supported by the NIH/NCI research grant CA1049820 to I.O.D.

Supporting Information Available: Elemental analysis data for all target compounds. This material is available free of charge via the Internet at http://pubs.acs.org.

References

(1) Guroff, G. A neutral, calcium-activated proteinase from the soluble fraction of rat brain. *J. Biol. Chem.* **1964**, *239*, 149−155.
(2) Suzuki, K. The structure of the calpains and the calpain gene. In *Intracellular calcium-dependent proteolysis*; Mellgren, R. L.; Murachi, T. , Eds.; CRC Press: Boca Raton, 1990; pp 25−35.
(3) Wang, K. K.; Yuen, P. W. Development and therapeutic potential of calpain inhibitors. *Adv. Pharmacol*, **1997**, *37*, 117−152.
(4) Santella, L.; Kyozuka, K.; Deiso, L.; Carafoli, E. Calcium, protease action, and the regulation of cell cycle. *Cell Calcium* **1998**, *2−3*, 123−130.
(5) Chan, S. L.; Mattson, M. P. Caspase and calpain substrates: roles in synaptic plasticity and cell death. *J. Neurosci. Res.* **1999**, *58*, 167−190.
(6) Utz, P. J.; Anderson, P. Life and death decisions: regulation of apoptosis by proteolysis of signaling molecules. *Cell Death Differ.* **2000**, *7*, 589−602.
(7) Vanderklish, P. W.; Bahr, B. A. The pathogenic activation of calpain: a marker and mediator of cellular toxicity and disease states. *Int. J. Exp. Pathol.* **2000**, *81*, 323−339.
(8) Carillo, S.; Pariat, M.; Steff, A. M.; Roux, P.; Etienne-Julan, M.; Lorca, T.; Piechaczyk, M. Differential sensitivity of fos and jun family members to calpains. *Oncogene* **1994**, *9*, 1679−1689.
(9) Gonen, H.; Shkedy, D.; Barnoy, S.; Kosower, N. S.; Ciechanover, A. On the involvement of calpains in the degradation of the tumor suppressor protein p53. *FEBS Lett.* **1997**, *406*, 17−22.
(10) Sorimachi, H.; Ishiura, S.; Suzuki, K. Structure and physiological function of calpains. *Biochem. J.* **1997**, *328*, 721−732.
(11) Atencio, I. A.; Ramachandra, M.; Shabram, P.; Demers, G. W. Calpain inhibitor 1 activates p53-dependent apoptosis in tumor cell lines. *Cell Growth Differ.* **2000**, *11*, 247−253.
(12) Gil-Parrado, S.; Fernandez-Montalvan, A.; Assfalg-Machleidt, I.; Popp, O.; Bestvater, F.; Holloschi, A.; Knoch, T. A.; Auerswald, E. A.; Welsh, K.; Reed, J. C.; Fritz, H.; Fuentes-Prior, P.; Spiess, E.; Salvesen, G. S.; Machleidt, W. Ionomycin-activated calpain triggers apoptosis. *J. Biol. Chem.* **2002**, *277*, 27217−27226.
(13) de la Fuente, M. T.; Casanova, B.; Garcia-Gila, M.; Silva, A.; Garcia-Pardo, A. Fibronectin interaction with α4β1 integrin prevents apoptosis in B cell chronic lymphocytic leukemia: correlation with Bcl-2 and Bax. *Leukemia* **1999**, *13*, 266−274.
(14) Kitada, S.; Anderson, J.; Akar, S., et al. Expression of apoptosis-regulating proteins in chronic lymphocytic leukemia: correlation with in vitro and in vivo chemoresponses. *Blood* **1998**, *91*, 3379−3389.
(15) Pepper, C.; Hoy, T.; Bentley, P. Elevated Bcl-2 and Bax are a consistent feature of apoptosis resistance in B-cell chronic lymphocytic leukemia and are correlated with in vivo chemoresistance. *Leuk. Lymphoma* **1998**, *28*, 355−361.
(16) Wood, D. E.; Thomas, A.; Devi, L. A.; Berman, Y.; Beavis, R. C.; Reed, J. C.; Newcomb, E. W. Bax cleavage is mediated by calpain during drug-induced apoptosis. *Oncogene* **1998**, *17*, 1069−1078.
(17) Squier, M. K.; Miller, A. C.; Malkinson, A. M.; Cohen, J. J. Calpain activation in apoptosis. *J. Cell Physiol.* **1994**, *159*, 229−237.
(18) Solay, E.; Eymin, B.; Droin, N.; Haugg, M. Proteases, proteolysis, and apoptosis. *Cell Biol. Toxicol.* **1998**, *14*, 121−132.
(19) Squier, M. K.; Sehnert, A. J.; Sellins, K. S.; Malkinson, A. M.; Takano, E.; Cohen, J. J. Calpain and calpastatin regulate neutrophil apoptosis. *J. Cell Physiol.* **1999**, *178*, 311−319.
(20) Sarin, A.; Adams, D. H.; Henkart, P. A. Protease inhibitors selectively block T cell receptor-triggered programmed cell death in a murine T cell hybridoma and activated peripheral T cells. *J. Exp. Med.* **1993**, *178*, 1693−1700.

(21) Zhu, D.-M.; Uckun, F. M. Calpain inhibitor II induces caspase-dependent apoptosis in human acute lymphoblastic leukemia and non-Hodgkin's lymphoma cells as well as some solid tumor cells. *Clin. Can. Res.* **2000**, *6*, 2456−2463.
(22) Witkowski, J. M.; Zmuda-Trezbiatowska, E.; Swiercz, J. M.; Cichorek, M.; Ciepluch, H.; Lewandowski, K.; Bryl, E.; Hellman, A. Modulation of the activity of calcium-activated neutral proteases (calpains) in chronic lymphocytic leukemia (B-CLL) cells. *Blood* **2002**, *100*, 1802−1809.
(23) Zhu, W.; Murtha, P. E.; Young, C. Y. Calpain inhibitor-induced apoptosis in human prostate adenocarcinoma cells. *Biochem. Biophys. Res. Commun*. **1995**, *214*, 1130−1137.
(24) Tripathy, R.; Gu, Z.-Q.; Dunn, D.; Senadhi, S. E.; Ator, M. A.; Chatterjee, S. P_2−Proline-derived inhibitors of calpain I. *Bioorg. Med. Chem. Lett*. **1998**, *19*, 2647−2652.
(25) Donkor, I. O.; Korukonda, R.; Huang, T. L.; LeCour, L., Jr. Peptidyl aldehyde inhibitors of calpain incorporating P_2-proline mimetics. *Bioorg. Med. Chem. Lett.* **2003**, *13*, 783−784.
(26) Donkor, I. O.; Zheng, X.; Han, J.; Lacy, C.; Miller, D. D. Significance of hydrogen bonding at the S_1' subsite of calpain I. *Bioorg. Med. Chem. Lett.* **2001**, *11*, 1753−1755.
(27) Harbeson, S. L.; Abelleira, S. M.; Akiyama, A.; Barrett, R. III; Carroll R. M.; Straub, J. A.; Tkacz, J. N.; Wu, C.; Musso, G. F. Stereospecific synthesis of peptidyl alpha-keto amides as inhibitors of calpain. *J. Med. Chem*. **1994**, *37*, 2918−2929.
(28) Donkor, I. O. A survey of calpain inhibitors. *Curr. Med. Chem.* **2000**, *7*, 1171−1188.
(29) Dixon, M. The graphical determination of K_m and K_i. *Biochem. J.* **1972**, *129*, 197−202.
(30) Chatterjee, S.; Iqbal, M.; Kauer, J. C.; Mallamo, J. P.; Senadhi, S. et al. Xanthene derived potent nonpeptidic inhibitors of recombinant human calpain I. *Bioorg. Med. Chem. Lett.* **1996**, *6*, 1619−1622.
(31) Shaw, E. Cysteinyl proteinases and their selective inactivation. *Adv. Enzymol*. **1990**, *63*, 271−347.
(32) Inoue, J.; Nakamura, M.; Cui, Y.-S.; Sakai, Y.; Sakai, O.; Hill, J. R.; Wang, K. K. W.; and Yuen, P.-W. Structure−activity relationship study and drug profile of *N*-(4-fluorophenylsulfonyl)-L-valyl-L-leucinal (SJA6017) as a potent calpain inhibitor. *J. Med. Chem*. **2003**, *46*, 868−871.
(33) Lubisch, W.; Beckenbach, E.; bop, S.; hofmann, H.-P.; Kartal, A.; Kastel, C.; Lindner, T.; Garrecht-Metz, M.; Reeb, J.; Ferdinand, R.; Vierling, M.; and Moller, A. Benzoylalanine-derived ketoamides carrying vinylbenzyl amino residues: Discovery of potent water-soluble calpain inhibitors with oral bioavailability. *J. Med. Chem*. **2003**, *46*, 2404−2412.
(34) Bruno, S.; Lassota, P.; Giaretti, W.; and Darzynkiewicz, Z. Apoptosis of rat thymocytes triggered by prednisolone, camptothecin, or teniposide is selective to G0 cells and is prevented by inhibitors of proteases. *Oncol. Res*. **1992**, *4*, 29−35.
(35) Sarin, A.; Clerici, M.; Blatt, S. P.; Hendrix, C. W.; Shearer, G. M.; and Henkart, P. A. Inhibition of activation-induced programmed cell death and restoration of defective immune responses of HIV+ donors by cysteine protease inhibitors. *J. Immunol*. **1994**, *153*, 862−872.
(36) Chatterjee, S.; Iqbal, M.; Mallya, S.; Senadhi, S. E.; O'Kane, T. M.; McKenna, B. A.; Bozyczko-Coyne, D.; Kauer, J. C.; Siman, R.; Mallamo, J. P. Exploration of the importance of the P_2−P_3 −NHCO- moiety in a potent di- or tripeptide inhibitor of calpain I: Insights into the development of nonpeptide inhibitors of calpain I. *Bioorg. Med. Chem*. **1998**, *6*, 509−522.
(37) Vanderklish, P. W.; Bahr, B. A. The pathogenic activation of calpain: a marker and mediator of cellular toxicity and disease states. *Int. J. Exp. Pathol*. **2000**, *81*, 323−339.
(38) Unpublished data.

JM050849W