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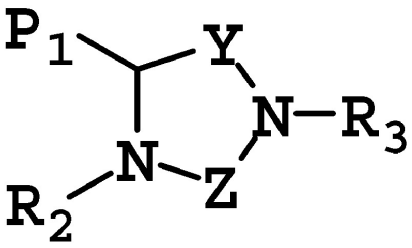

$Y=CH_2, Z=SO_2$ (I)

$CO, Z=SO_2$ (II)

$CO, Z=CH_2$ (III)

(P_1=primary specificity residue/diversity element

R_2, R_3=diversity elements)

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Potential Protease Inhibitors Based on a Functionalized Cyclic Sulfamide Scaffold

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Exploratory studies related to the design and synthesis of functionalized cyclic sulfamides (I) as potential inhibitors of proteolytic enzymes were carried out. The structural motif and three diversity sites embodied in the scaffold render it amenable to combinatorial parallel synthesis and the facile generation of lead discovery prospecting libraries. The scaffold was readily assembled starting with (DL) serine methyl ester, and a series of compounds was generated and screened against human leukocyte elastase. Modification of the P_1 recognition element, believed to be accommodated at the primary specificity site (S_1 subsite) of the enzyme, yielded compounds that inhibited the enzyme by an apparent hyperbolic partial mixed-type inhibition.

Introduction

Mammalian proteases are involved in a multitude of physiological processes, including blood coagulation, programmed cell death (apoptosis), signal transduction, and tissue remodeling.[1,2] The activity of proteases, such as human leukocyte elastase and proteinase 3, is ordinarily regulated by endogenous protein inhibitors; however, a mounting body of evidence suggests that the pathophysiology observed in a range of diseases, such as chronic obstructive pulmonary disease,[3−5] cancer,[6−8] and cystic fibrosis,[9,10] arises from an imbalance between the levels of these proteases and their physiological protein inhibitors. Agents capable of suppressing the aberrant activity of a protease are of potential value as probes for delineating the precise role of a protease in a particular disease and as potential therapeutic agents.[11−14]

Combinatorial chemistry is currently a powerful and integral component of the drug discovery process.[15,16] A critical factor associated with the construction of combinatorial libraries is the selection of an appropriate platform. The nature of the latter determines to a large extent the effectiveness and general applicability of a library in terms of lead identification and lead optimization. The use of a highly functionalized, nonpeptidyl template that provides an effective means for appending recognition elements in a specific vector relationship, binds to an enzyme (or receptor) in a predictable and substrate-like fashion, and is well-suited to using multiple binding sites to optimize potency and enzyme selectivity would offer several distinct advantages.

In previous studies, we have described the structure-based design of a novel heterocyclic scaffold (1,2,5-thiadiazolidin-3-one 1,1-dioxide) (II) (Figure 1) and have demonstrated that the scaffold is a general template suitable for the design of covalent and noncovalent inhibitors of mammalian and bacterial proteases and exhibits absolute selectivity between neutral, basic, and acidic serine proteases. We have furthermore demonstrated that scaffold II is amenable to the solution and solid-phase construction of libraries for lead identification and the rapid optimization of enzyme potency and selectivity.[17,18] More recently, we have used II as a prototype in the design of a range of surrogate scaffolds which mimic II in terms of the spatial orientation of the attached recognition elements. Thus, appropriate derivatives of 4-imidazolidinone (III) and cyclosulfamide (I) were found to inhibit human leukocyte elastase.[19,20] We describe herein the results of further exploratory studies related to the design and synthesis of highly functionalized cyclosulfamides as potential inhibitors of human leukocyte elastase and related proteolytic enzymes.

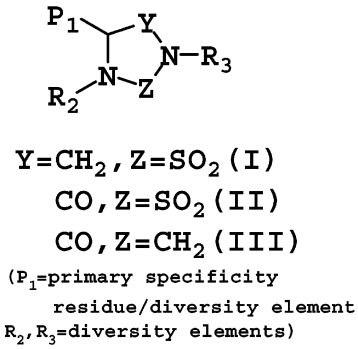

Figure 1.

Results and Discussion

Design Rationale. In designing scaffolds I−III, the X-ray crystal structure of the HLE-TOMI complex[21] (PDB 1HNL) was utilized. In this complex, the protein inhibitor occupies an extended binding region stretching from S_5 to S_3',[22] corresponding to residues −Pro-Ala-Cys-Thr-Leu-Glu-Tyr-Arg− of the inhibitor (Figure 2). The P_3−P_1 residues (−Cys-Thr-Leu−) of TOMI form an antiparallel β-sheet binding arrangement with the Ser-214 to Val-216 segment of HLE.

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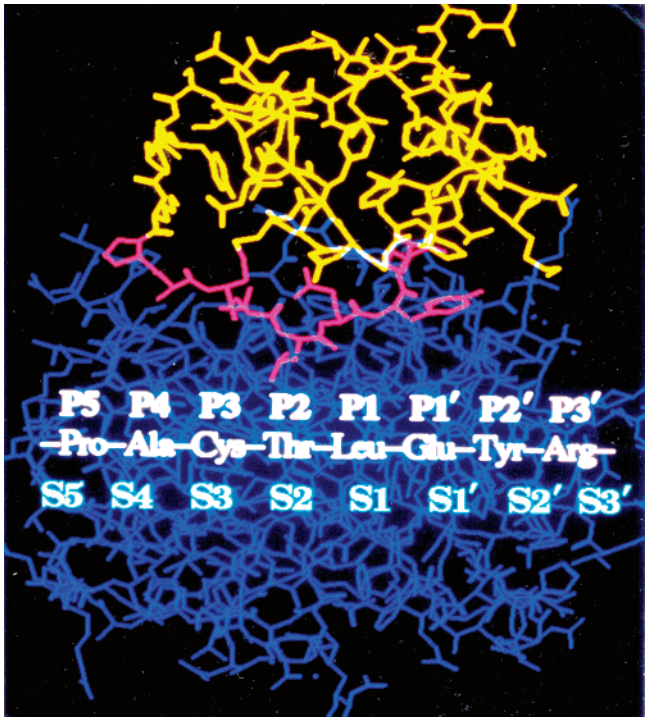


Figure 2. Human leukocyte elastase/turkey ovomucoid inhibitor complex. The enzyme and protein inhibitor are colored in blue and yellow, respectively. The active site of the enzyme interacts with the eight amino acid residues shown (colored in red).

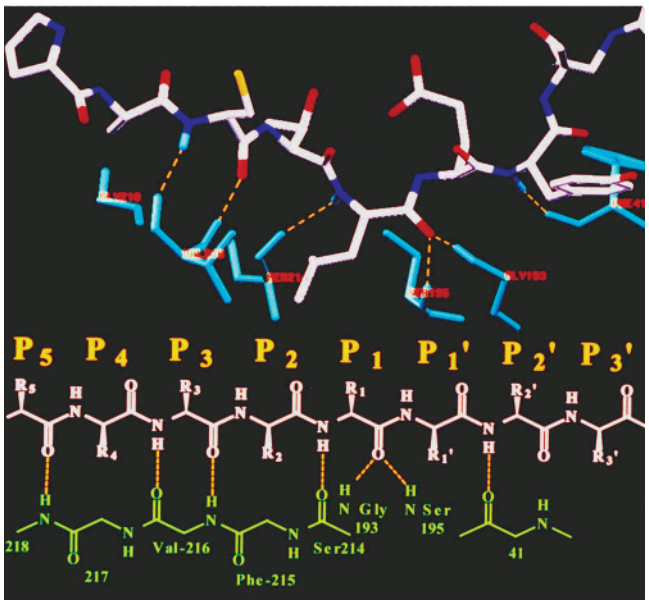


Figure 3. Network of hydrogen bonds and hydrophobic binding interactions between human leukocyte elastase and a peptidyl substrate.

Several hydrogen bonds are observed, including a pair of hydrogen bonds between the P_3 residue of the inhibitor and Val-216 of the enzyme.[23]

This is analogous to the network of hydrogen bonds and hydrophobic binding interactions observed between HLE and peptidyl substrates (Figure 3).[23] The backbone conformation of the inhibitor recognition loop (residues P_2-P_2') was locked by linking the nitrogen atoms of the P_1 and P_1' residues with an SO_2 or CH_2 group, thereby generating cyclic templates I−III (Figure 1). It was anticipated that the conversion of the flexible peptide inhibitor into rigid cyclic structures I−III would provide an entropic advantage that would more than compensate for the loss of one of the hydrogen bonds between HLE and TOMI. Other structural modifications included the attachment of nonpeptidyl recognition elements that would interact with the S and S′ subsites of the target enzyme and, in the case of template I, replace the carbonyl group with a methylene group to obtain *noncovalent* reversible competitive inhibitors of the enzyme.

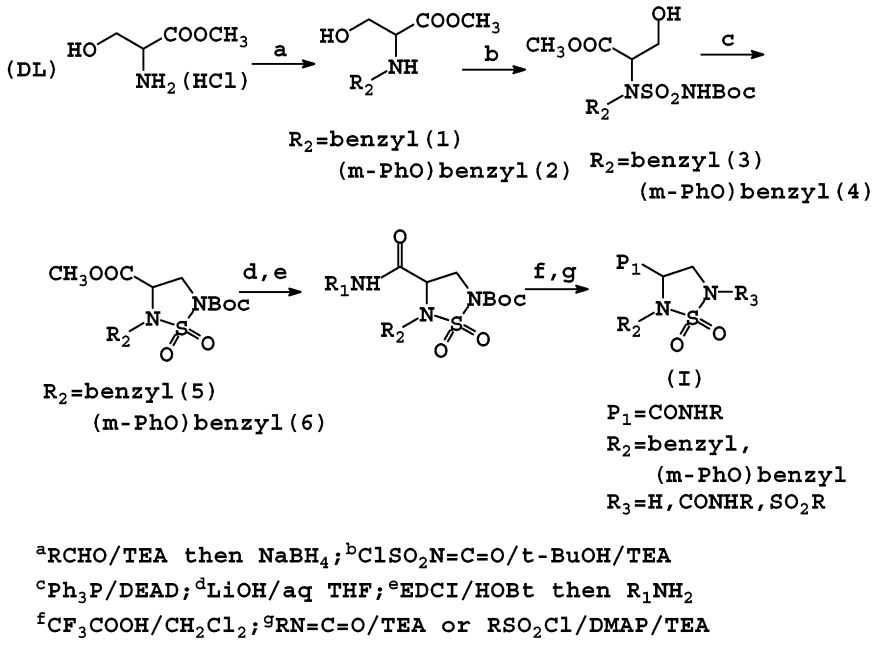
Importantly, sufficient flexibility is embodied in template I so that, in addition to diversity elements R_2 and R_3, the primary specificity residue P_1 was intended to serve both as a sensitive probe of the S_1 subsite and as a third diversity element (vide infra). As stated above, our initial general objective was the exploration of scaffold I and its use in the development of noncovalent inhibitors of HLE and related serine proteases.

The reaction sequence shown in Scheme 1 was used in the synthesis of compounds **8**−**26** (Table 1).[24] Thus, reductive amination of (DL) serine methyl ester hydrochloride with an appropriate aldehyde in the presence of sodium borohydride yielded the alkylated product.[25] The resulting N-substituted serine methyl ester was then reacted with the adduct of *N*-chlorosulfonyl isocyanate and *tert*-butyl alcohol (generated in situ) to give a substituted sulfamide, which was then used to generate the protected cyclic sulfamide using an intramolecular Mitsunobu reaction.The resulting intermediate was deblocked and elaborated further to give a series of functionalized cyclic sulfamides (listed in Table 1) that were screened for inhibitory activity toward HLE. The primary specificity residue (P_1), accommodated at the S_1 subsite of the enzyme, was used both as a diversity element and to probe the structural requirements of the S_1 subsite.

The inhibitory activity of compounds **8**−**26** toward HLE was then determined, and it was found to range from 0 to 66% under the conditions specified in Table 1. Compounds **15** and **25** were the most active (52 and 66% inhibition, respectively); consequently, a detailed kinetics study was undertaken to gain insight into the binding of these compounds to the active site of the enzyme. Both compounds showed curved Dixon plots.[26] A Lineweaver−Burk plot (Figures 4 and 5) of the kinetics data and a replot of the slope vs [I] and intercept vs [I] plot suggested that compounds **15** and **25** inhibit HLE via a hyperbolic mixed-type inhibition.[27] This would be in agreement with Scheme 2, where the inhibitor binds to either the initial ES complex or the free enzyme, and could also form a ternary ESI complex that still has the ability to turnover to produce product and EI complex with a different apparent K_p. According to Segel,[27] the pattern observed should result when a decrease in the apparent K_p, which increases the slope, competes with a decrease in the apparent K_s, which decreases the slope, with increasing inhibitor concentration. The decrease in apparent K_p is the dominant effect, resulting in the pattern shown in both Figures 4 and 5, which can be best described as hyperbolic partial mixed-type inhibition.[27]

However, the limit of solubility of these compounds precluded studying higher inhibitor concentrations to see if the slope of the inhibition curves obtained with compound

Scheme 1. Synthesis of Cyclosulfamide Derivatives (I)



[a]RCHO/TEA then $NaBH_4$;[b]$ClSO_2N{=}C{=}O$/t-BuOH/TEA
[c]Ph_3P/DEAD;[d]LiOH/aq THF;[e]EDCI/HOBt then R_1NH_2
[f]CF_3COOH/CH_2Cl_2;[g]$RN{=}C{=}O$/TEA or RSO_2Cl/DMAP/TEA

Table 1. Cyclosulfamide Derivatives (I)[a]

compd	P_1	R_2	R_3
8	$CONH(CH_2)_2Ph$	benzyl	Boc
9	$CONHCH_2CHPh_2$	benzyl	Boc
10	$CONH(CH_2)_2$-morpholino	benzyl	Boc
11	$CONH(CH_2)_2Ph$	benzyl	H
12	$CONHCH_2CHPh_2$	benzyl	H
13	$CONH(CH_2)_2$-morpholino	benzyl	H
14	$CONH(CH_2)_2Ph$	benzyl	$CONH(CH_2)_2Ph$
15	$CONHCH_2CHPh_2$	benzyl	$CONH(CH_2)_2Ph$
16	$CONH(CH_2)_2$-morpholino	benzyl	$CONH(CH_2)_2Ph$
17	$CONH(CH_2)_2Ph$	benzyl	SO_2CH_3
18	$CONHCH_2CHPh_2$	benzyl	SO_2CH_3
19	$CONH(CH_2)_2Ph$	benzyl	*p*-tolyl
20	$CONHCH_2CHPh_2$	benzyl	*p*-tolyl
21	$CONH(CH_2)_2$-morpholino	benzyl	*p*-tolyl
22	$COOCH_3$	(*m*-phenoxy)benzyl	
23	COOH	(*m*-phenoxy)benzyl	H
24	$CONHCH_2CHPh_2$	(*m*-phenoxy)benzyl	H
25	$CONHCH_2CHPh_2$	(*m*-phenoxy)benzyl	*p*-tolyl
26	$CONHCH_2CHPh_2$	(*m*-phenoxy)benzyl	$CONH(CH_2)_2Ph$

[a] Percent inhibitory activity was determined following incubation at an [I]/[E] of 500 for 30 min (see Results and Discussion for details). Compounds **15** and **25** were the most active and showed 52 and 66% inhibition, respectively.

s15 and **25** is actually approaching a limit. On the basis of the available data, it can be concluded that these compounds are inhibitors of HLE, have K_I's of 18 μM (compound **15)** and 10 μM (inhibitor **25)**, and that they most likely do not bind to the active site in a manner that excludes the substrate. The results are in sharp contrast to earlier findings that demonstrated that cyclosulfamide derivatives with P_1 = isobutyl and suitable recognition elements at R_2 and R_3 function as reversible competitive inhibitors of the enzyme.[20] Further exploratory studies aimed at delineating the structural determinants that influence the mode of binding in this series are currently in progress.

In conclusion, the cyclic sulfamide scaffold offers a means of generating high-diversity structures and libraries that can be used in lead identification by screening against a range of targets, including proteolytic enzymes.

Experimental Section

General. Melting points were recorded on a Mel-Temp apparatus and are uncorrected. ^{1}H NMR spectra of the synthesized compounds were recorded on Varian XL-300 or XL-400 spectrometers. Human leukocyte elastase was purchased from Elastin Products Co., Owensville, MO. Methoxysuccinyl Ala-Ala-Pro-Val *p*-nitroanilide was purchased from Sigma Chemicals Co., St Louis, MO. Aldrich 230−400 mesh silica gel was used for flash chromatography. Representative detailed synthetic procedures are described below.

Synthesis of 2-Benzylamino-3-hydroxy-propionic Acid Methyl Ester 1. A mixture of (DL) serine methyl ester hydrochloride (73.16 g; 475.5 mmol) in dry methanol (360 mL) was cooled in an ice bath and treated with triethylamine (66.8 mL; 0.476 mol). The ice bath was removed, and the

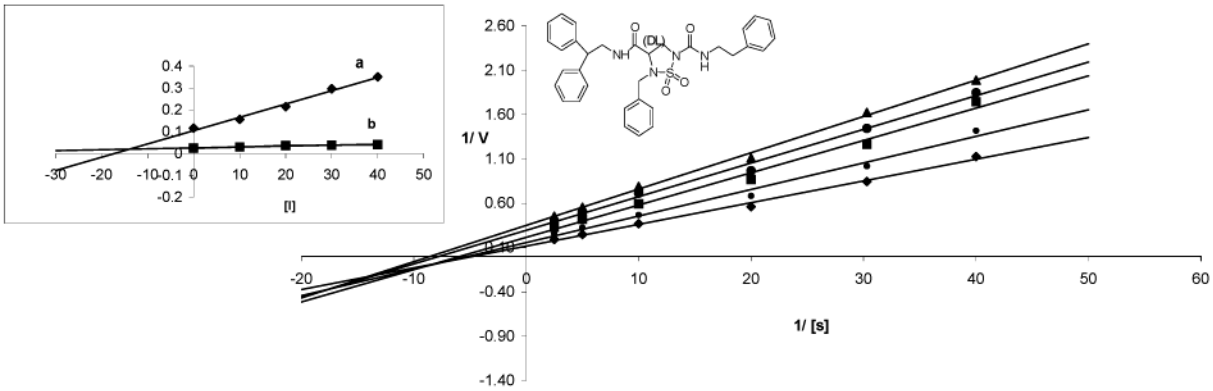


Figure 4. Lineweaver−Burk plot of compound **15** with HLE. As described in the Experimental Section, the inverse of the velocity of the reaction was graphed versus the inverse of the substrate concentration. The insert is the replot of the resulting slope (a) and intercept (b) against the inhibitor concentration. The K_I from the slope replot was found to be 18 μM, and the K_I from the intercept replot was found to be 63 μM.

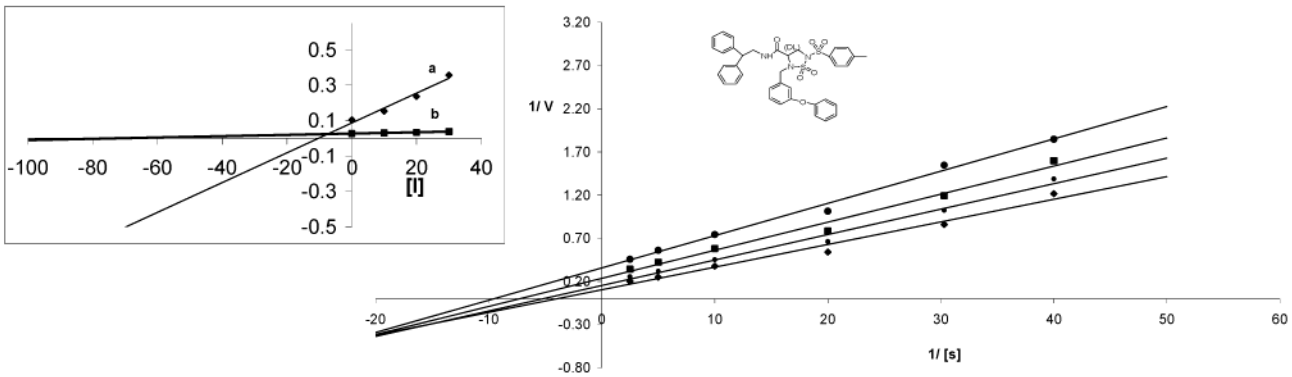
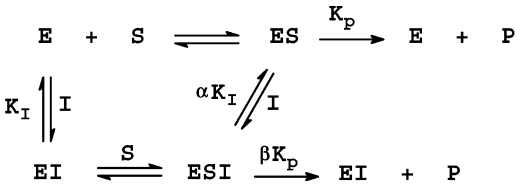


Figure 5. Lineweaver−Burk plot of compound **25** with HLE. As described in the Experimental Section, the inverse of the velocity of the reaction was graphed versus the inverse of the substrate concentration. The insert is the replot of the resulting slope (a) and intercept (b) against the inhibitor concentration. The K_I from the slope replot was found to be 10 μM, and the K_I from the intercept replot was found to be 70 μM.

Scheme 2



reaction mixture was stirred for 10 min. Benzaldehyde (48.6 mL; 0.476 mol) was added, and the reaction mixture was stirred for 2 h at room temperature. Sodium borohydride (33.8 g, 0.951 mol) was added over a period of 30 min. The solvent was removed to near dryness, and 5% aqueous HCl (500 mL) was added. The resulting solution was washed with ethyl ether (500 mL), and the aqueous phase was cooled in an ice bath and treated with a 10% aqueous NaOH solution to pH 9−10 with stirring. The aqueous solution was extracted with ethyl acetate (3 × 700 mL), and the combined organic extracts were dried over anhydrous sodium sulfate. The solvent was removed, and the crude product was purified by flash chromatography (silica gel/ethyl acetate/hexane) to give compound **1**[24] (67.97 g, 68% yield). ^{1}H NMR ($CDCl_3$): δ 3.42 (dd, 1H), 3.62 (dd, 1H), 3.68−3.80 (m, 5H), 3.87 (d, 1H), 7.20−7.40 (m, 5H).

Synthesis of 2-(*m*-Phenoxy)benzylamino-3-hydroxypropionic Acid Methyl Ester 2. A mixture of (DL) serine methyl ester hydrochloride (7.32 g; 48 mmol) in dry methanol (36 mL) was cooled in an ice bath and treated with triethylamine (4.90 g; 48 mmol). The ice bath was removed, and the mixture was stirred for 10 min. 3-Phenoxybenzaldehyde (9.9 g; 48 mmol) was added, and the reaction mixture was stirred for 2 h at room temperature. Sodium borohydride (3.40 g; 95 mmol) was then added over a period of 30 min, and the solution was stirred for another 2.5 h. The solvent was removed to near dryness, and 20% aqueous HCl (200 mL) was added. The resulting solution was extracted with ethyl ether (200 mL), and the aqueous phase was cooled in the ice bath and treated with 10% NaOH solution to pH ∼10 with stirring. The aqueous solution was extracted with ethyl acetate (3 × 70 mL), and the combined organic extracts were dried over anhydrous sodium sulfate. The solvent was removed, and the crude product was purified by flash chromatography (silica gel/ethyl acetate/hexane) to give the desired compound (9.73 g, 67% yield). ^{1}H NMR ($CDCl_3$): δ 2.30−2.40 (br s, 1H), 3.39−3.42 (dd, 1H), 3.59−3.64 (q, 2H), 3.66−3.90 (dd, 2H), 3.72−3.74 (s,3H), 6.84−7.40 (m, 9H). Anal. Calcd for $C_{17}H_{19}NO_4$: C, 69.62; H, 6.48; N, 4.78. Found: C, 69.81; H, 6.74; N, 4.77.

Synthesis of 3. A solution of *tert*-butyl alcohol (0.66 g, 8.89 mmol) in dry methylene chloride (6 mL) was added dropwise to a solution of *N*-chlorosulfonyl isocyanate (1.26 g; 8.89 mmol) in dry methylene chloride (15 mL) kept in an ice bath with stirring. After stirring for 15 min at 0 °C, the

resulting mixture was added dropwise to a solution of compound **1** (1.86 g; 8.89 mmol) and TEA (0.99 g; 14.8 mmol) in dry methylene chloride (18 mL) kept in an ice bath. The ice bath was removed, and the reaction mixture was stirred for 3 h. The reaction mixture was then washed with water (2 × 20 mL), and the organic phase was separated and dried over anhydrous sodium sulfate. The solvent was removed, and the crude product was purified by flash chromatography (silica gel/ethyl acetate/hexane) to give compound **3** (2.63 g, 76% yield). ^{1}H NMR ($CDCl_3$): δ 1.50 (s, 9H), 2.82 (t, 1H), 3.68 (s, 3H), 3.98 (t, 2H), 4.52−4.70 (m, 3H), 7.25−7.49 (m, 5H). Anal. Calcd for $C_{16}H_{24}N_2O_7S$: C, 49.48; H, 6.19; N, 7.22. Found: C, 49.36; H, 5.97; N, 7.02.

Synthesis of 4. A solution of *tert*-butyl alcohol (3.13 g; 42 mmol) in dry methylene chloride (15 mL) was added dropwise to a solution of *N*-chlorosulfonyl isocynate (6.0 g; 42 mmol) in dry methylene chloride (15 mL) kept in an ice bath with stirring. After 10 min at 0 °C, the resulting mixture was added to a solution of compound **2** (12.73 g; 42 mmol) and triethylamine (4.41 g; 42 mmol) in dry methylene chloride (20 mL) kept in an ice bath. The ice bath was removed, and the reaction mixture was stirred overnight. The reaction mixture was transferred to a separatory funnel and washed with 5% aqueous HCl (50 mL), 5% aqueous $NaHCO_3$ (50 mL), and brine (50 mL). The organic layer was dried over anhydrous sodium sulfate, and the solvent was removed, leaving a crude product which was purified by flash chromatography (silica gel/ethyl acetate/hexane) to give compound **4** (9.14 g, 53% yield). ^{1}H NMR ($CDCl_3$): δ 1.44−1.50 (s, 9H), 2.70−2.90 (br s,1H), 3.64−3.74 (s, 3H), 3.90−4.05 (dd, 2H), 4.48−4.70 (dd, 2H), 4.60−4.68 (m, 1H), 6.80−7.30 (m, 9H). Anal. Calcd for $C_{22}H_{28}N_2O_8S$: C, 55.00; H, 5.83; N, 5.83. Found: C, 55.29; H, 5.82; N, 5.86.

5-Benzyl-1,1-dioxo-1λ^6-[1,2,5]thiadiazolidin-2,4-dicarboxylic Acid 2-*tert*-Butyl Ester 4-Methyl Ester 5. A solution of compound **3** (3.36 g; 8.65 mmol) in dry THF (24 mL) was treated with triphenyl phosphine (4.58 g; 17.30 mmol) and diethyl azodicarboxylate (DEAD) (2.7 mL; 17.30 mmol). The reaction mixture was stirred for 3 h at room temperature. Removal of the solvent yielded a crude product which was purified by flash chromatography (silica gel/ethyl acetate/hexane) to give compound **5** (1.09 g; 34% yield). ^{1}H NMR ($CDCl_3$): $\boldsymbol{\delta}$ 1.55 (s, 9H), 3.81(s, 3 H), 3.89 (m, 2H), 4.03 (m, 1H), 4.49 (dd, 2H), 7.25−7.42 (m, 5H). Anal. Calcd for $C_{16}H_{22}N_2O_6S$: C, 51.89; H, 5.95; N, 7.57. Found: C, 51.76; H, 6.07; N, 7.52.

5-(*m*-Phenoxy)benzyl-1,1-dioxo-1λ^6-[1,2,5]thiadiazolidin-2,4-dicarboxylic Acid 2-*tert*-Butyl Ester 4-Methyl Ester 6. A solution of compound **4** (1.51 g; 3.7 mmol) in dry tetrahydrofuran (20 mL) under N_2 was treated with triphenylphosphine (1.95 g; 7.4 mmol) and diethyl azodicarboxylate (1.29 g; 7.4 mmol). The reaction mixture was stirred overnight. The solvent was removed, and the crude product was purified by flash chromatography (silica gel/ethyl acetate/hexane) to give compound **6** (1.16 g, 76% yield). ^{1}H NMR ($CDCl_3$): δ 1.53−1.57 (s, 9H), 3.71−3.74 (s, 3H), 3.90−3.93 (d, 2H), 4.0−4.09 (m, 1H), 4.37−4.55 (dd, 2H), 6.94−7.40 (m, 9H). Anal. Calcd for $C_{22}H_{26}N_2O_7S$. Found: C, 57.14; H, 5.63; N, 6.06. Found: C, 56.83; H, 5.94; N, 5.85.

5-Benzyl-1,1-dioxo-1λ^6-[1,2,5]thiadiazolidin-2,4-dicarboxylic Acid 2-*tert*-Butyl Ester 7. A solution of compound **5** (2.28 g; 6.99 mmol) in dry THF (15 mL) was cooled in an ice bath and treated with a solution of lithium hydroxide monohydrate (0.61 g; 13.98 mmol) in water (15 mL). The reaction mixture was stirred for 0.5 h at 0 °C. A solution of 5% aqueous HCl(100 mL) was added, and the mixture was extracted with ethyl acetate (3 × 200 mL). The organic extracts were combined and dried over anhydrous sodium sulfate. The solvent was removed, and the crude product was purified by flash chromatography (ethyl acetate/hexane) to give compound **7** (1.83 g; 73% yield). ^{1}H NMR (DMSO-d_6): δ 1.48 (s, 9H), 3.94 (m, 2H), 4.18 (dd, 1H), 4.44 (dd, 2H), 7.20−7.59 (m, 5H), 13.55 (br s, 1H). Anal. Calcd for $C_{15}H_{20}N_2SO_6$: C, 50.56; H, 5.62, N, 7.87. Found: C, 50.31; H, 5.67; N, 7.70.

Synthesis of Compounds 8−12. 5-Benzyl-1,1-dioxo-4-phenethylcarbamoyl-1λ^6-[1,2,5]thiadiazolidin-2-carboxylic Acid *tert*-Butyl Ester 8. A solution of compound **7** (1.70 g; 5.44 mmol) in dry DMF (17 mL) was treated with EDCI (1.15 g; 5.88 mmol), followed by *N*-hydroxybenzotriazole hydrate (1.50 g; 10.88 mmol) and 2-phenethylamine (0.69 g; 5.44 mmol). The reaction mixture was stirred for 4 h at room temperature. Ethyl acetate (150 mL) was added, and the resulting solution was washed with 5% aqueous HCl (2 × 20 mL), 5% aqueous $NaHCO_3$ (2 × 20 mL), and brine (2 × 20 mL). It was then dried over anhydrous sodium sulfate. The solvent was removed, and the crude product was purified by flash chromatography (ethyl acetate/hexane) to give compound **8** (1.99 g; 80% yield). ^{1}H NMR ($CDCl_3$): δ 1.55 (s, 9H), 2.73 (m, 2H), 3.38 (m, 1H), 3.49 (m, 1H), 3.78 (dd, 1H), 3.92 (t, 1H), 3.98 (dd, 1H), 4.16 (d, 1H), 4.35 (d, 1H), 6.60 (br s, 1H), 7.15−7.40 (m, 10H). Anal. Calcd for $C_{23}H_{29}N_3O_5S$: C, 60.13; H, 6.32; N, 9.15. Found: C, 60.25; H, 6.62; N, 9.09.

5-Benzyl-4-(2,2-diphenylethylcarbamoyl)-1,1-dioxo-1λ^6-[1,2,5]thiadiazolidin-2-carboxylic Acid *tert*-Butyl Ester 9. Compound **9** was synthesized using 2,2,-diphenylethylamine as the amine component and a similar procedure as the one used in the preparation of compound **8**. (1.48 g; 29% yield). ^{1}H NMR ($CDCl_3$): δ 1.55 (s, 9H), 3.69 (m, 1H), 3.85 (m, 4H), 3.98 (d, 1H), 4.09 (d, 1H), 4.17 (t, 1H), 6.65 (br s, 1H), 6.98−7.40 (m, 15H). Anal. Calcd for $C_{29}H_{33}N_3O_5S$: C, 65.05; H, 6.17; N, 7.85. Found: C, 64.93; H, 5.97; N, 7.74.

5-Benzyl-4-(2-morpholin-4-yl-ethylcarbamoyl)-1,1-dioxo-1λ^6-[1,2,5]thiadiazolidin-2-carboxylic Acid *tert*-Butyl Ester 10. Using 4-(2-aminoethyl)morpholine as the amine component, compound **10** was obtained in 27% yield (1.25 g). ^{1}H NMR ($CDCl_3$): δ 1.52 (s, 9H), 2.30−2.55 (m, 6H), 3.25 (m, 2H), 3.70 (m, 4H), 3.85 (dd, 1H), 3.95 (t, 1H), 4.18 (dd, 1H), 4.24 (d, 1H), 4.59 (d, 2H), 7.05−7.50 (m, 6H). Anal. Calcd for $C_{22}H_{32}N_4O_6S$: C, 55.00; H, 6.67; N, 11.67. Found: 55.10; H, 6.58; N, 11.50.

Synthesis of Compounds 11−13. 2-Benzyl-1,1-dioxo-1λ^6-[1,2,5]thiadiazolidin-3-carboxylic Acid Phenethylamide 11. Compound **8** (1.89 g; 4.11 mmol) in dry

methylene chloride (20 mL) was treated with TFA (5 mL), and the reaction mixture was stirred for 0.5 h at room temperature. The solvent was removed on the rotary evaporator, ethyl acetate (150 mL) was added, and the resulting solution was washed with 5% aqueous $NaHCO_3$ (2 × 30 mL) and brine (30 mL) and dried over anhydrous sodium sulfate. The solvent was removed, and the crude product was purified by flash chromatography (ethyl acetate/hexane) to give compound **11** (1.13 g; 76% yield). 1H NMR ($CDCl_3$) ***δ*** 2.65 (m, 2H), 3.19 (m, 1H), 3.20 (m, 1H), 3.48 (m, 1H), 3.61 (m, 1H), 3.77 (dd, 1H), 4.00 (d, 1H), 4.39(d, 1H), 4.50 (t, 1H), 6.50 (b, 1H), 7.10−7.21 (m, 10H). Anal. Calcd for $C_{18}H_{21}N_3O_3S$: C, 60.17; H, 5.85; N, 11.70. Found: C, 60.42; H, 5.69; N, 11.65.

2-Benzyl-1,1-dioxo-1λ⁶-[1,2,5]thiadiazolidin-3-carboxylic Acid (2,2-Diphenylethyl)-amide 12. Compound **9** (1.44 g; 2.69 mmol) was treated with 4 M HCl in 1,4-dioxane (50 mL; 200 mmol). The reaction mixture was stirred for 3 h at room temperature, and the solvent was removed on the rotary evaporator. Ethyl acetate (70 mL) was added to the residue, and the resulting solution was washed with 5% aqueous HCl (2 × 20 mL), 5% aqueous $NaHCO_3$ (2 × 20 mL), and brine (2 × 20 mL) and dried over anhydrous sodium sulfate. The solvent was removed to give crude compound **12**, which was purified using flash chromatography (1.23 g; 100% yield).[1]H NMR ($CDCl_3$): *δ* 3.1−3.2 (dd, 1H), 3.4−3.7 (m, 3H), 4.0−4.2 (m, 4H), 6.6 (br s, 1H), 7.2−7.4 (m, 15H). Anal. Calcd for $C_{24}H_{18}N_3O_3S$: C, 67.29; H, 4.21; N, 9.81. Found: C, 67.10; H, 4.26 N, 9.70.

2-Benzyl-1,1-dioxo-1λ⁶-[1,2,5]thiadiazolidin-3-carboxylic Acid (2-Morpholin-4-yl-ethyl)-amide 13. Compound **13** was synthesized using a procedure similar to that described above (1.38 g; 100% yield). [1]H NMR (DMSO-d_6): *δ* 3.01 (m, 4H), 3.22−3.46 (m, 5H), 3.48−3.59 (m, 3H), 3.88 (m, 5H), 4.15 (d, 1H), 4.29 (d, 1H), 7.20−7.60 (m, 5H), 8.22 (br s, 1H). Anal. Calcd for $C_{16}H_{24}N_4O_4S$: C, 52.17; H, 6.52; N, 15.22. Found: C, 52.27; H, 6.41; N, 15.11.

Synthesis of compounds 14−16. 5-Benzyl-1,1-dioxo-1λ⁶-[1,2,5]thiadiazolidin-2,4-dicarboxylic Acid Bis(phenethylamide) 14. A solution of compound **11** (0.30 g; 0.83 mmol) and TEA (0.09 g; 0.83 mmol) in dry methylene chloride (3 mL) was treated with 2-phenethyl isocyanate (0.17 g; 1.14 mmol). The reaction mixture was refluxed for 1.5 h with stirring. After removing the solvent on the Rotovac, 5% aqueous HCl (25 mL) was added, and the resulting mixture was then extracted with ethyl acetate (2 × 75 mL). The organic extracts were combined and dried over anhydrous sodium sulfate. The solvent was removed, and the crude product was purified by flash chromatography (ethyl acetate/hexane) to give compound **14** (0.09 g; 21% yield). [1]H NMR ($CDCl_3$): *δ* 2.65 (m, 2H), 3.90 (t, 3H), 3.24 (m, 1H), 3.45 (m, 1H), 3.80 (dd, 1H), 3.88 (m, 2H), 4.07 (dd, 1H), 4.25 (dd, 1H), 4.37 (m, 2H), 6.40 (b, 1H), 7.08−7.45 (m, 15H), 7.55 (br s, 1H). Anal. Calcd for $C_{27}H_{30}N_4O_4S$: C, 64.03; H, 5.93; N, 11.07. Found: C, 63.89; H, 5.98; N, 10.87.

Compounds **15** and **16** were synthesized using a similar procedure.

5-Benzyl-1,1-dioxo-1λ⁶-[1,2,5]thiadiazolidin-2,4-dicarboxylic Acid 4-[(2,2-Diphenyl-ethyl)-amide] 2-(Phenethylamide) 15. (0.43 g; 75% yield). [1]H NMR ($CDCl_3$): *δ* 2.82 (t, 2H), 3.40−3.61 (m, 2H), 3.67 (m, 2H), 3.85 (m, 2H), 3.99 (m, 2H), 4.10 (m, 2H), 5.92 (br s, 1H), 6.24 (br s,1H), 6.95−7.38 (m, 20H). Anal. Calcd for $C_{33}H_{34}N_4O_4S$: C, 68.04; H, 5.84; N, 9.62. Found: C, 67.89; H, 5.86; N, 9.50.

5-Benzyl-1,1-dioxo-1λ⁶-[1,2,5]thiadiazolidin-2,4-dicarboxylic Acid 4-[(2-Morpholin-4-yl-ethyl)-amide] 2-(Phenethylamide) 16. (0.24 g; 44% yield). [1]H NMR ($CDCl_3$): *δ* 2.30−2.50 (m, 6H), 2.84 (t, 2H), 3.18 (m,1H), 3.25 (m, 1H), 3.55 (m, 2H), 3.70 (m, 4H), 3.85 (dd, 1H), 4.01−4.20 (m, 3H), 4.56 (d, 1H), 5.99 (br s, 1H), 7.04 (br s, 1H), 7.17−7.25 (m, 10H). Anal. Calcd for $C_{25}H_{33}N_5O_5S$: C, 58.25; H, 6.41; N, 13.59. Found: C, 58.40; H, 6.30; N, 13.45.

2-Benzyl-5-methanesulfonyl-1,1-dioxo-1λ⁶-[1,2,5]thiadiazolidin-3-carboxylic Acid Phenethylamide 17. To a solution of TEA (0.42 g; 4.14 mmol) and 4-*N*,*N*-(dimethylamino)pyridine (0.03 g; 0.25 mmol) in dry acetonitrile (3 mL) was added compound **11** (0.33 g, 0.92 mmol). A solution of methanesulfonyl chloride (0.11 g; 0.92 mmol) in dry acetonitrile (3 mL) was added dropwise with stirring, and the reaction mixture was stirred for an additional 1.5 h at room temperature. The solvent was removed, ethyl acetate (100 mL) was added, and the resulting solution was washed with 5% aqueous HCl (2 × 25 mL), 5% aqueous $NaHCO_3$ (2 × 25 mL), and brine (2 × 25 mL). The organic phase was dried over anhydrous sodium sulfate. Removal of the solvent yielded a crude product which was purified by flash chromatography (ethyl acetate/hexane) to give compound **17** (0.24 g; 60% yield). [1]H NMR ($CDCl_3$): *δ* 2.68 (m, 2H), 3.18 (s, 3H), 3.28 (m,1H), 3.49 (m, 1H), 3.84 (dd, 1H), 4.04 (t, 1H), 4.09−4.23 (m, 2H), 4.55 (d, 1H), 6.39 (br s, 1H), 7.10−7.47 (m, 10H). Anal. Calcd for $C_{19}H_{23}N_3O_5S_2$: C, 52.66; H, 4.39; N, 16.17. Found: C, 52.38; H, 4.17; N, 16.32.

Compound **18** was synthesized using a similar procedure.

2-Benzyl-5-methanesulfonyl-1,1-dioxo-1λ⁶-[1,2,5]thiadiazolidin-3-carboxylic Acid (2,2-Diphenylethyl)-amide 18. (0.20 g; 50% yield). [1]H NMR (DMSO-d_6): *δ* 3.30 (s, 3H), 3.72 (m, 3H), 3.95 (m, 2H), 4.05−4.15 (m, 3H), 7.05−7.50 (m, 15H), 8.24 (br s, 1H). Anal. Calcd for $C_{25}H_{27}N_3O_5S_2$: C, 58.48; H, 5.26; N, 8.19. Found: C, 58.60; H, 5.31; N, 8.02.

Synthesis of Compounds 19−21. 2-Benzyl-1,1-dioxo-5-(toluene-4-sulfonyl)-1λ⁶-[1,2,5]thiadiazolidin-3-carboxylic Acid Phenethylamide 19. A solution of TEA (0.42 g; 4.14 mmol) and DMAP (0.03 g; 0.25 mmol) in dry acetonitrile (3 mL) was treated with compound **11** (0.33 g, 0.92 mmol). A solution of *p*-toluenesulfonyl chloride (0.18 g; 0.92 mmol) in dry acetonitrile (3 mL) was then added dropwise with stirring. The reaction mixture was stirred for an additional 1.5 h at room temperature. The solvent was removed, ethyl acetate (100 mL) was added, and the resulting solution was washed with 5% aqueous HCl (2 × 25 mL), 5% aqueous $NaHCO_3$ (2 × 25 mL), and brine (2 × 25 mL). The organic phase was dried over anhydrous sodium sulfate. Removal of the solvent yielded a crude product which was purified by flash chromatography (ethyl acetate/hexane) to give compound **19** (0.44 g; 89% yield). [1]H NMR ($CDCl_3$): *δ* 2.45 (s, 3H), 2.50 (m, 1H), 2.89 (m, 1H), 3.15 (m, 1H), 3.24 (m, 1H), 3.75 (dd, 1H), 3.84 (dd, 1H), 4.03 (m, 2H),

4.43 (d, 1H), 6.38 (br s, 1H), 7.05−7.45 (m, 12H), 7.92 (d, 2H). Anal. Calcd for $C_{25}H_{27}N_3O_5S_2$: C, 58.48; H, 5.26; N, 8.19. Found: C, 58.70; H, 5.12; N, 7.98.

Compounds **20** and **21** were prepared using a similar procedure.

2-Benzyl-1,1-dioxo-5-(toluene-4-sulfonyl)-1λ^6-[1,2,5]thiadiazolidin-3-carboxylic Acid (2,2-Diphenylethyl)-amide 20. (0.28 g; 61% yield). ^{1}H NMR ($CDCl_3$): δ 2.35 (s, 3H), 3.46−4.03 (m, 3H), 3.83 (dd, 1H), 3.93 (m, 3H), 4.14 (d, 1H), 6.37 (br s, 1H), 6.87 (d, 2H), 7.09−7.39 (m, 15H), 7.88 (d, 2H). Anal. Calcd for $C_{31}H_{31}N_2O_5S_2$: C, 64.70; H, 5.39; N, 4.87. Found: 64.82; H, 5.12; N, 4.80.

2-Benzyl-1,1-dioxo-5-(toluene-4-sulfonyl)-1λ^6-[1,2,5]thiadiazolidin-3-carboxylic Acid (2-Morpholin-4-yl-ethyl)-amide 21. (0.24 g; 66% yield). ^{1}H NMR ($CDCl_3$): δ 2.27 (m, 2H), 2.35 (m, 4H), 2.45 (s, 3H), 3.08 (dd, 2H), 3.19 (m, 4H), 3.59 (dd, 1H), 3.90−4.05 (m, 2H), 4.09 (d, 1H), 4.59 (d, 1H), 7.05 (br s, 1H), 7.25−7.45 (m, 7H), 7.89 (d, 2H). Anal. Calcd for $C_{23}H_{30}N_4O_6S_2$: C, 52.87; H, 5.75; N, 10.73. Found: C, 52.96; H, 5.82; N, 10.81.

2-(*m*-Phenoxy)benzyl-1,1-dioxo-1λ^6-[1,2,5]thiadiazolidin-3-carboxylic Acid Methyl Ester 22. A solution of compound **6** (1.96 g; 5.03 mmol) in dry methylene chloride (1.5 mL) was treated with trifluoroacetic acid (6 mL), and the reaction mixture was stirred for 2 h. The solvent was removed, and ethyl acetate (50 mL) was added. The solution was washed with saturated $NaHCO_3$ (20 mL) and brine (20 mL). The organic layer was dried over anhydrous sodium sulfate, and the solvent was removed, leaving a crude product which was purified by flash chromatography (silica gel/ethyl acetate/hexane) to give compound **22** (1.59 g, 99% yield). ^{1}H NMR ($CDCl_3$): δ 3.60−3.63 (m, 2H), 3.62−3.64 (s, 3H), 3.88−3.97 (dd, 1H), 4.37−4.38 (d, 2H), 4.70−4.80 (t, 1H), 6.90−7.39 (m, 9H). Anal. Calcd for $C_{17}H_{18}N_2O_5S$: C, 56.35; H, 4.97; N, 7.73. Found: C, 56.26; H, 4.80; N, 7.80.

2-(*m*-Phenoxy)benzyl-1,1-dioxo-1λ^6-[1,2,5]thiadiazolidin-3-carboxylic Acid 23. A solution of lithium hydroxide (70 mg; 2.8 mmol) in H_2O (3 mL) was added to a solution of compound **22** (0.41 g; 1.05 mmol) in tetrahydrofuran (3 mL) kept in an ice bath. The reaction mixture was stirred for 30 min. A solution of 5% HCl (50 mL) was added, the solution was extracted with ethyl acetate (3 × 20 mL), and the combined organic extracts were dried over anhydrous sodium sulfate. The solvent was removed, and the crude product was purified by flash chromatography (silica gel/ethyl acetate/hexane) to give compound **23** (0.37 g, 94% yield). ^{1}H NMR ($CDCl_3$): δ 3.52−3.73 (d, 2H), 3.82−3.94 (t, 1H), 4.29−4.52 (q, 2H), 4.98−5.62 (bs, 1H), 5.62−6.00 (br s, 1H), 6.90−7.39 (m, 9H). Anal. Calcd for $C_{16}H_{16}N_2O_4S$: C, 57.83; H, 4.82; N, 8.43. Found: C, 58.02; H, 4.73; N, 8.37.

2-(*m*-Phenoxy)benzyl-1,1-dioxo-1λ^6-[1,2,5]thiadiazolidin-3-carboxylic Acid (2,2-Diphenylethyl)-amide 24. A solution of compound **23** (2.4 g; 6.9 mmol) in dry DMF (25 mL) was treated with 1-[3-(dimethylamino)propyl]-3-ethylcarbodiimide hydrochloride (1.48 g; 7.6 mmol), followed by 1-hydroxybenzotriazole hydrate (1.9 g; 13.8 mmol) and 2,2-diphenylethylamine (1.4 g; 6.9 mmol). The reaction mixture was stirred for 5 h at room temperature. Ethyl acetate (220 mL) was added, and the organic layer was washed with 5% aqueous HCl (20 mL), 5% aqueous $NaHCO_3$ (20 mL), and brine (20 mL). The organic layer was dried over anhydrous sodium sulfate, and the solvent was removed, leaving a crude product which was purified by flash chromatography (silica gel/ethyl acetate/hexane) to give compound **24** (1.3 g, 36% yield). ^{1}H NMR ($CDCl_3$): δ 3.12−3.19 (dq, 1H), 3.43−3.54 (m, 2H), 3.65−3.69 (dd, 1H), 3.75−3.82 (dd, 1H), 3.97−4.14 (q, 2H), 4.16−4.22 (t, 1H), 6.65−6.71 (q, 1H), 6.62−7.39 (m, 19H). Anal. Calcd for $C_{30}H_{29}N_3O_4S$: C, 69.36; H, 5.59; N, 8.09. Found: C, 69.45; H, 5.50; N, 8.24.

2-(m-Phenoxy)benzyl-1,1-dioxo-1λ^6-[1,2,5]thiadiazolidin-2,4-dicarboxylic Acid 4-[(2,2-Diphenylethyl)-amide]-2-phenethylamide 25. A solution of compound **24** (0.40 g; 0.76 mmol) in dry acetonitrile (15 mL) was treated with triethylamine (0.16 g; 1.52 mmol) and DMAP (0.04 g). *p*-Toluenesulfonyl chloride (0.15 g; 0.76 mmol) in dry acetonitrile (15 mL) was added dropwise, and the reaction mixture was stirred for 3 h at room temperature. The solvent was removed, and the residue was dissolved in ethyl acetate (80 mL) and then washed with 5% aqueous HCl (50 mL), 5% aqueous $NaHCO_3$ (50 mL), and brine (50 mL). The organic layer was separated and dried over anhydrous sodium sulfate, and the solvent was removed, leaving a crude product that was purified by flash chromatography (silica gel/ethyl acetate/hexane) to give compound **25** (0.41 g, 83% yield). ^{1}H NMR ($CDCl_3$): δ 3.35−3.44 (s, 3H), 3.45−3.59 (m, 1H), 3.60−3.71 (m, 2H), 3.79−3.84 (dd, H), 3.90−3.94 (s, 2H), 3.94−4.02 (q, 2H), 6.37−6.48 (t, 1H), 6.57−7.93 (m, 23H). Anal. Calcd for $C_{35}H_{35}N_3O_6S_2$: C, 63.93; H, 5.33; N, 6.39. Found: C, 64.12; H, 5.54, N, 6.46.

2-[(*m*-Phenoxy)benzyl-1,1-dioxo-5-(toluene-4-sulfonyl)-1λ^6-[1,2,5]thiadiazolidin-3-carboxylic Acid (2,2-Diphenylethyl)-amide 26. A solution of compound **25** (0.42 g; 0.82 mmol) in dry methylene chloride (15 mL) was treated with triethylamine (0.08 g; 0.82 mmol) and phenethyl isocyanate (0.17 g; 1.15 mmol). The reaction mixture was refluxed for 1.5 h, and the solvent was removed. Ethyl acetate (50 mL) was added, and the organic layer was washed with 5% aqueous HCl (20 mL) and then dried over anhydrous sodium sulfate. The solvent was removed, and the crude product was purified by flash chromatography (silica gel/ethyl acetate/hexane) to give compound **26** (0.4 g, 72% yield). ^{1}H NMR ($CDCl_3$): δ 2.83−2.87 (t, 2H), 3.43−3.61 (m, 2H), 3.65−3.71 (q, 1H), 3.72−3.79 (q, 1H), 3.82−3.91 (m, 2H), 3.91−4.02 (m, 4H), 5.86−5.95 (t, 1H), 6.48−6.54 (t, 1H), 6.72−7.41 (m, 24H). Anal. Calcd for $C_{39}H_{38}N_4O_5S$: C, 69.44; H, 5.64; N, 8.31. Found: C, 69.49; H, 5.50; N, 8.24.

Biochemical Studies. The K_I's of compounds **15** and **25** with HLE were determined by constructing Lineweaver−Burk plots. The substrate, MeOSuc-Ala-Ala-Pro-Val *p*-nitroanilide, was varied from 25 to 400 μM for each inhibitor concentration (0−40 μM) using an HLE concentration of 70 nM in 0.1 M HEPES buffer; 0.5 M NaCl, pH 7.25; and a constant DMSO concentration of 10%. HLE and inhibitor were placed in a 1-cm cuvette in assay buffer and incubated at 25 °C for 20 min, then the reaction was initiated by the addition of substrate, and the increase in absorbance was monitored at 410 nm. The inverse of the velocity of the reaction was graphed versus the inverse of the substrate

concentration. The results are shown in Figures 3 and 4. The K_I's were determined by plotting the resulting slopes and intercepts against the inhibitor concentration.

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(22) Nomenclature used is that of Schechter, I.; Berger, A. *Biochem. Biophys. Res. Comm.* **1967**, *27*, 157−162, where S_1, S_2, S_3, ..., S_n and S_1', S_2', S_3', ..., S_n' correspond to the enzyme subsites on either side of the scissile bond. Each subsite accommodates a corresponding amino acid side chain designated P_1, P_2, P_3, ..., P_n and P_1', P_2', P_3', ..., P_n' of the substrate (or inhibitor). S_1 is the primary specificity site, and P_1-P_1' is the scissile bond.
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