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Utilization of the 1,2,3,5-thiatriazolidin-3-one 1,1-dioxide scaffold in the design of potential inhibitors of human neutrophil proteinase 3

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ABSTRACT

The S′ subsites of human neutrophil proteinase 3 (Pr 3) were probed by constructing diverse libraries of compounds based on the 1,2,3,5-thiatriazolidin-3-one 1,1-dioxide using combinational and click chemistry methods. The multiple points of diversity embodied in the heterocyclic scaffold render it well-suited to the exploration of the S′ subsites of Pr 3. Molecular modeling studies suggest that further exploration of the S′ subsites of Pr 3 using the aforementioned heterocyclic scaffold may lead to the identification of highly selective, reversible competitive inhibitors of Pr 3.

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1. Introduction

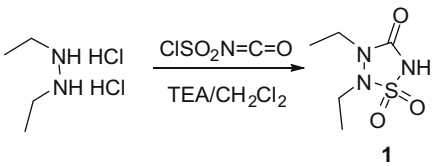
Several lines of evidence suggest that proteases stored in the azurophilic granules of neutrophils, such as human neutrophil elastase (HNE) and proteinase 3 (Pr 3), play an important role in the pathophysiology of a range of inflammatory diseases, including chronic obstructive pulmonary disease (COPD),[1–5] cystic fibrosis,[6–8] Wegener's granulomatosis,[9] and others.[10,11] In addition to their ability to degrade elastin and other components of the extracellular matrix,[12,13] these endopeptidases play an important role in regulating chronic inflammation by modulating the activity of pro-inflammatory cytokines and chemokines.[14,15]

COPD is characterized by an oxidant/antioxidant imbalance,[16,17] alveolar septal cell apoptosis,[18,19] chronic inflammation,[16,20] and a protease/antiprotease imbalance.[4,21] The molecular mechanisms which underlie the initiation and progression of the disorder are poorly understood. Furthermore, the precise role and actions of the proteases involved in COPD are not fully delineated, consequently there is a need for a better definition of which proteases and protease actions are of importance in COPD pathogenesis.[22] Elucidation of the role these proteases play in COPD requires the availability of highly specific substrates and inhibitors.

Pr 3 and HNE share a high sequence homology (57%) and their primary specificity sites S_1[23] are very similar, consequently, the design of covalent and non-covalent inhibitors that exhibit high specificity toward Pr 3 over HNE has been problematic.[24] We describe herein the results of exploratory studies related to the design and synthesis of potential non-covalent inhibitors of Pr 3 based on the 1,2,3,5-thiatriazolidin 1,1-dioxide scaffold that interact with and exploit key differences in the S′ subsites of the two enzymes.

2. Chemistry

The desired compounds were readily synthesized as shown in Schemes 1–4. Heterocyclic template **1** was assembled in one step by condensing commercially-available 1,2-diethyl hydrazine dihydrochloride with *N*-chlorosulfonyl isocyanate in the presence of excess triethylamine (TEA) (Scheme 1). Treatment of the resulting 2,3-diethyl 1,2,3,5-thiatriazolidin-3-one 1,1-dioxide intermediate **1** with TEA followed by the addition of *t*-butyl bromoacetate yielded the corresponding *t*-butyl ester which was readily deblocked and coupled to an array of structurally-diverse amines (Table 1) to yield compounds **4a–i** (Scheme 2, Table 2). Mitsunobu reaction of intermediate **1** with (DL) 3-phenyl-2-hydroxy-propionic acid methyl ester[25] followed by hydrolysis afforded acid **6** which was coupled to a diverse set of amine inputs (Table 1) to give



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Scheme 1.

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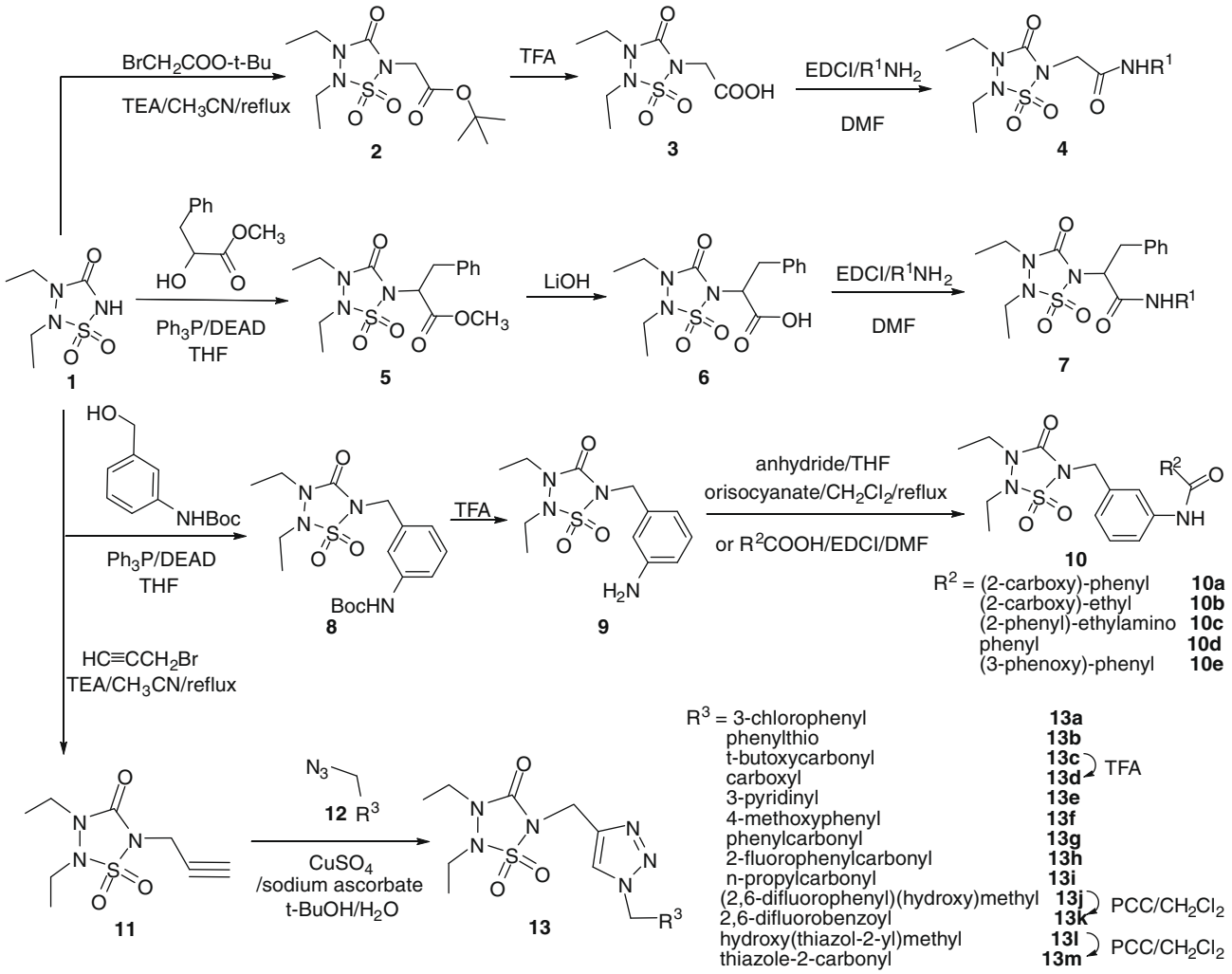
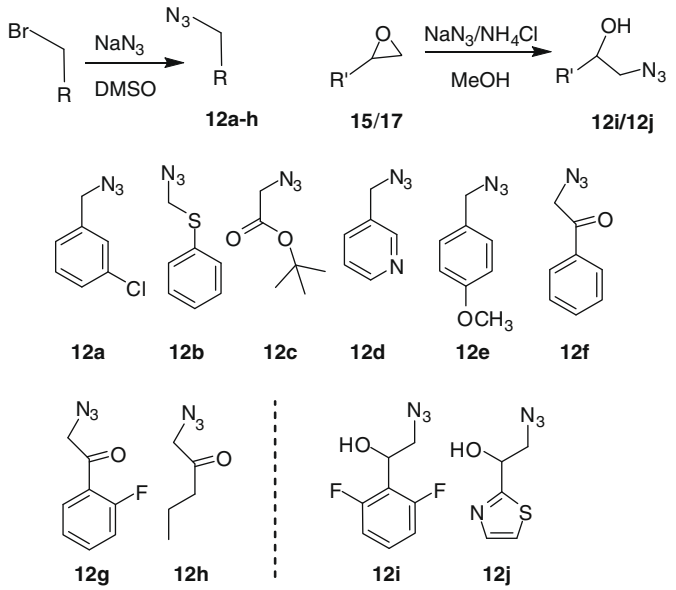


Scheme 2.

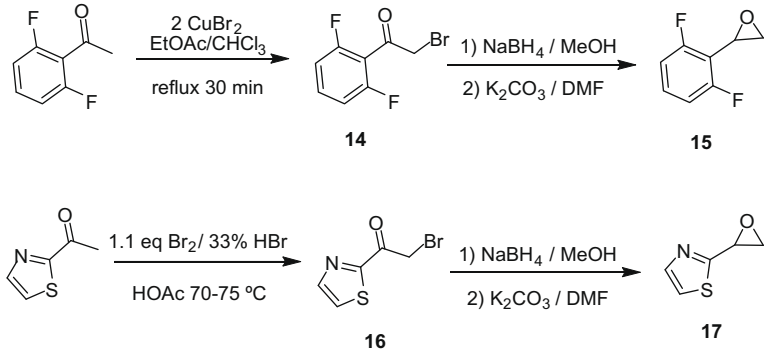
compounds **7a–e** (Scheme 2, Table 2). Likewise, alkylation of 2,3-diethyl 1,2,3,5-thiatriazolidin-3-one 1,1-dioxide (**1**) with *N*-Boc-*m*-aminobenzyl alcohol under Mitsunobu reaction conditions, followed by deprotection of the Boc group using TFA, yielded the corresponding aromatic amine which was further elaborated to yield compounds **10a–e** (Scheme 2). Lastly, alkylation of 2,3-diethyl 1,2,3,5-thiatriazolidin-3-one 1,1-dioxide (**1**) with propargyl bromide followed by click chemistry[26] with a diverse set of azide inputs **12a–j** (Scheme 3) gave compounds **13a–m** (Scheme 2, compounds **13d**, **13k** and **13m** were generated from **13c, 13j** and **13l**, respectively). The desired azides **12a–h** were readily prepared from the corresponding commercially-available halides[27] or halides prepared according to literature procedures[28] (Schemes 3 and 4). The azide precursors of compounds **13k** and **13m** could not be prepared directly from the corresponding α-bromoacetyl compounds, consequently an alternative method was used. This involved α-bromination of an appropriate methyl ketone followed by reduction and treatment with base to form the corresponding epoxide (Scheme 4) which was sequentially subjected to ring opening (Scheme 3: **12i**, **12j**), click chemistry and oxidation with pyridinium chloro-chromate (PCC) (Scheme 2). The X-ray crystal structure of a representative member of this class of compounds (compound **13a**) was determined (Fig. 1).



Scheme 3.

3. Biochemical studies

Enzyme assays and inhibition studies using human proteinase 3 and neutrophil elastase were conducted as previously described.[29]



Scheme 4.

Table 1

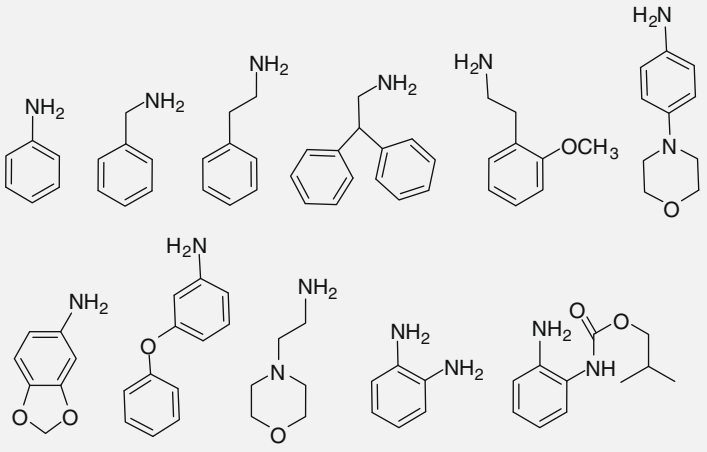
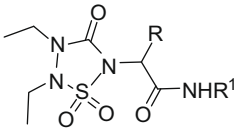


Table 2



Entry	R	R^1
4a	H	Phenyl
4b	H	Benzyl
4c	H	Phenethyl
4d	H	2,2-Diphenylethyl
4e	H	2-(2-Methoxyphenyl)ethyl
4f	H	4-Morpholinophenyl
4g	H	Benzo[*d*][1,3]dioxol-5-yl
4h	H	3-Phenoxyphenyl
4i	H	2-Morpholinoethyl
7a	bzl	2-(2-Methoxyphenyl)ethyl
7b	bzl	4-Morpholinophenyl
7c	bzl	Benzo[*d*][1,3]dioxol-5-yl
7d	bzl	2-Aminophenyl
7e	bzl	2-(Isobutoxycarbonylamino)phenyl

Compounds **7a–e** are DL isomers.

4. Results and discussion

4.1. Inhibitor design rationale

The biochemical rationale underlying the design of inhibitor (II) was based on the following considerations: (a) in previous studies

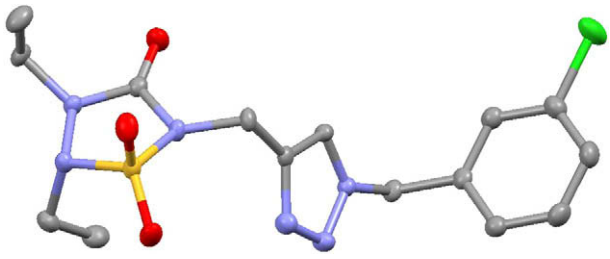


Figure 1. Mercury[53] drawing of compound **13a**, showing the 50% thermal ellipsoids. Hydrogen atoms have been omitted for clarity.

we have demonstrated that the heterocyclic scaffold (I) (1,2,5-thiadiazolidin-3-one 1,1-dioxide) (Fig. 2) is a highly versatile peptidomimetic that embodies a structural motif that renders the platform capable of binding to the active site of HNE and related

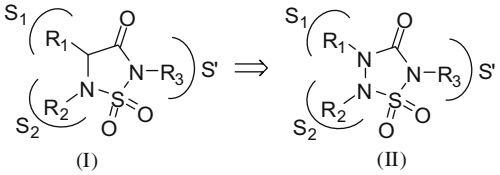


Figure 2. Inhibitor (II) design rationale.

chymotrypsin-like serine proteases in a substrate-like fashion, orienting recognition elements R_1 and R_2 toward the S_n subsites and R_3 toward the S_n' subsites.[24] Specifically, R_1 is accommodated at the primary substrate specificity subsite S_1 and its nature determines which subclass of serine proteases (neutral, basic, or acidic) will be inhibited, resulting in optimal enzyme selectivity;[30] (b) The encouraging results obtained with scaffold (I) suggested that it could serve as a prototype structure for the design of related scaffolds,[31,32] providing additional opportunities in terms of improving pharmacological and physicochemical properties. Thus, we reasoned that a functionalized surrogate scaffold (II) embellished with appropriate recognition elements could provide a structural framework for the rational design of non-covalent inhibitors of Pr 3 and related serine proteases. It was anticipated that the replacement of the α-carbon in scaffold (I) with nitrogen would decrease significantly the electrophilicity of the C=O carbon[33] and lead to a chemically robust ring system (II) that retained the substrate-like characteristics of (I) (Fig. 2); (c) Pr 3 shows a strong preference for small aliphatic P_1 residues (ethyl, *n*-propyl),[34–36] while HNE prefers medium size P_1 alkyl groups (isopropyl/*n*-propyl or isobutyl/*n*-butyl). This is because the size of the S_1 subsite in Pr 3 is smaller due to the replacement of Val190 (HNE) by Ile (Pr 3).[37] Unlike HNE, S′–P′ interactions beyond S_1' increase significantly the catalytic efficiency of Pr 3,[38] suggesting that S_n' interactions play an important role in substrate specificity.[39–42] Furthermore, the substitution of Leu143 (HNE) by Arg143 (Pr 3) and the presence of Asp61 in Pr 3 make the S_1'–S_3' subsites of Pr 3 more polar.[37]

Based on the aforementioned considerations, as well as modeling studies using the X-ray crystal structure of Pr 3,[37] it was envisaged that an entity based on scaffold (II) with attached recognition elements that can potentially interact with the S_n' subsites of the enzyme may function as non-covalent inhibitors of Pr 3. Thus, the S_n' subsites of Pr 3 were initially explored via the construction of focused libraries based on (II). Based on the initial assumption that inhibitor (II) would bind to the active site of Pr 3 in the same orientation as inhibitor (I), a ring nitrogen substitution (ethyl) was chosen that is congruent with the primary substrate specificity (S1) of Pr 3 and a diversity of amides and polar substituted triazoles were initially utilized to probe the S′ subsites. We speculated that a derivative of **4** having a lysine side chain on the α-carbon could potentially provide a favorable ion–ion interaction with Asp51 (see Fig. 3 for Pr 3 active site), however, Mitsunobu reaction of **1** with the α-hydroxyester of Cbz-L-lysine failed to give the expected product. Fortuitously, the Mitsunobu reaction with the α-hydroxyester of (DL) Phe was successful and made possible the synthesis of a wide range of derivatives of **7** and their subsequent use in the exploration of the S_2'–S_3' subsites along with compound **4**. The resulting compounds were either inactive or marginally active (selected examples are shown in Fig. 4). Then the nature, polarity, and geometry of the inhibitor component projecting toward the S′ subsites were changed by utilizing *meta*-substituted phenyl derivatives having a carboxyl group that could potentially interact with the Arg241 side chain located in the vicinity of the S_2' subsite of the enzyme. The inhibitory activity of compounds **10a–e** was also disappointingly low.

We then turned our attention to the use of click chemistry to generate a focused library of structurally-diverse electron-rich compounds having multiple sites capable of interacting with the S′ subsites of Pr 3. Molecular modeling studies using compound **13g** suggested that it fits into the Pr 3 active site well and engages in multiple interactions with the enzyme, including the following: (a) the phenyl ring binds to a hydrophobic pocket defined by Ile190, Phe192; (b) the triazole ring appears to accept H-bonds from both the backbone Val216 NH and from the Lys99 side chain; (c) the heterocyclic carbonyl O is well positioned to H-bond with the Lys99 side chain; (d) one of the sulfamide O's is capable of H-bonding with the backbone NH of Ile217; (e) the two ethyl groups interact with the second hydrophobic pocket, defined by side chains of Phe215, Ile217 and Trp218 (Fig. 3; the docked structure is derived from computational studies). Quite unexpectedly, inhibitor **13g** is predicted to bind to the active site in a reverse mode, namely, with the phenyl ring occupying the S1 pocket and

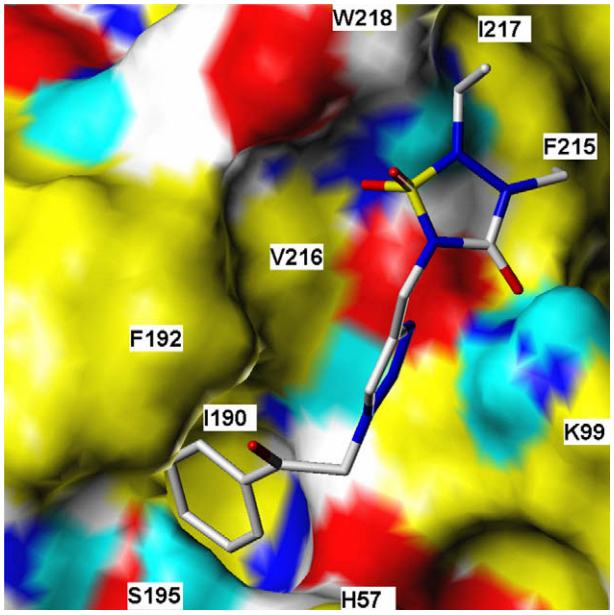


Figure 3. Compound **13g** bound to Pr3. The structure was generated from molecular simulation. Ligand rendered as CPK-colored sticks. Receptor surface colors correspond to: yellow = non-polar, white = polar alkyls, blue = polar N, cyan = polar H, red = O.

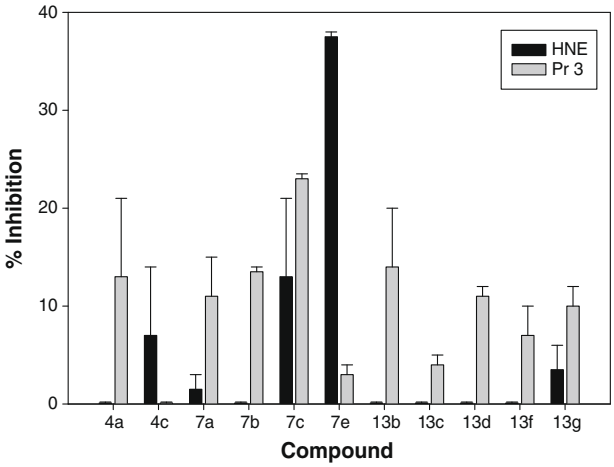


Figure 4. Inhibitory activity of selected compounds against human neutrophil elastase and proteinase 3.

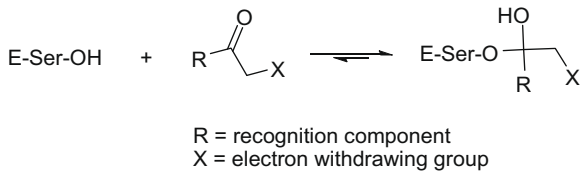


Figure 5. Mechanism of transition state inhibitor.

the rest of the molecule projecting toward the S′ subsites. The molecular modeling studies showed that the ketone carbonyl of **13g** is hydrogen bonded to the side chain hydroxyl of the catalytic Ser195 residue. We reasoned that the introduction of strongly electron-withdrawing substituents into the phenyl ring or replacement of the phenyl ring by a heteroaromatic five-membered ring would enhance the electrophilicity of the carbonyl carbon, potentially transforming **13g** into a transition state inhibitor of Pr 3 (Fig. 5). Thus, structural variants **13h** and **13k** having a 2-fluorophenyl or a 2,6-difluorophenyl group, respectively, were synthesized. The phenyl ring was also replaced by a thiazole ring (compound **13m**) for the same purpose.[43] The few compounds generated by these modifications were also devoid of any inhibitory activity. Future studies involving the generation of a focused library of potential inhibitors of Pr 3 incorporating in their structure a triazole scaffold are currently in progress and may provide a fruitful avenue of investigation for the development of potent and selective inhibitors of Pr 3.

5. Experimental

5.1. General

The ^{1}H spectra were recorded on a Varian XL-300 or XL-400 NMR spectrometer. A Hewlett–Packard diode array UV–vis spectrophotometer was used in the in vitro evaluation of the inhibitors. Human neutrophil elastase, proteinase 3 and Boc-Ala-Ala-Nva thiobenzyl ester were purchased from Elastin Products Company, Owensville, MO. Methoxysuccinyl Ala-Ala-Pro-Val *p*-nitroanilide and 5,5′-dithio-bis(2-nitrobenzoic acid) were purchased from Sigma Chemicals, St. Louis, MO. Melting points were determined on a Mel-Temp apparatus and are uncorrected. Reagents and solvents were purchased from various chemical suppliers (Aldrich, Acros Organics, TCI America, and Bachem). Silica gel (230–450 mesh) used for flash chromatography was purchased from Sorbent Technologies (Atlanta, GA). Thin layer chromatography was performed using Analtech silica gel plates. The TLC plates were visualized using iodine and/or UV light. The high resolution mass spectra were performed by the Mass Spectrometry Lab at the University of Kansas, Lawrence, KS.

5.2. Representative synthesis

5.2.1. 2,3-Diethyl 1,2,3,5-thiatriazolidin-3-one 1,1-dioxide 1

1,2-Diethylhydrazine dihydrochloride (33.0 g; 187 mmol) suspended in dry methylene chloride (300 mL) was cooled in an ice-bath under N_2 and treated with triethylamine (76.0 g; 747 mmol). After stirring for 20 min, a solution of *N*-chlorosulfonyl isocyanate (27.0 g; 187 mmol) in dry methylene chloride (100 mL) was added dropwise. The reaction mixture was stirred overnight at room temperature. The solvent was removed and 6 M HCl (150 mL) was added. The aqueous solution was extracted with ethyl acetate (3×200 mL) and the combined organic extracts were dried over anhydrous sodium sulfate. The drying agent was filtered and the solvent was removed, and then the crude product was purified by flash chromatography (silica gel/ethyl acetate/hexanes) to give compound **1** as a white solid (12.0 g; 33% yield), mp 64–66 °C. ^{1}H NMR ($CDCl_3$): δ 1.25–1.31 (m, 6H), 3.39–3.45 (q, 2H), 3.58–3.64 (q, 2H).

5.2.2. 2,2-Diethyl-5-carboxymethyl-1,2,3,5-thiatriazolidin-3-one 1,1-dioxide *t*-butyl ester 2

To a solution of compound **1** (14 g; 72.5 mmol) in dry *N*,*N*-dimethyl-formamide (70 mL) kept in an ice-bath was added sodium hydride (60%, w/w; 4.5 g; 112 mmol) and the reaction mixture was stirred for 15 min before adding *t*-butyl bromoacetate (18.5 g; 92.7 mmol). The reaction mixture was refluxed overnight with stirring. The solvent was removed in vacuo and the crude product was purified by flash chromatography (silica gel/ethyl acetate/hexanes) to give compound **2** as a colorless oil (17.7 g; 79% yield). ^{1}H NMR ($CDCl_3$): δ 1.22–1.28 (t, 3H), 1.28–1.34 (t, 3H), 1.47 (s, 9H), 3.39–3.44 (q, 2H), 3.60–3.65 (q, 2H), 4.10 (s, 2H).

5.2.3. 2,2-Diethyl-5-carboxymethyl-1,2,3,5-thiatriazolidin-3-one 1,1-dioxide 3

To a solution of compound **2** (3.34 g; 10.9 mmol) in dry methylene chloride (3 mL) was added trifluoroacetic acid (15 mL) and the reaction was stirred at room temperature for 1 h. The solvent and trifluoroacetic acid were removed and the residue was redissolved in ethyl acetate (200 mL). The organic solution was washed with saturated sodium bicarbonate (3×40 mL) and then brine (40 mL). The organic layer was dried over anhydrous sodium sulfate. The drying agent was filtered and the solvent was removed to give compound **3** as colorless oil (2.74 g; 100% yield). ^{1}H NMR ($CDCl_3$): δ 1.22–1.32 (m, 6H), 3.38–3.43 (q, 2H), 3.60–3.65 (q, 2H), 4.25 (s, 2H).

5.2.4. General coupling procedure for preparation of compounds 4a–i

A solution of acid **3** (2.4 mmol) in dry *N*,*N*-dimethylform-amide (5 mL) was treated with 1-[3-(dimethylamino)propyl]-3-ethylcarbodiimide hydrochloride (0.51 g; 2.64 mmol), followed by the addition of amine (2.4 mmol) from Table 1. The reaction mixture was stirred at room temperature overnight. The solvent was removed in vacuo and the residue was taken up with ethyl acetate (30 mL). The organic layer was washed sequentially with 5% HCl (3×10 mL), saturated $NaHCO_3$ (3×10 mL), and then brine (10 mL). The organic layer was dried over anhydrous sodium sulfate. The drying agent was filtered and the solvent removed on the rotary evaporator. The crude product was purified by flash chromatography (silica gel/ethyl acetate/hexanes) to give pure amide product (**4a–i**).

Compound **4a***:* White solid (52% yield), mp 108–110 °C. ^{1}H NMR ($CDCl_3$): δ 1.22–1.28 (t, 3H), 1.29–1.35 (t, 3H), 3.42–3.48 (q, 2H), 3.62–3.68 (q, 2H), 4.25 (s, 2H), 7.10–7.50 (m, 5H), 8.08 (s, 1H); HRMS (ESI) calculated for $C_{13}H_{18}N_4O_4SNa$ $[M+Na]^+$ 349.0946, found 349.0936.

Compound **4b***:* White solid (53% yield), mp 62–63 °C. ^{1}H NMR ($CDCl_3$): δ 1.18–1.25 (m, 6H), 3.34–3.42 (q, 2H), 3.58–3.66 (q, 2H), 4.20 (s, 2H), 4.46–4.50 (d, 2H), 6.28 (s, 1H), 7.22–7.36 (m, 5H); HRMS (ESI) calculated for $C_{14}H_{21}N_4O_4S$ $[M+H]^+$ 341.1284, found 341.1295; $C_{14}H_{20}N_4O_4SNa$ $[M+Na]^+$ 363.1103, found 363.1090.

Compound **4c***:* White solid (20% yield), mp 91–92 °C. ^{1}H NMR ($CDCl_3$): δ 1.18–1.25 (m, 6H), 2.80–2.85 (t, 2H), 3.22–3.28 (q, 2H), 3.56–3.63 (m, 4H), 4.10 (s, 2H), 5.98 (s, 1H), 7.18–7.32 (m, 5H); HRMS (ESI) calculated for $C_{15}H_{23}N_4O_4S$ $[M+H]^+$ 355.1440, found 355.1451; $C_{15}H_{22}N_4O_4SNa$ $[M+Na]^+$ 377.1259, found 377.1255.

Compound **4d***:* Oil (51% yield). ^{1}H NMR ($CDCl_3$): δ 1.10–1.18 (m, 6H), 3.02–3.08 (q, 2H), 3.52–3.58 (q, 2H), 3.92–3.96 (m, 2H), 4.08 (s, 2H), 4.10–4.18 (m, 1H), 5.96 (s, 1H), 7.20–7.32 (m, 10H); HRMS (ESI) calculated for $C_{21}H_{26}N_4O_4SNa$ $[M+Na]^+$ 453.1572, found 453.1575.

Compound **4e***:* Oil (39% yield). ^{1}H NMR ($CDCl_3$): δ 1.20–1.26 (m, 6H), 2.80–2.85 (t, 2H), 3.28–3.33 (q, 2H), 3.50–3.58 (q, 2H), 3.58–3.66 (q, 2H), 3.80 (s, 3H), 4.06 (s, 2H), 6.20 (s, 1H), 6.82–6.88 (m, 2H), 7.08–7.11 (m, 1H), 7.17–7.21 (m, 1H); HRMS (ESI) calculated for $C_{16}H_{24}N_4O_5SNa$ $[M+Na]^+$ 407.1365, found 407.1355.

Compound **4f***:* Yellow solid (32% yield), mp 135–137 °C. ^{1}H NMR ($CDCl_3$): δ 1.22–1.36 (m, 6H), 3.08–3.12 (t, 4H), 3.40–3.48 (q, 2H), 3.52–3.60 (q, 2H), 3.82–3.86 (t, 4H), 4.24 (s, 2H), 6.82–6.86 (d,

2H), 7.34–7.38 (d, 2H), 7.72 (s, 1H); HRMS (ESI) calculated for $C_{17}H_{26}N_5O_5S$ $[M+H]^+$ 412.1655, found 412.1643.

Compound **4g***:* Brown solid (35% yield), mp 83–85 °C. ^{1}H NMR ($CDCl_3$): δ 1.22–1.36 (m, 6H), 3.40–3.46 (q, 2H), 3.52–3.58 (q, 2H), 4.10 (s, 2H), 5.90 (s, 2H), 6.65–6.78 (dd, 2H), 7.10 (s, 1H), 8.22 (s, 1H); HRMS (ESI) calculated for $C_{14}H_{19}N_4O_6S$ $[M+H]^+$ 371.1025, found 371.0984; $C_{14}H_{18}N_4O_6SNa$ $[M+Na]^+$ 393.0845, found 393.0847.

Compound **4h***:* Oil (62% yield). ^{1}H NMR ($CDCl_3$): δ 1.22–1.36 (m, 6H), 3.42–3.48 (q, 2H), 3.62–3.68 (q, 2H), 4.25 (s, 2H), 6.74–7.37 (m, 9H), 7.95 (s, 1H); HRMS (ESI) calculated for $C_{19}H_{22}N_4O_5SNa$ $[M+Na]^+$ 441.1209, found 441.1206.

Compound **4i***:* Oil (45% yield). ^{1}H NMR ($CDCl_3$): δ 1.23–1.30 (t, 3H), 1.30–1.37 (t, 3H), 2.42–2.52 (m, 6H), 3.37–3.46 (m, 4H), 3.63–3.74 (m, 6H), 4.18 (s, 2H), 6.61 (s, 1H); HRMS (ESI) calculated for $C_{13}H_{26}N_5O_5S$ $[M+H]^+$ 364.1655, found 364.1635; $C_{13}H_{25}N_5O_5S$-Na $[M+Na]^+$ 386.1474, found 386.1490.

5.2.5. (DL) Methyl 2-(2,3-diethyl-1,1-dioxido-4-oxo-1,2,3,5-thiatriazolidin-5-yl)-3-phenylpropanoate 5

To a stirred solution of compound **1** (9.95 g; 55.2 mmol) and (DL) 3-phenyl-2-hydroxy propanoic acid methyl ester (10.6 g; 55.2 mmol) in dry tetrahydrofuran was added triphenyl phosphine (28.96 g; 110.4 mmol), followed by the dropwise addition of a solution of diethyl azodicarboxylate (97%; 19.9 g; 110.4 mmol) in dry tetrahydrofuran (30 mL). The reaction was stirred at room temperature overnight and the resulting precipitate was filtered off. The filtrate was evaporated to give the crude product which was purified by flash chromatography (silica gel/ethyl acetate/hexanes) to give compound **5** as a colorless oil (3.2 g; 16% yield). ^{1}H NMR ($CDCl_3$): δ 1.00–1.06 (t, 3H), 1.16–1.22 (t, 3H), 2.82–3.08 (m, 2H), 3.40–3.75 (m, 4H), 3.80 (s, 3H), 4.63–4.70 (m, 1H), 7.20–7.32 (m, 5H).

5.2.6. (DL) 2-(2,3-Diethyl-1,1-dioxido-4-oxo-1,2,3,5-thiatriazolidin-5-yl)-3-phenyl-propanoic acid 6

A solution of compound **5** (3.74 g; 10.5 mmol) in 1,4-dioxane (40 mL) was treated with 17.5 mL 6 N potassium hydroxide solution at room temperature for 0.5 h. The pH was adjusted to 7 by the addition of 5% hydrochloride solution and then the solvent was removed. The residue was acidified to pH 2 and extracted with ethyl acetate (3 × 50 mL). The combined organic extracts were dried over anhydrous sodium sulfate. The drying agent was filtered and the solvent was removed. The crude product was purified by flash chromatography (silica gel/ethyl acetate/hexanes) to give compound **6** as a colorless oil (2.4 g; 59% yield). ^{1}H NMR ($CDCl_3$): δ 1.00–1.06 (t, 3H), 1.16–1.22 (t, 3H), 2.76–2.86 (m, 1H), 2.94–3.04 (m, 1H), 3.39–3.75 (m, 4H), 4.65–4.73 (m, 1H), 7.20–7.32 (m, 5H).

5.2.7. Synthesis of amides 7a–e

The same coupling procedures were used as described above.

Compound **7a***:* Oil (15% yield). ^{1}H NMR ($CDCl_3$): δ 0.92–0.98 (t, 3H), 1.12–1.18 (t, 3H), 2.50–2.61 (m, 1H), 2.72–2.84 (m, 3H), 3.36–3.75 (m, 6H), 3.81 (s, 3H), 4.47–4.55 (m, 1H), 6.50 (s, 1H), 6.80–7.30 (m, 9H); HRMS (ESI) calculated for $C_{23}H_{30}N_4O_5SNa$ $[M+Na]^+$ 497.1835, found 497.1835.

Compound **7b***:* White solid (23% yield), mp 155–156 °C. ^{1}H NMR ($CDCl_3$): δ 0.92–0.98 (t, 3H), 1.12–1.18 (t, 3H), 2.60–2.68 (m, 1H), 2.85–2.94 (m, 1H), 3.08–3.12 (m, 4H), 3.38–3.78 (m, 4H), 3.91–3.95 (m, 4H), 4.62–4.68 (m, 1H), 6.81–6.85 (d, 2H), 7.20–7.35 (m, 5H), 7.36–7.40 (d, 2H), 8.25 (s, 1H); HRMS (ESI) calculated for $C_{24}H_{32}N_5O_5S$ $[M+H]^+$ 502.2124, found 502.2101; $C_{24}H_{31}N_5O_5SNa$ $[M+Na]^+$ 524.1944, found 524.1939.

Compound **7c***:* Brown solid (14% yield), mp 100–101 °C. ^{1}H NMR ($CDCl_3$): δ 0.94–1.00 (t, 3H), 1.16–1.20 (t, 3H), 2.60–2.68 (m, 1H), 2.86–2.94 (m, 1H), 3.38–3.77 (m, 4H), 4.62–4.68 (m, 1H), 5.93 (s, 2H), 6.68–6.78 (m, 2H), 7.18–7.30 (m, 6H), 8.32 (s, 1H); HRMS (ESI) calculated for $C_{21}H_{24}N_4O_6SNa$ $[M+Na]^+$ 483.1314, found 483.1306.

Compound **7d***:* White solid (33% yield), mp 128–130 °C. ^{1}H NMR ($CDCl_3$): δ 0.94–1.00 (t, 3H), 1.16–1.20 (t, 3H), 2.60–2.75 (m, 1H), 2.85–3.00 (m, 1H), 3.37–3.80 (m, 4H), 3.78 (br s, 2H), 4.69–4.77 (m, 1H), 6.68–7.34 (m, 9H), 7.90 (s, 1H); HRMS (ESI) calculated for $C_{20}H_{26}N_5O_4S$ $[M+H]^+$ 432.1706, found 432.1698; $C_{20}H_{25}N_5O_4S$-Na $[M+Na]^+$ 454.1525, found 454.1506.

Compound **7e***:* White solid (8% yield), mp 113–115 °C. ^{1}H NMR ($CDCl_3$): δ 0.94–1.02 (m, 9H), 1.16–1.20 (t, 3H), 1.90–2.01 (m, 1H), 2.69–2.80 (m, 1H), 2.89–3.00 (m, 1H), 3.40–3.80 (m, 4H), 3.92–3.96 (d, 2H), 4.68–4.74 (m, 1H), 7.00 (s, 1H), 7.04–7.32 (m, 9H), 8.20 (s, 1H); HRMS (ESI) calculated for $C_{25}H_{33}N_5O_6SNa$ $[M+Na]^+$ 554.2049, found 554.2053.

5.2.8. Synthesis of compound 8

To a stirred solution of compound **1** (0.77 g; 4 mmol) and 3-*N*-Boc amino benzyl alcohol (0.90 g; 4 mmol) in dry tetrahydrofuran (10 mL) was added triphenyl phosphine (2.12 g; 8 mmol) followed by the dropwise addition of a solution of diethyl azodicarboxylate (1.44 g; 8 mmol) in dry tetrahydrofuran (5 mL). The reaction was stirred at room temperature overnight. The solvent was removed under vacuum and the residue was taken up with ethyl acetate (40 mL), and then washed with water (20 mL). The organic layer was dried over anhydrous sodium sulfate. The solvent was evaporated and the crude product was purified by flash chromatography (silica gel/ethyl acetate/hexanes) to give compound **8** as colorless oil (0.49 g; 30% yield). ^{1}H NMR ($CDCl_3$): δ 1.12–1.30 (m, 6H), 1.50 (s, 9H), 3.25–3.40 (q, 2H), 3.50–3.65 (q, 2H), 4.60 (s, 2H), 6.72 (s, 1H), 6.98–7.40 (m, 4H).

5.2.9. Synthesis of compound 9

To a solution of compound **8** (0.47 g; 1.18 mmol) was added trifluoroacetic acid (5 mL) and the reaction was stirred at room temperature for 1 h. Trifluoroacetic acid was removed under vacuum and the residue was redissolved in 20 mL methylene chloride. The solvent was removed and the crude product was purified by flash chromatography (silica gel/ethyl acetate/hexanes) to give compound **9** as a colorless oil (0.30 g; 85% yield). ^{1}H NMR ($CDCl_3$): δ 1.16–1.24 (m, 6H), 3.26–3.36 (q, 2H), 3.54–3.64 (q, 2H), 4.58 (s, 2H), 5.25 (br s, 2H), 6.98–7.30 (m, 4H).

5.2.10. Synthesis of compounds 10a–b

A solution of free amine **9** (0.4 mmol) in dry methylene chloride (3 mL) kept in an ice-bath was treated with phthalic or succinic anhydride (0.4 mmol) and then the reaction mixture was refluxed for 1 h. The solvent was removed and the crude product was purified by flash chromatography (silica gel/ethyl acetate/hexanes) to give the corresponding product (**10a–b**).

Compound **10a***:* White solid (70% yield), mp 134–136 °C. ^{1}H NMR (DMSO-d_6): δ 1.10–1.20 (m, 6H), 3.32–3.40 (q, 2H), 3.55–3.63 (q, 2H), 4.64 (s, 2H), 7.02–7.70 (m, 7H), 7.72 (s, 1H), 7.88–7.90 (m, 1H), 10.40 (s, 1H); HRMS (ESI) calculated for $C_{20}H_{22}N_4O_6S$-Na $[M+Na]^+$ 469.1158, found 469.1161.

Compound **10b***:* White solid (85% yield), mp 109–111 °C. ^{1}H NMR (DMSO-d_6): δ 1.18–1.26 (m, 6H), 2.64–2.72 (t, 2H), 2.72–2.80 (t, 2H), 3.31–3.37 (q, 2H), 3.58–3.64 (q, 2H), 4.60 (s, 2H), 7.08–7.62 (m, 4H), 7.95 (s, 1H); HRMS (ESI) calculated for $C_{16}H_{22}N_4O_6SNa$ $[M+Na]^+$ 421.1158, found 421.1139.

Compound **10c***:* A solution of compound **9** (0.37 g; 0.81 mmol) and phenethyl isocyanate (0.18 g; 1.21 mmol) in dry methylene chloride (3 mL) was refluxed for 1 h. The solvent was removed under vacuum and ethyl acetate (80 mL) was added. The organic layer was washed with 5% HCl (20 mL) and dried over anhydrous

sodium sulfate. The drying agent was filtered and the solvent was removed. The crude product was purified by flash chromatography (silica gel/ethyl acetate/hexanes) to give compound **10c** as colorless oil (0.15 g; 42% yield). ^{1}H NMR ($CDCl_3$): δ 1.16–1.24 (m, 6H), 2.75–2.80 (t, 2H), 3.26–3.34 (q, 2H), 3.42–3.48 (q, 2H), 3.56–3.62 (q, 2H), 4.55 (s, 2H), 5.23 (s, 1H), 7.00–7.28 (m, 10H); HRMS (ESI) calculated for $C_{21}H_{27}N_5O_4SNa$ $[M+Na]^+$ 468.1681, found 468.1679.

Compounds **10d–e**: Amides **13d–e** were prepared from **9** and appropriate acids using a same coupling procedure described above.

Compound **10d**: Oil (76% yield). ^{1}H NMR ($CDCl_3$): δ 1.20–1.30 (m, 6H), 3.30–3.38 (q, 2H), 3.57–3.65 (q, 2H), 4.80 (s, 2H), 7.12–7.85 (m, 9H), 8.00 (s, 1H); HRMS (ESI) calculated for $C_{19}H_{22}N_4O_4SNa$ $[M+Na]^+$ 425.1259, found 425.1272.

Compound **10e**: Oil (10% yield). ^{1}H NMR ($CDCl_3$): δ 1.20–1.28 (m, 6H), 3.30–3.38 (q, 2H), 3.57–3.65 (q, 2H), 4.60 (s, 2H), 7.00–7.64 (m, 13H), 7.86 (s, 1H); HRMS (ESI) calculated for $C_{25}H_{26}N_4O_5SNa$ $[M+Na]^+$ 517.1522, found 517.1527.

5.2.11. Synthesis of compound 11

Compound **11**: Compound **1** (1.93 g; 10 mmol) was dissolved in 20 mL dry acetonitrile and triethylamine (1.01 g; 10 mmol) was added, followed by propargyl bromide (1.19 g; 10 mmol). The resulting mixture was refluxed overnight. The solvent was removed, and the residue was dissolved in 40 mL ethyl acetate. The organic solution was washed with 5% HCl (3×20 mL), 5% saturated $NaHCO_3$ (3×20 mL) and then brine (20 mL). The organic layer was dried over anhydrous sodium sulfate. The drying agent was filtered off and the filtrate was evaporated under vacuum to give compound **11** as a yellow oil (1.26 g; 54% yield). ^{1}H NMR ($CDCl_3$): δ 1.20–1.32 (m, 6H), 2.38–2.40 (t, 1H), 3.36–3.42 (q, 2H), 3.60–3.66 (q, 2H), 4.26–4.29 (d, 2H).

5.2.12. General procedure for preparation of azide 12a–h

To a solution of NaN_3 (1.44 g; 22 mmol) in 20 mL DMSO was added appropriate commercial or self-prepared halide (20 mmol), and the reaction was stirred at room temperature for overnight. The reaction mixture was added 70 mL H_2O under a water bath, and then extracted with 3×50 mL of diethyl ether. The combined organic layers were washed with 2×50 mL brine and dried over anhydrous sodium sulfate. Removal of solvent gave a pure product.

Compound **12a**: Oil (100% yield). ^{1}H NMR ($CDCl_3$): 4.35 (s, 2H), 7.20–7.38 (m, 4H).

Compound **12b**: Oil (98% yield). ^{1}H NMR ($CDCl_3$): 4.56 (s, 2H), 7.29–7.56 (m, 5H).

Compound **12c**: Oil (99% yield). ^{1}H NMR ($CDCl_3$): 1.51 (s, 9H), 3.77 (s, 2H).

Compound **12d**: Oil (90% yield). ^{1}H NMR ($CDCl_3$): 4.40 (s, 2H), 7.33–7.39 (m, 1H), 7.67–7.82 (m, 1H), 8.53–8.63 (m, 2H).

Compound **12e**: Oil (98% yield). ^{1}H NMR ($CDCl_3$): 3.81 (s, 3H), 4.25 (s, 2H), 6.88–6.94 (d, 2H), 7.23–7.29 (d, 2H).

Compound **12f**: Oil (95% yield). ^{1}H NMR ($CDCl_3$): 4.58 (s, 2H), 7.45–7.68 (m, 3H), 7.92–7.96 (m, 2H).

Compound **12g**: Oil (92% yield). ^{1}H NMR ($CDCl_3$): 4.52 (s, 2H), 7.10–7.30 (m, 2H), 7.57–7.64 (m, 1H), 7.97–8.02 (m, 1H).

Compound **12h**: Oil (76% yield). ^{1}H NMR ($CDCl_3$): 0.90–1.00 (t, 3H), 1.62–1.70 (q, 2H), 2.39–2.47 (t, 2H), 3.93 (s, 2H).

Azides **12i–j** were prepared using the following procedure:[44] Epoxide **15** or **17** (2.63 mmol) was dissolved in 15 mL methanol and then sodium azide (0.60 g; 9 mmol) and ammonium chloride (0.36 g; 6.6 mmol) were added. The resulting mixture was refluxed for 15 h. The solvent was removed and 15 mL of water was added. The aqueous solution was extracted with 2×15 mL ethyl acetate, and the combined organic layers were dried over anhydrous sodium sulfate. The drying agent was filtered and the filtrate was pumped to dryness, yielding a pure product.

Compound **12i**: Oil (96% yield). ^{1}H NMR ($CDCl_3$): 3.49–3.55 (m, 1H), 3.77–3.85 (m, 3H), 5.09–5.15 (m, 1H), 6.89–7.00 (m, 2H), 7.24–7.40 (m, 1H).

Compound **12j**: Oil (64% yield). ^{1}H NMR ($CDCl_3$): 3.63–3.80 (m, 2H), 4.72 (br s, 1H), 5.17–5.21 (m, 1H), 7.37–7.39 (d, 1H), 7.76–7.78 (d, 1H).

5.2.13. General alkyne–azide cycloaddition of compounds 13a–c, 13e–j and 13l

To a solution of **11** (1.8 mmol) and appropriate azide (1.8 mmol) in 5 mL of 1:1 *t*-BuOH and H_2O solution were added sodium ascorbate (0.04 g; 0.18 mmol) and copper sulfate pentahydrate (0.005 g; 0.018 mmol). The reaction mixture was stirred at room temperature overnight. The solvent was removed and the residue was taken up with ethyl acetate (30 mL) (any insoluble impurities were filtered off). The organic solution was washed with water (2×20 mL) and then dried over anhydrous sodium sulfate. The drying agent was filtered and the solvent was removed. The residue was purified by flash chromatography (silica gel/ethyl acetate/hexanes) to give pure compounds **13a–c, 13e–j** and **13l**.

Compound **13a**: White solid (75% yield), mp 83–84 °C. ^{1}H NMR ($CDCl_3$): 1.18–1.22 (t, 6H), 3.28–3.36 (q, 2H), 3.55–3.63 (q, 2H), 4.80 (s, 2H), 5.52 (s, 2H), 7.09–7.14 (t, 1H), 7.21 (s, 1H), 7.28–7.30 (d, 2H), 7.70 (s, 1H); HRMS (ESI) calculated for $C_{15}H_{20}ClN_6O_3S$ $[M+H]^+$ 399.1006, found 399.1016; $C_{15}H_{19}ClN_6O_3SNa$ $[M+Na]^+$ 421.0826, found 421.0824.

Compound **13b**: Oil (63% yield). ^{1}H NMR ($CDCl_3$): 1.20–1.25 (t, 6H), 3.30–3.38 (q, 2H), 3.58–3.66 (q, 2H), 4.80 (s, 2H), 5.60 (s, 2H), 7.28 (s, 5H), 7.64 (s, 1H); HRMS (ESI) calculated for $C_{15}H_{21}N_6O_3S_2$ $[M+H]^+$ 397.1117, found 397.1137; $C_{15}H_{20}N_6O_3S_2Na$ $[M+Na]^+$ 419.0936, found 419.0932.

Compound **13c**: White solid (52% yield), mp 96–97 °C. ^{1}H NMR ($CDCl_3$): 1.20–1.24 (t, 6H), 1.46 (s, 9H), 3.30–3.38 (q, 2H), 3.58–3.66 (q, 2H), 4.82 (s, 2H), 5.06 (s, 2H), 7.78 (s, 1H); HRMS (ESI) calculated for $C_{14}H_{24}N_6O_5SNa$ $[M+Na]^+$ 411.1427, found 411.1440.

Compound **13e**: White solid (63% yield), mp 85–86 °C. ^{1}H NMR ($CDCl_3$): 1.20–1.24 (m, 6H), 3.30–3.40 (q, 2H), 3.55–3.65 (q, 2H), 4.80 (s, 2H), 5.59 (s, 2H), 7.29–7.34 (m, 1H), 7.55–7.59 (m, 1H), 7.63 (s, 1H), 8.59–8.62 (m, 2H); HRMS (ESI) calculated for $C_{14}H_{20}N_7O_3S$ $[M+H]^+$ 366.1348, found 366.1354.

Compound **13f**: White solid (66% yield), mp 71–72 °C. ^{1}H NMR ($CDCl_3$): 1.18–1.22 (t, 6H), 3.28–3.36 (q, 2H), 3.55–3.63 (q, 2H), 3.80 (s, 3H), 4.78 (s, 2H), 5.43 (s, 2H), 6.85–6.89 (d, 2H), 7.18–7.22 (d, 2H), 7.51 (s, 1H); HRMS (ESI) calculated for $C_{16}H_{22}N_6O_4SNa$ $[M+Na]^+$ 417.1321, found 417.1321.

Compound **13g**: White solid (86% yield), mp 107–109 °C. ^{1}H NMR ($CDCl_3$): 1.20–1.26 (t, 6H), 3.34–3.40 (q, 2H), 3.60–3.66 (q, 2H), 4.86 (s, 2H), 5.85 (s, 2H), 7.52–7.60 (t, 2H), 7.64–7.72 (t, 1H), 7.82 (s, 1H), 7.98–8.02 (d, 2H); HRMS (ESI) calculated for $C_{16}H_{20}N_6O_4SNa$ $[M+Na]^+$ 415.1164, found 415.1164.

Compound **13h**: Oil (34% yield). ^{1}H NMR ($CDCl_3$): 1.20–1.28 (t, 6H), 3.32–3.39 (q, 2H), 3.58–3.65 (q, 2H), 4.85 (s, 2H), 5.79–5.83 (d, 2H), 7.20–7.37 (m, 2H), 7.62–7.70 (m, 1H), 7.80 (s, 1H), 7.95–8.02 (m, 1H); HRMS (ESI) calculated for $C_{16}H_{19}FN_6O_4SNa$ $[M+Na]^+$ 433.1070, found 433.1049.

Compound **13i**: Oil (76% yield). ^{1}H NMR ($CDCl_3$): 0.92–0.98 (t, 3H), 1.20–1.25 (t, 6H), 1.60–1.74 (m, 2H), 2.43–2.49 (t, 2H), 3.32–3.40 (q, 2H), 3.59–3.67 (q, 2H), 4.93 (s, 2H), 5.20 (s, 2H), 7.73 (s, 1H); HRMS (ESI) calculated for $C_{13}H_{22}N_6O_4SNa$ $[M+Na]^+$ 381.1321, found 381.1327.

Compound **13j**: White solid (77% yield), mp 127–129 °C. ^{1}H NMR ($CDCl_3$): 1.16–1.22 (t, 6H), 3.28–3.35 (q, 2H), 3.55–3.62 (q, 2H), 4.16–4.22 (m, 1H), 4.68–4.74 (m, 1H), 4.77 (s, 2H), 6.06–6.13 (m, 1H), 6.89–6.97 (t, 2H), 7.28–7.40 (m, 1H), 7.73 (s, 1H);

HRMS (ESI) calculated for $C_{16}H_{20}F_2N_6O_4SNa$ $[M+Na]^+$ 435.1133, found 453.1134.

Compound **13l***:* Oil (77% yield). ^1H NMR ($CDCl_3$): 1.19–1.25 (t, 6H), 3.28–3.36 (q, 2H), 3.55–3.63 (q, 2H), 4.60–4.71 (m, 1H), 4.75 (s, 2H), 4.96–5.04 (m, 1H), 5.43–5.51 (m, 1H), 5.56–5.61 (d, 1H), 7.34–7.36 (d, 1H), 7.73–7.76 (d, 2H); HRMS (ESI) calculated for $C_{13}H_{19}N_7O_4S_2Na$ $[M+Na]^+$ 424.0838, found 424.0827.

Compounds **13k**, **13m***:* To a suspension of pyridinium chlorochromate (PCC, 0.53 g; 2.5 mmol) in 5 mL methylene chloride was added compound **13j** or **13l** (1 mmol) and the reaction was stirred at room temperature for 3 days. The supernatant was decanted and the gummy residue was washed with 10 mL of methylene chloride. The combined organic solutions were concentrated and the residue was purified using flash chromatography (silica gel/ethyl acetate/hexanes) to give pure compound **13k** or **13m.**

Compound **13k***:* Oil (28% yield). ^1H NMR ($CDCl_3$): 1.20–1.26 (t, 6H), 3.32–3.40 (q, 2H), 3.59–3.67 (q, 2H), 4.86 (s, 2H), 5.70 (s, 2H), 7.01–7.08 (t, 2H), 7.51–7.60 (m, 1H), 7.81 (s, 1H); HRMS (ESI) calculated for $C_{16}H_{18}F_2N_6O_4SNa$ $[M+Na]^+$ 451.0976, found 451.0964.

Compound **13m***:* Oil (8% yield). ^1H NMR ($CDCl_3$): 1.20–1.26 (t, 6H), 3.32–3.40 (q, 2H), 3.59–3.67 (q, 2H), 4.87 (s, 2H), 6.03 (s, 2H), 7.82–7.84 (m, 2H), 8.15–8.15 (d, 1H); HRMS (ESI) calculated for $C_{13}H_{17}N_7O_4S_2Na$ $[M+Na]^+$ 422.0681, found 422.0686.

Compound **13d***:* Compound **13c** (0.11 g; 0.28 mmol) was added 3 mL 4 M HCl in dry 1,4-dioxane, and the reaction was stirred at room temperature for 2 h. The solvent was removed and the residue was washed with 1 mL chloroform, leaving the pure product **13d** as a white solid (0.09 g; 97% yield), mp 145–147 °C. ^1H NMR (CD_3OD): 1.18–1.22 (m, 6H), 3.30–3.40 (q, 2H), 3.60–3.70 (q, 2H), 4.80 (s, 2H), 5.26 (s, 2H), 8.03 (s, 1H); HRMS (ESI) calculated for $C_{10}H_{16}N_6O_5SNa$ $[M+Na]^+$ 355.0801, found 355.0802.

5.2.14. Synthesis of compound 14

Copper(II) bromide (4.46 g; 20 mmol) was ground and placed into a RB flask, ethyl acetate (10 mL) was added and the mixture was brought to boiling. 2′,6'-Difluoroacetylphenone (1.72 g; 11 mmol) in 10 mL chloroform was added through the top of the condenser. The reaction started immediately and the mixture was refluxed for 30 min whereupon a large amount of amber precipitate formed and gas release ceased. The reaction mixture was cooled down, 10 mL H_2O was added and stirred for 1 min. The precipitate was filtered and the filtrate was extracted with 30 mL ethyl acetate. The organic layer was washed with brine (2×20 mL) and dried over anhydrous sodium sulfate. The drying agent was filtered off and the filtrate was concentrated to dryness to give compound **14** as a colorless oil (2.35 g; 100% yield). ^1H NMR ($CDCl_3$): 4.39 (s, 2H), 6.95–7.05 (m, 2H), 7.42–7.55 (m, 1H).

5.2.15. Synthesis of compound 15

Compound **14** (2.35 g; 10 mmol) was dissolved in 30 mL methanol and sodium borohydride (0.40 g; 10 mmol) was added at 0 °C in small portions. The reaction was then stirred for 1 h at 0 °C. Two milliliters of 12 N sulfuric acid and 10 mL water were added and the reaction was stirred for 2 min before the solvent was removed. The residue was dissolved in 40 mL ethyl acetate, and the organic solution was washed with 30 mL water. The organic layer was separated and dried over anhydrous sodium sulfate. The drying agent was filtered and the filtrate was pumped to dryness. The residue was dissolved in 10 mL DMF, and solid potassium carbonate (3.45 g; 25 mmol) was added. The resulting mixture was stirred at room temperature overnight. Water (15 mL) was added and the solution was extracted ethyl acetate (2×40 mL). The combined organic layers were washed with brine (2×30 mL) and dried over anhydrous sodium sulfate. The drying agent was filtered off and the filtrate was concentrated to give pure compound **15** (0.82 g; 53% yield) as a colorless oil. ^1H NMR ($CDCl_3$): 3.14–3.18 (m, 1H), 3.32–3.36 (m, 1H), 4.01–4.04 (m, 1H), 6.82–6.95 (m, 2H), 7.21–7.33 (m, 1H).

5.2.16. Synthesis of compound 16

2-Acetylthiazole (1.27 g; 10 mmol) was dissolved in 8 mL glacial acetic acid, 33% HBr in acetic acid (2.5 mL; ~10 mmol) was added and the resulting mixture was placed in an ice-bath. Bromine (0.57 mL; 11 mmol) was added and then the reaction was allowed to warm to 70–75 °C for 2 h with stirring. The disappearance of reddish color indicated the reaction has completed. The reaction mixture was cooled to room temperature, and the precipitate was filtered and washed with 15 mL ethyl acetate. The filter cake was transferred into a round-bottom flask, 20 mL of water was added and pH was adjusted to ~10 using 6 N NaOH. The oily product was extracted with ethyl acetate (2×30 mL) and the combined organic layers were dried over anhydrous sodium sulfate. The drying agent was filtered and the filtrate was concentrated to dryness, yielding a pure compound **16** (1.63 g; 79% yield) as a reddish oil. ^1H NMR ($CDCl_3$): 4.73 (s, 2H), 7.78–7.80 (d, 1H), 8.05–8.07 (d, 1H).

5.2.17. Synthesis of compound 17

Compound **17** was prepared using the same procedure as compound **15**.

Compound **17***:* Oil (63% yield). ^1H NMR ($CDCl_3$): 3.01–3.05 (m, 1H), 3.24–3.28 (m, 1H), 4.28–4.32 (m, 1H), 7.32–7.34 (d, 1H), 7.77–7.79 (d, 1H).

5.3. Enzyme assays and inhibition studies

5.3.1. Human neutrophil proteinase 3

Twenty microliters of 32.0 mM 5,5′-dithio-bis(2-nitrobenzoic acid) in dimethyl sulfoxide and 10 μL of 3.45 μM human proteinase 3 in 0.1 M phosphate buffer/pH 6.50 (final enzyme concentration: 34.5 nM) were added to a cuvette containing a solution of 940 μL 0.1 M HEPES buffer/0.5 M NaCl/pH 7.25, 10 μL 862.5 μM inhibitor in dimethyl sulfoxide (final inhibitor concentration: 8.62 μM) and 20 μL 12.98 mM Boc-Ala-Ala-NVa-SBzl (final substrate concentration: 259.6 μM) at 25 °C. The change in absorbance was monitored at 410 nM for 2 min. A control (hydrolysis run) was also run under the same conditions by adding 5,5′-dithio-bis(2-nitrobenzoic acid) in dimethyl sulfoxide and 10 μL of 3.45 μM solution of human proteinase 3 in 0.1 M phosphate buffer/pH 6.50 (final enzyme concentration: 34.5 nM) to a cuvette containing a solution of 940 μL 0.1 M HEPES buffer/0.5 M NaCl/pH 7.25, 10 μL dimethyl sulfoxide and 20 μL 12.98 mM Boc-Ala-Ala-NVa-SBzl (final substrate concentration: 259.6 μM). The change in absorbance was monitored at 410 nM for 2 min. The percent inhibition of Pr 3 was determined using% inhibition = $(1 - v/v_o) \times 100$ and is the average of duplicate or triplicate determinations.

5.3.2. Human neutrophil elastase

Ten microliters of 7.0 μM human elastase in 0.05 mM sodium acetate buffer/0.5 M NaCl/pH 5.50 (final enzyme concentration: 70 nM) was added to a cuvette containing a solution of 970 μL 0.1 M HEPES buffer/0.5 M NaCl/pH 7.25, 10 μL of 3.5 mM solution of inhibitor in dimethyl sulfoxide (final inhibitor concentration: 35 μM) and 10 μL of 70 mM methoxysuccinyl-Ala-Ala-Pro-Val *p*-nitroaniline (final substrate concentration: 700 μM) at 25 °C. The change in absorbance was monitored at 410 nM for 2 min. A control (hydrolysis run) was also run under the same conditions by adding 10 μL of a 7.0 μM solution of human elastase (final enzyme concentration: 70 nM) to a cuvette containing 970 μL 0.1 M HEPES buffer/0.5 M NaCl/pH 7.25, 10 μL DMSO and 10 μL of 70 mM methoxysuccinyl-Ala-Ala-Pro-

Table 3
X-ray data collection and structure solution parameters

Molecular formula	$C_{15}H_{19}ClN_3O_6S$
Formula weight	398.87
Diffractometer	Bruker Kappa-Apex-II
Radiation/λ (Å)	Mo Kα/0.71073
Temp (K)	150
Color, habit	Colorless, needle
Crystal system	Monoclinic
Space group	$P2_1/n$
Crystal size (mm^3)	0.17 × 0.25 × 0.69
a (Å)	10.2729(12)
b (Å)	8.4972(9)
c (Å)	41.227(5)
β (°)	94.384(6)
V (Å^3)	3588.2(7)
Z	8
Calcd density (g cm^{-1})	1.477
Octants collected	$\pm h, \pm k, \pm l$
Max. h, k, l	12, 10, 50
Θ Range (°)	2.45–26.00
μ (mm^{-1})	0.359
Reflections/unique (R_{int})	80,192/7069 (0.0270)
Observed[>2σ]/parameters	7069/473
R_{obs}, R_{all}	0.0326/0.0383
Goodness-of-fit	1.083
ρ_{max}/ρ_{min} (e Å^{-3})	0.333, −0.466

Val *p*-nitroaniline (final substrate concentration: 700 μM). The change in absorbance was monitored at 410 nM for 2 min. The percent inhibition of HNE was determined using% inhibition = $(1 - v/v_o) \times 100$ and is the average of duplicate or triplicate determinations.

5.4. Computational method

Molecular docking simulation was performed using the SURFLEX program.[45] The structure of compound **13g** was constructed in SYBYL[46] and was structurally optimized to default convergence thresholds using the Tripos Force Field[47] and Gasteiger–Marsili partial atomic charges.[48] A receptor model was prepared for Pr 3 using the 1FUJ[37] crystal structure. This structure was protonated in SYBYL according to pH 7.0 protonation states, stripped of all water molecules and bound ligands, and electrostatically represented with Gasteiger–Marsili charges.

5.5. X-ray crystallography

A crystal was affixed to a nylon cryoloop using oil (Paratone-n, Exxon) and mounted in the cold stream of a Bruker Kappa-Apex-II area-detector diffractometer. The temperature at the crystal was maintained at 150 K using a Cryostream 700EX low-temperature apparatus (Oxford Cryosystems). The unit cell was determined from the setting angles of the reflections collected in 36 frames of data. Data were measured using graphite mono-chromated molybdenum Kα radiation (λ = 0.71073 Å) collimated to a 0.6 mm diameter and a CCD detector at a distance of 50 mm from the crystal with a combination of phi and omega scans. A scan width of 0.5° and scan time of 10 s were employed. Data collection, reduction, structure solution, and refinement were performed using the Bruker Apex2 suite (v2.0-2).[49] All available reflections to $2\theta_{max}$ = 52° were harvested and corrected for Lorentz and polarization factors with Bruker SAINT (v6.45).[50] Reflections were then corrected for absorption, interframe scaling, and other systematic errors with SADABS 2004/1.[51] The structure was solved (direct methods) and refined (full-matrix least-squares against F^2) with the Bruker SHELXTL package (v6.14-1).[52] All non-hydrogen atoms were refined using anisotropic thermal parameters. Hydrogen atoms were included at idealized positions and were not refined. Pertinent details are given in Table 3.

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