



Diversity oriented clicking delivers β -substituted alkenyl sulfonyl fluorides as covalent human neutrophil elastase inhibitors

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Diversity Oriented Clicking (DOC) is a discovery method geared toward the rapid synthesis of functional libraries. It combines the best attributes of both classical and modern click chemistries. DOC strategies center upon the chemical diversification of core “SuFExable” hubs—exemplified by 2-Substituted-Alkynyl-1-Sulfonyl Fluorides (SASFs)—enabling the modular assembly of compounds through multiple reaction pathways. We report here a range of stereoselective Michael-type addition pathways from SASF hubs including reactions with secondary amines, carboxylates, 1*H*-1,2,3-triazole, and halides. These high yielding conjugate addition pathways deliver unprecedented β -substituted alkenyl sulfonyl fluorides as single isomers with minimal purification, greatly enriching the repertoire of DOC and holding true to the fundamentals of modular click chemistry. Further, we demonstrate the potential for biological function – a key objective of click chemistry – of this family of SASF-derived molecules as covalent inhibitors of human neutrophil elastase.

Diversity Oriented Clicking | SuFEx | Michael addition | human neutrophil elastase | covalent inhibitor

Diversity Oriented Clicking (DOC) (1) is a unified click chemistry approach for the modular synthesis of functional molecules and achieving “diversity with ease” (2). DOC combines the essence of classical click philosophy and reactivity with modern sulfur(VI) fluoride exchange (SuFEx) technology, exemplified through the multiple click-cycloaddition pathways of 2-Substituted-Alkynyl-1-Sulfonyl Fluoride (SASF) connective hubs (3–5).

The family of existing “SuFExable” functionality is grouped into two distinct categories: 1) inorganic high oxidation state sulfur compounds that are heavily functionalized with exchangeable sulfur-fluoride bonds (6, 7), e.g., sulfonyl fluoride (SO₂F₂) (8) and thionyl tetrafluoride (SOF₄) (9), and 2) high oxidation state sulfur-fluoride containing head groups that are combined with organic functionality, e.g., alkyl/aryl sulfonyl fluorides, aromatic fluorosulfates, ethenesulfonyl fluoride (ESF) (10, 11), 1-bromoethenesulfonyl fluoride (BESF) (12–14), 2-substituted-alkynyl-1-sulfonyl fluorides (SASFs) (1), sulfamoyl fluorides (15–18), sulfonimidoyl fluorides (19) and sulfondiimidoyl fluorides (20) (selected examples shown in Fig. 1A). Each hub offers a multitude of pathways for synthesizing structurally diverse compounds with interesting functional properties, demonstrated by our recent report on the DOC of SASF hubs to deliver a library of distinct SuFExable pharmacophores (Fig. 1B) (1). Although β -functionalization of 2-substituted-alkenyl-1-sulfonyl fluorides (21) and 2-substituted-alkynyl-1-triflones (22) is known, the Michael addition chemistry of SASFs has not been previously reported.

SuFEx click chemistry affords the potential to modify proteins covalently; a technique with broad application in chemical biology (23), not least, bioconjugation (24, 25), target identification (26–28), inverse drug discovery (29), and activity-based protein profiling (30, 31). Examples of the selective *in vitro* or *in vivo* covalent capture of proteins by SuFEx-based probes are steadily growing (3). These events are made possible by environments that facilitate extraction of fluoride, such as proximal arginine side chains (27), and felicitous electrostatic and electric field effects that are distinct for all-natural proteins (32–35). In 2019 Sharpless, Wolan, and coworkers reported a new class of covalent inhibitors of human neutrophil elastase (hNE), identified through an agnostic screen of a library of sulfur-fluoride containing ligands (36). A member of the serine protease superfamily, hNE is aberrantly active in cystic fibrosis, chronic obstructive pulmonary disease, and inflammatory bowel diseases (37–44), and hence, an attractive target for drug discovery.

Through exploring the Michael addition pathways from SASF hubs with a range of nucleophiles, we report herein the synthesis of a diverse library of unprecedented β -substituted ethenesulfonyl derivatives (Fig. 1C). The nature of the β -substituent influences the electrophilicity

Significance

The ease of diversification of 2-Substituted-Alkynyl-1-Sulfonyl Fluorides through numerous reaction pathways further validates Diversity Oriented Clicking as an effective and robust method for lead discovery; one of the fundamental goals of click chemistry. The application of this approach is showcased by the discovery of a novel class of covalent inhibitors of the human neutrophil elastase, an attractive therapeutic target due to its implication in numerous chronic diseases. These molecules provide a prime example of the Sleeping Beauty effect in action, bio-compatible SuFEx reactions mediated by environmental factors unique to the “live” protein system, securing sulfur-fluoride-containing ligands as promising covalent therapeutics.

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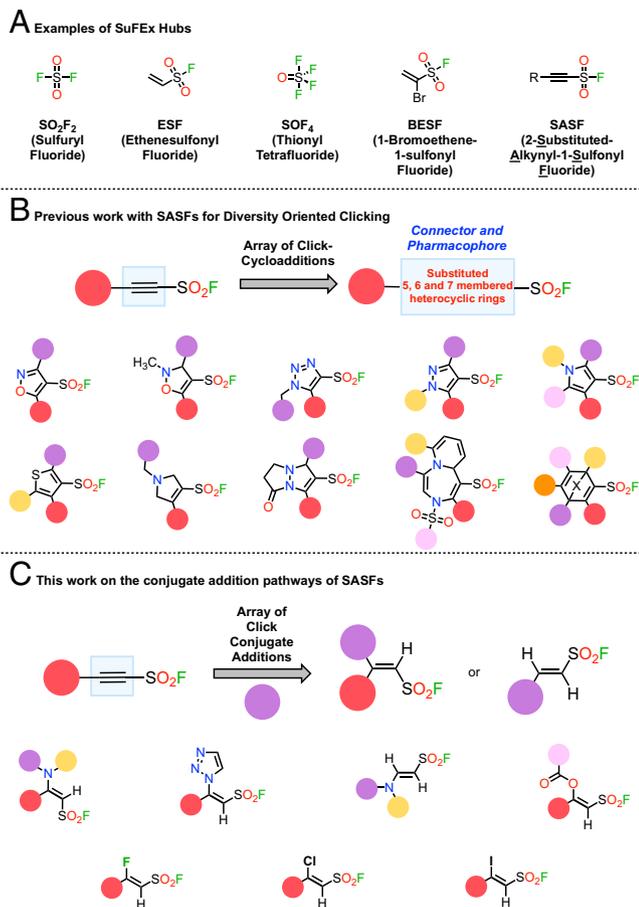


Fig. 1. (A) Examples of SuFEx-compatible hubs. (B) DOC of SASFs. (C) This work: DOC of SASF hubs through Michael addition pathways.

of the sulfonyl fluoride head group through a “push- and pull-like” distribution of electron density from the electron donating amine moiety on one end of the C=C double bond, and electron withdrawing sulfonyl fluoride group on the other end. The balance of these opposing forces fine tunes the reactivity of the β -substituted ESF derivatives toward incoming nucleophiles (Fig. 6). This ability to refine the electrophilicity of a reactive covalent group is highly desirable for drug discovery applications, and in-principle, should facilitate the design of covalent agents that are capable of selectively modifying target proteins through a “Sleeping Beauty” event, a perfect match-making with complete selectivity observed for living, but not denatured, biotargets (45). Through screening our library of novel ligands against hNE, we demonstrate the matched pair discovery of a new class of irreversible covalent inhibitors of hNE.

Results and Discussion

To assess the reactivity of SASFs toward Michael-type addition chemistry, morpholine (1.0 eq.) was added in one portion to a solution of Ph-SASF (**1**) in CH₃CN at room temperature. The stereoselective reaction reached complete conversion within minutes, generating exclusively the *E*-isomer addition product in >99% yield (Fig. 2A, **2-1**). An NOE correlation between H_A and H_B (Fig. 2B) was used to determine the stereochemical configuration of the product (*E*)-**2-1** and this assignment was corroborated by single-crystal X-ray crystallography. We attribute the observed stereoselectivity of this reaction to a protonation event (Fig. 2B).

After the initial attack at the triple bond, the available proton source is from the same amine that is attached to the double bond; therefore, intramolecular proton transfer occurs at the same side as the amine to generate the *E*-isomer exclusively. The enamine nature of the product results in a single addition, suggesting a stabilized “push-pull” state is achieved in the β -substituted ESF product.

We next explored the scope of the Michael-type click-addition chemistry of **1** with a collection of amines. In most cases, the reactions occurred rapidly and in high yield, selectively delivering the *E*-isomer of β -substituted ESFs as single products (Fig. 2A, **2-2** to **2-22**).¹ The reaction is tolerant of a wide range of functionality, including hydroxyl **2-8**, amide **2-9**, nitrile **2-16**, and carbonyl **2-18** groups. However, the presence of a carboxylic acid tended to slow the rate of conjugate addition, presumably due to the poor solubility of the corresponding salt in acetonitrile. When a 1:1 mixture of H₂O/CH₃CN was used as the solvent, the reaction of isonipecotic acid and **1** proceeded smoothly to afford the dual-addition product **2-22** in 95% yield, in which case both the amine and carboxylate groups had each reacted with an equivalent of the SASF. The reaction of piperazines proceeded smoothly (**2-11** to **2-13**) and even the sterically encumbered amine 1,3,3-trimethyl-6-azabicyclo[3.2.1]octane delivered product **2-14** in high yield (94%).

The SASFs were also found to be stable to thiols. For instance, reacting the 4-trifluoromethylphenyl SASF **3-1** with an equimolar amount of 1-hexanethiol in CH₃CN at ambient temperature for 12 h resulted in more than 80% recovery of the SASF species, with no Michael-type adduct or other products observed.

We next explored the Michael-type reactivity of substituted SASFs with a selection of nucleophiles (Fig. 3A and C, **4-1** to **4-29**). The addition of one equivalent of *N,N*-dipropargylamine to a variety of SASFs occurred readily to produce the corresponding ESF analogs in excellent yield (**4-1** to **4-9**). SASFs with alkyl substituents at the triple bond were less reactive compared with their aryl counterparts, requiring longer reaction times to achieve complete conversion (**4-7** ~30 min). The Michael addition of 1*H*-1,2,3-triazole to the SASFs gave exclusively the *Z*-products **4-15** to **4-23** in good overall yield. The *Z*-stereochemistry was determined by a combination of X-ray crystallography and NMR experiments. The crystal structure of **4-17** shows the triazole ring *cis* to the sulfonyl fluoride group, and out of plane with the π -system, whereas the NOESY spectrum of **4-16** reveals a strong NOE correlation between the alkenyl proton H_A and the 4-trifluoromethylbenzene ring (H_B), but not between H_A and the triazole ring protons (Fig. 3B). We posit that the *Z*-selectivity can be explained by a proton transfer process. After initial nucleophilic attack by the 1,2,3-triazole toward the triple bond, intramolecular proton transfer is not available, while the triazole itself is acidic enough to serve as the proton source for intermolecular protonation of the more stable anion intermediate, yielding the *Z*-isomer as the sole product and minimizing steric clash between the phenyl and SO₂F group (Fig. 3B).

The addition of secondary amines to the sterically hindered triisopropylsilyl (TIPS) SASF **5** was markedly slower than with other SASFs, resulting in synchronous protodesilylation to give the *E*-products **4-24** to **4-29** in good to moderate yield (Fig. 3C). This new synthesis compliments Hyatt and Krutak’s MnO₂ mediated dehydrogenation of β -fluorosulfonylethylamines (46) and the

¹Where yields were lower than expected, the mass balance could be accounted for in unreacted starting materials, especially when bulky amine substrates or SASFs with electron donating groups on the aromatic ring were utilized.

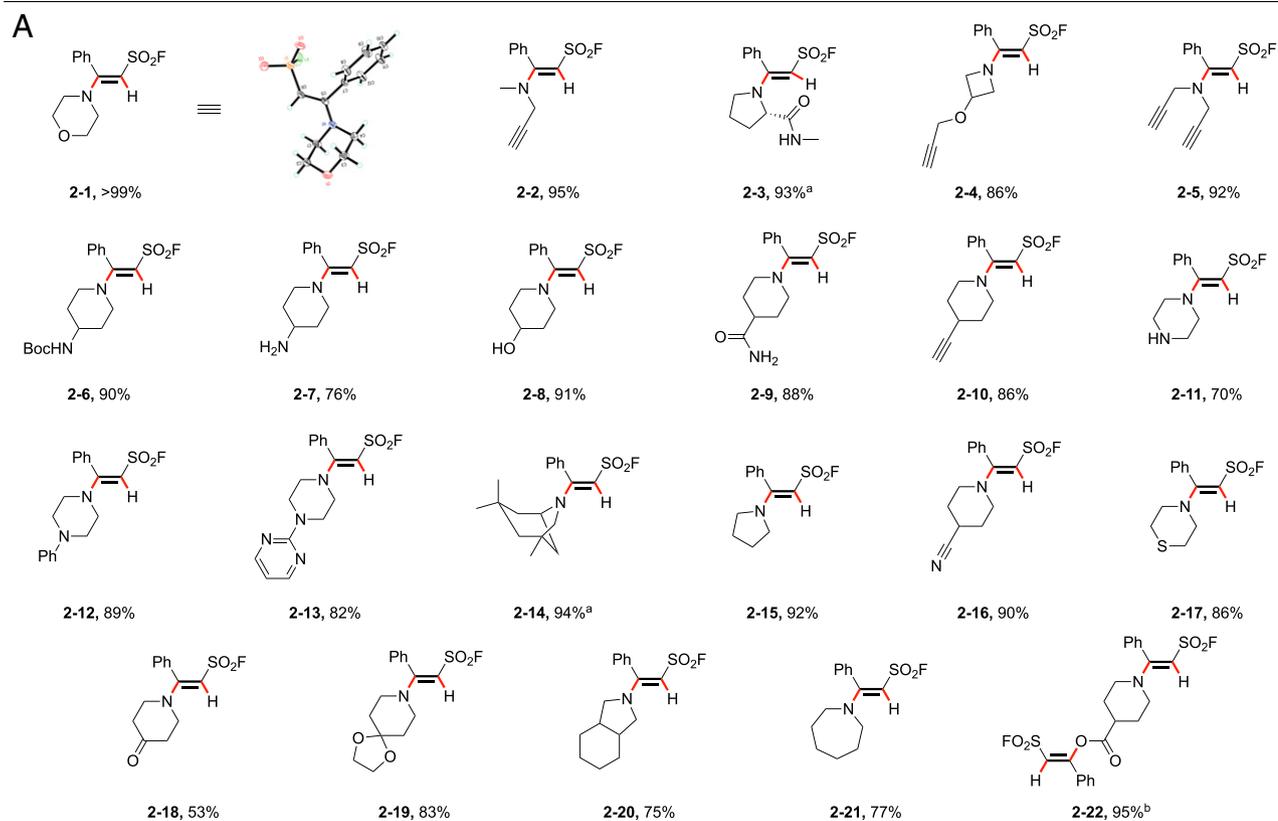
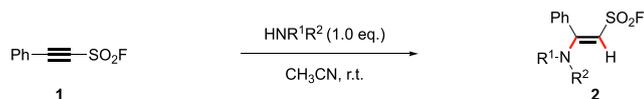


Fig. 2. Michael addition of amines to Ph-SASF (**1**): (A) Substrate scope. (B) Proposed mechanism and representative NOESY spectrum for (*E*)-**2-1**. General reaction conditions: The amine (1.0 eq.) was added to a solution of SASF (1.0 eq.) in CH_3CN , and the reaction was stirred at room temperature for 10 min or until complete conversion. Reactions were conducted on a 100 μmol scale unless stated otherwise in the *SI Appendix*. Isolated yields are reported. *a*. A pair of rotamers were observed. *b*. 0.5 eq. isonipetric acid was used, and 1:1 $\text{CH}_3\text{CN}/\text{H}_2\text{O}$ was used as the solvent.

reaction of the 1-bromo-2-triazolethene-1-sulfonyl fluoride connective hub with amines reported by Qin et al. (47). The assigned *E*-stereochemistry is consistent with the previously reported syntheses (46, 47) and supported by *trans*-coupling constants $J_{\text{H}_\text{A}-\text{H}_\text{B}}$ ranging from 12.3 to 12.7 Hz. Replacing the $-\text{SO}_2\text{F}$ group of **5** with the corresponding $-\text{SO}_2\text{CF}_3$ also led to desilylated products, ruling out the involvement of fluoride ions in the desilylation process. When *N,N*-dipropargylamine was used as the donor, >90% of unreacted starting material was recovered after 1 h. When stirred overnight at room temperature, no reaction occurred between TIPS-SASF and 3-isopropylaniline. In some instances, increasing the amount of the secondary amine to 2 equivalents improved the efficiency of the reaction (**4-29**).

Halides were also found to be viable nucleophiles for conjugate addition to SASFs (Fig. 4A). The reaction with

triethylamine trihydrofluoride ($\text{Et}_3\text{N}\cdot 3\text{HF}$) delivered the β -fluorinated ESF products **6-1** to **6-8** in high yield with *Z*-selectivity as suggested by the large $J_{\text{E-H}}$ value (29.2 Hz in the instance of (*Z*)-**6-4**) (48) and a significant NOE correlation between H_A and H_B (Fig. 4B). Similarly, triethylamine hydrochloride delivered the corresponding β -chlorinated ESF products in mostly excellent yields (**6-9** to **6-17**), although with comparatively longer reaction times (49). The β -iodinated ESF **6-18** was available using equimolar lithium iodide and acetic acid.

The addition of carboxylic acids to SASFs was next explored in the presence of 1.1 equivalents of triethylamine in CH_3CN , delivering the β -ester-substituted ESF products **7-1** to **7-17** in excellent yield with a short reaction time of 30 min (Fig. 5A). As with 1*H*-1,2,3-triazole, and halides, the addition of carboxylic acids proceeds with the *Z*-selectivity as determined by

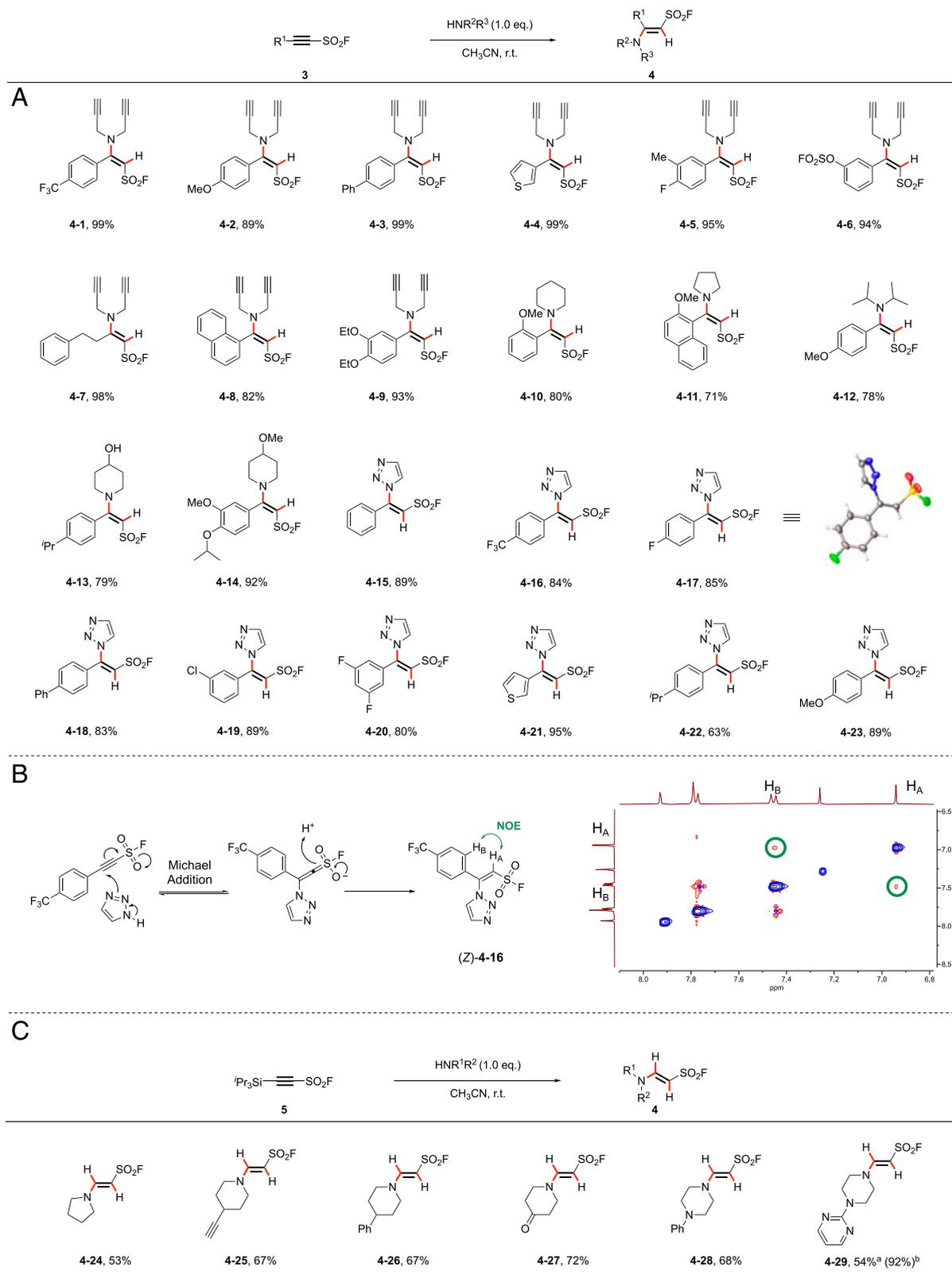


Fig. 3. Michael addition of amines and 1,2,3-triazoles to SASFs. (A) Substrate scope for aryl-SASF (**3**). (B) Proposed mechanism and representative NOESY spectrum for (*Z*)-**4-16**. (C) Substrate scope for trisopropylsilyl-SASF (**5**). General reaction conditions: The amine (1.0 eq.) was added to a solution of SASF (1.0 eq.) in CH_3CN , and the reaction was stirred at room temperature for 10 min or until full conversion. Scale of reactions outlined in the *General Procedures* and *SI Appendix*. Isolated yields are reported. *a*. 1.5 eq. amine was used; *b*. yield in parenthesis was obtained when 2.0 eq. amine was used.

NOESY experiments (Fig. 5B) and corroborated by single-crystal X-ray crystallography (7-11). For the addition of halides and carboxylates to the SASFs, there is no opportunity for an intramolecular proton transfer, therefore, an intermolecular

proton transfer occurs from the protonated triethylamine, favoring the *Z*-isomer (Fig. 4B and 5B).

We next studied the relative reactivity of the novel β -substituted ESFs. As expected, the electron-donating effects

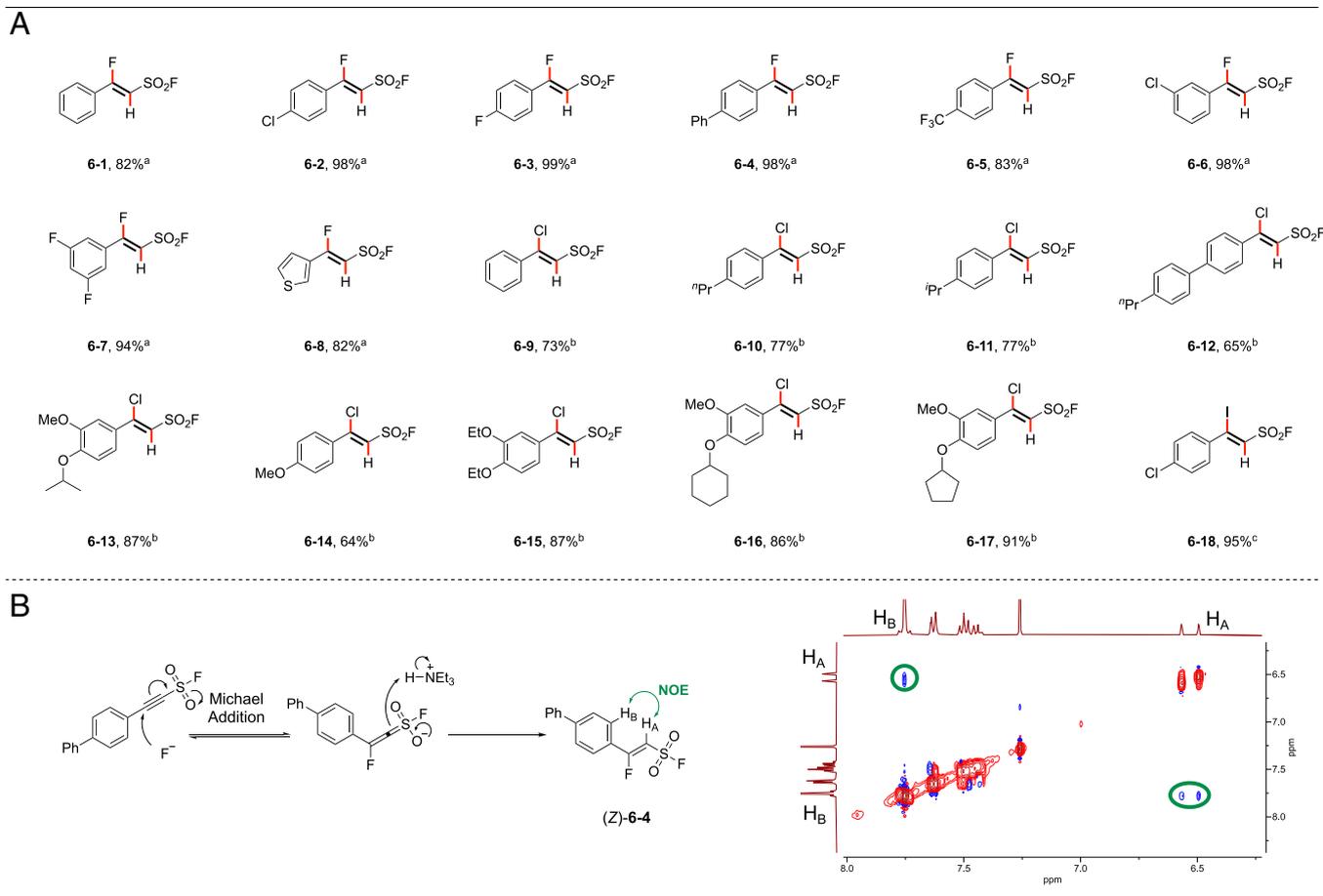
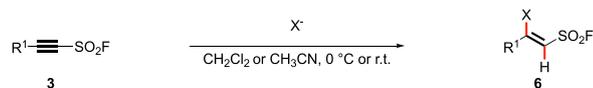


Fig. 4. Addition of halides to SASFs. (A) Substrate scope. (B) Proposed mechanism and representative NOESY spectrum for (Z)-6-4. General reaction conditions: halide source was added to a solution of SASF (1.0 eq.) in CH_3CN and the reaction was stirred at room temperature until complete conversion. Reactions were conducted on a 200 μmol scale unless stated otherwise in the *SI Appendix*. Isolated yields are reported. *a.* $\text{Et}_3\text{N}\cdot 3\text{HF}$ (0.5 eq.) was used. *b.* $\text{Et}_3\text{N}\cdot\text{HCl}$ (1.1 eq.) was used in CH_2Cl_2 at 0°C followed by stirring at room temperature for 16 h; *c.* Lil (1.0 eq.) and HOAc (1.0 eq.) were used.

of the secondary amine substituents strongly deactivated both the $-\text{SO}_2\text{F}$ group and double bond. Even when heated at 80°C for several hours, secondary amines did not undergo conjugate addition to β -amino-substituted ESFs. When PhOTBS (**8**) 1.1 eq. was reacted with the derivative **4-6** under standard SuFEx conditions [DBU (20 mol%), CH_3CN], the exchange reaction occurred exclusively at the fluorosulfate to give the product **9** in quantitative yield (Fig. 6A). In a separate experiment with the 1,2,3-triazole-substituted ESF **4-16** and PhOTBS (**8**), the $-\text{SO}_2\text{F}$ group showed higher SuFEx reactivity than that of β -amino-substituted ESFs. Here, the anticipated sulfonate product **10** was obtained in good yield (Fig. 6B).

One of the key objectives of click chemistry is the discovery of functional molecules. Therefore, with a collection of over 80 novel β -substituted ESF hubs in hand, we elected to screen the products for biological activity against hNE (36). First, the stability of the compounds was evaluated in an aqueous buffer solution at different pH values. The amine-substituted ESFs were unstable under acidic conditions, while β -fluorinated ESFs and 1,2,3-triazole-substituted ESFs decomposed within 1 h at pH 10. However, at pH 7.4, the compounds were stable over 6 h.

A kinetic assay using hNE was next employed to evaluate the inhibitory effects of the compounds. Pure hNE was incubated with compounds at different concentrations in phosphate-buffered saline (PBS, pH = 7.4) supplemented with 0.05%

(vol/vol) Nonidet P-40 for 10 min before the addition of substrate. The proteolytic activity of hNE was measured at 25°C for 30 min at 30-s intervals, and the IC_{50} values were obtained by fitting the data to dose-dependent inhibition. The fine tuning of SuFEx reactivity through the β -substituent effects is clearly demonstrated in the series of ligands: the β -amino-substituted and β -fluorinated ESFs showed no meaningful inhibition of hNE at the concentrations tested (>200 mM), while several 1,2,3-triazole-substituted ESFs and β -ester-substituted ESFs demonstrated good inhibition of this enzyme with IC_{50} values in the low micromolar range (Fig. 7A, see *SI Appendix* for full details). Next, representative compounds that showed hNE inhibition with IC_{50} of less than 10 μM were further analyzed by high-resolution electrospray ionization (ESI) mass spectrometry (refer to *SI Appendix* for full details). Fig. 7B depicts the ESI mass spectrum of compound **4-16** (exact mass 321 Da) incubated with hNE (25,198 Da), and a peak shift from the original protein mass by 301 Da consistent with covalent addition of one molecule of **4-16** and loss of one fluorine atom and one hydrogen (likely from the protease's catalytic serine). Likewise, other 1,2,3-triazole-substituted ESFs showed comparable peak shifts when incubated with hNE, consistent with covalent SuFEx modification of the protein.

Molecular docking simulations were performed to build a binding mode model of the most active compound (**4-16**,

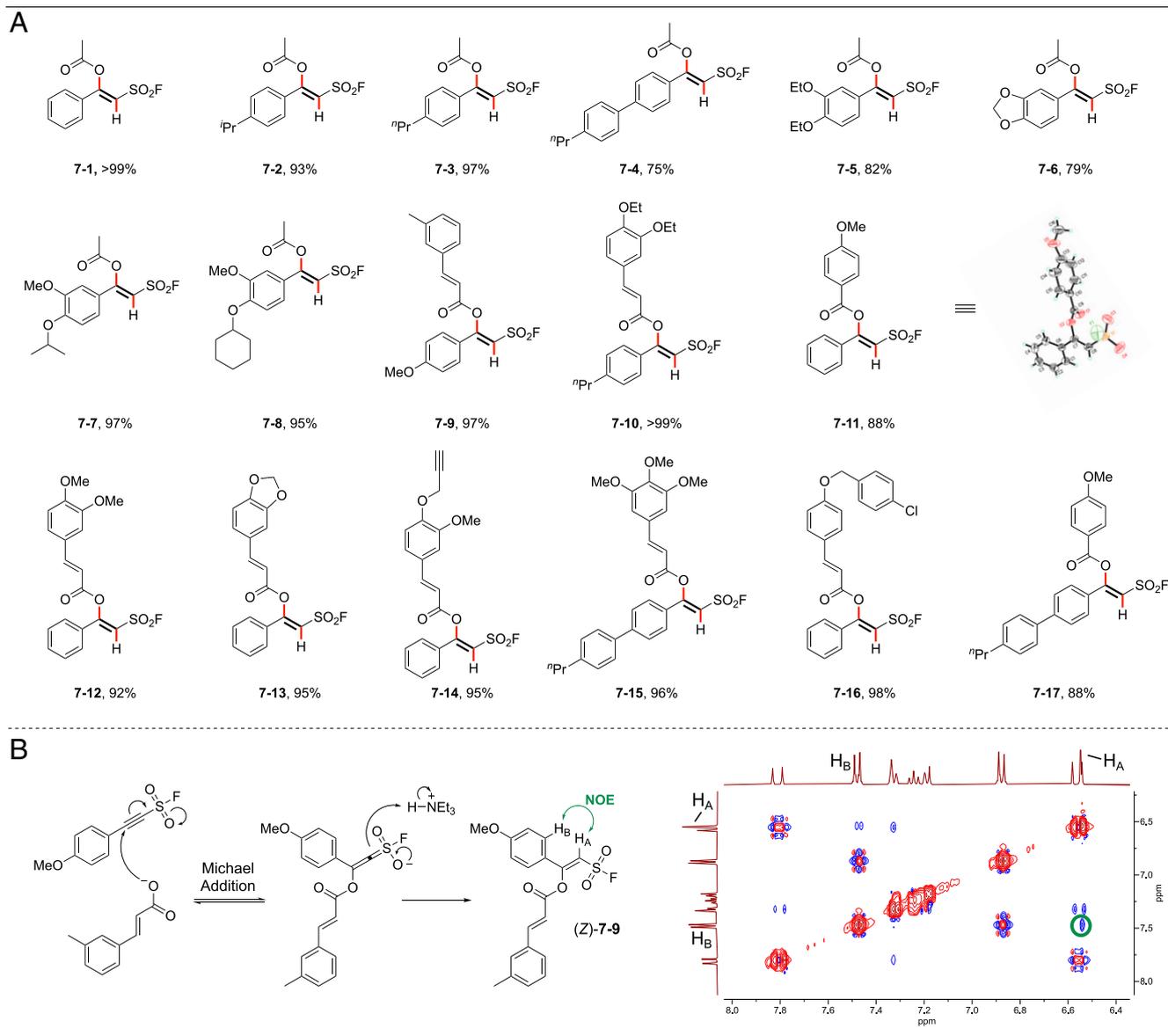
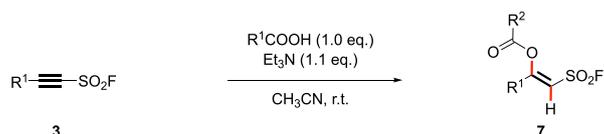


Fig. 5. Addition of carboxylic acids to SASFs. (A) Substrate scope. (B) Proposed mechanism and representative NOESY spectrum for (Z)-7-9. General reaction conditions: Et₃N (1.1 eq.) was added to a solution of SASF (1.0 eq.) and carboxylic acid (1.0 eq.) in CH₃CN, and the reaction was stirred at room temperature for 30 min. Reactions were conducted on a 100 μmol scale unless stated otherwise in the *SI Appendix*. Isolated yields are reported.

applying the reactive docking protocol (50) to model the ligand free and untethered with the intact warhead before the formation of the covalent adduct. In the predicted binding mode, the ligand **4-16** adopts an orientation similar to the SuFEx-derived hNE binders that we previously reported (Fig. 7C) (36). The hydrophobic pocket roughly defined by Ser214, Val216, and Val190 remains relevant to stabilizing the ligand, revealing significant engagement with the β-triazole moiety. This interaction suggests that the heterocycle could be stabilized by potential stacking with the peptide amides of Phe215 and Val216 backbones. The *cis* conformation orients the trifluorotoluene group of **4-16** such that it points toward the bulk solvent, reaching out to the side chains of Phe215 and Leu99. Finally, a potential hydrogen-bond interaction with the backbone amide of Gly193 appears to further stabilize one of the sulfone

oxygens in a conformation highly favorable to facilitate the formation of the nascent covalent bond between sulfur and Ser195 Oγ.

We have demonstrated DOC as a powerful method for discovering functional molecules by expanding the available click-pathways of SASF hubs to include the Michael addition of amines, 1*H*-1,2,3-triazole, halides, and carboxylic acids. Using the DOC approach, we have synthesized a collection of unprecedented β-substituted ESFs in excellent yield, and as single stereoisomers. The relative SuFEx reactivity of β-substituted ESFs can be readily tuned through judicious choice of β-substituent, and hence has potential application as a tool for developing covalent inhibitors. This is showcased through the discovery of a selection of covalent inhibitors of hNE, most notably the 1,2,3-triazole and carboxylic acid adducts, demonstrating the

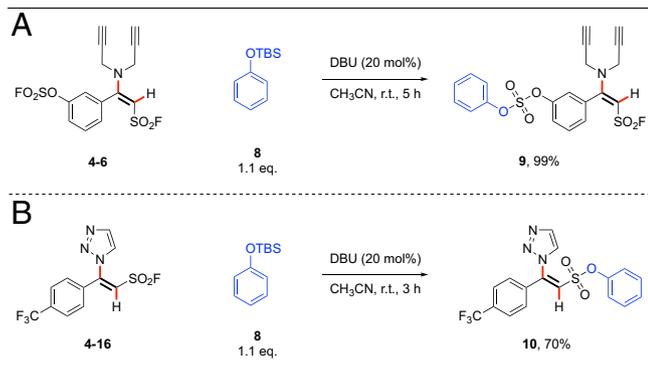


Fig. 6. SuFEx reactivity of β -disubstituted ESF. (A) Secondary amine adduct **4-6**. (B) 1,2,3-Triazole adduct **4-16**.

importance of the differing “push- and pull-like” distribution of electron density on ligand SuFExability. We advocate DOC as a valuable tool for discovery chemistry that will find wide

application in covalent enzyme inhibition, target identification, mapping of enzyme binding sites, and probing protein-protein interactions.

Materials and Methods

Synthesis of SASFs.

Method 1. To a solution of the required terminal alkyne (1.00 mmol) in diethyl ether (5.00 mL) at -78°C was added $t\text{BuLi}$ (1.05 eq.) dropwise. The resulting mixture was stirred at -78°C for 0.5 h before the addition of fluorosulfonic anhydride (1.50 mmol, 1.50 eq.). After stirring at -78°C for 0.5 h, the reaction was warmed to room temperature and the solvent was then removed under reduced pressure. The crude mixture was then purified by flash column chromatography (5% EtOAc in hexanes) to obtain the analytically pure product.

Method 2. To a solution of the required terminal alkyne (10.0 mmol) in anhydrous THF (10.0 mL) at -78°C was added $t\text{BuLi}$ (1.05 eq.) dropwise and stirred at -78°C for 1 h. To the cooled reaction mixture was added a balloon of SO_2 by cannula, first bubbled through conc. H_2SO_4 (20.0 mL) and stirred at -78°C for 1 h. The reaction mixture was warmed to room temperature and the remaining SO_2 removed by vacuum, purging with argon. The reaction mixture was

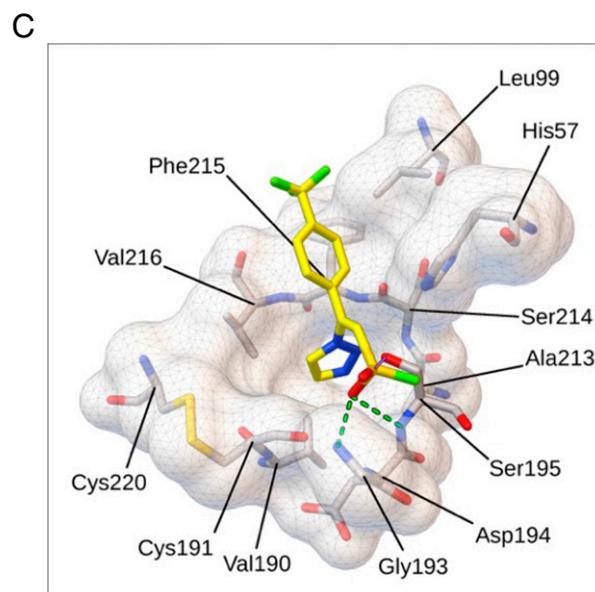
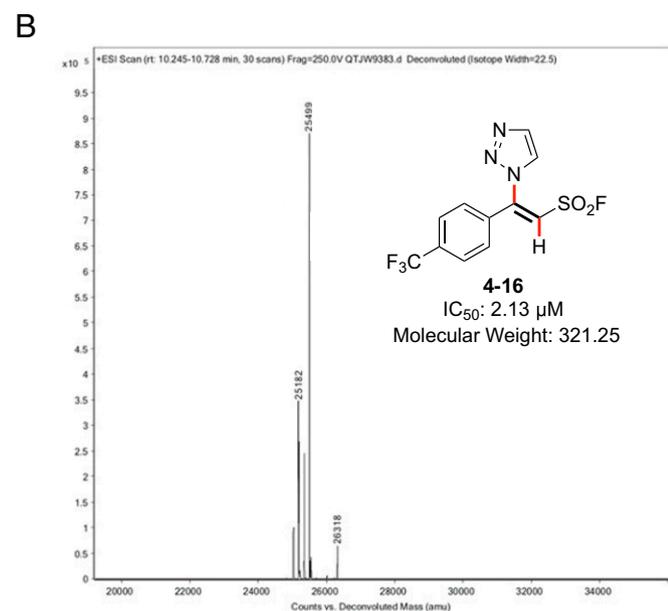
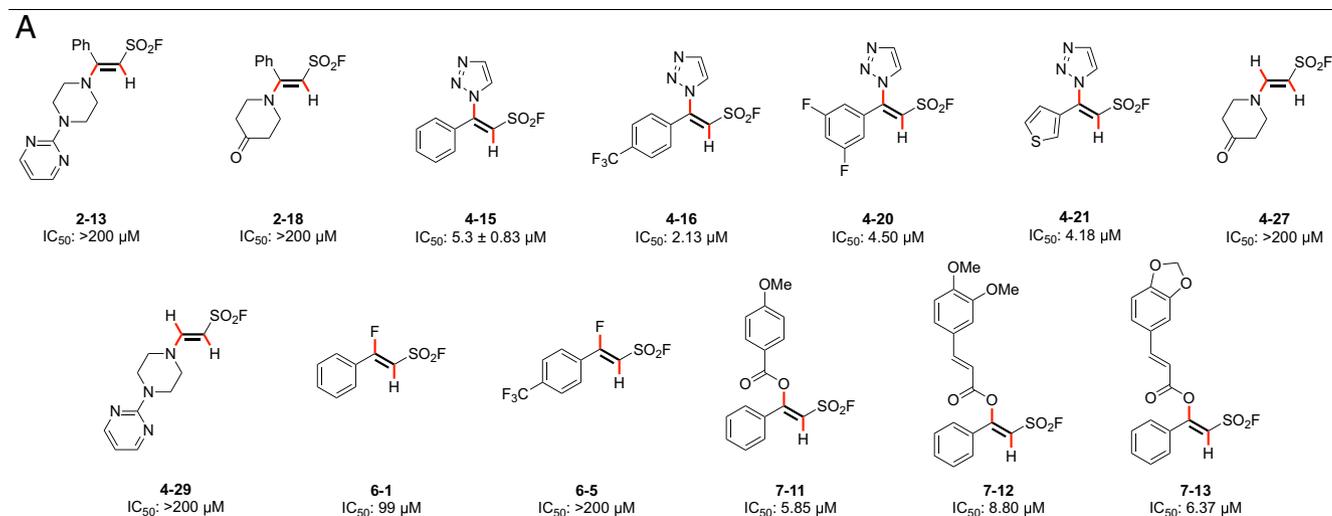


Fig. 7. (A) IC_{50} values of selected β -substituted ESFs against hNE; (B) ESI mass spectrum of the SuFEx-adduct of elastase and selected probe; (C) Molecular docking results: Residues defining the binding site are shown as *gray* sticks, and volumes represented as semitransparent surfaces. Ligand **4-16** is shown as *yellow* sticks; hydrogen bonds are shown as *green* dashed lines; incipient covalent bond is shown in *purple*.

then diluted with anhydrous THF (20.0 mL), cooled to -78°C and *N*-fluorobenzenesulfonamide (6.30 g, 20.0 mmol) was added in one portion and removed from the cold bath, and stirred at room temperature for 3 h. The mixture was then filtered, and the solvent removed under reduced pressure. The crude was redissolved in the minimum amount of diethyl ether, triturated with petroleum ether (50.0 mL), filtered, and the filtrate solvent was removed under reduced pressure. The crude mixture was purified by flash column to obtain the desired compound.

General Procedure A for the Preparation of Compounds 2-1 to 2-22 (Fig. 2A) and 4-1 to 4-23 (Fig. 3A). To a solution of the required SASF (100 μmol) in acetonitrile (2.00 mL) was added the required amine (100 μmol) and the reaction mixture was stirred at room temperature until full conversion was observed by Thin-layer chromatography (TLC). The reaction mixture was diluted with ethyl acetate, washed with brine and H_2O , dried over sodium sulfate, filtered, and the solvent removed under reduced pressure to obtain the desired compound (45 examples, 53 to 99% isolated yield).

General Procedure B for the Preparation of Compounds 4-23 to 4-29 (Fig. 3C). To a solution of 2-(triisopropylsilyl)ethyne-1-sulfonyl fluoride (200 μmol) in acetonitrile (2.00 mL) was added the required amine (300 μmol) and the reaction mixture was stirred at room temperature until full conversion was observed by TLC. The reaction mixture was diluted with ethyl acetate, washed with brine and H_2O , dried over sodium sulfate, filtered, and the solvent removed under reduced pressure to obtain the desired compound (6 examples, 53 to 72% isolated yield).

General Procedure C for the Preparation of Compounds 6-1 to 6-8 (Fig. 4A). To a solution of the required SASF (200 μmol) in acetonitrile (1.00 mL) was added triethylamine trihydrofluoride (100 μmol) and the reaction mixture was stirred at room temperature for 1 h. The reaction mixture was diluted with ethyl acetate, washed with brine and H_2O , dried over sodium sulfate, filtered, and the solvent removed under reduced pressure to obtain the desired compound (8 examples, 82 to 99% isolated yield).

General Procedure D for the Preparation of Compounds 6-9 to 6-17 (Fig. 4A). To a solution of the required SASF (100 μmol) in CH_2Cl_2 (500 μL) at 0°C was added triethylamine hydrochloride (110 μmol) and the reaction mixture was stirred at room temperature for 16 h. The reaction mixture was diluted with ethyl acetate, washed with brine and H_2O , dried over sodium sulfate, filtered, the solvent removed under reduced pressure and the crude product was purified by flash column chromatography to obtain the desired compound (9 examples, 64 to 91% isolated yield).

General Procedure E for the Preparation of Compound 6-18 (Fig. 4A). To a solution of the required SASF (100 μmol) in acetonitrile (1.00 mL) was added lithium iodide (100 μmol) and AcOH (100 μmol) and the reaction mixture was stirred at room temperature until full conversion was observed by TLC. The reaction mixture was diluted with ethyl acetate, washed with brine and H_2O , dried over sodium sulfate, filtered, and the solvent removed under reduced pressure to obtain the desired compound (1 example, 95% isolated yield).

General Procedure F for the Preparation of Compounds 7-1 to 7-17 (Fig. 5A). To a solution of the required SASF (100 μmol) and carboxylic acid (100 μmol) in acetonitrile (500 μL) was added Et_3N (110 μmol) and the reaction mixture was stirred at room temperature for 30 min. The reaction mixture was

diluted with ethyl acetate, washed with brine and H_2O , dried over sodium sulfate, filtered, and the solvent removed under reduced pressure to obtain the desired compound (17 examples, 79 to 98% isolated yield).

Protease Activity Assays [see Zheng et al. (36)]. hNE activity was measured in a total volume of 100 μL in a reaction buffer of PBS (pH 7.4) and 0.05% (vol/vol) Nonidet P 40 Substitute (Sigma). The final composition of each reaction was 5 nM hNE (Elastin Products Corp), 50 μM AAPV-aminomethylcoumarin (AMC) substrate (Millipore), $\sim 2.5\%$ dimethyl sulfoxide (Fisher), and various concentrations of compounds as inhibitors. hNE was incubated with compounds for 10 min at room temperature before the addition of AAPV-AMC. Residual proteolytic activity was measured kinetically at 25°C using an Envision microplate reader for a total of 30 min at 30-s intervals. Only data points "reflecting linear substrate conversion were used to determine relative protease activity. IC_{50} values were obtained by fitting the data to a dose-response inhibition, $\log(\text{inhibitor})$ vs. response-variable slope (four parameters) using GraphPad Prism.

Mass Spectrometry Analysis [see Zheng et al. (36)]. hNE was resuspended in 50 mM sodium acetate (NaOAc, pH 4.5), 100 mM NaCl to 0.2 mg/mL final concentration. DMSO solution of each compound (10 mM) was diluted 1:10 in the above buffer, then added in a compound:hNE ratio = 3:1 and incubated at room temperature for 1 h prior to analysis by MALDI-TOF mass spectrometry.

Molecular Modeling. The crystal structure of hNE was retrieved from the Protein Data Bank: (PDB id: 6e69). Hydrogens were added with Reduce, then the structure was prepared following the standard AutoDock protocol. A cubic docking grid box of $60 \times 60 \times 60$ points (22.5 \AA side) was defined around Ser195. Dockings were performed with AutoDock v4.2.6 generating 100 poses using the default LGA parameters. The top energy pose was selected and analyzed. Figures were generated using Python Molecular Viewer.

Data, Materials, and Software Availability. All study data are included in the article and/or supporting information.

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