“Sleeping beauty” phenomenon: SuFEx-enabled discovery of selective covalent inhibitors of human neutrophil elastase

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Abstract:

Sulfur-Fluoride Exchange (SuFEx) has emerged as the new generation of click chemistry. We report here a SuFEx-enabled approach exploiting the “sleeping beauty” phenomenon of sulfur fluoride compounds in the context of the serendipitous discovery of selective covalent human neutrophil elastase (hNE) inhibitors. Evaluation of an ever-growing collection of SuFExable compounds toward various biological assays unexpectedly yielded a selective and covalent hNE inhibitor, benzene-1,2-disulfonyl fluoride. Derivatization of the initial hit led to a better agent, 2-triflyl benzenesulfonyl fluoride, itself made through a SuFEx trifluoromethylation process, with IC_{50} = 1.1 μM and ~200-fold selectivity over the homologous neutrophil serine protease, cathepsin G. The optimized probe only modified active hNE and not its denatured form, setting another example of the “sleeping beauty” phenomenon of sulfur fluoride capturing agents for the discovery of covalent medicines.

Sulfur fluoride exchange (SuFEx)—the new generation click chemistry, since first introduced in 2014 (1), has quickly found diverse applications across an array of fields including chemical synthesis (2-12), material science (13-19), chemical biology (20-31), and drug discovery (32, 33). SuFEx creates robust intermolecular links between modules. The extreme fidelity stems from the ability of otherwise, very stable higher oxidation state sulfur fluorides (34-38) to exchange S–F with incoming nucleophiles under SuFEx catalysis conditions, forming stable and irreversible linkages united through a sulfur center. These SuFEx reactions are made possible by the unique requirements set to stabilize a departing fluoride ion in its transit away from a strong covalent bond, with "H^+" or "R_3Si^+" mediators, and especially favored by DBU-type amine catalysts (13, 39, 40), and also thought to involve bifluoride counter ion species (16, 17, 41).

In the context of selective *in vitro* or *in vivo* covalent capture of proteins by SuFExable[*] compounds, examples are accumulating rapidly (20-31), but we are still reluctant to make strong *a priori* inferences about the factors which determine a given capture's occurrence. This said, there is one remarkable fact shared by all SuFEx-based protein captures that the probes' special S–F links are among the most demure electrophiles known[†] (1, 34-38). Only a correctly folded and functionally active protein can serve as a catalyst[‡] for a SuFEx capture event upon one of its own nucleophilic amino acid sidechains (**Fig. 1A**). These bio-compatible SuFEx reactions are presumably mediated by environmental factors unique to the "live" system, thereby facilitating displacement of the otherwise stable sulfur-fluoride by the partner protein. In contrast, the denatured proteins are completely inert to the same S–F probes (24, 32). We name this special relationship between functional living proteins and SuFExable probes the "sleeping beauty" phenomenon[§], which mirrors very closely our earlier protein enabled Huisgen azide-alkyne cycloaddition (i.e. *in situ* click chemistry) (42, 43), in terms of the "live" enzyme requirement.

Taking inspiration from the original click chemistry manifesto (44); that molecular diversity can be achieved with ease through the connection of small modules, using just a few good reactions, in sequences of usually no more than three steps, we demonstrate here two distinct but logically

connected, sequential SuFEx-enabled entities: (1) efficient construction of a pool of SuFExable compounds *via* click chemistry principles, and (2) bioprospecting this library for covalent capture agents for important protein targets *via* “sleeping beauty” reactivity. The expectation, based on previous experience, is that evaluation of a SuFEx library will often result in multiple lead compounds. The latter can usually be followed up by easily deployed SAR-based (structure-activity-relationship-based) lead studies. This “compound-bank” mode of the SuFEx-enabled platform, itself contributes to the ever-growing library of SuFExable modules and candidates, and like “compounding-interest”, rewards the principle library with expanding opportunities for discovering new kinds of phenotypic modulators; including useful new functions for medicines in the long term (**Fig. *1*B**).

Over the past five years, we (1, 5, 8, 45) and others (12, 46-49) have developed a valuable collection of efficient methods to synthesize SuFExable compounds from either simple or complex organic molecules using highly connective SuFEx core electrophiles (e.g. SO_2F_2, $O{=}SF_4$, $CH_2{=}CHSO_2F$). Collective work within the Sharpless laboratory continues to build a library of SuFExable small modules. There are >1000 compounds in this collection[¶]. Thanks to the great stability of the SuFExable S–F links toward water and oxygen, the oldest library deposits (as DMSO solutions) are still pure. The individual compounds are dissolved as 10 mM DMSO stock solutions and stored in –20 ºC freezer, with most being stable at –20 ºC as evidenced by periodic inspection by LC-MS of randomly sampled compound over 2 years. To date, the library and/or its sub-libraries have been screened with collaborators at Scripps Research and other institutes against multiple targets. Here we report a case study using the SuFEx-enabled approach to the discovery of selective, covalent inhibitors of human neutrophil elastase (hNE).

Human neutrophil elastase; a member of the serine protease superfamily, is aberrantly active in cystic fibrosis (CF), chronic obstructive pulmonary disease (COPD), and inflammatory bowel diseases (IBD) (50-66). This protease is therefore a key target for the development of anti-inflammatory agents to combat these diseases. Alkyl and aryl sulfonyl fluorides have a long

history as rather promiscuous covalent inhibitors of serine proteases (67-76). In the late 1970's, the Powers group demonstrated the intrinsic reactivity differences across several serine proteases, including elastase, with 2-amido(peptido) benzenesulfonyl fluoride inhibitors (77, 78). Their results encouraged us to investigate our library of SuFExable compounds as potential covalent inhibitors of hNE.

Results and Discussion

A set of 105 compounds (**SI Appendix, Table S1**), many of which appear to be new compounds, were selected without bias, to form a primary library for the screen against hNE. The selected compounds can be categorized into four subsets by the nature of S–F functional groups (**Fig. *1*C**). Each group tends to have its own intrinsic zone of reactivity in acid-base environments. To give a general impression, the relative rate measured on a representative molecule of each subset under SuFEx catalysis are 28.5 (**I**), 1.0 (**II**, reference), 14.1 (**III**), and 4.1 (**IV**), respectively (**SI Appendix, Figure S6**). Aryl sulfonyl fluorides (**I**), despite their own extreme resistance toward nucleophiles[*], top the SuFEx reactivity hierarchy among the four subsets, while aryl fluorosulfates (**II**) lie at the bottom.

Elastase (5 nM) was incubated with each entry of the primary SuFEx library (final compound concentration 200 μM) for 10 min at room temperature prior to the addition of peptide substrate MeOSuc-AAPV-AMC (50 μM). Increase in fluorescence was measured for 30 min at 30 sec intervals. The assay was well behaved, as evidenced by a Z' of 0.86, signal-to-background of ~3000:1, and a hit cutoff of >95% inhibition. Reasoning that a covalent inhibitor, which presumably targets a catalytic residue, should completely inactivate the enzyme at high compound concentration (200 μM), a high threshold (95%) was chosen for hit identification. Under this criterion, the screen yielded 7 hits as probable covalent inhibitors (**Fig. 2A**, 6.7% overall hit rate and 23% hit rate within subset **I**). All 7 compounds belong to subset **I**, which suggests a rough cut off based on the S-link's inherent reactivity. After validation by NMR, LC-MS, and dose-dependent

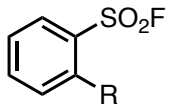
response, benzene-1,2-disulfonyl fluoride (**1**) proved to be the leading candidate, inhibiting hNE with IC_{50} = 3.3 ± 1.0 μM (**Fig. 2B**,**C**).

The covalent inhibition of hNE by **1** was examined by high-resolution MALDI-TOF mass spectrometry (**Fig. 3A**). Incubation of **1** (exact mass 242) with hNE yielded a peak shift from the protein mass by ~223 Da. Increased mass corresponds to: (1) a single molecule of **1** covalently captured by hNE; (2) the loss of one hydrogen from the protease (possibly from the catalytic serine); (3) the loss of one fluorine from **1**.

We also subjected hNE to co-crystallization with **1** in order to corroborate the covalent binding. The structure was determined using molecular replacement with PDB ID 5adw and the co-complex was refined to 2.33 Å resolution (**SI Appendix, Table S2**). Importantly the naïve Fo-Fc electron density maps contoured to 4σ clearly position **1**, as a result of the strong diffraction of sulfurs (**Fig. 3B**). The aryl group of **1** is nestled into a hydrophobic pocket consisting of residues Phe192 and Val216 and the compound is covalently bound to the catalytic Ser195, as highlighted by continuous electron density and a bond distance of ~1.6 Å (**Fig. 3C,D**). The covalent inhibition of hNE *via* sulfonylation by **1** appeared to be permanent—dialyzing away small molecules after incubation did not recover enzyme function (**SI Appendix, Figure S2**).

Considering the mono-covalent attachment mode of **1**, with the second $–SO_2F$ intact, it was envisaged that one of the two sulfonyl fluoride groups could be substituted so as to perhaps improve the capture rate, and/or selective binding. A set of benzenesulfonyl fluoride cores carrying *ortho*-substituents (**8**–**23**) was therefore examined. Compounds **8**–**18** were synthesized by the efficient aqueous potassium bifluoride exchange procedure from commercially available sulfonyl chlorides but showed poorer reactivity/binding with hNE[#]. Of the two improved compounds found in **Table 1**, **19**, the mono-vinylogous derivative of **1**, was a more active inhibitor with IC_{50} = 2.2 ± 0.7 μM. The *ortho*-sulfamoyl benzenesulfonyl fluorides (**20**, **21**) led to considerably lower activity. A recently developed potassium bifluoride catalyzed SuFEx trifluoromethylation of aryl sulfonyl fluorides (79) enabled us to quickly convert **1** to the

perfluoroalkyl sulfones (**22**, **23**). Compound **22** with the *ortho*-triflyl group emerged as the best lead molecule to date with IC_{50} = 1.1 ± 0.1 μM.



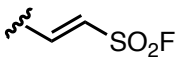
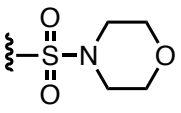
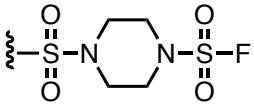
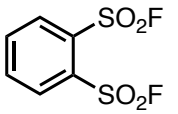
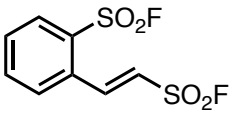
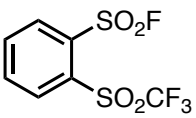
Compound	R	IC_{50} (μM)[ll]
1	SO_2F	3.3 ± 1.0
8	F	~120
9	Cl	82 ± 16
10	Br	20 ± 10
11	I	9.7 ± 1.2
12	Me	>200
13	OMe	73 ± 4
14	CN	13.3 ± 0.5
15	CF_3	60 ± 8
16	NO_2	20 ± 1
17	CO_2Me	37 ± 2
18	Ph	27 ± 1
19		2.2 ± 0.7
20		84.4 ± 0.6
21		>200
22	SO_2CF_3	1.1 ± 0.1
23	$SO_2(CF_2)_2CF_3$	48 ± 2

Table 1. Efforts of optimization of lead compound **1** with alternative ortho-substitutions.

High resolution MALDI-TOF mass spectrometry study supports the covalent inhibition mechanism of **22** to be sulfonylation of hNE (+ ~273 Da) possibly at its active site serine (**Fig. 4A,B**). In contrast, the incubation of compound **22** with inactive denatured hNE led to no detectable covalent modification of the enzyme (**Fig. 4C**). The requirement for the naturally folded enzyme represents another example of the unusual "sleeping beauty" phenomenon of SuFEx, wherein the sulfur fluoride probes are inert in biological fluids/buffer if the capture protein or proteins presented are denatured (24, 32).

Intriguingly, testing the lead compounds (**1**, **19**, **22**) against a panel of serine proteases, we found that two (**1**, **22**) among the three effective hNE inhibitors did not inactivate the homologous serine protease, human cathepsin G (hCG), which has 37% sequence identical to hNE and highly similar crystal structure (root-mean-square deviation (rmsd) = 0.82 Å, max rmsd = 5.89 Å for 180 out of 218 Cα residues of hNE). Unlike PMSF ($PhCH_2SO_2F$) long known for ablating the hydrolytic activity of almost all serine proteases, the compounds **1** and **22** identified in this study showed 58 and 182-fold specificity for hNE over hCG, respectively (**Table 2**). The selective inhibition of hNE could be partly attributed to a proximity factor as suggested by molecular modeling using a reactive docking protocol (**SI Appendix, Figure S5**).

Compound	Structure	hNE IC_{50} (μM)[ll]	hCG IC_{50} (μM)[ll]	S^{**}
1		3.3 ± 1.0	190 ± 40	58
19		2.2 ± 0.7	6.0 ± 0.7	2.7
22		1.1 ± 0.1	>200	>182

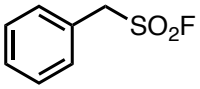
PMSF		24 ± 1	69 ± 6	2.5

Table 2. Selectivity of the most potent compounds **1**, **19**, **22** against hNE and hCG.

To conclude, we have demonstrated a SuFEx library-enabled approach to discover covalent deactivators of an enzyme's function, the protein at hand being human neutrophil elastase. Its structure is known, including complexed with reversible inhibitors in the active site, but the library of sulfonyl fluorides used in the screen was chosen without regard to any enzyme•potential ligand relationships. In other words, agnostic of structural considerations, the library yielded two more examples of the "sleeping beauty" phenomenon, and in this instance its successful outcome is distinguished by lack of known design elements around the pendant S–F electrophiles on the candidate "ligand" scaffolds. This successful sulfur fluoride library is being used and augmented regularly at Scripps Research, and it will hopefully contribute to future SuFEx-driven covalent drug discovery endeavors.

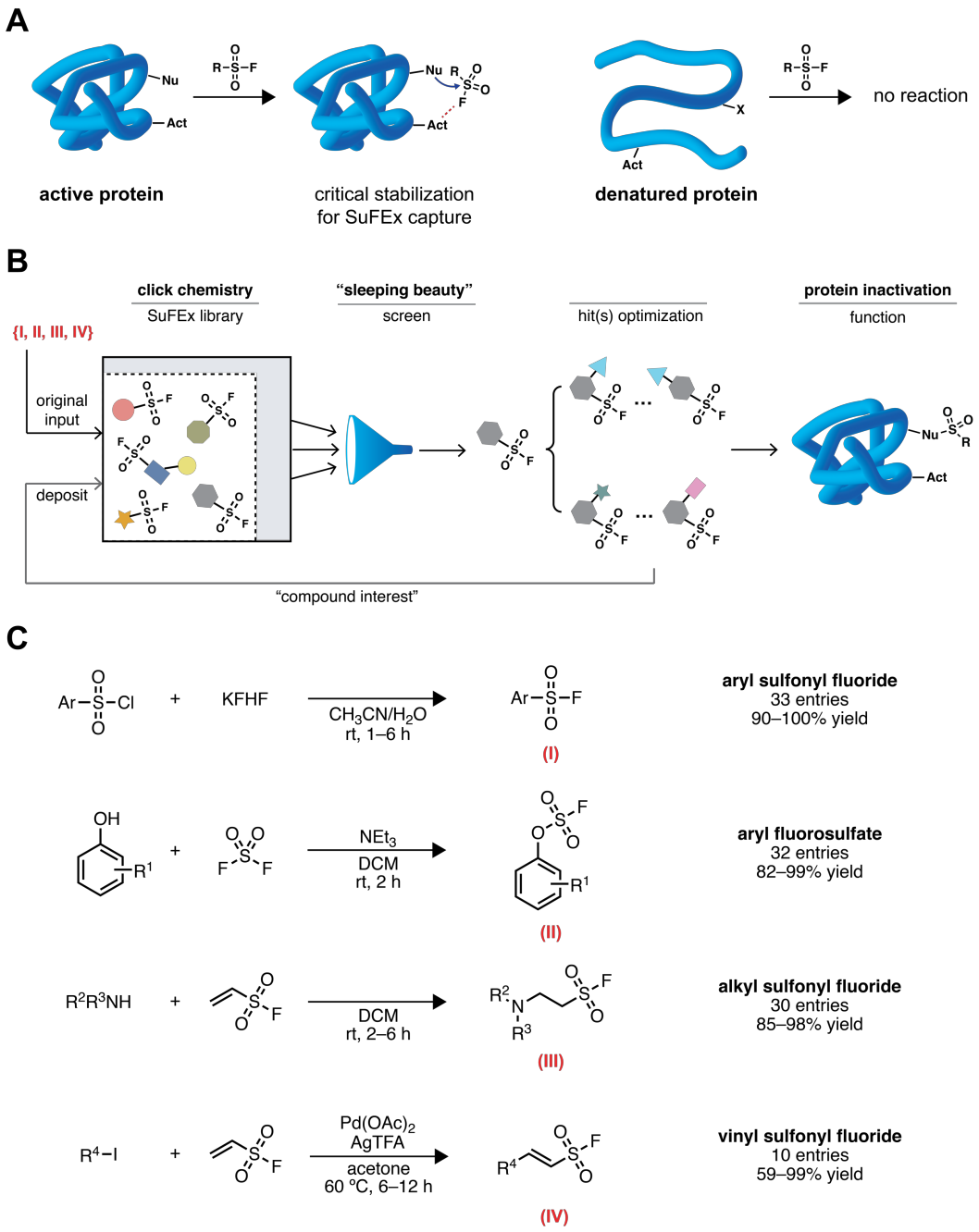


Fig. 1. Overview of SuFEx-enabled covalent drug discovery. (A) The "sleeping beauty" phenomenon: sulfur fluoride probes only capture a naturally folded protein where a nucleophilic sidechain (Nu) and activating sidechains (Act) are correctly positioned. (B) Cartoon schematic of the SuFEx-enabled covalent drug discovery process. (C) Efficient SuFEx derivatization of abundant building blocks yields a SuFExable library with four subset groups, categorized according to their sulfur-fluoride based functionalities.

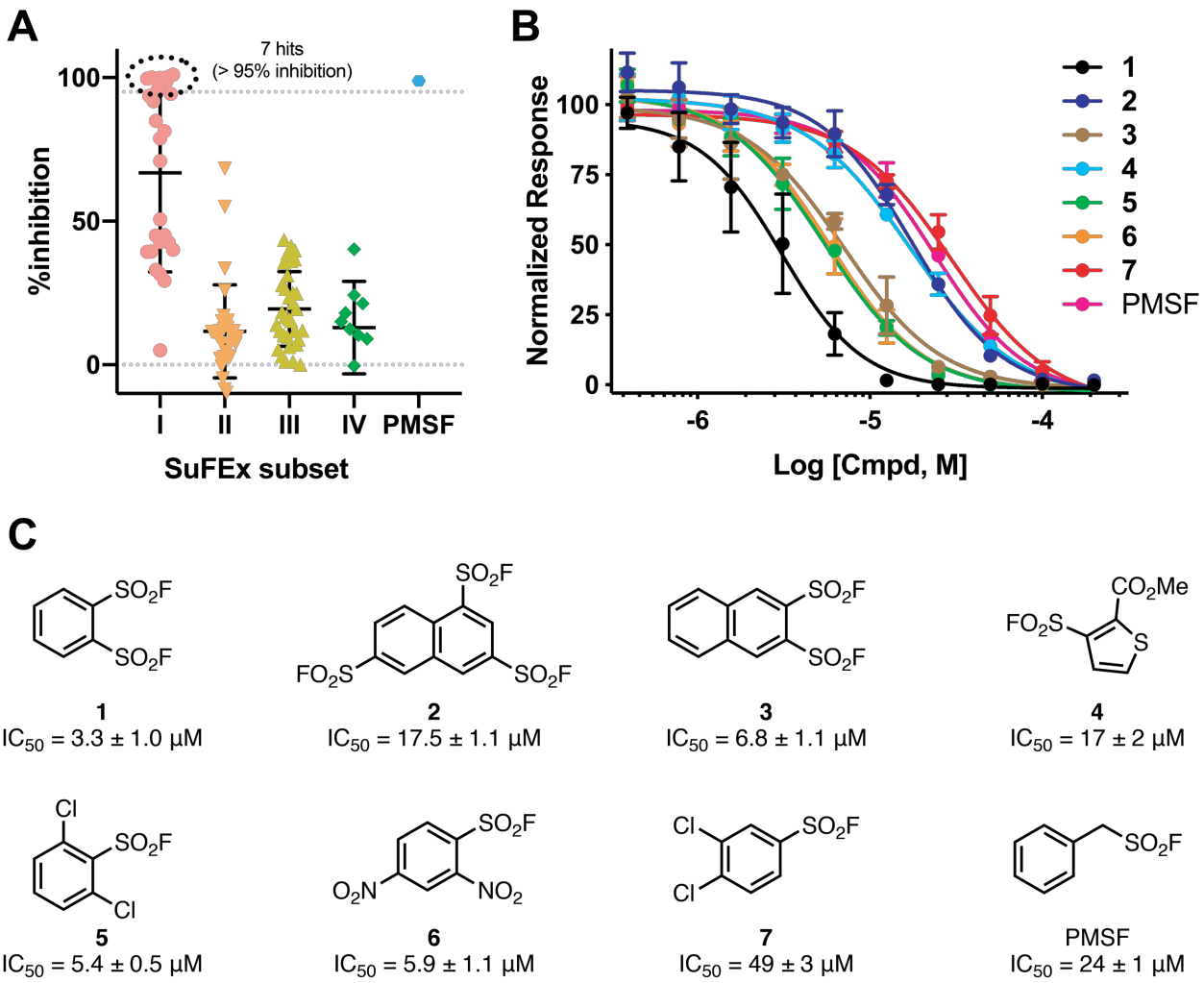


Fig. 2. Screen of the primary SuFEx library toward elastase inhibitory activity. (A) Initial screen with 105 SuFExable compounds yielded 7 hits with >95% inhibition at 200 μM. (B) Dose-response curves of hit compounds against hNE (AAPV-AMC fluorescence assay). Each compound was assessed over a two-fold logarithmic dilution series. (C) Structures and IC_{50} values of compounds **1**–**7** and PMSF (phenylmethane sulfonyl fluoride). IC_{50} values were measured based on 10 min incubation and are shown in mean ± SD (n ≥ 3).

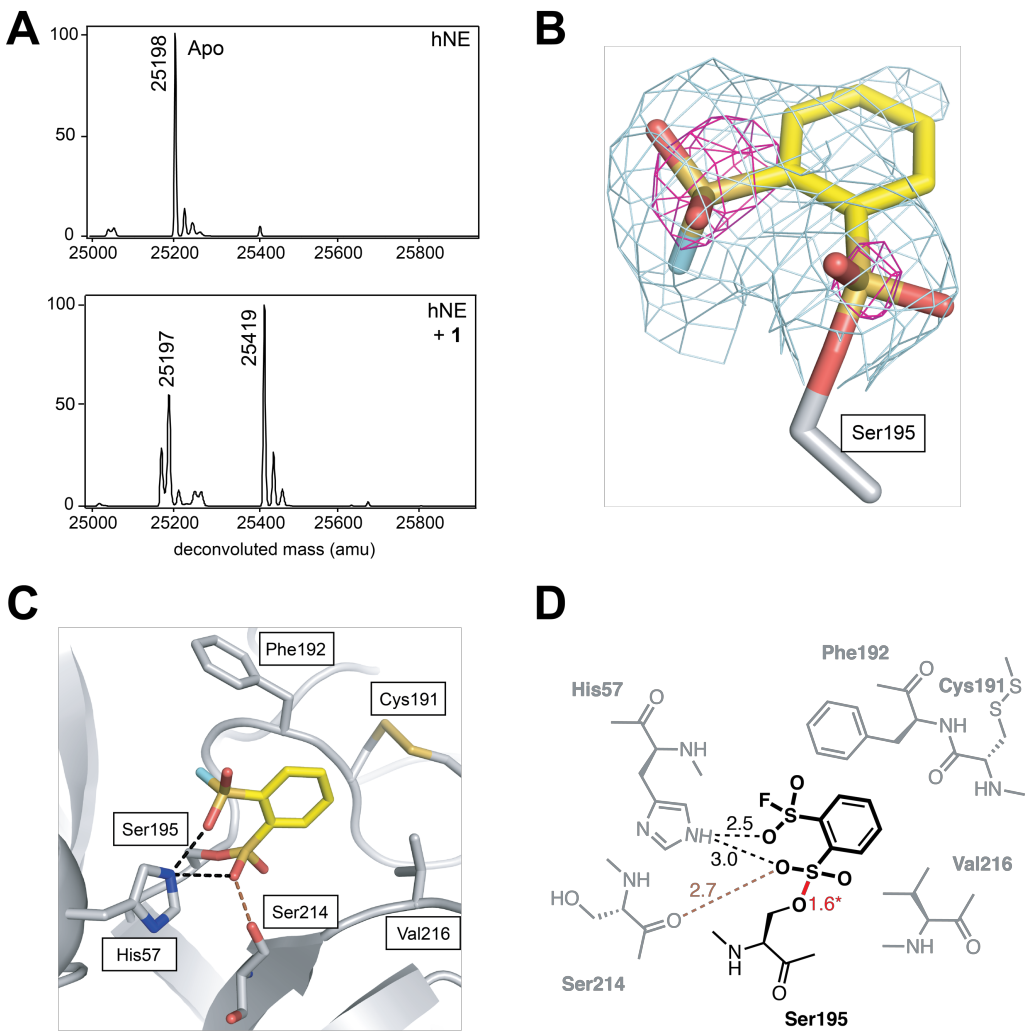


Fig. 3. Compound **1** is a covalent inhibitor of hNE. (A) MALDI-TOF mass spectrometry evidence of covalent complex **1**:hNE formation. (B) Naïve Fo-Fc map contoured at 1.5σ (green) and 4σ (magenta) clearly delineate the binding orientation of **1** to Ser195 and the specific location of the sulfur groups, respectively. Residue of **1** is shown as a stick model with yellow carbon, red oxygen, light blue fluorine, mustard sulfur. (C) Active site residues that provide potential hydrogen bonds (black dashes), repulsive interactions (brown dash), and hydrophobic residues that bind **1** (grey elastase carbon, nitrogen blue). (D) Schematic of bond distances between **1** and elastase with potential hydrogen bonds (black dashes) and negative repulsive interactions (brown dash) with bond distances in Å. The covalent bond (red) between Ser195 and the sulfur of **1** were set at 1.57 Å during structure refinement.

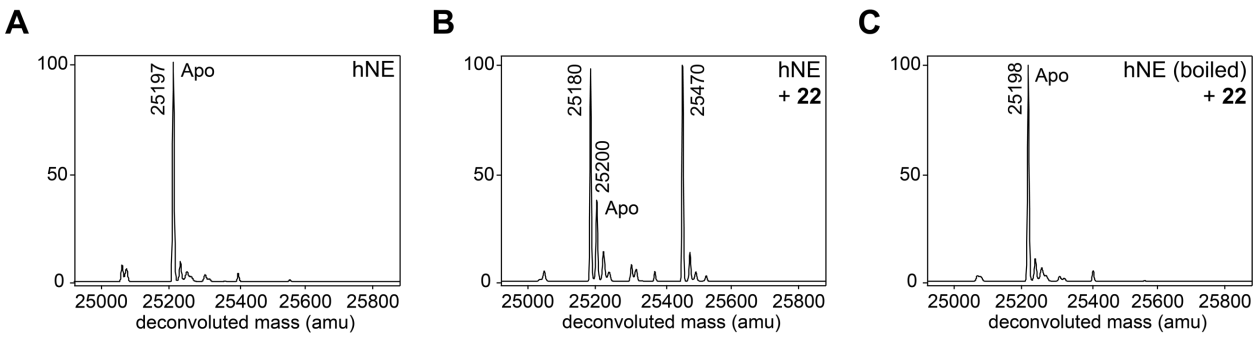


Fig. 4. The “sleeping beauty” phenomenon of sulfonyl fluoride **22** demonstrated by the MALDI-TOF mass spectra of (A) naturally folded hNE; (B) naturally folded hNE incubated with **22**; (C) denatured hNE (boiled) incubated with **22**.

Footnotes:

*SuFExable are defined to describe those compounds containing a sulfur fluoride link that can undergo SuFEx reaction.

†For example, no reaction occurred in the neat mixture of refluxing benzenesulfonyl fluoride (4 mL, ~33 mmol) and aniline (45 mL, ~500 mmol) at 184 ºC for 3 h. Under the same conditions, electrophiles commonly studied as covalent "warheads" in medicinal chemistry including epoxide, acrylamide, vinyl sulfone, chloroacetamide, chloromethyl ketone, β-lactam, maleimide, and fluorophosphate are not stable. (**SI Appendix, Table S3**).

‡As long as the accelerating AA sidechains (including but not limited to His57) and reactive moiety (Ser195) are separately considered, and the former remain overall unchanged, the intramolecular activation mode can be seen as "catalytic", even if the TON equals 1. For a similar case, see: Kassem S*, et al.* (2017) Stereodivergent synthesis with a programmable molecular machine. *Nature* 549(7672):374-378.

§"Sleeping beauty" here defines the phenomenon of the remarkable acceleration of a SuFEx reaction for otherwise very stable higher valent sulfur fluoride compounds, by the right, naturally folded protein catalyst for its own covalent capture. The term has been used by Izsvák and co-workers in molecular biology to describe the use of a specifically designed transposon system (i.e. Tc1/mariner-type transposase and transposon) that can selectively insert genes into the genomes of vertebrates. The genome of humans as well as of other mammals encodes for non-functional transposases and the introduction of an active transposase from lower vertebrate fish and amphibian species "awakens the system from an evolutionary sleep". For the original paper on sleeping beauty transposon, see: Ivics Z, Hackett PB, Plasterk RH, & Izsvak Z (1997) Molecular reconstruction of Sleeping beauty, a Tc1-like transposon from fish, and its transposition in human cells. *Cell* 91(4):501-510.

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[#]At the time of manuscript preparation, Murthy and co-workers reported a study on 2-nitrobenzenesulfonyl fluoride (**19**) and derivatives as covalent capture agents for new antibiotics development. Compound **17** showed only moderate inhibitory potency in our elastase activity assay. For Murthy's paper, see: Sadlowski C*, et al.* (2018) Nitro sulfonyl fluorides are a new pharmacophore for the development of antibiotics. *Mol Syst Des Eng* 3(4):599-603.

[||]IC_{50} values were measured based on 10 min incubation and are shown in mean ± SD (n ≥ 3).

***S* value denotes the selectivity, defined by the ratio of IC_{50} (hCG) over IC_{50} (hNE).

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Reference:

1. Dong JJ, Krasnova L, Finn MG, & Sharpless KB (2014) Sulfur(VI) Fluoride Exchange (SuFEx): Another Good Reaction for Click Chemistry. *Angew Chem Int Edit* 53(36):9430-9448.
2. Hanley PS, Ober MS, Krasovskiy AL, Whiteker GT, & Kruper WJ (2015) Nickel- and Palladium-Catalyzed Coupling of Aryl Fluorosulfonates with Aryl Boronic Acids Enabled by Sulfuryl Fluoride. *Acs Catal* 5(9):5041-5046.
3. Qin HL, Zheng QH, Bare GAL, Wu P, & Sharpless KB (2016) A Heck-Matsuda Process for the Synthesis of -Arylethenesulfonyl Fluorides: Selectively Addressable Bis-electrophiles for SuFEx Click Chemistry. *Angew Chem Int Edit* 55(45):14155-14158.
4. Barrow AS & Moses JE (2016) Synthesis of Sulfonyl Azides via Lewis Base Activation of Sulfonyl Fluorides and Trimethylsilyl Azide. *Synlett* 27(12):1840-1843.
5. Li SH, Wu P, Moses JE, & Sharpless KB (2017) Multidimensional SuFEx Click Chemistry: Sequential Sulfur(VI) Fluoride Exchange Connections of Diverse Modules Launched From An SOF4 Hub. *Angew Chem Int Edit* 56(11):2903-2908.
6. Schimler SD*, et al.* (2017) Nucleophilic Deoxyfluorination of Phenols via Aryl Fluorosulfonate Intermediates. *J Am Chem Soc* 139(4):1452-1455.
7. Zelli R, Tommasone S, Dumy P, Marra A, & Dondoni A (2016) A Click Ligation Based on SuFEx for the Metal-Free Synthesis of Sugar and Iminosugar Clusters. *Eur J Org Chem* (30):5102-5116.
8. Zha GF*, et al.* (2017) Palladium-Catalyzed Fluorosulfonylvinylation of Organic Iodides. *Angew Chem Int Edit* 56(17):4849-4852.
9. Ren GR, Zheng QH, & Wang H (2017) Aryl Fluorosulfate Trapped Staudinger Reduction. *Org Lett* 19(7):1582-1585.

10. Smedley CJ*, et al.* (2017) Sulfur-Fluoride Exchange (SuFEx)-Mediated Synthesis of Sterically Hindered and Electron-Deficient Secondary and Tertiary Amides via Acyl Fluoride Intermediates. *Chem-Eur J* 23(42):9990-9995.
11. Gao B, Li SH, Wu P, Moses JE, & Sharpless KB (2018) SuFEx Chemistry of Thionyl Tetrafluoride (SOF4) with Organolithium Nucleophiles: Synthesis of Sulfonimidoyl Fluorides, Sulfoximines, Sulfonimidamides, and Sulfonimidates. *Angew Chem Int Edit* 57(7):1939-1943.
12. Guo TJ*, et al.* (2018) A New Portal to SuFEx Click Chemistry: A Stable Fluorosulfuryl Imidazolium Salt Emerging as an "F-SO2+" Donor of Unprecedented Reactivity, Selectivity, and Scope. *Angew Chem Int Edit* 57(10):2605-2610.
13. Dong JJ, Sharpless KB, Kwisnek L, Oakdale JS, & Fokin VV (2014) SuFEx-Based Synthesis of Polysulfates. *Angew Chem Int Edit* 53(36):9466-9470.
14. Yatvin J, Brooks K, & Locklin J (2015) SuFEx on the Surface: A Flexible Platform for Postpolymerization Modification of Polymer Brushes. *Angew Chem Int Edit* 54(45):13370-13373.
15. Oakdale JS, Kwisnek L, & Fokin VV (2016) Selective and Orthogonal Post-Polymerization Modification using Sulfur(VI) Fluoride Exchange (SuFEx) and Copper-Catalyzed Azide-Alkyne Cycloaddition (CuAAC) Reactions. *Macromolecules* 49(12):4473-4479.
16. Gao B*, et al.* (2017) Bifluoride-catalysed sulfur(VI) fluoride exchange reaction for the synthesis of polysulfates and polysulfonates. *Nat Chem* 9(11):1083-1088.
17. Wang H*, et al.* (2017) SuFEx-Based Polysulfonate Formation from Ethenesulfonyl Fluoride-Amine Adducts. *Angew Chem Int Edit* 56(37):11203-11208.
18. Gahtory D*, et al.* (2018) Quantitative and Orthogonal Formation and Reactivity of SuFEx Platforms. *Chem-Eur J* 24(41):10550-10556.
19. Brooks K*, et al.* (2018) SuFEx Postpolymerization Modification Kinetics and Reactivity in Polymer Brushes. *Macromolecules* 51(2):297-305.

20. Grimster NP*, et al.* (2013) Aromatic Sulfonyl Fluorides Covalently Kinetically Stabilize Transthyretin to Prevent Amyloidogenesis while Affording a Fluorescent Conjugate. *J Am Chem Soc* 135(15):5656-5668.

21. Baranczak A*, et al.* (2015) A Fluorogenic Aryl Fluorosulfate for Intraorganellar Transthyretin Imaging in Living Cells and in Caenorhabditis elegans. *J Am Chem Soc* 137(23):7404-7414.

22. Narayanan A & Jones LH (2015) Sulfonyl fluorides as privileged warheads in chemical biology. *Chem Sci* 6(5):2650-2659.

23. Hett EC*, et al.* (2015) Rational Targeting of Active-Site Tyrosine Residues Using Sulfonyl Fluoride Probes. *Acs Chem Biol* 10(4):1094-1098.

24. Chen WT*, et al.* (2016) Arylfluorosulfates Inactivate Intracellular Lipid Binding Protein(s) through Chemoselective SuFEx Reaction with a Binding Site Tyr Residue. *J Am Chem Soc* 138(23):7353-7364.

25. Chen WT*, et al.* (2016) Synthesis of Sulfotyrosine-Containing Peptides by Incorporating Fluorosulfated Tyrosine Using an Fmoc-Based Solid-Phase Strategy. *Angew Chem Int Edit* 55(5):1835-1838.

26. Hoppmann C & Wang L (2016) Proximity-enabled bioreactivity to generate covalent peptide inhibitors of p53-Mdm4. *Chem Commun* 52(29):5140-5143.

27. Fadeyi O*, et al.* (2016) Chemoselective Preparation of Clickable Aryl Sulfonyl Fluoride Monomers: A Toolbox of Highly Functionalized Intermediates for Chemical Biology Probe Synthesis. *Chembiochem* 17(20):1925-1930.

28. Li SH*, et al.* (2016) Direct introduction of R-SO2F moieties into proteins and protein-polymer conjugation using SuFEx chemistry. *Polymer* 99:7-12.

29. Fadeyi OO*, et al.* (2017) Covalent Enzyme Inhibition through Fluorosulfate Modification of a Noncatalytic Serine Residue. *Acs Chem Biol* 12(8):2015-2020.

30. Wang NX*, et al.* (2018) Genetically Encoding Fluorosulfate-L-tyrosine To React with Lysine, Histidine, and Tyrosine via SuFEx in Proteins in Vivo. *J Am Chem Soc* 140(15):4995-4999.

31. Yang B*, et al.* (2018) Proximity-enhanced SuFEx chemical cross-linker for specific and multitargeting cross-linking mass spectrometry. *P Natl Acad Sci USA* 115(44):11162-11167.

32. Mortenson DE*, et al.* (2018) "Inverse Drug Discovery" Strategy To Identify Proteins That Are Targeted by Latent Electrophiles As Exemplified by Aryl Fluorosulfates. *J Am Chem Soc* 140(1):200-210.

33. Liu ZL*, et al.* (2018) SuFEx Click Chemistry Enabled Late-Stage Drug Functionalization. *J Am Chem Soc* 140(8):2919-2925.

34. Suter C (1944) Derivatives of Aromatic Sulfonic Acids. 1 Sulfonyl Halides, Esters, and Anhydrides. *The Organic Chemistry of Sulfur: Tetracovalent Sulfur Compounds*, (Wiley, New York), pp 452–458.

35. Steinkopf W (1927) Aromatic sulphuric flouride. *J Praktische Chemie* 117(1/3):1-82.

36. Steinkopf W (1930) On aromatic sulpho-fluoride. *J Praktische Chemie* 128(1/3):63-88.

37. Davies W & Dick JH (1932) Aliphatic sulphonyl flurorides. *J Chem Soc*:483-486.

38. Davies W & Dick JH (1932) Benzenesulphonyl flouride derivatives. *J Chem Soc*:2042-2046.

39. Gembus V, Marsais F, & Levacher V (2008) An efficient organocatalyzed interconversion of silyl ethers to tosylates using DBU and p-toluenesulfonyl fluoride. *Synlett* (10):1463-1466.

40. Choi EJ, Jung D, Kim JS, Lee Y, & Kim BM (2018) Chemoselective Tyrosine Bioconjugation through Sulfate Click Reaction. *Chem-Eur J* 24(43):10948-10952.

41. Hmissa T*, et al.* (2018) Autocatalytic Synthesis of Bifluoride Ionic Liquids by SuFEx Click Chemistry. *Angew Chem Int Edit* 57(49):16005-16009.

42. Lewis WG*, et al.* (2002) Click chemistry in situ: Acetylcholinesterase as a reaction vessel for the selective assembly of a femtomolar inhibitor from an array of building blocks. *Angew Chem Int Edit* 41(6):1053-+.

43. Agnew HD*, et al.* (2009) Iterative In Situ Click Chemistry Creates Antibody-like Protein-Capture Agents. *Angew Chem Int Edit* 48(27):4944-4948.

44. Kolb HC, Finn MG, & Sharpless KB (2001) Click chemistry: Diverse chemical function from a few good reactions. *Angew Chem Int Edit* 40(11):2004-+.

45. Zheng QH, Dong JJ, & Sharpless KB (2016) Ethenesulfonyl Fluoride (ESF): An On-Water Procedure for the Kilogram-Scale Preparation. *J Org Chem* 81(22):11360-11362.

46. Veryser C, Demaerel J, Bieliunas V, Gilles P, & De Borggraeve WM (2017) Ex Situ Generation of Sulfuryl Fluoride for the Synthesis of Aryl Fluorosulfates. *Org Lett* 19(19):5244-5247.

47. Zhou H*, et al.* (2018) Introduction of a Crystalline, Shelf-Stable Reagent for the Synthesis of Sulfur(VI) Fluorides. *Org Lett* 20(3):812-815.

48. Smedley CJ*, et al.* (2018) 1-Bromoethene-1-sulfonyl fluoride (BESF) is another good connective hub for SuFEx click chemistry. *Chem Commun* 54(47):6020-6023.

49. Leng J & Qin HL (2018) 1-Bromoethene-1-sulfonyl fluoride (1-Br-ESF), a new SuFEx clickable reagent, and its application for regioselective construction of 5-sulfonylfluoro isoxazoles. *Chem Commun* 54(35):4477-4480.

50. Birrer P*, et al.* (1994) Protease-antiprotease imbalance in the lungs of children with cystic fibrosis. *Am J Respir Crit Care Med* 150(1):207-213.

51. Cantin AM, Hartl D, Konstan MW, & Chmiel JF (2015) Inflammation in cystic fibrosis lung disease: Pathogenesis and therapy. *J Cyst Fibros* 14(4):419-430.

52. Gehrig S*, et al.* (2014) Lack of neutrophil elastase reduces inflammation, mucus hypersecretion, and emphysema, but not mucus obstruction, in mice with cystic fibrosis-like lung disease. *Am J Respir Crit Care Med* 189(9):1082-1092.

53. Gibson RL, Burns JL, & Ramsey BW (2003) Pathophysiology and management of pulmonary infections in cystic fibrosis. *Am J Respir Crit Care Med* 168(8):918-951.

54. Mayer-Hamblett N*, et al.* (2007) Association between pulmonary function and sputum biomarkers in cystic fibrosis. *Am J Respir Crit Care Med* 175(8):822-828.

55. Nakamura H, Yoshimura K, McElvaney NG, & Crystal RG (1992) Neutrophil elastase in respiratory epithelial lining fluid of individuals with cystic fibrosis induces interleukin-8 gene expression in a human bronchial epithelial cell line. *J Clin Invest* 89(5):1478-1484.

56. Nichols DP & Chmiel JF (2015) Inflammation and its genesis in cystic fibrosis. *Pediatr Pulmonol* 50 Suppl 40:S39-56.

57. Sagel SD, Chmiel JF, & Konstan MW (2007) Sputum biomarkers of inflammation in cystic fibrosis lung disease. *Proc Am Thorac Soc* 4(4):406-417.

58. Sagel SD, Wagner BD, Anthony MM, Emmett P, & Zemanick ET (2012) Sputum biomarkers of inflammation and lung function decline in children with cystic fibrosis. *Am J Respir Crit Care Med* 186(9):857-865.

59. Twigg MS*, et al.* (2015) The Role of Serine Proteases and Antiproteases in the Cystic Fibrosis Lung. *Mediators Inflamm* 2015:293053.

60. Wagner CJ, Schultz C, & Mall MA (2016) Neutrophil elastase and matrix metalloproteinase 12 in cystic fibrosis lung disease. *Mol Cell Pediatr* 3(1):25.

61. Barnes PJ (1994) Cytokines as mediators of chronic asthma. *Am J Respir Crit Care Med* 150(5 Pt 2):S42-49.

62. Barnes PJ (2004) Mediators of chronic obstructive pulmonary disease. *Pharmacol Rev* 56(4):515-548.

63. Pandey KC, De S, & Mishra PK (2017) Role of Proteases in Chronic Obstructive Pulmonary Disease. *Front Pharmacol* 8:512.

64. Qiu Y*, et al.* (2003) Biopsy neutrophilia, neutrophil chemokine and receptor gene expression in severe exacerbations of chronic obstructive pulmonary disease. *Am J Respir Crit Care Med* 168(8):968-975.

65. Belaaouaj A*, et al.* (1998) Mice lacking neutrophil elastase reveal impaired host defense against gram negative bacterial sepsis. *Nat Med* 4(5):615-618.

66. Motta JP*, et al.* (2012) Food-grade bacteria expressing elafin protect against inflammation and restore colon homeostasis. *Sci Transl Med* 4(158):158ra144.

67. Myers DK & Kemp A (1954) Inhibition of Esterases by the Fluorides of Organic Acids. *Nature* 173(4392):33-34.

68. Fahrney DE & Gold AM (1963) Sulfonyl Fluorides as Inhibitors of Esterases .1. Rates of Reaction with Acetylcholinesterase, Alpha-Chymotrypsin, and Trypsin. *J Am Chem Soc* 85(7):997-&.

69. Gold AM & Fahrney D (1964) Sulfonyl Fluorides as Inhibitors of Esterases .2. Formation + Reactions of Phenylmethanesulfonyl Alpha-Chymotrypsin. *Biochemistry-Us* 3(6):783-&.

70. Gold AM (1965) Sulfonyl Fluorides as Inhibitors of Esterases .3. Identification of Serine as Site of Sulfonylation in Phenylmethanesulfonyl Alpha-Chymotrypsin. *Biochemistry-Us* 4(5):897-&.

71. Baker BR & Hurlbut JA (1968) Irreversible Enzyme Inhibitors .114. Proteolytic Enzymes .4. Additional Active-Site-Directed Irreversible Inhibitors of Alpha-Chymotrypsin Derived from Phenoxyacetamides Bearing a Terminal Sulfonyl Fluoride. *J Med Chem* 11(2):241-&.

72. Baker BR & Hurlbut JA (1968) Irreversible Enzyme Inhibitors .113. Proteolytic Enzymes .3. Active-Site-Directed Irreversible Inhibitors of Alpha-Chymotrypsin Derived from Phenoxyacetamides with an N-Fluorosulfonylphenyl Substituent. *J Med Chem* 11(2):233-&.

73. Baker BR & Erickson EH (1968) Irreversible Enzyme Inhibitors .115. Proteolytic Enzymes .5. Active-Site-Directed Irreversible Inhibitors of Trypsin Derived from P-

(Phenoxyalkoxy)Benzamidines with a Terminal Sulfonyl Fluoride. *J Med Chem* 11(2):245-&.

74. Baker BR (1970) Specificic Irreversible Enzyme Inhibitors. *Annu Rev Pharmacolog* 10:35-+.

75. Laura R, Robison DJ, & Bing DH (1980) (Para-Amidinopheny)Methanesulfonyl Fluoride, an Irreversible Inhibitor of Serine Proteases. *Biochemistry-Us* 19(21):4859-4864.

76. Shannon DA*, et al.* (2012) Sulfonyl Fluoride Analogues as Activity-Based Probes for Serine Proteases. *Chembiochem* 13(16):2327-2330.

77. Lively MO & Powers JC (1978) Specificity and Reactivity of Human Granulocyte Elastase and Cathepsin-G, Porcine Pancreatic Elastase, Bovine Chymotrypsin and Trypsin toward Inhibition with Sulfonyl Fluorides. *Biochim Biophys Acta* 525(1):171-179.

78. Yoshimura T, Barker LN, & Powers JC (1982) Specificity and Reactivity of Human-Leukocyte Elastase, Porcine Pancreatic Elastase, Human Granulocyte Cathepsin-G, and Bovine Pancreatic Chymotrypsin with Arylsulfonyl Fluorides - Discovery of a New Series of Potent and Specific Irreversible Elastase Inhibitors. *J Biol Chem* 257(9):5077-5084.

79. Smedley CJ*, et al.* (2019) Bifluoride Ion Mediated SuFEx Trifluoromethylation of Sulfonyl Fluorides and Iminosulfur Oxydifluorides. *Angew Chem Int Ed Engl*.