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Discovery libraries targeting the major enzyme classes: The serine hydrolases

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ABSTRACT

Two libraries of modestly reactive ureas containing either electron-deficient acyl anilines or acyl pyrazoles were prepared and are reported as screening libraries for candidate serine hydrolase inhibitors. Within each library is a small but powerful subset of compounds that serve as a chemotype fragment screening library capable of subsequent structural diversification. Elaboration of the pyrazole-based ureas provided remarkably potent irreversible inhibitors of fatty acid amide hydrolase (FAAH, apparent K_i = 100–200 pM) complementary to those previously disclosed enlisting electron-deficient aniline-based ureas.

The serine hydrolases constitute a superfamily of enzymes representing more than 1% of the predicted proteins in the human genome for which at least 40–50% are still uncharacterized, lacking known roles or identified endogenous substrates.[1] Not only are they one of the largest and most diverse enzyme class in mammals, but they perform crucial roles in many biological processes.[1,2] There are approximately 240 human serine hydrolases composed of a series of lipases, peptidases, esterases, thioesterases, and amidases that hydrolyze small molecules, signaling lipids, peptides or post-translational ester and thioester protein modifications. They share a conserved mechanism that enlists a key active site serine nucleophile within a catalytic triad that is activated by a proton relay often involving an acidic (aspartate/glutamate) and basic residue (histidine/lysine). More than half of the serine hydrolases (>120 enzymes) remain poorly annotated, with no described physiological function or identified substrates, and an even greater number of these enzymes (>80%) lack selective inhibitors to aid in their characterization. In cases where a comprehensive understanding has been established, this has not only improved our fundamental understanding of biology, but it has also led to invaluable new therapies to treat disease.[1]

Herein, we report two distinct efforts designed to assemble a powerful screening library targeting the serine hydrolases. As a complement to our early ad hoc synthesis of a trifluoromethyl ketone library (ca. 300 compounds),[3] additional candidate inhibitors bearing activated electrophilic carbonyls,[4] and the more recent synthesis of an extensive series of α-ketoheterocycles[5] (ca. 1000 compounds) many of which target fatty acid amide hydrolase (FAAH),[6] the efforts herein provide two libraries of modestly reactive urea derivatives capable of irreversible[7] serine hydrolase inhibition by virtue of selective active site carbamoylation of the catalytic serine.[8] Such ureas, typically unreactive unless activated for nucleophilic acylation within a serine hydrolase active site, can be profiled against all serine hydrolases in the proteome[9] with confidence that they are not routinely reactive with enzymes or proteins outside the serine hydrolase family.[9,10] Because of their irreversible mechanism of action, those that are sufficiently selective may serve as initial in vivo pharmacological probes to define the function of an uncharacterized serine hydrolase,[11] used to confidently validate its potential as a therapeutic target, and optimized into drug candidates themselves.[1,12–15] First identified as a class of inhibitors effective for selective and potent inhibition of FAAH,[12–15] their utility for serine hydrolase inhibition requires a modestly reactive urea bearing an amine capable of behaving as an effective leaving group (e.g., electron-deficient aniline,[13–15] tetrazole,[16] triazole,[17] or imidazole[17]), was found often to be dependent on the nature, size, and substitution (e.g., tertiary vs secondary) of the second attached urea amine,[13–15] and can be exquisitely responsive to the recognition elements used to target the enzyme active site. Opportunities to create such comprehensive screening libraries for the serine hydrolases are enhanced with the availability and continued refinement of activity-based protein profiling (ABPP) proteomic screening technology[9–11] that offer the advantage of testing enzymes in their native state and eliminate the need for their recombinant expression, purification, and the development

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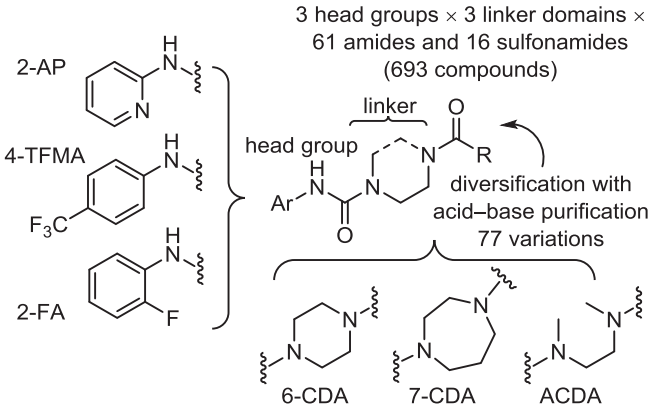
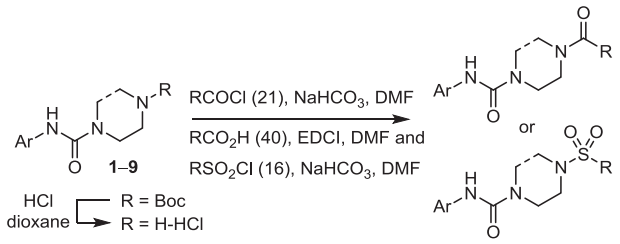


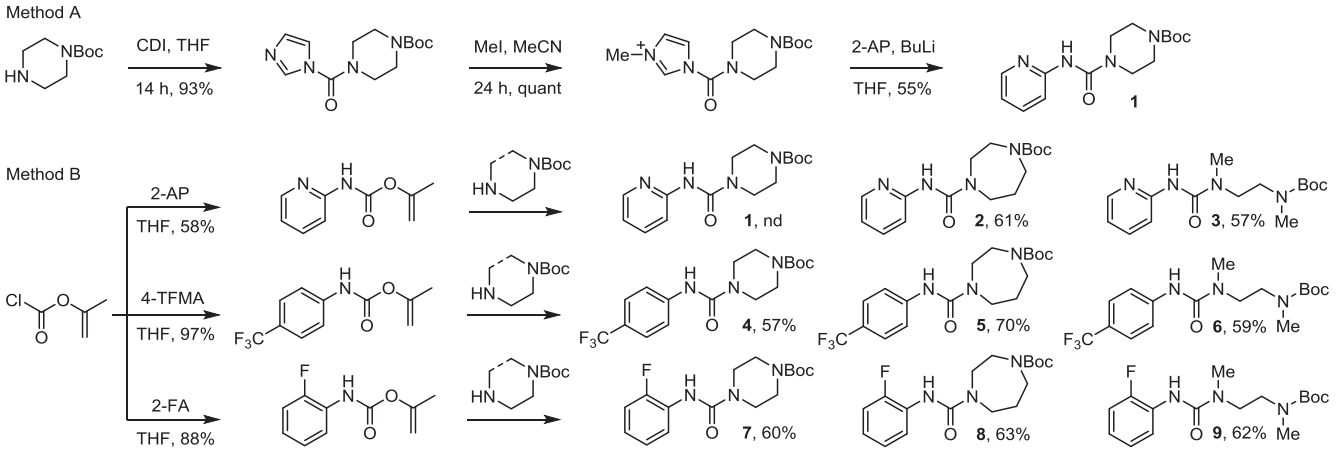
Figure 1. Aniline-based urea library of candidate serine hydrolase inhibitors.

of specific substrate assays. Because the inhibitors are screened against many enzymes in the proteome in parallel, both their potency and selectivity can be simultaneously evaluated.[18,19] Such libraries, in combination with use of this technology to rapidly assess proteome-wide selectivity, provide a powerful paradigm for discovery of selective chemical probes of new targets, for optimization of an enzyme inhibitor selectivity concurrent with target affinity,[19] and for the detection, identification, and characterization of new therapeutic targets.[1,2]



Scheme 2.

The first of the screening libraries detailed herein constitutes a 693-membered library of activated ureas that systematically vary the reactivity of an aniline leaving group (head group) as well as the structure and flexibility of the additional urea amine (linking unit) that bears a common resident functionalization site



Scheme 1.

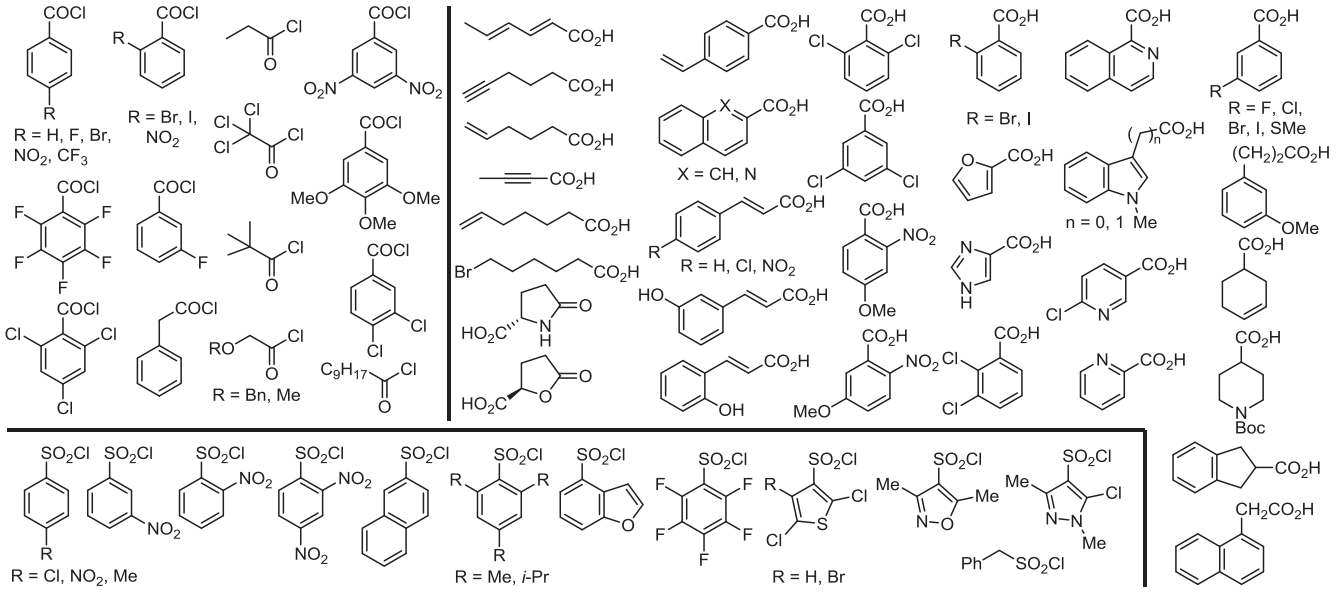


Figure 2. The 21 acid chlorides, 40 carboxylic acids, and 16 sulfonyl chlorides used in the library.