SUPPLEMENTARY INFORMATION

for

INTERACTION OF ANTITHROMBIN WITH SULFATED, LOW MOLECULAR WEIGHT LIGNINS
Opportunities for Potent, Selective Modulation of Antithrombin Function
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Computational Methods

Software/Hardware: SYBYL 7.2 (Tripos Associates, St. Louis, MO) was used for molecular visualization, minimization, and manipulation of protein structures from the Protein Data Bank (www.rcsb.org). Molecular modeling experiments were performed on an IRIX 6.5-based SGI Tezro graphical workstation. GOLD, version 3.2 (Jones, G., Willett, P., Glen. R. C., Leach, A. R., and Taylor, R. (1997) J. Mol. Biol. 267, 727-748), was used for docking and scoring experiments. Caffeic acid-based sulfated DHP oligomers (CDSO3) were built using SYBYL 7.2.

Energy Minimization: Energy minimization of modeled structures was performed to optimize the geometric conformation of CDSO3 oligomers and antithrombin. Except where stated, energy minimization was performed using the Tripos forcefield with Gasteiger-Hückel charges, a fixed dielectric constant of 80 and a non-bonded cutoff radius of 8 Å. Minimization was carried out for a maximum of 5,000 iterations subject to a termination gradient of 0.05 kcal/(mol·Å).

Protein Coordinates: The coordinates for the activated form of antithrombin were extracted from the crystal structure of the antithrombin-thrombin-pentasaccharide ternary complex (PDB entry 1TB6). Hydrogen atoms were added in SYBYL 7.2 and the structure was minimized with fixed heavy-atom coordinates using the Tripos force field for 1000 iterations subject to a termination gradient of 0.05 kcal/(mol·Å). Lys133, which was incompletely defined in the crystal structure (Li, W., Johnson, D.J., Esmo, C.T., and Huntington, J.A. (2004) Nat. Struct. Mol. Biol. 11, 857-862), was made appropriate modified to engineer the entire side chain and was then locally optimized through energy minimizations.

Atomic Coordinates for CDSO3: A library of selected β-5 CDSO3 oligomers was prepared using SYBYL 7.2. Sulfated caffeic acid is the basic monomeric unit constituting oligomeric CDSO3. The heterogeneous CDSO3 mixture used in the biochemical studies is
primarily made up of $\beta$-O-4 and $\beta$-5 inter-residue linkages with most oligomers less than 15 residues long. Other linkages, e.g., $\beta$-$\beta$ and 5-5, are also present, but in a much smaller proportion. Theoretically, CDSO3 can consist of 37,449 distinct sequences that are monomer to hexamer long in all possible stereochemical and inter-monomeric linkage combinations ($\beta$-O-4, $\beta$-5, $\beta$-$\beta$ and 5-5 linkages). The detailed screening of this library requires sophisticated programming as well as significant computation time. To assess whether there is selectivity in recognition of CDSO3 structure, a much smaller library of about 11 structures composed of $\beta$-5 (Figure S3) oligomers was prepared manually. Each molecule was energy minimized as described above.

**Docking of CDSO3 Oligomers:** CDSO3 oligomers were docked onto the activated form of antithrombin using GOLD v.3.2 (25). The binding site in antithrombin was defined as a collection of all atoms within 20Å from the $C^\varepsilon$ atom of Phe121 in the D helix of antithrombin. This definition of the binding site covers all known important heparin binding site residues including Lys11, Arg13, Arg46, Arg47, Trp49, Lys114, Phe121, Lys125, Arg129, Arg132, Lys133 and Lys136.

GOLD is a “soft docking” method that implicitly handles local protein flexibility by allowing a small degree of interpenetration, or van der Waals overlap, of the ligand and protein atoms. GOLD also optimizes the positions of hydrogen-bond donating atoms on Ser, Thr, Tyr, Lys and Arg residues as part of the docking process. CDSO3 oligomers were allowed complete rotational flexibility to search for an optimal binding mode in the protein binding site. Unless specified otherwise, default parameters were employed during the GOLD docking runs.

Docking was performed using a genetic algorithmic (GA) search with 300,000 iterations. In this search, GOLD starts with a population of 100 arbitrarily docked ligand orientations, evaluates them using a scoring function (the GA “fitness” function) and improves their average “fitness” by an iterative optimization procedure that is biased toward high scores. As the initial population is selected at random, several such GA runs are required to more reliably predict correct bound conformations. In this study 10 GA runs were performed on each molecule with the GOLD score as the “fitness” function. Collectively, these 10 GA runs are referred to as one docking experiment. The modified form of the GOLD scoring function, which utilizes hydrogen-bonding and van der Waals interactions (eq. V), was used to rank the final docked solutions. In this equation, $HB_{\text{ext}}$ and $VDW_{\text{ext}}$ are the “external” (non-bonded interactions taking place between the ligand and receptor) hydrogen bonding and van der Waals terms, respectively. Additional details on the use of this technique for identifying high affinity and high specificity sequences that recognize antithrombin are available from Raghuraman, A., Mosier, P. D., and Desai, U. R. (2006) *J. Med. Chem.* **49**, 3553-3562.

$$\text{GOLDScore}_{\text{mod}} = HB_{\text{ext}} + 1.375 \times VDW_{\text{ext}}$$

[V]
Figure S1: β-5 CDSO3 oligomer library for antithrombin docking studies. OS refers to –OSO$_3^-$ group. The cartoon, shown below each chemical structure, represents the chemical structure and is used in Table 4 of the main text.
Figure S1 (Contd): β-5 CDSO3 oligomer library for antithrombin docking studies. OS refers to –OSO$_3^-$ group. The cartoon, shown below each chemical structure, represents the chemical structure and is used in Table 4 of the main text.
**Figure S2: Reversibility of Inhibitory Effects of Sulfated DHPs.** To assess the reversibility of inhibitory effects of sulfated low molecular weight lignins, the inhibition of thrombin by FDSO3 was measured in the presence of 1.02 mg/mL protamine sulfate (Mₙ = 5,100 Da). Circles and triangles represent inhibition in the absence and presence of protamine sulfate, respectively. The bold black line represents the fit of the logistic function I described in the text, while the grey line is a linear trendline. The data show that FDSO3 inhibition of thrombin is essentially completely reversed by protamine sulfate.
Figure S3: The intrinsic tryptophan fluorescence of antithrombin decreases as the concentration of sulfated low molecular weight lignins increase at pH 7.4, I 0.15, 25 °C. Blue circles show the observed change in fluorescence at 340 nm for antithrombin – low molecular weight lignin complex and maroon squares show the inner filter corrected ΔFOBS/FO counts. Calculation of KD values with and without inner filter correction across different conditions shows that maximal correction was 1–3 % for CDSO3, 10–20 % for FDSO3 and 15–25 % for SDSO3.