Hydropathic interaction analyses of small organic activators binding to antithrombin

Gunnar T. Gunnarsson and Umesh R. Desai*

Department of Medicinal Chemistry, Virginia Commonwealth University, 410 N. 12th Street, PO Box 980540, Richmond, VA 23298, USA

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Abstract—Recently we designed the first small organic ligands, sulfated flavanoids and flavonoids, that act as activators of antithrombin for accelerated inhibition of factor Xa, a key proteinase of the coagulation cascade [Gunnarsson and Desai, Bioorg. Med. Chem. Lett. (2003) 13:579]. To better understand the binding properties of these activators at a molecular level, we have utilized computerized hydropathic interaction (HINT) analyses of the sulfated molecules interacting in two plausible electropositive regions, the pentasaccharide- and extended heparin-binding sites, of antithrombin in its native and activated forms. HINT analyses indicate favorable multi-point interactions of the activators in both binding sites of the two forms of antithrombin. Yet, HINT predicts better interaction of most activators, except for (−)-catechin sulfate, with the activated form of antithrombin than with the native form supporting the observation in solution that these molecules function as activators of the inhibitor. Further, whereas (+)-catechin sulfate recognized the activated form of antithrombin better in both the pentasaccharide- and extended heparin-binding sites, the native form was better recognized by (−)-catechin sulfate, thus explaining its weaker binding and activation potential in solution. A reasonable linear correlation between the overall HINT score and the solution free energy of binding of the sulfated activators was evident. This investigation indicates that HINT is a useful tool in understanding interactions of antithrombin with small sulfated organic ligands at a molecular level, has some good predictive properties, and is likely to be useful for rational design purposes.

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1. Introduction

Antithrombin, a plasma serine proteinase inhibitor, is a major physiologic regulator of the clotting process. The inhibitor inactivates several enzymes of the clotting cascade, especially factor Xa and thrombin, two enzymes that are common to both the extrinsic and intrinsic pathways of coagulation.1,2 However, antithrombin alone is a rather poor inhibitor of these proteinases. The inhibitor is greatly aided by heparin, a naturally occurring polysaccharide, which enhances its inhibitory activity several hundred-fold.2,3 Heparin is a highly sulfated, linear polydisperse molecule composed of alternating glucosamine and uronic acid residues that may range in number from ~10 to ~80.4,5 A specific five-residue sequence, called the pentasaccharide sequence, in polymeric heparin is involved in high-affinity binding to antithrombin.6–9 The binding of the pentasaccharide to antithrombin in the native state induces a conformational change in the inhibitor resulting in the formation of a high-affinity antithrombin-pentasaccharide complex, in which antithrombin exists in the activated state.10–13 The transition from the native to the activated state induced by the pentasaccharide is called the conformational activation of antithrombin. This conformational activation induces a ~300-fold faster reaction of the inhibitor with factor Xa, thus enabling physiologic regulation of enzymatic activity.

The sequence-specific pentasaccharide, or full-length heparin containing the pentasaccharide sequence, binds antithrombin in the pentasaccharide-binding site (PBS) formed by helices A and D.14,15 Electropositive residues Lys114, Lys125 and Arg129 that line the binding...
domain contribute most of the binding energy as well as play dominant functional roles in the recognition of the saccharide ligand and the conformational activation phenomenon.16–18 Full-length heparin but not the pentasaccharide, in addition, also interacts with an electro-positive region formed by Arg129, Arg132, Lys133 and Lys136 residues.19 This region, adjacent to the pentasaccharide-binding site, is disordered in the native state but forms the C-terminal end of helix D in the activated state of the inhibitor.14 and is called the extended heparin-binding site (EHBS). The EHBS contributes an ionic interaction in binding to full-length heparin and is presumed to play an important role in the process of the conformational transmission of heparin’s binding energy.19,20

To reduce the adverse effects associated with heparin therapy and induce oral bioavailability, we designed sulfated flavanoids (Fig. 1), small, synthetic, non-sugar molecules, as activators of antithrombin using rational molecular modeling involving hydropathic interaction (HINT) analysis.21 (−)-Epicatechin sulfate [(−)-ECS, Fig. 1], a sulfated flavan-3-ol derivative, was designed to interact with the PBS. While ECS was found to bind antithrombin and accelerate its reaction with factor Xa, the activation achieved was much smaller (∼10-fold).

Although HINT has been used to design organic ligands for a number of different proteins,22–25 its application in the design of small ligands that are highly negatively charged remains unexplored. Our first application of HINT to design ECS was based on the presumption that the activator binds in the PBS.21 Subsequent investigation on structurally related, but conformationally diverse, flavanoids and flavonoids (Fig. 1) showed that each of these sulfated molecules bind and activate antithrombin.26 Interestingly and contrary to our expectation, the activation potential of the sulfated molecules studied exhibited a rather narrow range of ∼2-fold variation. In addition, our recent biochemical studies suggest that (+)-CS, a diastereomer of (−)-ECS, does not bind in the PBS, but rather in the extended heparin-binding site.27

To probe in greater detail the interactions of our non-sugar organic activators with antithrombin, we modeled the binding of these sulfated molecules with the native and activated forms of the inhibitor in the pentasaccharide- and extended heparin-binding sites. The results show that HINT supports the conclusion that most sulfated flavanoids and flavonoids bind the activated form of antithrombin better than native form thereby leading to activation of the inhibitor for accelerated inhibition of factor Xa. Further, HINT supports the solution data that indicate preferential binding of (+)-CS to the EHBS. In combination, the results suggest that HINT is a useful technique to understand the interactions of small sulfated organic ligands with antithrombin and may be used for predictive purposes.

2. Methods

2.1. Modeling antithrombins

Sybyl 6.7 (Tripos Associates, St. Louis, MO) was used for computerized molecular modeling experiments. Structures of free antithrombin (accession number, 2ant28) and pentasaccharide-complexed antithrombin (accession number ‘1azx’14) was acquired from RCSB (http://www.rcsb.org/pdb/). In both these crystal structures, the inhibitor exists as a dimer of an inhibitory and a latent molecule. Chain I corresponding to the inhibitory monomer was extracted from each of these dimers and used as a model of the native (2ant) and activated (1azx) conformations. Residues 34–42 (2ant) and 1, 26–38 and 432 (1azx) are absent in the crystal structures. Previous biochemical studies do not implicate these absent amino acids in heparin binding1,29 and hence, the residues were not re-engineered into the polypeptide chain. More importantly, the residues known to be important were present in the activated inhibitory polypeptide chain. Individual atoms were assigned Gasteiger–Hückel charges and the polypeptide chain was minimized using Tripos forcefield until a terminating gradient of 0.5 kcal/mol-A2 was reached.

2.2. Docking in the pentasaccharide-binding domain

Structures of ECS, CS, MoS and QS (Fig. 1) were built using the small molecule builder module in Sybyl 6.7. The global minimum energy conformation of each molecule was searched by systematic variation of bond angles in all its rotatable bonds. The atom types for the oxygen atom in the SO3 groups were modified to ensure that the bond angles and bond lengths of the simulated sulfate group are equivalent to those observed in the co-crystal structure of pentasaccharide-antithrombin complex.14 The partial charge for each atom in the pentasaccharide was computed using the Gasteiger–Hückel method as implemented in Sybyl. The protocol assigns a charge of −0.46 to the oxygens of the SO3 groups in these sulfated molecules.

2.3. Docking in the pentasaccharide-binding domain

Flexidock module in Sybyl was used to dock the sulfated ligands on to the PBS of the activated form of

Figure 1. Structures of sulfated activators of antithrombin. Non-saccharide activator (−)-epicatechin sulfate [(−)-ECS] was designed to bind the pentasaccharide-binding site in antithrombin,18 while (−)-CS, MoS and QS were discovered in related structures query.21 ECS possesses two chiral centers at 2- and 3-positions. (−)- or (+)-catechin sulfate [(−)-CS or (−)-CS] differ from ECS in the orientation of only one group, either the aryl group at 2- or the sulfate at 3-position, respectively. ECS and CS are flavanoids. MoS and QS are flavonoids possessing unsaturation in the heterocyclic ring, which additionally bears a keto functionality. The number of sulfate groups remains equivalent in these structures.
antithrombin. Each molecule was pre-positioned in the binding site, defined as all residues within 4 Å distance from Arg47, Lys114, Lys125, Arg129.14 All basic amino acid residues lining the binding site were positively charged. Rotatable bonds of these residues, primarily the side chain single bonds, were allowed conformational flexibility in the docking process, while the backbone and remaining bonds were held rigid. Water was excluded from the simulation, although a distance dependent dielectric constant was used to simulate the presence of water. Docking was performed with a rigid ligand model (no ligand flexibility). No any additional constraints, such as explicit formation of hydrogen bond between selected donor-acceptor sites, were introduced. In general, Flexidock provides nearly 20 solutions for each docking experiment. Each of these structures was minimized, as described earlier, to eliminate bad electronic and/or steric contacts and the structure with lowest energy was used for HINT analysis.

2.4. Docking in the extended heparin-binding domain

Docking in the extended heparin-binding site region, defined by residues Arg132, Lys133 and Arg136, 19 was performed in a manner described above, except for positioning the activator in the EHBS in an arbitrary orientation. Biochemical results indicate that (+)-CS binds in the extended heparin-binding site.27 Because no crystal structure is available, we placed the flavanoids in an arbitrary orientation in the EHBS and used Flexidock to search for the best possible geometry within the binding site. This search is energy driven. Flexidock was performed for 100,000 iterations without any constraints to obtain 20 solutions that were further minimized and analyzed, as described above. Each molecule was placed initially in an equivalent orientation with respect to each other.

2.5. Scoring

Hydropathic interaction (HINT, EduSoft LC, Ashland, VA) analysis characterizes non-polar-non-polar, polar-polar, and non-polar-polar interactions between a ligand and its receptor.22–25 HINT is not a statistical method or a forcefield but employs parameters determined from solvent transfer experiments, and thus is expected to better correlate with the observed free energy of binding. In the HINT model, specific interactions between a small molecule and a macromolecule are described as a double sum over the atoms within each component:22–25

\[ B = \sum_{i=1}^{\text{atoms}} \sum_{j=1}^{\text{atoms}} b_{ij} = \sum \Sigma (S_i a_i S_j a_j R_{ij} T_{ij} + r_{ij}) \]  

(1)

where \( S \) is the solvent-accessible surface area, \( a \) is the hydrophobic atom constant, \( T \) is a descriptor function, and \( R \) and \( r \) are functions of the distance between atoms \( i \) and \( j \). From this equation, a binding score is calculated where \( b_{ij} \) describes specific interaction between atoms \( i \) and \( j \), and \( B \) describes the total interaction score between the two species. Detailed description of the HINT interaction analysis can be found in refs 22–25.

2.6. Scoring the interactions of sulfated flavanoids with antithrombin

Antithrombin was assigned HINT parameters from a dictionary of previously determined values. Only polar hydrogens were explicitly used in the partitioning of the protein and the ligand, following which an interaction score using eq 1 was calculated for each final docked structure. Because HINT parameters for the sulfur atoms in higher oxidation states including sulfates are not well established, sulfur interaction scores were eliminated. Three-dimensional maps that pictorially represent the non-covalent interactions deduced by HINT were calculated on a 1 Å grid.

3. Results and discussion

3.1. HINT analyses of antithrombin interacting with organic activators

We have previously used HINT to study the interaction of antithrombin with natural saccharide ligands, including the sequence-specific pentasaccharide.21,30 A reasonable linear correlation of the overall HINT score with the free energy of binding was noted for several mutants of antithrombin.21 The application of HINT to study the interaction of antithrombin with organic ligands remains unexplored and unexploited. This is especially important because of the possibility of designing novel non-sugar organic anticoagulants.

Our small activators (Fig. 1) have a common bicyclic-unicyclic skeleton with variations in the configuration of the pendant groups that allow wide conformational space to be screened.29 The activators were found to interact with an affinity (2–11 μM) comparable to a tri-saccharide (2 μM) on which was designed the first non-sugar small activator, (−)-ECS.21 Further, the sulfated molecules activate antithrombin, albeit much weakly (8–20-fold) in comparison to the reference tri-saccharide (~300-fold).21,31,32 To investigate whether HINT can enhance our understanding on the binding of these non-sugar activators, we simulated the interaction of (−)-ECS, (+)-CS, (−)-CS, MoS and QS in the pentasaccharide- and extended heparin-binding site of antithrombin in the native and activated states.

3.2. Favorable antithrombin interaction profile of all organic activators

The overall HINT score was found to be positive for all activators interacting with antithrombin. A priori this suggests the possibility that these ligands may interact favorably in either binding site (Table 1, see Fig. 2 for a HINT contour profile). Further, the scores are positive for interaction in both the native and the activated states. The HINT scores for (−)-ECS, (+)-CS, MoS,
and QS binding in either the PBS or EHBS of native antithrombin are lower than that for the activated form (Table 1, Fig. 3). In contrast, (−)-CS binds antithrombin in the native state better than in the activated state in both binding sites. These data suggest that (−)-ECS, (+)-CS, MoS and QS, but not (−)-CS, are likely to interact with antithrombin in the activated state more favorably than in the native state (Fig. 3). In other words, (−)-ECS, (+)-CS, MoS, and QS, but not (−)-CS, are all expected to shift the 'native→activated' equilibrium in antithrombin in favor of the activated form, and thus function as activators of antithrombin.

The simulation results described above are in line with the biochemical experiments in solution that show (−)-ECS, (+)-CS, MoS and QS act to activate antithrombin for inhibition of factor Xa nearly 10–20-fold. In contrast, (−)-CS, (+)-CS, MoS and QS, but not (−)-CS, are all expected to shift the 'native→activated' equilibrium in antithrombin in favor of the activated form, and thus function as activators of antithrombin.

### 3.3. Preferred site(s) of interaction in antithrombin of sulfated molecules

Detailed biochemical investigation with (+)-CS suggested that the activator does not bind in the penta-saccharide-binding site, but rather in the extended heparin-binding site.27 HINT score for (+)-CS binding in the EHBS of the activated state of antithrombin is 1038 higher than that for binding in the PBS supporting the solution phase preference (see Fig. 2). A HINT score difference of 1038 points corresponds to ~2.1 kcal/mol, assuming a conversion factor of 491 kcal/mol-HINT score deduced for saccharide–antithrombin interactions.21 Although small, this difference possibly explains the preference for EHBS considering the weak affinity of all activators studied (Table 1). Further, binding in the EHBS, rather than in the PBS, explains the much weaker activation observed with these sulfated activators.26

The HINT score for (−)-ECS and QS interacting in the EHBS is lower than that for the PBS by 472 and 997 points, respectively (Table 1). Although this simulation suggests that binding in the PBS is favored, it is likely that (−)-ECS and QS bind in the EHBS in solution. Two observations indicate the preference for EHBS: 1) It has been noted for saccharide ligands that engagement of the PBS leads to full conformational activation of antithrombin. This induces nearly 300-fold acceleration in rate of factor Xa inhibition by antithrombin–ligand complex. In contrast, (−)-ECS and QS accelerate factor Xa inhibition ~10–20-fold. 2) Antithrombin activation involves a movement of the N-terminal polypeptide during the process of heparin binding. Kinetic studies suggest that the polypeptide N-terminus, which sweeps over parts of the PBS, moves to expose remaining residues.33 This movement is followed by conformational alterations in the PBS to accommodate the incoming saccharide ligand.1,34,35 It is likely that these movements consume energy, which is made available by heparin binding. Because the binding energy released by the weak affinity of these sulfated molecules is low, movement of the N-terminal polypeptide and subsequent stabilizing changes in the PBS are most likely not favored.

Similarities in the sulfated activators studied also suggest that (−)-ECS, QS, and MoS probably bind in the EHBS. These include the overall charge density and the molecular size. In addition, EHBS appears to be preferred because of the presence of a highly electropositive domain formed by four neighboring positively charged residues, Arg129, Arg132, Lys133, and Lys136. Such a collection of residues in a small, compact area is not present anywhere in antithrombin, including the PBS.14,15

### 3.4. Parallel between observed and simulated free energy of binding

Figure 4 shows the relationship between the calculated free energy of binding ($\Delta G_{\text{calc}}^\circ$) for sulfated activators binding in the extended heparin-binding site of antithrombin in the activated form (from the overall HINT score using 491 HINT score per kcal/mol factor21,26) and QS binding in either the PBS or EHBS of native antithrombin.

#### Table 1. Overall HINT score, observed binding energies and acceleration in factor Xa inhibition for the interaction of sulfated flavanoids and flavonoids with antithrombin

<table>
<thead>
<tr>
<th>Pentasaccharide - Binding Site</th>
<th>Extended Heparin - Binding Site</th>
<th>$\Delta G_{\text{calc}}^\circ$* (kcal/mol)</th>
<th>Acceleration* $k_{\text{CAT}}/k_{\text{UNCAT}}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Native</td>
<td>Activated</td>
<td>Overall HINT Score</td>
<td>GO OBS</td>
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<tr>
<td>(−)-ECS</td>
<td>1449</td>
<td>2014</td>
<td>−6.8±0.1</td>
</tr>
<tr>
<td>(+)-CS</td>
<td>1886</td>
<td>2014</td>
<td>−7.5±0.2</td>
</tr>
<tr>
<td>(−)-CS</td>
<td>4458</td>
<td>1787</td>
<td>nd**</td>
</tr>
<tr>
<td>MoS</td>
<td>3382</td>
<td>2038</td>
<td>−7.8±0.2</td>
</tr>
<tr>
<td>QS</td>
<td>3317</td>
<td>2367</td>
<td>−6.5±0.2</td>
</tr>
</tbody>
</table>

* Taken from refs. 18 and 24.
** not measured. However, $\Delta G_{\text{obs}}$ and acceleration values of −6.3±0.1 kcal/mol and 8.5±1.2, respectively, for racemic (±)-CS (RCS) suggest that (−)-CS enantiomer has much weaker binding affinity and acceleration capability.
and the observed free energy of binding ($\Delta G_{\text{OBS}}^0$). Coupled with the simulated and observed data noted for (−)-CS and racemic CS (above), a reasonable correlation between the observed and calculated binding energy for sulfated flavanoids and flavonoids can be noted. Linear regression of the data ($r^2 = 0.9$) gives an intercept of $-4.8 \pm 0.6$ kcal/mol and a slope of $0.31 \pm 0.08$. The intercept of 4.8 kcal/mol may represent the free energy of binding arising from non-specific interactions with small molecules and is similar to $\sim 3.2$ kcal/mol noted for HINT analyses of antithrombin–oligosaccharide interaction. The slope of 0.31 is significantly different from an idealized slope of 1.0. This slope suggests that $\Delta G_{\text{OBS}}^0$ changes less per unit $\Delta G_{\text{CALC}}^0$, assuming a correlation factor derived from saccharide–antithrombin interactions. The lower slope, that is, $< 1.0$, may suggest greater sensitivity of the HINT technique for small organic molecules. Although the origin of this higher sensitivity is unclear at the present time and needs to be rigorously determined using an enhanced set of designed, structurally-diverse activators, it may be useful in the design of novel molecules.

3.5. HINT analysis at individual residue level

The study of HINT profile at a residue level offers an opportunity to understand the contribution and functional role of individual amino acid residues in the activation mechanism. In general, antithrombin activation is a two-step phenomenon in which the first step is the
formation of the initial recognition complex (AT:ACT) between the inhibitor in the native form (AT) and the activator (ACT) (Scheme 1). This is followed by a conformational change in the inhibitor to give the activated antithrombin–activator complex (AT*:ACT) that rapidly inactivates factor Xa.

This kinetic two-step conformational activation process is being simulated with activators binding to antithrombin in the native (AT, ‘2ant’) and activated states (AT*, ‘1azx’). Thus, the interactions derived from residue level HINT scores are likely to report on the initial recognition (step 1, Scheme 1) and conformational change steps (step 2). Detailed site-directed mutagenesis studies have shown that Lys114 and Arg129 play a major role in the conformational change process (step 2), while Lys125 is important for the initial recognition of the activator. Further, Lys114, Arg129 and Lys125 form a network of positively charged residues that modulate ligand recognition and antithrombin activation phenomenon.

The HINT profile at individual residue level (Table 2) suggests plausible explanation for absence of preferential binding in the PBS. Assuming that the activators bind in the PBS, favorable interactions are expected with important electropositive residues, Arg47, Lys114, Lys125, and Arg129, as noted for most activators. However, the binding affinity contributions predicted from the HINT profile, for example, of MoS (Lys114 > Lys125 > Arg129 = 0 kcal/mol), is at variance with the contributions established for saccharide ligands in solution (Lys114 > Lys125 > Arg129 = 3 kcal/mol). This observation is also true for individual residue HINT scores for (-)-ECS and QS. Further, although HINT consistently predicts high Arg47 contribution (~2–4 kcal/mol) for all synthetic activators, in solution this residue is known to contribute much less (~0.5 kcal/mol).

3.6. Rational design of a small organic activator in silico based on these studies

The above described HINT studies explain the binding properties of sulfated flavanoids/flavonoids. We reasoned that it should be possible to enhance the binding affinity of our potential activator by simultaneously engaging both the EHBS and PBS. This approach would involve chemically linking two molecules of a flavanoid/flavonoid so that each monomer could optimally interact with either site. We selected MoS, which was found to have the highest affinity for antithrombin among the sulfated flavanoids studied. Using its predicted binding mode in PBS and EHBS, we linked the 5-O-position of one monomer in the PBS with the 7-O-position of another in the EHBS—a ‘head to head’ arrangement—to create a dimeric MoS molecule. An ethylene bridge was found optimal for linking the two monomers. This structure was used to explore whether higher affinity flavanoid/flavonoid-based molecules could be designed. The MoS dimer was docked onto activated antithrombin structure in a manner similar to monomeric species and HINT calculations were performed on the docked complex.

The HINT contour map indicates favorable interactions across the entire length of the binding site (PBS and EHBS) (Fig. 5). The overall HINT score of the complex was found to be 7985 in comparison to ~3000–5000 observed for the monomeric species suggesting a binding affinity of 68 nM, using the conversion factors derived earlier. This predicted binding affinity is approximately 25-fold better than that for MoS monomer.

It is expected that MoS dimer functions as an activator of antithrombin because HINT profile shows strong

Figure 3. HINT analysis of sulfated flavanoids and flavonoids dependent activation of antithrombin. The minimum energy docked complex of (-)-ECS, (+)-CS and (-)-CS interacting with native (open) and activated (filled) forms of antithrombin in the pentasaccharide- (rectangles) and extended heparin- (ovals) binding sites was scored using HINT (see Table 1). The arrows show the HINT simulated transformation ‘native→activated’ for each sulfated molecule.

Figure 4. Parallel between observed and simulated free energy of binding of sulfated molecules with antithrombin. The overall HINT score (Table 1) of interaction in the extended heparin binding site of activated form of antithrombin was used to calculate the simulated binding energy. A correlation factor of 491HINT score per kcal/mol deduced for saccharide–antithrombin interaction was used.

Scheme 1.

AT + ACT \[\xrightarrow{k_1} \text{AT:ACT} \xrightarrow{k_2} \text{AT*:ACT}\]
interactions with amino acid residues important in the binding and activation process. For example, hydrogen bonding and ion-pair interactions are found between MoS dimer and Lys11 (HINT score 1449), Asn45 (HINT score 470), Lys125 (1725), Arg129 (1848), Arg132 (1461), Lys133 (1638), and Lys136 (1688) (Fig. 5). This is the first non-natural molecule that exhibits HINT profile resembling naturally occurring saccharide activators.\(^{18}\) The designed MoS dimer will be tested for its binding and activation potential.

Table 2. Residue level HINT scores* for the interaction of sulfated flavanoids and flavonoids with native or activated forms of antithrombin in the pentasaccharide- and extended heparin-binding site

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<th></th>
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<th>Activated</th>
<th>Native</th>
<th>Activated</th>
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*Residue level HINT scores were calculated by docking the sulfated molecule in either the pentasaccharide- or the extended heparin-binding site in the native and activated forms of antithrombin. See ‘Materials and methods’ for details.

+ Includes small contributions from residues, for example, V5, C8, A10, P12, D14, P41, E42, T44, V48, S112, F122, N135, G276, D277, E414, P416 and L417.

Figure 5. HINT map of MoS dimer binding to antithrombin. Dimeric MoS (orange balls and sticks, inset shows its structure) was placed in the heparin-binding site in antithrombin (cyan and purple ribbon) and optimal geometry of the complex searched using Flexidock in Sybyl. The EHBS includes the domain formed by residues Arg129, Arg132, Lys133, and Lys136, while the PBS includes the region formed by Lys114, Lys125 and Arg129. Side chains of these and other amino acid residues are shown in green capped sticks. The final complex was further minimized and scored using HINT. Blue ‘cloud’ represents the contours of the atomic level interactions. Contours at ±1.0 and −1.0 levels, representing either favorable (blue, positive) or unfavorable (red, negative) interactions, were mapped for better visualization. Side chains of amino acid residues are shown in green capped sticks.
In conclusion, HINT is successful in rationalizing the biochemical observations on antithrombin interacting with sulfated small organic activators. For selected sulfated flavanoids and flavonoids, interesting correlation is observed between the HINT score and the binding property. As more synthetic, non-sugar, organic activators become available, the applicability of HINT can be further tested. At the present time, HINT is likely to be useful in designing potential new organic antithrombin activators.

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References and notes