Bioorganic & Medicinal Chemistry Letters 23 (2013) 355–359

Contents lists available at SciVerse ScienceDirect

Bioorganic & Medicinal Chemistry Letters

journal homepage: www.elsevier.com/locate/bmcl

On scaffold hopping: Challenges in the discovery of sulfated small molecules as mimetics of glycosaminoglycans

Preetpal S. Sidhu [a], Philip D. Mosier [a], Qibing Zhou [b], Umesh R. Desai [a,*]

[a] Department of Medicinal Chemistry and Institute for Structural Biology and Drug Discovery, Virginia Commonwealth University, Richmond, VA 23219, United States
[b] Institute for Materia Medica, College of Life Science and Technology, Huazhong University of Science and Technology, Wuhan, Hunan 430074, PR China

ARTICLE INFO

Article history:
Received 26 August 2012
Revised 4 October 2012
Accepted 15 October 2012
Available online 24 October 2012

Keywords:
Allosteric inhibition
Anticoagulants
Scaffold hopping
Thrombin
Virtual screening

ABSTRACT

The design of sulfated, small, nonsaccharide molecules as modulators of proteins is still in its infancy as standard drug discovery tools such as library of diverse sulfated molecules and in silico docking and scoring protocol have not been firmly established. Databases, such as ZINC, contain too few sulfate-containing nonsaccharide molecules, which severely limits the identification of new hits. Lack of a generally applicable protocol for scaffold hopping limits the development of sulfated small molecules as synthetic mimetics of the highly sulfated glycosaminoglycans. We explored a sequential ligand-based (LBVS) and structure-based virtual screening (SBVS) approach starting from our initial discovery of monosulfated benzofurans to discover alternative scaffolds as allosteric modulators of thrombin, a key coagulation enzyme. Screening the ZINC database containing nearly 1 million nonsulfated small molecules using a pharmacophore developed from the parent sulfated benzofurans followed by a genetic algorithm-based dual-filter docking and scoring screening identified a group of 10 promising hits, of which three top-scoring hits were synthesized. Each was found to selectively inhibit human alpha-thrombin suggesting the possibility of this approach for scaffold hopping. Michaelis–Menten kinetics showed allosteric inhibition mechanism for the best molecule and human plasma studies confirmed good anticoagulation potential as expected. Our simple sequential LBVS and SBVS approach is likely to be useful as a general strategy for identification of sulfated small molecules hits as modulators of glycosaminoglycan–protein interactions.

Thrombin is a plasma serine protease that plays important roles in blood clotting.[1] It cleaves fibrinogen to fibrin resulting in clot formation, which is a natural defense mechanism to prevent excessive loss of blood from injury. Yet, under abnormal conditions, internal clot formation can results in heart attacks and strokes, which is typically prevented through the use of anticoagulants. Heparin, a highly sulfated glycosaminoglycan (GAG), is one such anticoagulant and has been in use essentially unchanged since the 1930s. It is also an anticoagulant with numerous adverse consequences, which has catalyzed the search for better anticoagulants.[2]

Majority of anticoagulant search efforts have focused on the discovery of direct inhibitors of coagulation enzymes, including thrombin and factor Xa. Dabigatran and rivaroxaban, small peptidomimetics, have been realized through these discovery efforts. Both of these are active site inhibitors. Major efforts are also in progress to discover active site inhibitors of other coagulation enzymes including factor VIIa and factor XIa.

A paradigm shifting idea is to discover small molecules that allosterically modulate coagulation enzymes. Allostery can offer major advantages such as fine control over a protein's activity and better specificity of action. Whereas active site inhibitors typically display an efficacy of 100%, allosteric inhibitors can induce less than quantitative inhibition, which in principle can afford greater tunability and regulatory control. With regard to the second point, higher specificity of action is possible from allostery because of greater structural differences in allosteric binding sites than in catalytic active sites.

Despite these advantages, the design of small molecule, allosteric inhibitors of coagulation remains uncharted. In fact, the possibilities for discovery of such molecules is high because most coagulation enzymes contain allosteric binding sites. For example, thrombin contains two anion-binding exosites, called exosites I and II, that are characterized by clusters of basic residues. Various co-factors and proteins interact with these exosites and regulate the function of thrombin.[3–5] Evidence accumulated over the past several decades indicates that the proteolytic function of thrombin can be altered through appropriate interaction with these exosites.[5] For example, heparin, a natural sulfated glycosaminoglycan, binds in exosite II and alters the active site of thrombin.[6] Likewise, thrombomodulin, an endothelial cell surface receptor, engages both exosites I and II to alter thrombin's specificity from fibrinogen to protein C.[1,7] Other naturally occurring allosteric modulators of thrombin include hirudin,[8] chondroitin sulfate,[9] and haemadin.[10]

* Corresponding author.
E-mail address: urdesai@vcu.edu (U.R. Desai).

0960-894X/$ - see front matter © 2012 Elsevier Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.bmcl.2012.10.079

Despite the availability of this natural avenue, few small molecules have been designed that exploit the natural allosteric mechanism of modulation of thrombin's activity.

Recently, our laboratory designed the first small molecules acting as allosteric inhibitors of thrombin.[11] A group of sulfated benzofurans containing a single sulfate group exhibited good inhibition of human α-thrombin under physiological conditions (Fig. 1). Enzyme kinetic studies showed an allosteric inhibition phenomenon, while later studies with site-directed thrombin mutants identified the site of binding to a hydrophobic region close to Arg173, a site encompassed within exosite II.[12] This site of binding is a new discovery and is likely to be a promising avenue for anticoagulant drug discovery.

To date monosulfated benzofuran scaffold is the only scaffold available for allosteric modulation. This is a serious limitation. It is important to discover alternative scaffolds to enhance structure–activity studies and improve the chance of clinical viability. Yet, how do we discover alternative sulfated small molecule scaffolds? A traditional technology is high-throughput screening (HTS) of a library of diverse molecules. Yet, such a library of molecules containing sulfate groups has not been developed nor is not commercially available. Another approach is virtual screening (VS). The primary goal here is to discover novel chemical structures that exhibit efficacy at the desired target. This is a more cost-effective solution with a reasonable level of hit discovery.[13,14]

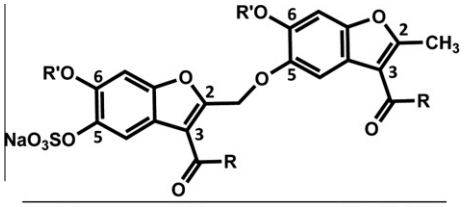
Nearly all VS approaches can be classified into either structure-based (SBVS) or ligand-based (LBVS) methods.[14–16] While a majority of discovery efforts have focused on one of the approaches, a growing trend has been to utilize both to enhance the probability of hit discovery.[17–22] We have previously explored both SBVS and LBVS, individually, to discover sulfated small molecules and understand their interaction with proteins.[11,12,23,24] Whereas SBVS of sulfated small molecules has proved to be challenging because of lack of robust scoring functions used in the docking, LBVS has been found to be beset with inability to consider the shape of the binding site.[25] LBVS is useful when applied to large libraries but such libraries are not available for sulfated small molecules. Likewise, SBVS with large libraries of sulfated small molecules, if available, is also difficult because it is computationally intensive and very time consuming.[26]

Despite these difficulties, VS is likely to be a useful approach to rapidly discover diverse sulfated small molecule scaffolds. To assess whether a VS-based approach can rapidly provide alternative allosteric thrombin inhibitors, we explored a sequential LBVS and SBVS approach. First we performed LBVS on a library of nearly 1 million nonsulfated molecules using a pharmacophore query developed on the basis of our monosulfated benzofuran results.[11,12] The hits generated from the LBVS study were then screened by docking onto thrombin to identify a smaller group of 10 hits. Of these, three top-scoring molecules were selected for thrombin inhibition studies. All three molecules based on a scaffold significantly different from that of benzofuran, except for the presence of sulfate group(s), were found to inhibit thrombin. The hits displayed allosteric inhibition mechanism in a manner similar to monosulfated benzofurans and plasma anticoagulation, as would be expected. The success achieved with sequential LBVS and SBVS bodes well for discovery of diverse sulfated small molecules as modulators of protein function.

Pharmacophore query generation. A pharmacophore was generated based on the SAR of monosulfated benzofuran library previously studied in our laboratory (Figs. 1 and 2).[11,27] Briefly, the potency of monosulfated benzofurans increased nearly a 1000-fold from a monomeric to a dimeric scaffold. Thus, at least two aromatic units were deemed as essential. The average distance between these aromatic units from their centroids was found to be 8.1 ± 0.5 Å. The SAR also indicated a strong requirement of a hydrophobic substituent at the 3 position of both aromatic units. These hydrophobic substituents were found to be 6.4 ± 1 Å from the aromatic centroid. Finally, the most difficult condition to be implemented in LBVS was the presence of a sulfate group at the 5 position of the first benzofuran ring (see Fig. 1). Commercial libraries do not contain molecules with a sulfate group. Hence, the pharmacophore query was devised with a hydroxyl group, instead of a sulfate group, under the assumption that it can be added later, both in silico and by synthesis. These features constituted the pharmacophore query for LBVS, which is shown in Figure 2.

Ligand-based virtual screening: LBVS was performed using UNITY on ZINC database (Shoichet Laboratory, USCF, CA), which contains structural information on nearly 1 million compounds (2006 version) that are all nonsulfated. The LBVS hits were also expected to satisfy three of four Lipinski's rules (MW ⩽500, H-Bond donors ⩽5, H-Bond acceptors ⩽10, and $-0.4 < \text{Log}P < +5.0$). In addition, no limit was placed on the number of rotatable bonds present in a hit. Using this 3D pharmacophore query, LBVS on the ZINC database identified 4560 hits. These hits were then processed computationally to introduce sulfate group(s) using an in-house programming script. The script identified free phenolic group(s) on the molecule and performed an in silico sulfation.

Structure-based virtual screening: Molecular docking studies were performed using the genetic algorithm-based dual-filter protocol developed earlier for heparin-based sequences.[28] In this approach, the first filter identified structures that interacted with the allosteric site on thrombin with a high GOLDScore, while the second filter was used to identify molecules that bound in the



R′	R	IC_{50} (μM)
$-CH_3$	—OEt	6.2
$-CH_3$	$-OCMe_3$	7.3
$-CH_3$	$-O(CH_2)_2OMe$	16.9
$-CHMe_2$	—OMe	1.25

Figure 1. Structures of some monosulfated benzofuran dimers discovered as first small molecular allosteric inhibitors of thrombin (Sidhu et al. *J. Med. Chem.* **2011**, *54,* 5522). Key units of this scaffold include: two aromatic rings, hydrophobic substituents at 3 positions and monosulfate at the 5 position. Pharmacophore query designed on the basis of this SAR is as shown in Figure 2.

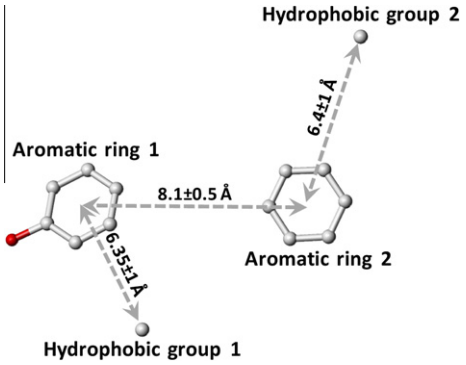


Figure 2. Three-dimensional (3D) pharmacophore query developed on the basis of SAR study with monosulfated benzofuran dimers (see Fig. 1).[11,27] The atom in red represents an oxygen of a hydroxyl group. See text for details.