3-15-2014

Design, synthesis, and biological evaluation of non-symmetric smal

Himabindu Anumala

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Design, Synthesis, and Biological Evaluation of Non-Symmetric Small Molecules as Inhibitors of Plasminogen Activator Inhibitor (PAI-1)

by

Himabindu Anumala

Thesis

Submitted to the Department of Chemistry
Eastern Michigan University
in partial fulfillment of the requirements
for the degree of

MASTER OF SCIENCE
in
Chemistry

Thesis Committee:
Cory Emal, PhD, Chair
Gregg Wilmes, PhD
Steven Pernecky, PhD

March 15, 2014
Ypsilanti, Michigan
Dedication

I would like to dedicate this work to three important people in my life so far; without them this would not have been completed. First I would like to thank my Mother, Sudha Mani Anumala, and my Father, Sambaiah Anumala, for their love, care and the trust they had in me in achieving my dreams.

The next important person who I would like to dedicate is to my Guru (Teacher in Sanskrit) Dr. Cory Emal for being my research advisor, mentor, and my motivation towards next higher step in my life.

I also owe an undying debt of gratitude to Dr. Cory Emal, for his commitment to teaching and the time he took to help me understand the complicated concepts and for providing me with an opportunity to present at national level conference.

Without him I would not have been able to work in a big company as a research associate and achieve this position as a researcher.
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I would like to thank my thesis committee, Dr. Gregg Wilmes and Dr. Steven Pernecky, for their comments that helped me in completion of my thesis.

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I would also like to thank our research collaborator Dr. Daniel Lawrence, University of Michigan, and to my previous and current research group members.

Finally, to those who have financially maintained me throughout my master’s program including National Institute of Health, Eastern Michigan University.
Abstract

Plasminogen activator inhibitor-1 (PAI-1) is a member of the serpin family of proteins, a primary inhibitor of both tissue-type and urokinase-type plasminogen activators in plasma, and is a well-established risk factor in various disease conditions. Increased levels of active PAI-1 in plasma are correlated with the development of atherosclerosis, diabetes, stroke, and other maladies. In the present study, we describe the synthesis of two new series of compounds that aim to reduce physiologically active PAI-1 levels. These molecules are related to a series of bis-arylsulfonimides and arylsulfonamides connected by short linking diamines, and to a series of hydrazine-based analogues. These studies resulted in the identification of small molecule inhibitors of PAI-1 that displayed \textit{in vitro} IC$_{50}$ values in the low micromolar range.
# Table of Contents

Dedication ........................................................................................................................................ ii  

Acknowledgements .......................................................................................................................... iii  

Abstract ........................................................................................................................................ iv  

List of Figures .................................................................................................................................. vi  

List of Tables .................................................................................................................................... vii  

Abbreviations ................................................................................................................................... viii  

Chapter I: Introduction ..................................................................................................................... 1  

References ........................................................................................................................................ 19  

Chapter II: Effect of varying the number and position of hydroxyl groups on the aromatic ring of sulfonimide-based PAI-1 inhibitor .................................................................................. 22  

Results and Conclusions .................................................................................................................. 24  

Experimental Methods and Data ...................................................................................................... 25  

Chapter III: Hydrazine-based small molecule inhibitors ................................................................. 31  

III-1 Effect of substituents on the aromatic ring of the hydrazine-based inhibitors ......................... 31  

III-2 Imine-based PAI-1 inhibitors .................................................................................................... 33  

III-3 Effect of replacing hydrazide moiety with different aromatic and alkyl substituted groups ................................................................................................................................. 34  

III-4 Effect of different substituents on “left hand” aromatic ring and varying the chain length between the carbonyl group and a 3, 4-dihydroxy aromatic ring ......................................................................................... 36  

III-5 Effect of replacing amide with sulfonamide ............................................................................. 39  

Results and Conclusions .................................................................................................................. 40  

Experimental Methods and Data ...................................................................................................... 40  

v
List of Figures

<table>
<thead>
<tr>
<th>Figure</th>
<th>Description</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Role of PAI-1 in Fibrinolysis</td>
<td>1</td>
</tr>
<tr>
<td>2</td>
<td>3-D Structure of Human Plasminogen Activator Inhibitor-1</td>
<td>4</td>
</tr>
<tr>
<td>3</td>
<td>Three Different Forms of PAI-1</td>
<td>5</td>
</tr>
<tr>
<td>4</td>
<td>Binding of PAI-1 with Vitronectin</td>
<td>6</td>
</tr>
<tr>
<td>5</td>
<td>PAI-1 Inhibitors by Xenova Limited</td>
<td>10</td>
</tr>
<tr>
<td>6</td>
<td>ZK4044, Menthol-based PAI-1 Inhibitor</td>
<td>11</td>
</tr>
<tr>
<td>7</td>
<td>PAI-1 Inhibitors by Izuhara et al</td>
<td>11</td>
</tr>
<tr>
<td>8</td>
<td>PAI-1 inhibitors that compete for same binding site as vitronectin</td>
<td>12</td>
</tr>
<tr>
<td>9</td>
<td>PAI-1 Inhibitors by Wyeth</td>
<td>13</td>
</tr>
<tr>
<td>10</td>
<td>Tannic Acid</td>
<td>14</td>
</tr>
<tr>
<td>11</td>
<td>Epigallocatechin Monogallate (EGCG) and Epigallocatechin-3, 5-Digallate</td>
<td>15</td>
</tr>
<tr>
<td>12</td>
<td>PAI-1 Inhibitors structurally similar to Tannic Acid</td>
<td>15</td>
</tr>
<tr>
<td>13</td>
<td>PAI-1 Inhibitors with ester or amide linkages</td>
<td>16</td>
</tr>
<tr>
<td>14</td>
<td>Structure which served as a basis for developing sulfonimide inhibitors</td>
<td>18</td>
</tr>
<tr>
<td></td>
<td>with different hydroxy arrangements</td>
<td></td>
</tr>
</tbody>
</table>
## List of Tables

<table>
<thead>
<tr>
<th>Table</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Sulfonamide and Sulfonimide-based inhibitors</td>
<td>17</td>
</tr>
<tr>
<td>2. Comparison of potencies of sulfonamide-based analogues to hydrazide analogues</td>
<td>24</td>
</tr>
<tr>
<td>3. Hydrazine analogues IC50 values versus PAI-1 in buffer (pH 7.4), buffer containing 1.5% BSA, and buffer containing human plasma</td>
<td>33</td>
</tr>
<tr>
<td>4. Lead molecule with different groups on the right hand side of the molecule</td>
<td>36</td>
</tr>
<tr>
<td>5. Assay results of structural analogues of CDE-330</td>
<td>38</td>
</tr>
</tbody>
</table>
### Abbreviations

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Definition</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aβ</td>
<td>Amyloid beta protein</td>
</tr>
<tr>
<td>µM</td>
<td>Micromolar</td>
</tr>
<tr>
<td>CNS</td>
<td>Central Nervous System</td>
</tr>
<tr>
<td>CDCl3</td>
<td>deuterated chloroform</td>
</tr>
<tr>
<td>CH2Cl2</td>
<td>Dichloromethane</td>
</tr>
<tr>
<td>DART</td>
<td>Direct Analysis in Real Time</td>
</tr>
<tr>
<td>DCM</td>
<td>Dichloromethane</td>
</tr>
<tr>
<td>DMF</td>
<td>Dimethylformamide</td>
</tr>
<tr>
<td>DMSO-d6</td>
<td>deuterated dimethylsulfoxide</td>
</tr>
<tr>
<td>ECM</td>
<td>extracellular matrix</td>
</tr>
<tr>
<td>EDC•HCl</td>
<td>N-(3-dimethylaminopropyl)-N'-ethylcarbodiimide hydrochloride</td>
</tr>
<tr>
<td>EGF</td>
<td>epidermal growth factor</td>
</tr>
<tr>
<td>EtOAc</td>
<td>ethyl acetate</td>
</tr>
<tr>
<td>H2O</td>
<td>Water</td>
</tr>
<tr>
<td>HCl</td>
<td>hydrochloric acid</td>
</tr>
<tr>
<td>HOBT</td>
<td>N-hydroxybenzotriazole</td>
</tr>
<tr>
<td>HRMS</td>
<td>high-resolution mass spectrometry</td>
</tr>
<tr>
<td>IC50</td>
<td>half-maximal inhibitory concentration</td>
</tr>
<tr>
<td>IL</td>
<td>Interleukin</td>
</tr>
<tr>
<td>J</td>
<td>coupling constant, in Hertz</td>
</tr>
<tr>
<td>KDa</td>
<td>Kilodalton</td>
</tr>
<tr>
<td>LRP</td>
<td>lipoprotein-related protein</td>
</tr>
<tr>
<td>Mg</td>
<td>Milligram</td>
</tr>
<tr>
<td>MgSO4</td>
<td>magnesium sulfate</td>
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<tr>
<td>MHz</td>
<td>Megahertz</td>
</tr>
<tr>
<td>MI</td>
<td>myocardial infarction</td>
</tr>
<tr>
<td>Ml</td>
<td>Milliliter</td>
</tr>
<tr>
<td>Mmol</td>
<td>Millimole</td>
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<tr>
<td>MS</td>
<td>mass spectrometry</td>
</tr>
<tr>
<td>NaHCO3</td>
<td>sodium bicarbonate</td>
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<td>NMR</td>
<td>nuclear magnetic resonance</td>
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<tr>
<td>PAI-1</td>
<td>plasminogen activator inhibitor-1</td>
</tr>
<tr>
<td>PDB</td>
<td>Protein Databank</td>
</tr>
<tr>
<td>PPARγ</td>
<td>peroxisome proliferator-activated receptor-g</td>
</tr>
<tr>
<td>RCL</td>
<td>reactive center loop</td>
</tr>
<tr>
<td>Serpin</td>
<td>serine protease inhibitor</td>
</tr>
<tr>
<td>SDS</td>
<td>Sodium dodecyl sulfate</td>
</tr>
<tr>
<td>TEA</td>
<td>Triethylamine</td>
</tr>
<tr>
<td>TGF- β</td>
<td>tissue growth factor-β</td>
</tr>
<tr>
<td>tPA</td>
<td>tissue-type plasminogen activator</td>
</tr>
<tr>
<td>uPA</td>
<td>urokinase-type plasminogen activator</td>
</tr>
<tr>
<td>uPAR</td>
<td>urokinase-type plasminogen activator receptor</td>
</tr>
</tbody>
</table>
VN
Vitronectin
Chapter I: Introduction

Plasminogen activator inhibitor-1 (PAI-1) is a single-chain glycoprotein with a molecular weight of 48 KDa (1). It belongs to a superfamily of proteins called serpins that inactivate tissue-type (tPA) and urokinase-type (uPA) plasminogen activators (1). These plasminogen activators regulate a process called fibrinolysis, which is the normal breakdown of blood clots within the human body and various mammals. Activation of plasminogen into its active form, plasmin, is an important step in fibrinolysis. PAI-1 inhibits plasmin generation by inhibiting uPA and tPA as shown in Figure 1 (2). Here it forms a sodium dodecyl sulfate-stable acyl enzyme complex, and this complex formation is highly specific and rapid (3).

Figure 1: Role of PAI-1 in Fibrinolysis

https://ahdc.vet.cornell.edu/clinpath/modules/coags/tertiary.htm

**Abbreviations:** tPA: tissue plasminogen activator; PAI: plasminogen activator inhibitor; PLG: Plasminogen; AP: Antiplasmin; FDPs: Fibrin(ogen) degradation products.

PAI-1 has both physiological and pathological roles in the human body. Normal levels of PAI-1 regulate processes such as angiogenesis, cell migration, and wound
healing. Elevated levels of PAI-1 have been associated with various diseases such as cardiovascular disease, cancer, atherosclerosis, and renal disease (1).

**PAI-1 and Diseases:**

The impairment of the fibrinolytic system results in a wide range of thrombotic conditions (4), including myocardial infarction (5), stroke (6), disseminated intravascular coagulation (7), peripheral artery disease (8), and deep vein thrombosis (9). Studies conducted by Diebold et al. suggest that there is a direct relationship between the over-expression of PAI-1 and vascular remodeling in arterial thrombus condition (10). Increased PAI-1 expression was observed in atherosclerotic lesions (11) and this increased amount of PAI-1 acts as an adipokine; adipokines are cytokines secreted by adipose tissue and have been proposed to play a major role in the atherogenic processes involving vascular injury, platelet, fibrin and lipid deposition (12).

Sawdey and Loskutoff reported that plasma PAI-1 levels were found to be greater in obese mice compared to those of lean mice due to over-expression of adipose tissue (13). PAI-1 expression is also regulated by endothelial cells that express functionally active PPARγ (14), which is associated with obesity, non-insulin-dependent diabetes mellitus, and insulin resistance (14). Tumor cells and endothelial cells that responded to inflammatory cytokines and inflammation mediators also synthesize and secrete excess PAI-1 (15). Over-expressed PAI-1 also accelerates angiogenesis (15), which is necessary for the progression of tumors from dormancy to malignancy. High levels of plasma PAI-1 are a biochemical prognostic marker in cancer (16).

The up-regulation of PAI-1 is associated with increased levels of amyloid beta protein (Aβ) in the brain. Increased Aβ levels cause amyloid plaques that are associated
with Alzheimer’s disease (17). Studies conducted by Liu et al. showed that knocking out the PAI-1 gene leads to increased levels of tPA and plasmin and decreased levels of Aβ accumulation (17). PAI-1 also enhances signal transmission between two neurons by stimulating them at the same time and has been shown to reverse cognitive defects in mice with Alzheimer's disease (18). PAI-1 plays a major role in both physiological and pathological processes of the brain such as neuronal migration, cerebral infarction, plasticity in neuroontogenesis and CNS neoplasia (19).

**PAI-1 as a Therapeutic Drug Target:**

From the previous studies we can conclude that inhibiting PAI-1 and lowering the levels of elevated plasma PAI-1 may be effective in treating or alleviating the symptoms of multiple diseases. Different approaches have been taken to inhibit the activity of PAI-1, including development of small molecules, peptides, and monoclonal antibodies. These act by inhibiting the synthesis and secretion of PAI-1 or by reducing the elevated levels of active PAI-1 in plasma. The development of inhibitors for PAI-1 has proven to be challenging due to the unique structure of PAI-1. PAI-1 does not catalyze a reaction and therefore is not an enzyme; it has no substrate or classical active site for binding of a drug molecule. The typical drug design for enzymatic inhibitors involves the development of a substrate analog, which mimics the structure of the substrate, thus competing with substrate for the active binding site and thereby inhibiting the activity of the enzyme. This strategy cannot be applied to design drug molecules for inhibiting PAI-1. Apart from the difficulties created by PAI-1’s structural features, the pathological role of PAI-1 is different for each disease condition.
PAI-1 Structure

PAI-1 is also known as endothelial PAI-1 or serpin E1, which is encoded by the SERPINE1 gene (20). The major tissues involved in the synthesis of PAI-1 are the liver, vascular, and adipose tissues (13). PAI-1 is a single chain glycoprotein composed of 379 amino acid residues; the secondary structure of active PAI-1 consists of 3 β-sheets, 8-9 α-helices, and a reactive center loop (RCL) (21), as shown in Figure 2 (22). The RCL contains 20 amino acid residues, which act as a pseudo-substrate towards its targeted serine protease (23).

FIGURE 2: 3-D Structure of Human Plasminogen Activator Inhibitor-1 (PDB) Protein Databank (PDB) (22)

PAI-1 exists in three different forms as shown in Figure 3 (24): the active, latent, and cleaved forms. In the active form of PAI-1, the RCL is solvent-exposed. The active form spontaneously converts into the latent form in which the reactive center loop is not solvent exposed and is buried in the β-sheets. This latent form cannot inhibit proteases
Conversion to the latent form has a half-life of 1 hour at pH 7.5 and exists in equilibrium with the active form (26). In the presence of the plasma protein vitronectin (VN), the latent form can be converted to the active form, but this conversion is very slow. This latent form can also be converted into the active form when treated with denaturants and negatively charged phospholipids (23). Interconversion of active and latent forms of PAI-1 is unique and distinguishes it from other serpins. The metastable active forms of other serpins have long term stability in the purified state whereas PAI-1 spontaneously changes to high energetic, reversible inactive latent form under physiological conditions (27). The third form is the cleaved form, which is the most stable form. Serine proteases (uPA or tPA) bind with the active form of PAI-1 through a covalent complex and cleave the RCL, thus forming an SDS-stable acyl-enzyme complex (28), making this cleaved form irreversible and inactive (29).
Interaction of PAI-1 with Vitronectin:

Vitronectin is a 75-kDa glycoprotein, belonging to the pexin family, found in human serum and the extracellular matrix, and promotes cell adhesion and motility (30). The interaction between PAI-1 and VN is highly specific and hydrophobic. The N-terminal somatomedin-B domain of VN interacts with the flexible joint region of active PAI-1 and stabilizes the active form of PAI-1 (31). This binding causes the central β-sheet to close, which prevents the insertion of the RCL and makes it available for target proteases (32).

Figure 4: Binding of PAI-1 with Vitronectin

The binding of VN to PAI-1 was found to have 1:1 stoichiometry Figure 4 (33). The binding sites of tPA and VN with PAI-1 overlap partially, and the binding is mutually exclusive, resulting in generation of binary equimolar complexes of tPA and PAI-1 (25). The stability of active form of PAI-1 when bound to VN is twice to that of unbound PAI-1 in solution and the half-life in extracellular matrix was found to be
greater than 24 h (30). VN regulates the activity of PAI-1 by maintaining the distribution of PAI-1 between the extracellular matrix and plasma and also alters the target specificity (30).

**Early PAI-1 Inhibitors:**

Previously reported inhibitors or modulators of PAI-1 activity include natural and synthetic peptides, small molecules, and monoclonal antibodies. Each of them has so far failed to develop into a drug molecule due to many issues such as poor bioavailability, IV administration for rapid onset of action, and difficulty in crossing blood-brain barrier; hence they are not clinically approved for human use.

NG-R1 (20(S)-protopanaxatriol notoginsenoside R1) is a dammarenene-type saponin, which is extracted and purified from the Chinese herb *Panax notoginseng*. NG-R1 has been shown to have the ability to increase tPA activity by two-fold and reduce PAI-1 activity by human endothelial cells (34). Astragaloside IV is a major constituent extracted from the roots of *Astragalus membranaceus*, which reduces PAI-1 expression by human endothelial cells (35). Synthesis and purification of these molecules is quiet challenging as the structure is complex with a high molecular weight. These two Chinese herbal medicines can inhibit PAI-1 synthesis and secretion by endothelial cells but failed with non-endothelial cells (hepatic, adipose) and thus limited its application in treating different diseases.

Lipid-lowering drug (fibrates) such as gemfibrozil (I-1) were found to lower the synthesis of PAI-1 in human hepatoma cell lines (36). 9-cis retinoic acid (I-2) has been shown to reduce the expression of PAI-1 in mammalian hepatocytes (37). However, the effect of fibrates on PAI-1 varies with the dosage and type of each fibrate molecule. T-
686 (I-3), a butadiene derivative, lowers plasma PAI-1 levels and atherosclerotic lesion area by 19% in rabbits with hypercholesteremia on oral administration. Poor oral bioavailability, water solubility, toxicity are the major issues associated with this butadiene derivative (38).

The inhibition of PAI-1 by the use of monoclonal antibodies was also reported. Levi et al. showed direct inhibition of binding of PAI-1 to tPA by using monoclonal antibodies. They observed rapid lysis of human thrombus and thrombus extension when inserted into rabbit jugular vein, with and without exogenous tPA. Antibody CLB-2C8 was shown to increase endogenous thrombolysis, inhibit the growth of thrombus in a rabbit model of venous thrombosis, and increase reperfusion and decrease re-occlusion in a canine model of coronary artery thrombosis (39). Berry et al. reported antithrombotic effects of monoclonal antibody MA33H1, which converts PAI-1 to a non-inhibitory substrate (40). Van Giezen et al. reported reduction in thrombus growth in a rat model of FeCl₃-induced carotid arterial thrombosis by the use of polyclonal antibody PRAP-1, the F₅ab fragment of PAI-1 (41). However, these antibodies require IV administration as they
are orally degraded by gastric and intestinal enzymes and cannot be used to act on chronic levels of plasma PAI-1.

The use of short peptides to inhibit PAI-1 was first observed in the mid 1990’s, when a tetradecapeptide was shown to accelerate fibrinolysis in vitro by inactivation of PAI-1 (42). Peptides Ac-TEASSSTA and Ac-TVASSSTA, which correspond to the P14-P7 residues of reactive center loop of PAI-1, form stable complexes with PAI-1, where the inhibitor is converted into substrate and is readily available for tPA to bind (43). The major problem with these peptides is that they are quickly inactivated upon oral administration and require subcutaneous or IV infusion for rapid onset of action. These peptides failed to treat brain related diseases as they have difficulty in crossing the blood-brain barrier.

To eliminate the drawbacks and undesirable effects of antibodies and peptides, many small molecule inactivators have been synthesized. Multiple researchers have focused on developing small molecules with high specificity and inhibitory activity towards PAI-1.

The first small molecule inhibitors of PAI-1 were reported by Xenova Limited and known as XR334 and XR330 (Figure 5). They are diketopiperazine-based inhibitors obtained from a natural product lead that inhibit thrombus formation in rats. Their in vitro IC$_{50}$ values against PAI-1 were found to be 51.7 µM and 30 µM respectively (44). On further optimization Xenova developed XR5118 (45) with an in vitro IC$_{50}$ of 3.5 µM and XR11211 (46) with an IC$_{50}$ of 0.20 µM. These molecules induce structural changes in PAI-1 and thereby prevent binding of PAI-1 with uPA/ tPA. They also help in conversion of active to latent forms of PAI-1. Poor physicochemical properties and isomerization
were the major issues with these diketopiperazines and thus failed to inhibit active PAI-1 in human plasma (46).

![Chemical structures of compounds I-4 to I-7](image)

**Figure 5: PAI-1 Inhibitors by Xenova Limited.**

Ye et al reported menthol-based molecules as PAI-1 inhibitors (47). ZK4044 (I-8), (Figure 6) was found to be effective and highly specific towards PAI-1; with an IC\textsubscript{50} of 0.644 \textmu M. ZK4044 binds to one of the two pockets, located between \(\beta\)-strands s3A and s5A of PAI-1. The major drawback of ZK4044 is that it is hydrophobic and needs to be further optimized to improve its water solubility and potency (48).
TM5007 is an orally active PAI-1 inhibitor reported by Izuhara et al that was identified through a virtual screen (Figure 7). TM5007 was found to be highly selective, stable and non-toxic with an IC\(_{50}\) of 29.2 µM, but its IC\(_{50}\) exceeded its peak plasma level in rodents, limiting its effectiveness as a drug (49). TM5275 (I-10) is an analog of TM5007 with an IC\(_{50}\) of 6.95 µM (Figure 7), which shows antithrombotic effects (50) and anti-fibrotic activity in a model of lung fibrosis (51).

In 2004, Wyeth researchers reported three molecules with low \textit{in vitro} IC\(_{50}\) values versus PAI-1 (52). WAY140312 was found to be orally effective in animal models of vascular disease. The IC\(_{50}\) of WAY140312 versus PAI-1 is 11.7 µM, and also prevents
thrombosis in a rat model of acute vascular injury (52). Tiplaxtinin is another potent inhibitor, which is orally effective with an IC₅₀ value of 2.7 µM. Tiplaxtinin is orally bioavailable, highly selective and less toxic (53). Tiplaxtinin impedes coronary occlusion (54) and reduces glucose stimulated PAI-1 mediated and nutritionally induced obesity (55). However, in the presence of protein VN, WAY140312 and tiplaxtinin failed to inhibit active PAI-1 in the blood, as vitronectin and these inhibitors compete for the same binding site on PAI-1 (56, 57).

![Figure 8](image)

**Figure 8: PAI-1 inhibitors that compete for same binding site as vitronectin.**

Wyeth also reported an oxadiazolidinedione-based inhibitor (I-13) with an IC₅₀ of 0.39 µM versus PAI-1 and a series of 2-carboxylic acid indole-based inhibitors with good anti- PAI-1 activity (58, 59) (Figure 9).

PAI-749 (I-15), a synthetic antagonist of PAI-1 was shown to have a dual mode of action. First, PAZ-749 acts by direct binding to PAI-1, thus, blocking the active site of tPA and preventing the formation of SDS-stable tPA/PAI-1 complex. Second, PAZ-749 binding to PAI-1, promotes plasmin-mediated proteolytic degradation of PAI-1. The antithrombotic activity of PAI-1 was effective, even in the presence of vitronectin, with an *in vitro* IC₅₀ of 0.157 µM and 0.087 µM for tPA and uPA respectively (60). However, these
molecules failed to develop into drug molecules due to poor pharmacokinetic properties, thus cannot inhibit active PAI-1 in human plasma.

Wyeth developed PAZ-417 (structure unrevealed) with an *in vitro* IC$_{50}$ of 0.6 µM and in transgenic models of Alzheimer’s disease it was shown to reduce Aβ levels in both plasma and the brain (61). Even though PAZ-417 has undergone clinical evaluation, the results of these studies have not been reported.

![Image](I-13.png) ![Image](I-14.png)

**Figure 9: PAI-1 Inhibitors by Wyeth.**

Many research groups have shown interest in the design, synthesis and development of small molecule inhibitors of PAI-1. This also attracted our own research group to develop small molecule inhibitors of PAI-1 with better potency, improved solubility, and more “drug-like” scaffolds that even act against vitronectin-bound PAI-1.
In 2007, we began our journey with the collaboration of Dr. Daniel Lawrence from University of Michigan Medical School.

Five polyphenolic compounds were identified from a high-throughput screen of the MicroSource SPECTRUM library (62). Out of these five polyphenolic compounds, two were natural products that provided potential starting points for structure–activity relationship due to similar structural features. These two natural molecules are tannic acid (Figure 10) and EGCDG (I-18), a compound extracted from green tea (Figure 11).

![Tannic Acid](image)

**Figure 10: Tannic Acid**

Tannic acid (TA), a naturally occurring polyphenolic compound with gallate (a salt or ester of gallic acid) and bigallate moieties (Figure 10), has an IC$_{50}$ value of 7 nM versus PAI-1 and is nearly 1000-fold more potent than tiplaxtinin. Due in part to its large molecular weight of ~2000 Da and its tendency to form aggregates at micromolar concentrations, it failed to perform well in *ex vivo* studies (61).
EGCG (I-17) and EGCDG (I-18) (Figure 11) are two other molecules containing galloyl moieties with anti-PAI-1 activity (61). EGCG and EGCDG are found in green tea, but their isolation and purification is highly expensive and laborious. Based on these galloyl moieties present in the library hits, structurally similar compounds with varied number of gallate and core moieties were synthesized by our group.

Figure 12: PAI-1 Inhibitors structurally similar to Tannic Acid.

Based on the library hits, a set of analogues were synthesized and the activity was tested both in *in vitro* and *ex vivo* assays. These molecules were also tested against anti-
thrombin (ATIII), a closely related serpin, which indicates the specificity of the inhibitor (62).

CDE-066 (I-19) and CDE-004 (I-20) are two inhibitors (Figure 12) similar to tannic acid with 5 gallates instead of digallates and with low nanomolar activity. These results encouraged us to synthesize molecules with even fewer number of gallate moieties that retained their activity against PAI-1. Further, we also synthesized molecules with different linking units between the two gallate moieties to investigate the role of the linking unit. Figure 13 shows compounds with different linker units with their IC$_{50}$ values.

![Molecules](image)

**Figure 13: PAI-1 Inhibitors with ester or amide linkages**

Unfortunately, the ester linkages are likely prone to hydrolysis in the presence of esterases limiting their use further in vivo. These ester bonds are also likely to be cleaved in the acidic environment of the stomach. To overcome this problem, sulfonamide- and sulfonimide-based linking groups were chosen in place of the ester linking units. There
are many reasons to switch to a more stable linking unit; many approved drugs are sulfonamide- or sulfonimide-based (such as antibacterials) and these groups also allow for increased structural diversity and are likely to be more stable *in vivo*.

<table>
<thead>
<tr>
<th>CDE- Code</th>
<th>Structure</th>
<th>IC₅₀ Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>CDE-032</td>
<td><img src="image1" alt="Structure" /></td>
<td>IC₅₀ = 9.45 µM</td>
</tr>
<tr>
<td>CDE-132</td>
<td><img src="image2" alt="Structure" /></td>
<td>IC₅₀ = 1384 µM</td>
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<tr>
<td>CDE-140</td>
<td><img src="image3" alt="Structure" /></td>
<td>IC₅₀ = 318 µM</td>
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<td>CDE-119</td>
<td><img src="image4" alt="Structure" /></td>
<td>IC₅₀ = 6134 µM</td>
</tr>
<tr>
<td>CDE-135</td>
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<td>IC₅₀ = 104 µM</td>
</tr>
<tr>
<td>CDE-165</td>
<td><img src="image6" alt="Structure" /></td>
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</tr>
<tr>
<td>CDE-183</td>
<td><img src="image7" alt="Structure" /></td>
<td>(IC₅₀ = 0.18 µM)</td>
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</table>

**Table1:** Sulfonamide and Sulfonimide-based inhibitors
Table 1 shows some of the sulfonamide and sulfonimide-based compounds with varied chain length and different substitution patterns on the aromatic rings. It was subsequently determined that molecules with 3,4-dihydroxy substitution patterns on the aryl sulfonyl moieties and shorter linking units were found to have good IC$_{50}$ values (63). Several symmetric and non-symmetric versions of this class were also generated and the effect of breaking symmetry was observed. Non-symmetric versions tend to have reduced activity, and molecules with two aryl sulfonyl moieties with hydroxyl groups were found to increase anti-PAI-1 activity.

![Chemical Structure]

CDE-141 (IC$_{50}$ = 0.086 µM)

**Figure 14:** Structure which served as a basis for developing sulfonimide inhibitors with different hydroxy arrangements

CDE-141 (Figure 14) was synthesized by Nadine El-Ayache and displayed an IC$_{50}$ of 0.086 µM versus PAI-1 and proved to be an effective inhibitor while maintaining specificity for PAI-1 over the related serpin anti-thrombin III (ATIII) (64). This inhibitor served as a basis for my project to synthesize sulfonamide-based inhibitors with different hydroxyl arrangements on the aromatic ring. The number and positioning of hydroxyls was altered in order to determine the ideal substitution of the aromatic ring and its effect on the inhibitory activity of PAI-1.
References

2. Tertiary Hemostasis Home Page
   https://ahdc.vet.cornell.edu/clinpath/modules/coags/tertiary.htm
40. Berry, C. N.; Lunven, C.; Lechaire, I.; Girardot, C.; and O’Connor, S. E. Br. J. Pharmacol. 1998, 125, 29-34.
Chapter II

Effect of varying the number and position of hydroxyl groups on the aromatic ring of sulfonimide-based PAI-1 inhibitors

CDE-141

My initial target was to synthesize a series of compounds analogous to compound CDE-141 and to examine the effect of number and position of hydroxyl groups on the aromatic ring. Each of these compounds synthesized have the central linking unit in common, which has an octyl side chain and two sulfonyl moieties attached to two aromatic rings. Compounds with varied number of hydroxyl groups on the aromatic ring were synthesized to observe their selectivity and activity against PAI-1. The position of hydroxyl groups on the aromatic ring was also altered to change the electronic properties of the aromatic ring and thus affect the interaction between the inhibitor and the binding site on the target protein.
Scheme 1 outlines the general synthetic route of sulfonimide-based inhibitors. Octylamine was treated with differently substituted methoxybenzene sulfonyl chlorides in the presence of a triethylamine (TEA) to form the N-substituted aryl sulfonamides. The appropriate methoxybenzenesulfonyl chloride was treated with N-substituted aryl sulfonamides to form corresponding bis-aryl sulfonimides in the presence of NaH in DMF. Boron tribromide in dichloromethane was employed to achieve the deprotection of the resulting sulfonimides to give the final products and would then be tested for potency versus PAI-1.

\[ \text{Scheme 1. Reagents and conditions: (a) Octylamine, triethylamine, ethyl acetate, 65-85\%; (b) NaH (60\% dispersion in oil), DMF, ArSO}_2\text{Cl 50-95\%; (c) 1M BBr}_3 \text{ in CH}_2\text{Cl}_2, CH_2Cl_2, 0^\circ\text{C to rt, 0-63\%.} \]
Table 2: Comparison of potencies of sulfonamide-based analogues to hydrazide analogues. *Compounds synthesized by Nadine El Ayache. CDE-293i and CDE-294i were found to be impure with starting material in it. NT corresponds to ‘not tested’ as the compound exhibits poor inhibitory activity in pH 7.4 buffer and/or 1.5% BSA (bovine serum albumin).

Results and Conclusions:

Based on the results it was determined that aryl sulfonimides with a 3,4-dihydroxybenzene substitution are more potent against PAI-1 than the analogous sulfonimides that do not contain 3,4-dihydroxybenzene moieties. Sulfonimides with a single hydroxyl group at either the 3-position or the 4-position (CDE-293i and CDE-294i) proved to be less potent than those sulfonimides, which have hydroxyl groups at both the 3- and 4- positions (CDE-292 and CDE-224). CDE-141 contains 3,4-dihydroxy substituted aromatic rings on either side of the sulfonimide, with an IC₅₀ of 0.086 µM.
CDE-146 has a 3,4-dihydroxy-substituted benzene on one side of the sulfonimide and no hydroxyl groups on the other aromatic ring but with better activity than that of CDE-292 and CDE-224. This demonstrates that at least one 3,4-dihydroxybenzene group is required for single-digit micro-molar potency versus PAI-1. Employing the phenol moiety on either side at 3, 3’ or 3, 4’ positions (293i and CDE-294i) results in decreasing the activity by 33- to 50-fold when compared to CDE-146. The 4,4’-disubstituted compound could not be synthesized, as after demethylation the compound was found to be cleaved, which precluded testing. Unfortunately, all of the compounds in this series showed reduced activity in human plasma when compared to their activity in buffer solutions. This reduced activity might be due to the binding with plasma proteins like albumin, which prohibits the molecules from being available to inhibit PAI-1.

**Experimental Methods and Data**

![Chemical structure](image)

**3-methoxy-N-octylbenzenesulfonamide (II-3):** To a solution of 3-methoxybenzenesulfonyl chloride (0.400g, 1.93mmol) and triethylamine (0.674mL, 4.84mmol) in ethyl acetate (5 mL), was added dropwise octylamine (0.319mL, 1.935mmol), and the resulting solution was stirred overnight under nitrogen. The reaction mixture was filtered, and the filtrate was washed with 1N HCl (2x), dried over MgSO₄,
filtered and concentrated under vacuum to afford 0.485 g (83.8%) of II-3 as a white solid.

$^1$H NMR (CDCl$_3$, 400 MHz): $\delta$ 7.41 (m, 3H), 7.08 (ddd, $J$ = 7.9, 2.6, 1.3 Hz, 1H), 4.77 (s, 1H), 3.83 (d, $J$ = 0.9 Hz, 3H), 2.92 (td, $J$ = 6.8, 4.6 Hz, 2H), 1.42 (q, $J$ = 6.9 Hz, 2H), 1.31 (m, 8H), 0.89 (m, 3H).

![Chemical Structure]

3,4-dimethoxy-N-((3-methoxyphenyl)sulfonyl)-N-octylbenzenesulfonamide (II-5):

To a stirring solution of II-3 (0.150 g, 0.56 mmol) in DMF (1.94 mL) was added portionwise NaH (0.0246 g, 0.616 mmol; 60% dispersion in oil). After 30 min, 3,4-dimethoxybenzenesulfonyl chloride (0.132 g, 0.560 mmol) was added to the reaction mixture. After an additional 1 h, the reaction solution was poured into water (15 mL), forming a white solid. The solid was filtered and recrystallized from methanol to afford 0.157 g (63.1%) of II-5 as a white solid. $^1$H NMR (CDCl$_3$, 400 MHz) $\delta$ 7.59 (dd, $J$ = 8.7, 2.3 Hz, 1H), 7.55 (d, $J$ = 9.1 Hz, 1H), 7.52 (d, $J$ = 2.3 Hz, 2H), 7.41 (m, 1H), 7.12 (dd, $J$ = 8.2, 2.3 Hz, 1H), 6.91 (d, $J$ = 8.6 Hz, 1H), 3.94 (s, 3H), 3.92 (s, 3H), 3.84 (s, 3H), 3.60 (m, 2H), 1.62 (m, 2H), 1.20 (m, 10H), 0.84 (m, 3H).
3,4-dihydroxy-N-((3-hydroxyphenyl)sulfonyl)-N-octylbenzenesulfonamide (CDE-292): To a solution of II-5 (0.100 g, 0.200 mmol) in CH₂Cl₂ (2.0 mL) at 0 °C was added dropwise boron tribromide (2.0 mL, 1M in CH₂Cl₂) under nitrogen. The resulting solution was allowed to stir overnight and warmed to room temperature. The reaction was quenched with a few drops of water and the precipitate formed was filtered. The solid was triturated with ethyl acetate and the filtrate was evaporated, affording 0.046 g (50.3%) of CDE-292 as a white solid. ¹H NMR (CDCl₃, 400 MHz) δ 7.24 (m, 7H), 3.61 (t, J = 6.9 Hz, 2H), 1.65 (m, 2H), 0.85 (t, J = 6.9 Hz, 3H); ¹³C NMR (CDCl₃, 100 MHz) δ 156.76, 149.99, 144.03, 140.72, 130.46, 130.32, 122.08, 121.45, 119.49, 115.49, 115.01, 114.88, 43.46, 31.79, 30.07, 29.55, 29.19, 29.02, 26.66, 14.15.

Impurity: ¹H NMR (CDCl₃, 400 MHz) δ 7.24 (m, 3H), 2.91 (t, J = 7.1 Hz, 2H), 1.40 (m, 2H), 1.22 (m, 10H), 0.83 (t, J = 6.8 Hz, 3H); ¹³C NMR (CDCl₃, 100 MHz) δ 140.63, 120.35, 118.99, 113.83, 60.73, 49.82, 31.79, 29.55, 29.14, 29.08, 21.17, 14.24.
3.4-dihydroxy-N-((4-hydroxyphenyl)sulfonyl)-N-octylbenzenesulfonamide (CDE-224): Synthesized following the procedure described in the synthesis of CDE-292 to afford 0.057 g (62.9%) of CDE-224 as a white solid. $^1$H NMR (400 MHz, acetone-d$_6$) δ 9.52 (bs, 1H), 9.01 (bs, 1H), 8.74 (bs, 1H), 7.81 (m, 2H), 7.43 (d, $J$ = 2.3 Hz, 1H), 7.34 (dd, $J$ = 8.4, 2.3 Hz, 1H), 6.98 (m, 3H), 3.61 (t, $J$=7.8 Hz, 2H), 1.62 (quin, $J$ = 7.8 Hz, 2H), 1.23 (m, 10H), 0.85 (t, $J$ = 6.9 Hz, 3H); $^{13}$C NMR (100 MHz, acetone-D$_6$): δ 162.12, 150.46, 145.01, 131.02, 130.96, 130.64, 121.41, 115.51, 115.05, 115.00, 49.02, 31.65, 29.81, 28.84, 26.38, 22.45, 13.52.

3-hydroxy-N-((3-hydroxyphenyl)sulfonyl)-N-octylbenzenesulfonamide (CDE-293i): Synthesized following the procedure, described in the synthesis of CDE-292 to afford 0.571 g (47.5%) of CDE-293i as a white solid. The carbon NMR also showed an
impurity presumed to be the cleaved sulfonamide of CDE-293i. $^1$H NMR (CDCl$_3$, 400 MHz): δ 7.55 (s, 2H), 7.44 (m, 2H), 7.37 (m, 2H), 7.10 (dd, J = 8.2, 1.8 Hz, 2H), 3.66 (m, 2H), 1.68 (m, 2H), 1.20 (m, 10H), 0.85 (m, 3H); $^{13}$C NMR (CDCl$_3$, 100 MHz): δ 156.55, 140.42, 130.53, 121.75, 119.69, 114.86, 113.77, 50.03, 31.78, 30.03, 29.17, 26.62, 26.55, 22.68, 14.16.

Presumed Cleaved compound: 156.56, 130.60, 120.51, 119.27, 113.77, 50.03, 29.79, 29.50, 29.12, 29.07, 26.55, 22.68, 14.16.

3-hydroxy-N-((3-hydroxyphenyl)sulfonyl)-N-octylbenzenesulfonamide (CDE-294i):

Synthesized following the procedure described in the synthesis of CDE-292 to afford 0.065 g (45.1%) of CDE-294i as a yellow solid. The proton NMR is assumed to have a cleaved sulfonamide. The compound was tested without isolation of solely the desired product. $^{13}$C NMR (CDCl$_3$, 100 MHz): δ 161.26, 156.85, 140.61, 130.08, 130.52, 130.32, 120.34, 118.93, 115.98, 43.45, 31.78, 29.56, 29.14, 29.09, 26.57, 22.68, 14.16.
4-hydroxy-\(N\)-((4-hydroxyphenyl)sulfonyl)-\(N\)-octylbenzenesulfonamide (HBA-I-101):

An attempt was made to synthesize HBA-I-101 following the procedure for the synthesis of CDE-292. The product obtained is an oily liquid and the NMR did not clearly indicate the intended product, which could not be isolated by various purification methods.
Chapter III: Hydrazine-based small molecule inhibitors

Symmetric and non-symmetric versions of sulfonamide and sulfonimide-based polyphenolic inhibitors of PAI-1 failed to inhibit PAI-1 effectively in assays containing human plasma because of the binding to various other plasma proteins (albumins). Our collaborators from the University of Michigan carried out a new screen against a different library of compounds and identified compound I-21, which is active against PAI-1 with an IC$_{50}$ value of 36 µM in a plasma-based assay. This small molecule PAI-1 inhibitor is highly specific and very economical to synthesize. We have developed a set of structural analogues of this lead molecule and have determined how these structural changes would have a significant effect on inhibitor potency.

![I-21](image)

**I-21**

**Lead Molecule**

IC$_{50}$ = 36 µM in plasma

III-1: Effect of substituents on the aromatic ring of the hydrazine-based inhibitors:

In order to investigate the effect of substituents on the left hand side of the aromatic ring, four compounds were synthesized, CDE-305, CDE-339, CDE-340, and CDE-341. The appropriate amines were treated with ethylchlorooxoacetate in the presence of pyridine and CH$_2$Cl$_2$. The resulting compound was treated with aqueous NH$_2$NH$_2$ in ethanol to yield the appropriate hydrazine-based inhibitors.
Scheme 2. Reagents and conditions: (a) Ethylchlorooxoacetate (1.05 eq), pyridine, CH₂Cl₂, 0 °C to rt, 77–100%; (b) NH₂NH₂ (2 eq), ethanol, 71-80%. All undefined R-groups are hydrogen.

III-1: R₃=F, R₄=CH₃
III-2: R₃=CF₃, R₅=CF₃
III-3: R₃=F, R₅=F
III-4: R₂=CH₃, R₅=F

III-5: R₃=F, R₄=CH₃
III-6: R₃=CF₃, R₅=CF₃
III-7: R₃=F, R₅=F
III-8: R₂=CH₃, R₅=F

CDE-305: R₃=F, R₄=CH₃
CDE-339: R₃=CF₃, R₅=CF₃
CDE-340: R₃=F, R₅=F
CDE-341: R₂=CH₃, R₅=F

III-1, III-2, III-3, III-4 are the amines with different substituents on the aromatic ring. III-5, III-6, III-7, III-8 are the intermediates with the appropriate substituents.

These four compounds synthesized contain CH₃, CF₃, and fluorine as substituent groups on the aromatic ring at R₂, R₃, R₄, or R₅. The effect of these groups on the inhibitor potency was determined. Scheme 2 outlines the general synthetic strategy of these structural analogues. Table 3 shows the results of the biological assays conducted on the hydrazide molecules.
Table 3: Hydrazine analogues IC_{50} values versus PAI-1 in buffer (pH 7.4), buffer containing 1.5% BSA, and buffer containing human plasma.

<table>
<thead>
<tr>
<th>Compound</th>
<th>R_2</th>
<th>R_3</th>
<th>R_4</th>
<th>R_5</th>
<th>IC_{50} (µM)</th>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>pH 7.4 1.5% BSA Plasma</td>
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<td>CDE-339</td>
<td>H</td>
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<td>H</td>
<td>CF_3</td>
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<tr>
<td>CDE-340</td>
<td>H</td>
<td>F</td>
<td>H</td>
<td>F</td>
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</tr>
<tr>
<td>CDE-341</td>
<td>CH_3</td>
<td>H</td>
<td>H</td>
<td>F</td>
<td>900 1460 1868</td>
</tr>
</tbody>
</table>

Substitution with different groups on R_3, R_4, R_5 (CDE-305, CDE-339, CDE-340) of the aromatic ring were found to have the best activity of this group of analogues.

Substitution on R_2 and R_5 of the aromatic ring as in CDE-341 decreased the inhibitor potency by about 30-fold as compared to the compound CDE-305. The IC_{50} of CDE-305 (62 µM) was 1.7-fold less compared to the IC_{50} of the lead molecule.

**III-2: Imine-based PAI-1 inhibitors:**

While working on the hydrazine class of PAI-1 inhibitors, CDE-339 and CDE-340 gave rise to new compounds CDE-312 and CDE-313i on acetone wash. The hydrazide moiety reacted with acetone to form an imine-based PAI-1 inhibitor. CDE-306
was synthesized by cyclizing CDE-305 with CDI. The IC$_{50}$ of CDE-313i was found to be 490 µM, and compound CDE-312 lost its activity in plasma. The IC$_{50}$ of cyclized imine was found to be 405 µM. This shows that left hand aromatic ring plays a major role in determining the activity of PAI-1 irrespective of right side imine group.

![CDE-306 (IC$_{50}$= 405 µM)](image1)

III-3: Effect of replacing hydrazide moiety with different aromatic and alkyl substituted groups:

We were interested in studying the effect of replacing the terminal hydrazide (-NHNH$_2$) moiety from our lead molecule with different aromatic and alkyl-substituted groups.
Scheme 3 outlines the synthesis of hydrazine-based derivatives. (4-chloro-3-(trifluoromethyl)phenyl)methanamine was treated with ethylchlorooxooacetate in pyridine and CH$_2$Cl$_2$ and then hydrolyzed with NaOH. The resulting compound was then treated with the appropriate amine to yield hydrazide-based derivatives and the effect of replacing the hydrazide moiety on inhibition of PAI-1 was determined. We observed a complete loss of the activity against PAI-1, even in the buffer systems. From the assay results obtained, we concluded that compounds with an unsubstituted hydrazide moiety have much higher potency versus PAI-1 across different buffer systems as compared to compounds containing different aromatic and alkyl substituents in place of the hydrazide.

Scheme 3. Reagents and conditions: (a) Ethylchlorooxooacetate (1.0eq), pyridine, CH$_2$Cl$_2$% (b) NaOH (c) RNHNH$_2$•HCl (1.3eq), NMM, HOBT, EDC, CH$_2$Cl$_2$. 
Table 4: Lead molecule with different groups on the right hand side of the molecule.

**III-4: Effect of different substituents on “left hand” aromatic ring and varying the chain length between the carbonyl group and a 3,4-dihydroxy aromatic ring.**

From the above results we noticed that removal of hydrazide moiety from the new class of inhibitors results in decreased or loss in activity against PAI-1. Hence, we were interested in synthesizing PAI-1 inhibitors with a different moiety on the right hand side.
and various halogen substituted amines on the left hand side of new class of PAI-1 inhibitors.

From our previous studies, we had PAI-1 inhibitors with catechol moieties (3,4-dihydroxybenzene) with different linking groups such as sulfonamides and esters that were effective in buffer but significantly lost activity in plasma. We thus decided to synthesize molecules with catechol moiety on the right side of our new class of inhibitors and thus see the effect on PAI-1 inhibition. CDE-330 was synthesized by Naga Guntaka, and exhibited an IC$_{50}$ value of 116 µM in plasma.

![CDE-330](image)

Following CDE-330, we synthesized various set of analogues with different halogen substituted benzylamines. I was interested in synthesizing molecules with varied chain length between the carbonyl and the catechol moiety on the right hand side to observe the effect of tether length on activity against PAI-1. Table 4 shows the assay results of structural analogues of CDE-330.

![Scheme 4](image)

**Scheme 4:** Synthesis of structural analogues of CDE-330.
<table>
<thead>
<tr>
<th>Compound</th>
<th>n</th>
<th>R₃</th>
<th>R₄</th>
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</table>

**Table 5:** Assay results of structural analogues of CDE-330.

CDE-366 is similar to the inhibitor CDE-390, but has a disubstituted amide nitrogen on the left hand side and was tested for PAI-1 inhibition. The IC₅₀ value of CDE-390 was 1.5-fold less potent than CDE-390 in buffer but both of these molecules lost their activity in plasma.
III-5: Effect of replacing amide with sulfonamide:

We were interested to study the effect of replacing the amide with a sulfonamide. The activity of CDE-303 was found to be almost 13 fold more potent than CDE-348 in 7.4 buffer, but similar to our previous sulfonamides CDE-303 lost activity in plasma. CDE-303 is synthesized as shown in Scheme 5.

Results and Conclusions:

The results obtained from the different sets of compounds suggested that molecules with hydrazide moiety on the right hand side exhibited an optimal activity of PAI-1 irrespective of the different substituents on the left hand aromatic ring. Replacing the terminal NHNH₂ with -NHNHR or -NHR diminishes the inhibitory activity against PAI-1. This data indicates that the hydrazide is likely interacting with PAI-1 and is important in inhibiting its activity.

The data also indicates that the inhibitory activity of 3,4-dihydroxybenzene substituted PAI-1 inhibitors varies with different spacer lengths while holding the substitution pattern constant and is also altered by different substitutions on the left hand aromatic ring while holding the spacer length constant.

Experimental Methods and Data

Ethyl 2-((3-fluoro-4-methylbenzyl) amino)-2-oxoacetate (III-5): To a solution of 3-fluoro-4-methyl-benzylamine (0.260 g, 1.87 mmol) and pyridine (0.30 mL, 3.66 mmol) in 5 mL of CH₂Cl₂ stirred in an ice bath, ethylchlorooxacetate (0.21 mL, 1.92 mmol) was added dropwise and the resulting mixture was allowed to stir overnight at room temperature. The reaction mixture was dissolved in 30 mL of EtOAc and washed with 0.1 N HCl (2x) and saturated aqueous NaHCO₃ (2x), and dried with anhydrous MgSO₄, filtered, and concentrated to afford 0.508 g (88%) of III-5 as a pale yellow solid.
NMR (CDCl$_3$, 400 MHz) $\delta$ 7.35 (s, 1H), 7.14 (t, $J = 7.9$ Hz, 1H), 6.95 (t, $J = 7.2$ Hz, 2H), 4.46 (d, $J = 6.1$ Hz, 2H), 4.35 (q, $J = 7.2$ Hz, 2H), 2.25 (s, 2H), 1.38 (t, $J = 7.1$ Hz, 3H).

$N$-(3-fluoro-4-methylbenzyl)-2-hydrazinyl-2-oxoacetamide (CDE-305): A solution of III-5 (0.508 g, 2.08 mmol) and hydrazine hydrate (0.26 mL, 4.17 mmol, ~50% in H2O) in 4.08 mL of ethanol was stirred overnight at room temperature. The reaction mixture was filtered and the solid was washed with ethanol. The solid sample was collected and dried in vacuo to provide 0.386 g (82.5%) of CDE-302 as a white solid. $^1$H NMR (CDCl$_3$, 400 MHz) $\delta$ 8.37 (m, 1H), 7.62 (m, 1H), 7.13 (m, $J = 7.3$ Hz, 1H), 6.93 (m, 2H), 4.44 (d, $J = 6.4$ Hz, 2H), 3.93 (bs, 2H), 2.25 (d, $J = 1.9$ Hz, 3H); $^{13}$C NMR (acetone-$d_6$, 100MHz) $\delta$ 161.3 (d, $J = 200$ Hz), 155.02, 155.02, 138.75 (d, $J = 7$ Hz), 131.52 (d, $J = 6$ Hz), 123.35 (d, $J = 3$ Hz), 114.28 (d, $J = 23$ Hz), 114.05 (d, $J = 23$ Hz), 42.24, 13.31 (d, $J = 4$ Hz). HRMS, DART calcd. for C$_{17}$H$_{22}$N$_1$O$_2$ [M+H]$^+$ 272. 165054, found: 271.164.

$N$-(3,5-bis(trifluoromethyl)benzyl)-2-hydrazinyl-2-oxoacetamide (CDE-339): The procedure for the synthesis of CDE-302 was followed with appropriate modifications, which afforded 0.056 g (57.2%) of CDE-339 as a white solid. $^1$H NMR (DMSO-$d_6$, 400 MHz) $\delta$ 10.05 (bs, 1H), 9.44 (t, $J = 6.4$ Hz, 1H), 7.91 (m, 3H), 4.50 (bs, 2H), 4.45 (d, $J =$
6.4 Hz, 2H); $^{13}$C NMR (DMSO-$d_6$, 100 MHz) δ 160.16, 157.68, 142.32, 130.11 (q, $J = 33$ Hz), 128.30, 123.30 (q, $J = 271$ Hz), 120.76, 41.46. HRMS, DART calcd. for C$_{11}$H$_9$F$_6$N$_3$O$_2$ [M+H]+ 330.06773, found: 330.06570.

$N$-(3,5-difluorobenzyl)-2-hydrazinyl-2-oxoacetamide (CDE-340): The procedure for the synthesis of CDE-302 was followed with appropriate modifications, which afforded 0.038 g (63.2%) of CDE-340 as a white solid. $^1$H NMR (DMSO-$d_6$, 400 MHz) δ 10.02 (bs, 1H), 9.30 (t, $J = 6.4$ Hz, 1H), 7.06 (tt, $J = 2.3$ Hz, 9.2, Hz, 1H), 6.92 (m, 2H), 4.50 (bs, 2H), 4.29 (d, $J = 6.4$ Hz, 2H); $^{13}$C NMR (DMSO-$d_6$, 100 MHz) δ 162.8 (dd, $J = 245$ Hz), 162.8 (dd, $J = 245$ Hz), 160.63, 158.38, 144.09 (t, $J = 9.5$ Hz), 110.90 (dd, $J = 18$ Hz), 110.73 (dd, $J = 18$ Hz), 102.86 (t, $J = 21$ Hz), 42.07. HRMS, DART calcd. for C$_9$H$_9$F$_2$N$_3$O$_2$ [M+H]+ 230.07411 found: 230.07359.

$N$-(5-fluoro-2-methylbenzyl)-2-hydrazinyl-2-oxoacetamide (CDE-341): The procedure for the synthesis of CDE-302 was followed with appropriate modifications, which afforded 0.047 g (76.7%) of CDE-341 as a white solid. $^1$H NMR (DMSO-$d_6$, 400 MHz) δ 10.01 (bs, 1H), 9.18 (t, $J = 6.4$ Hz, 1H), 7.14 (dd, $J = 5.9$ Hz, 1H), 6.92 (m, 2H), 4.50 (bs, 2H), 4.25 (d, $J = 6.3$ Hz, 2H), 2.22 (s, 3H); $^{13}$C NMR (DMSO-$d_6$, 100 MHz) δ 162.38, 159.57 (d, $J = 201$ Hz), 159.98, 139.47 (d, $J = 6.6$ Hz), 132.03 (d, $J = 7.6$ Hz),
131.83, 131.80, 114.29 (d, J = 21 Hz), 113.83 (d, J = 21 Hz), 40.40, 18.45. HRMS, DART calcd. for C_{10}H_{12}FN_{3}O_{2} [M+H]+ 226.09918, found: 226.09979.

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\text{N-(3,5-bis(trifluoromethyl)benzyl)-2-oxo-2-(2-(propan-2-ylidene)hydrazinyl)acetamide (CDE-312):} \quad \text{The liquid filtrate obtained during the extraction of CDE-339 was washed with acetone, resulted in the formation of CDE-312, which was dried and concentrated in vacuo to afford 0.039 g (8.6%) of yellowish white solid.} \]

\[\text{^1H NMR (DMSO-d}_6, 400 MHz) \delta 10.58 (bs, 1H), 9.62 (s, 1H), 7.96 (s, 3H), 4.49 (d, J = 6.4 Hz, 2H), 1.96 (s, 3H), 1.86 (s, 3H); ^{13}C NMR (DMSO-D}_6, 100 MHz) \delta 163.71, 160.94, 155.90, 142.68, 130.70 (q, J = 33 Hz), 128.95, 121.41, 42.23, 25.60, 18.25. HRMS, DART calcd. for C_{14}H_{13}F_{6}N_{3}O_{2} [M+H]+ 370.09901 found: 370.10141.}\]

\[
\text{N-(3,5-difluorobenzyl)-2-oxo-2-(2-(propan-2-ylidene)hydrazinyl)acetamide (CDE-313i):} \quad \text{The liquid filtrate obtained during the extraction of CDE-340 was washed with acetone, resulted in the formation of CDE-313i, which was dried and concentrated in vacuo to afford 0.093 g (26.4%) of yellowish white solid.} \]

\[\text{^1H NMR (DMSO-d}_6, 400 MHz) \delta 10.55 (bs, 1H), 9.51 (t, J = 6.4 Hz, 1H), 7.07 (m, 1H), 6.96 (m, 2H), 4.32 (d, J =} \]
6.4 Hz, 2H), 1.96 (s, 3H), 1.87 (s, 3H). HRMS, DART calcd. for C_{12}H_{13}F_{2}N_{3}O_{2} [M+H]^+ 270.10542, found: 270.09991.

N-(3-fluoro-4-methylbenzyl)-6-oxo-5,6-dihydro-4H-1,3,4-oxadiazine-2-carboxamide (CDE-306): To a solution of CDE-305 (0.100 g, 0.44 mmol) in THF (7.4 mL) was added CDI (0.086 g, 0.53 mmol) and the reaction mixture was allowed to stir for 24 h at room temperature. The reaction mixture was quenched with 1N HCl and extracted with ethyl acetate, followed by washing with brine, dried over MgSO₄, filtered and concentrated in vacuo. The solid obtained was triturated with acetone, filtered and dried in vacuo to afford 0.014 g (12.1%) of CDE-306 as a pale yellow solid. $^1$H NMR (acetone-$d_6$, 400 MHz) δ 8.47 (bs, 1H), 7.18 (t, $J = 7.8$ Hz, 1H), 7.07 (m, 2H), 4.51 (d, $J = 1$ Hz, 2H), 4.45 (m, 1H), 2.20 (d, $J = 1.7$ Hz, 3H). $^{13}$C NMR (acetone-$d_6$, 100 MHz) δ 161.20 (d, $J = 242$ Hz), 153.59, 153.37, 148.99, 138.63 (d, $J = 8$ Hz), 131.52 (d, $J = 5$ Hz), 123.37 (d, $J = 3$ Hz), 123.34 (d, $J = 18$ Hz), 114.13 (d, $J = 22$ Hz), 42.08 (d, $J = 11$ Hz), 13.30 (d, $J = 4$ Hz). HRMS, DART calcd. for C_{11}H_{10}FNO_{3} [M+H]^+ 252.07844, found: 252.07581.
**N-(4-chloro-3-(trifluoromethyl)benzyl)-2-oxo-2-(2-phenylhydrazinyl)acetamide (CDE-342):** To a solution of 2-((4-chloro-3-(trifluoromethyl)benzyl)amino)-2-oxoacetic acid (0.106 g, 0.376 mmol), phenylhydrazine hydrochloride (0.71 g, 0.49 mmol), N-methyl morpholine (0.05 mL, 0.49 mmol) and HOBT•H₂O (0.076 g, 1.5 mmol) in 5 mL of dry CH₂Cl₂ at 0°C, EDC•HCl (0.095 g, 1.65 mmol) was added portionwise and the reaction mixture was stirred overnight at room temperature. The reaction mixture was dissolved in 30 mL of EtOAc and washed with 1 N HCl (2x) and saturated aqueous NaHCO₃ (2x), and the organic phase was dried with anhydrous MgSO₄, filtered, and concentrated *in vacuo*. The solid obtained was triturated with chloroform, filtered and dried *in vacuo* to afford 0.077 g (55.5%) of **CDE-342** as a pale yellow solid. ¹H NMR (DMSO-d₆, 400 MHz) δ 10.59 (s, 1H), 9.45 (t, J = 6.3 Hz, 1H), 7.76 (s, 1H), 7.67 (d, J = 8.3 Hz, 1H), 7.57 (d, J = 8.4 Hz, 1H), 7.15 (m, 2H), 6.68 (m, 3H), 4.38 (d, J = 6.4 Hz, 2H), 4.27 (d, J = 6.5 Hz, 2H). ¹³C NMR (DMSO-d₆, 100 MHz) δ 160.01, 159.74, 148.34, 138.81 133.24, 131.62, 129.71, 128.65, 127.49 (d, J= 31 Hz), 126.86 (d, J=8 Hz), 124.01 (d, J=271 Hz), 118.78, 112.31, 41.95. HRMS, DART calcd. for C₁₆H₁₃ClF₃N₃O₂ [M+H]⁺ 372.07267, found: 372.07269.

![Chemical Structure](image)

**N-(4-chloro-3-(trifluoromethyl)benzyl)-2-(2-(2,4-difluorophenyl)hydrazinyl)-2-oxoacetamide (CDE-327):** Following the same procedure as described in the synthesis of **CDE-342**, substituting 2,4-difluorophenylhydrazine hydrochloride for
phenylhydrazine hydrachloride afforded 0.075 g (58.6%) of CDE-327 as a white solid.

$^1$H NMR (DMSO-$d_6$, 400 MHz) δ 9.48 (t, $J = 6.4$ Hz, 1H), 7.75 (d, $J = 1.8$ Hz, 1H), 7.70 (d, $J = 7.8$ Hz, 1H), 7.68 (d, $J = 8.3$ Hz, 1H), 7.57 (d, $J = 8.2$ Hz, 1H), 7.10 (m, 1H), 6.85 (m, 1H), 6.71 (m, 1H), 4.37 (d, $J = 6.4$ Hz, 2H), 4.01 (d, $J = 8$ Hz, 1H); $^{13}$C NMR (DMSO-$D_6$, 100 MHz) δ 164.30 (dd, $J = 295$), 162.40 (dd, $J = 295$), 160.43, 160.37, 139.33, 133.82, 132.18, 129.73, 127.89, 127.46 (q, $J = 30$ Hz), 124.75 (q, $J = 2.8$ Hz), 122.75 (q, $J = 270$ Hz), 114.90, (dd, $J = 12.4$ Hz), 111.57, (dd, $J = 2.4$ Hz), 104.23, (t, $J = 2.6$ Hz), 41.94. HRMS, DART calcd. for C$_{16}$H$_{11}$ClF$_5$N$_3$O$_2$ [M+H]+408.05382, found: 408.05249.

$N^1$-(4-chloro-3-(trifluoromethyl)benzyl)-$N^2$-(4-fluorobenzyl)oxalamide (CDE-338):

Following the same procedure as described in the synthesis of CDE-342 with appropriate modifications afforded 0.045 g (32.1%) of CDE-338 as a white solid. $^1$H NMR (DMSO-$d_6$, 400 MHz) δ 9.41 (t, $J = 6.4$ Hz, 1H), 9.31 (t, $J = 6.4$ Hz, 1H), 7.74 (d, $J = 1.8$ Hz, 1H), 7.64 (d, $J = 8.3$ Hz, 1H), 7.53 (d, $J = 8.3$ Hz, 1H), 7.25 (m, 2H), 7.09 (m, 2H), 4.35 (d, $J = 6.4$ Hz, 2H), 4.27 (d, $J = 6.5$ Hz, 2H); $^{13}$C NMR (DMSO-$d_6$, 100 MHz) δ 162.40 (d, $J = 295$), 159.94, 159.42, 136.16, 132.47, 132.26, 131.01, 129.93, 128.56, 127.08 (q, $J = 30$ Hz), 125.93 (q, $J = 4.8$ Hz), 121.56 (q, $J = 271$ Hz), 115.67, 42.25, 42.08. HRMS, DART calcd. for C$_{17}$H$_{13}$ClF$_4$N$_2$O$_2$ [M+H]+389.06801, found: 389.06589.
$N^4$-(4-chloro-3-(trifluoromethyl)benzyl)$-N^2$-(4-(trifluoromethyl)benzyl)oxalamide (CDE-337): Following the same procedure as described in the synthesis of CDE-342, substituting 4-(trifluoromethyl)benzyamine for phenylhydrazine hydrochloride afforded 0.026 g (16 %) of CDE-337 as a white solid. $^1$H NMR (DMSO-d$_6$, 400 MHz) $\delta$ 9.43 (t, $J$ = 5.9 Hz, 2H), 7.74 (d, $J$ = 1.8 Hz, 1H), 7.64 (d, $J$ = 6.8 Hz, 4H), 7.55 (d, $J$ = 7.8 Hz, 1H), 7.43 (s, 1H), 4.37 (t, $J$ = 6.4 Hz, 4H). $^{13}$C NMR (CDCl$_3$, 100 MHz) $\delta$ 159.78, 159.62, 141.12, 136.05, 132.24, 132.01, 128.81(q, $J$=31Hz), 128.09, 128.08, 127.68(q, $J$= 3.8Hz), 127.04(q, $J$= 31Hz), 125.71(q, $J$= 3.8Hz), 123.31 (q, $J$= 257Hz), 121.31 (q, $J$= 261 Hz), 43.35, 42.84. HRMS, DART calcd. for C$_{18}$H$_{13}$ClF$_6$N$_2$O$_2$ [M+H]$^+$ 439.06480, found: 439.05991

$N^1$-(4-chloro-3-(trifluoromethyl) benzyl)$-N^2$-(3,4-dimethoxyphenyl)oxalamide (CDE-326): Following the same procedure as described in the synthesis of CDE-342 substituting 3,4-dimethoxyaniline for phenylhydrazine hydrochloride afforded 0.097 g (63.1%) of CDE-326 as a white solid. $^1$H NMR (CDCl$_3$, 400 MHz) $\delta$ 9.12 (bs, 1H), 7.95 (t, $J$ = 5.7 Hz, 1H), 7.60 (m, 1H), 7.49 (d, $J$ = 8.2 Hz, 1H), 7.42 (dd, $J$ = 8.3, 1.7 Hz, 1H), 7.35 (d, $J$ = 2.4 Hz, 1H), 7.06 (dd, $J$ = 8.6, 2.8, Hz, 1H), 6.84 (d, $J$ = 8.7 Hz, 1H), 4.56 (d,
$J = 6.4 \text{ Hz}, 2\text{H}), 3.88 (\text{bs, 6H})$ $^{13}\text{C NMR (CDCl}_3, 100 \text{ MHz}) \delta 167.14, 166.07, 152.27, 151.73, 139.68, 132.01, 130.08, 129.77, 127.06, 128.30 (q, J = 36 \text{ Hz}), 126.30 (q, J = 6.4 \text{ Hz}), 122.30 (q, J = 240 \text{ Hz}), 112.04, 111.46, 104.30, 56.17, 56.06, 33.82. \text{ HRMS, DART calcd. for } C_{18}H_{16}ClF_3N_2O_4 [\text{M+H}^+] 417.08290, \text{ found: 417.08151}

![Chemical Structure](image)

$N^1$-(benzyloxy)-$N^2$-(4-chloro-3-(trifluoromethyl)benzyl)oxalamide(CDE-325): Following the same procedure as described in the synthesis of CDE-342 substituting O-benzylhydroxylamine for phenylhydrazine hydrochloride afforded 0.051g (41.6%) of CDE-325 as a white solid. 1H NMR (CDCl$_3$, 400 MHz) $\delta$ 9.51 (t, $J = 6.3 \text{ Hz}, 1\text{H}), 7.72 (s, 1\text{H}), 7.57 (d, J = 8.3 \text{ Hz}, 2\text{H}), 7.37 (d, J = 8.4 \text{ Hz}, 2\text{H}), 7.27 (m, 4\text{H}), 4.96 (s, 2\text{H}) 4.47 (d, J = 6.4 \text{ Hz}, 2\text{H}). \text{ $^{13}$C NMR (DMSO-}d_6, 100 \text{ MHz}) \delta 162.58, 158.31, 139.20, 134.90, 133.80, 132.20, 129.40, 128.90, 128.80 (q, J=31 \text{ Hz}), 127.49, 127.44, 126.50 (q, J=270 \text{ Hz}), 126.98 (q, J=3.8 \text{ Hz}), 78.54, 40.28 \text{ HRMS, DART calcd. for } C_{17}H_{14}ClF_3N_2O_3 [\text{M+H}^+] 387.07234, \text{ found: 387.07431.}

![Chemical Structure](image)

$N^1$-(4-chloro-3-(trifluoromethyl)benzyl)-$N^2$-methoxy-$N^2$-methyloxalamide(CDE-328): Following the same procedure as described in the synthesis of CDE-342 substituting $N,O$-dimethylhydroxylamine for phenylhydrazine hydrochloride afforded
0.081 g (69.2%) of **CDE-328** as a white solid. $^1$H NMR (DMSO-$d_6$, 400 MHz) δ 9.29 (t, $J = 5.9$ Hz, 1H), 7.70 (m, 2H), 7.55 (d, $J = 8.2$ Hz, 1H), 4.39 (d, $J = 5.9$ Hz, 2H), 3.62 (bs, 3H), 3.11 (bs, 3H); $^{13}$C NMR (DMSO-$d_6$, 100 MHz) 165.51, 164.27, 139.47, 133.35, 132.18, 129.61, 124.79 (q, $J = 9$ Hz), 126.96, (q, $J = 38$ Hz), 123.40 (q, $J = 272$ Hz), 62.41, 40.46, 39.63. HRMS, DART calcd. for C$_{12}$H$_{12}$ClF$_3$N$_2$O$_3$ [M+H]$^+$ 325.0568, found: 325.05920.

![Chemical structure](image)

$N^1$-(4-chloro-3-(trifluoromethyl)benzyl)-$N^2$,$N^2$-diisobutyloxalamide (CDE-343):

Following the same procedure as described in the synthesis of **CDE-342** substituting diisobutylamine for phenylhydrazine hydrochloride to afforded 0.096 g (75.3%) of **CDE-343** as a white solid. $^1$H NMR (CDCl$_3$, 400 MHz) δ 8.02 (t, $J = 6.4$ Hz, 1H), 7.59 (d, $J = 6.5$ Hz, 1H), 7.43 (m, 2H), 4.45 (d, $J = 6.3$ Hz, 2H), 3.17 (d, $J = 7.5$ Hz, 4H), 2.05 (m, 2H), 0.87 (d, $J = 6.7$ Hz, 12H); $^{13}$C NMR (CDCl$_3$, 100 MHz) δ 162.67 162.08, 136.95, 132.10, 131.28, 128.72 (q, $J = 32$ Hz), 126.82 (q, $J = 4$ Hz), 123.26 (q, $J = 272$ Hz), 55.58, 54.51, 42.17, 27.90, 26.40, 20.08, 19.73. HRMS, DART calcd. for C$_{18}$H$_{24}$ClF$_3$N$_2$O$_2$ [M+H]$^+$ 393.15565, found: 393.15509.
**N-(4-bromobenzyl)-3,4-dihydroxybenzamide (CDE-360):** To a solution of 3,4-dihydroxybenzoic acid (0.150 g, 0.97 mmol), 4-bromobenzylamine (0.217 g, 1.16 mmol), N-methyl morpholine (0.12 mL, 1.16 mmol) and HOBT•H₂O (0.178 g, 1.16 mmol) in 7 mL of dry CH₂Cl₂ and 1 mL of DMF at 0° C, EDC•HCl (0.222 g, 1.16 mmol) was added portionwise and the reaction mixture was stirred overnight at room temperature. The reaction mixture was dissolved in 4:1 mixture of ethyl acetate and hexane, washed with 0.1 N HCl (3x), followed by saturated aqueous NaHCO₃ (3x), and finally with brine solution. The organic phase was dried with anhydrous MgSO₄, filtered, and concentrated in vacuo. The solid obtained was triturated with chloroform, filtered and dried in vacuo to afford 0.138 g (44 %) of CDE-360 as a pale yellow solid. **¹H NMR** (DMSO-d₆, 400 MHz) δ 8.69 (t, J = 6.0 Hz, 1H), 7.46 (m, 2H), 7.27 (d, J = 2.3 Hz, 1H), 7.20 (m, 3H), 6.72 (d, J = 9.1 Hz, 1H), 4.33 (d, J = 5.9 Hz, 2H); **¹³C NMR** (DMSO-d₆, 100 MHz) δ 166.14, 148.47, 144.86, 139.58, 131.07, 129.40, 125.42, 119.58, 119.10, 115.12, 114.86, 39.71.

**N-(4-chlorobenzyl)-3,4-dihydroxybenzamide (CDE-361):** Following the same procedure as described in the synthesis of CDE-360 substituting 4-chlorobenzylamine for 4-bromobenzylamine afforded 0.138 g (51%) of CDE-361 as a white solid. **¹H NMR**
(DMSO-$d_6$, 400 MHz) δ 8.69 (t, $J = 5.9$ Hz, 1H), 7.30 (m, 4H), 7.19 (dd, $J = 8.2$, 2.1 Hz, 2H), 6.72 (d, $J = 8.2$ Hz, 1H), 4.35 (d, $J = 5.9$ Hz, 2H); $^{13}$C NMR (DMSO-$d_6$, 100 MHz) δ 166.14, 148.47, 144.87, 139.15, 131.13, 129.02, 128.15, 125.43, 119.10, 115.12, 114.86, 39.71. HRMS, DART calcd. for C$_{14}$H$_{12}$ClNO$_3$ [M+H]+ 278.05839, found: 278.05759.

3,4-dihydroxy-N-(4-(trifluoromethyl)benzyl)benzamide (CDE-363): Following the same procedure as described in the synthesis of CDE-360 substituting 4-(trifluoromethyl)benzylamine for 4-bromobenzylamine afforded 0.1571 g (52%) of CDE-363 as a white solid. $^1$H NMR (DMSO-$d_6$, 400 MHz) δ 8.76 (t, $J = 5.5$ Hz, 1H), 7.64 (d, $J = 8.2$ Hz, 2H), 7.46 (d, $J = 7.8$ Hz, 2H), 7.28 (d, $J = 1.8$ Hz, 1H), 7.21 (dd, $J = 8.2$, 1.8 Hz, 1H), 6.73 (d, $J = 8.2$ Hz, 1H), 4.45 (d, $J = 5.5$ Hz, 2H); $^{13}$C NMR (DMSO-$d_6$, 100 MHz) δ 166.26, 148.56, 145.00, 144.90, 127.78, 127.33 (q, $J = 31$ Hz), 125.31, 125.11 (q, $J = 4$ Hz), 124.38 (q, $J = 271$ Hz), 119.03, 115.12, 114.88, 39.70. HRMS, DART calcd. for C$_{14}$H$_{12}$ClNO$_3$ [M+H]+ 312.08476, found: 312.08551.

3,4-dihydroxy-N-(3-(trifluoromethyl)benzyl)benzamide (CDE-364): Following the same procedure as described in the synthesis of CDE-360 substituting 3-(trifluoromethyl)benzylamine for 4-bromobenzylamine afforded 0.150 g (50 %) of CDE-
364 as a white solid. $^1$H NMR (DMSO-$d_6$, 400 MHz) δ 8.77 (t, $J = 4.9$ Hz, 1H), 7.53 (m, 5H), 7.23 (m, 1H), 6.74 (d, $J = 8.2$ Hz, 1H), 4.46 (d, $J = 5.5$ Hz, 2H). $^{13}$C NMR (DMSO-$d_6$, 100 MHz) δ 166.28, 148.56, 144.91, 141.63, 131.36, 129.32, 128.95 (q, $J = 31$ Hz), 125.66, 123.48 (q, $J = 4$ Hz), 123.39 (q, $J = 4$ Hz), 124.13 (q, $J = 236$ Hz), 119.03, 115.12, 114.88, 42.67. HRMS, DART calcd. for C$_{15}$H$_{12}$F$_3$NO$_3$ [M+H]$^+$ 312.08476, found: 312.08530.

3-(3,4-dihydroxyphenyl)-N-(4-fluoro-3-methylbenzyl)propanamide(CDE-358):

Following the same procedure as described in the synthesis of CDE-360 substituting 4-fluoro-3-methyl-benzylamine for 4-bromobenzylamine, and 3,4-dihydroxycinnamic acid for 3,4-dihydroxybenzoic acid afforded 0.103 g (62%) of CDE-358 as a white solid. $^1$H NMR (DMSO-$d_6$, 400 MHz) δ 8.21 (t, $J = 5.9$ Hz, 1H), 6.99 (m, 2H), 6.92 (dd, $J = 5.3$, 2.3 Hz, 1H), 6.57 (d, $J = 7.9$ Hz, 1H), 6.54 (d, $J = 2.1$ Hz, 1H), 6.39 (dd, $J = 5.9$, 1.8 Hz, 1H), 4.14 (d, $J = 5.9$ Hz, 2H), 2.62 (t, $J = 7.4$ Hz, 2H), 2.31 (t, $J = 6.9$ Hz, 2H), 2.15 (d, $J = 1.4$ Hz, 3H); $^{13}$C NMR (DMSO-$d_6$, 100 MHz) δ 171.45, 159.57 (d, $J = 239$ Hz), 144.96, 143.31, 135.34, 132.02, 130.27 (d, $J = 4.7$ Hz), 126.30 (d, $J = 7.6$ Hz), 123.75 (d, $J = 18$ Hz), 118.79, 115.76, 115.37, 114.55 (d, $J = 21.9$ Hz), 41.23, 37.38, 30.50, 14.11. HRMS, DART calcd. for C$_{17}$H$_{18}$FNO$_3$ [M+H]$^+$ 304.13490, found: 304.14120.
N-(4-chlorobenzyl)-3-(3,4-dihydroxyphenyl)propanamide (CDE-359): Following the same procedure as described in the synthesis of CDE-360 substituting 4-chlorobenzylamine for 4-bromobenzylamine, and 3,4-dihydroxycinnamic acid for 3,4-dihydroxybenzoic acid afforded 0.80 g (47.6%) of CDE-359 as a white solid.

$^1$H NMR (DMSO-$d_6$, 400 MHz) $\delta$ 8.25 (t, $J = 5.6$ Hz, 1H), 7.27 (m, 2H), 7.07 (d, $J = 8.3$ Hz, 2H), 6.58 (d, $J = 8.2$ Hz, 1H) 6.54 (d, $J = 1.8$ Hz, 1H), 2H), 6.39 (dd, $J = 7.8$ Hz, 1.8 Hz, 1H), 4.18 (d, $J = 5.9$ Hz, 2H), 2.62 (t, $J = 7.8$ Hz, 2H), 2.32 (t, $J = 7.5$ Hz, 2H). $^{13}$C NMR (DMSO-$d_6$, 100 MHz) $\delta$ 171.58, 145.00, 143.36, 138.64, 131.96, 131.12, 128.86, 128.10, 118.89, 115.84, 115.40, 41.73, 37.96, 30.56. HRMS, DART calcd. for C$_{16}$H$_{16}$ClNO$_3$ [M+H]$^+$ 306.08790, found: 306.09009.

N-(4-fluorobenzyl)-3,4-dihydroxy-N-methylbenzamide (CDE-366): Following the same procedure as described in the synthesis of CDE-360 substituting 4-fluoro-N-methyl-benzylamine for 4-bromobenzylamine afforded 0.091 g (46.4%) of CDE-366 as a white solid. $^1$H NMR (Acetone-$d_6$, 400 MHz,) $\delta$ 7.34 (s, 2H), 7.10 (td, $J = 6.6$, 3.3 Hz, 2H), 6.97 (d, $J = 1.7$ Hz, 1H), 6.87 (m, 2H), 4.63 (s, 2H), 2.89 (s, 3H). $^{13}$C NMR (acetone-$d_6$, 100 MHz) $\delta$ 171.04, 163.31 (d, $J = 242$ Hz), 146.68, 144.83, 144.73, 134.06, 129.59 (d, $J = 7$ Hz), 128.20 (d, $J = 3$ Hz), 119.49, 115.27 (d, $J = 21$ Hz), 114.90, 114.74,
114.73, 29.21. HRMS, DART calcd. for C_{15}H_{14}FNO_{3} [M+H]+ 276.10359, found: 276.10391.

![Chemical structure](image)

**N-(4-chloro-3-(trifluoromethyl)benzyl)-3,4-dimethoxybenzenesulfonamide (CDE-302):** To a solution of 4-chloro-3-(trifluoromethyl)benzylamine (0.100 g, 0.48 mmol) and triethylamine (0.126 g, 1.19 mmol) in 2 mL of ethyl acetate, 3,4-dimethoxybenzenesulfonyl chloride was added and the resulting mixture was stirred overnight. The reaction mixture was quenched with water and washed with 5:1 ethyl acetate: hexane, dried over MgSO_{4}, filtered and concentrated in vacuo to afford 0.083 g (42.6%) of CDE-302 as a white solid. ¹H NMR (CDCl₃, 400 MHz) δ 7.44 (bs, 1H), 7.42 (s, 1H), 7.39 (d, J=2.3 Hz 1H), 7.36 (dd, J = 8.2, 1.8 Hz, 1H), 7.26 (d, J=1.8 Hz, 1H), 7.25 (d, J= 1.8 Hz), 6.89 (dd, J = 8.2, 1.8 Hz, 1H), 4.17 (d, J = 6.4 Hz, 2H), 3.93 (s, 3H), 3.90 (s, 3H). ¹³C NMR (CDCl₃, 100 MHz) δ 152.97, 149.37, 135.75, 132.27, 131.78, 131.27, 127.01 (J=31 Hz), 126.94 (J=8 Hz), 123.97 (J=270 Hz), 121.23, 110.60, 109.49, 56.29, 46.27, HRMS, DART calcd. for C_{16}H_{15}ClF_{3}NO_{3}S [M+H]+ 410.04001, found: 410.04408.
**N-(4-chloro-3-(trifluoromethyl)benzyl)-3,4-dihydroxybenzenesulfonamide (CDE-303):** To a solution of CDE-302 (0.050 g, 0.122 mmol) in CH$_2$Cl$_2$ (1.2 mL) at 0 °C was added dropwise, boron tribromide (1.2 mL, 1M in CH$_2$Cl$_2$) under nitrogen. The resulting solution was allowed to stir overnight and warmed to room temperature. The reaction was quenched with a few drops of water, the precipitate that formed was filtered. The solid was triturated with ethyl acetate and the filtrate was evaporated, affording 0.036 g (78.2%) of CDE-303 as white solid. $^1$H NMR (CDCl$_3$, 400 MHz) $\delta$ 7.72 (bs, 1H), 7.63 (s, 1H), 7.61 (d, $J$=2.3 Hz 1H), 7.52 (dd, $J$ = 8.2, 1.8 Hz, 1H), 7.10 (d, $J$=2.3 Hz, 1H), 7.03 (d, $J$=8.3, 2.3 Hz, 1H), 6.78 (dd, $J$ = 8.2, 1.8 Hz, 1H), 3.98 (d, $J$ = 5.9 Hz, 2H), $^{13}$C NMR (DMSO-$d_6$, 100 MHz) $\delta$ 149.99, 145.92, 138.90, 133.67, 131.91, 130.94, 129.95, 127.30 ($J$=31 Hz), 125.46 ($J$=9 Hz), 122.45 ($J$=271 Hz), 119.38, 115.73, 114.27, 45.38. HRMS, DART calcd. for C$_{14}$H$_{11}$ClF$_3$ NO$_4$S [M+H]+ 382.00931, found: 382.01277.