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Design, synthesis and evaluation of small molecules as inhibitors of plasminogen activator inhibitor-1

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Design, Synthesis and Evaluation of Small Molecules as Inhibitors of Plasminogen Activator Inhibitor-1

by

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Abstract

Plasminogen activator inhibitor type-1 (PAI-1) is a member of the serine protease inhibitor (serpin) superfamily. Excessive levels of PAI-1 inhibit urokinase-type plasminogen activator (uPA) and tissue-type plasminogen activator (tPA), which regulates fibrinolysis as well as the development of different pathological diseases like obesity, metabolic syndrome, tumor invasion and metastasis, and coronary heart disease. Currently, there is no Food and Drug Administration approval for inactivating higher levels of PAI-1. Therefore, PAI-1 is considered an attractive drug target. Due to PAI-1's different structural conformations and multiple binding domains, development of PAI-1 inhibitors is a challenging situation. In this research study, we describe the synthesis and evaluation of novel low molecular weight amides containing various moieties, including *para*-chlorobenzyl, polyphenol, oxindole, or isatin-based units. By changing the architectural scheme of these compounds we hope to effectively change the potency of our inhibitors, and will be able to develop a structure-activity relationship that will allow us to design a more potent small molecule as a PAI-1 inhibitor. Therefore, the synthesis and structure-activity relationship of those novel small molecules are discussed in this paper.

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Abbreviations

μM	micromolar
AcOH	acetic acid
BBr_3	borontribromide
calcd	calculated
$CDCl_3$	deuterated chloroform
CH_2Cl_2	dichloromethane
DART	Direct Analysis in Real Time
DMSO-d_6	deuterated dimethylsulfoxide
DMF	dimethylformamide
EDC•HCl	1-ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride
EtOAc	ethyl acetate
EtOH	ethanol
H_2O	water
HCl	hydrochloric acid
HOBT	1-hydroxybenzotriazole
HRMS	high-resolution mass spectrometry
IC_{50}	half-maximal inhibitory concentration
J	coupling constant, in Hertz
KDa	kilodalton
MA	monoclonal antibodies
mg	milligram
$MgSO_4$	magnesium sulfate
MHz	megahertz
ml	milliliter
mmol	millimole
MS	mass spectrometry
NaH	sodium hydride
$NaHCO_3$	sodium bicarbonate
NMM	*N*-methylmorpholine
NMR	nuclear magnetic resonance
NOE	nuclear Overhauser effect
PAI-1	plasminogen activator inhibitor-1
RCL	reactive center loop
serpin	serine protease inhibitor
tPA	tissue-type plasminogen activator
THF	tetrahydrofuran
uPA	urokinase-type plasminogen activator

Chapter I: Plasminogen Activator Inhibitor-1 (PAI-1)

I-1: Serpin Superfamily

The serine protease inhibitor (serpin) protein superfamily[1] has been identified with over 1500 members in different living sources such as animals, poxviruses, plants, bacteria, and archaea.[2,3,4] The products of these serpin genes are well known for the inhibition of serine proteases, but not all of them perform inhibitory functions. The non-inhibitory serpins are involved in different mechanisms such as hormone transportation. Among the vast number of serpin members, 36 are identified as human coded functional proteins.[5] Given below are a few different types of human serpins, including their functions and dysfunctions in the human system (**Table 1**).

Table 1: Human serpins and their function / dysfunction.[2,6,7]

Serpin Type	Protease Target	Disease Type Related to Abnormal Levels
Antitrypsin (SERPINA1)	Neutrophil elastase inhibition in extracellular matrices	Low levels: emphysema
Plasminogen activator inhibitor-1(PAI-1)(SERPIN1)	tPA/uPA, thrombin, and plasmin inhibition in extracellular matrices	Bleeding abnormality related diseases
PAI-2 (SERPINB2)	uPA inhibition in intracellular matrices	Cancer
PAI-3 (SERPINA5)	Active protein C inhibition in extracellular matrices	Deep-vein thrombosis, pulmonary embolism
Antithrombin (SERPINC1)	Thrombin and factor Xa inhibition in extracellular matrices	Low levels: thrombosis
Protein Z-dependent proteinase inhibitor (SERPINA10)	Activated factor Z and XI inhibition in extracellular matrices	Low levels: Venous thromboembolic disease

In early work concerning the PAI proteins, it was difficult and confusing to distinguish between the three plasminogen activator inhibitors, PAI-1, PAI-2, and PAI-3. This was mainly due to the unavailability of proper assays.[8] Studies were later conducted on PAI-1 using specific assays, first identified[7] and solidified in 1984,[9] after which PAI-1 became of interest for further study. Among these plasminogen activator inhibitors, PAI-2 plays an important role during pregnancy. Under normal conditions, the presence of PAI-2 in plasma is minimal; during pregnancy, PAI-2 is secreted at higher levels and specifically inhibits urokinase-type plasminogen activator (uPA) and 2-chain tissue-type plasminogen activator (tPA).[8,10] In addition, excess levels of PAI-2 are correlated with breast cancer.[7] PAI-3, also called protein C inhibitor, is secreted in several tissues (liver, kidneys, prostate, testes and pancreas), and it plays a major role in fertilization and acts as a protease inhibitor in the male reproductive organs. In addition, PAI-3 forms a complex with uPA to play a pivotal role in anticoagulation in the protein C pathway in human plasma.[6,11] Due to our key interest in PAI-1 inhibition, this introduction discusses background studies of PAI-1 structure, its role in blood coagulation and in fibrinolysis, early PAI-1 inhibitors and their problems, and current research regarding PAI-1 inhibitors.

I-2: Plasminogen Activator Inhibitor Type 1 (PAI-1) – Background and Roles in Diseases.

Plasminogen activator inhibitor type-1 (PAI-1) is the most important and rapid inhibitor of uPA and tPA.[12] According to the studies by Dieval and coworkers, deficiency of PAI-1 leads to a delay in bleeding disorder (hemorrhage / hyperfibrinolysis)[13] due to the high activity of tPA and insignificant amount of detectable tPA/PAI-1 complexes.[14] Low levels of active PAI-1 are correlated with high activity of tPA/uPA because the presence of PAI-1 reduces the amount of active tPA/uPA by producing covalently bonded tPA/PAI-1 or uPA/PAI-1 complexes. At normal

physiological levels, PAI-1 is involved in multiple tasks, including fibrinolysis (the normal breakdown of blood clots), which is required for a well-functioning mammalian system. PAI-1's main function is to regulate fibrinolysis by inhibiting the conversion of inactive plasminogen into an active plasmin by tPA or uPA (**Figure 1**). Plasmin then reacts with fibrin clots and converts into its degradation products. In contrast, elevated levels of PAI-1 inhibit uPA/tPA, which leads to hypofibrinolysis, an abnormal increase in the level of blood clots.[15-17] Studies have shown that a number of blood related diseases are associated with these excessive levels of PAI-1.[18,19]

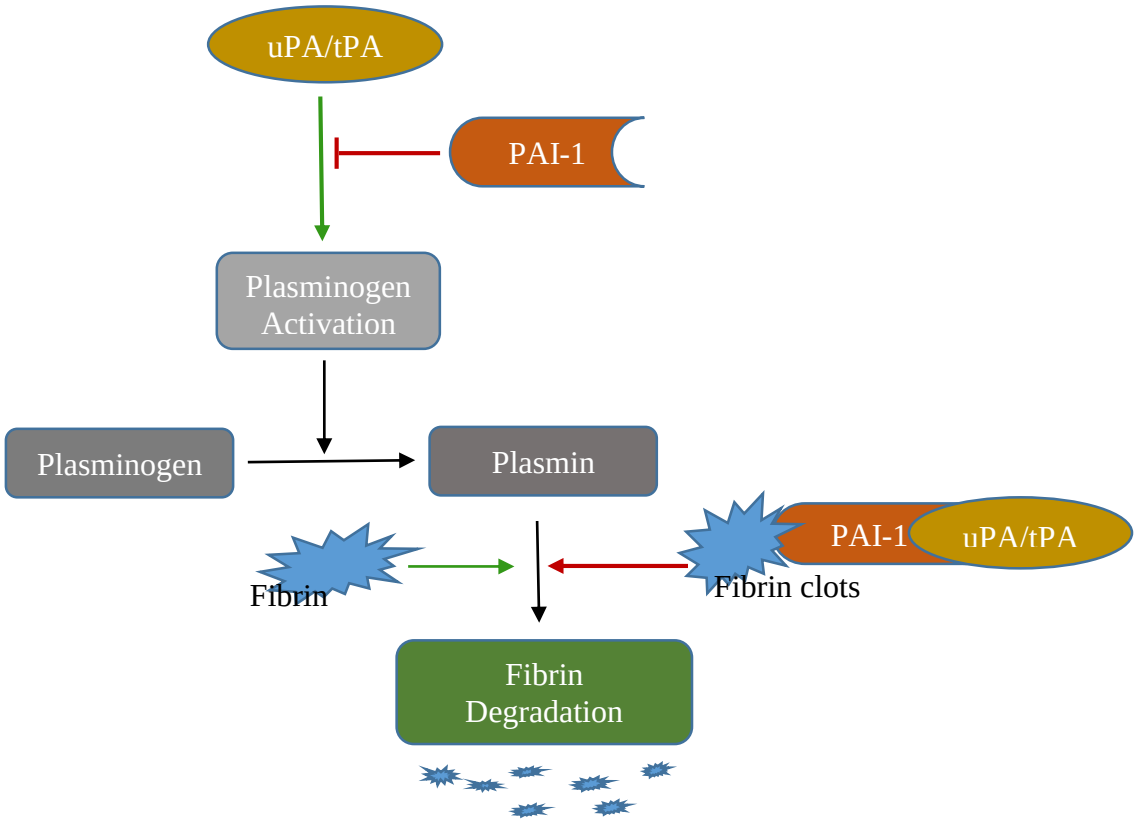


Figure 1: PAI-1's role in fibrinolysis. Green arrows denote stimulation and red arrows denote inhibition.

At the normal biological levels in a healthy person, PAI-1 plays a pivotal role in fibrinolysis, as well as in angiogenesis,[20] cell migration,[21] and wound healing.[22] It is expressed in vascular muscle cells, hepatocytes, adipose tissues, and platelets at low concentrations.[23,24] In

addition, due to different molecular mechanisms, excretion and storage of PAI-1 is varied by each cell type.[25,26] However, in pathological conditions, PAI-1 expresses in higher concentrations and is associated with an extensive number of pathological diseases such as obesity, metabolic syndrome, tumor invasion and metastasis, and coronary heart disease,[23,24] but the mechanism of PAI-1 involvement in the development of these diseases is not clear.[27]

PAI-1 is a 50 kDa glycoprotein[28] made up of an approximately 400 amino acids.[3,29] PAI-1 consists of three β-sheets (A, B, and C),[2,30] nine α-helixes (termed as A-I),[30] and a flexible reactive center loop (RCL) (**Figure 2-a**). The RCL comprises a 20 to 30 amino acid sequence (represented as $P_{16} – P'_{10}$)[27,31] that specifically targets certain proteases by serving as "bait". That part of the RCL contains the scissile bond at the location of P_1-P_1'[18,32] and is exposed to solvent. The target proteases uPA/tPA bind to the RCL in the active form of PAI-1 and lead to the formation of a non-covalent Michaelis complex[33,34] that acts as an intermediate between active PAI-1 and cleaved PAI-1. Once cleaved via proteolysis, the amino-terminal end of the RCL travels away from β-sheets B and C and flips nearly 180º to insert into β-sheet A. If the protease is able to complete its catalytic cycle and break its covalent bond to the RCL before the RCL inserts into β-sheet A, the cleaved form of PAI-1 and the regenerated active protease are formed (**Figure 2-d)**. If the protease is not able to complete its catalytic cycle and break its covalent bond to the RCL before the RCL inserts into β-sheet A, the inactive protease/PAI-1 covalent complex is formed[34] (**Figure 2-c**). At this stage, the serpin is kinetically stable and increases its thermal stability. Therefore, this conformational change is termed a "stressed" to "released" transition.[12,18,29]

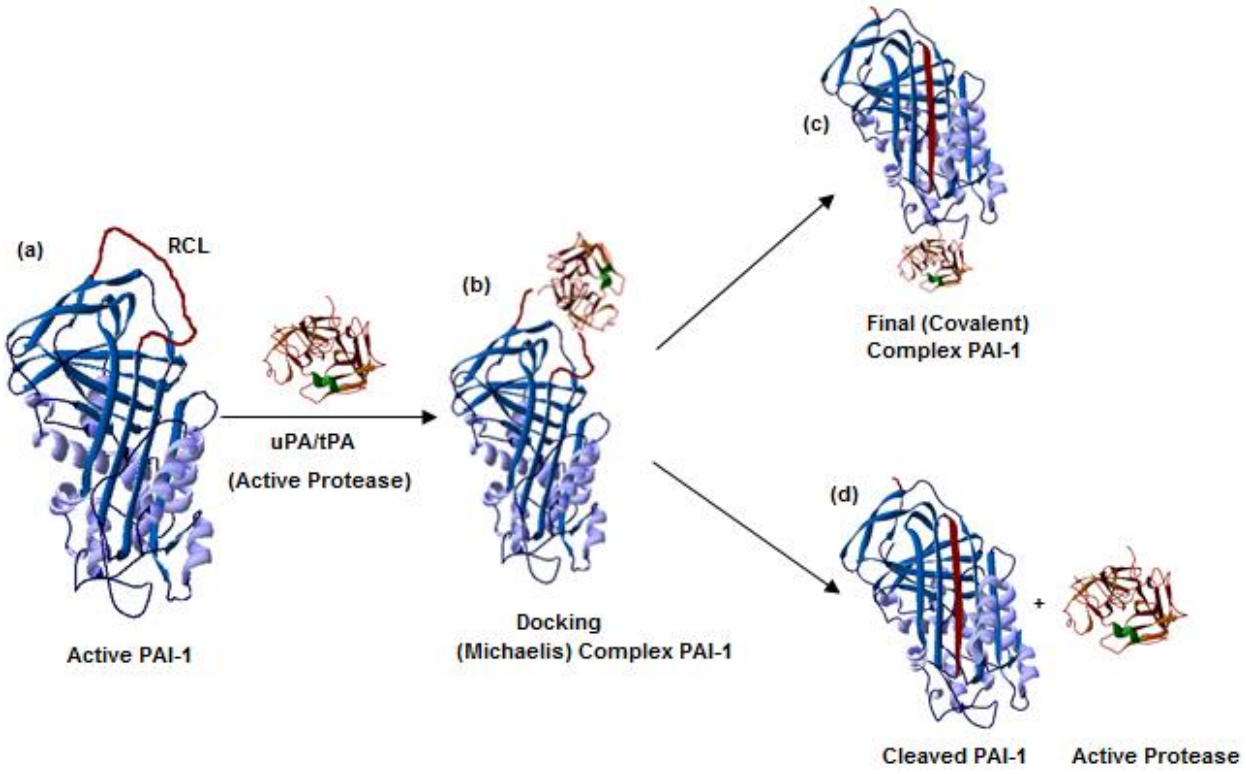


Figure 2: The PAI-1 structure and its mechanism: **(a)** The structure of the active PAI-1 RCL is in magenta, **(b)** Initial Michaelis complex; protease non-covalently bound to RCL of PAI-1. **(c)** The final covalent serpin-enzyme complex. **(d)** Inactive PAI-1 in its cleaved form and the detached active protease.

In addition, the spontaneous conversion of PAI-1 from the active form to the inactive latent form (**Figure 3b**) has a half-life of 1-2 hours at 37 ºC,[25,34,35] which prevents formation of a Michaelis complex with its target proteases. During this transition from the active to latent form, the RCL is inserted into β sheet A while releasing the first strand of β sheet C.[32] Additionally, PAI-1 activity may vary according to its environmental conditions. For example, vitronectin is a 70 kDa glycoprotein that binds with PAI-1 and is normally present in plasma and the extracellular matrix.[27,36] **(Figure 3c)**. When PAI-1 is bound to vitronectin, the active form of PAI-1 is stabilized by prolonging its half-life from 2-145 hours,[36] and allows greater interaction with its target proteases. Because of PAI-1's several different conformational structures (active,

latent, covalent complex, cleaved, and vitronectin-bound) the development of PAI-1 inhibitors is challenging.

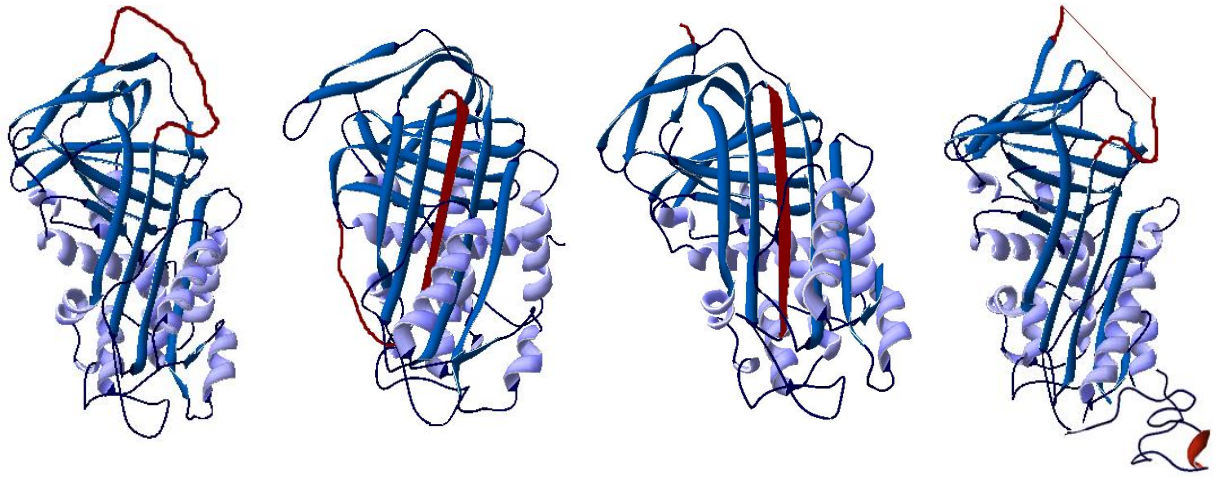


Figure 3: Different conversions of active serpin. **(a)** Structure of active PAI-1, **(b)** Spontaneously converted inactive latent form. **(c)** Inactive cleaved form **(d)** Vitronectin-bound PAI-1.

I-3: PAI-1 as a Drug Target

As a pharmacological target, designing an active PAI-1 inhibitor is an attractive goal. One method is to prevent the secretion of excess levels of PAI-1 from particular cells. However, PAI-1 production is varied according to different cell types; hence, it is difficult to develop PAI-1 inhibitors particularly for each cell type. Despite this challenge, some compounds have been reported for the inhibition of PAI-1 production in the endothelial cells.[26] For example, gemfibrozil is able to reduce the PAI-1 expression from endothelial cells.[37] Conversely, a more straightforward approach is to inhibit excessive levels of PAI-1 directly because drugs can easily access the blood stream and can immediately display antithrombotic activity.

The biological activity of a synthesized inhibitor is characterized by its half maximal inhibitor concentration (IC_{50}) value, which is the concentration of an inhibitor required to reduce its target's activity to 50%. In **Figure 4**, the graph represents the IC_{50} value of a synthesized compound in the lab. This graph was created by plotting percent serpin activity (Y axis) versus the concentration of a particular inhibitor (X axis), and the IC_{50} is determined by reading the inhibitor concentration reading at 50% serpin activity. The potency of an inhibitor increases as the IC_{50} value decreases. In other words, if the IC_{50} values are from mM to µM to nM range, inhibitors express minimal to moderate to excellent activity, respectively.

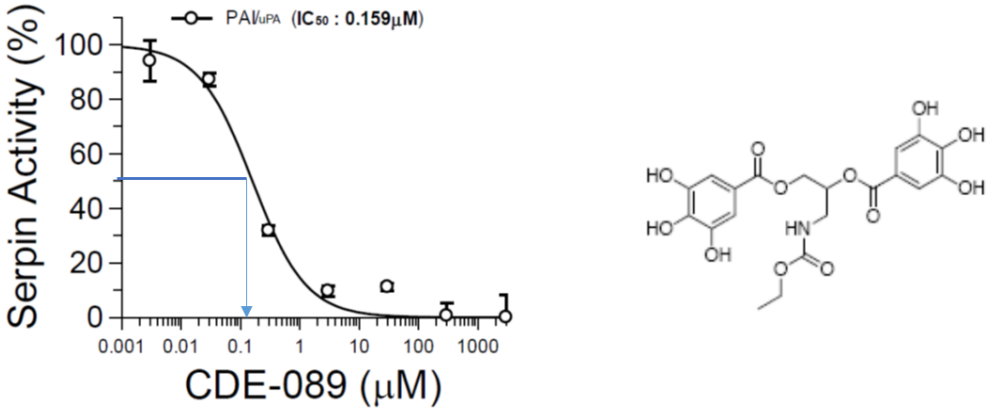
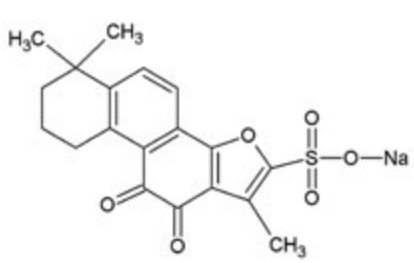


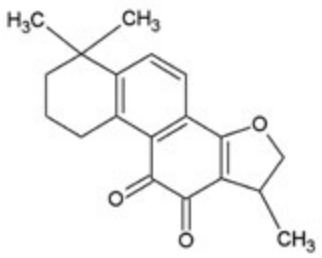
Figure 4: Plot of IC_{50} value for CDE-089

According to the theory of direct inhibition of PAI-1 in plasma, since the 1990s, a number of researchers have synthesized molecules as PAI-1 inhibitors. However, none of these compounds were able to be used as a clinical drug molecule because those compounds have complications and some major drawbacks *in vitro* as well as *in vivo*. Dried roots of *Salvia miltiorrhiza* (danshen), a traditional Chinese medicine, have been used to treat stroke that is mainly related to clot formation or thrombosis. Californian Indians had used *Salvia columbriae* for the similar treatment. Tanshinones and salvianolic acids are the major active ingredients in both natural products. In further studies, seven natural compounds were isolated from *Salvia*

miltiorrhiza extract and were analyzed against PAI-1 inhibitory activity. Those seven natural products are tanshinone I, tanshinone IIA, sodium tanshinone IIA sulfonate, cryptotanshinone, sodium danshensu, protocatechuic aldehyde, and β-sitosterol. Among those, sodium tanshinone IIA sulfonate **(I-1)** and cryptotanshinone **(I-2)** were identified with PAI-1 inhibitory activity in dose dependent manner, and it was confirmed that tanshinones are able to decrease the formation of the PAI-1/uPA complex and prevent fibrin clot formation.[38]



Sodium tanshinone IIA sulfonate
IC_{50} = 41 μM
I-1

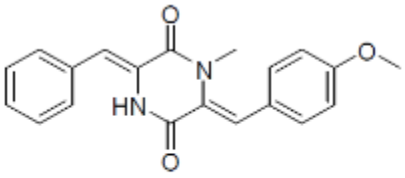


Cryptotanshinone
IC_{50} = 98 μM
I-2

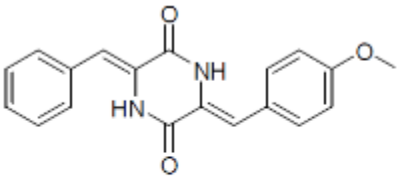
In addition to natural products, several research studies have reported monoclonal antibodies, peptides, and small molecules as effective PAI-1 inhibitors.[39] Monoclonal antibodies (MA) have become a topic of interest since the late 1980s. MAs are low molecular weight proteins with a molecular weight of approximately 150 kDa, which are designed to specifically bind to only one certain molecule; therefore, they are of potential therapeutic use as PAI-1 inhibitors[40] or as compounds that promote conversion to the latent form.[31] In 1998 Ngo and Declerck developed MA-124K1, which showed an approximately 60% PAI-1 neutralizing property *in vivo* using pre-treated endotoxin in rats.[40] MA-33H1F7, a monoclonal antibody studied in 1998, showed antithrombotic activity in humans and rat models by orienting PAI-1 into a non-inhibitory substrate;[41] however, MA-33H1F7 shows lower activity towards glycosylated PAI-1.[42] Most of the other available monoclonal antibodies were characterized

against recombinant non-glycosylated PAI-1 in animal models. For example, MA33B8 is able to prevent the recognition of target proteases of the PAI-1 RCL and prevent RCL cleavage by converting PAI-1 into the inactive latent form.[31] MA-8H9D4 and MA-55F4 were able to prevent formation of initial Michaelis complex with tPA/uPA by binding these monoclonal antibodies directly with RCL.[31] Even though these monoclonal antibodies are exciting therapeutic methods for the inhibition of excess PAI-1 levels, use of monoclonal antibodies requires the improvement of specific drug delivery methods because antibody-based drugs can be rapidly destabilized and show decreased efficacy by changes in its environment, such as in pH.

A number of studies have investigated the use of peptides as PAI-1 inhibitors. In 1995, Eitzman and coworkers used a SDS-PAGE analysis to show a 14-amino acid peptide (P1-P14) that could inhibit formation of the PAI-1/tPA complex, leading to the acceleration of fibrinolysis by rapid inhibition of PAI-1 *in vitro*.[28] In another study, paionin-4, identified from a phage-displayed peptide library, binds to PAI-1 in the same region that binds with MA33B8, and paionin-4 increases the rate of conversion from active to latent form. It is hypothesized that paionin-4 accelerates the conversion rate by stabilizing the transition state leading to RCL insertion.[30] However, similar to monoclonal antibodies, peptides are also not ideal drug candidates due to the lack of metabolic stability and efficacy *in vivo*.



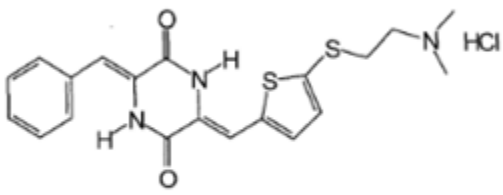
XR330
IC_{50} = 51 µM
I-3



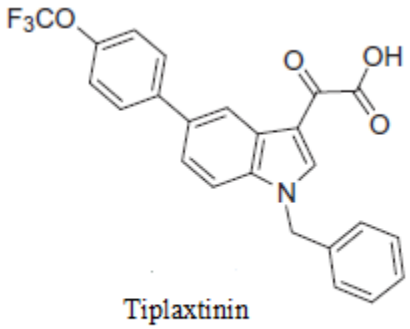
XR334
IC_{50} = 51 µM
I-4

Therefore, the design of orally effective small molecules was becoming of greater importance to overcome complications that were associated with monoclonal antibodies and

peptides. During the mid-1990s, the first reported small-molecule PAI-1 inhibitors were diketopiperazine derivatives by Xenova Limited named XR330 **(I-3)** and XR334 **(I-4)** that were isolated from the mycelium of an unidentified species of *Streptomyces.* XR330 and XR334 were able to inhibit PAI-1/tPA interaction *in vitro* with an IC_{50} value of 51 µM, and did not affect tPA activity. In addition, those compounds were found to have limited solubility. *Ex vivo* studies showed XR334 could enhance fibrinolysis and prolong the thrombus formation time in rats.[43] By modifying these lead molecules, XR5118 **(I-5)** was selected for further studies *in vivo* to seek the improvement of potency and solubility.[44] *In vitro* assay results (IC_{50} = 3.5 µM) and in a rat thrombosis model showed that inhibition of PAI-1 by XR5118 was selective over other serpins and enhanced fibrinolysis while increasing tPA activity in plasma, which indicated a possible treatment for thrombotic disease.[44] However, these diketopiperazine derivatives still experienced poor physiochemical properties.[18,45]



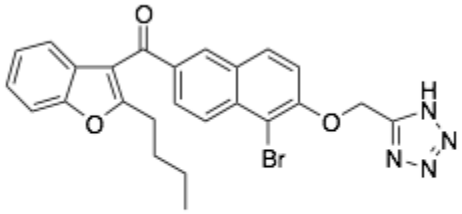
XR5118
IC_{50} = 3.5 µM
I-5



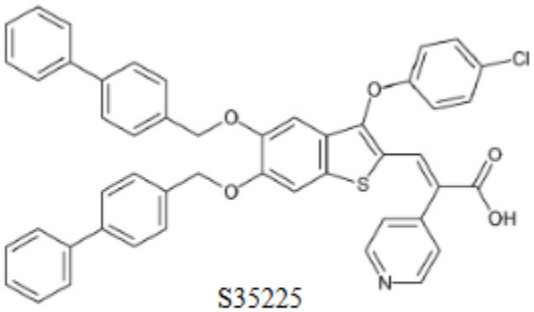
Tiplaxtinin
IC_{50} = 2.7 µM
I-6

Among the small molecule inhibitors, the most studied PAI-1 inhibitor to date is PAI-039 (also called tiplaxtinin) **(I-6)**, which has an IC_{50} value of 2.7µM versus PAI-1. Although it has activity against PAI-1 in animal models, it was not clinically approved due to low affinity to PAI-1, and the inability to inactivate PAI-1 in the presence of vitronectin.[23,46] Research has been

done to investigate PAI-039's mechanism of inhibition using SPR (surface plasmon resonance) analysis, and data shows that PAI-039 binds reversibly only to free PAI-1, and displays its anti-proteolytic activity against uPA/tPA with an IC_{50} value of 9-12 µM. Also, there is no binding observed with PAI-1 that is already bound to vitronectin or that exists in the latent conformation.[23]

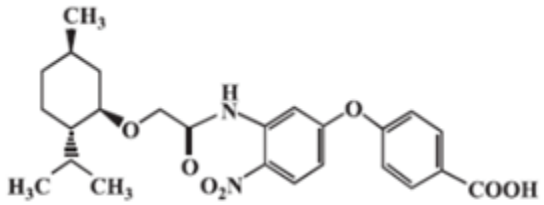


WAY-140312
IC_{50} = 11.7 µM
I-7



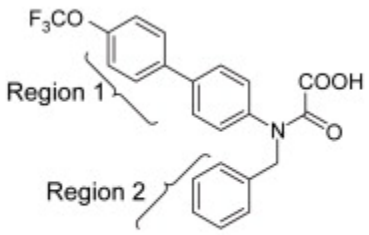
S35225
I-8

Wyeth also developed WAY-140312 **(I-7)**, which in the presence of tPA or uPA inhibits PAI-1 with an IC_{50} value of 11.7 µM. When tested in animal models of vascular injury, a 10 mg-kg^{-1} oral dose of WAY-140312 was required to reduce thrombotic diseases and displayed only a 29% bioavailability with a very low half-life (1 hour).[45] In 2008, Rupin and co-workers had tested S35225 **(I-8)**, a benzothiophene derivative, in *in vitro* and *in vivo* studies side-by-side with tiplaxtinin and WAY-140312. These studies showed that when they were intravenously administered, only S35225 inhibited PAI-1 activity in a dose-dependent fashion after 15 minutes in rat models, which indicated that this benzothiophene derivative is a direct PAI-1 inhibitor and in inhibits the development of clots. Even though S35225 is able to inhibit PAI-1 immediately after injection in the presence of vitronectin, S35225 is not useful for a long-term treatment due compared to tiplaxtinin and WAY-140312. Therefore, to increase the efficacy of direct PAI-1 inhibition in blood, S35225 led to further improvement.[47]

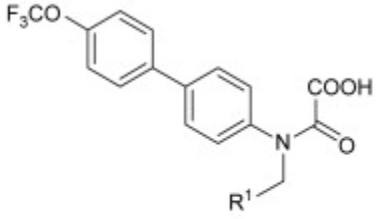


ZK4044
IC_{50} = 0.644 µM
I-9

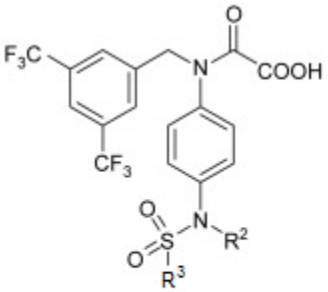
ZK4044 **(I-9)**, a menthol-based compound, was identified using high throughput library screen, and displayed an IC_{50} of 0.644 µM against PAI-1. ZK4044 selectively binds to PAI-1 over other serpins, such as antithrombin III, α-2 antitrypsin, and α-2 antiplasmin. In addition, this compound directly inhibits PAI-1 by preventing interaction with tPA/uPA. Furthermore, ZK4044 is able to prevent PAI-1's conversion from active to latent form.[26] However, it requires further optimization for improved water solubility because of its hydrophobic menthol based chemical structure.



Oxamic acid derivative
IC_{50} = 96 µM
I-10



I-11

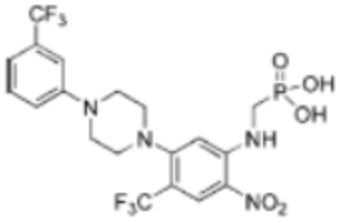


I-12

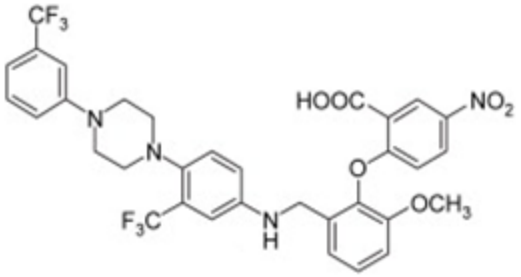
By utilizing the high throughput library screen, Jain *et.al*. identified an oxamic acid-based lead compound **(I-10)** with an IC_{50} value of 96 µM *in vitro*.[48] They were able to synthesize a series of structural analogs **(I-11, I-12)** by optimizing the aromatic substitution while retaining

the carboxylic acid functionality. Of the series of synthesized compounds, **I-12** showed good inhibitory activity compared to the lead molecule. However, due to the poor oral bioavailability of the derivatives, they displayed poor pharmacokinetics, and it was not further evaluated.[49]

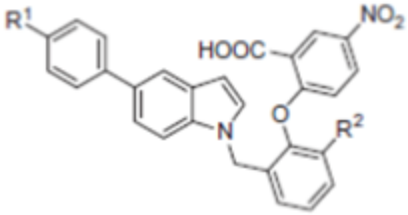
Another study reported piperazine derivative **I-13**, which was also identified from a high throughput library screen, with an IC_{50} value of 1.8 µM against PAI-1. Due to the phosphonic acid moiety, this compound is associated with poor oral bioavailability.[39] Therefore Ye e*t.al.* studied the structure-activity relationship using different analogs of **I-13** by modifying the structure in three different segments. Replacement of the aminomethyl phosphonic acid with a substituted benzoic acid moiety (**I-14)** showed improved potency against PAI-1, with an IC_{50} of 0.5 µM. Given **I-14's** selectivity for PAI-1 over other serpins, it was then tested in rats to determine its pharmacokinetic properties. The results showed that oral bioavailability improved to 43% with a moderate clearance rate of 10.4 ml/min/kg compared to the menthol based compound I-9; additionally, the inhibitor showed an extended half-life of 2.8 h.[39]



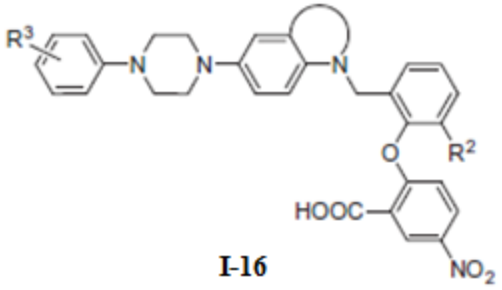
Piperazine derivative
IC_{50} = 1.8 µM
I-13



I-14



I-15



I-16

Pandiya and coworkers also synthesized structural analogs **(I-15 and I-16)** that are hybridized versions of the benzoic acid moiety of **I-14** with tiplaxtinin **(I-6)** and piperazine derivative **I-13.** These hybrid versions showed good PAI-1 inhibitory activity *in vitro* but failed or showed moderate antithrombotic activity in rat models.[49] Therefore, to improve the pharmacodynamics and pharmacokinetic properties, further efforts are in progress.

With the intention of developing more potent novel molecules with relatively high affinity for PAI-1 and the ability to inhibit vitronectin-bound PAI-1, our research group started a collaboration with Professor Daniel Lawrence's lab at the University of Michigan Medical School in 2007. Using a high throughput screen of the MicroSource SPECTRUM compound library, the Lawrence group was able to identify 19 structurally varied compounds that showed activity against PAI-1, three of which contained galloyl moieties (3,4,5-trihydroxybenzoates).[46]

These compounds allowed our group to develop a new class of novel polyphenolic PAI-1 inhibitors **(Figure 5)**. These compounds showed better PAI-1 inhibitory activity than tiplaxtinin, were able to block the formation of the initial Michaelis-like complex between PAI-1 and tPA/uPA, and also were shown to inhibit vitronectin-bound PAI-1. In addition, these compounds can reversibly bind PAI-1 with high affinity, which means the IC_{50} is not time dependent. However, none of the digallate analogs (CDE-008 **I-17**, CDE-031 **I-18**, CDE-034 **I-19**, CDE-056 **I-21**) (**Figure 5**) had an acceptable activity against PAI-1 in *ex vivo* experiments containing plasma proteins. On the other hand, compounds with more galloyl groups, such as CDE-066 (**I-20**) and CDE-082 (**I-22**), displayed better activity than the above digallate compounds against PAI-1 in plasma.[46] Generally, the trend was that the greater the number of galloyl groups, the better the inhibitory activity against PAI-1. However, CDE-066 **(I-20)** has a molecular weight nearly 2000 daltons and as such it cannot be considered as an ideal drug candidate. Also, in these polyphenolic compounds, the galloyl groups are attached via ester linkages, which are unstable in acidic media. If these are used as drug molecules, when orally administered, the ester can be easily hydrolyzed into the corresponding carboxylic acids and alcohols.

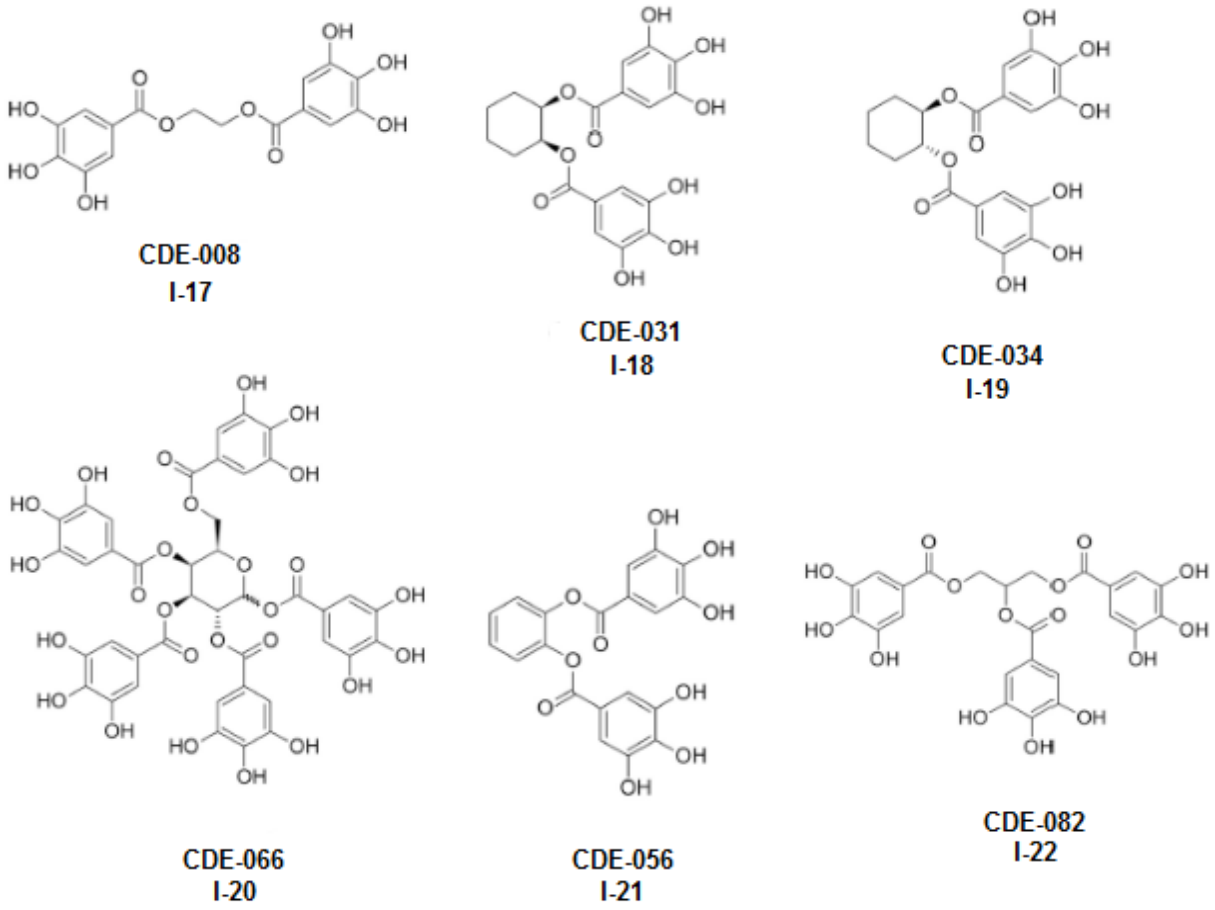


Figure 5: Second generation galloyl compounds[46]

Our research group then worked on designing a next generation of analogs of different polyphenolic compounds, which typically contain 3,4-dihydroxybenzene moieties linked with sulfonyl groups instead of ester linkages **(Figure 6).** Those bis-arylsulfonamides and arylsulfonimides are intended to be more biostable than the previous generation of polyphenolic compounds, as the sulfonamide linking unit is widely used in FDA-approved drugs. Different symmetric and non-symmetric molecules were designed by increasing the tether length between sulfonamide groups or by substituting an alkyl group for an acidic proton on nitrogen, and the resulting inhibitory activity against PAI-1 was determined. Many of these compounds showed better activity than tiplaxtinin, and showed high specificity for PAI-1 over anti-thrombin III (ATIII), a structurally related mammalian serpin.[50] However, even though the bis-

arylsulfonamides and arylsulfonimides selectively inhibit PAI-1, these compounds also lose their activity in plasma.

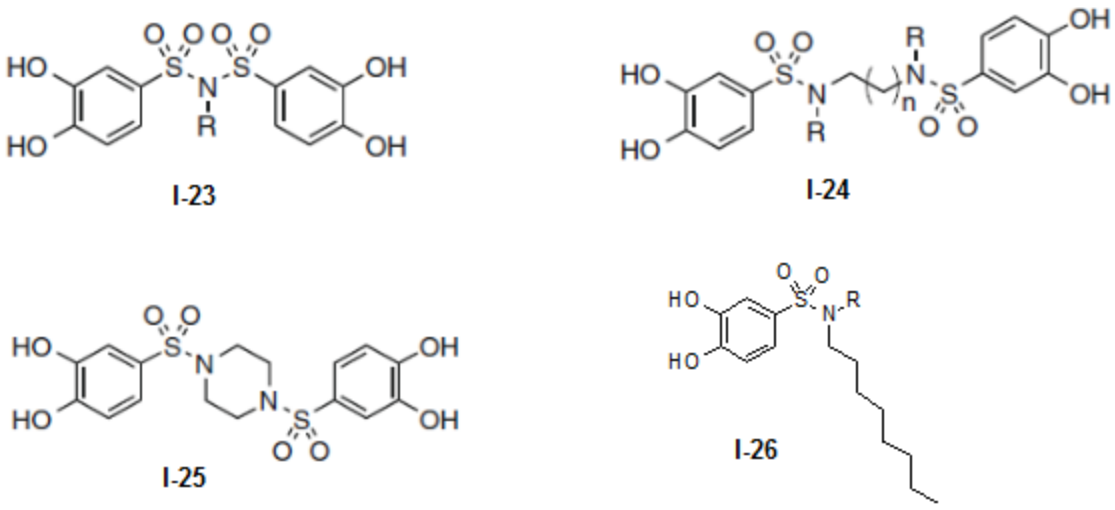


Figure 6: Third generation bis-arylsulfonamides and arylsulfonamides.

These studies showed that none of the compound were able to inhibit PAI-1 while providing all the requirements to be a potent PAI-1 inhibitor *in vitro* and *in vivo*. Hence, this provides the foundation for the design of novel group of inhibitors with a low IC_{50} value that are able to inhibit the activity of free PAI-1 as well as vitronectin-bound PAI-1.[12] Therefore, the Lawrence lab carried out a second library screen from University of Michigan Center for Chemical Genomics (CCG) library and identified a low molecular weight compound with a relatively low IC_{50} value versus PAI-1 in the presence of plasma (**Figure 7**). This compound inspired us to design next generation analogs of novel low molecular weight amides containing various replacement groups and moieties. From this collection of structurally diverse inhibitors, we set out to develop a structure-activity relationship for this class of compound that will allow us to design a more potent small molecule as a PAI-1 inhibitor.

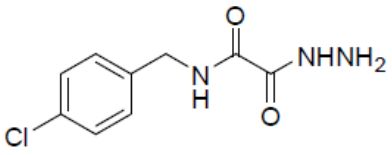


Figure 7: Second library screened leading molecule **(I-27)**.

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Chapter II: Next Generation Novel Small Molecules as PAI-1 Inhibitors

Our previous series of polyphenolic PAI-1 inhibitors, which contain galloyl moieties or 3,4-dihydroxybenzene sulfonamide groups, were able to provide good inhibitory activity versus PAI-1 in a standard buffer solution, but activity was destroyed when plasma was added. This is because of the poor availability of our inhibitors due to the preferential binding to other proteins present in plasma. Because of this, our focus is to improve the potencies of our inhibitors with PAI-1 in a plasma-containing assay. Therefore, our collaborators carried out a second library screen and identified a hydrazide (**I-27**) with an IC_{50} value of 36 µM in a plasma-containing assay.

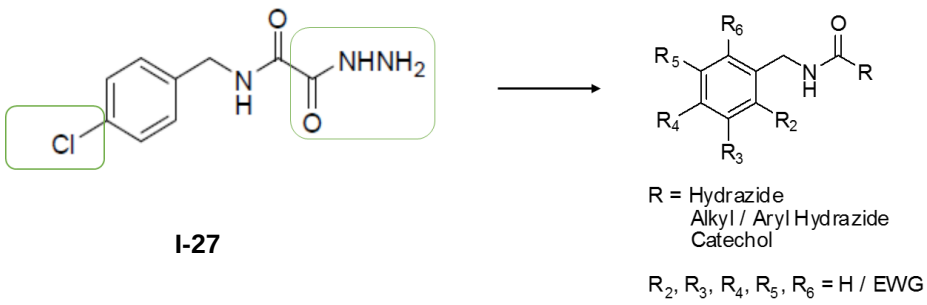
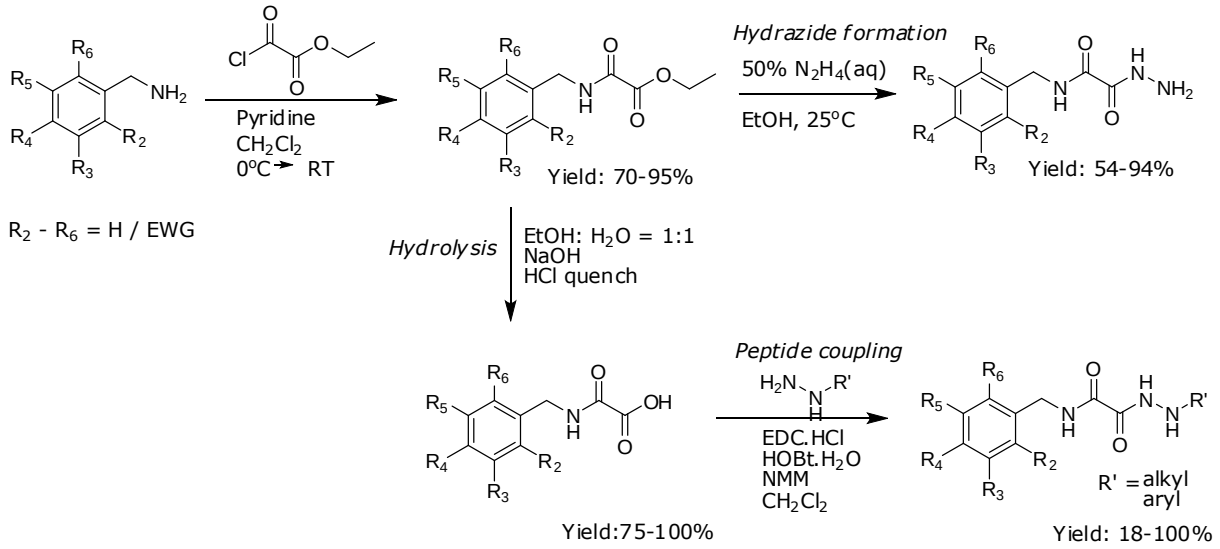


Figure 8: Structural modifications of lead molecule

This compound led us to synthesize structural analogs by modifying the substituents on the aromatic ring and/or varying the nature of the hydrazide group. We developed different esters, carboxylic acids, and substituted hydrazides, with various substituents on the aromatic ring. Also, to probe PAI-1 inhibitory activities we modified the hydrazide group by adding substituents or by replacement with a polyphenolic moiety **(Figure 8)**. By analysis of the effects of these key modifications on IC_{50} values, we were able to start to develop a structure-activity relationship for this class of molecules versus PAI-1.

As shown in **Scheme 1**, I chose differently substituted benzyl amines and treated them with ethyloxalyl chloride in the presence of pyridine to obtain the respective ester analogs. Our desired hydrazides were obtained by treating this ester compound with a 50% aqueous hydrazine solution. Also, the same ester compound was used to do the hydrolysis to attain the respective carboxylic acid, which was then coupled with differently substituted hydrazides to obtain the final diamides.



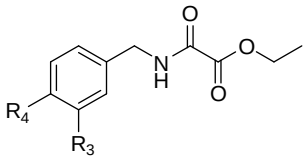
Scheme 1: General synthetic method for hydrazide analogs of lead molecule

II-1: Effect of changing aromatic ring substituents on ester, carboxylic acid, and hydrazide analogs.

Early in my research, my main focus was to synthesize different hydrazide compounds with variously substituted aromatic rings. The synthetic pathway **(Scheme 1)** proceeded through an ester intermediate. Some of the ester intermediates were also submitted for biological testing in order to compare the IC_{50} values of the esters with the corresponding hydrazides. In the ester compounds, mono-*para*-substituted aromatic rings with different halides or a nitrile showed

lower PAI-1 inhibitory activity compared to same substitution pattern in hydrazide compounds **(Table 2).** For example, considering ester compounds in a plasma-containing assay, the *para*-chloro substitution (CDE-444) exhibited an IC_{50} of 1799 µM. Replacement with bromine (CDE-259) yielded three-fold better inhibitory activity, whereas replacement with fluorine (CDE-247) lost activity in standard buffer and was therefore not tested in a plasma-based assay. However, if the benzene ring contains a substituent (CF_3 group) at the *meta* position in addition to the *para*-chloro substituent, the ester compound (CDE-256) and hydrazide compounds (CDE-251, CDE-261 and CDE-297) showed good inhibitory activity against PAI-1. Additionally, we tested two different carboxylic acids by altering the substituents at para position while keeping a CF_3 group at the *meta* position, but none of those compounds showed good inhibitory activity **(Table 3)**. As for the hydrazides **(Table 4)**, benzene moieties with *para* and *meta* substituted compounds show comparatively two- to three-fold higher IC_{50} values to our lead molecule and consistent values in each of the three different assays. These results indicated that EWGs at *meta* and *para* positions are beneficial for PAI-1 inhibitory activity. Therefore, we selected the *para*-chloro-*meta* trifluorobenzene moiety as the standard substitution pattern for synthesizing further small molecules.

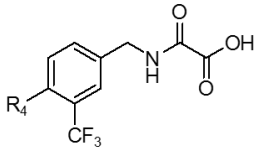
Table 2: Comparison of IC_{50} (µM) values of ester analogs.



Code	R_3	R_4	IC_{50} (µM)		
			Plasma	PAI-1/uPA pH 7.4	1.5% BSA
CDE-444	H	Cl	1799	2847	1062
CDE-247[a]	H	F	NT	>3000	NT
CDE-259[a]	H	Br	526	2998	694
CDE-319	H	CN	NT	>1000	NT
CDE-256[b]	CF_3	Cl	214	1353	235

[a]Compound synthesized by Naga Guntaka. [b]Compound synthesized by Greg Abernathy. NT: not tested.

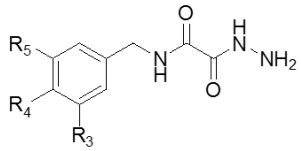
Table 3: Comparison of IC_{50} (µM) values of carboxylic acid analogs.



Code	R_4	IC_{50} (µM)		
		Plasma	PAI-1/uPA pH7.4	1.5% BSA
CDE-311	H	NT	>3000	NT
CDE-309[a]	Cl	>3000	1200	>3000

[a]Compound synthesized by Dan Van Strien. NT: not tested

Table 4: Comparison of IC_{50} (µM) values of hydrazide analogs.



Code	R_3	R_4	R_5	IC_{50} (µM)		
				Plasma	PAI-1/uPA pH 7.4	1.5% BSA
Lead	H	Cl	H	36	117	54
CDE-248[a]	H	F	H	478	1313	648
CDE-260	H	Br	H	52.5	166	50
CDE-251[b]	CF_3	Cl	H	108	76	123
CDE-261[b]	CF_3	F	H	69	76	102
CDE-297	CF_3	H	H	87	128	111
CDE-304	F	F	F	74	162	90
CDE-336	H	CN	H	851	5469	892

[a]Compound synthesized by Naga Guntaka. [b]Compound synthesized by Greg Abernathy. NT: not tested.

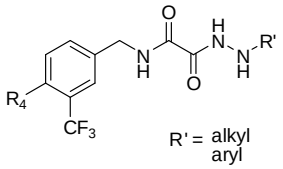
Some of the compounds were not tested in assays containing plasma or 1.5% BSA because those showed minimal potencies in simple buffer. As seen in **Tables 2-4**, even if we change the substitution pattern on the aromatic ring, the hydrazide analogs possess better inhibitory activity compared to ester and carboxylic acid analogs.

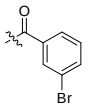
II-2: Effect of changing hydrazide substituents

Then we determined the IC_{50} values by designing compounds that replaced or substituted the hydrazide group with different moieties while keeping the left-hand side aromatic moiety constant. Substitution onto the terminal nitrogen atom of the hydrazide with different substituents **(Table 5)** did not show good inhibitory activity in simple buffer, except for CDE-334. In CDE-334, substituting with benzene sulfonyl group resulted in good inhibitory activity in simple buffer but activity was destroyed when plasma or 1.5% BSA was added to it. In contrast, if the entire terminal NH_2 group was replaced with different substituents **(Table 6)**, compounds containing a hydroxamate (CDE-280, CDE-320) showed IC_{50} values between 150-700 µM

range. However, if the entire hydrazide unit (ie. the carbonyl and both nitrogens) was removed, as in **Table 7**, and replaced with a group that does not contain a catechol unit, all the compounds lost any significant activity in a simple buffer solution. Due to this reason, those were not tested in the other assay systems.

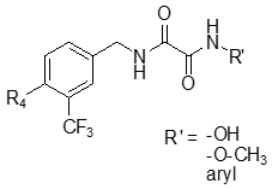
Table 5: Substituent on hydrazide group - substitute one hydrogen.

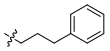


Code	R_4	R’	IC_{50} (µM)		
			Plasma	PAI-1/uPA pH 7.4	1.5% BSA
CDE-310	H		NT	>1000	NT
CDE-298[a]	Cl		NT	>1000	NT
CDE-333	Cl		NT	>100	NT
CDE-334	Cl		>300	189	>300

[a]Compound synthesized by Greg Abernathy. NT: not tested.

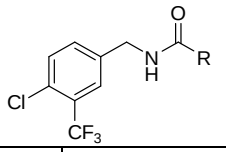
Table 6: Substituents on hydrazide group - substitute NH_2 group.

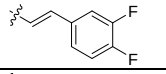
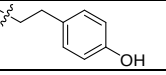
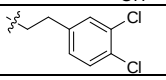


Code	R_4	R’	IC_{50} (µM)		
			Plasma	PAI-1/uPA pH 7.4	1.5% BSA
CDE-280[a]	Cl		168	203	159
CDE-320	Cl		693	329	513
CDE-335	Cl		NT	>100	NT

[a]Compounds synthesized by Greg Abernathy. NT: not tested.

Table 7: Substituents on hydrazide group - replace hydrazide and one carbonyl, with different substituents.

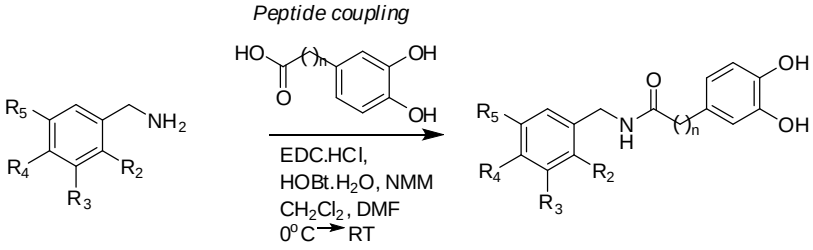


Code	R	IC_{50} (μM)		
		Plasma	PAI-1/uPA pH 7.4	1.5% BSA
CDE-349[a]		NT	>300	NT
CDE-354		NT	>300	NT
CDE-355		NT	>300	NT

[a]Compound synthesized by Naga Guntaka. NT: not tested.

II-3 Effect of increasing the chain length between carbonyl group and catechol unit

We also synthesized hybrids of the two major classes of PAI-1 inhibitors that our lab has studied by replacing the hydrazide unit in our above scaffold with a catechol group. These hybrids were synthesized according to the procedure shown in **Scheme 2**; potencies against PAI-1 are shown in **Table 8**.

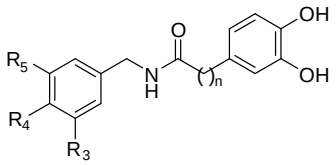


Scheme 2: Synthesis of peptide coupling products

Differently substituted benzylamines were treated with 3,4-dihydroxybenzoic acid or (3,4-dihydroxyphenyl)acetic acid or 3-(3,4-dihydroxyphenyl)propanoic acid under standard peptide-coupling conditions to yield the target amides with a varying tether length between the amide group and the catechol unit.

Table 8: Change of tether length comparison (a) IC_{50} values when n = 0, 1, 2 (b) comparison table.

(a)



Code	n	R_2	R_3	R_4	R_5	IC_{50} (µM)		
						Plasma	PAI-1/uPA pH 7.4	1.5% BSA
CDE-357	2	H	OCF_3	H	H	567	1370	1227
CDE-356	2	H	F	F	F	544	1100	1241
CDE-359[a]	2	H	H	Cl	H	974	3373	2084
CDE-454	2	H	H	Br	H	NT	738	607
CDE-344[b]	2	H	CF_3	H	H	866	1002	830
CDE-345[b]	2	H	H	CF_3	H	539	1469	673
CDE-330[c]	2	H	CF_3	Cl	H	115	365	112
CDE-382[d]	1	H	F	F	F	1371	6097	8553
CDE-370[d]	1	H	H	Cl	H	865	497	1079
CDE-371[d]	1	H	H	Br	H	1205	742	1188
CDE-383[d]	1	H	H	CF_3	H	355	1023	724
CDE-347[c]	1	H	CF_3	Cl	H	293	888	309
CDE-373	0	H	OCH_3	H	H	>3000	2508	>3000
CDE-374	0	OCF_3	H	H	H	1155	125	3864
CDE-387	0	H	OCF_3	H	H	961	462	809
CDE-376	0	H	H	OCF_3	H	998	320	1447
CDE-453	0	H	F	F	F	NT	1122	854
CDE-384	0	H	F	H	F	2433	1884	1774
CDE-390	0	H	H	F	H	>3000	4055	>3000
CDE-361[a]	0	H	H	Cl	H	851	232	2000
CDE-360[a]	0	H	H	Br	H	771	164	1670
CDE-364[a]	0	H	CF_3	H	H	533	475	898
CDE-388	0	H	H	CF_3	H	485	422	526
CDE-367	0	H	CF_3	F	H	260	367	572
CDE-348[c]	0	H	CF_3	Cl	H	240	99	454

(b)

R_3	R_4	R_5	Plasma IC_{50} (µM)		
			n=0	n=1	n=2
F	F	F	NT	1371	544
H	Br	H	771	1205	NT
H	Cl	H	851	865	974
CF_3	Cl	H	240	293	115
H	CF_3	H	485	355	539

[a]Compound synthesized by Himabindu Anumala. [b]Compound synthesized by Dan Strien. [c]Compound synthesized by Naga Guntaka. [d]Compound synthesized by Sarah Burke NT: not tested.

We synthesized a large number of potential inhibitors according to the above procedure and compared the change of tether length of these compounds. From our earlier studies of polyphenolic compounds, a 3,4-dihydroxybenzene (catechol) moiety exhibited similar activity to galloyl moiety. Therefore, we kept constant the catechol unit in the right hand side and placed differently substituted aromatic groups on the left hand side while changing the tether length between the carbonyl carbon and catechol unit. According to the comparison table **(Table 8-b)**, the *para*-bromo substituted compound showed varying IC_{50} values by increasing the chain length, that is an inhibitor with no spacer (CDE-360, n=0) exhibited an IC_{50} of 771 µM in plasma, an inhibitor with one methylene spacer (CDE-371, n=1) increased the IC_{50} value by ~two-fold (IC_{50} = 1205 µM) in plasma. The analogous inhibitor with two methylene spacers (CDE-454, n=2) showed IC_{50} of 738 µM in standard buffer and was not tested in plasma due to the loss of activity. However, considering the similar series of compounds that are substituted at the *para* position with a CF_3 group (CDE-388, CDE-383, CDE-345), all three values for n=0, 1, 2 showed similar activity, indicating that the length of the spacing unit was irrelevant in this series. As shown in **Figure 9**, we compared IC_{50} by changing the substitution pattern similar to our lead molecule (*para*-chlorobenzene) and to our standard aromatic substitution (*para*-chloro-*meta*-trifluorobenzene). Compounds in the *para*-chloro substituted series (CDE-361, CDE-370 and CDE-359) showed very similar activities of 851 µM, 865 µM, and 974 µM respectively in plasma as the tether length was increased. Counter to this, the series containing the *para*-chloro *meta*-trifluorobenzene moiety showed a lower IC_{50} value of 115 µM for n=2 compared to other two shorter inhibitors. Ultimately, it appears that any effect due to tether length is dependent on the substitution pattern on the aromatic ring.

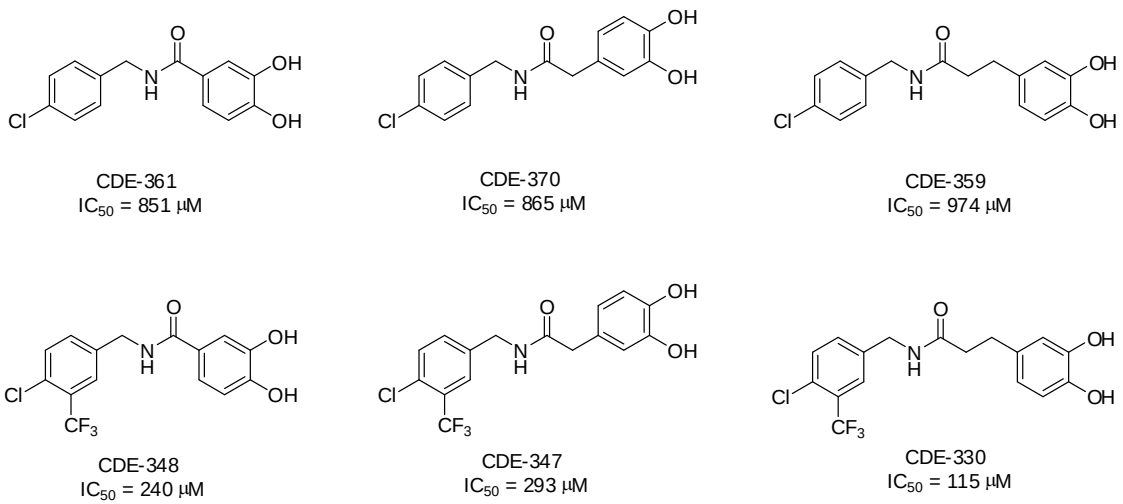
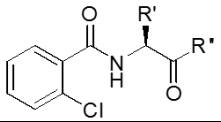


Figure 9: Comparison of tether length

II-4: Effect of changing amide position

We were also interested in what the effect would be of by changing the orientation of the left-hand amide. Instead of containing a benzylamide group, the carbonyl position was relocated to the benzylic carbon. Both ester and hydrazide versions were synthesized while adding a chiral position from the starting amino acid in two of the analogues, and our collaborators determined the IC_{50} values. Only CDE-401 showed a measureable IC_{50} value of 2502 μM in a simple buffer system, while other compounds did not show any activity at the concentrations used in the assay. Due to these higher values, none of the compounds were tested in assays containing 1.5% BSA or plasma.

Table 9: Change of amide position.



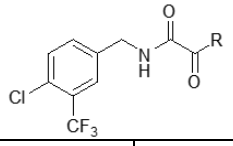
Code	R’	R’’	IC_{50} (µM)		
			Plasma	PAI-1/uPA pH 7.4	1.5% BSA
CDE-391	H		NT	>1000	NT
CDE-392	H		NT	>3000	NT
CDE-401			NT	2502	NT
CDE-393			NT	>3000	NT

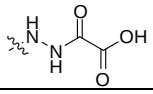
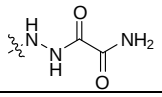
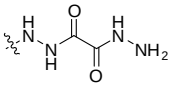
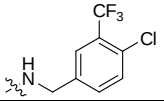
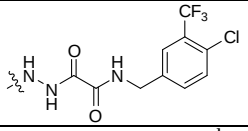
NT: not tested

II-5: Effect of extended versions of lead molecule

Finally, I wanted to observe the effect on activity of extended versions of the lead molecule because I thought that it would be a good comparison to our standard hydrazide compound. I held the substitution pattern on aromatic group constant (*para*-chloro-*meta*-trifluorobenzene) and extended the length of the molecule by using different functionalities in the R group (**Table 10**). In those compounds, the shortest version (CDE-431) and longest symmetric version (CDE-439) showed similar activity (IC_{50} ~ 58 µM) to our lead molecule. Also, the IC_{50} versus PAI-1 was increased by increasing the length of the inhibitor (CDE-251, CDE-445, and CDE-441 respectively). By contrast, CDE-323 was not tested in plasma due to poor solubility and inactivity in standard buffer.

Table 10: Extended versions of lead molecule.



Code	R	IC_{50} (μM)		
		Plasma	PAI-1/uPA pH 7.4	1.5% BSA
CDE-431		56.1	132	86.4
CDE-251[a]		108	76	123
CDE-440		1407	105	2972
CDE-445		538	108	692
CDE-441		1199	335	1087
CDE-323[b]		NT	>100	NT
CDE-439		58	150	76

[a]Compound synthesized by Greg Abernathy. [b]Compound synthesized by Sarah Burke. NT: not tested

II-6: Conclusion

Based upon the above results, the hydrazide analogs with substituted aromatic rings possess better inhibitory activity than similarly substituted carboxylic acid and ester analogs. The ester analogs are ten- to thirteen-fold less potent than corresponding hydrazide analogs. Additionally, 3,4-dihydroxybenzene (catechol moiety) and *para*-chloro-*meta*-trifluorobenzene are useful in designing potential PAI-1 inhibitors. In molecules containing both of these units, there is no predictable impact from changing the tether length between the carbonyl and the catechol unit. Also, we found that switching the carbonyl position of the left-hand amide destroys PAI-1 inhibitory activity. When considering extended versions of our lead molecule, the

longest symmetric compound and its monomer (the shortest molecule) displayed very good inhibitory activity in plasma containing assay, displaying IC_{50} values that were 1.5-fold higher than our lead molecule. However, we did not discover any compound that improved the potency of our lead molecule, although we did identify functional groups that can generally improve activity against PAI-1.

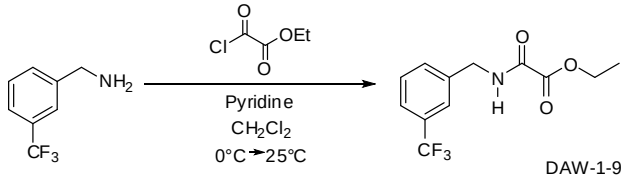
II-7: Experimental methods and data

Chemistry for general method:

Unless otherwise noted, all the reactions were performed in 120 ºC oven-dried glassware with magnetic stirring. All reagent grade solvents were used without purification for extraction, chromatography and reactions. Thin layer chromatography (TLC) was performed with 250 mm silica gel coated glass plates from Sorbent Technologies, and visualized with 254 nm UV light and/or aqueous $KMnO_4$ solution or ninhydrin solution. Column chromatography was executed using silica gel (Sorbent Technologies Premium R_f; 60 Å, 40-75 μM) as the stationary phase. ^1H and ^{13}C NMR spectra were obtained with a JEOL ECX-400 spectrometer with a probe temperature of 25 ºC using DMSO-d_6 (Cambridge Isotope Laboratories, Inc.) or $CDCl_3$ (Cambridge Isotope Laboratories, Inc.) as solvents. Chemical shifts were measured relative to the tetramethylsilane (TMS) peak and were recorded in δ (parts per million, ppm). The internal references were DMSO-d_6 (δ = 2.50 ppm) for ^1H NMR and DMSO-d_6 (δ = 39.5 ppm) for ^{13}C NMR; $CDCl_3$ (δ = 7.26 ppm) for ^1H NMR and $CDCl_3$ (δ = 77.0 ppm) for ^{13}C NMR. Coupling constants (*J*) are recorded in Hertz. Splitting patterns are labeled as follows: bs, broad singlet; s, singlet; d, doublet; t, triplet; q, quartet; p, pentet; m, multiplet. High-resolution mass spectroscopy data were provided by Prof. Ruth Ann Armitage, Eastern Michigan University,

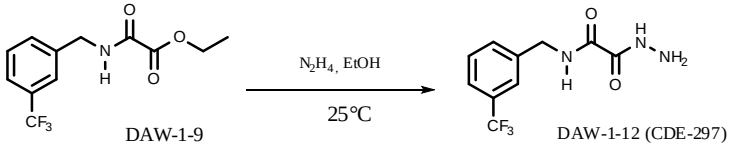
Ypsilanti, MI, and were recorded using Direct Analysis in Real Time (DART) on a JEOL AccuTOF DART instrument, JEOL USA, Inc., Peabody, Massachusetts.

Synthetic procedures:



Ethyl 2-oxo-2-((3-(trifluoromethyl)benzyl)amino)acetate [DAW-1-9]

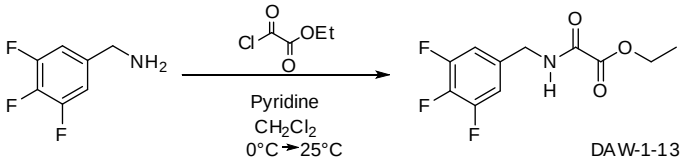
A solution of 3-(trifluromethyl)benzylamine (215 µl, 1.5 mmol), pyridine (364 µl, 4.5 mmol) and methylene chloride (6 ml) was cooled in an ice bath. Ethyl chlorooxoacetate (167 µl, 1.5 mmol) was added and the resulting solution was stirred 48 hours. The reaction mixture was diluted with ≈30 ml of ethyl acetate and washed with 0.2N HCl (2x) and saturated $NaHCO_3$ (2x), dried with $MgSO_4$, filtered, and concentrated *in vacuo* to obtain 0.3902 g (94.5%) of DAW-1-9 as a thick oil. ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.56 (m, 2H), 7.48 (m, 3H), 4.57 (d, *J*=5.9 Hz, 2H), 4.36 (q, *J*=7.3 Hz, 2H), 1.39 (t, *J*=7.4 Hz, 3H).



2-Hydrazinyl-2-oxo-*N*-(trifluoromethyl)benzyl)acetamide [DAW-1-12 (CDE-297)]

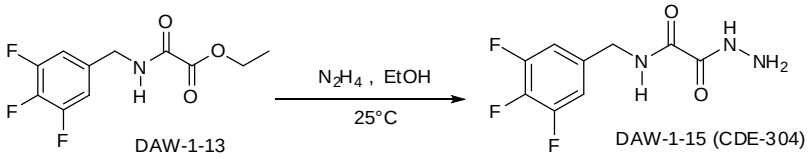
To a mixture of DAW-1-9 (110.1 mg, 0.41 mmol) in ethanol (4.2 ml) was added hydrazine hydrate (~50% solution with H_2O, 53.7 µl, 0.84 mmol). The milky reaction mixture was stirred overnight, filtered, and the solid was dried *in vacuo* to provide 0.1010g (94%) of DAW-1-12 as a white solid. ^{1}H NMR (DMSO-d_6, 400MHz) δ 10.04 (s, 1H), 9.36 (t, *J*= 6.4 Hz, 1H), 7.55 (m,

4H), 4.51 (bs, 2H), 4.35 (d, *J*=6.8 Hz, 2H); ^{13}C NMR (DMSO-d_6, 100 MHz) δ 160.55, 158.43, 140.85, 132.07, 129.90, 129.52 (q, *J*= 30.5 Hz), 124.76 (*J*=270.7 Hz), 124.41 (q, *J*=3.81 Hz), 124.19 (q, *J*=37.7 Hz); HRMS, DART calcd. for $C_{10}H_{11}F_3N_3O_2$ $[M+H]^+$ 262.08033, found: 262.07901.



Ethyl 2-oxo-2-((3,4,5-trifluorobenzyl)amino)acetate [DAW-1-13]

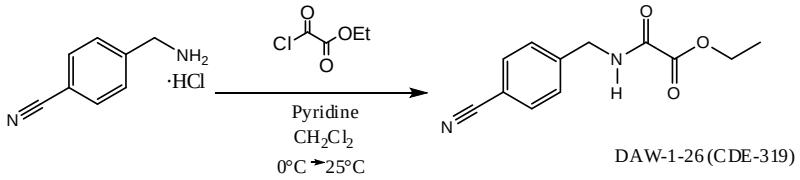
The solution of 3,4,5-trifluorobenzylamine (172.5 µl, 1.5 mmol), pyridine (364 µl, 4.5 mmol) and methylene chloride (6 ml) was cooled in an ice bath. Ethyl chlorooxoacetate (167 µl, 1.5 mmol) was added and the resulting yellow solution was stirred for 48 hours. The reaction mixture was diluted with ≈30 ml of ethyl acetate and washed with 0.2N HCl (2x) and saturated $NaHCO_3$ (2x), dried with $MgSO_4$, filtered, and concentrated *in vacuo* to obtain 0.3192 g (81.5%) of DAW-1-13 as a yellow solid. ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.47 (bs, 1H), 6.92 (m, 2H), 4.44 (d, *J*=6.4 Hz, 2H), 4.36 (q, *J*=7.3 Hz, 2H), 1.39 (t, *J*=6.9 Hz, 3H).



2-Hydrazinyl-2-oxo-*N*-(3,4,5-trifluorobenzyl)acetamide [DAW-1-15 (CDE-304)]

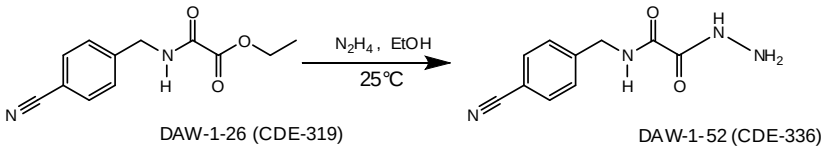
To a mixture of DAW-1-13 (104.9 mg, 0.40 mmol) in ethanol (4.2 ml), hydrazine hydrate (~50% solution with H_2O, 53.8 µl, 0.84 mmol) was added. The off-white reaction mixture was stirred for 48 hours, filtered, and the solid was dried *in vacuo* to provide 0.0537 g (54.3%) of DAW-1-15 as a white solid. ^{1}H NMR (DMSO-d_6, 400MHz) δ 10.02 (bs, 1H), 9.36 (t, *J*= 6.4 Hz, 1H), 7.15 (m, 2H), 4.50 (bs, 2H), 4.25 (d, *J*=6.4 Hz, 2H); ^{13}C NMR (DMSO-d_6, 100 MHz) δ 160.62,

158.31, 150.55 (ddd, *J*=245.1 Hz, *J*=9.5 Hz, *J*=3.8 Hz), 138.19 (dt, *J*=246 Hz, *J*=15.3 Hz), 136.79 (ddd, *J*=6.7 Hz, *J*=7.6 Hz, *J*=3.8Hz), 112.33 (dt, *J*=20.0 Hz, *J*=4.8 Hz), 41.73 ; HRMS, DART calcd. for $C_9H_9F_3N_3O_2$ $[M+H]^+$ 248.06468, found: 248.06619.



Ethyl 2-((4-cyanobenzyl)amino)-2-oxoacetate [DAW-1-26 (CDE-319)]

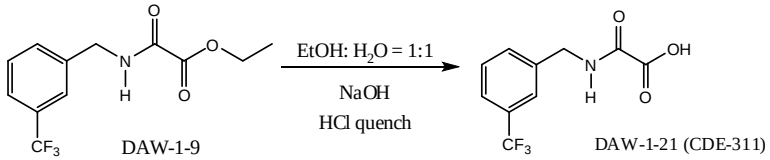
A mixture of 4-cyanobenzylamine hydrochloride (253.7 mg, 1.5 mmol), pyridine (365 µl, 4.5 mmol) and methylene chloride (6 ml) was cooled in an ice bath. Ethyl chlorooxoacetate (167 µl, 1.5 mmol) was added and the resulting solution was stirred 48 hours. The reaction mixture was filtered. The filtrate was diluted with ≈30 ml of ethyl acetate and washed with 0.2N HCl (2x) and saturated $NaHCO_3$ (2x), dried with $MgSO_4$, filtered, and concentrated *in vacuo* to obtain 0.2467 g (70.8%) of DAW-1-26 as a white solid. ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.63 (m, 2H), 7.48 (bs, 1H), 7.40 (m, 2H), 4.57 (d, *J*=6.4 Hz, 2H), 4.36 (q, *J*=7.3 Hz, 2H), 1.39 (t, *J*=7.4 Hz, 3H). ^{13}C NMR ($CDCl_3$, 100 MHz) δ 160.48, 156.86, 142.28, 132.69, 128.51, 118.55, 111.93, 63.61, 43.42, 14.05. HRMS, DART calcd. for $C_{12}H_{13}N_2O_3$ $[M+H]^+$ 233.09262, found: 233.09190.



***N*- (4-cyanobenzyl)-2-hydrazinyl-2-oxoacetamide [DAW-1-52 (CDE-336)]**

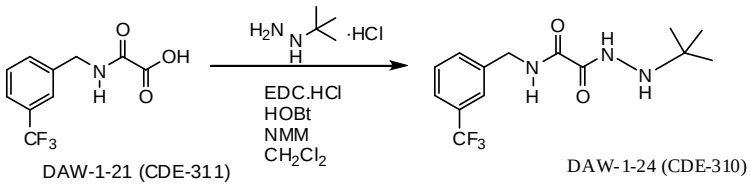
To a mixture of DAW-1-26 (99.5 mg, 0.43 mmol) in ethanol (4.2 ml), hydrazine hydrate (~50% solution with H_2O, 53.70 µl, 0.84 mmol) was added. The milky reaction mixture was stirred overnight, filtered, and the solid was dried *in vacuo* to provide 0.0781g (83.2%) of DAW-1-52 as

a white solid. ^{1}H NMR (DMSO-d_6, 400MHz) δ 10.27 (bs, 1H), 9.35 (t, J= 6.4 Hz, 1H), 7.74 (d, J=8.2 Hz, 2H), 7.39(d, J=8.3 Hz, 2H), 4.50 (s, 2H), 4.35 (d, J=6.4 Hz, 2H); ^{13}C NMR (DMSO-d_6, 100 MHz) δ 160.61, 158.37, 145.15, 132.80, 128.65, 119.37, 110.20, 42.53; HRMS, DART calcd. for $C_{10}H_{11}N_4O_2$ $[M+H]^+$ 219.08819, found: 219.08740.



2-Oxo-2-((3-(trifluoromethyl)benzyl)amino)acetic acid [DAW-1-21 (CDE-311)]

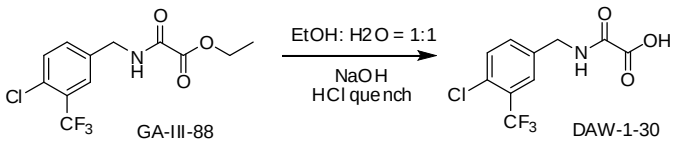
A solution of DAW-1-9 (237.1 mg, 0.86 mmol), 1 M NaOH (4 ml), H_2O (4 ml), and ethanol (8 ml) was stirred for 15 minutes. The reaction mixture was monitored by TLC (70% ethyl acetate : 30% hexane mobile phase) and the resulting solution was quenched with 1N HCl (12 ml) and allowed to cool in an ice bath for 1.5 hours, forming a precipitate. The solid was filtered and dried to provide 0.1603 g (75.4%) of DAW-1-21 as a white solid. ^{1}H NMR (DMSO-d_6, 400MHz) δ 9.42 (t, J=5.9 Hz, 1H), 7.56 (m, 4H), 4.36 (d, J=6.4 Hz, 2H); ^{13}C NMR (DMSO-d_6, 100 MHz) δ 162.49, 159.10, 140.57, 132.07, 129.96, 129.56 (q, J=30.5 Hz), 124.76 (q, J=270.8 Hz), 124.45 (q, J=3.8 Hz), 124.27 (q, J=3.2 Hz), 42.56; HRMS, DART calcd. for $C_{10}H_9F_3NO_3$ $[M+H]^+$ 248.05345, found: 248.05170.



DAW-1-21 (CDE-311) DAW-1-24 (CDE-310)

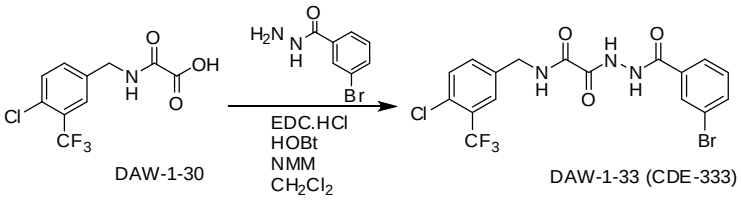
2-(2-(*tert*-Butyl)hydrazinyl)-2-oxo-*N*-(3-(trifluoromethyl)benzyl)acetamide [DAW-1-24 (CDE-310)]

To a mixture of DAW-1-21 (100.3 mg, 0.406 mmol), *tert*-butylhydrazine hydrochloride (60.5 mg, 0.486 mmol), 1-hydroxybenzotriazole [HOBt•H_2O] (76.1 mg, 0.486 mmol), and *N*-methylmorpholine [NMM] (53.5µl, 0.486 mmol) in methylene chloride (4 ml), 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride [EDC•HCl] (93.1 mg, 0.486 mmol) was added and stirred overnight. The reaction mixture was monitored by TLC (50% ethyl acetate: 50% hexane mobile phase) and the resulting solution was diluted with ≈20 ml of ethyl acetate and washed with 1N HCl (2x), saturated $NaHCO_3$ (2x), and brine solution, dried with $MgSO_4$, filtered, and concentrated *in vacuo* to obtain 0.0639 g (49.6%) of DAW-1-24 as a white solid. ^{1}H NMR ($CDCl_3$, 400 MHz) δ 8.53 (bs, 1H), 7.72 (bs, 1H), 7.56 (m, 2H), 4.55 (d, *J*=5.9 Hz, 2H), 4.47 (bs, 1H), 1.13 (s, 9H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ159.50, 157.92. 137.97, 131.24 (q, *J*=32.4 Hz), 131.22, 129.38, 124.78 (q, *J*=3.8 Hz), 124.65 (q, *J*=3.8 Hz), 123.97 (q, *J*=270.8 Hz), 55.70, 43.17, 27.20; HRMS, DART calcd. for $C_{14}H_{19}F_3N_3O_2$ $[M+H]^+$ 318.14293, found: 318.14240.



2-((4-Chloro-3-(trifluoromethyl)benzyl)amino)-2-oxoacetic acid [DAW-1-30]

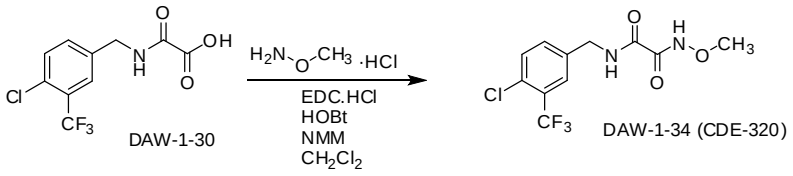
A solution of ethyl 2-oxo-2-((4-chloro-3-(trifluoromethyl)benzyl)amino)acetate [GA-III-88] (1.2757 g, 4.128 mmol), 1 M NaOH (16.5 ml), H_2O (16.5 ml), and ethanol (33 ml) was stirred for 15 minutes. The reaction mixture was monitored by TLC (70% ethyl acetate : 30% hexane mobile phase) and the resulting solution was quenched with 1 N H HCl (49.5 ml) and allowed to cool in an ice bath for 45 minutes, forming a precipitate. The solid was filtered and dried to provide 1.1967 g (quantitative yield) of DAW-1-30 as a white solid. ^{1}H NMR (DMSO-d_6, 400MHz) δ 9.42 (t, *J*=5.9 Hz, 1H), 7.73 (d, *J*=1.8 Hz, 1H), 7.65 (d, *J*=8.2 Hz, 1H), 7.54 (dd, *J*=6.4 Hz, *J*=1.8 Hz, 1H), 7.34 (d, *J*=6.4 Hz, 2H); ^{13}C NMR (DMSO-d_6, 100 MHz) δ 162.38, 159.10, 139.26, 133.70, 132.15, 129.70 (q, *J*=1.9 Hz), 127.39 (q, *J*=4.77 Hz), 126.95 (q, *J*=30.5 Hz), 123.4 (q, *J*=271.73 Hz), 42.09.



2-(2-(3-Bromobenzoyl)hydrazinyl)-*N*-(4-chloro-3-(trifluoromethyl)benzyl)-2-oxoacetamide [DAW-1-33 (CDE-333)]

To a mixture of DAW-1-30 (100.5 mg, 0.357 mmol), 3-bromobenzoic hydrazide (93.2 mg, 0.426 mmol), 1-hydroxybenzotriazole [HOBt•H_2O] (66.1 mg, 0.426 mmol), *N*-methylmorpholine [NMM] (47.0µl, 0.426 mmol) in methylene chloride (4 ml), 1-ethyl-3-(3-

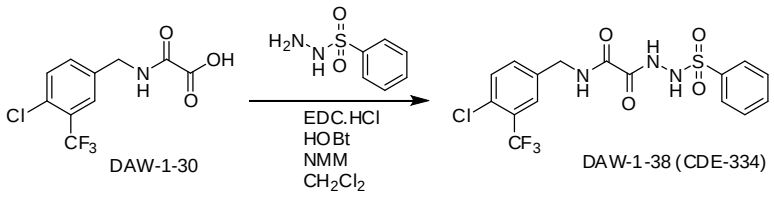
dimethylaminopropyl)carbodiimide hydrochloride [EDC•HCl] (83.0 mg, 0.426 mmol) was added and stirred for four days. The reaction mixture was filtered and the filtrate was diluted with ≈20 ml of ethyl acetate and washed with 1N HCl (2x), saturated $NaHCO_3$ (2x), and brine solution, and concentrated *in vacuo* to obtain 0.0365 g (21.3%) of DAW-1-33 as a yellow solid. ^{1}H NMR (DMSO-d_6, 400 MHz) δ 10.77 (bs, 1H), 10.64 (bs, 1H), 9.51 (t, *J*=4.5 Hz, 1H), 8.01(m, 1H), 7.84 (m, 1H), 7.76 (s, 1H), 7.68 (m, 2H), 7.57 (m, 1H), 4. 38 (d, *J*=13.3 Hz); ^{13}C NMR (DMSO-d_6, 100 MHz) δ 164.83, 160.36, 158.38, 139.41, 134.70, 133.78, 132.19, 131.19,130.60,129.72, 127.84 (q, *J*=43.8 Hz), 127.36 (q, *J*=6.6 Hz), 127.07 (q, *J*=6.6 Hz), 123.39 (q, *J*=271.7 Hz), 122.19, 120.32, 42.00; HRMS, DART calcd. for $C_{17}H_{13}BrClF_3N_3O_3$ $[M+H]^+$ 477.97810, found: 477.97821.



N^1-(4-Chloro-3-(trifluoromethyl)benzyl)-N^2-methoxyoxalamide [DAW-1-34 (CDE-320)]

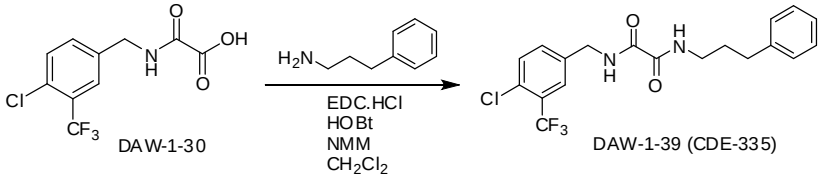
To a mixture of DAW-1-30 (103.8 mg, 0.369 mmol), methoxyamine hydrochloride (36.9 mg, 0.426 mmol), 1-hydroxybenzotriazole [HOBt•H_2O] (65.8 mg, 0.426 mmol), *N*-methylmorpholine [NMM] (47.0µl, 0.426 mmol) in methylene chloride (4 ml), 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride [EDC•HCl] (82.4 mg, 0.426 mmol) was added and stirred for four days. The reaction mixture was monitored by TLC(50% ethyl acetate: 50% hexane mobile phase) and the resulting solution was diluted with ≈20 ml of ethyl acetate and washed with 1N HCl (2x), saturated $NaHCO_3$ (2x), and brine solution, dried with $MgSO_4$, filtered, and concentrated *in vacuo* to obtain 0.0541 g (47.2%) of DAW-1-34 as a white solid. ^{1}H NMR (DMSO-d_6, 400 MHz) δ 12.11 (bs, 1H), 9.45 (t, *J*=5.9 Hz, 1H), 7.73 (d, *J*=1.8 Hz, 1H),

7.65 (d, *J*=8.2 Hz, 1H), 7.53 (dd, *J*=6.8 Hz, *J*=1.4 Hz, 1H), 4.33 (d, *J*=6.4 Hz, 2H), 3.59 (s, 3H); ^{13}C NMR (DMSO-d_6, 100 MHz) δ 160.17, 156.99, 139.26, 133.78, 132.16, 129.70, 127.52 (q, *J*=4.77 Hz), 126.92 (q, *J*=31.46 Hz), 223.38 (q, *J*=270.8 Hz), 63.67, 41.83; HRMS, DART calcd. for $C_{11}H_{11}ClF_3N_2O_3$ $[M+H]^+$ 311.04103, found: 311.03891.



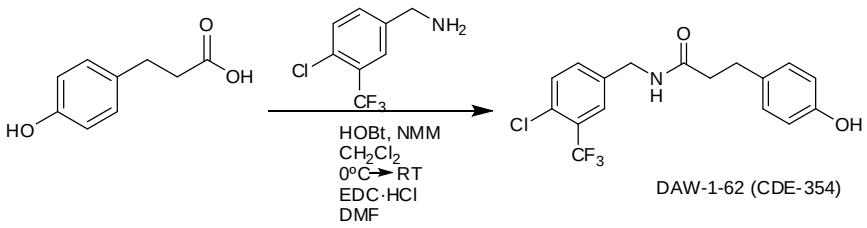
***N*-(4-Chloro-3-(trifluoromethyl)benzyl)-2-oxo-2-(2-(phenylsulfonyl)hydrazinyl)acetamide [DAW-1-38 (CDE-334)]**

To a mixture of DAW-1-30 (100.4 mg, 0.3565 mmol), benzenesulfonyl hydrazide (74.2 mg, 0.4263 mmol), 1-hydroxybenzotriazole [HOBt•H_2O] (66.2 mg, 0.426 mmol), *N*-methylmorpholine [NMM] (47.0µl, 0.426 mmol) in methylene chloride (4 ml), 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride [EDC•HCl] (82.3 mg, 0.426 mmol) was added and stirred overnight. The yellow reaction mixture was monitored by TLC (50% ethyl acetate: 50% hexane mobile phase) and the resulting solution was diluted with ≈20 ml of ethyl acetate and washed with 1N HCl (2x), saturated $NaHCO_3$ (2x), and brine solution (2x), dried with $MgSO_4$, filtered, and concentrated *in vacuo* to obtain 0.0563 g (36.3%) of DAW-1-38 as a white solid. ^{1}H NMR (DMSO-d_6, 400 MHz) δ 10.84 (d, *J*=2.3 Hz, 1H), 10.01 (d, *J*=2.7 Hz, 1H), 9.38 (t, *J*=5.9Hz, 1H), 7.78 (m, 3H), 7.57 (m, 5H), 4.28 (d, *J*=6.4 Hz, 2H); ^{13}C NMR (DMSO-d_6, 100 MHz) δ 159.65, 159.61, 139.75, 139.16, 133.69, 133.57, 132.16, 129.73, 129.38, 128.05, 127.34 (q, *J*=4.7 Hz), 126.95 (q *J*=30.5 Hz), 123.36 (q *J*=271.7 Hz), 41.87 ; HRMS, DART calcd. for $C_{16}H_{14}ClF_3N_3O_4S$ $[M+H]^+$ 436.03456, found: 436.03448.



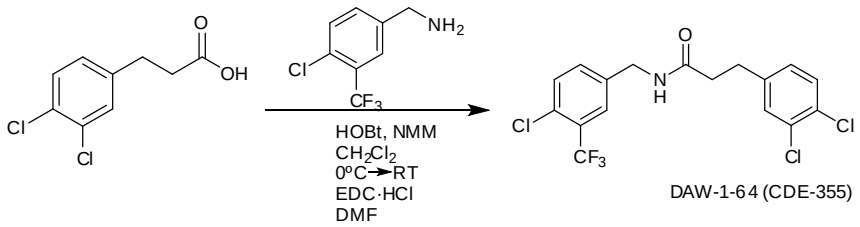
N^1-(4-Chloro-3-(trifluoromethyl)benzyl)-N^2-(3-phenylpropyl)oxalamide [DAW-1-39 (CDE-335)]

To a mixture of DAW-1-30 (101.6 mg, 0.361 mmol), 3-phenyl-1-propylamine (60.5 µl, 0.426 mmol), 1-hydroxybenzotriazole [HOBt•H_2O] (66.6 mg, 0.426 mmol), *N*-methylmorpholine [NMM] (47.0µl, 0.426 mmol) in methylene chloride (4 ml), 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride [EDC•HCl] (82.1 mg, 0.426 mmol) was added and stirred overnight. The brown reaction mixture was monitored by TLC (50% ethyl acetate: 50% hexane mobile phase) and the resulting solution was diluted with ≈20 ml of ethyl acetate and washed with 1N HCl (2x), saturated $NaHCO_3$ (2x), and brine solution (2x), and concentrated *in vacuo* to obtain 0.1285 g (89.3%) of DAW-1-39 as a white solid. ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.97 (t, *J*=20.6 Hz, 1H), 7.59 (d, *J*=1.3 Hz, 1H), 7.43 (m, 3H), 7.27 (m, 3H), 4.48 (d, *J*=6.4 Hz, 2H), 3.33 (q, *J*=6.84 Hz, 2H), 2.66 (t, *J*=7.3 Hz, 2H), 1.90 (p, *J*=7.3 Hz, 2H); ^{13}C NMR (DMSO-d_6, 100 MHz) δ 160.96,160.29, 142.08, 139.47, 133.75, 132.13, 129.66, 128.78, 127.46 (q, *J*=4.8 Hz), 126.85 (q, *J*=30.5 Hz), 126.26, 123.39 (q, *J*=270.8 Hz), 42.05, 39.16, 33.02, 30.89; HRMS, DART calcd. for $C_{19}H_{19}ClF_3N_2O_2$ $[M+H]^+$ 399.10872, found: 399.10779.



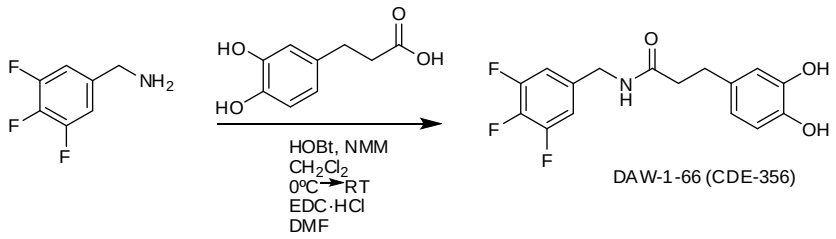
***N*-(4-Chloro-3-(trifluoromethyl)benzyl)-3-(4-hydroxyphenyl)propanamide [DAW-1-62 (CDE-354)]**

To a mixture of 3-(4-hydroxyphenyl)propanoic acid (182.9 mg, 1.097 mmol), 4-chloro-3-(trifluoromethyl)benzylamine (200 µl, 1.316 mmol), 1-hydroxybenzotriazole [HOBt•H_2O] (201.8 mg, 1.316 mmol), *N*-methylmorpholine [NMM] (145 µl, 1.316 mmol) in methylene chloride (5 ml) in an ice bath, 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride [EDC•HCl] (252.4 mg, 1.316 mmol) was added. The mixture was dissolved by adding dimethylformamide [DMF] (2 ml) and was stirred overnight. The yellow reaction mixture was reduced in volume *in vacuo* and the resulting solution was diluted with ≈15 ml of 4:1 of ethyl acetate:hexane and washed with 0.1N HCl (2x), saturated $NaHCO_3$ (2x), and brine solution (2x), dried over $MgSO_4$, and concentrated *in vacuo* to obtain 0.1623 g of DAW-1-62 as a dark yellow oil. The product was triturated with $CHCl_3$ to obtain 0.1090 g (27.8%) of pure DAW-1-62 as an off-white powder. ^{1}H NMR (DMSO-*d*$_6$, 400 MHz) δ 9.11 (s, 1H), 8.36 (t, *J*=5.8 Hz, 1H), 7.66 (s, 1H), 7.57 (d, *J*=8.2 Hz, 1H), 7.31 (d, *J*=7.3 Hz, 1H), 6.93 (d, *J*=8.2 Hz, 2H), 6.60 (d, *J*=8.2 Hz, 2H), 4.26 (d, *J*=5.9 Hz, 2H), 2.68 (t, *J*=7.4 Hz, 2H), 2.36 (t, *J*=7.3 Hz, 2H); ^{13}C NMR (DMSO-*d*$_6$, 100 MHz) δ 172.31, 156.02, 140.46, 133.28, 131.95, 131.67, 129.62, 129.30, 127.03 (q, *J*=4.7 Hz), 126.87 (q, *J*=30.5 Hz), 123.41 (q, *J*=271.7 Hz) 115.54, 41.57, 37.85, 30.76; HRMS, DART calcd. for $C_{17}H_{16}ClF_3NO_2$ $[M+H]^+$ 358.08217, found: 358.08139.



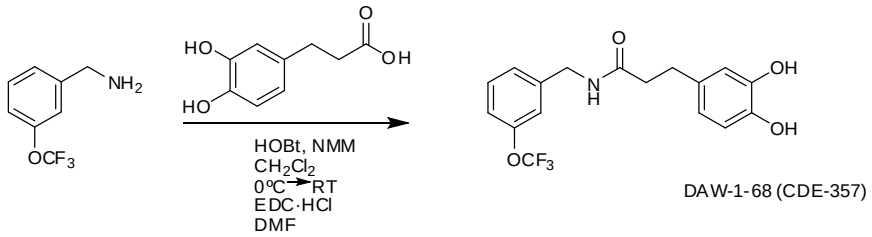
***N*-(4-Chloro-3-(trifluoromethyl)benzyl)-3-(3,4-dichlorophenyl)propanamide [DAW-1-64 (CDE-355)]**

To a mixture of 3-(3,4-dichlorophenyl)propanoic acid (240.8 mg, 1.097 mmol), 4-chloro-3-(trifluoromethyl)benzylamine (200 µl, 1.316 mmol), 1-hydroxybenzotriazole [HOBt•H_2O] (201.7 mg, 1.316 mmol), *N*-methylmorpholine [NMM] (145 µl, 1.316 mmol) in methylene chloride (5 ml) in an ice bath, 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride [EDC•HCl] (252.3 mg, 1.316 mmol) was added. The mixture was dissolved by adding dimethylformamide [DMF] (2 ml) and stirred overnight. The yellow reaction mixture was reduced in volume *in vacuo* and the resulting solution was diluted with ≈25 ml of 4:1 of ethyl acetate: hexane and washed with 0.1N HCl (2x), saturated $NaHCO_3$ (2x), and brine solution (1x), dried over $MgSO_4$, and concentrated *in vacuo* to obtain 0.240 g of DAW-1-64 as a yellow solid. The product was triturated with $CHCl_3$ to obtain 0.1825 g (40.5%) of pure DAW-1-64 as a white powder. ^{1}H NMR (DMSO-d_6, 400 MHz) δ 8.41 (t, *J*=5.5 Hz, 1H), 7.61 (d, *J*=1.8 Hz, 1H), 7.56 (d, *J*=8.2 Hz, 1H), 7.43 (d, *J*=8.2 Hz, 1H), 7.41 (d, *J*=1.8 Hz, 1H), 7.35 (dd, *J*=8.2 Hz, *J*=1.4 Hz, 1H), 7.14 (dd, *J*=8.3 Hz, *J*=2.3 Hz, 2H), 4.26 (d, *J*=5.9 Hz, 2H), 2.80 (t, *J*=7.3 Hz, 2H), 2.44 (t, *J*=7.3 Hz, 2H); ^{13}C NMR (DMSO-d_6, 100 MHz) δ 171.71, 142.89, 14350., 133.28, 131.93, 131.25, 130.86, 130.75, 129.34, 129.05, 127.05 (q, *J*=4.7 Hz), 126.92 (q, *J*=34.3 Hz), 123.36 (q, *J*=271.7 Hz), 41.60, 36.62, 30.37; HRMS, DART calcd. for $C_{17}H_{14}Cl_3F_3NO$ $[M+H]^+$ 410.00932, found: 410.00809.



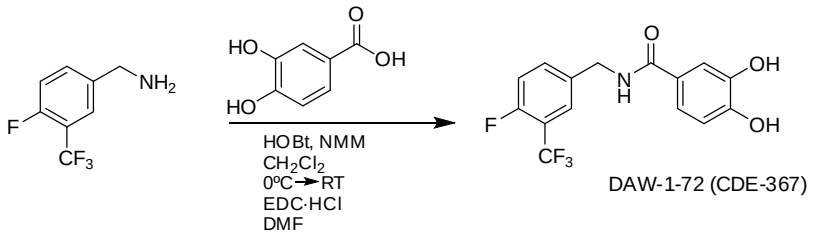
3-(3,4-Dihydroxyphenyl)-N-(3,4,5-trifluorobenzyl)propanamide [DAW-1-66 (CDE-356)]

To a mixture of 3,4,5-trifluorobenzylamine (201.8 µl, 1.647 mmol), 3-(3,4-dihydroxyphenyl)propanoic acid (250.5 mg, 1.372 mmol), 1-hydroxybenzotriazole [HOBt•H_2O] (252.9 mg, 1.647 mmol), *N*-methylmorpholine [NMM] (182.25 µl, 1.647 mmol) in methylene chloride (5 ml) in an ice bath, 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride [EDC•HCl] (316.2 mg, 1.647 mmol) was added. The mixture was dissolved by adding dimethylformamide [DMF] (2 ml) and stirred overnight. The dark yellow reaction mixture was reduced in volume *in vacuo* and the resulting solution was diluted with ≈25 ml of 4:1 of ethyl acetate: hexane and washed with 0.1N HCl (2x), saturated $NaHCO_3$ (2x), and brine solution (1x), dried over $MgSO_4$, and concentrated *in vacuo* to obtain 0.4753 g of DAW-1-66 as a brown oil. The product was triturated with $CHCl_3$ to obtain 0.4645 g (100%) of pure DAW-1-66 as a brown paste. ^{1}H NMR (DMSO-d_6, 400 MHz) δ 8.64 (s, 1H), 8.57 (s, 1H), 8.32 (t, *J*=5.9 Hz, 1H), 7.03 (m, 2H), 6.57 (m, 2H), 6.38 (dd, *J*=7.8 Hz, *J*=1.8 Hz, 1H), 4.18 (d, *J*=5.9 Hz, 2H), 2.62 (t, *J*=7.8 Hz, 2H), 2.34 (t, *J*=7.3 Hz, 2H); ^{13}C NMR (DMSO-d_6, 100 MHz) δ 172.38, 150.57 (ddd, *J*=246.0 Hz, *J*=9.5 Hz, *J*=3.8 Hz,), 145.52, 143.91, 137.94 (dt, *J*=245.0 Hz, *J*=16.2 Hz), 132.42, 119. 24, 116.20, 115.85, 111.94, 111.74, 41.44, 37.80, 30.92; HRMS, DART calcd. for $C_{16}H_{15}F_3NO_3$ $[M+H]^+$ 326.10042, found: 326.08871.



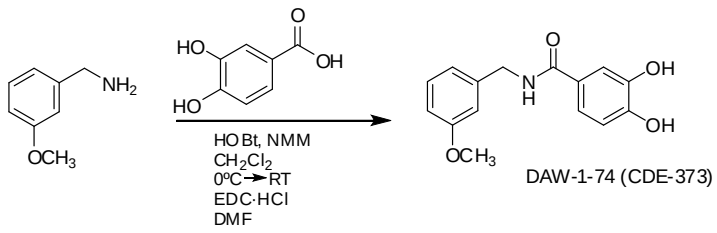
3-(3,4-Dihydroxyphenyl)-*N*-(3-(trifluoromethoxy)benzyl)propanamide [DAW-1-68 (CDE-357)]

To a mixture of 3-(triflouromethoxy)benzylamine (250 µl, 1.647 mmol), 3-(3,4-dihydroxyphenyl)propanoic acid (250.4 mg, 1.372 mmol), 1-hydroxybenzotriazole [HOBt•H_2O] (253.2 mg, 1.647 mmol), *N*-methylmorpholine [NMM] (182.25 µl, 1.647 mmol) in methylene chloride (5 ml) in an ice bath, 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride [EDC•HCl] (316.2 mg, 1.647 mmol) was added. The mixture was dissolved by adding dimethylformamide [DMF] (2 ml) and stirred overnight. The brown reaction mixture was reduced in volume *in vacuo* and the resulting solution was diluted with ≈25 ml of 4:1 of ethyl acetate: hexane and washed with 0.1N HCl (2x), saturated $NaHCO_3$ (2x), and brine solution (1x), dried over $MgSO_4$, and concentrated *in vacuo* to obtain DAW-1-68 as a brown oil. The product was triturated with $CHCl_3$ to obtain 0.2964 g (60.8%) of pure DAW-1-68 as a white powder. ^{1}H NMR (DMSO-d_6, 400 MHz) δ 8.62 (bs, 2H), 8.34 (t, *J*=5.9 Hz, 1H), 7.37 (dd, *J*=8.2 Hz, *J*=7.8 Hz, 1H), 7.16 (m, 2H), 7.10 (d, *J*=7.8 Hz, 1H), 6.56 (m, 2H), 6.39 (dd, *J*=8.2 Hz, *J*=1.8 Hz, 1H), 4.25 (d, *J*=5.9 Hz, 2H), 2.62 (t, *J*=7.32 Hz, 2H), 2.33 (t, *J*=7.4 Hz, 2H); ^{13}C NMR (DMSO-d_6, 100 MHz) δ 172.25, 148.90, 145.53, 143.88, 143.15, 132.51, 130.70, 126.62, 120.61 (q, *J*=254.6 Hz), 119.96, 119.62, 119.27, 116.67, 115.92, 41.91, 37.96, 31.08; HRMS, DART calcd. for $C_{17}H_{17}F_3NO_4[M+H]^+$ 356.11097, found: 356.10101.



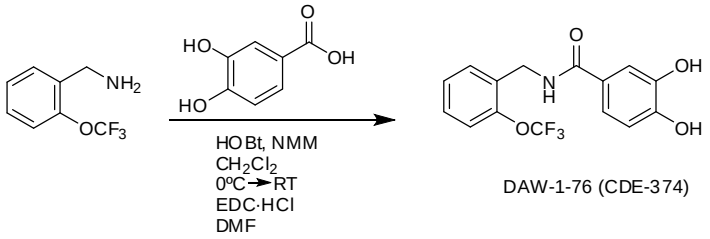
***N*-(4-Fluoro-3-(trifluoromethyl)benzyl)-3,4-dihydroxybenzamide [DAW-1-72 (CDE-367)]**

To a mixture of 4-fluoro-3-(trifluoromethyl)benzylamine (323.9mg, 1.647 mmol), 3,4-dihydroxybenzoic acid (211.8 mg, 1.372 mmol), 1-hydroxybenzotriazole [HOBt•H_2O] (252.9 mg, 1.647 mmol), *N*-methylmorpholine [NMM] (180.0 µl, 1.647 mmol) in methylene chloride (5 ml) in an ice bath, 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride [EDC•HCl] (317.5 mg, 1.647 mmol) was added. The mixture was dissolved by adding dimethylformamide [DMF] (2 ml) and stirred overnight. The yellow reaction mixture was reduced in volume *in vacuo* and the resulting solution was diluted with ≈25 ml of 4:1 of ethyl acetate: hexane and washed with 0.1N HCl (2x), saturated $NaHCO_3$ (2x), and brine solution (1x), dried over $MgSO_4$, and concentrated *in vacuo* to obtain 0.438 g of DAW-1-72 as a yellow solid. The product was triturated with $CHCl_3$ to obtain 0.226 g (50.0%) of pure DAW-1-72 as a yellow solid. ^{1}H NMR (DMSO-d_6, 400 MHz) δ 9.46 (s, 1H), 9.10 (s, 1H), 8.76 (t, *J*=5.9 Hz, 1H), 7.62 (m, 2H), 7.41 (dd, *J*=10.5 Hz, *J*=8.7 Hz, 1H), 7.27 (d, *J*=2.3 Hz, 1H), 7.18 (dd, *J*=8.2 Hz, *J*=2.3 Hz, 1H), 6.73 (d, *J*=8.2 Hz, 1H), 4.42 (d, *J*=5.9 Hz, 2H); ^{13}C NMR (DMSO-d_6, 100 MHz) δ 166.78, 158.26 (dq, *J*=250.8 Hz, *J*=1.9 Hz), 149.10, 145.43, 137.83 (d, *J*=3.8 Hz), 134.54 (d, *J*=8.6 Hz), 126.35 (q, *J*=4.8 Hz), 125.78, 123.23 (qd, *J*=269.8 Hz, *J*=9.5 Hz), 119.56, 117.56 (d, *J*=20.0 Hz), 116.65 (qd, *J*=32.4 Hz, *J*=12.4 Hz), 115.63, 42.11 ; HRMS, DART calcd. for $C_{15}H_{12}F_4NO_3[M+H]^+$ 330.07533, found: 330.06400.



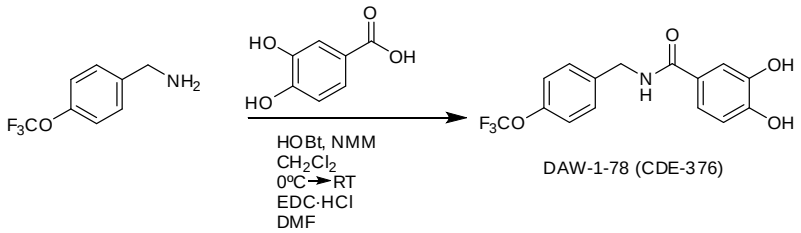
3,4-Dihydroxy-*N*-(3-methoxybenzyl)benzamide [DAW-1-74 (CDE-373)]

To a mixture of 3-methoxybenzylamine (220 µl, 1.647 mmol), 3,4-dihydroxybenzoic acid (211.5 mg, 1.372 mmol), 1-hydroxybenzotriazole [HOBt•H_2O] (251.7 mg, 1.647 mmol), *N*-methylmorpholine [NMM] (181 µl, 1.647 mmol) in methylene chloride (5 ml) in an ice bath, 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride [EDC•HCl] (316.6 mg, 1.647 mmol) was added. The mixture was dissolved by adding dimethylformamide [DMF] (2 ml) and stirred overnight. The light brown reaction mixture was diluted with ≈25 ml of 4:1 of ethyl acetate: hexane and washed with 0.1N HCl (2x), saturated $NaHCO_3$ (2x), and brine solution (1x), dried over $MgSO_4$, and concentrated *in vacuo* to obtain 0.1259 g of DAW-1-74 as a yellow crystals. The product was triturated with $CHCl_3$ to obtain 0.0694 g (18.5%) of pure DAW-1-74 as a yellow crystals. ^{1}H NMR (DMSO-d_6, 400 MHz) δ 9.40 (bs, 1H), 9.09 (bs, 1H), 8.63 (t, *J*=5.9 Hz, 1H), 7.27 (d, *J*=2.28 Hz, 1H), 7.18 (m, 2H), 6.81 (m, 2H), 6.73 (m, 2H), 4.35 (d, *J*=5.9 Hz, 2H), 3.39 (s, 3H); ^{13}C NMR (DMSO-d_6, 100 MHz) δ 166.64, 159.78, 148.93, 145.38, 142.25, 129.80, 126.15, 119.84, 119.51, 115.40, 113.42, 112.39, 55.47, 42.94; HRMS, DART calcd. for $C_{15}H_{16}NO_4$ $[M+H]^+$ 274.10792, found: 274.10089.



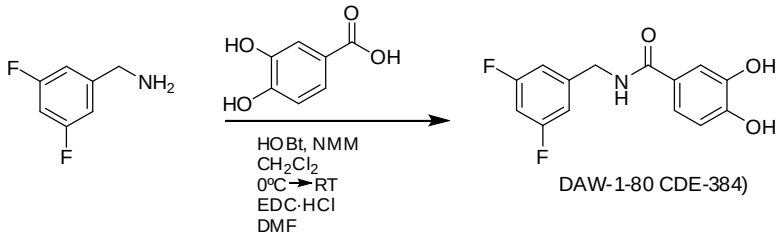
3,4-Dihydroxy-*N*-(2-(trifluoromethoxy)benzyl)benzamide [DAW-1-76 (CDE-374)]

To a mixture of 2-(trifluoromethoxy)benzylamine (246.25 µl, 1.647 mmol), 3,4-dihydroxybenzoic acid (211.4 mg, 1.372 mmol), 1-hydroxybenzotriazole [HOBt•H_2O] (252.9 mg, 1.647 mmol), *N*-methylmorpholine [NMM] (181 µl, 1.647 mmol) in methylene chloride (5 ml) in an ice bath, 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride [EDC•HCl] (315.8 mg, 1.647 mmol) was added. The mixture was dissolved by adding dimethylformamide [DMF] (2 ml) and stirred overnight. The light brown reaction mixture was diluted with ≈25 ml of 4:1 of ethyl acetate: hexane and washed with 0.1N HCl (2x). The aqueous layer was washed with saturated $NaHCO_3$ (2x), and a white solid formed, which was filtered and dried under vacuum to obtain 0.1226 g (27.3%) of DAW-1-76 as a white solid. ^{1}H NMR (DMSO-d_6, 400 MHz) δ 8.60 (bs, 1H), 7.33 (m, 4H), 7.23 (d, *J*=1.8 Hz, 1H), 7.16 (dd, *J*=8.2 Hz, *J*=2.3 Hz, 1H), 6.63 (d, *J*=8.2 Hz, 1H), 5.58 (bs, 2H), 4.44 (s, 2H); ^{13}C NMR (DMSO-d_6, 100 MHz) δ 167.08, 151.07, 146.57, 146.34, 132.97, 129.49, 128.94, 127.96, 124.46, 121.09, 120.81 (q, *J*=255.5 Hz), 119.48, 115.31, 115.02, 37.52; HRMS, DART calcd. for $C_{15}H_{13}F_3NO_4$ $[M+H]^+$ 328.07966, found: 328.07040.



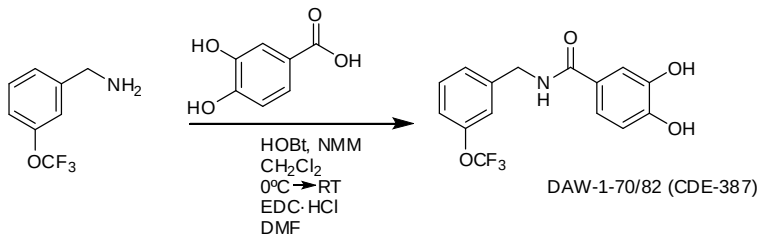
3,4-Dihydroxy-*N*-(4-(trifluoromethoxy)benzyl)benzamide [DAW-1-78 (CDE-376)]

To a mixture of 4-(trifluoromethoxy)benzylamine (251.5 µl, 1.647 mmol), 3,4-dihydroxybenzoic acid (211.6 mg, 1.372 mmol), 1-hydroxybenzotriazole [HOBt•H_2O] (252.2 mg, 1.647 mmol), *N*-methylmorpholine [NMM] (181 µl, 1.65 mmol) in methylene chloride (5 ml) in an ice bath, 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride [EDC•HCl] (315.8 mg, 1.647 mmol) was added. The mixture was dissolved by adding dimethylformamide [DMF] (2 ml) and stirred overnight. The brown reaction mixture was diluted with ≈25 ml of 4:1 of ethyl acetate: hexane and washed with 0.1N HCl (2x), saturated $NaHCO_3$ (2x), and brine solution (1x), dried over $MgSO_4$, and concentrated *in vacuo* to obtain 0.2477 g of DAW-1-78 as a white solid. The product was triturated with $CHCl_3$ to obtain 0.2271 g (50.6%) of pure DAW-1-78 as a grey crystals. ^{1}H NMR (DMSO-d_6, 400 MHz) δ 9.29 (bs, 1H), 8.71 (t, *J*=5.9 Hz, 1H), 7.36 (d, *J*=8.9 Hz, 2H), 7.26 (m, 3H), 7.20 (dd, *J*=8.2 Hz, *J*=2.3 Hz, 1H), 6.72 (d, *J*=8.3 Hz, 1H), 4.39 (d, *J*=5.3 Hz, 2H); ^{13}C NMR (DMSO-d_6, 100 MHz) δ 166.68, 149.03, 147.59, 145.41, 140.18, 129.48, 125.93, 121.41, 120.66 (q, *J*=258.4 Hz), 119.53, 115.66, 115.39, 42.38; HRMS, DART calcd. for $C_{15}H_{13}F_3NO_4$ $[M+H]^+$ 328.07966, found: 328.06619.



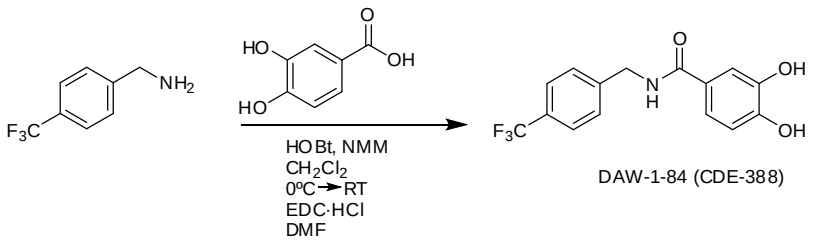
***N*-(3,5-Difluorobenzyl)-3,4-dihydroxybenzamide [DAW-1-80 (CDE-384)]**

To a mixture of 3,5-difluorobenzylamine (195.0 µl, 1.647 mmol), 3,4-dihydroxybenzoic acid (211.6 mg, 1.372 mmol), 1-hydroxybenzotriazole [HOBt•H_2O] (252.4 mg, 1.647 mmol), *N*-methylmorpholine [NMM] (181 µl, 1.65 mmol) in methylene chloride (5 ml) in an ice bath, 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride [EDC•HCl] (315.8 mg, 1.647 mmol) was added. The mixture was dissolved by adding dimethylformamide [DMF] (2 ml) and stirred overnight. The brown reaction mixture was diluted with ≈25 ml of 4:1 of ethyl acetate: hexane and washed with 0.1N HCl (2x), saturated $NaHCO_3$ (2x), and brine solution (1x), dried over $MgSO_4$, and concentrated *in vacuo* to obtain 0.2035 g of DAW-1-80 as a white solid. The product was triturated with $CHCl_3$ to obtain 0.1685 g (44%) of pure DAW-1-80 as a grey powder. ^{1}H NMR (DMSO-d_6, 400 MHz) δ 9.30 (s, 2H), 8.74 (t, *J*=5.9 Hz, 1H), 7.28 (d, *J*=1.8 Hz, 1H), 7.21 (dd, *J*=8.2 Hz, *J*=1.8 Hz, 1H), 7.03 (t, *J*=9.6 Hz, 1H), 6.94 (d, *J*=6.4 Hz, 2H), 6.73 (d *J*=8.3 Hz, 1H), 4.38 (d, *J*=5.9 Hz, 2H); ^{13}C NMR (DMSO-d_6, 100 MHz) δ 166.84, 162.87 (dd, *J*=231.6 Hz, *J*=13.4Hz), 149.13, 145.55 (t, *J*=8.5 Hz), 145.44, 125.73, 119.60, 115.63, 115.44, 110.54 (dd, *J*=19.07 Hz, *J*=6.7 Hz), 102.54 (t, *J*=25.71 Hz), 42.45; HRMS, DART calcd. for $C_{14}H_{12}F_2NO_3$ $[M+H]^+$ 280.07853, found: 280.06491.



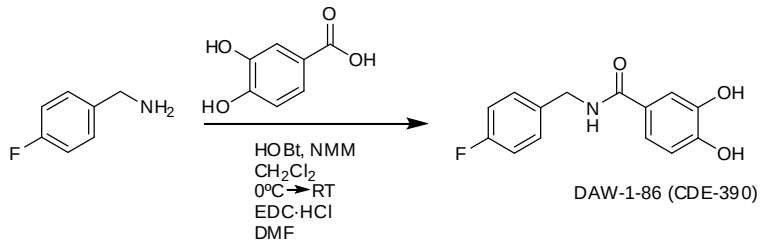
3,4-Dihydroxy-*N*-(3-(trifluoromethoxy)benzyl)benzamide [DAW-1-70/ 82 (CDE-387)]

To a mixture of 3-(trifluoromethoxy)benzylamine (247.0 µl, 1.647 mmol), 3,4-dihydroxybenzoic acid (211.8 mg, 1.372 mmol), 1-hydroxybenzotriazole [HOBt•H_2O] (252.9 mg, 1.647 mmol), *N*-methylmorpholine [NMM] (181.0 µl, 1.647 mmol) in methylene chloride (5 ml) in an ice bath, 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride [EDC•HCl] (315.8 mg, 1.647 mmol) was added. The mixture was dissolved by adding dimethylformamide [DMF] (2 ml) and stirred overnight. The brown reaction mixture was diluted with ≈25 ml of 4:1 of ethyl acetate: hexane and washed with 0.1N HCl (2x), saturated $NaHCO_3$ (2x), and brine solution (1x), dried over $MgSO_4$, and concentrated *in vacuo* to obtain 0.3098 g of DAW-1-82 as a white solid. The product was triturated with $CHCl_3$ to obtain 0.257 g (57.3%) of pure DAW-1-82 as a white powder. ^{1}H NMR (DMSO-d_6, 400 MHz) δ 9.30 (s, 2H), 8.74 (t, *J*=5.9 Hz, 1H), 7.41 (t, *J*=8.2 Hz, 1H), 7.22 (m, 5H), 6.73 (d, *J*=8.2 Hz, 1H), 4.43 (d, *J*=5.5 Hz, 2H); ^{13}C NMR (DMSO-d_6, 100 MHz) δ 166.79, 149.06, 148.96, 145.43, 143.62, 130.69, 126.69,125.89, 120.61 (q, *J*=255.5 Hz), 119.95, 119.57, 119.54, 115.63, 115.42, 42.54; HRMS, DART calcd. for $C_{15}H_{13}F_3NO_4$ $[M+H]^+$ 328.07966, found: 328.07059.



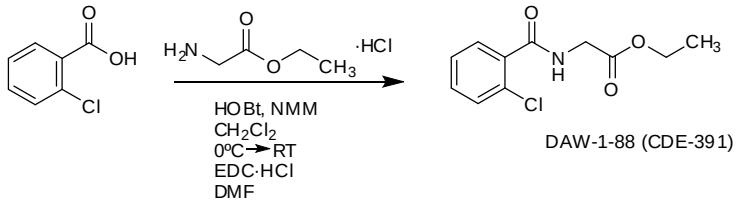
3,4-Dihydroxy-*N*-(4-(trifluoromethyl)benzyl)benzamide [DAW-1-84 (CDE-388)]

To a mixture of 4-(trifluoromethyl)benzylamine (235.0 µl, 1.647 mmol), 3,4-dihydroxybenzoic acid (211.7 mg, 1.372 mmol), 1-hydroxybenzotriazole [HOBt•H_2O] (252.9 mg, 1.647 mmol), *N*-methylmorpholine [NMM] (181.0 µl, 1.647 mmol) in methylene chloride (5 ml) in an ice bath, 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride [EDC•HCl] (315.9 mg, 1.647 mmol) was added. The mixture was dissolved by adding dimethylformamide [DMF] (2 ml) and stirred overnight. The brown reaction mixture was diluted with ≈25 ml of 4:1 of ethyl acetate: hexane and washed with 0.1N HCl (2x), saturated $NaHCO_3$ (2x), and brine solution (1x), dried over $MgSO_4$, and concentrated *in vacuo* to obtain 0.2817 g of DAW-1-84 as a white solid. The product was triturated with $CHCl_3$ to obtain 0.2467 g (57.8%) of pure DAW-1-84 as a white powder. ^{1}H NMR (DMSO-*d*$_6$, 400 MHz) δ 9.32 (s, 2H), 8.77 (t, *J*=5.5 Hz, 1H), 7.64 (d, *J*=8.2 Hz, 2H), 7.45 (d, *J*=7.8 Hz, 2H), 7.28 (d, *J*=2.3 Hz, 1H), 7.20 (dd, *J*=8.3 Hz, 1.8 Hz, 1H), 6.72 (d, *J*=8.2 Hz, 1H), 4.45 (d, *J*=5.5 Hz, 2H); ^{13}C NMR (DMSO-*d*$_6$, 100 MHz) δ 166.77, 149.08, 148.96, 145.55, 145.44, 128.31, 127.84 (q, *J*=32.4 Hz), 125.82, 125.63 (q, *J*=3.8 Hz), 124.91 (q, *J*=270.8 Hz), 119.56, 115.65, 115.41, 42.74; HRMS, DART calcd. for $C_{15}H_{13}F_3NO_3$ $[M+H]^+$ 312.08476, found: 312.07681.



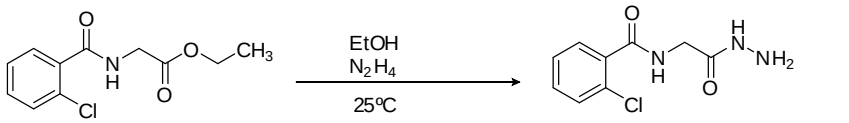
***N*-(4-(Fluorobenzyl)-3,4-dihydroxybenzamide [DAW-1-86 (CDE-390)]**

To a mixture of 4-fluorobenzylamine (188 µl, 1.65 mmol), 3,4-dihydroxybenzoic acid (211.3 mg, 1.372 mmol), 1-hydroxybenzotriazole [HOBt•H_2O] (252.0 mg, 1.647 mmol), *N*-methylmorpholine [NMM] (180.0 µl, 1.647 mmol) in methylene chloride (5 ml) in an ice bath, 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride [EDC•HCl] (315.2 mg, 1.647 mmol) was added. The mixture was dissolved by adding dimethylformamide [DMF] (2 ml) and stirred overnight. The brown reaction mixture was diluted with ≈25 ml of 4:1 of ethyl acetate: hexane and washed with 0.1N HCl (2x), saturated $NaHCO_3$ (2x), and brine solution (1x), dried over $MgSO_4$, and concentrated *in vacuo* to obtain 0.1910 g of DAW-1-86 as a white solid. The product was triturated with $CHCl_3$ to obtain 0.1524 g (42.5%) of pure DAW-1-86 as a white powder. ^{1}H NMR (DMSO-d_6, 400 MHz) δ 9.37 (s, 1H), 9.17 (s, 1H), 8.67 (t, *J*=3.7 Hz, 1H), 7.17 (m, 6H), 6.71 (dd, *J*=7.8 Hz, 4.1 Hz, 1H), 4.35 (s, 2H); ^{13}C NMR (DMSO-d_6, 100 MHz) δ 166.62, 161.59 (d, *J*=240.3 Hz), 148.94, 145.38, 136.77, 129.62 (d, *J*=8.6 Hz), 126.04, 119.52, 115.53, 115.51 (d, *J*=27.6 Hz), 115.32, 42.32; HRMS, DART calcd. for $C_{14}H_{13}FNO_3$ $[M+H]^+$ 262.08793, found: 262.08200.



Ethyl-2-(2-chlorobenzamide)acetate [DAW-1-88 (CDE-391)]

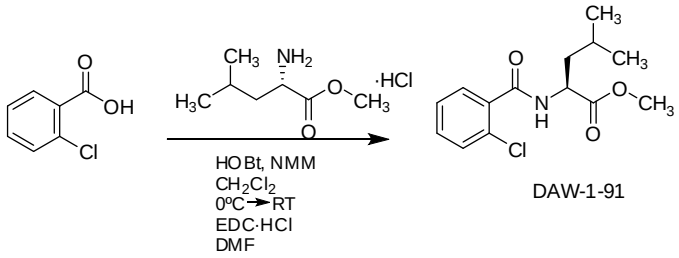
To a mixture of glycine ethyl ester hydrochloride (230.0 mg, 1.647 mmol), 2-chlorobenzoic acid (214.6 mg, 1.372 mmol), 1-hydroxybenzotriazole [HOBt•H_2O] (252.8 mg, 1.647 mmol), *N*-methylmorpholine [NMM] (181.0 µl, 1.647 mmol) in methylene chloride (5 ml) in an ice bath, 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride [EDC•HCl] (315.5 mg, 1.647 mmol) was added. The mixture was dissolved by adding dimethylformamide [DMF] (2 ml) and stirred overnight. The yellow reaction mixture was diluted with ≈25 ml of 4:1 of ethyl acetate: hexane and washed with 0.1N HCl (2x), saturated $NaHCO_3$ (2x), and brine solution (1x), dried over $MgSO_4$, and concentrated *in vacuo* to obtain 0.2929 g (88.34%) of DAW-1-86 as a yellow solid. ^{1}H NMR (DMSO-d_6, 400 MHz) δ 7.71 (dd, *J*=7.3 Hz, 1.8 Hz, 1H), 7.36 (m, 3H), 6.81 (s, 1H), 4.25 (m 4H), 1.31 (t, *J*=6.9 Hz, 3H); ^{13}C NMR (DMSO-d_6, 100 MHz) δ 169.70, 165.95, 134.23, 131.72, 131.01, 130.48, 130.46, 127.18, 61.82, 42.10, 14.25; HRMS, DART calcd. for $C_{11}H_{13}ClNO_3$ $[M+H]^+$ 242.05839, found: 242.05260.



2- Chloro-*N*-(2-hydrazinyl-2-oxoethyl)benzamide [DAW-1-90 (CDE-392)]

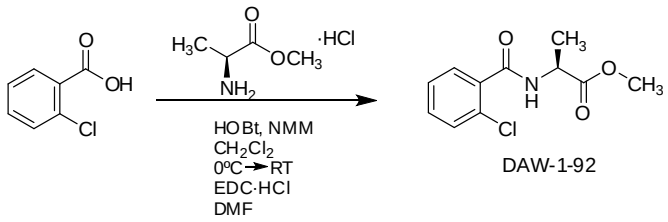
To a mixture of DAW-1-88 (101.1 mg, 0.42 mmol) in ethanol (4.2 ml), hydrazine hydrate (~50% solution in H_2O, 104.5 µl, 1.68 mmol) was added. The white reaction mixture was stirred for three days, filtered, and the solid was dried *in vacuo* to provide 0.0148 g (18.3%) of DAW-1-90

as a white solid. ^{1}H NMR (DMSO-d_6, 400MHz) δ 9.02 (s, 1H), 8.58 (t, *J*=5.5 Hz, 1H), 7.41 (m, 4H), 4.21 (s, 2H), 3.76 (d, *J*=5.9 Hz, 2H); ^{13}C NMR (DMSO-d_6, 100 MHz) δ 168.43, 167.08, 136.82, 131.45, 130.53, 130.18, 129.75, 127.52, 41.56; HRMS, DART calcd. for $C_9H_{11}ClN_3O_2$ $[M+H]^+$ 228.05398, found: 228.05170.



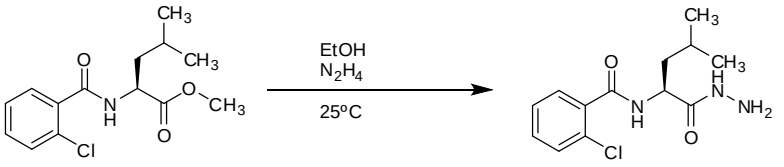
(*S*)-Methyl-2-(2-chlorobenzamido)-4-methylpentanoate [DAW-1-91]

To a mixture of *L*-leucine methyl ester hydrochloride (300.1 mg, 1.647 mmol), 2-chlorobenzoic acid (215.0 mg, 1.372 mmol), 1-hydroxybenzotriazole [HOBt•H_2O] (254.4 mg, 1.647 mmol), *N*-methylmorpholine [NMM] (181.0 µl, 1.647 mmol) in methylene chloride (5 ml) in an ice bath, 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride [EDC•HCl] (315.5 mg, 1.647 mmol) was added. The mixture was dissolved by adding dimethylformamide [DMF] (2 ml) and stirred for four days. The yellow reaction mixture was diluted with ≈25 ml of 4:1 of ethyl acetate: hexane and washed with 0.1N HCl (2x), saturated $NaHCO_3$ (2x), and brine solution (1x), dried over $MgSO_4$, and concentrated *in vacuo* to obtain 0.3800 g (97.6%) of DAW-1-91 as a yellow solid. ^{1}H NMR (DMSO-d_6, 400 MHz) δ 7.65 (m, 1H), 7.34 (m, 3H), 6.61 (d, *J*=7.3Hz, 1H), 4.84 (m, 1H), 3.76 (s, 3H), 1.72 (m, 3H), 0.99 (d, *J*=5.5 Hz, 3H), 0.96 (d, *J*=5.9 Hz, 3H); ^{13}C NMR (DMSO-d_6, 100 MHz) δ 173.24, 166.13, 134.61, 131.56, 130.86, 130.34, 127.14, 52.45, 51.41, 41.71, 52.01, 22.90, 22.05.



(*S*)-Methyl-2-(2-chlorobenzamido)propanoate [DAW-1-92]

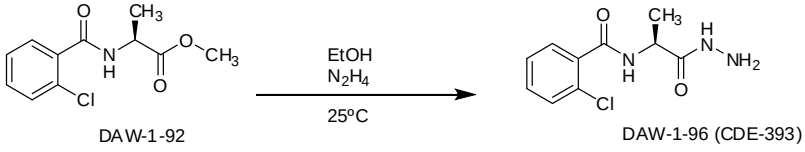
To a mixture of *L*-alanine methyl ester hydrochloride (230.3 mg, 1.647 mmol), 2-chlorobenzoic acid (215.0 mg, 1.372 mmol), 1-hydroxybenzotriazole [HOBt•H_2O] (253.0 mg, 1.647 mmol), *N*-methylmorpholine [NMM] (181.0 µl, 1.647 mmol) in methylene chloride (5 ml) in an ice bath, 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride [EDC•HCl] (315.8 mg, 1.647 mmol) was added. The mixture was dissolved by adding dimethylformamide [DMF] (2 ml) and stirred for four days. The yellow reaction mixture was diluted with ≈25 ml of 4:1 of ethyl acetate: hexane and washed with 0.1N HCl (2x), saturated $NaHCO_3$ (2x), and brine solution (1x), dried over $MgSO_4$, and concentrated *in vacuo* to obtain 0.3026 g (91.26%) of DAW-1-92 as a thick yellow oil. ^{1}H NMR (DMSO-d_6, 400 MHz) δ 7.67 (dd, *J*=7.3 Hz, 2.3 Hz, 1H), 7.36 (m, 3H), 6.83 (d, *J*=4.6 Hz, 1H), 4.81 (p, *J*=7.3 Hz, 1H), 3.79 (s, 3H), 1.53 (d, *J*=6.9 Hz, 3H); ^{13}C NMR (DMSO-d_6, 100 MHz) δ 173.31, 165.82, 134.51, 131.62, 130.95, 130.41, 130.35, 127.16, 52.69, 48.79, 18.59.



DAW-1-91 DAW-1-94 (CDE-401)

(*S*)-2- Chloro-*N*-(1-hydrazinyl-4-methyl-1-oxopentan-2-yl)benzamide [DAW-1-94 (CDE-401]

To a mixture of DAW-1-91 (150.5 mg, 0.529 mmol) in ethanol (2.1 ml), hydrazine hydrate (~50% solution in H_2O, 197.5 µl, 3.174 mmol) was added. The reaction mixture was stirred for three days. The brown reaction mixture was diluted with ≈15 ml of dichloromethane and washed with 0.1N HCl (2x), saturated NaHCO3 (2x), and brine solution (1x), dried over $MgSO_4$, and concentrated *in vacuo* to obtain 0.0396 g of DAW-1-94 as a light yellow solid. The product was triturated with CH_3OH to obtain 0.0281 g (18.72%) of pure DAW-1-94 as a pale yellow solid. 1H NMR (DMSO-d6, 400MHz) δ 11.01 (s, 1H), 8.55 (m, 1H), 7.40 (m, 5H), 5.21 (m, 5H), 4.40 (m, 1H), 1.84 (m, 2H), 1.58 (m, 1H), 0.86 (m, 6H); 13C NMR (DMSO-d6, 100 MHz) δ 170.25, 166.16, 145.57, 136.65, 130.68, 129.90, 128.99, 128.19, 49.35, 40.68, 24.47, 23.09, 21.25.

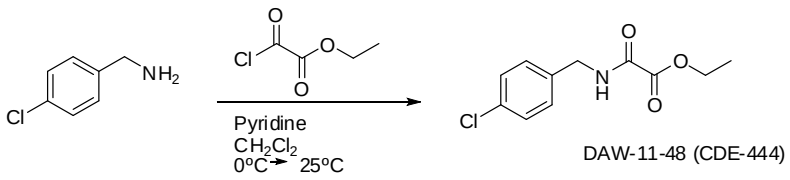


DAW-1-92 DAW-1-96 (CDE-393)

(*S*)-2- Chloro-*N*-(1-hydrazinyl-2-oxopropan-2-yl)benzamide [DAW-1-96 (CDE-393)]

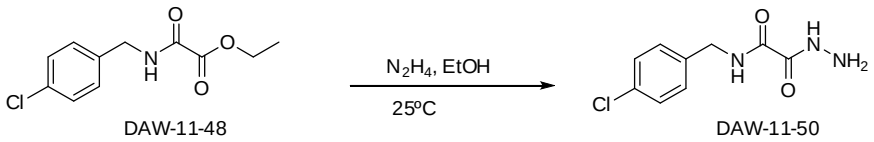
To a mixture of DAW-1-92 (127.9 mg, 0.529 mmol) in ethanol (2.0 ml), hydrazine hydrate (~50% solution in H_2O, 197.5 µl, 3.174 mmol) was added. The milky reaction mixture was stirred for three days, filtered, and the solid was dried *in vacuo* to provide 0.0608 g (47.56%) of DAW-1-96 as a white solid. ^{1}H NMR (DMSO-d_6, 400MHz) δ 9.06 (s, 1H), 8.51 (d, *J*=7.8 Hz,

1H), 7.39 (m, 4H), 4.37 (p, *J*=7.4 Hz, 1H), 4.21 (s, 2H), 1.23 (d, *J*=6.9 Hz, 3H); ^{13}C NMR (DMSO-d_6, 100 MHz) δ 171.78, 166.37, 136.98, 131.31, 130.51, 130.04, 129.69, 127.44, 47.92, 18.75; HRMS, DART calcd. for $C_{10}H_{13}ClN_3O_2$ $[M+H]^+$ 242.06962, found: 242.06590.



Ethyl-2-(4-chlorobenzylamino)-2-oxoacetate [DAW-11-48 (CDE-444)]

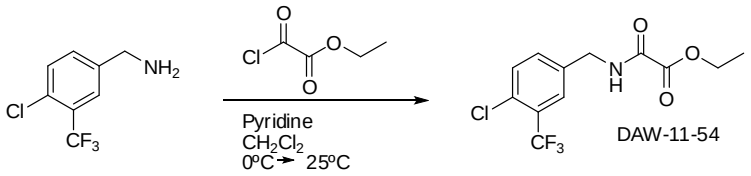
The solution of 4-chlorobenzylamine (547.5 µl, 4.5 mmol), dry pyridine (1.09 ml, 13.5 mmol) and methylene chloride (18 ml) was cooled in an ice bath. Ethyl oxalyl chloride (502.8 µl, 4.5 mmol) was added and the resulting milky solution was stirred for three days. The clear reaction mixture was concentrated to half volume, diluted with ≈50 ml of ethyl acetate and washed with 0.2N HCl (2x), saturated $NaHCO_3$ (2x), and brine (1x), dried with $MgSO_4$, filtered, and concentrated *in vacuo* to obtain 0.9790 g (90.02%) of DAW-11-48 as a pale yellow solid. ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.40 (s, 1H), 7.28 (m, 2H), 7.21 (m, 2H), 4.47 (d, *J*=6.4 Hz, 2H), 4.33 (q, *J*=6.9 Hz, 2H), 1.37 (t, *J*=6.9 Hz, 3H); HRMS, DART calcd. for $C_{11}H_{13}ClNO_3$ $[M+H]^+$ 242.05839, found: 242.05910.



***N*-(4-chlorobenzyl)-2-hydrazinyl-2-oxoacetamide [DAW-11-50]**

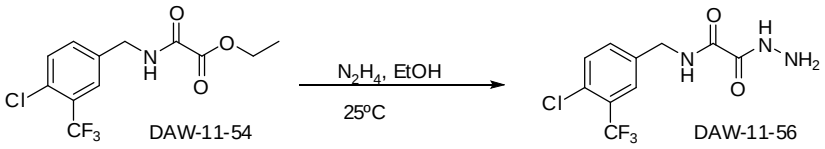
To a mixture of DAW-11-48 (956.1 mg, 3.956 mmol) in ethanol (44.0 ml), hydrazine hydrate (~50% solution in H_2O, 537.5 µl, 8.643 mmol) was added. The milky reaction mixture was stirred for two hours, filtered, and the solid was washed with ethanol, and dried *in vacuo* to

provide 0.7773 g (86.3%) of DAW-11-50 as a white solid. ^{1}H NMR (DMSO-d_6, 400MHz) δ 9.99 (s, 1H), 9.25 (t, *J*=5.9 Hz, 1H), 7.33 (d, *J*=8.2 Hz, 2H), 7.23 (d, *J*=8.2 Hz, 2H), 4.48 (s, 2H), 4.25 (d, *J*=6.4 Hz, 2H); ^{13}C NMR (DMSO-d_6, 100 MHz) δ 159.86, 157.94, 137.85, 131.42, 129.19, 128.19, 41.50.



Ethyl-2-(4-chloro-3-(trifluoromethyl)benzylamino)-2-oxoacetate [DAW-11-54]

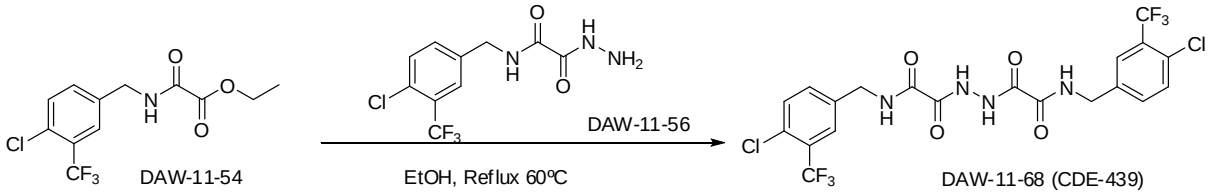
A solution of 4-chloro-3-(trifluoromethyl)benzylamine (3.04 ml, 20 mmol), pyridine (4.86 ml, 60 mmol) and methylene chloride (80 ml) was cooled in an ice bath. Ethyl oxalyl chloride (2.24 ml, 20 mmol) was added and the resulting milky solution was stirred 48 hours. The reaction mixture was concentrated, diluted with ≈75 ml of ethyl acetate and washed with 0.2N HCl (2x) and saturated $NaHCO_3$ (2x), and brine, dried with $MgSO_4$, filtered, and concentrated *in vacuo* to obtain 4.98 g (80.4%) of DAW-11-54 as a pale yellow solid. ^{1}H NMR ($CDCl_3$, 400MHz) δ 7.60 (d, *J*=1.8 Hz, 1H), 7.45 (m, 3H), 4.53 (d, *J*=6.4 Hz, 2H), 4.35 (q, *J*=7.3 Hz, 2H), 1.38 (t, *J*=7.4 Hz, 3H).



***N*-(4-Chloro-3-(trifluoromethyl)benzyl)-2-hydrazynil-2-oxoacetamide [DAW-11-56]**

To a mixture of DAW-1-54 (2.5009 g, 8.07 mmol) in ethanol (80.0 ml), hydrazine hydrate (~50% solution in H_2O, 1.034 ml, 16.15 mmol) was added. The reaction mixture was stirred for ~2 hours, filtered, triturated with ethanol, and the solid was dried *in vacuo* to provide 1.843 g

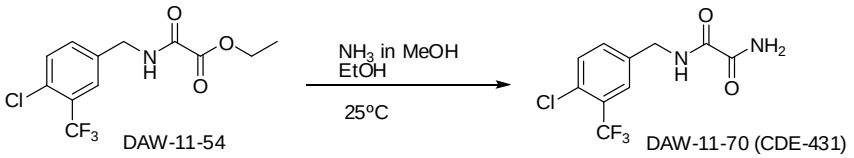
(77.3%) of DAW-11-56 as a white solid. ^{1}H NMR (DMSO-d_6, 400MHz) δ 10.03 (bs, 1H), 9.36 (t, *J*= 5.9 Hz, 1H), 7.72 (s, 1H), 7.64 (d, *J*=8.3 Hz, 1H), 7.52 (d, *J*=8.3 Hz, 1H), 4.52 (d, *J*=3.2 Hz, 2H), 4.33 (d, *J*=6.4 Hz, 2H); ^{13}C NMR (DMSO-d_6, 100 MHz) δ 160.03, 157.76, 138.98, 133.15, 131.57, 129.08, 126.79 (q, *J*=4.8 Hz), 126.35 (q, *J*=30.5 Hz), 122.83 (q, *J*=271.7 Hz), 41.28.



2,2^{1}-(Hydrazine-1,2-diyl)bis(*N*-(4-chloro-3-(trifluoromethyl)benzyl)-2-oxoacetamide)

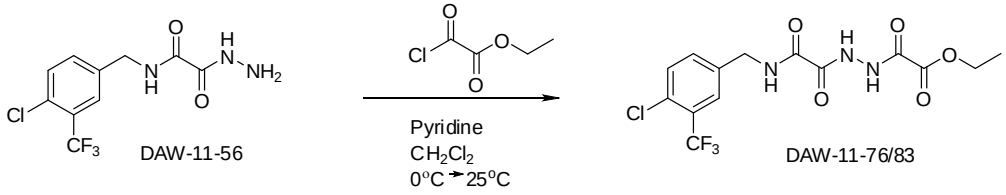
[DAW-11-68 (CDE-439)]

To a mixture of DAW-1-54 (77.4 mg, 0.25 mmol) in ethanol (2.0 ml), DAW-11-56 (73.9 mg, 0.25 mmol) was added. The reaction mixture was stirred for 6 days while monitoring by thin layaer chromatography [TLC] (60% EtOAc : 40% Hexane). Mixture was then refluxed at 60 ºC for 24 hours and visualized by TLC using same solvent ratio. After cooling, it was *in vacuo* and solid was triturated with chloroform-D to obtain 0.1235 g of crude DAW-11-68 as a white solid. It was purified by a column (eluent - 90% EtOAc : 10% hexane) to afford 0.0527 g (37.7%) of DAW-1-68 as a white solid. ^{1}H NMR ($CDCl_3$, 400MHz) δ 9.84 (bs, 2H), 7.92 (bs, 2H), 7.60 (d, *J*=1.8 Hz, 2H), 7.48 (d, *J*=8.2 Hz, 2H), 7.40 (dd, *J*=8.2 Hz, 2.3 Hz. 2H), 4.52 (d, *J*=6.4 Hz, 4H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 159.91, 159.57, 154.88, 135.92, 132.12, 131.12, 131.87, 128.78 (q, *J*=31.4 Hz), 126.96 (q, *J*=4.7 Hz), 122.56 (q, *J*=271.7 Hz), 42.66.



***N*[1]-(4-Chloro-3-(trifluoromethyl)benzyl)oxalamide [DAW-11-70 (CDE-431)]**

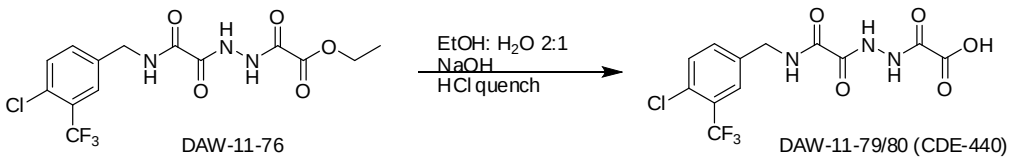
To a mixture of DAW-1-54 (100.1 mg, 0.323 mmol) in ethanol (2.0 ml), 7N ammonia (1 ml) was added. The mixture was stirred for two hours, filtered, and the solid was dried *in vacuo* to provide 0.0528 g (58.3%) of DAW-11-70 as a white solid. ^{1}H NMR (DMSO-d_6, 400MHz) δ 9.35 (t, *J*=6.4 Hz, 1H), 8.06 (s, 1H), 7.78 (s, 1H), 7.72 (d, *J*=1.8 Hz, 1H), 7.64 (d, *J*=8.2 Hz, 1H), 7.53 (dd, *J*=8.2 Hz, 1.4 Hz, 1H), 4.33 (d, *J*=6.4 Hz, 2H); ^{13}C NMR (DMSO-d_6, 100 MHz) δ 161.89, 160.63, 138.96, 133.16, 131.58, 129.07, 126.84 (q, *J*=5.7 Hz), 126.36 (q, *J*=29.6 Hz), 122.84 (q, *J*=271.7 Hz), 41.47; HRMS, DART calcd. for $C_{10}H_7ClF_3N_2O_2$ $[M+H]^+$ 281.03047, found: 281.02899.



Ethyl 2-(2-(2-(4-chloro-3-(trifluoromethyl)benzylamino)-2-oxoacetyl)hydrazinyl)-2-oxoacetate [DAW-11-76 / 83]

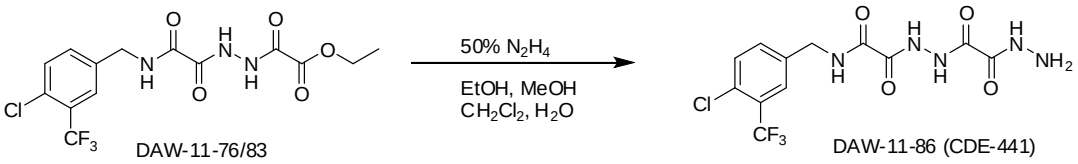
A solution of DAW-11-56 (1.0343 g, 3.5 mmol) in dimethyl formamide [DMF] (12.0 ml) and pyridine (0.850 ml, 10.5 mmol) was cooled in an ice bath. Ethyl oxalyl chloride (470.0 µl, 4.2 mmol) was added and the resulting mixture was stirred for five days and visualized by thin layer chromatography [TLC] (100% EtOAc). The reaction mixture was concentrated to remove pyridine, diluted with 4:1 mixture of ethyl acetate: hexane (≈120 ml) and washed with 0.2N HCl (2x) and saturated $NaHCO_3$ (2x), and brine, dried with $MgSO_4$, filtered, and concentrated *in*

vacuo to obtain 0.550 g (39.7%) of DAW-11-76/83 as an off white solid. ^{1}H NMR (DMSO-d_6, 400MHz) δ 10.58 (bs, 1H), 9.35 (bs, 1H), 7.72 (d, *J*=1.8 Hz, 1H), 7.64 (d, *J*=8.2 Hz, 1H), 7.53 (dd, *J*=8.2 Hz, 1.8 Hz, 1H), 4.34 (d, *J*=6.4 Hz, 2H), 4.07 (q, *J*=6.9 Hz, 2H), 1.19 (t, *J*=6.9 Hz, 3H).



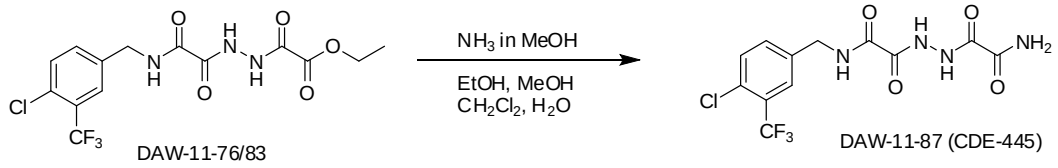
2-(2-(2-(4-Chloro-3-(trifluoromethyl)benzylamino)-2-oxoacetyl)hydrazinyl)-2-oxoacetic acid [DAW-11-79 / 80]

A solution of DAW-11-76 (81.8 mg, 0.207 mmol), 1 M NaOH (1.6 ml), H_2O (1.6 ml), and ethanol (3.25 ml) was stirred for 1 hour. The reaction mixture was monitored by TLC (100% ethyl acetate) and the resulting solution was quenched with 1N HCl (4 ml) and allowed to cool in an ice bath for 30 minutes, forming a precipitate. The solid was filtered and dried to provide 0.478 g (62.8%) of DAW-1-79/80 as a yellow solid. ^{1}H NMR (DMSO-d_6, 400MHz) δ 10.76 (s, 1H), 10.74 (s, 1H), 9.55 (t, *J*= 6.4 Hz, 1H), 7.74 (s, 1H), 7.66 (d, *J*=8.2 Hz, 1H), 7.55 (d, *J*=8.6 Hz, 1H), 4.36 (d, *J*=5.9 Hz, 2H); ^{13}C NMR (DMSO-d_6, 100 MHz) δ 160.95, 159.24, 158.52, 157.15, 138.68, 133.29, 131.66, 129.22, 126.97 (q, *J*=4.8 Hz), 126.69 (q, *J*=31.5 Hz), 122.83 (q, *J*=271.7 Hz), 41.44.



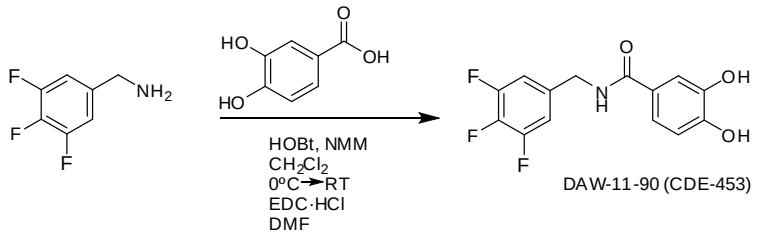
***N*-(4-Chloro-3-(trifluoromethyl)benzyl)-2-(2-(2-hydrazinyl-2-oxoacetyl)hydrazinyl)-2-oxoacetamide [DAW-11-86 (CDE-441)]**

To a mixture of DAW-11-76/83 (75.2 mg, 0.1895 mmol) in ethanol (1.9 ml), methanol (1.9 ml), dichloromethane (0.5 – 1.00 ml), and small amount of water were added to dissolve the compound and stirred for two days. Hydrazine hydrate (~50% solution in H_2O, 24.5 µl, 0.379 mmol) was added and the reaction mixture was stirred for another two days, turned into milky with fine powder, mixture was dried *in vacuo* to provide 0.0703 g (97.19%) of DAW-11-86 as a white solid. ^{1}H NMR (DMSO-d_6, 400MHz) δ 9.10 (bs, 1H), 7.71 (s, 1H), 7.63 (d, *J*=8.2 Hz, 1H), 7.53 (d, *J*=7.3 Hz, 1H), 4.33 (d, *J*=6.4 Hz, 2H); ^{13}C NMR (DMSO-d_6, 100 MHz) δ 162.33, 159.42, 156.93, 156.22, 139.33, 133.14, 131.54, 128.99, 126.76 (q, *J*=4.8 Hz), 126.34 (q, *J*=30.5 Hz), 122.86 (q, *J*=271.7 Hz), 41.35; HRMS, DART calcd. for $C_{12}H_{12}ClF_3N_5O_4$ $[M+H]^+$ 382.05299, found: 382.05020.



2-(2-(2-Amino-2-oxoacetyl)hydrazinyl)-*N*-(4-chloro-3-(trifluoromethyl)benzyl)-2-oxoacetamide [DAW-11-87 (CDE-445)]

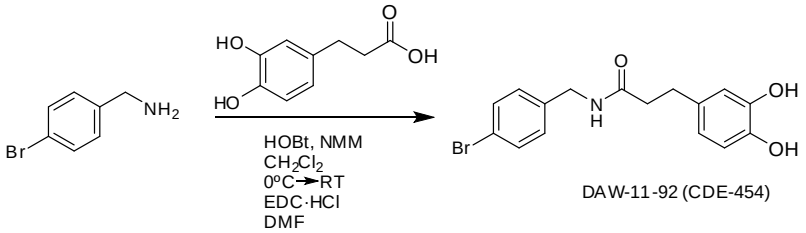
To a mixture of DAW-11-76/83 (75.2 mg, 0.1895 mmol) in ethanol (1.9 ml), methanol (1.9 ml), dichloromethane (0.5 – 1.00 ml), and small amount of water were added to dissolve the compound and stirred for five days. 7N ammonia (1 ml) was added and the reaction mixture was stirred for another few days, turned into milky with fine powder, mixture was dried *in vacuo* to provide 0.0694 g (99.87%) of DAW-11-87 as a white solid. ^{1}H NMR (DMSO-d_6, 400MHz) δ 9.67 (bs, 1H), 9.42 (bs, 1H), 9.21 (bs, 1H), 7.95 (bs, 1H), 7.72 (bs, 1H), 7.64 (m, 2H), 7.53 (d, *J*=8.3 Hz, 1H), 4.34 (d, *J*=6.4 Hz, 2H); ^{13}C NMR (DMSO-d_6, 100 MHz) δ 162.64, 161.60, 157.63, 157.14, 139.20, 133.17, 131.56, 129.02, 126.82 (q, *J*=4.7 Hz), 126.35 (q, *J*=30.5 Hz), 122.84 (q, *J*=271.7 Hz), 41.35; HRMS, DART calcd. for $C_{12}H_{11}ClF_3N_4O_4$ $[M+H]^+$ 367.04210, found: 367.04150.



3,4-Dihydroxy-N-(3,4,5-trifluorobenzyl)benzamide [DAW-11-90 (CDE-453)]

To a mixture of 3,4,5-trifluorobenzylamine (200.45 µl, 1.647 mmol), 3,4-dihydroxybenzoic acid (211.5 mg, 1.372 mmol), 1-hydroxybenzotriazole [HOBt•H_2O] (252.2 mg, 1.647 mmol), *N*-methylmorpholine [NMM] (181.08 µl, 1.647 mmol) in methylene chloride (5 ml) in an ice bath, 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride [EDC•HCl] (315.75 mg, 1.647

mmol) was added. The mixture was dissolved by adding dimethylformamide [DMF] (2 ml) and stirred overnight. The brown reaction mixture was reduced in volume in vacuo and the resulting solution was diluted with ≈25 ml of 4:1 of ethyl acetate: hexane, and washed with 0.1N HCl (2x), saturated $NaHCO_3$ (2x), and brine solution (1x), dried over $MgSO_4$, and concentrated *in vacuo* to obtain 0.3444 g of DAW-11-90 as a white solid. The product was triturated with $CHCl_3$ to obtain 0.2656 g (65.32%) of pure DAW-11-90 as an off white solid. ^{1}H NMR (DMSO-d_6, 400 MHz) δ 9.45 (bs, 1H), 9.12 (bs, 1H), 8.71 (t, *J*=5.9 Hz, 1H), 7.26 (d, *J*=1.8 Hz, 1H), 7.17 (m, 3H), 6.73 (d, *J*=8.2 Hz, 1H), 4.34 (d, *J*=5.9 Hz, 2H); ^{13}C NMR (DMSO-d_6, 100 MHz) δ 166.30, 150.04 (ddd, *J*=245.9 Hz, 9.53 Hz, 2.86 Hz), 148.60, 144.88, 137.95 (t, *J*=3.8 Hz), 137.43 (dt, *J*=245.9 Hz, 15.2 Hz), 125.14, 119.07, 115.09, 114.88, 111.46 (dd, *J*=15.2 Hz, 4.7 Hz), 41.58; HRMS, DART calcd. for $C_{14}H_{11}F_3NO_3$ $[M+H]^+$ 298.06910, found: 298.06500



***N*-(4-bromobenzyl)-3-(3,4-dihydroxyphenyl)propanamide [DAW-11-92 (CDE-454)]**

To a mixture of 4-bromobenzylamine (306.42 mg, 1.647 mmol), 3-(3,4-dihydroxyphenyl)propanoic acid (250.0 mg, 1.372 mmol), 1-hydroxybenzotriazole [HOBt•H_2O] (252.2 mg, 1.647 mmol), *N*-methylmorpholine [NMM] (181.1 µl, 1.647 mmol) in methylene chloride (5 ml) in an ice bath, 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride [EDC•HCl] (315.7 mg, 1.647 mmol) was added. The mixture was dissolved by adding dimethylformamide [DMF] (2 ml) and stirred overnight. The brown reaction mixture was reduced in volume *in vacuo* and the resulting solution was diluted with ≈25 ml of 4:1 of ethyl

acetate: hexane and washed with 0.1N HCl (2x), saturated $NaHCO_3$ (2x), and brine solution (1x), dried over $MgSO_4$, and concentrated *in vacuo* to obtain DAW-11-92 as a brown oil. The product was triturated with $CHCl_3$ to obtain 0.2570 g (53.5%) of pure DAW-1-68 as an off white solid. ^{1}H NMR (DMSO-d_6, 400 MHz) δ 8.67 (bs, 1H), 8.61 (s, 1H), 8.25 (t, *J*=5.9 Hz, 1H), 7.41 (d, *J*=8.2 Hz, 2H), 7.00 (d, *J*=8.2 Hz, 2H), 6.55 (m, 2H), 6.39 (dd, *J*=8.2 Hz, 2.3 Hz, 1H), 4.15 (d, *J*=5.9 Hz, 2H), 2.61 (t, *J*=8.4 Hz, 2H), 2.31 (t, *J*=7.8 Hz, 2H); ^{13}C NMR (DMSO-d_6, 100 MHz) δ 171.49, 144.96, 143.33, 139.05, 131.92, 130.98, 129.21, 119.53, 118.84, 115.79, 115.36, 41.22, 37.40, 30.52; HRMS, DART calcd. for $C_{16}H_{17}BrNO_3$ $[M+H]^+$ 350.03917, found: 350.03479.

Chapter III: Small molecule PAI-1 inhibitors using an isosteric replacement of catechol

In addition to the modification of the hydrazide-based lead molecule **(I-27)**, I chose to simultaneously work on a different project involving isosteres of catechol. Isosteres are electronically and/or sterically similar compounds that could improve an inhibitor's pharmacokinetic properties,[1]and lowering its toxicity while potentially providing the same opportunity for hydrogen bonding by using different functional groups.[1] The catechol was replaced with isosteric indole-based compounds such as oxindole and isatin **(Figure 10)**, as they are widely used in pharmaceuticals. It is hoped that they will improve the potency of our previous generation polyphenolic inhibitors towards PAI-1 in a plasma-based assay system. Therefore, the next step has been to combine the polyphenolic isosteres on different frameworks and investigate their structure-activity relationship based on the resulting IC_{50} values. Additionally, pro-drug versions of the hydrazide-based inhibitors using isatin were synthesized and were hoped to improve their performance *in vivo*.

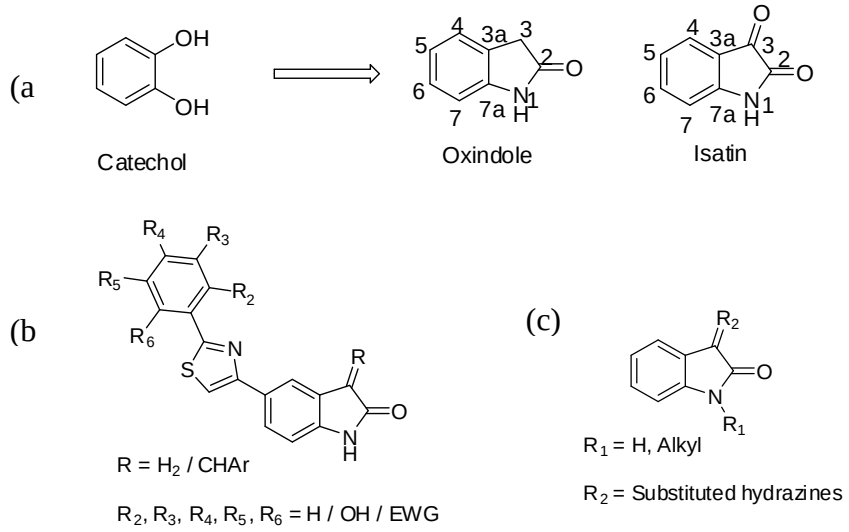


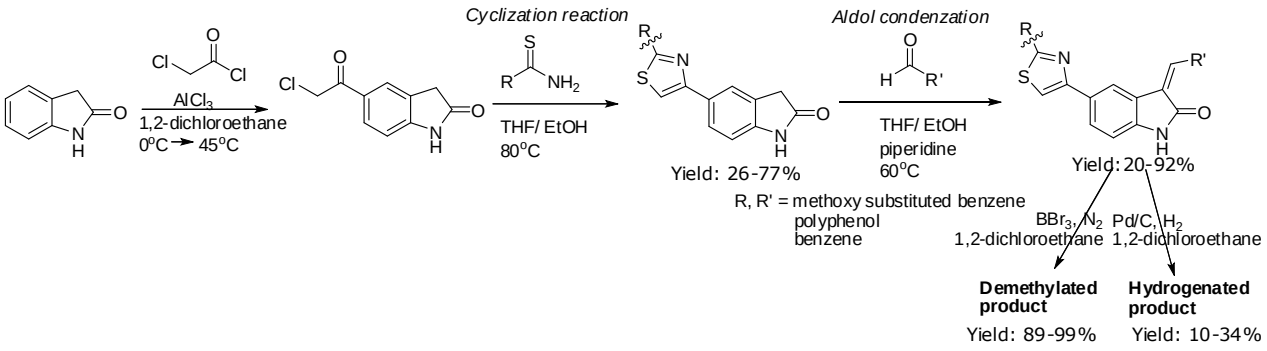
Figure 10: Isosteres and structural modification. (a) Isosteres of catechol. (b) Structural modification of oxindole. (c) Structural modification of isatin.

III-1: Oxindole-based compounds as inhibitors of PAI-1

Isosteres of catechol, such as oxindole and isatin, were used to synthesize thiazoles that could incorporate the polyphenols on a different central scaffold and allow changes to the substitution pattern at the left-hand aromatic moiety. Then the effect of substituting the C3 position of oxindole via an aldol condensation with various aldehydes was investigated. The aldol reaction did not work with hydroxyl-substituted benzaldehyde; therefore, the final hydroxyl-substituted product was accessed by demethylating the methoxy-substituted product from the previous step with boron tribromide. In addition, the effect that hydrogenation of the alkene formed in during the aldol reaction would had on PAI-1 activity was investigated.

Oxindole-based isosteres

As shown in **Scheme 3**, oxindole was treated with chloroacetyl chloride and $AlCl_3$ to substitute C5 position of oxindole. The resulting product (5-chloroacetyloxindole) was refluxed at 80 ºC with variously substituted thiobenzamides to yield the cyclization product[2]. The resulting thiazoles were refluxed at 60 ºC with differently substituted aldehydes in the presence of piperidine to yield the aldol products[2], which were then demethylated by treatment with boron tribromide or hydrogenated by treatment with H_2/Pd.

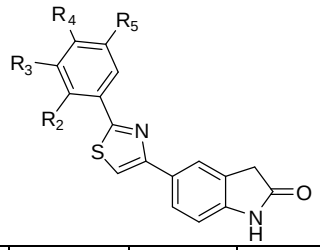


Scheme 3: General synthetic method for structural modification for oxindole based compounds

III-2: Effect of changing aromatic ring substituents

In the thiazole-based oxindoles we changed the substitution pattern on the left-hand aromatic ring with methoxy, hydroxy, methyl, CF_3, or halogen groups. Then we observed the effect on PAI-1 activity according to the change of substituents. We identified that compounds with only methoxy or hydroxy groups at the *meta* or *para* positions (CDE-227 and CDE-228) showed inhibitory activity in standard buffer but both lose activity when plasma is added to the assay. CDE-228, which contains a 3,4-dihydroxybenzene group, showed the best inhibitory activity with IC_{50} of 14.8 μM in standard buffer solution. None of the other compounds were tested in assays containing 1.5% BSA or plasma due to their lack of activity in standard buffer at the concentrations tested. In addition, all of these compounds became insoluble at concentrations above 100 μM.

Table 11: Cyclized product with substituted aromatic thiazole amines



Code	R_2	R_3	R_4	R_5	IC_{50} (μM)		
					Plasma	PAI-1/uPA pH 7.4	1.5% BSA
CDE-227[a]	H	OCH_3	OCH_3	H	>300	205	NT
CDE-228[a]	H	OH	OH	H	>300	14.8	NT
CDE-315[a]	H	H	F	H	NT	>100	NT
CDE-316[a]	CH_3	H	Br	H	NT	>100	NT
CDE-394	H	H	H	H	NT	>100	NT
CDE-395[b]	H	CF_3	H	H	NT	>100	NT
CDE-396[b]	H	H	CF_3	H	NT	>100	NT
CDE-397[b]	H	H	Br	H	NT	>100	NT
CDE-398[b]	H	Br	H	H	NT	>100	NT
CDE-399	H	H	Cl	H	NT	>100	NT
CDE-402	H	F	H	F	NT	>100	NT

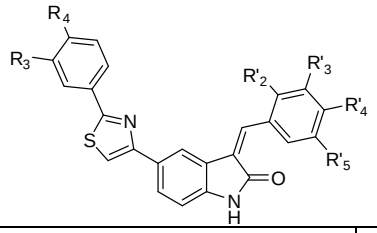
[a]Compound synthesized by Hasina Saraha. [b]Compound synthesized by Naga Guntaka. NT: not tested

III-3: Effect of changing substitution on both aromatic rings

Based on the previous results (**Table 11**), I chose only methoxy- or hydroxy-substituted benzaldehydes to run the aldol condensation because in the presence of other substituents such as halides or methyl groups, compounds did not show PAI-1 inhibitory activity. Due to the unsuccessful aldol reaction with the hydroxyl-substituted benzaldehyde, I used boron tribromide to demethylate the methoxy-substituted compounds. Even though this demethylation provided good yield, some of the final products were isolated with impurities (identified with an 'i' after the compound code). According to the IC_{50} values, only the polyphenolic compounds show PAI-1 inhibitory activity. The structure-activity relationship indicated that the PAI-1 inhibitory activity increased not only with the number of hydroxyl groups, but also is dependent on their

position on the aromatic ring. Substituting both the left and right hand sides moieties with a 3,4-dihydroxybenzene group provided the best IC_{50} (0.35 µM, **Table 12)**.

Table 12: Aldol condensation and demethylated products with substituted aromatic ring.



Code	Left Ar		Right Ar				IC_{50} (µM)		
	R_3	R_4	R_2'	R_3'	R_4'	R_5'	Plasma	PAI-1/uPA pH 7.4	1.5% BSA
CDE-400	H	H	H	H	OCH_3	H	NT	>100	NT
CDE-407	H	H	H	OCH_3	OCH_3	H	NT	>100	NT
CDE-416	H	H	H	OH	OH	H	>100	26.3	>100
CDE-408	OCH_3	OCH_3	H	OCH_3	OCH_3	H	NT	>100	NT
CDE-411i	OH	OH	H	OH	OH	H	>30	0.35	>30
CDE-417	OH	OH	H	OH	H	OH	>100	25.3	>100
CDE-418	OH	OH	OH	H	H	OH	>1000	27.6	>1000
CDE-419i	OH	OH	H	H	H	H	NT	>100	NT

NT: not tested

If at least one hydroxyl group was moved from the typical *meta* and *para* substitution pattern (CDE-416, CDE-417, CDE-418, CDE-419i), potency reduced approximately by 70 -80 fold, which were given between 25-27 µM (**Figure 11**). Comparison of IC_{50} values indicated that substituting both aromatic rings with 3,4-dihydroxybenzene were optimal. However, those compounds were not active in assays containing plasma or 1.5% BSA.

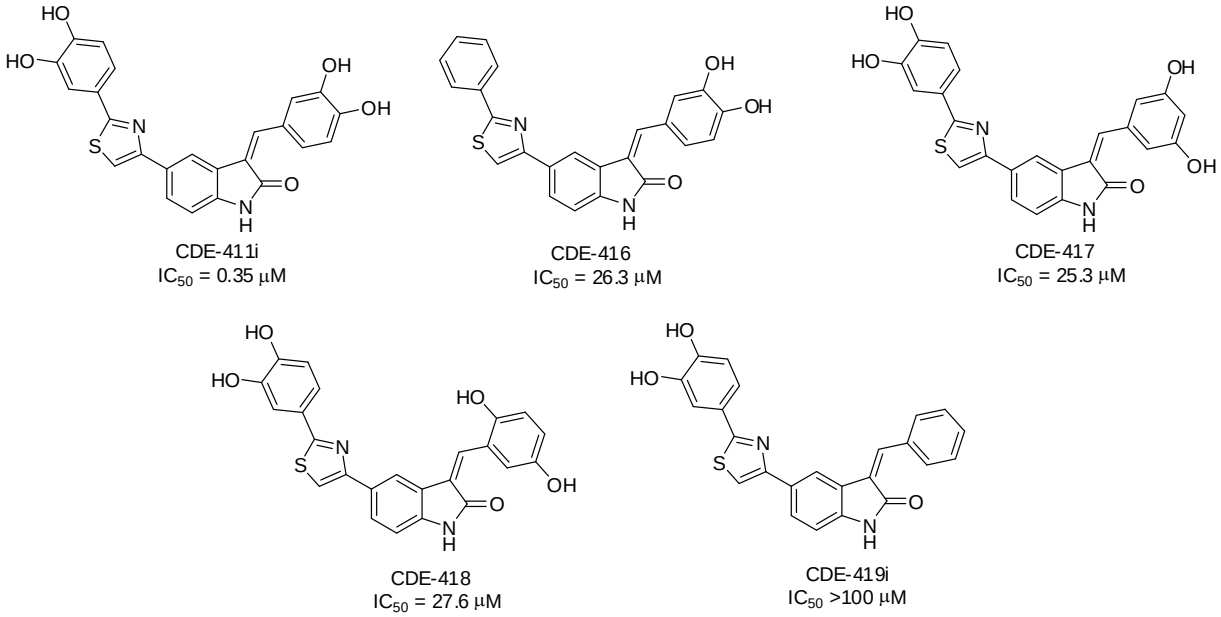
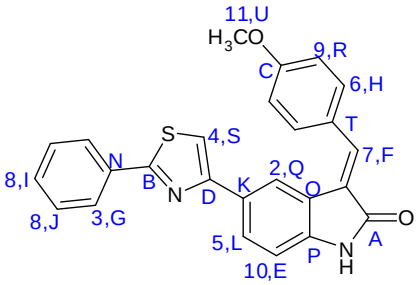


Figure 11: Comparison of hydroxyl substituents

Moreover, ^{1}H NMR and ^{13}C NMR indicated some of the aldol products **(Table 12)** were a mixture of *E*/Z isomers in a 3:1 or 4:1 ratio. Therefore, I decided it would be valuable to deduce the major component of the mixture. I selected CDE-400 for further analysis and ran an NOE experiment with a 5 second mixing time to identify the stereochemistry. According to that experiment, I was surprised to find that the final geometry of the CDE-400 sample was 100% *E* isomer **(figure 12-a)**. However, the NOE experiment was run 6 months after synthesis, giving the mixture time to equilibrate to a single isomer.

(a)



(b)

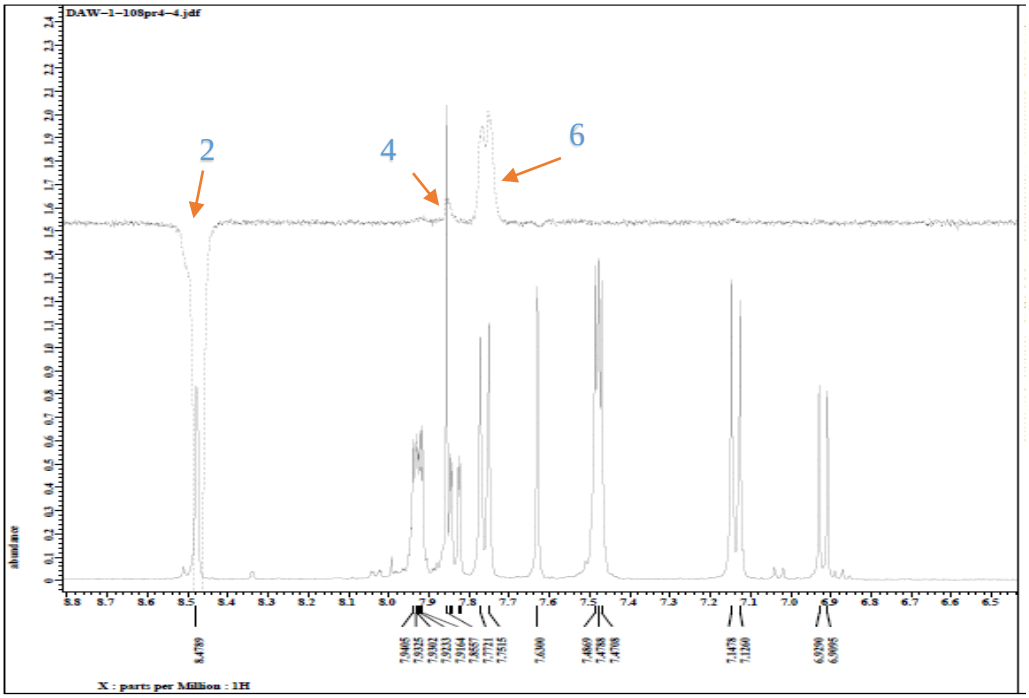
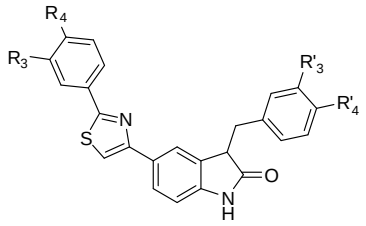


Figure 12: (a) Structure of CDE-400 with peak assignments. (b) NOE experiment for CDE-400. Proton 2 was irradiated. Signal transmitted to proton 4 and proton 6 through dipolar coupling.

III-4: Effect on reduction of alkene group

There was an attempt to reduce the alkene between the oxindole group and the right hand aromatic ring, but the final products were isolated with impurities. However, CDE-409i and CDE-410i was sent for biological testing and PAI-1 inhibitory activity was found to be >100 µM. Due to the difficulty of the purification process, low solubility, and inactivity in the standard buffer system, it was not pursued further.

Table 13: Hydrogenated products.

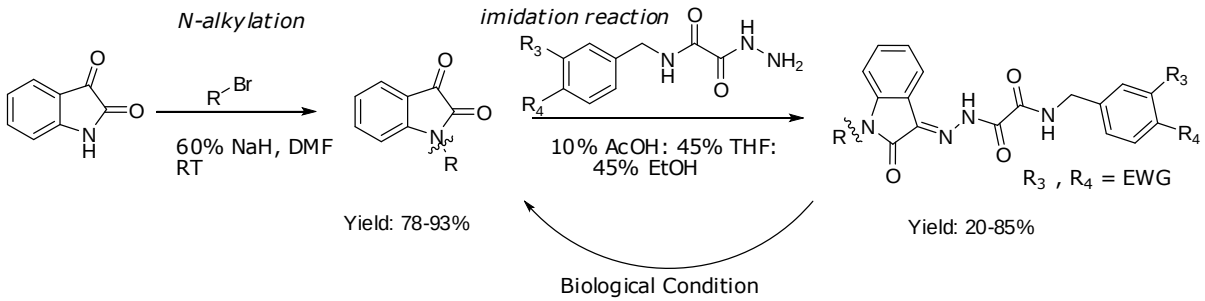


Code	Left Ar		Right Ar		IC_{50} (µM)		
	R_3	R_4	R_3'	R_4'	Plasma	PAI-1/uPA pH 7.4	1.5% BSA
CDE-409i	H	H	OCH_3	OCH_3	NT	>100	NT
CDE-410i	OCH_3	OCH_3	OCH_3	OCH_3	NT	>100	NT

NT: not tested, 'i': impurities

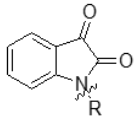
III-5: Isatin based pro-drug formation

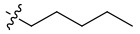
When working on the oxindole-based molecules, it was of interest to determine the effect on PAI-1 activity of substituting the acidic proton on the oxindole nitrogen atom. While searching the literature, there was a report[3] of the use of isatin as an isostere to catechol and oxindole (**Scheme 4**). The article provided an opportunity to pursue a new class of compounds by incorporating indole with our previously synthesized hydrazides, discussed in **Chapter II**. It is hoped that this new class of compounds can act as pro-drugs, which means they are metabolized in the body to produce the active drug. As stated in **Scheme 4**, isatin was treated with 60% sodium hydride, followed by the respective bromoalkane to obtain the *N*-alkylated product. The resulting alkylated product (or isatin itself, when approporiate) was treated with the previously synthesized hydrazide to obtain the desired pro-drug.



Scheme 4: General synthetic method for structural modification for isatin based compounds

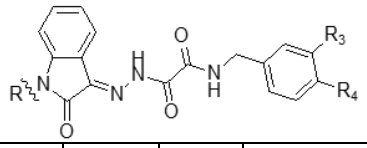
Table 14: IC_{50} of isatin and *N*-alkylated product. NT: not tested, NA: not active.

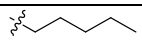
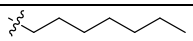


Code	R	IC_{50} (µM)		
		Plasma	PAI-1/uPA pH 7.4	1.5% BSA
Isatin	H	NT	>3000	NT
CDE-420		NT	>1000	NT

Different pro-drug analogues were synthesized by altering the alkyl substituents as well as the hydrazides **(Table 15)**. Using the lead compound as the basis for the pro-drug, the substitution of the nitrogen atom did not change the IC_{50} values in standard buffer solution. Therefore, those compounds were not tested in assays containing 1.5% BSA or plasma. The pro-drug of the hydrazide inhibitor that contains our preferred substitution pattern (*para*-chloro-*meta* trifluoro benzene moiety) with isatin (CDE-449) showed impressive inhibitory activity in standard buffer with an IC_{50} of 11 µM. Due to this result, our collaborators intend to test CDE-449 in transgenic mice that overexpress PAI-1, these studies will provide the impetus for further investigation of prodrugs of the hydrazide class of inhibitors.

Table 15: IC_{50} values of imidated isatin prodrugs



Code	R	R_3	R_4	IC_{50} (μM)		
				Plasma	PAI-1/uPA pH 7.4	1.5% BSA
CDE-421		H	Cl	NT	>100	NT
CDE-425		H	Cl	NT	>300	NT
CDE-426	H	H	Cl	NT	>100	NT
CDE-449	H	CF_3	Cl	>100	11	>100
CDE-427	H	CF_3	F	>300	139	>300

III-6: Conclusion

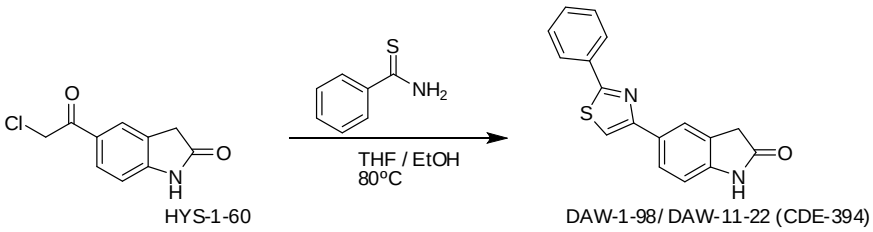
CDE-228 and CDE-411i showed that the 3,4-dihydroxybenzene moiety is very important for significant PAI-1 inhibitory activity, while alternate substitution patterns tend to lower inhibitory activity. In addition, the oxindole-based compounds have solubility issues and therefore they were not tested in assays containing 1.5% BSA or plasma. Therefore, the oxindole-based inhibitors need to be further improved to overcome these issues. Considering the isatin compounds, substituting the nitrogen atom with an alkyl group did not improve the activity against PAI-1. However, the pro-drug version showed improved inhibitory activity when both *meta* and *para* substituents are present. Also, the activity of CDE-449 confirmed that *para*-chloro-*meta*-trifluorobenzene moiety is beneficial for potential PAI-1 inhibitors, and we are evaluating the *in vivo* results of CDE-449 for further investigations of hydrazide pro-drugs.

III-7: Experimental methods and data

General synthetic methods:

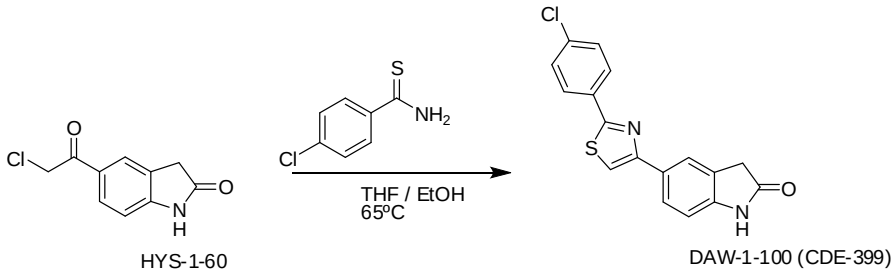
Unless otherwise noted, all the reactions were performed in 120 ºC oven-dried glassware with magnetic stirring. All reagent grade solvents used without purification for extraction, chromatography and reactions. Thin layer chromatography (TLC) was performed with 250 mm silica gel coated glass plates from Sorbent Technologies, and visualized with 254 nm UV light and/or aqueous $KMnO_4$ solution or ninhydrin solution. Column chromatography was executed using silica gel (Sorbent Technologies Premium R_f; 60 Å, 40-75 μM) as the stationary phase. ^1H and ^{13}C NMR spectra were obtained with a JEOL ECX-400 spectrometer with a probe temperature of 25 ºC using DMSO-d_6 (Cambridge Isotope Laboratories, Inc.) or $CDCl_3$ (Cambridge Isotope Laboratories, Inc.) as solvents. Chemical shifts were measured relative to the tetramethylsilane (TMS) peak and were recorded in δ (parts per million, ppm). The internal references were DMSO-d_6 (δ = 2.50 ppm) for ^1H NMR and DMSO-d_6 (δ = 39.5 ppm) for ^{13}C NMR; $CDCl_3$ (δ = 7.26 ppm) for ^1H NMR and $CDCl_3$ (δ = 77.0 ppm) for ^{13}C NMR. Coupling constants (*J*) are recorded in Hertz. Splitting patterns are labeled as follows: bs, broad singlet; s, singlet; d, doublet; t, triplet; q, quartet; p, pentet; m, multiplet. High-resolution mass spectroscopy data were provided by Prof. Ruth Ann Armitage, Eastern Michigan University, Ypsilanti, MI, and were recorded using Direct Analysis in Real Time (DART) from JEOL AccuTOF DART instrument, JEOL USA, Inc., Peabody, Massachusetts.

Synthetic procedures:



5-(2-Phenylthiazol-4-yl)indolin-2-one [DAW-1-98/ DAW-11-22 (CDE-394)]

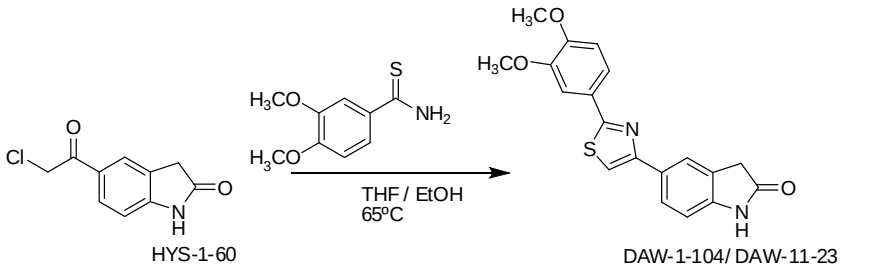
To a suspension of 5-chloroacetyloxindole [HYS-1-60] (764.1 mg, 3.64 mmol) in a mixture of THF/EtOH (9.2 ml/9.2 ml), thiobenzamide (500.7 mg, 3.64 mmol) was added. The mixture was refluxed at 80 ºC for 4 hr; the temperature was reduced to 65 ºC due to the evaporation of solvent and heated for another 16 hr. After cooling, it was visualized by thin layer chromatography (90% EtOAc:10% hexane), and concentrated *in vacuo* to obtain a solid, which was triturated with ethyl acetate to provide 0.6390 g (60.1%) of DAW-1-98 as a brown solid. ^{1}H NMR (DMSO-d_6, 400 MHz) δ 10.48 (s, 1H), 7.98 (m, 3H), 7.85 (m, 2H), 7.49 (m, 3H), 6.86 (d, J=8.2 Hz, 1H), 3.53 (s, 2H); ^{13}C NMR (DMSO-d_6, 100 MHz) δ 176.46, 166.62, 155.48, 143.79, 133.06, 130.29, 129.26, 127.88, 127.56, 127.25, 126.42, 126.13, 125.71, 122.38, 112.35, 109.22, 35.79; HRMS, DART calcd. for $C_{17}H_{13}N_2OS$ $[M+H]^+$ 393.07487, found: 393.06680.



HYS-1-60 DAW-1-100 (CDE-399)

5-(2-(4-Chlorophenyl)thiazol-4-yl)indolin-2-one [DAW-1-100 (CDE-399)]

To a suspension of 5-chloroacetyloxindole [HYS-1-60] (100.2 mg, 0.477 mmol) in a mixture of THF/EtOH (2.4 ml/2.4 ml), 4-chlorothiobenzamide (82.1 mg, 0.477 mmol) was added. The mixture was refluxed at 65 ºC for 20 hr. After cooling, it was visualized by thin layer chromatography (90% EtOAc: 10% hexane), and concentrated *in vacuo* to obtain a solid, which was triturated with ethyl acetate to provide 0.0559 g (35.9%) of DAW-1-100 as a brown solid. ^{1}H NMR (DMSO-d_6, 400 MHz) δ 10.49 (s, 1H), 8.00 (m, 3H), 7.85 (m, 2H), 7.55 (m, 2H), 6.86 (d, J=8.2 Hz, 1H), 3.53 (s, 2H); ^{13}C NMR (DMSO-d_6, 100 MHz) δ 177.00, 165.81, 156.16, 144.42, 135.32, 132.44, 129.84, 128.37, 127.95, 126.99, 126.29, 122.94, 113.38, 109.76, 36.33; HRMS, DART calcd. for $C_{17}H_{12}ClN_2OS$ $[M+H]^+$ 327.03590, found: 327.02701.

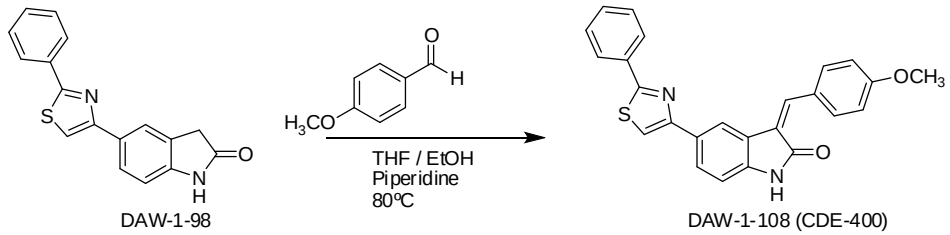


HYS-1-60 DAW-1-104/ DAW-11-23

5-(2-(3,4-Dimethylphenyl)thiazol-4-yl)indolin-2-one [DAW-1-104/DAW-11-23]

To a suspension of 5-chloroacetyloxindole [HYS-1-60] (531.2 mg, 2.535 mmol) in a mixture of THF/EtOH (12.75 ml/12.75 ml), 4-chlorothiobenzamide (500 mg, 2.535 mmol) was added. The mixture was refluxed at 65 ºC for 20 hr. After cooling, it was concentrated *in vacuo* to obtain a

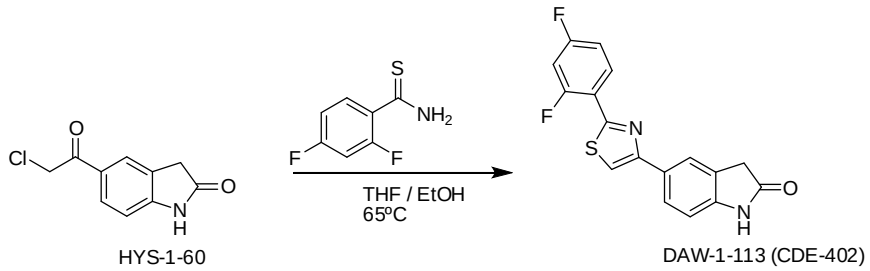
solid, which was triturated with ethyl acetate to provide 0.6897 g (77.2%) of DAW-1-104 as a brown solid. ^{1}H NMR (DMSO-d_6, 400 MHz) δ 10.47 (s, 1H), 7.86 (m, 3H), 7.50 (m, 2H), 7.04 (d, *J*=7.8 Hz, 1H), 6.86 (d, *J*=8.7 Hz, 1H), 3.84 (s, 3H), 3.79 (s, 3H), 3.53 (s, 2H); ^{13}C NMR (DMSO-d_6, 100 MHz) δ 176.46, 166.72, 155.14, 150.67, 149.03, 143.70, 127.67, 126.37, 126.03, 125.69, 122.35, 119.36, 111.92, 111.40, 109.19, 109.02, 55.63, 35.8.



DAW-1-98 DAW-1-108 (CDE-400)

(*Z*)-3-(4-Methoxybenzylidene)-5-(2-phenylthiazol-4-yl)indolin-2-one [DAW-1-108 (CDE-400)]

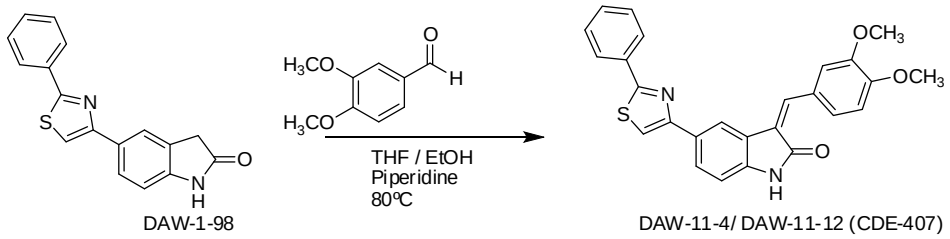
To a mixture of DAW-1-98 (100.1 mg, 0.342 mmol) and 4-methoxybenzaldehyde [*p*-anisaldehyde] (42.0 µl, 0.342 mmol) in THF/EtOH (1.72 ml/1.72 ml), piperidine (34.2 µl) was added. The mixture was refluxed at 80 ºC for 4 hr. After cooling, the precipitate was filtered and washed with ethanol to provide 0.0818 g (58.3%) of DAW-1-108 as a yellow solid. ^{1}H NMR (DMSO-d_6, 400 MHz) δ 10.67 (bs, 1H), 8.48 (d, *J*=1.4 Hz, 1H), 7.93 (m, 2H), 7.88 (s, 1H), 7.84 (dd, *J*=1.4 Hz, 8.2 Hz, 1H), 7.77 (d, *J*=8.7 Hz, 2H), 7.63 (s, 1H), 7.48 (m, 3H), 7.14 (d, *J*=8.7 Hz, 2H), 6.92 (d, *J*=8.2 Hz, 1H), 3.87 (s, 3H); ^{13}C NMR (DMSO-d_6, 100 MHz) δ 169.02, 167.58, 166.67, 166.50, 161.32, 160.77, 155.73, 154.94, 142.55, 140.25, 137.48, 136.61, 134.57, 133.10, 132.99, 131.65, 130.39, 130.31, 129.24, 129.21, 127.46, 127.38, 127.26, 126.97, 126.59, 126.50, 126.23, 126.00, 125.84, 125.81, 123.80, 121.60, 120.33, 117.22, 114.16, 113.82, 112.43, 112.39, 110.14, 109.34, 55.49, 55.39; (*E* and *Z* rotomers are present). HRMS, DART calcd. for $C_{25}H_{19}N_2O_2S$ $[M+H]^+$ 411.11671, found: 411.10870.



HYS-1-60 DAW-1-113 (CDE-402)

5-(2-(2,4-Difluorophenyl)thiazol-4-yl)indolin-2-one [DAW-1-113 (CDE-402)]

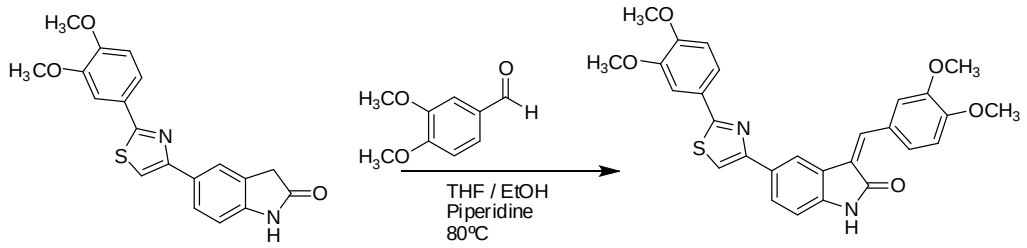
To a suspension of 5-chloroacetyloxindole [HYS-1-60] (99.9 mg, 0.477 mmol) in a mixture of THF/EtOH (2.4 ml/2.4 ml), 4-chlorothiobenzamide (82.9 mg, 0.477 mmol) was added. The mixture was refluxed at 80 ºC for two days. It was visualized by thin layer chromatography (80% EtOAc: 20% hexane), filtered, and the solid was washed with ethyl acetate to provide 0.0416 g (26.6%) of DAW-1-113 as a light pink solid. ^{1}H NMR (DMSO-d_6, 400 MHz) δ 10.48 (s, 1H), 8.37 (dt, *J*=8.7 Hz, 6.4 Hz, 1H), 8.09 (s, 1H), 7.87 (m, 2H), 7.49 (ddd, *J*=11.9 Hz, 9.2 Hz, 2.3 Hz, 1H), 7.27 (dt, *J*=8.2 Hz, 2.3 Hz, 1H), 6.86 (d, *J*=8.3 Hz, 1H), 3.53 (s, 2H); ^{13}C NMR (DMSO-d_6, 100 MHz) δ 176.45, 162.90 (dd, *J*=249.8 Hz, 12.4 Hz), 159.53 (dd, *J*=251.7 Hz, 12.4 Hz), 158.13 (d, *J*=4.8 Hz), 154.46, 143.90, 130.05 (dd, *J*=9.5 Hz, 3.8 Hz), 127.30, 126.45, 125.78, 122.44, 117.59 (dd, *J*=11.4 Hz, 3.8 Hz), 113.47 (d, *J*=7.6 Hz), 112.80 (dd, *J*=21.9 Hz, 2.9 Hz), 109.23, 104.92 (t, *J*=26.7 Hz), 35.78; HRMS, DART calcd. for $C_{17}H_{11}F_2N_2OS$ $[M+H]^+$ 329.05601, found: 329.04471.



DAW-1-98 DAW-11-4/ DAW-11-12 (CDE-407)

(*Z*)-3-(3,4-Dimethoxybenzylidene)-5-(2-phenylthiazol-4-yl)indolin-2-one [DAW-11-4/12 (CDE-407)]

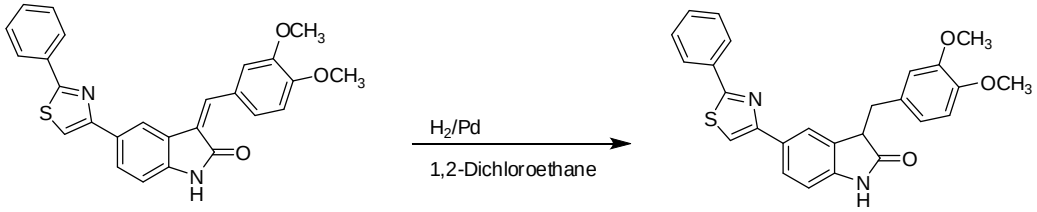
To a mixture of DAW-1-98 (100.4 mg, 0.342 mmol) and veratraldehyde (57.4 mg, 0.342 mmol) in THF/EtOH (1.71 ml/1.71 ml), piperidine (34.2 µl) was added. The mixture was refluxed at 80 ºC for two days. After cooling, the precipitate was filtered and washed with ethanol to provide 0.0555 g (36.8%) of DAW-11-4 as a red/yellow solid. ^{1}H NMR (DMSO-*d*$_6$, 400 MHz) δ 10.67 (s, 1H), 8.61 (d, *J*=0.9 Hz, 1H), 7.91 (m, 3H), 7.84 (dd, *J*=8.2 Hz, 1.4 Hz, 1H), 7.63 (s, 1H), 7.48 (m, 3H), 7.44 (d, *J*=1.8 Hz, 1H), 7.37 (dd, *J*=8.2 Hz, 1.8 Hz, 1H), 7.15 (d, *J*=8.3 Hz, 1H), 6.92 (d, *J*=8.2 Hz, 1H), 3.88 (s, 3H), 3.74 (s, 3H); ^{13}C NMR (DMSO-*d*$_6$, 100 MHz) δ 169.07, 166.63, 154.95, 150.57, 148.53, 142.61, 136.92, 132.91, 130.38, 129.24, 129.19, 127.29, 127.20, 126.66, 126.24, 126.05, 125.63, 123.70, 121.61, 120.54, 112.62, 112.38, 111.69, 110.19, 55.78, 55.36; HRMS, DART calcd. for $C_{26}H_{21}N_2O_3S$ $[M+H]^+$ 441.12730, found: 441.12711.



DAW-1-104 DAW-11-6/ DAW-11-13 (CDE-408)

3-(3,4-Dimethoxybenzylidene)-5-(2-(3,4-dimethoxyphenyl)thiazol-4-yl)indolin-2-one [DAW-11-6/13 (CDE-408)]

To a mixture of DAW-1-104 (100.0 mg, 0.286 mmol) and veratraldehyde (48.9 mg, 0.286 mmol) in THF/EtOH (1.5 ml/1.5 ml), piperidine (29.0 µl) was added. The mixture was refluxed at 80 ºC for two days. After cooling, the precipitate was filtered and washed with ethanol to provide 0.0701 g (49.0%) of DAW-11-6 as a red/yellow solid. ^{1}H NMR (DMSO-d_6, 400 MHz) δ 10.67 (s, 1H), 8.68 (d, *J*=1.8 Hz, 1H), 8.32 (d, *J*=1.4 Hz, 1H), 7.92 (s, 1H), 7.87 (m, 3H), 7.55 (m, 2H), 7.07 (m, 2H), 6.89 (d, *J*=8.2 Hz, 1H), 3.87 (s, 3H), 3.81 (m, 9H); ^{13}C NMR (DMSO-d_6, 100 MHz) δ 167.64, 166.80, 155.43, 151.25, 150.71, 149.06, 148.00, 140.10, 138.06, 127.59, 127.22, 126.46, 126.06, 125.87, 123.72, 119.49, 119.45, 117.11, 115.03, 111.95, 111.50, 111.16, 109.34, 109.28, 55.71, 55.66, 55.58, 55.40; (*E* and *Z* rotomers are present). HRMS, DART calcd. for $C_{28}H_{25}N_2O_5S$ $[M+H]^+$ 501.14630, found: 501.14842.

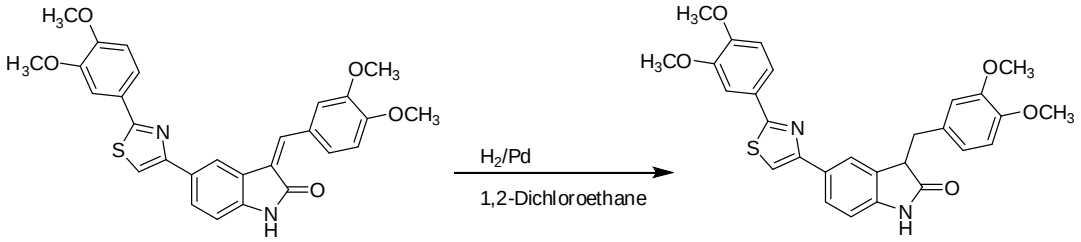


DAW-11-4 DAW-11-8 (CDE-409i)

3-(3,4-Dimethoxybenzyl)-5-(2-phenylthiazol-4-yl)indolin-2-one [DAW-11-8 (CDE-409i)]

To a stirring mixture of DAW-11-4 (29.5 mg, 0.0681 mmol) in 1,2-dichloroethane (3 ml), a small amount of 10% palladium on carbon was added under a hydrogen atmosphere. The

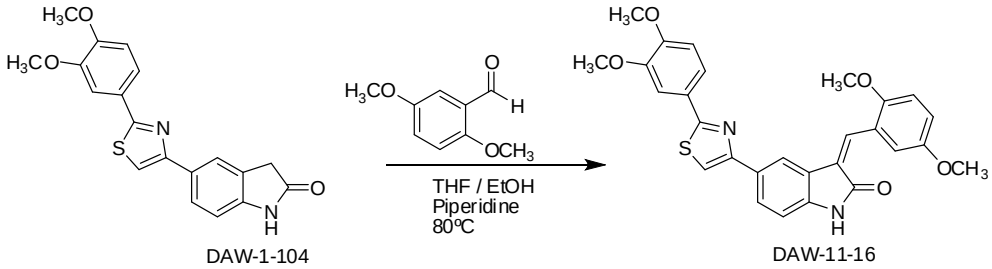
mixture was stirred for five days with regular replacement of hydrogen. Completion of the reaction was visualized by thin layer chromatography (80% EtOAc: 20% hexane), and the filtrate was concentrated *in vacuo* to obtain 10.3 mg (34.2%) of DAW-11-8 as a dark yellow solid. ^{1}H NMR (DMSO-*d*$_6$, 400 MHz) δ 8.66 (s, 1H), 7.96 (d, *J*=6.4 Hz, 2H), 7.82 (s, 2H), 7.44 (m, 4H), 6.96 (m, 4H), 4.11 (m, 9H); HRMS, DART calcd. for $C_{26}H_{23}N_2O_3S$ $[M+H]^+$ 443.14293, found: 443.14001.



DAW-11-6 DAW-11-10 (CDE-410i)

3-(3,4-Dimethoxybenzyl)-5-(2-(3,4-dimethoxyphenyl)thiazol-4-yl)indolin-2-one [DAW-11-10 (CDE-410i)]

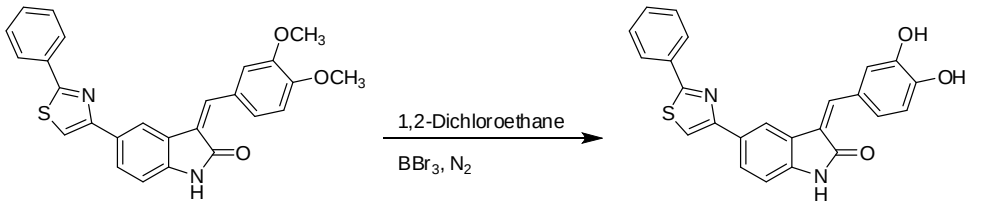
To a stirring mixture of DAW-11-6 (29.4 mg, 0.0681 mmol) in 1,2-dichloroethane (3.5 ml), small amount of palladium, 10% on carbon was added under a hydrogen atmosphere. The mixture was stirred for five days with regular replacement of hydrogen. Completion of the reaction was visualized by thin layer chromatography (80% EtOAc: 20% hexane), and the filtrate was concentrated *in vacuo* to obtain 3.0 mg (10.2%) of DAW-11-10 as a yellow solid. ^{1}H NMR (DMSO-*d*$_6$, 400 MHz) δ 7.84 (m, 2H), 7.56 (m, 5H), 6.95 (m, 3H), 6.70 (m, 1H), 3.87 (m, 15H); HRMS, DART calcd. for $C_{28}H_{27}N_2O_5S$ $[M+H]^+$ 503.16408, found: 503.15991.



DAW-1-104 DAW-11-16

3-(2,5-Dimethoxybenzylidene)-5-(2-(3,4-dimethoxyphenyl)thiazol-4-yl)indolin-2-one [DAW-11-16]

To a mixture of DAW-1-104 (225.4 mg, 0.639 mmol) and 2,5-dimethoxybenzaldehyde (108.2 mg, 0.639 mmol) in THF/EtOH (3.2 ml/3.2 ml), piperidine (63.9 µl) was added. The mixture was refluxed at 80 ºC for two days. After cooling, the precipitate was filtered and washed with ethanol to provide 0.0664 g (20.8%) of DAW-11-16 as a black solid. ^{1}H NMR (DMSO-*d*$_6$, 400 MHz) δ 10.69 (s, 1H), 8.42 (s, 1H), 7.84 (dd, *J*=8.3 Hz, 1.4 Hz, 1H), 7.80 (s, 1H), 7.67 (s, 1H), 7.45 (dd, *J*=8.2 Hz, 1.8 Hz, 1H), 7.38 (m, 2H), 7.08 (m, 3H), 6.92 (d, *J*=8.2 Hz, 1H), 3.81 (m, 12H).

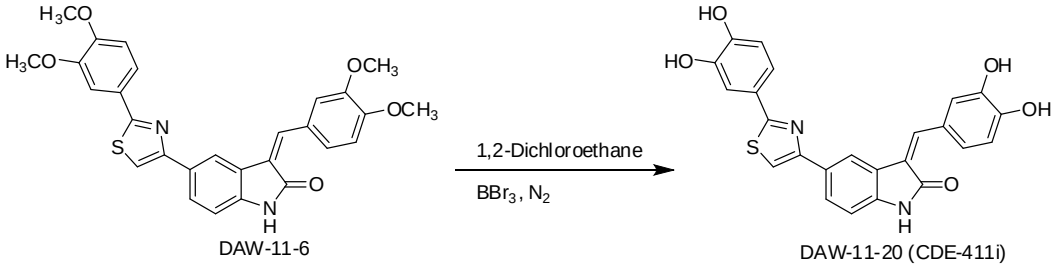


DAW-11-4 DAW-11-18 (CDE-416)

(*Z*)-3-(3,4-Dihydroxybenzylidene)-5-(2-phenylthiazol-4-yl)indolin-2-one [DAW-11-18 (CDE-416)]

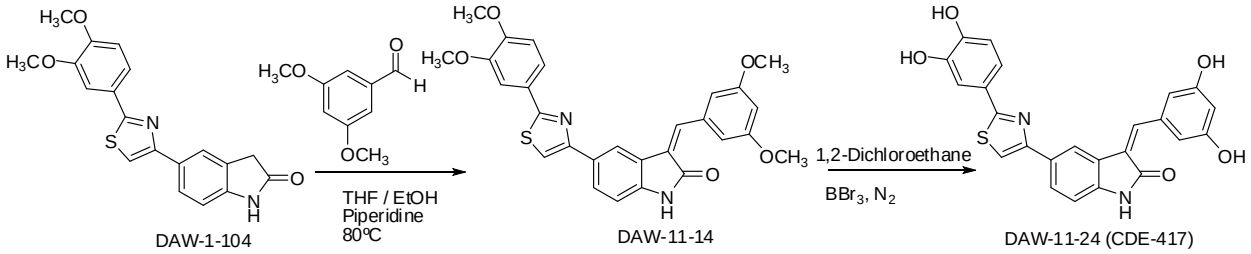
To a stirring solution of DAW-11-4 (44.0 mg, 0.1 mmol) in 1,2-dichloroethane (1 ml), boron tribromide (1.0 ml , 1M in CH_2Cl_2) was added dropwise under a nitrogen atmosphere. The resulting mixture was cooled in an ice bath and allowed to warm at room temperature overnight with stirring. The reaction was quenched with few drops of methanol, and the formed precipitate

was filtered and washed with ethyl acetate to provide 0.0378 g (91.7%) of DAW-11-18 as a yellowish orange solid. ^{1}H NMR (DMSO-d_6, 400 MHz) δ 10.64 (s, 1H), 8.31 (s, 1H), 8.20 (d, *J*=1.8 Hz, 1H), 8.02 (m, 3H), 7.85 (m, 1H) 7.75 (m, 2H), 7.49 (m, 3H), 6.85 (d, *J*=8.2 Hz, 1H), 6.79 (d, *J*=8.2 Hz, 1H), 4.02 (bs, 2H); ^{13}C NMR (DMSO-d_6, 100 MHz) δ 169.25, 166.54, 155.14, 148.03, 145.45, 142.33, 139.98, 137.64, 133.10, 132.94, 130.24, 129.33, 129.23, 127.28, 127.24, 126.36, 126.31, 126.08, 126.05, 125.34, 125.37, 124.48; HRMS, DART calcd. for $C_{24}H_{17}N_2O_3S$ $[M+H]^+$ 413.09599, found: 413.09500.



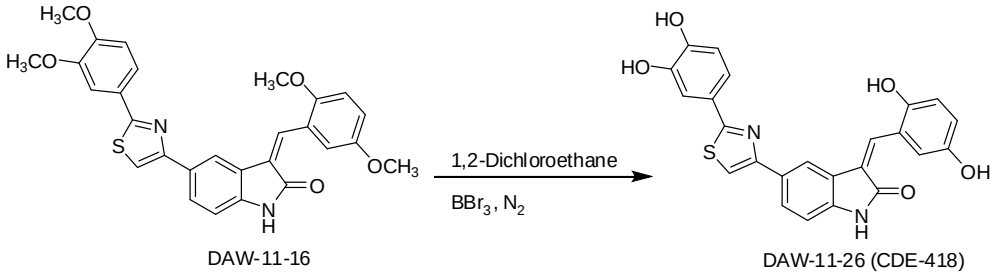
(*Z*)-3-(3,4-Dihydroxybenzylidene)-5-(2-(3,4-dimethoxyphenyl)thiazol-4-yl)indolin-2-one [DAW-11-20 (CDE-411i)]

To a stirring solution of DAW-11-6 (50.2 mg, 0.1 mmol) in 1,2-dichloroethane (1 ml), boron tribromide (1.0 ml , 1M in CH_2Cl_2) was added dropwise under a nitrogen atmosphere. The resulting mixture was cooled down in an ice bath and allowed to warm to room temperature overnight with stirring. The reaction was quenched with few drops of water, and the formed precipitate was filtered and washed with ethyl acetate to provide 0.0412 g (92.7%) of DAW-11-20 as a red solid. ^{1}H NMR (DMSO-d_6, 400 MHz) δ 10.62 (s, 1H), 8.45 (s, 1H), 8.23 (dd, *J*=19.2 Hz, 1.8 Hz, 1H), 7.81 (m, 2H), 7.62 (s, 1H), 7.31 (m, 2H), 6.85 (m, 4H), 4.76 (bs, 4H); HRMS, DART calcd. for $C_{24}H_{17}N_2O_5S$ $[M+H]^+$ 445.08583, found: 445.08411.



3-(3,5-Dihydroxybenzylidene)-5-(2-(3,4-dihydroxyphenyl)thiazol-4-yl)indolin-2-one [DAW-11-24 (CDE-417)]

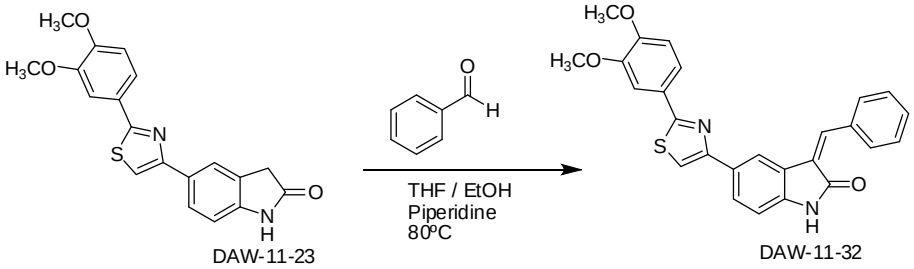
To a mixture of DAW-1-104 (225.1 mg, 0.639 mmol) and 3,5-dimethoxybenzaldehyde (106.4 mg, 0.639 mmol) in THF/EtOH (3.2 ml/3.2 ml), piperidine (63.9 µl) was added. The mixture was refluxed at 80 ºC for two days. After cooling, it was concentrated *in vacuo* to obtain a brown fine solid. The product was triturated with ethanol to provide 0.1400 g (43.8%) of DAW-11-14 as a brownish/red solid, which was used without further purification. To a stirring solution of DAW-11-14 (50.0 mg, 0.1 mmol) in 1,2-dichloroethane (1 ml), boron tribromide (1.0 ml , 1M in CH_2Cl_2) was added dropwise under a nitrogen atmosphere at 0 ºC. The resulting mixture was allowed to warm to room temperature overnight with stirring. The reaction was quenched with few drops of methanol. A fine solid was formed on addition of ethyl acetate; the mixture was concentrated *in vacuo* and triturated with ethyl acetate to provide 0.0398 g (89.5%) of DAW-11-24 as a light brown solid. 1H NMR (DMSO-d6, 400 MHz) δ 10.46 (s, 1H), 7.77 (m, 3H), 7.46-7.23 (m, 4H), 6.82 (m, 4H); No mass data (entire sample used for biological testing.



DAW-11-16 DAW-11-26 (CDE-418)

3-(2,5-Dihydroxybenzylidene)-5-(2-(3,4-dihydroxyphenyl)thiazol-4-yl)indolin-2-one [DAW-11-26 (CDE-418)]

To a 0 ºC stirring solution of DAW-11-16 (50.3 mg, 0.1 mmol) in 1,2-dichloroethane (1 ml), boron tribromide (1.0 ml, 1M in CH_2Cl_2) was added dropwise under a nitrogen atmosphere. The resulting mixture was allowed to warm to room temperature overnight with stirring. The reaction was quenched with few drops of methanol. A solid was formed on addition of ethyl acetate; the solid was filtered and triturated with ethyl acetate to provide 0.0440 g (98.9%) of DAW-11-24 as a light brown solid. 1H NMR (DMSO-d6, 400 MHz) δ 10.63 (s, 1H), 8.27 (s, 1H), 7.96 (m, 1H), 7.81 (dd, *J*=8.2 Hz, 1.8 Hz, 1H), 7.68 (s, 1H), 7.65 (s, 1H), 7.31 (m, 3H), 7.84 (m, 3H), 5.35 (bs, 4H); HRMS, DART calcd. for C24H17N2O5S [M+H]+ 445.08583, found: 445.08401.

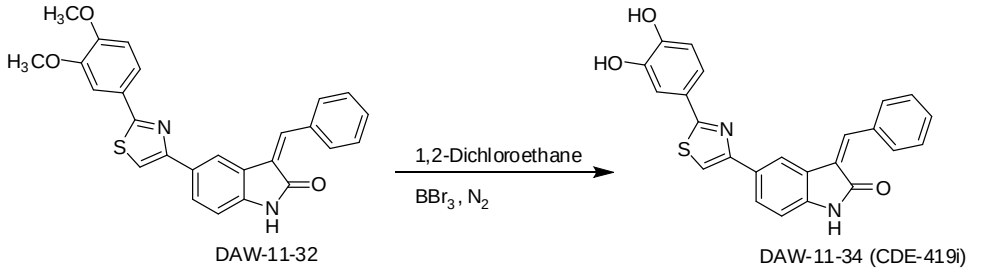


DAW-11-23 DAW-11-32

3-Benzylidene-5-(2-(3,4-dimethoxyphenyl)thiazol-4-yl)indolin-2-one [DAW-11-32]

To a mixture of DAW-11-23 (125.4 mg, 0.3547 mmol) and benzaldehyde (72.0 µl, 0.7094 mmol) in THF/EtOH (1.77 ml/1.77 ml), piperidine (35.5 µl) was added. The mixture was refluxed at 80 ºC for two days. The reaction was visualized by thin layer chromatography (80 %

EtOAc : 20 % hexane). After cooling, the brown solid was filtered and triturated with ethanol to provide 0.0832 g (53.3%) of DAW-11-32 as a black solid. ^{1}H NMR (DMSO-d_6, 400 MHz) δ 10.72 (s, 1H), 8.34 (s, 1H), 7.85 (dd, *J*=8.2 Hz, 1.8 Hz, 1H), 7.76 (m, 3H), 7.67 (s, 1H), 7.56 (t, *J*=7.3 Hz, 2H), 7.50 (d, *J*=7.3 Hz, 1H), 7.45 (dd, *J*=8.3 Hz, 1.8 Hz, 1H), 7.40 (d, *J*=1.8 Hz, 1H), 7.06 (d, *J*=8.7 Hz, 1H), 6.92 (d, *J*=8.2 Hz, 1H), 3.84 (s, 3H), 3.81 (s, 3H); ^{13}C NMR (DMSO-d_6, 100 MHz) δ 168.77, 166.78, 154.57, 150.78, 149.01, 142.82, 136.33, 134.42, 129.78, 129.48, 128.69, 127.98, 127.63, 127.47, 125.88, 121.22, 120.42, 119.39, 111.97, 11.56, 110.3, 108.85, 55.67.

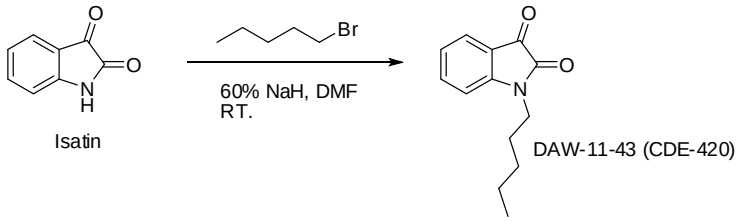


DAW-11-32 DAW-11-34 (CDE-419i)

3-Benzylidene-5-(2-(3,4-dihydroxyphenyl)thiazol-4-yl)indolin-2-one [DAW-11-34 (CDE-419i)]

To a 0 ºC stirring solution of DAW-11-32 (44.05 mg, 0.1 mmol) in 1,2-dichloroethane (1 ml), boron tribromide (1.0 ml, 1M in CH_2Cl_2) was added dropwise under a nitrogen atmosphere. The resulting mixture was allowed to warm to room temperature overnight with stirring. The reaction was quenched with few drops of methanol and few drops of water. The solid was filtered and dried under vacuum to provide 0.0457 g (quantitative yield) of DAW-11-34 as a dark yellow solid. A mixture of two *E/Z* isomers are present in the proton and carbon NMR. ^{1}H NMR (DMSO-d6, 400 MHz) for isomer A: δ 10.71(s, 1H), 8.38 (d, *J*=8.2 Hz, 1H), 8.27 (d, *J*=0.9 Hz, 1H), 7.85 (s, 1H), 7.80 (dd, *J*=8.2 Hz, 1.4 Hz, 1H), 7.74 (s, 1H), 7.61 (s, 1H), 7.56 (d, *J*=7.4 Hz, 2H), 7.34 (d, *J*=2.3 Hz, 2H), 7.21 (dd, *J*=8.3 Hz, 1.8 Hz, 1H), 6.92 (d, *J*=8.3 Hz, 1H), 6.82 (d,

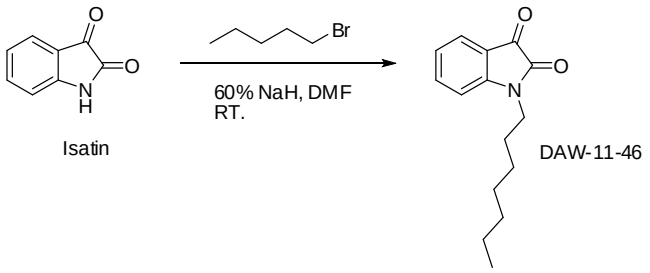
J=8.3 Hz, 1H), 4.04 (s, 2H) and for isomer B: δ 10.73(s, 1H), 8.37 (d, *J*=7.3 Hz, 1H), 8.32 (d, *J*=0.9 Hz, 1H), 7.92 (s, 1H), 7.87 (dd, *J*=7.8 Hz, 1.8 Hz, 1H), 7.76 (s, 1H), 7.68 (s, 1H), 7.53 (d, *J*=6.9 Hz, 2H), 7.44 (m, 2H), 7.30 (m, 1H), 6.88 (d, *J*=8.3 Hz, 1H), 6.81 (d, *J*=8.2 Hz, 1H), 4.04 (s, 2H); ^{13}C NMR (DMSO-d6, 100 MHz) δ 168.77, 167.34, 167.28, 167.19, 155.01, 154.36, 147.95, 147.91, 145.70, 142.70, 140.63, 137.32, 136.35, 134.43, 133.95, 131.99, 130.51, 129.86, 129.42, 128.77, 128.24, 127.89, 127.81, 127.64, 127.20, 126.65, 125.32, 124.79, 124.73, 121.29, 120.64, 118.18, 117.97, 117.70, 116.03, 115.97, 113.43, 113.37, 111.01, 110.77, 110.25, 109.49 ; HRMS, DART calcd. for C24H17N2O5S [M+H]+ 413.09599, found:413.09760.



1-Pentylindoline-2,3-dione [DAW-11-43 (CDE-420)]

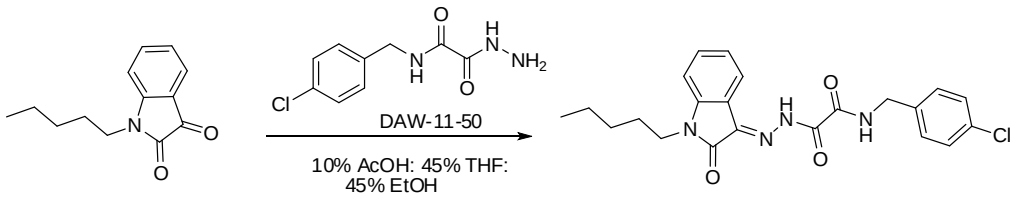
To a solution of isatin (176.9 mg, 1.2 mmol) in dimethylformamide (3.5 ml), 60% sodium hydride (52.9 mg, 1.3 mmol) was added portionwise and stirred for two hours. 1-Bromopentane (161.1 µl, 1.3 mmol) was added. The reaction mixture was stirred overnight at room temperature, and was visualized by thin layer chromatography (60% EtOAc: 40% hexane). The magenta reaction mixture was diluted with ≈24 ml of a 5:1 mixture of ethyl acetate:hexane and washed with 0.4N HCl (2x), H_2O (1x), dried over $MgSO_4$, and concentrated *in vacuo* to obtain 0.2405 g (92.3%) of DAW-11-43 as a red solid. ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.57 (m, 2H), 7.10 (t, *J*=7.4 Hz, 1H), 6.88 (d, *J*=8.2 Hz, 1H), 3.70 (t, *J*=7.3 Hz, 2H), 1.69 (p, *J*=7.3 Hz, 2H), 1.35 (m, 4H), 0,89 (t, *J*=6.8 Hz, 3H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ183.63, 158.10, 151.04, 138.27,

125.38, 123.55, 117.55, 110.12, 40.21, 28.93, 26.89, 22.24, 13.85; HRMS, DART calcd. for $C_{13}H_{16}NO_2$ $[M+H]^+$ 218.11810, found: 218.11330.



1-Heptylindoline-2,3-dione [DAW-11-46]

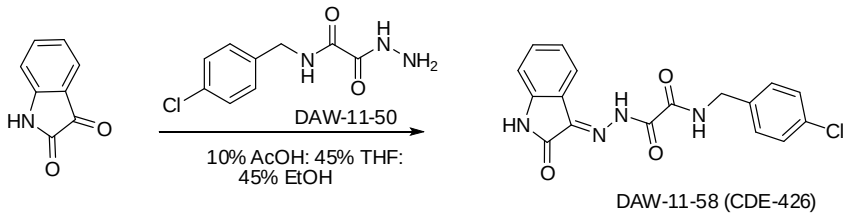
To a solution of isatin (176.7 mg, 1.2 mmol) in dimethylformamide (3.0 ml), 60% sodium hydride (54.2 mg, 1.6 mmol) was added portionwise and stirred for two hours. 1-Bromoheptane (205.0 µl, 1.3 mmol) was added. The reaction mixture was stirred overnight at room temperature and was visualized by thin layer chromatography (60% EtOAc: 40% hexane). The red reaction mixture was diluted with ≈24 ml of a 5:1 mixture of ethyl acetate:hexane and washed with 0.4N HCl (2x), H_2O (1x), dried over $MgSO_4$, and concentrated *in vacuo* to obtain 0.2321 g (78.8%) of DAW-11-46 as a red oil. ^{1}H NMR ($CDCl_3$, 400 MHz) δ7.57 (m, 2H), 7.10 (t, *J*=6.9 Hz, 1H), 6.88 (d, *J*=8.3 Hz, 1H), 3.70 (t, *J*=7.3 Hz, 2H), 1.68 (p, *J*=7.3 Hz, 2H), 1.30 (m, 8H), 0.86 (t, *J*=.6.9 Hz, 3H).



DAW-11-43 DAW-11-51 (CDE-421)

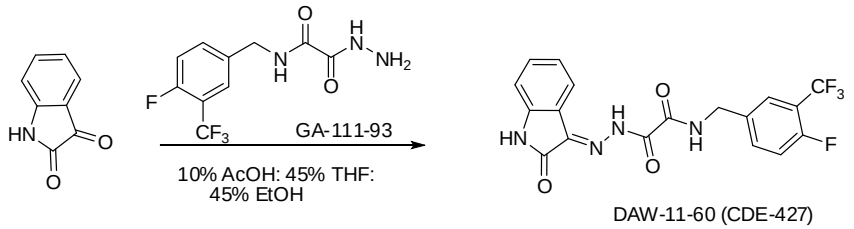
***N*-(4-Chlorobenzyl)-2-oxo-2-(2-(2-oxo-1-pentylindolin-3-ylidene)hydrazynil)acetamide [DAW-11-51 (CDE-421)]**

A mixture of DAW-11-43 (108.64 mg, 0.50 mmol) and DAW-11-50 (113.8 mg, 0.50 mmol) in a solution of acetic acid (10%), ethanol (45%), and tetrahydrofuran (45%, total of 6.5 ml) was stirred for 24 hr at room temperature. The reaction was visualized by thin layer chromatograpghy (50% EtOAc : 50% hexane), and concentrated *in vacuo* to obtain a solid, which was triturated with ethanol to provide 0.0936 g (43.9%) of DAW-11-51 as bright orange crystals. ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.80 (d, *J*=7.3 Hz, 1H), 7.74 (t, *J*=5.9 Hz, 1H), 7.40 (dt, *J*=0.9 Hz, 7.8 Hz, 1H), 7.30 (m, 2H), 7.25 (m, 3H), 7.11 (t, *J*=7.8 Hz, 1H), 6.87 (d, *J*=7.8 Hz, 1H), 4.52 (d, *J*=6.4 Hz, 2H), 3.74 (t, *J*=7.3 Hz, 2H), 1.70 (p, *J*=7.4 Hz, 2H), 1.35 (m, 4H), 0.89 (t, *J*=6.9 Hz, 3H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ161.04, 158.74, 157.27, 143.87, 140.70, 135.24, 133.82, 132.46, 129.38, 128.96, 123.38, 122.57, 119.23, 109.35, 43.09, 39.91, 28.98, 27.12, 22.27, 13.88; HRMS, DART calcd. for $C_{22}H_{24}ClN_4O_3$ $[M+H]^+$ 427.15370, found: 427.15201.



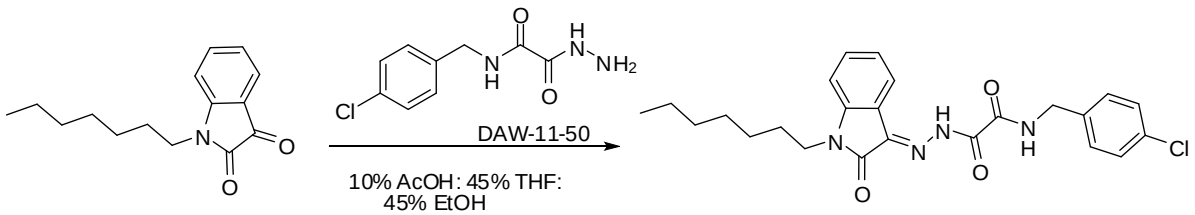
_N_-(4-Chlorobenzyl)-2-oxo-2-(2-(2-oxoindolin-3-ylidene)hydrazynil)acetamide [DAW-11-58 (CDE-426)]

A mixture of isatin (73.8 mg, 0.50 mmol) and DAW-11-50 (114.2 mg, 0.50 mmol) in a solution of acetic acid (10%), ethanol (45%), and tetrahydrofuran (45%, total of 6.5 ml) was stirred for 24 hr at room temperature. The reaction was visualized by thin layer chromatograpghy (80% EtOAc : 20% hexane), and then concentrated *in vacuo* to obtain a solid, which was triturated with ethanol to provide 0.1084 g (60.8%) of DAW-11-58 as a yellow solid. A mixture of *E/Z* isomers is present in the proton and carbon NMR. ^{1}H NMR (DMSO-d_6, 400 MHz) for isomer A: δ 11.58 (s, 1H), 10.86 (s, 1H), 9.84 (bs, 1H), 7.64 (d, *J*=7.3 Hz, 1H), 7.41 (m, 5H), 7.09 (t, *J*=7.8 Hz, 1H), 6.90 (d, *J*=7.8 Hz, 1H), 4.36 (d, *J*=5.9 Hz, 2H), and for isomer B: δ 11.26 (s, 1H), 10.86 (s, 1H), 9.76 (t, *J*=6.4 Hz, 1H), 7.55 (d, *J*=7.3 Hz, 1H), 7.32 (m, 5H), 7.06 (t, *J*=7.8 Hz, 1H), 6.90 (d, *J*=7.8 Hz, 1H), 4.33(d, *J*=6.4 Hz, 2H) ; ^{13}C NMR (DMSO-d_6, 100 MHz) δ 164.24, 162.52, 159.32, 159.04, 157.30, 156.78, 144.42, 143.88, 143.12, 140.51, 137.32, 133.70, 132.53, 131.69, 131.67, 129.43, 128.32, 125.99, 122.77, 122.20, 121.47, 119.54, 115.42, 111.28, 42.04; HRMS, DART calcd. for $C_{17}H_{14}ClN_4O_3$ $[M+H]^+$ 357.07545, found: 357.07620.



***N*-(4-Fluoro-3-(trifluoromethyl)benzyl)-2-oxo-2-(2-(2-oxoindolin-3-ylidene)hydrazynil)acetamide [DAW-11-60 (CDE-427)]**

A mixture of isatin (37.0 mg, 0.25 mmol) and GA-111-93 (69.9 mg, 0.25 mmol) in a solution of acetic acid (10%), ethanol (45%), and tetrahydrofuran (45%, total of 3.25 ml) was stirred for 24 hr at room temperature. The reaction was visualized by thin layer chromatography (80% EtOAc : 20% hexane), and then concentrated *in vacuo* to obtain a solid, which was triturated with ethanol to provide 0.0508 g (49.8%) of DAW-11-60 as a bright yellow solid. A mixture of *E/Z* isomers are present in the proton and carbon NMR. ^{1}H NMR (DMSO-d_6, 400 MHz) for isomer A: δ 11.58 (s, 1H), 10.86 (s, 1H), 9.87 (bs, 1H), 7.72 (m, 2H), 7.56 (d, *J*=7.8 Hz, 1H), 7.46 (m, 2H), 7.09 (t, *J*=8.2 Hz, 1H), 6.90 (d, *J*=7.8 Hz, 1H), 4.43 (d, *J*=6.4 Hz, 2H), and for isomer B: δ 11.26 (s, 1H), 10.86 (s, 1H), 9.80 (t, *J*=6.4 Hz, 1H), 7.65 (m, 3H), 7.39 (m, 2H), 7.05 (t, *J*=8.2 Hz, 1H), 6.90 (d, *J*=7.8 Hz, 1H), 4.40(d, *J*=6.4 Hz, 2H); ^{13}C NMR (DMSO-d_6, 100 MHz) δ 164.16, 162.47, 159.38, 159.22, 157.88 (d, *J*=245.0 Hz), 157.21, 157.16, 144.39, 143.91, 143.87, 143.08, 140.44, 135.46 (d, *J*=2.9 Hz), 134.46 (d, *J*=8.6 Hz), 133.63, 132.46, 126.38 (q, *J*=3.8 Hz), 125.99, 122.71, 122.62 (q, *J*=271.73 Hz), 122.11, 121.41, 119.51, 117.19 (d, *J*=21.0 Hz), 117.14 (d, *J*=20.0 Hz), 115.39, 111.21, 41.59; HRMS, DART calcd. for $C_{18}H_{13}F_4N_4O_3$ $[M+H]^+$ 409.09239, found: 409.09149.



DAW-11-46

Chemical Formula: $C_{15}H_{19}NO_2$
Molecular Weight: 245.32

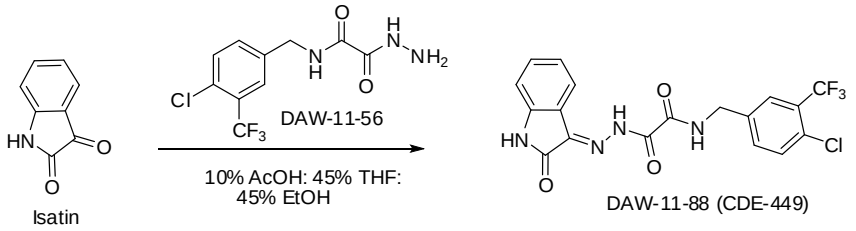
DAW-11-62

Chemical Formula: $C_{24}H_{27}ClN_4O_3$
Molecular Weight: 454.95

***N*-(4-Chlorobenzyl)-2-(2-(1-heptyl-2-oxoindolin-3-ylidene)hydrazynil)2-oxoacetamide**

[DAW-11-62 (CDE-425)]

A mixture of DAW-1-46 (122.2 mg, 0.50 mmol) and DAW-11-50 (113.8 mg, 0.50 mmol) in a solution of acetic acid (10%), ethanol (45%), and tetrahydrofuran (45%, total of 6.5 ml) was stirred for 24 hr at room temperature. The reaction was visualized by thin layer chromatography (80% EtOAc : 20% hexane), and then concentrated *in vacuo* to obtain a solid, which was triturated with ethanol to provide 0.0470 g (20.7%) of DAW-11-62 as bright orange crystals. ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.80 (d, *J*=7.3 Hz, 1H), 7.73 (t, *J*=5.9 Hz, 1H), 7.40 (t, *J*=7.8 Hz, 1H), 7.31 (d, *J*=8.2 Hz, 2H), 7.25 (m, 3H), 7.11 (t, *J*=7.8 Hz, 1H), 6.87 (d, *J*=7.8 Hz, 1H), 4.52 (d, *J*=5.9 Hz, 2H), 3.73 (t, *J*=6.9 Hz, 2H), 1.69 (p, *J*=7.3 Hz, 2H), 1.29 (m, 8H), 0.86 (t, *J*=6.8 Hz, 3H) ;^{13}C NMR ($CDCl_3$, 100 MHz) δ 161.04, 158.74, 157.28, 143.88, 140.72, 135.24, 133.84, 132.46, 129.39, 128.98, 123.38, 122.58, 119.25, 109.36, 43.09, 39.97, 31.62, 28.87, 27.44, 26.88, 22.54, 14.01; HRMS, DART calcd. for $C_{24}H_{28}ClN_4O_3$ $[M+H]^+$ 455.18498, found: 455.18329.



_N_-(4-chloro-3-(trifluoromethyl)benzyl)-2-oxo-2-(2-(2-oxoindolin-3-ylidene)hydrazinyl)acetamide [DAW-11-88 (CDE-449)]

A mixture of isatin (73.6 mg, 0.5 mmol) and DAW-11-56 (147.8 mg, 0.5 mmol) in a solution of acetic acid (10%), ethanol (45%), and tetrahydrofuran (45%, total of 6.5 ml) was stirred for three days at room temperature. The reaction was visualized by thin layer chromatography (80% EtOAc : 20% hexane), and then concentrated *in vacuo* to obtain a solid, which was triturated with ethanol to provide 0.1830 g (86.2%) of DAW-11-60 as a bright yellow solid. A 3:1 mixture of *E/Z* isomers are present in the proton and carbon NMR. ^{1}H NMR (DMSO-d_6, 400 MHz) δ 11.58 (s, 1H, isomer A), 11.26 (s, 1H, isomer B), 10.86 (s, 1H, mixture of A&B), 9.89 (t, *J*=5.9 Hz, 1H, isomer A), 9.81 (t, *J*=6.4 Hz, 1H, isomer B), 7.78 (m, 1H, mixture of A&B), 7.61 (m, 3H, mixture of A&B), 7.38 (m, 1H, mixture of A&B), 7.07 (m, 1H, mixture of A&B), 6.09 (d, *J*=7.8 Hz, 1H, mixture of A&B), 4.43 (d, *J*=6.4 Hz, 2H, mixture of A&B); ^{13}C NMR (DMSO-d_6, 100 MHz) δ 164.15, 162.46, 159.42, 159.16, 157.14, 156.61, 144.40, 143.94, 143.08, 140.46, 138.42, 133.64, 133.33, 132.46, 131.64, 131.61, 129.27, 127.02 (q, *J*=5.7 Hz), 126.41 (q, *J*=29.6 Hz), 125.99, 122.84 (q, *J*=271.7 Hz), 122.71, 122.12, 121.41, 119.50, 115.37, 11.22, 41.69; HRMS, DART calcd. for $C_{18}H_{13}ClF_3N_4O_3$ $[M+H]^+$ 425.06282, found: 425.06421.

References:

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3. Diaz, P.; Xu, J.; Astruc-Diaz, F.; Pan, H.; Brown, D.L.; Naguib, M. *J. Med. Chem.* **2008,** *51*, 4932-4947.