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Design and synthesis of oxadiazolidinediones as inhibitors of plasminogen activator inhibitor-1

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Abstract—A novel series of PAI-1 inhibitors containing an oxadiazolidinedione moiety were identified by high through-put screening. Optimization of substituents by parallel synthesis and the iterative design toward understanding structure–activity relationship to improve potency are described.

The serine protease inhibitor plasminogen activator inhibitor-1 (PAI-1)[1] is one of the primary inhibitors of the fibrinolytic system. The fibrinolytic system includes the proenzyme plasminogen, which is converted to the active enzyme, plasmin, by one of the tissue type plasminogen activators, urokinase plasminogen activator (uPA), and tissue plasminogen activator (tPA).[2] PAI-1 is the principal physiological inhibitor of uPA and tPA. One of plasmin's main responsibilities in the fibrinolytic system is to digest fibrin at the site of vascular injury. PAI-1 is acutely elevated in thrombotic events such as deep vein thrombosis following post-operative recovery from orthopedic surgery,[3] but is also associated with angiogenesis and vascular remodeling in chronic diseases such as atherosclerosis and cancer.[4] PAI-1 is synthesized by a number of different tissues, including hepatic, vascular, and adipose, and is stored in platelets to provide high local concentrations for clot stabilization.[5]

Because PAI-1 regulates plasminogen activation, it functions to regulate fibrinolysis not coagulation. Peptide inhibitors of PAI-1 have been shown to lyse clots in vitro without affecting clot formation and PAI-1 null mice have normal coagulation, but acceleration of clot lysis.[6] PAI-1 null humans have been identified and observed to have normal coagulation and abnormal bleeding only following trauma or surgery.[7] Thus, inhibition of PAI-1 would represent a useful strategy in treating a variety of cardiovascular diseases. A number of small molecules have been reported[8] to inhibit PAI-1 apart from antibodies[9] and peptides.[10] Some of the recent small molecule inhibitors are diketopiperazine analogues[11] **1**, benzothiophene derivatives[12] **2**, menthol based inhibitors[13] **3**, and piperazine analogues[14] **4** (Fig. 1).

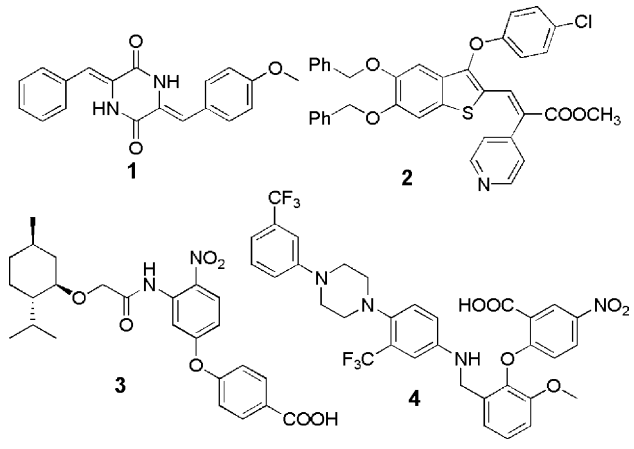

Figure 1. PAI-1 small molecule inhibitors.

Keywords: PAI-1 inhibitor; Oxadiazolidine diones; Parallel synthesis.
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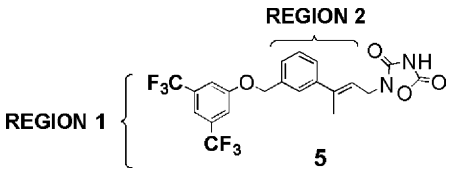
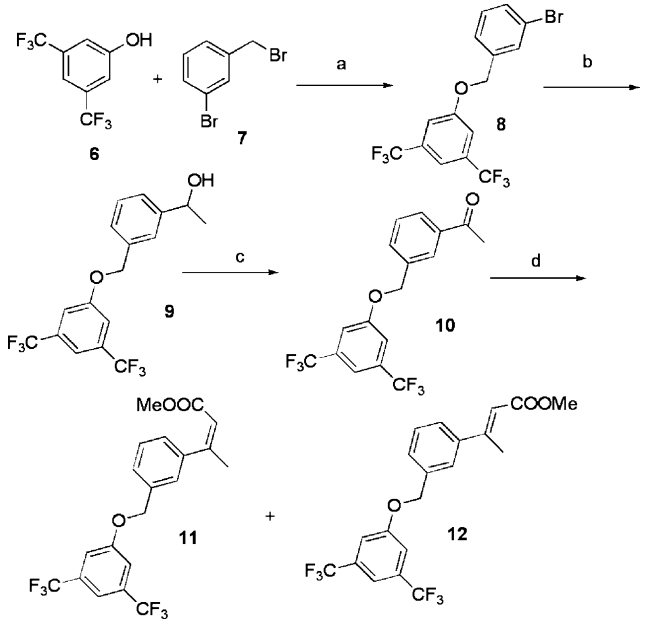


Figure 2.

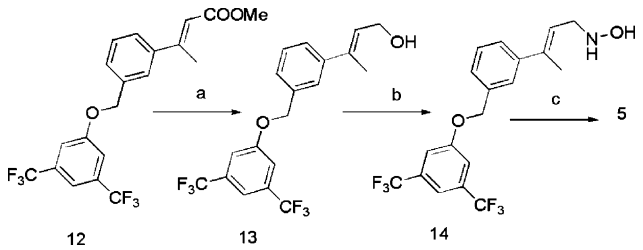
Our efforts toward identifying a PAI-1 inhibitor started with the high throughput screening of various compound libraries. The effort culminated in the identification of oxadiazolidinedione **5** (Fig. 2).

In an in vitro tPA activity assay,[15] **5** inhibited the PAI-1 inhibition of tPA with an IC_{50} of 5.29 μM, restoring enzymatic activation by tPA of its synthetic substrate. Using fluorescence spectroscopy,[16] the direct binding of **5** to PAI-1 indicated the inhibitor bound PAI-1 and the binding is consistent with the observed inhibition.

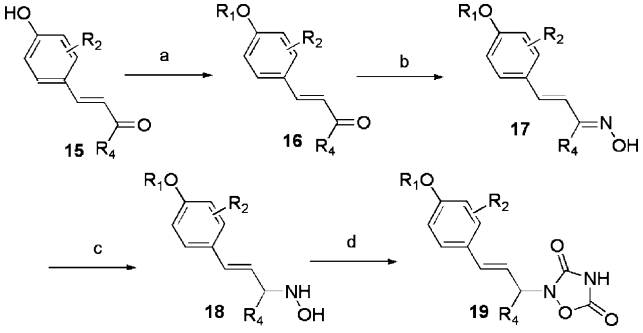
Oxadiazolidine **5** and analogues were prepared by following synthetic Schemes 1–3.[17] Benzylation of the phenol **6** with the bromide **7** gave the bromo compound **8**. Lithium exchange followed by reaction with acetaldehyde gave the benzyl alcohol **9**, which was oxidized to ketone **10** and subjected to olefination. A mixture of *cis* and *trans* isomers **11** and **12** were obtained in a 55:45 ratio (Scheme 1). The isomers were readily separated by column chromatography and individual isomers characterized by ^{1}H NMR were carried forward toward the synthesis of oxadiazolidinediones. As shown in Scheme 2 the ester was reduced to alcohol **13** using DIBAL. Mitsunobu reaction with bis Boc protected hydroxyl amine followed by deprotection gave the hydroxylamine



Scheme 1. Reagents and conditions: (a) K_2CO_3, acetone, reflux, 6 h, 74%; (b) (1) *n*-BuLi, −78 °C, 1 h; (2) acetaldehyde, 60 °C, 2 h, 63%; (c) Jones reagent, rt, 69%; (d) $(CF_3CH_2O)_2P(O)CH_2COOMe$, NaH, 0–25 °C, 12 h, 61%.



Scheme 2. Reagents and conditions: (a) DIBAL, THF, 0–25 °C, 2 h; (b) (1) (Boc)NHO(Boc), Ph_3P, DEAD, THF, rt, 85%; (2) TFA, DCM, rt, 82%; (c) ClCONCO, THF, rt, 95%.



Scheme 3. Reagents and conditions: (a) K_2CO_3, acetone, R_1X, reflux, 6 h; (b) NH_2OH, EtOH/Py, 60 °C, 2 h; (c) BH_3/Py, MeOH, 10% HCl, rt; (d) ClCONCO, THF, rt.

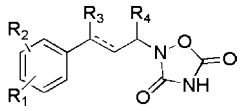
derivative **14**, which upon treatment with chlorocarbonyl isocyanate gave the desired oxadiazolidine dione derivative **5**.

Analogues **19** with substituents on the carbon adjacent to the oxadiazolidinedione ring were prepared as shown in Scheme 3. Alkylation of the α,β-unsaturated aldehyde or ketone **15** followed by condensation with hydroxylamine gave the oxime **17**. Borane reduction of the oxime and treatment of the resulting hydroxylamine **18** with chlorocarbonyl isocyanate gave the final targets. The synthetic sequence was found to be general and readily amenable for parallel synthesis.[18]

Our effort toward optimization of the lead **5** was focused on regions 1 and 2 (Fig. 2) while retaining the oxadiazolidinedione moiety intact. Since the HTS lead was the *trans* isomer it was of interest to see if the *cis* isomer **20** possessed any activity. The *cis* analogue synthesized was found to be equipotent in inhibiting the PAI-1 inhibition of tPA indicating that the stereochemistry of the double bond did not play a role in binding. Region 1 of the lead molecule has a very lipophilic phenyl ether tail. Switching the position of the oxygen atom and converting the lead to benzyl ether **21** retained the potency.

To explore the steric and lipophilic requirements for this region several analogues were made by parallel synthesis. The general trend observed is exemplified by analogues **21** through **29** in Table 1. Removal of the CF_3 groups to the unsubstituted benzyl ether **22** or substituting with a simple 3-methylbenzyl ether **23** lead to

Table 1. PAI-1 inhibitory activity of oxadiazolidinediones



Ex	R_1	R_2	R_3	R_4	Db/Sb[a] stereo	IC_{50} (μM % inhib[b])
5	3[(3,5-BisCF_3Ph)OCH_2]–	H	Me	H	*trans* Db	5.29
20	3[(3,5-BisCF_3Ph)OCH_2]–	H	Me	H	*cis* Db	7.2
21	3[(3,5-BisCF_3Ph)CH_2O]–	H	Me	H	*trans* Db	9.4
22	3($PhCH_2O$)–	H	Me	H	*trans* Db	24%
23	3[(3-MePh)CH_2O]–	H	Me	H	*trans* Db	37%
24	3[(2-Pyridyl)CH_2O]–	H	Me	H	*trans* Db	24.0
25	3[(2-Biphenyl)CH_2O]–	H	Me	H	*trans* Db	2.3
26	3,5[Bis(4-CF_3Ph)CH_2O]–	H	H	H	*trans* Db	2.5
27	3,5[Bis(4-ClPh)CH_2O]–	H	H	H	*trans* Db	2.1
28	4[(3,4-DiClPh)CH_2O]–	3-OMe	H	H	*trans* Db	3.22
29	5[(3,4-DiClPh)CH_2O]–	2-Cl	H	H	*trans* Db	3.8
30	4[(3,5-BisCF_3Ph)CH_2O]–	H	H	H	*trans* Db	14.9
31	4[(3,5-BisCF_3Ph)CH_2O]–	H	Et	H	*trans* Db	9.0
32	3[(3,5-DiClPh)O]–	H	Bu	H	*trans* Db	1.9
33	3[(3-MePh)O]–	H	Bu	H	Sb	2.2
34	4[(3-CF_3Ph)CH_2O]–	H	H	Me	*trans* Db	5.8
35	4[(3,4-DiClPh)CH_2O]–	H	H	Me	*trans* Db	6.1
36	4[(3,5-BisCF_3Ph)CH_2O]–	H	H	Ph	Sb	0.39
37	4[(3-ClPh)CH_2O]–	H	H	Ph	Sb	0.68

[a] Db: double bond; Sb: single bond.
[b] Inhibition @ 25 μM.

significant loss of potency. Similarly introducing a less lipophilic heteroaromatic tail piece like a 2-pyridylmethyl ether **24** showed reduced potency as well. To explore a nonhalogenated lipophilic tail piece, 2-biphenylmethyl ether **25** was synthesized and was found to show slight improvement. Similarly, bisbenzyloxy derivatives **26** and **27** showed slight improvement in potency although these analogues might occupy larger volume in the binding site. Adding yet another substituent R_2 on the central phenyl ring was not detrimental, but it did not afford any additional boost in activity as shown by examples **28** and **29**. From this preliminary SAR information it was clear that a highly lipophilic tail piece is preferred in region 1 and the region was flexible to accommodate bulky substituents.

In region 2, it was of importance to determine the contribution of the methyl substituent on the double bond toward activity. A twofold loss of potency was observed in the case of analogue **30**, which is devoid of the methyl group. However, increasing the size of the methyl group to ethyl **31** or *n*-butyl **32** led to a slight improvement in potency. In the case of butyl analogues reduction of the double bond to give the saturated analogue **33** retained the potency once again supporting the observation that the binding of this class of compounds is not affected by the rigidity provided by the double bond or its stereochemistry.

Moving the methyl group position from the double bond to the methylene adjacent to oxadiazolidinedione did not affect the potency as shown by examples **34** and **35**. However, an increase in activity was observed when the R_4 substituent was a phenyl group as shown in the saturated analogues **36** and **37**[19] with submicromolar inhibition. Thus the SAR for region 2 seems to show flexibility with respect to the linker, however a larger lipophilic group is preferred at R_4 or R_3 irrespective of the saturated or unsaturated nature of the linker.

In summary, we have explored the structure–activity relationships around the novel oxadiazolidinedione based PAI-1 inhibitor **5** by modifying regions 1 and 2. Initial optimization of the region 1 indicates the preference for lipophilic groups in that region and seems to be flexible in terms of steric requirements accommodating bulky substituents. Preliminary studies in region 2 indicated that the linker need not be a rigid double bond and is flexible with respect to the stereochemistry as well. As far as the substituents off of the linker, a lipophilic group like phenyl is favored over smaller alkyl substituent like methyl, resulting in submicromolar inhibitors.

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15. *Determination of PAI-1 inhibition of tPA activity*: Test compounds were dissolved in DMSO at a final concentration of 10 mM, then diluted 100 × in physiologic buffer. The inhibitory assay was initiated by the addition of test compound (1–100 μM final concentration, maximum DMSO concentration of 0.2%) in a pH 6.6 buffer containing 140 nM recombinant human plasminogen activator inhibitor-1 (PAI-1; *Molecular Innovations, Royal Oak, MI*). Following a 1 h incubation at room temperature, 70 nM of recombinant human tissue plasminogen activator (tPA) was added, and the combination of test compound, PAI-1 and tPA was incubated for an additional 30 min. Following the second incubation, Spectrozyme-tPA (*American Diagnostica, Greenwich, CT*), a chromogenic substrate for tPA, was added and absorbance read at 405 nm at 0 and 60 min. Relative PAI-1 inhibitory activity was equal to the residual tPA activity in the test compound/PAI-1 treatment. Control treatments include the complete inhibition of tPA by PAI-1 at the molar ratio employed (2:1), and the absence of any effect of the test compound on tPA alone.
16. *Direct binding to PAI-1*: Fluorescence spectroscopy was used to determine the binding of **5** to recombinant human PAI-1. The changes in the quantum yield of the protein were used to determine the binding affinity of **5** to PAI-1 using a Jobin Yvon fluorometer. A fixed concentration of the protein was serially titrated with increasing concentrations of **5**. The intrinsic fluorescence of the protein was measured at 335 nm using an excitation wavelength of 295 nm.
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18. Compounds were purified by HPLC and the purity was >90%. LC conditions: HP 1100, 23 °C, 10 μL injected; column: YMC-ODS-A 4.6 × 5.0 5 μm; gradient A: 0.05% TFA/water, B: 0.05% TFA/acetonitrile; time 0 and 1 min: 98% A and 2% B; 7 min: 10% A and 90% B; 8 min: 10% A and 90% B; 8.9 min: 98% A and 2% B; post time 1 min; flow rate 2.5 mL/min; detection: 215 and 254 nm, DAD. Semi-Prep HPLC: Gilson with Unipoint software; Column: Phenomenex C18 Luna 21.6 mm × 60 mm, 5 μM; solvent A: water (0.02% TFA buffer); solvent B: acetonitrile (0.02 % TFA buffer); solvent gradient: time 0: 5% B; 2.5 min: 5% B; 12 min: 95% B; hold 95% B 3 min; flow rate: 22.5 mL/min; detection: 215 and 254 nm.
19. During the reduction of the α,β-unsaturated oxime only saturated hydroxyl amines were obtained during the synthesis of analogues **36** and **37**.