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Novel Inhibitors of Plasminogen Activator Inhibitor-1: Development of New Templates From Diketopiperazines

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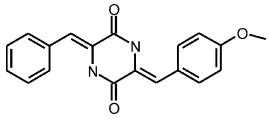
Abstract—Several isoquinoline-based templates were identified from the studies of the conformational effects of the diketopiperazine structures for PAI-1 inhibition. Moderate to good activity was retained with the elimination of unattractive characteristics in the diketopiperazine template.

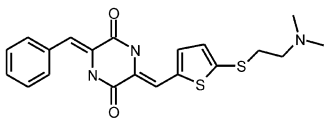
Plasminogen activator inhibitor-1 (PAI-1), a member of serine proteinase inhibitor family (serpin), is the major physiological inhibitor of tissue plasminogen activator (tPA) and urokinase plasminogen activator (uPA). These serine proteinases convert the zymogen plasminogen, to the active enzyme plasmin, which degrades fibrin clots.[1] Among the serpins, PAI-1 exhibits a unique conformational flexibility. Although it is synthesized as an active molecule, PAI-1 spontaneously converts to an inactive latent form[2] through the insertion of a large portion of the reactive centre loop[3] of the active form into β sheet A.[4] A third conformation of PAI-1, termed the substrate form, has been reported to be non-inhibitory towards various target proteinases.[3] X-ray structures of PAI-1 in the latent form,[4] and recently, the active conformation of a stable mutant form[5,6] are known. In addition, the conformation of the cleaved substrate form has also been identified.[3,7]

Epidemiological studies have shown that elevated circulating levels of PAI-1 are associated with coronary heart disease and possibly atherosclerosis.[8] These findings have led to considerable interest in the development of drugs that specifically inhibit PAI-1.[9] Initial PAI-1 inhibition studies with anti-sense oligonucleotides have given encouraging results[10,11] and inhibition of PAI-1 with a polyclonal antibody fragment[12] and inhibition with monoclonal antibodies,[13] resulted in enhancement of fibrinolysis. In addition, a synthetic peptide based on the reactive center loop of PAI-1 (P1–P14) was shown to inhibit PAI-1 in vitro.[14] These early approaches have been useful in demonstrating 'proof of principle'; however, they are unlikely to be clinically applicable.

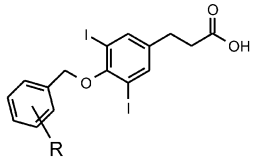
A number of small molecules have also been reported to inhibit PAI-1. The first compound was a DKP analogue XR334 (**1**).[15] More recently, XR5118 (**2**),[16] benzyloxy diiodophenyl alkanoic acid derivatives (**3**)[17] and AR-H029953XX (**4**)[18] have been described.



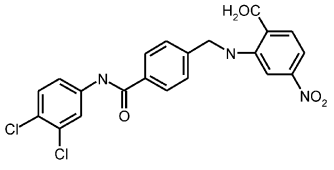
XR334 (1)



XR5118 (2)



(3)



AR-H029953XX (4)

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To date, XR5118 (**2**) and the related compound XR11211[19] are the most potent PAI-1 inhibitors reported, both in vitro[20] (XR5118 and XR11211) and in vivo[21] (XR5118). However, after extensive SAR work on the above DKP series, further development of this template was precluded by poor physicochemical properties due to their high lattice energies; in addition they were prone to isomerisation.[22] Moreover, due to the symmetrical nature of the template, the interpretation of SAR and the understanding of the pharmacophore proved to be difficult. Therefore, to find more drug-like PAI-1 inhibitors, we set out to design a series of new templates based on our understanding of the DKP series.

Upon further examination of the crystal structures of the DKP analogues,[23] it was noticed that the piperazinedione ring is almost planar while the two styryl groups on both side of the DKP template are significantly deviated from the DKP plane. This interesting conformation was identified as a potential opportunity for further investigation. Our initial studies were centred on introducing *N*-alkylation of one of the DKP amides to further disrupt the orientation of the styryl group on the left hand side and also forming an intramolecular H bond with either of the N−H of the DKP to flatten and rigidify the template. Table 1 exemplifies some of the data on analogues based on one of our more potent DKP inhibitors (**5**).

As the data in Table 1 illustrate, *N*-methylation of the potent compound (**5**) results in compound **6** with significant loss of PAI-1 inhibitory activity in our S2251 assay.[24] However, analogues with a flat conformation through internal H bond formation of the 'bottom' amide N–H and the embedded N atoms in the heteroaromatic

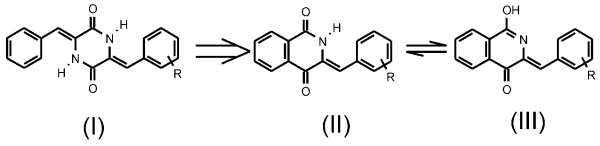


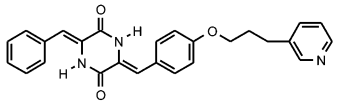
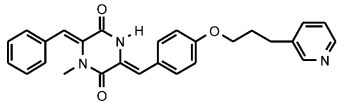
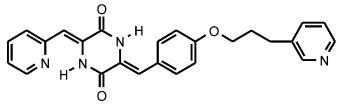
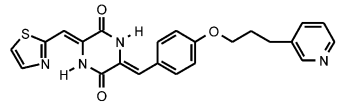
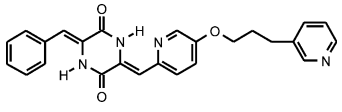
Figure 1.

rings on the left of the DKP give improved activity (**7** and **8**).[25] Interestingly, introduction of a similar internal H bond to the top half amide NH (**9**) failed to show any activity at 50 μM. The above dramatic results led us to assume tentatively that the bottom N–H may not be that crucial for binding while the top amide N–H may confer the inhibitory activity via an enol form. The retention of some degree of activity for *N*-methylated compound (**6**) and loss of activity for DKP analogues with *N*-alkylation at the top amide N–H (data not shown here) may also support such a hypothesis. Therefore, we proposed to fuse the styryl group on the left into the DKP ring to rigidify the template and eliminate the symmetry, which led to our first new template, the isoquinoline dione series (**II**)/(**III**) as shown in Figure 1.

Here, we disclose some of our initial findings in the form of embryonic SAR for some of the examples in this series in Table 2.

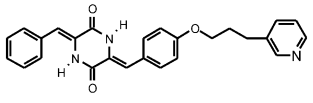
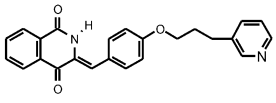
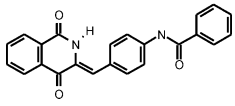
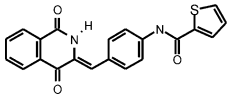
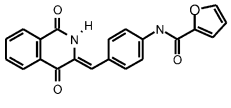
As the data in Table 2 illustrate, compound **10** derived from DKP (**5**) retains some of the inhibitory activity against PAI-1, while compounds **11**–**13** derived from the corresponding DKP analogues show more or less the same activity. Encouraged by these results, we intensified our efforts in modifying this template further to

Table 1. Studies on the conformational effects on PAI-1 activity for compounds with a DKP template

Compd[a]	Structure	IC_{50} (μM) (S2251)
5		1.0
6		12.0
7		0.30
8		0.60
9		> 50

[a]These DKP analogues are generally synthesized according to the reported procedures.[15,19]

Table 2. PAI-1 Inhibitory activity for isoquinoline dione analogues

Compd[a]	Structure	IC_{50} (μM) (S2251)
5		1.0
10		18.0
11		8.6
12		4.0
13		1.9

[a]These isoquinoline dione analogues are synthesized according to a one step procedure in literature.[26]

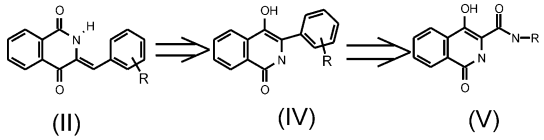
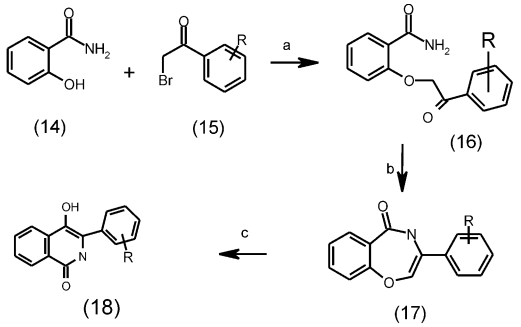


Figure 2.



Scheme 1. Reagents and conditions: (a) KOH, KI (cat.), acetone, rt, 3 h, 48–87%; (b) PTSA, tol. Dean–Stark reflux, 4 h, 55–90%; (c) $NaNH_2$, Dioxan, reflux, 1 h, 35–88%.

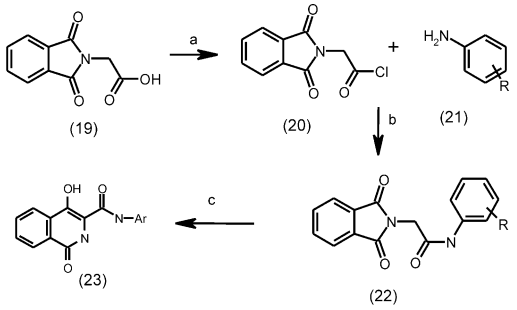
obtain more attractive and drug like templates. At this stage it was not our intention to fully optimize this particular series due to its similarities to the DKP template in many physicochemical characteristics. Therefore, we proposed to investigate the following two templates (**IV**) and (**V**) as shown in Figure 2, by the introduction of an enol functional group at the top of the template (**II**) combined with the information (hypothesis) gained in Table 1.

Literature precedent for general synthetic methods to the hydroxyisoquinoline template (**IV**) proved to be quite limited; we could only make a limited number of simple analogues in this series according to the procedure by Schenker[27] (Scheme 1). Salicylamide (**14**) was alkylated with a number of 2-bromoacetophenones (**15**) with KOH as base and catalytic amount of KI in acetone to give the intermediates (**16**) in good yields; subsequent acid (PTSA) catalyzed cyclization under Dean–Stark reflux conditions gave the compounds (**17**) in 55–90% yields. The final rearrangement reactions turned out to be capricious for some of the substituted analogues (**17**). The data on some of the compounds made in this series are summarized in Table 3.

As shown in Table 3, moderate PAI-1 inhibitory activity was observed for simple hydroxy isoquinolinone analogues **18a**–**18d** in S2251 assays, which was quite encouraging, considering that the corresponding DKP analogues do not show any appreciable activity at 50

Table 3. PAI-1 Inhibitory activity for an exploratory set of compounds **18**

Compd	R	IC_{50} (μM) (S2251)
18a	H	50.0
18b	*p*-F	39.5
18c	*p*-Cl	23.6
18d	*m*-OMe	9.8



Scheme 2. Reagents and conditions: (a) $SOCl_2$, toluene, reflux, 1 h, 100%; (b) $ArNH_2$, TEA, DCM, 50–85%; (c) Na, EtOH, reflux, 20%.

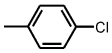
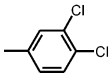
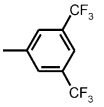
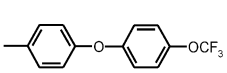
μM concentrations. Nevertheless, our efforts in exploring this series were hampered by the difficult chemistry. In order to confirm the importance of the enol form for activity, we turned our attention to the synthetically more amenable template (**V**), a hydroxy isoquinoline carboxylic amide series.

One of the synthetic approaches for the preparation of some of the analogues in this template series is outlined in Scheme 2.[28,29] *N*-Phthaloylglycine (**19**) was reacted with thionyl chloride under refluxing toluene for 1 h to give the acid chloride (**20**) as a white solid in quantitative yield. This compound was then coupled with a number of anilines (**21**) to give the corresponding amides (**22**). Subsequent treatment by NaOEt, generated in situ from sodium and ethanol gave the hydroxy isoquinoline carboxylic amides (**23**) in low yields. The PAI-1 activity data on some of the typical analogues in this series are shown in Table 4.

Moderate to good PAI-1 inhibitory activity are achieved with compounds **23a**–**23d,** as can be seen from the data in Table 4. Although the compounds prepared in this series still show poor solubility, we have achieved the goal of eliminating the symmetry in DKP template. More importantly, the enol as in the hydroxy carboxylic amide form was shown to be important for activity.

In summary, we have identified several structurally-different templates derived from the DKP template

Table 4. PAI-1 Inhibitory activity for the hydroxy isoquinoline carboxylic amide series

Compd	Ar	IC_{50} (μM) (S2251)
23a		24.0
23b		14.0
23c		13.7
23d		1.0

through our understanding and gradual modifications of the DKP structures. It is gratifying to find that moderate and good PAI-1 inhibitory activity from the evolved series are retained with the elimination of the symmetry of the DKP template and some of its chemical properties. Furthermore, the process and scope of the structural-based design have contributed to our understanding towards pharmacophore definition, which has laid the foundation for our further identification of more potent new templates.

Acknowledgements

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