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Synthesis and In Vitro Evaluation of a Series of Diketopiperazine Inhibitors of Plasminogen Activator Inhibitor-1

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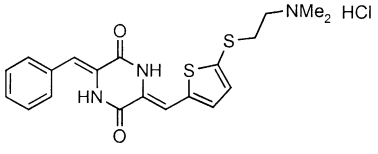
Abstract—We have synthesized and evaluated a series of diketopiperazine-based inhibitors of PAI-1. These studies resulted in the identification of **34** which inhibited PAI-1 in vitro with an $IC_{50} = 0.2\,\mu M$. The synthesis and SAR of these compounds are described.

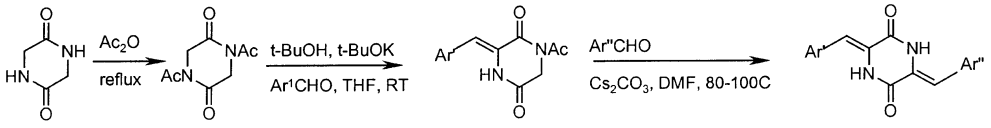
A key event in the regulation of thrombus formation and clearance is tissue plasminogen activator (tPA) generation of plasmin.[1] Plasminogen activator inhibitor-1 (PAI-1), a member of the serpin superfamily of proteinase inhibitors,[2,3] is the primary physiological regulator of tPA and is an essential regulatory protein of the fibrinolytic system. Several studies have linked increased PAI-1 activity with thromboembolic disease[4,5] and elevated levels of PAI-1 in transgenic mice are associated with severe venous thrombosis.[6] Furthermore, anti-PAI-1 antibodies have been shown to enhance fibrinolysis in vivo.[7] PAI-1 is also associated with a poor prognosis in a variety of cancers,[8] and is believed to play a role in angiogenesis,[9] metastasis[10] and invasion.[9]

PAI-1 is unique amongst serpins in that the protein can spontaneously convert from its active conformation into a latent, inactive form that is unable to irreversibly bind to tPA.[11] The reactive center loop (RCL), which is responsible for the inhibitory activity of PAI-1 is exposed in the active form, but is inserted into the major β-sheet A in the latent confirmation.[12] PAI-1 inhibition of tPA is mediated through a bait peptide bond (Arg346-Met347), present within the RCL, which mimics the natural substrate for tPA, plasminogen.[13]

We have previously reported XR5118 (**1**) which was identified from a medicinal chemistry program based on a natural product lead.[14] XR5118 has demonstrated PAI-1 inhibitory activity in vitro[15] and in vivo[16] and has been shown to attenuate the binding of a PAI-1 inhibiting monoclonal antibody CLB-2C8,[16] suggesting that binding of XR5118 to PAI-1 may occur at the epitope for CLB-2C8 which is the domain spanning amino acids 110–145. We describe here the synthesis and biological activities of a series of bis-arylidenediketopiperazines based on XR5118.



XR5118 (1)



2 **15** **1,16-26, 28, 35, 37**

Scheme 1.

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The general synthetic route used to prepare analogues of XR5118 is outlined in Scheme 1.[17–20] Glycine anhydride was bis-acetylated in acetic anhydride to yield the intermediate **2** (79% yield). Condensation of **2** with a variety of aromatic aldehydes using potassium *tert*-butoxide in THF yielded mono-arylidenes of general formula **15** in 60–80% yield. Performing this reaction in THF rather than DMF prevented formation of the bis-arylidene. Reaction of **15** with a second aldehyde using Cs_2CO_3 in DMF yielded the desired bis-arylidenediketopiperazines in reasonable yield (30–60%). NMR studies and comparison with known compounds confirmed that this route resulted in formation of only the 3*Z*,6*Z* isomer. The aldehydes used for both the first and second condensation were either available commercially or were prepared as shown in Scheme 2. Aldehydes **3**–**6** were conveniently prepared by reaction of 5-bromo-2-thiophene carboxaldehyde with the appropriate nucleophile. Direct reaction between the nucleophile and 5-bromo-2-thiophenecarboxaldehyde yielded **3**, **4** and **6**, whilst the preparation of **5** required protection of the aldehyde as the acetal, followed by Cu(I) catalyzed reaction with the sodium salt of *N*,*N*-dimethylethanolamine and then acid-catalyzed deprotection. To prepare aldehydes **7**–**10**, 4-nitrobenzaldehyde was protected as the acetal, which was then reduced using H_2/PtO_2 in THF to yield the corresponding amine. Reaction with the appropriate acid chloride followed by acid-catalyzed deprotection yielded the desired aldehydes. Reaction of the sodium salt of 4-hydroxybenzaldehyde with the appropriate alkylhalide ester afforded aldehydes **11**–**14**. Compound **27** was prepared by mCPBA oxidation of XR5118 in DCM/MeOH. Bis-arylidenediketopiperazines containing an ester functionality (e.g., **35**) were hydrolysed to their corresponding carboxylic acids (e.g., **34**) using NaOH in H_2O/THF/MeOH.

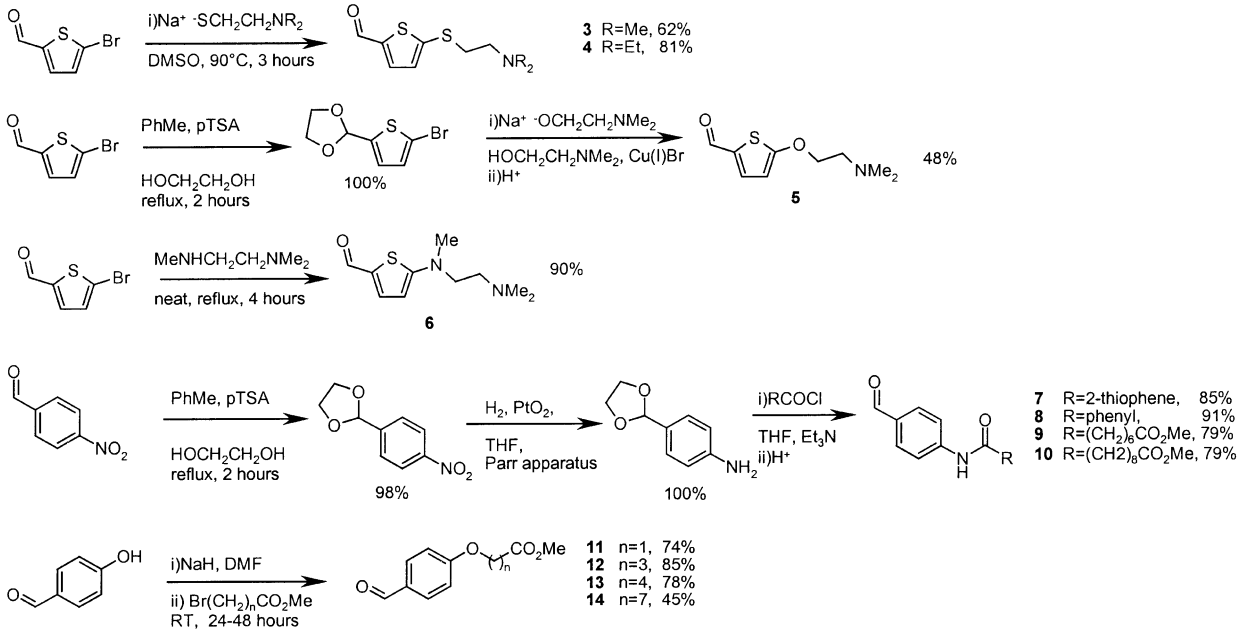
Compounds were tested in a plasmin generation chromogenic assay as reported previously,[21] utilizing the S2251 tripeptide as the chromogenic substrate.

Our initial investigations focused on the introduction of a variety of substituents onto the left-hand ring of XR5118 (Table 1).

In general, it was found that substitution on the left-hand ring with a variety of electron withdrawing and donating substituents was not well tolerated, with most analogues showing at least a 3-fold reduction in potency. However, increasing the size of the substituent, as with amides **24** and **25**, yielded compounds of similar activity to XR5118.

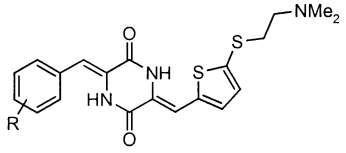
Modifications to the dimethylamino ethylsulfanyl side chain were also not tolerated (Table 2). Small changes to the linker and manipulation of the amine group resulted in a loss of activity indicating the sensitive nature of the side chain.

Björquist et al. have previously reported a series of site directed mutagenesis experiments which suggested that the binding site on PAI-1 for an anthranilic acid derivative, AR-H029953XX, was located in the region of the epitope for the antibody CLB-2C8.[22] It was also reported that the acidic moiety of AR-H029953XX was essential for activity, and it was postulated that there was an interaction between the carboxylic acid group and one or more of three arginines (Arg76, Arg115 and Arg118) present within the epitope region. As our own studies have suggested that this region on PAI-1 may be important for the binding of XR5118, we introduced an acidic group onto the diketopiperazine template to explore whether potency could be enhanced through a possible interaction with a putative arginine residue.



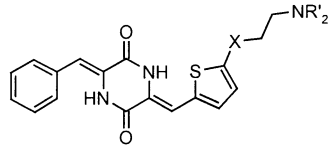
Scheme 2.

Table 1. PAI-1 inhibitory activity for compounds **1** and **16–25**



Compound no.	R	IC_{50} (μM)
1, XR5118	H	3.5±0.19
16	4-Me	12.4± 0.28
17	4-OMe	10.9±0.49
18	4-Br	13.1± 0.71
19	3-Cl	>25
20	4-CN	>25
21	2-Br	>25
22	4-CO_2Me	17.7±3.25
23	4-NMe2	>25
24	4-NHCOPh	6.1±1.48
25	4-NHCO(2-thiophene)	3.1±0.31

Table 2. PAI-1 inhibitory activity for compounds **1** and **26–28**



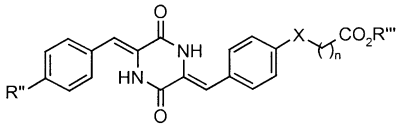
Compound no.	X	R′	IC_{50} (μM)
1, XR5118	S	Me	3.5±0.19
26	S	Et	>25
27	S(O)	Me	>25
28	O	Me	>25

Consequently carboxylic acid groups were introduced at varying distances from the diketopiperazine template.

Parallel studies had shown that the thiophenyl-thioether linkage of XR5118 could be successfully replaced by a phenyl-ether moiety, and for reasons of synthetic accessibility this grouping was used in these studies. Our initial efforts, in which the left-hand ring was left unsubstituted, produced compounds of very modest activity, with a marginal improvement in potency as the carboxylic acid functionality was extended away from the diketopiperazine ring (Table 3). However introduction of the thiophene 2-carbonylamino group, shown earlier to be tolerated, to the 4-position of the left-hand ring resulted in a 9-fold improvement in activity (cf. **32** and **33**). Extending the chain length from four to seven carbons yielded a further 9-fold improvement in potency (**34**). Exploration of the linker showed that replacement of the ether with an amide was well tolerated, and led to potent compounds such as **36** and **38**. The importance of the acidic functionality is clearly demonstrated by comparison of **34** and **36** with their corresponding methyl esters **35** and **37**.

On the basis of marginal superiority in potency and improved physicochemical properties, the ether **34** was selected for further assay studies.

Table 3. PAI-1 inhibitory activity for compounds **29–38**



Compound no.	R″	X	*n*	R‴	IC_{50} (μM)
29	H	—	—	H	>25
30	H	O	1	H	>25
31	H	O	3	H	20.0±0.27
32	H	O	4	H	15 (*n*=1)
33	NHCO(2-thiophene)	O	4	H	1.75±0.02
34	NHCO(2-thiophene)	O	7	H	0.20±0.015
35	NHCO(2-thiophene)	O	7	Me	1.93±0.25
36	NHCO(2-thiophene)	NHCO	6	H	0.52±0.035
37	NHCO(2-thiophene)	NHCO	6	Me	4.77±0.40
38	NHCO(2-thiophene)	NHCO	8	H	0.39±0.012

Table 4. Activity of **34** in various assay systems

Compound no.	Chromogenic assay IC_{50} (μM)	Fibrin plate assay IC_{50} (μM)	Complex assay IC_{50} (μM)
34	0.20±0.015	0.26±0.05	0.51±0.08

The excellent activity of **34** was confirmed in the fibrin plate assay and in a tPA/PAI-1 complex assay (Table 4). In the fibrin plate assay the PAI-1 inhibitory activity of the compounds is quantified by measuring the rate of fibrinolysis,[23] and in the solid-phase tPA/PAI-1 complex assay,[24] pre-incubation of PAI-1 with an inhibitor prevents tPA/PAI-1 complex formation in a concentration dependant manner.

In summary, we have developed several diketopiperazine-based inhibitors of PAI-1 showing a marked improvement in potency over XR5118. Compound **34** demonstrated excellent potency (0.2 μM) in a mechanistic plasmin generation assay. This level of activity was retained in a functional fibrinolysis assay, and in addition **34** prevented complex formation between tPA and PAI-1. We believe that **34** is the most potent inhibitor of PAI-1 reported to date, and this compound may prove useful in further evaluating the therapeutic potential of inhibiting PAI-1 activity.

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