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Design, Synthesis and In Vitro Evaluation of Potent, Novel, Small Molecule Inhibitors of Plasminogen Activator Inhibitor-1

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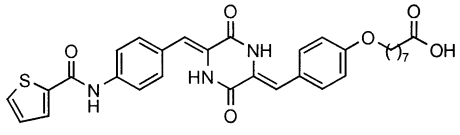
Abstract—We have synthesized and evaluated a series of tetramic acid-based and hydroxyquinolinone-based inhibitors of plasminogen activator inhibitor-1 (PAI-1). These studies resulted in the identification of several compounds which showed excellent potency against PAI-1. The design, synthesis and SAR of these compounds are described.

Plasminogen activators (PAs) are serine proteases that control the activation of the zymogen, plasminogen, to the active enzyme plasmin.[1] Plasminogen is converted to proteolytically active plasmin by either tissue-type plasminogen activator (tPA) or urokinase-type plasminogen activator (uPA).[1] Plasminogen activator inhibitor (PAI-1), a member of the serpin superfamily of protease inhibitors,[2,3] is the major physiological inhibitor of plasminogen activators. 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] PAI-1 is also associated with a poor prognosis in a variety of cancers,[7] and is believed to play a role in angiogenesis,[8] invasion[8] and metastasis.[9]

PAI-1 is produced in its active conformation, but can spontaneously convert into a latent, inactive form that is unable to bind to PAs.[10] PAI-1 inhibition of PAs is mediated through a bait peptide bond (Arg346-Met347), present on the reactive center loop (RCL), which mimics the natural substrate for PAs, plasminogen.[11] The RCL is exposed in the active form, but is inserted into the major β-sheet A in the latent conformation.[12]

We have previously reported XR11211, which demonstrated excellent potency (0.20 μM) in a mechanistic plasmin generation assay which used the S2251 tripeptide as the chromogenic substrate.[13] XR11211 also retained this level of activity in a functional fibrinolysis assay, and prevented complex formation between tPA and PAI-1.[13] However poor physicochemical properties were undesirable features of this series, and to overcome this issue we sought to identify alternative novel small molecule inhibitors that incorporated key structural features of the diketopiperazine template. This work resulted in the discovery of several novel templates, which demonstrated PAI-1 inhibitory activity including the tetramic acid series and the hydroxyquinolinone series. We describe here the design, synthesis and biological activities of these series.



XR11211

Our search for novel templates initially focused on the diketopiperazine nucleus. It was speculated that the biological activity of these compounds was exerted through one of diketopiperazine ring amides existing in the enol tautomer, whilst the other remained as a carboxamide.[14] This led to an extensive search for five-, six- and

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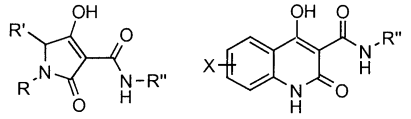
seven-membered rings that contained both enol and amide functionalities. We also wanted to replace the exocyclic double bond with a group that retained a degree of rigidity, and yet would not be prone to isomerisation. Several ring systems were identified which satisfied these criteria, and low levels of activity were seen in some of these series. The tetramic acid series (**A**) and the hydroxyquinolinone series (**B**) appeared to be the most attractive of these templates, and we sought to further develop these compounds.

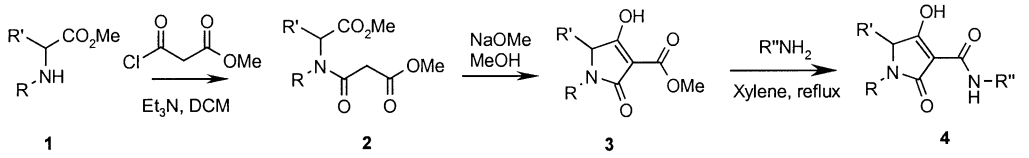


A **B**

The general synthetic route used to prepare tetramic acid derivatives is outlined in Scheme 1. Compounds **1** were prepared via derivatisation of the amine RNH_2 or from the appropriate amino acids. Reaction with methyl malonyl chloride yielded **2** in good yields, and Dieckmann cyclisation using sodium methoxide in methanol afforded the 3-methoxycarbonyl tetramic acids **3**. Reaction of **3** with the appropriate amine ($R''NH_2$) in refluxing xylene yielded the desired compounds **4**.

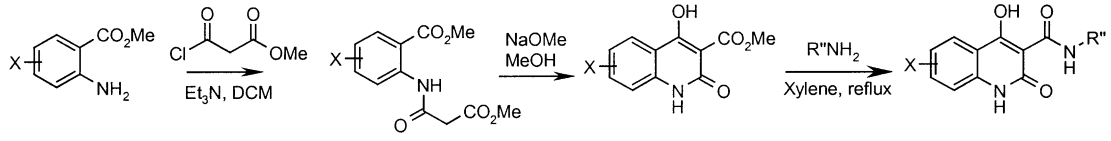
Scheme 2 outlines the route used to prepare hydroxyquinolinones. Substituted methyl anthranilates **5** were either commercially available, or were prepared by Suzuki–Miyaura coupling of the 4- and 5-bromo or iodo substituted methyl anthranilates with the appropriate arylboronic acid.[15] The 4- and 5-bromo or iodo substituted methyl anthranilates in turn were prepared by treatment of the appropriate isatoic anhydrides with methanol. Isatoic anhydrides were prepared from the appropriately substituted isatins.[16,17] Compounds **5** were then reacted with methyl malonyl chloride to yield **6** in good yields. Dieckmann cyclisation using sodium methoxide in methanol afforded **7**, and treatment of **7** with the appropriate amine ($R''NH_2$) in refluxing xylene yielded the desired compounds **8**.

Schemes 3 and 4 describe the routes used to synthesise anilines **9** and **10** used in this work. Reaction between 4-nitrophenol and 8-bromo-octanoic acid methyl ester, followed by reduction of the nitro group yielded **9** in good yield. To prepare **10**, 4-nitrophenol was alkylated



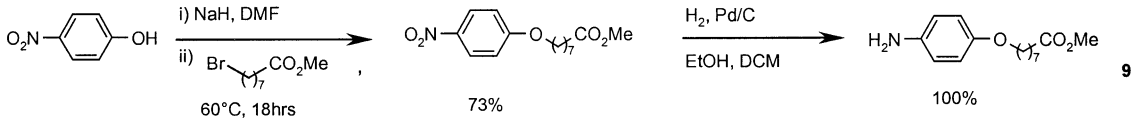
1 **2** **3** **4**

Scheme 1.

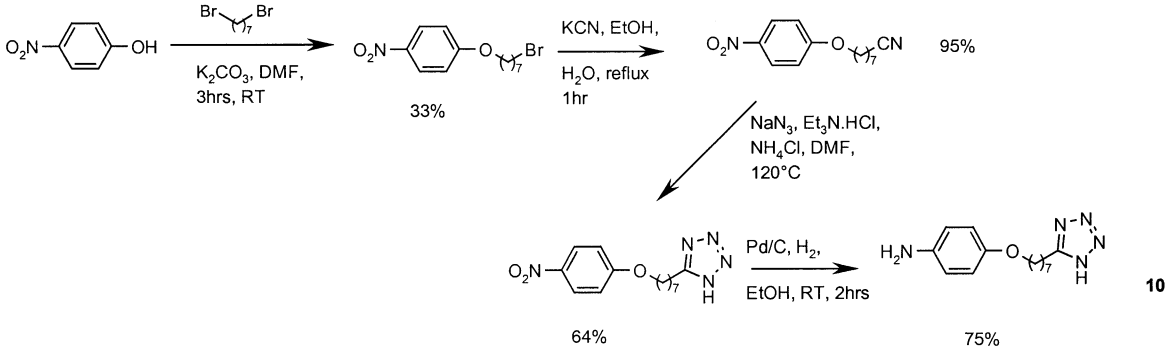


5 **6** **7** **8**

Scheme 2.



Scheme 3.



Scheme 4.

with 1,7-dibromoheptane and then treated with potassium cyanide to yield the corresponding nitrile. Reaction with sodium azide followed by reduction of the nitro group yielded **10** in satisfactory yield.

Compounds containing an ester functionality (e.g., **30**) were hydrolysed to their corresponding carboxylic acids (e.g., **17**) using NaOH in H_2O/THF/MeOH.

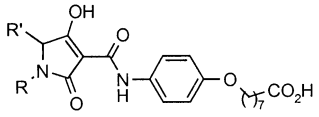
Compounds were tested in a PAI-1 mechanistic assay in which the ability of the compound to inhibit PAI-1 was quantified by measuring the activity of tPA using a chromogenic substrate.[18,19]

The versatile routes utilised in the synthesis of these series allowed for a diverse array of analogues to be prepared. It was rapidly established that in each of the series, introduction of the octanoic acid side chain, as used in XR11211, resulted in an improvement in activity. This result suggests that the tetramic acid series and the hydroxyquinolinone series inhibit PAI-1 in a similar manner to XR11211.

Once it had been established that the octanoic acid side chain was beneficial for activity within the tetramic acid series, our investigations focused on exploration of groups R and R′ (Table 1). It was found that substitution at R′ was not well tolerated as compounds with methyl (**12**), phenyl (**11**) and substituted benzyl (**13**) in this position showed poor activity, regardless of the substitution at R. At the R position, benzyl (**15**) was not tolerated but introduction of a phenyl group (**14**) to this position gave a compound of modest activity. This ring was then extensively explored with a wide variety of substituents. This work showed that substitution in the 4-position by small lipophilic groups gave a significant improvement in activity (e.g., **16**, **17**, **18**, **19**, **21**). Substitution in the 2-position was not well tolerated (**25**, **27**) possibly due to a steric interaction affecting the conformation of the phenyl ring. 3- and 3,4-substituted compounds (**24**, **29**) gave intermediate activity.

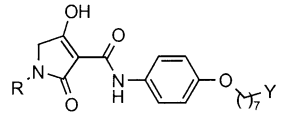
Replacement of the terminal carboxyl acid group with the isosteric tetrazole group resulted in compounds of

Table 1. PAI-1 inhibitory activity for compounds **11**–**29**



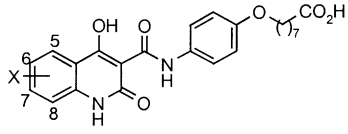
Compd	R	R′	IC_{50} (μM)
11	H	Ph	49.0
12	Ph	Me	>50
13	H	4-OH–$PhCH_2$	>50
14	Ph	H	21±13
15	Bn	H	>50
16	4-OMe–Ph	H	3.66±1.20
17	4-Cl–Ph	H	5.00±3.00
18	4-Me–Ph	H	1.95±0.37
19	4-Br–Ph	H	1.99±0.29
20	4-NO_2–Ph	H	4.25±0.96
21	4-*i*Pr–Ph	H	2.35±0.45
22	4-Morpholino–Ph	H	>50
23	4-NHAc–Ph	H	>50
24	3,4-diCl–Ph	H	13.0±7.0
25	1-Naphthyl	H	>50
26	2-Naphthyl	H	4.00±1.00
27	2-Cl–Ph	H	>50
28	2-OMe	H	>50
29	3-CO_2Me–Ph	H	17.9

Table 2. PAI-1 inhibitory activity for compounds **17**–**18**, **30**–**32**



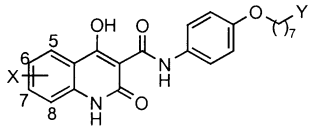
Compd	R	Y	IC_{50} (μM)
17	4-Cl–Ph	CO_2H	5.00±3.00
30	4-Cl–Ph	CO_2Me	>50
31	4-Cl–Ph	Tetrazole	0.69±0.06
18	4-Me–Ph	CO_2H	1.95±0.37
32	4-Me–Ph	Tetrazole	0.89±0.17

Table 3. PAI-1 inhibitory activity for compounds **33**–**56**



Compd	X	IC_{50} (μM)
33	Unsubstituted	1.85±0.21
34	5-I	7.0 ($n=1$)
35	5-NO_2	7.9±0.28
36	5-NH_2	4.25±1.1
37	5-Cl	3.0±1.4
38	6-Cl	1.95±1.34
39	6-F	4.9±1.27
40	6-NO_2	3.6±0.57
41	6-NH_2	>20
42	6-I	2.2±0.14
43	6-nC_5H_{11}	1.80±0.42
44	6-(4-Cl–Ph)	2.00±0.7
45	6-(2-Naphthyl)	0.67±0.13
46	6-(Benzo[*b*]thiophen-3-yl)	0.51±0.14
47	6-(benzo[*b*]thiophen-2-yl)	0.50±0.14
48	6,7-diF	2.35±0.50
49	6,7-diMeO	1.65±0.07
50	7-(Benzo[*b*]thiophen-2-yl)	0.30±0.14
51	7-CF_3	1.03±0.06
52	7-CN	0.90±0.28
53	7-NH_2	>30
54	7-(2-Naphthyl)	0.60±0.14
55	8-(1-Naphthyl)	>50
56	8-(4-MeO–Ph)	30.0±7.1

Table 4. PAI-1 inhibitory activity for **47**, **57**



Compd	X	Y	IC_{50} (μM)
47	6-(Benzo[*b*]thiophen-2-yl)	CO_2H	0.50±0.14
57	6-(Benzo[*b*]thiophen-2-yl)	Tetrazole	1.01±0.34

Table 5. Comparison of selected compounds in the complex and chromogenic assays

Compd	Complex assay IC_{50} (μM)	Chromogenic assay IC_{50} (μM)
XR11211	0.51 ± 0.08	0.20 ± 0.015
32	0.83 ± 0.26	0.89 ± 0.17
31	0.30 ($n = 1$)	0.69 ± 0.06
46	0.94 ± 0.41	0.51 ± 0.14
57	0.29 ± 0.15	1.01 ± 0.34

marginally improved potency (Table 2). Comparison of **17** with its corresponding methyl ester **30** clearly demonstrates the importance of the acidic functionality.

Within the hydroxyquinolinone series it was also quickly established that incorporation of the octanoic acid side chain was beneficial for activity. Our efforts in this series then focused on the introduction of various groups to the fused aryl ring (Table 3).

The hydroxyquinolinone system tolerated a wide variety of substituents in the 5-, 6- and 7-positions, although an amino group in the 6- and 7-positions was not tolerated. Substitution in the 8-position resulted in a loss of activity. Although many substituents were tolerated and gave good activities, larger lipophilic groups such as naphthyl (**45**, **54**) and benzothiophene (**46**, **47**, **50**) in the 6- and 7-positions appeared to enhance potency. Methylation of either amide NH abolished activity (data not shown) while tetrazole replacement of the carboxylic acid was also found to be tolerated in this series (Table 4).

The activities of **31**, **32**, **46** and **57** were confirmed in a tPA/PAI-1 complex assay (Table 5). In the solid-phase tPA/PAI-1 complex assay,[20] pre-incubation of PAI-1 with an inhibitor prevents tPA/PAI-1 complex formation in a concentration dependant manner.

In summary, this work has shown that we have been able to identify novel inhibitors of PAI-1 by incorporating certain key structural features present within the diketopiperazine series. This has led to the discovery and SAR development of the tetramic acid series and the hydroxyquinolinone series, several examples of which (e.g., **31**, **32**, **46**, **57**) show high levels of potency comparable to XR11211 in both the chromogenic and complex assays. These novel series did show some improvement in their physicochemical properties over the diketopiperazine series, although this improvement was not sufficient to warrant further development of these series. However, the levels of potency attained with these series have allowed us to increase our understanding of PAI-1 inhibition through further mechanistic studies.

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