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Synthesis and biological evaluation of piperazine-based derivatives as inhibitors of plasminogen activator inhibitor-1 (PAI-1)

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Abstract—Compound **2** was identified by high throughput screening as a novel PAI-1 inhibitor. Systematic optimization of the A, B, and C segments of **2** resulted in the identification of a more potent compound **39** with good oral bioavailability. The synthesis and SAR data are presented in this report.

A key step in the regulation of thrombus formation and clearance is the generation of plasmin through proteolytic cleavage of plasminogen by tissue plasminogen activator (tPA) and urokinase (uPA).[1] Plasmin dissolves fibrin clots by degrading insoluble fibrin molecules to small soluble fragments.[2] Plasminogen activator inhibitor-1 (PAI-1), a member of the serine protease inhibitor (serpin) family, is the major negative regulator of tPA and uPA.[3] PAI-1 is unique in the serpin family and its active conformation spontaneously rearranges into the inactive form through the insertion of a larger portion of the reactive center loop of the active form into β-sheet A in the middle of the molecule.[4] Recently, mechanisms by which PAI-1 interacts with small molecules[5] and its targeted protease such as tPA[6] have been reported. These studies provide important information on the three dimensional structure of PAI-1, which will help to design PAI-1 inhibitors.

Clinically, PAI-1 is considered to be a thrombotic risk factor.[7] Elevated levels of PAI-1 antigen and activity have been described to correlate with an increased risk of deep vein thrombosis,[8] atherosclerosis,[9] unstable angina and myocardial infarction.[3,7] In addition, elevated levels of PAI-1 are associated with a poor prognosis in cancer patients,[10] and PAI-1 may play a role in angiogenesis,[11] cancer invasion,[11] and metastasis.[12] Thus, inhibition of PAI-1 would represent a useful strategy in treating a variety of cardiovascular and cancer diseases. To date, several PAI-1 inhibitors including antibodies,[13] peptides,[14] and small molecules[3,15] have been reported.

We have previously reported a menthol-based inhibitor **1**, which demonstrates excellent potency in the primary enzymatic assay ($IC_{50} = 0.38$ μM) as well as the functional clot lysis assay ($IC_{50} = 0.01$ μM).[16] There are, however, some limitations such as poor solubility and low oral bioavailability associated with this series of compounds. In searching for an alternate template, high throughput screening of our compound library was performed, leading to the discovery of the structurally different compound **2** with an IC_{50} value of 1.8 μM in our primary assay.[16] Compound **2** has moderate potency, but poor oral bioavailability which is presumably at least partially attributed to the existence of the phosphonic acid moiety. Thus, an effort was made to replace the phosphonic acid group to improve the pharmacokinetic profile as well as increase potency. Structurally, compound **2** can be divided into three segments designated as **A**, **B**, and **C** (Fig. 1). Systematic modification of these segments is detailed in this paper.

We started the optimization with the replacement of the C segment (Table 1). The initial effort focused on direct replacement of the aminomethyl phosphonic acid with

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carboxylic acids. The isonipecotic acid adduct, compound **3** (Fig. 2) is slightly more potent than **2**. Switching the nitro and trifluoromethyl groups on the B segment gave compound **4**, which is equally potent as compound **3**. This is advantageous from a synthetic aspect, because a large number of analogues of **4** can be efficiently prepared via a common intermediate **7** through the displacement of the 2-chloro group with different nucleophiles (Scheme 1). Compound **7** was obtained as a sole product by the displacement of the 4-chloro group of compound **5** with 1-(3-trifluoromethylphenyl)piperazine at room temperature. This is because the reactivity of 4-chloride group of compound **7** is much higher than 2-chloride group based on the similar results reported by Welmaker et al.[17] Further displacement of the 2-chloro group with amino acids and hydroxyphenyl acids yielded analogues of compound **4**. It is observed that the distance between the carboxylic acid group of C segment to the central B-ring has an effect on potency. The closer the carboxylic acid group of C segment to the central B-ring, the less potent the compound is. For the amino-acid adduct series, **4** and **12** are equally potent since the distance of the carboxylic acid group of C segment in these two compounds to the central ring is almost identical. However, the distance for the carboxylic acid in **10** and **11** is shorter, and these compounds have lower potency accordingly. This is also true for the phenyl carboxylic acid adducts (**13**, **14**, **15**). The reason for this remains unclear. In general, compared with **4**, no significant potency improvement was observed for this series with most of the compounds having similar (**12, 13**) or lower activities (**10**, **11**, **15**).

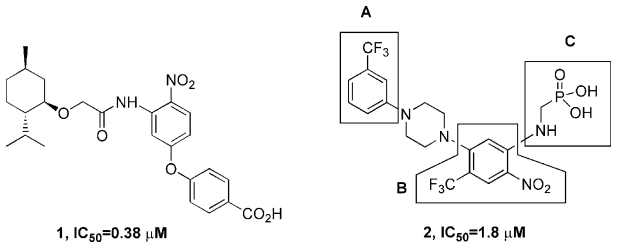
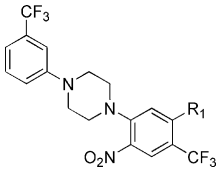
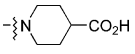
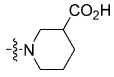
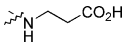
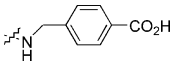
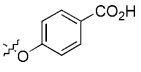
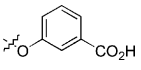


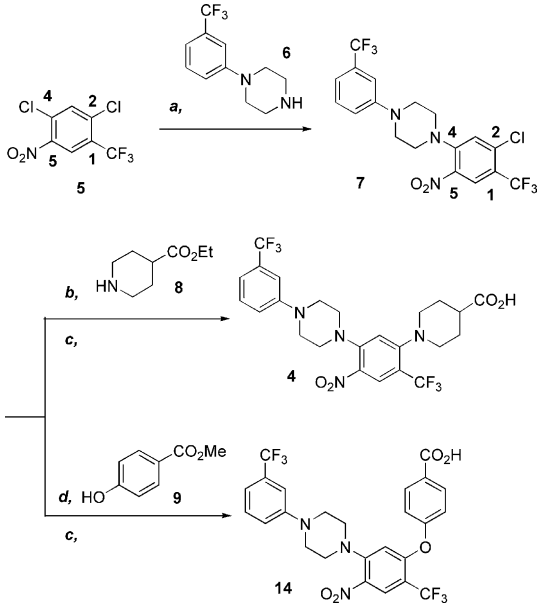
Figure 1. Menthol-based inhibitor **1** and new lead compound **2** from library.

Table 1. Replacement of phosphonic acid



Number	R_1	IC_{50} (μM)[a]
4		0.91
10		2.8
11		7.9
12		0.9
13		1.5
14		2.1
15		3.7

[a] IC_{50} values are averaged from multiple determinations ($n \geq 2$), and the standard deviations are $<30\%$ of the mean.



Scheme 1. Conditions: (a) DIEA, **6**, CH_3CN, rt, overnight; (b) DMSO, **8**, DIEA, 110 °C, 10 h; (c) LiOH, THF/H_2O, rt, 10 h; (d), NaH, DMSO, **9**, 110 °C, 4 h.

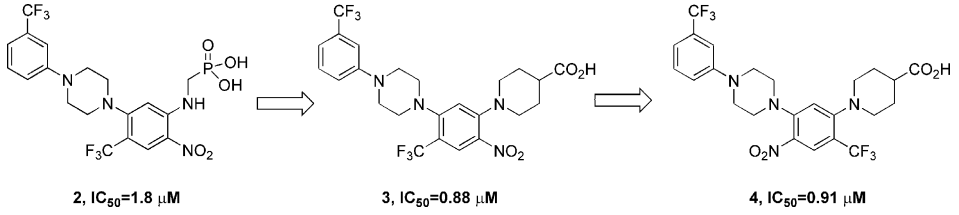
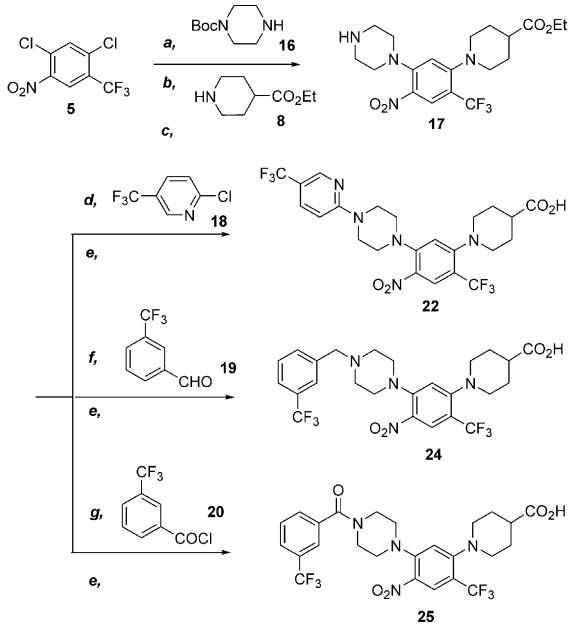


Figure 2. Initial replacement of phosphonic acid **2**.

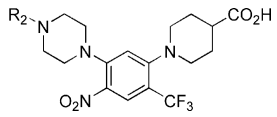
We next explored the optimization of the A segment while keeping isonipecotic acid as the C segment. The synthesis of the compounds in Table 2 is outlined in Scheme 2. Displacement of the 4-chloro group with *N*-Boc-piperazine **16** followed by addition of isonipecotic acid ethyl ester **8** and deprotection with TFA afforded **17**. Coupling of **17** to 2-chloro-5-(trifluoromethyl)pyridine **18** followed by saponification afforded **22**. Reductive-amination of **17** with 3-(trifluoromethyl)benzaldehyde **19** followed by saponification afforded **24**. Acylation of **17** with 3-(trifluoromethyl)benzoyl chloride **20** followed by saponification afforded **25**. The remaining compounds in Table 2 were prepared in a similar manner. Shifting the trifluoromethyl group from the *meta* to *para* position has no effect on potency (**4** vs **21**), whereas introduction of pyridine (**22**) decreases potency 3-fold. Insertion of methylene (**24**), carbonyl (**25**), or urea (**26**) spacers are also detrimental, and incorporation of lipophilic groups causes dramatic loss of potency (**23**, **27**).

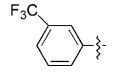
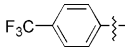
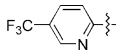
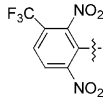
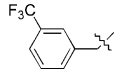
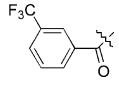
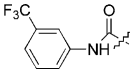
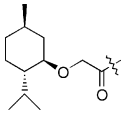
Next, we turned our attention to the replacement of the B segment. For this series, we chose 4-aminomethylbenzoic acid instead of isonipecotic acid as the C segment (Table 3). Replacement of trifluoromethyl with a



Scheme 2. Conditions: (a) DIEA, **16** CH_3CN, rt, overnight; (b) DMSO, **8**, DIEA, 110 °C, 10 h; (c), TFA, CH_2Cl_2; (d) DMSO, **18**, DIEA, 110 °C, 6 h; (e) LiOH, THF/H_2O, rt, 10 h; (f) $NaBH(OAc)_3$, **19**, $ClCH_2CH_2Cl$, rt, overnight; (g) Et_3N, **20**, CH_2Cl_2.

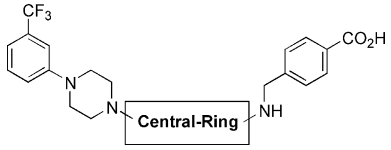
Table 2. Optimization of A segment

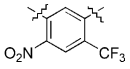
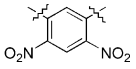
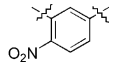
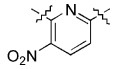
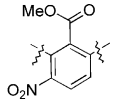
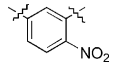
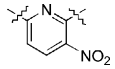


Number	R_2	IC_{50} (μM)[a]
4		0.91
21		1.1
22		3.2
23		>15
24		4.3
25		5.0
26		3.5
27		>15

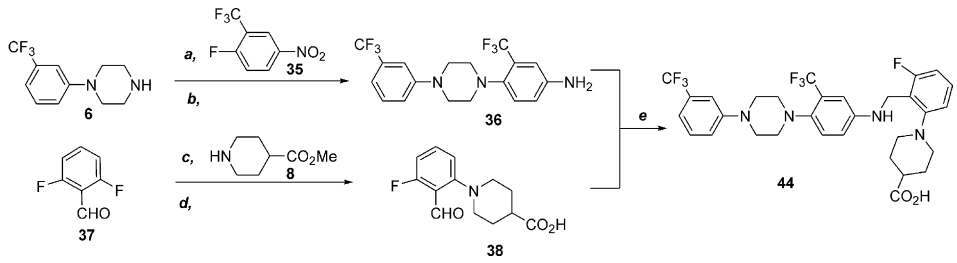
[a] IC_{50} values are averaged from multiple determinations ($n \geq 2$), and the standard deviations are <30% of the mean.

Table 3. Replacement of central ring



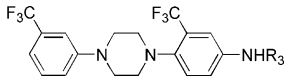
Number	Central ring	IC_{50} (μM)[a]
13		1.5
28		1.4
29		6.7
30		6.1
31		>15
32		2.8
33		3.4
34		1.7

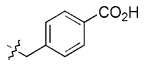
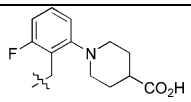
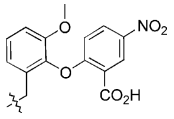
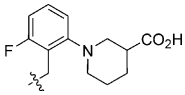
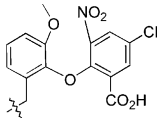
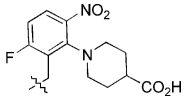
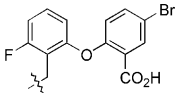
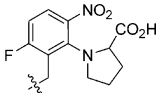
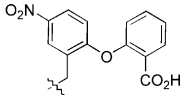
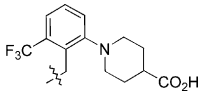
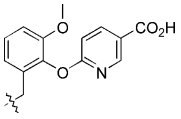
[a] IC_{50} values are averaged from multiple determinations ($n \geq 2$), and the standard deviations are <30% of the mean.



Scheme 3. Conditions: (a) DIEA, **35**, CH_3CN, rt, overnight; (b) Pd/C, H_2, EtOAc–MeOH, 2 h; (c) DIEA, **8**, CH_3CN, rt, overnight; (d) LiOH, THF/H_2O, rt, 2 h; (e) $NaBH_3CN$, MeOH–CH_2Cl_2–HOAC, rt, 2 h.

Table 4. Optimization of C segment



Number	R_3	IC_{50} (μM)[a]	Number	R_3	IC_{50} (μM)[a]
34		1.7	**44**		1.0
39		0.5	**45**		1.7
40		0.9	**46**		0.7
41		0.8	**47**		1.4
42		1.0	**48**		1.7
43		0.9			

[a] IC_{50} values are averaged from multiple determinations ($n \geq 2$), and the standard deviations are $<30\%$ of the mean.

nitro group retained potency (**13** vs **28**), but decreased potency was observed in most of the cases such as deletion of trifluoromethyl (**29**, **32**), and incorporation of methylcarboxylate (**31**). Changing the 1,3-substitution pattern of the B segment to 1,4-substitution and the removal of the nitro group yielded compound **34** which has equal potency to **13** but is less lipophilic based on the value of calculated LogP.[18]

Further optimization of the C segment was pursued keeping other parts of the template constant as shown in Table 4. Compounds listed were prepared according to Scheme 3. Addition of 1-(3-trifluoromethylphenyl)piperazine **6** to 2-fluoro-5-nitrobenzotrifluoride **35** followed by hydrogenation, afforded aniline **36**. Reductive-amination of **36** with 1-(3-fluoro-2-formylphenyl)-4-piperidinecarboxylic acid **38** afforded **44**. Intermediate **38** was prepared by addition of isonipecotic acid methyl ester **8** to 2,6-difluorobenzaldehyde **37** followed by hydrolysis. The starting aldehydes needed for the preparation of compounds **39**–**48** were either prepared in the same manner as **38** or as previously disclosed.[16] Compared with **34**, all the other compounds have similar or slightly improved potency (Table 4). Compound **39** was pursued further as it is about 4-fold more potent.

Most of the compounds were tested in a functional clot lysis assay which was described in a previous paper[16] (data not shown). Compound **39** was found to be the most potent inhibitor with an IC_{50} of 0.01 μM. The compound **39**, however, was less potent in the primary assay ($IC_{50} = 0.5$ μM). The reason for this apparent difference in potency in these assays is not clear, but par-

Table 5. Pharmacokinetic data for compound **1** and **39**[a]

Number	C_{max}[b] (μM)	T_{max}[b] (h)	$T_{1/2}$[b] (h)	Cl[c] (mL/min/kg)	Vdss[c] (L/kg)	F (%)[d]
1	0.16	3	3.0	4.84	0.4	23
39	0.48	3	2.8	10.4	1.33	43

[a] Compound administered in 94% PEG 300, 4% EtOH, and 2% water at 0.5 mg/kg iv, 2 mg/kg po.
[b] C_{max}, T_{max}, and $T_{1/2}$ for po.
[c] Cl and Vdss for iv.
[d] F% values are averaged from three rats.

tially may be attributed to the different assay systems because we observed that in the presence of lipid-containing plasma the compound was consistently more potent. To examine the possibility that compound **39** might inhibit other enzymes of the coagulation cascade, which could lead to enhanced clot lysis, we did test the compound in the clot lysis assay in the absence of PAI-1. The results showed that the compound **39** did not alter clot lysis time (data not shown). Thus, it is unlikely that compound **39** directly inhibits other enzymes in the blood-clotting cascade. Further pharmacokinetic studies of **39** were performed in rats, and the results are summarized in Table 5. Compound **39** has improved bioavailability (43%) compared with **1**, moderate clearance rate (10.4 mL/min/kg), and good half-life (2.8 h).

In summary, we have explored the structure–activity relationships around the novel PAI-1 inhibitor **2** by modifying the A, B, and C segments. Initial optimization of the C segment indicates that the aminomethyl phosphonic acid could be replaced with amino-acids. No improvement was seen in the modification of the A segment, but efforts to optimize the B segment resulted in the identification of the 2-trifluoromethylphenyl as the optimal group. Further optimization of the C segment led to the discovery of the most potent compound **39** in both the primary and functional assays. Currently, animal efficacy studies of **39** to validate the disease model are in progress.

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18. The calculated LogP value was obtained through the calculation of the compound via the software created by Advanced Chemistry Development, Inc, Canada. The cLog P value for **13** and **34** is 9.09 ± 0.64 and 6.97 ± 0.57, respectively.