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Identification of novel plasminogen activator inhibitor-1 inhibitors with improved oral bioavailability: Structure optimization of *N*-acylanthranilic acid derivatives

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ABSTRACT

Novel plasminogen activator inhibitor-1 (PAI-1) inhibitors with highly improved oral bioavailability were discovered by structure-activity relationship studies on *N*-acyl-5-chloroanthranilic acid derivatives. Because lipophilic *N*-acyl groups seemed to be important for the anthranilic acid derivatives to strongly inhibit PAI-1, synthesis of compounds in which 5-chloroanthranilic acid was bound to a variety of highly lipophilic moieties with appropriate linkers was investigated. As the result it appeared that some of the derivatives possessing aryl- or heteroaryl-substituted phenyl groups in the acyl chain had potent *in vitro* PAI-1 inhibitory activity. Oral absorbability of typical compounds was also evaluated in rats, and compounds **40**, **55**, **60** and **76** which have diverse chemical structure with each other were selected for further pharmacological evaluation.

Plasminogen activator inhibitor-1 (PAI-1), also known as SERPINE1, is a serine protease inhibitor belonging to the serpin superfamily. PAI-1 deactivates tissue-type plasminogen activator (tPA) and urokinase-type plasminogen activator (uPA) by covalently forming complexes with these serine protease enzymes which play central roles in the vascular thrombotic and thrombolytic system.[1–3] Inhibition of PAI-1 could therefore increase the activity of tPA and uPA to elevate the level of a fibrinolytic enzyme plasmin which is derived from its proenzyme plasminogen. Consequently, PAI-1 inhibitors are expected to be useful for treating various cardiovascular diseases[4,5] such as myocardial infarction, brain infarction, arteriosclerosis, deep vein thrombosis, and restenosis after coronary angioplasty. Many recent studies[6–9] have also revealed that PAI-1 is involved in various biological disorders including diabetes, tissue fibrosis (lung, kidney and liver), inflammation, cancer, Alzheimer's disease and so on. A number of small molecule PAI-1 inhibitors have been widely investigated as promising targets for new drug discovery,[5,10,11] while none of them is yet applied in a clinical setting. We have extensively studied small molecule PAI-1 inhibitors[12–15] and demonstrated a variety of intriguing biological properties suggestive of possible clinical applications not only as an anti-thrombotic agent devoid of risk of bleeding,[13] but also as drugs capable of preventing or treating senescence,[16] inflamation,[17] multiple sclerosis[18] and tumor.[19] Furthermore, inhibition of PAI-1 enhanced rapid and sustainable hematopoietic regeneration[20] suggesting a possible application of PAI-1 inhibitors in the field of regenerative medicine.

In the previous papers,[14,15] we have reported syntheses of *N*-acylanthranilic acid derivatives as new PAI-1 inhibitors which were designed based on the structure of original hits, TM5001[12] and TM5007[12] (Fig. 1), discovered as the outcome of a structure-based drug design followed by *in silico* screening focused on the binding affinity to the β-sheet A[21] of PAI-1 protein, a cleft which is thought to play an essential role in determining PAI-1's enzymatic activity.[22] Introduction of the 4-diphenylmethyl-1-piperazinyl moiety into the acyl side chain of 5-chloroanthranilic acid as in TM5275[15] (Fig. 1) was found to be an effective way to ameliorate oral bioavailability and pharmacokinetic (PK) profile of this series of PAI-1 inhibitors, since introduction of, for example, diphenylmethylamino, diphenylamino or *p*-chloroanilino group such as in compounds **1**, **2** or **3** (Fig. 1) in place of the 4-diphenylmethylpiperazin-1-yl group resulted in decrease of activity.[15] In spite of these imperfections observed, we continued to explore on anilide type compounds with more lipophilic substituents on the phenyl ring, expecting to identify new lead compounds with

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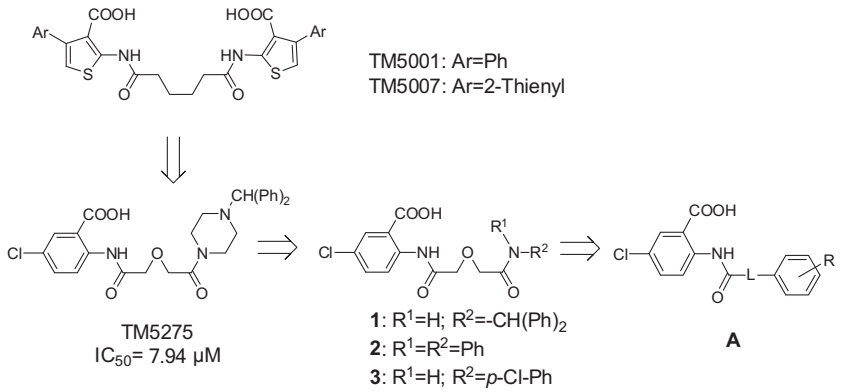
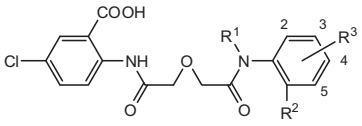


Fig. 1. Chemical modification of PAI-1 inhibitors TM5001 and TM5007.

more potent PAI-1 inhibitory activity as well as higher oral bioavailability than those of TM5275.

Thus, novel *N*-acyl-5-chloroanthranilic acid derivatives depicted as the general structure A (Fig. 1) were synthesized and evaluated as new PAI-1 inhibitors. Some of these compounds were found to exhibit potent PAI-1 inhibitory activity and excellent oral bioavailability in rats. This paper describes structure-optimization study on anthranilic acid derivatives (A).

Table 1
Profiles of new 5-chloroanthranilic acid derivatives.



Compd.	R^1	R^2	R^3	CLog P[a]	PAI-1 activity (%)[b]		Rat PK (50 mg/kg, *p.o.*)[c]		
					50 μM	20 μM	C_{max} (μM)[d]	T_{max} (h)	$T_{1/2}$ (h)
24	H	H	3-F	3.71	79.4 ± 12.3	99.7 ± 0.3	ND	ND	ND
25	H	H	3-CF_3	4.65	39.8 ± 6.3	93.2 ± 7.3	58.6 ± 10.9	1	0.8
26	H	H	2-(4F-Ph)	4.37	39.6 ± 3.3	82.4 ± 7.3	41.8 ± 7.0	1	0.8
27[e]	H	H	3-(4F-Ph)	5.40	12.4 ± 3.9	95.6 ± 5.8	62.8 ± 35.1	1	0.9
28[e]	H	H	4-(4F-Ph)	5.40	15.6 ± 3.3	92.2 ± 6.3	ND	ND	ND
29	H	Me	3-(4F-Ph)	5.25	8.1 ± 3.6	48.6 ± 9.0	92.7 ± 24.0	1	1.8
30	Et	Me	3-(4F-Ph)	6.21	8.0 ± 2.5	58.5 ± 5.8	0.82 ± 0.43[f]	1[f]	0.9[f]
31	H	Me	3-(2-MeO-Ph)	4.47	11.3 ± 4.9	73.7 ± 10.1	2.08 ± 0.83[f]	2[f]	2.5[f]
32	H	Me	3-(3-MeO-Ph)	5.01	6.9 ± 0.7	64.3 ± 7.5	9.4 ± 3.8[f]	2[f]	1.5[f]
33	H	Me	3-(4-MeO-Ph)	5.01	11.2 ± 2.5	61.5 ± 14.5	2.2 ± 0.5[f]	2[f]	1.0[f]
34	H	Me	3-(4-AcNH-Ph)	4.08	6.7 ± 1.7	41.4 ± 14.4	0.32 ± 0.1	2	1.4
35[g]	H	H	2-(4-Pyridyl)	2.85	24.3 ± 3.7	65.1 ± 10.5	3.5 ± 1.24	1	5.3
36	H	H	3-(4-Pyridyl)	3.88	12.8 ± 4.2	14.6 ± 6.2	18.0 ± 5.2	6	>18
37	H	H	4-(4-Pyridyl)	3.88	0.6 ± 1.0	28.7 ± 4.7	7.8 ± 1.7	6	>18
38[e]	H	H	3-(3-Pyridyl)	3.88	22.0 ± 2.7	74.2 ± 7.0	15.1 ± 3.73	2	>22
39[e]	H	H	3-(2-Pyridyl)	4.09	41.3 ± 8.5	76.7 ± 4.6	136.5 ± 23.0	1	2.4
40	H	H	3-(3-Furyl)	4.38	6.4 ± 2.6	16.2 ± 8.1	149.3 ± 34.8	1	2.1
							17.9 ± 6.4[f]	1[f]	2.3[f]
41	H	H	4-(3-Furyl)	4.38	19.2 ± 4.2	71.6 ± 2.2	17.59 ± 7.3[f]	2[f]	4.2[f]
42	H	H	3-(2-Furyl)	4.59	13.7 ± 2.0	69.9 ± 2.3	11.5 ± 1.9[f]	2[f]	4.0[f]
43	H	H	3-(3-Thienyl)	4.89	6.0 ± 1.4	79.0 ± 8.4	86.5 ± 20.4	2	1.9
44	H	H	3-(4-Pyrazolyl)	3.46	9.4 ± 3.5	20.7 ± 10.4	1.70 ± 1.75[f]	1[f]	3.1[f]
45	H	H	3-(1-Me-4-Pyrazolyl)	3.61	19.6 ± 1.2	96.1 ± 4.4	20.5 ± 6.0	2	9.9
46	H	H	3-(5-Oxazolyl)	3.26	63.3 ± 6.6	94.2 ± 10.3	ND	ND	ND
47	H	H	3-(5-Isoxazolyl)	3.56	36.6 ± 2.7	77.9 ± 5.9	14.0 ± 5.0[f]	1[f]	6.0[f]
48	H	H	3-(3-Isoxazolyl)	3.56	78.0[h]	100.4[i]	ND	ND	ND
49	H	H	3-(3,5-Me_2-1-Isoxazolyl)	3.29	35.8 ± 2.1	74.6 ± 12.9	2.0 ± 0.6[f]	2[f]	1.9[f]
50	H	H	3-(1-Imidazolyl)	3.53	59.6 ± 5.3	92.6 ± 4.9	ND	ND	ND
51	H	H	3-(1-Pyrrolyl)	4.75	14.5 ± 3.3	64.0 ± 3.2	7.4 ± 1.9[f]	2[f]	2.2[f]
TM5275[e,j]				3.37	6.0 ± 1.4	79.0 ± 8.4	34.2 ± 2.6	2.0	2.5

[a] CLogP was obtained from ChemDraw Ultra 10.0 for free carboxylic acid/amine.
[b] Remaining PAI-1 activity after incubation with test compound in Method A (see Ref. 23) is shown. Data are expressed as mean ± S.D.
[c] ND = not determined.
[d] Data are expressed as mean ± S.D.
[e] Na salt.
[f] Obtained at 5 mg/kg, *p.o.*
[g] HCl salt.
[h] Obtained from single experiment (n = 1) at 10 μM.
[i] Obtained from single experiment (n = 1) at 2.5 μM.
[j] See Ref. 15.