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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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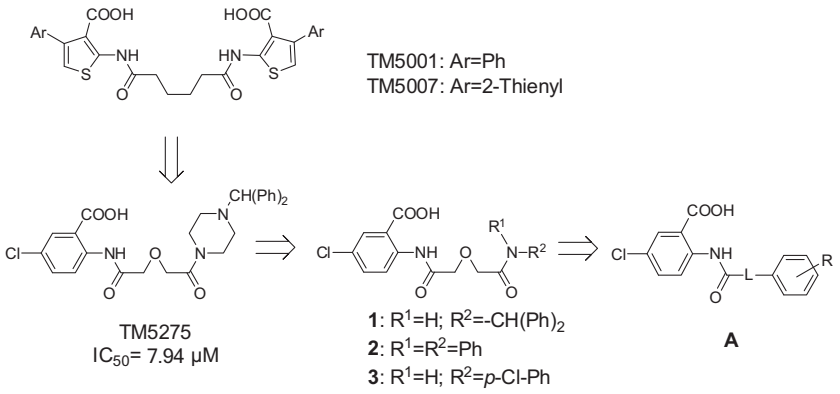
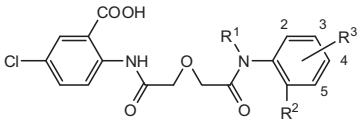


Fig. 1. Chemical modification of PAI-1 inhibitors TM5001 and TM5007.

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.



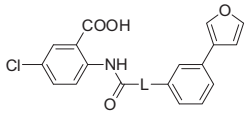
Compd.	R^1	R^2	R^3	CLog P[a]	PAI-1 activity (%)[b]		Rat PK (50 mg/kg, *p.o.*)[c]		
					50 μM	20 μM	C_{max} (μM)[d]	T_{max} (h)	$T_{1/2}$ (h)
24	H	H	3-F	3.71	79.4 ± 12.3	99.7 ± 0.3	ND	ND	ND
25	H	H	3-CF_3	4.65	39.8 ± 6.3	93.2 ± 7.3	58.6 ± 10.9	1	0.8
26	H	H	2-(4F-Ph)	4.37	39.6 ± 3.3	82.4 ± 7.3	41.8 ± 7.0	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 17.9 ± 6.4[f]	1 1[f]	2.1 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.

Chemistry[23]

5-Chloroanthranilic acid derivatives (Tables 1–3) were in general prepared by the methods shown in Scheme 1 starting with 5-chloroanthranilic acid esters (**4**). Compounds **6** which we prepared[15] previously by acylation of **4** with cyclic anhydrides (**5**) were coupled with various substituted-amines **7** and **8** to afford **9** and **12**, respectively. While **9** was then converted to **11** applying the Suzuki-Miyaura coupling followed by hydrolysis (Route A), a large variety of compounds (**13**) were synthesized directly and more conveniently by Route B (**6** → **12** → **13**). Using similar reaction procedures, compounds expressed by the general formula **18** were also synthesized easily *via* Route C (**4** → **15**→**17** → **18**) or D (**4** → **17** → **18**). Compound **57** (Table 2) was prepared in a different way (Route E); **4** was acylated with chloroacetyl chloride followed by reaction with 3-bromophenol (**20**) afforded **21** which was then subjected to the Suzuki-Miyaura coupling with 3-furyl-boronic acid (**22**) to yield **23**. Hydrolysis of **23** furnished

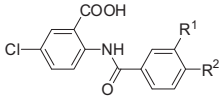
Table 2
N-Acyl-5-chloroanthranilic acid derivatives possessing *m*-(3-furyl)phenyl group combined by different linkers (L).



Compd.	L	CLog P[a]	PAI-1 activity (%)[b,c]		IC_{50} (μM)	Rat PK (5 mg/kg, *p.o.*)[c]		
			50 μM	20 μM		C_{max} (μM)[b]	T_{max} (h)	$T_{1/2}$ (h)
52	—$CH_2OCH_2CONHCH_2$—	4.41	17.7 ± 2.1	59.2 ± 9.3	7.45	14.0 ± 4.5	2	2.7
53	—CH_2SCH_2CONH—	4.75	47.3 ± 8.3	83.9 ± 13.9	ND	5.4 ± 7.0	1	2.6
54	—CH_2CONH—	4.63	25.7 ± 9.8	72.3 ± 9.8	ND	ND	ND	ND
55	—CONH—	3.09	8.4 ± 2.7	47.8 ± 2.1	3.56	22.5 ± 4.3	2	1.7
56	—$CONHCH_2$—	3.24	8.6 ± 3.6	46.9 ± 4.4	3.55	41.6 ± 25.5	2	>22
57	—CH_2O—	5.24	8.2 ± 4.7	39.9 ± 12.4	4.74	56.3 ± 4.1	2	1.9
58	—CH_2—	5.26	19.6 ± 2.7	63.7 ± 13.4	ND	43.1 ± 8.2	2	1.9
59	–[d]	5.21	8.7 ± 1.8	49.0 ± 4.0	7.39	29.4 ± 5.9	2	2.0

[a] CLog P was obtained from ChemDraw Ultra 10.0.
[b] Remaining PAI-1 activity after incubation with test compound in Method A (see Ref. 23) is shown.
[c] Data are expressed as mean ± S.D. ND = not determined.
[d] Direct bond.

Table 3
5-Chloro-*N*-(substituted)benzoylanthranilic acids.



Compd.	R^1	R^2	CLog P[a]	IC_{50} (μM)[b]	Rat PK (5mg/kg, *p.o.*)		
					C_{max} (μM)[c]	T_{max} (h)	$T_{1/2}$ (h)
60	Ph	H	6.04	2.82	17.0 ± 4.9	2	1.6
61	H	Ph	6.04	2.98	73.5 ± 13.0	2	10.4
62	H	^{i}PrO—	5.08	>10	22.3 ± 3.1	1	5.0
63	H	PhO—	6.25	5.52	64.2 ± 1.4	6	13.3
64	H	^{t}Bu	5.98	5.20	64.9 ± 22.9	1	3.4
65	Cyclohexyl	H	6.77	2.42	3.3 ± 2.8	1	1.4
66	1-Cyclohexenyl	H	6.47	2.31	5.20 ± 0.9	1	0.7
67	H	Cyclohexyl	6.77	2.40	15.2 ± 1.9	1	0.7
68	H	4-Morpholinyl	4.37	21.6	45.5 ± 0.8	1	1.8
69	H	PhCO—	5.39	4.11	35.0 ± 17.7	1	0.9
70[d]	H	1-Pyrrolyl	5.39	6.41	34.4 ± 25.8	2	11.3
71[d]	H	1-Pyrrolidinyl	4.62	7.80	26.7 ± 5.1	2	8.0
72	H	2-Thienyl	5.91	>20	38.4 ± 6.5	2	1.9
73	H	1-Adamantyl	8.44	1.17	13.8 ± 0.6	2	4.3
74	1-Adamantyl	H	8.44	1.86	4.4 ± 1.2	2	2.3
75[e]	4-Pyridyl	H	4.58	>40	5.2 ± 0.5	2	1.6
76[e]	8-Quinolinyl	H	5.92	3.63	22.2 ± 7.8	1	2.5
77[e]	3-Quinolinyl	H	5.96	1.67	1.1 ± 0.3	2	3.5
78[e]	5-Isoquinolinyl	H	5.71	1.84	1.2 ± 2.2	2	10.5
79[e]	4-Isoquinolinyl	H	5.71	3.19	1.0 ± 1.3	6	9.2
80	1-Naphthyl	H	7.21	0.90	6.3 ± 6.8	2	1.7

[a] CLog P was obtained from ChemDraw Ultra 10.0 for free carboxylic acid/amine.
[b] Calculated based on PAI-1 activities observed in Method B at various concentrations (see Ref. 23).
[c] Data are expressed as mean ± S.D. ND = not determined.
[d] HCl salt.
[e] Na salt.

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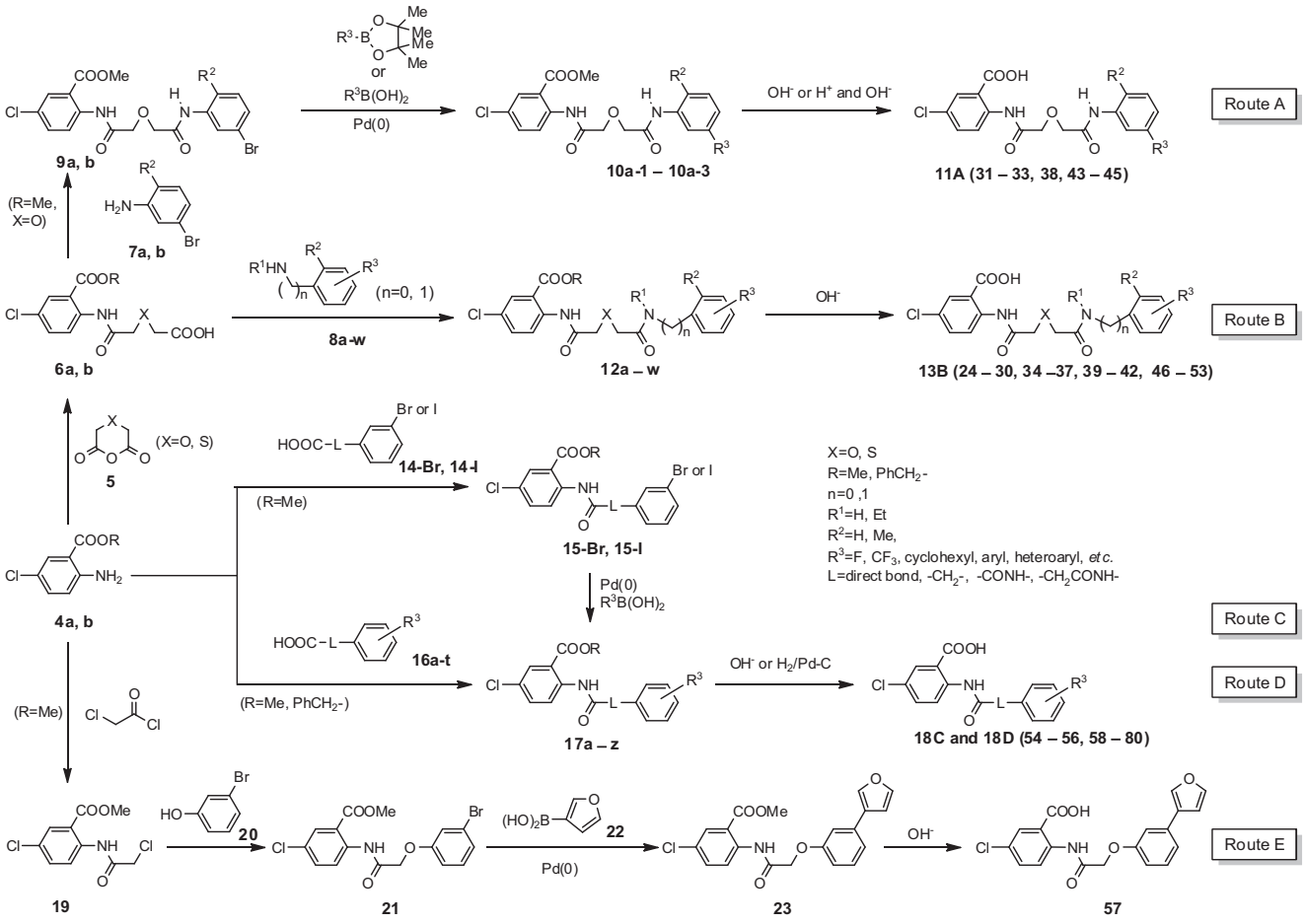

Scheme 1. Synthesis of 5-chloroanthranilic acid derivatives.

57. Biological properties of these compounds are summarized in Tables 1–3.

Results and discussion

As we have previously reported,[15] the strategy in our drug design was to combine substituted anthranilic acid and lipophilic moieties with proper length of acyl-type linkers, which led to the discovery of TM5275. The results from our series of the previous work[14,15] suggested that the carboxyl group in the anthranilic acid was essential to bind to the PAI-1 protein at the target cleft probably by ionic binding while the lipophilic group played a secondary effect to interact with a section of the cleft by van der Waals interactions or π-π stacking. Although co-crystallizing our inhibitors with PAI-1 for X-ray crystallography is still in progress to analyze their binding modes, computer-assisted docking simulation which we have reported on TM5001[12] and TM5275[24] denotes our speculation as being reasonable. Our subsequent study is directed towards finding smaller and simpler compounds than TM5275, expecting to induce high oral bioavailability sufficient to clearly exhibit *in vivo* biological action after oral administration.

Since the *in vitro* PAI-1 inhibitory activity[23] of simple anilides such as **24** and **25** (Table 1) was lower than that of TM5275 as was the case with compound **3**[15] (Fig 1), we next synthesized phenyl-substituted anilides, namely biphenylamide-type compounds, mimicking in part the structure of TM5275 which possesses two phenyl rings in the *N*-acyl side chain. While an *o*-biphenylamide derivative (**26**) failed to recover the activity of TM5275, almost complete recovery of the activity was observed in *m*- or *p*-biphenylamide derivatives (**27–34**) (Table 1). The results suggest that the cavity of PAI-1 protein can accommodate these types of compounds (**27–34**) better than **24–26**. Since compounds **27–34** have rather high CLogP values (Table 1), we synthesized compounds **35–51** in order to lower Clog P values by introducing heteroaryl groups instead of phenyl groups. However, CLogP itself does not seem to be is a significant determinant of *in vitro* potency as can be seen in Table 1. Among these compounds, **36**, **37**, and **40** were worthy of attention due to their favorable *in vitro* potencies.

Brief pharmacokinetic (PK) profiles[23] of some of the compounds were determined in rats by oral gavage (Table 1). Compound **40** which has *m*-(3-furyl)anilide structure was the most attractive in terms of both potent *in vitro* activity and high oral absorbability superior than TM5275.

In further studies, the linker section of **40** was replaced by various linkages (L) as shown in Table 2. While *in vitro* activities at 20 μM of compounds **52–59** thus prepared were significantly decreased relative to **40**, oral absorbability of these compounds was comparable or rather improved (Table 2). The length or the kinds of L were not major determinants both *in vitro* PAI-1 inhibitory activity and oral PK profile. Of particular interest, even the simplest compound **59**, which is the smallest in molecular size without any linker, showed considerably potent *in vitro* activity as well as high oral absorbability. Therefore, we next synthesized *N*-(substituted)benzoyl-5-chloroanthranilic acid derivatives (**60–80**, Table 3) possessing a different type of substituent at the

meta- or *para*-position on the benzoyl-benzene ring. The PAI-1 inhibitory activity (IC_{50}) and PK profiles in rats orally administered with 5 mg/kg of these test compounds are shown in Table 3.

Generally speaking, benzoyl derivatives (Table 3) with CLogP values of more than 6.0, such as compounds **60**, **61**, **63**, **65**, **66**, **67**, **73**, **74** and **80**, tended to exhibit high *in vitro* PAI-1 inhibitory activity irrespective of the position of substituents (R^1 or R^2). Conversely oral absorbability decreased in compounds **65**, **66**, **74** and **80** which have lipophilic substituents at the *meta*-position (R^1). Interestingly, highly lipophilic compounds **67** and **73** with cyclohexyl and 1-adamantyl group as a *para*-substituent, respectively, exhibited moderate oral absorbability. Although the CLogP values of compounds **76–79** possessing a quinoline or an isoquinoline moiety are lower than 6.0, these compounds, in contrast to the above-observed trends, exhibited potent *in vitro* activities and somewhat decreased oral absorbability with the exception of **76** which has the 8-quinolinyl moiety as a substituent at R^1. On the other hand, compound **75** with a 4-pyridyl moiety at R^1 surprisingly lost *in vitro* activity. Although structure-activity relationship studies on derivatives having nitrogen heterocycles as the benzoyl-phenyl substituents have not been studied in details, the current results suggest that the position of nitrogen atom is an important determinant of biological activities in this series of compounds.

While many of the compounds which had high potential with respect to both *in vitro* activity and PK profile as mentioned above violate Lipinski's Rule of Five[25] on drug-likeness in terms of CLogP, some fulfilled our criteria for further pharmacological evaluation, and are expected to exhibit high oral bioavailability in various animal models.

Conclusion

Novel 5-chloroanthranilic acid derivatives with lipophilic *N*-acyl groups were synthesized and evaluated as PAI-1 inhibitors *in vitro*. In addition, oral PK profiles of some of the compounds which exhibited potent *in vitro* activities were also evaluated in rats to select candidate compounds for further evaluation as potential new drugs. Taking diversity of chemical structure and relative ease of synthesis in consideration, compounds **40**, **55**, **60** and **76** were chosen as valuable tools to evaluate pharmacological profiles PAI-1 inhibitors in detail in order to develop useful drugs, in particular, with expectation of identifying unprecedentedly novel clinical applications.[16–21] Extensive pharmacological and toxicological studies on these compounds are in progress.

Our previous[14,15] and present investigations on *N*-acylanthranilic acid derivatives suggest that the cleft of the PAI-1 protein has considerably variable capacity in accommodating a variety of compounds which has a carboxylate group together with a proper lipophilic functionality in their molecular structures, because the relative differences in distance or position between the carboxylate and lipophilic moieties do not seem to affect PAI-1 inhibitory activity in a direct fashion. This fact may suggest that these anthranilic acid derivatives do not necessarily accommodate at the same site of the cavity of PAI-1, as we have observed a difference between TM5007 and TM5275[13]. Although the exact interaction modes for the newly identified inhibitors to associate with PAI-1 protein are uncertain at present, further structure-based studies on this series of compounds targeting not only the above-mentioned cleft but also another potential binding site such as the one proposed by Gorlatova et al.[26] for tiplaxtinin are going underway to unveil this issue with the aim of designing novel PAI-1 inhibitors.

In summary, structure-optimization starting with original hits (TM5001, TM5007)[12] to discover more drug-like compounds was achieved by identifying orally bioavailable 5-chloroanthranilic acid derivatives by our intense structure-activity-relationship studies, in particular, not only by converting the thiophene ring in the original hits to its isosteric benzene ring[15] but also by substantially diminishing molecular size.

A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at https://doi.org/10.1016/j.bmcl.2017.11.016.

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