Synthesis and Evaluation of Pyrrolin-2-one Compounds, a Series of Plasminogen Activator Inhibitor-1 Inhibitors

Hiroshi Miyazaki,*[,a] Tsuyoshi Ogiku,[a] Hiroshi Sai,[a] Yasunori Moritani,[a] Akio Ohtani,[b] and Hiroshi Ohmizu[a]

[a] *Medicinal Chemistry Laboratory, Mitsubishi Tanabe Pharma Co., Ltd.; 3–16–89 Kashima, Yodogawa, Osaka 532–8505, Japan: and* [b] *Pharmacology Laboratory, Mitsubishi Tanabe Pharma Co., Ltd.; 2–2–50 Kawagishi, Toda, Saitama 335–8505, Japan.* Received May 19, 2009; accepted June 16, 2009; published online June 18, 2009

A novel series of furan-2-one and pyrrolin-2-one derivatives having PAI-1 (plasminogen activator inhibitor-1) inhibitory activity were synthesized and evaluated for their antithrombotic activity in a rat arterial thrombosis model. Among the synthesized compounds, 5f (T-1776Na) was found to have good selectivity for PAI-1 over other enzymes and high antithrombotic activity.

Key words plasminogen activator inhibitor-1; inhibitor; antithrombotic drug; furan-2-one; pyrrolin-2-one

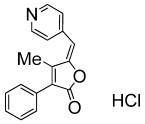
Plasminogen activator inhibitor-1 (PAI-1) is an effective fast-acting inhibitor of both tissue-type plasminogen activator (t-PA) and two-chain urokinase-type plasminogen activator (tcu-PA), and thus plays an important role in regulation of the fibrinolytic system.[1)] Elevated levels of PAI-1 in plasma have been observed in patients with deep vein thrombosis[2—4)] and unstable angina.[5)] Furthermore, a number of animal studies have shown that PAI-1 is responsible for fibrinolytic activity in thrombotic and prethrombotic states.[6—8)] Thus, inhibition of PAI-1 activity or reduction of its production may shift the balance between thrombogenesis and thrombolysis towards thrombolysis. In fact, it has been reported that an antibody against PAI-1 can enhance clot lysis and decrease thrombus growth in animal models of venous thrombosis[9)] and arterial thrombosis.[10)] Therefore, therapeutic inhibition of PAI-1 activity or reduction of its production may be useful for prevention and/or treatment of thrombotic disorders. Indeed, a number of small molecules that inhibit PAI-1 or reduce its production have recently been reported.[11—20)]

High-throughput screening of Mitsubishi Tanabe chemical libraries led to the discovery of 4-methyl-3-phenyl-5-[1-pyridin-4-ylmeth-(*E*)-ylidene]-5*H*-furan-2-one hydrochloride (**1a**) as a weak PAI-1 inhibitor (IC_{50}=24 μM, Fig. 1). To improve the inhibitory activity of **1a** towards PAI-1, we carried out a series of chemical modifications. In this paper, we describe the synthesis, structure–activity relationships (SAR), and antithrombotic activity of a series of furan-2-one and pyrrolin-2-one derivatives.

Chemistry

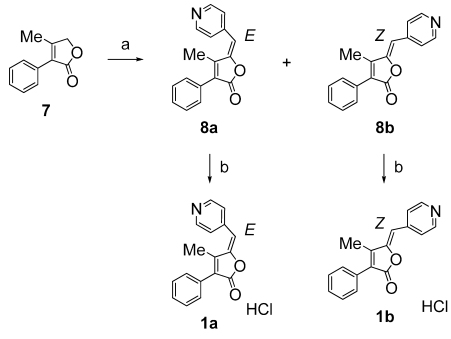
The synthetic route of furan-2-one derivatives is shown in Chart 1. The furan-2-one derivatives **1a** and **1b** were synthesized from 4-methyl-3-phenyl-2,5-dihydrofuran-2-one **7**.[21)] Treatment of **7** with lithium diisopropylamide (LDA) followed by 4-pyridinecarboxaldehyde and dehydration after methanesulfonylation of the hydroxyl group afforded the products **8a** (as a minor product) and **8b** (as a major product) in which the 5-pyridylmethylidene parts were introduced. Compounds **8a** and **8b** were separated by silica gel chromatography and transformed to their HCl salts **1a** and **1b**, respectively.

4-Methyllactam (pyrrolin-2-one) compounds were synthe-



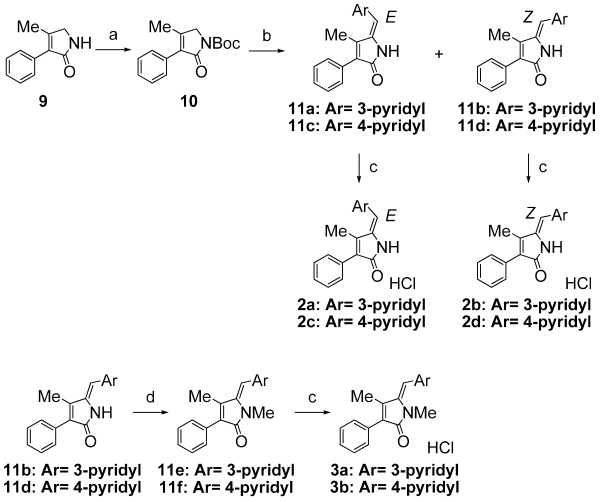
1a

Fig. 1. Hit Compound **1a**



Reagents: (a) i) LDA, 4-pyridinecarboxaldehyde, THF ii) MsCl, Et_3N, CH_2Cl_2, iii) DBU, CH_2Cl_2; (b) HCl, AcOEt, $CHCl_3$.

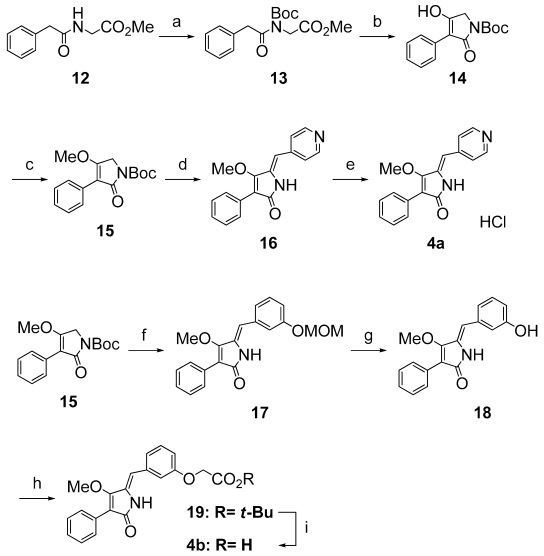
Chart 1



Reagents: (a) Boc_2O, DMAP, CH_2Cl_2; (b) i) LiHMDS, THF ii) 3- or-4-pyridinecarboxaldehyde, THF; (c) HCl, AcOEt, $CHCl_3$; (d) NaH, MeI, DMF.

Chart 2

* To whom correspondence should be addressed. e-mail: miyazaki.hiroshi@me.mt-pharma.co.jp



Reagents: (a) Boc_2O, DMAP, CH_3CN; (b) *t*-BuOK, DMF; (c) Me_2SO_4, K_2CO_3, acetone; (d) i) KHMDS, toluene, THF ii) 4-pyridinecarboxaldehyde, THF; (e) HCl, dioxane, THF; (f) i) KHMDS, toluene, THF ii) 3-methoxymethoxybenzaldehyde; (g) HCl aq., THF; (h) NaH, $BrCH_2CO_2t$-Bu, DMF; (i) TFA, CH_2Cl_2.

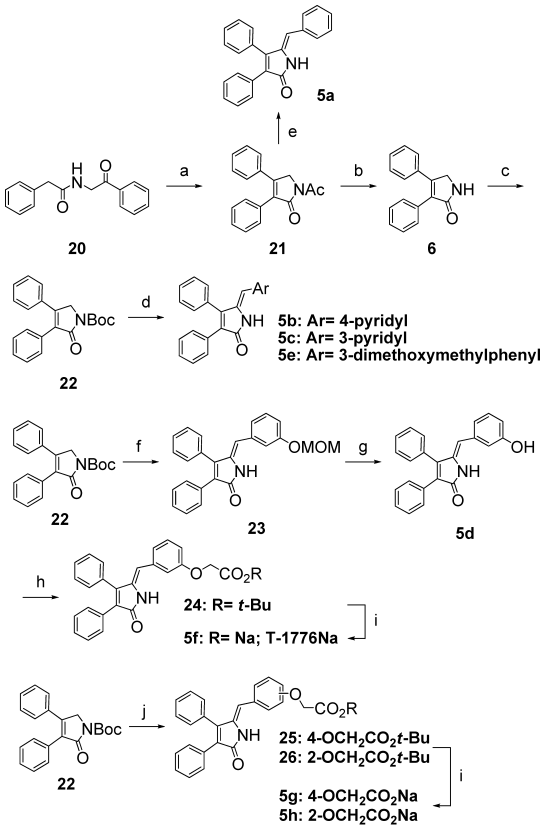
Chart 3

sized as shown in Chart 2. The acidic NH proton of **9**[22] was first protected with *tert*-butoxycarbonyl (Boc), and then the 5-pyridylmethylidene parts were introduced under basic conditions. These reactions proceeded with good *Z*-selectivity in cases of pyrrolin-2-one as well as furan-2-one, and separation of *E* and *Z*-products by silica gel chromatography followed by conversion to their HCl salts gave the 5-pyridylmethylidenepyrrolin-2-one compounds **2a**—**d**. Compounds **11b** and **11d** were then methylated by NaH and MeI, and converted to their HCl salts **3a** and **3b**, respectively.

The synthetic route of 4-methoxylactam (pyrrolin-2-one) compounds is shown in Chart 3. Compounds **4a** and **4b** were synthesized from the amide compound **12**. After **12** was protected with Boc, the product **13** was cyclized under basic conditions and transformed into the intermediate **15**. Compound **4a** was obtained from **15** by the same method as **2d** in two steps. In the case of compound **4b**, methoxymethyl (MOM)-protected 3-hydroxyphenyl was first introduced and the product **17** was converted to the 3-carboxymethoxyphenyl compound **4b** by a conventional method.

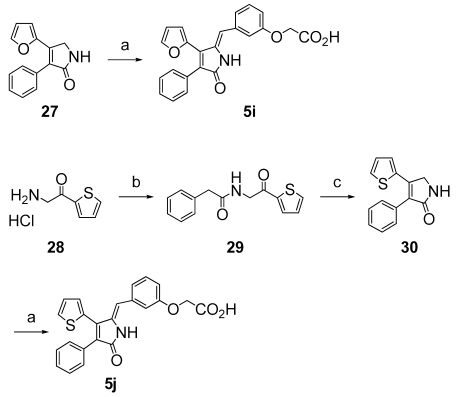
The 4-phenyllactam or 4-heteroaryllactam (pyrrolin-2-one) compounds **5a**—**j** and **6** were synthesized as follows (Chart 4, 5). 3,4-Diphenyl-1,5-dihydropyrrol-2-one **6** was synthesized in a similar manner as the literature.[23] *N*-(2-Oxo-2-phenylethyl)-2-phenylacetamide **20** was cyclized to give **21** by treatment with acetic anhydride, and cleavage of the *N*-acetyl group of **21** provided 3,4-diphenyl-1,5-dihydropyrrol-2-one **6**. After various substituents were introduced into the 5-position of the *N*-acetyl compound **21** or *N*-Boc protected compound **22**, several conventional conversions afforded the 4-phenyllactam compounds **5a**—**h**.

In the case of 2-furyl at the 4-position of pyrrolin-2-one, **5i** was obtained by introducing a substituent at the 5-position of **27**[24] without NH-Boc protection. The thienyl compound **5j** was provided in a similar manner as **5i** from the commercially available **28** in three steps.



Reagents: (a) Ac_2O, Et_3N; (b) NaOMe, MeOH; (c) Boc_2O, DMAP, CH_3CN; (d) i) KHMDS, toluene, THF ii) 4- or 3-pyridinecarboxaldehyde or 3-dimethoxymethylbenzaldehyde, THF; (e) i) KHMDS, toluene, THF ii) benzaldehyde, THF. (f) 3-methoxymethoxybenzaldehyde, KHMDS, toluene, THF; (g) HCl aq., THF; (h) $BrCH_2CO_2t$-Bu, NaH, DMF; (i) i) TFA, CH_2Cl_2 ii) NaOH aq., MeOH; (j) $OHCC_6H_4$(4- or 2-OCH_2CO_2t-Bu), KHMDS, toluene, THF;

Chart 4



Reagents: (a) $OHCC_6H_4$(3-OCH_2CO_2t-Bu), NaOH, MeOH, H_2O; (b) phenylacetic acid, CDI, Et_3N, CH_2Cl_2; (c) NaOMe, EtOH.

Chart 5

Biological Results and Discussion

The inhibitory activity of furan-2-one and pyrrolin-2-one derivatives for human PAI-1 is summarized in Tables 1 and 2. The *Z*-isomer **1b** showed better PAI-1 inhibitory activity than the *E*-isomer **1a**. However, these furan-2-one compounds (**1a**, **b**) were biologically unstable. This instability was believed to be due to their α,β-unsaturated lactone ring. As such, **1a** and **1b** were considered inappropriate for inhibition of thrombus formation *in vivo*. Thus, we tried to transform

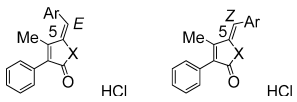
the lactone ring into a more stable scaffold. The pyrrolin-2-one compounds **2a**—**d**, having a 3 or 4-pyridine ring at the 5-position, were first evaluated. The 3-pyridine compound **2b** showed strong inhibitory activity for human PAI-1 (IC_{50}=9.8 μM). However, in case of the pyrrolin-2-ones, the inhibitory activity of the *E*-isomer **2a** and that of the 4-pyridine compounds **2c** and **2d** was low. Furthermore, the *N*-methylated compounds **3a** and **3b** had no PAI-1 inhibitory activity. This led us to examine the SAR of pyrrolin-2-one derivatives with no subsitituent at the 1-position.

Our search for more attractive templates focused on substituents at the 4-position of the pyrrolin-2-one ring (Table 2). In case of a methoxy group, compounds **4a** and **4b** exhibited no inhibitory activity. Next, a phenyl group was introduced. The phenylmethylidene compound **5a** and the 4-pyridylmethylidene compounds **5b** and **6**, having no substituent at the 5-position, did not inhibit human PAI-1. However, 3-pyridylmethylidene **5c** showed potent inhibitory activity for human PAI-1 (IC_{50}=12.6 μM). These findings suggest that hydrophilic groups at the *meta*-position of the aromatic ring, which extends from the 5-position, are favorable for PAI-1 inhibitory activity. Other hydrophilic substituents, such as alcohol (**5d**), dimethylacetal (**5e**) and carboxylic acid (**5f**), were also examined. Compound **5d** did not inhibit human PAI-1, while compound **5e** showed a moderate inhibitory activity (IC_{50}=7.9 μM). Particularly, compound **5f**, having a carboxylic acid group, was found to have potent PAI-1 inhibitory activity (IC_{50}=9.6 μM).

Next we focused our efforts on optimizing compound **5f**, having a phenoxy acetic acid group. However, compounds **5g** and **5h**, having a 4- or 2-phenoxy acetic acid moiety, and compounds **5i** and **5j**, having a furan or thiophene at the 4-position of pyrrolin-2-one ring, exhibited less potent inhibitory activity for human PAI-1 than compound **5f**.

In order to select the best compound, the synthesized pyrrolin-2-one compounds were evaluated for their antithrombotic activity in rat arterial thrombosis model. Compounds **2b**, **5c**, and **5e** could not be intravenously infused to rats due to their poor solubility. They were thus considered not to have the desired features for antithrombotic drug. On the other hand, compound **5f**, possessing a carboxylic acid group at the 5-position of the pyrrolin-2-one ring, had good solubility and was found to decrease thrombus weight at a dose of 0.5 μg/kg/min. Finally, we evaluated the selectivity of compound **5f** for rat PAI-1. Compound **5f** inhibited rat PAI-1 with an IC_{50} value of 10.3 μM, which is almost equal to its inhibition of human PAI-1. Compound **5f** showed no inhibitory effect against the serine proteases thrombin, plasmin, and trypsin, and the serpins antithrombin III, antiplasmin, and antitrypsin at 30 μM. It shows the antithrombotic activity of **5f** is thought to be based on its PAI-1 inhibition.

Table 1. Inhibitory Effect of Furan-2-one and Pyrrolin-2-one Derivatives against Human PAI-1 Activity[a)]



Compound	Configuration at C-5 position	X	Ar	Inhibitory activity[a)] IC_{50} (μM)
1a	*E*	O	4-Pyridyl	24 μM
1b	*Z*	O	4-Pyridyl	5.6 μM
2a	*E*	NH	3-Pyridyl	7% inhibition at 30 μM
2b	*Z*	NH	3-Pyridyl	9.8 μM
2c	*E*	NH	4-Pyridyl	5% inhibition at 30 μM
2d	*Z*	NH	4-Pyridyl	24% inhibition at 10 μM
3a	*Z*	NMe	3-Pyridyl	2% inhibition at 30 μM
3b	*Z*	NMe	4-Pyridyl	4% inhibition at 30 μM

a) See Experimental.

Conclusion

In this study, we examined the structure–activity relationships of a series furan-2-one derivatives. Initial modification led to identification of the pyrrolin-2-one template as a promising scaffold. Optimization of the pyrrolin-2-one derivatives resulted in identification of **5f** (T-1776Na) as a potent PAI-1

Table 2. Inhibitory Effect of Pyrrolin-2-one Derivatives against Human PAI-1 Activity[a)] and Their Antithrombotic Activity[b)]

Compound	R	Ar	Inhibitory activity[a)] IC_{50} (μM)	% decrease in thrombus weight[b)]
2b	Me	3-Pyridyl	9.8 μM	nt[c)]
4a	OMe	4-Pyridyl (HCl)	No effect at 30 μM	
4b	OMe	3-Carboxymehoxyphenyl	No effect at 30 μM	
5a	Ph	Ph	No effect at 15 μM	
5b	Ph	4-Pyridyl	No effect at 30 μM	
5c	Ph	3-Pyridyl	12.6 μM	nt[c)]
6	—	—	No effect at 15 μM	
5d	Ph	3-Hydroxyphenyl	No effect at 30 μM	
5e	Ph	3-Dimethoxymethylphenyl	7.9 μM	nt[c)]
5f	Ph	3-Carboxymehoxyphenyl (Na salt)	9.6 μM	43.4%
5g	Ph	4-Carboxymehoxyphenyl (Na salt)	No effect at 30 μM	
5h	Ph	2-Carboxymehoxyphenyl (Na salt)	32% inhibition at 30 μM	
5i	2-Furyl	3-Carboxymehoxyphenyl	30% inhibition at 30 μM	
5j	2-Thienyl	3-Carboxymehoxyphenyl	27% inhibition at 30 μM	

a) See Experimental. *b*) Compounds were intravenously infused at 0.5 μg/kg/min during electrical stimulation. See Experimental. *c*) Not tested due to low solubility.

inhibitor. Compound **5f** was selected for further biological evaluation and was found to possess good antithrombotic activity in rat model. Compound **5f** was also found to have good selectivity for PAI-1 over serine proteases and serpins. These findings indicate that pyrrolin-2-one derivatives are potential candidates for antithrombotic drugs. Accordingly, compound **5f** has been selected as lead compound and further research on its antithrombotic effects are on going.

Experimental

Melting points were measured using a Büchi 535 capillary melting point apparatus and are uncorrected. IR spectra were recorded on a PerkinElmer Spectrum One FT-IR spectrometer. ^{1}H-NMR spectra were recorded on a Bruker AC-200 spectrometer (200 MHz) or on a Bruker AVANCE 400 spectrometer (400 MHz) with Me_4Si as internal standard. Mass spectra were obtained on a ThermoFisher FINNIGAN LXQ or Q-TOF Ultima API mass spectrometer. Elemental analyses were obtained on a PerkinElmer 2400 II (C, H, N) and Dionex DX-320 (S).

4-Methyl-3-phenyl-5-[1-pyridin-4-ylmeth-(*E*)-ylidene]-5*H*-furan-2-one Hydrochloride (1a); 4-Methyl-3-phenyl-5-[1-pyridin-4-ylmeth-(*Z*)-ylidene]-5*H*-furan-2-one Hydrochloride (1b) 1.6 M *n*-BuLi in hexane (5.4 ml, 8.6 mmol) was slowly added to a solution of diisopropylamine (1.2 ml, 8.6 mmol) in tetrahydrofuran (THF) (15 ml) at −78 °C. The solution was stirred for 30 min at the same temperature and 4-methyl-3-phenyl-2,5-dihydrofuran-2-one **7**[21] (1.0 g, 5.7 mmol) in THF (15 ml) was added dropwise to the solution. The mixture was stirred for 30 min at −78 °C and 4-pyridinecarboxaldehyde (0.92 g, 8.6 mmol) in THF (5 ml) was added dropwise to the mixture. After the addition, the mixture was further stirred for 1 h at −78 °C. Water and ethyl acetate were added to the mixture and the organic layer was separated. The organic layer was then dried and concentrated *in vacuo*. The resulting residue was dissolved in CH_2Cl_2 (20 ml), and triethylamine (1.2 ml, 8.6 mmol) and methanesulfonyl chloride (0.67 ml, 8.6 mmol) were added to the solution at 0 °C. The mixture was stirred for 30 min at 0 °C and 1,8-diazabicyclo[5,4,0]undec-7-ene (DBU) (1.3 ml, 8.6 mmol) was added. After the addition, the reaction mixture was further stirred for 30 min at 0 °C. Water and $CHCl_3$ were then added to the mixture and the organic layer was separated. The organic layer was dried and concentrated *in vacuo*. The residue was chromatographed on silica gel to give 75 mg (5%) of 4-methyl-3-phenyl-5-[1-pyridin-4-ylmeth-(*E*)-ylidene]-5*H*-furan-2-one (**8a**) and 1.16 g (77%) of 4-methyl-3-phenyl-5-[1-pyridin-4-ylmeth-(*Z*)-ylidene]-5*H*-furan-2-one (**8b**) as a solid. mp 127—128 °C (**8a**), 189—190 °C (**8b**).

To a **8a** (200 mg, 0.76 mmol) solution in $CHCl_3$ (10 ml) was added 4 N HCl in ethyl acetate (0.19 ml) and the mixture was stirred for 10 min. The reaction mixture was then concentrated *in vacuo*. The resulting residue was triturated with diethyl ether and filtrated to afford **1a** (212 mg, 93%) as a solid. mp >230 °C. ^{1}H-NMR (400 MHz, DMSO-d_6) δ: 2.03 (3H, s), 7.21 (1H, s), 7.45—7.58 (5H, m), 8.05 (2H, d, J=6.4 Hz), 8.89 (2H, d, J=6.4 Hz). IR (ATR) cm^{-1}: 3045, 2948, 2382, 2117, 2011, 1754, 1652, 1627, 1598, 1494, 1178, 1058, 981, 789, 750, 696, 536, 481. MS (APCI): 264 $[M+H]^+$. HR-MS-ESI Calcd for $C_{17}H_{14}NO_2$ ($[M+H]^+$): 264.1025. Found: 264.1024.

1b, prepared from **8b** as described in the synthesis of **1a**, was obtained as a solid. Yield 94%. mp 279—280 °C (dec.). ^{1}H-NMR (400 MHz, DMSO-d_6) δ: 2.41 (3H, s), 6.90 (1H, s), 7.45—7.70 (5H, m), 8.22 (2H, d, J=6.7 Hz), 8.88 (2H, d, J=6.7 Hz). IR (ATR) cm^{-1}: 3059, 2969, 1772, 1651, 1629, 1584, 1496, 1478, 1445, 1186, 1153, 953, 871, 797, 786, 692, 546. MS (APCI): 264 $[M+H]^+$. HR-MS-ESI Calcd for $C_{17}H_{14}NO_2$ ($[M+H]^+$): 264.1025. Found: 264.1022.

4-Methyl-2-oxo-3-phenyl-2,5-dihydropyrrole-1-carboxylic Acid *tert*-Butyl Ester (10) To a solution of 4-methyl-3-phenyl-1,5-dihydropyrrol-2-one **9**[22] (5.1 g, 29.4 mmol) in CH_2Cl_2 (50 ml) were added $(Boc)_2O$ (9.6 g, 44.2 mmol) and *N*,*N*-dimethyl-4-aminopyridine (72 mg, 0.59 mmol) and the mixture was stirred at 40 °C for 30 min. After the reaction mixture was concentrated *in vacuo*, the resulting residue was chromatographed on silica gel to give 5.8 g (72%) of **10**. mp 127—128 °C. ^{1}H-NMR (400 MHz, DMSO-d_6) δ: 1.49 (9H, s), 2.11 (3H, s), 4.34 (2H, s), 7.34—7.40 (3H, m), 7.41—7.47 (2H, m). IR (ATR) cm^{-1}: 2980, 1748, 1362, 1302, 1161, 1093, 695. MS (APCI): 274 $[M+H]^+$. *Anal.* Calcd for $C_{16}H_{19}NO_3$: C, 70.31; H, 7.01; N, 5.12. Found: C, 70.28; H, 7.05; N, 5.09.

4-Methyl-3-phenyl-5-[1-pyridin-3-ylmeth-(*E*)-ylidene]-1,5-dihydropyrrol-2-one Hydrochloride (2a); 4-Methyl-3-phenyl-5-[1-pyridin-3-ylmeth-(*Z*)-ylidene]-1,5-dihydropyrrol-2-one Hydrochloride (2b) 1 M Lithium bis(trimethylsilyl)amide in THF (3.5 ml, 3.5 mmol) was slowly added to a solution of **10** (800 mg, 2.93 mmol) in THF (12 ml) at −78 °C. The mixture was stirred for 30 min at the same temperature and 4-pyridinecarboxaldehyde (380 mg, 3.5 mmol) in THF (5 ml) was added dropwise. After the addition, the mixture was further stirred for 30 min at −78 °C and for 16 h at room temperature. Water and ethyl acetate were added to the mixture and the organic layer was separated. The organic layer was dried and concentrated *in vacuo*. The resulting residue was chromatographed on silica gel to give 80 mg (10%) of 4-methyl-3-phenyl-5-[1-pyridin-3-ylmeth-(*E*)-ylidene]-1,5-dihydropyrrol-2-one (**11a**) and 570 mg (74%) of 4-methyl-3-phenyl-5-[1-pyridin-3-ylmeth-(*Z*)-ylidene]-1,5-dihydropyrrol-2-one hydrochloride (**11b**) as a solid. mp 180—182 °C (**11a**), 230—231 °C (**11b**).

To a solution of **11b** (150 mg, 0.57 mmol) in $CHCl_3$ (10 ml) was added 4 N HCl in ethyl acetate (0.16 ml) and the mixture was stirred for 10 min. The reaction mixture was concentrated *in vacuo*, and the resulting residue was triturated with diethyl ether and filtrated to afford **2b** (162 mg, 95%) as a solid. mp 311—314 °C (dec.). ^{1}H-NMR (400 MHz, DMSO-d_6) δ: 2.30 (3H, s), 6.54 (1H, s), 7.37—7.57 (5H, m) 7.95 (1H, dd, J=8.2, 5.5 Hz), 8.57 (1H, d, J=8.5 Hz), 8.73 (1H, d, J=5.5 Hz), 9.00 (1H, d, J=1.8 Hz), 10.64 (1H, s). IR (ATR) cm^{-1}: 3075, 2698, 1683, 1640, 1544, 1387, 1216, 789, 671, 435. MS (APCI): 263 $[M+H]^+$. HR-MS-ESI Calcd for $C_{17}H_{15}N_2O$ ($[M+H]^+$): 263.1184. Found: 263.1175.

2a, prepared from **11a** as described in the synthesis of **2b**, was obtained as a solid. Yield 81%. mp 302—304 °C (dec.). ^{1}H-NMR (400 MHz, DMSO-d_6) δ: 1.84 (3H, s), 6.60 (1H, s), 7.35—7.60 (5H, m) 7.88 (1H, dd, J=8.0, 5.5 Hz), 8.43 (1H, d, J=8.0 Hz), 8.76 (1H, d, J=5.5 Hz), 8.94 (1H, s), 10.46 (1H, s). IR (ATR) cm^{-1}: 3150, 2479, 1687, 1638, 1205, 832, 598. MS (APCI): 263 $[M+H]^+$. HR-MS-ESI Calcd for $C_{17}H_{15}N_2O$ ($[M+H]^+$): 263.1184. Found: 263.1180.

4-Methyl-3-phenyl-5-[1-pyridin-4-ylmeth-(*E*)-ylidene]-1,5-dihydropyrrol-2-one Hydrochloride (2c); 4-Methyl-3-phenyl-5-[1-pyridin-4-ylmeth-(*Z*)-ylidene]-1,5-dihydropyrrol-2-one Hydrochloride (2d) **2c** and **2d**, prepared from **10** and 4-pyridinecarboxaldehyde as described in the synthesis of **2a** and **2b**, were obtained as a solid. **2c** yield 6%. mp 331—335 °C (dec.). ^{1}H-NMR (400 MHz, DMSO-d_6) δ: 1.96 (3H, s), 6.64 (1H, s), 7.38—7.57 (5H, m) 8.01 (2H, d, J=6.6 Hz), 8.80 (2H, d, J=6.6 Hz), 10.59 (1H, s). IR (ATR) cm^{-1}: 3039, 1697, 1598, 1493, 1196, 1136, 856, 697. MS (APCI): 263 $[M+H]^+$. HR-MS-ESI Calcd for $C_{17}H_{15}N_2O$ ($[M+H]^+$): 263.1184. Found: 263.1173. **2d** yield 55%. mp 322—325 °C (dec.). ^{1}H-NMR (400 MHz, DMSO-d_6) δ: 2.31 (3H, s), 6.58 (1H, s), 7.40—7.58 (5H, m) 8.09 (2H, d, J=6.8 Hz), 8.81 (2H, d, J=6.8 Hz), 10.82 (1H, s). IR (ATR) cm^{-1}: 3050, 2734, 1698, 1617, 1597, 1205, 868, 802, 547. MS (APCI): 263 $[M+H]^+$. HR-MS-ESI Calcd for $C_{17}H_{15}N_2O$ ($[M+H]^+$): 263.1184.

1,4-Dimethyl-3-phenyl-5-[1-pyridin-3-ylmeth-(*Z*)-ylidene]-1,5-dihydropyrrol-2-one Hydrochloride (3a) 60% Sodium hydride in oil (25 mg, 0.63 mmol) was slowly added to a solution of **11b** (150 mg, 0.57 mmol) in DMF (3 ml). The mixture was stirred for 1 h at room temperature and methyl iodide (0.04 ml, 0.63 mmol) was added. After the addition, the mixture was further stirred for 1.5 h at room temperature. Water and ethyl acetate were added to the mixture and the organic layer was separated. The organic layer was washed with water, dried and concentrated *in vacuo*. The resulting residue was chromatographed on silica gel to give 100 mg (63%) of 1,4-dimethyl-3-phenyl-5-[1-pyridin-3-ylmeth-(*Z*)-ylidene]-1,5-dihydropyrrol-2-one (**11e**) as a solid. mp 129—130 °C.

To a solution of **11e** (90 mg, 0.33 mmol) in $CHCl_3$ (10 ml) was added 4 N HCl in ethyl acetate (0.09 ml) and the mixture was stirred for 10 min. The reaction mixture was then concentrated *in vacuo*. The resulting residue was triturated with diethyl ether and filtrated to afford **3a** (97 mg, 95%) as a solid. mp 213—215 °C. ^{1}H-NMR (400 MHz, DMSO-d_6) δ: 2.30 (3H, s), 2.84 (3H, s), 6.68 (1H, s), 7.37—7.55 (5H, m) 7.87 (1H, dd, J=8.0, 5.6 Hz), 8.34 (1H, d, J=8.0 Hz), 8.75 (1H, d, J=4.6 Hz), 8.90 (1H, d, J=1.5 Hz). IR (ATR) cm^{-1}: 3415, 2515, 1683, 1633, 1554, 1438, 788, 682, 547. MS (APCI): 277 $[M+H]^+$. HR-MS-ESI Calcd for $C_{18}H_{17}N_2O$ ($[M+H]^+$): 277.1341. Found: 277.1330.

1,4-Dimethyl-3-phenyl-5-[1-pyridin-4-ylmeth-(*Z*)-ylidene]-1,5-dihydropyrrol-2-one Hydrochloride (3b) This compound, prepared from **11d** as described in the synthesis of **3a**, was obtained as a solid. Yield 65%. mp 239—241 °C. ^{1}H-NMR (400 MHz, DMSO-d_6) δ: 2.31 (3H, s), 2.90 (3H, s), 6.74 (1H, s), 7.40—7.56 (5H, m) 7.94 (2H, d, J=6.5 Hz), 8.82 (2H, d, J=6.5 Hz). IR (ATR) cm^{-1}: 3499, 3374, 2615, 2073, 1689, 1621, 1606, 1505, 1385, 1242, 856, 632, 547. MS (APCI): 277 $[M+H]^+$. HR-MS-ESI Calcd for $C_{18}H_{17}N_2O$ ($[M+H]^+$): 277.1341. Found: 277.1330.

***tert*-Butoxycarbonylphenylacetylaminoacetic Acid Methyl Ester (13)**

To a solution of phenylacetylaminoacetic acid methyl ester **12** (500 mg, 2.41 mmol) in acetonitrile (20 ml) were added $(Boc)_2O$ (630 mg, 2.89 mmol) and *N*,*N*-dimethyl-4-aminopyridine (15 mg, 0.12 mmol) at 0 °C and the mixture was stirred at room temperature for 2 h. After the reaction mixture was concentrated *in vacuo*, water and ethyl acetate were added to the residue. The organic layer was separated, washed with brine, dried and concentrated *in vacuo*. The resulting residue was chromatographed on silica gel to give 405 mg (55%) of **13** as an oil. ^{1}H-NMR (200 MHz, $CDCl_3$) δ: 1.48 (9H, s), 3.73 (3H, s), 4.30 (2H, s), 4.47 (2H, s), 7.15—7.40 (5H, m). MS (SIMS): 308 $[M+H]^+$.

4-Hydroxy-2-oxo-3-phenyl-2,5-dihydropyrrole-1-carboxylic Acid *tert*-Butyl Ester (14) Potassium *tert*-butoxide (88 mg, 0.78 mmol) was slowly added to a solution of **13** (200 mg, 0.65 mmol) in DMF (10 ml) and the mixture was stirred for 10 min at room temperature. Aqueous saturated ammonium chloride solution, water and ethyl acetate were added to the reaction mixture. The organic layer was separated, washed with water, dried and concentrated *in vacuo*. The resulting residue was triturated with diethyl ether and filtrated to afford **14** (120 mg, 68%) as a solid. mp 152 °C. ^{1}H-NMR (400 MHz, DMSO-d_6) δ: 1.48 (9H, s), 4.27 (2H, s), 7.21 (1H, t, J=7.4 Hz), 7.35 (2H, t, J=7.6 Hz), 7.85 (2H, d, J=7.2 Hz), 12.36 (1H, br). IR (ATR) cm^{-1}: 3144, 1718, 1663, 1640, 1349, 1312, 1153, 694. MS (APCI): 276 $[M+H]^+$. *Anal.* Calcd for $C_{15}H_{17}NO_4$: C, 65.44; H, 6.22; N, 5.09. Found: C, 65.46; H, 6.22; N, 5.13.

4-Methoxy-2-oxo-3-phenyl-2,5-dihydropyrrole-1-carboxylic Acid *tert*-Butyl Ester (15) Potassium carbonate (30 g, 217.5 mmol) and dimethyl sulfate (27 ml, 290 mmol) were added to a solution of **14** in acetone (1 l) and the mixture was stirred for 3 h at reflux. Water and ethyl acetate were then added to the mixture and the organic layer was separated. The organic layer was dried and concentrated *in vacuo*. The resulting residue **15** (39 g, 93%) was used for the next reaction without further purification. mp 152 °C. ^{1}H-NMR (400 MHz, DMSO-d_6) δ: 1.50 (9H, s), 4.01 (3H, s), 4.62 (2H, s), 7.25 (1H, t, J=7.4 Hz), 7.37 (2H, t, J=7.6 Hz), 7.76 (2H, d, J=7.2 Hz). IR (ATR) cm^{-1}: 2982, 1707, 1629, 1315, 1153, 1012, 781, 697. MS (APCI): 290 $[M+H]^+$. *Anal.* Calcd for $C_{16}H_{19}NO_4$: C, 66.42; H, 6.62; N, 4.84. Found: C, 66.46; H, 6.62; N, 4.91.

4-Methoxy-3-phenyl-5-[1-pyridin-4-ylmeth-(*Z*)-ylidene]-1,5-dihydropyrrol-2-one Hydrochloride (4a) 0.5 M Potassium hexamethyldisilazide in toluene (8.9 ml, 4.45 mmol) was slowly added to a solution of **15** (1.0 g, 2.98 mmol) in THF (20 ml) at −78 °C and the reaction mixture was stirred for 20 min at the same temperature, then at 0 °C for 10 min. 4-Pyridinecarboxaldehyde (0.43 ml, 4.47 mmol) was slowly added to the reaction mixture at −78 °C and the mixture was stirred at 0 °C for 2 h. After aqueous saturated ammonium chloride solution was added to the reaction mixture, $CHCl_3$ was added. The organic layer was separated, washed with water and brine, dried and concentrated *in vacuo*. The resulting residue was triturated with diethyl ether and filtrated. The crystals obtained were recrystallized from THF to give 120 mg (14%) of 4-methoxy-3-phenyl-5-[1-pyridin-4-ylmeth-(*Z*)-ylidene]-1,5-dihydropyrrol-2-one (**16**) as crystals. mp 232—234 °C (dec.).

To a solution of **16** (120 mg, 0.43 mmol) in THF (20 ml) was added 4 N HCl in dioxane (0.15 ml) and the mixture was stirred for 10 min. The reaction mixture was then concentrated *in vacuo*. The resulting residue was triturated with diethyl ether and filtrated to afford **4a** (50 mg, 37%) as a solid. mp 227—228 °C (dec.). ^{1}H-NMR (400 MHz, DMSO-d_6) δ: 3.78 (3H, s), 6.47 (1H, s), 7.35—7.55 (5H, m), 8.11 (2H, d, J=6.8 Hz), 8.81 (2H, d, J=6.8 Hz), 10.72 (1H, s). IR (ATR) cm^{-1}: 3354, 3046, 2742, 2056, 1702, 1620, 1596, 1493, 1348, 1193, 1019, 870, 803, 698, 522. MS (APCI): 279 $[M+H]^+$. HR-MS-ESI Calcd for $C_{17}H_{15}N_2O_2$ ($[M+H]^+$): 279.1134. Found: 279.1126.

{3-[3-Methoxy-5-oxo-4-phenyl-1,5-dihydropyrrol-(2*Z*)-ylidenemethyl]phenoxy}acetic Acid (4b) 0.5 M Potassium hexamethyldisilazide in toluene (72 ml, 36 mmol) was slowly added to a solution of **15** (7.0 g, 24 mmol) in THF (200 ml) at −78 °C and the reaction mixture was stirred at −5 °C for 30 min. 3-Methoxymethoxybenzaldehyde (3.7 g, 22.3 mmol) was added slowly to the reaction mixture at −70 °C and the mixture was stirred at 0 °C for 2 h and at room temperature for 2 h. After aqueous saturated ammonium chloride solution was added to the reaction mixture, ethyl acetate was added. The organic layer was separated, dried and concentrated *in vacuo*. The resulting residue was triturated with diethyl ether and filtrated. The crystals obtained were recrystallized from ethyl acetate to give 3.3 g (44%) of 4-methoxy-5-[1-(3-methoxymethoxyphenyl)meth-(*Z*)-ylidene]-3-phenyl-1,5-dihydropyrrol-2-one (**17**) as crystals. mp 170—171 °C. ^{1}H-NMR (400 MHz, DMSO-d_6) δ: 3.39 (3H, s), 3.73 (3H, s), 5.27 (2H, s), 6.28 (1H, s), 6.96 (1H, dd, J=7.9, 2.3 Hz), 7.21 (1H, s), 7.24 (1H, d, J=7.9 Hz), 7.30 (1H, d, J=7.9 Hz), 7.32—7.47 (3H, m), 7.52—7.56 (2H, m), 10.09 (1H, s). IR (ATR) cm^{-1}: 3174, 1674, 1656, 1626, 1594, 1349, 1224, 1149, 1009, 780, 682, 647, 448. MS (APCI): 338 $[M+H]^+$.

To a **17** (2.8 g, 8.3 mmol) solution in THF (100 ml) was added 6 N aqueous HCl solution (10 ml) and the mixture was stirred at 50 °C for 2 h. After water and ethyl acetate were added to the mixture, the organic layer was separated, dried and concentrated *in vacuo*. The resulting residue was triturated with diethyl ether and filtrated to give 2.1 g (86%) of 5-[1-(3-hydroxyphenyl)meth-(*Z*)-ylidene]-4-methoxy-3-phenyl-1,5-dihydropyrrol-2-one (**18**) as a solid. mp 188—189 °C. ^{1}H-NMR (400 MHz, DMSO-d_6) δ: 3.73 (3H, s), 6.22 (1H, s), 6.72 (1H, dd, J=8.0, 2.3 Hz), 6.94 (1H, s), 7.06 (1H, d, J=8.0 Hz), 7.19 (1H, t, J=8.0 Hz), 7.32—7.46 (3H, m), 7.50—7.57 (2H, m), 9.46 (1H, s), 9.94 (1H, s). IR (ATR) cm^{-1}: 3314, 1656, 1594, 1451, 1361, 1231, 965, 738, 639. MS (APCI): 294 $[M+H]^+$.

60% Sodium hydride in oil (300 mg, 7.5 mmol) was slowly added to a solution of **18** (1.8 g, 6.1 mmol) in DMF (50 ml). The mixture was stirred at room temperature for 1 h and bromoacetic acid *tert*-butyl ester (2.3 g, 11.8 mmol) was added. After the addition, the mixture was further stirred at room temperature for 16 h. Water and ethyl acetate were added to the mixture and the organic layer was separated. The organic layer was washed with water, dried and concentrated *in vacuo*. The resulting residue was chromatographed on silica gel to give 1.2 g (43%) of {3-[3-methoxy-5-oxo-4-phenyl-1,5-dihydropyrrol-(2*Z*)-ylidenemethyl]phenoxy}acetic acid *tert*-butyl ester (**19**) as a solid. mp 167—168 °C. ^{1}H-NMR (200 MHz, $CDCl_3$) δ: 1.49 (9H, s), 3.78 (3H, s), 4.53 (2H, s), 6.34 (1H, s), 6.83 (1H, dd, J=8.2, 2.5 Hz), 6.95 (1H, br), 7.05 (1H, d, J=7.7 Hz), 7.32 (1H, d, J=8.2 Hz), 7.34—7.56 (5H, m), 7.71 (1H, br).

Trifluoroacetic acid (1 ml) was added to a solution of **19** (900 mg, 2.21 mmol) in CH_2Cl_2 (1 ml) and the reaction mixture was stirred at room temperature for 2 h. After the mixture was concentrated *in vacuo*, the resulting residue was triturated with diethyl ether and filtrated to give 720 mg (93%) of **4b** as a solid. mp 224—226 °C. ^{1}H-NMR (400 MHz, DMSO-d_6) δ: 3.73 (3H, s), 4.79 (2H, s), 6.29 (1H, s), 6.86 (1H, dd, J=8.2, 2.3 Hz), 7.09 (1H, s), 7.20 (1H, d, J=7.9 Hz), 7.30 (1H, t, J=7.9 Hz), 7.33—7.39 (1H, m), 7.43 (2H, t, J=7.0 Hz), 7.54 (2H, d, J=7.0 Hz), 10.14 (1H, s). IR (ATR) cm^{-1}: 3240, 2861, 2503, 1884, 1702, 1658, 1618, 1590, 1299, 1228, 973, 655, 506. MS (APCI): 352 $[M+H]^+$. HR-MS-ESI Calcd for $C_{20}H_{18}NO_5$ ($[M+H]^+$): 352.1185. Found: 352.1169. *Anal.* Calcd for $C_{20}H_{17}NO_5$: C, 68.37; H, 4.88; N, 3.99. Found: C, 68.28; H, 4.77; N, 4.05.

3,4-Diphenyl-1,5-dihydropyrrol-2-one (6) Triethylamine (150 ml, 1.08 mol) was slowly added to a suspension of *N*-(2-oxo-2-phenylethyl)-2-phenylacetamide **20** (32.4 g, 128 mmol) in acetic anhydride (150 ml) at 0 °C. After the reaction mixture was stirred at room temperature for 13 h, the mixture was concentrated *in vacuo*. Water and ethyl acetate were added to the residue, and the organic layer was separated, washed with aqueous saturated citric acid solution, aqueous saturated $NaHCO_3$ solution and brine, dried and concentrated *in vacuo*. Toluene (100 ml) was then added to the resulting residue, and the mixture was concentrated *in vacuo*. The residue was triturated with diethyl ether and filtrated to give 28.2 g (80%) of 1-acetyl-3,4-diphenyl-1,5-dihydropyrrol-2-one (**21**) as a solid. mp 154—155 °C. ^{1}H-NMR (400 MHz, DMSO-d_6) δ: 2.51 (3H, s), 4.81 (2H, s), 7.25—7.45 (10H, m). IR (ATR) cm^{-1}: 3059, 1707, 1688, 1639, 1371, 1333, 1291, 691. MS (APCI): 278 $[M+H]^+$.

28% Sodium methoxide in MeOH (13.6 ml, 65.1 mmol) was slowly added to a suspension of **21** (17.19 g, 62 mmol) in MeOH (400 ml) at 0 °C and the reaction mixture was stirred at the same temperature for 1 h. After slow addition of acetic acid (3.71 ml, 65.1 mmol) to the mixture, the mixture was concentrated *in vacuo*. Water and CH_2Cl_2 were added to the residue, and the organic layer was separated, washed with brine, dried and concentrated *in vacuo*. The resulting residue was triturated with diethyl ether and filtrated to give 14.01 g (96%) of **6** as a solid. mp 184—185 °C. ^{1}H-NMR (400 MHz, DMSO-d_6) δ: 4.37 (2H, s), 7.25—7.40 (10H, m), 8.52 (1H, s). IR (ATR) cm^{-1}: 3189, 3059, 1681, 1444, 1368, 762, 743, 702, 689. MS (APCI): 236 $[M+H]^+$. HR-MS-ESI Calcd for $C_{16}H_{14}NO$ ($[M+H]^+$): 236.1075. Found: 236.1067.

2-Oxo-3,4-dipenyl-2,5-dihydropyrrole-1-carboxylic Acid *tert*-Butylester (22) $(Boc)_2O$ (12.44 g, 57 mmol) and *N*,*N*-dimethyl-4-aminopyridine (348 mg, 2.85 mmol) were added to a suspension of **6** (6.71 g, 28.5 mol) in acetonitrile (300 ml) at 0 °C and the mixture was stirred at room temperature for 3 h. Water and ethyl acetate were then added to the mixture, and the organic layer was separated. The organic layer was washed with brine, dried and concentrated *in vacuo*. The resulting residue was chromatographed on silica gel to give 2.44 g (26%) of **22** as a solid. mp 144—146 °C. ^{1}H-NMR (400 MHz, DMSO-d_6) δ: 1.52 (9H, s), 4.78 (2H, s), 7.22—

7.27 (2H, m), 7.32—7.43 (8H, m). IR (ATR) cm^{-1}: 2978, 1764, 1349, 1297, 1160, 913, 691. MS (APCI): 336 $[M+H]^+$. *Anal.* Calcd for $C_{21}H_{21}NO_3$: C, 75.20; H, 6.31; N, 4.18. Found: C, 74.90; H, 6.29; N, 3.99.

3,4-Diphenyl-5-[1-phenylmeth-(*Z*)-ylidene]-1,5-dihydropyrrol-2-one (5a) 0.5 M Potassium hexamethyldisilazide in toluene (7.2 ml, 3.6 mmol) was slowly added to a solution of **21** (500 mg, 1.8 mmol) and benzaldehyde (0.27 ml, 2.7 mmol) in THF (20 ml) at −78 °C and the reaction mixture was stirred at −78 °C for 30 min and at room temperature for 12 h. After aqueous saturated ammonium chloride solution was added to the reaction mixture, ethyl acetate was added. The organic layer was separated, dried and concentrated *in vacuo*. The resulting residue was triturated with diisopropyl ether and filtrated. The crystals obtained were recrystallized from ethyl acetate, THF and diisopropyl ether to give 156 mg (27%) of **5a** as crystals. mp 243—244 °C. ^{1}H-NMR (400 MHz, DMSO-d_6) δ: 5.84 (1H, s), 7.23—7.34 (8H, m), 7.38 (2H, t, J=7.5 Hz), 7.44—7.49 (3H, m), 7.54 (2H, d, J=7.5 Hz), 10.63 (1H, s). IR (ATR) cm^{-1}: 3217, 1682, 1635, 1222, 795, 730, 683, 641, 517. MS (APCI): 324 $[M+H]^+$. HR-MS-ESI Calcd for $C_{23}H_{18}NO$ ($[M+H]^+$): 324.1388. Found: 324.1379.

3,4-Diphenyl-5-[1-pyridin-4-ylmeth-(*Z*)-ylidene]-1,5-dihydropyrrol-2-one (5b) This compound, prepared from **22** and 4-pyridinecaeboxaldehyde as described in the synthesis of **16**, was obtained as crystals. Yield 46%. mp 236—237 °C (dec.). ^{1}H-NMR (400 MHz, DMSO-d_6) δ: 5.78 (1H, s), 7.25—7.35 (7H, m), 7.45—7.52 (5H, m), 8.54 (2H, d, J=6.1 Hz), 10.80 (1H, s). IR (ATR) cm^{-1}: 3199, 3056, 1699, 1636, 1598, 1440, 1207, 692, 639, 481. MS (APCI): 325 $[M+H]^+$. HR-MS-ESI Calcd for $C_{22}H_{17}N_2O$ ($[M+H]^+$): 325.1341. Found: 325.1346.

3,4-Diphenyl-5-[1-pyridin-3-ylmeth-(*Z*)-ylidene]-1,5-dihydropyrrol-2-one (5c) This compound, prepared from **22** and 3-pyridinecaeboxaldehyde as described in the synthesis of **16**, was obtained as crystals. Yield 29%. mp 239—240 °C. ^{1}H-NMR (400 MHz, DMSO-d_6) δ: 5.86 (1H, s), 7.23—7.37 (7H, m), 7.40 (1H, dd, J=7.5, 4.7 Hz), 7.43—7.52 (3H, m), 8.00 (1H, d, J=8.2 Hz), 8.46 (1H, d, J=4.1 Hz), 8.66 (1H, s), 10.77 (1H, s). IR (ATR) cm^{-1}: 3199, 1677, 1442, 1217, 796, 731, 691, 515. MS (APCI): 325 $[M+H]^+$. HR-MS-ESI Calcd for $C_{22}H_{17}N_2O$ ($[M+H]^+$): 325.1341. Found: 325.1339.

5-[1-(3-Hydroxyphenyl)meth-(*Z*)-ylidene]-3,4-diphenyl-1,5-dihydropyrrol-2-one (5d) This compound, prepared from **22** and 3-methoxymethoxybenzaldehyde as described in the synthesis of **18**, was obtained as a solid. Yield 38%. mp 226—227 °C. ^{1}H-NMR (400 MHz, DMSO-d_6) δ: 5.75 (1H, s), 6.71 (1H, dd, J=7.9, 2.1 Hz), 6.86 (1H, s), 6.99 (1H, d, J=7.9 Hz), 7.18 (1H, t, J=7.9 Hz), 7.22—7.34 (7H, m), 7.43—7.49 (3H, m), 9.48 (1H, s), 10.51 (1H, s). IR (ATR) cm^{-1}: 3419, 3259, 3061, 1683, 1578, 1216, 727, 683, 642. MS (APCI): 340 $[M+H]^+$. HR-MS-ESI Calcd for $C_{23}H_{18}NO_2$ ($[M+H]^+$): 340.1338. Found: 340.1324.

5-[1-(3-Dimethoxymethylphenyl)meth-(*Z*)-ylidene]-3,4-diphenyl-1,5-dihydropyrrol-2-one (5e) This compound, prepared from **22** and 3-dimethoxymethylbenzaldehyde as described in the synthesis of **16**, was obtained as crystals. Yield 55%. mp 235—236 °C. ^{1}H-NMR (400 MHz, DMSO-d_6) δ: 3.25 (6H, s), 5.39 (1H, s), 5.85 (1H, s), 7.23—7.35 (8H, m), 7.40 (1H, t, J=7.8 Hz), 7.44—7.50 (4H, m), 7.57 (1H, d, J=7.8 Hz), 10.67 (1H, s). IR (ATR) cm^{-1}: 3209, 2948, 1682, 1361, 1049, 794, 734, 693, 642. MS (APCI): 398 $[M+H]^+$. HR-MS-ESI Calcd for $C_{26}H_{24}NO_3$ ($[M+H]^+$): 398.1756. Found: 398.1737. *Anal.* Calcd for $C_{26}H_{23}NO_3$: C, 78.57; H, 5.83; N, 3.52. Found: C, 78.55; H, 5.94; N, 3.62.

{3-[5-Oxo-3,4-diphenyl-1,5-dihydropyrrol-(2*Z*)-ylidenemethyl]phenoxy}acetic Acid Sodium Salt (5f) Following the synthetic method of **4b**, {3-[5-oxo-3,4-diphenyl-1,5-dihydropyrrol-(2*Z*)-ylidenemethyl]phenoxy}acetic acid (free form) was prepared from **5d** as crystals. Yield 61%. mp 247—248 °C. To a 397.5 mg (1 mmol) of free form solution in MeOH (10 ml) was added 2 N NaOH aqueous solution (0.5 ml). After the reaction mixture was stirred for 10 min at room temperature, the mixture was concentrated *in vacuo*. The resulting residue was triturated with diethyl ether and filtrated to give 419 mg (quant.) of **5f** as a solid. mp >320 °C. ^{1}H-NMR (400 MHz, DMSO-d_6) δ: 4.16 (2H, s), 5.78 (1H, s), 6.76 (1H, dd, J=8.1, 2.2 Hz), 6.90 (1H, s), 7.02 (1H, d, J=7.7 Hz), 7.21 (1H, t, J=8.1 Hz), 7.23—7.35 (7H, m), 7.42—7.49 (3H, m), 10.59 (1H, s). IR (ATR) cm^{-1}: 3242, 1690, 1601, 1426, 1219, 687. MS (APCI): 396 $[M-Na]^-$. HR-MS-ESI Calcd for $C_{25}H_{20}NO_4$ ($[M+H]^+$): 398.1392. Found: 398.1396.

{4-[5-Oxo-3,4-diphenyl-1,5-dihydropyrrol-(2*Z*)-ylidenemethyl]phenoxy}acetic Acid Sodium Salt (5g) This compound, prepared from **22** and 4-formylphenoxyacetic acid *tert*-butyl ester as described in the synthesis of **16** and **5f**, was obtained as a solid. Yield 46%. mp 270—275 °C (dec.). ^{1}H-NMR (400 MHz, DMSO-d_6) δ: 4.09 (2H, s), 5.78 (1H, s), 6.78 (2H, d, J=8.7 Hz), 7.21—7.32 (7H, m), 7.42—7.49 (5H, m), 10.53 (1H, s). IR (ATR) cm^{-1}: 3163, 1672, 1583, 1421, 1246, 1181, 1044, 691. MS (APCI): 396 $[M-Na]^-$. HR-MS-ESI Calcd for $C_{25}H_{20}NO_4$ ($[M+H]^+$): 398.1392. Found: 398.1392.

{2-[5-Oxo-3,4-diphenyl-1,5-dihydropyrrol-(2*Z*)-ylidenemethyl]phenoxy}acetic Acid Sodium Salt (5h) This compound, prepared from **22** and 2-formylphenoxyacetic acid *tert*-butyl ester as described in the synthesis of **16** and **5f**, was obtained as a solid. Yield 67%. mp 357—360 °C (dec.). ^{1}H-NMR (400 MHz, DMSO-d_6) δ: 4.52 (2H, s), 5.92 (1H, s), 6.86—6.94 (2H, m), 7.17—7.35 (9H, m), 7.40—7.47 (3H, m), 11.66 (1H, s). IR (ATR) cm^{-1}: 3196, 1683, 1624, 1576, 1495, 1442, 798, 744, 731, 695, 639. MS (APCI): 396 $[M-Na]^-$. HR-MS-ESI Calcd for $C_{25}H_{20}NO_4$ ($[M+H]^+$): 398.1392. Found: 398.1377.

{3-[3-Furan-2-yl-5-oxo-4-phenyl-1,5-dihydropyrrol-(2*Z*)-ylidenemethyl]phenoxy}acetic Acid (5i) This compound, prepared from 4-(2-furyl)-3-phenyl-1,5-dihydropyrrol-2-one **27**[24] and 3-formylphenoxyacetic acid *tert*-butyl ester as described in the synthesis of **5j**, was obtained as a solid. Yield 60%. mp 214—216 °C. ^{1}H-NMR (400 MHz, DMSO-d_6) δ: 4.80 (2H, s), 6.45 (1H, s), 6.68 (1H, dd, J=3.4, 1.8 Hz), 6.77 (1H, d, J=3.4 Hz), 6.90 (1H, dd, J=8.1, 2.4 Hz), 7.10—7.45 (8H, m), 7.82 (1H, d, J=1.8 Hz), 10.69 (1H, s). IR (ATR) cm^{-1}: 3237, 2504, 1702, 1641, 1300, 682. MS (APCI): 388 $[M+H]^+$. HR-MS-ESI Calcd for $C_{23}H_{18}NO_5$ ($[M+H]^+$): 388.1185. Found: 388.1184.

{3-[5-Oxo-4-phenyl-3-thiophen-2-yl-1,5-dihydropyrrol-(2*Z*)-ylidenemethyl]phenoxy}acetic Acid (5j) 1,1′-Carbonyldiimidazole (12.16 g, 75 mmol) was added to a suspension of phenylacetic acid (9.72 g, 71.4 mmol) in CH_2Cl_2 (70 ml) at room temperature and the mixture was stirred for 30 min. Triethylamine (11.94 ml, 85.68 mmol) and 2-amino-1-(2-thiophenyl)ethanone hydrochloride **28** (12.68 g, 71.4 mmol) were then added to the mixture at 0 °C. After the reaction mixture was stirred at room temperature for 14 h, aqueous 5% citric acid solution was added. The organic layer was separated, washed with aqueous saturated $NaHCO_3$ solution and brine, dried and concentrated *in vacuo*. The residue was triturated with EtOH and filtrated to give 12.34 g (67%) of *N*-[2-oxo-2-(2-thiophenyl)-ethyl]-2-phenylacetamide **29** as a solid. mp 90—92 °C. ^{1}H-NMR (400 MHz, DMSO-d_6) δ: 3.53 (2H, s), 4.54 (2H, d, J=5.6 Hz), 7.19—7.34 (6H, m), 8.04 (2H, d, J=4.6 Hz), 8.48 (1H, t, J=5.6 Hz). IR (ATR) cm^{-1}: 3380, 3097, 1650, 1512, 1415, 1236, 723, 513, 478. MS (APCI): 260 $[M+H]^+$. *Anal.* Calcd for $C_{14}H_{13}NO_2S$: C, 64.84; H, 5.05; N, 5.40; S, 12.37. Found: C, 64.46; H, 5.00; N, 5.36; S, 12.31.

95% Sodium methoxide (6.55 g, 115.2 mmol) was added to **29** (14.94 g, 57.6 mmol) in EtOH (50 ml) and the mixture was stirred at reflux for 30 min. After slow addition of acetic acid (6.57 ml, 115.2 mmol) to the mixture at 0 °C, the mixture was concentrated *in vacuo*. Water and CH_2Cl_2 were then added to the residue, and the organic layer was separated, washed with brine, dried and concentrated *in vacuo*. The resulting residue was triturated with MeOH and filtrated to give 7.91 g (57%) of 3-phenyl-4-(2-thiophenyl)-1,5-dihydropyrrol-2-one **30** as a solid. mp 224—227 °C. ^{1}H-NMR (400 MHz, DMSO-d_6) δ: 4.42 (2H, s), 7.07 (1H, dd, J=5.1, 3.8 Hz), 7.29—7.36 (3H, m), 7.38—7.48 (3H, m), 7.56 (1H, d, J=5.1 Hz), 8.38 (1H, s). IR (ATR) cm^{-1}: 3170, 3050, 1677, 725, 697. MS (APCI): 242 $[M+H]^+$.

A solution of NaOH (260 mg, 6.5 mmol) in water (1 ml) was added to a suspension of **30** (145 mg, 0.6 mmol) and 3-formylphenoxyacetic acid *tert*-butyl ester (118 mg, 0.5 mmol) in MeOH (2.5 ml). After stirring the mixture at room temperature for 16 h, 2 N HCl aqueous solution (3.25 ml) was added, and the reaction mixture was concentrated *in vacuo*. Water and $CHCl_3$ were then added to the residue. The organic layer was separated, washed with aqueous 5% citric acid solution, dried and concentrated *in vacuo*. The residue was triturated with MeOH and filtrated to give 175 mg (87%) of **5j** as a solid. mp 261—263 °C. ^{1}H-NMR (400 MHz, DMSO-d_6) δ: 4.79 (2H, s), 6.17 (1H, s), 6.88 (1H, dd, J=8.2, 2.3 Hz), 7.08 (1H, s), 7.16 (1H, d, J=7.6 Hz), 7.18—7.38 (8H, m), 7.75 (1H, d, J=4.9 Hz), 10.75 (1H, s). IR (ATR) cm^{-1}: 3218, 2499, 1890, 1705, 1646, 1622, 1307, 793, 687, 652, 442. MS (APCI): 404 $[M+H]^+$. HR-MS-ESI Calcd for $C_{23}H_{18}NO_4S$ ($[M+H]^+$): 404.0957. Found: 404.0960. *Anal.* Calcd for $C_{23}H_{17}NO_4S$: C, 68.47; H, 4.25; N, 3.47; S, 7.95. Found: C, 68.17; H, 4.33; N, 3.67.

Inhibitory Effect on the Reaction of Human PAI-1 with t-PA The experiment was performed according to the method of Keijer *et al.*[25] Inhibition of the interaction between t-PA and PAI-1 was determined by measuring residual t-PA activity. Twenty-five microliters of t-PA solution (final conc. 0.2 nM) and 25 μl of assay buffer or PAI-1 solution (PAI-1 was added so as to inhibit t-PA activity by 75% in the absence of test compound) were mixed and incubated with 2.5 μl of a test compound solution (dissolved in DMSO) at 25 °C for 15 min. Two hundred microliters of a chromogenic substrate solution (S-2288: H-D-iie-Pro-Arg-pNA) was then added to the mixture, and

the whole was incubated at 37 °C. The increase in absorbance at 405 nm was continuously (every 5 min) monitored for 2 h with a microplatereader and DA/D min was calculated. The IC_{50} value was estimated as the concentration that inhibits PAI-1 activity by 50%.

Antithrombotic Activity of 5f (T-1776Na) in a Rat Model of Arterial Thrombosis Thrombus was induced in the abdominal aorta by electrical stimulation.[26] On the day before the experiment, a catheter-type platinum electrode was implanted into the abdominal aorta through the left femoral artery under Nembutal (Abbot, 50 mg/kg, ip) anesthesia. After the end of the electrode was exteriorized at the back of the neck, a plate-type silver–silver chloride was implanted subcutaneously at the back of the neck at the opposite pole. After suturing the incisions, an electric cell and a variable resistance were placed on the back, each secured by adhesive tape, to act as a power source and fine-tuning the current, respectively. The rats were allowed to recover overnight in individual cages. In the experiment, each electrode was connected to the electric cell and a direct current (200 μA) was applied continuously for 4 h. Test compound or its vehicle was intravenously infused at the dose indicated during electrical stimulation. After current application was stopped, the rats were anesthetized again, and the thrombus formed was removed and its wet weight was measured with an electronic balance. Results are expressed as the means±S.E. of 10 rats.

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