Bioorganic & Medicinal Chemistry Letters 20 (2010) 546–548

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry Letters

journal homepage: www.elsevier.com/locate/bmcl

Evaluation of pyrrolin-2-one derivatives synthesized by a new practical method as inhibitors of plasminogen activator inhibitor-1 (PAI-1)

Hiroshi Miyazaki [a,*], Tsutomu Miyake [a], Yoshihiro Terakawa [a], Hiroshi Ohmizu [a], Tsuyoshi Ogiku [a], Akio Ohtani [b]

[a] Medicinal Chemistry Laboratory, Mitsubishi Tanabe Pharma Co., Ltd, 3-16-89, Kashima, Yodogawa, Osaka 532-8505, Japan
[b] Pharmacology Laboratory, Mitsubishi Tanabe Pharma Co., Ltd, 2-2-50, Kawagishi, Toda, Saitama 335-8505, Japan

ARTICLE INFO

Article history:
Received 1 September 2009
Revised 14 November 2009
Accepted 19 November 2009
Available online 23 November 2009

Keywords:
Plasminogen activator inhibitor-1
PAI-1 inhibitor
Antithrombotic agent
Pyrrolin-2-one

ABSTRACT

We describe in this Letter a new synthetic method for pyrrolin-2-ones as potent plasminogen activator inhibitor-1 (PAI-1) inhibitors. Pyrrolin-2-one derivatives synthesized from *N*-2-oxoethylamides and aldehydes in aqueous NaOH by one-pot were evaluated for their PAI-1 inhibitory activity. Among these derivatives, compounds **16** and **18** were found to possess potent PAI-1 inhibitory activity (compound **16**: IC_{50}: 0.69 μM, compound **18**: IC_{50}: 0.65 μM).

It is well known that plasminogen activator inhibitor-1 (PAI-1) is a specific inhibitor of both tissue-type plasminogen activator and urokinase-type plasminogen activator, and that PAI-1 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] and unstable angina.[3] Furthermore, a number of animal studies have shown that PAI-1 is the factor which disturbs fibrinolytic activity in both thrombotic and prethrombotic states.[4] Thus, inhibition of PAI-1 activity or reduction of its production is considered to shift the balance between thrombogenesis and thrombolysis towards thrombolysis. In fact, an antibody against PAI-1 has been shown to enhance clot lysis and decrease thrombus growth in animal models of venous thrombosis[5] and arterial thrombosis.[6] Recently, a number of small molecules have been evaluated for their ability to inhibit PAI-1 or reduce its production and for their potential to act as antithrombotic agents.[7]

We have previously reported the structure–activity relationship (SAR) of a series of pyrrolin-2-one derivatives for PAI-1 inhibition (Fig. 1)[8] and showed that compound **1**(T-1776Na) exhibited potent inhibitory activity for PAI-1 (IC_{50}: 9.6 μM), good antithrombotic efficacy in an animal thrombosis model, and high selectivity for PAI-1 over serine proteases and serpins. In the course of our search for a superior antithrombotic agent, we have found a facile new synthetic method for the pyrrolin-2-ones leading to a series of PAI-1 inhibitors. Herein, we disclose the development of this new synthetic method and evaluate the synthesized pyrrolin-2-ones for their inhibitory activity towards PAI-1.

The conventional synthetic route of **1**(T-1776Na) has some drawbacks (Scheme 1). Indeed, construction of the pyrrolin-2-one ring from the *N*-2-oxoethylamide **2** under basic conditions produces in some cases side reactions. In addition, stability of the pyrrolin-2-ones under preparation conditions remarkably changes depending on the chemical properties of 3,4-diaryl group, and a dimmer or the maleimide **3** are known to be obtained as major by-products.[9] Therefore, the synthesis of **1**(T-1776Na) requires construction of a pyrrolin-2-one ring from a phenacylamide under acidic conditions (Ac_2O) followed by Boc protection to introduce the phenylmethylidene part, and a total of eight steps are needed.[8] In order to overcome these problems, we anticipated that anion intermediates, generated in situ during construction of the pyrrolin-2-one ring under basic conditions, could be trapped with an aldehyde to directly afford the desired PAI-1 inhibitors.

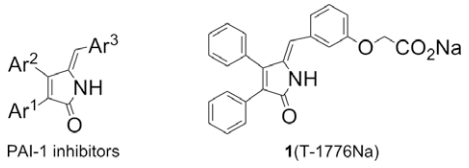
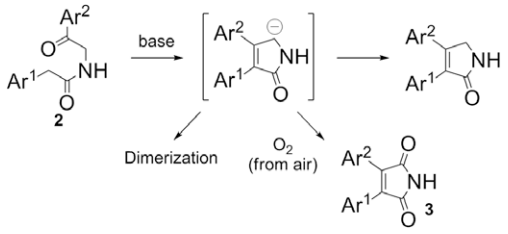


Figure 1. Structures of pyrrolin-2-one derivatives as PAI-1 inhibitors and their representative compound **1**(T-1776Na).

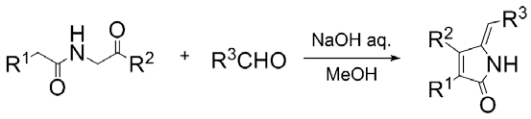
* Corresponding author. Tel.: +81 6 6300 2578; fax: +81 6 6300 2564.
E-mail address: miyazaki.hiroshi@me.mt-pharma (H. Miyazaki).

0960-894X/$ - see front matter © 2009 Elsevier Ltd. All rights reserved.
doi:10.1016/j.bmcl.2009.11.102



Scheme 1. Synthesis of pyrrolin-2-ones under basic conditions.

Table 1
Reaction of *N*-2-oxoethylamides with aldehydes[a]



R^3= 3-(*t*-BuO_2CCH_2O)Ph: **4**[b]
5-(HO_2C)furan-2-yl: **5**
5-[(*E*)-NCCH=CH]furan-2-yl: **6**
5-[(*E*)-EtO_2CCH=CH]furan-2-yl: **7**[b]
5-Methylfuran-2-yl: **8**

Entry	R^1	R^2	R^3	Temp (°C)	Yield[c] (%)
1	Ph	Ph	Ph	50	98 (**9**)
2[b]	Ph	Ph	**4**	rt	80 (**1**)
3	Ph	2-Furyl	2-Furyl	rt	93 (**10**)
4	Ph	2-Furyl	**5**	rt	71 (**11**)
5	Ph	2-Furyl	**6**	rt	83 (**12**)
6[b]	Ph	2-Furyl	**7**	rt	77 (**13**)
7	3,4-$(MeO)_2$Ph	2-Furyl	4-Pyridyl	50	30 (**14**)
8[b]	3,4-$(MeO)_2$Ph	2-Furyl	**7**	rt	70 (**15**)
9[b]	3,4-$(MeO)_2$Ph	2-Thienyl	**7**	rt	69 (**16**)
10	Ph	2-MeOPh	**8**	rt	72 (**17**)
11[b]	Ph	2-MeOPh	**7**	rt	83 (**18**)

[a] Reactions were carried using *N*-2-oxoethylamide, aldehyde (1.0 equiv) and 2 N NaOH aq (10 eqiuv) in MeOH.
[b] Products were obtained as the corresponding carboxylic acid instead of the ester residue.
[c] Numbers of reaction products are shown in parentheses.

The products of various reactions of *N*-2-oxoethylamides with aldehydes in aqueous NaOH using MeOH as a solvent are shown in Table 1. First, we examined the case where all substituents (R^1–R^3) were phenyl. An anion intermediate of the pyrrolin-2-one could be trapped with benzaldehyde, and only the *Z*-product was obtained in 98% by one-pot synthesis (entry 1).[10] Next, we applied this reaction to **1**(T-1776Na). In this case, a *Z*-product was also easily obtained as a carboxylic acid by acidification of the reaction mixture and filtration in 80% yield (entry 2). This *Z*-product could be quantitatively converted to **1**(T-1776Na). The reaction could also be used in the case where R^1–R^3 were substituted or nonsubstituted heteroaromatic rings (entries 3–11). In these cases, the *Z*-products were easily obtained as crystals or solids by acidification, (in some cases, extraction), and filtration. In all cases analytically pure compounds were obtained in good yield without further purification (silica gel chromatography or recrystallization).[11]

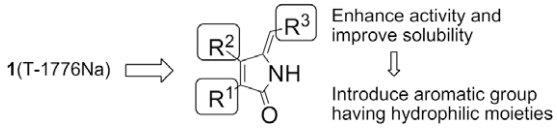
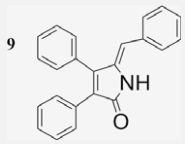
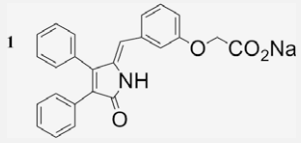
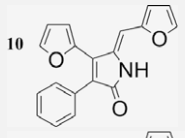
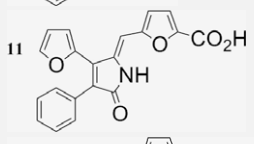
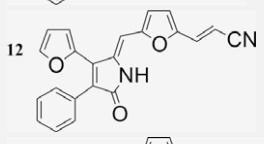
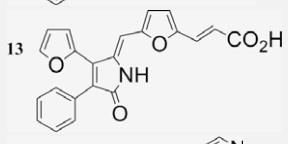
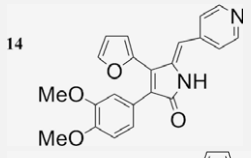
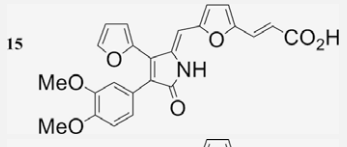
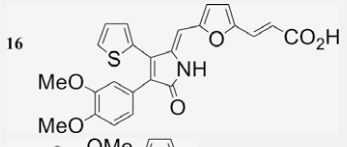
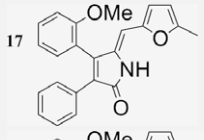
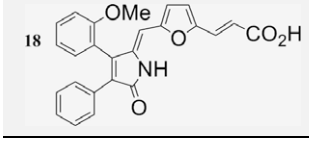


Figure 2. Optimization of substituents in the pyrrolin-2-one.

Thus, anion intermediates could be trapped with an aldehyde, which led us to achieve simple and practical synthesis of various pyrrolin-2-ones as PAI-1 inhibitors. The pyrrolin-2-one derivatives in entries 2–11 were designed to possess hydrophilic moieties at R^1–R^3, and make up for the scarce solubility of the pyrrolin-2-one scaffold (Fig. 2). These compounds are expected to have good potential as antithrombotic agents.

Table 2
Inhibitory effect of pyrrolin-2-one derivatives against human PAI-1 activity

Compound	Inhibitory activity
9 	No effect at 15 μM
1 	IC_{50}: 9.6 μM
10 	36% inhibition at 10 μM
11 	34% inhibition at 15 μM
12 	IC_{50}: 1.2 μM
13 	IC_{50}: 1.5 μM
14 	IC_{50}: 7.8 μM
15 	IC_{50}: 1.1 μM
16 	IC_{50}: 0.69 μM
17 	IC_{50}: 0.94 μM
18 	IC_{50}: 0.65 μM

Biologic evaluation of the synthesized pyrrolin-2-ones was performed by measuring the inhibitory effect on the reaction of human PAI-1 with t-PA, according to our previously published report[8] (Table 2). In that report, we showed that **9** was inactive against PAI-1 and that **1**(T-1776Na), having a phenoxy acetic acid group at R^3 of the pyrrolin-2-one, had potent PAI-1 inhibitory activity (IC_{50}: 9.6 μM). Various substituted pyrrolin-2-ones were next examined. First, a furan ring was adopted at R^2 of the pyrrolin-2-one as a hydrophilic equivalent to the benzene. Compounds **10**, **11**, having a furan or furan-2-carboxylic acid at R^3 showed weak PAI-1 inhibitory activity. However, in the case where the hydrophilic group (nitrile and carboxylic acid) was located far from the aromatic ring, the pyrrolin-2-ones exhibited good inhibitory activity against PAI-1 (**12**: IC_{50}: 1.2 μM, **13**: IC_{50}: 1.5 μM). Furthermore, to find PAI-1 inhibitors with decreased lipophilicity, 3,4-dimethoxyphenyl substituents were introduced at R^1. Although **14** did not inhibit PAI-1, **15** and **16** showed good PAI-1 inhibitory activity. We speculate that this is due to the hydrophilic moiety (carboxylic acid) in **15** and **16** being away from the aromatic ring in R^3. Finally, even when the 2-furyl or 2-thienyl at R^2 was converted into 2-methoxyphenyl, the inhibitory activity for PAI-1 was maintained. Compound **18** was found to be the most potent inhibitor of PAI-1 with an IC_{50} value of 0.65 μM.

In summary, we found a practical new synthetic method of pyrrolin-2-ones as potent PAI-1inhibitors. Using this new method, a series of pyrrolin-2-one derivatives were synthesized and their inhibitory activity for PAI-1 was evaluated. Some of the synthesized compounds were designed to possess a hydrophilic moiety at R^1–R^3, which led to the discovery of **16**[12] and **18**[13] with a PAI-1 inhibitory activity ten times more potent than that of **1**(T-1776Na). Further studies on these promising compounds are ongoing.

References and notes

1. Schneiderman, J.; Loskutoff, D. J. *Trends Cardiovasc. Med.* **1991**, *1*, 99.
2. (a) Nilsson, I. M.; Ljungner, H.; Tengborn, L. *Br. J. Med.* **1985**, *290*, 1453; (b) Wiman, B.; Ljungberg, S.; Chmielewska, J.; Urden, G.; Blomback, M.; Johnsson, H. *J. Lab. Clin. Med.* **1985**, *105*, 265; (c) Juhan-Vague, I.; Valadier, J.; Alessi, M. C.; Aillaud, M. F.; Ansaldi, J.; Philip-Joet, C.; Holvet, J.; Serradimigni, A.; Collen, D. *Thromb. Haemost.* **1987**, *57*, 67.
3. Wieczorek, I.; Ludlam, C. A.; Fox, K. A. A. *Am. J. Cardiol.* **1994**, *74*, 424.
4. (a) Krishnamurti, C.; Barr, C. F.; Hassett, M. A.; Young, G. D.; Alving, B. M. *Blood* **1987**, *69*, 798; (b) Reilly, C. F.; Fujita, T.; Mayer, E. F.; Siefried, M. E. *Arterioscler. Thromb.* **1991**, *11*, 1276; (c) Carmeliet, P.; Stassen, J. M.; Schoonjans, L.; Ream, B.; Van den Oord, J. J.; De Mol, M.; Mulligan, R. C.; Collen, D. *J. Clin. Invest.* **1993**, *92*, 2756.
5. Levi, M.; Biemond, B. J.; Van Zonnenveld, A. J.; Ten Cate, J. W.; Pannekoek, H. *Circulation* **1992**, *85*, 305.
6. Biemond, B. J.; Levi, M.; Coronel, R.; Janse, M. J.; Ten Cate, J. W.; Pannekoek, H. *Circulation* **1995**, *1*, 1175.
7. (a) Folkes, A.; Roe, M. B.; Sohal, S.; Golec, J.; Faint, R.; Brooks, T.; Charlton, P. *Bioorg. Med. Chem. Lett.* **2001**, *11*, 2589; (b) Folkes, A.; Brown, S. D.; Canne, L. E.; Chan, J.; Engelhardt, E.; Epshteyn, S.; Faint, R.; Golec, J.; Hanel, A.; Kearney, E.; Leahy, J. W.; Mac, M.; Matthews, D.; Prisbylla, M. P.; Sanderson, J.; Simon, R. J.; Tesfai, Z.; Vicker, N.; Wang, S.; Webb, R. R.; Charlton, P. *Bioorg. Med. Chem. Lett.* **2002**, *12*, 1063; (c) Wang, S.; Golec, J.; Miller, W.; Milutinovic, S.; Folkes, A.; Williams, S.; Brooks, T.; Hardman, K.; Charlton, P.; Wren, S.; Spencer, J. *Bioorg. Med. Chem. Lett.* **2002**, *12*, 2367; (d) De Nanteuil, G.; Lila-Ambroise, C.; Rupin, A.; Vallez, M. O.; Verbeuren, T. J. *Bioorg. Med. Chem. Lett.* **2003**, *13*, 1705; (e) Ye, B.; Bauer, S.; Buckman, B. O.; Ghannam, A.; Griedel, B. D.; Khim, S. K.; Lee, W.; Sacchi, K. L.; Shaw, K. J.; Liang, A.; Wu, Q.; Zhao, Z. *Bioorg. Med. Chem. Lett.* **2003**, *13*, 3361; (f) Ye, B.; Chou, Y. L.; Karanjawala, R.; Lee, W.; Lu, S. F.; Shaw, K. J.; Jones, S.; Lentz, D.; Liang, A.; Tseng, J. L.; Wu, Q.; Zhao, Z. *Bioorg. Med. Chem. Lett.* **2004**, *14*, 761; (g) Gopalsamy, A.; Kincaid, S. L.; Ellingboe, J. W.; Groeling, T. M.; Antrilli, T. M.; Krishnamurthy, G.; Aulabaugh, A.; Friedrichs, G. S.; Crandall, D. L. *Bioorg. Med. Chem. Lett.* **2004**, *14*, 3477; (h) Hu, B.; Jetter, J. W.; Wrobel, J. E.; Antrilli, T. M.; Bauer, J. S.; Di, L.; Polakowski, S.; Jain, U.; Crandall, D. L. *Bioorg. Med. Chem. Lett.* **2005**, *15*, 3514; (i) Elokdah, H.; Abou-Gharbia, M.; Hennan, J. K.; McFarlane, G.; Mugford, C. P.; Krishnamurthy, G.; Crandall, D. L. *J. Med. Chem.* **2004**, *47*, 3491; (j) Sai, H.; Ogiku, T.; Ohmizu, H.; Ohtani, A. *Chem. Pharm. Bull.* **2006**, *54*, 1686; (k) Miyazaki, H.; Ogiku, T.; Sai, H.; Ohmizu, H.; Murakami, J.; Ohtani, A.; Ohtani *Bioorg. Med. Chem. Lett.* **2008**, *18*, 6419.
8. Miyazaki, H.; Ogiku, T.; Sai, H.; Moritani, Y.; Ohtani, A.; Ohmizu, H. *Chem. Pharm. Bull.* **2009**, *57*, 979.
9. (a) Pal, M.; Swamy, N. K.; Hameed, P. S.; Padakanti, S.; Yeleswarapu, K. R. *Tetrahedron* **2004**, *60*, 3987; (b) Babu, P. R.; Balasubramanian, T. R. *Indian J. Chem., Sect. B* **1987**, *26B*, 63.
10. In the NOESY spectra ($CDCl_3$), the vinyl proton of compound **9** at δ 6.06 correlates with the protons of phenyl ring at R^2, while it does not correlate with the NH proton at δ 7.91. On the other hand, the NH proton correlates with the protons of phenyl ring at R^3. These data show the stereochemistry of compound **9** is *Z*.
11. *Typical procedure*: 2 N NaOH aq (2.5 ml) was added to a mixture of *N*-2-oxoethylamide (0.5 mmol) and aldehyde (0.5 mmol) in MeOH (5 ml) at room temperature (16–25 °C). After the reaction mixture was stirred for 14 h at room temperature, 2 N HCl aq (2.5 ml) was added at 0 °C. A precipitated solid was collected by filtration, washed with water and then MeOH, and dried to afford its products.
12. *Spectoscopic data of* **16**: ^{1}H NMR (400 MHz, DMSO-d_6) δ: 12.38 (br, 1H), 10.60 (s, 1H), 7.78 (t, *J* = 3.1 Hz, 1H), 7.52 (d, *J* = 15.9 Hz, 1H), 7.24 (d, *J* = 3.1 Hz, 2H), 7.19 (dd, *J* = 8.4, 1.5 Hz, 1H), 7.09 (d, *J* = 3.6 Hz, 1H), 6.99 (d, *J* = 3.6 Hz, 1H), 6.95 (d, *J* = 8.4 Hz, 1H), 6.88 (d, *J* = 1.5 Hz, 1H), 6.62 (d, *J* = 15.9 Hz, 1H), 5.99 (s, 1H), 3.76 (s, 3H), 3.51 (s, 3H). IR (ATR) cm^{-1}: 3279, 3103, 2835, 2479, 1681, 1637, 1600, 1256, 1146, 1024, 714, 625. MS (APCI): 450 $[M+H]^+$.
13. *Spectoscopic data of* **18**: ^{1}H NMR (400 MHz, DMSO-d_6) δ: 12.37 (br, 1H), 10.52 (s, 1H), 7.43–7.55 (m, 2H), 7.31–7.40 (m, 2H), 7.20–7.30 (m, 3H), 7.10–7.20 (m, 2H), 7.00–7.10 (m, 2H), 6.92 (d, *J* = 3.3 Hz, 1H), 6.61 (d, *J* = 15.9 Hz, 1H), 5.58 (s, 1H), 3.61 (s, 3H). IR (ATR) cm^{-1}: 3262, 3071, 2836, 1686, 1645, 1274, 1253, 1215, 1022, 983, 760, 713, 693, 639. Anal Calcd for $C_{25}H_{19}NO_5$: C, 72.63; H, 4.63; N, 3.39. Found: C, 72.35; H, 4.57; N, 3.40. MS (APCI): 414 $[M+H]^+$.