Available online at www.sciencedirect.com
SCIENCE DIRECT®
Bioorganic & Medicinal Chemistry Letters
Bioorganic & Medicinal Chemistry Letters 16 (2006) 2034–2036

Small molecule inhibitors of plasma kallikrein

Wendy B. Young,* Roopa Rai, William D. Shrader, Jana Burgess-Henry, Huiyong Hu, Kyle C. Elrod, Paul A. Sprengeler, Bradley A. Katz, Juthamas Sukbuntherng and Joyce Mordenti

Celera Genomics, 180 Kimball Way, South San Francisco, CA 94080, USA

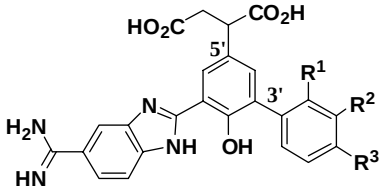
Received 10 November 2005; revised 13 December 2005; accepted 15 December 2005
Available online 18 January 2006

Abstract—Plasma kallikrein is a serine protease that is involved in pathways of inflammation, complement fixation, coagulation, and fibrinolysis. Herein, we describe the SAR and structural binding modes of a series of inhibitors of plasma kallikrein as well as the pharmacokinetics of a lead analog **11** in rat.

Plasma kallikrein (p-kallikrein) is a serine protease which plays a central role in the contact activation and kinin generation pathways.[1,2] Once p-kallikrein is activated from the zymogen precursor prekallikrein (Fletchor Factor),[3] it initiates surface-mediated activation of coagulation, fibrinolysis, and kinin generation. Once activated, p-kallikrein levels are controlled by endogenous C1 inhibitor as well as α_2-macroglobulin and antithrombin III.[4] An imbalance between p-kallikrein and its naturally occurring plasma inhibitors is associated with several disease states,[5] such as hereditary angioedema (HAE), inflammatory bowel disease (IBD), systemic lupus, rheumatoid arthritis (RA), allergic rhinitis, and others. Accordingly, research groups have pursued the development of inhibitors of p-kallikrein as potential therapeutics.[6,7] To date, no small molecule inhibitors have made it to market, although large proteins are currently in clinical trials for the treatment of HAE and for reduced blood loss during surgery.[7]

While developing inhibitors for our internal protease programs,[8] we routinely screened analogs against p-kallikrein. In doing so, we discovered many compounds in several different structural classes which were very potent against this enzyme. In particular, analogs of the 5-amidino-2-(2-hydroxy-biphenyl-3-yl)-benzimidazole (**1**) scaffold stood out as the most potent and selective.

Table 1. SAR of selected analogs in the 5-amidino-2-(2-hydroxy-biphenyl-3-yl)-benzimidazole series[9]



Compound	R^1	R^2	R^3	p-kall K_i (nM)	Selectivity for p-kall versus		
					Xa	VIIa	Trypsin
1	H	H	H	8	300	9	830
2	OH	H	H	13	460	1	1075
3	OMe	H	H	6	1190	22	2710
4	F	H	H	12	200	12	500
5	Me	H	H	6	110	10	140
6	H	OH	H	3	1200	30	2900
7	H	OMe	H	5	780	25	1430
8	H	$COCH_3$	H	6	375	5	1015
9	H	Me	H	4	600	10	1075
10	H	CN	H	10	150	7	530
11	H	$CONH_2$	H	0.5	10,300	200	23,600
12	H	Cl	H	3	530	22	1300
13	H	H	OH	65	190	14	135
14	H	H	OMe	27	250	85	75
15	H	H	Me	18	240	25	200
16	H	H	Cl	37	80	12	25

Table 1 outlines the SAR of 16 selected compounds with small substituents at the *ortho*, *meta*, and *para* positions on the 3′-phenyl ring of scaffold **1**.[9] The inhibition of all

Keywords: Plasma kallikrein; p-kallikrein; Serine protease; Coagulation; Fibrinolysis; Kinin generation; Small molecule inhibitor.
* Corresponding author. E-mail: wendy.young@yahoo.com

0960-894X/$ - see front matter © 2006 Elsevier Ltd. All rights reserved.
doi:10.1016/j.bmcl.2005.12.060

sixteen compounds for p-kallikrein is quite good, with inhibition constants (K_i) ranging from 0.5 nM (analog **11**) to 65 nM (analog **13**). This structural class also potently inhibits a related enzyme factor VIIa (fVIIa). Consequently, there is only a 1- to 200-fold separation between the activity for p-kallikrein and fVIIa within these selected analogs. The preference for p-kallikrein versus factor Xa (fXa) and trypsin is fairly impressive with roughly a 100- to 1000-fold potency separation for most analogs. Although not noted in Table 1, the selectivity versus another related enzyme, thrombin, was >1000-fold for all analogs, with most being >100,000-fold. Within this set of compounds, analog **11** is the most potent for p-kallikrein and has the best selectivity profile versus fXa, fVIIa, and trypsin.

We recently reported the first published crystal structures of human plasma kallikrein (complexed with benzamidine at 1.85 Å[10] and 1.40 Å[11] resolution two crystal forms).[12] P-kallikrein adopts a fold typical of a trypsin-like serine protease, with the catalytic triad, Asp102, His57, and Ser195 at the active site, and Asp189 at the S1 site (Fig. 1). Based on crystal structures of other trypsin-like proteases bound by inhibitors similar to those reported here,[8] we constructed a model of **11** in p-kallikrein (Fig. 1). In this model, the amidine makes hydrogen bonds with Asp189 at the S1 site, the phenolate with His57 and Ser195 at the active site, and the succinate with Lys192. From the model shown in Figure 1 or other models of **11** in fXa, fVIIa, and trypsin (not shown), it is not immediately apparent what elements are responsible for the differences in binding affinity between these enzymes for this scaffold. The excellent selectivity observed against fIIa, however, can be rationalized by the negative electronic interaction of the 5′-acid on the inhibitors with Glu192 of thrombin. In addition, the model suggests that the increased potency and selectivity observed for compound **11** is due to a specific interaction between the 3′ benzamide and Asp60 in p-kallikrein.

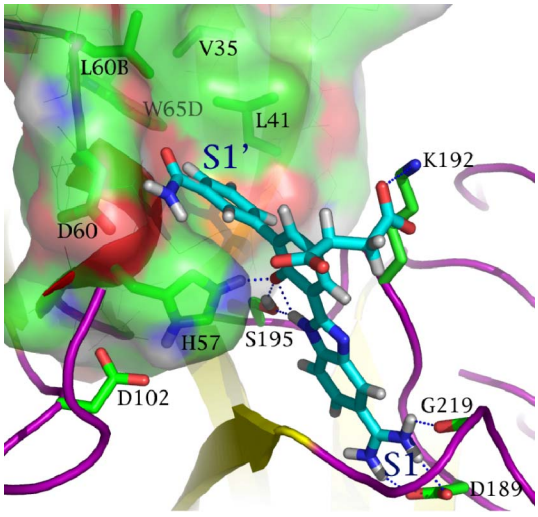


Figure 1. Model of **11** in plasma kallikrein.

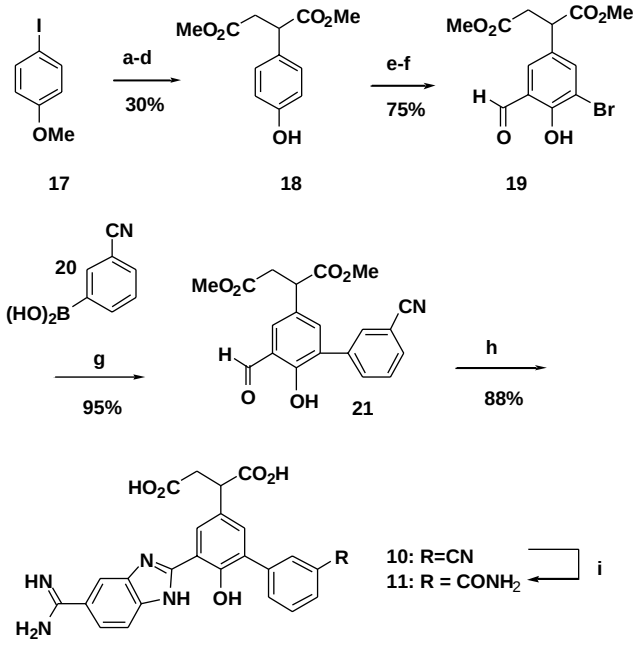
The pharmacokinetic properties of **11** were evaluated after intravenous (iv) bolus administration to male Sprague–Dawley rats (1 mg/kg).[13] The pharmacokinetic parameters of this evaluation are provided in Table 2. Analog **11** has impressive preclinical pharmacokinetics, displaying low plasma clearance, a small volume of distribution, and a long mean residence time (almost 10 h)-ideal characteristics for a drug targeting blood components. These data suggest that once-a-day dosing in human is possible.

Analog **11** was synthesized as outlined in Scheme 1. Starting from the commercially available 4-iodoanisole (**17**), the succinate moiety was inserted via a Heck

Table 2. Pharmacokinetics of **11** in male rats ($n = 3$) following iv bolus administration of 1 mg/kg[13]

Pharmacokinetic parameter[a]	Mean (±SD)
C_{max} (μM) at 2 min	17.8 (1.3)
CL (mL/min/kg)	0.54 (0.03)
V_c (mL/kg)	71 (6)
V_{ss} (mL/kg)	319 (5)
MRT (min)	591 (41)
AUC (μM*min)	3390 (183)
α-$t_{1/2}$ (min) [%AUC]	2 (0.1) [1]
β-$t_{1/2}$ (min) [%AUC]	30 (5) [8]
γ-$t_{1/2}$ (min) [%AUC]	449 (36) [91]

[a] C_{max}, maximum plasma concentration at first sampling time (2 min); CL, plasma clearance; V_c, volume of central compartment; V_{ss}, volume of distribution at steady state; MRT, mean residence time; AUC, area under the plasma concentration versus time curve; $t_{1/2}$, half life for the three exponential phases (α, β, γ); and %AUC, portion of AUC associated with each half life.



Scheme 1. Reagents and conditions: (a) dimethylfumarate, $PdCl_2$, NEt_3, MeCN; (b) $Pd(OH)_2/H_2$, MeOH; (c) HBr, reflux; (d) $SOCl_2$, MeOH; (e) paraformaldehyde, $MgCl_2$, TEA, MeCN; (f) *N*-bromosuccinimide, DMF; (g) $Pd(PPh_3)_4$, Na_2CO_3, toluene; (h) 3,4-diaminobenzamidine-HCl, O_2, EtOH; (i) 2 M NaOH.

reaction with dimethylfumarate and the resulting olefin was reduced by catalytic hydrogenation. Subsequent treatment with HBr followed by reesterification of the acid moieties led to diester **18**. Next, compound **18** was treated with magnesium chloride and paraformaldehyde followed by reaction with *n*-bromosuccinimide to give aryl bromide **19**. A Suzuki reaction of **19** and **20** afforded the biaryl analog **21**. The benzimidazole was then installed by treatment of **21** with 3,4-diaminobenzamidine to give **10**. Finally, the nitrile of **10** was hydrolyzed to the amide with sodium hydroxide and the resulting crude material was purified by reverse-phase HPLC to give **11** as a racemic mixture of enantiomers.

In conclusion, a series of potent 5-amidino-2-(2-hydroxy-biphenyl-3-yl)-benzimidazole inhibitors of plasma kallikrein were identified. Within this series, analog **11** demonstrated the best potency and selectivity profile for plasma kallikrein versus related serine proteases. The pharmacokinetic parameters after iv dosing to rat indicate that this compound is highly stable in vivo and could be further developed for the treatment of inflammatory or coagulation disorders.

Acknowledgments

The authors thank Robert Booth, Michael Green, Peter Young, and Gregory Jackson for helpful scientific discussions, and Nancy Fadis and Barbara Nielsen for aid in preparing the manuscript.

References and notes

1. Colman, R. W. In *Handbook of Proteolytic Enzymes*; Barret, A. J., Rawlings, N. D., Woessner, J. F., Eds., 2nd ed.; Elsevier: London, 2004; pp 1644–1651.
2. Kitchens, C. S. *Arch. Pathol. Lab. Med.* **2002**, *126*, 1382.
3. Hathaway, W. E.; Belhasen, L. P.; Hathaway, H. S. *Blood* **1965**, *26*, 521.
4. Bhoola, K. D.; Figuera, C. D.; Worthy, K. *Pharmacol. Rev.* **1992**, *44*, 1.
5. Devani, M.; Vecchi, M.; Ferrero, S.; Avesani, E. C.; Arizzi, C.; Chao, L.; Colman, R. W.; Cugno, M. *Dig. Liver Dis.* **2005**, *37*, 665; Isordia-Salas, I.; Pixley, R. A.; Sainz, I. M.; Martinez-Murillo, C.; Colman, R. W. *Arch. Med. Res.* **2004**, *35*, 369; Stadnicki, A.; Pastucha, E.; Nowaczyk, G.; Mazurek, U.; Plewka, D.; Machnik, G.; Wilczok, T.; Colman, R. W. *Am. J. Physiol. Gastrointest. Liver Physiol.* **2005**, *289*, G361; Davis, A. E. *Clin. Immunol.* **2005**, 3; Colman, R. W. *Immunopharmacol* **1999**, *43*, 103; Colman, R. W.; Sartor, R. B.; Adam, A. A.; DeLa Cadena, R. A.; Stadnick, A. *Clin. Rev. Allergy Immunol.* **1998**, *16*, 365.
6. Okada, Y.; Tsuda, Y.; Tada, M.; Wanaka, K.; Okamoto, U.; Hijikata-Okunomiya, A.; Okamoto, S. *Chem. Pharm. Bull.* **2000**, *48*, 1964.
7. As of this writing, Dyax and Genzyme are codeveloping DX-88, a proteinacious kallikrein inhibitor, which is in Phase II for the treatment of HAE and for reduced bleeding during Coronary Artery Bipass Graft (CABG) surgery.
8. Young, W. B.; Mordenti, J.; Torkelson, S.; Shrader, W. D.; Kolesnikov, A.; Rai, R.; Liu, L.; Hu, H.; Leahy, E. M.; Green, M. J.; Sprengeler, P. A.; Katz, B.; Yu, C.; Janc, J.; Elrod, K. Marzec, U.; Hanson, S. *Biorg. Med. Chem. Lett.* in press; Shrader, W. D.; Kolesnikov, A.; Burgess-Henry, J.; Rai, R.; Hendrix, J.; Hu, H.; Torkelson, S.; Ton, T.; Young, W. B.; Katz, B. A.; Yu, C.; Cabuslay, R.; Sanford, E.; Janc, J.; Sprengeler, P. *Biorg. Med. Chem. Lett.* in press; Young, W. B.; Kolesnikov, A.; Rai, R.; Sprengeler, P. A.; Leahy, E. M.; Shrader, W. D.; Sangalang, J.; Burgess-Henry, J.; Spencer, J.; Elrod, K.; Cregar, L. *Bioorg. Med. Chem. Lett.* **2001**, *11*(17), 2253; Katz, B. A.; Elrod, K.; Luong, C.; Rice, M.; Mackman, R. L.; Sprengeler, P. A.; Spencer, J.; Hatay, J.; Janc, J.; Link, J.; Litvak, J.; Rai, R.; Rice, K.; Sideris, S.; Verner, E.; Young, W. B. *J. Mol. Biol.* **2001**, *307*(5), 1451; Katz, B. A.; Elrod, K.; Verner, E.; Mackman, R. L.; Luong, C.; Shrader, W. D.; Sendzik, M.; Spencer, J. R.; Sprengeler, P. A.; Kolesnikov, A.; Tai, V. W.; Hui, H. C.; Breitenbucher, J. G.; Allen, D.; Janc, J. *J. Mol. Biol.* **2003**, *329*, 93.
9. Inhibition assays for factor Xa and thrombin were performed as described (Cregar, L.; Elrod, K. C.; Putnam, D.; Moore, W. R. *Arch. Biochem. Biophys.* **1999**, *366*, 125) with the pH adjusted to 7.4. The plasma kallikrein, trypsin, and fVIIa assays were performed and analyzed as in the above reference with the following additional details. Factor VIIa (Enzyme Research) was incubated at 7 nM and CH_3SO_2-D-CHA-But-Arg-pNA (Centerchem) was used as the substrate. The buffer for the factor VIIa assay was supplemented with 11 nM relipidated tissue factor and 5 mM $CaCl_2$. Trypsin (Athens Research Institute) was incubated at 10 nM with variable concentrations of inhibitor in 50 mM Tris (pH 7.4), 150 mM NaCl, 1.5 mM EDTA, 0.05% Tween 20, and 10% DMSO. The reaction was initiated with substrate, Tosyl-Gly-Pro-Lys-pNA (Centerchem), supplied at the Km (25 μM). Plasma Kallikrein (Athens Research Institute) was incubated at 540 pM with variable concentrations of inhibitor in 50 mM Tris (pH 7.4), 150 mM NaCl, 500 μM EDTA, 0.05% Tween 20, and 10% DMSO. The reaction was initiated with substrate, Pro-Phe-Arg-AMC (Bachem), supplied at the Km (350 μM).
10. The PDB access code for the X-ray coordinates is 2ANW.
11. The PDB access code for the X-ray coordinates is 2ANY.
12. Tang, J.; Yu, C.; Williams, S.R.; Springman, E.; Jeffreys, D.; Sprengeler, P.; Estevez, A.; Sampang, J.; Shrader, W.; Spencer, J.; Young, W.; McGrath, M.; Katz, B. *J. Mol. Biol.* in press.
13. Plasma concentrations of **11** were assayed by LC/MS/MS (LOQ = 0.00457 μM). Pharmacokinetic data were analyzed by WinNonlin-Pro (Pharsight Corp.), using a three-compartment model.