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Discovery of a potent and selective $\alpha_v\beta_3$ integrin antagonist with strong inhibitory activity against neointima formation in rat balloon injury model

Seiji Iwama,[a,*] Tomoko Kitano,[b] Fumiyo Fukuya,[b] Yayoi Honda,[b] Yuji Sato,[b] Mitsue Notake[b] and Toshiya Morie[a]

[a]*Chemistry Research Laboratories, Dainippon Pharmaceutical Co., Ltd, Enoki 33-94, Suita, Osaka 564-0053, Japan*
[b]*Pharmacology & Microbiology Research Laboratories, Dainippon Pharmaceutical Co., Ltd, Enoki 33-94, Suita, Osaka 564-0053, Japan*

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Abstract—A new series of phenylpiperazine-based derivatives with strong antagonistic activity for $\alpha_v\beta_3$ integrin was synthesized. Of these derivatives, the fluorine-substituted compound **8** showed strong inhibitory activity and high selectivity for $\alpha_v\beta_3$ integrin receptor ($IC_{50} = 0.055$ nM). In vivo evaluation of the antistenotic effects of **8** indicated that this compound significantly inhibits neointima formation in rat balloon injury model.

1. Introduction

Long-term success of percutaneous coronary interventions (PCIs) remains limited due to restenosis, which occurs within 6 months in 30–50% of patients who undergo balloon angioplasty and in 20–40% of patients who receive bare stent.[1] Therefore, reduction in the incidence of restenosis after angioplasty or stenting has been a research theme and clinical aim for a number of years. To date, numerous pharmacological agents have been evaluated in an attempt to reduce the rate of restenosis in patients who underwent PCIs. However, only a few compounds have been shown to influence restenosis after balloon angioplasty or stenting.[2] Pathologically, experimental evidence has suggested that restenosis is characterized by vascular smooth muscle cells (SMCs) migration, proliferation and extracellular matrix production in disease states.[3]

The $\alpha_v\beta_3$ integrin, also known as the vitronectin receptor, is expressed on a variety of cell types,[4] including endothelial cells, SMCs, macrophage, platelets, osteoclasts and various tumour cells, and is known to mediate a variety of biological events, including migration and proliferation of SMCs during restenosis,[5] adhesion of osteoclasts to bone matrix and angiogenesis.[6] The integrin $\alpha_v\beta_3$ binds to extracellular matrix proteins, such as vitronectin, fibronectin, osteopontin, fibrinogen and von Willebrand factor through interaction with the tripeptide Arg-Gly-Asp (RGD) sequence.

A vast number of cyclic peptide and nonpeptide antagonists with high affinity for $\alpha_v\beta_3$ integrin have been reported.[7] Nevertheless, only a few $\alpha_v\beta_3$ integrin antagonists have been shown to inhibit neointima formation in the arterial injury model.[5] Our interest in this field was prompted by the finding that $\alpha_v\beta_3$ antagonists inhibit migration of SMC into neointima and/or proliferation of SMC in neointima after arterial injury.

In early studies, Kessler and co-workers disclosed cyclic RGD peptides as selective $\alpha_v\beta_3$ integrin antagonists, and proposed an $\alpha_v\beta_3$ ligand-binding model. Their findings suggest that conformation of the RGD-sequence with $ArgC^{\alpha}/AspC^{\alpha}$-distance of about 500 pm and $ArgC^{\beta}/AspC^{\beta}$-distance of about 700 pm fits better in the $\alpha_v\beta_3$ receptor than in the $\alpha_{IIb}\beta_3$ receptor.[8] In our search for a new scaffold based on Kessler's model, we found 3-aminophenyl-piperazine moiety as a constrained scaffold for the nonpeptide $\alpha_v\beta_3$ integrin antagonists.

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* Corresponding author. Tel.: +81-663375901; fax: +81-663387656; e-mail: seiji-iwama@dainippon-pharm.co.jp

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Herein, we disclose our initial effort for the synthesis and structure–activity relationship of a new series of phenylpiperazine based $\alpha_v\beta_3$ integrin antagonists, which have strong inhibitory activity of neointima formation in rat balloon injury model.

2. Chemistry

Synthesis of the $\alpha_v\beta_3$ integrin antagonists **1**–**15** was accomplished by attaching the groups bearing the acidic and basic moieties in **1**–**15** to 3-aminophenyl-piperazine. As shown in Scheme 1, reaction of 3-fluoronitrobenzene with piperazine in DMSO at 100 °C,[9] followed by coupling with N_α-substituted glutamic acid *tert*-butyl ester (**17**) using EDCI and HOBt in DMF provided the coupled product **18**. Reduction of the nitro group in **18** with Fe and NH_4Cl in EtOH and H_2O gave the aniline **19**. Introduction of a cyclic guanidine moiety with $HgCl_2$ and diBoc thiourea derivatives (**20a**–**f**) using Kim's protocol,[10] followed by deprotection of both diBoc groups and the *tert*-butyl ester group provided the desired compounds **1**–**15**.

3. Results and discussion

3.1. Binding study

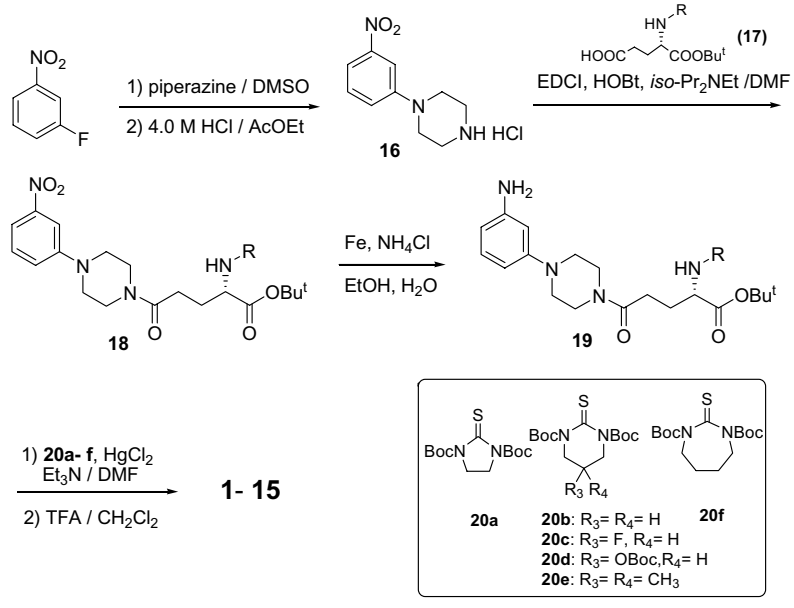
For in vitro screening, we examined the inhibitory activity of **1**–**15** for $\alpha_v\beta_3$ and $\alpha_{IIb}\beta_3$ integrins binding using human vitronectin and fibrinogen as ligands, respectively,[11,12] and for human vascular smooth muscle cells (HVSMC) proliferation.[13] As shown in Table 1, among the nonsubstituted six-membered guanidine analogues (**2**, **6**, **7**, **11**–**15**), the sulfonamide analogues (**11**, **12**) showed low selectivity for $\alpha_{IIb}\beta_3$ integrin. Likewise, the amide and urea analogues (**13**–**15**) showed low inhibitory activity for $\alpha_v\beta_3$ integrin, although their selectivity for $\alpha_{IIb}\beta_3$ integrin was modest. However, the benzylcarbamate analogue **2** showed high inhibitory activity for $\alpha_v\beta_3$ integrin ($IC_{50} = 0.058$ nM) and high selectivity for $\alpha_{IIb}\beta_3$ integrin. As for the effect of the guanidine moiety, the ring size [five-membered (**1**), six-membered (**2**) and seven-membered (**5**)] had no significant influence on both the inhibitory activity for $\alpha_v\beta_3$ integrin and inhibition of HVSMC proliferation. However, introduction of a fluorine atom on the six-membered cyclic guanidine moiety of **2** (yielding **3**) resulted in good improvement in inhibition of HVSMC proliferation. Likewise, the inhibitory activity of **8** for both $\alpha_v\beta_3$ integrin and HVSMC proliferation increased compared with that of **7**, **9** or **10**.

Among the compounds synthesized in this study, the fluorine-substituted analogues **3** and **8** having both high inhibitory activity for $\alpha_v\beta_3$ integrin (**3**: $IC_{50} = 0.063$ nM; **8**: $IC_{50} = 0.055$ nM) and potent inhibition of HVSMC proliferation (**3**: $IC_{50} = 0.26$ μM, **8**: $IC_{50} = 0.18$ μM) were selected as candidates for inhibition of neointima formation in rat balloon injury model.

3.2. Rat balloon injury model

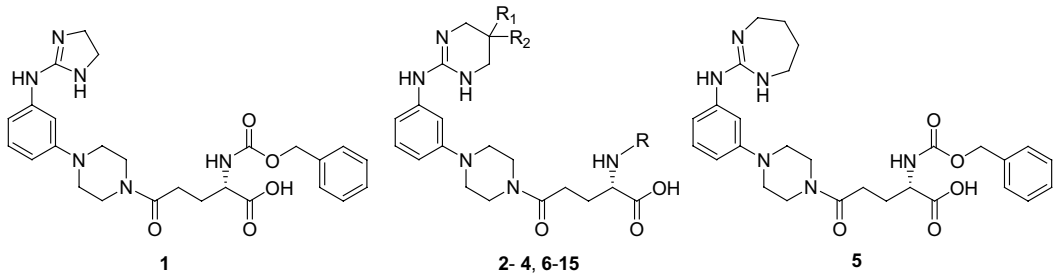
To evaluate the antistenotic effects of **3** and **8**, their ability to inhibit neointima formation in the carotid artery of rat balloon injury model was examined.[14]

For a period of 15 days starting 1 day before injury, compound **3** or **8** was infused to rats subcutaneously at a constant rate of 1.3 μmol/rat/day using an Alzet™ osmotic pump. Measurements of plasma levels of **8** indicated a steady state of plasma concentration exceeding the in vitro IC_{50} value for inhibition of HVSMC proliferation (**8**: ca. 0.35 μM, $IC_{50} = 0.18$ μM).



Scheme 1. Synthesis of $\alpha_v\beta_3$ integrin antagonists **1**–**15**.

Table 1. Inhibitory activity of phenyl-piperazine antagonists **1**–**15**



1 **2- 4, 6-15** **5**

Compound	R	R_1	R_2	$\alpha_v\beta_3$ IC_{50} (nM)	$\alpha_{IIb}\beta_3$ IC_{50} (nM)	$\alpha_v\beta_3/\alpha_{IIb}\beta_3$ ratio	HVSMC[a] proliferation assay IC_{50} (μM)
1	—	—	—	0.16	720	4500	3.0
2	$PhCH_2OCO$–	H	H	0.058	660	11,000	1.1
3	$PhCH_2OCO$–	F	H	0.063	540	8600	0.26
4	$PhCH_2OCO$–	OH	H	0.15	340	2300	3.5
5	—	—	—	0.056	>1000	>18,000	1.7
6	EtOCO–	H	H	0.59	>1000	>1700	2.1
7	*iso*-BuOCO–	H	H	0.32	>1000	>3100	0.91
8	*iso*-BuOCO–	F	H	0.055	>1000	>18,000	0.18
9	*iso*-BuOCO–	OH	H	0.30	>1000	>33,000	2.0
10	*iso*-BuOCO–	CH_3	CH_3	0.38	>1000	>2600	3.4
11	$PhSO_2$–	H	H	1.4	88	63	>10
12	Mes–[b]	H	H	0.22	30	140	>10
13	$PhCH_2CO$–	H	H	6.8	>1000	>150	>10
14	EtNHCO–	H	H	3.6	>1000	>280	>10
15	$PhCH_2NHCO$–	H	H	1.4	>1000	>710	>10

[a] HVSMC: human vascular smooth muscle cell.
[b] 2,4,6-Trimethylbenzenesulfonyl.

Table 2. Results of morphometrical examination of carotid cross sections

Compound	Number of animals	Intima area[a] (10^3 μm^2)	%Inhibition from control	Media area[a] (10^3 μm^2)	I/M ratio[a,b]	%Inhibition from control
Experiment no. 1						
Control	7	181 ± 16		156 ± 4	1.16 ± 0.09	
3	6	113 ± 13**	38	149 ± 6	0.76 ± 0.09**	34
Experiment no. 2						
Control	7	178 ± 17		171 ± 8	1.06 ± 0.10	
8	7	81 ± 9***	54	162 ± 7	0.50 ± 0.05***	53

** $p < 0.01$ and *** $p < 0.001$ compared with control.
[a] Values represent the mean ±SE.
[b] I/M ratio, intima area to media area ratio.

Rats carotid arteries were isolated 14 days after injury, cross-sectioned and morphometrically analyzed. As shown in Table 2, compounds **3** and **8** inhibited neointima formation by 38% ($p < 0.01$) and 54% ($p < 0.001$), respectively, compared to each control group, whereas no difference in media area was observed between compounds treated and untreated control rats. The ratios of intima area to media area (I/M) in the case of **8** were higher than those in the case of **3** (**3**: 34%, **8**: 53%). From these results, compound **8** was selected as a better inhibitor of neointima formation in rat balloon injury model. Representative cross sections of the arteries are shown in Figure 1. I/M ratios in the sections were close to the mean values in each group (control rat: 1.16, **8**-treated rat: 0.51). Following injury, the intima area in the control rat (Fig. 1b,e) remarkably increased compared with that in the uninjured normal rat (Fig. 1a,d). However, treatment with **8** inhibited neointima formation following injury (Fig. 1c,f). In addition, compound **8** exhibited a dose-dependent inhibition; I/M ratios of 21% and 38% at doses of 0.14 and 0.43 μmol/rat/day, respectively.

4. Conclusion

In summary, we synthesized highly selective $\alpha_v\beta_3$ integrin antagonists based on a phenyl-piperazine RGD peptidemimetic. Among the synthesized compounds, the fluorine-substituted **8** showed the strongest inhibitory activity with high selectivity for $\alpha_v\beta_3$ integrin. In vivo evaluation of the antistenotic effects of **8** indicated that

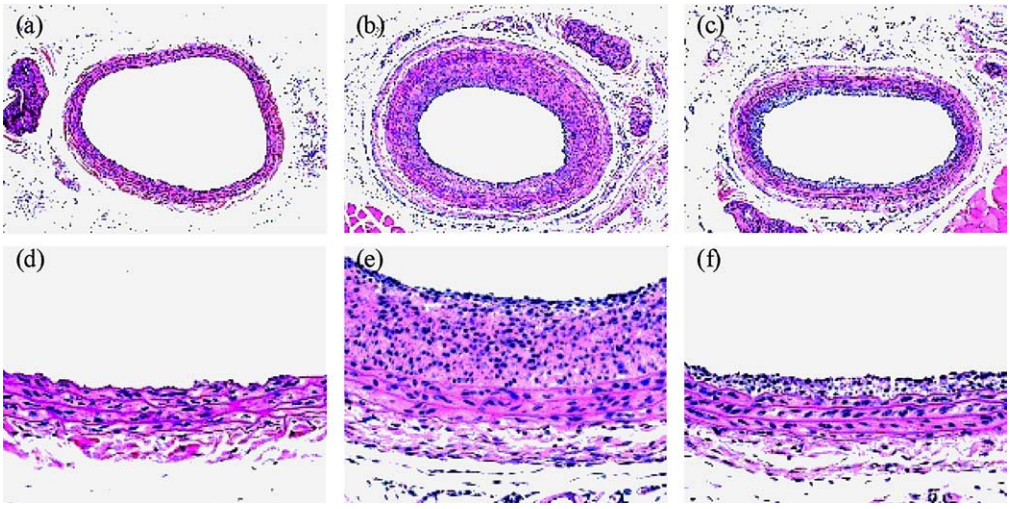


Figure 1. Typical cross sections of rats carotid arteries 14 days after balloon injury. (a) Normal, the right uninjured carotid artery (× 44). (b) Control, the left injured carotid artery in untreated rat (× 44). (c) Treated rat, the left injured carotid artery in **8**-treated rat (× 44). (d) High magnification (× 147) of panel (a). (e) High magnification (× 147) of panel (b). (f) High magnification (× 147) of panel (c).

this compound significantly inhibits neointima formation in rat balloon injury model. It is therefore suggested that compound **8** may offer a new entry for the treatment of restenosis after PCIs.

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References and notes

1. (a) The STRESS Investigators. *New Engl. J. Med.* **1994**, *331*, 496; (b) The BENESTENT Study Group. *New Engl. J. Med.* **1994**, *331*, 489; (c) Babapulle, M. N.; Eisenberg, M. J. *Circulation* **2002**, *106*, 2734.
2. (a) Johnson, G. J.; Griggs, T. R.; Badimon, L. *Thromb. Haemost.* **1999**, *81*, 835; (b) Gruberg, L.; Waksman, R.; Satler, L. F.; Pichard, A. D.; Kent, K. M. *Exp. Opin. Invest. Drugs* **2000**, *9*, 2555.
3. Ross, R. *Nature* **1993**, *362*, 801.
4. Byzova, T. V.; Rabbani, R.; D'Souza, S. E.; Plow, E. F. *Thromb. Haemost.* **1998**, *80*, 726.
5. Sajid, M.; Stouffer, G. A. *Thromb. Haemost.* **2002**, *87*, 187.
6. Brooks, P. C.; Clark, R. A. F.; Cheresh, D. A. *Science* **1994**, *264*, 569.
7. For recent reviews on $\alpha_v\beta_3$ integrin antagonist, see: (a) Miller, W. H.; Keenan, R. M.; Willette, R. N.; Lark, M. W. *Drug Discovery Today* **2000**, *5*, 397; (b) Duggan, M. E.; Hutchinson, J. H. *Exp. Opin. Ther. Patents* **2000**, *10*, 1367; (c) Hölzemann, G. *IDrugs* **2001**, *4*, 72; (d) Coleman, P. J.; Duong, L. T. *Exp. Opin. Ther. Patents* **2002**, *12*, 1009.
8. (a) Haubner, R.; Gratias, R.; Diefenbach, B.; Goodman, S. L.; Jonczyk, A.; Kessler, H. *J. Am. Chem. Soc.* **1996**, *118*, 7461; (b) Haubner, R.; Finsinger, D.; Kessler, H. *Angew. Chem., Int. Ed.* **1997**, *36*, 1374.
9. Belfield, A. J.; Brown, G. R.; Foubister, A. J.; Ratcliffe, P. D. *Tetrahedron* **1999**, *55*, 13285.
10. Kim, K. S.; Qian, L. *Tetrahedron Lett.* **1993**, *34*, 7677.
11. Inhibitory activity on human $\alpha_v\beta_3$ integrin binding was determined with the use of biotinylated human vitronectin as the ligand according to the methods described in Mousa, S. A.; Forsythe, M.; Lorelli, W.; Bozarth, J.; Xue, C.-B.; Wityak, J.; Sielecki, T. M.; Olson, R. E.; DeGrado, W.; Kapil, R.; Hussain, M.; Wexler, R.; Thoolen, M. J.; Reilly, T. M. *Coron. Artery Dis.* **1996**, *7*, 767.
12. Inhibitory activity on human $\alpha_{IIb}\beta_3$ integrin binding was determined with the use of biotinylated human fibrinogen as the ligand. The binding assay was carried out essentially in a similar manner as that for $\alpha_v\beta_3$ integrin binding, although the modified antigen capture ELISA procedure was used instead of immobilization of a purified $\alpha_{IIb}\beta_3$ integrin protein; see: (a) Kosugi, S.; Tomiyama, Y.; Shiraga, M.; Kashiwagi, H.; Mizutani, H.; Kanakura, Y.; Kurata, Y.; Matsuzawa, Y. *Thromb. Haemost.* **1996**, *75*, 339; (b) Kashiwagi, H.; Tomiyama, Y.; Tadokoro, S.; Honda, S.; Shiraga, M.; Mizutani, H.; Handa, M.; Kurata, Y.; Matsuzawa, Y.; Shattil, S. J. *Blood* **1999**, *93*, 2559.
13. Inhibitory activity on human VSMC proliferation was determined using the WST-1 cell proliferation assay; see: Ishiyama, M.; Shiga, M.; Sasamoto, K.; Mizoguchi, M.; He, P.-G. *Chem. Pharm. Bull.* **1993**, *41*, 1118.
14. Clowes, A. W.; Reidy, M. A.; Clowes, M. M. *Lab. Invest.* **1983**, *49*, 327.