Pergamon

Bioorganic & Medicinal Chemistry Letters, Vol. 7, No. 23, pp. 2935-2940, 1997

0960-894X/97 $17.00 + 0.00

PII: S0960-894X(97)10117-2

A NOVEL SERIES OF 4-PHENOXYQUINOLINES: POTENT AND HIGHLY SELECTIVE INHIBITORS OF PDGF RECEPTOR AUTOPHOSPHORYLATION

Kazuo Kubo,* Toshiyuki Shimizu, Shin-ichi Ohyama, Hideko Murooka, Tsuyoshi Nishitoba, Shinichiro Kato, Yoshiko Kobayashi, Mikio Yagi, Toshiyuki Isoe, Kazuhide Nakamura, Tatsushi Osawa and Toshio Izawa

Pharmaceutical Research Laboratory, KIRIN Brewery Co., Ltd., 3 Miyahara-cho, Takasaki-shi, Gunma 370-12, Japan

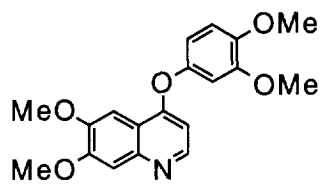
Abstract: A novel series of 4-phenoxyquinolines, some of which showed potent and highly selective inhibitory activities for PDGF receptor autophosphorylation, was discovered. Interestingly, their structures were very similar to those of the selective inhibitors for EGF receptor autophosphorylation.

Introduction

Platelet-derived growth factor (PDGF) is a potent mitogen for mesenchymal, glial and capillary endothelial cells.[1] There are three isoforms of PDGF, PDGF-AA, PDGF-BB and PDGF-AB, and they interact differentially with structurally related two kinds of receptors, PDGF α receptor (PDGF α-R) and PDGF β receptor (PDGF β-R).[2] PDGF participates in various physiological processes such as embryonal development and wound healing through its receptors autophosphorylation and subsequent signal transduction.[2] However, overexpression of PDGF and/or its receptors causes a number of pathophysiological processes including various forms of neoplasia, atherosclerosis, rheumatoid arthritis, pulmonary fibrosis, myelofibrosis, glomerulosclerosis and abnormal wound healing.[3]

Low molecular weight inhibitors of PDGF receptor (PDGF-R) autophosphorylation represent a novel class of therapeutic agents useful for the treatment of malignant and nonmalignant diseases involving excess cell proliferation. Some biological effects have been studied for several inhibitors, *e.g.*, tyrphostins,[4] 3-substituted quinoline derivatives,[5] 3-substituted quinoxaline derivatives,[6] and phenylaminopyrimidines.[7]

Recently, we screened in-house compounds and discovered a potent and highly selective inhibitor for PDGF-R autophosphorylation, 6,7-dimethoxy-4-(3,4-dimethoxyphenoxy)quinoline (**7o**: Ki6783),[8] whose structure is very similar to that of PD 153035 (**9d**),[9] a selective inhibitor of epidermal growth factor receptor (EGF-R) autophosphorylation. In this paper, we report our preliminary findings for a series of novel 4-phenoxyquinoline derivatives.



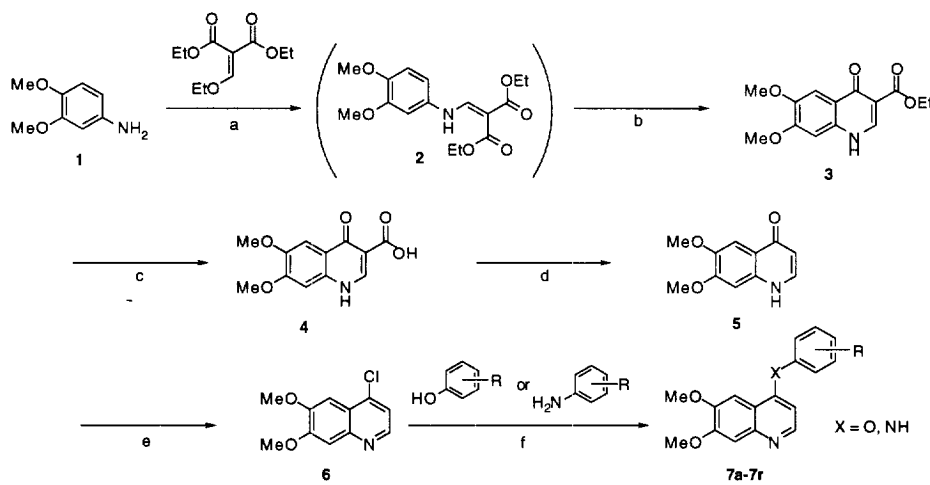
7o: Ki6783

Chemistry

Quinoline derivatives were prepared using general methods as shown in scheme 1.[10] Quinolone (**3**) was obtained by the condensation of 3,4-dimethoxyaniline (**1**) and diethyl ethoxymethylenemalonate. Basic hydrolysis and subsequent decarboxylation gave 6,7-dimethoxyquinolone (**5**), which was converted to 4-

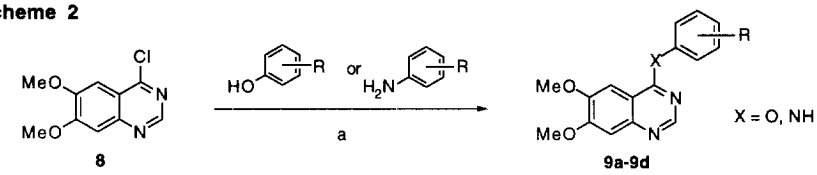
chloroquinoline (**6**) by a treatment with $POCl_3$. Finally, compounds **7a-7r** were prepared by nucleophilic replacement with the appropriate phenols and anilines. On the other hand, quinazoline derivatives (**9**) were also prepared by a similar method as shown in scheme 2.[11]

Scheme 1



(a) 120°C; (b) diphenyl ether/reflux; (c) 10% NaOH, MeOH/reflux; (d) diphenyl ether/reflux;
(e) $POCl_3$/reflux; (f) diglyme/170°C or DMAP/xylene/reflux

Scheme 2



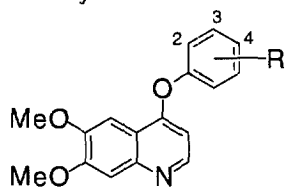
(a) diglyme/170°C or DMAP/xylene/reflux

Results and Discussion

The derivatives were examined for their inhibitory activity of PDGF β-R autophosphorylation stimulated by human recombinant PDGF-BB in rat mesangial cells.[12] The results are shown in Table 1. At first, several quinoline derivatives (**7a-7l**), which have a substituent on the phenyl ring, were examined. Several derivatves showed strong activities for PDGF-R autophosphorylation (**7a**, **7b**, **7c**, **7g** and **7l**). Then, we tried quantitative structure-activity relationship studies for 4-phenoxyquinoline derivatives including compounds **7a-7l** using physicochemical properties for steric, electronic and hydrophilic effects. Consequently, we found that

no features but a hydrophilicity, π value of the substituents on phenyl ring, had a correlation with the inhibitory activity ($r^2 = 0.725$).[13] Therefore, we focused on methoxy group having highly hydrophilicity and prepared some derivatives bearing two methoxy groups on the phenyl ring (**7m-7p**). Fortunately, we discovered that **7o**[14] had the strongest activity for PDGF-R autophosphorylation. We also investigated the inhibitory activities of **7o** on other receptors' autophosphorylation by intact cell assay and cell-free kinase assay.[15] **7o** showed the most selective inhibitory activities for PDGF-R autophosphorylation compared to those for other tyrosine and serine/threonine kinases (Table 2).

Table 1. Inhibitory Activity for PDGF-R Autophosphorylation[a]



		IC_{50} (μM)			IC_{50} (μM)
no.	R	PDGF-R	no.	R	PDGF-R
7a	H	0.76	**7i**	3-F	1.5
7b	2-OMe	0.60	**7j**	3-Br	8.4
7c	3-OMe	0.44	**7k**[b]	$3\text{-}NH_2$	1.7
7d	4-OMe	1.9	**7l**	$3\text{-}NO_2$	0.70
7e	3-Me	6.9	**7m**	$2,3\text{-}(OMe)_2$	4.4
7f	3-Et	8.4	**7n**	$2,6\text{-}(OMe)_2$	69.3
7g	3-OH	0.56	**7o** (Ki6783)	$3,4\text{-}(OMe)_2$	0.13
7h	3-OEt	12.9	**7p**	$3,5\text{-}(OMe)_2$	5.5

[a] See ref 8.
[b] **7k** was prepared by hydrogenation of **7l** using Pd catalyst.

Table 2. Inhibitory Activities for Various Protein Kinases of **7o** (Ki6783)

Intact Cell

Kinase	Cell	IC_{50}(μM)[a]
PDGF-R	rat mesangial cell	0.13
	NIH 3T3	0.07
	human AOSMC [b]	0.15
EGF-R	A431	>100
bFGF-R	NIH 3T3	>100
Insulin ß-R	HepG2	>100

Cell - Free

Kinase	IC_{50}(μM)[c]
PDGF ß-R	0.03
EGF-R	>10
c-Src	>10
Cdc2	>10
PKA	>10
PKC	>20
MAPK	>10
MAPKK	>10

[a] See ref 12.
[b] human AOSMC: human aortic smooth muscle cells
[c] See ref 15.

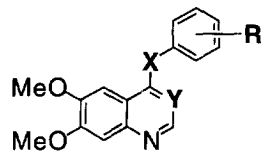


Figure 1

Table 3. Inhibitory Activities of Quinoline and Quinazoline Derivatives for PDGF-R and EGF-R Autophosphorylations[a]

				$IC_{50}(\mu M)$	
no.	X	Y	R	PDGF-R	EGF-R
7o (Ki6783)	O	CH	3,4-$(OMe)_2$	0.13	>100
9a	O	N	3,4-$(OMe)_2$	0.57	>100
7q	NH	CH	3,4-$(OMe)_2$	2.6	80.5
9b	NH	N	3,4-$(OMe)_2$	4.5	2.8
7j	O	CH	3-Br	8.4	2.5
7r	NH	CH	3-Br	21.5	0.37
9c	O	N	3-Br	14.8	0.07
9d (PD153035)	NH	N	3-Br	21.9	0.004

[a] See ref 12.

Although **7o** and **9d** have simular structures and find competitively to the receptor kinase with ATP,[8,9,16] it is very interesting that they have different selectivity for PDGF-R and EGF-R. In order to investigate more detail of their structure-activity relationships, we prepared their derivatives by individually exchanging three structural parts of them (X, Y and R positions in Figure 1). Their inhibitory activities (for PDGF-R and EGF-R) are listed in Table 3.

As shown in Table 3, when (X, Y) is (O, CH), the strongest activities and highest preference for PDGF-R were observed among the compounds with the same substituents on the phenyl ring, **7o** for 3,4-$(OMe)_2$ and **7j** for 3-Br, respectively. Substitution of the X position in **7o** by nitrogene (NH) to give **7q** resulted in a 20-fold loss of activity, and substitution of the Y position in **7q** by nitrogene (N) to give **9b** diminished the selectivity against the both receptors. On the other hand, when (X, Y) is (NH, N), the strongest activities and highest preference for EGF-R were obsereved, **9b** for 3,4-$(OMe)_2$ and **9d** for 3-Br. Substitution of the Y position in **9d** by carbon (CH) to give **7r** leads to a 100-fold loss of activity, and substitution of the X position in **7r** by oxygene (O) to give **7j** reduced the selectivity. These results showed that there would be different interactions between the two receptors and the atoms at the X and/or Y positions of these inhibitors. And moreover, as for the substituent (R) on the phenyl ring, the hydrophilic group would favor for the inhibitor of PDGF-R autophosphorylation, although it was reported that small lipophilic electron-withdrawing groups on the phenyl ring (ex. 3-Br) could favor for the EGF-R in 4-(phenylamino)quinazolines.[17] R groups on the phenyl ring would be recognized in different ways by the two tyrosine kinases due to teir distinct physicochemical properties in the highly conserved ATP binding site.[18] These observation gave us the important information that is useful to improve the specificity of the tyrosine kinase inhibitors.

In summary, we discovered 4-phenoxyquinolines which showed potent and highly selective inhibitory activity for PDGF-R autophosphorylation. Especially, Ki6783 showed the highest selectivity for PDGF-R

compared to other tyrosine and serine/threonine kinases.

Acknowledgment

We would like to thank Dr. Eisuke Nishida (Kyoto University) for providing us with recombinant MAPK and MAPKK.

References and Notes

1. Ross, R.; Raines. E. W.; Bowen-Pope, D. F. *Cell* **1986**, *46*, 155-169.
2. 1) Heldin, C. H. *J. Biol. Chem.* **1989**, *264*, 8905-8912. 2) Bishatee, S.; Majumdar, S.; Khire, J.; Das,M. *J. Biol. Chem.* **1989**, *264*, 11699-11705. 3) Seifert, R. A.; Hart, C. E.; Philips, P. E.; Forstrom, J. W.; Ross, R.; Murry,M. J.; Bowen-Pope, D. F. *J. Biol. Chem.* **1989**, *264*, 8771- 8778. 4) Heldin, C. H. *EMBO J.* **1992**, *11*, 4251-4259.
3. Heldin, C. H.; Westermark, B. *Transplan. Proc.* **1993**, *25*, 2074-2076.
4. Bilder, G. E.; Kasiewski, C. J.; Walczak, E. M.; Perrone, M. H. *Drug Dev. Res.* **1993**, *29*, 158-166.
5. 1) Maguire, M. P.; Sheets, K. R.; McVety, K.; Spada, A. P.; Zilberstein, A. *J. Med. Chem.* **1994**, *37*, 2129-2137. 2) Dolle, R. E.; Dunn, J. A.; Bobko, M; Singh, B; Kuster, J. E.; Baizman, E.; Harris, A. L.; Sawutz, D. G.; Miller, D.; Wang, S.; Faltynek, C. R.; Xie, W; Sarup, J.; Bode, D. C.; Pagani, E. d.; Silver, P. J. *J. Med. Chem.* **1994**, *37*, 2627-2629.
6. Kovalenko, M.; Gazit, A.; Bohmer, A.; Rorsman, C.; Ronnstrand, L.; Heldin, C-H.; Waltenberger, J.; Bohmer, F-D.; Levitzki, A. *Cancer Res.* **1994**, *54*, 6106-6114.
7. Buchdunger, E.; Zimmermann, J.; Mett, H.; Meyer, T.; Muller, M.; Regenass, U.; Lydon, N. B. *Proc. Natl. Acad. Sci. USA* **1995**, *92*, 2558-2562.
8. Yagi, M.; Kato, S.; Kobayashi, Y.; Kubo, K.; Ohyama, S.; Shimizu, T.; Nishitoba, T.; Isoe, T.; Nakamura, K.; Ohashi, H.; Kobayashi, N.; Iinuma, N.; Osawa, T.; Onose, R.; Osada, H. *Exp. Cell Res.* **1997**, *234*, 285-292.
9. Fry, D. W.; Kraker, A. J.; McMichael, A.; Ambroso, L. A.; Nelson, J. M.; Leopold, W. R.; Connors, R. W.; Bridges, A. J. *Science* **1994**, *265*, 1093-1095.
10. Gall-Istok, K.; Sterk, L.; Deak, Gy. *Acta Chim. Hung.*, **1983**, *112*, 241-247.
11. European patent application 566226 (15.1.1993).
12. Mesangial cells, NIH3T3 cells, and hAOSMC cells were used for the assay of PDGF β-R autophosphorylation in intact cells as discribed in ref 8. Tyrosine phosphorylation assay of other receptors was carried out by the same method as that for the PDGF-R. A431 cells, NIH3T3 cells, and HepG2 cells were used to study autophosphorylation of EGF-R, basic fibroblast growth factor receptor (bFGF-R) and insulin β-R induced by EGF, basic fibroblast growth factor (bFGF) and insulin, respectively.
13. Kubo, K.; Ohyama, S.; Murooka, H.; Shimizu, T.; Kato, S.; Tamura, Y.; Iijima, H. Nippon Yakugakkai Nenkai Koen Yoshishu **1996**, 116th (Pt 2), 29[C2]15-3, 275. π value was previously discdribed (Fujita, T.; Iwasa, J.; Hansch, C. *J. Am. Chem. Soc.* **1964**, *86*, 5175-5180).
14. A typical synthetic procedure is described as follows: 3,4-dimethoxyphenol (410 mg, 2.7 mmol) and 4-chloro-6,7-dimethoxyquinoline (**6**)[10] (200 mg, 0.9 mmol) were stirred at 170 °C for 20 min. After the usual work up, the crude product was chromatographed on silica gel to afford the corresponding quinoline (**7o**) (200 mg, 62%). **7o**: colorless plates; mp 188-190°C (hexane-AcOEt); ^{1}H NMR (500MHz, $CDCl_3$): δ 3.87 (s, 3H), 3.93 (s, 3H), 4.05 (s, 3H), 4.06 (s, 3H), 6.45 (d, *J*=4.9Hz, 1H), 6.75 (dd, *J*=2.4 and 9.2Hz, 1H), 6.75 (d, *J*=2.4Hz, 1H), 6.93 (d, *J*=9.2Hz, 1H), 7.42 (s, 1H), 7.57 (s, 1H), 8.48 (d, *J*=4.9Hz, 1H); FD-MS 341(M^+).
15. Cell-free receptor tyrosine kinase assays of Ki6783 for PDGF β-R, EGF-R, protein kinase A (PKA) and protein kinase C (PKC) were discribed in ref 8. MAPK and MAPKK activities were performed as previously described (Kosaka, H.; Nishida, E.; Gotoh, Y. *EMBO J.*, **1993**, *12*, 787-794). Determination of MAPK and MAPKK terminated reactions were spotted on phosphocellulose disc papers. Phosphorylated substrates on the paper were washed to remove free [γ-^{32}P]ATP and mesured by Cerenkov counting of incorporated radioactivity using the fluid scintillation counter. c-Src tyrosine kinase (UBI) activity was assayed using Src kinase substrate peptide (UBI). Cdc2 kinase (Seikagu co.) activity was assayed using the cdc2 kinase enzyme assay system (Amersham).
16. Ward, W. H. J.; Cook, P. N.; Slater, A. M.; Davies, D. H.; Holdgate, G. A.; Green, L. R. *Biochem. Phamacol.* **1994**, *48*, 659-666.
17. 1) Rewcastle, G. W.; Denny, W. A.; Bridges, A. J.; Hairong, Z.; Cody, D. R.; McMichael, A.; Fry, D. W. *J. Med. Chem.* **1995**, *38*, 3482-3487. 2) Thompson, A. M.; Bridges, A. J.; Fry, D. W.; Kraker, A. J.; Denny, W. A. *J. Med. Chem.* **1995**, *38*, 3780-3788.

18. 1) Hanks, S. K.; Quinn, A. M.; Hunter, T. *Science* **1988**, *241*, 42-52. 2) Burke, T. R. *Drugs Future* **1992**, *17*, 119-131.
19. All compounds were characterized by ^{1}H NMR and FD-MS analysis.

(*Received in Japan* 6 *February* 1997; *accepted* 16 *October* 1997)