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Bioorganic & Medicinal Chemistry Letters 13 (2003) 3001–3004

BIOORGANIC & MEDICINAL CHEMISTRY LETTERS

Potent and Selective Inhibitors of Platelet-Derived Growth Factor Receptor Phosphorylation. Part 4: Structure–Activity Relationships for Substituents on the Quinazoline Moiety of 4-[4-(*N*-Substituted(thio)carbamoyl)-1-piperazinyl]-6,7-dimethoxyquinazoline Derivatives

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Received 3 March 2003; accepted 14 April 2003

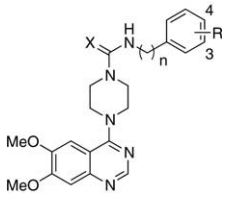
Abstract—Here, we investigated the structure–activity relationships of the 6,7-dimethoxyquinazoline moiety. With regard to exploration of positions and varieties of substituents on the quinazoline ring, 6,7-dialkoxy substitution was optimal. This study suggests the possibility of further modifications for this moiety.

Platelet-derived growth factor (PDGF) is known to act as a potent mitogen and chemotactic factor for various cells such as smooth muscle cells.[1–4] Abnormal PDGF-induced cell proliferation has been proposed which lead to the proliferative disorders (atherosclerosis, restenosis following PTCA, glomerulonephritis, glomerulosclerosis, liver cirrhosis, pulmonary fibrosis, leukemia and cancer).[5–9] In our previous publications, a series of 4-[4-(*N*-substituted (thio)carbamoyl)-1-piperazinyl]-6,7-dimethoxyquinazoline derivatives listed in Table 1 were found to be selective inhibitors for the PDGFR phosphorylation, and initial structure–activity relationships (SARs) of 4-nitrophenylcarbamoyl and piparazinyl moieties for KN1022 and several biological effects have been reported.[10–15]

In this paper, we report synthesis and SARs for inhibition of in vitro β-PDGFR phosphorylation focusing on the 6,7-dimethoxyquinazoline moiety, so as to examine the effect of position and series of substituents on quinazoline ring.

General synthetic procedures are outlined in Scheme 1. There are two approaches for obtaining the target molecule. Condensation of the 4-chloroquinazoline (**2**) with excess piperazine, followed by treatment with iso

Table 1. Inhibitors of β-PDGFR phosphorylation



No.	R	*n*	X	IC_{50}[a,b] (μmol/L)
KN1022	4-NO_2	0	O	0.70
1a	4-OPh	0	O	0.08
1b	H	1	S	0.55
KN734	3,4-OCH_2O–	1	S	0.09

[a]IC_{50} (μmol/mL) of β-PDGFR phosphorylation.
[b]Autophosphorylation was measured in intact cells using a two-site ELISA.[14,18]

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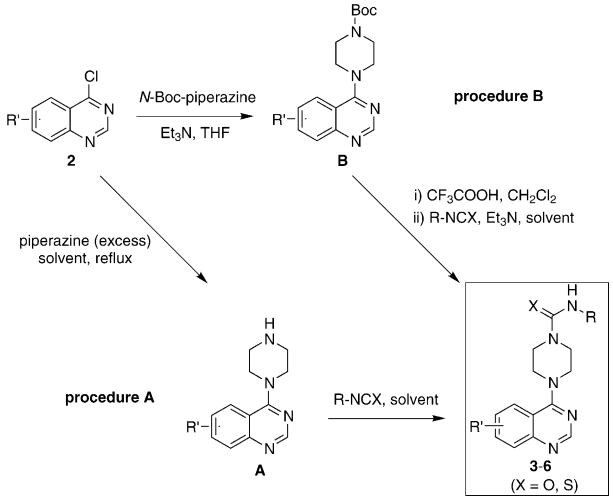
doi:10.1016/S0960-894X(03)00634-6



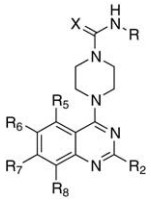
Scheme 1. General synthetic procedures.

(thio)cyanate provided the target molecule (procedure A). It was also obtained from the *N*-Boc-piperazine B, which was synthesized from **2** and *N*-Boc-piperazine, by deprotection with trifluoroacetic acid and condensation with iso(thio)cyanate (procedure B). Several analogues were synthesized by modification of the substituents. Synthetic procedures for all compounds that were evaluated for inhibition of β-PDGFR phosphorylation are described in Table 2.[16,17]

SARs for Inhibition of β-PDGFR Phosphorylation

All the analogues prepared were evaluated for their inhibition of β-PDGFR phosphorylation in accordance with known whole cell assay[14,18] and the resulting IC_{50} values are listed in Table 2. In the unsubstituted quinazolines, 4-phenoxyphenylurea analogue **3a** showed

Table 2. Inhibitory activity on β-PDGFR phosphorylation



No.	R_2	R_5	R_6	R_7	R_8	R	X	Procedure	IC_{50}[a,b] (μmol/L)
3a	H	H	H	H	H	4-PhOPh	O	Procedure A	0.38
3b	H	H	H	H	H	Bn	S	Procedure A	>30
4a	H	Me	H	H	H	4-PhOPh	O	Procedure A	6.25
4b	H	H	OMe	H	H	4-PhOPh	O	Procedure A	0.50
4c	H	H	Me	H	H	4-PhOPh	O	Procedure A	0.54
4d	H	H	F	H	H	4-PhOPh	O	Procedure A	0.35
4e	H	H	Br	H	H	4-PhOPh	O	Procedure A	1.38
4f	H	H	I	H	H	4-PhOPh	O	Procedure A	5.17
4g	H	H	NO_2	H	H	4-PhOPh	O	Procedure A	29.7
4h	H	H	H	OMe	H	4-PhOPh	O	Procedure A	0.82
4i	H	H	H	OiPr	H	4-PhOPh	O	Procedure A	1.12
4j	H	H	H	Me	H	4-PhOPh	O	Procedure A	0.10
4k	H	H	H	Cl	H	Bn	S	Procedure A	5.16
4l	H	H	H	NO_2	H	4-PhOPh	O	Procedure A	2.88
4m	H	H	H	COOMe	H	4-PhOPh	O	Procedure A	0.75
4n	H	H	H	COOH	H	4-PhOPh	O	Hydrolysis of **4m**[c]	>30
4o	H	H	H	H	OMe	4-PhOPh	O	Procedure A	10.7
5a	H	H	OMe	H	OMe	4-PhOPh	O	Procedure A	10.9
5b	H	H	H	OMe	OMe	4-PhOPh	O	Procedure A	>30
5c	H	H	F	F	H	4-PhOPh	O	Procedure B	0.97
5d	H	H	NO_2	Cl	H	4-PhOPh	O	Procedure B	1.32
5e	H	H	NO_2	Cl	H	Bn	S	Procedure B	>30
5f	H	H	NO_2	NH_2	H	4-PhOPh	O	Procedure A	1.16
5g	H	H	NO_2	NHEt	H	4-PhOPh	O	Procedure A	0.20
5h	H	H	NO_2	NHEt	H	Bn	S	Procedure A	>30
5i	H	H	NH_2	NHEt	H	Bn	S	Reduction of **5h**[d]	1.44
5j	H	H	NH_2	Cl	H	Bn	S	Reduction of **5e**[d]	0.16
5k	H	H	NHAc	Cl	H	Bn	S	Acetylation of **5j**[e]	>1
5l	H	H	OEt	OMe	H	4-PhOPh	O	Procedure B	0.04
5m	H	H	OEt	OMe	H	Bn	S	Procedure B	0.62
5n	H	H	OMe	OEt	H	4-PhOPh	O	Procedure B	0.01
5o	H	H	OMe	OEt	H	Bn	S	Procedure B	0.15
6a	H	H	OMe	OMe	OMe	4-PhOPh	O	Procedure A	>30
6b	Me	H	OMe	OMe	H	4-PhOPh	O	Procedure A	>30
6c	Cl	H	OMe	OMe	H	4-PhOPh	O	Procedure B	>30
6d	Morpholino	H	OMe	OMe	H	4-PhOPh	O	Procedure B	>30

[a]IC_{50} (μmol/mL) of β-PDGFR phosphorylation.
[b]Autophosphorylation was measured in intact cells using a two-site ELISA.[14,18]
[c]Hydrolysis was performed by LiOH in aqueous THF.
[d]Reduction was performed by Fe dust and $FeCl_3$ $6H_2O$ in aqueous ethanol under reflux condition.
[e]Acetylation was performed by Ac_2O in chloroform and triethylamine.

moderate activity, whereas the benzylthiourea analogue **3b** was devoid of any activity ($\geq$100-fold), despite the difference of activity for the corresponding 6,7-dimethoxy analogues (**1a** and **1b**) was approximately 10-fold. This discrepancy of activity indicates distinct SAR and it is unsuitable for assessing possibility to find a optimal replacement for the 6,7-dimethoxyquinazoline moiety, therefore we investigated the effect on combination of substituents on the quinazoline ring and *N*-substituted (thio)urea moiety for several potent analogues.

With regard to the quinazoline analogues with one substituent (compounds **4** series), 7-Me (**4j**) and 7-Cl (**4k**) enhanced activity comparing the unsubstituted quinazoline analogues (**3a** and **3b**); however the activity was weaker than corresponding initial dimethoxy analogues **1a** and **1b**, respectively. Other substitution (**4a**–**4i**, **4l**–**4o**) led to the retention or reduction in activity. Among methyl analogues, 7-Me (**4j**) with potent activity and 6-Me (**4c**) with moderate activity were observed, 5-Me analogue (**4a**) was a weak inhibitor. Among MeO-substituted analogues (**4b**, **4h**, **4o**), the order of potency is 6-position = 7-position»5-position. NO_2 analogues also showed similar SAR to methyl analogues, with 7-substitution (**4l**) being more potent than 6-substitution (**4g**); however, nitro group itself has a large detrimental effect for activity. Regarding the class of substituents, bulkiness had a negative effect for halogen analogues (**4d**–**4f**); however for alkoxy analogues (**4h**–**4i**) it had a relatively negligible effect on activity. Furthermore, 7-COOMe analogue (**4m**) showed modest activity, whereas 7-COOH analogue (**4n**) was inactive, suggesting that the polar substitution was not suitable, presumably due to low cellular penetration because of the whole cell assay.

Next, we investigated quinazoline analogues with two substituents (compounds **5** series). Since the marked decrease in activity was observed with the 6,8-$(OMe)_2$ (**5a**) and 7,8-$(OMe)_2$ (**5b**) analogues, 6,7-disubtitution was optimal for potent activity. The 6,7-F_2 (**5c**), 6-NO_2–7-Cl (**5d**–**5e**), and 6-NO_2–7-NH_2 (**5f**) analogues were weak inhibitors. Although the 6-NO_2–7-NHEt analogue (**5g**) showed potent activity, the discrepancy with benzylthiourea analogue (**5h**) similar to the unsubstituted quinazoline analogues (**3a** and **3b**) was observed. Reduction for nitro group of **5h** gave the 6-NH_2–7-NHEt analogue (**5i**) of modest activity. The similar enhancement was observed for 6-NO_2–7-Cl analogue (**5e**) and the resulting 6-NH_2–7-Cl analogue (**5j**), while acetylation of amino group (**5k**) reduced activity. Also, the SARs which 6-NO_2 substitution was detrimental (**5e**) and 6-NH_2 substitution was beneficial (**5j**) for 7-Cl analogue (**4k**) was clarified. Additionally, 6-OEt–7-OMe (**5l** and **5m**) and 6-OMe–7-OEt (**5n** and **5o**) analogues were equally potent compared to initial 6,7-$(OMe)_2$ analogue (**1a** and **1b**) without the discrepancy. These results indicate that 6-substitution of electron-donating group is suitable for potent activity and further modification of 6,7-dialkoxy group could be possible.[19]

Further addition of substituent on 6,7-dimethoxyquinazoline was devoid of activity. Placing an additional 8-OMe (**6a**), and 2-substitution by Me (**6b**), Cl (**6c**), and morpholine (**6d**) to 6,7-dimethoxyquinazoline also completely eliminated activity. Therefore, 6,7-dialkoxysubstitution on the quinazoline ring was optimal for the potent activity.

The 4-anilino-6,7-dimethoxyquinazolines are well-known as potent EGF receptor (EGFR) tyrosine kinase inhibitors by several groups.[20–22] The observed SARs with PDGFR and EGFR are almost similar. Both receptors preferred 6,7-dimethoxy and 6,7-diethoxy substitution and disfavoured 2-substitution and 8-substitution on quinazoline ring. These results indicate some clues for future understanding of the difference in interaction between PDGFR and EGFR with their inhibitors.

In summary, we investigated the SARs for position and variety of the substituents on quinazoline ring. 6,7-Dialkoxy substitution was optimal and further modifications of these substituents could be possible.

Acknowledgements

The technical assistance of Ms. Kumi Aoki, Miyuki Akimoto and Chika Okitsu is acknowledged. The authors gratefully appreciate Mrs. Yumiko Aono for her excellent technical assistance in chemical synthesis.

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16. (a) An example of procedure A: A mixture of 4-chloroquinazoline and excess anhydrous piperazine in 2-propanol was refluxed for 4 h. Evaporation, extraction with chloroform, and condensation with 4-phenoxyphenyl isocyanate in appropriate solvent provided the target molecule **3a**. (b) An example of procedure B: A mixture of 2,4-dichloro-6,7-dimethoxyquinazoline and *N*-*tert*-butoxycarbonylpiperazine (1.1 equiv) in THF and triethylamine was stirred overnight at room temperature. Evaporation, addition of water and NaCl provided 4-(2-chloro-6,7-dimethoxy-4-quinazolinyl)-1-piperazinecarboxylic acid *tert*-butyl ester in 98% yield. Removal of Boc group by trifluoroacetic acid in dichloromethane under ice-cooling, and then condensation with 4-phenoxyohenyl isocyanate in DMF and triethylamine provided the target molecule **6c**.
17. All final products had satisfactory ^{1}H NMR, Mass and CHN analyses.
18. CHO cells expressing wild-type β-PDGFR was grown to confluency in 96-well microtiter plates under standard tissue culture conditions. Quiescent cells were incubated at 37 °C with compound for 30 min followed by the addition of 8 nmol/L PDGF-BB for 10 min. Clarified lysates of cells by centrifugation were transfered into a second microtiter plate in which the wells were previously coated with anti-β-PDGFR mAb, and then incubated for 2 h at room temperature. After washing with binding buffer, rabbit polyclonal anti-phosphotyrosine antibody was added and plates were incubated at 37 °C for 60 min. Subsequently, each well was washed with binding buffer and incubated with horse radish peroxidase-conjugated anti-rabbit antibody at 37 °C for 60 min. Wells were washed prior to adding 2,2′-azino-bis(3-ethylbenzthiazoline-6-sulfonic acid, and the rate of substrate formation was monitored at 650 nm.
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