Structure-Activity Relationship of 4,6-Disubstituted Pyrimidines as EGFR and VEGFR-2 Tyrosine Kinase Inhibitors

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Dysregulation of the epidermal growth factor receptor (EGFR) signaling pathway by overexpression and persistent-activation has been observed in various cancers.[1] During tumor growth, angiogenesis is essential for supplying nutrients and oxygen.[2] Vascular endothelial growth factor receptor-2 (VEGFR-2) is the predominant mediator of tumor angiogenesis among many angiogenic factors.[3] For these reasons, EGFR and VEGFR-2 have been intensively explored as attractive molecular targets for anti-cancer therapy, and several tyrosine kinase inhibitors (TKIs) and monoclonal antibodies targeting EGFR or VEGFR-2 have been marketed.[1,2]

Cross-talk between the EGFR and VEGFR-2 signaling pathways can cause resistance to inhibitors of each signaling pathway.[4–6] Combination of bevacizumab (anti-VEGF-A) and erlotinib (EGFR TKI) has substantially increased progression-free survival of non-small cell lung cancer patients with EGFR-activating mutations compared to that achieved with erlotinib alone.[7] In addition, VEGFR contributes to the resistance to EGFR inhibitors in cancer cells.[8] Concurrent inhibition of EGFR and VEGFR signaling could prevent or impede resistance to EGFR TKIs, including that in patients with the EGFR T790M mutation.[9] Thus, a proper strategy for combined inhibition of EGFR and VEGFR-2 could be an effective approach for cancer treatment.

We have previously reported the structure-activity relationship (SAR) of indole tethered pyrimidines, which are able to concurrently inhibit angiokinases including EGFR and VEGFR-2.[10] Based on the previously reported **MKP** compounds (Figure 1), we further explored SAR of 4,6-disubstituted pyrimidines against EGFR and VEGFR-2. Herein, we describe the synthesis of 4,6-disubstituted pyrimidines and their inhibitory effects on EGFR and VEGFR-2 to investigate SAR.

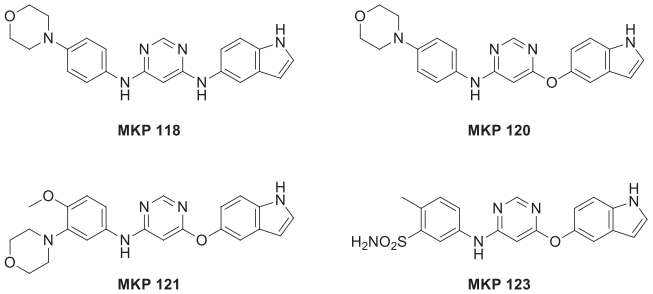


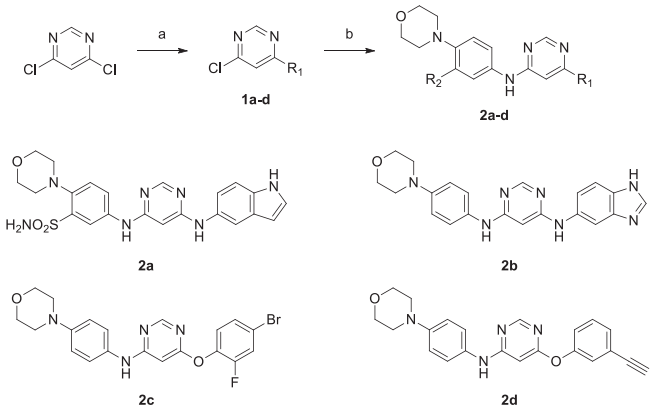
Figure 1. Structure of **MKP** compounds.[10]

The synthetic procedures for compounds **2a–d**, **4a–g**, and **6** are outlined in Schemes 1 and 2.

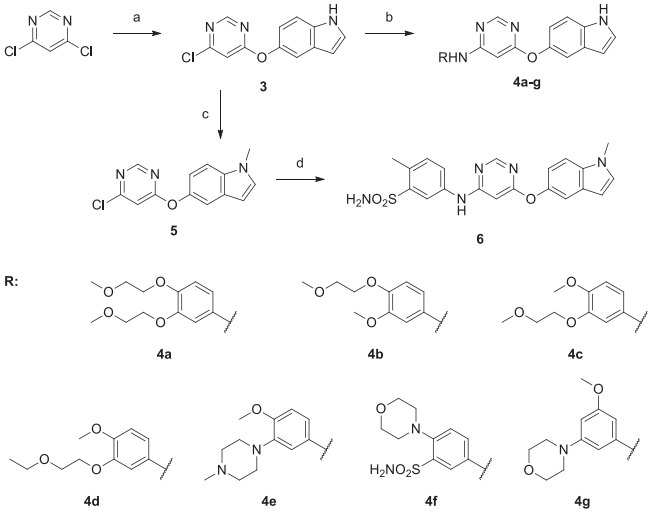
4,6-Dianilinopyrimidine derivatives **2a–b** and 4-anilino-6-phenoxy derivatives **2c–d** were prepared by two step sequence from 4,6-dichloropyrimidine (Scheme 1). Compounds **1a–d** were synthesized through the S_NAr reaction of 4,6-dichloropyrimidine with anilines or phenols. Subsequent addition of 4-morpholinoanilines to the 6-position of pyrimidine at high temperature using a microwave reactor yielded **2a–d**. 4-Anilino-6-(5-indolyloxy)-pyrimidine derivatives **4a–g** and **6** were synthesized through a similar method to the one used for preparation of **2c–d**.

All synthesized compounds were tested for inhibitory effects on EGFR and VEGFR kinase activities through the Eurofins kinase profiling service (Table 1).

Introduction of a benzimidazole instead of an indole moiety (**2b**) led to almost loss of activity against EGFR and VEGFR-2. Replacement of indole with 4-bromo-2-fluorobenzene and 3-ethynylbenzene (**2c–d**) also decreased the potency against both EGFR and VEGFR-2. Compared to that of **MKP 123**, *N*-methylated indole **6** displayed



Scheme 1. Reagents and conditions: (a) anilines or phenols, DBU, MeCN, RT, 12 h; (b) 4-morpholinoanilines, 1-BuOH, microwave, 200°C, approximately 0.5–1 h.



Scheme 2. Reagents and conditions: (a) 5-hydroxyindole, DBU, MeCN, RT, 12 h; (b) anilines, 1-BuOH, microwave, 200°C, approximately 0.5–1 h; (c) CH_3I, K_2CO_3, DMF, RT; (d) 5-amino-2-methyl benzenesulfonamide, 1-BuOH, microwave, 200°C, 0.5 h.

Table 1. Kinase activity of 4,6-disubstituted pyrimidines for EGFR and VEGFR-2.

	% inhibition at 1 μM		IC_{50} (nM)	
	EGFR	VEGFR-2	EGFR	VEGFR-2
MKP 118	100	22	7	1200
MKP 120	99	89	10	22
MKP 121	95	96	31	30
MKP 123	98	95	18	45
2a	98	98	26	357
2b	26	1	ND	ND
2c	84	38	155	155
2d	99	91	138	>3000
4a	99	92	63	74
4b	97	90	26	46
4c	95	90	27	57
4d	2	92	ND	90
4e	95	98	58	46
4f	81	95	305	37
4g	96	95	16	22
6	48	64	830	1345

ND, not determined.

strongly reduced inhibitory effect on both EGFR and VEGFR-2, indicating that the NH group of the indole ring is essential for the binding affinity of compounds to EGFR and VEGFR-2. It is assumed that the 5-hydroxyindole moiety is crucial for the ability of 4,6-disubstituted pyrimidines to bind to both EGFR and VEGFR-2.

Alkylether derivatives **4a–d** retained inhibitory effects on both targets except **4d** with a long side chain, which lost its activity for EGFR. This result suggested that the chain length could largely affect the inhibitory effect on EGFR kinase. The potency of **4e**, an *N*-methyl piperazine analog of **MKP121** slightly decreased against both EGFR and VEGFR-2 compared to those of the parent compound.

A morpholine derivative of **MKP123**, **4f** displayed a decreased activity for EGFR, but retained its activity for VEGFR-2. A regioisomer of **MKP121**, **4 g** showed more potent activities than those of **MKP121** for both kinases.

In summary, we have described the synthesis and biological evaluation of 4,6-disubstitutedpyrimidines as dual kinase inhibitors of EGFR and VEGFR-2 for extending SAR of indole substituted pyrimidines. In this scaffold, 5-indolyloxy moiety was important to show dual kinase activity. Regarding aniline moiety, various substituents were tolerable, but position and size of them affected activities and kinase selectivity. Further investigation is required to clarify the SAR of aniline moiety in detail.

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