Synthesis of (3-(2-Aminopyrimidin-4-yl)-4-hydroxyphenyl)phenyl Methanone Analogues as Inhibitors of Vascular Endothelial Growth Factor Receptor-2 Kinase

Kunal N. More and Jinho Lee*

*Department of Chemistry, Keimyung University, Daegu 42601, Korea. *E-mail: jinho@kmu.ac.kr*
Received August 30, 2016, Accepted November 23, 2016, Published online January 9, 2017

Angiogenesis is critical for tumor growth and mediated mainly by vascular endothelial growth factor (VEGF) signaling. Inhibition of the VEGF signaling pathway has emerged as one of the promising approaches for cancer therapy. VEGF receptor 2 (VEGFR-2) is considered as a major mediator of angiogenic effects of VEGF. We describe herein the discovery of a series of potent VEGFR-2 tyrosine kinase (KDR) inhibitors from a new 2-(2-aminopyrimidin-4-yl)phenol scaffold. The KDR activity was reduced appreciably by a series of compounds with a benzoyl group at position 4 of phenol ring. The structure–activity relationships for a series of (3-(2-aminopyrimidin-4-yl)-4-hydroxyphenyl)phenyl methanones revealed compound **9** (KDR IC_{50} = 25 nM) as the most potent inhibitor in the series. Compound **22** had a potent cellular activity on VEGF-induced HUVEC cell growth (IC_{50} < 0.1 μM) with high selectivity over HCT116.

Keywords: (3-(2-Aminopyrimidin-4-yl)-4-hydroxyphenyl)phenyl methanone, Antiangiogenesis, Inhibitor, KDR, Vascular endothelial growth factor receptor-2 kinase

Introduction

Angiogenesis, the growth of a new blood vessel from existing vessels, is essential for organ growth and wound healing,[1] and is also critical for tumor to grow beyond the size of the diffusion limit for oxygen. Cancer cells distant from blood vessels become hypoxic and secrete various proangiogenic factors such as vascular endothelial growth factor (VEGF), basic fibroblast growth factor (bFGF), platelet-derived growth factor (PDGF), and so on, to induce angiogenesis. Angiogenesis results in the proliferation and metastasis of cancer cells.[2] Pieces of evidence suggest that acquisition of functional blood supply is a rate-limiting step in establishment and metastasis of solid tumors. Therefore, inhibition of angiogenesis has emerged as a promising strategy for tumor therapy.[3]

The VEGF family includes VEGF-A, -B, -C, -D and placenta growth factor (PLGF). Among them, VEGF-A and its receptors are the best-characterized signaling pathways in developmental and tumor angiogenesis.[4] VEGF-A binds to receptors, VEGFR-1 and VEGFR-2. Of the two, VEGFR-2 is considered as the major mediator of mitogenic and angiogenic effects of VEGF-A.[5]

The VEGF pathway can be therapeutically inhibited by trapping the various VEGF ligands, targeting the extracellular domain of various VEGFRs, or inhibiting the intracellular signaling via small-molecule tyrosine kinase inhibitors which target the intracellular kinase domains of the VEGFR-2 (KDR). A number of small-molecule VEGFR-2 kinase inhibitors have been developed and have shown efficacy in the treatment of cancer patients (Figure 1).[6] Therapeutic outputs of drugs were found to be related to the selectivity of inhibitors over off-target kinases which was originated from structural characteristics. Thus, much research is directed toward identifying new inhibitors with different structural features which are useful for the treatment of tumors.

We tried to find a new scaffold for small molecule inhibitors of KDR using aminopyrimidine as a key moiety which had been frequently used for the development of small molecular drugs especially in the field of kinase inhibitor.[7] 2-(2-Aminopyrimidin-4-yl)phenol was chosen for a basic structure based on the report that showed 3-(2-aminopyrimidin-4-yl)-[1,1′-biphenyl]-4,4′-diol derivatives as strong CDK-2, CDK-5, and KDR inhibitors.[8] Molecular modeling studies revealed a hollow area around position 4 of phenol ring of 2-(2-aminopyrimidin-4-yl)phenol. Derivatization of the basic structure was performed through the introduction of benzoyl group at position 4 of phenol ring.[9]

Results and Discussion

The synthesis of 3-(2-aminopyrimidin-4-yl)-4-hydroxybenzophenone was performed according to Scheme 1. 2-Aminopyrimidine at position 3 of 4-hydroxybenzoate was synthesized by a series of reactions including acylation via Fries rearrangement, condensation with *N*,*N*-dimethylformamide diethyl acetal, and then cyclization with guanidine carbonate. Various phenyl groups (R^1) were

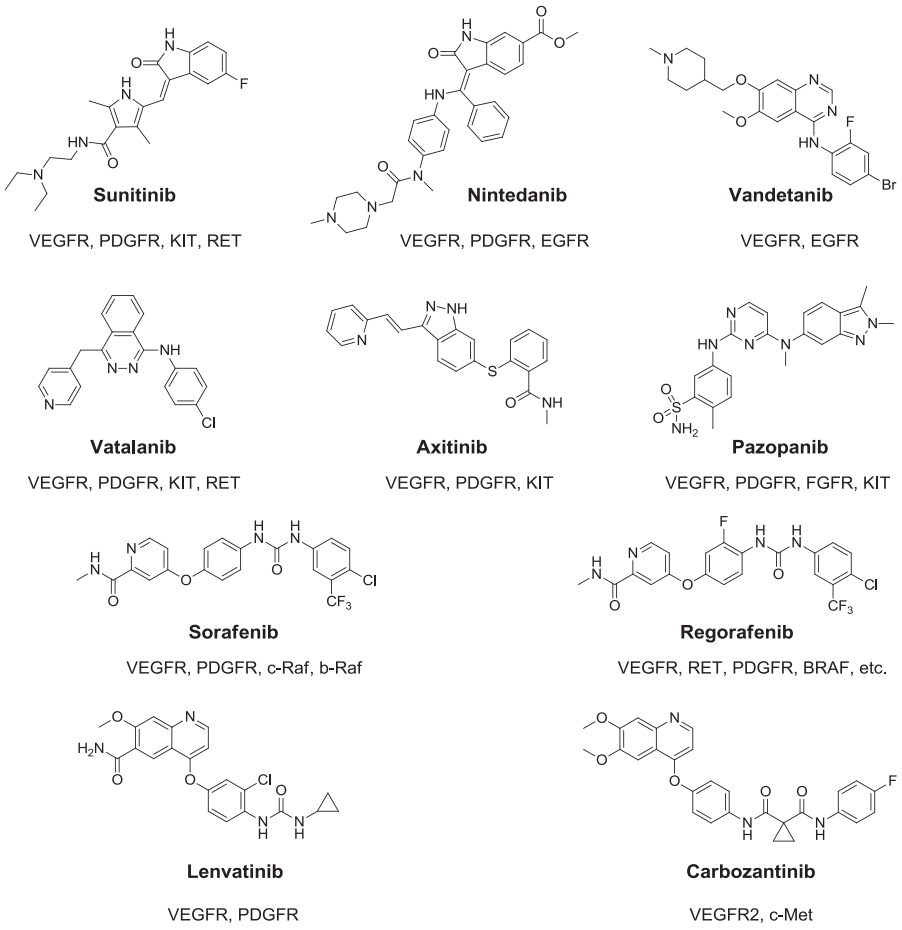


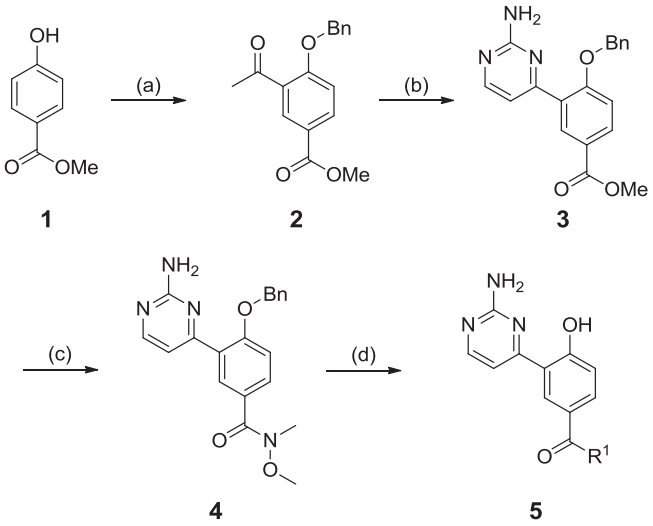
Figure 1. Structures and targets of FDA-approved VEGFR kinase inhibitors.

introduced by Weinreb–Nahm ketone synthesis[10] to make benzophenone moiety.

The introduction of a phenyl group in the form of benzophenone converted 2-(2-aminopyrimidin-4-yl)phenol, $IC_{50} > 10$ μM) to a potent KDR inhibitor (Table 1). Enzyme inhibitory activity was enhanced by 20-fold through the substitution of phenyl group of **5** with 2-fluoro-4-methylphenyl group as in **9**. 2,6-Difluorophenyl group of **7** improved the potency in growth inhibition of VEGF-induced human umbilical vein endothelial cell (HUVEC) by an order of magnitude. However, as the cellular potency increases, the selectivity over other cancer cell line decreases. Based on enzymatic, cellular inhibitory activity and selectivity data, 2-fluoro-4-methylphenyl group was chosen as the key substituent for the next optimization process.

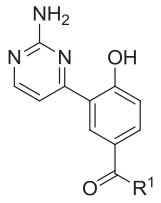
As an effort to improve the cellular potency and physicochemical properties, a substituent with hydrophilic moieties was appended at position 6 of the 2-aminopyrimidine ring. The substituent was attached at position 6 of the 2-aminopyrimidine by two different methods as shown in Scheme 2. Starting from aroylketene dithioacetal **17**, R^2-substituted aminopyrimidines were synthesized via either 6-chloro 2-aminopyrimidine **18** or 6-methylthio **19** intermediates.

Aminoalkyl linkages were used to introduce hydrophilic functionalities to the 2-aminopyrimidine ring. The structure of the hydrophilic group and the tether length had a significant effect on the cellular potency of the compound while its effect on enzymatic activity was minute (Table 2). The attachment of morpholinyl or 4-methylpiperazinyl group caused a slight reduction of enzyme inhibitory activity, **21**, **22**, **23** vs. **9**. However, those groups provided a significant improvement in the inhibitory activity of VEGF-induced HUVEC growth. Compound **22**, containing morpholinyl group linked by three methylene units, showed the highest potency in VEGF-induced HUVEC growth inhibition which was comparable to that of Sutent while more potent than Vandetanib and Vatalanib, all of which were approved KDR inhibitor. The interesting feature of compound **22** is that it shows more than two orders of magnitude selectivity over colon cancer cell line HCT116. It was suggested that a selective KDR inhibitor ultimately targets endothelial cells and should not inhibit the growth of cancer cells without VEGF receptors, such as HCT116, in the same concentration range as it inhibited VEGF-induced HUVEC growth.[11] These results suggest that a series of compounds shown in this report may have advantages over the developed inhibitors because it has



Scheme 1. Reagents and experimental conditions: (a) (i) AcCl, TEA, CH_2Cl_2, 0 °C to RT, (ii) $AlCl_3$, 180 °C, (iii) MeOH, 2.7 N HCl in EtOAc, reflux, (iv) BnBr, NaH, DMF; (b) (i) DMF · DEA, reflux, (ii) NH_2CNHNH_2 · 1/2 H_2CO_3, reflux; (c) (i) LiOH, H_2O: THF (1:1), reflux, (ii) CH_3NHOCH_3 · HCl, EDC, HOBt, TEA, DMF; (d) (i) R^1Br, n-BuLi, THF, −78 °C, (ii) BBr_3, CH_2Cl_2.

Table 1. Enzyme inhibitory and cell growth inhibitory activities of 3-(2-aminopyrimidin-4-yl)-4-hydroxybenzophenones (IC_{50} in μM).



	R^1	KDR[a]	HUVEC[b]	HCT116[c]
5	Phenyl	0.5	3.6	21
6	2-Fluorophenyl	0.14	2.5	>40
7	2,6-Difluorophenyl	0.52	0.18	3
8	2,4-Difluorophenyl	0.32	14.0	>40
9	2-Fluoro-4-methylphenyl	0.025	1.3	>40
10	4-Methylphenyl	0.12	2.0	>40
11	2,4-Dimethylphenyl	0.32	6.3	30
12	2-Fluoro-4-trifluoromethylphenyl	1.0	12.0	>40
13	4-Chlorophenyl	0.32	4.6	>40

[a] Biochemical KDR enzyme assays were performed according to the procedure described in Ref. 11.
[b] The inhibition of VEGF-induced proliferation of HUVEC was studied according to the procedure described in Ref. 11.
[c] HCT 116 cell growth inhibition assays were performed as described in Ref. 12.

distinctive structural features which provide not only strong enzyme inhibitory activities but also high selectivity on VEGF-induced HUVEC growth.

Molecular docking into the ATP-binding site of KDR was performed to speculate the binding mode of the compound. Compound **22** was used for docking study using AutoDock vina.[13] The crystal structure of KDR with PF-00337210 (2XIR) was obtained from protein data bank PDB.[14] Docking study revealed that compound **22** binds to the ATP-binding pocket as PF-00337210 where hydroxyl group interacts with backbone NH of Cys-919 in the hinge region and NH of position 6 of 2-aminopyrimidine ring interacts with backbone carbonyl oxygen of Leu-840 via hydrogen bonds (Figure 2).

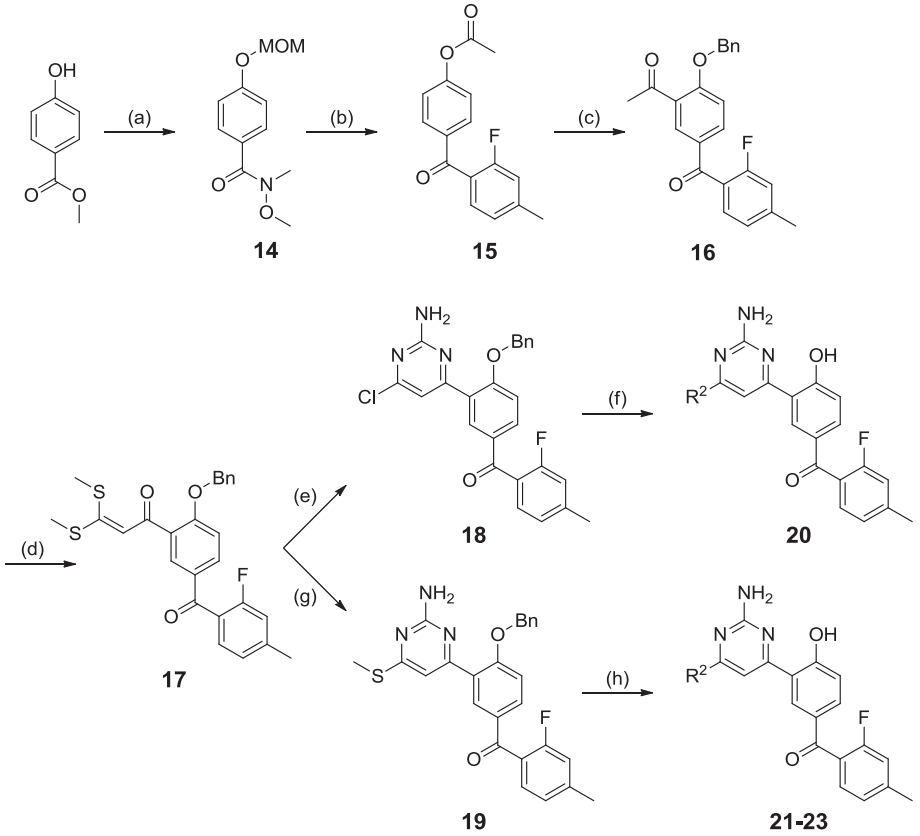
Conclusion

A series of compounds having 2-aminopyrimidine as a key structure were synthesized and evaluated for their inhibitory activity against KDR. The synthesized compound showed potent enzyme inhibitory activity as low as 0.025 μM. The inhibitory activity of a compound on VEGF-induced HUVEC growth was improved by the introduction of the hydrophilic substituent at position 6 of 2-aminopyrimidine. Compound **22** showed very potent growth inhibitory activity which was comparable to that of Sutent. As a conclusion, we found a new scaffold showing different structural feature and selectivity compared to the approved drugs. This result provides a chance that 2-aminopyrimidine derivatives can be used for the development of anticancer agent with antiangiogenic activity.

Experimental

General Methods for Synthesis. All reagents were commercially available and used as supplied without further purification. Proton magnetic resonance and carbon magnetic resonance were recorded on Bruker AVANCE 400 (^1H: 400 MHz, ^{13}C: 100 MHz) spectrometer. Mass spectra were obtained on a Jeol DX-300 instrument.

General Procedure for Compounds 5 to 13. 3-Acetyl-4-hydroxybenzoic acid: To 200 mL of dried dichloromethane (DCM) solution of 9.23 g (60.7 mmol) of methyl 4-hydroxybenzoate were added 12.7 mL (91.1 mmol) of triethylamine (TEA) and 5.2 mL (72.8 mmol) of acetyl chloride at 0 °C. After stirring for 1 h at room temperature (RT), the reaction mixture was washed with water, 1 N aq. HCl, and brine, sequentially, and dried over anhydrous $MgSO_4$. Removal of solvent *in vacuo* provided 11.10 g (57.2 mmol) of methyl 4-acetoxybenzoate which was used for next step without purification. To the above methyl 4-acetoxybenzoate was added 23 g of (170 mmol) of aluminum chloride and the mixture was stirred using mechanical stirrer at 180 °C. After 2 h, the remaining aluminum chloride was quenched with 50 mL of water, and then 50 mL of ethanol and 10 mL of 6 N aqueous HCl were added. After refluxing for 3 h, cooling to RT provided a precipitate. Solid thus obtained was separated to give 7.41 g (41.2 mmol) in 67.9% overall yield in two steps. ^1H NMR (DMSO-d_6, ppm); δ 12.89 (1H, br s),



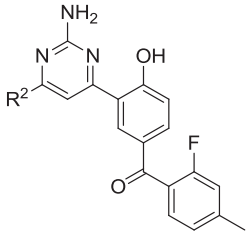
Scheme 2. Reagents and experimental conditions: (a) (i) MOMCl, K_2CO_3, DMF, (ii) LiOH, H_2O:THF (1:1), reflux, (iii) $CH_3NHOCH_3 \cdot HCl$, EDC, HOBt, TEA, DMF; (b) (i) 1-Br-2-F-4-MeC_6H_3, *n*-BuLi, THF, −78 °C, (ii) sat. HCl in EtOH, (iii) AcCl, TEA, CH_2Cl_2; (c) (i) $AlCl_3$, 180 °C, (ii) BnBr, NaH, DMF; (d) (i) LiHMDS, THF, −40 °C, CS_2, MeI; (e) (i) *p*TSA, EtOH:PhH (1:1), $NH_2CNHNH_2 \cdot 1/2\ H_2CO_3$, reflux, (ii) $POCl_3$, 100 °C; (f) (i) R^2NH_2, dioxane, reflux, (ii) BBr_3, CH_2Cl_2; (g) $NH_2CNHNH_2 \cdot HNO_3$, NaOH, DMF, 80 °C; (h) (i) *m*CPBA, CH_2Cl_2, (ii) R^2NH_2, AcCN, reflux, (iii) BBr_3, CH_2Cl_2.

12.22 (1H, s), 8.37 (1H, *J* = 2.4 Hz, d), 8.03 (1H, *J* = 8.8 Hz, 2.0 Hz, dd), 7.05 (1H, *J* = 8.8 Hz, d), 2.67 (3H, s). FAB MS (*m/e*) = 181 [M + l].

Methyl 3-acetyl-4-(benzyloxy)benzoate (2): To 7.41 g (41.2 mmol) of 3-acetyl-4-hydroxybenzoic acid in 150 mL of methanol was added 50 mL of 2.7 N HCl in ethyl acetate (EA) and the reaction mixture was refluxed for 5 h. After removal of solvent *in vacuo*, the residue was treated with EA and was washed with saturated aq. $NaHCO_3$ and brine, sequentially, then dried over anhydrous $MgSO_4$. Removal of solvent *in vacuo* provided 7.67 g (39.5 mmol) of methyl 3-acetyl-4-hydroxybenzoate and was used for next step without purification. To 60 mL of *N*,*N*-dimethylformamide (DMF) solution of methyl 3-acetyl-4-hydroxybenzoate was added 2.05 g (60%, 51.4 mmol) of sodium hydride and the reaction mixture was stirred for 10 min. After addition of 5.64 mL (47.4 mmol) of benzyl bromide, the reaction mixture was stirred for 2 h. The remaining sodium hydride was quenched with 5 mL of water and solvents were removed *in vacuo*. Residues were dissolved in 200 mL of EA and was washed with water and brine and then dried over anhydrous $MgSO_4$. After removal of solvent under reduced pressure, the crude product was purified by column chromatography (hexane/EA = 4/1, vol/vol) to give 9.31 g (32.8 mmol) in 79.6% overall yield in two steps. ^{1}H NMR ($CDCl_3$, ppm); δ 8.41 (1H, *J* = 2.4 Hz, d), 8.13 (1H, *J* = 8.8 Hz, 2.4 Hz, dd), 7.43–7.36 (5H, m), 7.07 (1H, *J* = 8.8 Hz, d), 5.23 (2H, s), 3.89 (3H, s), 2.60 (3H, s). FAB MS (*m/e*) = 285 [M + l].

Methyl 3-(2-aminopyrimidin-4-yl)-4-(benzyloxy)benzoate (3): Methyl 3-acetyl-4-(benzyloxy)benzoate (**2**) 5.39 g (19.0 mmol) was dissolved in 80 mL of *N*,*N*-dimethylformamide diethylacetal (DMF · DEA) and the resulting solution was heated to reflux for l5 h. After removal of excess DMF · DEA *in vacuo*, the residue was dissolved in 100 mL of methoxyethanol. After addition of 10.27 g (57 mmol) of guanidine carbonate, the reaction mixture was refluxed for 8 h. Methoxyethanol was removed *in vacuo* and the residue was dissolved in 200 mL of EA. The EA solution was washed with water and brine, and dried over anhydrous $MgSO_4$. The crude product thus obtained was purified by column chromatography (DCM/methanol = 95/5, vol/vol) to give 4.01 g (12.0 mmol) in 63.0% yield. ^{1}H NMR (DMSO-d_6, ppm); δ 8.49 (1H, *J* = 2.4 Hz, d), 8.19 (1H, *J* = 5.2 Hz, d), 8.11 (1H, *J* = 8.8 Hz, 2.4 Hz, dd), 7.48–7.29 (5H, m), 7.22 (1H, *J* = 8.8 Hz, d), 7.05 (1H, *J* = 5.2 Hz, d), 6.61 (2H, br s), 5.25 (2H, s), 2.65 (3H, s). FAB MS (*m/e*) = 336 [M + l].

Table 2. Enzyme inhibitory activities and cell growth inhibitory activities of compounds (IC_{50} in μM).



	R^2	KDR	HUVEC	HCT116
20	(3-(Dimethylamino) propyl)amino	0.11	2.0	7.3
21	(2-Morpholinoethyl) amino	0.09	2.2	36
22	(3-Morpholinopropyl) amino	0.09	<0.1	>40
23	(3-(4-Methylpiperazin-1-yl)propyl) amino	0.06	0.34	6.3
Sutent		0.03	0.04	4.0
Vandetanib		0.05	0.44	13.2
Vatalanib		0.06	0.19	>15

3-(2-Aminopyrimidin-4-yl)-4-(benzyloxy)-*N*-methoxy-*N*-methylbenzamide (4): To a mixture of 50 mL of water and 50 mL of tetrahydrofuran (THF) were added 2.60 g (7.76 mmol) of methyl 3-(2-aminopyrimidin-4-yl)-4-(benzyloxy)benzoate (3) and 977 mg (23.3 mmol) of lithium hydroxide. The resulting mixture was refluxed for 4 h and then THF was removed *in vacuo*. Acidification of the aqueous solution with 6 N HCl produced a white precipitate which was collected by filtration. To the 40 mL of DMF solution of 2.29 g (7.14 mmol) of the above white solid were added 1.04 g (10.7 mmol) of *N*,*O*-dimethylhydroxylamine hydrochloride, 2.74 g (14.3 mmol) of 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (EDC), 1.94 g (14.3 mmol) of 1-hydroxybenzotriazole hydrate (HOBT) and 1.49 mL (10.7 mmol) of TEA. After stirring for 3 h at RT, solvent was removed *in vacuo* and the residue was treated with 200 mL of EA. The EA solution was washed with water and brine, then dried over anhydrous $MgSO_4$. The crude product was purified by column chromatography (DCM/methanol = 95/5, vol/vol) to give 1.58 g (4.35 mmol) in 56.1 % yield. ^{1}H NMR (DMSO-d_6, ppm); δ 8.21 (1H, *J* = 5.2 Hz, d), 8.18 (1H, *J* = 2.4 Hz, d), 7.73 (1H, *J* = 8.8 Hz, 2.4 Hz, dd), 7.49–7.31 (5H, m), 7.28 (1H, *J* = 8.8 Hz, d), 7.15 (1H, *J* = 5.2 Hz, d), 6.64 (2H, s), 5.28 (2H, s), 3.56 (3H, s), 3.25 (3H, s). FAB MS (*m*/*e*) = 365 [M + 1].

(3-(2-Aminopyrimidin-4-yl)-4-hydroxyphenyl)(phenyl) methanone (5): To the 100 mL of dry THF solution of 727 mg (4.63 mmol) of monobromobenzene was added 1.85 mL (2.5 M, 4.6 mmol) of *n*-butyl lithium by dropwise at −78 °C under N_2 atmosphere. After 15 min, 3-(2-aminopyrimidin-4-yl)-4-(benzyloxy)-*N*-methoxy-*N*-methylbenzamide (**4**), 561 mg (1.54 mmol) of which was dissolved in 40 mL of dry THF, was added dropwise to the above reaction mixture and was stirred for 30 min at −78 °C. The reaction mixture was further stirred for 1 h at RT. Water was added to the reaction mixture to quench the residual lithium compounds, and the solvent was removed under reduced pressure. The residue was dissolved in 200 mL of EA and washed with water and brine, then dried over anhydrous $MgSO_4$. After removal of EA, a purification by column chromatography (DCM/methanol = 98/2, vol/vol) provided benzyl protected compound which was used for the next step. To the 50 mL of dry DCM solution of benzyl protected compound was added 2.66 mL (1.0 M, 2.66 mmol) of boron tribromide in DCM and the reaction mixture was stirred at RT for 3 h. After 5 mL of methanol was added to quench excess boron tribromide, solvent was removed *in vacuo*. The crude product was purified by column chromatography (DCM/methanol = 95:5, vol/vol) to give 213 mg (0.732 mmol) in 47.5% yield. ^{1}H NMR (DMSO-d_6, ppm); δ 14.86, (1H, s), 8.21 (1H, *J* = 5.2 Hz, d), 8.19 (1H, *J* = 2.0 Hz, d), 7.61 (1H, *J* = 8.8 Hz, 2.4 Hz, dd), 7.29 (2H, br s), 7.50 (2H, *J* = 7.2 Hz, br d), 7.44 (1H, *J* = 6.5 Hz, br t), 7.31 (3H, 1H, *J* = 5.3 Hz, q), 6.93 (1H, *J* = 5.2 Hz, d). FAB MS (*m*/*e*) = 292 [M + 1].

(3-(2-Aminopyrimidin-4-yl)-4-hydroxyphenyl)(2-fluorophenyl)methanone (6): ^{1}H NMR (DMSO-d_6, ppm); δ 14.78(1H, s), 8.39 (1H, *J* = 5.2 Hz, d), 8.34 (1H, *J* = 2.0 Hz, d), 7.72 (1H, *J* = 8.0 Hz, br d), 7.70–7.64 (1H,

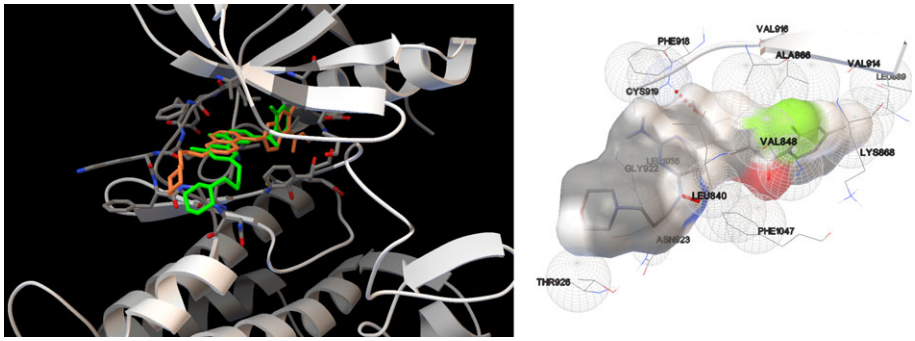


Figure 2. Docking of compound **22** (right panel, green colored) into the ATP-binding pocket of KDR (PF-00337210: orange colored). Right panel showed the interactions between compound **22** and KDR. Hydrogen bonds are shown in red.[15]

m), 7.58 (1H, J = 7.2 Hz, 1.6 Hz, td), 7.42–7.31 (2H, m), 7.31 (2H, br s), 7.18 (1H, J = 5.6 Hz, d), 7.05 (1H, J = 8.4 Hz, d). FAB MS (m/e) = 310 [M + l].

(3-(2-Aminopyrimidin-4-yl)-4-hydroxyphenyl)(2,6-difluorophenyl)methanone (7): ^{1}H NMR (DMSO-d_6, ppm); δ 14.97 (1H, s), 8.42 (1H, J = 5.4 Hz, d), 8.40 (1H, J = 2.0 Hz, d), 7.81 (1H, J = 8.8 Hz, 1.6 Hz, dd), 7.71–7.67 (1H, m), 7.32 (3H, J = 8.1 Hz, br t), 7.23 (1H, J = 5.6 Hz, d), 7.08 (1H, J = 8.8 Hz, d). FAB MS (m/e) = 328 [M + l].

(3-(2-Aminopyrimidin-4-yl)-4-hydroxyphenyl)(2,4-difluorophenyl)methanone (8): ^{1}H NMR (DMSO-d_6, ppm); δ 15.04 (1H, s), 8.41 (1H, J = 5.2 Hz, d), 8.39 (1H, J = 2.0 Hz, d), 8.06–8.00 (1H, m), 7.80 (1H, J = 8.8 Hz, 2.0 Hz, dd), 7.34 (2H, J = 8.4 Hz, 1.6 Hz, td), 7.32 (2H, br s), 7.29 (1H, J = 5.4 Hz, d), 7.07 (1H, J = 8.8 Hz, d). FAB MS (m/e) = 328 [M + l].

(3-(2-Aminopyrimidin-4-yl)-4-hydroxyphenyl)(2-fluoro-4-methylphenyl)methanone (9): ^{1}H NMR (DMSO-d_6, ppm); δ 15.10 (1H, s), 8.40 (1H, J = 5.2 Hz, d), 8.31 (1H, J = 2.0 Hz, d), 7.72 (1H, J = 8.8 Hz, 2.0 Hz, dd), 7.47 (1H, J = 6.2 Hz, t), 7.28 (2H, br s), 7.21 (2H, J = 11.2 Hz, t), 7.18 (1H, J = 5.2 Hz, d), 7.04 (1H, J = 8.8 Hz, d), 2.42 (3H, s). FAB MS (m/e) = 324 [M + l].

(3-(2-Aminopyrimidin-4-yl)-4-hydroxyphenyl)(4-methylphenyl)methanone (10): ^{1}H NMR (DMSO-d_6, ppm); δ 14.58, (1H, s), 8.39 (1H, J = 5.6 Hz, d), 8.30 (1H, J = 2.0 Hz, d), 7.75 (1H, J = 8.8 Hz, 2.0 Hz, dd), 7.65 (2H, J = 8.0 Hz, d), 7.37 (2H, J = 8.0 Hz, d), 7.28 (2H, br s), 7.19 (1H, J = 5.6 Hz, d), 7.05 (1H, J = 8.8 Hz, d), 2.42 (3H, s). FAB MS (m/e) = 306 [M + l].

(3-(2-Aminopyrimidin-4-yl)-4-hydroxyphenyl)(2,4-dimethylphenyl)methanone (11): ^{1}H NMR (DMSO-d_6, ppm); δ 15.06 (1H, s), 8.46 (1H, J = 5.6 Hz, d), 8.35 (1H, J = 2.0 Hz, d), 7.70 (1H, J = 8.8 Hz, 2.0 Hz, dd), 7.41 (1H, J = 5.6 Hz, d), 7.29 (2H, br s), 7.24–7.09 (4H, m), 2.36 (3H, s), 2.23 (3H, s). FAB MS (m/e) = 320 [M + l].

(3-(2-Aminopyrimidin-4-yl)-4-hydroxyphenyl)(2-fluoro-4-(trifluoromethyl)phenyl)methanone (12): ^{1}H NMR (DMSO-d_6, ppm); δ 15.01 (1H, s), 8.39 (1H, J = 5.6 Hz, d), 8.36 (1H, J = 2.0 Hz, d), 7.80–7.72 (4H, m), 7.27 (2H, br s), 7.18 (1H, J = 5.6 Hz, d), 7.03 (1H, J = 8.8 Hz, d). FAB MS (m/e) = 378 [M + l].

(3-(2-Aminopyrimidin-4-yl)-4-hydroxyphenyl)(4-chlorophenyl)methanone (13): ^{1}H NMR (DMSO-d_6, ppm); δ 14.96 (1H, s), 8.43 (1H, J = 5.6 Hz, d), 8.39 (1H, J = 2.0 Hz, d), 7.84 (1H, J = 8.8 Hz, 2.0 Hz, dd), 7.66 (2H, J = 8.0 Hz, d), 7.64 (2H, J = 8.0 Hz, d), 7.49 (1H, J = 5.6 Hz, d), 7.29 (2H, br s), 7.14 (1H, J = 8.8 Hz, d). FAB MS (m/e) = 326 [M + l].

General Procedure for Compounds 20 to 23. *N*-Methoxy-4-(methoxymethoxy)-*N*-methylbenzamide (14): To the 150 mL of DMF solution of 9.23 g (60.7 mmol) of methyl 4-hydroxybenzoate were added 16.8 g (91.1 mmol) of potassium carbonate and 6.00 mL (78.9 mmol) of chloromethyl methyl ether. After stirring for 3 h and removal of solvent, the residue was dissolved in 400 mL of EA. The EA solution was washed with water and brine, then dried over anhydrous $MgSO_4$. The crude product was purified by column chromatography (hexane/EA = 10/1, vol/vol) to give 8.56 g (43.7 mmol) of methyl 4-(methoxymethoxy)benzoate in 72.0% yield. To a mixture of 100 mL of water and 100 mL of THF were added 8.56 g (43.7 mmol) of methyl 4-(methoxymethoxy)benzoate and 2.75 g (65.6 mmol) of lithium hydroxide. After refluxing for 4 h, THF was removed *in vacuo*. Acidification of the aqueous solution with 6 N HCl produced a white precipitate which was collected by filtration. To the 100 mL of DMF solution of 6.60 g (36.3 mmol) of the above white solid were added 5.31 g (54.5 mmol) of *N*,*O*-dimethylhydroxyamine hydrochloride, 13.9 g (72.6 mmol) of EDC, 9.85 g (72.6 mmol) of HOBT, and 7.60 mL (54.5 mmol) of TEA. After 3 h of stirring at RT and removal of solvent, the residue was treated with 500 mL of EA. The EA solution was washed with water and brine, then dried over anhydrous $MgSO_4$. The crude product was purified by column chromatography (DCM/methanol = 95/5, vol/vol) to give 6.39 g (28.4 mmol) in 65.0% yield. ^{1}H NMR ($CDCl_3$, ppm); δ 7.84 (2H, J = 7.6 Hz, d), 6.88 (2H, J = 7.6 Hz, d), 6.12 (2H, s), 3.56 (3H, s), 3.49 (3H, s), 3.25 (3H, s). FAB MS (m/e) = 226 [M + l].

(2-Fluoro-4-methylphenyl)(4-(methoxymethoxy)phenyl)methanone: To the 50 mL of dry THF solution of 2.92 mL (23. l mmol) of 4-bromo-3-fluorotoluene was added 8.0 mL (2.5 M, 20 mmol) of *n*-butyl lithium by dropwise at −78 °C under N_2 atmosphere. After 15 min, *N*-methoxy-4-(methoxymethoxy)-*N*-methylbenzamide (**14**), 3.46 g (15.4 mmol) of which was dissolved in 30 mL of dry THF, was added dropwise to the above reaction mixture and stirred for 30 min at −78 °C. The reaction mixture was allowed to warm to RT over 1 h and then further stirred for 1 h. Water was added to the reaction mixture to quench the residual lithium compounds, and the solvent was removed *in vacuo*. The residue was dissolved in 200 mL of EA and washed with water and brine, then dried over anhydrous $MgSO_4$. After removal of solvent, a purification by column chromatography (hexane/EA = 10/1, vol/vol) to give 4.32 g (15.8 mmol) in 68.3% yield. ^{1}H NMR ($CDCl_3$, ppm); δ 7.84 (2H, J = 9.0 Hz, 1.0 Hz, dd), 7.45 (1H, J = 7.6 Hz, t), 7.14 (3H, J = 9.6 Hz, br t), 6.88 (1H, J = 12.0 Hz, br d), 5.27 (2H, s), 3.52 (3H, s), 2.45 (3H, s). FAB MS (m/e) = 275 [M + l].

4-(2-Fluoro-4-methylbenzoyl)phenyl acetate (15): To the 20 mL of ethanol, saturated with HCl, was added 4.99 g (18.2 mmol) of (2-fluoro-4-methylphenyl)-(4-(methoxymethoxy)phenyl)methanone and stirred at RT for 2 h. After removal of solvent, the residue was dissolved in 150 mL of DCM. To the solution were added 3.81 mL (27.3 mmol) of TEA and 1.56 mL (21.8 mmol) of acetyl chloride and the reaction mixture was stirred for 1 h. The reaction mixture was washed with water, 1 N HCl and brine, then dried over anhydrous $MgSO_4$. The crude product was purified by column chromatography (hexane/EA = 10/1, vol/vol) to give 4.65 g (17.1 mmol) in 93.9% yield. ^{1}H NMR ($CDCl_3$, ppm); δ 7.88 (2H, J = 8.6 Hz, 1.0 Hz,

dd), 7.48 (1H, J = 7.6 Hz, t), 7.22 (2H, J = 8.8 Hz, d), 7.09 (1H, J = 7.8 Hz, 0.6 Hz, dd), 6.98 (1H, J = 12 Hz, br d), 2.46 (3H, s), 2.35 (3H, s). FAB MS (m/e) = 273 [M + l].

1-(2-(Benzyloxy)-5-(2-fluoro-4-methylbenzoyl)phenyl) ethanone (16): To the 4.65 g (17.1 mmol) of the compound **15** was added 6.90 g (51.3 mmol) of aluminum chloride and the reaction mixture was stirred with a mechanical stirrer at 180 °C for 2 h. After quenching the reaction with 150 mL of water, 150 mL of ethanol and 50 mL of 6 N aqueous HCl were added and the reaction mixture was refluxed for 3 h. A yellow precipitate, formed while cooling the reaction mixture to RT, was collected by filtration. To the 70 mL of DMF solution of the obtained solid was added 850 mg (60%, 21 mmol) of sodium hydride and the reaction mixture was stirred for 10 min. To the above solution was added 2.20 mL (18.5 mmol) of benzyl bromide, and the mixture was stirred for 2 h. After addition of 5 mL of water, solvents were removed *in vacuo*. The residue was dissolved in 200 mL of EA and washed with water and brine, then dried over anhydrous $MgSO_4$. The crude product was purified by column chromatography (hexane/EA = 8/1, vol/vol) to give 4.53 g (12.5 mmol) at 73.1% yield. ^{1}H NMR (DMSO-d6, ppm); δ 7.96 (1H, s), 7.96 (1H, J = 7.2 Hz, d), 7.54 (2H, J = 7.2 Hz, d), 7.47–7.35 (5H, m), 7.22 (1H, J = 11.6 Hz, d), 7.20 (1H, J = 8.0 Hz, d), 5.37(2H, s), 2.52 (3H, s), 2.42 (3H, s). FAB MS (m/e) = 363 [M + l].

1-(2-(Benzyloxy)-5-(2-fluoro-4-methylbenzoyl)phenyl)-3,3-bis(methylsulfanyl)-2-propen-1-one (17): To 3.6 g (9.9 mmol) of the compound **16** in 20 mL of dry THF was added 18.8 mL (18.8 mmol) of 1.0 M lithium hexamethyldisilazide (in THF) at −40 °C. After stirring for 20 min, 0.56 mL (11.9 mmol) of CS_2 was added and then the mixture was stirred for 2 h. After addition of 1.54 mL (24. 7 mmol) of methyl iodide, the mixture was stirred for additional 4 h. After the completion of the reaction, 40 mL of hexane was added and then the reaction mixture was further stirred for 4 h to produce a yellow precipitate. The precipitate was obtained by filtration and was washed with water. Removal of water by blowing nitrogen gas gave 4.1 g (8.7 mmol) in 88% yield. ^{1}H NMR ($CDCl_3$, ppm); δ 8.23 (1H, J = 2.2 Hz, t), 8.08 (1H, J = 8.8 Hz, 2.4 Hz, dd), 7.47–7.36 (7H, m), 7.14 (1H, J = 8.8 Hz, d), 7.05 (1H, J = 7.6 Hz, d), 6.95 (1H, J = 10.8 Hz, d), 6.85 (1H, s), 5.20 (2H, s), 2.47 (3H, s), 2.42 (3H, s), 1.90 (3H , s). FAB MS (m/e) = 460 [M + l].

(3-(2-Amino-6-chloropyrimidin-4-yl)-4-(benzyloxy) phenyl)(2-fluoro-4-methylphenyl)methanone (18): To the mixture of ethanol and benzene (10 mL each) were added 960 mg (2.1 mmol) of the compound **17** and 1.96 g (10.2 mmol) of *p*-toluenesulfonic acid hydrate, and the mixture was refluxed for 6 h. After confirming that the starting material was fully dissolved, 1.85 g (10.2 mmol) of guanidine carbonate was added and the mixture was refluxed for 6 h. After removal of solvent, the residue was treated with DCM and washed with water. The purification of the crude product by column chromatography provided 2-amino-6-[2-(benzyloxy)-5-(2-fluoro-4-methylbenzoyl) phenyl]-4(3*H*)-pyrimidinone in 49% yield. Four-hundred milligrams (0.93 mmol) of the above compound was dissolved thoroughly in 4 mL of phosphorous oxychloride, then the solution was stirred for 10 min at 100 °C. The reaction mixture was concentrated and then neutralized with aqueous ammonia at 0 °C. A yellow precipitate thus obtained was filtered and dried to provide 310 mg (0.69 mmol) in 74% yield. ^{1}H NMR ($CDCl_3$, ppm); δ 8.39 (1H, J = 2.4 Hz, d), 7.91 (1H, J = 8.8 Hz, 2.0 Hz, dt), 7.43 (1H, J = 6.2 Hz, t), 7.40–7.36 (5H, m), 7.31(1H, s), 7.26 (1H, s), 7.10 (1H, J = 8.8 Hz, d), 7.06 (1H, J = 8.0 Hz, d), 6.96 (1H, J = 10.8 Hz, d), 5.26 (2H, s), 2.43 (3H, s). FAB MS (m/e) =448 [M + l].

(3-(2-Amino-6-((3-(dimethylamino)propyl)amino)pyrimidin-4-yl)-4-hydroxyphenyl)(2-fluoro-4-methylphenyl) methanone (20): To the 2 mL of dioxane solution of 36 mg (0.080 mmol) of the compound **18** was added 245 mg (1.70 mmol) of *N*,*N*-dimethyl-1,3-propandiamine, and the mixture was refluxed for 8 h. EA was added to the cooled reaction mixture and it was washed with water twice, then dried over anhydrous $MgSO_4$. The benzyl protected compound, obtained from above reaction, was dissolved in 2 mL of DCM, and 1 mL of 1 N boron tribromide (in DCM) was added thereto. After stirring for 4 h, methanol was added to the reaction mixture. A purification by column chromatography (chloroform/methanol/ammonium hydroxide = 100/10/1, vol/vol/vol) gave 11 mg in 28% yield. ^{1}H NMR ($CDCl_3$, ppm); δ 8.24 (1H, J = 2.4 Hz, d), 7.71 (1H, J =8.8 Hz, d), 7.39 (1H, J = 6.2 Hz, t), 7.15 (1H, J = 6.0 Hz, d), 6.86 (1H, J = 8.8 Hz, d), 6.92 (1H, J = 6.8 Hz, d), 6.24 (1H, s), 3.49 (2H, J = 7.2 Hz, t), 2.85 (2H, J = 7.2 Hz, t), 2.26 (6H, s), 2.44 (3H, s), 1.92 (2H, J = 6.2 Hz, quint). FAB MS (m/e) = 424 [M + l].

(3-(2-Amino-6-(methylsulfanyl)-4-pyrimidinyl)-4-(benzyloxy)phenyl)(2-fluoro-4-methylphenyl)methanone (19): To the 10 mL of DMF solution of 368 mg (3.02 mmol) of guanidine nitrate was added 161 mg (4.03 mmol) of NaOH. To the above mixture was added 940 mg (2.1 mmol) of the compound **17** and the mixture was heated to 80 °C for 4 h. The reaction mixture was poured into ice water and neutralized with 1 N HCl. A white precipitate thus obtained was filtered and dried to give 730 mg in 78% yield. ^{1}H NMR ($CDCl_3$, ppm); δ 8.41 (1H, J = 2.4 Hz, d), 7.89 (1H, J = 8.8 Hz, 2.0 Hz, dt), 7.47–7.33 (6H, m), 7.14 (1H, s), 7.10 (1H, J = 8.8 Hz, d), 7.06 (1H, J = 8.0 Hz, d), 6.96 (1H, J = 10.8 Hz, d), 5.22 (2H, s), 4.95 (2H, s), 2.43 (3H, s), 2.34 (3H, s). FAB MS (m/e) =460 [M + l].

(3-(2-Amino-6-(methylsulfonyl)pyrimidin-4-yl)-4-(benzyloxy)phenyl)(2-fluoro-4-methylphenyl)methanone: To the 20 mL of DCM solution of 360 mg (0.78 mmol) of the compound **19** was added 676 mg (3.92 mmol) of

3-chloroperbenzoic acid and the mixture was stirred for 6 h. After addition of isopropyl alcohol, the mixture was stirred for 20 min. After removal of solvent, the residue was treated with DCM and DCM solution was washed several times with saturated aqueous sodium bicarbonate and dried over anhydrous $MgSO_4$. The crude product was purified by column chromatography (hexane/EA = 1/1, vol/vol) to give 82 mg in 22 % yield. ^{1}H NMR ($CDCl_3$, ppm); δ 8.18 (1H, *J* = 2.4 Hz, d), 8.02 (1H, *J* = 8.8 Hz, 2.0 Hz, dt), 7.54 (1H, s), 7.45 (1H, *J* = 6.2 Hz, t), 7.43–7.31 (5H, m), 7.16 (1H, *J* = 8.8 Hz, d), 7.06 (1H, *J* = 8.0 Hz, d), 6.97 (1H, *J* = 10.8 Hz, d), 5.22 (2H, s), 3.13 (3H, s), 2.42 (3H, s). FAB MS (*m*/*e*) =492 [M + l].

(3-(2-Amino-6-((3-morpholinopropyl)amino)pyrimidin-4-yl)-4-(benzyloxy)phenyl)(2-fluoro-4-methylphenyl) methanone: To the 2 mL of acetonitrile solution of 30 mg (0.061 mmol) of (3-(2-amino-6-(methylsulfonyl)pyrimidin-4-yl)-4-(benzyloxy)phenyl)(2-fluoro-4-methylphenyl)methanone was added 0.18 mL of 4-(3-aminopropyl)morpholine and the reaction mixture was refluxed for 8 h. After solvent was removed, the residue was treated with EA and washed with water. The crude product was purified by column chromatography (chloroform/methanol/ammonium hydroxide = 100/10/1, vol/vol/vol) to give 26 mg in 76% yield. ^{1}H NMR ($CDCl_3$, ppm); δ 8.30 (1H, *J* = 2.4 Hz, d), 7.85 (1H, *J* = 8.8 Hz, 2.0 Hz, dt), 7.45–7.23 (6H, m), 7.06 (1H, *J* = 8.4 Hz, t), 6.94 (1H, *J* = 10.8 Hz, d), 6.30 (1H, s), 5.75 (1H, s), 5.21 (2H, s), 4.80 (2H, s), 3.78–3.62 (4H, m), 3.34–3.18 (2H, m), 2.42 (9H, s), l.65 (2H, *J* = 6.2 Hz, t). FAB MS (*m*/*e*) =556 [M + l].

(3-(2-Amino-6-((3-morpholinopropyl)amino)pyrimidin-4-yl)-4-hydroxyphenyl)(2-fluoro-4-methylphenyl)methanone (22): To the 2 mL of DCM solution of 26 mg (0.053 mmol) of (3-(2-amino-6-((3-morpholinopropyl)amino) pyrimidin-4-yl)-4-(benzyloxy)phenyl)(2-fluoro-4-methylphenyl)methanone was added 1 mL of 1 N boron tribromide (in DCM) and the mixture was stirred for 4 h. After methanol was added, the resultant mixture was concentrated and then purified by column chromatography (chloroform/methanol/ammonium hydroxide = 100/10/1, vol/vol/vol) to give 11 mg in 58% yield. ^{1}H NMR ($CDCl_3$, ppm); δ 8.31 (1H, *J* = 2.4 Hz, d), 7.71 (1H, *J* = 8.8 Hz, 2.0 Hz, dt), 7.42 (1H, *J* = 6.2 Hz, t), 7.07 (1H, *J* = 6.4 Hz, d), 6.98 (1H, *J* = 8.8 Hz, d), 6.94 (1H, *J* = 6.8 Hz, d), 6.22 (1H, s), 6.16 (1H, s), 4.80 (2H, s), 3.79–3.69 (4H, m), 3.58–3.38 (2H, m), 2.60–2.42 (6H, m), 2.44 (3H, s), l.72 (2H, *J* = 6.2 Hz, t). FAB MS (*m*/*e*) = 466 [M + l].

(3-(2-Amino-6-((2-morpholinoethyl)amino)pyrimidin-4-yl)-4-hydroxyphenyl)(2-fluoro-4-methylphenyl)methanone (21): ^{1}H NMR ($CDCl_3$, ppm); δ 8.39 (1H, *J* = 2.4 Hz, d), 7.64 (1H, *J* = 8.8 Hz, 2.0 Hz, dt), 7.43 (1H, *J* = 6.2 Hz, t), 7.07 (1H, *J* = 6.4 Hz, d), 6.98 (1H, *J* = 8.8 Hz, d), 6.92 (1H, *J* = 6.8 Hz, d), 6.34 (1H, s), 5.57 (1H, s), 4.82 (2H, s), 3.87–3.65 (4H, m), 3.49–3.43 (2H, m), 2.66–2.58 (2H, m), 2.55–2.47 (4H, m), 2.43 (3H, s). FAB MS (*m*/*e*) = 452 [M + l].

(3-(2-Amino-6-((3-(4-methylpiperazin-1-yl)propyl)amino) pyrimidin-4-yl)-4-hydroxyphenyl)(2-fluoro-4-methylphenyl) methanone (23): ^{1}H NMR ($CDCl_3$, ppm); δ 8.36 (1H, *J* =2.4 Hz, d), 7.64 (1H, *J* =8.8 Hz, 2.0 Hz, dt), 7.42 (1H, *J* = 6.2 Hz, t), 7.06 (1H, *J* = 6.4 Hz, d), 6.98 (1H, *J* = 8.8 Hz, d), 6.92 (1H, *J* = 6.8 Hz, d), 6.27 (2H, s), 4.80 (2H, s), 3.51–3.39 (2H, m), 2.53–2.47 (10H, m), 2.43 (3H, s), 2.32 (3H, s). FAB MS (*m*/*e*) = 479 [M + l].

Acknowledgment. This research was supported by the Bisa Research Grant (20130335) of Keimyung University in 2013.

References

1. P. Carmeliet, *Nature* **2005**, *438*, 932.
2. P. Carmeliet, *Nat. Med.* **2000**, *6*, 389.
3. P. Bhargava, M. Robinson, *Curr. Oncol. Rep.* **2011**, *13*, 103.
4. (a) H. F. Dvorak, *J. Thromb. Haemost.* **2005**, *3*, 1835; (b) M. J. Cross, J. Dixelius, T. Matsumoto, L. Claesson-Welsh, *Trends Biochem. Sci.* **2003**, *28*, 488.
5. A.-K. Olsson, A. Dimberg, J. Kreuger, L. Claesson-Welsh, *Nat. Rev. Mol. Cell Biol.* **2006**, *7*, 359.
6. (a) M. McTigue, B. W. Murray, J. H. Chen, Y.-L. Deng, J. Solowiej, R. S. Kania, *Proc. Natl. Acad. Sci. U.S.A.* **2012**, *109*, 18281; (b) A. Levitzki, *Annu. Rev. Pharmacool. Toxicol.* **2013**, *53*, 161; (c) P. Wu, T. E. Nielsen, M. H. Clausen, *Trends Pharmacol. Sci.* **2015**, *36*, 422.
7. L. Xing, B. Rai, E. Lunney, *J. Comput. Aided Mol. Des.* **2014**, *28*, 13.
8. S. Cao, P. Y. Bounaud, X. Chen, H. H. Chung, K. C. Sunil Kumar, C. Min, J. Yang, M. Long, Inhibitors of protein kinase for the treatment of disease, 2003, US20030187007.
9. J. Lee, H. J. Kim, S. Choi, H. G. Choi, S. Yoon, J.-H. Kim, K. Jo, S. Kim, S.-Y. Koo, M.-H. Kim, J. I. Kim, S.-Y. Hong, M. S. Kim, S. Ahn, H.-S. Yoon, H.-S. Cho, Cho, Novel 3-(2-amino-4-pyrimidinyl)-4-hydroxyphenyl ketone derivatives, 2004, WO2004080979
10. S. Nahm, S. M. Weinreb, *Tetrahedron Lett.* **1981**, *22*, 3815.
11. J. M. Wood, G. Bold, E. Buchdunger, R. Cozens, S. Ferrari, J. Frei, F. Hofmann, J. Mestan, H. Mett, T. O'Reilly, E. Persohn, J. Rösel, C. Schnell, D. Stover, A. Theuer, H. Towbin, F. Wenger, K. Woods-Cook, A. Menrad, G. Siemeister, M. Schirner, K.-H. Thierauch, M. R. Schneider, J. Drevs, G. Martiny-Baron, F. Totzke, D. Marmé, *Cancer Res.* **2000**, *60*, 2178.
12. P. M. Fresneda, S. Delgado, A. Francesch, I. Manzanares, C. Cuevas, P. Molina, *J. Med. Chem.* **2006**, *49*, 1217.
13. O. Trott, A. J. Olson, *J. Comput. Chem.* **2010**, *31*, 455.
14. T. Marrone, D. Hu-Lowe, M. Grazzini, M.-J. Yin, J. Chen, M. Hallin, K. Amundson, S. Yamazaki, D. Romero, A. McHarg, E. Blasi, Y. Hong, E. Tompkins, C. Palmer, J. Deal, B. Murray, J. Solowiej, M. McTigue, J. Wickersham, S. Bender, *Cancer Res.* **2007**, *67*, 3992.
15. M. F. Sanner, *J. Mol. Graph. Model.* **1999**, *17*, 57.

