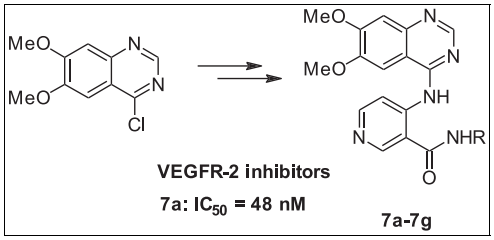
6,7-Dimethoxy-Quinazolin-4-yl-Amino-Nicotinamide Derivatives as Potent Inhibitors of VEGF Receptor II

Abhishek Ashok,[a]* Kannan Thanukrishnan,[a] Halehatty S. Bhojya Naik,[b] and Soma Ghosh[a]

[a]Anthem Biosciences Pvt. Ltd, 49 Bommasandra Industrial Area, Bommasandra, Bangalore 560099, Karnataka, India
[b]Department of PG Studies and Research in Industrial Chemistry, School of Chemical Sciences, Kuvempu University, Shankaraghatta, Karnataka 577451, India
*E-mail: abhickm@gmail.com

Additional Supporting Information may be found in the online version of this article.
Received March 23, 2016
DOI 10.1002/jhet.2750
Published online 00 Month 2016 in Wiley Online Library (wileyonlinelibrary.com).



The sprouting of new blood vessels, or angiogenesis, is necessary for any solid tumor to grow large enough to cause life-threatening disease. Vascular endothelial growth factor (VEGF) is one of the key promoters of tumor-induced angiogenesis. Inhibition of the VEGF signaling pathway has emerged as one of the most promising new approaches for cancer therapy. A series of 6,7-dimethoxy-quinazolin-4-yl-amino-nicotinamides were synthesized and evaluated as antagonists of VEGF receptor II (VEGFR-2). Many compounds display VEGFR-2 inhibitory activity, and compound 7a was found to be a potent inhibitor of VEGFR-2 in an homogeneous time-resolved fluorescence enzymatic assay with an IC_{50} as low as 48 nM (comparable activity to ZD-6474).

J. Heterocyclic Chem., **00,** 00 (2016).

INTRODUCTION

Tumor-induced angiogenesis is the term that describes the induction of blood vessels from existing vasculature of the site of a solid tumor, critical for tumor growth and dissemination [1,2]. Under hypoxic conditions, tumors induce angiogenesis by secreting vascular endothelial growth factor (VEGF). VEGF has been considered to play a major role in angiogenesis, the formation of new vasculature from an existing vascular network [3]. Endothelial cells lining the walls of neighboring blood vessels bind VEGF to cell surface VEGF receptor II (also known as KDR; murine version known as Flk-1), triggering a signaling cascade which leads to endothelial cell proliferation, migration and increasing vascular permeability in the tumor milieu [4,5]. VEGFR-2 is a membrane-bound tyrosine kinase receptor requiring ligand binding and dimerization for transphosphorylation of the receptors and subsequent substrates [6]. A number of small-molecule inhibitors affect VEFG/VEGFR signaling by directly competing with the ATP-binding site of the respective intracellular kinase domain. Of various VEGFR-2 inhibitors, the most successful marketed agent reportedly working via this mechanism is Avastin (Genentech, San Francisco, CA) [7,8]. Other drug candidates that exhibit this mechanism of action include *Novartis*' (Basel, Switzerland) PTK 787 A (Vatalanib) and *AstraZeneca*'s (Wilmington, DE) ZD6474 B (Vandetanib) (Fig. 1).

In addition, a number of nicotinamide derivatives have been detailed as potent inhibitors of VEGF [9–13]. We anticipated that one of the vital pharmacophore of ZD6474 and their analogues include the quinazoline ring and it was thought that it would be worthwhile to synthesize some new quinazoline derivatives bearing a nicotinamide derivative at the C-4 position of 6,7-dimethoxyquinazoline. Nevertheless, to the best of our knowledge, although several quinazoline carboxamide series have been developed as tyrosine kinase inhibitors, nicotinamide derivatives of 6,7-dimethoxyquinazoline have not been explored as VEGFR inhibitors. In this communication, we disclose a series of novel 6,7-dimethoxy-quinazolin-4-yl-amino-nicotinamide derivatives as potent inhibitors of VEGF-2.

RESULTS AND DISCUSSION

Chemistry. The new quinazoline derivatives described herein were synthesized as shown in Scheme 1. Previously described methods were used for synthesis of 6,7-dimethoxyquinazoline derivatives 2 [14], 3 [15] and 4 [16]. Ethyl-4-aminopyridine-3-carboxylate was commercially available. Reaction of known compound 4 with ethyl-4-aminopyridine-3-carboxylate in 2-propanol at 80°C yielded compound 5. Compound 5 was

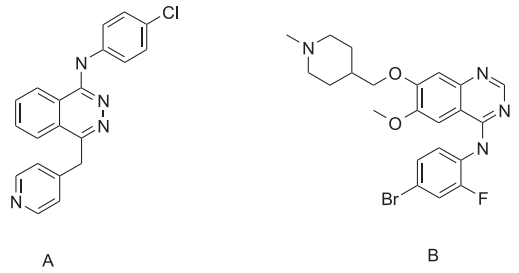


Figure 1. Chemical structures of PTK 787(A) and ZD 6474(B).

hydrolyzed by using lithium hydroxide to get the corresponding acid 6.

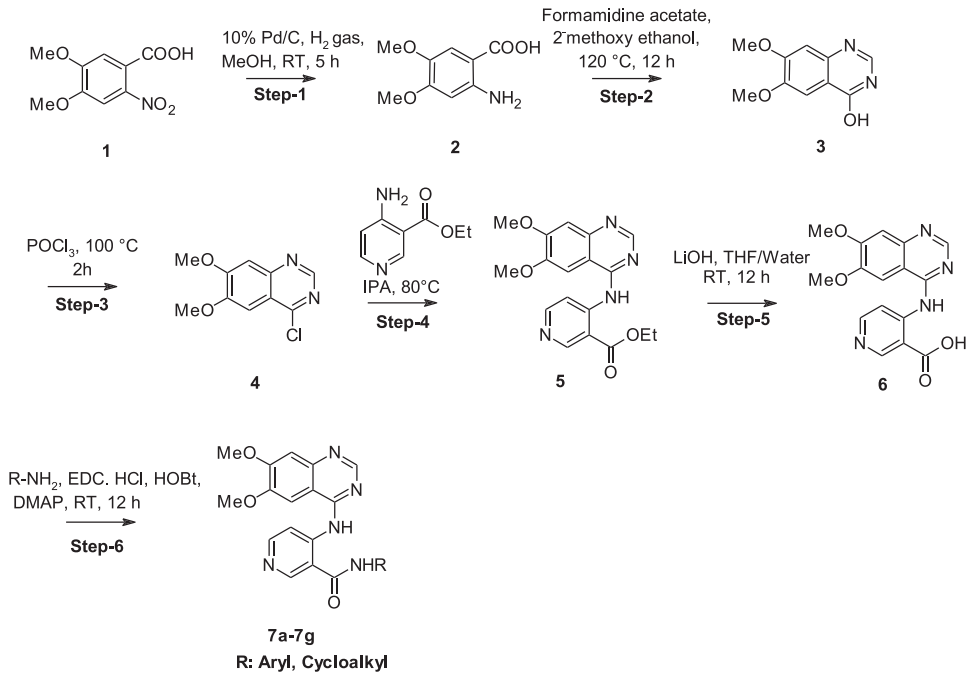
The synthesis of compound 7a was attempted by reaction of compound 6 with 3-bromo-aniline in the presence of *N*-ethyl-*N*′-(3-dimethylaminopropyl) carbodiimide HCl (EDC.HCl), 1-hydroxybenzotriazole and 4-dimethylaminopyridine at room temperature. Compound 7a was isolated in 75% yield after recrystallization by using hot 2-propanol. Employing similar conditions, compounds 7a–7g were synthesized in good yields. The reactions were complete within 12 h and the products were precipitated by adding water to the reaction mixture. Purification of all the compounds was achieved by recrystallization by using hot 2-propanol.

The structures of all the newly synthesized compounds were confirmed by IR, ^{1}H and ^{13}C NMR and LC mass (LCMS) spectral studies. The structure of compound 5 was interpreted by IR, ^{1}H NMR, and LC–MS analysis. The ^{1}H NMR showed a triplet at δ 1.31–1.36 and a quartet at δ 4.29–4.36, which corresponds to the ethyl ester group. Again, the doublets at δ 7.95 and δ 8.04 and singlet at δ 8.37 revealed the presence of pyridine group in the molecule. The IR spectrum of compound 5 showed the presence of –C=O group due to the appearance of strong band at 1633.91 cm^{-1}. Further, the LC–MS showed its molecular ion peaks at 355.4 (M + H), which are in accordance with its molecular formula $C_{18}H_{18}N_4O_4$.

The structure of compound 6 was interpreted by IR, ^{13}C NMR, and LC–MS analysis. The appearance of signal at 161.63 in ^{13}C NMR corresponds to –C=O group of the free carboxylic acid on the pyridine moiety, which again was confirmed by the presence of a strong band at 1669.61 cm^{-1} in the IR. Further, the LC–MS showed its molecular ion peaks at 327.1 (M + H), which are in accordance with its molecular formula $C_{16}H_{14}N_4O_4$.

The structure of compounds 7a–7g was confirmed by IR, ^{1}H and ^{13}C NMR, and LC–MS analysis. The ^{1}H NMR of 7a showed a doublet at δ 8.14, doublet of doublet at δ 8.7, and a singlet at δ 8.86, which are the characteristic peaks of the pyridine heterocycle present in the molecule. Also, the appearance of a doublet at δ 7.4, multiplet at δ 7.72–7.75, and a doublet at 8.16 confirmed the presence of 3-bromo aryl substituent at the amide group. IR spectrum showed the presence of –C=O group of amide at 1668.11 cm^{-1}, which again was confirmed by the appearance of signal at 158.08 in the ^{13}C NMR. The structure of 7a was again confirmed by LC–MS, which showed the molecular ion peaks at *m*/*z* 481.4 (M + H) corresponding to the molecular formula $C_{22}H_{18}BrN_5O_3$.

Scheme 1. Synthetic route to synthesize compounds 7a–7g.



Activity of compounds 7a–7g against VEGFR-2. Compounds 7a–7g were tested against isolated VEGRF-2 kinase in an enzymatic assay to evaluate their ability to inhibit tyrosine phosphorylation. Inhibition was determined by measuring the ability to inhibit phosphorylation level of biotinylated-polypeptide substrate (p-GAT, CIS Bio International, Cisbio China, Shanghai, China) in a homogeneous time-resolved fluorescence (HTRF) assay at an ATP concentration of 2 μM. The results were reported as a 50% inhibition concentration value (IC_{50}), a literature VEGFR-2 inhibitor also being included as an internal standard for quality control (ZD 6474; $IC_{50} = 0.045$ μM).

As can be seen from Table 1, a number of compounds exhibited low nanomolar potency against isolated VEGFR-2 enzyme. Of note were compounds that contained a halo-substituted aryl moiety at the amide position of the nictotinamide derivative. As such, the 3-bromo, 3-chloro-4-fluoro and 4-fluoro aryl substituted compounds gave rise to very active analogues with excellent inhibition against the isolated enzyme with an enzymatic IC_{50} of 48, 75, and 79 nM respectively (entries 1-2, 4). Indeed, 7a (entry 1), with an enzymatic IC_{50} of 48 nM performed comparably to the compound ZD-6474 in these assays ($IC_{50} = 45$ nM). Importantly, it was also observed that the thiophene-2-yl-methyl substituent at the amidic position (entry 5) also gave the active compound with enzymatic IC_{50} of 17 nM. Interestingly, a cyclohexyl substituent at the amidic position (entry 4) was tolerated giving rise to a moderately active compound ($IC_{50} = 0.39$ μM). Additionally, the 1H- indazole-6-yl compound also displayed reasonable activity (entry 6). The cyclopropyl substituent led to diminished potency against the enzyme (entry 7). In general, the aryl-substituted carboxamides (7a, 7b, 7d, and 7e and 7f) were much better inhibitors than the cycloalkyl-substituted carboxamides (7c and 7g).

Table 1

Activity of compounds (7a–7g) against VEGFR-2 tyrosine kinase.

Entry	Compound	R	Enzymatic, IC_{50} (μM)[a]
1	7a	3-Br Ph	0.048
2	7b	3-Cl, 4-F, Ph	0.075
3	7c	Cyclohexyl	0.39
4	7d	4-F Ph	0.079
5	7e	Thiophene-2-yl-methyl	0.017
6	7f	1H-indazole-6-yl	0.12
7	7g	Cyclopropyl	>10

[a]Determined against isolated enzyme for at least two independent experiments (at least 8 points per experiment). Positive control, ZD-6474 $IC_{50} = 0.045$ μM.

CONCLUSION

We have disclosed a series of 6,7-dimethoxy-quinazolin-4-yl-amino-nicotinamide derivatives as potent inhibitors of VEGFR-2. The lead compound from this series 7a, was found to be the most potent by displaying VEGFR-2 inhibitory activity with an IC_{50} as low as 48 nM in an HTRF enzymatic assay (comparable activity to ZD-6474). The compounds also provide an opportunity of laying the foundation for promising molecules of anticancer potency.

EXPERIMENTAL

Chemistry. Chemicals were obtained from Sigma-Aldrich Co. (Bengaluru, India) TLC experiments were performed on alumina-backed silica gel 40 F 254 plates (Merck, Darmstadt, Germany). The plates were illuminated under UV (254 nm) and $KMnO_4$. Melting points were determined by using Buchi B-540 (Buchi India (p) Ltd., Bengaluru, India) and are uncorrected. All ^1H and ^{13}C NMR spectra were recorded on a Bruker AM-300 and AM-400 (300 MHz, 400 MHz for ^1H NMR and 75, 100 MHz for ^{13}C NMR), Bruker BioSpin Corp., Germany. Molecular weights of unknown compounds were checked by LC–MS 6200 series Agilent Technology (Agilent, Bengaluru, India). Chemical shifts are reported in ppm (δ) with reference to internal standard TMS. The signals are designated as follows: s, singlet; d, doublet; t, triplet; dd, doublet of doublet; m, multiplet; bs, broad singlet. IR Spectra were recorded by using a Bruker Alpha FTIR spectrometer by using a diamond ATR single reflectance module (24 scans). Elemental analysis was carried out with a Perkin-Elmer model 240-C apparatus (Thermofisher, Bengaluru, India). The results of elemental analysis (C, H, and N) were within ±0.4% of the calculated amounts.

4-(6,7-Dimethyl-quinazolin-4-ylamino)-nicotinicacid ethylester (5). To a suspension of 4-Chloro-6,7-dimethoxyquinazoline 4 (2.0 g, 9 mmol) in 2-propanol (20 mL) was added ethyl-4-aminopyridine-3-carboxylate (1.47 g, 9 mmol) at 25°C. The reaction mixture was allowed to stir at 80°C for 4 h. After completion of reaction, reaction mixture was cooled to 0°C. The residue was filtered and dried to get product 5 as beige solid (3 g, 95%). mp: 298.5–298.7°C. IR (ATR, cm^{-1}) υ: 3385.22 (N–H), 3026.28 (Ar–CH), 1633.91 (C=O), 1569.74 (Ar–C=C), 1217.20 (C–O). ^1H NMR (DMSO-d_6 300 MHz) δ (ppm): 1.31–1.36 (t, $J = 7.2$ Hz, 3H, $–OCH_2CH_3$), 3.99 (s, 3H $–OCH_3$), 4.02 (s, 3H, $–OCH_3$), 4.29–4.36 (q, $J = 6.9$ Hz, 2H, $–OCH_2$), 7.37 (s, 1H, Ar–H), 7.95 (d, $J = 8.7$ Hz, 2H, Ar–H), 8.04 (d, $J = 8.4$ Hz, 2 H, Ar–H), 8.37 (s, 1H, pyridine H), 8.89 (s, 1H, –N=CH), 11.51 (bs, 1H, –NH). ^{13}C NMR (DMSO-d_6 100 MHz) δ: 14.68 ($–CH_3$, $–OCH_2CH_3$), 56.97 (CH_3, $–OCH_3$), 57.55 (CH_3,

–OCH_3), 61.22 (CH_2, –OCH_2CH_3), 100.47 (quinazoline C), 104.56 (quinazoline C), 108.19 (pyridine C), 124.45 (pyridine C), 127.12 (quinazoline C), 130.16 (quinazoline C), 136.76 (pyridine C), 142.10 (quinazoline C), 149.19 (pyridine C), 150.78 (quinazoline C), 156.93 (quinazoline, N=CHN), 158.45 (C=O), and 165.69 (quinazoline, N=C–NH). LC–MS (ESI, *m/z*): 355.4 (M + H).

4-(6,7-Dimethyl-quinazolin-4-ylamino)-nicotinic acid (6). Lithium hydroxide (0.71 g, 16.9 mmol) at 0°C was added to a solution of compound 5 (2.0 g, 5.6 mmol) in tetrahydrofuran (8 mL) and water (2 mL) and the reaction mixture was slowly warmed to room temperature (25°C) over a period of 12 h. After the completion of the reaction, water (50 mL) was added to the reaction mixture, and the volatiles were evaporated under reduced pressure. Aqueous layer was acidified with 1.5 N HCl to pH ~ 2 and was extracted with ethyl acetate (2 × 50 mL). Organic layer was washed with water (1 × 50 mL), saturated brine solution (1 X 50 mL) and dried over anhydrous sodium sulfate. Solvents were evaporated under reduced pressure to get product 6 as an off-white solid (1.32 g, 72%). mp: 270.3–270.5°C. IR (ATR, cm^{-1}) υ: 3168.49 (N–H), 2968.85 (Ar–CH), 1669.61 (C=O), 1598.39 (Ar–C=C), 1220.18 (C–O). ^{1}H NMR (DMSO-d_6 400 MHz) δ (ppm): 4.0 (s, 3H –OCH_3), 4.01 (s, 3H, –OCH_3), 7.29 (s, 1H, Ar–H), 7.90 (d, J = 8.8 Hz, 2H, Ar–H), 8.04 (d, J = 8.8 Hz, 2H, Ar–H), 8.15 (s, 1H, pyridine H), 8.85 (s, 1H, –N=CH), 11.02 (bs, 1H, –NH). ^{13}C NMR (DMSO-d_6 75 MHz) δ: 56.99 (CH_3, –OCH_3), 57.66 (CH_3, –OCH_3), 100.61 (quinazoline C), 104.77 (quinazoline C), 109.1 (pyridine C), 126.01 (pyridine C), 128.1 (quinazoline C), 131.1 (quinazoline C), 137.15 (pyridine C), 142.9 (quinazoline C), 149.93 (pyridine C), 150.7 (pyridine C), 151.03 (quinazoline C), 152.3 (pyridine C), 157.32 (quinazoline, N=CHN), 161.63 (C, -COOH), and 165.93 (quinazoline, N=C–NH). LC–MS (ESI, *m/z*): 327.1 (M + H).

General procedure for the synthesis of 4-(6,7-dimethoxy-quinazolin-4-ylamino)-*N*-nicotinamide derivatives (7a–7g). *N*-ethyl-*N*′-(3-dimethylaminopropyl)carbodiimide HCl (EDC.HCl) (442 mg, 2.3 mmol), 1-hydroxybenzotriazole (353 mg, 2.3 mmol), and 4-dimethylaminopyridine (37 mg, 0.3 mmol) at 0°C under nitrogen atmosphere were added to a solution of compound 6 (500 mg, 1.5 mmol) in *N*,*N*-dimethylformamide (2.5 mL). After 5 min, corresponding amine (1.5 mmol) was added to the reaction mixture at 0°C and the reaction mixture was slowly allowed to reach room temperature (25°C) and stirred at room temperature over a period of 12 h. The resulting reaction mass was poured into crushed ice to precipitate the product. The precipitated solid was filtered and dried under reduced pressure and was recrystallized with hot 2-propanol to yield the title compounds (7a–7g).

4-(6,7-Dimethoxy-quinazolin-4-ylamino)-N-(3-bromo-phenyl)-nicotinamide (7a). This compound was prepared by coupling of compound 6 with 3-bromo-aniline in the presence of EDC.HCl. It was obtained as an off-white solid (550 mg, 75%). mp: 211.7–220.8°C. IR (ATR, cm^{-1}) υ: 3437.19 (N–H), 3418.07 (amide N–H), 3010.82 (Ar–CH), 1668.11 (C=O), 1582.89 (Ar-C=C), 1234.20 (C–O). ^{1}H NMR (DMSO-d_6 300 MHz) δ (ppm): 3.94 (s, 3H –OCH_3), 4.09 (s, 3H, –OCH_3), 7.28 (s, 1H, Ar–H), 7.4 (d, J = 5.1 Hz, 1H, Ar–H), 7.55 (bs, 2H), 7.68 (s, 1H, Ar–H), 7.72–7.75 (m, 2H, Ar–H), 8.16 (d, J = 5.7 Hz, 1H, Ar–H), 8.41 (d, J = 6 Hz, 1H, pyridine H), 8.7 (dd, J_1 = 6.3 Hz, J_2 = 5.1 Hz, 1H, pyridine H), 8.86 (s, 1H, pyridine-H), 9.27 (s, 1H, –N=CH), 11.15 (bs, 1H, –NH). ^{13}C NMR (DMSO-d_6 100 MHz) δ: 56.22 (CH_3, –OCH_3), 57.17 (CH_3, –OCH_3), 101.05 (quinazoline C), 105.50 (quinazoline C), 107.42 (pyridine C), 113.71 (quinazoline C), 119.93 (pyridine C), 120.07 (Ar–C), 123.52 (Ar–C), 123.64 (Ar–C), 127.78 (Ar–C), 131.2 (Ar–C), 140.26 (Ar–C), 147.76 (quinazoline C), 149.12 (pyridine C), 151.98 (quinazoline C), 152.56 (pyridine C), 152.68 (pyridine C), 154.99 (quinazoline C), 156.43 (quinazoline, N=CHN), 158.08 (C, –CONH), and 163.69 (quinazoline, N=C–NH). LC–MS (ESI, *m/z*): 481.4 (M + H). *Anal*. Calcd. for $C_{22}H_{18}BrN_5O_3$ (480.32): C, 55.01; H, 3.78; N, 14.58. Found: C, 55.14; H 3.92; N, 14.72.

4-(6,7-Dimethoxy-quinazolin-4-ylamino)-N-(3-chloro-4-fluoro-phenyl)-nicotinamide (7b). This compound was prepared by coupling of compound 6 with 3-chloro-4-fluoro-aniline in the presence of EDC.HCl. It was obtained as an off-white solid (488 mg, 70%). mp: 240.6–241.7°C. IR (ATR, cm^{-1}) υ: 3432.75 (N–H), 3280.16 (amide N–H), 3091.39 (Ar–CH), 1666.66 (C=O), 1555.10 (Ar-C=C), 1236.13 (C–O). ^{1}H NMR (DMSO-d_6 300 MHz) δ (ppm): 3.94 (s, 3H –OCH_3), 4.09 (s, 3H, –OCH_3), 7.27 (s, 1H, Ar–H), 7.46 (s, 1H, Ar–H), 7.68 (s, 1H, Ar–H), 7.71–7.76 (m, 1H, Ar–H), 8.14 (dd, J_1 = 6.5 Hz, J_2 = 4.5 Hz, 1H, Ar–H), 8.43 (d, J = 6.3 Hz, 1H, pyridine), 8.74 (dd, J_1 = 6.3 Hz, J_2 = 4.8 Hz, 1H, pyridine-H), 8.88 (s, 1H, pyridine H), 9.27 (s, 1H, –N=CH), 11.27 (bs, 1H, –NH). ^{13}C NMR (DMSO-d_6 100 MHz) δ: 57.13 (CH_3, –OCH_3), 57.34 (CH_3, –OCH_3), 101.06 (quinazoline C), 105.6 (quinazoline C), 107.44 (pyridine C), 113.71 (quinazoline C), 115.5 (pyridine C), 117.55 (Ar–C), 119.59 (Ar–C), 121.80 (Ar–C), 122.93 (Ar–C), 135.82 (Ar–C), 147.73 (quinazoline C), 149.3 (pyridine C), 152 (quinazoline C), 152.11 (pyridine C), 152.32 (pyridine C), 152.57–152.67 (Ar–C, d, J = 10), 155.62 (quinazoline C), 156.43 (quinazoline, N=CHN), 158.08 (C, –CONH), and 163.58 (quinazoline, N=C–NH). LC–MS (ESI, *m/z*): 454.4 (M + H). *Anal*. Calcd. for $C_{22}H_{17}ClFN_5O_3$ (453.86): C, 58.22; H, 3.78; N, 15.43. Found: C, 58.04; H 3.59; N, 15.22.

4-(6,7-Dimethoxy-quinazolin-4-ylamino)-N-(cyclohexyl)-nicotinamide (7c). This compound was prepared by coupling of compound 6 with cyclohexylamine in the presence of

EDC.HCl. It was obtained as a pale yellow solid (356 mg, 57%). mp: 172.9–173.8°C. IR (ATR, cm^{-1}) υ: 3454.58 (N–H), 3274.94 (amide N–H), 3015.33 (Ar–CH), 1612.46 (C=O), 1554.2 (Ar-C=C), 1231.17 (C–O). ^{1}H NMR (DMSO-d_6 300 MHz) δ (ppm): 1.27–1.44 (m, 4H, cyclohexane), 1.71–1.87 (m, 4H, cyclohexane), 3.76–3.83 (m, 1H, cyclohexane), 3.92 (s, 3H, $–OCH_3$), 4.08 (s, 3H, $–OCH_3$), 7.25 (s, 1H, Ar–H), 7.59 (bs, 2H, –NH), 7.67 (s, 1H, Ar–H), 8.27 (d, J = 6.3 Hz, 1H, pyridine H), 8.67 (dd, J_1 = 6.0 Hz, J_2 = 4.8 Hz, 1H, pyridine H), 8.83 (s, 1H, pyridine H), 9.27 (s, 1H, −N=CH). ^{13}C NMR (DMSO-d_6 100 MHz) δ: 22.3 (cyclohexane C), 27.4 (cyclohexane C), 33.6 (cyclohexane C), 46.1 (cyclohexane C), 56.89 (CH_3, $–OCH_3$), 57.13 (CH_3, $–OCH_3$), 101.07 (quinazoline C), 106.1 (quinazoline C), 107.63 (pyridine C), 114.1 (quinazoline C), 119.73 (pyridine C), 147.79 (quinazoline C), 149.32 (pyridine C), 151.93 (quinazoline C), 152.61 (pyridine C), 152.73 (pyridine C), 155.01 (quinazoline C), 156.61 (quinazoline, N=CHN), 158.13 (C, –CONH), and 165.12 (quinazoline, N=C–NH). LC–MS (ESI, *m*/*z*): 408.5 (M + H). *Anal*. Calcd. for $C_{22}H_{25}N_5O_3$ (407.48): C, 64.85; H, 6.18; N, 17.19. Found: C, 64.87; H 6.07; N, 17.08.

4-(6,7-Dimethoxy-quinazolin-4-ylamino)-N-(4-fluoro-phenyl)-nicotinamide (7d). This compound was prepared by coupling of compound 6 with 4-fluoroaniline in the presence of EDC.HCl. It was obtained as an off-white solid (418 mg, 65%). mp: 266.2–270.3°C. IR (ATR, cm^{-1}) υ: 3426.82 (N–H), 3316.91 (amide N–H), 3044.05 (Ar–CH), 1665.87(C=O), 1511.32 (C=C–Ar), 1221.70 (C–O). ^{1}H NMR (DMSO-d_6 300 MHz) δ (ppm): 3.92 (s, 3H $–OCH_3$), 4.02 (s, 3H, $–OCH_3$), 7.27 (s, 1H, Ar–H), 7.66 (bs, 2H, –NH), 7.68 (s, 1H, Ar–H), 7.87 (d, J = 8.7 Hz, 2H, Ar–H), 8.02 (d, J = 8.4 Hz, 2H, Ar–H), 8.42 (d, J = 6.0 Hz, 1H, pyridine H), 8.72 (dd, J_1 = 6.3 Hz, J_2 = 4.8 Hz, 1H, pyridine H), 8.86 (s, 1H, pyridine H), 9.25 (s, 1H, –N=CH), 11.27 (bs, 1H, –NH). ^{13}C NMR (DMSO-d_6 100 MHz) δ: 56.82 (CH_3, $–OCH_3$), 57.17 (CH_3, $–OCH_3$–OCH_3), 101 (quinazoline C), 105.83 (quinazoline C), 107.48 (pyridine C), 112.9 (quinazoline C), 115.31 (Ar–C), 119.73 (pyridine C), 120.1 (Ar–C), 139.2 (Ar–C), 147.91 (quinazoline C), 149.41 (pyridine C), 150.01 (quinazoline C), 150.32–150.41 (Ar–C, d, J = 9), 152.66 (pyridine C), 152.91 (pyridine C), 154.33 (quinazoline C), 156.14 (quinazoline, N=CHN), 159.12 (C, –CONH), and 165 (quinazoline, N=C–NH). LC–MS (ESI, *m*/*z*): 420.4 (M + H). *Anal*. Calcd. for $C_{22}H_{18}FN_5O_3$ (419.42): C, 63.00; H, 4.33; N, 16.70. Found: C, 62.89; H 4.20; N, 16.86.

4-(6,7-Dimethoxy-quinazolin-4-ylamino)-N-(thiophen-2-yl-methyl)-nicotinamide (7e). This compound was prepared by coupling of compound 6 with thiophen-2-yl-methylamine in the presence of EDC.HCl. It was obtained as a pale brown solid (530 mg, 82%). mp: 176.6–180.6°C. IR (ATR, cm^{-1}) υ: 3479.40 (N–H), 3378.07 (amide N–H), 3015.48 (Ar–CH), 1655.83 (C=O), 1579.20 (C=C–Ar), 1235.34 (C–O). ^{1}H NMR (DMSO-d_6 300 MHz) δ (ppm): 3.92 (s, 3H $–OCH_3$), 4.08 (s, 3H, $–OCH_3$), 4.71 (d, J = 5.7 Hz, 2H, $–CH_2$ thiophene), 7.01–7.02 (m, 1H, thiophene-H), 7.12–7.17 (m, 1H, thiophene H), 7.27 (s, 1H, Ar–H), 7.67 (s, 1H, Ar–H), 7.72 (bs, 2H, –NH), 8.27 (d, J = 6.3 Hz, 1H, pyridine H), 8.67 (dd, J_1 = 6.3 Hz, J_2 = 4.8 Hz, 1H, pyridine H), 8.87 (s, 1H, pyridine H), 9.25 (s, 1H, –N=CH), 9.93 (bs, 1H, –NH). ^{13}C NMR (DMSO-d_6 100 MHz) δ: 38.11 ($–CH_2$, thiophene), 56.22 (CH_3, $–OCH_3$), 57.10 (CH_3, $–OCH_3$), 101.13 (quinazoline C), 105.49 (quinazoline C), 107.37 (pyridine C), 113.57 (quinazoline C), 115.90 (pyridine C), 125.73 (thiophene C), 126.40 (thiophene C), 127.19 (thiophene C), 141.60 (thiophene C), 148.43(quinazoline C), 149.15 (pyridine C), 151.92 (quinazoline C), 152.52 (pyridine C), 152.62 (pyridine C), 155.01 (quinazoline C), 156.32 (quinazoline, N=CHN), 158.04 (C, –CONH), 164.79 (quinazoline, N=C–NH). LC–MS (ESI, *m*/*z*): 422.4 (M + H). *Anal*. Calcd. for $C_{21}H_{19}N_5O_3S$ (421.48): C, 59.84; H, 4.54; N, 16.62. Found: C, 59.77; H 4.38; N, 16.54.

4-(6,7-Dimethoxy-quinazolin-4-ylamino)-N-(1H-indazole-6-yl)-nicotinamide (7f). This compound was prepared by coupling of compound 6 with 1H-indazole-6-ylamine in the presence of EDC.HCl. It was obtained as a brown solid (237 mg, 35%). mp: 211.7–215.5°C. IR (ATR, cm^{-1}) υ: 3428.78 (amide N–H), 3185.12 (N–H), 3159 (Ar–CH), 1664.98 (C=O), 1553.22 (C=C–Ar), 1231.25 (C–O). ^{1}H NMR (DMSO-d_6 300 MHz) δ (ppm): 3.96 (s, 3H $–OCH_3$), 4.09 (s, 3H, $–OCH_3$), 7.28 (s, 1H, Ar–H), 7.42–7.46 (m, 1H, Ar–H), 7.6 (bs, 2H, –NH), 7.68 (s, 1H, Ar–H), 7.7 (d, J = 8.4 Hz, 1H, Ar–H), 8.03 (s, 1H, Ar–H), 8.3 (s, 1H, indazole H), 8.49 (d, J = 6.0 Hz, 1H, pyridine H), 8.74 (dd, J_1 = 6.3 Hz, J_2 = 4.8 Hz, 1H, pyridine H), 8.94 (s, 1H, pyridine-H), 9.27 (s, 1H, –N=CH), 11.27 (bs, 1H, –NH), 13.14 (bs, 1H, –NH). ^{13}C NMR (DMSO-d_6 100 MHz) δ: 56.19 (CH_3, $–OCH_3$), 57.19 (CH_3, $–OCH_3$), 101.04 (quinazoline C), 101.58 (indazole C), 105.45 (quinazoline C), 107.40 (pyridine C), 113.70 (quinazoline C), 115.46 (pyridine C), 120.40 (indazole C), 121.04 (indazole C), 127.67 (indazole C), 133.76 (indazole C), 136.81 (indazole C), 144.61 (indazole C), 147.79 (quinazoline C), 149.09 (pyridine C), 151.92 (quinazoline C), 152.56 (pyridine C), 152.66 (pyridine C), 154.97 (quinazoline C), 156.42 (quinazoline, N=CHN), 158.05 (C, –CONH), and 163.63 (quinazoline, N=C–NH). LC–MS (ESI, *m*/*z*): 442.5 (M + H). *Anal*. Calcd. for $C_{23}H_{19}N_7O_3$ (441.45): C, 62.58; H, 4.34; N, 22.21. Found: C, 62.69; H 4.42; N, 22.30.

4-(6,7-Dimethoxy-quinazolin-4-ylamino)-N-(cyclopropyl)-nicotinamide (7g). This compound was prepared by coupling of compound 6 with cyclopropylamine in the presence of EDC.HCl. It was obtained as an off-white solid

(400 mg, 72%). mp: 212.0–221.8°C. IR (ATR, cm^{-1}) υ: 3403.34 (N–H), 3157.52 (amide N–H), 2997.85 (Ar–CH), 1632.01 (C=O), 1500 (C=C–Ar), 1234.47 (C–O). ^{1}H NMR (DMSO-d_6 300 MHz) δ (ppm): 0.63–0.70 (m, 2H, cyclopropane), 0.76–0.82 (m, 2H, cyclopropane), 2.91–2.97 (m, 1H, cyclopropane), 3.92 (s, 3H –OCH_3), 4.08 (s, 3H, –OCH_3), 7.24 (s, 1H, Ar–H), 7.6 5(bs, 2H, –NH), 7.67 (s, 1H, Ar–H), 8.25 (d, J = 6.0 Hz, 1H, pyridine H), 8.66 (dd, J_1 = 6.3 Hz, J_2 = 4.8 Hz, 1H, pyridine H), 8.85 (s, 1H, pyridine-H), 9.25 (s, 1H, –N=CH). ^{13}C NMR (DMSO-d_6 100 MHz) δ: 5.9 (cyclopropane C), 20.6 (cyclopropane C), 56.63 (CH_3, –OCH_3), 57.1 (CH_3, –OCH_3), 101.1 (quinazoline C), 105.8 (quinazoline C), 107.21 (pyridine C), 115.1 (quinazoline C), 118.79 (pyridine C), 147.76 (quinazoline C), 149.75 (pyridine C), 151.93 (quinazoline C), 152.81 (pyridine C), 152.76 (pyridine C), 155.04 (quinazoline C), 156.95 (quinazoline, N=CHN), 158.36 (C, –CONH), and 164.39 (quinazoline, N=C–NH). LC–MS (ESI, *m/z*): 366.5 (M + H). *Anal.* Calcd. for $C_{19}H_{19}N_5O_3$ (365.39): C, 62.46; H, 5.24; N, 19.71. Found: C, 62.58; H 5.32; N, 19.26.

HTRF assay. VEGFR tyrosine kinase inhibition is determined by measuring the phosphorylation level of poly-Glu-Ala-Tyr-biotin (pGAT-biotin, CIS Bio International) peptide in a HTRF assay. Two microliter per well of 25× compound in 100% DMSO (final concentration in the 50 μL kinase reaction is typically 1 nM to 10 μM) is added into a black 96-well Costar plate. Next, 38 μL of reaction buffer (25 mM Hepes, pH 7.5, 5 mM $MgCl_2$, 5 mM $MnCl_2$, 2 mM DTT, and 1 mg/mL BSA) containing 0.5 mmol pGAT-biotin and 3-4 ng KDR enzyme is added to each well. After 5–10 min preincubation, the kinase reaction is initiated by the addition of 10 μL of 10 μM ATP in the reaction buffer, after which the plate is incubated at room temperature for 45 min. The reaction is stopped by addition of 50 μL KF buffer (50 mM Hepes, pH 7.5, 0.5 M KF, and 1 mg/mL BSA) containing 100 mM EDTA and 0.36 μg/mL PY20K (Eu-cryptate labeled anti-phosphotyrosine antibody, CIS Bio International). After 30 min, 100 μL of 10 nM SV-XL (modified APC-labeled streptavidin, CIS Bio International) in KF buffer is added, and after additional 2 h incubation at room temperature, the plate is read in a RUBYstar HTRF Reader.

Acknowledgments. We are thankful to Anthem Biosciences management, Anthem Biosciences, Bangalore, India, for their invaluable support and allocation of resources for this work. We would like to thank analytical chemistry team, Department of Analytical Chemistry, Anthem Biosciences, Bangalore, India, for having carried out all the analytical work. Also, we would like to thank molecular biology team of Anthem Biosciences for executing the VEGFR-2 activity studies.

REFERENCES AND NOTES

[1] Carmeliet, P. Nature 2006, 438, 932.
[2] Folkman, J. N Engl J Med 1971, 285, 1182.
[3] Klagsbrun, M.; Moses, M. A. Chem Biol 1999, 6, R217.
[4] Cross, M. J.; Dixelius, J.; Matsumoto, T.; Claesson-Welsh, L. Trends Biochem Sci 2003, 28, 488.
[5] Dvorak, H. F. J Clin Oncol 2002, 20, 4368.
[6] Waltenberger, J.; Claesson-Welsh, L.; Siegbahn, A.; Shibuya, M.; Heldin, C. J Biol Chem 1994, 269, 26988.
[7] See Culy, C. Drugs Today 2005, 41, 23.
[8] Fine, S. L.; Martin, D. F.; Kirkpatrick, P. Nat Rev Drug Discov 2005, 4, 187.
[9] Choi, H. E.; Yoo, M. S.; Choi, J. H.; Lee, J. Y.; Kim, J. H.; Kim, J. H.; Lee, J. K.; Kim, G. I.; Park, Y.; Chi, Y. H.; Paik SHv Lee, J. H.; Lee, K. T. Bioorg Med Chem Lett 2011, 21, 6236.
[10] Celia, D.; Leon, S.; Qi, H.; Chester, Y.; Xiaohu, O.; Lynn, C.; Paul, C.; Joseph, K.; Timothy, H.; Rashid, S.; Tae-Seong, K.; Andrew, T.; Ling, W.; Michael, Z.; Angela, C.; James, B.; Charles, S.; Danlin, C.; Yongmei, G.; Sesha, N.; Gondi, K.; Anthony, P.; Richard, K. Bioorg Med Chem Lett 2007, 17, 6003.
[11] Paul William M.; Guido B. 2-amino-nicotinamide derivatives and their use as VEGF-receptor tyrosine kinase inhibitors, US 6624174.
[12] Dumasa, J.; Dixon, J. A. Expert Opin Ther Pat 2005, 15, 647.
[13] Hisashi, T.; Takahiro, H.; Kenji, K.; Yoshimasa, S.; Minoru, Y.; Masakazu, B.; Kazuyoshi, O.; Kenji, I.; Takaaki, I.; Yuriko, T.; Hiroyuki, A. Bioorg Med Chem Lett 2010, 20, 7234.
[14] Andrus, S.; Merritt, B.; Mettath Chun, S. J Org Chem 2002, 23, 8284.
[15] Jian-Wen, J.; Lin, Z.; Guang-Rong, M.; Jian-Hua, Z.; Qian, Z. Synth Commun 2014, 44, 346.
[16] Konakanchi, Durga Prasad. US 2012182390.