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# Discovery of *N*-(2-phenyl-1*H*-benzo[*d*]imidazol-5-yl)quinolin-4amine derivatives as novel VEGFR-2 kinase inhibitors



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#### ABSTRACT

Inhibition of the VEGF signaling pathway has become a valuable approach in the treatment of cancers. In this work, a series of *N*-(2-phenyl-1*H*-benzo[*d*]imidazol-5-yl)quinolin-4-amine derivatives were designed and identified as potent inhibitors of VEGFR-2 (KDR) kinase. These compounds with quinoline scaffold and benzimidazole moiety were synthesized and their biological activities against VEGFR-2 and two human cancer cell lines were evaluated. Among them, compound **7s** exhibited the most potent inhibitory activity against VEGFR-2 with IC<sub>50</sub> of 0.03  $\mu$ M and it also showed the highest anticancer activity against the tested cancer cell lines with IC<sub>50</sub> of 1.2  $\mu$ M against MCF-7 and 13.3  $\mu$ M against Hep-G2. Docking simulation supported the initial pharmacophoric hypothesis and suggested a common mode of interaction at the ATP-binding site of VEGFR-2, which demonstrates that compound **7s** is a potential agent for cancer therapy deserving further researching.

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# 1. Introduction

Angiogenesis, the formation of new capillary blood vessels from existing vasculature, is a normal physiological event that occurs during embryogenesis, wound healing, and the menstrual cycle [1]. It also contributes to a number of pathological disorders such as psoriasis, arthritis, endometriosis and diabetic retinopathy [2]. More importantly, it is required for the growth and metastasis of solid tumors [3–5]. In fact, in order to grow beyond a critical size, solid tumors need new blood capillaries to create their own nutrient supply to remove metabolic waste and to facilitate metastasis formation [6-8].

Among the many proangiogenic factors identified to date, vascular endothelial growth factor (VEGF) has been identified as the most common regulator of tumor angiogenesis and the increased expression of VEGF has been implicated in tumor growth and metastasis [9,10]. VEGF binds to VEGFR-2 (KDR), a receptor tyrosine kinase (RTK), which upon dimerization by VEGF undergoes autophosphorylation and initiates downstream signaling, ultimately leading to angiogenesis, vascular permeability enhancement, tumor proliferation, and tumor migration [11–14].

Inhibition of angiogenesis by blocking the VEGF signaling pathway has become a valuable approach in the treatment of cancers [15]. Several successful strategies for the inhibition of angiogenesis have been effectively demonstrated in preclinical and clinical settings. These approaches include VEGF soluble decoy receptors, antibodies directed against VEGF, and small molecules that inhibit KDR [16]. Bevacizumab, a monoclonal antibody to VEGF, has been approved by the FDA for the treatment of metastatic colorectal cancer and non-small-cell lung cancer [17,18]. In addition to biological-based antiangiogenics, small-molecule tyrosine kinase inhibitors of KDR, such as sorafenib [19], sunitinib [20], pazopanib [21], vandetanib [22], axitinib [23] and cabozantinib [24] have been approved for treatment of various types of cancers (Fig. 1).

Recently, a large number of VEGFR inhibitors have been reported or have entered clinical trials [6,25–32]. Some compounds containing quinoline or benzimidazole moiety exhibited potent antitumor activities [33–41]. We have recently identified a series of quinoline amides as effective inhibitors of KDR at the enzyme and cellular levels [42]. In an attempt to pursue new potent antitumor agents with KDR inhibitory activity, a series of new quinolin-4-amine derivatives containing quinoline and benzimidazole moieties were synthesized and their inhibitory activities against KDR and two cancer cell lines were evaluated. In addition, the structure–activity relationships and possible enzyme binding modes were also illustrated.



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Fig. 1. VEGFR inhibitors approved for clinical use.

# 2. Results and discussion

## 2.1. Chemistry

The route that enabled the synthesis of the title *N*-(2-phenyl-1*H*-benzo[*d*]imidazol-5-yl)quinolin-4-amine derivatives is outlined in Scheme 1. The synthesis of these benzimidazole quinolin-4-amines started from a series of commercially available substituted benzoic acids **2a**–**2u**. Condensation of **2a**–**2u** with 4-nitro-o-phenylenediamine (1) in polyphosphoric acid (PPA) at 120–150 °C for 5 h gave the 5-nitro-benzimidazole intermediates **3a**–**3u**. Hydrogenation of 5-nitro-benzimidazole intermediates **3a**–**3u** with Pd/C/H<sub>2</sub> under normal pressure at room temperature for 5 h or Fe/AcOH/EtOH under reflux for 2 h provided the 5-amino-benzimidazole intermediates **4a**–**4u**. Condensation of **4a**–**4u** with 4-chloroquinoline (**6**), which was prepared by quinolin-4-ol (**5**) in POCl<sub>3</sub> at 100 °C for 4 h, in isopropanol under reflux for 5 h gave the title benzimidazole quinolin-4-amines **7a–7u**.

# 2.2. Biological evaluation

#### 2.2.1. Antiproliferation assay

All the synthesized compounds **7a**–**7u** were evaluated for their anticancer activity against MCF-7 (human breast cancer) and Hep-G2 (human liver cancer) cell lines by MTT assay. They were also evaluated for cytotoxicity on a human normal liver cell line LO2. The results were summarized in Table 1. It was clear that all the tested compounds showed better activities against MCF-7 than against Hep-G2. It also indicated that these compounds exhibited weak cytotoxic activity against LO2. Among the tested compounds, compound **7s** showed the most potent activities with the IC<sub>50</sub> value of 1.2  $\mu$ M and 13.3  $\mu$ M against MCF-7 and HepG-2, respectively, which was better than the positive control sunitinib.

Structure–activity relationships (SARs) were inferred from data of cell proliferative experiments reported in Table 1. The type, number and position of substituents on the phenyl ring linking to the benzimidazole moiety played important roles in the antiproliferation activities. The inhibitory activities against MCF-7 of compounds **7a**–**7u** with different *para*-substituents increased in the following order: compound **7m** with *para*-chloro substituent and compound **7n** with *para*-bromo substituent exhibited potent inhibitory activities with IC<sub>50</sub> of 3.2  $\mu$ M and 3.4  $\mu$ M, respectively; compound **7a** with no substituent on *para*-position and compound **7j** with *para*-fluoro substituent displayed slightly weaker inhibitory activities with IC<sub>50</sub> of 4.6  $\mu$ M and 4.3  $\mu$ M, respectively; compound **7d** with *para*-methyl and compound **7g** with *para*-ethyl substituent showed much weaker inhibitory activities with IC<sub>50</sub> of 6.6  $\mu$ M and 8.0  $\mu$ M, respectively; compound **7g** with *para*-trifluoromethyl substituent exhibited the weakest inhibitory activity with IC50 of 18.9 µM, which was about one sixth of compound **7m**. The above results indicated that introduction of weakly electron-withdrawing substituent is favorable for the activity while introduction of strongly electron-withdrawing bulk substituent or strongly electron-donating bulk substituent causes obvious decrease of activity. This discipline was also observed in ortho-substituted and meta-substituted compounds. Compounds 7k, 7l and 7m with chloro substituent at different position showed distinct inhibitory activity against MCF-7. Compound 7m with para-chloro substituent showed more potent activity (IC<sub>50</sub> = 3.2  $\mu$ M) than compound **71** with *meta*-chloro substituent ( $IC_{50} = 6.3 \mu M$ ). The activity of the latter was higher than compound **7k** with *ortho*-chloro substituent  $(IC_{50} = 19.6 \ \mu M)$ . The result suggested that substituents in different positions led to different inhibitory activities (para- > meta-> ortho-). This rule was also found in other single substituted compounds. Comparing the activities of compounds 7r, 7s and 7t against MCF-7, we found that compound 7s with two chloro substituents at *para* and *meta* positions exhibited the most potent activity (IC<sub>50</sub> = 1.2  $\mu$ M), and compound **7t** with two chloro substituents at two meta positions displayed slightly weaker activity  $(IC_{50} = 2.3 \ \mu M)$ , however, introduction of two chloro substituents to two ortho positions caused an obvious decrease of activity (compound **7r**:  $IC_{50} = 24.7 \mu M$ ). It also demonstrated that introduction of certain substituents at para or meta position is more favorable than at ortho position.

All the above SARs were also suitable for the inhibitory activities of these compounds against Hep-G2, however, the inhibitory activities against Hep-G2 were much weaker than that against MCF-7.

## 2.2.2. VEGFR-2 inhibitory assay

The VEGFR-2 kinase inhibitory potency of the synthesized quinolin-4-amine derivatives containing benzimidazole moiety was examined and the results were also summarized in Table 1. Most of the tested compounds exhibited potent VEGFR-2 inhibitory activities. Among them, compound **7s** displayed the most potent inhibitory activity with IC<sub>50</sub> value of 0.03  $\mu$ M, which was similar to the positive control sunitinib. The SARs analysis result of VEGFR-2 inhibitory activities of the tested compounds were consistent with that of their anticancer activities, which suggested that the potent anticancer activities of the synthesized compounds were likely related to their VEGFR-2 inhibitory activities.

# 2.3. Molecular docking studies

In order to better understand the interaction between the synthesized compounds and VEGFR-2 kinase, molecular docking of the potent compound **7s** into the ATP binding site of VEGFR-2 kinase



Scheme 1. General procedure for the synthesis of *N*-(2-phenyl-1*H*-benzo[*d*]imidazol-5-yl)quinolin-4-amine derivatives. Reagents and conditions: (a) PPA, 120–150 °C, 5 h; (b) Pd/C, H<sub>2</sub>, CH<sub>3</sub>OH, r.t., 5 h; (c) Fe, AcOH, EtOH, reflux, 2 h; (d) POCl<sub>3</sub>, 100 °C, 4 h; (e) isopropanol, reflux, 5 h.

(PDB: 2QU5) was performed using the Discovery Studio 3.1/ CDOCKER protocol. The binding model is depicted in Fig. 2. In this binding model, compound **7s** is nicely bound to the ATP-binding cavity of VEGFR-2 via three hydrogen bonds, four  $\pi$ -cation interactions, two  $\pi - \pi$  interactions and one  $\sigma - \pi$  interaction. The nitrogen atom of quinoline forms a hydrogen bond (N···H-N: 2.24 Å, 128.79°) with the amino hydrogen atom of Cys919 and the phenyl ring of quinoline forms a  $\pi - \pi$  interaction (distance: 5.28 Å) with the phenyl ring of Phe918 and also forms a  $\sigma$ - $\pi$  interaction (distance: 2.83 Å) with the 4-position carbon atom of Leu840 while the pyridine ring of quinoline form another  $\pi - \pi$  interaction (distance: 6.01 Å) with the phenyl ring of Phe918, which suggests that the quinoline group plays an important role in the combination of the receptor and ligand. In addition, the nitrogen atom of benzimidazole forms a hydrogen bond (N···H–N: 1.94 Å, 164.27°) with the amino hydrogen atom of Asp1046 and the amino hydrogen atom of benzimidazole forms another hydrogen bond (N-H···O: 2.15 Å, 134.48°) with the carbonyl oxygen atom of Glu885. Both the imidazole ring and the phenyl ring of benzimidazole form  $\pi$ -cation interactions (distance: 4.38 Å for imidazole ring and 4.85 Å for phenyl ring) with the end amino cation of Lys868. The results indicated that the introduction of benzimidazole group to the quinoline group might reinforce the combination of compound 7s and the receptor, which might enhance the binding affinity, resulting in the increased anticancer activity of this compound. Furthermore, the amino cation linking quinoline and benzimidazole forms a  $\pi$ -cation interaction (distance: 4.78 Å) with the phenyl ring of Phe1047 while the phenyl ring with two chlorine atoms linking to benzimidazole forms another  $\pi$ -cation interaction (distance: 5.32 Å) with the imidazole amino cation of His1026.

# 3. Conclusions

A series of quinolin-4-amine derivatives containing benzimidazole moiety have been synthesized and discovered as novel VEGFR-2 inhibitors that displayed good antiproliferation activities against two tumor cell lines (MCF-7 and Hep-G2) and weak cytotoxic activities against the human normal live cell line L02. Compound **7s** exhibited the most potent inhibitory activity against VEGFR-2 with IC<sub>50</sub> of 0.03  $\mu$ M, which was equivalent to the commercial VEGFR-2 inhibitor sunitinib and also showed the greatest inhibitory activities against human breast cancer cell MCF-7 and human liver cancer cell Hep-G2 with IC<sub>50</sub> of 1.2  $\mu$ M and 13.3  $\mu$ M, respectively. Molecular docking of the most potent inhibitor **7s** into ATP binding site of VEGFR-2 kinase was performed and the result suggested that compound **7s** could bind well with the VEGFR-2 active site. The above results provided theoretical basis for further structural optimization of benzimidazole quinolin-4-amine derivatives as VEGFR-2 inhibitors and demonstrated that compound **7s** could be a potential anticancer agent.

#### 4. Experimental section

#### 4.1. Chemistry

Unless otherwise noted, all solvents and reagents were commercially available and used without further purification. Melting points (uncorrected) were determined on a RY-1 MP apparatus. ESI-MS spectra were recorded on an Agilent/HP 1100 Series LC/MSD Trap SL Mass spectrometer. <sup>1</sup>H NMR spectra and <sup>13</sup>C NMR were recorded on a Bruker AV-300 or AV-500 spectrometer at 25 °C with TMS and solvent signals allotted as internal standards. Chemical shifts were reported in ppm ( $\delta$ ). Elemental analyses were performed on a CHN–O-Rapid instrument.

# 4.1.1. General procedure for the preparation of the 5-nitro-2-phenyl-1H-benzo[d]imidazole derivatives **3a**–**3u**

A mixture of 4-nitro-o-phenylenediamine (3.22 g, 21 mmol) and substituted benzoic acid **2a–2u** (20 mmol) in PPA (40 mL) was stirred at 120–150 °C for 5 h. The reaction was quenched with water and the pH was adjusted to 6 with saturated NaOH. The filter cake was washed with water and recrystallized from ethyl acetate to give corresponding compounds **3a–3u**.

4.1.1.1. 5-Nitro-2-phenyl-1H-benzo[d]imidazole (**3a**). Pale orangered powder, yield: 88%, mp: 206–208 °C. <sup>1</sup>H NMR (300 MHz;  $d_6$ -DMSO): 7.48–8.04 (m, 4H); 8.04–8.69 (m, 4H); 13.64 (s, 1H). MS (ESI<sup>+</sup>) *m*/z 294.1 (M+H)<sup>+</sup>. Anal. Calcd for C<sub>13</sub>H<sub>9</sub>N<sub>3</sub>O<sub>2</sub>: C, 65.27; H, 3.79; N, 17.56; Found: C, 65.32; H, 3.82; N, 17.53.

4.1.1.2. 5-Nitro-2-o-tolyl-1H-benzo[d]imidazole (**3b**). Yellow powder, yield: 86%, mp: 146–148 °C. <sup>1</sup>H NMR (300 MHz;  $d_6$ -DMSO): 2.58 (s, 3H); 7.46 (s, 3H); 7.77–7.85 (m, 2H); 8.16 (s, 1H); 8.40–8.59 (m, 1H); 13.37 (s, 1H). MS (ESI<sup>+</sup>) *m*/*z* 254.1 (M+H)<sup>+</sup>. Anal. Calcd for C<sub>14</sub>H<sub>11</sub>N<sub>3</sub>O<sub>2</sub>: C, 66.40; H, 4.38; N, 16.59; Found: C, 66.34; H, 4.41; N, 16.64.

4.1.1.3. 5-Nitro-2-*m*-tolyl-1H-benzo[d]imidazole (**3c**). Yellow powder, yield: 99%, mp: 108–110 °C. <sup>1</sup>H NMR (300 MHz; *d*<sub>6</sub>-DMSO): 2.48 (s, 3H); 7.38–7.52 (m, 2H); 7.76 (d, *J* = 8.5 Hz, 1H); 8.00–8.15

#### Table 1

Enzymatic and cellular results for benzimidazole quinolin-4-amines.

Compound		VEGFR-2	Proliferative inhibition (IC <sub>50</sub> , $\mu$ M)		
No.	R	(IC <sub>50</sub> , μM)	Hep-G2	MCF-7	L02
7a	Н	2.48	116.0	4.6	>100
7b	2-CH <sub>3</sub>	>10	>100	54.2	>100
7c	3-CH <sub>3</sub>	6.80	59.7	18.9	>100
7d	4-CH <sub>3</sub>	4.65	54.1	6.6	>100
7e	2-OCH <sub>3</sub>	>10	>100	75.0	>100
7f	2-0H	9.53	52.5	11.4	>100
7g	4-CH <sub>2</sub> CH <sub>3</sub>	5.49	63.3	8.0	>100
7h	2-F	>10	>100	31.2	>100
7i	3-F	3.52	85.2	9.6	>100
7j	4-F	1.36	49.4	4.3	54.0
7k	2-Cl	>10	67.0	19.6	>100
71	3-Cl	2.40	38.0	6.3	>100
7m	4-Cl	0.24	45.4	3.2	65.8
7n	4-Br	0.18	42.8	3.4	92.5
7 <b>o</b>	2-CF <sub>3</sub>	>10	>100	51.1	>100
7p	3-CF <sub>3</sub>	>10	>100	42.8	>100
7q	4-CF <sub>3</sub>	>10	63.0	18.9	>100
7r	2,6-di-Cl	8.35	85.9	24.7	>100
7s	3,4-di-Cl	0.03	13.3	1.2	59.5
7t	3,5-di-Cl	0.08	26.8	2.3	82.3
7u	2-Br,5-F	7.82	62.7	20.4	>100
sunitinib		0.04	13.6	10.5	75.6

(m, 3H); 8.47 (s, 1H); 13.58 (s, 1H). MS (ESI<sup>+</sup>) m/z 254.2 (M+H)<sup>+</sup>. Anal. Calcd for C<sub>14</sub>H<sub>11</sub>N<sub>3</sub>O<sub>2</sub>: C, 66.40; H, 4.38; N, 16.59; Found: C, 66.45; H, 4.42; N, 16.53.

4.1.1.4. 5-*Nitro-2-p-tolyl-1H-benzo[d]imidazole* (**3d**). Yellow powder, yield: 81%, mp: 219–221 °C. <sup>1</sup>H NMR (300 MHz;  $d_6$ -DMSO): 2.32 (s, 3H); 7.29 (d, *J* = 7.9 Hz, 2H); 7.61 (d, *J* = 8.8 Hz, 1H); 7.98 (dd, *J* = 8.8, 2.2 Hz, 1H); 8.09 (d, *J* = 8.1 Hz, 2H); 8.35 (d, *J* = 2.1 Hz, 1H). MS (ESI<sup>+</sup>) *m*/*z* 254.1 (M+H)<sup>+</sup>. Anal. Calcd for C<sub>14</sub>H<sub>11</sub>N<sub>3</sub>O<sub>2</sub>: C, 66.40; H, 4.38; N, 16.59; Found: C, 66.52; H, 4.35; N, 16.65.

4.1.1.5. 2-(2-*Methoxyphenyl*)-5-*nitro*-1*H*-*benzo*[*d*]*imidazole* (**3***e*). Brown powder, yield: 97%, mp: 208–209 °C. <sup>1</sup>H NMR (300 MHz; *d*<sub>6</sub>-DMSO): 4.08 (s, 3H); 7.17 (t, J = 7.4 Hz, 1H); 7.30 (d, J = 8.3 Hz, 1H); 7.57 (t, J = 7.4 Hz, 1H); 7.81 (d, J = 8.9 Hz, 1H); 8.13 (d, J = 8.7 Hz, 1H); 8.39 (d, J = 7.4 Hz, 1H); 8.54 (s, 1H); 12.67 (s, 1H). MS (ESI<sup>+</sup>) *m/z* 270.1 (M+H)<sup>+</sup>. Anal. Calcd for C<sub>14</sub>H<sub>11</sub>N<sub>3</sub>O<sub>3</sub>: C, 62.45; H, 4.12; N, 15.61; Found: C, 62.66; H, 4.14; N, 15.67.

4.1.1.6. 2-(5-Nitro-1H-benzo[d]imidazol-2-yl)phenol (**3***f*). Dark brown powder, yield: 37%, mp:  $305-307 \circ C.^{1}H$  NMR (300 MHz;  $d_{6}$ -DMSO): 6.94–7.04 (m, 2H); 7.35 (t, J = 7.23 Hz, 1H); 7.71 (d, J = 8.8 Hz, 1H); 8.05 (dd, J = 8.8, 2.1 Hz, 1H); 8.26 (d, J = 7.0 Hz, 1H); 8.47 (d, J = 1.95 Hz, 1H). MS (ESI<sup>+</sup>) m/z 256.1 (M+H)<sup>+</sup>. Anal. Calcd for C<sub>13</sub>H<sub>9</sub>N<sub>3</sub>O<sub>3</sub>: C, 61.18; H, 3.55; N, 16.46; Found: C, 61.35; H, 3.51; N, 16.50.

4.1.1.7. 2-(4-Ethylphenyl)-5-nitro-1H-benzo[d]imidazole (**3**g). Orange powder, yield: 90%, mp: 84–86 °C. <sup>1</sup>H NMR (300 MHz;  $d_6$ -DMSO): 1.22 (t, J = 7.5 Hz, 3H); 2.68 (d, J = 7.5 Hz, 2H); 7.42 (d, J = 8.1 Hz, 2H); 7.72 (d, J = 9.0 Hz, 1H); 8.08–8.13 (m, 3H); 8.42 (s, 1H); 13.48 (s, 1H). MS (ESI<sup>+</sup>) m/z 268.1 (M+H)<sup>+</sup>. Anal. Calcd for C<sub>15</sub>H<sub>13</sub>N<sub>3</sub>O<sub>2</sub>: C, 67.40; H, 4.90; N, 15.72; Found: C, 67.52; H, 4.86; N, 15.68. 4.1.1.8. 2-(2-Fluorophenyl)-5-nitro-1H-benzo[d]imidazole (**3h**). Orange powder, yield: 26%, mp: 171–173 °C. <sup>1</sup>H NMR (300 MHz;  $d_6$ -DMSO): 7.41–7.52 (m, 2H); 7.60–7.68 (m, 1H); 7.81 (d, J = 8.7 Hz, 1H); 8.15 (dd, J = 8.7, 1.8 Hz, 1H); 8.27 (t, J = 7.2 Hz, 1H); 8.52 (s, 1H); 13.21 (s, 1H). MS (ESI<sup>+</sup>) m/z 258.1 (M+H)<sup>+</sup>. Anal. Calcd for C<sub>13</sub>H<sub>8</sub>FN<sub>3</sub>O<sub>2</sub>: C, 60.70; H, 3.13; N, 16.34; Found: C, 60.78; H, 3.12; N, 16.30.

4.1.1.9. 2-(3-Fluorophenyl)-5-nitro-1H-benzo[d]imidazole (3i). Pale yellow powder, yield: 42%, mp:  $205-207 \, ^{\circ}$ C. <sup>1</sup>H NMR (300 MHz;  $d_6$ -DMSO): 7.38–7.43 (m, 1H); 7.63–7.76 (m, 2H); 7.95–8.14 (m, 3H); 8.48 (s, 1H); 13.66 (s, 1H). MS (ESI<sup>+</sup>) *m/z* 258.1 (M+H)<sup>+</sup>. Anal. Calcd for C<sub>13</sub>H<sub>8</sub>FN<sub>3</sub>O<sub>2</sub>: C, 60.70; H, 3.13; N, 16.34; Found: C, 60.55; H, 3.15; N, 16.39.

4.1.1.10. 2-(4-Fluorophenyl)-5-nitro-1H-benzo[d]imidazole (**3***j*). Golden powder, mp: yield: 96%, mp: 258–260 °C. <sup>1</sup>H NMR (300 MHz;  $d_6$ -DMSO): 7.44 (t, J = 9.0 Hz, 2H); 7.75 (d, J = 8.7 Hz, 1H); 8.11 (dd, J = 9.0, 2.1 Hz, 1H); 8.23–8.27 (m, 2H); 8.45 (s, 1H); 13.59 (s, 1H). MS (ESI<sup>+</sup>) *m/z* 258.1 (M+H)<sup>+</sup>. Anal. Calcd for C<sub>13</sub>H<sub>8</sub>FN<sub>3</sub>O<sub>2</sub>: C, 60.70; H, 3.13; N, 16.34; Found: C, 60.84; H, 3.16; N, 16.38.



**Fig. 2.** (A) 2D molecular docking modeling of compound **7s** with VEGFR-2 kinase. The hydrogen bonds are displayed as green or blue dotted lines. The  $\pi$ -cation interactions,  $\pi$ - $\pi$  interactions and  $\sigma$ - $\pi$  interaction are shown as yellow line. (B) 3D model of the interaction between compound **7s** and VEGFR-2 kinase ATP binding site. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

4.1.1.11. 2-(2-Chlorophenyl)-5-nitro-1H-benzo[d]imidazole (**3k**). Brown powder, yield: 16%, mp: 168–170 °C. <sup>1</sup>H NMR (300 MHz;  $d_6$ -DMSO): 7.52–7.62 (m, 2H); 7.67–7.70 (m, 1H); 7.80 (d, J = 9.0 Hz, 1H); 7.94 (dd, J = 7.2, 2.1 Hz, 1H); 8.15 (dd, J = 8.7, 2.1 Hz, 1H); 8.53 (s, 1H); 13.42 (s, 1H). MS (ESI<sup>+</sup>) m/z 274.1 (M+H)<sup>+</sup>. Anal. Calcd for C<sub>13</sub>H<sub>8</sub>ClN<sub>3</sub>O<sub>2</sub>: C, 57.05; H, 2.95; N, 15.35; Found: C, 57.22; H, 2.97; N, 15.41.

4.1.1.12. 2-(3-Chlorophenyl)-5-nitro-1H-benzo[d]imidazole (31). Orange powder, yield: 83%, mp: 220–222 °C. <sup>1</sup>H NMR (300 MHz;  $d_6$ -DMSO): 7.61 (d, J = 5.1 Hz, 2H); 7.76 (d, J = 9.0 Hz, 1H); 8.10–8.17 (m, 2H); 8.22 (d, J = 0.9 Hz, 1H); 8.46 (d, J = 1.8 Hz, 1H); 13.69 (s, 1H). MS (ESI<sup>+</sup>) m/z 274.0 (M+H)<sup>+</sup>. Anal. Calcd for C<sub>13</sub>H<sub>8</sub>ClN<sub>3</sub>O<sub>2</sub>: C, 57.05; H, 2.95; N, 15.35; Found: C, 56.88; H, 3.01; N, 15.43.

4.1.1.13. 2-(4-Chlorophenyl)-5-nitro-1H-benzo[d]imidazole (**3m**). Yellow green powder, yield: 40%, mp: 301–303 °C. <sup>1</sup>H NMR (300 MHz; *d*<sub>6</sub>-DMSO): 7.66–7.77 (m, 3H); 8.13 (dd, J = 9.0, 2.1 Hz, 1H); 8.21 (d, J = 8.7 Hz, 2H); 8.49 (s, 1H); 13.66 (s, 1H). MS (ESI<sup>+</sup>) *m*/z 274.0 (M+H)<sup>+</sup>. Anal. Calcd for C<sub>13</sub>H<sub>8</sub>ClN<sub>3</sub>O<sub>2</sub>: C, 57.05; H, 2.95; N, 15.35; Found: C, 57.19; H, 2.92; N, 15.29.

4.1.1.14. 2-(4-Bromophenyl)-5-nitro-1H-benzo[d]imidazole (**3n**). Yellow powder, yield: 78%, mp: 296–298 °C. <sup>1</sup>H NMR (300 MHz;  $d_6$ -DMSO): 7.77–7.85 (m, 3H); 8.13–8.18 (m, 3H); 8.50 (s, 1H). MS (ESI<sup>+</sup>) *m*/z 318.0 (M+H)<sup>+</sup>. Anal. Calcd for C<sub>13</sub>H<sub>8</sub>BrN<sub>3</sub>O<sub>2</sub>: C, 49.08; H, 2.53; N, 13.21; Found: C, 49.24; H, 2.52; N, 13.26.

4.1.1.15. 5-*Nitro-2-(2-(trifluoromethyl)phenyl)-1H-benzo[d]imid-azole* (**30**). Pale orange powder, yield: 84%, mp: 190–191 °C. <sup>1</sup>H NMR (300 MHz; *d*<sub>6</sub>-DMSO): 7.80–7.91 (m, 4H); 7.98 (d, *J* = 7.5 Hz, 1H); 8.17 (d, *J* = 8.1 Hz, 1H); 8.54 (s, 1H), 13.52 (s, 1H). MS (ESI<sup>+</sup>) *m/z* 308.2 (M+H)<sup>+</sup>. Anal. Calcd for C<sub>14</sub>H<sub>8</sub>F<sub>3</sub>N<sub>3</sub>O<sub>2</sub>: C, 54.73; H, 2.62; N, 13.68; Found: C, 54.78; H, 2.64; N, 13.65.

4.1.1.16. 5-Nitro-2-(3-(trifluoromethyl)phenyl)-1H-benzo[d]imidazole (**3p**). Brown crystal, yield: 56%, mp: 229–230 °C. <sup>1</sup>H NMR (300 MHz; *d*<sub>6</sub>-DMSO): 7.76–7.93 (m, 3H); 8.12 (dd, J = 9.0, 2.1 Hz, 1H); 8.47–8.52 (m, 3H); 13.79 (s, 1H). MS (ESI<sup>+</sup>) m/z 308.1 (M+H)<sup>+</sup>. Anal. Calcd for C<sub>14</sub>H<sub>8</sub>F<sub>3</sub>N<sub>3</sub>O<sub>2</sub>: C, 54.73; H, 2.62; N, 13.68; Found: C, 54.86; H, 2.59; N, 13.72.

4.1.1.17. 5-*Nitro-2-(4-(trifluoromethyl)phenyl)-1H-benzo[d]imid-azole* (**3q**). Orange powder, yield: 21%, mp: 273–274 °C. <sup>1</sup>H NMR (300 MHz; *d*<sub>6</sub>-DMSO): 7.77 (d, J = 8.7 Hz, 1H); 7.94 (d, J = 8.7 Hz, 2H); 8.12 (dd, J = 9.0, 2.4 Hz, 1H); 8.37 (d, J = 8.1 Hz, 2H); 8.47 (d, J = 1.8 Hz, 1H); 13.78 (s, 1H). MS (ESI<sup>+</sup>) *m/z* 308.1 (M+H)<sup>+</sup>. Anal. Calcd for C<sub>14</sub>H<sub>8</sub>F<sub>3</sub>N<sub>3</sub>O<sub>2</sub>: C, 54.73; H, 2.62; N, 13.68; Found: C, 54.88; H, 2.60; N, 13.64.

4.1.1.18. 2-(2,6-Dichlorophenyl)-5-nitro-1H-benzo[d]imidazole (**3r**). Pale orange powder, yield: 80%, mp: 210–212 °C. <sup>1</sup>H NMR (300 MHz;  $d_6$ -DMSO): 7.60–7.71 (m, 3H); 7.83 (d, J = 9.0 Hz, 1H); 8.17 (dd, J = 9.0, 2.1 Hz, 1H); 8.58 (s, 1H); 13.65 (s, 1H). MS (ESI<sup>+</sup>) m/z 307.1 (M+H)<sup>+</sup>. Anal. Calcd for C<sub>13</sub>H<sub>7</sub>Cl<sub>2</sub>N<sub>3</sub>O<sub>2</sub>: C, 50.67; H, 2.29; N, 13.64; Found: C, 50.80; H, 2.31; N, 13.68.

4.1.1.19. 2-(3,4-Dichlorophenyl)-5-nitro-1H-benzo[d]imidazole (**3s**). Pale orange powder, yield: 42%, mp: 232–235 °C. <sup>1</sup>H NMR (300 MHz; *d*<sub>6</sub>-DMSO): 7.75 (d, J = 8.4 Hz, 1H); 7.82–7.88 (m, 1H); 8.03–8.15 (m, 2H); 8.37 (d, J = 2.1 Hz, 1H); 8.45 (s, 1H); 13.67 (s, 1H). MS (ESI<sup>+</sup>) *m*/*z* 307.1 (M+H)<sup>+</sup>. Anal. Calcd for C<sub>13</sub>H<sub>7</sub>Cl<sub>2</sub>N<sub>3</sub>O<sub>2</sub>: C, 50.67; H, 2.29; N, 13.64; Found: C, 50.82; H, 2.28; N, 13.60.

4.1.1.20. 2-(3,5-Dichlorophenyl)-5-nitro-1H-benzo[d]imidazole (**3t**). Pale orange powder, yield: 12%, mp: 290–292 °C. <sup>1</sup>H NMR (300 MHz; *d*<sub>6</sub>-DMSO): 7.77–7.82 (m, 2H); 8.14 (dd, J = 9.0, 2.1 Hz, 1H); 8.20 (d, J = 2.1 Hz, 2H); 8.49 (d, J = 2.1 Hz, 1H); 13.77 (s, 1H). MS (ESI<sup>+</sup>) *m*/z 307.0 (M+H)<sup>+</sup>. Anal. Calcd for C<sub>13</sub>H<sub>7</sub>Cl<sub>2</sub>N<sub>3</sub>O<sub>2</sub>: C, 50.67; H, 2.29; N, 13.64; Found: C, 50.53; H, 2.26; N, 13.57.

4.1.1.21. 2-(2-Bromo-5-fluorophenyl)-5-nitro-1H-benzo[d]imidazole (**3u**). Brown powder, mp: yield: 66%, mp: 218–220 °C. <sup>1</sup>H NMR (300 MHz;  $d_6$ -DMSO): 7.43 (dd, J = 8.4, 3.0 Hz, 1H); 7.70–7.92 (m, 3H); 8.18 (d, J = 9.0 Hz, 1H); 8.60 (s, 1H); 13.55 (s, 1H). MS (ESI<sup>+</sup>) m/z336.0 (M+H)<sup>+</sup>. Anal. Calcd for C<sub>13</sub>H<sub>7</sub>BrFN<sub>3</sub>O<sub>2</sub>: C, 46.45; H, 2.10; N, 12.50; Found: C, 46.53; H, 2.11; N, 12.55.

#### 4.1.2. General procedure for the preparation of the 2-phenyl-1Hbenzo[d]imidazol-5-amine derivatives **4a–4u**

Method A: A suspension of **3** (5.0 mmol,  $R = CH_3$ , OCH<sub>3</sub>, OH, CH<sub>2</sub>CH<sub>3</sub>, CF<sub>3</sub>) and 10% Pd/C (0.13 g) in methanol (30 mL) was hydrogenated under normal pressure for 5 h at room temperature. Filtration and evaporation gave corresponding compound **4**.

Method B: Compound **3** (5.0 mmol, R = F, Cl, Br) and iron (1.1 g, 20 mmol) were suspended in aqueous ethanol (120 mL, 70% v/v) containing acetic acid (2 mL, 30 mmol) and heated at reflux for 2 h. The reaction mixture was cooled to room temperature. Filtration and evaporation gave corresponding compound **4**.

4.1.2.1. 2-Phenyl-1H-benzo[d]imidazol-5-amine (**4a**). Pink crystal, yield: 99%, mp: 292–293 °C. <sup>1</sup>H NMR (300 MHz;  $d_6$ -DMSO): 3.17 (s, 2H); 6.54 (d, *J* = 8.5 Hz, 1H); 6.69 (s, 1H); 7.28 (d, *J* = 8.5 Hz, 1H); 7.41 (t, *J* = 7.0 Hz, 1H); 7.50 (t, *J* = 7.6 Hz, 2H); 8.07 (d, *J* = 7.8 Hz, 2H). MS (ESI<sup>+</sup>) *m*/z 210.1 (M+H)<sup>+</sup>. Anal. Calcd for C<sub>13</sub>H<sub>11</sub>N<sub>3</sub>: C, 74.62; H, 5.30; N, 20.08; Found: C, 74.48; H, 5.26; N, 20.14.

4.1.2.2. 2-o-Tolyl-1H-benzo[d]imidazol-5-amine (**4b**). Brown powder, yield: 64.28%, mp: 63–64 °C. <sup>1</sup>H NMR (300 MHz; CDCl<sub>3</sub>): 2.45 (s, 3H); 3.44 (s, 2H); 6.54 (t, J = 8.5 Hz, 1H); 7.08–7.46 (m, 6H). MS (ESI<sup>+</sup>) m/z 224.1 (M+H)<sup>+</sup>. Anal. Calcd for C<sub>14</sub>H<sub>13</sub>N<sub>3</sub>: C, 75.31; H, 5.87; N, 18.82; Found: C, 75.52; H, 5.91; N, 18.77.

4.1.2.3. 2-*m*-Tolyl-1H-benzo[d]imidazol-5-amine (**4c**). Yellow powder, yield: 82.33%, mp: 231–233 °C. <sup>1</sup>H NMR (300 MHz; CDCl<sub>3</sub>): 2.36 (s, 3H); 6.69 (dd, J = 8.7, 2.1 Hz, 1H); 6.85 (d, J = 1.8 Hz, 1H); 7.22 (d, J = 7.8 Hz, 1H); 7.32 (t, J = 7.5 Hz, 1H); 7.48 (d, J = 8.4 Hz, 1H); 7.79 (d, J = 7.8 Hz, 1H); 7.90 (s, 1H). MS (ESI<sup>+</sup>) m/z 224.1 (M+H)<sup>+</sup>. Anal. Calcd for C<sub>14</sub>H<sub>13</sub>N<sub>3</sub>: C, 75.31; H, 5.87; N, 18.82; Found: C, 75.38; H, 5.84; N, 18.86.

4.1.2.4. 2-*p*-Tolyl-1H-benzo[d]imidazol-5-amine (**4d**). Orange powder, yield: 89.4%, mp: 112–114 °C. <sup>1</sup>H NMR (300 MHz; CDCl<sub>3</sub>): 2.40 (s, 3H); 6.67 (dd, J = 8.7, 2.1 Hz, 1H); 6.84 (s, 1H); 7.88 (t, J = 1.8 Hz, 2H); 7.27 (s, 1H); 7.46 (d, J = 8.5 Hz, 1H); 7.88 (t, J = 1.8 Hz, 2H). MS (ESI<sup>+</sup>) m/z 224.1 (M+H)<sup>+</sup>. Anal. Calcd for C<sub>14</sub>H<sub>13</sub>N<sub>3</sub>: C, 75.31; H, 5.87; N, 18.82; Found: C, 75.05; H, 5.91; N, 18.79.

4.1.2.5. 2-(2-Methoxyphenyl)-1H-benzo[d]imidazol-5-amine (4e). Brown powder, yield: 97%, mp: 193–194 °C. <sup>1</sup>H NMR (300 MHz;  $d_6$ -DMSO): 3.99 (s, 3H); 6.54 (dd, J = 8.5, 1.6 Hz, 1H); 6.75 (s, 1H); 7.06 (t, J = 7.5 Hz, 1H); 7.18 (d, J = 8.3 Hz, 1H); 7.28 (d, J = 8.5 Hz, 1H); 7.37–7.42 (m, 1H); 8.24 (d, J = 7.7 Hz, 1H). MS (ESI<sup>+</sup>) m/z 240.1 (M+H)<sup>+</sup>. Anal. Calcd for C<sub>14</sub>H<sub>13</sub>N<sub>3</sub>O: C, 70.28; H, 5.48; N, 17.56; Found: C, 70.46; H, 5.50; N, 17.51.

4.1.2.6. 2-(5-Amino-1H-benzo[d]imidazol-2-yl)phenol (**4f**). Yellow powder, yield: 46%, mp: 250–251 °C. <sup>1</sup>H NMR (300 MHz; d<sub>6</sub>-DMSO): 6.61 (d, *J* = 8.3 Hz, 1H); 6.74 (s, 1H); 6.97 (t, *J* = 8.4 Hz, 2H); 7.28–7.36 (m, 2H); 7.93 (d, J = 7.7 Hz, 1H); 12.63 (s, 1H); 13.22 (s, 1H). MS (ESI<sup>+</sup>) m/z 226.1 (M+H)<sup>+</sup>. Anal. Calcd for C<sub>13</sub>H<sub>11</sub>N<sub>3</sub>O: C, 69.32; H, 4.92; N, 18.66; Found: C, 69.16; H, 4.95; N, 18.72.

4.1.2.7. 2-(4-Ethylphenyl)-1H-benzo[d]imidazol-5-amine (4g). Orange powder, yield: 71%, mp: 102–104 °C. <sup>1</sup>H NMR (300 MHz; CDCl<sub>3</sub>): 1.26 (t, J = 7.5 Hz, 3H); 2.67–2.71 (m, 2H); 5.30 (d, J = 0.5 Hz, 1H); 6.67 (dd, J = 8.5, 2.0 Hz, 1H); 6.84 (s, 1H); 7.28 (s, 1H); 7.45 (d, J = 8.0 Hz, 1H); 7.92 (d, J = 8.0 Hz, 2H). MS (ESI<sup>+</sup>) m/z 238.1 (M+H)<sup>+</sup>. Anal. Calcd for C<sub>15</sub>H<sub>15</sub>N<sub>3</sub>: C, 75.92; H, 6.37; N, 17.71; Found: C, 80.18; H, 6.33; N, 17.66.

4.1.2.8. 2-(2-Fluorophenyl)-1H-benzo[d]imidazol-5-amine (**4h**). White powder, yield: 61.9%, mp: 214–215 °C. <sup>1</sup>H NMR (300 MHz; CDCl<sub>3</sub>): 6.72 (dd, J = 8.4, 2.1 Hz, 1H); 6.88 (s, 1H); 7.16–7.23 (m, 1H); 7.30–7.43 (m, 2H); 7.51 (d, J = 6.6 Hz, 1H); 8.46 (dd, J = 7.8, 1.8 Hz, 1H). MS (ESI<sup>+</sup>) m/z 228.1 (M+H)<sup>+</sup>. Anal. Calcd for C<sub>13</sub>H<sub>10</sub>FN<sub>3</sub>: C, 68.71; H, 4.44; N, 18.49; Found: C, 68.56; H, 4.43; N, 18.45.

4.1.2.9. 2-(3-Fluorophenyl)-1H-benzo[d]imidazol-5-amine (4i). Pale orange powder, yield: 76%, mp: 245–246 °C. <sup>1</sup>H NMR (300 MHz;  $d_6$ -DMSO): 4.98 (s, 2H); 6.54 (d, J = 8.4 Hz, 1H); 6.67 (s, 1H); 7.19–7.30 (m, 2H); 7.49–7.56 (m, 1H); 7.81–7.91 (m, 2H); 12.32 (s, 1H). MS (ESI<sup>+</sup>) m/z 228.1 (M+H)<sup>+</sup>. Anal. Calcd for C<sub>13</sub>H<sub>10</sub>FN<sub>3</sub>: C, 68.71; H, 4.44; N, 18.49; Found: C, 68.89; H, 4.46; N, 18.44.

4.1.2.10. 2-(4-Fluorophenyl)-1H-benzo[d]imidazol-5-amine (**4j**). Orange crystal, yield: 73.86%, mp: 68–69 °C. <sup>1</sup>H NMR (300 MHz; CDCl<sub>3</sub>): 3.50 (s, 2H); 6.66 (d, J = 8.4 Hz, 1H); 6.80 (s, 1H); 7.06 (t, J = 8.4 Hz, 2H); 7.42 (d, J = 8.4 Hz, 1H); 9.97 (m, 2H). MS (ESI<sup>+</sup>) m/z 228.1 (M+H)<sup>+</sup>. Anal. Calcd for C<sub>13</sub>H<sub>10</sub>FN<sub>3</sub>: C, 68.71; H, 4.44; N, 18.49; Found: C, 68.93; H, 4.41; N, 18.55.

4.1.2.11. 2-(2-Chlorophenyl)-1H-benzo[d]imidazol-5-amine (**4k**). Brown powder, yield: 76.58%, mp: 87–89 °C. <sup>1</sup>H NMR (500 MHz; CDCl<sub>3</sub>): 6.73 (dd, J = 8.0, 2.0 Hz, 1H); 6.89 (s, 1H); 7.33–7.41 (m, 2H); 7.47 (dd, J = 8.0, 1.5 Hz, 1H); 7.52 (d, J = 8.0 Hz, 1H); 8.39 (dd, J = 7.5, 1.5 Hz, 1H). MS (ESI<sup>+</sup>) m/z 244.1 (M+H)<sup>+</sup>. Anal. Calcd for C<sub>13</sub>H<sub>10</sub>ClN<sub>3</sub>: C, 64.07; H, 4.14; N, 17.24; Found: C, 64.25; H, 4.16; N, 17.26.

4.1.2.12. 2-(3-Chlorophenyl)-1H-benzo[d]imidazol-5-amine (41). Orange powder, yield: 38%, mp: 72–74 °C. <sup>1</sup>H NMR (300 MHz; CDCl<sub>3</sub>): 6.69 (dd, J = 8.7, 2.1 Hz, 1H); 6.82 (d, J = 1.8 Hz, 1H); 7.33–7.35 (m, 2H); 7.47 (d, J = 8.4 Hz, 1H); 7.84–7.88 (m, 1H); 7.97–7.99 (m, 1H). MS (ESI<sup>+</sup>) m/z 244.1 (M+H)<sup>+</sup>. Anal. Calcd for C<sub>13</sub>H<sub>10</sub>ClN<sub>3</sub>: C, 64.07; H, 4.14; N, 17.24; Found: C, 63.88; H, 4.17; N, 17.29.

4.1.2.13. 2-(4-Chlorophenyl)-1H-benzo[d]imidazol-5-amine (4m). Brown powder, yield: 57%, mp: 88–90 °C. <sup>1</sup>H NMR (300 MHz; CDCl<sub>3</sub>): 2.17 (s, 2H); 6.63 (dd, J = 8.7, 2.1 Hz, 1H); 6.73 (d, J = 2.1 Hz, 1H); 7.31 (dd, J = 6.9, 2.4 Hz, 2H); 7.37 (d, J = 8.7 Hz, 1H); 8.85 (dd; J = 5.4, 1.8 Hz, 2H). MS (ESI<sup>+</sup>) m/z 244.2 (M+H)<sup>+</sup>. Anal. Calcd for C<sub>13</sub>H<sub>10</sub>ClN<sub>3</sub>: C, 64.07; H, 4.14; N, 17.24; Found: C, 64.23; H, 4.12; N, 17.28.

4.1.2.14. 2-(4-Bromophenyl)-1H-benzo[d]imidazol-5-amine (4n). Orange powder, yield: 64.45%, mp: 104–106 °C. <sup>1</sup>H NMR (300 MHz;  $d_6$ -DMSO): 6.54 (d, J = 8.4 Hz, 1H); 6.70 (s, 1H); 7.25 (d, J = 8.4 Hz, 1H); 7.64 (d, J = 8.4 Hz, 2H); 8.06 (d, J = 8.4 Hz, 2H). MS (ESI<sup>+</sup>) m/z288.0 (M+H)<sup>+</sup>. Anal. Calcd for C<sub>13</sub>H<sub>10</sub>BrN<sub>3</sub>: C, 54.19; H, 3.50; N, 14.58; Found: C, 54.34; H, 3.51; N, 14.62. 4.1.2.15. 2-(2-(*Trifluoromethyl*)phenyl)-1H-benzo[d]imidazol-5amine (**40**). Brown powder, yield: 93%, mp: 153–155 °C. <sup>1</sup>H NMR (300 MHz; *d*<sub>6</sub>-DMSO): 5.12 (s, 2H); 6.50 (d, *J* = 7.4 Hz, 1H); 6.80 (s, 1H); 7.45 (d, *J* = 8.4 Hz, 1H); 7.61–7.66 (m, 2H); 8.54–8.71 (m, 2H). MS (ESI<sup>+</sup>) *m*/*z* 278.2 (M+H)<sup>+</sup>. Anal. Calcd for C<sub>14</sub>H<sub>10</sub>F<sub>3</sub>N<sub>3</sub>: C, 60.65; H, 3.64; N, 15.16; Found: C, 60.58; H, 3.69; N, 15.20.

4.1.2.16. 2-(3-(*Trifluoromethyl*)phenyl)-1H-benzo[d]imidazol-5amine (**4p**). Brown crystal, yield: 76%, mp: 93–95 °C. <sup>1</sup>H NMR (300 MHz; *d*<sub>6</sub>-DMSO): 5.02 (s, 2H); 6.58 (d, J = 8.4 Hz, 1H); 6.70 (s, 1H); 7.33 (d, J = 8.3 Hz, 1H); 7.71–7.76 (m, 2H); 8.35–8.41 (m, 2H). MS (ESI<sup>+</sup>) m/z 278.1 (M+H)<sup>+</sup>. Anal. Calcd for C<sub>14</sub>H<sub>10</sub>F<sub>3</sub>N<sub>3</sub>: C, 60.65; H, 3.64; N, 15.16; Found: C, 60.47; H, 3.61; N, 15.22.

4.1.2.17. 2-(4-(*Trifluoromethyl*)*phenyl*)-1*H*-*benzo*[*d*]*imidazo*1-5*amine* (**4q**). White powder, yield: 82%, mp: 208–209 °C. <sup>1</sup>H NMR (300 MHz; *d*<sub>6</sub>-DMSO): 5.09 (s, 2H); 6.57 (dd, J = 8.5, 1.6 Hz, 1H); 6.67 (s, 1H); 7.35 (d, J = 8.5 Hz, 1H); 7.86 (d, J = 8.1 Hz, 2H); 8.26 (d, J = 8.1 Hz, 2H); 12.51 (s, 1H). MS (ESI<sup>+</sup>) *m*/z 278.1 (M+H)<sup>+</sup>. Anal. Calcd for C<sub>14</sub>H<sub>10</sub>F<sub>3</sub>N<sub>3</sub>: C, 60.65; H, 3.64; N, 15.16; Found: C, 60.83; H, 3.67; N, 15.13.

4.1.2.18. 2-(2,6-Dichlorophenyl)-1H-benzo[d]imidazol-5-amine (**4r**). Brown crystal, yield: 64.25%, mp: 137–139 °C. <sup>1</sup>H NMR (300 MHz;  $d_6$ -DMSO): 6.56 (dd, J = 8.7, 2.1 Hz, 1H); 6.67 (s, 1H); 7.28 (d, J = 8.4 Hz, 1H); 7.51–7.63 (m, 3H). MS (ESI<sup>+</sup>) *m/z* 278.0 (M+H)<sup>+</sup>. Anal. Calcd for C<sub>13</sub>H<sub>9</sub>Cl<sub>2</sub>N<sub>3</sub>: C, 56.14; H, 3.26; N, 15.11; Found: C, 56.28; H, 3.23; N, 15.15.

4.1.2.19. 2-(3,4-Dichlorophenyl)-1H-benzo[d]imidazol-5-amine (**4s**). Orange crystal, yield: 71%, mp: 100–102 °C. <sup>1</sup>H NMR (300 MHz;  $d_6$ -DMSO): 6.57 (d, *J* = 8.5 Hz, 1H); 6.68 (s, 1H); 7.31 (d, *J* = 8.5 Hz, 1H); 7.77 (d, *J* = 8.4 Hz, 1H); 8.03 (d, *J* = 8.4 Hz, 1H); 8.27 (s, 1H). MS (ESI<sup>+</sup>) *m*/z 278.1 (M+H)<sup>+</sup>. Anal. Calcd for C<sub>13</sub>H<sub>9</sub>Cl<sub>2</sub>N<sub>3</sub>: C, 56.14; H, 3.26; N, 15.11; Found: C, 56.32; H, 3.24; N, 15.15.

4.1.2.20. 2-(3,5-Dichlorophenyl)-1H-benzo[d]imidazol-5-amine (**4t**). Brown powder, yield = 51%, mp: 241–243 °C. <sup>1</sup>H NMR (300 MHz;  $d_6$ -DMSO): 6.57 (d, J = 8.4 Hz, 1H); 6.60 (s, 1H); 7.30 (d, J = 8.4 Hz, 1H); 7.62 (s, 1H); 8.06 (s, 2H); 12.50 (s, 1H). MS (ESI<sup>+</sup>) m/z 278.0 (M+H)<sup>+</sup>. Anal. Calcd for C<sub>13</sub>H<sub>9</sub>Cl<sub>2</sub>N<sub>3</sub>: C, 56.14; H, 3.26; N, 15.11; Found: C, 56.30; H, 3.29; N, 15.08.

4.1.2.21. 2-(2-Bromo-5-fluorophenyl)-1H-benzo[d]imidazol-5-amine (**4u**). Yellow powder, yield: 92%, mp: 96–97 °C. <sup>1</sup>H NMR (300 MHz; CDCl<sub>3</sub>): 6.74 (dd, J = 8.4, 2.1 Hz, 1H); 6.86 (d, J = 4.5 Hz, 1H); 7.01 (dd, J = 8.7, 3.0 Hz, 1H); 7.54–7.64 (m, 2H); 8.08 (dd, J = 9.9, 3.3 Hz, 1H). MS (ESI<sup>+</sup>) m/z 306.0 (M+H)<sup>+</sup>. Anal. Calcd for C<sub>13</sub>H<sub>9</sub>FBrN<sub>3</sub>: C, 51.00; H, 2.96; N, 13.73; Found: C, 51.15; H, 2.99; N, 13.70.

## 4.1.3. Synthesis of 4-chloroquinoline (6)

A suspension of quinolin-4-ol (**5**) (2 g) in POCl<sub>3</sub> (30 mL) was heated at 100 °C for 4 h. After cooling, the mixture was concentrated under reduced pressure and the ice was added to the residue. The pH was adjusted to 6 with ammonia to allow precipitation. The filter cake was washed with water and dried to give 4-chloroquinoline (**6**). White powder, yield: 77%, mp: 28–31 °C. <sup>1</sup>H NMR (300 MHz; CDCl<sub>3</sub>): 7.41 (d, J = 4.7 Hz, 1H); 7.54–7.60 (m, 1H); 7.69–7.74 (m, 1H); 8.09–8.17 (m, 2H); 8.73 (d, J = 4.7 Hz, 1H). MS (ESI<sup>+</sup>) m/z 164.0 (M+H)<sup>+</sup>. Anal. Calcd for C<sub>9</sub>H<sub>6</sub>ClN: C, 66.07; H, 3.70; N, 8.56; Found: C, 66.18; H, 3.68; N, 8.52.

4.1.4. General procedure for the preparation of the N-phenyl-1Hbenzo[d]imidazol-5-yl)quinolin-4-amine derivatives **7a**-**7u** 

A mixture of 4-chloroquinoline (**6**, 5 mmol) and appropriate substituted anilines (**4a**–**4u**, 6 mmol) in isopropanol (40 mL) was stirred at reflux for 5 h. The reaction mixture was concentrated under reduced pressure and the solid residue was purified by column chromatography on silica gel, eluting with  $CH_2Cl_2/CH_3OH$  (10/1) to furnish title compound **7a**–**7u**.

4.1.4.1. *N*-(2-phenyl-1H-benzo[d]imidazol-6-yl)quinolin-4-amine (**7a**). Yellow powder, yield: 47%, mp: 323–325 °C. <sup>1</sup>H NMR (300 MHz; *d*<sub>6</sub>-DMSO): 6.77 (d, *J* = 6.9 Hz, 1H); 7.31 (dd, *J* = 8.7, 1.8 Hz, 1H); 7.51–7.62 (m, 3H); 7.73–7.84 (m, 3H); 8.01–8.11 (m, 2H); 8.25 (dd, *J* = 8.1, 1.5 Hz, 2H); 8.47 (d, *J* = 6.9 Hz, 1H); 8.85 (d, *J* = 8.7 Hz, 1H); 11.09 (s, 1H); 13.44 (s, 1H). <sup>13</sup>C NMR (300 MHz; *d*<sub>6</sub>-DMSO): 99.7, 117.0, 120.2, 123.6, 126.6, 136.9, 129.0, 129.8, 130.2, 133.7, 138.2, 142.5, 152.8, 155.6. MS (ESI<sup>+</sup>) *m*/z 337.2 (M+H)<sup>+</sup>. Anal. Calcd for C<sub>22</sub>H<sub>16</sub>N<sub>4</sub>: C, 78.55; H, 4.79; N, 16.66; Found: C, 78.81; H, 4.81; N, 16.62.

4.1.4.2. *N*-(2-o-tolyl-1H-benzo[d]imidazol-6-yl)quinolin-4-amine (**7b**). Bright yellow crystal, yield: 56%, mp: 218–220 °C. <sup>1</sup>H NMR (300 MHz; CDCl<sub>3</sub>): 2.52 (s, 3H); 6.58 (d, J = 6.4 Hz, 1H); 7.05–7.63 (m, 10H); 7.97–8.04 (m, 1H); 8.67 (d, J = 8.2 Hz, 1H). <sup>13</sup>C NMR (300 MHz;  $d_6$ -DMSO): 21.9, 100.6, 118.1, 122.0, 124.4, 126.8, 127.3, 130.3, 130.5, 132.1, 134.0, 137.9, 144.1, 154.2, 155.7. MS (ESI<sup>+</sup>) *m*/z 351.1 (M+H)<sup>+</sup>. Anal. Calcd for C<sub>23</sub>H<sub>18</sub>N<sub>4</sub>: C, 78.83; H, 5.18; N, 15.99; Found: C, 79.02; H, 5.14; N, 16.03.

4.1.4.3. N-(2-m-tolyl-1H-benzo[d]imidazol-6-yl)quinolin-4-amine(**7c**). Bright yellow crystal, yield: 75%, mp: 237–239 °C. <sup>1</sup>H NMR (300 MHz; CDCl<sub>3</sub>): 2.43 (s, 3H); 6.76 (d, *J* = 6.9 Hz, 1H); 7.28–7.36 (m, 2H); 7.46 (t, *J* = 7.5 Hz, 1H); 7.71–7.83 (m, 3H); 8.00-8.12 (m, 4H); 8.46 (d, *J* = 6.9 Hz, 1H); 8.86 (d, *J* = 8.7 Hz, 1H); 11.09 (s, 1H); 13.04 (s, 1H). <sup>13</sup>C NMR (300 MHz; *d*<sub>6</sub>-DMSO): 21.0, 99.7, 117.1, 120.6, 123.8, 126.7, 127.1, 128.9, 129.7, 130.7, 133.5, 138.2, 142.8, 152.8, 155.2. MS (ESI<sup>+</sup>) *m/z* 351.1 (M+H)<sup>+</sup>. Anal. Calcd for C<sub>23</sub>H<sub>18</sub>N<sub>4</sub>: C, 78.83; H, 5.18; N, 15.99; Found: C, 78.95; H, 5.21; N, 15.95.

4.1.4.4. *N*-(2-*p*-tolyl-1*H*-benzo[*d*]imidazol-6-*y*l)quinolin-4-amine (**7d**). Yellow powder, yield: 73%, mp: 363–365 °C. <sup>1</sup>H NMR (300 MHz; CDCl<sub>3</sub>): 2.41 (s, 3H); 6.76 (s, 1H); 7.29 (d, *J* = 8.4 Hz, 1H); 7.4 (d, *J* = 8.1 Hz, 2H); 7.74–7.85 (m, 3H); 8.01–8.06 (m, 2H); 8.12 (d, *J* = 7.8 Hz, 2H); 8.47 (d, *J* = 6.9 Hz, 1H); 8.8 (d, *J* = 8.4 Hz, 1H); 11.01 (s, 1H); 13.24 (s, 1H). <sup>13</sup>C NMR (300 MHz; *d*<sub>6</sub>-DMSO): 21.0, 99.6, 117.0, 120.3, 123.6, 126.6, 126.8, 127.1, 129.5, 133.6, 138.4, 139.9, 142.5, 152.9, 155.5. MS (ESI<sup>+</sup>) *m/z* 351.1 (M+H)<sup>+</sup>. Anal. Calcd for C<sub>23</sub>H<sub>18</sub>N<sub>4</sub>: C, 78.83; H, 5.18; N, 15.99; Found: C, 78.67; H, 5.22; N, 16.04.

4.1.4.5. N-(2-(2-methoxyphenyl)-1H-benzo[d]imidazol-6-yl)quino-lin-4-amine (7e). Dark brown crystal, yield: 78%, mp: 220–223 °C. <sup>1</sup>H NMR (300 MHz; d<sub>6</sub>-DMSO): 6.06 (s, 3H); 6.77 (d, J = 6.9 Hz, 1H); 7.12–7.18 (m, 1H); 7.27–7.31 (m, 2H); 7.50–7.56 (m, 1H); 7.74–7.83 (m, 3H); 8.00–8.11 (m. 2H); 8.36 (dd, J = 7.8, 1.5 Hz, 1H); 8.47 (d, J = 6.9 Hz, 1H); 8.86 (d, J = 8.4 Hz, 1H); 11.10 (s, 1H); 12.42 (s, 1H). <sup>13</sup>C NMR (300 MHz; d<sub>6</sub>-DMSO): 56.6, 100.7, 113.4, 118.0, 120.0, 124.3, 127.5, 132.8, 134.3, 144.0, 145.4, 158.7. MS (ESI<sup>+</sup>) m/z 367.1 (M+H)<sup>+</sup>. Anal. Calcd for C<sub>23</sub>H<sub>18</sub>N<sub>4</sub>O: C, 75.39; H, 4.95; N, 15.29; Found: C, 75.48; H, 4.91; N, 15.34.

4.1.4.6. 2-(6-(Quinolin-4-ylamino)-1H-benzo[d]imidazol-2-yl)phenol (**7***f*). Yellow crystal, mp: yield: 10%, mp: 241–243 °C. <sup>1</sup>H NMR (300 MHz;  $d_6$ -DMSO): 6.79 (s, 1H); 7.00–7.07 (m, 2H); 7.33–7.42 (m, 2H); 7.75 (t, J = 6.9 Hz, 3H); 7.92–8.02 (m, 2H); 8.12 (d,

 $J = 7.8 \text{ Hz}, 1\text{H}; 8.46 (d, J = 6.6 \text{ Hz}, 1\text{H}); 8.71 (d, J = 8.4 \text{ Hz}, 1\text{H}); 10.61 (s, 1\text{H}); 12.89 (s, 1\text{H}); 13.50 (s, 1\text{H}). {}^{13}\text{C} \text{ NMR} (300 \text{ MHz}; d_6\text{-DMSO}): 100.4, 112.8, 116.1, 117.8, 121.9, 124.5, 129.2, 132.2, 135.0, 139.0, 147.2, 153.4, 155.6. MS (ESI<sup>+</sup>) <math>m/z$  353.1 (M+H)<sup>+</sup>. Anal. Calcd for C<sub>22</sub>H<sub>16</sub>N<sub>4</sub>O: C, 74.98; H, 4.58; N, 15.90; Found: C, 74.75; H, 4.60; N, 15.95.

4.1.4.7. *N*-(2-(4-ethylphenyl)-1*H*-benzo[d]imidazol-6-yl)quinolin-4amine (**7g**). Yellow powder, yield: 10%, mp: 351–353 °C. <sup>1</sup>H NMR (500 MHz; *d*<sub>6</sub>-DMSO): 1.23 (t, *J* = 7.5 Hz, 3H); 2.69 (q, *J* = 4.5 Hz, 2H); 6.76 (s, 1H); 7.26 (d, *J* = 8.5 Hz, 1H); 7.40 (d, *J* = 8.0 Hz, 2H); 7.76 (t, *J* = 7.5 Hz, 3H); 7.97 (t, *J* = 7.5 Hz, 1H); 8.04 (d, *J* = 8.5 Hz, 1H); 8.14 (d, *J* = 8.0 Hz, 2H); 8.44 (d, *J* = 6.5 Hz, 1H); 8.77 (d, *J* = 8.5 Hz, 1H); 10.76 (s, 1H); 13.24 (s, 1H). <sup>13</sup>C NMR (300 MHz; *d*<sub>6</sub>-DMSO): 15.3, 28.0, 99.9, 117.6, 123.2, 126.1, 126.6, 127.4, 128.3, 132.5, 144.6, 146.0, 152.7. MS (ESI<sup>+</sup>) *m*/*z* 365.2 (M+H)<sup>+</sup>. Anal. Calcd for C<sub>24</sub>H<sub>20</sub>N<sub>4</sub>: C, 79.10; H, 5.53; N, 15.37; Found: C, 79.28; H, 5.54; N, 15.41.

4.1.4.8. *N*-(2-(2-fluorophenyl)-1H-benzo[d]imidazol-6-yl)quinolin-4amine (**7h**). Yellow powder, yield: 68%, mp: 337–339 °C. <sup>1</sup>H NMR (300 MHz; *d*<sub>6</sub>-DMSO): 6.76 (s, 1H); 7.32 (dd, J = 8.4, 1.8 Hz, 1H); 7.39–7.49 (m, 2H); 7.56–7.63 (m, 1H); 7.77–7.82 (m, 3H); 7.99–8.09 (m, 2H); 8.23–8.29 (m, 1H); 8.45 (d, J = 6.9 Hz, 1H); 8.84 (d, J = 8.4 Hz, 1H); 11.10 (s, 1H); 12.91 (s, 1H). <sup>13</sup>C NMR (300 MHz; *d*<sub>6</sub>-DMSO): 99.7, 116.4, 116.8, 120.1, 123.7, 125.1, 126.9, 130.2, 132.1, 133.7, 134.3, 138.2, 142.5, 155.7, 161.1. MS (ESI<sup>+</sup>) *m/z* 355.1 (M+H)<sup>+</sup>. Anal. Calcd for C<sub>22</sub>H<sub>15</sub>FN<sub>4</sub>: C, 74.56; H, 4.27; N, 15.81; Found: C, 74.69; H, 4.30; N, 15.77.

4.1.4.9. *N*-(2-(3-fluorophenyl)-1H-benzo[d]imidazol-6-yl)quinolin-4amine (**7i**). Dark yellow powder, yield: 46%, mp: 342–343 °C. <sup>1</sup>H NMR (300 MHz; *d*<sub>6</sub>-DMSO): 6.75 (d, *J* = 6.6 Hz, 1H); 7.30–7.40 (m, 2H); 7.59–7.83 (m, 4H); 8.02–8.10 (m, 4H); 8.46 (d, *J* = 7.2 Hz, 1H); 8.81 (d, *J* = 8.4 Hz, 1H); 11.07 (s, 1H); 13.54 (s, 1H). <sup>13</sup>C NMR (300 MHz; *d*<sub>6</sub>-DMSO): 99.0, 113.1, 113.4, 116.7, 117.0, 120.1, 120.5, 122.7, 123.7, 126.8, 131.1, 132.0, 133.7, 138.2, 142.4, 151.5, 155.5, 160.8, 164.0. MS (ESI<sup>+</sup>) *m/z* 355.1 (M+H)<sup>+</sup>. Anal. Calcd for C<sub>22</sub>H<sub>15</sub>FN<sub>4</sub>: C, 74.56; H, 4.27; N, 15.81; Found: C, 74.35; H, 4.29; N, 15.85.

4.1.4.10. N-(2-(4-fluorophenyl)-1H-benzo[d]imidazol-6-yl)quinolin-4-amine (**7***j*). Bright yellow powder, yield: 62%, mp: 353–355 °C. <sup>1</sup>H NMR (300 MHz; d<sub>6</sub>-DMSO): 6.73 (s, 1H); 7.29–7.40 (m, 3H); 7.70–7.76 (m, 3H); 8.00–8.07 (m, 2H); 8.29–8.44 (m, 3H); 8.85 (s, 1H); 11.12 (s, 1H); 13.55 (s, 1H). <sup>13</sup>C NMR (300 MHz; d<sub>6</sub>-DMSO): 99.7, 115.9, 116.2, 116.9, 120.1, 123.6, 126.4, 126.8, 129.0, 133.7, 138.2, 142.4, 151.9, 155.6. MS (ESI<sup>+</sup>)*m/z*355.1 (M+H)<sup>+</sup>. Anal. Calcd for C<sub>22</sub>H<sub>15</sub>FN<sub>4</sub>: C, 74.56; H, 4.27; N, 15.81; Found: C, 74.37; H, 4.25; N, 15.76.

4.1.4.11. *N*-(2-(2-chlorophenyl)-1*H*-benzo[d]imidazol-6-yl)quinolin-4-amine (**7k**). Brown crystal, yield: 56%, mp: 330–332 °C. <sup>1</sup>H NMR (500 MHz; *d*<sub>6</sub>-DMSO): 6.76 (s, 1H); 7.32 (d, J = 8.0 Hz, 1H); 7.51–7.57 (m, 2H); 7.65–7.76 (m, 4H); 7.94–7.98 (m, 2H); 8.07 (d, J = 8.5 Hz, 1H); 8.44 (d, J = 7.0 Hz, 1H); 8.84 (d, J = 8.5 Hz, 1H); 10.90 (s, 1H); 13.06 (s, 1H). <sup>13</sup>C NMR (300 MHz; *d*<sub>6</sub>-DMSO): 99.8, 117.3, 121.4, 123.6, 126.4, 127.5, 129.6, 130.4, 131.4, 131.6, 132.0, 133.0, 143.5, 150.4, 154.7. MS (ESI<sup>+</sup>) *m*/*z* 371.1 (M+H)<sup>+</sup>. Anal. Calcd for C<sub>22</sub>H<sub>15</sub>ClN<sub>4</sub>: C, 71.25; H, 4.08; N, 15.11; Found: C, 71.48; H, 4.12; N, 15.15.

4.1.4.12. N-(2-(3-chlorophenyl)-1H-benzo[d]imidazol-6-yl)quinolin-4-amine (71). Yellow powder, yield: 54%, mp: 323–325 °C. <sup>1</sup>H NMR (500 MHz; d<sub>6</sub>-DMSO): 6.77 (s, 1H); 7.32 (d, <math>J = 8.0 Hz, 1H); 7.57–7.63 (m, 2H); 7.77 (t, J = 7.5 Hz, 3H); 7.99 (t, J = 7.0 Hz, 1H); 8.09 (d, J = 8.5 Hz, 1H); 8.24 (d, J = 7.5 Hz, 1H); 8.32 (s, 1H); 8.46 (d,

J = 7.0 Hz, 1H); 8.85 (d, J = 8.5 Hz, 1H); 10.93 (s, 1H); 13.67 (s, 1H). <sup>13</sup>C NMR (300 MHz;  $d_6$ -DMSO): 100.6, 121.8, 124.4, 126.0, 127.0, 127.4, 130.6, 131.8, 132.7, 134.1, 134.6, 144.0, 154.5, 163.4. MS (ESI<sup>+</sup>) m/z 371.1 (M+H)<sup>+</sup>. Anal. Calcd for C<sub>22</sub>H<sub>15</sub>ClN<sub>4</sub>: C, 71.25; H, 4.08; N, 15.11; Found: C, 71.46; H, 4.05; N, 15.06.

4.1.4.13. *N*-(2-(4-chlorophenyl)-1*H*-benzo[d]imidazol-6-yl)quinolin-4-amine (**7m**). Yellow powder, yield: 55%, mp: 345–347 °C. <sup>1</sup>H NMR (300 MHz; *d*<sub>6</sub>-DMSO): 6.76 (s, 1H); 7.32 (d, *J* = 7.8 Hz; 1H); 7.65–7.83 (m, 5H); 8.00–8.10 (m, 2H); 8.26 (d, *J* = 8.7 Hz, 2H); 8.47 (d, *J* = 6.9 Hz, 1H); 8.83 (d, *J* = 8.7 Hz, 1H); 11.06 (s, 1H); 13.53 (s, 1H). <sup>13</sup>C NMR (300 MHz; *d*<sub>6</sub>-DMSO): 99.8, 117.0, 120.2, 123.4, 123.7, 126.9, 128.4, 130.2, 132.6, 133.8, 134.9, 138.2, 142.5, 146.3, 151.8, 155.6. MS (ESI<sup>+</sup>) *m/z* 371.2 (M+H)<sup>+</sup>. Anal. Calcd for C<sub>22</sub>H<sub>15</sub>ClN<sub>4</sub>: C, 71.25; H, 4.08; N, 15.11; Found: C, 71.07; H, 4.10; N, 15.14.

4.1.4.14. *N*-(2-(4- bromophenyl)-1*H*-benzo[d]imidazol-6-yl)quinolin-4-amine (**7n**). Pale yellow powder, yield: 62%, mp: 359–361 °C. <sup>1</sup>H NMR (500 MHz; *d*<sub>6</sub>-DMSO): 6.76 (m, 1H); 7.31 (s, 1H); 7.66–7.80 (m, 5H); 8.02 (t, *J* = 8.0 Hz, 1H); 8.11 (d, *J* = 8.5 Hz, 1H); 8.21 (d, *J* = 8.0 Hz, 2H); 8.46 (d, *J* = 6.5 Hz, 1H); 8.86 (d, *J* = 8.5 Hz, 1H); 11.06 (s, 1H); 13.62 (s, 1H). <sup>13</sup>C NMR (300 MHz; *d*<sub>6</sub>-DMSO): 100.2, 116.5, 120.6, 123.7, 126.5, 128.5, 129.1, 131.9, 133.7, 135.0, 139.4, 144.2, 151.6, 155.4. MS (ESI<sup>+</sup>) *m*/*z* 415.1 (M+H)<sup>+</sup>. Anal. Calcd for C<sub>22</sub>H<sub>15</sub>BrN<sub>4</sub>: C, 63.63; H, 3.64; N, 13.49; Found: C, 63.85; H, 3.60; N, 13.53.

4.1.4.15. N-(2-(2-(trifluoromethyl)phenyl)-1H-benzo[d]imidazol-6-yl)quinolin-4-amine (**70**). Dark yellow powder, yield: 34%, mp: 244–246 °C. <sup>1</sup>H NMR (300 MHz;*d*<sub>6</sub>-DMSO): 6.73–6.78 (m, 1H); 7.34 (s, 1H); 7.66–7.87 (m, 6H); 7.95–8.08 (m, 3H); 8.46 (d,*J*= 6.9 Hz, 1H); 8.83 (d,*J*= 8.4 Hz, 1H); 11.06 (s, 1H); 13.15 (s, 1H). <sup>13</sup>C NMR (300 MHz;*d*<sub>6</sub>-DMSO): 100.9, 120.5, 123.6, 126.7, 130.4, 132.2, 132.5, 133.6, 145.8, 149.2, 163.1. MS (ESI<sup>+</sup>)*m/z*405.1 (M+H)<sup>+</sup>. Anal. Calcd for C<sub>23</sub>H<sub>15</sub>F<sub>3</sub>N<sub>4</sub>: C, 68.31; H, 3.74; N, 13.85; Found: C, 68.16; H, 3.78; N, 13.81.

4.1.4.16. N-(2-(3-(trifluoromethyl)phenyl)-1H-benzo[d]imidazol-6yl)quinolin-4-amine (**7p**). Brown powder, yield: 60%, mp: 243–245 °C. <sup>1</sup>H NMR (300 MHz;  $d_6$ -DMSO): 6.74–6.78 (m, 1H); 7.33 (d, J = 6.3 Hz, 1H); 7.69–7.87 (m, 5H); 7.99–8.05 (m, 2H); 8.45–8.57 (m, 3H); 8.82 (d, J = 8.4 Hz, 1H); 11.05 (s, 1H); 13.68 (s, 1H). <sup>13</sup>C NMR (300 MHz;  $d_6$ -DMSO): 100.6, 119.3, 123.7, 124.5, 126.4, 127.0, 130.5, 132.4, 133.8, 138.2, 143.8, 152.2, 162.5. MS (ESI<sup>+</sup>) m/z 405.1 (M+H)<sup>+</sup>. Anal. Calcd for C<sub>23</sub>H<sub>15</sub>F<sub>3</sub>N<sub>4</sub>: C, 68.31; H, 3.74; N, 13.85; Found: C, 68.08; H, 3.72; N, 13.80.

4.1.4.17. N-(2-(4-(trifluoromethyl)phenyl)-1H-benzo[d]imidazol-6yl)quinolin-4-amine (**7q**). Dark brown powder, yield: 21%, mp: 258–260 °C. <sup>1</sup>H NMR (300 MHz;  $d_6$ -DMSO): 6.76–6.85 (m, 1H); 7.35 (d, J = 7.5 Hz, 1H); 7.71–8.11 (m, 7H); 8.48 (d, J = 7.4 Hz, 3H); 8.85 (d, J = 8.3 Hz, 1H); 11.02 (s, 1H); 13.77 (s, 1H). <sup>13</sup>C NMR (300 MHz;  $d_6$ -DMSO): 101.1, 120.2, 123.4, 125.9, 126.5, 127.2, 130.4, 133.7, 142.1, 154.6, 161.7. MS (ESI<sup>+</sup>) m/z 405.3 (M+H)<sup>+</sup>. Anal. Calcd for C<sub>23</sub>H<sub>15</sub>F<sub>3</sub>N<sub>4</sub>: C, 68.31; H, 3.74; N, 13.85; Found: C, 68.55; H, 3.77; N, 13.89.

4.1.4.18. N-(2-(2,6-dichlorophenyl)-1H-benzo[d]imidazol-6-yl)quinolin-4-amine (**7r**). Pale yellow powder, yield: 52%, mp:354–356 °C. <sup>1</sup>H NMR (300 MHz; d<sub>6</sub>-DMSO): 6.79 (s, 1H); 7.35 (d,<math>J = 9.0 Hz, 1H); 7.60–7.85 (m, 6H); 8.03 (d, J = 3.9 Hz, 2H); 8.47 (d, J = 6.9 Hz, 1H); 8.80 (d, J = 8.4 Hz, 1H); 11.03 (s, 1H); 13.19 (s, 1H). <sup>13</sup>C NMR (300 MHz; d<sub>6</sub>-DMSO): 100.6, 117.8, 121.0, 124.5, 127.7, 129.2, 133.4, 134.6, 135.8, 139.0, 143.4, 153.2, 156.5. MS (ESI<sup>+</sup>) m/z 405.2 (M+H)<sup>+</sup>. Anal. Calcd for C<sub>22</sub>H<sub>14</sub>Cl<sub>2</sub>N<sub>4</sub>: C, 65.20; H, 3.48; N, 13.82; Found: C, 65.38; H, 3.46; N, 13.85.

4.1.4.19. N-(2-(3,4-dichlorophenyl)-1H-benzo[d]imidazol-6-yl)quinolin-4-amine (**7s**). Yellow powder, yield: 56%, mp: 247–249 °C. <sup>1</sup>H NMR (300 MHz; *d*<sub>6</sub>-DMSO): 6.74 (s, 1H); 7.32 (d, J = 8.1 Hz, 1H); 7.68–7.87 (m, 4H); 7.99–8.06 (m, 2H); 8.21 (dd, J = 8.4, 1.8 Hz, 1H); 8.46–8.47 (m, 2H); 8.80 (d, J = 8.7 Hz, 1H); 11.06 (s, 1H); 13.61 (s, 1H). <sup>13</sup>C NMR (300 MHz; *d*<sub>6</sub>-DMSO): 101.6, 119.2, 121.8, 123.1, 124.7, 128.0, 129.2, 130.3, 131.5, 133.0, 134.1, 134.9, 135.8, 140.4, 144.0, 153.5, 158.5. MS (ESI<sup>+</sup>) *m/z* 405.2 (M+H)<sup>+</sup>. Anal. Calcd for C<sub>22</sub>H<sub>14</sub>Cl<sub>2</sub>N<sub>4</sub>: C, 65.20; H, 3.48; N, 13.82; Found: C, 65.41; H, 3.45; N, 13.78.

4.1.4.20. N-(2-(3,5-dichlorophenyl)-1H-benzo[d]imidazol-6-yl)quinolin-4-amine (7t). Dark yellow powder, yield: 18%, mp:349–350 °C. <sup>1</sup>H NMR (300 MHz; d<sub>6</sub>-DMSO): 6.75 (s, 1H); 7.25–7.40(m, 1H); 7.63–7.92 (m, 4H); 7.95–8.15 (m, 2H); 8.26 (s, 2H); 8.46 (d,<math>J = 6.9 Hz, 1H); 8.80 (d, J = 8.1 Hz, 1H); 11.05 (s, 1H); 13.65 (s, 1H). <sup>13</sup>C NMR (300 MHz; d<sub>6</sub>-DMSO): 100.5, 114.9, 120.7, 122.6, 124.4, 125.8, 127.7, 129.9, 133.9, 134.4, 135.7, 142.6, 150.6, 161.1. MS (ESI<sup>+</sup>) m/z 405.1 (M+H)<sup>+</sup>. Anal. Calcd for C<sub>22</sub>H<sub>14</sub>Cl<sub>2</sub>N<sub>4</sub>: C, 65.20; H, 3.48; N, 13.82; Found: C, 65.42; H, 3.52; N, 13.86.

4.1.4.21. *N*-(2-(2-bromo-5-fluorophenyl)-1H-benzo[d]imidazol-6-yl) quinolin-4-amine (**7u**). Brown crystal, yield: 40%, mp: 238–239 °C. <sup>1</sup>H NMR (300 MHz; *d*<sub>6</sub>-DMSO): 6.73 (s, 1H); 7.32–7.43 (m, 2H); 7.69–7.90 (m, 5H); 7.99–8.07 (m, 2H); 8.45 (d, *J* = 6.9 Hz, 1H); 8.82 (d, *J* = 8.4 Hz, 1H); 10.95 (s, 1H); 11.17 (s, 1H). <sup>13</sup>C NMR (300 MHz; *d*<sub>6</sub>-DMSO): 100.4, 119.7, 120.0, 124.2, 127.2, 129.0, 132.7, 136.3, 139.6, 141.4, 151.6, 160.2. MS (ESI<sup>+</sup>) *m/z* 433.0 (M+H)<sup>+</sup>. Anal. Calcd for C<sub>22</sub>H<sub>14</sub>BrFN<sub>4</sub>: C, 60.99; H, 3.26; N, 12.93; Found: C, 61.21; H, 3.29; N, 12.97.

#### 4.2. Cell proliferative assay

The antiproliferative activities of the prepared *N*-(2-phenyl-1*H*benzo[*d*]imidazol-5-yl)quinolin-4-amine derivatives against Hep-G2, MCF-7 and L02 cells were evaluated by MTT assay. Briefly, target tumor cells were grown to log phase in RPMI 1640 medium supplemented with 10% fetal bovine serum. After diluting to  $2 \times 10^4$  cells mL<sup>-1</sup> with the complete medium, 100 µL of the obtained cell suspension was added to each well of 96-well culture plates. The subsequent incubation was permitted at 37 °C, 5% CO<sub>2</sub> atmosphere for 24 h before the antiproliferative assessments. Tested samples at pre-set concentrations were added to 6 wells. After 48 h exposure period, 40 µL of PBS containing 2.5 mg mL<sup>-1</sup> of MTT was added to each well. Four hours later, 100 µL extraction solution (10% SDS-5% isobutyl alcohol-0.010 M HCl) was added. After an overnight incubation at 37 °C, the optical density was measured at a wavelength of 570 nm on an ELISA microplate reader.

#### 4.3. In vitro kinase assay

In vitro kinase assay was tested using HTScan VEGF Receptor 2 Kinase Assay Kit (purchased from Cell Signaling Technology, Inc.) by colorimetric ELISA assay according to the instructions. Reaction cocktail containing VEGFR2 was incubated with various concentrations of tested compounds or DMSO (0.1%) for 5 min at room temperature, and then ATP/substrate peptide cocktail was added to the pre-incubated reaction cocktail. After incubation at room temperature for 30 min, the reaction was stopped and transferred to a 96-well streptavidin-coated plate, and incubated for 1 h at room temperature. Primary antibody [phosphorylated tyrosine monoclonal antibody [(pTyr-100), 1:1000 in PBS/T with 1% bovine serum albumin (BSA)] was added into per well until the wells were washed thrice with PBS/T. After incubated at room temperature for 1 h, phosphorylation of the substrate was monitored with HRP-labeled anti-mouse IgG antibody (1:500 in PBS/T with 1% BSA), followed by a chromogenic reaction. Finally, the VEGFR2 kinase assay was detected at 450 nm with microplate reader. The reaction processed with only DMSO (0.1%) served as a vehicle control. The results were expressed as percent kinase activity of the vehicle control, and IC<sub>50</sub> was defined as the compound concentration that resulted in 50% inhibition of enzyme activity. The kinase assay was performed thrice independently.

# 4.4. Molecular docking

Molecular docking of compound **7s** into the three dimensional X-ray structure of VEGFR-2 kinase (PDB code: 2QU5) was carried out using the Discovery Stutio (version 3.1) as implemented through the graphical user interface DiscoveryStudio CDOCKER protocol.

The three-dimensional structure of the compound **7s** was constructed using ChemBio 3D Ultra 11.0 software [Chemical Structure Drawing Standard; Cambridge Soft corporation, USA (2008)], then it was energetically minimized by using MMFF94 with 5000 iterations and minimum RMS gradient of 0.10. The crystal structures of VEGFR-2 kinase were retrieved from the RCSB Protein Data Bank (http://www.rcsb.org/pdb/home/home.-do). All bound waters and ligands were eliminated from the protein and the polar hydrogen was added. The whole 2QU5 was defined as a receptor and the site sphere was selected based on ATP binding site of 2QU5. Compound **7s** were placed during the molecular docking procedure. Types of interactions of the docked protein with ligand were analyzed after the end of molecular docking.

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# Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.ejmech.2014.07.071.

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