Novel Quinolinylaminoisoquinoline Bioisosteres of Sorafenib as Selective RAF1 Kinase Inhibitors: Design, Synthesis, and Antiproliferative Activity against Melanoma Cell Line

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Design and synthesis of a new series of quinolinylaminoisoquinoline derivatives as conformationally restricted bioisosteres of Sorafenib are described. Their *in vitro* antiproliferative activity against A375P melanoma cell line was tested. Compounds 1b, 1d, 1g, and 1j showed the highest potency against A375P cell line with IC_{50} values in sub-micromolar scale. In addition, compound 1d exerted high selectivity towards RAF1 serine/threonine kinase with 96.47% inhibition at 10 μM, and IC_{50} of 0.96 μM. This compound can possess antiproliferative activity against melanoma cells through inhibition of RAF1 kinase.

Key words bioisostere; melanoma; quinolinylaminoisoquinoline; Sorafenib; antiproliferative activity

The RAS/RAF/MEK/extraccellular signal-regulated kinase (ERK) (ERK pathway) is a well-known cell signaling network essential for cell survival, growth, and proliferation.[1] The RAS proteins are membrane-bound small G-protein, whereas RAF, MEK, and ERK are cytosolic protein kinases which compose a sequential signaling cascade. Among these four kinases, RAF kinases have been the most studied drug targets since mutations of the RAF protein were found in approximately 7% of human cancers[2,3] with particularly high frequency in melanoma (50–70%), ovarian cancer (35%), thyroid cancer (30%), and colorectal cancer (10%). Due to high incidence of RAF overexpression in melanoma, it has been a potential drug target for treatment of melanoma.

Melanoma is a malignant tumor of melanocytes. It is considered as the most aggressive form of skin cancer. The major risk factors for melanoma development include exposure to solar UV irradiation, fair skin, dysplastic nevi syndrome, and a family history of melanoma. Melanomas can metastasize either by the lymphatic or by the hematogenous route.[4] Early stage melanoma (stage I/II) can be cured surgically with more than 95% success rate. But melanoma metastasizing to major organs (stage IV) is virtually incurable.[5] Patients with advanced melanoma have a median survival time of less than one year, and the estimated 5-year survival rate is less than 15%.[6,7] With the incidence of melanoma rapidly rising in the United States and other developed countries, there has been an urgent need to develop more effective drugs.

Sorafenib (Nexavar®, Fig. 1) targets ERK pathway. It inhibits its basal phosphorylation of ERK (pERK) in numerous cancer cell lines *in vitro*, including melanoma cell lines, independent of their K-RAS and B-RAF mutational status.[8] Numerous compounds with structural similarity to Sorafenib have been recently reported as potential antiproliferative agents against melanoma.[9–23] In addition, amino-heterocycles as urea bioisosteres have been reported as RAF kinase inhibitors. The 2-aminoquinazoline and 2-aminoquinoline derivatives illustrated in Fig. 1 are bioisosteres of Sorafenib with RAF kinase inhibitory activity.[24] In the present investigation, the target compounds were designed as conformationally restricted bioisosteres of Sorafenib. The pyridine-2-carboxamide moiety of Sorafenib was replaced with 8′-aminoquinoline nucleus in our target compounds. The oxygen linker of Sorafenib was isosterically replaced with NH. And the phenylurea scaffold of Sorafenib was replaced with substituted 1′-aminoisoquinoline moiety (Fig. 1). The antiproliferative activity of the target compounds was tested over A375P human melanoma cell line. And one potential compound was examined for inhibitory effect over a panel of 38 kinases. The procedures and results are reported in details.

Results and Discussion

Chemistry The target compounds **1a**–**o** and **2a**–**m** were synthesized by the pathways illustrated in Charts 1 and 2. Nitration of 1-chloroisoquinoline (**3**) using a mixture of nitric acid and sulfuric acid yielded 1-chloro-5-nitroisoquinoline (**4**).[25] Nucleophilic substitution of the chloro group of compound **4** with different (hetero)aromatic amines was carried out by heating in the presence of 2,2′-bis(diphenylphosphino)-1,1′-binaphthyl (BINAP), palladium acetate, and anhydrous potassium carbonate to produce compounds **5a**–**o**. Reduction of the nitro group of **5a**–**o** to the corresponding amino compounds **6a**–**o** was done using Pd/C in hydrogen atmosphere. Nitration of 4-chloroquinoline (**9**) was carried out in the same way as preparation of compound **4** using a mixture of nitric and sulfuric acids to produce 4-chloro-8-nitroquinoline (**7**).[9,26] Reaction between the amino compounds **6a**–**o** and the chloro compound **7** afforded the quinolinylaminoisoquinoline compounds **8a**–**o**. Finally, the nitro group of **8a**–**o** was reduced with Pd/C in hydrogen atmosphere to give the final compounds **1a**–**o** (Chart 1).

The authors declare no conflict of interest.

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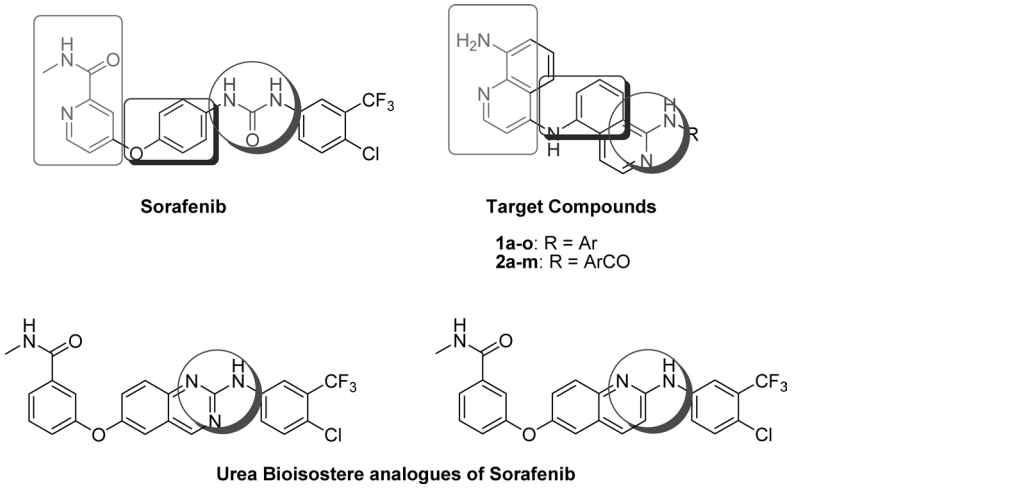
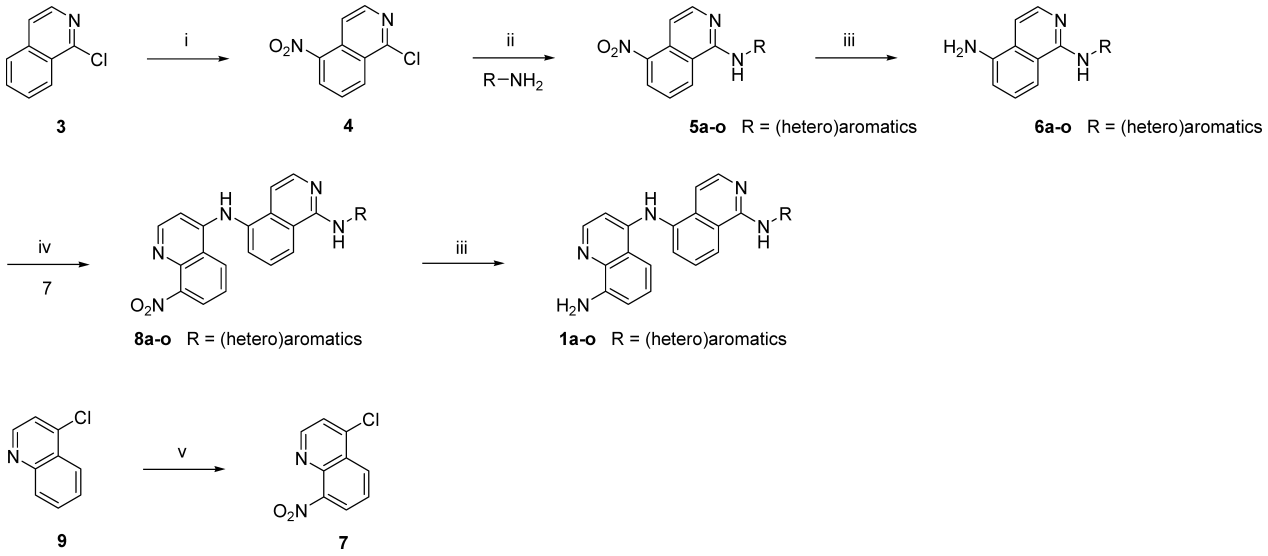


Fig. 1. Rational Design of the Target Compounds



Reagents and reaction conditions: (i) HNO_3, H_2SO_4, rt, 3 h; (ii) BINAP, $Pd(OAc)_2$, K_2CO_3, dioxane, 100°C, 40 h; (iii) Pd/C, H_2, MeOH, rt, 2 h; (iv) BINAP, $Pd(OAc)_2$, K_2CO_3, dioxane, 100°C, 19 h; (v) HNO_3, H_2SO_4, rt, 4 h.

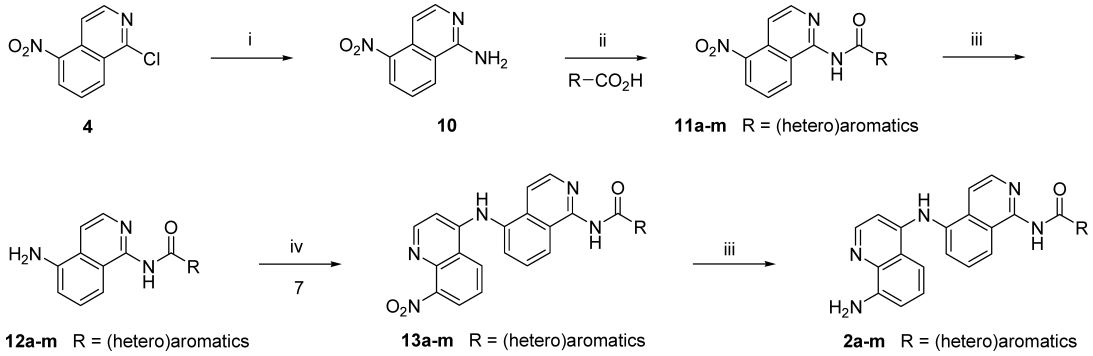
Chart 1. Synthesis of Compounds **1a**–**o**

For synthesis of the diarylamide target compounds **2a**–**m**, the pathway drawn in Chart 2 was utilized. Substitution of chloro group of compound **4** with ethanolic ammonia yielded 1-amino-5-nitroisoquinoline (**10**). Condensation of the amino compound **10** with the appropriate aromatic carboxylic acids in the presence of *O*-benzotriazole-*N*,*N*,*N*′,*N*′-tetramethyl-uronium-hexafluoro-phosphate (HBTU) and diisopropylethyl-amine (Hünig's base) produced the diarylamides **11a**–**m** possessing nitro group. Reduction of nitro group of **11a**–**m** to the corresponding amino analogues **12a**–**m** was achieved using Pd/C in hydrogen atmosphere. Similar to preparation of compounds **8a**–**o**, the amino compounds **12a**–**m** were heated with 4-chloro-8-nitroquinoline (**7**) in the presence of BINAP, palladium acetate, and anhydrous potassium carbonate to afford compounds **13a**–**m**. The nitro group of **13a**–**m** was reduced to amino with Pd/C in hydrogen atmosphere to produce the final compounds **2a**–**m**.

Biological Evaluation. Antiproliferative Activity against A375P Human Melanoma Cell Line The antiproliferative activity of the target compounds was tested against A375P human melanoma cell line. Their ability to inhibit the growth of A375P cell line is summarized in Tables 1 and 2. Sorafenib was utilized as a reference standard due to structural similarity with the target compounds, and it has shown high potency against different melanoma cell lines.[27)]

The results showed that compounds **1c**, **d** with amine linker at position 1 of the isoquinoline nucleus were more potent than the corresponding amide analogues **2d**, **e**. This may be rationalized to appropriate fitting of the amine derivatives at the receptor site. The amine derivatives are exact bioisosteres of Sorafenib, but amide analogues possess additional carbonyl group. Among all the target compounds, compounds **1b**, **1d**, **1g**, and **1j** with amine linker were the most potent with IC_{50} values in sub-micromolar scale. Compounds **2c**, **2e**, and **2k** with amide linker, and compound **1c** with amine linker showed also superior potency to Sorafenib but with IC_{50} values in micromolar scale.

Upon investigating the influence of the terminal aryl ring



Reagents and reaction conditions: (i) NH_3 (7N solution in EtOH), 80°C, 40 h; (ii) HBTU, DIPEA, THF, 80°C, 30 h; (iii) Pd/C, H_2, MeOH, rt, 2 h; (iv) BINAP, $Pd(OAc)_2$, K_2CO_3, dioxane, 100°C, 40 h.

Chart 2. Synthesis of Compounds **2a**–**m**

on potency, it was found that heteroaromatics were unfavorable for activity of compounds **1k**–**o** and **2l**. For instance, compound **1c** with 3′-(trifluoromethyl)phenyl terminal moiety showed higher potency than the corresponding pyrimidine isostere **1m**. The increased bulkiness on that terminal aryl ring as in compounds **1h**, **i**, **2h**–**j**, and **2m** was unfavorable for activity. Compound **1b** with 4′-(trifluoromethyl)phenyl terminal ring demonstrated higher potency than **1e** possessing 2′,4′-bis(trifluoromethyl)phenyl terminal moiety. Opposite finding was encountered in case of compounds **2d** and **2g**. In addition, compound **2g** with 3′,5′-bis(trifluoromethyl)phenyl terminal ring was more potent than its positional isomer **2f** containing 2′,4′-bis(trifluoromethyl)phenyl terminal moiety.

In case of compounds with NH linker, compounds **1b**–**d** and **1g** with electron-withdrawing groups on the terminal aryl ring were more potent than compound **1a** with 3′,4′-dimethylphenyl terminal moiety. This can be attributed to steric and/or electronic differences between the substituents on terminal aryl ring, and their influence(s) on affinity and potency. And in case of diarylamide derivatives, dimethoxyphenyl (compounds **2a**, **b**) and benzo[*b*]dioxine (compound **2i**) terminal moieties were unfavorable for activity.

***In Vitro* Kinase Screening** In order to study the mechanism of action at molecular level and the kinase inhibitory profile of this series of compounds, compound **1d** was selected as a representative example due to its high potency against A375P human melanoma cell line, and due to high structural similarity to the lead compound, Sorafenib, especially at the terminal moiety. Compound **1d** was tested at a single dose concentration of 10 μM over a panel of 38 oncogenic kinases. As illustrated in Fig. 2, it exerted strong inhibitory effect, 96.47%, with high selectivity against RAF1 kinase. So it can be concluded that this compound may inhibit melanoma cell proliferation due to RAF1 kinase inhibition.

Compound **1d** was further tested over RAF1 and B-RAF (V600E) kinases in order to determine its IC_{50}, where a 10-doses IC_{50} mode with threefold serial dilutions starting at 2 μM concentration was applied. Table 3 illustrates the IC_{50} values of compound **1d** over RAF1 and V600E B-RAF kinases. It was 9.8 times more potent against RAF1 kinase than B-RAF (V600E). So compound **1d** could inhibit melanoma cell growth through RAF1 kinase inhibition rather than B-RAF (V600E) kinase inhibition.

The selectivity of compound **1d** towards RAF1 kinase may be attributed to restricted conformation in both aminoquinoline and aminoisoquinoline nuclei. The difference in geometry between the binding pocket of RAF1 (C-RAF) serine/threonine kinase and other kinases may enable the selectivity of compound **1d** for RAF1.

Conclusion

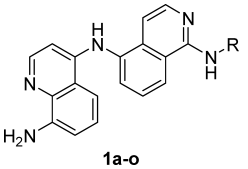
A new series of quinolinylaminoisoquinoline derivatives was designed as conformationally restricted bioisosteres of Sorafenib. They were synthesized as a continuation of our ongoing anticancer development research project. The target compounds were evaluated for antiproliferative activity against A375P human melanoma cell line. A structure–activity relationship (SAR) study has been made to correlate between the compounds structures and antiproliferative activities. Compounds **1b**, **1d**, **1g**, and **1j** possessing amine linker showed the highest potency against A375P cell line with IC_{50} values in sub-micromolar scale. Their terminal aryl rings were 4′-(trifluoromethyl)phenyl, 4′-chloro-3′-(trifluoromethyl)phenyl, 2′-morpholino-5′-(trifluoromethyl)-phenyl, and benzo[*d*][1,3]dioxole moieties, respectively. So these terminal aryl moieties together with amine linker are the most optimum for antiproliferative activity of this series of compounds. In addition, compounds **1c**, **2c**, **2e**, **2g**, and **2k** exerted higher potency than Sorafenib against A375P, but with IC_{50} values in micromolar scale.

In order to examine the possible mechanism of antiproliferative activity of this series of compounds at molecular level, compound **1d** was tested for inhibitory activity against 38 oncogenic kinase panel. It demonstrated high selectivity towards RAF1 serine/threonine kinase over the other 37 tested kinases. Compound **1d** inhibited the activity of RAF1 kinase by 96.47% at 10 μM, and its IC_{50} value was 0.96 μM. So this compound can inhibit melanoma cell proliferation through inhibition of RAF1 kinase which is overexpressed in most of melanoma cases. This compound can be utilized for future development of selective RAF1 inhibitors for treatment of melanoma.

Experimental

Chemistry ^{1}H- and ^{13}C-NMR spectra were recorded on a Bruker Avance 400 spectrometer (400 MHz for ^{1}H and 100 MHz for ^{13}C) using tetramethylsilane as an internal standard. LC-mass spectra were determined on a Waters Quattro Micro System. Column chromatography was carried out using silica gel (230–400 mesh). Solvents and liquid reagents were

Table 1. Antiproliferative Activity of Quinolinylaminoisoquinolinylamines **1a**–**o**



1a-o

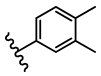
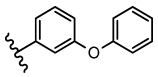
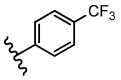
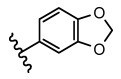
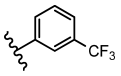
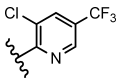
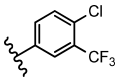
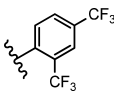
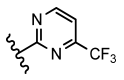
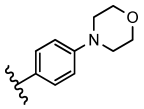
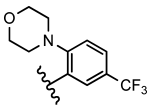
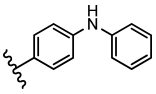
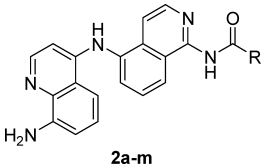
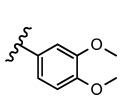
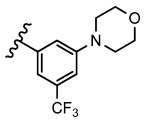
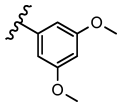
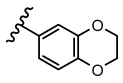
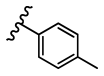
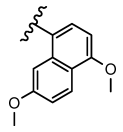
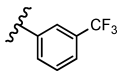
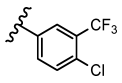
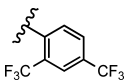
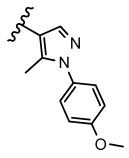
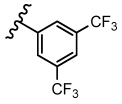
Comp. No.	R	A375P (IC_{50}, μM)	Comp. No.	R	A375P (IC_{50}, μM)
1a		>10	**1i**		>10
1b		0.94	**1j**		0.30
1c		1.0	**1k**		>10
1d		0.99	**1l**		>10
1e		>10	**1m**		>10
1f		>10	**1n**		>10
1g		0.98	**1o**		>10
1h		>10		Sorafenib	3.4

Table 2. Antiproliferative Activity of Quinolinylaminoisoquinolinylamides **2a**–**m**



2a-m

Comp. No.	R^3	A375P (IC_{50}, μM)	Comp. No.	R^3	A375P (IC_{50}, μM)
2a		>10	**2h**		>10
2b		7.0	**2i**		>10
2c		2.7	**2j**		>10
2d		>10	**2k**		2.6
2e		1.4	**2l**		>10
2f		>10	**2m**		7.0
2g		2.8		Sorafenib	3.4

transferred using hypodermic syringes. Purity % of all the target compounds were determined by LC-MS and found to be > 95%. All solvents and reagents were commercially available and used without further purification.

1-Chloro-5-nitroisoquinoline (4) To a solution of 1-chloroisoquinoline (**3**, 1.0 g, 6.11 mmol) in sulfuric acid (5.0 mL, 84.4 mmol) was added nitric acid (0.9 mL, 21.4 mmol) dropwise at 0°C. The mixture was stirred at room temperature for 3 h. Upon completion, the reaction mixture was cooled to 0°C and neutralized with 10% $NaHCO_3$ aqueous solution. The resulting precipitate was collected by filtration and dried to give the title compound (1.26 g, 99%) as a white solid. ^{1}H-NMR (DMSO-d_6) δ: 8.74–8.69 (m, 2H), 8.53 (d, J=6.1 Hz, 1H), 8.29 (d, J=6.1 Hz, 1H), 8.00 (t, J=8.1 Hz, 1H); ^{13}C-NMR (DMSO-d_6) δ: 152.6, 145.3, 144.8, 133.3, 130.3, 128.9, 127.5, 127.0, 115.8.

General Procedure for the Preparation of 5-Nitro-*N*-substituted Isoquinolin-1-amines (5a–o) To a mixture of 4-chloro-5-nitroisoquinoline (**3**, 1.0 g, 4.8 mmol), BINAP (62 mg, 0.10 mmol), palladium(II) acetate (22 mg, 0.10 mmol), and anhydrous potassium carbonate (0.263 g, 1.9 mmol) in dioxane, the appropriate amine (0.48 mmol) was added. The mixture was refluxed for 40 h. Upon completion, the reaction mixture was filtered through celite. The filtrate was evaporated under reduced pressure, and the resulting residue was purified by column chromatography to afford the corresponding compounds **5a**–**o**.

N-(3,4-Dimethylphenyl)-5-nitroisoquinolin-1-amine (**5a**): Brown solid, yield 50%; ^{1}H-NMR (DMSO-d_6) δ: 9.38 (s, 1H), 8.92 (d, J=8.5 Hz, 1H), 8.49 (dd, J=0.7, 0.8 Hz, 1H), 8.16 (d, J=6.2 Hz, 1H), 7.77 (t, J=8.1 Hz, 1H), 7.55–7.51 (m, 2H), 7.49 (d, J=6.1 Hz, 1H), 7.11 (d, J=8.8 Hz, 1H), 2.22 (d, J=11.0 Hz, 6H).

5-Nitro-*N*-(4-(trifluoromethyl)phenyl)isoquinolin-1-amine (**5b**): Orange solid, yield 49%; ^{1}H-NMR (DMSO-d_6) δ: 9.86 (s, 1H), 8.96 (d, J=8.5 Hz, 1H), 8.55 (d, J=7.8 Hz, 2H), 8.28 (d,

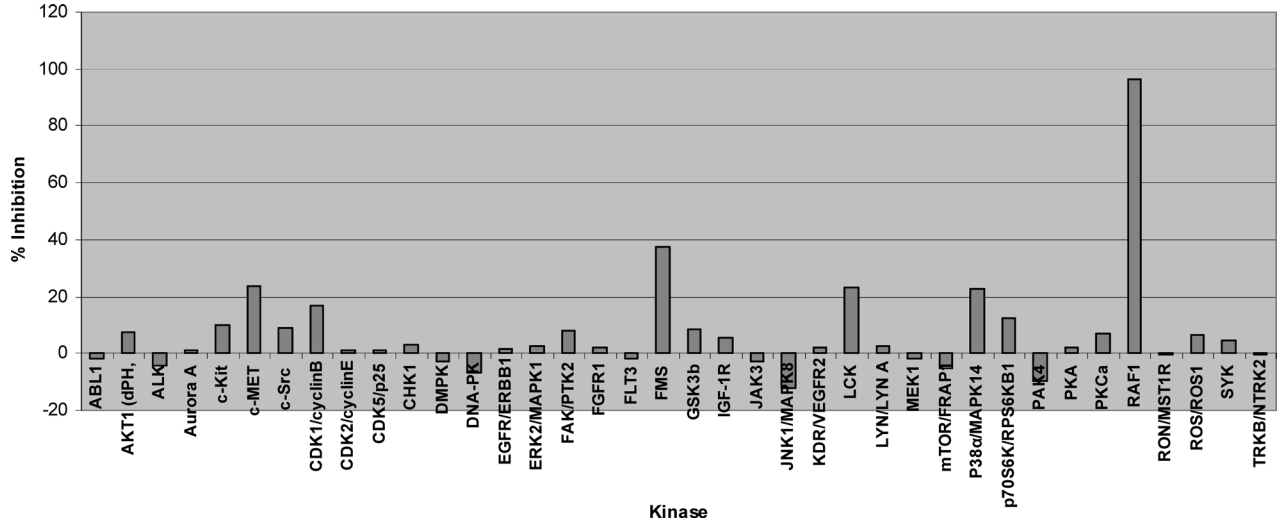


Fig. 2. Inhibition Percentages of Compound **1d** at a Single Dose Concentration of 10 μM over a Panel of 38 Kinases

% Activity in each enzyme is the mean of two different readings; test compound was used in a single dose concentration of 10 μM; 100% activity refers to enzyme activity in negative control (DMSO); % inhibition was calculated by subtracting % activity from 100.

Table 3. Enzymatic Inhibitory Activities of Compound **1d**

Kinase	IC_{50} (μM)
B-RAF (V600E)	9.4
RAF1	0.96

J=6.1 Hz, 1H), 8.08 (d, *J*=8.5 Hz, 2H), 7.85 (t, *J*=8.1 Hz, 1H), 7.72–7.67 (m, 2H).

5-Nitro-*N*-(3-(trifluoromethyl)phenyl)isoquinolin-1-amine (**5c**): Brown solid, yield 44%; ^{1}H-NMR (DMSO-d_6) δ: 9.80 (s, 1H), 8.96 (d, *J*=8.3 Hz, 1H), 8.54 (d, *J*=7.8 Hz, 1H), 8.29–8.25 (m, 2H), 8.19 (d, *J*=8.3 Hz, 1H), 7.85 (t, *J*=8.1 Hz, 1H), 7.63 (d, *J*=6.2 Hz, 1H), 7.59 (t, *J*=8.0 Hz, 1H), 7.37 (d, *J*=7.7 Hz, 1H).

N-(4-Chloro-3-(trifluoromethyl)phenyl)-5-nitroisoquinolin-1-amine (**5d**): Brown solid, yield 60%; ^{1}H-NMR (DMSO-d_6) δ: 9.89 (s, 1H), 8.94 (d, *J*=8.3 Hz, 1H), 8.55 (d, *J*=7.7 Hz, 1H), 8.42 (s, 1H), 8.27 (d, *J*=6.0 Hz, 2H), 7.86 (t, *J*=8.2 Hz, 1H), 7.70–7.67 (m, 2H).

N-(2,4-Bis(trifluoromethyl)phenyl)-5-nitroisoquinolin-1-amine (**5e**): Brown solid, yield 40%; ^{1}H-NMR (DMSO-d_6) δ: 9.32–9.27 (m, 2H), 9.13 (d, *J*=7.9 Hz, 1H), 8.59 (d, *J*=7.9 Hz, 1H), 8.14 (s, 1H), 8.02–7.96 (m, 3H), 7.85 (d, *J*=7.8 Hz, 1H).

N-(4-Morpholinophenyl)-5-nitroisoquinolin-1-amine (**5f**): Brown solid, yield 54%; ^{1}H-NMR (DMSO-d_6) δ: 9.38 (s, 1H), 8.90 (d, *J*=8.4 Hz, 1H), 8.49 (t, *J*=7.9 Hz, 2H), 8.11 (d, *J*=6.1 Hz, 1H), 8.01 (d, *J*=6.2 Hz, 1H), 7.75 (t, *J*=8.2 Hz, 1H), 7.44 (d, *J*=6.2 Hz, 1H), 6.96 (d, *J*=9.0 Hz, 2H), 3.75 (t, *J*=4.6 Hz, 4H), 3.08 (t, *J*=4.6 Hz, 4H).

N-(2-Morpholino-5-(trifluoromethyl)phenyl)-5-nitroisoquinolin-1-amine (**5g**): Orange solid, yield 50%; ^{1}H-NMR (DMSO-d_6) δ: 9.29 (s, 1H), 8.75 (d, *J*=8.4 Hz, 1H), 8.55 (d, *J*=7.5 Hz, 1H), 8.47 (d, *J*=1.8 Hz, 1H), 8.24 (d, *J*=6.3 Hz, 1H), 7.89 (t, *J*=8.1 Hz, 1H), 7.59 (d, *J*=6.1 Hz, 1H), 7.46 (dd, *J*=1.7, 1.8 Hz, 1H), 7.40 (d, *J*=8.4 Hz, 1H), 3.67 (t, *J*=4.3 Hz, 4H), 2.94 (t, *J*=4.5 Hz, 4H).

N^1-(5-Nitroisoquinolin-1-yl)-N^4-phenylbenzene-1,4-diamine (**5h**): Brown solid, yield 43%; ^{1}H-NMR (DMSO-d_6) δ: 9.42 (s, 1H), 8.91 (d, *J*=8.4 Hz, 1H), 8.49 (t, *J*=9.0 Hz, 2H), 8.35–8.31 (m, 2H), 8.13 (d, *J*=6.1 Hz, 1H), 8.07 (s, 1H), 8.01 (d, *J*=6.1 Hz, 1H), 7.76 (t, *J*=8.3 Hz, 1H), 7.69–7.64 (m, 2H), 7.21 (t, *J*=7.5 Hz, 3H), 6.90 (d, *J*=8.4 Hz, 1H).

5-Nitro-*N*-(3-phenoxyphenyl)isoquinolin-1-amine (**5i**): Orange solid, yield 28%; ^{1}H-NMR (DMSO-d_6) δ: 9.58 (s, 1H), 8.91 (d, *J*=8.4 Hz, 1H), 8.51 (d, *J*=7.8 Hz, 1H), 8.19 (dd, *J*=1.4, 1.5 Hz, 1H), 7.79 (t, *J*=8.3 Hz, 1H), 7.67 (d, *J*=8.1 Hz, 1H), 7.63–7.60 (m, 1H), 7.56 (d, *J*=6.2 Hz, 1H), 7.38–7.32 (m, 3H), 7.15 (t, *J*=7.2 Hz, 1H), 7.10–7.06 (m, 2H), 6.69 (d, *J*=8.0 Hz, 1H).

N-(Benzo[*d*][1,3]dioxol-5-yl)-5-nitroisoquinolin-1-amine (**5j**): Brown solid, yield 22%; ^{1}H-NMR (DMSO-d_6) δ: 9.43 (s, 1H), 8.89 (d, *J*=8.4 Hz, 1H), 8.49 (d, *J*=7.7 Hz, 1H), 8.15 (d, *J*=6.2 Hz, 1H), 7.77 (t, *J*=8.2 Hz, 1H), 7.49 (d, *J*=6.2 Hz, 1H), 7.45 (d, *J*=2.0 Hz, 1H), 7.16 (dd, *J*=2.1, 2.2 Hz, 1H), 6.91 (d, *J*=8.4 Hz, 1H), 6.01 (s, 2H).

N-(3-Chloro-5-(trifluoromethyl)pyridin-2-yl)-5-nitroisoquinolin-1-amine (**5k**): Brown solid, yield 35%; ^{1}H-NMR (DMSO-d_6) δ: 9.09 (s, 1H), 8.76–8.71 (m, 2H), 8.59–8.52 (m, 3H), 8.02 (t, *J*=8.1 Hz, 1H), 7.80 (t, *J*=8.0 Hz, 1H).

N-(4,6-Dimethylpyrimidin-2-yl)-5-nitroisoquinolin-1-amine (**5l**): Brown solid, yield 48%; ^{1}H-NMR (DMSO-d_6) δ: 10.15 (s, 1H), 9.57–8.53 (m, 2H), 8.45 (s, 1H), 7.99 (s, 1H), 7.74 (t, *J*=8.1 Hz, 1H), 6.75 (s, 1H), 2.25 (s, 6H).

5-Nitro-*N*-(4-(trifluoromethyl)pyrimidin-2-yl)isoquinolin-1-amine (**5m**): Brown solid, yield 33%; ^{1}H-NMR (DMSO-d_6) δ: 11.08 (s, 1H), 8.44 (d, *J*=4.9 Hz, 1H), 8.65–8.61 (m, 2H), 8.45 (brs, 1H), 8.12 (brs, 1H), 7.78–7.75 (m, 1H), 7.40 (d, *J*=4.9 Hz, 1H).

5-Nitro-*N*-(pyrazin-2-yl)isoquinolin-1-amine (**5n**): Brown solid, yield 45%; ^{1}H-NMR (DMSO-d_6) δ: 9.89 (s, 1H), 9.28 (brs, 1H), 9.0 (d, *J*=8.38 Hz, 1H), 8.6 (d, *J*=7.72 Hz, 1H), 8.41–8.38 (m, 1H), 8.26 (d, *J*=2.6 Hz, 2H), 7.80 (t, *J*=8.1 Hz, 1H), 7.66 (brs, 1H).

4-Methyl-*N*-(5-nitroisoquinolin-1-yl)thiazol-2-amine (**5o**): Orange solid, yield 45%; ^{1}H-NMR (DMSO-d_6) δ: 12.29 (s, 1H), 9.10 (d, *J*=8.2 Hz, 1H), 8.52 (d, *J*=7.8 Hz, 1H), 8.32 (d, *J*=5.4 Hz, 1H), 7.77 (t, *J*=8.0 Hz, 1H), 7.62 (s, 1H), 6.65 (s, 1H), 2.30 (s, 3H).

General Procedure for the Preparation of N^1-Substituted isoquinolin-1,5-diamines (6a–o) A mixture of the appropriate nitroisoquinolinylamine **5a–o** (0.27 mmol) and 5% Pd/C (40 mg) in MeOH (5 mL) was stirred in hydrogen atmosphere at room temperature for 2 h. Upon completion, the reaction mixture was filtered through celite. The filtrate was evaporated under reduced pressure, and the resulting residue was purified by column chromatography to afford the corresponding compounds **6a–o** in 14–91% yield.

4-Chloro-8-nitroquinoline (7) To a solution of 4-chloroisoquinoline (**9**, 5.34 g, 32.7 mmol) in sulfuric acid (24.0 mL, 84.4 mmol) was added nitric acid (4.8 mL, 114.6 mmol) dropwise at 0°C. The mixture was stirred at room temperature for 4 h. Upon completion, the reaction mixture was cooled to 0°C and neutralized with 10% $NaHCO_3$ aqueous solution. The resulting precipitate was collected by filtration and dried to give **7** (4.21 g, 62%) as a white solid. ^{1}H-NMR (DMSO-d_6) δ: 8.97 (d, J=4.7 Hz, 1H), 8.48 (d, J=8.5 Hz, 1H), 8.40 (d, J=7.5 Hz, 1H), 8.00 (d, J=4.7 Hz, 1H), 7.93 (t, J=8.3 Hz, 1H); ^{13}C-NMR ($CDCl_3$) δ: 152.0, 149.0, 143.2, 140.8, 128.2, 127.4, 126.3, 124.3, 122.9.

General Procedure for the Preparation of N^5-(8-Nitroquinolin-4-yl)-N^1-substituted Isoquinolin-1,5-diamines (8a–o) According to the procedure described for **5a–o**, compounds **8a–o** were obtained from the corresponding **6a–o**. **8a**: 46%; **8b**: 86%; **8c**: 48%; **8d**: 59%; **8e**: 44%; **8f**: 41%; **8g**: 60%; **8h**: 44%; **8i**: 35%; **8j**: 24%; **8k**: 21%; **8l**: 14%; **8m**: 26%; **8n**: 16%; **8o**: 8%.

General Procedure for the Preparation of Target Compounds (1a–o) A mixture of the appropriate nitroquinolinylisoquinolinylamine **8a–o** (0.20 mmol) and 5% Pd/C (30 mg) in MeOH (5 mL) was stirred in hydrogen atmosphere at room temperature for 2 h. Upon completion, the reaction mixture was filtered through celite. The filtrate was evaporated under reduced pressure, and the resulting residue was purified by column chromatography to afford the corresponding compounds **1a–o**.

N^1-(3,4-Dimethylphenyl)-N^5-(8-nitroquinolin-4-yl)-isoquinoline-1,5-diamine (**1a**): Pale yellow solid, yield 83%; MS m/z: 406 $[M+1]^+$; ^{1}H-NMR (DMSO-d_6) δ: 9.12 (s, 1H), 8.88 (s, 1H), 8.50 (t, J=4.7 Hz, 1H), 8.17 (d, J=5.1 Hz, 1H), 7.89 (d, J=5.9 Hz, 1H), 7.69–7.64 (m, 2H), 7.62–7.56 (m, 3H), 7.24 (t, J=7.9 Hz, 1H), 7.08 (d, J=8.7 Hz, 1H), 6.97 (d, J=5.9 Hz, 1H), 6.85 (d, J=7.4 Hz, 1H), 6.05 (d, J=5.1 Hz, 1H), 5.80 (s, 2H), 2.22 (d, J=13.2 Hz, 6H); ^{13}C-NMR (DMSO-d_6) δ: 153.4, 149.7, 147.1, 145.1, 141.0, 138.5, 137.9, 135.8, 135.7, 134.0, 129.8, 129.2, 127.9, 126.3, 125.6, 122.3, 121.3, 119.6, 119.3, 118.6, 109.1, 108.0, 107.4, 101.8, 19.6, 18.7.

N^4-(1-(4-(Trifluoromethyl)phenylamino)isoquinolin-5-yl)-quinoline-4,8-diamine (**1b**): Pale green solid, yield 59%; MS m/z: 446 $[M+1]^+$; ^{1}H-NMR (DMSO-d_6) δ: 9.63 (s, 1H), 8.91 (s, 1H), 8.52 (d, J=5.3 Hz, 1H), 8.18 (d, J=4.9 Hz, 1H), 8.14 (d, J=8.2 Hz, 2H), 8.02 (d, J=5.6 Hz, 1H), 7.23 (s, 2H), 7.68 (d, J=8.2 Hz, 2H), 7.61 (d, J=8.2 Hz, 1H), 7.26 (t, J=7.4 Hz, 1H), 7.16 (d, J=5.5 Hz, 1H), 6.86 (d, J=7.3 Hz, 1H), 6.08 (d, J=4.8 Hz, 1H), 5.81 (s, 2H); ^{13}C-NMR (DMSO-d_6) δ: 152.5, 149.7, 147.1, 145.0, 144.8, 140.5, 137.9, 136.0, 134.1, 128.1, 126.8, 126.0, 125.7, 125.6, 123.3, 121.5, 121.3, 120.0, 119.6, 119.3, 109.3, 109.2, 108.0, 101.9.

N^4-(1-(3-(Trifluoromethyl)phenylamino)isoquinolin-5-yl)-quinoline-4,8-diamine (**1c**): Pale green solid, yield 86%; MS m/z: 446 $[M+1]^+$; ^{1}H-NMR (DMSO-d_6) δ: 9.58 (s, 1H), 8.91 (s, 1H), 8.53 (d, J=6.9 Hz, 1H). 8.37 (s, 1H), 8.25 (d, J=8.0 Hz, 1H), 8.18 (d, J=4.8 Hz, 1H), 8.00 (d, J=5.7 Hz, 1H), 7.75–7.70 (m, 2H), 7.61 (d, J=8.2 Hz, 1H), 7.56 (t, J=7.6 Hz, 1H), 7.32 (d, J=7.4 Hz, 1H), 7.25 (t, J=7.8 Hz, 1H), 7.13 (d, J=5.7 Hz, 1H), 6.86 (d, J=7.3 Hz, 1H), 6.08 (d, J=4.5 Hz, 1H), 5.81 (s, 2H); ^{13}C-NMR (DMSO-d_6) δ: 153.2, 150.1, 147.5, 145.8, 142.5, 141.0, 138.6, 136.6, 134.6, 129.9, 128.4, 127.1, 126.1, 124.0, 123.6, 121.8, 120.3, 119.9, 118.1, 116.5, 110.3, 109.5, 109.3, 108.4, 102.5.

N^4-(1-(4-Chloro-3-(trifluoromethyl)phenylamino)-isoquinolin-5-yl)quinoline-4,8-diamine (**1d**): Pale green solid, yield 64%; MS m/z: 480 $[M+1]^+$; ^{1}H-NMR (DMSO-d_6) δ: 9.78 (s, 1H), 8.93 (s, 1H), 8.52 (s, 2H), 8.34 (d, J=6.5 Hz, 1H), 8.18 (s, 1H), 8.00 (s, 1H), 7.73 (s, 2H), 7.66 (d, J=9.0 Hz, 1H), 7.61 (d, J=7.4 Hz, 1H), 7.25 (s, 1H), 7.16 (s, 1H), 6.86 (d, J=6.1 Hz, 1H), 6.07 (s, 1H), 5.81 (s. 2H); ^{13}C-NMR (DMSO-d_6) δ: 158.5, 152.4, 145.5, 144.0, 140.7, 135.4, 134.0, 131.5, 129.8, 128.4, 126.7, 126.4, 126.1, 124.4, 121.9, 121.6, 119.8, 119.1, 118.6, 110.5, 109.1, 108.7, 108.2, 107.0, 101.5.

N^4-(1-(2,4-Bis(trifluoromethyl)phenylamino)isoquinolin-5-yl)quinoline-4,8-diamine (**1e**): Brown solid, yield 49%; MS m/z: 514 $[M+1]^+$; ^{1}H-NMR (DMSO-d_6) δ: 9.57 (s, 1H), 9.20 (s, 1H), 9.08–9.06 (m, 2H), 8.72 (d, J=7.6 Hz, 1H), 8.22 (d, J=5.1 Hz, 1H), 8.11 (s, 1H), 7.90 (t, J=7.6 Hz, 2H), 7.84 (d, J=7.6 Hz, 1H), 7.63 (d, J=8.3 Hz, 1H), 7.35 (d, J=7.6 Hz, 1H), 7.29 (t, J=7.9 Hz, 1H), 6.88 (d, J=7.5 Hz, 1H), 6.22 (d, J=5.0 Hz, 1H), 5.84 (s, 2H).

N^4-(1-(4-Morpholinophenylamino)isoquinolin-5-yl)-quinoline-4,8-diamine (**1f**): Pale yellow solid, yield 25%; MS m/z: 463 $[M+1]^+$; ^{1}H-NMR (DMSO-d_6) δ: 9.09 (s, 1H), 8.85 (s, 1H), 8.48–8.46 (m, 1H), 8.17 (d, J=4.3 Hz, 1H), 7.85 (d, J=5.5 Hz, 1H), 7.72–7.64 (m, 4H), 7.61 (d, J=8.7 Hz, 1H), 7.24 (t, J=7.4 Hz, 1H), 6.94 (t, J=7.9 Hz, 3H), 6.85 (d, J=7.1 Hz, 1H), 6.05 (d, J=4.1 Hz, 1H), 5.80 (s, 2H), 3.75 (t, J=4.3 Hz, 4H), 3.07 (t, J=4.5 Hz, 4H); ^{13}C-NMR (DMSO-d_6) δ: 153.6, 149.6, 147.0, 146.6, 145.3, 141.1, 138.1, 135.8, 134.1, 133.3, 127.7, 126.0, 125.5, 122.4, 121.4, 119.5, 119.3, 115.4, 108.7, 107.8, 107.0, 101.8, 66.2, 49.2.

N^4-(1-(2-Morpholino-5-(trifluoromethyl)phenylamino)-isoquinolin-5-yl)quinoline-4,8-diamine (**1g**): Pale yellow solid, yield 53%; MS m/z: 531 $[M+1]^+$; ^{1}H-NMR (DMSO-d_6) δ: 9.24 (s, 1H), 8.98 (s, 1H), 8.93 (s, 1H), 8.28–8.23 (m, 2H), 8.09 (d, J=5.9 Hz, 1H), 7.88 (t, J=7.7 Hz, 1H), 7.79 (d, J=7.4 Hz, 1H), 7.67 (d, J=8.3 Hz, 1H), 7.52 (d, J=8.2 Hz, 1H), 7.45 (d, J=8.2 Hz, 1H), 7.31 (t, J=7.9 Hz, 1H), 7.19 (d, J=5.9 Hz, 1H), 6.92 (d, J=7.5 Hz, 1H), 6.17 (d, J=5.1 Hz, 1H), 5.87 (s, 2H), 3.88 (t, J=4.0 Hz, 4H), 3.04 (t, J=4.3 Hz, 4H); ^{13}C-NMR (DMSO-d_6) δ: 152.0, 149.4, 147.0, 145.7, 145.3, 140.7, 138.1, 136.6, 135.3, 133.8, 127.7, 127.4, 126.3, 125.6, 124.9, 124.4, 122.7, 121.1, 119.7, 119.5, 119.0, 116.5, 108.8, 107.9, 102.2, 66.7, 51.4.

N^4-(1-(4-(Phenylamino)phenylamino)isoquinolin-5-yl)-quinoline-4,8-diamine (**1h**): Brown solid, yield 64%; MS m/z: 469 $[M+1]^+$; ^{1}H-NMR (DMSO-d_6) δ: 9.16 (s, 1H), 8.87 (s, 1H), 8.49 (t, J=4.7 Hz, 1H), 8.17 (d, J=5.1 Hz, 1H), 8.03 (s, 1H), 7.86 (d, J=6.0 Hz, 1H), 7.71 (d, J=8.8 Hz, 2H), 7.66 (d, J=4.4 Hz, 2H), 7.61 (d, J=8.3 Hz, 1H), 7.21 (m, 3H), 7.09 (d, J=8.8 Hz, 2H), 7.02 (d, J=7.8 Hz, 2H), 6.94 (d, J=6.0 Hz, 1H), 6.85 (d, J=6.9 Hz, 1H), 6.75 (t, J=7.2 Hz, 1H), 6.06 (d, J=5.2 Hz, 1H), 5.80 (s, 2H); ^{13}C-NMR (DMSO-d_6) δ: 153.5,

149.6, 147.0, 144.5, 141.1, 138.1, 137.8, 135.8, 134.1, 129.1, 127.8, 126.1, 125.5, 122.5, 122.1, 121.4, 119.5, 119.3, 118.6, 118.3, 118.1, 115.4, 108.8, 107.8, 107.1, 101.9.

N^4-(1-(3-Phenoxyphenylamino)isoquinolin-5-yl)-quinoline-4,8-diamine (**1i**): Pale brown solid, yield 68%; MS *m/z*: 470 $[M+1]^+$; ^{1}H-NMR (DMSO-d_6) δ: 9.35 (s, 1H), 8.89 (s, 1H), 8.49 (s, 1H), 8.17 (d, J=4.2 Hz, 1H), 7.92 (d, J=5.3 Hz, 1H), 7.74–7.67 (m, 4H), 7.61 (d, J=8.0 Hz, 1H), 7.42–7.37 (m, 2H), 7.32 (t, J=7.9 Hz, 1H), 7.25 (t, J=7.4 Hz, 1H), 7.13 (t, J=6.7 Hz, 1H), 7.10–7.05 (m, 3H), 6.85 (d, J=6.8 Hz, 1H), 6.64 (d, J=7.3 Hz, 1H), 6.06 (d, J=4.1 Hz, 1H), 5.80 (s, 2H); ^{13}C-NMR (DMSO-d_6) δ: 157.3, 157.1, 152.3, 150.3, 147.5, 145.8, 144.2, 143.3, 141.1, 139.4, 136.5, 134.6, 130.5, 130.0, 128.4, 126.9, 126.0, 123.7, 120.3, 120.0, 119.0, 115.8, 112.2, 110.9, 109.2, 108.9, 108.4, 102.4.

N^4-(1-(Benzo[*d*][1,3]dioxol-5-ylamino)isoquinolin-5-yl)quinoline-4,8-diamine (**1j**): Pale green solid, yield 94%; MS *m/z*: 422 $[M+1]^+$; ^{1}H-NMR (DMSO-d_6) δ: 9.20 (s, 1H), 8.89 (s, 1H), 8.48 (d, J=4.1 Hz, 1H), 8.16 (d, J=4.9 Hz, 1H), 7.87 (d, J=5.9 Hz, 1H), 7.64–7.58 (m, 4H), 7.24 (t, J=7.8 Hz, 2H), 6.97 (d, J=5.9 Hz, 1H), 6.89–6.85 (m, 2H), 6.04 (d, J=5.1 Hz, 1H), 6.00 (s, 2H), 5.80 (s, 2H); ^{13}C-NMR (DMSO-d_6) δ: 153.4, 149.6, 147.0, 146.8, 145.3, 142.2, 140.9, 138.1, 135.9, 135.4, 134.0, 127.8, 126.2, 125.5, 121.3, 119.5, 119.4, 113.8, 108.8, 107.9, 107.7, 107.5, 103.5, 101.9, 100.7.

N^4-(1-(3-Chloro-5-(trifluoromethyl)pyridin-2-ylamino)-isoquinolin-5-yl)quinoline-4,8-diamine (**1k**): Pale green solid, yield 47%; MS *m/z*: 481 $[M+1]^+$; ^{1}H-NMR (DMSO-d_6) δ: 8.90 (s, 1H), 8.75 (s, 1H), 8.55 (s, 1H), 8.26–8.22 (m, 2H), 7.73–7.64 (m, 5H), 7.26 (s, 1H), 6.87 (m, 2H), 6.11 (s, 1H), 5.82 (s, 2H).

N^4-(1-(4,6-Dimethylpyrimidin-2-ylamino)isoquinolin-5-yl)-quinoline-4,8-diamine (**1l**): Pale green solid, yield 27%; MS *m/z*: 408 $[M+1]^+$; ^{1}H-NMR (DMSO-d_6) δ: 9.89 (s, 1H), 8.94 (s, 1H), 8.20–8.16 (m, 2H), 8.07 (d, J=8.2 Hz, 1H), 7.70 (d, J=7.5 Hz, 1H), 7.65–7.61 (m, 2H), 7.42 (d, J=5.8 Hz, 1H), 7.26 (t, J=7.7 Hz, 1H), 6.86 (d, J=7.0 Hz, 1H), 6.70 (s, 1H), 6.08 (d, J=5.1 Hz, 1H), 5.81 (s, 2H), 2.25 (s, 6H).

N^4-(1-(4-(Trifluoromethyl)pyrimidin-2-ylamino)-isoquinolin-5-yl)quinoline-4,8-diamine (**1m**): Pale yellow solid, yield 80%; MS *m/z*: 448 $[M+1]^+$; ^{1}H-NMR (DMSO-d_6) δ: 10.89 (s, 1H), 8.96 (s, 1H), 8.76 (d, J=4.8 Hz, 1H), 8.20 (d, J=5.1 Hz, 2H), 8.16 (brs, 1H), 7.74 (d, J=6.8 Hz, 1H), 7.68 (t, J=8.1 Hz, 1H), 7.62 (d, J=8.3 Hz, 1H), 7.45 (brs, 1H), 7.31 (d, J=4.8 Hz, 1H), 7.26 (t, J=7.9 Hz, 1H), 6.86 (d, J=7.5 Hz, 1H), 6.11 (d, J=5.1 Hz, 1H), 5.82 (s, 2H).

N^4-(1-(Pyrazin-2-ylamino)isoquinolin-5-yl)quinoline-4,8-diamine (**1n**): Orange solid, yield 74%; MS *m/z*: 380 $[M+1]^+$; ^{1}H-NMR (DMSO-d_6) δ: 10.15 (s, 1H), 9.47 (s, 1H), 8.93 (s, 1H), 8.56 (d, J=4.6 Hz, 1H), 8.38 (s, 1H), 8.23 (s, 1H), 8.18 (d, J=5.0 Hz, 1H), 8.07 (d, J=5.6 Hz, 1H), 7.74–7.71 (m, 2H), 7.61 (d, J=8.8 Hz, 1H), 7.27 (d, J=5.7 Hz, 2H), 6.86 (d, J=7.4 Hz, 1H), 6.09 (s, 1H), 5.82 (s, 2H).

N^4-(1-(4-Methylthiazol-2-ylamino)isoquinolin-5-yl)-quinoline-4,8-diamine (**1o**): Pale green solid, yield 38%; MS *m/z*: 399 $[M+1]^+$; ^{1}H-NMR (DMSO-d_6) δ: 11.76 (s, 1H), 8.91 (s, 1H), 8.70 (s, 1H), 8.17 (s, 1H), 8.09 (brs, 1H), 7.71 (s, 2H), 7.60 (d, J=7.5 Hz, 1H), 7.25 (t, J=6.8 Hz, 1H), 7.16 (brs, 1H), 6.86 (d, J=6.5 Hz, 1H), 6.66 (s, 1H), 6.06 (s, 1H), 5.81 (s, 2H), 2.32 (s, 3H); ^{13}C-NMR (DMSO-d_6) δ: 158.7, 151.4, 150.0, 147.5, 145.8, 141.7, 140.9, 138.6, 136.5, 134.3, 128.6, 127.5, 126.1, 121.9, 119.9, 109.3, 108.3, 106.9, 102.5, 17.3.

5-Nitroisoquinolin-1-amine (10) 1-Chloro-5-nitroisoquinoline (**4**) (500 mg, 2.40 mmol) was added to ammonia (7 N solution in EtOH) (10 mL), and the mixture was refluxed for 40 h. After completion of the reaction, the solvent was removed under reduced pressure. The residue was purified by column chromatography (silica gel, ethyl acetate–hexane 2 : 1 v/v) to give **10** (350 mg, 77%) as an orange solid. ^{1}H-NMR ($CDCl_3$) δ: 8.41 (d, J=7.8 Hz, 1H), 8.16–8.13 (m, 2H), 7.76 (d, J=6.3 Hz, 1H), 7.59 (t, J=7.7 Hz, 1H), 5.32 (s, 2H); ^{13}C-NMR (100 MHz, DMSO-d_6) δ: 167.4, 157.8, 146.1, 144.4, 129.3, 127.6, 118.0, 104.1, 102.2.

General Procedure for the Preparation of *N*-(5-Nitroisoquinolin-1-yl)-substituted 2-Carboxamides (11a–m) A mixture of compound **10** (21 mg, 0.11 mmol), HBTU (102 mg, 0.27 mmol), and *N*,*N*-diisopropylethylamine (71 mg, 0.55 mmol) in dry THF (5 mL) was stirred at 0°C for 30 min. The appropriate acid (0.22 mmol) was then added. After stirring under reflux for 30 h, the reaction mixture was diluted with saturated aqueous $NaHCO_3$, and extracted with ethyl acetate. The organic layer was washed with brine, dried over anhydrous $MgSO_4$, and concentrated *in vacuo*. The residue was purified by column chromatography to afford the corresponding compounds **11a**–**m**.

3,4-Dimethoxy-*N*-(5-nitroisoquinolin-1-yl)benzamide (**11a**): Yellow solid, yield 29%; ^{1}H-NMR (DMSO-d_6) δ: 11.09 (s, 1H), 8.63 (s, 1H), 8.33–8.30 (m, 1H), 7.81–7.74 (m, 4H), 7.44 (s, 1H), 7.12 (s, 1H), 3.86 (s, 6H).

3,5-Dimethoxy-*N*-(5-nitroisoquinolin-1-yl)benzamide (**11b**): Yellow solid, yield 41%; ^{1}H-NMR (400 MHz, DMSO-d_6) δ: 10.92 (s, 1H), 8.64 (d, J=7.7 Hz, 1H), 7.93–7.88 (m, 2H), 7.46–7.41 (m, 2H), 7.31 (s, 1H), 6.77 (t, J=4.5 Hz, 1H), 6.41 (d, J=2.2 Hz, 1H), 3.76 (s, 6H).

4-Methyl-*N*-(5-nitroisoquinolin-1-yl)benzamide (**11c**): Yellow solid, yield 47%; ^{1}H-NMR (DMSO-d_6) δ: 11.14 (s, 1H), 8.64 (d, J=6.2 Hz, 2H), 8.42 (d, J=8.3 Hz, 1H), 8.24 (d, J=6.0 Hz, 1H), 8.00 (d, J=7.8 Hz, 2H), 7.84 (t, J=8.3 Hz, 1H), 7.38 (d, J=7.7 Hz, 2H), 2.42 (s, 3H).

N-(5-Nitroisoquinolin-1-yl)-3-(trifluoromethyl)benzamide (**11d**): Yellow solid, yield 31%; ^{1}H-NMR (DMSO-d_6) δ: 8.66 (dd, J=0.9, 1.0 Hz, 2H), 8.49 (s, 3H), 8.02 (d, J=7.5 Hz, 2H), 7.89 (d, J=8.0 Hz, 1H), 8.85 (d, J=5.3 Hz, 1H), 7.81 (d, J=7.7 Hz, 1H).

4-Chloro-*N*-(5-nitroisoquinolin-1-yl)-3-(trifluoromethyl)-benzamide (**11e**): Yellow solid, yield 65%; ^{1}H-NMR ($CDCl_3$) δ: 15.3 (brs, 1H), 9.38 (s, 1H), 8.76 (s, 1H), 8.58 (d, J=6.9 Hz, 1H), 8.50 (d, J=8.1 Hz, 1H), 7.91 (d, J=15.0 Hz, 1H), 7.82 (t, J=8.1 Hz, 1H), 7.63 (d, J=8.1 Hz, 2H).

N-(5-Nitroisoquinolin-1-yl)-2,4-bis(trifluoromethyl)-benzamide (**11f**): Pale yellow solid, yield 35%; ^{1}H-NMR (DMSO-d_6) δ: 11.72 (s, 1H), 8.83–8.81 (m, 1H), 8.66 (d, J=7.7 Hz, 1H), 8.60 (brs, 1H), 8.22 (s, 2H), 8.14 (s, 2H), 7.91 (t, J=8.1 Hz, 1H).

N-(5-Nitroisoquinolin-1-yl)-3,5-bis(trifluoromethyl)-benzamide (**11g**): Yellow solid, yield 69%; ^{1}H-NMR ($CDCl_3$) δ: 15.63 (s, 1H), 9.40 (d, J=8.1 Hz, 1H), 8.86 (s, 2H), 8.61 (dd, J=1.3, 1.4 Hz, 1H), 8.04 (s, 1H), 7.93 (d, J=6.9 Hz, 1H), 7.86–7.83 (m, 1H), 7.67–7.65 (m, 1H).

3-Morpholino-*N*-(5-nitroisoquinolin-1-yl)-5-(trifluoromethyl)benzamide (**11h**): Yellow solid, yield 45%; ^{1}H-NMR (DMSO-d_6) δ: 11.41 (s, 1H), 8.66 (s, 2H), 8.46 (s, 1H), 8.28 (s, 1H), 7.87–7.82 (m, 3H), 7.46 (s, 1H), 3.79 (t,

J=4.4 Hz, 4H), 3.32 (t, *J*=4.8 Hz, 4H).

N-(5-Nitroisoquinolin-1-yl)-2,3-dihydrobenzo[*b*][1,4]-dioxine-6-carboxamide (**11i**): Yellow solid, yield 32%; ^{1}H-NMR (DMSO-d_6) δ: 11.05 (s, 1H), 8.63 (d, *J*=7.2 Hz, 1H), 8.17 (d, *J*=8.4 Hz, 1H), 7.89 (d, *J*=8.3 Hz, 1H), 7.80 (dd, *J*=2.1, 2.2 Hz, 1H), 7.74 (d, *J*=2.1 Hz, 1H), 7.66 (d, *J*=7.5 Hz, 1H), 7.54–7.51 (m, 1H), 7.18 (d, *J*=8.5 Hz, 1H), 4.44–4.36 (m, 4H).

4,7-Dimethoxy-*N*-(5-nitroisoquinolin-1-yl)-1-naphthamide (**11j**): Yellow solid, yield 23%; ^{1}H-NMR (DMSO-d_6) δ: 11.41 (s, 1H), 8.65 (d, *J*=7.6 Hz, 1H), 8.56 (brs, 1H), 8.18 (d, *J*=9.2 Hz, 3H), 8.06 (brs, 1H), 7.90–7.85 (m, 2H), 7.22 (dd, *J*=2.5, 2.6 Hz, 1H), 6.98 (d, *J*=8.2 Hz, 1H), 4.05 (s, 3H), 3.80 (s, 3H).

2,5-Dimethyl-*N*-(5-nitroisoquinolin-1-yl)furan-3-carboxamide (**11k**): Pale yellow solid, yield 17%; ^{1}H-NMR (DMSO-d_6) δ: 10.65 (s, 1H), 8.65–8.61 (m, 2H), 8.38 (d, *J*=8.4 Hz, 1H), 8.23 (d, *J*=6.0 Hz, 1H), 7.85 (t, *J*=8.1 Hz, 1H), 6.76 (s, 1H), 2.49 (s, 3H), 2.30 (s, 3H).

N-(5-Nitroisoquinolin-1-yl)thiazole-4-carboxamide (**11l**): Yellow solid, yield 31%; ^{1}H-NMR (DMSO-d_6) δ: 11.11 (s, 1H), 9.33 (s, 1H), 8.66 (d, *J*=7.5 Hz, 2H), 8.61 (s, 1H), 8.47 (d, *J*=8.3 Hz, 1H), 8.27 (d, *J*=6.0 Hz, 1H), 7.86 (t, *J*=8.0 Hz, 1H).

1-(4-Methoxyphenyl)-*N*-(5-nitroisoquinolin-1-yl)-5-(trifluoromethyl)-1*H*-pyrazole-4-carboxamide (**11m**): Yellow solid, yield 50%; ^{1}H-NMR (DMSO-d_6) δ: 11.42 (s, 1H), 8.64 (d, *J*=7.6 Hz, 1H), 8.54–8.47 (m, 3H), 8.46 (s, 1H), 7.88 (t, *J*=8.0 Hz, 1H), 7.48 (d, *J*=8.7 Hz, 2H), 7.12 (d, *J*=8.8 Hz, 2H).

General Procedure for the Preparation of *N*-(5-Aminoisoquinolin-1-yl)-substituted 2-Carboxamides (12a–m) According to the procedure described for **6a**–**o**, compounds **12a**–**m** were obtained from the corresponding **11a**–**m** in 38–85% yield.

General Procedure for the Preparation of *N*-(5-((8-Nitroquinolin-4-yl)amino)isoquinolin-1-yl)-substituted 2-Carboxamides (13a–m) According to the procedure described for **5a**–**o**, compounds **13a**–**m** were obtained from the corresponding **12a**–**m**. **13a**: 27%; **13b**: 25%; **13c**: 22%; **13d**: 25%; **13e**: 23%; **13f**: 28%; **13g**: 19%; **13h**: 22%; **13i**: 25%; **13j**: 31%; **13k**: 26%; **13l**: 20%; **13m**: 17%.

General Procedure for the Preparation of Target Compounds (2a–m) The target compounds **2a**–**m** were prepared from the corresponding **13a**–**m** as described for **1a**–**o**.

N-(5-(8-Aminoquinolin-4-ylamino)isoquinolin-1-yl)-3,4-dimethoxybenzamide (**2a**): Brown solid, yield 16%; MS *m*/*z*: 466 $[M+1]^+$; ^{1}H-NMR (DMSO-d_6) δ: 10.91 (s, 1H), 8.99 (s, 1H), 8.20 (d, *J*=5.1 Hz, 1H), 7.79–7.71 (m, 7H), 7.62 (d, *J*=8.4 Hz, 1H), 7.27 (t, *J*=7.8 Hz, 1H), 7.12 (d, *J*=8.5 Hz, 1H), 6.87 (d, *J*=6.9 Hz, 1H), 6.12 (d, *J*=5.1 Hz, 1H), 5.82 (s, 2H), 3.86 (s, 6H); ^{13}C-NMR (DMSO-d_6) δ: 167.0, 162.7, 155.8, 150.3, 146.1, 140.8, 134.2, 130.8, 130.1, 129.6, 129.0, 128.5, 127.3, 127.1, 126.9, 126.6, 125.2, 125.0, 124.6, 123.7, 123.3, 122.9, 121.0, 115.8, 109.8, 56.5.

N-(5-(8-Aminoquinolin-4-ylamino)isoquinolin-1-yl)-3,5-dimethoxybenzamide (**2b**): Brown solid, yield 9%; MS *m*/*z*: 466 $[M+1]^+$; ^{1}H-NMR (DMSO-d_6) δ: 10.97 (s, 1H), 8.96 (s, 1H), 8.20 (d, *J*=5.1 Hz, 2H), 7.75–7.71 (m, 3H), 7.62 (d, *J*=8.0 Hz, 1H), 7.35 (s, 1H), 7.26 (t, *J*=7.7 Hz, 1H), 7.08–7.06 (m, 1H), 6.87 (d, *J*=7.6 Hz, 1H), 6.73 (s, 1H), 6.11 (d, *J*=5.1 Hz, 1H), 5.82 (s, 2H); ^{13}C-NMR (DMSO-d_6) δ: 166.9, 161.6, 160.8, 157.7, 152.6, 150.0, 148.0, 146.0, 144.7, 140.6, 140.0, 138.3, 135.4, 132.6, 128.1, 126.8, 125.0, 124.4, 122.5, 118.5, 117.2, 108.8, 105.7, 56.9.

N-(5-(8-Aminoquinolin-4-ylamino)isoquinolin-1-yl)-4-methylbenzamide (**2c**): Brown solid, yield 27%; MS *m*/*z*: 420 $[M+1]^+$; ^{1}H-NMR (DMSO-d_6) δ: 10.94 (s, 1H), 8.99 (s, 1H), 8.34 (s, 1H), 8.21 (d, *J*=4.8 Hz, 1H), 8.04–8.01 (m, 1H), 7.93 (d, *J*=8.5 Hz, 1H), 7.73–7.66 (m, 5H), 7.40–7.36 (m, 2H), 7.27 (t, *J*=6.8 Hz, 1H), 6.87 (d, *J*=7.4 Hz, 1H), 6.13 (s, 1H), 5.82 (s, 2H), 2.42 (s, 3H); ^{13}C-NMR (DMSO-d_6) δ: 166.9, 160.5, 152.6, 150.0, 149.0, 146.6, 144.7, 142.0, 139.0, 138.0, 134.0, 130.6, 129.8, 127.7, 126.2, 125.0, 124.6, 123.7, 122.5, 118.2, 117.6, 112.6, 107.7, 22.7.

N-(5-(8-Aminoquinolin-4-ylamino)isoquinolin-1-yl)-3-(trifluoromethyl)-benzamide (**2d**): Green solid, yield 57%; MS *m*/*z*: 474 $[M+1]^+$; ^{1}H-NMR (DMSO-d_6) δ: 8.95 (s, 1H), 8.57–8.52 (m, 2H), 8.48 (brs, 1H), 8.19 (t, *J*=8.4 Hz, 2H), 8.05 (d, *J*=5.2 Hz, 1H), 7.92–7.88 (m, 2H), 7.75–7.70 (m, 2H), 7.63 (d, *J*=7.3 Hz, 1H), 7.26 (t, *J*=7.8 Hz, 2H), 6.86 (d, *J*=7.3 Hz, 1H), 6.08 (d, *J*=4.9 Hz, 1H), 5.81 (s, 2H); ^{13}C-NMR (DMSO-d_6) δ: 166.3, 149.6, 147.0, 145.3, 138.1, 135.7, 135.1, 134.2, 132.4, 131.5, 129.6, 129.3, 128.9, 128.8, 127.8, 127.0, 126.1, 125.6, 125.3, 124.8, 124.6, 124.1, 119.4, 108.8, 107.9, 101.9.

N-(5-(8-Aminoquinolin-4-ylamino)isoquinolin-1-yl)-4-chloro-3-(trifluoromethyl)benzamide (**2e**): Brown solid, yield 91%; MS *m*/*z*: 508 $[M+1]^+$; ^{1}H-NMR ($CDCl_3$) δ: 15.61 (s, 1H), 8.98 (d, *J*=7.8 Hz, 1H), 8.81 (s, 1H), 8.55 (d, *J*=8.9 Hz, 1H), 8.38 (d, *J*=5.2 Hz, 1H), 7.83 (d, *J*=7.5 Hz, 1H), 7.78–7.75 (m, 2H), 7.63 (d, *J*=8.0 Hz, 1H), 7.41–7.36 (m, 2H), 7.17 (d, *J*=7.6 Hz, 1H), 6.98 (d, *J*=7.4 Hz, 1H), 6.65 (s, 1H), 6.32 (s, 1H), 5.06 (s, 2H); ^{13}C-NMR (DMSO-d_6) δ: 166.8, 165.1, 150.2, 149.3, 146.3, 142.6, 140.8, 139.8, 137.9, 135.9, 133.0, 130.8, 128.5, 127.6, 125.3, 122.9, 122.0, 119.7, 116.6, 112.8, 110.8, 108.2, 106.8, 104.2, 103.9, 102.2.

N-(5-(8-Aminoquinolin-4-ylamino)isoquinolin-1-yl)-2,4-bis(trifluoromethyl)benzamide (**2f**): Yellow solid, yield 79%; MS *m*/*z*: 542 $[M+1]^+$; ^{1}H-NMR (DMSO-d_6) δ: 12.06 (s, 1H), 9.18 (s, 1H), 8.29 (d, *J*=6.7 Hz, 1H), 8.23–8.16 (m, 3H), 8.11 (d, *J*=4.8 Hz, 2H), 7.79–7.76 (m, 2H), 7.61 (d, *J*=8.2 Hz, 1H), 7.46 (s, 1H), 7.26 (t, *J*=7.6 Hz, 1H), 6.86 (d, *J*=7.3 Hz, 1H), 6.10 (d, *J*=4.9 Hz, 1H), 5.82 (s, 2H); ^{13}C-NMR (DMSO-d_6) δ: 167.7, 149.5, 147.0, 145.3, 138.1, 136.2, 134.2, 130.3, 130.1, 129.7, 129.5, 128.5, 127.6, 127.2, 126.9, 126.8, 125.6, 125.0, 125.0, 123.5, 123.4, 123.3, 121.3, 119.5, 108.8, 107.9, 102.1.

N-(5-(8-Aminoquinolin-4-ylamino)isoquinolin-1-yl)-3,5-bis(trifluoromethyl)benzamide (**2g**): Pale brown solid, yield 92%; MS *m*/*z*: 542 $[M+1]^+$; ^{1}H-NMR ($CDCl_3$) δ: 14.31 (s, 1H), 8.97 (d, *J*=7.9 Hz, 1H), 8.89 (s, 2H), 8.35 (d, *J*=5.0 Hz, 1H), 8.00 (s, 1H), 7.83 (d, *J*=7.1 Hz, 1H), 7.78 (d, *J*=8.0 Hz, 1H), 7.46 (d, *J*=7.1 Hz, 1H), 7.35–7.31 (m, 2H), 7.20 (d, *J*=6.9 Hz, 1H), 6.96 (d, *J*=6.2 Hz, 1H), 6.81 (s, 1H), 6.30 (d, *J*=4.9 Hz, 1H), 5.06 (s, 2H); ^{13}C-NMR (DMSO-d_6) δ: 167.3, 165.8, 152.3, 152.0, 148.0, 145.7, 144.0, 140.5, 137.9, 135.0, 132.8, 130.9, 127.7, 125.9, 125.5, 123.6, 122.9, 120.6, 119.9, 115.7, 110.0, 107.8, 106.8, 102.9.

N-(5-(8-Aminoquinolin-4-ylamino)isoquinolin-1-yl)-3-morpholino-5-(trifluoromethyl)benzamide (**2h**): Brown solid, yield 21%; MS *m*/*z*: 559 $[M+1]^+$; ^{1}H-NMR (DMSO-d_6) δ: 8.95 (s, 1H), 8.23 (d, *J*=5.2 Hz, 1H), 8.12 (brs, 1H), 8.02 (s, 1H), 7.91 (s, 1H), 7.71 (m, 4H), 7.62 (d, *J*=8.2 Hz, 1H), 7.42–7.37 (m, 2H), 7.26 (t, *J*=7.8 Hz, 1H), 6.86 (d, *J*=7.4 Hz, 1H), 6.09 (d, *J*=5.0 Hz, 1H), 5.82 (s, 2H), 3.80 (t, *J*=4.2 Hz, 4H), 3.32 (t, *J*=3.8 Hz, 4H); ^{13}C-NMR (DMSO-d_6) δ: 165.9, 164.8, 150.8,

149.5, 146.9, 145.8, 140.3, 138.7, 136.2, 134.3, 130.6, 130.2, 129.6, 129.2, 128.2, 128.1, 127.3, 126.6, 126.1, 124.4, 124.1, 123.3, 120.0, 118.4, 112.8, 107.8, 66.8. 54.5.

N-(5-(8-Aminoquinolin-4-ylamino)isoquinolin-1-yl)-2,3-dihydrobenzo[*b*][1,4]dioxine-6-carboxamide (**2i**): Pale brown solid, yield 42%; MS *m/z*: 464 $[M+1]^+$; ^{1}H-NMR (DMSO-d_6) δ: 10.71 (s, 1H), 8.97 (s, 1H), 8.19 (d, J=4.8 Hz, 2H), 8.17–8.15 (m, 1H), 7.77–7.70 (m, 5H), 7.62 (d, J=8.4 Hz, 1H), 7.26 (t, J=8.0 Hz, 1H), 6.99 (d, J=8.2 Hz, 1H), 6.86 (d, J=7.3 Hz, 1H), 6.10 (d, J=4.9 Hz, 1H), 5.82 (s, 2H), 4.36–4.29 (m, 4H); ^{13}C-NMR (DMSO-d_6) δ: 166.5, 164.7, 149.6, 147.1, 146.8, 145.3, 143.0, 142.9, 138.1, 136.5, 135.6, 134.2, 127.5, 127.4, 127.0, 125.6, 124.3, 122.5, 119.4, 117.3, 116.7, 114.8, 110.0, 109.0, 108.8, 107.9, 102.0.

N-(5-(8-Aminoquinolin-4-ylamino)isoquinolin-1-yl)-4,7-dimethoxy-1-naphthamide (**2j**): Pale brown solid, yield 31%; MS *m/z*: 516 $[M+1]^+$; ^{1}H-NMR (DMSO-d_6) δ: 11.01 (s, 1H), 9.01 (s, 1H), 8.30 (d, J=7.2 Hz, 1H), 8.23–8.16 (m, 3H), 8.08–8.01 (m, 3H), 7.81–7.75 (m, 2H), 7.64 (d, J=8.3 Hz, 1H), 7.27 (t, J=7.7 Hz, 1H), 7.22 (dd, J=2.2, 2.4 Hz, 1H), 6.98 (d, J=8.1 Hz, 1H), 6.87 (d, J=7.6 Hz, 1H), 6.13 (d, J=5.1 Hz, 1H), 5.83 (s, 2H), 4.05 (s, 3H), 3.83 (s, 3H); ^{13}C-NMR (DMSO-d_6) δ: 169.8, 165.7, 160.8, 158.7, 152.6, 150.0, 145.1, 140.9, 138.0, 135.7, 134.4, 132.4, 131.5, 129.7, 129.1, 128.9, 128.8, 127.2, 127.0, 126.6, 125.4, 125.3, 124.0, 123.6, 122.6, 120.4, 115.6, 110.3, 105.9, 56.8, 55.5.

N-(5-(8-Aminoquinolin-4-ylamino)isoquinolin-1-yl)-2,5-dimethylfuran-3-carboxamide (**2k**): Brown solid, yield 32%; MS *m/z*: 424 $[M+1]^+$; ^{1}H-NMR (DMSO-d_6) δ: 10.43 (s, 1H), 8.96 (s, 1H), 8.19 (d, J=5.0 Hz, 2H), 7.74–7.67 (m, 3H), 7.61 (d, J=8.3 Hz, 1H), 7.34 (brs, 1H), 7.26 (t, J=8.0 Hz, 1H), 6.86 (d, J=7.4 Hz, 1H), 6.68 (brs, 1H), 6.09 (d, J=5.0 Hz, 1H), 5.82 (s, 2H), 2.52 (s, 3H), 2.29 (s, 3H); ^{13}C-NMR (DMSO-d_6) δ: 165.8, 161.8, 159.0, 155.6, 153.9, 150.87, 148.1, 146.8, 142.3, 137.8, 135.0, 131.3, 128.7, 127.9, 125.7, 123.2, 120.5, 118.6, 117.7, 113.3, 110.0, 108.3, 107.9, 13.2, 11.6.

N-(5-(8-Aminoquinolin-4-ylamino)isoquinolin-1-yl)-thiazole-4-carboxamide (**2l**): Pale yellow solid, yield 17%; MS *m/z*: 413 $[M+1]^+$; ^{1}H-NMR (DMSO-d_6) δ: 10.89 (s, 1H), 9.33 (s, 1H), 8.99 (s, 1H), 8.59 (s, 1H), 8.34 (s, 1H), 8.21 (d, J=5.1 Hz, 1H), 7.97 (d, J=7.3 Hz, 1H), 7.77–7.72 (m, 2H), 7.69–7.66 (m, 1H), 7.62 (d, J=8.9 Hz, 1H), 7.27 (t, J=7.8 Hz, 1H), 6.87 (d, J=7.6 Hz, 1H), 6.13 (d, J=5.2 Hz, 1H), 5.82 (s, 2H); ^{13}C-NMR (DMSO-d_6) δ: 162.6, 155.4, 149.0, 146.5, 140.5, 135.2, 130.8, 129.9, 129.4, 128.9, 127.6, 127.1, 126.1, 126.0, 125.2, 124.9, 124.0, 123.8, 122.4, 120.6, 114.7, 108.7.

N-(5-(8-Aminoquinolin-4-ylamino)isoquinolin-1-yl)-1-(4-methoxyphenyl)-5-(trifluoromethyl)-1*H*-pyrazole-4-carboxamide (**2m**): Pale brown solid, yield 18%; MS *m/z*: 570 $[M+1]^+$; ^{1}H-NMR (DMSO-d_6) δ: 12.72 (s, 1H), 8.98 (s, 1H), 8.43 (s, 1H), 8.32 (s, 1H), 8.20 (d, J=5.1 Hz, 1H), 8.07–8.04 (m, 1H), 7.78 (d, J=7.9 Hz, 2H), 7.61 (d, J=8.2 Hz, 1H), 7.49 (d, J=8.9 Hz, 2H), 7.27 (t, J=7.8 Hz, 1H), 7.13 (d, J=8.9 Hz, 2H), 7.10–7.07 (m, 1H), 6.87 (d, J=7.5 Hz, 1H), 6.12 (d, J=5.1 Hz, 1H), 5.82 (s, 2H), 3.86 (s, 3H); ^{13}C-NMR (DMSO-d_6) δ: 167.3, 160.5, 155.1, 152.6, 150.0, 149.0, 146.1, 142.5, 138.4, 134.6, 130.3, 129.9, 129.5, 128.0, 127.7, 127.3, 126.9, 126.1, 125.2, 125.1, 124.8, 123.7, 123.7, 122.0, 120.6, 118.0, 115.6, 108.4, 56.2.

Evaluation of the Antiproliferative Activity A375P cells were purchased from American Type Culture Collection (ATCC, Rockville, MD, U.S.A.) and maintained in Dulbecco's modified Eagle's medium (DMEM, Welgene, Daegu, Korea) supplemented with 10% foetal bovine serum (FBS, Welgene, Daegu, Korea) and 1% penicillin/streptomycin (Welgene, Daegu, Korea) in a humidified atmosphere with 5% CO_2 at 37°C. A375P cells were taken from culture substrate with 0.05% trypsin–0.02% ethylenediaminetetraacetic acid (EDTA) and plated at a density of 5×10^3 cells/well in 96 well plates and then incubated at 37°C for 24 h in a humidified atmosphere with 5% CO_2 prior to treatment with various concentrations (3-fold serial dilution, 12 points) of test compounds. The cells were incubated for 48 h after treatment with the test compounds. The A357P cell viability was assessed by the conventional 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) reduction assay. MTT assays were carried out with CellTiter 96® (Promega) according to the manufacturer's instructions. The absorbance at 590 nm was recorded using EnVision 2103 (Perkin Elmer, Boston, MA, U.S.A.). The IC_{50} was calculated using GraphPad Prism 4.0 software.

Kinase Screening Reaction Biology Corp. Kinase HotSpot℠ service[28] was used for screening of compound **1d**, and IC_{50} Profiler Express for IC_{50} measurement. Assay protocol: In a final reaction volume of 25 μL, kinase (5–10 mU) is incubated with 25 mM Tris pH 7.5, 0.02 mM ethylene glycol-bis(2-aminoethylether)-*N*,*N*,*N*′,*N*′-tetraacetic acid (EGTA), 0.66 mg/mL myelin basic protein, 10 mM magnesium acetate and [γ^{33}P-ATP] (specific activity approx. 500 cpm/pmol, concentration as required). The reaction is initiated by the addition of the Mg-ATP mix. After incubation for 40 min at room temperature, the reaction is stopped by the addition of 5 μL of a 3% phosphoric acid solution. Ten microliter of the reaction is then spotted onto a P30 filtermat and washed three times for 5 min in 75 mM phosphoric acid and once in methanol prior to drying and scintillation counting.

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