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Design and synthesis of new 2-anilinoquinolines bearing *N*-methylpicolinamide moiety as potential antiproliferative agents

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Abstract

A series of new 2-anilinoquinolines **6a–o** possessing the substantial *N*-methylpicolinamide motif at C5 has been designed and synthesized as sorafenib analogues. The antiproliferative activities of the target compounds were preliminary appraised against a panel of 3 human cancer cell lines (MCF-7, SK-BR3 and HCT116), and a selected array was further tested over a panel of approximately 60 cancer cell lines at NCI at 10 μM concentration. Interestingly, compounds **6c**, **6d**, **6j**, **6k** and **6l** showed promising selective anticancer activities (growth inhibition > 80%) towards certain cancer cells at 10 μM testing dose. Compounds **6d** and **6j** were advanced to 5-dose testing mode to determine their GI_{50} values, and

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compared with our previously reported ureidoquinoline **B** and sorafenib as reference compounds. The 4-chloro-3-trifluoromethylaniline derivative **6j** manifested superior potency than both compound **B** and sorafenib over 11 and 8 cell lines, respectively. It showed GI_{50} values of 0.36 μM, 0.66 μM, 0.68 μM and 0.60 μM against the breast MDA-MB-468, renal A498 and melanoma SK-MEL-5 and UACC-62 cell lines, respectively. Moreover, both **6d** and **6j** exerted low cytotoxic effects against HFF-1 normal cell line. Furthermore, compounds **6d** and **6j** were tested against both B-Raf^{V600E} and C-Raf kinases, and displayed modest inhibitory activities, which were justified by molecular docking study. Compound **6j** could serve as a promising candidate for further development of potent anticancer chemotherapeutics.

Keywords

Antiproliferative activity; 2-Anilinoquinoline; *N*-methylpicolinamide; RAF kinase

Introduction

Cancer is still one of the main leading causes of mortalities all over the globe, and researches dedicated for the understanding of various molecular mechanisms involved in cancer and the development of new cancer treatments still constitute an urgent research issue. In the last few decades, great efforts have been made to circumvent the major problem of low selectivity of conventional anticancer drugs and ameliorate their potencies (1). Few years ago, a substantial shift in anticancer drug development has been noticed, where much attention have been paid towards the identification of new small molecules capable of targeting specific proteins, such as kinases whose activities are more specifically correlated with cancerous cells. A plenty of targeted anticancer agents are currently being developed in order to achieve better anticancer activity with fewer side effects (2).

Sorafenib (Nexavar®), a diaryl urea derivative, is the first reported RAF kinase inhibitor (3–5), that has been approved by FDA for treatment of advanced renal cell carcinoma (RCC) (6) and hepatocellular carcinoma (7). Besides RAF suppressing activity of sorafenib, it showed multikinase inhibitory effect over a number of oncogenic kinases, such as the proangiogenic vascular endothelial growth factor receptor-1 (VEGFR-1), VEGFR-2, VEGFR-3, and platelet-derived growth factor receptor-β (PDGFR- β) (8, 9), which account for its broad spectrum anticancer activity. Nevertheless, sorafenib still has certain drawbacks, like its poor physicochemical properties (10), weak therapeutic efficacy towards malignant melanoma (11), and accompanying toxicities (12, 13). Therefore, extensive structural modification have been carried out on sorafenib for both exploration and optimization purposes (14–20).

Among these efforts, Ramurthy et al. (15) conserved the picolinamide motif of sorafenib and changed its central ureidophenyl moiety into different bicyclic structures, exemplied by the anilinoquinazoline derivative **A** (Figure 1). Such modifications resulted in potent and selective RAF kinase inhibitors on the biochemical level, but devoid of cellular potency. On the other hand, our group has recently disclosed a series of *N*-methylpicolinamide based 2-ureidoquinolines (16), represented by compound **B** (Figure 1), which displayed broad spectrum anticancer activity in the cell based assay, as well as favorable RAF kinase inhibitory effects (potency and selectivity).

Accordingly, and with the purpose to further explore the SAR of those 2,5-disubstituted quinolines (16), a new series of 2-anilinoquinolines featuring the *N*-methylpicolinamide motif **6a–o** has been designed and synthesized (Figure 1). Our main objective in this study is to explore the impact of replacing the longer spacer, urea, in compound **B** with the shorter amine on the cellular anticancer activity. Moreover, various anilines were installed on C2 of quinoline to interrogate their influence on the antineoplastic activity (Figure 1). From another perspective, our target compounds **6a–o** could be considered as positional isomers to compound **A**, where the oxypicolinamide moiety is changed from C6 to C5 of quinoline. The anticancer activities of all final compounds were initially assessed over a panel of 3 human cancer cell lines (MCF-7, SK-BR3 and HCT116), and a selected group of 10 compounds was further examined against a panel of approximately 60 human cancer cell lines at NCI.

Materials and Methods

Chemistry

General

All solvents and reagents were commercially available and used without further purification. IR spectra (KBr disks) were recorded with a Bruker FT-IR instrument (Bruker Bioscience, Billerica, MA, USA). ^{1}H and ^{13}C NMR spectra were recorded on a Bruker Avance 300 or 400 MHz spectrometer, using appropriate deuterated solvents, as indicated. Chemical shifts (δ) are recorded in parts per million (ppm) upfield from tetramethylsilane (TMS) as internal standard, and s, d, t, and m are designated as singlet, doublet, triplet and multiplet, respectively. Coupling constants (*J*) are reported in hertz (Hz). Mass spectra were recorded on Waters Acquity UPLC/Synapt G2 QTOF MS mass spectrometer. The reaction progress was monitored on TLC plate (Merck, silica gel 60 F_{254}), and flash column chromatography was performed using silica gel (Merck, 230–400 mesh) and the indicated solvent system. Solvents and liquid reagents

were transferred using hypodermic syringes. Compounds **1**–**3** were synthesized following the literature method (16).

General procedure for synthesis of compounds 4a–o

A mixture of compound **3** (0.2 g, 1.03 mmol) and the appropriate aniline derivative (1.03–1.55 mmol) were fused at 160 °C for 5–60 min. The reaction mixture was cooled, and either dissolved in DCM and concentrated under reduced pressure to afford the desired product in pure form, or diluted with water and extracted with ethyl acetate (3×10 mL). The combined organic layers were washed with brine, dried over Na_2SO_4, filtered and concentrated under reduced pressure. The obtained residue was purified by silica gel chromatography using the proper eluent.

N-(3-Fluorophenyl)-5-methoxyquinolin-2-amine (4a)

The compound was purified by flash column chromatography using (Hexane:ethyl acetate, 4:1 v/v). Yield: 85%, ^{1}H NMR (300 MHz, $CDCl_3$) δ 11.17 (br. s, 1H), 8.59 (d, *J* = 9.6 Hz, 1H), 7.69 (t, *J* = 8.2 Hz, 1H), 7.50–7.41 (m, 2H), 7.19–7.06 (m, 4H), 6.89 (d, *J* =8.1 Hz, 1H), 4.02 (s, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 164.86, 161.56, 156.05, 152.77, 139.57, 137.17, 134.72, 131.49, 120.51, 114.99, 113.53, 112.07, 110.36, 108.07, 105.27, 56.20.

N-(3-Trifluoromethylphenyl)-5-methoxyquinolin-2-amine (4b)

The compound was purified by flash column chromatography using (Hexane:ethyl acetate, 4:1 v/v). Yield: 73%, ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.42 (d, *J* = 8.8 Hz, 1H), 8.09 (s, 1H), 7.84 (d, *J* = 8.0 Hz, 1H), 7.55 (t, *J* = 8.0 Hz, 1H), 7.47 (t, *J* = 8.8 Hz, 2H), 7.31 (t, *J* = 7.6 Hz, 1H), 6.89 (d, *J* = 8.8 Hz, 1H), 6.73 (d, *J* = 7.6 Hz, 1H), 4.01 (s, 3H).

N-(3-Chlorophenyl)-5-methoxyquinolin-2-amine (4c) (21)

Yield: 100%, ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.54 (d, *J* = 9.5 Hz, 1H), 7.67 (t, *J* = 8.3 Hz, 1H), 7.47–7.37 (m, 3H), 7.32–7.30 (m, 2H), 7.00 (d, *J* = 9.5 Hz, 1H), 6.84 (d, *J* = 8.1 Hz, 1H), 4.01 (s, 3H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 155.91, 152.82, 138.63, 138.50, 137.09, 135.34, 133.99, 130.88, 127.00, 124.03, 122.12, 113.82, 111.52, 108.64, 104.84, 56.11.

N-(3-(Tert-butyl)phenyl)-5-methoxyquinolin-2-amine (4d) (21)

The compound was purified by flash column chromatography using (0–3% MeOH in DCM). Yield: 91%, ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.38 (dd, *J* = 9.2, 0.8 Hz, 1H), 7.64 (t, *J* = 1.6 Hz, 1H), 7.52 (t, *J* = 8.4 Hz, 1H), 7.38–7.32 (m, 3H), 7.18 (dt, *J* = 7.4, 1.2 Hz, 2H), 7.01 (d, *J* = 9.2 Hz, 1H), 6.69 (dd, *J* = 8.0, 0.8 Hz, 1H), 4.00 (s, 3H), 1.39 (s, 9H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 155.50, 155.09, 152.46, 148.75, 139.92, 132.45, 129.79, 128.82, 120.32, 119.21, 118.25, 117.98, 115.86, 110.23, 101.81, 55.60, 34.80, 31.36.

N-(4-Isopropylphenyl)-5-methoxyquinolin-2-amine (4e) (21)

Yield: 100%, ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.30 (d, *J* = 9.1 Hz, 1H), 7.49–7.40 (m, 3H), 7.34 (d, *J* = 11.3 Hz, 1H), 7.22 (d, *J* = 8.4 Hz, 2H), 6.93 (d, *J* = 9.1 Hz, 1H), 6.78 (br. s, 1H), 6.63 (d, *J* = 7.8 Hz, 1H), 3.94 (s, 3H), 2.95–2.86 (m, 1H), 1.26 (d, *J* = 7.0 Hz, 6H). ^{13}C NMR (75 MHz, $CDCl_3$): δ 155.53, 155.19, 148.80, 144.13, 137.76, 132.46, 129.78, 127.19, 121.29, 119.10, 115.86, 109.93, 101.75, 55.60, 33.62, 24.13.

N-(4-Ethylphenyl)-5-methoxyquinolin-2-amine (4f)

The compound was purified by flash column chromatography using (hexane:ethyl acetate, 3:1 v/v). Yield: 95%, ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.31 (d, *J* = 9.0 Hz, 1H), 7.50–7.33 (m, 4H), 7.19 (d, *J* = 8.1 Hz, 2H), 6.93 (d, *J* = 9.0 Hz, 1H), 6.83 (br. s, 1H), 6.64 (d, *J* = 7.5 Hz, 1H), 3.95 (s, 3H), 2.65 (q, *J* = 7.5 Hz, 2H), 1.25 (t, *J* = 6.0 Hz, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 155.51, 155.15, 148.79, 139.53, 137.67, 132.47, 129.80, 128.65, 121.33, 119.11, 115.84, 109.88, 101.74, 55.61, 28.35, 15.77.

5-Methoxy-N-(4-phenoxyphenyl)quinolin-2-amine (4g)

The compound was purified by flash column chromatography using (ethyl acetate:methanol, 9:1 v/v). Yield: 71%, ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.25 (d, *J* = 9.0 Hz, 1H), 7.97 (br. s, 1H), 7.52–7.26 (m, 6H), 7.07–6.82 (m, 6H), 6.59 (d, *J* = 7.3 Hz, 1H), 3.88 (s, 3H); ^{13}C NMR (75 MHz, $CDCl_3$); δ 157.78, 155.55, 154.78, 153.00, 147.14, 135.32, 133.22, 130.39, 129.79, 123.03, 122.96, 121.16, 119.96, 118.43, 118.01, 117.27, 115.43, 110.23, 102.26, 55.66.

N-([1,1'-Biphenyl]-4-yl)-5-methoxyquinolin-2-amine (4h)

Yield: 100%, ^{1}H NMR (300 MHz, $CDCl_3$) δ 10.64 (br. s, 1H), 8.49 (d, *J* = 9.3 Hz, 1H), 7.67–7.57 (m, 4H), 7.46–7.38 (m, 7H), 7.03 (d, *J* =7.8 Hz, 1H), 6.82 (d, *J* =7.8 Hz, 1H), 3.99 (s, 3H); ^{13}C NMR (75 MHz, $CDCl_3$): δ 156.01, 153.02, 147.39, 146.10, 140.65, 139.71, 138.86, 137.70, 134.36, 128.98, 128.59, 127.83, 127.01, 125.17, 115.38, 113.42, 110.74, 108.50, 104.97, 56.13.

5-Methoxy-N-(4-morpholinophenyl)quinolin-2-amine (4i)

The compound was purified by flash column chromatography using (ethyl acetate:hexane, 0–35%). Yield: 60%, ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.27 (d, *J* = 9.1 Hz, 1H), 7.48–7.30 (m, 4H), 6.92 (d, *J* = 8.6 Hz, 3H), 6.84 (d, *J* = 9.1 Hz, 1H), 6.62 (d, *J* = 7.7 Hz, 1H), 3.94 (s, 3H), 3.86 (s, 4H), 3.12 (s, 4H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 155.77, 155.52, 148.85, 148.03, 132.71, 132.39, 129.77, 123.46, 118.99, 116.77, 115.68, 109.64, 101.58, 66.98, 55.59, 49.91.

N-(4-Chloro-3-(trifluoromethyl)phenyl)-5-methoxyquinolin-2-amine (4j) (21)

Yield: 97%, ^{1}H NMR (400 MHz, $CDCl_3$) δ 11.31 (br. s, 1H), 8.58 (d, *J*=9.6 Hz, 1H), 7.66 (t, *J* = 10.0 Hz, 2H), 7.58 (d, *J*=8.4 Hz, 1H), 7.51 (dd, *J*=8.4, 2.4 Hz, 1H), 7.39 (d, *J*=8.4 Hz, 1H), 6.97 (d, *J* = 9.6 Hz, 1H), 6.85 (d, *J*=8.4 Hz, 1H), 3.98 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 156.07, 152.56, 140.05, 137.23, 134.99, 134.23, 133.23, 131.05, 128.97, 123.89, 123.84, 120.67, 113.80, 110.41, 107.68, 105.45, 56.21.

N-(4-Chloro-3-fluorophenyl)-5-methoxyquinolin-2-amine (4k) (21)

Yield: 100%, ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.37 (d, *J* = 9.0 Hz, 1H), 7.94 (dd, *J* = 11.4, 2.1 Hz, 1H), 7.52 (t, *J* = 7.5 Hz, 1H), 7.43 (d, *J* = 8.1 Hz, 1H), 7.29–7.25 (m, 1H), 7.16 (d, *J* = 8.1 Hz, 1H), 6.83–6.68 (m, 3H), 3.97 (s, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 155.42, 153.43, 148.25, 140.55, 132.78, 130.37, 130.07, 119.58, 116.13, 115.36, 115.32, 111.06, 107.83, 107.48, 102.51, 55.66.

N-(3-Chloro-4-fluorophenyl)-5-methoxyquinolin-2-amine (4l)

Yield: 100%, ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.36 (d, *J* = 9.0 Hz, 1H), 7.87 (dd, *J* = 6.3, 2.1 Hz, 1H), 7.51 (t, *J* = 8.1 Hz, 1H), 7.44–7.37 (m, 2H), 7.11 (t, *J* = 8.7 Hz, 1H), 6.82 (d, *J* = 9.0 Hz, 1H), 6.70–6.50 (m, 2H), 3.97 (s, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 155.47, 153.97, 148.29, 137.07, 132.84, 130.05, 122.05, 119.74, 119.65, 119.35, 116.83, 116.54, 116.05, 110.49, 102.32, 55.65.

N-(4-Bromo-3-fluorophenyl)-5-methoxyquinolin-2-amine (4m)

The compound was purified by flash column chromatography using (ethyl acetate:hexane, 0–35%). Yield: 47%, ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.37 (d, *J* = 8.7 Hz, 1H), 7.92 (dd, *J* = 11.1, 2.4 Hz, 1H), 7.55–7.40 (m, 3H), 7.13 (dd, *J* = 8.4, 1.4 Hz, 1H), 6.83–6.68 (m, 3H), 3.97 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 155.42, 153.39, 148.23, 141.47, 133.16, 132.79, 130.08, 119.57, 116.14, 115.78, 111.11, 107.68, 107.31, 102.54, 100.30, 55.66.

N-(3,4-Difluorophenyl)-5-methoxyquinolin-2-amine (4n) (21)

Yield: 98%, ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.40 (d, *J* = 8.8 Hz, 1H), 7.85 (dq, *J* = 7.7, 2.4 Hz, 1H), 7.55 (t, *J* = 8.4 Hz, 1H), 7.44 (d, *J* = 8.4 Hz, 1H), 7.18–7.13 (m, 2H), 6.85 (d, *J* = 9.2 Hz, 1H), 6.73 (d, *J* = 7.6 Hz, 1H), 4.01 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 155.46, 153.81, 151.59, 147.58, 133.15, 130.30, 118.83, 117.38, 117.20, 115.84, 115.69, 110.50, 109.74, 109.53, 102.48, 55.66.

N-(3,4-Dimethylphenyl)-5-methoxyquinolin-2-amine (4o)

Yield: 100%, ^{1}H NMR (300 MHz, $CDCl_3$) δ 10.81 (br. s, 1H), 8.46 (d, *J* = 9.6 Hz, 1H), 7.63 (t, *J* = 8.3 Hz, 1H), 7.35 (d, *J* = 8.3 Hz, 1H), 7.20 (d, *J* = 7.8 Hz, 1H), 7.08–7.05 (m, 2H), 6.99 (d, *J* = 9.6 Hz, 1H), 6.86 (d, *J* = 8.1 Hz, 1H), 3.99 (s, 3H), 2.26 (s, 6H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 155.84, 152.85, 138.68, 138.52, 136.95, 136.46, 134.18, 132.26, 130.81, 125.83, 122.00, 112.90, 109.80, 108.41, 104.96, 56.12, 19.66, 19.27.

General procedure for synthesis of compounds 5a–o

To a stirred solution of the appropriate methoxyquinoline **4** (1.55 mmol) in anhydrous DCM (18 mL) at 0° C, 1M solution of BBr_3 in DCM (5.5 mL, 4.65 mmol) was added dropwise. After complete addition, the reaction mixture was gradually warmed to rt and stirred for 9 h. Water was added and the resulting solution was neutralized with saturated solution of $NaHCO_3$. The aqueous layer was extracted with ethyl acetate (3×50 mL), the organic layers were combined, washed with brine, dried over Na_2SO_4, filtered and concentrated under reduced pressure to afford the desired product, unless stated otherwise, in pure form.

N-(3-Fluorophenyl)-5-hydroxyquinolin-2-amine (5a)

Yield: 69%, ^{1}H NMR (400 MHz, Acetone-d_6) δ 9.10 (br. s, 1H), 8.38 (d, *J* = 9.0 Hz, 1H), 8.32 (dt, *J* = 12.4, 2.2 Hz, 1H), 7.55 (dd, *J* = 8.8, 1.2 Hz, 1H), 7.43 (t, *J* = 8.2 Hz, 1H), 7.33 (t, *J* = 8.0 Hz, 2H), 7.04 (d, *J* = 9.1 Hz, 1H), 6.81 (dd, *J* = 7.6, 0.7 Hz, 1H), 6.74 (dt, *J* = 8.3, 2.0 Hz, 1H).

N-(3-Trifluoromethylphenyl)-5-hydroxyquinolin-2-amine (5b)

The compound was purified by flash column chromatography (hexane:ethyl acetate, 3:1 then hexane:ethyl acetate, 1:2 v/v). Yield: 100%, ^{1}H NMR (300 MHz, Acetone-d_6) δ 9.01 (br. s, 2H), 8.75 (s, 1H), 8.38 (d, *J* = 9.0 Hz, 1H), 8.15 (d, *J* = 7.8 Hz, 1H), 7.54 (t, *J* = 7.8 Hz, 1H), 7.43 (t, *J* = 8.1 Hz, 1H), 7.33–7.27 (m, 2H), 7.03 (d, *J* = 9.0 Hz, 1H), 6.78 (dd, *J* = 7.5, 0.6 Hz, 1H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 154.18, 153.24, 148.47, 142.52, 132.04, 129.81, 129.36, 121.62, 118.30, 117.06, 115.06, 114.70, 112.11, 106.48.

N-(3-Chlorophenyl)-5-hydroxyquinolin-2-amine (5c) (21)

Yield; 93.6%; ^{1}H NMR (300 MHz, Acetone-d_6) δ 8.90 (br. s, 2H), 8.47 (t, *J* = 2.0 Hz, 1H), 8.38 (d, *J* = 9.0 Hz, 1H), 7.80 (dt, *J* = 8.2, 1.2 Hz, 1H), 7.46–7.28 (m, 3H), 7.02–6.97 (m, 2H), 6.79 (d, *J* = 7.5 Hz, 1H); ^{13}C NMR (75 MHz, $CDCl_3$): δ 154.21, 153.24, 148.57, 143.22, 133.78, 131.96, 129.85, 129.78, 120.64, 118.33, 118.12, 116.74, 115.04, 112.14, 106.45.

N-(3-(Tert-butyl)phenyl)-5-hydoxyquinolin-2-amine (5d) (21)

The compound was purified by flash column chromatography using (0–2% methanol in DCM). Yield: 48%; ^{1}H NMR (400 MHz, Acetone-d_6) δ 8.55 (br. s, 1H), 8.33 (dd, *J* = 9.0, 0.7 Hz, 1H), 8.21 (t, *J* = 2.0 Hz, 1H), 7.77 (dq, *J* = 8.0, 1.0 Hz, 1H), 7.39 (t, *J* = 7.7 Hz, 1H), 7.30–7.24 (m, 2H), 7.07 (dq, *J* = 7.8, 1.0 Hz, 1H), 7.01 (d, *J* = 9.0 Hz, 1H), 6.75 (dd, *J* = 7.6, 1.0 Hz, 1H), 1.40 (s, 9H); ^{13}C NMR (100 MHz, Acetone-d_6): δ 154.76, 153.19, 151.44, 148.99, 141.47, 131.46, 129.40, 128.08, 118.33, 118.25, 116.35, 116.12, 114.85, 111.89, 106.01, 34.41, 30.83.

N-(4-Isopropylphenyl)-5-hydroxyquinolin-2-amine (5e) (21)

Yield: 81%, ^{1}H NMR (300 MHz, Acetone-d_6) δ 8.43 (br. s, 1H), 8.15 (d, *J* = 9.1 Hz, 1H), 7.74 (d, *J* = 7.9 Hz, 2H), 7.20 (t, *J* = 7.5 Hz, 1H), 7.12 (d, *J* = 8.4 Hz, 1H), 7.04 (d, *J* = 7.9 Hz, 2H), 6.82 (d, *J* = 9.1 Hz, 1H), 6.56 (d, *J* = 7.5 Hz, 1H), 2.76–2.67 (m, 1H), 1.07 (d, *J* = 6.9 Hz, 6H); ^{13}C NMR (75 MHz, Acetone-

d_6): δ 154.78, 153.26, 149.02, 141.74, 139.45, 131.56, 129.51, 126.35, 119.12, 118.16, 114.77, 111.86, 105.92, 33.37, 23.66.

2-((4-Ethylphenyl)amino)quinolin-5-ol (5f)

The compound was purified by flash column chromatography (hexane-ethyl acetate, 3:1 v/v). Yield: 77%, ^{1}H NMR (300 MHz, Acetone-d_6) δ 8.76 (br. s, 2H), 8.38 (d, *J* = 9.0 Hz, 1H), 7.93 (d, *J* = 8.5 Hz, 2H), 7.43–7.32 (m, 2H), 7.17 (d, *J* = 8.4 Hz, 2H), 7.01 (d, *J* = 9.1 Hz, 2H), 6.76 (dd, *J* = 7.3, 1.1 Hz, 1H), 2.62 (q, *J* = 7.7 Hz, 2H), 1.24 (t, *J* = 7.6 Hz, 3H); ^{13}C NMR (75 MHz, Acetone-d_6) δ 154.86, 153.35, 149.05, 139.33, 137.25, 131.72, 129.62, 127.93, 119.33, 118.15, 114.87, 111.84, 106.03, 28.04, 15.53.

2-((4-Phenoxyphenyl)amino)quinolin-5-ol (5g)

The compound was purified by flash column chromatography (hexane-ethyl acetate, 3:1 v/v). Yield: 38%, ^{1}H NMR (300 MHz, Acetone-d_6) δ 8.80 (br. s, 2H), 8.35 (d, *J* = 9.0 Hz, 1H), 8.08 (d, *J* = 9.0 Hz, 2H), 7.41–7.29 (m, 4H), 7.10–6.98 (m, 6H), 6.75 (d, *J* = 7.5 Hz, 1H); ^{13}C NMR (75 MHz, Acetone-d_6) δ 158.51, 154.61, 153.25, 150.87, 148.88, 137.84, 131.69, 129.72, 129.59, 122.50, 120.34, 119.72, 118.21, 117.70, 114.84, 111.92, 106.06.

N-([1,1'-Biphenyl]-4-yl)-5-hydroxyquinolin-2-amine (5h)

The compound was purified by flash column chromatography (hexane-ethyl acetate, 1:2 v/v). Yield: 90.5%, ^{1}H NMR (400 MHz, Acetone-d_6) δ 8.88 (br. s, 2H), 8.36 (dd, *J* = 8.8, 0.4 Hz, 1H), 8.18 (dt, *J* = 8.8, 2.4 Hz, 2H), 7.70–7.65 (m, 4H), 7.48–7.40 (m, 3H), 7.36–7.30 (m, 2H), 7.04 (d, *J* = 8.8 Hz, 1H), 6.77 (dd, *J* = 7.2, 0.8 Hz, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 154.50, 153.23, 148.88, 141.29, 140.89, 133.60, 131.67, 129.60, 128.76, 126.94, 126.50, 126.26, 118.97, 118.31, 114.87, 112.15, 106.12.

5-Hydroxy-N-(4-morpholinophenyl)quinolin-2-amine (5i)

The compound was purified by flash column chromatography (hexane-ethyl acetate, 1:2 v/v). Yield: 67.3%, ^{1}H NMR (300 MHz, Acetone-d_6) δ 8.90 (br. s, 1H), 8.27 (d, *J* = 9.1 Hz, 1H), 7.89 (d, *J* = 9.0 Hz, 2H), 7.35 (t, *J* = 8.3 Hz, 1H), 7.23 (d, *J* = 8.3 Hz, 1H), 6.95 (t, *J* = 9.0 Hz, 3H), 6.70 (dd, *J* = 7.6, 0.8 Hz, 1H), 3.79 (t, *J* = 4.7 Hz, 4H), 3.09 (t, *J* = 4.8 Hz, 4H).

N-(4-Chloro-3-(trifluoromethyl)phenyl)-5- hydroxyquinolin -2-amine (5j) (21)

Yield: 99%, ^{1}H NMR (300 MHz, Acetone-d_6) δ 9.10 (br. s, 2H), 8.84 (s, 1H), 8.41(d, *J* = 8.9 Hz, 1H), 8.21 (d, *J* = 7.8 Hz, 1H), 7.54–7.32 (m, 3H), 7.03 (d, *J* = 9.0 Hz, 1H), 6.82 (d, *J* = 7.4 Hz, 1H); ^{13}C NMR (75 MHz, Acetone-d_6): δ 153.89, 153.30, 148.17, 141.06, 132.32, 131.57, 129.99, 125.16, 122.67, 121.91, 118.21, 117.28, 117.21, 115.19, 112.09, 106.76.

2-((4-Chloro-3-fluorophenyl)amino)quinolin-5-ol (5k) (21)

The compound was purified by flash column chromatography using (hexane:ethyl acetate, 3:1 v/v). Yield: 54%, ^{1}H NMR (400 MHz, Acetone-d_6) δ 9.02 (br. s, 2H), 8.57 (dd, *J* = 12.8, 2.4 Hz, 1H), 8.39 (dd, *J* = 8.8, 0.4 Hz, 1H), 7.58 (dq, *J* = 8.8, 0.8 Hz, 1H), 7.47–7.36 (m, 3H), 7.01 (d, *J* = 9.2 Hz, 1H), 6.81 (dd, *J* = 7.2, 1.2 Hz, 1H); ^{13}C NMR (100 MHz, Acetone-d_6) δ 153.98, 153.24, 148.42, 142.42, 142.31, 132.09, 129.96, 129.86, 118.35, 115.10, 115.08, 115.05, 112.13, 106.60, 106.32.

2-((3-Chloro-4-fluorophenyl)amino)quinolin-5-ol (5l)

The compound was purified by flash column chromatography (hexane:ethyl acetate, 3:1 v/v). Yield: 40%, ^{1}H NMR (400 MHz, Acetone-d_6) δ 8.92 (br. s, 2H), 8.57 (dd, *J* = 6.8, 2.4 Hz, 1H), 8.38 (dd, *J* = 8.8, 0.4 Hz, 1H), 7.82 (dq, *J* = 4.0, 2.4 Hz, 1H), 7.43 (t, *J* = 7.6 Hz, 1H), 7.34 (dt, *J* = 8.0, 0.8 Hz, 1H), 7.24 (t, *J* = 8.8 Hz, 1H), 6.98 (d, *J* = 8.8 Hz, 1H), 6.79 (dd, *J* = 7.6, 0.8 Hz, 1H); ^{13}C NMR (100 MHz, Acetone-d_6) δ 154.12, 153.26, 148.48, 138.95, 132.01, 129.80, 119.92, 118.44, 118.38, 118.24, 116.36, 116.14, 115.04, 111.99, 106.44.

N-(4-Bromo-3-fluorophenyl)-5-hydoxyquinolin-2-amine (5m)

Yield: 100%, ^{1}H NMR (300 MHz, Acetone-d_6) δ 9.05 (br. s, 1H), 8.55 (d, *J* = 11.2 Hz, 1H), 8.37 (d, *J* = 9.2 Hz, 1H), 7.54–7.52 (m, 3H), 7.43–7.33 (m, 2H), 7.02 (d, *J* = 9.0 Hz, 1H), 6.80 (d, *J* = 7.9 Hz, 1H).

2-((3,4-Difluorophenyl)amino)quinolin-5-ol (5n) (21)

The compound was purified by flash column chromatography (hexane:ethyl acetate, 3:1 v/v). Yield: 40%, ^{1}H NMR (400 MHz, Acetone-d_6) δ 8.95 (br. s, 2H), 8.54 (dq, *J* = 10.0, 2.4 Hz, 1H), 8.37 (dd, *J* = 8.8, 0.4 Hz, 1H), 7.55-7.51 (m, 1H), 7.43 (t, *J* = 8.0 Hz, 1H), 7.35 (d, *J* = 8.0 Hz, 1H), 7.25 (q, *J* = 8.8 Hz, 1H), 6.98 (d, *J* = 8.8 Hz, 1H), 6.79 (dd, *J* = 7.2, 0.8 Hz, 1H); ^{13}C NMR (100 MHz, Acetone-d_6) δ 154.14, 153.25, 148.49, 131.97, 129.79, 118.26, 116.86, 116.67, 115.02, 114.26, 114.24, 112.00, 107.52, 107.29, 106.43.

2-((3,4-Dimethylphenyl)amino)quinolin-5-ol (5o)

The compound was purified by flash column chromatography (hexane:ethyl acetate, 3:1 v/v). Yield: 55%, ^{1}H NMR (300 MHz, Acetone-d_6) δ 8.61 (br. s, 2H), 8.35 (d, *J* = 9.1 Hz, 1H), 7.77 (dd, *J* = 8.0, 1.9 Hz, 1H), 7.68 (d, *J* = 1.4 Hz, 1H), 7.41–7.29 (m, 2H), 7.09 (d, *J* = 8.1 Hz, 1H), 7.02 (d, *J* = 9.1 Hz, 1H), 6.75 (dd, *J* = 7.4, 0.9 Hz, 1H), 2.26 (s, 3H), 2.21 (s, 3H); ^{13}C NMR (75 MHz, Acetone-d_6) δ 154.89, 153.36, 148.71, 139.17, 136.42, 131.84, 129.70, 129.59, 120.84, 117.80, 117.11, 114.72, 111.74, 106.06, 19.32, 18.32.

General procedure for synthesis of compounds 6a–n

A suspension of the appropriate 5-hydroxyl anilinoquinoline **5** (0.185 mmol) and anhydrous K_2CO_3 (0.56 mmol) in DMF (2 mL) was stirred at r.t. for 30 minutes. Then, a solution of 4-(3-chloropropyl)morpholine (0.030 g, 0.185 mmol) or 4-(3-chloropropyl)methyl piperazine (0.063 g, 0.36 mmol) in DMF (1 mL) was added, followed by NaI (0.67 mmol). The reaction mixture was heated at 95 °C for 4–24 h, then cooled down to r.t. Water was added, and the aqueous layer was extracted with ethyl acetate (3×30 mL). The combined organic layers were washed with brine, dried over Na_2SO_4, filtered, concentrated under reduced pressure and the resultant residue was purified by flash column chromatography using the appropriate eluent.

4-((2-((3-Fluorophenyl)amino)quinolin-5-yl)oxy)-N-methylpicolinamide (6a)

The compound was purified by flash column chromatography using (0–35% ethyl acetate in hexane) and then crystallized from aq. MeOH to afford the entitled compound as pure yellowish white solid (11% yield); mp 265–266 °C, IR (KBr) *ν*/cm^{-1}: 3393, 3327 (2NH), 1668 (C=O), ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.83 (s, 1H), 8.80 (q, *J* = 4.8 Hz, 1H), 8.55 (d, *J* = 5.6 Hz, 1H), 8.22 (dd, *J* = 4.0, 12.5 Hz, 1H), 8.03 (d, *J* = 9.1 Hz, 1H), 7.75–7.69 (m, 2H), 7.56 (d, *J* = 7.8 Hz, 1H), 7.41–7.34 (m, 2H), 7.22–7.18 (m, 2H), 7.10 (d, *J* = 9.0 Hz, 1H), 6.81 (dt, *J* = 8.5, 2.5 Hz, 1H), 2.79 (d, *J* = 4.7 Hz, 3H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 166.41, 164.18, 154.88, 153.08, 151.07, 149.20, 148.83, 143.35, 143.24, 131.23, 130.60, 130.40, 125.07, 117.31, 115.55, 114.95, 114.81, 114.42, 109.36, 105.72, 105.46, 26.47, HRMS (ESI-TOF) *m/z* calcd for $C_{22}H_{16}FN_4O_2$ $[M-H]^+$: 387.1258, found: 387.1255.

N-Methyl-4-((2-((3-(trifluoromethyl)phenyl)amino)quinolin-5- yl)oxy picolin- amide (6b)

The compound was purified by flash column chromatography using (0–35% EA in Hex) to afford the entitled compound as pure yellowish white solid (20.2% yield); mp 235–236 °C, ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.43 (d, *J* = 5.6 Hz, 1H), 8.16 (s, 1H), 8.06–8.01 (m, 2H), 7.92 (d, *J* = 8.1 Hz, 1H), 7.79 (d, *J* = 2.4 Hz, 1H), 7.76 (d, *J* = 8.5 Hz, 1H), 7.60 (t, *J* = 7.8 Hz, 1H), 7.50 (t, *J* = 7.9 Hz, 1H), 7.35 (d, *J* = 7.7 Hz, 1H), 7.12 (br. s, 1H), 7.03 (dd, *J* = 7.6, 0.8 Hz, 1H), 7.00 (q, *J* = 2.6 Hz, 1H), 6.87 (d, *J* = 9.0 Hz, 1H), 3.05 (d, *J* = 5.1 Hz, 3H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 166.56, 164.48, 153.88, 152.50, 149.90, 148.91, 131.79, 129.82, 129.54, 125.09, 122.58, 119.24, 117.75, 116.35, 114.39, 113.85, 112.90, 110.33, 26.15.

4-((2-((3-Chlorophenyl)amino)quinolin-5-yl)oxy)-N-methylpicolinamide (6c)

The compound was purified by flash column chromatography using (0–1% MeOH in DCM) and then crystallized from aq. MeOH to afford the entitled compound as pure yellow solid (27.5% yield); mp 246–247 °C, IR (KBr) ν/cm^{-1}: 3360, 3323 (2NH), 1667 (C=O), ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.80 (s, 1H), 8.80 (q, *J* = 4.4 Hz, 1H), 8.55 (d, *J* = 5.6 Hz, 1H), 8.33 (t, *J* = 2.0 Hz, 1H), 8.03 (d, *J* = 8.8 Hz, 1H), 7.81 (dq, *J* = 8.0, 0.4 Hz, 1H), 7.72 (m, 2H), 7.42 (d, *J* = 2.4 Hz, 1H), 7.37 (t, *J* = 8.0 Hz, 1H), 7.22–7.18 (m, 2H), 7.10 (d, *J* = 9.2 Hz, 1H), 7.04 (ddd, *J* = 7.6, 0.8 Hz, 1H), 2.79 (d, *J* = 4.8 Hz, 3H), ^{13}C NMR (100 MHz, DMSO-d_6) δ 166.40, 164.17, 154.83, 153.09, 151.07, 149.22, 148.80, 142.99, 133.53, 131.28, 130.73, 130.43, 125.01, 121.34, 118.28, 117.40, 117.34, 115.55, 114.97, 114.42, 109.37, 26.47, HRMS (ESI-TOF) m/z calcd for $C_{22}H_{17}ClN_4O_2Na$ $[M+Na]^+$: 427.0938, found: 427.0937.

4-((2-((3-(Tert-butyl)phenyl)amino)quinolin-5-yl)oxy)-N-methylpicolinamide (6d)

The compound was purified by flash column chromatography using (0–50% EA in Hex) to afford the entitled compound as pure yellow solid (33% yield); mp 92–95 °C, IR (KBr) ν/cm−1: 3380, 3323 (2NH), 1668 (C=O), ^{1}H NMR (400 MHz, CDCl3) δ 8.41 (d, *J* = 5.5 Hz, 1H), 8.06 (q, *J* = 4.9 Hz, 1H), 7.99 (dd, *J* = 9.2, 0.5 Hz, 1H), 7.81 (d, *J* = 2.4 Hz, 1H), 7.71 (d, *J* = 8.5 Hz, 1H), 7.64 (t, *J* = 1.8 Hz, 1H), 7.59 (t, *J* = 7.7 Hz, 1H), 7.39 (dq, *J* = 8.0, 1.2 Hz, 1H), 7.33 (t, *J* = 7.7 Hz, 1H), 7.20 (dt, *J* = 7.7, 1.6 Hz, 1H), 7.00–6.96 (m, 3H), 3.04 (d, *J* = 5.1 Hz, 3H), 1.37 (s, 9H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 166.52, 164.48, 155.07, 152.60, 149.89, 149.41, 139.09, 131.92, 129.96, 128.91, 123.96, 121.03, 118.66, 118.46, 117.33, 113.87, 113.80, 112.17, 110.48, 34.81, 31.33, 26.16; HRMS (ESI-TOF) m/z calcd for $C_{26}H_{25}N_4O_2$ $[M-H]^+$: 425.1978, found: 425.1977.

4-((2-((4-Isopropylphenyl)amino)quinolin-5-yl)oxy)-N-methylpicolinamide (6e)

The compound was purified by flash column chromatography using (0–50% EA in hexane) to afford the entitled compound as pure yellow solid (36% yield); mp 165–166 °C, IR (KBr) ν/cm^{-1}: 3392, 3332

(2NH), 1672 (C=O), 1H NMR (400 MHz, $CDCl_3$) δ 8.40 (d, *J* = 5.6 Hz, 1H), 8.06 (q, *J* = 5.0 Hz, 1H), 7.94 (dd, *J* = 9.1, 0.6 Hz, 1H), 7.81 (d, *J* = 2.4 Hz, 1H), 7.69 (d, *J* = 8.5 Hz, 2H), 7.57 (t, *J* = 7.7 Hz, 1H), 7.50 (d, *J* = 8.5 Hz, 2H), 7.25 (d, *J* = 8.4 Hz, 2H), 7.14 (br. s, 1H), 6.98–6.93 (m, 3H), 3.03 (d, *J* = 5.1 Hz, 3H), 2.97–2.90 (m, 1H), 1.29 (d, *J* = 6.9 Hz, 6H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 166.62, 164.53, 155.31, 152.46, 149.86, 149.44, 149.33, 144.45, 137.32, 131.45, 129.61, 127.18, 124.57, 121.38, 117.47, 113.73, 113.67, 112.10, 110.47, 33.61, 26.16, 24.09.

4-((2-((4-Ethylphenyl)amino)quinolin-5-yl)oxy)-N-methylpicolinamide (6f)

The compound was purified by flash column chromatography (silica gel, hexane-ethyl acetate, 2:1 then 1:2 v/v) to afford the entitled compound as yellow solid (56% yield); mp 158–159 °C, IR (KBr) ν/cm^{-1}: 3394, 3336 (2NH), 1674 (C=O), ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.38 (d, *J* = 5.6 Hz, 1H), 8.09 (q, *J* = 5.0 Hz, 1H), 7.92 (d, *J* = 9.1 Hz, 1H), 7.82 (d, *J* = 2.5 Hz, 1H), 7.68 (d, *J* = 8.5 Hz, 1H), 7.56–7.49 (m, 3H), 7.33 (br. s, 1H), 7.21 (d, *J* = 8.4 Hz, 2H), 6.96–6.91 (m, 3H), 3.03 (d, *J* = 9.2 Hz, 1H), 2.80 (d, *J* = 5.1 Hz, 3H), 2.66 (q, *J* = 7.6 Hz, 2H), 1.27 (t, *J* = 7.6 Hz, 3H), ^{13}C NMR (100 MHz, $CDCl_3$) δ 166.62, 164.56, 155.37, 152.46, 149.87, 149.46, 149.31, 139.68, 137.37, 131.36, 129.56, 128.58, 124.58, 121.36, 117.43, 113.72, 113.61, 112.24, 110.47, 28.33, 26.18, 15.72.

N-Methyl-4-((2-((4-phenoxyphenyl)amino)quinolin-5-yl)oxy)picolinamide (6g)

The compound was purified by flash column chromatography (silica gel, hexane:ethyl acetate, 2:1 then 1:3 v/v) to afford the entitled compound as yellow solid (44% yield); mp 190–191 °C, IR (KBr) ν/cm^{-1}: 3383, 3334 (2NH), 1671 (C=O), ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.40 (d, *J* = 5.5 Hz, 1H), 8.06 (q, *J* = 5.0 Hz, 1H), 7.96 (dd, *J* = 9.0, 0.5 Hz, 1H), 7.81 (d, *J* = 2.4 Hz, 1H), 7.70 (d, *J* = 8.5 Hz, 1H), 7.63–7.55 (m, 3H), 7.39–7.34 (m, 2H), 7.14-7.10 (m, 2H), 7.08–7.04 (m, 4H), 6.99–6.96 (m, 2H), 6.88 (d, *J* = 9.0 Hz, 1H), 3.03 (d, *J* = 5.1 Hz, 3H), ^{13}C NMR (100 MHz, $CDCl_3$) δ 166.61, 164.53, 157.74, 155.11, 152.98, 152.47, 149.87, 149.34, 149.32, 135.38, 131.51, 129.73, 129.67, 124.65, 122.99, 122.96, 119.96, 118.39, 117.49, 113.80, 113.78, 112.27, 110.41, 26.17.

4-((2-([1,1'-Biphenyl]-4-ylamino)quinolin-5-yl)oxy)-N-methylpicolinamide (6h)

The compound was purified by flash column chromatography using (0–2% MeOH in DCM) and then crystallized from aq. MeOH to afford the entitled compound as pure light yellow solid (41% yield); mp 224–226 °C, IR (KBr) ν/cm^{-1}: 3397, 3330 (2NH), 1670 (C=O), ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.75 (s, 1H), 8.82 (q, *J* = 4.6 Hz, 1H), 8.56 (d, *J* = 5.6 Hz, 1H), 8.12 (d, *J* = 8.7 Hz, 2H), 8.00 (d, *J* = 9.1 Hz, 1H), 7.71–7.69 (m, 6H), 7.47 (t, *J* = 7.5 Hz, 2H), 7.42 (d, *J* = 2.5 Hz, 1H), 7.33 (t, *J* = 7.3 Hz, 1H), 7.22 (dd, *J* = 5.6, 2.6 Hz, 1H), 7.17 (dd, *J* = 6.9, 1.8 Hz, 1H), 7.12 (d, *J* = 9.2 Hz, 1H), 2.79 (d, *J* = 4.8 Hz,

3H), ^{13}C NMR (100 MHz, DMSO-d_6) δ 166.45, 164.17, 155.05, 153.07, 151.07, 149.18, 149.13, 141.03, 140.46, 133.48, 130.97, 130.30, 129.36, 127.34, 127.16, 126.53, 124.97, 119.42, 117.19, 115.64, 114.66, 114.42, 109.31, 26.48.

N-Methyl-4-((2-((4-morpholinophenyl)amino)quinolin-5-yl)oxy)picolinamide (6i)

The compound was purified by flash column chromatography using (0–2.5% MeOH in DCM) to afford the entitled compound as pure yellow solid (65% yield); mp 112–115 °C, IR (KBr) ν/cm^{-1}: 3318 (NH), 1668 (C=O), ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.37 (d, *J* = 5.4 Hz, 1H), 8.02 (d, *J* = 4.5 Hz, 1H), 7.94 (dd, *J* = 9.1, 0.6 Hz, 1H), 7.81 (d, *J* = 2.4 Hz, 1H), 7.69 (d, *J* = 8.5 Hz, 2H), 7.90 (d, *J* = 9.0 Hz, 1H), 7.76 (d, *J* = 2.1 Hz, 1H), 7.64 (d, *J* = 8.4 Hz, 1H), 7.54 (t, *J* = 7.5 Hz, 1H), 7.43–7.40 (m, 3H), 6.94–6.92 (m, 4H), 6.84 (d, *J* = 9.0 Hz, 1H), 3.87 (t, *J* = 4.5 Hz, 4H), 3.14 (t, *J* = 4.2 Hz, 4H), 3.00 (d, *J* = 4.8 Hz, 3H);^{13}C NMR (75 MHz, $CDCl_3$) δ 166.64, 164.53, 155.88, 152.45, 149.86, 149.49, 149.37, 148.32, 132.36, 132.09, 131.51, 129.68, 124.38, 123.66, 123.53, 117.39, 116.70, 113.56, 111.56, 110.43, 66.94, 49.81, 26.18.

4-((2-((4-Chloro-3-(trifluoromethyl)phenyl)amino)quinolin-5-yl)oxy)-N-methylpicolinamide (6j)

The compound was purified by flash column chromatography using (0–30% EA in Hex) to afford the entitled compound as pure yellow solid (43.2% yield); mp 220–223 °C, IR (KBr) ν/cm^{-1}: 3392, 3316 (2NH), 1665 (C=O), ^{1}H NMR(400 MHz, DMSO-d_6) δ 10.09 (s, 1H), 8.80 (q, *J* = 4.9 Hz, 1H), 8.76 (d, *J* = 2.6 Hz, 1H), 8.55 (d, *J* = 5.6 Hz, 1H), 8.22 (dd, *J* = 8.8, 2.5 Hz, 1H), 8.07 (d, *J* = 9.2 Hz, 1H), 7.76–7.67 (m, 3H), 7.42 (d, *J* = 2.6 Hz, 1H), 7.23–7.21(m, 2H), 7.11 (d, *J* = 9.2 Hz, 1H), 2.79 (d, *J* = 4.8 Hz, 3H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 166.37, 164.15, 154.57, 153.09, 151.07, 149.23, 148.53, 140.98, 132.34, 131.59, 130.61, 127.21, 126.90, 125.00, 123.40, 121.80, 117.50, 117.36, 115.50, 115.28, 114.43, 109.36, 26.47; HRMS (ESI-TOF) m/z calcd for $C_{23}H_{15}ClF_3N_4O_2$ $[M-H]^+$: 471.0836, found: 471.0832.

4-((2-((4-Chloro-3-fluorophenyl)amino)quinolin-5-yl)oxy)-N-methylpicolinamide (6k)

The compound was purified by flash column chromatography (silica gel, hexane-ethyl acetate, 1:1 then 2:1 v/v) to afford the entitled compound as yellow solid (32% yield); mp 278–280 °C, IR (KBr) ν/cm^{-1}: 3367, 3320 (2NH), 1665 (C=O); ^{1}H NMR (300 MHz, DMSO-d_6) δ 9.94 (br. s, 1H), 8.78 (d, *J* = 4.6 Hz, 1H), 8.53 (d, *J* = 5.6 Hz, 1H), 8.43 (d, *J* = 12.8 Hz, 1H), 8.04 (d, *J* = 9.0 Hz, 1H), 7.77–7.68 (m, 2H), 7.60–7.48 (m, 2H), 7.40 (br. s, 1H), 7.20 (d, *J* = 5.8 Hz, 2H), 7.09 (d, *J* = 9.0 Hz, 1H), 2.78 (d, *J* = 4.6 Hz, 3H); ^{13}C NMR (75 MHz, DMSO-d6) δ 166.38, 164.15, 155.93, 154.62, 153.09, 151.07, 149.19, 148.66, 142.19, 142.04, 131.44, 130.69, 130.49, 125.12, 117.43, 115.89, 115.51, 115.17, 114.43, 110.98, 110.75, 26.47; HRMS (ESI-TOF) m/z calcd for $C_{22}H_{16}ClFN_4O_2Na$ $[M+Na]^+$: 445.0844, found: 445.0841.

4-((2-((3-Chloro-4-fluorophenyl)amino)quinolin-5-yl)oxy)-N-methylpicolinamide (6l)

The compound was purified by flash column chromatography (silica gel, hexane:ethyl acetate, 1:1 then 2:1 v/v) to afford the entitled compound as yellow solid (33% yield); mp 259–260 °C, IR (KBr) ν/cm^{-1}: 3380, 3317 (2NH), 1667 (C=O) ^{1}H NMR (300 MHz, DMSO-d_6) δ 9.79 (br. s, 1H), 8.78 (d, *J* = 4.7 Hz, 1H), 8.54 (d, *J* = 5.4 Hz, 1H), 8.44 (d, *J* = 3.8 Hz, 1H), 8.01 (d, *J* = 9.3 Hz, 1H), 7.81–7.69 (m, 3H), 7.41 (t, *J* = 9.1 Hz, 2H), 7.19 (d, *J* = 4.1 Hz, 2H), 7.06 (d, *J* = 8.9 Hz, 1H), 2.78 (d, *J* = 4.4 Hz, 3H); ^{13}C NMR (75 MHz, DMSO-d_6) δ 166.40, 164.15, 154.71, 153.09, 151.07, 149.21, 148.73, 138.80, 131.32, 130.45, 124.95, 120.00, 119.52, 119.16, 119.07, 117.34, 117.10, 115.41, 114.95, 114.42, 109.33, 26.46.

4-((2-((4-Bromo-3-fluorophenyl)amino)quinolin-5-yl)oxy)-N-methylpicolinamide (6m)

The compound was purified by flash column chromatography using (0–50% EA in Hex) to afford the entitled compound as pure yellow solid (17.7% yield); mp 283–285 °C, ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.97 (s, 1H), 8.80 (q, *J* = 4.9 Hz, 1H), 8.55 (d, *J* = 5.6 Hz, 1H), 8.42 (dd, *J* = 12.2, 2.4 Hz, 1H), 8.05 (d, *J* = 9.1 Hz, 1H), 7.78–7.70 (m, 2H), 7.63 (t, *J* = 8.6 Hz, 1H), 7.54 (dd, *J* = 8.8, 2.3 Hz, 1H), 7.41 (d, *J* = 2.6 Hz, 1H), 7.23–7.21 (m, 2H), 7.10 (d, *J* = 9.2 Hz, 1H), 2.79 (d, *J* = 4.9 Hz, 3H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 166.40, 164.16, 154.64, 153.18, 151.17, 149.19, 148.59, 142.64, 133.48, 131.57, 130.51, 125.19, 117.24, 116.46, 116.39, 115.54, 115.00, 114.44, 109.35, 106.83, 106.04, 26.47, HRMS (ESI-TOF) m/z calcd for $C_{22}H_{15}BrFN_4O_2$ $[M-H]^+$: 465.0363, found: 465.0370.

4-((2-((3,4-Difluorophenyl)amino)quinolin-5-yl)oxy)-N-methylpicolinamide (6n)

The compound was purified by flash column chromatography (silica gel, hexane-ethyl acetate, 1:1 then 2:1 v/v) to afford the entitled compound as yellow solid (40% yield); mp 248–251 °C, IR (KBr) ν/cm−1: 3381, 3325 (2NH), 1667 (C=O); ^{1}H NMR (300 MHz, DMSO-d_6) δ 9.82 (br. s, 1H), 8.78 (d, *J* = 4.7 Hz, 1H), 8.54 (d, *J* = 5.6 Hz, 1H), 8.39 (dq, *J* = 7.4, 2.5 Hz, 1H), 8.00 (d, *J* = 9.1 Hz, 1H), 7.74–7.66 (m, 2H), 7.52 (br. s, 1H), 7.44–7.35 (m, 2H), 7.19 (dt, *J* = 8.6, 1.5 Hz, 2H), 7.06 (d, *J* = 9.2 Hz, 1H), 2.78 (d, *J* = 4.7 Hz, 3H); ^{13}C NMR (75 MHz, DMSO-d_6) δ 166.40, 164.16, 154.72, 153.09, 151.06, 149.19, 148.75, 138.76, 138.61, 131.29, 130.41, 125.02, 117.85, 117.61, 117.33, 115.41, 114.95, 114.42, 109.33, 107.80, 107.80, 107.50, 26.46. HRMS (ESI-TOF) m/z calcd for $C_{22}H_{16}F_2N_4O_2Na$ $[M+Na]^+$: 429.1139, found: 429.1142.

4-((2-((3,4-Dimethylphenyl)amino)quinolin-5-yl)oxy)-N-methylpicolinamide (6o)

The compound was purified by flash column chromatography (hexane-ethyl acetate, 2:1 then 1:2 v/v) to afford the entitled compound as yellow solid (52% yield); mp 207–208 °C, IR (KBr) ν/cm^{-1}: 3390, 3338

(2NH), 1672 (C=O), ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.41 (s,1H), 8.79 (q, *J* = 4.6 Hz, 1H), 8.54 (d, *J* = 5.6 Hz, 1H), 7.93 (d, *J* = 9.2 Hz, 1H), 7.77 (dd, *J* = 8.1, 2.0 Hz, 1H), 7.68–7.65 (m, 3H), 7.41 (d, *J* = 2.6 Hz, 1H), 7.20 (q, *J* = 2.6 Hz, 1H), 7.12–7.10 (m, 2H), 7.05 (d, *J* = 9.2 Hz, 1H), 2.80 (d, *J* = 4.8 Hz, 3H), 2.26 (s, 3H), 2.21 (s, 3H), ^{13}C NMR (100 MHz, DMSO-d_6) δ 166.46, 164.18, 155.30, 153.07, 151.02, 149.31, 149.19, 139.20, 136.57, 130.66, 130.09, 130.05, 129.65, 124.83, 120.65, 116.96, 116.90, 115.41, 114.38, 114.25, 109.32, 26.46, 20.27, 19.21, HRMS (ESI-TOF) m/z calcd for $C_{24}H_{22}N_4O_2Na$ $[M+Na]^+$: 421.1641, found: 421.1634.

Preliminary anticancer screening over 3 human cancer cell lines

The antineoplastic activity of the target compounds was examined over 3 human cancer cell lines using the (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) tetrazolium (MTT) assay. HCT-116 (Human colorectal carcinoma), MCF-7 (breast cancer cells) and SK-BR3 (breast cancer cells) were supplied from the Korea Cell Line Bank (KCLB). All cell lines were grown in RPMI 1640/DMEM (Gibco BRL) supplemented with 10% (v/v) heat inactivated Fetal Bovine Serum (FBS) and maintained at 37 °C in a humidified atmosphere with 5% CO_2. The cells (5 × 104 cells/mL) were seeded into 96-well plate. Various concentrations of samples were added to each well in duplicate, then incubated at 37 °C with 5% CO_2 for 2 days such that time cells are in the exponential phase of growth at the time of compound addition. Add 15 μL of the Dye Solution (Promrga, Cell Titer 96) to each well. Incubate the plate at 37 °C for up to 4 h in a humidified, 5 % CO_2 atmosphere. After incubation, add 100 μL of the solubilization Solution/Stop Mix (Promrga, Cell Titer 96) to each well. Allow the plate to stand overnight in a sealed container with a humidified atmosphere at room temperature to completely solubilize the formazan crystals. The optical density was measured using a microplate reader (Versamax, Molecular Devices) with a 570 nm wavelength.

NCI anticancer screening over 60 human cancer cell lines

The cancer cell screening over full panel of 60 human cancer cell lines was applied at the National Cancer Institute (NCI), Bethesda, Maryland, USA (22) following the standard procedure (23).

In vitro cytotoxicity evaluation against HFF-1 cell line

It was conducted using MTT assay following the same protocol described above.

In vitro enzyme assay for compounds 6d and 6j

Reaction Biology Corporation Kinase HotSpot[SM] service (24) was used for screening of compounds **6d** and **6j**. The assay protocol is briefly described as following: 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 tetracetic acid (EGTA), 0.66 mg/mL myelin basic protein, 10 μM 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. 10 μL of the reaction solution 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.

Molecular docking

The docking study was performed using the X-ray co-crystal structure of B-Raf^{V600E}, in its DFG-out conformation, with sorafenib (PDB: 1UWJ) (25) and C-Raf homology model. For docking study with DFG-out conformation of C-Raf (amino acid code in Uniprot: P04049) , the homology model of C-Raf copying with sorafenib was generated from a B-RAFV600E X-ray crystal structure using Modeller module implemented in Discovery Studio program. The homology model was selected with low PDF energy, and the proteins and ligands were respectively prepared using Protein Prepare Wizard and Ligprep module in Maestro 9.7 (Schrödinger, LLC, New York). After adding hydrogens, the protein was neutralized and then optimized with energy minimization for hydrogens only. The ligand was prepared through protonation at pH value of 7.4 and energy minimization. Gold suite ver. 5.2 (CCDC, Cambridge, UK) with gold score was employed to predict the binding mode of the ligand into B-Raf^{V600E} and C-Raf. The poses with the highest gold score were selected as the best docking poses and used for analysis of binding mode.

Results and Discussion

Synthesis

Synthesis of the key intermediate 3

The synthetic pathway for preparation of compound **3** was reported and discussed in details (Scheme 1) (16).

Synthesis of the new intermediates 4a–o and 5a–o and the target compounds 6a–o

As depicted in Scheme 2, the introduction of aniline motif at C2 of quinoline was the first step for the preparation of the target compounds **6a–o**. Nucleophilic substitution of 2-chloro-5-methoxyquinoline (**3**) with various substituted anilines was efficiently achieved under neat conditions at 160 °C for 5–60 minutes to afford the 5-methoxyanilinoquinolines **4a–o** in moderate to excellent yield (47–100%) (21). Demethylation of the produced methoxy derivatives **4a–o** with boron tribromide in DCM at 0 °C furnished the corresponding hydroxyl derivatives **5a–o** (21). The installation of the second functionality, *N*-methylpicolinamide, at C5 of quinoline skeleton was accomplished through *O*-arylation of the hydroxyquinolines **5a–o** with 4-chloro-*N*-methylpicolinamide utilizing cesium carbonate as a base in DMSO at 135 °C for 4 hours.

Biological evaluation

In vitro anticancer activity evaluation of the target compounds against 3 human cancer cell lines

All final compounds were subjected to preliminary assessment for their antiproliferative activity against three cancer cell lines, MCF-7 & SK-BR3 (breast cancer cells) and HCT-116 (Human colorectal carcinoma) by MTT assay method, using sorafenib as a reference compound. The compounds were tested at two concentrations of 100 μM & 10 μM, and the percentage of growth inhibition (GI) was recorded as listed in Table 1. The results revealed that all compounds showed comparable (slightly inferior or superior) anticancer activity at 100 μM. Of special interest, compounds **6d** and **6j,** that displayed high GI values against all cell lines (82.77–86.71 and 76.25–90.81 %, respectively), similar to sorafenib.

Antiproliferative activity of the target compounds over NCI-60 cell line panel

The primary screening demonstrated the anticancer potential of our compounds that is worthy to be broadly scouted. Therefore, their structures were submitted to National Cancer Institute (NCI) (22), and 10 compounds were selected on the basis of degree of structural variation and computer modeling techniques for assessment of their *in vitro* antineoplastic activity against a panel of 60 cancer cell lines representing 9 different tissues (blood, lung, colon, CNS, skin, ovary, kidney, prostate and breast). The compounds were tested at a single dose concentration of 10 μM, and the mean percentages of growth (%G), GI, range of growth, as well as number of responsive cells were determined as shown in Table 2.

Based on the overall anticancer activity parameter, mean GI%, the potencies of the tested compounds could be generally ranked in the following order; 3,4-disubstituted anilines compounds, **6j–l** (41.1–44.9%) > 3-substituted anilines, **6c** and **6d** (36.7–37.7%) > 4-substituted anilines, **6e**–**h** (19.2–31.3%). The only exception to this order is the 3,4-difluoroaniline derivative, **6n,** that showed mean GI value of 32.3%. In terms of the positive cytostatic activity parameter, it is noticed that all of the tested compounds possess selective cytostatic anticancer activities (blocking tumor cell proliferation) towards certain cell lines, in contrast to the known broad cytotoxic effects (killing of cancer cell) of sorafenib. For example, compound **6f** demonstrated remarkable GI (>50%) against 2 cell lines out of 59, while compound **6k** showed strong growth inhibitory effects over 17 cell lines out of 58.

Regarding the anticancer activity towards individual cell lines (Table 3), the prostate PC-3 cell line was found to be highly susceptible (%G < 40%) to most of the tested compounds. Concerning the 3-substituted anilines **6c** and **6d** showed selective potency against leukemia cancer cell MOLT-4 with GI values of 67.57 and 65.56%, respectively. Meanwhile, colon cancer cell HT29 proved to be selectively sensitive to **6d** (GI = 80.3%). Moreover, compound **6c**, with chlorine moiety, exerted significant activity towards the colon cancer cells HCT-15 and SW-620, melanoma cells SK-MEL-5 and UACC-62, and breast cancer cell MDA-MB-468 with GI values of 71.32, 79.25, 80.48, 75.30 and 82.61 %, respectively. On the other hand, the 4-substituted anilines, **6e**–**h,** displayed weak to moderate inhibitory activity against most of the cell lines. It seems that the variation of substituents at 4-position of aniline has not shown remarkable change in the antitumor activity over the individual cell lines. In respect to the 3,4-disubstitiuted anilines, compounds **6j** with 4-chloro-3-trifluoromethylphenyl, and **6l** with 3-chloro-4-fluorophenyl exerted selective potency against the leukemia K-562, melanoma SK-MEL-5, prostate PC-3 and breast MDA-MB-468 cell lines, with GI values of 69.32–74.00, 87.69–90.31, 69.22–84.70 and 89.47–93.49 %, respectively. It is interesting to find that the reversal of substituents of compound **6l**, as in its positional isomer **6k** led to noticeable shift of the anticancer activity toward different cell lines. Compound **6k** exerted lethal effect (GI = 111.72%) against the CNS cancer cell SNB-75, beside its pronounced inhibitory effect (GI values of 90.52, 98.64, 91.89 and 91.81%) over the lung cancer HOP-92, melanoma MALME-3M, ovarian OVCAR-4 and renal RXF-393 cell lines, respectively. The full growth inhibitory activities of both **6d** and **6j** over 58 cell lines is illustrated in Figure 2.

After this initial single dose screening of the 10 selected compounds, compounds **6d** and **6j** that satisfied the threshold inhibition criteria of NCI were further tested in a five-dose testing mode to determine their GI_{50} values (the molar concentration causing half-maximal growth inhibition). The GI_{50} values of **6d** and **6j** in comparison to both compound **B** (16) and sorafenib (26) are showed in Table 4.

As shown in Table 4, most of the GI_{50} data for both **6d** and **6j** were less than 10 μM. In comparison to sorafenib, it was found that compound **6d** is less potent than sorafenib over all cell lines. On the contrary, compound **6j** (with 4-chloro-3-trifluoromethylphenyl moiety) displayed superior anticancer potency than both compound **B** and sorafenib over 11 and 8 cell lines, respectively, with GI_{50} values of 0.356–1.81μM (Figure 3). Among those sensitive cell lines are the melanoma cells SK-MEL-5, UACC-257 and UACC-62, in which the mutant B-Raf^{V600E} is overexpressed (27, 28). Towards them, compound **6j** showed GI_{50} values of 0.678 μM, 1.71 μM and 0.6 μM, respectively. Such interesting finding was an apparent indication that **6j** may possess B-Raf^{V600E} inhibitory effect. Moreover, compound **6j** displayed potent antiproliferative activity against the breast cancer cell MDA-MB-468 with GI_{50} value of 0.356 μM, being 10 and 6 times more potent than both **B** and sorafenib, respectively. In respect to the other cell lines, both **6d** and **6j** seemed to be less potent than compound **B** and sorafenib. From these results, we can conclude that either structural rigidification of sorafenib or spacer change of compound **B** from urea to amine resulted in generation of selective anticancer agents, which target only certain cell lines with better potency.

Evaluation of cytotoxicity against the HFF-1 normal cell

To get some insights about the differential cytotoxicity of this new set of compounds, the most active members **6d** and **6j** have been tested against the human foreskin fibroblast (HFF-1) normal cell line using the (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) tetrazolium (MTT) assay (Table 5). Interestingly, both compounds **6d** and **6j** showed low growth inhibitory effects as indicated by their high GI_{50} values (> 10.0 μM), which point out their selective growth inhibitory activities towards human cancer cells rather normal cell lines.

In vitro kinase inhibitory activity of compounds 6d and 6j

In order to explore the impact of our structural modifications on the RAF kinase inhibitory activity, compounds **6d** and **6j** were tested against both B-Raf^{V600E} and C-RAF kinases (Table 6). Unfortunately, both **6d** and **6j** showed modest inhibitory activity. Upon comparison with the ureidoquinoline **B** (16), we can conclude that replacement of the urea linker with amine has a negative impact on RAF kinase inhibition. Such findings reflect the significance of urea motif at C2 position of quinoline scaffold in terms of RAF kinase activity.

Moreover, kinase screening of both compounds **6d** and **6j** over a number of receptor and non-receptor protein kinases was performed (Table 7). The results revealed that **6d** and **6j** do not possess considerable inhibitory effects against the tested kinases. Relatively, compound **6j** exhibited a moderate inhibitory activity against Pim3 kinase with 38.9% inhibition at 10 μM. These biochemical assay results denote that the selective cellular potency of our anilinoquinolines is not due to protein kinase inhibition, which suggest the existence of other leading mechanism(s) responsible for this antineoplastic effect. Accordingly, further screening of compound **6j** over a broad array of potential molecular targets is currently under processing.

Molecular docking study

In attempt to warrant the modest RAF kinase inhibitory activities of this type of compounds, represented by compound **6j**, a molecular docking study was performed. The anilinoquinoline **6j** as well as its ureidoquinoline homolog **B** were docked in the catalytic kinase domain of B-Raf^{V600E} (PDB accession code 1UWJ) (24), and C-Raf homology model. As shown in Figure 4, both compounds **6j** and **B** could bind with the hinge region residue Cys532 (B-Raf^{V600E})/Cys424 (C-Raf) by two hydrogen bonds *via* their picolinamide moiety. Also, their quinoline scaffold was engaged in multiple hydrophobic and electrostatic interactions with various amino acid residues (like Lys483 and Thr529 (B-Raf^{V600E})) in the gatekeeper region. However, within the DFG loop, only the ureidoquinoline **B** was able to bind with the backbone NH of Asp594 (B-Raf^{V600E})/Asp486 (C-Raf) *via* its carbonyl oxygen of urea, while compound **6j** missed this key interaction. Also, an additional difference between the binding modes of compounds **6j** and **B** was observed in the allosteric site of RAF kinase. The urea spacer of compound **B** enabled its terminal 4-chloro-3-trifluoromethylphenyl group to be deeply inserted within the hydrophobic allosteric site adjacent to the ATP binding site. Such hydrophobic interactions were lost in case of the anilinoquinoline derivative **6j**, mainly because of its short amine spacer. The aforementioned observations could provide some insights about the unpretentious RAF kinase inhibitory effects of compound **6j**, as an example for this new series of 2-anilinoquinolines.

Conclusion

In conclusion, a series of novel 2-anilinoquinolines possessing 5-oxypicolinamide moiety has been designed, synthesized, and evaluated for its anticancer activity over a panel of 60 cancer cell lines. All of the tested anilinoquinolines displayed selective antineoplastic activities against a number of cancer cells. Compound **6j** proved to be the most active member in this series with superior potency than its corresponding ureidoquinoline **B** and sorafenib across certain cell lines, with submicromolar or single

digit micro-molar GI_{50} values. In addition, **6j** showed low cytotoxic activity against the HFF-1 normal cell line. The modest RAF inhibitory activity of these derivatives suggest the presence of other underlying mechanism(s) responsible for their anticancer activities. Accordingly, further screening of compound **6j** over a large group of prospective molecular targets is currently in progress.

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Supplementary data

Supplementary data (^{1}H NMR, ^{13}C NMR, and HRMS data for representative compounds and NCI cell based assays) associated with this article was submitted.

Conflict of interest

The authors declare no conflict of interest.

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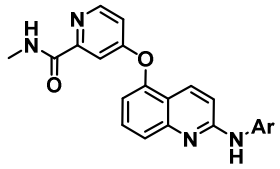
Table 1. Preliminary antiproliferative activity of the target compounds against a panel of 3 human cancer cell lines.[a]



Compound No.	Ar	% Growth inhibition					
		HCT116		MCF-7		SK-BR-3	
		100 μM	10 μM	100 μM	10 μM	100 μM	10 μM
6a	3-F-C_6H_4	**71.30**	60.28	48.71	17.17	**66.61**	12.66
6b	3-CF_3-C_6H_4-	**73.20**	20.17	33.05	39.08	58.31	45.58
6c	3-Cl-C_6H_4-	**69.04**	30.51	52.38	21.06	**70.63**	17.56
6d	3-$(CH_3)_3$C-C_6H_4-	**85.58**	8.83	**82.77**	-2.02	**86.71**	6.78
6e	4-$(CH_3)_2$CH-C_6H_4-	**79.33**	-16.00	**83.74**	11.91	**83.68**	12.05
6f	4-Et-C_6H_4-	**67.55**	29.08	60.27	13.85	**66.99**	5.20
6g	4-OPh-C_6H_4-	64.39	1.03	55.30	10.21	**65.51**	21.58
6h	4-Ph-C_6H_4-	53.64	15.65	50.03	10.58	46.63	16.66
6i	4-Morpholin-C_6H_4-	**69.55**	-5.49	57.94	7.96	**65.77**	12.72
6j	4-Cl-3-CF_3-C_6H_3-	**90.81**	57.52	**77.45**	24.85	**76.25**	24.53
6k	4-Cl-3-F-C_6H_3-	55.91	22.31	46.08	34.04	64.87	56.38
6l	3-Cl-4-F-C_6H_3-	**68.33**	25.18	58.54	50.08	**79.33**	**69.53**
6m	4-Br-3-F-C_6H_3-	**75.67**	52.84	37.37	33.18	59.48	35.94
6n	3,4-F_2-C_6H_3-	**71.61**	30.74	56.09	49.53	**68.98**	61.85
6o	3,4-$(CH_3)_2$-C_6H_3-	26.48	13.85	**69.16**	17.69	**72.68**	24.43
Sorafenib		**97.32**	48.41	**96.06**	40.45	**93.12**	48.87

[a] Compounds were tested in duplicate mode at two concentrations of 100 μM and 10 μM.

Table 2. Overview of the preliminary anticancer assay at single dose concentration of 10 μM.

Compound No.	NSC code	60 cell lines assay in single dose (10 μM)				
		Mean growth %	Mean Growth inhibition %	Range of growth %	The most sensitive cell line	Positive cytostatic effect[a]
6c	**778307**	63.3	36.7	17.4 to 97.7	MDA-MB-468 (breast)	16/57
6d	**776931**	62.3	37.7	19.7 to 100.7	HT29 (colon)	18/58
6e	**776933**	80.8	19.2	31.5 to 122.3	PC-3 (prostate)	3/58
6f	**780447**	79.8	20.2	37.7 to 102.9	SR (leukemia)	2/59
6g	**780448**	68.7	31.3	30.2 to 99.9	SR (leukemia)	9/59
6h	**776934**	75.6	24.4	32.5 to 104.9	PC-3 (prostate)	4/58
6j	**776932**	57.7	42.3	9.69 to 93.7	SK-MEL-5 (melanoma)	20/58
6k	**780446**	58.9	41.1	−11.7 to 99.5	SNB-75 (CNS)	17/59
6l	**782196**	55.1	44.9	6.51 to 97.0	MDA-MB-468 (breast)	23/58
6n	**782194**	67.7	32.3	29.4 to 98.5	OVCAR-4 (ovarian)	11/60

[a] The ratio between number of cell lines with percent growth from 0 to 50 and total number of cell lines.

Table 3. The growth inhibition percentages of the tested compounds over the most sensitive cell lines at 10 μM concentration.[a,b]

		% Growth inhibition									
Cancer type	**Cell line/ NSC code**	**6c 778307**	**6d 776931**	**6e 776933**	**6f 780447**	**6g 780448**	**6h 776934**	**6j 776932**	**6k 780446**	**6l 782196**	**6n 782194**
Leukemia	K-562	57.16	**73.24**	57.37	39.95	59.02	51.15	**69.32**	16.24	**74.00**	50.73
	MOLT-4	**67.57**	**65.56**	46.80	49.08	57.33	42.85	**66.34**	30.79	NT	47.80
	SR	**67.56**	**60.25**	57.60	**62.28**	**69.77**	55.30	**66.04**	37.79	NT	37.50
Lung Cancer	HOP-92	14.87	51.66	19.36	–	28.76	28.54	25.61	**90.52**	22.12	25.72
	NCI-H522	22.33	40.85	23.57	32.18	37.33	40.25	55.37	47.32	**60.04**	39.01
Colon Cancer	HCT-116	**67.38**	**61.88**	30.74	32.5	52.0	36.09	**63.75**	54.61	**82.44**	64.49
	HCT-15	**71.32**	50.70	41.21	48.06	45.3	31.28	49.21	40.68	**83.55**	**68.47**
	HT29	36.54	**80.30**	37.07	27.64	37.51	17.98	35.08	17.47	18.63	37.80
	SW-620	**79.25**	37.81	–	–	12.22	3.33	52.69	18.02	**79.67**	42.49
CNS Cancer	SNB-75	15.33	15.49	20.80	25.6	32.19	21.58	38.16	**111.72**	12.44	56.76
	U251	**61.64**	48.75	33.26	27.95	34.42	33.63	51.81	46.74	**73.25**	55.79
Melanoma	MALME-3M	54.87	19.35	10.05	11.26	21.77	13.65	45.69	**98.64**	59.75	37.98
	SK-MEL-5	**80.48**	52.66	21.26	37.63	**63.74**	29.02	**90.31**	55.24	**87.69**	48.06

	UACC-62	**75.30**	36.83	37.85	39.72	37.55	46.68	**64.88**	47.69	**74.73**	56.19
Ovarian Cancer	OVCAR-4	NT	39.83	17.26	25.26	47.94	29.07	51.61	**91.89**	59.90	**70.65**
Renal Cancer	RXF 393	27.48	15.49	–	10.01	–	–	22.19	**91.81**	24.74	17.32
	UO-31	18.51	57.82	22.60	23.52	42.07	39.38	38.60	18.38	31.31	17.32
Prostate Cancer	PC-3	**61.05**	**76.37**	**68.53**	55.84	**64.59**	**67.54**	**84.70**	33.91	**69.22**	**62.65**
Breast Cancer	T-47D	25.26	54.66	21.26	38.95	56.72	42.78	41.71	48.68	34.04	19.92
	MDA-MB-468	**82.61**	55.11	27.17	23.44	36.33	33.09	**89.47**	48.72	**93.49**	32.41

[a] Bold figures indicate GI > 60%.
[b] –; GI < 10%, NT; not tested.

Table 4. GI_{50} values (μM) of compounds **6d**, **6j**, **B** and sorafenib over NCI-60 cell line panel.[a,b]

Cell lines	GI_{50} value (μM)				Cell lines	GI_{50} value (μM)			
	6d	**6j**	**B**	**Sorafenib**		**6d**	**6j**	**B**	**Sorafenib**
Leukemia					**Melanoma**				
CCRF-CEM	3.44	4.44	>100	2.0	M14	3.24	6.46	5.00	2.0
HL-60(TB)	6.04	50.8	NT	1.58	MDA-MB-435	4.60	3.94	3.12	1.58
K-562	3.97	6.28	>100	3.16	SK-MEL-2	NT	NT	7.18	1.58
MOLT-4	3.33	**1.74**	>100	3.16	SK-MEL-28	NT	NT	4.59	2.0
RPMI-8226	3.86	5.32	NT	1.58	SK-MEL-5	3.51	**0.678**	2.62	1.58
SR	3.80	3.97	3.22	3.16	UACC-257	4.39	**1.71**	>100	2.0
Non-Small Cell Lung Cancer					UACC-62	3.93	**0.60**	1.97	2.32
A549/ATCC	5.00	6.59	2.83	3.16	**Ovarian Cancer**				
EKVX	NT	NT	NT	2.51	IGROV1	12.8	17.9	12.3	2.51
HOP-62	30.7	13.9	2.76	2.0	OVCAR-3	6.26	6.70	2.84	3.16
HOP-92	2.45	3.81	1.51	1.58	OVCAR-4	7.70	3.39	1.17	3.16
NCI-H226	7.58	**1.22**	3.73	2.0	OVCAR-5	NT	NT	3.85	3.16
NCI-H23	7.07	6.66	4.81	2.0	OVCAR-8	8.28	14.6	3.47	2.51
NCI-H322M	> 100	23.7	3.91	2.51	NCI/ADR-RES	5.98	10.6	3.88	2.51
NCI-H460	4.01	5.36	2.79	2.51	SK-OV-3	49.1	19.1	3.78	2.51
NCI-H522	9.84	6.04	14.7	2.0	**Renal Cancer**				
Colon Cancer					786-0	3.80	11.6	1.89	3.16
COLO 205	NT	NT	4.36	2.0	A498	6.17	**0.66**	1.36	2.51
HCC-2998	NT	NT	>100	3.16	ACHN	NT	NT	1.66	3.16
HCT-116	3.17	1.81	2.28	1.58	CAKI-1	5.85	16.8	4.28	3.16

HCT-15	3.04	6.47	NT	2.51	RXF 393	4.94	7.13	1.89	3.16
HT29	3.26	8.54	2.04	2.0	SN12C	4.66	18.9	2.62	2.51
KM12	NT	NT	9.94	1.58	TK-10	16.9	12.6	2.17	3.98
SW-620	3.86	3.02	NT	2.51	UO-31	3.46	25.4	2.62	2.51
CNS Cancer					**Prostate Cancer**				
SF-268	12.8	14.7	3.84	2.51	PC-3	3.05	**1.22**	57.7	2.0
SF-295	3.90	8.66	2.16	1.58	DU-145	14.7	15.7	4.74	3.16
SF-539	8.35	14.3	2.60	1.58	**Breast Cancer**				
SNB-19	7.61	3.70	5.06	3.16	MCF7	2.83	2.75	5.91	2.51
SNB-75	5.15	4.97	1.09	3.16	MDA-MB-231/ATCC	3.50	4.97	1.67	1.26
U251	12.8	14.7	3.12	2.0	HS 578T	15.8	9.24	1.73	2.51
Melanoma					BT-549	3.28	5.43	4.67	3.16
LOX IMVI	4.21	9.84	2.32	1.58	T-47D	3.59	4.79	1.47	1.58
MALME-3M	3.18	9.01	2.61	2.0	MDA-MB-468	2.46	**0.356**	3.56	2.0

[a] Bold figures indicate superior potency than compound **B** and sorafenib, bold underlined figures refer to submicromolar GI_{50} values. [b] NT, not tested.

Table 5. Cytotoxicity evaluation of compounds **6d** and **6j** against HFF-1 normal cell line.[a]

Tested concentrations	% Growth inhibition	
	6d	**6j**
10 μM	17.17±4.44	30.27±2.18
1.0 μM	5.28±4.62	7.94±0.57
0.1 μM	6.88±4.41	2.66±0.83
GI_{50} (μM)	> 10.0	> 10.0

[a] % Growth inhibition was recorded for each compound at 3 different concentrations.

Table 6. *In vitro* enzymatic activity of compounds **6d**, **6j** and **B** over B-Raf^{V600E} and C-Raf kinases.[a]

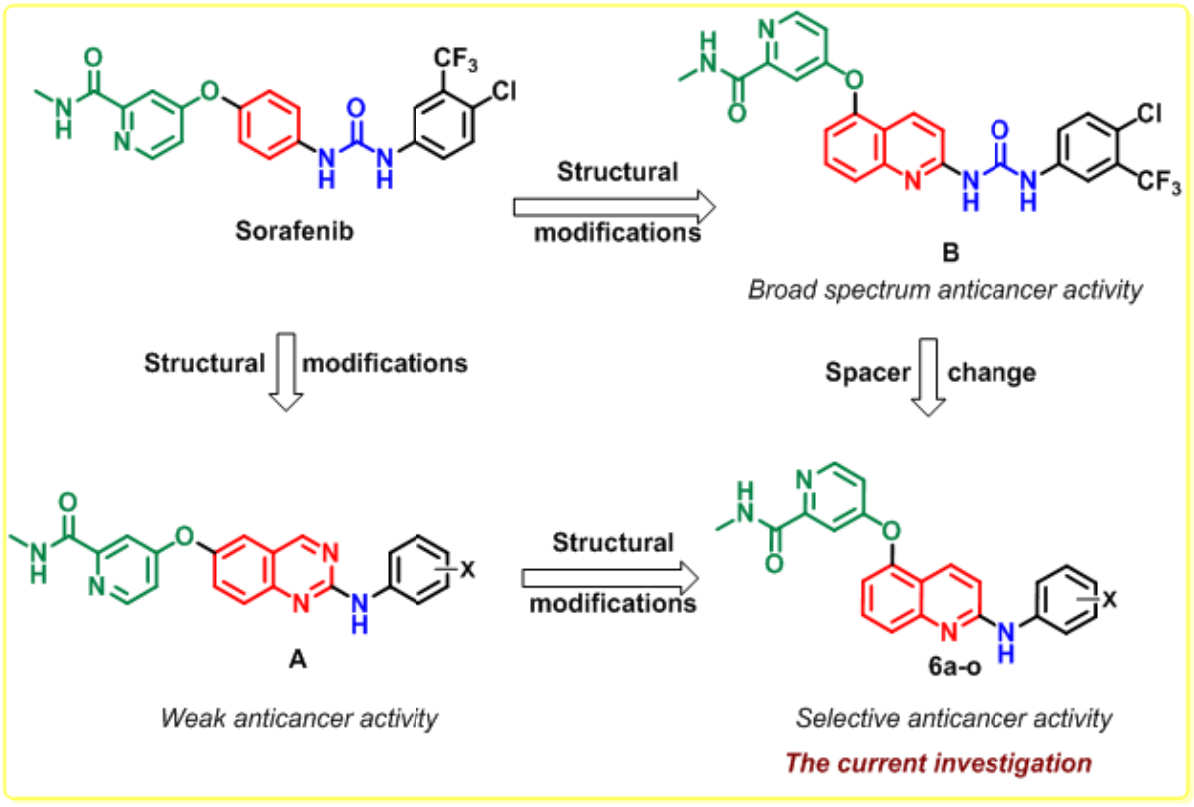
Compound No.	BRAF^{V600E}		C-RAF	
	% Inhibition[a]	IC_{50} (μM)[b]	% Inhibition[a]	IC_{50} (μM)[b]
6d	13.9	> 100	37.3	92.6
6j	9.09	> 100	−5.83	> 100
B[c]	81.8	0.316	96.3	0.061

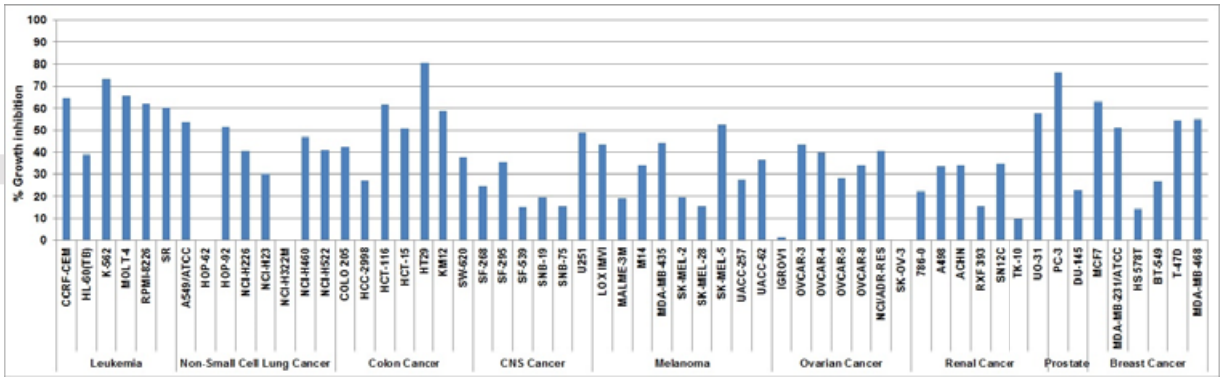
[a] Compounds were tested in single dose duplicate mode at a concentration of 10 μM.
[b] Compounds were tested in a 10-dose IC_{50} mode with 3-fold serial dilution starting at 100 μM.
[c] The assay results were retrieved from reference 16.

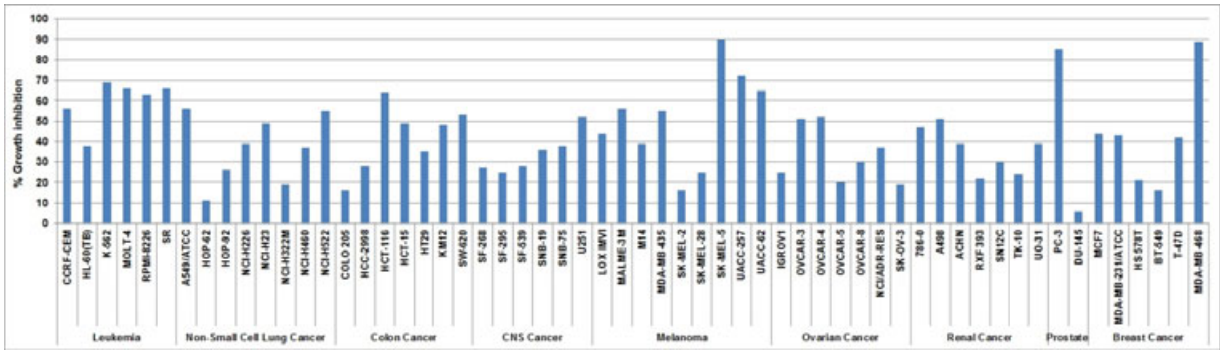
Table 7. *In vitro* enzymatic screening of compounds **6d** and **6j** against certain protein kinases.[a]

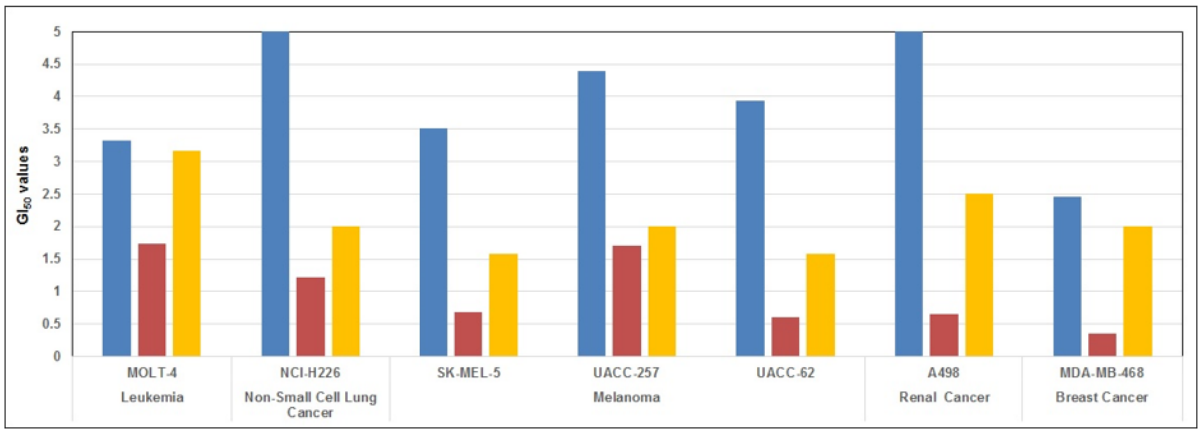
Kinases	% Enzymatic inhibition	
	6d	**6j**
ABL1	−7.77	5.14
ABL^{T315I}	NT	22.6
c-Kit	NT	−1.04
FLT3	−2.94	3.52
FMS	9.13	−0.87
P38a/MAPK14	NT	−36.0
LCK	NT	−8.77
VEGFR2	−65.4	3.93
Pim3	−74.6	38.9
TrkA	12.3	6.39

[a] Compounds were tested in single dose duplicate mode at a concentration of 10 μM.

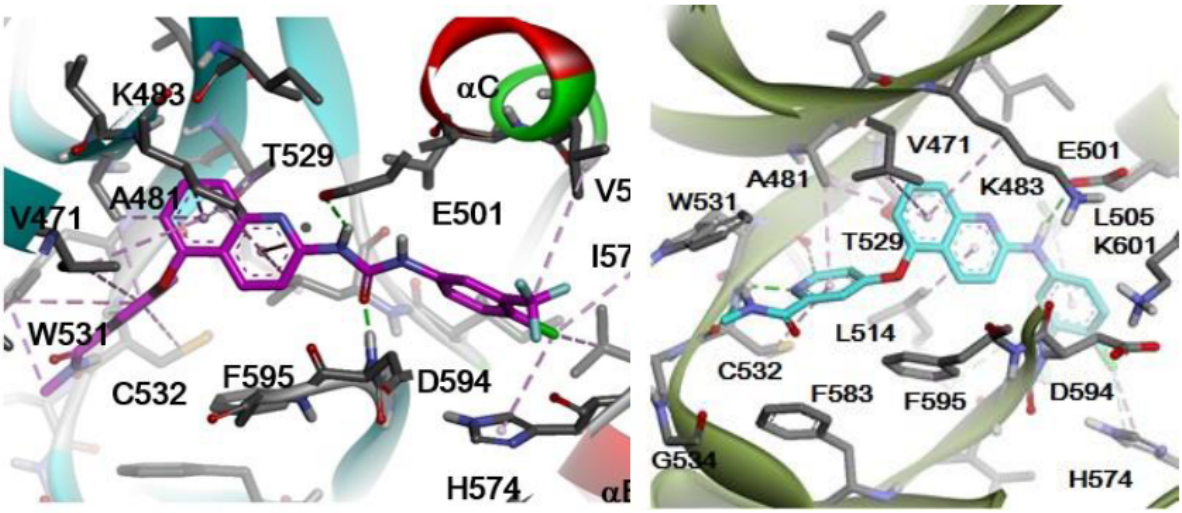








(A)



(B)