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Broad-Spectrum Antiproliferative Activity of Diarylureas and Diarylamides Possessing Pyrrolo[3,2-c]Pyridine Scaffold

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Abstract: A series of diarylureas and diarylamides possessing pyrrolo[3,2-*c*]pyridine scaffold was designed and synthesized. Their *in vitro* antiproliferative activities were tested against a panel of 49 cell lines of eight different cancer types at the NCI and compared with Sorafenib as a reference compound. Most of the compounds showed strong and broad-spectrum antiproliferative activities with superior potencies to Sorafenib. Compounds 8a, 9d and 9f showed lethal effect with mean %inhibition more than 100% over the 49 tested cell lines. In addition, the mean %inhibition results of compounds 8d, 8e, 9e, 9g and 9h were more than 80%. And most of the IC_{50} values of the target compounds were in submicromolar scale. Compounds 8a, 9b and 9e demonstrated high selectivity towards cancer cell lines compared with NIH3T3 fibroblasts.

Key words: Anticancer, antiproliferative, diarylamide, diarylurea, pyrrolo[3,2-*c*]pyridine.

1. Introduction

Cancer is a major leading cause of death worldwide. According to the American Cancer Society Report, 577,190 cancer patients died, and more than 1.6 million new cancer cases were identified in 2012 only in USA [1]. More than 70% of all cancer deaths have occurred in low and middle-income countries. Deaths from cancer worldwide are estimated to exceed 13 million in 2030 according to the WHO (World Health Organization) report [2]. Despite of the extensive efforts and investment in research, the management of human cancers still constitutes a major challenge for contemporary medicinal chemistry. There has been an urgent need for development of more efficient anticancer agents with minimal side effects.

Diarylureas and diarylamides have been highlighted as potential antiproliferative agents against a variety of cancer cell lines [3-25]. Sorafenib (Nexavar®, Fig. 1) is an example of anticancer diarylureas that has been approved by the U.S. FDA (Food and Drug Administration) for treatment of advanced renal cancer [26]. It has also been approved in Europe for treatment of HCC (hepatocellular carcinoma) [27]. Sorafenib is currently subjected to clinical trials for other types of cancer such as metastatic breast, advanced gastric, HCC, thyroid, NSCLC (non-small cell lung cancer), pancreatic, prostate, bladder, metastatic colorectal, ovarian, brain, esophageal/gastroesophageal, leukemia, glioblastoma,

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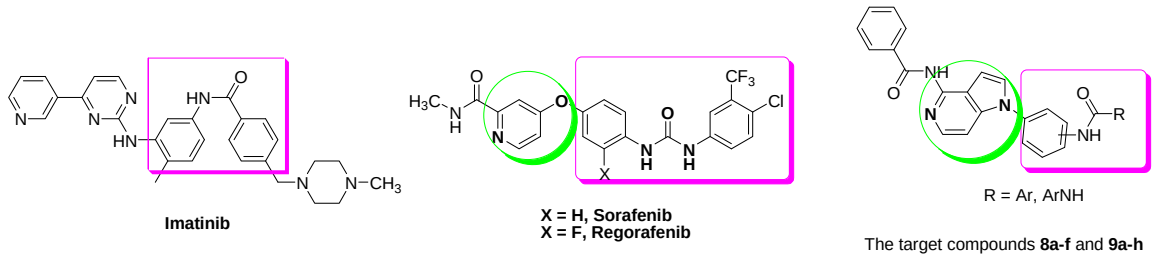


Fig. 1 Structures of Imatinib, Sorafenib, Regorafenib and the target compounds.

Hodgkin's lymphoma, skin/ocular melanoma and neuroendocrine cancers [28]. Regorafenib (BAY73-4506, Stivarga®) is the fluoro analogue of Sorafenib (Fig. 1) which was approved by the FDA in 2012 for treatment of metastatic colorectal cancer [29]. In 2013, the FDA expanded the approved use for treatment of patients with GIST (gastrointestinal stromal tumors) [30]. In addition, many clinical trials have been conducted for RCC (Regorafenib against renal cell carcinoma), HCC (in combination with Sorafenib), NSCLC, angiosarcoma, biliary tract carcinoma, liposarcoma, osteogenic sarcoma and Ewing/Ewing-like sarcomas [31]. Imatinib (Gleevec®, Fig. 1) is an example of diarylamides which is used for treatment of CML (chronic myeloid leukemia) with diminished side effects [32]. Imatinib has been tested in clinical trials for treatment of GIST, thyroid cancer, breast cancer, meningioma, ovarian cancer, and NSCLC in combination with other drugs [33].

In the present investigation, the target compounds were designed as conformationally restricted analogs of Sorafenib and Regorafenib by isosteric replacement of the 4-phenoxypyridine moiety of the reference drug with a pyrrolo[3,2-*c*]pyridine nucleus (Fig. 1). They showed promising antiproliferative activity against melanoma cell lines [12, 16]. The broad-spectrum anticancer activities expressed by Sorafenib and Regorafenib encouraged us to extend the biological investigations of our target compounds against other types of cancer other than melanoma. They were tested for antiproliferative activities over leukemia, NSCLC, colon, CNS, ovarian, renal, prostate and breast cancer cell line panels at the NCI (National Cancer Institute), Bethesda, Maryland, USA [34]. The results are reported in details.

2. Materials and Methods

2.1 General

The target compounds were purified by column chromatography using silica gel (0.040-0.063 mm, 230-400 mesh) and technical grade solvents. All melting points were obtained on a Walden Precision Apparatus Electrothermal 9,300 apparatus and are uncorrected. NMR (nuclear magnetic resonance) spectroscopy was performed using a Bruker ARX-300, 300 MHz spectrometer (Bruker Bioscience, Billerica, MA, USA) with TMS (tetramethylsilane) as an internal standard. MS (Mass spectra) and purities of the target compounds (> 95%) were determined by LC-MS analysis using the following system: Waters 2,998 Photodiode Array Detector, Waters 3100 Mass Detector, Waters SFO System Fluidics Organizer, Waters 2545 Binary Gradient Module, Waters Reagent Manager, Waters 2767 Sample Manager, Sunfire^{TM} C_{18} column (4.6 × 50 mm, 5 µm particle size); Solvent gradient = 95% A at 0 min, 1% A at 5 min; solvent A: 0.035% TFA (trifluoroacetic acid) in water; solvent B: 0.035% TFA in CH_3OH; flow rate = 3.0 mL/min; the AUC was calculated using Waters MassLynx 4.1 software. The % purity was determined by dividing the AUC of the compound by the total AUC. All reagents and solvents were purchased from Aldrich chemical Co. and TCI (Tokyo Chemical Industry) Co., and used without further purification.

2.2 Synthesis

2.2.1 7-Hydroxy-1H-pyrrolo[2,3-b]pyridinium 3-chlorobenzoate (2)

It was prepared following the literature procedure [35, 36]. Mp: 141-143 °C (Lit. mp: 144.1-146 °C) [36].

2.2.2 4-Chloro-7-azaindole (3)

It was prepared following the literature procedure [36]. Mp: 176-177 °C (Lit. mp: 175.3-177 °C) [36].

Procedures for Synthesis of Para-disubstituted Benzene Derivatives 4a, 5a, 6a, 8a,b and 9a-c.

They were reported in our previously published article [12].

2.2.3 1-(3-Nitrophenyl)-1H-pyrrolo[3,2-c]pyridin-4-amine hydrochloride (4b)

A mixture of compound 3 (230 mg, 2.0 mmol) and 3-nitroaniline (1.38 g, 10.0 mmol) was fused at 180 ºC for 2-5 h with stirring. The reaction mixture was cooled to room temperature and dissolved in ethanol (150 mL). The resulting suspension was filtered to remove the insoluble material, and the filtrate was evaporated under reduced pressure. The residue was purified by column chromatography (silica gel, ethyl acetate-methanol 10:1 v/v then switching to ethyl acetate-methanol 5:1 v/v) to obtain the desired purified product. Yield 9.5%; 1H NMR (CD_3OD): δ 8.33 (t, 1H, J = 2.0 Hz), 8.25 (dd, 1H, J = 1.1, 6.1 Hz), 7.94-7.91 (m, 1H), 7.81-7.76 (m, 1H), 7.63 (d, 1H, J = 2.2 Hz), 7.45 (d, 1H, J = 3.4 Hz), 6.90 (d, 1H, J = 3.4 Hz), 6.85 (d, 1H, J = 6.3 Hz); ^{13}C NMR (CD_3OD): δ 155.1, 150.4, 144.5, 140.3, 132.2, 131.0, 127.2, 122.6, 119.7, 115.0, 113.3, 104.5, 98.6; MS *m/z*: 255.95 (M^+ + 2), 254.65 (M^+); purity: 97.5%.

2.2.4 N-(1-(3-Nitrophenyl)-1H-pyrrolo[3,2-c]pyridin-4-yl) benzamide (5b)

To a stirred solution of compound 4b (0.465 g, 1.6 mmol) in acetonitrile (25 mL) at room temperature, diisopropylamine (0.7 mL, 4.0 mmol) was slowly added under nitrogen atmosphere. Benzoyl chloride (0.2 mL, 1.9 mmol) was slowly added and the reaction mixture was stirred at room temperature for 8 h. The reaction mixture was concentrated under reduced pressure, and water (20 mL) and CH_2Cl_2 (20 mL) were added to the residue. The organic layer was separated and the aqueous layer was extracted with CH_2Cl_2 (2 × 20 mL). The combined organic layer extracts were washed with brine, 1 N HCl, and then aqueous $NaHCO_3$, dried over anhydrous $MgSO_4$, and filtered. The organic solvent was evaporated under reduced pressure and the residue was purified by column chromatography (silica gel, hexanes-ethyl acetate 3:1 v/v) to give the target product. Yield 15%; ^{1}H NMR (DMSO-d_6): δ 10.90 (brs, 1H), 8.41 (t, 1 H, J = 2.1 Hz), 8.30 (d, 1 H, J = 8.0 Hz), 8.16-8.09 (m, 4H), 7.93-7.82 (m, 2H), 7.62-7.52 (m, 4H), 6.74 (brs, 1H); MS m/z: 359.11 (M^+ + 1), 358.24 (M^+); purity: 95.3%.

2.2.5 N-(1-(3-Aminophenyl)-1H-pyrrolo[3,2-c]pyridin-4-yl) benzamide (6b)

A mixture of compound 5b (0.466 g, 1.3 mmol) and Pd/C (10%) in anhydrous THF (20 mL) was stirred in hydrogen atmosphere at room temperature for 2 h. The reaction mixture was filtered through celite, and the filtrate was concentrated under reduced pressure. The residue was purified by column chromatography (silica gel, hexanes-ethyl acetate 1:2 v/v) to give the purified desired product. Yield 32%; 1H NMR (DMSO-d_6): δ 10.84 (brs, 1H), 8.11-8.09 (m, 3H), 7.62 (d, 2H, J = 3.2 Hz), 7.54 (d, 2H, J = 7.5 Hz), 7.42 (brs, 1H), 7.22 (t, 1H, J = 7.9 Hz), 6.77 (brs, 1H), 6.69-6.62 (m, 3H), 5.50 (brs, 2H); MS *m/z*: 330.24 (M^+ + 2), 329.21 (M^+ + 1); purity: 96.0%.

2.2.6 1-(3-(4-Benzamido-1H-pyrrolo[3,2-c]pyridin-1-yl) phenyl)-3-(3, 4-dichloro phenyl) urea (8c)

To a solution of compound 6b (22.0 mg, 0.06 mmol) in anhydrous THF (10 mL), 3, 4-dichlorophenyl isocyanate (13.7 mg, 0.06 mmol) was added. The reaction mixture was stirred under nitrogen

atmosphere at room temperature for 8 h. The solvent was evaporated under reduced pressure and the residue was purified by column chromatography (silica gel, hexanes-ethyl acetate 3:1 v/v then switching to hexanes-ethyl acetate 1:1 v/v) to obtain the purified product 8a (2.3 mg, 6.7%). Mp: > 280 °C; 1H NMR (DMSO-d_6): δ 10.86 (brs, 1H), 9.98 (brs, 1H), 8.77 (brs, 1H), 8.15-8.07 (m, 3H), 7.90 (brs, 1H), 7.73 (d, 1H, J = 3.5 Hz), 7.60-7.45 (m, 6H), 7.35-7.32 (m, 3H), 7.26 (d, 1H, J = 7.8 Hz), 6.78-6.69 (m, 1H); MS m/z: 518.38 (M^+ + 2), 517.42 (M^+ + 1), 516.45 (M^+); purity: 96.2%.

Synthesis of compounds 8d, e was achieved by the same procedure as described for compound 8c.

2.2.7 1-(3-(4-Benzamido-1H-pyrrolo[3,2-c]pyridin-1-yl) phenyl)-3-(4-chloro-3-(trifluoromethyl) phenyl) urea (8d)

^{1}H NMR (DMSO-d_6): δ 10.88 (brs, 1H), 9.85 (brs, 1H), 9.73 (brs, 1H), 8.15-8.09 (m, 3H), 7.90-7.87 (m, 1H), 7.72-7.69 (m, 2H), 7.59 (d, 2H, J = 9.3 Hz), 7.55-7.48 (m, 4H), 7.25-7.19 (m, 1H), 6.87 (brs, 1H), 6.68 (d, 1H, J = 3.8 Hz); MS *m/z*: 552.22 (M^+ + 2), 551.18 (M^+ + 1), 550.14 (M^+); purity: 98.0%.

2.2.8 1-(3, 5-Bis (trifluoromethyl) phenyl)-3-(3-(4-benzamido-1H-pyrrolo[3,2-c]pyridine -1-yl) phenyl) urea (8e)

^{1}H NMR (DMSO-d_6): δ 10.89 (brs, 1H), 10.36 (brs, 1H), 9.71 (brs, 1H), 8.30-8.25 (m, 5H), 8.14 (brs, 2H), 7.90 (brs, 1H), 7.80 (d, 1H, J = 2.4 Hz), 7.63 (d, 1H, J = 6.2 Hz), 7.58-7.33 (m, 5H), 7.18 (d, 1H, J = 2.4 Hz); MS *m/z*: 584.52 (M^+ + 1), 583.56 (M^+); purity: 97.8%.

2.2.9 1-(3-(4-Benzamido-1H-pyrrolo[3,2-c]pyridin-1-yl) phenyl)-3-(4-((4-ethylpiperazin-1-yl) methyl)-3-(trifluoromethyl) phenyl) urea (8f)

A mixture of 4-((4-ethylpiperazin-1-yl) methyl)-3-(trifluoromethyl) aniline (7, 17.5 mg, 0.06 mmol), triethylamine (0.014 mL, 0.1 mmol), and *p*-nitrophenyl chloroformate (12.4 mg, 0.06 mmol) in 1,4-dioxane (5 mL) was heated at 60 °C for 2 h. A solution of 6 (20 mg, 0.06 mmol) in 1,4-dioxane (5 mL) was slowly added thereto. The reaction mixture was heated at 90 °C overnight. The reaction mixture was concentrated under reduced pressure and then partitioned between water (5 mL) and ethyl acetate (5 mL). The organic layer was separated and the aqueous layer was then extracted with ethyl acetate (3 × 3 mL). The combined organic extracts were washed with brine and dried over anhydrous Na_2SO_4. After evaporation of the organic solvent, the residue was purified by column chromatography (silica gel, hexanes-ethyl acetate 3:1 v/v then switching to hexanes-ethyl acetate 1:1 v/v) to yield compound 8f. ^{1}H NMR (DMSO-d_6): δ 10.90 (brs, 1H), 9.32-9.23 (m, 2H), 8.24-8.20 (m, 3H), 8.03-7.98 (m, 2H), 7.75-7.68 (m, 5H), 7.82-7.73 (m, 5H), 7.60 (dd, 1H, J = 6.3 Hz, J = 5.7 Hz), 2.74 (s, 4H), 2.39-2.29 (m, 6H), 0.99 (q, 2 H, J = 7.2 Hz), 0.84 (t, 3H, J = 7.3 Hz); MS m/z: 642.75 (M^+ + 1), 641.72 (M^+); purity: 96.8%.

2.2.10 N-[3-(4-Benzamido-1H-pyrrolo[3,2-c]pyridin-1-yl)-p henyl]-3, 4-dichlorobenzamide (9d)

A mixture of compound 6b (20.0 mg, 0.06 mmol), 3, 4-dichlorobenzoic acid (23.11 mg, 0.12 mmol), HOBt (18.1 mg, 0.13 mmol), and EDCI (29.1 mg, 0.15 mmol) in dry DMF (1.0 mL) was cooled to 0 °C under nitrogen atmosphere. To the reaction mixture, triethylamine (0.002 mL, 0.015 mmol) was added at 0 °C. The mixture was then stirred at 80 °C for 12 h. The reaction mixture was cooled and then partitioned between water (5 mL) and ethyl acetate (5 mL), and the organic layer was separated. The aqueous layer was then extracted with ethyl acetate (3 × 3 mL), and the combined organic extracts were washed with brine and dried over anhydrous Na_2SO_4. After evaporation of the organic solvent, the residue was purified by column chromatography (silica gel, hexanes-ethyl acetate 3:1 v/v then switching to hexanes-ethyl acetate 1:1 v/v) to yield compound 9d. 1H NMR (DMSO-d_6): δ 10.86 (brs, 1H), 10.68 (brs, 1H), 8.26 (d, 1H, J = 2.0

Hz), 8.15-8.11 (m, 4H), 7.98 (dd, 1H, *J* = 2.0, 2.1 Hz), 7.88-7.85 (m, 2H), 7.59-7.52 (m, 3H), 7.45-7.39 (m, 3H), 6.76-6.63 (m, 2H); MS m/z: 501.50 (M^+ + 1, 500.46 (M^+); purity: 97.0%.

Synthesis of compounds 9e-h was achieved by the same procedure as described for compound 9d.

2.2.11

N-[3-(4-Benzamido-1H-pyrrolo[3,2-c]pyridin-1-yl)-phenyl]-3-(trifluoromethyl) benzamide (9e)

^{1}H NMR (DMSO-d_6): δ 10.89 (brs, 1H), 10.77 (brs, 1H), 8.31 (d, 2H, *J* = 9.1 Hz), 8.18-8.09 (m, 2H), 8.02 (d, 1H, *J* = 7.6 Hz), 7.88-7.80 (m, 2H), 7.76 (d, 1H, *J* = 3.3 Hz), 7.68-7.58 (m, 4H), 7.59 (d, 2H, *J* = 8.6 Hz), 7.44-7.39 (m, 2H), 6.70-6.67 (m, 1H); MS m/z: 503.01 (M^+ + 2), 502.00 (M^+ + 1), 501.00 (M^+); purity: 97.7%.

2.2.12

N-[3-(4-Benzamido-1H-pyrrolo[3,2-c]pyridin-1-yl)-phenyl]-4-chloro-3-(trifluoromethyl) benzamide (9f)

^{1}H NMR (DMSO-d_6): *δ* 10.90 (brs, 1H), 10.83 (brs, 1H), 8.43 (d, 1H, *J* = 1.8 Hz), 8.31 (d, 1H, *J* = 8.5 Hz), 8.13-8.10 (m, 4H), 7.97 (d, 1H, *J* = 8.3 Hz), 7.83 (d, 1H, *J* = 7.8 Hz), 7.75 (d, 1H, *J* = 3.3 Hz), 7.63 (t, 2H, *J* = 8.1 Hz), 7.57-7.53 (m, 3H), 7.42-7.39 (m, 1H), 6.71-6.67 (m, 1H); MS *m/z*: 536.42 (M^+ + 2), 535.41 (M^+ + 1); purity: 97.3%.

2.2.13

N-[3-(4-Benzamido-1H-pyrrolo[3,2-c]pyridin-1-yl)-phenyl]-3-morpholino-5-(trifluoromethyl) benzamide (9g)

^{1}H NMR (DMSO-d_6): δ 10.90 (brs, 1H), 10.66 (brs, 1H), 8.44-8.27 (m, 3H), 8.00-7.95 (m, 1H), 7.83 (d, 2H, *J* = 8.6 Hz), 7.75 (d, 2H, *J* = 3.0 Hz), 7.69 (brs, 1H), 7.65-7.55 (m, 4H), 7.43-7.35 (m, 1H), 7.26-7.19 (m, 1H), 7.06-7.04 (m, 1H), 6.70 (brs, 1H), 3.77 (t, 4H, *J* = 3.1 Hz), 2.93 (t, 4H, *J* = 3.3 Hz); ^{13}C NMR (DMSO-d_6): δ 164.5, 154.9, 141.1, 140.2, 136.4, 134.3, 133.8, 133.5, 132.3, 130.9, 129.5, 129.1, 128.8, 128.6, 127.5, 125.8, 124.8, 124.3, 123.0, 122.0, 119.7, 118.4, 117.2, 114.7, 104.6, 66.9, 53.6; MS m/z: 586.60 (M^+ + 1), 585.61 (M^+); purity: 96.0%.

2.2.14

N-[3-(4-Benzamido-1H-pyrrolo[3,2-c]pyridin-1-yl)-phenyl]-3-(4-methyl-1H-imidazol-1-yl)-5-(trifluoromethyl) benzamide (9h)

^{1}H NMR (DMSO-d_6): δ 10.91 (brs, 1H), 10.82 (brs, 1H), 8.49 (brs, 1H), 8.44 (brs, 1H), 8.29 (brs, 1H), 8.21-8.16 (m, 3H), 8.10 (d, 2H, *J* = 7.2 Hz), 7.84 (d, 1H, *J* = 8.2 Hz), 7.76 (d, 2H, *J* = 3.8 Hz), 7.69-7.61 (m, 2H), 7.58-7.56 (m, 3H), 7.43 (d, 1H, *J* = 7.2 Hz), 6.71 (d, 1H, *J* = 3.1 Hz), 2.54 (s, 3H); ^{13}C NMR (DMSO-d_6): δ 169.1, 152.7, 141.1, 140.6, 139.7, 137.0, 135.5, 134.8, 133.9, 133.5, 132.6, 131.4, 129.8, 129.2, 128.7, 128.5, 127.9, 125.3, 124.6, 124.1, 123.4, 121.7, 119.6, 118.2, 117.4, 116.1, 110.6, 104.8, 19.4; MS m/z: 582.47 (M^+ + 2), 581.45 (M^+ + 1), 580.43 (M^+); purity: 98.0%.

2.3 Cancer Cell Line Screening at the NCI

Screening against the cancer cell lines was carried out at the NCI (National Cancer Institute), Bethesda, Maryland, USA [34] applying the standard protocol of the NCI [37, 38].

2.4 Evaluation of the Antiproliferative Activity against NIH3T3 Fibroblasts

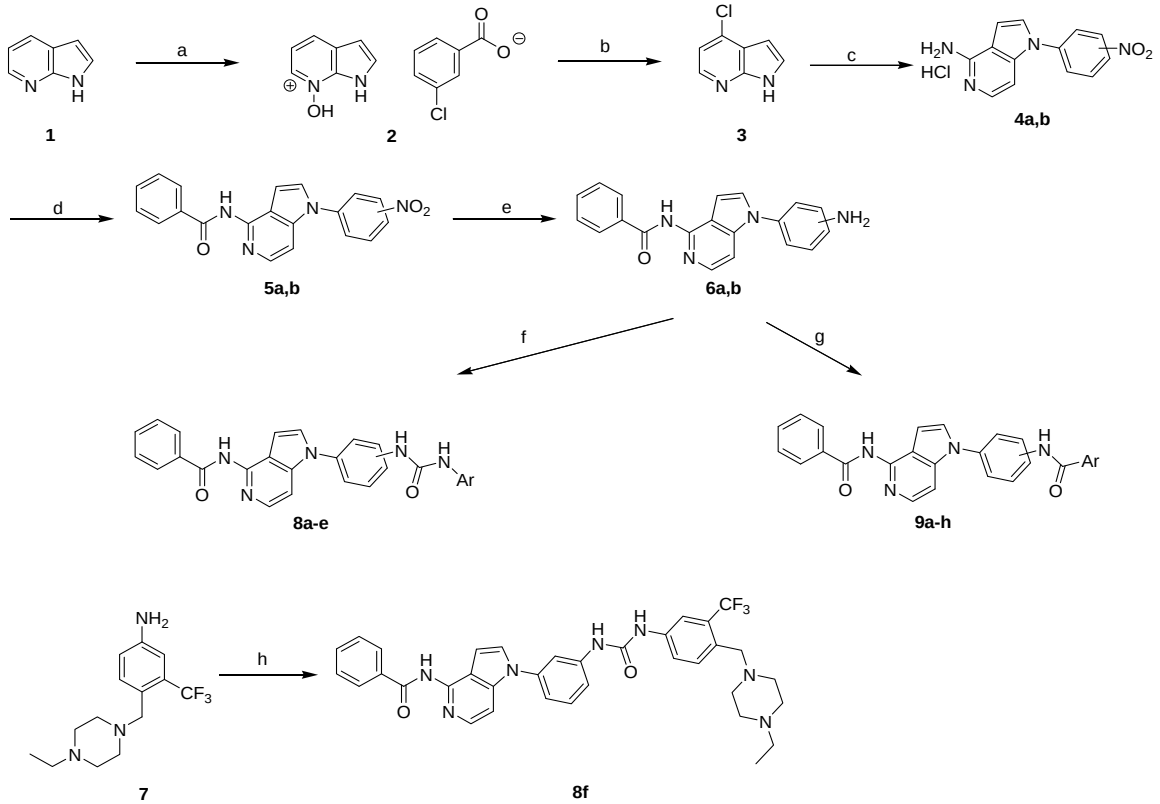
NIH3T3 fibroblasts were purchased from ATCC (American Type Culture Collection, Rockville, MD, USA) and maintained in DMEM (Dulbecco's modified eagle medium), Welgene, Daegu, Republic of Korea) supplemented with BCS (bovine calf serum, Welgene, Daegu, Republic of Korea) and 1% penicillin/streptomycin (Welgene, Daegu, Republic of Korea) in a humidified atmosphere with 5% CO_2 at 37 ^{o}C. NIH3T3 fibroblasts were taken from culture substrate with 0.05% trypsin-0.02% EDTA and plated at a density of 5 × 10^3 cells/well in 96 well plates and then incubated at 37 ^{o}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 the tested compounds. The cells were incubated for 48 h after treatment with the test

compounds. The NIH3T3 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, USA). The IC_{50} values were calculated using GraphPad Prism 4.0 software. Triplicate testing was performed for each test compound.

3. Results and Discussion

3.1 Chemistry

The target compounds 8a-f and 9a-h were synthesized by the pathway illustrated in Scheme 1. 7-Hydroxy-1H-pyrrolo[2,3-b]pyridinium 3-chlorobenzoate (2) was prepared by reacting 7-azaindole (1) with 3-chloroperbenzoic acid [35, 36]. Compound 2 was then heated with phosphorus oxychloride to produce 4-chloro-7-azaindole (3) [33]. Compound 4 was prepared according to the literature procedure [12, 39, 40]. Fusion of 3 with p-or m-nitroaniline neat led to nucleophilic displacement of the 4-chloro group by the aromatic amino group, followed by rearrangement of the resulting secondary amine to give the amine hydrochloride salts 4a, b, respectively. Reaction of the amino groups of compounds 4a, b with benzoyl chloride in the presence of diisopropylamine as a base furnished the benzamido derivatives 5a, b. Reduction of the nitro groups of 5a, b using Pd-C/H_2 gave the corresponding amino compounds, N-(1-(4-aminophenyl)-1H-pyrrolo[3,2-c]pyridin-4-yl) benzamide (6a) and N-(1-(3-aminophenyl)-1H-pyrrolo[3,2-c]pyridin-4-yl)



Scheme 1 Reagents and conditions: (a) 3-chloroperoxybenzoic acid, DME:heptane (1:2), rt, 2.5 h, 89.2%; (b) $POCl_3$, 55 °C then rt then 85-90 °C, 18 h, 80%; (c) appropriate nitroaniline, 180 °C, 2-5 h, 18%(*para*); 9.5% (*meta*); (d) benzoyl chloride, diisopropylamine, CH_3CN, rt, 8 h, 85% (*para*); 15% (*meta*); (e) Pd/C, H_2, THF, rt, 2 h, 48% (*para*); 32% (*meta*); (f) aryl isocyanate, THF, rt, 8 h, 14~53%; (g) benzoic acid derivative, HOBt, EDCI, TEA, DMF, 80 °C, 12 h, 9~26%; (h) (i) 4-nitrophenyl chloroformate, TEA, 1,4-dioxane, 60 °C, 2 h, (ii) 6 in 1,4-dioxane, 90 °C, overnight, 45%.

benzamide (6b). Reaction of 6a, b with the appropriate aryl isocyanates produced the corresponding diarylurea derivatives 8a-e. The bisamide derivatives 9a-h were obtained by condensation of the amino intermediates 6a, b with the corresponding benzoic acid derivatives in the presence of HOBt/EDCI/triethylamine.

The N-ethylpiperazinyl diarylurea derivative 8f was synthesized by a modified method through heating the aniline derivative 7 with p-nitrophenyl chloroformate in the presence of triethylamine as a base to form the corresponding carbamate intermediate and subsequent heating with compound 6b. Structures of the target compounds, and their yield percentages and melting points are summarized in Table 1.

3.2 Antiproliferative Activities of the Target Compounds

3.2.1 Single-dose Testing

Structures of the target compounds were submitted to NCI, Bethesda, Maryland, USA [34], and they were selected for *in vitro* anticancer assay against a panel of six leukemia, nine NSCLC, seven colon, six CNS, seven ovarian, seven renal, two prostate, and five breast cancer cell lines. The compounds were tested at a single dose concentration of 10 μM, and the percentages of growth inhibition over the 49 tested cell lines were determined. The mean inhibition percentages for each of the tested compounds over the full panel of cell lines are illustrated in Fig. 2.

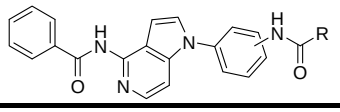
The results showed that the meta-disubstituted benzene compounds 9e and 9f were more active than the corresponding *para* positional isomers 9a and 9b. This can be attributed to the influence of the substituents orientation at the receptor site on the activity. The amide derivatives 9a, 9d and 9f demonstrated higher activities than the corresponding urea analogues 8b-d. This illustrated the relationship between the linker length, and compound fitting and affinity at the receptor site, and hence antiproliferative activity.

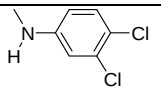
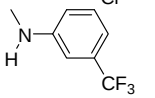
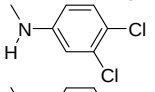
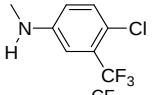
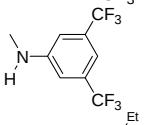
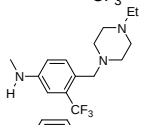
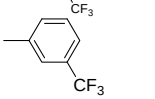
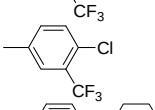
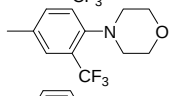
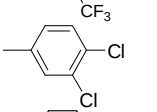
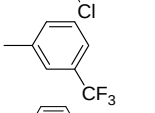
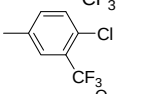
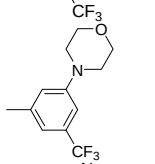
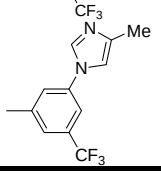
The effect of substituents on the terminal phenyl ring was also studied. Compounds 8a and 9d with the highest mean %inhibition values share the same terminal ring, 3, 4-dichlorophenyl. So it was optimal for antiproliferative activity of this series of compounds. Compounds 8d, 9b and 9f with mean %inhibition values of 94.16%, 80.72%, and 130.04%, respectively, which possess 4-chloro-3-(trifluoromethyl)phenyl moiety same as Sorafenib and Regorafenib. So this moiety is also optimal for activity. Compound 9f showed higher activity than 9e possessing 3-(trifluoromethyl)phenyl terminal moiety. This can be rationalized that the *para*-chloro group may contribute to stronger hydrophobic interaction at the receptor site. Compounds 9g and 9h with 3-morpholino-5-(trifluoromethyl) phenyl and 3-(4-methyl-1H-imidazol-1-yl)-5-(trifluoromethyl) phenyl moieties, respectively, demonstrated slightly higher mean %inhibition values than compound 9e possessing 3-(trifluoromethyl)phenyl ring. This may be attributed to the enhanced binding affinity produced by morpholino or methylimidazole substituents to the target molecule. Compound 8f expressed the lowest %inhibition results, and it is the bulkiest derivative among this series. So there may be an inverse relationship between bulkiness and antiproliferative activity. Similarly, the shorter amide linker was more favorable for activity than the longer urea spacer.

Upon comparing the results the target pyrrolo[3,2-c]pyridine compounds with their isosteric pyrrolo[2,3-d]pyrimidine analogues [25], compounds 8a, 8d, and 9a showed higher mean %inhibition results than the corresponding pyrrolo [2,3-d]pyrimidine derivatives.

The %inhibitions of the best urea compound 8a and the best amide analogue 9d over each cell line of the panel are illustrated in Fig. 3. At 10 μM concentration, both compounds showed lethal effects (> 100% inhibition) over 35 and 37 cell lines, respectively.

Table 1 Structures of the target compounds, and their yield percentages and melting points.



Compound No.	Site of attachment to benzene ring	R	Yield (%)	Melting point (ºC)
8a	*Para*		14	172-5
8b	*Para*		35	158-60
8c	*Meta*		26	205-8
8d	*Meta*		53	143-5
8e	*Meta*		28	> 280
8f	*Meta*		45	269-72
9a	*Para*		16.4	166-9
9b	*Para*		21	133-6
9c	*Para*		11	220-3
9d	*Meta*		15	190-1
9e	*Meta*		26	142-4
9f	*Meta*		12.6	200-1 (dec.)
9g	*Meta*		9	249-52
9h	*Meta*		15	211-4 (dec.)

Both compounds demonstrated broad-spectrum antiproliferative activities over all the eight tested cancer types. But compound 9d was more active than 8a against 31 cell lines.

3.2.2 Five-dose Testing

Compounds 8a, 8b, 8d, 8e, and 9a-h with promising results in single-dose testing were further tested in a

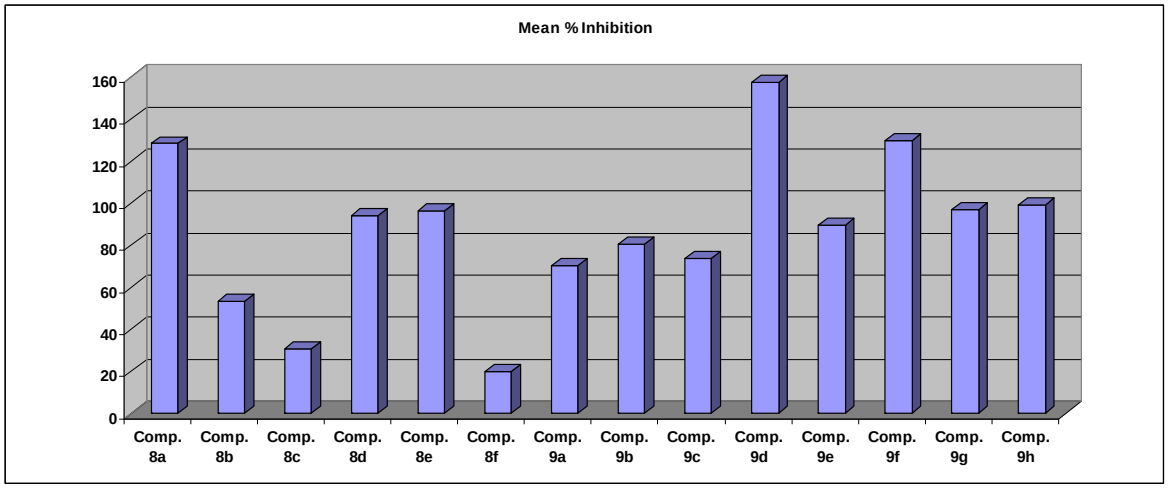
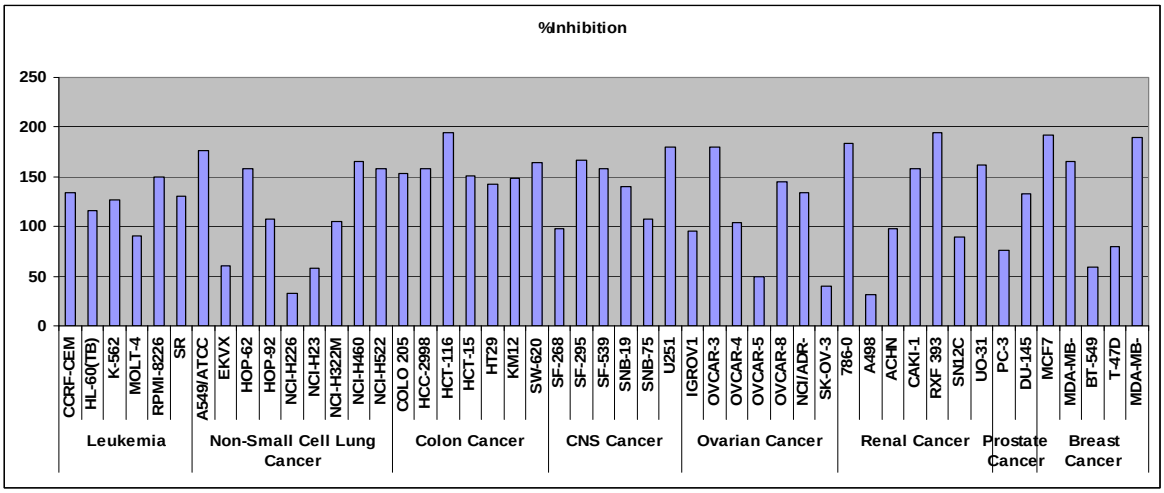
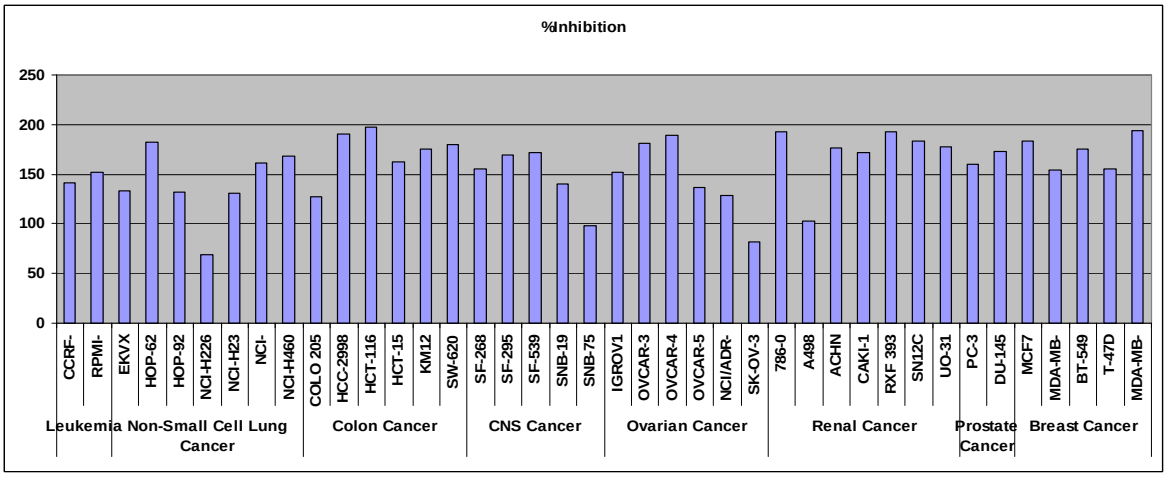


Fig. 2 Mean inhibition percentages observed with the final compounds in single-dose (10 µM) 49-cancer cell line screening. Mean %inhibition represents the mean inhibition percentages over the 49 cell lines. The inhibition percentages were calculated by subtracting the growth percentages from 100.



(a)



(b)

Fig. 3 %Inhibition expressed by compounds (a) 8a and (b) 9d at a single-dose concentration of 10 µM over the cell line panel of eight different cancer types.

five-dose testing mode, in order to determine their IC_{50} values over the 49 cancer cell lines. The mean IC_{50} values of these twelve compounds over the eight cancer types are shown in Table 2.

As shown in Table 2, most of the results were in sub-micromolar scale. Amide derivatives were more potent than the urea analogues. And all the mean IC_{50} results of compounds 8d, 9d-f and 9h were in sub-micromolar range over all the eight subpanels. The *meta* derivatives 9e and 9f were more potent than the corresponding *para* positional isomers 9a and 9b. The mean IC_{50} values of compound 9e possessing amide linker and 3-(trifluoromethyl)phenyl terminal moiety were in 2-digit nanomolar scale over all the eight subpanels.

The IC_{50} values of the twelve compounds tested in five-dose mode over the most sensitive cell line of each subpanel are summarized in Table 3. All the eight cell lines were highly sensitive to the target compounds. Diarylamides were generally more potent than diarylureas. For compounds 9a, 9b and 9d-h, all their IC_{50} values were in sub-micromolar scale, and most of them were in 2-digit nanomolar range. Of special interest, the amide compounds 9d and 9e demonstrated 2-digit nanomolar IC_{50} values over all the cell lines. Moreover, most of the results showed that the twelve tested compounds were more potent than Sorafenib against all the eight cell lines. The results of Sorafenib were obtained from NCI datawarehouse index [39] and are inserted in Table 3. And the highest potencies were shown by compound 9c against SR leukemia cell line and HCT-15 colon cancer cell lines with IC_{50} values less than 10 nm.

It is noteworthy that the diarylurea compounds 8a and 8d were more potent than the corresponding pyrrolo[2,3-d]pyrimidine analogues [25] against HCT-15 colon cancer cell line, U251 CNS cancer cell line, and PC-3 prostate cancer cell line. The diarylamide compound 9a was equipotent against U251 cell line, and more potent over PC-3 cell line, compared with its pyrrolo[2,3-d]pyrimidine isostere.

Some representative compounds, 8a, 9a-c and 9e-h, were tested against NIH3T3 fibroblasts in order to determine their selectivity for cancer cells compared with normal cells. The highest selectivity indices were expressed by the urea derivative 8a and the amide analogues 9b and 9e. Of special interest, the selectivity index of compound 9e was 174. Those compounds are promising leads for design of highly potent and highly selective antiproliferative agents.

Table 2 Mean IC_{50} values (μM) of the tested compounds over *in vitro* subpanel cancer cell lines[a].

Compound No.	Subpanel cancer cell lines[b]							
	I	II	III	IV	V	VI	VII	VIII
8a	3.13	4.29	0.93	0.84	1.31	1.46	1.07	0.77
8b	2.28	5.65	3.54	6.14	6.59	7.97	3.00	6.24
8d	0.45	0.59	0.36	0.45	0.41	0.66	0.37	0.86
8e	1.66	3.57	1.64	1.84	1.73	1.71	1.63	3.73
9a	0.31	2.33	0.73	0.48	0.95	0.82	1.23	2.17
9b	0.40	4.42	0.50	0.35	2.82	2.74	3.62	4.06
9c	0.06	2.10	0.30	0.37	1.42	1.30	0.65	1.94
9d	0.21	0.35	0.16	0.19	0.21	0.03	0.16	0.40
9e	0.04	0.06	0.03	0.04	0.05	0.06	0.04	0.03
9f	0.12	0.25	0.11	0.13	0.14	0.15	0.11	0.22
9g	0.24	0.42	0.11	0.12	0.77	0.13	0.11	20.07
9h	0.34	0.54	0.32	0.38	0.58	0.59	0.38	0.48

[a] Mean IC_{50} values were calculated by dividing the summation of IC_{50} values of the compound over cell lines of the same cancer type by the number of cell lines in the subpanel.

[b] I: Leukemia; II: Non-small cell lung cancer; III: Colon cancer; IV: CNS cancer; V: Ovarian cancer; VI: Renal cancer; VII: Prostate cancer; VIII: Breast cancer.

Table 3 IC_{50} values (μM) of the tested compounds over the most sensitive cell line of each subpanel, and NIH3T3 fibroblasts.

Compound No.	Cancer cell lines								NIH3T3	Selectivity idex[i]
	SR[a]	NCI-H522[b]	HCT-15[c]	U251[d]	OVCAR-3[e]	RXF 393[f]	PC-3[g]	MCF7[h]		
8a	0.19	1.02	0.11	0.39	1.36	0.58	0.59	1.01	11.48	8.44
8b	1.49	1.69	1.03	2.04	2.93	2.24	1.80	6.60	-	-
8d	0.35	0.21	0.33	0.50	0.24	0.21	0.39	0.35	-	-
8e	1.72	0.83	1.07	1.63	1.28	1.16	1.58	1.65	-	-
9a	0.22	0.34	0.04	0.13	0.93	1.02	0.81	0.39	0.05	0.05
9b	0.02	0.99	0.03	0.06	0.78	0.78	0.56	0.31	5.65	5.71
9c	< 0.01	1.58	< 0.01	0.05	0.19	0.24	1.19	0.06	1.74	1.10
9d	0.04	0.07	0.08	0.09	0.06	0.07	0.07	0.04	-	-
9e	0.03	0.02	0.03	0.04	0.02	0.02	0.04	0.03	6.96	174.0
9f	0.07	0.03	0.03	0.10	0.08	0.07	0.10	0.03	0.02	0.20
9g	0.08	0.02	0.03	0.06	0.03	0.03	0.10	0.03	0.013	0.16
9h	0.06	0.50	0.28	0.26	0.30	0.18	0.41	0.33	0.68	1.36
Sorafenib	3.16	2.00	2.51	2.00	3.16	3.16	2.00	2.51	-	-

[a]Leukemia cell line; [b]non-small cell lung cancer cell line; [c]colon cancer cell line; [d]CNS cancer cell line; [e]ovarian cell cancer line; [f]renal cancer cell line; [g]prostate cancer cell line; [h]breast cancer cell line; [i]selectivity index was calculated by dividing the IC_{50} value against NIH3T3 by the IC_{50} value against the least sensitive cell line.

4. Conclusions

A series of diarylureas and diarylamides possessing pyrrolo[3,2-c]pyridine scaffold was designed with structural similarity to Sorafenib, Regorafenib and synthesized. The fourteen final compounds were tested at a single-dose concentration of 10 μM at the NCI over 49 cancer cell line panel of eight different cancer types, and the most active twelve of them were subsequently tested in five-dose testing mode. Compounds 8a, 8d and 9a-h showed broad-spectrum anticancer activities with strong potencies. Among them, the diarylamides 9a-h exerted higher potencies than the diarylureas. So the amide linker is more favorable for antiproliferative activity of this series of compounds. Compounds 8a, 8d and 9a showed higher activity and stronger potencies than the corresponding pyrrolo[2,3-d]pyrimidine isosteres. Meta-disubstituted benzene derivatives were generally more potent than *para*-disubstituted benzene positional isomers. 3, 4-dichlorophenyl and 4-chloro-3-(trifluoromethyl) phenyl terminal moieties were found to be optimum for activity. So it can be concluded that those terminal moieties together with pyrrolo[3,2-c]pyridine nucleus carrying benzamido moiety at position 4 and *meta*-disubstituted benzene ring at position 1 and amide linker constitute the pharmacophore of this series of compound. The target compounds showed more promising potencies than Sorafenib. So the target compounds, especially diarylamides, can be considered as promising leads for future development of potential anticancer agents with high potency. Compounds 8a, 9b and 9e showed superior selectivity towards cancer cells than NIH3T3 fibroblasts. These three compounds are potential leads for design and development of highly potent and highly selective antiproliferative agents.

Acknowledgements

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