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Mohammed I. El-Gamal, Byung-Jun Park, Chang-Hyun Oh

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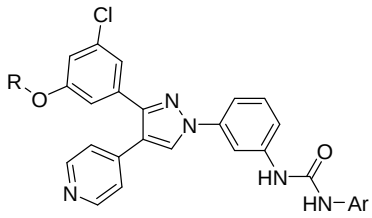
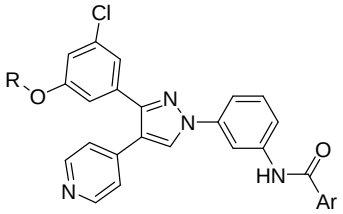
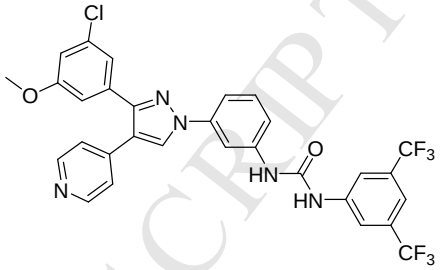


Ten urea target compounds



Ten amide target compounds

Biological testing



Compound 1e (the most promising hit)

Synthesis, *in vitro* antiproliferative activity, and kinase inhibitory effects of pyrazole-containing diarylureas and diarylamides

Mohammed I. El-Gamal[1,2,3], Byung-Jun Park[4], and Chang-Hyun Oh[4,5,*]

[1] Department of Medicinal Chemistry, College of Pharmacy, University of Sharjah, Sharjah 27272, United Arab Emirates.

[2] Sharjah Institute for Medical Research, University of Sharjah, Sharjah 27272, United Arab Emirates.

[3] Department of Medicinal Chemistry, Faculty of Pharmacy, University of Mansoura, Mansoura 35516, Egypt.

[4] Center for Biomaterials, Korea Institute of Science and Technology, PO Box 131, Cheongryang, Seoul 130-650, Republic of Korea.

[5] Department of Biomolecular Science, University of Science and Technology, 113 Gwahangno, Yuseong-gu, Daejeon 305-333, Republic of Korea.

***** Corresponding author.
E-mail addresses: choh@kist.re.kr; Phone No.: +82 2 958 5160; fax: +82 2 958 5308.
Address: Center for Biomaterials, Korea Institute of Science and Technology, PO Box 131, Cheongryang, Seoul 130-650, Republic of Korea [C.-H. Oh].

Abstract

Twenty pyrazole-containing diarylureas and diarylamides were designed and synthesized. They were tested for *in vitro* antiproliferative activity over a 58-cancer cell line panel at the NCI, USA. The diarylurea derivatives **1b-e** and **1g** exerted the strongest antiproliferative activity. Among them, compound **1e** possessing 3,5-bis(trifluoromethyl)phenyl terminal ring and 3`-methoxy-5`-chlorophenyl ring attached to the central pyrazole ring was the most potent. Its IC_{50} values were in sub-micromolar range against most of the tested cell lines. It showed superior potency than sorafenib, a reference diarylurea drug, over all the tested cell lines. It was also extremely selective towards cancer cells than non-cancerous cells (IC_{50} against RAW 264.7 macrophages was higher than 100 µM). At molecular level, compound **1e** selectively inhibited V600E mutated B-RAF kinase (IC_{50} = 0.39 µM). It also stimulated caspase 3/7 enzymes in RPMI-8226 leukemia cells (2.79 fold increase at 10 µM concentration, EC_{50} = 1.52 µM). So compound **1e** may kill cancer cells through induction of apoptosis. This promising candidate can be considered further for development of new efficient anticancer agents.

Keywords: Amide; Antiproliferative; Caspase; Kinase, Pyrazole; Urea.

1. Introduction

Cancer is one of the major issues that requires every research efforts in order to develop more efficient and safer drug candidates. It comes at the second rank after cardiovascular diseases as a leading cause of death worldwide. The World Health Organization (WHO) expects significant increase of the number of newly discovered cancer cases to exceed 15 million new cases worldwide every year [1,2]. Kinases are among the most interesting molecular targets for anticancer agents. They are over-expressed in cancer cells. Kinase inhibition deprives the tumor cell from very essential growth factors, and hence leads to cancer cell death.

Numerous pyrazole derivatives have been reported as anticancer agents [3-13]. Moreover, several diarylurea and diarylamide derivatives have been highlighted with promising anticancer activity [6-8, 10, 14-30]. Sorafenib and imatinib are examples of clinically-used diarylureas and diarylamides, respectively (Figure 1). Sorafenib has been approved by the U.S. Food and Drug Administration (FDA) for treatment of advanced renal cancer. It has also been under investigation in clinical trials against other types of cancer such as hepatocellular carcinoma (HCC), Hodgkin's lymphoma, metastatic colorectal, esophageal/gastroesophageal, brain, glioblastoma, ovarian, leukemia, metastatic breast, pancreatic, advanced gastric, thyroid, non-small cell lung cancer (NSCLC), prostate, bladder, skin/ocular melanoma, and neuroendocrine cancers [31,32]. Imatinib is used for treatment of chronic myeloid leukemia (CML) with minimal adverse effects [33]. Imatinib has been evaluated in clinical trials for treatment of thyroid cancer, breast cancer, gastrointestinal stromal tumors (GIST), ovarian cancer, meningioma, and non-small cell lung cancer (NSCLC) in combination with other drugs [34].

In this study, the target molecules were designed as hybrids of pyrazole and diarylurea/diarylamide moieties. Twelve of them were selected for *in vitro* antiproliferative screening over a panel of 58 human cancer cell lines of nine different cancer types at the National Cancer Institute (NCI, Bethesda, Maryland, USA). The most potent compound was further tested against a panel of kinases, and tested for ability to induce apoptosis in cancer cells. The results are presented in details in the next sections.

[Figure 1]

2. Results and discussion

2.1. Chemistry

Scheme 1 shows the synthetic pathway utilized to prepare the target compounds **1a-j** and **2a-j**. 3,5-Dichlorobenzoic acid (**3**) was heated with sodium methoxide in hexamethylphosphoric triamide (HMPA) to obtain 3-chloro-5-methoxybenzoic acid (**4**). Treatment of the acid **4** with methyl alcohol in presence of acetyl chloride gave the corresponding methyl ester **5** [35]. Compound **5** was treated with lithium bis(trimethylsilyl)amide (LiHMDS) and 4-picoline to produce the ketone **6**. The product **6** was reacted with dimethylformamide dimethylacetal (DMF-DMA), and the intermediate was treated with hydrazine to produce the 3,4-diarylpyrazole intermediate **7** [3, 17, 36, 37]. Compound **7** was heated with 1-iodo-3-nitrobenzene using cuprous iodide, potassium carbonate, and L-proline as catalysts to get the *N*-(*m*-nitrophenyl)pyrazole intermediate **8**, which was subsequently reduced to amino using hydrogen gas in presence of palladium over carbon. Treatment of the aniline derivative **9** with five different aryl isocyanate reagents yielded the corresponding diarylurea derivatives **1a-e**, while heating compound **9** with five different aryl carboxylic acids in presence of 1-hydroxybenzotriazole (HOBt), 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide (EDCI), and triethylamine (TEA) afforded the corresponding diarylamide derivatives **2a-e**. BBr_3 was used further to demethylate the methoxy compounds **1a-e** and **2a-e** to the corresponding hydroxyl derivatives **1f-j** and **2f-j** [3-12, 37]. Table 1 represents the exact structure of each target molecule and the yield percentages.

[Scheme 1 & Table 1]

2.2. Biological screening

2.2.1. In vitro antiproliferative activity

The NCI (Bethesda, Maryland, USA) selected twelve out of the twenty target compounds for testing their *in vitro* antiproliferative activity against a panel 58 cancer cell lines taken from 9 cancer types. They were initially tested at a single-dose concentration of 10 µM to find the percentage of growth inhibition effect on the cell lines. The mean inhibition percentages of the tested compounds over the NCI-58 panel are depicted in Figure 2.

The results indicated that the urea derivatives **1b**, **1c**, and **1e** were more active than the corresponding amide analogues **2b**, **2c**, and **2e**. This can be attributed to one or both of the following effects; the terminal *NH* of the urea linker might contribute to stronger affinity at the receptor site through additional hydrogen bond formation, and the longer linker may allow better molecular fitting at the receptor site. Moreover, three hydroxyl compounds (**1g**, **2i**, and **2j**) exerted better activity than the corresponding methoxy analogues (**1b**, **2d**, and **2e**), but the methoxy compound **1d** showed higher activity compared to its hydroxyl analogue **1i**. Upon investigating the terminal ring effect on the activity, it was found that compounds **1d**, **1e** and **1g** possessing 4`-chloro-3`-(trifluoromethyl)phenyl, 3`,5`-bis(trifluoromethyl)phenyl, and 3`,4`-dichlorophenyl, respectively, were the most active. These terminal rings carrying hydrophobic, bulky, and electron-withdrawing substituents may affect the activity through stronger affinity at the receptor site, enhancing the ability to penetrate the cancer cells, or through electronic effect. One or more of these effects can rationalize the stronger activity of those derivatives. Compound **2i** possessing 4`-morpholino-3`-(trifluoromethyl)phenyl terminal ring was the most active among the diarylamide derivatives. This can be rationalized that the steric and/or electronic effect(s) of these substituents in addition to their ability to form additional hydrogen bond(s) might enhance the potency.

[Figure 2]

The activities of each of the most active compounds **1d**, **1e**, and **1g** against each individual cell line of the tested panel are illustrated in Figure 3. The three compounds showed broad-spectrum activity against all the nine tested cancer types. At 10 µM, compounds **1d**, **1e**, and **1g** demonstrated lethal effects (≥ 100% growth inhibition) over 22, 30, and 35 cell lines, respectively.

[Figure 3]

Out of the twelve tested compounds at one-dose concentration, the most active ten molecules **1b-e**, **1g**, **1i**, **2c**, **2d**, **2i**, and **2j** were selected for further testing in a 5-dose assay in order to investigate their efficacy and potency. The mean IC_{50} values over the nine tested subpanels are summarized in Table 2. As discussed above, the urea derivatives are significantly more potent than the amides. All the urea analogues exerted sub-micromolar to one-digit micromolar mean IC_{50} values. Compound **1e**

with methoxy, urea, and bis(trifluoromethyl)phenyl moieties showed the strongest potency. All its mean IC_{50} values were in the sub-micromolar range. Its dose-response curves over all the tested cell lines of the nine different cancer types are shown in Figure 4. It showed very promising antiproliferative activity to lethal effect and broad-spectrum anticancer activity.

Upon comparison of the activity of compound **1e** with its positional isomer, 4`-chloro-3`-methoxyphenyl analogue [8], it is found that compound **1e** was more potent against all the nine cancer subpanels. Moreover, compound **1e** was more potent against most of the individual cell lines. So the presence of chloro and methoxy groups at positions 3` and 5` might be more optimal for stronger affinity at the receptor site.

[Table 2 & Figure 4]

For more elaboration on the antiproliferative activity of the target compounds, the IC_{50} values of the tested compounds over the most sensitive cell line of each cancer type are depicted in Table 3. The results were compared with those of sorafenib, diarylurea multikinase anticancer drug, as a reference standard. The urea analogues are again much more potent than the amides, and showed superior potencies than sorafenib. The strongest potency was given by compound **1e** with submicromolar IC_{50} values over the nine cell lines.

[Table 3]

The IC_{50} and TGI values of compound **1e** and sorafenib are summarized in Table 4. Compound **1e** was more potent than sorafenib against all the 58 tested cell lines. Most of its IC_{50} values were in the sub-micromolar scale (on 52 cell lines), while sorafenib did not show any sub-micromolar IC_{50} value. In addition, the TGI values of compound **1e** were less than those of sorafenib against most of the 58 tested cell lines. These findings indicate superior potency and efficacy of compound **1e** over the tested cell line panel. It is noteworthy that compound **1e** is super-selective towards cancer cells than non-cancerous cells. Its IC_{50} value against RAW 264.7 macrophages was higher than 100 µM [37].

[Table 4]

2.2.2. Kinase profiling

Due to the structural similarity between compound **1e** and sorafenib (both are diarylurea derivatives), and due to our previous reports of pyrazole derivatives as kinase inhibitors [6, 9-13], we decided to test it over a panel of 27 kinases as a part of our efforts to investigate its molecular mechanism of action. The inhibition percentage values at a single dose of 10 µM are summarized in Table 5. The compound is interestingly selective against V600E mutated B-RAF kinase with 94.72% inhibition. Its IC_{50} value is 0.39 µM. So inhibition of V600E-B-RAF kinase could be, at least in part, a mechanism of antiproliferative activity of compound **1e** at molecular level. Since V600E-B-RAF is over-expressed and is considered as an essential growth factor in several types of cancer such as melanoma, colorectal cancer, lung cancer, brain tumor, and many other types of cancer [10, 38], this can account for the broad-spectrum antiproliferative activity of compound **1e**.

[Table 5]

2.2.3. Caspase 3/7 assay

Compound **1e** was further tested for apoptotic activity in RPMI-8226 leukemia cells. We measured the caspase 3/7 enzyme levels in the cells after treatment with 10 different concentrations of the tested compound. The dose-response curve is illustrated in Figure 5. It showed promising induction of caspase 3/7 enzymes (2.79-fold increase at 10 µM concentration, EC_{50} = 1.52 µM). So caspase activity stimulation and induction of apoptosis could be another mechanism of the antiproliferative activity of compound **1e**.

[Figure 5]

3. Conclusion

The present article reports the design, synthesis, and biological evaluation at cellular and molecular levels of a series of triarylpyrazoles containing urea or amide linker. We found that the urea linker and 3`-chloro-5`-methoxyphenyl moieties are optimum for strong potency of this series of compounds. The results of this study led to discovery of a very promising compound, **1e**. It showed superior selectivity towards cancer over non-cancer cells. It was much more potent than sorafenib against the 58 tested cancer cell lines. Most of its IC_{50} values over the tested cancer cell lines were in sub-micromolar scale, while all the results of sorafenib were in the micromolar range. Furthermore, it is potent and selective against V600E-B-RAF

kinase (IC_{50} = 0.39 μM), and induced apoptosis in the tested leukemia cells. This compound is a promising candidate for future design and development of selective kinase inhibitors as potential anticancer agents. Further structural modification and optimization of this series of compounds is currently in progress.

4. Experimental

4.1. General

The target compounds **1a-j** and **2a-j** were purified by flash column chromatography using silica gel (0.040-0.063 mm, 230-400 mesh) and technical grade solvents. ^{1}H NMR and ^{13}C NMR analyses were conducted on a Bruker Avance 400 or 300 spectrometer using tetramethylsilane as an internal standard. Melting points were measured on a Stuart melting point apparatus (Staffordshire, UK), and are uncorrected. LC-MS analysis was conducted using the following system: Waters 2998 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} C18 column (4.6 x 50 mm, 5 μm particle size); Solvent gradient = 95% A at 0 min, 1% A at 5 min; solvent A: 0.035% trifluoroacetic acid (TFA) 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. Elemental analysis was done using FlashSmart elemental analyzer (Thermo Fisher Scientific, Waltham, MA, USA). The solvents and liquid reagents were transferred using hypodermic syringes. All the solvents and reagents were purchased from commercial companies, and used as such.

*4.2. Synthesis of the amine intermediate **9***

It was achieved through the synthetic pathway reported in the literature [37].

*4.3. Synthesis of the diarylurea derivatives **1a-e***

To a solution of compound **9** (50 mg, 0.1 mmol) in anhydrous THF (1 mL), a solution of the appropriate aryl isocyanate (0.1 mmol) in THF (1 mL) was added dropwise at room temperature under nitrogen atmosphere. The reaction mixture was stirred at room temperature overnight. The solvent was evaporated under reduced pressure, and the residue was purified by column chromatography (silica gel, hexane-ethyl acetate mobile phase system) to yield the title compounds.

Compound **1a**: mp: 226-9 ºC; ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.99 (s, 1H), 8.96 (s, 1H), 8.73 (s, 1H), 8.57 (d, 2H, *J* = 6.0 Hz), 8.17 (s, 1H), 7.53 (d, 1H, *J* = 7.6 Hz), 7.49-7.46 (m, 3H), 7.43 (d, 1H, *J* = 4.8 Hz), 7.38 (dd, 2H, *J* = 4.8 Hz, 1.2 Hz), 7.30 (t, 2H, *J* = 7.8 Hz), 7.11 (s, 1H), 7.11 (t, 1H, *J* = 2.0 Hz), 6.99 (d, 1H, *J* = 7.2 Hz), 6.97-6.96 (m, 1H), 3.75 (s, 3H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 160.6, 153.0, 150.4, 148.7, 141.5, 140.2, 139.9, 135.7, 134.5, 130.4, 129.7, 129.3, 123.2, 122.5, 120.6, 120.1, 118.9, 117.1, 114.4, 113.5, 112.3, 109.1, 56.1; LC-MS: 496.0 (M^+ + 1); elemental analysis: calculated C:67.81%, H:4.47%, N:14.12%; found: C:67.53%, H:4.49%, N:14.18%..

Compound **1b**: mp: 210-3 ºC; ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.14 (s, 1H), 9.06 (s, 1H), 8.95 (s, 1H), 8.56 (d, 2H, *J* = 6.0 Hz), 8.16 (s, 1H), 7.90 (d, 1H, *J* = 2.4 Hz), 7.57-7.51 (m, 2H), 7.48-7.40 (m, 2H), 7.38-7.35 (m, 3H), 7.13-7.10 (m, 2H), 6.95 (dd, 1H, *J* = 2.0 Hz, 1.2 Hz), 3.74 (s, 3H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 160.6, 152.8, 150.4, 148.7, 141.1, 140.3, 140.2, 139.9, 135.6, 134.5, 131.5, 131.0, 130.5, 129.7, 123.8, 123.2, 120.6, 120.2, 120.0, 119.0, 117.4, 114.4, 113.6, 112.7, 109.4, 56.1; LC-MS: 564.9 (M^+ + 2), 563.9 (M^+ + 1); elemental analysis: calculated C:59.54%, H:3.57%, N:12.40%; found: C:59.70%, H:3.34%, N:12.33%.

Compound **1c**: mp: 240-3 ºC; ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.16 (brs, 2H), 8.97 (s, 1H), 8.57 (d, 2H, *J* = 6.0 Hz), 8.17 (s, 1H), 8.03 (s, 1H), 7.62 (d, 1H, *J* = 8.0 Hz), 7.57-7.47 (m, 3H), 7.45 (s, 1H), 7.38 (d, 2H, *J* = 5.6 Hz), 7.32 (d, 1H, *J* = 7.6 Hz), 7.14 (s, 1H), 7.11 (t, 1H, *J* = 1.8 Hz), 6.96 (s, 1H), 3.75 (s, 3H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 160.6, 153.0, 150.4, 148.7, 141.1, 140.9, 140.2, 139.9, 135.7, 134.5, 130.5, 130.4, 129.7, 123.2, 122.5, 120.6, 120.1, 117.4, 114.8, 114.4, 113.5, 112.7, 109.4, 56.1; LC-MS: 564.0 (M^+ + 1); elemental analysis: calculated C:61.76%, H:3.75%, N:12.42%; found: C:61.96%, H:3.62%, N:12.29%.

Compound **1d**: mp: 213-5 ºC; ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.68 (brs, 2H), 8.95 (s, 1H), 8.56 (d, 2H, *J* = 5.6 Hz), 8.18 (s, 1H), 8.13 (d, 1H, *J* = 2.4 Hz), 7.71 (dd, 1H, *J* = 8.4 Hz, 1.6 Hz), 7.61 (d, 1H, *J* = 8.8 Hz), 7.55 (d, 1H, *J* = 4.0 Hz), 7.45 (d, 2H, *J* = 4.4 Hz), 7.38 (d, 2H, *J* = 6.0 Hz), 7.13-7.11 (m, 2H), 6.96 (s, 1H), 3.75 (s, 3H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 160.6, 153.1, 150.4, 148.7, 141.2, 140.2, 139.9, 135.7, 134.5, 132.4, 130.4, 129.7, 123.7, 123.2, 120.6, 120.1, 117.5, 117.4, 114.4, 113.5,

112.7, 109.5, 56.1; LC-MS: 598.8 (M^+ + 2), 597.8 (M^+ + 1); elemental analysis: calculated C:58.21%, H:3.37%, N:11.70%; found: C:58.24%, H:3.45%, N:11.45%.

Compound **1e**: mp: 221-4 ºC; ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.53 (brs, 2H), 8.90 (s, 1H), 8.56 (d, 2H, *J* = 6.0 Hz), 8.22-8.20 (m, 3H), 7.58 (s, 1H), 7.51 (d, 2H, *J* = 6.4 Hz), 7.43 (d, 1H, *J* = 8.0 Hz), 7.38 (d, 2H, *J* = 5.6 Hz), 7.13-7.10 (m, 2H), 6.96 (s, 1H), 3.74 (s, 3H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 160.6, 153.4, 150.3, 148.7, 141.4, 140.2, 139.8, 135.7, 134.5, 131.2, 130.9, 130.2, 129.6, 125.2, 123.1, 122.5, 120.6, 120.1, 118.3, 117.6, 114.4, 113.5, 112.7, 109.6, 56.1; LC-MS: 632.9 (M^+ + 2), 631.9 (M^+ + 1); elemental analysis: calculated C:57.02%, H:3.19%, N:11.08%; found: C:56.94%, H:3.08%, N:11.22%.

*4.4. Synthesis of the diarylamide derivatives **2a-e***

A mixture of the amine compound **9** (50 mg, 0.1 mmol), appropriate benzoic acid derivative (0.2 mmol), HOBt (36 mg, 0.3 mmol), and EDCI (38 mg, 0.2 mmol) in DMF (1.0 mL) was cooled to 0 °C under nitrogen atmosphere. Triethylamine (0.03 mL, 0.2 mmol) was added to the reaction mixture at the same temperature. The mixture was then stirred at 80 °C for 12 h. The reaction mixture was cooled and then partitioned between saturated saline (10 mL) and ethyl acetate (10 mL). The organic layer was separated, and the aqueous layer was extracted again with ethyl acetate (3 x 5 mL). The organic extract was washed with saturated saline (3 x 15 mL), and dried using anhydrous sodium sulfate. The organic solvent was evaporated, and the crude residue was purified by column chromatography (silica gel, hexane-ethyl acetate mobile phase system) to yield the title products.

Compound **2a**: mp: 173-5 ºC; ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.48 (brs, 1H), 8.97 (s, 1H), 8.55 (dd, 2H, *J* = 4.4 Hz, 1.6 Hz), 8.45 (t, 1H, *J* = 2.0 Hz), 7.99 (d, 2H, *J* = 6.8 Hz), 7.83 (dd, 1H, *J* = 8.0 Hz, 1.2 Hz), 7.65 (dd, 1H, *J* = 8.0 Hz, 1.6 Hz), 7.62-7.58 (m, 1H), 7.55-7.51 (m, 3H), 7.36 (dd, 2H, *J* = 4.4 Hz, 1.6 Hz), 6.94 (s, 1H), 3.72 (s, 3H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 166.2, 160.6, 150.4, 148.8, 140.9, 140.2, 139.7, 135.6, 135.1, 134.5, 132.3, 130.3, 129.7, 128.9, 128.2, 123.1, 120.6, 120.2, 119.1, 114.4, 114.0, 113.6, 111.3, 56.1; LC-MS: 481.1 (M^+ + 1); elemental analysis: calculated C:69.92%, H:4.40%, N:11.65%; found: C:69.75%, H:4.31%, N:11.70%.

Compound **2b**: mp: 242-5 ºC; ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.60 (s, 1H), 8.98 (s, 1H), 8.55 (d, 2H, *J* = 5.6 Hz), 8.40 (s, 1H), 8.25 (s, 1H), 7.96 (dd, 1H, *J* = 8.4 Hz, 2.0 Hz), 7.82 (d, 2H, *J* = 8.4 Hz), 7.67 (d, 1H, *J* = 8.0 Hz), 7.52 (t, 1H, *J* = 8.2 Hz), 7.36 (d, 2H, *J* = 5.6 Hz), 7.13 (s, 1H), 7.09 (s, 1H), 6.93 (s, 1H), 3.72 (s, 3H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 163.8, 160.6, 150.4, 140.5, 140.2, 139.7, 135.6, 135.3, 135.1, 131.8, 131.3, 130.4, 130.1, 129.7, 128.6, 123.1, 120.6, 120.2, 119.2, 114.4, 113.6, 111.3, 56.1; LC-MS: 549.0 (M^+ + 1); elemental analysis: calculated C:61.16%, H:3.48%, N:10.19%; found: C:61.05%, H:3.51%, N:10.10%.

Compound **2c**: mp: 193-5 ºC; ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.71 (brs, 1H), 9.01 (s, 1H), 8.57 (d, 2H, *J* = 4.8 Hz), 8.43 (s, 1H), 8.35 (s, 1H), 8.31 (d, 1H, *J* = 7.6 Hz), 7.99 (d, 1H, *J* = 7.6 Hz), 7.87 (d, 1H, *J* = 8.6 Hz), 7.81 (t, 1H, *J* = 8.0 Hz), 7.71 (d, 1H, *J* = 8.0 Hz), 7.56 (t, 1H, 8.0 Hz), 7.38 (d, 2H, *J* = 4.8 Hz), 7.15 (d, 1H, *J* = 1.2 Hz), 7.11 (s, 1H), 6.96 (s, 1H), 3.74 (s, 3H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 164.7, 160.6, 150.4, 148.8, 140.5, 140.1, 139.8, 135.9, 135.6, 134.5, 132.4, 130.4, 130.3, 129.7, 124.8, 124.7, 123.1, 120.6, 120.2, 119.3, 114.4, 114.3, 113.6, 111.4, 56.1; LC-MS: 549.1 (M^+ + 1); elemental analysis: calculated C:63.45%, H:3.67%, N:10.21%; found: C:63.41%, H:3.54%, N:10.33%.

Compound **2d**: mp: 250-3 ºC; ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.58 (brs, 1H), 8.99 (s, 1H), 8.55 (dd, 2H, *J* = 4.6 Hz, 1.4 Hz), 8.41 (t, 1H, 2.0 Hz), 8.28-8.26 (m, 2H), 7.83 (dd, 1H, *J* = 8.0 Hz, 1.2 Hz), 7.66 (t, 2H, *J* = 9.0 Hz), 7.53 (t, 1H, *J* = 8.2 Hz), 7.37 (dd, 2H, *J* = 4.4 Hz, 1.6 Hz), 7.13 (t, 1H, *J* = 1.6 Hz), 7.10 (t, 1H, 2.2 Hz), 6.94 (dd, 1H, *J* = 2.0 Hz, 1.2 Hz), 3.72-3.71 (m, 7H), 2.96 (t, 4H, *J* = 4.4 Hz); ^{13}C NMR (100 MHz, DMSO-d_6) δ 164.5, 160.6, 150.4, 150.3, 148.8, 140.7, 140.2, 139.7, 135.6, 134.5, 133.5, 130.7, 130.4, 129.7, 124.3, 123.1, 120.6, 120.2, 114.4, 113.6, 111.3, 66.9, 56.1, 53.6; LC-MS: 634.1 (M^+ + 1); elemental analysis: calculated C:62.51%, H:4.29%, N:11.05%; found: C:62.33%, H:4.18%, N:11.06%.

Compound **2e**: mp: 255-7 ºC; ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.88 (s, 1H), 9.01 (s, 1H), 8.65 (s, 2H), 8.55 (d, 2H, *J* = 6.0 Hz), 8.40 (t, 1H, *J* = 1.8 Hz), 8.38 (s, 1H), 7.87 (d, 1H, *J* = 8.0 Hz), 7.72 (dd, 1H, *J* = 8.0 Hz, 1.6 Hz), 7.57 (t, 1H, *J* = 8.2 Hz), 7.37 (d, 2H, *J* = 6.0 Hz), 7.14 (s, 1H), 7.10 (t, 1H, *J* = 2.0 Hz), 6.94 (s, 1H), 3.73 (s, 3H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 163.2, 160.6, 150.4, 148.9, 140.2, 140.1, 139.8, 137.3,

135.6, 134.5, 131.2, 130.8, 130.5, 129.7, 129.1, 124.9, 123.1, 122.2, 120.6, 120.3, 119.3, 114.5, 114.4, 113.6, 111.5, 56.1; LC-MS: 617.1 (M^+ + 1); elemental analysis: calculated C:58.40%, H:3.10%, N:9.08%; found: C:58.51%, H:3.13%, N:8.98%.

*4.5. Synthesis of the demethylated derivatives **1f-j** and **2f-j***

The methoxy compound **1a-e** or **2a-e** (0.1 mmol) was dissolved in anhydrous dichloromethane (1 mL), and boron tribromide (0.08 mL of a 1M solution in methylene chloride, 1.2 mmol) was added thereto dropwise at -78 °C under nitrogen atmosphere. The reaction mixture was stirred at the same temperature for 30 min, then stirred overnight at room temperature. The mixture was quenched with saturated aqueous sodium carbonate. Ethyl acetate (15 mL) was added, the mixture was stirred, and the organic layer was separated. The aqueous layer was extracted again with ethyl acetate (2 x 10 mL). The combined ethyl acetate extracts were washed with saturated saline solution, and then dried with anhydrous sodium sulfate. The organic solvent was evaporated under reduced pressure, and the remained crude product was purified by short column chromatography (silica gel, hexane-ethyl acetate mobile phase system) to yield the title compounds.

Compound **1f**: mp: 233-5 ºC; ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.10 (s, 1H), 8.98 (s, 1H), 8.95 (s, 1H), 8.71 (s, 1H), 8.57 (s, 2H), 8.18 (t, 1H, *J* = 2.0 Hz), 7.54-7.49 (m, 1H), 7.48 (d, 1H, *J* = 1.2 Hz), 7.46 (d, 1H, *J* = 0.8 Hz), 7.43 (d, 1H, *J* = 8.0 Hz), 7.39-7.37 (m, 3H), 7.30 (t, 2H, *J* = 8.0 Hz), 6.99-6.98 (m, 2H), 6.87-6.85 (m, 2H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 159.0, 153.0, 150.2, 149.0, 141.5, 140.4, 140.0, 135.6, 134.2, 130.4, 129.7, 129.3, 123.2, 122.5, 120.0, 119.0, 118.9, 117.0, 115.8, 114.5, 112.3, 109.0; LC-MS: 482.0 (M^+ + 1); elemental analysis: calculated C:67.29%, H:4.18%, N:14.53%; found: C:67.10%, H:4.01%, N:14.65%.

Compound **1g**: mp: 249-52 ºC; ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.11 (s, 1H), 9.18 (s, 1H), 9.10 (s, 1H), 8.94 (s, 1H), 8.56 (dd, 2H, *J* = 4.4 Hz, 1.6 Hz), 8.18 (t, 1H, *J* = 2.0 Hz), 7.91 (d, 1H *J* = 2.4 Hz), 7.56-7.52 (m, 2H), 7.46 (t, 1H, *J* = 8.0 Hz), 7.40-7.36 (m, 4H), 6.98 (t, 1H, *J* = 1.6 Hz), 6.87 (dt, 2H, *J* = 6.8 Hz, 1.9 Hz); ^{13}C NMR (100 MHz, DMSO-d_6) δ 159.0, 152.8, 150.4, 149.0, 141.1, 140.3, 140.2, 140.0, 135.6, 134.1, 131.5, 131.0, 130.4, 129.7, 123.8, 123.1, 120.0, 119.9, 119.0, 118.9, 117.3, 115.8, 114.5, 112.6, 109.3; LC-MS: 551.6 (M^+ + 2), 550.6 (M^+ + 1); elemental

analysis: calculated C:58.87%, H:3.29%, N:12.71%; found: C:58.81%, H:3.33%, N:12.62%.

Compound **1h**: mp: 205-6 ºC; ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.10 (brs, 1H), 9.15 (d, 2H, *J* = 6.8 Hz), 8.95 (s, 1H), 8.57 (d, 2H, *J* = 5.6 Hz), 8.18 (s, 1H), 8.03 (s, 1H), 7.62 (d, 1H, *J* = 8.0 Hz), 7.55 (t 1H, *J* = 6.4 Hz), 7.51-7.40 (m, 3H), 7.38 (d, 2H, *J* = 5.6 Hz), 7.33 (d, 1H, *J* = 7.6 Hz), 6.98 (s, 1H), 6.86 (dd, 2H, *J* = 4.8 Hz, 2.0 Hz); ^{13}C NMR (100 MHz, DMSO-d_6) δ 159.0, 153.0, 150.4, 149.0, 141.1, 140.9, 140.2, 140.0, 135.6, 134.2, 130.5, 130.4, 129.7, 123.1, 122.5, 120.0, 119.0, 118.8, 117.4, 115.8, 114.8, 114.5, 112.6, 109.3; LC-MS: 550.1 (M^+ + 1); elemental analysis: calculated C:61.15%, H:3.48%, N:12.73%; found: C:61.09%, H:3.45%, N:12.80%.

Compound **1i**: mp: 225-8 ºC; ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.32-10.28 (m, 3H), 8.90 (s, 1H), 8.53 (dd, 2H, *J* = 4.6 Hz, 1.4 Hz), 8.18-8.15 (m, 2H), 7.74 (d, 1H, *J* = 8.8 Hz), 7.59 (d, 1H, *J* = 8.8 Hz), 7.52-7.46 (m, 3H), 7.43 (d, 1H, *J* = 8.0 Hz), 7.38 (dd, 2H, *J* = 4.4 Hz, 1.6 Hz), 6.75-6.74 (m, 3H); LC-MS: 584.0 (M^+ + 1); elemental analysis: calculated C:57.55%, H:3.10%, N:11.98%; found: C:57.48%, H:3.02%, N:12.15%.

Compound **1j**: mp: 181-4 ºC; ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.09 (brs, 1H), 9.49 (brs, 1H), 9.36 (brs, 1H), 8.96 (s, 1H), 8.57 (d, 2H, *J* = 4.8 Hz), 8.17 (brs, 2H), 7.67 (s, 1H), 7.58 (d, 1H, *J* = 7.2 Hz), 7.50-7.44 (m, 2H), 7.38 (d, 2H, *J* = 5.6 Hz), 6.98 (s, 1H), 6.87-6.84 (m, 2H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 159.0, 152.9, 150.4, 149.0, 142.2, 140.8, 140.2, 140.0, 135.6, 134.2, 131.4, 130.5, 129.7, 123.1, 122.4, 120.0, 119.0, 118.7, 117.8, 115.8, 114.5, 113.0, 109.7; LC-MS: 618.1 (M^+ + 1); elemental analysis: calculated C:56.37%, H:2.94%, N:11.33%; found: C:56.21%, H:2.80%, N:11.55%.

Compound **2f**: mp: >265 ºC; ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.51 (s, 1H), 10.11 (s, 1H), 8.99 (s, 1H), 8.58 (dd, 2H, *J* = 4.8 Hz, 1.6 Hz), 8.49 (t, 1H, *J* = 2.0 Hz), 8.03-8.01 (m, 2H), 7.85 (dd, 1H, *J* = 8.2 Hz, 1.0 Hz), 7.68 (dd, 1H, *J* = 8.2 Hz, 1.4 Hz), 7.64-7.53 (m, 4H), 7.40 (dd, 2H, *J* = 4.4 Hz, 1.6 Hz), 7.01 (t, 1H, *J* = 1.6 Hz), 6.88-6.86 (m, 2H); ^{13}C NMR (75 MHz, DMSO-d_6) δ 166.4, 159.0, 149.3, 148.9, 140.8, 139.6, 135.4, 135.0, 134.2, 132.3, 130.4, 130.0, 128.9, 128.1, 123.4, 119.7, 119.3,

119.1, 115.9, 114.6, 114.1, 111.4; LC-MS: 467.1 (M^+ + 1); elemental analysis: calculated C:69.45%, H:4.10%, N:12.00%; found: C:69.61%, H:3.95%, N:11.87%.

Compound **2g**: mp: 234-6 ºC; ^{1}H NMR (300 MHz, DMSO-d_6) δ 10.62 (s, 1H), 8.98 (s, 1H), 8.56 (dd, 2H, *J* = 4.5 Hz, 1.6 Hz), 8.43 (d, 1H, *J* = 1.8 Hz), 8.27 (d, 1H, *J* = 2.0 Hz), 7.98 (dd, 1H, *J* = 2.0 Hz, 8.4 Hz), 7.84 (d, 2H, *J* = 8.4 Hz), 7.69 (d, 1H, *J* = 7.7 Hz), 7.54 (t, 1H, *J* = 8.1 Hz), 7.38 (dd, 2H, *J* = 4.5 Hz, 1.6 Hz), 6.99 (s, 1H), 6.87-6.84 (m, 2H); ^{13}C NMR (75 MHz, DMSO-d_6) δ 163.8, 159.1, 150.4, 149.1, 140.5, 140.2, 139.8, 135.3, 134.1, 131.8, 131.3, 130.4, 130.1, 129.7, 128.6, 123.1, 120.1, 119.1, 118.9, 115.8, 114.6, 111.3; LC-MS: 535.0 (M^+ + 1); elemental analysis: calculated C:60.52%, H:3.20%, N:10.46%; found: C:60.41%, H:3.15%, N:10.57%.

Compound **2h**: mp: 178-81 ºC; ^{1}H NMR (300 MHz, DMSO-d_6) δ 10.76 (brs, 1H), 8.98 (s, 1H), 8.55-8.53 (m, 2H), 8.43 (d, 1H, *J* = 1.8 Hz), 8.35-8.30 (m, 2H), 7.98 (1H, d, *J* = 6.9 Hz), 7.88-7.77 (m, 2H), 7.69 (d, 1H, *J* = 7.5 Hz), 7.54 (t, 1H, *J* = 8.1 Hz), 7.38-7.36 (m, 2H), 6.89-6.80 (m, 3H); LC-MS: 535.0 (M^+ + 1); elemental analysis: calculated C:62.87%, H:3.39%, N:10.47%; found: C:62.95%, H:3.48%, N:10.26%.

Compound **2i**: mp: 216-8 ºC; ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.61 (s, 1H), 8.84 (s, 1H), 8.49 (dd, 2H, *J* = 4.6 Hz, 1.4 Hz), 8.34 (s, 1H), 8.24-8.19 (m, 2H), 7.71 (d, 1H, *J* = 8.0 Hz), 7.63-7.50 (m, 3H), 7.35 (dd, 2H, *J* = 4.8 Hz, 1.6 Hz), 6.93 (t, 1H, *J* = 1.6 Hz), 6.86 (t, 1H, *J* = 2.0 Hz), 6.80 (t, 1H, *J* = 1.6 Hz), 3.74 (t, 4H, *J* = 4.2 Hz), 2.98 (t, 4H, *J* = 4.2 Hz); ^{13}C NMR (100 MHz, DMSO-d_6) δ 165.0, 158.8, 150.0, 149.2, 140.5, 140.2, 139.6, 135.4, 134.3, 133.4, 130.3, 129.5, 124.1, 123.3, 122.9, 120.0, 119.1, 115.9, 114.5, 111.5, 66.8, 53.4; LC-MS: 620.1 (M^+ + 1); elemental analysis: calculated C:61.99%, H:4.06%, N:11.30%; found: C:62.04%, H:4.01%, N:11.20%.

Compound **2j**: mp: >265 ºC; ^{1}H NMR (300 MHz, DMSO-d_6) δ 10.87 (s, 1H), 9.00 (s, 1H), 8.67 (s, 2H), 8.57 (d, 2H, *J* = 4.8 Hz), 8.41 (d, 2H, *J* = 10.7 Hz), 7.88 (d, 1H, *J* = 7.9 Hz), 7.73 (d, 1H, *J* = 8.0 Hz), 7.57 (t, 1H, *J* = 8.1 Hz), 7.38 (d, 2H, *J* = 4.9 Hz), 6.97 (s, 1H), 6.86 (d, 2H, *J* = 4.9 Hz); ^{13}C NMR (75 MHz, DMSO-d_6) δ 163.2, 159.2, 150.4, 149.2, 140.2, 139.8, 137.3, 135.5, 134.1, 131.2, 130.8, 130.4, 129.6, 129.1, 125.4, 123.1, 121.8, 120.1, 119.2, 118.8, 115.9, 114.6, 114.4, 111.4; LC-MS: 603.0

(M^+ + 1); elemental analysis: calculated C:57.77%, H:2.84%, N:9.29%; found: C:57.49%, H:2.74%, N:9.50%.

4.6. Antiproliferative screening

It was conducted at the National Cancer Institute (NCI, Bethesda, Maryland, USA) following their standard protocol [https://dtp.cancer.gov/discovery_development/nci-60/methodology.htm].

4.7. Kinase screening

It was carried out at Reaction Biology Corp. (Malvern, PA, USA) [http://www.reactionbiology.com] following the procedure previously reported in our published article [39].

4.8. Caspase 3/7 assay

It was conducted at Reaction Biology Corp. (Malvern, PA, USA) [http://www.reactionbiology.com] following the procedure previously reported in our published article [13].

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Supplementary file

^{1}H NMR, ^{13}C NMR, and LC-MS spectra as well as the NCI result charts of the target compounds can be found at

The authors have declared no conflict of interest

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Illustration captions:

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

Table 2. Mean IC_{50} values (μM) of the tested compounds against NCI cell line subpanels.

Table 3. IC_{50} values (μM) of the tested compounds and sorafenib against the most sensitive cell line of each subpanel.

Table 4. IC_{50} and TGI values (μM) of compound **1e** and sorafenib over the NCI cancer cell line panel.

Table 5. Inhibitory effects of compound **1e** against a panel of 27 kinases.

Figure 1. Design rationale of the target compounds **1a-j** and **2a-j**.

Figure 2. Mean inhibition percentage of the twelve tested compounds against the NCI-58 cancer cell line panel.

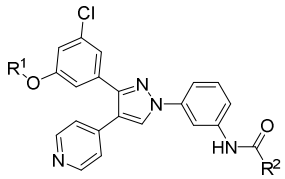
Figure 3. %inhibition exerted by a single-dose concentration of 10 μM of each of compound **1d** (Fig. 3a), compound **1e** (Fig. 3b), and compound **1g** (Fig. 3c) against all the NCI-58 cancer cell lines.

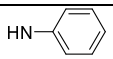
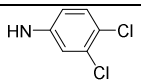
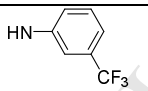
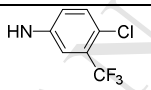
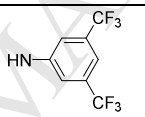
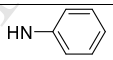
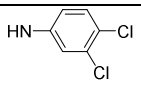
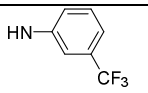
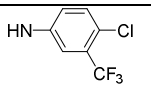
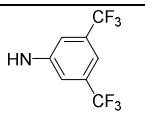
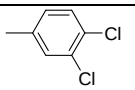
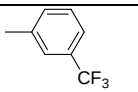
Figure 4. Dose-response curves of compound **1e** over the NCI-58 cancer cell line panel of the nine cancer types.

Figure 5. Caspase-3/7 activity in RPMI-8226 leukemia cells after treatment with different concentrations of compound **1e**.

Scheme 1. Reagents, reaction conditions, and yield percentages: a) 1) sodium methoxide, HMPA, 115-120 °C, 15 h, 2) aq. HCl, 54%; b) acetyl chloride, CH_3OH, rt, 15 h, 89%; c) 4-picoline, LiHMDS, THF, rt, overnight, 77%; d) DMF-DMA, rt, 18 h; e) hydrazine monohydrate, C_2H_5OH, rt, overnight, 72%; f) 1-iodo-3-nitrobenzene, K_2CO_3, CuI, L-proline, DMSO, 90 °C, 8 h, 59%; g) H_2 (g), 10% Pd/C, THF, rt, 2 h, 87%; h) appropriate aryl isocyanate, THF, rt, 12 h, 24~85%; i) BBr_3, CH_2Cl_2, -78 °C, 30 min; rt, 1 h, 24~89% **(1f-j)** and 28~79% (**2f-j**); j) appropriate benzoic acid derivative, HOBt, EDCI, TEA, DMF, 80 °C, 12 h, 28~79%.

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



Compound No.	R^1	R^2	Yield%
1a	CH_3		24%
1b	CH_3		60%
1c	CH_3		62%
1d	CH_3		85%
1e	CH_3		82%
1f	H		24%
1g	H		89%
1h	H		62%
1i	H		79%
1j	H		72%
2a	CH_3		65%
2b	CH_3		65%
2c	CH_3		59%

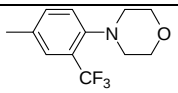
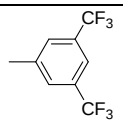
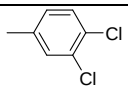
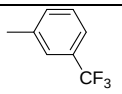
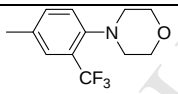
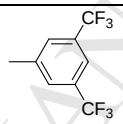
2d	CH_3		79%
2e	CH_3		58%
2f	H		28%
2g	H		65%
2h	H		58%
2i	H		79%
2j	H		75%

Table 2. Mean IC_{50} values (μM) of the tested compounds against NCI cell line subpanels[a]

Cancer Subpanel	No. of cell line in each subpanel	Compound No.									
		1b	**1c**	**1d**	**1e**	**1g**	**1i**	**2c**	**2d**	**2i**	**2j**
Leukemia	6	1.67	1.11	**0.92**	**0.32**	2.84	2.36	10.56	3.54	**0.63**	2.48
NSCLC[b]	8	2.48	1.64	1.60	**0.49**	2.08	2.50	45.36	39.85	5.99	6.47
Colon	7	2.46	1.35	1.27	**0.44**	2.20	2.62	22.11	11.49	4.13	2.89
CNS	6	2.49	1.78	1.68	**0.66**	2.06	2.34	52.68	35.51	2.67	4.47
Melanoma	8	2.23	1.69	1.66	**0.61**	1.77	1.98	49.12	19.86	1.64	3.53
Ovarian	7	3.05	2.20	1.89	**0.69**	2.65	3.26	59.99	48.26	20.43	6.70
Renal	8	2.48	1.67	1.77	**0.55**	1.89	2.64	22.06	19.50	4.67	3.64
Prostate	2	2.49	1.51	1.10	**0.38**	2.25	2.69	51.36	51.65	3.37	3.74
Breast	6	2.58	1.94	1.89	**0.86**	2.20	2.43	35.75	21.59	3.14	3.57

[a] The 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] Non-Small Cell Lung Cancer.

Table 3. IC_{50} values (μM) of the tested compounds and sorafenib against the most sensitive cell line of each subpanel.

		Cancer Cell Lines								
		RPMI-8226[a]	A549/ATCC[b]	HCT-116[c]	U251[d]	UACC-62[e]	OVCAR-3[f]	UO-31[g]	PC-3[h]	MDA-MB-468[i]
Compound No.	**1b**	**0.65**	**1.48**	2.02	**1.70**	2.40	**1.56**	**1.89**	**1.13**	**1.74**
	1c	**0.43**	**0.73**	**0.75**	**0.88**	**1.47**	**0.59**	**1.03**	**0.42**	**1.05**
	1d	**0.40**	**0.68**	**0.63**	**0.73**	**1.27**	**0.45**	**1.66**	**0.34**	**0.99**
	1e	**0.24**	**0.37**	**0.33**	**0.32**	**0.36**	**0.33**	**0.26**	**0.30**	**0.34**
	1g	**1.44**	**1.96**	1.74	**1.44**	1.69	**1.96**	**1.91**	**1.69**	**1.71**
	1i	1.95	**2.33**	1.96	**1.72**	2.08	**2.34**	**2.10**	2.10	2.08
	2c	3.13	3.62	3.17	5.56	8.04	7.69	2.71	2.71	4.26
	2d	**1.50**	9.15	9.64	5.03	9.10	53.10	4.50	3.30	12.10
	2i	**0.40**	**1.64**	1.96	**1.40**	1.72	7.20	**1.41**	**1.09**	3.42
	2j	2.50	**2.62**	3.23	3.23	3.79	3.28	**2.50**	2.28	3.10
	Sorafenib	1.58	3.16	1.58	2.00	1.58	3.16	2.51	2.00	2.00

[a] Leukemia cell line; [b] non-small cell lung cancer cell line; [c] colon cancer cell line; [d] CNS cancer cell line; [e] melanoma cell line; [f] ovarian cancer cell line; [g] renal cancer cell line; [h] prostate cancer cell line; [i] breast cancer cell line.
Bold figures indicate superior potency than the reference compounds.

Table 4. IC_{50} and TGI values (μM) of compound **1e** and sorafenib over the NCI cancer cell line panel

Cancer Type	Cell Line	Comp. 1e		Sorafenib	
		IC_{50}[a]	TGI[b]	IC_{50}[a]	TGI[b]
Leukemia	CCRF-CEM	0.27	1.33	2.00	5.01
	HL-60(TB)	0.33	3.24	1.58	100
	K-562	0.35	5.84	3.16	NT[c]
	MOLT-4	0.31	1.64	3.16	100
	RPMI-8226	0.24[d]	0.76	1.58[d]	3.16
	SR	0.45	3.31	3.16	63.10
NSCLC	A549/ATCC	0.37[d]	4.68	3.16[d]	7.94
	HOP-62	0.65	2.39	2.00	3.16
	HOP-92	0.23	0.78	1.58	5.01
	NCI-H226	0.70	5.17	2.00	3.16
	NCI-H23	0.33	1.60	2.00	5.01
	NCI-H322M	0.93	10.90	2.51	5.01
	NCI-H460	0.38	1.76	2.51	5.01
	NCI-H522	0.35	1.76	2.00	5.01
Colon Cancer	COLO 205	0.45	1.88	2.00	3.16
	HCC-2998	0.62	4.95	3.16	NT[c]
	HCT-116	0.33[d]	1.27	1.58[d]	2.51
	HCT-15	0.35	2.25	2.51	3.16
	HT29	0.45	2.21	2.00	5.01
	KM12	0.51	2.75	1.58	2.51
	SW-620	0.39	2.00	2.51	5.01
CNS Cancer	SF-268	0.62	3.25	2.51	3.98
	SF-295	0.33	1.44	1.58	6.31
	SF-539	1.41	3.49	1.58	2.51
	SNB-19	0.42	11.30	3.16	3.98
	SNB-75	0.85	5.83	3.16	3.98
	U251	0.32[d]	1.31	2.00[d]	3.16
Melanoma	MALME-3M	0.53	3.57	2.00	39.81

	M14	0.44	2.16	2.00	3.16
	MDA-MB-435	0.43	2.03	1.58	5.01
	SK-MEL-2	0.66	2.70	2.00	6.31
	SK-MEL-28	0.90	4.93	2.51	2.51
	SK-MEL-5	1.14	2.39	1.58	2.51
	UACC-257	0.41	1.84	2.00	5.01
	UACC-62	0.36[d]	1.50	1.58[d]	2.51
Ovarian Cancer	IGROV1	0.57	12.60	2.51	10.00
	OVCAR-3	0.33[d]	1.41	3.16[d]	3.98
	OVCAR-4	0.50	21.40	3.16	25.12
	OVCAR-5	1.46	12.80	3.16	6.31
	OVCAR-8	0.39	2.19	3.16	12.59
	NCI/ADR-RES	0.82	9.98	2.51	7.94
	SK-OV-3	0.77	5.75	2.51	5.01
Renal Cancer	786-0	0.57	2.17	3.16	5.01
	A498	0.39	3.59	2.51	3.16
	ACHN	0.40	12.50	2.51	10.00
	CAKI-1	0.42	4.75	3.16	12.59
	RXF 393	1.26	3.92	3.16	5.01
	SN12C	0.39	1.98	2.51	3.16
	TK-10	0.68	14.60	3.98	10.00
	UO-31	0.26[d]	1.58	2.51[d]	6.31
Prostate Cancer	PC-3	0.30[d]	1.63	2.00[d]	5.01
	DU-145	0.47	10.60	3.16	7.94
Breast Cancer	MCF7	0.38	9.32	2.51	7.94
	MDA-MB-231/ATCC	0.46	1.86	1.26	3.16
	HS 578T	1.40	7.26	2.51	3.98
	BT-549	2.22	7.14	3.16	3.98
	T-47D	0.39	4.16	1.58	5.01
	MDA-MB-468	0.34[d]	2.52	2.00[d]	5.01

[a] IC_{50} is the concentration producing 50% inhibition.

[b] TGI is the concentration producing 100% inhibition.

[c] NT means that the compound was not tested by the NCI against this cell line.

[d] Data taken from Table 3.

Table 5. Inhibitory effects of compound **1e** against a panel of 27 kinases.

Kinase	% inhibition at 10 µM dose[a]
ABL1	-12.53±0.37
AKT1	-5.92±1.11
ALK	-3.77±0.09
ARAF	49.22±1.10
Aurora A	-15.10±0.34
B-RAF (wild type)	39.10±0.47
c-Kit	1.25±0.81
c-MET	5.66±2.96
c-RAF	57.77±1.36
DNA-PK	3.50±0.40
EGFR	-7.47±0.18
ERBB2/HER2	-14.82±1.23
ERBB4/HER4	0.90±0.77
ERK2/MAPK1	-8.66±2.25
FGFR1	-1.71±1.06
FLT3	-2.82±0.81
FMS	-0.94±1.71
JAK1	10.70±0.20
JAK2	-2.88±0.03
JNK1	2.27±0.68
KDR/VEGFR2	-5.83±3.20
MEK1	-7.99±0.30
mTOR/FRAP1	-15.11±0.64
P38α/MAPK14	-5.41±5.28
PDGFRb	-9.26±0.27
PI3K (p110a/p85a)	16.78±0.21
V600E-B-RAF	94.72±0.22 **(IC_{50} = 0.39 µM)**

[a] Inhibition percentage values expressed as a mean of duplicate measurements ± S.E.M.

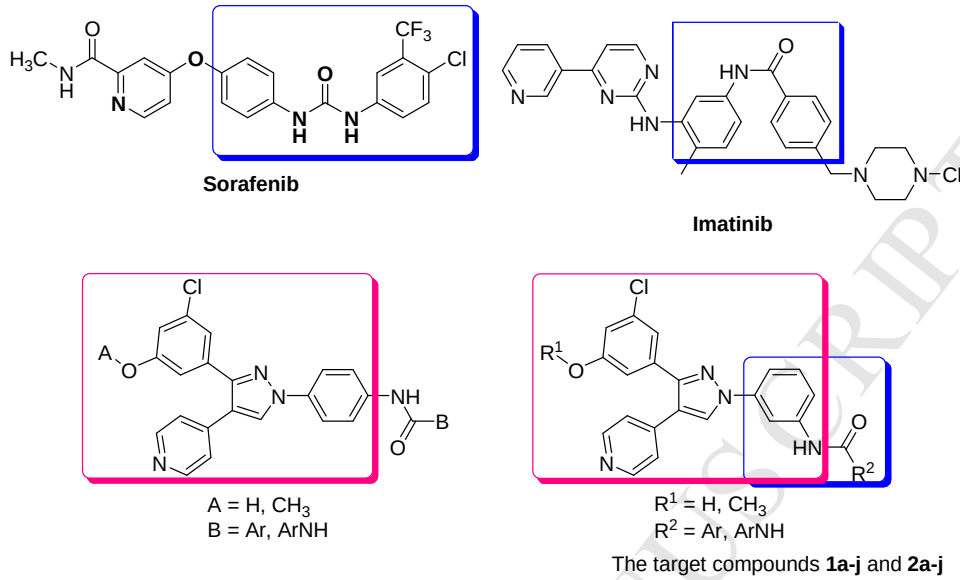


Figure 1. Design rationale of the target compounds **1a-j** and **2a-j**.

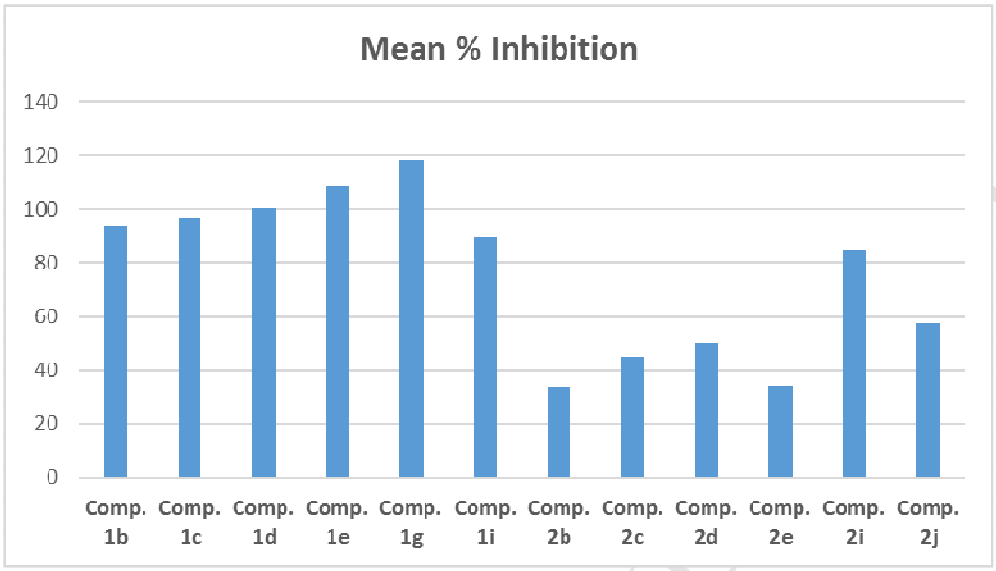
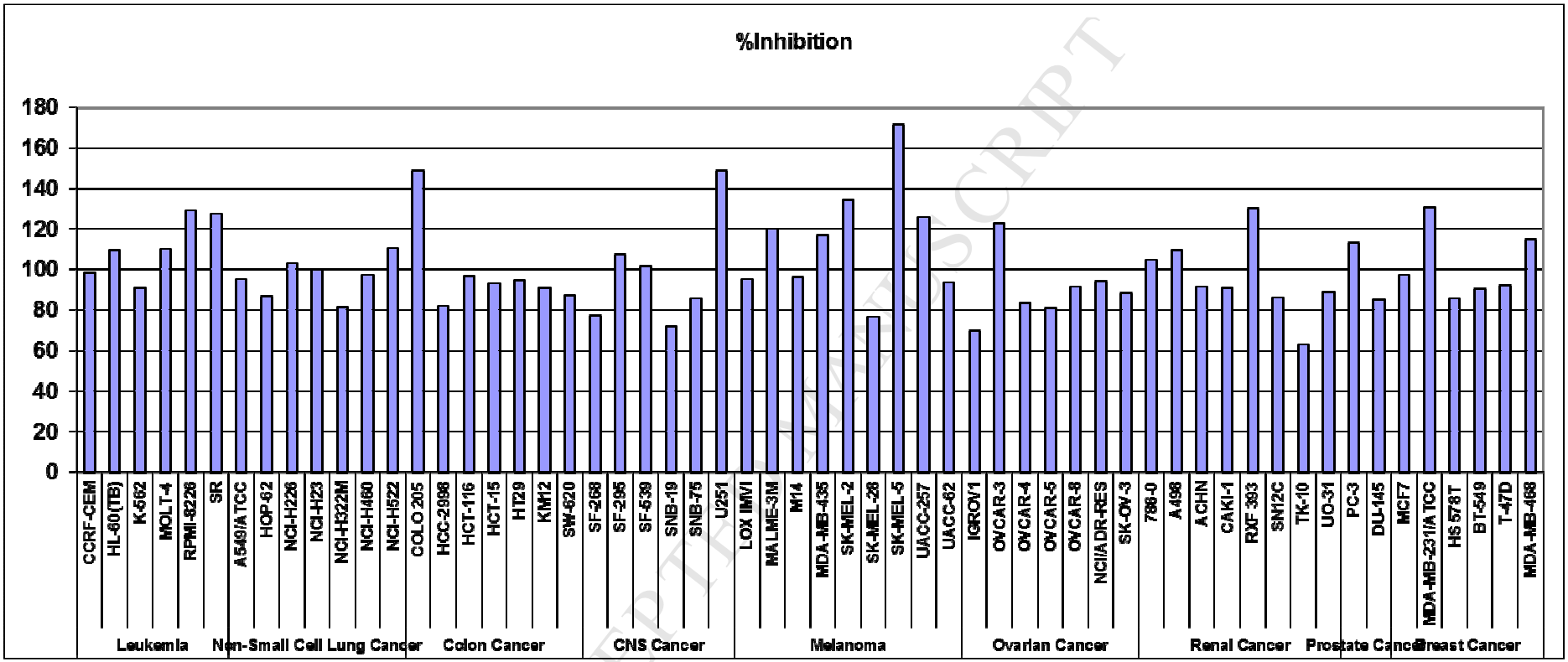
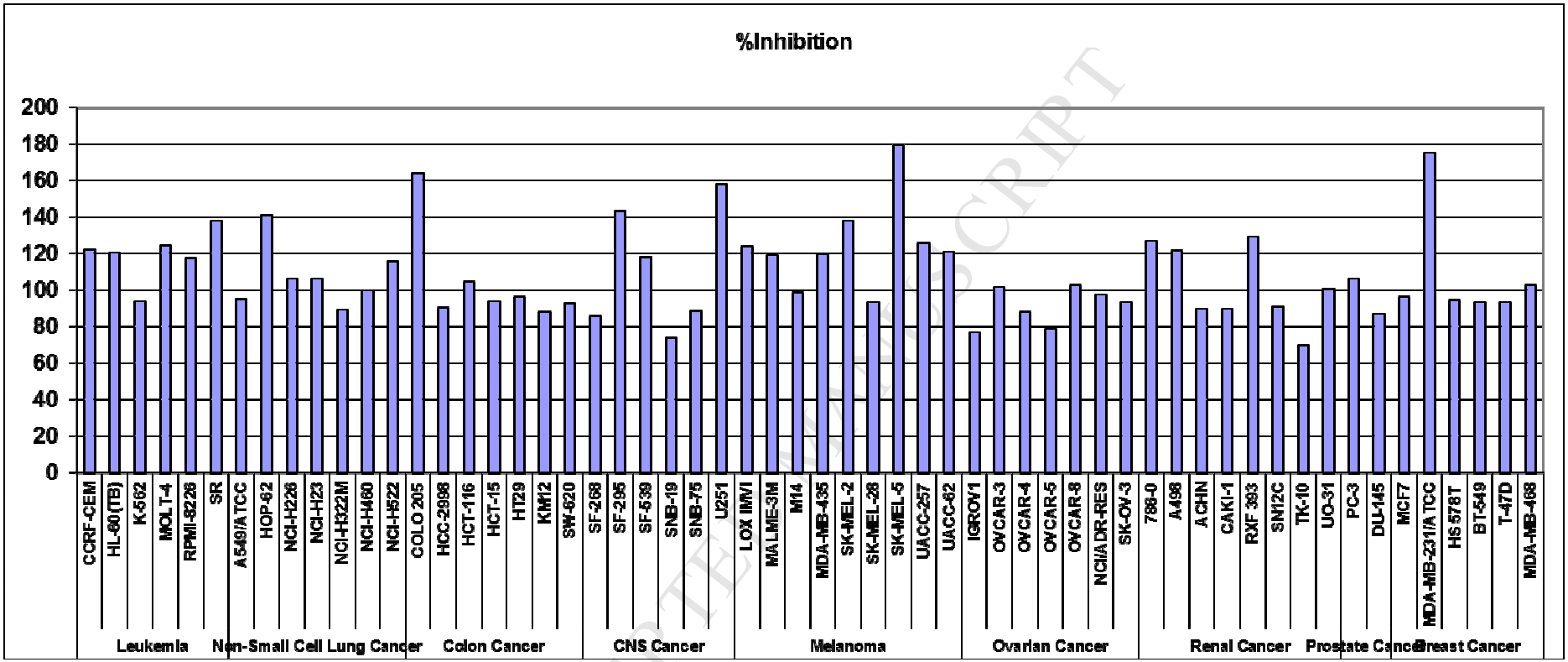


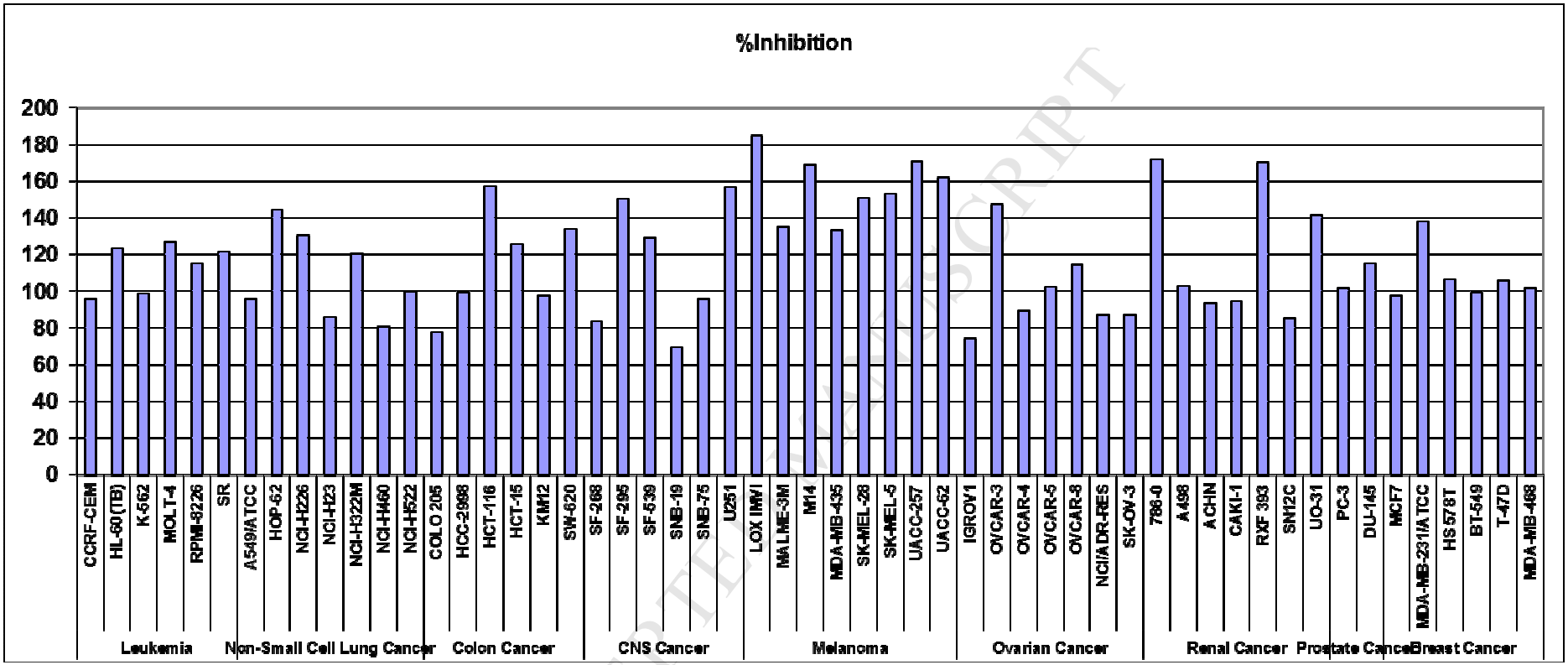
Figure 2. Mean inhibition percentage of the twelve tested compounds against the NCI-58 cancer cell line panel.



(a) Compound **1d**



(b) Compound **1e**



(c) Compound **1g**

Figure 3. %inhibition exerted by a single-dose concentration of 10 µM of each of compound **1d** (Fig. 3a), compound **1e** (Fig. 3b), and compound **1g** (Fig. 3c) against all the NCI-58 cancer cell lines.

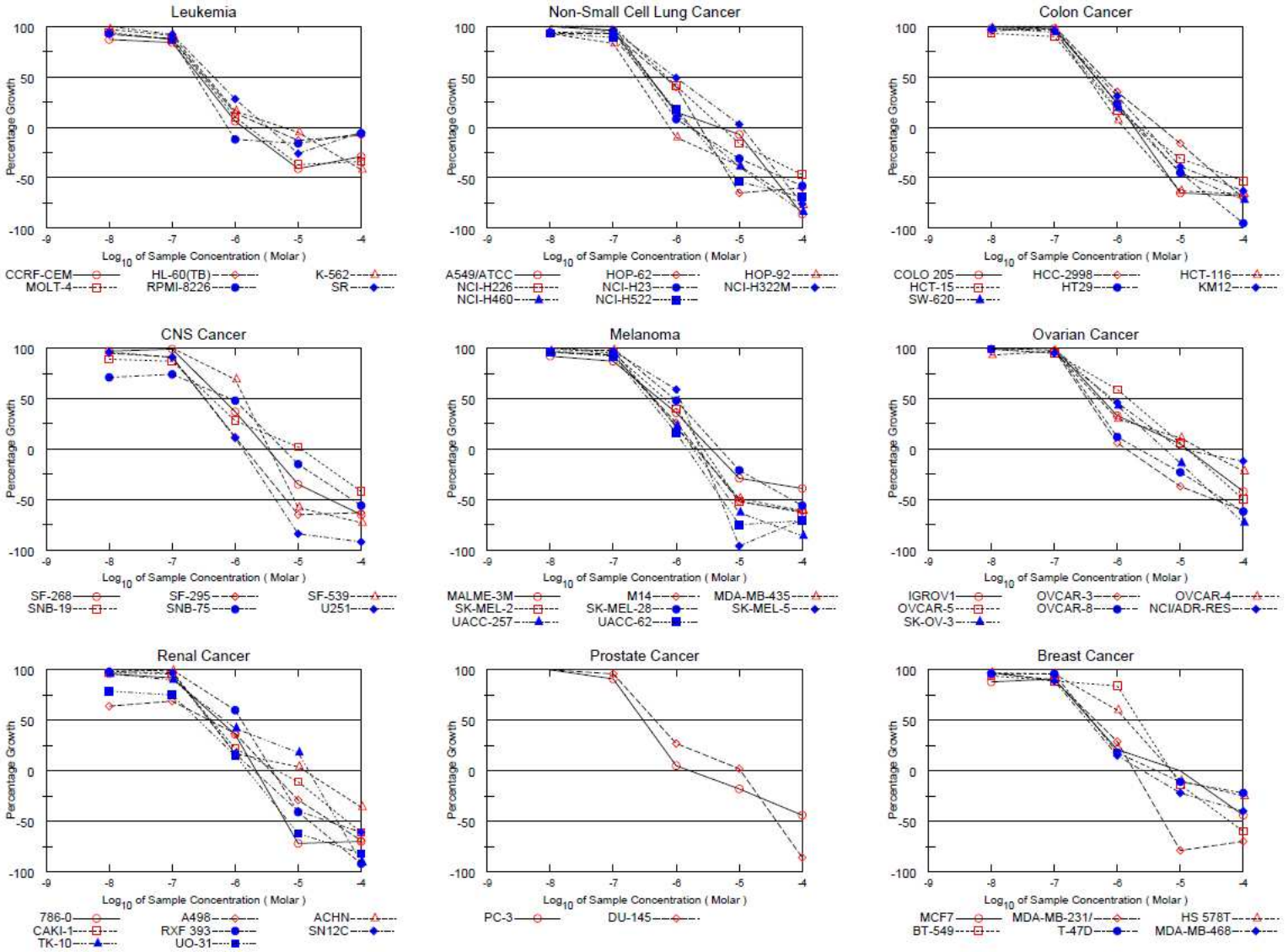


Figure 4. Dose-response curves of compound **1e** over the NCI-58 cancer cell line panel of the nine cancer types.

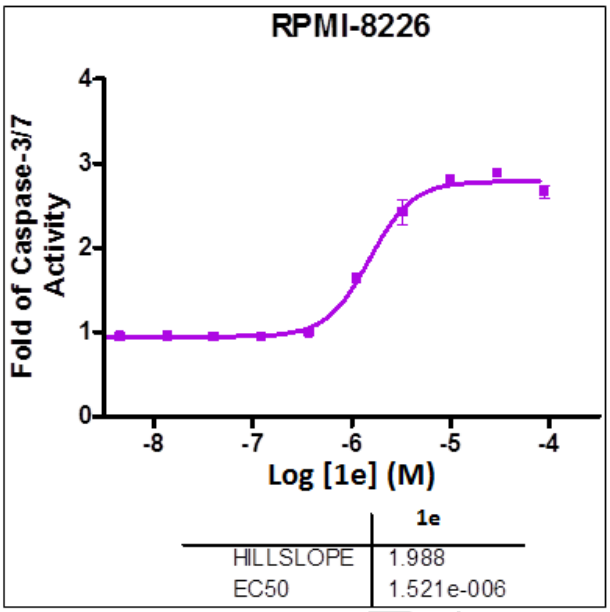
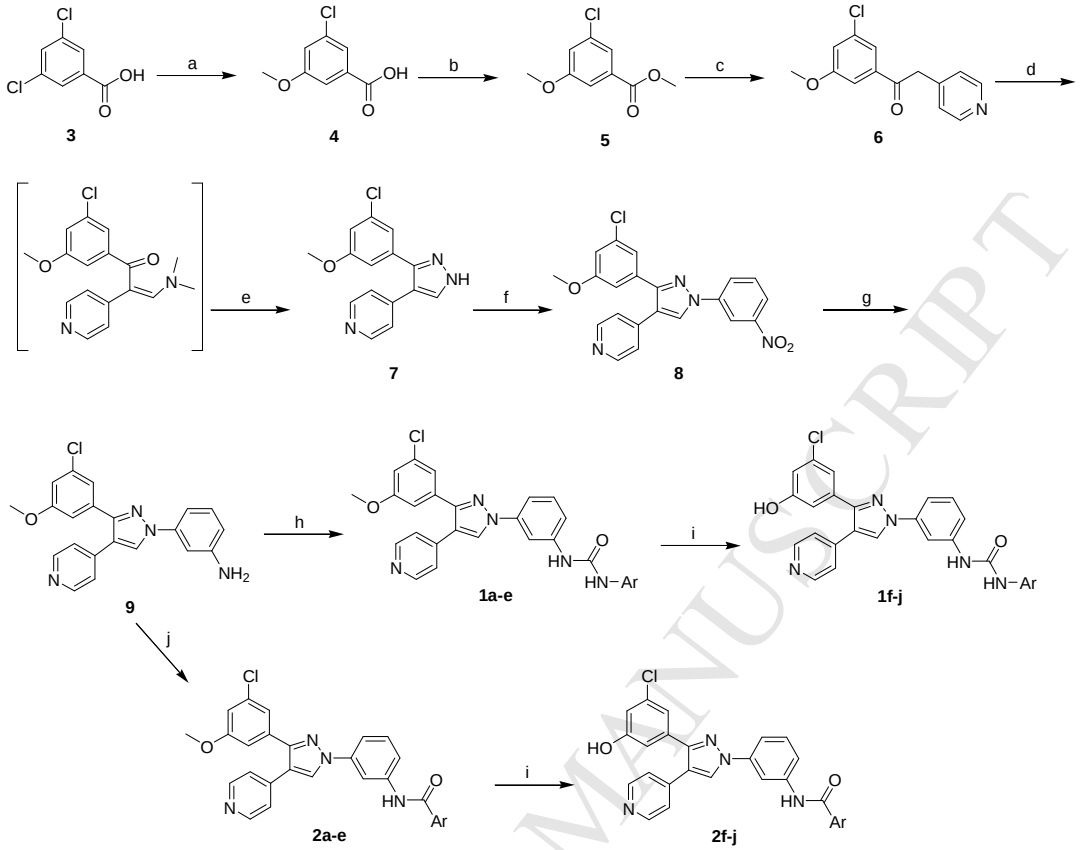


Figure 5. Caspase-3/7 activity in RPMI-8226 leukemia cells after treatment with different concentrations of compound **1e**.



Scheme 1. Reagents, reaction conditions, and yield percentages: a) 1) sodium methoxide, HMPA, 115-120 °C, 15 h, 2) aq. HCl, 54%; b) acetyl chloride, CH_3OH, rt, 15 h, 89%; c) 4-picoline, LiHMDS, THF, rt, overnight, 77%; d) DMF-DMA, rt, 18 h; e) hydrazine monohydrate, C_2H_5OH, rt, overnight, 72%; f) 1-iodo-3-nitrobenzene, K_2CO_3, CuI, $_L$-proline, DMSO, 90 °C, 8 h, 59%; g) H_2 (g), 10% Pd/C, THF, rt, 2 h, 87%; h) appropriate aryl isocyanate, THF, rt, 12 h, 24~85%; i) BBr_3, CH_2Cl_2, -78 °C, 30 min; rt, 1 h, 24~89% **(1f-j)** and 28~79% (**2f-j**); j) appropriate benzoic acid derivative, HOBt, EDCI, TEA, DMF, 80 °C, 12 h, 28~79%.

► Synthesis and *in vitro* antiproliferative activities of new pyrazole derivatives are reported.

► Compounds **1c-e** and **2i** were the most potent antiproliferative agents.

► Compound **1e** showed superior potency than sorafenib against ALL the 58 tested cancer cell lines.

► The IC_{50} values of compound **1e** were in submicromolar scale against most of the tested cancer cell lines.

► IC_{50} of compound **1e** against V600E-B-RAF kinase was 0.39 μM.

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