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MEDICINAL CHEMISTRY RESEARCH

ORIGINAL RESEARCH

Synthesis, characterization, and in vitro anticancer evaluation of 2-substituted 5-arylsulfonyl-1,3-oxazole-4-carbonitriles

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Abstract

In this series, six new 2-substituted 5-arylsulfonyl-1,3-oxazole-4-carbonitriles were synthesized and characterized by IR, ^{1}H NMR, ^{13}C NMR spectroscopy, elemental analysis and chromato-mass-spectrometry. The anticancer activities of the compounds were evaluated via single high dose (10^{-5}M) against 60 cancer cell lines by the National Cancer Institute according to its own screening protocol. In the next phase, the compounds have been selected for five-dose assay. All synthesized compounds displayed growth inhibitory (GI50) and cytostatic activities (TGI) against the most sensitive cell lines at submicromolar (0.2–0.6 μM) and micromolar concentrations (1–3 μM), respectively. Cytotoxic activity (LC_{50}) of these compounds, with the exception of **4d**, against the most sensitive cell lines was also high (5–6 μM). All compounds exhibit high selectivity towards leukemia cell lines, and among them, **4e** and **4f** showed the best antiproliferative and cytostatic selectivity. Compounds **4c** and **4f** displayed considerable cytotoxic selectivity towards the renal and breast cancer subpanels. Our results provided evidence for anticancer activities of novel 2-substituted 5-arylsulfonyl-1,3-oxazole-4-carbonitriles which could be useful for developing new anticancer drugs. These substances could also be used as an excellent framework in anticancer research that may lead to discovery of potent antitumor agents.

Keywords 2-Substituted 5-arylsulfonyl-1,3-oxazole-4-carbonitriles · Synthesis · Anticancer activity · Selectivity

Introduction

Cancer is a general term for malignant diseases characterized by uncontrolled and abnormal cell growth. The development of new anticancer therapeutic agents is one of the fundamental goals in medicinal chemistry. Despite the crucial role of cancer chemotherapy, the lack of antitumor selectivity has become one of the main barriers for developing effective anticancer drugs. There are still significant challenges with resistance to existing therapies, a need for new targets, and a deeper understanding over molecular mechanisms. Therefore, it is great interest for the search of newer and safer anticancer agents (Narang and Desai 2009; Semenyuta et al. 2013; Semenyuta et al. 2014).

Oxazole derivatives together with naturally occurring oxazoles have a wide range of pharmacological applications as antipathogenic (Suh et al. 2015; Jin 2016; Joshi et al. 2017; Pouramiri et al. 2017) and anticancer agents (Liu et al. 2010; El-All et al. 2015; Zhou et al. 2016). Some 1,3-oxazoles can interact with the colchicine site of β-tubulin resulting in microtubule polymerization stopping and inhibition of cell proliferation (Semenyuta et al. 2013, 2014, 2016; Romagnoli et al. 2017). In addition, some aryloxazoles are effective against cancerous cells resistant to other anticancer drugs (Schobert et al. 2010). Since a mechanism action of these compounds has not been reported completely, further studies on biological activity of different 1,3-oxazole derivatives are essential to find more potent anticancer compounds.

In this paper, we described the synthesis and anticancer activity of a novel class of 1,3-oxazole derivatives such as 2-substituted 5-arylsulfonyl-1,3-oxazole-4-carbonitriles. The synthesized compounds were screened for their anticancer activities against full NCI 60 cell line panel.

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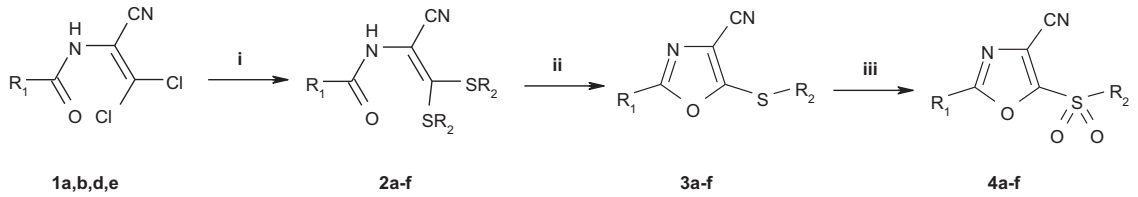


a: R_1 = Ph, R_2 = 4-BrC_6H_4
b: R_1 = 4-FC_6H_4, R_2 = Ph
c: R_1 = 4-FC_6H_4, R_2 = 4-MeC_6H_4
d: R_1 = thiophen-2-yl, R_2 = Ph
e: R_1 = ^{t}Bu, R_2 = Ph
f: R_1 = ^{t}Bu, R_2 = 4-BrC_6H_4

Scheme 1 Synthesis of target compounds **4a–f**. Reaction conditions and reagents: (i) arenethiol, Et_3N, MeCN, rt, 8 h; (ii) Ag_2CO_3, MeCN, reflux, 8 h; (iii) H_2O_2, HAc, reflux, 2 h

Material and Methods

Chemistry

The methodology of synthesis of compound **4** (Scheme 1) was described previously (Pil'o et al. 2002). The three-stage reaction sequence involves treatment of 2-acylamino-3,3-dichloroacrylonitriles **1** with arenethiols in the presence of triethylamine to obtain 2-acylamino-3,3-bis(arylsulphanyl) acrylonitriles **2** followed by cyclization in presence of silver carbonate to form 5-arylsulphanyl-1,3-oxazole-4-carbonitriles **3**. Compounds **3** were converted into the corresponding sulfonyl derivatives **4** by oxidation with hydrogen peroxide.

Data of synthesized novel 1,3-oxazole derivatives **4a–f** are presented in Experimental part. NMR (^{1}H NMR and ^{13}C NMR), chromato-mass and elemental analysis confirm reliably the structure of the obtained compounds. The intensive absorption bands of SO_2-group appeared at 1154–1164 and 1327–1357 cm^{-1} in the IR spectra as well as intensive band at 2246–2252 cm^{-1} corresponded to CN group were observed.

The synthesized compounds were submitted for in-vitro anticancer assay at National Cancer Institute (NCI), USA against full NCI 60 cell lines panel and granted NCS codes shown in Table 1.

General chemistry methods

^{1}H (300 or 400 MHz) and ^{13}C (100 or 125 MHz) NMR spectra were recorded on a Varian Mercury and Bruker Avance DRX 500 spectrometer in DMSO-d_6 solution. IR spectra were recorded on a Vertex 70 spectrometer from KBr pellets. The melting points were estimated on a Fisher-Johns instrument. The chromatomass spectra were recorded on an Agilent 1100 Series high performance liquid chromatograph equipped with a diode matrix with an Agilent LC/MS mass selective detector allowing a fast switching the positive/negative ionization modes. The reaction progress was monitored by the TLC method on Silica gel 60 F_{254} Merck.

General procedure for the synthesis of compounds 2a–f

To a solution of appropriate 2-acylamino-3,3-dichloroacrylonitriles (**1a**, **b**, **d**, **e**) (0.01 mol) in 30 ml of acetonitrile, triethylamine (0.02 mol) and an appropriate arenethiol (0.02 mol) were added, and the mixture was stirred at room temperature for 8 h. The precipitate was filtered off and all volatiles were removed in vacuo. The residue was treated with water, separated, dried and used in the next step without purification.

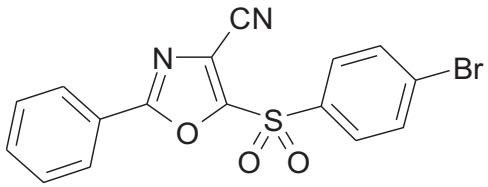
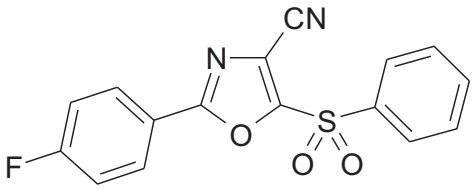
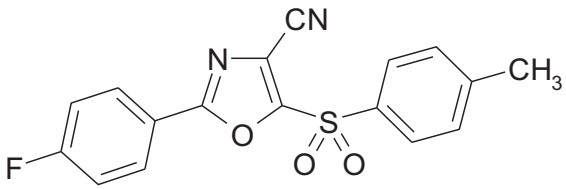
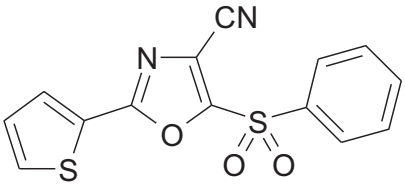
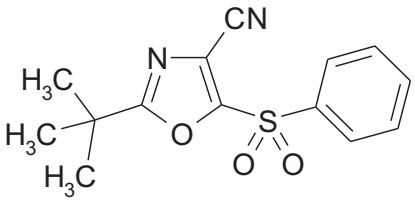
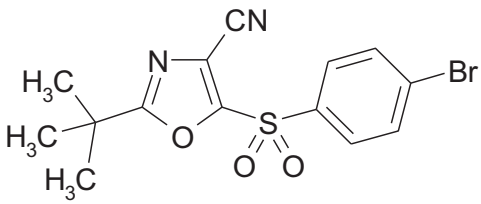
General procedure for the synthesis of compounds 3a–f

Suspension of 2-acylamino-3,3-bis(arylsulfanyl)acrylonitriles (0.01 mol) **2a–f** and dry silver carbonate (0.03 mol) in 40 ml of acetonitrile was stirred at reflux for 8–10 h, then kept at room temperature for 8 h. The precipitate was filtered off. All volatiles were removed in vacuo and water was added to residue. The precipitate formed was filtered, dried and used in the next step without purification.

General procedure for the synthesis of compounds 4a–f

Solution of appropriate 5-arylsulfanyl-1,3-oxazole-4-carbonitrile (0.005 mol) **3a–f** in glacial acetic acid (10 ml) was heated to reflux. Three portions of 30 % H_2O_2 of 1 ml each were added during 2 h. The mixture was kept at room temperature for 8 h. The precipitate was filtered and purified by recrystallization.

Table 1 Chemical structures of compounds **4a–f**

Compound	NCI code NSC	Molecular weight	Chemical structure	Chemical name
4a	762315	389.23		5-[(4-bromophenyl)sulfonyl]-2-phenyl-1,3-oxazole-4-carbonitrile
4b	762267	328.32		2-(4-fluorophenyl)-5-(phenylsulfonyl)-1,3-oxazole-4-carbonitrile
4c	762311	342.35		2-(4-fluorophenyl)-5-(toluene-4-sulfonyl)-1,3-oxazole-4-carbonitrile
4d	762317	316.36		5-benzenesulfonyl-2-thiophen-2-yl-1,3-oxazole-4-carbonitrile
4e	762314	290.34		2-(*tert*-butyl)-5-(phenylsulfonyl)-1,3-oxazole-4-carbonitrile
4f	765447	369.24		5-((4-bromophenyl)sulfonyl)-2-(*tert*-butyl)-1,3-oxazole-4-carbonitrile

2-(4-Bromophenyl)-5-(phenylsulfonyl)-1,3-oxazole-4-carbonitrile (4a) White solid (66%); mp (glacial acetic acid) 163–165 °C; IR (KBr) ν_{max}/cm^{-1} 1070, 1154 (SO_2), 1272, 1332 (SO_2), 1353, 1448, 1477, 1548, 1570, 1604, 2246

(CN). ^{1}H NMR (400 MHz, $(CD_3)_2SO$) δ 7.58–7.71 (3H, m, ArH), 7.97–8.06 (6H, m, ArH). ^{13}C NMR (125 MHz, $(CD_3)_2SO$) δ 110.84 (CN), 119.01 (C^4_{oxazol}), 124.45 (C_{Ph}), 128.00 ($2C_{Ph}$), 130.01 ($2C_{Ph}$), 130.80 ($2C_{Ph}$), 130.98 (C_{Ph}), 133.89 (C_{Ph}), 133.97 ($2C_{Ph}$), 136.69 (C_{Ph}), 152.45 (C^5_{oxazol}), 164.74 (C^2_{oxazol}). LCMS, *m/z*: 389 $[M+1]^+$. Anal.calcd for $C_{16}H_9BrN_2O_3S$: C, 49.37; H, 2.33; N, 7.20; S, 8.24. Found: C, 49.35; H, 2.31; N, 7.12; S, 8.13.

2-(4-Fluorophenyl)-5-(phenylsulfonyl)-1,3-oxazole-4-carbonitrile (4b) White solid (73%); mp (ethanol) 155–160 °C; IR (KBr) ν_{max}/cm^{-1} 1076, 1157 (SO_2), 1273, 1328 (SO_2), 1354, 1448, 1493, 1554, 1605, 2252 (CN). ^{1}H NMR (400 MHz, $(CD_3)_2SO$) δ 7.40–7.58 (7H, m, ArH), 8.03–8.05 (2H, m, ArH). ^{13}C NMR (125 MHz, $(CD_3)_2SO$) δ 112.39 (CN), 117.08 (C^4_{oxazol}), 119.64(C^4_{oxazol}), 122.08 (C_{Ph}), 129.50 ($2C_{Ph}$), 129.95 ($2C_{Ph}$), 130.02 ($2C_{Ph}$), 130.57 ($2C_{Ph}$), 131.11 ($2C_{Ph}$), 152.92 (C^5_{oxazol}), 163.56 (C^2_{oxazol}). LCMS, *m/z*: 329 $[M+1]^+$. Anal.calcd for $C_{16}H_9FN_2O_3S$: C, 58.53; H, 2.76; N, 8.53; S, 9.77. Found: C, 58.50; H, 2.74; N, 8.45; S, 9.69.

2-(4-Fluorophenyl)-5-(toluene-4-sulfonyl)-1,3-oxazole-4-carbonitrile (4c) White solid (74%); mp (ethanol) 185–188 °C; IR (KBr) ν_{max}/cm^{-1} 1076, 1156 (SO_2), 1271, 1327 (SO_2), 1347, 1415, 1493, 1554, 1601, 2251 (CN). ^{1}H NMR (400 MHz, $(CD_3)_2SO$) δ 2.43 (3H, s, CH_3), 7.42–7.46 (2H, m, ArH), 7.57 (2H, d, $J = 8$ Hz, ArH), 8.02 (2H, d, $J = 8$ Hz, ArH), 8.05–8.08 (2H, m, ArH). ^{13}C NMR (100 MHz, $(CD_3)_2SO$) δ 21.78 (CH_3), 110.86 (CN), 117.26 (C^4_{oxazol}), 117.48 (C_{Ph}), 118.34 (C_{Ph}), 121.17 (C_{Ph}), 128.93 ($2C_{Ph}$), 130.86 (C_{Ph}), 130.95 (C_{Ph}), 131.36 ($2C_{Ph}$), 134.54 (C_{Ph}), 147.68 (C_{Ph}), 153.40 (C^5_{oxazol}), 163.70 (C^2_{oxazol}), 164.14 (C_{Ph}). LCMS, *m/z*: 343 $[M+1]^+$. Anal.calcd for $C_{17}H_{11}FN_2O_3S$: C, 59.64; H, 3.24; N, 8.53; S, 9.77. Found: C, 59.61; H, 3.21; N, 8.57; S, 9.72.

5-Benzenesulfonyl-2-thiophen-2-yl-1,3-oxazole-4-carbonitrile (4d) Yellow solid (76%); mp (ethanol) 165–168 °C; IR (KBr) ν_{max}/cm^{-1} 1072, 1156 (SO_2), 1289, 1328 (SO_2), 1351, 1444, 1502, 1554, 1586, 2247 (CN). ^{1}H NMR (300 MHz, $(CD_3)_2SO$) δ 7.27 (1H, dd, $J_1 = 3.9$ Hz, $J_2 = 0.9$ Hz, thiophene), 7.74–8.12 (7H, m, ArH, thiophene). ^{13}C NMR (125 MHz, $(CD_3)_2SO$) δ 110.23 (CN), 118.43 (C^4_{oxazol}), 125.46 ($C_{thiophene}$), 128.28 ($2C_{Ph}$), 129.24 ($C_{thiophene}$), 130.40 ($2C_{Ph}$), 132.75 ($C_{thiophene}$), 134.35 ($C_{thiophene}$), 135.98 (C_{Ph}), 137.04(C_{Ph}), 151.64 (C^5_{oxazol}), 160.23 (C^2_{oxazol}). LCMS, *m/z*: 317 $[M+1]^+$. Anal.calcd for $C_{14}H_8N_2O_3S_2$: C, 53.15; H, 2.55; N, 8.85; S, 20.27. Found: C, 53.13; H, 2.53; N, 8.82; S, 20.24.

2-(Tert-butyl)-5-(phenylsulfonyl)-1,3-oxazole-4-carbonitrile (4e) White solid (68%); mp (ethanol) 118–120 °C; IR (KBr) ν_{max}/cm^{-1} 1162 (SO_2), 1267, 1354 (SO_2), 1451, 1550, 2251 (CN). ^{1}H NMR (400 MHz, $(CD_3)_2SO$) δ 1.29 (9H, s, $3CH_3$), 7.75–7.91 (3 H, m, ArH), 8.07 (2H, d, $J =$ 7.6, ArH). ^{13}C NMR (125 MHz, $(CD_3)_2SO$) δ 28.00 ($3CH_{3tbutyl}$), 34.82 (C_{tbutyl}), 110.76 (CN), 117.09 (C^4_{oxazol}), 128.58 ($2C_{Ph}$), 130.90 ($2C_{Ph}$), 136.47 (C_{Ph}), 137.49 (C_{Ph}), 153.02 (C^5_{oxazol}), 175.48 (C^2_{oxazol}). LCMS, *m/z*: 291 $[M+1]^+$. Anal.calcd for $C_{14}H_{14}N_2O_3S$: C, 57.92; H, 4.86; N, 9.65; S, 11.04. Found: C, 57.90; H, 4.84; N, 9.55; S, 10.92.

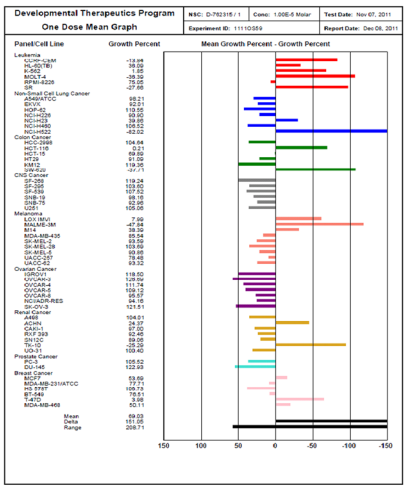
5-((4-Bromophenyl)sulfonyl)-2-(tert-butyl)-1,3-oxazole-4-carbonitrile (4f) White solid (73%); mp (ethanol) 93–95 °C; IR (KBr) ν_{max}/cm^{-1} 1164 (SO_2), 1263, 1357 (SO_2), 1466, 1550, 1572, 2251 (CN). ^{1}H NMR (400 MHz, $(CD_3)_2SO$) δ 1.30 (9H, s, $3CH_3$), 7.98 (4H, s, Ar). ^{13}C NMR (125 MHz, $(CD_3)_2SO$) δ 27.98 ($3CH_{3tbutyl}$), 34.78 (C_{tbutyl}), 110.73 (CN), 117.39 (C^4_{oxazol}), 130.55 ($2C_{Ph}$), 130.94 ($2C_{Ph}$), 133.97(C_{Ph}), 136.66 (C_{Ph}), 152.40 (C^5_{oxazol}), 175.61 (C^2_{oxazol}). LCMS, *m/z*: 367 $[M-1]^-$. Anal.calcd for $C_{14}H_{13}BrN_2O_3S$: C, 45.54; H, 3.55; N, 7.59; S, 8.68. Found: C, 45.51; H, 3.54; N, 7.50; S, 8.60.

In vitro Anticancer Screening of the synthesized compounds

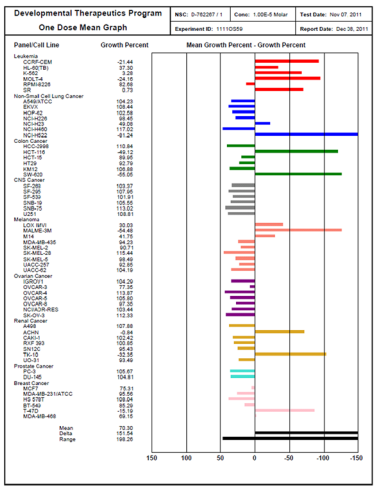
One doses full NCI 60 cell panel assay

Synthesized compounds **4a–f** were submitted to National Cancer Institute NCI, Bethesda, Maryland, U.S.A. under the Developmental Therapeutic Program DTP. The cell line panel engaged a total of 60 different human tumor cell lines derived from nine cancer types, including lung, colon, melanoma, renal, ovarian, brain, leukemia, breast and prostate.

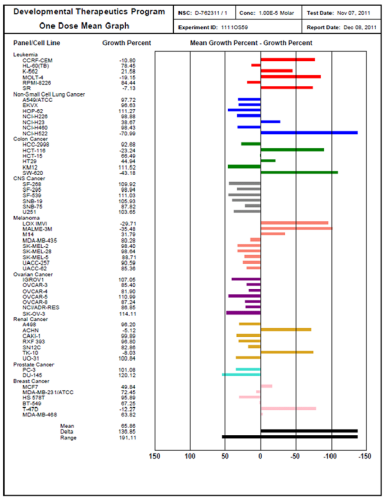
Primary in vitro one dose anticancer screening was initiated by cell inoculating of each 60 panel lines into a series of standard 96-well microliter plates at 5000–40000 cells/well in RPMI 1640 medium containing 5% fetal bovine serum and 2 mM L-glutamine (day 0), and then preincubated in absence of drug at 37 °C and 5% CO_2 for 24 h. Test compounds were then added into the plates at one concentration of 10^{-5} M (day 1) followed to incubation for a further 48 h at the same conditions. Then the media were removed, the cells were fixed *in situ*, washed, and dried (day 3). The sulforhodamine B assay was used for cell density determination, based on the measurement of cellular protein content. After an incubation period, cell monolayers were fixed with 10% (wt/vol) trichloroacetic acid and stained for 30 min, after which the excess dye was removed by washing repeatedly with 1% (vol/vol) acetic acid. The bound stain was resolubilized in 10 mM Tris base solution and measured spectrophotometrically on automated microplate readers for OD determination at 510 nm.



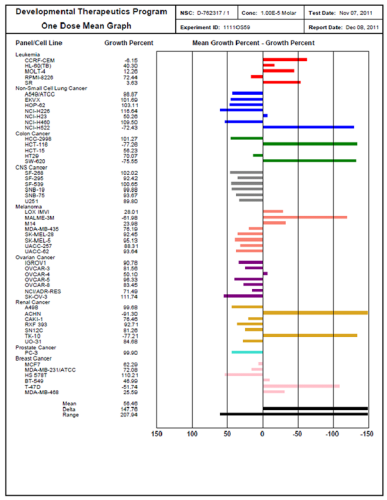
Compound **4a**



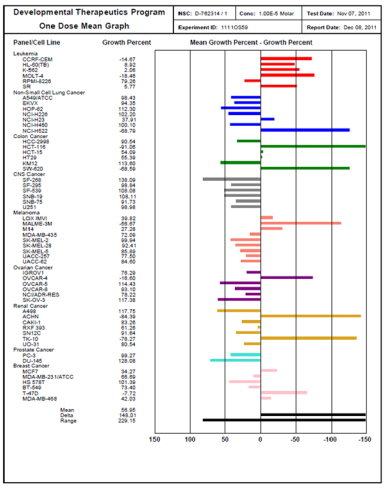
Compound **4b**



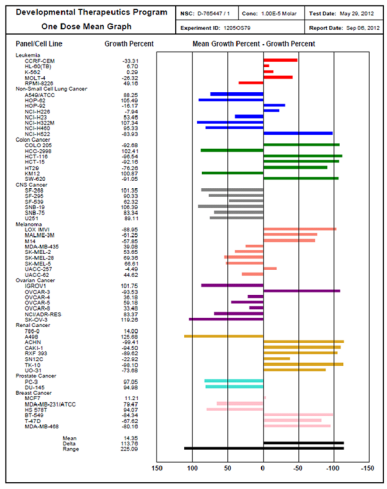
Compound **4c**



Compound **4d**



Compound **4e**



Compound **4f**

Fig. 1 One dose mean graph for 2-substituted 5-arylsulfonyl-1,3-oxazole-4-carbonitriles against the NCI 60 human cancer cell lines at 10 µM

Five doses full NCI 60 cell panel assay

Cells of all 60 lines, representing nine cancer subpanels, were incubated at five different concentrations (0.01, 0.1, 1, 10 and 100 μM) of the tested compounds. The outcomes were used to create $\log_{10}$ concentration *versus* percentage growth inhibition curves and three response parameters (GI_{50}, TGI and LC_{50}) were calculated for each cell line. The GI_{50} value (growth inhibitory activity) corresponds to the concentration of the compound causing 50% decrease in net cell growth. The TGI value (cytostatic activity) is the concentration of the compound resulting in total growth inhibition. The LC_{50} value (cytotoxic activity) is the concentration of the compound causing net 50% loss of initial cells at the end of the incubation period of 48 h.

The three dose–response parameters GI_{50}, TGI and LC_{50} were calculated for each experimental compound. Data calculations were made according to the method described by the NCI/NIH Development Therapeutics Program (https://dtp.cancer.gov/discovery_development/nci-60/default.htm).

The % growth curve is calculated as:

$$[(T - T_0)/(C - T_0)] \times 100,$$

where: T_0 is the cell count at day 0, C is the vehicle control (without drug) cell count (the absorbance of the SRB of the control growth). T is the cell count at the test concentration at day 3.

The GI_{50} and TGI values are determined as the drug concentrations result in a 50 and 0% growth at 48 h drug exposure. Growth inhibition of 50% (GI_{50}) is calculated from:

$$[(T - T_0)/(C - T_0)] \times 100 = 50.$$

The TGI is the concentration of test drug where:

$$100 \times (T - T_0)/(C - T_0) = 0.$$

Thus, the TGI signifies a cytostatic effect.

The LC_{50}, which signifies a cytotoxic effect, is calculated as:

$$[(T - T_0)/T_0] \times 100 = -50,$$

when $T < T_0$.

Selectivity index (SI) of the compounds is calculated as:

$$SI = MID_p/MID_{sp},$$

where MID_p – the average sensitivity of all cell lines towards the test agent, MID_{sp} – the average sensitivity of all cell lines of a particular subpanel towards the test agent.

Results and Discussion

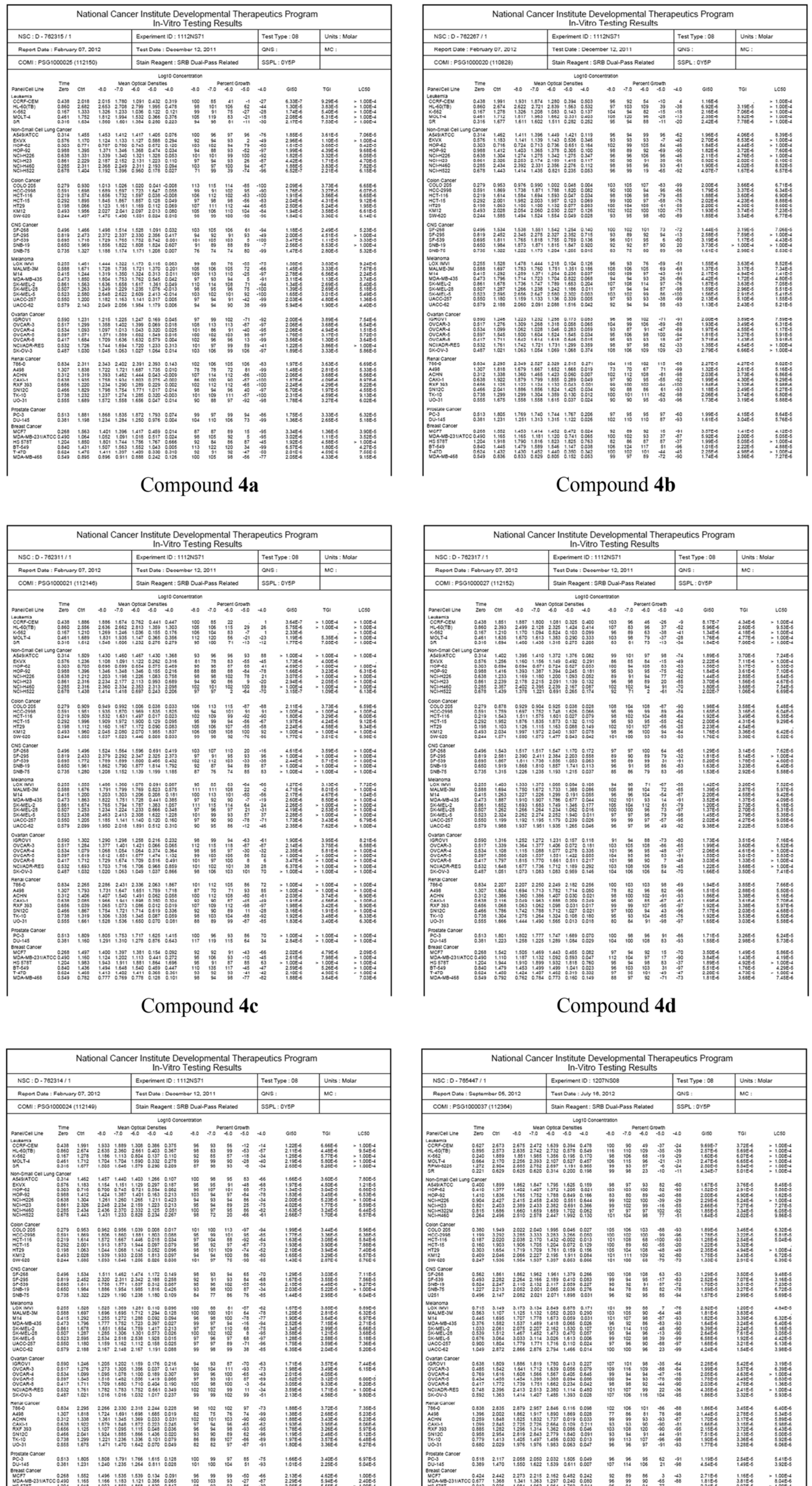
The one dose assay

The tumor growth inhibition properties of the synthesized compounds were screened on human cancer cell lines at the NIH, Bethesda, Maryland, USA, under the drug discovery program of the NCI, for one dose anti-cancer assay. Results for each compound were reported as a mean graph of the percent growth of the treated cells when compared to the untreated control cells. The synthesized compounds showed a distinctive sensitivity against individual cell lines (Fig. 1).

Anticancer data reveals that compound **4a** showed the growth percent ranging between—82.02 and 126.69%. The most sensitive cell lines were NCI-H522 (Non-Small Cell Lung Cancer, lethality is 82.02%,), MALME-3M (Melanoma, −47.84%), SW-620 (Colon Cancer, −37.71%), MOLT-4, SR and CCRF-CEM (Leukemia, −36.39, 27.66 and 13.84%, respectively), and TK-10 (Renal Cancer, −25.29%). It also exhibited the cell proliferation inhibition against Colon Cancer HCT-116 (99.79%), Leukemia K-562 and HL-60(TB) (98.15 and 64.0%, respectively), Breast Cancer T-47D (96.02%), Melanoma LOX IMVI and M14 (82.01 and 61.61%, respectively), and Renal Cancer ACHN (75.63%) cell lines in one dose primary assay.

Compound **4b** showed the growth percent ranging from −81.24 to 117.02%, and displayed the best cytotoxicity against NCI-H522 (lung cancer), SW-620, HCT-116 (colon cancer), and MALME-3M (melanoma) cell lines with the cell proliferation of −81.24, −55.05, −49.12, and −54.48%, respectively. This compound also showed the cytotoxic effect against Renal cancer TK-10 and ACHN (−32.5 and 0.84%, respectively), Leukemia MOLT-4 and CCRF-CEM (−24.16 and −21.44%, respectively), and Breast Cancer T-470 (−15.19%) cell lines. In addition, compound **4b** shows the cell proliferation inhibition of Leukemia SR and K-562 (99.27 and 96.72%), Melanoma LOX IMVI and M14 (69.97 and 58.25%), and Non Small Cell Lung Cancer NCI-H23 (50.92%) cell lines.

Compound **4c** showed broad spectrum of lethality against the human cancer cell lines: Non-Small Cell Lung Cancer NCI-H522 (70.0%), Colon Cancer SW-620 and HCT-116 (43.18 and 23.24%, respectively), Melanoma MALME-3M and LOX IMVI (35.49 and 29.71%, respectively), Leukemia CCRF-CEM (19.15, 10.80 and 7.13%), Breast Cancer T-47D (12.27%), Renal Cancer TK-10 and ACHN (8.03 and 5.12%, respectively). Apart from this, compound **4c** also exhibited the cell growth inhibition against Leukemia K-562 (78.42%), Melanoma M14 (68.21%), Colon Cancer HT-29 (55.06%), and Non-Small Cell Lung Cancer NCI-H23 (61.33%) cell lines in one dose primary assay.



Compound **4a** Compound **4b** Compound **4c** Compound **4d** Compound **4e** Compound **4f**

Fig. 2 The anticancer activity of the synthesized compounds against the NCI 60 human cancer cell lines (five-dose assay). *Note*. The first column describes the subpanel and cell line involved. The next two columns list the mean optical densities (MOD) of cells at day 0 and the vehicle control, the next five columns list the MOD test for each of five different concentrations. Each concentration is expressed as the log10 (molar). The next five columns list the calculated PGs for each concentration. The response parameters GI_{50}, TGI and LC_{50} were interpolated values representing the concentrations at which the PG is +50, 0 and −50 respectively. Sometimes these response parameters cannot be obtained by interpolation. If, for instance, all of the PGs in a given row exceed + 50, then none of the three parameters can be obtained by interpolation. In such a case, the value given for each response parameter is the highest concentration tested and preceded by a ">" sign

Compound **4d** showed the growth percent ranging from −91.30 to 116.64%, and displayed the best cytotoxicity against Renal cancer ACHN and TK-10 (−91.30 and −77.21%, respectively), Colon Cancer HCT-116 and SW-620 (−77.26 and −75.66, respectively), Non-Small Cell Lung Cancer NCI-H522 of −72.43%, Melanoma MALME-

3M of −61.98%, and Breast Cancer T-470 of −51.74% cell lines. This compound also showed the minor cytotoxic effect against Leukemia CCRF-CEM cell line of −6.15%. In addition, compound **4d** demonstrated the cell proliferation inhibition of Leukemia SR, MOLT-4 and HL-60(TB) (96.37, 87.74 and 59.70%, respectively), Melanoma M14 and LOX IMVI (76.02 and 71.99%) and Breast Cancer MDA-MB-468 and BT-549 (74.41 and 53.01%, respectively) cell lines.

Compound **4e** showed the growth percent ranges from −91.06 to 138.09%, and displayed cytotoxicity against Colon Cancer HCT-116 and SW-620 (−91.06 and −88.59%, respectively), Renal Cancer ACHN and TK-10 (−84.39 and −78.27%, respectively), Non-Small Cell Lung Cancer NCI-H522 (−68.79%), Melanoma MALME-3M (−56.67%), Ovarian Cancer OVCAR-4 (−16.60%), Leukemia MOLT-4 and CCRF-CEM (−18.46 and −14.67%, respectively) and Breast Cancer T-470 (−7.72 %) cell lines. This compound showed the cell proliferation inhibition of Leukemia K-562, SR and HL-60(TB) (97.94, 94.23 and 91.08%, respectively), Melanoma M14 and LOX IMVI (72.22 and 60.12%), Breast Cancer MCF-7 and MDA-MB-468 (64.72 and 57.97%, respectively), and Non-Small Cell Lung Cancer NCI-H23 (62.09%) cell lines.

Compound **4f** showed the growth percent ranges from −91.06 to 138.09%, and displayed the cytotoxicity against Renal Cancer ACHN, TK-10, CAKL-1, RXF-393, UO-31 and SN12C (−99.41, −98.10, −94.50, −89.62, −73.68 and −22.92%, respectively), Ovarian Cancer OVCAR-3 (−93.53%), Colon Cancer HCT-116, COLO-205, HCT-15, SW-620 and HT-29 (−96.54, −92.68, −92.16, −91.05 and −76.26%, respectively), Melanoma LOX IMVI, MALME-3M and N14 (−88.95, −61.25 and −57.85%), Breast Cancer BT-549, VDA-MB-469 and T-470 (−84.34, 80.16 and 67.62%, respectively), Leukemia CCRF-CEM and MOLT-4 (−33.31 and −26.32%, respectively), Non-Small Cell Lung Cancer NCI-H522, HOP-92, NCI-H226 (−83.93, −16.17 and −7.04%, respectively) cell lines. This compound showed the cell proliferation inhibition of Leukemia K-562 and HL-60(TB) (99.71 and 93.30%, respectively), Breast Cancer MCF-7 (88.79%), Renal Cancer 786-0 (86%), Melanoma MDA-MB-435 (60.92%), and Ovarian Cancer OVCAR-8 and OVCAR-4 (−66.52 and −63.72%, respectively) cell lines.

CNS Cancer, Ovarian Cancer, and Prostate Cancer cell lines were least sensitive to the synthesized compounds.

The five-dose assay

All synthesized compounds satisfied the pre-determined threshold inhibition criteria of the NCI-60 One-Dose Screening were tested against the panels of 60 cancer cell lines of NCI. Figure 2 represents the results of the five-dose assay for anticancer activity of these compounds against each cancer cell line.

Compound **4a** showed GI50 values ranging from 0.63 (Leukemia CCRF-CEM cell line) to 25.6 μM (CNS Cancer SNB-19 cell line), TGI—from 2.2 (Non-Small Cell Lung Cancer NCI-H522 cell line) to 85.3 μM (CNS Cancer SNB-19 cell line), and LC50—from 6.1 (Non-Small Cell Lung Cancer NCI-H522) to 84.2 μM (Non-Small Cell Lung Cancer HOP-62 cell line). LC50 of compound 4a for Leukemia subpanel, EKVX (lung cancer), SF-295 and SNB-19 (CNS cancer), NCI/ADR-RES (ovarian cancer) and HS 578 T (breast cancer) cell lines exceeded 100 μM.

Compound **4b** showed GI_{50} values ranging from 0.41 (Non-Small Cell Lung Cancer NCI-H522 cell line) to 37.3 μM (CNS Cancer SNB-19 cell line), TGI – from 1.67 (Non-Small Cell Lung Cancer NCI-H522 cell line) to 75.9 μM (CNS Cancer SF-395 cell line), and LC_{50}—from 6.0 (Renal Cancer RXF-393 cell line) to 83.9 μM (Non-Small Cell Lung Cancer A549/ATCC cell line). LC_{50} of compound **4b** for Leukemia subpanel, EKVX, HOP-62 and NCI-H226 (lung cancer), SF-295 and SNB-19 (CNS cancer), NCI/ADR-RES and SK-OV-3 (ovarian cancer), HS-578T and T-47D (breast cancer) cell lines exceeded 100 μM. TGI for SNB-19 (CNS cancer) cell line was also more than 100 μM.

Compound **4c** showed GI_{50} values ranging from 0.31 (Non-Small Cell Lung Cancer NCI-H522 cell line) to 46.9 μM (Non-Small Cell Lung Cancer HOP-62 cell line) with the exception of cancer lines with $LC_{50} > 100$ μM, TGI—from 2.86 (Non-Small Cell Lung Cancer NCI-H522 cell line) to 41.5 μM (Breast Cancer HS-578T cell line) with the same exception. Typical locate of LC_{50} tend to be in the short range of 6.0 to 10 μM with the exception of Breast Cancer MCF7 cell line (21 μM), and cancer lines with $LC_{50} > 100$ μM (Table 1).

Compound **4d** showed GI_{50} values ranging from 0.20 (Non-Small Cell Lung Cancer NCI-H522 cell line) to 19.4 μM (Renal Cancer 786-0), TGI – from 1.1 (Non-Small Cell Lung Cancer NCI-H522 cell line) to 51.4 μM (CNS Cancer SF-295 cell line), and LC_{50} – from 40.9 (Non-Small Cell Lung Cancer NCI-H522) to 95.3 μM (Leukemia HL-60(TB) cell line). LC_{50} of compound **4d** for Leukemia subpanel with the exception of HL-60(TB), and EKVX (lung cancer), SF-295 (CNS cancer), OVCAR-4, OVCAR-8 and NCI/ADR-RES (ovarian cancer), HS 578 T and T47D (breast cancer) cell lines exceeded 100 μM.

Compound **4e** showed GI_{50} values ranging from 0.27 (Non-Small Cell Lung Cancer NCI-H522 cell line) to 21.3 μM (CNS Cancer SNB-19 cell line), TGI – from 1.7 (Non-Small Cell Lung Cancer NCI-H522 cell line) to 55.5 μM (Breast Cancer HS 578 T cell line), and LC_{50}—from 5.8 (Colon Cancer SW-620 cell line) to 98.0 μM (Ovarian Cancer SK-OV-3 cell line). LC_{50} of compound **4e** for Leukemia subpanel with the exception of HL-60(TB), and

Table 2 Selectivity indices of the synthetic compounds towards the particular subpanels

Indices	Leukemia	Non-Small Cell Lung Cancer	Colon Cancer	CNS Cancer	Melanoma	Ovarian Cancer	Renal Cancer	Prostate Cancer	Breast Cancer
Compound **4a**									
GI_{50}	2.50	0.82	1.23	0.55	1.02	1.00	1.30	0.54	1.38
TGI	1.42	0.85	1.50	0.49	1.05	1.03	1.46	0.64	1.17
LC_{50}	–	0.73	1.48	0.80	0.91	1.22	1.45	0.64	0.92
Compound **4b**									
GI_{50}	3.15	0.85	1.41	0.49	1.00	0.96	1.32	0.53	1.31
TGI	1.59	0.81	1.62	0.561	1.04	0.86	1.50	0.58	1.09
LC_{50}	–	0.80	1.51	0.66	0.86	1.51	1.36	0.52	1.01
Compound **4c**									
GI_{50}	2.45	0.40	2.82	1.58	0.78	1.72	2.61	0.20	2.50
TGI	1.05	2.52	1.69	0.31	0.75	1.45	1.04	—	1.23
LC_{50}	–	1.26	1.03	—	1.08	1.06	1.23	—	0.56
Compound **4d**									
GI_{50}	3.32	0.83	1.24	0.57	0.96	1.03	1.23	0.48	1.31
TGI	1.89	0.87	1.45	0.54	0.99	1.07	1.34	0.56	1.01
LC_{50}	0.40	0.81	1.49	0.63	0.99	1.04	1.45	0.64	1.01
Compound **4e**									
GI_{50}	3.83	0.78	1.17	0.54	1.15	1.01	1.07	0.54	1.38
TGI	2.66	0.74	1.38	0.54	1.10	1.01	1.24	0.58	1.22
LC_{50}	3.68	0.84	1.51	0.65	0.99	0.69	1.34	0.59	2.13
Compound **4f**									
GI_{50}	3.40	0.57	0.96	0.47	1.64	1.26	1.45	0.73	1.08
TGI	2.43	0.64	1.21	0.52	1.44	1.28	1.67	0.72	0.67
LC_{50}	–	0.54	1.22	0.54	1.20	1.48	2.00	0.75	3.52

cell lines NCI-H23 (lung cancer), SNB-19 (CNS cancer), NCI/ADR-RES (ovarian cancer), and HS 578 T (breast cancer) exceeds 100 μM.

Compound **4f** showed GI_{50} values ranging from 0.43 (Leukemia SR cell line) to 22.4 μM (Breast Cancer HS 578 T cell line), TGI—from 2.5 (Colon Cancer HCT-116 cell line) to 37.6 μM (Non-Small Cell Lung Cancer A549/ATCC cell line), and LC_{50} – from 5.0 (Colon Cancer HCT-116) to 98.6 μM (Non-Small Cell Lung Cancer NCI-H460 cell line). LC_{50} of compound **4f** for Leukemia subpanel, NCI-H226 (lung cancer), HT29 (colon cancer), OVCAR-4 and NCI/ADR-RES (ovarian cancer), SF-295 and SNB-19 (CNS cancer), OVCAR-4 and NCI/ADR-RES (ovarian cancer), MCF-7, MDA-MB-231/ATCC and T-47D (breast cancer) cell lines exceeded 100 μM.

Thus, all the compounds displayed growth inhibitory (GI_{50}), and cytostatic activities (TGI) against the most sensitive cell lines at submicromolar (0.2–0.6 μM) and micromolar concentrations (1–3 μM), respectively. Cytotoxic activity (LC_{50}) of these compounds, with the exception of **4d**, against the most sensitive cancer cell lines was also high (5–6 μM).

Table 2 demonstrates selectivity of the synthesized compounds towards the particular cancer subpanels.

Discussion

Thus, all compounds exhibited high antiproliferative selectivity towards leukemia cell lines, and among them, **4e** and **4f** showed the best antiproliferative and cytostatic selectivity. These compounds displayed the considerable cytotoxic selectivity towards Renal (**4f**) and Breast Cancer (**4e** and **4f**) subpanels. But high antiproliferative selectivity towards these cancer subpanels demonstrated compound **4c** only (Table 2).

The anticancer activity results showed that the presence of a hydrophobic *tert*-butyl moiety, stabilizing a molecule conformation, at 2 position of 1–3-oxazol ring (compounds **4e** and **4f**) instead of phenyl one (compounds **4a** and **4b**)

appreciably enhances their anticancer activity towards Leukemia, while displacement of phenyl moiety at 5 position of one on *p*-tolyl group (compound **4c**) enhances its anticancer activity against Renal and Breast Cancer.

The present human tumor cell line in vitro screen provides preliminary data of anticancer activity of new compounds. This assay was designed only to select compounds for a secondary, more comprehensive, in vivo testing.

Conclusion

The novel series of 2-substituted 5-arylsulfonyl-1,3-oxazole-4-carbonitriles have been synthesized in good yields and displayed high anticancer activity. Differently substituted oxazoles have different activity. Indeed the obtained results indicate that compounds **4e** and **4f** showed higher anticancer activity towards Leukemia, whereas compound **4c** displays considerable cytotoxic selectivity towards Renal Cancer and Breast Cancer subpanels. The present studies reveal that the 2-substituted 5-arylsulfonyl-1,3-oxazole-4-carbonitriles provides a valuable new therapeutic intervention for the treatment of cancer diseases, and the **4e** and the **4f** are the potent lead compounds for anticancer drug discovery and further research.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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