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MEDICINAL CHEMISTRY RESEARCH

ORIGINAL RESEARCH

Synthesis of ester and amide derivatives of 1-phenyl-3-(thiophen-3-yl)-1*H*-pyrazole-4-carboxylic acid and study of their anticancer activity

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Abstract A series of novel thiophene containing 1,3-diarylpyrazole derivatives were synthesized and the structures were determined by IR, ^{1}H-NMR, and HRMS analysis. The anticancer activity of the title compounds against MCF7, MDA-MB-231, HeLa, Raji, and HL60 human cancer cells growth were investigated by MTT assay. Interestingly, Raji and HL60 cells exhibited more sensitivity to synthesized compounds. (4-Benzyl-piperidin-1-yl)-(1-phenyl-3-thiophen-3-yl-1*H*-pyrazol-4-yl)-methanone (**4c**) possessed the highest growth inhibitory effect on Raji and HL60 cancer cells (GI_{50} 25.2 ± 3.2 and 28.3 ± 1.53 μM, respectively). Compound **4f**, 4-trifluoromethylphenyl piperazine at the amide part, also showed potent activity on Raji and HL60 cancer cell lines with a GI_{50} value of 32.0 ± 1.27 and 36.7 ± 2.15 μM, respectively. Physicochemical properties of the synthesized compounds were evaluated in silico, and it was found that all compounds should present good passive oral absorption. All synthesized compounds demonstrated good drug-likeness values. The data suggested that these compounds might be promising for further development.

Keywords Synthesis · Anticancer activity · 1*H*-Pyrazoles · Thiophene · MTT

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Introduction

Cancer is one of the most serious threats against human health in the world, and the clinical prognosis remains relatively poor. Chemotherapy is a major form of cancer treatment. However, the majority of cancers are either resistant to chemotherapy or acquire resistance during treatment. As a result, the design and discovery of non-traditional, efficient, and safe chemical classes of agents are the prime targets in contemporary medicinal chemistry (Cozzi, 2003; Johnston *et al.,* 2005).

Pyrazoles constitute an important heterocyclic family containing nitrogen in their five-membered ring. Several pyrazole derivatives have been found to possess significant biological activities, stimulating the research activity in medicinal chemistry and chemical biology (Silveira *et al.,* 1993; Sridhar *et al.,* 2004; Nagarapu *et al.,* 2011; Padmaja *et al.,* 2009; Gaston *et al.,* 1996). Many pyrazole derivatives are well acknowledged to possess a wide range of anticancer bioactivities (Riyadh *et al.,* 2010; Anzaldi *et al.,* 2009; El-Shafei *et al.,* 2009; Zheng *et al.,* 2010; Xie *et al.,* 2010; Lian *et al.,* 2009). Literature survey revealed that some pyrazoles have been implemented as antileukemic (Daidone *et al.,* 2004; Chou *et al.,* 2007), antitumor (Li *et al.,* 2006; Xia *et al.,* 2007), and anti-proliferative (Schenone *et al.,* 2004) agents, beside their capability to exert remarkable anticancer effects through inhibiting different types of enzymes which play important roles in cell division (Warshakoon *et al.,* 2006; Huang *et al.,* 2007). Although the skeleton of pyrazole plays an important role in its biological effects, the type of peripheral substituents is also crucial. Wei *et al.* demonstrated that ethyl 1-(2′-hydroxy-3′-aroxypropyl)-3-aryl-1*H*-pyrazole-5-carboxylate derivatives suppressed lung cancer cell growth (Wei *et al.,* 2006). They found that ethyl 1-arylmethyl-3-aryl-1*H*-pyrazole-5-carboxylate derivatives

promoted human umbilical vein endothelial cell (HUVEC) apoptosis to a certain extent at the concentrations of 5–20 μM and the effect on the cell viability was dose dependent (Ding *et al.,* 2007). Pyrazole-fused heterocycles such as dihydropyrazoloxazin-4-one and pyrazolopyrazinone derivatives also showed anticancer activity (Wei *et al.,* 2007; Xie *et al.,* 2008; Zhang *et al.,* 2008; Pan *et al.,* 2008).

It is well known that the incorporation of heterocyclic rings into prospective pharmaceutical candidates is a major tactic to gain activity and safety advantages. Heterocyclic rings such as thiophene are important pharmacophores in search of molecules with antitumor activity (Micale *et al.,* 2002; Insuasty *et al.,* 2008; Leite *et al.,* 2004; Al-Obaid *et al.,* 2009; Basnet *et al.,* 2010; Barresi *et al.,* 2002). N-substituted 2-pyrazolines bearing thiophene substituent showed important activity against different cancer cell lines with remarkable values in leukemia, and renal and prostate cancer panels. It is also reported that terthiophene derivatives, bioisosteres of terpyridine, resulted considerable protein kinase C (PKC) inhibitory activity and antitumor cytotoxicities against several human cancer cell lines (Kim *et al.,* 1998).

In the present study, Compounds **A** (Zheng *et al.,* 2009) and **B** (Insuasty *et al.,* 2011) were considered as the parent compounds for the design of our target compounds (Fig. 1). In our effort to discover and develop potential new anticancer agents, we synthesized a series of novel 1,3-diarylpyrazole derivatives bearing thiophene functionality and evaluated their anticancer activities. The title compounds **4a–m** were designed by inserting thiophene-3-yl residue and introduction of the carboxamide side chain at 4 position of pyrazole ring. Herein, we report the synthesis and antiproliferative activity of compounds against five different human cancer cell lines. In silico evaluations of title compounds are also reported.

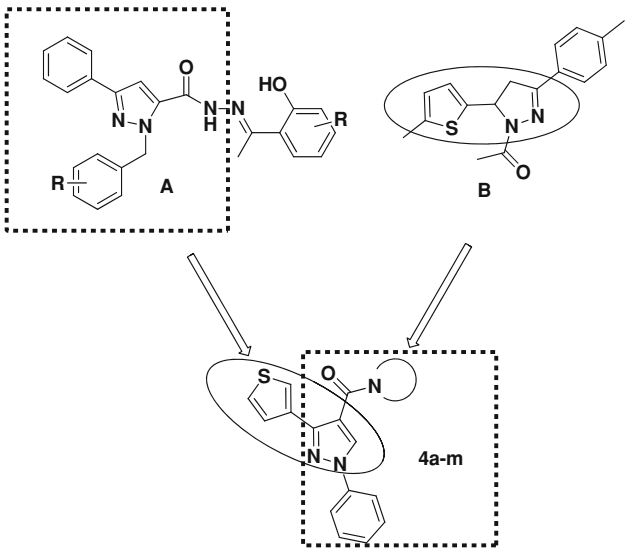


Fig. 1 Design of the title compounds

Results

Chemistry

We focused our synthetic efforts on diaryl heterocyclic ring systems as illustrated in Scheme 1. The starting compound, the hydrazone derivative **1**, was generated by carrying out condensation reaction in the presence of phenyl hydrazine and acetic acid in refluxing ethanol. This hydrazone derivative was then reacted with $POCl_3$ and DMF resulting in 1,3-diaryl pyrazole **2** with aldehyde group at 4 position. 1-Phenyl-3-thiophen-3-yl-1*H*-pyrazole-4-carbaldehyde **2** was oxidized to carboxylic acid derivative **3** in the presence of potassium permanganate in acetone–water mixture. By treatment of **3** with appropriate amines or phenols in the presence of triethylamine and ethyl chloroformate, which was used as the carboxylate activator, the amide and ester derivatives **4** were prepared in good yield (40–88 %) (Table 1). The chemical structures of these compounds were elucidated by their IR, HRMS, and ^{1}H-NMR spectral data.

The IR spectrum of hydrazone **1** has shown the disappearance of the carbonyl peak belonging to acetyl group of 3-acetyl-thiophene and the secondary N–H stretching band was observed at 3349 cm^{-1} as singlet. The IR spectra of compound **2** exhibited a characteristic strong absorption for aldehyde carbonyl group at 1667 cm^{-1}. In the IR spectra of compound **3,** strong absorption band at 1690 cm^{-1} and intense O–H stretching absorption in the region of 3300–2500 cm^{-1} for carboxylic acid were observed. Final amide and ester derivatives exhibited a characteristic strong absorption in the area of 1744–1615 cm^{-1} attributable to the C=O of amide or ester group.

In the ^{1}H-NMR spectra of compound **2**, two singlets were observed due to the aldehyde group at 10.10 ppm and pyrazole-H_5 at 8.50 ppm, each showing the integration for one proton. In the ^{1}H-NMR spectra of compounds **3**, signal of the carboxylic acid was not observed, but signal of the pyrazole-H_5 was seen at 8.59 ppm. In the ^{1}H-NMR spectra of final amide and ester derivatives **4a–m,** pyrazole-H_5 gave a singlet at about 9.00–8.04 ppm.

Biological evaluations

Effects of the compounds on the viability of cancer cells

Synthesized 1-phenyl-3-thiophen-3-yl-1*H*-pyrazole-4-carboxylic acid **3** and its amide and ester derivatives **4a–m** were screened against five human cancer cell lines (HeLa, MCF7, MDA-MB-231, Raji, and HL60) using 3-(4,5-dimethyldiazol-2-yl)-2,5-diphenyltetrazolium bromide

Scheme 1 **a** Phenyl hydrazine, acetic acid, ethanol, 2 h reflux. **b** Dimethyl formamide, $POCl_3$, 50 °C, 4 h. **c** Potassium permanganate, acetone–water (3:2), 2.5 h reflux. **d** Appropriate amine or phenol derivative, ethyl chloroformate, triethylamine, dichloromethane, rt, overnight

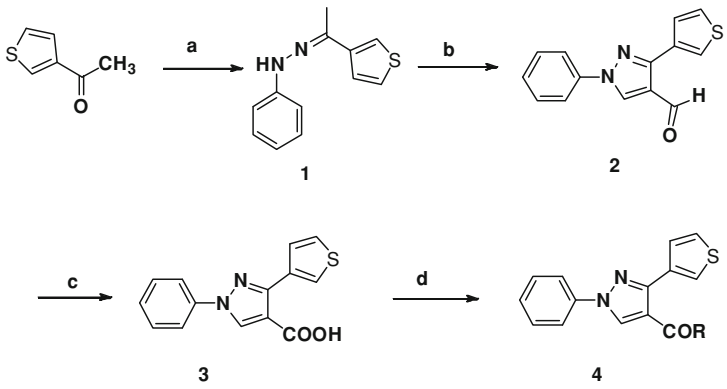
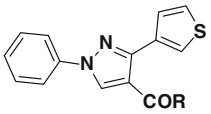
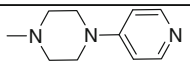
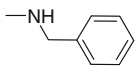
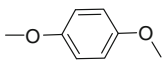
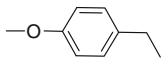
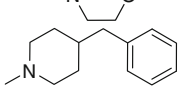
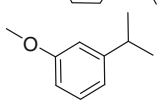
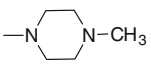
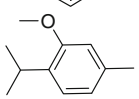
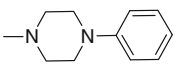
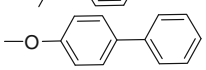
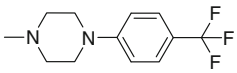
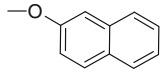


Table 1 Characterization data of 1,3-diarylpyrazole derivatives (**3, 4a–m**)



Compnd.	R	Melting point (°C)	% of yield	Compnd.	R	Melting point (°C)	% of yield
3	OH	201-203	87	**4g**		243-245	41
4a		128-130	40	**4h**		153-155	49
4b		147-149	70	**4i**		75-77	54
4c		78-80	61	**4j**		65-68	88
4d		110-112	42	**4k**		116-118	43
4e		179-181	40	**4l**		146-148	56
4f		143-145	43	**4m**		129-131	64

(MTT) assay. The MTT cell proliferation assay has been widely accepted as a reliable way to measure the cell proliferation rate, and conversely when metabolic events lead to apoptosis or necrosis (Holst-Hansen and Brünner, 1998; Reile *et al.,* 1990, Kueng *et al.,* 1989; Senaratne *et al.,* 2000). After incubation with compounds at the concentration of 50 μM for 48 h, the cells were treated with MTT to measure their growth/viability (% of the untreated control) by a spectrophotometric method as described previously (Mosmann, 1983; Denizot and Lang, 1986). Figure 2 indicates the cytotoxic effects of the compounds on the viability of the tested cancer cells that were incubated for 48 h. For example, exposure of HeLa cells to compounds **4a**, **4c**, **4e**, and **4f** at 50 μM for 48 h resulted in cell viability decrease from 100 to 82.3, 71.8, 67.9, and 80.7 %, respectively. For MDA-MB-231 cells, compounds **3**, **4a**, **4b**, **4c**, **4e, 4f**, and **4g** decreased the cell viability from 100 to 80.9, 85.0, 77.6, 82.8, 81.4, 84.2, and 87.4 %, respectively. Furthermore, only compounds **4c** and **4f** exhibited poor activity toward the MCF7 cell line (cell viability reduced from 100 to 90.7 % for **4c** and from 100 to 85.9 % for **4f**). On the other hand, compound **4c** showed the ever highest growth inhibitory potential against Raji cell line. Additionally, compound **4c** was able to display a significant activity against HL60 tumor cell lines, reducing cell viability from 100 to 39.1 %.

Among all compounds, **4c** showed the most potent inhibitory effect on Raji and HL60 cells growth (GI_{50} 25.2 ± 3.2 and 28.3 ± 1.53 μM, respectively), followed by **4f** (GI_{50} 32.0 ± 1.27 and 36.7 ± 2.15 μM, respectively) (Table 2).

Lipinski's rule-of-five and drug-likeness profile

The synthesized compounds **(4a–m)** were submitted to an in silico evaluation using a molecular modeling approach. In order to predict drug-like properties of the synthesized compounds, we analyzed these derivatives according to the rule-of-five developed by Lipinski *et al.* 2001. Predictions of ADME properties for the compounds are given in Table 3. Calculated physicochemical properties (http://www.molinspiration.com/cgi-bin/properties) showed that most of the compounds fulfilled the Lipinski "rule-of-five." The compound **4f** violated the criteria for molecular weight and logP. Compounds **4c**, **4i, 4j, 4k, 4l**, and **4m** had very large *c*logP values, which might be disadvantageous with regard to pharmacokinetic properties of these molecules in biological systems. Rest of the compounds exhibited favorable *c*log P values. The polar surface areas of the synthesized compounds were relatively small in comparison with the average value for acceptable drug molecules (<90Å^2).

Currently, there are many approaches to assess a compound's drug-likeness based on topological descriptors, fingerprints of molecular drug-likeness structure keys (Tetko, 2005). In this work, we used the Osiris program (http://www.organic-chemistry.org/prog/peo) for calculating the fragment-based drug-likeness of the compounds (Table 3). The most active compound **4c** demonstrated good drug-likeness value (=3.5).

Table 2 The GI_{50} values of the **4c** and **4f**[a] for cell viability in Raji and HL60 cell lines

Cell line	GI_{50} (μM)	
	4c	**4f**
Raji	25.2 ± 3.2	28.3 ± 1.53
HL60	32.0 ± 1.27	36.7 ± 2.15

[a] Rest of the compounds have IC_{50} values of >100 μM. Results are presented as mean ± SE; ($n = 4$. * $P < 0.05$ vs control; ** $P < 0.01$ vs control; *** $P < 0.001$ vs control)

Discussion

Cancer is the second leading cause of death in developed countries, accounting for nearly one in five deaths. A key feature of cancer cells is their uncontrolled proliferation; thus, inhibition of proliferative pathways is believed to be an effective strategy to fight cancer and much attention has recently been paid to the discovery and development of new, more selective anticancer agents (Lee *et al.,* 2007; Kemnitzer *et al.,* 2008; Hu *et al.,* 2008). The design and synthesis of new pyrazole derivatives has been a subject of consistent interest. Pyrazole derivatives have occupied a unique position in drug discovery due to their broad range of biological activities, including anti-inflammatory, anti-angiogenic, antibacterial, antimicrobial, anticancer, anti-depressant, antioxidant, anti-influenza, and analgesic activities (Tewari *et al.,* 2010; Christodoulou *et al.,* 2010; Lv *et al.,* 2010; Gouda *et al.,* 2010; Chou *et al.,* 2010; Abdel-Aziz *et al.,* 2009; Bandgar *et al.,* 2009; Shih *et al.,* 2010; Machado *et al.,* 2009). In the present study, 1,3-diarylpyrazole derivatives bearing thiophene moiety showed moderate anticancer activity. It is possible to relate the structural characteristics of the compounds to their

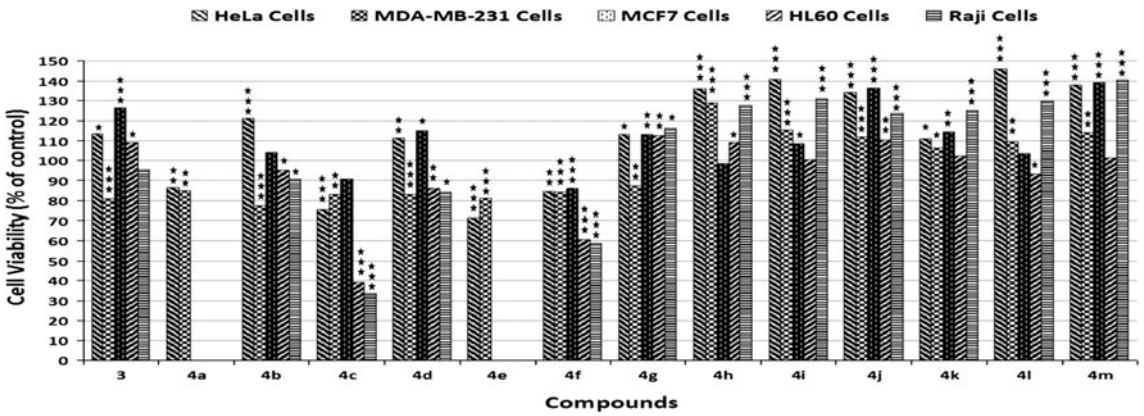


Fig. 2 Effects of the compounds on cervical carcinoma (HeLa), breast cancer (MDA-MB-231 and MCF7), Burkitt lymphoma (Raji), and human promyelocytic leukemia (HL60) cells viability. HeLa, MDA-MB-231, MCF7, Raji, and HL60 cells were treated with compounds **3, 4a–m** at concentration of 50 μM or left untreated (control) for 48 h. Cell viability was analyzed by MTT assay and illustrated in column figures. Results are presented as mean ± SE; ($n = 4$; * $P < 0.05$ vs control; ** $P < 0.01$ vs control; *** $P < 0.001$ vs control); Compounds **4a** and **4e** were not tested against MCF7, Raji, and HL60 cells due to poor solubility

Table 3 Calculated physicochemical properties of the synthesized compounds

Compound	Predicted oral bioavailability						Drug-likeness
	HBA[a]	HBD[b]	M.W[c]	clogP[d]	logS[e]	TPSA[f]	
3	4	1	296.35	2.642	−3.41	55.125	0.69
4a	4	1	385.49	3.901	−4.73	46.924	0.25
4b	5	0	365.45	2.594	−3.11	47.369	1.58
4c	4	0	453.61	5.325	−5.29	38.135	3.5
4d	5	0	378.50	2.639	−2.61	41.373	6.94
4e	5	0	440.57	4.336	−4.46	41.373	7.06
4f	5	0	508.56	5.232	−5.23	41.373	−3.32
4g	6	0	441.56	3.047	−3.66	54.265	5.29
4h	5	0	402.47	4.42	−4.89	53.365	−1.83
4i	4	0	400.50	5.278	−5.37	44.131	−1.64
4j	4	0	414.53	5.852	−5.74	44.131	−2.65
4k	4	0	428.55	5.779	−6.08	44.131	−4.89
4l	4	0	448.54	6.159	−6.96	44.131	−1.42
4m	4	0	422.50	5.547	−6.48	44.131	−3.73

[a] Number of hydrogen-bond acceptor

[b] Number of hydrogen-bond donor

[c] Molecular weight

[d] Calculated lipophilicity

[e] Solubility parameter

[f] Topological polar surface area (Å^2)

antitumor activity. The data obtained by MTT assay showed that amide derivatives **4a–g** had inhibitory effects on the growth of tested human cancer cells as shown in Fig. 2. When chemical structure of the compounds are taken into consideration, introduction of benzylpiperidin moiety at the amide portion led to **4c** with enhanced activity against all the cancer cell lines with the best results against Raji and HL60. The compound **4b** with morpholine was inactive against all tested tumor cell lines except against MDA-MB-231 which showed poor inhibitor effect on cell growth. Piperazine amide derivatives **4d**, **4e**, and **4g** displayed diminished activity. Interestingly, introduction of p-fluoromethylphenyl piperazine group in amide portion led to **4f** with improved activity against especially Raji and HL60 cells. Taken together, compound **4c** was the most effective compound in suppressing Raji and HL60 cells growth (GI_{50} 25.2 ± 3.2 and 28.3 ± 1.53 μM, respectively). Ester derivatives of 1-phenyl-3-(thiophen-3-yl)-1*H*-pyrazole-4-carboxylic acid **4h–m** did not affect the growth of HeLa, MCF7, MDA-MB-231, Raji, and HL60 cells.

We then determined the suitability of compounds for anticancer purposes where a good absorption after oral administration is obligatory. LogP, the measure of the compound's solubility and permeability, is believed to be very important. Very high lipophilicity and the resulted large logP values cause poor absorption or permeation and should be avoided. Physicochemical properties of the synthesized compounds were evaluated in silico, and found that all compounds should present good passive oral absorption. All the synthesized compounds demonstrated good drug-likeness values.

Conclusion

A series of novel 1,3-diarylpyrazole derivatives has been successfully synthesized. Structures of all products were determined by IR, ^{1}H-NMR, and HRMS. We found that compounds **4c** and **4f** could suppress Raji and HL60 cancer cell growth. Compound **4c** bearing benzylpiperidine group in the amide portion showed significant inhibitory effect against Raji and HL60 cell lines. Owing to the significant results obtained, chemical studies are being conducted to modify structures to improve the antitumor activity of such compounds.

Experimental

Chemistry

The chemicals were purchased from the commercial vendors and were used without purification. Thin-layer

chromatography (TLC) was performed on Merck 60F254 plates. Reactions were monitored by TLC on silica gel, with detection by UV light (254 nm) or charring Dragendorff reagent (Stahl, 1969). Melting points were determined with an SMP-II Digital Melting Point Apparatus and are uncorrected (Schorpp Geaetetechnik, Germany). IR spectra were obtained in-house using a Perkin Elmer Spectrum 400 FTIR/FTNIR spectrometer equipped with a universal ATR Sampling Accessory. ^{1}H-NMR spectra were recorded in $CDCl_3$ or DMSO-d_6 on a Varian Mercury 400 MHz High Performance Digital FT-NMR spectrometer using tetramethylsilane as the internal standard at the NMR facility of Faculty of Pharmacy, Ankara University. All chemical shifts were recorded as δ (ppm). High-resolution mass spectra data (HRMS) were collected in-house using a Waters LCT Premier XE Mass Spectrometer (high-sensitivity orthogonal acceleration time-of-flight instrument) operating in either ESI (+) or ESI (−) methods, also coupled to an AQUITY Ultra Performance Liquid Chromatography system (Waters Corporation, Milford, MA, USA). Flash chromatography was performed with a Combiflash® Rf automated flash chromatography system with RediSep columns (Teledyne-Isco, Lincoln, NE, USA) using dichloromethane-methanol solvent gradients.

N-Phenyl-N′-(1-thiophen-3-yl-ethylidene)hydrazine ***1***

A solution of acetylthiophene (0.052 mol), phenyl hydrazine (0.058 mol), and acetic acid (2 ml, 0.035 mol) in ethanol was stirred for 2 h at reflux, and then evaporated. The precipitated was filtered off and dried. Yield 80 %; IR (FT-IR/ATR): 3349 cm^{-1} (N–H), 1933 cm^{-1} (C=N). ^{1}H-NMR (DMSO-d_6) δ: 9.13 (1H, s, N–N<u>H</u>), 7.64 (1H, m, ArH), 7.59 (1H, m, ArH), 7.51 (1H, m, ArH), 7.20 (4H, m, ArH), 6.73 (1H, m, ArH), 2.23 (3H, s, C–C<u>H</u>$_3$).

1-Phenyl-3-(thiophen-3-yl)-1H-pyrazole-4-carbaldehyde ***2***

In a dry flask, phosphoroxy chloride ($POCl_3$) (16.77 ml, 0.18 mol) was added dropwise to an ice-cold stirred solution of hydrazone derivative (0.06 mol) in 100 ml DMF. The reaction mixture was allowed to attain room temperature, and then heated at 50 °C for about 5 h. The resulting mixture was poured onto crushed ice, neutralized with dilute NaOH, and left overnight. The precipitate obtained was purified by crystallization in acetone–water mixture. Yield 82 %, mp 124 °C (Lit. mp: 120 °C (Arbaciauskiene *et al.,* 2011); IR (FT-IR/ATR): 1667 cm^{-1} (C=O). ^{1}H-NMR ($CDCl_3$) δ: 10.10 (1H, s, CHO), 8.50 (1H, s, pyrazole H-5), 8.19 (1H, m, ArH), 7.80–7.77 (2H, m, ArH), 7.70 (1H, m, ArH), 7.54–7.50 (2H, m, ArH), 7.44–7.38 (2H, m, ArH). HRMS $C_{14}H_{11}N_2OS$ $[M + H]^+$ *Calc*. 255.0592, Found m/z 255.0586.

1-Phenyl-3-(thiophen-3-yl)-1H-pyrazole-4-carboxylic acid ***3***

To a solution of aldehyde (2 g, 7.86 mmol) in 100 ml acetone–water mixture (3:2) potassium permanganate (2.48 g, 15.7 mmol) was added and the corresponding dark violet reaction mixture was stirred for 2.5 h to achieve full conversion. The solvents were removed under vacuum and the dark suspension was quenched upon addition of 20 ml saturated sodium sulfite solution. The violet solid was filtered off and washed with water. The turbid light brown mother liquor was treated with HCl adjusting the pH to 1 and CH_2Cl_2 was added. Organic phase was washed with water, dried over sodium sulfate, and evaporated under vacuum. The precipitate obtained was purified by crystallization in acetone–water mixture. IR (FT-IR/ATR): 1690 cm^{-1} (C=O), 2618 cm^{-1} (C=C). ^{1}H-NMR ($CDCl_3$) δ: 8.59 (1H, s, pyrazole H-5), 8.32 (1H, m, ArH), 7.79 (3H, m, ArH), 7.51 (2H, m, ArH), 7.37 (2H, m, ArH). HRMS $C_{14}H_{11}N_2O_2S$ $[M + H]^+$ *Calc*. 271.0541, Found m/z 271.0535.

General procedure for the preparation of amide/ester derivatives of 1-phenyl-3-(thiophen-3-yl)-1H-pyrazole-4-carboxylic acid ***4***

To the solution of acid derivatives (1 mmol) triethylamine (2 mmol) and ethyl chloroformate (1 mmol) were added, followed by stirring at 0 °C for 30 min. After addition of the appropriate amine or phenol derivative (1.2 mmol), mixture was stirred for an additional 1 h at 0 °C. Then, the reaction mixture was warmed to room temperature and kept stirring overnight. After the solvent was evaporated under reduce pressure, acetone was added, filtered, and evaporated. Residue was dissolved in DCM and the organic phase was washed with a 1 N HCl and 1 % $NaHCO_3$ solutions and brine, dried over Na_2SO_4, and evaporated under vacuum. The residue was purified by automated flash column chromatography (Combiflash® Rf) using DCM-MeOH (0–10 %) as eluents.

N-Benzyl-1-phenyl-3-(thiophen-3-yl)-1H-pyrazole-4-carboxamide ***4a*** IR (FT-IR/ATR): 1639 cm^{-1} (C=O), 3318 cm^{-1} (N–H). ^{1}H-NMR ($CDCl_3$) δ: 8.45 (1H, s, pyrazole H-5), 7.73 (2H, m, ArH), 7.68 (1H, m, ArH), 7.48–7.44 (2H, m, ArH), 7.40–7.21 (8H, m, ArH), 6.13 (1H, s, N<u>H</u>), 4.54 (2H, d, J = 5.6 Hz, NH–C<u>H</u>$_2$). HRMS $C_{21}H_{18}N_3OS$ $[M + H]^+$ *Calc*. 360.1171, Found m/z 360.1162.

Morpholin-4-yl[1-phenyl-3-(thiophen-3-yl)-1H-pyrazol-4-yl]methanone ***4b*** IR (FT-IR/ATR): 1630 cm^{-1} (C=O). ^{1}H-NMR ($CDCl_3$) δ: 8.09 (1H, s, pyrazole H-5), 7.73 (2H, m, ArH), 7.65 (1H, m, ArH), 7.48 (1H, m, ArH), 7.47 (2H,

m, ArH), 7.40 (1H, m, ArH), 7.35 (1H, m, ArH), 3.70 (4H, m, morpholine H-2,6), 3.29 (4H, m, morpholine H-3,5). HRMS $C_{18}H_{18}N_3O_2S$ $[M + H]^+$ *Calc*. 340.1120, Found m/z 340.1103.

(4-Benzylpiperidin-1-yl)(1-phenyl-3-(thiophen-3-yl)-1H-pyrazol-4-yl)-methanone ***4c*** IR (FT-IR/ATR): 1618 cm^{-1} (C=O). ^{1}H-NMR ($CDCl_3$) δ: 8.04 (1H, s, pyrazole H-5), 7.73 (2H, m, ArH), 7.64 (1H, m, ArH), 7.50–7.44 (3H, m, ArH), 7.36–7.23 (4H, m, ArH), 7.19 (1H, m, ArH), 7.08 (2H, m, ArH), 4.75–2.70 (4H, m, piperidin H-2,6), 2.46 (2H, s, CH_2), 1.73–1.20 (4H, m, piperidin H-3,5), 0.71 (1H, m, piperidin H-4). HRMS $C_{26}H_{26}N_3OS$ $[M + H]^+$ *Calc*. 428.1797, Found m/z 428.1790.

(4-Methylpiperazin-1-yl)[1-phenyl-3-(thiophen-3-yl)-1H-pyrazol-4-yl]methanone ***4d*** IR (FT-IR/ATR): 1619 cm^{-1} (C=O). ^{1}H-NMR ($CDCl_3$) δ: 8.06 (1H, s, pyrazole H-5), 7.73 (2H, d, $J = 7.2$ Hz, ArH), 7.64 (1H, m, ArH), 7.50–7.44 (3H, m, ArH), 7.37 (1H, m, ArH), 7.33 (1H, m, ArH), 3.80 (2H, m, piperazine H-5), 3.29 (2H, m, piperazine H-3), 2.42 (2H, m, piperazine H-2), 2.23 (3H, s, $C\underline{H}_3$), 2.06 (2H, m, piperazine H-6). HRMS $C_{19}H_{21}N_4OS$ $[M + H]^+$ *Calc*. 353.1436, Found m/z 353.1432.

[1-Phenyl-3-(thiophen-3-yl)-1H-pyrazol-4-yl)(4-phenylpiperazin-1-yl)methanone ***4e*** IR (FT-IR/ATR): 1636 cm^{-1} (C=O). ^{1}H-NMR ($CDCl_3$) δ: 8.11 (1H, s, pyrazole H-5), 7.73 (2H, m, ArH), 7.67 (1H, m, ArH), 7.52–7.46 (3H, m, ArH), 7.37 (2H, m, ArH), 7.25 (2H, m, ArH), 6.90 (3H, m, ArH), 3.93 (2H, s, piperazine H-5), 3.43 (2H, s, piperazine H-3), 3.20 (2H, s, piperazine H-2), 2.81 (2H, s, piperazine H-6). HRMS $C_{24}H_{23}N_4OS$ $[M + H]^+$ *Calc*. 415.1593, Found m/z 415.1576.

[1-Phenyl-3-(thiophen-3-yl)-1H-pyrazol-4-yl)[4-(4-trifluoromethylphenyl)-piperazin-1-yl]-methanone ***4f*** IR (FT-IR/ATR): 1615 cm^{-1} (C=O). ^{1}H-NMR ($CDCl_3$) δ: 8.12 (1H, s, pyrazole H-5), 7.75 (2H, d, $J = 8$ Hz, ArH), 7.66 (1H, m, ArH), 7.51–7.46 (5H, m, ArH), 7.38 (2H, m, ArH), 6.86 (2H, d, $J = 8.4$ Hz, ArH), 3.92 (2H, m, piperazine H-5), 3.43–3.30 (4H, m, piperazine H-2,3), 2.89 (2H, m, piperazine H-6). HRMS $C_{25}H_{22}F_3N_4OS$ $[M + H]^+$ *Calc*. 483.1466, Found m/z 483.1467.

[1-Phenyl-3-(thiophen-3-yl)-1H-pyrazol-4-yl)(4-pyridin-4-yl-piperazin-1-yl)-methanone ***4g*** IR (FT-IR/ATR): 1630 cm^{-1} (C=O). ^{1}H-NMR ($CDCl_3$) δ: 8.28 (2H, d, $J = 6.8$ Hz, pyridine H-2,6), 8.12 (1H, s, pyrazole H-5), 7.74 (2H, m, ArH), 7.66 (1H, m, ArH), 7.50–7.46 (3H, m, ArH), 7.38–7.32 (2H, m, ArH), 6.58 (2H, d, $J = 6.8$ Hz, pyridine H-3,5), 3.88–2.99 (8H, m, piperazine H-2,3,5,6). HRMS $C_{23}H_{22}N_5OS$ $[M + H]^+$ *Calc*. 416.1545, Found m/z 416.1534.

4-Methoxyphenyl 1-phenyl-3-(thiophen-3-yl)-1H-pyrazole-4-carboxylate ***4h*** IR (FT-IR/ATR): 1738 cm^{-1} (C=O). ^{1}H-NMR ($CDCl_3$) δ: 8.67 (1H, s, pyrazole H-5), 8.39 (1H, m, ArH), 7.83 (3H, m, ArH), 7.53 (2H, m, ArH), 7.40–7.32 (2H, m, ArH), 7.13 (2H, m, ArH), 6.95 (2H, m, ArH), 3.82 (3H, s, CH_3). HRMS $C_{21}H_{17}N_2O_3S$ $[M + H]^+$ *Calc*. 377.0960, Found m/z 377.0956.

4-Ethylphenyl 1-phenyl-3-(thiophen-3-yl)-1H-pyrazole-4-carboxylate ***4i*** IR (FT-IR/ATR): 1714 cm^{-1} (C=O). ^{1}H-NMR ($CDCl_3$) δ: 8.67 (1H, s, pyrazole H-5), 8.39 (1H, m, ArH), 7.81 (3H, m, ArH), 7.53–7.49 (2H, m, ArH), 7.40–7.36 (1H, t, $J = 7.6$ Hz, ArH), 7.32 (1H, m, ArH), 7.25 (2H, d, $J = 8.8$ Hz, ArH), 7.10 (2H, d, $J = 8.4$ Hz, ArH), 2.69–2.63 (2H, q, $J = 7.6$ Hz, $C\underline{H}_2$–CH_3), 1.26 (3H, t, $J = 7.2$ Hz, CH_2–$C\underline{H}_3$). HRMS $C_{22}H_{19}N_2O_2S$ $[M + H]^+$ *Calc*. 375.1167, Found m/z 375.1150.

3-Isopropylphenyl 1-phenyl-3-(thiophen-3-yl)-1H-pyrazole-4-carboxylate ***4j*** IR (FT-IR/ATR): 1744 cm^{-1} (C=O). ^{1}H-NMR ($CDCl_3$) δ: 8.67 (1H, s, pyrazole H-5), 8.39 (1H, m, ArH), 7.82 (3H, m, ArH), 7.51 (2H, m, ArH), 7.40–7.31 (3H, m, ArH), 7.13 (1H, m, ArH), 7.05 (2H, m, ArH), 2.93 (1H, m, $C\underline{H}$–$(CH_3)_2$), 1.27 (6H, s, CH–$(C\underline{H}_3)_2$). HRMS $C_{23}H_{21}N_2O_2S$ $[M + H]^+$ *Calc*. 389.1324, Found m/z 389.1325.

2-Isopropyl-5-methyl-phenyl 1-phenyl-3-(thiophen-3-yl)-1H-pyrazole-4-carboxylate ***4k*** IR (FT-IR/ATR): 1729 cm^{-1} (C=O). ^{1}H-NMR ($CDCl_3$) δ: 8.67 (1H, s, pyrazole H-5), 8.40 (1H, m, ArH), 7.82 (3H, m, ArH), 7.54 (2H, m, ArH), 7.41 (1H, m, ArH), 7.32 (1H, m, ArH), 7.23 (1H, m, ArH), 7.08 (1H, m, ArH), 6.94 (1H, s, ArH), 3.07 (1H, m, $C\underline{H}$–$(CH_3)_2$), 2.34 (3H, s, CH_3), 1.21–1.19 (6H, s, CH–$(C\underline{H}_3)_2$). HRMS $C_{24}H_{23}N_2O_2S$ $[M + H]^+$ *Calc*. 403.1480, Found m/z 403.1465.

Biphenyl-4-yl 1-phenyl-3-(thiophen-3-yl)-1H-pyrazole-4-carboxylate ***4l*** IR (FT-IR/ATR): 1698 cm^{-1} (C=O). ^{1}H-NMR ($CDCl_3$) δ: 9.00 (1H, s, pyrazole H-5), 8.70 (1H, m, ArH), 8.13 (3H, m, ArH), 7.94–7.89 (2H, m, ArH), 7.89–7.69 (8H, m, ArH), 7.58–7.49 (2H, m, ArH). HRMS $C_{26}H_{19}N_2O_2S$ $[M + H]^+$ *Calc*. 423.1167, Found m/z 423.1174.

Naphthalen-2-yl 1-phenyl-3-(thiophen-3-yl)-1H-pyrazole-4-carboxylate ***4m*** IR (FT-IR/ATR): 1732 cm^{-1} (C=O). ^{1}H-NMR ($CDCl_3$) δ: 8.96 (1H, s, pyrazole H-5), 8.61 (1H, d, $J = 2.8$ Hz, ArH), 8.13–8.03 (6H, m, ArH), 7.89 (1H, d,

$J = 2.4$ Hz, ArH), 7.76–7.69 (4H, m, ArH), 7.63–7.55 (3H, m, ArH). HRMS $C_{24}H_{17}N_2O_2S$ $[M + H]^+$ *Calc.* 397.1011, Found m/z 397.1008.

Anticancer activity

Cell lines and cell culture

The human cancer cell lines, cervical carcinoma (HeLa), estrogen receptor positive breast carcinoma (MCF7), estrogen receptor negative breast carcinoma (MDA-MB-231), Burkitt's lymphoma (Raji), and human promyelocytic leukemia (HL60), were obtained from ATCC.

Cytotoxicity assay

MDA-MB-231 and MCF7 cells were cultured in DMEM; whereas, HeLa, HL60, and Raji cells were grown in RPMI-1640 medium in a humidified atmosphere containing 5 % CO_2 at 37 °C. Both DMEM and RPMI-1640 medium were supplemented with 10 % fetal bovine serum (FBS), 200 mM L-glutamine, 100 IU/ml penicillin, and 100 μg/ml streptomycin (all from Hyclone Laboratories, Logan, UT, USA). Cell viability was determined using MTT (3-[4,5-dimethyl-2-thiazolyl]-2,5-diphenyl-2H-tetrazolium bromide) assay (Cell Proliferation Kit I, Roche, Germany). Briefly, cells were seeded in a 96-well plate at 10.000 cells per well and cultured for overnight in growth medium containing 1 % FBS. Then cells were treated with 50 μM of the test compounds for 48 h. As a solvent control, cells were also treated with dimethyl sulfoxide (DMSO) at a final concentration of 0.1 %. At the end of the incubation time, MTT reagent at the final concentration of 0.5 mg/ml was added to each well and incubated for an additional 4 h. After formation of blue formazan crystals, medium containing MTT was discarded and DMSO was added to the wells to dissolve crystals. The absorbance of samples was measured with Spectra Max M3 micro plate reader (Molecular Devices, Sunnyvale, CA, USA) at a wavelength of 570 nm. Average absorbance values from quadruplicate replicates per test compound and solvent control (DMSO) were calculated. Mean solvent control values were set to 100 % of viability and then the effects of the test compounds to the cell viability were calculated by comparing mean values obtained from compound treated culture wells with those of the solvent controls. As the compounds exhibited to reduction on cell viability more than 50 % at 50 μM, the GI_{50} values were also calculated from the concentration-response curves using the PRISM 5, GraphPad Software (GraphPad Prism Version 5.04, 2010).

Statistical significance of the effects of compounds to the cell viability was analyzed by one-way ANOVA and Tukey's post hoc test using SigmaStat v3.5 software. P values <0.05 were considered to be statistically significant. (* $P \leq 0.05$, ** $P \leq 0.01$, *** $P \leq 0.001$).

Lipinski's rule-of-five and drug-likeness profile

In order to explore the bioavailability of synthesized derivatives, theoretical calculations were carried out to predict some physicochemical properties of the synthesized compounds. The bioavailability of the compounds was assessed using ADME (absorption, distribution, metabolism, and elimination) prediction methods. In particular, we calculated the compliance of compounds to the Lipinski's rule-of-five (Lipinski *et al.,* 2001). Lipinski's "rule-of-five" and the later addition of other parameters such as polar surface area (PSA) (Ertl *et al.,* 2000) describes molecular properties important for a drug pharmacokinetics in the human body. This approach has been widely used as a filter for substances that would likely be further developed in drug design programs. Poor absorption and permeation are more likely to occur when there are more than five hydrogen-bond donors (HBD), more than ten hydrogen-bond acceptors (HDA), the molecular mass (MM) is greater than 500, or the log P value (clogP) is greater than five. The topological polar surface area (TPSA) should be smaller than 90 Å (Brueggemeier *et al.,* 2005). Molecules violating more than one of these rules may have problems with bioavailability.

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References

Abdel-Aziz M, Abuo-Rahma GEA, Hassan AA (2009) Synthesis of novel pyrazole derivatives and evaluation of their antidepressant and anticonvulsant activities. Eur J Med Chem 44:3480–3487

Al-Obaid AM, Abdel-Hamide SG, El-Kashef HA, Abdel-Aziz AA-M, El-Azab AS, Al-Khamees HA, El-Subbagh HI (2009) Substituted quinazolines, part 3. Synthesis, in vitro antitumor activity and molecular modeling study of certain 2-thieno-4(3H)-quinazolinone analogs. Eur J Med Chem 44:2379–2391

Anzaldi M, Macciò C, Mazzei M, Bertolotto M, Ottonello L, Dallegri F, Balbi A (2009) Antiproliferative and proapoptotic activities of a new class of pyrazole derivatives in HL-60 cells. Chem Biodivers 6:1674–1687

Arbaciauskiene E, Martynaitis V, Krikstolaityte S, Holzer W, Sackus A (2011) Synthesis of 3-substituted 1-phenyl-1H-pyrazole-4-carbaldehydes and the corresponding ethanones by Pd-catalyzed cross-coupling reactions. ARKIVOC 11:1–21

Bandgar BP, Gawande SS, Bodade RG, Gawande NM, Khobragade CN (2009) Synthesis and biological evaluation of a novel series of pyrazole chalcones as anti-inflammatory, antioxidant and antimicrobial agents. Bioorg Med Chem 17:8168–8173

Barresi V, Condorelli DF, Fortuna CG, Musumarra G, Scire S (2002) In vitro antitumor activities of 2,6-di-[2-(Heteroaryl)vinyl]pyridines and pyridiniums. Bioorg Med Chem 10:2899–2904

Basnet A, Thapa P, Karki R, Choi H, Choi JH, Yun M, Jeong B-S, Jahng Y, Na Y, Cho W-J, Kwon Y, Lee C-S, Lee E-S (2010) 2,6-Dithienyl-4-furyl pyridines: synthesis, topoisomerase I and II inhibition, cytotoxicity, structure-activity relationship, and docking study. Bioorg Med Chem Lett 20:42–47

Brueggemeier RW, Hackett JC, Diaz-Cruz ES (2005) Aromatase inhibitors in the treatment of breast cancer. Endocr Rev 26: 331–345

Chou L-C, Huang L-J, Yang J-S, Lee F-Y, Teng C-M, Kuo S-C (2007) Synthesis of furopyrazole analogs of 1-benzyl-3-(5-hydroxymethyl-2-furyl)indazole (YC-1) as novel anti-leukemia agents. Bioorg Med Chem 15:1732–1740

Chou LC, Huang LJ, Hsu MH, Fang MC, Yang JS, Zhuang SH, Lin HY, Lee FY, Teng CM, Kuo SC (2010) Synthesis of 1-benzyl-3-(5-hydroxymethyl-2-furyl)selenolo[3,2-c]pyrazole derivatives as new anticancer agents. Eur J Med Chem 45:1395–1402

Christodoulou MS, Liekens S, Kasiotis KM, Haroutounian SA (2010) Novel pyrazole derivatives: synthesis and evaluation of anti-angiogenic activity. Bioorg Med Chem 18:4338–4350

Cozzi P (2003) The discovery of a new potential anticancer drug: a case history. Farmaco 58:213–220

Daidone G, Maggio B, Raffa D, Plescia S, Schillaci D, Raimondi MV (2004) Synthesis and in vitro antileukemic activity of new 4-triazenopyrazole derivatives. Farmaco 59:413–417

Denizot F, Lang R (1986) Rapid colorimetric assay for cell growth and survival. Modifications to the tetrazolium dye procedure giving improved sensitivity and reliability. J Immunol Methods 89:271–277

Ding X-L, Meng N, Xia Y, Wang L-J, Zhang X-F, Zhao B-X, Miao J-Y (2007) Synthesis of 3-phenyl-1-[(aryl)methyl]-1H-pyrazole-5-carboxylic acid ethyl ester derivatives and determination of their activity as promoters of cell apoptosis. Youji Huaxue 27:1542–1546

El-Shafei A, Fadda AA, Khalil AM, Ameen TAE, Badria FA (2009) Synthesis, antitumor evaluation, molecular modeling and quantitative structure-activity relationship (QSAR) of some novel arylazopyrazolodiazine and triazine analogs. Bioorg Med Chem 17:5096–5105

Ertl P, Rohde B, Selzer P (2000) Fast calculation of molecular polar surface area as a sum of fragment-based contributions and its application to the prediction of drug transport properties. J Med Chem 43:3714–3717

Gaston MA, Dias LRS, Freitas ACC, Miranda ALP, Barreiro EJ (1996) Synthesis and analgesic properties of new 4-arylhydrazone 1-H pyrazole [3,4-b]pyridine derivatives. Pharm Acta Helv 71:213–219

GraphPad Prism Version 5.04 for Windows (2010) GS, San Diego. www.graphpad.com

Gouda MA, Berghot MA, Shoeib AI, Khalil AM (2010) Synthesis and antimicrobial activity of new anthraquinone derivatives incorporating pyrazole moiety. Eur J Med Chem 45:1843–1848

Holst-Hansen C, Brünner N (1998) MTT-cell proliferation assay. In: Celis JE (ed) Cell Biology: a Laboratory Handbook, 2nd edn. Academic Press, San Diego, pp 16–18

http://www.molinspiration.com/cgi-bin/properties

http://www.organic-chemistry.org/prog/peo

Hu WP, Yu HS, Chen YR, Tsai YM, Chen YK, Liao CC, Chang LS, Wang JJ (2008) Synthesis and biological evaluation of thiobenzanilides as anticancer agents. Bioorg Med Chem 16:5295–5302

Huang S, Lin R, Yu Y, Lu Y, Connolly PJ, Chiu G, Li S, Emanuel SL, Middleton SA (2007) Synthesis of 3-(1H-benzimidazol-2-yl)-5-isoquinolin-4-ylpyrazolo[1,2-b]pyridine, a potent cyclin dependent kinase 1 (CDK1) inhibitor. Bioorg Med Chem Lett 17:1243–1245

Insuasty B, Orozco F, Quiroga J, Abonia R, Nogueras M, Cobo J (2008) Microwave induced synthesis of novel 8,9-dihydro-7H-pyrimido[4,5-b][1,4]diazepines as potential antitumor agents. Eur J Med Chem 43:1955–1962

Insuasty B, Chamizo L, Munoz J, Tigreros A, Quiroga J, Abonia R, Nogueras M, Cobo J (2011) Synthesis of 1-substituted 3-aryl-5-aryl(hetaryl)-2-pyrazolines and study of their antitumor activity. Arch Pharm Chem Life Sci 345:275–286

Johnsto SRD, Ford H, Ross P (2005) In the royal marsden hospital hand book of cancer chemotherapy. Elsevier, London, New York, Oxford

Kemnitzer W, Jiang S, Wang Y, Kasibhatla S, Crogan-Grundy C, Bubenik M, Labrecque D, Denis R, Lamothe S, Attardo G, Gourdeau H, Tseng B, Drewe J, Cai SX (2008) Discovery of 4-aryl-4H-chromenes as a new series of apoptosis inducers using a cell- and caspase-based HTS assay. Part 5. Modifications of the 2- and 3-positions. Bioorg Med Chem Lett 18:603–607

Kim DSHL, Ashendel CL, Zhou Q, Chang CT, Lee E-S, Chang CJ (1998) Novel protein kinase c inhibitors: α-terthiophene derivatives. Bioorg Med Chem Lett 8:2695–2698

Kueng W, Silber E, Eppenberger U (1989) Quantification of cells cultured on 96-well plates. Anal Biochem 182:16–19

Lee ER, Kang YJ, Choi HY, Kang GH, Kim JH, Kim BW, Han YS, Nah SY, Paik HD, Park YS, Cho SG (2007) Induction of apoptotic cell death by synthetic naringenin derivatives in human lung epithelial carcinoma A549 cells. Biol Pharm Bull 30: 2394–2398

Leite ACL, da Silva KP, de Souza IA, de Araujo JM, Brondani DJ (2004) Synthesis, antitumor and antimicrobial activities of new peptidyl derivatives containing the 1,3-benzodioxole system. Eur J Med Chem 39:1059–1065

Li J, Zhao YF, Zhao XL, Yuan XY, Gong P (2006) Synthesis and anti-tumor activities of novel pyrazolo[1,5-a]pyrimidines. Arch Pharm Chem Life Sci 339:593–597

Lian S, Su H, Zhao B-X, Liu W-Y, Zheng L-W, Miao J-Y (2009) Synthesis and discovery of pyrazole-5-carbohydrazide N-glycosides as inducer of autophagy in A549 lung cancer cells. Bioorg Med Chem 17:7085–7092

Lipinski CA, Lombardo F, Dominy BW, Feeney PJ (2001) Experimental and computational approaches to estimate solubility and permeability in drug discovery and development settings. Adv Drug Deliv Rev 46:3–26

Lv PC, Sun J, Luo Y, Yang Y, Zhu HL (2010) Design, synthesis, and structure-activity relationships of pyrazole derivatives as potential FabH inhibitors. Bioorg Med Chem Lett 20:4657–4660

Machado P, Campos PT, Lima GR, Rosa FA, Flores AFC, Bonacorso HG, Zanatta N, Martins MAP (2009) Experimental and calculated structural parameters of 5-trihalomethyl-4,5-dihydro-1H-pyrazole derivatives, novel analgesic agents. J Mol Struct 917: 176–182

Micale N, Zappala M, Grasso S (2002) Synthesis and antitumor activity of 1,3-benzodioxole derivatives. Farmaco 57:853–859

Mosmann T (1983) Rapid colorimetric assay for cellular growth and survival: application to proliferation and cytotoxicity assays. J Immunol Methods 65:55–63

Nagarapu L, Mateti J, Gaikwad HK, Bantu R, Rani MS, Subhashini NJP (2011) Synthesis and anti-inflammatory activity of some novel 3-phenyl-N-[3-(4-phenylpiperazin-1yl)propyl]-1H-pyrazole-5-carboxamide derivatives. Bioorg Med Chem Lett 21: 4138–4140

Padmaja A, Payani T, Reddy DG, Padmavathi V (2009) Synthesis, antimicrobial and antioxidant activities of substituted pyrazoles, isoxazoles, pyrimidine and thioxopyrimidine derivatives. Eur J Med Chem 44:4557–4566

Pan XH, Liu X, Zhao BX, Xie YS, Shin DS, Zhang SL, Zhao J, Miao JY (2008) 5-Alkyl-2-ferrocenyl-6,7-dihydropyrazolo[1,5-

a]pyrazin-4(5H)-one derivatives inhibit growth of lung cancer A549 cell by inducing apoptosis. Bioorg Med Chem 16: 9093–9100

Reile H, Birnbock H, Bernhardt G, Spruss T, Schonenberger H (1990) Computerized determination of growth kinetic curves and doubling times from cells in microculture. Anal Biochem 187:262–267

Riyadh SM, Farghaly TA, Abdallah MA, Abdallah MM, Abd El-Aziz MR (2010) New pyrazoles incorporating pyrazolylpyrazole moiety: synthesis, anti-HCV and antitumor activity. Eur J Med Chem 45:1042–1050

Schenone S, Bruno O, Ranise A, Bondavalli F, Brullo C, Fossa P, Mosti L, Menozzi G, Carraro F, Naldini A, Bernini C, Manettic F, Botta M (2004) New pyrazolo[3,4-d]pyrimidines endowed with A431 antiproliferative activity and inhibitory properties of Src phosphorylation. Bioorg Med Chem Lett 14:2511–2517

Senaratne SG, Pirianov G, Mansi JL, Arnett TR, Colston KW (2000) Bisphosphonates induce apoptosis in human breast cancer cell lines. Br J Cancer 82:1459–1468

Shih SR, Chu TY, Reddy GR, Tseng SN, Chen HL, Tang WF, Wu MS, Yeh JY, Chao YS, Hsu JT, Hsieh HP, Horng JT (2010) Pyrazole compound BPR1P0034 with potent and selective anti-influenza virus activity. J Biomed Sci 17:13–21

Silveira IAFB, Paulo LG, Miranda ALP, Rocha SO, Freita ACC, Barreiro EJ (1993) New pyrazolylhydrazone derivatives as inhibitors of platelet aggregation. J Pharm Pharmacol 45: 646–649

Sridhar R, Perumal PT, Etti S, Shanmugam G, Ponnuswamy MN, Prabavathy VR, Mathivanan N (2004) Design, synthesis and antimicrobial activity of 1H-pyrazolecarboxylates. Bioorg Med Chem Lett 14:6035–6040

Stahl E (1969) Thin-layer chromatography. Springer, New York

Tetko IV (2005) Computing chemistry on the web. Drug Discov Today 10:1497–1500

Tewari AK, Srivastava P, Singh VP, Singh A, Goel RK, Monhan CG (2010) Novel anti-inflammatory agents based on pyrazole based dimeric compounds; design, synthesis, docking and in vivo activity. Chem Pharm Bull 58:634–638

Warshakoon NC, Wu S, Boyer A, Kawamoto R, Renock S, Xu K, Pokross M, Evdokimov AG, Zhou S, Winter C, Walter R, Mekel M (2006) Design and synthesis of a series of novel pyrazolyl-pyridines as HIF 1-α prolyl hydroxylase inhibitors. Bioorg Med Chem Lett 16:5687–5690

Wei F, Zhao B-X, Huang B, Zhang L, Sun C-H, Dong W-L, Shin D-S, Miao J-Y (2006) Design, synthesis, and preliminary biological evaluation of novel ethyl 1-(2′-hydroxy-3′-aroxypropyl)-3-aryl-1H-pyrazole-5-carboxylate. Bioorg Med Chem Lett 16:6342–6347

Wei F, Zhao BX, Dong WL, Hua Z, Shin DS, Wang DW, Xia Y, Ge YQ (2007) Microwave-assisted rapid and straightforward synthesis of novel 6-(aroxymethyl)-2-aryl-6,7-dihydropyrazolo[5,1-c][1,4]oxazin-4-one derivatives. Synth Commun 37:4415–4424

Xia Y, Dong Z-W, Zhao B-X, Ge X, Meng N, Shin D-S, Miao J-Y (2007) Synthesis and structure-activity relationships of novel 1-arylmethyl-3-aryl-1H-pyrazole-5-carbohydrazide derivatives as potential agents against A549 lung cancer cells. Bioorg Med Chem 15:6893–6899

Xie YS, Pan XH, Zhao BX, Liu JT, Shin DS, Zhang JH, Zheng LW, Zhao J, Miao JY (2008) Synthesis, structure characterization and preliminary biological evaluation of novel 5-alkyl-2-ferrocenyl-6,7-dihydropyrazolo[1,5-a]pyrazin-4(5H)-one derivatives. J Organomet Chem 693:1367–1374

Xie Y-S, Zhao H-L, Su H, Zhao B-X, Liu J-T, Li J-K, Lv H-S, Wang B-S, Shin D-S, Miao J-Y (2010) Synthesis, single-crystal characterization and preliminary biological evaluation of novel ferrocenyl pyrazolo[1,5-a]pyrazin-4(5H)-one derivatives. Eur J Med Chem 45:210–218

Zhang JH, Fan CD, Zhao BX, Shin DS, Dong WL, Xie YS, Miao JY (2008) Synthesis and preliminary biological evaluation of novel pyrazolo[1,5-a]pyrazin-4(5H)-one derivatives as potential agents against A549 lung cancer cells. Bioorg Med Chem 16:0165–10171

Zheng LW, Wub LL, Zhao BX, Dong WL, Miao JY (2009) Synthesis of novel substituted pyrazole-5-carbohydrazide hydrazone derivatives and discovery of a potent apoptosis inducer in A549 lung cancer cells. Bioorg Med Chem 17:1957–1962

Zheng L-W, Li Y, Geb D, Zhao B-X, Liu Y-R, Lv H-S, Ding J, Miao J-Y (2010) Synthesis of novel oxime-containing pyrazole derivatives and discovery of regulators for apoptosis and autophagy in A549 lung cancer cells. Bioorg Med Chem Lett 20: 4766–4770