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MEDICINAL CHEMISTRY RESEARCH

ORIGINAL RESEARCH

Synthesis, cytotoxicity, and molecular properties prediction of novel 1,3-diarylpyrazole derivatives

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Abstract A novel combinatorial library of ester and amide derivatives of 1,3-diarylpyrazoles was designed and synthesized. Anticancer activities of these compounds were assessed against MCF7, MDA-MB-231, HeLa, Raji, and HL60 human cancer cells by MTT assay. Out of these, compounds **4c** and **5f** were found as the most promising anticancer agents with IC_{50} values of 8.12 and 9.63 μM in Raji cells, respectively. All compounds exhibited suitable drug-like characteristics according to Lipinski's rule.

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

Introduction

Cancer is the second leading cause of death in developed countries, accounting for nearly one in five deaths. Chemotherapy is a major form of cancer treatment. However, majority of cancers are either resistant to chemotherapy or acquire resistance during treatment. Several important drugs including 5-flurouracil (5FU), doxorubicin, celecoxib, and mitoxantrone with different structures and mechanisms of antitumor activities, fail to end these problems completely. Due to several side effects, drug resistance and failure of antitumor drugs in certain cases of cancer, the design and discovery of non-traditional, efficient and safe chemical classes of agents are prime targets in contemporary medicinal chemistry (Cozzi, 2003; Johnston *et al*., 2005). 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.

Pyrazoles constitute an important heterocyclic family containing nitrogen in their five-membered ring. Several pyrazole derivatives have been found to possess considerable biological activities, which have stimulated numerous research activities in medicinal chemistry or chemical biology (Silveira *et al*., 1993; Sridhar *et al*., 2004; Nagarapu *et al*., 2011; Padmaja *et al*., 2009; Gaston *et al*., 1996, Baytas *et al*., 2012; Banoglu *et al*., 2007). 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). The literature survey has 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, besides their capabilities to exert remarkable anticancer effects through inhibiting different types of enzymes that play important roles in cell division (Warshakoon *et al.*, 2006; Huang *et al.*, 2007). Although the skeleton of the pyrazole is an important building block in biological effects, the type of peripheral substituents is also crucial. The studies concerning structure–activity relationships have shown that the cytotoxic potency of the compounds has been highly dependent on the substitution types and patterns on the aryl ring. Among the reported studies,

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pyrazole-5-carboxylate (Wei *et al.*, 2006; Zheng *et al.*, 2010), pyrazole-5-carboxamide (Ding *et al.*, 2009), and pyrazole-5-carbohydrazide (Lian *et al.*, 2009; Xia *et al.*, 2007; Xia *et al.*, 2008; Zheng *et al.*, 2009) derivatives have been shown to have significant anticancer activities against A549 lung cancer cell lines by inducing apoptosis or autophagy. 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 certain extents at concentrations of 5–20 μM (Ding *et al.*, 2007). Oxime-containing pyrazole derivatives (**1A** in Fig. 1) were synthesized by Zheng *et al.*, and it was found that their effects on dose- and time-dependent inhibition of proliferation were mainly attributed to the autophagy induction in lung cancer cells (Zheng *et al.*, 2010). The effects of 3-aryl-1-(4-*tert*-butylbenzyl)-1*H*-pyrazole-5-carbohydrazide hydrazone derivatives on A549 cell growth were investigated and it was found that **1B** (Fig. 1) possessed the highest growth inhibitory effect and induced apoptosis with IC_{50} value of 0.28 μM (Zheng *et al.*, 2009). It was suggested that the potency of 3-aryl-1-arylmethyl-1*H*-pyrazole-5-carbohydrazide hydrazone against A549 lung cancer cells related with the lipophilicity of the compound and depended on the capacity of compound chelating metal ions, because it was recognized that metal ions have important roles in the cell growth (Xia *et al.*, 2008). In another study, Huang *et al.* determined that *N*-((1,3-diphenyl-1*H*-pyrazol-4-yl)methyl)aniline derivatives showed potent anti-CDK2/cyclin E activity (Huang *et al.*, 2012). Compound **2** showed the most potent inhibition activity which inhibited the growth of MCF7 and B16-F10 cell lines with IC_{50} values of 1.88 and 2.12 μM and inhibited the CDK2/cyclin E holoenzyme activities with IC_{50} of 0.98 μM (Fig. 1).

In our previous work, 1,3-diarylpyrazole derivatives containing thiophene were synthesized and the effects of the compounds on MCF7, MDA-MB-231, HeLa, Raji, and HL60 human cancer cells growth were investigated. We found that (4-benzyl-piperidin-1-yl)-(1-phenyl-3-thiophen-3-yl-1*H*-pyrazol-4-yl)methanone **3** (Fig. 1) possessed the highest growth inhibitory effect on Raji and HL60 cancer cells (Inceler *et al.*, 2013). On the basis of all those observations, we considered to design and synthesize a new type of 1,3-diarylpyrazole, wherein potent 3-/4-pyridine moiety was linked to biologically active phenyl pyrazole moiety at C-3 position and different ester or amide groups at C-4 position on the basis of combinatorial synthesis, which is the current trend being practiced in most of the drug discoveries. In this article, we are also interested in exploring the biological activities of such molecules through structural modifications.

Results and discussions

Chemistry

We focused our synthetic efforts on diaryl heterocyclic ring systems as illustrated in Scheme 1. First, the hydrazone

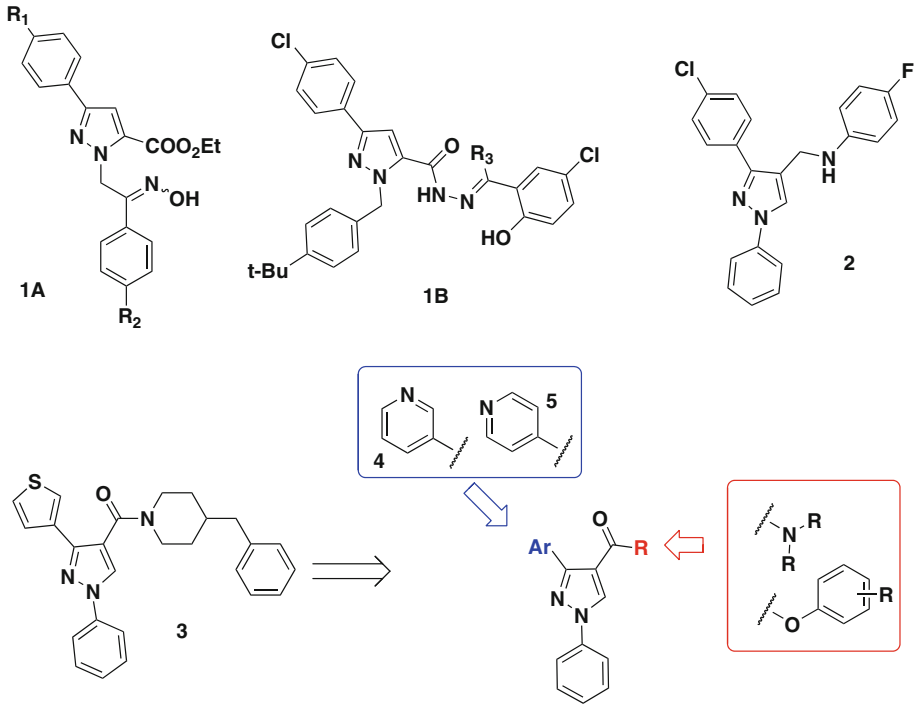
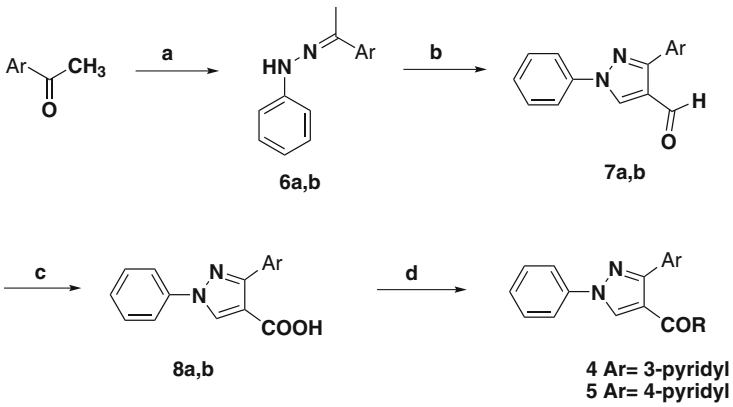


Fig. 1 Structure of the some pyrazole derivatives with anticancer activity (*1A*, *1B*, and *2*), the lead compound (*3*) and general structure of the synthesized compounds



Scheme 1 (*a*) Phenyl hydrazine, acetic acid, ethanol, 2 h reflux; (*b*) *Method A* dimethyl formamide, $POCl_3$, 50 °C, 4 h; *Method B* dimethyl formamide, $POCl_3$, MW irradiation, 210 W, 50 °C, 20 min; (*c*) potassium permanganate, acetone–water (3:2), 1 h reflux; (*d*) appropriate amine or phenol derivative, ethyl chloroformate, triethylamine, dichloromethane, rt, overnight

derivative **6** was generated by condensing 3-/4-acetylpyridine and phenylhydrazine in the presence of acetic acid in refluxing ethanol. The IR spectra of hydrazone **6** showed disappearance of the carbonyl peak that belonged to acetyl group of acetylpyridine derivatives. In the IR spectra of hydrazones **6a** and **6b**, secondary N–H stretching bands were observed at 3169 and 3274 cm^{-1} as unspiked, respectively. This hydrazone derivative was then reacted with phosphoroxy chloride ($POCl_3$) and DMF resulting in 1,3-diaryl pyrazole **7** with aldehyde group at 4 position. We focused our attention on the microwave-assisted synthesis technique after obtaining compound **7**. Microwave-assisted organic synthesis is a technique which can be used to rapidly explore the "chemistry space" and to increase the diversity of the compounds produced. Nowadays, it is possible to perform many of previous conventional heated reactions, using this technique (Lidstrom *et al.*, 2001). Microwave irradiation dominates over usual conventional methods due to its non-hazardous and eco-friendly nature. The microwave-induced organic reaction received considerable attention due to its simplicity and operational convenience. The use of microwave irradiation as a heat source in synthetic chemistry offers a promising alternative (Jyothi *et al.*, 2007; Kalluraya *et al.*, 2008). So, these reactions were performed in a microwave oven due to its low cost and availability. In a typical experiment, in order to synthesize compound **7**, $POCl_3$ was added dropwise to an ice-cold stirred solution of hydrazone **6** in DMF. The reaction mixture was allowed to attain room temperature, and then heated at 50 °C for 4 h. Following the work-up, desired compounds were obtained. Comparing the two methods, microwave-assisted synthesis technique dramatically cut down reaction time (conventional method time 4 h, microwave-assisted synthesis time 20 min), and increased product yields as shown in the "Experimental" section (conventional method yield 80 %, microwave-assisted synthesis yield 92 %). The IR spectra of compounds **7a**, **7b** characteristically exhibited strong absorptions for aldehyde carbonyl group at 1673 and 1669 cm^{-1}, respectively. The ^{1}H-NMR spectrum of the compounds **7a** and **7b** showed disappearance of the methyl proton signal and N–N–H signal. In the ^{1}H-NMR spectra of compound **7**, two bands were displayed due to aldehydic C–H (at 10.06 ppm for **7a**; at 10.04 ppm for **7b**) and pyrazole (at 8.58 for **7a**; at 9.43 ppm for **7b**), each denoting one proton, after integration. 1-Phenyl-3-pyridin-3-/4-yl-1*H*-pyrazole-4-carbaldehyde **7** was oxidized to carboxylic acid derivative **8** in the presence of potassium permanganate in acetone–water mixture. In the IR spectra of compounds **8a**, **8b** strong absorption bands at 1678 and 1709 cm^{-1}, respectively, and intense O–H stretching absorption in the region of 3300–2500 cm^{-1} for carboxylic acid, were observed. In the ^{1}H-NMR spectrum of compound **8a**, signal of the carboxylic acid appeared at 12.74 ppm, but for **8b**, signal of the carboxylic acid was not observed due to proton exchange. The signals of the pyrazole C–H were seen at 9.16 and 9.14 ppm for **8a** and **8b**, respectively. By treatment of **8** with appropriate amines or phenols in the presence of ethyl chloroformate, which was used as the carboxylate activator, and triethylamine in dichloromethane resulting amide and ester derivatives **4a**–**4m** and **5a**–**5n** were prepared in good yield (43–82 %). Compounds were purified by automated flash chromatography and were checked for purity using a UPLC before they were tested in biological assays (purity was >97 %). The structures of these compounds were confirmed by high resolution mass spectrometry (HRMS), IR and ^{1}H- and ^{13}C-NMR spectral data. Final amide and ester derivatives characteristically exhibited strong absorption in the area of 1597–1746 cm^{-1} attributable to the C=O of amide or ester groups. In the ^{1}H-NMR spectra of final compounds **4a**–**4m** and **5a**–**5n**, pyrazole C–H gave singlets in the 9.03–8.08 ppm range.

Effects of the compounds on the viability of cancer cells

Synthesized 1-phenyl-3-(pyridine-3-/4-yl)-1*H*-pyrazole-4-carboxylic acid **8a**, **8b** and its amide and ester derivatives **4a**–**4m** and **5a**–**5n** 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 (MTT) assay. The MTT cell proliferation assay is widely accepted as a reliable method to measure the cell proliferation rate (Holst-Hansen and Brünner, 1998; Reile *et al.*, 1990; Kueng *et al.*, 1989; Senaratne *et al.*, 2000). In order to measure the growth/viability (% of the untreated control), a spectrophotometer was used as described previously (Mosmann, 1983; Denizot and Lang, 1986). The IC_{50} values (Table 1) were calculated from the concentration–response curves by means of the PRISM 5, GraphPad Software (GraphPad Prism Version 5.04 for Windows, 2010).

In human cervical carcinoma cells (HeLa), IC_{50} values for the compounds **4c**, **4f**, **5c**, and **5k** were in the range of 25.26–33.56 μM (Table 1). Rest of the compounds did not show 50 % inhibition in HeLa cells, even at a concentration of 100 μM. Solubility problems occurred with compounds **4a**, **4l**, **4m**, **5i**, **5j**, and **5m**, hence they were not evaluated in MCF7, Raji, and HL60 cell lines. Present study revealed that among the breast cancer cell lines tested, human estrogen receptor positive breast adenocarcinoma cells (MCF7) are more sensitive to tested compounds than estrogen-independent breast cancer cells (MDA-MB-231). Compounds **4c**, **5k**, and **5n** exhibited moderate activity towards the MCF7 cell line (IC_{50} 32.24, 32.25, and 44.53 μM, respectively). Compounds **4f**, **4i**, **4k**, and **5c** were found less active against MCF7 cells, and their IC_{50} values were calculated as higher than 50 μM. Tested ester and amide derivatives of 1-phenyl-3-(pyridine-3-/4-yl)-1*H*-pyrazole-4-carboxylic acid **4a**–**4m** and **5a**–**5n** did not affect the growth of MDA-MB-231 cells except for compound **5k**, which is an *tert*-buthylphenyl ester of pyrazol-4-carboxylate (IC_{50} 33.56 μM). The tested compounds were found more effective in B lymphocyte cell line (Raji). In Raji cells, IC_{50} values for the compounds **4a**–**4m** were in the range of 8.12 and 75.64 μM. Compound **4c** (IC_{50} 8.12 μM, carrying 3-pyridyl moiety on pyrazole ring and benzyl piperidine at amide portion) followed by **5f** (IC_{50} 9.63 μM, carrying 4-pyridyl moiety on pyrazole ring and *tert*-buthylphenylpiperazine at amide portion) displayed significant antitumor activities. Compounds **4f**, **4i**, **4k**, **5c**, **5l**, and **5n** also showed significant cytotoxic activity in Raji cells (IC_{50} 15.12, 11.57, 20.56, 21.3, 10.59, and 12.05 μM, respectively). The efficiencies of compounds **4e**, **4j**, and **5k** were found to be moderate with IC_{50} values of 32.84, 42.26, and 33.27 μM, respectively. Finally, for HL60 leukemia cell line, **4c** (IC_{50} 16.14 μM), **4f** (IC_{50} 16.91 μM), and **5c** (IC_{50} 16.35 μM) followed by **5k** (IC_{50} 18.72 μM) and **5l** (IC_{50} 17.84 μM) displayed significant antitumor activity at 48 h. Compounds **4e**, **4i**, and **4k** were showed equipotent inhibitory activity having the considerable growth inhibition at the end time point (IC_{50} 24.52, 23.69, and 22.65 μM, respectively).

It is possible to relate the structural characteristics of the compounds to their antitumor activity. The data obtained by MTT assay showed that the amide derivatives have shown better inhibitory effects on the growth of tested human cancer cells than ester derivatives as indicated by the results in Table 1. When chemical structures of the compounds are taken into consideration, introduction of benzylpiperidin moiety at amide part of pyrazole-3-carboxamide core in both 3- and 4-pyridin series, led to **4c** and **5c** with enhanced activities against all the cancer cell lines with the best results against Raji cells. In our previous study, 1,3-diarylpyrazole derivatives containing thiophene moiety were synthesized and their anticancer effects were evaluated. It was found that the (4-benzyl-piperidin-1-yl)-(1-phenyl-3-thiophen-3-yl-1*H*-pyrazol-4-yl)methanone (**3** in Fig. 1) possessed the highest growth inhibitory effect on Raji and HL60 cancer cells (IC_{50} 25.2 and 28.3 μM, respectively). In present work, especially against Raji cells, **4c**, bearing 4-benzyl-piperidin moiety, displayed inhibitory activity on cell viability at 48 h, being the most efficient derivative with IC_{50} value of 8.12 μM. Compound **5c** was also found to be very potent inhibitor on cell viability against Raji and HL60 cancer cells (IC_{50} 21.3 and 16.3 μM, respectively). Taken these results into consideration, we can conclude that 4-benzyl-piperidin moiety at amide part of 1,3-diarylpyrazole-4-carboxamide core enhances anticancer activity, complying with our previous findings. By introducing *tert*-buthylphenylpiperazine to amide part, compounds **4f** (IC_{50} 15.12 μM) and **5f** (IC_{50} 9.63 μM) were obtained, and both showed significant anticancer activities.

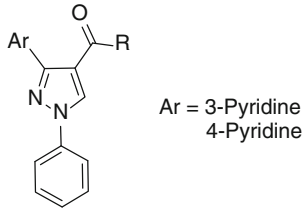
The enhanced activities of compounds **4c**, **4f**, **4i**, **5c**, **5f**, **5l**, and **5n** were mainly attributed to the presence of biologically active groups like 4-benzyl-piperidin and *tert*-buthylphenylpiperazine groups at amide part of the 1,3-diarylpyrazole-4-carboxamide core. For ester derivatives, 3-isopropylphenoxy, 2-isopropyl-5-methylphenoxy, and 2-naphthyloxy groups seem effective in anticancer activities in these types of compounds. This activity was attributed to the presence of electronegative groups in the molecules which increase the lipophilicity and affect the partitioning of molecules into membranes and facilitate hydrophobic interactions of the molecules with specific binding sites on either receptor or enzymes. Many anticancer drugs are effective against MCF7 and A549 cells, through causing apoptosis. Hence like the cytotoxic drugs, the synthesized compounds can be effective anticancer drugs through a similar mechanism (Leong *et al.*, 2003; Mizutani, 2007).

Lipinski's rule of five and drug-likeness profile

Physicochemical properties of synthesized compounds

The synthesized compounds **8a**, **8b**, **4a**–**4m**, and **5a**–**5n** were submitted to an in silico evaluation using a molecular modeling approach. To predict drug-like properties of synthesized compounds, we analyzed these derivatives

Table 1 In vitro cytotoxic activity of the synthesized compounds (**8a**, **8b**; **4a**–**4m**; and **5a**–**5n**)



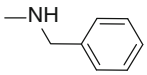
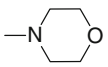
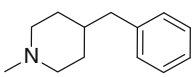
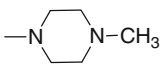
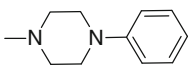
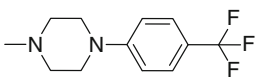
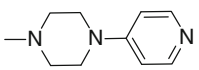
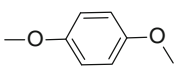
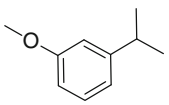
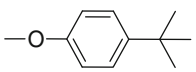
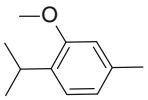
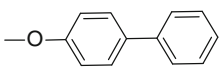
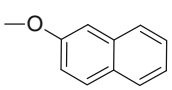
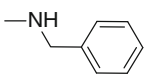
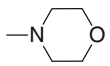
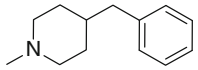
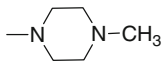
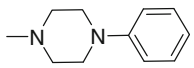
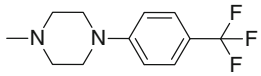
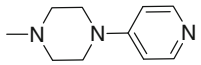
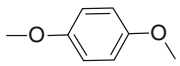
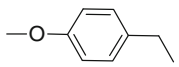
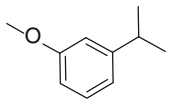
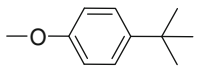
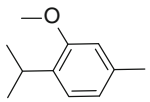
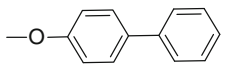
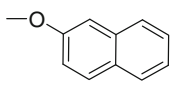
Compounds	Ar	R	IC_{50} (μM)[a]				
			HeLa	MDA-MB-231	MCF7	Raji	HL60
8a	3-Pyridine	OH	>100	>100	>100	>100	>100
8b	4-Pyridine	OH	>100	>100	>100	>100	>100
4a	3-Pyridine		>100	>100	NT	NT	NT
4b	3-Pyridine		>100	>100	>100	>100	>100
4c	3-Pyridine		**25.26 ± 2.23**	>100	**32.24 ± 1.39**	**8.12 ± 1.23**	**16.14 ± 1.47**
4d	3-Pyridine		>100	>100	>100	**75.64 ± 2.93**	>100
4e	3-Pyridine		>100	>100	>100	**32.84 ± 5.33**	**24.52 ± 3.51**
4f	3-Pyridine		**28.55 ± 5.12**	>100	**75.34 ± 3.90**	**15.12 ± 0.88**	**16.91 ± 0.12**
4g	3-Pyridine		>100	>100	>100	>100	>100
4h	3-Pyridine		>100	>100	>100	>100	>100
4i	3-Pyridine		>100	>100	**75.52 ± 2.65**	**11.57 ± 1.87**	**23.69 ± 2.53**
4j	3-Pyridine		>100	>100	>100	**42.26 ± 4.39**	>100
4k	3-Pyridine		>100	>100	**84.20 ± 2.23**	**20.56 ± 2.24**	**22.65 ± 1.59**
4l	3-Pyridine		>100	>100	NT	NT	NT
4m	3-Pyridine		>100	>100	NT	NT	NT
5a	4-Pyridine		>100	>100	>100	>100	>100

Table 1 continued

Compounds	Ar	R	IC_{50} (μM)[a]				
			HeLa	MDA-MB-231	MCF7	Raji	HL60
5b	4-Pyridine		>100	>100	>100	>100	>100
5c	4-Pyridine		**32.81 ± 1.11**	>100	**56.42 ± 1.33**	**21.3 ± 1.03**	**16.35 ± 0.82**
5d	4-Pyridine		>100	>100	>100	>100	>100
5e	4-Pyridine		>100	>100	>100	>100	>100
5f	4-Pyridine		>100	>100	>100	**9.63 ± 3.59**	>100
5g	4-Pyridine		>100	>100	>100	>100	>100
5h	4-Pyridine		>100	>100	>100	>100	>100
5i	4-Pyridine		>100	>100	NT	NT	NT
5j	4-Pyridine		>100	>100	NT	NT	NT
5k	4-Pyridine		**35.43 ± 3.62**	**34.52 ± 3.58**	**32.25 ± 0.82**	**33.27 ± 1.63**	**18.72 ± 2.65**
5l	4-Pyridine		>100	>100	>100	**10.59 ± 1.77**	**17.84 ± 3.44**
5m	4-Pyridine		>100	>100	NT	NT	NT
5n	4-Pyridine		>100	>100	**44.53 ± 4.97**	**12.05 ± 3.62**	>100

NT not tested

Compounds with IC_{50} values lower than 100 μM are given in bold

[a] Each experiment was independently performed four times and expressed as mean ± SD

according to the rule-of-five developed by Lipinski *et al.* (2001). Lipinski's "rule-of-five" and the later addition of other parameters such as PSA (Ertl *et al.*, 2000) describes molecular properties important for a drug's pharmacokinetics in the human body. PSA has been shown to be a very good descriptor characterizing drug absorption, including intestinal absorption, bioavailability, Caco-2 permeability, and blood–brain barrier penetration. This approach has been widely used as a filter for substances that are likely be further developed in drug design programs. Log *P*, the measure of the compound's solubility and permeability, is believed to be very important. Very high lipophilicity and the resulted large log *P* values cause poor absorption or permeation and should be avoided.

Predictions of ADME properties for these compounds are given in Table 2. Calculated physicochemical properties (http://www.molinspiration.com) showed that most of the compounds fulfilled the Lipinski's "rule-of-five". Theoretically, these compounds should present good passive oral absorption and differences in their bioactivity cannot

be attributed to this property. Compounds **4i**, **4j**, **4k**, **4l**, **5j**, **5k**, **5l**, and **5m** had very large *c*log *P* values of 5.20, 5.42, 5.15, 5.51, 4.99, 5.20, 4.91, and 5.29, respectively, which might be disadvantageous with regard to pharmacokinetic properties of these molecules in biological systems. The rest of the compounds exhibited favorable *c*log *P* values. Along with this, compounds **4c** and **5f**, which showed good antitumor screening results (Raji cells, IC_{50} 8.12 and 9.63 μM, respectively), have optimal *c*log *P* values, compared to other compounds in the series. Our results indicated that compounds possessing lipophilicity with log *P* values in the range of 3.15–5.31 (**4c**, **4e**, **4f**, **4i**, **4k**, **5c**, **5f**, **5k**, **5l**, and **5n**) demonstrated better inhibitory effects on the growth of selected cancer cells. Our results showed good correlations with previously published anticancer activities of similar pyrazole derivatives in which they showed growth in inhibitory effect on A549 lung cancer cells with log *P* values in the range of 3.12–4.94 (Xia *et al.*, 2007, 2008). The total polar surface area (TPSA) was calculated based on the methodology published by Ertl *et al.* (2000) as sums of O- and N-centered polar fragments contributions. The PSA is closely related to the hydrogen bonding potential of a compound. The TPSAs of the synthesized compounds were relatively small in comparison with the average value for acceptable drug molecules (<90 $Å^2$). It has to be kept in mind that log *P* and PSA values are the most important two features, although not sufficient for predicting oral absorption of a drug.

*Drug-likeness, drug score and toxicity risk profile of compounds (**8a, 8b; 4a–4m; 5a–5n**)*

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 study, we used the Osiris program (http://www.organic-chemistry.org/prog/peo.) for calculating the physicochemical properties (solubility, drug-likeness, and drug score) and the toxicity risks (mutagenicity, tumorogenicity, irritation, reproduction) of the compounds **8a**, **8b**; **4a–4m**; **5a–5n** (Table 3). Aqueous solubility of a compound significantly affects its absorption and distribution characteristics. Typically, a low solubility goes along with a bad absorption and therefore the general, the aim is to avoid poorly soluble compounds. More than 80 % of the drugs on the market have a (estimated) log *S* value of greater than −4. The log *S* values of most of our compounds **8a**, **8b**; **4a–4m**; **5a–5n**, are around −4. Drug-likeness may be defined as a complex balance of various molecular properties and structural features which determine whether a particular molecule is similar to known drugs. These properties influence the behavior of a molecule in a living organism, including bioavailability, transport properties, affinity to proteins, reactivity, toxicity, metabolic stability, and many others. It is interesting that, most of our compounds demonstrated good drug-likeness values (from 8.99 to 0.32). A positive value states that the molecule contains predominantly fragments which are frequently present in commercial drugs. Activities of all the compounds were analyzed under the four known criteria for successful drug activities in GPCR ligand, ion channel modulation, kinase inhibition, and nuclear receptor ligand activities. Results for all compounds are shown in Table 2. Values between −1.00 and 1.50 state that molecules may be active.

Moreover, we used the Osiris program for prediction of overall toxicity of the derivatives as it may point to the presence of some fragments generally responsible for the mutagenic, tumorigenic, irritant, or reproductive effects in these molecules. The toxicity risk predictor locates fragments within a molecule, which indicate a potential toxicity risk. Toxicity risk alert is an indication that the drawn structure may be harmful concerning the risk category specified. Only compounds containing 3-isopropyl-phenyl residue (compounds **4i** and **5j**) and naphthalen-2-yl moiety (**4m** and **5n**) showed in silico irritant and mutagenic properties, respectively. The rest of the compounds presented low in silico toxicity risk profile. In this study, we also examined the drug score. The drug score combines drug-likeness, *c*log *P*, log *S*, molecular weight, and toxicity risks in one handy value, and may be used to judge the compound's overall potential to qualify for a drug. Our results showed that the all synthesized compounds including the two of most potent ones demonstrated good drug score values (Table 3).

Conclusion

This study demonstrates the synthesis of a novel combinatorial library of a series of 1,3-diarylpyrazoles and the biological evaluation of their cytotoxic activities. Physicochemical properties of synthesized compounds were also 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 preliminary antitumor studies revealed that these agents exhibited significant antitumor activity in inhibiting various human tumor cell growths. The cytotoxic study results revealed that the compounds **4c** and **5f** were the most promising cytotoxic agents with IC_{50} values of 8.12 and 9.63 μM, respectively, in Raji cells. The present studies generated a series of new potent antitumor agents, which have potential for further antitumor drug development.

Experimental

Chemistry

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

Table 2 Calculated physicochemical properties of the synthesized compounds

Compounds	Predicted oral bioavailibility						Bioactivity scores					
	HBA[a]	HBD[b]	M.M[c]	*c*log *P*[d]	Volume	TPSA[e]	GPCRL[f]	ICM[g]	KI[h]	NRL[i]	PI[j]	EI[k]
8a	5	1	265.2	1.76	230	68.01	−0.10	−0.08	−0.04	−0.16	−0.44	−0.02
8b	5	1	265.2	1.54	230	68.01	−0.15	−0.12	−0.11	−0.19	−0.48	−0.10
4a	5	1	354.4	2.78	323	59.81	0.00	−0.13	0.02	−0.27	−0.17	−0.12
4b	6	0	334.3	1.00	301	60.26	0.01	−0.22	0.05	−0.32	−0.22	−0.16
4c	5	0	422.5	3.73	397	51.02	0.14	−0.02	−0.02	−0.20	−0.07	−0.08
4d	6	0	347.4	1.05	321	54.26	0.09	−0.11	0.09	−0.33	−0.20	−0.12
4e	6	0	409.5	2.74	376	54.26	0.05	−0.14	0.04	−0.29	−0.22	−0.16
4f	6	0	477.5	3.64	407	54.26	0.09	−0.07	0.07	−0.16	−0.18	−0.14
4g	7	0	410.4	1.45	372	67.15	0.09	−0.10	0.08	−0.29	−0.18	−0.12
4h	6	0	371.3	3.77	328	66.25	−0.18	−0.16	−0.07	−0.20	−0.37	−0.18
4i	5	0	383.4	5.20	353	57.02	−0.15	−0.14	−0.12	−0.11	−0.34	−0.15
4j	5	0	397.4	5.42	369	57.02	−0.11	−0.07	−0.06	−0.07	−0.32	−0.13
4k	5	0	397.4	5.15	369	57.02	−0.18	−0.20	−0.18	−0.12	−0.42	−0.19
4l	5	0	417.4	5.51	374	57.02	−0.11	−0.09	−0.02	−0.12	−0.27	−0.11
4m	5	0	391.4	4.90	347	57.02	−0.11	−0.10	0.00	−0.11	−0.29	−0.10
5a	5	1	354.4	2.56	323	59.81	−0.03	−0.017	−0.03	−0.29	−0.20	−0.17
5b	6	0	334.3	0.78	301	60.26	−0.03	−0.26	−0.01	−0.34	−0.25	−0.22
5c	5	0	422.5	3.51	397	51.02	0.11	−0.05	−0.06	−0.22	−0.10	−0.12
5d	6	0	347.4	0.83	321	54.26	0.05	−0.14	0.04	−0.35	−0.24	−0.18
5e	6	0	409.5	2.52	376	54.26	0.03	−0.16	0.00	−0.31	−0.25	−0.21
5f	6	0	477.2	3.42	407	54.26	0.07	−0.10	0.03	−0.17	−0.21	−0.18
5g	7	0	410.4	1.23	372	67.15	0.06	−0.13	0.04	−0.31	−0.21	−0.17
5h	6	0	371.3	3.55	328	66.25	−0.21	−0.20	−0.12	−0.22	−0.40	−0.23
5i	5	0	369.4	4.41	336	57.02	−0.17	−0.15	−0.17	−0.16	−0.36	−0.20
5j	5	0	383.4	4.99	353	57.02	−0.18	−0.17	−0.17	−0.13	−0.37	−0.20
5k	5	0	397.4	5.20	369	57.02	−0.14	−0.10	−0.11	−0.09	−0.35	−0.18
5l	5	0	397.4	4.91	369	57.02	−0.21	−0.23	−0.23	−0.14	−0.45	−0.24
5m	5	0	417.4	5.29	374	57.02	−0.13	−0.12	−0.05	−0.13	−0.30	−0.15
5n	5	0	391.4	4.68	347	57.02	−0.14	−0.13	−0.05	−0.13	−0.33	−0.15

[a] Number of hydrogen-bond acceptor

[b] Number of hydrogen-bond donor

[c] Molecular mass

[d] Calculated lipophilicity

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

[f] GPCR ligand

[g] Ion channel modulator

[h] Kinase inhibitor

[i] Nuclear receptor ligand

[j] Protease inhibitor

[k] Enzyme inhibitor

(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 by 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, values are

Table 3 Drug-likeness, drug score and toxicity risks of the synthesized compounds (**8a**, **8b**; **4a**–**4m**; **5a**–**5n**)

Compounds	Toxicity risk				Bioavailability and drug score		
	MUT[a]	TUM[b]	IRRIT[c]	RE[d]	log *S*[e]	Drug-likeness	Drug score
8a	Low	Low	Low	Low	−2.35	−0.03	0.69
8b	Low	Low	Low	Low	−2.35	−1.16	0.57
4a	Low	Low	Low	Low	−3.66	2.15	0.75
4b	Low	Low	Low	Low	−2.04	3.38	0.89
4c	Low	Low	Low	Low	−4.23	5.32	0.62
4d	Low	Low	Low	Low	−1.55	8.89	0.9
4e	Low	Low	Low	Low	−3.39	8.99	0.76
4f	Low	Low	Low	Low	−4.17	−1.46	0.36
4g	Low	Low	Low	Low	−2.6	7.22	0.82
4h	Low	Low	Low	Low	−3.82	0.72	0.63
4i	Low	Low	Medium	Low	−4.68	−0.16	0.35
4j	Low	Low	Low	Low	−4.96	−1.73	0.3
4k	Low	Low	Low	Low	−5.02	−2.4	0.29
4l	Low	Low	Low	Low	−5.89	1.14	0.38
4m	Medium	Low	Low	Low	−5.41	−1.15	0.26
5a	Low	Low	Low	Low	−3.66	1.24	0.7
5b	Low	Low	Low	Low	−2.04	2.61	0.87
5c	Low	Low	Low	Low	−4.23	4.59	0.62
5d	Low	Low	Low	Low	−1.55	8.16	0.9
5e	Low	Low	Low	Low	−3.39	8.27	0.76
5f	Low	Low	Low	Low	−4.17	−2.19	0.33
5g	Low	Low	Low	Low	−2.6	6.55	0.82
5h	Low	Low	Low	Low	−3.82	−0.12	0.55
5i	Low	Low	Low	Low	−4.31	0.08	0.51
5j	Low	Low	Medium	Low	−4.68	−1.01	0.3
5k	Low	Low	Low	Low	−4.96	−2.48	0.28
5l	Low	Low	Low	Low	5.02	−3.25	0.28
5m	Low	Low	Low	Low	−5.89	0.32	0.34
5n	Medium	Low	Low	Low	−5.41	−2.06	0.23

[a] Mutagenic
[b] Tumorigenic
[c] Irritant
[d] Reproductive effective
[e] Solubility

given in δ (ppm) and J values are in Hz. ^{13}C-NMR spectra were recorded in $CDCl_3$ or DMSO-d_6 on a Bruker Ultrashield 300 MHz NMR spectrometer using tetramethylsilane as the internal standard at the NMR facility of Gazi University, values are given in 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 ESI (+) method, also coupled to an AQUITY ultra performance liquid chromatography system (Waters Corporation, Milford, MA, USA). Flash chromatography was performed using a Combiflash® Rf automated flash chromatography system with RediSep columns (Teledyne-Isco, Lincoln, NE, USA) using dichloromethane–methanol solvent gradients. Elemental analyzes (C, H, N) were determined on a Leco CHNS 932 instrument and gave values within ±0.4 % of the theoretical values. Microwave-assisted reactions were carried out with a Milestone MicroSYNTH Microwave Synthesis System. Calculation of important physicochemical properties (log *P*, number of hydrogen-bond donors and acceptors, and TPSA) was performed using Molinspiration Cheminformatics Software at URL http://www.molinspiration.com. Determining of toxicity risk, drug-likeness and drug score was done using Osiris program at URL http://www.organic-chemistry.org/prog/peo.

General procedure for the preparation of *N*-Phenyl-*N*'-(1-pyridin-3/4-yl-ethylidene)-hydrazine (**6a**, **6b**)

Compounds **6a**, **6b** were synthesized as previously described (Lokhande *et al.*, 2011; Rathelot *et al.*, 2002). A solution of acetylpyridine derivative (52 mmol), phenyl hydrazine (6.27 g, 58 mmol), and acetic acid (2 ml, 35 mmol) in ethanol was stirred for 2 h at reflux, and then evaporated. The precipitate was filtered off and dried.

*N-Phenyl-N'-(1-pyridin-3-yl-ethylidene)-hydrazine (**6a**)*

mp 139–140 °C (Huck *et al.*, 2004).

*N-Phenyl-N'-(1-pyridin-4-yl-ethylidene)-hydrazine (**6b**)*

mp 147 °C [Lit. mp (Chu and Teague 1958): 148–149 °C].

General procedure for the preparation of 1-phenyl-3-(pyridin-3-/4-yl)-1*H*-pyrazole-4-carbaldehyde derivatives (**7a**, **7b**)

*Method A (*Rathelot *et al.*, 2002; Bratenko *et al.*, 2002)

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 **(6a** or **6b)** (0.06 mol) in 100 ml DMF. This reaction mixture was allowed to attain room temperature, and then heated at 50 °C for about 4 h. The resulting mixture was poured onto crushed ice, neutralized with diluted NaOH and left overnight. The precipitate obtained was purified by crystallization in acetone–water mixture.

Method B

In a dry flask, $POCl_3$ (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 flask was placed in MicroSYNTH Microwave Synthesis System and irradiated at 210 W for 20 min while the temperature was set to 50 °C. The resulting mixture was poured onto crushed ice, neutralized with diluted NaOH and left standing overnight. The yellow precipitate obtained was purified by crystallization in acetone–water mixture.

*1-Phenyl-3-(pyridin-3-yl)-1H-pyrazole-4-carbaldehyde (**7a**)* Method A: yield 85 %, Method B: 98 %, mp 159–160 °C (Huck *et al.*, 2004).

*1-Phenyl-3-(pyridin-4-yl)-1H-pyrazole-4-carbaldehyde (**7b**)* Method A: yield 12 g (80 %), Method B: yield 13.8 (92 %), mp 147–149 °C (Badadhe *et al.*, 2011).

General procedure for the preparation of 1-phenyl-3-(pyridin-3/4-yl)-1*H*-pyrazole-4-carboxylic acid derivatives (**8**)

Potassium permanganate (24 mmol) was added to a solution of aldehyde (12 mmol) in 100 ml acetone–water mixture (3:2) 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 obtained was quenched upon addition of 20 ml saturated sodium sulfite solution. The violet solid was then 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.

*1-Phenyl-3-(pyridin-3-yl)-1H-pyrazole-4-carboxylic acid (**8a**)*

Yield 82 %, mp 239–242 °C [Lit. mp (Bratenko *et al.*, 2001); 236–238 °C].

*1-Phenyl-3-(pyridin-4-yl)-1H-pyrazole-4-carboxylic acid (**8b**)*

Yield 79 %, mp 233–235 °C; IR (FTIR/FTNIR-ATR): 1709 cm^{-1} (C=O) ^{1}H-NMR (DMSO-d_6) δ: 9.16 (1H, s), 8.67 (2H, s), 8.01 (2H, d, $J = 8$ Hz), 7.88 (2H, s), 7.56 (2H, t, $J = 7.6$ Hz), 7.42 (1H, m). HRMS $C_{15}H_{12}N_3O_2$ $[M+H]^+$ *Calc*. 266.0930, Found *m/z* 266.0933.

General procedure for the preparation of amide and ester derivatives of 1-phenyl-3-(pyridin-3/4-yl)-1*H*-pyrazole-4-carboxylic acids (**4a**–**4m**; **5a**–**5n)**

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

*1-Phenyl-3-(pyridin-3-yl)-1H-pyrazole-4-carboxylic acid benzylamide (**4a**)*

Yield 56 %, mp 162–164 °C; IR (FTIR/FTNIR-ATR): 1637 cm^{-1} (C=O), 3266 cm^{-1} (N–H). ^{1}H-NMR ($CDCl_3$) δ: 8.97 (1H, d, $J = 2$ Hz), 8.62–8.59 (1H, m), 8.42 (1H, s), 8.03 (1H, td, $J = 2$ Hz, $J = 8$ Hz), 7.71 (2H, d, $J = 7.6$ Hz), 7.49–7.45 (2H, m), 7.37–7.22 (7H, m), 5.97 (1H, s), 4.53 (2H, d, $J = 5.6$ Hz). ^{13}C-NMR ($CDCl_3$) δ: 167.8, 148.0, 147.5, 139.9, 138.0, 133.5, 133.3, 134.2, 129.5, 129.3, 128.7, 127.3, 126.9, 126.3, 124.4, 120.2,

114.9, 43.5, HRMS $C_{22}H_{19}N_4O$ $[M+H]^+$ *Calc*. 355.1559, Found *m/z* 355.1545.

*Morpholin-4-yl-[1-phenyl-3-(pyridin-3-yl)-1H-pyrazol-4-yl]methanone (**4b**)*

Yield 56 %, mp 128–130 °C; IR (FTIR/FTNIR-ATR): 1607 cm^{-1} (C=O). ^{1}H-NMR ($CDCl_3$) δ: 8.99 (1H, d, $J = 1.6$ Hz), 8.63 (1H, dd, $J = 1.6$ Hz, $J = 4.8$ Hz), 8.16 (1H, s), 8.06 (1H, td, $J = 2$ Hz, $J = 8$ Hz), 7.74 (2H, d, $J = 8$ Hz), 7.53–7.50 (2H, m), 7.39–7.34 (2H, m), 3.68–3.23 (8H, m). ^{13}C-NMR ($CDCl_3$) δ: 169.3, 148.0, 147.5, 139.9, 133.5, 133.3, 134.2, 129.5, 129.3, 126.3, 124.4, 120.2, 114.9, 67.3, 46.7. HRMS $C_{19}H_{19}N_4O_2$ $[M+H]^+$ *Calc*. 335.1508, Found *m/z* 335.1495.

*(4-Benzyl-piperidin-1-yl)-[1-phenyl-3-(pyridin-3-yl)-1H-pyrazol-4-yl]methanone (**4c**)*

Yield 69 %, mp 158–160 °C; IR (FTIR/FTNIR-ATR): 1601 cm^{-1} (C=O). ^{1}H-NMR ($CDCl_3$) δ: 8.99 (1H, d, $J = 2.4$ Hz), 8.62 (1H, dd, $J = 1.6$ Hz, $J = 4.8$ Hz), 8.17 (1H, s), 8.07–8.03 (1H, m), 7.75 (2H, d, $J = 8.4$ Hz), 7.49 (2H, t, $J = 8$ Hz), 7.36–7.33 (2H, m), 7.27–7.23 (2H, m), 7.22–7.17 (1H, m), 7.06 (2H, d, $J = 7.2$ Hz), 4.73–3.61 (2H, m), 2.69–2.42 (4H, m), 1.71–1.62 (3H, m), 1.35–0.63 (2H, m). ^{13}C-NMR ($CDCl_3$) δ: 173.4, 148.0, 147.5, 139.9, 139.7, 133.5, 133.3, 134.2, 129.5, 129.3, 128.8, 128.3, 126.3, 125.9, 124.4, 120.2, 114.9, 45.3, 41.8, 37.4, 29.5. HRMS $C_{27}H_{27}N_4O$ $[M+H]^+$ *Calc*. 423.2185, Found *m/z* 423.2179.

*(4-Methyl-piperazin-1-yl)-[1-phenyl-3-(pyridin-3-yl)-1H-pyrazol-4-yl]methanone (**4d**)*

Yield 78 %, mp 137–139 °C; IR (FTIR/FTNIR-ATR): 1613 cm^{-1} (C=O). ^{1}H-NMR ($CDCl_3$) δ: 9.00 (1H, d, $J = 2$ Hz), 8.65–8.59 (1H, m), 8.13 (1H, s), 8.08–8.03 (1H, m), 7.78–7.73 (2H, m), 7.52–7.49 (2H, m), 7.36–7.31 (2H, m), 3.79 (2H, s), 3.25 (2H, s), 2.41 (2H, s), 2.20 (3H, s), 1.98 (2H, s). ^{13}C-NMR ($CDCl_3$) δ: 169.3, 148.0, 147.5, 139.9, 133.5, 133.3, 134.2, 129.5, 129.3, 126.3, 124.4, 120.2, 114.9, 51.7, 49.5, 46.8. HRMS $C_{20}H_{22}N_5O$ $[M+H]^+$ *Calc*. 348.1824, Found *m/z* 348.1810.

*(4-Phenyl-piperazin-1-yl)-[1-phenyl-3-(pyridin-3-yl)-1H-pyrazol-4-yl]methanone (**4e**)*

Yield 73 %, mp 150–152 °C; IR (FTIR/FTNIR-ATR): 1597 cm^{-1} (C=O). ^{1}H-NMR ($CDCl_3$) δ: 9.03 (1H, d, $J = 1.2$ Hz), 8.60 (1H, dd, $J = 1.6$ Hz, $J = 4.8$ Hz), 8.17 (1H, s), 8.07 (1H, td, $J = 2$ Hz, $J = 8$ Hz), 7.75 (2H, d, $J = 8.4$ Hz), 7.52–7.49 (2H, m), 7.37–7.33 (2H, m), 7.26–7.22 (2H, m), 6.88 (1H, t, $J = 7.2$ Hz), 6.83 (2H, d, $J = 8$ Hz), 3.93–3.88 (2H, m), 3.42–3.37 (2H, m), 3.40–3.37 (2H, m), 2.79–2.74 (2H, m). ^{13}C-NMR ($CDCl_3$) δ: 169.3, 149.8, 148.0, 147.5, 139.9, 133.5, 133.3, 134.2, 129.8, 129.5, 129.3, 126.3, 124.4, 122.2, 120.2, 114.9, 114.6, 53.6, 49.5. HRMS $C_{25}H_{24}N_5O$ $[M+H]^+$ *Calc*. 410.1981, Found *m/z* 410.1977.

*[1-Phenyl-3-(pyridin-3-yl)-1H-pyrazol-4-yl]-[4-(4-trifluoromethyl-phenyl)-piperazin-1-yl]methanone (**4f**)*

Yield 71 %, mp 178–180 °C; IR (FTIR/FTNIR-ATR): 1619 cm^{-1} (C=O). ^{1}H-NMR ($CDCl_3$) δ: 9.01 (1H, d, $J = 1.6$ Hz), 8.62 (1H, dd, $J = 1.6$ Hz, $J = 4.8$ Hz), 8.19 (1H, s), 8.09 (1H, td, $J = 2$ Hz, $J = 8$ Hz), 7.75 (2H, d, $J = 7.6$ Hz), 7.52–7.49 (2H, m), 7.45 (2H, d, $J = 8.8$ Hz), 7.39–7.35 (2H, m), 6.83 (2H, d, $J = 8.8$ Hz), 3.93–3.87 (2H, m), 3.40–3.28 (4H, m), 2.87–2.83 (2H, m). ^{13}C-NMR ($CDCl_3$) δ: 169.3, 153.2, 148.0, 147.5, 139.9, 133.5, 133.3, 134.2, 129.5, 129.3, 126.3, 126.0, 124.4, 124.1, 120.2, 114.9, 113.6, 53.6, 49.5. HRMS $C_{26}H_{23}N_5OF_3$ $[M+H]^+$ *Calc*. 478.1855, Found *m/z* 478.1835.

*[1-Phenyl-3-(pyridin-3-yl)-1H-pyrazol-4-yl]-(4-pyridin-4-yl-piperazin-1-yl)methanone (**4g**)*

Yield 44 %, mp 196–198 °C; IR (FTIR/FTNIR-ATR): 1630 cm^{-1} (C=O). ^{1}H-NMR ($CDCl_3$) δ: 9.00 (1H, d, $J = 2$ Hz), 8.61 (1H, dd, $J = 1.6$ Hz, $J = 4.8$ Hz), 8.27 (2H, d, $J = 6.4$ Hz), 8.19 (1H, s), 8.07 (1H, td, $J = 2$ Hz, $J = 8$ Hz), 7.76 (2H, d, $J = 7.2$ Hz), 7.53–7.47 (2H, m), 7.39–7.35 (2H, m), 6.56 (2H, d, $J = 6.4$ Hz), 3.88–2.93 (8H, m). ^{13}C-NMR ($CDCl_3$) δ: 169.3, 152.2, 150.5, 148.0, 147.5, 139.9, 133.5, 133.3, 134.2, 129.5, 129.3, 126.3, 124.4, 120.2, 114.9, 107.2, 53.6, 49.5. HRMS $C_{24}H_{23}N_6O$ $[M+H]^+$ *Calc*. 411.1933, Found *m/z* 411.1936.

*1-Phenyl-3-(pyridin-3-yl)-1H-pyrazole-4-carboxylic acid 4-methoxy-phenyl ester (**4h**)*

Yield 76 %, mp 158–160 °C; IR (FTIR/FTNIR-ATR): 1726 cm^{-1} (C=O). ^{1}H-NMR ($CDCl_3$) δ: 9.13 (1H, d, $J = 1.6$ Hz), 8.71 (1H, s), 8.65–8.61 (1H, m), 8.31–8.25 (1H, m), 7.85–7.79 (2H, m), 7.55–7.51 (2H, m), 7.43–7.32 (2H, m), 7.09–7.06 (2H, m), 6.93–6.87 (2H, m), 3.80 (3H, s). ^{13}C-NMR ($CDCl_3$) δ: 165.5, 157.6, 148.0, 147.5, 142.0, 139.9, 133.5, 133.3, 134.2, 129.5, 129.3, 126.3, 124.4, 122.9, 120.2, 115.5, 114.9, 55.9. HRMS $C_{22}H_{18}N_3O_3$ $[M+H]^+$ *Calc*. 372.1348, Found *m/z* 372.1330.

*1-Phenyl-3-(pyridin-3-yl)-1H-pyrazole-4-carboxylic acid 3-isopropyl-phenyl ester (**4i**)*

Yield 58 %, mp 63–65 °C; IR (FTIR/FTNIR-ATR): 1716 cm^{-1} (C=O). ^{1}H-NMR ($CDCl_3$) δ: 9.14 (1H, d, $J = 2.4$ Hz), 8.72

(1H, s), 8.65–8.61 (1H, m), 8.30 (1H, td, $J = 2$ Hz, $J = 7.6$ Hz), 7.83 (2H, d, $J = 8$ Hz), 7.57–7.52 (2H, m), 7.43–7.29 (3H, m), 7.13–6.97 (3H, m), 2.94–2.91 (1H, m), 1.25 (6H, s). ^{13}C-NMR ($CDCl_3$) δ: 165.5, 153.5, 150.7, 148.0, 147.5, 139.9, 133.5, 133.3, 134.2, 129.5, 129.3, 129.0, 126.3, 124.4, 123.2, 120.2, 119.8, 119.0, 115.5, 33.5, 23.5. HRMS $C_{24}H_{22}N_3O_2$ $[M+H]^+$ *Calc*. 384.1712, Found *m/z* 384.1697.

*1-Phenyl-3-(pyridin-3-yl)-1H-pyrazole-4-carboxylic acid 4-tert-butyl-phenyl ester (**4j**)*

Yield 66 %, mp 138–141 °C; IR (FTIR/FTNIR-ATR): 1744 cm^{-1} (C=O). ^{1}H-NMR ($CDCl_3$) δ: 9.13 (1H, d, $J = 1.6$ Hz), 8.71 (1H, s), 8.62 (1H, dd, $J = 1.6$ Hz, $J = 5.2$ Hz), 8.29 (1H, td, $J = 2$ Hz, $J = 8$ Hz), 7.85–7.81 (2H, m), 7.55–7.48 (2H, m), 7.42–7.31 (4H, m), 7.09–7.06 (2H, m), 1.31 (9H, s). ^{13}C-NMR ($CDCl_3$) δ: 165.5, 148.3, 148.0, 147.5, 146.5, 139.9, 133.5, 133.3, 134.2, 129.5, 129.3, 126.3, 125.6, 124.4, 121.5, 120.2, 115.5, 34.5, 31.5. HRMS $C_{25}H_{24}N_3O_2$ $[M+H]^+$ *Calc*. 398.1869, Found *m/z* 398.1854.

*1-Phenyl-3-(pyridin-3-yl)-1H-pyrazole-4-carboxylic acid 2-isopropyl-5-methyl-phenyl ester (**4k**)*

Colorless oil. Yield 51 %; IR (FTIR/FTNIR-ATR): 1736 cm^{-1} (C=O). ^{1}H-NMR (DMSO-d_6) δ: 9.49 (1H, s), 9.00–8.97 (1H, m), 8.63 (1H, dd, $J = 1.6$ Hz, $J = 4.8$ Hz), 8.22 (1H, td, $J = 1.6$ Hz, $J = 8.4$ Hz), 8.06 (2H, d, $J = 7.6$ Hz), 7.62–7.57 (2H, m), 7.51–7.45 (2H, m), 7.26 (1H, d, $J = 8$ Hz), 7.08 (1H, d, $J = 7.6$ Hz), 7.03–7.00 (1H, m), 2.99–2.96 (1H, m), 2.29 (3H, s), 1.09 (6H, s). ^{13}C-NMR (DMSO-d_6) δ: 165.5, 148.0, 147.5, 147.5, 139.9, 137.5, 136.3, 133.5, 133.3, 134.2, 129.5, 129.3, 126.3, 125.7, 125.3, 124.4, 122.6, 120.2, 115.5, 27.5, 23.5, 21.4. HRMS $C_{25}H_{24}N_3O_2$ $[M+H]^+$ *Calc*. 398.1869, Found *m/z* 398.1872.

*1-Phenyl-3-(pyridin-3-yl)-1H-pyrazole-4-carboxylic acid biphenyl-4-yl ester (**4l**)*

White solid. Yield 78 %, mp 169–172 °C; IR (FTIR/FTNIR-ATR): 1718 cm^{-1} (C=O). ^{1}H-NMR ($CDCl_3$) δ: 9.19–9.16 (1H, m), 8.76 (1H, s), 8.67–8.63 (1H, m), 8.35–8.30 (1H, m), 7.87–7.83 (2H, m), 7.64–7.60 (2H, m), 7.58–7.35 (9H, m), 7.27–7.23 (2H, m). ^{13}C-NMR ($CDCl_3$) δ: 165.5, 148.5, 148.0, 147.5, 140.8, 139.9, 137.6, 133.5, 133.3, 134.2, 129.7, 129.5, 129.3, 128.1, 127.9, 126.3, 124.4, 122.3, 120.2, 115.5, HRMS $C_{27}H_{20}N_3O_2$ $[M+H]^+$ *Calc*. 418.1556, Found *m/z* 418.1556.

*1-Phenyl-3-(pyridin-3-yl)-1H-pyrazole-4-carboxylic acid naphthalen-2-yl ester (**4m**)*

White solid. Yield 44 %, mp 184–186 °C; IR (FTIR/FTNIR-ATR): 1727 cm^{-1} (C=O). ^{1}H-NMR ($CDCl_3$) δ: 9.19–9.16 (1H, m), 8.77 (1H, s), 8.65–8.61 (1H, m), 8.29 (1H, td, $J = 2$ Hz, $J = 8$ Hz), 7.88–7.79 (6H, m), 7.64 (1H, d, J = 2.4 Hz), 7.55–7.28 (6H, m). ^{13}C-NMR ($CDCl_3$) δ: 165.5, 153.5, 148.0, 147.5, 139.9, 134.7, 134.2, 133.5, 133.3, 130.2, 129.5, 129.3, 129.1, 128.1, 126.9, 126.7, 126.3, 124.4, 124.0, 120.2, 117.8, 115.5, 109.7. HRMS $C_{25}H_{18}N_3O_2$ $[M+H]^+$ *Calc*. 392.1399, Found *m/z* 392.1391.

*1-Phenyl-3-(pyridin-4-yl)-1H-pyrazole-4-carboxylic acid benzylamide (**5a**)*

White solid. Yield 50 %, mp 184–187 °C; IR (FTIR/FTNIR-ATR): 1639 cm^{-1} (C=O) ^{1}H-NMR ($CDCl_3$) δ: 8.60 (2H, d, $J = 6$ Hz), 8.39 (1H, s), 7.72 (2H, d, $J = 7.6$ Hz), 7.67 (2H, d, $J = 6.4$ Hz), 7.48 (2H, t, $J = 8$ Hz), 7.38–7.25 (6H, m), 5.99 (1H, s, NH), 4.55 (2H, d, $J = 6$ Hz). ^{13}C-NMR ($CDCl_3$) δ: 168.1, 150.2, 140.5, 139.9, 138.2, 133.5, 129.5, 129.3, 128.9, 127.2, 126.9, 126.3, 121.5, 120.2, 114.0, 43.5. HRMS $C_{22}H_{19}N_4O$ $[M+H]^+$ *Calc*. 355.1559, Found *m/z* 355.1559.

*Morpholin-4-yl-[1-phenyl-3-(pyridin-4-yl)-1H-pyrazol-4-yl]methanone (**5b**)*

White solid. Yield 50 %, mp 158–161 °C; IR (FTIR/FTNIR-ATR): 1627 cm^{-1} (C=O) ^{1}H-NMR ($CDCl_3$) δ: 8.67 (2H, d, $J = 6$ Hz), 8.12 (1H, s), 7.73 (2H, d, $J = 7.2$ Hz), 7.68 (2H, d, $J = 6$ Hz), 7.53–7.49 (2H, m), 7.37–7.33 (1H, m), 3.78–3.70 (4H, m), 3.31–3.24 (4H, m). ^{13}C-NMR ($CDCl_3$) δ: 169.3, 150.2, 140.5, 139.9, 133.5, 129.5, 129.3, 126.3, 121.5, 120.2, 114.0, 67.3, 46.7. HRMS $C_{19}H_{19}N_4O_2$ $[M+H]^+$ *Calc*. 335.1508, Found *m/z* 335.1519.

*(4-Benzyl-piperidin-1-yl)-[1-phenyl-3-(pyridin-4-yl)-1H-pyrazol-4-yl]methanone (**5c**)*

White solid. Yield 72 %, mp 91–93 °C; IR (FTIR/FTNIR-ATR): 1621 cm^{-1} (C=O) ^{1}H-NMR ($CDCl_3$) δ: 8.65 (2H, d, $J = 6.4$ Hz), 8.08 (1H, s), 7.73 (2H, d, $J = 7.6$ Hz), 7.68 (2H, d, $J = 6$ Hz), 7.53–7.48 (2H, m), 7.37–7.33 (1H, m), 7.29–7.25 (2H, m), 7.19–7.16 (1H, m), 7.06 (2H, d, $J = 8$ Hz), 4.78–3.59 (2H, m), 2.73–2.43 (4H, m), 1.75–1.66 (3H, m), 1.41–0.71 (2H, m). ^{13}C-NMR ($CDCl_3$) δ: 173.4, 150.2, 140.5, 139.9, 133.5, 129.5, 129.3, 128.9, 128.4, 126.3, 121.5, 120.2, 114.0, 45.3, 41.8, 37.4, 29.5.

HRMS $C_{27}H_{27}N_4O$ $[M+H]^+$ *Calc*. 423.2185, Found *m/z* 423.2175.

*(4-Methyl-piperazin-1-yl)-[1-phenyl-3-(pyridin-4-yl)-1H-pyrazol-4-yl]methanone (**5d**)*

White solid. Yield 66 %, mp 136–138 °C; IR (FTIR/FTNIR-ATR): 1616 cm^{-1} (C=O) ^{1}H-NMR ($CDCl_3$) δ: 8.66 (2H, d, $J = 6$ Hz), 8.10 (1H, s), 7.74 (2H, d, $J = 8.4$ Hz), 7.68 (2H, d, $J = 6$ Hz), 7.52–7.47 (2H, m), 7.39–7.34 (1H, m), 3.83–3.80 (2H, m), 3.29–3.25 (2H, m), 2.45–2.41 (2H, m), 2.22 (3H, s), 2.06–2.02 (2H, m). ^{13}C-NMR ($CDCl_3$) δ: 169.3, 150.2, 140.5, 139.9, 133.5, 129.5, 129.3, 126.3, 121.5, 120.2, 114.0, 51.7, 49.5, 46.8. HRMS $C_{20}H_{22}N_5O$ $[M+H]^+$ *Calc*. 348.1824, Found *m/z* 348.1817.

*(4-Phenyl-piperazin-1-yl)-[1-phenyl-3-(pyridin-4-yl)-1H-pyrazol-4-yl]methanone (**5e**)*

White solid. Yield 54 %, mp 185–187 °C; ^{O}C IR (FTIR/FTNIR-ATR): 1636 cm^{-1} (C=O) ^{1}H-NMR ($CDCl_3$) δ: 8.66 (2H, d, $J = 6$ Hz), 8.15 (1H, s), 7.76 (2H, d, $J = 7.2$ Hz), 7.71 (2H, d, $J = 6$ Hz), 7.53–7.49 (2H, m), 7.39–7.35 (1H, m), 7.27–7.23 (2H, m), 6.91–6.83 (3H, m), 3.99–3.94 (2H, m), 3.43–3.38 (2H, m), 3.25–3.21 (2H, m), 2.87–2.82 (2H, m). ^{13}C-NMR ($CDCl_3$) δ: 169.3, 150.2, 149.9, 140.5, 139.9, 133.5, 129.9, 129.5, 129.3, 126.3, 122.3, 121.5, 120.2, 114.6, 114.0, 53.6, 49.5. HRMS $C_{25}H_{24}N_5O$ $[M+H]^+$ *Calc*. 410.1981, Found *m/z* 410.1986.

*[1-Phenyl-3-(pyridin-4-yl)-1H-pyrazol-4-yl]-[4-(4-trifluoromethyl-phenyl)-piperazin-1-yl]methanone (**5f**)*

White solid. Yield 56 %, mp 172–174 °C; IR (FTIR/FTNIR-ATR): 1615 cm^{-1} (C=O) ^{1}H-NMR ($CDCl_3$) δ: 8.67 (2H, d, $J = 6$ Hz), 8.16 (1H, s), 7.76 (2H, d, $J = 7.6$ Hz), 7.71 (2H, d, $J = 6$ Hz), 7.53–7.49 (2H, m), 7.48 (2H, d, $J = 8.8$ Hz), 7.41–7.38 (1H, m), 6.85 (2H, d, $J = 8.4$ Hz), 3.97–3.93 (2H, m), 3.41–3.31 (4H, m), 2.93–2.91 (2H, m). ^{13}C-NMR ($CDCl_3$) δ: 169.3, 153.2, 150.2, 140.5, 139.9, 133.5, 129.5, 129.3, 126.3, 124.7, 121.5, 120.2, 114.0, 113.6, 53.6, 49.5. HRMS $C_{26}H_{23}N_5OF_3$ $[M+H]^+$ *Calc*. 478.1855, Found *m/z* 478.1855.

*[1-Phenyl-3-(pyridin-4-yl)-1H-pyrazol-4-yl]-(4-pyridin-4-yl-piperazin-1-yl)methanone (**5g**)*

White solid. Yield 56 %, mp 207–209 °C; IR (FTIR/FTNIR-ATR): 1632 cm^{-1} (C=O) ^{1}H-NMR ($CDCl_3$) δ: 8.66 (2H, d, $J = 6.4$ Hz), 8.27 (2H, d, $J = 6.8$ Hz), 8.16 (1H, s), 7.73 (2H, d, $J = 8$ Hz), 7.69 (2H, d, $J = 6$ Hz), 7.52–7.48 (2H, m), 7.40–7.37 (1H, m), 6.58 (2H, d, $J = 6.8$ Hz), 3.93–3.87 (2H, m), 3.41–3.37 (4H, m), 3.03–2.99 (2H, m). ^{13}C-NMR ($CDCl_3$) δ: 169.3, 152.4, 150.6, 150.2, 140.5, 139.9, 133.5, 129.5, 129.3, 126.3, 121.5, 120.2, 114.0, 107.2, 53.6, 49.5. HRMS $C_{24}H_{23}N_6O$ $[M+H]^+$ *Calc*. 411.1933, Found *m/z* 411.1915.

*1-Phenyl-3-(pyridin-4-yl)-1H-pyrazole-4-carboxylic acid 4-methoxy-phenyl ester (**5h**)*

White solid. Yield 67 %, mp 160–162 °C; IR (FTIR/FTNIR-ATR): 1728 cm^{-1} (C=O) ^{1}H-NMR ($CDCl_3$) δ: 8.71 (1H, s), 8.67 (2H, d, $J = 5.6$ Hz), 7.92 (2H, d, $J = 6$ Hz), 7.82 (2H, d, $J = 7.2$ Hz), 7.55–7.51 (2H, m), 7.43–7.37 (1H, m), 7.09 (2H, d, $J = 7.2$ Hz), 6.92 (2H, d, $J = 7.2$ Hz), 3.80 (3H, s). ^{13}C-NMR ($CDCl_3$) δ: 165.5, 157.7, 150.2, 142.0, 140.5, 139.9, 133.5, 129.5, 129.3, 126.3, 122.9, 121.5, 120.2, 114.9, 115.5, 114.9, 55.9. HRMS $C_{22}H_{18}N_3O_3$ $[M+H]^+$ *Calc*. 372.1348, Found *m/z* 372.1330.

*1-Phenyl-3-(pyridin-4-yl)-1H-pyrazole-4-carboxylic acid 4-ethyl-phenyl ester (**5i**)*

White solid. Yield 60 %, mp 101–103 °C; IR (FTIR/FTNIR-ATR): 1728 cm^{-1} (C=O) ^{1}H-NMR ($CDCl_3$) δ: 8.74 (1H, s), 8.67 (2H, d, $J = 6$ Hz), 7.98 (2H, d, $J = 5.6$ Hz), 7.84 (2H, d, $J = 7.6$ Hz), 7.56–7.41 (3H, m), 7.25 (2H, d, $J = 8.8$ Hz), 7.09 (2H, d, $J = 8.8$ Hz), 2.68 (2H, q, $J = 7.6$ Hz), 1.24 (3H, t, $J = 7.6$ Hz). ^{13}C-NMR ($CDCl_3$) δ: 165.5, 150.2, 146.9, 141.4, 140.5, 139.9, 133.5, 129.5, 129.3, 126.3, 121.8, 121.5, 120.2, 115.5, 29.3, 14.8. HRMS $C_{23}H_{20}N_3O_2$ $[M+H]^+$ *Calc*. 370.1556, Found *m/z* 370.1543.

*1-Phenyl-3-(pyridin-4-yl)-1H-pyrazole-4-carboxylic acid 3-isopropyl-phenyl ester (**5j**)*

White solid. Yield 48 %, mp 128–130 °C; IR (FTIR/FTNIR-ATR): 1725 cm^{-1} (C=O) ^{1}H-NMR ($CDCl_3$) δ: 9.03 (1H, s), 8.99 (2H, d, $J = 6.4$ Hz), 8.24 (2H, d, $J = 6.4$ Hz), 8.13 (2H, d, $J = 7.2$ Hz), 7.87–7.83 (2H, m), 7.75–7.71 (1H, m), 7.63 (1H, t, $J = 7.6$ Hz), 7.43 (1H, d, $J = 8$ Hz), 7.34–7.28 (2H, m), 3.25–3.21 (1H, m), 1.57 (6H, s). ^{13}C-NMR ($CDCl_3$) δ: 165.5, 153.5, 150.7, 150.2, 140.5, 139.9, 133.5, 129.5, 129.3, 129.1, 126.3, 123.2, 121.5, 120.2, 119.9, 119.0, 115.5, 33.5, 23.5. HRMS $C_{24}H_{22}N_3O_2$ $[M+H]^+$ *Calc*. 384.1712, Found *m/z* 384.1703.

*1-Phenyl-3-(pyridin-4-yl)-1H-pyrazole-4-carboxylic acid 4-tert-butyl-phenyl ester (**5k**)*

White solid. Yield 62 %, mp 160–162 °C; IR (FTIR/FTNIR-ATR): 1746 cm^{-1} (C=O) ^{1}H-NMR ($CDCl_3$) δ: 8.71 (1H, s), 8.67 (2H, d, $J = 6.4$ Hz), 7.92 (2H, d, $J = 6$ Hz), 7.80 (2H,

d, $J = 7.6$ Hz), 7.55–7.50 (2H, m), 7.43–7.37 (3H, m), 7.09 (2H, d, $J = 8.8$ Hz), 1.31 (9H, s). ^{13}C-NMR ($CDCl_3$) δ: 165.5, 150.2, 148.5, 146.7, 140.5, 139.9, 133.5, 129.5, 129.3, 126.3, 125.9, 121.5, 120.2, 115.5, 34.5, 31.5. HRMS $C_{25}H_{24}N_3O_2$ $[M+H]^+$ *Calc*. 398.1869, Found *m/z* 398.1861.

*1-Phenyl-3-(pyridin-4-yl)-1H-pyrazole-4-carboxylic acid 2-isopropyl-5-methyl-phenyl ester (**5l**)*

White solid. Yield 43 %, mp 142–144 °C; IR (FTIR/FTNIR-ATR): 1729 cm^{-1} (C=O) ^{1}H-NMR ($CDCl_3$) δ: 8.74 (1H, s), 8.68 (2H, d, $J = 6$ Hz), 7.97 (2H, d, $J = 6$ Hz), 7.84 (2H, d, $J = 7.6$ Hz), 7.57–7.42 (3H, m), 7.25 (H, d, $J = 8$ Hz), 7.06 (1H, d, $J = 7.6$ Hz), 6.92 (1H, s), 3.05–3.01 (1H, m), 2.34 (3H, s), 1.20 (6H, s). ^{13}C-NMR ($CDCl_3$) δ: 165.5, 150.2, 147.7, 140.5, 139.9, 137.6, 136.5, 133.5, 129.5, 129.3, 126.3, 126.0, 125.6, 121.5, 120.2, 115.5, 27.5, 23.5, 21.4. HRMS $C_{25}H_{24}N_3O_2$ $[M+H]^+$ *Calc*. 398.1869, Found *m/z* 398.1869.

*1-Phenyl-3-(pyridin-4-yl)-1H-pyrazole-4-carboxylic acid biphenyl-4-yl ester (**5m**)*

White solid. Yield 50 %, mp 195–197 °C; IR (FTIR/FTNIR-ATR): 1733 cm^{-1} (C=O) ^{1}H-NMR ($CDCl_3$) δ: 8.77 (1H, s), 8.71 (2H, d, $J = 6.4$ Hz), 7.98 (2H, d, $J = 6$ Hz), 7.85 (2H, d, $J = 8.8$ Hz), 7.65–7.62 (2H, d, $J = 8.8$ Hz), 7.59–7.53 (4H, m), 7.47–7.42 (3H, m), 7.39–7.35 (1H, m), 7.26 (2H, d, $J = 8.8$ Hz). ^{13}C-NMR ($CDCl_3$) δ: 165.5, 150.2, 148.5, 141.3, 140.5, 139.9, 137.9, 133.5, 129.8, 129.5, 129.3, 128.3, 127.9, 126.3, 122.5, 121.5, 120.2, 115.5. HRMS $C_{27}H_{20}N_3O_2$ $[M+H]^+$ *Calc*. 418.1556, Found *m/z* 418.1551.

*1-Phenyl-3-(pyridin-4-yl)-1H-pyrazole-4-carboxylic acid naphthalen-2-yl ester (**5n**)*

White solid. Yield 53 %, mp 182–184 °C; IR (FTIR/FTNIR-ATR): 1734 cm^{-1} (C=O) ^{1}H-NMR ($CDCl_3$) δ: 8.79 (1H, s), 8.68 (2H, d, $J = 6.4$ Hz), 7.95 (2H, d, $J = 6.4$ Hz), 7.90–7.81 (5H, m), 7.66 (1H, d, $J = 2.4$ Hz), 7.57–7.41 (5H, m), 7.35–7.30 (1H, m). ^{13}C-NMR ($CDCl_3$) δ: 165.5, 153.4, 150.2, 148.5, 141.3, 140.5, 139.9, 137.9, 134.9, 133.5, 130.3, 129.8, 129.5, 129.3, 128.3, 128.0, 127.9, 126.9, 126.7, 126.3, 124.3, 122.5, 121.5, 120.2, 117.9, 115.5, 109.7. HRMS $C_{25}H_{18}N_3O_2$ $[M+H]^+$ *Calc*. 392.1399, Found *m/z* 392.1384.

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-2*H*-tetrazolium bromide) assay (Cell Proliferation Kit I, Roche, Germany). Briefly, cells were seeded in a 96-well plate at 10,000 cells per well, cultured overnight in growth medium containing 1 % FBS. Then the cells were treated with 50 μM of test compounds for 48 h. As for 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 was 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 the crystals. The absorbance values of samples were 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 test compounds to the cell viability were calculated by comparing mean values obtained from compound treated culture wells with those of the solvent controls. This experiment was performed in quadruplicate. The IC_{50} values were calculated from the concentration–response curves by means of the PRISM 5, GraphPad Software (GraphPad Prism Version 5.04 for Windows 2010).

Statistical significances of the effects of compounds to the cell viabilities were analyzed by one-way ANOVA andTukey's post hoc test using SigmaStat v3.5 software. $p < 0.05$ were considered statistically significant (* $p \leq 0.05$, ** $p \leq 0.01$, *** $p \leq 0.001$ compared with control).

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 synthesized compounds. The bioavailabilities of the compounds were 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). Poor absorption and permeation are more likely to occur when there are more than 5 hydrogen-bond donors (HBD), more than 10 hydrogen-bond acceptors (HDA), when the molecular mass (MM) is greater than 500, or the log *P* value (*c*log *P*) is greater than 5. The TPSA should be smaller than 90 Å (Brueggemeier *et al.*, 2005). The method for calculation of molecule volume developed by Molinspiration is based on group contributions. These have been obtained by fitting sum of fragment contributions to "real" 3D volume for a training set of about twelve thousand, mostly drug-like molecules. Molecules violating more than one of these rules may have problems in terms of bioavailability.

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