Design, Synthesis, and Evaluation of Some Novel Heterocycles Bearing Pyrazole Moiety as Potential Anticancer Agents

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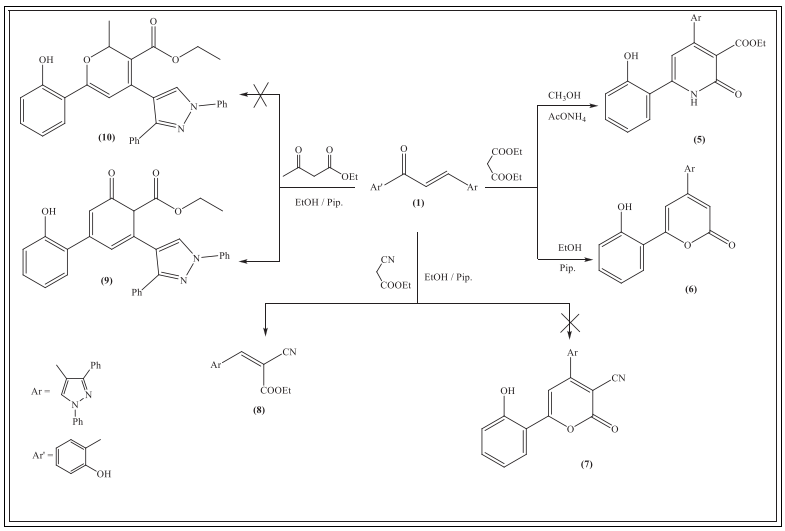

1,3-Diphenylpyrazole-4-carboxylaldehyde and *o*-hydroxyacetophenone were exploited as starting materials for the synthesis of novel substituted chalconated pyrazole derivative. The proclivity of this compound towards carbon and nitrogen nucleophiles such as malononitrile, diethyl malonate, ethyl cyanoacetate, ethyl acetoacetate, semicarbazide, thiosemicarbazide, and hydroxylamine has been investigated. The structures of all synthesized compounds were ascertained by analytical and spectral data. The antitumor activity of the target synthesized compounds was tested against a panel of two human tumor cell lines, namely, hepatocellular carcinoma (liver) HepG2 and mammary gland breast MCF-7.

INTRODUCTION

In the last few decades, anticancer drugs have been sophisticated from chemically synthesized compounds. Recently, a large number of interesting pyrazole derivatives have been synthesized [1]. The pyrazole-tethered heterocyclic compounds have received senior attention owing to their diverse chemotherapeutic potential [2–4]. The important pyrazole-based drugs available in the market are apixaban, celecoxib, deracoxib, tolpiprazole, fipronil, lonazolac, remogliflozin etabonate, and many more. Ruxolitinib and crizotinib are important pyrazole-tethered anticancer drugs [5]. Some pyrazole-based drugs are depicted in Figure 1. Pyrazole derivatives show anticancer activity due to their inhibition of various targets such as EGFR [6], IGF-1R [7], tubulin [8], B-raf [9,10], mTOR [11], HDAC [12,13], ALK [14], topoisomerase II [15], JAK2 [16], ROS 1 [17], among others. Hence, incorporation of the pyrazole moiety is an important synthetic strategy in rational drug development process. The chalcones are α,β-unsaturated ketones that acts as a central core for a variety of pharmacologically important compounds. Their true importance may be explained in two branches: the biological activity associated with them and their synthetic utility for the preparation of pharmacologically important heterocyclic derivatives. Chalcones of various classes have been extensively studied owing to their wide range of pharmacological activities such as antileishmanial [18], antimalarial [19,20], antifungal [21], invasive [22], antitrypanosomal [23], antibacterial [24], anti-inflammatory [25], and anticancer activity [26,27]. Chalcones are widely spread in nature as in fruits, species, soy-based food stuff, vegetable, and tea. Their 2′-hydroxy derivatives play an important role in the flavonoid synthesis and biosynthesis as both precursors and products [28].

RESULTS AND DISCUSSION

As a continuation of our efforts in respect of synthesizing biologically active heterocyclic compounds

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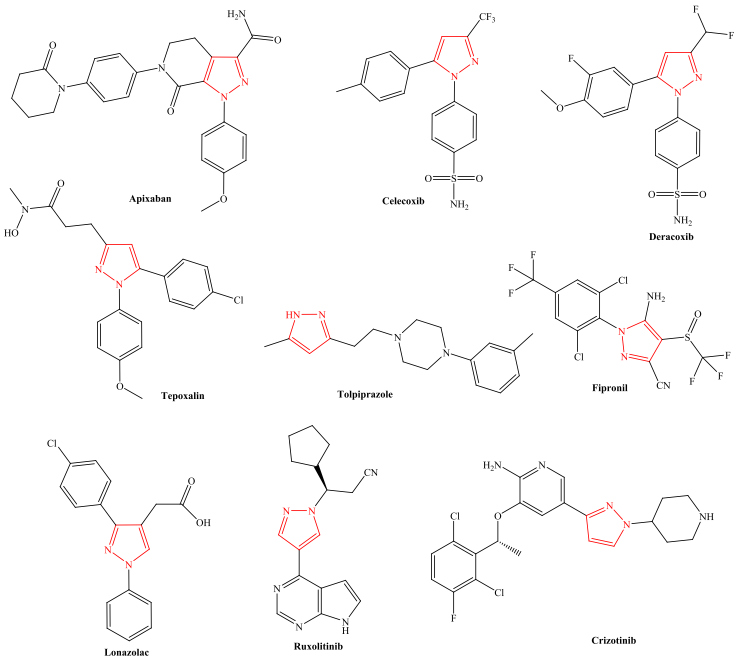
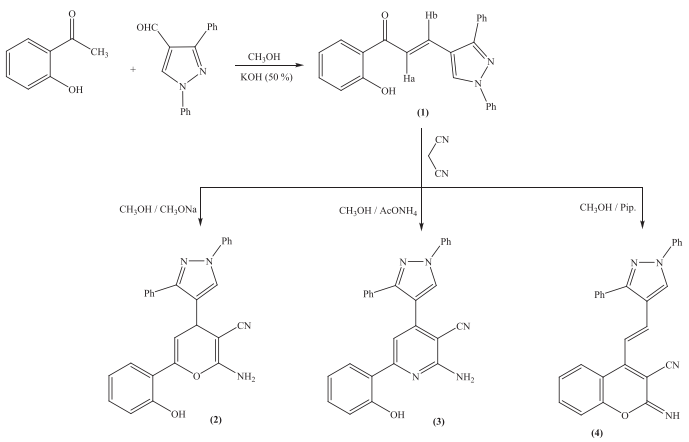


Figure 1. Pyrazole-based drugs. [Color figure can be viewed at wileyonlinelibrary.com]

especially those with anticipated cytotoxic activity [29,30], we aimed to synthesize new series of pyran, chromone, and pyridine derivatives bearing pyrazole moiety in the hope that new anticancer agents might be detected. The substituted chalconated pyrazole derivative **1** was synthesized by Claisen–Schmidt condensation reaction [31]. Thus, the reaction of *o*-hydroxyacetophenone with 1,3-diphenylpyrazole-4-carboxylaldehyde in methanolic potassium hydroxide at room temperature for 12 h furnished (*E*)-3-(1,3-diphenyl-1*H*-pyrazol-4-yl)-1-(2-hydroxyphenyl)prop-2-en-1-one **1** (Scheme 1). The structure of compound **1** was substantiated from the complete analysis of IR and mass spectra beside the correct analytical data. Further support for the suggested structure of compound **1** was gained from its ^{1}H NMR spectrum that showed signals for OH, olefinic, and aromatic protons. The higher coupling constant (*J*) for the olefinic protons H_a and H_b is in a good agreement with its existence as the *E*-configurated isomer (cf. experimental section). Because chalcones are versatile synthon for the preparation of five-membered and six-membered ring systems [32], the proclivity of compound **1** with different carbon nucleophiles such as malononitrile, diethyl malonate, ethyl cyanoacetate, and ethyl acetoacetate has been investigated. Therefore, cyclic condensation of **1** with malononitrile under different reaction conditions afforded pyran and pyridine derivatives **2**–**4** (Scheme 1).

Reaction of pyrazole derivative **1** with malononitrile in refluxing methanol in the presence of sodium methoxide

Scheme 1



yielded pyran derivative **2**. The structure of compound **2** was confirmed by complete analysis of IR, ^{1}H NMR, and mass spectra beside the correct elemental analysis. Thus, the IR spectrum showed absorption bands for NH_2 at 3347, 3224 cm^{-1} and C≡N at 2214 cm^{-1}. Moreover, ^{1}H NMR spectrum of **2** (DMSO-d_6) showed signals that is consistent with the proposed structure at δ (ppm): 11.20 (br s, 1H, OH, exchangeable with D_2O), 8.97 (s, C_5H pyrazole), 7.97–7.15 (m, $14H_{arom.}$), 6.32 (s, 2H, NH_2, exchangeable with D_2O), 5.31 (d, 1H, C_5H pyran), 3.91 (d, 1H, C_4H pyran).

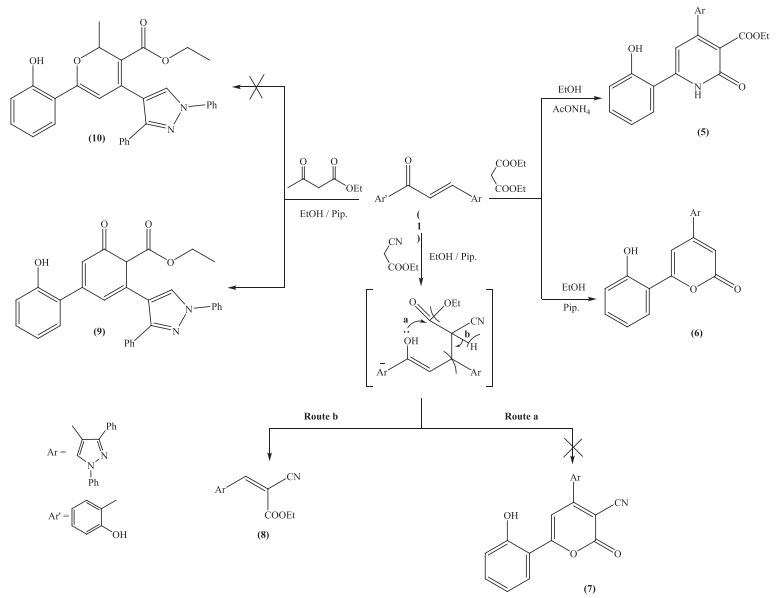
While refluxing the reaction mixture of **1** and malononitrile in methanol in the presence of ammonium acetate afforded the corresponding pyridine derivative **3**. Studying the spectroscopic data of compound **3** confirmed its proposed structure. Thus, the IR spectrum displayed broad ν_{OH} at 3404 cm^{-1} with absorption band for NH_2 at 3340, 3236 cm^{-1}, $\upsilon_{C\equiv N}$ at 2214 cm^{-1} and $\upsilon_{C=N}$ at 1649 cm^{-1}. ^{1}H NMR spectrum of compound **3** (DMSO-d_6) displayed singlet at δ (ppm) 13.35 integrated for one proton of OH that disappeared with D_2O, singlet for one proton at δ 8.95 corresponding to C_5-H of the pyrazole moiety, the deshielded aromatic protons (15H) was shown downfield at δ 7.96–6.90 ppm as well as a broad singlet at δ 7.15 ppm integrated for two protons of NH_2 that is exchangeable with D_2O. On the other hand, cyclic condensation of α-enone **1** with malononitrile in refluxing methanol in the presence of catalytic amount of piperidine produced 2-imino-2*H*-chromene derivative **4** (Scheme 1). Structure of the synthesized compound **4** was ascertained from its analytical and spectral data.

In this study, reaction of chalconated pyrazole derivative **1** with diethyl malonate under two different reaction conditions was also investigated as shown in Scheme 2. Therefore, reaction of **1** with diethyl malonate in boiling ethanol in the presence of ammonium acetate afforded the corresponding pyridinone derivative **5**. Screening of ^{1}H NMR spectrum (DMSO-d_6) of compound **5** revealed the presence of signals downfield for (NH) proton as a singlet at 12.62 ppm, (OH) proton as a singlet at 9.31 ppm, C_5-H of the pyrazole moiety as a singlet at 8.50 ppm, aromatic protons (15H) as a multiplet in the region of δ 8.01–7.31 ppm besides the existence of quartet signal at 4.32 ppm and triplet signal at 1.21 ppm characteristic for ethyl protons of ester group that is consistent with the structure **5**.

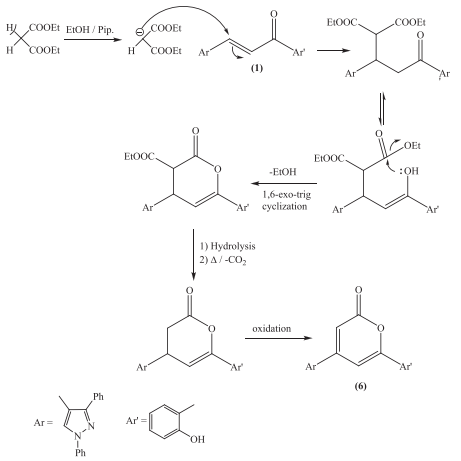
In contrast, 4-(1,3-diphenyl-1*H*-pyrazol-4-yl)-6-(2-hydroxyphenyl)-2*H*-pyran-2-one **6** was obtained as a sole product in a fairly good yield upon treatment of compound **1** with diethyl malonate in boiling ethanol in the presence of catalytic amount of piperidine. The formation of **6** could be postulated as follows (Scheme 3).

Refluxing of compound **1** with ethyl cyanoacetate in boiling ethanol in the presence of a catalytic amount of piperidine afforded on a hot crystalline product with the molecular formula $C_{21}H_{17}N_3O_2$ and not the pyrone derivative. The carbanion underwent nucleophilic addition reaction to the double bond of compound **1** *via* Michael type addition reaction to give a substance where structure should be either compound **7** (route a) or compound **8** (route b). The actual structure of the product was assigned as compound **8** based on the spectroscopic data (Scheme 2). The ^{1}H NMR spectrum of this product revealed the absence of phenolic OH proton and the presence of a triplet signal at 1.28 ppm and a quartet signal at 4.30 ppm characteristic for ethyl protons of ester

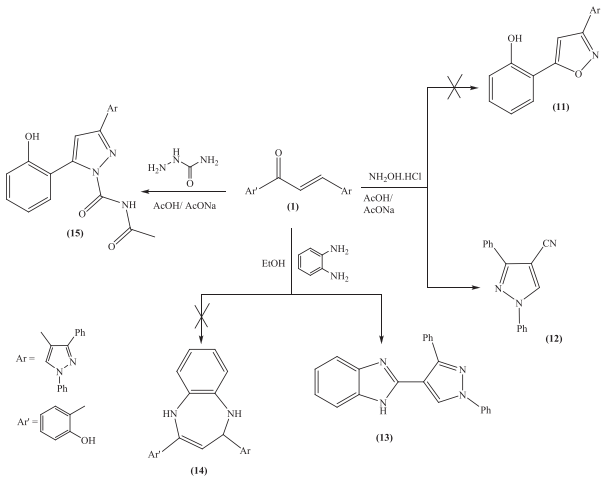
Scheme 2



Scheme 3



Scheme 4



group that ruled out the expected structure **7** and confirm the structure **8**. Moreover, the IR spectrum of this product displayed the absorption bands for $\upsilon_{C\equiv N}$ (sharp band) at 2220 cm^{-1} and $\upsilon_{C=O}$ (ester) at 1723 cm^{-1}. Also, the highest recorded peak in the mass spectrum at m/z = 343 attributable for the correct molecular ion peak for molecular formula $C_{21}H_{17}N_3O_2$. Furthermore, the strong evidence for the structure **8** is forthcoming from authentic sample prepared from the reaction of 1,3-diphenylpyrazole-4-carboxylaldehyde with ethyl cyanoacetate in boiling ethanol in the presence of a catalytic amount of piperidine (TLC, mp, and IR comparison).

Treatment of **1** with ethyl acetoacetate in boiling ethanol with few drops of piperidine afforded an unexpected product with molecular formula $C_{32}H_{28}N_2O_4$ (M_r = 476) that was identified as ethyl 5-(1,3-diphenyl-1*H*-pyrazol-4-yl)-2′-hydroxy-3-oxo-3,4-dihydro-[1,1′-biphenyl]-4-carboxylate **9**. Furthermore, the strong clue for this structure is forthcoming from ^{1}H NMR spectrum that completely accord with the assigned structure **9** and ruled out **10** as shown in Figure 2.

As a precursor to construct more heterocyclic compounds incorporating pyrazole moiety, compound **1** was allowed to react with different nitrogen nucleophiles, namely, hydroxylamine, *o*-phenylenediamine, and semicarbazide hydrochloride as shown in Scheme 4.

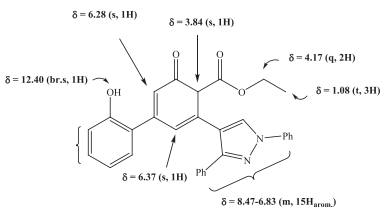
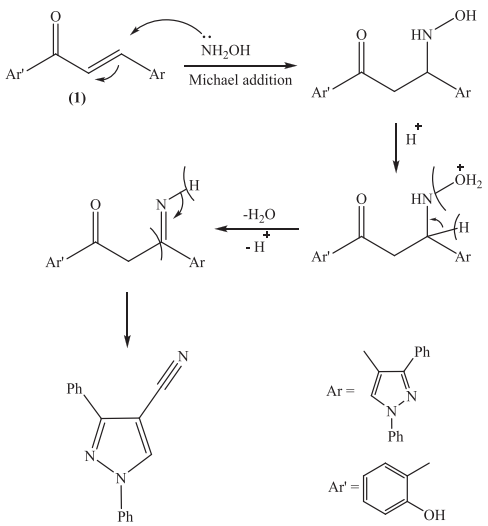


Figure 2. ^{1}H NMR spectrum of compound **9**.

It was reported that the reaction of chalcone derivatives with hydroxylamine, *o*-phenylenediamine, and/or thiosemicarbazide yielded the corresponding oxazole, pyrazole, and benzodiazepine derivatives, respectively. Thus, when compound **1** was subjected to react with hydroxylamine in boiling acetic acid in the presence of fused sodium acetate afforded the unexpected compound that identified as 1,3-diphenyl-1*H*-pyrazole-4-carbonitrile **12** [33] rather than **11**. The spectroscopic data are in good agreement with the proposed structure. Thus, the appearances of a sharp absorption band at 2224 cm^{-1} for CN in the IR spectrum confirms the unexpected structure of compound **12**. The conversion of compounds **1** to **12** could be explained according the following mechanism (Scheme 5).

The reaction of α-enone **1** with *o*-phenylenediamine in boiling ethanol furnished the unexpected product 2-(1,3-diphenyl-1*H*-pyrazol-4-yl)-1*H*-benzo[*d*]imidazole **13**

Scheme 5



instead of benzodiazepine derivative **14**. The spectroscopic data are in accordance with the assigned structure (cf. experimental section). On the other hand, treatment of **1** with semicarbazide hydrochloride in the presence of fused sodium acetate in refluxing acetic acid gave pyrazole derivative **15** (Scheme 4). Formation of **15** can be explained *via* Michael addition reaction of α,β-unsaturated carbonyl, cyclization followed by acylation [34]. The IR spectrum of **15** exhibited ν_{NH} at 3177 cm^{-1} and ν_{CO} at 1676 cm^{-1}. Moreover, ^1H NMR and mass spectra were in consistent with the assigned structure.

Meanwhile, reaction of **1** with thiosemicarbazide in boiling dioxane also yielded the unexpected product with molecular formula $C_{32}H_{28}N_2O_4$ that was identified as 2-(1,3-diphenyl-1*H*-pyrazol-4-yl)chroman-4-one **16** (Scheme 6) that obtained *via* intramolecular addition of phenolic OH group on β-carbon of α,β-unsaturated carbonyl group. Furthermore, IR and ^1H NMR spectra are completely matched with the assigned structure **16** and lack of **17** (cf. experimental section).

Protection of phenolic OH group *via* alkylation of the chalconated pyrazole **1** with ethyl iodide in dimethylformamide in the presence of anhydrous potassium carbonate yielded the *O*-alkylated product **18** (Scheme 6). The structure of compound **18** was confirmed from the analytical and spectroscopic data. The ^1H NMR spectrum of **18** is in accordance with the suggested structure as it revealed the absence of phenolic proton and showed signals corresponding to ethyl, olefinic, and aromatic protons (cf. experimental section).

Another attempt to construct pyrazole derivative through reaction of compound **18** with thiosemicarbazide in boiling dioxane was not successful because it afforded Schiff-like base **19** rather than **20** as shown in Scheme 6. The ^1H NMR spectrum of compound **19** revealed the presence of signals downfield for (NH) proton as a singlet at 11.33 ppm, C_5-H of the pyrazole moiety as a singlet at 9.18 ppm, (CH=) proton as a singlet at 8.22 ppm, aromatic protons (10H) as a multiplet in the region of 7.91–7.36 ppm and (NH_2) protons as a broad singlet at 7.69 ppm that ruled out the expected structure **20** and confirm the structure **19**. Moreover, the IR spectrum of this product showed the absorption bands for NH_2 at 3330, 3257 cm^{-1}, NH at 3159 cm^{-1}, and C=N at 1618 cm^{-1}. Furthermore, the ample evidence for the structure **19** is forthcoming from authentic sample prepared from the reaction of 1,3-diphenylpyrazole-4-carboxylaldehyde with thiosemicarbazide in boiling dioxane (TLC, mp, and IR comparison).

Cytotoxicity and antitumor evaluation. *In vitro* cytotoxicity of all the synthesized compounds was determined by the MTT [(3-(4,5-dimethyl-2-thiazolyl) 2,5-diphenyl-2*H*-tetrazolium bromide)] assay against a panel of two different human cancer cell lines, namely, MCF-7 (human breast) and hepatocellular carcinoma (HePG-2). From the IC_{50} values that represent the compounds concentrations required to produce a 50% inhibition of cell growth after 72 h of incubation, compared with untreated controls (Table 1). All the tested compounds showed cytotoxic activity ranged from very strong to weak activity. As for the activity against hepatocellular carcinoma (HePG-2), the highest cytotoxic activity was displayed by compounds **3**, **9**, and **16** that showed IC_{50} 8.96 ± 0.7, 6.93 ± 0.5, and 10.27 ± 1.0 μM/mL, respectively. Remarkably strong inhibitory activity was also seen for compounds **2** and

Scheme 6

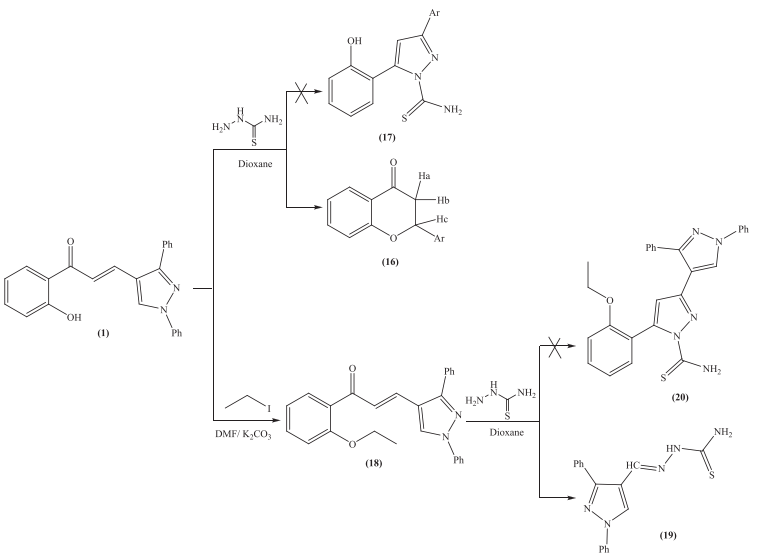


Table 1

Cytotoxicity (IC_{50}) of the tested compounds on different cell lines.

Compound	*In vitro* cytotoxicity IC_{50} (μM)•	
	HePG2	MCF-7
1	56.97 ± 3.2	61.91 ± 3.8
2	18.23 ± 1.6	5.87 ± 0.4
3	8.96 ± 0.7	11.82 ± 1.2
4	94.83 ± 5.2	79.62 ± 4.3
5	27.46 ± 2.1	19.28 ± 1.7
6	31.50 ± 2.4	22.62 ± 1.9
8	21.78 ± 1.8	29.95 ± 2.5
9	6.93 ± 0.5	7.38 ± 0.7
12	75.31 ± 4.1	68.34 ± 4.0
15	42.11 ± 2.9	34.06 ± 2.9
16	10.27 ± 1.0	15.17 ± 1.4
DOX	4.50 ± 0.3	4.17 ± 0.2

IC_{50} (μg/mL): 1–10 (very strong), 11–20 (strong), 21–50 (moderate), 51–100 (weak), above 100 (non-cytotoxic). DOX, doxorubicin

16. Also, compounds **5**, **6**, **8**, and **15** showed moderate activity, and compounds **1**, **4**, and **12** had weak activity.

As for the activity against mammary gland (breast) MCF-7 cell line, compound **2** showed very strong activity with IC_{50} 5.87 ± 0.4 μM/mL, while compounds **3**, **5**, and **16** showed strong activity towards with IC_{50} 11.82 ± 1.2, 19.28 ± 1.7, and 15.17 ± 1.4 μM/mL, respectively. On the other hand, compounds **6**, **8**, and **15** showed moderate activity, while compounds **1**, **4**, and **12** showed weak activity as shown in Figure 3.

Structure–activity relationship. By comparing the experimental cytotoxicity of the compounds reported in this study to their structures, the following structure–activity relationship was postulated.

- All the tested compounds showed cytotoxic activity ranged from very strong to weak activity; this is due to the presence of pyrazole ring.
- Compound **2** showed very strong activity against MCF-7 and strong activity against HePG-2; this is due to the presence of NH_2 group available to form hydrogen bond with either one of the nucleobases of the DNA and causes it damage.
- Compound **9** showed very strong activity against MCF-7 and HePG-2 due to the introduction of cyclohexa-2,4-dien-1-one ring and ester groups.
- Compound **3** showed very strong activity against HePG-2 and strong activity against MCF-7 due to the presence of CN, OH, and NH_2 groups.
- Compound **16** showed strong activity against both MCF-7 and HePG-2; this is due to the presence of chromanone and pyrazole rings.
- Compound **5** showed strong activity against MCF-7; this is due to the presence of OH and NH and also the formation of intramolecular hydrogen bond between the ester group and the NH group of the DNA that increases its lipophilicity.

EXPERIMENTAL

Chemistry. All melting points were taken on a Griffin and Georgy melting point apparatus and are uncorrected. IR spectra were recorded on Pye Unicam SP1200 spectrophotometer using the KBr wafer technique. ^1H NMR experiments were run at 300 on a Varian Mercury VX-300 NMR spectrometer using tetramethylsilane as internal standard in dimethyl sulfoxide (DMSO-d_6). Chemical shifts are quoted as δ. EI–MS were measured on a Schimadzu GC–MS operating at 70 eV. Elemental analyses were carried out at the Microanalytical Unit, Faculty of Science, Ain Shams University, using a Perkin-Elmer 2400 CHN elemental analyzer, and satisfactory analytical data (±0.4) were obtained for all

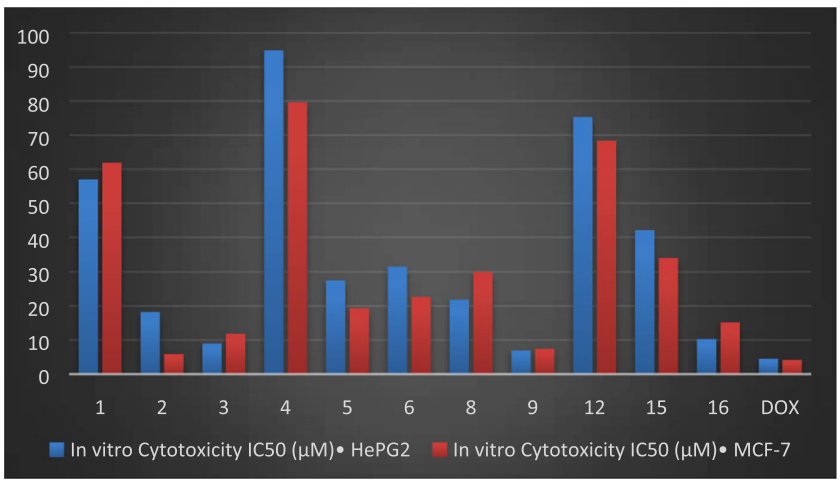


Figure 3. Cytotoxic activity of the tested compounds on different cell lines. [Color figure can be viewed at wileyonlinelibrary.com]

compounds. The homogeneity of the synthesized compounds was controlled by thin layer chromatography using aluminum sheet silica gel F_{254} (Merck). The pharmacological activity assays were carried out at Pharmacology Department, Faculty of Pharmacy, El-Mansoura University, El-Mansoura, Egypt.

(E)-3-(1,3-Diphenyl-1H-pyrazol-4-yl)-1-(2-hydroxyphenyl) prop-2-en-1-one (1). A mixture of 1,3-diphenylpyrazole-4-carboxylaldehyde (1 g, 10 mmol) and *o*-hydroxyacetophenone (0.55 g, 10 mmol) was stirred in absolute methanol (20 mL) in the presence of potassium hydroxide (50%) for 12 h. The precipitated solid was filtered off, washed several times with cooled water, dried, and then recrystallized from benzene to give **1** as yellow crystals; mp 170–172°C, yield: 95%. IR (ν/cm^{-1}): br 3443 (OH), 1672 (C=O), 1636 (C=N). ^{1}H NMR (DMSO-d_6) δ (ppm): 6.99–7.98 (m, 14$H_{arom.}$), 7.80 (d, 1H, H_a, J = 15.3), 8.35 (d, 1H, H_b, J = 14.4), 9.46 (s, 1H, C_5-$H_{pyrazole}$), 12.57 (br s, 1H, OH, exchangeable with D_2O). MS *m/z* (%): 366 ($M^{+\cdot}$, 54). *Anal*. Calcd for $C_{24}H_{18}N_2O_2$ (366.42): C, 78.67; H, 4.95; N, 7.65. Found: C, 78.31; H, 5.22; N, 7.52.

2-Amino-4-(1,3-diphenyl-1H-pyrazol-4-yl)-6-(2-hydroxyphenyl)-4H-pyran-3-carbonitrile (2). A mixture of **1** (1 g, 10 mmol), malononitrile (0.18 g, 10 mmol) in methanol (20 mL), and sodium methoxide (0.5 g Na in 30 mL methanol) was refluxed for 8 h. The reaction mixture was poured onto ice and acidified with dilute acetic acid. The precipitated solid was filtered off, washed several times with cooled water, and then recrystallized from toluene to give **2** as brown crystals; mp 125–127°C, yield: 73%, IR (ν/cm^{-1}): 3347, 3224 (NH_2), 2214 (C≡N). ^{1}H NMR (DMSO-d_6) δ (ppm): 3.91 (d, 1H, C_4H_{pyran}), 5.31 (d, 1H, C_5H_{pyran}), 6.32 (br s, 2H, NH_2, exchangeable with D_2O), 7.15–7.97 (m, 14$H_{arom.}$), 8.97 (s, 1H, $C_5H_{pyrazole}$), 11.20 (br s, 1H, OH, exchangeable with D_2O). MS *m/z* (%): 430 ($M^{+\cdot}$−2, 40). *Anal*. Calcd for $C_{27}H_{20}N_4O_2$ (432.48): C, 74.98; H, 4.66; N, 12.95. Found: C, 75.03; H, 4.75; N, 13.11.

2-Amino-4-(1,3-diphenyl-1H-pyrazol-4-yl)-6-(2-hydroxyphenyl)nicotinonitrile (3). A mixture of **1** (1 g, 10 mmol) and malononitrile (0.18 g, 10 mmol) in methanol (20 mL) in the presence of ammonium acetate (0.7 g, 10 mmol) was refluxed for 10 h. The precipitated solid was filtered off, washed several times with cooled water, dried, and then recrystallized from dioxane to give **3** as buff crystals; mp 315–317°C, yield: 75%. IR (ν/cm^{-1}): br 3404 (OH), 3340, 3236 (NH_2), 2214 (C≡N), 1649 (C=N). ^{1}H NMR (DMSO-d_6) δ (ppm): 6.90–7.96 (m, 15$H_{arom.}$), 7.15 (br s, 2H, NH_2, exchangeable with D_2O), 8.95 (s, 1H, C_5-$H_{pyrazole}$), 13.35 (br s, 1H, OH, exchangeable with D_2O). MS *m/z* (%): 429 ($M^{+\cdot}$, 100). *Anal*. Calcd for $C_{27}H_{19}N_5O$ (429.48): C, 75.51; H, 4.46; N, 16.31. Found: C, 75.21; H, 4.27; N, 16.65.

(E)-4-(2-(1,3-Diphenyl-1H-pyrazol-4-yl)vinyl)-2-imino-2H-chromene-3-carbonitrile (4). A mixture of **1** (1 g, 10 mmol) and malononitrile (0.18 g, 10 mmol) in methanol (20 mL) in the presence of catalytic amount of piperidine was refluxed for 8 h. The reaction mixture was poured onto ice and acidified with dilute acetic acid. The precipitated solid was filtered off, washed several times with cooled water, and then recrystallized from ethanol to give **4** as yellow crystals; mp 274–276°C, yield: 36%. IR (ν/cm^{-1}): 3163 (NH), 2203 (C≡N), 1636 (C=N). ^{1}H NMR (DMSO-d_6) δ (ppm): 7.1–8.12 (m, 14$H_{arom.}$ + 1H, NH), 7.55 (d, 1H, H_a, J = 16.4), 8.68 (d, 1H, H_b, J = 17.1), 9.22 (s, 1H, C_5-$H_{pyrazole}$). MS *m/z* (%): 415 ($M^{+\cdot}$ + 1, 23). *Anal*. Calcd for $C_{27}H_{18}N_4O$ (414.47): C, 78.24; H, 4.38; N, 13.52. Found: C, 78.11; H, 4.27; N, 13.64.

Ethyl 4-(1,3-diphenyl-1H-pyrazol-4-yl)-6-(2-hydroxyphenyl)-2-oxo-1,2-dihydropyridine-3-carboxylate (5). A mixture of **1** (1 g, 10 mmol), diethylmalonate (0.44 g, 10 mmol), and ammonium acetate (0.5 g) in absolute ethanol (20 mL) was refluxed for 10 h. The reaction mixture was poured onto ice and acidified with dilute acetic acid. The precipitated solid was filtered off, washed several times with cooled water, and then recrystallized benzene to give **5** as yellow crystals; mp 180–182°C, yield: 62%, IR (ν/cm^{-1}): 3400 (OH), 3149 (NH), 1720 (C=O_{ester}), 1674 (C=O_{lactam}). ^{1}H NMR (DMSO-d_6) δ (ppm): 1.23 (t, 3H, $CH_2\underline{CH_3}$), 4.32 (q, 2H, $\underline{CH_2}CH_3$), 7.31–8.50 (m, 15$H_{arom.}$), 9.31 (s, 1H, $C_5H_{pyrazole}$), 9.99 (br s, 1H, NH, exchangeable with D_2O), 12.60 (s, 1H, OH, exchangeable with D_2O). MS *m/z* (%): 478 ($M^{+\cdot}$ + 1, 12). *Anal*. Calcd for $C_{29}H_{23}N_3O_4$ (477.52): C, 72.94; H, 4.86; N, 8.80. Found: C, 72.87; H, 4.89; N, 8.79.

4-(1,3-Diphenyl-1H-pyrazol-4-yl)-6-(2-hydroxyphenyl)-2H-pyran-2-one (6). A mixture of **1** (1 g, 10 mmol) and diethylmalonate (0.44 g, 10 mmol) in absolute ethanol (20 mL) in the presence of catalytic amount of piperidine was refluxed for 8 h. The reaction mixture was poured onto ice and acidified with dilute acetic acid. The precipitated solid was filtered off, washed several times with cooled water, and then recrystallized from ethanol to give **6** as buff crystals; mp 275–277°C, yield: 58%. IR (ν/cm^{-1}): 3451 (OH), 1662 (C=O). ^{1}H NMR (DMSO-d_6) δ (ppm): 7.28–8.12 (m, 16$H_{arom.}$), 8.34 (s, 1H, $C_5H_{pyrazole}$), 9.60 (br s, 1H, OH, exchangeable with D_2O). MS *m/z* (%): 404 ($M^{+\cdot}$−2, 10). *Anal*. Calcd for $C_{26}H_{18}N_2O_3$ (406.44): C, 76.83; H, 4.46; N, 6.89. Found: C, 76.79; H, 4.50; N, 6.92.

Ethyl (Z)-2-cyano-3-(1,3-diphenyl-1H-pyrazol-4-yl)acrylate (8). A mixture of **1** (1 g, 10 mmol) and ethyl cyanoacetate (0.31 g, 10 mmol) in ethanol (20 mL) in the presence of catalytic amount of piperidine was refluxed for 10 h. The white solid that separated on hot was dried and then recrystallized from petroleum 60–80°C to give **8**

as white crystals; mp 130–132°C, yield: 33%. IR (ν/cm^{-1}): 1723 (C=O), 2220 (C≡N). ^{1}H NMR (DMSO-d_6) δ (ppm): 1.28 (t, 3H, $CH_2\underline{CH_3}$), 4.28 (q, 2H, $\underline{CH_2}CH_3$), 7.57–7.95 (m, 10H$_{arom.}$), 8.13 (s, 1H, $CH_{olefinic}$), 9.20 (s, 1H, $C_5H_{pyrazole}$). MS *m/z* (%): 343 ($M^{+\cdot}$, 15). *Anal*. Calcd for $C_{21}H_{17}N_3O_2$ (343): C, 73.45; H, 4.99; N, 12.24. Found: C, 73.52; H, 5.03; N, 12.36.

Ethyl 5-(1,3-diphenyl-1H-pyrazol-4-yl)-2′-hydroxy-3-oxo-3,4-dihydro-[1,1′-biphenyl]-4-carboxylate (9). A mixture of **1** (1 g, 10 mmol) and ethyl acetoacetate (0.35 mL, 10 mmol) in absolute ethanol (20 mL) in the presence of catalytic amount of piperidine was refluxed for 8 h. The buff solid that separated after slow evaporation of the solvent was dried and then recrystallized from ethanol to give **9** as buff crystals; mp 253–255°C, yield: 78%, IR (ν/cm^{-1}): br 3441 (OH), 2918, 2849 (CH_3, CH_2), 1731 ($C=O_{ester}$), 1657 ($C=O_{cyclohex\text{-}2,4\text{-}dienone}$). ^{1}H NMR (DMSO-$d_6$) δ (ppm): 1.08 (t, 3H, $CH_2\underline{CH_3}$), 4.17 (q, 2H, $\underline{CH_2}CH_3$), 3.84 (s, 1H, $C_2H_{cyclohex\text{-}2,4\text{-}dienone}$), 6.28 (s, 1H, $C_6H_{cyclohex\text{-}2,4\text{-}dienone}$), 6.37 (s, 1H, $C_4H_{cyclohex\text{-}2,4\text{-}dienone}$), 6.83–8.47 (m, 15H$_{arom.}$), 12.40 (br s, 1H, OH, exchangeable with D_2O). MS *m/z* (%): 476 ($M^{+\cdot}$, 22). *Anal*. Calcd for $C_{30}H_{24}N_2O_4$ (476.53): C, 75.62; H, 5.08; N, 5.88. Found: C, 75.83; H, 5.11; N, 5.97.

1,3-Diphenyl-1H-pyrazole-4-carbonitrile (12). A mixture of **1** (1 g, 10 mmol) and hydroxylamine hydrochloride (0.69 g, 10 mmol) in acetic acid (20 mL) in the presence of fused sodium acetate (0.5 g) was heated under reflux for 6 h. The solid product that deposited was collected by filtration, washed with cold water, dried, and then recrystallized from petroleum 60–80°C to give **12** as yellow crystals; mp 120–122°C, yield 81%. IR (ν/cm^{-1}): 2224 (C≡N), 1597 (C=N). ^{1}H NMR (DMSO-d_6) δ (ppm): 7.42–8.00 (m, 10H$_{arom.}$), 9.43 (s, 1H, $C_5H_{pyrazole}$). MS *m/z* (%): 247 ($M^{+\cdot}$ + 2, 34). *Anal*. Calcd for $C_{16}H_{11}N_3$ (245.29): C, 78.35; H, 4.52; N, 17.13. Found: C, 78.45; H, 4.57; N, 17.26.

2-(1,3-Diphenyl-1H-pyrazol-4-yl)-1H-benzo[d]imidazole (13). A mixture of **1** (1 g, 10 mmol) and *o*-phenylenediamine (0.3 mL, 10 mmol) in ethanol was heated under reflux for 8 h. The solid product that deposited was collected by filtration, washed with cold water, dried, and then recrystallized from toluene to give **13** as pale yellow crystals; mp 270–272°C, yield 67%. IR (ν/cm^{-1}): 3130 (NH), 1528 (C=N). ^{1}H NMR (DMSO-d_6) δ (ppm): 7.19–8.00 (m, 14H$_{arom.}$), 9.09 (s, 1H, $C_5H_{pyrazole}$), 12.60 (br s, 1H, NH, exchangeable with D_2O). MS *m/z* (%): 336 ($M^{+\cdot}$, 17). *Anal*. Calcd for $C_{22}H_{16}N_4$ (336.40): C, 78.55; H, 4.79; N, 16.66. Found: C, 78.52; H, 4.83; N, 16.57.

N-Acetyl-5-(2-hydroxyphenyl)-1′,3′-diphenyl-1H,1′H-[3,4′-bipyrazole]-1-carboxamide (15). A mixture of **1** (1 g, 10 mmol) and semicarbazide hydrochloride (0.4 g, 10 mmol) in acetic acid (20 mL) in the presence of fused sodium acetate (1 g) was heated under reflux for 7 h. The solid product that deposited was collected by filtration, washed with cold water, dried, and then recrystallized from toluene to give **15** as yellow crystals; mp 177–179°C, yield 75%. IR (ν/cm^{-1}): 3177 (NH), 2979 (CH_3), br 1676 (C=O). ^{1}H NMR (DMSO-d_6) δ (ppm): 2.12 (s, 3H, $CO\underline{CH_3}$), 7.34–8.22 (m, 15H$_{arom.}$), 8.89 (s, 1H, $C_5H_{pyrazole}$), 10.99 (br s, 1H, NH, exchangeable with D_2O), 11.17 (br s, 1H, OH, exchangeable with D_2O). MS *m/z* (%): 462 ($M^{+\cdot}$−1, 30). *Anal*. Calcd for $C_{27}H_{21}N_5O_3$ (463.50): C, 69.97; H, 4.57; N, 15.11. Found: C, 70.01; H, 4.61; N, 15.25.

2-(1,3-Diphenyl-1H-pyrazol-4-yl)chroman-4-one (16). A mixture of **1** (1 g, 10 mmol) and thiosemicarbazide (0.3 g, 10 mmol) in dioxane was heated under reflux for 6 h. The solid product that deposited was collected by filtration, washed with cold water, dried, and then recrystallized from petroleum 60–80°C to give **16** as brown crystals; mp 182–184°C, yield 81%. IR (ν/cm^{-1}): 2920, 2850 (CH_2, CH), 1681 (C=O). ^{1}H NMR (DMSO-d_6) δ (ppm): 2.98 (d, 1H, H_a, J = 11.4 Hz), 3.44 (dd, 1H, H_b, J = 13.2 Hz), 5.75 (d, 1H, H_c, J = 10.5 Hz), 7.06–7.94 (m, 14H$_{arom.}$), 8.86 (s, 1H, $C_5H_{pyrazole}$). MS *m/z* (%): 338 ($M^{+\cdot}$ + 2, 5). *Anal*. Calcd for $C_{24}H_{18}N_2O_2$ (336.42): C, 78.67; H, 4.95; N, 7.65. Found: C, 78.62; H, 4.97; N, 7.73.

(E)-3-(1,3-Diphenyl-1H-pyrazol-4-yl)-1-(2-ethoxyphenyl) prop-2-en-1-one (18). A mixture of **1** (1 g, 10 mmol) and ethyl iodide (0.4 g, 10 mmol) in dimethylformamide (20 mL) in the presence of anhydrous potassium carbonate (1 g) was stirred in an ice bath for 15 h. The solid product that deposited was collected by filtration, washed with cold water, dried, and then recrystallized from petroleum 60–80°C to give **18** as buff crystals; mp 90–92°C, yield 63%. IR (ν/cm^{-1}): 2972, 2866 (CH_2, CH_3), 1672 (C=O). ^{1}H NMR (DMSO-d_6) δ (ppm): 1.28 (t, 3H, $CH_2\underline{CH_3}$), 4.10 (q, 2H, $\underline{CH_2}CH_3$), 7.33–8.00 (m, 14H$_{arom.}$ + 2H$_{olefinic}$), 9.22 (s, 1H, $C_5H_{pyrazole}$). MS *m/z* (%): 394 ($M^{+\cdot}$, 12). *Anal*. Calcd for $C_{26}H_{22}N_2O_2$ (394.47); C, 79.17; H, 5.62; N, 7.10. Found: C, 79.26; H, 5.84; N, 7.15.

(E)-2-((1,3-Diphenyl-1H-pyrazol-4-yl)methylene)hydrazine-1-carbothioamide (19). A mixture of **18** (3.9 g, 10 mmol) and thiosemicarbazide (0.9 g, 10 mmol) in dioxane (30 mL) was heated under reflux for 5 h. The solid product that deposited was collected by filtration, washed with cold water, dried, and then recrystallized from ethanol to give **19** as yellow crystals; mp 224–226°C, yield 53%. IR (ν/cm^{-1}): 3330, 3257, 3159 (NH_2, NH), 1618 (C=N). ^{1}H NMR (DMSO-d_6) δ (ppm): 7.36–7.91 (m, 10H$_{arom.}$), 7.69 (br s, 2H, NH_2, exchangeable with D_2O), 8.22 (s, 1H, CH=), 9.18 (s, 1H, $C_5H_{pyrazole}$), 11.33 (br s, 1H, NH, exchangeable with D_2O). MS *m/z* (%): 322 ($M^{+\cdot}$ + 1, 25). *Anal*. Calcd for $C_{17}H_{15}N_5S$ (321.40):

C, 63.53; H, 4.70; N, 21.79; S, 9.98. Found: C, 63.64; H, 4.81; N, 21.75; S, 9.91.

Cytotoxicity assay. The cytotoxic activity of 11 compounds was tested against two human tumor cell lines, namely, hepatocellular carcinoma (liver) HePG-2 and mammary gland (breast) MCF-7. The cell lines were obtained from the ATCC *via* the Holding Company for Biological Products and Vaccines (VACSERA, Cairo, Egypt). Doxorubicin was used as a standard anticancer drug for comparison. The reagents used were RPMI-1640 medium, MTT, DMSO, 5-fluorouracil (Sigma Co., St. Louis, MO, USA), and fetal bovine serum (Gibco, Paisley, UK).

The different cell lines [35,36] mentioned earlier were used to determine the inhibitory effects of compounds on cell growth using the MTT assay. This colorimetric assay is based on the conversion of the yellow tetrazolium bromide (MTT) to a purple formazan derivative by mitochondrial succinate dehydrogenase in viable cells. The cells were cultured in RPMI-1640 medium with 10% fetal bovine serum. Antibiotics added were 100 units/mL penicillin and 100 μg/mL streptomycin at 37°C in a 5% CO_2 incubator. The cell lines were seeded [37] in a 96-well plate at a density of 1.0×10^4 cells per well at 37°C for 48 h under 5% CO_2 incubator. After incubation, the cells were treated with different concentration of compounds and incubated for 24 h. After 24 h of drug treatment, 20 μL of MTT solution at 5 mg/mL was added and incubated for 4 h. Dimethyl sulfoxide (DMSO) in a volume of 100 μL was added into each well to dissolve the purple formazan formed. The colorimetric assay is measured and recorded at absorbance of 570 nm using a plate reader (EXL 800, BioTech, Winoosky, VT, USA). The relative cell viability in percentage was calculated as (A_{570} of treated samples/A_{570} of untreated sample) × 100.

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