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Molecular modeling and synthesis of new 1,5-diphenylpyrazoles as breast cancer cell growth inhibitors

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Abstract: New pyrazoles have been synthesized and evaluated as breast cancer cell growth inhibitors. Condensation of the substituted pyrazole-4-carbaldehyde **1** with acetophenone and chloroacetophenone afforded α, β-unsaturated ketones **2** and **3**, respectively. Compounds **2** and **3** were subjected to different reactions using hydrazine hydrate, substituted hydrazine hydrate, hydroxylamine, *o*-phenylenediamine, malononitrile under different conditions affording 4-substituted pyrazole derivatives **4–28**. Structure elucidation of these compounds was conducted using IR, ^{1}H NMR, ^{13}C NMR, mass spectral data and elemental analysis. Antitumor activity of target compounds was tested against MCF-7 cell line (human breast cancer). Compounds **4**, **10** and **20** show significant antitumor activity against breast cancer. Docking was performed with protein 1UYK to study the binding mode of the designed compounds.

Keywords: anticancer; chalcone; pyrazole; pyridine.

Introduction

Breast cancer is the most popular malignancy and the leading cause of death in women worldwide [1]. It has been shown that inhibition of the enzyme aromatase is one of the mechanisms of the most antitumor drugs especially in the treatment of breast cancer [2–4]. Heat shock proteins (HSP) are members of the molecular chaperones which play an important role in the folding of a large number of cellular proteins [5, 6]. The proliferative activity of breast cancer cells leads to elevated HSP-90 expression [7, 8]. It has been found that the levels of HSP-90 decreased in patients with clinical and biological response to aromatase inhibitors therapy [9].

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A literature survey has revealed the importance of pyrazole derivatives as potent anticancer agents [10–16] including celecoxib (I in Figure 1) that is used in treatment of breast cancer [17–24]. A variety of 1,5-diphenylpyrazole derivatives (Figure 1) have been synthesized and their cytotoxic properties evaluated [25–27]. Chalcone derivatives (Figure 2) exhibit significant biological properties

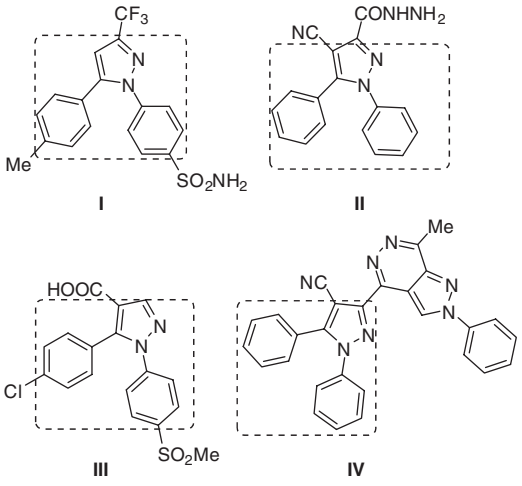


Figure 1 1,5-Diphenylpyrazole derivatives **I–IV** as anticancer agents.

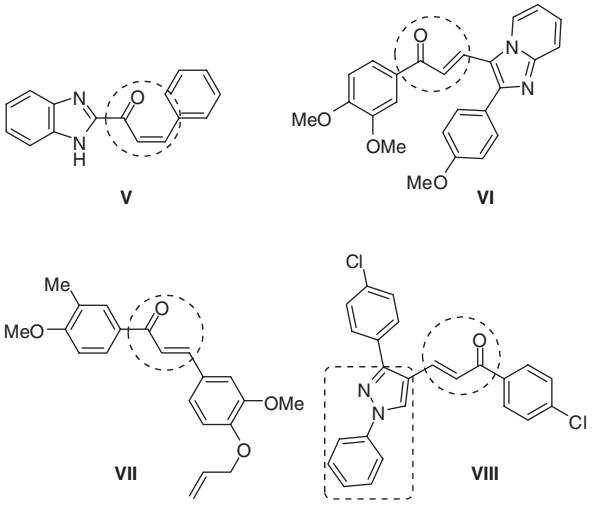


Figure 2 Chalcone derivatives **V–VIII** with known anticancer activity.

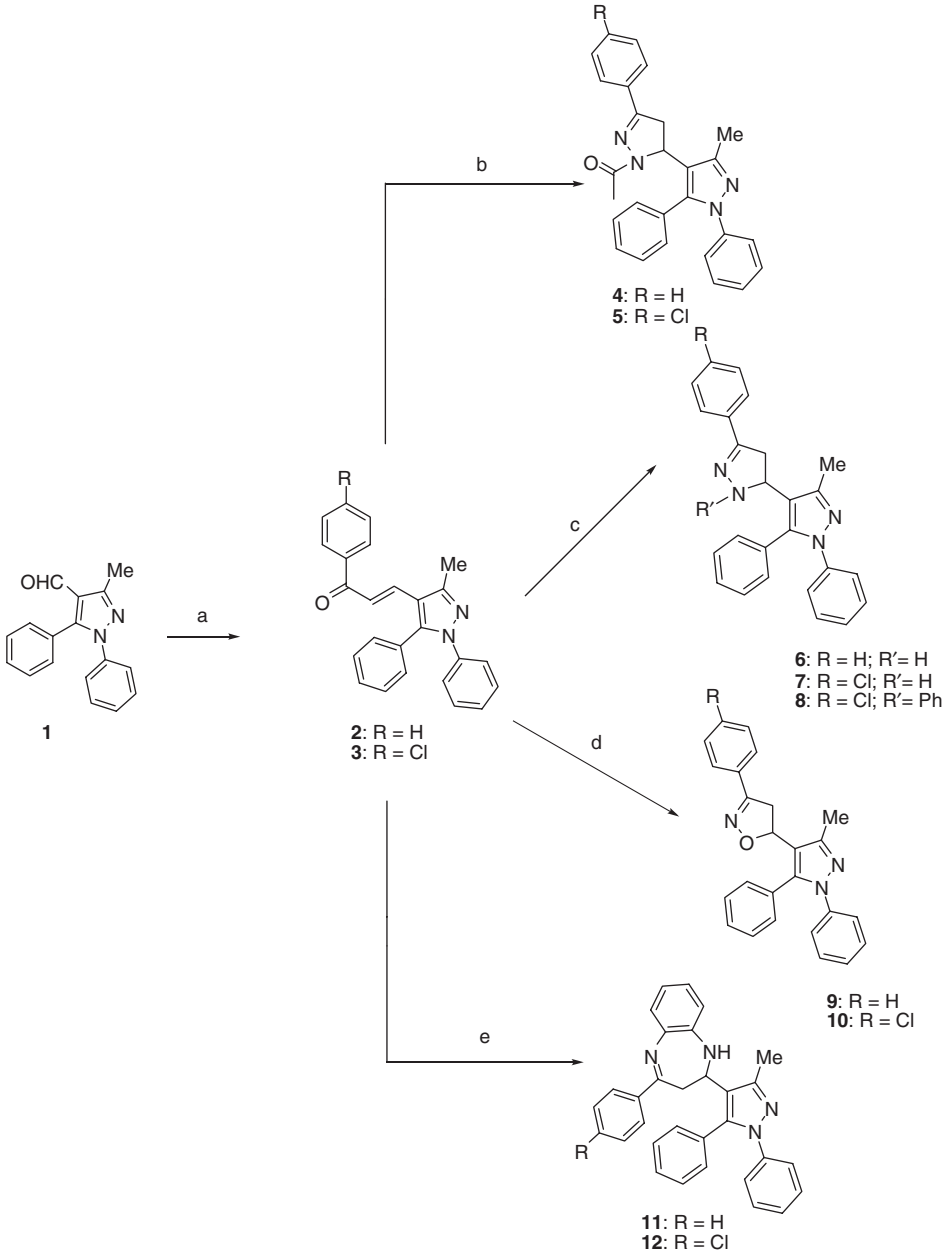
including antiproliferative activities [28–32]. It has been reported that many heterocyclic compounds have a significant antitumor activity when linked to the pyrazole system [33–40].

Inspired by these finding, and in order to develop new anticancer therapeutic agents, we were encouraged to integrate a 1,5-diphenylpyrazole moiety as a main scaffold with a chalcone moiety to form new hybrid molecules [41–43].

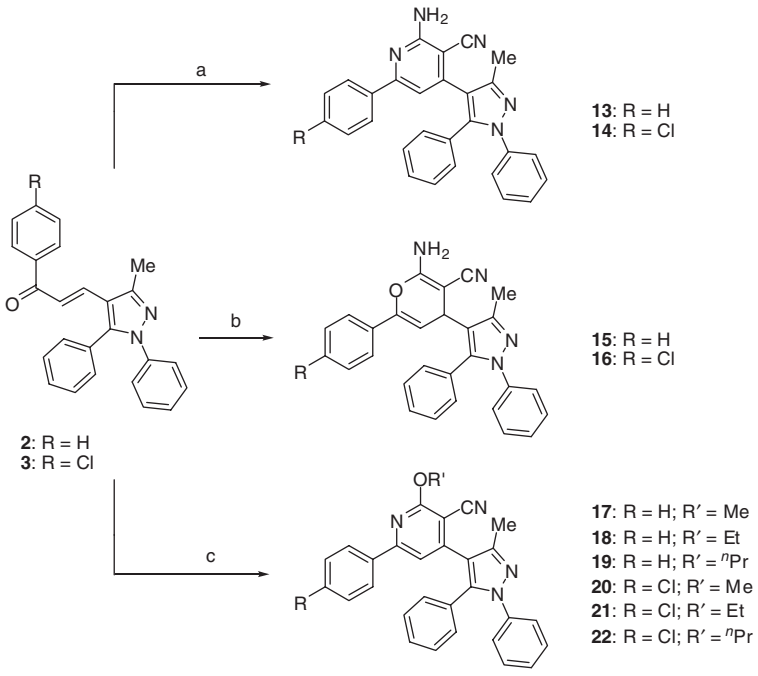
Results and discussion

Chemistry

The synthetic pathways adopted for the preparation of the target new compounds are illustrated in Schemes **1** and **2**. The starting material **1** was obtained via Vilsmeir Haack's formylation using $POCl_3$ [44, 45]. The α, β-unsaturated carbonyl derivatives **2** and **3** were synthesized via



Scheme 1 The synthesis of **3–12**: (a) acetophenone or 4-chloroacetophenone, NaOH, absolute ethanol; (b) NH_2NH_2, AcOH; (c) R′-$NHNH_2$, absolute ethanol; (d) $NH_2OH{\cdot}HCl$, KOH; (e) *o*-phenylenediamine, Et_3N, absolute ethanol.



Scheme 2 The synthesis of **13–22**: (a) malononitrile, ammonium acetate, absolute ethanol; (b) malononitrile, DMF, pyridine; (c) malononitrile, sodium methoxide or sodium ethoxide or sodium propoxide, methanol, ethanol and n-propanol.

Table 1 Molecular modeling results of **3–20** with amino acids of the enzyme 1UYK and their biological screening results against breast cancer cell line (MCF-7).

Comp. no.	E[c] of interaction ligand-protein	% inhibition	IC_{50}[b], µg/mL	Comp. no.	E[c] of interaction ligand-protein	% inhibition	IC_{50}[b], µg/mL
3	-40.264	33.982		**13**	-31.271	55.288	
4	-34.761	78.174[a]	11.7	**14**	-29.997	54.672	
5	-37.322	30.857		**15**	-42.784	48.686	
6	-30.629	25.53		**16**	-33.386	40.597	
7	-35.633	67.877[a]	25.9	**17**	-30.134	43.615	
8	-32.328	56.28		**18**	-29.425	37.013	
9	-33.727	16.848		**19**	-29.530	25.861	
10	-39.095	50.244		**20**	-30.324	66.437[a]	29.5
11	-29.938	58.489		**21**	-29.072	41.855	
12	-35.397	38.773		**22**	-36.324	35.488	

[a]Compounds showing significant antitumor activity; [b]IC_{50} for active antitumor compounds; [c]energy (kJ/mol).

Claisen-Schmidt condensation [46, 47] of compound **1** with a ketone (Scheme 1). The IR spectrum of **3** reveals a C=O band of carbonyl group at 1658 cm^{-1}. The reaction of compounds **2** and **3** with hydrazine hydrate in the presence of acetic acid yielded *N*-acetylpyrazole derivatives **4** and **5**, respectively. A similar reaction conducted in ethanol gave the *N*-substituted pyrazoles **6** and **7**. Moreover, reaction of chalcone **3** with phenylhydrazine afforded compounds **8**. ^{1}H NMR spectrum of **4** shows a doublet at 3.85 ppm and a triplet at 5.12 ppm corresponding to the pyrazoline CH_2 and CH, respectively. ^{1}H NMR spectrum of **7** shows an exchangeable signal at 7.63 ppm for NH group. The mass spectrum of compound **8** shows a molecular ion peak at *m/z* 489 corresponding to the molecular formula $C_{31}H_{25}ClN_4$ and a peak at 490 (M^++2) due to the presence of the isotopic chlorine atom. Hydroxylamine hydrochloride was also allowed to react with chalcones **2** and **3** to form isoxazoline derivatives **9** and **10**, respectively. The ^{1}H NMR spectrum of the compound **9** is characterized by the presence of a doublet at 3.5 ppm for CH_2 and a triplet at 5.9 ppm for CH of the isoxazoline ring. Reaction of *o*-phenylenediamine with chalcones **2** and **3** gave

the corresponding 1,5-benzodiazepines **11** and **12**. The IR spectrum of **11** shows a significant band at 3350 cm^{-1} attributed to N-H group and the disappearance of an absorption band assigned to the carbonyl group. ^{13}C NMR spectrum of **12** shows signals at 38.7, 40.4, 121.1, 124.8 ppm corresponding to the benzodiazepine carbon atoms and a signal at 14.2 ppm corresponding to the CH_3 group.

Pyridine-3-carbonitriles and pyran-3-carbonitriles were prepared as shown in Scheme 2. Malononitrile was allowed to react with compounds **2** and **3** in the presence of ammonium acetate to give pyridine-3-carbonitrile derivatives **13** and **14**, respectively. A similar reaction conducted in DMF in the presence of piperidine, afforded the corresponding pyran-3-carbonitriles derivatives **15** and **16**. Furthermore, reaction of chalcones **2** and **3** with malononitrile in the presence of sodium alkoxide in an alcohol gave the corresponding 2-alkoxypyridine-3-carbonitriles derivatives **17–22**. IR spectrum of **13** reveals the presence of a stretching band at 3415 cm^{-1} attributed to NH_2 group. The ^1H NMR of **14** displays a signal at 6.27 ppm corresponding to NH_2 group while compound **15** shows characteristic 2 signals at 6.56 and 6.83 ppm corresponding to the pyran protons.

Antitumor activity

All synthesized compounds were tested for their cytotoxic activity against MCF-7 (human breast cancer cell line) using the method of Skehan [48, 49]. The results are presented in Table 1. A series of 1,5-diphenylpyrazoles was synthesized and evaluated *in vitro* for antitumor activity against MFC-7 cell line. Compounds **4**, **7** and **20** exhibit strong activity (Table 1). Compounds **5**, **8**, **11**, **13**–**17** and **22** show moderate activity. Compounds **12** and **18** show weak activity. Compounds **9** and **19** show very weak activity. 1,5-Benzodiazepine derivative **11**, pyridine-3-carbonitriles **13** and **14** and pyran derivatives **15** and **16** are moderately active in comparison to the starting compounds **2** and **3**. The introduction of a propyl chain in compounds **19**, **22** causes reduction in activity in comparison with the other pyridine-3-carbonitriles **17**, **18**, **20** and **21.**

Molecular modeling

The molecular operating environment (MOE) [50] based molecular docking was done for the target compounds

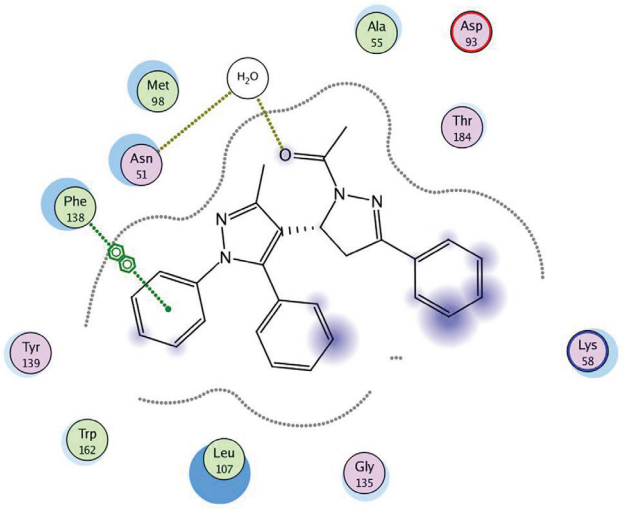


Figure 3 Docking of **4** in 1UYK binding side.

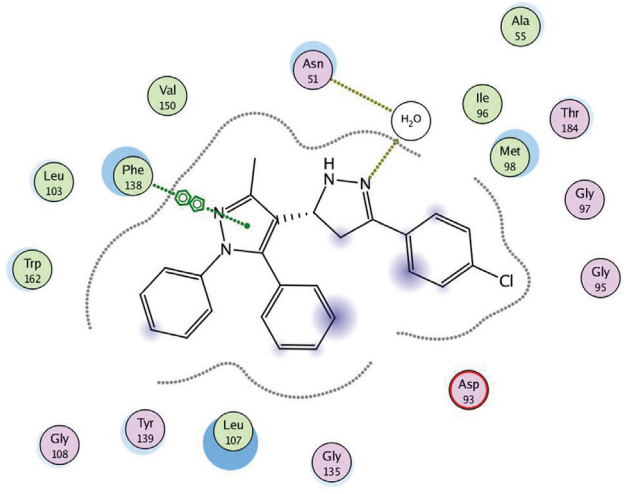


Figure 4 Docking of **7** in 1UYK binding side.

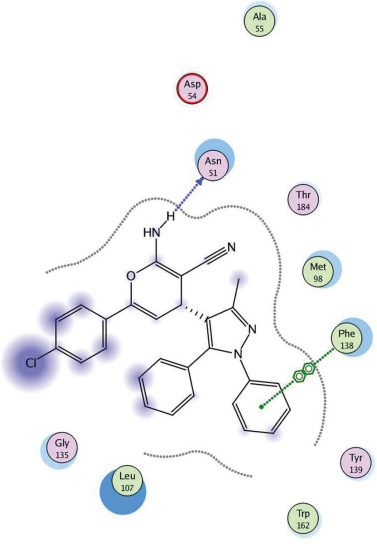


Figure 5 Docking of **16** in 1UYK binding side.

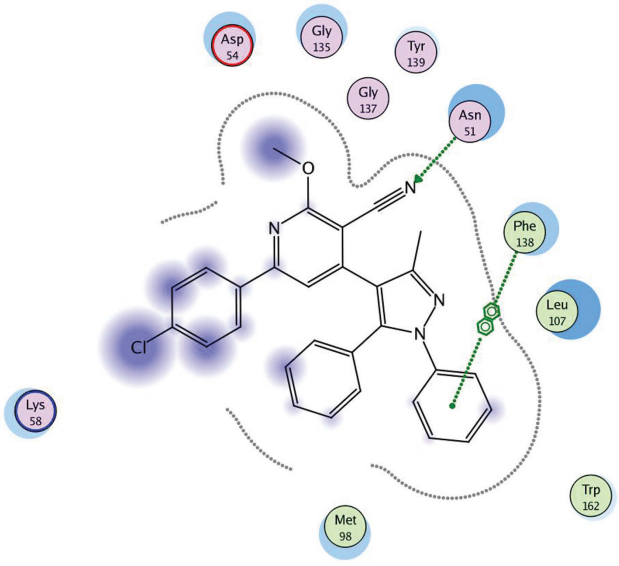


Figure 6 Docking of **20** in 1UYK binding side.

3–22 on heat shock proteins (HSP) obtained from the protein data bank (code, 1UYK.pdb) with the help of PharmMapper software [51]. When examining the protein-ligand interaction for the protein molecule 1UYK [52], it was found that the main amino acids involved in binding to the ligand are Asn (51), Asp (93) and Phe (138). The selected complexes calculated as part of this work are shown in Figures 3–6.

Conclusion

There is a strong correlation between molecular modeling and biological screening results which confirm that the structural modification of the lead structure affects the activity in a predictable manner.

Experimental

Chemistry

Melting points are uncorrected and were recorded in open capillaries on an electro-thermal melting point apparatus. IR spectra were recorded on a Mattson 5000 FT-IR spectrometer in KBr disks at the Faculty of Science, Mansoura University. ^{1}H NMR (200 MHz) and ^{13}C NMR (50 MHz) spectra were obtained on a Gemini Varian spectrometer using TMS as internal standard, at the Micro-analytical Unit of Cairo University. Mass spectral analyses were performed on a JOEL JMS-600H spectrometer at Cairo University. Microanalyses were performed at the Micro-analytical Unit of Cairo University. All reagents were purchased from the Aldrich Chemical Company. Compounds **1**, **2** were synthesized according to reported methods [53, 54].

1-(4-Chlorophenyl)-3-[(3-methyl)-1,5-diphenyl-1*H*-pyrazol-4-yl] prop-2-en-1-one (3) A mixture of compound **1** (2.6 g, 0.01 mol), chloroacetophenone (0.01 mol) and sodium hydroxide (0.025 mol) in absolute ethanol (20 mL) was heated under reflux for 2 h. The solid obtained was collected by filtration, dried and crystallized from ethanol: light brown solid; yield 85%; mp 155–156°C; IR: 1590 (C=N), 1606 (C=C), 1658 cm^{-1} (C=O); ^{1}H NMR: δ 2.41 (s, 3H, CH_3), 6.8 (d, 1H, J = 16.3 Hz), 7.2 (d, 1H, J = 16.3 Hz), 7.17–7.44 (m, 14H, Ar-H); MS: m/z 399 [M$^+$].

General procedure for the preparation of compounds 4 and 5

A solution of compound **2** or **3** (0.001 mol) and hydrazine hydrate (0.1 mL, 0.002 mol) in glacial acetic acid (15 mL) was heated under reflux for 6–9 h. Cold water was added to the hot mixture and the product extracted using ethyl acetate was crystallized from ethanol.

1-[5-(3-Methyl-1,5-diphenyl-1*H*-pyrazol-4-yl)-3-phenyl-4,5-dihydro-1*H*-pyrazol-1-yl]ethan-1-one (4) Yield 70%; mp 118–120°C; IR: 1645 (C=N), 1675 cm^{-1} (C=O); ^{1}H NMR: δ 1.92 (s, 3H, CH_3), 2.03 (s, 3H, $COCH_3$), 3.85 (d, 2H, CH_2 pyrazoline), 5.12 (t, 1H, CH pyrazoline), 7.15–7.57 (m, 15H, Ar-H); MS: m/z 421 [M$^+$+1]. Anal. calcd for $C_{27}H_{24}N_4O$: C, 77.12; H, 5.75; N, 13.32. Found: C, 77.03; H, 5.53; N, 13.12.

1-[3-(4-Chlorophenyl)-5-(3-methyl-1,5-diphenyl-1*H*-pyrazol-4-yl)-4,5-dihydro-1*H*-pyrazol-1-yl]ethan-1-one (5) Yield (67%); mp 122–124°C; IR: 1620 (C=N), 1680 cm^{-1} (C=O); ^{1}H NMR: δ 1.89 (s, 3H, CH_3), 2.32 (s, 3H, $COCH_3$), 3.57 (d, 2H, CH_2 pyrazoline), 4.56 (t, 1H, CH pyrazoline), 7.10–7.97 (m, 14H, Ar-H); MS: m/z 455 [M$^+$+1], 456 [M$^+$+2]. Anal. calcd for $C_{27}H_{23}ClN_4O$: C, 71.28; H, 5.10; N, 12.30. Found: C, 71.16; H, 5.36; N, 12.04.

Preparation of 3-methyl-1,5-diphenyl-4-[3-(substituted phenyl)-4,5-dihydropyrazol-5-yl)]-1*H*-pyrazoles 6–8

A mixture of compound **2** or **3** (0.005 mol) with hydrazine hydrate (0.02 mol) or phenylhydrazine (0.005 mol) in absolute ethanol was heated under reflux for a period of time indicated below and then concentrated *in vacuo*. On cooling the separated solid was filtered and crystallized from ethanol.

3-Methyl-1,5-diphenyl-4-(3-phenyl-4,5-dihydro-1*H*-pyrazol-5-yl)-1*H*-pyrazole (6) Compound **6** was prepared from **2** and hydrazine hydrate, reaction time 6 h: yield 56%; mp 135–137°C; IR: 1599 (C=N), 3305 cm^{-1} (N-H); ^{1}H NMR: δ 1.8 (s, 3H, CH_3), 2.7 (d, 2H, CH_2 pyrazoline), 3.3 (t, 1H, CH pyrazoline), 6.7–8 (m, 15H, Ar-H), 7.63 (s, 1H, NH D_2O exchangeable); MS: m/z 378 [M$^+$]. Anal. calcd for $C_{25}H_{22}N_4$: C, 79.34; H, 5.86; N,14.80. Found: C, 79.71; H, 5.66; N, 14.64.

4-[3-(4-Chlorophenyl-4,5-dihydro-1*H*-pyrazol-5-yl]-3-methyl-1,5-diphenyl-1*H*-pyrazole (7) Compound **7** was prepared from **3** and hydrazine hydrate, reaction time 6 h: yield 69%; mp 183–185°C; IR: 1593 (C=N), 3365 cm^{-1} (N-H); ^{1}H NMR: δ 1.9 (s, 3H, CH_3), 2.5 (d, 2H, CH_2 pyrazoline), 3.9 (t, 1H, CH pyrazoline), 7.00–8.02 (m, 14H, Ar-H), 7.36 (s, 1H, NH D_2O exchangeable); MS: m/z 413 [M$^+$+1], 414 [M$^+$+2]. Anal. calcd for $C_{25}H_{21}ClN_4$: C, 72.72; H, 5.13; N, 13.57. Found: C, 72.47; H, 5.37; N, 13.34.

4-[3-(4-Chlorophenyl)-1-phenyl-4,5-dihydro-1*H*-pyrazol-5-yl]-3-methyl-1,5-diphenyl-1*H*-pyrazole (8) Compound **8** was prepared from **3** and phenylhydrazine, reaction time 20 h; yield 63%; mp 110–112°C; IR: 1599 (C=N), 1605 cm^{-1} (C=C); ^1H NMR: δ 11.37 (s, 3H, CH_3), 3.35 (d, 2H, CH_2 pyrazoline), 5.55 (t, 1H, CH pyrazoline), 6.46–8.02 (m, 19H, Ar-H); MS: *m/z* 489 [M^{+1}], 490 [M^+]. Anal. calcd for $C_{31}H_{25}ClN_4$: C, 76.14; H, 5.15; N, 11.46. Found: C, 76.34; H, 5.39; N, 11.74.

Preparation of 5-(3-methyl-1,5-diphenyl-1*H*-pyrazol-4-yl)-3-(substituted phenyl)-4,5-dihydroisoxazoles 9 and 10

A mixture of compound **2** or **3** (0.03 mol), absolute ethanol (15 mL), hydroxylamine hydrochloride (0.2 g, 0.003 mol) and potassium hydroxide (0.2 g, 0.004 mol) was heated under reflux for 10 h. After cooling, the precipitate was filtered and crystallized from ethanol.

5-(3-Methyl-1,5-diphenyl-1*H*-pyrazol-4-yl)-3-phenyl-4,5-dihydroisoxazole (9) Yield 60%; mp 103–105°C; ^1H NMR: δ 2.5 (s, 3H, CH_3), 3.5 (d, 2H, CH_2 isoxazoline H-4), 5.9 (t, 1H, CH isoxazoline), 7.03–7.89 (m, 15H, Ar-H); ^{13}C NMR: δ 21.2, 46.6, 80.4, 119.4, 121.2, 123.7, 124.0, 126.7, 129.5, 131.6, 135.0, 136.5, 139.4, 142.7, 144.3, 146.7, 149.4, 153.4, 159.2; MS: *m/z* 379[M^+]. Anal. calcd for $C_{27}H_{23}ClN_4O$: C, 79.13; H, 5.58; N, 11.07. Found: C, 79.45; H, 5.76; N, 11.37.

3-(4-Chlorophenyl)-5-(3-methyl-1,5-diphenyl-1*H*-pyrazol-4-yl)-4,5-dihydroisoxazole (10) Yield 54%; mp 113–115°C; ^1H NMR: δ 2.1 (s, 3H, CH_3), 3.3(d, 2H, CH_2 isoxazoline), 5.2 (t, 1H, CH isoxazoline), 7.01–7.52 (m, 14H, Ar-H); MS: *m/z* 414 [M^++1], 415 [M^++2]. Anal. calcd for $C_{25}H_{20}ClN_3O$: C, 72.55; H, 4.87; N, 10.15. Found: C, 72.20; H, 4.63; N, 10.42.

Preparation of compounds 11 and 12

A mixture of **2** or **3** (2.44 g, 0.01 mol), *o*-phenylenediamine (1.08 g, 0.01 mol) and triethylamine (3 mL) in absolute ethanol (15 mL) was heated under reflux for 15 h. The reaction mixture was cooled to 0°C and the resultant precipitate was filtered and crystallized from ethanol.

2-(3-Methyl-1,5-diphenyl-1*H*-pyrazol-4-yl)-4-phenyl-2,3-dihydro-1*H*-1,5 benzodiazepine (11) Yield 59%; mp 178–180°C; IR: 1593 (C=N), 3350 cm^{-1} (N-H); MS: *m/z* 454 [M^+. Anal. calcd for $C_{31}H_{26}N_4$: C, 81.91; H, 5.77; N, 12.33. Found: C, 81.78; H, 5.47; N, 12.13.

4-(4-Chlorophenyl)-2-(3-methyl-1,5-diphenyl-1*H*-pyrazol-4-yl)-2,3-dihydro-1*H*-1,5-benzodiazepine (12) Yield 63%; mp 186–188°C; IR: 1592 (C=N), 3375 cm^{-1} (N-H); ^{13}C NMR: δ 14.2, 38.7, 40.4, 115.7, 119.2, 121.1, 124.8, 126.1, 127.7, 128.6, 130.0, 130.1, 135.7, 136.4, 137.7, 138.8, 140.2, 144.9, 146.4, 148.7, 153.2, 165.4; MS: *m/z* 489 [M^++1], 490 [M^++2]. Anal. calcd for $C_{31}H_{25}ClN_4$: C, 76.14; H, 5.15; N, 11.46. Found: C, 76.34; H, 5.37; N, 11.27.

Preparation of compounds 13 and 14

A solution of chalcone **2** or **3** (0.005 mol), malononitrile (0.005 mol) and ammonium acetate (0.04 mol) in ethanol was heated under reflux for 6 h. On cooling, the precipitated solid was filtered, dried and crystallized from ethanol.

2-Amino-4-(3-methyl-1,5-diphenyl-1*H*-pyrazol-4-yl)-6-phenylpyridine-3-carbonitrile (13) Yield 64%; mp 131–133°C; IR: 1598 (C=N), 2227 (CN), 3415 cm^{-1} (NH_2); ^1H NMR: δ 2.41(s, 3H, CH_3), 6.32 (s, 2H, NH_2), 7.19 (s, 1H, H-pyridine), 7.31–8.30 (m, 15H, Ar-H); ^{13}C NMR: 19.3, 89.1, 103.4, 116.7, 121.4, 123.7, 124.8, 126.7, 128.5, 130.7, 132.4, 134.6, 136.3, 140.0, 143.8, 150.2, 154.7, 156.3, 163.7; MS: *m/z* 428 [M^++1]. Anal. calcd for $C_{28}H_{21}N_5$: C, 78.67; H, 4.95; N, 16.38. Found: C, 78.87; H, 4.65; N, 16.63.

2-Amino-6-(4-chlorophenyl)-4-(3-methyl-1,5-diphenyl-1*H*-pyrazol-4-yl)pyridine-3-carbonitrile (14) Yield 70%; mp 146–148°C; IR: 1630 (C=N), 2223 (CN), 3350 cm^{-1} (NH_2); ^1H NMR: δ 2.34 (s, 3H, CH_3), 6.27 (s, 2H, NH_2), 7.11 (s, 1H, H-pyridine), 7.29–8.37 (m, 14H, Ar-H); MS: *m/z* 462 [M^++1], 463 (M^++2], 3.4%). Anal. calcd for $C_{28}H_{20}ClN_5$: C, 72.80; H, 4.36; N, 15.16. Found: C, 72.53; H, 4.10; N, 15.42.

Preparation of compounds 15 and 16

A mixture of malononitrile (0.001 mol) compound **2** or **3** (0.001 mol), and a few drops of piperidine in DMF (15 mL) was stirred at room temperature. The precipitated product was washed with DMF and crystallized from ethanol.

2-Amino-4-(3-methyl-1,5-diphenyl-1*H*-pyrazol-4-yl)-6-phenyl-4*H*-pyran-3-carbonitrile (15) Yield 56%; mp 120–122°C; IR: 1593 (C=N), 2230 (CN), 3400 cm^{-1} (NH_2); ^1H NMR: δ 2.41(s, 3H, CH_3), 6.30 (s, 2H, NH_2), 6.56 (d, 1H, *J* = 3.8 Hz), 6.83 (d, 1H, *J* = .8 Hz), 7.31–8.30 (m, 15H, Ar-H); MS: *m/z* 430 [M^+. Anal. calcd for $C_{28}H_{22}N_4O$: C, 78.12; H, 5.15; N, 13.01. Found: C, 78.43; H, 5.36; N, 13.31.

2-Amino-6-(4-chlorophenyl)-4-(3-methyl-1,5-diphenyl-1*H*-pyrazol-4-yl)-4*H*-pyran-3-carbonitrile (16) Yield 59%; mp 126–128°C; IR: 1587 (C=N), 2218 (CN), 3365 cm^{-1} (NH_2); ^1H NMR: δ 2.34 (s, 3H, CH_3), 6.11 (s, 2H, NH_2), 6.34 (d, 1H, *J* = 3.6 Hz), 6.75 (d, 1H, *J* = 3.6 Hz), 7.25–8.20 (m, 14H, Ar-H); MS: *m/z* 464 [M^+], 466 [M^++2]. Anal. calcd for $C_{28}H_{21}ClN_4O$: C, 72.33; H, 4.55; N, 12.05. Found: C, 72.63; H, 4.67; N, 12.35.

Preparation of compounds 17–22

A mixture of chalcone **2** or **3** (0.001 mol), malononitrile (0.001 mol) and a solution of sodium alkoxide (15 mL, 0.014 mol of sodium in 100 mL of the appropriate alcohol, namely, methanol, ethanol or *n*-propanol) was stirred at room temperature for the period of time indicated below. The product was crystallized from ethanol/DMF.

2-Methoxy-4-(3-methyl-1,5-diphenyl-1*H*-pyrazol-4-yl)-6-phenylpyridine-3-carbonitrile (17) Compound **17** was prepared from **2** and malononitrile in sodium methoxide and methanol, reaction time 2 h; yield 70%; mp 105–107°C; IR: 1560 (C=N), 2220 cm^{-1} (CN);^1H NMR: δ 2.47 (s, 3H, CH_3), 4.27 (s, 3H, OCH_3), 7.48 (s, 1H, pyridine-H), 6.73–7.88 (m, 15H, Ar-H); MS: *m/z* 443 [M^++1]. Anal. calcd for $C_{29}H_{22}N_4O$: C, 78.71; H, 5.01; N, 12.66. Found: C, 78.48; H, 5.39; N, 12.48.

2-Ethoxy-4-(3-methyl-1,5-diphenyl-1*H*-pyrazol-4-yl)-6-phenylpyridine-3-carbonitrile (18) Compound **18** was prepared from **2** and malononitrile in sodium ethoxide and ethanol, reaction time 3 h; yield 67%; mp 101–103°C; IR: 1569 (C=N), 2227 cm^{-1} (CN); ^1H NMR: δ 1.36 (t, 3H, O-$CH_2\underline{CH_3}$), 2.01 (s, 3H, CH_3), 4.10 (q, 2H, O-$\underline{CH_2}CH_3$), 7.49

(s, 1H, pyridine-H), 7.23–7.85 (m, 15H, Ar-H); MS: *m/z* 457 [M⁺+1]. Anal. calcd for $C_{30}H_{24}N_4O$: C, 78.92; H, 5.30; N, 12.27. Found: C, 78.69; H, 5.67; N, 12.42.

4-(3-Methyl-1,5-diphenyl-1*H*-pyrazol-4-yl)-6-phenyl-2-propoxypyridine-3-carbonitrile (19) Compound **19** was prepared from **2** and malononitrile in sodium propoxide and *n*-propanol, reaction time 4 h; yield 64%; mp 138–140°C; IR: 1567 (C=N), 2230 cm^{-1} (CN); ^1H NMR: δ 0.8 (t, 3H, $OCH_2CH_2\underline{CH_3}$), 1.89 (m, 2H, $OCH_2\underline{CH_2}CH_3$), 2.16 (s, 3H, CH3), 4.56 (t, 2H, $O\underline{CH_2}CH_2CH_3$), 7.58 (s, 1H, pyridine-H), 7.13–8.08 (m, 15H, Ar-H); MS: *m/z* 471 [M⁺+1]. Anal. calcd for $C_{31}H_{26}N_4O$: C, 79.12; H, 5.57; N, 11.91. Found: C, 79.37; H, 5.32; N, 11.61.

6-(4-Chlorophenyl)-2-methoxy-4-(3-methyl-1,5-diphenyl-1*H*-pyrazol-4-yl)pyridine-3-carbonitrile (20) Compound **20** was prepared from **3** and malononitrile in sodium methoxide and methanol, reaction time 1 h; yield 61%; mp 123–125°C; IR: 1570 (C=N), 2219 cm^{-1} (CN); ^1H NMR: δ 2.41 (s, 3H, CH_3), 4.34 (s, 3H, OCH_3), 7.44 (s, 1H, pyridine-H), 6.88–7.37 (m, 14H, Ar-H); MS: *m/z* 476 [M⁺], 478 [M⁺+2]. Anal. calcd for $C_{29}H_{21}ClN_4O$: C, 73.03; H, 4.44; N, 11.75. Found: C, 73.37; H, 4.28; N, 11.98.

6-(4-Chlorophenyl)-2-ethoxy-4-(3-methyl-1,5-diphenyl-1*H*-pyrazol-4-yl)pyridine-3-carbonitrile (21) Compound **21** was prepared from **3** and malononitrile in sodium ethoxide and ethanol, reaction time 2.5 h; yield 66%; mp 140–142°C; IR: 1564 (C=N), 2217 cm^{-1} (CN); ^1H NMR: δ 1.15 (t, 3H, O-$CH_2\underline{CH_3}$), 2.15 (s, 3H, CH_3), 4.32 (q, 2H, O-$\underline{CH_2}CH_3$), 7.68 (s, 1H, pyridine-H), 7.11–7.52 (m, 14H, Ar-H); MS: *m/z* 491 [M⁺+1], [M⁺+2]. Anal. calcd for $C_{30}H_{23}ClN_4O$: C, 73.39; H, 4.72; N, 11.41. Found: C, 73.70; H, 4.58; N, 11.69.

6-(4-Chlorophenyl)-4-(3-methyl-1,5-diphenyl-1*H*-pyrazol-4-yl)-2-propoxy pyridine-3-carbonitrile (22) Compound **22** was prepared from **3** and malononitrile in sodium propoxide and *n*-propanol, reaction time 6 h; yield 62%; mp 164–166°C; IR: 1561 (C=N), 2224 cm^{-1} (CN); ^1H NMR: δ 0.9 (t, 3H, $OCH_2CH_2\underline{CH_3}$), 1.74(m, 2H, $OCH_2\underline{CH_2}CH_3$), 2.31 (s, 3H, CH3), 4.46 (t, 2H, $O\underline{CH_2}CH_2CH_3$), 7.88 (s, 1H, pyridine-H), 7.10–8.10 (m, 14H, Ar-H); MS: *m/z* 504 [M⁺], 506 [M⁺+2]. Anal. calcd for $C_{31}H_{25}ClN_4O$: C, 73.73; H, 4.99; N, 11.09. Found: C, 73.59; H, 4.69; N, 11.29.

Biology

All synthesized compounds were evaluated for their antitumor activity against human breast cancer cell line (MCF-7) adopting the sulforhodamine B (SRB) assay [48]. All materials were obtained from Sigma Chemical Co. (USA). The cell line was obtained frozen in liquid nitrogen (-180°C) from the American Type Culture Collection. It was maintained by serial sub-culturing in 75 cm^2 cell culture flasks (Fisher Scientific, Pittsburgh, PA, USA) at 37°C in atmosphere of 5% CO_2 using 10 mL of RPMI-1640 [supplemented with 1% (2 mM) glutamic acid, 10% unheated fetal bovine serum (FBS) 100 μg/mL penicillin and 100 μg/mL streptomycin]. Using 96-well microtiter plates at a concentration of 5×10^4–10^5 cell/well in a fresh medium, cells were seeded and left to attach to the plates for 24 h. Treatment with the test compound allowed attachment of cell to the wall of the plate. Monolayer cells were incubated with the compounds for 48 h at 37°C in a humidified incubator with 5% CO_2. Cells were fixed with trichloroacetic acid and stained for 30 min with 0.4% (wt/vol) sulforhodamine B (SRB) stain dissolved in 1% acetic acid. Unbound dye was washed with 1% acetic acid and protein bound dye was extracted with Tris EDTA (Meter Tech. Σ 960, USA). The optical density (O.D.) of each well was measured spectrophotometrically at 564 nm with an ELIZA microplate reader (Meter Tech. Σ 960, USA). The percentage of cell survival was calculated as follows: Survival fraction=O.D. (treated cells)/O.D. (control cells). The IC_{50} values were calculated using different concentrations of the tested compounds. The relation between surviving fraction and compound concentration was plotted to obtain the survival curve (Table 1).

Molecular docking

The docking studies and modeling calculations were done using 'MOE version 2008.10 release of Chemical Computing Group's' which was operated under Windows XP operating system installed on an Intel Pentium IV PC with a 2.8 MHz processor and 512 RAM. The tested compounds were built in 2D using ChemBiooffice suite and geometric optimization was done using Hyperchem, then subjected to docking simulation. The X-ray crystallographic structure of heat shock protein enzyme was obtained from the Protein Data Bank; code '1UYK.pdb'.

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References

[1] Ferlay, J.; Shin, H. R.; Bray, F.; Forman, D.; Mathers, C.; Parkin, D. M. Estimates of worldwide burden of cancer in 2008: GLOBOCAN 2008. *Int. J. Cancer.* **2010**, *127*, 2893–2917.
[2] Mokbel, K. The evolving role of aromatase inhibitors in breast cancer. *Int. J. Clin. Oncol.* **2002**, *7*, 279–283.
[3] Goss, P. E.; Powles, T. J.; Dowsett, M.; Hutchison, G.; Brodie, A. M.; Gazet, J. C. Treatment of advanced postmenopausal breast cancer with an aromatase inhibitor, 4-hydroxyandrostenedione: phase II report. *Cancer Res.* **1986**, *46*, 4823–4826.
[4] Simpson, E. R. Sources of estrogen and their importance. *J. Steroid Biochem. Mol. Biol.* **2003**, *86*, 225–230.
[5] Bukau, B.; Weissman, J.; Horwich, A. Molecular chaperones and protein quality control. *Cell* **2006**, *125*, 443–451.
[6] Lindquist, S.; Craig, E. A. The heat shock proteins. *Ann. Rev. Genet.* **1988**, *22*, 631–637.
[7] Zagouri, F.; Bournakis, E.; Koutsoukos, K.; Papadimitriou, C. A. Heat shock protein 90 (hsp90) expression and breast cancer. *Pharmaceuticals (Basel)* **2012**, *5*, 1008–1020.
[8] Che, H. L.; Hong, H. M.; Chang, Y. Y.; Chang, W. W. Inhibition of heat shock protein (Hsp) 27 potentiates the suppressive effect of Hsp90 inhibitors in targeting breast cancer stem-like cells. *Biochimie* **2012**, *94*, 1382–1389.
[9] Yiu, C. C.; Chanplakorn, N.; Chan, M. S.; Loo, W. T.; Chow, L. W.; Toi, M.; Sasano, H. Down-regulation of heat-shock protein 70 (HSP-70) correlated with responsiveness to neoadjuvant aromatase inhibitor therapy in breast cancer patients. *Anticancer Res.* **2010**, *30*, 3465–3472.

[10] Bouabdallah, I.; M'Barek, L. A.; Zyad, A.; Ramdani, A.; Zidane, I.; Melhaoui, A. New pyrazolic compounds as cytotoxic agents. *Nat. Prod. Res.* **2007**, *21*, 298–302.
[11] Havrylyuk, D.; Zimenkovsky, B.; Vasylenko, O.; Zaprutko, L.; Gzella, A.; Lesyk, R. Synthesis of novel thiazolone-based compounds containing pyrazoline moiety and evaluation of their anticancer activity. *Eur. J. Med. Chem.* **2009**, *44*, 1396–1404.
[12] Shaharyar, M.; Abdullah, M. M.; Bakht, M. A.; Majeed, J. Pyrazoline bearing benzimidazoles: search for anticancer agent. J. *Eur. J. Med. Chem.* **2010**, *45*, 114–119.
[13] Perchellet, E. M.; Ward, M. M.; Skaltsounis, A. L.; Kostakis, I. K.; Pouli, N.; Marakos, P.; Perchellet, J. H. Antiproliferative and proapoptotic activities of pyranoxanthenones, pyranothioxanthenones and their pyrazole-fused derivatives in HL-60 cells. *Anticancer Res.* **2006**, *26*, 2791–2804.
[14] Koca, I.; Ozgur, A.; Coskun, K. A.; Tutar, Y. Synthesis and anticancer activity of acyl thioureas bearing pyrazole moiety. *Bioorg. Med. Chem.* **2013**, *21*, 3859–3865.
[15] Caliskan, B.; Yilmaz, A.; Evren, I.; Menevse, S.; Uludag, O.; Banoglu, E. Synthesis and evaluation of analgesic, anti-inflammatory, and anticancer activities of new pyrazole-3(5)-carboxylic acid derivatives. *Med. Chem. Res.* **2013**, *22*, 782–793.
[16] Zheng, L. W.; Li, Y.; Ge, D.; Zhao, B. X.; Liu, Y. R.; Lv, H. S.; Ding, J.; Miao, J. Y. Synthesis of novel oxime-containing pyrazole derivatives and discovery of regulators for apoptosis and autophagy in A549 lung cancer cells. *Bioorg. Med. Chem. Lett.* **2010**, *20*, 4766–4770.
[17] Dang, C. T.; Dannenberg, A. J.; Subbaramaiah, K.; Dickler, M. N.; Moasser, M. M.; Seidman, A. D.; D'Andrea, G. M.; Theodoulou, M.; Panageas, K. S.; Norton, L.; et al. Phase II study of celecoxib and transtuzumab in metastatic breast cancer patients who have progressed after prior transtuzumzb-based treatments. *Cancer Res.* **2004**, *10*, 4062–4067.
[18] Canney, P. A.; Machin, M. A.; Curto, J. A feasibility study of the efficacy and tolerability of the combination of exemestane with the COX-2 inhibitor celecoxib in post-menopausal patients with advanced breast cancer. *Eur. J. Cancer* **2006**, *42*, 2751–2756.
[19] Prosperi, R. J.; Robertson, F. M. Cyclooxygenase-2 directly regulates gene expression of P450 Cyp19 aromatase promoter regions pII, pI.3 and pI.7 and estradiol production in human breast tumor cells. *Prostag. Oth. Lipid M.* **2006**, *81*, 55–70.
[20] Singh, B.; Berry, J. A.; Shoher, A.; Lucci, A. COX-2 induces IL-11 production in human breast cancer cells. *J. Surg. Res.* **2006**, *131*, 267–275.
[21] Chow, L. W. C.; Yip, A. Y. S.; Loo, W. T. Y.; Toi, M. Evaluation of neoadjuvant inhibition of aromatase activity and signal transduction in breast cancer. *Cancer Lett.* **2008**, *262*, 232–238.
[22] Chow, L. W.; Cheng, C. W.; Wong, J. L.; Toi, M. Serum lipid profiles in patients receiving endocrine treatment for breast cancer–the results from the Celecoxib Anti-Aromatase Neoadjuvant (CAAN) Trial. *Biomed. Pharmacother.* **2005**, *59*, 302–305.
[23] Brodie, A. M. Aromatase, its inhibitors and their usein breast cancer treatment. *Pharmacol. Ther.* **1993**, *60*, 501–515.
[24] Miller, W. L. Molecular biology of steroid hormone synthesis. *Endocr. Rev.* **1988**, *9*, 295–318.
[25] Insuasty, B.; Tigreros, A.; Orozco, F.; Quiroga, J.; Abonia, R.; Nogueras, M. Sanchez, A.; Cobo, J. Synthesis of novel pyrazolic analogues of chalcones and their 3-aryl-4-(3-aryl-4,5-dihydro-1*H*-pyrazol-5-yl)-1-phenyl-1*H*-pyrazole derivatives as potential antitumor agents. *Bioorg. Med. Chem.* **2010**, *18*, 4965–4974.
[26] Farag, A. M.; Ali, A. K.; El-Debss, M. A.; Mayhoub, A. S.; Amr, A. E.; Abdel-Hafez, N. A.; Abdulla, M. M. Design, synthesis and structure activity relationship study of novel pyrazole-based heterocycles as potential antitumor agents. *Eur. J. Med. Chem.* **2010**, *45*, 5887–5898.
[27] Zhang, D.; Wang, G.; Zhao, G.; Xu, W.; Huo, L. Synthesis and cytotoxic activity of novel 3-(1H-indol-3-yl)-1H-pyrazole-5-carbohydrazide derivatives. *Eur. J. Med. Chem.* **2011**, *46*, 5868–5877.
[28] Modzelewska, A.; Catherine, P.; Geetha, A. and Nancy, E. Anticancer activities of novel chalcone and bis-chalcone derivatives, *Bioorg. Med. Chem. Lett.* **2006**, *14*, 3491–3495.
[29] Ngameni, B.; Kuete, V.; Ambassa, P.; Justin, k.; Marlyse, M. L.; Tchoukoua, A.; Roy, R.; Ngadjui, B. T.; Tetsuya, M. Synthesis and evaluation of anticancer activity of O-allylchalcone derivatives. *Med. Chem.* **2013**, *3*, 233–237.
[30] Juvale, K.; Pape, V. F.; Wiese, M. Investigation of chalcones and benzochal-cones as inhibitors of breast cancer resistance protein. *Bioorg. Med. Chem.* **2012**, *20*, 346–355.
[31] Kamal, A.; Reddy, J. S.; Ramaiah, M. J.; Dastagiri, D.; Bharathi, E. V.; Prem Sagar, M. V. Synthesis and biological evaluation of imidazopyridine/pyrimidine-chalcone derivatives as potential anticancer agents. *Med. Chem. Commun.* **2010**, *1*, 355–360.
[32] Nofal, Z. M.; Soliman, E. A.; Abd El-karim, S. S.; El-zahar, M. I.; Srour, A. M.; Sethumadhavan, S.; Maher, T. J. Novel benimidazole derivatives as expected anticancer agents. *Acta Poloniae Pharm. Drug Res.* **2011**, *68*, 519–534.
[33] Thabit, M. G.; Atta, S. A.; Nasr, M. N. Synthesis and biological evaluation of new 3-(4-subsituted phenyl)aminoquinoxaline derivatives as anticancer agents. *Heterocycl. Commun.* **2015**, *21*, 25–35.
[34] Farag, A. M., Mayhoub, A. S.; Eldebss, M. A.; Amr, A. E.; Ali, A.K.; Abdel-Hafez, N. A.; Abdulla, M. M. Synthesis and structure-activity relationship studies of pyrazole-based heterocycles as antitumor agents. *Arch. Pharm. Chem. Life Sci.* **2010**, *343*, 384–396.
[35] Balbi, A.; Anzaldi, M. Macciò, C.; Aiello, C.; Mazzei, M.; Gangemib, R.; Castagnola, P. Miele, M.; Rosanod, C.; Viale, M. Synthesis and biological evaluation of novel pyrazole derivatives with anticancer activity. *Eur. J. Med. Chem.* **2011**, *46*, 5293–5309.
[36] Nitulescu, G. M.; Draghici, C.; Missir, A. V. Synthesis of new pyrazole derivatives and their anticancer evaluation. *Eur. J. Med. Chem.* **2010**, *45*, 4914–4919.
[37] Moa, W. Y.; Liang, Y. J.; Gu, Y. C.; Fu, L. W.; He, H. W. Synthesis and cytotoxicity of 8-cyano-3-substitutedalkyl-5-methyl-4-methylene-7-methoxy-3,4-dihydropyrido[4,3-d]pyrimidines. *Bioorg. Med. Chem. Lett.* **2011**, *21*, 5975–5977.
[38] Ghorab, M. M.; El-Gazzar, M. G.; Alsaid; M. S. Synthesis, characterization and anti-breast cancer activity of new 4-aminoantipyrine-based heterocycles. *Int. J. Mol. Sci.* **2014**, *15*, 7539–7553.
[39] Ansari, F. L.; Iftikhar, F.; Ul-Haq, I.; Mirza, B.; Baseer, M.; Rashid, U. Solid-phase synthesis and biological evaluation of a parallel library of 2,3-dihydro-1,5-benzothiazepines. *Bioorg. Med. Chem.* **2008**, *16*, 7691–7697.
[40] Bariwal, J. B.; Upadhyay, K. D.; Manvar, A. T.; Trivedi, J. C.; Singh, J. S.; Jain, K. S.; Shah, A. K. 1,5-Benzothiazepine, a

versatile pharmacophore: a review. *Eur. J. Med. Chem.* **2008**, *43*, 2279–2290.
[41] Viegas, J. C.; Danuello, A.; Silva, B. V.; Barreiro, E. J.; Fraga, C. A. Molecularhybridization: a useful tool in the design of new drug prototypes. *Curr. Med. Chem.* **2007**, *14*, 1829–1852.
[42] Walsh, J. J.; Bell, A. Hybrid drugs for malaria. *Curr. Pharm. Des.* **2009**, *15*, 2970–2985.
[43] Anand, N.; Singh, P.; Sharma, A.; Tiwari, S.; Singh, V.; Singh, D. K. Synthesis and evaluation of small libraries of triazolylmethoxy chalcones, flavanones and 2-aminopyrimidines as inhibitors of mycobacterial FAS-II and PknG. *Bioorg. Med. Chem.* **2012**, *20*, 5150–5163.
[44] Yoneda, F.; Tsukuda, K.; Kawazoe, M.; Sone, A. Synthesis and properties of 1-benzothiopyrano[2,3-*o*]-pyrimidine-2,4-(3*H*)-diones (10-thia-5-deazaflavins). *J. Heterocycl. Chem.* **1981**, *18*, 1329–1333.
[45] Chan, W. L.; Ho, D. D.; Lau, C. P.; Wat, K. H.; Kong, Y. C.; Cheng, K. F.; Wong, T. T.; Chan, T. Y. Structure function relationship study of yuehchukene. I. Anti-implantation and estrogenic activities of substituted yuehchukene derivatives. *Eur. J. Med. Chem.* **1991**, *26*, 387–390.
[46] Pool, W. O.; Harwood, H. J.; Ralston, A. W. 2-Alkyl benzimidazoles as derivatives for identification of aliphatic acids. *J. Amer. Chem. Soc.* **1937**, *1*, 178–179.
[47] Albogami, A. S.; Karama, U.; Mousa, A. A.; khan, M. Simple and efficient one step synthesis of functionalized flavanones and chalcones. *Orient. J. Chem.* **2012**, *28*, 619–626.
[48] Skehan, P.; Storeng, R.; Scudiero, D.; Monks, A.; McMahon, J.; Vistica, D.; Warren, J. T.; Bokesch, H.; Kenney, S.; Boyd, M. R. New colorimetric cytotoxicity assay for anticancer drug screening. *Natl. Cancer Inst.* **1990**, *82*, 1107–1112.
[49] Vichai, V.; Kirtikara, K. Sulforhodamine B colorimetric assay for cytotoxicity screening. *Nat. Protoc.* **2006**, *1*, 1112–1116.
[50] Molecular Operating Environment (MOE), 2013.08; Chemical Computing Group Inc., 1010 Sherbooke St. West, Suite #910, Montreal, QC, Canada, H3A 2R7, 2015.
[51] Liu, X.; Ouyang, S.; Yu, B.; Huang, K.; Liu, Y.; Gong, J.; Zheng, S.; Li, Z.; Li, H.; Jiang, H. PharmMapper Server: a web server for potential drug target identification via pharmacophore mapping approach. *Nucleic Acids Res.* **2010**, *38*, 609–614.
[52] Wright, L.; Barril, X.; Dymock, B.; Sheridan, L.; Surgenor, A.; Beswick, M.; Drysdale, M.; Collier, A.; Massey, A.; Davies, N.; et al. Structure-activity relationships in purine-based inhibitor binding to HSP90 isoforms. *Chem. Biol.* **2004**, *11*, 775–785.
[53] Genin, M. J.; Biles, C.; Keiser, B. J.; Poppe, S. M.; Swaney, S. M.; Tarpley, W. G.; Yagi, Y.; Romero, D. L. Novel 1,5-diphenylpyrazole non nucleoside HIV-1 reverse transcriptase inhibitors with enhanced activity versus the delavirdine-resistant P236L mutant: lead identification and SAR of 3- and 4-subsituted derivatives. *J. Med. Chem.* **2000**, *43*, 1034–1039.
[54] Finar, I. L.; Manning, M. The Preparation and Some Reactions of 4-Formyl-1-phenylpyrazoles. *J. Chem. Soc.* **1961**, 2737–2741.