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Original article

Design, synthesis and structure–activity relationship study of novel pyrazole-based heterocycles as potential antitumor agents

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Structure–activity relationship (SAR)

ABSTRACT

The versatile *hitherto* unreported 3-[(*E*)-3-(dimethylamino)acryloyl]-1,5-diphenyl-1*H*-pyrazole-4-carbonitrile (**3**) was prepared *via* the reaction of 3-acetyl-1,5-diphenyl-1*H*-pyrazole-4-carbonitrile (**1**) with dimethylformamid-dimethylacetal (DMF-DMA). The latter product and 3-((*E*)-3-morpholin-4-yl-acryloyl)-1,5-diphenyl–1*H*-pyrazole-4-carbonitrile (**4**) underwent regioselective 1,3-dipolar cycloaddition with nitrilimines to afford the corresponding pyrazole derivatives. *In vivo* anti-estrogenic activity and acute toxicity after single oral dose of the newly synthesized compounds were evaluated. *In vitro* disease-oriented primary antitumor screening utilizing 14 cell lines of breast and ovarian tumor subpanels has been also carried out. All tested compounds showed anti-estrogenic properties equipotent or superior to the reference drug, letrozole. 3-[3-(4-Cyano-1,5-diphenyl-1*H*-pyrazole-3-yl)-1-(4-methylphenyl)-1*H*-pyrazole-4-carbonyl]-1,5-diphenyl-1*H*-pyrazole-4-carbonitrile (**27c**) and 3-(3-acetyl-1-phenyl-1*H*-pyrazole-4-carbonyl)-1,5-diphenyl-1*H*-pyrazole-4-carbonitrile (**8a**) showed a significant cytotoxic activity in a nanomolar range against certain types of breast and ovarian tumors with tolerable toxicity.

1. Introduction

Celecoxib (Fig. 1a) is a non-steroidal anti-inflammatory (NSAID) drug approved for treatment of the signs and symptoms of osteoarthritis, rheumatoid arthritis, management of acute pain, treatment of primary dysmenorrhea and to reduce the number of adenomatous colorectal polyps in Familial Adenomatous Polyposis (FAP). New indication of celecoxib was also approved for treatment of signs and symptoms of ankylosing spondylitis [1]. It was categorized as selective cyclooxygenase-2 (COX-2) inhibitor.

On the other hand, several authors have reported the inhibitory effects of celecoxib on breast cancer [2–6], recurrent malignant glioma [7], advanced non-small cell lung cancer [8–10], refractory multiple myeloma [11], advanced colorectal cancer [12] and advanced pancreatic cancer [13].

It was proved that celecoxib exerts its antitumor action *via* inhibition of aromatase, an enzyme complex consisting of a cytochrome P-450 hemoprotein and a flavoprotein, which converts C-19 androgens, such as testosterone and androstenedione, to C-18 estrogen such as estradiol and estrone [14–17]. Therefore, it is useful in controlling the progression of tumors with estrogen receptors; i.e. breast and ovarian tumors.

In continuation of our recent work aiming at the synthesis of a variety of heterocyclic ring systems with remarkable biological importance [18–28] and that related to the structure of celecoxib and other 1,5-diphenylpyrazole with known antineoplastic effects [29], we report here our procedure for a regioselective synthesis of a series of pyrazole-based heterocyclic ring systems with a new substitution pattern which could reinforce the interaction of 1,5-diphenylpyrazole skeleton with aromatase enzyme (Fig. 1c).

All the newly synthesized compounds were designed to contain cyano group at C4 of the pyrazole ring, as it is common in different aromatase inhibitors such as anastrozole [30,31], fadrozole [32] and letrozole [33,34] (Fig. 1c). In addition, the bulky polycyclic structures at C1, 3 and 5 of the pyrazole ring provide favorable

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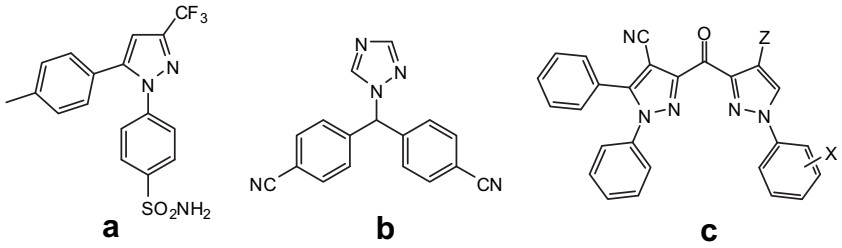


Fig. 1. Heterocyclic ring systems related to pyrazoles.

interaction with the hydrophobic residues in the largely nonpolar active sites of the cytochrome isozymes [35].

2. Results and discussion

2.1. Chemistry

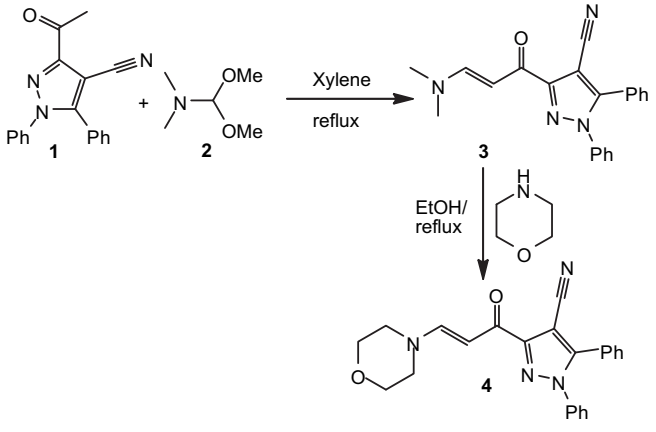
In the course of our investigation, we have found that the 3-[(*E*)-3-(dimethylamino)acryloyl]-1,5-diphenyl-1*H*-pyrazole-4-carbonitrile (**3**) is an excellent building block for the synthesis of a variety of heterocyclic ring systems incorporating a pyrazole moiety. The enaminone derivative **3** was obtained from the reaction of 3-acetyl-1,5-diphenyl-1*H*-pyrazole-4-carbonitrile (**1**) with dimethylformamid-dimethylacetal (DMF-DMA) (Scheme 1). The structure of compound **3** was confirmed by its elemental analysis and spectral data (see Section Experimental).

Treatment of the enaminone **3** with morpholine under reflux condition affords 3-((*E*)-3-morpholin-4-yl-acryloyl)-1,5-diphenyl–1*H*-pyrazole-4-carbonitrile (**4**) (Scheme 1). The IR spectrum of the latter product exhibited characteristic bands at 1680 and 2230 cm^{-1} corresponding to a carbonyl and a nitrile group respectively. Its mass spectrum revealed a peak at *m*/*z* 384 corresponding to its molecular ion.

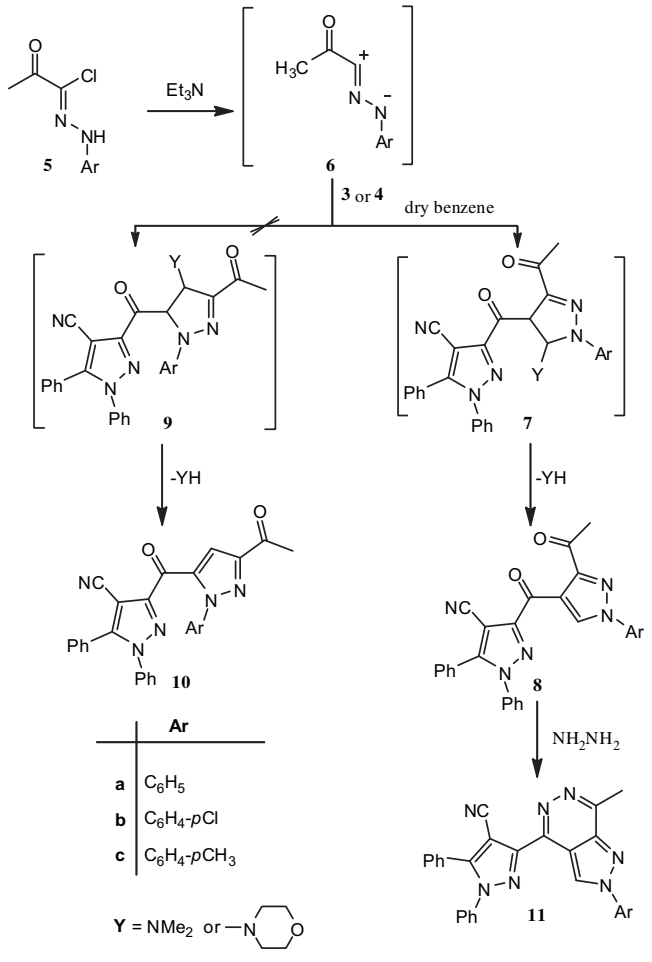
When either compound **3** or **4** was allowed to react with the nitrilimines **6**, **13**, **18**, **22** and **26** [liberated *in situ* from the corresponding hydrazonyl halides **5**, **12**, **17**, **21** and **25**, respectively, with triethylamine in refluxing benzene], it afforded the new pyrazole derivatives **8a–c**, **14a–c**, **19a–c**, **23a–c** and **27a–c**, respectively (Schemes 2–7).

Thus, nitrilimines **6a–c** [generated *in situ* by the action of triethylamine on 2-oxo-*N*-arylpropane hydrazonyl chlorides **5a–c**] reacted with either compound **3** or **4**, in refluxing benzene, and afforded, in each case, only one isolable product. The reaction products were identified as the pyrazole structures **8a–c** (Scheme 2). The latter products were assumed to be formed *via* initial 1,3-dipolar cycloaddition of the nitrilimines **6a–c** to the activated double bond in compound **3** or **4** to afford the non-isolable cycloadducts **7a–c** which undergoes loss of dimethylamine or morpholine molecule yielding the final pyrazole derivatives **8a–c**.

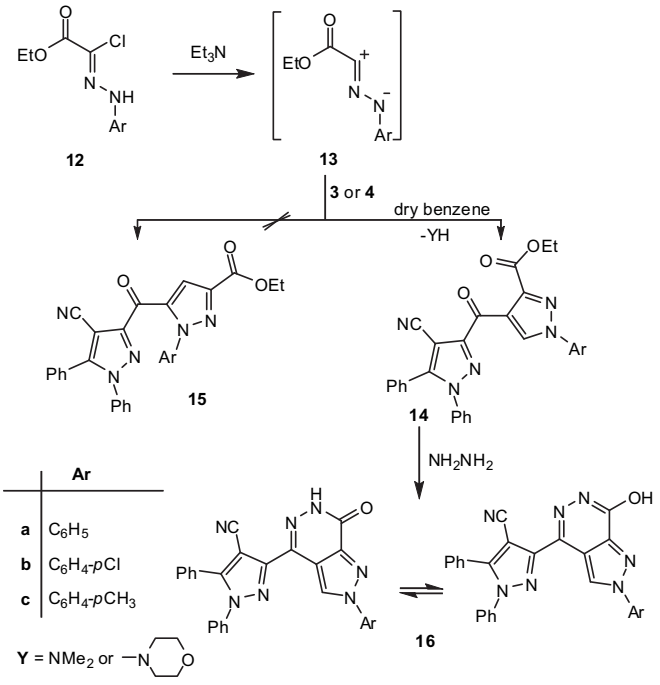
The other possible regioisomer **9** was excluded on the basis of the spectral data of the isolated products. For example, in the pyrazole ring system, C-4 is the most electron-rich carbon, thus H-4 is expected to appear in the NMR spectra at higher field, typically near 6.0 ppm. On the other hand, H-5 is linked to the carbon attached to a nitrogen atom and thus it is deshielded to appear near 8.0 ppm. The ^{1}H NMR spectra of the isolated products **8a–c** revealed, in each case a singlet signal in the region of 8.54–8.67 which indicates the presence of the pyrazole H-5 rather than H-4.



Scheme 1.



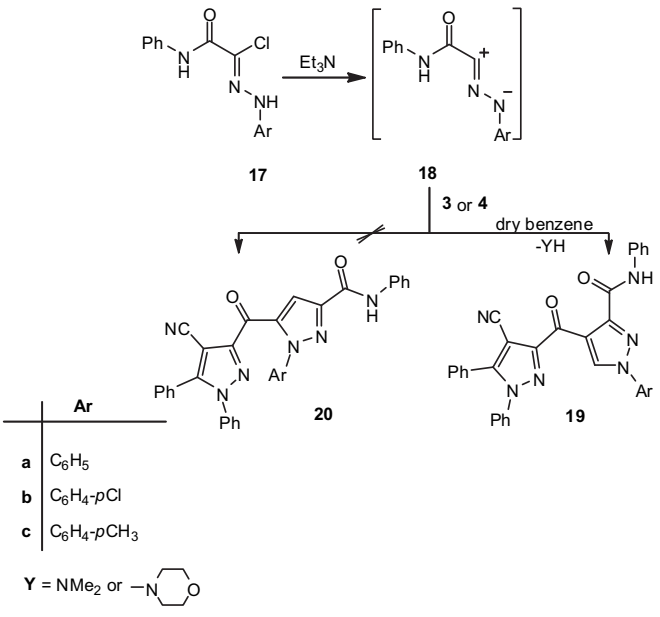
Scheme 2.



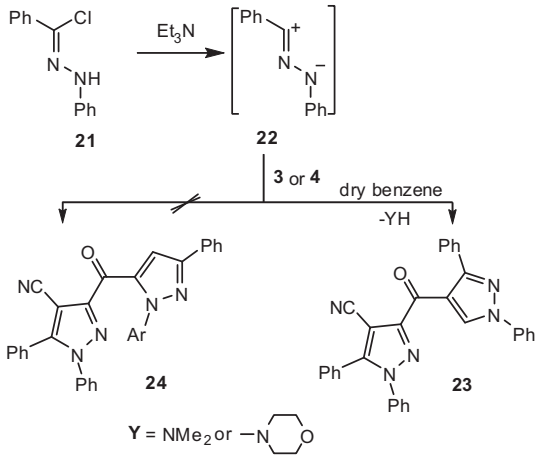
Scheme 3.

This conclusion was further confirmed chemically by the reaction compounds **8a**–**c** with hydrazine hydrate, to afford fused pyrazolopyridazine ring systems **11a**–**c** (Scheme 2).

Similarly, when of the enaminone **3** or the morpholinyl derivative **4** was treated with the nitrilimine **13** [generated *in situ* by the action of triethylamine on the hydrazonyl chlorides **12a**–**c**] they afforded the corresponding pyrazole derivatives **14a**–**c** (Scheme 3). The structures of the isolated products **14a**–**c** were established on the basis of their elemental analysis and spectral data. For example, the ^{1}H NMR spectra of compounds **14a**–**c** displayed, in each case, the pyrazole H-5 in the region of 8.61–8.67 ppm which is in accordance with the proposed structure. Moreover, the IR spectra



Scheme 4.

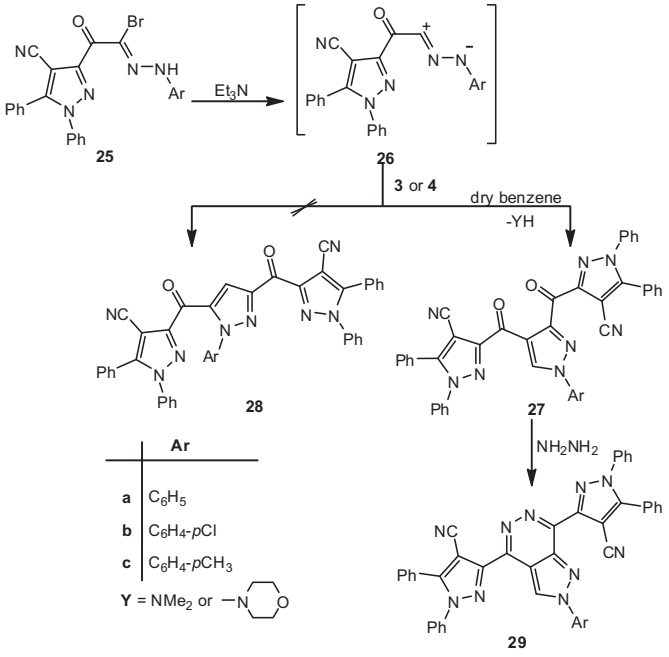


Scheme 5.

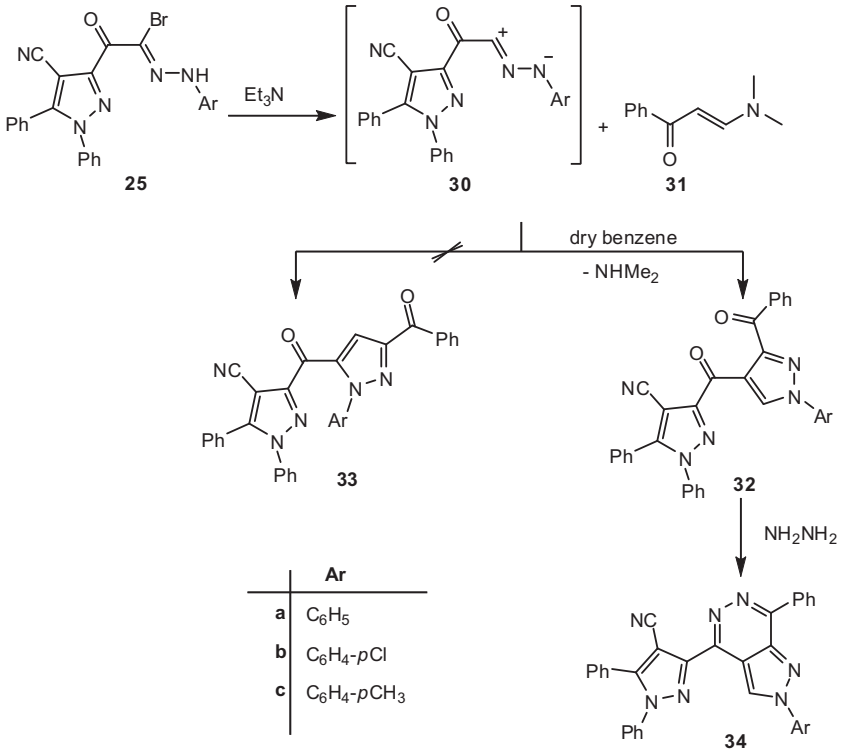
of the isolated products **14a**–**c** showed, in each case, two strong absorption bands in the region 1720–1660 cm^{-1} corresponding to two carbonyl groups. A further confirmation of the proposed structure came from the reaction of products **14a**–**c** with hydrazine hydrate, in refluxing ethanol, which afforded the corresponding fused ring systems **16a**–**c**. The structures of the products **16a**–**c** were confirmed on the basis of their elemental analysis and spectral data (see Section Experimental).

In a similar manner, phenylcarbamolyl-*N*-arylnitrilimines **18a**–**c** (liberated *in situ* from phenylcarbomoylhydrazonoyl chlorides **17a**–**c** by the action of triethylamine) react with the enaminone derivative **3** or **4** to furnish the corresponding pyrazole derivatives **19a**–**c** (Scheme 4).

The IR spectra of the isolated products **14a**–**c** exhibited, in each case, two strong absorption bands near 1650 and 1690 cm^{-1} corresponding to two carbonyl groups. The ^{1}H NMR spectrum of compound **19a**, taken as a atypical example of the prepared series, revealed a signal corresponding to pyrazole H-5 proton at 8.50 ppm in addition to an aromatic multiplet in the region 7.20–7.37 ppm



Scheme 6.



Scheme 7.

and a signal at 10.80 ppm (D_2O-exchangeable) characteristic for NH proton.

Similarly, when the enaminone **3** or **4** was treated with nitrileimine **22** [generated *in situ* by the action of triethylamine on *N*-phenylbenzohydrazonoyl chloride **21**], it afforded 3-(1,3-diphenyl-1*H*-pyrazole-4-carbonyl)-1,5-diphenyl-1*H*-pyrazole-4-cabonitrile (**23**) (Scheme 5).

The mass spectrum of the product **23** exhibited a peak corresponding to its molecular ion at *m*/*z* 491 and its ^{1}HNMR spectrum revealed a singlet signal at 8.60 ppm corresponding to pyrazole H-5 proton, in addition to an aromatic multiplet in the region 7.20–7.37 ppm.

Treatment of the enaminone derivative **3** or **4** with the nitrileimine derivatives **26a**–**c** [generated *in situ* by the action of triethylamine on the pyrazole hydrazonyl bromides **25a**–**c**] afforded 3-[3-(4-cyano-1,5-diphenyl-1*H*-pyrazole-3-yl)-1-aryl-1*H*-pyrazole-4-caronyl]-1,5-diphenyl-1*H*-pyrazole-4-carbonitriles **27a**–**c** (Scheme 6).

When the products **27a**–**c** were treated with hydrazine hydrate, they afforded the corresponding 3-[7-(4-cyano-1,5-diphenyl-pyrazole-3-yl)-2-aryl-2*H*-pyrazolo[3,4-*d*]pyridazin-4-yl]-1,5-diphenyl-1*H*-pyrazole-4-carbonitrile derivatives **29a**–**c**. The IR spectra of isolated products **29a**–**c** showed, in each case, the disappearance of bands corresponding to carbonyl groups.

The nitrilimine intermediates **26a**–**c** react also with *N*,*N*-dimethylamino-1-phenylpropenone (**31**) to afford the corresponding 3-(4-benzoyl-1-aryl-1*H*-pyrazole-3-carbonyl)-1,5-diphenyl-1*H*-pyrazole-4-carbonitriles **32a**–**c**. Treatment of the later products with hydrazine hydrate, afforded the corresponding **34a**–**c** (Scheme 7).

2.2. Pharmacology and toxicology

2.2.1. Anti-estrogenic activity

The anti-estrogenic activity of the synthesized compounds and letrozole (Femara®), as a reference drug, was evaluated in the Pharmacology and Toxicology Department of National Research Center, Dokki, Egypt.

In the used method, the increase of uterine weight in castrated female rats, induced by repeated administration of estradiol, is antagonized by anti-estrogenic compounds. The test compounds (>99% pure) in carboxymethylcellulose (CMC) are administered orally by gavage to groups of immature ovarectomized rat. On the 8th day the animals are sacrificed and the uterine weights were determined.

The mean value of % reduction in uterine weight was calculated relative to control group, which was treated with estradiol alone, and relative potency to reference drug was calculated as depicted in Table 1.

2.2.2. Acute toxicity (LD_{50})

The rats were dosed by oral gavage with different doses of aqueous suspensions of a very fine powder of the tested compounds. Under the conditions of these gavage studies, there was a great safety for albino rates in comparison with letrozole as a reference drug as shown in Table 1.

The result showed that, the anti-estrogenic effect of all tested compounds was found similar or superior to those of reference drug (Table 1 and Fig. 2). However, the safety varied, some are safer others are more toxic than letrozole (Table 1 and Fig. 3). The type of function group at C4 in the pyrazole ring B (Fig. 4) is of great influence on both anti-estrogenic pharmacological effect and acute toxicity of the tested compound. Compounds that carry phenyl-carbamoyl moiety were found 1.6 times to be more potent than the reference drug (Table 1 and Fig. 3). Removal of NH from such group has dramatic increase in potency with the *p*-chloro derivative as shown in compound **32c** (Table 1 and Fig. 2). In contrast, removal of the carbamoyl moiety decreased the anti-estrogenic activity as shown in compound **23** (Table 1 and Fig. 2). Also, chlorinated derivatives are more active than non-halogenated derivatives as shown in compounds **14c**, **19c** and **32c** (Table 1 and Fig. 2).

Table 1
Mean anti-estrogenic activity and acute toxicity (LD_{50}) of the synthesized compounds and letrozole*.

Compound	% Reduction in uterine weight[a]	Relative potency to letrozole[b]	LD_{50} (mg/kg)[c]
Letrozole	21.65	1	252.61 ± 0.11
3	41.98	1.93	231.90 ± 0.14
4	43.43	2	463.11 ± 0.18
8a	32,45	1.49	323.71 ± 0.11
11a	23.97	1.1	32.54 ± 0.15
14a	23.98	1.1	121.73 ± 0.11
14b	34.86	1.61	142.69 ± 0.12
14c	42.83	1.96	273.61 ± 0.11
16a	32.86	1.51	114.18 ± 0.14
16b	23.76	1.09	322.00 ± 0.10
19b	34.87	1.61	101.34 ± 0.13
19c	34.65	1.60	121.91 ± 0.14
23	23.54	1.08	121.91 ± 0.14
27c	32.43	1.49	211.19 ± 0.13
29a	34.87	1.61	222.00 ± 0.10
29b	43.41	2	111.52 ± 0.12
32b	23.87	1.1	552.81 ± 0.16
32c	43,98	2.16	452.81 ± 0.14
34a	32.59	1.5	13 1.9 ± 0.14
34c	21.87	1.01	452.81 ± 0.12

*Statistical comparison of the difference between estradiol control group and treated groups was done by one-way ANOVA and Duncan's multiple comparison test $^{*}P < 0.05$.
[a] Value represents mean of twelve rats.
[b] Potency was calculated as regards the percentage change of the letrozole.
[c] Value represents mean values ± SE of five mice per group.

Compounds that carry ethyl carboxylate, phenylcarbamoyl or phenyl moieties at C4 of the pyrazole ring B (Fig. 4) are more toxic than letrozole; while those carrying acetyl or phenylcarbonyl moieties are safer than the reference drug (Table 1 and Fig. 3).

2.2.3. In vitro disease-oriented primary antitumor screening

Twenty samples of the newly synthesized compounds were selected by the National Cancer Institute (NCI), Bethesda, Maryland, USA, to evaluate their *in vitro* antitumor activity using 14 breast and ovarian cell lines. The results revealed high degree of antitumor selectivity against both utilized breast and ovarian cancer cell lines (Tables 2 and 3). In other words, compounds that revealed good effect against breast tumor cell lines such as **4**, **8a**, **11a**, **14a**, **16a**, **19a**, **29b** and **32b** (Table 2) have a moderate to weak activity against ovarian tumor cell lines (Table 3). In contrast, compounds such as **14c**, **16b**, **29a**, **32c** and **34c** showed remarkable activity against ovarian tumors cell lines and a moderate to weak activity against breast tumor cell lines (Tables 2 and 3). However, compounds **27c** and **34b** exhibited an excellent activity against both tumor cell lines (Tables 2 and 3). With exception of compound **27c**, all chloro derivatives either excluded by NCI or revealed weak activity against breast tumor cell lines (Table 2).

2.2.3.1. Effect of the newly synthesized compounds on breast cancer. Detailed interpretation of the obtained results showed that compound **8a** has activity against MiDA-N tumor cell line in nanomolar range (GI_{50} value is 77 nM) (Table 2). The enaminone **4** and the pyrazole ethyl ester **14a** are nearly equipotent with almost the same range of activity against MCF-7 tumor cell line (GI_{50} values are 5.49 μM) (Table 2). Compounds **8a**, **11a** and **19a** showed remarkable activity against HS-578T cell line (GI_{50} values are 6.4, 1.4 and 8.8 μM, respectively) (Table 2). GI_{50} of compound **16a** is less than 3 μM against T-47D and NCI/ADR-RES cell lines (Table 2). Both phenylcarbamoyl **19a** and its *p*-tolyl derivatives and **19b** showed GI_{50} against MDA/MB-435 cell lines around 2.1 μM (Table 2). Compound **19b** revealed good activity against MiDA-N cell line (Table 2). Removal of NH or carbamoyl moieties from the latter compound is associated with an increase in potency against the same cell line as shown in compounds **23** and **32b** (GI_{50} values are 5.5, 3.3 and 2.7 μM, respectively) (Table 2). BT 549 tumor cell line was found to be sensitive to compounds **23**, **27c** and **29b** (GI_{50} values are 1.1, 2.4 and 1.0 μM, respectively) (Table 2).

Replacement of phenyl ring in compounds **19** with 4-cyano-1,5-dipehnylpyrazolyl moiety as in case of compound **27c** produces the most active member in this study which revealed antitumor activity in a nanomolar range against MCF-7 tumor cell line (GI_{50} value is 61 nM) (Table 2).

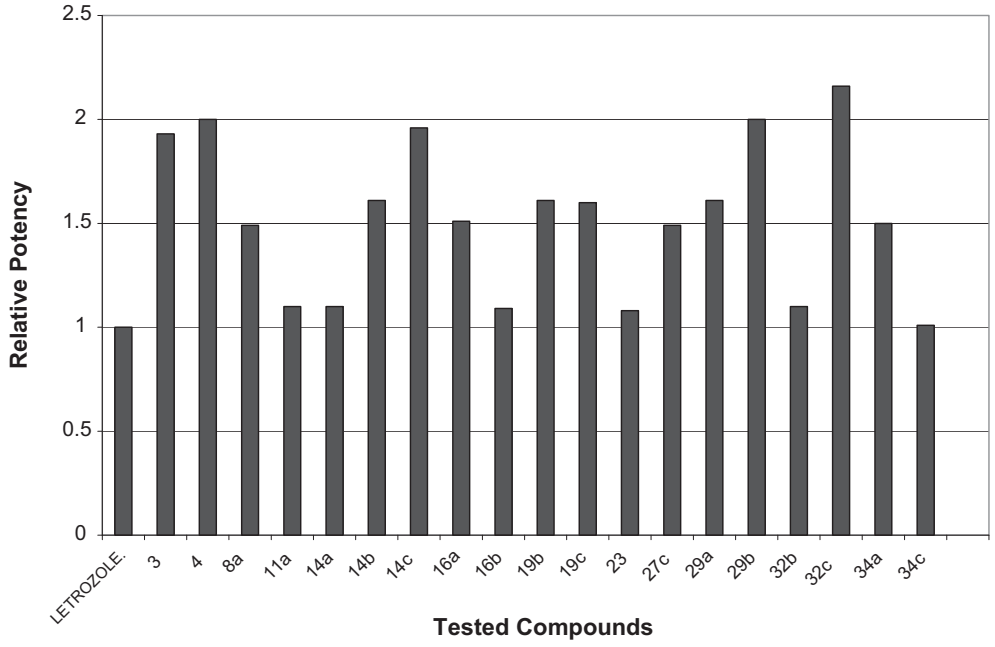


Fig. 2. Anti-estrogenic potency of the tested compounds relative to letrozole.

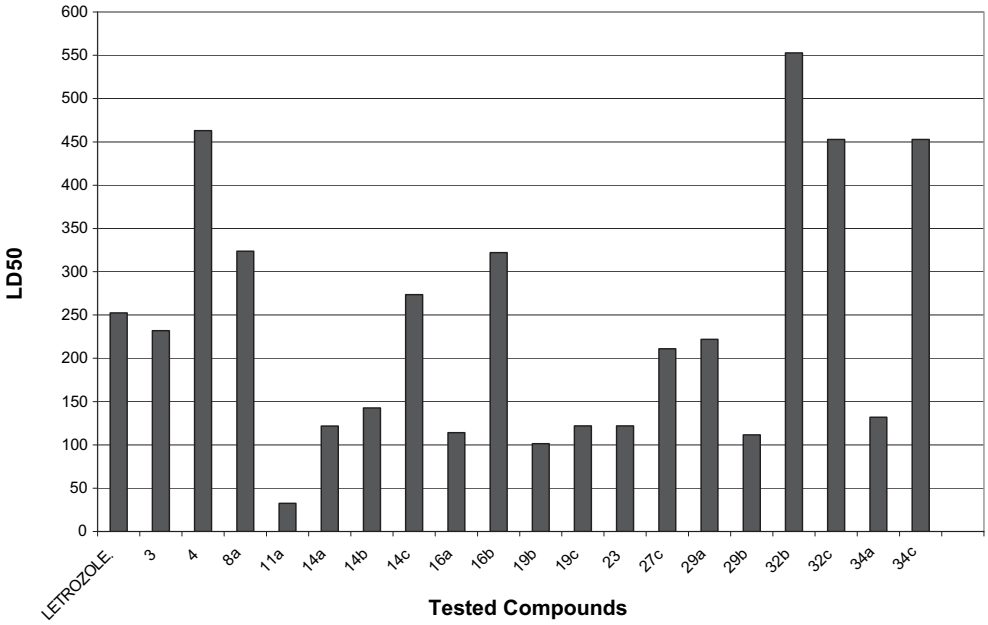


Fig. 3. LD_{50} of tested compounds and letrozole after single oral dose.

The benzoyl derivative **32b** showed remarkable activity against six tumor cell lines (GI_{50} values between 2.6 and 7.1 μM) (Table 2), while its *p*-chloro isostere **32c** has no significant antitumor activity against the same breast tumor cell lines (Table 2). The pyridazine derivative **34b** maintained the activity against MDA/MB. 231 ATTC, NCI/ADR-RES and MDA/MB-435 tumor cell lines with little variations (Table 2). At the same time, it increased the cytotoxic activity against MCF-7, HS-578T and BT 549 tumor cell lines. Finally, the cytotoxic activity was decreased against T-47D, MDA/MB-435 and MiDA-N tumor cell lines (Table 2). The *p*-chloro derivative **34c** showed weak activity against all tested tumor cell lines (Table 2).

2.2.3.2. Effect of the newly synthesized compounds on ovarian cancer. In general, the effects of the tested compounds against ovarian tumor cell lines are much less than their effect on breast tumor cell lines (Table 3). However, compound **27c** has an excellent effect in a nanomolar range against IGROVI ovarian tumor cell line (GI_{50} value is 22 nM) (Table 3). Enaminones **3**, **4** and acetylpyrazole derivatives **8** have a moderate to weak activity against different ovarian tumor cell lines (Table 3). The *p*-chloro derivative **14c** revealed good activity against OVCAR-4 and OVCAR-8 tumor cell lines (GI_{50} values are 5.4 and 9.1 μM, respectively) (Table 3). Replacement of ethoxy group with phenyl moiety maintains the antitumor activity against OVCAR-4 tumor cell line (GI_{50} value id 5.4) as shown in compound **32c** (Table 3).

The hydroxypyridazine derivative **16b** showed good activity against OVCAR-4 and OVCAR-8 tumor cell lines (GI_{50} value are 3.2 and 4.3 μM, respectively) (Table 3). The increase of lipophilicity of the latter compound, by replacement of hydroxy group with phenyl moiety, maintains GI_{50} values against OVCAR-4 and OVCAR-8 cell lines below 10 μM, in addition to an increase in cytotoxic activity against OVCAR-5 cell line as shown in compound **34b** (Table 3).

In addition, compound **23** showed GI_{50} below 10 μM against used OVCAR tumor cell lines. The broad spectrum activity was observed in both compounds **19b** and **29a** since they possess remarkable activity against all tested ovarian cell lines (Table 3).

Finally, compound **34c** revealed significant activity against all used tumor cell lines except IGROVI (Table 3).

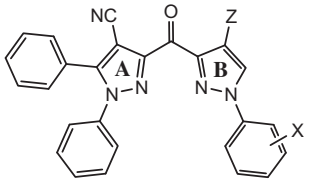


Fig. 4. Compounds carrying ethyl carboxylate, phenylcarbamoyl or phenyl moieties at C4 of the pyrazole ring.

3. Conclusion

The objective of the present study is the synthesis and evaluation of two pharmacological activities, in addition to acute toxicity study. The first is *in vivo* investigation of anti-estrogen activity. The second is *in vitro* investigation of the antitumor activity on breast and ovarian cell lines of new 1,5-diphenylpyrazoles incorporating cyano pharmacophore group, structurally related to well-documented antineoplastic agents. This aim has been verified by the synthesis of hybrid compounds comprising the above-mentioned pharmacophores substituted essentially with a carbonylphenylpyrazole counterpart at the pyrazole-C3 moiety having the general structures shown in Fig. 1c.

The highest active derivative in present study is compound **27c** which showed significant cytotoxic activity in a nanomolar range against certain types of breast and ovarian tumors with tolerable toxicity ($LD_{50} > 200$ mg/kg). In addition, its anti-aromatase activity was found one and half times more than letrozole.

The oral LD_{50} values in this study revealed that most of the tested compounds are relatively nontoxic, the LD_{50} of 11 compounds were found more than 200 mg/kg.

Finally, the remarkable antitumor activity associated with significant anti-aromatase activity and relatively high safety margin displayed by these compounds will be of interest for future structure optimization with the hope of finding more active and selective antitumor agents.

Table 2
Growth inhibitory concentration (GI_{50}, μM) of tested compounds on different Breast Cancer's cell lines.

Compound	Cell lines							
	MDA, MB. 231, ATTC	MCF-7	T-47D	NCI, ADR-RES	HS-578T	MDA, MB-435	MiDA-N	BT 549
3	50.4	39.8	30.2	90.6	I.A.[a]	I.A.[a]	45.4	31.1
4	I.A.[a]	5.49	I.A.[a]	I.A.[a]	I.A.[a]	I.A.[a]	38.4	41.4
8a	I.A.[a]	25.7	36.8	I.A.[a]	6.46	I.A.[a]	0.077	I.A.[a]
11a	I.A.[a]	8.46	9.14	I.A.[a]	1.39	I.A.[a]	38.4	96.8
14a	N.T.[b]	5.49	I.A.[a]	I.A.[a]	N.T.[b]	I.A.[a]	38.4	31.8
14b	I.A.[a]	I.A.[a]	I.A.[a]	I.A.[a]	65.0	71.8	62.3	39.8
14c	I.A.[a]	I.A.[a]	I.A.[a]	I.A.[a]	I.A.[a]	I.A.[a]	I.A.[a]	I.A.[a]
16a	6.21	I.A.[a]	2.95	2.76	46.8	21.8	13.8	44.8
16b	I.A.[a]	I.A.[a]	I.A.[a]	I.A.[a]	I.A.[a]	I.A.[a]	I.A.[a]	I.A.[a]
19a	I.A.[a]	I.A.[a]	33.0	I.A.[a]	8.84	2.03	I.A.[a]	11.8
19b	6.27	34.4	30.8	2.48	63.2	2.23	5.49	I.A.[a]
23	49.8	33.3	28.0	98.0	I.A.[a]	61.0	3.36	1.12
27c	I.A.[a]	0.061	25.7	I.A.[a]	37.4	I.A.[a]	26.8	2.47
29a	52.4	17.8	81.0	24.5	94.4	I.A.[a]	I.A.[a]	11.8
29b	7.90	9.42	16.0	7.46	9.72	9.20	2.26	1.02
32b	6.12	I.A.[a]	7.18	2.58	I.A.[a]	2.65	2.74	I.A.[a]
32c	I.A.[a]	I.A.[a]	I.A.[a]	I.A.[a]	I.A.[a]	I.A.[a]	I.A.[a]	I.A.[a]
34a	8.84	9.53	21.8	I.A.[a]	31.8	31.8	31.2	21.8
34b	6.42	43.8	38.0	2.28	16.8	23.8	63.8	38.8
34c	52.1	41.6	42.6	74.7	38.6	46.3	I.A.[a]	I.A.[a]
Letrozole	0.15	0.7	0.44	1.16	2.17	0.067	1.61	0.89

[a] I.A., inactive; GI_{50} value > 100 μM.
[b] N.T., not tested.

4. Experimental

4.1. Chemistry

4.1.1. General

All melting points were measured on a Gallenkamp melting point apparatus (Weiss-Gallenkamp, London, UK). The infrared spectra were recorded in potassium bromide disks on a pye Unicam SP 3300 and Shimadzu FT IR 8101 PC infrared spectrophotometers (Pye Unicam Ltd. Cambridge, England and Shimadzu, Tokyo, Japan, respectively). The NMR spectra were recorded on a Varian Mercury VX-300 NMR spectrometer (Varian, Palo Alto, CA, USA). ^{1}H spectra were run at 300 MHz and ^{13}C spectra were run at 75.46 MHz in deuterated chloroform ($CDCl_3$) or dimethyl sulphoxide (DMSO-d_6). Chemical shifts are given in parts per million and were related to that of the solvent. Mass spectra were recorded on a Shimadzu GCMS-QP 1000 EX mass spectrometer (Shimadzu) at 70 eV. Elemental analyses were carried out at the Micro-analytical Center of Cairo University, Giza, Egypt.

2-Oxo-*N*-arylpropanehydrazonoyl chlorides **5a**–**c** [36], ethyl 2-(2-arylhydrazono)-2-chloroaceatate derivatives **12a**–**c** [37] phenylcarbamoylarylhydrazonoyl chlorides **17a**–**c** [38], *N*-phenylbenzenecarbohydrazonyl chloride (**21**) [39], 3-acetyl-1,5-diphenyl-1*H*-pyrazole-4-carbonitrile (**1**) and 4-cyano-α-oxo-1,5-diphenyl-*N*-aryl-1*H*-pyrazole-3-ethanehydrazonoyl bromide derivatives **25a**–**c** [40], and 3-dimethylamino-1-phenylpropenone (**31**) [41] were prepared following the procedures reported in the literature.

Table 3
Growth inhibitory concentration (GI_{50}, μM) of tested compounds on different ovarian cancer's cell lines.

Compound	Cell lines					
	IGROVI	OVCAR-3	OVCAR-5	OVCAR-8	OVCAR-4	SK-OV-3
3	98.0	98.8	I.A.[a]	75.8	63.8	23.8
4	88.0	21.5	90.8	I.A.[a]	62.8	33.8
8a	96.8	66.8	34.8	62.8	69.8	39.8
11a	92.0	96.8	I.A.[a]	I.A.[a]	56.4	36.8
14a	I.A.[a]	N.T.[b]	N.T.[b]	I.A.[a]	I.A.[a]	N.T.[b]
14b	93.2	I.A.[a]	61.2	65.8	45.8	30.8
14c	94.8	91.8	I.A.[a]	9.18	5.46	I.A.[a]
16a	92.2	I.A.[a]	61.8	61.5	26.38	36.8
16b	91.34	33.4	32.8	3.23	4.38	30.8
19a	99.5	23.8	I.A.[a]	I.A.[a]	28.4	16.8
19b	16.6	7.66	9.72	9.52	7.56	9.42
23	I.A.[a]	4.94	6.23	9.83	5.49	I.A.[a]
27c	0.022	99.8	36.8	20.3	30.4	38.8
29a	13.3	7.26	9.12	9.82	7.46	5.72
29b	98.0	95.8	I.A.[a]	61.8	43.8	36.8
32b	I.A.[a]	38.4	36.8	I.A.[a]	63.8	36.8
32c	I.A.[a]	63.8	36.8	I.A.[a]	5.49	I.A.[a]
34a	94.8	93.8	I.A.[a]	68.0	63.0	32.8
34b	93.4	I.A.[a]	8.94	9.23	5.49	I.A.[a]
34c	93.4	I.A.[a]	7.94	9.23	7.66	9.72
Letrozole	0.095	5.87	1.62	4.50	0.88	1.09

[a] I.A., inactive; GI_{50} value > 100 μM.
[b] N.T., not tested.

4.1.2. 3-[(E)-3-(Dimethylamino)acryloyl]-1,5-diphenyl-1H-pyrazole-4-carbonitrile (**3**)

A mixture of the acetylpyrazole **1** (5.74 g, 20 mmol) and dimethylformamide dimethylacetal (DMF-DMA) (2.66 mL, 20 mmol) in dry xylene (30 mL) was refluxed for 3 h, then allowed to cool. The orange yellow precipitate was filtered off, washed with petroleum ether (60/80 °C), dried and crystallized from ethanol/DMF to afford compound **3**. Yield 92%; mp 225–227 °C. IR (KBr) ν cm^1: 2230 (C≡N), 1693 (C=O). ^{1}H NMR (DMSO-d_6) δ 2.55 (s, 6H, $2CH_3$), 5.85 (d, 1H, CH, $J = 12.5$ Hz), 7.39 (m, 10H, ArH's), 7.68 (d, 1H, CH, $J = 12.5$ Hz). MS *m*/*z* (%): 342 (M^+, 22.5), 272 (16.3), 180 (9.0), 123 (55.4), 77 (100). Anal. Calcd for $C_{21}H_{18}N_4O$ (342.40): C, 73.67; H, 5.30; N, 16.36%. Found: C, 73.73; H, 5.33; N, 16.35%.

4.1.3. 3-((E)-3-Morpholin-4-yl-acryloyl)-1,5-diphenyl–1H-pyrazole-4-carbonitrile (**4**)

A solution of the enaminone **3** (3.42 g, 10 mmol) and morpholine (0.84, 10 mmol) in ethanol (15 mL) was heated under reflux condition for 2 h. The reaction mixture was concentrated in vacuum. The solid product obtained upon cooling was filtered off and crystallized from ethanol/DMF to give compound 3-((*E*)-3-

morpholin-4-yl-acryloyl)-1,5-diphenyl-1*H*-pyrazole-4-carbonitrile (**4**). Yield 70%; mp 172 °C. IR (KBr) ν cm^{-1}: 2230 (C≡N), 1680 (C=O). ^{1}H NMR (DMSO-d_6) δ 2.87 (t, 4H, 2CH$_2$), 3.98 (t, 4H, CH$_2$), 5.81 (d, 1H, CH, J = 12.6 Hz), 7.41–7.69 (m, 10H, ArH's), 7.89 (d, 1H, CH, J = 12.6 Hz). MS m/z (%): 384 (M$^+$, 72.5), 367 (100), 337 (52.8), 297 (16.6), 272 (50.5), 180 (32.1), 141 (18.1), 112 (35.1), 77 (57.1). Analysis for: $C_{23}H_{20}N_4O_2$ (384.44): C, 71.86; H, 5.24; N, 14.57; S,%. Found: C, 71.90; H, 5.30; N, 14.53%.

*4.1.4. 3-(3-Acetyl-1-aryl-1H-pyrazole-4-carbonyl)-1,5-diphenyl-1H-pyrazole-4-carbonitriles **8a–c***

4.1.4.1. General procedure. To a mixture of the enaminone derivative **3** or **4** (10 mmol) and the appropriate 2-oxo-*N*-arylpropanehydrazonoyl chloride **5** (10 mmol) in benzene (20 mL), an equivalent amount of triethylamine was added. The reaction mixture was heated under reflux for 8 h. The solvent was distilled off at reduced pressure and the residual brown viscous liquid was taken in methanol and the resulting solid was collected by filtration washed thoroughly with ethanol, dried and finally crystallized from ethanol to afford corresponding 3-(3-acetyl-1-aryl-1*H*-pyrazole-4-carbonyl)-1,5-diphenyl-1*H*-pyrazole-4-carbonitrile derivative **8**. The synthesized compounds **8a–c** together with their physical and spectral data are listed below.

*4.1.4.2. 3-(3-Acetyl-1-phenyl-1H-pyrazole-4-carbonyl)-1,5-diphenyl-1H-pyrazole-4-carbonitrile (**8a**).* Yield 74%; mp 160 °C. IR (KBr) ν cm^{-1}: 2223 (C≡N), 1708, 1670 (2C=O). ^{1}H NMR (DMSO-d_6) δ 2.10 (s, 3H, CH$_3$), 8.67 (s, 1H, py H-5), 7.39–7.49 (m, 15H, ArH's); ^{13}C NMR (DMSO-d_6) δ 27.67 (COCH$_3$), 112.90, 113.85 (pyrazole carbons), 119.67 (C≡N), 121.00, 125.58, 126.30, 128.18, 128.77, 129.18, 129.30, 133.09, 138.35, 149.68, 150.22, 150.62 (aromatic and pyrazole carbons), 180.06, 193.43 (C=O carbons). MS m/z (%): 457 (M$^+$, 5.4), 442 (54.4), 213 (20.8), 77 (100). Anal. Calcd for $C_{28}H_{19}N_5O_2$ (457.50): C, 73.52; H, 4.19; N, 15.31%. Found: C, 73.59; H, 4.23; N, 15.33%.

*4.1.4.3. 3-[3-Acetyl-1-(4-chlorophenyl)-1H-pyrazole-4-carbonyl]-1,5-diphenyl-1H-pyrazole-4-carbonitrile (**8b**).* Yield 70%; mp 221–222 °C. IR (KBr) ν cm^{-1}: 2230 (C≡N), 1700, 1663 (2C=O). MS m/z (%): 493 (M$^+$, 9.8), 491 (M$^+$, 30.2), 476 (46.5), 272 (24.3), 247 (25.3), 205 (88.9), 180 (26.9), 141 (36.5), 77 (100). Anal. Calcd for $C_{28}H_{18}N_5O_2Cl$ (491.93): C, 68.36; H, 3.68; N, 14.23%. Found: C, 68.41; H, 3.70; N, 14.26%.

*4.1.4.4. 3-[3-Acetyl-1-(4-methylphenyl)-1H-pyrazole-4-carbonyl]-1,5-diphenyl-1H-pyrazole- 4-carbonitrile (**8c**).* Yield 80%; mp 230–231 °C. IR (KBr) ν cm^{-1}: 2231 (C≡N), 1700, 1662 (2C=O) ^{1}HNMR (DMSO-d_6) δ 2.42 (s, 3H, CH$_3$), 2.58 (s, 3H, CH$_3$), 7.22–7.63 (m, 14H, ArH's), 8.54 (s, 1H, py H-5). MS m/z (%): 471 (M$^+$, 19.1), 456 (34.2), 227 (15.7), 77 (100). Anal. Calcd for $C_{29}H_{21}N_5O_2$ (471.52): C, 73.87; H, 4.48; N, 14.85%. Found: C, 73.90; H, 4.50; N, 14.82%.

*4.1.5. Reaction of 3-(3-acetyl-1-aryl-1H-pyrazole-4-carbonyl)-1,5-diphenyl-1H-pyrazole-4-carbonitriles **8a–c** with hydrazine hydrate*

4.1.5.1. General procedure. Hydrazine hydrate (80%, 2 mL) was added to a solution of the appropriate compound **8** (5 mmol) in ethanol (10 mL). The reaction mixture was heated under reflux for 1 h, concentrated in vacuum, and diluted with water. The precipitate obtained was filtered off, washed with ice-cold water, dried and crystallized from ethanol. The synthesized compounds **11a–c** together with their physical and spectral data are listed below.

*4.1.5.2. 3-(7-Methyl-2-phenyl-2H-pyrazolo[3,4-d]pyridazin-4-yl)-1,5-diphenyl-1H-pyrazole-4-carbonitrile (**11a**).* Yield 70%; mp 280–282 °C. IR (KBr) ν cm^{-1}: 2226 (C≡N). ^{1}H NMR (DMSO-d_6) δ 2.15 (s, 3H, CH$_3$), 7.20–7.37 (m, 15H, ArH's), 8.67 (s, 1H, pyrazole-H-5); ^{13}C NMR (DMSO-d_6) δ 18.04 (*C*H3), 113.69, 115.58 (pyrazole carbons), 117.95 (C≡N), 120.46, 120.53, 121.77, 125.13, 125.57, 128.80, 128.97, 129.15, 129.34, 129.69, 130.29, 138.07 (aromatic carbons), 152.91, 153.98 (pyridazine C=N carbons). MS m/z (%): 453 (M$^+$, 100), 284 (57.1), 77 (72.4). Anal. Calcd for $C_{28}H_{19}N_7$ (453.50): C, 74.16; H, 4.22; N, 21.62%. Found: C, 74.20; H, 4.23; N, 21.59%.

*4.1.5.3. 3-[7-Methyl-2-(4-chlorophenyl)-2H-pyrazolo[3,4-d]pyridazin-4-yl]-1,5-diphenyl-1H-pyrazole-4-carbonitrile (**11b**).* Yield 80%; mp: >300 °C. IR (KBr) ν cm^{-1}: 2233 (CN). ^{1}H NMR (DMSO-d_6) δ 2.17 (s, 3H, CH$_3$), 7.30–7.41 (m, 14H, ArH's), 8.85 (s, 1H, pyrazole-H-5). MS m/z: 489 (M$^+$, 31.9), 487 (M$^+$, 100), 284 (43.4), 244 (32.1), 77 (56.4). Anal. Calcd for $C_{28}H_{18}N_7Cl$ (487.95): C, 68.92; H, 3.72; N, 20.09%. Found: C, 68.95; H, 3.75; N, 20.11%.

*4.1.5.4. 3-[7-Methyl-2-(4-methylphenyl)-2H-pyrazolo[3,4-d]pyridazin-4-yl]-1,5-diphenyl-1H-pyrazole-4-carbonitrile (**11c**).* Yield 70%; mp: 261–262 °C. IR (KBr) ν cm^{-1}: 2229 (C≡N). ^{1}HNMR (DMSO-d_6) δ 2.14 (s, 3H, CH$_3$), 2.35 (s, 3H, CH$_3$), 7.19–7.38 (m, 14H, ArH's), 8.71 (s, 1H, py H-5). MS m/z: 467 (M$^+$, 100), 284 (50.0), 244 (21.7), 77 (80.6). Anal. Calcd for $C_{29}H_{21}N_7$ (467.53): C, 74.50; H, 4.52; N, 20.97%. Found: C, 74.51; H, 4.55; N, 20.8%.

*4.1.6. 3-(1-Aryl-3-ethoxycarbonyl-4-carbonyl)-1,5-diphenyl-1H-pyrazole-4-carbonitriles **14a–c***

4.1.6.1. General procedure. To a mixture of the appropriate enaminone derivative **3** or **4** (10 mmol) and the corresponding chloro (arylhydrazono)ethyl acetate **12** (10 mmol) in benzene (20 mL), an equivalent amount of triethylamine was added, and the reaction mixture was heated under reflux for 5 h. The solvent was distilled off under reduced pressure and the residual viscous liquid was taken in methanol. The resulting solids were collected by filtration, washed with ethanol, dried and finally crystallized from ethanol to afford the compounds **14a–c**. The physical and spectral data for obtained product are listed below.

*4.1.6.2. 3-(1-Phenyl-3-ethoxycarbonyl-4-carbonyl)-1,5-diphenyl-1H-pyrazole-4-carbonitrile (**14a**).* Yield 73%; mp 205–206 °C. IR (KBr) ν cm^{-1}: 2239 (C≡N), 1732, 1650 (2C=O). ^{1}H NMR (CDCl$_3$) δ 1.23 (t, 3H, CH$_3$, J = 7.2 Hz), 4.29 (q, 2H, CH$_2$, J = 7.2 Hz), 7.20–7.64 (m, 15H, ArH's), 8.67 (s, 1H, pyrazole H-5). MS m/z (%): 487 (M$^+$, 47), 443 (68.6), 272 (27.5), 215 (41.9), 180 (22.0), 77 (100). Anal. Calcd for $C_{29}H_{21}N_5O_3$ (487.51): C, 71.44; H, 4.34; N, 14.36%. Found: C, 71.41; H, 4.33; N, 14.39%.

*4.1.6.3. 3-(1-(4-Chlorophenyl)-3-ethoxycarbonyl-4-carbonyl)-1,5-diphenyl-1H-pyrazole-4-carbonitrile (**14b**).* Yield 70%; mp 215–216 °C. IR (KBr) ν cm^{-1}: 2230 (C≡N), 1738, 1660 (2C=O). ^{1}H NMR (DMSO-d_6) δ 1.26 (t, 3H, CH$_3$, J = 7.2 Hz), 4.25 (q, 2H, CH$_2$, J = 7.2 Hz), 7.25–7.61 (m, 14H, ArH's), 8.62 (s, 1H, pyrazole H-5). MS m/z (%): 523 (7.2), 521 (M$^+$, 23.1), 249 (21), 180 (19), 138 (45), 77 (100). Anal. Calcd for $C_{29}H_{20}N_5O_3Cl$ (521.97): C, 66.73; H, 3.86; N, 13.41%. Found: C, 66.75; H, 3.88; N, 13.40%.

*4.1.6.4. 3-[1-(4-Methylphenyl)-3-ethoxycarbonyl-4-carbonyl]-1,5-diphenyl-1H-pyrazole-4-carbonitrile (**14c**).* Yield 72%; mp 185–186 °C. IR (KBr) ν cm^{-1}: 2231(C≡N), 1730, 1665 (2C=O). ^{1}H NMR (DMSO-d_6) δ 1.23 (t, 3H, CH$_3$, J = 7.2 Hz), 2.40 (s, 3H, CH $_3$), 4.29 (q, 2H, CH$_2$, J = 7.2 Hz), 7.23–7.64 (m, 14H, ArH's), 8.61 (s, 1H, pyrazole-H-5); ^{13}C NMR (DMSO-d_6) δ 13.66 (*C*H$_3$CH$_2$), 20.47 (Ar-4-*C*H$_3$), 61.26 (CH$_3$*C*H$_2$), 112.76, 113.84 (pyrazole carbons), 119.64 (*C*≡N), 120.56, 125.56, 126.31, 128.77, 129.05, 129.18, 129.26, 129.379, 129.57, 132.69, 136.08, 137.70, 138.16, 144.90, 150.28, 150.70 (pyrazole and aromatic carbons), 161.59 (ester C=O),

179.13 (ketone C=O).. MS *m/z* (%): 501 (M^+, 12.3), 456 (71.1), 428 (23.5), 272 (20.1), 77 (100). Anal. Calcd for $C_{30}H_{23}N_5O_3$ (501.55): C, 71.84; H, 3.72; N, 13.96%. Found: C, 71.89; H, 3.70; N, 14.00%.

4.1.7. Reaction of 3-(1-aryl-3-ethoxycarbonyl-4-carbonyl)-1,5-diphenyl-1H-pyrazole-4-carbonitriles ***14a–c*** *with hydrazine hydrate*

4.1.7.1. General procedure. Hydrazine hydrate (80%, 3 mL) was added to a solution of the appropriate 3-(1-aryl-3-ethoxycarbonyl-4-carbonyl)-1,5-diphenyl-1*H*-pyrazole-4-carbonitrile **14** (5 mmol) in ethanol (10 mL). The reaction mixture was heated under reflux for 1 h, concentrated in vacuum, cooled, and diluted with water. The precipitate obtained was filtered, washed with ice-cold water, dried and crystallized from ethanol. The synthesized compounds **16a–c** together with their physical and spectral data are listed below.

*4.1.7.2. 3-(7-Hydroxy-2-phenyl-2H-pyrazolo[3,4-d]pyridazin-4-yl)-1,5-diphenyl-1H-pyrazole-4-carbonitrile (****16a****).* Yield 70%; mp 249–250 °C. IR (KBr) *ν* cm^{-1}: 3350 (OH), 3205 (NH), 2229 (C≡N), 1678 (C=O). ^{1}H NMR (DMSO-d_6) *δ* 2.98 (br s, 1H, OH, D_2O-exchangeable), 7.17–7.39 (m, 15H, ArH's), 8.64 (s, 1H, pyrazole H-5), 11.00 (br s, 1H, NH, D_2O-exchangeable). MS *m/z* (%): 455 (M^+, 100), 398 (33.7), 295 (14.1), 180 (11.1), 77 (95.9). Anal. Calcd for $C_{27}H_{17}N_7O$ (455.92): C, 73.83; H, 3.76; N, 21.52%. Found: C, 73.85; H, 3.77; N, 21.55%.

*4.1.7.3. 3-[7-Hydroxy-2-(4-chlorophenyl)-2H-pyrazolo[3,4-d]pyridazin-4-yl]-1,5-diphenyl-1H-pyrazole-4-carbonitrile (****16b****).* Yield 72%; mp 288–290 °C. IR (KBr) *ν* cm^{-1}: 3371 (OH), 3230 (NH), 2228 (C≡N), 1680 (C=O). ^{1}HNMR (DMSO-d_6) *δ* 3.18 (br s, 1H, OH, D_2O-exchangeable), 7.29–7.50 (m, 14H, ArH's), 8.71 (s, 1H, pyrazole H-5), 11.01 (br s, 1H, NH, D_2O-exchangeable). MS *m/z* (%): 491 (M^+, 30.8), 489 (M^+, 100), 434 (10.7), 432 (29.9), 77 (44.2). Anal. Calcd for $C_{27}H_{16}N_7OCl$ (489.92): C, 66.19; H, 3.29; N, 20.01%. Found: C, 66.21; H, 3.32; N, 20.03%.

*4.1.7.4. 3-[7-Hydroxy-2-(4-methylphenyl)-2H-pyrazolo[3,4-d]pyridazin-4-yl]-1,5-diphenyl-1H-pyrazole-4-carbonitrile (****16c****).* Yield 75%; mp 295–297 °C. IR (KBr) *ν* cm^{-1}: 3354 (OH), 3200 (NH), 2225 (C≡N), 1672 (C=O). ^{1}HNMR (DMSO-d_6) *δ* 2.5 (s, 3H, CH_3), 3.10 (br s, 1H, OH, D_2O-exchangeable), 7.20–7.37 (m, 14H, ArH's), 8.60 (s, 1H, pyrazole-H-5), 10.51 (br s, 1H, NH, D_2O-exchangeable). MS *m/z* (%): 469 (100), 412 (33.5), 244 (8.2), 77 (60.0). Anal. Calcd for $C_{28}H_{19}N_7O$ (469.51): C, 71.63; H, 4.07; N, 20.88%. Found: C, 71.68; H, 4.08; N, 20.86%.

4.1.8. 4-(4-Cyano-1,5-diphenyl-1H-pyrazole-3-carbonyl)-1-aryl-1H-pyrazole-3-carboxylic acid phenylamids ***19a–c***

4.1.8.1. General procedure. To a mixture of the appropriate enaminone derivative **3** or **4** (10 mmol) and the corresponding phenylcarbamoylarylhydrazonoyl chloride **17** (10 mmol) in benzene (30 mL), an equivalent amount of triethylamine was added and the reaction mixture was heated under reflux for 6 h. The solvent was removed under reduced pressure and the residual viscous liquid was taken in methanol. The resulting solid was collected by filtration washed with ethanol, dried and finally recrystallized from ethanol to afford the compounds **19a–c**. The physical and spectral data for obtained product are listed below.

*4.1.8.2. 4-(4-Cyano-1,5-diphenyl-1H-pyrazole-3-carbonyl)-1-phenyl-1H-pyrazole-3-carboxylic acid phenylamid (****19a****).* Yield 73%; mp 160–161 °C. IR (KBr) *ν* cm^{-1}: 3261 (NH), 2223 (C≡N), 1690, 1650 (2C=O). ^{1}H NMR (DMSO-d_6) *δ* 7.20–7.37 (m, 20H, ArH's), 8.50 (s, 1H, pyrazole H-5), 10.80 (br s, 1H, NH, D_2O-exchangeable). MS *m/z* (%): 534 (M^+, 19.1), 442 (100), 272 (22.4), 141 (13.3), 77 (50.6). Anal. Calcd for $C_{33}H_{22}N_6O_2$ (534.57): C, 74.14; H, 4.14; N, 15.72%. Found: C, 74.18; H, 4.11; N, 15.75%.

*4.1.8.3. 4-(4-Cyano-1,5-diphenyl-1H-pyrazole-3-carbonyl)-1-(4-chlorophenyl)-1H-pyrazole-3-carboxylic acid phenylamid (****19b****).* Yield 80%; mp 224–225 °C. IR (KBr) *ν* cm^{-1}: 3265 (NH), 2233 (C≡N), 1685, 1650 (2C=O). ^{1}HNMR (DMSO-d_6) *δ*: 7.25–7.90 (m, 19H, ArH's), 9.25 (s, 1H, pyrazole-H-5), 11.47 (br s, 1H, NH, D_2O-exchangeable). MS *m/z* (%): 570 (M^+, 6.8), 568 (M^+, 19.1), 476 (88.1), 272 (22.1) 180 (8.5), 141 (25), 77 (100). Anal. Calcd for $C_{33}H_{21}N_6O_2Cl$ (569.02): C, 69.65; H, 3.72; N, 14.77%. Found: C, 69.69; H, 3.70; N, 14.76%.

*4.1.8.4. 4-(4-Cyano-1,5-diphenyl-1H-pyrazole-3-carbonyl)-1-(4-methlphenyl)-1H-pyrazole-3-carboxylic acid phenyl amid (****19c****).* Yield 72%; mp 240–242 °C. IR (KBr) *ν* cm^{-1}: 3260 (NH), 2230 (C≡N), 1683, 1645 (2C=O). ^{1}HNMR (DMSO-d_6) *δ* 2.40 (s, 3H, CH_3), 7.15–7.93 (m, 19H, ArH's), 9.20 (s, 1H, pyrazole-H-5), 11.55 (br s, 1H, NH, D_2O-exchangeable). MS *m/z* (%): 548 (M^+, 5.5), 456 (100), 428 (17.6), 244 (43.9), 77 (87.2). Anal. Calcd for $C_{34}H_{24}N_6O_2$ (548.61): C, 74.44; H, 4.41; N, 15.32%. Found: C, 74.47; H, 4.45; N, 15.36%.

*4.1.9. 3-(1,3-Diphenyl-1H-pyrazole-4-carbonyl)-1,5-phenyl-1H-pyrazole-4-carbonitrile (****23****)*

To a mixture of the appropriate enaminone derivative **3** or **4** (10 mmol) and *N*-phenylbenzenecarbohydrazonoyl chloride (**21**) (10 mmol) in benzene (30 mL), an equivalent amount of triethylamine was added and the reaction mixture was heated under reflux for 8 h. The solvent was removed under reduced pressure and the residual viscous liquid was taken in methanol. The resulting solid was collected by filtration washed with ethanol, dried and finally crystallized from ethanol to afford the compound **23**. Yield 55%; mp 225–226 °C. IR (KBr) *ν* cm^1: 2228 (C≡N), 1671 (C=O). ^{1}H NMR (DMSO-d_6) *δ* 7.20–7.37 (m, 20H, ArH's), 8.60 (s, 1H, pyrazole H-5); ^{13}C NMR (DMSO-d_6) *δ* 113.01, 118.77 (*C*≡N), 120.61, 125.80, 126.29, 127.64, 128.66, 129.18, 129.28, 129.71, 130.55, 135.28, 138.60, 143.66, 151.2 (pyrazole and aromatic carbons), 178.85 (ketone C=O). MS *m/z* (%): 491 (M^+, 29.1), 247 (26.2), 116 (10.8), 77 (100). Anal. Calcd for $C_{32}H_{21}N_5O$ (491.56): C, 78.19; H, 4.30; N, 14.24%. Found: C, 78.14; H, 4.33; N, 14.26%.

4.1.10. 3-[3-(4-Cyano-1,5-diphenyl-1H-pyrazole-3-yl)-1-aryl-1H-pyrazole-4-carbonyl]-1,5-diphenyl-1H-pyrazole-4-carbonitriles ***27a–c***

4.1.10.1. General procedure. To a mixture of the appropriate enaminone derivative **3** or **4** (10 mmol) and the corresponding pyrazolehydazonyl bromide derivative **25** (10 mmol) in benzene (30 mL), an equivalent amount of triethylamine was added. The reaction mixture was heated under reflux for 9 h. The solvent was removed under reduced pressure. The residual brown viscous liquid was taken in methanol then the resulting solid was collected by filtration washed with ethanol, dried and finally crystallized from ethanol/DMF to afford the corresponding 3-[3-(4-cyano-1,5-diphenyl-1*H*-pyrazole-3-yl)-1-aryl-1*H*-pyrazole-4-carbonyl]-1,5-diphenyl-1*H*-pyrazole-4-carbonitrile derivatives **27**. The physical and spectral data for obtained products are listed below.

*4.1.10.2. 3-[3-(4-Cyano-1,5-diphenyl-1H-pyrazole-3-yl)-1-phenyl-1H-pyrazole-4-carbonyl]-1,5-diphenyl-1H-pyrazole-4-carbonitrile (****27a****).* Yield 73%; mp 279–280 °C. IR (KBr) *ν* cm^{-1}: 2223, 2231 (2CN), 1678, 1663 (2C=O). ^{1}H NMR (DMSO-d_6) *δ* 7.18–7.40 (m, 25H, ArH's), 7.93 (s, 1H, pyrazole H-5); ^{13}C NMR (DMSO-d_6) *δ* 112.42, 112.53, 119.94 (*C*≡N), 123.27, 125.12, 125.44, 128.37, 128.90, 129.20, 129.59, 132.63, 138.29, 149.89, 150.29, 150.349 (pyrazole and aromatic carbons),

179.013, 180.46 (C=O carbons). MS *m/z* (%): 686 (M^+, 4.2) 442 (40.3), 414 (13.2), 272 (50.1), 244 (18.2), 77 (100). Anal. Calcd for $C_{43}H_{26}N_8O_2$ (686.73): C, 75.30; H, 3.81; N, 16.31%. Found: C, 75.33; H, 3.79; N, 16.30%.

*4.1.10.3. 3-[3-(4-Cyano-1,5-diphenyl-1H-pyrazole-3-yl)-1-(4-chlorophenyl)-1H-pyrazole-4-carbonyl]-1,5-diphenyl-1H-pyrazole-4-carbonitrile (**27b**).* Yield 80%; mp 250–252 °C. IR (KBr) *ν* cm^{-1}: 2230, 2220 (2CN), 1680, 1631 (2C=O). ^1H NMR (DMSO-d_6) *δ* 7.25–7.48 (m, 24H, ArH's), 7.94 (s, 1H, pyrazole H-5). MS *m/z* (%): 722 (M^+, 10.8), 720 (M^+, 33.4), 478 (8.5), 476 (19.8), 272 (72.9), 180 (24.6), 141 (54.1), 77 (100). Anal. Calcd for $C_{43}H_{25}N_8O_2Cl$ (720.17): C, 71.61; H, 3.49; N, 15.53%. Found: C, 71.63; H, 3.48; N, 15.55%.

*4.1.10.4. 3-[3-(4-Cyano-1,5-diphenyl-1H-pyrazole-3-yl)-1-(4-methylphenyl)-1H-pyrazole-4-carbonyl]-1,5-diphenyl-1H-pyrazole-4-carbonitrile (**27c**).* Yield 73%; mp 281–282 °C. IR (KBr) *ν* cm^{-1}: 2231, 2220 (2C≡N), 1680, 1651 (2C=O). ^1H NMR (DMSO-d_6) *δ* 2.41 (s, 3H, CH_3), 7.20–7.37 (m, 24H, ArH's), 8.67 (s, 1H, pyrazole H-5); ^{13}C NMR (DMSO-d_6) *δ* 20.33 (Ar-4-*C*H_3), 112.20, 116.73, 116.75 (pyrazole carbons), 119.73 (*C*≡N), 120.53, 125.31, 125.94, 128.03, 128.48, 128.78, 128.86, 128.97, 130.53, 132.26, 137.35, 137.89, 148.23, 150.17 (pyrazole and aromatic carbons), 180.78, 182.31 (C=O). MS *m/z* (%): 700 (3.3), 456 (9.9), 428 (32.5), 272 (63.2), 244 (23.2), 77 (100). Anal. Calcd for $C_{44}H_{28}N_8O_2$ (700.77): C, 75.42; H, 4.03; N, 15.99%. Found: C, 75.50; H, 4.07; N, 16.10%.

*4.1.11. Reaction of 3-[3-(4-cyano-1,5-diphenyl-1H-pyrazole-3-yl)-1-aryl-1H-pyrazole-4-carbonyl]-1,5-diphenyl-1H-pyrazole-4-carbonitriles **27a–c** with hydrazine hydrate*

4.1.11.1. General procedure. Hydrazine hydrate (80%, 3 mL) was added to a solution of the appropriate 3-[3-(4-cyano-1,5-diphenyl-1*H*-pyrazole-3-yl)-1-aryl-1*H*-pyrazole-4-carbonyl]-1,5-diphenyl-1*H*-pyrazole-4-carbonitrile **27** (5 mmol) in ethanol (10 mL). The reaction mixture was heated under reflux for 1 h, concentrated in vacuum, cooled, and diluted with water, the precipitate obtained was filtered off, washed with ice-cold water, dried and crystallized from ethanol. The synthesized compounds **29a–c** together with their physical and spectral data are listed below.

*4.1.11.2. 3-[7-(4-Cyano-1,5-diphenyl-1H-pyrazole-3-yl)-2-phenyl-2H-pyrazolo[3,4-d]pyridazin-4-yl]-1,5-diphenyl-1H-pyrazole-4-carbonitrile (**29a**).* Yield 68%; mp >300 °C. IR (KBr) *ν* cm^{-1}: 2225, 2235 (2C≡N). ^1H NMR (DMSO-d_6) *δ* 7.20–7.47 (m, 25H, ArH's), 8.60 (s, 1H, pyrazole H-5); ^{13}C NMR (DMSO-d_6) *δ* 111.87, 115.87 (pyrazole carbons), 118.88 (*C*≡N), 120.61, 120.6, 122.67, 125.41, 125.74, 127.04, 129.28, 129.50, 131.76, 133.38, 140.7, 150.09 (pyrazole and aromatic carbons), 152.68, 159.65 (pyridazine C=N carbons). MS *m/z* (%): 682 (M^+, 63), 180 (29), 141 (22.1), 77 (100). Anal. Calcd for $C_{43}H_{26}N_{10}$ (682.74): C, 75.64; H, 3.83; N, 20.51; %. Found: C, 75.63; H, 3.81; N, 20.51%.

*4.1.11.3. 3-[7-(4-Cyano-1,5-diphenyl-1H-pyrazole-3-yl)-2-(4-chlorophenyl)-2H-pyrazolo[3,4-d]pyridazin-4-yl]-1,5-diphenyl-1H-pyrazole-4-carbonitrile (**29b**).* Yield 72%; mp >300 °C. IR (KBr) *ν* cm^{-1}: 2230, 2220 (2C≡N). ^1H NMR (DMSO-d_6) *δ* 7.26–7.52 (m, 24H, ArH's), 8.71 (s, 1H, pyrazole H-5); ^{13}C NMR (DMSO-d_6) *δ* 113.80, 114.21 (pyrazole carbons), 119.90 (*C*≡N), 123.73, 125.51, 125.75 126.13, 127.83, 129.20, 128.91, 129.29, 129.46, 129.68, 130.38, 135.05, 135.71, 138.22, 144.89 (pyrazole and aromatic carbons), 156.13, 157.58 (pyridazine C=N carbons). MS *m/z* (%): 718 (M^+, 25.2), 716 (M^+, 77), 180 (33), 77 (100). Anal. Calcd for $C_{43}H_{25}N_{10}Cl$ (717.19): C, 72.01; H, 3.51; N, 19.53%. Found: C, 72.10; H, 3.60; N, 19.50%.

*4.1.11.4. 3-[7-(4-Cyano-1,5-diphenyl-1H-pyrazole-3-yl)-2-(4-methylphenyl)-2H-pyrazolo[3,4-d]pyridazin-4-yl]-1,5-diphenyl-1H-pyrazole-4-carbonitrile (**29c**).* Yield 70%; mp >300 °C; IR (KBr) *ν* cm^{-1}: 2238, 2215 (2 CN). ^1H NMR (DMSO-d_6) *δ* 2.43 (s, 3H, CH_3), 7.25–7.47 (m, 24H, ArH's), 9.15 (s, 1H, py H-5). MS *m/z* (%): 696 (M^+, 12.6), 244 (3.5), 77 (100). Anal. Calcd for $C_{44}H_{28}N_{10}$ (696.77): C, 75.84; H, 4.05; N, 20.10%. Found: C, 75.83; H, 4.08; N, 20.12%.

*4.1.12. 3-(4-Benzoyl-1-aryl-1H-pyrazole-3-carbonyl)-1,5-diphenyl-1H-pyrazole-4-carbonitriles **32a–c***

4.1.12.1. General procedure. To a mixture of the enaminone **31** (10 mmol) and appropriate hydrazonyl bromide **25** (10 mmol) in benzene (30 mL), an equivalent amount of triethylamine was added. The reaction mixture was heated under reflux for 9 h. The solvent was distilled off under reduced pressure. The residual viscous liquid was taken in methanol and the resulting solid was collected by filtration washed with ethanol, dried and finally crystallized from ethanol/DMF to afford the corresponding of 3-(4-benzoyl-1-aryl-1*H*-pyrazole-3-carbonyl)-1,5-diphenyl-1*H*-pyrazole-4-carbonitrile derivatives **32a–c**. The physical and spectral data for obtained products are listed below.

*4.1.12.2. 3-(4-Benzoyl-1-phenyl-1H-pyrazole-3-carbonyl)-1,5-diphenyl-1H-pyrazole-4-carbonitrile (**32a**).* Yield 80%; mp 179–180 °C. IR (KBr) *ν* cm^{-1}: 2237 (CN), 1680, 1650 (2C=O). ^1H NMR (DMSO-d_6) *δ* 7.23–7.50 (m, 20H, ArH's), 8.89 (s, 1H, pyrazole H-5). MS *m/z* (%): 519 (M^+, 13.0), 443 (49.7), 272 (20.7), 141 (13.2), 77 (100). Anal. Calcd for $C_{33}H_{21}N_5O_2$ (519.55): C, 76.29; H, 4.07; N, 13.48%. Found: C, 76.30; H, 4.10; N, 13.50%.

*4.1.12.3. 3-[4-Benzoyl-1-(4-chlorophenyl)-1H-pyrazole-3-carbonyl]-1,5-diphenyl-1H-pyrazole-4-carbonitrile (**32b**).* Yield 70%; mp 183–184 °C. IR (KBr) *ν* cm^{-1}: 2233 (CN), 1678, 1643 (2C=O). ^1H NMR (DMSO-d_6) *δ* 7.32–7.87 (m, 19H, ArH's), 9.11 (s, 1H, pyrazole H-5); ^{13}C NMR (DMSO-d_6) *δ* 112.35 (pyrazole), 119.68 (*C*≡N), 124.86, 125.28, 125.34, 125.84, 127.08, 128.68, 128.78, 129.19, 129.29, 129.44, 129.53, 13.67, 133.13, 136.25, 137.44, 137.77, 137.81, 149.20, 150.01, 150.42, 154.08 (pyrazole and aromatic carbons), 180.46, 188.16 (C=O carbons). MS *m/z* (%): 555 (M^+, 31.5), 553 (M^+, 100), 180 (31.9), 141 (14.8), 77 (86.9). Anal. Calcd for $C_{33}H_{20}N_5O_2Cl$ (553.01): C, 71.54; H, 3.63; N, 12.64%. Found: C, 71.55; H, 3.65; N, 12.60%.

*4.1.12.4. 3-[4-Benzoyl-1-(4-methylphenyl)-1H-pyrazole-3-carbonyl]-1,5-diphenyl-1H-pyrazole-4-carbonitrile (**32c**).* Yield 73%; mp 142–143 °C. IR (KBr) *ν* cm^{-1}: 2233 (CN), 1678, 1650 (2C=O). ^1H NMR (DMSO-d_6) *δ* 2.45 (s, 3H, CH_3), 7.22–7.60 (m, 19H, ArH's), 9.02 (s, 1H, py H-5); ^{13}C NMR (DMSO-d_6) *δ* 26.24 (Ar-4-*C*H_3), 112.23 (pyrazole), 119.46 (*C*≡N), 124.46, 124.93, 125.23, 125.55, 125.75, 127.95, 128.67, 128.86, 129.20, 129.36, 129.52, 130.39, 132.40, 133.11, 137.26, 137.38, 137.58, 149.57, 150.01, 150.18, 153.98 (pyrazole and aromatic carbons), 180.31, 187.92 (C=O carbons). Analysis for: $C_{34}H_{23}N_5O_2$ (533.58): C, 76.53; H, 4.34; N, 13.13%. Found: C, 76.55; H, 4.31; N, 13.15%.

*4.1.13. Reaction of 3-(4-benzoly-1-aryl-1H-pyrazole-3-carbonyl)-1,5-diphenyl-1H-pyrazole-4-carbonitriles **32a–c** with hydrazine hydrate*

4.1.13.1. General procedure. Hydrazine hydrate (80%, 4 mL) was added to a solution of appropriate 3-(4-benzoly-1-aryl-1*H*-pyrazole-3-carbonyl)-1,5-diphenyl-1*H*-pyrazole-4-carbonitrile **32** (5 mmol) in ethanol (10 mL). The reaction mixture was heated under reflux for 1 h, concentrated in vacuum, cooled, and diluted with water. The precipitate obtained was filtered off, washed with water, dried and crystallized from ethanol. The synthesized

compounds **34a**–**c** together with their physical and spectral data are listed below.

*4.1.13.2. 3(2,4-Diphenyl-2H-pyrazolo[3,4-d]pyridazin-7-yl)-1,5-diphenyl-1H-pyrazole-4-carbonitrile (**34a**).* Yield 68%; mp 289–291 °C. IR (KBr) ν cm^{-1}: 2320 (C≡N). ^1H NMR (DMSO-d_6) δ 7.20–7.54 (m, 20H, ArH's), 8.71 (s, 1H, pyrazole H-5). MS *m/z*: 515 (M^+, 9.8), 244 (20.3), 77 (100). Anal. Calcd for $C_{33}H_{21}N_7$ (515.57): C, 76.87; H, 4.10; N, 19.01%. Found: C, 76.90; H, 4.13; N, 19.03%.

*4.1.13.3. 3(2-(4-Chlorophenyl)-4-phenyl-2H-pyrazolo[3,4-d]pyridazin-7-yl)-1,5-diphenyl-1H-pyrazole-4-carbonitrile (**34b**).* Yield 79%; mp >300 °C. IR (KBr) ν cm^{-1}: 2240 (CN). ^1H NMR (DMSO-d_6) δ 7.45–7.88 (m, 19H, ArH's), 9.34 (s, 1H, pyrazole H-5). MS *m/z*: 551 (M^+, 13.1), 549 (M^+, 35.1), 244 (13.0), 77 (100). Anal. Calcd for $C_{33}H_{20}N_7Cl$ (550.01): C, 72.06; H, 3.67; N, 17.83%. Found: C, 72.02; H, 3.70; N, 17.80%.

*4.1.13.4. 3(2-(4-methylphenyl)-2-phenyl-2H-pyrazolo[3,4-d]pyridazin-7-yl)-1,5-diphenyl-1H-pyrazole-4-carbonitrile (**34c**).* Yield 74%; mp >300 °C. IR (KBr) ν cm^{-1}: 2228 (C≡N). ^1H NMR (DMSO-d_6) δ 2.42 (s, 3H, CH_3), 7.18–7.52 (m, 19H, ArH's), 8.78 (s, 1H, pyrazole H-5). MS *m/z*: 529 (M^+). Anal. Calcd for $C_{34}H_{23}N_7$ (529.60): C, 77.11; H, 4.37; N, 18.51%. Found: C, 77.15; H, 4.35; N, 18.54%.

4.2. Pharmacology

4.2.1. Antagonism of uterus weight increase due to estrogen treatment (anti-estrogenic activity)

4.2.1.1. Animals. Immature female Sprague-Dawley rats weighing about 55 g were obtained from Animal House Laboratory, Nile Company, Cairo, Egypt and were acclimatized for 1 week in the animal facility that has 12 h light/dark cycles with the temperature controlled at 21–23 °C. Normal rat chow and water were made available.

The animals were housed individually in stainless steel cages in temperature-controlled and humidity-monitored quarters. Test animals were provided continuous access to tap water.

4.2.1.2. Procedure. Groups of 12 animals were injected daily for 7 days with estradiol 0.03–0.05 μg per animal S.C. and various doses 0.0–0.06 μg per animal S.C. of the tested compounds and letrozole as reference standard or estradiol alone.

The tested compound was administered in 0.5% solution of carboxymethylcellulose (CMC) orally. On the 8th day, the animals were sacrificed and the uterine weights were determined.

4.2.1.3. Evaluation. Mean values of each group were calculated and expressed as % reduction of uterine weight compared to controls treated with estradiol alone.

4.2.2. Evaluation of acute toxicity following single dose administration

4.2.2.1. Animals. 600 Adult mice of both sexes weighing 25 ± 3 g were obtained from Animal House Laboratory, Nile company, Cairo, Egypt and acclimatized for 1 week in the animal facility that has 12 h light/dark cycles with the temperature controlled at 21–23 °C. Normal rat chow and water were made available.

4.2.2.2. Procedure. LD_{50} was measured on 30 mice. Animals were fasted for 12 h prior to dosing. Rats were divided into 6 groups, 5 animals in each group. Treatment rats were dosed by oral gavage, using a curved, balltipped stainless steel feeding needle, with aqueous suspensions of very fine powder of the tested compound. Animals, in each group, were given doses of 100, 160, 256, 409, 655 and 1050 mg/km B.W. After 24 h results were recorded. The controls received tap water by gavage in the same volume.

4.2.2.3. Observation. All animals were monitored continuously for 10 h after dosing for signs of toxicity. For the remainder of the 14 days study period, animals were monitored for mortality. At the end of the study the number of dead animals was expressed in percentage and the LD_{50} value was calculated according to Weill (1952) method [42].

4.2.2.4. Statistical analysis. The data were evaluated for homogeneity of variances and normality by Bartlett's test. Bartlett's test indicated homogeneous variances, treated and control groups were compared using a one-way analysis of variance (ANOVA), followed by comparison of the treated groups to the control groups by Dunnett's *t*-test for multiple comparisons [43], where variances were considered significantly different by Bartlett's test.

4.2.3. In vitro antitumor screening

20 Compounds were subjected to the National Cancer Institute *in vitro* disease-oriented primary antitumor screening. 14 Cell lines of breast and ovarian tumor cell lines were utilized. The human tumor cell lines of the cancer screening panel were grown in RPMI 1640 medium containing 5% fetal bovine serum and 2 mM L-glutamine. For a typical screening experiment, cells were inoculated into 96-well microtiter plates in 100 mL at plating densities ranging from 5000 to 40,000 cells/well depending on the doubling time of individual cell lines. After cell inoculation, the microtiter plates were incubated at 37 °C, 5% CO_2, 95% air, and 100% relative humidity for 24 h prior to addition of experimental drugs. After 24 h, two plates of each cell line were fixed *in situ* with TCA, to represent a measurement of the cell population for each cell line at the time of drug addition. Experimental drugs were solubilized in DMSO at 400-fold the desired final maximum test concentration and stored frozen prior to use. At the time of drug addition, an aliquot of frozen concentrate was thawed and diluted to twice the desired final maximum test concentration with complete medium containing 50 mg mL^{-1} gentamicin. Additional four 10-fold or 1/2 log serial dilutions were made to provide a total of five drug concentrations plus control. Aliquots of 100 mL of these different drug dilutions were added to the appropriate microtiter wells already containing 100 mL of medium, resulting in the required final drug concentrations. Following drug addition, the plates were incubated for an additional 48 h at 37 °C, 5% CO_2, 95% air, and 100% relative humidity. For adherent cells, the assay was terminated by the addition of cold TCA. Cells were fixed *in situ* by the gentle addition of 50 mL of cold 50% (w/v) TCA (final concentration, 10% TCA) and incubated for 60 min at 4 °C. The supernatant was discarded, and the plates were washed five times with tap water and air dried. Sulforhodamine B (SRB) solution (100 mL) at 0.4% (w/v) in 1% acetic acid was added to each well, and plates were incubated for 10 min at room temperature. After staining, unbound dye was removed by washing five times with 1% acetic acid and the plates were air dried. Bound stain was subsequently solubilized with 10 mM trizma base, and the absorbance was read on an automated plate reader at a wavelength of 515 nm. For suspension cells, the methodology was the same except that the assay was terminated by fixing settled cells at the bottom of the wells by gently adding 50 mL of 80% TCA (final concentration, 16% TCA). The parameter used here is GI_{50} which is the $\log_{10}$ concentration at which PG is +50, was calculated for each cell line [44–46].

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