Synthesis and antibreast cancer activity of new 3-methy-1,5-diphenyl pyrazole derivatives

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Abstract: New series of 3-methyl-1,5-diphenyl pyrazole derivatives possessing different substitutions as pyridine, hydrazinylidenemethyl, 1,3-benzoxazole, amine phenyl, oxime, N- phenylacetamide moieties and were synthesized. The structures of synthesized compounds were established using IR, 1H NMR, 13C NMR, elemental and mass spectral analyses. In-vitro antitumor screening using breast cancer cell line (MCF-7) has been carried out. Among the compounds tested, compounds 7 and 16 were found to be the most active candidates of the synthesized series. Based on pharmacophore mapping of the established derivatives, potential anticancer target (1UYK) was chosen to perform docking process. Some of the synthesized compounds showed a good docking score toward it.
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Keywords: Pyrazole; anticancer; oxime; N-phenylacetamide; pyridine

1. Introduction

Malignancy is caused by abnormalities in cells. This may be due to inherited genes or caused by outside exposure to different chemicals, radiation, or infectious agents (Liott, Steeg and Steller-Stevenson, 1991; Harris and Hollstein, 1993; Mignatti and Rifkin, 1993; Alison, 2001). There are different types of cancer which can affect people and cause death, but breast cancer is one of the important causes of mortality worldwide with over a million cases each year (Tsang *et al.,* 2001; Parkin *et al.,* 2005; Ferlay *et al.,* 2010). Although there is an extensive effort in research, the cure of human malignancies still represents a major challenge to discover and find new anticancer agents with less side effects and more efficient (Lisurek, 2010; Reymond, 2010; Kumar *et al.,* 2013; Mok *et al.,* 2013). Nitrogen contained heterocycles compounds have given much attention due to their interesting biological activities (Katritzky *et al.,* 1996; Kidwai, 2002; Katritzky, 2006; Moa, 2011; Tantawy *et al.,* 2013; Thabit *et al.,* 2015). In particular, pyrazole ring displayed a wide range of biological activities as anticancer (Dayam *et al.,* 2006; Abdel-Aziz *et al.,* 2010; Farag *et al.,* 2010; Liu *et al.,* 2012; Vujasinović *et al.,* 2012), fungistatic (Sridhar *et al.,* 2004), antidepressant, anticonvulsant, anti-HIV, antimicrobial, anti-inflammatory activities (Zuhal *et al.,* 2007; Ali *et al.,* 2007; Revanasiddappa *et al.,* 2010; Babasaheb *et al.,* 2012). 4-functionally substituted N-arylpyrazole derivatives play an important role in cancer therapy (Abdel-Aziz, El-Zahabi and Dawood, 2010; Liu *et al.,* 2012). Furthermore, celecoxib and other 1,5-diphenyl derivatives (Figure 1) were reported to be active as anticancer agents (Singh *et al.,* 2006; Farag *et al.,* 2010; Insuasty *et al.,* 2010; Zhang *et al.,* 2011).

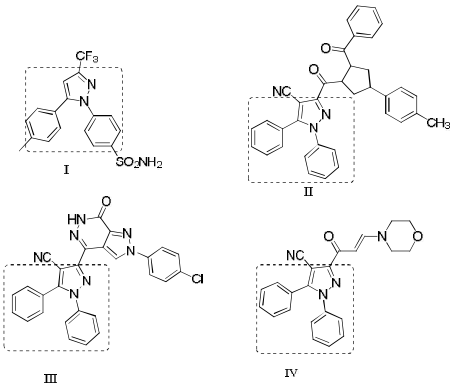


Figure 1. I) celecoxib, II-IV) 1,5-diphenylpyrazole derivatives as antibreast cancer agents.

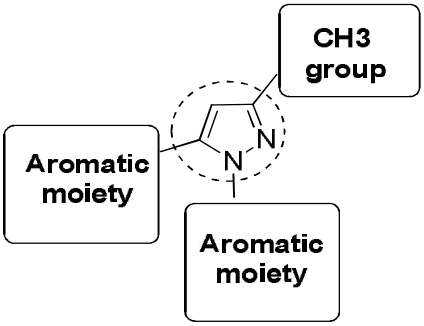


Figure 2. Main scaffold (A) of our new compounds

So, and as a part of drug discovery effort toward finding novel drugs for the treatment of breast cancer, and by using multicomponent reactions which were

reported as a powerful tool to synthesize diverse and complex heterocyclic compounds using simpler, energy savings, and reduced waste procedures (Sinkkonen *et al.,* 2002; Jain and Vederas, 2004). It was proposed to prepare a series of 3-methyl-1,5-diphenyl-1*H*-pyrazole (Figure 2) of the general structure (**A**).

In order to overcome the in-vivo instability of the aldehyde functional group, several modifications on the formyl group were carried out as the formation of oximes, hydrazones, aniline derivatives that proved to possess high stability and good antimitotic activity (Kaufmann *et al.,*2007; Pojarová *et al.,* 2007; Vogel *et al.,* 2008; El-Nakkady *et al.,* 2012). No one can deny the important role of benzoxazole as a good pharmacophore for anticancer activity (Xiang *et al.,* 2012; Jiang *et al.,* 2010; Aiello *et al.,* 2008). Chalcones, as well, exhibit different significant activities including the antiproliferative ones (Modzelewska *et al.,* 2006; Kamal *et al.,* 2010; Nofal *et al.,* 2011; Juvale *et al.,* 2012; Ngameni *et al.,* 2013). Moreover, pyridine has an appreciable activity as antimicrobial and anticancer activity (Prachayasittikul *et al.,* 2009; Nassar, 2010). N-phenylacetamide derivatives were readily established as potent antitumor agents (Aliabadi *et al.,*2013). So, based on the previous findings, different reactions, on the 4-poistion of scaffold **A**, were performed in order to increase their antitumor activities as shown in Figure 3.

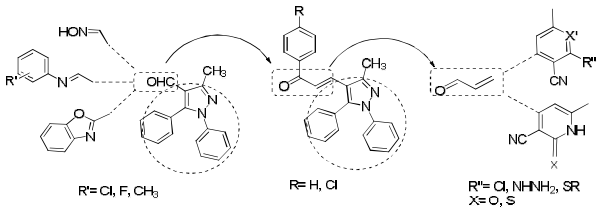
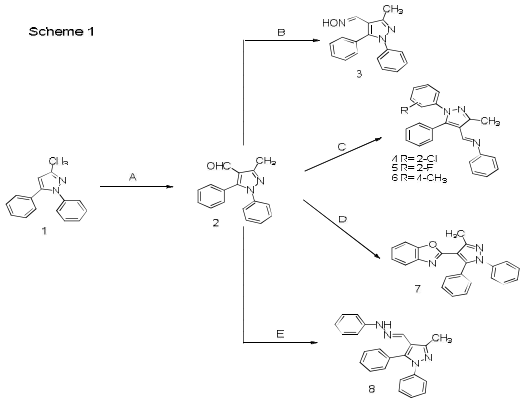


Figure 3. Design of the target compounds via different reactions

2. Material and Methods

2.1. Chemistry

The target compounds were obtained via the adopted synthetic approach illustrated in Scheme 1. The starting material 3-methyl-1,5-diphenyl-1*H*-pyrazole-4-carbaldehyde **(2)** was prepared according to literature procedure (Genin *et al.,* 2000) by Vilsmeiere Haack reaction of 3-methyl-1,5-diphenyl-1*H*-pyrazole **(1) (**(Genin *et al.,* 2000). Condensation of the starting compound **2** with hydroxylamine hydrochloride yielded the carboxaldehyde oxime derivative **3**. ^{1}H NMR of **3** showed a new exchangeable signal at 8.91 ppm corresponding to OH proton of the oxime group. Different substituted anilines were allowed to react with compound **2** yielding amine phenyl derivatives **4-6**. The reaction of the substituted pyrazole-4-carbaldehyde derivative **2** with the o-aminophenol gave the 4-substituted pyrazole derivative **7**. The mass spectral data of the given compound displayed molecular ion peaks which confirmed its molecular weight.The hydrazone dervative **8** was obtained by the reaction of compound **2** with phenyhydrazine in absolute ethanol. IR spectrum of compound **8** revealed the presence of stretching band at 3305 cm^{-1}assigned for NH group



Scheme 1. The synthesis of 1-8: A) POCl3, DMF; B) NH2OH, pyridine, absolute ethanol; C) R-C6H4-NH2, glacial acetic acid, absolute ethanol; D) o-aminophenol, absolute ethanol; E) phenyhydrazine, absolute ethanol.

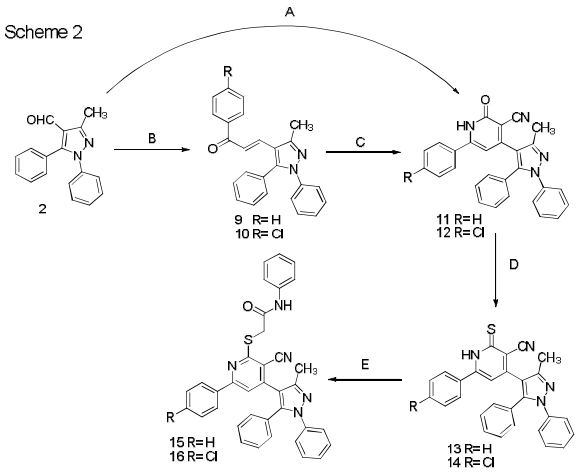
Other targeted *N*-phenylacetamide derivatives were prepared as shown in Scheme 2. *Via* Claisen-Schmidt condensation of the starting material 3-methyl-1,5-diphenyl-1*H*-pyrazole-4-carbaldehyde **(2)** with equimolar amount of acetophenone and its 4-chloro derivative under basic catalyzed reaction to yield the chalcone derivatives compounds **9** (Finar and Manning, 1961) and **10**, respectively.

The cyclization of the chalcones **9** and **10** with ethyl cyanoacetate yielded 2-oxo-1,2-dihydropyridine-3-carbonitrile **11** and **12,** respectively by 2 different methods; method A was by one pot three component cyclocondensation reaction of 3-methyl-1,5-diphenyl-1H-pyrazole-4-carbaldehyde **(2)**, ethylcyanoacetate, ammonium acetate and acetophenone or 4-chloroacetophenone, but method B showed stepwise synthesis including formation of the chalcones derivatives **9** and **10** firstly, and then the reaction of the latter comounds **9** and **10** with ethylcyanoacetate in the presence of ammonium acetate to yield compounds **11** and **12,** respectively. It was observed that method A produced higher yield than method B. The structure of the newly synthesized compound **11** was confirmed using IR spectra which showed characteristic

absorption bands at 3449 cm^{-1} for NH group , at 2217 cm^{-1} for CN group and at 1669 cm^{-1} corresponding to C=O group. In addition, ^1H NMR of **12** revealed the appearance of an exchangeable signal at 7.59 ppm due to NH of the pyridine ring.

Phosphorus pentasulphide was allowed to react with compounds **11** and **12** in the presence of pyridine afforded 2-sulfanylidene-pyridine-3-carbonitriles **13** and **14**, respectively. IR spectrum of **12** revealed a band of the cyano group at 2218 cm^{-1} and absorption band at 3313 cm^{-1} attributed to the amine group and the absence of the carbonyl group band. The corresponding N-phenylacetamide derivatives **15** and **16** were finally obtained by treating compounds **13** and **14** with 2-chloro-N-phenylacetamide in DMF, respectively.

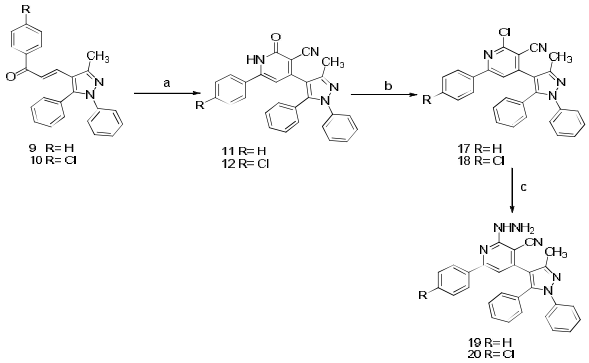
Scheme 2



Scheme 2. The synthesis of 9-16: A) acetophenone or 4-chloroacetophenone, ethylcyanoacetate, ammonium acetate, absolute ethanol; B) acetophenone or 4-chloroacetophenone, NaOH, absolute ethanol; C) ethylcyanoacetate, ammonium acetate, absolute ethanol; D) P_2S_5, Pyridine; E) 2-chloro-*N*-phenylacetamide, DMF.

The formation of chloro derivatives **17** and **18** from the 2-oxo-pyridine carbonitriles was done by reaction of compounds **11** and **12** with phosphorous oxychloride. The mass spectrum of compound **18** confirmed the presence of the chlorine atom through appearance of a molecular ion peak at m/z = 481 corresponding to the molecular formula $C_{28}H_{18}Cl_2N_4$ and a peak at 482 (M^{+2}) due to the presence of the isotopic chlorine atom. Reaction of compounds **17** and **18** with hydrazine hydrate afforded the corresponding 2-hydrazinylpyridine-3-carbonitrile derivatives **19** and **20**. IR spectrum of **19** showed the presence of significant bands at 2217 cm^{-1} for CN group, at 3300 cm^{-1} for NH group and at 3426 cm^{-1} for NH_2 group. ^1H NMR of **20** revealed the appearance of new exchangeable signals at 2.19 and 4 ppm corresponding to NH_2, NH groups, respectively. (Scheme 3).

Scheme 3



Scheme 3. The synthesis of 17-20: A) ethyl cyanoacetate, ammonium acetate, absolute ethanol; B) POCl3, N, N-DEA; C) Hydrazine hydrate, absolute ethanol.

2.2. Biology

The antitumor activity of all the newly synthesized compounds was evaluated against human breast cancer cell line (MCF-7) using the sulforhodamine B (SRB) assay (Skehan et al., 1990) obtaining the materials from Sigma Chemical Co. (USA). The used cell line, which obtained from the American Type Culture Collection, was frozen in liquid nitrogen (-180 ^0C) and maintained by serial sub-culturing in 75 cm^2 cell culture flasks (Fisher Scientific, Pitts burgh, PA) at 37 ^0C in atmosphere of 5% CO_2 using 10 ml of RPMI-1640 [supplemented with 1% (2mM) glutamic acid, 10% unheated Fetal Bovine Serum (FBS) 100 µg/ml Penicillin and 100 µg/ml Streptomycin]. Seeding the cells in 96-well microtiter plates at a concentration of $5x10^4$-10^5 cell/well using a fresh medium was done and the plates were left for 24 h. To allow the attachment of cells to the wall of the plates, it was treated with the test compounds. Incubation of the monolayer cells with the compounds for 48h at 37°C by a humidified incubator with 5% CO_2. Fixation of the cells with trichloroacetic acid, then staining, for 30 minutes, was occurred with 0.4% (wt/vol) Sulforhodamine B (SRB) stain which was dissolved in 1% acetic acid. Washing the unbound dye with 1% acetic acid, and for the protein bound dye, it was extracted with Tris EDTA (Meter tech. Σ 960, USA). The optical density (O.D.) of each well was measured spectrophotometrically at 564 nm using an ELIZA microplate reader (Meter tech. Σ 960, U.S.A.). Calculation of the percentage of cell survival was done as follows: Survival fraction = O.D. (treated cells)/ O.D. (control cells) as shown in table 1. By using different concentrations of the tested

compounds, the IC_{50} values were calculated and to obtain the survival curve, the relation between the surviving fraction was plotted against the concentration of the tested compound.

2.3. Molecular docking

The docking studies and modeling calculations were done using 'Molecular Operating Environment (MOE) (Molecular Operating Environment, 2012) 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 Chem Biooffice (Chem Bio Office version 13) suite and geometric optimization was done using Hyberchem (HyperChem™ Professional 7.51) then subjected to docking simulation. Our designed compounds had to go through 30 runs of docking process with 0.01 kcal/mol RMS gradient and 0.1 Å RMS distance. Studying of the docking results for each compound was done, separately, to determine the best fitting compounds with the used protein molecule. The X-ray crystallographic structure of heat shock protein enzyme was obtained from the Protein Data Bank; code "1UYK.pdb" (Wright et al., 2004). The enzyme was prepared for docking studies by removing the ligand molecule from the enzyme active site, and then hydrogen atoms were added, the protein molecule was kept rigid with flexible binding active site, which was isolated by the Alpha site finder tool using the binding amino acids as key elements in isolation, with dummies around it.

3. Results

3.1. Antitumor activity

The antitumor activity for all the synthesized compounds **3-20** was tested against human breast cancer cell line (MCF-7).

In light of the biological results, the following considerations could be made:

A series of 3-methyl-1,5-diphenylpyrazole **3-20** were synthesized and evaluated for their in vitro antitumor activity against breast cancer cell line (MCF-7). As shown from table 1, Compounds **3**, **6**, **7**, **8**, **15**, **16**, **17**, **19** and **20** exhibited strong activity. Compounds **4**, **11** and **13** showed moderate activity. Compounds **5**, **10**, **12,** **14** and **18** showed lower activity. The synthesis of the carboxaldehyde oxime derivative **3** increased the activity compared with the start compound. Also, synthesis of the hydrazone dervative **8** and N-phenylacetamide derivatives **15** and **16** greatly increased the antitumor activity.

Otherwise the introduction of 4-chloro or 4-fluro aniline derivatives in compounds **4**, **5** caused reduction in activity in comparable with the other 4-methyl substituted derivative **6.** Otherwise, the formation of 2-sulfanylidene-1,2-dihydropyridine-3-carbonitriles **13** and **14** didn't greatly affect the antitumor activity as in compound **14.** However, cyclization of chalcones **9** and **10** into either pyrazoylethan-2-ene-1-one derivative **17** or its 2-hydrazinyl pyridine-3-carbonitrile derivatives **19** and **20** increased the activity greatly.

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.	% surviving	% inhibition	IC_{50}[b], µg/mL	E of interaction ligand-protein
3	35.803	74.197	15.2	-27.114
4	62.623	47.377		-35.713
5	69.384	30.616		-36.241
6	33.983	66.017	18.4	-36.872
7	18.912	81.088	9.2	-34.953
8	22.818	77.182	13.4	-32.335
10	78.018	33.982		-40.264
11	55.476	44.524		-41.187
12	76.832	33.168		-42.156
13	75.072	42.928		-38.536
14	81.971	28.029		-39.650
15	34.862	65.138	19.6	-38.639
16	37.699	82.301	8.7	-47.183
17	35.763	65.237	32.0	-38.522
18	86.430	33.57		-39.773
19	29.736	70.264	23.5	-39.226
20	26.340	73.66	20.2	-40.313

3.2. Molecular modeling study

For evaluation of their recognition profile at the binding pocket, molecular docking simulations were performed for the synthesized compounds with the target enzyme using the pharmacophore mapping approach to investigate their interaction with the designed compounds with the target protein. The synthesized compounds **3-20** were comparatively evaluated in terms of estimated free energy of binding (kcal/mol). The used enzyme (1UYK) contains a natural ligand which was used as a reference for docking process.[55] Docking simulations were carried out with the aid of Docking Server (MOE).[56] It was found that the main amino acids involved in binding to the ligand; Asn (51), Asp (93) and Phe (138) as shown in figure 4.

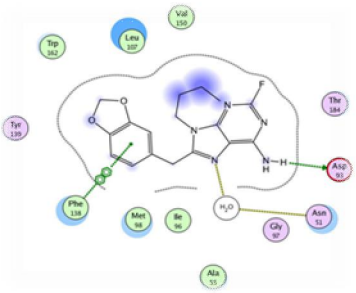


Figure 4. Binding site of docked ligand with 1UYK protein.

There is a strong match, in most of compounds, between the obtained results from the docking studies and those of the biological screening; for examples, binding of compound **8** with the protein site is afforded through the amino group which is bound to amino acid Asn 51, the pyrazole ring is bound to the amino acid Asp 93 and the phenyl substitution with amino acid Phe 138 afforded another hydrophobic attraction. Another example is observed for compound **20** with the protein site where the Asn 51 amino acid binds with the cyano group on the pyridine ring. Good hydrophobic attraction between the pyrazole ring and the amino acid Phe 138, also the amino acid Asp 93 binds with the hydrazine group on the pyridine ring providing more binding of the ligand with the receptor site. Other examples for the docked structures are shown in figure 5.

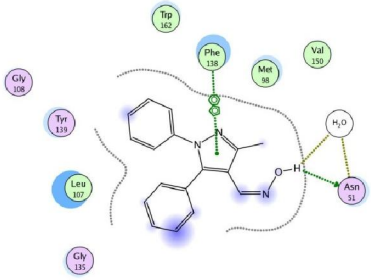


Figure 5. Docking of 3 in 1UYK binding side.

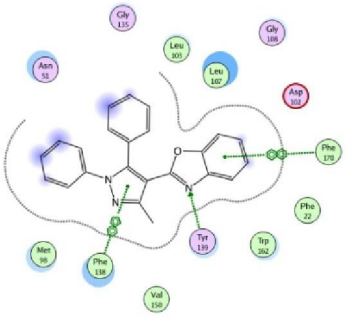


Figure 6. Docking of 7 in 1UYK binding side.

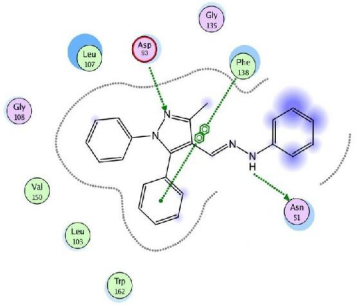


Figure 7. Docking of 8 in 1UYK binding side.

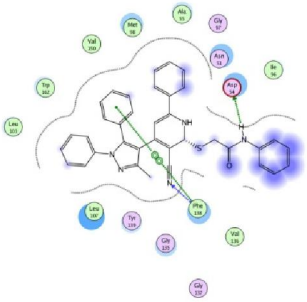


Figure 8. Docking of 15 in 1UYK binding side.

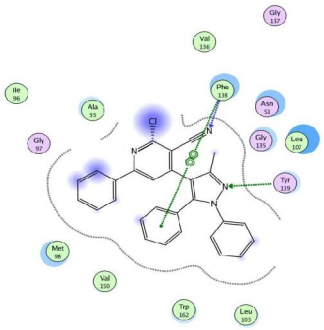


Figure 9. Docking of 17 in 1UYK binding side.

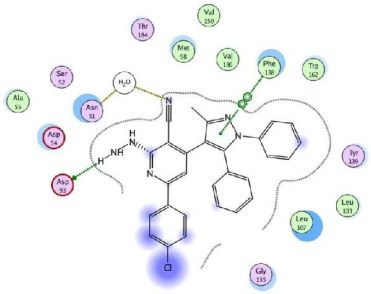


Figure 10. Docking of 20 in 1UYK binding side.

4. Discussion

4.1. Chemistry

Melting points are uncorrected. They were recorded by Open Capillary tube method using on Electro-thermal Melting Point apparatus. IR spectra were recorded on Mattson 5000 FT-IR spectrometer

(υ in cm^{-1}) using KBr disk at Faculty of Science, Mansoura University. ^{1}H-NMR and ^{13}C-NMR spectra were obtained on NMR spectrometer (200 MHz) Gemini Varian using TMS as internal standard, (chemical shifts in ppm, δ units) microanalytical centre, Cairo University. Mass spectral analyses were performed on a JOEL JMS-600H spectrometer at Cairo University. Microanalyses (C, H, N) were performed at Micro-analytical Unit, Cairo University, and were in agreement with the proposed structures within ±0.4 of the calculated values. All reagents were purchased from the Aldrich Chemical Company. Substrates **1** (Genin *et al.,* 2000), **2** (Genin *et al.,* 2000), **9** (Finar and Manning, 1961) were synthesized according to reported methods.

3-Methyl-1,5-diphenyl-1H-pyrazole-4-carbaldehyde oxime (3)

Heating 3-methyl-1,5-diphenyl-1H-pyrazole-4-carbaldehyde (**2)** (4.22 mmol) and hydroxylamine hydrochloride (14.50 mmol) under reflux for 10 h in absolute ethanol (20 ml) and 3 ml pyridine. Pouring onto cold water, filtration and crystallization of the obtained precipitate from petroleum ether yielded the titled compound: Yield (90%); mp 196-198 ^{0}C; IR: 1597 (C=N), 3410 cm^{-1} (OH); ^{1}H NMR: δ 2.39 (s, 3H, CH3), 7.21-7.51 (m, 10H, Ar-H) 8.38 (s, 1H, CH=N), 8.91 (s, 1H, OH); MS: m/z 277 [M^+]. Anal. calcd. for $C_{17}H_{15}N_3O$ (277): C, 73.63; H, 5.45; N, 15.15. Found: C, 73.34; H, 5.69; N, 15.43.

General procedure for the preparation of compounds 4 – 6

A mixture of compound **2** (17.53 g, 0.05 mol), the appropriate aromatic amine; 2-chloroaniline or 2-fluroaniline or 4-methylaniline (0.05 mol) in absolute ethanol (20 mL) and glacial acetic acid (0.5 mL) were heated under reflux for 5 hr. The precipitate after evaporation of the resulting clear solution was crystallized from aqueous ethanol.

2-Chloro-N-[(3-methyl-1,5-diphenyl-1*H*-pyrazol-4-yl)methylene]aniline (4): Yield (52%); mp 147-149 ^{0}C; ^{1}H NMR: δ 2.41 (s, 3H, CH_3), 7.12-7.80 (m, 14H, Ar-H), 8.63 (s, 1H, CH=N); MS: m/z 372 [M^++1], 373 [M^++2]. Anal. Calcd for $C_{23}H_{18}ClN_3$ (371): C, 74.29; H, 4.88; N, 11.30. Found: C, 74.32; H, 4.67; N, 11.63.

2-Fluoro-N-[(3-methyl-1,5-diphenyl-1*H*-pyrazol-4-yl)methylene]aniline (5): Yield (65%); mp 153-155 ^{0}C; ^{1}H NMR: δ 2.39 (s, 3H, CH_3), 7.32-8.10 (m, 14H, Ar-H), 8.45 (s, 1H, CH=NH); MS: m/z 356 [M^++1], 357 [M^++2]. Anal. Calcd for $C_{23}H_{18}FN_3$ (355): C, 77.73; H, 5.10; N, 11.82. Found: C, 77.59; H, 5.39; N, 11.64.

4-Methyl-N-[(3-methyl-1,5-diphenyl-1*H*-pyrazol-4-yl)methylene]aniline (6): Yield (69%); mp 174-176 ^{0}C; ^{1}H NMR: δ 2.34 (s, 3H, CH_3), 2.53 (s, 3H, CH_3), 7.14-7.83 (m, 14H, Ar-H), 8.72 (s, 1H, CH=NH); ^{13}C NMR: 19.36, 25.66, 115.14, 123.02, 125.32, 126.39, 128.36, 129.63, 130.84, 133.12, 136.69, 139.57, 140.06, 142.65, 146.38, 153.06, 158.88, and 163.93; MS: m/z 351 [M^+]. Anal. Calcd for $C_{24}H_{21}N_3$ (351): C, 82.02; H, 6.02; N, 11.96. Found: C, 82.36; H, 6.25; N, 11.79**.**

2-(3-Methyl-1,5-diphenyl-1*H*-pyrazol-4-yl)-1,3-benzoxazole (7)

Compound **2** (0.004 mol) and o-aminophenol (0.006 mol) were heated under reflux in absolute ethanol (30 ml) at 70 ^{0}C for 7 h, then poured onto cold water, filtered and the precipitate was crystallized from petroleum ether giving compound **7**: Yield (64 %); mp 151-153 ^{0}C; ^{1}H NMR: δ 2.36 (s, 3H, CH_3), 6.62-8.11 (m, 14H, Ar-H); MS: m/z 352 [M^+]. Anal. Calcd for $C_{23}H_{17}N_3O$ (351): C, 78.61; H, 4.88; N, 11.96. Found: C, 78.47; H, 4.59; N, 11.78.

3-Methyl-1,5-diphenyl-4-[1-phenyl(hydrazinylidenemethyl)]-1*H*-pyrazole (8)

Compound **2** (0.004 mol), phenyl hydrazine (0.005 mol) and 2-3 drops of glacial acetic acid were heated under reflux in absolute ethanol for 4 h. Cold water was added to the hot mixture and the formed precipitate was filtered and crystallized from petroleum ether; brown compound; Yield (71%); mp 123-125 ^{0}C; IR: 1593 (C=N), 3305 cm^{-1} (N-H); ^{1}H NMR: δ 2.40 (s, 3H, CH_3), 8.20 (s, 1H, CH=N), 11.44 (s, 1H, NH D_2O exchangeable); MS: m/z 352 [M^++1], 353 [M^++2]. Anal. Calcd for $C_{23}H_{20}F_4$ (352): C, 78.38; H, 5.72; N, 15.90. Found: C, 78.16; H, 5.59; N, 15.63.

1-(4-Chlorophenyl)-3-[(3-methyl)-1,5-diphenyl-1*H*-pyrazol-4-yl]prop-2-en-1-one 10

Compound **2** (2.6 g, 0.01 mol), chloroacetophenone (0.01 mol) in ethanol (30 ml) and sodium hydroxide (0.025 mol) in ethanol (20 ml) were stirred at room temperature for 2 h. Filteration and washing the preciptate with cold water then crystallization from ethanol: Yield (85%); mp 155-157 ^{0}C; IR: 1658 (C=O), 1590 cm^{-1} (C=N); ^{1}H NMR: δ 2.41 (s, 3H, CH_3), 6.8 (d, 1H, J=16.3), 7.2 (d, 1H, J= 16.3), 7.17-7.44 (m, 14H, Ar-H); MS: m/z 399 [M^++1], 400 [M^++2].

General procedure for the preparation of compounds 11 and 12

Method A: A mixture of acetophenone or 4-chloroacetophenone (0.002 mol), 3-methyl-1,5-diphenyl-1H-pyrazole-4-carbaldehyde (**2)** (0.002 mol), ethylcyanoacetate (2.5 mmol) and ammonium acetate (20 mmol) was heated under reflux in ethanol (50 ml). The formed precipitate was filtered and crystallized from DMF/ethanol 1:2, respectively yielding compounds **11** and **12**, respectively, but in method B: Ethylcyanoacetate (0.005 mol), ammonium acetate (0.04 mol) in ethanol (30 ml) were heated under reflux with compounds **9**[52] and **10**

(0.005 mol) for 6 h. The precipitated solid, on cooling, was filtered, dried and recrystallized to give compounds **11** and **12**, respectively. It was observed that method A produced higher yield than method B.

4-(3-Methyl-1,5-diphenyl-1*H*-pyrazol-4-yl)-2-oxo-6-phenyl-1,2 dihydropyridine-3-carbonitrile (11): Yield (86%); mp 110-112 ^{0}C. IR: 1635 (C=N), 1560 (C=C), 1669 (C=O), 2217 (CN), 3449 cm^{-1} (N-H); ^{1}H NMR: δ 2.42 (s, 3H, CH_3), 7.18-8.11 (m, 15H, Ar-H), 7.20 (s, 1H, H-pyridine), 7.36 (NH D_2O exchangeable); MS: m/z (%): 428 [M^+]. Anal. Calcd for $C_{28}H_{20}N_4O$ (428): C, 78.49; H, 4.70; N, 13.08. Found: C, 78.28; H, 4.97; N, 13.39.

6-(4-Chlorophenyl)-4-(3-methyl-1,5-diphenyl-1*H*-pyrazol-4-yl)-2-oxo-1,2-dihydropyridine-3-carbonitrile (12): Yield (88%); mp 174-176 ^{0}C; IR: 1649 (C=N), 1556 (C=C), 1710 (C=O), 2216 (CN), 3356 cm^{-1} (N-H); ^{1}H NMR: 2.50 (s, 3H, CH_3), 6.34 (s, 1H, H-pyridine), 7.32-8.22 (m, 14H, Ar-H), 7.59 (NH D_2O exchangeable). ^{13}C NMR: δ 22.32, 110.21, 117.36, 122.45, 124.32, 125.93, 127.45, 128.34, 129.47, 131.05, 132.54, 137.30, 140.58, 144.78, 151.34, 155.42, 159.34, 162.74; MS: m/z 463 [M^++1], 464 [M^++2]. Anal. Calcd for $C_{28}H_{19}ClN_4O$: C, 72.65; H, 4.14; N, 12.10. Found: C, 72.47; H, 4.37; N, 12.34.

General procedure for the preparation of compounds 13 and 14: Heating compounds **11** or **12** (0.01 mol), phosphorous pentasulphide (4.44 g, 0.01 mol) in pyridine (25 ml) under reflux for 6 h, then filtrated the formed preciptate, dried and recrystallized from DMF-water to give compounds **13** and **14,** respectively.

4-(3-Methyl-1,5-diphenyl-1*H*-pyrazol-4-yl)-6-phenyl-2-sulfanylidene-1,2-dihydropyridine-3-carbonitrile (13): Yield (65%); mp 210-212 ^{0}C; IR: 1576 (C=N), 2219 (CN), 3320 cm^{-1} (N-H); ^{1}H NMR: δ 2.24 (s, 3H, CH_3), 6.16-7.89 (m, 15H, Ar-H), 6.81 (s, 1H, H-pyridine), 7.52 (NH D_2O exchangeable); MS: m/z (%): 444 [M^+]. Anal. Calcd for $C_{28}H_{20}N_4S$ (444): C, 75.65; H, 4.53; N, 12.60. Found: C, 75.36; H, 4.69; N, 12.89.

6-(4-Chlorophenyl)-4-(3-methyl-1,5-diphenyl-1*H*-pyrazol-4-yl)-2-sulfanylidene-1,2-dihydropyridine-3-carbonitrile (14): Yield (82%); mp 183-185 ^{0}C; IR: 1570 (C=N), 2218 (CN), 3313 cm^{-1} (N-H); ^{1}H NMR: δ 2.56 (s, 3H, CH_3), 6.32-8.01 (m, 14H, Ar-H), 6.74 (s, 1H, H-pyridine), 7.60 (NH D_2O exchangeable); MS: m/z (%): 478 [M^++1], 480 [M^++2]. Anal. Calcd for $C_{28}H_{19}ClN_4S$ (478): C, 70.21; H, 4.00; N, 11.70. Found: C, 70.54; H, 4.25; N, 11.96.

General procedure for the preparation of compounds 15 and 16

2-Chloro-N-phenylacetamide (0.003 mol), compounds **13** or **14** (0.003 mol) in DMF (15 ml) were heated under reflux for 10 h. On cooling, the formed precipitate was filtrated and recrystallized from DMF-water yielding the corresponding titled compounds **15** and **16.**

2-{[3-Cyano-4-(3-methyl-1,5 diphenyl-1*H*-pyrazol-4-yl)-6 phenyl-pyridin-2-yl]sulfany}N-phenylacetamide (15): Yield (67%); mp 253-255 ^{0}C; IR: 1652 (C=O), 2214 (CN), 3330 cm^{-1} (N-H); ^{1}H NMR: δ 2.1 (s, 3H, CH_3), 4.19(s, 2H, CH_2), 7.12-8.32 (m, 20H, Ar-H), 7.53 (NH D_2O exchangeable), 8.67 (s, 1H, Ar-H); MS: m/z (%): 577 [M^+]. Anal. Calcd for $C_{36}H_{27}N_5OS$ (577): C, 74.85; H, 4.71; N, 12.12. Found: C, 74.78; H, 4.93; N, 12.31.

2-{[6-(4-Chlorophenyl)-3-cyano-4-(3-methyl-1,5-diphenyl-1*H*-pyrazol-4-yl)-pyridin-2-yl]sulfanyl}N-phenylacetamide (16): Yield (69%); mp 194-196 ^{0}C; IR: 1659 (C=O), 2218 (CN), 3339 (N-H); ^{1}H NMR: δ 2.5 (s, 3H, CH_3), 4.36 (s, 2H, CH_2), 7.21-8.35 (m, 19H, Ar-H), 7.44 (NH D_2O exchangeable), 8.51 (s, 1H, Ar-H); MS: m/z (%): 612 [M^++1], 613 [M^++2]. Anal. Calcd for $C_{36}H_{26}ClN_5OS$ (612): C, 70.63; H, 4.28; N, 11.44. Found: C, 70.69; H, 4.43; N, 11.70.

General procedure for the preparation of compounds 17 and 18

A mixture of compound **11** or **12** (0.0013 mol), phosphorous oxychloride (0.026 mol) and N,N-diethylaniline (0.1 ml) were refluxed for 6 h. Crushed ice was added, the product was collected by filteration, dried and extracted with ether (20 ml), the ethereal layer was dried afforded the title compounds.

2-Chloro-4-(3-methyl-1,5-diphenyl-1*H*-pyrazol-4-yl)-6-phenylpyridine-3-carbonitrile (17): Yield (66%); mp 98-100 ^{0}C; IR:1645 (C=N), 2232 cm^{-1} (CN); ^{1}H NMR: δ 2.4 (s, 3H, CH_3), 7.14-7.57 (m, 15H, Ar-H), 8.35 (s, 1H, H-pyridine); MS: m/z 446 [M^+]. Anal. Calcd for $C_{28}H_{19}ClN_4$: C, 75.25; H, 4.28; N, 12.54. Found: C, 75.45; H, 4.57; N, 12.35.

2-Chloro-6-(4-chlorophenyl)-4-(3-methyl-1,5-diphenyl-1*H*-pyrazol-4-yl)pyridine-3-carbonitrile (18): Yield (63%); mp 130-132 ^{0}C; IR: 1589 (C=N), 2223 cm^{-1} (CN); ^{1}H NMR: δ 2.2 (s, 3H, CH_3), 6.57-7.67 (m, 14H, Ar-H), 8.14 (s, 1H, H-pyridine); MS: m/z 481 [M^++1], 482 [M^++2]. Anal. calcd. for $C_{28}H_{18}Cl_2N_4$: C, 69.86; H, 3.77; N,11.64. Found: C, 69.68; H, 3.63; N, 11.82.

General procedure for the preparation of compounds 19 and 20

Hydrazine hydrate (0.7 g, 0.02 mol) was added to compounds **17** or **18** (0.001 mol) in ethanol. The mixture was heated under reflux for 12 h. The solvent was concentrated in vacuo. The separated solids were recrystallized from methanol to afford the title compounds.

2-Hydrazinyl-4-(3-methyl-1,5-diphenyl-1*H*-pyrazol-4-yl)-6-phenylpyridine-3-carbonitrile

(19): Yield (61%); mp 179-180 ^{0}C; IR: 1573 (C=N), 2217 (CN), 3300 (NH), 3426 cm^{-1}(NH_2); ^{1}H NMR: δ 2.10 (d, 2H, NH_2, D_2O exchangeable), 2.42 (s, 3H, CH_3), 4.36 (t, 1H, NH), 6.08-8.31 (m, 15H, Ar-H), 7.25 (s, 1H, H-pyridine); MS: m/z 442 [M^+]. Anal. Calcd for $C_{28}H_{22}N_6$: C, 76.00; H, 5.01; N, 18.99. Found: C, 76.34; H, 5.28; N, 18.63.

6-(4-Chlorophenyl)-2-hydrazinyl-4-(3-methyl-1,5-diphenyl-1*H*-pyrazol-4-yl) pyridine-3-carbonitrile (20): Yield (56%); mp 109-111 ^{0}C; IR: 1579 (C=N), 2215 (CN), 3365 (NH), 3350 cm^{-1} (NH_2); ^{1}H NMR: δ 2.19 (d, 2H, NH_2, D_2O exchangeable), 2.36 (s, 3H, CH_3), 4 (t, 1H, NH), 6.77-8.14 (m, 14H, Ar-H), 7.27 (s, 1H, H-pyridine); MS: m/z 477 [M^++1], 478 [M^++2]. Anal. Calcd for $C_{28}H_{21}ClN_6$: C, 70.51; H, 4.44; N, 17.62. Found: C, 70.79; H, 4.74; N, 17.32.

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