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 were established 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.

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](image1.png) Figure 1. I) celecoxib, II-IV) 1,5-diphenylpyrazole derivatives as antibreast cancer agents.

![Figure 2](image2.png) 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-1H-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 benzoazole 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 (Alabadi 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-1H-pyrazole-4-carbaldehyde (2) was prepared according to literature procedure (Genin et al., 2000) by Vilsmeiere Haack reaction of 3-methyl-1,5-diphenyl-1H-pyrazole (1) (Genin et al., 2000). Condensation of the starting compound 2 with hydroxylamine hydrochloride yielded the carboxaldehyde oxime derivative 3. 1H 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 derivative 8 was obtained by the reaction of compound 2 with phenyldrazine 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) POCI₃, DMF; B) NH₂OH, pyridine, absolute ethanol; C) R-C₆H₄-NH₂, glacial acetic acid, absolute ethanol; D) o-aminophenol, absolute ethanol; E) phenyldrazine, 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-1H-pyrazole-4-carbaldehyde (2) with equimolar amount of acetoephene 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 acetoephene 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 compounds 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, \(^1\)H 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-sulfonylidene-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.

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\(_2\)N\(_4\)S and a peak at 482 (M\(^+\)) 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. \(^1\)H 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. The synthesis of 17-20: A) ethyl cyanoacetate, ammonium acetate, absolute ethanol; B) POCl\(_3\), N, N-DEA; C) Hydrazine hydrate, absolute ethanol.](image)

### 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 °C) and maintained by serial sub-culturing in 75 cm\(^2\) cell culture flasks (Fisher Scientific, Pitts burgh, PA) at 37 °C 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\textsubscript{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 Hyperchem (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 hydrazonel derivative 8 and N-phenylacetamide derivatives 15 and 16 greatly increased the antitumor activity.

Otherwise the introduction of 4-chloro or 4-fluoro 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-sulfanyliden-1,2-dihydropyridine-3-carboxaldehyde oxime derivative 7 increased the activity greatly.

#### 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.\textsuperscript{55} Docking simulations were carried out with the aid of Docking Server (MOE).\textsuperscript{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.

### 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).

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<th>Comp. No.</th>
<th>% surviving</th>
<th>% inhibition</th>
<th>IC\textsubscript{50}, µg/mL</th>
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\textsuperscript{55} Docking simulations were carried out with the aid of Docking Server (MOE).

\textsuperscript{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.
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.

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.
(o in cm⁻¹) using KBr disk at Faculty of Science, Mansoura University. 1H-NMR and 13C-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 I (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 174-176°C.

### 2-Chloro-N-[(3-methyl-1,5-diphenyl-1H-pyrazol-4-yl)methylene]aniline (4): Yield (52%); mp 147-149°C; IR: 1597 (C=O), 1590 cm⁻¹ (C=N); 1H NMR: δ 2.39 (s, 3H, CH₃), 7.12-7.80 (m, 14H, Ar-H); MS: m/z 372 [M+1], 373 [M+2]. Anal. Caled for C₇H₁₃N₂O (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 h. The precipitate after evaporation of the resulting clear solution was crystallized from aqueous ethanol.

### 2-Chloro-N-[3-methyl-1,5-diphenyl-1H-pyrazol-4-yl]methylene]aniline (4): Yield (52%); mp 147-149°C; IR: 1597 (C=O), 1590 cm⁻¹ (C=N); 1H NMR: δ 2.41 (s, 3H, CH₃), 7.12-7.80 (m, 14H, Ar-H), 8.63 (s, 1H, CH=N); MS: m/z 372 [M+1], 373 [M+2]. Anal. Caled for C₇H₁₃N₂O (277): 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-1H-pyrazol-4-yl]methylene]aniline (5): Yield (65%); mp 153-155°C; IR: 1658 (C=O), 1590 cm⁻¹ (C=N); 1H NMR: δ 2.32 (s, 3H, CH₃), 7.32-8.10 (m, 14H, Ar-H), 8.45 (s, 1H, CH=N); MS: m/z 356 [M+1], 357 [M+2]. Anal. Caled for C₇H₁₃FNO (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-1H-pyrazol-4-yl]methylene]aniline (6): Yield (69%); mp 174-176°C; IR: 1597 (C=O), 3410 cm⁻¹ (OH); 1H NMR: δ 2.34 (s, 3H, CH₃), 7.14-7.83 (m, 14H, Ar-H), 8.72 (s, 1H, CH=N); 13C 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. Caled for C₁₇H₁₃N₂O (351): C, 78.02; H, 6.02; N, 11.96. Found: C, 82.36; H, 6.25; N, 11.79.

### 2-(3-Methyl-1,5-diphenyl-1H-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°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°C; IR: 1593 (C=O), 3305 cm⁻¹ (N-H); 1H NMR: δ 2.36 (s, 3H, CH₃), 6.62-8.11 (m, 14H, Ar-H); MS: m/z 352 [M⁺]. Anal. Caled for C₁₇H₁₃N₂O (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)-1H-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°C; IR: 1593 (C=O), 3305 cm⁻¹ (N-H); 1H NMR: δ 2.40 (s, 3H, CH₃), 8.20 (s, 1H, CH=N), 11.44 (s, 1H, NH D₂O exchangeable); MS: m/z 352 [M⁺+1], 353 [M⁺+2]. Anal. Caled for C₁₇H₁₃N₂O (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-1H-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 (30 ml) at 70°C were stirred at room temperature for 2 h. Filtration and washing the precipitate with cold water then crystallization from ethanol: Yield (85%); mp 155-157°C; IR: 1658 (C=O), 1590 cm⁻¹ (C=N); 1H NMR: δ 2.41 (s, 3H, CH₃), 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-pyrazol-4-carbaldehyde (2) (0.002 mol), ethylcyanooacetate (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: Ethylcyanooacetate (0.005 mol), ammonium acetate (0.04 mol) in ethanol (30 ml) were heated under reflux with compounds 952 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-1H-pyrazol-4-yl)-2-oxo-1,2-dihydropyridine-3-carbonitrile (11): Yield (86%); mp 110-112 °C; IR: 1635 (C=N), 1560 (C=C), 1669 (C=O), 2217 (CN), 3449 cm⁻¹ (N-H); ¹H NMR: δ 2.42 (s, 3H, CH₃), 7.18-8.11 (m, 15H, Ar-H), 7.20 (s, 1H, H-pyridine), 7.36 (NH D₂O exchangeable); MS: m/z (%): 428 [M⁺]. Anal. Calcd for C₂₅H₂₀N₅O (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-1H-pyrazol-4-yl)-2-oxo-1,2-dihydropyridine-3-carbonitrile (12): Yield (88%); mp 174-176 °C; IR: 1649 (C=N), 1556 (C=C), 1710 (C=O), 2216 (CN), 3356 cm⁻¹ (N-H); ¹H NMR: 2.50 (s, 3H, CH₃), 6.34 (s, 1H, H-pyridine), 7.32-8.22 (m, 14H, Ar-H), 7.59 (NH D₂O exchangeable). ¹³C 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 C₂₅H₂₀ClN₅O: 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 filtered the formed precipitate, dried and recrystallized from DMF-water to give compounds 13 and 14, respectively.

4-(3-Methyl-1,5-diphenyl-1H-pyrazol-4-yl)-6-phenyl-2-sulfanylidene-1,2-dihydropyridine-3-carbonitrile (13): Yield (65%); mp 210-212 °C; IR: 1576 (C≡N), 2219 (CN), 3320 cm⁻¹ (N-H); ¹H NMR: δ 2.24 (s, 3H, CH₃), 6.16-7.89 (m, 15H, Ar-H), 6.81 (s, 1H, H-pyridine), 7.52 (NH D₂O exchangeable); MS: m/z (%): 444 [M⁺]. Anal. Calcd for C₂₅H₂₀N₅S (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-1H-pyrazol-4-yl)-2-sulfanylidene-1,2-dihydropyridine-3-carbonitrile (14): Yield (82%); mp 183-185 °C; IR: 1570 (C≡N), 2218 (CN), 3313 cm⁻¹ (N-H); ¹H NMR: δ 2.56 (s, 3H, CH₃), 6.32-8.01 (m, 14H, Ar-H), 6.74 (s, 1H, H-pyridine), 7.60 (NH D₂O exchangeable); MS: m/z (%): 478 [M⁺+1], 480 [M⁺+2]. Anal. Calcd for C₂₅H₂₀ClN₅S (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 filtered and recrystallized from DMF-water yielding the corresponding titled compounds 15 and 16.

2-{[3-Cyano-4-(3-methyl-1,5-diphenyl-1H-pyrazol-4-yl)-6-phenyl-pyridin-2-yl]sulfanyl}N-phenylacetamide (15): Yield (67%); mp 253-255 °C; IR: 1652 (C=O), 2214 (CN), 3330 cm⁻¹ (N-H); ¹H NMR: δ 2.1 (s, 3H, CH₃), 4.19 (s, 2H, CH₂), 7.12-8.32 (m, 20H, Ar-H), 7.53 (NH D₂O exchangeable), 8.67 (s, 1H, Ar-H); MS: m/z (%): 577 [M⁺]. Anal. Calcd for C₃₆H₂₇N₅OS (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-1H-pyrazol-4-yl)-pyridin-2-yl)sulfanyl]N-phenylacetamide (16): Yield (69%); mp 194-196 °C; IR: 1659 (C=O), 2218 (CN), 3339 (N-H); ¹H NMR: δ 2.5 (s, 3H, CH₃), 4.36 (s, 2H, CH₂), 7.21-8.35 (m, 19H, Ar-H), 7.44 (NH D₂O exchangeable), 8.51 (s, 1H, Ar-H); MS: m/z (%): 612 [M⁺+1], 613 [M⁺+2]. Anal. Calcd for C₃₆H₂₇ClN₅OS (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 filtration, dried and extracted with ether (20 ml), the etherial layer was dried afforded the title compounds. 2-Chloro-4-(3-methyl-1,5-diphenyl-1H-pyrazol-4-yl)-6-phenylpyridine-3-carbonitrile (17): Yield (66%); mp 98-100 °C; IR: 1645 (C≡N), 2232 cm⁻¹ (CN); ¹H NMR: δ 2.8 (s, 3H, CH₃), 7.14-7.57 (m, 15H, Ar-H), 8.35 (s, 1H, H-pyridine); MS: m/z 446 [M⁺]. Anal. Calcd for C₂₅H₁₆ClN₅S (446): C, 75.25; H, 4.43; N, 12.35.

2-Chloro-6-(4-chlorophenyl)-4-(3-methyl-1,5-diphenyl-1H-pyrazol-4-yl)pyridine-3-carbonitrile (18): Yield (63%); mp 130-132 °C; IR: 1589 (C≡N), 2223 cm⁻¹ (CN); ¹H NMR: δ 2.2 (s, 3H, CH₃), 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 C₂₅H₁₈Cl₂N₅: 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-1H-pyrazol-4-yl)-6-phenylpyridine-3-carbonitrile
(19): Yield (61%); mp 179-180 0C; IR: 1573 (C=N), 2217 (CN), 3365 (NH), 3350 cm-1(NH); 1H NMR: δ 2.10 (d, 2H, NH), 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 C28H28ClN6: C, 76.34; H, 5.28; N, 18.63. Found: C, 76.34; H, 5.28; N, 18.39. 

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