Synthesis and *In Vitro* Cytotoxicity of New 3-(5-methyl-1-aryl-1*H*-1,2,3-triazol-4-yl)-1-phenyl-1*H*-pyrazoles

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Abstract: In this paper we present the synthesis of a novel series of 3-(5-methyl-1-aryl-1*H*-1,2,3-triazol-4-yl)-1-phenyl-1*H*-pyrazoles **3-6** by employing Vilsmeier-Haack reaction followed by Knoevenagel condensation on 1-aryl-3-acetyl-4-methyl-1,2,3-triazoles **1a,b.** The cytotoxicity and *in vitro* anticancer evaluation of the prepared compounds have been assessed against four different human cancer cell lines including breast MCF-7; liver HepG2 and lung cancer A549 as well as human normal melanocyte HFB4. The results revealed that the prepared compounds exert their actions in MCF-7 and HepG2. Compounds **4a** and **6a** revealed promising anticancer activity compared to the activity of the commonly used anticancer drug, doxorubicin. MCF-7 cells are slightly more sensitive to the tested compounds than HepG2 cells.

Keywords: 1,2,3-triazoles, Vilsmeier-Haack reaction, Knoevenagel condensation, anticancer, MCF-7, HepG2, A549, HFB4.

1. INTRODUCTION

Cancer comprises a group of complicated diseases characterized by the unregulated growth of abnormal cells that have the capability of invading normal tissues, metastasizing to other organs and spreading to other body parts [1, 2]. As a leading cause of death and disability, cancer is responsible for nearly 7.6 million deaths per year [3]. Chemotherapy is one of the important approaches used in cancer therapy, but severe side effects resulting from toxicity of chemotherapeutics on normal cells remains an important obstacle in clinical application [4, 5]. The pyrazolyl-1,2,3-triazoles have been the subject of considerable research due to its usefulness in synthetic organic chemistry and because of the pharmacological properties shown by some of its derivatives. Many pyrazolyl-1,2,3-triazoles are found to be more potent anticancer activities [6-8]. In the same direction and in continuing effort to find more potent and selective biologically active heterocyclic compounds [9-11], herein, we designed and synthesized a series of pyrazolyl-1,2,3-triazole compounds. Their biological activities against four different human tumor cell lines including breast cancer MCF-7, liver cancer HepG2 and lung cancer A549 cell lines were evaluated. Additionally, we estimated the cytotoxicity effect of the prepared compounds against the normal cell line (human normal melanocyte, HFB4).

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2. RESULTS AND DISCUSSION

2.1. Chemistry

1-Aryl-3-acetyl-4-methyl-1,2,3-triazoles **1a,b** were prepared according to the reported method [12] and **1c** was synthesized based on literature procedure [12]. Condensation of **1a-c** with phenyl hydrazine in absolute ethanol in the presence of drops of glacial acetic acid gave 5-methyl-1-aryl-4-(1-(2-phenylhydrazono)ethyl)-1*H*-1,2,3-triazoles **2a-c**. Application of Vilsmeier-Haack reaction on hydrazones **2a-c**,by treatment of this hydrazones with phosphorusoxy chloride and dimethylformamide (1:2) afforded the target 3-(5-methyl-1-aryl-1*H*-1,2,3-triazol-4-yl)-1-phenyl-1*H*-pyrazole-4-carbaldehydes **3a-c** (Scheme **1**).

The structures of the new compounds **3a-c** were confirmed by the spectral data. In the IR spectrum a band in the range 1690-1700 cm^{-1} characteristic of the CHO group was observed, while the ^{1}H NMR spectrum showed a singlet signal for one proton at δ 9.25 ppm due to the pyrazole fragment and a singlet for one proton at δ 10.56 ppm due to the CHO fragment.

The carboxaldehydes **3a-c** are considered to be a useful starting materials for further synthesis, thus **3a-c** were treated with benzoylaceteonitrile, 3-methyl-1-phenyl-1*H*-pyrazol-5(4*H*)-one and 1*H*-indene-1,3(2*H*)-dione in absolute ethanol and in the presence of a catalytic amount of piperidine (Knoevenagel condensation) to afforded the corresponding products **4a-c**, **5a-c** and **6a-c** respectively (Scheme **2**).

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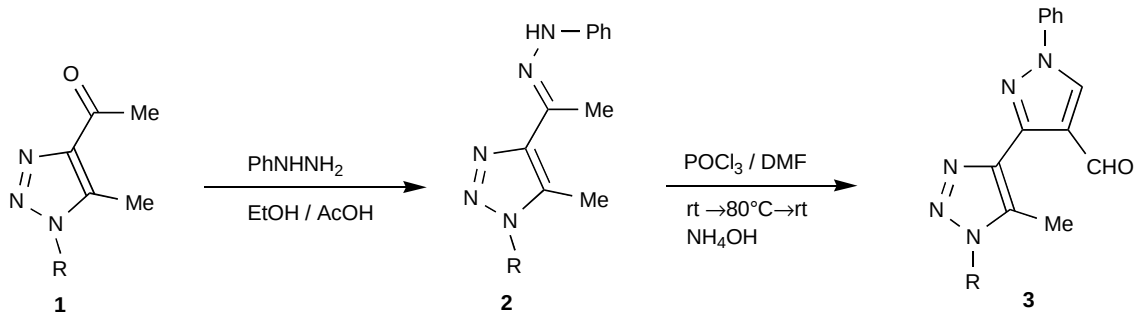
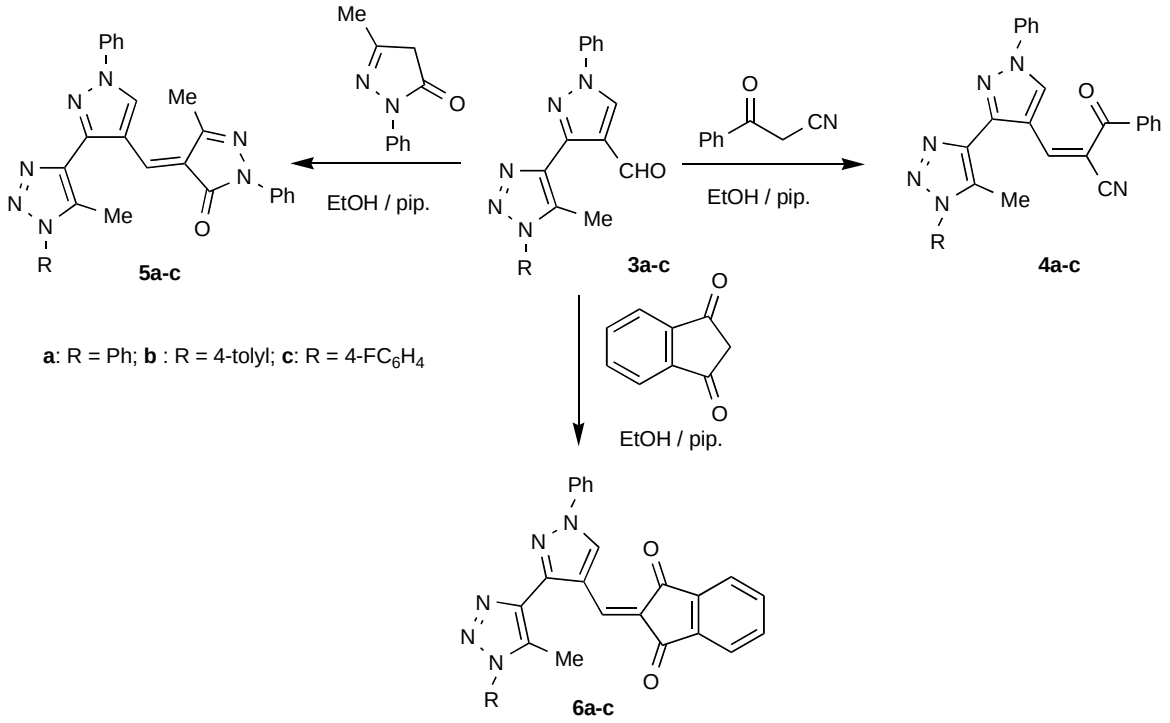


1 **2** **3**

a: R = Ph; **b**: R = 4-tolyl; **c**: R = 4-FC_6H_4

Scheme 1:



5a-c **3a-c** **4a-c**

a: R = Ph; **b** : R = 4-tolyl; **c**: R = 4-FC_6H_4

6a-c

Scheme 2:

The IR spectra of compounds **4a-c** there are bands in the range 2198-2217 cm^{-1}due to the presence of carbonitrile group (CN). Also there is a band at 1685 cm^{-1} due to the carbonyl group (C=O). The ^{1}H NMR spectra of **5a-c** showed a singlet at 9.26 ppm for olefinic protons.

X-ray crystallography of **5c** (crystallized from DMF) confirmed the anticipated structure (Figure **1**).

2.2. *In Vitro* Cytotoxicity Activity

As shown in Table **1**, the cytotoxicity of the synthetic compounds was tested using SRB assay in MCF7, HepG2, and A549 cell lines as well as normal cell line (human normal melanocyte, HFB4). For comparison, doxorubicin was also tested. While treatment with DMSO was used as control cancer cells.

The results revealed that all compounds did not exert any activity against lung cancer A549 cell line. Moreover, the tumor cells showed normal growth in culture system and DMSO did not seem to have any noticeable effect on cellular growth. Additionally, all the synthetic compounds exhibited lower toxicity to the normal HFB4 cell line.

Studying the anticancer activity of the new compounds against MCF-7 and HepG2 cell lines, revealed that although compounds **2b, 3b** and **4b** showed no anticancer activity in MCF-7 and HepG2 cell lines, most of the compounds were found to be

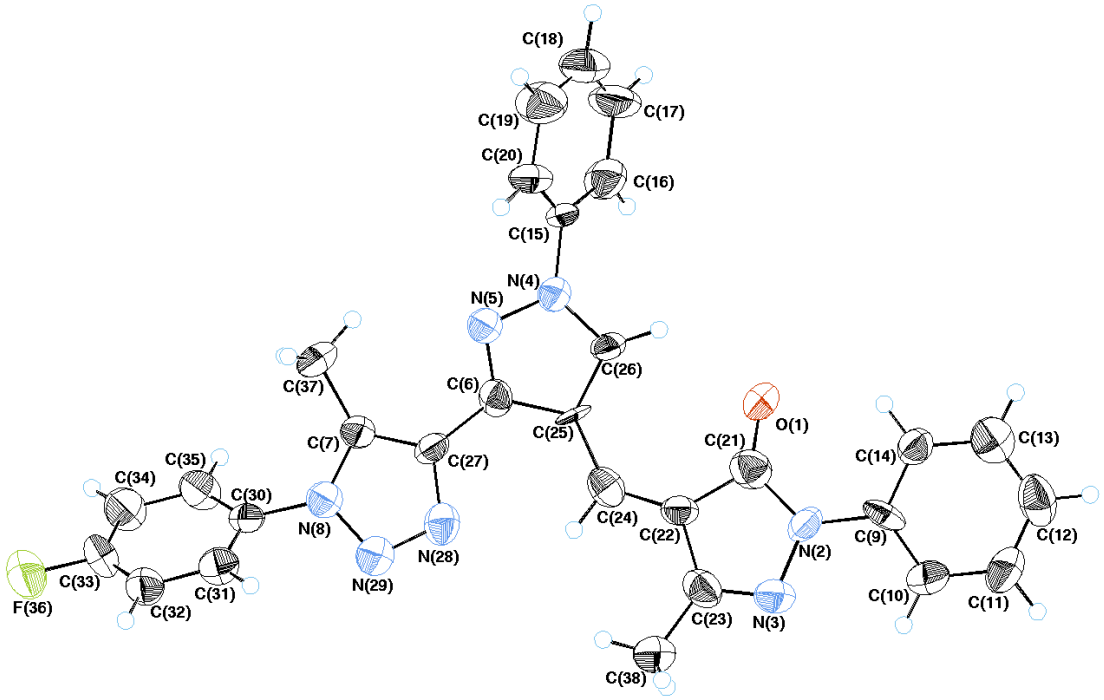


Figure 1:

potent against both cell lines with IC_{50} values ranging from 2.50- 16.10 µg/ml for MCF-7 and ranging from 3.50 – 17.50 µg/ml for HepG2. Moreover, the results showed that compound **4a** was found to be potent anticancer agent had IC_{50} value (5.25±0.55 and 6.10±0.70 µg/ml respectively) near to the standard drug, doxorubicin (2.90±0.27 and 3.70±0.35µg/ml respectively) against MCF-7 and HepG2 cell lines. On the other hand, compound **6a** was found to be more potent than doxorubicin as anticancer agents with IC_{50} values of 2.70±0.30 and 2.50±0.28 µg/ml for compound **6a** versus 2.90±0.27µg/ml for doxorubicin in case of MCF-7. While in case of HepG2, compound **6a** had IC_{50} values of 3.00±0.33 and 3.50±0.40 µg/ml versus 3.70±0.35 µg/ml for doxorubicin.

In conclusion, The tested compounds exert anti-carcinogenic activity in breast MCF-7 and hepatic HepG2 cancer cell lines through reduce the cell proliferation and resulted in significant growth inhibitory, especially, compounds **4a** and **6a** which revealed promising activity compared to the activity of the commonly used anticancer drug, doxorubicin. The present study reveals that among the human cancer cell lines tested, MCF-7 cells are slightly more sensitive to the tested compounds than HepG2 cells.

2.3. Structure Activity Relation Ship

The structure-anticancer activity relationship of the synthesized compounds against four different human cancer cell lines including breast MCF-7; liver HepG2; lung cancer A549 and human normal melanocyte HFB4 revealed that, 2-benzoyl-3-(3-(5-methyl-1-phenyl-1*H*-1,2,3-triazol-4-yl)-1-phenyl-1*H*-pyrazol-4-yl)acrylonitrile **4a** and 2-((3-(5-methyl-1-phenyl-1*H*-1,2,3-triazol-4-yl)-1-phenyl-1*H*-pyrazol-4-yl)methylene)-1*H*-indene-1,3(2*H*)-dione **6a** have promising anticancer activity compared to other synthesized compounds. The compounds which have 1-subs.phenyl-1*H*-1,2,3-triazole **4b,c** and **6b,c** have low anticancer activity than 1- phenyl-1*H*-1,2,3-triazoles.

3. EXPERIMENTAL

3.1. Chemistry

All melting points were determined on Electrothermal IA 9000 series digital melting point apparatus. Elemental analytical data were carried out at the microanalytical unit, Cairo University, Giza, Egypt. The IR spectra were recorded in potassium bromide disks on a JASCO FT/IR-6100. 1H-NMR and ^{13}C-NMR spectra were run on JOEL-ECA 500 MHz in (DMSO-d6). Chemical shifts values (δ) are given in parts per million (ppm). The mass spectra were performed using Shimadzu Qp-2010 plus. Fetal bovine serum (FBS) and L-glutamine, were obtained from Gibco Invitrogen Company (Scotland, UK). Dulbecco's modified Eagle's (DMEM) medium was provided from Cambrex (New Jersey, USA). Doxorubicin, penicillin,

Table 1: *In Vitro* Cytotoxicity Activity of the Synthesized Compounds on Different Cell Lines

Compound	IC_{50} (µg/ml)			
	MCF-7	HepG2	A549	HFB4
Doxo.	2.90±0.27	3.70±0.35	4.30±0.40	74.80±8.50
DMSO	N.A.	N.A.	N.A.	84.30±7.70
2a	12.60±1.30	15.00±1.62	N.A.	76.66±8.20
2b	N.A.	N.A.	N.A.	70.43±8.00
2c	16.10±1.60	17.50±1.80	N.A.	110.20±12.30
3a	11.20±1.20	14.00±1.50	N.A.	94.40±10.00
3b	N.A.	N.A.	N.A.	60.10±6.73
3c	11.20±1.20	14.00±1.50	N.A.	94.40±10.00
4a	5.25±0.55	6.10±0.70	N.A.	44.55±4.90
4b	N.A.	N.A.	N.A.	55.90±6.40
4c	14.70±1.75	16.95±1.70	N.A.	60.90±7.20
5a	3.70±0.55	5.90±0.60	N.A.	64.40±6.00
5b	9.20±0.90	17.20±1.80	N.A.	102.00±11.00
5c	12.80±1.40	14.40±1.50	N.A.	88.50±8.86
6a	2.70±0.30	3.00±0.33	N.A.	77.40±7.50
6b	N.A.	N.A.	N.A.	71.30±7.90
6c	N.A.	N.A.	N.A.	49.90±5.50

Data were expressed as Mean ± Standard error (S.E.) of six independent experiments.
N.A. is no activity.

and streptomycin were obtained from Sigma Chemical Company (Saint Louis, MO, USA).

1-(1-(4-Fluorophenyl)-5-methyl-1*H*-1,2,3-triazol-4-yl)ethanone (1c) was synthesized based on literature procedure [12].

Yield 78 %; m.p. 154-156°C; IR (KBr) νmax/cm^{-1} 1669 (C=O); ^{1}H NMR (DMSO-d_6) δ 1.98, 2.39 (2s, 6H, 2 CH_3), 7.38-7.82 (m, 4 H, Ar-H); MS m/z (%): 219 (M^+, 60), 77 (100); Anal. Calcd for $C_{11}H_{10}FN_3O$ (219.22): C, 60.27; H, 4.60; N, 19.17;%. Found: C, 60.38; H, 4.73; N, 19.33%.

3.1.3. 5-Methyl-1-aryl-4-(1-(2-phenylhydrazono) ethyl)-1H-1,2,3-triazoles (2a-c)

Appropriate 1-(5-methyl-1-aryl-1*H*-1,2,3-triazol-4-yl)ethanones**1a-c** (10 mmol), phenylhydrazine (1.1g, 10.1 mmol) in absolute ethanol (20 mL) and glacial acetic acid (0.5 mL) were heated at reflux temperature for 2.5 h. The formed colorless solid was filtered and drayed to yield **2a-c**.

5-Methyl-1-phenyl-4-(1-(2-phenylhydrazono)ethyl)-1H-1,2,3-triazole (2a)

Yield 82 %; m.p. 220-222°C; IR (KBr) νmax/cm^{-1} 3318 (NH), 1628 (C=N); ^{1}H NMR (DMSO-d_6) δ 1.91, 2.50 (2s, 6H, 2 CH_3), 7.44-7.97 (m, 10 H, Ar-H), 11.15 (s, 1H, 1 NH, D_2O exchangeable); MS m/z (%): 291 (M^+, 57), 144 (100), 91 (97), 65 (90); Anal. Calcd for $C_{17}H_{17}N_5$ (291.35): C, 70.08; H, 5.88; N, 24.04 %. Found: C, 70.21; H, 5.92; N, 24.32%.

5-Methyl-4-(1-(2-phenylhydrazono)ethyl)-1-p-tolyl-1H-1,2,3-triazole (2b)

Yield 87 %; m.p. 201-203°C; IR (KBr) νmax/cm^{-1} 3298 (NH), 1632 (C=N); ^{1}H NMR (DMSO-d_6) δ 1.91, 2.49, 2.62 (3s, 9H, 3 CH_3), 7.46-7.94 (m, 9 H, Ar-H), 11.18 (s, 1H, 1 NH, D_2O exchangeable); MS m/z (%): 306 [$(M+1)^+$, 58)], 305 (M^+, 10), 144 (100), 91 (97), 65 (90); Anal. Calcd for $C_{18}H_{19}N_5$ (305.38): C, 70.80; H, 6.27; N, 22.93 %. Found: C, 70.93; H, 6.41; N, 22.99%.

1-(4-Fluorophenyl)-5-methyl-4-(1-(2-phenylhydrazono)ethyl)-1H-1,2,3-triazole (2c)

Yield 83 %; m.p. 203-204°C; IR (KBr) νmax/cm^{-1} 3295 (NH), 1625 (C=N); ^{1}H NMR (DMSO-d_6) δ 1.90, 2.48 (2s, 6H, 2 CH_3), 7.45-7.93 (m, 9 H, Ar-H), 11.20 (s, 1H, 1 NH, D_2O exchangeable); MS m/z (%): 310 [$(M+1)^+$, 49)], 309 (M^+, 22), 144 (100), 91 (97), 65 (90); Anal. Calcd for $C_{17}H_{16}FN_5$ (309.34): C, 66.01; H, 5.21; N, 22.64%. Found: C, 66.23; H, 5.40; N, 22.78%.

3-(5-Methyl-1-aryl-1H-1,2,3-triazol-4-yl)-1-phenyl-1H-pyrazole-4-carbaldehydes (3a-c)

Phosphorus oxychloride (20 mL, 0.2 mol) was added dropwise with stirring to dimethyl formamide (150 mL) at 0–5 °C. Then appropriate 5-methyl-1-aryl-4-(1-(2-phenylhydrazono)ethyl)-1*H*-1,2,3-triazoles **2a-c** (0.094 mol) was added portion-wise with continuous stirring, left overnight at room temperature, poured onto ice-cold water and neutralized with ammonium hydroxide solution (5 %). The formed precipitate was filtered, dried and recrystallized from ethanol (95 %) to give **3a-c**.

3-(5-Methyl-1-phenyl-1H-1,2,3-triazol-4-yl)-1-phenyl-1H-pyrazole-4-carbaldehyde (3a)

Yield 78 %; m.p. 229-230°C; IR (KBr) νmax/cm^{-1} 1690 (C=O); ^{1}H NMR (DMSO-d_6) δ 2.42 (s, 3H, CH_3), 7.38-8.01 (m, 10 H, Ar-H), 9.25 (s, 1H, pyrazole-H), 10.56 (s, 1H, CHO); ^{13}C-NMR (DMSO-d_6) δ 9.75, 20.66, 38.87, 39.16, 39.45, 39.72, 118.97, 123.05,124.95, 127.55, 129.54, 130.00, 132.98, 133.08, 137.11, 138.42, 139.53, 145.83, 186.66; MS m/z (%): 330 [(M+1)$^+$, 100)], 329 (M$^+$, 10), 77 (98); Anal. Calcd for $C_{19}H_{15}N_5O$ (329.36): C, 69.29; H, 4.59; N, 21.26 %. Found: C, 69.36; H, 4.66; N, 21.60%.

3-(5-Methyl-1-p-tolyl-1H-1,2,3-triazol-4-yl)-1-phenyl-1H-pyrazole-4-carbaldehyde (3b)

Yield 83 %; m.p. 194-196°C; IR (KBr) νmax/cm^{-1} 1701 (C=O); ^{1}H NMR (DMSO-d_6) δ 2.42, 2.60 (2s, 6H, 2 CH_3), 7.40-7.99 (m, 9 H, Ar-H), 9.26 (s, 1H, pyrazole-H), 10.55 (s, 1H, CHO); MS m/z (%): 344 [(M+1)$^+$, 100)], 343 (M$^+$, 12), 77 (98); Anal. Calcd for $C_{20}H_{17}N_5O$ (343.38): C, 69.96; H, 4.99; N, 20.40 %. Found: C, 70.07; H, 5.11; N, 20.68%.

3-(1-(4-Fluorophenyl)-5-methyl-1H-1,2,3-triazol-4-yl)-1-phenyl-1H-pyrazole-4-carbaldehyde (3c).

Yield 77 %; m.p. 248-249°C; IR (KBr) νmax/cm^{-1} 1699 (C=O); ^{1}H NMR (DMSO-d_6) δ 2.41 (s, 3H, CH_3), 7.39-7.98 (m, 9 H, Ar-H), 9.24 (s, 1H, pyrazole-H), 10.53 (s, 1H, CHO); MS m/z (%): 348 [(M+1)$^+$, 100)], 347 (M$^+$, 17), 77 (98); Anal. Calcd for $C_{19}H_{14}FN_5O$ (347.35): C, 65.70; H, 4.06; N, 20.16%. Found: C, 65.31; H, 4.15; N, 20.31%.

2-Benzoyl-3-(3-(5-methyl-1-phenyl-1H-1,2,3-triazol-4-yl)-1-phenyl-1H-pyrazol-4-yl)acrylonitrile (4a-c)

To a round-bottom flask were added appropriate 3-(5-methyl-1-aryl-1*H*-1,2,3-triazol-4-yl)-1-phenyl-1*H*-pyrazole-4-carbaldehydes **3a-c** (2 mmol), 3-oxo-3-phenylpropanenitrile (0.3 g, 2 mmol),absolute ethanol (10 mL), and piperidine (1 mL). The resulting suspension was heated at for 5 h. After completion, the formed solid was filtered and dryad to afford **4a-c**.

2-Benzoyl-3-(3-(5-methyl-1-phenyl-1H-1,2,3-triazol-4-yl)-1-phenyl-1H-pyrazol-4-yl)acrylonitrile (4a)

Yield 54 %; m.p. 230-231°C; IR (KBr) νmax/cm^{-1} 1680 (C=O), 2198 (CN); ^{1}H NMR (DMSO-d_6) δ 2.43 (s, 3H, CH_3), 7.44-7.96 (m, 15 H, Ar-H), 9.13 (s, 1H, pyrazole-H), 9.33 (s, 1H, CH-olefinic); MS m/z (%): 456 (M$^+$, 56), 302 (97), 77(100); Anal. Calcd for $C_{28}H_{20}N_6O$ (456.50): C, 73.67; H, 4.42; N, 18.41%. Found: C, 73.73; H, 4.55; N, 18.64%.

2-Benzoyl-3-(3-(5-methyl-1-p-tolyl-1H-1,2,3-triazol-4-yl)-1-phenyl-1H-pyrazol-4-yl)acrylonitrile (4b)

Yield 52 %; m.p. 198-200°C; IR (KBr) νmax/cm^{-1} 1687 (C=O), 2210 (CN); ^{1}H NMR (DMSO-d_6) δ 2.49, 2.62 (2s, 6H, 2 CH_3), 7.46-7.97 (m, 14 H, Ar-H), 9.14 (s, 1H, pyrazole-H), 9.33 (s, 1H, CH-olefinic); MS m/z (%): 470 (M$^+$, 52), 302 (96), 77(100); Anal. Calcd for $C_{29}H_{22}N_6O$ (470.52): C, 74.03; H, 4.71; N, 17.86 %. Found: C, 74.27; H, 4.82; N, 17.99%.

2-Benzoyl-3-(3-(1-(4-fluorophenyl)-5-methyl-1H-1,2,3-triazol-4-yl)-1-phenyl-1H-pyrazol-4-yl)acrylonitrile (4c)

Yield 49 %; m.p. 227-229°C; IR (KBr) νmax/cm^{-1} 1685 (C=O), 2217 (CN); ^{1}H NMR (DMSO-d_6) δ 2.47 (s, 3H, CH_3), 7.45-7.97 (m, 14 H, Ar-H), 9.13 (s, 1H, pyrazole-H), 9.32 (s, 1H, CH-olefinic); MS m/z (%): 474 (M$^+$, 60), 302 (97), 77(100); Anal. Calcd for C28H19FN6O (474.49): C, 70.88; H, 4.04; N, 17.71%. Found: C, 70.99; H, 4.22; N, 17.87%.

3-Methyl-4-((3-(5-methyl-1-aryl-1H-1,2,3-triazol-4-yl)-1-phenyl-1H-pyrazol-4-yl)methylene)-1-phenyl-1H-pyrazol-5(4H)-one (5a-c)

A solution of appropriate 3-(5-methyl-1-aryl-1*H*-1,2,3-triazol-4-yl)-1-phenyl-1*H*-pyrazole-4-carbaldehydes **3a-c** (2 mmol), in absolute ethanol (20 mL) containing piperidine (0.1 mL) was treated with 3-methyl-1-phenyl-1*H*-pyrazol-5(4*H*)-one (0.35 g, 2 mmol), and the resulting mixture was then refluxed for 4 h. After cooling to r.t., it was filtered and dryad to furnish **5a-c**.

3-Methyl-4-((3-(5-methyl-1-phenyl-1H-1,2,3-triazol-4-yl)-1-phenyl-1H-pyrazol-4-yl)methylene)-1-phenyl-1H-pyrazol-5(4H)-one (5a)

Yield 61 %; m.p. 267-268°C; IR (KBr) νmax/cm^{-1} 1662 (C=O); ^{1}H NMR (DMSO-d_6) δ 2.49, 2.64 (2s, 6H,2 CH_3), 7.37-7.69 (m, 15 H, Ar-H), 8.76 (s, 1H, pyrazole-

H), 9.29 (s, 1H, CH-olefinic); MS m/z (%): 485 (M^+, 27), 456 (25), 298 (32), 77(100); Anal. Calcd for $C_{29}H_{23}N_7O$ (485.54): C, 71.74; H, 4.77; N, 20.19%. Found: C, 71.85; H, 4.83; N, 20.23%.

3-Methyl-4-((3-(5-methyl-1-p-tolyl-1H-1,2,3-triazol-4-yl)-1-phenyl-1H-pyrazol-4-yl)methylene)-1-phenyl-1H-pyrazol-5(4H)-one (5b)

Yield 58 %; m.p. 273-275°C; IR (KBr) νmax/cm^{-1} 1668 (C=O); ^{1}H NMR (DMSO-d_6) δ 2.49, 2.51, 2.64 (3s, 9H,3 CH_3), 7.37-7.93 (m, 14 H, Ar-H), 8.77 (s, 1H, pyrazole-H), 9.30 (s, 1H, CH-olefinic); MS m/z (%): 485 (M^+, 27), 456 (25), 298 (32), 77(100); Anal. Calcd for $C_{30}H_{25}N_7O$ (499.57): C, 72.13; H, 5.04; N, 19.63 %. Found: C, 72.26; H, 5.18; N, 19.74%.

4-((3-(1-(4-Fluorophenyl)-5-methyl-1H-1,2,3-triazol-4-yl)-1-phenyl-1H-pyrazol-4-yl)methylene)-3-methyl-1-phenyl-1H-pyrazol-5(4H)-one (5c)

Yield 62 %; m.p. 252-253°C; IR (KBr) νmax/cm^{-1} 1657 (C=O); ^{1}H NMR (DMSO-d_6) δ 2.49, 2.51 (2s, 6H,2 CH_3), 7.41-7.93 (m, 14 H, Ar-H), 8.76 (s, 1H, pyrazole-H), 9.29 (s, 1H, CH-olefinic); MS m/z (%): 503 (M^+, 21), 456 (25), 298 (32), 77(100); Anal. Calcd for $C_{29}H_{22}FN_7O$ (503.53): C, 69.17; H, 4.40; N, 19.47%. Found: C, 69.24; H, 4.57; N, 19.63%.

2-((3-(5-Methyl-1-aryl-1H-1,2,3-triazol-4-yl)-1-phenyl-1H-pyrazol-4-yl)methylene)-1H-indene-1,3(2H)-dione (6a-c)

Appropriate 3-(5-methyl-1-aryl-1*H*-1,2,3-triazol-4-yl)-1-phenyl-1*H*-pyrazole-4-carbaldehydes **3a-c** (2 mmol) and 1H-indene-1,3(2H)-dione (0.29 g, 2 mmol) were dissolved in ethanol (20 mL) containing piperidine (0.1 ml) and heated at reflux. After 3 h, the obtained mixture was cooled to r.t. and the formed solid was filtered and dryad to yield **6a-c**.

2-((3-(5-methyl-1-phenyl-1H-1,2,3-triazol-4-yl)-1-phenyl-1H-pyrazol-4-yl)methylene)-1H-indene-1,3(2H)-dione (6a)

Yield 58 %; m.p. 276-277°C; IR (KBr) νmax/cm^{-1} 1720 (C=O); ^{1}H NMR (DMSO-d_6) δ 2.49 (s, 3H, CH_3), 7.48-7.97 (m, 13 H, Ar-H), 8.81 (s, 1H, pyrazole-H), 10.05 (s, 1H, CH-olefinic); MS m/z (%): 457 (M^+, 48), 77(100); Anal. Calcd for $C_{28}H_{19}N_5O_2$ (457.48): C, 73.51; H, 4.19; N, 15.31 %. Found: C, 73.63; H, 4.28; N, 15.60%.

2-((3-(5-methyl-1-p-tolyl-1H-1,2,3-triazol-4-yl)-1-phenyl-1H-pyrazol-4-yl)methylene)-1H-indene-1,3(2H)-dione (6b)

Yield 61 %; m.p. 287-288°C; IR (KBr) νmax/cm^{-1} 1723 (C=O); ^{1}H NMR (DMSO-d_6) δ 2.49, 2.63 (2s, 6H, $2CH_3$), 7.51-7.98 (m, 12 H, Ar-H), 8.82 (s, 1H, pyrazole-H), 10.06 (s, 1H, CH-olefinic); MS m/z (%): 471 (M^+, 51), 77(100); Anal. Calcd for $C_{29}H_{21}N_5O_2$ (471.51): C, 73.87; H, 4.49; N, 14.85 %. Found: C, 73.93; H, 4.38; N, 15.02%.

2-((3-(1-(4-fluorophenyl)-5-methyl-1H-1,2,3-triazol-4-yl)-1-phenyl-1H-pyrazol-4-yl)methylene)-1H-indene-1,3(2H)-dione (6c)

Yield 62 %; m.p. 291-292°C; IR (KBr) νmax/cm^{-1} 1718 (C=O); ^{1}H NMR (DMSO-d_6) δ 2.50(s, 3H, CH_3), 7.48-7.96 (m, 12 H, Ar-H), 8.81 (s, 1H, pyrazole-H), 10.07 (s, 1H, CH-olefinic); MS m/z (%): 475 (M^+, 49), 77(100); Anal. Calcd for $C_{28}H_{18}FN_5O_2$ (475.47): C, 70.73; H, 3.82; N, 14.73 %. Found: C, 70.90; H, 3.97; N, 14.91%.

3.2. Anticancer Activity

Cell Lines and Culturing

Anticancer activity screening for the tested compounds utilizing 4 different human tumor cell lines including breast cancer MCF-7, liver cancer HepG2 and lung cancer A549 as well as the normal cell line (human normal melanocyte, HFB4) were obtained from the American Type Culture Collection (Rockville, MD, USA). The tumor cells were maintained in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% heat inactivated fetal calf serum (GIBCO), penicillin (100 U/ml) and streptomycin (100 µg/ml) at 37 °C in humidified atmosphere containing 5% CO_2. Cells at a concentration of 0.50 x 106 were grown in a 25 cm^2 flask in 5 ml of complete culture medium.

In Vitro Cytotoxicity Assay

The cytotoxicity activity was measured *in vitro* using the Sulfo-Rhodamine-B stain (SRB) assay according to the previous reported standard procedure [2]. Cells were inoculated in 96-well microtiter plate (104 cells/ well) for 24 h before treatment with the tested compounds to allow attachment of cell to the wall of the plate. The tested compounds were dissolved in DMSO at 1 mg/ml immediately before use and diluted to the appropriate volume just before addition to the cell culture. Different concentration of tested compounds and doxorubicin were added to the cells. Triplicate wells were prepared for each individual dose. Cells were incubated with the compounds for 48 h. at 37°C and in atmosphere of 5% CO_2. After 48 h cells were fixed, washed, and stained for 30 min with 0.4% (w/v) SRB dissolved in 1% acetic acid. Unbound dye was removed by four washes with 1% acetic acid, and attached stain was recovered with Tris-EDTA buffer. Color intensity was measured in an ELISA reader. The

relation between surviving fraction and drug concentration is plotted to get the survival curve for each cell line after the specified time. The concentration required for 50% inhibition of cell viability (IC50) was calculated and the results are given in Table 1. The results were compared to the antiproliferative effects of the reference control doxorubicin [13].

Statistical Analysis

The results are reported as Mean ± Standard error (S.E.) for at least six times experiments.

3.3. X-Ray Crystallography

A single crystal of compound **5c** was obtained by slow evaporation at room temperature, from dimethylformamide (DMF). The crystal structure was solved and refined using MaXus (Bruker Nonius, Deflt and MacScience, Japan) [14] Mo K_α radiation (λ = 0.71073Å) and a graphite monochromator were used for data collection. The chemical formula and ring labeling system is shown in Figure **1**. Crystal data for compound **5c**: $C_{29}H_{22}FN_7O$, Mr, 503.53; system, orthorhombic; Space group, $P\bar{1}$; unit cell dimensions, a, 8.5977 (4) Å; b, 11.9783 (6)Å; c, 13.5204 (9) Å; α, 65.121 (2)°; β, 77.827 (2)°; γ, 86.839 (3)°; V, 1233.90 (12)Å^3; Z, 2; D_x, 1.129 Mg m^{-3}; θ range for data collection, 27.57 °; μ (Mo- K_α), 0.07 mm^{-1}; T = 298 K; independent reflections, 7293; measured reflections, 5622; observed reflections, 780; R_{int}, 0.117; R(all), 0.360; R(gt), 0.037; wR(ref), 0.092; wR(all), 0.194; wR(gt), 0.092; S(ref), 1.463; S(all), 1.233; S(gt), 1.463; Δ/σ_{max}, 0.029, $\Delta\rho_{max}$, 1.12 eÅ^3; $\Delta\rho_{min}$-1.23eÅ^3.

Crystallographic data for the structures **5c** have been deposited with the Cambridge Crystallographic Data Center (CCDC) under the number 1047557. Copies of the data can be obtained, free of charge, on application to CCDC 12 Union Road, Cambridge CB2 1EZ, UK [Fax: +44-1223-336033; e-mail: deposit@ ccdc.cam.ac.uk or at www.ccdc.cam.ac.uk].

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