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Design and Synthesis of Some New Oxadiazole Derivatives as Anticancer Agents

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Abstract

In this work, some new oxadiazole derivatives have been prepared, by reacting phenyl hydrazine and acetic anhydride together, which furnished 2,4-dimethyl-4-phenyloxadiazole. This product was reacted with a series of aromatic aldehydes, to obtain a series of oxadiazole derivatives. These derivatives were characterized by TLC, melting points, infrared red, proton nuclear magnetic resonance, carbon thirteen nuclear magnetic resonance and mass spectroscopy. Finally, these synthetized derivatives were tested for antiproliferative activity by two different cell lines. MCF-7 (Breast cancer cell line) and HepG2 (Liver cancer cell line) were used to assess the antiproliferative activity of the prepared compounds.

Keywords

Phenyl Hydrazine, Oxadiazole, Aromatic Aldehydes, Acetic Anhydride, Benzylidene Synthesis, Cytotoxic Assay, Anticancer, HepG2 and MCF-7

1. Introduction

Cancer is one of the most challenging public health diseases that face humankind [1] [2]. It is characterized by the development of abnormal cells that divide uncontrollably and have the ability to infiltrate and destroy normal body tissues [1] [2]. The disease is characterized by high morbidity and mortality rates [3]. In many countries, it has become the second largest killer after cardiovascular diseases [3]. In 2012, there were 14 million new cases and 8.2 million deaths [4]. Among men, lung cancer was the most predominant, while among women, it was breast cancer. It was reported that there were 24 million cancer cases annually and 14.6 million annual deaths by the end of 2015 [4]. The previous data led the researchers globally to combat this disease, searching for new anticancer

agents having heterocyclic nucleus is having a worldwide attention at various laboratories [5] [6] [7] [8]. It was reported that heterocyclic compounds could have anti-antiproliferative activities, with different proposed mechanisms of action [5] [9]. The anticancer activity of these compounds may be due to their intercalating properties or covalent binding abilities to DNA [9] or cell membrane interaction [10]. Many of the drugs being used in chemotherapy have heterocycles as their basic structure, for example, pyrrole, pyrrolidine, pyridine, imidazole, pyrimidines, pyrazole, indole, quinoline, oxadiazole, azole, benzimidazole, etc. as the key building blocks to develop active biological compounds [11]. This research is based on the reaction of phenyl hydrazine with acetic anhydride to obtain 2,4-dimethyl-4-phenyloxadiazole. This product was reacted with a series of aromatic aldehydes, to obtain a series of oxadiazole derivatives. The reaction was performed at one site of the two active methylene groups and this is most likely because of the steric hindrance maintained by the phenyl group of the phenyl hydrazine [12]-[27].

2. Materials

2.1. Reagents

All solvents and reagents were obtained from commercial sources and were used without further purification except petroleum ether and ethyl acetate. phenyl Hydrazine was purchased from Sigma Aldrich (Cairo, Egypt). Series of aromatic aldehydes were acquired from Sigma Aldrich (Cairo, Egypt). Absolute ethanol, ethanol 95%, acetic anhydride, ethyl acetate and petroleum ether were purchased from Piochem (Cairo, Egypt). Distilled water was used for the experiments.

2.2. Instruments

Progress of chemical reactions was observed using TLC (Merck, silica gel plates 60 F254) and visualized using a UV-Vis spectrometer at 254 nm. Melting points were determined by Mel-Temp apparatus. NMR spectra were performed in Chloroform (7.26 ppm), with trimethyl silane as an internal standard, using Bruker Avance 500 spectrometer at ambient temperature, at drug discovery unit, Faculty of Pharmacy, Ain Shams University (ASU, Cairo, Egypt). All chemical shifts were expressed in parts per million (δ), and coupling constants (J) in Hz. FTIR spectra were recorded using KBr pellets on a model 883 double beam infrared spectrophotometer Bruker in 200 - 4000 cm^{-1}, at drug discovery unit, Faculty of Pharmacy, Ain Shams University (ASU, Cairo, Egypt). MS spectra were recorded using a Bruker Esquire 2000 by APC or ES ionization, at drug discovery unit, Faculty of Pharmacy, Ain Shams University (ASU, Cairo, Egypt).

2.3. Cell Culture: HepG2, MCF-7

Cell line was obtained from Nawah Scientific Inc., (Mokatam, Cairo, Egypt). Cells were maintained in DMEM media supplemented with 100 mg/mL of streptomycin, 100 units/mL of penicillin and 10% of heat-inactivated fetal bovine se-

rum in humidified, 5% (v/v) CO_2 atmosphere at 37˚C [28] [29].

2.4. Cytotoxicity Assay: HepG2, MCF-7

Cell viability was assessed by SRB assay. Aliquots of 100 µL cell suspension (5×10^3 cells) were in 96-well plates and incubated in complete media for 24 h. Cells were treated with another aliquot of 100 µL media containing drugs at various concentrations. After 72 h of drug exposure, cells were fixed by replacing media with 150 µL of 10% TCA and incubated at 4˚C for 1 h. The TCA solution was removed, and the cells were washed 5 times with distilled water. Aliquots of 70 µL SRB solution (0.4% w/v) were added and incubated in a dark place at room temperature for 10 min. Plates were washed 3 times with 1% acetic acid and allowed to air-dry overnight. Then, 150 µL of TRIS (10 mM) was added to dissolve protein-bound SRB stain; the absorbance was measured at 540 nm using a BMG LABTECH®-FLUOstar Omega microplate reader (Ortenberg, Germany) [28] [29].

3. Chemistry and Scheme

3.1. Scheme

General scheme for the synthesis of compound (2) and compounds (14-22), illustrated in Figure 1.

3.2. Procedure and Synthesis of Compound (2): 2,5-Dimethyl-3-Phenyl-1,3,4-Oxadiazolidine

Mixture of phenyl hydrazine (20 ml, 22 gm, 0.202 mole) and acetic anhydride were stirred together for 24 hours under reflux conditions at 125˚C as described in (Figure 1). TLC was made by 3:2 Petroleum Ether: Ethyl Acetate system. Precipitate was obtained by the concentration of acetic anhydride layer. Then it was

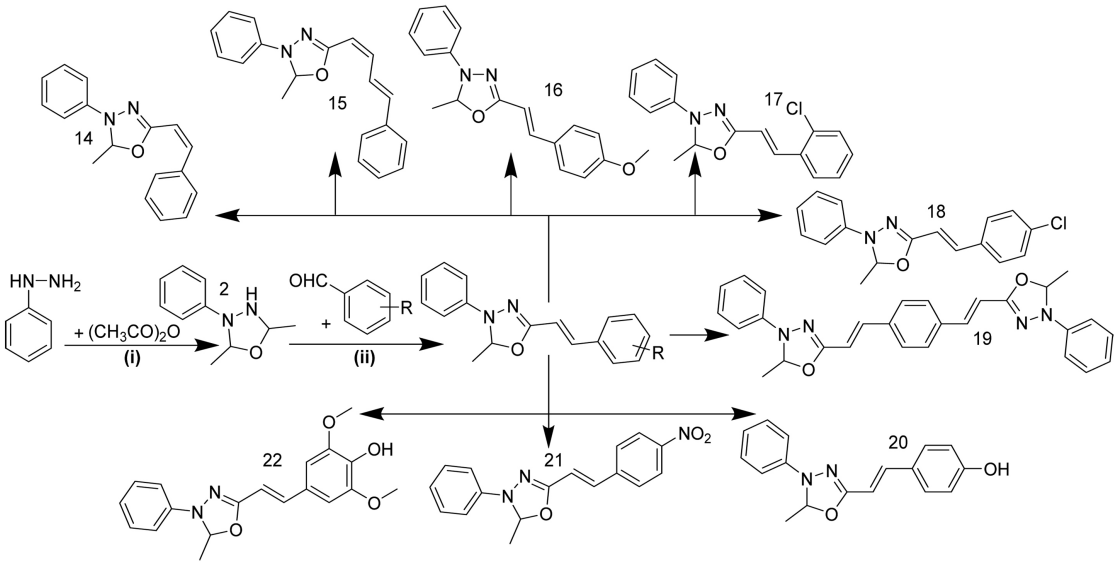

Figure 1. General scheme. Reagents and conditions: (i) acetic anhydride (reactant & solvent), refluxing at 125˚C, 24 hrs. (ii) series of aromatic aldehydes, conc. sulfuric acid, refluxing absolute ethanol, 105˚C, 12 - 29 Hrs.

crystallized by using absolute ethanol. Yield 88%. m.p = 122˚C. ^{1}HNMR (400 MHz, $CDCl_3$): δ 1.25 ppm (d, CH_3), 1.79 ppm (d, CH_3), 4.5 ppm (q, CH), 4.92 ppm (q, CH), 6.87 - 7.29 ppm (m, aromatic protons) and 10.3 ppm (d, -NH-). ^{13}CNMR (100 MHz, $CDCl_3$): δ C1 (154.4 ppm), C2 (130.2 ppm), C3 (114.2 ppm), C4 (123.8 ppm), C5 (114.2 ppm), C6 (130.2 ppm), C7 (104.6 ppm), C8 (95.7 ppm), C9 (23.1 ppm) and C10 (27.4 ppm).

3.3. Procedure and Synthesis of Compounds (14-22)

Mixture of **2** (2 gm, 0.011 mole) and series of aromatic aldehydes were mixed together for 12 - 29 hours under refluxing absolute ethanol at 105˚C. With the presence of 0.5 ml H_2SO_4 as a catalyst as mentioned in (**Figure 1**). TLC was made by 1:2 Petroleum Ether: Ethyl Acetate system. Product was obtained from the organic layer, later on, water was added which retrieved more amounts of the product. Crystallization was performed by ethanol to obtain pure crystallized product.

3.3.1. Compound 14: (Z)-2-Methyl-3-Phenyl-5-Styryl-2,3-Dihydro-1,3,4-Oxadiazole

Yield: 85%. m.p = 200˚C - 202˚C. ^{1}HNMR (400 MHz, $CDCl_3$): δ 3.1 ppm (d, CH_3), 4.9 ppm (d, -CH-), 5.3 ppm (d, -CH-), 6.8 ppm (q, -CH-O) and 6.95 - 7.7 ppm (m, aromatic). ^{13}CNMR (100 MHz, $CDCl_3$): δ C1 (131.5 ppm), C2 (129.5 ppm), C3 (127.8 ppm), C4 (137 ppm), C5 (127.8 ppm), C6 (129.5 ppm), C7 (142.5 ppm), C8 (121.3 ppm), C9 (149.2 ppm), C10 (90.9 ppm), C11 (38.6 ppm), C1 (147.5 ppm), C2 (115 ppm), C3 (130 ppm), C4 (125 ppm), C5 (130 ppm) and C6 (115 ppm).

3.3.2. Compound 15: 2-Methyl-3-Phenyl-5-((1Z,3E)-4-Phenylbuta-1,3-Dien-1-Yl)-2,3-Dihydro-1,3,4-Oxadiazole

Yield: 82.9%. m.p = 210˚C - 212˚C. IR: 672 cm^{-1} (C-H, bending), 699.52 cm^{-1} (aromatic, bending), 1002 cm^{-1} (C-O, stretching), 1067.33 cm^{-1} (C-N, stretching), 1397 cm^{-1} (C-H, bending), 1547 cm^{-1} (C=C, aromatic), 1630 cm^{-1} (C=C, stretching), 1689 cm^{-1} (C=N, stretching), 2432 cm^{-1} (aromatic, overtone), 2878 cm^{-1} (C-H, stretching), 3048 cm^{-1} (C-H, aromatic) and 3085 cm^{-1} (C-H, stretching). ^{1}HNMR (400 MHz, $CDCl_3$): δ 2.8 ppm (d, CH3), 5.1 (q, -CH-O-), 5.4 ppm (d, -CH-), 5.9 ppm (t, -CH-), 6.5 ppm (t, -CH-), 6.6 ppm (d, -CH-) and 6.8 - 7.6 ppm (m, aromatic). ^{13}CNMR (100 MHz, $CDCl_3$): δ C1 (132.5 ppm), C2 (129.5 ppm), C3 (128 ppm), C4 (136.8 ppm), C5 (128 ppm), C6 (129.5 ppm), C7 (143.5 ppm), C8 (123.3 ppm), C9 (146.2 ppm), C10 (119.8 ppm), C11 (149.5 ppm), C12 (90.2 ppm), C13 (22.4), C1 (146.5 ppm), C2 (116.7 ppm), C3 (129.4 ppm), C4 (125 ppm), C5 (129.4 ppm) and C6 (116.7 ppm).

3.3.3. Compound 16: (E)-5-(4-Methoxystyryl)-2-Methyl-3-Phenyl-2,3-Dihydro-1,3,4-Oxadiazole

Yield 86%. m.p = 223˚C. ^{1}HNMR (400 MHz, $CDCl_3$): δ 2.1 ppm (d, CH_3), 3.8

ppm (s, CH_3), 4.9 (q, -CH-O-), 5.9 ppm (d, -CH-), 6.5 ppm (d, -CH-), and 6.7 - 7.9 ppm (m, aromatic). ^{13}CNMR (100 MHz, $CDCl_3$): δC1 (55.8) ppm), C2 (156.5 ppm), C3 (120.1 ppm), C4 (128.7 ppm), C5 (126.8 ppm), C6 (128.7 ppm), C7 (120.1 ppm), C7 (143.5 ppm), C8 (142.1 ppm), C9 (119.6 ppm), C10 (149.5 ppm), C11 (84.6 ppm), C12 (29.4), C1 (146.1 ppm), C2 (118.7 ppm), C3 (126.4 ppm), C4 (121 ppm), C5 (126.4 ppm) and C6 (118.7 ppm).

3.3.4. Compound 17: (E)-5-(2-Chlorostyryl)-2-Methyl-3-Phenyl-2,3-Dihydro-1,3,4-Oxadiazole

Yield 83%. m.p = 205˚C. ^{1}HNMR (400 MHz, $CDCl_3$): δ1.7 ppm (d, CH_3), 4.7 (q, -CH-O-), 5.8 ppm (d, -CH-), 6.9 ppm (d, -CH-), and 6.9 - 7.5 ppm (m, aromatic). ^{13}CNMR (100 MHz, $CDCl_3$): δC1 (129.9 ppm), C2 (129.1 ppm), C3 (137 ppm), C4 (135.8 ppm), C5 (127.8 ppm), C6 (126.5 ppm), C7 (140.5 ppm), C8 (125.3 ppm), C9 (149.3 ppm), C10 (89.2 ppm), C11 (27.9 ppm), C1 (145.5 ppm), C2 (116.1 ppm), C3 (129.9 ppm), C4 (120.8 ppm), C5 (129.9 ppm) and C6 (116.1 ppm). MS: m/z: 298.07 (100.0%), (M + 1) 299.06 (87.2%), (M + 2) 297.05 (12.8%).

3.3.5. Compound 18: (E)-5-(4-Chlorostyryl)-2-Methyl-3-Phenyl-2,3-Dihydro-1,3,4-Oxadiazole

Yield 92%. m.p = 199˚C. IR: 590 cm^{-1} (chloride sub., bending), 669 cm^{-1} (C-H, bending), 800.52 cm^{-1} (aromatic, bending), 710 cm^{-1} (mono sub., bending), 1050 cm^{-1} (C-O, stretching), 1249.33 cm^{-1} (C-N, stretching), 1350 cm^{-1} (C-H, bending), 1540 cm^{-1} (C=C, aromatic), 1658 cm^{-1} (C=C, stretching), 1678 cm^{-1} (C=N, stretching), 2413 cm^{-1} (aromatic, overtone), 2857 cm^{-1} (C-H, stretching), 3035 cm^{-1} (C-H, aromatic) and 3095 cm^{-1} (C-H, stretching. ^{1}HNMR (400 MHz, $CDCl_3$): δ1.6 ppm (d, CH_3), 4.3 (q, -CH-O-), 5.2 ppm (d, -CH-), 6.7 ppm (d, -CH-), and 6.8 - 7.9 ppm (m, aromatic). ^{13}CNMR (100 MHz, CDCl3): δC1 (130.9 ppm), C2 (129.4 ppm), C3 (133.3 ppm), C4 (133.9 ppm), C5 (133.3 ppm), C6 (129.4 ppm), C7 (142.5 ppm), C8 (122.3 ppm), C9 (153.6 ppm), C10 (85.2 ppm), C11 (29.9 ppm), C1 (142.6 ppm), C2 (116.4 ppm), C3 (129.9 ppm), C4 (121.8 ppm), C5 (129.9 ppm) and C6 (116.4 ppm). MS: m/z: 298.07 (100.0%), (M + 1) 299.06 (63.7%), (M + 2) 297.05 (36.3%).

3.3.6. Compound 19: 1,4Bis((2)-(5-Methyl-4-Phenyl-4,5-Dihydro-1,3,4-Oxadiazole-2-Yl) Vinyl)Benzene

Yield 76%. m.p = 295˚C - 297˚C. ^{1}HNMR (400 MHz, $CDCl_3$): δ2.4 ppm (d, CH_3), 4.89 (q, -CH-O-), 5.95 ppm (d, -CH-), 6.5 ppm (d, -CH-), and 6.6 - 8.1 ppm (m, aromatic). ^{13}CNMR (100 MHz, $CDCl_3$): δC1 (122.9 ppm), C2 (129.1 ppm), C3 (119.3 ppm), C4 (133.9 ppm), C5 (119.3 ppm), C6 (129.1 ppm), C7 (89.6 ppm), C8 (32.1 ppm), C9 (149.6 ppm), C10 (122.8 ppm), C11 (148.7 ppm), C12 (134.4 ppm) and C13 (129.9 ppm).

3.3.7. Compound 20: (E)-4-(2-(5-Methyl-4-Phenyl-4,5-Dihydro-1,3,4-Oxadiazol-2-Yl) Vinyl)Phenol

Yield 39%. m.p = 233˚C. IR: 659 cm^{-1} (C-H, bending), 798 cm^{-1} (aromatic, bending), 708 cm^{-1} (mono sub., bending), 1049 cm^{-1} (C-O, stretching), 1218 cm^{-1} (C-OH, stretching), 1310 cm^{-1} (C-N, stretching), 1367 cm^{-1} (C-H, bending), 1535 cm^{-1} (C=C, aromatic), 1608 cm^{-1} (C=C, stretching), 1658 cm^{-1} (C=N, stretching), 2460 cm^{-1} (aromatic, overtone), 2832 cm^{-1} (C-H, stretching), 3055 cm^{-1} (C-H, aromatic), 3095 cm^{-1} (C-H, stretching) and 3507 cm^{-1} (OH, Stretching). ^{1}HNMR (400 MHz, $CDCl_3$): δ 1.93 ppm (d, CH_3), 4.83 (q, -CH-O-), 5.9 ppm (d, -CH-), 6.1 ppm (d, -CH-), 6.9 - 7.5 ppm (m, aromatic) and 9.8 (s, OH). ^{13}CNMR (100 MHz, $CDCl_3$): δC1 (159.1 ppm), C2 (135.8 ppm), C3 (119.3 ppm), C4 (129.9 ppm), C5 (119.3 ppm), C6 (135.8 ppm), C7 (149.5 ppm), C8 (123.8 ppm), C9 (149.6 ppm), C10 (90.9 ppm), C11 (21.1 ppm), C1 (145.6 ppm), C2 (120.4 ppm), C3 (109.3 ppm), C4 (121.8 ppm), C5 (109.3 ppm) and C6 (120.4 ppm).

3.3.8. Compound 21: (E)-2-Methyl-5-(4-Nitrostyryl)-3-Phenyl-2,3-Dihydro-1,3,4-Oxadiazole

Yield 62%. m.p = 225˚C. ^{1}HNMR (400 MHz, $CDCl_3$): δ 1.24 ppm (d, CH_3), 4.63 (q, -CH-O-), 6.4 ppm (d, -CH-), 6.8 ppm (d, -CH-) and 6.9 - 8.9 ppm (m, aromatic). ^{13}CNMR (100 MHz, $CDCl_3$): δ C1 (155.1 ppm), C2 (132.8 ppm), C3 (125.6 ppm), C4 (143.9 ppm), C5 (125.6 ppm), C6 (132.8 ppm), C7 (144.7 ppm), C8 (129.8 ppm), C9 (149.6 ppm), C10 (90.9 ppm), C11 (27.1 ppm), C1 (143.6 ppm), C2 (125.4 ppm), C3 (115.3 ppm), C4 (121.8 ppm), C5 (115.3 ppm) and C6 (125.4 ppm).

3.3.9. Compound 22: (E)-2,6-Dimethoxy-4-(2-(5-Methyl-4-Phenyl-4,5-Dihydro-1,3,4-Oxadiazol-2-Yl) Vinyl) Phenol

Yield 65%. m.p = 280˚C. ^{1}HNMR (400 MHz, CDCl3): δ 1.98 ppm (d, CH_3), 3.8 (s, CH_3), 4.6 (q, -CH-O-), 5.9 ppm (d, -CH-), 6.7 ppm (d, -CH-) and 7.1 - 7.9 ppm (m, aromatic). ^{13}CNMR (100 MHz, $CDCl_3$): δ C1 (139.6 ppm), C2 (149.9 ppm), C3 (114.6 ppm), C4 (126.9 ppm), C5 (114.6 ppm), C6 (14998 ppm), C7 (55.1 ppm), C8 (55.1 ppm), C9 (141.2 ppm), C10 (139.1 ppm), C11 (150.6 ppm), C12 (88 ppm), C13 (22.5 ppm), C1 (143.7 ppm), C2 (129.2 ppm), C3 (116.3 ppm), C4 (120.8 ppm), C5 (116.3 ppm) and C6 (129.2 ppm).

4. Results

4.1. Cytotoxicity Results of MCF-7

MCF-7 cell line was used to assay the antiproliferative activity of compounds 14, 15, 16, 17 and 18, compound 18 was the most potent in this group with IC_{50} value of 3.54 µm and compound 16 was the lowest in potency with IC_{50} value of 52.67 µm (**Figure 2**). Microscopical examination of the tested compounds in the cell line

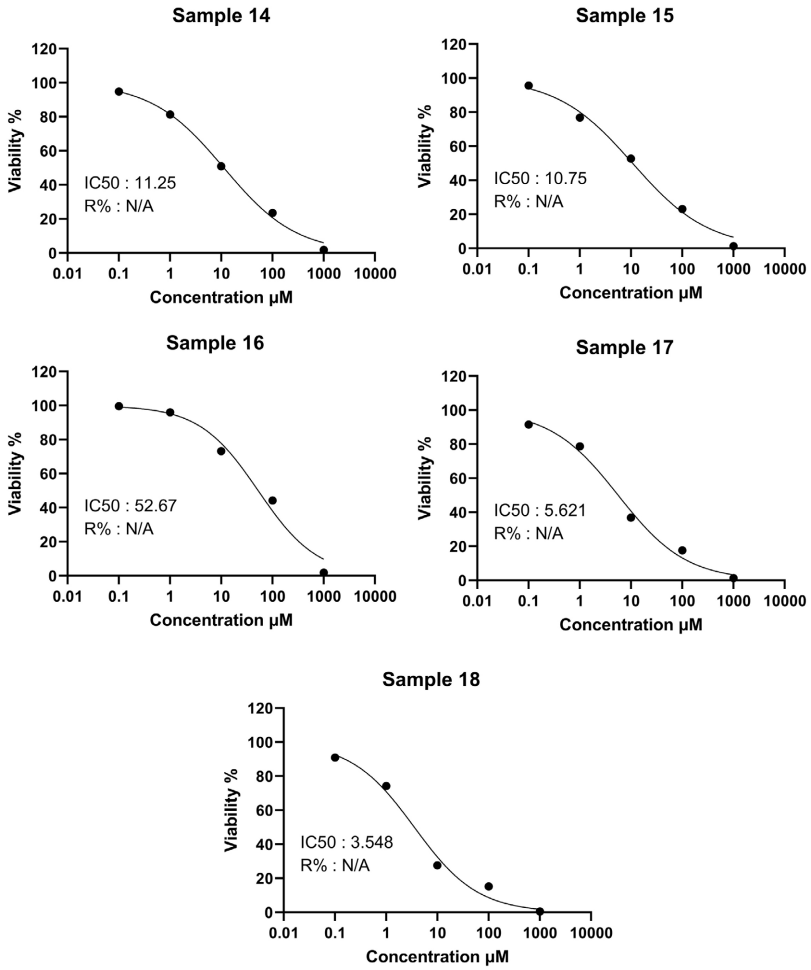

Figure 2. IC_{50} Values of compounds 14 - 18 against MCF-7 Cell line [28] [29].

at 100 µm used to confirm the calculation of the IC_{50} (**Figure 3**).

4.2. Cytotoxicity Results of HepG2

HepG2 cell line was used to assay the antiproliferative activity of compounds 19, 20, 21 and 22, compound 19 was the most potent in this group with IC_{50} value of 9.38 µm and compound 21 was the lowest in potency with IC_{50} value of 32.39 µm (**Figure 4**). Microscopical examination of the tested compounds in the cell line at concentration of 100 µm was used to confirm the calculation of the IC_{50} (**Figure 5**).

5. Conclusion

From the above findings, we concluded that all assayed compounds have potential antiproliferative activity on both cell lines which were tested. Generally, it was found that the cyclized phenyl hydrazine derivatives (oxodiazoles) are more potent than derivatives with open side chains (not cyclized) [5]. For MCF-7 cell line, compound 18 was found to be the most potent compound in the group scoring 3.548 mm, compound 16 was the lowest in potency scoring 52.67 mm. For HepG2 cell line, compound 19 was found to be the most potent compound

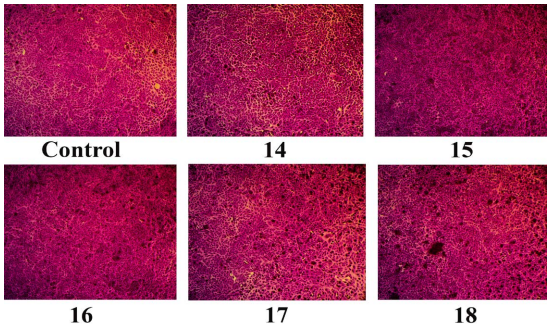

Figure 3. MCF-7 cell line under microscopic examination of control and compounds 14 - 18 at 100 μm concentration [28] [29].

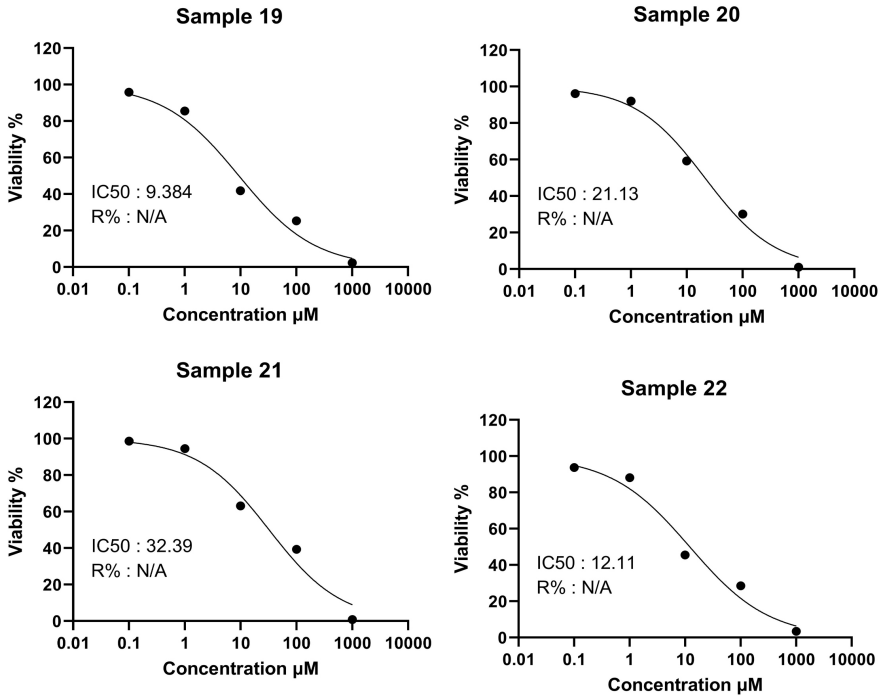

Figure 4. IC_{50} Values of compounds 19 - 22 against HepG2 Cell line [28] [29].

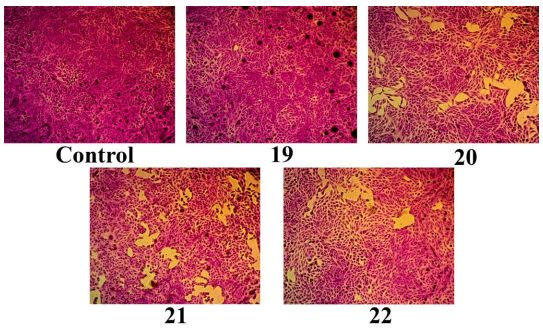

Figure 5. HepG2 cell line under microscopic examination of control and compounds 19 - 22 at 100 μm concentration [28] [29].

among the other compounds scoring 9.384 mm and compound 21 was the lowest in potency in this group, scoring 32.39 mm (**Figure 6**).

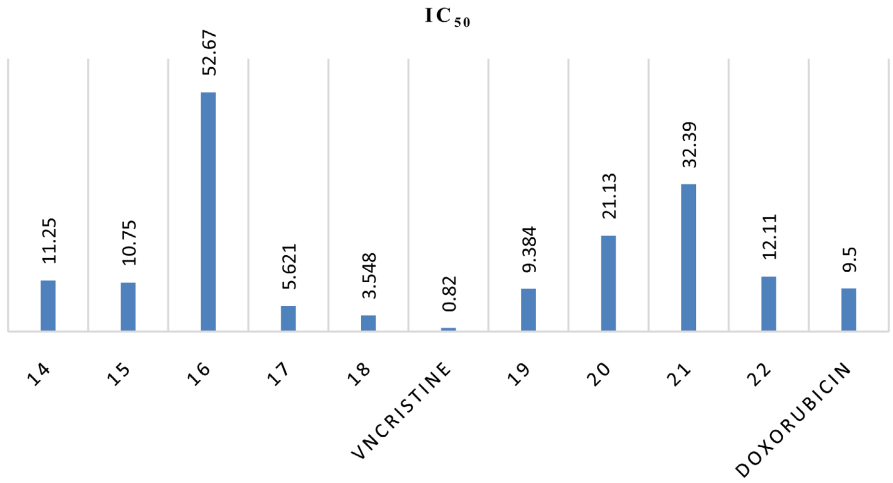

Figure 6. Summary of the cytotoxic assay results of all compounds against standard drugs.

Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

References

[1] Bridges, A.J. (2001) Chemical Inhibitors of Protein Kinases. *Chemical Reviews*, **101**, 2541-2572. https://doi.org/10.1021/cr000250y

[2] Wang, J.D., Miller, K., Boschelli, D.H., Ye, F., Wu, B., Floyd, M.B., Powell, D.W., Wissner, A., Weber, J.M. and Boschelli, F. (2000) Inhibitors of Src Tyrosine Kinase: The Preparation and Structure Activity Relationship of 4-anilino-3-cyanoquinolines and 4-anilinoquinazolines. *Bioorganic & Medicinal Chemistry Letters*, **10**, 2477-2480. https://doi.org/10.1016/S0960-894X(00)00493-5

[3] Cancer: Factsheet No. 297 (2015, February). https://www.cancer.org/research/cancer-facts-statistics/all-cancer-facts-figures/cancer-facts-figures-2015.html

[4] International Agency for Cancer Research and World Health Organization (2014) World Cancer Factsheet 2014. https://www.cancer.org/research/cancer-facts-statistics/all-cancer-facts-figures/cancer-facts-figures-2014.html

[5] Salem, M., Ayyad, R., Sakr, H. and Gaafer, A. (2022) Design and Synthesis of New Compounds Derived from Phenyl Hydrazine and Different Aldehydes as Anticancer Agents. *International Journal of Organic Chemistry*, **12**, 28-39. https://doi.org/10.4236/ijoc.2022.121003

[6] Cai, S.X. (2007) Small Molecule Vascular Disrupting Agents: Potential New Drugs for Cancer Treatment. *Recent Patents on Anti-Cancer Drug Discovery*, **2**, 79-101. https://doi.org/10.2174/157489207779561462

[7] Shi, L.M., Fan, Y., Lee, J.K., Waltham, M., Andrews, D.T., Scherf, U., Paull, K.D. and Weinstein, J.N. (2000) Mining and Visualizing Large Anticancer Drug Discovery Databases. *Journal of Chemical Information and Modeling*, **40**, 367-379. https://doi.org/10.1021/ci990087b

[8] Monks, A., Scudiero, D., Skehan, P., Shoemaker, R., Paull, K., Vistica, D., Hose, C.,

Langley, J., Cronise, P., Wolff, V.A., Goodrich, M.G., Campbell, H., Mayo, J. and Boyd, M.J. (1991) Feasibility of a High-Flux Anticancer Drug Screen Using a Diverse Panel of Cultured Human Tumor Cell Lines. *Journal of the National Cancer Institute*, **83**, 757-766. https://doi.org/10.1093/jnci/83.11.757

[9] Palmer, B.D., Rewcastle, G.W., Atwell, G.J., Baguley, B.C. and Denny, W.A. (1988) Potential Antitumor Agents. 54. Chromophore Requirements for *in Vivo* Antitumor Activity among the General Class of Linear Tricyclic Carboxamides. *Journal of Medicinal Chemistry*, **31**, 707-712. https://doi.org/10.1021/jm00399a003

[10] Denny, W.A., Rewcastle, G.W. and Baguley, B.C. (1990) Potential Antitumor Agents. 59. Structure-Activity Relationships for 2-Phenylbenzimidazole-4-Carboxamides, a New Class of Minimal DNA-Intercalating Agents Which May Not Act via Topoisomerase II. *Journal of Medicinal Chemistry*, **33**, 814-819. https://doi.org/10.1021/jm00164a054

[11] Lang, D.K., Kaur, R., Arora, R. Saini, B. and Arora S. (2020). Nitrogen-Containing Heterocycles as Anticancer Agents: An Overview. *Anti-Cancer Agents in Medicinal Chemistry*, **20**, 2150-2168. https://doi.org/10.2174/1871520620666200705214917

[12] Sakr, H.M., Ayyad R.R., Mahmoud, K., Mansour, A.M. and Ahmed, A.G. (2021) Design Synthesis of Analgesics and Anticancer of Some New Derivatives of Benzimidazole. *International Journal of Organic Chemistry*, **11**, 144-169. https://doi.org/10.4236/ijoc.2021.113011

[13] Khalifa, M.M., Sakr, H.M., Ibrahim, A., Mansour A.M. and Ayyad R.R. (2022) Design and Synthesis of New Benzylidene-Quinazolinone Hybrids as Potential Anti-Diabetic Agents: *In Vitro* α-Glucosidase Inhibition, and Docking Studies. *Journal of Molecular Structure*, **1250**, Article ID: 131768. https://doi.org/10.1016/j.molstruc.2021.131768

[14] El-Helby, A., Sakr, H.M., Ayyad R.R. and Mahdi, H. (2020) Design, Synthesis, Molecular Modeling, *in Vivo* Studies and Anticancer Activity Evaluation of New Phthalazine Derivatives as Potential DNA Intercalators and Topoisomerase II Inhibitors. *Bioorganic Chemistry*, **103**, Article ID: 104233. https://doi.org/10.1016/j.bioorg.2020.104233

[15] Ayyad, R.R. (2012) Synthesis and Biological Evaluation of Novel Iodophthalazinedione Derivatives as Anticonvulsant Agents. *Al-Azhar Journal of Pharmaceutical Sciences*, **45**, 1-13. https://doi.org/10.21608/ajps.2012.7146

[16] El-Dehna, W.M. Abou-Seri, S., El-Kerdawy, A. and Ayyad, R.R. (2016) Increasing the Binding Affinity of VEGFR-2 Inhibitors by Extending Their Hydrophobic Interaction with the Active Site: Design, Synthesis and Biological Evaluation of 1-Substituted-4-(4-Methoxybenzyl) Phthalazine Derivatives. *European Journal of Medicinal Chemistry*, **113**, 50-62. https://doi.org/10.1016/j.ejmech.2016.02.029

[17] El-Helby, A., Ayyad, R.R., Sakr, H.M. and Abdul-Rahim, A.S. (2017) Design, Synthesis, Molecular Modeling and Biological Evaluation of Novel 2,3-Dihydrophthalazine-1,4-Dione Derivatives as Potential Anticonvulsant Agents. *Journal of Molecular Structure*, **1130**, 333-351. https://doi.org/10.1016/j.molstruc.2016.10.052

[18] El-Helby, A., Ayyad, R.R., Sakr, H.M., El-Adl, K. and Ali, M.M. (2017) Design, Synthesis, Molecular Docking, and Anticancer Activity of Phthalazine Derivatives as VEGFR-2 Inhibitors. *Archiv der Pharmazie*, **350**, Article ID: 1700240. https://doi.org/10.1002/ardp.201700240

[19] El-Helby, A., Ayyad, R.R., El-Adl, K. and El-Kady H. (2019) Phthalazine-1, 4-dione Derivatives as Non-Competitive AMPA Receptor Antagonists: Design, Synthesis, Anticonvulsant Evaluation, ADMET Profile and Molecular Docking. *Molecular Diversity*, **23**, 283-298. https://doi.org/10.1007/s11030-018-9871-y

[20] Eissa, I.H., Metwally, A.M., Belal, A. and Mehany A.B. (2019) Discovery and Anti-proliferative Evaluation of New Quinoxalines as Potential DNA Intercalators and Topoisomerase II Inhibitors. *Archiv der Pharmazie*, **352**, Article ID: 1900123. https://doi.org/10.1002/ardp.201900123

[21] Kamal, I.M., Abdul-Rahman, A.A., Ayyad R.R. and El-Adl, K. (2013) Design and Synthesis of Some Novel 2-(3-methyl-2-oxoquinoxalin-1(2H)-yl)-N-(4-(substituted)phenyl) Acetamide Derivatives for Biological Evaluation as Anticonvulsant Agents. *Bulletin of Faculty of Pharmacy, Cairo University*, **51**, 101-111. https://doi.org/10.1016/j.bfopcu.2012.11.003

[22] El-Helby, A., Ayyad, R.R. and Zayed, M.F. (2011) Synthesis and Biological Evaluation of Some Novel Quinoxaline Derivatives as Anticonvulsant Agents. *Arzneimittelforschung*, **61**, 379-381. https://doi.org/10.1055/s-0031-1296214

[23] El-Helby, A., Ayyad R.R., El-Adl, K. and El-Wan, A. (2017) Quinoxalin-2 (1H)-One Derived AMPA-Receptor Antagonists: Design, Synthesis, Molecular Docking and Anticonvulsant Activity. *Medicinal Chemistry Researchs*, **26**, 2967-2984. https://doi.org/10.1007/s00044-017-1996-5

[24] El-Helby, A., Ayyad R.R., Zayed, M.F. and Abou-El-Kheer H.S. (2019) Design, Synthesis, *in Silico* ADMET Profile and GABA-A Docking of Novel Phthalazines as Potent Anticonvulsants. *Archiv Der Pharmazie*, **352**, Article ID: 1800387. https://doi.org/10.1002/ardp.201800387

[25] El-Helby, A., Ayyad, R.R., Sakr, H.M. and El-Adl, K. (2018) Design, Synthesis, *In Vitro* Anti-Cancer Activity, ADMET Profile and Molecular Docking of Novel Triazolo [3,4-a] Phthalazine Derivatives Targeting VEGFR-2 Enzyme. *Anti-Cancer Agents in Medicinal Chemistry*, **18**, 1184-1196. https://doi.org/10.2174/1871520618666180412123833

[26] Ekhlass, M.N., Abdul Razek F.M., Ayyad R.R. and El-Farargy, A.F. (2016) Synthesis and Some Reactions of 1-aryl-4-acetyl-5-methyl-1, 2, Triazole Derivatives with Anticonvulsant Activity. *Mini-Reviews in Medicinal Chemistry*, **16**, 926-936. https://doi.org/10.2174/1389557516666160118105505

[27] Ayyad R.R. (2014) Synthesis and Anticonvulsant Activity of 6-Iodo Phthalazinedione Derivatives. *Al-Azhar Journal of Pharmaceutical Sciences*, **50**, 43-54. https://doi.org/10.21608/ajps.2016.6930

[28] Skehan, P., Storeng, R., Scudiero, D., Monks, A., McMahon, J. and Vistica, D. (1990) New Colorimetric Cytotoxicity Assay for Anticancer-Drug Screening. *Journal of the National Cancer Institute*, **82**, 1107-1112. https://doi.org/10.1093/jnci/82.13.1107

[29] Allam, R.M., Al-Abd, A.M., Khedr, A. and Sharaf, O.A. (2018) Fingolimod Interrupts the Cross Talk between Estrogen Metabolism and Sphingolipid Metabolism within Prostate Cancer Cells. *Toxicology Letters*, **11**, 77-85. https://doi.org/10.1016/j.toxlet.2018.04.008