SYNTHESIS, STRUCTURAL CHARACTERIZATION OF SOME PYRAZOLO [1-5A] PYRIMIDINE AND IMIDAZO[1,2-B]- PYRAZOLE DERIVATIVES AS ANTI-CANCER ACTIVITY

**Usama Fathy^{1,✉}, Ameen A. Abu-Hashem², Rasha S. Gouhar³, Hanem M.
Awad⁴ and Abdelbaset M. Elgamal⁵**

¹Applied Organic Chemistry Department, National Research Centre, 33 El Bohouth st. (former EL Tahrir st.)-Dokki-Giza-Egypt-P.O.12622.

²Photochemistry Department (Heterocyclic Unit), National Research Centre, 33 El Bohouth st. (former EL Tahrir st.)-Dokki-Giza-Egypt-P.O.12622.

³Therapeutic Chemistry Department, National Research Centre, 33 El Bohouth st. (former EL Tahrir st.)-Dokki-Giza-Egypt-P.O.12622.

⁴Tanning Materials and Leather Technology Department, National Research Centre, 33 El Bohouth st. (former EL Tahrir st.)-Dokki-Giza-Egypt-P.O.12622.

⁵Chemistry of Natural and Microbial Products Department, National Research Centre, 33 El Bohouth st. (former EL Tahrir st.)-Dokki-Giza-Egypt-P.O.12622.

✉Corresponding Author: usamafathy2000@yahoo.com

ABSTRACT

For anticancer cell line evaluation, a new sequence of pyrazolo [1-5a] pyrimidine and imidazo[1,2-b] pyrazole derivatives were synthesized. The reaction of 5-aminopyrazolo with appropriate functionalized enaminones derivatives and 2-bromoacetophenone to afford, respectively. In vitro tests were performed on the newly synthesized compounds against HepG-2 and MCF-7 cancer cells to assay their cytotoxicity. A few compounds showed promising activity as anticancer agents against MCF-7 cancer cell.

Keywords: Pyrazole, Pyrimidine, Imidazole, Enaminone, Anticancer Activity.

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INTRODUCTION

Cancer is a term used to describe a group of cancerous diseases that can affect various parts of the body.¹ The liver can develop primary cancer, in which it forms itself, or to cancer that develops elsewhere in the body and then is transferred to the liver (secondary or metastatic cancer). In addition, breast cancer is cancer that forms in the cells of the breasts. As a result, there is a constant need for new chemotherapeutic medicines for cancer care. Pyrazolo[1,5-a] pyrimidine is one of the pyrazolepyrimidine isomers, they are purine analogue². They have a wide variety of biological activity such as anti-proliferative agents³, antifungal⁴, antimicrobial^{5, 6}, chronic myeloid leukemia inhibitors⁷, antioxidant^{8,9}, cathepsin K inhibitors^{10,11}, anti-tubercular¹², anti-inflammatory¹³, antiviral¹⁴. Currently, they reported strong cytotoxicity against several human cancer cell lines.¹⁵⁻²⁰

For example, compound **I** showed the highest activity against HepG2, MCF7, HCT116 and HeLa cell lines with IC₅₀ values 1.88, 0.47, 0.54 and 0.40 µg/ml, respectively²¹. Also, Compound **II** was found to be the most active, with IC₅₀ values of 1.98, 2.20, and 2.61 M, respectively, against MCF-7, BT474, and A549 cancer cell lines. Compounds **III** and **IV** were also shown to have anticancer activity against four separate cell lines (HepG2, MCF-7, A549 and Caco2).²²

Imidazo[1,2-b] pyrazole derivatives were synthesized previously by many authors.²³⁻²⁵ In the last years, it was reported as an anticancer agent.^{26,27} For example, compounds **V**, **VI** and **VII** show promise as an anticancer agent with an IC₅₀ less than 10 mM in the 6 cell lines tested.²⁷

Fig.-1: Structure of Some Anticancer Pyrazolo[1-5a]pyrimidin and Imidazo[1,2-b]pyrazole Derivatives

By screening HepG-2 and MCF-7 for anticancer activity, compounds **10a-l** and **12a-c** derivatives carrying a phenyldiazenyl moiety have been prepared to produce new potent anticancer agents.

EXPERIMENTAL

Synthesis of 1-phenyl-2-bromoethanone (2-bromoacetophenone) (2)

Add anhydrous AlCl₃ (0.5 g) to a stirred solution of acetophenone (12.0 g, 0.1 mol) in dry ether (100 ml). Bromine (16.0 g, 0.2 mol) was added drop-wise over 30 min. duration with stirring for 20 min. to the resulting solution, the reaction mixture was poured on cold water. Separated the organic layer, washed twice with water, then with a solution of NaHCO₃ (5 %, 150 ml) and gradually dried over anhydrous Na₂SO₄, then filtered. Under reduced pressure, the solvent was extracted and the solid residue was recrystallized from ether to provide compound **2**.

Synthesis of 3-oxo-3-phenylpropanenitrile (3)

KCN solution (6.5 g, 0.1 mol in 30 ml H₂O) was added to a solution of phenacyl bromide (10 g, 0.05 mol) in benzene (60 ml) and the reaction mixture was heated to 70 °C with intensive shaking until the aqueous layer become deep orange. The reaction mixture was cooled and the aqueous layer was removed, benzene washed and HCl acidified. Filtration, collected the solid precipitate, washed with cold water, dried and finally recrystallized from hot H₂O to produce compound **3**.

Synthesis of 2-oxo-N,2-diphenylacetohydrazonoyl cyanide (6)

In EtOH (100 ml) CH₃COONa.3H₂O (13.6 g, 0.1 mol) was introduced to a stirred solution of benzoylacetonitrile (**3**) (14.5g, 0.1 mol). The mixture was chilled at 0°C after stirring for 15 min and treated with a cold solution of the required arylamine (0.1 mol) in 6 M HCl (6 ml) with NaNO₂ (0.7 g, 10 mmol) in 5 ml H₂O. The diazonium salt was added and stirred at 0-5°C for an additional 2 h, then placed in a refrigerator (4°C) for 8 h. The resulting solid was stored, washed thoroughly with H₂O and dried by filtration. To give the corresponding acetohydrazonoyl cyanide (**6**), the crude product was crystallized from EtOH.

Synthesis of 3-phenyl-4-(phenyldiazenyl)-1H-pyrazol-5-amine derivatives (7a-c)

Compounds (**7a-c**) were obtained through the reaction of acetohydrazonoyl cyanide derivatives (**6a-c**) with 98 % NH₂-NH₂.H₂O in n-BuOH and 4 h reflux. To afford compounds (**7a-c**), the product obtained was filtered, dried and crystallized by EtOH.

3-phenyl-4-(phenyldiazenyl)-1H-pyrazol-5-amine (7a)

Yield (71%), m.p. 217°C; IR (KBr, ν cm⁻¹): 3330-3195 (NH+NH₂), ¹H NMR (DSMO-*d*₆, ppm) δ 6.2 (s, 2H, D₂O-exchangeable, NH₂), 7.30-7.51 (m, 6H, 3H Ar+3H ph), 7.73 (d, J = 7.8 Hz, 2H (*o*), Ar), 8.13 (d, J = 8.5 Hz, 2H (*o*), ph), 12.09 (s, 1H, D₂O-exchangeable, NH); ¹³C NMR (DSMO-*d*₆, ppm) 95.35, 126.30, 127.39, 127.93, 128.33, 128.34, 128.89(2C), 129.14(2C), 132.02, 139.86, 145.22, 149.84, 153.51; MS (C₁₅H₁₃N₅), (m/z , 100%) = 263 (M⁺).

4-((4-chlorophenyl)diazenyl)-3-phenyl-1H-pyrazol-5-amine (7b)

Yield (78%); m.p. 230 °C; IR (KBr, ν cm⁻¹): 3335-3197(NH+NH₂); ¹H NMR (DSMO-*d*₆, ppm) δ 6.31 (s, 2H, D₂O-exchangeable, NH₂), 7.38-7.53 (m, 4H, Ar), 7.74 (t, J = 8.7 Hz, 3H (*p*, *m*), ph), 8.10 (d, J = 8.5 Hz, 2H (*o*), ph), 12.11 (s, 1H, D₂O-exchangeable, NH); ¹³C NMR (DSMO-*d*₆, ppm) 96.50, 126.27, 127.42, 127.99, 128.34, 128.47(2C), 128.83, 128.93, 129.15, 131.81, 132.90, 141.18, 145.47, 151.88; MS (C₁₅H₁₂ClN₅), (m/z , %) = 297 (M⁺, 35), 399 (M⁺+2, 11).

4-((4-fluorophenyl)diazenyl)-3-phenyl-1H-pyrazol-5-amine (7c)

Yield (70%), m.p. 225°C; IR (KBr, ν cm⁻¹): 3335-3200 (NH+NH₂), ¹H NMR (DSMO-*d*₆, ppm) δ 6.2 (s, 2H, D₂O-exchangeable, NH₂), 7.28-7.47 (m, 4H (*m*, *o*), Ar-), 7.77 (m, 3H (*p*, *m*) ph), 8.11 (d, J = 8.5 Hz, 2H (*o*), ph), 12.08 (s, 1H, D₂O-exchangeable, NH); ¹³C NMR (DSMO-*d*₆, ppm) 95.60, 115.73, 116.04, 122.52, 127.37, 127.87, 128.26, 128.86, 130.55, 131.88, 133.02, 140.99, 147.42, 149.84, 158.40 ; MS (C₁₅H₁₂FN₅), (m/z , 100%) = 281 (M⁺).

Synthesis of 2,7-diphenyl-3-(phenyldiazenyl) pyrazolo[1,5-*a*] pyrimidine derivatives (10a-l)

A mixture of compound **7** ($\approx$ 0.3 g; 0.001 mol) and the corresponding enaminones (**8**), (0.001 mol) in 20 ml CH₃COOH was refluxed for 1-2 h, left to cool. The product obtained was washed, dried, and crystallized from EtOH to give **10a-l** compounds, respectively.

2,7-diphenyl-3-(phenyldiazenyl)pyrazolo[1,5-*a*]pyrimidine (10a)

Yield (72%), m.p. 190°C; IR (KBr, ν cm⁻¹): 1660 (C=N), ¹H NMR (DSMO-*d*₆, ppm) δ 7.45-7.65 (m, 10H,), 7.80 (d, J = 7.5 Hz, 2H), 8.15 (d, J = 6.5 Hz, 2H), 8.20 (d, J = 1.5 Hz, 2H), 8.86 (d, J = 4.5 Hz, 1H of *pyrimidine*); ¹³C NMR (DSMO-*d*₆, ppm) 110.93, 120.85, 121.81, 124.81, 128.63(2C), 128.69(2C), 129.45(2C), 129.50(2C), 129.59(2C), 129.99(2C), 131.50, 131.80, 139.62, 144.68, 146.13, 153.55, 153.78, 153.83 ; MS (C₂₄H₁₇N₅), (m/z , 100%) = 375 (M⁺).

7-(4-chlorophenyl)-2-phenyl-3-(phenyldiazenyl)pyrazolo[1,5-*a*]pyrimidine (10b)

Yield (72%), m.p. 203°C; IR (KBr, ν cm⁻¹): 1665 (C=N), ¹H NMR (DSMO-*d*₆, ppm) δ 7.48-7.59 (m, 7H,), 7.70 (d, J = 8.1 Hz, 2H), 7.79 (d, J = 8.1 Hz, 2H), 8.16 (d, J = 7.5 Hz, 2H), 8.22 (d, J = 8.4 Hz, 2H), 8.87 (d, J = 4.2 Hz, 1H of *pyrimidine*); ¹³C NMR (DSMO-*d*₆, ppm) 110.73, 120.56, 120.60, 121.63, 124.68, 128.46, 128.63, 128.63, 129.27, 129.32(2C), 129.46(2C), 129.90(2C), 131.55 (2C), 131.67, 136.15, 139.44, 144.76, 153.28, 153.58, 153.62; MS (C₂₄H₁₆ClN₅), (m/z , 100%) = 409 (M⁺, 28), 411 (M⁺+2, 9).

2-phenyl-3-(phenyldiazenyl)-7-(*p*-tolyl)pyrazolo[1,5-*a*]pyrimidine (10c)

Yield (72%), m.p. 180°C; IR (KBr, ν cm⁻¹): 1664 (C=N), ¹H NMR (DSMO-*d*₆, ppm) δ 2.45 (s, 3H, CH₃), 7.46-7.60 (m, 9H,), 7.80 (d, J = 7 Hz, 2H), 8.13 (d, J = 8.1 Hz, 2H), 8.17 (d, J = 1.2 Hz, 2H), 8.87 (d, J = 4.2 Hz, 1H of *pyrimidine*); ¹³C NMR (DSMO-*d*₆, ppm) 20.97, 110.30, 120.62, 121.53(2C), 126.93, 128.38(2C), 129.05(2C), 129.20(2C), 129.24(2C), 129.34(2C), 129.68(2C), 129.75 (2C), 131.64, 141.50, 146.00, 153.51, 153.60; MS (C₂₅H₁₉N₅), (m/z , 100%) = 389 (M⁺).

2-Phenyl-3-(phenyldiazenyl)-7-(thiophen-2-yl)pyrazolo[1,5-*a*]pyrimidine (10d)

Yield (72%), m.p. 140°C; IR (KBr, ν cm⁻¹): 1668 (C=N), ¹H NMR (DSMO-*d*₆, ppm) δ 7.41-7.58 (m, 7H,), 7.81 (d, J = 1.5 Hz, 2H), 7.98 (d, J = 4.8 Hz, 1H), 8.20 (d, J = 4.8 Hz, 1H), 8.37 (d, J = 7.5 Hz, 2H), 8.60 (d, J = 3.6 Hz, 1H of *pyrimidine*), 8.82 (d, J = 4.8 Hz, 1H of *pyrimidine*); ¹³C NMR (DSMO-*d*₆, ppm) 21.17,

106.82, 121.82, 124.73, 127.97, 128.85(2C), 129.45(2C), 129.48(2C), 129.85(2C), 130.05, 131.71, 133.17, 136.24, 139.44, 139.53, 152.90, 153.18, 153.80, 172.14; MS ($C_{22}H_{15}N_5S$), (m/z , 100%) = 381 (M^+).

3-((4-chlorophenyl)diazenyl)-2,7-diphenylpyrazolo[1,5-a]pyrimidine (10e)

Yield (72%), m.p. 170°C; IR (KBr, ν cm^{-1}): 1665 (C=N), 1H NMR (DSMO- d_6 , ppm) δ 7.53-7.68 (m, 8H), 7.79 (d, J = 7.5 Hz, 2H), 7.79 (d, J = 8.1 Hz, 2H), 8.14 (d, J = 7.5 Hz, 2H), 8.17 (d, J = 1.8 Hz, 1H of pyrimidine), 8.90 (d, J = 1.2 Hz, 1H of pyrimidine); ^{13}C NMR (DSMO- d_6 , ppm) 111.02, 114.95, 123.25, 124.64, 128.30, 128.54, 128.57(2C), 129.41(2C), 129.54(2C), 129.67(2C), 129.89(2C), 131.42 (2C), 131.52, 134.18, 146.15, 152.23, 153.67, 153.98; MS ($C_{24}H_{16}ClN_5$), (m/z , 100%) = 409 (M^+ , 23), 411 (M^+ +2, 7).

7-(4-chlorophenyl)-3-((4-chlorophenyl)diazenyl)-2-phenylpyrazolo[1,5-a]pyrimidine (10f)

Yield (72%), m.p. 198°C; IR (KBr, ν cm^{-1}): 1670 (C=N), 1H NMR (DSMO- d_6 , ppm) δ 7.50 (d, J = 8.1 Hz, 2H, *o*-Ar₁), 7.60 (d, J = 4.5 Hz, 2H, *m*-Ar₁), 7.72-7.75 (m, 5H, 2H Ar₂+3H ph), 7.76 (d, J = 8.1 Hz, 2H, *o*-ph), 8.12 (d, J = 7.5 Hz, 2H *o*-Ar₂), 8.21 (d, J = 8.1 Hz, 1H of pyrimidine), 8.86 (d, J = 0.9 Hz, 1H pyrimidine); ^{13}C NMR (DSMO- d_6 , ppm) 110.92, 123.23, 124.65, 128.29, 128.49, 128.58, 128.63(2C), 129.36(2C), 129.54(2C), 131.43(2C), 131.54(2C), 131.74(2C), 134.21, 136.21, 144.84, 152.16, 153.55, 153.84; MS ($C_{24}H_{15}Cl_2N_5$), (m/z , 100%) = 443 (M^+ , 21), 445 (M^+ + 2, 12), 447 (M^+ + 4, 2.5).

3-((4-chlorophenyl)diazenyl)-2-phenyl-7-(*p*-tolyl)pyrazolo[1,5-a]pyrimidine (10g)

Yield (72%), m.p. 180°C; IR (KBr, ν cm^{-1}): 1665 (C=N), 1H NMR (DSMO- d_6 , ppm) δ 2.45 (s, 3H, CH₃-), 7.47-7.64 (m, 9H), 7.80 (d, J = 8.7 Hz, 2H), 8.13 (d, J = 8.1 Hz, 2H), 8.18 (d, J = 2.1 Hz, 1H of pyrimidine), 8.89 (d, J = 4.5 Hz, 1H of pyrimidine); ^{13}C NMR (DSMO- d_6 , ppm) 21.14, 110.73, 120.66, 120.73, 121.26, 123.29, 126.96, 128.34, 128.60(2C), 128.72, 128.88, 129.21(2C), 129.42, 129.48, 129.61, 129.89, 131.58, 134.20, 141.72, 145.43, 145.54, 146.27, 152.27, 153.98; MS ($C_{25}H_{18}ClN_5$), (m/z , 100%) = 423 (M^+ , 26), 425 (M^+ + 2, 8).

3-((4-chlorophenyl)diazenyl)-2-phenyl-7-(thiophen-2-yl)pyrazolo[1,5-a]pyrimidine (10h)

Yield (72%), m.p. 230°C; IR (KBr, ν cm^{-1}): 1668 (C=N), 1H NMR (DSMO- d_6 , ppm) δ 7.42-7.65 (m, 6H), 7.82 (d, J = 8.1 Hz, 2H of Ar₁), 8.04 (d, J = 4.8 Hz, 1H of thiophen), 8.23 (d, J = 5.1 Hz, 1H of thiophen-), 8.36 (d, J = 7.8 Hz, 2H of ph), 8.63 (d, J = 3.9 Hz, 1H of pyrimidine), 8.85 (d, J = 5.1 Hz, 1H of pyrimidine); ^{13}C NMR (DSMO- d_6 , ppm) 94.30, 106.98, 120.69, 123.34, 127.99, 128.85(2C), 129.29(2C), 129.41(2C), 129.53(2C), 129.90, 130.64, 131.49, 133.28, 134.27, 136.38, 144.48, 152.31, 153.14; MS ($C_{22}H_{14}ClN_5S$), (m/z , 100%) = 415 (M^+ , 16), 425 (M^+ + 2, 5).

3-((4-fluorophenyl)diazenyl)-2,7-diphenylpyrazolo[1,5-a]pyrimidine (10i)

Yield (72%), m.p. 120°C; IR (KBr, ν cm^{-1}): 1670 (C=N), 1H NMR (DSMO- d_6 , ppm) δ 7.37-7.68 (m, 8H), 7.84 (d, J = 5.4 Hz, 2H), 7.87 (d, J = 5.4 Hz, 2H), 8.14 (d, J = 7.5 Hz, 2H), 8.19 (d, J = 2.5 Hz, 1H of pyrimidine), 8.88 (d, J = 4.5 Hz, 1H of pyrimidine); ^{13}C NMR (DSMO- d_6 , ppm) 94.24, 110.87, 115.64, 116.07, 116.38, 121.78, 123.65, 124.47, 128.20, 128.53, 128.57, 128.82, 129.35, 129.49, 129.65, 129.86, 131.40, 131.57, 139.45, 146.06, 150.35, 150.67, 161.22, 164.50 (C-F); MS ($C_{24}H_{16}FN_5$), (m/z , 100%) = 393 (M^+).

7-(4-Chlorophenyl)-3-((4-fluorophenyl)diazenyl)-2-phenylpyrazolo[1,5-a]pyrimidine (10j)

Yield (72%), m.p. 130°C; IR (KBr, ν cm^{-1}): 1673 (C=N), 1H NMR (DSMO- d_6 , ppm) δ 7.34 (dd, J = 2.1 Hz, 2H), 7.37 (dd, J = 8.4 Hz, 2H), 7.69 (d, J = 1.5 Hz, 2H), 8.14 (d, J = 7.5 Hz, 2H), 7.81 (d, J = 1.8 Hz, 2H), 8.12 (d, J = 5.4 Hz, 2H), 8.21 (d, J = 6.9 Hz, 2H), 8.85 (d, J = 4.8 Hz, 1H of pyrimidine); ^{13}C NMR (DSMO- d_6 , ppm) 95.12, 110.80, 116.05, 116.35, 123.68, 123.89, 124.53, 128.51, 128.65, 128.82, 129.37, 129.51, 129.65, 129.86, 131.53, 131.75, 136.21, 139.43, 144.79, 150.34, 153.37, 153.66, 161.26, 164.50 (C-F); MS ($C_{24}H_{15}ClFN_5$), (m/z , 100%) = 427 (M^+ , 22), 429 (M^+ + 2, 7).

3-((4-Fluorophenyl)diazenyl)-2-phenyl-7-(p-tolyl)pyrazolo[1,5-a]pyrimidine (10k)

Yield (72%), m.p. 183°C; IR (KBr, ν cm⁻¹): 1665 (C=N), ¹H NMR (DSMO-*d*₆, ppm) 2.44 (s, 3H, CH₃-), 7.35-7.65 (m, 9H), 7.82 (d, *J* = 8.5 Hz, 2H), 8.15 (d, *J* = 7.5 Hz, 2H), 8.17 (d, *J* = 2.1 Hz, 1H of pyrimidine), 8.85 (d, *J* = 4.5 Hz, 1H of pyrimidine); ¹³C NMR (DSMO-*d*₆, ppm) 21.03, 110.62, 120.13, 121.50, 123.45, 126.96, 128.51, 128.71(2C), 128.80, 129.32(2C), 129.45, 129.63, 129.88, 129.92, 131.72, 135.20, 141.13, 144.86, 145.66, 146.53, 153.21, 163.98; MS (C₂₄H₁₈FN₅), (*m/z*, 100%) = 407 (M⁺).

3-((4-Fluorophenyl)diazenyl)-2-phenyl-7-(thiophen-2-yl)pyrazolo[1,5-a]pyrimidine (10l)

Yield (72%), m.p. 200°C; IR (KBr, ν cm⁻¹): 1670 (C=N), ¹H NMR (DSMO-*d*₆, ppm) δ 7.38-7.62 (m, 6H), 7.82 (d, *J* = 5.4 Hz, 2H), 7.95 (d, *J* = 4.8 Hz, 1H), 8.18 (d, *J* = 5.1 Hz, 1H), 8.32 (d, *J* = 7.2 Hz, 2H), 8.56 (d, *J* = 3.9 Hz, 1H of pyrimidine), 8.78 (d, *J* = 4.8 Hz, 1H of pyrimidine); ¹³C NMR (DSMO-*d*₆, ppm) 106.66, 116.04, 116.34, 123.66, 123.78, 124.42, 127.80(2C), 128.67(2C), 129.32(2C), 129.68(2C), 130.52(2C), 131.54(2C), 133.00, 136.05, 139.37, 144.13, 150.37, 152.70, 153.03, 161.23, 164.52, 171.99; MS (C₂₂H₁₄FN₅S), (*m/z*, 100%) = 309 (M⁺).

Synthesis of 1*H*-imidazo[1,2-*b*]pyrazole Derivatives (12a-c)

For a mixture of compound **7a-c** (0.26 g, 1 mmol), K₂CO₃ (0.45, 3mM) and compound **2** (0.357 g, 1 mmol) in acetone (25 ml), the reaction mixture was refluxed for 4 h. and cooled at room temperature. The product was filtered, washed, dried, and recrystallized from EtOH to give **13a-b** compounds, respectively.

2,6-Diphenyl-7-(phenyldiazenyl)-1*H*-imidazo[1,2-*b*]pyrazole (12a)

Yield (72%), m.p. 250°C; IR (KBr, ν cm⁻¹): 3360 (NH), ¹H NMR (DSMO-*d*₆, ppm) δ 7.15-7.65 (m, 10H), 7.77 (d, *J* = 5.4 Hz, 2H), 7.95 (d, *J* = 4.8 Hz, 2H), 8.45 (s, 1H of imidazo pyrazole), 12.32 (s, 1H, D₂O-exchangeable, NH), ¹³C NMR (DSMO-*d*₆, ppm) 98.13, 119.85, 125.41, 125.97, 127.12(2C), 128.22, 128.36(2C), 128.78(2C), 128.97(2C), 129.53(2C), 129.98(2C), 130.52(2C), 133.11, 139.50, 141.27, 149.15; MS (C₂₃H₁₇N₅), (*m/z*, 100%) = 363 (M⁺).

7-((4-Chlorophenyl)diazenyl)-2,6-diphenyl-1*H*-imidazo[1,2-*b*]pyrazole (12b)

Yield (72%), m.p. 270°C; IR (KBr, ν cm⁻¹): 3370 (NH), ¹H NMR (DSMO-*d*₆, ppm) δ 7.35-7.67 (m, 10H), 7.80 (d, *J* = 5.4 Hz, 2H), 7.96 (d, *J* = 4.8 Hz, 2H), 8.48 (s, 1H of imidazo pyrazole), 12.52 (s, 1H, D₂O-exchangeable, NH), ¹³C NMR (DSMO-*d*₆, ppm) 98.15, 120.30, 127.21, 128.14(2C), 128.44, 128.50, 128.78(2C), 128.95(2C), 128.97(2C), 129.65(2C), 130.88(2C), 131.51, 132.62, 134.71, 140.72, 141.80, 150.10; MS (C₂₃H₁₆ClN₅), (*m/z*, 100%) = 397(M⁺, 26), 399 (M⁺ + 2, 8).

7-((4-Fluorophenyl)diazenyl)-2,6-diphenyl-1*H*-imidazo[1,2-*b*]pyrazole(12c)

Yield (72%), m.p. >300°C; IR (KBr, ν cm⁻¹): 3375 (NH), ¹H NMR (DSMO-*d*₆, ppm) δ 7.45-7.68 (m, 10H), 7.82 (d, *J* = 5.4 Hz, 2H), 7.95 (d, *J* = 4.8 Hz, 2H), 8.50 (s, 1H of imidazo pyrazole), 12.78 (s, 1H, D₂O-exchangeable, NH), ¹³C NMR (DSMO-*d*₆, ppm) 98.22, 116.50, 120.31, 123.7, 127.92 (2C), 128.15, 128.33, 128.47, 128.85(2C), 128.97, 129.65(2C), 130.88(2C), 131.71(2C), 134.50, 140.88, 141.80, 150.10, 161.12; MS (C₂₃H₁₆ClN₅), (*m/z*, 100%) = 381(M⁺).

General Procedure

The melting points were uncorrected and determined using an Electrothermal IA 9100 (Shimadzu, Japan) apparatus. Vario El-Mentar apparatus (Shimadzu, Japan), National Research Centre, Cairo, Egypt, carried out microanalytical research. IR spectra were reported on a Perkin-Elmer 1650 Spectrophotometer, National Research Centre, Cairo, Egypt as potassium bromide pellets using KBr disc technique. NMR experiments in Deuterated Dimethylsulphoxide (DMSO-*d*₆) were calculated on a Varian -300 MHz and chemical changes were expressed as parts per million; ppm (π values) against TMS as an internal standard, Faculty of Science, University of Cairo, Cairo, Egypt. Mass spectra were recorded on the 70 eV mass spectrometer at Shimadzu GCMS-QP-1000EX, University of Cairo, Cairo, Egypt.

Biological Evaluation

Cell Culture Conditions

The American Model Culture Collection (Rockville, MD) obtained the cells of human liver carcinoma (HepG-2), and human breast adenocarcinoma (MCF-7). The cytotoxicity activities on the lines of human cancer cells HepG-2 and MCF-7 were calculated using MTT assay, which was based on the reduction of tetrazolium salt in viable cells by mitochondrial dehydrogenases.²⁸⁻³⁰ The absorbance was measured at 570 nm, using a microplate reader from SpectraMax® Paradigm® Multi-Mode. The relative viability of the cells was expressed as the mean percentage of viable cells compared to the control cells not treated.

Statistical Analysis

Both tests were carried out in triplicate and replicated on three separate days. All the values were as mean $\pm$ SD. SPSS probit testing software system (SPSS Inc., Chicago, IL) calculated the IC₅₀.

RESULTS AND DISCUSSION

Chemistry

In the first scheme, the phenacyl bromide was obtained by bromination of acetophenone, then reacted with KCN in dry benzene to afford compound **3**, which allowed to reacting with 1-chloro-2-phenyldiazene (**5**) to yield compound **6**, when it reacting with NH₂.NH₂.H₂O yield 5-aminopyrazole derivatives (**7a-c**). The IR spectrum of compound **7a-c** displayed the characteristic sharp absorption bands of NH and NH₂ at $\approx$ 3335, 3200 cm⁻¹, respectively (Scheme-1). The ¹H-NMR spectrum of **7a-c** exhibited the singlet signals of protons NH₂ and NH appear at 6.2-6.31 and 12.08-12.11 ppm, respectively. 5-aminopyrazole derivatives (**7a-c**) as an intermediate key for synthesis the synthesis of compounds **10a-l** and **12a-c** derivatives as shown in Scheme-2, and 3.

Scheme-1: The Mechanistic Pathway for the Synthesis of 5-amino phenyldiazenyl-1H-pyrazol Derivatives (7a-c)

In Scheme-2, mating occurs between 5-amino phenyldiazenyl-1H-pyrazol (**7a-c**) and functionalized enaminones (**8**) by refluxing in glacial CH₃COOH, the addition of nucleophilic amino group to the olefinic double bond and releasing dimethylamine led to the formation of the corresponding pyrazolo [1-5a] pyrimidine (**10a-l**) (Scheme-2). The ¹H-NMR spectrum of **10a-l** exhibited the disappearance of the singlet as duplet signals at $\delta \approx$ 8.17-8.56 and $\delta \approx$ 8.85-8.90 ppm, respectively. The ¹³C-NMR spectrum of **10a-l**

signals of protons NH₂ and NH and revealed deshielding of H-5, H-6 of pyrazolo [1-5a] pyrimidine appear exhibited a high chemical shift of F-C where it appears at about 164-171 ppm.

10	Ar ₁	Ar ₂
a	Ph	Ph
b	Ph	4-ClC ₆ H ₄
c	Ph	4-MeC ₆ H ₄
d	Ph	2-Thienyl
e	4-ClC ₆ H ₄	Ph
f	4-ClC ₆ H ₄	4-ClC ₆ H ₄
g	4-ClC ₆ H ₄	4-MeC ₆ H ₄
h	4-ClC ₆ H ₄	2-Thienyl
i	4-FC ₆ H ₄	Ph
j	4-FC ₆ H ₄	4-ClC ₆ H ₄
k	4-FC ₆ H ₄	4-MeC ₆ H ₄
l	4-FC ₆ H ₄	2-Thienyl

Scheme-2: Synthesis of Phenyldiazenyl pyrazolo[1, 5-a]pyrimidine Derivatives (10a-l)

Also, 5-amino phenyldiazenyl-1H-pyrazole (**7a-c**) were refluxed with 2-bromo acetophenone (**2**) in the presence of K₂CO₃ in acetone to afford the correspond imidazo[1,2-b]pyrazole derivatives (**12a-c**). The IR spectrum of compound **12a-c** showed a sharp absorption band of NH at 3370 cm⁻¹. Also, the ¹H-NMR spectrum of **12a-c** exhibited H protons of H-3 and NH of imidazo[1,2-b]pyrazole appear as singlet signals at δ ≈ 8.45-8.50 and δ ≈ 12.32-12.78 ppm, respectively (Scheme-3).

Biological Activity

Cytotoxicity Activity

The attained results showed that all compounds had dose-dependent cytotoxicity against both cell types. (Table-1, Figs.-2 and 3). The constructed deduction from these outcomes is that, in assessment with the positive control doxorubicin, compounds **7a-c** with (IC₅₀ ≈ 29.4 μM) and **10c-d** (IC₅₀ ≈ 29.9 μM) had comparable activities against HepG-2; compounds **10a, 10b, 10e, 10f, 10g, 10h, 10k, 10l, 12a and 12b** had slightly moderate activities; compounds **12c, 10j** and **10i**, respectively, had fewer activities relative to the positive control, regarding human liver cancer (HepG-2) (Fig.-2 and Table-1). Regarding breast cancer cells (MCF-7); all the compounds were more potent relative to the positive control. In addition to **7a** and **12a**

showed better anticancer activities against MCF-7 where IC_{50} for both compounds $\approx 6.1 \mu M$ when compared with doxorubicin ($IC_{50} = 10.3 \mu M$) (Fig.-3 and Table-1).

Scheme-3: Synthesis of imidazo[1,2-b]pyrazole Derivatives (12a-c)

Fig.-2: The Compounds had Anticancer Activity against the HepG-2 Human Cancer Cell Line at Various Concentrations

Fig.-3: The Compounds had Anticancer Activity against MCF-7 Human Cancer Cell Line at Various Concentrations

In general, from the SAR point of view, the pyrazolo [1-5a] pyrimidine and imidazo[1,2-b]pyrazole derivatives bearing phenyldiazenyl moiety have a significant anticancer agent. The combination of enaminones and 2-bromoacetophenone with 5-aminopyrazole are given benefits. The activity of methyl group and 2-thinyl afford **10c-d** which exhibited the highest anticancer activity against A549 cancer cell, respectively.

Table-1: The Cytotoxic IC₅₀ Values of the Compounds were evaluated using the MTT Assay on Two Different Cell Lines

Compound	IC ₅₀ (μM) ± SD	
	HepG-2	MCF-7
7a	29.6± 2.9	6.1± 0.5
7b	29.2± 3.1	8.9± 0.7
7c	29.4± 2.5	8.5± 0.4
10a	30.5± 1.9	7.7± 0.5
10b	31.1± 2.6	8.7± 1.1
10c	29.1± 3.1	9.5± 1.1
10d	29.9± 2.4	9.1± 0.9
10e	32.5± 3.1	8.2± 0.5
10f	30.8± 3.1	9.6± 0.8
10g	32.1 ± 2.9	8.7± 1.1
10h	33.1 ± 3.2	8.6± 0.6
10i	36.7± 3.5	9.0 ± 0.4
10j	34.5± 3.1	10.3± 1.1
10k	31.0 ± 2.8	7.5± 0.4
10l	32.0 ± 3.4	7.3± 0.5
12a	31.9± 2.7	6.1± 0.4
12b	30.3± 2.5	8.0 ± 0.5
12c	34.0 ± 3.3	7.4± 0.3
Doxorubicin	28.5± 1.9	10.3± 0.8

CONCLUSION

New pyrimidine and imidazo[1,2-b] pyrazole derivatives bearing phenyldiazenyl moiety were built and screened for anticancer activity. A few compounds exhibited good anticancer activities against HepG-2 and MCF-7. The compounds **7a-c** and **10c-d** are the most potent compound against MCF-7 cancer cells.

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