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Synthesis, *In Vitro* Antiproliferative Evaluation and Molecular Docking of New tetrazole-chalcone and tetrazole-pyrazoline Hybrids

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ABSTRACT

New hybrids of tetrazole moiety with different chalcone derivatives were synthesized. The reaction of these chalcones with hydrazine hydrate resulted in the formation of tetrazole-pyrazoline hybrids. Evaluation of the *in vitro* antiproliferative activity of all newly synthesized hybrids against three cancer cell lines and *Vero-B* normal cell line, using MTT-based assay, was performed. Most of the chalcone derivatives exerted superior activity against colon *HCT-116* and prostate *PC-3* cell lines, in comparison with cisplatin (IC_{50} = 20 and 5 µg/ml) and 5-FU (IC_{50} = 17.3 and 21.4 µg/ml), respectively. Compound **5a** was found to be the most active antiproliferative agent against colon *HCT-116* and prostate *PC-3* cell lines (IC_{50} = 0.6 and 1.6 µg/ml) with high selectivity indices (SI = 6.66 and 2.50), respectively. Compound **8f**, in particular, displayed a wider spectrum of activity that included, in addition, excellent effect against breast *MCF-7* cell lines with SI = 2.75. The available docking results revealed a good binding of **5b** with HDAC2 and CYP17A1 that endorses the *in vitro* biological activity against the tested colon and prostate cell lines.

INTRODUCTION

Cancer designates a group of diseases characterized by malignant cells that grow and divide uncontrollably. The abnormal cells have the capacity to spread and invade surrounding tissues via a metastasis process (Vogelstein *et al*., 2013); and the abnormal mutations to DNA within cells may be either inherited or caused by carcinogenic agents (Fischer *et al*., 2005). The traditional cancer treatment includes surgery, chemotherapy and radiation therapy; and the ideal goal of chemotherapy is to specifically target cancer cells without affecting normal cells, to avoid toxic side effects. Searching for new compounds with potent and selective anticancer activities is highly demanded. Literature survey highlighted the importance of tetrazole-containing compounds (A; Figure 1) as antitumor agents (Bayomi *et al*., 2016; Arshad *et al*., 2014; Jedhe *et al*., 2013; Bhaskar and Mohite, 2010). On the other hand, compounds with Chalcone-based structure (B; Figure 1) were described as cytotoxic agents that act via different mechanisms including induction of apoptosis and inhibition of cell proliferation (Sankappa Rai *et al*., 2015; Bayomi *et al*., 2015; Bayomi *et al*., 2013; de Vasconcelos *et al*., 2013; Sharma *et al*., 2013; Murad *et al*., 2012). Some pyrazoline derivatives (Yuan *et al*., 2014; Viale *et al*., 2013; Nitulescu *et al*., 2013) were also reported (C; Figure 1) to inhibit different enzymes that are involved in the cell division.

In view of the previous findings, our research group got interested to design new hybrid molecules, where the tetrazole nucleus and chalcone or pyrazoline functionality were incorporated into the single compound to be evaluated for antiproliferative activity (Figure 1). We thought that co-occurrence of tetrazole and chalcone/pyrazoline pharmacophores should result in new derivatives with the promising biological profile.

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The antiproliferative activity of the newly synthesized compounds was carried out using MTT-based assay against human colon cancer (*HCT-116*), prostate cancer (*PC-3*) and human breast cancer (*MCF-7*) cell lines along with the normal kidney of African green monkey (*Vero B*) cell line. A molecular docking simulation was performed to explore the binding interactions with different enzymes involved in cell proliferation, namely: histone deacetylase 2 enzyme (HDAC2), cytochrome P450 17A1 and 5,10-methenyltetrahydrofolate synthetase (MTHFS).

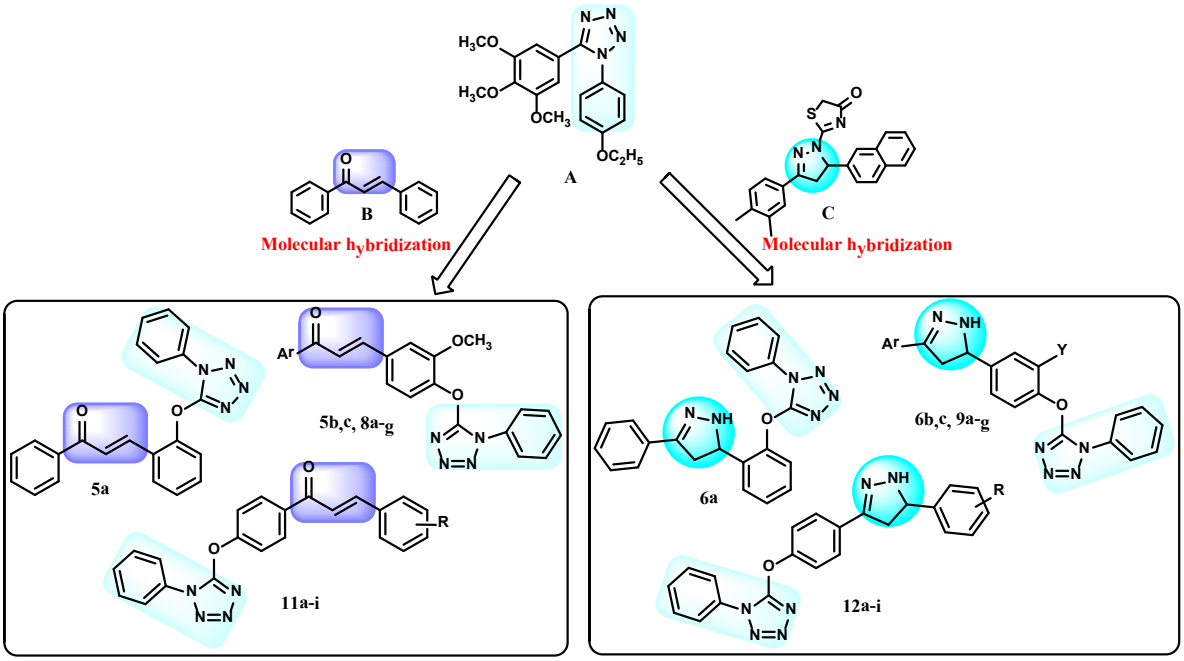


Fig. 1: Reported antitumor compounds (A-C); and the designed tetrazole-chalcone and tetrazole-pyrazoline hybrids.

EXPERIMENTAL PROTOCOLS

Chemistry

Melting points (ºC, uncorrected) were measured using *Fisher-Johns* apparatus. Elemental analyses (C, H, N) were carried out at the microanalytical unit, Cairo University. IR spectra (KBr) were attained using Mattson 5000 FT-IR spectrometer (ν in cm^{-1}) at Faculty of Pharmacy, Mansoura University and the results were expressed in wave number (cm^{-1}). ^{1}H NMR and ^{13}C NMR spectra were obtained on FT-NMR spectrometer (300 MHz) Gemini Varian, using TMS as internal standard and DMSO as a solvent (chemical shifts in δ units, ppm), at Cairo University. MS measurements were performed on JEOL JMS-600H spectrometer, Cairo University. The purities of the compounds were checked by thin layer chromatography (TLC) on silica gel G (Merck) and spots were visualized by irradiation with ultraviolet light (UV; 254 nm). All chemicals, reagents, and solvents were purchased from Aldrich Chemical Company and used as received. The known compounds, *E*-3-(2-Hydroxyphenyl)-1-phenylprop-2-en-1-one **(3a)** (Pan *et al.*, 2013), *E*-1-(3,4-Dimethoxyphenyl)-3-(4-hydroxyphenyl)prop-2-en-1-one **(3b)** (Cai *et al.*, 2017), *E*-1-(3,4-Dimethoxyphenyl)-3-(4-hydroxy-3-methoxyphenyl) prop-2-en-1-one **(3c)** (Pathak *et al.*, 2014), 3-Methoxy-4-((1-phenyl-1*H*-tetrazol-5-yl)oxy) benzaldehyde **(7)** (Buonerba *et al.*, 2017), 4-((1-phenyl-1*H*-tetrazol-5-yl)oxy)acetophenone **(10)** (Eisa *et al.*, 1990), were prepared according to the reported procedures.

General procedure for synthesis of (E)-1-phenyl-3-(2-((1-phenyl-1H-tetrazol-5-yl)oxy)phenyl)prop-2-en-1-one ***(5a)*** *and (E)-1-(3,4-dimethoxyphenyl)-3-(4-((1-phenyl-1H-tetrazol-5-yl)oxy)-3-(un)substituted phenyl)prop-2-en-1-ones* ***(5b,c)*** *(Scheme 1)*

5-Chloro-1-phenyltetrazole **(4)** (1.8 g, 10 mmol) was added, with stirring, to a mixture of the chalcone derivative **(3a-c)** (10 mmol) and anhydrous K_2CO_3 (2.0 g, 15 mmol) in DMF (15 mL). The reaction mixture was stirred for 24 hours at room temperature and then diluted with water (30 mL). The precipitated solid product was collected by filtration, washed with water, dried and crystallized from ethanol to give compounds **5a-c**.

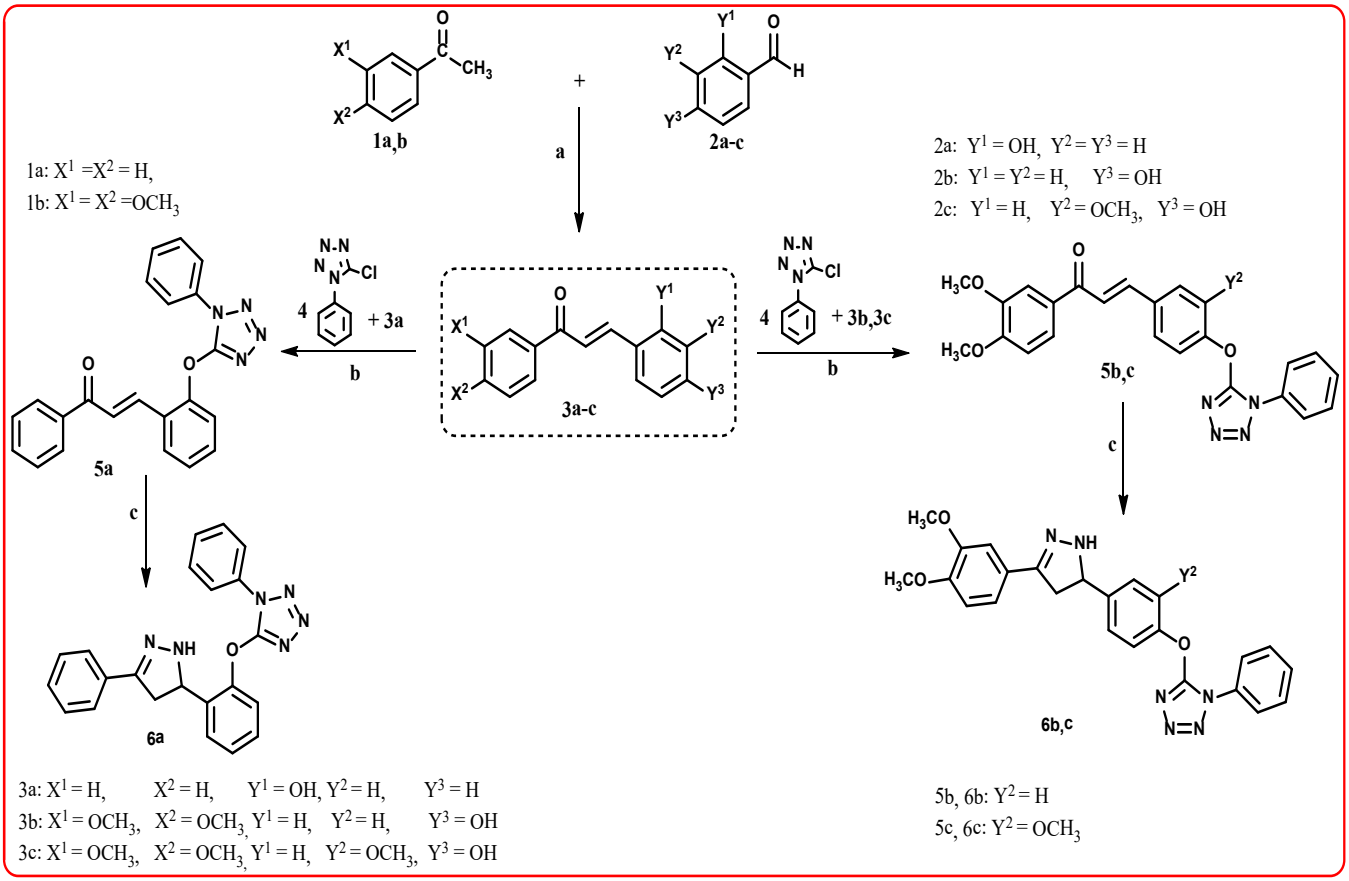
E-1-phenyl-3-(2-((1-phenyl-1H-tetrazol-5-yl)oxy)phenyl)prop-2-en-1-one ***(5a)***

Yield 68%; mp 151-152°C; IR; 1665 (C=O), 1604 (C=C), 1182 (C-O). ^{1}H NMR; 6.81-7.59 (m, 11H, ArH), 7.71 (d, 1H, CO-CH=CH, *J* = 14), 7.77-7.98 (m, 3H, ArH), 8.12 (d, 1H, CO-CH=CH, *J* = 14). Anal. Calcd for $C_{22}H_{16}N_4O_2$ (%): C, 71.73; H, 4.38; N, 15.21. Found: C, 72.03; H, 4.62; N, 15.38.

E-1-(3,4-Dimethoxyphenyl)-3-(4-((1-phenyl-1H-tetrazol-5-yl) oxy)phenyl)prop-2-en-1-one ***(5b)***

Yield 61%; mp 118-120°C; IR; 1657 (C=O), 1593 (C=C), 1161 (C-O). ^{1}H NMR; 3.80 (s, 6H, OCH_3), 6.87-7.50 (m, 9H, ArH), 7.60 (d, 1H, CO-CH=CH, *J* = 15), 7.66-7.69 (m, 3H, ArH), 7.78 (d, 1H, CO-CH=CH, *J* = 15), ^{13}C NMR; 188.32 (C=O), 159.27, 153.48, 150.27, 149.37, 145.53, 143.69, 132.57,

131.13, 130.92, 129.91, 129.71, 129.38, 124.48, 123.17, 122.80 (2C), 121.94, 122.59, 121.21, 112.59, 110.85, 56.16 ($O\underline{C}H_3$), 56.13 ($O\underline{C}H_3$). Anal. Calcd. for $C_{24}H_{20}N_4O_4$(%): C, 67.28; H, 4.71; N, 13.08. Found: C, 66.98; H, 4.76; N, 13.32.



Scheme 1: Reagents and conditions: (a) 2.5% NaOH, C_2H_5OH, RT.; (b) K_2CO_3, DMF, 24 h, RT. (c) $NH_2NH_2.H_2O$, C_2H_5OH, reflux, 10-15 h.

E-1-(3,4-Dimethoxyphenyl)-3-(3-methoxy-4-((1-phenyl-1H-tetrazol-5-yl)oxy)phenyl)prop-2-en-1-one ***(5c)***

Yield 61%; mp 132-133°C; IR; 1658 (C=O), 1597 (C=C), 1155 (C-O). ^{1}H NMR; 3.80 (s, 3H, OCH_3), 3.87 (s, 6H, OCH_3), 7.17-7.78 (m, 8H, ArH), 7.87 (d, 1H, CO-C$\underline{H}$=CH, *J* = 14.5), 7.93-8.00 (m, 3H, ArH), 8.03 (d, 1H, CO-CH=C$\underline{H}$, *J* = 14.5). ^{13}C NMR; 191.79 (C=O), 159.27, 154.47, 153.31, 150.27, 148,82, 146.09, 143.23, 141.62, 130.49, 130.42, 129.94, 129.82, 128.63, 123.46 (2C), 123.57, 122.92, 122.56, 121.94, 113.41 110.88, 56.42 ($O\underline{C}H_3$), 55.77 ($O\underline{C}H_3$), 55.60 ($O\underline{C}H_3$). Anal. Calcd for $C_{25}H_{22}N_4O_5$ (%): C, 65.49; H, 4.84; N, 12.22. Found: C, 65.68; H, 4.52; N, 11.95.

General Procedure for Synthesis of 1-phenyl-5-(2-(3-phenyl-4,5-dihydro-1H-pyrazol-5-yl)phenoxy)-1H-tetrazole ***(6a)*** *and 5-(4-(3-(3,4-dimethoxyphenyl)-4,5-dihydro-1H-pyrazol-5-yl)-2-(un)substituted-phenoxy)-1-phenyl-1H-tetrazoles* ***(6b)*** *and* ***(6c)*** *(Scheme 1)*

A mixture of the chalcone derivative **5a-c** (10 mmol) and hydrazine monohydrate (95%) (1.2 mL, 20 mmol) in ethanol (30 mL) was heated at reflux temperature for 10-15 hours. The reaction mixture was cooled, poured onto the crushed ice, and the obtained solid was collected by filtration, washed with water, dried and crystallized from ethanol to afford products **6a-c**.

1-Phenyl-5-(2-(3-phenyl-4,5-dihydro-1H-pyrazol-5-yl)phenoxy)-1H-tetrazole ***(6a)***

Yield 60%; mp 170-172°C; IR; 3336 (NH), 1590 (C=N), 1178 (C-O). ^{1}H NMR; 3.12 (dd, 1H, C_4 pyrazole, *J* = 11.8, *J* = 7.4), 3.88 (dd, 1H, C_4 pyrazole, *J* = 11.8, *J* = 10.9), 4.86 (dd, 1H, C_5 pyrazole, *J* = 10.9, *J* = 7.4), 6.77-7.78 (m, 14H, ArH), 9.21 (s, 1H, N-H). MS m/z (%); 382.41 (M^+, 2.11), 380.41 (1.95), 378.94 (1.12), 282.09 (27.24), 265.26 (100), 256.20 (84.51), 236.98 (11.68). Anal. Calcd for $C_{22}H_{18}N_6O$: (%): C, 69.10; H, 4.74; N, 21.98. Found: C, 69.48; H, 4.51; N, 21.67.

5-(4-(3-(3,4-Dimethoxyphenyl)-4,5-dihydro-1H-pyrazol-5-yl) phenoxy)-1-phenyl-1H-tetrazole ***(6b)***

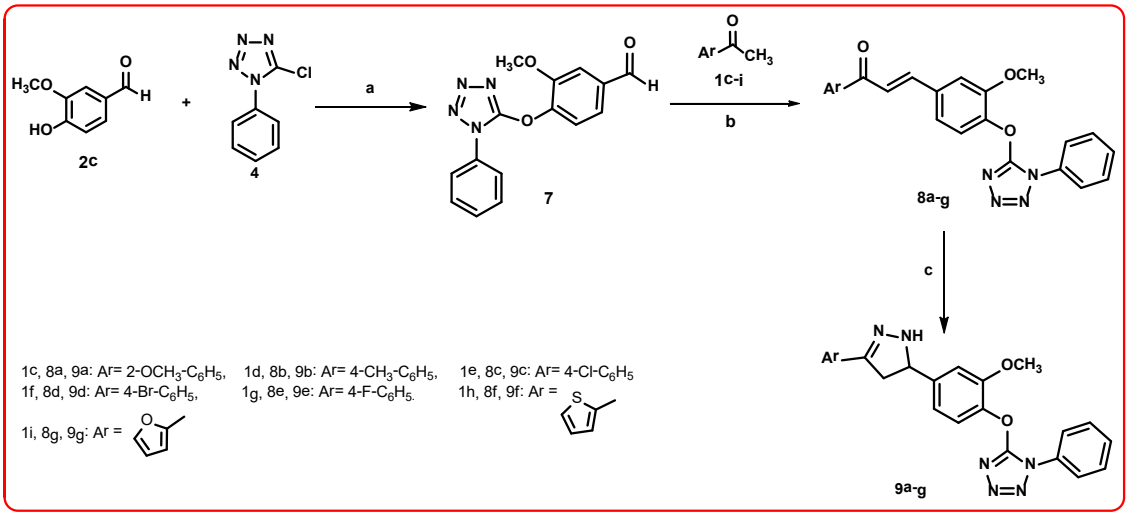
Yield 76%; mp 162-164°C; IR; 3347 (NH), 1604 (C=N), 1141 (C-O). ^{1}H NMR; 2.75 (dd, 1H, C_4 pyrazole, *J* = 10.8, *J* = 8.7), 3.31 (s, 6H, 2 $OC\underline{H}_3$), 3.84 (dd, 1H, C_4 pyrazole, *J* = 8.7, *J* = 4.4), 4.70 (dd, 1H, C_5 pyrazole, *J* = 10.8, *J* = 4.4), 6.70-7.77 (m, 12H, ArH), 9.20 (s, 1H, N-H). MS m/z (%); 442.45 (M^+, 1.56), 440.45 (2.05), 328.14 (13.92), 118.13 (16.68), 55.03 (100), 43.11 (55.63). Anal. Calcd for $C_{24}H_{22}N_6O_3$ (%): C, 65.15; H, 5.01; N, 18.99. Found: C, 65.53; H, 5.31; N, 18.67.

5-(4-(3-(3,4-Dimethoxyphenyl)-4,5-dihydro-1H-pyrazol-5-yl)-2-methoxyphenoxy)-1-phenyl-1H-tetrazole ***(6c)***

Yield 61%; mp 150-152°C; IR; 3347(NH), 1604 (C=N), 1141 (C-O). ^{1}H NMR; 2.79 (dd, 1H, C_4 pyrazole, J = 18.1, J = 11.7), 3.12 (dd, 1H, C_4 pyrazole, J = 18.1, J = 5.1), 3.38 (s, 6H, 2 OC$\underline{H}_3$), 3.63 (s, 6H, 2 OC$\underline{H}_3$), 4.87 (dd, 1H, C_5 pyrazole, J = 11.7, J = 5.1), 6.73-7.76 (m, 11H, ArH), 9.18 (s, 1H, D_2O exchangeable, N-H). MS m/z (%); 472.00 (M^+, 2.47), 470.00 (1.45), 264.25 (100), 152.13 (41.72), 115.13 (55.63), 71.07 (98.41), 57.03 (76.17). Anal. Calcd for $C_{25}H_{24}N_6O_4$ (%): C, 63.55; H, 5.12; N, 17.79 Found: C, 63.43; H, 5.44; N, 18.07.

General Procedure for Synthesis of E-1-(aryl or heteroaryl)-3-(3-methoxy-4-((1-phenyl-1H-tetrazol-5-yl)oxy)phenyl)prop-2-en-1-ones ***(8a-g)*** *(Scheme 2)*

3-Methoxy-4-((1-phenyl-1*H*-tetrazol-5-yl)oxy) benzaldehyde **(7)** (2.96 g, 10 mmol) was added, while stirring, to a solution of the appropriate ketone **(1c-i)** (10 mmol) in ethanolic NaOH solution (2.5%, 10 mL). Stirring was continued at room temperature for 2 hours. The reaction mixture was then acidified with dilute HCl and the separated product was collected by filtration, washed with water, dried and crystallized from ethanol, to give the required chalcone derivatives **8a-g**.



Scheme 2: Reagents and conditions: (a) K_2CO_3, DMF, 24 h, RT. (b) 2.5% NaOH, C_2H_5OH, 2 h (c) NH_2NH_2. H_2O, C_2H_5OH, reflux, 10-15 h.

3-(3-Methoxy-4-((1-phenyl-1H-tetrazol-5-yl)oxy)phenyl)-1-(2-methoxyphenyl)prop-2-en-1-one ***(8a)***

Yield 65%; mp 102-103°C; IR; 1660 (C=O), 1596 (C=C), 1146 (C-O). ^{1}H NMR; 3.78 (s, 3H, OCH_3), 3.94 (s, 3H, OCH_3), 7.27 (d, 1H, CO-C$\underline{H}$=CH, J = 15), 7.43-7.54 (m, 3H, ArH), 7.64 (d, 1H, CO-CH=C$\underline{H}$, J = 15), 7.78-8.02 (m, 9H, ArH). Anal. Calcd. for $C_{24}H_{20}N_4O_4$ (%): C, 67.28; H, 4.71; N, 13.08. Found: C, 67.46; H, 5.09; N, 13.37.

3-(3-Methoxy-4-((1-phenyl-1H-tetrazol-5-yl)oxy)phenyl)-1-(p-tolyl)prop-2-en-1-one ***(8b)***

Yield 69%; mp 142-144°C; IR; 1665 (C=O), 1606 (C=C), 1162 (C-O). ^{1}HNMR; 2.33 (s, 3H, CH_3), 3.87 (s, 3H, OCH_3), 7.23-7.59 (m, 4H, ArH), 7.64 (d, 1H, CO-C$\underline{H}$=CH, J = 16), 7.68-7.87 (m, 6H, ArH), 8.01 (d, 1H, CO-CH=C$\underline{H}$, J = 16), 8.10-8.12 (m, 2H, ArH). Anal. Calcd for $C_{24}H_{20}N_4O_3$ (%): C, 69.89; H, 4.89; N, 13.58. Found: C, 70.13; H, 4.54; N, 13.78.

1-(4-Chlorophenyl)-3-(3-methoxy-4-((1-phenyl-1H-tetrazol-5yl) oxy)phenyl)prop-2-en-1-one ***(8c)***

Yield 82%; mp 183-185°C; IR; 1666 (C=O), 1602 (C=C), 1456, (C-O). ^{1}H NMR; 3.89 (s, 3H, OCH_3), 7.62 (d, 1H, CO-C$\underline{H}$=CH, J = 16.5), 7.68-7.88 (m, 9H, ArH), 7.98 (d, 1H, CO-CH=C$\underline{H}$, J = 16.5), 8.02-8.23 (m, 3H, ArH). ^{13}C NMR; 191.93 (C=O), 159.05, 156.64, 150.32 (O-$\underline{C}$-N), 146.17, 143.51 (C-Cl), 138.07, 138.21, 136.12, 130.49, 130.05 (2C), 129.97, 129.73 (2C), 128.91, 128.75 (2C), 123.65 (2C), 122.65, 121.02, 56.42 (O$\underline{C}H_3$). Anal. Calcd for $C_{23}H_{17}ClN_4O_3$ (%): C, 63.82; H, 3.96; N, 12.94. Found: C, 63.51; H, 4.20; N, 13.25.

1-(4-Bromophenyl)-3-(3-methoxy-4-((1-phenyl-1H-tetrazol-5-yl) oxy)phenyl)prop-2-en-1-one ***(8d)***

Yield 62%; mp 190-192°C; IR; 1664 (C=O), 1602 (C=C), 1164 (C-O). ^{1}H NMR; 3.81 (s, 3H, OCH_3), 7.18-7.55 (m, 9H, ArH), 7.57 (d, 1H, CO-C$\underline{H}$=CH, J = 15.5), 7.68-7.78 (m, 3H, ArH), 7.81 (d, 1H, CO-CH=C$\underline{H}$, J = 15.5). ^{13}C NMR; 189.12 (C=O), 159.67, 156.74, 150.78, 144.06, 143.99, 141.45, 136.73, 133.23 (2C), 132.15, 132.02, 130.06, 129.73, 129.40, 129.29 (2C), 128.11, 122.49 (2C), 122.06, 121.52, 56.35 (O$\underline{C}H_3$). Anal. Calcd for $C_{23}H_{17}BrN_4O_3$ (%): C, 57.88; H, 3.59; N,11.74. Found: C, 58.12; H, 4.25; N, 11.45.

1-(4-Fluorophenyl)-3-(3-methoxy-4-((1-phenyl-1H-tetrazol-5-yl) oxy)phenyl)prop-2-en-1-one ***(8e)***

Yield 78%; mp 155-157°C; IR; 1676 (C=O), 1601 (C=C), 1154 (C-O). ^{1}H NMR; 3.88 (s, 3H, OCH_3), 7.11 (d, 1H, CO-C$\underline{H}$=CH, J = 15), 7.24-7.87 (m, 9H, ArH), 8.04 (d, 1H, CO-CH=C$\underline{H}$, J = 15), 8.09-8.31 (m, 3H, ArH). Anal. Calcd for $C_{23}H_{17}FN_4O_3$ (%): C, 66.34; H, 4.12; N, 13.45. Found: C, 66.04; H, 4.45; N, 13.15.

3-(3-Methoxy-4-((1-phenyl-1H-tetrazol-5-yl)oxy)phenyl)-1-(thiophen-2-yl)prop-2-en-1-one ***(8f)***

Yield 68%; mp 110-112°C; IR; 1652 (C=O), 1599 (C=C), 1115 (C-O). ^{1}H NMR; 3.90 (s, 3H, OCH_3), 7.34 (d, 1H, CO-C<u>H</u>=CH, *J* = 16.5), 7.37-7.62 (m, 8H, ArH), 7.80 (d, 1H, CO-CH=C<u>H</u>, *J* = 16.5), 7.83-7.90 (m, 11H, ArH). ^{13}C NMR; 189.98 (C=O), 159.73, 151.21, 150.75, 146.82, 143.88, 143.53, 139.42, 135.77, 134.85, 133.26, 132.02, 129.74, 129.41 (2C), 128.42, 123.14 (2C), 122.19, 121.54, 56.36 (O<u>C</u>H$_3$). Anal. Calcd for $C_{21}H_{16}N_4O_3S$ (%): C, 62.36; H, 3.99; N, 13.85; S, 7.89. Found: C, 62.68; H, 4.31; N, 14.23; S, 7.49.

1-(Furan-2-yl)-3-(3-methoxy-4-((1-phenyl-1H-tetrazol-5-yl)oxy) phenyl)prop-2-en-1-one ***(8g)***

Yield 73%; mp 94-95°C; IR; 1657 (C=O), 1603 (C=C), 1159 (C-O). ^{1}H NMR; 3.81 (s, 3H, OCH_3), 7.19-7.29 (m, 4H, ArH), 7.35 (d, 1H, CO-C<u>H</u>=CH, *J* = 16), 7.46 (d, 1H, CO-CH=C<u>H</u>, *J* = 16), 7.48-7.92 (m, 7H, ArH). ^{13}C NMR; 190.62 (C=O), 159.31, 153.62, 151.21, 152.84, 146.83, 146.64, 144.53, 142.68, 133.13, 129.76, 129.51 (2C), 129.38, 124.83 (2C), 122.00, 121.55, 117.75, 112.69, 56.35 (O<u>C</u>H$_3$). Anal. Calcd for $C_{21}H_{16}N_4O_4$ (%): C, 64.94; H, 4.15; N,14.43. Found: C, 65.18; H, 4.31; N, 14.23.

General Procedure for Synthesis of 5-(2-methoxy-4-(3-aryl or heteroaryl-4,5-dihydro-1H-pyrazol-5-yl)phenoxy)-1-phenyl-1H-tetrazoles ***(9a-g)*** *(Scheme 2)*

A mixture of a chalcone derivative **(8a-g)** (10 mmol) and hydrazine monohydrate (95%) (1.2 mL, 20 mmol) in ethanol (30 mL) was heated at reflux temperature for 10-15 hours. The reaction mixture was cooled, poured onto crushed ice and the obtained solid product was collected by filtration, washed with water, dried and crystallized from ethanol, to furnish the pyrazolines **9a-g**.

5-(2-Methoxy-4-(3-(2-methoxyphenyl)-4,5-dihydro-1H-pyrazol-5-yl)phenoxy)-1-phenyl-1H-tetrazole ***(9a)***

Yield 70%; mp 201-202°C; IR; 3347 (NH), 1574 (C=N), 1174 (C-O). ^{1}H NMR; 2.45-2.51 (m, 1H, C_4 pyrazole), 3.30-3.40 (m, 2H, C_4 pyrazole), 3.75 (s, 6H, 2 OCH_3), 4.56-4.62 (m, 1H, C_5 pyrazole), 6.70-7.76 (m, 12H, ArH), 9.21 (s, 1H, N-H). MS m/z (%); 442.16 (M^+, 2.35), 440.16 (1.11), 256.32 (7.78), 152.13 (13.90), 98.11 (100), 83.09 (23.35), 55.03 (80.62). Anal. Calcd for $C_{24}H_{22}N_6O_3$ (%): C, 65.15; H, 5.01; N, 18.99. Found: C, 65.43; H, 5.34; N, 18.77.

5-(2-Methoxy-4-(3-(p-tolyl)-4,5-dihydro-1H-pyrazol-5-yl) phenoxy)-1-phenyl-1H-tetrazole ***(9b)***

Yield 66%; mp 122-124°C; IR; 3350 (NH), 1604 (C=N), 1177 (C-O). ^{1}H NMR; 2.27 (s, 3H, CH_3), 2.35-2.41 (m, 1H, C_4 pyrazole), 3.31-3.40 (m, 1H, C_4 pyrazole), 3.86 (s, 3H, OCH_3), 4.55-4.61 (m, 1H, C_5 pyrazole), 6.73-7.77 (m, 12H, ArH), 9.23 (s, 1H, N-H). ^{13}C NMR; 159.34, 151.83, 149.30, 147.94, 146.13, 140.18, 137.89, 134.17, 129.72 (2C), 129.53, 129.31 (2C), 125.82 (2C), 125.44 (2C), 121.94, 117.74, 111.23, 56.14 (O<u>C</u>H$_3$), 56.02, 41.12 (<u>C</u>H$_2$), 21.35 (<u>C</u>H$_3$). MS m/z (%); 426.19 (M^+, 1.45), 424.19 (2.22), 135.01 (19.46), 111.19 (62.83), 71.07 (35.03), 57.03 (100), 51.01 (30.02). Anal. Calcd for $C_{24}H_{22}N_6O_2$ (%): C, 67.59; H, 5.20; N, 19.71. Found: C, 67.43; H, 5.34; N, 19.87.

5-(4-(3-(4-Chlorophenyl)-4,5-dihydro-1H-pyrazol-5-yl)-2-methoxyphenoxy)-1-phenyl-1H-tetrazole ***(9c)***

Yield 69%; mp 194-196°C; IR; 3347 (NH), 1574 (C=N), 1174 (C-O). ^{1}H NMR; 2.60-2.69 (m, 1H, C_4 pyrazole), 3.32-3.42 (m, 1H, C_4 pyrazole), 3.91 (s, 3H, OCH_3), 4.56-4.62 (m, 1H, C_5 pyrazole), 6.71-8.18 (m, 12H, ArH), 9.22 (s, 1H, N-H). ^{13}C NMR; 159.41, 153.73, 151.83, 149.30, 146.13, 140.18, 137.89, 134.17, 129.72, 129.53 (2C), 129.31 (2C), 125.82 (2C), 124.34 (2C), 121.94, 117.74, 111.23, 56.14 (O<u>C</u>H$_3$), 56.02, 41.12 (<u>C</u>H$_2$). MS m/z (%); 448.90 (M^++2, 1.11), 447.90 (M^++1, 2.78), 446.90 (M^+, 3.34), 264.12 (57.82), 117.00 (72.84), 109.12 (100). Anal. Calcd for $C_{23}H_{19}ClN_6O_2$ (%): C, 61.82; H, 4.29; N, 18.81. Found: C, 61.43; H, 4.34; N, 19.07.

5-(4-(3-(4-Bromophenyl)-4,5-dihydro-1H-pyrazol-5-yl)-2-methoxyphenoxy)-1-phenyl-1H-tetrazole ***(9d)***

Yield 64%; mp 184-185°C; IR; 3347 (NH), 1578 (C=N), 1175 (C-O). ^{1}H NMR; 2.52-2.60 (m, 1H, C_4 pyrazole), 3.34-3.39 (m, 1H, C_4 pyrazole), 3.79 (s, 3H, OCH_3), 4.32-4.40 (m, 1H, C_5 pyrazole), 6.71-8.10 (m, 12H, ArH), 9.24 (s, 1H, N-H). ^{13}C NMR; 159.32,151.82, 150.40, 148.47, 146.01, 140.17, 137.43, 130.90 (2C), 130.72, 129.30 (2C), 127.49 (2C), 127.39, 124.87 (2C), 121.94, 117.73, 112.23, 56.31 (O<u>C</u>H$_3$), 55.32, 40.57 (<u>C</u>H$_2$). MS m/z (%); 493.06 (M^++2, 2.82), 492.06 (M^++1, 1.67), 491.06 (M^+, 2.53), 331.95 (60.61), 253.08 (80.62), 145.01 (65.05), 55.03 (100). Anal. Calcd for $C_{23}H_{19}BrN_6O_2$ (%): C, 56.22; H, 3.90; N, 17.10. Found: C, 56.43; H, 4.34; N, 17.07.

5-(4-(3-(4-Fluorophenyl)-4,5-dihydro-1H-pyrazol-5-yl)-2-methoxyphenoxy)-1-phenyl-1H-tetrazole ***(9e)***

Yield 77%; mp 114-115°C; IR; 3347 (NH), 1603 (C=N), 1141 (C-O). ^{1}H NMR; 2.61-2.70 (m, 1H, C_4 pyrazole), 3.40-3.48 (m, 1H, C_4 pyrazole), 3.79 (s, 3H, OCH_3), 4.70-4.81 (m, 1H, C_5 pyrazole), 6.71-7.89 (m, 12H, ArH), 9.20 (s, 1H, N-H). ^{13}CNMR; 164.59 (C-F), 159.32, 151.82, 150.42, 148.46, 146.15, 140.16, 131.84, 129.30 (2C), 127.84, 127.78 (2C), 124.43 (2C), 121.94, 117.73, 115.80, 115.68, 111.23, 56.30 (O<u>C</u>H$_3$), 56.02, 41.09 (<u>C</u>H$_2$). Anal. Calcd for $C_{23}H_{19}FN_6O_2$ (%): C, 64.18; H, 4.45; N, 19.52. Found: C, 64.43; H, 4.34; N, 19.17.

5-(2-Methoxy-4-(3-(thiophen-2-yl)-4,5-dihydro-1H-pyrazol-5-yl) phenoxy)-1-phenyl-1H-tetrazole ***(9f)***

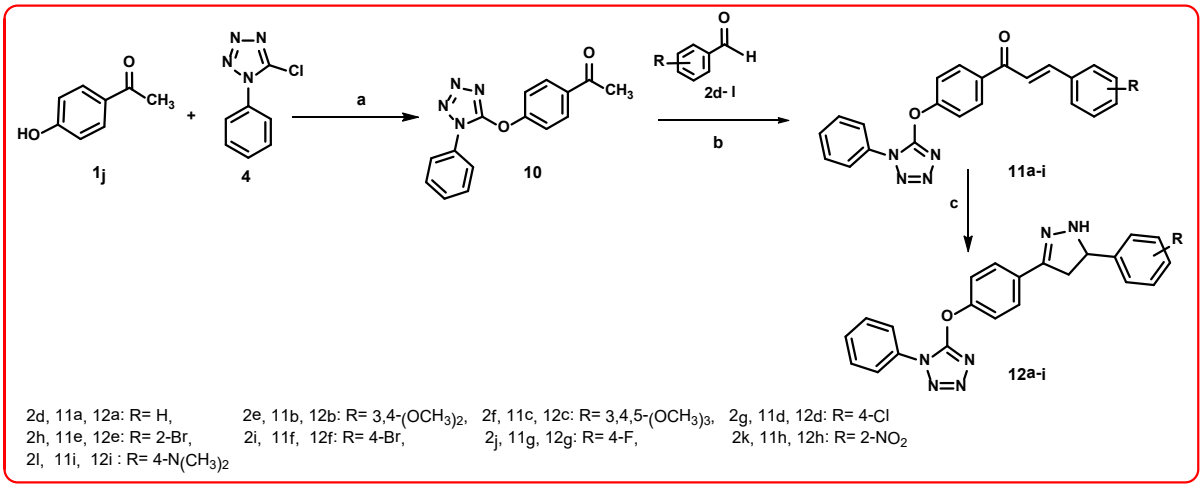
Yield 62%; mp 134-135°C; IR; 3348 (NH), 1604 (C=N), 1112 (C-O). ^{1}H NMR; 2.48-2.52 (m, 1H, C_4 pyrazole), 3.35-3.41 (m, 1H, C_4 pyrazole), 3.88 (s, 3H, OCH_3), 4.70-4.81 (m, 1H, C_5 pyrazole), 6.69-8.03 (m, 11H, ArH), 9.23 (s, 1H, N-H). ^{13}C NMR; 159.24, 151.82, 150.26, 148.45, 146.19, 140.16, 129.31, 129.16 (2C), 127.95, 126.68, 126.55, 126.47, 124.59 (2C), 121.95, 117.73, 111.22, 56.30 (O<u>C</u>H$_3$), 56.03, 41.93 (<u>C</u>H$_2$). MS m/z (%); 420.11 (M^++2, 4.32), 418.11 (M^+, 2.23), 258.07 (100), 135.07 (26.69), 115.07 (22.24). Anal. Calcd for $C_{21}H_{18}N_6O_2S$ (%): C, 60.27; H, 4.34; N, 20.08; S, 7.66. Found: C, 60.43; H, 4.35; N, 19.78; S, 8.02.

5-(4-(3-(Furan-2-yl)-4,5-dihydro-1H-pyrazol-5-yl)-2-methoxyphenoxy)-1-phenyl-1H-tetrazole ***(9g)***

Yield 61%; mp 193-195°C; IR; 3347 (NH) 1604 (C=N), 1143 (C-O). ^{1}H NMR; 2.50-2.58 (m, 1H, C_4 pyrazole), 3.36-3.42 (m, 2H, C_4 pyrazole), 3.90 (s, 3H, OCH_3), 4.71-4.76 (m, 1H, C_5 pyrazole), 6.59-7.92 (m, 11H, ArH), 9.19 (s, 1H, N-H). MS m/z (%); 402.40 (M^+, 2.76), 400.40 (2.22), 281.03 (15.01), 207.02 (47.26), 111.13 (100), 67.06 (85.07), 53.08 (52.26). Anal. Calcd for $C_{21}H_{18}N_6O_3$ (%): C, 62.68; H, 4.51; N, 20.88. Found: C, 62.43; H, 4.34; N, 19.07.

General Procedure for Synthesis of E-1-(4-((1-phenyl-1H-tetrazol-5-yl)oxy)phenyl)-3-(un) substituted phenylprop-2-en-1-ones ***(11a-i)*** *(Scheme 3)*

Compound 4-((1-Phenyl-1H-tetrazol-5-yl)oxy) acetophenone **(10)** (2.8 g, 10 mmol) was added, while stirring, to the appropriate benzaldehyde **(2d-i)** (10 mmol) in ethanolic NaOH solution (2.5%, 10 mL). Stirring was continued for additional 2 hours. The reaction mixture was then acidified with dilute HCl and the separated compounds were collected by filtration, washed with water, dried and crystallized from ethanol to give **11a-i**.



Scheme 3: Reagents and conditions: (a) K_2CO_3, DMF, 24 h, RT. (b) 2.5% NaOH, C_2H_5OH, 2 h (c) NH_2NH_2. H_2O, C_2H_5OH, reflux, 10-15 h.

E-3-Phenyl-1-(4-((1-phenyl-1H-tetrazol-5-yl)oxy)phenyl)prop-2-en-1-one ***(11a)***

Yield 71%; mp 103-105°C; IR; 1679 (C=O), 1598 (C=C), 1163(C-O). ^{1}H NMR; 7.48-7.69 (m, 11H, ArH), 7.73 (d, 1H, CO-C<u>H</u>=CH, *J* = 13.5), 7.75-7.82 (m, 3H, ArH), 7.89 (d, 1H, CO-CH=C<u>H</u>, *J* = 13.5). ^{13}C NMR δ (ppm); 187.89 (C=O), 159.92, 159.01, 156.48, 144.34, 134.59, 132.76, 131.49, 130.78, 129.92, 129.83, 129.72, 129.29, 128.95, 128.90, 128.76 (2C), 123.31 (2C), 122.45 (2C), 121.86. Calcd for $C_{22}H_{16}N_4O_2$ (%): C, 71.73; H, 4.38; N, 15.21. Found: C, 71.53; H, 4.29; N, 15.54.

E-3-(3,4-Dimethoxyphenyl)-1-(4-((1-phenyl-1H-tetrazol-5-yl) oxy)phenyl) prop-2-en-1-one ***(11b)***

Yield 69%; mp 76-78°C; IR; 1680 (C=O), 1595 (C=C), 1160, (C-O). ^{1}H NMR; 3.88 (s, 6H, 2O<u>C</u>H$_3$), 6.95-7.69 (m, 12H, ArH), 7.74 (d, 1H, CO-C<u>H</u>=CH, *J* = 13), 8.02 (d, 1H, CO-CH=C<u>H</u>, *J* = 13). ^{13}C NMR; 190.84 (C=O), 160.05, 158.67, 154.48, 149.59, 149.29, 145.56, 133.41, 130.92, 130.59, 129.81, 129.71, 129.52, 126.78, 123.40, 122.34 (2C), 121.51, 119.26 (2C), 110.49, 110.24, 55.96 (2 O<u>C</u>H$_3$). Anal. Calcd. for $C_{24}H_{20}N_4O_4$ (%): C, 67.28; H, 4.71; N, 13.08. Found: C, 67.58; H, 4.98; N, 13.38.

E-3-(3,4,5-Trimethoxyphenyl)-1-(4-((1-phenyl-1H-tetrazol-5-yl) oxy)phenyl) prop-2-en-1-one ***(11c)***

Yield 61%; mp 80-82°C; ^{1}H NMR; 3.79 (s, 3H, OCH_3), 3.88 (s, 6H, 2O<u>C</u>H$_3$), 7.55 (d, 1H, CO-C<u>H</u>=CH, *J* = 14), 7.61-8.12 (m, 11H, ArH), 8.32 (d, 1H, CO-CH=C<u>H</u>, *J* = 14). ^{13}C NMR; 191.83 (C=O), 159.92, 159.05, 156.42, 153.31, 153.09, 144.88, 139.87, 132.76, 130.73, 130.40, 129.92, 129.83, 129.73, 129.29, 123.31 (2C), 122.45 (2C), 121.02, 106.72, 106.67, 56.13 (O<u>C</u>H$_3$), 56.03 (O<u>C</u>H$_3$), 55.71 (O<u>C</u>H$_3$). Anal. Calcd. for $C_{25}H_{22}N_4O_5$ (%): C, 65.49; H, 4.84; N, 12.22. Found: C, 65.05; H, 4.68; N, 12.50.

E-3-(4-Chlorophenyl)-1-(4-((1-phenyl-1H-tetrazol-5-yl)oxy) phenyl)prop-2-en-1-one ***(11d)***

Yield 85%; mp 105-107°C; IR; 1662 (C=O), 1603 (C=C), 1166 (C-O). ^{1}H NMR; 7.24-7.89 (m, 11H, ArH), 7.96 (d, 1H, CO-C<u>H</u>=CH, *J* = 14), 7.99-8.04 (m, 2H, ArH), 8.32 (d, 1H, CO-CH=C<u>H</u>, *J* = 14). Anal. Calcd. for $C_{22}H_{15}ClN_4O_2$ (%): C, 65.06; H, 3.75; N, 13.91. Found: C, 65.36; H, 3.98; N, 13.78.

E-3-(2-Bromophenyl)-1-(4-((1-phenyl-1H-tetrazol-5-yl)oxy) phenyl)prop-2-en-1-one ***(11e)***

Yield 81%; mp 168-170°C; IR; 1665 (C=O), 1603 (C=C), 1169, (C-O). ^{1}H NMR; 7.41 (d, 1H, CO-C<u>H</u>=CH, *J* = 14), 7.49-8.03 (m, 11H, ArH), 8.22 (d, 1H, CO-CH=C<u>H</u>, *J* = 14), 8.33-8.35 (m, 2H, ArH). Anal. Calcd for $C_{22}H_{15}BrN_4O_2$ (%): C, 59.08; H, 3.38; N, 12.53. Found: C, 59.36; H, 3.58; N, 12.84.

E-3-(4-Bromophenyl)-1-(4-((1-phenyl-1H-tetrazol-5-yl)oxy) phenyl)prop-2-en-1-one ***(11f)***

Yield 77%; mp 92-94°C; IR; 1664 (C=O), 1602 (C=C), 1166, (C-O). ^{1}H NMR; 7.18-7.65 (m, 6H, ArH), 7.69 (d, 1H, CO-C<u>H</u>=CH, *J* = 12.2), 7.73-7.89 (m, 4H, ArH), 7.98 (d, 1H, CO-CH=C<u>H</u>, *J* = 12.2), 8.02-8.34 (m, 3H, ArH). Anal. Calcd. for

$C_{22}H_{15}BrN_4O_2$ (%): C, 59.08; H, 3.38; N,12.53. Found: C, 59.36; H, 3.58; N, 12.84.

E-3-(4-Fluorophenyl)-1-(4-((1-phenyl-1H-tetrazol-5-yl)oxy) phenyl)prop-2-en-1-one ***(11g)***

Yield 89%; mp 93-95°C; IR; 1670 (C=O), 1598 (C=C), 1163, (C-O). ^{1}H NMR; 6.96-7.26 (m, 2H, ArH), 7.33 (d, 1H, CO-C<u>H</u>=CH, *J* = 16.5), 7.59-7.69 (m, 4H, ArH), 7.75 (d, 1H, CO-CH=C<u>H</u>, *J* = 16.5), 7.81-8.34 (m, 7H, ArH). Anal. Calcd. for $C_{22}H_{15}FN_4O_2$ (%): C, 68.39; H, 3.91; N, 14.51. Found: C, 68.09; H, 3.56; N, 14.12.

E-3-(2-Nitrophenyl)-1-(4-((1-phenyl-1H-tetrazol-5-yl)oxy) phenyl)prop-2-en-1-one ***(11h)***

Yield 79%; mp 170-172°C; IR; 1670 (C=O), 1549, 1340 (NO_2), 1602 (C=C), 1168 (C-O), ^{1}H NMR; 7.42 (d, 1H, CO-C<u>H</u>=CH, *J* = 15), 7.51-8.24 (m, 13H, ArH), 8.33 (d, 1H, CO-CH=C<u>H</u>, *J* = 15). ^{13}C NMR; 187.61 (C=O), 159.32, 158.91, 156.57, 141.45, 135.15, 133.79, 132.19, 130.86, 130.37, 129.85, 129.75, 128.75 (2C), 127.98, 127.77, 124.65 (2C), 123.25, 121.25, 120.03, 119.81. Anal. Calcd for $C_{22}H_{15}N_5O_4$ (%): C, 63.92; H, 3.66; N, 16.94. Found: C, 64.09; H, 3.70; N, 17.12.

E-3-(4-(Dimethylamino)phenyl)-1-(4-((1-phenyl-1H-tetrazol-5-yl)oxy)phenyl) prop-2-en-1-one ***(11i)***

Yield 77%; mp 68-70°C; ^{1}H NMR; 3.43 (s, 6H, 2CH_3), 7.55-7.73 (m, 6H, ArH), 7.75 (d, 1H, CO-C<u>H</u>=CH, *J* = 15.3), 7.85-8.25 (m, 5H, ArH), 8.33 (d, 1H, CO-CH=C<u>H</u>, *J* = 15.3). ^{13}C NMR; 187.71 (C=O), 159.86, 158.90, 156.63, 156.39, 148.73, 133.67, 130.94, 130.33, 129.86, 129.76, 129.66, 129.47, 129.23, 124.63, 123.25 (2C), 122.38, 120.05 (2C), 119.83 (2C), 39.94 (<u>C</u>H_3), 39.78 (<u>C</u>H_3). Anal. Calcd for $C_{24}H_{21}N_5O_2$ (%): C, 70.06; H, 5.14; N, 17.02. Found: C, 70.36; H, 5.45; N, 17.32.

General Procedure for Synthesis of 5-(4-(5-(substituted phenyl)-4,5-dihydro-1H-pyrazol-3-yl)phenoxy)-1-phenyl-1H-tetrazoles ***(12a-i)*** *(Scheme 3)*

A mixture of a chalcone derivative **(11a-i)** (10 mmol) and hydrazine monohydrate (95%) (1.2 mL, 20 mmol) in ethanol (30 mL) was heated at reflux temperature for 10-15 hours. The reaction mixture was cooled, poured onto crushed ice and the obtaining solid product was collected by filtration, washed with water and crystallized from ethanol to give the pyrazoline compounds **12a-i**.

1-Phenyl-5-(4-(5-phenyl-4,5-dihydro-1H-pyrazol-3-yl)phenoxy)-1H-tetrazole ***(12a)***

Yield 61%; mp 200-202°C; IR; 3347(NH), 1604 (C=N), 1141 (C-O). ^{1}H NMR; 2.51-2.60 (m, 1H, C_4 pyrazole), 3.34-3.40 (m, 1H, C_4 pyrazole), 4.45-4.51 (m, 1H, C_5 pyrazole), 6.69-7.77 (m, 14H, ArH), 9.17 (s, 1H, N-H). MS m/z (%); 382.25 (M^+, 1.67), 380.25 (1.12), 97.11 (19.46), 69.06 (38.92), 109.12 (100), 43.11 (41.72). Anal. Calcd. for $C_{22}H_{18}N_6O$ (%): C, 69.10; H, 4.74; N, 21.98. Found: C, 69.43; H, 4.34; N, 22.07.

5-(4-(5-(3,4-Dimethoxyphenyl)-4,5-dihydro-1H-pyrazol-3-yl) phenoxy)-1-phenyl-1H-tetrazole ***(12b)***

Yield 63%; mp 204-206°C; IR; 3348 (NH), 1603 (C=N), 1140 (C-O). ^{1}H NMR; 2.50-2.58 (m, 1H, C_4 pyrazole), 3.33-3.43 (m, 1H, C_4 pyrazole), 3.84 (s, 6H, 2 OCH_3), 4.46-4.54 (m, 1H, C_5 pyrazole), 6.69-7.78 (m, 12H, ArH), 9.18 (s, 1H, N-H). MS m/z (%); 442.10 (M^+, 2.34), 440.10 (1.12), 151.26 (100), 137.13 (4.45), 109.19 (25.58), 83.09 (22.24), 67.06 (21.13). Anal. Calcd for $C_{24}H_{22}N_6O_3$ (%): C, 65.15; H, 5.01; N, 18.99. Found: C, 65.43; H, 5.34; N, 19.07.

5-(4-(5-(3,4,5-Trimethoxyphenyl)-1-phenyl-4,5-dihydro-1H-pyrazol-3-yl)phenoxy)-1H-tetrazole ***(12c)***

Yield 63%; mp 194-196°C; ^{1}H NMR; 2.52-2.61 (m, 1H, C_4 pyrazole), 3.32-3.40 (m, 1H, C_4 pyrazole), 3.80 (s, 3H, OCH_3), 3.87 (s, 6H, OCH_3), 4.72-4.79 (m, 1H, C_5 pyrazole), 6.69-7.77 (m, 11H, ArH), 9.16 (s, 1H, N-H). MS m/z (%); 472.51 (M^+, 2.12), 470.51 (1.12), 97.11 (25.58), 69.00 (49.48), 55.03 (100), 43.11 (55.04), 41.11 (36.14). Anal. Calcd. for $C_{25}H_{24}N_6O_4$ (%): C, 63.55; H, 5.12; N, 17.79. Found: C, 63.03; H, 5.34; N, 18.00.

5-(4-(5-(4-Chlorophenyl)-4,5-dihydro-1H-pyrazol-3-yl)phenoxy)-1-phenyl-1H-tetrazole ***(12d)***

Yield 62%; mp 174-176°C; IR; 3332 (NH), 1602 (C=N), 1172 (C-O). ^{1}H NMR; 2.53-2.63 (m, 1H, C_4 pyrazole), 3.31-3.42 (m, H, C_4 pyrazole), 4.62-4.70 (m, 1H, C_5 pyrazole), 6.71-7.79 (m, 13H, ArH), 9.14 (s, 1H, N-H). ^{13}C NMR; 158.43, 154.62, 151.86, 150.53, 141.70, 131.80, 130.84, 129.38, 129.31 (2C), 128.59 (2C), 128.43, 128.33, 127.22 (2C), 123.45 (2C), 121.98 (2C), 53.54 (H-<u>C</u>-NH), 40.63 (<u>C</u>H_2). MS m/z (%); 419.00 (M^++2, 1.39), 416.95 (M^+, 3.98), 258.07 (89.51), 223.06 (45.59), 121.07 (100), 64.99 (55.62). Anal. Calcd for $C_{22}H_{17}ClN_6O$ (%): C, 63.39; H, 4.11; N, 20.16. Found: C, 63.43; H, 4.34; N, 20.07.

5-(4-(5-(2-Bromophenyl)-4,5-dihydro-1H-pyrazol-3-yl)phenoxy)-1-phenyl-1H-tetrazole ***(12e)***

Yield 71%; mp 185-186°C; IR; 3325 (NH), 1601 (C=N), 1171 (C-O). ^{1}H NMR; 2.51-2.59 (m, 1H, C_4 pyrazole), 3.34-3.45 (m, 1H, C_4 pyrazole), 4.71-4.78 (m, 1H, C_5 pyrazole), 6.69-7.78 (m, 13H, ArH), 9.17 (s, 1H, N-H). ^{13}C NMR; 158.45, 154.63, 151.83, 150.58, 140.17, 132.60, 130.61, 130.47, 129.64, 129.54 (2C), 129.29 (2C), 128.41, 127.59, 124.42 (2C), 121.95 (2C), 119.96, 53.43 (H-<u>C</u>-NH), 40.52 (<u>C</u>H_2). MS m/z (%); 463.05 (M^++2, 2.78), 462.05 (M^++1, 1.67), 461.05 (M^+, 2.56), 264.19 (83.96), 167.13 (100), 148.20 (50.04), 123.19 (87.84). Anal. Calcd for $C_{22}H_{17}BrN_6O$ (%): C, 57.28; H, 3.71; N, 18.22. Found: C, 57.43; H, 3.34; N, 18.07.

5-(4-(5-(4-Bromophenyl)-4,5-dihydro-1H-pyrazol-3-yl)phenoxy)-1-phenyl-1H-tetrazole ***(12f)***

Yield 60%; mp 148 –150°C; IR; 3328 (NH), 1604 (C=N), 1170 (C-O). ^{1}H NMR; 2.49-2.53 (m, 1H, C_4 pyrazole), 3.32-3.41 (m, 1H, C_4 pyrazole), 4.77-4.80 (m, 1H, C_5 pyrazole), 6.69-8.09 (m, 13H, ArH), 9.14 (s, 1H, N-H). ^{13}C NMR; 158.43 (Ar″-<u>C</u>-N), 154.43 (Ar′-<u>C</u>-O), 151.83 (O-<u>C</u>-N), 150.56 (Ar′-<u>C</u>=N), 140.19, 132.08, 131.68 (2C), 129.44, 129.38 (2C), 128.50 (2C), 127.51 (2C), 124.54 (2C), 121.95 (2C), 118.82, 53.43 (H-<u>C</u>-NH), 40.52 (<u>C</u>H_2). Anal. Calcd for $C_{22}H_{17}BrN_6O$ (%): C, 57.28; H, 3.71; N, 18.22. Found: C, 57.06; H, 3.90; N, 18.35.

5-(4-(5-(4-Fluorophenyl)-4,5-dihydro-1H-pyrazol-3-yl)phenoxy)-1-phenyl-1H-tetrazole ***(12g)***

Yield 65%; mp 170-171°C; IR; 3333 (NH), 1603 (C=N), 1154 (C-O). ^{1}H NMR; 2.48-2.55 (m, 1H, C_4 pyrazole), 3.30-3.42 (m, 1H, C_4 pyrazole), 4.84-4.98 (m, 1H, C_5 pyrazole), 6.66-8.21 (m, 13H, ArH), 9.08 (s, 1H, N-H). ^{13}C NMR; 160.88, 158.39, 154.32, 151.87, 150.53, 139.55, 130.46, 129.31, 129.28 (2C), 127.62 (2C), 127.07 (2C), 124.67 (2C), 121.99 (2C), 115.63, 115.43, 53.21, 41.48 ($\underline{C}H_2$). Anal. Calcd for $C_{22}H_{17}FN_6O$ (%): C, 65.99; H, 4.28; N, 20.99. Found: C, 66.06; H, 3.90; N, 21.35.

5-(4-(5-(2-Nitrophenyl)-4,5-dihydro-1H-pyrazol-3-yl)phenoxy)-1-phenyl-1H-tetrazole ***(12h)***

Yield 64%; mp 170-172°C; IR; 3269 (NH), 1623 (C=N), 1174 (C-O). ^{1}H NMR; 2.50-2.57 (m, 1H, C_4 pyrazole), 3.34-3.42 (m, 1H, C_4 pyrazole), 4.51-4.59 (m, 1H, C_5 pyrazole), 6.69-8.38 (m, 13H, ArH), 9.11 (s, 1H, N-H). ^{13}C NMR; 159.57, 156.55, 151.83, 148.05, 140.18, 137.28, 130.16, 130.05, 129.30, 129.27 (2C), 128.18 (2C), 127.01 (2C), 126.25, 123.48 (2C), 121.95 (2C), 53.54, 40.62. MS m/z (%); 427.12 (M^+, 2.22), 111.13 (26.69), 97.11 (37.25), 69.06 (45.04), 55.03 (100), 43.05 (43.37). Anal. Calcd for $C_{22}H_{17}BrN_6O$ (%): C, 61.82; H, 4.01; N, 22.94. Found: C, 62.06; H, 3.90; N, 23.30.

N,N-Dimethyl-4-(3-(4-((1-phenyl-1H-tetrazol-5-yl)oxy) phenyl)4,5-dihydro-1H-pyrazol-5-yl)aniline ***(12i)***

Yield 59%; mp 185-186°C; ^{1}H NMR; 2.54-2.62 (m, 1H, C_4 pyrazole), 3.05 (s, 6H, CH_3), 3.36-3.43 (m, 1H, C_4 pyrazole), 5.00-5.15 (m, 1H, C_5 pyrazole), 6.69-7.77 (m, 13H, ArH), 9.07 (s, 1H, N-H). Anal. Calcd. for $C_{24}H_{23}N_7O$ (%): C, 67.75; H, 5.45; N, 23.04. Found: C, 67.99; H, 5.13; N, 23.30.

Biological evaluation

Cytotoxicity screening was evaluated by MTT assay, as previously reported (Ibrahim *et al*., 2014; Alarif *et al*., 2013). The used cells include human colon cancer (*HCT-116*), prostate cancer (*PC-3*), breast cancer (*MCF-7*) and normal kidney of African green monkey (*Vero B*) cell lines. The cell lines were obtained from American Type Culture Collection (ATCC). The cells were cultivated at 37°C and 10% CO_2 in a medium supplemented with 10% fetal bovine serum, 100 IU/ml penicillin and 100 µg/ml streptomycin. Cisplatin (*cis*-diamineplatinum (II) dichloride) and 5-FU (5-fluorouracil) were obtained from Sigma and dissolved in 0.9% saline then stored as 8 mM stock solution at −20°C. Then the synthesized compounds were solubilized in DMSO and stored at −20°C. The cells were seeded in 96-well plate as 5×10^4 cells/mL (100 µL/well). Serial dilutions of the tested compounds, Cisplatin and 5-FU (100, 30, 10, 3.3, 1.1 or 0.36 ug/ml) were added after overnight incubation of the cells at 37°C and 5% CO_2. DMSO was added to each well, and the solubilized formazan product was spectrophotometrically quantified with the help of a microplate reader, PowerWave XS (BioTek, Winooski, VT, USA), at 540 nm. Each experiment was repeated three times and IC_{50} was calculated as the concentration that causes 50% inhibition of cell growth using GraphPad Prism *(version 5.0, La Jolla, CA)*. The results were compared to the untreated cells (DMSO without the tested compounds), and to cisplatin and 5-Fluorouracil (5-FU) as positive controls (Table 1). In order to find out whether the tested compounds are toxic to normal cells, their anti-proliferative activity against *Vero-B* normal cell line was evaluated. Selectivity index was calculated by dividing the IC_{50} against cancer cell line over the IC_{50} against the normal cell line (Table 2).

$$SI = (IC_{50}) \text{ normal}/(IC_{50}) \text{ cancer.}$$

Molecular docking methodology

Docking process was done on MOE software (MOE 2013.08 of Chemical Computing Group ULC). The newly prepared hybrids were built and optimized geometrically using ChemBioOffice, and a database file for the structures to be docked was prepared. Protein files were downloaded from the protein data bank (website: http://www.rcsb.org) and prepared for the docking process by keeping the binding sites flexible and adding water of solvation. PharmMapper server (Liu *et al*., 2010) was found helpful to determine the most suitable PDB files, where histone deacetylase 2 enzyme (HDAC2), cytochrome P450 17A1 and 5,10-methenyltetrahydrofolate synthetase (MTHFS) were chosen. Docking of twelve compounds to prove their binding mode with the selected proteins was done using 100 runs for each compound and Triangle marcher technique for placement stage, affinity dG for rescoring and forcefield method for refining. Docking scores were represented in Tables 3-5, and binding interactions of some representative compounds are shown in Figures 2-5.

RESULTS AND DISCUSSION

Chemistry

The reaction sequence used for the synthesis of the desired compounds is shown in Schemes 1, 2 and 3.

Synthesis of compounds ***5a-c*** *and* ***6a-c*** *(Scheme 1)*

The reported chalcone derivatives **(3a-c)** were prepared *via* Claisen-Schmidt condensation of substituted acetophenone derivatives **1a** or **1b** with the appropriate hydroxyl benzaldehyde **(2a-c)** in the presence of ethanolic NaOH (2.5%). The targeted tetrazole-containing hybrids **(5a-c)** were obtained *via* the well-known Williamson ether synthesis by reacting 5-chloro-1-phenyl-1*H*-tetrazole **(4)** with the prepared hydroxyl chalcone derivatives **(3a-c)** at room temperature for 24 hours while stirring in DMF and in the presence of anhydrous K_2CO_3. The IR spectra of these products **(5a-c)** showed carbonyl stretching vibrations of the enone fragments at 1665-1593 cm^{-1} and absorption bands at 1182-1155 cm^{-1} for ether link (C–O). ^{1}H NMR spectrum of compound **5b** demonstrated characteristic singlet peak assigned to 6 protons of two methoxy groups at δ 3.80 ppm. The vinylic protons resonated as two doublets within the aromatic region with *J* value 15 Hz. This observation indicates the *trans* configuration of the enone chalcone structure (Silverstein *et al*., 1991). The structure of compound **5b** was supported by ^{13}C NMR spectroscopy that showed a signal at 188.32 ppm for the carbonyl carbon, while the two methoxy carbons appeared as two signals at 56.16 and 56.13.

Table 1: *In vitro* antiproliferative activities of the designed compounds.

Compound NO.	IC_{50} (µg/ml)[a]				Compound NO.	IC_{50} (µg/ml)[a]			
	HCT-116[b]	PC-3[c]	MCF-7[d]	Vero B[e]		HCT-116[b]	PC-3[c]	MCF-7[d]	Vero B[e]
5a	0.6	1.6	na	4.0	**6a**	16.0	35.0	38.1	na
5b	3.7	3.0	na	8.0	**6b**	na	na	na	na
5c	1.6	2.2	na	8.0	**6c**	na	na	27.3	na
8a	3.0	6.0	na	7.0	**9a**	na	na	na	na
8b	na	2.5	na	9.0	**9b**	na	na	24.5	na
8c	4.0	6.0	na	12.8	**9c**	na	na	38.1	na
8d	6.0	7.0	na	40	**9d**	8.0	16.5	15.6	na
8e	2.5	6.0	na	8.0	**9e**	na	na	na	na
8f	2.5	5.0	2.9	8.0	**9f**	na	na	na	na
8g	3.0	6.0	31.4	9.0	**9g**	na	na	na	na
11a	na	18.0	na	na	**12a**	na	na	na	na
11b	40.0	25.0	na	17.5	**12b**	na	na	na	na
11c	na	na	na	na	**12c**	na	na	na	na
11d	12.0	40.0	na	50.0	**12d**	na	na	45.3	na
11e	na	na	na	na	**12e**	na	na	na	na
11f	40.0	40.0	na	na	**12f**	na	na	na	na
11g	25.0	na	42.4	na	**12g**	na	na	na	na
11h	12.0	17.6	na	na	**12h**	na	na	na	na
11i	na	na	na	na	**12i**	na	na	na	na
Cisplatin	20.0	5.0	20.0	34.4	**5-FU**	17.3	21.4	9.74	12.8

[a] IC_{50}: Compound concentration required to inhibit cell proliferation by 50%.
[b] Colon cancer cell line; [c] Prostate cancer cell line; [d] Breast cancer cell line; [e] Normal African green monkey kidney cell line.
na: not active till 50 µg/ml.

Table 2: Selectivity indices of the active compounds.

Compound NO.	Selectivity index (SI)[a]			Compound NO.	Selectivity index (SI)[a]		
	HCT-116[b]	PC-3[c]	MCF-7[d]		HCT-116[b]	PC-3[c]	MCF-7[d]
5a	6.66	2.50	–	**8g**	3.00	1.50	0.28
5b	2.16	2.66	–	**9b**	–	–	>2.04
5c	5.00	3.63	–	**9c**	–	–	>1.31
6a	>3.12	>1.42	>1.31	**9d**	>6.25	>3.03	>3.20
6c	–	–	>1.83	**11a**	–	>2.77	–
8a	2.33	1.16	–	**11b**	0.43	0.70	–
8b	–	3.60	–	**11d**	4.16	1.25	–
8c	3.20	2.13	–	**11f**	>1.25	>1.25	–
8d	6.66	5.71	–	**11g**	>2.00	–	>1.17
8e	3.20	1.33	–	**11h**	>4.16	>2.84	–
8f	3.20	1.60	2.75	**12d**	–	–	>1.10
Cisplatin	1.72	6.88	1.72	**5-FU**	0.73	0.59	1.31

[a] Selectivity index: SI = (IC_{50}) normal/(IC_{50}) cancer.
[b] Colon cancer cell line; [c] Prostate cancer cell line; [d] Breast cancer cell line.

Table 3: Binding scores (Kcal/mol) of the twelve docked compounds with HDAC2 in comparison to reference inhibitor N-(2-aminophenyl)benzamide.

Compound No.	Binding scores	Compound No.	Binding scores
5a	−8.54584217	**6a**	−6.35466719
5b	−11.0169601	**6b**	−10.7547264
8d	−11.2120924	**9d**	−10.7752962
8e	−9.7349453	**9e**	−9.6193018
8g	−9.52019119	**9g**	−8.99661255
11a	−11.9166155	**Reference ligand.**	−10.7447565
11b	−8.08154964		

Table 4: Binding scores (Kcal/mol) of the twelve docked compounds with CY-P17A1 in comparison to reference drug TOK-001.

Compound No.	Binding scores	Compound No.	Binding scores
5a	−7.758	**6a**	−7.483
5b	−10.088	**6b**	−8.740
8d	−9.4827	**9d**	−9.414
8e	−8.312	**9e**	−9.356
8g	−9.072	**9g**	−8.870
11a	−8.418	**Reference ligand.**	−8.518
11b	−8.472		

Table 5: Binding scores (Kcal/mol) for the twelve docked compounds with MTHFS in comparison to reference 10-formyltetrahydrofolate.

Compound No.	Binding scores	Compound No.	Binding scores
5a	−9.943	**6a**	−9.395
5b	−9.173	**6b**	−10.523
8d	−10.218	**9d**	−9.726
8e	−10.294	**9e**	−12.534
8g	−10.646	**9g**	−10.819
11a	−8.911	**Reference ligand.**	−11.102
11b	−9.161		

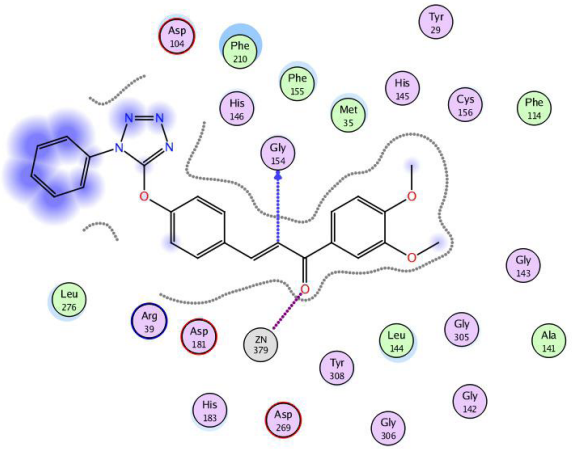


Fig. 2: Binding of HDAC2 with compound (**5b**).

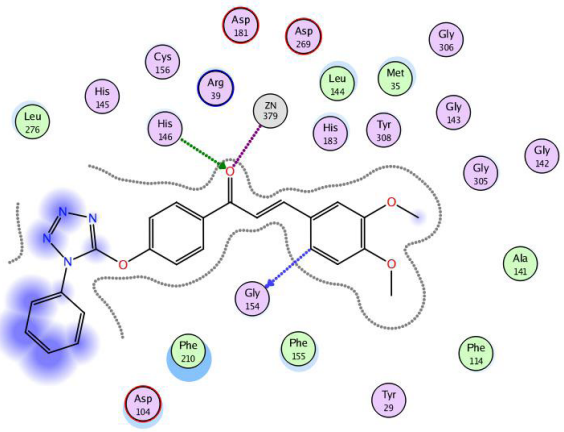


Fig. 3: Binding of HDAC2 with compound (**11b**).

Compounds **6a-c** were prepared by reacting α,β-unsaturated ketones **(5a-c)** with an excess of hydrazine hydrate (95%) in ethanol, at reflux temperature. The successful synthesis of pyrazoline derivatives was verified by the micro-analytical and spectral data. IR spectra of **6a-c** showed the disappearance of the carbonyl stretching vibration of compounds **5a-c** and the appearance of absorption bands at 3347-3336 cm^{-1} and 1604-1590 cm^{-1} associated with NH and C=N groups of pyrazoline ring. The ^1H NMR spectrum of compound **6c**, as a representative example, showed the chiral proton at C_5 of the pyrazoline ring as a doublet of doublet at 4.87 with a coupling constant 11.7 and 5.1 Hz due to vicinal coupling with the methylene protons (CH_2) at C_4 of the pyrazoline ring. The CH_2 protons appeared as two distinct doublets of doublet at δ 2.79 and 3.12 ppm. Each proton has a germinal and vicinal coupling with coupling constant of 18.1 and 11.7 Hz for one proton and 18.1 and 5.1 Hz for the other. In addition, a D_2O exchangeable NH proton singlet was observed at δ 9.18. Mass spectrum of the pyrazoline derivatives displayed molecular ion peaks inconsistent with the assigned molecular weight. For compound **6b**, a molecular ion peak was observed at m/z, 442.45 in agreement with its formula $C_{24}H_{22}N_6O_3$.

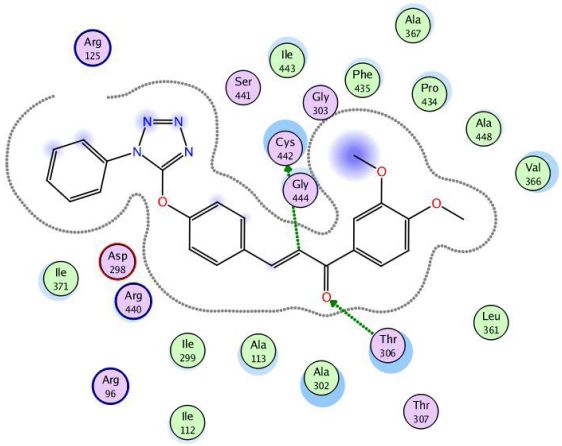


Fig. 4: Ligand interactions in the binding site of CYP17A1 with compound (**5b**).

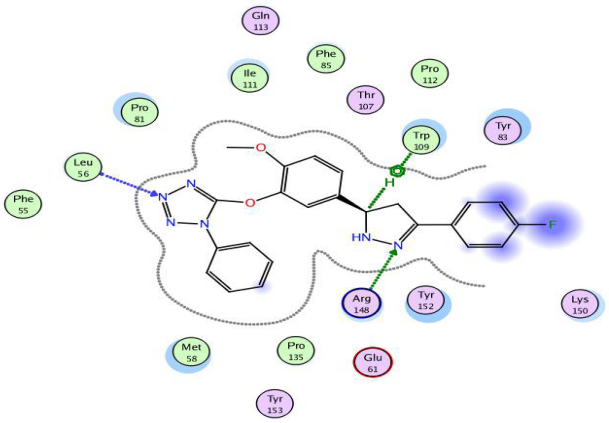


Fig. 5: Ligand interactions in the binding site of MTHFS with compound (**9e**).

*Synthesis of compounds **8a-g** and **9a-g** (Scheme 2)*

The reported compound 3-methoxy-4-((1-phenyl-1*H*-tetrazol-5-yl)oxy) benzaldehyde **(7)** was obtained via *O*-alkylation of 4-hydroxy-3-methoxybenzaldehyde (vanillin) **(2b)** with 5-chloro-1-phenyl-1*H*-tetrazole **(4)** in DMF in the presence of anhydrous K_2CO_3. Compound **7** was then reacted with 1 equivalent of substituted acetophenones, 2-acetylthiophene or 2-acetylfuran **(1c-i)** in an ethanolic solution of NaOH (2.5%) followed by acidification with HCl to afford α,β-unsaturated carbonyl compounds **(8a-g)**. Nucleophilic cycloaddition reaction of compounds **(8a-g)** with hydrazine monohydrate (95%) in ethanol gave the corresponding pyrazolines **(9a-g).** The IR spectra of the later compounds showed bands at 3350-3347 cm^{-1} of the (N-H) group and 1604-1574 cm^{-1} of (C=N) group. ^{1}H NMR spectrum of compound **9b** revealed the presence of a broad singlet at δ 9.23 ppm of the N-H proton; and three multiplet bands at δ 2.35-2.41, 3.31-3.40 and 4.55-4.61 ppm integrated for the two C_4 protons at and the C_5 proton of the pyrazoline ring, respectively. A molecular ion peak was detected at m/z 442.16, in accordance with the molecular formula of compound **9a**.

*Synthesis of compounds **11a-i** and **12a-i** (Scheme 3)*

The starting compound 1-(4-((1-phenyl-1H-tetrazol-5-yl)oxy)phenyl) ethan-1-one **(10)** was prepared as previously reported, by reacting 5-chloro-1-phenyl-1*H*-tetrazole **(4)** and 4-hydroxyacetone **(1j)** in DMF in the presence of anhydrous K_2CO_3 as a catalyst. Claisen-Schmidt condensation of differently substituted benzaldehydes **(2d-i)** with compound **10** was carried out in ethanolic NaOH (2.5%), followed by acidification with HCl afforded the target compounds 1-(4-((1-phenyl-1*H*-tetrazol-5-yl) oxy)phenyl)-3-(un)substituted phenylprop-2-en-1-ones **(11a-i).** In ^{1}H NMR spectrum of compound **11c**, the protons of three methoxy groups resonated as two singlets at δ 3.79 (OCH_3) and 3.88 ($2OCH_3$). These methoxy carbons appeared as three signals in ^{13}C NMR spectrum at 56.13, 56.03 and 55.71 ppm. Also, the structure of compound **11i** was confirmed by its ^{1}H NMR spectroscopy that showed a singlet peak assigned to two methyl groups at δ 3.43 ppm representing the dimethylamino moiety. The pyrazoline compounds **12a-i** were prepared by reacting α,β-unsaturated ketones **(11a-i)** with hydrazine hydrate (95%) in refluxing ethanol. Again, microanalytical and spectral data of these pyrazolines were inconsistent with the proposed structures. A molecular ion peak at m/z 472.51 represented the molecular weight of compound **12c** was observed in its mass spectrum.

BIOLOGICAL ACTIVITY

In vitro antiproliferative screening

Several approaches have been utilized to measure the viability of cells and determine cell growth via staining with different vital dyes. In the present work, the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT assay) was used to assess the antiproliferative activity of the newly synthesized tetrazole-chalcone/pyrazoline hybrids. This assay depends on the ability of viable cells to reduce the yellow tetrazolium salt (MTT) to a purple product. Three mammalian cell lines, namely: the colon cancer (*HCT-116*), prostate cancer (*PC-3*) and breast cancer (*MCF-7*) cell lines, and the African green monkey kidney (*Vero-B*) normal cell line were used. The IC_{50} of the tested compounds along with that of the reference drugs Cisplatin and 5-Fluorouracil are shown in Table 1 and selectivity indices of the active compounds are shown in Table 2.

Regarding the antiproliferative activity against colon *HCT-116* cell line, eleven chalcone derivatives showed higher activity, with IC_{50} ranging from 0.6 to 12 µg/ml, than that of cisplatin (IC_{50} = 20 µg/ml) and 5-FU (IC_{50} = 17.3 µg/ml). Compounds **5a** and **5c** were identified as the most potent compounds at IC_{50} = 0.6 and 1.6 µg/ml with selectivity index = 6.66 and 5.00, respectively. Compounds **8e** and **8f** showed their effect against colon *HCT-116* cell line at IC_{50} = 2.5 µg/ml that represents eight and seven times the activity of the reference drugs cisplatin and 5-FU, respectively, while compounds **8a** and **8g** (IC_{50} = 3 µg/ml) were proved to be six and five times these reference drugs. Other derivatives that showed better IC_{50} values than both cisplatin and 5-FU included **5b**, **8c, 8d, 11d,** and **11h** (IC_{50} of 3.6-12 µg/ml). On the other hand, only two pyrazoline derivatives, **6a** and **9d**, displayed equal or better activity against colon *HCT-116* cell lines than the reference drugs, with IC_{50} values of 16 and 8 µg/ml. In addition, both compounds **6a** and **9d** have no cytotoxic activities against the normal *Vero B* cell line till a concentration of 50 µM.

Regarding the activity against prostate *PC-3* cell line, compounds **5a-c** and **8b** exhibited IC_{50} values of 1.6−3 µg/ml, better than that of the reference drugs cisplatin (IC_{50} = 5 µg/ml) and 5-FU (IC_{50} = 21.4 µg/ml) with selectivity indices ranging from 2.50 to 3.63. In addition, compounds, **8a** and **8c-g** showed nearly comparable activities, as revealed from their IC_{50} (5−7 µg/ml), to cisplatin (IC_{50} = 5 µg/ml), but higher than 5-FU (IC_{50} = 21.4 µg/ml). On the other hand, none of the pyrazoline derivatives displayed comparable activity with cisplatin; but only **9d** displayed a better effect than that of 5-FU with no effect on the normal *Vero* B cell line.

Among the tested compounds, only the chalcone derivative **8f** showed remarkable antiproliferative activity against breast *MCF-7* cell line with IC_{50} = 2.9 µg/ml and SI = 2.75; while the pyrazoline **9d** displayed an IC_{50} = 15.6 µg/ml and showed selectivity against breast *MCF-7* cancer cell line versus the normal *V*ero B cell line.

The above mentioned biological results indicated the presence of antiproliferative activity in the chalcone compounds,

rather than their corresponding pyrazoline derivatives and most of the active compounds exerted high selectivity indices. Eight of the tested chalcone compounds (**5a-c**, **8a**, **8c**, **8e-g**) were proved to be of better or equal effect than the reference drugs against two tested cell lines, while only two pyrazoline derivatives (**6a** and **9d**), showed only weak or modest activity against the three tested cancer cell lines. The activity emerged mostly with chalcone-tetrazole hybrids, where the chalcone enone system appears essential, and tetrazole moiety is connected at the β-carbon of the enone system.

In such a group of active compounds **5a-c** and **8a-g**, the substitution pattern at phenyl A of chalcone system did not show great prevalence, except that the presence of a CH_3 group, with both hydrophobic and electron-donating properties, at *para* position, negatively affected the activity against colon *HCT-116* cancer cell lines.

Compound **5a**, in which the 1-phenyl tetrazolyloxy moiety is incorporated at *ortho* position of the phenyl ring linked to the β-carbon of the enone system, exerted superior activity relative to the other tested chalcone derivatives, against the tested cell lines; while compound **5c** is the second most potent compound, where three methoxy groups are present at the two chalcone phenyl rings.

Interchanging the enone system in compound **5b** to give **11b** resulted in a negative impact on the activity. The same pattern was observed with all other reversed chalcone derivatives **11a** and **11c-i**.

The growth inhibition activity against breast *MCF-7* cell lines appeared with compound **8f** indicating the beneficial effect of the 2-thienyl groups on the action against this type of cancer cell lines. Compound **8f** has a broad spectrum of activity against the three tested cancer cell lines.

DOCKING STUDIES

Molecular docking studies were carried out for twelve compounds (biologically active and inactive), to help to elucidate which portions of the molecules are critical for antiproliferative activity.

Docking with histone deacetylase 2 enzyme (HDAC2) (PDB: 3MAX)

Compounds were tested for binding with HDAC2 that is important in colon cancer cells (Bressi *et al*., 2010). The HDAC2 active site consists of the catalytic machinery, a lipophilic 'tube' which leads from the surface to this machinery, and a 'foot pocket' immediately adjacent to the machinery. The overall binding scores of the tested compounds with HDAC2 in comparison to reference inhibitor N-(2-aminophenyl)benzamide are shown in Table 3. Compounds **5b** and **8d** displayed higher binding scores than the reference ligand. Figure 2 shows the importance of showing the importance of the α-β unsaturated carbonyl moiety, in compound **5b**, in binding to Zn atom and amino acid Gly154 of the binding site of HDAC2.

However, compound **11a**, which is a reversed chalcone structure of compounds **5** and **8**, exhibited higher binding score than other derivatives, though it was proved inactive in the *in vitro* biological testing. Figure 3 represents the binding of compound **11b** to the active site of HDAC2, where no interaction between the electron rich double bond and Gly154 was observed, but instead a hydrophobic interaction with the adjacent phenyl group, and binding of the carbonyl group with Zn atom and His146. In the biological evaluation, compound **11b** displayed weak activity against colon *HCT-116* cell lines.

Docking with Cytochrome P450 17A1 (CYP 17A1) (PDB: 3SWZ)

Cytochrome P450 17A1 catalyzes the biosynthesis of androgens in humans (DeVore and Scott, 2012); and prostate cancer cells proliferate in response to androgen steroids. The overall binding scores of the twelve compounds with CYP17A1 in comparison to reference drug TOK-001are displayed in Table 4, where the highest binding score of −10.088 was observed with compound **5b**. Interaction of chalcone tested compound **5b** in the binding site of CYP17A1 is shown in Figure 4, where binding of the α-β unsaturated carbonyl moiety was observed with Cys442 and Thr306 residues. This pattern of interaction goes consistently with the observed in vitro biological activity.

Docking with 5,10-Methenyltetrahydrofolate synthetase (MTHFS) (PDB: 3HY3)

MTHFS regulates the flow of carbon through the one-carbon metabolic network, which supplies essential components for the growth and proliferation of cells. Inhibition of MTHFS in human MCF-7 breast cancer cells has been shown to arrest the growth of cells (Wu *et al*., 2009). The overall binding scores for the tested compounds with MTHFS in comparison to reference 10-formyltetrahydrofolate are shown in Table 5, where the pyrazoline compound **9e** displayed the highest negative binding score (−12.534) among the tested compounds. Ligand interaction in the binding site of MTHFS with this pyrazoline (**9e**) is shown in Figure 5, where the tetrazole and pyrazole rings interact with Leu56, Trp109, and Arg148. Yet, this compound (**9e**) was proved inactive against *MCF-7* cell lines in the *in vitro* antiproliferative evaluation.

CONCLUSION

Nineteen chalcone-tetrazole hybrids and their cyclic pyrazoline-tetrazole structures were prepared and biologically evaluated. Eight of the tested chalcone compounds (**5a-c**, **8a**, **8c**, **8e-g**) showed significant antiproliferative activities, with IC_{50} values better than, or comparable to, that of the reference drugs, cisplatin, and 5-FU, against *HCT-116* and *PC-3* cell lines with high selectivity towards cancer cell lines.

The activity appeared mostly with chalcone-tetrazole hybrids, where the chalcone enone system appears essential; and tetrazole moiety is connected at the β-carbon of the enone system. Interchanging the enone system in compounds **5** and **8** to give **11** or its cyclization into a pyrazoline structure (**6** and **9**) has, generally, a negative impact on the activity.

The substitution pattern at phenyl A of chalcone system, in the active compounds **5** and **8** did not show great prevalence. However, the presence of a 2-thienyl group (**8f**) has a beneficial effect on the growth inhibition activity against breast *MCF-7* cell lines. Compound **8f** has a broad spectrum of activity against the three tested cancer cell lines.

Compound **5b** binds to HDAC2 and CYP17A1 enzymes with a binding score of −11.017 and −10.088 Kcal/mol

respectively, inconsistent with its antiproliferative activity against colon *HCT-116*, and prostate *PC-3* cell lines (IC_{50} = 3.7 and 3.0). On the other hand, no interaction between the electron rich double bond of compound **11b**, a reversed chalcone structure, and Gly154 in the active site of HDAC2 was observed.

Although the pyrazoline compound **9e** didn't show any activity in the present *in vitro* study, it had good binding scores with the amino acids residues in the MTHFS enzyme.

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