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Design, synthesis, and biological evaluation of a novel series of thiazole derivatives based on pyrazoline as anticancer agents

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Abstract

This study deals with the design and synthesis of new pyrazoline-thiazole scaffold as promising anti-cancer agents, with the goal of building new compounds with combinations of various heterocyclic moieties. The pyrazoline-thiazole scaffold was synthesized via cyclization of the chalcone derivative with thiosemicarbazide to afford the corresponding thioamide 5, which was the key precursor for synthesis of the compounds 7-15. Three cancer cell lines (MCF-7, HepG-2 and A549) were used to examine in *vitro* anticancer activities of the recently synthesized compounds. Three compounds 7c, 9c, and 11d were found to show the most promising anti-cancer activity against three cell lines.

Keywords: Chalcone; thioamide; pyrazoline; thiazole; haloketone; anti-cancer agents

1. Introduction

Cancer is the second largest cause of death in the world, it is a broad group of diseases that can begin in practically any organ or tissue of the body. These diseases are brought on when abnormal cells grow out of control, cross their normal boundaries to infect nearby body parts, and/or move to other organs. The latter process, known as metastasising, is a major factor in cancer-related death. Lung, prostate, colorectal, stomach, and liver cancers are the most prevalent in men, while breast, colorectal, lung, cervical, and thyroid cancers are the most prevalent in women. The cancer burden continues to grow globally, causing tremendous physical, emotional, and financial stress on individuals, families, communities, and health systems. In countries where health systems are strong, survival rates for many cancers are improved by easy access to early detection, quality treatment, and survivors' care [1-3]. The pyrazoline heterocyclic is a special scaffold possessing myriad activities. It has become a helpful pharmacophore in the development of successful anticancer therapeutics [4-6]. In addition to, the thiazole is one of the most effective motifs for the stated target activity, according to the drug design field. Thiazoles have greater anticancer action due to their improved binding domain, decreased cytotoxicity in physiological cells, and site-specific mobility toward cancer cells (pathological cells). Additionally, it has been observed that thiazole compounds have cytotoxic effects on a number of cancer types [7-11].

For decades, due to its numerous therapeutic applications, pyrazoline-thiazole hybrid had attracted a great interest as an essential scaffold [12, 13] in various drugs, such as Ruxolitinib (I), Crizotinib (II), and Tozasertib (III). The compound I was used for treatment myelofibrosis and photovoltaic by blocking the impulses that allow cancer cells to grow [14]; the compound II was used for treatment metastatic non-

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small cell lung cancer [15] and the compound III was used for the treatment of solid tumors and hematopoietic cancers [16-18] (Fig. 1). Also, many therapeutically accessible thiazole-containing antiproliferative agents such as exhibited their anticancer activity profile via a variety of mechanisms, including, Tiazofurin (IV), Dasatinib (V) Dabarafenib (VI) [19-21] (Fig. 1).

Recently, some of the reported pyrazolines and thiazoles showed a range of biological activities, including, antimicrobial [22-25], antioxidant and anti-inflammatory agent [26, 27], anti-proliferative activities [23, 28, 29], as dual EGFR and HER-2 inhibitors [30], antiviral [31] and anti-cancer agents [22, 32-36].

Based on the previous aspects, it was of interest to develop new derivatives of pyrazolyl-thiazole scaffold bearing various functional groups that may exhibit more potent anti-cancer activities, we combined the molecules of pyrazoline and thiazole in one component, and based on the pharmacological action profiles, we concentrated on structural modification to improve the activity of the pyrazolines-thiazole and assessment as anti-cancer activity (Fig. 2).

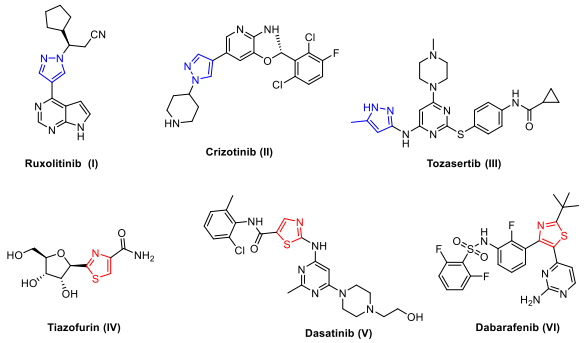


Fig. 1: Some structure of pyrazoline and thiazole bearing anti-cancer drugs

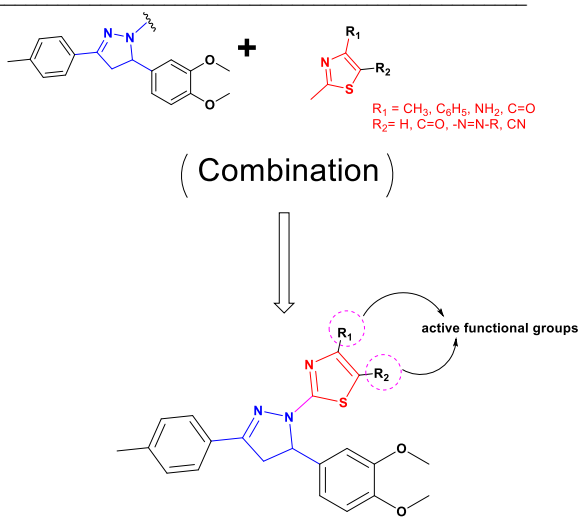


Fig. 2 : Target Compounds 7(a-d), 9 (a-c),11(a-f),15

2. Experimental

2.1. Chemistry

The melting points were determined using an Electro-thermal IA 9100 instrument without correction (Shimadzu, Japan). On a Perkin-Elmer 1650 Spectrophotometer at the National Research Centre in Cairo, Egypt, IR spectra were captured as KBr pellets using the KBr disc technique. Chemical shifts were measured using a Varian-500 MHz in deuterated DMSO-d6 and measured as ppm against TMS as an internal reference at the National Research Centre, Cairo, Egypt. Mass spectra were recorded on Shimadzu GCMS-QP-1000EX mass spectrometer at 70 eV.

Synthesis of 3-(3,4-dimethoxyphenyl)-1-(p-tolyl)prop-2-en-1-one (3)

To a mixture of 4-methylacetophenone (**1**) (13.4g, 0.1 mol) and veratraldehyde **2** (16.6g, 0.1 mol) in absolute EtOH (25 mL), NaOH solution (10%, 5 mL) was added. The mixture was stirred for 4 hrs and the formed solid was filtered and recrystallized from EtOH to yield the chalcone derivative **3**.

Yield 90-95%; m.p. 87-90 °C, IR (KBr, υ cm^{-1}): 1675 (C=O), 1645 (C=C); ^{1}H NMR (DMSO-d_6, ppm): δ 2.36 (s, 3H, -C<u>H</u>$_3$), 3.78 (s, 3H, -OC<u>H</u>$_3$), 3.84 (s, 3H, -OC<u>H</u>$_3$), 6.97 (d, 1H Ar, J = 8.5 Hz), 7.32-7.35 (m, 3H, Ar-H), 7.51 (s, 1H, Ar-H), 7.65 (d, IH, J = 15.25 Hz, -C<u>H</u>=CH-), 7.78 (d, IH, J = 15.25 Hz, -C<u>H</u>=CH-), 8.03 (d, 2H, J = 7.6 Hz, Ar-H); ^{13}C NMR (DMSO-d_6, ppm): δ 21.13 (-CH$_3$), 56.1, 56.2 (2C of 2OC<u>H</u>$_3$), 108.4, 111.2, 121.3, 128.5, 128.7, 128.9, 129.1, 129.2, 131.2, 135.5, 141.5, 144.1, 148.2, 148.4, 185.5 (C=O); MS, m/z (%): 282 (M^+., 78). Analysis for $C_{18}H_{18}O_3$ (282.34) Calcd.: C, 76.57; H, 6.43; O, 17.00%. Found: C, 76.49; H, 6.34; O, 17.01%.

Synthesis of 5-(3,4-dimethoxyphenyl)-3-(p-tolyl)-4,5-dihydro-1H-pyrazole-1-carbothioamide (5)

To a solution of NaOH (1g, 25 mmol) and the chalcone derivative **3** (0.28g, 1 mmol) in absolute EtOH (25 mL), thiosemicarbazide (**4**) (0.27g, 3 mmol) was added under stirring. The reaction mixture was refluxed for 6 hrs. The resulting solid was allowed to cool, filtered, washed, dried, and crystallized from EtOH to produce the thioamide derivative **5**.

Yield 75-77%; m.p. 180-183 °C, IR (KBr, υ cm^{-1}): 3448 (NH_2), 1255 (C=S); ^{1}H NMR (DMSO-d_6, ppm): δ 2.31 (s, 3H, -C<u>H</u>$_3$), 3.36 (d, 1H, J = 13.5, Hz, -CH_2 of pyrazoline), 3.67 (s, 3H, -OC<u>H</u>$_3$), 3.73 (s, 3H, -OC<u>H</u>$_3$), 3.95 (d, 1H, J = 13.7 Hz, -C<u>H</u>$_2$ of pyrazoline), 4.59 (s, 2H, NH_2, D_2O exchangeable), 5.55 (dd, 1H, J = 8.5, 4,1 Hz, -C<u>H</u> of the chiral carbon of pyrazoline), 6.87 (d, 2H, J = 8.5, 2.6 Hz, Ar-H), 7.29 (s, 1H, Ar-H), 7.31 (d, 2H, J = 8.0, 1.3 Hz, Ar-H), 7.54 (d, 2H, J = 8.0, 1.8 Hz, Ar-H); ^{13}C NMR (DMSO-d_6, ppm): δ 21.3 (CH_3), 46.1 (CH_2 of pyrazoline), 56.0, 56.1 (2O<u>C</u>H$_3$), 59.2 (chiral carbon), 109.9, 111.2, 127.2, 127.3, 127.6, 128.2, 128.7, 129.1, 138.9, 141.5, 148.2, 148.4, 152.5 (Ar-C), 180.2 (C=S); MS, m/z (%): 355 (M^+., 67). Analysis for $C_{19}H_{21}N_3O_2S$ (355.46) Calcd.: C, 64.20; H, 5.96; N, 11.82; O, 9.00; S, 9.02 %. Found: C, 64.35; H, 5.87; N, 11.85; O, 9.10; S, 9.15 %.

Synthesis of pyrazolyl-phenyl thiazole derivatives 7a-d

To a suspension of the thioamide derivative **5** (0.35 g, 1 mmol) in absolute EtOH (25 mL), the appropriate phenacyl bromide derivatives **6a-d** (1 mmol) (namely: 2-bromo-1-phenylethan-1-one, 2-bromo-1-(4-chlorophenyl)ethan-1-one, 2-bromo-1-(4-bromophenyl)ethan-1-one and 2-bromo-1-(p-tolyl)ethan-1-one) were added and the mixture was refluxed for 4 hrs. The reaction solution was cooled and the formed precipitate was filtered and recrystallized from EtOH to yield the corresponding pyrazolyl-thiazole derivatives **7a-d**.

2-(5-(3,4-Dimethoxyphenyl)-3-(p-tolyl)-4,5-dihydro-1H-pyrazol-1-yl)-4-phenylthiazole (7a)

Yield 80-83%; m.p. 160-163 °C, IR (KBr, υ cm^{-1}): 1620 (C=N); ^{1}H NMR (DMSO-d_6, ppm): δ 2.33 (s, 3H, -C<u>H</u>$_3$), 3.31 (dd, 1H, J = 18.0, 9.1 Hz, -CH_2 of pyrazoline), 3.67 (s, 3H, -OC<u>H</u>$_3$), 3.72 (s, 3H, -OC<u>H</u>$_3$), 3.92 (dd, 1H, J = 18.0, 12,4 Hz, -C<u>H</u>$_2$ of pyrazoline), 5.55 (dd, 1H, J = 6.65, 11,5 Hz, -C<u>H</u> of the chiral carbon of pyrazoline), 6.87 (d, 2H, J = 8.5 Hz, Ar-H), 7.04 (s, 1H of thiazole, H_5), 7.22-7.33 (m, 5H, Ar-H), 7.37 (s, 1H, Ar-H), 7.65 (d, 2H, J = 7.5 Hz, Ar-H), 7.72 (d, 2H, J = 7.5 Hz, Ar-H); ^{13}C NMR (DMSO-d_6, ppm): δ 21.3 (CH_3), 46.1 (CH_2), 56.0, 56.2 (2C,OCH_3), 63.1 (chiral carbon), 102.2, 109.9, 111.2, 126.8, 127.2, 127.3, 127.8, 128.2, 128.4, 129.1, 131.4, 138.9, 141.5, 148.2, 148.3, 148.6, 152.5, 159.2 (Ar-C); MS, m/z (%): 455 (M^+., 65). Analysis for $C_{27}H_{25}N_3O_2S$ (455.58) Calcd.: C, 71.18; H, 5.53; N, 9.22; O, 7.02; S, 7.04%. Found: C, 71.25; H, 5.49; N, 9.27; O, 7.12; S, 7.10 %.

4-(4-Chlorophenyl)-2-(5-(3,4-dimethoxyphenyl)-3-(p-tolyl)-4,5-dihydro-1H-pyrazol-1-yl)thia-zole (7b)

Yield 80-82%; m.p. 180-182 °C, IR (KBr, υ cm^{-1}): 1622 (C=N); ^{1}H NMR (DMSO-d_6, ppm): δ 2.33 (s, 3H, -C<u>H</u>$_3$), 3.33 (dd, 1H, J = 13.5, 8,1 Hz, -CH_2 of pyrazoline), 3.67 (s, 3H, OC<u>H</u>$_3$), 3.72 (s, 3H, -OC<u>H</u>$_3$), 3.93 (dd, 1H, J = 13.7, 4,3 Hz, -CH_2 of pyrazoline), 5.55 (dd, 1H, J = 8.5, 4,1 Hz, -C<u>H</u> of the chiral carbon of pyrazoline), 6.86 (d, 2H, J = 9.5 Hz, Ar-H), 7.02 (s, 1H, thiazole, H_5), 7.26 (d, 2H, J = 6.5 Hz, Ar-H), 7.36 (s, 1H, Ar-H), 7.38 (d, 2H, J = 5.5 Hz, Ar-H), 7.65 (d, 2H, J = 6.5 Hz, Ar-H), 7.73 (d, 2H, J = 6.5 Hz, Ar-H); ^{13}C NMR (DMSO-d_6, ppm): δ 21.3 (CH_3), 46.1 (CH_2), 56.0, 56.2 (2C,OCH_3), 63.1 (chiral carbon), 102.2, 109.9, 111.2, 126.8, 127.2, 127.3, 127.8, 128.2, 128.4 , 129.1, 131.4, 138.9, 141.5, 148.2, 148.3, 148.6, 152.5, 159.2 (Ar-C); MS, m/z (%): 489 (M^+., 48), 491 (M + 2, 15). Analysis for $C_{27}H_{24}ClN_3O_2S$ (489.02) Calcd.: C, 66.18; H, 4.94; Cl, 7.23; N, 8.58; O, 6.53; S, 6.54 %. Found: C, 66.25; H, 4.91; Cl, 7.27; N, 8.56; O, 6.55; S, 6.51 %.

4-(4-Bromophenyl)-2-(5-(3,4-dimethoxyphenyl)-3-(p-tolyl)-4,5-dihydro-1H-pyrazol-1-yl)-thiazole (7c)

Yield 80-82%; m.p. 190-192 °C, IR (KBr, υ cm^{-1}): 1625 (C=N); ^{1}H NMR (DMSO-d_6, ppm): δ 2.34 (s, 3H, -CH_3), 3.33 (dd, 1H, J = 12.5, 8.1 Hz, -CH_2 of pyrazoline), 3.67 (s, 3H, OCH_3), 3.73 (s, 3H, -OCH_3), 3.93 (dd, 1H, J = 13.7, 4.3 Hz, -CH_2 of pyrazoline), 5.57 (dd, 1H, J = 8.5, 4.1 Hz, -CH of the chiral carbon of pyrazoline), 6.87 (d, 2H, J = 9.5 Hz, Ar-H), 7.05 (s, 1H, thiazole, H_5), 7.28 (d, 2H, J = 6.5 Hz, Ar-H), 7.37 (s, 1H, Ar-H), 7.40 (d, 2H, J = 5.5 Hz, Ar-H), 7.66 (d, 2H, J = 6.5 Hz, Ar-H), 7.73 (d, 2H, J = 6.5 Hz); ^{13}C NMR (DMSO-d_6, ppm): δ 21.3 (CH_3), 46.1 (CH_2), 56.0, 56.2 (2C,OCH_3), 63.3 (chiral carbon), 102.3, 110.1, 111.2, 122.3, 126.8, 127.2, 127.3, 127.8, 128.3, 128.5, 129.1, 131.4, 138.8, 141.6, 148.4, 148.6, 148.5, 152.5, 159.4 (Ar-C); MS, m/z (%): 534 (M^+., 52), 536 ($M^{+\cdot}$ + 2, 47). Analysis for $C_{27}H_{24}BrN_3O_2S$ (534.47) Calcd.: C, 60.68; H, 4.53; Br, 14.95; N, 7.86; O, 5.99; S, 6.00 %. Found: C, 60.63; H, 4.51; Br, 14.89; N, 7.87; O, 5.97; S, 5.98 %.

2-(5-(3,4-Dimethoxyphenyl)-3-(p-tolyl)-4,5-dihydro-1H-pyrazol-1-yl)-4-(p-tolyl)thiazole (7d)

Yield 80-82 %; m.p. 168-170 °C, IR (KBr, υ cm^{-1}): 1620 (C=N); ^{1}H NMR (DMSO-d_6, ppm): δ 2.34 (s, 3H, -CH_3), 2.35 (s, 3H, -CH_3), 3.33 (dd, 1H, J = 12.5, 8,1 Hz, -CH_2 of pyrazoline), 3.67 (s, 3H, OCH_3), 3.71 (s, 3H, -OCH_3), 3.92 (dd, 1H, J = 13.7, 4.3 Hz, -CH_2 of pyrazoline), 5.57 (dd, 1H, J = 8.5, 4.1 Hz, -CH of the chiral carbon of pyrazoline), 6.85 (d, 2H, J = 9.5 Hz, Ar-H), 7.03 (s, 1H, thiazole, H_5), 7.27 (d, 2H, J = 6.5 Hz, , Ar-H), 7.35 (s, 1H, Ar-H), 7.40 (d, 2H, J =

5.5 Hz, Ar-H), 7.65 (d, 2H, *J* = 6.5 Hz, , Ar-H), 7.72 (d, 2H, *J* = 6.5 Hz, , Ar-H); ^{13}C NMR (DMSO-d_6, ppm): δ 21.3, 21.4 (2C of CH_3), 46.2 (CH_2), 56.0, 56.2 (2C,OCH_3), 63.1 (chiral carbon), 102.3, 110.1, 111.2, 122.3, 126.8, 127.2, 127.3, 127.8, 128.3, 128.5, 129.1, 131.4, 138.8, 141.6, 148.4, 148.6, 148.5, 151.8, 159.2 (Ar-C); MS, m/z (%): 469 (M^+, 48). Analysis for $C_{28}H_{27}N_3O_2S$ (469.60) Calcd.: C, 71.62; H, 5.80; N, 8.95; O, 6.81; S, 6.83 %. Found: C, 71.60; H, 5.83; N, 8.91; O, 6.79; S, 6.81 %.

General procedure for preparation of compounds 9a-c

The compounds 9a-c were prepared by the reaction of equimola amounts of the thioamide derivative 5 with the appropriate 3-chloropentane-2,4-diones (8a-c) in EtOH and refluxed for 4 hrs. The reaction solution was cooled and the formed precipitate was filtered and recrystallized from EtOH to yield the corresponding pyrazolyl-thiazole derivatives 9a-c.

1-(2-(5-(3,4-Dimethoxyphenyl)-3-(p-tolyl)-4,5-dihydro-1H-pyrazol-1-yl)-4-methylthiazol-5-yl) ethan-1-one (9a)

Yield 80-85%; m.p. 185-188 ˚C, IR (KBr, υ cm^{-1}): 1665 (C=O), 1620 (C=N); ^{1}H NMR (DMSO-d_6, ppm): δ 2.32 (s, 3H, -CH_3), 2.35 (s, 3H, -CH_3), 2.36 (s, 3H, -CH_3), 3.33 (dd, 1H, *J* = 12.5, 8.1 Hz, -CH_2 of pyrazoline), 3.67 (s, 3H, OCH_3), 3.69 (s, 3H, -OCH_3), 3.93 (dd, 1H, *J* = 13.7, 4.3 Hz, -CH_2 of pyrazoline), 5.63 (dd, 1H, *J* = 8.5, 11.5 Hz, -CH of the chiral carbon of pyrazoline), 6.65 (d, 1H, *J* = 8.5 Hz, Ar-H), 6.84 (d, 2H, *J* = 8.5 Hz, , Ar-H), 6.87 (s, 1H, Ar-H), 7.26 (d, 2H, *J* = 7.5 Hz, Ar-H), 7.66 (d, 2H, *J* = 7.5 Hz, Ar-H); ^{13}C NMR (DMSO-d_6, ppm): δ 15.7 (CH_3) 21.4(CH_3), 26.7(CH_3) 46.5 (CH_2), 56.2, 56.3 (2C,OCH_3), 63.2 (chiral carbon), 109.9, 111.2, 127.2, 127.3, 128.2, 129.1, 129.3, 131.4, 138.9, 141.5, 148.2, 148.4, 149.2, 152.5 (Ar-C), 159.2 (C=N), 180.1(C=O); MS, m/z (%):435 (M^+, 35). Analysis for $C_{24}H_{25}N_3O_3S$ (435.54) Calcd.: C, 66.19; H, 5.79; N, 9.65; O, 11.02; S, 7.36 %. Found: C, 66.23; H, 5.73; N, 9.61; O, 11.05; S, 7.31 %.

1-(2-(5-(3,4-Dimethoxyphenyl)-3-(p-tolyl)-4,5-dihydro-1H-pyrazol-1-yl)-4-ethoxythiazol-5-yl) ethan-1-one (9b)

Yield 80-85%; m.p. 197-200 ˚C, IR (KBr, υ cm^{-1}): 1675 (C=O), 1625 (C=N); ^{1}H NMR (DMSO-d_6, ppm): δ 1.24 (t, 3H, *J* = 7.5 Hz, -CH_2CH_3), 2.29 (s, 3H, -CH_3), 2.36 (s, 3H, -CH_3), 3.31 (dd, 1H, *J* = 12.5, 8.1 Hz, -CH_2 of pyrazoline), 3.68 (s, 3H, OCH_3), 3.78 (s, 3H, -OCH_3), 3.93 (dd, 1H, *J* = 13.7, 4.3 Hz, -CH_2 of pyrazoline), 4.47 (q, 2H, -CH_2CH_3), 5.63 (dd, 1H, *J* = 11.5 Hz, -CH of the chiral carbon), 6.65 (d, 1H, *J* = 8.5 Hz, Ar-H), 6.86 (d, 1H, *J* = 8.1 Hz, Ar-H), 6.89 (s, 1H, Ar-H), 7.15 (d, 2H, *J* = 8.5 Hz, Ar-H), 7.69 (d, 2H, *J* = 8.5 Hz, Ar-H); ^{13}C NMR (DMSO-d_6, ppm): δ 15.3 (CH_3) 21.5 (CH_3), 26.5(CH_3) 46.1 (CH_2), 56.0, 56.1 (2C, OCH_3), 63.1 (chiral carbon), 65.2, 110.3, 111.3, 127.3, 127.5, 128.2, 128.5, 129.4, 129.7, 131.6, 138.9, 143.5, 148.5, 148.9, 152.5, 156.2 (Ar-C), 159.2 (C=N), 181.8 (C=O); MS, m/z (%): 465 (M^+, 43). Analysis for $C_{25}H_{27}N_3O_4S$ (465.57) Calcd.: C, 64.50; H, 5.85; N, 9.03; O, 13.75; S, 6.89 %. Found: C, 64.47; H, 5.81; N, 9.05; O, 13.77; S, 6.85 %.

1-(2-(5-(3,4-Dimethoxyphenyl)-3-(p-tolyl)-4,5-dihydro-1H-pyrazol-1-yl)-4-(phenylamino)-thiazole-5-yl)ethan-1-one (9c)

Yield 80-85%; m.p. 218-220 ˚C, IR (KBr, υ cm^{-1}): 3360 (NH), 1680 (C=O), 1625 (C=N); ^{1}H NMR (DMSO-d_6, ppm): δ 2.25 (s, 3H, -CH_3), 2.28 (s, 3H, -CH_3), 3.32 (dd, 1H, *J* = 10.5, 6.5 Hz, -CH_2 of pyrazoline), 3.68 (s, 3H, OCH_3), 3.77 (s, 3H, -OCH_3), 3.90 (dd, 1H, *J* = 13.7, 4.3 Hz, -CH_2 of pyrazoline), 5.54 (dd, 1H, *J* = 11.5 Hz, -CH of the chiral carbon), 6.77-6.91 (m, 4H, Ar-H), 7.12-7.29 (m, 6H, Ar-H), 7.42 (d, 2H, *J* = 8.5 Hz, Ar-H). 9.80 (s, 1H, NH, D_2O-exchangeable); ^{13}C NMR (DMSO-d_6, ppm): δ 21.3(CH_3), 26.7(CH_3), 46.1 (CH_2), 56.0, 56.1 (2C, OCH_3), 63.2 (chiral carbon), 109.8, 111.2, 119.7, 119.9, 127.2, 127.3, 127.8, 128.1, 128.2, 128.3, 128.7, 129.1, 131.6, 138.2, 141.5, 148.2, 148.4, 150.1, 152.5, 159.2 (Ar-C), 181.8 (C=O); MS, m/z (%):512 (M^+, 61). Analysis for $C_{29}H_{28}N_4O_3S$ (512.63) Calcd.: C, 67.95; H, 5.51; N, 10.93; O, 9.36; S, 6.25 %. Found: C, 67.91; H, 5.54; N, 10.90; O, 9.39; S, 6.21 %.

Synthesis of pyrazolyl-diazenyl thiazole derivatives 11a-f

An equimolar mixture of the thioamide derivative 5 (1.0 mmol) and the appropriate of hydrazonoyl chloride (namely: 2-oxo-N-phenyl propanehydrazonoyl chloride, N-(4 chloro/bromo/fluro/methyl/ methoxy phenyl)-2-oxopropanehydrazonoyl chloride) (10a-f) (1.0 mmol) in absolute EtOH (25 mL) was refluxed for 4 hrs. After cooling, the formed solid was filtered, washed, dried and recrystallized from EtOH to afford the corresponding hydrazones 11a-f.

2-(5-(3,4-Dimethoxyphenyl)-3-(p-tolyl)-4,5-dihydro-1H-pyrazol-1-yl)-4-methyl-5-(phenyl-diazenyl)thiazole (11a)

Yield 78-80%; m.p. 198-200 ˚C, IR (KBr, υ cm^{-1}): 1620 (C=N), 1600 (N=N); ^{1}H NMR (DMSO-d_6, ppm): δ 2.31 (s, 3H, -CH_3), 2.46 (s, 3H, -CH_3), 3.38 (dd, 1H, *J* = 10.5, 6.5 Hz, -CH_2 of pyrazoline), 3.68 (s, 3H, OCH_3), 3.71 (s, 3H, -OCH_3), 3.98 (dd, 1H, *J* = 6.5 Hz, -CH_2 of pyrazoline), 5.74 (dd, 1H, *J* = 15.2 Hz, -CH of the chiral carbon), 6.67-6.85 (m, 3H, Ar-H), 6.90 (d, 2H, *J* = 8.5 Hz, Ar-H), 6.95 (s, 1H, Ar-H), 7.25-7.70 (m, 6H, Ar-H); ^{13}C NMR (DMSO-d_6, ppm): δ 14.1(CH_3), 21.2(CH_3), 46.1, 56.0 (2C,OCH_3), 63.1 (chiral carbon), 109.4, 111.2, 122.2, 127.2, 127.3, 127.6, 128.2, 128.4, 128.7, 129.1,

133.4, 138.7, 141.5, 148.2, 148.4, 149.4, 151.7, 152.5, 159.2(Ar-C); MS, m/z (%):497 (M^+, 67). Analysis for $C_{28}H_{27}N_5O_2S$ (497.62) Calcd.: C, 67.58; H, 5.47; N, 14.07; O, 6.43; S, 6.44 %. Found: C, 67.53; H, 5.41; N, 14.15; O, 6.45; S, 6.39 %.

5-((4-Chlorophenyl)diazenyl)-2-(5-(3,4-dimethoxyphenyl)-3-(p-tolyl)-4,5-dihydro-1H-pyrazol-1-yl)-4-methylthiazole (11b)

Yield 80-82%; m.p. 210-213 °C, IR (KBr, υ cm^{-1}): 1625 (C=N), 1608 (N=N); ^{1}H NMR (DMSO-d_6, ppm): δ 2.33 (s, 3H, -CH_3), 2.47 (s, 3H, -CH_3), 3.40 (dd, 1H, J = 10.5, 6.5 Hz, -CH_2 of pyrazoline), 3.68 (s, 3H, OCH_3), 3.71 (s, 3H, -OCH_3), 3.99 (dd, 1H, J = 6.5 Hz, -CH_2 of pyrazoline), 5.75 (dd, 1H, J = 15.2 Hz, -CH of the chiral carbon), 6.68 (d, 1H, J = 8.5 Hz, Ar-H), 6.86 (d, 1H, J = 8.5 Hz, Ar-H), 6.92 (s, 1H, Ar-H), 7.27 (d, 2H, J = 7.5 Hz, Ar-H), 7.48 (d, 2H, J = 8.5 Hz, Ar-H), 7.64 (d, 2H, J = 8.5 Hz, Ar-H), 7.69 (d, 2H, J = 7.5 Hz, Ar-H); ^{13}C NMR (DMSO-d_6, ppm): δ 14.2(CH_3), 21.3(CH_3), 46.1 (CH_2), 56.1 (2C,OCH_3), 63.5 (chiral carbon), 109.4, 111.7, 117.7, 127.2, 127.3, 128.2, 128.9, 129.1, 129.3, 133.6, 133.8, 138.9, 141.5, 148.4, 148.8, 149.5, 151.9, 152.5, 159.2 (Ar-C); MS, m/z (%):531 (M^+, 65), 533 (M + 2, 21). Analysis for $C_{28}H_{26}ClN_5O_2S$ (531.06) Calcd.: C, 63.21; H, 4.93; Cl, 6.66; N, 13.16; O, 6.01; S, 6.03 %. Found: C, 63.17; H, 4.91; Cl, 6.61; N, 13.21; O, 6.03; S, 6.01 %.

5-((4-Bromophenyl)diazenyl)-2-(5-(3,4-dimethoxyphenyl)-3-(p-tolyl)-4,5-dihydro-1H-pyrazol-1-yl)-4-methylthiazole (11c)

Yield 85-90%; m.p. 238-240 °C, IR (KBr, υ cm^{-1}): 1625 (C=N), 1610 (N=N); ^{1}H NMR (DMSO-d_6, ppm): δ 2.34 (s, 3H, -C$\underline{H}_3$), 2.47 (s, 3H, -C$\underline{H}_3$), 3.36 (dd, 1H, J = 10.5, 6.5 Hz, -CH_2 of pyrazoline), 3.68 (s, 3H, OC$\underline{H}_3$), 3.71 (s, 3H, -OC$\underline{H}_3$), 3.99 (dd, 1H, J = 6.5 Hz, -C$\underline{H}_2$ of pyrazoline), 5.76 (dd, 1H, J = 11.50 Hz, -C$\underline{H}$ of the chiral carbon), 6.68 (d, 1H, J = 8.5 Hz, Ar-H), 6.86 (d, 1H, J = 8.5 Hz, Ar-H), 6.92 (s, 1H, Ar-H), 7.28 (d, 2H, J = 7.5 Hz, Ar-H), 7.57 (d, 2H, J = 8.5 Hz, Ar-H), 7.62 (d, 2H, J = 8.5 Hz, Ar-H), 7.70 (d, 2H, J = 6.5 Hz, Ar-H); ^{13}C NMR (DMSO-d_6, ppm): δ 14.1(CH_3), 21.2(CH_3), 46.2 (CH_2), 56.2 (2C,OCH_3), 63.2 (chiral carbon), 109.9, 111.2, 117.7, 122.3, 127.3, 127.5, 128.2, 129.3, 131.7, 133.7, 138.9, 141.5, 148.2, 148.4, 149.5, 151.9, 152.5, 159.6 (Ar-C); MS, m/z (%):575 (M^+, 54), 577 (M + 2, 49). Analysis for $C_{28}H_{26}BrN_5O_2S$ (575.51) Calcd.: C, 58.33; H, 4.55; Br, 13.86; N, 12.15; O, 5.55; S, 5.56 %. Found: C, 58.29; H, 4.50; Br, 13.83; N, 12.21; O, 5.58; S, 5.53 %.

2-(5-(3,4-Dimethoxyphenyl)-3-(p-tolyl)-4,5-dihydro-1H-pyrazol-1-yl)-5-((4-fluoro-phenyl-diazenyl)-4-methylthiazole(11d)

Yield 85-90%; m.p. 260-262 °C, IR (KBr, υ cm^{-1}): 1625 (C=N), 1605 (N=N); ^{1}H NMR (DMSO-d_6, ppm): δ 2.34 (s, 3H, -C$\underline{H}_3$), 2.48 (s, 3H, -C$\underline{H}_3$), 3.37 (dd, 1H, J = 10.5, 6.5 Hz, -CH_2 of pyrazoline), 3.68 (s, 3H, OC$\underline{H}_3$), 3.72 (s, 3H, -OC$\underline{H}_3$), 3.99 (dd, 1H, J = 6.5 Hz, -C$\underline{H}_2$ of pyrazoline), 5.77 (dd, 1H, J = 11.50 Hz, -C$\underline{H}$ of the chiral carbon), 6.67 (d, 1H, J = 8.5 Hz, Ar-H), 6.88 (d, 1H, J = 8.5 Hz, Ar-H), 6.94 (s, 1H, Ar-H), 7.29 (d, 2H, J = 7.5 Hz, Ar-H), 7.62 (d, 2H, J = 8.5 Hz, Ar-H), 7.68 (d, 2H, J = 8.5 Hz, Ar-H), 7.71 (d, 2H, J = 6.5 Hz, Ar-H); ^{13}C NMR (DMSO-d_6, ppm): δ 14.2(CH_3), 21.3(CH_3), 46.2 (CH_2), 56.1 (2C,OCH_3), 63.2 (chiral carbon), 109.8, 111.2, 115.6, 117.8, 127.2, 127.3, 128.2, 129.1, 133.7, 138.9, 141.5, 148.5, 148.2, 149.7, 151.8, 152.6, 162.5 (Ar-C); MS, m/z (%): 515 (M^+, 63). Analysis for $C_{28}H_{26}FN_5O_2S$ (515.61) Calcd.: C, 65.23; H, 5.08; F, 3.68; N, 13.58; O, 6.21; S, 6.22 %. Found: C, 65.19; H, 5.03; F, 3.65; N, 13.63; O, 6.25; S, 6.19 %.

2-(5-(3,4-Dimethoxyphenyl)-3-(p-tolyl)-4,5-dihydro-1H-pyrazol-1-yl)-4-methyl-5-(p-tolyl-diazenyl)thiazole (11e)

Yield 85-90%; m.p. 164-166 °C, IR (KBr, υ cm^{-1}): 1624 (C=N), 1605 (N=N); ^{1}H NMR (DMSO-d_6, ppm): δ 2.31 (s, 3H, -C$\underline{H}_3$), 2.33 (s, 3H, -C$\underline{H}_3$), 2.46 (s, 3H, -C$\underline{H}_3$), 3.37 (dd, 1H, J = 12.5, 6.5 Hz, -CH_2 of pyrazoline), 3.68 (s, 3H, OC$\underline{H}_3$), 3.73 (s, 3H, -OC$\underline{H}_3$), 3.99 (dd, 1H, J = 6.5 Hz, -C$\underline{H}_2$ of pyrazoline), 5.77 (dd, 1H, J = 10.5 Hz, -C$\underline{H}$ of the chiral carbon), 6.77 (d, 1H, J = 8.5 Hz, Ar-H), 6.85 (d, 1H, J = 7.5 Hz, , Ar-H), 6.91 (s, 1H, , Ar-H), 7.29 (d, 2H, J = 7.5 Hz, , Ar-H), 7.45 (d, 2H, J = 8.5 Hz, , Ar-H), 7.65 (d, 2H, J = 8.5 Hz, , Ar-H), 7.68 (d, 2H, J = 6.5 Hz, , Ar-H); ^{13}C NMR (DMSO-d_6, ppm): δ 14.2(CH_3), 21.3(CH_3), 21.4 (CH_3), 46.1 (CH_2), 56.1 (2C,OCH_3), 63.3 (chiral carbon), 109.7, 111.2, 122.2, 127.2, 127.3, 128.3, 129.1, 129.6, 133.7, 138.2, 141.5, 141.7, 148.2, 148.4, 149.5, 151.6, 152.5, 159.4 (Ar-C); MS, m/z (%): 511 (M^+, 58). Analysis for $C_{29}H_{29}N_5O_2S$ (511.64) Calcd.: C, 68.08; H, 5.71; N, 13.69; O, 6.25; S, 6.27 %. Found: C, 68.13; H, 5.67; N, 13.71; O, 6.18; S, 6.21 %.

2-(5-(3,4-Dimethoxyphenyl)-3-(p-tolyl)-4,5-dihydro-1H-pyrazol-1-yl)-5-((4-methoxyphenyl)-diazenyl)-4-methylthiazole (11f)

Yield 85-90%; m.p. 190-192 °C, IR (KBr, υ cm^{-1}): 1625 (C=N), 1608 (N=N); ^{1}H NMR (DMSO-d_6, ppm): δ 2.35 (s, 3H, -C$\underline{H}_3$), 2.47 (s, 3H, -C$\underline{H}_3$), 3.38 (dd, 1H, J = 10.5, 6.5 Hz, -CH_2 of pyrazoline), 3.72 (s, 3H, OC$\underline{H}_3$), 3.80 (s, 3H, -OC$\underline{H}_3$), 3.82 (s, 3H, -OC$\underline{H}_3$), 3.98 (dd, 1H, J = 6.5 Hz, -C$\underline{H}_2$ of pyrazoline), 5.56 (dd, 1H, J = 11.50 Hz, -C$\underline{H}$ of the chiral carbon), 6.76 (d, 1H, J = 8.5 Hz, Ar-H), 6.77 (d, 1H, J = 8.5 Hz, Ar-H), 6.79 (s, 1H, Ar-H), 6.97 (d, 2H, J = 7.5 Hz, Ar-H), 7.13 (d, 2H, J = 8.5 Hz, , Ar-H), 7.44 (d, 2H, J = 8.5 Hz, Ar-H), 7.72 (d, 2H, J = 6.5 Hz, Ar-H); ^{13}C NMR (DMSO-d_6, ppm): δ 14.1(CH_3), 21.3(CH_3), 46.2 (CH_2), 56.1 (2C,OCH_3),

56.3 (OCH_3) 63.4 (chiral carbon), 109.9, 111.2, 114.5, 124. 1, 127.3, 127.5, 128.2, 129.2, 133.7, 138.9, 141.7, 148.2, 148.4, 149.7, 151.7, 152.5, 159.8 (Ar-C); MS, m/z (%): 527 (M^+, 63). Analysis for $C_{29}H_{29}N_5O_3S$ (527.64) Calcd.: C, 66.01; H, 5.54; N, 13.27; O, 9.10; S, 6.08 %. Found: C, 65.97; H, 5.53; N, 13.31; O, 9.13; S, 6.01 %.

Synthesis of the amino thiazole derivatives 13a-b

To a suspension of the thioamide derivative 5 (0.35 g, 1 mmol) in absolute EtOH (25 mL), the appropriate 2-bromoacetonitrile (12a) or 2-bromomalonitrile (12b) (1.1 mmol) was added and the mixture was refluxed for 4 hrs. The reaction was cooled and the formed solid was filtered, washed, dried and recrystallized from EtOH to afford the corresponding amino thiazole derivatives 13a-b.

2-(5-(3,4-Dimethoxyphenyl)-3-(p-tolyl)-4,5-dihydro-1H-pyrazol-1-yl)thiazol-4-amine (13a)

Yield 85-90%; m.p. 215-218 °C, IR (KBr, υ cm^{-1}): 3345-3350 (NH_2), 1624 (C=N); ^{1}H NMR (DMSO-d_6, ppm): δ 2.30 (s, 3H, -C<u>H</u>$_3$), 3.12 (dd, 1H, J = 10.5, 6.5 Hz, -CH_2 of pyrazoline), 3.76 (s, 3H, OC<u>H</u>$_3$), 3.78 (s, 3H, -OC<u>H</u>$_3$), 3.80 (dd, 1H, J = 6.5 Hz, -C<u>H</u>$_2$ of pyrazoline), 5.81 (dd, 1H, J = 10.5 Hz, -C<u>H</u> of the chiral carbon), 5.91 (s, 1H, H_5 thiazole), 6.21 (s, 2H, NH_2, D_2O-exchangeable), 6.56 (d, 2H, J = 8.5 Hz, Ar-H), 6.74 (s, 1H, Ar-H), 6.81 (d, 2H, J = 7.5 Hz, Ar-H), 7.22 (d, 2H, J = 8.5 Hz, Ar-H); ^{13}C NMR (DMSO-d_6, ppm): δ 21.3(CH_3), 46.1 (CH_2), 56.1 (3C,OCH_3), 63.2 (chiral carbon), 109.7, 111.2, 125.7, 127.2, 127.3, 128.2, 128.9, 138.6, 141.5, 148.2, 148.4, 150.1, 152.5, 159.2 (Ar-C); MS, m/z (%): 394 (M^+, 71). Analysis for $C_{21}H_{22}N_4O_2S$ (394.49) Calcd.: C, 63.94; H, 5.62; N, 14.20; O, 8.11; S, 8.13 %. Found: C, 63.89; H, 5.58; N, 14.41; O, 8.08; S, 8.09 %.

4-Amino-2-(5-(3,4-dimethoxyphenyl)-3-(p-tolyl)-4,5-dihydro-1H-pyrazol-1-yl)thiazole-5-carbonitrile (13b)

Yield 85-90%; m.p. 270-273 °C, IR (KBr, υ cm^{-1}): 3355-3360 (NH_2), 2230 (C≡N), 1625 (C=N); ^{1}H NMR (DMSO-d_6, ppm): δ 2.31 (s, 3H, -C<u>H</u>$_3$), 3.13 (dd, 1H, J = 12.5, 7.5 Hz, -CH_2 of pyrazoline), 3.77 (s, 3H, OC<u>H</u>$_3$), 3.79 (s, 3H, -OC<u>H</u>$_3$), 3.90 (dd, 1H, J = 6.5 Hz, -C<u>H</u>$_2$ of pyrazoline), 5.79 (dd, 1H, J = 10.5 Hz, -C<u>H</u> of the chiral carbon), 6.27 (s, 2H,NH_2, D_2O-exchangeable), 6.77 (d, 2H, J = 8.5 Hz, Ar-H), 6.90 (s, 1H, Hz, Ar-H), 7.12 (d, 2H, J = 8.5 Hz, Ar-H), 7.42 (d, 2H, J = 8.5 Hz, Ar-H); ^{13}C NMR (DMSO-d_6, ppm): δ 21.2(CH_3), 46.2 (CH_2), 56.1, 56.3 (2C,OCH_3), 63.5 (chiral carbon), 109.8, 111.2, 113.5, 127.3, 127.5, 128.2, 128.5, 129.1, 131.6, 138.9, 141.5, 148.2, 148.2, 150.1, 152.5, 159.2 (Ar-C); MS, m/z (%): 419 (M^+, 65). Analysis for $C_{22}H_{21}N_5O_2S$ (419.50) Calcd.: C, 62.99; H, 5.05; N, 16.69; O, 7.63; S, 7.64 %. Found: C, 62.95; H, 5.07; N, 16.72; O, 7.59; S, 7.61 %.

Synthesis of the pyrazolyl-thiazol-4-one derivatives 15

A suspension of the thioamide derivative 5 (0.35 g, 1 mmol) in absolute EtOH (25 mL) and the bromoacetic acid (14) (1.1 mmol) was added and the mixture was refluxed for 4 hrs. The reaction was cooled and the solid formed product was filtered, washed, dried and recrystallized from EtOH to afford the corresponding thiazolone 15.

2-(5-(3,4-Dimethoxyphenyl)-3-(p-tolyl)-4,5-dihydro-1H-pyrazol-1-yl)thiazol-4(5H)-one (15)

Yield 85-90%; m.p. 285-290 °C, IR (KBr, υ cm^{-1}): 1685 (C=O), 1625 (C=N); ^{1}H NMR (DMSO-d_6, ppm): δ 2.30 (s, 3H, -C<u>H</u>$_3$), 3.15 (dd, 1H, J = 10.5, 6.5 Hz, -CH_2 of pyrazoline), 3.78 (s, 3H, OC<u>H</u>$_3$), 3.80 (s, 3H, -OC<u>H</u>$_3$), 3.90 (dd, 1H, J = 6.5 Hz, -C<u>H</u>$_2$ of pyrazoline), 4.25 (s, 2H, CH_2 of thiazolone ring), 5.79 (dd, 1H, J = 10.50 Hz, -C<u>H</u> of the chiral carbon), 6.76 (d, 2H, J = 7.5 Hz, Ar-H), 6.78 (s, 1H, Ar-H), 7.14 (d, 2H, J = 8.5 Hz, Ar-H), 7.56 (d, 2H, J = 8.5 Hz, Ar-H); ^{13}C NMR (DMSO-d_6, ppm): δ 21.3(CH_3), 38.9 (CH_2), 46.1 (CH_2), 56.1, (2C,OCH_3), 63.3 (chiral carbon), 109.7, 111.2, 127.2, 127.3, 128.2, 128.4, 129.1, 129.5, 138.7, 141.4, 148.2, 148.4, 152.5(Ar-C), 177.5 (C=O); MS, m/z (%): 395 (M^+, 65). Analysis for $C_{21}H_{21}N_3O_3S$ (395.48) Calcd.: C, 63.78; H, 5.35; N, 10.63; O, 12.14; S, 8.11 %. Found: C, 63.72; H, 5.37; N, 10.69; O, 12.01; S, 8.04 %.

2.2. Biological Assessment

2.2.1. Cell culture

MCF-7: Breast cancer cell, HepG-2: Hepatocellular carcinoma and A-549: Lung cancer cell were obtained from Nawah Scientific (Mokatam, Cairo, Egypt). The cells were cultured in Dulbecco's modified Eagle medium (DMEM) fortified with 10% inactivated fetal bovine serum, 100 units/mL of penicillin, and 100 mg/mL of streptomycin. The cells were kept in a humidified atmosphere at 37 oC with 5% CO2.

2.2.2. Cytotoxicity Assay

The cytotoxic activities of the new compounds were investigated using SRB assay was performed on the tested cell lines. About 3000–5000 Cells were seeded in 96-well plates contained in a 100 µl complete growth medium. After 24 h, the cells were attached to one another in the 100 µl containing the tested compounds with a serial concentration of (0.01, 0.1, 1, 10, and 100 µM). After 72 h of incubating the cells with the treated compounds, the growth medium was discarded, and the cells were fixed by adding TCA (10%W/V) to each well and incubated for 1 h at 4 °C. After washing, 70 µL SRB

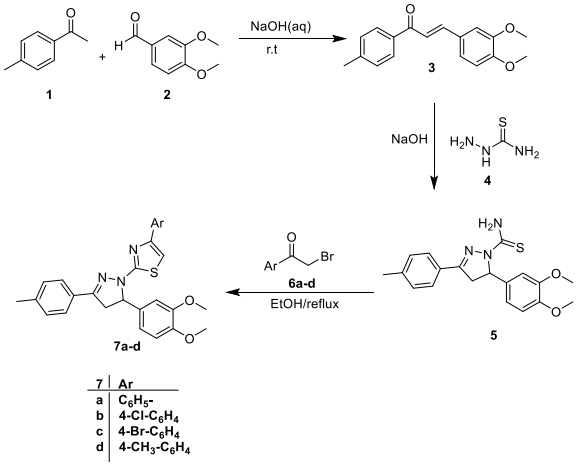
solution (0.4% w/v) was added to each well and incubated at 20 °C. After 10 min, the plates were washed with acetic acid (1%V/V) and allowed to dry. The absorbance of the bounded SRB was measured at 540 nm using a BMG LABTECH®-FLUOstar Omega microplate reader (Ortenberg, Germany) after adding TRIS buffer (10 mM) to dissolve protein-bound SRB stain. The dose-response curve was fitted for each compound using non-linear regression and the SI = IC50 value normal cell/IC50 value cancer cell [35, 36].

Statistical Analysis

All experiments were independently conducted at least three times as mean ± SD. All IC50 values were computed using Graph Pad Prism version 8.0.1., San Diego, California USA.

3. Results and Discussion

The Schemes 1-3 illustrate the synthetic pathway for the synthesis of pyrazolyl-aryl thiazole derivatives (target compounds). In Scheme 1, the chalcone 3 was prepared by stirring of p-methyl acetophenone (1) with veratraldehyde 2 in the presence of NaOH in EtOH. IR spectrum of the compound 3 showed absorption bands at 1675 and 1635 cm-1 due to the presence of C=O and C=C groups, respectively. Also, 1H NMR spectrum of the chalcone 3 showed three singlet signals at δ 2.3, 3.78, and 3.84 ppm confirming the existence of CH3 and 2 OCH3 groups, respectively. In addition, the vinylic protons of -CH=CH- appeared as two doublets at δ 7.65 ppm and 7.78 ppm with a high coupling constant, J = 15.25 Hz, which was an evidence of E configuration around the double bond. Also, 13C NMR spectrum displayed signals for the carbons CH3 and 2 OCH3 at δ 21.1 and 56.1, 56.2 ppm, respectively, as well as a signal for C=O at δ 185.5 ppm.

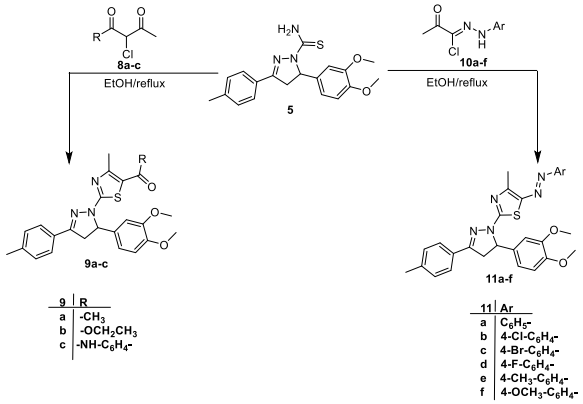


Scheme 1: Synthesis of pyrazolyl-phenylthiazole derivatives (7a-d)

The chalcone 3 underwent cyclization to form the pyrazoline thioamide derivative 5 by refluxing with thiosemicarbazide (4) in the presence of NaOH in absolute EtOH. IR analysis showed NH2 and C=S bands at 3428 cm-1 and 1273 cm-1, respectively. The 1H NMR spectrum showed three H of the pyrazoline ring of thioamide 5 allocated to the pyrazoline ring's HA, HB, and HX as ABX system, where 2H of the pyrazoline AB appeared as a doublet of doublet at δ 3.36 ppm and 3.93 ppm, respectively, and 1H (X) of the chiral carbon appeared at 5.56 ppm as a doublet of doublet. In addition, 13C NMR confirmed the presence of CH2, CH (chiral carbon), and C=S at δ 46.1, 59.2, and 180.2 ppm, respectively. The thioamide derivatives 5 was taken as a key starting material for the synthesis of various pyrazoline-arylthiazole derivatives 7a-c (target compounds) as shown in Scheme 1.

The thioamide 5 reacted as a nucleophilic reagent with different substituted phenacyl bromide 6a-d as a smooth reaction to afford the corresponding pyrazolyl thiazole derivatives 7a-d. Through the Hantzsch thiazole synthesis type, the reaction was carried out via the nucleophilic sulfur atom of the thioamide to produce imidothioate derivatives, which underwent cyclization to give the cyclic hydroxy intermediates, followed by dehydration to yield the end products 7a-d [37, 38].

The IR spectra of 7a-d revealed the disappearance of the NH2 function band and showed the formation of C=N bands around at 1620-1625 cm-1. 1H NMR spectra represented an increase in the aromatic integration at the aromatic region assignable to the new phenyl ring protons next to the thiazole proton (Scheme 1). 1H NMR spectra of 7a-d showed three signals of pyrazoline as ABX plus 1H as a singlet signal assignable to the thiazole proton around δ 7.02-7.05 ppm.

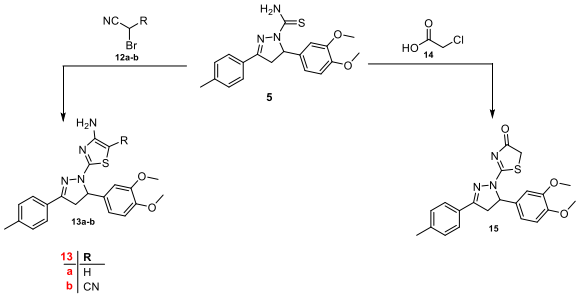


Scheme 2: Synthesis of thiazolyl-pyrazolines derivatives 9a-b and 11a-f

In Scheme 2, the thioamide 5 reacted with different substituted 3-chloropentane-2,4-diones 8a-c in absolute EtOH to afford the corresponding pyrazolyl thiazole derivatives 9a-c. IR spectra of 9a-c showed a band around at 1655-1680 cm-1 due to the presence of C=O group. In addition, IR spectrum of the compound 9c exhibited a band at 3360 cm-1 due to the presence of NH group. 1H NMR spectrum of 9a as an example, proved the disappearance of NH2 signal and appearance of three new singlet signals at δ 2.32, 2.35, and 2.36 ppm due to 3CH3 groups. 13C NMR spectrum of 9a proved the presence of three signals at δ 152.5, 159.2 and 180.1 ppm due to the presence of two C=N and C=O groups, respectively (Scheme 2). 1H NMR spectrum of 9c showed the presence of NH proton (D2O-exchangeable) as a singlet signal at δ 9.80 ppm, along with the signals of pyrazoline ABX protons.

Similarly, the thioamide 5 reacted with electrophilic reagent such as various hydrazonoyl chloride derivatives 10a-f and refluxed in EtOH to afford the corresponding pyrazolyl-phenyl diazenyl-thiazoles 11a-f. The reaction was carried out according to the Hantzsch mechanism to produce thiohydrazonates, followed by cyclized to produce cyclic hydroxy intermediates, followed by dehydrated to produce the final products 11a-f [37, 38]. IR spectra of the compounds 11a–f showed the disappearance of the bands corresponding to the NH2 group and exhibited a characteristic band at 1600-1610 cm-1 due to N=N group. 1H NMR spectra of 11a–f proved the aromatic integration was increased in the aromatic region assigned to the new phenyl ring protons plus three H left as ABX system of pyrazoline ring, in addition to two CH3 groups of phenyl and thiazole rings around at δ 2.31-2.34 and δ 2.46-2.48 ppm (Scheme 2). 1H NMR of the compounds 11e and 11f exhibited the presence of new signals at 2.33 and 3.82 ppm due to the new CH3 and OCH3 groups, respectively. An extra CH3 or OCH3 carbons at the phenyl-diazenyl ring were indicated in the 13C NMR spectra as singlet signals around at δ 21.1-21.4 and δ 56.1-56.3 ppm, respectively (Scheme 2).

In Scheme 3, the thioamide 5 reacted with 2-bromoacetonitrile (12a) or 2-bromomalonitrile (12b) and refluxed in EtOH to afford the corresponding 4-amino thiazole derivatives 13a-b. IR spectra of 13a-b exhibited the presence of NH2 group around at δ 3350-3360 cm-1, in addition to C≡N band for the compound 13b at 2230 cm-1. 1H NMR spectra of 13a-b showed NH2 group as a singlet signal around at δ 6.21-6.27 ppm, in addition to thiazole proton H5 as a singlet signal at δ 5.91 ppm for compound 13a. Additionally, 13C NMR for the compound 13b showed a signal at δ 113.5 ppm due to the presence of C≡N group.



Scheme 3: Synthesis of aminothiazole 13a-b and thiazolone derivatives 15

Finally, the thioamide 5 reacted with bromoacetic acid 14 to afford the pyrazolyl thiazolone derivative 15. IR spectrum of the thiazolone 15 exhibited C=O and C=N groups as characteristic bands at 1685 and 1625 cm-1, respectively. 1H NMR spectrum showed singlet signal at δ 4.25 due the CH2 protons of the thiazolone ring. 13C NMR spectrum of the thiazolone derivative 15 showed two signals at δ 38.9 and 177.5 ppm due to the CH2 and C=O carbons of the thiazolone ring, respectively.

3.2. Biological assessment

The SRB assay was employed to examine the cytotoxicity of the new 18 different compounds on three different solid tumour cell lines (MCF-7, HepG-2 and A549).

Three compounds, 7c, 9c and 11d, were found to be the most potent anticancer activity against the three cell lines. The compound 7c has IC50 values of 24.6, 18.5 and 34.2 µg/mL, respectively against the three cell lines. On the other hand, 9c is active against MCF-7 and HepG-2 only and produced IC50 values 329.5 and 4.6 µg/mL, respectively. Also, the compound 11d was active against all the tested cell lines and has IC50 values 15.4, 16.5 and 35.2 µg/mL, respectively. These three compounds 7c, 9c and 11d were considered as promising anticancer agents. On the other hand, the sex compounds 5, 7b, 9a, 11b, 11c, and 13b exhibited moderate cytotoxicity where they have IC50 values < 100 µg/mL as compared with doxorubicin as standard drug against the three cell lines (Table 1, Fig. 3-5). The rest of the compounds produced minor activity against all cell lines where they exhibited IC50 values > 100 µM.

The SAR studies showed the presence of electron-withdrawing groups such as Cl, Br, F, and NH-ph on the pyrazoline-thiazole scaffold enhance the compound's anticancer activity as compounds 7c, 9c, and 11d. As a result, the pyrazoline-thiazoles scaffold provides a promising framework for the screening of new anti-cancer medications.

Table 1: The anticancer IC50 values of synthesized compounds against 3 cell lines.

Compound	IC50 µM		
	MCF-7	**HepG-2**	**A549**
3	>100	>100	>100
5	69.5±2.8	>100	>100
7a	>100	>100	87.4±3.8
7b	81.4±3.2	78.2±2.9	>100
7c	24.6±2.2	18.5±1.5	34.2±2.6
7d	>100	>100	>100
9a	85.5±3.4	>100	>100
9b	>100	>100	>100
9c	29.5±1.8	34.6 ±2.1	>100
11a	>100	>100	>100
11b	48.2±2.5	>100	>100
11c	38.4±2.3	51.5±2.5	68.6±2.75
11d	15.4±1.6	16.5±0.8	35.2±2.25
11e	>100	>100	84.3±3.4
11f	>100	>100	>100
13a	>100	>100	>100
13b	75.6±2.8	59.7±2.4	65.5±3.2
15	>100	>100	>100
Doxorubicin	14.50	10.55	12

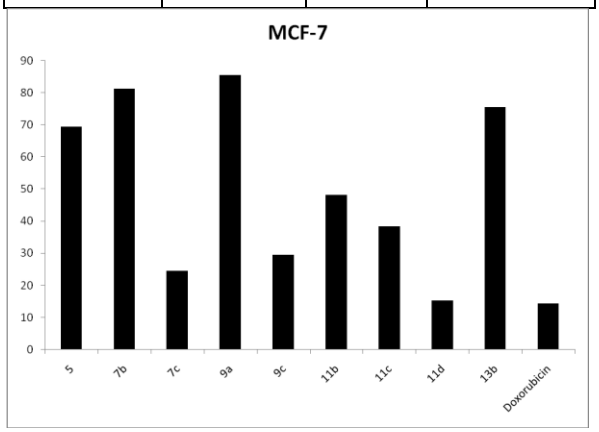


Fig. 3: Anticancer activity of the active compounds against MCF-7 cell line.

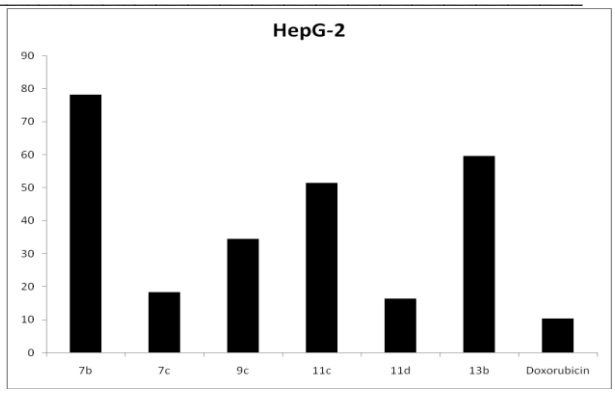


Fig. 4: Anticancer activity of the active compounds against HepG-2 cell line.

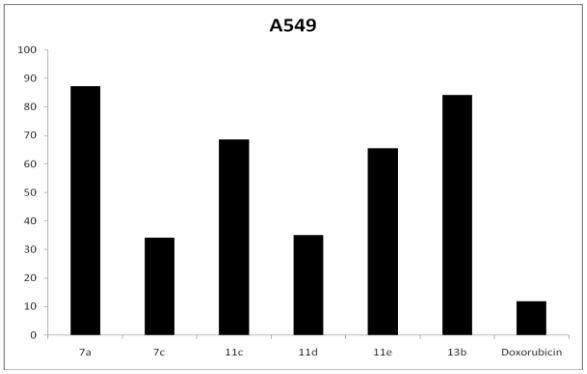


Fig. 5: Anticancer activity of the active compounds against A549 cell line.

4. Conclusion

Novel series of heterocyclic compounds bearing the pyrazoline-thiazole moieties were synthesized and assessment for their anti-cancer activity against three cell lines (MCF-7, HepG-2, and A549). The starting thioamide 5 play an important role in the synthesis of the various pyrazoline-thiazole scaffolds 7-15. Three compounds 7c, 9c, and 11d were showed the most promising anti-cancer activity against three cell lines.

5. Conflicts of interest

"There are no conflicts to declare".

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