Design, efficient synthesis and molecular docking of some novel thiazolyl-pyrazole derivatives as anticancer agents

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Abstract
Pyrazoles, thiazoles and fused thiazoles have been reported to possess many biological activities. 3-Methyl-5-oxo-4-(2-arylhydrazono)-4,5-dihydro-1H-pyrazole-1-carbothioamides 3a,b (obtained from the reaction of ethyl 3-oxo-2-(2-arylhydrazono)butanoates 1a,b with thiosemicarbazide) could be transformed into a variety of thiazolyl-pyrazole derivatives 6a–h, 10a–c, 15a–c, 17, 19 and 21 via their reaction with a diversity hydrazonoyl chlorides as well as bromoacetyl derivatives. Moreover, the computational studies were carried out for all new compounds. The results indicated that five compounds showed promising binding affinities (10a: −3.4 kcal/mol, 6d: −3.0 kcal/mol, 15a: −2.2 kcal/mol, 3a: −1.6 kcal/mol, and 21: −1.3 kcal/mol) against the active site of the epidermal growth factor receptor kinase (EGFR). The cytotoxicity of the potent products 3a, 6d, 10a, 15a, and 21 was examined against human liver carcinoma cell line (HepG-2) and revealed activities close to Doxorubicin standard drug. There was an understanding between the benefits of restricting affinities and the data obtained from the practical anticancer screening of the tested compounds.

Keywords: Coupling reaction, Cyclocondensation, Hydrazonoyl halides, Pyrazolones, Anticancer activity, Molecular docking

Introduction
The pyrazole nucleus has many biological activities, including several pharmaceuticals currently on the market. Moreover, pyrazole derivatives have found numerous applications in fluorescent substances, dyes, agrochemicals, and more. Therefore, the interest in pyrazole chemistry is still ongoing [1–6]. Thiazoles are additionally significant class of heterocyclic compounds, found in numerous powerful biologically active drugs such as Ritonavir (antiretroviral drug), Sulfathiazole (antimicrobial drug), Tiazofurin (antineoplastic drug), and Abafungin (antifungal drug) [7]. Compounds containing thiazole show many biological activities such as antihypertensive, antimicrobial and antifungal, anti-HIV, anticonvulsant and anti-inflammatory activities [8–12].

Thiazole derivatives are also known to possess several anticancer activities [13–15]. There are several mechanisms for the antitumor action of thiazole derivatives, acting on cancer biotargets, such as inosine monophosphate dehydrogenase (IMPDH) [16], tumor necrosis factor TNF-α [17] and apoptosis inducers. The biological profiles of these new generations of thiazole would represent a productive matrix for further advancement of better anticancer specialists. Drug design part guarantees that thiazole is best particle for the said target activity. Thiazoles have better action as an anticancer just as it demonstrates better binding domain and they have less cytotoxicity to normal cell (physiological cell) however alongside that it has site explicit movement to malignant growth cell (pathological cell). We can seek after the superior to best treatment for malignant growth
treatment since it limit side and unfriendly impact and it additionally indicates target oriented action.

Thiazolyl-pyrazole hybrids have displayed antitubercular [18, 19], anti-inflammatory, antimicrobial [20], antimycobacterial activities [21], and FabH inhibitors [22]. Moreover, thiazolyl-pyrazole compounds were identified as as potential anticancer agents [23, 24] (Fig. 1). The EGFR PTKs have been identified as interesting targets for medicinal chemistry programs especially in cancer therapy [25]. Compounds that inhibit the kinase activity of EGFR after binding its cognate ligand are of potential interest as new therapeutic antitumor agents [26]. Lv et al. [27] reported that thiazolyl-pyrazole analogues showed modest to potent EGFR TK inhibitory and potential anticancer activities. The molecular docking results indicated that thiazolyl-pyrazolines were nicely bound to the EGFR kinase. Over the most recent two decades we have been associated with a program planning to orchestrate practically substituted heterocyclic compounds with foreseen biological activities that can be utilized as biodegradable agrochemicals from shoddy research facility accessible beginning materials [28–37]. In the edge of this program, it appeared important to synthesize a new class of compounds amassing the thiazole and the pyrazole moieties in one entity that may result in upgraded biological activity because of the synergistic impact of the two rings. A β-Ketoester seemed suitable starting material to fulfill this objective (Fig. 1).

Results and discussion

Chemistry

In the present work we used ethyl acetoacetate. To avoid interference of the active methylene in the following reactions ethyl acetoacetate was coupled with aryl diazonium salts to afford the hydrazo derivatives 1a,b; which are the starting compounds for the synthesis of target thiazolyl-pyrazole derivatives. Thus, compounds 1a,b were allowed to react with thiosemicarbazide to afford 3-methyl-4-oxo-4-(2-arylhydrazono)-4,5-dihydro-1H-pyrazole-1-carbothioamides 3a,b presumably via the intermediacy of the thiosemicarbzones 2a,b which eliminate ethanol (Scheme 1).

The pyrazole derivatives 3a,b react with the hydrazonyl chlorides 4 to afford assumingly the thiohydrazonate intermediates 5a–h which lose water to afford the final isolable thiazolyl-pyrazole derivatives 6a–h. Spectral and analytical data of these compounds are in complete agreement with their proposed structures (cf. Scheme 1 and “Experimental”). A further evidence of the structure was deduced when the hydrazone 1a reacted with the thiazolyl hydrazine 7 to afford a product which was found to be typically identical to 6a.

The pyrazole derivative 3a reacts with the hydrazonyl chloride esters 8a–c to afford the thiazolyl- pyrazole derivatives 10a–c via the thiodyrazonate intermediates 9a–c respectively. Structures 10a–c are all supported by spectral and analytical data (cf. Scheme 2 and

Fig. 1 Anticancer activity of some reported thiazolyl-pyrazoles a–c and the target compounds. 
A further evidence of the structures 10a–c was deduced from alternative synthesis when the pyrazole 3a reacted with ethyl chloroacetate to afford the thiazolyl-pyrazole derivative 11 which couples with the benzene diazonium chloride 12 to afford products which were found to be typically identical to 10a. The identity was deduced from matching melting points, tlc and IR spectra.

The pyrazole derivative 3a reacts also with the bromoacetetyl derivatives 13a–c, 16, 18 and 20 to afford the corresponding thiazolyl-pyrazole derivatives 15a–c, 17, 19 and 21 respectively.

Spectral data and elemental analyses of these products are in complete concurrence with the proposed structures (cf. Scheme 3 and “Experimental”).

**Molecular docking studies**

The docking parameters were validated by redocking the native co-crystal ligand in order to determine the ability of MOE to reproduce the orientation and position of the native ligand in crystal structure, to ensure that the poses of the docked compounds represent a valid potential binding mode. The redocking of ligands with its targets revealed an RMSD ≤ 2.3 Å between the original ligand position and the docked poses which was RMSD = 1.3 Å this affirmed the ligands bound near the genuine pocket and adaptation of their objectives, this shows the dependability of conventions and parameters of docking. Molecular modeling studies were performed with MOE 2014, 0901, software available from Chemical Computing Group Inc., 1010...
Preparation of the ligand

The ligands (3a, 3b, 6a, 6b, 6c, 6d, 10a, 11, 15a, 15b, 15c, 17, 19, 21) coordinates were built and modeled using the Chemsketch software (http://www.acdlabs.com/resources/freeware/).

Next, the right atom types (including hybridization states) and right bond types were characterized, hydrogen atoms were added, charges were relegated to each atom, lastly the structures were vitality limited by utilizing MOE program (MMFF94x, gradient: 0.01) [39]. The energies of ligand structures were minimized using the semiempirical AM1 strategy [40] with MOE program.

Selection of protein crystal structures

Crystallographic structures of EGFR with its Ligand is available in the Protein DataBank [41]. In this study, EGFR kinase crystal structure 1M17 is tested and selected for docking [42]. The errors of the protein were revised by the structure arrangement process in MOE.
Reasonable protein structure is created by the task of hydrogen positions based on default rules. Water molecules contained have been expelled from the initially restored protein. Finally, partial charges were calculated by the Gasteiger methodology, and the active site of the ensemble has been characterized as the collection of residues within 10.0 Å of the bound inhibitor and comprised the union of all ligands of the ensemble. All atoms located less than 10.0 Å from any ligand atom were considered.

From the results obtained from docking studies as shown in Table 1, compounds 10a, 6d, 3a, 21, and 15a were the most favorable compounds which meant by its lower binding energy (Binding energy = −3.4, −3.0, −1.6, −1.3 and −2.2 kcal/mol, respectively), hydrogen bonding (number of H-bonds = 6, 3, 1, 2, and 3, respectively), and other hydrophobic interactions with the active site of the of EGFR kinase that might be one of the reasons for the good activities shown by these compounds in vitro studies (Figs. 2a, b, 3a, b, 4a, b, 5a, b, 6a, b).

**Anticancer evaluation**

The cytotoxicity of five of the synthesized products 3a, 6d, 10a, 15a and 21 was evaluated against human liver carcinoma cell line (HepG-2) using the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-tetrazolium bromide (MTT) assay and doxorubicin was used as a reference drug (IC$_{50}$ value of doxorubicin = 3.07 ± 0.27 μg/mL). Data generated were used to plot a dose response curve of which the concentration of test compounds required to kill 50% of cell population (IC$_{50}$) was determined. Cytotoxic activity was expressed as the mean IC$_{50}$ of three independent experiments. The results are represented in Table 2 and Figs. 7 and 8.

| Table 1 The interactions of the synthesized compounds with active sites of EGFR kinase |
|-----------------------------------------------|------------------------------|-----------------|-----------------|
| Compound | Binding energy (Kcal/mol) | No. of H-bonds | Length of H-bonds | Formed amino acids with H-bonds |
|-----------------------------------------------|------------------------------|-----------------|-----------------|
| Reference legend: erlotinib ((6,7-bis (2-methoxy-ethoxy) quinazoline-4-yl)-(3-ethynylphenyl)amine) | −3.3 | 3 | 3.15 Å | GLN 767 |
| 3a | −1.6 | 1 | 3.88 Å | CYS 773 |
| 3b | −0.3 | 1 | 4.26 Å | VAL 702 |
| 6a | −1.3 | 1 | 4.46 Å | LYS 721 |
| 6b | −1.3 | 1 | 4.83 Å | LYS 721 |
| 6c | −1.4 | 4 | 4.16 Å | MET 742 |
| 6d | −3.0 | 3 | 4.49 Å | ASP 776 |
| 10a | −3.4 | 6 | 3.50 Å | MET 769 |
| 11 | −1.1 | 3 | 4.05 Å | MET 742 |
| 15a | −2.2 | 3 | 4.51 Å | LYS 721 |
| 15b | −0.8 | 1 | 3.75 Å | GLY 772 |
| 15c | −1.1 | 1 | 4.32 Å | LYS 721 |
| 17 | −1.1 | 1 | 4.46 Å | LYS 721 |
| 19 | −1.3 | 2 | 3.60 Å | GLY 772 |
| 21 | −1.3 | 1 | 375 Å | LYS 721 |
Examination of the SAR leads to the following conclusions

- The descending order of activity of the new compounds was as follows: \(10a > 6d > 3a > 21 > 15a\).
- The phenylhydrazo-thiazolone \(10a\) showed higher antitumor inhibitory activities against HepG-2 cell lines (IC\(_{50}\) value of 2.20 ± 0.13 μg/mL) than the standard doxorubicin drug (IC\(_{50}\) value of 3.07 ± 0.27 μg/mL). Accordingly, compound \(10a\) might be considered as a promising scaffold anti-liver cancer chemotherapeutic and deserves further optimization and in-depth biological studies.
- The 4-pyridyl-thiazole derivative \(21\) has higher antitumor activity than the 4-phenyl-thiazole derivative \(15a\).
- Presence of two methyl groups (electron donating group) at position 3 and 5 of the phenyl ring in the arylhydrazo-pyrazolone moiety in compound \(6d\)
increases its activity than the un-substituted compounds 6a–c.

**Experimental Chemistry**

**General**

Melting points were recorded in open capillaries using an electrothermal Gallenkamp apparatus and are uncorrected. Elemental analyses were carried out by the microanalytical center at Cairo University. The $^1$H NMR spectra were recorded on a Varian Mercury VXR-300 spectrometer and the chemical shifts were related to that of the solvent DMSO-$_d_6$. The mass spectra were recorded on a GCMSQ1000-EX Shimadzu spectrometers. The IR spectra were measured on a Pye-Unicam SP300 instrument.

**Synthetic procedures**

*Synthesis of 3-methyl-5-oxo-4-(2-arylhydrazono)-4,5-dihydro-1H-pyrazole-1-carbothioamides 3a,b* A catalytic amount of HCl was added to a mixture of ethyl 3-oxo-2-(2-
arylhydrazono)butanoate derivatives 1a or 1b (10 mmol) and thiosemicarbazide (0.91 g, 10 mmol) in EtOH (40 mL) then the solution was refluxed for 6 h as indicated by TLC. The formed precipitate from reaction mixture was separated by filtration, washed with EtOH and recrystallized from dioxane to give pyrazole-1-carbothioamides 3a and 3b, respectively.

3-Methyl-5-oxo-4-(2-phenylhydrazono)-4,5-dihydro-1H-pyrazole-1-carbothioamide (3a) Orange solid, (78% yield), mp 230–232 °C (AcOH) (Lit. mp 224 °C [34]); IR (KBr) ν = 3429–3263 (NH2 and NH), 3070, 2940 (C–H), 1684 (C=O), 1590 (C=N) cm\(^{-1}\); \(^1\)H NMR (DMSO-\(d_6\)) δ: 2.25 (s, 3H, CH\(_3\)), 7.22–7.65 (m, 5H, Ar–H), 8.91 (br s, 1H, NH), 9.43 (br s, 1H, NH), 13.06 (br s, 1H, SH). Anal. Calcd. For C\(_{11}\)H\(_{11}\)N\(_5\)OS (261.30): C, 50.56; H, 4.24; N, 26.80. Found: C, 50.49; H, 4.13; N, 26.63%.

4-(2-(3,5-Dimethylphenyl)hydrazono)-3-methyl-5-oxo-4,5-dihydro-1H-pyrazole-1-carbothioamide (3b) Orange solid, (72% yield), mp 200–202 °C; IR (KBr) ν = 3423–3294 (NH2 and NH), 3093, 2970 (C–H), 1662 (C=O), 1607 (C=N) cm\(^{-1}\); \(^1\)H NMR (DMSO-\(d_6\)) δ: 2.32 (s, 3H, CH\(_3\)), 2.37 (s, 6H, 2CH\(_3\)), 6.70 (s, 1H, Ar–H), 6.95 (s, 1H, Ar–H), 7.06 (s, 1H, Ar–H), 7.28 (br s, 2H, NH\(_2\)), 12.87 (br s, 1H, NH); MS m/z (%): 289 (M\(^+\), 10), 230 (100), 214 (57), 140 (21), 105 (81), 91 (96), 77 (85). Anal. Calcd. For C\(_{13}\)H\(_{15}\)N\(_5\)OS (289.36): C, 53.96; H, 5.23; N, 24.20. Found: C, 53.74; H, 5.14; N, 24.06%.

**General method for synthesis of 5-(aryldiazenyl)thiazol-2-yl)-4-(2-phenylhydrazono)-1H-pyrazol-5(4H)-ones 6a-h and 5-oxo-4-(2-phenylhydrazono)-4,5-dihydro-1H-pyrazol-1-yl)-5-(2-phenylhydrazono)thiazol-5(4H)-one 11a–c** Triethylamine (0.1 g, 1 mmol) was added to a cold mixture of 4,5-dihydro-1H-pyrazole-1-carbothioamides 3a or 3b (1 mmol) and appropriate hydrazonoyl halides 4 or 8 (1 mmol) in dioxane (15 mL). The formed solution was refluxed until complete reaction (3–6 h as monitored by TLC). Methanol was added to the residue formed after removing the

### Table 2 IC\(_{50}\) values of tested compounds ± standard deviation against HepG-2

| Compound No. | IC\(_{50}\) (μg/mL) | Compound No. | IC\(_{50}\) (μg/mL) |
|--------------|-------------------|--------------|-------------------|
| Doxorubicin  | 3.07±0.27         | 10a          | 2.20±0.13         |
| 3a           | 4.39±0.47         | 15a          | 12.5±0.97         |
| 6d           | 3.90±0.41         | 21           | 4.80±0.56         |

![Fig. 6](image)

**Fig. 6** a 2D interaction between 21 and amino acids of EGFR kinase, b 3D model of hydrogen bond interaction of 21 with EGFR kinase

![Fig. 7](image)

**Fig. 7** Cytotoxic activities of tested compounds against HepG-2
excess solvent and the product separated was filtered, washed with methanol, dried and recrystallized from the proper solvent to give compounds 6a–h and 10a–c, respectively. The products 6a–h and 10a–c together with their physical constants are listed below.

3-Methyl-1-(4-methyl-5-(phenyldiazenyl)thiazol-2-yl)-4-(2-phenylhydrazono)-1H-pyrazol-5(4H)-one (6a) Orange solid, (72% yield), mp 215–217 °C (EtOH); IR (KBr) ν = 3428 (NH), 3050, 2980 (C–H), 1690 (C=O), 1597 (C=N) cm⁻¹; ¹H NMR (DMSO-d₆) δ: 2.25 (s, 3H, CH₃), 2.47 (s, 3H, CH₃), 7.10–7.75 (m, 10H, Ar–H), 11.69 (br s, 1H, NH); ¹³C-NMR (DMSO-d₆): δ 12.08, 15.13 (CH₃), 117.17, 121.41, 123.63, 126.70, 126.83, 126.8, 130.07, 132.53, 129.8, 134.0, 139.79, 150.16, 153.27, 157.16 (Ar–C and C=N), 177.39 (C=O); MS m/z (%): 403 (M⁺, 19), 330 (10), 202 (5), 180 (14), 118 (19), 104 (100), 91 (50), 77 (98), 65 (64). Anal. Calcd. For C₂₀H₁₇N₇OS (403.46): C, 59.54; H, 4.25; N, 24.30. Found: C, 59.49; H, 4.21; N, 24.18%.

3-Methyl-1-(4-methyl-5-(p-tolyldiazenyl)thiazol-2-yl)-4-(2-phenylhydrazono)-1H-pyrazol-5(4H)-one (6b) Orange solid, (76% yield), mp 208–210 °C (EtOH); IR (KBr) ν = 3429 (NH), 3066, 2925 (C–H), 1658 (C=O), 1599 (C=N) cm⁻¹; ¹H NMR (DMSO-d₆) δ: 2.13 (s, 3H, CH₃), 2.25 (s, 3H, CH₃), 2.47 (s, 3H, CH₃), 7.14–7.51 (m, 9H, Ar–H), 11.53 (br s, 1H, NH); MS m/z (%): 417 (M⁺, 3), 261 (34), 202 (67), 125 (94), 93 (61), 77 (43), 65 (100). Anal. Calcd. For C₂₁H₁₉N₇OS (417.49): C, 60.42; H, 4.59; N, 23.49. Found: C, 60.26; H, 4.39; N, 23.18%.

1-(5-(3-Chlorophenyl)diazenyl)-4-methylthiazol-2-yl)-3-methyl-4-(2-phenylhydrazono)-1H-pyrazol-5(4H)-one (6c) Orange solid, (76% yield), mp 218–220 °C (DMF); IR (KBr) ν = 3420 (NH), 3050, 2985 (C–H), 1669 (C=O), 1625 (C=N) cm⁻¹; ¹H NMR (DMSO-d₆) δ: 2.25 (s, 3H, CH₃), 2.48 (s, 3H, CH₃), 7.14–7.65 (m, 9H, Ar–H), 11.53 (br s, 1H, NH); MS m/z (%): 437 (M⁺, 3), 261 (34), 202 (67), 125 (94), 93 (61), 77 (43), 65 (100). Anal. Calcd. For C₂₀H₁₆ClN₇OS (437.91): C, 54.86; H, 3.68; N, 22.39. Found: C, 54.67; H, 3.49; N, 22.26%.

4-(2-(3,5-Dimethylphenyl)hydrazono)-3-methyl-1-(4-methyl-5-(phenyldiazenyl)thiazol-2-yl)-1H-pyrazol-5(4H)-one (6d) Yellow solid, (71% yield), mp 195–197 °C (EtOH); IR (KBr) ν = 3410 (NH), 3024, 2960 (C=O), 1607 (C=N) cm⁻¹; ¹H NMR (DMSO-d₆) δ: 2.32 (s, 3H, CH₃), 2.43 (s, 3H, CH₃), 6.85 (s, 1H, Ar–H), 6.92 (s, 1H, Ar–H), 7.06–7.28 (m, 6H, Ar–H), 11.50 (br s, 1H, NH); MS m/z (%): 431 (M⁺, 11), 430 (39), 325 (19), 230 (13), 105 (37), 91 (34), 77 (100), 67 (37). Anal. Calcd. For C₂₂H₂₁N₇OS (431.51): C, 61.23; H, 4.91; N, 22.72. Found C, 61.15; H, 4.75; N, 22.65%.

4-(2-(3,5-Dimethylphenyl)hydrazono)-3-methyl-1-(4-methyl-5-(p-tolyldiazenyl)thiazol-2-yl)-1H-pyrazol-5(4H)-one (6e) Yellow solid, (71% yield), mp 181–183 °C (EtOH); IR (KBr) ν = 3413 (NH), 3040, 2958 (C=O), 1663 (C=N) cm⁻¹; ¹H NMR (DMSO-d₆) δ: 2.35 (s, 3H, CH₃), 2.43 (s, 3H, CH₃), 2.50 (s, 3H, CH₃), 7.88–7.74 (m, 8H, Ar–H), 11.50 (br s, 1H, NH); MS m/z (%): 431 (M⁺, 11), 430 (39), 325 (19), 230 (13), 105 (37), 91 (34), 77 (100), 67 (37). Anal. Calcd. For C₂₂H₂₁N₇OS (431.51): C, 61.23; H, 4.91; N, 22.72. Found C, 61.15; H, 4.75; N, 22.65%. 

4-(2-(3,5-Dimethylphenyl)hydrazono)-3-methyl-1-(4-methyl-5-(p-tolyldiazenyl)thiazol-2-yl)-1H-pyrazol-5(4H)-one (6f) Yellow solid, (71% yield), mp 218–220 °C (EtOH); IR (KBr) ν = 3420 (NH), 3050, 2985 (C–H), 1669 (C=O), 1625 (C=N) cm⁻¹; ¹H NMR (DMSO-d₆) δ: 2.25 (s, 3H, CH₃), 2.48 (s, 3H, CH₃), 7.14–7.65 (m, 9H, Ar–H), 11.53 (br s, 1H, NH); MS m/z (%): 431 (M⁺, 11), 430 (39), 325 (19), 230 (13), 105 (37), 91 (34), 77 (100), 67 (37). Anal. Calcd. For C₂₂H₂₁N₇OS (431.51): C, 61.23; H, 4.91; N, 22.72. Found C, 61.15; H, 4.75; N, 22.65%.
Ar–H), 11.47 (br s, 1H, NH); MS m/z (%): 445 (M^+; 36), 340 (15), 230 (31), 121 (26), 105 (45), 91 (100), 77 (48). Anal. Calcd. For C_{23}H_{19}Cl_{2}N_{7}O_{2}S (500.40): C, 52.80; H, 3.81; N, 19.59. Found: C, 52.63; H, 3.81; N, 19.46%.

Alternative synthesis of 6a To a solution of ethyl 3-oxo-4-(2-phenylhydrazono)butanoate (4a) (0.234 g, 1 mmol) in 2-propanol (10 mL), 2-hydrazinyl-4-methyl-5-(phenyl diazenyl)thiazole (7) (0.233 g, 1 mmol) was added. The mixture was refluxed for 3 h then cooled to room temperature. The solid precipitated was filtered off, washed with water, dried and recrystallized from EtOH to give the corresponding product, 6a which were identical in all aspects (m.p., mixed m.p. and IR spectra) with those obtained from reaction of 3a with 4a.

2-(3-Methyl-5-oxo-4-(2-phenylhydrazono)-4,5-dihydro-1H-pyrazol-1-yl)-5-(2-(m-tolyl)hydrazono)thiazol-4(5H)-one (10b) Orange solid, (68% yield), mp 212–214 °C (EtOH); IR (KBr) ν = 3429, 2659 (NH), 3051, 2935 (C–H), 1682, 1660 (C=C), 1600 (C=N) cm^{-1}; ¹H NMR (DMSO-d_6) δ: 2.25 (s, 3H, CH₃), 2.47 (s, 3H, CH₃), 2.74–7.67 (m, 6H, Ar−H), 9.43 (br s, 1H, NH), 11.46 (br s, 1H, NH); MS m/z (%): 419 (M^+; 51), 261 (23), 202 (73), 125 (100), 93 (65), 65 (94), 51 (38). Anal. Calcd. For C_{20}H_{17}N_{5}O_{2}S (419.46): C, 57.27; H, 4.09; N, 23.37. Found: C, 57.09; H, 4.02; N, 23.22%.

Alternate method for 10a Synthesis of 2-(3-methyl-5-oxo-4-(2-phenylhydrazono)-4,5-dihydro-1H-pyrazol-1-yl)-5-(2-(m-tolyl)hydrazono)thiazol-4(5H)-one (11) To a mixture of pyrazole-1-carbothioamide 3a (2.61 g, 10 mmol) in ethanol (30 mL) containing anhydrous sodium acetate (3.3 g, 40 mmol), ethyl chloroacetate (1.22 g, 10 mmol) was added. The mixture was refluxed for 4–8 h (monitored by TLC), then left to cool. The solid product was filtered off, washed with ethanol and recrystallized from EtOH to afford the thiazolone derivative 11 as pale yellow solid (69% yield); mp 193–195 °C (EtOH); IR (KBr) ν = 3263 (NH), 3060, 2924 (C–H), 1688, 1659 (C=C), 1583 (C=N) cm^{-1}; ¹H NMR (DMSO-d_6) δ: 2.25 (s, 3H, CH₃), 4.14 (s, 2H, CH₂), 7.17–7.63 (m, 9H, Ar−H); MS m/z (%): 293 (M^+; 24), 133 (20), 125 (100), 93 (73), 65 (94), 43 (51). Anal. Calcd. For C_{19}H_{15}N_{5}O_{2}S (398.49): C, 56.48; H, 4.10; N, 23.22%.
$5H, Ar=H$), 9.45 (br s, 1H, NH); $^{13}$C-NMR (DMSO-$d_6$): δ 12.07 (CH$_3$), 31.52 (CH$_2$), 117.12, 126.67, 126.78, 130.03, 141.75, 150.14, 157.16 (Ar=C and C=N), 172.47, 177.40 (C=O); MS, m/z (%) 301 (M$^+$, 64), 261 (56), 202 (73), 125 (100), 93 (79), 77 (53), 65 (84). Anal. Calcd for C$_{13}$H$_{11}$N$_5$O$_2$S (301.32): C, 51.82; H, 3.68; N, 23.24. Found C, 51.71; H, 3.60; N, 23.08%.

**Coupling of thiazolone derivative** 11 **with benzenediazonium chloride** 12 A cold solution of benzenediazonium chloride 12 was added portionwise to a cold solution of 11 (0.301 g, 1 mmol) in pyridine (20 mL). After complete addition of the diazonium salt, the solid that separated was filtered off, washed with water and finally recrystallized from EtOH to give a product proved to be identical in all respects (IR spectra, mp and mixed mp) with compound 10a which was resulted from reaction of 3a with 8a.

**Synthesis of 3-methyl-4-(2-phenylhydrazono)-1-(4-aryl(hetaryl)thiazol-2-yl)-1H-pyrazol-5(4H)-ones 15a-c, 17, 19 and 21** General procedure: An ethanolic solution of 3-methyl-5-oxo-4-(2-phenylhydrazono)-4,5-dihydro-1H-pyrazole-1-carbothioamide (3a) (0.261 g, 1 mmol) and α-bromoacetyl derivatives 13a-c or 16 or 18 or 20 (1 mmol) was refluxed for 4–8 h, then left to cool. The solid product was filtered off, washed with ether and recrystallized from dioxane to afford the thiazole derivatives 15a-c, 17, 19 and 21, respectively.

**3-Methyl-4-(2-phenylhydrazono)-1-(4-phenylthiazol-2-yl)-1H-pyrazol-5(4H)-one (15a)** Orange solid (70% yield); mp 277–279 °C (DMF); IR (KBr): v/cm$^{-1}$ 3429 (NH), 3061, 2958 (C–H), 1685 (C=O), 1583 (C=N); $^1$H-NMR (DMSO-$d_6$): δ 2.44 (s, 3H, CH$_3$), 7.28 (s, 1H, thiazole H5), 7.30–7.51 (m, 10H, Ar–H), 9.06 (br s, 1H, NH); $^{13}$C-NMR (DMSO-$d_6$): δ 12.08 (CH$_3$), 111.75, 122.01, 126.68, 128.11, 129.11, 130.06, 134.50, 141.78, 148.00, 150.15, 152.04, 155.15, 157.16, (Ar=C and C=N), 171.40 (C=O); MS m/z (%): 361 (M$^+$, 31), 284 (24), 202 (89), 125 (100), 93 (83), 77 (62). Anal. calcd for C$_{19}$H$_{16}$N$_6$OS (376.43): C, 59.42; H, 3.69; N, 23.11%. Found: C, 63.55; H, 4.14; N, 22.16%.

1-(4-(4-Aminophenyl)thiazol-2-yl)-3-methyl-4-(2-phenylhydrazono)-1H-pyrazol-5(4H)-one (15c) Orange solid (78% yield); mp 215–217 °C (DMF/ETOH); IR (KBr): v/cm$^{-1}$ 3428 (NH), 3069, 2959 (C–H), 1686 (C=O), 1582 (C=N); $^1$H-NMR (DMSO-$d_6$): δ 2.43 (s, 3H, CH$_3$), 7.28 (s, 1H, thiazole H5), 7.39 (d, J = 8.4 Hz, 2H, Ar–H), 7.47–7.49 (m, 5H, Ar–H), 7.92 (d, J = 8.4 Hz, 2H, Ar–H), 9.05 (br s, 1H, NH); MS m/z (%): 395 (M$^+$, 80), 318 (66), 261 (52), 202 (57), 125 (76), 93 (41), 77 (58), 67 (100). Anal. calcd for C$_{19}$H$_{18}$N$_6$OS (395.87): C, 57.65; H, 3.56; N, 17.69. Found: C, 57.39; H, 3.38; N, 17.60%.

**3-Methyl-1-(4-(2-oxo-2H-chromen-3-yl)thiazol-2-yl)-4-(2-phenylhydrazono)-1H-pyrazol-5(4H)-one (17)** Orange solid (75% yield); mp 239–241 °C (DMF); IR (KBr): v/cm$^{-1}$ 3412 (NH), 3054, 2920 (C–H), 1715, 1689 (C=C), 1596 (C=N); $^1$H-NMR (DMSO-$d_6$): δ 2.42 (s, 3H, CH$_3$), 7.28 (s, 1H, thiazole H5), 7.30–7.69 (m, 9H, Ar–H), 8.35 (s, 1H, Coumarine H4), 8.87 (br s, 1H, NH); MS m/z (%): 429 (M$^+$, 100), 401 (27), 352 (53), 271 (19), 255 (50), 171 (9), 77 (37), 67 (80). Anal. calcd for C$_{23}$H$_{15}$N$_5$O$_5$S (492.45): C, 61.53; H, 3.52; N, 16.31. Found: C, 61.42; H, 3.37; N, 16.22%.

**3-Methyl-1-(4-(3-oxo-3H-benzo[f]chromen-2-yl)thiazol -2-yl)-4-(2-phenylhydrazono)-1H-pyrazol-5(4H)-one (19)** Orange solid (78% yield); mp 280–282 °C (DMF); IR (KBr): v/cm$^{-1}$ 3429 (NH), 3057, 2957 (C–H), 1722, 1675 (C=O), 1629 (C=N); $^1$H-NMR (DMSO-$d_6$): δ 2.41 (s, 3H, CH$_3$), 7.28 (s, 1H, thiazole H5), 7.46–7.50 (m, 6H, Ar–H), 7.61 (d, J = 7.2 Hz, 1H, Ar–H), 7.75 (t, J = 8.00 Hz, 1H, Ar–H), 7.92 (d, J = 5.6 Hz, 1H, Ar–H), 8.00 (d, J = 8.00 Hz, 1H, Ar–H), 8.42 (s, 1H, Coumarine H4), 8.61 (d, J = 8.4 Hz, 1H, Ar–H), 9.06 (br s, 1H, NH); MS m/z (%): 479 (M$^+$, 100), 451 (27), 305 (48, 222 (9), 139 (7), 77 (39), 67 (80). Anal. calcd for C$_{26}$H$_{17}$N$_5$O$_5$S (475.51): C, 65.12; H, 3.57; N, 14.61. Found: C, 65.02; H, 3.41; N, 14.39%.

**3-Methyl-4-(2-phenylhydrazono)-1-(4-(pyridin-2-yl)thiazol-2-yl)-1H-pyrazol-5(4H)-one (21)** Orange solid (73% yield); mp 240–242 °C (DMF); IR (KBr): v/cm$^{-1}$ 3428 (NH), 3069, 2959 (C–H), 1686 (C=O), 1627 (C=N); $^1$H-NMR (DMSO-$d_6$): δ 2.42 (s, 3H, CH$_3$), 7.28 (s, 1H, thiazole H5), 7.30–7.78 (m, 7H, Ar–H), 8.40 (d, 1H, Ar–H), 8.72 (d, 1H, Ar–H), 9.06 (br s, 1H, NH); MS m/z (%): 362 (M$^+$, 8), 261 (51), 202 (77), 125 (100), 93 (78), 77 (45), 65 (19). Anal. calcd for C$_{19}$H$_{18}$N$_6$OS (362.41): C, 59.65; H, 3.89; N, 23.19. Found: C, 59.42; H, 3.69; N, 23.11%.
Molecular modeling
Docking Study was performed using the MOE 2014.09 software. Regularization and optimization for protein and ligand were performed. Each docked compound was assigned a score according to its fit in the ligand binding pocket (LBP) and its binding mod.

Cytotoxic activity
In this study, the newly synthesized compounds were subjected to cytotoxic evaluation on human tumour cell line [43].

Materials and methods
Chemicals All chemicals used in this study are of high analytical grade. They were obtained from (either Sigma-Alderich or Biorad).

Human tumor cell lines The tumour cell lines were obtained frozen in liquid nitrogen (−180 °C) from the American Type Culture Collection (ATCC) and was maintained at the National Cancer Institute, Cairo, Egypt, by serial subculturing.

Measurement of potential cytotoxic activity The cytotoxic activity was measured in vitro on human cancer cell line (HEPG2) using Sulforhodamine-B stain (SRB) assay.

Cells were plated in 96 multi well plates for 24 h before treatment with the compounds to allow attachment of the cells to the wall of the plate.

- Different concentrations of the compound under test (0, 6.25, 12.5, 25, 50 and 100 µg/mL) were added to the cell monolayer. Triplicate wells were prepared for each individual dose.
- Monolayer cells were incubated with the compounds for 48 h at 37 °C and in atmosphere of 5% CO₂.
- After 48 h cell was fixed, washed and stained with Sulforhodamine B stain.
- Excess stain was washed with acetic acid and attached stain was recovered with Tris EDTA buffer.
- Colour intensity was measured in an ELISA reader.
- The relation between surviving fraction and drug concentration was plotted and IC₅₀ (the concentration required for 50% inhibition of cell viability) was calculated for each compound by Sigmaplot software.

Conclusions
We portrayed a convenient and efficient synthesis of numerous diversely substituted thiazolyl-pyrazole derivatives from cheap laboratory accessible starting materials. Since a variety of thiazolyl-pyrazole derivatives 6a–h, 10a–c, 15a–c, 17, 19 and 21 were synthesized from reaction of 3-methyl-5-oxo-4-(2-arylhdyrazono)-4,5-dihydro-1H-pyrazole-1-carbothioamides 3a,b with a diversity hydrazonyl chlorides as well as bromoacetyl derivatives. Simple synthetic routes were pursued and no risky solvents, catalysts or substantial metals were included. Moreover, the computational studies were carried out for all the products and the results revealed that four new compounds showed promising binding affinities against EGFR. The cytotoxicity of the potent products was tested against HepG-2 using Doxorubicin standard drug. There was an agreement between the benefits of binding affinities and the data obtained from the practical anticancer screening of the tested compounds.

Abbreviations
TEA: methylamine; TLC: thin layer chromatography; HepG2: human hepatocellular carcinoma; EOCH: ethanol; m.p.: melting point; IR: infra-red; ATCC: American Type Culture Collection; EGFR: the epidermal growth factor receptor kinase.

Authors’ contributions
SMG and FMA designed research; SMG and MSF performed research; All authors wrote the final version of the manuscript. All authors read and approved the final manuscript.

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Availability of data and materials
The datasets and samples of the compounds used during the current study are available from the corresponding author on reasonable request.

Competing interests
The authors declare that they have no competing interests.

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