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Original article

Synthesis of some new pyrazole-based 1,3-thiazoles and 1,3,4-thiadiazoles as anticancer agents

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ABSTRACT

N-(4-(Pyrazol-4-yl)thiazol-2-yl)-*N*′-phenylthiourea derivative **2** was synthesized and then treated with variety of hydrazonoyl chlorides under basic condition at reflux to afford the corresponding 2-(4-(pyrazol-4-yl)thiazol-2-ylimino)-1,3,4-thiadiazole derivatives **6**, **10a**–**e** and **17a**–**e**. Reaction of 2 with ethyl chloroacetate and with 3-chloro-2,4-pentanedione gave the thiazolidin-4-one **22** and 1,3-thiazole **25** derivatives, respectively. Condensation of thiazolidin-4-one **22** with aldehydes gave their 5-arylidene derivatives **23a**–**f**. Most of the synthesized compounds were tested for anticancer activity against human hepatocelluar carcinoma HepG2, human breast cancer MCF-7 and human lung cancer A549. Their SAR was studied and variously affected by the electronic factor of electron donating and withdrawing groups. Many of the tested compounds showed moderate to high anticancer activity.

1. Introduction

Pyrazole derivatives have been receiving several applications in the field of medicinal chemistry and pharmaceuticals. They have potent anticancer [1–6], antibacterial [7], and fungistatic [8] activities. In addition, 1,3,4-thiadiazoles were recently reported by us and others as highly anti-inflammatory [9–11], and anticonvulsant [9,11] as well as anticancer agents [12], reporting their inhibitory profile against human hepatocelluar carcinoma cell line, HepG2 [12].

Furthermore, several 1,3-thiazole scaffolds have been reported as potent anticancer agents (Fig. 1). For example, 1,3-thiazole structures S3U937 (I) [13] and S8A375 (II) [14] exhibited potential anticancer activity against various cancer types [15]. An excellent effectiveness of the pyrazolylthiazole derivative N2 (III) in fighting cancer was also reported, *via* EGFR TK inhibition that plays an important role in cell growth regulation [8]. In addition, thiazolidin-4-ones are important class of heterocyclic compounds that possess various types of biological activities [16] Therefore, thiazolidin-4-ones are well established to have antitubercular [17,18], anticancer [19–22], anti-inflammtory [23,24] and anticonvulsant [25,26] activities. Interestingly, the potential anticancer activity of a lead compound, 4-thiazolidinone derivative (IV) (Fig. 1) was appreciated in (NCI) Bethesda, USA exploring its ability to fight different types of cancer cell lines *via* JSP-1 inhibition [27].

In the light of the above findings and in continuation of authors' efforts to synthesize new anticancer agents [1,28–30], this work considered the putative scaffold thiazolylpyrazole. This scaffold was attached by different isosteric moieties aiming for synthesis of some novel azole derivatives of potential anticancer activity.

2. Results and discussion

2.1. Chemistry

4-(2-Aminothiazol-4-yl)-2,3-dimethyl-1-phenylpyrazol-5-one (**1**) was prepared following the literature procedure [31]. Reaction of 4-(2-aminothiazol-4-yl)pyrazol-5-one derivative **1** with phenylisothiocyanate in dimethylformamide, in the presence of potassium hydroxide, at room temperature afforded 1-(4-(2,5-dihydro-2,3-dimethyl-5-oxo-1-phenyl-1*H*-pyrazol-4-yl)thiazol-2-yl)-3-phenylthiourea (**2**) in 67% yield (Scheme 1). The IR spectrum of compound **2** revealed two bands at 3456, 3172 and 1628 cm^{-1}

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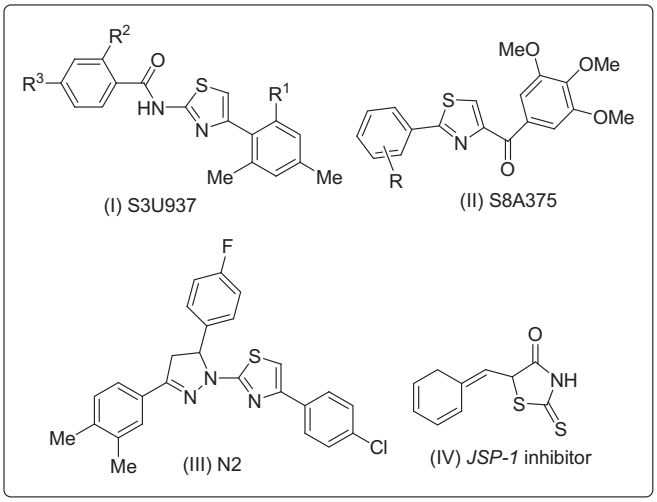


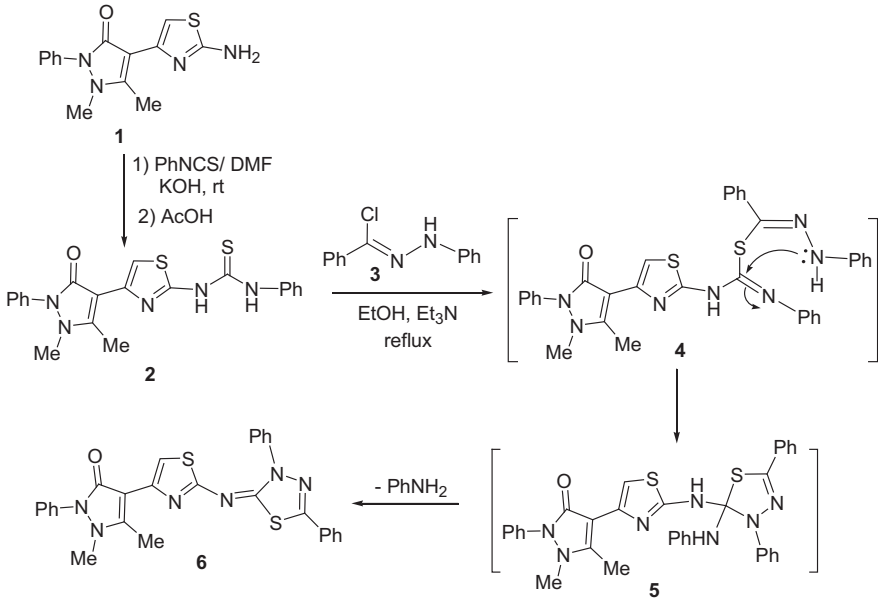
Fig. 1. Lead compounds among thiazole derivatives with anticancer activity.

assignable to two NH and C=O functions, respectively. Its ^{1}H NMR spectrum displayed two singlet signals at δ 2.50 and 3.50 corresponding to two methyl protons.

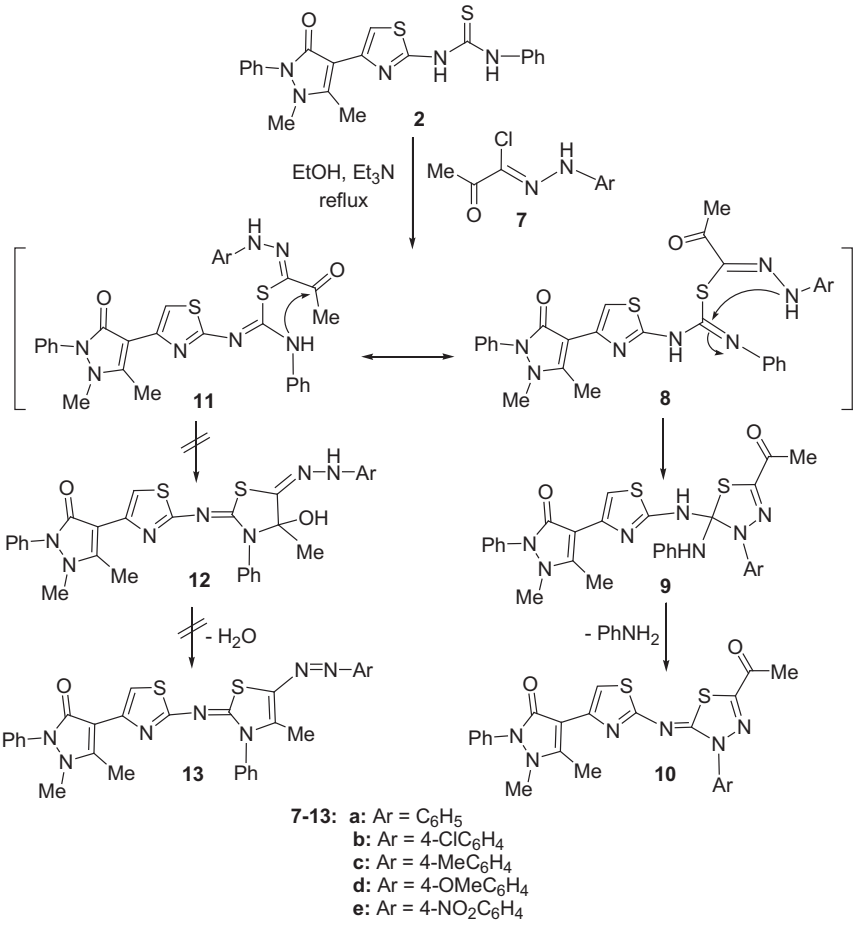
Next, the reactivity of the disubstituted thiourea derivative **2** with various types of hydrazonoyl chlorides was evaluated. Thus, treatment of the disubstituted thiourea derivative **2** with the hydrazonoyl chloride **3** in refluxing ethanol in the presence of triethylamine afforded only one isolable product. Elemental analyses and spectral data (IR, MS, ^{1}H and ^{13}C NMR spectra) confirmed the reaction product as 3,5-diphenyl-2-(4-(2,3-dimethyl-1-phenyl-5-oxo-pyrazol-4-yl)thiazol-2-ylimino)-1,3,4-thiadiazole (**6**) as shown in Scheme 1. Formation of **6** proceeded *via* loss of HCl followed by loss of aniline molecules from the intermediates **4** and **5**, respectively. The IR spectrum of compound **6** was free of any band due to NH function and its ^{1}H NMR spectrum revealed the presence of three singlet signals at δ 2.53, 3.53 and 7.24 assignable to two methyl and thiazole-5-CH protons, respectively. In addition, the ^{13}C NMR spectrum of **6** has 22 carbon-signals and its MS spectrum showed a peak at *m/z* 522 corresponding to its molecular ion (M^+).

In a similar manner, when the thiazolylthiourea derivative **2** was allowed to react with the acetyl hydrazonoyl chlorides **7a-e** in refluxing ethanol in the presence of triethylamine, it afforded in each case a single isolable product. There are two expected cyclization routes leading to either the 1,3,4-thiadiazolyl structure **10** or 1,3-thiazolyl structure **13** can be suggested for the reaction product *via* loss of either aniline or water molecules from the intermediates **8** and **11**, respectively, as outlined in Scheme 2. However in all cases, the reaction proceeded *via* loss of aniline similar to the mechanism shown in Scheme 1, and the reaction product was proved, in each case, to be 1,3,4-thiadiazole **10,** where the spectral data (IR, MS, ^{1}H NMR and ^{13}C NMR spectra) were completely agree with structure **10** and not **13**. Mass spectrum of the reaction products **10a-e** showed, in each case, a peak corresponding to their molecular ions. Furthermore, ^{1}H NMR spectrum of **10a**, for example, exhibited three characteristic singlet signals at 2.51, 2.76 and 3.50 assignable to CH_3, $COCH_3$ and NCH_3 protons, respectively, in addition to an aromatic multiplet in the region 7.68–7.32. The ^{13}C NMR spectrum of **10a** displayed three aliphatic carbon peaks at 9.1, 22.9 and 31.7 in addition to a characteristic acetyl (C=O) peak at 191.7 among a total 20 carbon-signals.

To generalize the above findings, the reactivity of the thiazolythiourea derivative **2** with several ester hydrazonoyl chlorides **14a–e** was also conducted. At first, treatment of compound **2** with *C*-ethoxycarbonyl *N*-phenylhydrazonoyl chloride **14a** in absolute ethanol, in the presence of a catalytic amount of triethylamine, at reflux afforded a single product for which structures **17a** and **20a** can be postulated (Scheme 3). It was expected that loss of ethanol from the assumed intermediate **18a** may be easier than the loss of aniline from **15a**, however the spectroscopic analyses of the isolated product ascertained its structure as ethyl 3-phenyl-2-(4-(2,3-dimethyl-1-phenyl-5-oxo-pyrazol-4-yl)thiazol-2-ylimino)-1,3,4-thiadiazole-5-carboxylate (**17a**) (Scheme 3). As can be seen in the experimental part, the IR, ^{1}H NMR and ^{13}C NMR spectra of **17a** showed the presence of the ester $CO_2CH_2CH_3$ group and disappearance of the hydrazone-NH function ruling out the other thiazolidin-4-one structure **20a**. The same results were obtained when the thiazolythiourea derivative **2** was treated with further ester



Scheme 1. Synthesis of 4-(pyrazol-4-yl)thiazol-2-ylimino-1,3,4-thiadiazole derivative **6**.



Scheme 2. Synthesis of 5-acetyl-4-(pyrazol-4-yl)thiazol-2-ylimino-1,3,4-thiadiazoles **10a**–**e**.

hydrazonoyl chlorides **14b**–**e** under similar experimental conditions resulting in the formation of 1,3,4-thiadiazole-2-carboxylate esters **17b**–**e** and there was no evidence for the formation of the thiazolidin-4-one structures **20b**–**e** (Scheme 3).

Then, reaction of the thiazolylthiourea derivative **2** with ethyl chloroacetate in refluxing ethanol in the presence of triethylamine furnished a single product that was identified as 2-(4-(2,3-dimethyl-1-phenyl-5-oxo-pyrazol-4-yl)thiazol-2-ylimino)-3-phenyl-1,3-thiazolidin-4-one (**22**) *via* intramolecular cyclization with loss of ethanol molecule from the intermediate **21** (Scheme 4). The elemental analyses and spectral data (IR, MS, ^{1}H NMR and ^{13}C NMR) of the reaction product were in consistence with the assigned structure. Thereafter, the thiazolidin-4-one derivative **22** underwent a condensation reaction when treated with aromatic aldehydes in refluxing ethanol in the presence of catalytic amount of piperidine to furnish the corresponding 5-benzylidene-thiazolidin-4-one derivatives **23a**–**e** (Scheme 4). The structures of the reaction products were confirmed from their elemental and spectral data. For example, the NMR spectra of **23a**–**e** were free of the aliphatic 5-CH_2 protons and carbons of the starting substrate **22** at δ 4.46 (^{1}H NMR) and at δ 34.3 (^{13}C NMR), respectively, and revealed instead singlet signals around δ 8.2 (^{1}H NMR) and around δ 140 (^{13}C NMR) due to the methine =*CH* proton and carbons, respectively. The exocyclic C=CH bond in **23a**–**e** was assigned as *Z*-configuration on the basis of ^{1}H NMR spectroscopy where the methine proton, deshielded by the adjacent C=O, was observed around δ 8.2 which is close to analogous (*Z*)-5-arylidene-thiazolidin-4-ones [32,33]. However, in the *E*-configuration such proton resonates at lower chemical shift values ($\delta < 7.5$) [34].

Reaction of the thiazolylthiourea derivative **2** with 3-chloropentane-2,4-dione in refluxing ethanol in the presence of triethylamine resulted in the formation of 2-(thiazolylimino)thiazole derivative **25** (Scheme 4). Formation of **25** proceeded *via* intramolecular cyclization with loss of water from the intermediate **24**. The structure of the product **25** was established on the basis of its elemental analysis and spectral data (see Experimental part).

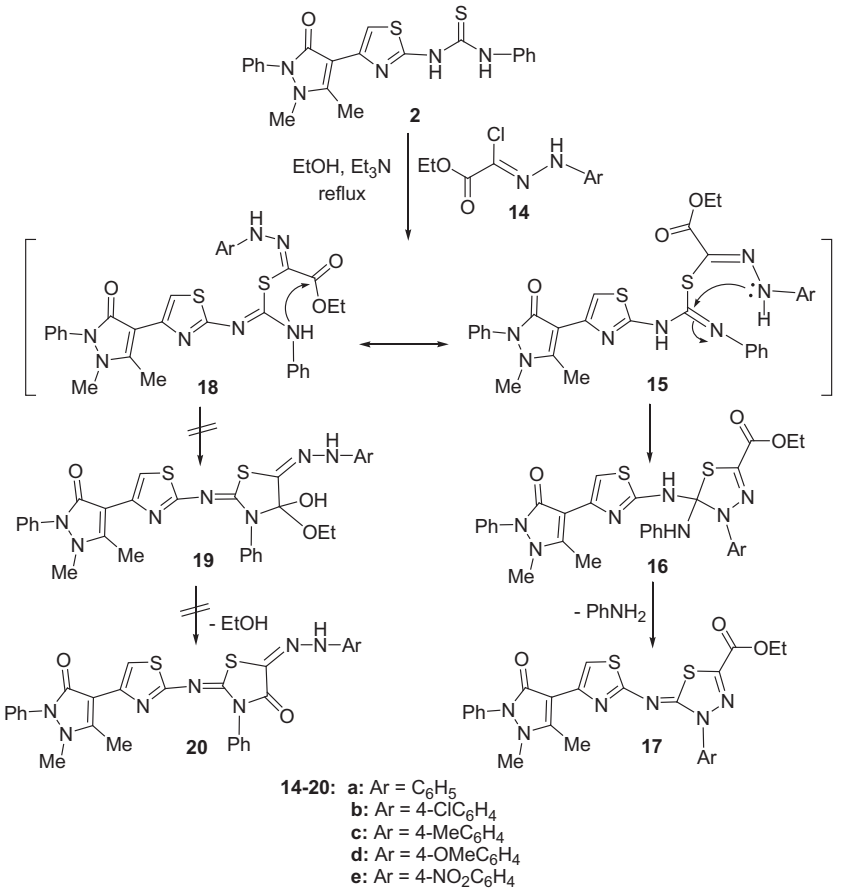
2.2. Pharmacology

2.2.1. Antitumour activity

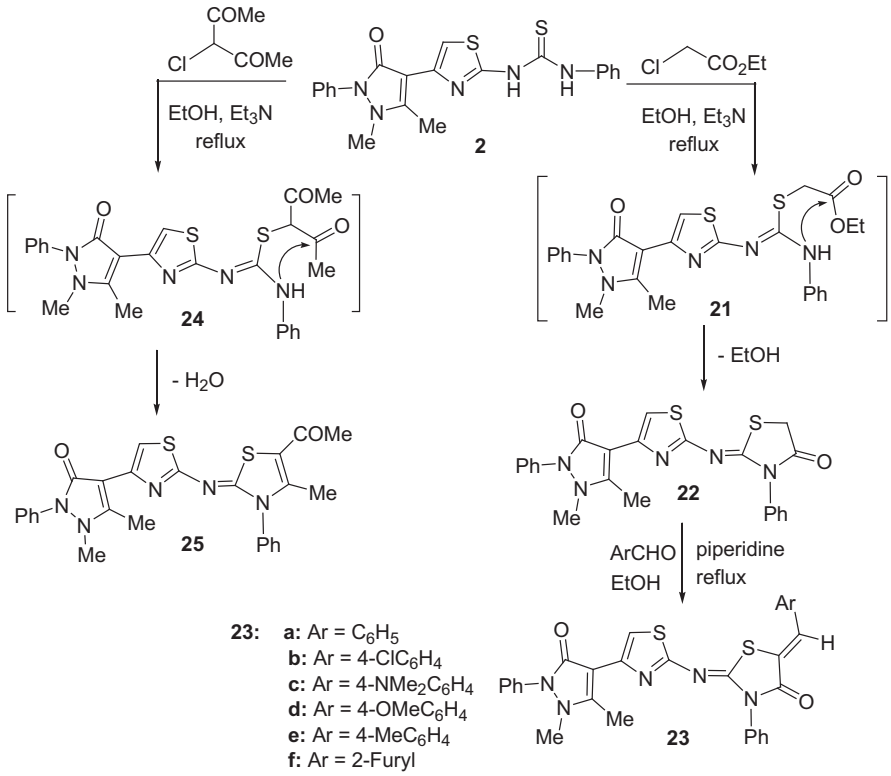
The in-vitro anti-tumour activity of the test compounds was achieved in the cell culture Lab, college of pharmacy, Ain Shams University, Cairo, Egypt. Compounds **2**, **6**, **10a**–**e**, **17a**–**e**, **22** and **23a**–**f** were tested for their in-vitro antitumour activity against human hepatocellular carcinoma cell (HepG2), human breast cancer cells (MCF-7) and human lung cancer cells (A549). Doxorubicin was used as a reference standard and showed IC_{50} 0.877, 1.172 and 0.411 μM against cancer cell lines, respectively.

The anticancer HepG2 profile suggested that, test compounds showed a great variable activity compared to reference drug as shown in Table 1. Among the test compounds, compounds **2** and **17e** were the most potent pyrazole derivatives, displaying IC_{50} values of 8.438 and 8.107 uM respectively compared to reference standard.

Concomitantly, thiazolylpyrazole derivatives **17d** and **23d** showed equipotent anticancer activity against HepG2 of IC_{50} values 12.91 and 13.17 uM, respectively. Also, compound **23f** exerted appreciated activity of IC_{50} 16.65 uM. Further, **10c** and **10d**



Scheme 3. Synthesis of ethyl 4-(pyrazol-4-yl)thiazol-2-ylimino-1,3,4-thiadiazole-5-carboxylates **17a**–**e**.



Scheme 4. Synthesis of 4-(pyrazol-4-yl)thiazol-2-ylimino-1,3-thiazolidines **22**, **23**, **25**.

Table 1
IC_{50} of the test set of compounds against human hepatocellular carcinoma cell line HepG2, human breast cancer cells MCF-7 and human lung cancer cells A549.

	IC_{50} (μM) HepG2 cell line	IC_{50} (μM) MCF-7 cell line	IC_{50} (μM) A549 cell line
Compound No. Series A			
6	>100	>100	>100
10a	67.11	>100	>100
10b	>100	>100	>100
10c	31.66	56.83	20.74
10d	41.25	64.50	26.51
10e	>100	>100	>100
17a	>100	>100	>100
17b	>100	>100	>100
17c	>100	>100	>100
17d	12.91	15.88	13.06
17e	8.107	10.03	8.68
Compound No. Series B			
22	70.49	>100	>100
23a	63.42	>100	>100
23b	>100	>100	>100
23c	>100	>100	>100
23d	13.17	26.08	9.10
23e	>100	>100	>100
23f	16.65	22.35	13.67
SP 2[a]	8.348	10.08	7.743
Doxorubicin	0.877	1.172	0.411

[a] SP: starting precursor.

displayed pronounced activity, affording IC_{50} 31.66 and 41.25 uM respectively. On the other hand, pyrazole derivatives **10a**, **22** and **23a** showed mild activity against HepG2 (IC_{50}; 67.11, 70.49 and 63.42 uM, respectively). Finally, human hepatocellular carcinoma cells, HepG2 showed resistance for the rest of the test compounds. Interestingly, compounds **2**, **17d**, **17e** and **23d** displayed the most pronounced activity against HepG2 cell line compared to doxorubicin (Figs. 3 and 4).

On the other hand, MCF-7 profile as shown in Table 1, explored seven azole derivatives **2**, **10c**, **10d**, **17d**, **17e**, **23d** and **23f** with considering anticancer activity compared to Doxorubicin. Noticeably, compounds **2** and **17e** were the most potent derivatives, exerting IC_{50} values of 10.08 and 10.03 uM, respectively. Similarly, the thiazolylpyrazole derivatives **17d**, **23d** and **23f** exerted appreciated activity of IC_{50} 15.88, 26.08 and 22.35 uM, respectively. On the other hand, the azole derivatives **10c** and **10d** showed mild activity against MCF-7 (IC_{50} 56.83 and 64.50 uM, respectively). In addition, MCF-7 cell lines showed high resistance to the rest of the test compounds. Compounds **2** and **17e** displayed the most pronounced activity against MCF-7 cell line compared to doxorubicin (Fig. 5).

Considering the human lung cancer cell line (A549), the seven azole derivatives **2**, **10c**, **10d**, **17d**, **17e**, **23d** and **23f** displayed a remarkable activity compared to Doxorubicin as shown in Table 1. Interestingly, compounds **2**, **17e** and **23d** were the most potent anticancer agents with IC_{50} values of 7.743, 8.68 and 9.10 uM, respectively. Concomitantly, the derivatives **17d** and **23f** showed almost equipotent activity of IC_{50} values, 13.06 and 13.67 uM, respectively. Finally, the azole derivatives **10c** and **10d** showed appreciated anticancer activity of IC_{50} 20.74 and 26.51 uM, respectively compared to the reference standard. However, A549 cell line showed high resistance to the rest of the test compounds. Compounds **2**, **17e** and **23d** displayed the most pronounced activity against A549 cell line compared to doxorubicin (Fig. 6).

The antitumour activity of the test compounds **2**, **6**, **10a**–**e**, **17a**–**e**, **22** and **23a**–**f** against HepG2, MCF7 and A549 cell lines was attributed to two series, *series A* and *series B*. In both series, imine linker connected the putative scaffold thiazolylpyrazole to thiadiazole ring (*Series A*) or thiazolidine ring (*Series B*) (Fig. 2). Herein, the structure similarity of *Series A* and *B*, was considered focussing on isosteric relations between thiadiazole and thiazolidine rings, respectively [35,36]. In *Series A*, compounds **6**, **10a**–**e** and **17a**–**e**, the heterocyclic thiadiazole moiety was substituted at N3 and C5 by alternating aryl and alkyl groups. Similarly, in *Series B*, compounds **2**, **22** and **23a**–**f** the isosteric heterocyclic thiazolidin-4-one ring was also substituted at N3 and C5.

2.2.2. Structure activity relationship (SAR)

Concerning *Series A*, compounds **10a**–**e** that carry acetyl group at C5 concomitantly with different aryl moieties at N3, alternative SAR was explored. At first, SAR was exemplified by substituting N3 phenyl moiety of **10a** (IC_{50} 67.11 uM) by electron donating group e.g. *p*-CH_3 and *p*-OCH_3 groups affording **10c** and **10d** analogues (IC_{50} 31.66 and 41.25 uM, respectively), this substitution effectively increased the anti-cancer activity against HepG2. On the other hand, substituting N3 phenyl moiety of **10a** by electron withdrawing group e.g. *p*-Cl-phenyl and *p*-NO_2-phenyl groups affording compounds **10b** and **10e**, respectively, diminished the activity (IC_{50} > 100 uM). Simultaneously, C5-acetyl group of **10a** was replaced by phenyl moiety, to afford 3,5-diphenyl analogue **6** (IC_{50} > 100 uM). This replacement produced negative impact on the anti-cancer activity.

Secondly, SAR was explored in structural replacement between **10c**–**e** and **17c**–**e** analogues where the acetyl group at C5 of thiadiazole ring in **10c**–**e** was replaced by ethoxycarbonyl moiety in **17c-e**. Among the latter analogues, **17e** (IC_{50} 8.107 uM) that has *p*-NO_2 phenyl moiety at N3, exerted the most potent anticancer activity of all tested compounds. In contrary, replacing N3 *p*-NO_2 phenyl moiety with electron donating group *p*-OCH_3 phenyl, in **17d**, decreased the activity (IC_{50} 12.91 uM). Further, the C5 ethoxycarbonyl group when coupled with *p*-CH_3 phenyl moiety at N3, producing **17c**, the activity have been almost abolished (IC_{50} > 100 uM).

Compatible anticancer activities against human breast cancer cells MCF-7 were afforded by *Series A* (compounds **6**, **10a**–**e** and **17a**–**e**). Focussing on the derivatives displayed remarkable activity, one can find that coexistence of *p*-electron donating substituted phenyl groups at N3 with acetyl group at C5 of **10c** and **10d** analogues (IC_{50} 56.83 and 64.50 uM, respectively), positively enhanced the activity against MCF-7 cell line. This is obvious upon comparing compounds **10c** and **10d** either with N3 phenyl analogue **10a** or with *p*-electron withdrawing phenyl analogues **10b** and **10e**

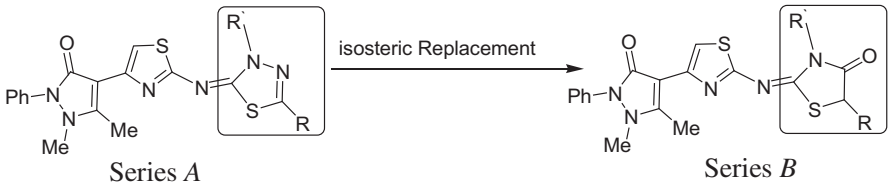


Fig. 2. Design of the new azoles based on isosteric relation between *series A* and *series B* using thiazolylpyrazole scaffold.

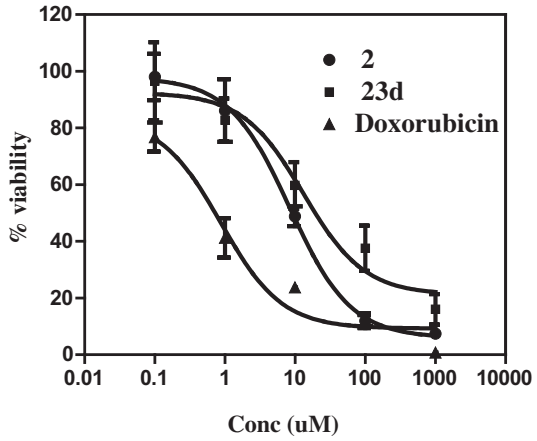


Fig. 3. Dose–response profiles for compounds **2**, **23d** (*Series B*) and doxorubicin against human hepatocellular carcinoma cell line (HepG2).

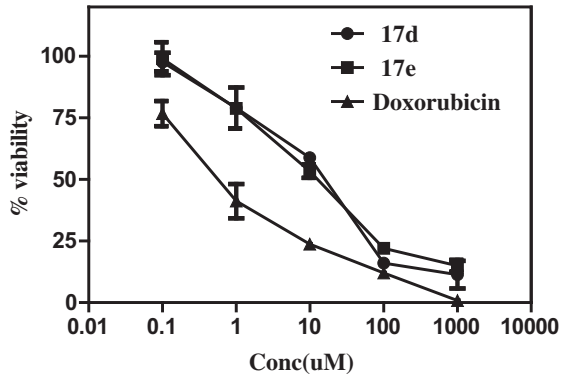


Fig. 4. Dose–response profiles for compounds **17d**, **17e** (*Series A*) and doxorubicin against human hepatocellular carcinoma cell line (HepG2).

(IC_{50} > 100 uM for each). Also, it is notable that the diphenyl analogue **6** (IC_{50} > 100 uM) did not succeed to exert remarkable effect against MCF-7 cell line. On the other hand, the C5 ethoxycarbonyl analogues **17d** and **17e** explored another outcome, where both N3 *p*-substituted electron donating **17d** and withdrawing phenyl analogues **17e,** respectively, showed remarkable anti MCF-7 activity (IC_{50} 15.88 and 10.03 uM, respectively), without ignoring that electron donating analogue **17d** is slightly less effective than the electron withdrawing analogue **17e**.

Going to human lung cancer cells A549, interestingly the recorded data consolidated the former one. Acetyl analogues carried N3 *p*-CH_3 and *p*-OCH_3 phenyl groups e.g. **10c** and **10d** (IC_{50} 15.88 and 10.03 uM, respectively), predominated over the electron withdrawing analogues **10b** and **10e** as well as diphenyl analogue **6** (IC_{50} > 100 uM). Similarly, the ethoxycarbonyl analogues **17d** and **17e** exhibited remarkable anti A549 activity (IC_{50} 13.06 and 8.68 uM, respectively), considering that they possess electron donating and withdrawing groups, respectively.

Mapping the activity of the most potent analogues in *Series A*; compounds **10c**, **10d**, **17d** and **17e**, it is noteworthy to mention that the electronic effect plays an essential role in controlling the activity. *p*-Methylphenyl derivative **10c** was more potent than its *p*-methoxyphenyl analogue **10d** against the three tested cell lines, HepG2, MCF-7 and A549. Similarly, N3 *p*-NO_2 phenyl analogue **17e** was more potent than *p*-OCH_3 phenyl analogue **17d** against all tested cell lines.

In a similar manner, the anti-cancer activity of *series B* (compounds **2**, **22** and **23a**–**f**) was evaluated based on the alternative replacement between electron donating and withdrawing groups. In *Series B*, the unsymmetrical disubstituted thiourea derivative **2** (IC_{50} 8.438 uM) was employed as a pivotal compound. For example, inoculation of thiourea moiety in **2** (IC_{50} 8.438 uM) into thiazolidin-4-one ring in **22** analogue (IC_{50} 70.49 uM), greatly diminished the anti-cancer activity. Substituting C5 of thiazolidin-4-one ring in **22** by plain benzylidine moiety to afford **23a** (IC_{50} 63.42 uM) did not effectively enhance the anticancer activity. In contrary, the activity was increased to almost five folds of **22**, *via* introducing *p*-OCH_3 benzylidine group at C5, producing **23d** (IC_{50} 13.17 uM). Similarly, the activity was also enhanced in **23f** (IC_{50} 16.65 uM) that carried 2-furanylidene moiety at C5. Noticeably, HepG2 cancer cell exerted high resistance towards **23b, 23c** and **23e** (IC_{50} > 100 uM of each). A diagrammatic comparison of IC_{50} against HepG2 of the most active compounds in *Series A* and *B* is depicted in Fig. 7.

MCF-7 profile of *Series B* explored only two derivatives with remarkable anticancer activity; **23d** and **23f**. Recorded data explored that replacing *p*-OCH_3 benzylidene moiety at C5 of **23d** by 2-furanylidene moiety in **23f** (IC_{50} 26.08 and 22.35 uM, respectively) slightly decrease the anticancer activity. Comparatively, anti MCF-7 activity was reduced to its half value upon cyclizing the thiourea derivative **2** (IC_{50} 10.08 uM) into the corresponding **23d** and **23f** derivatives.

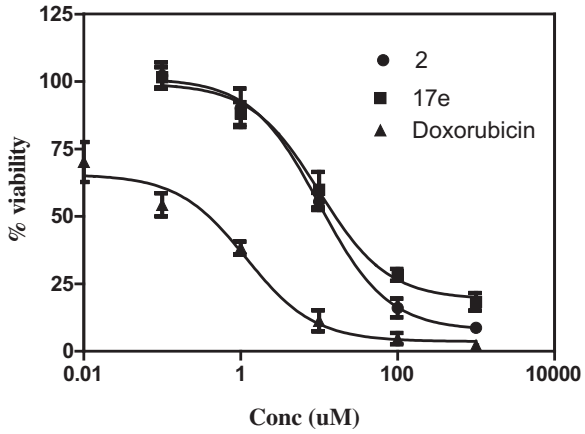


Fig. 5. Dose–response profiles for compounds **2**, **17e** (*Series A &B*) and doxorubicin against human breast cancer cells (MCF7).

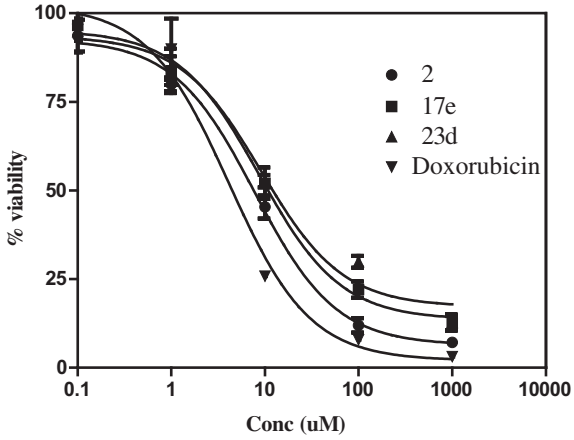


Fig. 6. Dose–response profiles for compounds **2**, **17e**, **23d** (*Series A & B*) and doxorubicin against human lung cancer cells (A549).

Human lung cancer cells A549 displayed different results where cyclizing thiourea moiety in **2** (IC_{50} 7.743 uM) into the corresponding benzylidene derivative **23d** (IC_{50} 9.10 uM) almost keep the recorded anticancer activity. On the contrary, cyclizing **2** (IC_{50} 7.743 uM) into the corresponding furanylidene analogue **23f** almost diminished the activity to its half value (IC_{50} 13.67 uM).

3. Conclusion

In conclusion, several *hitherto* unreported tri-heterocyclic systems, incorporating pyrazole, thiazole and thiadiazole moieties, were synthesized in a facile method and their structures were established by all possible spectral data. Most of the synthesized compounds were tested for anticancer activity against human hepatocelluar carcinoma HepG2 cell line. The SAR of the test compounds was controlled by isosteric replacement between *Series A* and *B*, using 4-(thiazol-4-yl)-1,2-dihydro-2,3-dimethyl-1-phenylpyrazol-5-one as scaffold. SAR was variously affected by the electronic factor, represented in alternative replacement of electron donating and withdrawing groups. Among all the test compounds, disubstituted thiourea **2** and 1,3,4-thiadiazole **17e** of *Series B* and *A*, respectively, were almost equipotent, exerting the highest anticancer activity. Also, human lung cancer cell line A549 was the most sensitive cancer cell line towards the corresponding test compounds.

4. Experimental

4.1. General

All melting points were measured on a Gallenkamp electrothermal melting point apparatus. The infrared spectra were recorded for potassium bromide pellets on a Pye Unicam SP 3-300 and FT IR 8101 PC Schimadzu infrared spectrophotometers. The ^{1}H NMR spectra were recorded in trifluoroacetic acid and dimethylsulfoxide-d_6 at 300 MHz on Bruker AC-300 or Varian Mercury NMR spectrometers. Mass spectra were recorded at 70eV on a GCMS–QP 1000 EX Shimadzu mass spectrometer (for GC/MS) and on Agilent GC 6890N coupled with an Agilent Mass Selective Detector 5973 (for ESI-MS). Elemental analyses were carried out at the Microanalytical Center of Cairo University. Hydrazonoyl chlorides **3** [37], **7a–e** [38,39], and **14a–e** [40] were prepared according to procedures reported in the literature.

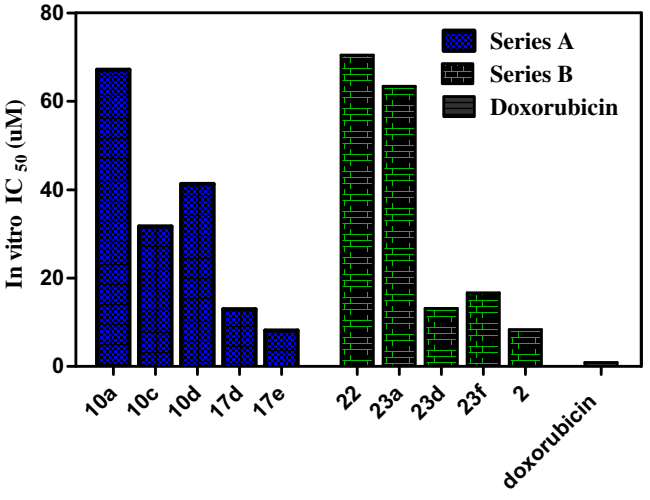


Fig. 7. Diagrammatic comparison of the in-vitro IC_{50} of the test compounds **10a**, **10c**, **10d**, **17d** and **17e** (*Series A*) and **2**, **22**, **23a**, **23d** and **23f** (*Series B*) with Doxorubicin against human Hepatocellular Carcinoma HepG2.

4.2. Chemistry

4.2.1. Synthesis of N-[4-(2,5-dihydro-2,3-dimethyl-5-oxo-1-phenyl-1H-pyrazol-4-yl)thiazol-2-yl]-N′-phenylthiourea (*2*)

A mixture of the aminothiazole derivative **1** (2 mmol, 0.572 g), KOH (2 mmol, 0.112 g) in DMF (10 mL) was stirred for 10 min. Then, phenylisothiocyanate (2 mmol, 0.27 g) was added and stirring was continued for 6 h at room temperature. The reaction mixture was then diluted with water (10 mL) and acidified with few drops of acetic acid. The solid formed was filtered off, washed with ethanol, dried and finally recrystallized from acetic acid to afford the corresponding thiazolylthiourea derivative **2** as pale yellow powder. Yield (0.57 g, 67%), mp. 199–200 °C; IR (KBr) ν (cm^{-1}): 3456, 3172 (2NH), 1628 (C=O), 1593 (C=C); ^{1}H NMR (TFA/DMSO-d_6) δ 2.5 (s, 3H, CH_3), 3.5 (s, 3H, NCH_3), 7.08 (s, 1H, thiazole-5-CH), 7.24–7.40 (m, 7H, ArH), 7.55–7.67 (m, 3H, ArH), 9.4 (br. s, 2H, 2NH, D_2O-exchangeable); MS *m/z* (%): 421 (M^+, 41.7), 286 (50), 225 (41.7), 155 (41.7), 101 (75), 86 (66.7), 72 (100), 59 (100). Anal. Calcd for $C_{21}H_{19}N_5OS_2$ (421.54): C, 59.83; H, 4.54; N, 16.61; S, 15.21%. Found: C, 59.75; H, 4.50; N, 16.55; S, 15.11%.

4.2.2. Synthesis of thiazolylimino-1,3,4-thiadiazole derivatives ***6***, ***10a–e*** and ***17a–e***

General procedure: To a mixture of the thiazolylthiourea derivative **2** (1 mmol, 0.421 g) and the appropriate hydrazonoyl chlorides **3**, **7a–e** and **14a–e** (1 mmol) in absolute ethanol (10 mL), triethylamine (0.1 mL) was added and the resulting mixture was refluxed for 6–10 h, then left to cool to room temperature. The solid formed was collected by filtration, washed with ethanol, dried and finally recrystallized from DMF/EtOH to afford the corresponding thiazolylimino-1,3,4-thiadiazole derivatives **6**, **10a–e** and **17a–e**, respectively. The physical properties and spectral data of the obtained products are listed below.

*4.2.2.1. 3,5-Diphenyl-2-(4-(2,3-dimethyl-1-phenyl-5-oxo-pyrazol-4-yl)thiazol-2-ylimino)-1,3,4-thiadiazole (**6**).* Pale yellow crystals, yield (0.31 g, 58%), mp. 252–254 °C (EtOH/DMF); IR (KBr) ν (cm^{-1}): 1666 (C=O), 1589 (C=C); ^{1}H NMR (TFA/DMSO-d_6) δ 2.53 (s, 3H, CH_3), 3.53 (s, 3H, NCH_3), 7.24 (s, H, thiazole-5-CH), 7.36–7.47 (m, 2H, ArH), 7.49–7.68 (m, 11H, ArH), 7.85 (d, 2H, $J = 6.99$ Hz); ^{13}C NMR (TFA/DMSO-d_6) δ 9.2, 31.7, 94.3, 108.5, 112.4, 116.2, 124.2, 125.7, 126.5, 127.6, 128.0, 128.3, 128.4, 128.6, 129.6, 129.9, 132.3, 136.1, 146.8, 155.9, 164.2, 168.9; MS *m/z* (%): 522 (M^+, 47.5), 402 (6.1), 373 (3.3), 296 (28.4), 261 (54.0), 215 (13.3), 91 (79.1), 77 (100), 56 (89). Anal. Calcd for $C_{28}H_{22}N_6OS_2$ (522.64): C, 64.35; H, 4.24; N, 16.08; S, 12.27. Found: C, 62.88; H, 4.07; N, 15.57; S, 12.05.

*4.2.2.2. 5-Acetyl-2-(4-(2,3-dimethyl-1-phenyl-5-oxo-pyrazol-4-yl) thiazol-2-ylimino)-3-phenyl-1,3,4-thiadiazole (**10a**).* Orange-red crystals, yield (0.33 g, 68%), mp. 256-8 °C (EtOH/DMF); IR (KBr) ν (cm^{-1}): 1656 (C=O), 1557 (C=C); ^{1}H NMR (TFA/DMSO-d_6) δ 2.51 (s, 3H, CH_3), 2.76 (s, 3H, $COCH_3$), 3.50 (s, 3H, NCH_3), 7.68–7.32 (m, 11H, ArH + thiazole-5-CH); ^{13}C NMR (TFA/DMSO-d_6) δ 9.1, 22.9, 31.7, 93.9, 109.7, 121.4, 127.6, 128.1, 128.5, 129.3, 129.4, 129.9, 130.0, 132.2, 135.6, 146.8, 152.4, 166.4, 169.2, 191.7; MS *m/z* (%): 488 (M^+, 27.9), 487 ($M^+ - 1$, 48.8), 312 (25.6), 242 (16.3), 161 (16.3), 149 (20.9), 119 (27.9), 91 (39.5), 73 (55.8), 56 (100). Anal. Calcd for $C_{24}H_{20}N_6O_2S_2$ (488.58): C, 59.00; H, 4.13; N, 17.20; S, 13.13. Found: C, 59.12; H, 4.11; N, 17.17; S, 13.04.

*4.2.2.3. 5-Acetyl-3-(4-chlorophenyl)-2-(4-(2,3-dimethyl-1-phenyl-5-oxo-pyrazol-4-yl)thiazol-2-ylimino)-1,3,4-thiadiazole (**10b**).*

Yellowish orange crystals, yield (0.31 g, 59%), mp. 240–242 °C (EtOH/DMF); IR (KBr) ν (cm^{-1}): 1656 (C=O); ^1H NMR (TFA/DMSO-d6) δ 2.68 (s, 3H, CH_3), 2.91 (s, 1H, $COCH_3$), 3.67 (s, 1H, NCH_3), 7.45–7.81 (m, 10H, ArH + thiazole-5-CH); MS *m/z* (%): 523 (M^+, 30.7), 430 (5.2), 295 (6), 261 (7.9), 152 (9.4), 111 (13.5), 73 (58.1), 56 (100). Anal. Calcd for $C_{24}H_{19}ClN_6O_2S_2$ (523.03): C, 55.11; H, 3.66; N, 16.07; S, 12.26. Found: C, 55.42; H, 3.61; N, 16.15; S, 12.30.

*4.2.2.4. 5-Acetyl-2-(4-(2,3-dimethyl-1-phenyl-5-oxo-pyrazol-4-yl) thiazol-2-ylimino)-3-(4-tolyl)-1,3,4-thiadiazole (**10c**).* Yellow crystals, yield (0.32 g, 63%), mp. 250–252 °C (ETOH/DMF); IR (KBr) ν (cm^{-1}): 1688, 1644 (2 C=O), 1592 (C=C); ^1H NMR (TFA/DMSO-d_6) δ 2.33 (s, 3H, CH_3), 2.5 (s, 3H, CH_3), 2.75 (s, 3H, CH_3), 3.5 (s, 3H, CH_3), 7.14–7.67 (m, 10H, ArH + thiazole-5-CH); MS *m/z* (%): 502 (M^+, 17.7), 328 (4.1), 243 (3.3), 158 (3.1), 132 (11.2), 107 (16.9), 91 (21.0), 80 (42.9), 77 (19.5), 64 (100). Anal. Calcd for $C_{28}H_{22}N_6O_2S_2$ (502.61): C, 59.74; H, 4.41; N, 16.72; S, 12.76. Found: C, 59.57; H, 4.35; N, 16.79; S, 12.66.

*4.2.2.5. 5-Acetyl-3-(4-anisyl)-2-(4-(2,3-dimethyl-1-phenyl-5-oxo-pyrazol-4-yl)thiazol-2-ylimino)-1,3,4-thiadiazole (**10d**).* Yellow crystals, yield (0.21 g, 41%), mp. 264–266 °C (DMF); IR (KBr) ν (cm^{-1}): 1658 (C=O), 1555 (C=C); ^1H NMR (TFA/DMSO-d_6) δ 2.50 (s, 3H, CH_3), 2.74 (s, 3H, $COCH_3$), 3.50 (s, 3H, NCH_3), 3.90 (s, 3H, OCH_3), 7.08 (d, 2H, ArH, J = 8.97 Hz), 7.31–7.36 (m, 3H, ArH), 7.55–7.67 (m, 5H, ArH + thiazole-5-CH); ^{13}C NMR (TFA/DMSO-d_6) δ 22.9, 39.8, 54.5, 108.0, 111.8, 114.0, 115.5, 119.3, 125.8, 127.6, 129.6, 129.9, 132.2, 152.3, 159.2, 160.3, 160.8, 161.4, 162.0, 166.2, 191.6; MS *m/z* (%): 518 (M^+, 100), 426 (7.4), 292 (14.2), 259 (10.5), 152 (14.2), 121 (13.6), 77 (36.4), 73 (56.2), 56 (95.7). Anal. Calcd for $C_{25}H_{22}N_6O_3S_2$ (518.61): C, 57.90; H, 4.28; N, 16.20; S, 12.37. Found: C, 57.99; H, 4.34; N, 16.31; S, 12.35.

*4.2.2.6. 5-Acetyl-2-(4-(2,3-dimethyl-1-phenyl-5-oxo-pyrazol-4-yl) thiazol-2-ylimino)-3-(4-nitrophenyl)-1,3,4-thiadiazole (**10e**).* Yellowish brown crystals, yield (0.30 g, 56%), mp. 290–292 °C (DMF); IR (KBr), ν (cm^{-1}), 1654 (C=O), 1555 (C=C); ^1H NMR (TFA/DMSO-d_6) δ 2.53 (s, 3H, CH_3), 2.77 (s, 3H, $COCH_3$), 3.51 (s, 3H, NCH_3), 7.33 (d, 2H, ArH, J = 8.4 Hz), 7.45 (s, thiazole-5-CH), 7.56–7.67 (m, 3H, ArH), 8.07 (d, 2H, ArH, J = 9.19 Hz), 8.39 (d, 2H, ArH, J = 9.19 Hz); MS *m/z* (%): 533 (M^+, 46.4), 441 (9.3), 307 (9.3), 286 (10.3), 266 (10.3), 215 (11.3), 160 (11.3), 124 (20.6), 77 (41.2), 56 (100). Anal. Calcd for $C_{24}H_{19}N_7O_4S_2$ (533.58): C, 54.02; H, 3.59; N, 18.38; S, 12.02. Found: C, 54.11; H, 3.70; N, 18.18; S, 12.11.

*4.2.2.7. Ethyl 3-phenyl-2-(4-(2,3-dimethyl-1-phenyl-5-oxo-pyrazol-4-yl)thiazol-2-ylimino)-1,3,4-thiadiazole-5-carboxylate (**17a**).* Reddish-orange solid, yield (0.32 g, 61%), mp. 228–230 °C (EtOH/DMF); IR (KBr) ν (cm^{-1}): 1739, 1658 (2 C=O), 1538 (C=C); ^1H NMR (TFA/DMSO-d_6) δ 1.45 (t, 3H, CH_2CH_3, J = 7.14 Hz), 2.52 (s, 3H, CH_3), 3.52 (s, 3H, N–CH_3), 4.60 (q, 2H, CH_2CH_3, J = 7.14 Hz), 7.33–7.36 (m, 2H, ArH), 7.39 (s, 1H, thiazole-5-CH), 7.46–7.51 (m, 3H, ArH), 7.55–7.69 (m, 5H, ArH); ^{13}C NMR (TFA/DMSO-d_6) δ 9.2, 11.4, 31.8, 65.6, 93.9, 109.5, 124.0, 127.6, 128.1, 128.6, 129.3, 129.9, 130.1, 132.3, 135.5, 144.3, 146.8, 127.9, 165.4, 169.0; MS *m/z* (%): 519 (M^++1, 20.6), 518 (M^+, 63.4), 426 (3.7), 386 (3.5), 292 (6.6), 259 (5.8), 215 (4.4), 161 (6.1), 144 (7.6), 91 (19.2), 77 (53.6), 56 (100); Anal. Calcd for $C_{25}H_{22}N_6O_3S_2$ (518.61): C, 57.90; H, 4.28; N, 16.20; S, 12.37. Found: C, 57.76; H, 4.25; N, 16.29; S, 12.27.

*4.2.2.8. Ethyl 3-(4-chlorophenyl)-2-(4-(2,3-dimethyl-1-phenyl-5-oxo-pyrazol-4-yl)thiazol-2-ylimino)-1,3,4-thiadiazole-5-carboxylate (**17b**).* Yellow crystals, yield (0.43 g, 77%), mp. 114–115 °C (DMF); IR (KBr) ν (cm^{-1}): 1711, 1663 (2 C=O),1558 (C=C); ^1H NMR (TFA/DMSO-d_6) δ 1.42 (t, 3H, CH_2CH_3, J = 7.17 Hz), 2.5 (s, 3H, CH_3), 3.49 (s, 3 H, NCH_3), 4.59 (q, 2H, CH_2CH_3, J = 7.17 Hz), 7.31–43 (m, 5H, ArH + thiazole-5-CH), 7.54–7.66 (m, 5H, ArH); ^{13}C NMR (TFA/DMSO-d_6) δ 9.17, 11.34, 31.8, 65.6, 93.8, 109.5, 125.2, 127.6, 128.1, 128.7, 129.4, 129.9, 132.2, 133.9, 136.7, 144.3, 146.7, 155.9, 157.7, 165.2, 168.99; ESI-MS *m/z*: 553 (M^+), 486, 407, 274, 212. Anal. Calcd for $C_{25}H_{21}ClN_6O_3S_2$ (553.06): C, 54.29; H, 3.83; N, 15.20; S, 11.60. Found: C, 54.20; H, 3.76; N, 15.00; S, 11.70.

*4.2.2.9. Ethyl 2-(4-(2,3-dimethyl-1-phenyl-5-oxo-pyrazol-4-yl)thiazol-2-ylimino)-3-(4-tolyl)-1,3,4-thiadiazole-5-carboxylate (**17c**).* Yellowish orange crystals, yield (0.39 g, 74%), mp. 225–226 °C (DMF); IR (KBr) ν (cm^{-1}): 1739, 1652 (2 C=O), 1589 (C=C); ^1H NMR (TFA/DMSO-d_6) δ 1.43 (t, 3H, CH_2CH_3, J = 7.02 Hz), 2.33 (s, 3H, CH_3), 2.51 (s, 3H, CH_3), 3.51 (s, 3H, NCH_3), 4.59 (q, 2H, CH_2CH_3, J = 7.02 Hz), 7.25–7.43 (m, 7H, ArH + thiazole-5-CH), 7.55–7.67 (m, 3H, ArH); ^{13}C NMR (TFA/DMSO-d_6) δ 9.2, 11.4, 18.6, 31.8, 65.6, 94.0, 109.7, 123.8, 127.7, 128.0, 129.1, 129.9, 132.3, 132.9, 141.6, 144.2, 146.8, 157.9, 165.3, 168.9; ESI-MS *m/z*: 533 (M^+ +H), 477, 402, 320, 212, 150. Anal. Calcd for $C_{26}H_{24}N_6O_3S_2$ (532.64): C, 58.63; H, 4.54; N, 15.78; S, 12.04. Found: C, 58.52; H, 4.50; N, 15.66; S, 12.12.

*4.2.2.10. Ethyl 3-(4-anisyl)-2-(4-(2,3-dimethyl-1-phenyl-5-oxo-pyrazol-4-yl)thiazol-2-ylimino)-1,3,4-thiadiazole-5-carboxylate (**17d**).* Yellow crystals, yield (0.43 g, 78%), mp. 179–181 °C (DMF); IR (KBr) ν (cm^{-1}): 1715, 1654 (2 C=O), 1559 (C=C); ^1H NMR (TFA/DMSO-d_6) δ 1.41 (t, 3H, CH_2CH_3, J = 7.14 Hz), 2.49 (s, 3H, CH_3), 3.48 (s, 3H, CH_3), 3.87 (s, 3H, CH_3), 4.57 (q, 2H, CH_2CH_3, J = 7.14 Hz), 7.04 (d, 2H, ArH, J = 9.06 Hz), 7.30–7.35 (m, 3H, ArH + thiazole-5-CH), 7.52–7.64 (m, 5H, ArH); MS *m/z* (%): 548 (M^+, 88.6), 547 (M^+ − 1, 100), 322 (12.0), 134 (17.7), 121 (19.4), 77 (32.6), 56 (86.9); Anal. Calcd. for $C_{26}H_{24}N_6O_4S_2$ (548.64): C, 56.92; H, 4.41; N, 15.32; S, 11.69. Found: C, 57.01; H, 4.51; N, 15.44; S, 11.65.

*4.2.2.11. Ethyl 2-(4-(2,3-dimethyl-1-phenyl-5-oxo-pyrazol-4-yl)thiazol-2-ylimino)-3-(4-nitrophenyl)-1,3,4-thiadiazole-5-carboxylate (**17e**).* Yellow crystals, yield (0.43 g, 78%), mp. 179–181 °C (DMF); IR (KBr) ν (cm^{-1}): 1713, 1662 (2 C=O), ^1H NMR (TFA/DMSO-d_6) δ 1.45 (t, 3H, CH_2CH_3, J = 7.14 Hz), 2.55 (s, 3H, CH_3), 3.52 (s, 3H, CH_3), 4.62 (q, 2H, CH_2CH_3, J = 7.14 Hz), 7.34–7.41 (m, 2H, ArH), 7.46 (s, 1H, thiazole-5-CH), 7.57–7.68 (m, 3H, ArH), 8.07 (d, 2H, ArH, J = 9.06 Hz), 8.38 (d, 2H, ArH, J = 9.09 Hz); MS *m/z* (%): 563 (M^+, 7.8), 517 (18.2), 312 (9.1), 286 (16.9), 185 (10.4), 138 (15.6), 112 (18.2), 97 (32.5), 69 (55.8), 60 (100); Anal. Calcd for $C_{25}H_{21}N_7O_5S_2$ (563.61): C, 53.28; H, 3.76; N, 17.40; S, 11.38. Found: C, 53.10; H, 3.75; N, 17.44; S, 11.25.

*4.2.3. Synthesis of 2-(4-(2,3-dimethyl-1-phenyl-5-oxo-pyrazol-4-yl)thiazol-2-ylimino)-3-phenyl-1,3-thiazolidin-4-one (**22**)*

To a solution of the thiazolylthiourea derivative **2** (1 mmol, 0.421 g) in absolute ethanol (10 mL), ethyl chloroacetate (1.2 mmol, 0.13 mL) was added followed by triethylamine (0.1 mL). The reaction mixture was heated at reflux for 6 h then left to cool to room temperature. The solid formed was filtered off, washed with ethanol, dried and finally recrystallized from DMF/EtOH to afford the thiazolidin-4-one derivative **22** in 0.22 g (46% yield) as pale brown powder. Mp. 258–260 °C (DMF/EtOH); IR (KBr) ν (cm^{-1}): 1729, 1641 (2 C=O), 1562 (C=C); ^1H NMR (TFA/DMSO-d_6) δ 2.43 (s, 3H, CH_3), 3.43 (s, 3H, NCH_3), 4.46 (s, 2H, SCH_2CO), 7.10–7.12 (m, 2H, ArH), 7.26 (d, 2H, ArH, J = 7.62 Hz), 7.37–7.40 (m, 3H, ArH), 7.48 (s, 1H, thiazole-5-CH), 7.50–7.59 (m, 3H, ArH); ^{13}C NMR (TFA/DMSO-d_6) δ 9.1, 31.7, 34.3, 93.3, 113.2, 126.3, 127.6, 127.9, 129.0, 129.9, 130.0, 130.1, 131.5, 132.2, 146.7, 156.0, 168.5, 172.9, 173.7; MS *m/z* (%): 461 (M^+, 57.1), 369 (12.7), 235 (12.7), 210 (16), 152 (21), 119 (31.7), 93 (31.7), 77 (66.7), 56 (100). Anal. Calcd for $C_{23}H_{19}N_5O_2S_2$ (461.56): C, 59.85; H, 4.15; N, 15.17; S, 13.89. Found: C, 59.91; H, 4.11; N, 15.09; S, 13.91.

4.2.4. Synthesis of 5-arylidene-2-(4-(2,3-dimethyl-1-phenyl-5-oxo-pyrazol-4-yl)thiazol-2-ylimino)-3-phenyl-1,3-thiazolidin-4-ones ***23a–f***

To a solution of the thiazolidin-4-one derivative **22** (1 mmol, 0.462 g) in absolute ethanol (10 mL), the appropriate aldehyde derivatives (1 mmol) was added followed by adding piperidine (0.1 mL) drop-wise and the mixture was allowed to heat at reflux for 4–6 h then left to cool to room temperature. The solid product that formed was filtered off, washed with ethanol, dried and finally recrystallized from DMF to afford the corresponding arylidene derivatives **23a–f**. The physical properties and spectral data of the obtained products are listed below.

*4.2.4.1. 5-Benzylidene-2-(4-(2,3-dimethyl-1-phenyl-5-oxo-pyrazol-4-yl)thiazol-2-ylimino)-3-phenyl-1,3-thiazolidin-4-one (**23a**).* Yellow crystals, yield (0.29 g, 52%), mp. 297–298 °C; IR (KBr) ν (cm^{-1}): 1700, 1660 (2 C=O), 1560 (C=C); ^{1}H-(TFA/DMSO-d_6) δ 2.51 (s, 3H, CH_3), 3.50 (s, 3H, NCH_3), 7.24–7.39 (m, 4H, ArH), 7.49–7.68 (m, 12H, ArH + thiazole-5-CH), 8.33 (s, 1H, C=*CH*); MS *m/z* (%): 549 (M^+, 0.70), 281 (1.28), 98 (3.23), 80 (88.0), 139 (1.22), 68 (2.0), 64 (100). Anal. Calcd for $C_{30}H_{23}N_5O_2S_2$ (549.67): C, 65.55; H, 4.22; N, 12.74; S, 11.67. Found: C, 65.42; H, 4.25; N, 12.83; S, 11.80.

*4.2.4.2. 5-(4-Chlorobenzylidene)-2-(4-(2,3-dimethyl-1-phenyl-5-oxo-pyrazol-4-yl)thiazol-2-ylimino)-3-phenyl-1,3-thiazolidin-4-one (**23b**).* Yellowish-orange crystals, yield (0.47 g, 81%), mp. >300 °C; IR (KBr) ν (cm^{-1}): 1708, 1653 (2 C=O), 1592 (C=C); ^{1}H NMR (TFA/DMSO-d_6) δ 2.5 (s, 3H, CH_3), 3.5 (s, 3H, NCH_3), 7.23–7.26 (m, 2H, ArH), 7.31–7.46 (m, 2H, ArH), 7.41–7.60 (m, 5H, ArH + thiazole-5-CH), 7.62–7.67 (m, 6H, ArH), 8.26 (s, 1H, C=*CH*); ESI-MS *m/z*: 584 (M^+), 533. Anal. Calcd for $C_{30}H_{22}ClN_5O_2S_2$ (584.11): C, 61.69; H, 3.80; N, 11.99; S, 10.98. Found: C, 61.53; H, 3.89; N, 11.88; S, 11.00.

*4.2.4.3. 5-(4-(N,N-dimethylamnio)benzylidene)-2-(4-(2,3-dimethyl-1-phenyl-5-oxo-pyrazol-4-yl)thiazol-2-ylimino)-3-phenyl-1,3-thiazolidin-4-one (**23c**).* Orange crystals, yield (0.43 g, 73%), mp. >300 °C; IR (KBr) ν (cm^{-1}): 1693, 1654 (2 C=O), 1563 (C=C); ^{1}H NMR (TFA/DMSO-d_6) δ 2.50 (s, 3H, CH_3), 3.40 (s, 6H, $N(CH_3)_2$), 3.47 (s, 3H, NCH_3), 7.23–7.31 (m, 4H, ArH), 7.46–7.65 ((m, 7H, ArH + thiazole-5-CH), 7.76 (d, 2H, ArH, J = 8.73 Hz), 7.84 (d, 2H, ArH, J = 8.73 Hz), 8.26 (s, 1H, C=*CH*); ^{13}C NMR (TFA/DMSO-d_6) δ 9.2, 31.7, 46.4, 93.4, 112.9, 113.5, 119.8, 120.6, 126.4, 127.6, 128.0, 129.2, 129.9, 130.3, 131.0, 131.3, 131.9, 132.2, 134.3, 137.0, 143.0, 146.7, 165.3, 166.3, 167.9; MS *m/z* (%): 593 (M^++1, 11.3), 592 (M^+, 25.6), 563 (23.1), 460 (3.8), 388 (7.5), 296 (10.6), 261 (20.6), 218 (10.6), 177 (41.3), 119 (18.8), 77 (38.8), 56 (100). Anal. Calcd for $C_{32}H_{28}N_6O_2S_2$ (592.73): C, 64.84; H, 4.76; N, 14.18; S, 10.82. Found: C, 64.73; H, 4.72; N, 14.08; S, 10.71.

*4.2.4.4. 5-(4-Methylbenzylidene)-2-(4-(2,3-dimethyl-1-phenyl-5-oxo-pyrazol-4-yl)thiazol-2-ylimino)-3-phenyl-1,3-thiazolidin-4-one (**23d**).* Yellowish orange crystals, yield (0.44 g, 76%), mp. >300 °C; IR (KBr) ν (cm^{-1}): 1704, 1656 (2 C=O), 1563 (C=C); ^{1}H NMR (TFA/DMSO-d_6) δ 2.51 (s, 3H, CH_3), 3.50 (s, 3H, NCH_3), 3.93 (s, 3H, OCH_3), 7.12 (d, 2H, ArH, J = 8.76 Hz), 7.23–7.34 (m, 4H, ArH), 7.47–7.67 (m, 9H, ArH + thiazole-5-CH), 8.28 (s, 1H, C=*CH*); ^{13}C NMR (TFA/DMSO-d_6) δ 9.2, 31.7, 54.3, 93.6, 112.4, 113.1, 114.6, 114.8, 124.2, 124.3, 126.5, 127.6, 128.1, 129.1, 129.9, 130.2, 131.6, 132.2, 133.0, 142.0, 146.7, 162.7, 166.7, 167.3, 167.9; MS *m/z* (%): 580 (M^++1, 27.9), 579 (M^+, 75.2), 487 (5.8), 387 (10.2), 289 (8.8), 215 (7.5), 164 (26.2), 149 (18.4), 77 (49.7), 56 (100). Anal. Calcd for $C_{31}H_{25}N_5O_3S_2$ (579.69): C, 64.23; H, 4.35; N, 12.08; S, 11.06. Found: C, 64.19; H, 4.31; N, 12.01; S, 11.00.

*4.2.4.5. 5-(4-Methoxybenzylidene)-2-(4-(2,3-dimethyl-1-phenyl-5-oxo-pyrazol-4-yl)thiazol-2-ylimino)-3-phenyl-1,3-thiazolidin-4-one (**23e**).* Yellow crystals, yield (0.42 g, 75%), mp. >300 °C; IR (KBr) ν (cm^{-1}): 1706, 1655 (2 C=O), 1561 (C=C); ^{1}H NMR (TFA/DMSO-d_6) δ 2.37 (s, 3H, CH_3), 2.50 (s, 3H, CH_3), 3.50 (s, 3H, NCH_3), 7.23–7.51 (m, 6H, ArH), 7.54–7.67 (m, 9H, ArH + thiazole-5-CH), 8.29 (s, 1H, C=*CH*); ^{13}C NMR (TFA/DMSO-d_6) δ 9.2, 19.3, 31.8, 40.0, 112.2, 113.0, 113.4, 113.5, 114.4, 115.3, 126.5, 127.7, 128.0, 129.1, 129.6, 129.9, 130.2, 130.6, 131.6, 132.3, 142.7, 145.3, 146.7, 166.9, 168.0; MS *m/z* (%): 563 (M^+, 29.7), 461 (18.9), 312 (16.2), 231 (13.5), 119 (56.8), 93 (54.1), 77 (62.2), 56 (100); Anal. Calcd for $C_{31}H_{25}N_5O_2S_2$ (563.69): C, 66.05; H, 4.47; N, 12.42; S, 11.38. Found: C, 66.22; H, 4.38; N, 12.34; S, 11.27.

*4.2.4.6. 5-(2-Furyl)methylene-2-(4-(2,3-dimethyl-1-phenyl-5-oxo-pyrazol-4-yl)thiazol-2-ylimino)-3-phenyl-1,3-thiazolidin-4-one (**23f**).* Reddish brown crystals, yield (0.34 g, 63%): mp. 288–289 °C; IR (KBr) ν (cm^{-1}): 1700, 1644 (2 C=O), 1600 (C=C); ^{1}H NMR (TFA/DMSO-d_6) δ 2.51 (s, 3H, CH_3), 3.50 (s, 3H, NCH_3), 6.67–6.69 (dd, 1H, furan-4-CH, J = 3.66, 3.65 Hz), 7.16 (d, 1H, furan-3-CH, J = 3.66 Hz), 7.21–7.33 (m, 4H, ArH), 7.43–7.67 (m, 7H, ArH + thiazole-5-CH), 7.83 (d, 1H, furan-5-CH, J = 1.53 Hz), 8.01 (s, 1H, C=*CH*); ^{13}C NMR (TFA/DMSO-d_6) δ 9.2, 31.8, 112.1, 112.9, 113.2, 113.6, 114.3, 115.0, 115.1, 123.9, 125.9, 126.5, 127.6, 128.0, 129.1, 129.9, 130.2, 131.6, 132.3, 146.8, 148.2, 149.4, 167.9, 168.1; MS *m/z* (%): 539 (M^+, 66.7), 263 (33), 150 (40), 135 (60), 129 (53.3), 97 (100), 85 (80.0), 64 (60.0), 56 (53.3). Anal. Calcd for $C_{28}H_{21}N_5O_3S_2$ (539.63): C, 62.32; H, 3.92; N, 12.98; S, 11.88. Found: C, 62.17; H, 3.89; N, 12.81; S, 11.81.

*4.2.5. Synthesis of 5-acetyl-2-(4-(2,3-dimethyl-1-phenyl-5-oxo-pyrazol-4-yl)thiazol-2-ylimino)-2,3-dihydro-4-methyl-3-phenylthiazole (**25**)*

To a mixture of the thiazolylthiourea derivative **2** (1 mmol, 0.421 g) and 3-chloro-2,4-pentanedione (1 mmol, 0.135 g) in absolute ethanol (10 mL), triethylamine (1 mmol, 0.1 mL) was added and the resulting mixture was refluxed for 4 h then left to cool to room temperature. The formed solid product was filtered off, washed with ethanol, dried and finally recrystallized from DMF to afford the thiazolylimino-thiazole derivative **25** in 0.40 g (80% yield) as yellowish grey crystals. Mp. 265–266 °C; IR (KBr) ν (cm^{-1}): 1655, 1629 (2 C=O); ^{1}H NMR (TFA/DMSO-d_6) δ 2.27 (s, 3H, CH_3), 2.49 (s, 3H, CH_3), 2.90 (s, 3H, $COCH_3$), 3.22 (s, 3H, NCH_3), 7.36–7.63 (m, 11H, ArH + thiazole-5-CH); MS *m/z* (%): 502 (M^++1, 28.8) 501 (M^+, 89), 275 (23.2), 258 (18.5), 243 (6.8), 201 (5.2), 110 (14.7), 77 (57.8), 56 (100); Anal. Calcd for $C_{26}H_{23}N_5O_2S_2$ (501.62): C, 62.25; H, 4.62; N, 13.96; S, 12.78. Found: C, 62.22; H, 4.61; N, 13.99; S, 12.80.

4.3. In vitro antitumour assay

4.3.1. Methodology: cell culture

Both HepG2 human hepatocellular carcinoma cells and MCF-7 human breast cancer cells were grown in RPMI medium, while A549 human lung cancer cells was grown in DMEM. All cell lines were supplemented with 10% heat inactivated FBS, 50 units/mL of penicillin and 50 mg/mL of streptomycin and maintained at 37 °C in a humidified atmosphere containing 5% CO_2. The cells were maintained as “monolayer culture” by serial subculturing.

4.3.2. Cell growth inhibitory assay

Cytotoxicity was determined using SRB method as previously described by Skehan et al. [41]. Exponentially growing cells were collected using 0.25% Trypsin-EDTA and seeded in 96-well plates at 1000–2000 cells/well in RPMI supplemented medium. After 24 h, cells were incubated for 72 h with various concentrations of the tested compounds. Following 72 h treatment, the cells will be fixed with 10% trichloroacetic acid for 1 h at 4 °C. Wells were stained for

10 min at room temperature with 0.4% SRB dissolved in 1% acetic acid. The plates were air dried for 24 h and the dye was solubilized with Tris–HCl for 5 min on a shaker at 1600 rpm. The optical density (OD) of each well was measured spectrophotometrically at 564 nm with an ELISA microplate reader (ChroMate-4300, FL, USA). The IC_{50} values were calculated according to the equation for Boltzman sigmoidal concentration–response curve using the nonlinear regression fitting models (Graph Pad, Prism Version 5).

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