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## SYNTHESIS OF SOME NOVEL THIAZOLE, THIADIAZOLE AND 1,4-PHENYLENE-BIS-THIAZOLE DERIVATIVES AS POTENT ANTITUMOR AGENTS

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**Abstract** – A novel series of 2-ethylidenehydrazono-5-arylazothiazoles **5a-h** and 2-ethylidenehydrazono-5-arylazothiazolones **9a-d** were prepared by cyclocondensation of hydrazonyl halides **3a-h** and **7a-d** with ethylidenethiosemicarbazide **2**. In addition, reaction of **2** with *N*-phenyl-carbohydrazonyl chloride (**14**), afforded 1,3,4-thiadiazole derivative **17** as the end product. Moreover, the thiosemicarbazide derivative **2** was reacted with various bromoacetyl compounds **19a-d** and 1,1'-(1,4-phenylene)bis(2-bromoethanone) (**21**) furnished the respective thiazole derivatives **20a-d** and 1,4-phenylene-*bis*-thiazole derivative **22**. The structures of the newly synthesized compounds were established on the basis of spectroscopic evidences and their alternative syntheses. The newly synthesized compounds were evaluated for their antitumor activities against hepatocellular carcinoma (HepG2) cell line and the results revealed promising activities of compounds **5h**, **5d**, **5g**, **5f** and **5e** with IC<sub>50</sub> equal 2.23 ± 0.28, 2.48 ± 0.34, 2.49 ± 0.24, 4.03 ± 0.11, and 5.32 ± 0.27 μM, respectively.

Functionalized thiazole derivatives have received much attention due to their diverse biological activities such as antimicrobial,<sup>1</sup> antiviral,<sup>2</sup> cytotoxic and antitumor,<sup>3-6</sup> and HIV-protease inhibitory agents.<sup>7</sup> Compounds incorporating two thiazole rings either directly connected as in bisthiazoles or through a linker unit as in bisthiazolyl compounds, show pronounced biological activity as DNA replication inhibitors in the tumor cells<sup>8</sup> and HIV-protease inhibitors.<sup>7-9</sup>

Pyridine derivatives also play a key role catalyzing both biological and chemical systems. In many enzymes of living organisms it is the prosthetic pyridine nucleotide (NADP) that is involved in various

oxidation-reduction processes. Pyridine nucleus is the main constituent in the important vitamins niacin and pyridoxine (vitamin B6) and also in highly toxic alkaloids such as nicotine. In the pharmaceutical industry, pyridine forms the nucleus of over 7000 existing drugs. Pyridine ring system is very widely distributed in nature, especially in plant kingdom. Many important alkaloids such as atropine from *Atropa belladonna*, Deadly nightshade, contains saturated pyridine nucleus.<sup>10,11</sup>

In the last two decades we have been involved in a program aiming at the synthesis of functionally substituted heterocyclic compounds from cheap laboratory available starting materials with anticipated biological activities.<sup>12-20</sup> Recently, in the frame of our program, some new functionally substituted thiazoles and / or bisthiazoles were required to be screened for their biological activity as antitumor agents. It seemed to us that the combination of a thiazole ring with a pyridine ring in one entity may lead to enhanced biological activity due to the synergistic effect of both rings. 4-Acetylpyridine seemed a suitable starting material to fulfill this objective.

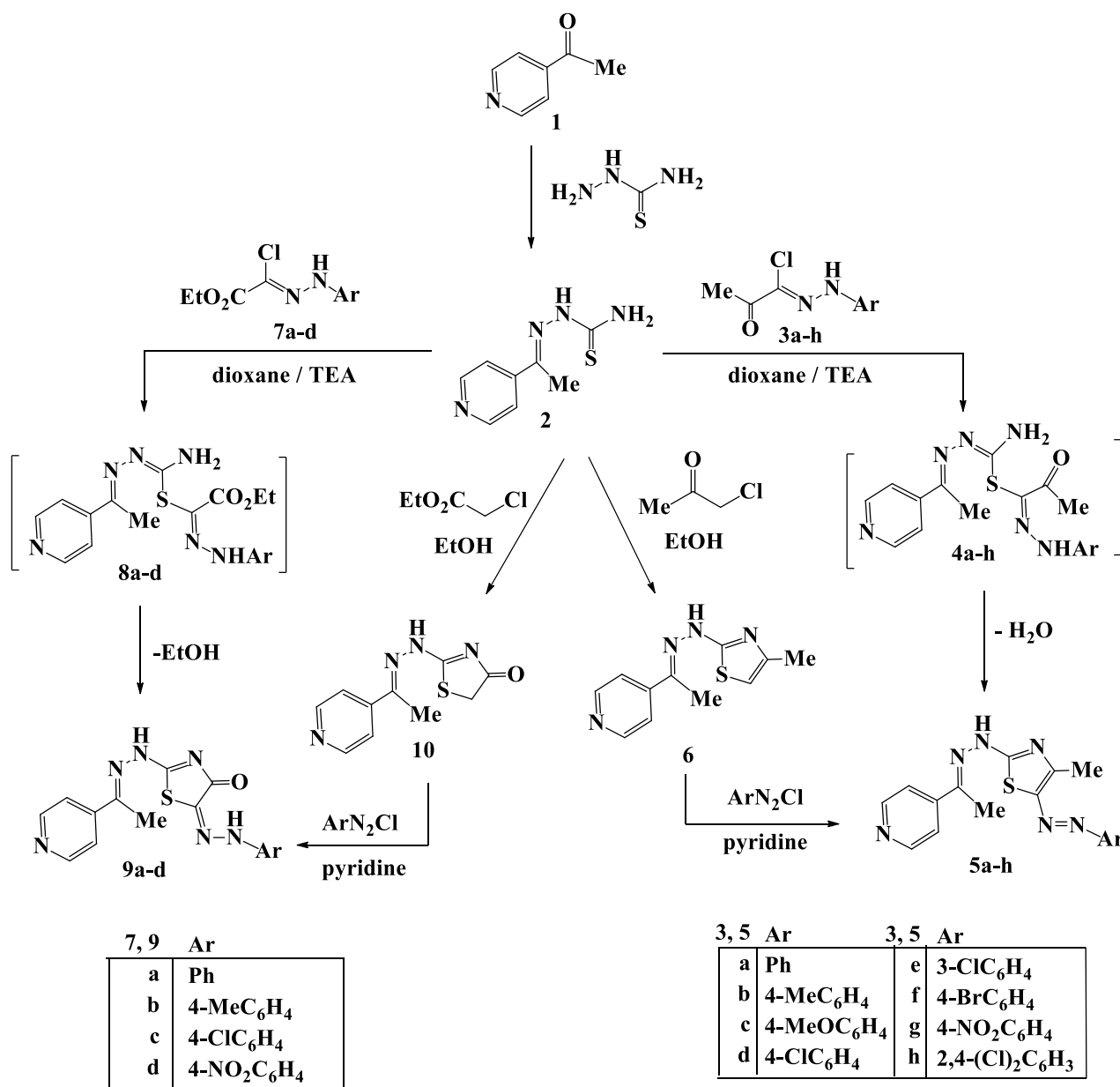
We commenced our study on the reactions of ethylidenethiosemicarbazone **2**<sup>21</sup> with  $\alpha$ -keto hydrazonoyl halides **3a-h** in refluxing dioxane (6-10 h) in the presence of a basic catalyst such as TEA (Scheme 1). The structures of isolated products **5a-h** were evidenced by spectral data together with elemental analyses. For instance, the IR spectra of products displayed in each case the absorption bands in the region 3431-3397 and 1596-1590  $\text{cm}^{-1}$  due to the (NH) and (C=N) groups, respectively. In  $^1\text{H}$  NMR spectra all the products have characteristic singlet signals in the region  $\delta$  11.20-10.85 ppm ( $\text{D}_2\text{O}$  exchangeable) assignable to the (NH) protons. On the basis of the foregoing results, the isolated products from the reactions of **2** with **3a-h** can be assigned 4-methyl-5-(aryldiazenyl)-2-(2-(1-(pyridin-4-yl)ethylidene)hydrazinyl)thiazoles **5a-h** (Scheme 1).

Authentic samples of **5a-h** could be prepared through alternative synthetic procedures. Thus, ethylidenethiosemicarbazone **2** was reacted with chloroacetone in EtOH under thermal conditions to give thiazole derivative **6**. Coupling of the latter product **6** with arene diazonium chloride in pyridine afforded the respective authentic samples **5a-h** (Scheme 1).

In a similar manner, when thiosemicarbazone derivative **2** was allowed to react with the ethyl (*N*-arylhydrazono)-chloroacetates **7a-d** in refluxing dioxane in the presence of triethylamine, it afforded in each case a single isolable product, namely, 5-(2-arylhydrazono)-2-(2-(1-(pyridin-4-yl)ethylidene)hydrazinyl)thiazol-4(5*H*)-ones **9a-d** (Scheme 1). The structures of compounds **9a-d** were confirmed on the bases of spectral data and elemental analyses (see Experimental part).

For example, the IR spectra of the products showed, in each case, one carbonyl band at 1695-1710  $\text{cm}^{-1}$  and two NH bands in the regions 3432-3425 and 3258-3251  $\text{cm}^{-1}$ . Their mass spectra of the latter products revealed in each case, the molecular ion peaks at the expected  $m/z$  values and their elemental analysis data were consistent with their assigned structures.

4-Thiazolidinone compound **10** was obtained by reaction of thiosemicarbazone derivative **2** with ethyl chloroacetate in EtOH and in the presence of anhydrous sodium acetate. Coupling of the latter product **10** with arenediazonium chloride in pyridine afforded the respective authentic samples **9a-d** (Scheme 1).

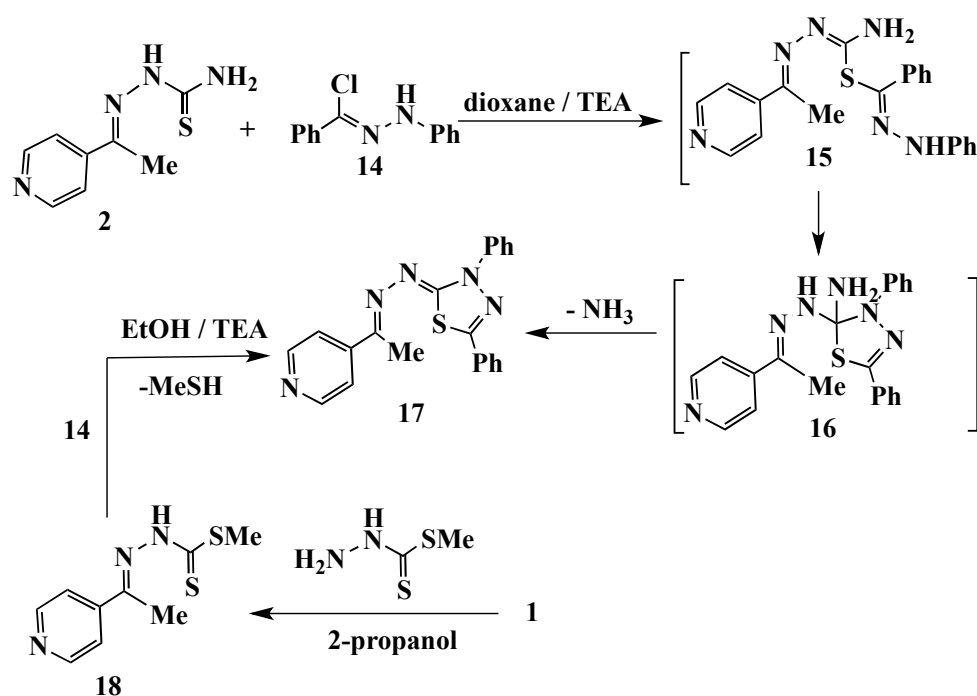


Scheme 1. Synthesis of the arylazothiazoles **5a-h** and **9a-d**

Next, the reactivity of thiosemicarbazone derivative **2** towards hydrazonoyl halide, bereft of  $\alpha$ -keto group, was examined. In the present study, we have established that reaction of ethylidenethiosemicarbazone **2** with *N*-phenylcarbohydrazonoyl chloride (**14**) gave the 1,3,4-thiadiazole **17** as the end product (Scheme 2). The reaction proceeded *via* *S*-alkylation,<sup>22</sup> with removal of hydrogen chloride, to give *S*-alkylated intermediates **15** followed by intramolecular Michael<sup>23</sup> type addition under

the employed reaction conditions, gave cycloadduct **16**. Elimination of ammonia from **16** afforded the same final product **17** (Scheme 2).

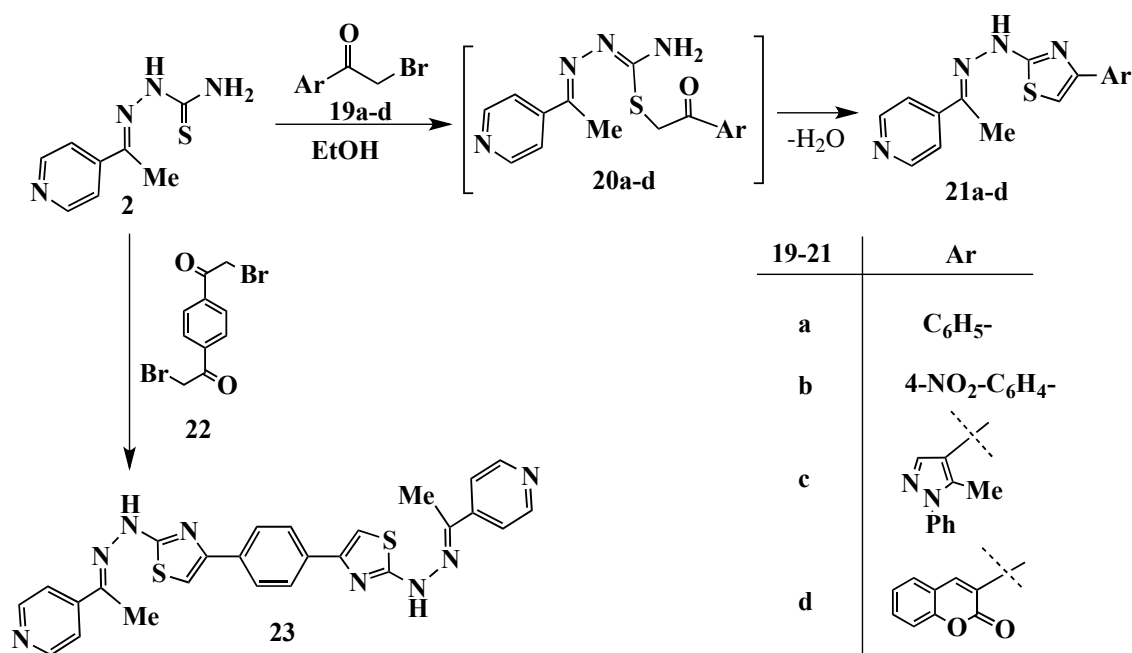
Structure **17** was confirmed by elemental analysis, spectral data, and alternative synthesis route. Thus, methyl 2-(1-(pyridin-4-yl)ethylidene)hydrazinecarbodithioate (**18**) (prepared from refluxing 4-acetylpyridine (**1**) with methyl hydrazinecarbodithioate in 2-propanol for 4 h) was reacted with **14** in ethanol in the presence of TEA at room temperature for 2 h afforded a product identical in all aspects (mp, mixed mp, and spectra) with **17**.



**Scheme 2.** Synthesis of 1,3,4-thiadiazole **17**

Moreover, the thiosemicarbazone derivative **2** was reacted with various bromoacetyl compounds **19a-d** to furnish the respective thiazole derivatives **21a-d** through the formation of intermediate **20a-d** (Scheme 3).  $^1\text{H}$  NMR spectrum for **21a** showed a singlet signal of the methine proton of thiazole ring at  $\delta$  7.73 ppm and the NH proton appeared as another singlet at  $\delta$  12.12 ppm. Mass spectrum of compound **21a** revealed the presence of the molecular ion peak at  $m/z$  294. The IR spectrum of compound **21d** showed an absorption peak at  $1702\text{ cm}^{-1}$  due to  $\text{C}=\text{O}$  stretching vibrations.

Finally, compound **2** was reacted with 1,1'-(1,4-phenylene)bis(2-bromoethanone) (**21**) to give 1,4-phenylene-*bis*-thiazole derivative **22** (Scheme 3). Structure **22** was elucidated *via* elemental analysis and spectral data.  $^1\text{H}$  NMR spectrum of **22** showed three singlet signals at  $\delta$  2.39, 7.33 and 11.73 due to methyl group, thiazole H5 and NH group, respectively. Its mass spectrum revealed a peak corresponding to its molecular ion at  $m/z$  510 [cf. Experimental section].



**Scheme 3.** Synthesis of the thiazoles **20a-d** and bis-thiazole **22**

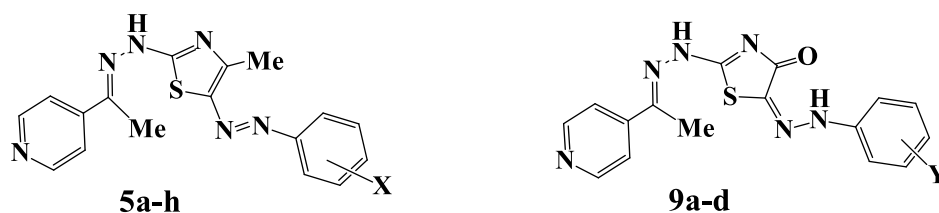
### ANTICANCER ACTIVITY

The anticancer activity of some newly synthesized compounds was determined against a liver carcinoma cell line HepG2, using doxorubicin as a reference drug. Data generated were used to plot a dose-response curve of which the concentration ( $\mu\text{M}$ ) of test compounds required to kill 50% of cell population ( $\text{IC}_{50}$ ) was determined. The cytotoxic activity was expressed as the mean  $\text{IC}_{50}$  of three independent experiments (Table 1) and the results revealed that all the tested compounds showed inhibitory activity to the tumor cell lines in a concentration dependent manner. The small values of  $\text{IC}_{50}$  for the selected compounds indicate that, for more anticancer effect higher concentrations can be used.

The results are represented in Table 1 showed that:

- The *in vitro* inhibitory activities of tested compounds against the human liver carcinoma (HepG2) have the descending order as follow: **5h** > **5d** > **5g** > **5f** > **5e** > **9c** > **9d** > **5a** > **9a** > **5b** > **9b** > **5c**.
- The thiazole ring has *in vitro* inhibitory activity than the thiazolone ring (**5a** > **9a**, **5b** > **9b**, **5d** > **9c**, **5g** > **9d**).
- The introduction of electron-withdrawing group (chlorine atom, bromine atom and nitro group) at the 4-position of phenyl group at position 5 in the thiazole ring enhances the antitumor activity. In contrast, introduction of electron-donating group (methyl group and methoxy group) decreases the antitumor activity.

**Table 1.** The *in vitro* inhibitory activity of tested compounds against a liver carcinoma cell line HepG2 expressed as IC<sub>50</sub> values (μM) ± standard deviation from six replicates



Compound No.	X (or Y)	IC <sub>50</sub> (μM)
<b>Doxorubicin</b>	-	0.72 ± 0.16
<b>5a</b>	H	19.3 ± 0.25
<b>5b</b>	4-Me	47.6 ± 0.14
<b>5c</b>	4-MeO	>100
<b>5d</b>	4-Cl	2.48 ± 0.34
<b>5e</b>	3-Cl	5.32 ± 0.27
<b>5f</b>	4-Br	4.03 ± 0.11
<b>5g</b>	4-NO <sub>2</sub>	2.49 ± 0.24
<b>5h</b>	2,4-(Cl) <sub>2</sub>	2.23 ± 0.28
<b>9a</b>	H	21.8 ± 0.19
<b>9b</b>	p-Me	49.6 ± 0.16
<b>9c</b>	p-Cl	7.98 ± 0.30
<b>9d</b>	4-NO <sub>2</sub>	11.3 ± 0.22

In our present work, a new series of 2-ethylidenehydrazono-5-arylazothiazoles and 2-ethylidenehydrazono-5-arylazothiazolones were synthesized from reaction of ethylidene-thiosemicarbazide with various hydrazonoyl halides. The structures of the newly synthesized compounds were established on the basis of spectroscopic evidences and their synthesis by alternative methods. The *in vitro* growth inhibitory activity of the synthesized compounds against hepatocellular carcinoma (HepG2) cell line was investigated in comparison with doxorubicin as a standard drug using MTT assay and the results revealed promising activities of compounds **5h**, **5d**, **5g**, **5f** and **5e** with IC<sub>50</sub> equal 2.23 ± 0.28, 2.48 ± 0.34, 2.49 ± 0.24, 4.03 ± 0.11, and 5.32 ± 0.27 μM, respectively.

## EXPERIMENTAL

All melting points were determined on an electrothermal Gallenkamp apparatus and are uncorrected. Solvents were generally distilled and dried by standard literature procedures prior to use. The IR spectra

were measured on a Pye-Unicam SP300 instrument in potassium bromide discs. The  $^1\text{H-NMR}$  and  $^{13}\text{C-NMR}$  spectra were recorded on a Varian Mercury VXR-300 spectrometer (300 MHz for  $^1\text{H-NMR}$  and 75 MHz for  $^{13}\text{C-NMR}$ ) and the chemical shifts were related to that of the solvent  $\text{DMSO-}d_6$ . The mass spectra were recorded on a GCMSQ1000-EX Shimadzu and GCMS 5988-A HP spectrometers, the ionizing voltage was 70 eV. Elemental analyses and spectral measurements were carried out by the microanalytical center at Cairo University and the Analytical Laboratory of the Institute of Organic Chemistry, Technical University of Dresden, Germany. Antitumor activity was evaluated at the Regional Center for Mycology and Biotechnology at Al-Azhar University, Cairo, Egypt. Hydrazonoyl halides **3a-h**, **7a-d** and **14** were prepared as reported in the literature.<sup>24</sup>

### Reactions of 2-(1-(pyridin-4-yl)ethylidene)hydrazinecarbothioamide (**2**) with hydrazonoyl chlorides **3a-h**

**General procedure.** A mixture of 2-(1-(pyridin-4-yl)ethylidene)hydrazinecarbothioamide (**2**) (0.194 g, 1 mmol) and appropriate hydrazonoyl halides **3a-h** (1 mmol) in dioxane (15 mL) containing triethylamine (0.1 g, 1 mmol) was refluxed until all the starting materials were consumed (3–6 h as monitored by TLC). Excess of solvent was removed under reduced pressure and the reaction mixture was triturated with MeOH. The product separated was filtered, washed with MeOH, dried and recrystallized from the proper solvent to give compounds **5a-h**. The products **5a-h** together with their physical constants are listed below.

**4-Methyl-5-(phenyldiazenyl)-2-(2-(1-(pyridin-4-yl)ethylidene)hydrazinyl)thiazole (**5a**).** Red solid (68% yield); mp 170-172 °C (DMF); IR (KBr):  $\nu/\text{cm}^{-1}$  3426 (NH), 3052, 2923 (CH), 1595 (C=N);  $^1\text{H-NMR}$  ( $\text{DMSO-}d_6$ ):  $\delta$  2.54 (s, 3H,  $\text{CH}_3$ ), 2.65 (s, 3H,  $\text{CH}_3$ ), 7.05-8.94 (m, 9H, Ar-H), 11.12 (s, 1H, NH); MS  $m/z$  (%): 336 ( $\text{M}^+$ , 13). Anal. Calcd for  $\text{C}_{17}\text{H}_{16}\text{N}_6\text{S}$  (336.12): C, 60.69; H, 4.79; N, 24.98. Found C, 60.53; H, 4.71; N, 24.75%.

**4-Methyl-2-(2-(1-(pyridin-4-yl)ethylidene)hydrazinyl)-5-(*p*-tolyl diazenyl)thiazole (**5b**).** Red solid (70% yield); mp 192-194 °C (DMF); IR (KBr):  $\nu/\text{cm}^{-1}$  3415 (NH), 3047, 2934 (CH), 1596 (C=N);  $^1\text{H-NMR}$  ( $\text{DMSO-}d_6$ ):  $\delta$  2.30 (s, 3H,  $\text{CH}_3$ ), 2.52 (s, 3H,  $\text{CH}_3$ ), 2.61 (s, 3H,  $\text{CH}_3$ ), 6.87-8.94 (m, 8H, Ar-H), 11.03 (s, 1H, NH); MS  $m/z$  (%): 350 ( $\text{M}^+$ , 61). Anal. Calcd for  $\text{C}_{18}\text{H}_{18}\text{N}_6\text{S}$  (350.13): C, 61.69; H, 5.18; N, 23.98. Found C, 61.49; H, 5.05; N, 23.77%.

**5-((4-Methoxyphenyl)diazenyl)-4-methyl-2-(2-(1-(pyridin-4-yl)ethylidene)hydrazinyl)thiazole (**5c**).** Red solid (66% yield); mp 210-212 °C (DMF); IR (KBr):  $\nu/\text{cm}^{-1}$  3397 (NH), 3051, 2927 (CH), 1594 (C=N);  $^1\text{H-NMR}$  ( $\text{DMSO-}d_6$ ):  $\delta$  2.49 (s, 3H,  $\text{CH}_3$ ), 2.58 (s, 3H,  $\text{CH}_3$ ), 2.78 (s, 3H,  $\text{OCH}_3$ ), 6.98-8.83 (m, 8H, Ar-H), 11.01 (s, 1H, NH); MS  $m/z$  (%): 366 ( $\text{M}^+$ , 46). Anal. Calcd for  $\text{C}_{18}\text{H}_{18}\text{N}_6\text{OS}$  (366.13): C, 59.00; H, 4.95; N, 22.93. Found C, 58.86; H, 4.92; N, 22.79%.

**5-((4-Chlorophenyl)diazenyl)-4-methyl-2-(2-(1-(pyridin-4-yl)ethylidene)hydrazinyl)thiazole (5d).**

Red solid (73% yield); mp 178-180 °C (DMF); IR (KBr):  $\nu/\text{cm}^{-1}$  3404 (NH), 3071, 2971 (CH), 1596 (C=N);  $^1\text{H-NMR}$  (DMSO- $d_6$ ):  $\delta$  2.54 (s, 3H, CH<sub>3</sub>), 2.65 (s, 3H, CH<sub>3</sub>), 7.31-8.95 (m, 8H, Ar-H), 11.06 (s, 1H, NH); MS  $m/z$  (%): 372 ( $\text{M}^+ + 2$ , 42), 370 ( $\text{M}^+$ , 13). Anal. Calcd for C<sub>17</sub>H<sub>15</sub>ClN<sub>6</sub>S (370.08): C, 55.06; H, 4.08; N, 22.66. Found C, 54.91; H, 4.01; N, 22.47%.

**5-((3-Chlorophenyl)diazenyl)-4-methyl-2-(2-(1-(pyridin-4-yl)ethylidene)hydrazinyl)thiazole (5e).**

Red solid (67% yield); mp 187-189 °C (DMF \ EtOH); IR (KBr):  $\nu/\text{cm}^{-1}$  3418 (NH), 3046, 2977 (CH), 1592 (C=N);  $^1\text{H-NMR}$  (DMSO- $d_6$ ):  $\delta$  2.55 (s, 3H, CH<sub>3</sub>), 2.63 (s, 3H, CH<sub>3</sub>), 7.04-8.88 (m, 8H, Ar-H), 10.93 (s, 1H, NH); MS  $m/z$  (%): 372 ( $\text{M}^+ + 2$ , 20), 370 ( $\text{M}^+$ , 6). Anal. Calcd for C<sub>17</sub>H<sub>15</sub>ClN<sub>6</sub>S (370.08): C, 55.06; H, 4.08; N, 22.66. Found C, 55.00; H, 4.13; N, 22.57%.

**5-((4-Bromophenyl)diazenyl)-4-methyl-2-(2-(1-(pyridin-4-yl)ethylidene)hydrazinyl)thiazole (5f).**

Red solid (72% yield); mp 172-174 °C (DMF); IR (KBr):  $\nu/\text{cm}^{-1}$  3407 (NH), 3080, 2967 (CH), 1590 (C=N);  $^1\text{H-NMR}$  (DMSO- $d_6$ ):  $\delta$  2.49 (s, 3H, CH<sub>3</sub>), 2.60 (s, 3H, CH<sub>3</sub>), 7.33-8.82 (m, 8H, Ar-H), 10.85 (s, 1H, NH); MS  $m/z$  (%): 416 ( $\text{M}^+ + 2$ , 16), 414 ( $\text{M}^+$ , 14). Anal. Calcd for C<sub>17</sub>H<sub>15</sub>BrN<sub>6</sub>S (414.03): C, 49.16; H, 3.64; N, 20.24. Found C, 49.12; H, 3.58; N, 20.17%.

**4-Methyl-5-((4-nitrophenyl)diazenyl)-2-(2-(1-(pyridin-4-yl)ethylidene)hydrazinyl)thiazole (5g).**

Brown solid (69% yield); mp 219-221 °C (EtOH); IR (KBr):  $\nu/\text{cm}^{-1}$  3425 (NH), 3052, 2914 (CH), 1592 (C=N);  $^1\text{H-NMR}$  (DMSO- $d_6$ ):  $\delta$  2.49 (s, 3H, CH<sub>3</sub>), 2.65 (s, 3H, CH<sub>3</sub>), 7.51-8.75 (m, 8H, Ar-H), 11.09 (s, 1H, NH); MS  $m/z$  (%): 381 ( $\text{M}^+$ , 38). Anal. Calcd for C<sub>17</sub>H<sub>15</sub>N<sub>7</sub>O<sub>2</sub>S (381.10): C, 53.53; H, 3.96; N, 25.71. Found C, 53.48; H, 3.91; N, 25.66%.

**5-((2,4-Dichlorophenyl)diazenyl)-4-methyl-2-(2-(1-(pyridin-4-yl)ethylidene)hydrazinyl)-thiazole (5h).**

Red solid (73% yield); mp 141-143 °C (DMF); IR (KBr):  $\nu/\text{cm}^{-1}$  3431 (NH), 3054, 2975 (CH), 1590 (C=N);  $^1\text{H-NMR}$  (DMSO- $d_6$ ):  $\delta$  2.52 (s, 3H, CH<sub>3</sub>), 2.62 (s, 3H, CH<sub>3</sub>), 7.69-8.81 (m, 7H, Ar-H), 11.13 (s, 1H, NH); MS  $m/z$  (%): 404 ( $\text{M}^+$ , 19). Anal. Calcd for C<sub>17</sub>H<sub>14</sub>Cl<sub>2</sub>N<sub>6</sub>S (404.04): C, 50.38; H, 3.48; N, 20.74. Found C, 50.26; H, 3.39; N, 20.64%.

**Alternate method for 5a-h****A. Synthesis of 4-methyl-2-(2-(1-(pyridin-4-yl)ethylidene)hydrazinyl)thiazole (6)**

To a solution of thiosemicarbazone **2** (1.98 g, 10 mmol) in EtOH (20 mL), chloroacetone (0.92 g, 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 EtOH and recrystallized from dioxane to afford the thiazole derivative **6** as yellow solid (69% yield); mp 184-186 °C; IR (KBr):  $\nu/\text{cm}^{-1}$  3423 (NH), 3050, 2920 (CH), 1592 (C=N);  $^1\text{H-NMR}$  (DMSO- $d_6$ ):  $\delta$  2.17 (s, 3H, CH<sub>3</sub>), 2.27 (s, 3H, CH<sub>3</sub>), 6.35 (s, 1H, thiazole-H5), 7.66 (d,  $J = 5.1$  Hz, 2H, pyridine-H3,H5), 8.57 (d,  $J = 5.1$  Hz, 2H, pyridine-H2,H6), 11.32 (s, 1H, NH); MS  $m/z$  (%): 232



(M<sup>+</sup>, 100). Anal. Calcd for C<sub>11</sub>H<sub>12</sub>N<sub>4</sub>S (232.08): C, 56.87; H, 5.21; N, 24.12. Found C, 56.75; H, 5.19; N, 24.00%.

### B. Coupling of thiazole 6 with arenediazonium chlorides

To a solution of **6** (0.464 g, 2 mmol) in pyridine (20 mL), cooled to 0-5 °C in an ice bath, was added portionwise a cold solution of arenediazonium chloride [prepared by diazotizing aniline derivatives (2 mmol) dissolved in hydrochloric acid (6 M, 2 mL) with a solution of sodium nitrite (0.14 g, 2 mmol) in water (3 mL)]. After complete addition of the diazonium salt, the reaction mixture was stirred for a further 30 min in an ice bath. The solid that separated was filtered off, washed with water and finally recrystallized from DMF to give products proved to be identical in all respects (mp, mixed mp and IR spectra) with compounds **5a-h** which obtained from reaction of **2** with **3a-h**.

### Reactions of 2-(1-(pyridin-4-yl)ethylidene)hydrazinecarbothioamide (2) with hydrazonoyl chlorides

**7a-d. General procedure.** A mixture of 2-(1-(pyridin-4-yl)ethylidene)hydrazinecarbothioamide (**2**) (0.194 g, 1 mmol) and appropriate hydrazonoyl halides **7a-d**, (1 mmol) in dioxane (15 mL) containing TEA (0.1 g, 1 mmol) was refluxed until all the starting materials were consumed (3–6 h as monitored by TLC). Excess of solvent was removed under reduced pressure and the reaction mixture was triturated with MeOH. The product separated was filtered, washed with MeOH, dried and recrystallized from EtOH to give compounds **9a-d**. The products **9a-d** together with their physical constants are listed below.

**5-(2-Phenylhydrazono)-2-(2-(1-(pyridin-4-yl)ethylidene)hydrazinyl)thiazol-4(5H)-one (9a).** Yellow solid (70% yield); mp 175-177 °C; IR (KBr):  $\nu/\text{cm}^{-1}$  3427, 3258 (2NH), 3043, 2974 (CH), 1695 (C=O), 1593 (C=N); <sup>1</sup>H-NMR (DMSO-*d*<sub>6</sub>):  $\delta$  2.33 (s, 3H, CH<sub>3</sub>), 6.93-8.85 (m, 9H, Ar-H), 10.24, 12.18 (2s, 2H, 2NH); MS *m/z* (%): 338 (M<sup>+</sup>, 73). Anal. Calcd for C<sub>16</sub>H<sub>14</sub>N<sub>6</sub>OS (338.09): C, 56.79; H, 4.17; N, 24.84. Found C, 56.64; H, 4.10; N, 24.79%.

**2-(2-(1-(Pyridin-4-yl)ethylidene)hydrazinyl)-5-(2-(p-tolyl)hydrazono)thiazol-4(5H)-one (9b).** Yellow solid (71% yield); mp 160-162 °C; IR (KBr):  $\nu/\text{cm}^{-1}$  3433, 3253 (2NH), 3051, 2922 (CH), 1708 (C=O), 1619 (C=N); <sup>1</sup>H-NMR (DMSO-*d*<sub>6</sub>):  $\delta$  2.21 (s, 3H, CH<sub>3</sub>), 2.37 (s, 3H, CH<sub>3</sub>), 7.04-8.70 (m, 8H, Ar-H), 10.42, 12.23 (2s, 2H, 2NH); MS *m/z* (%): 338 (M<sup>+</sup>, 73). Anal. Calcd for C<sub>17</sub>H<sub>16</sub>N<sub>6</sub>OS (352.11): C, 57.94; H, 4.58; N, 23.85. Found C, 57.84; H, 4.47; N, 23.75%.

**5-(2-(4-Chlorophenyl)hydrazono)-2-(2-(1-(pyridin-4-yl)ethylidene)hydrazinyl)thiazol-4(5H)-one (9c).** Yellow solid (72% yield); mp 210-212 °C; IR (KBr):  $\nu/\text{cm}^{-1}$  3425, 3166 (2NH), 3101, 2974 (CH), 1708 (C=O), 1597 (C=N); <sup>1</sup>H-NMR (DMSO-*d*<sub>6</sub>):  $\delta$  2.34 (s, 3H, CH<sub>3</sub>), 7.10-8.70 (m, 8H, Ar-H), 10.40, 12.15 (2s, 2H, 2NH); MS *m/z* (%): 372 (M<sup>+</sup>, 29). Anal. Calcd for C<sub>16</sub>H<sub>13</sub>ClN<sub>6</sub>OS (372.06): C, 51.54; H, 3.51; N, 22.54. Found C, 51.38; H, 3.44; N, 22.46%.

**5-(2-(4-Nitrophenyl)hydrazono)-2-(2-(1-(pyridin-4-yl)ethylidene)hydrazinyl)thiazol-4(5H)-one (9d).** Yellow solid (68% yield); mp 161-163 °C; IR (KBr):  $\nu/\text{cm}^{-1}$  3432, 3251 (2NH), 3054, 2930 (CH), 1710

(C=O), 1594 (C=N);  $^1\text{H-NMR}$  (DMSO- $d_6$ ):  $\delta$  2.29 (s, 3H, CH<sub>3</sub>), 7.37-8.69 (m, 8H, Ar-H), 10.39, 12.09 (2s, 2H, 2NH); MS  $m/z$  (%): 383 (M<sup>+</sup>, 57). Anal. Calcd for C<sub>16</sub>H<sub>13</sub>N<sub>7</sub>O<sub>3</sub>S (383.08): C, 50.12; H, 3.42; N, 25.57. Found C, 50.03; H, 3.39; N, 25.46%.

#### Alternate method for 9a-d

##### A. Synthesis of 2-(2-(1-(pyridin-4-yl)ethylidene)hydrazinyl)thiazol-4(5H)-one (10)

To a mixture of thiosemicarbazone **2** (0.198 g, 1 mmol) and anhydrous sodium acetate (0.33 g, 4 mmol) in EtOH (20 mL) was added, ethyl chloroacetate (1.22 g, 10 mmol) and then mixture was refluxed for 4-8 h (monitored by TLC), then left to cool. The solid product was filtered off, washed with water and recrystallized from EtOH to afford the thiazolone derivative **10** as pale brown solid (69% yield); mp 230-232 °C; IR (KBr):  $\nu/\text{cm}^{-1}$  3431 (NH), 3052, 2975 (CH), 1712 (C=O), 1597 (C=N);  $^1\text{H-NMR}$  (DMSO- $d_6$ ):  $\delta$  2.29 (s, 3H, CH<sub>3</sub>), 3.97 (s, 2H, CH<sub>2</sub>), 7.73 (d,  $J = 5.1$  Hz, 2H, pyridine-H<sub>3</sub>,H<sub>5</sub>), 8.64 (d,  $J = 5.1$  Hz, 2H, pyridine-H<sub>2</sub>,H<sub>6</sub>), 10.39 (s, 1H, NH);  $^{13}\text{C-NMR}$  (DMSO- $d_6$ ):  $\delta$  15.91 (CH<sub>3</sub>), 33.10 (CH<sub>2</sub>), 123.25, 148.10, 154.73, 163.23, 165.18 (Ar-C), 174.26 (C=O) ppm; MS  $m/z$  (%): 234 (M<sup>+</sup>, 63). Anal. Calcd for C<sub>10</sub>H<sub>10</sub>N<sub>4</sub>OS (234.06): C, 51.27; H, 4.30; N, 23.91. Found C, 51.19; H, 4.27; N, 23.84%.

##### B. Coupling of thiazolone derivative 10 with arenediazonium chlorides

To a solution of **10** (0.468 g, 2 mmol) in pyridine (20 mL), cooled to 0-5 °C in an ice bath, was added portionwise a cold solution of arenediazonium chloride [prepared by diazotizing aniline derivatives (2 mmol) dissolved in hydrochloric acid (6 M, 2 mL) with a solution of sodium nitrite (0.14 g, 2 mmol) in water (3 mL)]. After complete addition of the diazonium salt, the reaction mixture was stirred for a further 30 min in an ice bath. The solid that separated was filtered off, washed with water and finally recrystallized from DMF to give products proved to be identical in all respects (mp, mixed mp and IR spectra) with compounds **10a-d** which obtained from reaction of **2** with **7a-d**.

##### Reactions of ethylenethiosemicarbazide 2 with *N'*-phenylbenzohydrazonoyl chloride (14)

A mixture of 2-(1-(pyridin-4-yl)ethylidene)hydrazinecarbothioamide (**2**) (0.194 g, 1 mmol) and *N'*-phenylbenzohydrazonoyl chloride (**14**) (0.230 g, 1 mmol) in dioxane (10 mL) containing triethylamine (0.1 g, 1 mmol) was refluxed for 5 h. Excess of solvent was removed under reduced pressure and the reaction mixture was triturated with MeOH. The product separated was filtered, washed with MeOH, dried and recrystallized from DMF to give 3,5-diphenyl-2-((1-(pyridin-4-yl)ethylidene)hydrazono)-2,3-dihydro-1,3,4-thiadiazole **17** as white solid (70% yield); mp 147-149 °C; IR (KBr):  $\nu/\text{cm}^{-1}$  3051, 2929 (CH), 1593 (C=N);  $^1\text{H-NMR}$  (DMSO- $d_6$ ):  $\delta$  2.46 (s, 3H, CH<sub>3</sub>), 7.24-7.92 (m, 14H, Ar-H); MS  $m/z$  (%): 371 (M<sup>+</sup>, 63). Anal. Calcd for C<sub>21</sub>H<sub>17</sub>N<sub>5</sub>S (371.12): C, 67.90; H, 4.61; N, 18.85. Found C, 67.83; H, 4.44; N, 18.70%.

#### Alternate method for 17

##### A. Synthesis of methyl 2-(1-(pyridin-4-yl)ethylidene)hydrazinecarbodithioate (18)

To a solution of 4-acetylpyridine (**1**) (0.242 g, 2 mmol) in 2-propanol (20 mL) was added methyl hydrazinecarbodithioate (0.244 g, 10 mmol). The mixture was refluxed for 3 h then left to cool. The solid product was filtered off and finally recrystallized from EtOH to afford compound **18** as yellow (66% yield); mp 212-214 °C; IR (KBr):  $\nu/\text{cm}^{-1}$  3356 (NH), 3051, 2981 (CH), 1597 (C=N);  $^1\text{H-NMR}$  (DMSO- $d_6$ ):  $\delta$  2.26 (s, 3H, CH<sub>3</sub>), 2.41 (s, 3H, CH<sub>3</sub>), 8.11 (d,  $J = 5.1$  Hz, 2H, pyridine-H<sub>3</sub>,H<sub>5</sub>), 8.87 (d,  $J = 5.1$  Hz, 2H, pyridine-H<sub>2</sub>,H<sub>6</sub>), 11.12 (s, 1H, NH); MS  $m/z$  (%): 386 (M<sup>+</sup>, 21), 273 (55), 92 (26), 77 (100). Anal. Calcd for C<sub>9</sub>H<sub>11</sub>N<sub>3</sub>S<sub>2</sub> (225.04): C, 47.97; H, 4.92; N, 18.65. Found C, 47.88; H, 4.78; N, 18.5%.

#### B. Reaction of **18** with *N'*-phenylbenzohydrazonoyl chloride (**14**)

A mixture of methyl 2-(1-(pyridin-4-yl)ethylidene)hydrazinecarbodithioate (**18**) (0.225 g, 1 mmol) and *N'*-phenylbenzohydrazonoyl chloride (**14**) (0.230 g, 1 mmol) in EtOH (10 mL) containing triethylamine (0.1 g, 1 mmol) was stirred at room temperature for 2 h. The product separated was filtered, washed with MeOH, dried and finally recrystallized from dioxane to give product proved to be identical in all respects (mp, mixed mp, and IR spectra) with compound **17** which obtained from reaction of **2** with **14**.

#### Synthesis of thiazoles **21a-d**

**General procedure.** To a solution of 2-(1-(pyridin-4-yl)ethylidene)hydrazinecarbothioamide (**2**) (0.194 g, 1 mmol) in EtOH (20 mL), a proper bromoacetyl derivative **19a-d** (1 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 EtOH and recrystallized from proper solvent to afford the thiazole derivative **21a-d**. The products **21a-d** together with their physical constants are listed below.

**4-Phenyl-2-(2-(1-(pyridin-4-yl)ethylidene)hydrazinyl)thiazole (21a).** Yellow solid (72% yield); mp 226-228 °C (EtOH); IR (KBr):  $\nu/\text{cm}^{-1}$  3429 (NH), 3057, 2922 (CH), 1595 (C=N);  $^1\text{H-NMR}$  (DMSO- $d_6$ ):  $\delta$  2.37 (s, 3H, CH<sub>3</sub>), 7.73 (s, 1H, thiazole H<sub>5</sub>), 7.41-7.52 (m, 5H, Ar-H), 7.87 (d,  $J = 5.1$  Hz, 2H, pyridine-H<sub>3</sub>,H<sub>5</sub>), 8.76 (d,  $J = 5.1$  Hz, 2H, pyridine-H<sub>2</sub>,H<sub>6</sub>), 12.12 (s, 1H, NH); MS  $m/z$  (%): 294 (M<sup>+</sup>, 51). Anal. calcd for C<sub>16</sub>H<sub>14</sub>N<sub>4</sub>S (294.09): C, 65.28; H, 4.79; N, 19.03. Found: C, 65.14; H, 4.66; N, 18.93%.

**4-(4-Nitrophenyl)-2-(2-(1-(pyridin-4-yl)ethylidene)hydrazinyl)thiazole (21b).** Brown solid (76% yield); mp 281-283 °C (DMF); IR (KBr):  $\nu/\text{cm}^{-1}$  3434 (NH), 3049, 2965 (CH), 1594 (C=N);  $^1\text{H-NMR}$  (DMSO- $d_6$ ):  $\delta$  2.38 (s, 3H, CH<sub>3</sub>), 7.86 (s, 1H, thiazole H<sub>5</sub>), 8.06- 8.18 (m, 4H, Ar-H), 8.27 (d,  $J = 5.1$  Hz, 2H, pyridine-H<sub>3</sub>,H<sub>5</sub>), 8.83 (d,  $J = 5.1$  Hz, 2H, pyridine-H<sub>2</sub>,H<sub>6</sub>), 12.17 (s, 1H, NH); MS  $m/z$  (%): 339 (M<sup>+</sup>, 65). Anal. calcd for C<sub>16</sub>H<sub>13</sub>N<sub>5</sub>O<sub>2</sub>S (339.08): C, 56.63; H, 3.86; N, 20.64. Found: C, 56.51; H, 3.69; N, 20.53%.

**4-(5-Methyl-1-phenyl-1H-pyrazol-4-yl)-2-(2-(1-(pyridin-4-yl)ethylidene)hydrazinyl)thiazole (21c).** Yellow solid, (70% yield); mp 206-208 °C (DMF); IR (KBr):  $\nu/\text{cm}^{-1}$  3344 (NH), 3050, 2982 (CH), 1602 (C=N);  $^1\text{H-NMR}$  (DMSO- $d_6$ ):  $\delta$  2.37 (s, 3H, CH<sub>3</sub>), 2.55 (s, 3H, CH<sub>3</sub>), 7.03-7.98 (m, 6H, Ar-H and thiazole H<sub>5</sub>), 7.57 (d,  $J = 5.1$  Hz, 2H, pyridine-H<sub>3</sub>,H<sub>5</sub>), 8.41 (s, 1H, pyrazole H<sub>3</sub>), 8.90 (d,  $J = 5.1$  Hz, 2H,

pyridine-H2,H6), 11.47 (s, 1H, NH); MS  $m/z$  (%): 374 ( $M^+$ , 39). Anal. calcd for  $C_{20}H_{18}N_6S$  (374.13): C, 64.15; H, 4.85; N, 22.44. Found: C, 64.04; H, 4.69; N, 22.27%.

**3-(2-(2-(1-(Pyridin-4-yl)ethylidene)hydrazinyl)thiazol-4-yl)-2H-chromen-2-one (21d).** Yellow solid (70% yield); mp 263-265 °C (dioxane); IR (KBr):  $\nu/cm^{-1}$  3423 (NH), 3063, 2959 (CH), 1702 (C=O), 1600 (C=N);  $^1H$ -NMR (DMSO- $d_6$ ):  $\delta$  2.36 (s, 3H,  $CH_3$ ), 7.37-7.69 (m, 4H, Ar-H), 7.83 (d,  $J = 5.1$  Hz, 2H, pyridine-H3,H5), 8.34 (s, 1H, Coumarine H4), 8.58 (s, 1H, thiazole H5), 8.78 (d,  $J = 5.1$  Hz, 2H, pyridine-H2,H6), 12.07 (s, 1H, NH); MS  $m/z$  (%): 362 ( $M^+$ , 39). Anal. calcd for  $C_{19}H_{14}N_4O_2S$  (362.08): C, 62.97; H, 3.89; N, 15.46. Found: C, 62.83; H, 3.79; N, 15.30%.

### Synthesis of 1,4-bis(2-(2-(1-(pyridin-4-yl)ethylidene)hydrazinyl)thiazol-4-yl)benzene (23)

To a solution of thiosemicarbazone **2** (0.198 g, 1 mmol) in EtOH (20 mL), 1,1'-(1,4-phenylene)bis(2-bromoethanone) (**21**) (0.634 g, 2 mmol) were added. The mixture was refluxed for 4-8 h (monitored by TLC), then left to cool. The solid product was filtered off, washed with EtOH and recrystallized from DMF to afford the bis-thiazole derivative **22** as a white solid (68% yield); mp 327-329 °C; IR (KBr):  $\nu/cm^{-1}$  3423 (NH), 3063, 2975 (CH), 1593 (C=N);  $^1H$ -NMR (DMSO- $d_6$ ):  $\delta$  2.39 (s, 6H, 2 $CH_3$ ), 7.33 (s, 2H, 2 thiazole H5), 8.02 (d,  $J = 5.1$  Hz, 4H, 2 pyridine-H3,H5), 8.14 (s, 4H, Ar-H), 8.82 (d,  $J = 5.1$  Hz, 4H, 2 pyridine-H2,H6), 11.73 (s, 2H, 2NH); MS  $m/z$  (%): 510 ( $M^+$ , 17). Anal. Calcd for  $C_{26}H_{22}N_8S_2$  (510.14): C, 61.15; H, 4.34; N, 21.94. Found C, 61.08; H, 4.329; N, 21.76%.

### Antitumor Activity

Human liver carcinoma (HepG-2) cell lines were obtained from the American Type Culture Collection (ATCC, Rockville, MD). The cells were grown on RPMI-1640 medium supplemented with 10% inactivated fetal calf serum and 50  $\mu$ g/mL entamycin. The cells were maintained at 37 °C in a humidified atmosphere with 5%  $CO_2$  and were subcultured two to three times a week.

For antitumor assays, the tumor cell lines were suspended in medium at concentration  $5 \times 10^4$  cell/well in Corning® 96-well plates (six replicates) to achieve eight concentrations for each compound. Six vehicle controls with media or 0.5% DMSO were run for each 96 well plate as a control. After incubating for 24 h, the numbers of viable cells were determined by the MTT test. Briefly, the media was removed from the 96 well plate and replaced with 100  $\mu$ L of fresh culture RPMI 1640 medium without phenol red then 10  $\mu$ L of the 12 Mm MTT stock solution (5 mg of MTT in 1 mL of PBS) to each well including the untreated controls. The 96 well plates were then incubated at 37 °C and 5%  $CO_2$  for 4 h. An 85  $\mu$ L aliquot of the media was removed from the wells, and 50  $\mu$ L of DMSO was added to each well and mixed thoroughly with the pipette and incubated at 37 °C for 10 min. then, the optical density was measured at 590 nm with the microplate reader (SunRise, TECAN, Inc, USA) to determine the number of viable cells and the percentage of viability was calculated as  $(1-(OD_t/OD_c)) \times 100\%$  where  $OD_t$  is the mean optical

density of untreated cells. The relation between surviving cells and drug concentration is plotted to get the survival curve of each tumor cell line after treatment with the specified compound. The 50% inhibitory concentration (IC<sub>50</sub>), the concentration required to cause toxic effects in 50% of intact cells, was estimated from graphic plots of the dose response curve for each conc. Using Graphad Prism software (San Diego, CA, USA).<sup>25-28</sup>

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