

## SYNTHETIC UTILITY OF ETHYLIDENETHIOSEMICARBAZIDE: SYNTHESIS AND ANTI-CANCER ACTIVITY OF 1,3-THIAZINES AND THIAZOLES WITH IMIDAZOLE MOIETY

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**Abstract** – Reactions of ethylidenethiosemicarbazide **3** with DMAD **4** or substituted methylenemalononitriles **8** gave thiazolidin-4-one **6** or 1,3-thiazine derivatives (**10**, **11**), respectively. Also, treatment of **3** with hydrazonoyl halides **12a-i**,  $\alpha$ -haloketones **15a-d**, and chloroacetic acid **18** afforded the corresponding arylazothiazoles **14a-i**, thiazoles **17a-d**, and thiazolin-4-one derivative **20**, respectively. The structures of the synthesized products were confirmed by IR,  $^1\text{H}$  NMR,  $^{13}\text{C}$  NMR and mass spectral techniques. The anti-cancer activity of the selected products against the colon carcinoma cell line (HCT-116) was determined and the results revealed promising activity of compound **6**.

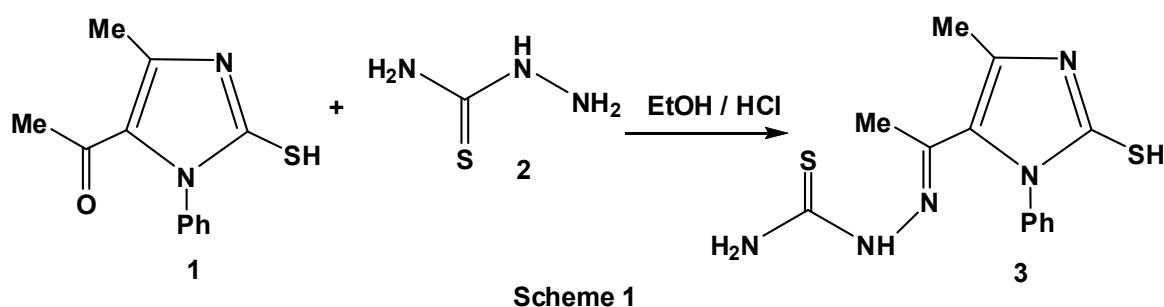
## INTRODUCTION

Recent literature is enriched with synthesis and biological activities of alkylidenethiosemicarbazides. 1-(1-Arylethylidene)thiosemicarbazides exhibited potent inhibitory activities against mushroom tyrosinase<sup>1,2</sup> (multifunctional copper-containing enzyme cause dermatological disorders). Also, 1-[1-(heterocyclic)ethylidene]thiosemicarbazides and their metal complexes have been investigated as potential anticancer agents.<sup>3-5</sup> The cytotoxic activity of thiosemicarbazones against human tumor cell lines has been attributed to their ability to inhibit ribonucleoside diphosphate reductase (RDR), a rate-limiting enzyme in DNA syntheses that catalyzes the conversion of ribonucleotides into deoxyribonucleotides.<sup>4</sup> On the other hands, alkylidenethiosemicarbazides are reactive building blocks for construction of bioactive heterocycles such as 1,3,4-thiadiazoles,<sup>6</sup> imidazolinones,<sup>7</sup> thiazoles,<sup>8-11</sup> and thiazolidin-4-one.<sup>9</sup> As a part of our research interest towards developing new routes for the synthesis of a

variety of heterocyclic systems with promising biological and pharmacological activities,<sup>12-16</sup> we report in the present work the synthesis of a new series of 1,3-thiazines and thiazoles bearing imidazole moiety.

## RESULTS AND DISCUSSION

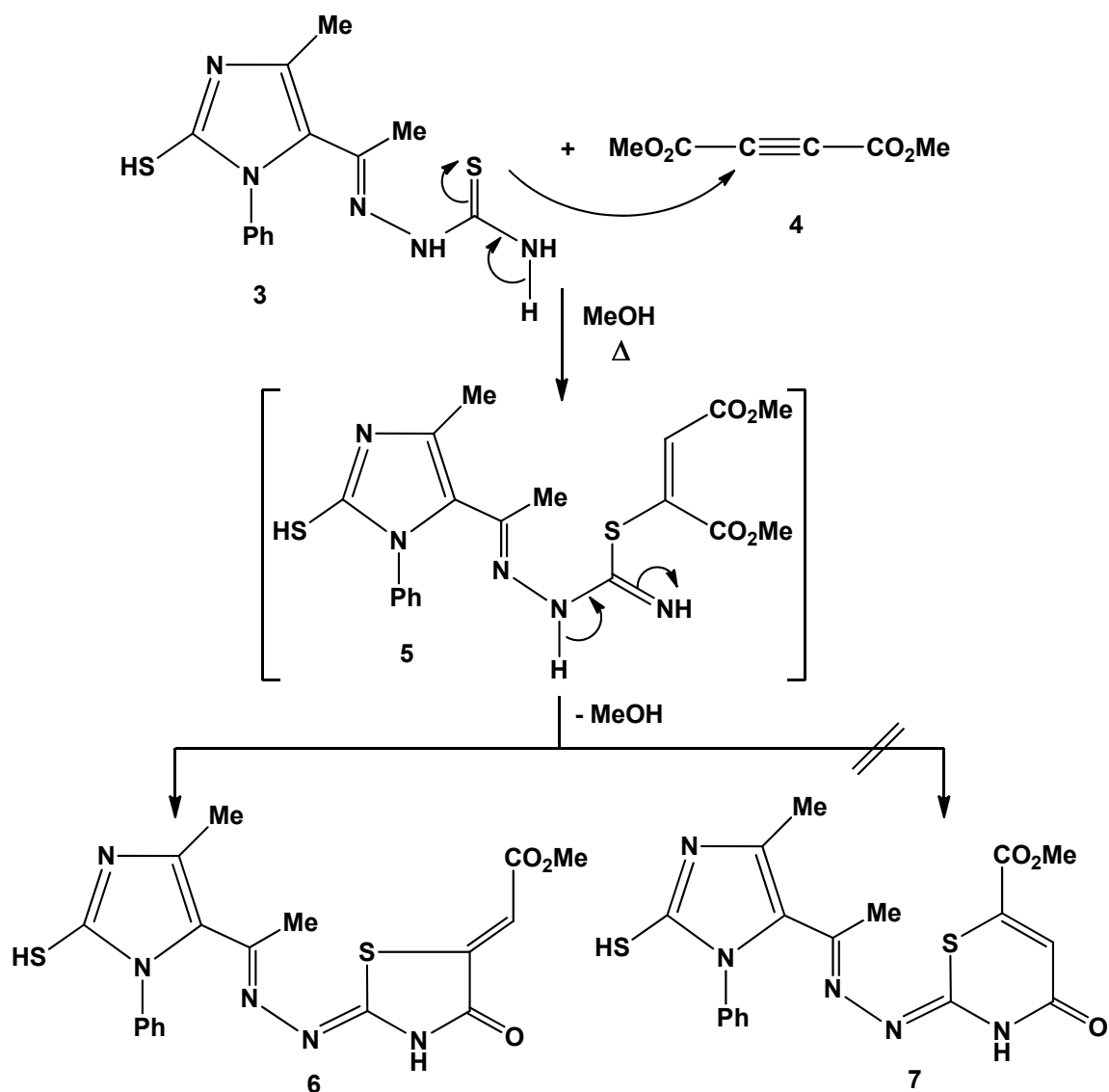
1-[1-(2-Mercapto-4-methyl-1-phenyl-1*H*-imidazol-5-yl)ethylidene]thiosemicarbazide (**3**) was prepared via condensation of 5-acetyl-2-mercapto-4-methyl-1-phenyl-1*H*-imidazole (**1**)<sup>17</sup> with thiosemicarbazide (**2**) in absolute ethanol in the presence of a catalytic amount of HCl as depicted in Scheme 1.



The structure elucidation of the product **3** was substantiated through spectral data. Product **3** can be existed in two geometric structures (*E*, and *Z*). In <sup>1</sup>H NMR, when N-H signal is revealed at  $\delta = 10.79$ -10.90 ppm suggesting the *E* configuration, in which N-H is hydrogen bonded to the solvent.<sup>18-20</sup> On the other hand, if N-H signal is revealed at  $\delta = 14.69$ -14.66 ppm indicates the presence of the *Z* configuration, in which N-H is hydrogen bonded to the nitrogen of the ring.<sup>18-20</sup> in our case, NH signal was revealed at  $\delta = 10.98$  ppm which confirms the *E*-form. Also another signal was observed at  $\delta = 10.45$  ppm characteristic to mercapto (SH)<sup>21</sup> group of imidazole ring.

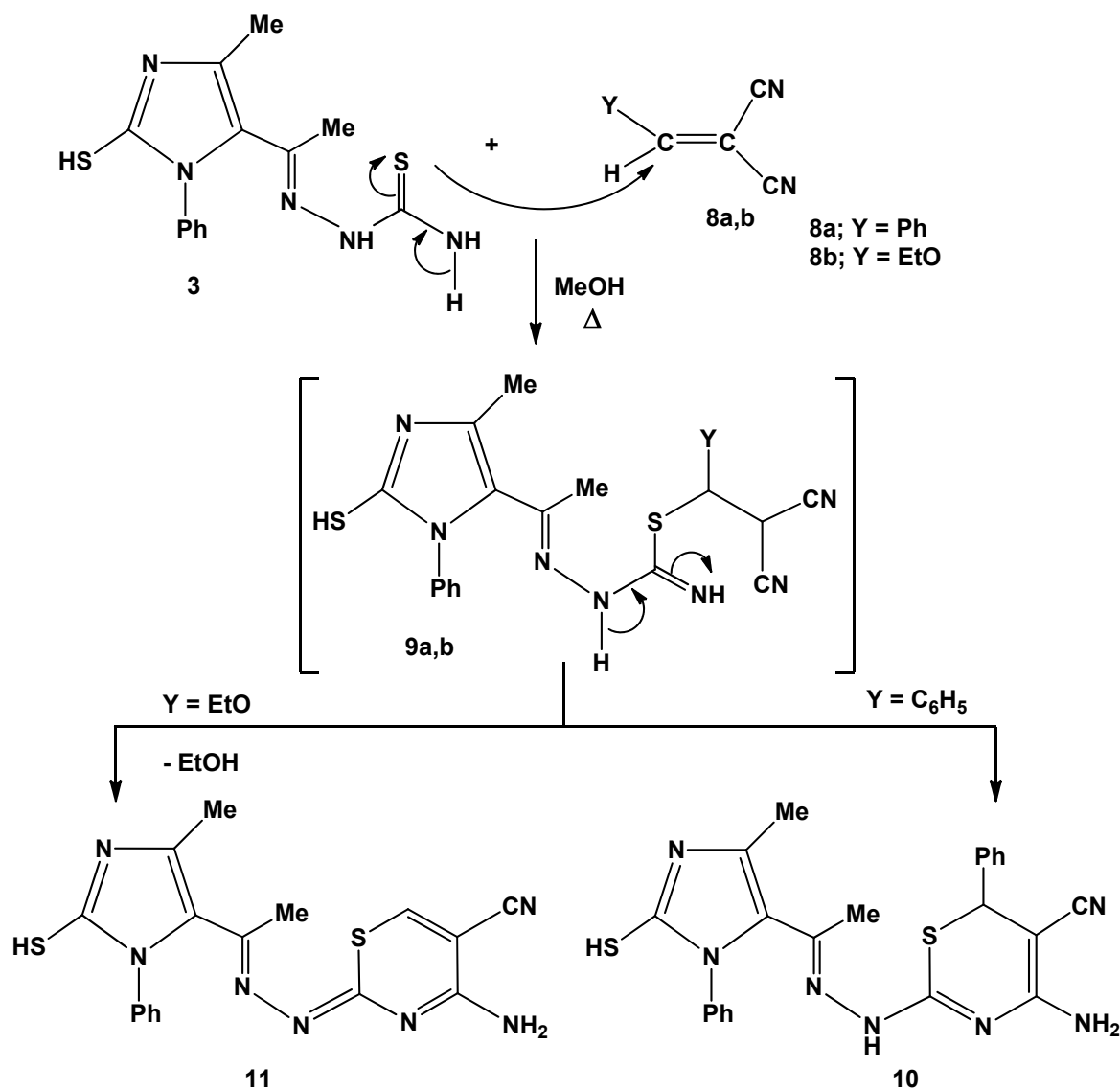
We commenced our study on the reaction of ethylenedithiosemicarbazide with activated triple bond. It was reported that, reactions of alkylidenedithiosemicarbazides with dimethyl acetylenedicarboxylate (DMAD) afforded either thiazolidin-4-ones<sup>22-24</sup> or 1,3-thiazin-4-ones<sup>22,23,25</sup> according to the reaction conditions. Thus, treatment of 1-[1-(2-mercapto-4-methyl-1-phenyl-1*H*-imidazol-5-yl)ethylidene]-thiosemicarbazide (**3**) with DMAD in methanol gave the corresponding thiazolidin-4-one derivative **6** or its isomeric structure 1,3-thiazin-4-one derivative **7** (Scheme 2). The structure of the isolated product was inferred from its elemental analysis and spectral data [IR and <sup>1</sup>H NMR]. Its IR spectrum showed absorption bands at  $\nu = 3247$  (NH), 1704, 1694 (2C=O), and 1606 (C=N) cm<sup>-1</sup>, its <sup>1</sup>H NMR spectrum revealed singlet signal at  $\delta$  12.77 ppm (D<sub>2</sub>O-exchangeable) assignable to (NH) group and another singlet signal at  $\delta$  6.64 ppm due to vinylic-H.<sup>23,26</sup> Revealing of the latter signal of vinylic-H excludes 1,3-thiazin-4-one structure **7**. To account for the formation of product **6** we assumed that the reaction initially proceeds *via* addition of thiol group in thiosemicarbazone moiety into triple bond to give the non-isolable intermediate **5**. Elimination of methanol molecule from the latter intermediate afforded the

final product **6** (Scheme 2).



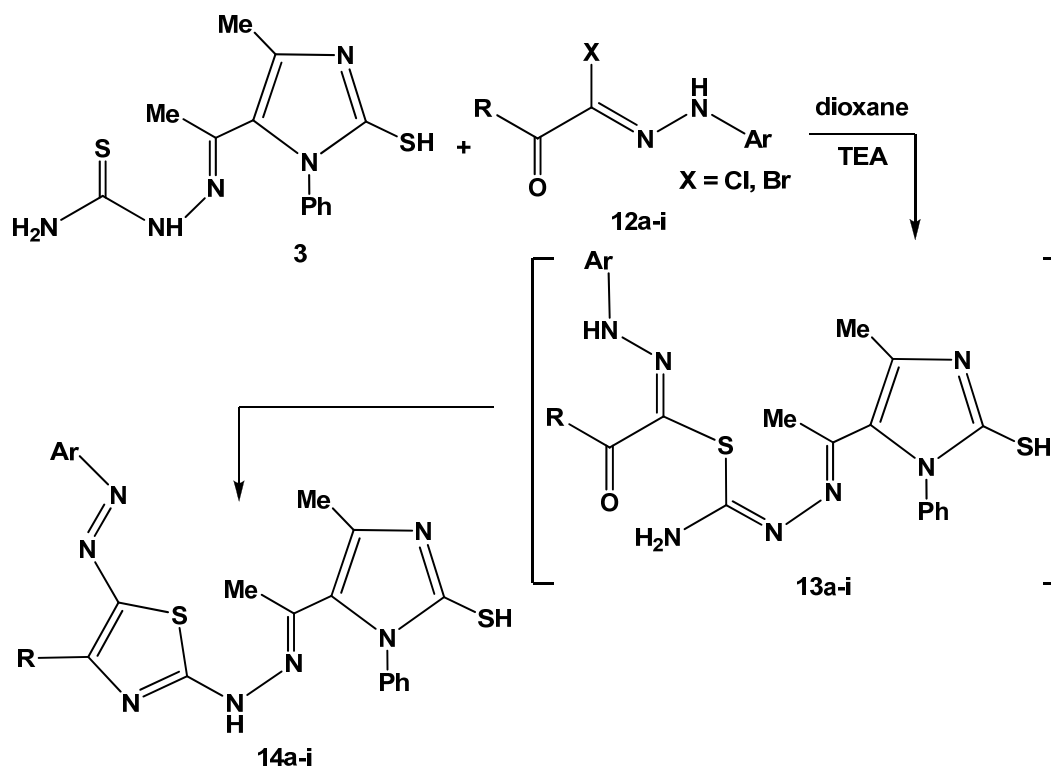
Scheme 2

Next, we investigated the behavior of ethylenedithiosemicarbazide **3** towards substituted methylenemalononitriles, as activated double bond. Thus, treatment of compound **3** with benzylidenemalononitrile **8a** or ethoxymethylenemalononitrile **8b** in refluxing methanol afforded the respective 1,3-thiazines (**10** or **11**) (Scheme 3). The reactions proceeded through addition of thiol group in thiosemicarbazone moiety into activated double bond to give the non-isolable intermediates **9a,b**. Intramolecular cyclization of intermediate **9a** via addition of amino group into nitrile group<sup>27</sup> afforded 6*H*-1,3-thiazine derivative **10**. On the other hand, intramolecular addition of intermediate **9b** followed by elimination of ethanol molecule gave the respective 2*H*-1,3-thiazine derivative **11** (cf. Scheme 3). The structures of products **10**, **11** were established based on analytical and spectral data. The structures of 1,3-thiazines in agreement with literature reports<sup>28,29</sup> concerning the reactions of thiosemicarbazides with substituted methylenemalononitriles.



Scheme 3

We extended our study on the reactivity of ethylidenethiosemicarbazide **3** towards halogenated compounds, such as hydrazonoyl halides,  $\alpha$ -halocarbonyl compounds and chloroacetic acid. Reaction of **3** with each of the hydrazonoyl halides **12a-i** in dioxane under thermal condition in the presence of triethylamine for 6-8 hrs yielded in each case one isolable product as evidenced by TLC analysis. The structures of isolated products were evidenced by spectral data together with elemental analyses. For instance, the IR spectra of products display in each case the absorption bands in the region 3213-3171 and 1604-1584  $\text{cm}^{-1}$  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  $\delta$  10.83-10.54 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 **3** with **12a-i** can be assigned 2-[2-{1-(2-mercapto-4-methyl-1-phenyl-1H-imidazol-5-yl)ethylidene}hydrazono]-4-substituted-5-arylthiazole (**14a-i**) (Scheme 4).



Compd No.	R	Ar	Compd No.	R	Ar
12a, 14a	Me	Ph	12f, 14f	Me	4-NO <sub>2</sub> C <sub>6</sub> H <sub>4</sub>
12b, 14b	Me	4-MeC <sub>6</sub> H <sub>4</sub>	12g, 14g	Ph	Ph
12c, 14c	Me	4-MeOC <sub>6</sub> H <sub>4</sub>	12h, 14h	Ph	4-ClC <sub>6</sub> H <sub>4</sub>
12d, 14d	Me	4-ClC <sub>6</sub> H <sub>4</sub>	12i, 14i	C <sub>4</sub> H <sub>3</sub> S	Ph
12e, 14e	Me	4-BrC <sub>6</sub> H <sub>4</sub>			

Scheme 4

Products **14a-i** can be present in three tautomeric forms **14A**, **14B**, and **14C** as shown in Figure 1. To elucidate the actual tautomeric form of these compounds, their electronic absorption was measured. The spectra of these compounds (**14a**, **14g**, and **14i**) in dioxane showed in each case pair of absorption bands in the regions 442-447 and 292-296 nm, analogous for the azo chromophore<sup>30</sup> and another pair in the regions 366-373 and 298-306 nm analogous for the hydrazone chromophore<sup>31</sup> (Table 1). These finding excluded tautomeric form **14B**. Furthermore, the aromatic stability of thiazole ring excluded tautomeric form **14C**.

Table 1: UV Spectra of compounds **14a**, **14g**, and **14i**

Compound no.	$\lambda_{\text{max}}$ (log $\epsilon$ )	$\lambda_{\text{max}}$ (log $\epsilon$ )
<b>14a</b>	442 (4.83), 292 (4.94)	366 (4.25), 298 (4.82)
<b>14g</b>	444 (4.80), 296 (4.77)	373 (4.35), 306 (4.84)
<b>14i</b>	447 (4.61), 295 (4.68)	369 (4.50), 298 (5.01)

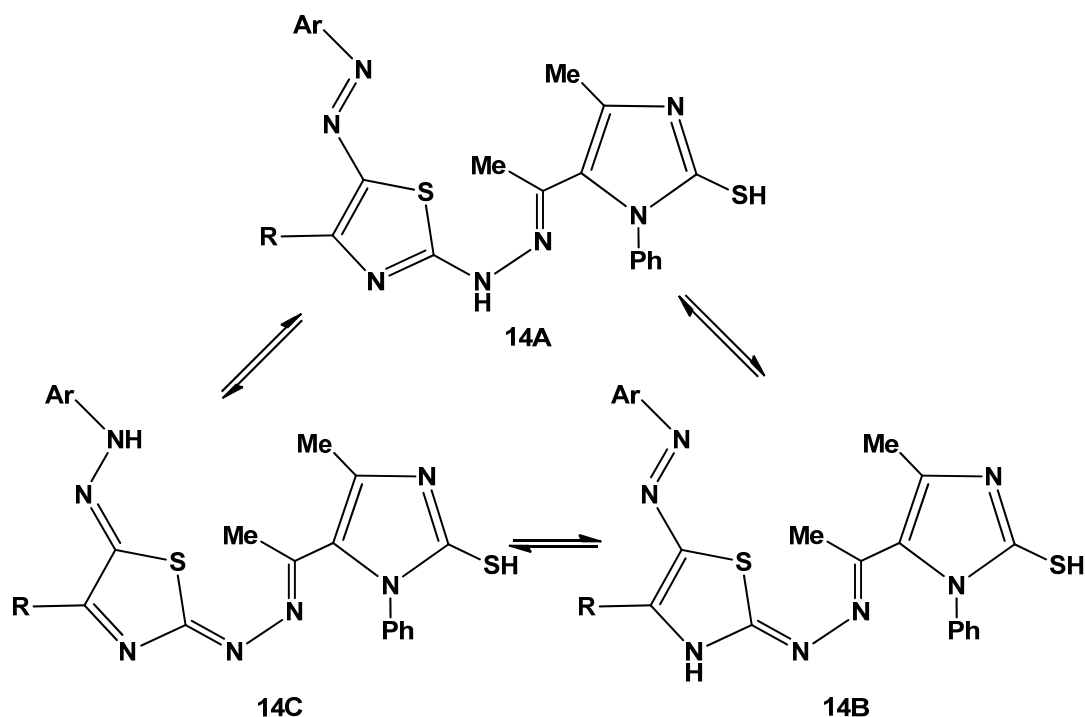


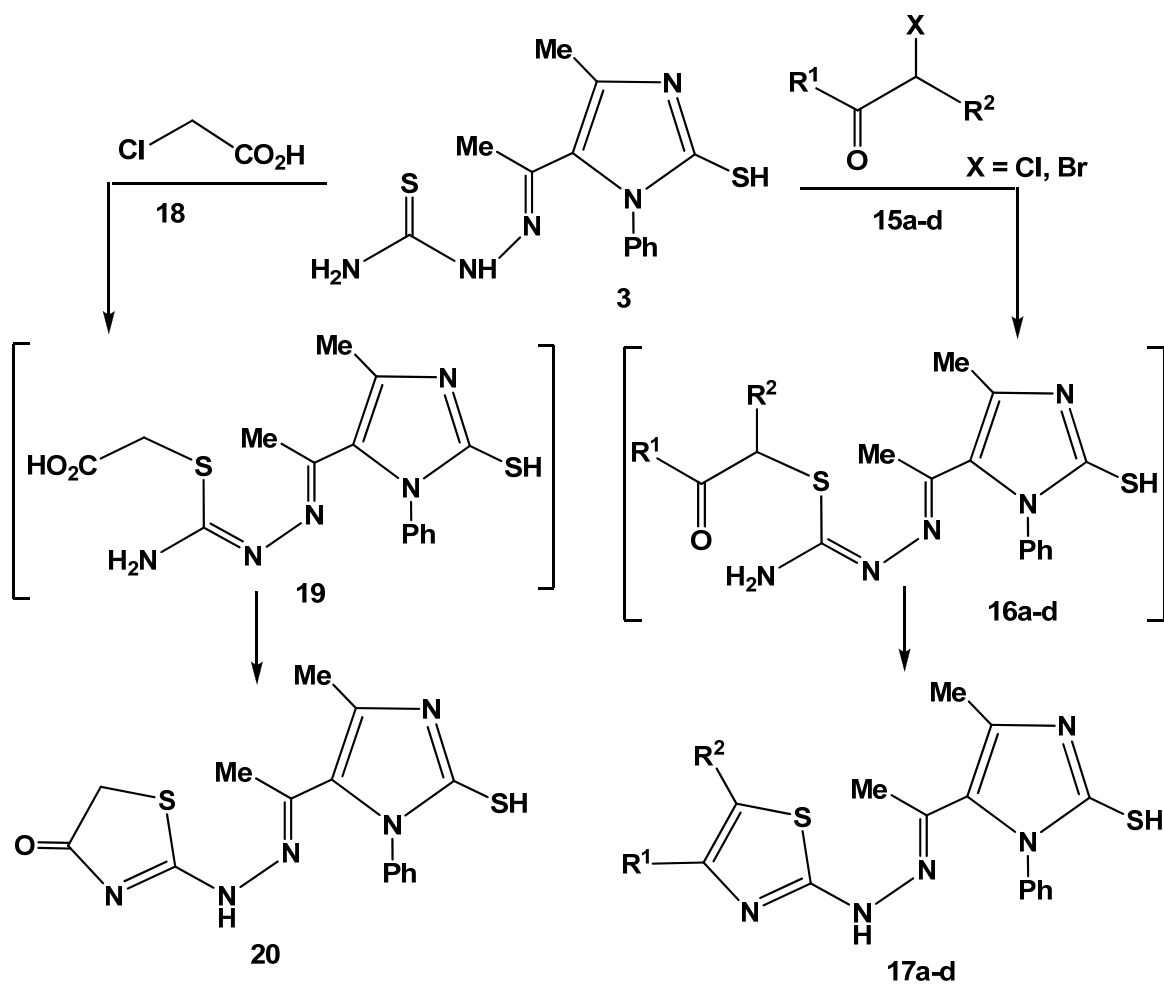
Figure 1

Finally, ethyldithiosemicarbazide **3** reacted with  $\alpha$ -halocarbonyl compounds, namely chloroacetone **15a**, phenacyl bromide **15b**, 3-chloro-2,4-pentanedione **15c**, *N*-phenyl 2-chloro-3-oxobutanamide **15d**, and chloroacetic acid **18** under thermal conditions in dioxane to afford 2-[2-{1-(2-mercapto-4-methyl-1-phenyl-1*H*-imidazol-5-yl)ethylidene}hydrazono]-4-substituted thiazoles (**17a-d**), and 2-[2-{1-(2-mercapto-4-methyl-1-phenyl-1*H*-imidazol-5-yl)ethylidene}hydrazono]-4-oxo-4,5-dihydrothiazole **20**, respectively (Scheme 5). Analytical and spectroscopic data were consistent with the final products **17a-d** and **20**.

## ANTI-CANCER EVALUATION

The cytotoxicity of synthesized products **6**, **10**, **11**, **14a**, **14g** and **20** was evaluated against human colon carcinoma cell line (HCT-116) using the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay and doxorubicin was used as a reference drug ( $IC_{50}$  value of doxorubicin =  $0.469 \pm 0.03$   $\mu\text{g/mL}$ ). 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 Tables 2, 3 and Figure 2.

The results revealed that thiazolidin-4-one derivative **6** ( $IC_{50}$  = 1.9  $\mu\text{g/mL}$ ) has promising antitumor activity against colon carcinoma (HCT-116) while 1,3-thiazines (**10** and **11**) and arylazothiazoles (**14a** and **14g**) have moderate activity ( $IC_{50}$  = 3.9-4.6  $\mu\text{g/mL}$ ). On the other hand, thiazolin-4-one **20** has poor inhibitory activity against (HCT-116) ( $IC_{50}$  = 33  $\mu\text{g/mL}$ ).



Compd No.	R <sup>1</sup>	R <sup>2</sup>	Compd No.	R <sup>1</sup>	R <sup>2</sup>
15a, 17a	Me	H	15c, 17c	Me	MeCO
15b, 17b	Ph	H	15d, 17d	Me	PhNHCO

Scheme 5

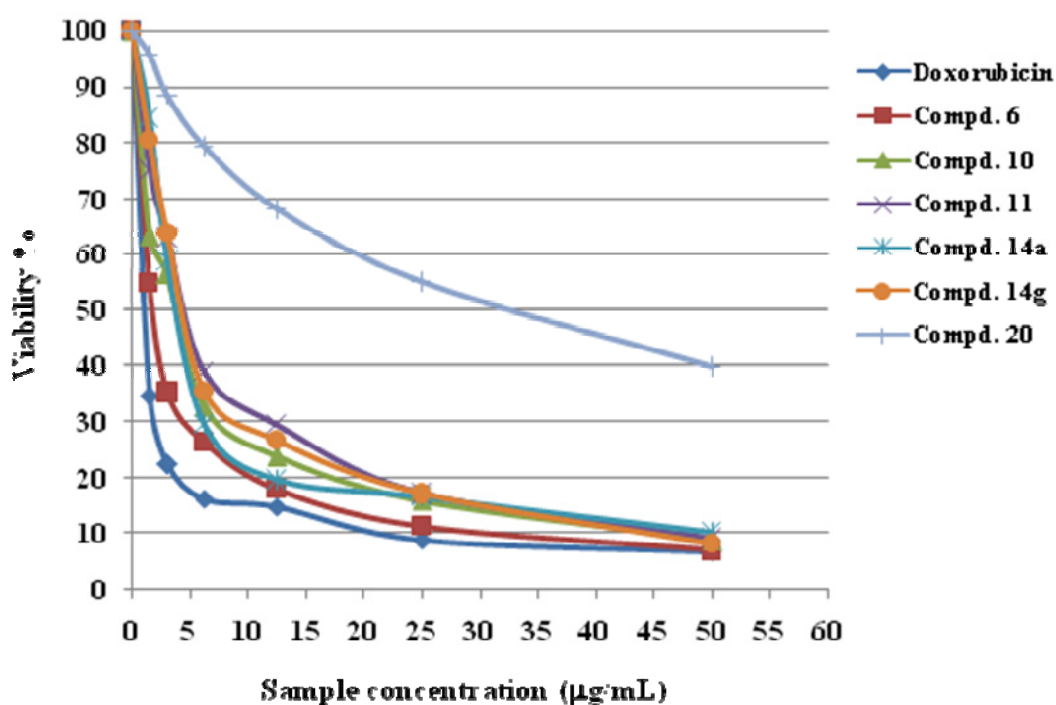
Table 2. Viability values of tested compounds against HCT-116

Sample conc. (µg/mL)	Viability %						
	Doxorubicin	6	10	11	14a	14g	20
50	6.82	6.97	8.71	9.04	10.38	8.24	39.61
25	8.89	11.28	15.94	17.12	16.53	17.08	54.92
12.5	14.83	17.94	23.62	29.46	19.46	26.71	68.19
6.25	16.16	26.25	32.46	38.84	29.33	35.35	79.24
3.125	22.28	35.12	56.28	62.93	58.72	63.87	88.36
1.56	34.64	54.83	63.31	75.1	84.51	80.54	95.80
0	100	100	100	100	100	100	100

**Table 3. IC<sub>50</sub> values of tested compounds  $\pm$  standard deviation against HCT-116**

Compound No.	IC <sub>50</sub>	Compound No.	IC <sub>50</sub>
Doxorubicin	0.469 $\pm$ 0.03	14a	4.1 $\pm$ 0.07
6	1.9 $\pm$ 0.05	14g	4.6 $\pm$ 0.016
10	3.9 $\pm$ 0.02	20	33 $\pm$ 0.18
11	4.8 $\pm$ 0.08		

**Figure 2. Dose-response curves for the cytotoxicity of compounds 6, 10, 11, 14a, 14g, and 20**



## CONCLUSION

New thiazolidin-4-one, 1,3-thiazines, arylazothiazoles, thiazoles, and thiazolin-4-one have been synthesized using ethylenedithiosemicarbazide as starting material under thermal conditions.

## EXPERIMENTAL

Melting points were measured on an Electrothermal IA 9000 series digital melting point apparatus. IR spectra were recorded in potassium bromide discs on Pye Unicam SP 3300 and Shimadzu FTIR 8101 PC infrared spectrophotometers. NMR spectra were recorded on a Varian Mercury VX-300 NMR spectrometer operating at 300 MHz (<sup>1</sup>H NMR) or 75 MHz (<sup>13</sup>C NMR) and run in deuterated



dimethylsulfoxide (DMSO-*d*<sub>6</sub>). Chemical shifts were related to that of the solvent. Mass spectra were recorded on a Shimadzu GCeMS-QP1000 EX mass spectrometer at 70 eV. Elemental analyzes were measured by using a German made Elementar vario LIII CHNS analyzer. Antitumor activity was evaluated by the Regional Center for Mycology and Biotechnology, Al-Azhar University, Cairo, Egypt. Hydrazonoyl halides<sup>32,33</sup> were prepared as previously reported in the respective literature.

***Synthesis of 1-[1-(2-mercapto-4-methyl-1-phenyl-1*H*-imidazol-5-yl)ethylidene]thiosemicarbazide (3).***

5-Acetyl-2-mercapto-4-methyl-1-phenyl-1*H*-imidazole (**1**) (11.5 g, 50 mmol) was dissolved in 100 mL of EtOH and stirred with an equimolar quantity of thiosemicarbazide for 24 h at room temperature with catalytic amounts of HCl. The desired thiosemicarbazone precipitated from reaction mixture was filtered, washed with EtOH and recrystallized from acetic acid to give pure product of compound **3**.

Yellow solid (12.2 g, 80%); mp 229 °C; IR (KBr):  $\nu$  3439, 3236 (NH<sub>2</sub>), 3155 (NH), 1587 (C=N) cm<sup>-1</sup>; <sup>1</sup>H-NMR (DMSO-*d*<sub>6</sub>):  $\delta$  2.35 (s, 3H, CH<sub>3</sub>), 2.96 (s, 3H, CH<sub>3</sub>), 4.31 (s, br, 2H, NH<sub>2</sub>), 6.99-7.66 (m, 5H, Ar-H), 10.45 (s, 1H, SH), 10.98 (s, 1H, NH); MS *m/z* (%): 305 (M<sup>+</sup>, 100), 230 (45), 91 (82), 77 (27). Anal. Calcd for C<sub>13</sub>H<sub>15</sub>N<sub>5</sub>S<sub>2</sub> (305.08): C, 51.12; H, 4.95; N, 22.93; S, 21.00. Found C, 51.31; H, 5.18; N, 23.12; S, 21.09%.

***Synthesis of methyl 2-[2-{1-(2-mercapto-4-methyl-1-phenyl-1*H*-imidazol-5-yl)ethylidene}hydrazono]-4-oxo-thiazolidin-5-ylidenethanoate (6).***

To a solution of 1-[1-(2-mercapto-4-methyl-1-phenyl-1*H*-imidazol-5-yl)ethylidene]thiosemicarbazide (**3**) (0.305 g, 1 mmol) in dry MeOH (20 mL) was added dimethyl acetylenedicarboxylate (0.142 g, 1 mmol). The solution was refluxed for 2 h. The precipitate was filtered, washed with MeOH, and recrystallized from EtOH to give product **6**.

Canary yellow solid (0.31 g, 75%); mp 352 °C; IR (KBr):  $\nu$  3247 (NH), 1704, 1694 (2C=O), 1606 (C=N) cm<sup>-1</sup>; <sup>1</sup>H-NMR (DMSO-*d*<sub>6</sub>):  $\delta$  2.53 (s, 3H, CH<sub>3</sub>), 3.35 (s, 3H, CH<sub>3</sub>), 3.77 (s, 3H, COOCH<sub>3</sub>), 6.64 (s, 1H, C=CH), 6.96-7.66 (m, 5H, Ar-H), 10.41 (s, 1H, SH), 12.77 (s, 1H, NH); MS *m/z* (%): 415 (M<sup>+</sup>, 68), 229 (68), 118 (68), 77 (100). Anal. Calcd for C<sub>18</sub>H<sub>17</sub>N<sub>5</sub>O<sub>3</sub>S<sub>2</sub> (415.08): C, 52.03; H, 4.12; N, 16.86; S, 15.43. Found C, 52.21; H, 4.18; N, 16.72; S, 15.29%.

***Reactions of 1-[1-(2-mercapto-4-methyl-1-phenyl-1*H*-imidazol-5-yl)ethylidene]thiosemicarbazide (3) with benzylidenemalononitrile (8a) and ethoxymethylenemalononitrile (8b).***

To a solution of 1-[1-(2-mercapto-4-methyl-1-phenyl-1*H*-imidazol-5-yl)ethylidene]thiosemicarbazide (**3**) (0.305 g, 1 mmol) in dry MeOH (20 mL) was added benzylidenemalononitrile (**8a**) or ethoxymethylenemalononitrile (**8b**) (1 mmol). The solution was refluxed for 2 h. The precipitate was

filtered, washed with methanol, and recrystallized from EtOH to give product **10** or **11**, respectively.

**2-[2-{1-(2-Mercapto-4-methyl-1-phenyl-1H-imidazol-5-yl)ethylidene}hydrazono]-4-amino-6-phenyl-6H-1,3-thiazine-5-carbonitrile (10).**

Yellow solid (0.32 g, 70%); mp 214 °C; IR (KBr):  $\nu$  3446-3196 (NH<sub>2</sub> + NH), 2264 (C $\equiv$ N), 1596 (C=N) cm<sup>-1</sup>; <sup>1</sup>H-NMR (DMSO-*d*<sub>6</sub>):  $\delta$  2.32 (s, 3H, CH<sub>3</sub>), 3.35 (s, 3H, CH<sub>3</sub>), 3.77 (s, 1H, thiazine-H), 4.65 (s, br, 2H, NH<sub>2</sub>), 7.04-7.75 (m, 10H, Ar-H), 10.41 (s, 1H, SH), 11.37 (s, 1H, NH); <sup>13</sup>C-NMR (DMSO-*d*<sub>6</sub>):  $\delta$  9.31 (CH<sub>3</sub>), 16.38 (CH<sub>3</sub>), 52.17 (CH), 108.51 (CN), 117.19, 118.63, 120.62, 120.91, 122.82, 123.45, 123.88, 124.02, 126.18, 128.79, 128.96, 129.52, 139.81, 142.32, 143.45 (Ar-C); MS *m/z* (%): 459 (M<sup>+</sup>, 52), 229 (100), 118 (31), 77 (83). Anal. Calcd for C<sub>23</sub>H<sub>21</sub>N<sub>7</sub>S<sub>2</sub> (459.13): C, 60.11; H, 4.61; N, 21.33; S, 13.95. Found C, 60.21; H, 4.48; N, 21.42; S, 13.99%.

**2-[2-{1-(2-Mercapto-4-methyl-1-phenyl-1H-imidazol-5-yl)ethylidene}hydrazono]-4-amino-1,3-thiazine-5-carbonitrile (11).**

Canary yellow solid (0.27 g, 72%); mp 212 °C; IR (KBr):  $\nu$  3429, 3238 (NH<sub>2</sub>), 2258 (C $\equiv$ N), 1598 (C=N) cm<sup>-1</sup>; <sup>1</sup>H-NMR (DMSO-*d*<sub>6</sub>):  $\delta$  2.38 (s, 3H, CH<sub>3</sub>), 3.05 (s, 3H, CH<sub>3</sub>), 4.64 (s, br, 2H, NH<sub>2</sub>), 6.99 (s, 1H, thiazine-H), 7.04-7.68 (m, 5H, Ar-H), 10.63 (s, 1H, SH); <sup>13</sup>C-NMR (DMSO-*d*<sub>6</sub>):  $\delta$  9.29 (CH<sub>3</sub>), 16.34 (CH<sub>3</sub>), 108.41 (CN), 117.09, 118.32, 120.61, 120.66, 122.82, 126.22, 128.89, 128.99, 129.32, 139.84, 142.11, 143.75 (Ar-C); MS *m/z* (%): 381 (M<sup>+</sup>, 6), 288 (97), 230 (63), 127 (58), 77 (100). Anal. Calcd for C<sub>17</sub>H<sub>15</sub>N<sub>7</sub>S<sub>2</sub> (381.08): C, 53.52; H, 3.96; N, 25.70; S, 16.81. Found C, 53.41; H, 4.08; N, 25.62; S, 16.91%.

**Synthesis of 2-[2-{1-(2-mercapto-4-methyl-1-phenyl-1H-imidazol-5-yl)ethylidene}hydrazono]-4-substituted-5-arylazothiazoles (14a-i).**

*General procedure:*

A mixture of 1-[1-(2-mercapto-4-methyl-1-phenyl-1H-imidazol-5-yl)ethylidene]thiosemicarbazide (**3**) (0.305 g, 1 mmol) and appropriate hydrazonoyl halides **12a-i** (1 mmol) in dioxane (30 mL) containing triethylamine (0.1 g, 1 mmol) was refluxed for 6-8 h. (monitored by TLC). The formed precipitate was isolated by filtration, washed with MeOH, dried and recrystallized from appropriate solvent to give products **14a-i**.

**2-[2-{1-(2-Mercapto-4-methyl-1-phenyl-1H-imidazol-5-yl)ethylidene}hydrazono]-4-methyl-5-phenyl-azothiazole (14a).**

Red solid (0.36 g, 80%); mp 266 °C; IR (KBr):  $\nu$  3213 (NH), 1594 (C=N) cm<sup>-1</sup>; <sup>1</sup>H-NMR (DMSO-*d*<sub>6</sub>):  $\delta$

2.48 (s, 3H, CH<sub>3</sub>), 3.29 (s, 3H, CH<sub>3</sub>), 3.57 (s, 3H, CH<sub>3</sub>), 6.97-7.63 (m, 10H, Ar-H), 10.50 (s, 1H, SH), 10.54 (s, 1H, NH); <sup>13</sup>C-NMR (DMSO-*d*<sub>6</sub>): δ 9.42 (CH<sub>3</sub>), 13.77 (CH<sub>3</sub>), 15.01 (CH<sub>3</sub>), 114.15, 118.45, 120.22, 121.15, 121.85, 123.25, 124.33, 125.75, 127.16, 127.31, 129.73, 131.61, 133.73, 138.61, 143.13 (Ar-C); MS *m/z* (%): 447 (M<sup>+</sup>, 41), 229 (61), 77 (100). Anal. Calcd for C<sub>22</sub>H<sub>21</sub>N<sub>7</sub>S<sub>2</sub> (447.13): C, 59.04; H, 4.73; N, 21.91; S, 14.33. Found C, 59.21; H, 4.88; N, 21.82; S, 14.19%.

***2-[2-{1-(2-Mercapto-4-methyl-1-phenyl-1H-imidazol-5-yl)ethylidene}hydrazono]-4-methyl-5-(4-methylphenylazo)thiazole (14b).***

Red solid (0.38 g, 82%); mp 254 °C; IR (KBr): ν 3281 (NH), 1601 (C=N) cm<sup>-1</sup>; <sup>1</sup>H-NMR (DMSO-*d*<sub>6</sub>): δ 2.25 (s, 3H, CH<sub>3</sub>), 2.57 (s, 3H, CH<sub>3</sub>), 3.29 (s, 3H, CH<sub>3</sub>), 3.56 (s, 3H, CH<sub>3</sub>), 7.03-7.62 (m, 9H, Ar-H), 10.47 (s, 1H, SH), 10.58 (s, 1H, NH); MS *m/z* (%): 461 (M<sup>+</sup>, 12), 229 (77), 91 (100). Anal. Calcd for C<sub>23</sub>H<sub>23</sub>N<sub>7</sub>S<sub>2</sub> (461.15): C, 59.84; H, 5.02; N, 21.24; S, 13.89. Found C, 59.71; H, 4.98; N, 21.32; S, 13.74%.

***2-[2-{1-(2-Mercapto-4-methyl-1-phenyl-1H-imidazol-5-yl)ethylidene}hydrazono]-4-methyl-5-(4-methoxyphenylazo)thiazole (14c).***

Red solid (0.37 g, 78%); mp 248 °C; IR (KBr): ν 3194 (NH), 1603 (C=N) cm<sup>-1</sup>; <sup>1</sup>H-NMR (DMSO-*d*<sub>6</sub>): δ 2.45 (s, 3H, CH<sub>3</sub>), 3.29 (s, 3H, CH<sub>3</sub>), 3.56 (s, 3H, CH<sub>3</sub>), 3.87 (s, 3H, OCH<sub>3</sub>), 7.06-7.72 (m, 9H, Ar-H), 10.48 (s, 1H, SH), 10.62 (s, 1H, NH); MS *m/z* (%): 477 (M<sup>+</sup>, 19), 229 (71), 91 (100). Anal. Calcd for C<sub>23</sub>H<sub>23</sub>N<sub>7</sub>OS<sub>2</sub> (477.14): C, 57.84; H, 4.85; N, 20.53; S, 13.43. Found C, 57.72; H, 4.91; N, 20.33; S, 13.24%.

***2-[2-{1-(2-Mercapto-4-methyl-1-phenyl-1H-imidazol-5-yl)ethylidene}hydrazono]-4-methyl-5-(4-chlorophenylazo)thiazole (14d).***

Red solid (0.39 g, 75%); mp 262 °C; IR (KBr): ν 3191 (NH), 1590 (C=N) cm<sup>-1</sup>; <sup>1</sup>H-NMR (DMSO-*d*<sub>6</sub>): δ 2.47 (s, 3H, CH<sub>3</sub>), 3.30 (s, 3H, CH<sub>3</sub>), 3.62 (s, 3H, CH<sub>3</sub>), 7.09-7.83 (m, 9H, Ar-H), 10.51 (s, 1H, SH), 10.80 (s, 1H, NH); MS *m/z* (%): 483 (M<sup>+</sup>+2, 12), 481 (M<sup>+</sup>, 30), 229 (100), 111 (50). Anal. Calcd for C<sub>22</sub>H<sub>20</sub>ClN<sub>7</sub>S<sub>2</sub> (481.09): C, 54.82; H, 4.18; N, 20.34; S, 13.30. Found C, 54.78; H, 4.28; N, 20.52; S, 13.19%.

***2-[2-{1-(2-Mercapto-4-methyl-1-phenyl-1H-imidazol-5-yl)ethylidene}hydrazono]-4-methyl-5-(4-bromophenylazo)thiazole (14e).***

Red solid (0.39 g, 75%); mp 270 °C; IR (KBr): ν 3171 (NH), 1584 (C=N) cm<sup>-1</sup>; <sup>1</sup>H-NMR (DMSO-*d*<sub>6</sub>): δ 2.48 (s, 3H, CH<sub>3</sub>), 3.31 (s, 3H, CH<sub>3</sub>), 3.62 (s, 3H, CH<sub>3</sub>), 7.07-7.84 (m, 9H, Ar-H), 10.50 (s, 1H, SH),

10.83 (s, 1H, NH); MS  $m/z$  (%): 527 ( $M^+ + 2$ , 12), 525 ( $M^+$ , 13), 229 (65), 77 (100). Anal. Calcd for  $C_{22}H_{20}BrN_7S_2$  (525.04): C, 50.19; H, 3.83; N, 18.62; S, 12.18. Found C, 50.28; H, 3.78; N, 18.82; S, 12.29%.

***2-[2-{1-(2-Mercapto-4-methyl-1-phenyl-1H-imidazol-5-yl)ethylidene}hydrazono]-4-methyl-5-(4-nitro-phenylazo)thiazole (14f).***

Dark red solid (0.34 g, 70%); mp 224 °C; IR (KBr):  $\nu$  3190 (NH), 1590 (C=N)  $cm^{-1}$ ;  $^1H$ -NMR (DMSO- $d_6$ ):  $\delta$  2.48 (s, 3H, CH<sub>3</sub>), 3.33 (s, 3H, CH<sub>3</sub>), 3.71 (s, 3H, CH<sub>3</sub>), 7.12-7.93 (m, 9H, Ar-H), 10.51 (s, 1H, SH), 10.92 (s, 1H, NH); MS  $m/z$  (%): 492 ( $M^+$ , 35), 229 (97), 118 (76), 77 (100). Anal. Calcd for  $C_{22}H_{20}N_8O_2S_2$  (492.12): C, 53.64; H, 4.09; N, 22.75; S, 13.02. Found C, 53.78; H, 4.18; N, 22.62; S, 13.15%.

***2-[2-{1-(2-Mercapto-4-methyl-1-phenyl-1H-imidazol-5-yl)ethylidene}hydrazono]-4-phenyl-5-phenyl-azothiazole (14g).***

Red solid (0.36 g, 70%); mp 352 °C; IR (KBr):  $\nu$  3192 (NH), 1602 (C=N)  $cm^{-1}$ ;  $^1H$ -NMR (DMSO- $d_6$ ):  $\delta$  3.24 (s, 3H, CH<sub>3</sub>), 3.29 (s, 3H, CH<sub>3</sub>), 6.99-8.29 (m, 15H, Ar-H), 10.53 (s, 1H, SH), 10.65 (s, 1H, NH);  $^{13}C$ -NMR (DMSO- $d_6$ ):  $\delta$  13.31 (CH<sub>3</sub>), 15.77 (CH<sub>3</sub>), 114.17, 115.93, 118.15, 119.33, 120.42, 121.17, 122.15, 123.25, 123.87, 124.36, 125.79, 126.11, 127.26, 127.41, 129.72, 131.57, 133.83, 137.62, 142.13 (Ar-C); MS  $m/z$  (%): 509 ( $M^+$ , 9), 231 (31), 77 (100). Anal. Calcd for  $C_{27}H_{23}N_7S_2$  (509.15): C, 63.63; H, 4.55; N, 19.24; S, 12.58. Found C, 63.51; H, 4.68; N, 19.12; S, 12.49%.

***2-[2-{1-(2-Mercapto-4-methyl-1-phenyl-1H-imidazol-5-yl)ethylidene}hydrazono]-4-phenyl-5-(4-chloro-phenylazo)thiazole (14h).***

Red solid (0.38 g, 70%); mp 316 °C; IR (KBr):  $\nu$  3193 (NH), 1604 (C=N)  $cm^{-1}$ ;  $^1H$ -NMR (DMSO- $d_6$ ):  $\delta$  3.26 (s, 3H, CH<sub>3</sub>), 3.32 (s, 3H, CH<sub>3</sub>), 7.03-8.29 (m, 14H, Ar-H), 10.54 (s, 1H, SH), 10.68 (s, 1H, NH); MS  $m/z$  (%): 545 ( $M^+ + 2$ , 3), 543 ( $M^+$ , 9), 231 (54), 111 (50), 77 (100). Anal. Calcd for  $C_{27}H_{22}ClN_7S_2$  (543.11): C, 59.60; H, 4.08; N, 18.02; S, 11.79. Found C, 59.41; H, 4.18; N, 18.12; S, 11.89%.

***2-[2-{1-(2-Mercapto-4-methyl-1-phenyl-1H-imidazol-5-yl)ethylidene}hydrazono]-4-(2-thienyl)-5-phenyl-azothiazole (14i).***

Red solid (0.36 g, 70%); mp 268 °C; IR (KBr):  $\nu$  3198 (NH), 1597 (C=N)  $cm^{-1}$ ;  $^1H$ -NMR (DMSO- $d_6$ ):  $\delta$  3.28 (s, 3H, CH<sub>3</sub>), 3.31 (s, 3H, CH<sub>3</sub>), 7.01-8.03 (m, 13H, Ar-H), 10.50 (s, 1H, SH), 10.62 (s, 1H, NH);  $^{13}C$ -NMR (DMSO- $d_6$ ):  $\delta$  13.33 (CH<sub>3</sub>), 15.87 (CH<sub>3</sub>), 113.77, 114.93, 118.15, 119.35, 120.72, 121.13, 122.19, 123.35, 123.82, 124.36, 125.77, 126.18, 127.26, 127.91, 129.79, 132.57, 133.81, 136.61, 143.63

(Ar-C); MS  $m/z$  (%): 515 ( $M^+$ , 9), 231 (31), 77 (100). Anal. Calcd for  $C_{25}H_{21}N_7S_3$  (515.10): C, 58.23; H, 4.10; N, 19.01; S, 18.65. Found C, 58.41; H, 4.18; N, 19.16; S, 18.49%.

**Reactions of 1-[1-(2-mercapto-4-methyl-1-phenyl-1H-imidazol-5-yl)ethylidene]thiosemicarbazide (3) with  $\alpha$ -haloketones and  $\alpha$ -haloacid.**

**General procedure:**

A mixture of **3** (0.305 g, 1 mmol) and  $\alpha$ -haloketones (**15a-d**) or chloroacetic acid (**18**) (1 mmol) in dioxane (30 mL) was refluxed for 4-6 h. (monitored by TLC). The product started to separate out during the course of reaction. The solid product was filtered, washed with water, dried and recrystallized from DMF to give the corresponding compounds **17a-d** or **20**, respectively.

**2-[2-{1-(2-Mercapto-4-methyl-1-phenyl-1H-imidazol-5-yl)ethylidene}hydrazono]-4-methylthiazole (17a).**

Yellow solid (0.28 g, 82%); mp 154 °C; IR (KBr):  $\nu$  3197 (NH), 1606 (C=N)  $cm^{-1}$ ;  $^1H$ -NMR (DMSO- $d_6$ ):  $\delta$  2.28 (s, 3H, CH<sub>3</sub>), 3.31 (s, 3H, CH<sub>3</sub>), 3.57 (s, 3H, CH<sub>3</sub>), 6.18 (s, 1H, thiazole-H), 6.92-7.62 (m, 5H, Ar-H), 10.30 (s, 1H, SH), 10.94 (s, 1H, NH);  $^{13}C$ -NMR (DMSO- $d_6$ ):  $\delta$  9.45 (CH<sub>3</sub>), 13.47 (CH<sub>3</sub>), 15.33 (CH<sub>3</sub>), 118.48, 120.53, 121.23, 123.15, 124.33, 127.21, 129.73, 131.61, 133.63, 138.65, 143.43 (Ar-C); MS  $m/z$  (%): 343 ( $M^+$ , 53), 231 (100), 113 (90), 77 (94). Anal. Calcd for  $C_{16}H_{17}N_5S_2$  (343.09): C, 55.95; H, 4.99; N, 20.39; S, 18.67. Found C, 55.81; H, 4.88; N, 20.22; S, 18.59%.

**2-[2-{1-(2-Mercapto-4-methyl-1-phenyl-1H-imidazol-5-yl)ethylidene}hydrazono]-4-phenylthiazole (17b).**

Yellow solid (0.30 g, 75%); mp 192 °C; IR (KBr):  $\nu$  3296 (NH), 1616 (C=N)  $cm^{-1}$ ;  $^1H$ -NMR (DMSO- $d_6$ ):  $\delta$  3.34 (s, 3H, CH<sub>3</sub>), 3.61 (s, 3H, CH<sub>3</sub>), 6.22 (s, 1H, thiazole-H), 7.12-7.92 (m, 10H, Ar-H), 10.34 (s, 1H, SH), 10.84 (s, 1H, NH);  $^{13}C$ -NMR (DMSO- $d_6$ ):  $\delta$  13.33 (CH<sub>3</sub>), 15.81 (CH<sub>3</sub>), 117.15, 119.33, 120.41, 121.17, 122.15, 123.25, 123.87, 124.36, 126.15, 127.24, 127.25, 131.59, 133.73, 137.52, 142.33 (Ar-C); MS  $m/z$  (%): 405 ( $M^+$ , 52), 231 (78), 176 (91), 134 (100), 113 (55), 77 (96). Anal. Calcd for  $C_{21}H_{19}N_5S_2$  (405.11): C, 62.19; H, 4.72; N, 17.27; S, 15.81. Found C, 62.11; H, 4.88; N, 17.42; S, 15.69%.

**2-[2-{1-(2-Mercapto-4-methyl-1-phenyl-1H-imidazol-5-yl)ethylidene}hydrazono]-4-methyl-5-acetylthiazole (17c).**

Yellow solid (0.29 g, 75%); mp 112 °C; IR (KBr):  $\nu$  3214 (NH), 1708 (C=O), 1602 (C=N)  $cm^{-1}$ ;  $^1H$ -NMR (DMSO- $d_6$ ):  $\delta$  2.31 (s, 3H, CH<sub>3</sub>), 2.42 (s, 3H, COCH<sub>3</sub>), 3.31 (s, 3H, CH<sub>3</sub>), 3.57 (s, 3H, CH<sub>3</sub>), 6.96-7.64

(m, 5H, Ar-H), 10.30 (s, 1H, SH), 10.90 (s, 1H, NH); MS  $m/z$  (%): 385 ( $M^+$ , 38), 230 (45), 189 (45), 148 (42), 112 (51), 77 (100). Anal. Calcd for  $C_{18}H_{19}N_5OS_2$  (385.10): C, 56.08; H, 4.97; N, 18.17; S, 16.64. Found C, 55.91; H, 4.88; N, 18.22; S, 16.59%.

**2-[2-{1-(2-Mercapto-4-methyl-1-phenyl-1H-imidazol-5-yl)ethylidene}hydrazono]-4-methyl-5-(N-phenyl-carbamoyl)thiazole (17d).**

Yellow solid (0.32 g, 70%); mp 185 °C; IR (KBr):  $\nu$  3233 (NH), 1671 (C=O), 1600 (C=N)  $cm^{-1}$ ;  $^1H$ -NMR (DMSO- $d_6$ ):  $\delta$  2.31 (s, 3H, CH<sub>3</sub>), 3.29 (s, 3H, CH<sub>3</sub>), 3.49 (s, 3H, CH<sub>3</sub>), 7.06-7.81 (m, 10H, Ar-H), 10.30 (s, 1H, SH), 10.90 (s, 1H, NH), 11.08 (s, 1H, NH); MS  $m/z$  (%): 462 ( $M^+$ , 13), 343 (26), 252 (98), 141 (100), 77 (69). Anal. Calcd for  $C_{23}H_{22}N_6OS_2$  (462.13): C, 59.72; H, 4.79; N, 18.17; S, 13.86. Found C, 59.91; H, 4.85; N, 18.12; S, 13.79%.

**2-[2-{1-(2-Mercapto-4-methyl-1-phenyl-1H-imidazol-5-yl)ethylidene}hydrazono]-4-oxo-4,5-dihydro-thiazole (20).**

Yellow solid (0.26 g, 75%); mp 278 °C; IR (KBr):  $\nu$  3246 (NH), 1697 (C=O), 1614 (C=N)  $cm^{-1}$ ;  $^1H$ -NMR (DMSO- $d_6$ ):  $\delta$  2.35 (s, 3H, CH<sub>3</sub>), 3.54 (s, 3H, CH<sub>3</sub>), 3.68 (s, 2H, CH<sub>2</sub>), 6.93-7.63 (m, 5H, Ar-H), 10.35 (s, 1H, SH), 10.84 (s, 1H, NH); MS  $m/z$  (%): 345 ( $M^+$ , 20), 344 (100), 229 (47), 118 (44), 77 (78). Anal. Calcd for  $C_{15}H_{15}N_5OS_2$  (345.07): C, 52.15; H, 4.38; N, 20.27; S, 18.56. Found C, 52.21; H, 4.28; N, 20.15; S, 18.49%.

## CYTOTOXIC ACTIVITY

Potential cytotoxicity of the compounds was tested using the method of Skehan *et al.*,<sup>34</sup> using Sulfo-Rhodamine-B stain (SRB). Cells were plated in 96-multiwell plates ( $10^4$  cells/well) for 24 h before treatment with the tested compound to allow attachment of cell to the wall of the plate. Different concentrations of the compound under test (0, 1.56, 3.125, 6.25, 12.5, 25, and 50  $\mu g/mL$ ) were added to the cell monolayer in triplicate wells individual dose, monolayer cells were incubated with the compounds for 48 h at 37 °C and in atmosphere of 5% CO<sub>2</sub>. After 48 h, cells were fixed, washed and stained with SRB stain, excess stain was washed with acetic acid and attached stain was recovered with *tris*-EDTA buffer, color intensity was measured in an ELISA reader. The relation between surviving fraction and drug concentration is plotted (Figure 2).

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