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Synthesis of some *p*-toluenesulfonyl-hydrazinothiazoles and hydrazino-bis-thiazoles and their anticancer activity

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ABSTRACT

A series of novel *p*-toluenesulfonyl-hydrazinothiazoles and hydrazino-bis-thiazoles derivatives (**2a**–**f**, **3a**–**f** and **5**–**8**) were synthesized by initial condensation of *p*-toluenesulfonylthiosemicarbazide **1** with a series of α-halogenocarbonyls in acetone or dimethylformamide (DMF)/acetone, mixture. All our synthesized compounds were submitted for further acylation reaction in the presence of acetic anhydride. The structures of newly synthesized derivatives **2a**–**f**, **3a**–**f** and **5**–**8** were confirmed by IR, ^{1}H-NMR, EIMS spectral data and elemental analysis. Compounds **2a**, **2c**, **2d**, **2e** and **3a** showed significant anticancer activities ($IC_{50} < 10$ μM) on both prostate DU-145 and hepatocarcinoma Hep-G2 cancer cell lines.

1. Introduction

The experimental research led to the identification of biologically active compounds with the sulphonyl-hydrazinic group. The thiazolyl group is also of great importance in treating biological systems. Compounds of this functional group showed antimicrobial [1,2], antitumoural [2–6], analgesic [1,7], anti-inflammatory and antipyretic [1] properties. Also, some synthetic thiazoles exhibited a wide range of biological activities, such as antitumor, antifilarial, antibacterial, antifungal, and anti-inflammatory [8]. The activities of hydrazinothiazole compounds, on Monoamine Oxidase Inhibitors (MAOI) [9], and the anti-thrombotic activities [10] of aryliden-thiosemicarbazole compounds were reported. Recent studies demonstrated significant antimicrobial and anticancer potencies of new synthetic thiazoles [11,12]. In the previous work, acid hydrazides were documented as important compounds, due to their high reactivity usefulness in heterorganic synthesis, as key starting materials to form various classes of biologically and pharmacologically active candidates [8,13]. Holla et al. [14] synthesized a series of aryliden-hydrazinothiazoles and 2-furanyliden-hydrazinothiazoles with antimicrobial and anti-inflammatory activities. Bhat and Holla [15] also used a series of acyl-thiosemicarbazide in the synthesis of thiazolo[2,3-c][1,2,4]triazoles. Such results encouraged us to study several-hydrazinothiazoles [16–19], for their biological properties. In view of these reports and in continuation of our previous works in heterocyclic chemistry, the present study was designed to synthesize new hydrazinothiazoles derivatives and investigate their cytotoxic activities. Compared with the activity of the reference antineoplastic compound (doxorubicin) on both prostate DU-145 and hepatocarcinoma Hep-G2 cancer cell lines, some of the compounds can be considered as good anticancer compounds. Compounds **3a**, **2e**, **2c**, **2d**, **2a** and **1** showed 100% inhibition of DU-145 proliferation respectively at 34.1; 35.2; 36.2; 38.5; 44.2 and 102 μM (Fig. 1). Total inhibition at 34.1; 35.2; 36.2; 44.2; 51 and 72.5 μM of Hep-G2 proliferation was also recorded with **3a**, **2e**, **2a**, **1** and **2c** respectively

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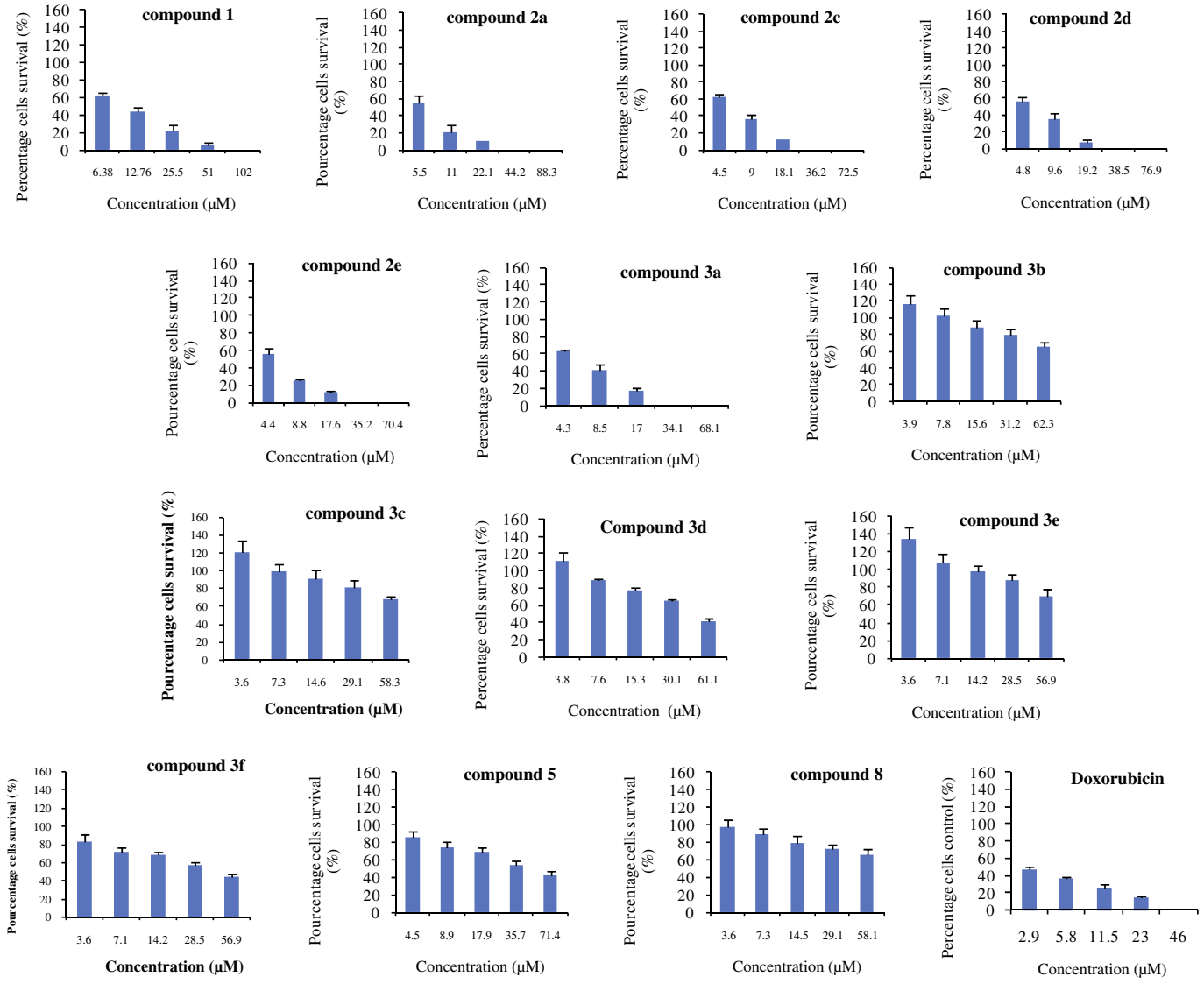


Fig. 1. Effects of the compounds on DU-145.

(Fig. 2). Though compound **1** (the substrate) was active on both DU-145 and Hep-G2, it can be noted that some of the synthesized derivatives were more effective, as they presented lower IC_{50} values. This includes compound **2a** and **2e** on the two cancer cell lines. Though not as active as doxorubicin, compounds **2a** ($IC_{50} < 5.65$ μM on Hep-G2) and **2e** (IC_{50} of 4.68 μM on DU-145 and below 4.51 μM on Hep-G2) showed the best anticancer activities (Table 1). When regarding the structure–activity relationship, it can mainly be deduced from the results obtained with compounds from series **2** (Compounds **2a**, **2c**, **2d**, **2e**, **2f**) and **3** (Compounds **3a**, **3c**, **3d**, **3e**, **3f**) that acetylation significantly reduced the anticancer activity. This hypothesis is confirmed when analysing the results of compounds **5** and **8** on the two cancer cell lines.

2. Results and discussion

For the synthesis of these organic compounds, we used the Hantzsch type condensation reaction with a *p*-toluenesulfonylthiosemicarbazide **1** as reagents, obtained by acylation of thiosemicarbazide with the *p*-toluenesulfochloride. The reaction was done in the presence of both NaOH and pyridine. The best result was obtained using the NaOH method.

The derivatives of *p*-toluenesulfonyl-hydrazinothiazole **2a**–**f** were synthesized with the condensation reaction of *p*-toluenesulfonylthiosemicarbazide **1** [20] and a series of α-halogenocarbonyls (chloroacetone; 1,3-dichloroacetone; α-bromoacetophenone; 3-chloroacetylacetone; ethyl α-bromoacetoacetate and ethyl γ-bromoacetoacetate), in acetone or DMF/acetone, mixture (Scheme 1). Compounds **3a**–**f** were obtained by the acylation reaction in the presence of acetic anhydride.

A series of compounds were also synthesized that were linked by two thiazole nucleus separated by the carbonylhydrazine (–CO–NH–NH–) group. For the synthesis of these organic compounds, the 4-(2-phenyl-thiazol-4-carbonyl)-thiosemicarbazide **4** was condensed with 1,3-dichloroacetone and ethyl γ-bromoacetoacetate respectively (Scheme 2).

The thiazolic compounds reacted with acetic anhydride by the acylation reaction (Scheme 2). In the synthesis of sulfonyl-hydrazinothiazole derivatives (Scheme 1), the hydrazinic group takes part in a double acylation with acetic anhydride, but in the case of thiazolyl-carbonylhydrazinothiazole, there was instead a monoacylation reaction with acetic anhyride.

No condensation and acylation reaction studies on synthesized *p*-toluenesulfonyl-hydrazinothiazole **2a**–**f**, **3a**–**f and** thiazolyl-carbonylhydrazinothiazole **5**–**8** derivatives, was previously

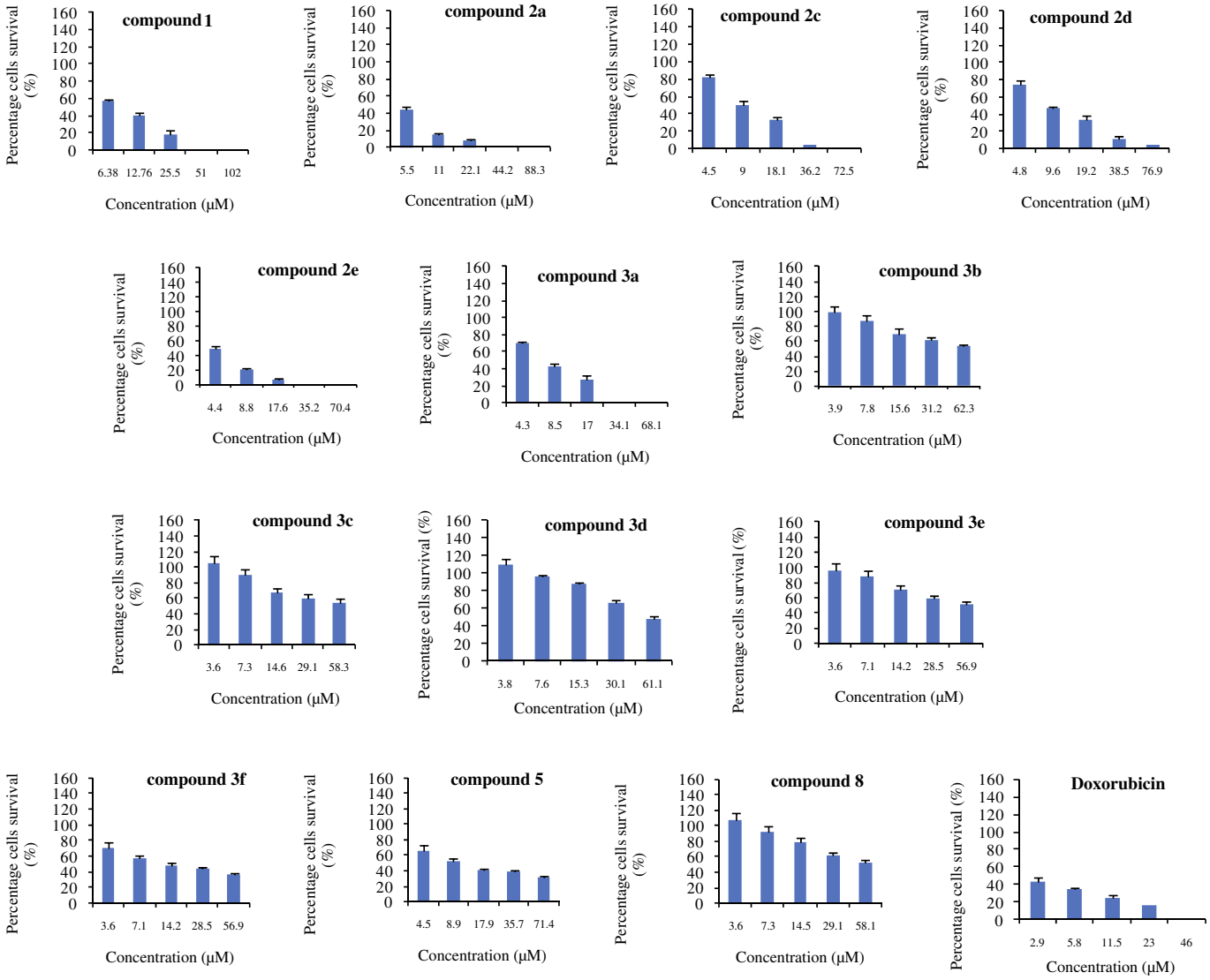


Fig. 2. Effects of the compounds on Hep-G2 cells survivals.

reported. The adduct (**2a**–**f**) and acylated (**3a**–**f**, **5**–**8**) products were isolated here for the first time via total synthesis by transformation of synthetics condensate **1** and **4** as a starting material. The structures of the synthesized compounds were assigned on the bases of its spectral data, mainly mass, elemental analysis, IR and ^{1}H-NMR (cf. Section 3).

In conclusion, a series of new *p*-toluenesulfonyl-hydrazinothiazoles and hydrazino-bis-thiazoles derivatives have been made conveniently available by direct condensation and acylation of a *p*-toluenesulfonylthiosemicarbazide with a series of α-halogenocarbonyls and acetic anhydride, respectively. Prostate carcinoma appears as the most common tumor in men [21]. Human liver tumors, particularly hepatocellular carcinoma, are among the most common malignancies worldwide [22], with an estimated annual incidence of 1 million cases, mainly in Africa and Asia [23]. This explains the used of both cell lines in the present work. The cut-off point for good cytotoxic compound has also been defined as 10 μM [24]. Therefore, the target compounds **2a**, **2c**, **2d**, **2e** and **3a** showed significant anticancer activities on both prostate DU-145 and hepatocarcinoma Hep-G2 cancer cell lines with IC_{50} values below 10 μM. However, the assays on normal cell lines are still to be done to confirm the significance of such compounds as potential anticancer drugs. The studies on the *p*-toluenesulfonyl-hydrazinothiazoles and hydrazino-bis-thiazoles derivatives as anticancer compounds are currently under investigation in our laboratory.

3. Experimental

3.1. Chemistry

Melting points were determined on open glass capillaries using an Electrothermal IA 9000 digital melting point apparatus and are uncorrected. The ^{1}H-NMR spectra were recorded with Bruker WM-300 and Bruker WM-400 spectrometer in the $CDCl_3$ or acetone-d_6. The mass spectra were recorded using a Varian MAT-311A and the IR spectra with IR-ART Spectrophotometer. The quantitative elemental analysis were recorded by the Vario EL analyser.

3.1.1. *p*-Toluenesulfonylthiosemicarbazide (**1**)

Ten millimoles of thiosemicarbazide was dissolved in 5 ml of pyridine. In the solution obtained, 10 mmol of *p*-toluenesulfonyl-chloride (with cooling and shaking), and continuous shaking at room temperature for 24 h. The same volume of water was added and then acidified with conc. CH_3COOH. The precipitate obtained, was filtered, purified by re-crystallization in ethanol. Yield 75%;

Table 1
Activity of the some of the compounds and doxorubicin on DU-145 and Hep-G2 cancer cell lines.

Samples	DU-145	Hep-G2
	IC_{50} (μM)	IC_{50} (μM)
1	8.98	8.98
2a	6.96	<5.65
2c	9.07	9.16
2d	9.84	8.77
2e	4.68	<4.51
3a	7.63	7.36
3b	>62.34	>62.34
3c	>58.27	>58.27
3d	53.49	57.40
3e	>56.95	>56.95
3f	46.36	10.96
5	49.23	11.23
8	>58.14	>58.14
Doxorubicin	<2.94	<2.94

white crystals; mp = 198–199 °C (mp = 192 °C lit. [25], mp = 214–215 °C lit. [26]); IR (KBr cm^{-1}): 3453, 3338 (νNH), 3188, 2993 (νCH), 1339, 1158 (νSO2), 1280 (νC═S); EIMS: *m/z*: 245 (M^+), 156, 139, 107, 91 (100%), 77, 61, 45, 39; ^{1}H-NMR ($CDCl_3$) δ(ppm): 2.43 (s, 3H, *CH_3*), 7.31 (d, 2H, *J* = 8.3 Hz, *C_6H_4-meta*), 7.82 (d, 2H, *J* = 8.3 Hz, *C_6H_4-ortho*), 8.48 (l.b., 2H, *–NH–NH–*), 8.57 (s, 2H, *–NH_2*) (lit. [27]); Anal. Calcd. for $C_8H_{11}N_3O_2S_2$: C, 41.52%; H, 5.42%; N, 16.14%; S, 24.63%. Found: C, 40.39%; H, 5.03%; N, 15.99%; S, 23.87%.

3.1.2. General procedure for the synthesis of arylsulfonyl-hydrazinothiazole (**2a–f**)

Two millimoles of arylsulfonyl-thiosemicarbazide were dissolved in a mixture of DMF anh. (5 ml) and acetone anh.(5 ml) and then 2.2 mmol α-halogenocarbonyl-derivative were added. The reaction mixture was stirred at room temperature for 24 h. The reaction progress was monitored by TLC. The reaction mixture was cooled with ice and neutralized with $NaHCO_3$ solution. The precipitate obtained was filtered and purified by re-crystallization in ethanol.

*3.1.2.1. 4-Methyl-2-[2-(p-toluenesulfonyl)-hydrazino]-thiazole (**2a**).* Yield 60.23%; white-yellow crystals; mp = 162–164 °C; IR (KBr cm^{-1}): 3248, 3206 (νNH), 3078, 2921, 2857 (νCH), 1314, 1169 (νSO_2); EIMS: *m/z*: 283 (M^+), 278, 252, 246, 232, 220, 156, 139, 128 (100%), 99, 91, 71, 65, 45, 39; ^{1}H-NMR ($CDCl_3$) δ(ppm): 2.07 (s, 3H, *CH_3*), 2.40 (s, 3H, *CH_3*), 6.71 (s, 1H, Th-*CH*), 7.38 (d, 2H, *J* = 8.4 Hz, *C_6H_4-meta*), 7.86 (d, 2H, *J* = 8.4 Hz, *C_6H_4-ortho*), 9.27 (s, 1H, *NH*) 9.31 (s, 1H, *NH*).

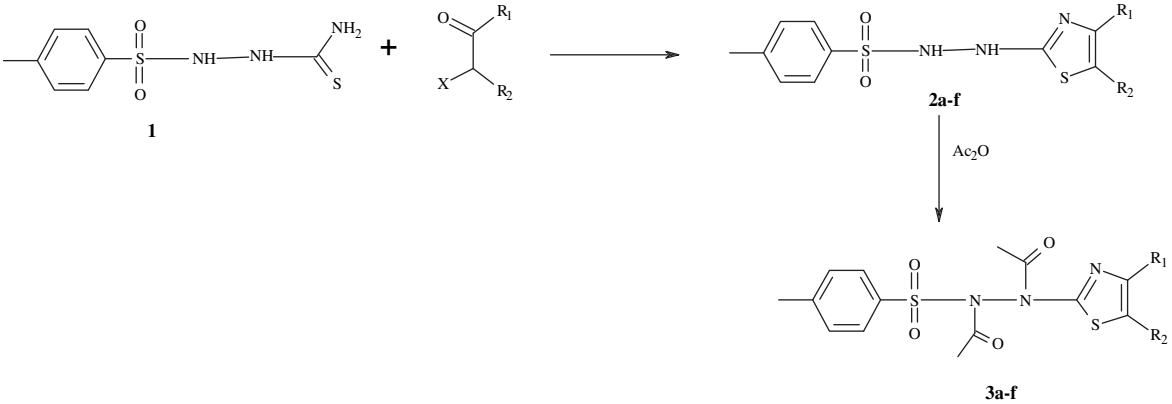
Anal. Calcd. for $C_{11}H_{13}N_3O_2S_2$: C, 46.63%; H, 4.62%; N, 14.83%; S, 22.63%. Found: C, 46.55%; H, 4.56%; N, 14.73%; S, 22.95%.

*3.1.2.2. 4-Chloromethyl-2-[2-(p-toluenesulfonyl)-hydrazino]-thiazole (**2b**).* Yield 66.23%; white-yellow crystals; mp = 188–189 °C; IR (KBr cm^{-1}): 3269, 3217 (νNH), 3086, 2955, 2858 (νCH), 1343, 1165 (νSO_2); EIMS: *m/z*: 317 (M^+), 319 (M + 2), 253, 162, 156, 143, 139, 132, 107, 98, 91 (100%), 77, 71, 65, 59, 45, 39; ^{1}H-NMR ($CDCl_3$) δ (ppm): 2.35 (s, 3H, *CH_3*), 4.39 (s, 2H, *CH_2Cl*), 6.82 (s, 1H, Th-*CH*), 7.33 (d, 2H, *J* = 8.42 Hz, *C_6H_4-meta*), 7.75 (d, 2H, *J* = 8.42 Hz, *C_6H_4-ortho*), 9.05 (s,1H, *NH*), 9.37 (s, 1H, *NH*); Anal. Calcd. for $C_{11}H_{12}ClN_3O_2S_2$: C, 34.21%; H, 2.87%; N, 9.97%; S, 15.22%. Found: C, 34.20%; H, 2.78%; N, 9.92%; S, 15.18%.

*3.1.2.3. 4-Phenyl-2-[2-(p-toluenesulfonyl)-hydrazino]-thiazole (**2c**).* Yield 66.23%; white-yellow crystal; mp = 179–180 °C; IR (KBr cm^{-1}): 3261, 3177 (νNH), 3117, 3078, 2950, 2867 (νCH), 1331, 1164 (νSO_2); EIMS: *m/z*: 345 (M^+), 281, 252, 189, 161, 134, 102 (100%), 91, 77, 65, 63, 45, 39; ^{1}H-NMR (CDCl3) δ(ppm): 2.41 (s, 3H, *CH_3*), 7.26–7.34 (m, 5H, Ar*H*), 7.72–7.81 (m, 4H, Ar*H*), 7.17 (s, 1H, Th-*CH*), 9.16 (l.b.,1H, *NH*), 9.34 (s, 1H, *NH*); Anal. Calcd. for $C_{16}H_{15}N_3O_2S_2$: C, 55.63%; H, 4.38%; N, 12.16%; S, 18.56%. Found: C, 55.60%; H, 4.33%; N, 12.12%; S, 18.45%.

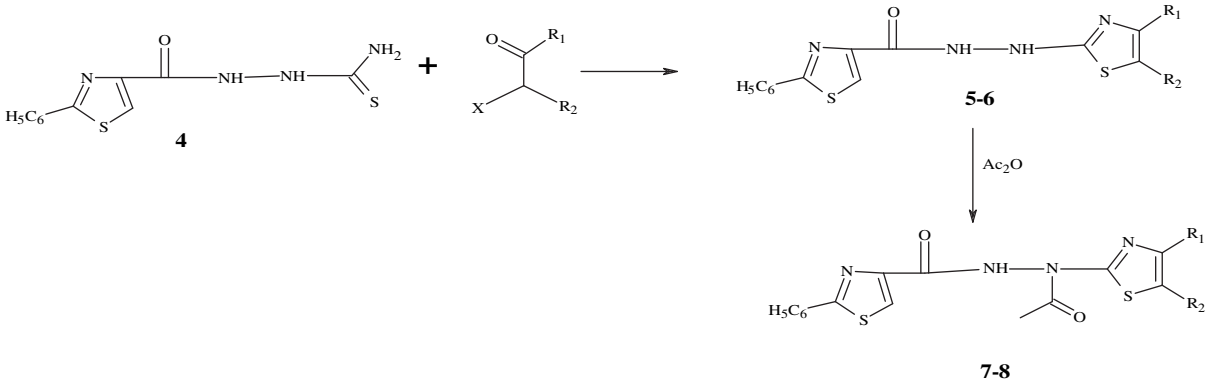
*3.1.2.4. 5-Acetyl-4-methyl-2-[2-(p-toluenesulfonyl)-hydrazino]-thiazole (**2d**).* Yield 58.62%; white-yellow crystal; mp = 210–211 °C; IR (KBr cm^{-1}): 3056, 2857 (νCH), 1598 (νCO), 1325, 1164 (νSO_2); EIMS: *m/z*: 325 (M^+), 262, 232, 227, 169, 156, 140, 126, 106, 91, 83, 65, 43 (100%), 39; ^{1}H-NMR (acetone) δ(ppm): 2.38 (s, 3H, *CH_3*), 2.44 (s, 3H, *CH_3*), 2.46 (s, 3H, *CH_3*), 7.31 (d, 2H, *J* = 8.4 Hz, *C_6H_4-meta*), 7.76 (d, 2H, *J* = 8.4 Hz, *C_6H_4-ortho*), 9.07 (s, 1H, *NH*), 9.12 (s, 1H, *NH*);Anal. Calcd. for $C_{13}H_{15}N_3O_3S_2$: C, 47.98%; H, 4,65%; N, 12.91%; S, 19.70%. Found: C, 47.90%; H, 4.33%; N, 12.62%; S, 19.48%.

*3.1.2.5. 5-Ethoxycarbonyl-4-methyl-2-[2-(p-toluenesulfonyl)-hydrazino]-thiazole (**2e**).* Yield 66.43%; white-yellow crystal; mp = 180–81 °C; IR (KBr cm^{-1}): 3170 (νNH), 2937, 2864 (νCH), 1681 (νCO ester), 1333, 1168 (νSO_2); EIMS: *m/z*: 355 (M^+), 309, 292, 261, 200, 171, 155, 138, 126, 107, 91 (100%), 85, 67, 45; ^{1}H-NMR ($CDCl_3$) δ (ppm): 1.35 (t, 3H, *J* = 6.9 Hz, *CH_3*), 2.34 (s, 3H, *CH_3*), 2.37 (s, 3H,



	2a, 3a	2b, 3b	2c, 3c	2d, 3d	2e, 3e	2f, 3f
R_1	CH_3	CH_2Cl	C_6H_5	CH_3	CH_3	$CH_2COOC_2H_5$
R_2	H	H	H	$COCH_3$	$COOC_2H_5$	H

Scheme 1. Synthesis of compounds **3a–f**.



	5, 7	6, 8
R_1	CH_2Cl	$CH_2COOC_2H_5$
R_2	H	H

Scheme 2. Synthesis of compound **5**–**8** deriviatives.

CH$_3$), 4.28 (q, 2H, *J* = 6.9 Hz, *CH*$_2$), 7.33 (d, 2H, *J* = 8.4 Hz, C$_6$*H*$_4$-*meta*), 7.75 (d, 2H, *J* = 8.4 Hz, C$_6$*H*$_4$-*ortho*), 9.12 (s, 1H, *NH*), 9.16 (s, 1H, *NH*); Anal. Calcd. for $C_{14}H_{17}N_3O_4S_2$: C, 47.31%; H, 4.82%; N, 11.82%; S, 18.04%. Found: C, 47.30%; H, 4.80%; N, 11.82%; S, 18.04%.

*3.1.2.6. 4-Ethyl acetate-2-[2-(p-toluenesulfonyl)-hydrazino]-thiazole (**2f**).* Yield 60.64%; white cyrstals; mp = 192–193 °C; IR (KBr cm^{-1}): 3113, 2982, 2936, 2903 (*ν*CH), 1732, 1707 (*ν*CO ester), 1364, 1168 (*ν*SO$_2$); EIMS: *m*/*z*: 355 (M$^+$), 309, 284, 200, 171, 107, 91 (100%), 65, 43; ^{1}H-NMR (CDCl$_3$) *δ*(ppm): 1.07 (t, 3H, *J* = 7.2 Hz, *CH*$_3$), 2.35 (s, 3H, C*H*$_3$), 3.55 (s, 2H, C*H*$_2$), 4.01 (q, 2H, *J* = 7.2 Hz, C*H*$_2$), 6.83 (s, 1H, Th-CH), 7.49 (d, 2H, *J* = 8.4 Hz, C$_6$*H*$_4$-*meta*), 7.72 (d, 2H, *J* = 8.4 Hz, C$_6$*H*$_4$-*ortho*), 8.97 (s, 1H, *NH*), 9.14 (s, 1H, *NH*); Anal. Calcd. for $C_{14}H_{17}N_3O_4S_2$: C, 47.31%; H, 4.82%; N, 11.82%; O, 18.01%; S, 18.04%. Found: C, 48.10%; H, 4.48%; N, 11.31%, S, 17.40%.

*3.1.3. General procedure for the synthesis of [2-(2-phenyl-1,3-thiaz olo-4-carbonyl)-hydrazino]-thiazoles (**5**,**6**)*

Two millimoles of 4-(2-phenyl-1,3-thiazolo-4-carbonyl)-thiosemicarbazide [20], were dissolved in a mixture of DMF anh. (3 ml) and acetone anh.(5 ml) and then 2.2 mmol α-halogenocarbonyl-derivative were added. The reaction mixture was stirred at room temperature for 48 h. The reaction progress was monitored by TLC. The reaction mixture was cooled with ice and neutralized with $NaHCO_3$ solution. The precipitate obtained was filtered and purified by re-crystallization in ethanol.

*3.1.3.1. 4-Chloromethyl-2-[2-(2-phenyl-1,3-thiazolo-4-carbonyl)-hyd razino]-thiazole (**5**).* Yield 58.63%; white-yellow crystals; mp = 220–221 °C; IR (KBr cm^{-1}): 3272, 3170 (*ν*NH), 3113, 3091, 3062, 2950, 2857 (*ν*CH), 1673 (*ν*CO amide); EIMS: *m*/*z*: 350 (M$^+$) 352 (M + 2), 204, 187, 160, 121, 101, 94, 77, 71, 57, 36 (100%); ^{1}H-NMR (CDCl$_3$) *δ*(ppm): 4.5 (s, 2H, *CH*$_2$*Cl*), 7.21 (s, 1H, Th-*CH*), 7.46–7.48 (m, 3H, *ArH*), 8.02–8.06 (m, 2H, *ArH*), 8.31 (s, 1H, Th-*CH*), 9.29 (s, 1H, *NH*), 10.42 (s, 1H, *NH*); Anal. Calcd. for $C_{14}H_{11}ClN_4OS_2$: C, 47.93%; H, 3.16%; N, 15.97%; S, 18.28%. Found: C, 47.90%; H, 3.10%; N, 15.65%; S, 18.29%.

*3.1.3.2. 4-Ethyl acetate-2-[2-(2-phenyl-1,3-thiazolo-4-carbonyl)-hydrazino]-thiazole (**6**).* Yield 53.41%; white-yellow crystal; mp = 204–205 °C; IR (KBr cm^{-1}): 3270, 3168 (*ν*NH), 3113, 3091, 3062, 2950, 2857 (*ν*CH), 1707 (*ν*CO ester), 1673 (*ν*CO amide); EIMS: *m*/*z*: 388 (M$^+$), 301, 218, 203 (100%), 200, 187, 160, 121, 101, 94, 87, 77, 71, 57; ^{1}H-NMR (CDCl$_3$) *δ*(ppm): 1.07 (t, 3H, *J* = 7.2 Hz, C*H*$_3$), 3.56 (s, 2H, *CH*$_2$), 3.99 (q, 2H, *J* = 7.2 Hz, *CH*$_2$), 6.82 (s, 1H, Th-*CH*), 7.43–7.45 (m, 3H, *ArH*), 7.93–7.96 (m, 2H, *ArH*), 8.29 (s, 1H, Th-*CH*), 9.24 (s, 1H, *NH*), 10.32 (s, 1H, *NH*); Anal. Calcd. for $C_{17}H_{16}N_4O_3S_2$: C, 52.56%; H, 4.15%; N, 14.42%; S, 16.51%. Found: C, 52.96%; H, 4.12%; N, 14.03%; S, 17.19%.

3.1.4. General procedure of acetylation

Two millimoles of hydrazinothiazole were treated with 2 ml of acetic anhydride and two drops (catalytic amounts) of pyridine. The resulting mixture was heated for 5 min at 139 °C and then stirred at room temperature for 24 h. Before adding the ethanol solution on the reaction mixture, acetic anhydride was evaporated under reduced pressure. The obtained solid was re-crystallized from ethanol to afford compounds **3a**–**f** and **7**–**8**, respectively.

*3.1.4.1. 4-Methyl-2-[2-(p-toluenesulfonyl)-N,N-diacetyl-hydrazino]-thiazole (**3a**).* Yield 61.64%; white-yellow crystal; mp = 128–130 °C; IR (KBr cm^{-1}): 3110, 2922 (*ν*CH), 1695, 1698 (*ν*CO amide), 1367, 1162 (*ν*SO$_2$); EIMS: *m*/*z*: 367 (M$^+$), 325, 212, 170, 128, 100, 91, 65, 43 (100%), 39; ^{1}H-NMR (CDCl$_3$) *δ*(ppm): 2.23 (s, 3H, *CH*$_3$), 2.42 (s, 3H, *CH*$_3$), 2.46 (s, 3H, *CH*$_3$), 2.48 (s, 3H, C*H*$_3$), 6.91 (s, 1H, Th-*CH*), 7.39 (d, 2H, *J* = 8.2 Hz, C$_6$*H*$_4$-*meta*), 8.04 (d, 2H, *J* = 8.2 Hz, C$_6$*H*$_4$-*ortho*); Anal. Calcd. for $C_{15}H_{17}N_3O_4S_2$: C, 49.03%; H, 4.66%; N, 11.44%; S, 17.45%. Found: C, 49.10%; H, 4.48%; N, 11.31%; S, 17.40%.

*3.1.4.2. 4-Chloromethyl-2-[2-(p-toluenesulfonyl)-N,N-diacetyl-hydrazino]-thiazole (**3b**).* Yield 65.43%; white-yellow crystal; mp = 152–153 °C; IR (KBr cm^{-1}): 3108, 3074, 3058 (*ν*CH), 1702 (*ν*CO amide), 1358, 1166 (*ν*SO$_2$); EIMS: *m*/*z*: 401 (M$^+$), 403 (M + 2), 359, 342, 316, 246, 203, 161, 154, 139, 133, 119, 107, 91, 65, 43 (100%), 39; ^{1}H-NMR (CDCl$_3$) *δ*(ppm): 2.23 (s, 3H, *CH*$_3$), 2.41 (s, 3H, *CH*$_3$), 2.53 (s, 3H, *CH*$_3$), 4.51 (s, 2H, *CH*$_2$*Cl*), 6.93 (s, 1H, Th-*CH*), 7.34 (d, 2H, *J* = 8.2 Hz, C$_6$*H*$_4$-*meta*), 8.04 (d, 2H, *J* =8.2 Hz, C$_6$*H*$_4$-*ortho*); Anal. Calcd. for $C_{15}H_{16}ClN_3O_4S_2$: C, 44.83%; H, 4.01%; N, 10.46%; S, 15.95%. Found: C, 44.83%; H, 4.01%; N, 10.46%; S, 15.95%.

*3.1.4.3. 4-Phenyl-2-[2-(p-toluenesulfonyl)-N,N-diacetyl-hydrazino]-thiazole (**3c**).* Yield 56.39%; white crystal; mp = 142–144 °C; IR (KBr cm^{-1}): 3117, 3048, 2925 (*ν*CH), 1724, 1704 (*ν*CO amide), 1370, 1169 (*ν*SO$_2$); EIMS: *m*/*z*: 429 (M$^+$), 387, 274, 232 (100%), 190, 162, 134, 102, 91, 77, 65, 43; ^{1}H-NMR (CDCl$_3$) *δ*(ppm): 2.23 (s, 3H, *CH*$_3$), 2.32 (s, 3H, *CH*$_3$), 2.43 (s, 3H, *CH*$_3$), 7.05 (s, 1H, Th-*CH*), 7.32–7.41 (m, 4H, Ar*H*), 7.81–7.86 (m, 5H, Ar*H*); Anal. Calcd. for $C_{20}H_{19}N_3O_4S_2$: C, 55.93%; H, 4.46%; N, 9.78%; S, 14.93%. Found: C, 55.93%; H, 4.46%; N, 9.85%; S, 14.90%.

*3.1.4.4. 5-Acetyl-4-methyl-2-[2-(p-toluenesulfonyl)-N,N-diacetyl-hydrazino]-thiazole (**3d**).* Yield 65.64%; white crystal; mp = 150–151 °C; IR (KBr cm^{-1}): 3078, 3025, 2922 (νCH), 1731, 1719, 1649 (νCO cetone, amide), 1373, 1165 (νSO_2); EIMS: *m/z*: 409 (M^+), 367, 253, 212, 170, 142, 91, 83, 65, 43 (100%); ^{1}H-NMR ($CDCl_3$) δ(ppm): 2.36 (s, 3H, *CH*$_3$), 2.38 (s, 3H, *CH*$_3$), 2.41 (s, 3H, *CH*$_3$), 2.45 (s, 3H, C*H*$_3$), 2.46 (s, 3H, C*H*$_3$), 7.45 (d, 2H, J = 8.2 Hz, C$_6$*H*$_4$-*meta*), 7.79 (d, 2H, J = 8.2 Hz, C$_6$*H*$_4$-*ortho*); Anal. Calcd. for $C_{17}H_{19}N_3O_5S_2$: C, 49.87%; H, 4.68%; N, 10.26%; S, 15.66%. Found: C, 49.70%; H, 4.48%; N, 10.31%; S, 15.40%.

*3.1.4.5. 5-Ethoxycarbonyl-4-methyl-2-[2-(p-toluensulfonyl)-N,N-diacetyl-hydrazino]-thiazole (**3e**).* Yield 61.43%; white-yellow crystals; mp = 162–163 °C; IR (KBr cm^{-1}): 3106, 3072, 3025, 2981, 2905 (νCH), 1730, 1683 (νCO ester, amide), 1370, 1169 (νSO_2); EIMS: *m/z*: 439 (M^+), 396, 380, 284, 242, 200, 172, 154, 143, 138, 91, 65, 43 (100%); ^{1}H-NMR ($CDCl_3$) δ(ppm): 1.38 (t, 3H, J = 7.0 Hz, *CH*$_3$), 2.24 (s, 3H, *CH*$_3$), 2.41 (s, 3H, *CH*$_3$), 2.44 (s, 3H, C*H*$_3$), 2.46 (s, 3H, C*H*$_3$), 4.31 (q, 2H, J = 7.0 Hz, *CH*$_2$), 7.38 (d, 2H, J = 8.2 Hz, C$_6$*H*$_4$-*meta*), 7.81 (d, 2H, J = 8.2 Hz, C$_6$*H*$_4$-*ortho*); Anal. Calcd. for $C_{18}H_{21}N_3O_6S_2$: C, 47.31%; H, 4.82%; N, 11.82%; S, 18.04%. Found: C, 47.30%; H, 4.80%; N, 11.82%; S, 18.04%.

*3.1.4.6. 4-Ethyl acetate-2-[2-(p-toluenesulfonyl)-N,N-diacetyl-hydrazino]-thiazole (**3f**).* Yield 60.64%; white crystal; mp = 128–30 °C; IR (KBr cm^{-1}): 3113, 2982, 2936, 2903 (νCH), 1732, 1683 (νCO ester, amide), 1364, 1168 (νSO_2); EIMS: *m/z*: 439 (M^+), 396, 380, 366, 324, 284, 242 (100%), 200, 171, 139, 91, 65, 43; ^{1}H-NMR ($CDCl_3$) δ(ppm): 1.20 (t, 3H, J = 7.2 Hz, *CH*$_3$), 2.24 (s, 3H, *CH*$_3$), 2.41 (s, 3H, *CH*$_3$), 2.47 (s, 3H, *CH*$_3$), 3.74 (s, 2H, C*H*$_2$), 4.22 (q, 2H, J = 7.2 Hz, C*H*$_2$), 6.91 (s, 1H, Th-*CH*), 7.48 (d, 2H, J = 8.2 Hz, C$_6$*H*$_4$-*meta*), 7.93 (d, 2H, J = 8.2 Hz, C$_6$*H*$_4$-*ortho*); Anal. Calcd. for $C_{18}H_{21}N_3O_6S_2$: C, 50.03%; H, 4.66%; N, 10.44%; S, 17.45%. Found: C, 49.10%; H, 4.48%; N, 11.31%; S, 17.40%.

*3.1.4.7. 4-Chloromethyl-2-[2-(2-phenyl-1,3-thiazolo-4-carbonyl)-N-acetyl-hydrazino]-thiazole (**7**).* Yield 54.33%; white-yellow crystals; mp = 181–182 °C; IR (KBr cm^{-1}): 3108, 3082, 3050, 2984, 2961, 2905 (νCH), 1700 (νCO amide); EIMS: *m/z*: 392 (M^+), 341 (M + 2), 260, 232, 203, 188, 160, 132, 116, 57, 43 (100%); ^{1}H-NMR ($CDCl_3$) δ (ppm): 2.38 (s, 3H, *CH*$_3$), 4.52 (s, 2H, *CH*$_2$*Cl*), 7.32 (s, 1H, Th-*CH*), 7.47–7.49 (m, 3H, *ArH*), 8.04–8.09 (m, 2H, *ArH*), 8.35 (s, 1H, Th-*CH*) 9.67 (s, 1H, *NH*); Anal. Calcd. for $C_{16}H_{13}ClN_4O_2S_2$: C, 48.91%; H, 3.34%; Cl, 9.02%; N, 14.26%; O, 8.14%; S, 16.32%. Found: C, 48.9%; H, 3.15%; N, 14.34%; S, 16.41%.

*3.1.4.8. 4-Ethylacetate-2-[2-(2-phenyl-1,3-thiazolo-4-carbonyl)-N-acetyl-hydrazino]-thiazole (**8**).* Yield 58.63%; white-yellow crystals; mp = 160–162 °C; IR (KBr cm^{-1}): 3108, 3082, 3050, 2984, 2961, 2905 (νCH), 1724, 1700 (νCO ester, amide); EIMS: *m/z*: 430 (M^+), 387, 356, 342, 314, 200, 188, 160, 116, 57, 43 (100%); ^{1}H-NMR ($CDCl_3$) δ(ppm): 1.06 (t, 3H, J = 7.2 Hz, *CH*$_3$), 2.38 (s, 3H, *CH*$_3$), 3.58 (s, 2H, *CH*$_2$), 4.12 (q, 2H, J = 7.2 Hz, *CH*$_2$), 6.98 (s, 1H, Th-*CH*), 7.43–7.46 (m, 3H, *ArH*), 7.93–7.99 (m, 2H, *ArH*), 8.31 (s, 1H, Th-*CH*), 9.67 (s, 1H, –NH); Anal. Calcd. for $C_{19}H_{18}N_4O_4S_2$: C, 47.93%; H, 3.16%; N, 15.97%; S, 18.28%. Found: C, 47.90%; H, 3.10%; N, 15.65%; S, 18.29%.

3.2. Cytotoxicity assay

3.2.1. Antitumor assay on Human DU-145 prostate carcinoma and Hepatoma Hep-G2 cells

The cytotoxic activity of samples were tested on Human DU-145 (androgen-insensitive prostate cancer cells) and Hepatocarcinoma Hep-G2, following the XTT (2,3-bis[2-methoxy-4-nitro-5-sulfophenyl]-2H-tetrazolium-5-carboxyanilide inner salt) assay [28–30].

Briefly, cells were cultured in Dulbecco's Minimum Essential Medium (DMEM) supplemented with 5% Foetal Calf Serum (FCS), gentamicin sulphate (0.004%), glucose (0.57%) and $NaHCO_3$ (0.12%). Cells were seeded into 96-well flat-bottomed plates at a concentration of 3.0×10^5 cells per ml. After 24 h, cells were treated with samples, which were diluted with culture medium to a final concentration of 25 μg/ml [29, 30]. XTT labelling reagent (50:1) was added and the absorbance (560 nm) read after 72 h [29]. Experiments were carried out three times in triplicate. Active samples (with less than 50% survival) were after an exposure time of 72 h were serially diluted in a concentration range of 3.12–50 μg/ml and tested. The concentration of the sample that inhibited 50% cell proliferation (IC_{50}) was determined graphically. Doxorubicin, a known antitumor agent, was used as positive control. The cells survival percentage was determined using the formula: % Survival Cell = $(OD_T/OD_C) \times 100$; OD_T and OD_C being the absorbance of the test sample-treated group and the control group (0.1% DMSO) respectively.

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