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SYNTHESIS AND ANTICANCER ACTIVITY OF NOVEL 1,3-THIAZOLE DERIVATIVES

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ABSTRACT

2,5-disubstituted and 2,4-disubstituted thiazoles (**2**) and (**3**) were obtained from the reaction of 2,3-dihydroxybenzaldehyde thiosemicarbazone with *p*-chlorophenacyl bromide in different conditions. Acylation and alkylation of compound **(2)** and **(3)** with acetic anhydride and methyl iodide yielded the corresponding triacetyl derivatives (**4** and **7**) and *N*-methyl derivatives (**5** and **8**), respectively. Condensation of compound **3** with aromatic aldehyde in the presence of piperidine gave the corresponding aryl vinyl azo derivative (**10a,b**). Some thiazole derivatives were evaluated for their anticancer activity against MCF-7 and Hep-G2 cell lines. Among the tested compounds highest activity against liver carcinoma cells (Hep-G2) cell lines than the breast cancer (MCF-7) cell lines. The results showed that the investigated compounds **(2,3,5,8)** and **(10a)** had a significantly greater cytotoxic effect against human hepatocellualr carcinoma cells (Hep-G2) compared to that of other compounds **(4)** and **(6)**.

KEYWORD: *p*-chlorophenacyl, piperidine, hepatocellular.

INTRODUCTION

Thiazoles are important class of five membered heterocyclic compounds, found in many potent biologically active molecules such as sulfathiazole (antimicrobial drug), Ritonavir (antiretroviral drug), Abafungin (antifungal drug) with trade name Abasol cream and Bleomycin and taizofurin (antineoplastic drug).[1]

Recently, thiazolidinone research area unexpectedly becomes interesting and promising for Oncology. In-depth study of PPARS allowed putting forward and validating the concept of anticancer potential existence of PPAR agonists including thiazolidinone. While applying the research strategy through the past few years we succeed in gaining a number of interesting synthetic results that make possible to extend the field of the chemistry of thiazolidinone and related heterocycles, especially in the scope of drug-like molecule design.[2]

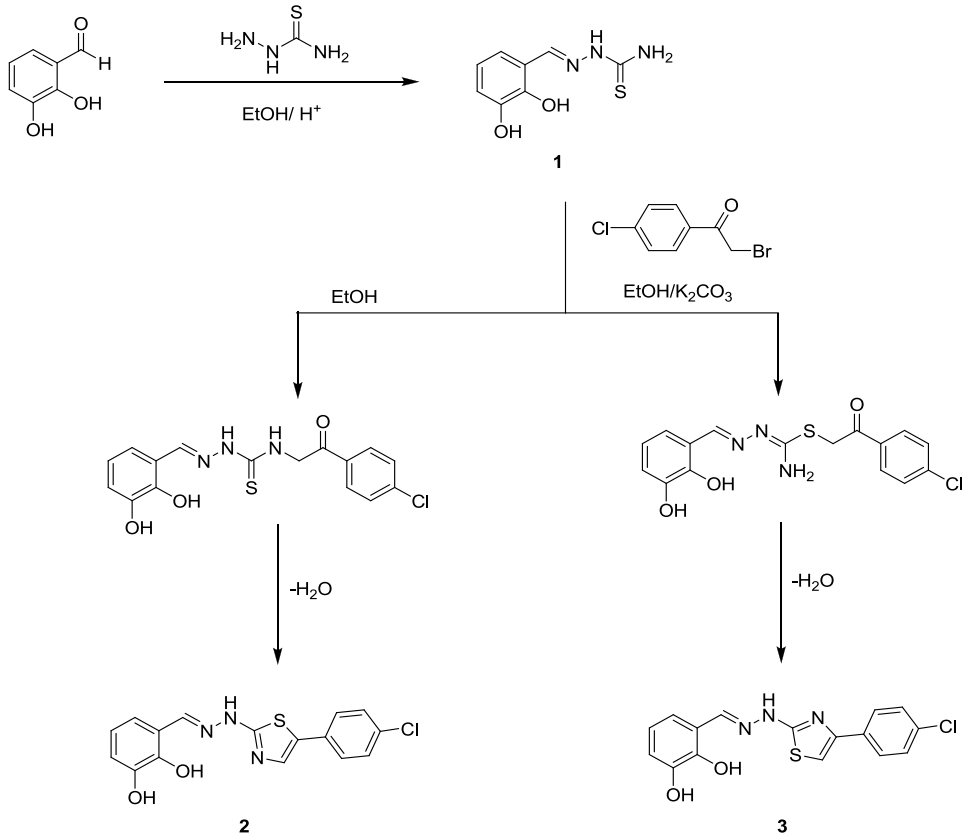
Anticancer activity evaluation of thiazolidinones and related heterocyclic systems and efficient approaches to interpretation of structure activity relationship.[3]

Keeping in view the importance of thiazole derivatives and in continuation of our research on a biologically active molecules[4-9], we herein report the synthesis of some 2,5-disubstituted thiazole derivatives and evaluated them for anticancer activity.

RESULTS AND DISCUSSION

Chemistry

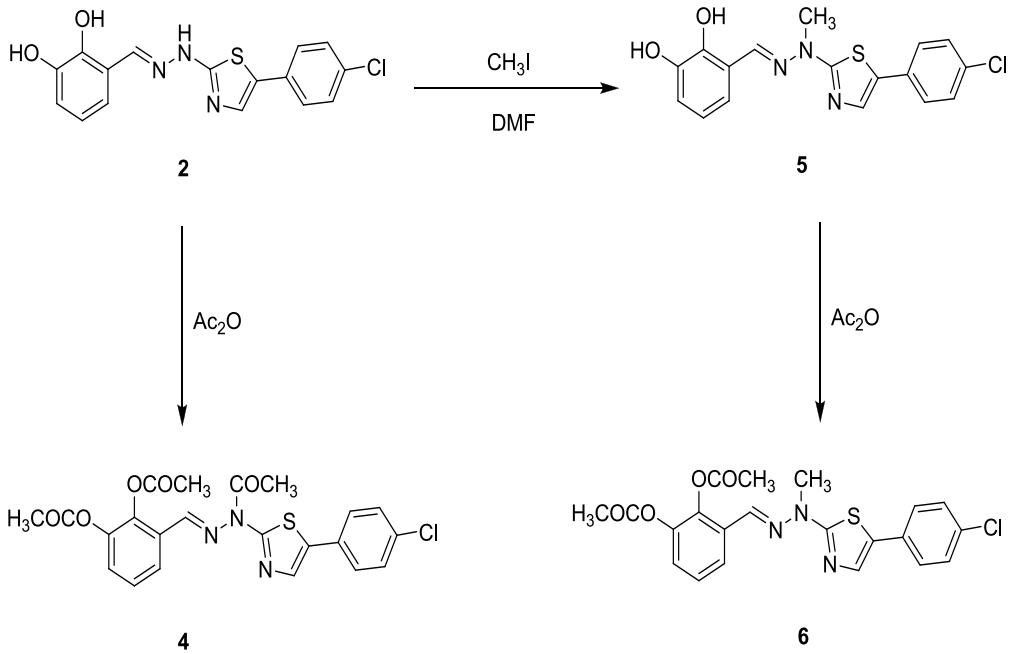
A new series of 2-[(2,3-dihydroxybenzylidene)amino]-5-(*p*-chlorophenyl) and/or 4-(*p*-chlorophenyl)-thiazoles (**2**) and (**3**) were synthesized in a two steps synthetic process (**scheme-1**). In the first step 2,3-dihydroxybenzaldehyde thiosemicarbazone (**1**) was prepared by the condensation of 2,3-dihydroxybenzaldehyde with thiosemicarbazide in ethanol in the presence of catalytic amount of acetic acid. In the second step, compound **(1**) was reacted with p-chlorophenacyl bromide in refluxing ethanol only to yield the corresponding 2-[(2,3-dihydroxybenzylidene)amino]-5-(*p*-chlorophenyl)-thiazoles **(2).** While, the refluxing of compound **(1)** with *p*-chlorophenacyl bromide in ethanol in the presence of anhydrous potassium carbonate afforded the corresponding 2-[(2,3-dihydroxybenzylidene)amino]-4-(*p*-chlorophenyl)-thiazoles **(3)**.



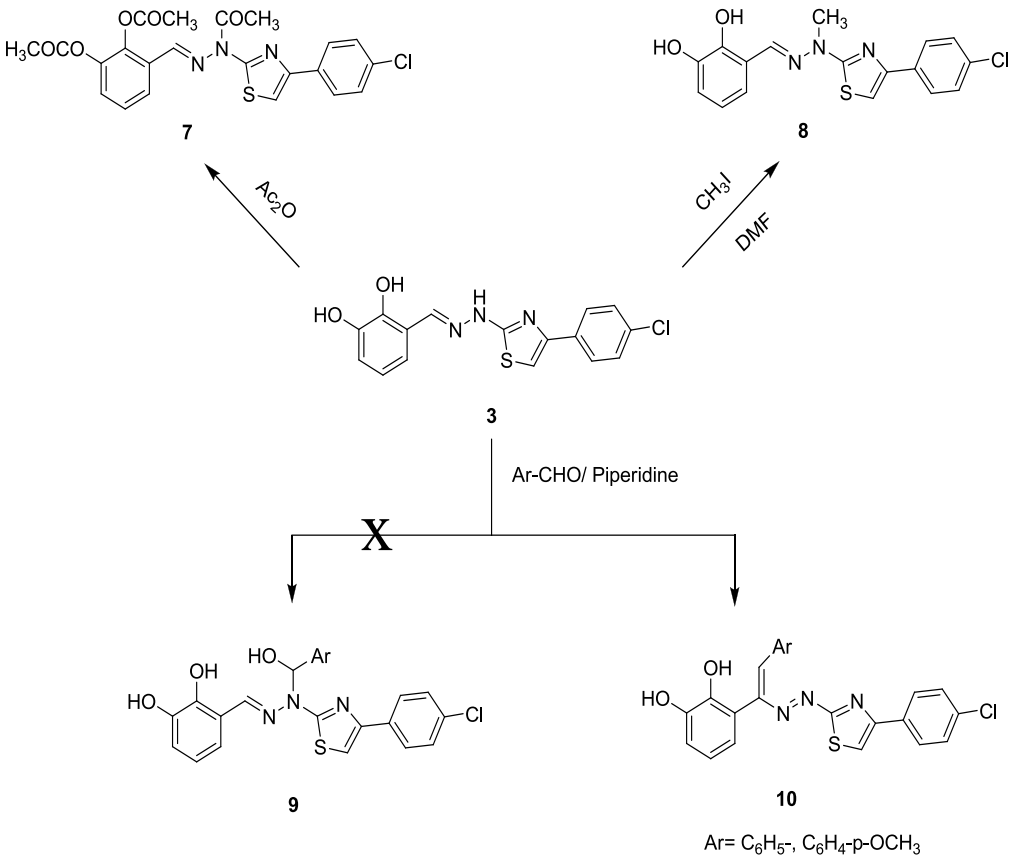
Scheme 1

Acetylation of thiazole derivatives **(2)** and **(3)** with acetic anhydride under reflux leads to the formation 2-[(2,3-diacetoxybenzylidene)-acetylamino)-5-(p-chlorophenyl)-thiazole **(4)** and 2-[(2,3-diacetoxybenzylidene)-acetylamino]-4-(p-chlorophenyl)-thiazole **(7)**, respectively. Alkylation of compound **2** and **3** with methyl iodide in dimethyl formamide under reflux yielded the corresponding 2-[(2,3-dihydroxybenzylidene)-methyl amino]-5-(p-chlorophenyl) thiazole **(5)** and 2-[2,3-dihydroxybenzylidene)methylamino)-4-(p-chlorophenyl) thiazole **(8)**. acylation of compound **5** under reflux gives 2-[(2,3-diacetoxybenzylidene)-methylamino]-5-(p-chlorophenyl)thiazole **(6)**, (**scheme 2, 3**).

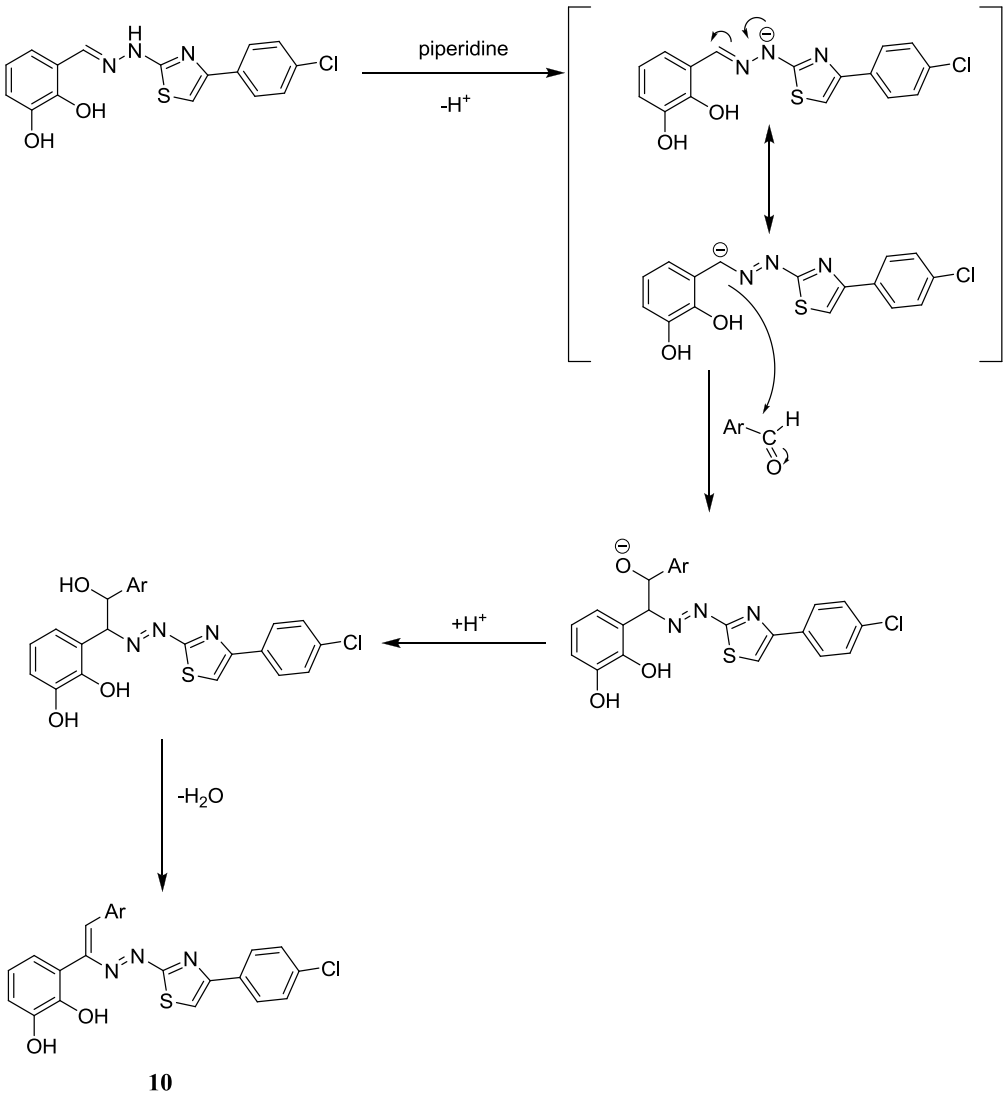
It was also reported that,[10] the reaction of 2-[(2,3-dihydroxybenzylidene)amino] -4-(p-chlorophenyl]thiazole **(3)** with aromatic aldehydes (namely, benzaldehyde and anisaldehyde) in the presence of catalytic amount of piperidine under fusion gave the expected structure **(9)**. But in our case only the corresponding unexpected 2-[(□-2,3-dihydroxyphenyl-□-arylvinyl)azo]-4-(p-chlorophenyl)thiazole **(10)**, (**scheme 3**). The compound **(10)** may be formed by the carbon nucleophile attack at the carbonyl group in the aromatic aldehydes, followed by elimination of water molecule as shown in **scheme 4**.



Scheme 2



Scheme 3



Scheme 4: Mechanism of Formation of Compound **10**

Antitumor activity

The *in vitro* antitumor activity of the tested thiazole derivatives was achieved in the cell culture lab., Cancer Biology Department and Pharmacology Unit, National Cancer Institute, Cairo University, Cairo, Egypt.

Compound **(2-6**, **8)** and **(10a)** were tested for their *invitro* antitumor activity against human breast carcinoma cells (MCF-7) and human hepatocellular carcinoma cells (Hep-G2) using the method of Skehan *et al* 1990[11]. Doxorubicin was used as a reference standard. The inhibitory activity against breast cancer cells (MCF-7) and liver carcinoma cells (Hep-G2) was detected by using different concentration of the tested samples (50, 25, 12.5, 5.00 and 0.00 □g/ml) and surviving fraction (%) was determined by colorimetric method. The IC_{50} was calculated from **Table 1, 2**.

Table 1: Cytotoxixcity of some thiazole derivatives (2-6, 8) and (10a) against human breast cancer (MCF-7) cell lines.

Conc. (□g/ml)	2	3	4	5	6	8	10a	Dox.
0.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
5.00	0.641	0.494	0.577	0.641	0.986	0.500	0.673	0.381
12.50	0.458	0.391	0.451	0.429	0.599	0.261	0.513	0.365
25.00	0.549	0.404	0.289	0.423	0.514	0.324	0.481	0.322
50.00	0.669	0.513	0.310	0.378	0.365	0.472	0.513	0.299

Table 2: Cytotoxixcity of some thiazole derivatives (2-6, 8) and (10a) against human hepatocellular carcinoma (Hep-G2) cell lines.

Conc. (□g/ml)	2	3	4	5	6	8	10a	Dox.
0.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
5.00	0.291	0.227	0.536	0.368	0.682	0.314	0.368	0.507
12.50	0.205	0.218	0.379	0.336	0.500	0.323	0.309	0.388
25.00	0.236	0.273	0.350	0.341	0.323	0.300	0.282	0.319
50.00	0.409	0.327	0.264	0.350	0.214	0.308	0.355	0.314

The result of 50% inhibitory concentration (IC_{50}) data is summarized in **Table 3.**

Table 3: IC_{50} (□g/ml) values of thiazole derivatives after 48 h continuous exposure of tumor cell lines compared with control.

Compound No.	Tumor cell types	
	MCF-7	Hep-G2
2	10.80	3.60
3	4.80	3.30
4	9.60	6.70
5	10.0	3.80
6	27.4	12.5
8	5.0	3.60
10a	17.0	3.80
Doxorubicin	3.83	5.18

The antitumor results of thiazole derivatives are listed in **table 3**, Doxorubicin was used as controls, as shown in **table 3**, some of thiazole derivatives, compounds **2, 3, 5, 8** and **10a** showed good inhibitory activity against Hep-G2 cells compare with Doxorubicin drug as control. These data of compounds **2, 3, 5, 8** and **10a** in **table 3** suggest that these compounds provide good models for further design of potent antitumor agents for treatment of liver cancer.

These data in **Table 3** showed that; all thiazole derivatives lower activity against breast cancer than that of doxorubicin drug as control.

CONCLUSION

A new series of thiazole derivatives were prepared in good yield. The structure of the prepared compounds was confirmed by IR, ^{1}H-NMR, ^{13}C-NMR, MS and elemental analysis. The antitumor activity of thiazole derivatives were evaluated on human breast and liver cancer cell lines. As a result of the cell culture studies, all of the prepared compounds have shown anticancer activity for breast and cancer cells. In conclusion, novel thiazole derivatives might be potentially useful in the field of cancer treatment. Finally, the thiazole derivatives (**2, 3, 5, 8** and **10**) can be suggested as potent candidates for liver cancer drug.

Experimental

Instruments

Chemicals and solvents were purchased from commercial sources in analytical grade purity. Melting points were determined in open capillary on a melt-Temp. Apparatus and were uncorrected. The IR spectra were recorded on a SHIMADZA IR spectrometer as KBr pellets and the wave number are given in cm^{-1}. The ^{1}H- and ^{13}C-NMR spectra were recorded in DMSO-d_6 on a Brucker-400 spectrometer (400MHz). The molecular weight of the final compound were determined by electron ionization (EI) mass spectrometry performed using a probe Agilent MSD 5975 spectrometer operating at 70 eV. The elemental analysis was performed on a Perkin Elmer 2400 series II CHN elemental analyzer.

Synthesis

Synthesis of 2-[(2,3-dihydroxybenzylidene)amino]-5-(p-chlorophenyl) thiazole (2)

A mixture of 2,3-dihydroxybenzaldehyde thiosemicarbazone (**1**, 0.01 mol) and *p*-chlorophenacyl bromide (0.01 mol) in ethanol (30 mL) was heated under reflux for 4h. The solid formed after cooling was filtered off, washed with ethanol and dried and purified by ethanol to give compound (**2)** as pale yellow crystals, yield: 73%, m.p. 229ºC. IR (KBr): 3460-2850 (br. OH), 3248 (NH), 1625 (C=N), 1610, 1585 (C=C), 1091, 1051, 1010 (C-O) cm^{-1}. ^{1}H-NMR (DMSO-d_6): 6.69-7.87 (m, 8H, Ar-H and H-thiazole), 8.34 (s, 1H, CH=N), 9.43-9.46 (br. 2H, OH), 12.16 (s, 1H, NH) ppm. ^{13}C-NMR (DMSO-d_6): 168.5 (S-C=N), 149.8, 146.1 (2x C-OH), 145.0 (C=N), 141.37, 133.9, 132.5, 131.7, 129.7, 129.1, 127.7, 120.9, 119.8, 117.7, 116.8, 104.5 (C-aromatic and thiazole ring) ppm. MS (m/z, %)= 347

(M^++2), 345 (M^+). Anal. Calcd for $C_{16}H_{12}N_3ClO_2S$: C, 55.65; H, 3.48; N, 12.17. Found: C, 55.47; H, 3.38; N, 12.08.

Synthesis of 2-[(2,3-dihydroxybenzylidene)amino]-4-(p-chlorophenyl) thiazole (3)

A mixture of 2,3-dihydroxybenzaldehyde thiosemicarbazone (**1**, 0.01 mol) and anhydrous potassium carbonate (0.03 mol) in ethanol was heated for 30 min., then added 4-chlorophenacyl bromide (0.01 mol). The reaction mixture was heated under reflux for 3 h, then cooled and neutralized with dilute hydrochloric acid (2%). The resulting solid was filtered off, washed with water, dried and purified by recrystallization from ethanol to give compound 3 as pale yellow crystals. yield: 71%, m.p. 195ºC, IR (KBr): 3560-3100 (br. OH), 3248 (NH), 1610 (C=N), 1577, 1571 (C=C), 1091, 1053, 1012 (C-O) cm^{-1}. ^{1}H-NMR (DMSO-d_6): δ 6.69-7.88 (m, 8H, Ar-H and H-thiazole ring), 8.33 (s, 1H, CH=N), 9.14 (s 1H, OH), 9.45 (s 1H, OH), 12.16 (s, 1H, NH) ppm. ^{13}C-NMR (DMSO-d_6): δ 168.5 (S-C=N), 146.1, 145.2 (C-OH), 141.3 (C=N), 144.9, 132.5, 129.1, 127.7, 120.9, 119.8, 117.6, 116.8, 104.5 (C-aromatic and C-thiazole ring) ppm. MS (m/z, %)= 347 (M^++2), 345 (M^+). Anal. Calcd for $C_{16}H_{12}N_3ClO_2S$: C, 55.65; H, 3.48; N, 12.17. Found: C, 55.51; H, 3.36; N, 12.09.

Synthesis of 2-[(2,3-diacetoxybenzylidene)acetylamino]-4- and/or 5-(p-chlorophenyl) thiazoles (4, 7)

A solution of **(2)** and **(3)** (0.01 mol) in acetic anhydride (30 mL) was heated under reflux for 2 h. The reaction mixture was cooled and poured into ice-water. The resultant solid was filtered off, washed with water, dried and recrystallized to give (**4)** and **(7)**.

Compound **(4)** as colorless crystals; yield: 63%, m.p. 185ºC, IR (KBr): 1770 (C=O of ester), 1693 (C=O of acetyl), 1625 (C=N), 1605, 1575 (C=C), 1211, 1161, 1010 (C-O) cm^{-1}. ^{1}H-NMR (DMSO-d_6): δ 2.28 (s, 3H, $COCH_3$), 7.45-8.12 (m, 8H, Ar-H and H-thiazole ring), 8.99 (s, 1H, CH=N) ppm. ^{13}C-NMR (DMSO-d_6): δ 147.9 (C=N), 143.3 (C=N), 142.2, 133.2, 133.1, 129.3, 129.1, 128.1, 127.9, 127.5, 126.8, 124.8, 114.6 (C-aromatic and C-thiazole ring), 22.9, 20.8, 20.3 (3x $COCH_3$) ppm. MS (m/z, %)= 347 (M^++2), 345 (M^+). Anal. Calcd for $C_{22}H_{18}N_3ClO_5S$: C, 56.05; H, 3.82; N, 8.92. Found: C, 55.59; H, 3.66; N, 8.78.

Compound **(7)** as colorless crystals; yield: 61%, m.p. 142 ºC, IR (KBr): 1770, 1750 (C=O of ester), 1695 (C=O of acetyl), 1647 (C=N), 1587, 1577 (C=C), 1181, 1089, 1012 (C-O) cm^{-1}. ^{1}H-NMR (DMSO-d_6): δ 2.01 (s, 3H, $COCH_3$), 2.30 (s, 3H, $COCH_3$), 2.50 (s, 3H, $COCH_3$), 7.00-8.01 (m, 8H, Ar-H and H-thiazole ring), 9.01 (s, 1H, CH=N) ppm. ^{13}C-NMR (DMSO-

d_6): δ 172.5, 168.8, 156.2 (C=O), 148.8, 147.4 (C-O), 143.6 (C=N), 140.6, 133.2, 132.9, 132.8, 129.3, 128.9, 128.1, 127.9, 127.7, 126.8, 125.9, 125.5, 125.2, 124.8, 114.9, 112.7 (C-aromatic and C-thiazole ring), 22.9, 21.5, 20.5 (3x $COCH_3$) ppm. MS (m/z, %)= 473 (M^++2, 11.4), 472 (M^++1, 10.0), 471 (M^+, 27.50), 431 (22.9), 430 (17.60), 429 (57.0), 389 (30.20), 388 (22.20), 387 (77.10), 347 (17.7), 346 (15.40), 345 (46.20), 344 (18.70), 330 (26.40), 329 (24.0), 328 (71.20), 327 (29.10), 249 (7.7), 293 (5.8), 292 (18.8), 269 (34.10), 268 (17.0), 267 (89.9), 253 (17.60), 251 (5.30), 239 (5.80), 238 (4.8), 237 (4.8), 236 (8.60), 227 (16.90), 226 (10.60), 225 (46.9), 224 (13.40), 212 (35.50), 211 (20.7), 210 (100), 209 (20.1), 208 (10.60), 196 (12.70), 181 (15.10), 174 (29.0), 173 (9.50), 170 (15.0), 168 (40.60), 139 (19.40), 138 (29.7), 137 (44.60), 136 (24.50), 135 (11.90), 134 (16.90), 133 (12.90), 123 (5.90), 122 (6.60), 114 (2.30), 113 (2.80), 109 (7.8), 108 (12.90), 89 (8.50), 79 (5.0), 77 (3.90), 75 (7.1), 65 (6.8), 64 (4.8), 63 (6.7), 55 (5.10), 52 (5.9), 51 (8.0). Anal. Calcd for $C_{22}H_{18}N_3ClO_5S$: C, 56.05; H, 3.82; N, 8.92. Found: C, 55.87; H, 3.71; N, 8.71.

Synthesis of 2-[(2,3-dihydroxybenzylidene)-N-methylamino]-5 and/or 4-(p-chlorophenyl)thiazole (5, 8)

To a solution of **(2)** and **(3)** (0.01 mol) in dimethyl formamide (30 mL) was added methyl iodide (0.01 mol) and refluxed for 4 h. after the reaction was complete, the reaction was cooled and poured into water (50 mL) and PH was adjusted to 5.5 with dilute hydrochloric acid (2 mol/L). The resulting solid was filtered to isolate compound **(5)** and **(8)**, respectively.

2-[(2,3-dihydroxybenzylidene)-N-methylamino]-5-(p-chlorophenyl) thiazole ***(5)*** as pale yellow crystals; yield: 67%, m.p. 200 ºC, IR (KBr): 3550-2887 (br. OH), 1624 (C=N), 1605, 1578 (C=C), 1091, 1053 (C-O) cm^{-1}. ^{1}H-NMR (DMSO-*d_6*): δ 3.46 (s, 3H, CH_3), 6.82-7.80 (m, 8H, Ar-H and H-thiazole ring), 8.36 (s, 1H, CH=N), 9.16 (s 1H, OH), 9.56 (s 1H, OH) ppm. MS (m/z, %)= 361 (M^++2, 7.20), 359 (M^+, 14.20), 347 (15.20), 345 (17.20), 344 (35.20), 292 (5.0), 267 (15.2), 225 (15.20), 210 (18.20), 209 (7.20), 196 (13.20), 195 (3.20), 174 (16.20), 137 (12.20), 136 (6.20), 132 (6.50), 75 (4.20). Anal. Calcd for $C_{17}H_{14}N_3ClO_2S$: C, 56.82; H, 3.90; N, 11.70. Found: C, 56.56; H, 3.72; N, 11.56.

2-[(2,3-dihydroxybenzylidene)-N-methylamino]-4 -(p-chlorophenyl) thiazole ***(4)*** as pale yellow crystals; yield: 65%, m.p. 171ºC, IR (KBr): 3360-2985 (br. OH), 1624 (C=N), 1603, 1576 (C=C), 1091, 1049, 1010 (C-O) cm^{-1}. ^{1}H-NMR (DMSO-*d_6*): δ 3.69 (s, 3H, CH_3), 6.81-7.88 (m, 8H, Ar-H and H-thiazole ring), 8.36 (s, 1H, CH=N), 9.17 (s 1H, OH), 9.63 (s 1H, OH) ppm. ^{13}C-NMR (DMSO-*d_6*): δ 168.5, 148.9, 146.2, 145.3, 141.5, 133.4, 131.5, 129.5,

128.7, 121.3, 119.8, 117.6, 117.1, 116.8, 104.6 and 32.9 (N-CH_3) ppm. Anal. Calcd for $C_{17}H_{14}N_3ClO_2S$ (359): C, 56.82; H, 3.90; N, 11.70. Found: C, 56.61; H, 3.62; N, 11.42.

Synthesis of 2-[(2,5-diacetoxybenzylidene)-methylamino]-5-(p-chlorophenyl) thiazoles (6)

To a solution of **5** (0.1 mol) in acetic anhydride (25 mL) and refluxed for 2h. The solvent was evaporated under vacuo. The residue was crystallized from ethanol to give **(6**) as white solid. yield: 63%, m.p. 160ºC, IR (KBr): 1774 (C=O), 1623 (C=N), 1604, 1577 (C=C), 1161, 1091, 1014 (C-O) cm^{-1}. ^{1}H-NMR (DMSO-d_6): δ 2.3 (s, 3H, $COCH_3$), 2.51 (s, 3H, $COCH_3$), 3.61 (s, 3H, N-CH_3), 6.92-8.21 (m, 8H, Ar-H and H-thiazole ring), 9.01 (s, 1H, CH=N) ppm. ^{13}C-NMR (DMSO-d_6): δ 172.18, 168.4 (C=O), 156.2, 148.8, 147.9 (C-O), 143.3, 142.2 (C=N), 133.2, 133.0, 129.3, 129.1, 128.1, 127.9, 127.9, 127.7, 127.5, 126.8, 124.8, 114.6, 111.1 (C-aromatic and C-thiazole ring), 22.9 (N-CH_3), 20.8, 20.3 (2x $COCH_3$) ppm. MS (m/z, %)= 445 (M^++2, 11.20), 443 (M^+, 32.20), 431 (30.31), 430 (21.81), 429 (60.32), 428 (48.64), 403 (10.20), 401 (15.20), 3.89 (34.95), 387 (100), 386 (21.20), 370 (21.56), 369 (9.63), 345 (31.82), 344 (18.12), 330 (29.00), 329 (19.68), 328 (69.39), 327 (17.10), 210 (17.0), 209 (12.32), 197 (13.20), 196 (3.20), 174 (7.23), 173 (6.20), 137 (7.50), 135 (5.20), 108 (2.50), 75 (2.50). Anal. Calcd for $C_{21}H_{18}N_3ClO_5S$ (443): C, 56.88; H, 4.06; N, 9.48. Found:.

Synthesis of 2-[(□-2,3-dihydroxyphenyl-□-arylvinyl)azo]-4-(p-chloron phenyl) thiazoles (10a,b)

A mixture of **(3)** (0.1 mol), aromatic aldehydes (namely, benzaldehyde and anisaldehyde (0.01 mol) and piperidine (1 mL), the mixture was fused on hot plate at 120ºC for 1h. the reaction mixture was added ethanol (50 mL) and refluxed for 2 h, cooled and neutralized with dilute hydrochloric acid (2%). The resulting product was filtered off, washed with water, dried and recrystallized from ethanol to give,

2-[(□-2,3-dihyydroxyphenyl-□-phenylvinyl)azo]-4- (p-chloro phenyl) thiazoles ***(10a***) as orange crystals, yield: 71%, m.p. 155ºC. IR (KBr): 3510-2980 (br. OH), 1622 (C=N), 1610, 1595 (C=C), 1178, 1093 (C-O) cm^{-1}. ^{1}H-NMR (DMSO-d_6): 6.88-7.98 (m, 13H, Ar-H and H-thiazole), 8.91 (s, 1H, H-olefinic), 9.41 (br. s, 1H, OH), 10.49 (br. s, 1H, OH) ppm. MS (m/z%): 445 (M^++2, 16.2), 443 (M^+, 32.50). Anal. Calcd for $C_{23}H_{16}N_3ClO_2S$: C, 63.74; H, 3.695; N, 9.70. Found: C, 63.63; H, 3.35; N, 9.51.

2-[(□-2,3-dihyydroxyphenyl-□-(p-methoxyphenyl)vinyl)azo]-4- (p-chloro phenyl) thiazoles ***(10b)*** as orange crystals, yield: 74%, m.p. 178ºC. IR (KBr): 3551-2960 (br. OH), 1623

(C=N), 1610, 1585 (C=C), 1174, 1161, 1093 (C-O) cm^{-1}. ^{1}H-NMR (DMSO-d_6): 3.84 (s, 3H, OCH_3), 6.80-7.94 (m, 12H, Ar-H and H-thiazole ring), 8.87 (s, 1H, H-olefinic), 9.8 (br. s, 1H, OH), 10.16 (br. s, 1H, OH) ppm. ^{13}C-NMR (DMSO-d_6): δ 172.18, 168.4 (C=O), 164.7, 162.7, 161.9 (C-O), 148.3 (C=N), 136.8, 134.6, 132.9, 132.3, 132.2, 132.0, 131.5, 129.9, 129.5, 128.7, 127.3, 121.6, 120.8, 119.9, 119.6, 115.4, 114.9 (C-aromatic and C-thiazole ring), 55.9 (O-CH_3) ppm. MS (m/z, %): 465 (M^++2, 12.10), 464 (M^++1, 9.80), 463 (M^+, 30.7), 448 (9.50), 447 (14.10), 446 (47.10), 347 (11.0), 346 (7.0), 345 (31.60), 330 (15.0), 329 (16.20), 328 (32.40), 327 (12.80), 315 (1.5), 313 (3.30), 306 (4.8), 305 (2.8), 304 (11.70), 299 (7.8), 298 (4.90), 297 (17.30), 289 (6.90), 288 (4.90), 287 (17.30), 257 (4.70), 256 (2.90), 255 (10.30), 238 (8.60), 237 (11.70), 236 (20.30), 212 (38.70), 211 (18.20), 210 (100), 209 (13.30), 198 (2.60), 197 (2.2), 196 (6.50), 176 (9.10), 175 (9,80), 174 (27.40), 170 (18.0), 169 (7.1), 168 (47.40), 165 (15.90), 164 (14.70), 163 (29.20), 163 (29.20), 151 (8.0), 150 (6.10), 149 (15.0), 148 (5.40), 141 (11.70), 140 (8.9), 139 (37.70), 138 (23.5), 137 (42.20), 136 (48.30), 135 (66.10), 134 (22.20), 133 (23.30), 123 (6.7), 121 (26.20), 120 (7.30), 113 (14.90), 112 (11.0), 111 (34.90), 108 (13.60), 107 (16.40), 105 (5.20), 102 (8.40), 93 (5.7), 92 (19.0), 91 (10.10), 90 (10.60), 89 (15.0), 85 (5.10), 84 (13.40), 80 (9.50), 79 (10.0), 77 (33.20), 76 (13.40), 75 (20.50), 73 (22.20), 69 (11.20), 65 (13.60), 64 (13.90), 63 (20.60), 55 (10.10), 52 (11.70), 51 (15.8), 50 (12.0). Anal. Calcd for $C_{29}H_{18}N_3ClO_5S$ (463): C, 62.20; H, 3.89; N, 9.07. Found: C, 62.02; H, 3.71; N, 8.97.

Anticancer Evaluation

Two different human cancer cell lines MCF-7 and Hep-G2 were obtained from national cancer institute (Cairo, Egypt) and cells were plated in 96 multi well plate (104 cells/well) for 24 h before treatment with the tested compounds to allow attachment of the cell to the well of the plate. Different concentration of the compounds under test (5.00, 12.50, 25.00 and 50.00 □g/ml) were added to the cell monolayer triplicate wells were prepared from each individual dose monolayer cells were incubated with the test compounds for 48 h at 37ºc and in atmosphere of 5% CO_2. After 48 h, cells were fixed, washed and stained with sulforhodamine-B stain. Excess stain was washed with acetic acid and attached stain was recovered with Tri EDTA buffer.

Color intensity was measured in ELISA reader. The relation between surviving fraction and compound concentration is plated to get the survival curve of each tumor cell line after the specified compound.

REFERENCES

1. Khalil, A. M.; Berghot, M. A.; Gouda, M. A.; *Eur. J. Med. Chem*., 2009; 44: 4434.
2. Aridoss, G.; Amirthaganesan, S.; Kim, M. S.; Kim, J. T.; Jlong, Y. T.; *Eur. J. Med. Chem*., 2009; 44: 4199.
3. Leoni, A.; Locatelli, A; Morigi, R; Rambaldi, M. A.; patent review (2008-2012, part 2). *Exp. Opin. Ther. Pat*, 2014.
4. Tung, T. T.; Qanh, D. T. K.; Dung, P.T.P.; Hue, V. T. M.; Park, S. H.; Han, B. W.; Xin, J.; Hong, J. T.; Han, S. B.; Nam, N. H.; *Med. Chem.*, 2013; 9: 1051-1057.
5. Ghorab, M. M.; Al-Said, M. S.; Pharm. Research, 2012; 35: 956-973.
6. Li, S. R.; Li, D. D.; Wang, R. R.; Sun, J; Dang, J. J; Du, Q. R.; Fang, R.; Zhang, W. M.; Zhu, H. L.; *Eur. J. Med. Chem*., 2014; 75: 438-447.
7. Geranikaki, A.; Hadjipaulou-Litina, D.; Zablotskaya, A.; Segal, I.; *Bioinorg. Chem. Appl, 2007*.
8. Barradas, J. S.; Errea, M. I., Daccorso, N. B.; Sepulveda, C. S.; Damonte, E. B.; *Eur. J. Med. Chem.*, 2011; 46: 259-264.
9. Chawla, A.; Chawla, P.; Sunnaina, S. M.; Kaur, K.; Dhawan, R. K.; *Int. J. Pharmacol Pharm. Sci*., 2015; 2: 1-8.
10. Hassan, K. I.; Sayed, H-El-Tamany; El-Shaarawy, F. R.; El-Deen, I. M.; *J. Chem. Eng.*, 2008; 65-69.
11. Skehan, P.; Storeng, R.; New colorimetric cytotoxicity assay for anticancer drug screening, *J. Nat. Cancer Instit*, 1990; 82: 1107-1112.