Thiazolidinedione or Rhodanine: A Study on Synthesis and Anticancer Activity Comparison of Novel Thiazole Derivatives

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ABSTRACT - Purpose: A new series of thiazolyl-2,4-thiazolidinedione / rhodanine compounds **T1-T23** was synthesized and tested for their anticancer activities. Hepatocellular carcinoma cell lines were chosen due to their strong drug resistance to test the new compounds. **Methods**: All compounds were synthesized via Knoevenagel Condensation reaction and thiazolidinedione ester compounds (**T3,T9,T15,T20**) were hydrolyzed for obtaining the acidic compounds (**T6,T12,T17,T23**). All compounds were firstly screened for their anticancer activity against two hepatocellular carcinoma (HCC) cell lines, Huh7 and Plc/Prf/5 (Plc) cell lines by sulforhodamine B assay. Further IC_{50} values were calculated for three candidates (**T4**, **T15**, **T21**) in five different HCC (Huh7, Plc, Snu449, HepG2, Hep3B) and one breast cancer (Mcf7) cell line. **Results**: Compounds **T4**, **T15**, **T21** had very strong anticancer effects even though their 10 μM concentration in Huh7 cell line. According to IC_{50} values, **T21** was the most effective compound with IC_{50} values in a range from 2 to 16 μM in 6 cancer cell lines. In terms of cytotoxicity **T21** mostly affected Huh7 and interestingly it was less effective against Plc. **Conclusions**: Considering these results it can be suggested that compounds **T4**, **T15** and **T21** may lead to the development of more potent anticancer drugs in the future.

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INTRODUCTION

Hepatocellular carcinoma (HCC) is one of the deadliest type of cancers with a 5-year survival rate below 15% (1). The treatment of HCC has been a challenge so far because of its unusual resistance to chemotherapeutic agents. Sorafenib is the only drug, approved by Food and Drug Administration (FDA, United States) for targeted HCC treatment, but its effect on patient survival is minimal. So, there is a need to find novel efficient molecules acting against HCC cells.

Taking into account of the rising trend of the incidence of various organ cancers, effective therapies are urgently needed to control human malignancies. However, almost all of the chemotherapy drugs which are currently on the market cause serious side effects (2).

Thiazolidinedione (TZD) and rhodanine analog compounds have become very important groups of heterocyclic compounds in drug design and discovery. The PPAR-gamma (PPAR-γ) activating TZD medications are a class of drugs used to improve lipid and glucose metabolism in type-2 diabetes. More interestingly, numerous compounds containing the TZD ring have been developed as potential anticancer agents (3-4). TZDs, which are anti-cancer therapeutics for the most common types of cancers including, lung, breast, and colon, have been explored for the PPAR-γ-dependent and -independent mechanisms by which TZDs exert their anti-tumor effects (5). Rhodanines have been reported to possess antibacterial, antifungal, antiviral, antimalarial, insecticidal, herbicidal, antitumor, anti-inflammatory and cardiotonic activities (6-7). Besides, rhodanine derivatives are still broadly evaluated for their anticancer activity against different cancer cell lines, often exhibiting selective toxicity against normal cell lines (8-10).

Thiazoles are ubiquitous building blocks in medicinal chemistry and they can be found in numerous natural products (e.g., epothilone) and biologically important compounds including the anticancer drug dasatinib (11). On the other hand, triazoles have occupied an important role, not only in organic chemistry but also in medicinal chemistry due to their easy synthesis and attractive features as well as numerous biological activities. Furthermore, this heterocycle has a high dipole moment and it is capable of hydrogen bonding, which could be favorable in the binding

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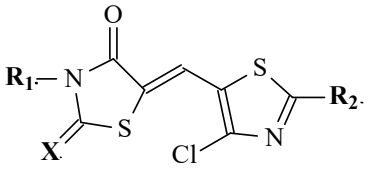
of biomolecular targets. In recent years, by combining triazole with other pharmacophores, a number of compounds with potent antitumor activity were synthesized (12).

Inspired by the biological importance of these ring systems, we described the synthesis of a new series of thiazolyl-2,4-thiazolidinediones / rhodanines (**T1-T23**) as lead structures in developing anticancer agents (Figure 1). Compounds **T13-T23** have five membered triazole ring on the second position of thiazole ring. Instead, the other compounds (**T1-T12**) have five membered pyrrolidine and six membered heterocyclic morpholine rings which have been used by organic and medicinal chemists in different structural combinations. Thus, the contribution to anticancer activity of these parts will be able to evaluate.



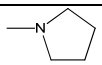
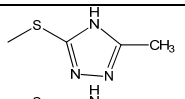
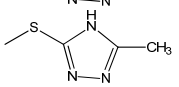
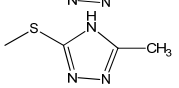
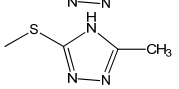
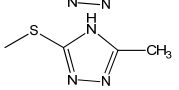
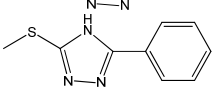
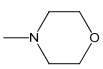
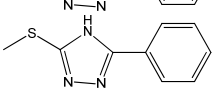
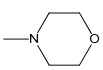
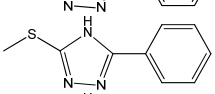
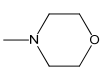
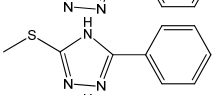
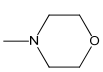
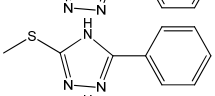
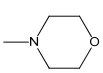
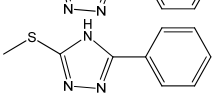
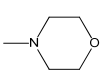
Compound No	X	Substituents R1	Substituents R2	Compound No	X	Substituents R1	Substituents R2
T1	O	H		**T13**	O	H	
T2	S	H		**T14**	S	H	
T3	O	$CH_2COOC_2H_5$		**T15**	O	$CH_2COOC_2H_5$	
T4	S	$CH_2COOC_2H_5$		**T16**	S	CH_2COOH	
T5	S	CH_2COOH		**T17**	O	CH_2COOH	
T6	O	CH_2COOH		**T18**	O	H	
T7	O	H		**T19**	S	H	
T8	S	H		**T20**	O	$CH_2COOC_2H_5$	
T9	O	$CH_2COOC_2H_5$		**T21**	S	$CH_2COOC_2H_5$	
T10	S	$CH_2COOC_2H_5$		**T22**	S	CH_2COOH	
T11	S	CH_2COOH		**T23**	O	CH_2COOH	
T12	O	CH_2COOH					

Figure 1. Formula of the compounds **T1-T23.**

METHODS and MATERIALS

Chemistry

Melting points were measured on an Electrothermal 9100 type apparatus (Electrothermal Engineering, Essex, UK) and uncorrected. All instrumental analyses were performed in Central Laboratory of Pharmacy Faculty of Ankara University. ^{1}H NMR and ^{13}C NMR spectra were determined with a VARIAN Mercury 400 FT-NMR spectrometer (Varian Inc, Palo Alto, CA, USA) in $CDCl_3$ and DMSO-d_6. All chemical shifts were reported as δ (ppm) values. Mass spectra were recorded on Waters

Micromass ZQ (Waters Corporation, Milford, MA, USA) by using ESI (+) method. Elementary analyses were performed on a Leco CHNS 932 analyzer (Leco, St. Joseph, USA) and satisfactory results ±0.4% of calculated values (C, H, N) were obtained. For the chromatographic analysis Merck Silica Gel 60 (230-400 mesh ASTM) was used. The chemical reagents used in synthesis were purchased from E. Merck (Darmstadt, Germany) and Aldrich (Milwaukee, MI, USA). Rhodanine (**Ic)** and 2-(4-oxo-2-thioxo-thiazolidin-3-yl) acetic acid (**Id**) were purchased from Aldrich (Milwaukee, MI, USA). 5-((4-chloro-2-(morpholin-4-yl)-1,3-thiazol-5-yl)methylidene) thiazolidine-2,4-dione (**T7**), (Z)-Ethyl 2-(5-((4-chloro-2-(morpholin-4-yl)-1,3- thiazol-5-yl)methylidene)- 2,4-dioxothiazolidin-3-yl)acetate (**T9**), (Z)-2-(5-((4-chloro-2-(morpholin-4-yl)-1,3-thiazol-5-yl) methylidene)- 2,4-dioxothiazolidin-3-yl)acetic acid (**T12**) were synthesized according to the literature (13).

Synthesis of ethyl 2-(4-oxo-2-thioxo-thiazolidin-3-yl) acetate (Ie)

A mixture of 2-(4-oxo-2-thioxo-thiazolidin-3-yl)acetic acid (**Id**) (0.50 g (2.63 mmol)), ethanol (5 ml) and H_2SO_4 (0.5 ml) was refluxed for 7h. The crude product was crystallized from ethanol. Yield: 0.445 g, 77.21%; m.p.: 60 ºC. Spectroscopic analysis: ^{1}H-NMR (δ ppm; $CDCl_3$): 1.29 (t, 3H, CH_3), 4.23 (q, 2H, CH_2), 4.08 (s, 2H, CH_2), 4.72 (s, 2H, CH_2). Anal. Calc. for $C_7H_9NO_3S_2$: C: 38.34, H: 4.14, N: 6.39, S: 29.25% , found; C:38.53, H:4.19, N:6.47, S:29.58%

General procedure for the synthesis of 2-substituted-4-chlorothiazole-5-carbaldehydes (IIIa-d)

To a stirred suspension of 2,4-dichlorothiazole-5-carbaldehyde **(II)** (5.5 mmol), prepared from 2,4-TZD (**Ia**), and sodium carbonate (5.5 mmol) in acetonitrile (25 ml) was added heterocyclic secondary amine or thiol (5.5 mmol), followed by stirring for 18h at room temperature. The salts were filtered, washed with acetonitrile and the filtrate was evaporated in vacuo to dryness. The residue was purified by column chromatography using dichloromethane: ethyl acetate (10:1) as eluant.

4-Chloro-2-(pyrrolidin-1-yl)thiazole-5-carbaldehyde (IIIa)

This compound was obtained as colorless needles. Yield: 84%, mp 118 ºC (Ref.14; m.p.:118 ºC).

4-Chloro-2-(morpholin-4-yl)-thiazole-5-carbaldehyde (IIIb)

This compound was obtained as shining crystals. Yield: 1.3 g, 93.0%, m. p.: 200 ºC (Ref.13; m.p.: 200 ºC).

4-Chloro-2-(5-methyl-4H-1,2,4-triazol-3-ylthio)thiazole-5-carbaldehyde (IIIc)

This compound was obtained as yellow crystals. Yield: 52.5%, m. p.: 178 º C. (Ref.15; m. p.: 178 ºC).

4-Chloro-2-(5-phenyl-4H-1,2,4-triazol-3-ylthio)thiazole-5-carbaldehyde (IIId)

This compound was obtained as cream colored crystals. Yield: 61.97%, m. p.: 210 º C. Spectroscopic analysis: ^{1}H-NMR (DMSO, 400 MHz, δ, ppm): 7.56-7.59 (m, 3H, Ar-H), 8.00-8.03 (m, 2H, Ar-H), 9.83 (s, 1H, CHO), 15.35 (s, 1H, NH); MS (ESI+) *m/z* (rel. intensity) : 355.1 (M+H, 100%)

Synthesis of compounds T1-T5, T8, T10, T11, T13-T15,T16, T18-T22

A mixture of 2-substituted-4-chloro-thiazole-5-carbaldehyde (**IIIa-d**) (0.001 mol) and **Ia-e** (0.001 mol) was heated at 100-110 ºC in the presence of 0.5 ml acetic acid glacial and sodium acetate (0.001 mol). The reaction mixture was extracted with $CHCl_3$ (3X50 ml) and the organic layer was washed with water, dried over anhydrous Na_2SO_4 and evaporated to dryness.

(Z)-5-((4-chloro-2-(pyrrolidin-1-yl)thiazol-5-yl)methylene) thiazolidine-2,4-dione (T1)

Reaction time: 4h. The crude product was purified by column chromatography on using dichloromethane : EtOAc (5:1) as eluant. Yield: 34.0%, m.p.: 290 ºC; ^{1}H NMR, δ, ppm (400 MHz, $CDCl_3$): 2.10-2.14 (m, 4H, $2CH_2$), 3.55 (broad s, 4H, $2NCH_2$), 7.97 (s, 1H, =CH); ^{13}C NMR, δ ppm (100 MHz, DMSO-d_6): 166.6 (CO), 166.3 (CO), 143.1 (C), 120.9 (C), 116.5 (C), 111.7 (CH), 49.6 (N-CH_2), 24.9 (CH_2); MS (ESI+) *m/z* (rel. intensity): 315.9 (M+H, 100%); Anal. Calc. for $C_{11}H_{10}ClN_3O_2S_2$: C: 41.84, H: 3.19, N: 13.31, S: 19.33%, found; C: 42.02, H: 3.10, N: 13.10, S: 19.70%

(Z)-5-((4-chloro-2-(pyrrolidine-1-yl)thiazol-5-yl)methylene)-2-thioxo-thiazolidine-4-one (T2)

Reaction time: 4h. The crude product was purified by column chromatography using dichloromethane : EtOAc (5:1) as eluant. Yield: 59.0%, m.p.: 281 ºC; ^{1}H NMR, δ, ppm (400 MHz, DMSO-d_6): 2.00 (broad s, 4H, $2CH_2$), 3.45 (broad s, 4H, $2NCH_2$), 7.42 (s, 1H, =CH), 13.64 (s, 1H, NH); ^{13}C NMR, δ ppm (100 MHz, DMSO-d_6):

193.1 (CS), 168.3 (CO), 166.9 (C), 144.6 (C), 120.6 (C), 118.2 (C), 112.4 (CH), 49.8 (N-CH_2), 25.0 (CH_2); MS (ESI+) *m/z* (rel. intensity) : 331.6 (M+H, 100%); Anal. Calc. for $C_{11}H_{10}ClN_3OS_3$: C: 39.81, H: 3.04, N: 12.66, S: 28.98%, found; C: 39.93, H: 2.90, N: 12.68, S: 28.70%

(Z)-Ethyl 2-(5-((4-chloro-2-(pyrrolidin-1-yl)thiazol-5-yl)methylene)-2,4-dioxothiazolidin-3-yl)acetate (T3)

Reaction time: 4h. The crude product was purified by column chromatography using hexane : EtOAc (3:1) as eluant. Yield: 54.5%, m.p.: 192 ºC; ^{1}H NMR, δ, ppm (400 MHz, $CDCl_3$): 1.29 (t, 3H, J= 7.00 Hz, $CH_2\underline{CH_3}$), 2.10-2.14 (m, 4H, $2CH_2$), 3.56 (broad s, 4H, $2NCH_2$), 4.24 (q, 2H, J= 7.20 Hz, $\underline{CH_2}CH_3$), 4.45 (s, 2H, $\underline{CH_2}CO$), 8.05 (s, 1H, =CH); ^{13}C NMR, δ ppm (100 MHz, $CDCl_3$): 167.4 (CO), 166.7 (CO), 166.4 (CO), 164.9 (C), 145.5 (C), 124.9 (C), 113.7 (C), 112.9 (CH), 62.0 ($\underline{CH_2}CH_3$), 49.9 (N-CH_2), 42.2 ($\underline{CH_2}CO$), 25.6 (CH_2), 14.1 ($CH_2\underline{CH_3}$); MS (ESI+) *m/z* (rel. intensity) : 401.7 (M+H, 100%); Anal. Calc. for $C_{15}H_{16}ClN_3O_4S_2$: C: 44.83, H: 4.01, N: 10.46, S: 15.96%, found; C: 44.44, H: 3.90, N: 10.20, S: 15.62%

(Z)-Ethyl 2-(5-((4-chloro-2-(pyrrolidin-1-yl)thiazol-5-yl)methylene)-4-oxo-2-thioxothiazolidin-3-yl) acetate (T4)

Reaction time: 4h. The crude product was purified by column chromatography using hexane : EtOAc (3:1) as eluant. Yield: 54.48%, m.p.: 191 ºC; ^{1}H NMR, δ, ppm (400 MHz, $CDCl_3$): 1.28 (t, 3H, J= 7.00 Hz, $CH_2\underline{CH_3}$), 2.13 (broad s, 4H, $2CH_2$), 3.56 (broad s, 4H, $2NCH_2$), 4.23 (q, 2H, J= 7.20 Hz, $\underline{CH_2}CH_3$), 4.83 (s, 2H, $\underline{CH_2}CO$), 7.91 (s, 1H, =CH); ^{13}C NMR, δ ppm (100 MHz, $CDCl_3$): 191.2 (CS), 179.9 (CO), 167.9 (CO), 165.9 (C), 146.6 (C), 124.1 (C), 115.4 (C), 113.8 (CH), 61.9 ($\underline{CH_2}CH_3$), 50.0 (N-CH_2), 45.0 ($\underline{CH_2}CO$), 25.5 (CH_2), 14.1 ($CH_2\underline{CH_3}$); MS (ESI+) *m/z* (rel. intensity) : 418.0 (M+H, 100%); Anal. Calc. for $C_{15}H_{16}ClN_3O_3S_3$: C: 43.11, H: 3.86, N: 10.05, S: 23.02%, found; C: 43.55, H: 4.05, N: 10.41, S: 23.18%

(Z)-2-(5-((4-chloro-2-(pyrrolidin-1-yl)thiazol-5-yl)methylene)-4-oxo-2-thioxothiazolidin-3-yl)acetic acid (T5)

Reaction time: 4h. The crude product was purified by column chromatography using dichloromethane : EtOAc: acetic acid (5:1:0.1) as eluant. Yield: 16.85%, m.p.: 280 ºC; ^{1}H NMR, δ, ppm (400 MHz, DMSO-d_6): 2.03 (broad s, 4H, $2CH_2$), 3.36 (broad s, 4H, $2NCH_2$), 4.60 (s, 2H, $\underline{CH_2}CO$), 7.67 (s, 1H, =CH); MS (ESI-) *m/z* (rel. intensity) : 388.2 (M-H, 100%); Anal. Calc. for $C_{13}H_{12}ClN_3O_3S_3$–H_2O : C: 38.33, H: 3.46, N: 10.32, S: 23.56%, found; C: 38.23, H: 3.31, N: 10.17, S: 23.35%

(Z)-5-((4-chloro-2-morpholinothiazol-5-yl)methylene)-2-thioxothiazolidine-4-one (T8)

Reaction time: 2h. The crude product was crystallized from ethanol. Yield: 68.58%, m.p.: 287 ºC; ^{1}H NMR, δ, ppm (400 MHz, DMSO-d_6): 3.58 (t, 4H, J= 5.00 Hz, $2NCH_2$), 3.73 (t, 4H, J= 4.60 Hz, $2OCH_2$), 7.44 (s, 1H, =CH), 13.68 (s, 1H, NH); ^{13}C NMR, δ ppm (100 MHz, DMSO-d_6): 193.1 (CS), 170.6 (CO), 168.3 (C), 143.7 (C), 120.1 (C), 119.5 (C), 113.1 (CH), 65.0 (O-CH_2), 47.8 (N-CH_2); MS (ESI+) *m/z* (rel. intensity) : 347.9 (M+H, 80%); Anal. Calc. for $C_{11}H_{10}ClN_3O_2S_3$: C: 37.98, H: 2.90, N: 12.08, S: 27.65%, found; C: 38.09, H: 2.90, N: 12.20, S: 27.43%

(Z)-Ethyl 2-(5-((4-chloro-2 morpholinothiazol-5-yl)methylene)-4-oxo-2-thioxothiazolidin-3-yl)acetate (T10)

Reaction time: 3h. The crude product was purified by column chromatography using dichloromethane as eluant. Yield: 71.06%, m.p.: 206 ºC; ^{1}H NMR, δ, ppm (400 MHz, $CDCl_3$): 1.29 (t, 3H, J= 7.00 Hz, $CH_2\underline{CH_3}$), 3.64 (t, 4H, J= 4.60 Hz, $2NCH_2$), 3.84 (t, 4H, J= 4.80 Hz, $2OCH_2$), 4.24 (q, 2H, J= 7.00 Hz, $\underline{CH_2}CH_3$), 4.83 (s, 2H, $\underline{CH_2}CO$), 7.91 (s, 1H, =CH); ^{13}C NMR, δ ppm (100 MHz, $CDCl_3$): 191.1 (CS), 171.2 (CO), 166.3 (CO), 165.8 (C), 145.8 (C), 123.4 (C), 116.9 (C), 114.5 (CH), 65.8 (O-CH_2), 61.9 ($\underline{CH_2}CH_3$), 48.2 (N-CH_2), 45.0 ($\underline{CH_2}CO$), 14.1 ($CH_2\underline{CH_3}$); MS (ESI+) *m/z* (rel. intensity) : 433.6 (M+H, 40%); Anal. Calc. for $C_{15}H_{16}ClN_3O_4S_3$-0,4H_2O : C: 40.89, H: 3.84, N: 9.54, S: 21.78%, found; C: 40.84, H: 3.70, N: 9.56, S: 21.43%

(Z)-2-(5-((4-chloro-2-morpholinothiazol-5-yl)methylene)-4-oxo-2-thioxothiazolidin-3-yl)acetic acid (T11)

Reaction time: 2h. The crude product was purified by column chromatography using hexane : EtOAc (3:1) as eluant. Yield: 38.70%, m.p.: 255 ºC; ^{1}H NMR, δ, ppm (400 MHz, DMSO-d_6): 3.61 (t, 4H, J= 4.40 Hz, $2NCH_2$), 3.74 (t, 4H, J= 4.60 Hz, $2OCH_2$), 4.69 (s, 2H, $\underline{CH_2}CO$), 7.65 (s, 1H, =CH); ^{13}C NMR, δ ppm (100 MHz, DMSO-d_6): 190.8 (CS), 171.1 (CO), 167.2 (CO), 165.6 (C), 145.5 (C), 122.4 (C), 115.1 (C), 113.1 (CH), 65.1 (O-CH_2), 48.1 ($\underline{CH_2}CO$), 45.1 (N-CH_2); MS (ESI+) *m/z* (rel. intensity) : 405.6 (M+H, 60%); Anal. Calc. for $C_{13}H_{12}ClN_3O_4S_3$: C: 38.33, H: 3.46, N: 10.32, S: 23.56%, found; C: 38.23, H: 3.31, N: 10.17, S: 23.35%

(Z)-5-((4-chloro-2-(5-methyl-4H-1,2,4-triazol-3-ylthio)thiazol-5-yl)methylene)thiazolidine-2,4-dione (T13)

Reaction time: 5h. The crude product was crystallized from DMF-ethanol. Yield: 49.83%, m.p.: 288 ºC, ^{1}H-NMR, δ ppm (400 MHz, DMSO-d_6): 2.46 (s, 3H, CH_3), 7. 63 (s, 1H, =CH), 14.48 (s, 1H, NH); ^{13}C NMR, δ ppm (100 MHz, DMSO-d_6): 167.8 (CO), 166.5 (CO), 165.6 (C), 155.7 (C), 151.6 (C), 140.8 (C), 127.5 (C), 126.7 (C), 117.5 (CH), 11.7 (CH_3); MS (ESI+) *m/z* (rel. intensity): 360.1 (M+H, 100%); Anal. Calc. for $C_{10}H_6ClN_5O_2S_3$.0,5H_2O: C: 32.60, H: 1.90, N:19.02, S:26.08%, found; C: 32.47, H: 1.87, N:18.89, S:25.92%

(Z)-5-((4-chloro-2-(5-methyl -4H-1, 2, 4-triazol-3-ylthio)thiazol-5-yl)methylene)-2-thioxothiazolidine-4-one (T14)

Reaction time: 1h. The crude product was crystallized from dimethylformamide-ethanol. Yield: 58.28%, m.p.: 304 ºC, ^{1}H-NMR, δ ppm (400 MHz, DMSO-d_6): 2.47 (s, 3H, CH_3), 7. 46 (s, 1H, =CH); ^{13}C NMR, δ ppm (100 MHz, DMSO-d_6): 193.4 (CS), 168.6 (CO), 166.9 (C), 155.7 (C), 151.5 (C), 141.8 (C), 128.1 (C), 126.4 (C), 117.4 (CH), 11.7 (CH_3); MS (ESI+) *m/z* (rel. intensity): 376.0 (M+H, 100%); Anal. Calc. for $C_{10}H_6ClN_5OS_4$: C: 32.01, H: 1.61, N: 18.67, S: 34.11%, found; C: 32.06, H: 1.68, N:18.38, S:33.93%

(Z)-Ethyl-2-(5-((4-chloro-2-(5-methyl -4H-1, 2, 4-triazol-3-ylthio)thiazol-5-yl)methylene)-2,4-dioxothiazolidin-3-yl)acetate (T15)

Reaction time: 7h. The crude product was purified by column chromatography using dichloromethane : EtOAc (5:1) as eluant. Yield: 53.57%, m.p.: 169 ºC, ^{1}H-NMR, δ ppm (400 MHz, DMSO-d_6): 1.20 (t, 3H, J=7.20 Hz, $CH_2\underline{CH_3}$), 1.99 (s, 3H, CH_3), 4.17 (q, 2H, J=7.20 Hz, $\underline{CH_2}CH_3$), 4.50 (s, 2H, $\underline{CH_2}CO$), 7.82 (s, 1H, =CH); ^{13}C NMR, δ ppm (100 MHz, DMSO-d_6): 167.1 (CO), 166.4 (CO), 165.1 (CO), 163.9 (C), 155.7 (C), 151.5 (C), 142.4 (C), 125.7 (C), 122.4 (C), 120.7 (CH), 61.7 ($\underline{CH_2}CH_3$), 42.5 ($\underline{CH_2}CO$), 13.9 ($CH_2\underline{CH_3}$), 11.7 (CH_3); MS (ESI+) *m/z* (rel. intensity) : 446.0 (M+H, 100%); Anal. Calc. for $C_{14}H_{12}ClN_5O_4S_3$-0,3$C_4H_8O_2$: C: 38.69, H: 3.08, N: 14.85, S: 20.35%, found; C: 38.72, H: 3.12, N:14.92, S:20.35%

(Z)-2-(5-((4-Chloro-2-(5-methyl-4H-1,2,4-triazol-3-ylthio)thiazol-5-yl)methylene)-4-oxo-2-thioxothiazolidin-3-yl)acetic acid (T16)

Reaction time: 1h. The crude product was crystallized from dimethylformamide-ethanol. Yield: 42.07 %, m.p.: 288 ºC; ^{1}H NMR, δ, ppm (400 MHz, DMSO-d_6): 2.40 (s, 3H, CH_3), 4.37 (s, 2H, $\underline{CH_2}CO$), 7.60 (s, 1H, =CH); ^{13}C NMR, δ ppm (100 MHz, DMSO-d_6): 190.7 (CS), 167.8 (CO), 166.9 (CO), 165.4 (C), 155.8 (C), 142.8 (C), 126.1 (C), 123.5 (C), 119.8 (CH), 45.2 ($\underline{CH_2}CO$), 11.7 (CH_3); MS (ESI+) *m/z* (rel. intensity) : 434.1 (M+H, 100%); Anal. Calc. for $C_{12}H_8ClN_5O_3S_4$: C: 33.26, H: 1.86, N: 16.17, S: 29.54%, found; C: 33.44, H: 1.98, N: 16.05, S: 29.86%

(Z)-5-((4-Chloro-2-(5-phenyl-4H-1,2,4-triazol-3-ylthio)thiazol-5-yl)methylene)thiazolidine-2,4-dione (T18)

Reaction time: 3h. The crude product was crystallized from dimethylformamide-ethanol. Yield: 51.52%, m.p.: 286 ºC, ^{1}H-NMR, δ ppm (400 MHz, DMSO-d_6): 7.58-7.61 (m, 3H, Ar-H), 7. 65 (s, 1H, =CH), 8.05-8.07 (m, 2H, Ar-H); ^{13}C NMR, δ ppm (100 MHz, DMSO-d_6): 166.5 (CO), 165.9 (CO), 164.7 (C), 156.4 (C), 152.2 (C), 141.0 (C), 130.7 (C), 129.0 (C), 126.4 (C), 126.1 (C), 118.1 (CH); MS (ESI+) *m/z* (rel. intensity) : 422.0 (M+H, 100%); Anal. Calc. for $C_{15}H_8ClN_5O_2S_3$-H_2O: C: 41.00, H: 2.30, N: 15.95%, S: 21.85, found; C: 40.74, H: 2.45, N: 15.68, S:21.71%

(Z)-5-((4-Chloro-2-(5-phenyl-4H-1,2,4-triazol-3-ylthio)thiazol-5-yl)methylene)-2-thioxothiazolidine-4-one (T19)

Reaction time: 1h. The crude product was crystallized from dimethylformamide-ethanol. Yield: 70.77%, m.p.: 297 ºC, ^{1}H-NMR, δ ppm (400 MHz, DMSO-d_6): 7. 44 (s, 1H, =CH), 7.58-7.59 (m, 3H, Ar-H), 8.06-8.08 (m, 2H, Ar-H); MS (ESI+) *m/z* (rel. intensity) : 438.1 (M+H, 100%); Anal. Calc. for $C_{15}H_8ClN_5OS_4$-0,3H_2O: C: 40.69, H: 1.96, N: 15.83, S: 28.91%, found; C: 40.70, H: 2.19, N: 15.78, S:28.76%

(Z)-Ethyl 2-(5-((4-chloro-2-(5-phenyl-4H-1,2,4-triazol-3-ylthio)thiazol-5-yl)methylene)-2,4-dioxothiazolidin-3-yl)acetate (T20)

Reaction time: 2h. The crude product was purified by column chromatography using hexane : EtOAc (3:1) as eluant. Yield: 60.37%, m.p.: 188 ºC, ^{1}H-NMR, δ ppm (400 MHz, DMSO-d_6): 1.18 (t, 3H, J= 7.00 Hz, $CH_2\underline{CH_3}$), 4.14 (q, 2H, J= 7.00 Hz, $\underline{CH_2}CH_3$), 4.47 (s, 2H, $\underline{CH_2}CO$), 7.56-7.59 (m, 3H, Ar-H), 7. 82 (s, 1H, =CH), 8.03-8.06 (m, 2H, Ar-H); ^{13}C NMR, δ ppm (100 MHz, DMSO-d_6): 166.2 (CO), 164.8 (CO), 163.7 (CO), 142.1 (C), 130.8 (C), 129.0 (C), 126.1 (C), 125.8 (C), 122.3 (C), 120.6 (CH), 61.5 ($\underline{CH_2}CH_3$), 42.4 ($\underline{CH_2}CO$), 13.7 ($CH_2\underline{CH_3}$); MS (ESI+) *m/z* (rel. intensity) : 508.0 (M+H, 100%); Anal. Calc. for $C_{19}H_{14}ClN_5O_4S_3$: C: 44.92, H: 2.78, N: 13.79, S:

18.94%, found; C: 44.68, H: 2.89, N: 13.72, S:18.85%

(Z)-Ethyl 2-(5-((4-chloro-2-(5-phenyl-4H-1,2,4-triazol-3-ylthio)thiazol-5-yl)methylene)-4-oxo-2-thioxothiazolidin-3-yl)acetate (T21)

Reaction time: 1h. The crude product was crystallized from dimethylformamide-ethanol. Yield: 64.01%, m.p.: 249 ºC, ^{1}H-NMR, δ ppm (400 MHz, DMSO-d_6): 1.20 (t, 3H, J= 7.20 Hz, $CH_2\underline{CH_3}$), 4.17 (q, 2H, J= 7.00 Hz, $\underline{CH_2}CH_3$), 4.81 (s, 2H, $\underline{CH_2}CO$), 7.59-7.60 (m, 3H, Ar-H), 7. 68 (s, 1H, =CH), 8.07-8.10 (m, 2H, Ar-H); ^{13}C NMR, δ ppm (100 MHz, DMSO-d_6): 190.5 (CS), 165.5 (CO), 165.1 (CO), 142.7 (C), 130.8 (C), 128.9 (C), 126.1 (C), 123.2 (C), 119.9 (CH), 61.5 ($\underline{CH_2}CH_3$), 45.0 ($\underline{CH_2}CO$), 13.8 (CH_3); MS (ESI+) *m/z* (rel. intensity): 524.0 (M+H, 70%); Anal. Calc. for $C_{19}H_{14}ClN_5O_3S_4$: C: 43.55, H: 2.69, N: 13.36, S: 24.45%, found; C: 43.30, H: 2.60, N: 13.26, S: 24.03%

(Z)-2-(5-((4-chloro-2-(5-phenyl-4H-1,2,4-triazol-3-ylthio)thiazol-5-yl)methylene)-4-oxo-2-thioxothiazolidin-3-yl)acetic acid (T22)

Reaction time: 1h. The crude product was crystallized from dimethylformamide-ethanol. Yield: 49.85%, m.p.: 355 ºC (decomp.). ^{1}H-NMR, δ ppm (400 MHz, DMSO-d_6): 4.71 (s, 2H, $\underline{CH_2}CO$), 7.59-7.60 (m, 3H, Ar-H), 7. 67 (s, 1H, =CH), 8.08-8.10 (m, 2H, Ar-H); MS (ESI+) *m/z* (rel. intensity) : 496.1 (M+H, 60%); Anal. Calc. for $C_{17}H_{10}ClN_5O_3S_4$: C: 41.17, H: 2.03, N: 14.12, S: 25.86%, found; C: 40.86, H: 2.06, N: 144.52, S: 26.11%

General synthesis of compounds T6, T17, T23

A mixture of acetic acid ester compound **T4 / T15 / T20** (0.2 mmol), glacial acetic acid (4 mL) and HCl 12 N (1 mL) was refluxed for 2h. After evaporation in vacuo, the residue was refluxed again with glacial acetic acid (4 mL) and HCl 12 N (1 mL) for 2h after evaporation to dryness in vacuo.

(Z)-2-(5-((4-chloro-2-(pyrrolidin-1-yl)thiazol-5-yl)methylene)-2,4-dioxothiazolidin-3-yl)acetic acid (T6)

The crude solid was crystallized from ethanol providing pure carboxylic acid. Yield: 37.63%, m.p.: 280 ºC; ^{1}H NMR, δ, ppm (400 MHz, DMSO-d_6): 1.98-2.02 (m, 4H, $2CH_2$), 3.48 (broad s, 4H, $2NCH_2$), 4.32 (s, 2H, $\underline{CH_2}CO$), 7.79 (s, 1H, =CH); ^{13}C NMR, δ ppm (100 MHz, DMSO-d_6): 167.9 (CO), 166.7 (CO), 165.8 (CO), 164.3 (C), 144.5 (C), 122.9 (C), 112.9 (C), 111.3 (CH), 49.9 (N-CH_2), 42.4 ($\underline{CH_2}CO$), 25.1 (CH_2); MS (ESI+) *m/z* (rel. intensity) : 373.7 (M+H, 100%); Anal. Calc. for $C_{13}H_{12}ClN_3O_4S_2$-0,6HCl: C: 39.54, H: 3.22, N: 10.65, S: 16.21%, found; C: 39.64, H: 3.40, N: 10.78, S: 16.29%

(Z)-2-(5-((4-Chloro-2-(5-methyl-4H-1,2,4-triazol-3-ylthio)thiazol-5-yl)methylene)-2,4-dioxothiazolidin-3-yl)acetic acid (T17)

The crude solid was crystallized from acetic acid glacial providing pure carboxylic acid. Yield: 60.71%, m.p.: 274 ºC; ^{1}H NMR, δ, ppm (400 MHz, DMSO-d_6): 2.47 (s, 3H, CH_3), 4.38 (s, 2H, $\underline{CH_2}CO$), 7.82 (s, 1H, =CH); ^{13}C NMR, δ ppm (100 MHz, DMSO-d_6): 167.6 (CO), 166.9 (CO), 165.1 (CO), 164.0 (C), 155.7 (C), 142.2 (C), 125.7 (C), 122.5 (C), 120.4 (CH), 42.6 ($\underline{CH_2}CO$), 11.7 (CH_3); MS (ESI+) *m/z* (rel. intensity) : 418.0 (M+H, 100%); Anal. Calc. for $C_{12}H_8ClN_5O_4S_3$-0,2 CH_3COOH: C: 34.69, H: 2.08, N: 16.32, S: 22.36%, found; C: 34.94, H: 2.32, N: 16.15, S: 22.20%

(Z)-2-(5-((4-chloro-2-(5-phenyl-4H-1,2,4-triazol-3-ylthio)thiazol-5-yl)methylene)-2,4-dioxothiazolidin-3-yl)acetic acid (T23)

The crude solid was crystallized from ethanol providing pure carboxylic acid. Yield: 57.33%, m.p.: 275 ºC, ^{1}H-NMR, δ ppm (400 MHz, DMSO-d_6): 4.37 (s, 2H, $\underline{CH_2}CO$), 7.58-7.60 (m, 3H, Ar-H), 7. 82 (s, 1H, =CH), 8.05-8.08 (m, 2H, Ar-H); ^{13}C NMR, δ ppm (100 MHz, DMSO-d_6): 167.6 (CO), 165.9 (CO), 165.0 (C), 164.1 (C), 156.8 (C), 151.9 (C), 141.9 (C), 130.8 (C), 129.1 (C), 126.1 (C), 122.8 (C), 120.2 (CH), 43.2 ($\underline{CH_2}CO$); MS (ESI-) *m/z* (rel. intensity) : 478.1 (M-H, 70%); Anal. Calc. for $C_{17}H_{10}ClN_5O_4S_3$-$2H_2O$: C: 39.61, H: 2.74, N: 13.60, S: 18.63%, found; C: 39.78, H: 2.76, N:13.65, S:18.74%

Biological Part

Preparation of Stock Solutions

All tested drug solutions were prepared as 5 mM in DMSO and stored in -20 ºC until to use. Further dilutions of the compounds were prepared in respective media used for each cell line. DMSO was used as the control vehicle for all experiments and doxorubicin was used as positive anticancer drug control to check SRB assay efficiency.

Cancer Cell Lines

Two HCC cell lines Huh7 and Plc/Prf/5 (Plc) were used for initial screening experiments. Five hepatocellular carcinoma (HCC) cell lines [Huh7, Hep3B, Snu449, Plc and HepG2] and one breast cancer cell line Mcf7 were used for secondary screening. Cells were cultured at 37 ^{0}C with 5% CO_2 in DMEM completed with 10% FBS, 1x

NEA, 2 mM L-Glutamine and 100 units penicillin/ streptomycine except Snu449 that was cultured in completed RPMI 1640.

Sulforhodamine B (SRB) Assay for Cytotoxicity

SRB assay was carried out as described elsewhere with some modifications (16). Shortly, cells were cultured in 96 well plates for 24h. After the incubation, fresh media with tested compounds were added onto the cells. When a 72h incubation period was completed, media was discarded. Cells were washed with 1xPBS once and fixed by 10% trichloroacetic acid (TCA) for 1h at 4 ºC. Overnight, dried plates were stained with 0.4% SRB in 1% acetic acid for 10 minutes at room temperature. To wash the excess of SRB, dye plates were washed with 1% acetic acid 5 times by tapping. Unbuffered 10 mM trisma base solution was added into the wells to resolubilize the SRB dye. Optic density (OD) of each well was measured by µ-Quant microplate reader in a 405 to 515 nm wave length range. Each concentration of the compounds was tested as triplicate. Average of OD values was used for calculation of cell survival percentages and IC_{50} values.

RESULTS

Chemistry

Thiazolyl-2,4-thiazolidinedione / rhodanine compounds **T1-T23** were synthesized according to the synthetic pathway described in Scheme 1. 2,4-dichlorothiazole-5-carbaldehyde (**II**) (17) was obtained with 2,4-TZD (**Ia**) (18) and N,N-dimethylformamide in phosphoryl chloride. Morpholine / pyrrolidine / triazole substituted-4-chloro-thiazole-5-carbaldehydes (**IIIa-d**) were synthesized with 2,4-dichlorothiazole-5-carbaldehyde (**II**) and appropriate heterocyclic amine in sodium carbonate/ acetonitrile. Ethyl 2,4-dioxothiazolidin-3-yl acetate (**Ib**) was prepared via N-alkylation of 2,4-TZD with ethyl bromoacetate in THF / NaH (19). Ethyl 2-(4-oxo-2-thioxo-thiazolidin-3-yl) acetate (**Ie**) was obtained from 2-(4-oxo-2-thioxo-thiazolidin-3-yl) acetic acid (**Id**) by esterification reaction in ethanol / H_2SO_4.

The condensation of thiazolyl carbaldehydes (**IIIa-d)** with 2,4-TZD (**Ia**) / ethyl 2,4-dioxothiazolidine-3-ylacetate **Ib** / rhodanine (**Ic) /** 2-(4-oxo-2-thioxo-thiazolidin-3-yl) acetic acid (**Id**) / ethyl 2-(4-oxo-2-thioxo-thiazolidin-3-yl) acetate (**Ie**) in the presence of sodium acetate / acetic acid glacial by Knoevenagel reaction, led to thiazolyl-2,4-TZDs / rhodanines (**T1-T5, T7-T11, T13-T15, T16, T18-T22)**.

The acidic hydrolysis of TZD-ester compounds **T3, T9, T15** and **T20** provided corresponding carboxylic acids **T6, T12, T17** and **T23**.

The structure of the synthesized compounds was elucidated by elementary analysis, ^{1}H NMR, ^{13}C NMR and mass spectral data. All spectral data were in accordance with assumed structures. It was reported that by using unsubstituted imidazolidinediones and benzaldehydes in acidic medium, the main product was the Z isomer (20). In our previous papers related with thiazolyl TZD and our other TZD compounds, all our synthesized compounds were seen in Z isomeric form (13,21-24). Besides one of our compounds (**T9)** was found as Z isomer (13). In this study, we used the same reaction conditions for obtaining the compounds and also, in ^{1}H NMR spectra, methyne protons (=CH) of the compounds **T1-T23** were observed between 7.26-8.07 ppm as a singlet which correspond to the results in our recently published papers. According to our previous data, we can say that our compounds **T1-T23** were formed in Z configuration. ^{13}C NMR data of all new compounds was reported except compounds **T5, T19, T22** because of their poor solubility.

Mass analysis of compounds was performed by using ESI (+) method. All the compounds have M+H ion peaks except compounds **T5** and **T23.** Thus, their mass analysis was performed by using ESI (-) method and their M-H ion peaks were observed.

2.2. Biology

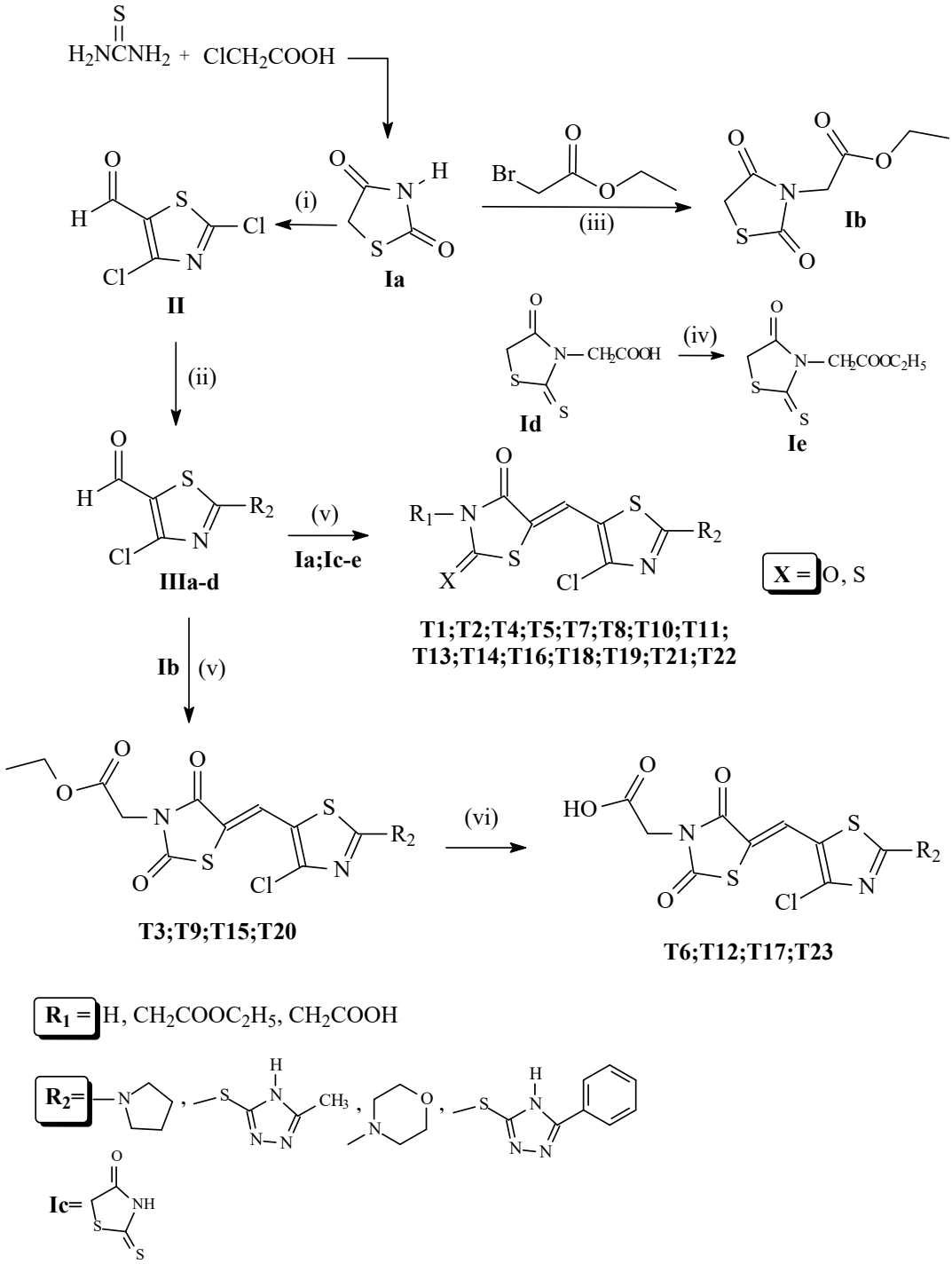
Anticancer activity

We synthesized a new series of thiazolyl-2,4-thiazolidinediones / rhodanines having pyrrolidine, morpholine, triazole ring systems for investigation of their anticancer activities. All the synthesized compounds have TZD or its isostere rhodanine ring which contains hydrogen atom, acetic acid and acetic acid ethyl ester groups on their N-3 position.

First of all, 10 µM concentrations of 23 TZD and analogue compounds were screened by sulforhodamine B assay in two HCC cell line, Huh7 and Plc (Figure 2). Three compounds (**T4**, **T15** and **T21**) showed very strong cytotoxic activity against Huh7 cell line compared to Plc cell line (Table 1). In the second screening of these three compounds in 6 different cancer cell lines (5 HCC, 1 breast), it was carried out with at least 5 different concentrations (2,5 to 40µM) of the compounds. We found that **T21** was the most effective anticancer agent. As it can be seen in

Figure 3, IC_{50} values of **T21** were from 5 to 16μM.

According to cell survival plots of these compounds (Figure 4a, 4b, 4c), **T4** was the most tolerable agent for HCC cell lines, except Huh7 (Figure 5a). Huh7 was the most affected cell line by all three compounds and a very sharp decrease of the plotlines in Figure 4 and Figure 5a indicate that. When we compare the different cell densities at various concentrations, Plc was relatively resistant to **T21** than other cell lines (Figure 4c) while Snu449 was strongly resistant to **T4** and **T15** (Figure 5b). As expected, **T4, T15,** and **T21** stopped the well-known hyper-chemosensitive Mcf7 cells (Figure 5c), however not as much as Huh7.



Scheme 1. (i) = $POCl_3$ / DMF; (ii) = R_2-H / Na_2CO_3 / CH_3CN; (iii) = NaH / THF ; (iv) = Ethanol / H_2SO_4 (v) = CH_3COOH / CH_3COONa; (vi) = CH_3COOH / HCl.

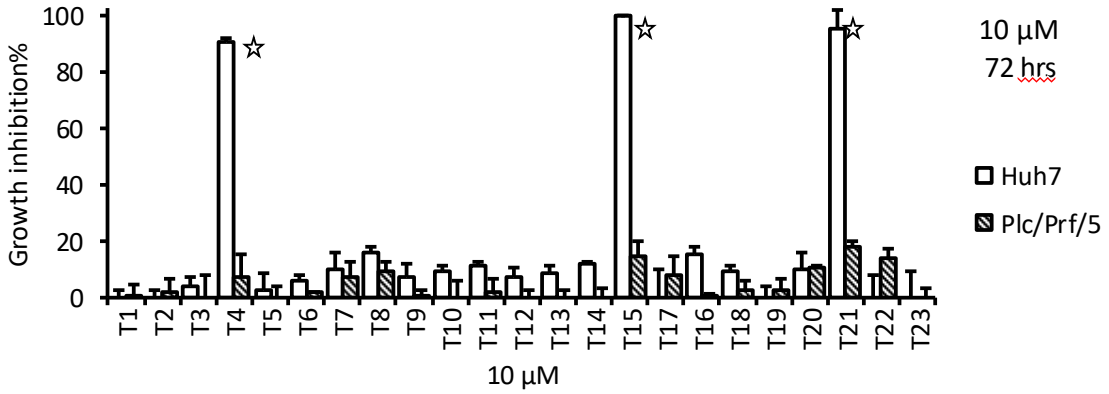


Figure 2. ***Initial cytotoxicity screening of TZDs***. Huh7 and Plc/Prf/5 cells were treated by 10 µM concentration of each TZD for 72 hours. At the end of the treatment, cells were fixed by 10% TCA for 1 hour at 4°C, dark. Cells were washed by ddH2O twice and the plates were left for dry. 0.4% sulforhodamine B (in 0.1% acetic acid) was used for staining protein content of treated/untreated cells. DMSO was used as vehicle control. After washing excessive SRB dye, well dried plates were resolublized by 100 µl unbuffered 10mM Trisma solution per well. Optical density values, reflecting cell densities, were measured at in a 405 to 515 nm wave length range. Growth inhibition percentages were calculated by comparing the average OD values in the wells of the vehicle (DMSO) and TZDs. Growth inhibition percentage and their standard deviation values of each treatment were used for the plot. Star symbol shows the most effective compounds.

Table 1. IC_{50} values of the most promising TZDs for 72 hours treatment.

		T4		***T15***		***T21***		***Doxorubicine***	
Type of Cancer	***Cell line***	**IC_{50} (µM)**	**± SD**	**IC_{50} (µM)**	**± SD**	**IC_{50} (µM)**	**± SD**	**IC_{50} (µM)**	**± SD**
HCC	***Huh7***	3.16	0.88	5.43	1.09	4.67	0.93	0.0016	0.0002
HCC	***Hep3B***	8.89	0.80	9.61	0.96	6.02	1.20	1.59	0.0159
HCC	***HepG2***	32.30	7.43	12.75	2.04	13.69	2.05	0.03	0.0021
HCC	***Plc/Prf/5***	32.97	5.60	17.37	1.91	16.49	3.46	1.02	0.02
HCC	***Snu449***	non-toxic		12.10	2.42	8.16	1.14	19.16	0.13
Breast	***Mcf7***	9.33	0.7	9.41	0.75	2.30	0.32	<0.0001	-

DISCUSSION

In this series of thiazolyl-2,4-thiazolidinediones / rhodanines, most potent compounds were compounds **T4**, **T15** and **T21** which have pyrrolidine, methyl substituted triazole and phenyl substituted triazole ring, respectively. Among active compounds, pyrrolidino thiazole compound (**T4)** and phenyl substituted triazolo thiazole compound (**T21)** have rhodanine ring, in contrast, a methyl substituted triazole compound (**T15**) has TZD ring. All these compounds (**T4**, **T15** and **T21**) contain acetic acid ethyl ester group on N-3 position of TZD or its isostere rhodanine ring. In this series, TZD compounds, differing by only one atom from rhodanine (S to O), can produce similar anticancer effects for Huh7, Hep3B cell lines. On the other hand, phenyl substituted triazolo thiazolyl rhodanine compound (**T21)** is more potent than pyrrolidino thiazolyl rhodanine compound (**T4)** and methyl substituted triazolo thiazolyl TZD (**T15**) against Mcf7 line. Besides, it was not seen the difference of anticancer activity against Mcf7 between rhodanine compound **T4** and TZD compound **T15** (Figure 5c). **T15** and **T21** are more potent than **T4** against Plc and HepG2 cell lines. In the meantime, **T4** is not toxic against Snu449 while **T15** and **T21** have similar activity. **T20** is a TZD compound and analogue of **T21**. Interestingly, **T20** was nonresponsive compound. When their structures are compared, **T20** and **T21** differ only one atom from each other (O to S). This

difference has destroyed completely the activity of **T20**.

Surprisingly, none of the thiazolyl TZD or rhodanine compounds containing six membered morpholine ring have anticancer potency.

As a result, we can say that TZD and its isostere ring rhodanine can produce about similar anticancer effects for Huh7, Hep3B cell lines. Besides, it is important to declare that compounds having lipofilic acetic acid ethyl ester group instead of acetic acid or imidic hydrogen with acidic character on the N-3 position of TZD and rhodanine rings; and also, five membered heterocyclic rings such as triazole and pyrrolidine on thiazole ring have been significant for anticancer potency.

CONCLUSION

The goal of this study was synthesizing a new series of pyrrolidino / morpholino / triazolo thiazolyl-2,4-thiazolidinediones / rhodanines and then investigating their anticancer activities in hepatocellular carcinoma (HCC) cell lines. All 23 compounds were initially screened in Huh7 and Plc/Prf/5 (Plc) cell lines by sulforhodamine B assay. **T4**, **T15**, **T21** were three candidates that had very strong anticancer effects even though their 10 µM concentration in Huh7 cell line, in contrast they had no effect on Plc cell line. We chose these compounds for further experiments to calculate IC_{50} values in five different HCC and one breast cancer cell lines. According to IC_{50} values, **T21** was the most effective compound with IC_{50} values in a range from 2 to 16 µM in 6 cancer cell lines.

Compounds **T4**, **T15** and **T21** have pyrrolidine, methyl substituted triazole and phenyl substituted triazole rings, respectively. Pyrrolidino thiazole compound **T4** and phenyl substituted triazolo thiazole compound **T21** have rhodanine ring, while methyl substituted triazolo thiazole **T15** has TZD ring. All these compounds (**T4**, **T15** and **T21**) contain acetic acid ethyl ester group on N-3 position of TZD or its isostere rhodanine ring.

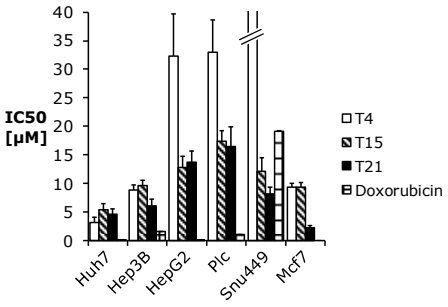


Figure 3. Cell type dependent IC_{50} differences of TZDs.

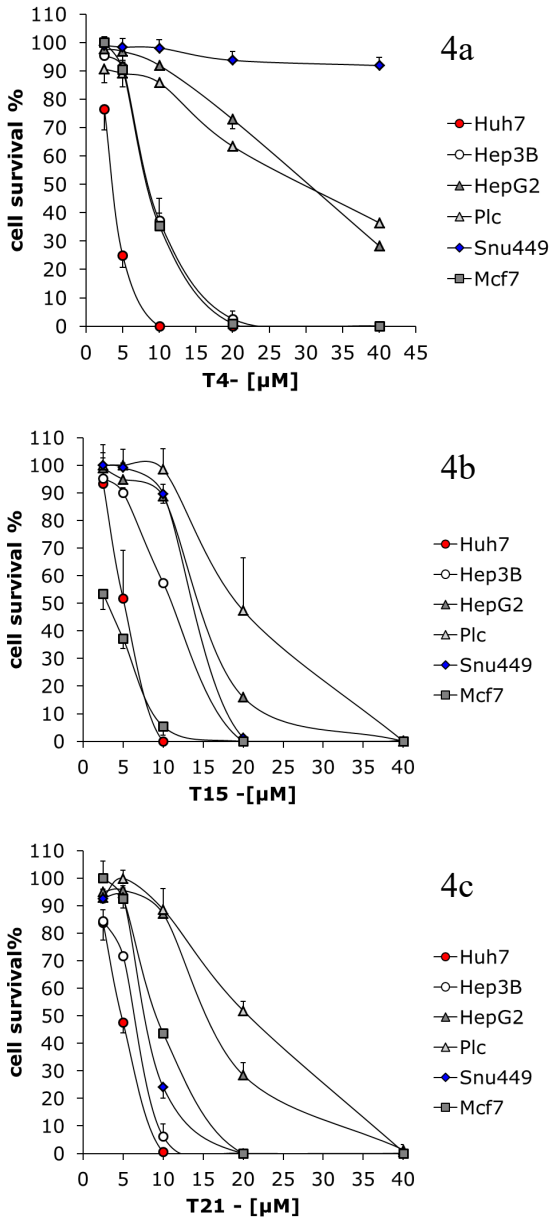
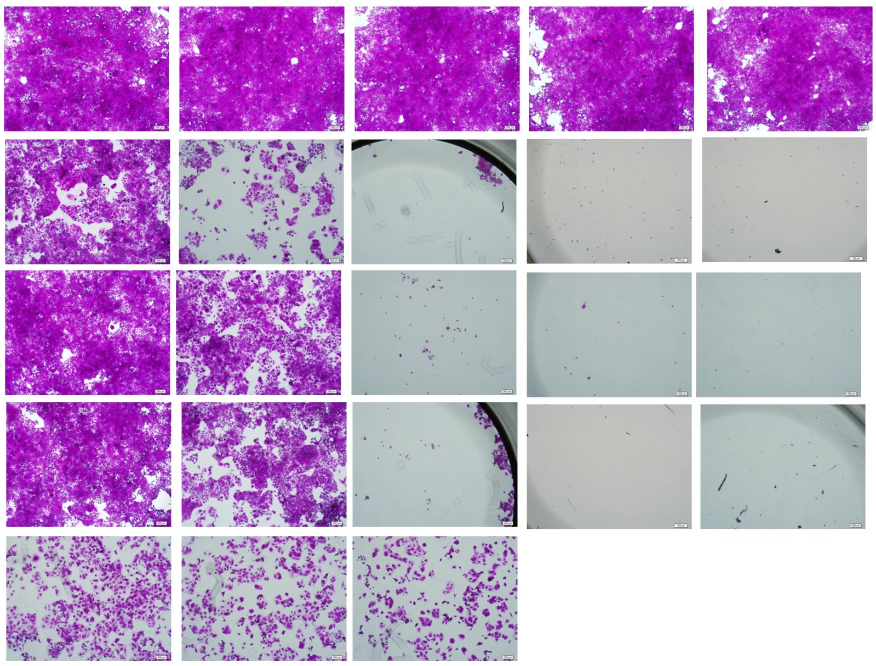


Figure 4. TZD Dose-Response Curves for Huh7, Hep3B, HepG2, Plc, Snu449 and Mcf7 cell lines. (a) **T4**, 72h treatment plot, (b) **T15,** 72h treatment plot, (c) **T21** 72h treatment plot. Cell survival percentage and standard deviation values were used.

TZD and its isostere rhodanine compounds can produce similar anticancer effects for Huh7, Hep3B cell lines and as a conclusion, thiazole compounds bearing five membered heterocyclic rings with lipofilic group on N-3 position of TZD and rhodanine ring have been significant for anticancer potency.

The overall data presented in this study provide compounds **T4**, **T15** and **T21** as an excellent class of novel anticancer agents that may lead to the development of more potent anticancer drugs in the future.

5(a) Huh7



DMSO*

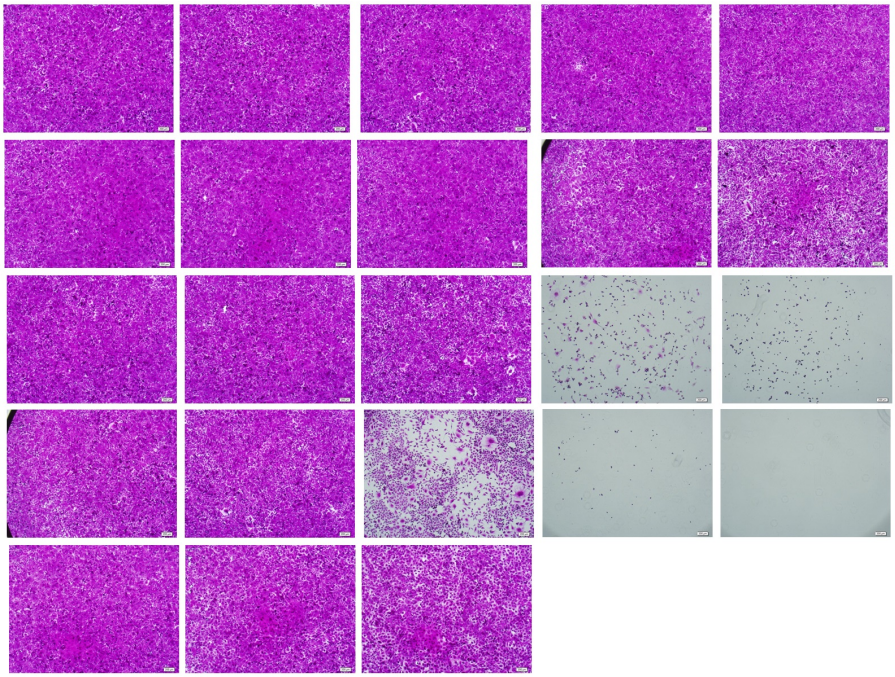
T4
2.5- 5.0- 10.0- 20.0- 40.0 µM

T15
2.5- 5.0- 10.0- 20.0- 40.0 µM

T21
2.5- 5.0- 10.0- 20.0- 40.0 µM

Doxorubicine
1.0 - 2.0, 4.0 µM

5(b) Snu449



DMSO*

T4
2.5- 5.0- 10.0- 20.0- 40.0 µM

T15
2.5- 5.0- 10.0- 20.0- 40.0 µM

T21
2.5- 5.0- 10.0- 20.0- 40.0 µM

Doxorubicine
1.0 - 2.0, 4.0 µM

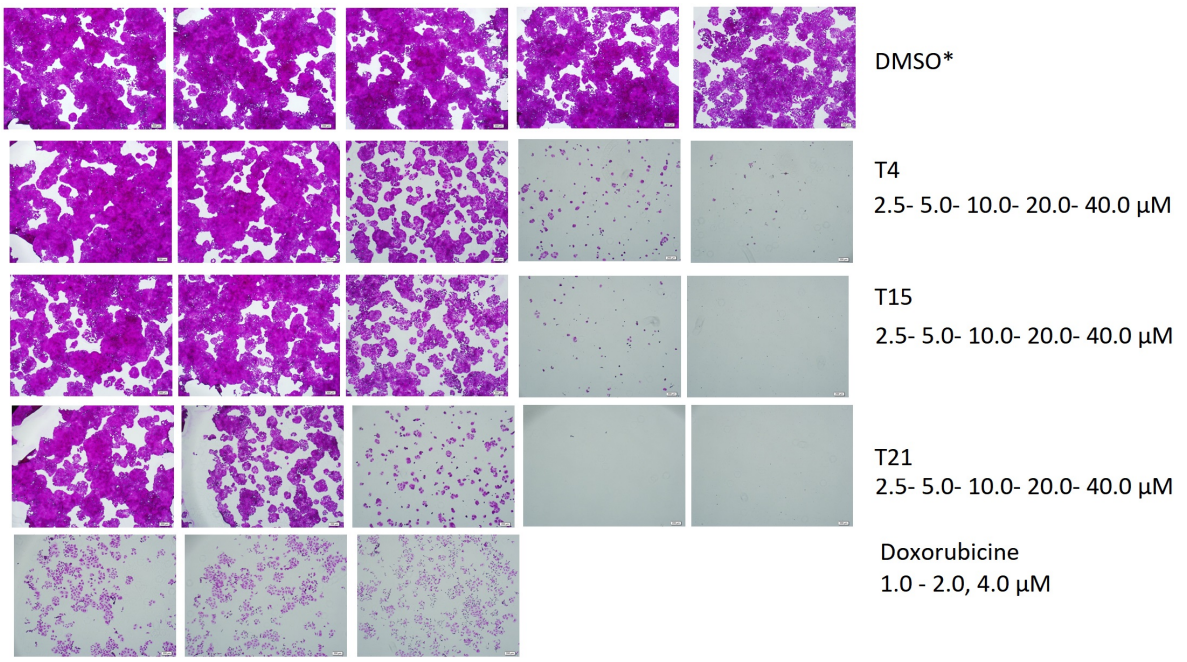


Figure 5. Concentration dependent cell density changes after TZD treatments. Huh7(a), Snu449 (b) and Mcf7 cells were treated with 2.5, 5, 10, 20 and 40 µM of TZDs, respective amount of DMSO and 1, 2, 4 µM doxorubicine for 72h. SRB stained cells were photographed under phase-contrast inverted microscope before resolubilization step of cytotoxicity assay.

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