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 IC50 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 IC50 values, T21 was the most effective compound with IC50 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).

Thiazolinedione (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 antitumor 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...
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.

![Figure 1. Formula of the compounds T1-T23.](image)

| Compound No | X | R1 | Substituents | Compound No | X | Substituents |
|-------------|---|----|--------------|-------------|---|--------------|
| T1          | O | H  |              | T13         | O | H  |  
| T2          | S | H  |              | T14         | S | H  |  
| T3          | O | CH2COOC2H5 |  | T15         | O | CH2COOC2H5 |  
| T4          | S | CH2COOC2H5 |  | T16         | S | CH2COOH |  
| T5          | S | CH2COOH |  | T17         | O | CH2COOH |  
| T6          | O | CH2COOH |  | T18         | O | H  |  
| T7          | O | H  |  | T19         | S | H  |  
| T8          | S | H  |  | T20         | O | CH2COOC2H5 |  
| T9          | O | CH2COOC2H5 |  | T21         | S | CH2COOC2H5 |  
| T10         | S | CH2COOC2H5 |  | T22         | S | CH2COOH |  
| T11         | S | CH2COOH |  | T23         | O | CH2COOH |  
| T12         | O | CH2COOH |  |              |   |    |  

**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. 1H NMR and 13C NMR spectra were determined with a VARIAN Mercury 400 FT-NMR spectrometer (Varian Inc, Palo Alto, CA, USA) in CDCl3 and DMSO-d6. 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-((4-chloro-2-(morpholin-4-yl)-1,3-thiazol-5-yl)methylidene)-2,4-dioxothiazolidine-3-yl)acetate (T9), (Z)-2-((4-chloro-2-(morpholin-4-yl)-1,3-thiazol-5-yl) methylidene)-2,4-dioxothiazolidine-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 H2SO4 (0.5 ml) was refluxed for 7h. The crude product was crystallized from ethanol. Yield: 0.445 g, 77.21%; m.p.: 60 °C.

Spectroscopic analysis: 1H-NMR (δ ppm; CDCl3): 1.29 (t, 3H, CH3), 4.23 (q, 2H, CH2), 4.08 (s, 2H, CH2), 4.72 (s, 2H, CH2). Anal. Calc. for C7H9NO3S2: 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 °C (Ref.14; m.p.:118 °C).

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 °C (Ref.13; m.p.: 200 °C).

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 °C. (Ref.15; m.p.: 178 °C).

4-Chloro-2-(5-phenyl-4H-1,2,4-triazol-3-ylthio)thiazole-5-carbaldehyde (IIIId)
This compound was obtained as cream colored crystals. Yield: 61.97%, m. p.: 210 °C. Spectroscopic analysis: 1H-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 °C in the presence of 0.5 ml acetic acid glacial and sodium acetate (0.001 mol). The reaction mixture was extracted with CHCl3 (3X50 ml) and the organic layer was washed with water, dried over anhydrous Na2SO4 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 using dichloromethane : EtOAc (5:1) as eluant. Yield: 34.0%, m.p.: 290 °C; 1H NMR, δ, ppm (400 MHz, CDCl3): 2.10-2.14 (m, 4H, 2CH2), 3.55 (broad s, 4H, 2NCH2), 7.97 (s, 1H, =CH); 13C NMR, δ ppm (100 MHz, DMSO-d6): 166.6 (C=O), 166.3 (C=O), 143.1 (C), 120.9 (C), 116.5 (C), 111.7 (CH), 49.6 (N-CH2), 24.9 (CH2); MS (ESI+) m/z (rel. intensity): 315.9 (M+H, 100%); Anal. Calc. for C11H10ClN3O2S2: C: 41.84, H: 3.19, N: 13.31, S: 19.33%, found; C:38.53, H:4.19, N:6.47, S:29.58%

(Z)-5-((4-chloro-2-(pyrrolidine-1-yl)thiazol-5-yl)methylene) thiazolidine-2,4-dione (T1)
Reaction time: 4h. The crude product was purified by column chromatography using dichloromethane : EtOAc (5:1) as eluant. Yield: 59.0%, m.p.: 281 °C; 1H NMR, δ, ppm (400 MHz, DMSO-d6): 2.10-2.14 (m, 4H, 2CH2), 3.55 (broad s, 4H, 2NCH2), 7.97 (s, 1H, =CH); 13C NMR, δ ppm (100 MHz, DMSO-d6): 166.6 (CO), 166.3 (CO), 143.1 (C), 120.9 (C), 116.5 (C), 111.7 (CH), 49.6 (N-CH2), 24.9 (CH2); MS (ESI+) m/z (rel. intensity): 315.9 (M+H, 100%); Anal. Calc. for C11H10ClN3O2S2: 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)-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: 54.53%, m.p.: 192 °C; 1H NMR, δ ppm (400 MHz, CDCl3): 1.29 (t, 3H, J = 7.00 Hz, CH3CH2), 2.10-2.14 (m, 4H, 2CH2), 3.56 (broad s, 4H, 2NCH2), 4.24 (q, 2H, J = 7.20 Hz, CH2CH3), 4.45 (s, 2H, CH2CO), 8.05 (s, 1H, =CH). 13C NMR, δ ppm (100 MHz, CDCl3): 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 (CH2CH3), 49.9 (N-CH2), 42.2 (CH2CO), 25.6 (CH2), 14.1 (CH2CH3); MS (ESI+) m/z (rel. intensity): 401.7 (M+H, 100%); Anal. Calc. for C15H16ClN3O4S3: C: 43.11, H: 3.86, N: 10.05, S: 23.80%.

(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 °C; 1H NMR, δ ppm (400 MHz, CDCl3): 1.18 (t, 3H, J = 7.00 Hz, CH2CH3), 2.13 (broad s, 4H, 2CH2), 3.56 (broad s, 4H, 2NCH2), 4.23 (q, 2H, J = 7.20 Hz, CH2CH3), 4.83 (s, 2H, CH2CO), 7.91 (s, 1H, =CH). 13C NMR, δ ppm (100 MHz, CDCl3): 191.2 (CS), 179.9 (CO), 167.9 (CO), 165.9 (C), 146.6 (C), 124.1 (C), 113.5 (C), 113.8 (CH), 61.9 (CH2CH3), 50.0 (N-CH2), 45.0 (CH2CO), 25.5 (CH2), 14.1 (CH2CH3); MS (ESI+) m/z (rel. intensity): 418.0 (M+H, 100%); Anal. Calc. for C15H10ClN3O3S3: C: 37.98, H: 2.90, N: 12.08, S: 23.80%.

(Z)-2-((4-chloro-2-pyrrolidin-1-yl)thiazol-5-yl)methylen)-4-oxo-2-thioxothiazolidin-3-yl)acetate (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 °C; 1H NMR, δ ppm (400 MHz, DMSO-d6): 2.03 (broad s, 4H, 2CH2), 3.36 (broad s, 4H, 2NCH2), 4.60 (s, 2H, CH2CO), 7.67 (s, 1H, =CH); MS (ESI-) m/z (rel. intensity): 388.2 (M-H, 100%); Anal. Calc. for C13H12ClN3S2-O2H: 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 purified by column chromatography using dichloromethane: ethyl acetate (8:2) as eluant. Yield: 68.84%, m.p.: 106 °C; 1H NMR, δ ppm (400 MHz, DMSO-d6): 1.99 (t, 3H, J = 7.00 Hz, CH2CH3), 2.12-2.16 (m, 4H, 2CH2), 3.57 (broad s, 4H, 2NCH2), 3.70 (t, 2H, J = 7.20 Hz, CH2CH3), 4.45 (s, 2H, CH2CO), 8.05 (s, 1H, =CH). 13C NMR, δ ppm (100 MHz, DMSO-d6): 190.2 (CO), 170.2 (CO), 165.7 (C), 145.2 (C), 124.9 (C), 113.7 (C), 112.9 (CH), 62.0 (CH2CH3), 49.9 (N-CH2), 42.2 (CH2CO), 25.6 (CH2), 14.1 (CH2CH3); MS (ESI+) m/z (rel. intensity): 347.9 (M+H, 80%); Anal. Calc. for C15H10ClN3O3S3: 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)-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 °C; 1H-NMR, δ ppm (400 MHz, DMSO-d6): 2.46 (s, 3H, CH3), 7.63 (s, 1H, =CH), 14.48 (s, 1H, NH); 13C NMR, δ ppm (100 MHz, DMSO-d6): 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 (CH3); MS (ESI+) m/z (rel. intensity): 376.0 (M+H, 100%); Anal. Calc. for C10H6ClN5OS4: C: 32.01, H: 1.61, N: 18.67, S: 33.93%. Found: C: 32.06, H: 1.68, N: 18.38, S: 33.91%.

Yield: 42.07%, m.p.: 288 °C; 1H NMR, δ ppm (400 MHz, DMSO-d6): 2.40 (s, 3H, CH3), 4.37 (s, 2H, CH2CO), 7.60 (s, 1H, =CH); 13C NMR, δ ppm (100 MHz, DMSO-d6): 190.7 (CO), 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 (CH2CO), 11.7 (CH3); MS (ESI+) m/z (rel. intensity): 434.1 (M+H, 100%); Anal. Calc. for C12H9ClN5O4S5: C: 40.69, H: 2.30, N: 13.79, S: 25.92%.

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

Reaction time: 1h. The crude product was crystallized from dimethylformamide-ethanol. Yield: 58.28%, m.p.: 304 °C; 1H NMR, δ ppm (400 MHz, DMSO-d6): 2.47 (s, 3H, CH3), 7.46 (s, 1H, =CH); 13C NMR, δ ppm (100 MHz, DMSO-d6): 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 (CH3); MS (ESI+) m/z (rel. intensity): 360.1 (M+H, 100%); Anal. Calc. for C10H6ClN5O2S3: 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-phenyl-4H-1,2,4-triazol-3-ylthio)thiazol-5-yl)methylene)-2-thioxothiazolidine-4-one (T17)

Reaction time: 3h. The crude product was purified by column chromatography using hexane : EtOAc (3:1) as eluant. Yield: 60.37%, m.p.: 188 °C; 1H NMR, δ ppm (400 MHz, DMSO-d6): 7.44 (s, 1H, =CH), 7.58-7.59 (m, 3H, Ar-H), 8.06-8.08 (m, 2H, Ar-H); 13C NMR, δ ppm (100 MHz, DMSO-d6): 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 C13H11ClN5O2S4: C: 40.69, 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-thioxothiazolidin-3-yl)acetate (T18)

Reaction time: 3h. The crude product was crystallized from dimethylformamide-ethanol. Yield: 51.52%, m.p.: 286 °C; 1H-NMR, δ ppm (400 MHz, DMSO-d6): 7.58-7.61 (m, 3H, Ar-H), 7.65 (s, 1H, =CH), 8.05-8.07 (m, 2H, Ar-H); 13C NMR, δ ppm (100 MHz, DMSO-d6): 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): 438.1 (M+H, 100%); Anal. Calc. for C13H11ClN5O2S4: 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)-5-((4-Chloro-2-((5-phenyl-4H-1,2,4-triazol-3-ylthio)thiazol-5-yl)methylene)-4-oxothiazolidin-3-yl)acetate (T19)

Reaction time: 1h. The crude product was purified by column chromatography using hexane : EtOAc (3:1) as eluant. Yield: 70.77%, m.p.: 297 °C; 1H-NMR, δ ppm (400 MHz, DMSO-d6): 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 C13H11ClN5O2S4: 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-methyl-4H-1,2,4-triazol-3-ylthio)thiazol-5-yl)methylene)-2-dioxothiazolidin-3-yl)methylene)-4-oxothiazolidin-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 °C; 1H-NMR, δ ppm (400 MHz, DMSO-d6): 7.44 (s, 1H, =CH), 7.58-7.61 (m, 3H, Ar-H), 8.06-8.08 (m, 2H, Ar-H); 13C NMR, δ ppm (100 MHz, DMSO-d6): 166.2 (CO), 165.8 (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): 508.0 (M+H, 100%); Anal. Calc. for C15H14ClN5O4S3: C: 40.69, H: 2.30, N: 15.95, S: 21.85, found: C: 40.74, H: 2.45, N: 15.68, S: 21.71%.
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. 1H-NMR, δ ppm (400 MHz, DMSO-d6): 1.20 (t, 3H, J= 7.20 Hz, CH2CH3), 4.17 (q, 2H, J= 7.00 Hz, CH2CH3), 4.81 (s, 2H, CH2CO), 7.59-7.60 (m, 3H, Ar-H), 7. 68 (s, 1H, =CH), 8.07-8.10 (m, 2H, Ar-H); 13C NMR, δ ppm (100 MHz, DMSO-d6): 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 (CH2CH3), 45.0 (CH2CO), 13.8 (CH3); MS (ESI+) m/z (rel. intensity): 524.0 (M+H, 70%); Anal. Calc. for C19H14ClN5O3S4: 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.). 1H-NMR, δ ppm (400 MHz, DMSO-d6): 4.71 (s, 2H, CH2CO), 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 C17H10ClN5O3S4: C: 41.17, H: 2.03, N: 14.12, S: 25.86%, found; C: 40.86, H: 2.06, N: 14.42, 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; 1H NMR, δ ppm (400 MHz, DMSO-d6): 1.98-2.02 (m, 4H, 2CH2), 3.48 (broad s, 4H, 2NCH2), 4.32 (s, 2H, CH2CO), 7.79 (s, 1H, =CH); 13C NMR, δ ppm (100 MHz, DMSO-d6): 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-CH2), 42.4 (CH2CO), 25.1 (CH2); MS (ESI+) m/z (rel. intensity): 373.7 (M+H, 100%); Anal. Calc. for C19H18ClN3O4S2: 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 ethanol providing pure carboxylic acid. Yield: 60.71%, m.p.: 274 ºC; 1H-NMR, δ ppm (400 MHz, DMSO-d6): 2.47 (s, 3H, CH3), 4.38 (s, 2H, CH2CO), 7.82 (s, 1H, =CH); 13C NMR, δ ppm (100 MHz, DMSO-d6): 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 (CH2CO), 11.7 (CH3); MS (ESI+) m/z (rel. intensity): 418.0 (M+H, 100%); Anal. Calc. for C12H8ClN5O4S3-0.2 CH3COOH: 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 carbonylic acid. Yield: 57.33%, m.p.: 275 ºC; 1H NMR, δ ppm (400 MHz, DMSO-d6): 4.37 (s, 2H, CH2CO), 7.58-7.60 (m, 3H, Ar-H), 7. 82 (s, 1H, =CH); 13C NMR, δ ppm (100 MHz, DMSO-d6): 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 (CH2CO); MS (ESI-) m/z (rel. intensity): 478.1 (M-H, 70%); Anal. Calc. for C17H10ClN5O4S3-2H2O: 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 ºC with 5% CO2 in DMEM completed with 10% FBS, 1x
NEA, 2 mM L-Glutamine and 100 units penicillin/~streptomycin 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-chlorothiazole-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 / $\text{H}_2\text{SO}_4$.

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.
**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.** IC₅₀ values of the most promising TZDs for 72 hours treatment.

| Type of Cancer | Cell line | T4  IC₅₀ (µM) ± SD | T15 IC₅₀ (µM) ± SD | T21 IC₅₀ (µM) ± SD | Doxorubicine IC₅₀ (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/Prf5 (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 IC50 values in five different HCC and one breast cancer cell lines. According to IC50 values, T21 was the most effective compound with IC50 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.

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

5(b) Snu449
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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REFERENCES

1. Jemal A, Siegel R, Ward E, Hao Y, Xu J, Murray T, Thun MJ. Cancer Statistics. CA: Cancer J Clin, 2008; 58(2):71-96.
2. Nussbaumer S, Bonnabry P, Veuthey J-L, Fleury-Souverain S. Analysis of Anticancer Drugs: A Review. Talanta, 2011; 85:2265-2289.
3. Liu K, Rao W, Parikh H, Li Q, Guo TL, Grant S, Kellogg GE, Zhang S. 3,5-Disubstituted-thiazolidine-2,4-dione analogs as anticancer agents: Design, synthesis and biological characterization. Eur J Med Chem, 2012; 47:125-137.
4. Yang X, So W-Y, Maa RCW, Yu LWL, Kong APS, Lee HM, Xu G, Ozaki R, Ko GTC, Chan JCN. Use of thiazolidinedione and cancer risk in Type 2 diabetes: The Hong Kong diabetes registry. Diabetes Res Clin Pract, 2012; 97:e13-e17.
5. Blanquicett C, Roman J, Hart CM. Thiazolidinediones as anti-cancer agents. Cancer Ther, 2008; 6(A): 25-34.
6. Tomasic T, Peterlin Masic L. Rhodanine as a privileged scaffold in drug discovery. Curr Med Chem, 2009; 16:1596-1629.
7. Tomasic T, Peterlin Masic L. Rhodanine as a scaffold in drug discovery: a critical review of its biological activities and mechanisms of target modulation. Expert Opin Drug Discov, 2012; 7:549-560.
8. Havrylyuk D, Mosula L, Zimenkovsky B, Vasylenko O, Gzella A, Lesyk R. Synthesis and anticancer activity evaluation of 4-thiazolidinones containing benzothiazole moiety. Eur J Med Chem, 2010; 45(11):5012-5021.
9. Moorthy BT, Ravi S, Srivastava M, Chiruvela KK, Hemlal H, Joy O, Raghavan SC. Novel rhodanine derivatives induce growth inhibition followed by apoptosis. Bioorg Med Chem Lett, 2010; 20(21):6297-6301.
10. Li W, Zhai X, Zhong Z, Li G, Pu Y, Gong P. Design, Synthesis and Evaluation of Novel Rhodanine-containing Sorafenib Analogs as Potential Antitumor Agents. Arch Pharm, 2011; 344(6):349-357.
11. Guernon JM, Wu Y-J. 3-Bromocyclohexane-1,2-dione as a useful reagent for Hantzsch synthesis of
thiazoles and the synthesis of related heterocycles. Tetrahedron Lett, 2011; 52(28):3633-3635.

12. Duan YC, Ma YC, Zhang E, Shi XJ, Wang MM, Ye XW, Liu HM. Design and synthesis of novel 1,2,3-triazole-dithiocarbamate hybrids as potential anticancer agents. Eur J Med Chem, 2013; 62:11-19.

13. Ezer M, Tatar Yildirim L, Bayro O, Verspohl EJ, Bozdag-Dundar O. Synthesis and antidiabetic activity of morpholinothiazolyl-2,4-thiazolidindione derivatives. J Enzyme Inhib Med Chem, 2012; 27(3):419-427.

14. Debski N, Hanefeld W, Schlitzen M. Improved synthesis of 2-substituted 4-chlorothiazole-5-carbaldehydes. J Heterocycl Chem, 1997; 34:1427-1429.

15. Mentese A, Ceylan-Unlusoy M, Bozdag-Dundar O, Altanlar N, Ertan R. Synthesis and antimicrobial activity of some novel thiazolidine-2,4-dione derivatives. Arzneim-Forsch/Drug Res, 2009; 59(12):659-665.

16. Vichai V, Kirtikara K. Sulforhodamine B colorimetric assay for cytotoxicity screening. Nat Protoc, 2006; 1(3):1112-1116.

17. Athmani S, Farhat MF, Iddon B. Azoles. Part 9. Synthesis of derivatives of thiophene [2,3-d]thiazole, 4H-pyrrolo-[2,3-d]thiazole, 2H-pyrazolo[3,4-d]thiazole and isoxazole [3,4-d]thiazole from thiazolidine-2,4-dione. J Chem Soc Perkin Trans 1, 1992; 6:973-977.

18. DeLima MCA, Costa DDLB, Goes AJS, Galdino SL, Pitta IR, Luu-Duc C. Synthèse et activité antimicrobienne de derives chlorobenzyl benzylidène imidazolidinediones et thiazolidinediones substituées. Pharmazie, 1992; 47(3):182-183.

19. Rida SM, Salama HM, Laboute IM, Ghany YS. Synthesis of Some 3-(Benzimidazol-2-ylmethyl)thiazolidinone Derivatives as Potential Antimicrobial Agents. Pharmazie, 1985; 40:727-728.

20. Tan SF, Ang KP, Fong YF. (Z)- and (E)-5-arylmethylenehydantoins: spectroscopic properties and configuration assignment. J Chem Soc Perkin Trans 2, 1986; 12:1941-1944.

21. Ozgen O, Ceylan-Unlusoy M, Bozdag-Dundar O, Ertan R, Kendi E. 3-(4-Chlorobenzyl)-5-(4-o xo-4H-chromen-3-ylmethylene)-1,3-thiazolidine-2,4-dione. Acta Crystallogr Sect E, 2005; E61:o870-o872.

22. Ozgen O, Bozdag-Dundar O, Ertan R, Kendi E. 5-[4-Chloro-2-(4-chlorobenzylsulfonyl)thiazol-5- ylmethylene]-3-(2,4-dichlorobenzyl)thiazolidine-2,4-dione. Acta Crystallogr Sect E, 2007; E61:o2355-o2356.

23. Bozdag-Dundar O, Ozgen O, Mentese A, Altanlar N, Atlı O, Kendi E, Ertan R. Synthesis and antimicrobial activity of some new thiazolyl thiazolidine-2,4-dione derivatives. Bioorg Med Chem, 2007; 15:6012-6017.

24. Ceylan-Unlusoy M, Kazak C, Bayro O, Verspohl EJ, Ertan R, Bozdag-Dundar O. Synthesis and antidiabetic activity of 2,4-thiazolidindione, imidazolidinedione and 2-thioxo-imidazolidine-4-one derivatives bearing 6-methyl chromonyl pharmacophore. J Enzyme Inhib Med Chem, 2013; 28(6):1205-1210.