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SYNTHESIS AND EVALUATION OF CYTOTOXIC ACTIVITY ON MCF-7 CELL LINE OF SOME DIESTERS DERIVED FROM 5-(HYDROXYBENZYLIDENE)THIAZOLIDINE-2,4-DIONES

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Abstract: Knoevenagel condensation of thiazolidine-2,4-dione, which were prepared from chloroacetic acid and thioure by 1,3 dipolar cycloaddition reaction, with 2-hydroxybenzaldehyde, 5-bromo-2-hydroxybenzaldehyde, 3-hydroxybenzaldehyde, 4-hydroxybenzaldehyde and 4-hydoxy-3-methoxybezaldehyde gave five corresponding 5-(hydroxybenzylidene)thiazolidine-2,4-dione compounds. The reaction of 5-(hydroxybenzylidene)thiazolidine-2,4-diones and ethyl chlorofomate or ethyl chloroacetate occurred at both NH and OH centers and gave ten corresponding diesters. The structures of the diesters were confirmed by IR, MS, ^{1}H and ^{13}C-

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NMR spectral data. However, in test for cytotoxic activity against MCF-7 cells, none of the diester compounds exhibited significant activity.

***Keywords*:** diester, 5-(hydroxybenzylidene)thiazolidine-2,4-diones, thiazolidine-2,4-dione, cytotoxic activity

Introduction

Thiazolidine-2,4-dione (TZD) is an important and attractive heterocyclic system to study properties and applications. In the last time, TZD and some TZDs derivatives were concerned by many scientists all over the world because of their biological activities such as anticancer,[1] antidiabetic,[1,2] antibacterial,[3,4] antioxidant,[3] anti-hyperglycemic[5] or inhibiting Pan-Pim kinases[6] etc.

Structural modifications of TZD core have been carried out using substitutions at free –NH– and $–CH_2–$ moieties of TZD molecule. In the alkaline medium, the free –NH– moiety of TZD has been alkylated or acylated using alkyl/ aryl halides or chloride acids. Absolute substitution at the $–CH_2–$ moiety with aldehydes or ketones led to the formation of arylidene derivatives, via Knoevenagel condensation. Some 5-(hydroxybenzylidene)thiazolidine-2,4-dione compounds were synthesized by Knoevenagel condensation of thiazolidine-2,4-dione with suitable hydroxybenzaldehydes. Normally, although containing two nucleophilic centers (OH and NH), but treating 5-(hydroxybenzylidene)thiazolidine-2,4-dione compounds with alkyl chloroacetate in the presence of K_2CO_3, only OH center reacted to obtain corresponding *O*-alkylation products.[3,5,7] The same results were recorded while treating these compounds with ethyl chloroformate and *O*-acylation products were formed.[3,7,8] In order to prevent the nucleophilic attack of hydroxyl group, Rosanna Maccari *et. all* added substrate (chloroacetamide) dropwise to a solution of appropriate 2,4-TZDs

containing hydroxy group and K_2CO_3 in acetonitrile[9] and the formation of some *N*-alkylation products was recorded. The alkylation at only OH group or both OH and NH groups of 5-(4-hydroxybenzylidene)thiazolidine-2,4-diones was mentioned in the literature[1] in which 1.2 or 2.2 equivalents of propargyl bromide were used with 1.0 equivalence the TZD, respectively. The alkylation at both OH and NH groups of 5-(hydroxybenzylidene)thiazolidine-2,4-diones was mentioned in case of treating 5-(4-hydroxy-3-methoxybenzylidene)thiazolidine-2,4-dione or 5-(3-hydroxy-4-methoxybenzylidene)thiazolidine-2,4-dione with methyl bromoacetate.[10] However, physical and spectral properties of the diesters were not described.

In this study, we presented the results of the synthesis and characterization of various diester derivatives of 5-(hydroxybenzylidene)thiazolidine-2,4-diones which were prepared by alkylation or acylation of 5-(hydroxybenzylidene)thiazolidine-2,4-diones at both OH and NH centers with ethyl chloroacetate or ethyl chloroformate. The selective cytotoxicity of synthesized diesters were tested on the against MCF-7 cells.

Results and Discussion

Thiazolidine-2,4-dione was prepared by 1,3 dipolar cycloaddition according to known method[2,3] and its structure was confirmed by comparing the melting point and spectral data with those of the same compound presented in the literatures.[2,3]

- Reaction of thiazolidine-2,4-dione with hydroxybenzaldehydes afforded 5-(hydroxybenzylidene)thiazolidine-2,4-dione compounds. The synthesis of the five 5-(hydroxybenzylidene)thiazolidine-2,4-dione compounds from

thiazolidine-2,4-dione and corresponding hydroxybenzaldehydes including 2-hydroxybenzaldehyde,[8,11] 5-bromo-2-hydroxybenzaldehyde,[6] 3-hydroxybenzaldehyde,[5,9] 4-hydroxybenzaldehyde[1,3,7,9,11] and 4-hydoxy-3-methoxybezaldehyde (vaniline)[7,10,11,12] was reported in the literatures but we have not had information on both physical and spectral properties of the 5-(5-bromo-2-hydroxybenzylidene)thiazolidine-2,4-dione compound which was remarked as (**2b**) in this report. In this work, the 5-(hydoxybenzylidene)thiazolidine-2,4-dione compounds (**2a-e**) were synthesized according to the previous publications[5,7,9] and their structures were confirmed by the IR, NMR, MS spectra, as well as by comparing their physical properties and spectral data with those of the similar compounds presented in the literatures.[5,9,11]

In this research, the reaction of 5-(hydoxybenzylidene)thiazolidine-2,4-diones with ethyl chloroforamate or ethyl chloroacetate, 2.0 equivalents of K_2CO_3 with 2.0 equivalents of the ethyl esters were used for 1.0 equivalent of 5-(hydroxybenzylidene)thiazolidine-2,4-dione compounds. In this condition, both OH and NH centers of the 5-(hydroxybenzylidene)thiazolidine-2,4-dione compounds reacted contemporaneously to afford the corresponding diesters. The structures of the diesters were confirmed by the IR, NMR, MS spectra.

The IR spectra of the final products appeared some bands at 2800-3000 cm^{-1} and some peaks in the range of 1380-1400 cm^{-1} which were represented of valence and bending vibrations of the Csp^3-H bonds. The presence of the carbonyl groups was recognized easily by the intense absorption bands at around 1700-1800 cm^{-1}. The ^{1}H-NMR spectra of the final products did not appear any characteristic broad *single* signal in the range of 9.80-12.70 ppm of protons in the N-H and O-H groups. The

formation of the diester products was confirmed by the appearance of the signals of protons in two ethyl groups, which were assigned as two *quartet* (with the intensity of 2H for each signal, *J*=7.0Hz) around 4.46-5.02 ppm and two *triplet* (with the intensity of 3H for each signal, *J*=7.0Hz) around 1.20-1.40 ppm. The *singlet* signals for methylene groups between heteroatom and carbonyl group of the **4a-e** compounds appeared around 4.46-5.02 ppm. In the ^{13}C-NMR spectra, 4 signals appearing between 148.5-168.5 ppm were assigned to 4 carbonyl carbons including 2 carbonyl carbons in the thiazolidine-2,4-dione ring and 2 carbonyl carbons in functional group of ester; 4 signals appearing in the aliphatic region of the spectra of **3a-e** compounds were attributed to 4 carbons of two ethyl groups while the spectra of the **4a-e** compounds appeared more than 2 signals in this region due to the presence of 2 methylene groups, each of them was between heteroatom and carbonyl group. Besides that, the spectra of **3b** and **4b** compounds showed additional signals of the carbon in the methoxy group at 55.72 ppm and 56.10 ppm, respectively. The MS spectra of final products also showed molecular ion peaks being accordance to the assumed structures. The IR, NMR, MS spectral data indicated the diesters containing TZD ring.

The selective cytotoxicity on breast cancer cells (MCF-7): The synthesized compounds were tested their selective cytotoxicity on breast cancer cells (MCF-7 cells) via SRB (sulforhodamine B) assay. The selective cytotoxicity result on breast cancer cells of diesters containing TZD ring (**3a-e**, **4a-e**) at a concentration of 100 µg/mL was shown in Table 1 (The **3d** compound did not dissolve well in DMSO, so it was not tested the selective cytotoxicity on breast cancer cells).

Although all tested compounds have not shown good cytotoxic activity on MCF-7 cells but ethyl 5-(((ethoxycarbonyl)oxy)benzylidene)-2,4-dioxothiazolidine-3-carboxylate compounds were more cytotoxic than ethyl 2-(5-(4-(2-ethoxy-2-oxoethoxy)benzylidene)-2,4-dioxothiazolidin-3-yl)acetate compounds.

Table 1. Selective cytotoxicity on breast cancer cells of the synthesized compounds

Sample	Percentage of cytotoxicity (%)			
	1st	2nd	3rd	Avg ± SD
3a	24.43	10.58	16.77	17.26 ± 6.94
3b	27.58	27.25	25.63	26.82 ± 1.04
3c	18.39	13.36	14.57	15.44 ± 2.63
3d	-	-	-	-
3e	27.71	24.34	24.93	25.66 ± 1.80
4a	7.18	1.98	-1.40	2.59 ± 4.32
4b	5.54	5.69	-2.52	2.90±4.70
4c	2.90	5.16	-0.28	2.59±2.73
4d	4.03	5.16	-1.12	2.69±3.35
4e	1.13	-1,72	2.52	0.64±2.16
Camptothecin	53.84	50.64	54.46	52.98±3.81

(Note: Avg: average, SD: standar deviation)

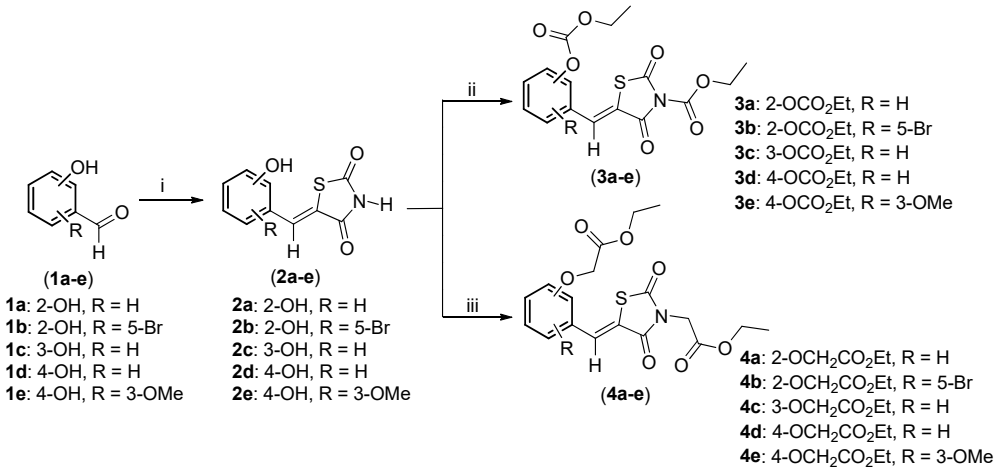
Experimental

The reactions were monitored by TLC. Melting points were measured by using Gallenkamp apparatus and capillary tubes. The IR spectra of synthesized compounds were recorded on FTIR-8400S-SHIMADZU spectrometer using KBr pellets. ^{1}H-NMR was recorded on Bruker Avance spectrometer at 500 MHz using a DMSO-d_6 as a solvent and tetramethylsilane (TMS) as an internal standard, as well as the ^{13}C-NMR spectra were recorded at 125 MHz. The HR-MS spectra were recorded on a Bruker micrOTOF–Q 10187 spectrometer with conditions: Source: ESI, Ion mode: positive, Capillary: 4000 V, Nebulizer: 1.2 Bar, Drying gas flow:

9.0 L/min, Drying heater temperature: 200 °C, Scan range: 100-3000 amu. Biological activity was tested by the selective cytotoxicity on breast cancer cells (MCF-7 cells) via SRB (sulforhodamine B) assay.

All reagents were of commercial quality and were used without any further purification.

The synthesis of diesters was performed following the steps shown in Scheme 1.



Scheme 1. Pathway for synthesis of diesters containing TZD ring. Reagents: (i) thiazolidine-2,4-dione, $MeCO_2H$, piperidine, toluene; (ii) $ClCO_2Et$, K_2CO_3, acetone; (iii) $ClCH_2CO_2Et$, K_2CO_3, acetone.

Thiazolidine-2,4-dione was prepared by condensing equimolar quantities of urea and chloroacetic acid as the reported procedures.[2,3] To a flask containing a solution of chloroacetic acid (47.25 g, 0.5 mol) in 50 mL of water, was added a solution of thiourea (38.0 g, 0.5 mol) dissolved in 50 mL of water. The mixture was stirred for 15 min to form a white precipitate, accompanied by considerable cooling. To the contents of the flask was then added slowly 50 mL of concentrated HCl dropwise and the reaction mixture was refluxed with stirring for 12 hrs at 100-110 °C. After cooling to room temperature, the obtained product was filtered and washed with cold water

to remove traces of hydrochloric acid and then was purified by recrystallization from water. Yield 50.25 g (86%), mp. 122-123 ^{o}C (water); IR (ν, cm^{-1}): 3472, 2947, 2816, 1744, 1690, 1227; ^{1}H-NMR (δ, ppm and *J*, Hz): 12.03 (1H, *s*, –NH–), 4.15 (2H, *s*, –CH_2–).

5-(Hydoxybenzylidene)thiazolidine-2,4-dione compounds (**2a-e**) were prepared by Knoevenagel condensation of suitable hydroxybenzaldehydes (**1a-e**) with thiazolidine-2,4-dione using weakly basic amine as a catalyst (piperidine) and toluene as a solvent.[5,7,9] A solution of substituted benzaldehyde i.e. salicylaldehyde (1.22 g, 10 mmol) or 5-bromosalicylaldehyde (2.01 g, 10 mmol), 3-hydroxybenzaldehyde (1.22 g, 10 mmol) or 4-hydroxybenzaldehyde (1.22 g, 10 mmol) or vanillin (1.52 g, 10 mmol) in 50 mL of dried toluene was refluxed with thiazolidine-2,4-dione (1.17 g, 10 mmol), glacial acetic acid (0.8 mL) and piperidine (0.5 mL) for 8.0 hrs. The yellow precipitate finally obtained was filtered, washed well with water and then recrystallized from ethanol to give **2a-2e** compounds, respectively.

5-(2-Hydroxybenzylidene)thiazolidine-2,4-dione (**2a**): yield 1.28 g (58%), mp. 278-280 ^{o}C (ethanol); IR (ν, cm^{-1}): 3426, 3032, 1728, 1667, 1589,1450, 1242; ^{1}H-NMR (δ, ppm and *J*, Hz): 12.23 (1H, *s*, –NH–), 8.17 (1H, *s*,=CH-Ar), 7.76 (1H, *d-d*, ^{3}J = 8.0, ^{4}J = 1.0, ArH), 7.62 (1H, *d-d*, ^{3}J = 8.0, ^{3}J = 8.0, ArH), 7.49 (1H, *d*, ^{3}J = 8.0, ArH), 7,38 (1H, *d-d-d*, ^{3}J = 8.0, ^{3}J = 8.0, ^{4}J = 1.0, ArH).

5-(5-Bromo-2-hydroxybenzylidene)thiazolidine-2,4-dione (**2b**): yield 1.71 g (57%), mp. 256-258 ^{o}C (ethanol); IR (ν, cm^{-1}): 3194, 1728, 1636, 1589, 1498, 1281, 602; ^{1}H-NMR (δ, ppm and *J*, Hz): 12.63 (1H, *s*, –NH–), 10.89 (1H, *s*, -OH), 7.89 (1H, *s*,=CH-Ar), 7.49 (1H, *d-d*, ^{3}J = 8.5, ^{4}J = 2.5, ArH), 7.41 (1H, *d*, ^{4}J = 2.5, ArH), 6.95 (1H, *d*, ^{3}J = 8.5, ArH); ^{13}C-

NMR (δ, ppm): 167.7, 167.3, 134.4, 130.3, 125.5, 123.9, 122.3, 118.3, 110.5.

5-(3-Hydroxybenzylidene)thiazolidine-2,4-dione (**2c**): yield 1.13 g (51%), mp. 290-293 ^{o}C (ethanol); IR (ν, cm^{-1}): 3302, 3163, 3063, 1751, 1690, 1589, 1450, 1288; ^{1}H-NMR (δ, ppm and *J*, Hz): 12.61 (1H, *s*, –NH–), 9.85 (1H, *s*, -OH), 7.70 (1H, *s*,=CH-Ar), 7.35 (1H, *d-d*, ^{3}J = 8.0, ^{3}J = 8.0, ArH), 7.05 (1H, *d*, ^{3}J = 8.0, ArH), 7.00 (1H, *d*, ^{4}J = 2.0, ArH), 6.90 (1H, *d-d*, ^{3}J = 8.0, ^{4}J = 2.0, ArH).

5-(4-Hydroxybenzylidene)thiazolidine-2,4-dione (**2d**): yield 1.24 g (56%), mp. 318-320 ^{o}C (ethanol); IR (ν, cm^{-1}): 3403, 3133, 1728, 1682, 1574, 1512, 1281; ^{1}H-NMR (δ, ppm and *J*, Hz): 12.46 (1H, *s*, –NH–), 10.31 (1H, *s*, -OH), 7.70 (1H, *s*,=CH-Ar), 7.46 (2H, *d*, ^{3}J = 8.5, ArH), 6.92 (2H, *d*, ^{3}J = 8.5, ArH).

5-(4-Hydroxy-3-methoxybenzylidene)thiazolidine-2,4-dione (**2e**): yield 1.36 g (54%), mp. 242-245 ^{o}C (ethanol); IR (ν, cm^{-1}): 3464, 3186, 1728, 1682, 1574, 1520, 1288; ^{1}H-NMR (δ, ppm and *J*, Hz): 12.46 (1H, *s*, –NH–), 9.96 (1H, *s*, -OH), 7.72 (1H, *s*,=CH-Ar), 7.17 (1H, *d*, ^{4}J = 2.0, ArH), 7.09 (1H, *d-d*, ^{3}J = 8.0, ^{4}J = 2.0, ArH), 6.94 (1H, *d*, ^{3}J = 8.0, ArH), 3.83 (1H, *s*, -OCH_3).

General procedure for synthesis of diesters containing TZD heterocycle (**3a-e**, **4a-e**): A mixture of 5 mmol a definite 5-(hydroxybenzylidene)thiazolidine-2,4-dione and anhydrous K_2CO_3 (1.38 g, 10 mmol) in 50 mL acetone was magnetic stirred and refluxed for 3.0 hrs, then ethyl chloroformate (1.09 g, ~10 mmol - for synthesis of **3a-e** compounds) or ethyl chloroacetate (1.23 g, ~10 mmol - for synthesis of **4a-e** compounds) in 10 mL acetone was added dropwise for 1.5 hrs while stirring. The reaction mixture was refluxed and stirred for 10 hrs and the

reaction was monitored by TLC. After that, the solution was cooled, poured in crushed ice, the solid separated was filtered, washed with water and recrystallized from ethanol. As the result, yellow crystals were obtained in all the cases.

Ethyl 5-(2-((ethoxycarbonyl)oxy)benzylidene)-2,4-dioxothiazolidine-3-carboxylate (**3a**): yield 2.30 g (63%), mp. 122-123 ^{o}C (ethanol); IR (ν, cm^{-1}): 2986, 1790, 1759, 1705, 1319, 1249; ^{1}H-NMR (δ, ppm and *J*, Hz): 7.83 (1H, *s*, =CH-Ar), 7.64 (1H, *d-d*, ^{3}J = 8.0, ^{3}J = 8.0, ArH), 7.60 (1H, *d*, ^{3}J = 8.0, ArH), 7.52 (1H, *d-d*, ^{3}J = 8.0, ^{3}J = 8.0, ArH), 7.50 (1H, *d*, ^{3}J = 8.0, ArH), 4.45 (2H, *q*, ^{3}J = 7.0, -CH_2-OOC-O-), 4.31 (2H, *q*, ^{3}J = 7.0, -CH_2-OOC-N–), 1.33 (3H, *t*, ^{3}J = 7.0, -CH_3), 1.32 (3H, *t*, ^{3}J = 7.0, -CH_3); ^{13}C-NMR (δ, ppm): 164.3, 162.5, 152.9, 150.1, 147.5, 132.8, 128.9, 127.7, 126.8, 125.7, 123.8, 123.7, 66.0, 65.8, 14.4, 14.2; MS: *m/z* 388.0426 $(M+Na)^{+}$, calculated for $C_{16}H_{15}NO_7SNa$: 388.0467.

Ethyl 5-(5-bromo-2-((ethoxycarbonyl)oxy)benzylidene)-2,4-dioxothiazolidine-3-carboxylate (**3b**): yield 3.24 g (73%), mp. 126-127 ^{o}C (ethanol); IR (ν, cm^{-1}): 2986, 2924, 1759, 1705, 1612, 1489; 1319, 1188; ^{1}H-NMR (δ, ppm and *J*, Hz): 7.81 (1H, *d-d*, ^{3}J = 8.5, ^{4}J = 2.0, ArH), 7.76 (1H, *s*,=CH-Ar), 7.65 (1H, *d*, ^{4}J = 2.0, ArH), 7.49 (1H, *d*, ^{3}J = 8.5, ArH), 4.45 (2H, *q*, ^{3}J = 7.0, -CH_2-OOC-O-), 4.30 (2H, *q*, ^{3}J = 7.0, -CH_2-OOC-N–), 1.33 (3H, *t*, ^{3}J = 7.0, -CH_3), 1.31 (3H, *t*, ^{3}J = 7.0, -CH_3); ^{13}C-NMR (δ, ppm): 163.3, 161.7, 151.9, 148.5, 146.9, 134.7, 130.6, 127.7, 125.5, 125.4, 125.1, 119.1, 65.6, 65.5, 13.9, 13.7; MS: *m/z* 465.9566/467.9548 $(M+Na)^{+}$, calculated for $C_{16}H_{14}BrNO_7SNa$: 465.9572.

Ethyl 5-(3-((ethoxycarbonyl)oxy)benzylidene)-2,4-dioxothiazolidine-3-carboxylate (**3c**): yield 2.26 g (62%), mp. 131-132 ^{o}C (ethanol); IR (ν, cm^{-1}): 3063, 2986, 1798, 1759, 1613, 1312, 1235; ^{1}H-NMR (δ, ppm and

J, Hz): 7.98 (1H, *s*,=CH-Ar), 7.63 (1H, *d-d*, $^3J_1 = {}^3J_2$ = 8.0, ArH), 7.55 (2H, *m*, ArH), 7.42 (1H, *ddd*, ^3J = 8.0, $^4J_1 = {}^4J_2$ = 1.0, ArH), 4.46 (2H, *q*, ^3J = 7.0, -CH_2-OOC-O-), 4.29 (2H, *q*, ^3J = 7.0, -CH_2-OOC-N–), 1.35 (3H, *t*, ^3J = 7.0, -CH_3), 1.32 (3H, *t*, ^3J = 7.0, -CH_3); ^{13}C-NMR (δ, ppm): 163.8, 162.1, 152.7, 151.2, 147.1, 134.2, 133.0, 130.7, 127.5, 123.8, 123.0, 121.5, 65.5, 64.9, 13.9, 13.7; MS: *m/z* 388.0448 $(M+Na)^+$, calculated for $C_{16}H_{15}NO_7SNa$: 388.0467.

Ethyl 5-(4-((ethoxycarbonyl)oxy)benzylidene)-2,4-dioxothiazolidine-3-carboxylate (**3d**): yield 2.41 g (66%), mp. 139-140 °C (ethanol); IR (ν, cm^{-1}): 2986, 1798, 1751, 1705, 1697, 1597, 1304, 1234; ^{1}H-NMR (δ, ppm and *J*, Hz): 8.00 (1H, *s*,=CH-Ar), 7.73 (H, *d*, ^3J = 9.0, ArH), 7.46 (2H, *d*, ^3J = 9.0, ArH), 4.46 (2H, *q*, ^3J = 7.0, -CH_2-OOC-O-), 4.29 (2H, *q*, ^3J = 7.0, -CH_2-OOC-N–), 1.34 (3H, *t*, ^3J = 7.0, -CH_3), 1.31 (3H, *t*, ^3J = 7.0, -CH_3); ^{13}C-NMR (δ, ppm): 163.9, 162.2, 152.5, 152.2, 147.2, 133.3, 131.8, 130.6, 122.4, 120.2, 65.6, 65.0, 14.0, 13.9; MS: *m/z* 388.0451 $(M+Na)^+$ calculated for $C_{16}H_{15}NO_7SNa$: 388.0467.

Ethyl 5-(4-((ethoxycarbonyl)oxy)-3-methoxybenzylidene)-2,4-dioxothiazolidine-3-carboxylate (**3e**): yield 2.77 g (70%), mp. 127-128 °C (ethanol); IR (ν, cm^{-1}): 2986, 1790, 1751, 1705, 1605, 1312, 1257; ^{1}H-NMR (δ, ppm and *J*, Hz): 7.99 (1H, *s*,=CH-Ar), 7.46 (1H, *d*, ^4J = 2.0, ArH), 7.41 (1H, *d*, ^3J = 8.5, ArH), 7.23 (1H, *dd*, ^3J = 8.5, ^4J = 2.0, ArH), 4.46 (2H, *q*, ^3J = 7.0, -CH_2-OOC-O-), 4.26 (2H, *q*, ^3J = 7.0, -CH_2-OOC-N–), 3.87 (3H, *s*, -OCH_3), 1.34 (3H, *t*, ^3J = 7.0, -CH_3), 1.30 (3H, *t*, ^3J = 7.0, -CH_3); ^{13}C-NMR (δ, ppm): 163.9, 162.1, 152.1, 151.3, 147.2, 141.1, 133.6, 131.9, 123.5, 122.3, 120.5, 115.2, 65.6, 65.0, 56.1, 13.9, 13.7; MS: *m/z* 418.1560 $(M+Na)^+$, calculated for $C_{17}H_{17}NO_8SNa$: 418.0573.

Ethyl 2-(5-(2-(2-ethoxy-2-oxoethoxy)benzylidene)-2,4-dioxothiazolidin-3-yl)acetate (**4a**): yield 1.97 g (50%), mp. 111-112 °C (ethanol); IR (ν, cm^{-1}): 8.24 (1H, *s*,=CH-Ar), 7.51 (1H, *d*, ^3J = 8.0, ArH), 7.50 (1H, *dd*, $^3J_1 = {}^3J_2$ = 8.0, ArH), 7.17 (1H, *dd*, $^3J_1 = {}^3J_2$ = 8.0, ArH), 7.12 (1H, *d*, ^3J = 8.0, ArH), 5.00 (2H, *s*, -O-CH_2-(C=O)), 4.50 (2H, *s*, –N-CH_2-(C=O)), 4.17 (4H, *m*, -CH_2COO), 1.22 (6H, *m*, -CH_3); ^{13}C-NMR (δ, ppm): 168.4, 167.1, 166.8, 165.0, 156.7, 132.8, 128.9, 128.8, 121.9, 121.6, 120.9, 113.2, 65.2, 61.7, 60.9, 42.2, 14.0, 13.9; MS: *m/z* 416.0757 $(M+Na)^+$, calculated for $C_{18}H_{19}NO_7SNa$: 416.0780.

Ethyl 2-(4-bromo-2-((3-(2-ethoxy-2-oxoethyl)-2,4-dioxothiazolidin-5-ylidene)methyl)phenoxy)acetate (**4b**): yield 2.41 g (51%), mp. 118-119 °C (ethanol); IR (ν, cm^{-1}): 2986, 2924, 2854, 1736, 1690, 1597, 1381, 1219, 602; ^{1}H-NMR (δ, ppm and *J*, Hz): 8.10 (1H, *s*,=CH-Ar), 7.68 (1H, *d-d*, ^3J = 9.0, ^4J = 3.0, ArH), 7.60 (1H, *d*, ^4J = 3.0, ArH), 7.13 (1H, *d*, ^3J = 9.0, ArH), 5.02 (2H, *s*, -O-CH_2-(C=O)), 4.51 (2H, *s*, –N-CH_2-(C=O)), 4.21 (2H, *q*, -COO-CH_2-), 4.18 (2H, *q*, -COO-CH_2-), 1.24 (3H, *t*, -CH_3), 1.22 (3H, *t*, -CH_3); ^{13}C-NMR (δ, ppm): 168.1, 166.7, 166.6, 164.8, 155.7, 134.8, 131.1, 127.6, 123.9, 122.9, 115.5, 113.1, 65.4, 61.7, 60.9, 42.3, 14.0, 13.9; MS: *m/z* 472.0065 $(M+H)^+$ and 474.0043 $(M+2+H)^+$ calculated for $C_{18}H_{19}BrNO_7S$: 472.0060.

Ethyl 2-(5-(3-(2-ethoxy-2-oxoethoxy)benzylidene)-2,4-dioxothiazolidin-3-yl)acetate (**4c**): yield 2.24 g (57%), mp. 96-97 °C (ethanol); IR (ν, cm^{-1}): 2986, 2916, 1744, 1697, 1605, 1373, 1211; ^{1}H-NMR (δ, ppm and *J*, Hz): 8.00 (1H, *s*,=CH-Ar), 7.51 (1H, *d-d*, ^3J = 8.0, ^3J = 8.0, ArH), 7.28 (1H, *d*, ^3J = 8.0, ArH), 7.23 (1H, *d*, ^4J = 2.0, ArH), 7.13 (1H, *d-d*, 3*J* = 8.0, ^4J = 2.0, ArH), 4.88 (2H, *s*, -O-CH_2-(C=O)), 4.51 (2H, *s*, –N-CH_2-(C=O)), 4.22 (2H, *q*, -COO-CH_2-), 4.19 (2H, *q*, -COO-CH_2-), 1.25

(3H, *t*, -CH_3), 1.23 (3H, *t*, -CH_3); ^{13}C-NMR (δ, ppm): 168.5, 166.8, 166.7, 164.9, 158.0, 134.1, 133.9, 130.6, 122.8, 121.0, 117.4, 116.0, 64.7, 61.7, 60.7, 42.2, 14.0, 13.9; MS: *m/z* 416.0745 $(M+Na)^+$, calculated for $C_{18}H_{19}NO_7SNa$: 416.0780.

Ethyl 2-(5-(4-(2-ethoxy-2-oxoethoxy)benzylidene)-2,4-dioxothiazolidin-3-yl)acetate (**4d**): yield 2.44 g (62%), mp. 125-126 °C (ethanol); IR (ν, cm^{-1}): 2978, 1744, 1690, 1589, 1381, 1211; ^{1}H-NMR (δ, ppm and *J*, Hz): 7.88 (1H, *s*,=CH-Ar), 7.49 (2H, *d*, ^3J = 9.0, ArH), 7.00 (2H, *d*, ^3J = 9.0, ArH), 4.67 (2H, *s*, -O-CH_2-(C=O)-), 4.46 (2H, *s*, –N-CH_2-(C=O)), 4.28 (2H, *q*, ^3J = 7.0, -COO-CH_2-), 4.23 (2H, *q*, ^3J = 7.0, -COO-CH_2-), 1.31 (6H, *m*, -CH_3); ^{13}C-NMR (δ, ppm): 168.2; 167.5, 166.3, 165.7, 159.7, 134.1, 132.3, 126.8, 118.8, 115.4, 65.2, 62.2, 61.6, 41.2, 14.2, 14.1; MS: *m/z* 416.0762 $(M+Na)^+$, calculated for $C_{18}H_{19}NO_7SNa$: 416.0780.

Ethyl 2-(5-(4-(2-ethoxy-2-oxoethoxy)-3-methoxybenzylidene)-2,4-dioxothiazolidin-3-yl)acetate (**4e**): yield 2.28 g (54%), mp. 95-96 °C (ethanol); IR (ν, cm^{-1}): 2986, 2909, 1728, 1690, 1589, 1373, 1211; ^{1}H-NMR (δ, ppm and *J*, Hz): 7.70 (1H, *s*, =CH-Ar), 7.31 (1H, *d*, ^4J = 2.0, ArH), 7.23 (1H, *d-d*, ^3J = 8.5, ^4J = 2.0 ArH), 7.07 (1H, *d*, ^3J = 8.5, ArH), 4,90 (2H, *s*, -O-CH_2(C=O)), 4.49 (2H, *s*, –N-CH_2-(C=O)), 4.20 (4H, *q*, -COO-CH_2-), 1.24 (6H, *t*, -CH_3); ^{13}C-NMR (δ, ppm):168.3, 167.0, 166.8, 165.0, 149.4, 149.1, 134.3, 126.2, 123.6, 117.9, 114.2, 113.5, 65.0, 61.7, 60.8, 55.7, 42.2, 14.1, 14.0; MS: *m/z* 446.0867 $(M+Na)^+$, calculated for $C_{19}H_{21}NO_8SNa$: 446.0886.

Procedure SRB assay for testing biological activity of the diesters containing TZD ring (**3a-e**, **4a-e**): The assay was performed as previously described with some modifications.[13] Cells MCF-7, which seeded at a density of 10,000 cells/well in 96-well plates, were cultured for 24 hrs

before being incubated with solution of diester (**3a-c**, **3e** or **4a-e**) at 100 µg/mL concentration for 48 hrs. The treated cells were fixed with the cold trichloroacetic acid solution (Merck, 50 % (w/v)) for 1-3 hrs, washed, and stained with SRB (Sigma, 0.2 % (w/v)) for 20 min. After five times of washing by acetic acid solution (Merck, 1 %), protein-bound dye was solubilized in Tris base solution (Promega, 10 mM). Optical density values were determined by a 96-well micro-titer plate reader (Synergy HT, Biotek Instruments) at the wavelengths of 492 nm and 620 nm. The percentage of growth inhibition (Inh %) was calculated according to the formula: Inh % = (1-[ODt/ODc] x 100) %, in which ODt and ODc were the optical density values of the test sample and the control sample, respectively. Camptothecin (Calbiochem) was used as a positive control.

Conclusions

Treating 5-(hydroxybenzylidene)thiazolidine-2,4-dione compounds with ethyl chloroformate or ethyl chloroacetate in the alkaline medium gave ethyl 5-(4-((ethoxycarbonyl)oxy)benzylidene)-2,4-dioxothiazolidine-3-carboxylates and ethyl 2-(5-((2-ethoxy-2-oxoethoxy)benzylidene)-2,4-dioxothiazolidin-3-yl)acetates, respectively. The diesters were formed by the reactions at both OH and NH centers in the molecule of the 5-(hydroxybenzylidene)thiazolidine-2,4-dione compounds. The structures of the ten synthesized diester compounds were verified by IR, ^{1}H-NMR, ^{13}C-NMR and MS spectral data.

The result of testing selective cytotoxicity toward MCF-7 cells of nine diesters (**3a-c**, **3e** and **4a-e**) showed that the cytotoxic activity of ethyl 5-(4-((ethoxycarbonyl)oxy)benzylidene)-2,4-dioxothiazolidine-3-carboxylate compounds was higher than this of ethyl 2-(5-((2-ethoxy-2-

oxoethoxy)benzylidene)-2,4-dioxothiazolidin-3-yl)acetate compounds. However, these diesters did not have the significant cytotoxic activity against MFC-7 cells.

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