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Benzilic acid based new 2-aryl-1,3-thiazolidin-4-one derivatives: Synthesis and anticancer activity

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ABSTRACT: Some new derivatives of 2-aryl-1,3-thiazolidin-4-one derivatives were produced (**3a-p** and **4a-p**) in the search of potentially active new molecules with antitumor features. Compounds were obtained by cyclocondensation of 2-hydroxy-2,2-diphenyl-N-[(substituted phenyl)methylene]acetohydrazides (**2**) and mercaptoacetic acid or 2-mercaptopropionic acid. Identification and characterization of 32 new 2-aryl substituted thiazolidin-4-ones were performed with spectral and elemental analyzes. Compound **3c**, **3g**, **3j**, **4g**, **4n**, and **4p** were chosen as prototypes and assayed for their anticancer activity against diverse several cancer cell lines with *in vitro* primary anticancer test in the National Cancer Institute. Compound **4g** exhibited significant anticancer activity with the inhibition value 84.19% against a leukemia cell line MOLT-4 while compound **4p** had a remarkable inhibition ratio (72.11%) against the growth of a CNS (central nervous system) cancer cell line SF-295 in the primary screen. These preliminary and important results indicated that the compounds carried 2-aryl-1,3-thiazolidin-4-one scaffold can be evaluated as potentially promising anticancer agents.

KEYWORDS: 1,3-Thiazolidin-4-one; benzilic acid; mercaptoacetic acid; 2-mercaptopropionic acid; anticancer agents.

1. INTRODUCTION

One of the most common death cause is cancer globally and remains a life-threatening health problem all over the world. According to the GLOBOCAN (Global Cancer Observatory) data, an estimated 18.1 million cases and 9.6 million deaths in 2018 caused by different types of cancers are recorded [1]. In 2020, the number of new cases is expected to increase to 15 million [2]. It is known that the most common cancer cases and causes of cancer deaths are closely related to the degree of socio-economic levels of countries, distribution of main risk factors for cancers, aging, and growth of the population and lifestyles [3]. Cancer treatment can be collected under three main methods: Resection of the primary tumor, chemotherapy, or radiotherapy, which could have varying success or cause a different range of short and long-term adverse health effects [4]. Today, cancer researches focus on finding new and more effective treatment techniques like "targeted" therapy, which targets particularly single or a class of molecular targets in cancer cells [5]. Therefore it is a remarkable application area to develop cancer cell-targeted and selective new anticancer agents in medicinal chemistry.

Heterocyclic structures which have sulfur and nitrogen are a known class of molecules which provide numerous possibility for synthesis of new physiologically active compounds [6,7]. Especially 4-thiazolidinone scaffold takes great attention due to its several biological properties like antimicrobic, antiparasitic, antioxidant, anticonvulsant, anti-HIV, anti-inflammatory, anti-tubercular, antifungal, and particularly anticancer [8–11]. In recent decades, investigation of 4-thiazolidinone derivatives for its antitumor activity on colon, breast, renal, lung, prostate, ovarian, melanoma, leukemia, and CNS (central nervous system) cancers cell lines have an increasing trend for anticancer drug discovery [12,13]. Due to its wide range of biological potentials, in our previous work, some derivatives of 2-aryl-1,3-thiazolidin-4-one were synthesized and assayed for their antimycobacterial and anticancer activity [14]. Herein, we communicate the synthesis and characterization of two series of 2-aryl-4-thiazolidinone derivatives (**3** and **4**) in search of finding potent compounds for cancer treatments. The six chosen thiazolidinone derivatives were assayed for their anticancer activity against various diverse cancer cell lines.

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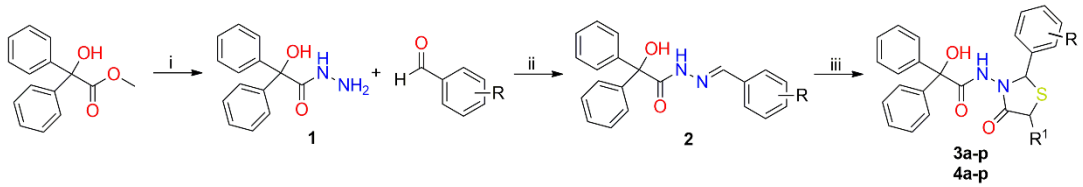
2. RESULTS AND DISCUSSIONS

2.1. Chemistry

The synthetic synthesis pathway of series **3a-p** and **4a-p** proceed in three successive steps (Figure 1). The reaction of methyl-2-hydroxy-2,2-diphenylacetate with hydrazine hydrate in the ethanol for six hours resulted in 2-hydroxy-2,2-diphenylacetohydrazide (**1**). Compound **1** refluxed with different aromatic aldehydes for four hours to prepare various phenyl substituted Schiff bases (**2**). At the third step 2-hydroxy-N-(4-oxo-2-substitutedphenyl-1,3-thiazolidin-3-yl)-2,2-diphenylacetamides (**3a-p**) and 2-hydroxy-N-(5-methyl-4-oxo-2-substitutedphenyl-1,3-thiazolidin-3-yl)-2,2-diphenylacetamides (**4a-p**) were gained from the treatment of **2** with mercaptoacetic acid or 2-mercaptopropionic acid, respectively [14,15].



Compounds **3a-p:**

R^1=H	3a	3b	3c	3d	3e	3f	3g	3h	3i	3j	3k	3l	3m	3n	3o	3p
R	2-CH_3	3-CH_3	4-CH_3	2-OH	4-OH	3-OC_2H_5 4-OH	4-OCH_3	3,4-OCH_3	2,5-OCH_3	4-$COOCH_3$	2-NO_2	3-NO_2	4-NO_2	2,4-NO_2	3-NO_2 4-Cl	4-$N(CH_3)_2$

Compounds **4a-p**:

R^1=CH_3	4a	4b	4c	4d	4e	4f	4g	4h	4i	4j	4k	4l	4m	4n	4o	4p
R	2-CH_3	3-CH_3	4-CH_3	2-OH	4-OH	3-OC_2H_5 4-OH	3-OCH_3 4-OH 5-NO_2	4-OCH_3	3,4-OCH_3	2,5-OCH_3	4-$COOCH_3$	2-NO_2	3-NO_2	4-NO_2	3-NO_2 4-Cl	4-$N(CH_3)_2$

Figure 1. The synthetic procedures for the synthesis of compounds **3** and **4** derivatives. Reagents: i) $NH_2NH_2.H_2O$, ethanol, reflux, 6h; ii) Ethanol, reflux; iii) Mercaptoacetic acid/2-mercaptopropionic acid, dry benzene, reflux, 6h.

Together with elemental analysis, newly synthesized thiazolidin-4-one based compounds were defined with spectral methods including IR, ^{1}H-NMR, ^{13}C-NMR -APT, ESI-MS (-). Absorption bands for N-H and O-H of the new derivatives were signaled at between 3208-3461 cm^{-1} in the IR spectra. The stretching vibration of the typical amide C=O group was observed between 1661-1687 cm^{-1}, as expected [15,16]. In addition, the expected ring closure for 4-thiazolidinon ring of compounds **3** and **4** is supported by the appearance of the second strong C=O stretching bands at 1694-1727 cm^{-1} [17,18].

^{1}H-NMR spectral analysis of the compounds **3a-p** demonstrated that the SCH_2 (methylene) protons of thiazolidinone ring were signaled between δ 3.63-3.96 ppm, as two singlets or doublets. For **4a-p**, the $SCHCH_3$ (methine) protons resonated at between 3.85-4.21 ppm, as two quartets. Both observed signals are specific for the protons at 5-position of the 2,3 disubstituted thiazolidin-4-one rings [7]. For both series, peaks exhibited between δ 5.72-6.81 ppm, δ 6.65-6.95 ppm, and δ 10.09-10.46 ppm were ascribed to the C_2-H, C-OH, and CONH protons respectively.[15,19].

^{13}C-APT spectra of the new compounds **3c**, **3e**, **3f**, **4n**, and **4p** authenticated the characteristic carbon chemical shifts of the 4-thiazolidinones. Signals of characteristic thiazolidin-4-one C_2, C_4 (lactam C=O), C_5 (**3a-p**) and C_5 (**4a-p**) exhibited at δ 56.76-62.51, 172.03-172.23, 30.21-30.31 and 38.41-39.31 ppm, respectively [20,21]. Carbon resonances of C-OH and CONH (amide C=O) appeared in the order of δ 81.16-81.84 and 169.16-171.84 ppm. The signals of the aromatic carbons were detected in the expected areas.

APCI(-) mass spectral analysis of **3c**, **3e**, **3f**, **4n**, and **4p** revealed molecular ions $(M-H)^-$ with greatest relative intensity (100%) at m/z 417, 419, 463, 462 and 460, respectively . This confirmed the molecular weights of the compounds and showed that the expected structures were successfully achieved [22]. It is also obvious that the $[(M-H)-SCH_2CO]^-$ fragments of the compounds showed the main fragmentation pattern of the 4-thiazolidinone system [23]. Detailed spectral data presented in the experimental section.

2.2. Anticancer activity

The National Cancer Institute (NCI) determined six of the synthesized compounds, **3c**, **3g**, **3j**, **4g**, **4n**, and **4p** to test their possible anticancer activity. To conduct the primary anticancer assays, the Drug Evaluation

Branch of the National Cancer Institute protocol was applied [24–26]. Using 10^{-5}M single dose in an *in vitro* analysis, the cytotoxicity and /or the inhibitory features of the new thiazolidin-4-one were measured using 60 different human cancer cell lines originating from 9 neoplastic diseases. For observing the growth percentage, spectrophotometric methods were performed, and for comparison test agents that were used not added to control. The sulphorhodamine B (SRB) test was employed for predicting cell growth/viability estimation and as a procedural method, the drug was exposed continuously for two days.

Non-small cell lung cancer (NSCL), leukemia, CNS cancer, renal, and melanoma cell lines were chosen for screening in the NCI. For individual effects, mean graphs were produced and the variance of every cell line from the total mean value was shown with bars for all the cells studied. The mean of total growth inhibition, expressed in percentage, was evaluated as the center point of the graph. Bars are viewed with a higher inhibition than the average on the right side of the cell lines, while those on the left side have a lower inhibition than the average. In Figure 2-7, single dose Mean Graphs for compounds **3c**, **3g**, **3j**, **4g**, **4n**, and **4p** for leukemia, CNS, renal, melanoma, and NSCL cancer cell lines are declared respectively.

Against the aforementioned tumour cell lines, the selected compounds showed remarkable inhibition percents (Table 1). Compared with compounds **3c** and **4p**, compound **3g** which bearing a methoxy substitution at the *para* position showed the best but similar antitumor activity for leukemia RPMI-8226 cell lines. Compound **4p** is one of the compounds with high growth inhibition percentage (72.11%). Accordingly, a methyl substitution at position 5 of the thiazolidin-4-one ring and a *para*-dimethylamino group at the phenyl ring seems to be favorable for inhibition of CNS SF-295 cell lines. Compound **4n** also showed good anticancer activity towards to NSCL cancer cell line HOP-92, with an inhibition percent of 67.51%. For compound **4g**, which have the best inhibition percent (84.19%), it was exhibited that combined with a methoxy at position 3, a hydroxy at position 4, and nitro at position 5 on the phenyl, a growth of 15.81% was recorded for a leukemia cell line MOLT-4. (Table 1).

Table 1. *In vitro* tumor cell growth inhibition of **3c**, **3g**, **3j**, **4g**, **4n,** and **4p**.

Compound	Panel	Cell Line	Growth Percent	Inhibition Percent
3c	Leukemia	K-562	37.43	62.57
		RPMI-8226	59.85	40.15
	CNS Cancer	SF-295	49.30	50.70
3g	Leukemia	RPMI-8226	56.92	43.08
	NSCL	A549/ATCC	58.42	41.58
	CNS Cancer	SF-295	42.89	57.00
3j	Melanoma	SK-MEL-5	60.23	39.77
	Renal Cancer	A498	51.38	48.62
4g	Leukemia	HL-60(TB)	37.95	62.05
		MOLT-4	15.81	**84.19**
4n	NSCL	HOP-92	32.49	67.51
	CNS Cancer	SF-295	43.30	56.70
4p	Leukemia	RPMI-8226	60.11	39.89
	CNS Cancer	SF-295	27.89	**72.11**
	Renal Cancer	UO-31	47.70	52.30

4. CONCLUSION

32 new 2-aryl-4-thiazolidinones have been synthesized and some compounds (**3c**, **3g**, **3j**, **4g**, **4n**, and **4p**) were assayed for their antitumor activity against a broad array of cancer cell lines by the NCI. Compound **4g** and **4p**, both have 5-methyl substitution at the thiazolidinone ring showed significant antitumor activity with high inhibition percents (84.19% and 72.11%, respectively**)** against different cancer cell lines. The best inhibitory activity observed for **4g** which furnished with 3-methoxy, 4-hydroxy, and 5-nitro substitutions at the 2-phenyl ring. According to the present study, it can be said that derivatives of 2-aryl-1,3-thiazolidin-4-one incorporating benzilic acid present a potential candidate against different cancer cell lines. Further research and modification will be done on compounds **4g** and **4p** to investigate their relationship with the known therapeutic targets in the studied cancer cell lines.

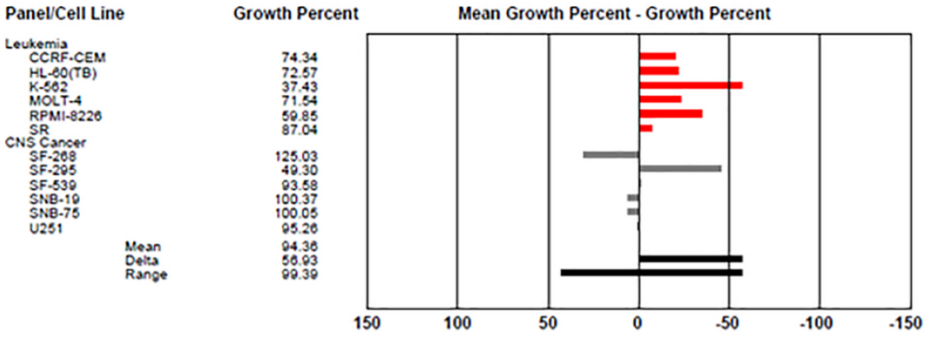


Figure 2. One dose Mean Graph for leukemia and CNS cancer cell lines of **3c**.

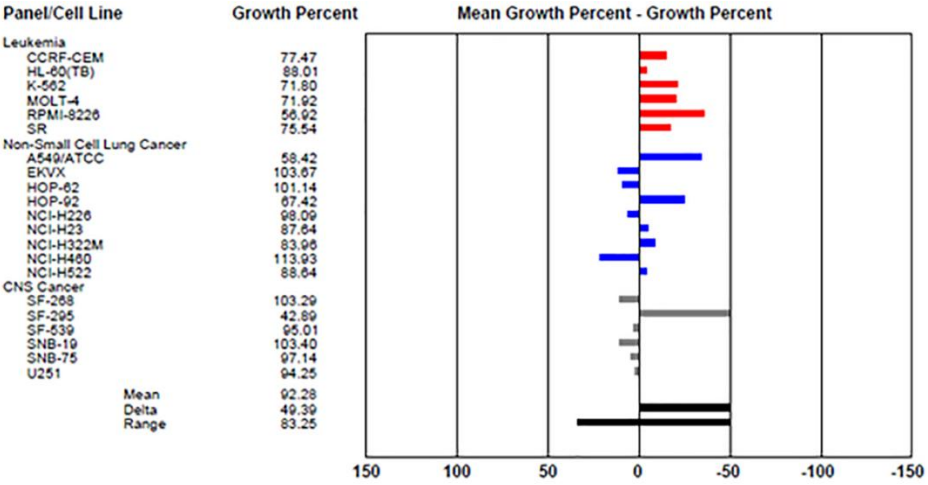


Figure 3. One dose Mean Graph for leukemia, NSCL and CNS cancer cell lines of **3g**.

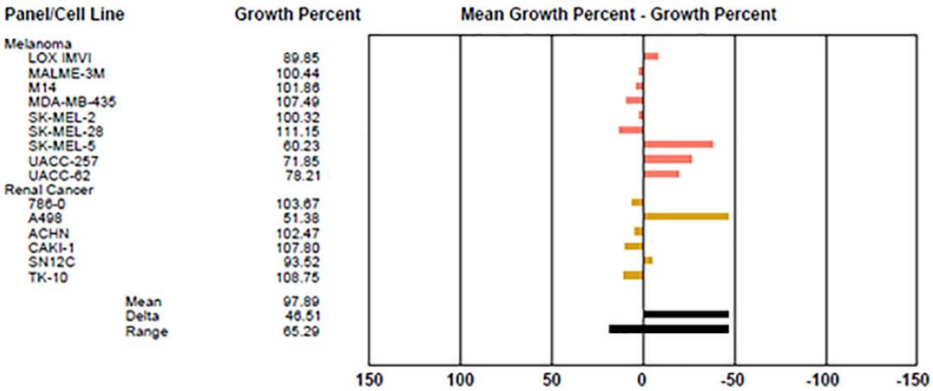


Figure 4. One dose Mean Graph for melanoma and renal cancer cell lines of **3j.**

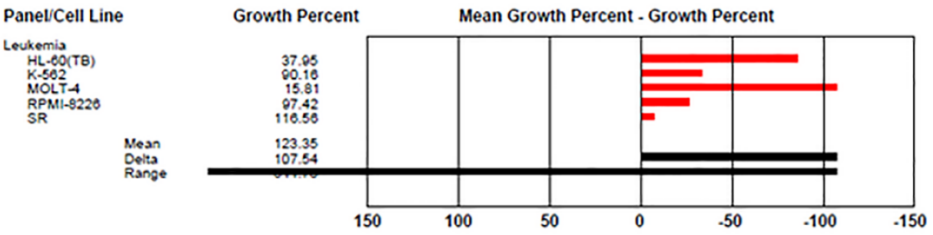


Figure 5. One dose Mean Graph for leukemia cell lines of **4g**.

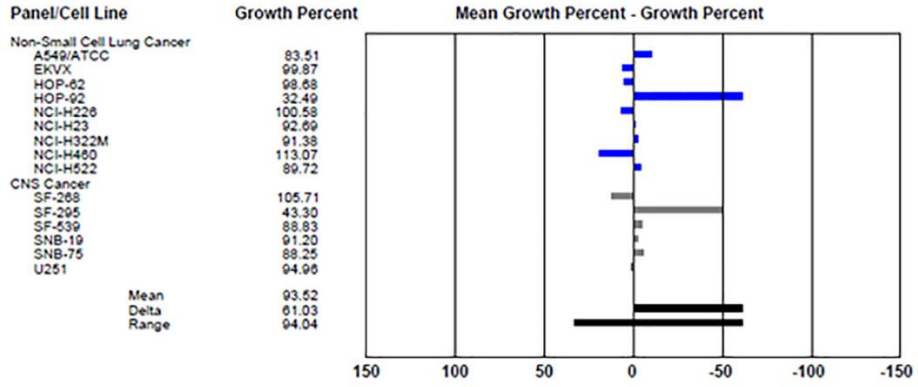


Figure 6. One dose Mean Graph for NSCL and CNS cancer cell lines of **4n**.

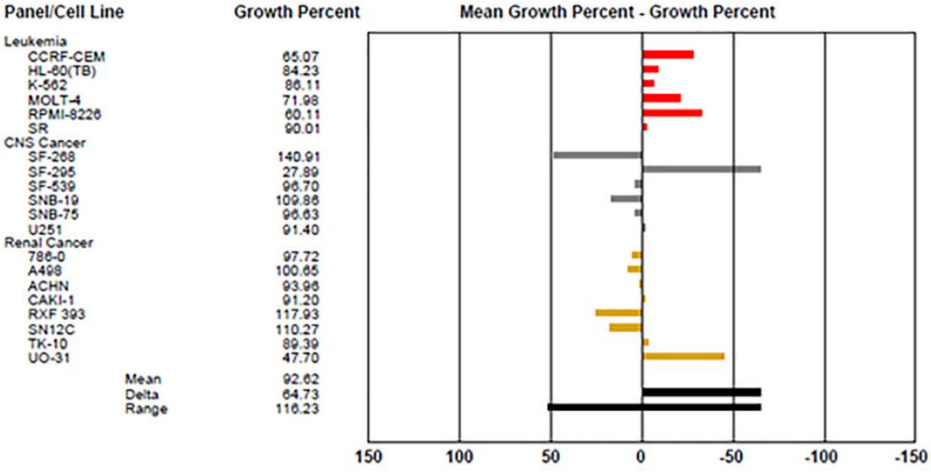


Figure 7. One dose Mean Graph for leukemia, CNS cancer, and renal cancer cell lines of **4p**.

5. MATERIALS AND METHODS

5.1. Chemistry

Melting points measurement was conducted with Buchi 540 apparatus by an open capillary method. TLC was carried out to verify the purity of the synthesized compounds. Infrared spectral analyses of the compounds were conducted with KBr disk using Perkin-Elmer 1600 FTIR. The observed values were recorded as υ_{max} cm^{-1}. The 500 MHz NMR Varian Unity INOVA Spectrometer was used to conduct ^{1}H-NMR and ^{13}C-NMR(APT) analyses using TMS as reference and DMSO-d_6 as the solvent and all the signals observed are expressed in ppm. Thermo Finnigan Flash EA 1112 was used to conduct the elemental analysis (C, H, N) of the novel compounds and the results were found to be in ± 0.4%. Mass spectral data (LC/MS-ESI) were measured with Finnigan Trace DSQ Mass Spectrometer.

5.1.1. *Synthesis of 2-hydroxy-2,2-diphenylacetohydrazide (**1**)*

2-hydroxy-2,2-diphenylacetic acid ester (0.05 mol) was refluxed with 12 ml of hydrazine hydrate for 12 h. After the reaction was completed, it was poured into a crystallizing dish and left open. The crystals formed thereof were further recrystallized from C_2H_5OH [27,28].

5.1.2. *General procedure for preparation of 2-hydroxy-2,2-diphenyl-N'-[(substituted phenyl)methylene]acetohydrazides (**2**)*

A solution of compound **1** (6 mmol) in ethyl alcohol (30 mL) was refluxed with a suitable aromatic aldehyde (6.6 mmol) for 4 h. The obtained raw precipitate was recrystallized from/washed with ethyl alcohol to yield the desired clean compounds [27–30].

5.1.3. *General procedure for preparation of 2-hydroxy-N-(4-oxo-2-substitutedphenyl-1,3-thiazolidin-3-yl)-2,2-diphenylacetamide/2-Hydroxy-N-(5-methyl-4-oxo-2-substitutedphenyl-1,3-thiazolidin-3-yl)-2,2-diphenylacetamides (**3a-p** and **4a-p**)*

With a Dean-Stark trap, 5 mmol of the purified compound **2** was suspended in dry benzene (30 ml) and refluxed for 6 h with 5 eq of either mercaptoacetic acid or 2-mercaptopropionic acid. Evaporation of excess benzene is done in vacuo. By using saturated $NaHCO_3$ solution, the gained residue was triturated until CO_2 evolution stopped and refrigerated at night. The yielded product washed with H_2O, dried, and purified by recrystallization from ethyl alcohol or water [14].

*N-[2-(2-methylphenyl)-4-oxo-1,3-thiazolidin-3-yl]-2-hydroxy-2,2-diphenylacetamide (**3a**)*

Yield 63%; M.p.: 192-193 °C; IR (KBr) ($\bar{\nu}$, cm^{-1}): 3275 (O-H/N-H), 1679, 1711 (C=O); ^{1}H-NMR (DMSO-d_6, 500 MHz) δ (ppm): 2.16 (3H, s, 2-CH_3), 3.73, 3.82 (2H, 2d, *J*=16.10 Hz, thiazolidine C_5-H), 6.10 (1H, s, thiazolidine C_2-H), 6.71 (1H, s, C-OH), 7.13 (1H, d, *J*=7.81 Hz, Ar-H), 7.19-7.27 (12H, m, Ar-H), 7.58 (1H, d, *J*=4.88 Hz, Ar-H), 10.29 (1H, s, CONH). Anal. Calcd. for $C_{24}H_{22}N_2O_3S$ (418.51): C, 68.88; H, 5.30; N, 6.69. Found: C, 68.79; H, 5.53; N, 6.53.

*N-[2-(3-Methylphenyl)-4-oxo-1,3-thiazolidin-3-yl]-2-hydroxy-2,2-diphenylacetamide (**3b**)*

Yield 62%; M.p.: 189-190 °C; IR (KBr) ($\bar{\nu}$, cm^{-1}): 3280 (O-H/N-H), 1675, 1711 (C=O); ^{1}H-NMR (DMSO-d_6, 500 MHz) δ (ppm): 2.26 (3H, s, 3-CH_3), 3.69, 3.82 (2H, 2d, *J*=16.11 Hz, thiazolidine C_5-H), 5.79 (1H, s, thiazolidine C_2-H), 6.71 (1H, s, C-OH), 7.11-7.17 (3H, m, Ar-H), 7.20-7.27 (11H, m, Ar-H), 10.22 (1H, s, CONH). Anal. Calcd. for $C_{24}H_{22}N_2O_3S$ (418.51): C, 68.88; H, 5.30; N, 6.69. Found: C, 68.62; H, 5.42; N, 6.62.

*N-[2-(4-Methylphenyl)-4-oxo-1,3-thiazolidin-3-yl]-2-hydroxy-2,2-diphenylacetamide (**3c**)*

Yield 56%; M.p.: 217-218 °C; IR (KBr) ($\bar{\nu}$, cm^{-1}): 3284 (O-H/N-H), 1680, 1714 (C=O); ^{1}H-NMR (DMSO-d_6, 500 MHz) δ (ppm): 2.32 (3H, s, 4-CH_3), 3.69, 3.82 (2H, 2dd, *J*=15.62, 1.46 Hz, thiazolidine C_5-H), 5.78 (1H, s, thiazolidine C_2-H), 6.69 (1H, s, C-OH), 7.10 (4H, d, *J*=7.81 Hz, 4-methylphenyl $C_{2,3,5,6}$-H), 7.18-7.24 (5H, m, Ar-H), 7.25-7.27 (3H, m, Ar-H), 7.30 (2H, d, *J*=8.30 Hz, Ar-H), 10.20 (1H, s, CONH); ^{13}C-NMR (APT) (DMSO-d_6/125 MHz) δ (ppm): 21.54 (CH_3), 30.21 (C_5), 62.42 (C_2), 81.23 (C-OH), 127.94, 127.99, 128.15, 128.28, 128.94, 129.53 (ar. CH), 135.18, 139.05, 144.12, 144.16 (ar. C), 169.27 (amide C=O), 172.06 (lactam C=O); LC/MS: *m/z* 417 $(M-H)^-$. Anal. Calcd. for $C_{24}H_{22}N_2O_3S$ (418.51): C, 68.88; H, 5.30; N, 6.69. Found: C, 68.50; H, 5.45; N, 6.62.

*N-[2-(2-Hydroxyphenyl)-4-oxo-1,3-thiazolidin-3-yl]-2-hydroxy-2,2-diphenylacetamide (**3d**)*

Yield 37%; M.p.: 226-228 °C; IR (KBr) ($\bar{\nu}$, cm^{-1}): 3308 (O-H/N-H), 1678, 1711 (C=O); ^{1}H-NMR (DMSO-d_6, 500 MHz) δ (ppm): 3.63, 3.76 (2H, 2dd, *J*=16.11, 1.95 Hz, thiazolidine C_5-H), 6.16 (1H, s, thiazolidine C_2-H), 6.75 (1H, s, C-OH), 6.77-6.78 (2H, m, 2-hydroxyphenyl $C_{3,5}$-H), 7.14 (1H, td, *J*=7.32, 1.95 Hz, 2-hydroxyphenyl C_4-H), 7.22-7.33 (11H, m, Ar-H), 9.85 (1H, s, 2-OH), 10.19 (1H, s, CONH). Anal. Calcd. for $C_{23}H_{20}N_2O_4S$ (420.48): C, 65.70; H, 4.79; N, 6.66. Found: C, 65.50; H, 4.56; N, 7.02.

*N-[2-(4-Hydroxyphenyl)-4-oxo-1,3-thiazolidin-3-yl]-2-hydroxy-2,2-diphenylacetamide (**3e**)*

Yield 29%; M.p.: 223-224 °C; IR (KBr) ($\bar{\nu}$, cm^{-1}): 3243 (O-H/N-H), 1665, 1694 (C=O); ^{1}H-NMR (DMSO-d_6, 500 MHz) δ (ppm): 3.68, 3.77 (2H, 2dd, *J*=16.11, 1.95 Hz, thiazolidine C_5-H), 5.72 (1H, s, thiazolidine C_2-H), 6.68 (1H, s, C-OH), 6.70 (2H, dd, *J*=6.84, 1.95 Hz, 4-hydroxyphenyl $C_{3,5}$-H), 7.07 (2H, brs, 4-hydroxyphenyl $C_{2,6}$-H), 7.20-7.25 (7H, m, Ar-H), 7.26-7.28 (3H, m, Ar-H), 9.60 (1H, s, 4-OH), 10.12 (1H, s, CONH); ^{13}C-NMR (APT) (DMSO-d_6/125 MHz) δ (ppm): 30.27 (C_5), 62.51 (C_2), 81.27 (C-OH), 115.72, 127.86, 127.97, 127.99, 128.10, 128.14, 128.36, 130.61 (ar. CH), 144.10, 144.23 (ar. C), 158.86 (ar. C-OH), 169.16 (amide C=O), 172.03 (lactam C=O); LC/MS: *m/z* 419 $(M-H)^-$. Anal. Calcd. for $C_{23}H_{20}N_2O_4S$ (420.48): C, 65.70; H, 4.79; N, 6.66. Found: C, 65.51; H, 5.00; N, 6.68.

*N-[2-(3-Ethoxy-4-hydroxyphenyl)-4-oxo-1,3-thiazolidin-3-yl]-2-hydroxy-2,2-diphenylacetamide (**3f**)*

Yield 51%; M.p.: 198-200 °C; IR (KBr) ($\bar{\nu}$, cm^{-1}): 3347 (O-H/N-H), 1673, 1712 (C=O); ^{1}H-NMR (DMSO-d_6, 500 MHz) δ (ppm): 1.30 (3H, t, *J*=6.83 Hz, 3-OCH_2CH_3), 3.87-3.96 (2H, m, 3-OCH_2CH_3), 3.68,3.78 (2H, 2d, *J*=15.62 Hz, thiazolidine C_5-H), 5.76 (1H, s, thiazolidine C_2-H), 6.71 (1H, d, *J*=7.81 Hz, C-OH), 7.01 (1H, d, *J*=1.95 Hz, Ar-H), 7.13 (2H, brs, Ar-H), 7.22-7.25 (4H, m, Ar-H), 7.27 (6H, s, Ar-H), 9.04 (1H, brs, Ar-OH), 10.16 (1H, s, CONH); ^{13}C-NMR (APT) (DMSO-d_6/125MHz) δ (ppm): 15.43 (OCH_2CH_3), 30.31 (C_5), 56.76 (C_2), 64.42 (OCH_2CH_3), 81.27 (C-OH), 115.51, 121.96, 128.01, 128.15, 128.29 (ar. CH), 144.15, 144.23 (ar. C), 147.47, 148.34

(ar. C-OR), 169.23 (amide C=O), 172.05 (lactam C=O); LC/MS: *m/z* 463 $(M-H)^-$. Anal. Calcd. for $C_{25}H_{24}N_2O_5S$ (464.53): C, 64.64; H, 5.21; N, 6.03. Found: C, 64.34; H, 5.38; N, 5.92.

*N-[2-(4-Methoxyphenyl)-4-oxo-1,3-thiazolidin-3-yl]-2-hydroxy-2,2-diphenylacetamide (**3g**)*

Yield 68%; M.p.: 158-159 °C; IR (KBr) ($\bar{\nu}$, cm^{-1}): 3276 (O-H/N-H), 1673, 1714 (C=O); ^{1}H-NMR (DMSO-d_6, 500 MHz) δ (ppm): 3.77 (3H, s, 4-OCH_3), 3.69, 3.80 (2H, 2dd, *J*=15.62, 1.46 Hz, thiazolidine C_5-H), 5.79 (1H, s, thiazolidine C_2-H), 6.69 (1H, s, C-OH), 6.86 (2H, dd, *J*=8.79, 1.95 Hz, 4-methoxyphenyl $C_{3,5}$-H), 7.08 (2H, d, *J*=5.86 Hz, Ar-H), 7.19 (2H, t, *J*=7.32 Hz, Ar-H), 7.22-7.28 (6H, m, Ar-H), 7.34 (2H, d, *J*=8.79 Hz, 4-methoxyphenyl $C_{2,6}$-H), 10.17 (1H, s, CONH). Anal. Calcd. for $C_{24}H_{22}N_2O_4S$ (434.51): C, 66.34; H, 5.10; N, 6.45. Found: C, 66.38; H, 5.52; N, 6.15.

*N-[2-(3,4-Dimethoxyphenyl)-4-oxo-1,3-thiazolidin-3-yl]-2-hydroxy-2,2-diphenylacetamide (**3h**)*

Yield 61%; M.p.: 115-116 °C; IR (KBr) ($\bar{\nu}$, cm^{-1}): 3303 (O-H/N-H), 1676, 1698 (C=O); ^{1}H-NMR (DMSO-d_6, 500 MHz) δ (ppm): 3.67 (3H, s, OCH_3), 3.76 (3H, s, OCH_3), 3.69, 3.80 (2H, 2dd, *J*=16.11, 1.46 Hz, thiazolidine C_5-H), 5.80 (1H, s, thiazolidine C_2-H), 6.71 (1H, s, C-OH), 6.84 (1H, d, *J*=8.30 Hz, 3,4-dimethoxyphenyl C_5-H), 6.88 (1H, dd, *J*=8.30, 1.95 Hz, 3,4-dimethoxyphenyl C_6-H), 7.06 (1H, d, *J*=1.95 Hz, 3,4-dimethoxyphenyl C_2-H), 7.20 (2H, t, *J*=6.83 Hz, Ar-H), 7.22-7.27 (7H, m, Ar-H), 10.23 (1H, s, CONH). Anal. Calcd. for $C_{25}H_{24}N_2O_5S$ (464.54): C, 64.64; H, 5.21; N, 6.03. Found: C, 64.35; H, 5.69; N, 5.79.

*N-[2-(2,5-Dimethoxyphenyl)-4-oxo-1,3-thiazolidin-3-yl]-2-hydroxy-2,2-diphenylacetamide (**3i**)*

Yield 62%; M.p.: 177-178 °C; IR (KBr) ($\bar{\nu}$, cm^{-1}): 3303 (O-H/N-H), 1676, 1698 (C=O); ^{1}H-NMR (DMSO-d_6, 500 MHz) δ (ppm): 3.67 (3H, s, OCH_3), 3.69 (3H, s, OCH_3), 3.78,3.82 (2H, 2d, *J*=1.70 Hz, thiazolidine C_5-H), 6.15 (1H, s, thiazolidine C_2-H), 6.77 (1H, s, COH), 6.85 (1H, dd, *J*=9.03, 3.17 Hz, Ar-H), 6.93 (1H, d, *J*=9.28 Hz, Ar-H), 7.18 (1H, d, *J*=3.42 Hz, Ar-H), 7.26-7.29 (10H, m, Ar-H), 10.36 (1H, s, CONH). Anal. Calcd. for $C_{25}H_{24}N_2O_5S$ (464.54): C, 64.64; H, 5.21; N, 6.03. Found: C, 64.20; H, 5.55; N, 5.96.

*N-[2-(4-Methoxycarbonylphenyl)-4-oxo-1,3-thiazolidin-3-yl]-2-hydroxy-2,2-diphenylacetamide (**3j**)*

Yield 61%; M.p.: 151-152 °C; IR (KBr) ($\bar{\nu}$, cm^{-1}): 3333 (O-H/N-H), 1672, 1722 (C=O); ^{1}H-NMR (DMSO-d_6, 500 MHz) δ (ppm): 3.88 (3H, s, 4-$COOCH_3$), 3.74, 3.90 (2H, 2dd, *J*=15.13, 1.46 Hz, thiazolidine C_5-H), 5.89 (1H, s, thiazolidine C_2-H), 6.72 (1H, s, C-OH), 7.09 (2H, d, *J*=7.32 Hz, Ar-H), 7.17 (2H, t, *J*=6.83 Hz, Ar-H), 7.20-7.23 (3H, m, Ar-H), 7.24-7.26 (3H, m, Ar-H), 7.56 (2H, d, *J*=8.30 Hz, 4-methoxycarbonylphenyl $C_{2,6}$-H), 7.86 (2H, d, *J*=8.79 Hz, 4-methoxycarbonylphenyl $C_{3,5}$-H), 10.34 (1H, s, CONH). Anal. Calcd. for $C_{25}H_{22}N_2O_5S$ (462.52): C, 64.92; H, 4.79; N, 6.06. Found: C, 64.99; H, 4.72; N, 6.10.

*N-[2-(2-Nitrophenyl)-4-oxo-1,3-thiazolidin-3-yl]-2-hydroxy-2,2-diphenylacetamide (**3k**)*

Yield 32%; M.p.: 208-209 °C; IR (KBr) ($\bar{\nu}$, cm^{-1}): 3303, 3493 (O-H/N-H), 1674, 1719 (C=O); ^{1}H-NMR (DMSO-d_6, 500 MHz) δ (ppm): 3.70, 3.92 (2H, 2d, *J*=15.62 Hz, thiazolidine C_5-H), 6.17 (1H, s, thiazolidine C_2-H), 6.79 (1H, s, C-OH), 7.16-7.19 (2H, m, Ar-H), 7.20-7.25 (8H, m, Ar-H), 7.60 (1H, t, *J*=7.81 Hz, 2-nitrophenyl C_4-H), 7.73 (1H, t, *J*=7.81 Hz, 2-nitrophenyl C_6-H), 8.00 (2H, t, *J*=8.30 Hz, 2-nitrophenyl $C_{3,5}$-H), 10.46 (1H, s, CONH). Anal. Calcd. for $C_{23}H_{19}N_3O_5S$ (449.48): C, 61.46; H, 4.26; N, 9.35. Found: C, 61.13; H, 4.09; N, 8.99.

*N-[2-(3-Nitrophenyl)-4-oxo-1,3-thiazolidin-3-yl]-2-hydroxy-2,2-diphenylacetamide (**3l**)*

Yield 78%; M.p.: 185-186 °C; IR (KBr) ($\bar{\nu}$, cm^{-1}): 3366 (O-H/N-H), 1680, 1728 (C=O); ^{1}H-NMR (DMSO-d_6, 500 MHz) δ (ppm): 3.76, 3.95 (2H, 2dd, *J*=15.62, 1.46 Hz, thiazolidine C_5-H), 5.94 (1H, s, thiazolidine C_2-H), 6.74 (1H, s, C-OH), 7.08 (2H, d, *J*=7.32 Hz, Ar-H), 7.16 (2H, t, *J*=6.83 Hz, Ar-H), 7.19-7.26 (6H, m, Ar-H), 7.56 (1H, t, *J*=7.81 Hz, 3-nitrophenyl C_5-H), 7.87 (1H, dd, *J*=7.81, 1.46 Hz, 3-nitrophenyl C_6-H), 8.15 (1H, dd, *J*=8.30, 2.44 Hz, 3-nitrophenyl C_4-H), 8.27 (1H, t, *J*=1.95 Hz, 3-nitrophenyl C_2-H), 10.40 (1H, s, CONH). Anal. Calcd. for $C_{23}H_{19}N_3O_5S$ (449.48): C, 61.46; H, 4.26; N, 9.35. Found: C, 61.68; H, 4.16; N, 9.12.

*N-[2-(4-Nitrophenyl)-4-oxo-1,3-thiazolidin-3-yl]-2-hydroxy-2,2-diphenylacetamide (**3m**)*

Yield 40%; M.p.: 232-235 °C; IR (KBr) ($\bar{\nu}$, cm^{-1}): 3107, 3360 (O-H/N-H), 1676, 1719 (C=O); ^{1}H-NMR (DMSO-d_6, 500 MHz) δ (ppm): 3.75, 3.93 (2H, 2dd, *J*=16.11, 1.95 Hz, thiazolidine C_5-H), 5.93 (1H, s, thiazolidine C_2-H), 6.76 (1H, s, C-OH), 7.11 (2H, dd, *J*=7.81, 0.98 Hz, Ar-H), 7.14-7.19 (3H, m, Ar-H), 7.22-7.27 (5H, m, Ar-H), 7.68 (2H, dd, *J*=8.79, 1.95 Hz, 4-nitrophenyl $C_{2,6}$-H), 8.08 (2H, dd, *J*=8.79, 1.95 Hz, 4-nitrophenyl $C_{3,5}$-H),

10.43 (1H, s, CONH). Anal. Calcd. for $C_{23}H_{19}N_3O_5S$ (449.48): C, 61.46; H, 4.26; N, 9.35. Found: C, 61.76; H, 3.97; N, 9.71.

*N-[2-(2,4-Dinitrophenyl)-4-oxo-1,3-thiazolidin-3-yl]-2-hydroxy-2,2-diphenylacetamide (**3n**)*

Yield 47%; M.p.: 206-207 °C; IR (KBr) ($\bar{\nu}$, cm^{-1}): 3213, 3372 (O-H/N-H), 1676, 1709 (C=O); ^{1}H-NMR (DMSO-d_6, 500 MHz) δ (ppm): 3.76, 3.93 (2H, 2dd, *J*=16.11, 1.95 Hz, thiazolidine C_5-H), 5.94 (1H, brs, thiazolidine C_2-H), 6.76 (1H, s, C-OH), 7.10-7.27 (10H, m, Ar-H), 7.68 (1H, d, *J*=8,79 Hz, 2,4-dinitrophenyl C_6-H), 8.06-8.09 (2H, m, 2,4-dinitrophenyl $C_{2,5}$-H), 10.41 (1H, s, CONH). Anal. Calcd. for $C_{23}H_{18}N_3O_7S$ (494.47): C, 55.87; H, 3.67; N, 11.33. Found: C, 55.48; H, 3.55; N, 11.18.

*N-[2-(4-Chloro-3-nitrophenyl)-4-oxo-1,3-thiazolidin-3-yl]-2-hydroxy-2,2-diphenylacetamide (**3o**)*

Yield 45%; M.p.: 187-188 °C; IR (KBr) ($\bar{\nu}$, cm^{-1}): 3365 (O-H/N-H), 1678, 1724 (C=O); ^{1}H-NMR (DMSO-d_6, 500 MHz) δ (ppm): 3.64, 3.85 (2H, 2d, *J*=15.84 Hz, thiazolidine C_5-H), 5.79 (1H, d, *J*=5.79 Hz, thiazolidine C_2-H), 6.67 (1H, s, C-OH), 6.96-7.17 (10H, m, Ar-H), 7.54,7.62 (2H, 2d, *J*=8.36 Hz, Ar-H), 8.02, 8.12 (1H, 2d, *J*=2.00 Hz, Ar-H), 10.36 (1H, s, CONH). Anal. Calcd. for $C_{23}H_{18}ClN_3O_5S$ (483.92): C, 57.08; H, 3.75; N, 8.68. Found: C, 56.85; H, 3.83; N, 8.38.

*N-[2-(4-Dimethylaminophenyl)-4-oxo-1,3-thiazolidin-3-yl]-2-hydroxy-2,2-diphenylacetamide (**3p**)*

Yield 43%; M.p.: 196-198 °C; IR (KBr) ($\bar{\nu}$, cm^{-1}): 3208 (O-H/N-H), 1687, 1727 (C=O); ^{1}H-NMR (DMSO-d_6, 500 MHz) δ (ppm): 2.93 (6H, s, 2 x CH_3), 3.66, 3.75 (2H, 2dd, *J*=16.11, 1.46 Hz, thiazolidine C_5-H), 5.72 (1H, s, thiazolidine C_2-H), 6.64 (2H, d, *J*=8.79 Hz, 4-dimethylaminophenyl $C_{3,5}$-H), 6.67 (1H, s, COH), 7.07 (2H, brs, 4-dimethylaminophenyl $C_{2,6}$-H), 7.18 (2H, t, *J*=7.32 Hz, Ar-H), 7.21-7.27 (8H, m, Ar-H), 10.09 (1H, s, CONH). Anal. Calcd. for $C_{25}H_{25}N_3O_3S$ (447.54): C, 67.09; H, 5.63; N, 9.39. Found: C, 67.40; H, 5.37; N, 9.65.

*N-[2-(2-Methylphenyl)-5-methyl-4-oxo-1,3-thiazolidin-3-yl]-2-hydroxy-2,2-diphenylacetamide (**4a**)*

Yield 45%; M.p.: 224-226 °C; IR (KBr) ($\bar{\nu}$, cm^{-1}): 3288, 3371 (O-H/N-H), 1675, 1714 (C=O); ^{1}H-NMR (DMSO-d_6, 500 MHz) δ (ppm): 1.48 (3H, d, *J*=6.83 Hz, 5-CH_3), 2.14 (3H, s, C_2-CH_3), 3.98, 4.03 (1H, 2br s, thiazolidine C_5-H), 6.11 (1H, s, thiazolidine C_2-H), 6.72 (1H, s, C-OH), 7.11 (2H, d, *J*=6.83 Hz, Ar-H), 7.20 (12H, br s, Ar-H), 10.31 (1H, s, CONH). Anal. Calcd. for $C_{25}H_{24}N_2O_3S$ (432.53): C, 69.42; H, 5.59; N, 6.48. Found: C, 69.22; H, 5.62; N, 6.39.

*N-[2-(3-Methylphenyl)-5-methyl-4-oxo-1,3-thiazolidin-3-yl]-2-hydroxy-2,2-diphenylacetamide (**4b**)*

Yield 65%; M.p.: 178-179 °C; IR (KBr) ($\bar{\nu}$, cm^{-1}): 3296, 3388 (O-H/N-H), 1682, 1714 (C=O); ^{1}H-NMR (DMSO-d_6, 500 MHz) δ (ppm): 1.51-1.53 (3H, m, 5-CH_3), 2.27 (3H, s, C_3-CH_3), 3.99, 4.09 (1H, 2q, *J*=6.83 Hz, thiazolidine C_5-H), 5.79 (1H, s, thiazolidine C_2-H), 6.70 (1H, s, C-OH), 7.16-7.18 (3H, m, Ar-H), 7.21-7.25 (6H, m, Ar-H), 7.27 (5H, d, *J*=5.37 Hz, Ar-H), 10.24 (1H, s, CONH). Anal. Calcd. for $C_{25}H_{24}N_2O_3S$ (432.53): C, 69.42; H, 5.59; N, 6.48. Found: C, 69.16; H, 5.72; N, 6.44.

*N-[2-(4-Methylphenyl)-5-methyl-4-oxo-1,3-thiazolidin-3-yl]-2-hydroxy-2,2-diphenylacetamide (**4c**)*

Yield 35%; M.p.: 187-188 °C; IR (KBr) ($\bar{\nu}$, cm^{-1}): 3397 (O-H/N-H), 1682, 1717 (C=O); ^{1}H-NMR (DMSO-d_6, 500 MHz) δ (ppm): 1.50 (3H, d, *J*=3.42 Hz, 5-CH_3), 2.31 (3H, d, *J*=3.42 Hz, C_4-CH_3), 3.97, 4.07 (1H, 2qd, *J*=6.83, 0.93 Hz, thiazolidine C_5-H), 5.74 (1H, s, thiazolidine C_2-H), 6.67 (1H, s, C-OH), 7.12 (2H, d, *J*=7.81 Hz, 4-methylphenyl $C_{3,5}$-H), 7.19 (2H, t, *J*=6.83, Ar-H), 7.22-7.27 (8H, m, Ar-H), 7.30 (2H, d, *J*=8.30, 3.42 Hz, 4-methylphenyl $C_{2,6}$-H), 10.23 (1H, s, CONH). Anal. Calcd. for $C_{25}H_{24}N_2O_3S$ (432.53): C, 69.42; H, 5.59; N, 6.48. Found: C, 69.38; H, 5.87; N, 6.28.

*N-[2-(2-Hydroxyphenyl)-5-methyl-4-oxo-1,3-thiazolidin-3-yl]-2-hydroxy-2,2-diphenylacetamide (**4d**)*

Yield 58%; M.p.: 234-235 °C; IR (KBr) ($\bar{\nu}$, cm^{-1}): 3278 (O-H/N-H), 1673, 1705 (C=O); ^{1}H-NMR (DMSO-d_6, 500 MHz) δ (ppm): 1.46 (3H, d, *J*=6.83 Hz, 5-CH_3), 3.92, 4.00 (1H, 2q, *J*=6.83 Hz, thiazolidine C_5-H), 6.18 (1H, s, thiazolidine C_2-H), 6.73 (1H, s, C-OH), 6.78 (1H, d, *J*=7.81 Hz, 2-hydroxyphenyl C_3-H), 6.81 (2H, dd, *J*=8.30, 0.98 Hz, 2-hydroxyphenyl $C_{5,6}$-H), 7.13 (1H, td, *J*=7.32, 1.46 Hz, 2-hydroxyphenyl C_4-H), 7.21-7.25 (10H, m, Ar-H), 9.83 (1H, s, 2-OH), 10.23 (1H, s, CONH). Anal. Calcd. for $C_{24}H_{22}N_2O_4S$ (434.50): C, 66.34; H, 5.10; N, 6.45. Found: C, 65.99; H, 5.01; N, 6.54.

*N-[2-(4-Hydroxyphenyl)-5-methyl-4-oxo-1,3-thiazolidin-3-yl]-2-hydroxy-2,2-diphenylacetamide (**4e**)*

Yield 54%; M.p.: 235-236 °C; IR (KBr) ($\bar{\nu}$, cm^{-1}): 3372 (O-H/N-H), 1661, 1710 (C=O); ^{1}H-NMR (DMSO-d_6, 500 MHz) δ (ppm): 1.50 (3H, d, *J*=6.34 Hz, 5-CH_3), 3.94, 4.03 (1H, 2q, *J*=6.83 Hz, thiazolidine C_5-H), 5.70, 5.73 (1H, 2s, thiazolidine C_2-H), 6.70-6.73 (3H, m, COH and Ar-H), 7.10 (2H, brs, Ar-H), 7.21-7.24 (5H, m, Ar-H), 7.27-7.28 (5H, m, Ar-H), 10.12 (1H, s, CONH). Anal. Calcd. for $C_{24}H_{22}N_2O_4S$ (434.50): C, 66.34; H, 5.10; N, 6.45. Found: C, 66.55; H, 5.32; N, 6.32.

*N-[2-(3-Ethoxy-4-hydroxyphenyl)-5-methyl-4-oxo-1,3-thiazolidin-3-yl]-2-hydroxy-2,2-diphenylacetamide (**4f**)*

Yield 65%; M.p.: 202-203 °C; IR (KBr) ($\bar{\nu}$, cm^{-1}): 3381 (O-H/N-H), 1672, 1713 (C=O); ^{1}H-NMR (DMSO-d_6, 500 MHz) δ (ppm): 1.28 (3H, t, *J*=7.32 Hz, 3-OCH_2CH_3), 1.48 (3H, d, *J*=6.83 Hz, 5-CH_3), 3.85-3.99 (3H, m, thiazolidine C_5-H and 3-OCH_2CH_3), 5.75 (1H, s, thiazolidine C_2-H), 6.95 (1H, s, C-OH), 6.67-6.73 (2H, m, 3-ethoxy-4-hydroxyphenyl $C_{5,6}$-H), 6.95 (1H, s, 3-ethoxy-4-hydroxyphenyl C_2-H), 7.18-7.27 (10H, m, Ar-H), 8.49 (1H, s, 4-OH), 10.20 (1H, s, CONH). Anal. Calcd. for $C_{26}H_{26}N_2O_5S$ (478.56): C, 65.25; H, 5.48; N, 5.85. Found: C, 65.16; H, 5.60; N, 5.49.

*N-[2-(4-Hydroxy-3-methoxy-5-nitrophenyl)-5-methyl-4-oxo-1,3-thiazolidin-3-yl]-2-hydroxy-2,2-diphenylacetamide (**4g**)*

Yield 70%; M.p.: 238-239 °C; IR (KBr) ($\bar{\nu}$, cm^{-1}): 3346 (O-H/N-H), 1673, 1706 (C=O); ^{1}H-NMR (DMSO-d_6, 500 MHz) δ (ppm): 1.39 (3H, t, *J*=6.73Hz, 5-CH_3), 3.55 (3H, d, *J*=5.70 Hz, OCH_3), 3.85,3.94 (1H, 2q, *J*=7.00 Hz, thiazolidine C_5-H), 5.63 (1H, s, thiazolidine C_2-H), 6.76 (1H, brs, C-OH), 7.10-7.14 (10H, m, Ar-H), 7.30-7.32 (2H, m, Ar-H), 10.16 (1H, s, CONH). Anal. Calcd. for $C_{25}H_{23}N_3O_7S$ (509.53): C, 58.93; H, 4.55; N, 8.25. Found: C, 58.65; H, 4.72; N, 8.44.

*N-[2-(4-Methoxyphenyl)-5-methyl-4-oxo-1,3-thiazolidin-3-yl]-2-hydroxy-2,2-diphenylacetamide (**4h**)*

Yield 68%; M.p.: 175-176 °C; IR (KBr) ($\bar{\nu}$, cm^{-1}): 3300,3401 (O-H/N-H), 1687, 1715 (C=O); ^{1}H-NMR (DMSO-d_6, 500 MHz) δ (ppm): 1.51 (3H, d, *J*=7.08 Hz, 5-CH_3), 3.78 (3H, d, *J*=2.45 Hz, C_4-OCH_3), 3.97, 4.06 (1H, 2q, *J*=6.83 Hz, thiazolidine C_5-H), 5.77 (1H, s, thiazolidine C_2-H), 6.67 (1H, brs, C-OH), 6.86-6.89 (2H, m, Ar-H), 7.11-7.12 (2H, m, Ar-H), 7.18-7.29 (8H, m, Ar-H), 7.33-7.36 (6H, m, Ar-H), 10.18 (1H, s, CONH). Anal. Calcd. for $C_{25}H_{24}N_2O_4S$ (448.54): C, 66.95; H, 5.39; N, 6.25. Found: C, 66.60; H, 5.65; N, 6.18.

*N-[2-(3,4-Dimethoxyphenyl)-5-methyl-4-oxo-1,3-thiazolidin-3-yl]-2-hydroxy-2,2-diphenylacetamide (**4i**)*

Yield 31%; M.p.: 138-139 °C; IR (KBr) ($\bar{\nu}$, cm^{-1}): 3337 (O-H/N-H), 1674, 1707 (C=O); ^{1}H-NMR (DMSO-d_6, 500 MHz) δ (ppm): 1.51 (3H, d, *J*=4.39 Hz, 5-CH_3), 3.67 (3H, s, OCH_3), 3.76 (3H, s, OCH_3), 3.97, 4.05 (1H, 2qd, *J*=6.83, 1.46 Hz, thiazolidine C_5-H), 5.75 (1H, s, thiazolidine C_2-H), 6.70 (1H, brs, C-OH), 7.05 (1H, s, 3,4-dimethoxyphenyl C_2-H), 6.84 (1H, dd, *J*=8.30, 3.42 Hz, 3,4-dimethoxyphenyl C_6-H), 6.88 (1H, td, *J*=8.30, 1.95 Hz, 3,4-dimethoxyphenyl C_5-H), 7.09-7.26 (10H, m, Ar-H), 7.33-7.36 (6H, m, Ar-H), 10.24 (1H, s, CONH). Anal. Calcd. for $C_{26}H_{26}N_2O_5S$ (478.56): C, 65.25; H, 5.48; N, 5.85. Found: C, 65.47; H, 5.75; N, 5.71.

*N-[2-(2,5-Dimethoxyphenyl)-5-methyl-4-oxo-1,3-thiazolidin-3-yl]-2-hydroxy-2,2-diphenylacetamide (**4j**)*

Yield 54%; M.p.: 172-173 °C; IR (KBr) ($\bar{\nu}$, cm^{-1}): 3342 (O-H/N-H), 1675, 1710 (C=O); ^{1}H-NMR (DMSO-d_6, 500 MHz) δ (ppm): 1.47 (3H, d, *J*=6.83 Hz, 5-CH_3), 3.64 (3H, s, OCH_3), 3.66 (3H, s, OCH_3), 3.93, 4.03 (1H, 2qd, *J*=6.83, 1.95 Hz, thiazolidine C_5-H), 6.14 (1H, s, thiazolidine C_2-H), 6.76 (1H, s, C-OH), 6.84 (1H, dd, *J*=2.93, 1.46 Hz, 2,4-dimethoxyphenyl C_3-H), 6.92 (1H, dd, *J*=9.27, 1.46 Hz, 2,4-dimethoxyphenyl C_5-H), 7.20 (1H, d, *J*=82.93 Hz, 2,4-dimethoxyphenyl C_6-H), 7.24-7.27 (10H, m, Ar-H), 10.29 (1H, s, CONH). Anal. Calcd. for $C_{26}H_{26}N_2O_5S$ (478.56): C, 65.25; H, 5.48; N, 5.85. Found: C, 64.95; H, 5.32; N, 5.78.

*N-[2-(4-Methoxycarbonylphenyl)-5-methyl-4-oxo-1,3-thiazolidin-3-yl]-2-hydroxy-2,2-diphenylacetamide (**4k**)*

Yield 77%; M.p.: 167-168 °C; IR (KBr) ($\bar{\nu}$, cm^{-1}): 3322 (O-H/N-H), 1669, 1704 (C=O); ^{1}H-NMR (DMSO-d_6, 500 MHz) δ (ppm): 1.52 (3H, d, *J*=7.32 Hz, 5-CH_3), 3.88 (3H, s, 4-$COOCH_3$), 4.03, 4.15 (1H, 2qd, *J*=6.83, 1.46 Hz, thiazolidine C_5-H), 5.86 (1H, s, thiazolidine C_2-H), 6.71 (1H, s, C-OH), 7.07-7.11 (2H, m, Ar-H), 7.14-7.18 (2H, m, Ar-H), 7.19-7.22 (3H, m, Ar-H), 7.24-7.27 (3H, m, Ar-H), 7.56 (2H, dd, *J*=8.30, 1.95 Hz, 4-methoxycarbonylphenyl $C_{2,6}$-H), 7.86 (2H, d, *J*=8.30 Hz, 4-methoxycarbonylphenyl $C_{3,5}$-H), 10.34 (1H, s, CONH). Anal. Calcd. for $C_{26}H_{24}N_2O_5S$ (476.55): C, 65.53; H, 5.08; N, 5.88. Found: C, 65.60; H, 4.96; N, 5.88.

*N-[2-(2-Nitrophenyl)-5-methyl-4-oxo-1,3-thiazolidin-3-yl]-2-hydroxy-2,2-diphenylacetamide (**4l**)*

Yield 50%; M.p.: 164-165 °C; IR (KBr) ($\bar{\nu}$, cm^{-1}): 3263, 3369 (O-H/N-H), 1681, 1717 (C=O) ^{1}H-NMR (DMSO-d_6, 500 MHz) δ (ppm): 1.50 (3H, d, *J*=6.83 Hz, 5-CH_3), 4.02, 4.15 (1H, 2qd, *J*=6.83, 1.46 Hz, thiazolidine C_5-H), 6.16 (1H, s, thiazolidine C_2-H), 6.80 (1H, s, C-OH), 7.14-7.25 (10H, m, Ar-H), 7.60 (1H, dd, *J*=6.83, 1.46 Hz, 2-nitrophenyl C_4-H), 7.75 (1H, dd, *J*=7.81, 0.98 Hz, 2-nitrophenyl C_6-H), 8.01-8.04 (2H, m, 2-nitrophenyl $C_{3,5}$-H), 10.33 (1H, s, CONH). Anal. Calcd. for $C_{24}H_{21}N_3O_5S$ (463.50): C, 62.19; H, 4.57; N, 9.07. Found: C, 62.57; H, 4.37; N, 8.82.

*N-[2-(3-Nitrophenyl)-5-methyl-4-oxo-1,3-thiazolidin-3-yl]-2-hydroxy-2,2-diphenylacetamide (**4m**)*

Yield 53%; M.p.: 198-199 °C; IR (KBr) ($\bar{\nu}$, cm^{-1}): 3329, 3385 (O-H/N-H), 1676, 1724 (C=O); ^{1}H-NMR (DMSO-d_6, 500 MHz) δ (ppm): 1.55 (3H, d, *J*=7.32 Hz, 5-CH_3), 4.06, 4.21 (1H, 2qd, *J*=7.32, 1.46 Hz, thiazolidine C_5-H), 5.93 (1H, s, thiazolidine C_2-H), 6.73 (1H, s, C-OH), 7.06-7.10 (2H, m, Ar-H), 7.14-7.25 (8H, m, Ar-H), 7.57 (1H, t, *J*=8.30 Hz, 3-nitrophenyl C_5-H), 7.86 (1H, d, *J*=7.81 Hz, 3-nitrophenyl C_6-H), 8.15 (1H, d, *J*=7.80 Hz, 3-nitrophenyl C_4-H), 8.28 (1H, q, *J*=1.95 Hz, 3-nitrophenyl C_2-H), 10.41 (1H, s, CONH). Anal. Calcd. for $C_{24}H_{21}N_3O_5S$ (463.50): C, 62.19; H, 4.57; N, 9.07. Found: C, 62.35; H, 4.84; N, 9.10.

*N-[2-(4-Nitrophenyl)-5-methyl-4-oxo-1,3-thiazolidin-3-yl]-2-hydroxy-2,2-diphenylacetamide (**4n**)*

Yield 70%; M.p.: 121-122 °C; IR (KBr) ($\bar{\nu}$, cm^{-1}): 3274, 3461 (O-H/N-H), 1673, 1714 (C=O); ^{1}H-NMR (DMSO-d_6, 500 MHz) δ (ppm): 1.52,1.54 (3H, 2d, *J*=7.32 Hz, 5-CH_3), 4.07,4.19 (1H, 2q, *J*=6.83Hz, thiazolidine C_5-H), 5.92-5.93 (1H, m, thiazolidine C_2-H), 6.73 (1H, s, COH), 7.10-7.21 (5H, m, Ar-H), 7.23-7.28 (5H, m, Ar-H), 7.69 (2H, d, *J*=8.30 Hz, 4-nitrophenyl $C_{2,6}$-H), 8.08 (2H, d, *J*=8.05 Hz, 4-nitrophenyl $C_{3,5}$-H), 10.40,10.41 (1H, s, CONH); ^{13}C-NMR (APT) (DMSO-d_6/125 MHz) δ (ppm): 20.40 (5-CH_3), 38.41 (C_5), 60.44 (C_2), 81.16 (C-OH), 123.95, 127.95, 128.14, 128.23, 130.17, 130.50 (Ar. CH), 144.07, 144.10, 145.33 (Ar. C), 148.40 (Ar. C-NO_2), 171.69 (amide C=O), 172.23 (lactam C=O); LC/MS: *m/z* 462 $(M-H)^-$. Anal. Calcd. for $C_{24}H_{21}N_3O_5S$ (463.50): C, 62.19; H, 4.57; N, 9.07. Found: C, 62.53; H, 4.46; N, 8.88.

*N-[2-(4-Chloro-3-nitrophenyl)-5-methyl-4-oxo-1,3-thiazolidin-3-yl]-2-hydroxy-2,2-diphenylacetamide (**4o**)*

Yield 46%; M.p.: 180-181 °C; IR (KBr) ($\bar{\nu}$, cm^{-1}): 3387 (O-H/N-H), 1674, 1721 (C=O); ^{1}H-NMR (DMSO-d_6, 500 MHz) δ (ppm): 1.52 (3H, d, *J*=6.83 Hz, 5-CH_3), 4.05, 4.20 (1H, 2q, *J*=6.83 Hz, thiazolidine C_5-H), 5.90 (1H, s, thiazolidine C_2-H), 6.79 (1H, brs, C-OH), 7.12-7.15 (2H, m, Ar-H), 7.19-7.26 (8H, m, Ar-H), 7.64 (1H, d, *J*=8.30 Hz, 4-chloro-3-nitrophenyl C_5-H), 7.72 (1H, dd, *J*=8.30, 1.95 Hz, 4-chloro-3-nitrophenyl C_6-H), 8.10 (1H, s, 4-chloro-3-nitrophenyl C_2-H), 10.46 (1H, s, CONH). Anal. Calcd. for $C_{24}H_{20}ClN_3O_5S$ (497.95): C, 57.89; H, 4.05; N, 8.44. Found: C, 57.55; H, 3.98; N, 7.30.

*N-[2-(4-Dimethylaminophenyl)-5-methyl-4-oxo-1,3-thiazolidin-3-yl]-2-hydroxy-2,2-diphenylacetamide (**4p**)*

Yield 33%; M.p.: 184-185 °C; IR (KBr) ($\bar{\nu}$, cm^{-1}): 3264 (O-H/N-H), 1674, 1709 (C=O); ^{1}H-NMR (DMSO-d_6, 500 MHz) δ (ppm): 1.51 (3H, d, *J*=6.83 Hz, 5-CH_3), 2.93 (6H, d, *J*=1.46 Hz, $N(CH_3)_2$), 3.94, 4.02 (1H, 2q, *J*=6.83 Hz, thiazolidine C_5-H), 5.69,5.73 (1H, 2s, thiazolidine C_2-H), 6.65 (1H, d, *J*=7.32 Hz, C-OH), 7.11 (2H, d, *J*=5.37 Hz, Ar-H), 7.19-7.23 (6H, m, Ar-H), 7.25-7.28 (6H, m, Ar-H), 10.10 (1H, s, CONH); ^{13}C-NMR (APT) (DMSO-d_6/125 MHz) δ (ppm): 20.89 (5-CH_3), 39.31 (C_5), 40.28, 40.44 ($N(CH_3)_2$), 61.37 (C_2), 81.24 (C-OH), 112.39, 112.44, 127.94, 127.96, 128.08, 128.12, 128.16, 128.38, 128.41, 130.28 (Ar. CH), 144.14, 144.17, 144.28 (ar. CH), 171.84 (amide C=O), 172.04 (lactam C=O). LC/MS: *m/z* 460 $(M-H)^-$. Anal. Calcd. for $C_{26}H_{27}N_3O_3S$ (461.57): C, 67.65; H, 5.90; N, 9.10. Found: C, 67.96; H, 5.97; N, 8.98.

5.2. *In vitro* evaluation of anticancer activity

To provide growth on the cancer screening panel for the cell lines used in the study, RPMI medium with 5% fetal bovine serum and 2mM L-glutamine was used. Inoculation of cells into 96 well microtiter plates in 100 µL was done according to the doubling time of each single cell line. The plating cell density ranged between 5,000 - 40,000 cells per well. Following this, a 24 h condition for the incubation process of microtiter plates was set at 37 °C, 95% air, 5% CO_2, and 100% relative humidity and the experimental drugs were eventually added at time Tz. To determine the cell population for individual cell lines at time Tz, two plates of individual cell lines were bounded *in situ* using trichloroacetic acid. To provide two-fold of the wanted maximum final concentration that contains a complete medium of gentamicin (50 µg/ml) at time Tz, a frozen portion of the concentrate was defrosted and diluted. In addition to the control, a further series of dilutions were conducted to obtain a concentration of 1/2 log, fourfold, or 10 fold giving a sum of five drug

concentrations. To each suitable microtiter well that accommodates the medium (100 µl), a 100 µl portion of each of the various drug concentrations were added to obtain the necessary final drug concentrations. The incubation was continued for another two days under the same conditions as mentioned above. The assay for the adherent cells was discontinued after adding a cold TCA. Subsequently, 50 µl of cold 50% (w/v) trichloroacetic acid was gently added to bind the cells *in situ* and at 4°C, the incubation continued for another 1 h. The plates were thoroughly washed with H_2O and left to dry after discarding the supernatant. At room temperature, plates were incubated for 10 minutes after the addition of 0.4% (w/v) 100 µl solution of Sulforhodamine B (SRB) in 1% acetic acid to each well. By using 1% acetic acid, the excess dye was removed at the end of the staining process. This was done by washing five times, after which the plates were dried. Dissolving bound stain in 10 mM trizma base, an automated plate reader was used to measure the absorbance at a wavelength of 515 nm. The aforesaid methodology was applied for suspension cells, however, the termination of the assay was done by soft addition of 50 µl of 80% TCA This helped in fixing the settled cells at the bottom of the wells. The calculation of the growth percentage was done by using seven absorbance records for each drug concentration. The percentage of growth inhibition was calculated using the equations (Eq. 1, Eq. 2) below:

$$\frac{Ti-Tz}{C-Tz} \times 100, \ when\ Ti \geq Tz \qquad \text{Eq. 1}$$

$$\frac{Ti-Tz}{Tz} \times 100, \ when\ Ti < Tz \qquad \text{Eq.2}$$

Tz=Time zero, C = Control growth, Ti = Test growth in the presence of drugs at the five concentration levels

For each experimental agent, the three dose-response parameters were calculated. 50% growth inhibition (GI_{50}), the drug concentration during the drug incubation measured by SRB staining and resulted in a 50% decrease in the net protein increase in control cells, was determined with the formula $\frac{Ti-Tz}{C-Tz} \times 100 = 50$. Total growth inhibition from drug concentration was determined using Ti=Tz. The concentration of the drug that results in a 50% reduction (LC_{50})in the measured protein, was compared at the end of the drug treatment. The initial concentration (beginning) and that of the end of the treatment showed a net loss of cells following treatment. This was calculated using the formula $\frac{Ti-Tz}{Tz} \times 100 = -50$. For each of these parameters, values were calculated, if the level of the activity was reached. However, the values are expressed as greater or less than the maximum or minimum tested concentration, if the effect was not reached or exceeded.

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Appendix A. Supplementary Material

Supplementary material related to this article can be accessed at https://dx.doi.org/10.29228/jrp.21 .

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