

Ultrasound-Assisted Facile Synthesis and anticancer evaluation of Novel N-(2-substituted phenyl-4-oxathiazolidine-3-carbonothioyl) benzamide

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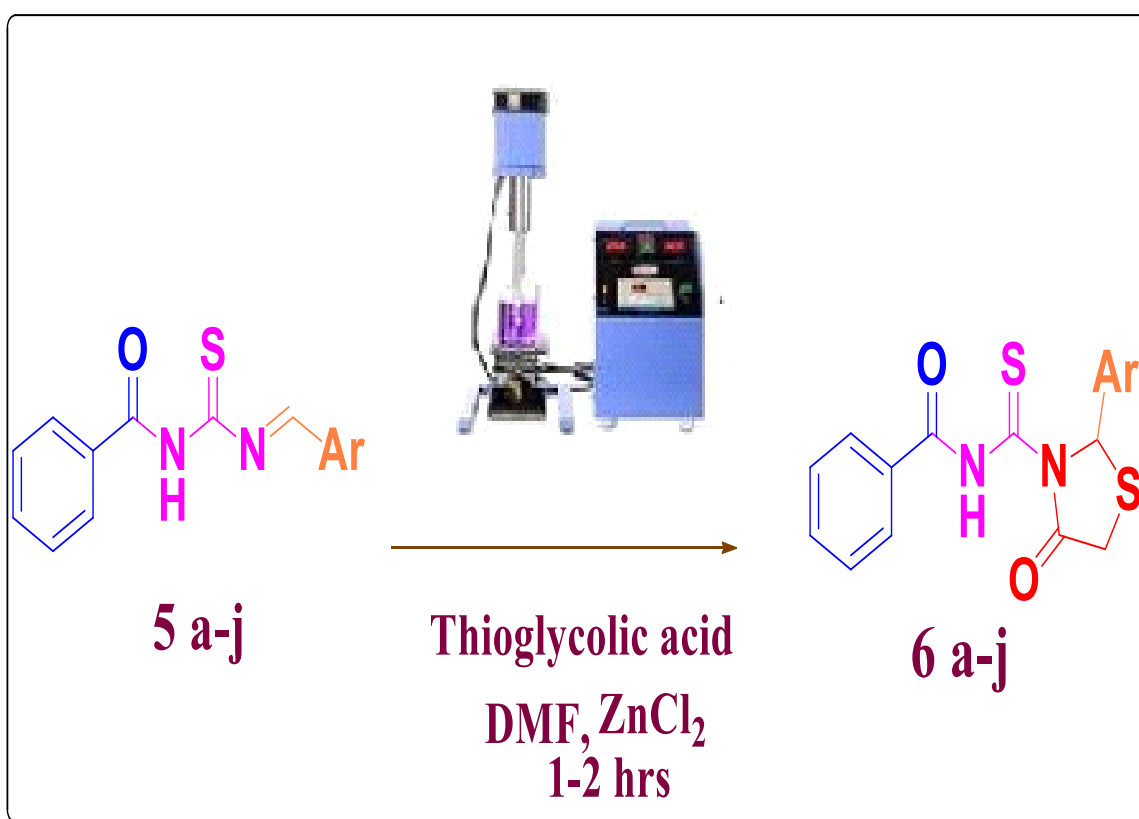
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Graphical Abstract:



Abstract: The work reports synthesis of ten novel derivatives of N-(2-substituted phenyl)- 4-oxothiazolidine-3-carbonothioyl benzamide derivatives **6(a-j)** were synthesized by cyclization of key compounds N-(benzylidenecarbamothioyl)-benzamide using thioglycolic acid in solvent DMF and catalyst anhydrous ZnCl₂ using ultra-sonicator as an eco-friendly synthetic route. The nitrogen and sulphur containing heterocycle such as thiazolidinone has attracted continuing interest because of its varied biological activities. With the coupling of benzamide, the anticancer activity of thiazolidin-4-one derivatives is enhanced. Out of the 10 derivatives, **6a** and **6c** were found to have potential activity against MCF7 cell line whereas **6d** and **6h** exhibited potential activity against both the cell lines MCF7 and HeLa cell line. As the synthesized derivatives showed good anticancer activity this moiety can be further studied and modified to help in development of anticancer drug. The structures of the synthesized compounds were confirmed by spectral characterization such as IR, ¹H NMR, ¹³CNMR and Mass spectral studies.

Keywords: Thiazolidin-4-one, Benzamide, Green chemistry, Ultrasound assisted reaction, Anticancer activity.

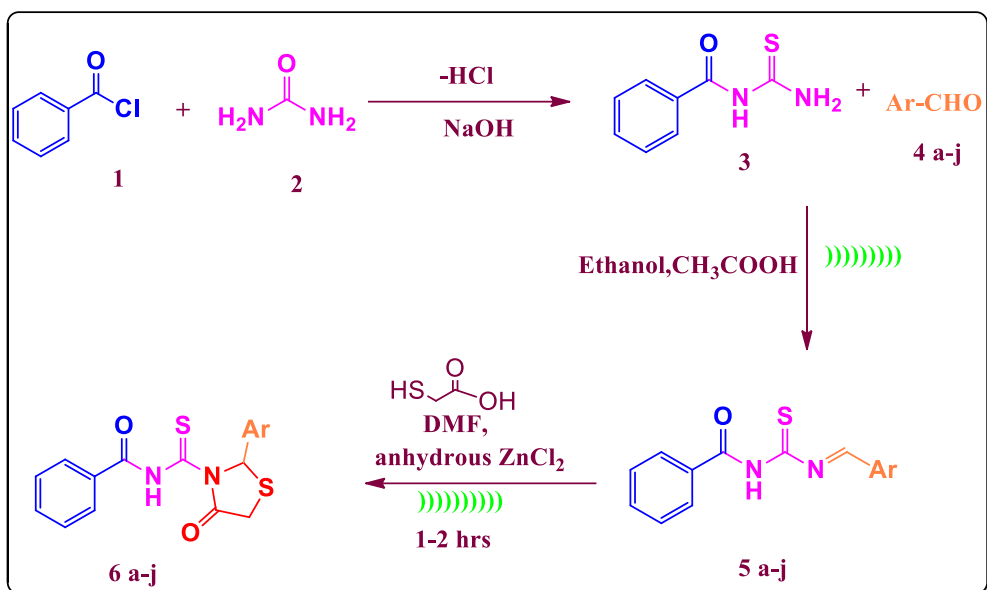
Introduction: Cancer is continuing to be a major health problem worldwide. The development of new anticancer therapeutic agents is one of the fundamental goals in medicinal chemistry as cancer causes about 13% of all the death [1]. The number of new cases is expected to rise by about 70% over the next two decades. According to paper published in American cancer society journal 1,735,350 new cancer cases were diagnosed in 2018[2]. Surpassing cardiovascular diseases, it is taking the position number one killer due to various factors [3]. Also the treatment of cancer is associated with various side effects, which include bone marrow depression, alopecia, drug-induced cancer, hepatotoxicity, and many more. Because of the need and value of anticancer drugs, many laboratories are intensively investigating the chemistry and biology of novel anticancer agents. Also the development of resistance against the existing anticancer drugs and cytotoxicity. Genotoxicity of anticancer drugs to the normal cells are other major problems in cancer therapy, keeping research window open in search for newer anticancer molecules [4]. Around the world, tremendous resources are being invested in prevention, diagnosis, and treatment of cancer. Development of anticancer drugs with fewer or no side effects is important for the treatment of cancer. The search for such potential anticancer drugs have led to the discovery of synthetic small molecules with anti-carcinogenic activity and limited harmful side effects particularly with respect to the immune system [5].

Thiazolidin-4-one, a saturated form of thiazole with carbonyl group on fourth carbon, has been considered as a moiety of choice as it possesses a broad spectrum of pharmacological activities against several targets. This array of biological response profile has attracted the attention of worldwide scientists to further investigate the potential of this organic motif. Thiazolidin-4-one ring system is a core structure in various synthetic compounds displaying broad spectrum of biological activities [6], such as an anticancer and antimicrobial activity [7-11]. The combination of two pharmacophores into a single molecule is an effective and commonly used direction in modern medicinal chemistry for the exploration of novel and highly active compounds [12]. The use of ultrasound to promote chemical reactions is called sono-chemistry. Ultrasonic-assisted organic synthesis (UAOS) is a green synthetic approach and it is a powerful technique towards the increase in reaction rate [13-15]. It can also be considered as important tool for conservation of energy and minimization of waste as compared to the conventional techniques [16-17]. This research work is introducing ultrasound- promoted synthesis of thiazolidin-4-one derivative from the readily available starting materials under mild and selective conditions.

Results and discussion

2.1. Chemistry

All the final compounds **6(a-j)** were synthesized by following the procedure depicted in **Scheme 1**. The starting material N-carbamothioylbenzamide (**3**) was synthesized by the reaction of thiourea (**2**) with benzyl chloride (**1**) in NaOH. The compound (**3**) obtained in good yield in step I is was refluxed with substituted aromatic aldehydes (0.01 mol) (**4**) with catalytic amount of glacial acetic acid to give N-(benzylidenecarbamothioyl) benzamide i.e. Schiff's bases **5(a-j)**. Schiff's bases, thioglycolic acid and anhydrous zinc chloride were taken in DMF and the reaction mixture was kept inside an Ultrasonicator for about 1-2 hours.



Scheme 1 Synthesis of N-(2-substituted phenyl-4-oxathiazolidine-3-carbonothioyl) benzamide

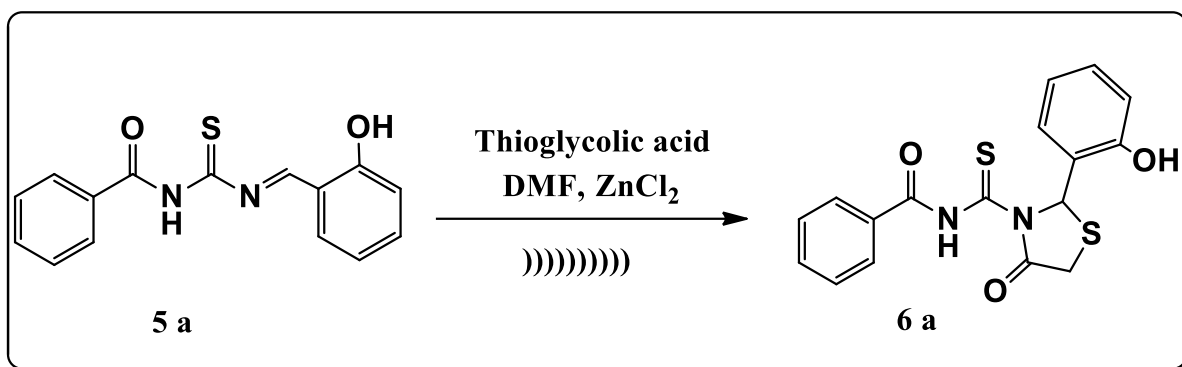
Table 1. The physical characterization data of the synthesized compounds **6(a-j)**.

Entry	R	Molecular formula	Molecular weight	Yield (%)	M.P °C
6a	2-Hydroxyphenyl	C ₁₇ H ₁₄ N ₂ O ₃ S ₂	358.43	89	156-158
6b	4- Hydroxyphenyl	C ₁₇ H ₁₄ N ₂ O ₃ S ₂	358.43	85	130-132
6c	4-Methoxyphenyl	C ₁₈ H ₁₆ N ₂ O ₃ S ₂	372.46	85	176-178
6d	4-Chlorophenyl	C ₁₇ H ₁₃ ClN ₂ O ₂ S ₂	376.88	80	110-112
6e	3,4-Dimethoxyphenyl	C ₁₉ H ₁₈ N ₂ O ₄ S ₂	402.49	75	152-154
6f	4-Hydroxy-3-methoxyphenyl	C ₁₈ H ₁₆ N ₂ O ₄ S ₂	388.46	78	124-126
6g	3-Ethoxy -4- Hydroxyphenyl	C ₁₉ H ₁₈ N ₂ O ₄ S ₂	402.49	75	178-180
6h	3,4-DichloroPhenyl	C ₁₇ H ₁₂ Cl ₂ N ₂ O ₂ S ₂	411.33	80	102-104
6i	Furan-2-yl	C ₁₅ H ₁₂ N ₂ O ₃ S ₂	332.40	82	155-157
6j	Thiophen-2-yl	C ₁₅ H ₁₂ N ₂ O ₂ S ₃	348.46	65	148-150

For the optimization of reaction conditions, the model reaction was carried out by cyclization of intermediate (E)-N-((2-hydroxybenzylidene) carbamothioyl) benzamide with thioglycolic acid and anhydrous zinc chloride in DMF to give the derivative N-(2-(2-hydroxyphenyl)-4-oxothiazolidine-3-carbonothioyl) benzamide **6a**, in various solvents, by using catalyst with conventional refluxing and ultrasound assisted modern green technique. Various solvents were tried and on the basis of yield of the product obtained dimethyl form amide (DMF) was selected

as solvent for this reaction. No product was obtained without use of catalyst; hence zinc chloride was selected as catalyst which gave desired products with better yield with solvent DMF. **Table 2.** shows optimization of reaction conditions for 4-(benzyloxy)-N-(3-chloro-2-(4-hydroxyphenyl)-4-oxoazetidin-1-yl) benzamide **6a** derivative using various solvent with and without use of catalyst.

Table 2. Optimization of reaction conditions for **6a**.



Entry	Catalyst	Solvent	Method A Conventional Reflux		Method B Ultrasound Assisted	
			Time (h)	Yield(%)	Time (h)	Yield (%)
1	No Catalyst	Benzene	22	-	6	-
2	No Catalyst	1,4-Dioxane	15	-	6	-
3	No Catalyst	DMF	12	-	5	-
4	Anhydrous zinc chloride	Benzene	16	55	4	65
5	Anhydrous zinc chloride	1,4-Dioxane	12	60	3	75
6	Anhydrous zinc chloride	DMF	08	70	1	89

The synthesis of all derivatives of N-(2-substituted phenyl-4-oxathiazolidine-3-carbonothioyl) benzamide **6(a-j)** was carried out by refluxing and ultrasonic irradiation methods for comparison of conventional and modern green chemistry tool, using ultra-sonication. The conventional method requires 7-8 hrs refluxing in benzene in special Dean Stark elaborate glass apparatus. Benzene is proven carcinogenic solvent and also harm surrounding. The conventional method also need to remove continuously benzene and water azeotrope to drive the reaction to completion. The current methodology uses benign and eco-friendly solvent DMF which is also water soluble. The reaction is eco-friendly neat and clean carried out in simple borosil beaker. The time required for completion of reaction with yield in percent is mentioned in **Table 3**.

Table 3. Comparison of reaction kinetics of conventional refluxing and ultrasonic irradiation methods for the synthesized compounds **6(a-j)**.

Entry	Conventional Refluxing		Ultrasonic Irradiation	
	Time(hrs)	Yield(%)	Time(hrs)	Yield(%)
6a	08.00	70	01.00	89
6b	06.15	65	02.00	85
6c	06.50	72	01.10	85
6d	07.10	70	01.20	80
6e	07.50	71	01.15	75
6f	08.00	65	02.00	78
6g	07.30	67	02.00	75
6h	07.30	61	01.30	80
6i	08.00	66	02.00	82
6j	08.00	65	02.00	70

2.2. *IN VITRO* ANTICANCER ACTIVITY

The *in vitro* anticancer activity of the N-(2-substituted phenyl-4-oxathiazolidine-3-carbonothioyl) benzamide **6(a-j)** derivatives were examined against two human cancer cell lines. Cell line MCF 7 for human breast cancer and HeLa cell line for human cervical cancer was incubated in culture medium with varying concentrations of the compounds and the effect

was measured by a MTT assay, using the anticancer drug ADR as positive control. The GI_{50} values with the selected cell lines and molecular docking results obtained are summarized in **Table 4**. Some of the synthesized derivatives exhibited good anticancer activity against the tested cell lines. Compound **6a**, **6c**, **6d** and **6h** are the most active among the synthesised series. Compound **6a** bearing polar donating hydroxy group at *ortho* position of phenyl ring and compound **6c** having methoxy as polar donating group at *para* position of phenyl ring exhibited excellent anti-cancer activity against MCF7 breast cancer cell line with $GI_{50} < 10 \mu M$. Compound **6d** showing chloro at *para* position of phenyl ring exhibited excellent anti-cancer activity against MCF7 breast cancer cell line and HeLa cervical cell line with $GI_{50} < 10 \mu M$ and $18 \mu M$ respectively. Compound **6h** bearing two chloro at *meta* and *para* position of phenyl ring also exhibited excellent anti-cancer activity against MCF7 breast cancer cell line with $GI_{50} < 10 \mu M$ and HeLa cervical cell line with GI_{50} and $12 \mu M$. All the remaining derivatives gives moderate anticancer activity on both the cell line.

Table 4. *In vitro* anticancer activity of synthesized compounds.

Sr. No	$GI_{50} \mu M$ MCF-7	$GI_{50} \mu M$ HeLa
6a	<10	>80
6b	>80	>80
6c	<10	>80
6d	<10	18
6e	>80	>80
6f	>80	>80
6g	>80	>80
6h	<10	12
6i	>80	>80
6j	>80	>80
ADR	<10	<10
PNU-142372	NA	NA

GI_{50} : The concentrations required to cause 50 % inhibition of cancer cell growth; MCF-7: Human breast cancer cell lines; HeLa: Human cervical cell line.

3. Materials and Methods

3.1. General Information

All the chemicals used for the synthesis were procured from Merck (Mumbai, India), Sigma (Mumbai, India), HiMedia (Mumbai, India) or Qualigens (Mumbai, India) and used without further purification. The ultrasound sonicator (Sonics Vibra-cell, Model no. VCX 500, Newtown, CT, USA) equipped with solid synthetic probe, 13 mm in tip diameter, operating at 20 kHz with a maximum power output of 500 W, was used for synthesis of final title compounds. The progress of each reaction was monitored by ascending thin layer chromatography (TLC) using pre-coated silica gel F254 Alumina TLC Plates (Merck) and the spots were visualized with UV light and iodine vapour. Elemental analyses (C, H, and N) were done with a Flash EA 112 analyser (Shimadzu, Mumbai, India) and all analyses were consistent (within 0.4%) with theoretical values. IR spectra were recorded on a PS 4000 FTIR instrument (Jasco, Tokyo, Japan) using KBr pellets. ^1H and ^{13}C -NMR (400 MHz) spectra were recorded in DMSO-*d*₆ on an Avance 400 NMR spectrometer (Bruker, Billerica, MA, USA) fitted with an Aspect 3000 computer and all the chemical shifts (δ ppm) were referred to internal TMS for ^1H and the solvent signal for ^{13}C -NMR. ^1H -NMR data are reported in the order of chemical shift, multiplicity (s, singlet; d, doublet; t, triplet; q, quartet; br, broad; br s, broad singlet; m, multiplet and/or multiple resonance), number of protons. A Micro TOF-Q-II (Bruker Daltonics, Billerica, MA, USA) with electron spray ionization (ESI) was used to obtain the HRMS data.

3.2. Experimental section:

a) Synthesis of N-carbamothioylbenzamide (3):

Thiourea (0.33mol) (**2**) was taken in 250 ml of 10% NaOH solution and dissolved in conical flask. Benzoyl chloride (**1**) (45 ml) was added in 5 portions to the solution. The vessel was stoppered and shaken vigorously after each addition until all the chloride ions was reacted. The solution was transferred to a beaker and the conical flask was rinsed with little water. A few grams of crushed ice were placed in the solution, conc. HCl was added slowly while stirring until the mixture was acidic. The resulting crystalline precipitate was collected upon a Buchner funnel, washed with cold water and drained well. The solid was placed in beaker with 100 ml of CCl₄, the beaker was covered with a water glass and boiled gently for 10 min. It was allowed to cool slightly, filtered under suction. The product was washed on filter paper with 10-20 ml of CCl₄. The dried product was recrystallized from boiling water (about 500 ml) with addition

of little decolourising charcoal if necessary, filtered through hot water funnel and allowed to recrystallize.

b) Synthesis of General procedure for Synthesis of N- (substituted benzylidenecarbamothioyl) benzamide 5(a-j):

Equimolar quantity of N-carbamothioylbenzamide (**3**) and substituted aromatic aldehydes (0.01 mol) **4(a-j)** were taken and to this mixture 0.01 mol of glacial acetic acid was added. The progress of the reaction was checked with the help of TLC. The product was filtered and washed with the freshly prepared solution of sodium meta bisulphite, to wash away any traces of unreacted aldehyde. The solid was filtered and allowed to dry.

c) General procedure for Synthesis of N-(2-substituted phenyl-4-oxathiazolidine-3-carbonothioyl) benzamide 6(a-j):

Equimolar quantity of respective Schiff's base and thioglycolic acid (0.01mol) and anhydrous Zinc Chloride (0.04 mol) were taken in DMF (20 ml). The beaker containing reaction mixture was kept inside an Ultrasonicator for about 1-2 hours during the reaction at room temperature. After completion of the reaction (monitored by TLC), the mixture was poured into ice-cold water. Then this mixture was washed with sodium bicarbonate to wash away unreacted thioglycolic acid and allowed to settle for 10 min. Then ethyl acetate was poured in the mixture and it was separated using a separating funnel. Ethyl acetate layer is poured in a beaker and sodium nitrite is added to it. The sodium nitrite absorbs the water. Now the ethyl acetate containing the product is taken in petri plate and allowed to evaporate.

Structures of the synthesized derivatives **6 a-j** were confirmed by spectral studies as reported below:

N-(2-(2-hydroxyphenyl)-4-oxothiazolidine-3-carbonothioyl) benzamide **6a**:

IR (KBr) ν_{\max} (cm⁻¹): 3457.24 OH stretching, 3440 NH stretching, 3118-3019 aromatic CH stretching, 1635.56 C=O stretching of amide, 1250 C=S stretching; ¹H-NMR(DMSO-*d*₆), δ ppm: 3.91,3.82(dd,2H, -CH₂-), 5.38(s,1H, -OH), 5.90 (s,1H of thiazolidinone), 8.01 (s,1H of NH), 6.81 to 8.05 (m,5H,4H of two aromatic rings); ¹³C-NMR(DMSO-*d*₆), δ ppm: 33.4, 57.3, 115.6, 118.2, 121.4, 127.3(2), 128.0, 128.3, 128.6(2), 132.0, 132.3, 153.4, 165.2, 171.4 and 179.1; MS (ESI) m/z: 362.05 [M+4]⁺; Molecular Formula: C₁₇H₁₄N₂O₃S₂; Elemental Analysis: Calculated (C, H, N, S) 56.96, 3.94, 7.82, 17.89.Found: 56.92, 3.90, 7.75, 17.78.

N-(2-(4-hydroxyphenyl)-4-oxothiazolidine-3-carbonothioyl) benzamide **6b**:

IR (KBr) ν_{\max} (cm⁻¹): 3487.24 OH stretching, 3410 NH stretching, 3104-3005 aromatic CH stretching, 1633.56 C=O stretching of amide, 1252 C=S stretching; ¹H-NMR(DMSO-*d*₆), δ ppm: 3.93, 3.86(dd,2H, -CH₂-), 5.34(s,1H, -OH), 5.93(s,1H of thiazolidinone), 8.1 (s,1H of

NH), 6.62-8.85 (m,5H,4H of two aromatic rings); $^{13}\text{C-NMR(DMSO-}d_6\text{)}$, δ ppm: 33.5, 63.2, 115.6(2), 127.4(2), 128.6(2), 130.2, (2), 131.7, 132.2, 133.4, 156.7, 165.2, 171.0 and 179.0; MS (ESI) m/z: 362.05 $[\text{M}+4]^+$; Molecular Formula: $\text{C}_{17}\text{H}_{14}\text{N}_2\text{O}_3\text{S}_2$; Elemental Analysis: Calculated (C, H, N, S) 56.96, 3.94, 7.82, 17.89. Found: 56.35, 2.85, 8.29, 17.96.

N-(2-(4-methoxyphenyl)-4-oxothiazolidine-3-carbonothioyl) benzamide **6c**:

IR (KBr) ν_{max} (cm^{-1}): 3430 NH stretching, 3108-3009 aromatic CH stretching, 1630.56 C=O stretching of amide, 1254 C=S stretching; $^1\text{H-NMR(DMSO-}d_6\text{)}$, δ ppm: 3.75(s,3H, $-\text{OCH}_3$), 3.82, 3.92(dd,2H, $-\text{CH}_2-$), 5.73(s,1H of thiazolidinone), 6.82-8.15(m,5H,4H of two aromatic rings),8.26 (s,1H, $-\text{NH}$); $^{13}\text{C-NMR(DMSO-}d_6\text{)}$, δ ppm: 32.75, 59.29, 69.75, 114.75(2), 127.31, 127.69, 128.21, 128.63, 129.30(2), 130.29, 132.75, 134.30, 159.29, 162.75, 172.75 and 179.30; MS (ESI) m/z: 376.14 $[\text{M}+4]^+$; Molecular Formula: $\text{C}_{18}\text{H}_{16}\text{N}_2\text{O}_3\text{S}_2$; Elemental Analysis: Calculated (C, H, N, S) 58.04, 4.33, 7.52, 17.22. Found: 57.98, 4.21, 7.60, 17.32.

N-(2-(4-chlorophenyl)-4-oxothiazolidine-3-carbonothioyl) benzamide **6d**:

IR (KBr) ν_{max} (cm^{-1}): 3415 NH stretching, 3112-3013 aromatic CH stretching, 1620.56 C=O stretching of amide, 1255 C=S stretching, 760 C-Cl stretching; $^1\text{H-NMR(DMSO-}d_6\text{)}$, δ ppm: 3.75, 3.92(dd,2H, $-\text{CH}_2-$), 5.78(s, 1H of thiazolidinone), 7.57-8.12(m, 5H, 4H of two aromatic rings) 8.24(s,1H, $-\text{NH}$); $^{13}\text{C-NMR(DMSO-}d_6\text{)}$, δ ppm: 34.75, 62.75, 127.69(2), 128.63(2), 129.63(2), 130.29(2), 132.29, 132.75, 134.30, 138.63, 162.75, 172.75 and 179.30; MS (ESI) m/z: 382.88 $[\text{M}+6]^+$; Molecular Formula: $\text{C}_{17}\text{H}_{13}\text{ClN}_2\text{O}_2\text{S}_2$; Elemental Analysis: Calculated (C, H, Cl, N, S) 54.18, 3.48, 9.41, 7.43, 17.02. Found: 54.11, 3.35, 9.49, 7.32, 17.89.

N-(2-(3,4-dimethoxyphenyl)-4-oxothiazolidine-3-carbonothioyl) benzamide **6e**:

IR (KBr) ν_{max} (cm^{-1}): 3416 NH stretching, 3118-3019 aromatic CH stretching, 1640.56 C=O stretching of amide, 1240 C=S stretching; $^1\text{H-NMR(DMSO-}d_6\text{)}$, δ ppm: 3.79(s,6H, $-\text{OCH}_3$), 3.91,3.80(dd,2H, $-\text{CH}_2-$), 5.91(s, 1H of thiazolidinone), 6.69-8.0(m,5H,3H of two aromatic rings), 8.1 (s,1H, $-\text{NH}$); $^{13}\text{C-NMR(DMSO-}d_6\text{)}$, δ ppm: 33.3, 56.3(2), 63.5, 112.1, 113.9, 122.0, 127.3(2), 128.7(2), 132.3, (2), 132.7, 148.4, 149.5, 165.2, 171.3 and 179.1; MS (ESI) m/z: 406.07 $[\text{M}+4]^+$; Molecular Formula: $\text{C}_{19}\text{H}_{18}\text{N}_2\text{O}_4\text{S}_2$; Elemental Analysis: Calculated (C, H, N, S) 56.70, 4.51, 6.96, 15.93. Found: 56.76, 4.49, 6.85, 15.99.

N-(2-(4-hydroxy-3-methoxyphenyl)-4-oxothiazolidine-3-carbonothioyl) benzamide **6f**:

IR (KBr) ν_{max} (cm^{-1}): 3433 NH stretching, 3178-3089 aromatic CH stretching, 1612.56 C=O stretching of amide, 1251 C=S stretching; $^1\text{H-NMR(DMSO-}d_6\text{)}$, δ ppm: 3.79(s,3H $-\text{CH}_3$), 3.91, 3.80(dd,2H, $-\text{CH}_2-$), 5.31(s,1H, -OH), 5.90(s,1H of thiazolidinone), 8.01(s,1H, $-\text{NH}$), 6.69-8.01(m,5H,3H of two aromatic rings); $^{13}\text{C-NMR(DMSO-}d_6\text{)}$, δ ppm: 33.5, 56.2, 63.5, 114.4, 115.6, 122.3, 127.3(2), 128.9(2), 132.3, 132.9, 133.4, 147.3(2), 165.6, 171.4 and 179.0; MS

(ESI) m/z: 392.06 [M+4]⁺; Molecular Formula: C₁₈H₁₆N₂O₄S₂; Elemental Analysis: Calculated (C, H, N, S): 55.65, 4.15, 7.21, 16.51. Found: 55.61, 4.21, 7.33, 16.45.

N-(2-(3-ethoxy-4-hydroxyphenyl)-4-oxothiazolidine-3-carbonothioyl) benzamide **6g**:

IR (KBr) ν_{\max} (cm⁻¹): 3415 NH stretching, 3104-3005 aromatic CH stretching, 1614 C=O stretching of amide, 1252 C=S stretching; ¹H-NMR(DMSO-*d*₆), δ ppm: 1.28(s,3H, -CH₃), 3.89-3.81(dd,2H,-CH₂-), 4.01(s,2H, -OCH₂-), 5.30(s,1H, -OH), 5.99(s, 1H of thiazolidinone), 6.71-8.0 (m,5H,3H of two aromatic rings),8.01(s,1H -NH);¹³C-NMR(DMSO-*d*₆), δ ppm: 14.5, 33.2, 63.5, 64.7, 114.2, 115.2, 121.5, 127.7(2), 128.5(2), 132.3(3), 147.3, 148.3, 165.2, 171.4 and 179.0; MS (ESI) m/z: 406.07 [M+4]⁺; Molecular Formula: C₁₉H₁₈N₂O₄S₂; Elemental Analysis: Calculated (C, H, N, S) 56.70, 4.51, 6.96, 15.93. Found: 56.61, 4.56,6.83, 15.95.

N-(2-(3,4-dichlorophenyl)-4-oxothiazolidine-3-carbonothioyl) benzamide **6h**:

IR (KBr) ν_{\max} (cm⁻¹): 3445 NH stretching, 3118-3000 aromatic CH stretching, 1612 C=O stretching of amide, 1256 C=S stretching, 740 C-Cl stretching; ¹H-NMR(DMSO-*d*₆), δ ppm: 3.99,3.91(dd,2H,-CH₂-), 5.87(s,1H of thiazolidinone), 8.1(s,1H, -NH), 7.01-8.07(m,5H,3H of two aromatic rings);¹³C-NMR(DMSO-*d*₆), δ ppm: 33.4, 62.7, 127.3(2), 128.4, 128.9(2), 129.7, 130.3, 131.8(3), 132.2(2), 165.3, 171.1 and 179.0; MS (ESI) m/z: 419.97 [M+8]⁺; Molecular Formula: C₁₇H₁₂Cl₂N₂O₂S₂; Elemental Analysis: Calculated (C,H,Cl,N,S) 49.64,2.94,17.24,6.81, 15.59. Found: 49.56,2.97,17.31,6.87, 15.65.

N-(2-(furan-2-yl)-4-oxothiazolidine-3-carbonothioyl) benzamide **6i**:

IR (KBr) ν_{\max} (cm⁻¹): 3435 NH stretching, 3100-3000 aromatic CH stretching, 1630 C=O stretching of amide, 1258 C=S stretching; ¹H-NMR(DMSO-*d*₆), δ ppm: 3.89,3.78(dd,2H, -CH₂-), 6.09(s,1H of thiazolidinone), 8.03(s,1H, -NH), 6.17-8.11(m,5H,3H of two aromatic rings);¹³C-NMR(DMSO-*d*₆), δ ppm: 31.3, 63.3, 107.1, 110.4, 127.7(2), 128.6(2), 132.2, 133.5, 142.5, 151.7, 165.2, 171.3 and 179.0; MS (ESI) m/z: 336.03 [M+4]⁺; Molecular Formula: C₁₅H₁₂N₂O₃S₂; Elemental Analysis: Calculated (C, H, N, S) 54.20, 3.64, 8.43, 19.29 Found: 54.11,3.53, 8.51, 19.13.

N-(4-oxo-2-(thiophen-2-yl) thiazolidine-3-carbonothioyl) benzamide **6j**:

IR (KBr) ν_{\max} (cm⁻¹): 3438 NH stretching, 3112-3014 aromatic CH stretching, 1615 C=O stretching of amide, 1260 C=S stretching; ¹H-NMR(DMSO-*d*₆), δ ppm: 3.85,3.79(dd,2H, -CH₂-), 5.96(s,1H of thiazolidinone), 8.01(s,1H, -NH), 6.78-8.09(m,5H, 3H of two aromatic rings); ¹³C-NMR(DMSO-*d*₆), δ ppm: 33.4, 62.3, 125.3, 126.5, 127.1, 127.7(2), 128.9(2), 132.2, 133.4, 139.5, 165.3, 171.4 and 179.2; MS (ESI) m/z: 352.01 [M+4]⁺; Molecular

Formula: C₁₅H₁₂N₂O₂S₃; Elemental Analysis: Calculated (C, H, N, S) 51.70, 3.47, 8.04, 27.61
Found: 51.67, 3.54, 8.13, 27.77.

3.3. BIOLOGICAL EVALUATION

IN VITRO ANTICANCER SCREENING

Based on the literature survey and prediction of biological activity software Way2drugs (www.pharmaexpert.ru/passonline/) the compounds were predicted to show anticancer activity therefore the synthesized derivatives were evaluated for anticancer activity.

The target compounds **6(a-j)** were evaluated for *in vitro* anticancer activity against two cancer cell line viz. human breast cancer cell line (MCF-7), and human cervical cancer cell line (HeLa). The GI₅₀ values (concentration required to Growth inhibition of 50 %) for the synthesized compounds were determined by MTT assays method, using Adriamycin as standard drug. Selected cancer cells were cultured in a 96-well plate (Nalge Nunc International, Denmark) at a density of 2×10^4 cells per well, incubated for 24 hrs. The cells were then treated with varying concentrations of compounds and ADR. After 24 hrs, the cells were washed and treated with 0.01 mL MTT per well. Plates were incubated at 37 °C in a 5% CO₂ atmosphere for 4 hrs, and 0.1 mL of the extraction buffer (10% sodium dodecyl sulfate in 0.01% HCl) was added. After an overnight incubation at 37 °C, the absorbance was measured at 595 nm using an ELISA reader (Bio-Rad) and was compared with the control cultures without compound. To determine cell viability, Percent viability was calculated as

$$[(\text{absorbance of drug-treated sample}) / (\text{control absorbance})] \times 100.$$

4. Conclusion:

Ten novel N-(2-substituted phenyl-4-oxathiazolidine-3-carbonothioyl) benzamide **6(a-j)** derivatives were successfully synthesized using ultrasound irradiation giving excellent yield of 70%–89% in shorter reaction times of 1–2 hrs in contrast to conventional reactions which require 7-8 hrs refluxing in Dean Stark elaborate glass apparatus.

The remarkable benefits of ultra-sonication as a green synthetic strategy are as follows: (1) reactions were carried at room temperature (2) required much less time for completion of reaction as compared to conventional refluxing hence the ultrasound methodology saves time and electricity (3) highly accelerated reaction rate (4) the use of very less amount of benign solvent DMF (5) and shortened and clean work-up procedure (6) eco-friendly as the reactions are carried out in closed acoustic chamber. The synthesized compounds were evaluated for *in*

in vitro anticancer activity on MCF-7 human breast cancer cell line and HeLa human cervical cancer cell line.

Some of the synthesized compounds exhibited excellent activity against MCF7 and HeLa cancer cell lines. Compound **6d** showed excellent *in vitro* anticancer activity against a breast (MCF7) and cervical HeLa cancer cell line with concentrations of <10 μM and 18 μM . Compound **6h** showed excellent *in vitro* anticancer activity against breast (MCF7) and cervical HeLa cancer cell line with concentrations of <10 μM and 12 μM respectively. In conclusion, compounds **6d** and **6h** shows growth inhibition concentration 50% (GI_{50}) anticancer activity even at a very low concentration <10 μM . As compared to the standard drug ADR, these derivatives were found to be the competent moieties with potent anticancer activity. Thus, on the basis of *in vitro* anticancer activity data it can be concluded that the N-(2-substituted phenyl-4-oxathiazolidine-3-carbonothioyl) benzamide possesses very good potential for development as novel cytotoxic agents and can prove a benchmark for the development of potential anticancer agents.

Acknowledgements:

The authors are thankful to the Mrs. Fatima Rafiq Zakaria Chairman Maulana Azad Educational Trust, Principal Maulana Azad Postgraduate & Research Centre, Aurangabad and Dr. Zahid Zaheer, Principal, Y.B. Chavan College of Pharmacy, Dr. Rafiq Zakaria Campus, Aurangabad 431 001 (M.S.), India for providing the laboratory facility. UDN is very much thankful to Dr. Babasaheb Ambedkar Research and Training Institute (BARTI), Pune for Financial Support.

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