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

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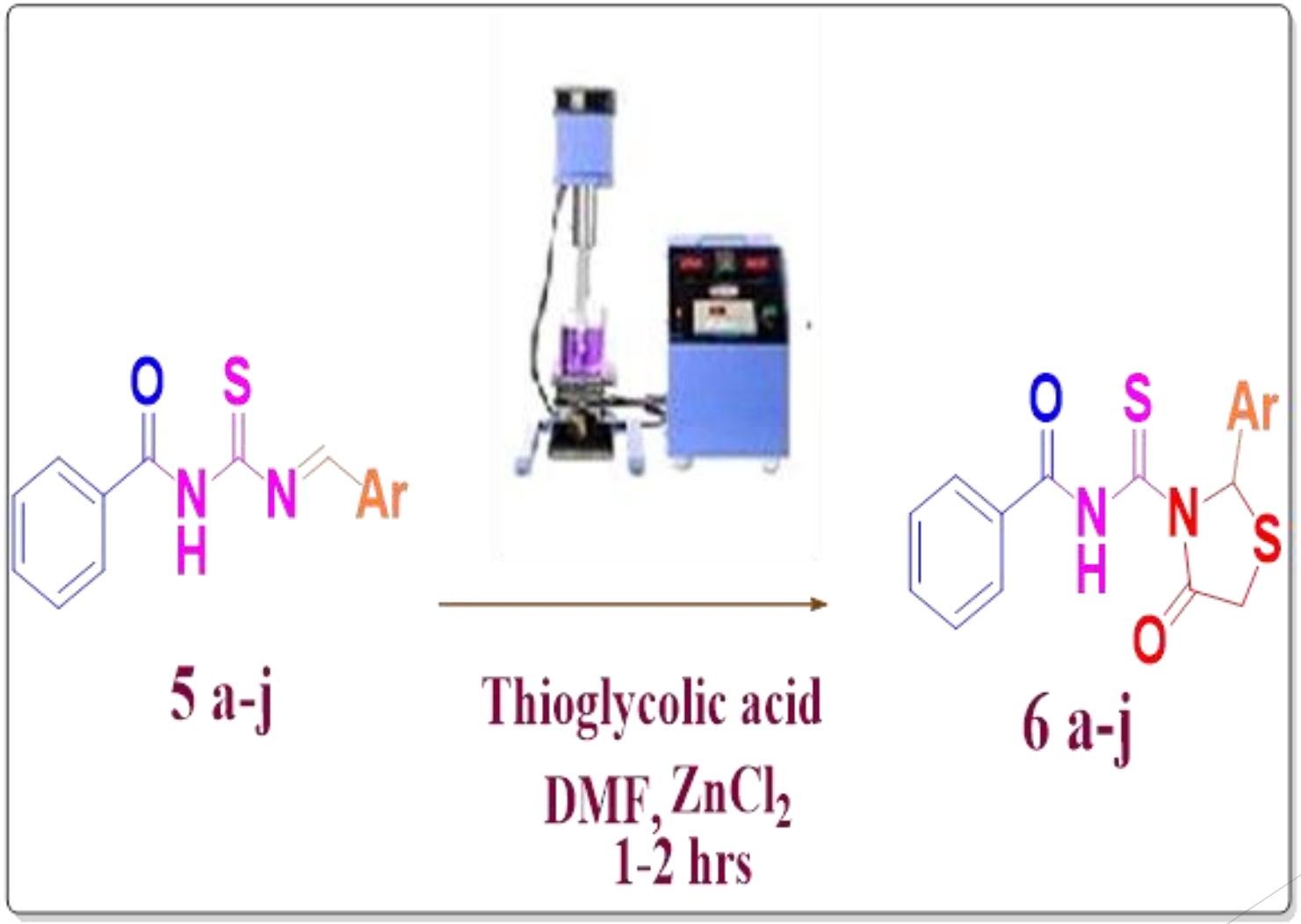
Graphical Abstract:



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

- **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 $ZnCl_2$ 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, ^{1}H NMR, ^{13}CNMR and Mass spectral studies.

Contents:

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 thiazolinedin-4-one derivative from the readily available starting materials under mild and selective conditions.

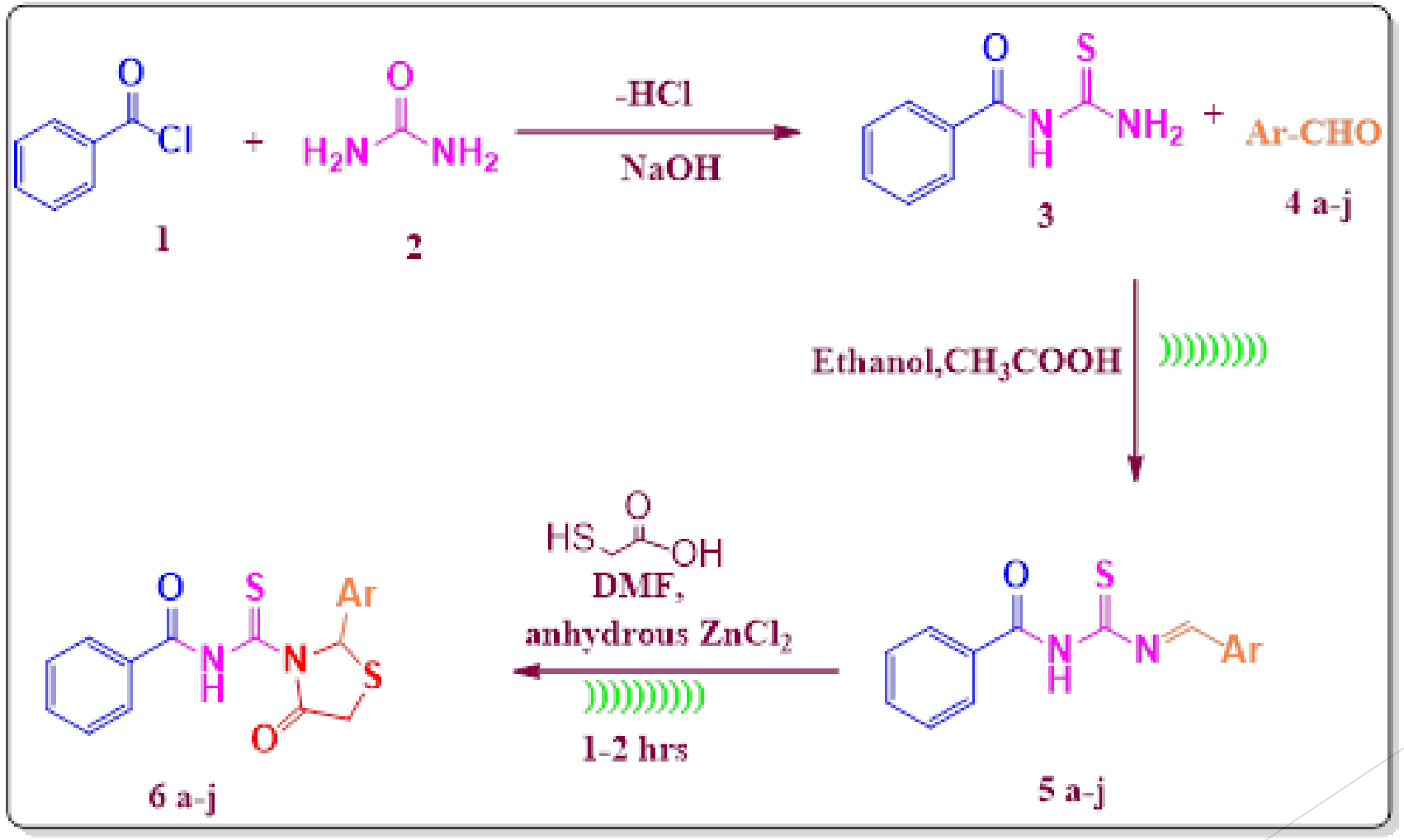
NEED OF STUDY

- Cancer is one of the leading global health burden and most serious clinical problems in the world with increasing incidences every year. According to data from the World Health Organization (WHO), more than 13 % of all deaths worldwide are directly caused by cancer every year, making cancer one of the most public-threatening diseases [9, 10]. As per the annual report of the U.S. NIH on the status of cancer from 1975–2014, though cancer death rates continue to decrease in the United States, even then, progress in reducing death rates and improving survival is limited for several cancer types, underscoring the need for intensified efforts to discover new strategies for its prevention, early detection and treatment and to apply proven preventive measures broadly and equitably. This necessitates continued cancer drug discovery efforts [11].
- Chemotherapy is still the main treatment for cancer and existing chemotherapeutic agents are accompanied by various detrimental side effects. This clearly motivates the crucial need to design novel chemotherapeutic agents with more compelling antitumor activities and reduced side effects. According to the statistics of the National Cancer Institute, it can be observed that cancer disease is jumping into the first place of the death reasons in the modern world.

OBJECTIVE OF STUDY

- To design and synthesize novel heterocyclic compounds containing benzamide coupled with thiazolidin-4-one ring.
- To use green chemistry tool for reaction i.e. ultrasound promoted synthesis.
- To confirm structures of the synthesized intermediates and final derivatives by chemical and spectral studies such as IR, Mass, ^{1}HNMR, ^{13}CNMR and elemental analysis.
- To study *in vitro* anticancer activity of the synthesized compounds against selected cancer cell lines such as MCF7 human breast cancer cell line and HeLa human cervical cancer cell line.

SCHEME OF SYNTHESIS



1 + 2 → 3 + 4 a-j

3 → 5 a-j (Ethanol, CH_3COOH,))))))))

5 a-j → 6 a-j (HS–CH(=O)–OH, DMF, anhydrous $ZnCl_2$,))))))))), 1-2 hrs

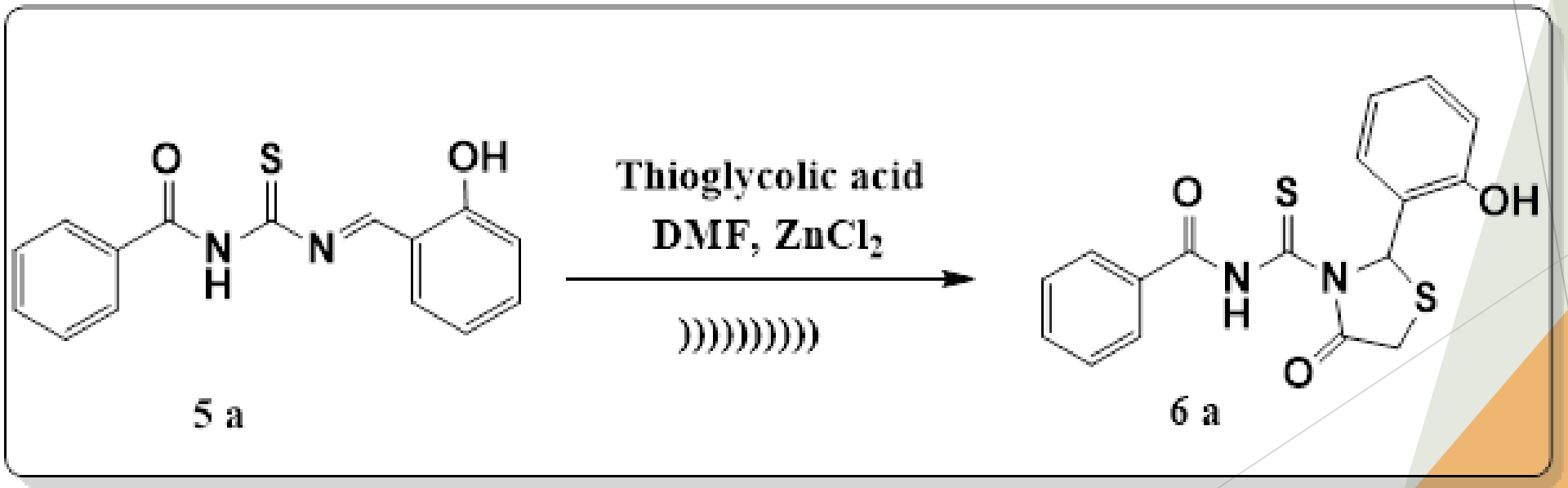
Results and discussion

- **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.

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

Entry	R	Molecular formula	Molecular weight	Yield (%)	M.P °C
6a	2-Hydroxyphenyl	$C_{17}H_{14}N_2O_3S_2$	358.43	89	156-158
6b	4- Hydroxyphenyl	$C_{17}H_{14}N_2O_3S_2$	358.43	85	130-132
6c	4-Methoxyphenyl	$C_{18}H_{16}N_2O_3S_2$	372.46	85	176-178
6d	4-Chlorophenyl	$C_{17}H_{13}ClN_2O_2S_2$	376.88	80	110-112
6e	3,4-Dimethoxyphenyl	$C_{19}H_{18}N_2O_4S_2$	402.49	75	152-154
6f	4-Hydroxy-3-methoxyphenyl	$C_{18}H_{16}N_2O_4S_2$	388.46	78	124-126
6g	3-Ethoxy -4- Hydroxyphenyl	$C_{19}H_{18}N_2O_4S_2$	402.49	75	178-180
6h	3,4-DichloroPhenyl	$C_{17}H_{12}Cl_2N_2O_2S_2$	411.33	80	102-104
6i	Furan-2-yl	$C_{15}H_{12}N_2O_3S_2$	332.40	82	155-157
6j	Thiophen-2-yl	$C_{15}H_{12}N_2O_2S_3$	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.



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 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

- ***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}<10µM.
- 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}<10µM and 18 µM 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}<10µM and HeLa cervical cell line with GI_{50} and 12µM. 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} µM MCF-7	GI_{50} µM 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.

MATERIALS AND METHODS:

- 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 Flashea 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. ^{1}H and ^{13}C-NMR (400 MHz) spectra were recorded in DMSO-*d6* 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 ^{1}H and the solvent signal for ^{13}C-NMR. ^{1}H-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.

EXPERIMENTAL WORK

- **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 CCl_4, 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 CCl_4. 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.

Spectral Characterization:

- *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; ^{1}H-NMR(DMSO-*d6*), δ ppm: 3.91,3.82(dd,2H, $-CH_2-$), 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); ^{13}C-NMR(DMSO-*d6*),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: $C_{17}H_{14}N_2O_3S_2$; 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; ^{1}H-NMR(DMSO-*d6*), δ ppm: 3.93, 3.86(dd,2H, $-CH_2-$), 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}C-NMR(DMSO-*d6*),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 $[M+4]^+$; Molecular Formula: $C_{17}H_{14}N_2O_3S_2$; 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}H-NMR(DMSO-*d6*), δ ppm: 3.75(s,3H, $-OCH_3$), 3.82, 3.92(dd,2H,$-CH_2-$), 5.73(s,1H of thiazolidinone), 6.82-8.15(m,5H,4H of two aromatic rings),8.26 (s,1H,–NH);^{13}C-NMR(DMSO-*d6*),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 $[M+4]^+$; Molecular Formula: $C_{18}H_{16}N_2O_3S_2$; 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}H-NMR(DMSO-*d6*), δ ppm: 3.75, 3.92(dd,2H, $–CH_2–$), 5.78(s, 1H of thiazolidinone), 7.57-8.12(m, 5H, 4H of two aromatic rings) 8.24(s,1H, –NH);^{13}C-NMR(DMSO-*d6*),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 $[M+6]^+$; Molecular Formula: $C_{17}H_{13}ClN_2O_2S_2$; 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}H-NMR(DMSO-*d6*), δ ppm: 3.79(s,6H, $–OCH_3$), 3.91,3.80(dd,2H, $–CH_2–$), 5.91(s, 1H of thaizolidinone), 6.69-8.0(m,5H,3H of two aromatic rings), 8.1 (s,1H, –NH);^{13}C-NMR(DMSO-*d6*),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 $[M+4]^+$; Molecular Formula: $C_{19}H_{18}N_2O_4S_2$; 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}H-NMR(DMSO-*d6*), δ ppm: 3.79(s,3H $–CH_3$), 3.91, 3.80(dd,2H, $–CH_2–$), 5.31(s,1H, -OH), 5.90(s,1H of thiazolidinone), 8.01(s,1H, –NH), 6.69-8.01(m,5H,3H of two aromatic rings);^{13}C-NMR (DMSO-*d6*),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: $C_{18}H_{16}N_2O_4S_2$; 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; ^{1}H-NMR(DMSO-*d6*), δ ppm: 1.28(s,3H, $–CH_3$), 3.89-3.81(dd,2H,$–CH_2–$), 4.01(s,2H, $–OCH_2–$), 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);^{13}C-NMR(DMSO-*d6*),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: $C_{19}H_{18}N_2O_4S_2$; 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; ^{1}H-NMR(DMSO-*d6*), δ ppm: 3.99,3.91(dd,2H,–CH_2–), 5.87(s,1H of thiazolidinone), 8.1(s,1H, –NH), 7.01-8.07(m,5H,3H of two aromatic rings);^{13}C-NMR(DMSO-*d6*),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: $C_{17}H_{12}Cl_2N_2O_2S_2$; 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; ^{1}H-NMR(DMSO-*d6*), δ ppm: 3.89,3.78(dd,2H, –CH_2–), 6.09(s,1H of thiazolidinone), 8.03(s,1H, –NH), 6.17-8.11(m,5H,3H of two aromatic rings);^{13}C-NMR(DMSO-*d6*),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: $C_{15}H_{12}N_2O_3S_2$; 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; ^{1}H-NMR(DMSO-*d6*), δ ppm: 3.85,3.79(dd,2H, –CH_2–), 5.96(s,1H of thiazolidinone), 8.01(s,1H, –NH), 6.78-8.09(m,5H, 3H of two aromatic rings); ^{13}C-NMR(DMSO-*d6*),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: $C_{15}H_{12}N_2O_2S_3$; Elemental Analysis: Calculated (C, H, N, S) 51.70, 3.47, 8.04, 27.61 Found: 51.67, 3.54, 8.13, 27.77.

- **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 GI_{50} 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% CO_2 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 [(absorbance of drug-treated) sample / (control absorbance)] × 100.

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 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.

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