Synthesis and Invitro Anti-Cancer Evaluation of Some Novel 2, 3 Disubstituted Thiazolidinones

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Abstract

Thiazolidinone and its derivatives have high pharmacological relevance since they are available in both natural products and pharmaceutical compounds. The main synthetic routes to thiazolidinones comprising three components such as an amine, a carbonyl group and mercapto acid. The classical method of synthesis reported may be either a one-pot three-component condensation method or a two-step process. Synthesis and anticancer activity evaluation of thiazolidinones containing benzothiazole moiety. These compounds were screened for in-vitro anticancer activity. The activity data exhibits that all compounds were found to show potent anticancer activity. Various substituents at C-2 and C-3 of thiazolidinone results in potent anticancer activity. Prompted by these reports, we aimed to prepare the following series of 2, 3-disubstituted-Thiazolidinone derivatives as potent anticancer agents.

Key words: Thiazolidiones, amines, carbonyl group, mercapto acid, one pot three component condensation, anticancer, benzothiazole moiety, C-2 and C-3, 2,3 –disubstituted

1. INTRODUCTION

Thiazolidinones possess a wide spectrum of biological and pharmacological activity due to the presence of nitrogen and sulfur which is considered to be responsible for the structural features to impart their activities. Despite the optimal use of available anticancer drugs (ACDs), many patients fail to experience therapeutic efficacy and others do so only at the expense of significant toxic side effects. The limitations with the conventional ACDs highlighted the need for developing newer anti-cancer agents with new, less toxic and more effective drugs are required. Thiazolidinones are five membered ring system containing sulphur and nitrogen atom, received a much attention of medicinal chemists due to their potential biological activities. Various substituents’ at C-2 and C-3 of thiazolidinone results in potent anticancer activity. Prompted by these reports, we aimed to prepare the following series of 2, 3-disubstituted- Thiazolidinone derivatives as potent anticancer agents.

Hence the specific aims and objectives of the present study are,

- To synthesize a series of novel 2, 3-disubstituted thiazolidinones.
- To characterize the synthesized compounds by IR, NMR, Mass spectra and elemental analysis.
- To evaluate the test compounds for anti-cancer activity by using human cervical cancer cell line (HeLa) by MTT assay method.

The title compounds are planned to synthesize by using the following synthetic routes mentioned in the following Schemes.

Scheme

Synthesis of 2-(3- (4- (4-aminophenylsulfonyl) phenyl)-2- (2-phenylsubstituted)-4 oxothiazolidin-5-yl) acetic acid (TD1-7).

Materials and methods

Melting points (mp) were taken in open capillaries on Thomas Hoover melting point apparatus and are uncorrected. The IR spectra were recorded in film or in potassium bromide disks on a Perkin-Elmer 398 spectrometer. The 1H spectra were recorded on a DPX-500 MHz Bruker FT-NMR spectrometer. The chemical shifts were reported as parts per million (δ ppm) tetramethylsilane (TMS) as an internal standard. Mass spectra were obtained on a JEOL-SX-102 instrument using fast atom bombardment (FAB positive). Elemental analysis was performed on a Perkin-Elmer 2400 C, H, N analyzer and values were within the acceptable limits of the calculated values. The progress of the reaction was monitored on readymade silica gel plates (Merek) using chloroform-methanol (9:1) as a solvent system. Iodine was used as a developing agent. Spectral data (IR, NMR and mass spectra) confirmed the structures of the synthesized compounds.
compounds and the purity of these compounds was ascertained by micro analysis. Elemental (C,H,N) analysis indicated that the calculated and observed values were within the acceptable limits (± 0.4%). All chemicals and reagents were obtained from Aldrich (USA), Lancaster (UK) or Spectrochem Pvt.Ltd (India) and were used without further purification.

**General procedure for synthesis of 2-(5-amino-1,3,4-thiadiazol-2-yl)phenol (TD1-7)**

4-(aminophenylsulfonyl) benzenamine (2.48gm) (0.01mol) and substituted benzaldehydes (1.47gm) (0.01mol) were dissolved in alcohol (30ml) in a 250ml round bottom flask. To this concentrated sulphuric acid (0.5ml) and dry dioxane (12ml) was added with constant stirring. To this mixture, 2-mercapto succinic acid (1.5 gm) (0.01mol) in 12ml of dry dioxane was added slowly and refluxed for 3 hr at 80°C with occasional shaking. The reaction completion was monitored by thin layer chromatography. The solid mass separated was poured in to ice cold water and filtered. The solid was neutralized with one percent sodium carbonate solution, filtered and dried. The residue was recrystallized from methanol.

1. **Synthesis of 3-(4-(4-aminophenylsulfonyl) phenyl)-2-(2-nitrophenyl)-4- oxothiazolidin-5-yl) acetic acid (TD1)**

2. **Synthesis of 3-(4-(4-aminophenylsulfonyl)phenyl)-2-(4-dimethylamino)phenyl)-4-oxothiazolidin-5-yl)acetic acid (TD 2).**

3. **Synthesis of 3-(4-(4-aminophenylsulfonyl)phenyl)-2-(4-methoxyphenyl)-4-oxothiazolidin-5-yl) acetic acid (TD 3).**
4. Synthesis of (3-(4-(4-aminophenylsulfonyl)phenyl)-2-(4-hydroxyphenyl)-4-oxothiazolidin-5-yl)acetic acid (TD 4).

Yield : 2.68 g; 79.0 %
Melting Point : 227-229 °C
RF Value : 0.78 (benzene: ethyl acetate, 8:2)
Molecular Formulas : C₂₉H₂₈N₂O₈S₂
Molecular Weight : 498.5
IR (KBr) cm⁻¹ : 3316 (OH), 3290 (NH₂), 3045 (Ar-CH), 1622 (C=O, S=O), 765 (C-S-C), 2916 (OCH₃).
¹H NMR (CDCl₃) δ ppm : 3.73(s, 3H, CH₃), 3.80 (d, 1H, CH₃), 4.01 (s, 2H, NH₂), 6.63 (d, J = 8.0 Hz, 2H, Ar-H), 7.27 (d, J = 7.5Hz, 2H, Ar-H), 7.65 (d, J = 7.0 Hz, 2H, Ar-H), 7.95 (d, J = 7.0 Hz, 2H, Ar-H).

Elemental Analysis
Calculated : C, 57.82; H, 4.45; N, 5.62
Found : C, 57.86; H, 4.45; N, 5.61

5. Synthesis of (3-(4-(4-aminophenylsulfonyl)phenyl)-4-oxo-2-styrylthiazolidin-5-yl)acetic acid (TD 5).

Yield : 2.47 g; 81.0 %
Melting Point : 197-199 °C
RF Value : 0.76 (benzene: ethyl acetate, 8:2)
Molecular Formulas : C₃₀H₂₅N₂O₇S
Molecular Weight : 485.5
IR (KBr) cm⁻¹ : 3310 (OH, broad), 3287 (NH₂), 3045 (Ar-CH), 1619 (C=O, S=O), 765 (C-S-C).
¹H NMR (CDCl₃) δ ppm : 2.82-3.07 (d, 2H, CH₃), 4.01 (s, 2H, NH₂), 6.63 (d, J = 8.0 Hz, 2H, Ar-H), 7.65 (d, J = 7.5 Hz, 2H, Ar-H), 7.95 (d, J = 7.0 Hz, 2H, Ar-H).

Elemental Analysis
Calculated : C, 57.82; H, 4.45; N, 5.62
Found : C, 57.86; H, 4.45; N, 5.61

6. Synthesis of (3-(4-(4-aminophenylsulfonyl)phenyl)-2-(4-aminophenyl)-4-oxothiazolidin-5-yl)acetic acid (TD 6).

Yield : 2.12 g; 64.6 %
Melting Point : 212-214 °C
RF Value : 0.65 (benzene: ethyl acetate, 8:2)
Molecular Formulas : C₂₉H₂₆N₃O₇S
Molecular Weight : 603.5
IR (KBr) cm⁻¹ : 3302 (OH), 3296 (NH₂), 3038 (Ar-CH), 1613 (C=O, S=O), 764 (C-S-C).
¹H NMR (CDCl₃) δ ppm : 2.82-3.07 (d, 2H, CH₃), 6.63 (d, J = 8.0 Hz, 2H, Ar-H), 7.27 (d, J = 7.5 Hz, 2H, Ar-H), 7.65 (d, J = 7.0 Hz, 2H, Ar-H), 7.95 (d, J = 7.0 Hz, 2H, Ar-H).

Elemental Analysis
Calculated : C, 57.81; H, 4.68; N, 5.66
Found : C, 57.89; H, 4.67; N, 5.65

7. Synthesis of (3-(4-(4-aminophenylsulfonyl)phenyl)-2-(4-chlorophenyl)-4-oxothiazolidin-5-yl)acetic acid (TD 7).

Yield : 2.32 g; 76.0 %
Melting Point : 187-189 °C
RF Value : 0.72 (benzene: ethyl acetate, 8:2)
Molecular Formulas : C₂₉H₂₅N₂O₇S
Molecular Weight : 485.5
IR (KBr) cm⁻¹ : 3310 (OH, broad), 3287 (NH₂), 3045 (Ar-CH), 1619 (C=O, S=O), 765 (C-S-C).
¹H NMR (CDCl₃) δ ppm : 2.82-3.07 (d, 2H, CH₃), 6.63 (d, J = 8.0 Hz, 2H, Ar-H), 7.65 (d, J = 7.5 Hz, 2H, Ar-H), 7.95 (d, J = 7.0 Hz, 2H, Ar-H), 10.86 (s, 1H, OH).

Elemental Analysis
Calculated : C, 57.13; H, 4.38; N, 8.69
Found : C, 57.11; H, 4.37; N, 8.68

**Chromatography Studies Of Synthesized Compounds**

**Thin Layer Chromatography**

Thin Layer Chromatography or TLC is a solid-liquid form of chromatography where the stationary phase is a polar absorbent and the mobile phase can be a single solvent or combination of solvents. TLC is an expensive technique and quick that can be used for determining the number of components in a mixture, verify a substance’s identity, monitor the process of a reaction, determine appropriate conditions for column chromatography, analyze the fractions obtained from column chromatography.
MATERIALS AND METHODS

1. Preparation of plates
Silicagel G was mixed in a glass mortar to smooth consistency with the requisite amount of water and slurry was quickly transferred to despresser. The mixtures have been spread over the plates in thickness of 0.2mm and allow setting in to a suitable holder and after 30 minutes; plates were dried at 120°C, for further activation of the absorbant.

2. Sample application
About 2 mm of absorbent from the edge of plate was removed to give sharply defined edges. 2-5μl volumes of synthesized compounds were spotted with the help of capillary tubes, just above 1cm of the bottom of coated plates.

3. Development chamber
The chromatographic chamber was lined with filter paper dipping into mobile phase so as to maintain the atmospheric saturation with solvent vapors in the chamber. The solvent front was allowed to rise to distance of about 12cm from the baseline on the plate was removed from the tank and allowed to dry in the air.

4. Solvent system
The choice of best developing solvent is one of the most important decisions in practical TLC by review of literature survey on by knowing nature of compounds, this solvent system used is benzene: ethyl acetate (8:2).

5. Detection of components
The spots were visualized under Iodine chamber.

Column Chromatography
Purification of synthesized derivatives was done by column chromatography.

Materials
1. Glass column of size 45cm x 3cm.
2. Silicagel for column chromatography 60-120 mesh size.
3. Eluting solvent system benzene :ethylacetate (8:2).

Preparation of column
The silica gel 60-120 mesh size was made in to slurry with the above solvent system. The bottom of the column was plugged with little glass wool. Then the slurry was poured in to the column, which is filled with solvent after two third of the column areas were filled with slurry. It was set aside for 30 minutes and eluting solvent was passed through column for several time ensure good packing of the column. After the adsorbents are settled, a filter paper was kept to prevent disturbance of the two player of the adsorbent as fresh mobile phase to be added to column for the process of elution. The fractions were collected for every 5m land analyzed for the presence of different of similar compound by running TLC and then allow evaporating to get the residue.

Pharmacological Screening

In-Vitro Anti-Cancer Activity
Tissue culture has been used to screen may anti-cancer drugs since there is clear correlation between the in vitro and in vivo activities of potential chemotherapeutic agents. There is scientific justification for cytotoxicity testing in tissue, since animal models are in many ways in adequate for predicting the effects of chemicals on humans since there are many metabolic differences between species61-63. Cytotoxicity studies involve the analysis of morphological damage or inhibition of zone of outgrowth induced by the chemicals tested.

Assay For Proliferation Studies

In Vitro Anti Cancer Activity
The human cervical cancer cell line (HeLa) was obtained from national center for cell science (NCCS) pune. The HeLa cells were grown in Eagles Minimum Essential Medium containing 10% fetal bovine serum (FBS) and maintained at 37°C, 5% CO2, 95% air and 100% relative humidity. Maintenance cultures were passaged weekly, and the culture medium was changed twice a week. Toxicity of test compound in cells was determined by MTT assay based on mitochondrial reduction of yellow MTT tetrazolium dye to a highly colored blueoarmazan product.

Assay for Proliferation Studies - MTT Assay

Principle
MTT [(3-(4,5-dimethyl thiazol-2yl)-2,5diphenyl tetrazolium bromide] measures the metabolic activity of the viable cells. The assay can be performed entirely in a microtiterplate (MTP). It is suitable for measuring cell proliferation, Cell viability or Cytotoxicity. The reaction between MTT and mitochondrial dehydrogenase produces water-insoluble formazan salt. This method involves culturing the cells in a 96 well microtiterplate and then incubating with MTT solution for approximately 2 hours. During incubation period, viable cells convert MTT to a water insoluble formazan dye. The formazan dye in the MTP is solubilized and quantified with an ELISA plate reader. The absorbance directly correlates with the cell number. This is applicable for adherent cells cultured in MTP.

Materials for MTT assay

- The human cervical cancer cell line (HeLa) Eagles Minimum Essential Medium containing 10% fetal bovine serum (FBS).
- Phosphate buffered saline (PBS)
- Dimethyl sulphoxide (DMSO)
- MTT [(3-(4,5-dimethylthiazol-2yl)-2,5 di phenyl tetrazolium bromide] CO2 incubator (WTC Binder, Germany)
- Laminar air flow cabin (Klenzaid, Chennai, India).
- Refrigerated centrifuge ( Biofuge fresco, Heraeus, Germany).
- ELISA-reader ( For MTP ) Anthos 2010, Germany).
- Deep freezer (Polar Angelontioni Industries, Italy).
- Ultrasonic bath ( Transonic [ 460/H ], by Elma, Germany).
- Vaccum pump ( Zenith [model: PDF-2-2.5], Mumbai, India).
- Pipettes (Eppendof, Hamburg, Germany).
Cell treatment procedure

The cells were detached with trypsin-ethylenediamine tetraacetic acid (EDTA) to make single cell suspensions and viable cells were counted using a hemocytometer and diluted with medium with 5% FBS to give final density of 1x10^5 cells/ml. One hundred microlitres per well of cell suspension were seeded into 96-well plates at plating density of 10,000 cells/well and incubated to allow for cell attachment at 37°C, 5% CO₂, 95% air and 100% relative humidity.

After 24 h the cells were treated with serial concentrations of the extracts and fractions. They were initially dissolved in neat dimethylsulfoxide (DMSO) and further diluted in serum free medium to produce five concentrations. One hundred microlitres per well of each concentration was added to plates to obtain final concentrations of 100, 10, 1.0 and 0.1 μM. The final volume in each well was 200 μl and the plates were incubated at 37°C, 5% CO₂, 95% air and 100% relative humidity for 48h. The medium containing without samples were served as control. Triplicate was maintained for all concentrations.

Procedure

**In-vitro anticancer screening**

The human cervical cancer cell line (HeLa) was obtained from National Centre for Cell Science (NCCS), Pune. The cells were grown in Eagles Minimum Essential Medium containing 10% fetal bovine serum (FBS). For screening experiment, the cells were seeded into 96-well plates in 100μl of medium containing 5% FBS, at plating density of 10,000 cells/well and incubated at 37°C, 5% CO₂, 95% air and 100% relative humidity for 24 hours prior to addition of samples. The samples were solubilized in Dimethylsulfoxide and diluted in serum free medium. After 24 hours, 100 μl of the medium containing the samples at various concentration (eg: 0.063, 0.125, 0.25, 0.5, 1.0 mM etc...) was added and incubated at 37°C, 5% CO₂, 95% air and 100% relative humidity for 48 hours. Triplicate was maintained and the medium containing without samples were served as control.

After 48 hours, 15μl of MTT (5mg/ml) in phosphate buffered saline (PBS) was added to each well and incubated at 37°C for 4 hours. The medium with MTT was then flicked off and the formed formazan crystals were solubilized in 100μl of DMSO and then measured the absorbance at 570 nm using micro plate reader. The % cell inhibition was determined using the following formula

$$\text{% cell Inhibition} = 100 - \frac{\text{Abs(sample)}}{\text{Abs(control)}} \times 100.$$
Table No.3: IC_{50} Values of Synthesized Compounds (TD1–TD7)

<table>
<thead>
<tr>
<th>COMPOUND CODE</th>
<th>IC_{50} (MICRO MOLAR)</th>
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<tr>
<td>TD1</td>
<td>45.70 µM</td>
</tr>
<tr>
<td>TD2</td>
<td>96.23 µM</td>
</tr>
<tr>
<td>TD3</td>
<td>75.25 µM</td>
</tr>
<tr>
<td>TD4</td>
<td>92.36 µM</td>
</tr>
<tr>
<td>TD5</td>
<td>08.23 µM</td>
</tr>
<tr>
<td>TD6</td>
<td>48.60 µM</td>
</tr>
<tr>
<td>TD7</td>
<td>&gt;100 µM</td>
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</table>
Table No.4 TD1

<table>
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<tr>
<th>Concentration (uM)</th>
<th>% Growth Inhibition</th>
<th>IC_{50}</th>
<th>R^2</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.1uM</td>
<td>1.3442</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1uM</td>
<td>12.0079</td>
<td>45.70</td>
<td>0.0005</td>
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<tr>
<td>10uM</td>
<td>35.1791</td>
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<tr>
<td>100uM</td>
<td>74.4578</td>
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Table No.10 TD3

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<td>0.1uM</td>
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<td>1uM</td>
<td>14.2646</td>
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<tr>
<td>10uM</td>
<td>25.0772</td>
<td>75.26</td>
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<td>100uM</td>
<td>55.0587</td>
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Table No.5 TD2

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<tr>
<td>0.1uM</td>
<td>1.9424</td>
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<td>1uM</td>
<td>10.447</td>
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<td>10uM</td>
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<td>100uM</td>
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Table No.11 TD4

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<td>1uM</td>
<td>19.6137</td>
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<tr>
<td>10uM</td>
<td>35.4004</td>
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<td>0.0016</td>
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<tr>
<td>100uM</td>
<td>83.8273</td>
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### Table 5: TD5

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<td>1µM</td>
<td>12.5979</td>
<td>0.825</td>
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<td>10µM</td>
<td>30.9561</td>
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<tr>
<td>100µM</td>
<td>60.8174</td>
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### Table 6: TD7

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<th>IC₅₀</th>
<th>R²</th>
</tr>
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<tr>
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<td>3.4112</td>
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<td>10µM</td>
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<td>100µM</td>
<td>74.8578</td>
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### Table 7: TD6

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<th>% Growth Inhibition</th>
<th>IC₅₀</th>
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<tr>
<td>0.1µM</td>
<td>2.2342</td>
<td>48.60</td>
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<td>1µM</td>
<td>12.5979</td>
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<td>10µM</td>
<td>32.9161</td>
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<tr>
<td>100µM</td>
<td>73.8178</td>
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### Graphs:

- Graph showing % Cell Inhibition vs Concentration in µM for TD5.
- Graph showing % Cell Inhibition vs Concentration in µM for TD7.
- Graph showing % Cell Inhibition vs Concentration in µM for TD6.
RESULTS AND DISCUSSION

5.1. Chemical work:
The results of the present work are discussed under the following heads.
Scheme:2-(3-(4-(4-amino phenyl sulfonyl) phenyl)-4-oxo-2- (4-substituted-phenyl thiazolidin-5-yl) acetic acid.

5.1.1 Synthesis of 2-(3-(4-(4-amino phenyl sulfonyl) phenyl) -4-oxo-2-(4-substituted-phenyl thiazolidin-5-yl) acetic acid.
Synthetic route depicted in scheme outline the chemistry part of the present work. 2-(3-(4-(4-amino phenyl sulfonyl) phenyl) -4-oxo-2-(4-substituted-phenyl thiazolidin-5-yl) acetic acid (TD1-7) were obtained by the condensation of 4-(4-amino phenyl sulfonyl) benzenamine with substituted benzaldehydes in presence of dry dioxane, concentrated sulphuric acid and ethanol. The formation of the substituted thiazolidinone was confirmed by the presence of characteristic peaks in the IR spectra. It showed characteristic peaks at around 3400 cm\(^{-1}\) for NH2 stretching and peak around 2900 cm\(^{-1}\) due to the presence of N=CH stretching. The NMR spectrum of the compounds TD1-7 showed the characteristic peak around \(\delta\) 2.70 ppm for CH3 group, \(\delta\) 3.00 ppm for CH2 and \(\delta\) 5.70 ppm for NCH and also shows multiplet in the range of \(\delta\) 6.80-8.30 ppm owing to aromatic protons. The appearance of peak due to chlorine in IR spectra around 700 -800 cm\(^{-1}\) and formation M+2 peak in the mass spectra. Data from the elemental analyses and molecular ion recorded in the mass spectra further confirmed the assigned structure.

5.2. Pharmacological Investigation
The anticancer screening of title compounds (TD1-7) were evaluated against human cervical cancer cell line (HeLa) by MTT assay method. In this assay the effective ranges of anticancer activity for compounds TD1-7 were in the concentration of 0.1, 1.0, 10, 100 \(\mu\)M respectively in the human cervical cancer cell line (HeLa). Triplicate was maintained and the medium containing without samples were served as control. TD1 (p-nitrophenyl) produced IC50 value 45.70 \(\mu\)M in case of the human cervical cancer cell line (HeLa). Relatively less value of IC50 indicates the sample has more anticancer activity. The compounds TD1 (p-nitro phenyl) had shown the percentage of cell inhibition was 74.85 against the human cervical cancer cell line (HeLa) in the highest concentration, which have p-nitrophenyl group in the thiazolidinone nucleus. The result indicates that TD1 (p-nitrophenyl group) showed a significant anticancer activity against the human cervical cancer cell line (HeLa), when compared to that control. TD2 (dimethyl amino group) produced IC50 value 66.23 \(\mu\)M in case of the human cervical cancer cell line (HeLa). Relatively less value of IC50 indicates the sample has more anticancer activity. The compound TD2 (dimethyl amino group) had shown the percentage of cell inhibition was 52.89 against the human cervical cancer cell line (HeLa), which have dimethylamino group in the thiazolidinone nucleus. The results indicate that TD2 (dimethyl amino group) showed a moderate anticancer activity against the human cervical cancer cell line (HeLa), when compared to that of control. TD3 (methoxyl group) produced IC50 value 75.26\(\mu\)M in case of the human cervical cancer cell line (HeLa). Relatively less value of IC50 indicates the sample has more anticancer activity. The compound TD3 (methoxyl group) had shown the percentage of cell inhibition was 52.25 against the human cervical cancer cell line (HeLa), which have dimethyl amino group in the thiazolidinone nucleus. The results indicate that TD3 (methoxyl group) showed a less anticancer activity against the human cervical cancer cell line (HeLa), when compared to that of control. TD4 (Hydroxyl group) produced IC50 value 92.36 \(\mu\)M in case of the human cervical cancer cell line (HeLa). Relatively less value of IC50 indicates the sample has more anticancer activity. The compound TD4 (Hydroxyl group) had shown the percentage of cell inhibition was 63.82 against the human cervical cancer
cell line (HeLa) in the highest concentration, which have imidazole group in the thiadiazole nucleus. The results indicate that \textbf{TD4} (Hydroxyl group) showed a moderate significant anticancer activity against the human cervical cancer cell line (HeLa), when compared to that of control. \textbf{TD5} (vinyl group) produced IC50 value 75.26 μM in case of the human cervical cancer cell line (HeLa). Relatively less value of IC50 indicates the sample has more anticancer activity. The compound \textbf{TD5} (vinyl group) had shown the percentage of cell inhibition was 55.05 against the human cervical cancer cell line (HeLa) in the highest concentration, which have vinyl group in the thiazolidinone nucleus. The results indicate that \textbf{TD5} (vinyl group) showed a moderate significant anticancer activity against the human cervical cancer cell line (HeLa), when compared to that of control. \textbf{TD6} (p-amino group) produced IC50 value 48.60 μM in case of the human cervical cancer cell line (HeLa). Relatively high value of IC50 indicates the sample has more anticancer activity. The compound \textbf{TD6} (p-amino group) had shown the percentage of cell inhibition was 73.85 against the human cervical cancer cell line (HeLa) in the highest concentration, which have p-amino group in the thiadiazole nucleus. The results indicates that \textbf{TD6} (p-amino group) showed a good significant anticancer activity against the human cervical cancer cell line (HeLa), when compared to that of control. \textbf{TD7} (p-chloro group) produced IC50 value > 100 μM in case of the human cervical cancer cell line (HeLa). Relatively high value of IC50 indicates the sample has more and significant anticancer activity. The compound \textbf{TD7} (p-chloro group) had shown the percentage of cell inhibition was 55.05 against the human cervical cancer cell line (HeLa), when compared to that of control. \textbf{TD7} (p-chloro group) produced IC50 value > 100 μM in case of the human cervical cancer cell line (HeLa). Relatively high value of IC50 indicates the sample has more and significant anticancer activity. The compound \textbf{TD7} (p-chloro group) had shown the percentage of cell inhibition was 73.85 against the human cervical cancer cell line (HeLa) in the highest concentration, which have p-chloro group in the thiadiazole nucleus. The results indicates that \textbf{TD7} (p-chloro group) showed a more significant anticancer activity against the human cervical cancer cell line (HeLa), when compared to that of control. The best mean IC50 values were achieved with compound (\textbf{TD3, TD4, TD5 and TD7}) with slight difference among them. Title compounds (\textbf{TD1-7}) were found to exhibit mild to moderate anticancer activities in cell lines and the results were summarized below:

\begin{itemize}
  \item Compound \textbf{TD1} (p-nitrophenyl group) shows less activity against the HeLa (IC50 = 47.50) cancer cell lines.
  \item Compound \textbf{TD2} (dimethylamino group) shows moderate activity against the HeLa (IC50 = 66.23) cancer cell lines.
  \item Compound \textbf{TD3} (methoxyl group) shows high significant activity against the HeLa (IC50 = 72.56) cancer cell lines.
  \item Compound \textbf{TD4} (4-hydroxy group) shows more & potent significant against the HeLa (IC50 = 92.36) cancer cell lines.
  \item Compound \textbf{TD5} (vinyl group) shows the moderate activity against the HeLa (IC50 = 68.25) cancer cell lines.
  \item Compound \textbf{TD6} (p-amino group) shows less significant activity against the HeLa (IC50 = 48.60) cancer cell lines.
  \item Compound \textbf{TD7} (p-chloro) shows very high and potent significant activity against the HeLa (IC50 > 100) cancer cell lines.
\end{itemize}

Among the test compounds, compound 3-(4-(4-aminophenylsulfonyl)phenyl)-2-(4-chlorophenyl)-4-oxothiazolidin-5-yl) acetic acid (TD7) was found to be the most active agent which showed 74.85 percentage of cell inhibition against the human cervical cancer cell line (HeLa) in the highest concentration, which have p-chloro group in the thiazolidinone nucleus.

\section*{SUMMARY AND CONCLUSION}

In summary, a new series of 2-(3-(4-(4-aminophenylsulfonyl) phenyl)-4-oxo-2-(4-substituted-phthiazolidin-5-yl) acetic acid were synthesized. These title compounds containing seven different substituents at C-2 and C-3 were screened for their anticancer agents. Most of the test compounds were found to exhibit significant anticancer activity against the human cervical cancer cell line (HeLa) in the highest concentration. Among the substituents at C-2, p-chloro phenyl substituent and at C-5 4-amino phenyl sulfonyl substituent showed maximum potency, while 4-methoxy phenyl, 4-hydroxy phenyl and 4-nitro phenyl substituent showed equipotent activity but the dimethylaminophenyl, vinyl and 4-amino phenyl substituent at C-2 exhibited least activity when compare to other substituents.

The order of activity at C-2 is p-chloro phenyl ≥ 4-hydroxy phenyl ≥ 4-methoxy phenyl ≥ 4-nitro phenyl ≥ 4-amino phenyl ≥ dimethylaminophenyl ≥ vinyl substituents. Among the test compounds, compound 3-(4-(4-aminophenylsulfonyl)phenyl)-2-(4-chlorophenyl)-4-oxothiazolidin-5-yl) acetic acid (TD7) was found to be the most active agent which showed 74.85 percentage of cell inhibition against the human cervical cancer cell line (HeLa) in the highest concentration, which have p-chlorophenyl group in the thiazolidinone nucleus. Hence this molecule can be selected as a lead molecule of the present study for further exploitation.

\section*{REFERENCE}

2. Schmidt D and Loscher W, Epilepsia, 46, 2005, 858.
33. Berseneva VS, Tkachev AV, Morzherin YY, Dehaen W, Luyten I,
32. Souza MV, Ferreira SB, Mendonca JS, Costa M, Rebello FR, Quim.
30. Aquino TM, Liesen AP, Lima JG, Silva RE, Lima VT, Araújo JM,
29. Tenorio RP, Carvalho CS, Pessanha CS, Lima JG, de Faria AR, 
28. Graciet JC, Niddam, V, Gamberoni, M,Trabaud, C, Dessolin J,
26. Andrade AMC, Lima WT, Rocha MPA, Lima MCA, Galdino SL,
25. Akerblom E, 2-aminothiazolin-4-one and 2-iminothiazolidin-4-one
24. Andrade AMC, Lima WT, Rocha MPA, Lima MCA, Galdino SL,
17. Mali JR, Pratap UR, Netankad PD, Mane RA, Tetrahedron Lett, 50, 
16. Pratap UR, Jawale DV, Bhosle MR, Mane RA, Tetrahedron Lett, 52, 
15. Bolognese A, Correale G, Manfra M, Lavecchia A, Novellino E, 
14. Neuenfeldt, PD, Drawanz BB, Siqueira GM, Gomes CRB, Wardell 
12. Souza MV, Ferreira SB, Mendonca JS, Costa M, Rebello FR, Quim.
10. Pratap UR, Jawale DV, Bhosle MR, Mane RA, Tetrahedron Lett, 52,
9. Graciet JC, Niddam, V, Gamberoni, M,Trabaud, C, Dessolin J,
7. Mali JR, Pratap UR, Netankad PD, Mane RA, Tetrahedron Lett, 50,
6. Mali JR, Pratap UR, Netankad PD, Mane RA, Tetrahedron Lett, 50, 
5. Mali JR, Pratap UR, Netankad PD, Mane RA, Tetrahedron Lett, 50,
4. Mali JR, Pratap UR, Netankad PD, Mane RA, Tetrahedron Lett, 50,
3. Mali JR, Pratap UR, Netankad PD, Mane RA, Tetrahedron Lett, 50,
2. Mali JR, Pratap UR, Netankad PD, Mane RA, Tetrahedron Lett, 50,
1. Mali JR, Pratap UR, Netankad PD, Mane RA, Tetrahedron Lett, 50,


76. Maity TK, Nagalakshmi G, Maiti BC, the synthesis, characterization and antiproliferative activity of 2-(substituted phenyl)-5-methyl-3-pyrind-4-yl-1, 3-thiazolidinones, Int J Pharm Tech Res, 3(2), 2011, 707-718.


81. Suroor Ahmad Khan, Mohammad Imran, Mohammad Shahar, synthesis of 2-substituted phenyl-3-(1-naphthyl)-1, 3-thiazolyl-amino -4-oxo thiazolidin-4-one acid derivatives as anti-hyperglycemic activity, Acta Poloniae Pharmaceutica, 66(1), 2009, 51-56.


83. Birendra Shrivastava, Pankaj Sharma, Lamba HS, Jaya Sharma, Lokesh Sharma,Synthesis of 5-substituted-1, 3-thiazolidin-4-ones as anti-hyperglycemic activity, Pharmacie Globale (IJCP), 3(9),2010,1-6.


88. Dharmaraj, Perumal Yogiweswari, Ashok Kumar TG, Microwave-assisted synthesis and anti-YFV activity of 2, 3-diyarl-1, 3-thiazolidin-4-ones, J Pharm Pharm Sci, 8(3), 2005, 426-429.

89. Ravindra K Rawl, Ashutoosh Kumar, Mohammad Imran Siddidi and Setu B Katti, Molecular docking studies on 4-thiazolidinones as HIV-1 RT inhibitors, J Mol Model, 13, 2007, 155-161.


100. Setu B Katti, Molecular docking studies on 4-thiazolidinones as HIV-1 RT inhibitors, J Med Chem, 45, 2010, 1242-1251.


