European Journal of Molecular & Clinical Medicine

ISSN 2515-8260 Volume 10 Issue 8, 2023

DESIGN, SYNTHESIS AND CHARACTERIZATION OF THIAZOLIDINE-2, 4-DIONE ANALOGUES AS ANTI-CANCER AGENTS

Ganesh Prasad Patel[1*], Akash Yadav[2], Naveen Gupta[3], Dharmendra Singh Rajput[4]

[1*,2,3,4]Patel College of Pharmacy, Madhyanchal Professional University, Ratibad-462044, Bhopal, Madhya Pradesh.

***Corresponding Author:** Ganesh Prasad Patel
*Patel College of Pharmacy, Madhyanchal Professional University, Ratibad-462044, Bhopal, Madhya Pradesh.

Abstract
Compounds with halogen group substitution on aromatic ring exhibited promising activity. Compound 3i with the 2,3-dichloro group at the phenyl ring attached to heterocyclic thiazolidine-2,4-dione showed potent activity against HeLa cells when compared with reference drug adriamycin. Substitution at R with electron withdrawing groups such as chloro, bromo, iodo in compounds 3a, 3b, 3f, 3g and 3i showed increase in activity. The electron donating groups such as methoxy, ethoxy, methyl, ethyl and hydroxy substituted compounds 3c, 3e, 3j, 3l, 3m, 3o, 3p, 3r showed decrease in activity. The compound 3i with 2, 3-dichloro substitution on the phenyl ring exhibited IC_{50} value of 0.007 μM better than other substitutions. Compounds 3n with 3-NO_2 groups showed significant improvement in activity. Compound 3h with 3-CN group substitution on the phenyl ring exhibited intermediate potency in the series. Constitutively, 3j and 3k containing 3-hydroxy and 3,4-di hydroxy groups attached to phenyl ring showed decrease in activity compared to other derivatives of the series, attributed to the presence electron withdrawing group decrease the potency.

Key-words: Thiazolidine-2, 4 dione, Anticancer, SRB Assay, Chloroacetic Acid, Chemotherapeutic Agents.

INTRODUCTION
Cancer is the second leading cause of death globally and was responsible for around 8.8 million deaths in 2015. Globally, nearly 1 in 6 deaths is due to cancer. Approximately 70% of deaths from cancer occur in low and middle-income countries. Presently in India, it is a major cause of morbidity and mortality. Cancer cases, as well as mortality, are increasing rapidly among Indian women, primarily because of low awareness and late detection. Data showed that India accounts for the third highest number of cancer cases among women after China and the US, with a 4.5-5% growth rate annually [1, 2]. When a cell becomes uncontrolled with irregular growth may be responsible for cancer. It can affect almost any part of the body. External factors such as tobacco, infectious organisms, and an unhealthy diet, and internal factors, such as inherited genetic mutations, hormones, and immune conditions are the main cause of cancer. These factors may act mutually or in a series to cause cancer.
Data (2012-2014) collected from population-based cancer registries (PBCR) and hospital based registries showed that breast and cervical cancers are prominently found in Indian women. The northeast part of the country reported the highest number of cancer cases in both males and females. Breast cancer is the number one cancer, which estimates about 1.5 lakh (over 10 percent of all cancers) new cases in 2016. Tobacco users accounted for about 30 percent of all cancers in males and females [3].
Despite the huge efforts to implement novel chemotherapeutic strategies for the treatment of different types of cancer, this disease remains one of the major concerns worldwide. Consequently, there is an urgent need to find unexplored classes of substances with selective action against cancer cells. The regulation of the cell proliferations and apoptotic pathways associated with cell death is known as an important approach to understand a great variety of medical illnesses, including cancer

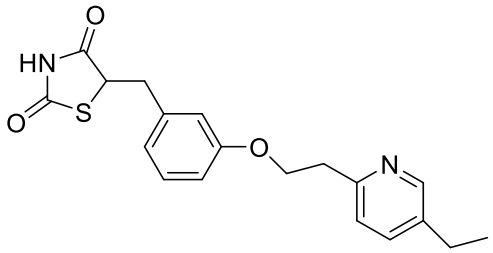
ISSN 2515-8260 Volume 10 Issue 8, 2023

[4, 5]. Therefore, the identification of cell-cycle regulators and apoptotic stimuli to combat cancer cells represents an attractive strategy to the discovery and development of potential antitumor agents [6, 7].

As the crucial role of the medicinal chemist is to provide new molecules to the pharmaceutical industry or to refine the existing moieties so that its duration of action and potency increases, at the same time the toxic effects/side effects of the drugs are reduced. The other important role of medicinal chemist is the modification of chemical structures having known pharmacological or physiological effects.

Cancer is the second leading cause of death after the heart diseases across the globe which affected 8.2 million lives in the year 2015. Chemotherapy is one of the primary options among all the currently available cancer treatment strategies. Cancer treatment often fails when cancer becomes resistant to anti-cancer drugs. Thus, there is a continuous need to develop novel anticancer drugs that are efficacious, well-tolerated, nontoxic, and orally available, to overcome the problem of drug resistance and give patients a better chance to survive.

The literature survey revealed that thiazolidine-2, 4-dione (TZD) and rhodanine analogs have been recognized as the privileged templates in drug design and discovery[203,204] and numerous compounds containing the TZD ring have been developed as potential anticancer agents, such as Pioglitazone found in the treatment of Stage IA to IIB Non-Small Cell Lung Cancer (NSCLC).



Pioglitazone

It is firmly believe that there is still more research work to be done in this field to find a novel thiazolidine-2,4-dione derivatives with potent anticancer activity to inhibit the growth of multiple cancer cell lines to induce apoptosis as well as to interfere with the cell cycle transition, having more potency and less side effects. The present project is related with the design and synthesis of novel thiazolidine-2, 4-dione derivatives and their biological evaluation to produce better anticancer activity.

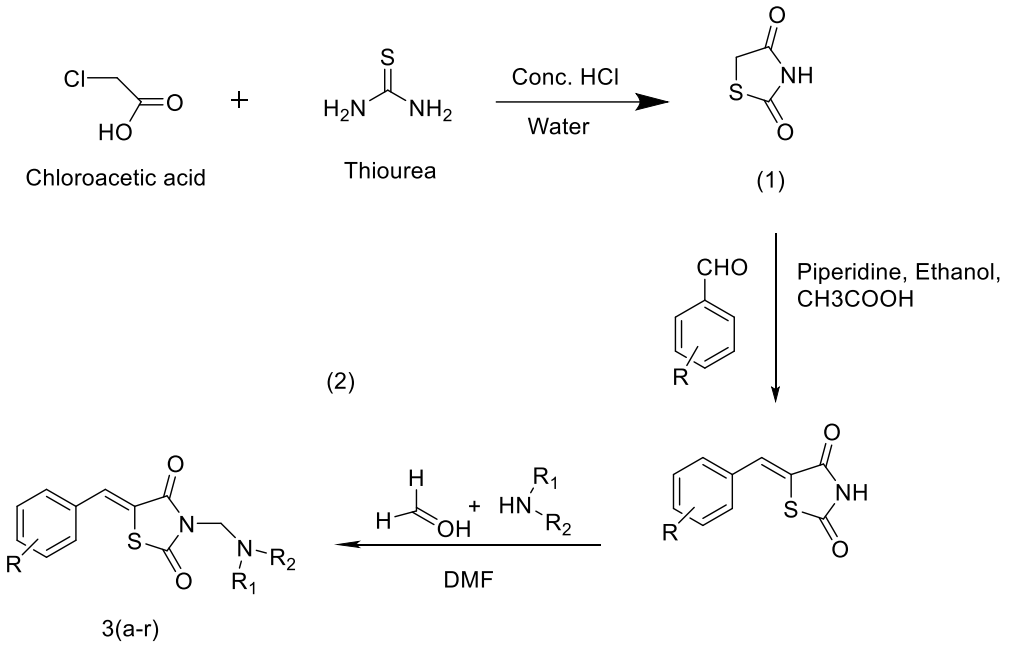
In literature review, several methods has been reported for the synthesis of 2,4-thiazolidine-2,4-diones, but most of them are very complicated, having longer reaction time and require very advanced synthetic technology. The chemicals & reagents which are required for the synthesis are not easily available and too expensive. So in the present investigation, it is decided to synthesize different 2, 4-thiazolidinedione derivatives by efficient, cost effective, environmentally friendly technique.

MATERIALS AND METHODS

Scheme for synthesis

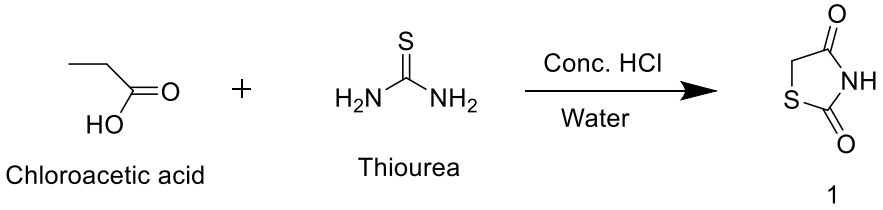
The synthetic work carried out during present investigation has been described in the following scheme.

Reaction Scheme



Compd. No.	R	R1	R2
3a	4-Cl	H	H
3b	4-F	CH_3	CH_3
3c	-4OCH_3	H	H
3d	2,5 di OCH_3	CH_3	CH_3
3e	4$C_6H_5CH_2O$-	CH_3	CH_3
3f	3-Br	C_2H_5	C_2H_5
3g	4-Br	CH_3	C_2H_5
3h	3-CN	H	H
3i	2,3di-Cl	H	H
3j	3-OH	H	H
3k	3,4di-OH	CH_3	CH_3
3l	4-Me	H	H
3m	3,4di Me	CH_3	CH_3
3n	3-NO_2	H	H
3o	2-OH,3-OMe	CH_3	CH_3
3p	4-NH_2	H	H
3q	2-OH,5-Cl	CH_3	CH_3
3r	-4OC_2H_5	CH_3	CH_3

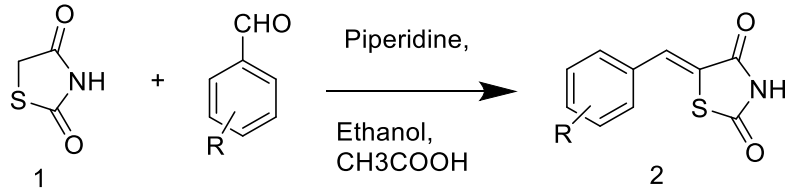
Synthesis of thiazolidine-2, 4-dione (1)



In a 250 ml three-necked flask, a solution containing 56.4g (0.6M) of chloroacetic acid in 60 ml of water and 45.6g (0.6M) of thiourea was dissolved in 60ml of water. The mixture was stirred for 15 minute till occurrence of white precipitates. To the contents of flask was now added slowly 60 ml of conc. hydrochloric acid from dropping funnel to dissolve the precipitates, after which the reaction mixture was stirred and refluxed for 10-12 hrs at 100-110˚C, on cooling the contents of flask were

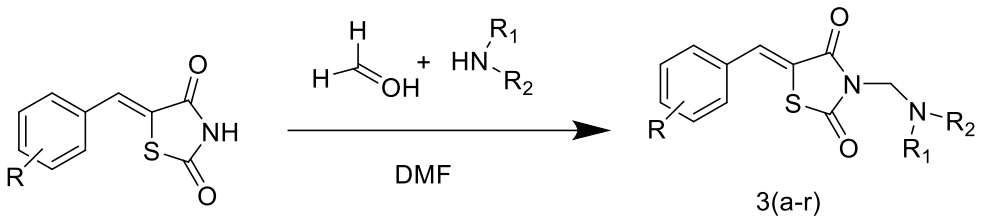
solidified to a mass of clusters of white needles. The product was filtered and washed with water to remove traces of hydrochloric acid and dried. It was recrystallised from ethanol.

Synthesis of (Z)-5-benzylidenethiazolidine-2,4-dione (2)



A mixture of 2,4-thiazolidinedione 1 (2.4 g, 20 mmol), benzaldehyde derivative 2 (20 mmol), piperidine (1.4 g, 16 mmol) and ethanol (150 ml) was refluxed for 16–24 h. The reaction mixture was poured into H_2O and acidified with AcOH to give 3a–3d as solids, which were recrystallized from methanol. Completion of reaction has been confirmed using TLC using Benzene: Ethyl acetate as solvent system (3:7).

Synthesis target compounds (3a to 3r)



To a solution of 2, 4-thiazolidinedione (0.1M) in DMF, formaldehyde (0.2M) was added under stirring. The reaction mixture was stirred at room temperature for 0.5hrs to complete the reaction of formaldehyde. To the solution of secondary amine in DMF was added drop wise and reflux for several hrs to complete the reaction. The completion of reaction monitored by TLC using solvent system chloroform: methanol (9:1). After the completion of reaction was poured in an ice cold water and filtered off and wash with hot water. Finally it was recrystallised from chloroform, ethanol to give final compound.

Biological evaluation

Thiazolidine-2, 4-dione showed verities of pharmacological effects. It has been implicated in a wide variety of cancers of hematological and epithelial origin, such as bladder, gastric, leukaemia, colorectal and prostate carcinomas [145,146]. Studies suggested that expression induced by the response in HeLa (Cervical cancer cells), HCT-8(Colon carcinoma)cells[239]. (HeLa)

All 18 synthesized thiazolidine-2,4-dione derivatives were evaluated for their anticancer activity against selected human cancer cell lines of HeLa (Cervical cancer cells), HCT-8(Colon carcinoma)using sulforhodamine B (SRB) method. The results of anticancer activity are expressed in terms of growth inhibition fifty ($IC_{50}\mu M$) values.

Protocol for SRB assay

The synthesized derivatives were evaluated for their anti-cancer activity against selected human cancer cell line of Cervical cancer cells (HeLa) and HCT-8(Colon carcinoma)using sulforhodamine B (SRB) assay. The results of anti-cancer activity were expressed in terms of growth inhibition ($IC_{50}\mu M$) values and are presented in **Table 7.6**. RPMI 1640 medium (10% fetal bovine serum and 2 mM L-glutamine) was used for maintainingthe cell lines. The cells were inoculated into 96 well microtiter plates in 90 µL at 5000 cells per well. Before addition of testing compounds, the microtiter plates were incubated at 37°C, 95% air, 5%CO_2 and 100 % relative humidity for 24 h.

ISSN 2515-8260 Volume 10 Issue 8, 2023

The testing compounds were diluted in DMF for the preparation of stock solution of 10^{-2} concentration. During experiment four 10-fold serial dilutions were prepared using complete medium. Aliquots of 10 µl dilutions of different testing compounds were added into microtiter plates for preparing final drug concentration. These microtiter plates were incubated for 48 hours at standard conditions and finally assay was terminated after addition of cold TCA. The cells were fixed by the addition of cold TCA [50 µl, 30 % (w/v)] and then incubated for further 60 minutes at 4°C. The supernatant was discarded; the plates were washed at least five times by using tap water and dried in air. The prepared sulforhodamine B (SRB) solution [(50 µl) at 0.4 % (w/v) in 1 % acetic acid] was added in each wells and then incubated at room temperature for 20 minutes. After staining the prepared plates, unbound dye was recovered and residual dye was removed by washing five times using 1 % acetic acid. After staining, unbound dye was recovered and the residual dye was removed by washing five times with 1 % acetic acid and dried in air. Bound stain was subsequently eluted with 10 mM trizma base, and the absorbance was read on an *Elisa* plate reader at a wavelength of 540 nm with 690 nm reference wavelength [147,148].

Percent growth was calculated on a plate-by-plate basis for test wells relative to control wells. Percent Growth was expressed as the ratio of average absorbance of the test well to the average absorbance of the control wells X 100. Using the six absorbance measurements [time zero (Tz), control growth (C), and test growth in the presence of drug at the four concentration levels (Ti)], the percentage growth was calculated at each of the drug concentration levels. The dose response parameters were calculated for each test article. Growth inhibition of 50 % (IC_{50}) was calculated from [(Ti-Tz)/(C-Tz)] x 100 = 50, which is the drug concentration resulting in a 50% reduction in the net protein increase (as measured by SRB staining) in control cells during the drug incubation. Values were calculated for each parameter if the level of activity was reached; however, if the effect was not reached or was exceeded, the values for that parameter were expressed as greater or less than the maximum or minimum concentration tested.

RESULTS AND DISCUSSION

In the present study, 18 thiazolidine-2,4-dione derivatives have been synthesized which are outlined in **scheme 1**. The starting material thiazolidine-2,4-dione (1) was prepared by the reaction of chloroacetic acid with thiourea in the presence of hydrochloric acid. The compound 5-benzylidenethiazolidine-2,4-dione (2) was prepared by the reaction of 2,4-thiazolidinedione 1 (2.4 g, 20 mmol), benzaldehyde derivative 2 (20 mmol), piperidine (1.4 g, 16 mmol) and ethanol (150 ml) was refluxed for 16–24 h. The general procedure for the synthesis of final compounds (3a-3r) was reaction by 2, 4-thiazolidinedione (0.1M) in DMF, formaldehyde (0.2M) and secondary amine derivatives. The structures assigned to the compounds were supported by the results of IR, ^{1}H NMR, ^{13}C NMR and mass spectral data. **Table** described the **s**tructure and properties such as molecular weight, yield, melting point and R_f value of synthesized compounds 3(a–r).

Description of physical or chemical properties:

The synthesized compounds showed molecular weight within the range of Lipinski rule of five. The variation in molecular weights confirms the identity of compounds. Compound 3f showed the highest mol. wt. of the series which is 369. All compounds find with good percent yield that confirm the strength of synthetic procedures. Compounds such as 3b,, 3c, 3f, 3g, 3i, 3j, 3l, 3o, 3p, 3q and 3r showed highest percentage yield (70-90%) compared to other compounds of the series. Melting point represents the important physical properties of the compounds which show in the range of 250-350. R_f (retardation factor) value determine the fraction of an analyte in the mobile phase of a chromatographic system. The synthesized compounds show R_f value in the range 0.6 to 0.8 (**Table 7.5**).

ISSN 2515-8260 Volume 10 Issue 8, 2023

Description of IR spectra:

FT-IR spectra of synthesized compound 1, thiazolidine-2,4-dione showed characteristic peaks at υ cm^{-1} 3130 (N-H), 3047 (Ar C-H), 1737 (C=O), 619 (C-S-C), whereas compound 2 showed peaks at υ cm^{-1} 3323, 3170 (N-H), 3049 (Ar C-H), 1649 (C=O). The FT-IR spectra of compound 3a showed peaks at υ cm^{-1} 3206 (N-H), 3055 (Ar C-H), 1695 (C=O), 1546 (C=N), and 1482 (C=C). The target compound 3b showed characteristic IR peaks at υ cm^{-1} 3182 (N-H), 3062 (Ar C-H), 1658 (C=O), 1546 (C=C) and 756 (C-F). The target compound 3c showed characteristic IR peaks at υ cm^{-1} 3285 (N-H), 3057 (Ar C-H), 1678 (C=O), 1535 (C=C). The compound 3d showed characteristic υ cm^{-1}3032 (Ar C-H), 1697 (C=O), 1546 (C=C), 1278 (C-O-C). The compound 3e showed characteristic peaks at υ cm^{-1} 3032 (Ar C-H), 1658 (C=O), 1546 (C=C), 1278 (C-O-C). The compound 3f showed characteristic peaks at υ cm^{-1} 3061 (Ar C-H), 1681 (C=O), 1502 (C=C), 1276 (C-O-C), 867 (C-Br). The compound 3g showed characteristic peaks at υ cm^{-1} 3030 (Ar C-H), 1697 (C=O), 1546 (C=C), 1070 (C-Br). The compound 3h showed characteristic peaks at υ cm^{-1} 3205 (-NH_2), 3032 (Ar C-H), 1658 (C=O), 1546 (C=C). The compound 3i showed characteristic peaks at υ cm^{-1} 3240 (N-H), 3030 (Ar C-H), 2993 (Ali C-H), 1681 (C=O). The compound 3j showed characteristic peaks at υ cm^{-1} 3140 (N-H)3028 (Ar C-H), 2922 (Ali C-H), 1668 (C=O), 1543 (C=C). The compound 3k showed characteristic peaks at υ cm^{-1} 3032 (Ar C-H), 2980 (Ali C-H), 1655 (C=O), 1544 (C=C). The compound 3l showed characteristic peaks at υ cm^{-1} 3150 (N-H), 3080, 3062 (Ar C-H), 1680 (C=O), 1286 (C-O-C). The compound 3m showed characteristic peaks at υ cm^{-1} 3086 (Ar C-H), 2993 (Ali C-H), 1670 (C=O), 1546 (C=C). The compound 3n showed characteristic peaks at υ cm^{-1} 3161 (N-H), 3072 (Ar C-H), 2978 (Ali C-H), 1670 (C=O), 1533 (C=C). The compound 3o showed characteristic peaks at υ cm^{-1} 3032 (Ar C-H), 2883 (Ali C-H), 1670 (C=O), 1546 (C=C), 1280 (C-O-C). The compound 3p showed characteristic peaks at υ cm^{-1} 3113 (N-H), 3080 (Ar C-H), 2968 (Ali C-H), 1691 (C=O), 1529(C=C). The compound 3q showed characteristic peaks at υ cm^{-1} 3168 (N-H), 3084 (Ar C-H), 2941 (Ali C-H), 1680 (C=O), 1535(C=C), 1286 (C-O-C). The compound 3r showed characteristic peaks at υ cm^{-1} 3055 (Ar C-H), 2949 (Ali C-H), 1693 (C=O), 1546 (C=C), 1247 (C-O-C), 742, 680 (C-Cl)

Description of NMR spectra:

^{1}H NMR spectra peaks of the respective protons of the synthesized compounds were verified on the basis of their chemical shifts (δ), multiplicities, and coupling constants (*J*). Compounds showed two doublet at around δ 8.152, 7.952 which could be accounted for two C-H groups of *para* substituted benzene, two singlet at around δ 10.255 and 8.259 indicative of thiazolidine-2,4-dione N-H hydrogen and benzylidene C-H hydrogen. The compound 3a showed chemical shift δ 8.26 (s, 1H, =C-H), 8.15 (d, *J* = 1.76 Hz, 2H, ArH), 7.95 (d, *J* = 8.40 Hz, 2H, ArH), 5.11(s, 2H, NH_2), 4.23(s, 2H, -CH_2). The compound 3b showed peak around δ 8.60 (s, 1H, =C-H), 8.14 (d, *J* = 8.36 Hz, 2H, ArH), 7.94 (d, *J* = 8.40 Hz, 2H, ArH), 4.55 (s, 2H, -CH_2), 2.26 (s, 6H, -CH_3). The compound 3c showed peak around δ 7.95 (s, 1H, =C-H), 8.08 (d, *J* = 6.52 Hz, 2H, ArH), 7.89 (d, *J* = 6.44 Hz, 2H, ArH), 5.22 (s, 2H, -NH_2), 4.4 (s,2H, CH_2), 3.83 (s, 3H, -CH3). The compound 3d showed peak around δ 8.22 (s, 1H, =C-H), 8.18 (d, *J* = 8.92 Hz, 2H, ArH), 7.91 (d, *J* = 7.16 Hz, 1H, ArH), 7.61 (d, *J* = 7.16 Hz, 1H, ArH), 4.55 (s, 2H, CH_2), 3.83 (s, 6H, -CH_3), 2.26 (s, 6H, CH_3). The compound 3e showed peak around δ 7.95 (s, 1H, =C-H), 8.06 (d, *J* = 9.96 Hz, 2H, ArH), 7.94 (d, *J* = 6.36 Hz, 2H, ArH), 7.92 - 7.57 (m, *J* = 5.92 Hz, 5H, ArH), 4.55 (s, 2H, CH_2), 2.26 (s, 6H, CH_3). The compound 3f showed peak around δ 7.95 7.96 (s, 1H, =C-H), 8.00 (d, *J* = 9.88 Hz, 2H, ArH), 7.93 (d, *J* = 6.88 Hz, 2H, ArH), 7.51 (d, *J* = 6.32 Hz, 2H, ArH), 7.12 (d, *J* = 8.00 Hz, 2H, ArH), 4.55 (s, 2H, CH_2), 2.64 (s, 4H, CH_3), 1.02 (s, 6H, CH_3). The compound 3g showed peak around δ 7.84 (s, 1H, =C-H), 8.14 (d, *J* = 7.2 Hz, 2H, ArH), 7.99 (d, *J* = 7.76 Hz, 2H, ArH), 4.55 (s, 2H, CH_2), 2.64 (s, 2H, CH_2), 2.26 (s, 3H, CH_3), 1.02 (s, 3H, CH_3). The compound 3h showed peak around δ 8.89 (s, 1H, =C-H), 8.29 (s, 1H), 8.12 (d, *J* = 7.28 Hz, 1H, ArH), 7.93 (d, *J* = 6.76 Hz, 1H, ArH), 7.64 (d, *J* = 9.44Hz, 1H, ArH), 7.55 (t, *J* = 9.68 Hz, 1H, ArH), 5.11 (s, 2H, NH_2), 4.84 (s, 2H, CH_2). The

compound 3i showed peak around δ 8.84 (s, 1H, =C-H), 8.45 (d, *J* = 7.88 Hz, 1H, ArH), 8.36 (d, *J* = 6.80 Hz, 1H, ArH), 8.28 (d, *J* = 6.32 Hz, 2H, ArH), 5.11 (s, 2H, NH_2), 4.84 (s, 2H, CH_2). The compound 3j showed peak around δ 8.28 (s, 1H, =C-H), 8.05 (d, *J* = 6.56 Hz, 2H, ArH), 7.98 (d, *J* = 7.88 Hz, 1H, ArH), 7.86 (d, *J* = 7.92 Hz, 2H, ArH), 7.50 (t, *J* = 7.44 Hz, 1H, ArH), 5.11 (s, 2H, NH_2), 4.84 (s, 2H, CH_2). The compound 3k showed peak around δ 8.28 (s, 1H, =C-H), 8.23 (d, *J* = 8.48 Hz, 2H, ArH), 8.05 (d, *J* = 9.08 Hz, 1H, ArH), 7.71 (d, *J* = 7.32 Hz, 1H, ArH), 5.35 (s, 2H, OH), 4.55 (s, 2H, CH_2), 2.26 (s, 6H, CH_3). The compound 3l showed peak around δ 8.70 (s, 1H, =C-H), 8.13 (d, *J* = 12.88 Hz, 2H ArH,), 8.02 (d, *J* = 6.08 Hz, 2H, ArH), 5.11 (s, 2H, NH_2), 4.84 (s, 2H, CH_2), 2.34 (s, 3H, CH_3). The compound 3m showed peak around δ 8.28 (s, 1H, =C-H), 8.24 (d, *J* = 8.52 Hz, 1H, ArH), 8.09 (d, *J* = 8.44 Hz, 1H, ArH), 7.99 (d, *J* = 8.6 Hz, 1H, ArH), 4.55 (s, 2H, CH_2), 2.34 (s, 6H, CH_3), 2.26 (s, 6H, CH_3). The compound 3n showed peak around δ 8.30 (s, 1H, =C-H), 8.06 (s, 1H, ArH), 7.98 (d, *J* = 7.88 Hz, 2H, ArH), 7.88 (d, *J* = 8 Hz, 1H, ArH), 7.62 (t, *J* = 7.64 Hz, 1H, ArH), 5.11 (s, 2H, NH_2), 4.84 (s, 2H, CH_2). The compound 3o showed peak around δ 8.25 (s, 1H, =C-H), 8.05 (d, *J* = 9.8 Hz, 2H, ArH), 7.88 (d, *J* = 8.44 Hz, 2H, ArH), 7.78 (d, *J* = 8.36 Hz, 1H, ArH), 5.35 (s, 1H, OH), 4.55 (s, 2H, CH_2), 3.83 (s, 3H, CH_3), 2.26 (s, 6H, CH_3). The compound 3p showed peak around δ 8.38 (s, 1H, =C-H), 8.32 (d, *J* = 4.2 Hz, 2H, ArH), 8.30 (d, *J* = 4.24 Hz, 2H, ArH), 6.72 (s, 2H, NH_2), 5.11 (s, 2H, NH_2), 4.84 (s, 2H, CH_2). The compound 3q showed peak around δ 9.24 (s, 1H, =C-H), 8.53 (d, *J* = 8.92 Hz, 1H, ArH), 8.22 (d, *J* = 8.48 Hz, 1H, ArH), 8.40 (s, 1H, ArH), 5.35 (s, 1H, OH), 4.55 (s, 2H, CH_2), 2.6 (s, 6H, CH_3). The compound 3r showed peak around δ 8.81 (s, 1H, =C-H), 8.10 (d, *J* = 8.28 Hz, 2H, ArH), 7.95 (d, *J* = 6.12 Hz, 1H, ArH), 4.55 (s, 2H, CH_2), 4.09 (q, 2H, CH_2), 2.6 (s, 6H, CH_3), 1.32 (t, 3H), CH_3)

Description of Mass spectra:

The compounds showed m/z values in their respective range. The compound 2a showed peak at *m/z* 165 and base peak at *m/z* 105. The compound 3a showed molecular ion peak at *m/z*268 and base peak at *m/z*179. The base peak of compound 3a was (((4-ethoxyphenyl)ethynyl)sulfonium). Compound 3b showed molecular ion peak at *m/z*280 with base peak at 179 and other fragment peaks at *m/z*173, 145, 125. Similarly, other derivatives of the series showed their respective *m/z* value peaks according to molecular mass of the compounds. Compound 3c showed molecular ion peak at m/z 264 with base peak at 179. Compound 3d showed molecular ion peak at m/z 322 with base peak at 179.

Table 1: Structure and properties such as molecular weight, yield, melting point and R_f value of synthesized compounds 3(a–r).

S. No.	Compd. No.	Mol. Wt.	Yield (%)	Melting point (˚C)	R_f
1	3a	268.717	65	220	0.7
2	3b	280.316	75	244	0.6
3	3c	264.298	86	236	0.9
4	3d	322.378	56	212	0.7
5	3e	354.423	47	190	0.6
6	3f	369.275	86	185	0.8
7	3g	355.249	76	178	0.8
8	3h	259.282	57	234	0.5
9	3i	303.162	86	207	0.7
10	3j	250.272	78	240	0.7
11	3k	294.325	65	216	0.8
12	3l	248.299	85	187	0.8
13	3m	290.379	67	227	0.7
14	3n	279.27	57	230	0.5
15	3o	308.351	86	239	0.7
16	3p	249.287	86	175	0.7

17	3q	312.77	85	198	0.7
18	3r	306.379	84	228	0.7

Biological Activity

The synthesized compounds 3a-r were evaluated for their anti-cancer activity against selected human cancer cell line of Cervical cancer cells (HeLa) and Colon carcinoma (HCT-8) using sulforhodamine B (SRB) method. The results of anti-cancer activity are expressed in terms of growth inhibition fifty (IC50 μM) values and are shown in Table 7.8 and Table 7.9.

Compound 3i was the most potent derivative of the series against both cell lines HeLa and HCT-8 with IC50 value of 0.007 μM and 0.001 μM, consecutively. By comparing the activities of 3i with other derivatives, it was revealed that the presence of chloro group at C-2 and C-3 positions of phenyl ring confers the highest cytotoxic activity against cervical cancer and Colon carcinoma cell lines.

Furthermore, compound 3a and 3b showed good inhibitory activity against HeLa cells with IC50 value of 0.032 μM and 0.028, consecutively. Compounds 3f, 3g, 3n, 3q showed significant improvement in activity with IC50 values of 0.028, 0.055, 0.066, 0.032, 0.148 μM, consecutively, against HeLa cells. Compounds 3d, 3h, 3k, 3l, 3m, 3p and 3r showed the lowest potency in the series with IC50 values of 3.012, 3.087, 2.016, 3.087, 4.651, 7.139 and 2.253 μM, respectively against HeLa cells. Whereas, compounds 3c, 3e, 3i, 3j and 3o showed intermediate inhibitory activities.

HCT-8 cell line study revealed that compounds 3a, 3g, 3i, 3n showed potential activity with IC50 values of 0.097, 0.012, 0.001 and 0.023 μM, respectively. Compounds 3d, 3k, 3l, 3m and 3p and showed lesser activity with IC50 values of 10.034, 8.012, 7.045, 8.034 and 10.012 μM, consecutively, against HCT-8 cell line. The structure activity relationships (SARs) indicate that compounds having phenyl ring attached to (3a-r) thiazolidine-2,4-dione ring were important for anti-cancer activity.

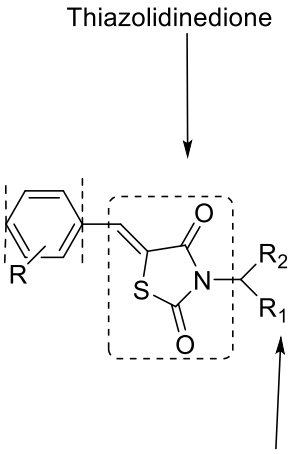
Table 2 *In vitro* antiproliferative activity (IC_{50})of synthesized compounds 3(a–r) against Cervical cancer cells (HeLa) and Colon carcinoma (HCT-8).

S.No.	Compd. No.	HeLa (μm) (Cervical cancer cells)	HCT-8 (μm) (Colon carcinoma)
1	3a	0.012	0.097
2	3b	0.028	> 25
3	3c	1.028	1.085
4	3d	3.012	10.034
5	3e	1.097	> 25
6	3f	0.055	0.056
7	3g	0.066	0.012
8	3h	3.087	0.123
9	3i	0.007	0.011
10	3j	1.572	4.065
11	3k	2.016	8.012
12	3l	3.087	7.045
13	3m	4.651	8.034
14	3n	0.032	0.023
15	3o	1.031	3.076
16	3p	7.139	10.012
17	3q	0.148	0.234
18	3r	2.253	5.012
Adriamycin		0.0001	0.0023

*ADR = Adriamycin, positive control compound

DISCUSSION

The whole molecule divided into three parts where one thiazolidine-2, 4-dione ring connected with di-substituted amino group. The literature survey revealed that when thiazolidine-2, 4-dione connected with di-arylamino group important for activity.



Compounds with halogen group substitution on aromatic ring exhibited promising activity. Compound 3i with the 2,3-dichloro group at the phenyl ring attached to heterocyclic thiazolidine-2,4-dione showed potent activity against HeLa cells when compared with reference drug adriamycin. Substitution at R with electron withdrawing groups such as chloro, bromo, iodo in compounds 3a, 3b, 3f, 3g and 3i showed increase in activity. The electron donating groups such as methoxy, ethoxy, methyl, ethyl and hydroxy substituted compounds 3c, 3e, 3j, 3l, 3m, 3o, 3p, 3r showed decrease in activity. The compound 3i with 2,3-dichloro substitution on the phenyl ring exhibited IC50 value of 0.007 μM better than other substitutions. Compounds 3n with 3-NO_2 groups showed significant improvement in activity. Compound 3h with 3-CN group substitution on the phenyl ring exhibited intermediate potency in the series. Constitutively, 3j and 3k containing 3-hydroxy and 3,4-di hydroxy groups attached to phenyl ring showed decrease in activity compared to other derivatives of the series, attributed to the presence electron withdrawing group decrease the potency.

Here compound 3i which consist halogen group substitution on aromatic ring showed potent activity against HCT-8 cells when compared with reference drug adriamycin.

Substitution at R with electron withdrawing groups such as 4-Cl, 3-Br, 4-Br and 2,3di-Cl (0.097, 0.056, 0.012, 0.011 μM, consecutively). Substitution at R with electron donating groups such as -4OCH_3, 2, 5 di OCH_3, 4C_6H_5O-, 3-OH, 3,4-di-OH, 4-Me, 3,4di Me, 4-NH_2 and -4OC_2H_5 were showed decreased in activity against HCT-8 cells.

REFERENCES

1. A. Nefzi, J.M. Ostresh, R.A. Houghten, Chem. Rev. 97 (1997) 449.
2. M. Yu, M. Tsuyoshi, Y. Tohru, K. Mitsuru, O. Hiroyuki, I. Hitoshi, S. Takashi, Novel 5-substituted 2,4- thiazolidinedione and 2,4-oxazolidinedione derivatives as insulin sensitizers and anti-diabetic activities. J Med Chem. 45 (2002) 1518-1534.
3. C.D. Barros, A.A. Amato, T.B. Oliveira, K.B.R. Iannini, A.L. Silva, T.G. Silva, E.S. Leite, M.Z. Hernandes, M.C.A. Lima, S.L. Galdino, F.A.R. Neves, I.R. Pitta, Synthesis and anti-inflammatory activity of new arylidene-thiazolidine-2,4-dione as PPAR ligands, Bioorg. Med. Chem. 18 (2010) 3805-3811.

4. N. Swathi, Y. Ramu, C.V.S. Subrahmanyam, K. Satyanarayana, Synthesis, quantum mechanical calculation and biological evaluation of 5-(4-substituted aryl/hetero aryl methylidene)-1,3-thiazolidine-2,4-diones, Int. J. Pharm. Sci. 4 (2012) 561-566.
5. S.R. Pattan, K.R. Alagwadi, A.R. Bhat, V.V.K. Reddy, J.S. Pattan, A.B. Khade, K.G. Bhatt, Synthesis and evaluation of some 5-[1-{4-4-substituted phenyl amino}-meth-(Z)-ylidene]-thiazolidine-2,4-dione for antitubercular activity, Indian Drugs 45 (2008) 532-535.
6. B. Oya, O. Ozen, M. Arzu, K. Engin, E. Rahmiye, Synthesis and antimicrobial activity of some new thiazolyl thiazolidine-2,4-dione derivatives, Bioorg. Med. Chem. 15 (2007) 6012-6017.
7. S.A.H. El-Feky, Synthesis and anticonvulsant properties of some novel quinazolinone thiazolidine and 4-thiazolidone derivatives, Pharmazie 48 (1993) 894-896.
8. G.A. Shankar, R.A. Kallanagouda, New thiazolidinedione-5-acetic acid amide derivatives: synthesis, characterization and investigation of antimicrobial and cytotoxic properties, Med. Chem. Res. 21 (2012) 816-824.
9. B. Meunier, Hybrid molecules with a dual mode of action: dream or reality? Acc. Chem. Res. 41 (2008) 69-77.
10. L. Xu, J. Vagner, J. Josan, R.M. Lynch, D.L. Morse, B. Baggett, H. Han, E.A. Mash, V.J. Hruby, J. Robert, Enhanced targeting with heterobivalent ligands, Mol. Cancer Ther. 8(8) (2009) 2356-2365.
11. C. Blanquicett, J. Roman, C.M. Hart, Thiazolidinediones as anti-cancer agents, Cancer Ther. 6(A) (2008) 25-34.
12. L.A. Plastas, J.M. Stewart, The crystal structure of 2-imino-5-phenyl-4-thiazolidinone, Chem. Comm. (1969) 811.
13. V. Amirthalingam; K.V. Muralidharan, The crystal and molecular structure of 2-amino-4-thiazolidinone-5-acetic acid, Chem. Comm. (1969) 986.
14. V.S. Jain, D.K.Vora, C.S.Ramaa, Thiazolidine-2,4-diones: Progress towards multifarious applications, 21 (2013) 1599-1620.
15. Z. S. Safi, A theoretical study on the structure of thiazolidine-2,4-dione and its 5-substituted derivatives in the gas phase. Implications for the thiazolidine-2,4-dione -water complex, 9 (206) 616-625.
16. I.I. Soronovich, V. Vladzimirskaya, M. Turkevich, Thiazolidine-2,4-dione derivatives with an so group outside the ring, Chem HeterocyclCompd (1981) 17: 38. https://doi.org/10.1007/BF00507087
17. A.R.A. Raouf, M.T. Omar, M.M. Habashy, Studies on 4-thiazolidinones. I. Cleavage of 3-Phenyl-2,4-thiazolidinediones with benzylamine and hydrazine, 315 (3) (1973) 492-496.
18. B.R. Prashantha Kumar, M.J. Nanjan, QSAR Study on Thiazolidine-2,4-dione Derivatives for Antihyperglycemic Activity. Indian Journal of Pharmaceutical Sciences. 2008;70(5):565-571.
19. S. Vijaya Laxmi, P. Anil, G. Rajitha, Asha Jyothi Rao, Peter A. Crooks, and B. Rajitha, Synthesis of thiazolidine-2,4-dione derivatives: anticancer, antimicrobial and DNA cleavage studies, J Chem Biol. 9(4) (2016) 97–106.
20. H.M. Randall, R.G. Fowler, N. Fuson, J.R. Dangl, Infrared Determination Of Organic Structures. D. Van Nostrand Company, Inc., New York 1949.
21. G. Bruno, L. Costantino, C. Curinga, R. Maccari, F. Monforte, F. Nicolo, R. Ottana, M.G. Vigorita, Bioorg. Med. Chem. 10 (2002) 1077.
22. R. Ottana, R. Maccari, M.L. Barreca, G. Bruno, A. Rotondo, A. Rossi, G. Chiricosta, R.D. Paola, L. Sautebin, S. Cuzzocrea, M.G. Vigorita, Bioorg. Med. Chem. 13 (2005) 4243.
23. M.M. Ip, P.W. Sylvester, L. Schenkel, Antitumor efficacy in rats of CGP 19984,a thiazolidinedione derivative that inhibits luteinizing hormone secretion, Cancer Res, 46 (1986) 1735-1740.
24. T. Fujita, Y. Sugiyama, S. Taketomi, T. Sohda, Y. Kawamatsu, H. Iwatsuka, Z. Suzuoki,Reduction of insulin resistance in obese and/or diabetic animals by 5-[4-(1-

methylcyclohexylmethoxy)benzyl]-thiazolidine-2,4-dione (ADD-3878, U-63,287, ciglitazone), a new antidiabetic agent, Diabetes, 32 (1983) 804-810.

25. L.F. da Rocha Junior, M.J. Rego, M.B. Cavalcanti, M.C. Pereira, M.G. Pitta, P.S. de Oliveira, S.M. Goncalves, A.L. Duarte, C. de Lima Mdo, R. Pitta Ida, Synthesis of a novel thiazolidinedione and evaluation of its modulatory effect on IFN- gamma , IL-6, IL-17A, and IL-22 production in PBMCs from rheumatoid arthritis patients, Biomed Res Int, 2013 (2013) 926060.
26. A.M. Youssef, M.S. White, E.B. Villanueva, I.M. El-Ashmawy, A. Klegeris, Synthesis and biological evaluation of novel pyrazolyl-2,4-thiazolidinediones as anti-inflammatory and neuroprotective agents, Bioorg Med Chem, 18 (2010) 2019-2028.
27. U. Albrecht, D. Gordes, E. Schmidt, K. Thurow, M. Lalk, P. Langer, Synthesis and structureactivity relationships of 2-alkylidenethiazolidine-4,5-diones as antibiotic agents, Bioorg Med Chem, 13 (2005) 4402-4407.