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SYNTHESIS AND CHARACTERIZATION OF NOVEL 3-(AMINOMETHYL)-5-BENZYLIDENETHIAZOLIDINE-2,4-DIONE DERIVATIVES AS ANTICANCER AGENTS

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ABSTRACT

Thiazolidine-2, 4-dione (TZD) is a vital nucleus in heterocyclic chemistry. Novel 3-(aminomethyl)-5-benzylidenethiazolidine-2,4-dione derivatives were synthesized and characterized by ^{1}H NMR, IR and Mass spectroscopy. The compounds wer synthesized by knoevenagel condensation with cyclization reaction and evaluated for anticancer activity against human cancer cell lines of HeLa (Cervical cancer cells) and HCT-8 (Colon carcinoma) using sulforhodamine B (SRB) method. Compound 3i was the most potent derivative of the series against both cell lines HeLa and HCT-8 with IC_{50} value of 0.007 μM and 0.001 μM, consecutively. By comparing the activities of potent compounds (3a, 3b, 3f, 3g, 3i, 3n, 3q) with other derivatives, it was revealed that the presence of electronegative groups at C-2 and C-3 positions of phenyl ring confers the highest cytotoxic activity against Cervical cancer and Colon carcinoma cell lines. The physicochemical parameters of these compounds followed the Lipinski rule of five.

Keywords: Thiazolidine-2, 4-dione, Cancer cell lines, Synthesis, Characterization.

1. INTRODUCTION

Cancer is the one of the major health issue in the present world. After heart disease it becomes the second leading cause of death. In a WHO report, it is suspected that about 8.2 million people may be die due to cancer in 2021. Lung, liver, stomach, and bowel cancer are the most common causes of human deaths worldwide, accounting for nearly a half of all cancer deaths. The five most common types of disease diagnosed in 2012 were lung, prostate, colorectal, stomach, and liver cancer among men; and breast, colorectal, lung, cervix, and stomach cancer among women [1]. Despite enormous efforts aimed at the implementation of new treatment strategies of chemotherapeutic agents, treatment results in most cases are unsatisfactory [2]. Therefore, there is an urgent need to find new classes of substances with selective action against tumour cells.

Heterocyclic compounds play an important role in cancer therapy. Among them, the derivatives of thiazolidine-2, 4-dione are found useful [3-6]. Researchers' interest in the derivatives of thiazolidine-2,4-dione has increased recently, the main reason being a wide spectrum of biological properties shown by these derivatives. It has been confirmed by numerous reviews on the activity and mechanisms of action of thiazolidine-2,4-diones [2, 7, 8]. Thiazolidine-2,4-diones are known as antidiabetic drugs and include rosiglitazone, pioglitazone, and darglitazone. Moreover, thiazolidine-2,4-dione derivatives possess biological activities such as aldose reductase inhibitory [9], antibacterial [6, 10-13], antifungal [10], antitubercular [14], and antiinflammatory activity [15], etc.

Regulation of both cell proliferation and pathways of apoptosis connected with cell death is important in understanding various diseases including malignancies [16]. Therefore, identification of regulators of the cell cycle and apoptosis stimulators is an attractive strategy to explore potential anticancer agents [17].

A number of derivatives of 2,4-dioxothiazolidine-5-acetic acid with the ring of 5-substituted-2-amino-1,3,4-thiadiazole showed cytotoxic effects *in vitro* against four human tumour cell lines (cervical carcinoma - HeLa, colorectal cancer - HT29, lung cancer - A549, and breast cancer - MCF-7). Among the 14 derivatives, 2-(2,4-dioxo-1,3-thiazolidin-5-yl)-N-[5-(3,4,5-trimethoxyphenyl)-1,3,4-thiadiazol-2-yl]acetamide showed significant inhibitory activity against the tested cell lines [18].

The aim of the present research was to synthesise new

thiazolidine-2,4-dione derivatives and to evaluate *in vitro* their potential as anticancer and antibacterial agents. As thiosemicarbazone [19, 20] and acylhydrazone derivatives [20-24] present anticancer activity, which is similar to the activity of the above mentioned thiazolidine-2,4-diones, it was assumed that the structure modification of the thiazolidine-2,4-dione ring in position 5 by thiosemicarbazide and hydrazide derivatives can extend the biological activity of the new compounds.

2. EXPERIMENTAL

2.1. Material and methods

Melting points were determined using Fisher-Johns apparatus (Fisher Scientific, Schwerte, Germany) and were not corrected. The ^{1}H NMR and ^{13}C NMR spectra were recorded by a Bruker Avance 300 MHz instrument using DMSO-d_6 as solvent and TMS as an internal standard. Chemical shifts were expressed as δ (ppm). MS using atmospheric pressure chemical ionisation (APCI) was recorded on a Bruker MicroTOF II mass spectrometer. APCI settings were as follows: vaporiser temperature, 350°C; drying gas temperature, 180°C; drying gas flow, 4 l/min; and nebuliser pressure, 2 bar. The purity of the compounds was checked by TLC on plates with silica gel Si 60 F_{254}, produced by Merck Co. (Darmstadt, Germany). Elemental analyses were performed by AMZ 851 CHX analyser and the results were within ±0.4% of the theoretical value.

The experimental work included the synthesis and spectral characterization of thiazolidine-2,4-dione derivatives. All the reagents and solvents were obtained from S Merck India Ltd., CDH (central drug house), Sigma -Aldrich, SD fine etc. Melting points were determined in open glass capillaries using melting point apparatus and are uncorrected. Progress of the reactions was monitored by using TLC plates (silica gel G), hexane:ethyl acetate (5:5 and 1:1, v/v) and hexane: acetone (7:3, v/v) used as solvent systems. The spots were located by exposure to iodine vapors or under UV-light. IR spectra were recorded on FT/IR, Shimadzu Fourier transform spectrophotometer (model no. 8400S) using KBr pellets of the compounds and found to be in the range 4000-400 cm^{-1}. ^{1}H NMR and ^{13}C NMR spectra of the synthetic compounds were recorded on Bruker 400 MHz instrument in solvent (DMSO-d_6/$CHCl_3$); chemical shift (δ) values reported in parts per million (ppm) using tetramethylsilane as internal reference. The splitting pattern abbreviations are as follows: s, singlet; bs, broad singlet d, doublet; dd, double doublet; t, triplet; m, multiplet. Mass spectra were recorded on LC-MS Spectrometer, Model Q-ToF Micro, Waters.

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 1).

2.2. 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 minutes till occurrence of white precipitates. To the contents of flask was 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, R_f value: 0.64, Solubility: Product was soluble in water and ethyl alcohol, Yield: 90%, M.P.: 123-125°C, IR (KBr) υ cm^{-1} 3156 (N-H), 3058 (Ar C-H), 1746 (C=O), 623 (C-S-C).

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

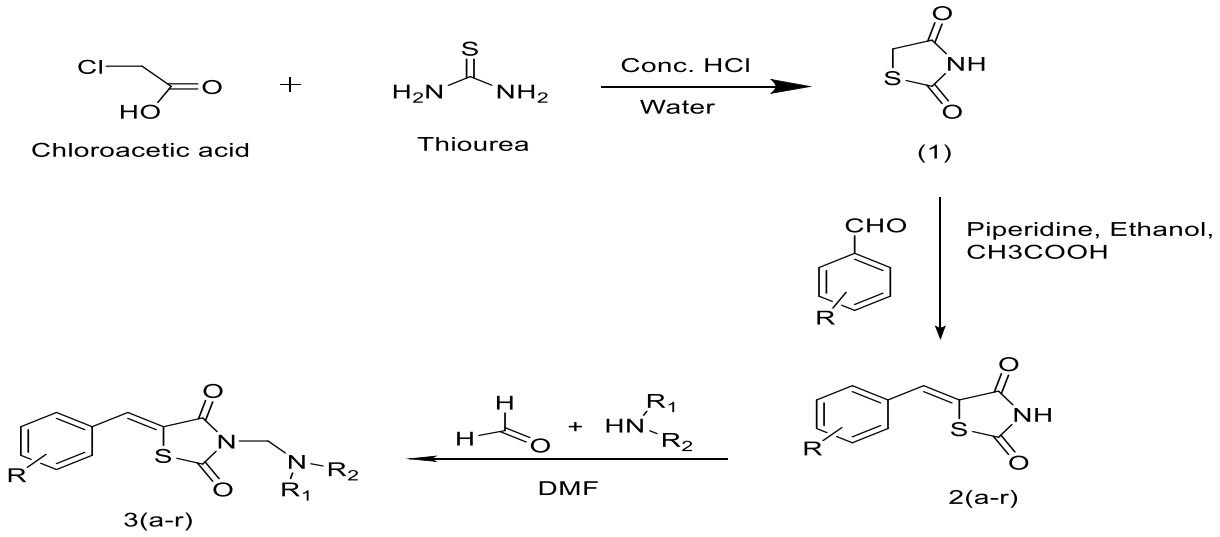
A mixture of 2,4-thiazolidinedione 1 (2.4 g, 20 mmol), benzaldehyde derivative (20 mmol), piperidine (1.4 g, 16 mmol) and ethanol (150 ml) was refluxed for 16-24 h. The reaction mixture was poured into H_2O and acidified with AcOH to give a solid compounds 2(a-r), which were recrystallized from methanol. Completion of reaction has been confirmed using TLC using Benzene: Ethyl acetate as solvent system (3:7), R_f Value = 0.8, Yield: 85%, M.P.: 173-175°C, IR (KBr) υ cm^{-1} 3323, 3170 (N-H), 3049 (Ar C-H), 1649 (C=O);

1H NMR (400 MHz, DMSO-d_6) δ 9.71 (s, 1H, -CHO), 8.25 (s, 1H, -N-H), 7.72 (d, J = 9.3 Hz, 2H, ArH), 7.50 (d, J = 8.0 Hz, 2H, ArH), 4.91 (s, 2H, NH_2); ESI-MS m/z: 165 [$(M + 1)^+$, (13%)], 133 (24%, $C_8H_9O_2$), 105 (100%, C_7H_5O), 79 (22%, C_5H_3O), 63 (49%, C_5H_3).

2.4. 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 was monitored by TLC using solvent system chloroform: methanol (9:1). After the completion of reaction, the mixture was poured in ice cold water, filtered off and washed with hot water. Finally it was recrystallised from chloroform, ethanol to give final compound.

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



Scheme for synthesis

S. No.	Compd. No.	R	R1	R2
1	**3a**	4-Cl	H	H
2	**3b**	4-F	CH_3	CH_3
3	3c	-4OCH_3	H	H
4	**3d**	2,5 di OCH_3	CH_3	CH_3
5	3e	4C_6H_5O-	CH_3	CH_3
6	**3f**	3-Br	C_2H_5	C_2H_5
7	**3g**	4-Br	CH_3	C_2H_5
8	**3h**	3-CN	H	H
9	3i	2,3di-Cl	H	H
10	3j	3-OH	H	H
11	**3k**	3,4di-OH	CH_3	CH_3
12	**3l**	4-Me	H	H
13	**3m**	3,4di Me	CH_3	CH_3
14	**3n**	3-NO_2	H	H
15	3o	2-OH,3-OMe	CH_3	CH_3
16	**3p**	4-NH_2	H	H
17	**3q**	2-OH,5-Cl	CH_3	CH_3
18	**3r**	-4OC_2H_5	CH_3	CH_3

2.5. *In vitro* cytotoxicity

The synthesized derivatives were evaluated for their anticancer activity against selected human cancer cell line of Cervical cancer and Colon carcinoma using sulforhodamine B (SRB) assay. The results of anticancer activity were expressed in terms of growth inhibition

(GI_{50} mM) values and are presented in table 1. RPMI 1640 medium (10% fetal bovine serum and 2 mM L-glutamine) was used for maintaining the cell lines. The cells were inoculated into 96 well microtiter plates in 90 ml 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. 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 ml dilutions of different testing compounds were added into microtiter plates for preparing final drug concentration. These microtiter plates were incubated for 48 h at standard conditions and finally assay was terminated after addition of cold TCA. The cells were fixed by the addition of cold TCA [50 ml, 30% (w/v)] and then incubated for further 60 min 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 ml) at 0.4% (w/v) in 1% acetic acid] was added in each wells and then incubated at room temperature for 20 min. 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 [30, 31]. The growth percentage was calculated by average absorbance of the test wells to the average absorbance of the control wells multiplied by 100. The growth inhibition values (GI_{50}) were observed by the using of formula [(Ti-Tz)/(C-Tz)] x 100 ¼ 50. Where GI_{50} represents the 50% reduction in the net protein increase (as measured by SRB staining) in control cells during the drug incubation, Tz represents for time zero, C for control growth, and Ti for test growth in the presence of drug at the four concentration levels [25, 26].

3. RESULTS AND DISCUSSION

3.1. Chemistry

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-thiazolidine-dione 1 (2.4 g, 20 mmol), benzaldehyde derivatives (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.

3.1.1. Characterization of (5Z)-3-(aminomethyl)-5-(4-chlorobenzylidene)-1,3-thiazolidine-2,4-dione (3a)

R_f Value = 0.7, Yield: 65 %, M.P.: 218-220°C, IR (KBr) υ cm^{-1} 3206 (N-H), 3055 (Ar C-H), 1695 (C=O), 1546 (C=N), and 1482 (C=C); ^{1}H NMR (400 MHz, DMSO-d_6) 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); ESI-MS *m/z*: 268 [(M + 1)$^+$, (10%)]. Anal. Calcd. For $C_{11}H_9ClN_2O_2S$ (%):C, 49.17; H, 3.38; Cl, 13.19; N, 10.42; O, 11.91; S, 11.93; Found: C, 49.17; H, 3.38; Cl, 13.19; N, 10.42; O, 11.91; S, 11.93.

3.1.2. Characterization of (5Z)-3-[(dimethylamino) methyl]-5-(4-fluorobenzylidene)-1,3-thiazolidine-2,4-dione (3b)

R_f Value = 0.6, Yield: 75 %, M.P.: 241-244°C, IR (KBr) υ cm^{-1} IR (KBr) υ cm^{-1} 3182 (N-H), 3062 (Ar C-H), 1658 (C=O), 1546 (C=C) and 756 (C-F); ^{1}H NMR (400 MHz, DMSO-d_6) 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); ESI-MS *m/z*: 280 [(M + 1)$^+$, (10%)], 216 (100%, $C_{11}H_6NO_2S$); Anal. Calcd. For $C_{13}H_{13}ClN_2O_2S$ (%):C, 52.61; H, 4.42; Cl, 11.95; N, 9.44; O, 10.78; S, 10.80. Found: C, 52.61; H, 4.42; Cl, 11.95; N, 9.44; O, 10.78; S, 10.80.

3.1.3. Characterization of (5Z)-3-(aminomethyl)-5-(4-methoxybenzylidene)-1,3-thiazolidine-2,4-dione (3c)

R_f Value = 0.9, Yield: 86 %, M.P.: 234-236°C. , IR (KBr) υ cm^{-1} 3285 (N-H), 3057 (Ar C-H), 1678 (C=O), 1535 (C=C); ^{1}H NMR (400 MHz, DMSO-d_6) 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); ESI-MS *m/z*: 264

[(M + 1)$^+$, (20%)], 216 (86%, $C_{11}H_6NO_2S$); Anal. Calcd. For $C_{12}H_{12}N_2O_3S$ (%):C, 54.53; H, 4.58; N, 10.60; O, 18.16; S, 12.13. Found: C, 54.53; H, 4.58; N, 10.60; O, 18.16; S, 12.13.

3.1.4. Characterization of (5Z)-5-(2,5-dimethoxybenzylidene)-3-[(dimethylamino)methyl]-1,3-thiazolidine-2,4-dione (3d)

R_f Value = 0.7, Solubility: Product was partially soluble in chloroform and soluble in DMF., Yield: 56 %, M.P.: 210-212ºC, IR (KBr) υ cm^{-1} 3032 (Ar C-H), 1697 (C=O), 1546 (C=C), 1278 (C-O-C); ^{1}H NMR (400 MHz, DMSO-d_6) 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); ESI-MS *m/z*: 322 [(M + 1)$^+$, (10%)], 216 (100%, $C_{11}H_6NO_2S$); Anal. Calcd. For $C_{15}H_{18}N_2O_4S$ (%):C, 55.88; H, 5.63; N, 8.69; O, 19.85; S, 9.95; Found C, 55.88; H, 5.63; N, 8.69; O, 19.85; S, 9.95.

3.1.5. Characterization of (5Z)-3-[(dimethylamino)methyl]-5-(4-phenoxybenzylidene)-1,3-thiazolidine-2,4-dione (3e)

R_f Value = 0.6, Yield: 47 %, M.P.: 187-190ºC, IR (KBr) υ cm^{-1} 3032 (Ar C-H), 1658 (C=O), 1546 (C=C), 1278 (C-O-C); ^{1}H NMR (400 MHz, DMSO-d_6) 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); ESI-MS *m/z*: 354 [(M + 1)$^+$, (10%)], 216 (100%, $C_{11}H_6NO_2S$); Anal. Calcd. For $C_{19}H_{18}N_2O_3S$ (%): C, 64.39; H, 5.12; N, 7.90; O, 13.54; S, 9.05. Found: C, 64.39; H, 5.12; N, 7.90; O, 13.54; S, 9.05.

3.1.6. Characterization of (5Z)-5-(3-bromobenzylidene)-3-[(diethylamino)methyl]-1,3-thiazolidine-2,4-dione (3f)

R_f Value = 0.8, Solubility: Product was partially soluble in chloroform and soluble in DMF, Yield: 86 %, M.P.: 183-185ºC, IR (KBr) υ cm^{-1} 3061 (Ar C-H), 1681 (C=O), 1502 (C=C), 1276 (C-O-C), 867 (C-Br); ^{1}H NMR (400 MHz, DMSO-d_6) δ 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); ESI-MS *m/z*: 368 [(M + 1)$^+$, (10%)], 216 (100%, $C_{11}H_6NO_2S$); Anal. Calcd. For $C_{15}H_{17}BrN_2O_2S$ (%):C, 48.79; H, 4.64; Br, 21.64; N, 7.59; O, 8.67; S, 8.68. Found: C, 48.79; H, 4.64; Br, 21.64; N, 7.59; O, 8.67; S, 8.68.

3.1.7. Characterization of (5Z)-5-(4-bromobenzylidene)-3-{[ethyl(methyl)amino]methyl}-1,3-thiazolidine-2,4-dione (3g)

R_f Value = 0.8, Yield: 76 %, M.P.: 176-178ºC, IR (KBr) υ cm^{-1} 3030 (Ar C-H), 1697 (C=O), 1546 (C=C), 1070 (C-Br). ^{1}H NMR (400 MHz, DMSO-d_6) 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); ESI-MS *m/z*: 354 [(M$^+$ + 1), (12%)], 253 (13%, $C_{15}H_{10}FN_2O$), 216 (100%, $C_{11}H_6NO_2S$). Anal. Calcd. For $C_{14}H_{15}BrN_2O_2S$ (%):C, 47.33; H, 4.26; Br, 22.49; N, 7.89; O, 9.01; S, 9.03. Found: C, 47.33; H, 4.26; Br, 22.49; N, 7.89; O, 9.01; S, 9.03.

3.1.8. Characterization of 3-{(Z)-[3-(aminomethyl)-2,4-dioxo-1,3-thiazolidin-5-ylidene]methyl}benzonitrile (3h)

R_f Value = 0.5, Yield: 57 %, M.P.: 232-234ºC, IR (KBr) υ cm^{-1} 3205 (-NH_2), 3032 (Ar C-H), 1658 (C=O), 1546 (C=C); ^{1}H NMR (400 MHz, DMSO-d_6) 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); ESI-MS *m/z*: 259 [(M +1)$^+$, 47%], 244 (15%, $C_{11}H_6N_3O_2S$), 216 (100%, $C_{11}H_6NO_2S$). Anal. Calcd. For $C_{12}H_9N_3O_2S$ (%): C, 55.59; H, 3.50; N, 16.21; O, 12.34; S, 12.37. Found: C, 55.59; H, 3.50; N, 16.21; O, 12.34; S, 12.37.

3.1.9. Characterization of (5Z)-3-(aminomethyl)-5-(2,3-dichlorobenzylidene)-1,3-thiazolidine-2,4-dione (3i)

R_f Value = 0.7, Yield: 86 %, M.P.: 205-207ºC, IR (KBr) υ cm^{-1} IR (KBr) υ cm^{-1} 3240 (N-H), 3030 (Ar C-H), 2993 (Ali C-H), 1681 (C=O); ^{1}H NMR (400 MHz, DMSO-d_6) 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); ESI-MS *m/z*: 303 [(M +1)$^+$, 47%], 244 (15%, $C_{11}H_6N_3O_2S$), 216 (100%, $C_{11}H_6NO_2S$). Anal. Calcd. For $C_{11}H_8C_{l2}N_2O_2S$ (%):C, 43.58; H, 2.66; Cl, 23.39; N, 9.24; O, 10.55; S, 10.5. Found: C, 43.58; H, 2.66; Cl, 23.39; N, 9.24; O, 10.55; S, 10.5.

3.1.10. Characterization of (5Z)-3-(aminomethyl)-5-(3-hydroxybenzylidene)-1,3-thiazolidine-2,4-dione (3j)

R_f Value = 0.7, Yield: 78 %, M.P.: 238-240°C, IR (KBr) υ cm^{-1}, 3140 (N-H)3028 (Ar C-H), 2922 (Ali C-H), 1668 (C=O), 1543 (C=C); ^{1}H NMR (400 MHz, DMSO-d_6) 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); ESI-MS m/z: 250 [$(M + 1)^+$, (26%)], 289 (8%, $C_{17}H_9N_2OS$), 103 (100%, C_8H_7). Anal. Calcd. For $C_{11}H_{10}N_2O_3S$ (%):C, 52.79; H, 4.03; N, 11.19; O, 19.18; S, 12.81. Found: C, 52.79; H, 4.03; N, 11.19; O, 19.18; S, 12.81.

3.1.11. Characterization of (5Z)-5-(3,4-dihydroxybenzylidene)-3-[(dimethylamino)methyl]-1,3-thiazolidine-2,4-dione (3k)

R_f Value = 0.8, Solubility: Product was partially soluble in chloroform and soluble in DMF, Yield: 65 %, M.P.: 214-216°C, IR (KBr) υ cm^{-1} 3032 (Ar C-H), 2980 (Ali C-H), 1655 (C=O), 1544 (C=C); ^{1}H NMR (400 MHz, DMSO-d_6) 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); ESI-MS m/z: 294 [$(M + 1)^+$, (26%)], 289 (8%, $C_{17}H_9N_2OS$), 103 (100%, C_8H_7); Anal. Calcd. For $C_{13}H_{14}N_2O_4S$ (%):C, 53.05; H, 4.79; N, 9.52; O, 21.74; S, 10.89. Found: C, 53.05; H, 4.79; N, 9.52; O, 21.74; S, 10.89.

3.1.12. Characterization of (5Z)-3-(aminomethyl)-5-(4-methylbenzylidene)-1,3-thiazolidine-2,4-dione (3l)

R_f Value = 0.8, Yield: 85 %, M.P.: 185-187°C, IR (KBr) υ cm^{-1} 3150 (N-H), 3080, 3062 (Ar C-H), 1680 (C=O), 1286 (C-O-C); ^{1}H NMR (400 MHz, DMSO-d_6) 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); ESI-MS m/z: 248 [$(M + 1)^+$, (26%)], 289 (8%, $C_{17}H_9N_2OS$), 103 (100%, C_8H_7); Anal. Calcd. For $C_{12}H_{12}N_2O_2S$ (%): C, 58.05; H, 4.87; N, 11.28; O, 12.89; S, 12.91. Found: C, 58.05; H, 4.87; N, 11.28; O, 12.89; S, 12.91.

3.1.13. Characterization of (5Z)-3-[(dimethylamino)methyl]-5-(3,4-dimethylbenzylidene)-1,3-thiazolidine-2,4-dione (3m)

R_f Value = 0.7, Yield: 67 %, M.P.: 225-227°C, IR (KBr) υ cm^{-1} 3086 (Ar C-H), 2993 (Ali C-H), 1670 (C=O), 1546 (C=C); ^{1}H NMR (400 MHz, DMSO-d_6) 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); ESI-MS m/z: 290 [$(M + 1)^+$, (26%)], 289 (8%, $C_{17}H_9N_2OS$), 103 (100%, C_8H_7); Anal. Calcd. For $C_{15}H_{18}N_2O_2S$ (%):C, 62.04; H, 6.25; N, 9.65; O, 11.02; S, 11.04. Found: C, 62.04; H, 6.25; N, 9.65; O, 11.02; S, 11.04.

3.1.14. Characterization of (5Z)-3-(aminomethyl)-5-(3-nitrobenzylidene)-1,3-thiazolidine-2,4-dione (3n)

R_f Value = 0.5, Yield: 57 %, M.P.: 228-230°C, IR (KBr) υ cm^{-1} 3161 (N-H), 3072 (Ar C-H), 2978 (Ali C-H), 1670 (C=O), 1533 (C=C); ^{1}H NMR (400 MHz, DMSO-d_6) 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); ESI-MS m/z: 279 [$(M + 1)^+$, (26%)], 289 (8%, $C_{17}H_9N_2OS$), 103 (100%, C_8H_7); Anal. Calcd. For $C_{11}H_9N_3O_4S$ (%): C, 47.31; H, 3.25; N, 15.05; O, 22.92; S, 11.48. Found: C, 47.31; H, 3.25; N, 15.05; O, 22.92; S, 11.48.

3.1.15. Characterization of (5Z)-3-[(dimethylamino)methyl]-5-(2-hydroxy-3-methoxybenzylidene)-1,3-thiazolidine-2,4-dione (3o)

R_f Value = 0.7, Yield: 86 %, M.P.: 237-239°C., IR (KBr) υ cm^{-1} 3032 (Ar C-H), 2883 (Ali C-H), 1670 (C=O), 1546 (C=C), 1280 (C-O-C); ^{1}H NMR (400 MHz, DMSO-d_6) 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); ESI-MS m/z: 308 [$(M + 1)^+$, (26%)], 289 (8%, $C_{17}H_9N_2OS$), 103 (100%, C_8H_7); Anal. Calcd. For $C_{14}H_{16}N_2O_4S$ (%): C, 54.53; H, 5.23; N, 9.08; O, 20.75; S, 10.40. Found: C, 54.53; H, 5.23; N, 9.08; O, 20.75; S, 10.40.

3.1.16. Characterization of (5Z)-5-(4-aminobenzylidene)-3-(aminomethyl)-1,3-thiazolidine-2,4-dione (3p)

R_f Value = 0.7, Yield: 86 %, M.P.: 173-175°C, IR (KBr) υ cm^{-1} 3113 (N-H), 3080 (Ar C-H), 2968 (Ali C-H), 1691 (C=O), 1529 (C=C); ^{1}H NMR (400 MHz, DMSO-d_6) 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); ESI-MS m/z: 250 [$(M+1)^+$, (26%)], 289 (8%, $C_{17}H_9N_2OS$), 103 (100%, C_8H_7); Anal. Calcd. For $C_{11}H_{11}N_3O_2S$ (%):C, 53.00; H, 4.45; N, 16.86; O, 12.84; S, 12.86. Found: C, 53.00; H, 4.45; N, 16.86; O, 12.84; S, 12.86.

3.1.17. Characterization of (5Z)-5-(5-chloro-2-hydroxybenzylidene)-3-[(dimethylamino) methyl]-1,3-thiazolidine-2,4-dione (3q)

R_f Value = 0.7, Yield: 85 %, M.P.: 196-198°C, IR (KBr) υ cm^{-1} 3168 (N-H), 3084 (Ar C-H), 2941 (Ali C-H), 1680 (C=O), 1535(C=C), 1286 (C-O-C); ^{1}H NMR (400 MHz, DMSO-d_6) 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); ESI-MS m/z: 312 [$(M+1)^+$, (26%)], 289 (8%, $C_{17}H_9N_2OS$), 103 (100%, C_8H_7); Anal. Calcd. For $C_{13}H_{13}ClN_2O_3S$ (%):C, 49.92; H, 4.19; Cl, 11.34; N, 8.96; O, 15.35; S, 10.25. Found: C, 49.92; H, 4.19; Cl, 11.34; N, 8.96; O, 15.35; S, 10.25.

3.1.18. Characterization of (5Z)-3-[(dimethylamino)methyl]-5-(4-ethoxybenzylidene)-1,3-thiazolidine-2,4-dione (3r)

R_f Value = 0.7, Yield: 84 %, M.P.: 226-228°C, IR (KBr) 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); ^{1}H NMR (400 MHz, DMSO-d_6) 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); ESI-MS m/z: 306 [$(M+1)^+$, (26%)], 289 (8%, $C_{17}H_9N_2OS$), 103 (100%, C_8H_7); Anal. Calcd. For $C_{15}H_{18}N_2O_3S$ (%): C, 58.80; H, 5.92; N, 9.14; O, 15.67; S, 10.47. Found: C, 58.80; H, 5.92; N, 9.14; O, 15.67; S, 10.47.

Table 1: Structure and Physical properties 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

3.2. Biology

The compounds (3a-r) synthesized in the present work 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) assay. The results of anti-cancer activity are expressed in terms of inhibitory concentration fifty (IC_{50} μM) values and are shown in Table 2.

Compound 3i was the most potent derivative of the series against both cell lines HeLa and HCT-8 with IC_{50} 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 IC_{50} value of 0.032 μM and 0.028, consecutively. Compounds 3f,

3g, 3n, 3q showed significant improvement in activity with IC_{50} 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 IC_{50} 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 IC_{50} 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 IC_{50} 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. The whole molecule divided into three parts where one thiazolidine-2,4-dione ring connected with disubstituted amino group. The literature survey revealed that when thiazolidine-2,4-dione connected with di-arylamino group important for activity.

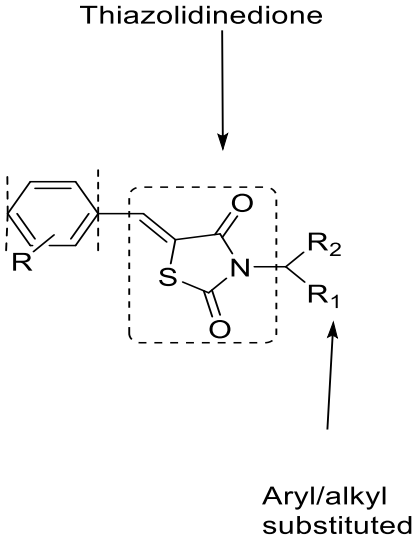


Table 2: *In vitro* antiproliferative activity (IC_{50}) of synthesized compounds 3(a-r) against Cervical cancer cells (HeLa) and Colon carcinoma (HCT-8).

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*

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.

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.

3.3. Computational studies

3.3.1. In silico prediction of physicochemical properties

Physicochemical properties of the synthesized compounds 3a-r were calculated using QikProp module of Schrodinger software. In the present study different *in silico* values of synthesized compounds were predicted such as Mol. Wt., polar surface area (PSA), QPlogPo/w, predicted apparent Caco-2 permeability (QPPCaco), predicted brain/blood partition coefficient (QPlogBB), predicted apparent MDCK cell permeability (QPPMDCK), solvent accessible surface area (SASA) and percent human oral absorption. These values were essential for correlating the biological activity with its physicochemical properties. The predicted values are presented in table 3.

Lipophilicity is one of the basic molecular property and important parameter in the design of drugs. Lipophilicity, assure the ability of a molecule to mix with an oily phase rather than with water, is usually measured as partition coefficient, *P*, between the two phases and is often expressed as log *P*. In the present study, we have calculated the logP by Schrodinger software to identify the relationship of thiazolidine-2,4-diones and anticancer activity. Here the compound 3e was the most lipophilic compound of the series whereas 3p was identified as less lipophilic. Furthermore, 3i showed IC_{50} value around 0.007 μM which may be a cause for its intermediate activity against Cervical cancer cell line.

Table 3: Molecular descriptors of thiazolidine-2,4-dione derivatives.

Compounds	Mol. Wt.	PSA	QPlog Po/w	QPP Caco	QPlog BB	QPP MDCK	SASA	PercentHuman OralAbsorption
3a	268.72	159.28	1.94	79.19	-0.43	132.80	479.79	72.30
3b	280.32	169.62	2.02	387.64	0.15	540.08	518.85	85.11
3c	264.30	151.76	1.56	80.62	-0.66	54.85	492.06	70.19
3d	322.38	101.81	1.96	386.05	-0.13	298.94	590.69	84.69
3e	354.42	342.07	3.40	391.74	-0.17	302.43	644.87	93.25
3f	369.28	157.84	3.05	541.51	0.22	1127.12	578.13	93.74
3g	355.25	158.82	2.75	441.63	0.19	912.24	563.39	90.39
3h	259.28	173.63	0.73	16.72	-1.36	10.02	493.78	53.10
3i	303.16	130.84	2.33	80.29	-0.32	249.30	496.44	74.68
3j	250.27	165.44	0.72	24.03	-1.14	14.83	468.78	55.89
3k	294.33	129.41	0.71	41.95	-1.03	27.01	532.99	60.14
3l	248.30	150.90	1.76	80.37	-0.61	54.73	487.33	71.35
3m	290.38	112.96	2.39	387.34	0.01	295.21	564.42	87.24
3n	279.27	149.75	0.77	9.87	-1.54	5.56	492.31	49.26
3o	308.35	122.30	1.51	155.04	-0.50	111.33	558.81	74.99
3p	249.29	161.37	0.53	21.02	-1.19	12.82	470.36	53.72
3q	312.77	126.05	1.85	143.09	-0.29	249.82	543.83	76.37
3r	306.38	151.76	2.32	387.32	-0.15	298.43	593.78	86.85

Molecular weight and polar surface area of compounds were in the range of Lipinski rule of 5, optimum with respect to number of hydrogen bonding donors and acceptors. Molecules with a polar surface area of greater

than 140 Å squared responsible for poor cell membranes permeability. Most of the compounds of the series showed the value below 140 Å. Compound 3i showed potential anticancer activity with PSA value of 130 Å squared.

Descriptors like QPPCaco represent the apparent permeability across the Caco-2 cell membrane. QPPCaco value less than <25 showed poor permeability whereas >500 represents for better permeability. Compounds 3h, 3j, 3n and 3p were showed poor Caco values whereas compound 3f showed good value.

QPlogBB value represents the brain/blood partition coefficient (0.22 to -0.43) and QPPMDCK value represents the apparent permeability across the MDCK cells which can be considered as good mimic for blood brain barrier (BBB) by non-active transport. Compound 3i showed best QPlogBB and QPPMDCK values. SASA represents the total solvent accessible surface area in square angstroms using a probe with a 1.4 Å radius. The values of SASA should be in the range of 300.0-1000.0. In summary, Qikprop predictions suggests that thiazolidine-2,4-dione derivatives have optimum parameters for anticancer activity and can be considered as lead molecule for further modifications.

4. CONCLUSION

In the present study an attempt has been made to identify the necessary structural requirements of thiazolidine-2,4-dione derivatives for potential anticancer activity. In summary, the atom-based 3D-QSAR studies has been performed on thiazolidine-2,4-dione derivatives. The common pharmacophore model predicted by 3D-QSAR method implies the presence of several important pharmacophore features such as the presence of electron withdrawing group, aromaticity, EDG and hydrophobic long chain along with the presence of H-bond donors and acceptors for their inhibitory potencies towards cancer. Resemblance of these pharmacophore features as predicted by 3DQSAR study with the experimentally outlined structural features in the thiazolidine-2,4-dione analogs indicates the suitability of 3D-QSAR approach to validate the experimental results. This approach led us to short-list most active derivatives with the incorporation of more than one structural feature in a single molecule. The description of 3D QSAR study showed the thiazole ring substituted with hydrogen bond acceptor is important for activity. Phenyl ring substituted with other heterocyclic rings with electron withdrawing groups may increase the anticancer activity. Furthermore, hydrophobic group substitution on thiazole ring may also increase the anticancer activity. From the overall analyses, we conclude that the Model 1 (AADHR_1) pharmacophore truly reflects the features of potent inhibitors and this pharmacophore could be used as fast and accurate tool to assist discovery of anticancer agents.

On the basis of this pharmacophore several thiazolidine-2,4-dione derivatives has been logically generated and proceed for synthesis. The compounds were taken for synthesis by efficient synthetic route. Through this scheme we have identified a novel class of thiazolidine-2,4-dione derivatives as potent and selective inhibitors for anticancer activity. These derivatives showed potential activity against Cervical cancer cells (HeLa) and Colon carcinoma (HCT-8). Among the synthesized compounds, 3i was the most potent derivative of the series against both cell lines HeLa and HCT-8 with IC_{50} value of 0.007 μM and 0.001 μM, consecutively. The SAR study revealed that phenyl ring is essential for anti-cancer activity whereas replacement of phenyl ring with chloro group at C-2 and C-3 positions of phenyl ring confers the highest cytotoxic activity against leukaemia and Colon carcinoma cell lines. Similarly, substitution of electron donating groups on phenyl ring produced decreased anti-cancer activity. In conclusion, the structural features of compound 3i may be considered for the development of newer anti-cancer agents.

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Conflict of interest

None declared

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