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Design, synthesis and characterization of novel benzothiazole derivatives containing thiazolidine-2, 4-dione for anti-cancer screening

Aleti Rajareddy and M Srinivas Murthy

Abstract
A novel synthetic methodology for preparation of Novel benzothiazole derivatives containing thiazolidine-2, 4-dione were prepared by knoevenagel condensation reaction, Schiff's base and manich base reaction. The newly synthesized compound was characterized using 1H-NMR, FT-IR and MASS spectrometry. The anticancer activity of the synthesized compounds was evaluated by tube dilution method. All the synthesised compounds were carried out the *in vitro* Anti-cancer activity by MTT Assay. The compounds 4a, 4f and 4h exhibited good anticancer activity against breast (MCF-7) cancer cell lines at a concentration of 0.5 mg / mL^{-1}.

Keywords: 2-Amino benzothiazole, Terephthalaldehyde, thiazolidine-2, 4-dione, Anti-cancer activity

1. Introduction
Heterocyclic compounds containing nitrogen and sculpture have considerably a lot of attention due to wide application of pharmacological activity. Since the 1990s, various pharmacological investigations of newly synthesized benzothiazoles demonstrated interesting pharmacological activities and led to the development of new medications for treating diseases. The substituted benzothiazole derivatives have antitumor [1], antitubercular [2], antifungal [3], CNS [4-5] activities. In addition to their diverse biological activities, in association with other heterocyclics, benzothiazole are known to play a crucial role in several processes of chemical and pharmacological importance as therapeutics in clinical applications. In the view of having a wide scope to find new potentially active agents, we have synthesized a new series of benzothiazole thiazolidine-2,4-dione derivatives (4a-4l) which is an extension of our previous reported work on biological studies of novel 2-(4-sub-stitutedbenzylthio)-5-amino-6-(benzo[d]thiazol-2-yl)-7-(4-chlo-rophenyl)pyrido[2,3-d]-pyrimidin-4(3H)-one. Moreover benzothiazole [6-8] is alternative vital pharmacodynamic heterocyclic nuclei that once incorporated in several heterocyclic templates have currently been possessing wide spectrum of activities.
Thiazolidine-2, 4-dione (TZD) is a vital nucleus in heterocyclic chemistry. TZD shows multidirectional pharmacological activities such as antioxidant [9], antihyperglycemic (glitazone drugs) [10, 11], antibacterial and anti-fungal [12], anticancer [13], antimicrobial, antitubercular [14], anti-arthritic [15], diabetic & diabetic complications [16, 17] and anti-inflammatory activity [18]. Due to various pharmacological actions of TZD derivatives, researchers keep on huge interest in synthesis of new TZD derivatives by using various synthetic methods and carry out clinical trials for achieving lead target. Structures of the products were characterized by IR, 1HNMR and LC-MS mass spectrometry. Results of biological activities indicate that some compounds possess potential Antibacterial activity and anticancer activity.

2. Materials and methods
In this Investigation chemicals were purchased from local dealer with S.D fine make was used. The synthesized compounds were screened for anti-cancer activities. Melting points were determined in an open capillary and are uncorrected. The IR spectra were recorded on Schimadzu FTIR-8400S. 1H-NMR spectra were recorded in DMSO-d_6 and chemical shifts (δ) on Bruker DRX-300 MHz. spectrometer using TMS as internal reference with their values expressed in δ ppm. Purity of all the synthesized compounds were routinely checked by TLC on silica gel G in the solvent system (n-Hexane: Ethyl acetate (7:3)).

2.2 General procedures

2.1 General procedure for thiazolidine-2, 4-Dione (1).

The equimolar quantity (1:1) of chloroacetic acid (56.4 g, 0.6mol) in 60 ml of water was added to the solution of thiourea (45.6 g, 0.6mol) in 60 ml of water. The mixture was stirred for 15 min. and precipitates were obtained after cooling. Then add slowly 60 ml of concentrated hydrochloric acid from a dropping funnel. Once the mixture got converted to solution form, it was refluxed for 8-10 hour at 100-110°C. On cooling, the contents of the flask solidified to a cluster of white needles. The product was filtered and washed with water to remove traces of hydrochloric acid and dried. It was purified by recrystallization from ethyl alcohol. Yield: 85%; m.p.:123-125°C.

2.2 General procedure for the synthesis of 5-substituted-1, 3-thiazolidine-2, 4-dione (2)

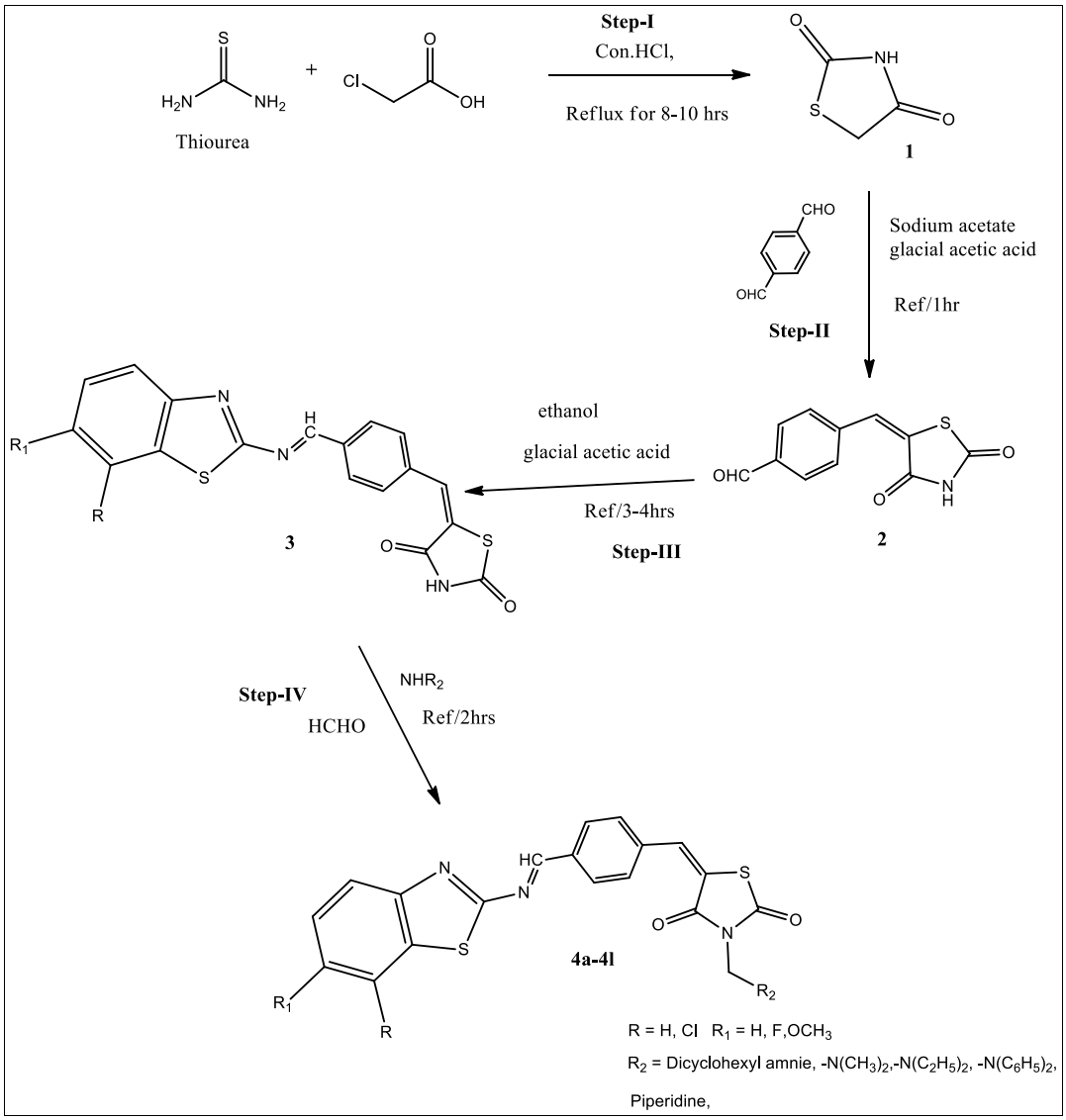
A mixture of 2,4-thiazolidinedione 1 (1.17g, 0.01mol), substituted Benzaldehyde (0.01mol), glacial acetic acid (25mL) and fused sodium acetate (0.18g) was refluxed for 1 hr with occasional shaking. Cool, then the reaction mixture was cooled to room temperature and it was poured in water (250mL), the product obtained was filtered, washed with water, alcohol and ether and was recrystallized with glacial acetic acid.

2.3 General procedure for the synthesis of 5-((E)-4-((E)-(benzo[d]thiazol-2-ylimino) methyl) benzylidene) thiazolidine-2, 4-dione (3)

A mixture of equimolar quantity of 2-amino benzothiazole (0.01mol) and Compound 2 was dissolved in 20ml of ethanol, refluxed for 3-4hrs in the presence of few drops of (2ml) glacial acetic acid. The progress of the reaction was monitored by TLC (n-Hexane: EtoAc 7:3). The reaction mixture was cooled to room temperature and keep in refrigerator for overnight to get precipitate. A solid was obtained, which was filtered off and recrystallized from methanol or ethanol to give crystalline solid.

2.3 General procedure for the synthesis of 5-((E)-4-((E)-((benzo[d]thiazol-2-yl) imino) benzylidene)-3-(piperazin-1-ylmethyl) thiazolidine-2, 4-dione (4a-4l)

5-((E)-4-((E)-(benzo[d]thiazol-2-ylimino) methyl) benzylidene) thiazolidine-2, 4-dione (3) (0.01 mol) in alcohol (30 ml) was stirred with aq. formaldehyde (0.02 mol) at room temperature for 1hr. The reaction mixture was then heated under reflux with an appropriate secondary amine (0.02 mol) for about 3 hrs. The alcohol was removed; the residue was cooled and left overnight in a refrigerator. The resultant product was triturated with crushed ice. The product was filtered under suction, washed 2 or 3 times with small portions of ice-cold water and dried. The compound was purified by recrystallisation from aq. Alcohol.



Scheme

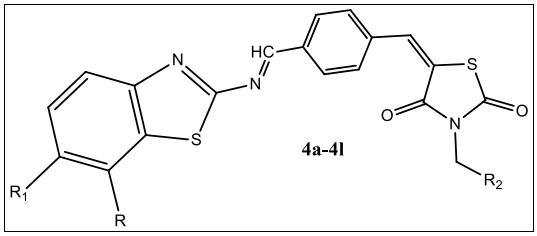
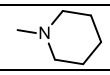
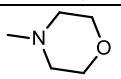
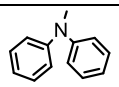
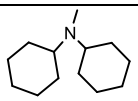
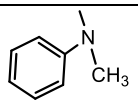
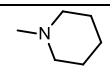


Table 2: Physical data of Compounds 5a-5o

Compound	Molecular Formula	R	R_1	R_2	Molecular Weight (gms)	M.P (^{0}C)
4a	$C_{21}H_{18}N_4O_2S_2$	H	H	$-N(CH_3)_2$	422	247-249
4b	$C_{23}H_{22}N_4O_2S_2$	H	H	$-N(C_2H_5)_2$	450	261-263
4c	$C_{24}H_{22}N_4O_2S_2$	H	H		462	217-219
4d	$C_{23}H_{20}N_4O_3S_2$	H	H		464	271-273
4e	$C_{31}H_{27}N_4O_2S_2$	H	H		546	261-263
4f	$C_{31}H_{34}N_4O_2S_2$	H	H		558	205-207
4g	$C_{26}H_{20}N_4O_2S_2$	H	H		484	231-233
4h	$C_{21}H_{16}ClFN_4O_2S_2$	F	Cl	$-N(CH_3)_2$	474	260-261
4i	$C_{23}H_{20}ClFN_4O_2S_2$	F	Cl	$-N(C_2H_5)_2$	502	217-219
4j	$C_{24}H_{20}N_4O_3S_2$	$-OCH_3$	H	$-N(CH_3)_2$	452	196-198
4k	$C_{24}H_{24}N_4O_3S_2$	$-OCH_3$	H	$-N(C_2H_5)_2$	480	213-215
4l	$C_{25}H_{24}N_4O_3S_2$	$-OCH_3$	H		492	270-272

4a.5-((E)-4-((E)-benzo[d]thiazol-2-yl) imino) methyl) benzylidene)-3-dimethylamino-1-yl- methyl) thiazolidine-2, 4-dione. Mol. formula: $C_{22}H_{20}N_4O_2S_2$, Rf value: 5.8, yield 80%, IR (ν cm−1): 3143, 3054(C-H *Str*, Ar), 2930, 2891, 2793(C–H *Str*, Aliphatic), 2311 (C-S-C *Str*), 1684(C=O *Str*, Indole), 1588 (C=N *Str*), 1515 (C=CH *Str*), 1431 (C=C *Str*, Ar). ^{1}H-NMR (DMSO) $\delta\delta$ ppm: 8.38-8.27(d, 2H, Ar-H), 8.11-7.88(d, 2H, Ar-H), 7.84-7.77(t, 3H, Ar-H), 7.69-7.67 (d, 2H, Ar-H), 7.55-7.54 (d, 2H, Ar-H), 7.51-7.41 (t, 3H, Ar-H). 3.33(S, 3H, -CH_3); Mass (ESI-MS): m/z 422(M), 423(M + 1, 100%).

4b: (5-((E)-4-((E)-benzo[d]thiazol-2-yl)imino)methyl)benzylidene)-3-diethylamino-1-yl-methyl) thiazolidine-2,4-dione. $C_{23}H_{22}N_4O_2S_2$, Rf value: 6.7, yield 74%, IR (ν cm−1): 3037, 2932(C-H *Str*, Ar), 2872(C–H *Str*, Aliphatic), 2346 (C-S-C *Str*), 1721(C=O *Str*, Indole), 1555(C=N *Str*), 1520(C=CH *Str*), 1432 (C=C *Str*, Ar), 771(C-Cl *Str*, Ar). ^{1}H-NMR (DMSO) $\delta\delta$ ppm: 8.37-8.28(t, 3H, Ar-H), 7.88-7.84(t, 3H, Ar-H), 8.10(s, 1H, Ar-H), 7.83-7.68(d, 4H, Ar-H), 7.58-7.57(d, 2H, Ar-H), 7.55-7.51 (d, 2H, Ar-H), 3.39(s, 3H, -CH_3); Mass (ESI-MS): m/z 450(M), 451(M + 1, 100%).

4c.5-((E)-4-((E)-benzo[d]thiazol-2-yl)imino)methyl)benzylidene)-3-piperidine-1-methyl) thiazolidine-2,4-dione. $C_{24}H_{22}N_4O_2S_2$, Rf value: 7.2, yield 86%,. IR (ν cm−1): IR (ν cm−1): 3100 (C-H *Str*, Ar), 2987,2882(C–H *Str*, Aliphatic), 2336(C-S-C *Str*), 1705(C=O *Str*, Indole), 1663, 1546(C=N *Str*), 1506(C=CH *Str*), 1459(C=C *Str*, Ar), 797(C-Cl *Str*, Ar), 588(C-F *Str*, Ar). ^{1}H-NMR (DMSO) $\delta\delta$ ppm: 8.57-8.35(d, 4H, Ar-H), 8.06-8.04(d, 4H, Ar-H), 7.94-7.92(t, 2H, Ar-H), 7.82-7.75(t, 2H, Ar-H), 3.31(s, 3H, -CH_3), 1.97-1.94(s, 3H, -CH_3); Mass (ESI-MS): m/z 462(M), 463(M + 1, 100%).

4d: 5-((E)-4-((E)-benzo[d]thiazol-2-yl)imino)methyl)benzylidene)-3-morpholine-1-methyl) thiazolidine-2,4-dione $C_{23}H_{20}N_4O_3S_2$, Rf value: 5.3, yield 81%,. IR (ν cm−1): 3093(C-H *Str*, Ar), 2976, 2884(C–H *Str*, Aliphatic), 2383(C-S-C *Str*), 1699(C=O *Str*, Indole), 1578(C=N *Str*), 1565(C=CH *Str*), 1476(C=C *Str*, Ar), 803(C-Cl *Str*, Ar). ^{1}H-NMR (DMSO) $\delta\delta$ ppm: 8.47-8.37(d, 2H, Ar-H), 7.95-7.88(d, 4H, Ar-H), 7.40-7.39(t, 2H, Ar-H), 7.35(s, 1H, Ar-H), 7.35-7.34(d, 2H, Ar-H), 3.34-3.30(s, 3H, -CH_3), 1.986-1.982(s, 3H, -CH_3); Mass (ESI-MS): m/z 464(M), 465(M + 1, 100%).

4e: 5-((E)-4-((E)-benzo[d]thiazol-2-yl) imino)methyl)benzylidene)-3-diphenyl amine-1-methyl) thiazolidine-2,4-dione. $C_{31}H_{22}N_4O_2S_2$, Rf value: 6.8, yield 74%, IR (ν cm−1): 3065(C-H *Str*, Ar), 2987, 2894(C–H *Str*, Aliphatic), 2365(C-S-C *Str*), 1716C=O *Str*, Indole), 1584(C=N *Str*), 1543(C=CH *Str*), 1467(C=C *Str*, Ar), ^{1}H-NMR (DMSO) $\delta\delta$ ppm: 7.98-7.90(d, 2H, Ar-H), 7.78-7.80(d, 2H, Ar-H), 7.68-7.67(d, 2H, Ar-H), 7.40-7.39(t, 2H, Ar-H),

7.29-7.28(t, 3H, Ar-H), 7.14-7.10(t, 2H, Ar-H), 2.14-2.10(s, 3H, -CH_3); Mass (ESI-MS): m/z 546(M), 547(M + 1, 100%).

3. Pharmacological activity: Anticancer activity [8-10]

3.2. Antibacterial Studies: All the compounds (4a–4l) have been screened for antibacterial activity using cup-plate agar diffusion method by measuring the inhibition zone in mm. Streptomycin (50 µg/mL) used as a standard drugs for antimicrobial activity. The compounds were screened for antibacterial activity against *Salmonella paratyphi, P.auregunosa, E.Coli, P.mirabilis, L.bacillus, S.pyrogenus* species in nutrient agar medium. All values are expressed as Zone of Inhibition in mm, Bore size = 6mm; *Compounds showed maximum activity against respective Fungal; Zone size 9-11 = Poor activity; Zone size 12-18 = Moderate activity, Concentration of test compounds is 50µg/ml. Antibacterial activity values among the test compounds are presented in Table No.1. All the test compounds (4a–4l) showed a varied degree of antibacterial activity with broad spectrum of activity against the entire Gram negative and Gram positive bacterial strains employed. However, among this series of compounds 4b, 4d, 4e and 4i had good Antibacterial activity showed high activity.

3.2. Anticancer activity

Cell viability was evaluated by the MTT Assay with three independent experiments with six concentrations of compounds in triplicates. Cells were trypsinized and perform the trypan blue assay to know viable cells in cell suspension. Cells were counted by hemocytometer and seeded at density of 5.0 X 10^3 cells / well in 100 µl media in 96 well plate culture medium and incubated overnight at 37 ^{0} C. After incubation, take off the old media and add fresh media 100 µl with different concentrations of test compound in labelled wells in 96 plates. After 48 hrs., Discard the drug solution and add the fresh medic with MTT solution (0.5 mg / $mL^{-1)}$ was added to each well and plates were incubated at 37 ^{0} C for 3 hrs. At the end of incubation time, precipitates are formed as a result of the reduction of the MTT salt to chromophore Formosan crystals by the cells with metabolically active mitochondria. The optical density of solubilised crystals in DMSO was measured at 570 nm on a microplate reader. The percentage growth inhibition was calculated using the following formula and concentration of test drug needed to inhibit cell growth by 50 % values is generated from the dose-response curves for each cell line using with origin software.

$$\% \text{ Inhibition} = \frac{100\ (\text{Control} - \text{Treatment})}{\text{Control}}$$

The results of this study revealed that all synthesized compounds significantly cytotoxic in a concentration dependent manner and results were shown in table 2. The IC50 values of all test compounds were found to be between 17.79 - 72.72 mM. All the synthesized compounds have shown moderate cytotoxic activity.

4. Results and discussion

4.1 Synthesis: A novel synthetic methodology for preparation of Novel benzothiazole derivatives containing thiazolidine-2,4-dione were prepared by knoevenagel condensation reaction, Schiff's base and manich base reaction between thiazolidine-2,4-dione with Terephthalaldehyde, which on reacted with substituted aldehyde and finally it reacts with secondary amines to give Novel benzothiazole title compounds.

4.2 Spectroscopy

The structures of all the newly synthesized compounds were characterized as 4a-4l on the basis of satisfactory analytical and spectral data including IR, LC-MASS and ^{1}H NMR data.

Table 3: Antibacterial activity by Zone of Inhibition (in mm)

S. No	Micro Organism	Zone of inhibition (mm)					
		Salmonella paratyphi	***P. auregunosa***	***E.Coli***	***P.mirabilis***	***L.bacillus***	***S.pyrogenus***
1	4a	10	19	17	13	14	15
4	4b	23	12	15	19	24	17
3	4c	14	17	14	15	16	13
4	4d	21	25	14	0	10	0
5	4e	9	24	13	13	25	11
6	4f	11	0	0	11	10	24
7	4g	9	0	10	10	0	0
8	4h	16	14	18	11	10	0
9	4i	20	19	18	26	23	16
10	4j	10	21	15	19	16	15
11	4k	19	20	16	18	13	0
12	4l	0	0	0	0	13	15
13	Streptomycin	30	32	32	33	28	30

Table 2: Cytotoxic Activity of Novel benzothiazole derivatives on MCF-7 Cells

Sample Description	Test Parameters IC_{50} (µg)
	MCF 7
4a	17.98
4b	24.07
4c	50.75
4d	52.6
4e	25.35
4f	21.88
4g	72.72
4h	17.79

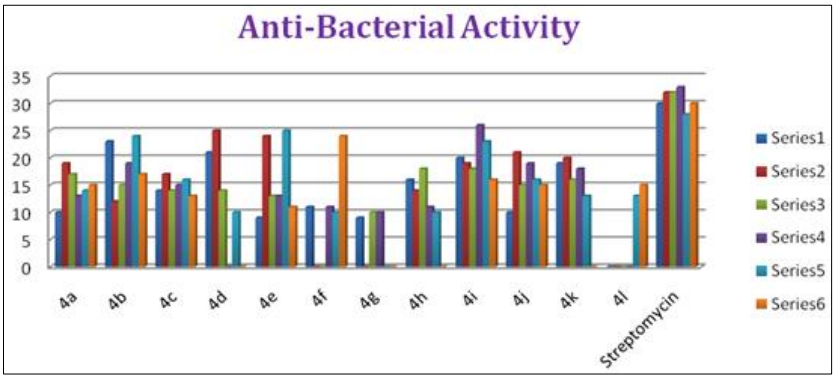


Fig 1: Graphical representation of antibacterial activity of Novel benzothiazole derivatives

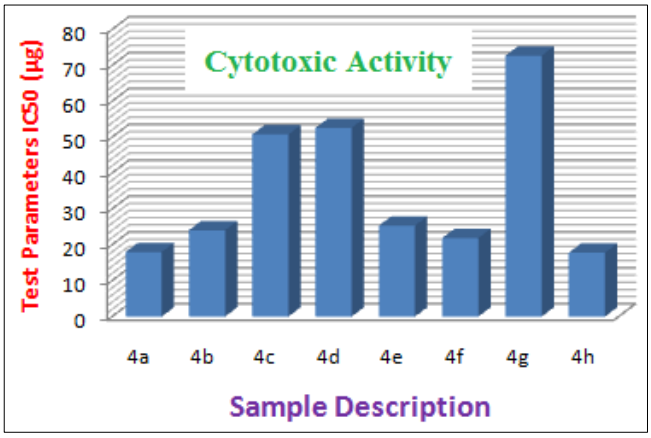


Fig 2: Graphical representation of Cytotoxic Activity of Novel benzothiazole derivatives on MCF-7 Cells

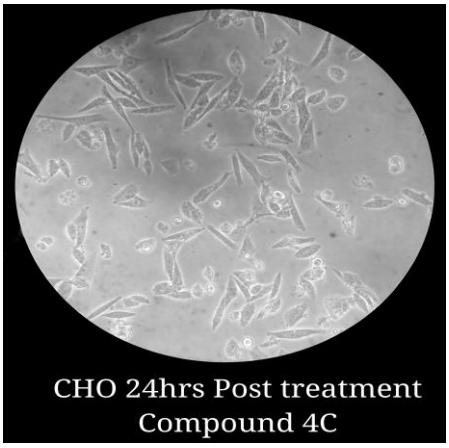
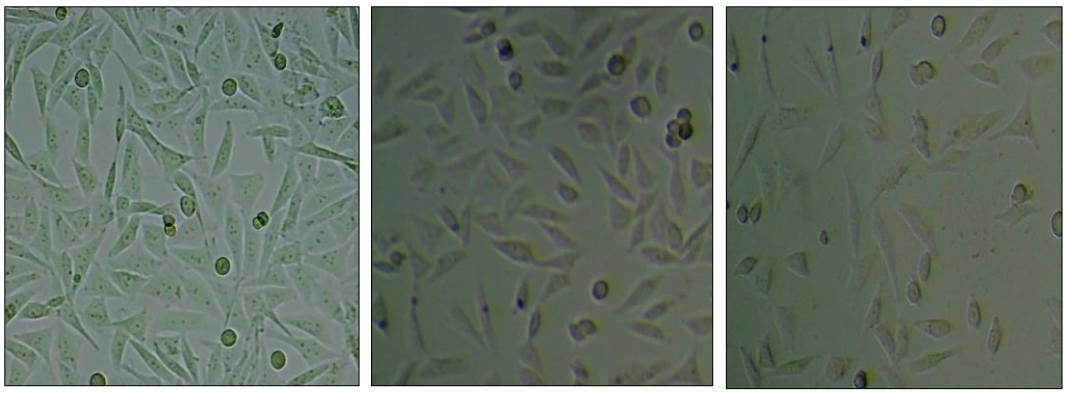
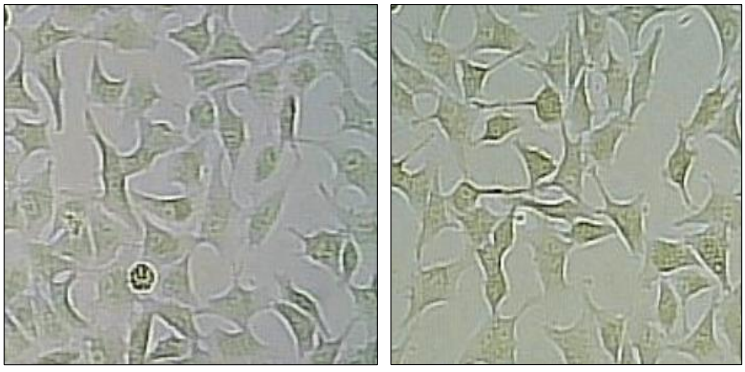


Fig 3: Photograps of Cytotoxic Activity of Novel benzothiazole derivatives on MCF-7 Cells with different concentration

MRD-1



5µg 10 µg 25 µg



50 µg 100µg

5. Conclusion

In current research work, we successfully synthesized and characterized newly synthesized structural analogs of novel benzothiazole derivatives containing thiazolidine 2, 4 moieties. The yield of the synthesized compounds was found to be in the range from 68-85 %. In conclusion, the present study highlights the importance of benzothiazole derivatives having various heterocyclic moiety features responsible for the antimicrobial and anticancer activities and may serve as a lead molecule for further modification to obtain clinically useful novel entities.

6. Acknowledgements

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7. Author's contributions

Corresponding author has done all the work, interpreted the data, and written the manuscript.

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