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Synthesis, Characterization and Biological Evaluation of Substitutedthiazolidin-4-Ones as Anticancer Agents

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Abstract: **A new series of substituted thiazolidin-4-ones were synthesized and evaluated for anticancer activity by means of MTT assay method for improved anticancer activity .The structures of these synthesized compounds were established by means of IR,H NMR analysis.All the compounds were evaluated for their anticancer activity .Compounds TH10 & TH19 were found most active due to presence of electron withdrawing groups at appropriate position.**

Keywords: **Anticancer activity, MTT assay, Thiazolidin-4-ones.**

1. INTRODUCTION

Hetrocyclic compounds comprise the major family of organic compounds. Thiazole derivatives are an important class of hetrocyclic compounds. Theextencive synthetic possibilities of these hetrocyclic due to the presence of several reaction sites. Thiazoles and their derivatives have attracted continuing interest over the years because of their varied biological activities and are enormously essential with wide range of synthetic ,pharmaceutical and industrial applications. Approximately 90% of new drugs contain heterocyclic moieties. So far, modifications of thiazole ring have proven highly effective with improved potency and lesser toxicity. The high therapeutic properties of these hetrocycles have encouraged the medicinal chemists to synthesize a large number of novel chemotherapeutic agents.[(1,2)]

Mostly researches have maintained their interest in nitrogen and sulphur containing hetrocyclic compounds through decades of historical development of organic synthesis[(3,4)]. In the continuation of our drug research program[(5)], the present work is aimed towards the construction of novel hetrocyclic compounds of anticipated utility as anticancer agents. Design of new lead structures employed as antitumor agents is one of the most urgent research areas in contemporary medicinal chemistry. Cancer is a second leading cause of death.[(6)] and is characterized by the uncontrolled proliferation of cells, which may be rapid or slow depending on the particular cancer. It poses a serious human health problem despite much progress in understanding its biology and pharmacology.[(7)] Thiazolidin-4-ones have attracted a great deal of interest owing to their antimicrobial 8, anti-inflammatory[9], CNS depresent[10], antitubercular[11], antitumor[12], anthelmintic[13], sedative[14], antiretroviral properties[15] and antineoplastic[16] activity.

2. EXPERIMENT

Melting point were recorded on a cintexm.p. apparatus, in open capillaries and are uncorrected. I.R. spectra recorded in KBr on Thermoscientific, Nicolet-155.H1-NMR spectra on JNM-ECS400 300MHz spectrophotometer using TMS as internal standard(chemical shift in – ppm)

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General Procedure for synthesis of Substituted thiazolidin-4-ones.

Step1-prepration of ethyl-4-aryl-benzoate (2)

To the substituted benzoic acids in absolute ethanol add1 ml H2SO4 dropwise and refluxed for 3.5 hrs.The solvent was evaporated on water bath and the precipitate washed with cooled water and recrystallized with ethanol.

Step2-prepration of aryl substituted benzohydrazide(3)

To the equimolar quantities of (2) and hydrazine-hydrate in ethanol refluxed for 3hrsThe solvent was evaporated on water bath and the precipitate washed with cooled water and recrystallized with ethanol.

Step3-prepration of aryl-N-substituted phenyl-methylidene-benzohydrazide (4)

To the equimolar quantities of(3) andaromatic substituted benzaldehyde in methanol refluxed for 3hrs.The solvent was evaporated on water bath and the precipitate washed withdiethyl ether and recrystallized with ethanol.

Step4-prepration of aryl-N[2-(substituted phenyl)-4-oxo-1,3-thiazolidin-3-yl]-benzamide(5)

To I mol of (4) add 0.9 mol of thioglycollic acid in DMF with a pinch of ZnCl2 refluxed for 6 hrs .The product so formed cooled& poured on crushed ice.The solid product filtered,washed&recrystallized from ethanol.

3. SCHEME

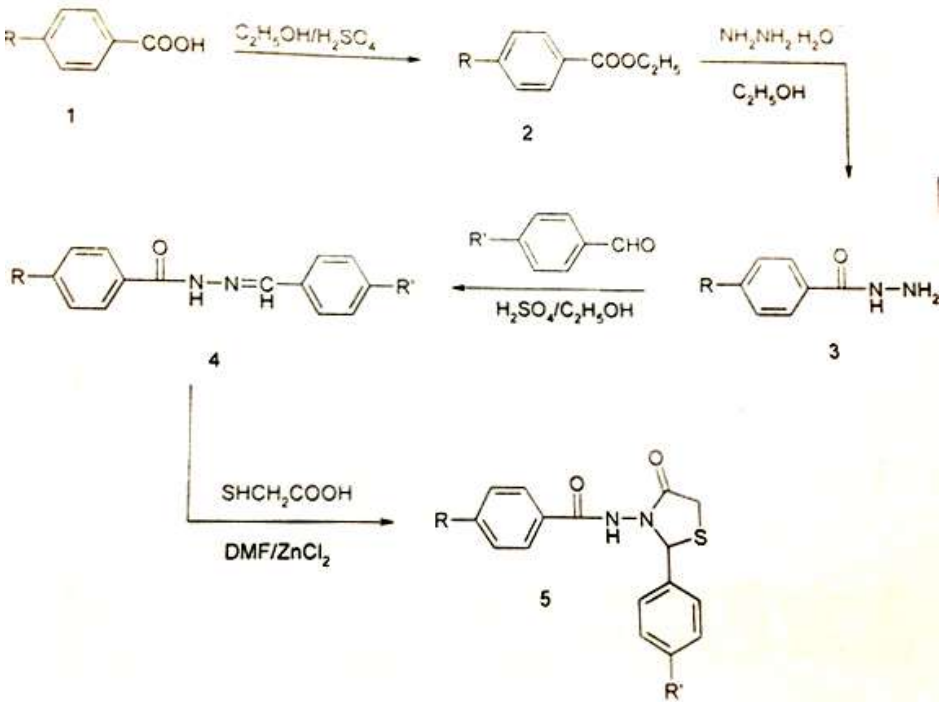

Table-I: Physicochemical characteristics of synthesized substitutedthiazolidin-4-ones derivatives

Compound	-R	-R`	MOLECULAR FORMULA	MOLECULAR WEIGHT	M.P. (C`)	Rf	%yield
TH01	-H	-H	C16H14N2O2 S	298.37	136-138	0.56	54
TH02	-H	-NO2	C16H14N3O4S	343.36	133-135	0.54	45
TH03	-H	-Cl	C16H13N2O2SCl	332.81	141-143	0.70	51
TH04	-H	-OH	C16H14N2O3S	314.37	138-140	0.53	60
TH05	-OH	-H	C16H14N2O3 S	314.37	1142-143	0.65	65
TH06	-OH	-NO2	C16H14N3O4S	359.36	133-135	0.68	70
TH07	-OH	-Cl	C16H13ClN2O2 S	348.81	134-136	0.72	45
TH08	-OH	-OH	C16H14N2O4S	330.36	131-133	0.49	60
TH09	-Cl	-H	C16H12ClN2O2 S	332.81	140-142	0.39	60

TH10	-Cl	-NO2	C16H12ClN3O4 S	377.81	132-134	0.59	45
TH11	-Cl	-Cl	C16H12ClN2O2 S	367.26	136-138	0.56	85
TH12	-Cl	-OH	C16H13ClN2O3 S	348.81	140-142	0.72	65
TH13	-NH2	-H	C16H15N3O2S	313.38	132-134	0.66	45
TH14	-NH2	-NO2	C16H14N4O4S	358.38	131-133	0.47	45
TH15	-NH2	-Cl	C16H14ClN3O2 S	347.38	134-135	0.65	70
TH16	-NH2	-OH	C16H15N3O3 S	329.38	132-134	0.58	55
TH17	-NO2	-H	C16H13N3O4 S	343.36	131-133	0.52	60
TH18	-NO2	-NO2	C16H12N4O6 S	388.36	138-140	0.62	75
TH19	-NO2	-Cl	C16H12ClN3O4 S	377.81	138-140	0.35	75
TH20	-NO2	-OH	C16H13N3O5 S	359.36	139-140	0.56	55

4. CHARACTERIZATION OF SUBSTITUTEDSYNTHESIZED1, 3-THIAZOLIDIN-4-ONES

TH02-IR(KBr):1684(C=O str.,ketonic),3058(C-H str.,aromatic),3147(N-H str.),768(C-S str.,aromatic),1520(N=O str.,aromatic) and 1245 (C-N str.)

H-NMR:2.64(s,2H,-S-CH2),7.86-6.52 (m,5H,ArH),1.91(s,1H,-CHN-),4.74(s,1H,-CONH-),6.84-6.82(d,2H,ArH);6.54-6.52(d,2H,ArH).

TH07-IR(KBr):1665(C=O str.,ketonic),2874(C-H str.,aromatic),3138(O-H str.),2973(N-H str.),759,837(C-Sstr.,aromatic) and 1125 (C-N str.).

H-NMR:8.09(s, 1H,OH),2.85(s,2H,-S-CH2),1.25(s,1H,-CHN-),4.38(s,1H,-CONH-),8.094-8.089(d,2H,ArH);7.585-7.582(d,2H,ArH),7.581-7.447(d,2H,ArH);7.427-7.408(d,H,ArH).

TH09-IR(KBr):1685(C=O str.,ketonic),2873(C-H str.,aromatic),3412(O-H str.),3284(N-H str.),886(C-S str.,aromatic),510(C-1 str., aromatic).

H-NMR:2.503(s,2H,-S-CH2),,1.18(s,1H,-CHN-),3.165(s,1H,-CONH-),6.98-6.94(m,5H,ArH);8.18-8.07(d,2H,ArH),7.64-763(d,2H,ArH).

TH10-IR(KBr):1673(C=O str.,ketonic),2994(C-H str.,aromatic),3285(N-H str.),524(Clstr.,aromatic),745 (C-S str. Aromatic), 1168 (C-N str.)and 542 (N=O str. Aromatic).

H-NMR:3.36(s,2H,-S-CH2),1.27(s,1H,-CHN-),2.5(s,1H,-CONH-),8.24-8.08(d,2H,ArH);8.02-7.84(d,2H,ArH),7.45-7.43 (d,2H,ArH);6.84-6.82(d,H,ArH).

TH13-IR(KBr):1745(C=O str.,ketonic),2953(C-H str.,aromatic),3137(N-H str.), 734 (C-S str. Aromatic), 1087 (C-N str.).

H-NMR:3.808(s,2H,-S-CH2),1.103(s,1H,-CHN-),2.5(s,1H,-CONH-),5.624(m,5H,ArH);7.89-7.87(d,2H,ArH),7.00-6.91(d,2H,ArH).

TH15-IR(KBr):1783(C=O str.,ketonic),3084(C-H str.,aromatic),3515(N-H str.),735 (C-S str. Aromatic), 1268 (C-N str.)and 514 (Cl str.).

H-NMR:1.137(s,2H,-S-CH2),0.75(s,1H,-CHN-),3.016(s,1H,-CONH-),3.845(s,2H,-NH2-)7.745-7.722(d,2H,ArH);7.488-7.411(d,2H,ArH),6.928-6.904(d,2H,ArH);6.84-6.85(d,H,ArH).

TH16-IR(KBr):1725(C=O str.,ketonic),3168(C-H str.,aromatic),3478(O-H str.),2927(N-H str.),794(C-S str.,aromatic).

H-NMR:2.385(s,2H,-S-CH2),,1.258(s,1H,-CHN-),4.225(s,1H,-CONH-),3.363(s,2H,NH2-),8.394-8.380(m,5H,ArH),7.2(s,1H,OH),6.917-6.874(d,2H,ArH),7.670-7.650(d,2H,ArH).

TH17-IR(KBr):1759(C=O str.,ketonic),3065(C-H str.,aromatic),1365(N=O str.),3476(N-H str.),748(C-S str.,aromatic), 1048(C-N str.) .

H-NMR:2.5(s,2H,-S-CH2),,1.194(s,1H,-CHN-),3.478(s,1H,-CONH-),7.763-7.742(d,2H,ArH),7.29-7.20(d,2H,ArH), 6.938-6.917(d,2H,ArH),6.755-6.734(d,2H,ArH).

TH19-IR(KBr):1835(C=O str.,ketonic),1246(C-Nstr.)3154(C-H str.,aromatic),1586(N=O str.),3438(N-H str.),826(C-S str.,aromatic),694(Cl) .

H-NMR:2.5(s,2H,-S-CH2),2.3(s,1H,-CHN-),3.35(s,1H,-CONH-),8.18-8.02(d,2H,ArH),7.64-7.63(d,2H,ArH),7.13-7.11(d,2H,ArH),6.98-6.96(d,2H,ArH).

TH20-IR(KBr):1726(C=O str.,ketonic),1175(C-Nstr.)3154(C-H str.,aromatic),1586(N=O str.),3495(N-H str.),826(C-S str.,aromatic),3152(OH str.) .

H-NMR:2.5(s,2H,-S-CH2),1.3(s,1H,-CHN-),3.91(s,1H,-CONH-),7.514(s,1H,OH),8.29-8.16(d,2H,ArH),8.141-7.951(d,2H,ArH),7.636-7.635(d,2H,ArH),7.494-7.475(d,2H,ArH).

5. ANTICANCER ACTIVITY

Cancer is general term for the group of diseases occur when there is an uncontrolled growth of abnormal cells in one or more organs of tissues of the body. Cancer occurs when old or damaged cells continue to divide and multiply uncontrollably. This results in the development of malignant tumor or other abnormalities that interfere with the functioning of the effected organs or tissues.

A- Cell lines and culture medium:

A-549 (Lung carcinoma), HT-29(Colon carcinoma) and HeLa(Cervix carcinoma) cell lines were procured. Stock cells were cultured in DMEM supplemented with 10% inactivated Fetal Bovine Serum (FBS), pencillin (100 IU/ml).streptomycin (100□g/ml) and amphotericin B(5□g/ml) in an humidified atmosphere of 5% CO2 at 37oC until confluent. The cells were dissociated with TPVG solution (0.2% trypsin, 0.02% EDTA,0.05% glucose in PBS).The stock cultures were grown in 25cm^2 culture flasks and all experiments were carried out in 96 microlitres plates.

B- Prepration of Test Solutions:

For cytotoxicity studies, each weighed test drugs were separately dissolved in distilledDMSO and volume was made up with DMEM supplemented with 2% inactivated FBS to obtain a stock solution of 1mg/ml concentration and sterilized by filteration. Serial two fold dilutions were prepared from this for carrying out cytotoxic studies.

C- Determination of cell viability by MTT assay:

Principle-The ability of the cells to survive a toxic insult has been the basis of most cytotoxicity assay.This assay is based on the assumption that dead cells or their products do not reduce tetrazolium.The assay depends both on the number of cells present andon the mitochondrial activity per cell.The principle involved is the cleavage of tetrazolium salt 3-(4,5 dimethyl thiazole-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) into a blue coloured product (formazan) by mitochondrial enzyme succinate dehydrogenase.The number of cells was found to be proportional to the extent of formazan production by the cells used.

Procedure-The monolayer cell culture was trypsinzed and the cell count was adjusted to1.0 to 105 cells/ml using DMEM containing 10%FBS.To each well of the 96 well microlite plate, 0.1 ml of the diluted cell suspension (approximately 10,000 cells) was added.After 24 hrs, when a partial monolayer was formed, the supernatant was flicked off, washed the monolayer once with medium and 100ul of different test concentrations of test drugs were added on the partial monolayer in microliteplates. The plates were then incubated at 37 degree Celsius for 3 days in 5% carbon dioxid atmosphere and microscopic examination was carried out and observations were noted every 24 hrs interval. After 72 hrs, the drug solutions in the wells were discarded and 50 ml of propanol was added and the plates were gently shaken to solubilize the formed formazan. The absorbance was measured using a microplate reader at a wavelength of 540 nm. The percentage growth inhibition was calculated using the following formula and concentration of test drug needed to inhibit cell growth by 50% (CTC50)VALUES IS GENERATED FROM THE DOSE-RESPONSE CURVES FOR EACH CELL LINE.

$$\%\ \text{Growth inhibition} = 100 - \frac{(\text{Mean OD of individual test group}}{\text{Mean OD of control group}} \text{x}100)$$

In-vitro cytotoxity activity of 1,3-thiazolidin-4-one derivatives against cancer cell lines CTC50 (□g/ml)

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compounds	A-549 (Lung carcinoma)	HT-29 (colon carcinoma)	HeLa (cervix carcinoma)
TH01	103±0.79	54±0.13	44±0.91
TH02	88±0.87	44±0.30	37±0.28
TH03	95±0.20	51±0.43	42±0.26
TH04	112±0.10	67±1.40	49±0.46
TH05	162±0.50	74±0.08	64±0.20
TH06	138±0.66	61±1.40	57±0.66
TH07	197±0.25	69±0.68	62±0.36
TH08	189±0.92	118±0.28	67±0.18
TH09	84±0.43	39±0.36	34±0.74
TH10	79±0.66	32±0.33	29±0.22
TH11	82±0.43	37±0.39	30±0.82
TH12	84±0.66	42±0.38	37±0.22
TH13	136±0.63	64±0.30	54±0.36
TH14	108±0.79	51±0.53	41±0.45
TH15	120±0.25	54±0.38	47±0.40
TH16	141±0.87	127±0.28	61±0.65
TH17	87±0.70	42±0.53	37±0.56
TH18	83±1.31	35±0.18	32±0.46
TH19	80±1.31	39±0.13	34±0.38
TH20	90±0.87	45±0.35	41±0.38
Standard drug(5-FU)	54±0.22	49±0.33	32±0.43

6. RESULTS

The synthesized compounds were screened for in-vitro anticancer activity against A-549(lung carcinoma), HT-29(lung carcinoma) and HeLa (cervix carcinoma) cell lines using MTT assay method. The results of anticancer activity of synthesized compounds are shown in the table. Compounds TH10 and TH19 were showed highest activity against all cell lines, it may be due to presence of electron withdrawing groups at both –R and-R' positions. Compound TH08 was substituted with electron donating groups at both side of –R and –R' positions. This may be the reason for lower activity of these compounds. From the tested compounds, some were found to exhibit much higher inhibitory effects towards the tomor cell lines than the reference drug 5-FU.

7. CONCLUSION

The results obtained from anticancer activity showed that Compounds TH10 and TH19 were showed highest activity against all cell lines ,it may be due to presence of electron withdrawing groups at both –R and-R' positions. Compound TH08 was substituted with electron donating groups at both side of –R and –R' positions. This may be the reason for lower activity of these compounds. So we can say that –R and –R' positions when substituted with electron withdrawing groups shows maximum anticancer activity.

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