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NOVEL 2,4-THIAZOLIDINEDIONE ANALOGUES AS CYTOTOXIC AGENTS: SYNTHESIS AND BIOLOGICAL SCREENING

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Abstract: Synthesis of novel Thiazolidinedione derivatives was performed from reaction of N-benzoyl substituted aryl alkyl halide with N-substituted 2, 4-thiazolidinedione derivatives and screened for their in vitro cytotoxic activity by MTT assay. The cell lines used were MCF 7 (Breast cancer cell), HELA (Cervical cancer cell line), HEK (Normal epidermal kidney cell line) HEP (Laryngeal cancer cell line) MDA MB 468 (Brest cancer cell line). Result of screening on cell line showed moderate to good cytotoxic activity for all the compounds. Compound DGS27 (IC_{50} = 3.63 µM, HEP), DGS27 (IC_{50} = 1.07 µM, HEK), DGS7 (IC_{50} = 1.09 µM, HELA), DGS7 (IC_{50} = 1.989 µM, M468) and DGS7 (IC_{50} = 2.5 µM, MCF7) was found to be most active compared to standard Methotrexate (IC_{50} = 0.22 µM HEP, IC_{50} =0.072 µM HEK, IC_{50} = 0.102 µM HELA, IC_{50} = 0.152 µM M468, IC_{50} = 0.100 µM MCF7). Structure activity relationship of synthesized analogs suggested that the attachment of electron withdrawing groups like Chloro at R2 position and Acetyl group at R1 gave better cytotoxic activity. Activity by Hydrogen and Acetyl substitution at R1 position linked with N substituted 2, 4 - Thiazolidinedione scaffold gave better activity in the order of H > CH_2COOH. Our findings may impart new direction to medicinal chemists and biochemists for further investigations of 2, 4- Thiazolidinedione containing cytotoxic agents.

Keywords: 2,4 - Thiazolidinedione, Cytotoxic, MTT assay

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INTRODUCTION

Cancer is a class of diseases in which a cell, or a group of cells display uncontrolled growth (division beyond the normal limits), invasion (intrusion on and destruction of adjacent tissues), and sometimes metastasis (spread to other locations in the body via lymph or blood). Malignancy is caused by abnormalities in cells, which might be due to inherited genes or caused by outside exposure of the body to chemicals, radiation, or even infectious agents[i].

HDAC1 was identified using the HDAC inhibitor trapoxin as an affinity tag from nuclear extracts in 1996. It turned out that HDAC1 shares high sequence homology with yeast Rpd3, a global gene regulator and transcriptional co-repressor with histone deacetylase activity[10]. Subsequently, 18 HDAC family members have been identified in the human genome. Following recombinant expression or purification of the HDAC proteins, it was only recently possible to characterize the inhibitory profile of HDAC inhibitors, which have already been widely applied in cell culture and animal models. It turned out that most of the currently used HDAC inhibitors act rather unselective and inhibit either all or at least several members of the HDAC family.

Unselective HDAC inhibitors are now being evaluated in clinical trials and show promising results in adult patients with leukaemia's and solid tumors. Vorinostat (SAHA) was the first HDAC inhibitor to be approved by the US Food and Drug Administration for cutaneous T-cell lymphoma in 2006[11]. However, first phase I and II studies demonstrate that pan-HDAC inhibitors may also cause numerous side effects such as bone marrow depression, diarrhea, weight loss, taste disturbances, electrolyte changes, disordered clotting, fatigue, and cardiac arrhythmias[12]

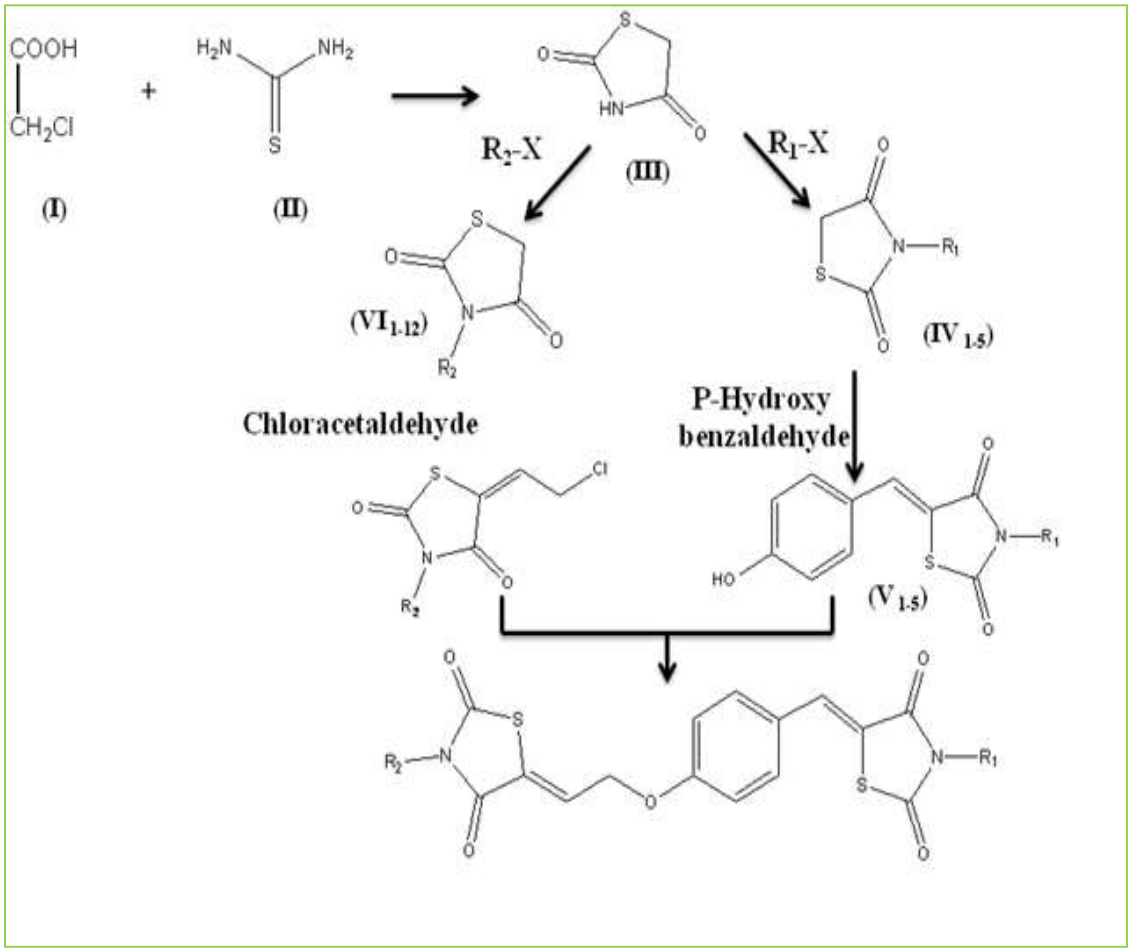
These observations are not surprising if one considers the central role of HDACs as key regulators of chromatin structure and posttranslational modifiers of numerous key proteins in any cell type and tissue. Thus, the question arises whether future drug development in the field should focus on selective targeting of individual HDAC family members, which possess a critical oncogenic function in cancer cells but have no such function in normal cells. Here, we discuss the potential of individual HDACs as drug targets in cancer therapy. To this aim, it was reviewed the role of individual HDACs not only in cancer, but also in normal physiology and development in order to understand the potential side effects associated with their inhibition

Thiazolidine-2,4-diones (TZD) are the derivatives of thiazolidine, which belongs to an important group of heterocyclic compounds. TZD and rhodanine analogs have been recognized as the privileged templates in drug design and discovery and numerous compounds containing the TZD ring have been developed as potential anticancer agents. More interestingly, some TZD analogs such as the anti-diabetic drug troglitazone have been shown to exhibit anticancer activities through the inhibition of the HDAC inhibitor..

MATERIAL AND METHODS:

Melting points of all synthesized compounds were determined in open capillaries using Veego melting point apparatus, Model VMP-D (Veego India ltd., Mumbai, India) and were uncorrected. Infrared spectra were recorded using KBr pellets on SHIMADZU-FT-IR 8400S instrument. Mass spectra were recorded on PerkinElmer LC–MS PE Sciex API/65 Spectrophotometer. The ^{1}H NMR spectra were recorded on Brucker Avance-300 (300 MHz) model spectrophotometer in $CDCl_3$ using DMSO as solvent and TMSi as internal standard with ^{1}H resonant frequency of 300 MHz. The TLC was performed on precoated alumina silica gel 60 F_{254} (Merck). The mobile phase was benzene:methanol (9:1) and detection was made using UV light. The resulting compounds were purified by recrystallization using suitable solvent. The elemental analyses were done on elementar Vario EL 3 Carlo erba 1108 and were well in accordance with the structures assigned to the compound. Synthetic grade chemicals procured from SD fine chemicals, Baroda, India were used for the synthesis of the target compounds. All the compounds were prepared according to the literature procedures with some minor modifications[11,12] General synthetic procedures used for the preparation of the target compound are as follows:

SYNTHETIC PATHWAY



R_2=	R_1=
1) 2,3-dichlorobenzoyl	a) -H
2) 2,4-dicholorobenzoyl	b) $-CH_2COOH$
3) 4-nitrobenzoyl	c) $-CH_2COOC_2H_5$
4) Benzoyl	d) $-CH_2CH_2COOCH_3$
5) 4-chlorobenzoyl	e) $-CH_2CH_2COOC_2H_5$
6) 2-chlorobenzoyl	

Synthesis of 2,4-thiazolidinedione (Microwave)(1A)

Add Chloracetic acid 56.5 gm (0.6 mol) in 60 ml of water andthiourea 45.6 gm (0.6 mol) in 60 ml of water were mixed and transferred in 250 ml round bottom flask.The mixture was stirred for 15 minute and cooled which yields white precipitates. To the content of the flask 60 ml of conc. HCl was added slowly from dropping funnel.The mixture was refluxed for 15 min at 750watt at microwave. On cooling the content of the flask solidified into a cluster of white needles. The product was filtered. The product was purified by recrystallization from ethyl alcohol

Synthesis of 3- aryl substituted 2,4-thiazolidinedione (microwave)(2A-2F)

Add 2,4 –thiazolidinedione (0.01 mole) in 20 mL of 5% aqueous sodium hydroxide in a well-corked flask. Add Substituted benzoyl chloride (0.01 mole) about 0.5 mL at a time, with constant shaking. Add 10 ml Ethanol to flask and reflux for 5-7 min at 750 watt in Microwave. Filter off the solid derivative, wash with a little cold water and recrystalize from ethanol.

Synthesis Of 5 - (4-Hydroxy Benzylidine) 3- Aryl Substituted 2,4-Thiazolidinedione (3A-3F)

To a solution of 4-hydroxy benzaldehyde (0.25M) and 3-Aryl substituted 2,4thiazolidinedione (0.25M) in hot glacial acid (50mL), fused sodium acetate (1.8g) was added. It was refluxed for 1 hr with occasional shaking. It was poured in water (500mL), then product obtain was filtered, The product was recrystalize with glacial acetic acid.

Synthesis of 3- substituted 5 - (4-hydroxy benzylidine) 2,4 –thiazolidinedione (conventional): (4A-4E)

Add 5-(4-hydroxy benzylidine)2,4 –thiazolidinedione (0.01 mole) in 20 mL Acetone in a well-corked flask. Add Different substituted alkyl halide (0.01 mole) about 0.5 mL at a time, with constant shaking. Add 0.25 gm Potassium Carbonate to that mixture and stir vigorously for 15 min. Allow it to cool for 10 minutes and then reflux it for 4 hours. Filter off the solid derivative, wash with a little cold water and recrystalize from ethanol.

Synthesis of 3- substituted 5 - (4-hydroxy benzylidine) 2,4 –thiazolidinedione (microwave): (5A-5E)

5-(4-hydroxy benzylidine)2,4 –thiazolidinedione (0.01 mole) was added in 20 mL Acetone in a well-corked flask. Different substituted alkyl halide (0.01 mole) about 0.5 mL were added at a time, with constant shaking. 0.25 gm Potassium Carbonate added to that mixture and stir vigorously for 15 min. Allow it to cool for 10 minutes and then reflux it for 5-6 min at 730 Watt in Microwave.Filter off the solid derivative, wash with a little cold water and recrystalize from ethanol.

SYNTHESIS OF 3- substituted benzoyl-5-(2-(4-((3-methyl-2,4-dioxothiazolidin-5-ylidene) methyl) phenoxy) ethylidene)thiazolidine-2,4-dione: (DGS1 TO DGS30)

Equimolar quantity of 3- substituted 5 - (4-hydroxy benzylidine) 2,4 –thiazolidinedione derivatives (3A-3F) and 3-substituted 5-(4-hydroxybenzylidine) 2,4–thiazolidinedione (4a-4q) in acetonitrile were reacted in presence of glacial acetic acid. The reaction mixture was allowed to reflux and completion of reaction was checked by TLC. Precipitate was obtained after pouring the reaction mixture to crushed ice and finally compounds were recrystallized using ethanol.

Cytotoxic Study

All the synthesized compounds were screened for their cytotoxic activity on MCF-7 (human mammary gland adrenocarcinoma cell line), HEK (epidermal kidney cancer cell line), HELA (Brest cancer cell line), M468 (Brest cancer cell line) and MCF7 (Laryngeal cancer cell line) by MTT assay. The screening experiments were carried out at Microbiology unit of Alpaa Research Laboratory. Cultures were observed using an inverted microscope to assess the degree of viability, and the absence of bacterial and fungal contaminants was confirmed. Cell monolayer was washed with PBS without ca^{++}/mg^{++} using a volume equivalent to half the volume of culture medium. Trypsin/EDTA was added on to the washed cell monolayer using 1 ml per 25 cm^2 of surface area. Flask was rotated to the cover monolayer with trypsin and moved to the incubator and left for 2-4 minutes. The cells were examined using an inverted microscope to ensure that all the cells were detached and floated. The cells were resuspended in a small volume of fresh serum containing medium and 100-200 µl was removed to perform cell count. The required number of cells were transferred to a new labeled flask containing prewarmed HCT-15 and incubated as appropriate for the cell line. All the cytotoxicity experiments were carried out in 96 well plates. Gefitinib was used as a reference standard for cytotoxic activity. All solutions of test compound were prepared using DMSO. IC_{50} values were calculated. It is a drug concentration causing 50 % inhibition of cell proliferation. Statistical significance of the data (expressed as mean ± SEM) was demonstrated by performing one-way ANOVA test followed by Dennett comparison of IC_{50} of the entire compounds against Methotrexate using Graph pad Prism (Version 5.0) software. Results of the biological screening and statistical analysis are shown in Table 1.

RESULTS:

The target molecules were designed by joining two different moieties i.e., two 2,4-Thiazolidinedione linked with phenoxy molecule. These novel derivatives were synthesized with different aryl substitution at 3^{rd} position of Thiazolidinedione ring and different substituted alkyl at 3^{rd} position of second Thiazolidinedione and phenoxy at 5^{th} position of thiazolidinedione. Synthesis of target molecule was carried out as per scheme. The structure of

all synthesized compound were confirmed by physical characterization i.e., melting point, R_f value, IR, MASS and NMR spectroscopy which are as bellow.

3-benzoyl-5-(2-(4-((2,4-dioxothiazolidin-5-ylidene)methyl)Phenoxy)ethylidene) thiazolidine -2,4-dione(DGS1)

Light brown product; R_f value 0.50, Yield 44%; M.p. 282-287 °C; IR (KBr, υ_{max}, cm^{-1}): 1755(C=O), 3513(NH); ^1H NMR (300 MHz, δ ppm, DMSO): 10.1 (s, 1H, Imid), 7.96-6.76 (m, 9H, Ar-H), 6.41 (s, 1H, Ethylene), 4.61 (s, 2H, Methylene) ppm; and MS: m/z 490 (M + Na)

3-benzoyl-5-(2-(4-((3-acetyl-2,4-dioxothiazolidin-5-ylidene)methyl)Phenoxy)ethylidene) thiazolidine -2,4-dione(DGS2)

Light brown product; R_f value 0.53, Yield 36%; M.p. 304-307 °C; IR (KBr, υ_{max}, cm^{-1}): MS: m/z 525 (M)

3-benzoyl-5-(2-(4-((3-butyl-2,4-dioxothiazolidin-5-ylidene)methyl)Phenoxy)ethylidene) thiazolidine -2,4-dione(DGS3)

Dark yellow product; R_f value 0.54, Yield 30%; M.p. 290-292 °C; IR (KBr, υ_{max}, cm^{-1}): MS: m/z 554 (M + 1)

3-benzoyl-5-(2-(4-((3-isobutyl-2,4-dioxothiazolidin-5-ylidene)methyl)Phenoxy)ethylidene) thiazolidine -2,4-dione(DGS4)

Light Yellow product; R_f value 0.51, Yield 44%; M.p. 268-270 °C; IR (KBr, υ_{max}, cm^{-1}): MS: m/z 553 (M)

3-benzoyl-5-(2-(4-((3-pentyl-2,4-dioxothiazolidin-5-ylidene)methyl)Phenoxy)ethylidene) thiazolidine -2,4-dione(DGS5)

Yellow product; R_f value 0.49, Yield 41%; M.p. 292-295 °C; IR (KBr, υ_{max}, cm^{-1}) MS: m/z 567(M)

3-2,3-dichlorobenzoyl-5-(2-(4-((2,4-dioxothiazolidin-5- ylidene) methyl)Phenoxy)ethylidene) thiazolidine -2,4-dione(DGS6)

Light Yellow product; R_f value 0.42, Yield 52%; M.p. 348-350 °C; ^1H NMR (300 MHz, δ ppm, DMSO): 10.15 (s, 1H, Imid), 8.42-7.67 (m, 7H, Ar-H), 6.68 (s, 1H, Ethylene), 4.85 (s, 2H, Methylene) ppm; MS: m/z 534 (M + 1)

3-2,3-dichlorobenzoyl-5-(2-(4-((3-acetyl2,4-dioxothiazolidin-5-ylidene) ethyl) Phenoxy) ethylidene) thiazolidine -2,4-dione(DGS7)

Dark Brown product; R_f value 0.48, Yield 35%; M.p. 337-340 °C; IR (KBr, υ_{max}, cm^{-1}): 1683 (C=O), 3010 (CH), 3100(NH); MS: m/z 597 (M+4)

3-2,3-dichlorobenzoyl-5-(2-(4-((3-n-butyl-2,4-dioxothiazolidin-5-ylidene) methyl) Phenoxy) ethylidene) thiazolidine -2,4-dione(DGS8)

White product; R_f value 0.45, Yield 46%; M.p. 324-327 °C; IR (KBr, υ_{max}, cm^{-1}): 1679 (C=O), 2952 (CH), 3120(NH); MS: m/z 623 (M + 2)

3-2,3-dichlorobenzoyl-5-(2-(4-((3-iso-butyl-2,4-dioxothiazolidin-5-ylidene) methyl) Phenoxy) ethylidene) thiazolidine -2,4-dione(DGS9)

White product; R_f value 0.58, Yield 38%; M.p. 310-315 °C; IR (KBr, υ_{max}, cm^{-1}): 1733 (C=O), 2895 (CH), 3096(NH); MS: m/z 625 (M+4)

3-2,3-dichlorobenzoyl-5-(2-(4-((3-pentyl-2,4-dioxothiazolidin-5-ylidene) methyl) Phenoxy) ethylidene) thiazolidine -2,4-dione(DGS10)

Light Brown product; R_f value 0.52, Yield 46%; M.p. 351-359 °C; IR (KBr, υ_{max}, cm^{-1}): 1705 (C=O), 3425(NH); MS: m/z 636 (M)

3-(2,4-dichlorobenzoyl)-5-(2-(4(2,4-dioxothiazolidin-5-ylidene)methyl) phenoxy) ethylidene) thiazolidine-2,4-dione (DGS11)

Light Brown product; R_f value 0.63, Yield 75%; M.p. 140-141 °C; IR (KBr, υ_{max}, cm^{-1}): 1682 (C=O), 3529(NH), 2912 (CH); ^{1}H NMR (300 MHz, δ ppm, DMSO): 9.706 (s, 1H, NH), 7.97-7.41 (m, 7H, Ar-H), 5.52 (s, 2H, Methylene), 7.109-6.51 (m, 2H, Ethylene) ppm; and MS: m/z 539 (M + 4)

3-(2,4-dichlorobenzoyl)-5-(2-(4(3-acetyl-2,4-dioxothiazolidin-5-ylidene)methyl) phenoxy) ethylidene) thiazolidine-2,4-dione (DGS12)

Light Brown product; R_f value 0.49, Yield 51%; M.P. 352-358 °C; IR (KBr, υ_{max}, cm^{-1}): 1683 (C=O), 2964 (CH), 3049(NH); MS: m/z 595 (M + 2)

3-(2,4-dichlorobenzoyl)-5-(2-(4(3-n-butyl-2,4-dioxothiazolidin-5-ylidene)methyl) phenoxy) ethylidene) thiazolidine-2,4-dione (DGS13)

Brown product; R_f value 0.59, Yield 56%; M.p. 386-388 °C; IR (KBr, υ_{max}, cm^{-1}): 1658 (C=O), 3024 (CH), 3578(NH); MS: m/z 622 (M)

3-(2,4-dichlorobenzoyl)-5-(2-(4(3-iso-butyl-2,4-dioxothiazolidin-5-ylidene)methyl) phenoxy) ethylidene) thiazolidine-2,4-dione (DGS14)

Yellow product; R_f value 0.52, Yield 39%; M.p. 412-415 °C; IR (KBr, υ_{max}, cm^{-1}): 1661 (C=O), 3010 (CH), 3530(NH); MS: m/z 622 (M)

3-(2,4-dichlorobenzoyl)-5-(2-(4(3-pentyl-2,4-dioxothiazolidin-5-ylidene)methyl) phenoxy) ethylidene) thiazolidine-2,4-dione (DGS15)

Light Brown product; R_f value 0.5, Yield 41%; M.p. 418-421 °C; IR (KBr, υ_{max}, cm^{-1}): 1672 (C=O), 2950 (CH), 3393(NH); MS: m/z 635 (M + 1)

3-(3,5-dichlorobenzoyl)-5-(2-(4-(2,4-dioxothiazolidin-5-ylidene)methyl) phenoxy) ethylidene) thiazolidine-2,4-dione. (DGS16)

Light Brown product; R_f value 0.39, Yield 70%; M.p. 432-435 °C; IR (KBr, υ_{max}, cm^{-1}): 1682 (C=O), 3529(NH), 3031 (CH); MS: m/z 637.5 (M + 4)

3-(3,5-dichlorobenzoyl)-5-(2-(4-3-acetyl-(2,4-dioxothiazolidin-5-ylidene)methyl) phenoxy) ethylidene) thiazolidine-2,4-dione. (DGS17)

Light Brown product; R_f value 0.35, Yield 46%; M.p. 438-440 °C; IR (KBr, υ_{max}, cm^{-1}): 1635 (C=O), 3015 (CH), 3431(NH); MS: m/z 594 (M + 1)

3-(3,5-dichlorobenzoyl)-5-(2-(4-3-n-butyl-(2,4-dioxothiazolidin-5-ylidene)methyl) phenoxy) ethylidene) thiazolidine-2,4-dione. (DGS18)

Light Yellow product; R_f value 0.46, Yield 52%; M.p. 425-429 °C; IR (KBr, υ_{max}, cm^{-1}): 1665 (C=O), 3042 (CH), 3310(NH); MS: m/z 623 (M + 2)

3-(3,5-dichlorobenzoyl)-5-(2-(4-3-iso-butyl-(2,4-dioxothiazolidin-5-ylidene)methyl) phenoxy) ethylidene) thiazolidine-2,4-dione. (DGS19)

Light yellow product; R_f value 0.49, Yield 50%; M.p. 395-401 °C; IR (KBr, υ_{max}, cm^{-1}): 1673 (C=O), 2960 (CH), 3240(NH); MS: m/z 621 (M)

3-(3,5-dichlorobenzoyl)-5-(2-(4-3-pentyl-(2,4-dioxothiazolidin-5-ylidene)methyl) phenoxy) ethylidene) thiazolidine-2,4-dione. (DGS20)

Light brown product; R_f value 0.41, Yield 51%; M.p. 382-385 °C; IR (KBr, υ_{max}, cm^{-1}): 1711 (C=O), 3020 (CH), 3430(NH); MS: m/z 635 (M)

3-(4-nitrobenzoyl)-5-(2-(4-(2,4-dioxothiazolidin-5-ylidene)methyl) phenoxy) ethylidene) thiazolidine -2,4-dione (DGS21)

Light Yellow product; R_f value 0.70, Yield 70%; M.p. 171-173 °C; IR (KBr, υ_{max}, cm^{-1}): 1652 (C=O), 3429(NH), 3012 (CH); ^1H NMR (300 MHz, δ ppm, DMSO): 10.12 (s, 1H, NH), 8.37-6.77 (m, 8H, Ar-H), 4.728 (s, 2H, Methylene), 7.18-6.68 (m, 2H, Ethylene) ppm; and MS: m/z 535 (M + Na)

3-(4-nitrobenzoyl)-5-(2-(4-3-acetyl-(2,4-dioxothiazolidin-5-ylidene)methyl)phenoxy) ethylidene) thiazolidine -2,4-dione (DGS22)

Light Brown product; R_f value 0.64, Yield 58%; M.p. 382-384 °C; IR (KBr, υ_{max}, cm^{-1}): 1665 (C=O), 3055 (CH), 3421(NH); MS: m/z 570 (M)

3-(4-nitrobenzoyl)-5-(2-(4-n-butyl-(2,4-dioxothiazolidin-5-ylidene)methyl) phenoxy) ethylidene) thiazolidine -2,4-dione (DGS23)

Light Yellow product; R_f value 0.53, Yield 36%; M.p. 352-355 °C; MS: m/z 598 (M)

3-(4-nitrobenzoyl)-5-(2-(4-iso-butyl-(2,4- dioxothiazolidin-5- ylidene) methyl) phenoxy) ethylidene) thiazolidine -2,4-dione (DGS24)

Light Brown product; R_f value 0.48, Yield 48%; M.p. 362-365 °C; MS: m/z 597 (M)

3-(4-nitrobenzoyl)-5-(2-(4-(2,4-dioxothiazolidin-5-ylidene)methyl) phenoxy) ethylidene) thiazolidine -2,4-dione (DGS25)

Light yellow product; R_f value 0.44, Yield 49%; M.p. 342-345 °C; MS: m/z 612 (M)

3-(4 chloro benzoyl)-5-(2-(4-(2,4-dioxo thiazolidin-5-ylidene)methyl) phenoxy) ethylidene) thiazolidine-2,4-dione (DGS26)

Light Yellow product; R_f value 0.54, Yield 38%; M.p. 442-445 °C; IR (KBr, υ_{max}, cm^{-1}): 1661 (C=O), 3017 (CH), 3521(NH); ^1H NMR (300 MHz, δ ppm, DMSO): 10.03 (s, 1H, NH), 7.89-6.38 (m, 8H, Ar-H), 4.62 (s, 2H, Methylene), 7.17-6.68 (m, 2H, Ethylene) ppm; and MS: m/z 503.6 (M+2)

3-(4 chloro benzoyl)-5-(2-(4-acetyl-(2,4-dioxo thiazolidin-5-ylidene)methyl) phenoxy) ethylidene) thiazolidine-2,4-dione (DGS27)

Light Brown product; R_f value 0.43, Yield 42%; M.p. 438-441 °C; MS: m/z 561 (M + 2)

3-(4 chloro benzoyl)-5-(2-(4-n-butyl-(2,4-dioxo thiazolidin-5-ylidene)methyl) phenoxy) ethylidene) thiazolidine-2,4-dione (DGS28)

Light Brown product; R_f value 0.49, Yield 41%; M.p.402-405 °C; MS: m/z 589 (M+2)

3-(4 chloro benzoyl)-5-(2-(4-iso-butyl-(2,4-dioxo thiazolidin-5-ylidene)methyl) phenoxy) ethylidene) thiazolidine-2,4-dione (DGS29)

Light Brown product; R_f value 0.64, Yield 58%; M.p. 402-405 °C; MS: m/z 587 (M)

3-(4 chloro benzoyl)-5-(2-(4-pentyl-(2,4-dioxo thiazolidin-5-ylidene)methyl) phenoxy) ethylidene) thiazolidine-2,4-dione (DGS27)

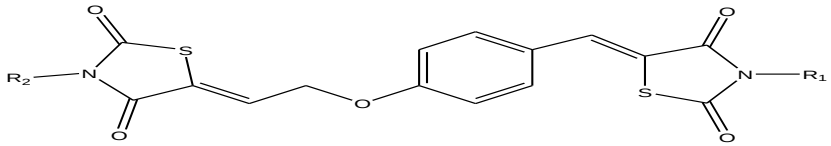
Light Yellow product; R_f value 0.50, Yield 45%; M.p. 412-415 °C; MS: m/z 603 (M + 2)

CYTOTOXIC ACTIVITY-

All the synthesized compound and standard drugs Methotrexate were subjected to cytotoxic activity on 5 different cell line like MCF 7 (Breast cancer cell line), HEK (Human Epidermal kidney cancer cell line) HELA (Cervical cancer cell line) HEP (Laryngeal cancer cell line) M468 (Brest cancer cell line). In HEP 2 cell line, total eight compounds DGS11, DGS27, DGS7, DGS12, DGS2, DGS21, DGS1, DGS16 were active which having high activity as compare to standard drug Methotrexate. In HEK cell line, total eight compounds DGS27, DGS11, DGS6, DGS12, DGS1, DGS21, DGS2, DGS26 were active which having high activity as compare to standard drug Methotrexate. In HELA cell line, total eight compounds DGS7, DGS27, DGS6, DGS11, DGS12, DGS1, DGS2, DGS17 were active which having high activity as compare to standard drug Methotrexate. In MDA-MB cell line, total seven compounds DGS27, DGS7, DGS6, DGS2, DGS12, DGS16, DGS11, DGS17 were active which having high activity as compare to standard drug Methotrexate. In MCF 7 cell line, total eight compounds DGS7, DGS6, DGS27, DGS2, DGS1, DGS11, DGS17, DGS12, DGS16 were active which having high activity as compare to standard drug Methotrexate From above all cell line study, Compounds DGS 7, DGS 27, DGS 11, DGS 17 and DGS 6 have shown low or similar % inhibition value as to Methotrexate so, it may be define as substitution on R1 and R2 position play an important role in binding with receptor which results in potent Cytotoxic activity.

DISCUSSION:

The size and liphophilicity of the substitution on the substituted 2,4-Thiazolidinedione moiety were considered to be key factors in determining cytotoxic activity. Thus, the introduction of bulky group was expected to decrease the biological activity of the corresponding to Thiazolidinedione moeity. The characteristics of the substituted functional groups at Thiazolidinedione ring system have strong influence on the spectrum and extent of cytotoxic activity. Electron withdrawing groups such as chloro at R2 and Hydrogen or Acetyl substitution at R1 produced greater/comparable cytotoxic activity than the electron releasing groups as substituent such as methyl. This indicates that electron withdrawing group increases activity while electron releasing group slightly rise in activity while unsubstituted derivatives are active as well.



Compound Code	R_1	R_2	Mean IC_{50}(μM)				
			HEP	HEK	HELA	M468	MCF 7
DGS-01	-H	Benzoyl	22.779	1.476	45.58	16.39	13.23
DGS-02	$-CH_2COOH$	Benzoyl	11.54	2.603	60.15	8.350	11.38
DGS-03	$-CH_2COOC_2H_5$	Benzoyl	63.41	65.56	66.38	71.56	29.84
DGS-04	$-CH_2CH_2COOCH_3$	Benzoyl	52.71	44.28	55.76	52.74	16.88
DGS-05	$-CH_2CH_2COOC_2H_5$	Benzoyl	97.54	>100	107.7	34.91	82.76
DGS-06	-H	2,3-dicholorobenzoyl	13.637	4.467	1.091	9.821	5.72
DGS-07	$-CH_2COOH$	2,3-dicholorobenzoyl	42.23	12.46	3.345	1.989	2.5
DGS-08	$-CH_2COOC_2H_5$	2,3-dicholorobenzoyl	59.41	39.59	59.49	38.01	39.49
DGS-09	$-CH_2CH_2COOCH_3$	2,3-dicholorobenzoyl	81.00	32.53	141.1	59.77	29.99
DGS-10	$-CH_2CH_2COOC_2H_5$	2,3-dicholorobenzoyl	83.55	5.018	>100	80.43	97.44
DGS-11	-H	2,4-dicholorobenzoyl	5.1808	5.018	1.145	5.712	14.67
DGS-12	$-CH_2COOH$	2,4-dicholorobenzoyl	28.48	5.267	11.70	30.68	15.952
DGS-13	$-CH_2COOC_2H_5$	2,4-dicholorobenzoyl	13.45	71.47	54.42	38.49	30.44
DGS-14	$-CH_2CH_2COOCH_3$	2,4-dicholorobenzoyl	27.70	70.48	179.7	66.90	52.79
DGS-15	$-CH_2CH_2COOC_2H_5$	2,4-dicholorobenzoyl	32.84	50.23	6.56	29.41	87.44
DGS-16	-H	3,5-dichlorobenzoyl	21.61	3.184	13.44	20.25	21.74
DGS-17	$-CH_2COOH$	3,5-dichlorobenzoyl	87.22	5.9	33.97	27.77	19.69
DGS-18	$-CH_2COOC_2H_5$	3,5-dichlorobenzoyl	>100	65.96	8.43	37.19	60.91
DGS-19	$-CH_2CH_2COOCH_3$	3,5-dichlorobenzoyl	>100	18.48	10.42	>100	85.44
DGS-20	$-CH_2CH_2COOC_2H_5$	3,5-dichlorobenzoyl	>100	55.76	8.83	32.69	64.99
DGS-21	-H	4-nitrobenzoyl	19.176	2.315	14.7	19.24	11.55
DGS-22	$-CH_2COOH$	4-nitrobenzoyl	22.779	1.752	11.75	14.28	12.355
DGS-23	$-CH_2COOC_2H_5$	4-nitrobenzoyl	16.33	12.74	74.28	55.001	>100
DGS-24	$-CH_2CH_2COOCH_3$	4-nitrobenzoyl	>100	30.34	25.76	>100	>100
DGS-25	$-CH_2CH_2COOC_2H_5$	4-nitrobenzoyl	25.76	9.41	110.41	99.63	94.75
DGS-26	-H	4-Chlorobenzoyl	4.54	2.54	2.439	5.947	6.854
DGS-27	$-CH_2COOH$	4-Chlorobenzoyl	3.6378	1.072	1.238	4.632	3.854
DGS-28	$-CH_2COOC_2H_5$	4-Chlorobenzoyl	80.86	36.30	9.13	19.82	31.22
DGS-29	$-CH_2CH_2COOCH_3$	4-Chlorobenzoyl	26.93	20.54	105.1	34.89	25.80
DGS-30	$-CH_2CH_2COOC_2H_5$	4-Chlorobenzoyl	19.83	60.72	>100	19.74	23.71
Std (Methotrexate)			0.2216	1.51	0.1016	0.1528	0.1002

Table 1: In vitro cytotoxic screening data of synthesized Thiazolidinedione derivatives compared against the standard drug Methotrexate

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