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SYNTHESIS AND EVALUATION OF ANTI-PROLIFERATIVE ACTIVITY OF NOVEL THIAZOLIDINONE DERIVATIVES

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Keywords:

Rhodanine, 2,4-thiazolidine-dione, Anti-proliferative activity, Leukemic cell lines

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ABSTRACT: A series of, 2-thioxo-4-thiazolidinone (rhodanine) and 2,4-thiazolidine-dinone derivatives were synthesized by reacting 4-thiazolidine-dione/rhodanine moieties with 2-(4-formylphenoxy)-N-substituted-phenyl-acetamide, in equimolar proportions. The authentication of synthesized compounds was done by FTIR, ^{1}HNMR, and mass spectrophotometry. The derivatives, N-(phenyl) -2- {4-[(4-oxo-2-thioxo-1, 3-thiazolidin-5-ylidene) methyl]phenoxy}acetamide and 2-{4-[(2,4-dioxo-1,3-thiazolidin-5-ylidene)-methyl]phenoxy}-N-(phenyl)-acetamide, were further screened for their anticancer properties against CEM cell lines. Cell viability was determined by trypan blue dye exclusion assay and cytotoxic effect of the compounds by MTT assay. The two derivatives, N-(phenyl)-2-{4-[(4-oxo-2-thioxo-1,3-thiazolidin-5-ylidene)methyl]phenoxy} acetamide and 2-{4-[(2,4-dioxo-1,3-thiazolidin-5-ylidene)-methyl]phenoxy}-N-(phenyl)-acetamide were found to be anti-proliferative in nature at 75 - 100 µM and 100 - 250 µM concentrations, respectively. Thus, it can be established that the rhodanine derivatives, N-(phenyl) -2- {4- [(4-oxo- 2- thioxo-1, 3-thiazolidin-5-ylidene) methyl]- phenoxy}acetamide and 2-{4-[(2,4-dioxo-1,3-thiazolidin-5-ylidene) methyl] phenoxy}-N-(phenyl)-acetamide have anti-proliferative property against leukemic cell lines (lymphoblastic leukemia) and can be further optimized to improve its efficacy and safety.

INTRODUCTION: Search of remedies for diseases has been the reason for research from the very beginning of human existence, which led to the discovery of new drugs or derivatives of known and tested drugs with more potency and less toxicity. The synthesis of derivatives has been an important part, as it is aimed at modifying the action of drugs, particularly to reduce its side effects [1].

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Furthermore; the scope of synthetic medicinal chemistry is broadening in recent times, as more than 60% of drugs used in practice are synthesized derivatives [2].

Cancer involves unregulated cell growth. The chemotherapeutic agents treat it by regulating or modifying the growth of cancer cells. As chemotherapy affects cell division, tumors with high growth fractions such as, acute myelogenous leukemia are more sensitive to chemotherapy as compared to those with slower growth rates (*e.g.*, indolent lymphomas). The chemotherapeutic agents are usually divided into alkylating agent, anti-metabolites, anthracyclines, plant alkaloids and other antitumor agents [3]. Irrespective of the class they represent, these agents are known to affect the

cell division or DNA synthesis in cancer cells, directly or indirectly. However, limited selectivity and high toxicity are some of the major limitations of the existing drugs [3]. Rhodanine, a derivative of thiazolidine, can be prepared by the reaction of carbon disulfide, ammonia and chloroacetic acid [4]. Rhodanines, thiazolidine-2, 4-diones, and pseudo-thiohydantoins have become a promising class of heterocyclic compounds since the introduction of various glitazones and epalrestat for the treatment of type-II diabetes mellitus and its complications. Chemical modifications of these heterocyclic compounds constantly result in drugs with a wide spectrum of pharmacological activities.

Additionally, synthesis of substituted rhodanines based on high throughput screening hits often leads to potent and selective modulators of targeted enzymes or receptors, which exert their pharmacological activities through different mechanisms of action. Thus, as a result of various possibilities of chemical derivatization of the rhodanine ring, the rhodanine-based compounds will probably remain a privileged scaffold in drug discovery process. The current study has been conducted to synthesize novel derivatives of rhodanine and characterize by physical and spectral analysis along with the evaluation of their anti-proliferative properties.

MATERIALS AND METHODS:

Chemicals: Aniline, triethylamine and 4-hydroxy benzaldehyde were purchased from Merck Specialities Pvt., Ltd., Qualigens Fine Chemicals and Spectrochem Private Limited, Mumbai, respectively. Glacial acetic acid, potassium carbonate, acetone, dichloromethane, methanol, ethanol, chloroform and dimethylformamide (DMF) were procured from S. D. Fine Chemicals Limited, Mumbai. The silica gel G, used for the preparation of thin layer chromatography, was also purchased from S. D. fine chemicals, Mumbai. Dimethyl sulfoxide (DMSO), trypan blue, and 3-(4, 5- dimethylthiazol-2-yl) -2, 5-diphenyltetrazolium bromide (MTT), used for evaluating cell proliferation, were purchased from Sigma-Aldrich, USA.

Synthesis of 2-(4-formylphenoxy)-N-substituted-phenyl-acetamide (III):

Synthesis of 2-chloro-*N*-substituted-acetamide (II): The reaction mixture was prepared by drop wise addition of 2-chloroacetyl chloride (24 mM) into a solution containing aryl amine/alkyl amine I (20 mM) and triethylamine (24 mM) prepared in anhydrous dichloromethane (20 mL) at 0 ºC. The reaction mixture was warmed to room temperature followed by stirring for approximately 20h. Residue obtained after the removal of solvent (under pressure) was filtered and washed with ice-cold water. The crude product was subsequently purified by crystallization from a mixture of methanol and water **Fig. 1**.

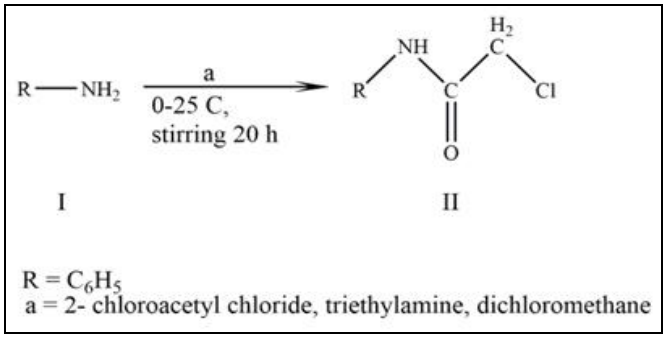

FIG. 1: SYNTHESIS OF 2-chloro-*N*-substituted-acetamide

Synthesis of 2-(4-formylphenoxy)-*N*-substituted-phenyl- acetamide (III) from 2- chloro -*N*-substituted-acetamide (II): Anhydrous potassium carbonate (20 mM), 4-hydroxybenzaldehyde (11mM), and 2-chloro-*N*-substituted acetamide II (10 mM) were dissolved in anhydrous acetone (30ml). The reaction mixture was refluxed for 24 h following the addition of potassium iodide (11mM) and cooled to room temperature. Potassium carbonate was filtered and acetone was removed under pressure to yield the crude product. Purification of the crude product was done by re-crystallization using DMF-ethanol mixture **Fig. 2** [5].

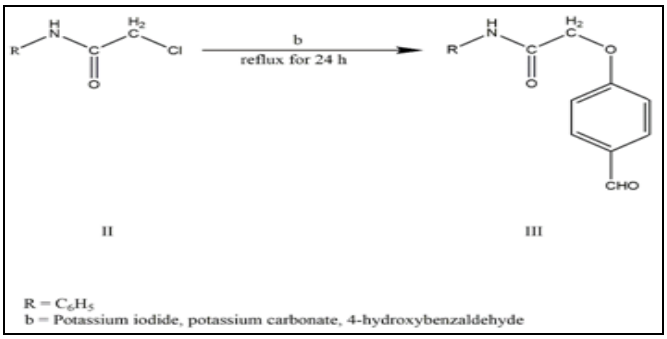

FIG. 2: SYNTHESIS OF 2-(4-formylphenoxy)-*N*-substituted-phenyl-acetamide

Synthesis of Derivatives from 2-thioxo-4-thiazolidinone (IV) and 2, 4-thiazolidinedione (V): Rhodanine (3 mM) / 2, 4-thiazolidine-dinone

and ammonium acetate (8.9 mM) were added to glacial acetic acid (4.5-10 ml) followed by the gradual addition of carbonylic substrate / aldehyde III (3.3 mM). The reaction mixture was refluxed for 1-12 h and cooled to room temperature, followed by addition of distilled water to get the precipitate. The product obtained was purified by re-crystallized with DMF-ethanol mixture **Fig. 3** [6].

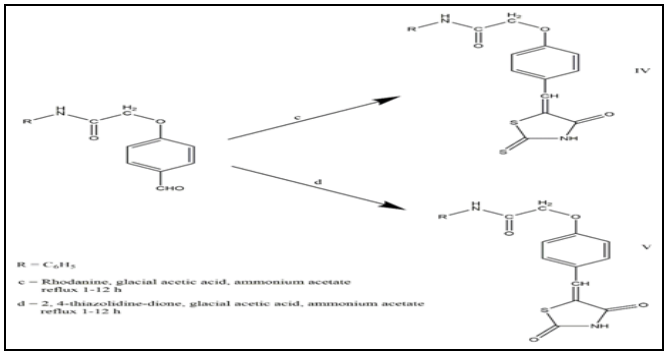

FIG. 3: SYNTHESIS OF DERIVATIVES FROM 2-thioxo -4-thiazolidinone AND 2, 4-thiazolidinedione

Characterization of Synthesized Compounds: The purity and structure of newly synthesized derivatives (N-(phenyl)-2-{4-[(4-oxo-2-thioxo-1,3-thiazolidin-5-ylidene)methyl] phenoxy}acetamide and 2-{4-[(2, 4-dioxo-1, 3-thiazolidin-5-ylidene) methyl] phenoxy}-N-(phenyl)-acetamide) were confirmed by TLC, FTIR, ^{1}H NMR and mass spectrometry. The melting point (MP) was determined by open capillary method in paraffin bath and reported uncorrected. The FTIR spectra were recorded on JASCO 460-plus spectrometer using KBr powder by diffuse reflectance technique. The range of IR region preferred for the study was between 4000-400 cm^{-1}. Readings for ^{1}HNMR spectra were recorded on a Bruker Ultraspec AMX 400 MHz spectrometer using TMS as internal standard and mass spectra were recorded on triple quadrapole LC/MS-6410 (Agilent technologies). TLC was performed by using pre-coated silica gel-G plates from S. D. Fine Chem., using toluene: ethyl acetate (1:1) as mobile phase.

Anti - proliferative Activity of Rhodanine Derivatives: Trypan blue dye exclusion and MTT assays were performed on CEM cell line (human T-cell leukemia cells). DMSO and 5-fluorouracil-treated cells were used as vehicle and positive controls, respectively. The assays were repeated three times and the values obtained were plotted against respective time points.

Trypan Blue Dye Exclusion Assay: The CEM cells were seeded at a density of 1×10^5 cells/well followed by incubation for 24 h. The test compounds were subsequently added into cells at concentrations of 10, 50, 100 and 250 µM. These cells were re-suspended in 0.4% trypan blue after harvesting at an interval of 24 h. The viable cells were counted using hemocytometer and IC_{50} values (50% inhibition of cell growth) were estimated after 48 and 72 h of treatment [7].

MTT Assay: Effect of test compounds were analyzed by treating the CEM cells with increasing concentrations of compounds (10, 50, 100 and 250 µM) followed by their incubation (24, 48, and 72 h). Subsequent estimation of proliferating cells was done based on their color change, following the addition of MTT reagent (5 mg/ml) [8].

RESULTS AND DISCUSSION: In the current study, derivatives were synthesized by reacting 2-(4-formylphenoxy) - N-substituted-phenyl-acetamide (III) with rhodanine (IV) and 2, 4-thiazolidine. The derivatives were confirmed and characterized with the help of physical and spectral analysis. The primary compounds -2- chloro- N- substituted-acetamide is a brown-colored compound; yield 80-85%; MP 95-100 ºC and 2-(4-formylphenoxy)-N-substituted-phenylacetamide is a brown colored compound; yield 80-85%; MP 70 - 75 ºC.

Spectral Analysis of Derivatives: N-(phenyl)-2-{4- [(4-oxo- 2-thioxo -1, 3-thiazolidin- 5-ylidene) methyl] phenoxy} acetamide: yellow - colored compound; molecular weight 370.04 g; yield 80-85%; MP 250-255 ºC; R_f 0.57; IR (, cm^{-1}): 3365.17 (-N-H), 3021.91 (aromatic -C-H), 2840.63 (aliphatic -C-H), 1649.8 (-C=O), 1451.17 (-C=C); ^{1}H-NMR: 10.12-13.76 (1H, s, N-H), 7.56-7.57 (6H, m, aromatic), 7.29-7.34 (2H, m, aromatic), 7.14-7.17 (2H, d, aromatic), 7.05-7.09 (1H, m, -C-H), 4.8 (1H, s, $-CH_2$), **Fig. 4a, b** and **c**.

2-{4- [(2, 4-dioxo-1, 3-thiazolidin -5- ylidene) methyl]phenoxy}-N-(phenyl)acetamide: Yellow-colored compound; molecular weight 354.07; yield 80-85%; MP 234 - 240 ºC; R_f 0.65; IR (cm^{-1}): 3387.35 (-N-H), 3150.15 (aromatic -C-H), 2917.77 (aliphatic -C-H), 1687.41 (-C=O), 1443.46 (-C=C); ^{1}H-NMR: 12.49 (1H, s, -N-H), 10.116 (1H, s, -N-H), 7.03-7.76 (10H, m, aromatic), 4.77-4.79 (2H, m, $-CH_2$), **Fig. 5**.

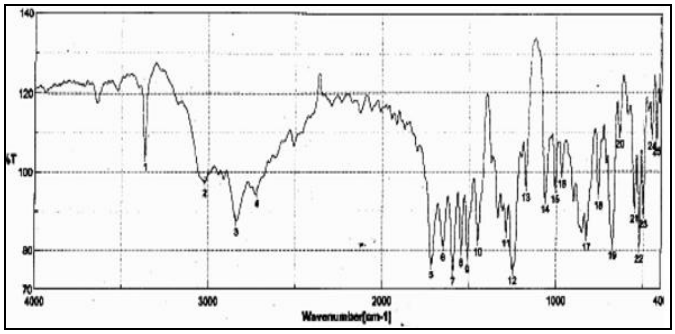

FIG. 4a: INFRARED SPECTRA OF *N*-(phenyl)-2-{4-[(4-oxo-2-thioxo-1, 3-thiazolidin-5-ylidene) methyl]-phenoxy}-acetamide

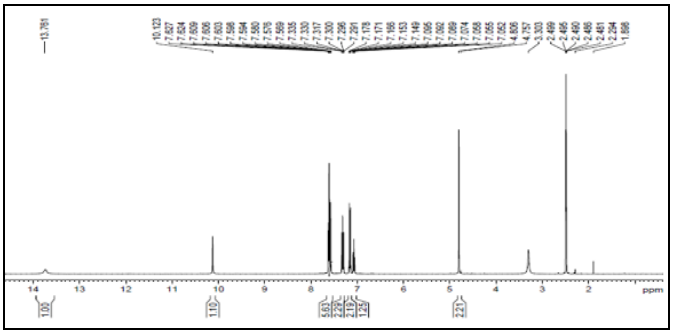

FIG. 4b: NMR SPECTRA OF *N*-(phenyl)-2-{4-[(4-oxo-2-thioxo-1, 3-thiazolidin-5-ylidene) methyl] phenoxy}-acetamide

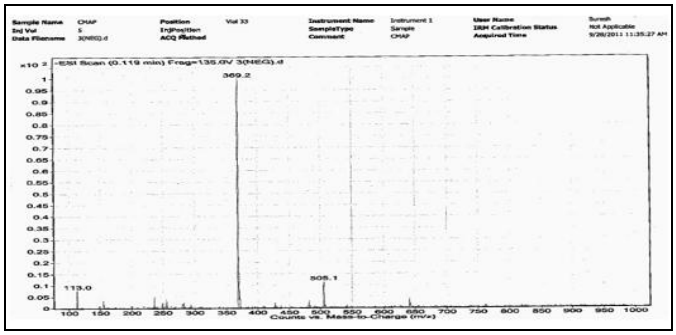

FIG. 4c: MASS SPECTRA OF *N*-(phenyl)-2-{4-[(4-oxo-2-thioxo-1, 3-thiazolidin-5-ylidene) methyl] phenoxy}acetamide

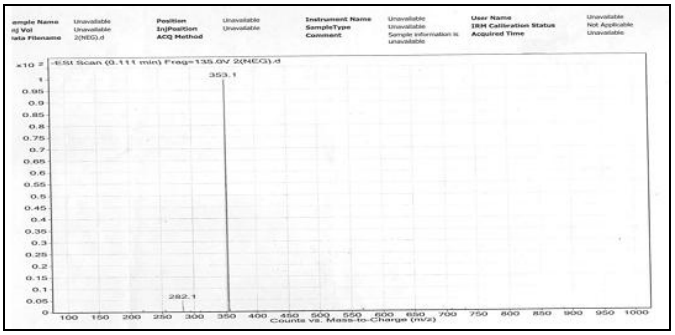

FIG. 5: MASS SPECTRA OF 2-{4-[(2, 4-dioxo-1, 3-thiazolidin-5-ylidene)-methyl]phenoxy}-*N*-(phenyl) acetamide

Rhodanine is a bioactive molecule having anti-inflammatory, antibacterial, antifungal, antiviral, antidiabetic, anticancer and antitubercular activities [9]. A study by Havrylyuk *et al*., [10] reported the synthesis and anticancer activity of 4-thiazolidinones containing benzothiazole moiety. In this study, reactions of hydrazine with tri-thiocarbonyl diglycolic acid or 6-methyl-2-aminobenzothiazole with 2-carbethoxymethylthio-2-thiazoline-4-one yielded 3- or 2-substituted-4-thiazolidinones, which was further condensed for obtaining a series of 5-arylidene derivatives having anticancer activity.

Anti-Proliferative Activity: In the current study, CEM cells were treated with increasing concentrations of the compounds. Cell viability and cytotoxic effect of the compounds was determined by trypan blue and MTT assay respectively. Since the compound was dissolved in DMSO, cells treated with DMSO alone served as vehicle control, cell counts were taken at an interval of 24 h.

Results obtained showed that treatment of CEM cells with test compounds affected viability of cells in a dose-dependent manner. *N*-(phenyl)-2-{4-[(4-oxo -2- thioxo-1, 3-thiazolidin-5-ylidene) methyl]-pheno-xy}-acetamide displayed a decrease in cell viability at a concentration of 100-250 µM after 24 h and at 75-100 µM after 72 h. On performing MTT assay, decrease in cell proliferation was observed at a concentration of 100-250 µM, even after 72 h of incubation.

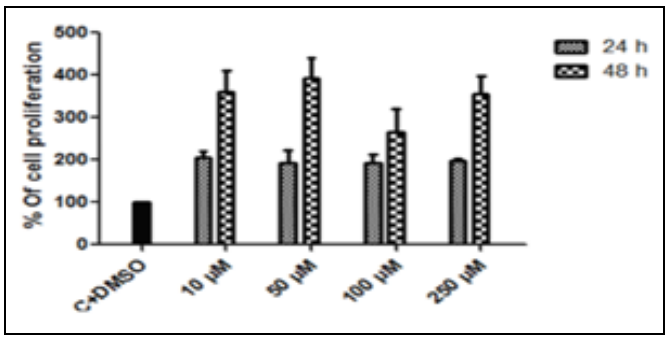

Fig. 6: TRYPAN BLUE ASSAY FOR N-(phenyl)-2-{4-[(4-oxo-2-thioxo-1, 3-thiazolidin-5-ylidene) methyl] phenoxy} acetamide

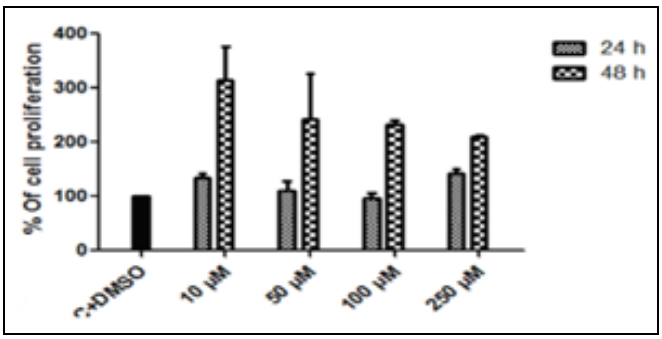

FIG. 7: TRYPAN BLUE ASSAY FOR 2-{4-[(2, 4-dioxo-1, 3-thiazolidin-5-ylidene) methyl] phenoxy}-*N*-(phenyl)-acetamide

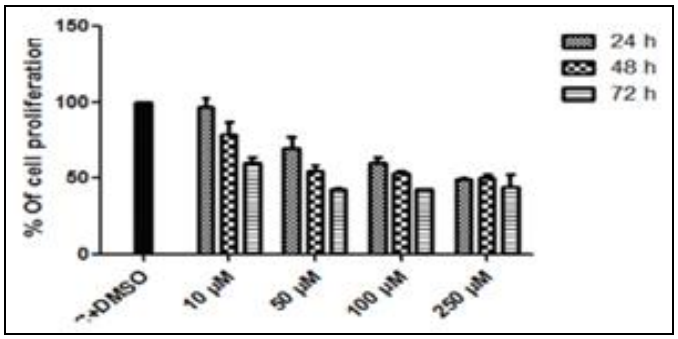

FIG. 8: MTT ASSAY FOR *N*-(phenyl)-2-{4-[(4-oxo-2-thioxo-1, 3-thiazolidin-5-ylidene)methyl] phenoxy}-acetamide

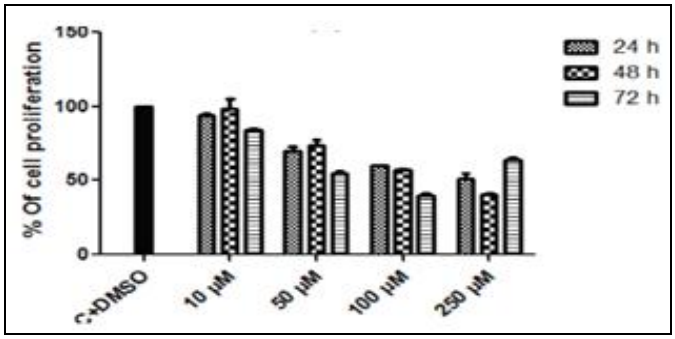

FIG. 9: MTT ASSAY FOR 2-{4-[(2,4-dioxo-1, 3-thiazolidin-5-ylidene) methyl]phenoxy}-*N*-(phenyl)-acetamide

Similar results were also exhibited by 2-{4-[(2, 4-dioxo-1-3-thiazolidin-5-ylidene) methyl]phenoxy}-*N*-(phenyl) acetamide. However, upon comparison of MTT assay after 72 h of incubation, 2-{4-[(2, 4-dioxo-1,3-thiazolidin-5-ylidene) methyl] phenoxy}-*N*-(phenyl)-acetamide exhibited a marginal increase in the percentage of cell proliferation than *N*-(phenyl)-2-{4-[(4-oxo-2-thioxo-1, 3- thiazolidin- 5-ylidene)- methyl] phenoxy}-acetamide **Fig. 6 - 9**.

CONCLUSION: It can be established that the rhodanine derivatives, *N*-(phenyl)-2-{4-[(4-oxo-2-thioxo-1, 3-thiazolidin-5-ylidene)methyl] phenoxy} acetamide and 2-{4-[(2,4-dioxo-1,3-thiazolidin-5-yli dene) methyl] phenoxy}-*N*-(phenyl)-acetamide have anti-proliferative activity on leukemic lines (lymphoblastic leukemia) and therefore can further be studied for improving its efficacy and safety.

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CONFLICT OF INTEREST: The authors declare that they have no conflict of interest.

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