ORIGINAL RESEARCH ARTICLE

Evaluation of genotoxicity of (4-fluorophenyl) thiazolidin-4-one in CHO-K1 cells

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Abstract

4-thiazolidinones are five-membered heterocyclic ring compounds with diverse pharmacological impacts. In a previous study, we reported a series of newly synthesized derivatives of 4-thiazolidinones with different functional groups, which exhibited anticancer activity against ovarian (SKOV3) and cervical (HeLa) cancer cell lines. Among these derivatives, (4-fluorophenyl) thiazolidin-4-one (4-TH) demonstrated potent cytotoxic activity against SKOV3, with an IC_{50} value of 12.3 µM. However, it was also found to be extremely toxic to normal cells (CHO-K1) with an IC_{50} of 7.5 µM. Before considering its use in cancer research, it is crucial to gain a comprehensive understanding of its potential genotoxic effects on normal cells. In this study, we aimed to assess the *in vitro* cytogenetic toxicity of 4-TH using normal Chinese hamster ovary cells (CHO-K1). Referring to the IC_{50} of 4-TH, we selected three sub-lethal concentrations (2, 5, and 7.5 µM) and treated CHO-K1 cells for 24 h (one cell cycle duration) to estimate its dose-dependent induction of chromosome aberrations, and examine the effect of 4-TH on cell division, micronucleus induction potential and cell cycle arrest properties following standard protocols. The results showed that 4-TH was highly toxic to normal cells, as all three sublethal concentrations caused a statistically significant increase in the number of chromosomal aberrations ($P < 0.001$), formation of micronuclei ($P < 0.01$), and changes in the rate of cell division (mitotic index) ($P < 0.05$) compared to control. In addition, there was a significant increase in the number of cells in the G1 phase, indicating that all concentrations of 4-TH tested induced apoptosis. The evaluation of the cytotoxic, clastogenic, and aneugenic properties of 4-TH, a potent cytotoxic agent, will undoubtedly provide critical information for determining its safety and potential as an anticancer drug.

1. Introduction

Heterocyclic compounds hold significant pharmacological importance[1,2]. Recently, there has been increasing industrial and biological interest in the chemistry of 4-thiazolidinones due to their diverse bioactive properties[3]. Thiazolidinones are five-membered heterocyclic compounds with a sulfur atom at position 1, a nitrogen atom at position 3, and a carbonyl group

at position 2, 4, or 5. Depending on the position of the oxo group or keto group, they are classified as 2-thiazolidinones, 4-thiazolidinones, and 5-thiazolidinones[4]. The applications of 4-thiazolidinone compounds are particularly diverse and can be found in a variety of clinically employed drugs. These compounds exhibit antitubercular[4], antimicrobial[5,6], anticonvulsant[7-9], anti-inflammatory and analgesic[10], and anti-cancer[11-13] properties. In addition, they have been reported as cox-1 inhibitors[14], bacterial enzyme inhibitors, and non-nucleoside inhibitors of HIV Type 1 reverse transcriptase[15-20]. Some synthetic 4-thiazolidinone (2-thioxo-4-thiazolidinone) derivatives have demonstrated potent cytotoxic against renal cancer, non-small cell lung cancer, breast cancer, melanoma, and ovarian cancer cell lines[21]. The versatility of 4-thiazolidinone derivatives has made them widely utilized pharmaceuticals and agrochemicals[22]. They have also been identified as optical brighteners[23], antioxidants[24], corrosion inhibitors[25], and additives with various additional properties. The popularity of heterocyclic molecules can be attributed to their ease of molecular structural modification, allowing for specific customization[26]. The reason these heterocyclic compounds perform so well is that they can act as molecules that can control protein synthesis, transmission of energy of nerve impulses, sight, and metabolism, all of which depend on chemical involvement of heterocyclic molecules in the form of vitamins, enzymes, co-enzymes, nucleic acids, ATP, and serotonin[27].

Earlier toxicological assessments were performed on various synthetic 4-thiazolidinone derivatives employing different methodologies, including acute toxicity, analgesic, and anti-inflammatory studies using mice models, which reported that the compounds did not cause any morbidity and mortality[28]. In line with this, we previously synthesized novel thiazolidine-4-one derivatives that demonstrated significant anticancer and antibacterial activities[29]. Among these derivatives, (4-fluorophenyl) thiazolidin-4-one (4-TH) showed potent anticancer activity against ovarian cancer cells (SKOV3) as well as toxicity to normal Chinese hamster ovary cells (CHO-K1). However, further investigation is needed to fully explore the safety and possible toxic effects of these compounds, particularly in the context of their use as chemotherapeutic agents. Hence, in this study, we employed 4-TH to assess its detailed genotoxic potential (clastogenic, mitotoxic, and aneugenic potentials) as well as its impact on the cell cycle in normal CHO-K1 cells.

2. Materials and methods

2.1. Test chemical

(4-fluorophenyl) thiazolidin-4-one (4-TH) was used in this study to assess its possible genotoxic potential *in vitro*. Its chemical structure and IUPAC name are as follows:
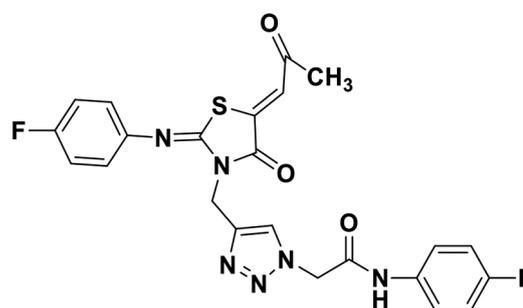
Molecular weight: 496.49

IUPAC name: N-(4-fluorophenyl)-2-(4-(((2Z,5Z)-2-((4-fluorophenyl)imino)-4-oxo-5-(2-oxopropylidene) thiazolidin-3-yl)methyl)-1H-1,2,3-triazol-1-yl)acetamide (4-TH)

2.2. DPPH assays

2,2-diphenyl-1-picryl-hydrazyl-hydrate (DPPH) is an electron transfer-based free radical technique that creates a violet solution in methanol. This experiment was carried out following our prior published work[30]. The DPPH assay is a simple and rapid approach to investigate antioxidant properties. The violet color of the DPPH solution changes to yellow as the molar absorptivity of DPPH radicals at 517 nm decreases from 9660 to 1640. During the assay, the odd electron of the DPPH radical is coupled with a hydrogen atom from a free radical scavenging antioxidant, resulting in the formation of the reduced DPPH-H. The subsequent decolorization of the solution is stoichiometric in terms of electron capture.

2.3. Cell line

The CHO-K1 cell line, a normal mammalian cell line, was obtained from ATCC (Bethesda, MD, USA). It was maintained in DMEM medium supplemented with 10% FBS, 2 mM L-glutamine, 100 µg/ml penicillin, and 100 µg/ml streptomycin, incubated at 37°C in a 5% CO_2 incubator. Cell viability, chromosome aberration, mitotic index, and micronucleus (MN) assays were conducted following the Organisation for Economic Co-operation and Development guidelines and our earlier published work[31,32].

2.4. Cell assays

2.4.1. Cytotoxicity and in vitro cytogenetic toxicity

MTT assay is a cell viability test commonly used to determine the cytotoxicity potential of chemicals. In the present study, CHO-K1 cells were seeded in 96-well culture plates. Once all of the cells attached to the plate surface, different concentrations of 4-Thiozolidone, ranging from

1 to 20 µM, were added in triplicates and incubated for 24 h. The media was discarded carefully to avoid cell dislodgement, and serum-free media containing MTT was added. The plate was then kept in a CO_2 incubator for 2 – 3 h to allow drying. Next, 100 µl of dimethylsulfoxide (DMSO) was added to each well to dissolve the crystals, and OD readings were taken at 570 nm to calculate the percentage of cell viability.

Based on the cytotoxicity results from the MTT assay, three sub-lethal concentrations of the (4-TH) (2, 5, and 7.5 µM) were selected for further cytogenetic toxicity studies, including chromosome aberration, mitotic index, and MN assays. All these tests were performed following our previously published work[31,33].

2.4.2. Chromosomal aberration assay

Chromosome aberration assay is performed to assess the clastogenic effects of compounds. In the present study, mitomycin-C and DMSO were used as the positive and vehicle controls, respectively, for comparison. The assay procedure followed our earlier work with some minor modifications, which are described briefly in the following. Cells were cultured and categorized into five different test groups. One group was treated with only DMSO as a vehicle control, and another with mitomycin-C (2.5 µM) as a positive control. The remaining three groups were treated with three different concentrations of 4-TH. All the treated cells were incubated for 24 h in 5% CO_2 at 37°C, then transferred to fresh media, and treated with 0.02% (w/v) colchicine for 30 – 40 min to arrest the cells in metaphase. The collected cells were washed with phosphate-buffered saline (PBS) and trypsinized. After hypotonic treatment with 0.5% KCl, cells were incubated in a 37°C incubator for 20 – 30 min and then centrifuged at 2000 rpm for 5 min. The cell pellet was collected and fixed with a fixative (1:3 v/v methanol and acetic acid). Chromosome slides were prepared using the flame drying method and stained with 10% Giemsa. At least 100 well-spread chromosome spreads in triplicates were scored from each concentration of 4-TH and from both control groups. During the count, each metaphase spread was screened to determine the clastogenic effects of the chemical by observing chromatid and chromosome aberrations, such as gaps, breaks, fragments, minutes, pulverization, and translocations.

2.4.3. Mitotic index assay

Mitotic index assay is performed to assess the effect of a compound on the proliferation rate of the cells and provides information on the rate of cell division in the presence of a toxicant. In the present study, slides were prepared following a similar procedure to the chromosome aberration assay, and the mitotic index was obtained from the ratio of the number of dividing cells (prophase to telophase) to the total number of cells observed. At least 2000 cells per test concentration were screened in triplicates.

2.4.4. MN assay

MN assay is a method used to assess both the clastogenic and aneugenic potency of a test chemical. In this study, CHO-K1 cells were treated with three different concentrations of 4-TH for 24 h. After treatment, the cells were washed with PBS and treated with cytochalasin-B (3 µg/ml) for another 24 h to obtain bi-nucleated cells. Subsequently, the cells were collected and incubated with 0.9% (v/v) sodium citrate for 10 min at 8°C. Following this, the cells were centrifuged at 1200 rpm for 5 min, and the pellet was collected. To this pellet, 0.5 ml of fresh sodium citrate was added, and smears were prepared on clean, grease-free glass slides. The slides were then kept overnight at 37°C in an incubator under 85% relative humidity. Finally, the slides were stained with 0.5% of Giemsa stain to visualize and score the presence of MN. At least 2000 cells were scored in triplicate to calculate the percentage of MN for each concentration, which was then compared with the control groups.

2.4.5. Cell cycle analysis

The effect of 4-TH on different phases of the cell cycle was assessed using flow cytometry analysis, following our previously reported protocol[33]. Briefly, CHO-K1 cells were treated with three different concentrations of 4-TH (2, 5, and 7.5 µM). Following treatment, the cells were trypsinized, collected in ice-cold 1 × PBS, and then washed twice. Subsequently, they were fixed overnight in 70% ethanol at −20°C. The following day, the sample was washed with 1×PBS, and the cells were stained using a propidium iodide (PI) solution supplemented with RNAse and triton-X for 45 min at room temperature. After staining, the cells were washed with 1 × PBS to remove any unbound PI, and cell cycle analysis was performed using the BD fluorescence-activated cell sorting (FACS) Canto system (USA).

2.5. Statistical analysis

Each experiment was carried out in triplicate. The results were analyzed using Student's t-test, followed by one-way analysis of variance tests (Dennett's post-test was performed using GraphPad Prism version 8, GraphPad Software, San Diego, CA). The mean differences were compared with the corresponding control samples to determine the level of significance.

3. Results and discussions

Despite the numerous bioactive properties reported for 4-thiazolidinone derivatives, a comprehensive

safety assessment is essential for their further clinical development[34]. Therefore, in this study, we conducted a detailed cytogenetic investigation on 4-TH using a normal mammalian cell line.
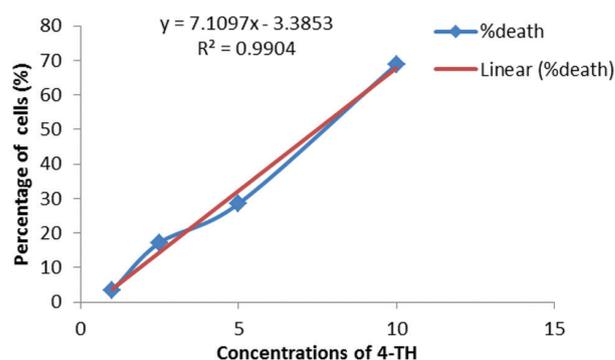
3.1. Cytotoxic and antioxidant effects of 4-TH

The cytotoxicity of 4-TH was tested at various concentrations ranging from 0.1 µM to 100 µM on the CHO-K1 cell line, and 50% cell death was observed at 7.5 µM concentration (Figure 1), indicating a high cytotoxic nature of the compound. Using this IC_{50} value as a reference, we selected three sub-lethal concentrations and conducted tests on normal CHO-K1 cells to assess their cytogenetic toxicity and effects on the cell cycle. In addition, we investigated whether the toxic effects were due to radical generation or direct interaction with the genetic material by evaluating the antioxidant properties of 4-TH using the DPPH assay.

The DPPH assay conducted on 4-TH demonstrated significantly lower antioxidant activity compared to the standard compound butylated hydroxytoluene. These observed antioxidant properties of 4-TH were consistent with the previous results on various thiazolidinone derivatives of 1,3-thiazole and 1,3,4-thiadiazole[35]. In general, the phenolic hydroxyl group is associated with antioxidant activities due to hydrogen donation and free radical stabilization through resonance. The results showed that the scavenging effect increased with increasing 4-TH concentrations, with the EC_{50} value found to be 50 µM (Figure 2). The antioxidant activity could be attributed to the presence of phenolic hydroxy groups in the structures. However, IC_{50} in CHO-K1 cells revealed that even at 7.5 µM, it induced 50% cell death. This high toxicity in the presence of 4-TH could be due to an increase in oxidant radical generation, even at low concentrations. These radicals may interact with cell biomolecules and genetic material, causing more cytotoxic effects in CHO-K1 cells, even at very low concentrations.

3.2. Effect of 4-TH on cell cycle phases

We performed FACS to evaluate changes in cell cycle phases and understand the toxic effect of 4-TH on CHO-K1 cells. As shown in Figure 3, all three tested concentrations of 4-TH (2.5, 5, and 7.5 µM) induced apoptotic cells in the sub-G1 phase, with percentages of 8.7%, 5.9%, and 7.5%, respectively. In addition, a higher accumulation of cells was observed in G0/G1 phase. This arrest of cells at the G0/G1 phase could be attributed to the DNA repair process, indicating that the cells experienced a highly toxic exposure to the compound and required time to pass through S-phase. Thereby there was a reduction in cell population in the S phase. Moreover, during the G2/M

Figure 1. Percentage of cytotoxic effect (MTT assay) of 4-TH on CHO-K1 cell line.
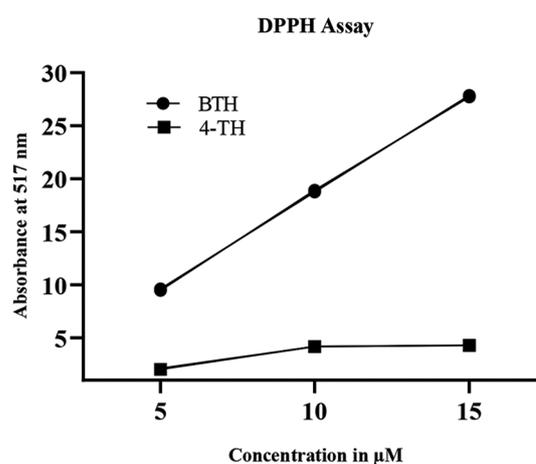
Figure 2. Antioxidant activity of 4-TH. Data are presented as the mean of two independent replications ± standard error of the mean (SE); EC_{50} value of 4-TH = 50 µM.

phase, a higher number of cells accumulated compared to other phases, suggesting that cells were undergoing DNA repair during mitotic cell division. This study provides evidence that 4-TH has the ability to induce DNA damage, leading to the activation of apoptosis pathways in the cells. It is also assumed that the unrepaired DNA-damaged cells from the G1 phase finally arrest at the G2/M phase for further DNA repair before entering the next cell division. Hence, 4-TH is likely to induce cell death, possibly through apoptosis, and the toxicity caused is independent of the S phase.

3.3. Cytogenetic toxicity of 4-TH

3.3.1. Chromosomal aberration assay

Chromosomal aberrations were assessed in CHO-K1 cell lines after 24 h of post-treatment with DMSO, mitomycin-C, and 4-TH at different concentrations (2.5, 5, and 7.5 µM). DMSO-treated cells exhibited 26
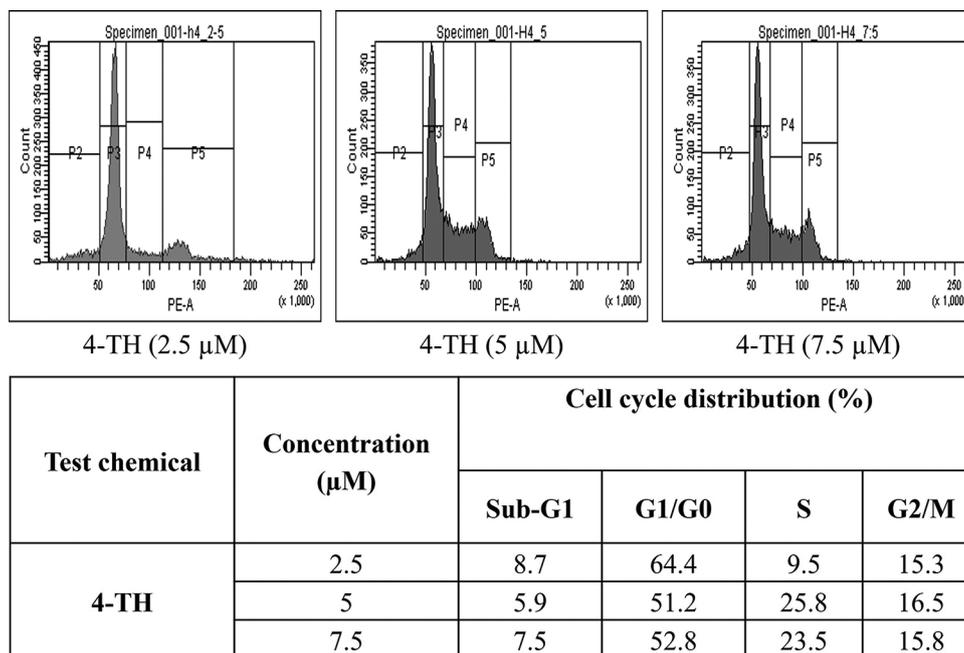
Figure 3. The effect of 4-TH on the cell cycle distribution in CHO-K1 cells.

Test chemical	Concentration (µM)	Cell cycle distribution (%)			
		Sub-G1	G1/G0	S	G2/M
4-TH	2.5	8.7	64.4	9.5	15.3
	5	5.9	51.2	25.8	16.5
	7.5	7.5	52.8	23.5	15.8

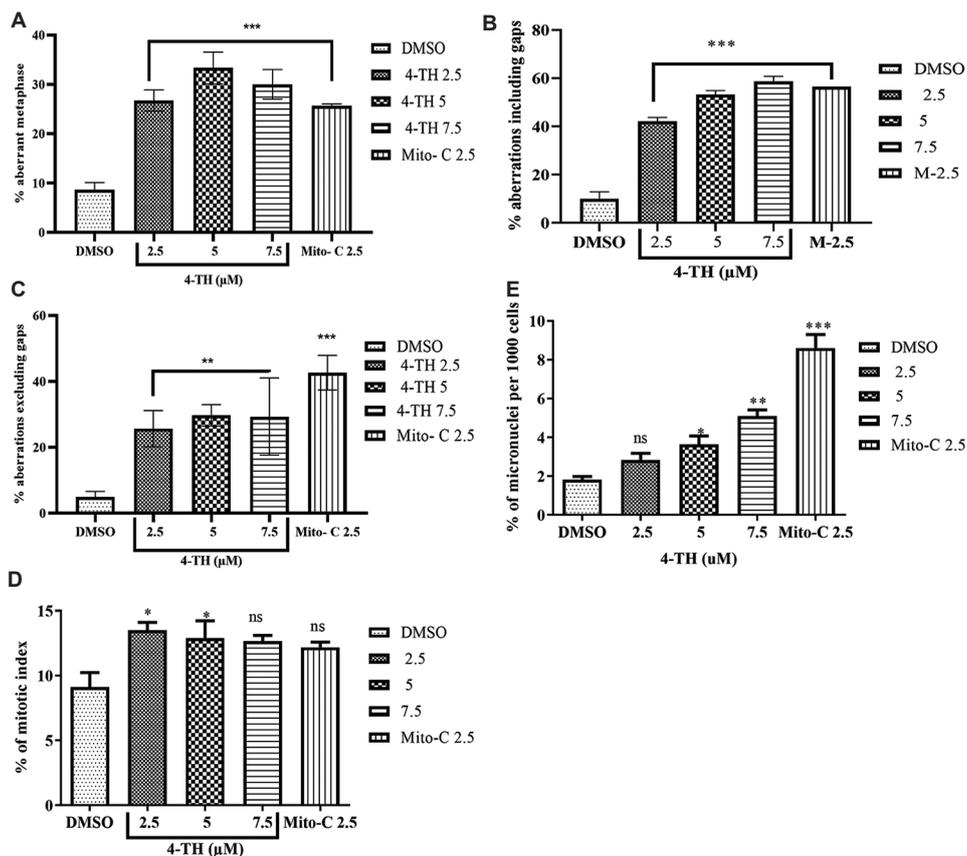Figure 4. Cytogenetic toxicity of 4-TH in different concentrations on Chinese hamster ovary cells. (A) Percentage of aberrant metaphases; (B) total number of aberrations including gaps; and (C) total number of aberrations excluding gaps. (D) Changes in mitotic index and (E) induction of micronucleus. ***$P < 0.001$, **$P < 0.01$, and *$P < 0.05$ compared to dimethylsulfoxide (vehicle control) using Dunnett's multiple comparison test.

aberrant metaphases (8.63%) out of 301 metaphases, with 9.95% of aberrations including gaps and 4.9% excluding gaps. Mitomycin-C induced 90 aberrant metaphases (25.63%) out of 351 metaphases, with 56.41% of aberrations including gaps, and 40.48% excluding gaps. During the evaluation, mitomycin-C treated cells induced a greater number of complex chromosomal abnormalities like triradial and dicentric chromosomes. The induction of chromosomal aberrations was found to be statistically significant at $P < 0.001$ level compared to the group treated with DMSO (Table 1). 4-TH compound at 2.5 μM induced 73 aberrant metaphases (26.76%) out of 270 metaphases, with 42.22% of aberrations including gaps, which were statistically significant compared to DMSO-treated cells ($P < 0.001$). Moreover, the percentage of aberrations excluding gaps was 26.29%, which showed statistical significance when compared with the vehicle control ($P < 0.01$). At an intermediate concentration of 5 μM, 101 aberrant metaphases (33.33%) were observed out of 301 metaphases, with 53.15% aberrations including gaps ($P < 0.001$), and 29.90% of aberrations excluding gaps ($P < 0.01$) compared to DMSO-treated cells. The highest concentration of 7.5 μM induced 90 aberrant metaphases (30%), with 58.66% aberrations including gaps, which were statistically significant when compared to the vehicle control ($P < 0.001$). In addition, the total number of aberrations excluding gaps (29.33%) was statistically significant ($P < 0.01$) when compared to the vehicle control (Figure 4A-C).

DNA strand-breaks induced by the toxic agents can manifest as various forms of chromosome aberrations, depending on the phase of the cell cycle. For example, DNA damage occurring during the G1 phase mostly leads to chromosome aberrations such as chromosome gaps, chromosome breaks, dicentric chromosomes, or ring chromosomes. On the other hand, when the agent causes DNA damage during the S-phase of the cell cycle, it primarily results in chromatid aberrations, including chromatid gaps, breaks, fragments, or minutes[31,36]. Among all the tested concentrations of 4-TH, the highest concentration (7.5 μM) induced a higher number of translocations, ring chromosomes, and other complex chromosomal aberrations compared to other concentrations. In addition, this chromosome aberration test confirms that 4-TH at all three tested concentrations exhibits highly clastogenic properties (Table 1).

3.3.2. Mitotic index

To understand the rate of cell division in the presence of different concentrations of 4-TH, the mitotic index was analyzed. The cells treated with DMSO exhibited 277 dividing cells (9.12%) out of a total of 6039 cells observed. In contrast,

Table 1. Frequency of chromosome aberrations induced by 4-TH at three different concentrations in CHO-K1 cells 24-h post-treatment

Compound	Dose (μM)	No. of metaphases observed	No. of aberrant metaphases	Chromatid type of aberrations G	B	Chromosome type of aberrations G	B	F	M	Tr	Qr	Dic	% of aberrant metaphase	Total no. of aberrations	% of aberrations including gaps	% of aberrations excluding gaps
DMSO (vehicle control)	1%	301	26	15	13	0	1	0	0	0	0	0	8.63±0.85	30	9.95±1.69	4.96±0.96
4-TH	2.5	270	73	33	17	5	3	2	4	1	1	16	26.76±1.24***	114	42.22±0.93***	26.29±0.96**
	5	301	101	48	26	11	5	2	2	1	0	24	33.33±1.85***	160	53.15±1.01***	29.90±1.87**
	7.5	300	90	44	14	22	8	8	1	2	0	20	30.00±1.73***	176	58.66±1.20***	29.33±6.76**
Mitomycin-C (positive control)	2.5	351	90	34	46	11	5	4	7	9	0	25	25.63±0.23***	198	56.41±3.53***	40.48±3.03***

Notes: DMSO and mitomycin-C as vehicle and positive controls, respectively. Types of chromosomal aberrations: G: Gaps; B: Breaks; F: Fragment; M: Minute; Tri: Triradial; Qr: Quadra radial; Dic: Dicentric. The level of significance determined in the treated group was compared with the vehicle control using Dunnett's multiple comparison test. ***$P<0.001$; **$P<0.01$; ±: Mean±SEM.

Table 2. Changes in the mitotic index observed after 24-h post-treatment with three different concentrations of 4-TH in CHO-K1 cells

Compound	Dose (μM)	Total number of cells	Total number of dividing cells observed	Percentage of mitotic index
DMSO	--	3035	277	9.12±1.12
4-TH	2.5	3007	406	13.50±0.60*
	5	3027	390	12.88±1.33*
	7.5	3028	383	12.64±0.45 (ns)
Mitomycin- C	2.5	3051	371	12.15±0.42 (ns)

Notes: DMSO and mitomycin-C as vehicle and positive controls, respectively. ±: SEM; ns: Not significance and *$P<0.05$ compared to DMSO (vehicle control) using Dunnett's multiple comparison test

Table 3. Induction of micronuclei in CHO-K1 cells after treatment with three different concentrations of 4-TH for 24 h

Chemical used	Dose (μM)	Total number of cells scored	Number of micronucleus observed	Percentage of micronuclei/1000 cells
DMSO	-	6039	11	1.82±0.16
4-TH	2.5	6016	17	2.82±0.34 (ns)
	5	6048	22	3.63±0.43*
	7.5	6077	31	5.10±0.31**
Mitomycin-C	2.5	6046	52	8.60±0.70***

Notes: DMSO and mitomycin-C as vehicle and positive controls, respectively. ±: SEM; ns: Not significant, ***$P<0.001$, **$P<0.01$, *$P<0.05$ compared to DMSO (vehicle control) using Dunnett's multiple comparison test.

the positive control, the cells treated with mitomycin-C, showed 371 (12.15%) dividing cells out of 3051 cells, and the mitotic index was statistically not significant (Table 2). Similarly, in CHO-K1 cells treated with 4-TH at 2.5 μM and 5 μM concentrations, the rate of dividing cells was significantly higher compared to the vehicle control ($P<0.05$). However, no significant difference in the rate of dividing cells was observed when the cells were treated with 4-TH at a concentration of 7.5 μM. The lowest and intermediate concentrations of 4-TH induced a significant increase in cell division compared to control DMSO ($P<0.05$). The highest concentration of 4-TH induced a lower percentage of dividing cells, and it was not statistically significant compared to DMSO-treated cells (Figure 4D). Interestingly, all these three concentrations of 4-TH induced a greater number of dividing cells compared to the positive control group. These results are also correlated with the cell cycle study, where a greater number of cells accumulated in the G/M phase.

3.3.3. Micronuclei

In the DMSO-treated CHO-K1 cells, 11 micronuclei (1.82%) were observed out of 6039 cells. However, cells treated with mitomycin-C (2.5 μM) had a significantly higher percentage of MN (8.60%), with 51 micronuclei observed out of 6046 cells ($P<0.001$). Among the 4-TH treatment groups, cells treated with 2.5 μM concentration exhibited 17 MN (2.82%) out of 6016 cells, 5 μM concentration exhibited 22 micronuclei (3.63%) out of 6048 cells, and 7.5 μM concentration exhibited 31 MN (5.10%) out of 6077 cells. The lowest concentration of

2.5 μM did not show any statistical significance, while the intermediate concentration of 5 μM showed statistical significance at $P<0.05$ level, and the highest concentration of 7.5 μM showed statistical significance at $P<0.01$ level when compared to the vehicle control. Among all the tested concentrations, 7.5 μM showed the highest percentage of MN induction (Table 3). The formation of a greater number of MN is also correlated with the observed chromosome aberrations, such as breaks, fragments, and minutes, which could not participate during the anaphase stage and ultimately form MN[37,38]. Hence, based on this MN test, it is confirmed that all the tested concentrations of 4-TH exhibit a highly aneugenic nature.

4. Conclusion

Our *in vitro* genotoxicity assessments on the mammalian cell line system revealed that 4-TH possesses high clastogenic and aneugenic properties, induces apoptosis, and significantly affects the proliferation rate of normal cells. These findings raise concerns about the potential carcinogenic and mutagenic effects of 4-TH on normal cells, which could pose health risks, including the recurrence of secondary cancers post-treatment with this compound as a drug. Therefore, further investigations are imperative to ensure the safety of 4-TH.

Acknowledgments

Conflict of interest

All the authors declare that they have no competing interests.

Author contributions

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Investigation: Jhansi Mamilla, Kalpana Javvaji, Kavya Lekha Sunkara, Umesh B. Kosurkar
Methodology: Sunil Misra, Ravindra M. Kumbhare
Writing – original draft: Kalpana Javvaji, Sunil Misra
Writing – review & editing: Sunil Misra, Ravindra M. Kumbhare

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Availability of data

Supporting data can be obtained from corresponding author following formal request.

References

1. Brown FC, 1961, 4-Thiazolidinones. *Chem Rev*, 61: 463–521.

2. Jain AK, Vaidya A, Ravichandran V, *et al.*, 2012, Recent developments and biological activities of thiazolidinone derivatives: A review. *Bioog Med Chem*, 20: 3378–3395.

    https://doi.org/10.1016/j.bmc.2012.03.069

3. Tripathi AC, Gupta SJ, Fatima G, *et al.*, 2014, 4-Thiazolidinones: The advances continue. *Eur J Med Chem*, 72, 52–77.

    https://doi.org/10.1016/j.ejmech.2013.11.017

4. Thomas B, LS A, Harindran J, 2014, Novel Mannich bases of 4-thiazolidinone derivatives as antitubercular agents. *Int J Res Pharm Chem*, 4: 351–359.

    https://doi.org/10.1016/j.jsps.2010.05.002

5. Patel NB, Shaikh FM, 2010, Synthesis and antimicrobial activity of new 4-thiazolidinone derivatives containing 2-amino-6-methoxybenzothiazole. *Saudi Pharm J*, 18: 129–136.

6. Vicini P, Geronikaki A, Anastasia K, *et al.*, 2006, Synthesis and antimicrobial activity of novel 2-thiazolylimino-5-arylidene-4-thiazolidinones. *Bioorg Med Chem*, 14: 3859–3864.

    https://doi.org/10.1016/j.bmc.2006.01.043

7. Ulusoy N, Ergenç N, Ekinci AC, *et al.*, 1996, Synthesis and anticonvulsant activity of some new arylidenehydrazides and 4-thiazolidinones. *Mon Chem/Chem Mon*, 127: 1197–1202.

    https://doi.org/10.1007/bf00844695

8. Archana, Srivastava VK, Kumar A, 2002, Synthesis of newer thiadiazolyl and thiazolidinonyl quinazolin-4 3H-ones as potential anticonvulsant agents. *Eur J Med Chem*, 37: 873–882.

    https://doi.org/10.1016/s0223-5234(02)01389-2

9. Shiradkar MR, Ghodake M, Bothara KG, *et al.*, 2007, Synthesis and anticonvulsant activity of clubbed thiazolidinone-barbituric acid and thiazolidinone-triazole derivatives. *Arkivoc*, 14: 58-74.

    https://doi.org/10.3998/ark.5550190.0008.e08

10. Ottana R, Maccari R, Barreca ML, *et al.*, 2005, 5-Arylidene-2-imino-4-thiazolidinones: Design and synthesis of novel anti-inflammatory agents. *Bioorg Med Chem*, 13: 4243–4252.

    https://doi.org/10.1016/j.bmc.2005.04.058

11. Boyd MR, Paull KD, 1995, Some practical considerations and applications of the national cancer institute *in vitro* anticancer drug discovery screen. *Drug Dev Res*, 34: 91–109.

    https://doi.org/10.1002/ddr.430340203

12. Carter PH, Scherle PA, Muckelbauer JA, *et al.*, 2001, Photochemically enhanced binding of small molecules to the tumor necrosis factor receptor-1 inhibits the binding of TNF-alpha. *Proc Natl Acad Sci*, 98: 11879–11884.

    https://doi.org/10.1073/pnas.211178398

13. Cutshall NS, O'Day C, Prezhdo M, 2005, Rhodanine derivatives as inhibitors of JSP-1. *Bioorg Med Chem Lett*, 15: 3374–3379.

    https://doi.org/10.1016/j.bmcl.2005.05.034

14. Look GC, Schullek JR, Holmes CP, *et al.*, 1996, The identification of cyclooxygenase-1 inhibitors from 4-thiazolidinone combinatorial libraries. *Bioorg Med Chem Let*, 6: 707–712.

    https://doi.org/10.1016/0960-894x(96)00097-2

15. Barreca ML, Chimirri A, De Luca L, *et al.*, 2001, Discovery of 2,3-diaryl-1,3-thiazolidin-4-ones as potent anti-HIV-1 agents. *Bioorg Med Chem Lett*, 11: 1793–1796.

    https://doi.org/10.1016/s0960-894x(01)00304-3

16. Rao A, Carbone A, Chimirri A, *et al.*, 2002, Synthesis and anti-HIV activity of 2,3-diaryl-1,3-thiazolidin-4-(thi)one

derivatives. *Il Farmaco*, 57: 747–751.

https://doi.org/10.1016/S0014-827X(02)01268-5

17. Rao A, Balzarini J, Carbone A, *et al.*, 2004, Synthesis of new 2,3-diaryl-1,3-thiazolidin-4-ones as anti-HIV agents. *Il Farmaco*, 59: 33–39.

https://doi.org/10.1016/j.farmac.2003.09.001

18. Balzarini J, Orzeszko B, Maurin JK, *et al.*, 2007, Synthesis and anti-HIV studies of 2-adamantyl-substituted thiazolidin-4-ones. *Eur J Med Chem*, 42: 993–1003.

https://doi.org/10.1016/j.ejmech.2007.01.003

19. Rao A, Chimirri A, Ferro S, *et al.*, 2004, Microwave-induced synthesis of benzimidazole and thiazolidinone derivatives as HIV-1 RT inhibitors. *Arkivoc,* 5: 147–155.

https://doi.org/10.3998/ark.5550190.0005.514

20. Ravichandran V, Kumar BP, Sankar S, 2009, Predicting anti-HIV activity of 1,3,4-thiazolidinone derivaties: 3D-QSAR approach. *Eur J Med Chem*, 44: 1180–1187.

https://doi.org/10.1016/j.ejmech.2008.05.036

21. Mosula L, Zimenkovsky B, Havrylyuk D, *et al.*, 2009, Synthesis and antitumor activity of novel 2-thioxo-4-thiazolidinones with benzothiazole moieties. *Farmacia*, 57: 321–330.

22. Dua R, Shrivastava S, Sonwane SK, *et al.*, 2011, Pharmacological significance of synthetic heterocycles scaffold: A review. *Adv Biol Res*, 5: 20–144.

23. Zahradnik M, 1983, The Production and Application of Fluorescent Brightening Agents. Translated by Wilkinson Procharzka ZF. Chichester: Wiley-Interscience, p147.

24. Naganna MG, Rohini YR, 2014, Synthesis antimicrobial and antioxidant evaluation of 3-(2-(4-fluorobenzylthio) pyrimidin-4-yl-amino)-2-(3-substituted phenyl) thiazolidin-4-ones. *World J Pharm Pharm Sci*, 3: 1094–1109.

25. Liu HL, Li Z, Anthonsen T, 2000, Synthesis and fungicidal activity of 2-imino-3-(4-arylthiazol-2-yl)-thiazolidin-4-ones and their 5-arylidene derivatives. *Molecules*, 5: 1055–1061.

https://doi.org/10.3390/50901055

26. Radin NS, 2008, Drug design: Hiding in full view. *Drug Dev Res*, 69: 15–25.

https://doi.org/10.1002/ddr.20223

27. Thomas AB, Nanda RK, Kothapalli LP, *et al.*, 2013, Synthesis, biological activity, molecular modelling studies and 3D-QSAR Investigations of N-[2-(aryl/substituted aryl)-4-oxo-1, 3-thiazolidin-3-yl] pyridine-4-carboxamides. *Open Conf Proc J*, 4: 99–112.

https://doi.org/10.2174/2210289201304010099

28. Taranalli AD, Bhat AR, Srinivas S, *et al.*, 2008, Antiinflammatory,analgesic and antipyretic activity of certain thiazolidinones. *Indian J Pharm Sci*, 70: 159.

https://doi.org/10.4103/0250-474X.41448

29. Kosurkar UB, Mamilla J, Dadmal TL, *et al.*, 2023, Synthesis of novel Thiazolidine-4-One derivatives, their cytotoxicity, antifungal properties, molecular docking and molecular dynamics. *Rus J Bioorg Chem*, 49, 314–323.

https://doi.org/10.1134/S1068162023020127

30. Gandhi B, Juliya J, Dileep V, *et al.*, 2021, Antioxidant and biological activities of novel structured monoacylglycerol derivatives with phenolic acids. *Eur J Lipid Sci Technol*, 123: 2100055.

https://doi.org/10.1002/ejlt.202100055

31. Deshpande SS, Veeragoni D, Rachamalla HK, *et al.*, 2022, Anticancer properties of ZnO-Curcumin nanocomposite against melanoma cancer and its genotoxicity profiling. *J Drug Deliv Sci Tech*, 75: 103703.

https://doi.org/10.1016/j.jddst.2022.103703

32. Choudante PC, Nethi SK, Díaz-García D, *et al.*, 2022, Tin-loaded mesoporous silica nanoparticles: Antineoplastic properties and genotoxicity assessment. *Biomater Adv*, 137: 212819.

https://doi.org/10.1016/j.bioadv.2022.212819

33. Javvaji K, Mamilla J, Kongari L, *et al.*, 2023, *In vitro* cytogenetic toxicity and cell cycle arrest profiling of fluorinated trifluoromethyl 4-Thiazolidinone on CHO-K1 cells. *Arch Clin Toxicol*, 5: 1–8.

https://doi.org/10.46439/toxicology.5.018

34. Bhat M, Poojary B, Kalal BS, *et al.*, 2018, Synthesis and evaluation of thiazolidinone-pyrazole conjugates as anticancer and antimicrobial agents. *Future Med Chem*, 10: 1017–1036.

https://doi.org/10.4155/fmc-2017-0191

35. Djukic M, Fesatidou M, Xenikakis I, *et al.*, 2018, *In vitro* antioxidant activity of thiazolidinone derivatives of 1,3-thiazole and 1,3,4-thiadiazole. *Chem Biol Interact*, 286: 119–131.

https://doi.org/10.1016/j.cbi.2018.03.013

36. Dillehay LE, Denstman SC, Williams JR, 1987, Cell cycle dependence of sister chromatid exchange induction by DNA topoisomerase II inhibitors in Chinese hamster V79 cells. *Can Res*, 47: 206–209.

37. Çelik A, Eke D, 2011, The assessment of cytotoxicity and genotoxicity of tetracycline antibiotic in human blood lymphocytes using CBMN and SCE analysis, *in vitro*. *Int J Hum Gen*, 11: 23–29.

https://doi.org/10.1080/09723757.2011.11886119

38. Hagmar L, Bonassi S, Strömberg U, *et al.*, 1998, Chromosomal aberrations in lymphocytes predict human cancer: A report from the European Study Group on Cytogenetic Biomarkers and Health (ESCH). *Can Res*, 58: 4117–4121.