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Synthesis of 4-thiazolidinone derivatives and assessment of their toxicological properties

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Abstract

The current study was designated to assess the potential toxicological effects of six newly synthesized 4-thiazolidinone derivatives by using Ames-Salmonella, Escherichia coli WP2, Zea mays seed germination and random amplified polymorphic DNA (RAPD) assay systems. The bacterial tester strains S. typhimurium TA1535, TA1537, TA1538, TA98, TA100 and E. coli WP2uvrA were chosen to check the gene mutation causing capabilities of the test materials in prokaryotic systems and Z. mays seeds for determination of toxicological potentials in eukaryotic systems. Furthermore, OPA-4 and OPY-13 primers were used in RAPD analysis to determine genotoxic activities on the eukaryotic genomes. According to the results, none of the test materials showed significant mutagenic activity on the bacterial tester strains at tested concentrations. On the other hand, all of them showed significant inhibition on the germination of Z. mays seeds in a dose dependent manner from 2 mM to 6 mM concentrations. The germination was completely inhibited at 6 mM and higher dose levels. The RAPD analysis results also showed a parallelism with the germination assay results and the test materials significantly changed the RAPD profiles for both primers when compared with the control groups. In a conclusion, these newly synthesized 4-thiazolidinone derivatives were determined as genotoxically suspect compounds according to the presented results, and they should analyze with multiple toxicology test systems before performing other types of biological activity determination studies.

Keywords: 4-Thiazolidinone, Genotoxicity, Ames-*Salmonella* assay, *E. coli* WP2 assay, *Z. mays* seed germination, RAPD.

1. Introduction

Synthetic chemicals play a major role to meet increasing industrial and medicinal demands in the developing world. Recently thiazolidine derivatives have attracted attentions with their versatile properties and they have been one of the important research focuses. 4-thiazolidinones are the derivatives of thiazolidine, which belong to an important group of heterocyclic compounds. Diverse biological activities such as antibacterial, pesticidal, antifungal, insecticidal, anticonvulsant, tuberculostatic, anti-inflammatory, antithyroidal, antiviral, SHP-2 inhibitor and calcium antagonist, potentiation of pentobarbital-induced sleeping time, etc., have been found to be associated with thiazolidinone derivatives [1-4].

Due to their importance in biological systems, 4-thiazolidinone derivatives have a wide-range usage potential in especially human health-care applications [1-4]. On the other hand, this situation necessitates the determination of toxicological properties for newly synthesized derivatives before more complicated biological activity studies [5].

In vitro bacterial reversion assay systems are frequently used for determining the genotoxic properties of natural or newly synthesized synthetic materials because of their cheap, reproducible, validated and fast-responsive applications. Ames-*Salmonella* and *E. coli* WP2 are among the most preferred bacterial test systems for mutagenicity researches focused on the determination of various substances causing gene mutations [6, 7].

On the other hand, germination tests performing with isogenic seeds of several higher plants and comparative RAPD profile analysis with known primers of the germinated seedlings may be used for determining the toxicological properties of test materials on eukaryotic systems with more complex metabolic pathways than prokaryotic cells [8-11].

Thus, the present study was designed to assess the toxicological potency of six newly synthesized 4-thiazolidinone derivatives by using Ames-*Salmonella*, *Escherichia coli* WP2, *Zea mays* seed germination and random amplified polymorphic DNA (RAPD) assay systems.

2. Materials and Methods

Materials

Direct acting mutagens sodium azide (NaN_3), 4-nitro-*o*-phenilenediamine (4NPD), 9-aminoacridine (9-AA) and *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine (MNNG) from ABCR GmbH & Co. KG, Sigma-Aldrich and Merck. Other solvents and pure chemicals, including magnesium sulfate ($MgSO_4$), sodium ammonium phosphate ($Na_2NH_2PO_4$), D-glucose, D-biotin, sodium chloride (NaCl), L-histidine HCl, L-tryptophane, sodium phosphate-dibasic (Na_2HPO_4), crystal violet, citric acid monohydrate, potassium phosphate-dibasic (K_2HPO_4), sodium phosphate-monobasic (NaH_2PO_4) were also obtained from Difo, Fluka, Merck and Sigma.

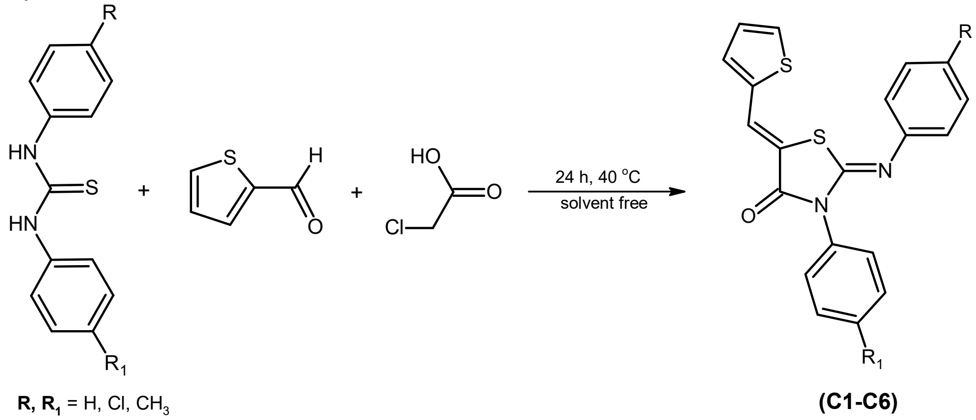
S. typhimurium TA1535 (ATCC® Number: 29629), TA1537 (ATCC® Number: 29630), TA1538 (ATCC® Number: 29631) strains were provided by The American Type Culture Collection – Bacteria Department of Georgetown University, Washington, USA, *S. typhimurium* TA98 (71-098L) and TA100 (71-100L) were obtained from Moltox Inc, USA, and *E. coli* WP2*uvrA* (ATCC® Number: 49979) strain was provided by LGC standards Middlesex, UK. All strains were stored at -80 ºC. Working cultures were prepared by inoculating nutrient broth with the frozen cultures, followed by an overnight incubation at 37 ºC with gentle agitation [5].

Methods

Synthesis of 4-thiazolidinone derivatives

A mixture of the appropriate amine (1 mmol) and substitute phenylisothiocyanate (1.2 mmol) was stirred in CH_2Cl_2 at room temperature for 24 h. The crude product was concentrated under vacuum and recrystallized from ethanol. The appropriate thiourea (1 mmol), chloroacetic acid (1.2 mmol) and thiophene-2-carbaldehyde (1 mmol) were stirred with a magnetic stirrer at 40 ºC for 24 h. The crude product was purified by column chromatography ($CHCl_3$ or CH_2Cl_2) [12]. Thus, six 4-thiazolidinone derivatives were obtained in the end of the process (Figure 1, Table 1).

Figure 1. Synthesis of 4-thiazolidinone derivatives



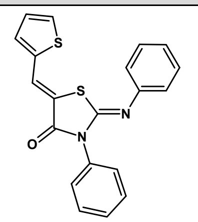
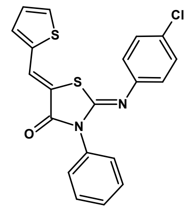
3-Phenyl-2-(phenylimino)-5-(thiophen-2-ylmethylidene)-1,3-thiazolidin-4-one (C-1): Yellow solid, m.p. 214-5 ^{0}C; FTIR (near) $\gamma_{max/cm^{-1}}$**:** 3096, 1707, 1635, 1591, 1493, 1364, 1353, 857; ^{1}H NMR (500MHz, CDCl$_3$)**:** δ **(**ppm) 6.99 (brd, 2H), 7.15 (dd, 1H), 7.19 (m, 1H), 7.37 (brd, 2H), 7.38 (d, 1H), 7.46 (dd, 2H), 7.48 (m, 1H), 7.54 (dd, 2H), 7.53 (d, 1H), 8.01 (s, 1H); ^{13}C NMR (100MHz, CDCl$_3$)**:** δ **(**ppm) 119.72, 121.32, 124.40, 125.18, 128.28, 128.68, 129.17, 129.55, 131.07, 132.42, 135.04, 138.31, 148.41, 150.72, 166.47; MS (m/z)**:** 364 M^{+2}, 363 M^{+1}, 362 M$^+$, 361 M^{-1}, 194, 140, 96, 77.

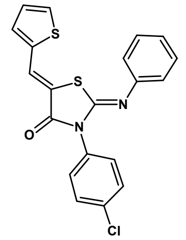
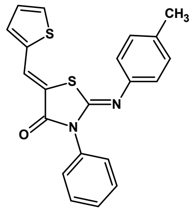
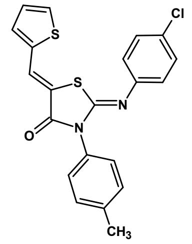
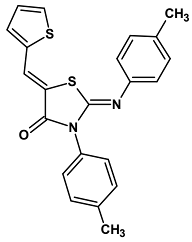
2-[(4-Chlorophenyl)imino]-3-phenyl-5-(thiophen-2-ylmethylidene)-1,3-thiazolidin-4-one (C-2): Yellow crystal, m.p. 162-3 ^{0}C; FTIR (near) $\gamma_{max/cm^{-1}}$**:** 3092, 1705, 1641, 1597, 1495, 1368, 1352, 850; ^{1}H NMR (500MHz, CDCl$_3$)**:** δ **(**ppm) 6.92 (dt, 2H), 7.16 (dd, 1H), 7.33 (dt,2H), 7.36 (brd, 1H), 7.45 (brd, 3H), 7.54 (brd, 2H), 7.59 (brd, 1H), 8.02 (s, 1H); ^{13}C NMR (100MHz, CDCl$_3$)**:** δ **(**ppm) 119.01, 122.55, 124.61, 128.02, 128.53, 129.08, 129.44, 130.31, 131.09, 132.46, 134.66, 137.95, 146.76, 151.28, 166.14; MS (m/z) **:** 398 M^{+2}, 397 M^{+1}, 396 M$^+$, 228, 168, 140, 125, 96, 77.

3-(4-Chlorophenyl)-2-(phenylimino)-5-(thiophen-2-ylmethylidene)-1,3-thiazolidin-4-one (C-3): Yellow crystal, m.p. 155-6 ^{0}C; FTIR (near) $\gamma_{max/cm^{-1}}$**:** 3089, 1710, 1637, 1592, 1498, 1361, 1354, 847; ^{1}H NMR (500MHz, CDCl$_3$) **:** δ **(**ppm) 6.98 (dd, 2H), 7.15 (dd, 1H), 7.19 (brd, 1H), 7.35 (brd, 1H), 7.36 (d, 1H), 7.38 (d, 1H), 7.43 (dt, 2H), 7.51 (dt, 2H), 7.57 (brd, 1H), 8.01 (s, 1H); ^{13}C NMR (100MHz, CDCl$_3$)**:** δ **(**ppm) 119.07, 121.04, 124.55, 125.11, 128.52, 129.39, 129.56, 131.07, 132.42, 133.17, 134.76, 137.96, 147.79, 150.23, 165.95; MS (m/z)**:** 398 M^{+2}, 397 M^{+1}, 396 M$^+$, 228, 168, 140, 125, 96, 77.

2-[(4-Methylphenyl)imino]-3-phenyl-5-(thiophen-2-ylmethylidene)-1,3-thiazolidin-4-one (C-4): Yellow crystal, m.p. 175-6 ^{0}C; FTIR (near) $\gamma_{max/cm^{-1}}$**:** 3023, 2916, 1709, 1634, 1597, 1497, 1360, 1346, 1260, 1172, 1112, 857, 811, 750; ^{1}H NMR (500MHz, CDCl$_3$)**:** δ **(**ppm) 2.37 (s, 3H), 6.80 (dd, 2H), 7.00 (brd, 1H), 7.14 (dd, 1H), 7.34 (brd, 1H), 7.48 (dd, 2H), 7.52 (dd, 2H), 7.54 (dd, 1H), 7.58 (brd, 2H), 8.00 (s, 1H); ^{13}C NMR (100MHz, CDCl$_3$)**:** δ **(**ppm) 21.15, 119.79, 120.10, 121.14, 124.45, 125.21, 127.36, 128.25, 129.48, 131.21, 132.45, 133.13, 134.67, 137.66, 144.51, 149.82, 166.50; MS (m/z) **:** 378 M^{+2}, 377 M^{+1}, 376 M$^+$, 208, 168, 140, 98, 91, 77.

Table 1. The chemical properties of the test materials.

Structure	Formula	Name	FW
 (C-1)	$C_{20}H_{14}N_2OS_2$	3-phenyl-2-(phenylimino)-5-(thiophen-2-yl methylidene)-1,3-thiazolidin-4-one	362.468 g/mol
 (C-2)	$C_{20}H_{13}ClN_2OS_2$	2-[(4-chlorophenyl)imino]-3-phenyl-5-(thiophen-2-yl methylidene)-1,3-thiazolidin-4-one	396.913 g/mol

 (C-3)	$C_{20}H_{13}ClN_2OS_2$	3-(4-chlorophenyl)-2-(phenylimino)-5-(thiophen-2-yl methylidene)-1,3-thiazolidin-4-one	396.913 g/mol
 (C-4)	$C_{21}H_{16}N_2OS_2$	2-[(4-methylphenyl)imino]-3-phenyl-5-(thiophen-2-yl methylidene)-1,3-thiazolidin-4-one	376.494 g/mol
 (C-5)	$C_{21}H_{15}ClN_2OS_2$	2-[(4-chlorophenyl)imino]-3-(4-methylphenyl)-5-(thiophen-2-yl methylidene)-1,3-thiazolidin-4-one	410.940 g/mol
 (C-6)	$C_{22}H_{18}N_2OS_2$	3-(4-methylphenyl)-2-[(4-methyl phenyl)imino]-5-(thiophen-2-yl methylidene)-1,3-thiazolidin-4-one	390.521 g/mol

2-[(4-Chlorophenyl)imino]-3-(4-methylphenyl)-5-(thiophen-2-ylmethylidene)-1,3-thiazolidin-4-one (C-5): Yellow crystal, m.p. 166-7 ^{0}C; FTIR (near) $\gamma_{max/cm}{}^{-1}$**:** 3071, 2916, 1710, 1631, 1590, 1499, 1369, 1353, 1273, 1154, 1117, 851, 820, 762; ^{1}H NMR (500MHz, $CDCl_3$)**:** δ **(**ppm) 2.37 (s, 3H), 6.96 (dd, 2H), 7.14 (dd, 1H), 7.19 (dd, 2H), 7.34 (d, 1H), 7.34 (dd, 2H), 7.35 (dd, 2H), 7.55 (d,1H), 8.00 (s, 1H) ppm; ^{13}C NMR (100MHz, $CDCl_3$)**:** δ **(**ppm) 21.48, 118.92, 121.36, 123.58, 127.68, 128.19, 129.32, 129.87, 130,55, 132.54, 134.25, 138.20, 143.84, 145,98, 148.61, 150.27, 166.29; MS (m/z)**:** 412 (M+2), 411 (M+1), 410 (M^+), 242, 140, 96, 65.

3-(4-Methylphenyl)-2-[(4-methylphenyl)imino]-5-(thiophen-2-ylmethylidene)-1,3-thiazolidin-4-one (C-6): Yellow solid, m.p. 146-7 ^{0}C; FTIR (near) $\gamma_{max/cm}{}^{-1}$**:** 3065, 2917, 1703, 1634, 1595, 1505, 1353, 863; ^{1}H NMR (500MHz, $CDCl_3$) **:** δ **(**ppm) 2.35 (s, 3H), 2.41 (s, 3H), 6.87 (brd, 2H), 7.13 (dd, 1H), 7.15 (brd, 2H), 7.33(brd, 1H), 7.34 (brs, 4H), 7.54 (brd, 1H), 7.99 (s, 1H); ^{13}C NMR (100MHz, $CDCl_3$)**:** δ **(**ppm) 21.03, 21.35, 119.74, 120.96, 123.83, 127.76, 128.42, 129.86, 130.06, 130.69, 132.04, 132.33, 134. 38, 138.13, 138.79, 145.74, 150.20, 166.41; MS (m/z) **:** 392 M^{+2}, 391 M^{+1}, 390 M^+, 222, 195, 140, 96, 41.

In vitro bacterial reversion assays

The toxicity of chemicals toward *S. typhimurium* TA1535, TA1537, TA1538, TA98, TA100 and *E. coli* WP2*uvrA* tester strains was determined as described in detail elsewhere. These tests confirmed that there was normal growth of the background lawn, spontaneous colony numbers within the regular range, and no significant reduction in cell survival. Thus, for the concentrations and conditions reported here, no toxicity or other adverse effects were observed [5].

The bacterial genotoxicity assays with the tester strains were performed according to the described by in detail elsewhere [6, 7]. The known mutagens sodium azide (NaN_3 in water - 5 µg/plate) for *S. typhimurium* TA1535 and TA100, 4-nitro-*o*-phenilenediamine (4NPD in DMSO - 2.5 µg/plate) for *S. typhimurium* TA1538 and TA98, 9-aminoacridine (9-AA in methanol - 50 µg/plate) for *S. typhimurium* TA1537, and *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine (MNNG in 10% DMSO - 1 µg/plate) for *E. coli* WP2*uvrA* were used as positive controls and 10% DMSO was used as negative control in these studies [6, 7].

In the genotoxicity test performed with *S. typhimurium* tester strains, 100 µl of the overnight bacterial culture, 50 µl of the test compounds at different concentrations (0.2, 0.4, 0.6, 0.8 and 1 mM/plate in 10% DMSO), and 500 µl of buffer solution were added to 2 ml of the top agar containing 0.5 mM histidine/biotin. The mixture was poured onto minimal glucose agar plates. Histidine independent revertant colonies and viable cells were scored on plates after incubation at 37 ºC for 48 h.

The procedure of the genotoxicity test described for the Ames-*Salmonella* assay is applicable to the *E. coli* WP2 reverse mutation assay. The only procedural difference is the addition of limited tryptophan (0.01 mM) instead of histidine to the top agar [7].

The plate incorporation method was used to assess the results of genotoxicity and antigenotoxicity assays [13].

In this procedure, the mutagenic index was calculated for each concentration, which is the average number of revertants per plate divided by the average number of revertants per plate with the negative (solvent) control. A sample is considered genotoxic when a dose-response relationship was observed and a two-fold increase in the number of mutants with at least one concentration was observed [5]. The results are presented as the average and standard deviation of three experiments with triplicate plates/dose experiment [14, 15].

Seed germination

Z. mays seeds with uniform genophond characteristics were obtained from Faculty of Agriculture - Atatürk University. For germination studies, the seeds were soaked in test material solutions at varying concentrations (2, 3, 4, 5, 6 mM) for 48 h in the dark at room temperature with gentle shaking on an orbital shaker at 150 rpm to get well mixing. After the incubation period, the seeds were washed with distilled water and transferred to 10-mm Petri dishes containing a piece of filter paper (90 mm) and 5 mL of distilled water. The seeds were tested for germination in a growth chamber under a range of conditions established by the OECD guidelines: temperature, 24 ºC; humidity, 70 ± 25%; photoperiod, 18-h light; and light intensity, 300 µE m^{-2} s^{-1} with protection from drying. In this experiment, each Petri dish (n=10) contained 5 seeds, and germination rates were investigated for 3 days. Distilled water and 10% DMSO solution are also used as negative control and vehicle control respectively [11].

RAPD Analysis

Healthy looking *Z. mays* seedlings after seed germination assay were chosen for DNA extraction. Genomic DNA was extracted from the root tips of the seedlings after liquid nitrogen treatment and powdering according to a modified method described by Lin et al. in 2001 [16]. In this method, Approximately 10-15 mg tissue samples were placed in 2 ml Eppendorf tubes; 1000 µl DNA extraction buffer [100 mM Tris-HCl (pH 8.0); 50 mM EDTA (pH 8.0); 500 mM NaCl; 2% SDS (w/v); 2% 2-mercaptoethanol (v/v); 1% PVP (w/v)] was added and mixed well. The tube was placed in a water bath at 65 ºC and was incubated for 40 min and shaken briefly at 5-min intervals. The sample was centrifuged at 12 000 × g for 15 min at 4 ºC. The supernatant was transferred into a new 1.5 ml tube and mixed with equal volume of phenol: chloroform:isoamylalcohol (25:24:1), and then centrifuged. The supernatant was transferred and mixed with 1/10 volume 10% CTAB-0.7 M NaCl in a new tube. After centrifugation, the supernatant was added with equal volume of chloroform: isoamylalchol (24:1). The DNA was precipitated by the addition of 0.6 volume of freezer-cold isopropanol, left at -20 ºC for 10 min. The DNA was pelleted by centrifugation (12 000 × g, 10 min) and the isopropanol was poured off carefully; the sample was allowed to air-dry before being dissolved in 100 ml of TE buffer. Then samples were screened for RAPD variation using standard 10-base primers supplied by Operon (Operon Technologies, Inc., Alameda, California, USA). Thirty microliters of reaction cocktail was prepared as follows: 10× buffer (3.0 µl), deoxynucleoside triphosphates (10 mM; 1.2 µl), magnesium chloride (25 mM; 1.2 µl), primer (5 µM; 2.0 µl), Taq polymerase (5 units; 0.4 µl), water (19.2 µl), and sample DNA (3.0 µl; 100 ng/µl). 2 primers were selected and used for amplification studies. Sequences (5'→3') of primers were AATCGGGCTG (OPA-4) and GGGTCTCGGT (OPY-13). The thermal cycle was as follows: 2 minutes at 95 ºC; 2 cycles of 30 seconds at 95 ºC, 1 minute at 37 ºC, 2 minutes at 72 ºC; 2 cycles of 30 seconds at 95 ºC, 1 minute at 35 ºC, 2 minutes at 72 ºC; and 41 cycles of 30 seconds at 94 ºC, 1 minute at 35 ºC, 2 minutes at 72 ºC, followed by a final 5-minute extension at 72 ºC, then brought down to 4 ºC [10].

The PCR products (27 µl) were mixed with 6× gel loading buffer (3 µl) and loaded onto an agarose gel (1.5% w/v), electrophoresis was done in 0.5× TBE (Trise-Borate-EDTA) buffer at 70 V for 150 min. Amplification products separated by gel was stained in ethidium bromide solution (2 µl EtBr/100 ml 1× TBE buffer) for 40 min [10]. The amplified DNA product was detected by using the Bio Doc Image Analysis System with Uvisoft analysis package (Cambridge, UK). Each experiment in this study is repeated at least twice and only reproducible results were scored [10]. RAPD patterns were evaluated by using TotalLab TL120 computer software.

3. Results and Discussion

Synthetic derivatives of 4-thiazolidinones, which belong to the thiazolidine derivatives, possess important biochemical and pharmacological properties. These compounds appears to offer promising a potential for medical applications with their diverse biological activities including antibacterial, pesticidal, antifungal, insecticidal, anticonvulsant, tuberculostatic, anti-inflammatory, antithyroidal, antiviral, SHP-2 inhibitor and calcium antagonist, potentiation of pentobarbital-induced sleeping time, etc., effects [1-4].

Due to this wide-range use potential of 4-thiazolidinones, determination of toxicological properties of newly synthesized derivatives has become a very important strategy for the improvement of human health-care applications [1-4, 17-19].

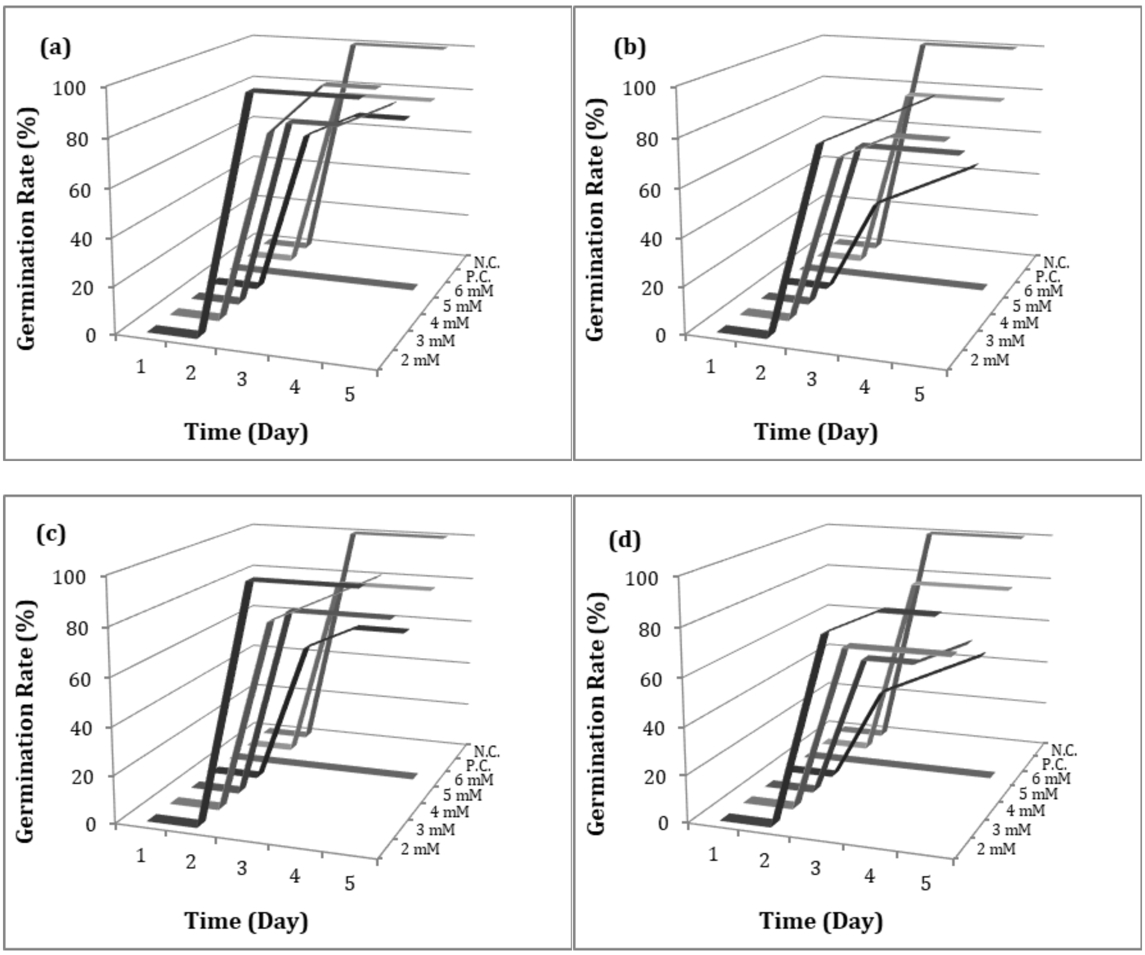
Thus, the aim of the current study was determination of the toxicological potency of six newly synthesized 4-thiazolidinone derivatives by using Ames-*Salmonella*, *Escherichia coli* WP2, *Zea mays* seed germination and random amplified polymorphic DNA (RAPD) assay systems.

In the *in vitro* bacterial assay, diverse tester strains were chosen to examine the genotoxic properties acting with various mechanisms of the test materials. *S. typhimurium* TA 1535 and TA100 strains with a single base substitution mutation were chosen to detect NaN_3 like mutagenic agents that cause base substitutions. *S. typhimurium* TA1537 strain with +1 type frame-shift mutation was for 9-AA like induced mutagenesis caused by base insertions into the DNA sequence; *S. typhimurium* TA 1538 and TA98 strains with -1 type frame-shift mutation were for 4NPD like induced mutagenesis caused by base deletions [6]. Moreover, *E. coli* WP2*uvrA* strain was also used to determine of the mutagenesis potentials arisen from oxidative damages of the test materials [7]. According to the bacterial assay results, any test materials did not show mutagenic effect on the tester strains at tested concentrations up to 1 mM/plate (Table 2). Thus, the test materials were considered as genotoxically safe for the test concentrations in the matter of mentioned mechanisms.

Z. mays seeds with uniform genophond characteristics were also chosen as a model for examining the toxicological properties of the test materials in eukaryotic systems [8-11]. In this experiment, all test materials showed significant inhibition on the seed germination at tested concentrations ranged from 2 mM to 6 mM in a dose dependent manner. The complete inhibition was observed at 6 mM for all test compounds (Figure 2). So, milder concentrations with inhibition activity (2, 3, 4 and 5 mM) were selected for performing RAPD analysis as a further toxicological investigation.

RAPD is a PCR-based technique that checks genomic material damages and mutational events caused by various external agents [20-21]. RAPD analysis also showed a parallelism with the germination assay results. According to the data, the test materials caused significant changes in the RAPD profiles for both primers. These changes included loss of normal bands and appearance of new bands in comparison to the positive and negative control groups (Figure 3 and 4). The genomic materials from *Z. mays* seedlings were affected by the treatment of test materials at various concentrations ranging from 2 to 5 mM. These results referred that one possible reason for the inhibition of the germination may be genotoxic activities of the test materials, which cause changes in DNA sequences of the seeds and result in differentiations in the RAPD profiles [10, 20-21].

Figure 2. Germination rates of test materials: Compound **1** (a), **2** (b), **3** (c), **4** (d), **5** (e) and **6** (f). NaN_3 in distilled water and 10% DMSO were positive and negative controls respectively.



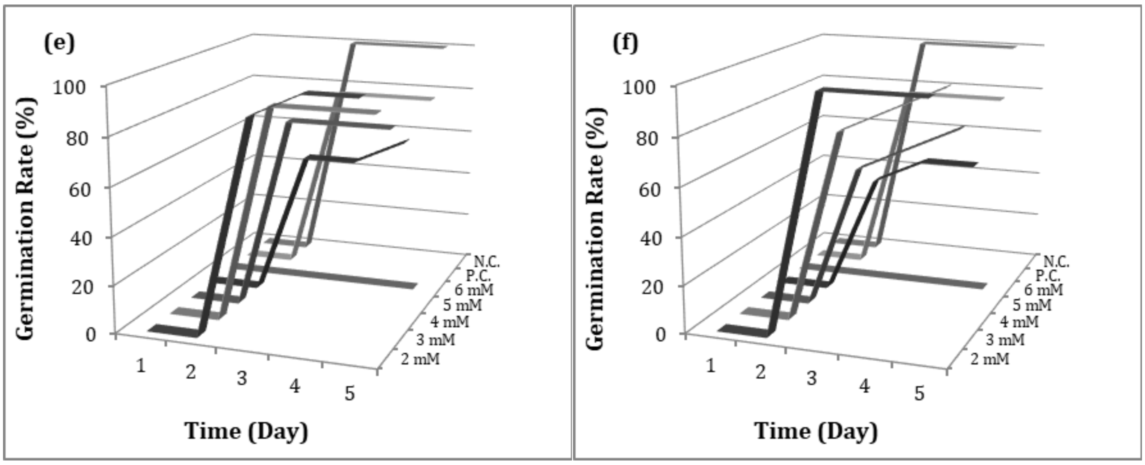


Figure 3. RAPD analysis results of Compound **1** (a), **2** (b), **3** (c), **4** (d), **5** (e) and **6** (f) for OPA-4 primer. (M: Wide range DNA marker 50-10000 bp; PC: positive control; NC: negative control; A, B, C and D: 2 mM, 3 mM, 4 mM and 5 mM treatment of the test compounds; RC: PCR reaction control)

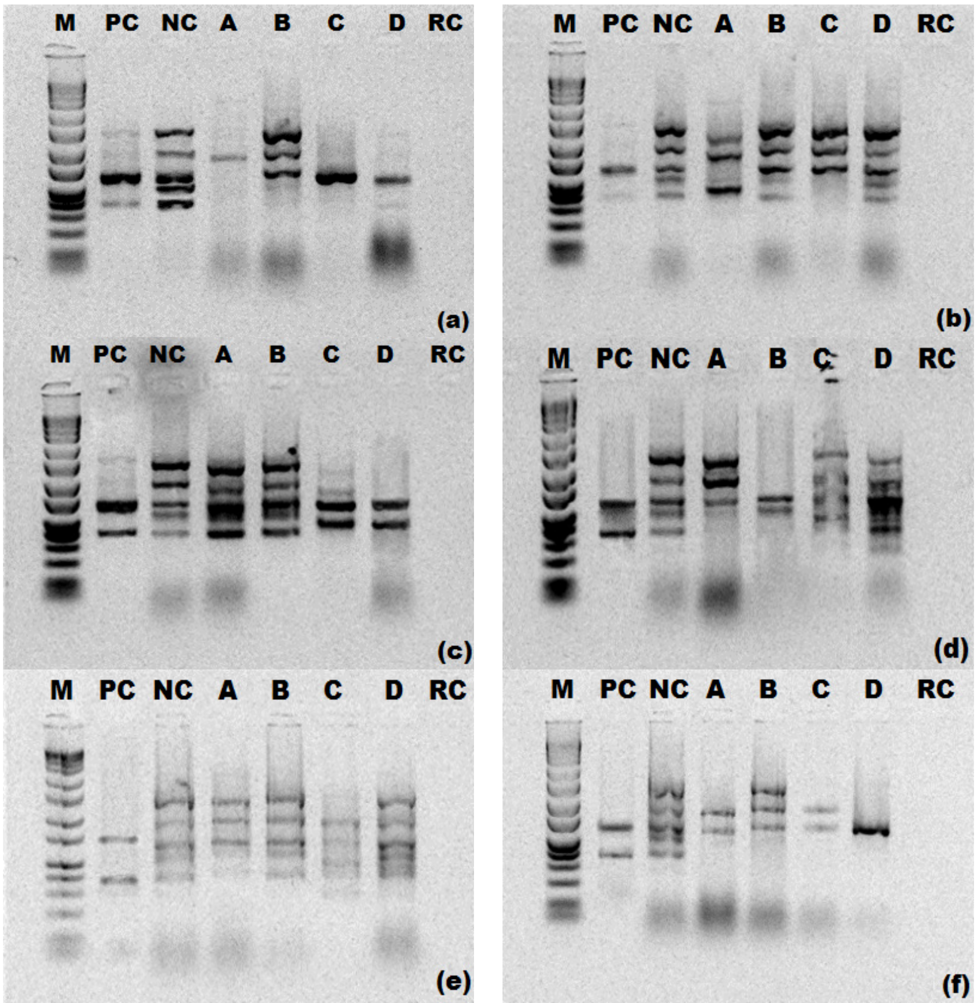


Figure 4. RAPD analysis results of Compound **1** (a), **2** (b), **3** (c), **4** (d), **5** (e) and **6** (f) for OPY-13 primer. (M: Wide range DNA marker 50-10000 bp; PC: positive control; NC: negative control; A, B, C and D: 2 mM, 3 mM, 4 mM and 5 mM treatment of the test compounds; RC: PCR reaction control)

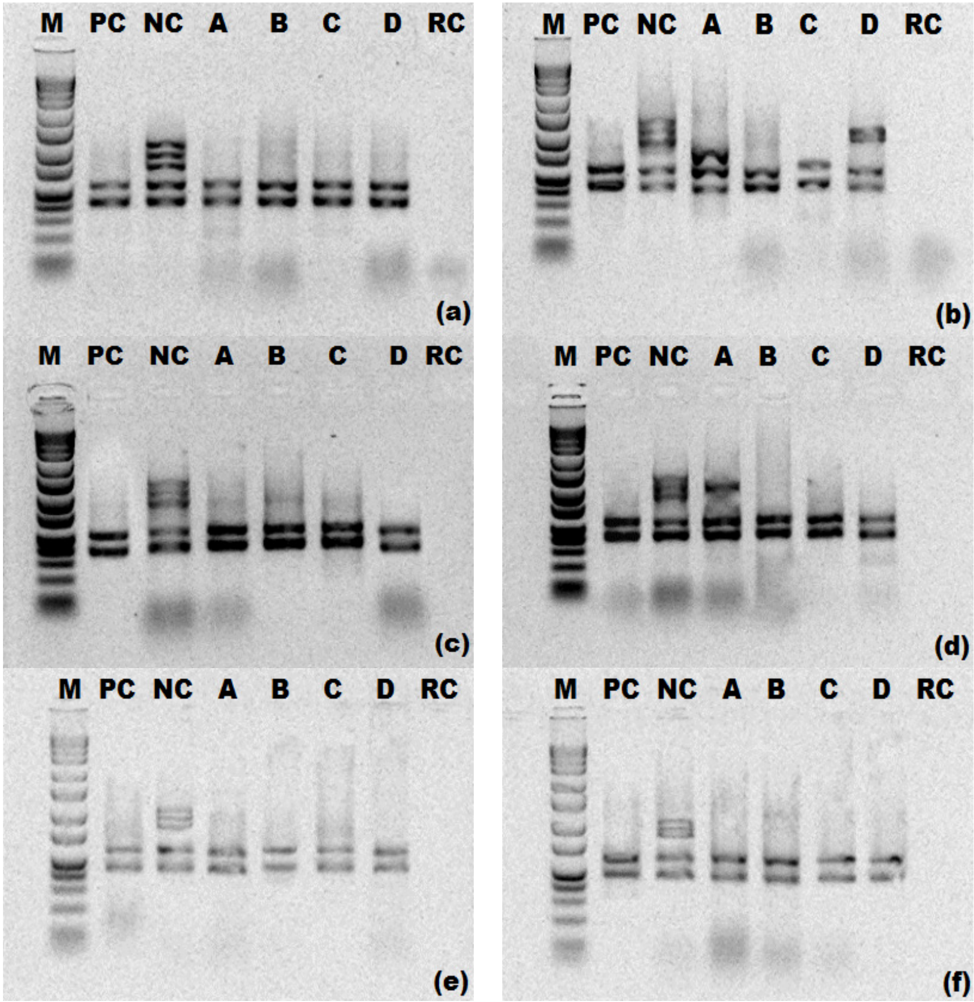


Table 2. The mutagenicity assay results of the test materials for bacterial tester strains.

Test Items	Dose (mM/plt)	Count of Revertant Colonies					
		S. typhimurium TA1535	*S. typhimurium* TA1537	*S. typhimurium* TA1538	*S. typhimurium* TA98	*S. typhimurium* TA100	*E. coli* WP2*uvrA*
		Mean ± S.D.	Mean ± S.D.	Mean ± S.D.	Mean ± S.D.	Mean ± S.D.	Mean ± S.D.
C1							
	0.2	24.2 ± 2.54	26.5 ± 3.20	16.8 ± 3.24	35.7 ± 4.23	34.5 ± 3.59	31.7 ± 1.70
	0.4	25.0 ± 1.63	24.2 ± 2.11	19.2 ± 2.85	39.5 ± 2.22	30.2 ± 3.29	27.8 ± 1.67
	0.6	25.0 ± 2.08	26.2 ± 2.27	19.8 ± 2.27	34.7 ± 3.30	29.7 ± 3.99	28.8 ± 2.41
	0.8	22.8 ± 2.67	26.0 ± 3.00	18.7 ± 3.15	37.5 ± 3.25	33.0 ± 2.77	29.7 ± 2.21
	1.0	22.8 ± 2.54	23.2 ± 2.27	17.0 ± 2.52	35.3 ± 2.75	33.5 ± 3.59	27.5 ± 2.63
C2							
	0.2	23.0 ± 2.88	24.0 ± 3.11	17.0 ± 2.65	37.2 ± 3.08	31.3 ± 2.43	29.0 ± 3.83
	0.4	22.5 ± 2.36	24.3 ± 2.81	16.3 ± 2.21	39.8 ± 2.41	33.8 ± 3.85	23.8 ± 2.48
	0.6	24.3 ± 2.21	23.8 ± 2.80	17.2 ± 1.68	34.5 ± 2.14	33.0 ± 2.52	25.8 ± 2.27
	0.8	22.2 ± 2.67	24.8 ± 2.48	16.3 ± 2.81	37.8 ± 2.19	30.7 ± 3.99	26.3 ± 2.81
	1.0	23.7 ± 2.13	23.0 ± 2.83	18.5 ± 1.61	39.3 ± 2.75	34.8 ± 1.95	25.3 ± 0.94

C3	0.2	24.2 ± 1.77	24.0 ± 2.38	17.7 ± 1.37	34.7 ± 3.60	33.3 ± 3.09	26.5 ± 2.69
	0.4	22.5 ± 2.87	23.8 ± 2.61	19.5 ± 1.71	35.2 ± 2.19	34.6 ± 3.04	24.8 ± 2.67
	0.6	23.2 ± 1.57	25.0 ± 3.37	18.2 ± 2.54	34.7 ± 2.69	32.3 ± 2.21	24.5 ± 2.36
	0.8	23.5 ± 2.63	24.0 ± 2.89	17.3 ± 1.70	38.5 ± 1.71	36.7 ± 1.89	24.2 ± 2.03
	1.0	23.5 ± 2.57	26.3 ± 2.69	18.8 ± 1.07	35.2 ± 2.41	33.0 ± 1.91	25.8 ± 3.72
C4	0.2	23.3 ± 2.75	24.5 ± 3.64	18.3 ± 2.05	37.2 ± 3.39	34.8 ± 2.79	28.5 ± 3.82
	0.4	21.8 ± 2.67	24.8 ± 2.11	18.0 ± 1.73	35.8 ± 2.11	35.2 ± 3.44	28.0 ± 2.31
	0.6	24.3 ± 0.94	24.2 ± 2.34	22.5 ± 3.95	36.3 ± 2.75	34.5 ± 1.26	25.8 ± 2.97
	0.8	23.3 ± 2.81	24.2 ± 2.11	17.7 ± 1.80	34.6 ± 3.73	35.7 ± 2.81	24.3 ± 2.62
	1.0	22.2 ± 2.27	25.3 ± 1.97	16.5 ± 2.36	37.7 ± 3.35	36.0 ± 4.16	26.7 ± 3.14
C5	0.2	24.2 ± 2.67	24.7 ± 2.87	18.0 ± 1.52	35.5 ± 2.50	36.3 ± 3.25	26.2 ± 2.27
	0.4	23.8 ± 2.27	27.7 ± 1.49	17.5 ± 2.50	35.3 ± 2.49	34.0 ± 2.83	25.0 ± 2.52
	0.6	23.7 ± 1.11	24.7 ± 3.90	16.5 ± 3.04	39.5 ± 1.26	34.5 ± 2.99	26.8 ± 1.95
	0.8	22.3 ± 1.70	27.2 ± 3.24	16.7 ± 2.13	34.5 ± 2.43	35.3 ± 1.70	27.5 ± 1.89
	1.0	23.5 ± 1.71	25.0 ± 3.11	18.5 ± 1.61	38.0 ± 3.61	31.7 ± 2.49	25.2 ± 2.34
C6	0.2	23.3 ± 1.11	24.5 ± 3.86	18.3 ± 2.29	39.0 ± 3.42	35.2 ± 2.91	25.7 ± 3.35
	0.4	22.3 ± 3.99	25.5 ± 2.22	17.2 ± 2.27	35.3 ± 2.62	35.3 ± 2.62	26.7 ± 3.64
	0.6	23.8 ± 2.11	29.7 ± 3.04	17.5 ± 1.26	35.3 ± 2.56	34.8 ± 1.07	28.5 ± 1.38
	0.8	21.5 ± 1.71	24.3 ± 3.50	17.2 ± 2.19	35.2 ± 4.14	30.7 ± 2.81	26.7 ± 2.43
	1.0	22.3 ± 1.89	25.8 ± 1.95	16.8 ± 1.21	36.8 ± 1.57	34.5 ± 3.15	27.5 ± 3.30
NaN_3 **9-AA** **4NPD** **MNNG** **(µg/plate)**	5 50 2.5 1	430.0 ± 7.43	533.5 ± 13.61	359.0 ± 19.98	670.7 ± 14.79	612.7 ± 11.64	347.7 ± 13.24
DMSO (µl/plate)	100	25.5 ± 1.38	26.2 ± 2.54	19.2 ± 2.11	39.2 ± 4.56	34.5 ± 2.69	31.7 ± 2.69

Consequently, the toxicological properties of six newly synthesized 4-thiazolidinones derivatives were investigated in the present study and they can be thought as genotoxically safe up to 1 mM/plate concentrations because they do not show any mutagenic activity on bacterial tester strains. However, all test materials showed significant inhibition on *Z. mays* seed germination at higher concentrations up to 6 mM and further RAPD profile analysis of the treated seedlings indicated that a reason for the inhibition might be potential mutagenic activities of the test compounds. Thus, these results give us valuable preliminary information about possible toxicological effects of six newly synthesized 4-thiazolidinones derivatives and underline the necessity of performing more complicate assay systems before use of these molecules for human consumption or health applications.

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