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Radioprotective Effects of 2-Imino-3-[(chromone-2-yl)carbonyl] thiazolidines against γ-Irradiation in Mice

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Radioprotectors / Chromone / 2-Iminothiazolidine / γ-Irradiation

A series of 2-imino-3-[(chromone-2-yl) carbonyl]thiazolidines substituted at the C-5 and / or C-7 positions of a chromone ring were synthesized. The *in vivo* toxicity and radioprotective efficacy of these agents were evaluated in male NMRI mice against cobalt-60 γ-rays. The LD_{50} values as determined by a Probit analysis, were 659, 1216 and 790 mg/kg for compounds, 2, 3 and 4, respectively. For studying radioprotective effects, one half of the toxic LD_{50} values were used, namely 330, 605 and 395 mg/kg for compounds 2, 3 and 4, respectively. The dose reduced factor (DRF) was determined by dividing the $LD_{50/30}$ values obtained from the radiation survival curve in the presence of a radioprotective agent by the $LD_{50/30}$ value obtained from a control radiation survival curve. A compound with a hydroxyl group substituent at the C-5 position afforded better radioprotective activity than those without this substituent. The radioprotective effect of chromone having a hydroxyl group at only the C-7 position was similar to that of the unsubstituted chromone. The most active compound has hydroxyl groups at the C-5 and C-7 positions of the chromone ring; it had a DRF of 1.48.

INTRUDUCTION

A radioprotector is a chemical or biological compound capable of modifying the normal response of a biological system to radiation-induced toxicity or lethality. The aminothiols, such as *S*-2-(3-aminopropylamino)ethyl phosphorothioic acid (WR-2721), are potent radioprotectors[1)]. Using on *in vivo* electron spine resonance study, Miura Y. et al recently showed that WR 2721 has powerful radioprotective activity and lower protective effects compared to 5-HT and Trolox[2)]. However, its toxicity has limited its applications in medicine or in hazardous radiation environments[3)]. For this reasons the search for more effective and less toxic radioprotectans has spurred interest in the development of different compounds. 2-Aminothiazoline (2-AT, Fig. 1) showed protective activity in both mice and rats[4)]. Several organic and inorganic salts as well as derivatives of 2-AT were synthesized as potential protective agents against radiation injury[4,5)]. We recently reported on the radioprotective activity of 2-iminothiazolidine derivatives against γ-radiation[6)]. The percentage survival of mice at 30 days for the latter compared to control mice was 10 to 30% when injected before γ-irradiation; these compounds had significant protective effects compared to the control[6)]. Some natural antioxidants (e.g. flavonoids) are widely disturbed in fruits and vegetables. Flavonoids are benzo-γ-pyrone derivatives which are able

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to suppress the formation of free radicals and have antioxidant effects[7–12]. Some of these compounds are currently being used as antioxidant food additives[13]. The radioprotective effects of structurally different flavonoids have been investigated against γ-irradiation in mice, and have shown protective activity[14–16]. Along this line, we have incorporated 2-AT with chromone having a hydroxyl moiety at the C-5 and / or C-7 positions. In this paper the toxicity and radioprotective activity of three 2-imino-3-[(chromone-2-yl)carbonyl]thiazolidines (Fig. 1: 2, 3 and 4), which are structurally similar to flavonoids, against gamma radiation are reported.

MATERIALS AND METHODS

Chemicals

All chemicals were purchased from Sigma-Aldrich (Steinheim-Germany). 2-AT was prepared according to our previously reported method[6]. Melting points (m.p.) were determined using a Kofler hot stage apparatus. ^{1}H NMR spectra were recorded on a Bruker FT-80 Spectrometer. TMS was used as an internal standard. Infrared spectra were acquired on a Nicolet 550-FT spectrometer. Mass spectra were measured with a Finnigan TSQ-70 spectrometer at 70 eV. The purity of all compounds was confirmed by TLC.

Preparation of 4-oxo-4H-1-benzopyran 2-carboxylic acid (8)

A mixture of 2-hydroxyacetophenone (1g, 7.34 mmol) and diethyl oxalate (2.5 ml, 18.4 mmol) was added to sodium (0.5 g, 2.17 mmol) in absolute ethanol (10 ml). The mixture was heated at 50°C for 3 h, cooled, added to 25% sulfuric acid (25 ml) and heated under reflux for 1.5 h. After the reaction mixture was cooled, an insoluble solid was collected. The resulting solid was recrystallized from aqueous ethanol (ethanol-water, 80:20) to give 1.25 g (90%) of chromone-2-carboxylic acid (8) as a pale-yellow powder; m.p.= 242–243°C ; IR (KBr), ν (cm^{-1}): 3100 (aromatic), 1741 (C=O, COOH), 1631 (C=O, pyrane); ^{1}H NMR (DMSO-d_6) δ (ppm): 7.5–7.12 (m, 4H, aromatic), 6.90 (s, 1H, H_3); MS (m/z, %): 190 (M^+, 100), 162 (60), 145 (20), 92 (60), 89 (30).

Anal. Calcd for $C_{10}H_6O_4$: C, 63.16; H, 3.16. Found: C, 63.38; H, 3.02.

Preparation of 7-hydroxy-4-oxo-4H-1-benzopyran 2-carboxylic acid (9)

This compound was prepared in a way similar to compound 8 in 60% yield. It was crystallized from water-ethanol (50:50); m.p.=316–318°C.

Anal. Calcd for $C_{10}H_6O_5$: C, 58.25; H, 2.91. Found; C, 58.46; H, 2.73.

Preparation of 5, 7-dihydroxy-4-oxo-4H-1-benzopyran 2-carboxylic acid (10)

To a stirred solution of 2, 4, 6-trihydroxyacetophenone monohydrate (7.2 g, 12 mmol) in 25 ml of dry pyridine, cooled in an ice bath, ethoxalyl chloride (7.5 ml) was added dropwise. After the mixture was allowed to come to room temperature it was stirred overnight. A slurry was poured into water and extracted five times with $CHCl_3$ (3×50 ml). The combined $CHCl_3$ layer was washed with 10% HCl, followed by water. The solvent was removed under reduced pressure. After the residue was taken up in 50 ml of ethanol, 100 ml of 5% Na_2CO_3 was added and mixture was stirred at 60–70°C for 2 h. The ethanol was removed *in vacuo* and the residue was acidified. The precipitate was collected and recrystallized from ethyl acetate-methanol (90:10) to give 0.9 g (34%) of compound 10; m.p.=305–308°C.

Anal. Calcd for $C_{10}H_6O_6$: C, 54.05; H, 2.70. Found: C, 53.86; H, 2.88

Preparation of 2-imino-3-[(4-oxo-4H-1-benzopyrane-2-yl)carbonyl]thiazolidine (2)

N-N′-carbonyldiimidazole (1.27 g, 7.9 mmol) was added to a stirred solution of compound 8 (1 g, 5.3 mmol) in dry DMF (5 ml). After the mixture was stirred at 40–45°C for 1 h, 2-AT (0.64 g, 6.3 mmol) was added. Following further stirring overnight, the mixture was filtered and the filtrate was added to water (40 ml). Then, after 24 h at 0°C the solid was collected and recrystallized from n-hexane-chloroform (60:40)

to give compound 2 (1.1 g, 75%); m.p.=247–250°C; IR (KBr), ν (cm^{-1}): 3291 (C-H, aromatic), 1643 (C=0), 1635 (C=O); ^{1}H NMR ($CDCl_3$+CF_3COOH), δ(ppm): 8.25 (d, J=8 Hz, 1H, H-5), 7.44–8.05 (m, 3H, aromatic), 7.38 (s, 1H, H-3), 4.26 (t, J=9.2 Hz, CH_2), 3.59 (t, J=9.2 Hz, CH_2); MS (m/z%): 274 (M^+, 10), 145 (10), 129 (90), 89 (100).
Anal. Calcd for $C_{13}H_{10}N_2O_5S$: C, 50.98; H, 3.27; N, 9.15. Found: C, 50.81; H, 3.09; N, 9.34.

Preparation of 2-imino-3-[(7-hydroxy-4-oxo-4H-1-benzopyran-2-yl) carbonyl]thiazolidine (3)

This compound was prepared in a similar way to compound 2 in 80% yield, and then recrystallized from THF-MeOH (30:70); m.p.=272–274°C; IR (KBr), ν (cm^{-1}): 3252 (OH), 3180 (C-H, aromatic), 1644 (C=O), 1615 (C=O); ^{1}H NMR (DMSO-d_6), δ (ppm): 7.88 (d, J=9.6 Hz, 1H, H-5), 6.93 (d, J=9.6, 1H, H-6), 6.90 (s, 1H, H-8), 6.68 (s, 1H, H-3), 3.68 (t, J=6.4 Hz, 2H, CH_2), 3.32 (t, J=6.4 Hz, 2H, CH_2); MS (m/z %): 290 (M^+, 10), 234 (10), 187 (20), 161 (30), 129 (80), 101 (100).
Anal. Calcd for $C_{13}H_{10}N_2O_4S$: C, 53.79; H, 3.45; N, 9.66. Found: C, 53.62; H, 3.64; N, 9.78.

Preparation of 2-imino-3-[(5,7-dihydroxy-4-oxo-4H-1-benzopyran-2-yl) carbonyl]thiazolidine (4)

After compound 10 (1g, 4.5 mmol) was dissolved in dry THF (30 ml), *N, N′*-carbonyldiimidazole (1.45 g, 9 mmol) was added under argon. The mixture was heated at 60–70°C for 4 h. Following coolding, 2-AT (0.7 g, 6.75 mmol) in dry THF (15 ml) was added. After further stirring for 24 h, the resulting precipitate was filtered and recrystallized from ethanol to give compound 4 (0.7 g, 50%); m.p.=256–258°C; IR (KBr), ν (cm^{-1}): 3416(OH), 1639 (C=O), 1582 (C=O); ^{1}H NMR (DMSO-d_6), δ (ppm): 6.60 (s, 1H, H_3), 6.40 (d, J= 2.4 Hz, 1H, H-8), 6.18 (d, J=2.4 Hz, 1H, H-6), 3.85 (t, J=6.4 Hz, 2H, CH_2), 3.52 (t, J=6.4 Hz, 2H, CH_2); MS (m/z %): 306 (M^+, 10), 279 (10), 248 (15), 222 (100).
Anal. Calcd for $C_{13}H_{10}N_2O_3S$: C, 50.93; H, 3.65; N, 10.22. Found: C, 50.81; H, 3.69; N, 9.98.

Animals

Eight-week old male NMRI mice (Pasteur Institute of Iran) weighing 28±3 g were used. A standardized pellete diet was given and tap water was *ad libitum*. The animals were housed in groups of seven for one week in a quarantine facility. All of the mice were kept under controlled lighting conditions (light: dark, 12:12h) and temperature (22±1°C) in the university animal house. Experiments were conducted according to principles outlined in "The Guide for The Care and Use of Laboratory Animals" prepared by Tehran University of Medical Sciences.

Toxicity studies

For toxicity studies the compounds were suspended in sterile distilled water having 0.2% polysorbate 80 (Tween 80) and were injected intraperitoneally (*i. p.*) in mice. Male NMRI mice were injected with a geometric progression of doses through the dose-response range for lethal toxicity. Ten animals were used for each subgroup dose, and four doses were used for determining of each LD_{50}. Death observations were analyzed by Probit analysis to determine the toxic LD_{50}[17] (Fig. 2).

Radioprotective effect studies

Whole-body irradiation was performed with a cobalt-60 γ-radiation source (Teratron 780, Canada). Mice were placed in ventilated plexiglass cages and irradiated. in groups of ten mice simultaneously. The source-to-skin distance was 80 cm with a dose rate of 77.5 cGy/min at room temperature (23±2°C). The mice were injected the compounds, which had been suspended in sterile distilled water having 0.2% Tween 80. For radioprotective studies, one half of a dose of LD_{50} was administered 30 min before whole-body irradiation with a geometric progression of of gamma-rays doses. The control received an equal volume of sterile distilled water having 0.2% Tween 80 in the same manner. Ten mice were used for each subgroup dose of γ-rays. A total of 40 mice were used for each compound. Survival was monitored on a daily basis, and the number of animals 30 days post irradiation was recorded. The $LD_{50/30}$ and 95% confidence

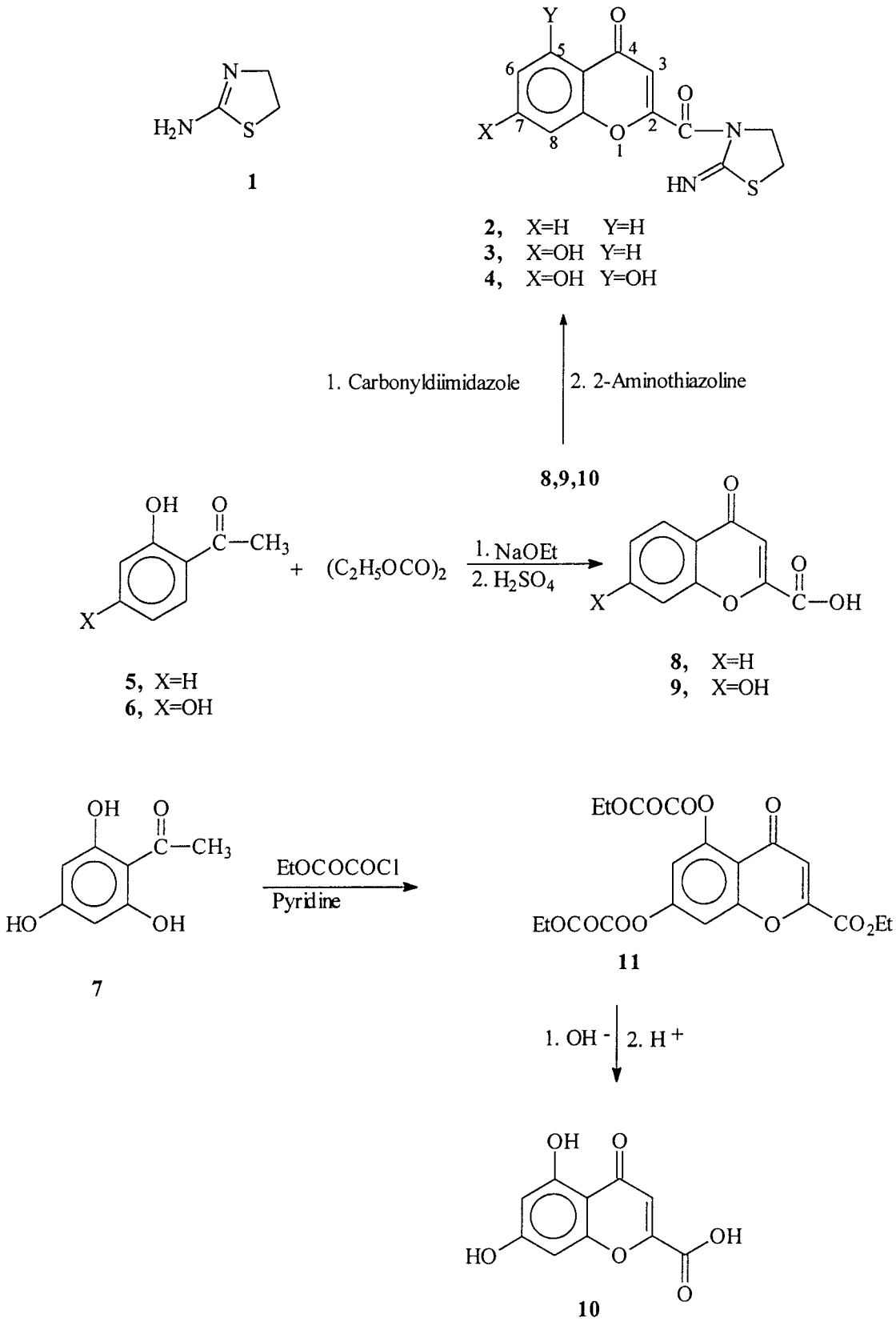

Fig. 1 Synthesis and structures of 2-aminothiazoline (**1**) and 2-amino-3[(chormone-2-yl)carbonyl]thiazolines (**2**, **3** and **4**).

limits were determined from Probit curve fitting of the 30-day mortality data, and were fitted to Probit curves[17]. Each DRF was computed as the ratio of the radiation $LD_{50/30}$ value with the radioprotector to that without a radioprotector.

Statistical analysis

The LD_{50} values were determined using the Probit statistical analysis[17]; the values were expressed as the mean ±95% confidence limits. The significance of the difference between the mean values obtained for various study end points was calculated using the student's *t* test. The values were considered to be significantly different if $p < 0.05$.

RESULTS

Chromone 2-carboxylic acids were synthesized according to Fig. 1. Compounds 8 and 9 were prepared from 2-hydroxyacetophenone (5) or 2, 4-dihydroxycetophenone (6) and subsequent addition of 25% sulfuric acid, respectively. 5, 7-Dihydroxychromone-2-carboxylic acid (10) was prepared from 2, 4, 6-trihydroxyacetophenone (7) and ethoxalyl chloride in dry pyridine. 2-Imino-3-[(chromone-2-yl)carbonyl] thiazolidines were prepared from chromone 2-carboxylic acids (8, 9, 10), *N, N′*-carbonyldiimidazole and 2-AT. The final products were purified by recrystallization. The toxicity effects of compounds 2, 3 and 4 were determined *in vivo* against male NMRI mice. Suspensions of compounds 2, 3 and 4 in sterile distilled water were injected *i.p.* in mice. These compounds showed a concentration-dependent effect on the survival in mice. The LD_{50} values for these compounds were determined by the Probit analysis method[17], and were found to be 659, 1216 and 790 mg/kg for compounds 2, 3 and 4, respectively (Table1). To evaluate the of radioprotective activity, one half of the toxic LD_{50} values were used, namely

Table 1. Toxicity and radioprotective effects of 2-imino-3-[(chromone-2-yl) carbonyl]thiazolidines.

Compound	LD_{50}±95% Confidence limits (mg/kg)	Dose injected (mg/kg)[a]	LD_{50}±95% Confidence limits (cGy)[b]	DRF[c]
Control	–	–	654 (508, 700)**	–
WR-2721[d]	609 (545, 673)*	300	1066 (886, 1246)	1.63
2	659 (487, 744)	330	767 (482, 867)	1.17
3	1216 (1032, 1420)	605	771 (556, 850)	1.18
4	790 (668, 897)	395	968 (823, 1109)	1.48

[a] The dose was based on one-half the LD50 value for the compounds.

[b] Male NMRI mice were injected ip with a suspension of compounds 30 minutes before cobalt-60 (-radiation. 30-day survival was used to determine the LD50/30 value.

[c] The dose reduced factor (DRF) was determined by dividing the LD50/30 value obtained from the radiation survival curve in the presence of a radioprotective agent by the LD50/30 value obtained from the control radiation survival curve.

[d] WR-2721 was dissolved in sterile distilled water.

*Based on LD50 values: Compound 3 versus compound 2 ($p<0.005$), compound 3 versus compound 4 ($p<0.05$), compound 4 versus WR-2721 ($p>0.05$), compound 2 versus compound 4 ($p<0.01$), compound 2 versus WR-2721 ($p>0.05$).

**Based on LD50/30 values: compound 2 versus control ($p>0.05$), compound 3 versus control ($p>0.05$), compound 4 versus control ($p<0.05$), WR-2721 versus control ($p<0.05$), compound 2 versus compound 3 ($p>0.05$), compound 2 versus compound 4 ($p<0.05$), compound 2 versus WR-2721 ($p<0.01$), compound 4 versus WR-2721 ($p<0.05$), compound 3 versus compound 4 ($p<0.01$) and compound 3 versus WR-2721 ($p<0.01$).

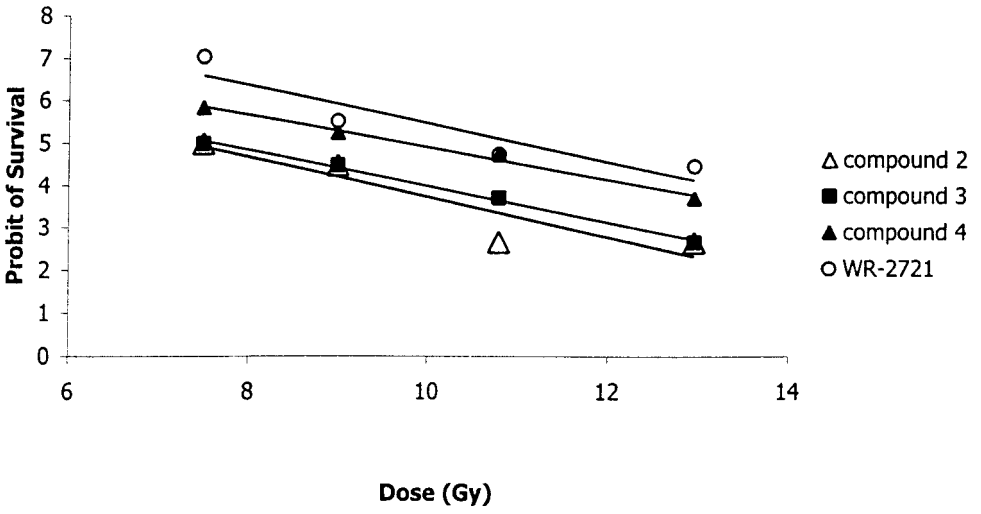

Fig. 2. Effects of different radioprotectors given by the $LD_{50/30}$ of whole-body γ-irradiation mice. Ten mice per radiation dose were used, and a total of 40 mice were used to calculate each $LD_{50/30}$. Compound 2 versus compound 3 ($p > 0.05$), compound 2 versus compound 4 ($p < 0.05$), compound 2 versus WR-2721 ($p < 0.01$), compound 4 versus WR-2721 ($p < 0.05$), compound 3 versus compound 4 ($p < 0.01$), compound 3 versus WR-2721 ($p < 0.01$).

330, 605 and 395 mg/kg for compounds 2, 3 and 4. The compounds were evaluated *in vivo* for their radioprotective effects using male NMRI mice against cobalt-60 gamma rays. We found that the radiation dose for the $LD_{50/30}$ for control mice to be 654 cGy (Table 1). The $LD_{50/30}$ values for compounds 2, 3 and 4 were 767, 771 and 968 cGy when these compounds were injected 30 minutes prior to gamma radiation. The dose reduced factor (DRF) was calculated from the radiation-survival curve generated for each compound tested (Table 1, Fig. 2). Compounds 2, 3 and 4 had DRF values of 1.17, 1.18 and 1.48, respectively. Although the radioprotective effects of compounds 2 and 3 were the same, compound 4 had a DRF of 1.48, more than that of compounds 2 and 3. The radioprotective effects of new synthesized compounds were compared to WR-2721. WR-2721 had a DRF of 1.63 when injected 30 minutes before irradiation (Table 1 and Fig. 2).

DISCUSSION

Flavonoids exhibit radioprotective activity *in vivo* and *in vitro*[14–16]. 2-AT and its derivatives showed protective activity in mice and rats against gamma radiation[4,5,6]. Along this line we have synthesized compounds having 2-AT and chromone containing a hydroxyl moiety at the C-5 and / or C-7 positions. The hydroxyl moiety was selected because the hydroxyl group is important for the antioxidant activity of flavonoids[7,11,14]. The three 2-imino-3-[(chromone-2-yl)carbonyl]thiazolidines prepared in this study were structurally similar to flavonoids. The LD_{50} values obtained for compound 2 were lower than those of compounds 3 and 4 (Table 1). The data indicated that apparently the substitution of a hydroxyl group at the 7- position of the chromone ring reduced the toxicity. However, the presence of two hydroxyl groups at the C-5 and C-7 positions of the chromone ring reduced the LD_{50} value. All compounds were less toxic than 2-AT (LD_{50} 150 mg/kg) in mice[18]. To evaluate the radioprotective activity, one half of the toxic LD_{50} values were used, namely 330, 605 and 395 mg/kg for compounds 2, 3 and 4 respectively, and 300 mg/kg for WR-2721. The dose reduced factor (DRF) was calculated from the radiation-survival curve generated for each compound tested (Table 1 and Fig. 2). The higher was the DRF value, the greater was the radioprotective effect. Compound 4 with a DRF of 1.48 exhibited the highest radioprotective effect. Compounds 2 and 3, having DRF values of 1.17 and 1.18 respectively, were

less effective. It should be noted that compounds 3 and 4 have one or two hydroxyl groups on the chromone ring, while compound 2 lacks a hydroxyl group. This observation demonstrated that 2-imino-3-[(chromone-2-yl) carbonyl]thiazolidines have radioprotective activity. Compound 3, having a hydroxyl group at the C-7 position, had the same radioprotective effect as compound 2 without a hydroxyl group. Compound 4, having a hydroxyl group at the C-5 and C-7 positions, slowed a significantly greater radioprotective effect than the other compounds (2 and 3). Therefore, the presence of a hydroxyl group at the C-5 position is important for the radioprotective effect. This finding is in agreement with others reports that the hydroxyl moiety at the chromone ring enhances the antioxidant and radioprotective effects of flavonoids[7,14]. The substitution of hydroxyl groups in the chromone ring increases their free-radical scavenging ability by binding heavy metal ions, which are known to catalyze many processes leading to the appearance of free radicals.[7,10,19]. It is probable that the hydroxyl group at the C-5 position with the 4-oxo function can chelate metals which may be able to suppress the formation of free radicals that are generated from gamma radiation. WR-2721 administered *i.p.* gave significant radioprotection to normal tissues. Thirty-day survival was evaluated by treating mice *i.p.* with 300 mg/kg of drugs 30 min before exposure to irradiation, it had a DRF value of 1.63.Therefore, although WR-2721 is more effective than the newly synthesized chromone derivatives, WR-2721 still had a number of undesirable side effects[20,21]. The structure of compound 4 is different from WR-2721, but similar to that of natural flavonoids. It had a DRF value of 1.48, close to that of WR-2721. Although we have not yet obtained the LD50/30 and DRF values of the compound which has an OH group at C-5 and a H at C-7, the presence of a hydroxyl group at the C-5 position is probably more important than the thiol for radioprotection. The thiol seems to be the major toxic principle of sulfur-containing protectors; also, cysteine thiol is the a toxic mediator[22]. This agent probably has a lower side effects than WR-2721 because its structure is similar to that of natural flavonoids. In conclusion, the combination of a chromone ring with 2-aminothiazoline resulted in 2-imino-3-[(chromone-2-yl)carbonyl]thiazolidines with moderate-to-good radioprotective activities. The most active derivative was compound 4, having hydroxyl groups at the C-5 and C-7 positions of the chromone ring.

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REFERENCE

1. Brown, D. Q., Graham III, J. W., Mackenzie, L. J. (1988) Can WR-2721 be improved upon? Pharmacol. Ther. **39**: 157–168.
2. Miura, Y., Anzai, K., Ueda, J., Ozawa, T. (2000) Novel approach to *in vivo* screening for radioprotective activity in whole mice: *in vivo* electron spin resonance study probing the redox reaction of nitoxyl. J. Radiat. Res. **41**: 103–111.
3. Turrisi, A. T., Glover, D. G., Hurwitz, S. (1986) The final reports of the phase I trail of single dose WR-2721, s-2(aminopropylamino)ethylphosphorothioic acid, intravenous injection. Cancer Treat. Rep. **70**: 1389–1393.
4. Gogatyrev, G. P., Goncharenko, E. N., Graevskaya, E. E. (1977) Study of the antiradiation effectiveness of 2-amino-2-thiazoline and its 5-substituted derivatives *in vivo* and in vitro. Mekh. Prir. Modif. Radiochustvitel`nosti **3**: 80–88.
5. Taira, K., Katsuhara, R., Kubota, M. (1965) Chemical protector against radiation injury. I. Radioprotective effect of 2-aminothiazoline salts against lethal doses of γ-radiation. Boei. Eisei. **12**: 99–105.
6. Hosseinimehr, S. J., Shafiee, A., Mozdarani, H., Akhlaghpour, S. (2001) Radioprotective effects of 2-iminothiazolidine derivatives against lethal dose of gamma radiation in mice. J. Radiat. Res. **42**: 401–408.
7. Bors,W., Heller, W., Michel, C., Saran, M.(1990) Flavonoids as antioxidants: Determination of radical scavenging efficiencies. Methods in Enzymol. **186**: 343–355.

8. Hodinck, W. F., Kung, F. S., Rotteger, W. J. (1986) Inhibition of mitochondrial respiration and production of toxic oxygen radicals by flavonoids, structure-activity study. Biochem. Pharmacol. **35**: 2345–2357.
9. Steven. J. F., Miranda, C. L., Buhler, D. R. (1998) Chemistry and biology of hop flavonoids. J. Am. Soc. Brew. Chem. **56**: 136–145.
10. Afanasev, I. B., Dorozho, A. I., Brodskii, A. V.(1989) Chelating and free radical scavenging mechanism of inhibitory action of rutin and querectin in lipid peroxidation. Biochem. Pharmacol. **38**: 1763–1769.
11. Larson, R. A., (1988) The antioxidants of higher plants. Phytochem. **27**: 969–978.
12. Robak, J., Gryglewski, R. J. (1988) Flavonoids are scavengers of superoxide anions. Biochem. Pharmacol. **37**: 837–841.
13. Koleva, I., Vanbeek, T. A., Lissen, J. P. (2002) Screening of plant extracts for antioxidant activity: a comparative study on three testing methods. Phytochemical Anal. **13**: 8–17.
14. Castillo, J., Gracia. O. B., Lorente, J. (2000) Antioxidant activity and radioprotective effects against chromosomal damage induced *in vivo* by x-rays of flavan-3-ols (procyanidins) from grape seeds (Vitis Vinifera): comparative study versus other phenolic and organic compounds. J. Agric. Food Chem. **48**: 1738–1745.
15. Shimoi, K., Masuda, S., Furugori, M. (1994) Radioprotective effects of antioxidative flavonoids in γ-ray irradiated mice. Carcinogen. **15**: 2669–2672.
16. Nakamura, T., Nakazawa, Y., Onizuka, S. (1997) Antimutagenicity of Tochu tea (an aqueous extract of Eucommia Ulmoides leaves): 1. The clastogen-suppressing effects of Tochu tea in CHO cells and mice. Mutat. Res. Gen. Toxicol. **388**: 7–20.
17. Finney, D. J. (1971) Probit Analysis. (Eds. D. J. Finney), pp. 1–99, Cambridge, Cambridge university.
18. Shashkov, V. S., Fedoseev, V. M. (1961) The antiradiation activity of new isothiuronium derivatives. Med. Radiol. **6**: 25–29.
19. Havsteen, B. (1983) Flavonoids, a class of natural products of high pharmacological potency. Biochem. Pharmacol. **32**: 1141–1148.
20. Yuhas, J. M., Spellman, J. M., Culo, F. (1980) The role of WR-2721 in radiotherapy and /or chemotherapy. Cancer Clin.Trails. **3**: 211–216.
21. Kligerman, M. M., Glover, D. J., Thuris, A. T., Norflett, A. L. Yuhas, J. M., Coia, L. R., Goodman, R. L. (1984) Toxicity of WR-2721 administered in single and multiple dose. Int. J. Radiat. Oncol. **10**: 1773–1776.
22. Koch, K. E., Roberts, J. C., Lubec, G. (1997) Radiation protection by alpha-methyl-homocysteine thiolactone in vitro. Life Sciences. **60**: 341–350.

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