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RESEARCH ARTICLE

Synthesis and evaluation of antioxidant activity of new quinoline-2-carbaldehyde hydrazone derivatives: bioisosteric melatonin analogues

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Abstract

Overproduction of reactive oxygen species results in oxidative stress that can cause fatal damage to vital cell structures. It is known that the use of antioxidants could be beneficial in the prevention or delay of numerous diseases associated with oxidative stress. Melatonin (MLT) is known as a powerful free-radical scavenger and antioxidant. It was found that indole ring of MLT can be employed by bioisosteric replacement by other aromatic rings. Quinoline derivatives constitute an important class of compounds for new drug development. Owing to quinoline and hydrazones appealing physiological properties and are mostly found in numerous biologically active compounds a series of quinoline-2-carbaldehyde hydrazone derivatives were synthesized as bioisosteric analogues of MLT, characterized and *in vitro* antioxidant activity was investigated by evaluating their reducing effect against oxidation of a redox-sensitive fluorescent probe. Cytotoxicity potential of all compounds was investigated both by lactate dehydrogenase leakage assay and by MTT assay.

Keywords

Antioxidant, hydrazone, lactate dehydrogenase leakage, melatonin, MTT, quinoline

History

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Introduction

The balance between free radicals and the antioxidant system of the body has a vital importance in maintaining the health status of organism[1–3]. Reactive oxygen species (ROS) and reactive nitrogen species are well known as both harmful and beneficial species. Oxidative stress that gives increase to generation of mainly ROS has been connected with an extensive range of diseases including those of cardiovascular, inflammatory, neurodegenerative, and autoimmune origin[4,5].

In recent years, various biological activities of melatonin (MLT) have been defined resulting in much attention in the development of synthetic derivatives possessing antioxidant activity[6–9]. These derivatives have structural resemblance to MLT, being derivatives of either substituted or bioisosteric moieties of the indole ring[10,11].

In our ongoing study, we have synthesized various series of indole derivatives, with some modifications on MLT (Figure 1)[4,6–9]. The synthesized bioisosteric MLT analogues have been evaluated for their potential radical scavenging activity and protective effect against oxidative damage[4,6–9]. A review on the structure activity relationship of several of these derivatives has been previously published by Suzen[12]. Most of the compounds exhibited noteworthy antioxidant activity in DPPH scavenging, superoxide dismutase, and lipid peroxidation assays. To determine the structural parameters of the antioxidant activity substitutions on the second position of the indole ring and of the alkyl chain of the acyl group have been underlined. Substitution of the indole ring of MLT with a naphthalene or quinolone ring leads to compounds of similar behavior[13]. Quinoline and their derivatives play important roles in the synthesis of natural products and as therapeutic agents, and constitute an important class of compounds for new drug development. They have been synthesized by many researchers as model compounds[14] and found to exhibit different pharmacological activities covering anti-cancer, anti-mycobacterial, anti-microbial, anti-convulsant, anti-inflammatory, and cardiovascular activities[15–19].

It has been experimentally established that some quinolone derivatives can function as free-radical scavengers[20–22]. Synthetic quinoline derivatives such as 2-chloroquinoline-3-carboxaldehydes[14], spiro-substituted 4-hydroxypyranoquinolinones[21], and pyrimido quinoline derivatives[23] have shown significant antioxidant activity. In this study, quinoline derivatives were synthesized in order to investigate whether the quinoline is a substantial heterocyclic bioisoster of the MLT indole moiety. A series of quinoline-2-carboxaldehydes hydrazones (**3a–q**) were synthesized (Figure 2 and Table 1), characterized, and their antioxidant activity was investigated *in vitro* by evaluating their reducing effect against oxidation of a redox-sensitive fluorescent probe,

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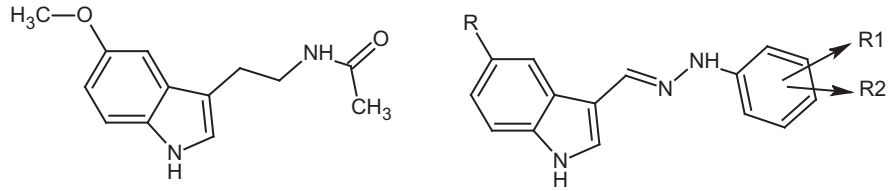

Figure 1. (a) Melatonin, (b) melatonin analogue indole-3-carboxaldehyde hydrazones that have higher antioxidant activity than MLT[4,6–9].

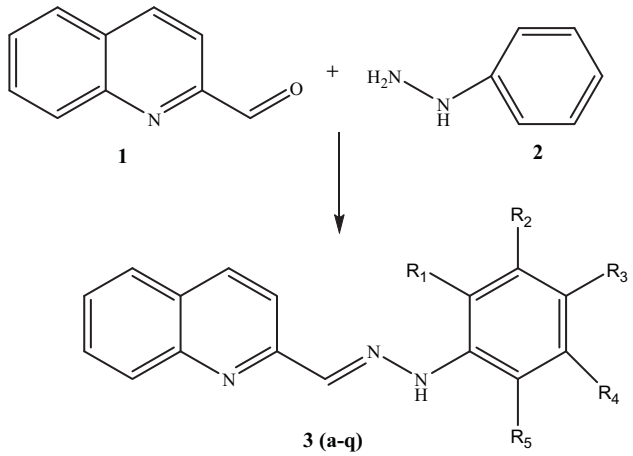

Figure 2. Synthesis of quinoline-2- carboxaldehyde hydrazones.

Table 1. Quinoline-2 carboxaldehyde hydrazones.

Compounds	R_1	R_2	R_3	R_4	R_5
3a	H	H	H	H	H
3b	F	H	H	H	H
3c	H	F	H	H	H
3d	H	H	F	H	H
3e	F	H	F	H	H
3f	F	H	H	F	H
3g	H	F	H	F	H
3h	Cl	H	H	H	H
3i	H	Cl	H	H	H
3j	H	H	Cl	H	H
3k	Cl	H	H	Cl	H
3l	H	Cl	Cl	H	H
3m	H	Cl	H	Cl	H
3n	Br	H	H	H	H
3o	H	Br	H	H	H
3p	H	H	Br	H	H
3q	NO_2	H	NO_2	H	H

2′,7′-dichlorofluorescin-diacetate (DCFH-DA), in the presence of cumene hydroperoxide. The results were compared with MLT, as the parent compound. The cytotoxicity of all compounds was investigated in two assays in Chinese hamster ovary (CHO-K1) cells; the lactate dehydrogenase (LDH) leakage assay and 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay. Seventeen compounds were characterized on the basis of ^{1}H and ^{13}C NMR, Mass, and FT-IR spectra, and elemental analysis. Compounds **3a–d**[24] were published previously.

Materials and methods

In this study, a series of new quinoline-2-carbaldehyde hydrazones (**3a–q**) was developed (Figure 2) as bioisosteric analogues of MLT. The target hydrazones derived from quinoline-2-carboxaldehyde **1** and the appropriate hydrazine derivatives **2** (Table 1) using simple reaction strategies that have been adopted[25]. Phenyl hydrazine derivatives and quinoline-2-carboxaldehyde were heated in the presence of ethanol. The chemical reagents used in synthesis were purchased from Sigma (Steinheim, Germany) and Aldrich (St. Louis, MO). Uncorrected melting points were determined with a Büchi SMP-20 apparatus (Sigma, Steinheim, Germany). The ^{1}H and ^{13}C NMR spectra were recorded with a Varian 400 MHz using TMS as an internal standard and DMSO-d_6 as a solvent. ESI mass spectra were determined on a Waters micromass ZQ spectrometer (Waters, Milford, MA), FT-IR spectra were recorded on a Jasco 420Fourier instrument (Jasco, Portland, OR). Elemental analyses were performed using CHNS-932 (LECO, St. Joseph, MI). All spectral analysis was performed at Ankara University, Faculty of Pharmacy, Central Laboratory.

General procedure for the synthesis of compounds 3a–q

Quinoline-2-carboxaldehyde **1** (0.1 mmol) was reacted with phenyl hydrazine or its derivatives (0.13 mmol) in 10 ml of EtOH, in the presence of 0.5 g CH_3COONa on the hot water bath. Upon cooling, the precipitate was collected and washed with cold EtOH. The crude product was purified by column chromatography using chloroform: *i*-propanol (100:1) as an eluent to give **3a–r**. The final compounds were obtained as a mixture of *E*/*Z* *stereoisomers*.

2-[2-(2′,4′-difluorophenyl)hydrazinylidene]methyl}quinoline (**3e**): Yield 57.8%, m.p. 156–157 °C; ^{1}H-NMR 7.04 (1H, m, H-6′), 7.27 (1H, m, H-3′), 7.55 (1H, m, H-5′), 7.63 (1H, m, H-8), 7.73 (1H, m, H-5), 7.94 (2H, t, H-6, H-7), 8.13 (1H, d, H-3), 8.26 (1H, s, CH), 8.30 (1H, d, H-4), 10.81 (1H, s, NH); EIMS *m*/*z*, 284 (M + H, %100). Anal. Calcd. for $C_{16}H_{11}F_2N_3$: C, 67.84%; H, 3.91%; N, 14.83%; found: C, 67.51%; H, 4.31%; N, 14.93%.

2-[2-(2′,5′-difluorophenyl)hydrazinylidene]methyl}quinoline (**3f**): Yield 73.5%, m.p. 183–184 °C; ^{1}H-NMR 6.61 (1H, m, H-4′), 7.22 (1H, m, H-6′), 7.40 (1H, m, H-3′), 7.56 (1H, m, H-8), 7.73 (1H, m, H-5), 7.95 (2H, t, H-6, H-7), 8.20 (1H, d, H-3), 8.31 (1H, s, CH), 8.32 (1H, d, H-4), 11.02 (1H, s, NH); EIMS *m*/*z*, 284 (M + H, %100). Anal. Calcd. for $C_{16}H_{11}F_2N_3$: C, 67.84%; H, 3.91%; N, 14.83%; found: C, 68.24%; H, 4.14%; N, 14.87%.

2-[2-(3′,5′-difluorophenyl)hydrazinylidene]methyl}quinoline (**3g**): Yield 59.0%, m.p. 218–220 °C; ^{1}H-NMR 6.58 (1H, m, H-4′), 6.79 (2H, dd, H-2′; H-6′), 7.56 (1H, m, H-8), 7.73 (1H, m, H-5), 7.95 (2H, m, H-6, H-7), 8.03 (1H, s, CH), 8.17 (1H, d, H-3), 8.31 (1H, d, H-4), 11.22 (1H, s, NH); EIMS *m*/*z*, 284 (M + H, %100). Anal. Calcd. for $C_{16}H_{11}F_2N_3$: C, 67.84%; H, 3.91%; N, 14.83%; found: C, 68.08%; H, 3.88%; N, 14.87%.

2-[2-(2′-chlorophenyl)hydrazinylidene]methyl}quinoline (**3h**): Yield 33.0%, m.p. 136–138 °C; ^{1}H-NMR 6.86 (1H, m, H-5′), 7.30 (1H, t, H-4′), 7.36 (1H, dd, H-8), 7.55 (1H, m, H-6′), 7.72 (2H, m, H-5, H-3′), 7.96 (2H, dd, H-6, H-7), 8.15 (1H, d, H-3), 8.31 (1H, d, H-4), 8.47 (1H, s, CH), 10.49 (1H, s, NH); ^{13}C-NMR 106.99, 115.46, 117.62, 121.72, 127.02, 127.7, 128.27, 128.99, 129.16, 130.27, 133.23, 136.65, 141.33 (=CH), 142.26, 147.90, 155.62; EIMS *m*/*z*, 282 (M + H, %100); 284.4 (M + H + 2). Anal. Calcd. for $C_{16}H_{12}ClN_3$• 0.1H_2O: C, 67.77%; H, 4.34%; N, 14.82%; found: C, 67.42%; H, 4.24%; N, 14.73%.

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2-[2-(3′-chlorophenyl)hydrazinylidene]methyl}quinoline (**3i**): Yield 78.1%, m.p. 214–215 °C; ^{1}H-NMR 6.84 (1H, dd, H-4′), 7.09 (1H, dd, H-6′), 7.25 (1H, s, H-2′), 7.30 (1H, t, H-5′), 7.57 (1H, t, H-8), 7.75 (1H, t, H-5), 7.97 (2H, t, H-6, H-7), 8.06 (1H, s, CH), 8.16 (1H, d, H-3), 8.33 (1H, d, H-4), 11.08 (1H, s, NH); ^{13}C-NMR 111.94, 112.44, 118.04, 119.93, 127.11, 128.04, 128.60, 129.18, 130.49, 131.61, 134.70, 136.88, 138.96 (=CH), 146.68, 148.13, 155.38; EIMS *m/z*, 282 (M + H, %100); 284.3 (M + H + 2). Anal. Calcd. for $C_{16}H_{12}ClN_3$• 0.1H_2O: C, 67.77%; H, 4.34%; N, 14.82%; found: C, 67.41%; H, 4.43%; N, 14.72%.

2-[2-(4′-chlorophenyl)hydrazinylidene]methyl}quinoline (**3j**): Yield 60.3%, m.p. 205–207 °C; ^{1}H-NMR 7.20 (2H, d, H-3′, H-5′), 7.33 (2H, d, H-2′, H-6′), 7.56 (1H, t, H-8), 7.74 (1H, t, H-5), 7.96 (2H, t, H-6, H-7), 8.04 (1H, s, CH), 8.13 (1H, d, H-3), 8.32 (1H, d, H-4), 11.04 (1H, s, NH); ^{13}C-NMR 114.64, 117.93, 123.80, 127.02, 127.97, 128.58, 129.14, 129.77, 130.47, 136.78, 138.29 (=CH), 144.14, 148.15, 155.54; EIMS *m/z*, 282 (M + H, %100); 284.3 (M + H + 2). Orh 1: 281.74. Anal. Calcd. for $C_{16}H_{12}ClN_3$• 0.5H_2O: C, 66.09%; H, 4.51%; N, 14.45%; found: C, 65.67%; H, 4.83%; N, 14.35%.

2-[2-(2′,5′-dichlorophenyl)hydrazinylidene]methyl}quinoline (**3k**): Yield 37.7%, m.p. 181–183 °C; ^{1}H-NMR 6.92 (1H, dd, H-4′), 7.43 (1H, d, H-3′), 7.60 (1H, m, H-8), 7.68 (1H, d, H-6′), 7.77 (1H, m, H-5), 8.00 (2H, t, H-6, H-7), 8.21 (1H, d, H-3), 8.36 (1H, d, H-4), 8.51 (1H, s, CH), 10.67 (1H, s, NH); ^{13}C-NMR 114.12, 115.73, 118.00, 120.44, 127.18, 127.99, 128.39, 129.13, 130.32, 131.47, 133.39, 136.83 (=CH), 142.46, 147.88, 154.92; EIMS *m/z*, 316 (M^+, %100); 318 (M + 2); 320 (M + 4). Orh 7: Anal. Calcd. for $C_{16}H_{11}Cl_2N_3$: C, 60.77%; H, 3.51%; N, 13.29%; found: C, 60.50%; H, 3.69%; N, 13.31%.

2-[2-(3′,4′-dichlorophenyl)hydrazinylidene]methyl}quinoline (**3l**): Yield 57.6%, m.p. 219–221 °C; ^{1}H-NMR 7.07 (1H, dd, H-6′), 7.36 (1H, d, H-2′), 7.45 (1H, d, H-5′), 7.54 (1H, t, H-8), 7.71 (1H, t, H-5), 7.93 (2H, t, H-6, H-7), 8.02 (1H, s, CH), 8.13 (1H, d, H-3), 8.29 (1H, d, H-4), 11.11 (1H, s, NH); ^{13}C-NMR 113.52, 114.11, 118.08, 121.32, 127.20, 128.09, 128.60, 129.21, 130.51, 131.76, 132.49, 136.90, 139.61(=CH), 145.29, 148.12, 155.19; EIMS *m/z*, 316 (M^+, %100); 318 (M + 2); 320 (M + 4). Anal. Calcd. for $C_{16}H_{11}Cl_2N_3$: C, 60.77%; H, 3.51%; N, 13.29%; found: C, 60.47%; H, 3.66%; N, 13.24%.

2-[2-(3′,5′-dichlorophenyl)hydrazinylidene]methyl}quinoline (**3m**): Yield 49.1%, m.p. 242–244 °C; ^{1}H-NMR 6.94 (1H, t, H-4′), 7.12 (2H, d, H-2′, H-6′), 7.56 (1H, t, H-8), 7.73 (1H, t, H-5), 7.95 (2H, dd, H-6, H-7), 8.03 (1H, s, CH), 8.16 (1H, d, H-3), 8.31 (1H, d, H-4), 11.19 (1H, s, NH); ^{13}C-NMR 111.43, 118.21, 119.16, 127.33, 128.17, 128.63, 129.26, 130.56, 135.52, 137.02, 140.39 (=CH), 147.51, 148.10, 155.02; EIMS *m/z*, 316 (M^+, %100); 318 (M + 2); 320 (M + 4). Anal. Calcd. for $C_{16}H_{11}Cl_2N_3$• 0,5H_2O: C, 59.10%; H, 3.72%; N, 12.92%; found: C, 58.79%; H, 3.79%; N, 12.99%.

2-[2-(2′-bromophenyl)hydrazinylidene]methyl}quinoline (**3n**): Yield 35.3%, m.p. 144–146 °C; ^{1}H-NMR 6.81 (1H, t, H-4′), 7.34 (1H, t, H-5′), 7.52 (1H, dd, H-3′), 7.57 (1H, d, H-6′), 7.56 (1H, d, H-8), 7.73 (1H, t, H-5), 7.96 (2H, dd, H-6, H-7), 8.15 (1H, d, H-3), 8.32 (1H, d, H-4), 8.48 (1H, s, CH), 10.26 (1H, s, NH); ^{13}C-NMR 107.04, 115.66, 117.82, 121.82, 126.98, 127.87, 128.37, 129.07, 129.16, 130.27, 133.23, 136.65, 141.03 (=CH), 142.36, 147.90, 155.32; EIMS *m/z*, 326 (M^+, %100); 328 (M + 2). Anal. Calcd. For $C_{16}H_{12}BrN_3$• 0.25H_2O : C, 58.11%; H, 3.81%; N, 12.71%; found: C, 57.86%; H, 3.95%; N, 12.65%.

2-[2-(3′-bromophenyl)hydrazinylidene]methyl}quinoline (**3o**): Yield 54.3%, m.p. 218–220 °C; ^{1}H-NMR 7.00 (1H, d, H-6′), 7.13 (1H, d, H-4′), 7.24 (1H, t, H-5′), 7.38 (1H, s, H-2′), 7.57 (1H, t, H-8), 7.75 (1H, t, H-5), 8.00 (2H, q, H-6, H-7), 8.05 (1H, s, CH), 8.15 (1H, d, H-3), 8.33 (1H, d, H-4), 11.07 (1H, s, NH); ^{13}C-NMR 112.06, 115.037, 117.80, 122.59, 123.02, 126.89, 127.81, 128.37, 128.96, 130.27, 131.69, 136.65, 138.77 (=CH), 146.55, 147.91, 155.13; EIMS *m/z*, 326 (M^+, %100); 328 (M + 2). Anal. Calcd. for $C_{16}H_{12}BrN_3$: C, 58.91%; H, 3.71%; N, 12.88%; found: C, 58.53%; H, 3.71%; N, 12.79%.

2-[2-(4′-bromophenyl)hydrazinylidene]methyl}quinoline (**3p**): Yield 55.2%, m.p. 210–212 °C; ^{1}H-NMR 7.13 (2H, dd, H-5′, H-3′), 7.42 (2H, dd, H-2′, H-6′), 7.54 (1H, m, H-8), 7.72 (1H, m, H-5), 7.94 (2H, q, H-6, H-7), 8.01 (1H, s, CH), 8.11 (1H, d, H-3), 8.29 (1H, d, H-4), 11.02 (1H, s, NH); ^{13}C-NMR 111.22, 114.89, 117.70, 126.81, 127.75, 128.36, 128.92, 130.25, 132.38, 136.56, 138.17 (=CH), 144.27, 147.92, 155.28; EIMS *m/z*, 326 (M^+, %100); 328 (M + 2). Anal. Calcd. for $C_{16}H_{12}BrN_3$• 0.25H_2O: C, 58.11%; H, 3.81%; N, 12.71%; found: C, 57.79%; H, 3.75%; N, 12.61%.

2-[2-(2′,4′-dinitrophenyl)hydrazinylidene]methyl}quinoline (**3q**): Yield 11.9%, m.p. 255–257 °C; ^{1}H-NMR 7.63 (1H, t, H-8), 7.78 (1H, t, H-5), 8.01 (2H, t, H-6, H-7), 8.22 (2H, t, H-4, H-3), 8.38 (1H, d, H-6′), 8.43 (1H, d, H-5′), 8.83 (2H, s, H-3′), 11.93 (1H, s, NH); EIMS *m/z*, 338 (M + H, %100). Anal. Calcd. for $C_{16}H_{11}N_5O_4$: C, 56.97%; H, 3.29%; N, 20.76%; found: C, 57.20%; H, 3.22%; N, 20.40%.

Biological activity studies

CHO-K1 cells were cultured in Ham's F12 medium supplemented with 10% fetal bovine serum, 1% of a 100 U/ml penicillin–streptomycin solution, 2 mM (final concentration) L-glutamine, and 1 mM (final concentration) sodium pyruvate at 37 °C, in 5% CO_2 atmosphere. This medium was used in all cell incubations.

Antioxidant activity on ROS-induced DCFH-DA oxidation

Cells were seeded at a density of 5×10^3 cells/well in black 96-well plates and incubated for 24 h at 37 °C in a humid atmosphere containing 5% CO_2 for cell attachment. The medium in the wells was removed and then the cells were incubated with DCFH-DA (20 μM) containing medium for 1 h. Cells were washed with PBS to remove excess DCFH-DA. 10 μM-synthesized compounds and 1 μM cumene hydroperoxide were added into medium. The production of fluorescent DCF was evaluated by monitoring the fluoresence intensity at 488 nm excitation, and 530 nm emission wavelength for 60 min[26].

Cytotoxic effect via LDH assay

CHO cells were seeded at a density of 10×10^3 cells/well in 96-well plates and incubated for 24 h at 37 °C in a humid atmosphere containing 5% CO_2 for cell attachment. Cytotoxic effects of the compounds at 10 μM concentration and for 24 h incubation were evaluated via the LDH activity assay according to the method of Hassoun et al.[27] with a minor modification. The activity of LDH in 100 μl of media was determined by direct calculation based on the decrease in absorbance[28].

Cytotoxic effect via MTT assay

Cells were seeded at a density of 5×10^3 cells/well in 96-well plates and incubated at 37 °C in a humid atmosphere containing 5% CO_2 for 24 h for cell attachment. The cells were treated with the newly synthesized compounds (10 μM) for 24 h. Control (medium only) and positive control (15 μM Triton X-100) were included in every experiment. Following the exposure period, the medium was removed, cells were washed with PBS, and then incubated with MTT at a final concentration of 0.5 mg/ml for 4 h at 37 °C. The medium was then removed and formed formazan crystals were dissolved in 150 μl of dimethyl sulfoxide. The absorbance was recorded at 550 nm on a microplate reader. Each

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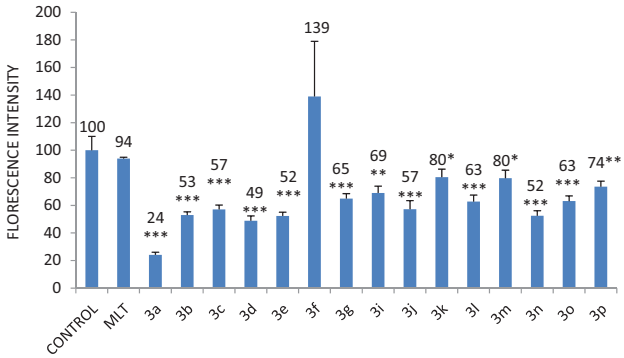

Figure 3. Oxidation of DCFH via ROS in CHO cells after the incubation with 10 μM MLT or 10 μM quinoline derivatives for 60 min. Values are mean ± SD of three individual experiments. Values above the bars are % control values. $*p<0.05$, $**p<0.005$, $***p<0.0005$ (MLT, melatonin).

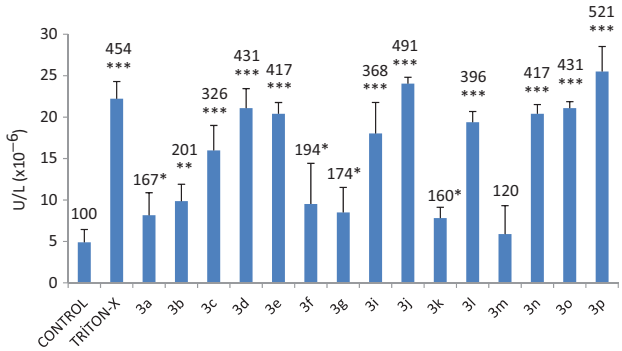

Figure 4. Lactate dehydrogenase (LDH) activity in the medium leaked from the cytosol of CHO-K1 cells incubated with 10 μM quinolone derivatives. Values are mean ± SD of three individual experiments. Values above the bars are % control values. $*p<0.05$, $**p<0.005$, $***p<0.0005$, compared with control values.

experiment was repeated on three separate days. The ratio of the absorbance of treated samples to the absorbance of control (taken as %100) was expressed as % cell viability.

Results and discussion

Effects of synthesized hydrazone derivatives on cellular ROS

For the estimation of ROS inside cells, DCFH-DA was used as a probe. In cellular systems, non-fluorescent probe DCFH-DA readily crosses the cell membrane and undergoes hydrolysis by intracellular estrases to non-fluorescent 2′,7′-dichlorofluorescin (DCFH). DCFH is then rapidly oxidized in the presence of ROS to highly fluorescent 2′,7′- dichlorofluorescein (DCF)[29].

The protective effect of the newly synthesized MLT analogues against DCFH-DA oxidation was determined in CHO cells that were preloaded with the fluorescent probe. Oxidation of the probe located in the cytosol was screened at various time intervals up to 60 min. It is observed that all the newly synthesized hydrazone derivatives, except **3f**, have potent antioxidant activity on DCFH oxidation, even higher than the parent compound MLT. Compound **3a**, the only compound bearing no halogen on the aromatic ring, was found to be the most potent antioxidant (%24 decreased the control oxidation values) among all tested compounds. According to the structure activity relationship, mono-halogenated derivatives were found to have better antioxidant effect compared with the di-halogenated derivatives (Figure 3).

Cytotoxic effect of the synthesized hydrazone derivatives

LDH is a cytoplasmic enzyme and its leakage from injured cells into the culture medium has been shown to be useful as an indicator of cellular membrane damage. In the present study, we determined LDH activity in the culture medium in order to assess effect of hydrazone derivatives on membrane integrity.

The MTT assay relies on the ability of live but not dead cells to reduce a water-soluble yellow dye, MTT, to water-insoluble purple formazan crystals. Since the substrate and the product absorb at very different wavelengths, no washing step is required which is a clear advantage of this assay and makes it a useful tool for drug screening studies.

The cells were incubated with the compounds (10 μM) for 24 h in LDH assay. As can be seen in Figure 4, most of hydrazone derivatives induced cell membrane damage. LDH leakage into the medium was statistically insignificant with **3m** and **3a**. The most cytotoxic hydrazone derivative was found to be **3p** which has *p*-bromine substitution on aromatic ring. In contrast, **3e**, **3g**

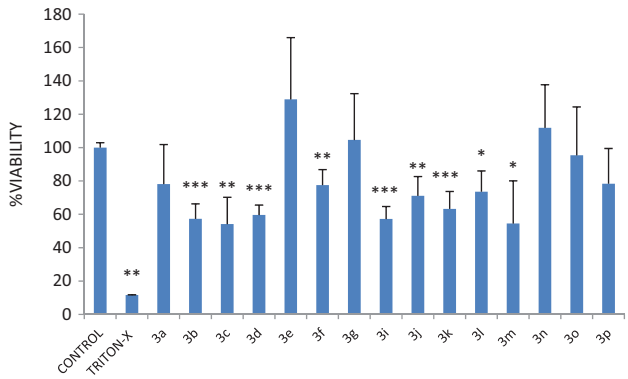

Figure 5. Effect of quinolone derivatives (10 μM) on cell viability of CHO-K1 cells evaluated by MTT assay. Values are mean ± SD of four individual experiments. Triton X-100 was used as the cytotoxic control. $*p<0.05$, $**p<0.005$, $***\ p<0.0005$, groups compared with the control group.

and **3n** were detected to have no cytotoxic effect in MTT assay. The possible reason for it is the diversity of parameters between assays. Results indicate that except **3a** and **3m**, other hydrazone derivatives have strong membrane damaging effect with longer exposure periods (24 h).

10 μM of **3b**, **3c**, **3d**, **3f**, **3i**, **3j**, **3k**, **3l**, and **3m** decreased cell viability in MTT assay after incubation with CHO-K1 cells for 24 h (Figures 4 and 5). This effect was found to be related to the type of aromatic halogen substitution; mono-F and mono-Cl substances decreased cell viability while mono-Br did not show such an effect. Among dihalogenated compounds *p*-dihalogened ones were found to be more cytotoxic than *m*- and *o*-dihalogenated compounds. The only substance without any halogene substitution on aromatic ring, **3a**, did not have any negative effect on cell viability.

Declaration of interest

The authors report no conflicts of interest. The chemical compounds of this work were supported by The Scientific and Technological Research Council of Turkey (TÜBİTAK) Research and Development Grant 112S599. The cell culture facility was established by the support of TUBITAK 108S202 Grant.

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