ORIGINAL RESEARCH

Synthesis and evaluation of cytotoxic activities of some substituted isoxazolone derivatives

Sevim Rollas[1], Şila Kokyan[1], Bedia Koçyiğit-Kaymakçıoğlu[1], Suna Özbaş-Turan[2], Jülide Akbuğa[2]

ABSTRACT: Several isoxazolone derivatives were synthesized, starting from substituted 1,3,4-thiadiazoles and 1,2,4-triazole-3-thione. In the first part of the research, compounds 2-(4-aminophenyl)-5-alkyl/arylamino-1,3,4-thiadiazoles (4a-e) and 5-(4-aminophenyl)-4-substitude-2,4-dihydro-3H-1,2,4-triazole-3-thiones (5a-c) were prepared from ethyl 4-aminobenzoate. In the second part, compounds, which were prepared by coupling the diazonium salts of aromatic primary amines with ethyl acetoacetat (6a-e, 7a-c) were cyclized with hydroxylamine hydrochloride and sodium acetate in ethanol and yielded 3-methyl-4-[2-{4-[5 alkyl/arylamino)-1,3,4-thiadiazol-2-yl]phenyl} hydrazinylidene]isoxazol-5(4H)-one (8a-e) 3-methyl-4-[2-{4-[4-(4-alkyl/aryl)-5-thioxo-4,5-dihydro-1H-1,2,4-triazol-3-yl]phenyl}hydrazinylidene]isoxazol-5(4H)-one (8f-g). The structures of the synthesized compounds were confirmed by elemental analysis (C,H,N,S), UV, IR, 1H-NMR and mass spectroscopic methods. Cytotoxicity of these compounds were evaluated by using HEK293 cell line of MTT [3-(4,5-dimethylthiazole-2-yl)-2,5-diphenyltetrazolium bromide] assay. The highest inhibitions were confirmed as %45.72 for the compound 3-methyl-4-[2-(4-{5-[(4-methoxyphenyl) amino]-1,3,4-thiadiazol-2-yl}phenyl)hydrazinylidene]isoxazol-5(4H)-one (8e) and %33.07 for the compound 3-methyl-4-[2-(4-{5-[(4-methylphenyl)amino]-1,3,4-thiadiazol-2-yl}phenyl)hydrazinylidene]isoxazol-5(4H)-one (8a).

KEYWORDS: 1,2,4-Triazole-3-thione, 1,3,4-thiadiazole, isoxazolone, hydrazone, cytotoxic activity

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1. INTRODUCTION

Isoxazoles have a long history of application in pharmaceutical and agrochemical industry (1). Isoxazole-5-one derivatives are also known for their biological activity as anti-androgenic, antitubercular, cytotoxic and antibacterial agents (2-6).

The recognition of the pharmacological activity of some isoxazole derivatives such as Gantricin, a sulfa drug from amino isoxazole and cycloserine a simple derivative of 3-isoxazolidone as antibiotics has aroused a new interest in this field (7). Many synthesis methods are reported about the cyclization of isoxazolones derivatives. One of the most useful method to obtain isoxazolones relies on the cyclocondensation of β-keto esteres or α,β unsaturated esters with hydrazines or hydroxylamines, respectively (8-11). In the present study, isoxazole derivatives have been synthesized by the reaction of 1,3-dicarbonyl compounds with hydroxylamines in basic medium (12-13). On the other hand, it has been reported that 1,2,4-triazole derivatives possess a wide spectrum of chemotherapeutic activities including anti-inflammatory, anticancer, antidepressant, antibacterial, anticonvulsant and as well as antifungal properties (14-22). In fact, some of their derivatives are active constituents of currently used drugs such as Fluconazole and Itraconazole as potent antifungal agents (23). Furthermore, various 1,3,4-thiadiazole derivatives were shown to possess excellent pharmacological properties such as antimicrobial, analgesic and anti-inflammatory activities (24-27). On the basis of these observations, we aimed to synthesize a new class of heterocyclics, wherein potent

1,2,4-triazole and 1,3,4-thiadizaole moiety is attached to position 4 of the isoxazolone ring. Therefore, a series of new 3-methyl-4-(2-{4-[5 alkyl/arylamino)-1,3,4-thiadiazol-2-yl]phenyl} hydrazinylidene)isoxazol-5(4H)-one and 3-methyl-4-(2-{4-[4-(4-aryl)-5-thioxo-4,5-dihydro-1H-1,2,4-triazol-3-yl]phenyl} hydrazinylidene) isoxazol-5(4H)-one were synthesized and their structures were confirmed by means of UV, IR, ^{1}H-NMR, mass spectroscopy and elemental analysis. All the synthesized compounds were screened for their cytotoxic activities by using HEK293 cell line of MTT [3-(4,5-dimethylthiazole-2-yl)-2,5-diphenyltetrazolium bromide] Assay.

2. EXPERIMENTAL

2.1. Chemistry

All chemicals and solvents were purchased from Merck, Aldrich, or Fluka. Melting points were determined by a "Schmelzpunktbestimmer" SMP II and were uncorrected. The reactions were monitored on Merck pre-coated aluminium TLC plates 60F-254 and the products were visualized by UV-light using chloroform and acetone (50:50 v/v) as solvent system. The UV spectra were recorded on a Schimadzu UV-1601 spectrometry. The IR spectra were recorded on a Schimadzu FTIR 8400S spectrometry. ^{1}H-NMR spectra were recorded in DMSO on a Bruker Avance-DPX-400 spectrometer in DMSO-d_6 and chemical shifts were given in δ ppm with tetramethylsilane. The splitting patterns of ^{1}H-NMR were designed as follows: s: singlet, d: doublet, t: triplet, q: quartet, m: multiplet. Mass spectrometry was performed using an Agilent 1100 MSD spectrometer. Elemental analysis was performed using a Vario MICROV1.5.7

Synthesis of ethyl 4-(benzoylamino)benzoate (**1**). Benzocaine (0.03 mol) was dissolved in 36 mL ether. Benzoylchloride (3.6 mL diluted in 3.6 mL ether.) was added drop by drop and stirred, after evaporating ether, the solid product was washed with distilled water until the smell of benzoyl chloride disappeared and then crystallized from ethanol.

Synthesis of 4-(benzoylamino)benzoylhydrazine (**2**). 9 mL hydrazine hydrate was added to ethyl 4-(benzoylamino)benzoate (28). The mixture was refluxed at 110-130°C for 30 minutes. After adding 15 mL ethanol, the mixture was heated in water bath for 45 minutes. The residue was filtered, washed with water and crystallized from ethanol.

General procedure for the preparation of 1-[4-(benzoylamino)benzoyl]-4-alkyl/-arylthiosemicarbazide (**3a-h**). 0.005 mol alkyl/arylisothiocyanate was added to the solution of 0.005 mol 4-(benzoylamino)benzoylhydrazine in 40 mL ethanol. The mixture was refluxed on water bath for 2-2.5 h. The solid product, obtained on cooling, was washed with distilled water and crystallized from ethanol.

General procedure for the preparation of 2-(4-aminophenyl)-5-alkyl/arylamino-1,3,4-thiadiazole (**4a-e**). 15-20 mL 50% H_2SO_4 was added to 3a-f (0.006 mol) and refluxed at 110-150°C for 5 h. The mixture was neutralized with 2N NaOH. The precipitate was filtered, washed with water and crystallized from ethanol (28).

General procedure for the preparation of 5-(4-aminophenyl)-4-alkyl/aryl-1,2,4-triazole-3-thione (**5a-c**). 15-20 mL 2N NaOH was added to 3a-f (0.005 mol) and refluxed on water bath for 4 h. The reaction mixture was neutralized with 10% HCl. The precipitate was filtered, washed with water and crystallized from ethanol (28).

General procedure for the preparation of coupling products (**6a-e, 7a-c**).

1 ml of an ice-cold solution of sodium nitrite (10%) containing 0.2 ml hydrochloric acid (37%) is added drop by drop to 0.001 mol 2-(4-aminophenyl)-5-alkyl/arylamino-1,3,4-thiadiazolles or 5-(4-aminophenyl)-4-alkyl/arylamino-1,2,4-triazole-3-thiones. The reaction mixture is then poured into a mixture of 0.001 mol ethylacetoacetat and 5 g sodium acetate in ethanol (5 mL) by vigorous stirring. The precipitate was filtered, washed with water, dried and crystallized from appropriate solvent (28).

General procedure for the preparation of 5-isoxazolone derivatives (**8a-g**). 0.001 mol coupling product, 0.001 mol hydroxylamine hydrocloride, 0.5 g sodium acetate were solved in 5 mL ethanol and refluxed on water bath for 3-3.5 h. The precipitate was filtered, washed with water and crystallized from ethanol.

3-methyl-4-[2-(4-{5-[(4-methylphenyl)amino]-1,3,4-thiadiazol-2-yl}phenyl)hydrazinylidene]isoxazol-5(4*H*)-one **(8a):** Yield 69 %, mp 240-242 °C**.** UV $\lambda_{max.}$ (EtOH) (nm)**:** 415.5, 327.5, 249.5. IR ($\bar{\upsilon}_{max}$, cm^{-1}): 3194 (N-H); 1716 (C=O); 1553, 1516 (C=N). ^{1}H NMR (400 MHz) (DMSO-d_6/TMS) δ ppm: 2.23 (s, 3H, -CH_3); 2.30 (s, 3H, -CH_3); 7.20 (2H, d, J: 8 Hz, Ar-H); 7.51 (2H, d, J: 8 Hz, Ar-H); 7.93 (2H, d, J: 9 Hz, Ar-H); 8.0 (2H, d, J: 9 Hz, Ar-H); 10.52 (s, 1H, -NH); 12.91 (s, 1H, =C-N=NH). Anal. Calcd. for $C_{19}H_{16}N_6O_2S$; C, 58.15; H, 4.11; N, 21.42; S, 8.15 Found: C, 57.74; H, 4.12; N, 20.71; S, 7.90.

3-methyl-4-[2-{4-[5-(methylamino)-1,3,4-thiadiazol-2-yl] phenyl}hydrazinylidene]isoxazol-5(4*H*)-one **(8b):):** Yield 80 %, mp 209-211 °C. UV $\lambda_{max.}$ (EtOH) (nm): 421.5, 304, 220. IR ($\bar{\upsilon}_{max}$, cm^{-1}): 3207 (N-H); 1716 (C=O); 1556 (C=N). ^{1}H NMR (400 MHz), (DMSO-d_6/TMS) δ ppm: 2.27 (s, 3H, -CH_3); 2.95 (s, 3H, -CH_3); 7.83 (2H, d, J: 9 Hz, Ar-H); 7.85 (2H, d, J: 9 Hz, Ar-H), 8.19 (s, 1H, -NH-); 12.82 (s, 1H, =C-N=NH-). Anal. Calcd. for $C_{13}H_{12}N_6O_2S$. 1 H_2O; C, 46.6; H, 4.2; N, 25.1; S, 9.5 Found: C, 45.7; H, 4.1; N, 24.2; S, 9.2.

3-methyl-4-[2-{4-[5-(propylamino)-1,3,4-thiadiazol-2-yl]phenyl}hydrazinylidene]isoxazol-5(4*H*)-one

(8c):): Yield 64 %, mp 214 °C. UV $\lambda_{max.}$ (EtOH) (nm)**:** 423.5, 302.5. IR ($\bar{\upsilon}_{max}$, cm^{-1}): 3200 (N-H); 1720 (C=O); 1552 (C=N). ^{1}H NMR (400 MHz), (DMSO-d_6/TMS) δ ppm: 0.92 (t, 3H, CH_3); 1.57 (m, 2H, CH_2); 2.27 (s, 3H, -CH_3); 3.26 (t, 2H, -CH_2); 7.74 (2H, d, J: 9 Hz, Ar-H), 7.81 (2H, d, J: 9 Hz, Ar-H), 7.98 (s, 1H, -NH-), 12.82 (s, 1H, =C-N=NH-). CI-MS, m/z (%): 345 (M^++1, 100). Anal. Calcd. For $C_{15}H_{16}N_6O_2S$: C, 52.3; H, 4.7; N, 24.4; S, 9.3 Found: C, 51.9; H, 4.6; N, 24.1; S, 9.0.

3-methyl-4-[2-(4-{5-[(4-ethylphenyl)amino]-1,3,4-thiadiazol-2-yl}phenyl)hydrazinylidene]isoxazol-5(4*H*)-one **(8d):** Yield 60%, mp 231-233 °C. UV $\lambda_{max.}$ (EtOH) (nm)**:** 414, 321, 251. IR ($\bar{\upsilon}_{max}$, cm^{-1}) : 3192 (N-H); 1716 (C=O); 1548, 1506 (C=N). ^{1}H NMR (400 MHz), (DMSO-d_6/TMS) δ ppm: 1.16 (t, 3H, -CH_3); 2.28 (s, 3H, -CH_3); 2.55 (m, 2H, -CH_2); 7.19 (2H, d, J: 8 Hz, Ar-H); 7.54 (2H, d, J: 8 Hz, Ar-H); 7.71 (2H, d, J: 9 Hz, Ar-H); 7.91 (2H, d, J: 9 Hz, Ar-H); 10.49 (s, 1H, -NH); 12.83 (s, 1H, =C-N=NH-). Anal. Calcd. For $C_{20}H_{18}N_6O_2S$ .1H_2O: C , 56.5; H, 4.7; N, 19.7; S, 7.5 Found: C, 56.9; H, 4.4; N, 19.0; S, 7.6.

3-methyl-4-[2-(4-{5-[(4-methoxyphenyl)amino]-1,3,4-thiadiazol-2-yl}phenyl)hydrazinylidene] isoxazol-5(4*H*)-one **(8e):** Yield 50 %, mp 229-231 °C. UV $\lambda_{max.}$ (EtOH) (nm)**:** 410.0, 249.5

251. IR ($\bar{\upsilon}_{max}$, cm^{-1}): 3190 (N-H); 1716 (C=O); 1549, 1506 (C=N). ^{1}H-NMR (400 MHz), (DMSO-d_6/TMS) δ ppm: 2.28 (s, 3H, -CH_3); 3.83 (s, 3H, -OCH_3); 6.85 (2H, d, J: 9 Hz, Ar-H); 7.05 (2H, d, J: 9 Hz, Ar-H); 7.57 (2H, d, J: 8 Hz, Ar-H); 7.79 (d, J: 8 Hz, 2H, Ar-H); 10.22 (s, 1H, -NH); 10.36 (s, 1/4 H_2SO_4-OH) 12.83 (s, 1H, =C-N=NH-). CI-MS, m/z (%): 409.0 (M^++1, 20.2). Anal. Calcd. For $C_{19}H_{16}N_6O_3S$.¼H_2SO_4 : C, 52.60; H, 3.81; N, 19.30; S, 9.24. Found: C, 52.20; H, 3.56; N, 18.18; S, 9.26.

3-methyl-4-{2-[4-(4-ethyl-5-thioxo-4,5-dihydro-1*H*-1,2,4-triazol-3-yl)phenyl]hydrazinylidene} isoxazol-5(4*H*)-one **(8f):** Yield 59 %, mp 222 °C; Lit. mp 220 °C (13). ^{1}H-NMR (400 MHz), (DMSO-d_6/TMS) δ ppm: 1.05 (t, 3H, CH_2-CH_3); 2.27 (s,3H, -CH_3); 4.04 (m, 2H, -CH_2); 7.73 (d, 2H, J. 9 Hz, Ar-H), 7.80 (d, J: 9 Hz, 2H, Ar-H); 12.81 (s, 1H, =C-N=NH-); 13.95 (s, 1H, -NH-).

3-methyl-4-(2-{4-[4-(4-methoxyphenyl)-5-thioxo-4,5-dihydro-1*H*-1,2,4-triazol-3-yl]phenyl}hydra-zinylidene)isoxazol-5(4*H*)-one (**8g**)**:** Yield 63 %, mp 230-232 °C. UV $\lambda_{max.}$ (EtOH) (nm)**:** 399.0, 259.0, 231.0. IR ($\bar{\upsilon}_{max}$, cm^{-1}): 3031 (N-H); 1716 (C=O); 1553, 1520 (C=N). ^{1}H-NMR (400 MHz), (DMSO-d_6/TMS) δ ppm: 2.31 (s, 3H, -CH_3); 3.78 (s, 3H, -OCH_3); 7.03 (d, J: 9 Hz, 2H, Ar-H); 7.27 (dd, J: 9 Hz, 4H, Ar-H); 7.61 (d, J: 9 Hz, 2H, Ar-H); 12.72 (s, 1H, =C-N=NH-); 14.09 (s, 1H, -NH). CI-MS, m/z (%): 409.0 (M^++1, 100). Anal. Calcd. For $C_{19}H_{16}N_6O_3S$ .1 H_2O: C, 53.4; H, 4.2; N, 19.7; S, 7.5 Found: C, 53.8; H, 3.9; N, 18.1; S, 7.6.

2.2 Cytotoxic activity

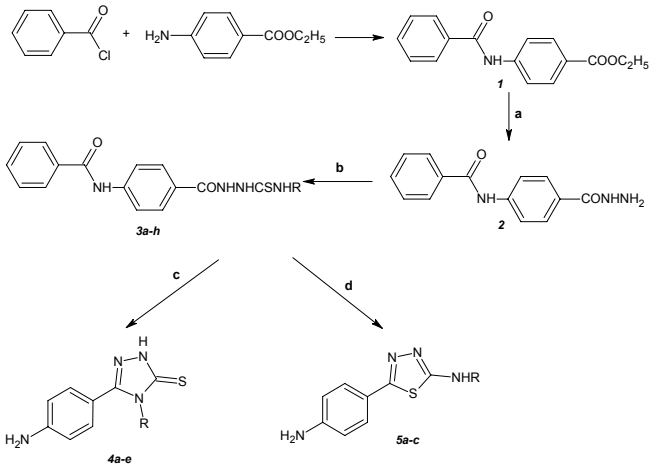
The synthesized compounds were tested for their cytotoxic activities. Cell viability and cytotoxic activity profile of the compounds were analyzed using the MTT assay (29, 30, 31). MTT is cleaved to formazan by the "succinate-tetrazolium reductase" system (EC 1.3.99.1) which belongs to the mitochondrial respiratory chain and is active only in viable cells.

HEK293 cell line was used for the determination of cytotoxic activity (ATCC® CRL-1573™). Cytotoxicity testing in vitro was done by the method of modified Woerdenbag et al (30, 31). The MTT metabolic assay was carried out in seeded at the density of 1x10^4 cells/well in 96-well flat-bottom cell culture plates well plates with 200 µL of opti-MEM (invitrogen, USA) and incubated for 24 hours at 37°C, 5% CO_2. The following day, media was aspirated and the compounds were dissolved in DMSO and diluted with medium before they were added to the cell cultures at the concentrations of 5µg/mL and 10µg/mL. Cells were incubated for 48 hrs at 37°C, 5%CO_2. After the incubation period 10µL of the MTT labeling reagent [final concentration 0.5 µg/mL (Cell proliferation kit MTT, Roche, Germany)] was added to each well. The cultures was incubated for 4-12 hours in a humidified atmosphere (e.g. 37°C, 5.0% CO_2) and 100µL of the solubilization buffer was added into each well. The plate was allowed to stand overnight in the incubator in a humidified atmosphere (e.g. 37°C, 5.0% CO_2), the formazan precipitates were solubilized. Absorbance of the formazan product was measured spectrophotometrically at 550 and 690 nm.

Statistical analyses were done using unpaired Student's t-test using Prism 3.0 (GraphPad Software, San Diego; CA; USA).

3. RESULTS AND DISCUSSION

In our research, starting from substituted 1,3,4-thiadiazole and 1,2,4-triazole-3-thione, several isoxazolone derivatives **(8a-g)** were synthesized. In the first part of this study, compounds 2-(4-aminophenyl)-5-alkyl/arylamino-1,3,4-thiadiazoles (**4a-e**) and 5-(4-aminophenyl)-4-substitude-2,4-dhydro-3H-1,2,4-triazole-3-thions (**5a-c**) were prepared from ethyl 4-aminobenzoate according to the literature [13, 32, 33] (Scheme 1). In the second part, compounds, which were prepared by coupling the diazonium salts of aromatic primary amines with ethyl acetoacetat (**6a-e, 7a-c**) (**6a-e, 7a-c**) were cyclized with hydroxylamine hydrochloride and sodium acetate in ethanol and yielded 3-methyl-4-[2-{4-[5-alkyl/arylamino)-1,3,4-thiadiazol-2-yl]phenyl}hydrazinylidene]isoxazol-5(4*H*)-one (**8a-e**) 3-methyl-4-[2-{4-[4-(4-aryl)-5-thioxo-4,5-dihydro-1*H*-1,2,4-triazol-3-yl]phenyl}hydrazinylidene)] isoxazol-5(4*H*)-one (**8f, g**) (Scheme 2 and 3). Purity of the synthesized isoxazolone compounds (**8a-g**) were checked by thin layer chromatography.

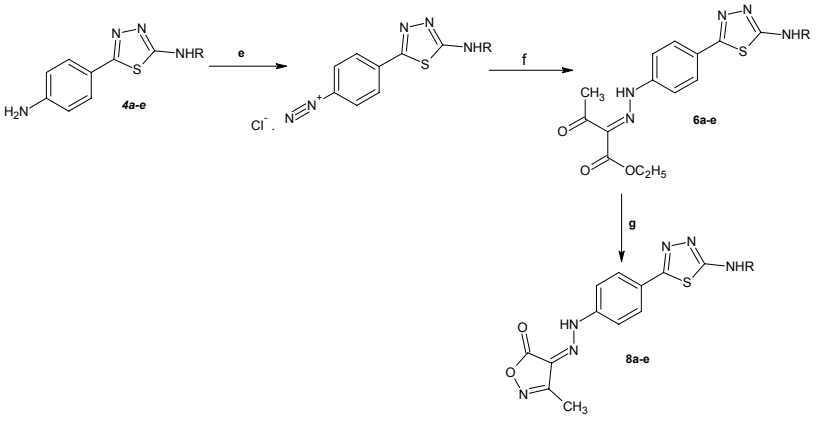


SCHEME 1. Synthesis of intermediate products of 1,3,4-thiadiazole (4a-e) and 1,2,4-triazole-3-thione (5a-c) derivatives

R= methyl, ethyl, propyl, 4-methylphenyl, 4-methoxyphenyl

Reaction condition: a: $N_2H_4.H_2O$; b: RNCS; c: H_2SO_4, D; d: NaOH, D.

The structures of new compounds (**8a-g**) were confirmed by UV, IR, ^{1}H-NMR, mass spectroscopic (for compound **8c, 8e** and **8g**) methods and elemental analysis.



SCHEME 2. Synthesis of 5-isoxazolone derivatives (8a-e)

R= methyl, ethyl, propyl, 4-methylphenyl, 4-methoxyphenyl

Reaction condition: e: $NaNO_2$, HCl, 0-5°C; f: $CH_3COCH_2COOC_2H_5$, CH_3COONa; g: NH_2OH, CH_3COONa.

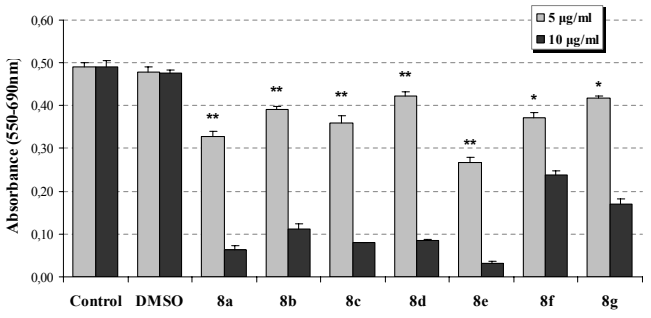
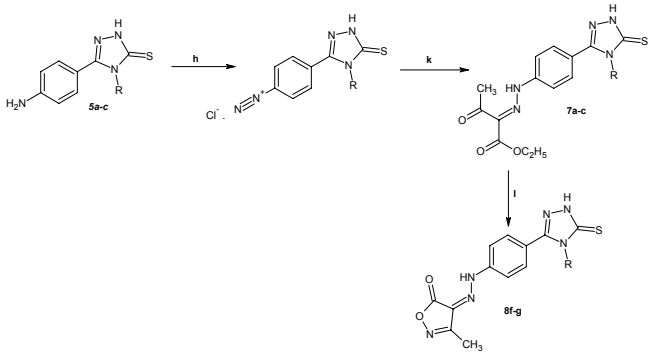


FIGURE 1. Cytotoxic activity profile of the compounds using MTT assay (*p<0.05, **p<0.001).



SCHEME 3. Synthesis of 5-isoxazolone derivatives (8f-g)
R= methyl, ethyl, propyl, 4-methylphenyl, 4-methoxyphenyl
Reaction condition: h: $NaNO_2$, HCl, 0-5°C; k: $CH_3COCH_2COOC_2H_5$, CH_3COONa; l: NH_2OH, CH_3COONa.

The cyclization of 5-isoxazolone ring for compounds **8a-g** was evidenced by its ^{1}H-NMR and IR spectra. The ^{1}H-NMR spectra of compounds **8a-g** showed a sharp singlet at 2.27-2.31 ppm due to methyl protons of 5-isoxazolones. Also, the IR spectrum of compounds **8a-g**, isoxazolone C=O stretching bands were determined at 1716-1720 cm^{-1}. On the other hand, the hydrazone N-H streching bands were observed at 3032-3207 cm^{-1}. Hydrazone and isoxazolone C=N bands were detected at 1506-1553 cm^{-1}.

The ^{1}H-NMR spectra of compounds containing 1,3,4-thiadiazole moiety (**8a-g**) displayed NH resonances at 7.98-10.52 ppm assigned for secondary amine NH protons (34). The ^{1}H-NMR spectra of compounds containing 1,2,4-triazole-3-thione, displayed triazole NH resonances at 13.95-14.09 ppm. All of compounds displayed hydrazone NH resonances at 12.72-12.91 ppm (35).

Cytotoxicity of the seven compounds were evaluated by using HEK293 cell line according to procedures of MTT [3-(4,5-dimethylthiazole-2-yl)-2,5-diphenyltetrazolium bromide] assay. All the compounds were dissolved in dimethylsulfoxide (2.5%) and no cytotoxic effect was observed when compared with the control group. Two different concenterations (5.0 and 10.0 mg/mL) were used. Cell viability and cytotoxic activity profile of the compounds were analyzed using the MTT assay.

Cytotoxic activity results were presented in Figures 1, 2 and 3. Among the tested compounds, two compounds showed 10-20%, four compounds showed 20-35% and one compound showed 45.72% cytotoxic activity. The highest inhibitions were confirmed as 45.72% for the compound 3-methyl-4-[2-(4-{5-[(4-methoxyphenyl)amino]-1,3,4-thiadiazol-2-yl}phenyl)hydrazinylidene]isoxazol-5(4*H*)-one **(8e)** and 33.07% for the compound 3-methyl-4-[2-(4-{5-[(4-methylphenyl)amino]-1,3,4-thiadiazol-2-yl}phenyl)hydrazinylidene]isoxazol-5(4*H*)-one (**8a**). As these values were under 50% we can conclude that neither compound displayed cytotoxic activity at 5.0 µg/mL concentration. All the compounds were tested at concentrations of 5.0 and 10.0 mg/mL and they showed a dose-related effect at these concentrations (p<0.001 for compound **8a**, **8b**, **8c**, **8d** and **8e**; p<0.05 for compound **8f** and **8g**).

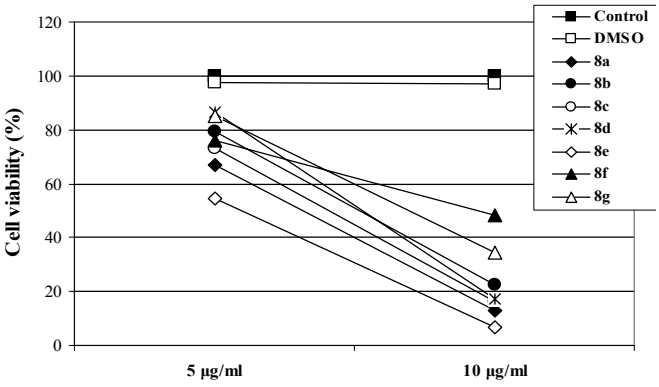


FIGURE 2. Cell viability (%) of compounds 8a-g

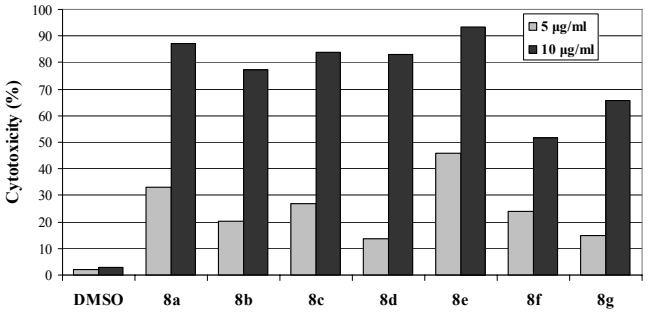


FIGURE 3. Cytotoxic activity (%) of compounds 8a-g

4. CONCLUSION

A series of 5-isoxazolone derivatives have been synthesized starting from substituted 1,3,4-thiadiazoles and 1,2,4-triazole-3-thiones and screened for their cytotoxic activities against HEK293 cell line. The cytotoxicity screening indicated that among the tested compounds, 5-isoxazolone derivatives carrying 5-methyl/methoxyphenylamino-1,3,4-thiadiazole ring (compound **8a** and **8e**) exhibited noteworthy activity.

5. ACKNOWLEDGEMENT

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Sübstitüe izoksazolon türevlerinin sentezi ve sitotoksik aktivitelerinin değerlendirilmesi

ÖZET: Sübstitüe 1,3,4-tiyadiazol ve 1,2,4-triazol-3-tiyon bileşiklerinden hareketle bazı izoksazolon türevi bileşikler sentezlenmiştir. Araştırmanın birinci bölümünde, 2-(4-aminofenil)-5-alkil/arilamino-1,3,4-tiyadiazol yapısındaki bileşikler ve 5-(4-aminofenil)-4-sübstitüe-2,4-dihidro-3H-1,2,4-triazol-3 tiyon yapısındaki bileşikler, etil 4-amino benzoat'tan hareketle literatür yöntemine göre sentezlenmiştir. Araştırmanın ikinci bölümünde triazol ve tiyadiazollerin etil asetoasetat ile kenetlenmeleriyle oluşan ürünlerin hidroksilamin hidroklorür ve sodyum asetatla, etanollü ortamda siklizasyonu sonucunda 3-metil-4-{2-[4-(5-alkil/arilamino-1,3,4-tiyadiazol-2-il)fenil]hidraziniliden}-izoksazol-5(4H)-on ve 3-metil-4-{2-[4-alkil/aril-5-tiyokso-4,5-dihidro-1H-1,2,4-triazol-3-il)fenil]hidraziniliden}izoksazol-5(4H)-on' lar elde edilmiştir. Sentezlenen izoksazolon bileşiklerinin yapıları elementel analiz (C,H,N,S), UV, IR, 1H-NMR ve kütle spektroskopik yöntemleri kullanılarak aydınlatılmıştır. Sentezlenen bileşiklerin sitotoksik aktiviteleri HEK293 hücre hattı kullanılarak MTT [3-(4,5-dimetiltiyazol-2-il)-2,5-difeniltetrazolyum bromür] testi ile tayin edilmiştir. En yüksek düzeyde sitotoksik etki gösteren bileşikler; % 45.72 ile 3-metil-4-[2-(4-{5-[(4-metoksifenil)amino]-1,3,4-tiyadiazol-2-il}fenil)hidraziniliden]isoksazol-5(4H)-on (8e) ve % 33.07 ile 3-metil-4-[2-(4-{5-[(4-metilfenil)amino]-1,3,4-tiyadiazol-2-il}fenil)hidraziniliden]isoksazol-5(4H)-on (8a) bileşikleridir.

ANAHTAR KELİMELER: 1,2,4-Triazol-3-tiyon, 1,3,4-tiyadiazol, izoksazolon, hidrazon, sitotoksik aktivite

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