Research Article

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Synthesis and biological evaluation of some new pyrimidine bearing 2,5-disubstituted 1,3,4-oxadiazole derivatives as cytotoxic agents

Pirimidin içeren bazı yeni 2,5-disübstitüe 1,3,4-oksadiazol türevlerinin sentezi ve sitotoksik ajan olarak değerlendirilmesi

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Abstract

Objective: As a result of adverse effects including drug-resistance, toxicity and low bioavailability, there has been a crucial need for novel anticancer agents. In this present study, some novel 2,5-disubstituted 1,3,4-oxadiazole derivatives bearing pyridine moiety were synthesized and their potential cytotoxic activities were examined.

Materials and methods: A series of seven new compounds of 2\{(5-(3-(pyrimidin-2-yl)thio)propyl)-1,3,4-oxadiazol-2-yl)thio\}|1-(4-substituted)ethan-1-one derivatives were synthesized by reacting 5-{(3-(pyrimidin-2-yl)thio)propyl}-1,3,4-oxadiazole-2-thiol and 4-substituted phenacyl bromide derivatives in acetone with potassium carbonate. The structures of the obtained compounds were elucidated using FT-IR, 1H-NMR and MS spectral data and elemental analyses. In vitro cytotoxic activity of the compounds was evaluated by MTT assay.

Results: Among the tested compounds, compound 4a was found to be the most active cytotoxic agent against A549 cells, in compared with cisplatin as standard drug.

Conclusions: It was determined that some of synthesized compounds had considerable anticancer activity against the A549 cell lines. Compound 4a including phenyl moiety was the most active compound against the A549 cell line and was identified as a lead moiety. Besides, compound 4c including 4-metoxy phenyl moiety exhibited cytotoxic activity against A549 cells. Consequently, compounds possess phenyl and 4-methoxy phenyl moieties have been determined to be important for cytotoxic activity of these compounds.

Keywords: 1,3,4-Oxadiazole; Pyrimidine; Cytotoxic activity.

Özet

Amaç: Kansere karşı günümüzde kullanılan ilaçların ilaç direnci, toksisite ve düşük biyoyararlanım gibi advers etkilerden dolayı, tedavide yeni antikanser ajanların varlığına ihtiyaç duymaktadır. Bu çalışmada pirimidin içeren bazı yeni 2,5-disübstitüe 1,3,4-oksadiazol türevleri sentezenmiştir ve potansiyel sitotoksik etkileri incelenmiştir.

Metod: 2\{(5-(3-(pirimidin-2-ıyl)thio)propil)-1,3,4-oksadiazol-2-ıyl)thio\}|1-(4-sübstitüe)etan-1-on türevlerinin 7 bileşikten oluşan serisi, 5-{(3-(pirimidin-2-ıyl)thio)propil}-1,3,4-oxadiazol-2-thiol ve 4-sübstitüe phenacyl bromide türevlerinin aseton içinde potasyum karbonat eşliğinde reaksiyona sokulmasıyla elde edilmiştir. Elde edilen bileşiklerin yapıları FT-IR, 1H-NMR ve MS spektrum verileri ve elementel analiz kullanarak aydınlatılmıştır. Bileşiklerin in vitro sitotoksik aktivitesi MTT metodu kullanılarak ölçülmuştur.

Bulgular: Test edilen bileşikler arasında, 4a bileşiği A549 hücrelerine karşı, standart ilaç sisplatine göre, en aktif sitotoksik ajan olarak bulunmuştur.

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**Introduction**

Cancer is described as a large group of disease that uncontrolled growth and expansion of abnormal cells included. Because of being responsible from 8.2 million deaths in 2012, cancer is one of the main causes of death in the world. It is presumed that within the next two decades the number of people with cancer will arise by about 70% [1]. The causes of cancer formation are generally using tobacco and alcohol, unhealthy diet, chemicals, radiation, immune system, hormones, infectious organisms [2]. Chemo- and alcohol, unhealthy diet, chemicals, radiation, immune system, hormones, infectious organisms [2].

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**Sonuç:** Sentezlenen bileşiklerin bir kısmının A549 hücrelerine karşı önemli sitotoksik aktivite gösterdiği belirlenmiştir. Fenil içeren 4a bileşği en yüksek sitotoksik aktivite göstererek öncü bileşik olarak kabul edilmiştir. Ayrıca 4-metoksi fenil içeren 4c bileşği de sitotoksik aktivite göstermiştir. Sonuç olarak, fenil ve 4-metoksi kısımlarının bu bileşiklerde sitotoksik aktivite açısından önemli olduğu belirlenmiştir.

**Anahtar kelimeler:** 1,3,4-Oksadiazol; Pirimidin; Sitotoksik aktivite.

Another significant ring in medicinal chemistry is pyrimidine. Pyrimidine moiety has proven to display anti HIV [27], antimicrobial [28], anti-inflammatory [29], antinociceptive [30], antioxidant [31] and antitumor activity [32, 33]. Moreover there are some drugs bearing pyrimidine moiety such as methotrexate, 5-fluorouracil (5-FU), tegafur and thioguanine. Also some 2,4,6-trisubstituted 5-cyano-pyrimidine derivatives have shown immunosuppressive activity [34]. In another study, pyrimidine–benzimidazole hybrids have found to show cytotoxic activity against MCF-7, MGC-803, EC-9706 and SMMC-7721 human cancer cell lines [35]. Further some thiazole-pyrimidine derivatives exhibited important cytotoxic activity [36].

In the light of this information, we synthesized seven compounds (4a–g) possessing 1,3,4-oxadiazole and pyrimidine moieties and tested their antiproliferative activity against A549 lung cancer cells. The antiproliferative activity of the new compounds was evaluated by MTT method. The cytotoxic potency of compounds 4a–g was studied in comparison with the known anticancer drug cisplatin.

**Materials and methods**

**Chemicals**

All chemicals were purchased from Sigma-Aldrich Chemical Co (Sigma-Aldrich Corp., St. Louis, MO, USA) and Merck
Elemental analyses were performed on a Leco TruSpec Micro LC/MS/MS system (AB Applied Biosystems Co., MA, USA). Spectroscopic data were recorded with the following instruments: IR, Shimadzu 8400S spectrophotometer (Shimadzu, Tokyo, Japan); NMR, Bruker DPX 500 NMR spectrometer (Bruker Bioscience, Billerica, MA, USA), in DMSO-$_d_6$, using TMS as internal standard; M+1 peaks were determined by AB Sciex-3200 Q-TRAP LC/MS/MS system (AB Applied Biosystems Co., MA, USA). Elemental analyses were performed on a Leco TruSpec Micro CHN/CHNS elemental analyzer (Leco, MI, USA).

**Synthesis of the compounds**

**Ethyl 4-[(pyrimidin-2-yl)thio]butanoate (1)** Pyrimidine-2-thiol (0.04 mol) was dissolved in acetonitrile (250 mL). Potassium carbonate (0.04 mol) was added. After addition of 4-chlorobutanoate, the reaction mixture was refluxed for 5 h. After completion of the reaction, the solvent was evaporated under reduced pressure and then water was added to wash the resulting solid and the mixture was filtered to give compound 1.

**4-[(Pyrimidin-2-yl)thio]butano hydrazide (2)** Ethyl 4-[(pyrimidin-2-yl)thio]butanoate (0.03 mol) was dissolved in ethanol (250 mL). Hydrazine hydrate (0.03 mol) dissolved in ethanol, was added gradually and the mixture stirred at room temperature. After completion of the reaction, the solvent was evaporated under reduced pressure then water was added to wash the resulting solid and the mixture was filtered, dried and recrystallized from ethanol to give compound 2.

**5-[3-((Pyrimidin-2-yl)thio)propyl]-1,3,4-oxadiazole-2-thiol (3)** 4-[(Pyrimidin-2-yl)thio]butano hydrazide (0.02 mol) was dissolved in ethanol (250 mL). Potassium hydroxide (0.02 mol) was dissolved in ethanol (80 mL) with constant stirring. The second solution was added to first one. After carbon disulfide (0.02 mol) was added to this, the mixture was refluxed for 5 h. After completion of the reaction, dilute HCl was added to remove the salt form of the compound. Then water was added and the mixture was filtered, dried and recrystallized from ethanol to give compound 3.

**General procedure for the synthesis of 1-(4-substitutedphenyl)-2-[5-(3-((pyrimidin-2-yl)thio)propyl)-1,3,4-oxadiazol-2-yl]thio]ethan-1-one derivatives (4a–g)** 5-[3-(Pyrimidin-2-yl)thio]propyl]-1,3,4-oxadiazole-2-thiol (10 mmol) was dissolved in acetonitrile (100 mL). Potassium carbonate (10 mmol) was added. After that, appropriate phenacyl bromide derivatives were added and stirred for 12 h at room temperature. After TLC screening, the solvent was evaporated under reduced pressure then water was added to wash the resulting solid and the mixture was filtered, dried and recrystallized from ethanol to give the final compounds 4a–g.

1-Phenyl-2-[5-(3-((pyrimidin-2-yl)thio)propyl)-1,3,4-oxadiazol-2-yl]thio]ethan-1-one (4a) Yield 74%–76%, m.p. 53–56°C. IR $\nu_{\text{max}}$ (cm$^{-1}$): 3107.32–3062.96 (Aromatic C-H), 2949.16–2914.44 (Aliphatic C-H), 1672.28 (C=O ketone), 1564.27–1448.54 (C=C, C=N), 1197.18–1165.00 (C-O). $^1$H-NMR (500 MHz, DMSO$_d_6$, ppm) $\delta$ 2.05–2.11 (m, 2H, C-CH$_2$-C), 2.98 (t, 2H, J: 7.36 Hz, CH$_2$-Ar), 3.19 (t, 2H, J: 7.16 Hz, S-CH$_2$), 5.08 (s, 2H, CO-CH$_2$), 7.23 (t, H, J: 4.89 Hz, Ar-H), 7.59 (t, 2H, J: 7.73 Hz, Ar-H), 7.72 (t, H, J: 7.39 Hz, Ar-H), 8.05 (d, 2H, J: 7.41 Hz, Ar-H), 8.63 (d, 2H, J: 4.82 Hz, Ar-H). Anal. For C$_{18}$H$_{18}$N$_4$O$_2$S$_2$ calculated: C, 55.94; H, 4.69; N, 14.50; O, 8.57; S, 17.25; MS [M$^+$]$: m/z$ 373.

1-(4-Methylphenyl)-2-[5-(3-((pyrimidin-2-yl)thio)propyl)-1,3,4-oxadiazol-2-yl]thio]ethan-1-one (4b) Yield 65%–67%, m.p. 127–130°C. IR $\nu_{\text{max}}$ (cm$^{-1}$): 3061.03–3034.03 (Aromatic C-H), 2929.87–2897.08 (Aliphatic C-H), 1685.79 (C=O ketone), 1568.13–1489.05 (C=C, C=N), 1192.01–1143.79 (C-O). $^1$H-NMR (500 MHz, DMSO$_d_6$, ppm) $\delta$ 2.05–2.11 (m, 2H, C-CH$_2$-C), 2.41 (s, 3H, Ar-CH$_3$), 2.98 (t, 2H, J: 7.37 Hz, CH$_2$CH$_2$-Ar), 3.19 (t, 2H, J: 7.17 Hz, S-CH$_2$), 5.04 (s, 2H, CO-CH$_2$), 7.23 (t, 1H, J: 4.86 Hz, Ar-H), 7.39 (d, 2H, J: 8.18 Hz, Ar-H), 7.94 (d, 2H, J: 8.16 Hz, Ar-H), 8.63 (d, 2H, J: 4.77 Hz, Ar-H). Anal. For C$_{18}$H$_{18}$N$_4$O$_2$S$_2$ calculated: C, 55.94; H, 4.69; N, 15.04; O, 8.59; S, 17.22; found: C, 54.90; H, 4.33; N, 15.08; O, 8.57; S, 17.25; MS [M$^+$]$: m/z$ 387.

1-(4-Methoxyphenyl)-2-[5-(3-((pyrimidin-2-yl)thio)propyl)-1,3,4-oxadiazol-2-yl]thio]ethan-1-one (4c) Yield 73%–75%, m.p. 68–74°C. IR $\nu_{\text{max}}$ (cm$^{-1}$): 3115.04–3074.53 (Aromatic C-H), 2968.45–2837.29 (Aliphatic C-H), 1672.28 (C=O ketone), 1598.99–1483.26 (C=C, C=N), 1257.59–1159.22 (C-O). $^1$H-NMR (500 MHz, DMSO$_d_6$, ppm) $\delta$ 2.05–2.11 (m, 2H, C-CH$_2$-C), 2.98 (t, J: 7.37 Hz, 2H, CH$_2$CH$_2$-Ar), 3.19 (t, 2H, J: 7.17 Hz, S-CH$_2$), 3.88 (s, 3H, O-CH$_3$), 5.02 (s, 2H, CO-CH$_2$), 7.10 (d, 2H, J: 8.87 Hz, Ar-H), 7.23 (t, 1H, J: 4.85 Hz, Ar-H) 8.02 (d, 2H, J: 8.86 Hz, Ar-H), 8.63 (d, 2H, J: 4.84 Hz, Ar-H). Anal. For C$_{18}$H$_{18}$N$_4$O$_2$S$_2$ calculated: C, 53.71; H, 4.51; N, 13.92; O, 11.93; S, 15.93; found: C, 53.76; H, 4.49; N, 13.95; O, 11.90; S, 15.96; MS [M$^+$]$: m/z$ 403.
1-(4-Chlorophenyl)-2-[(5-(3-((pyrimidin-2-yl)thio)propyl)-1,3,4-oxadiazol-2-yl)thio]ethan-1-one (4d)
Yield 68%–70%, m.p. 96–97°C. IR \(\nu\) max (cm\(^{-1}\)): 3057.37–3039.81 (Aromatic C-H), 2933.73–2899.01 (Aliphatic C-H), 1681.93 (C=O ketone), 1585.49–1485.19 (C=C, C=N), 1193.94–1085.92 (C-O). \(^1\)H-NMR (500 MHz, DMSO-\(d_6\), ppm) \(\delta\) 2.05–2.11 (m, 2H, C-CH\(_2\)-C), 2.98 (t, 2H, \(J=7.37\) Hz, CH\(_2\)-CH\(_3\)-Ar), 3.19 (t, 2H, J=7.16 Hz, S-CH\(_2\)), 5.14 (s, 2H, CO-CH\(_2\)), 7.23 (t, 1H, J=4.85 Hz, Ar-H), 7.66 (d, 2H, J=8.50 Hz, Ar-H), 8.06 (d, 2H, J=8.52 Hz, Ar-H), 8.63 (d, 2H, J=4.85 Hz, Ar-H). Anal. For C\(_{17}\)H\(_{15}\)ClN\(_4\)O\(_2\)S\(_2\) calculated: C, 50.18; H, 3.72; Cl, 8.71; N, 13.79; O, 7.85; S, 15.79; MS \([M+1]^+\): m/z 407.

1-(4-Chlorophenyl)-2-[(5-(3-((pyrimidin-2-yl)thio)propyl)-1,3,4-oxadiazol-2-yl)thio]ethan-1-one (4e)
Yield 71%–73%, m.p. 86–89°C. IR \(\nu\) max (cm\(^{-1}\)): 3061.03–3020.53 (Aromatic C-H), 2933.73–2900.94 (Aliphatic C-H), 1683.86 (C=O ketone), 1579.06–1485.19 (C=C, C=N), 1195.87–1149.57 (C-O). \(^1\)H-NMR (500 MHz, DMSO-\(d_6\), ppm) \(\delta\) 2.05–2.11 (m, 2H, C-CH\(_2\)-C), 2.98 (t, 2H, J=7.37 Hz, CH\(_2\)-CH\(_3\)-Ar), 3.19 (t, 2H, J=7.16 Hz, S-CH\(_2\)), 5.07 (s, 2H, CO-CH\(_2\)), 7.23 (t, 1H, J=4.84 Hz, Ar-H), 7.66 (d, 2H, J=8.50 Hz, Ar-H), 8.06 (d, 2H, J=8.52 Hz, Ar-H), 8.63 (d, 2H, J=4.85 Hz, Ar-H). Anal. For C\(_{17}\)H\(_{15}\)ClN\(_4\)O\(_2\)S\(_2\) calculated: C, 52.29; H, 3.87; F, 4.87; N, 14.35; O, 8.20; S, 16.45; MS \([M+1]^+\): m/z 391.

MTT assay
In order to examine cytotoxicity of the compounds against A549 (human lung adenocarcinoma cells) cell line, MTT assay [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide] was applied in accordance with the reported data [37, 38]. A549 Human lung adenocarcinoma cells were incubated in 90% RPMI supplemented with 10% fetal bovine serum (Gibco, Paisley, Scotland) and cells were incubated at 37°C in a humidified atmosphere of 95% air and 5% CO\(_2\). Then, growing cells were plated at 2 \times 10\(^4\) cells/mL into 96-well microtiter tissue culture plates (Nunc, Denmark) and incubated for 24 h. After this time, the synthesized compounds and cisplatin (reference drug) were dissolved in DMSO severally and added to give final concentration in the range 3.9–500 \(\mu\)g/mL and the cells were incubated for 24 h. At the end of this period, MTT was added to a final concentration of 0.5 mg/mL and the cells were incubated for 4 h at 37°C. Purple formazan crystals formed by MTT metabolism arose at the end of the process and crystals were dissolved in 200 mL DMSO. The absorbance was read at 540 nm with a microtiter plate spectrophotometer (Bio-Tek plate reader). The percentage of viable cells was calculated on the grounds that the medium control. IC\(_{50}\) values were defined as the drug concentrations that diminished absorbance to 50% of control values.

Results and discussion
Target molecules (4a–g) were synthesized in four steps as shown in Figure 1. In the first step, ethyl 4-[(pyrimidin-2-yl)thio]butanoate (1) was synthesized via bimolecular nucleophilic substitution (SN2) reaction. Pyrimidine-2-thiol and ethyl 4-chlorobutanoate was refluxed in acetone with potassium carbonate to obtain an irritant intermediate product (2). In the second step, 4-[(pyrimidin-2-yl)thio]butanoic acid was obtained from ethyl 4-[(pyrimidin-2-yl)thio]butanoate with excess of hydrazine hydrate in ethanol. 5-[(3-(Pyrimidin-2-yl)thio)propyl]-1,3,4-oxadiazole-2-thiol (3) was synthesized by the ring closure reaction of 4-[(pyrimidin-2-yl)thio]butanoic acid with carbon disulfide in ethanolic KOH. Finally, 5-[(3-(pyrimidin-2-yl)thio)propyl]-1,3,4-oxadiazole-2-thiol was reacted with...
appropriate α-bromoacetophenone derivatives via bimolecular nucleophilic substitution (SN₂) reaction to give the intended 1-(4-substitutedphenyl)-2-[(5-(3-(pyrimidin-2-ylthio)propyl)-1,3,4-oxadiazol-2-yl)thio]ethan-1-one derivatives (4a–g).

1-Phenyl-2-[(5-(3-(pyrimidin-2-yl)thio)propyl)-1,3,4-oxadiazol-2-yl)thio]ethan-1-one

Structures of the obtained compounds were elucidated by spectral data. In the IR spectra, significant stretching bands belonging to C=O were observed in between 1687 and 1672 cm⁻¹, bands belonging to C=N and C=C were observed in between 1602 and 1448 cm⁻¹ and bands belonging to C-O were observed at about 1257 –1085 cm⁻¹. In the NMR spectra of the final compounds, a singlet peak at about 5.07 ppm was observed belonging to -CH₂ of the oxoethyl moiety. A triplet peak about 3.19 ppm was observed belonging to the -SCH₂ structure. Hydrogen atoms close to the nitrogen atom were observed at 8.63 ppm. The other hydrogen atom in pyrimidine was observed at 7.23 ppm in the NMR spectra. The other peaks belonging to aromatic and aliphatic protons were observed at the estimated areas.

Cytotoxic activities of the synthesized compounds (4a–g) were compared against positive controls by using A549 (human non-small cell lung cancer). Tested concentrations for compounds were in between 3.9 and 500 mg/mL and for control (cisplatin) were in between 0.98 and 500 mg/mL. The corresponding IC₅₀ values are listed in Table 1.

Table 1: IC₅₀ values (mg/mL) for compounds 4a–g in A549 cancer cell lines.

| Compounds | A549         |
|-----------|--------------|
| 4a        | 68.33 ± 7.6  |
| 4b        | 116.67 ± 29.3|
| 4c        | 95 ± 8.7     |
| 4d        | 136.67 ± 28.9|
| 4e        | 106.67 ± 5.8 |
| 4f        | 143.33 ± 66.6|
| 4g        | > 500        |
| Cisplatin | 13.67 ± 2.9  |

*Cytotoxicity of the compounds. Incubation for 2A h. IC₅₀ is the drug concentration required to inhibit 50% of the cell growth. The values represent mean ± SD of triplicate determinations.

Conclusions

The synthesis and cytotoxic activity of seven 1-(4-substitutedphenyl)-2-[(5-(3-(pyrimidin-2-ylthio) propyl)-1,3,4-oxadiazol-2-yl)thio]ethan-1-one derivatives...
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