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Original article

Synthesis of Some Hydrazone Derivatives Bearing Purine Moiety as Anticancer Agents

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In the present study, some hydrazone derivatives were synthesized via the reaction of 6-hydrazino-9-(β-D-ribofuranosyl)-9H-purine with various benzaldehydes. The structures of the compounds were elucidated by spectroscopic techniques such as ^{1}H-NMR, ^{13}C-NMR and ES-MS and elemental analyses. Anti-proliferative activity tests were performed on human lung (A549) and breast (MCF-7) cancer cell lines using MTT assay. Some derivatives were found to be effective against A549 and MCF-7 cell lines among these compounds. They exhibited time and dose dependent anti-proliferative activity against A549 and MCF-7 cancer cell lines.

Key words: Hydrazone, Purine, Anti-proliferative activity, Cancer

Antikanser Ajanlar olarak Pürin Parçası Taşıyan Bazı Hidrazon Türevlerinin Sentezi

Bu çalışmada, 6-hidrazino-9-(β-D-ribofuranosil)-9H-pürinin farklı benzaldehitler ile reaksiyonu sonucu bazı hidrazon türevleri sentezlenmiştir. Bileşiklerin yapıları ^{1}H-NMR, ^{13}C-NMR ve ES-MS gibi spektroskopik teknikler ve elemental analiz ile aydınlatılmıştır. MTT deneyi kullanılarak insan akciğer (A549) ve meme (MCF-7) kanser hücreleri üzerinde anti-proliferatif etki testleri gerçekleştirilmiştir. Bu bileşikler arasında, bazı türevlerin A549 ve MCF-7 hücrelerine karşı etkili olduğu bulunmuştur. Bu bileşikler A549 ve MCF-7 kanser hücrelerine karşı zamana ve doza bağımlı anti-proliferatif etki göstermişlerdir.

Anahtar kelimeler: Hidrazon, Pürin, Anti-proliferatif etki, Kanser

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INTRODUCTION

Cancer, which is characterized by abnormal and uncontrolled cell division, is the second leading cause of death in most countries after cardiovascular disease. Although there are many drugs currently available for cancer, the treatment of cancer is still a major problem due to the resistance and adverse effects accompanying the long-term use of these drugs (1-6).

In recent years, the search for new effective anticancer agents has gained great importance. One important approach to anticancer agents is the design of a compound whose structure is related to those of pyrimidines and purines involved in the biosynthesis of DNA. These analogues interfere with the formation or utilization of one of these essential nucleobases (1-11).

Purine nucleoside analogues (PNAs) remain an important class of drugs used in the treatment of cancer. Fludarabine and cladribine, which have chemical structures similar to adenosine or deoxyadenosine, are antineoplastic drugs currently used in the treatment of hematological malignancies belonging to the group of PNAs (1-11).

In a previous study, N^6-amino-adenosine (6-hydrazino-9-(β-D-ribofuranosyl)-9*H*-purine) was found to be a potent antitumor agent against five cancer cell lines, namely human myelogenous leukemia (K562), cervical carcinoma (HeLa), colon carcinoma (HT-29), colon adenocarcinoma (Caco-2), and breast carcinoma (MCF-7) cell lines with GI_{50} and GI_{100} values in the low micromolar or submicromolar range. In the same study, its mechanism of action was also investigated and the study demonstrated that its antitumor activity is related in part to ribonucleotide reductase inhibition (7).

Medicinal chemists have also carried out considerable research for novel antitumor agents bearing a hydrazone moiety. Many studies have confirmed that hydrazone derivatives possess antitumor activity (12-14).

On the basis of these findings, we became interested in biological evaluation of purine analogues as antitumor agents. Herein, we described the synthesis of some hydrazone derivatives of N^6-amino-adenosine and focused on their potential anti-proliferative effects against human lung (A549), and breast (MCF-7) cancer cell lines.

EXPERIMENTAL

Chemistry

All reagents were purchased from commercial suppliers and were used without further purification. Melting points (m.p.) were determined on a Electrothermal 9100 melting point apparatus (Weiss-Gallenkamp, Loughborough, UK) and are uncorrected. Proton nuclear magnetic resonance (^{1}H-NMR) spectra were recorded on a Bruker 400 MHz spectrometer (Bruker, Billerica, MA, USA). Carbon nuclear magnetic resonance (^{13}C-NMR) spectra were recorded on a Bruker 100 MHz spectrometer (Bruker, Billerica, MA, USA). Chemical shifts were expressed in parts per million (ppm) and tetramethylsilane was used as an internal standard. Mass spectra were recorded on a VG Quattro Mass spectrometer (Agilent, Minnesota, USA). Elemental analyses were performed on a Perkin Elmer EAL 240 elemental analyser (Perkin-Elmer, Norwalk, CT, USA).

General procedure for the synthesis of the compounds

6-Hydrazino-9-(β-D-ribofuranosyl)-9H-purine

A mixture of 6-chloro-9-(β-D-ribofuranosyl)-9*H*-purine (0.02 mol) and hydrazine (2 mL) in ethanol was stirred at room temperature for 3 hours and filtered. The residue was crystallized from ethanol (9).

6-(N^2-(4-substituted benzylidene)hydrazino)-9-(β-D-ribofuranosyl)-9H-purines **(1a-j)**

A mixture of 6-hydrazino-9-(β-D-ribofuranosyl)-9*H*-purine (0.001 mol) and appropriate benzaldehyde (0.001 mol) in ethanol (10 mL) was refluxed for 3 hours and filtered. The residue was crystallized from ethanol (15-18).

6-(N^2-(4-nitrobenzylidene)hydrazino)-9-(β-D-ribofuranosyl)-9H-purine **(1a)**

^{1}H-NMR (400 MHz, DMSO-d_6): 3.59-3.63 (2H, m), 3.67-3.73 (1H, m), 3.97-4.01 (1H, m), 4.17-4.21 (1H, m), 4.60-4.64 (1H, m), 5.24-5.30 (1H, m), 5.52-5.54 (1H, m), 5.98-6.01

(1H, m), 7.99-8.03 (2H, d, *J*= 8.82 Hz), 8.29-8.33 (2H, d, *J*= 8.92 Hz), 8.44-8.47 (2H, d, *J*= 7.68 Hz), 8.62 (1H, s), 12.19 (1H, s).
^{13}C-NMR (100 MHz, DMSO-d_6): 61.40 (CH_2), 70.42 (CH), 73.65 (CH), 85.72 (CH), 87.69 (CH), 119.21 (C), 124.07 (2CH), 127.37 (2CH), 141.29 (C, CH), 141.63 (C, CH), 147.22 (C), 152.13 (C, CH).
For $C_{17}H_{17}N_7O_6$, calculated: C, 49.16; H, 4.13; N, 23.61; found: C, 49.15; H, 4.14; N, 23.61.
MS (ES): $[M+1]^+$: m/z 416

6-(N^2-(4-methylbenzylidene)hydrazino)-9-(β-D-ribofuranosyl)-9H-purine (**1b**) (15)

^{1}H-NMR (400 MHz, DMSO-d_6): 2.34 (3H, s), 3.56-3.62 (2H, m), 3.69-3.74 (1H, m), 3.98-4.01 (1H, m), 4.17-4.20 (1H, m), 4.63 (1H, m), 5.25 (1H, m), 5.53 (1H, m), 5.97-5.99 (1H, m), 7.26-7.33 (2H, d, *J*= 8.03 Hz), 7.65-7.68 (2H, d, *J*= 8.10 Hz), 8.35-8.40 (2H, d, *J*= 8.80 Hz), 8.57 (1H, s), 11.80-12.00 (1H, br).
^{13}C-NMR (100 MHz, DMSO-d_6): 20.99 (CH_3), 61.46 (CH_2), 70.48 (CH), 73.64 (CH), 85.77 (CH), 87.72 (CH), 118.80 (C), 126.76 (2CH), 129.33 (2CH), 129.47 (C), 132.02 (C), 139.18 (C, CH), 141.06 (C, CH), 151.68 (CH).
For $C_{18}H_{20}N_6O_4$, calculated: C, 56.24; H, 5.24; N, 21.86; found: C, 56.25; H, 5.24; N, 21.85.
MS (ES): $[M+1]^+$: m/z 385.

6-(N^2-(4-cyanobenzylidene)hydrazino)-9-(β-D-ribofuranosyl)-9H-purine (**1c**)

^{1}H-NMR (400 MHz, DMSO-d_6): 3.59-3.63 (2H, m), 3.68-3.71 (1H, m), 3.98-4.00 (1H, m), 4.17-4.21 (1H, m), 4.60-4.65 (1H, m), 5.24-5.31 (1H, m), 5.52-5.54 (1H, d, *J*= 6.08 Hz), 5.98-6.00 (1H, d, *J*= 5.83 Hz), 7.89-7.96 (2H, m), 8.37 (2H, s), 8.45 (2H, s), 8.61 (1H, s), 12.10 (1H, s).
^{13}C-NMR (100 MHz, DMSO-d_6): 59.47 (CH_2), 68.48 (CH), 71.72 (CH), 83.76 (CH), 85.75 (CH), 109.01 (C), 116.87 (C), 117.24 (C), 125.15 (2CH), 130.71 (2CH), 137.46 (C), 139.61 (CH), 140.08 (CH), 148.94 (C), 149.74 (C), 150.14 (CH).
For $C_{18}H_{17}N_7O_4$, calculated: C, 54.68; H, 4.33; N, 24.80; found: C, 54.66; H, 4.35; N, 24.79.
MS (ES): $[M+1]^+$: m/z 396.

6-(N^2-(4-hydroxybenzylidene)hydrazino)-9-(β-D-ribofuranosyl)-9H-purine (**1d**) (15)

^{1}H-NMR (400 MHz, DMSO-d_6): 3.57-3.60 (2H, m), 3.68-3.73 (1H, m), 3.98-4.01 (1H, m), 4.18 (1H, m), 4.62-4.64 (1H, m), 5.24-5.25 (1H, m), 5.52-5.54 (1H, m), 5.92-5.98 (1H, m), 6.84-6.86 (2H, d, *J*= 8.62 Hz), 7.58-7.61 (2H, d, *J*= 8.63 Hz), 8.33-8.46 (2H, m), 8.53 (1H, s), 9.89 (1H, s), 11.00-11.50 (1H, br).
^{13}C-NMR (100 MHz, DMSO-d_6): 60.59 (CH_2), 69.60 (CH), 72.68 (CH), 84.87 (CH), 86.84 (CH), 114.70 (2CH), 117.81 (C), 124.87 (C), 127.55 (2CH), 139.88 (C, 2CH), 150.49 (C), 150.96 (CH), 157.99 (C).
For $C_{17}H_{18}N_6O_5$, calculated: C, 52.85; H, 4.70; N, 21.75; found: C, 52.86; H, 4.72; N, 21.74.
MS (ES): $[M+1]^+$: m/z 387.

6-(N^2-(4-isopropylbenzylidene)hydrazino)-9-(β-D-ribofuranosyl)-9H-purine (**1e**) (16)

^{1}H-NMR (400 MHz, DMSO-d_6): 1.19-1.21 (6H, m), 2.88-2.94 (1H, m), 3.57-3.61 (2H, m), 3.69-3.73 (1H, m), 3.98-4.01 (1H, m), 4.17-4.21 (1H, m), 4.61-4.66 (1H, m), 5.24-5.25 (1H, m), 5.51-5.53 (1H, m), 5.97-5.99 (1H, d, *J*= 5.97 Hz), 7.32-7.34 (2H, d, *J*= 8.25 Hz), 7.67-7.70 (2H, d, *J*= 8.22 Hz), 8.35-8.40 (2H, d, *J*= 8.25 Hz), 8.55 (1H, s), 11.76 (1H, br).
^{13}C-NMR (100 MHz, DMSO-d_6): 23.67 ($2CH_3$), 33.32 (CH), 61.49 (CH_2), 70.49 (CH), 73.60 (CH), 85.76 (CH), 87.72 (CH), 118.88 (C), 126.67 (2CH), 126.79 (2CH), 132.51 (C), 140.96 (2CH), 144.75 (C), 149.91 (2C), 152.08 (CH).
For $C_{20}H_{24}N_6O_4$, calculated: C, 58.24; H, 5.87; N, 20.38; found: C, 58.25; H, 5.85; N, 20.40.
MS (ES): $[M+1]^+$: m/z 413.

6-(N^2-(4-dimethylaminobenzylidene)hydrazino)-9-(β-D-ribofuranosyl)-9H-purine (**1f**) (16)

^{1}H-NMR (400 MHz, DMSO-d_6): 2.93-3.03 (6H, m), 3.60-3.62 (2H, m), 3.69-3.73 (1H, m), 3.99-4.01 (1H, m), 4.17-4.21 (1H, m), 4.62-4.65 (1H, m), 5.22-5.23 (1H, m), 5.50-5.52 (1H, m), 5.96-5.98 (1H, d, *J*= 6.02 Hz), 6.77-6.79 (2H, d, *J*= 8.97 Hz), 7.57-7.59 (2H, d, *J*= 8.85 Hz), 8.35 (2H, s), 8.50-8.51 (1H, s), 11.50 (1H, br).
^{13}C-NMR (100 MHz, DMSO-d_6): 41.30 ($2CH_3$), 62.06 (CH_2), 71.06 (CH), 74.07 (CH), 86.32 (CH), 88.30 (CH), 112.16 (2CH), 119.21 (C), 122.76 (C), 128.57 (2CH), 141.09 (C, CH), 150.58 (C), 151.63 (CH), 152.60 (CH), 160.30 (C).
For $C_{19}H_{23}N_7O_4$, calculated: C, 55.20; H, 5.61;

N, 23.72; found: C, 55.21; H, 5.60; N, 23.72.
MS (ES): $[M+1]^+$: m/z 414.

6-(N^2-(4-chlorobenzylidene)hydrazino)-9-(β-D-ribofuranosyl)-9H-purine (**1g**) (15)

^{1}H-NMR (400 MHz, DMSO-d_6): 3.58-3.63 (2H, m), 3.69-3.73 (1H, m), 3.99-4.02 (1H, m), 4.18-4.21 (1H, m), 4.61-4.65 (1H, m), 5.23-5.24 (1H, m), 5.51-5.53 (1H, m), 5.98-6.00 (1H, d, *J*= 5.87 Hz), 7.52-7.55 (2H, m), 7.78-7.80 (2H, d, *J*= 8.49 Hz), 8.35 (1H, s), 8.42 (1H, s), 8.58 (1H, s), 11.88 (1H, s).

^{13}C-NMR (100 MHz, DMSO-d_6): 61.97 (CH_2), 70.98 (CH), 74.14 (CH), 86.25 (CH), 88.24 (CH), 119.48 (C), 128.75 (2CH), 129.33 (2CH), 134.18 (C), 134.31 (C), 141.75 (CH), 143.49 (CH), 151.14 (C), 152.18 (C), 152.68 (CH).

For $C_{17}H_{17}ClN_6O_4$, calculated: C, 50.44; H, 4.23; N, 20.76; found: C, 50.45; H, 4.24; N, 20.75.

MS (ES): $[M+1]^+$: m/z 405, $[M+3]^+$: m/z 407, $[M+4]^+$: m/z 408.

6-(N^2-(4-bromobenzylidene)hydrazino)-9-(β-D-ribofuranosyl)-9H-purine (**1h**)

^{1}H-NMR (400 MHz, DMSO-d_6): 3.60-3.63 (2H, m), 3.69-3.73 (1H, m), 3.99-4.01 (1H, m), 4.18-4.20 (1H, m), 4.63-4.64 (1H, m), 5.23-5.24 (1H, m), 5.51-5.52 (1H, m), 5.98-6.00 (1H, m), 7.65-7.68 (2H, m), 7.72-7.74 (2H, m), 8.34 (1H, s), 8.42 (1H, s), 8.58 (1H, s), 11.89 (1H, s).

^{13}C-NMR (100 MHz, DMSO-d_6): 61.97 (CH_2), 70.98 (CH), 74.14 (CH), 86.25 (CH), 88.24 (CH), 119.49 (C), 122.94 (C), 129.00 (2CH), 132.24 (2CH), 134.66 (C), 141.76 (CH), 143.57 (CH), 151.11 (C), 152.18 (C), 152.66 (CH).

For $C_{17}H_{17}BrN_6O_4$, calculated: C, 45.45; H, 3.81; N, 18.71; found: C, 45.45; H, 3.80; N, 18.70.

MS (ES): $[M+1]^+$: m/z 451, $[M+2]^+$: m/z 452, $[M+3]^+$: m/z 453.

6-(N^2-(4-fluorobenzylidene)hydrazino)-9-(β-D-ribofuranosyl)-9H-purine (**1i**)

^{1}H-NMR (400 MHz, DMSO-d_6): 3.59-3.61 (2H, m), 3.66-3.74 (1H, m), 3.99-4.02 (1H, m), 4.20 (1H, m), 4.63-4.64 (1H, m), 5.24-5.31 (1H, m), 5.51-5.53 (1H, m), 5.95-6.00 (1H, m), 7.26-7.36 (2H, m), 7.82-7.84 (2H, m), 8.37-8.45 (2H, m), 8.52-8.62 (1H, m), 11.84 (1H, br).

^{13}C-NMR (100 MHz, DMSO-d_6): 61.98 (CH_2), 70.99 (CH), 74.15 (CH), 86.26 (CH), 88.24 (CH), 116.17 (CH), 116.39 (CH), 119.46 (C), 129.31 (C), 131.96 (CH), 131.99 (CH), 141.60 (CH), 143.96 (C, CH), 150.93 (C), 152.42 (CH), 164.44 (C).

For $C_{17}H_{17}FN_6O_4$, calculated: C, 52.58; H, 4.41; N, 21.64; found: C, 52.55; H, 4.40; N, 21.64.

MS (ES): $[M+1]^+$: m/z 389, $[M+2]^+$: m/z 390, $[M+3]^+$: m/z 391.

6-(N^2-(4-methoxybenzylidene)hydrazino)-9-(β-D-ribofuranosyl)-9H-purine (**1j**)

^{1}H-NMR (400 MHz, DMSO-d_6): 3.58-3.61 (2H, m), 3.70-3.77 (2H, m), 3.82-3.87 (3H, m), 4.01 (1H, m), 4.64 (1H, m), 5.24-5.32 (1H, m), 5.52 (1H, m), 5.97-5.99 (1H, d, *J*= 5.88 Hz), 7.03-7.07 (2H, m), 7.72-7.84 (2H, m), 8.34-8.39 (2H, m), 8.55 (1H, s), 11.75 (1H, br).

^{13}C-NMR (100 MHz, DMSO-d_6): 55.75 (CH_3), 62.00 (CH_2), 71.01 (CH), 74.14 (CH), 86.29 (CH), 88.27 (CH), 114.75 (2CH), 119.29 (C), 127.04 (C), 130.46 (2CH), 141.43 (CH), 145.43 (C, CH), 150.61 (C), 151.82, 152.26 (CH), 162.15 (C).

For $C_{18}H_{20}N_6O_5$, calculated: C, 54.00; H, 5.03; N, 20.99; found: C, 54.00; H, 5.00; N, 21.00.

MS (ES): $[M+1]^+$: m/z 401.

Pharmacology

Cell culture and treatment

A549 human lung and MCF-7 human breast adenocarcinoma cell lines were obtained from ATCC. The cells were grown in RPMI 1640 medium supplemented with 2 mM L-glutamine and 10% fetal bovine serum, 1% penicillin/streptomycin at a temperature of 37°C in a humidified incubator with a 5% CO_2 atmosphere. The synthesized compounds (**1a-j**) and mitoxantrone (antineoplastic agent) were dissolved in dimethyl sulfoxide (DMSO) as a stock solution and the stock solutions were diluted to the required concentrations in cell culture media. The final DMSO concentration was 0.1% in each well.

A total of 70-80% confluent cells (after 24 h) were treated with mitoxantrone and the synthesized compounds (**1a-j**) at concentrations 50, 100, 200, 300 and 400 µM for 24 and 48 h incubations in growth medium.

Cell proliferation assay/cytotoxicity

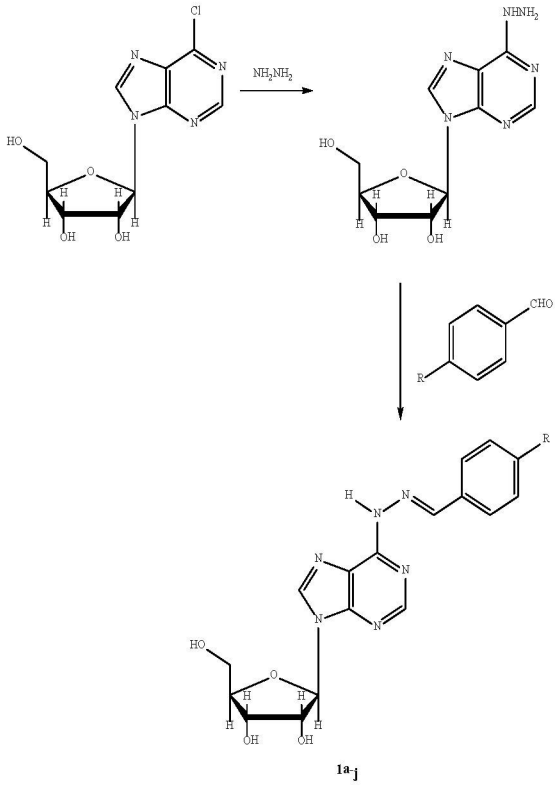
The proliferations of A549 and MCF-7 cells were assessed by MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) assay, which is based on the reduction of MTT by the mitochondrial dehydrogenase of intact cells to a purple formazan product. Yellow MTT is reduced to purple formazan in the mitochondria of living cells. This reduction takes place only when mitochondrial reductase enzymes are active, and therefore conversion can be directly related to the number of viable (living) cells (19).

Cells were inoculated into 96-well culture plates at densities of 3 x 10^3 cells per well. After 24 h, they were treated with different concentrations (50, 100, 200, 300 and 400 µM) of the compounds (**1a-j**) and mitoxantrone for 24 and 48 h. After the incubations, MTT solution (5 mg/mL) was added to each well and incubated for 3 h at 37°C. At the end of the incubations, the purple MTT-formazan crystals were dissolved by adding 100 µL DMSO to each well. The absorbance of the samples was measured with an ELISA (wavelength 570 nm). In the experiment, each concentration was performed in seven wells. The data were mean values from three different experiments. MTT reduction was used to estimate cell proliferation or cytotoxicity at the end of the assay. The data were expressed as a mean ± standard error (SEM). The cytotoxic effects of the synthesized compounds (**1a-j**) on the proliferation of cancer cells were expressed as percent cell proliferation, using the following formula:

Percent cell proliferation = OD of drug treated sample/OD of none treated sample (include 0.1% DMSO alone = solvent control) x100 (20,21).

Statistical analysis

The results were reported as mean ± standard error of mean (SEM). Statistical differences between the experimental groups were determined by one-way analysis of variance (ANOVA) and Tukey's test. In this study, the experimental groups were evaluated by comparing with the control group (solvent group= 0.1% DMSO) Differences were considered significant if $p < 0.05$ ($^*p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$).



Scheme 1. The synthesis of the compounds (1a-j)

Hydrazino-9-(β-D-ribofuranosyl)-9H-purine with various benzaldehydes (15-18). The reaction is presented in Scheme 1 and some properties of the compounds (1a-j) are given in Table 1.

RESULTS AND DISCUSSION

6-Hydrazino-9-(β-D ribofuranosyl)-9*H*-purine was synthesized as described in the literature (9). Some hydrazone derivatives (**1a-j**) were obtained by the reaction of 6-hydrazino-9-(β-D-ribofuranosyl)-9*H*-purine with various benzaldehydes (15-18). The reaction is presented in Scheme 1 and some properties of the compounds (**1a-j**) are given in Table 1.

The structures of all compounds were confirmed by ^{1}H-NMR, ^{13}C-NMR, mass spectral data and elemental analysis. In the ^{1}H-NMR spectra of the compounds (**1a-j**), N-H proton belonging to hydrazone moiety was observed in the region 11-12 ppm. The signal due to N=CH proton appeared at 8.5-8.6 ppm. In the ^{13}C-NMR spectra of the compounds (**1a-j**), the hydrazone carbon

Table 1. Some properties of the compounds (**1a-j**)

Compound	R	Yield (%)	M.p. (°C)	Molecular formula	Molecular weight
1a	NO_2	84	245.4	$C_{17}H_{17}N_7O_6$	415
1b	CH_3	69	163.5	$C_{18}H_{20}N_6O_4$	384
1c	CN	71	246.6	$C_{18}H_{17}N_7O_4$	395
1d	OH	65	214.9	$C_{17}H_{18}N_6O_5$	386
1e	$CH(CH_3)_2$	63	158	$C_{20}H_{24}N_6O_4$	412
1f	$N(CH_3)_2$	72	181.7	$C_{19}H_{23}N_7O_4$	413
1g	Cl	76	166.5	$C_{17}H_{17}ClN_6O_4$	404
1h	Br	80	170.9	$C_{17}H_{17}BrN_6O_4$	449
1i	F	75	205.6	$C_{17}H_{17}FN_6O_4$	388
1j	OCH_3	66	157	$C_{18}H_{20}N_6O_5$	400

was observed in the region 139-145 ppm. The mass spectra of all compounds (**1a-j**) showed M+1 peaks, in agreement with their molecular formula. In the mass spectra of halogen substituted derivatives (**1g-i**), M+2 and M+3 peaks were also observed. All compounds (**1a-j**) gave satisfactory elemental analysis.

The concentration and time-dependent cytotoxic effects of the compounds (**1a-j**) were tested on human lung (A549) and breast (MCF-7) cancer cell lines using MTT assay (Figures 1-4 and Tables 2-5). Mitoxantrone, an anthracenedione antineoplastic agent, was used as a reference drug.

After 24 h incubation, percentages of A549 cell proliferation for mitoxantrone according to concentration increase were determined as 80.58%, 68.84%, 61.84%, 58.22%, and 45.02% ($p<0.01$ and $p<0.001$), respectively. Although there was a decrease in cell proliferation associated with concentration increase in most of the test compounds, it was apparent that the most significant decrease in cell proliferation occured at the mitoxantrone concentrations. The anti-proliferative effects of compounds **1e**, **1f**, **1g** and **1h** for 24 h were noteworthy and greater cytotoxicity was observed when compared with control group at the concentrations of 300 and 400 μM ($p<0.001$ and $p<0.01$). Cell proliferation values for compounds **1e**, **1f**, **1g** and **1h** were observed as 71.24%, 68.36%, 69.81%, 79.51% at the concentration of 300 μM, whereas percentages of cell proliferation were determined as 67.80%, 62.36%, 64.79%, 64.42% at the concentration of 400 μM, respectively (Table 2).

After 48 h incubation, A549 cell proliferation values for mitoxantrone according to concentration increase were determined as 60.49%, 52.28%, 48.88%, 37.30%, and 30.65% ($p<0.001$), respectively. Most of the test compounds caused a decrease in cell proliferation associated with time and concentration (Table 3). Although the test compounds brought about significant decrease in A549 cell proliferation after 48 h incubation when compared with 24 h incubation, it was clear that the most cytotoxic activity occured at the mitoxantrone concentrations.

After 48 h incubation, compounds **1d**, **1e** and **1f** caused higher cytotoxic activity in A549 cells at the concentrations of 200, 300 and 400 μM when compared with the control ($p<0.001$). Percentages of cell proliferation for compounds **1d**, **1e** and **1f** were observed as 62.20%, 55.93%, 54.94% at the concentration of 200 μM, 60.02%, 44.49%, 59.46% at the concentration of 300 μM and 53.00%, 40.61%, 50.26% at the concentration of 400 μM, respectively. The compound bearing bromo group (**1h**) brought about significant decrease in cell proliferation particularly at the concentrations of 300 and 400 μM when compared with the control ($p<0.001$).

After 24 h incubation, percentages of MCF-7 cell proliferation for mitoxantrone according to concentration increase were determined as 79.57%, 68.94%, 63.62%, 52.77%, and 49.57% ($p<0.001$ and $p<0.01$), respectively. Although there was a decrease in cell proliferation associated with concentration increase in most of the test compounds, the most significant decrease in cell proliferation was observed at the mitoxantrone concentration (Table 4).

The effects of compounds **1b**, **1f** and **1g** on MCF-7 cell proliferation for 24 h were

noteworthy and higher cytotoxic activity was determined at the concentrations of 200, 300 and 400 µM when compared with the control (p<0.001).

Percentages of cell proliferation values for compounds **1b** and **1f** were observed as 69.72% and 64.40% at the concentration of 200 µM, 65.20% and 60.99% at the concentration of 300 µM, and 60.05% and 59.13% at the concentration of 400 µM, respectively. Compounds **1g** and **1h** also caused significant decrease in cell proliferation particularly at the concentrations of 300 µM (69.85% and 64.95%, respectively) and 400 µM (56.28% and 67.71%, respectively) when compared with the control (p<0.001).

After 48 h incubation, percentages of MCF-7 cell proliferation for mitoxantrone according to concentration increase were determined as 68.70%, 60.45%, 59.22%, 38.81%, and 27.79%, respectively (Table 5).

In almost all compounds, decrease in MCF-7 cell proliferation was observed due to concentration increase after 48 h incubation when compared with 24 h incubation.

After 48 h incubation, compounds **1b**, **1f** and **1g** caused higher cytotoxic activity against MCF-7 cells at the concentrations of 100, 200, 300 and 400 µM when compared with the control (p<0.001). Especially, the highest cytotoxic activity was determined at the concentration of 400 µM of compounds **1b, 1e**, **1f** and **1g.**

As shown in the Figure 5, MCF-7 cell proliferation values for compounds **1b, 1f** and **1g** were observed as 60.05%, 59.13%

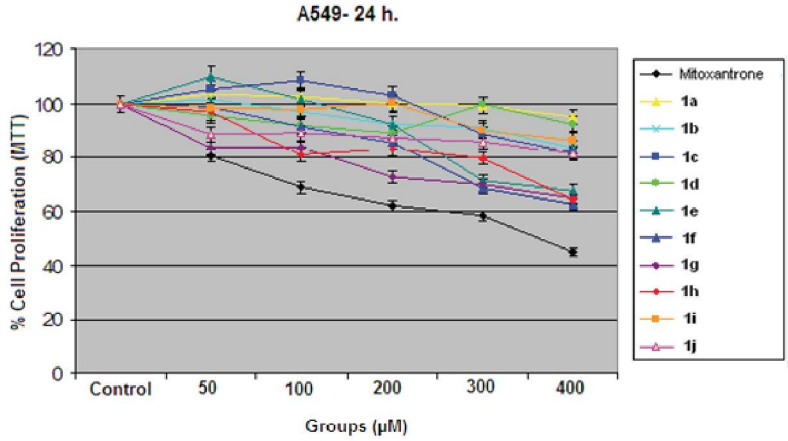


Figure 1. Effects of compounds **1a-j** on A549 cell proliferation after 24 hours of incubation. Cel proliferation was determined by the MTT assay, and was calculated as a ratio (percentage) o numbers of viable cells in the experimental wells to that in the control well (control 100 %) Data points represent means ± SEM for three independent experiments of seven independent wells (n = 7).

Table 2. Statistical analysis of effects of compounds **1a-j** on A549 cell proliferation after 24 hours.

Compounds	Statistically Analysis (A549 /24 h)				
	50 µM	100 µM	200 µM	300 µM	400 µM
Mitoxantrone	p<0.01	p<0.001	p<0.001	p<0.001	p<0.001
1a	ns	ns	ns	ns	ns
1b	ns	ns	ns	ns	ns
1c	ns	ns	ns	ns	p<0.05
1d	ns	ns	ns	ns	ns.
1e	ns	ns	ns	p<0.001	p<0.001
1f	ns	ns	ns	p<0.001	p<0.001
1g	ns	ns	p<0.01	p<0.001	p<0.001
1h	ns	ns	p<0.05	p<0.01	p<0.001
1i	ns	ns.	ns.	n.s	ns.
1j	ns	ns	ns	ns	p<0.05

p< 0.05*, p< 0.01**, p< 0.001***, significantly different from control.
ns.: not statistically significant (p > 0.05).

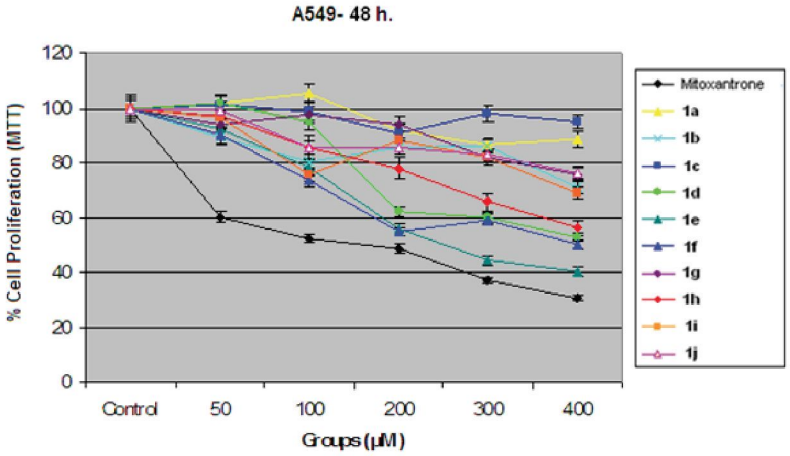


Figure 2. Effects of compounds **1a-j** on A549 cell proliferation after 48 hours of incubation. Cell proliferation was determined by the MTT assay, and was calculated as a ratio (percentage) of numbers of viable cells in the experimental wells to that in the control well (control 100%). Data points represents means ± SEM for three independent experiments of seven independent wells (n = 7).

Table 3. Statistical analysis of effects of compounds **1a-j** on A549 cell proliferation after 48 hours.

Compounds	Statistical Analysis (A549 /48 h)				
	50 µM	100 µM	200 µM	300 µM	400 µM
Mitoxantrone	p<0.001	p<0.001	p<0.001	p<0.001	p<0.001
1a	ns	ns	ns	ns	ns
1b	ns	ns	ns	ns	p<0.01
1c	ns	ns	ns	ns	ns
1d	ns	ns	p<0.001	p<0.001	p<0.001
1e	ns	p<0.01	p<0.001	p<0.001	p<0.001
1f	ns	p<0.01	p<0.001	p<0.001	p<0.001
1g	ns	ns	ns	p<0.05	p<0.01
1h	ns	ns	p<0.05	p<0.001	p<0.001
1i	ns.	p<0.05	ns.	p<0.05	p<0.01
1j	ns.	ns.	ns.	p<0.05	p<0.01

p< 0.05*, p< 0.01**, p< 0.001***, significantly different from control.
ns.: not statistically significant (p > 0.05).

and 62.19% at the concentration of 300 µM, whereas percentages of cell proliferation were determined as 51.01%, 64.40%, and 56.28% at the concentration of 400 µM, respectively. It was found that compound **1e** caused significant decrease (55.91%) in MCF-7 cell proliferation at the concentration of 400 µM when compared with the control (p<0.001).

CONCLUSION

In conclusion, we described the synthesis of some hydrazone derivatives (**1a-j**) bearing purine moiety, which were tested for anti-proliferative activity against A549 and MCF-7 cancer cell lines. These observations clearly indicated that functional groups at the *para* position on the phenyl ring have a considerable influence on anti-proliferative activity. It was determined that compounds **1b, 1e, 1f, 1g** and **1h** showed higher cytotoxic activity when compared with other compounds. It can be attributed to the effects of methyl, isopropyl, dimethylamino, chloro, and bromo substituents on anticancer activity. Among these derivatives, compounds **1f** and **1g** exhibited significant inhibitory activity against A549 and MCF-7 cancer cell lines associated with time and concentration. Compound **1e** caused more

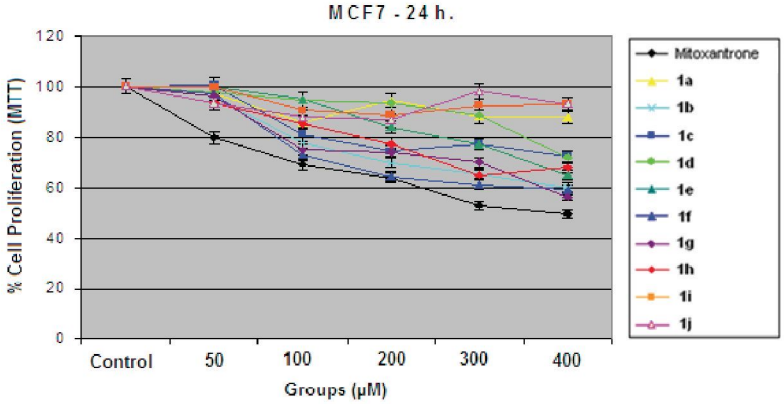


Figure 3. Effects of compounds **1a-j** on MCF-7 cell proliferation after 24 hours of incubation. Cell proliferation was determined by the MTT assay, and was calculated as a ratio (percentage) of numbers of viable cells in the experimental wells to that in the control well (control 100%). Data points represent means± SEM for three independent experiments of seven independent wells (n = 7).

Table 4. Statistical analysis of effects of compounds **1a-j** on MCF-7 cell proliferation after 24 hours.

	Statistical Analysis (MCF-7 / 24 h)				
Compound	**50 µM**	**100 µM**	**200 µM**	**300 µM**	**400 µM**
Mitoxantrone	p<0.01	p<0.001	p<0.001	p<0.001	p<0.001
1a	ns.	p<0.05	ns.	ns.	ns.
1b	ns.	p<0.01	p<0.001	p<0.001	p<0.001
1c	ns.	p<0.01	p<0.001	p<0.01	p<0.001
1d	ns.	ns.	ns.	ns.	p<0.001
1e	ns.	ns.	p<0.05	p<0.01	p<0.001
1f	ns.	p<0.01	p<0.001	p<0.001	p<0.001
1g	ns.	p<0.01	p<0.01	p<0.001	p<0.001
1h	ns.	ns.	p<0.01	p<0.001	p<0.001
1i	ns.	ns.	ns.	ns.	ns.
1j	ns.	ns.	ns.	ns.	ns.

p< 0.05*, p< 0.01**, p< 0.001***, significantly different from control.
ns.: not statistically significant (p > 0.05).

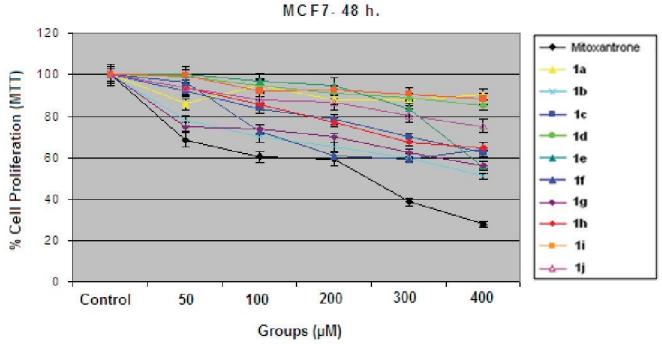


Figure 4. Effects of compounds **1a-j** on MCF-7 cell proliferation after 48 hours of incubation. Cell proliferation was determined by the MTT assay, and was calculated as a ratio (percentage) of numbers of viable cells in the experimental wells to that in the control well (control 100%). Data points represent means ± SEM for three independent experiments of seven independent wells (n = 7).

Table 5. Statistical analysis of effects of compounds **1a-j** on MCF-7 cell proliferation after 48 hours.

	Statistical Analysis (MCF-7 / 48 h)				
Compounds	50 µM	100 µM	200 µM	300 µM	400 µM
Mitoxantrone	p<0.001	p<0.001	p<0.001	p<0.001	p<0.001
1a	ns	ns	ns	ns	ns.
1b	p<0.01	p<0.001	p<0.001	p<0.001	p<0.001
1c	ns	ns	p<0.05	p<0.01	p<0.001
1d	ns	ns	ns	ns	ns
1e	ns	ns	ns	ns	p<0.001
1f	ns	p<0.001	p<0.001	p<0.001	p<0.001
1g	p<0.01	p<0.001	p<0.001	p<0.001	p<0.001
1h	ns	ns	p<0.01	p<0.001	p<0.001
1i	ns	ns	ns	ns	ns
1j	ns	ns	ns	ns	p<0.05

$p< 0.05$*, $p< 0.01$**, $p< 0.001$***, significantly different from control.
ns.: not statistically significant ($p > 0.05$).

significant decrease in A549 cell proliferation for 48 h, in MCF-7 cell proliferation for 24 h.

One of the most important findings, compound **1b** caused significant decrease in MCF-7 cell proliferation at high concentrations. It can be concluded that aliphatic groups (methyl and isopropyl) on phenyl ring have an important impact on antiproliferative activity against A549 and MCF-7 cancer cell lines.

REFERENCES

1. Gupta SP, Quantitative Structure-Activity Relationship Studies on Anticancer Drugs, Chem Rev 94, 1507-1551, 1994.
2. Galmarini CM, Mackey JR, Dumontet C, Nucleoside analogues: mechanisms of drug resistance and reversal strategies, Leukemia 15, 875-890, 2001.
3. Galmarini CM, Mackey JR, Dumontet C, Nucleoside analogues and nucleobases in cancer treatment. Lancet Oncol 3, 415-424, 2002.
4. Silverman RB, The Organic Chemistry of Drug Design and Drug Development, pp. 298-302, Elsevier Academic Press, Burlington, 2004.
5. Robak T, Lech-Maranda E, Korycka A, Robak E, Purine Nucleoside Analogs as Immunosuppressive and Antineoplastic Agents: Mechanism of Action and Clinical Activity, Curr Med Chem 13, 3165-3189, 2006.
6. Singh S, Saxena AK, 2D-QSAR of purine-scaffold novel class of Hsp90 binders that inhibit the proliferation of cancer cells, Med Chem Res 17, 290-296, 2008.
7. Cappellacci L, Petrelli R, Franchetti P, Vita P, Kusumanchi P, Kumar M, Jayaram HN, Zhou, B, Yen Y, Grifantini M, Synthesis and biological activity of novel N^6-substituted and 2,N^6-disubstituted adenine ribo- and 3'-C-methyl-ribonucleosides as antitumor agents, Eur J Med Chem 46, 1499-1504, 2011.
8. Franchetti P, Cappellacci L, Pasqualini M, Petrelli R, Vita P, Jayaram HN, Horvath Z, Szekeres T, Grifantini M, Antitumor Activity of C-Methyl-β-D-ribofuranosyladenine Nucleoside Ribonucleotide Reductase Inhibitors, J Med Chem 48, 4983-4989, 2005.
9. Johnson JA, Thomas HJ, Schaeffer HJ, Synthesis of Potential Anticancer Agents. XIIL Ribosides of 6-Substituted Purines, J Am Chem Soc 80, 699-702, 1958.
10. Parker WB, Enzymology of Purine and Pyrimidine Antimetabolites Used in the Treatment of Cancer. Chem Rev 109, 2880-2893, 2009.
11. Robak T, Emerging drugs for rarer chronic lymphoid leukemias, Expert Opin 13(1), 95-118, 2008.
12. Rollas S, Küçükgüzel SG, Biological Activities of Hydrazone Derivatives, Molecules 12, 1910-1939, 2007.
13. Terzioğlu N, Gürsoy A, Synthesis and anticancer evaluation of some new hydrazone derivatives of 2,6-dimethylimidazo[2,1-b]-[1,3,4] thiadiazole-5-carbohydrazide, Eur J Med Chem 38, 781-786, 2003.
14. Vicini P, Incerti M, Doytchinova I, La Colla P, Busonera B, Loddo R, Synthesis and antiproliferative activity of benzo[d]isothiazole hydrazones, Eur J Med Chem 41, 624-632, 2006.

15. Chen H, Shang Z, Chang J, Novel Synthesis of 7-β-D-Ribofuranosyl-7H-1,2,4-triazolo[3,4-i] purines With Use of NBS, Synthetic Commun 36, 445-450, 2006.
16. Marumoto R, Yoshioka Y, Kawazoe K, Honjo M, Hypotensive nebularinylhydrazones. Ger Offen DE 2163790 A 19720720, 1972.
17. Nagamatsu T, Yamasaki H, Fujita T, Endo K, Machida H, Novel xanthine oxidase inhibitor studies. Part 2. Synthesis and xanthine oxidase inhibitory activities of 2-substituted 6-alkyl-idenehydrazine- or 6-arylmethylidenehydrazino-7H-purines and 3- and/or 5-substituted 9H-1,2,4-triazolo[3,4-i] purines, J Chem Soc Perkin Trans 1, 3117-3125, 1999.
18. Nagamatsu T, Yamasaki H, Akiyama T, Hara S, Mori K, Kusakabe H, Facile and general syntheses of 3-and/or 5-substituted 7-b-D-ribofuranosyl-7H[1,2,4]triazolo[3,4-i]purines as a new class of potential xanthine oxidase inhibitors, Synthesis 4, 655-663, 1999.
19. Fotakis G, Timbrell JA, In vitro cytotoxicity assays: Comparison of LDH, neutral red, MTT and protein assay in hepatoma cell lines following exposure to cadmium chloride. Toxicol Lett 160, 171-177, 2006.
20. Dikmen M, Canturk Z, Ozturk Y, Tunali Y, Investigation of the apoptotic effect of curcumin in human leukemia HL-60 cells by using flow cytometry, Cancer Biother Radiopharm 25(6), 749-755, 2010.
21. Kim MJ, Kim YJ, Park HJ, Chung JH, Leem KH, Kim HK, Apoptotic effect of red wine polyphenols on human colon cancer SNU-C4 cells, Food Chem Toxicol 44, 898-902, 2006.

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