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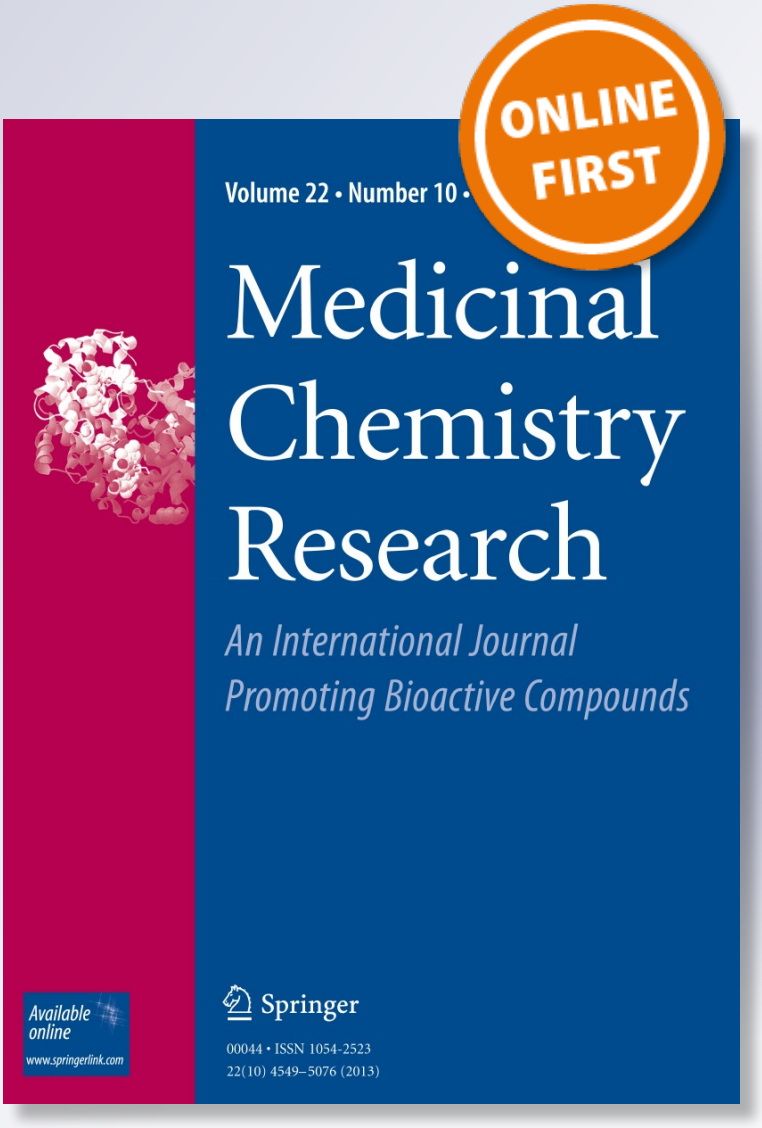

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MEDICINAL CHEMISTRY RESEARCH

ORIGINAL RESEARCH

Synthesis and antiproliferative activities of some pyrazole-sulfonamide derivatives

Samet Mert · Ayşe Şahin Yağlıoğlu · Ibrahim Demirtas · Rahmi Kasımoğulları

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Abstract In this study, a series of pyrazole-sulfonamide derivatives were designed and synthesized from 1-(4-aminophenyl)-4-benzoyl-5-phenyl-*N*-(5-sulfamoyl-1,3,4-thiadiazol-2-yl)-1*H*-pyrazole-3-carboxamide (**1**). The newly synthesized sulfonamides were characterized by FT-IR, ^{1}H NMR, ^{13}C NMR, and elemental analyses. The compounds were tested for their in vitro antiproliferative activities against HeLa and C6 cell lines. The tests were carried out as dose-dependent assay starting from 5 to 100 μg/mL. All compounds showed especially cell selective effect against rat brain tumor cells (C6). Some of the tested compounds (**3**) and (**7**) showed promising broad spectrum antitumor activity comparable to the activities of the commonly used anticancer drugs, 5-fluorouracil and cisplatin.

Keywords Sulfonamide · Pyrazole · HeLa cell lines · C6 cell lines · Antiproliferative activity

Introduction

Cancer is one of the most fatal health problems faced by mankind today (Sondhi *et al.*, 2010; Byakodi *et al.*, 2012; Schwartz and Hulka, 1990; Giri *et al.*, 2001; Landis *et al.*, 1999). This is a group of diseases in which a number of cells display uncontrolled growth, invasion, and sometimes metastasis, which is the spreading of cells to other tissues of body via lymph or blood (Alvarez and Besa, 2000; Gao *et al.*, 2010; Gediya and Njar, 2009; Li *et al.*, 2012; Yilmaz and Ayan, 2011). Various drugs are available for the treatment of cancer but many of them have side effects. Because chemotherapy drugs have a wide range of non-specific effects, there is an urgent need to develop safe and cost effective anticancer agents (Da Settimo *et al.*, 1998). Sulfonamides possess many types of biological activities such as antibacterial (Drews, 2000), hypoglycemic (Boyd, 1988), diuretic (Supuran and Scozzafava, 2000; Maren, 1976), and antiglaucoma (Supuran and Scozzafava, 2001; Kasimogullari *et al.*, 2009). Recently, many structurally novel sulfonamide derivatives have been reported to show significant antitumor activity (Abbate *et al.*, 2004; Ismail *et al.*, 2006; Supuran *et al.*, 2004). Also pyrazole-containing compounds have received considerable attention owing to their diverse chemotherapeutic potentials (Ghorab *et al.*, 2012; Rostom, 2010; Lv *et al.*, 2010; Strocchi *et al.*, 2012; Faidallah *et al.*, 2007). For example, Celecoxib is a non-steroidal anti-inflammatory drug and selective COX-2 inhibitor which includes sulfonamide and pyrazole scaffolds in the same structure. Recently, some studies have been reported about antitumor effect of this drug (Harris *et al.*, 2000; Trifan *et al.*, 2002; Cianchi *et al.*, 2006; Masferrer *et al.*, 2000; Shaik *et al.*, 2006). Literature survey showed that aryl/heteroaryl sulfonamides may act as antitumor agents through several mechanisms (Al-Said *et al.*, 2010). The most remarkable mechanism is the inhibition of carbonic anhydrase isoenzymes (CA) (Abbate *et al.*, 2004; Casini *et al.*, 2002). Previously we studied about synthesis and biological activity of some sulfonamide containing pyrazole-3-carboxamides (Kasimogullari *et al.*, 2011; Sen *et al.*, 2013). We observed many compounds have inhibition potential on human carbonic anhydrase isoenzymes

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(CA I and CA II). These findings prompted us to synthesis some pyrazole-sulfonamide derivatives and determine their antiproliferative effects as in vitro.

In this research, we have aimed to synthesize, characterize, and determine the antiproliferative effects of various sulfonamide derivatives of pyrazole-3-carboxylic acid against some tumour cells [brain tumor cells (C6), uterus cancer cells (Hela)] with in vitro studies. Four of the compounds (**2**–**4**) and (**6**) described here previously synthesized and characterized in our laboratory and their inhibition effects were investigated on human CA I and CA II isoenzymes (Kasimogullari *et al.*, 2011). Compounds (**5**, **7**–**9**) were synthesized in this study are novel and were not previously synthesized. Structural elucidation of synthesized compounds has been performed by means of ^{1}H NMR, ^{13}C NMR, IR, and elemental analysis.

Results and discussion

Chemistry

The synthetic route used to synthesize the target 1,3,4-thiadiazole-2-sulfonamide containing pyrazole-3-carboxamides (**1**–**9**) is outlined in Scheme 1. First an indol derivative (**2**) prepared by the cyclization reaction of (**1**) with chloroacetone. Then diazotization of (**1**) with sodium nitrite followed by treatment with KI afforded (**3**). Also some coupling products (**4**–**7**) were obtained by the condensation of phenol, salicylic acid, and 1,3-dicarbonyl compounds with the diazonium salt solution of (**1**). Finally acetamide (**8**) and carbamate (**9**) derivatives were synthesized from the direct reaction of (**1**) with acetyl chloride and ethyl chloroformate in the presence of triethylamine. Analytical and spectral data (^{1}H NMR, ^{13}C NMR, IR, and elemental analysis) of the synthesized compounds were in full agreement with the proposed structures. IR spectra of the target pyrazole-sulfonamides showed NH and C=O stretching bands at 3,264–3,184 and 1,736–1,656 cm^{-1}, respectively. S=O; asymmetric and symmetric stretching bands were observed at around ~1,365 and ~1,170 cm^{-1}, respectively. The peak appearing only in the spectrum of (**5**) at 3,358 cm^{-1} is attributed to characteristic OH stretching. Also IR spectrum of (**5**) showed a broad absorption band from 2,500 to 3,500 cm^{-1} due to OH stretching of COOH. The absorption bands associated with other functional groups appeared in the expected regions and the absorption values were consistent with our previous reports and literature (Sen *et al.*, 2013; Jennings and Lovely, 1991; Patel and Agravat, 2009; Kasimogullari *et al.*, 2010; Lodeiro *et al.*, 2003).

Pyrazole-sulfonamides (**2**–**9**) were characterized by disappearance of a broad singlet at 6.51 ppm due to NH_2 group of (**1**) (Kasimogullari *et al.*, 2011). The resonances between ~7.80 and ~6.60 ppm can be assigned to aromatic protons. Compound (**5**) characterized by two broad exchangeable singlets in the 12.79 and 7.50 ppm due to COOH and OH groups, respectively. In the ^{1}H NMR spectrum of (**7**), NH proton belonging to hydrazinyl group (Ar–NH–N=C) was appeared at 11.51 ppm. Based on this data we can say that compound (**7**) prefers hydrazo tautomeric structure instead of azo form (Ar–N=N–C). In general, CONH protons appear at around ~13.7 ppm. Compound (**8**) also shows a CONH peak at 10.17 ppm attributed to acetamide proton. The other peaks appeared at the expected chemical shifts and integral values. In addition to the ^{1}H NMR spectra, ^{13}C resonance assignments have also been carried out. The peaks of C=O for alkyl ketones are observed around ~196.00 ppm while the peaks of Ph-C=O and O=C–NH are around ~191.00 and ~165.00 ppm, respectively. There are two interesting peaks at 162.88 ppm corresponding to the ester C=O belonging to (**7**) and at 153.77 ppm corresponding to the carbamate C=O of (**9**). The peak resonances at 177.55 ppm corresponds to the acid carbonyl of (**5**).

Biological activity

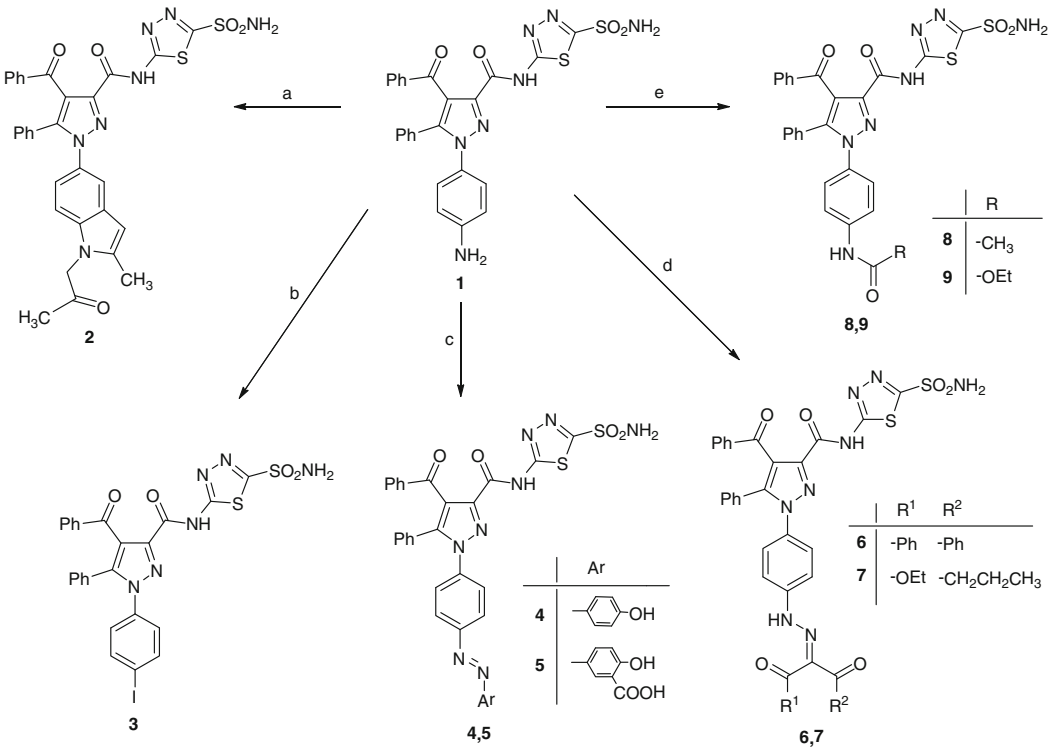
Antiproliferation activities of (**2**–**9**) were determined against human cervix carcinoma (HeLa) and rat brain tumor cells (C6) using the BrdU cell proliferation ELISA assay (Demirtas *et al.*, 2009; Demirtas and Sahin, 2013). 5-Fluorouracil and cisplatin were used as standards. The activities of samples and standards were investigated at eight different concentrations (5, 10, 20, 30, 40, 50, 75, and 100 μg/mL). The IC_{50} and IC_{75} values of the most effective compounds against HeLa and C6 were given in Table 1.

*Antiproliferative activity of compound (**2**) against HeLa and C6 cell lines*

Activity studies showed that compound (**2**) demonstrates antiproliferative activity against HeLa and C6 cell lines in a concentration-dependent manner (Fig. 1b). However, compound (**2**) exhibits weak antiproliferative activity as compare with the standards. The potency of inhibition for HeLa cells is in the order of; 5-FU ~ cisplatin > **2** and for C6; cisplatin > 5-FU > **2**.

*Antiproliferative activity of compound (**3**) against HeLa and C6 cell lines*

Compound (**3**) exhibits antiproliferative activity against HeLa and C6 cell lines in a concentration-dependent manner (Fig. 2a, b). In addition, compound (**3**) has better



Scheme 1 Synthetic route for the target compounds. Reagents and conditions: (*a*) chloroacetone, DMF, 60 °C, 72 h; (*b*) $NaNO_2$/HCl, 0 °C then KI; (*c*) $NaNO_2$/HCl, 0 °C then phenol or salicylic acid; (*d*) $NaNO_2$/HCl, 0 °C then 1,3-dicarbonyl compounds; (*e*) CH_3COCl or EtOCOCl, $(Et)_3N$, THF, reflux, 5 h

activity against HeLa to compare with cisplatin and 5-FU on 100 μg/mL concentration. Moreover, compound (**3**) has usually shown weak activity at lower concentrations. The activity of compound (**3**) was determined as cell selectively against C6 cell line and was observed higher antiproliferative activity than standards at high concentrations (40–100 μg/mL). The potency of inhibition for HeLa is in the order of 5-FU ∼ cisplatin > **3** and for C6 3 > cisplatin > 5-FU.

*Antiproliferative activity of compounds (**4**) and (**5**) against HeLa and C6 cell lines*

Compounds (**4**) and (**5**) show antiproliferative activity against HeLa and C6 cell lines in a concentration-dependent manner (Fig. 3a, b). Compound (**4**) has high antiproliferative activity at a concentration of 100 μg/mL against HeLa. However, the other doses does not affect any activities. Compounds (**4**) and (**5**) have higher activities than standards against C6 cell lines on 100 μg/mL. In addition, compound (**4**) also exhibits strong activity at the concentration of 75 μg/mL against C6 cell lines. At the same time, compound (**4**) has cell selective effect against C6. The potency of inhibition for HeLa is in the order of 5-FU ∼ cisplatin > **4** > **5** and for C6 cisplatin > 5-FU ∼ **4** > **5**.

*Antiproliferative activities of compounds (**6**) and (**7**) against HeLa and C6 cell lines*

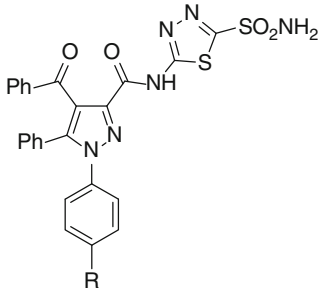
The antiproliferative activity of compound (**7**) and standard compounds are almost the same values on 40–100 μg/mL concentration against HeLa cells. Compound (**6**) exhibits the best activity on 100 μg/mL concentration against HeLa cells. The antiproliferative activities of compounds (**6**) and (**7**) against HeLa and C6 cell lines are in a concentration-dependent manner (Fig. 4a, b).

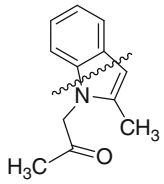
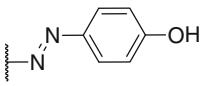
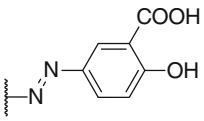
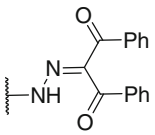
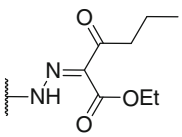
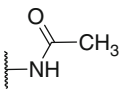
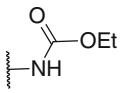
According to Fig. 4b, both compounds (**6**) and (**7**) demonstrate strong activities against C6 to compare with cisplatin and 5-FU (for compound (**6**) on 50–100 μg/mL and for compound (**7**) on 30–100 μg/mL concentrations). The potency of inhibition for HeLa is in the order of 5-FU ∼ cisplatin > **7** > **6** and for C6, **6** ∼ **7** > cisplatin > 5-FU.

*Antiproliferative activities of compounds (**8**) and (**9**) against HeLa and C6 cell lines*

Compound (**8**) exhibits weak activity against HeLa and C6 (Fig. 5a, b). Compound (**9**) is also exhibits weak activity against HeLa, but the same compound shows strong activity against C6 to compare with cisplatin and 5-FU (on 75–100 μg/mL concentrations). The potency of inhibition for HeLa is in the order of 5-FU ∼ cisplatin > **9** > **8** and for C6, **9** > cisplatin > 5-FU > **8**.

Table 1 IC_{50} and IC_{75} values of pyrazole-sulfonamide derivatives (**2–9**)



Compounds	*R*	Hela		C6	
		IC_{50} (mM)	IC_{75} (mM)	IC_{50} (mM)	IC_{75} (mM)
2		76.94	91.61	70.07	86.21
3		78.41	101.41	21.26	52.40
4		85.98	111.32	35.13	62.00
5		118.92	126.91	84.72	101.82
6		67.09	92.73	45.31	64.16
7		40.71	59.97	33.60	53.56
8		155.24	143.33	104.78	120.90
9		83.13	105.46	46.81	69.70

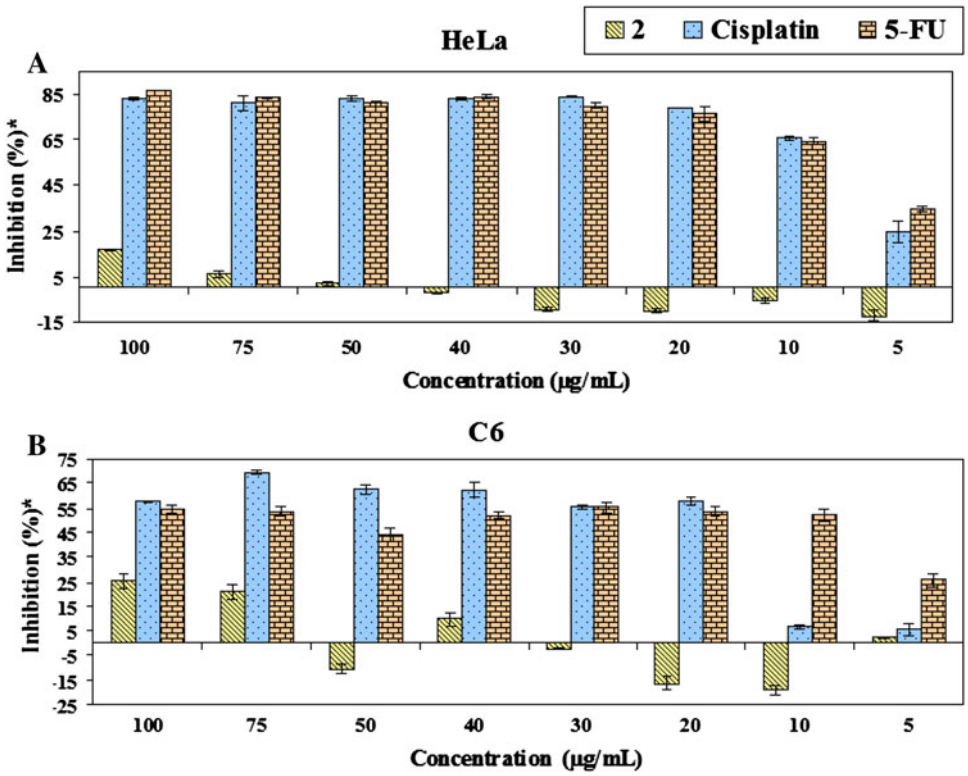


Fig. 1 Antiproliferative activity of (**2**) and standards against HeLa (**a**) and C6 (**b**) cell line. *Asterisks* each substance was tested twice in triplicates against cell lines. Data show average of two individual experiments ($p < 0.01$)

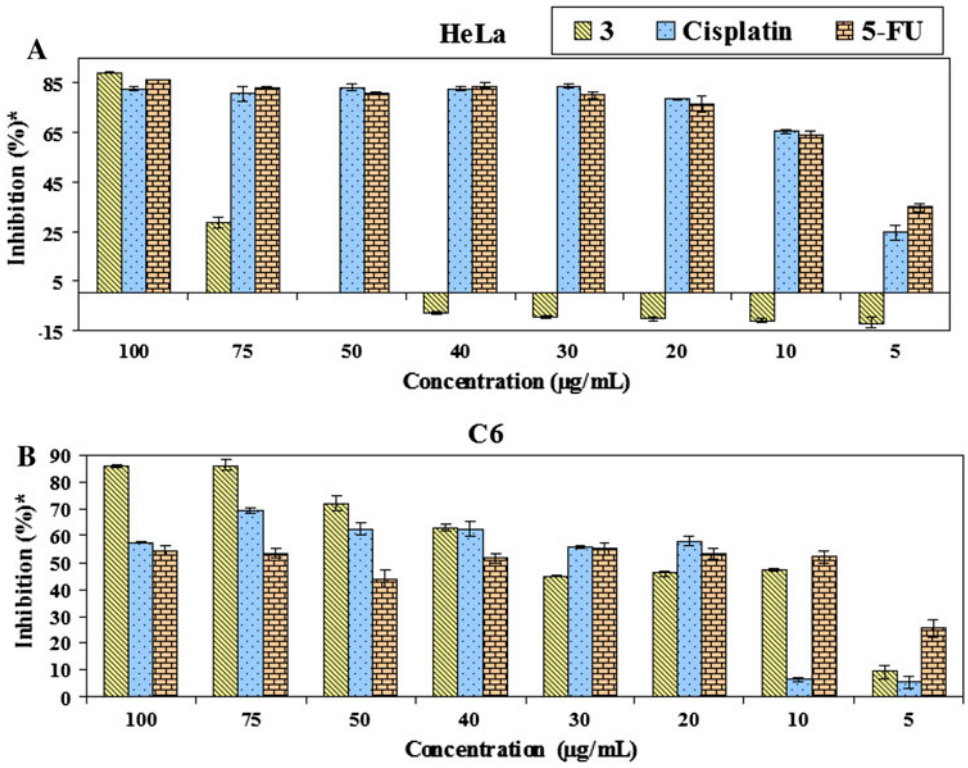


Fig. 2 Antiproliferative activity of (**3**) and standards against HeLa (**a**) and C6 (**b**) cell line. *Asterisks* each substance was tested twice in triplicates against cell lines. Data show average of two individual experiments ($p < 0.01$)

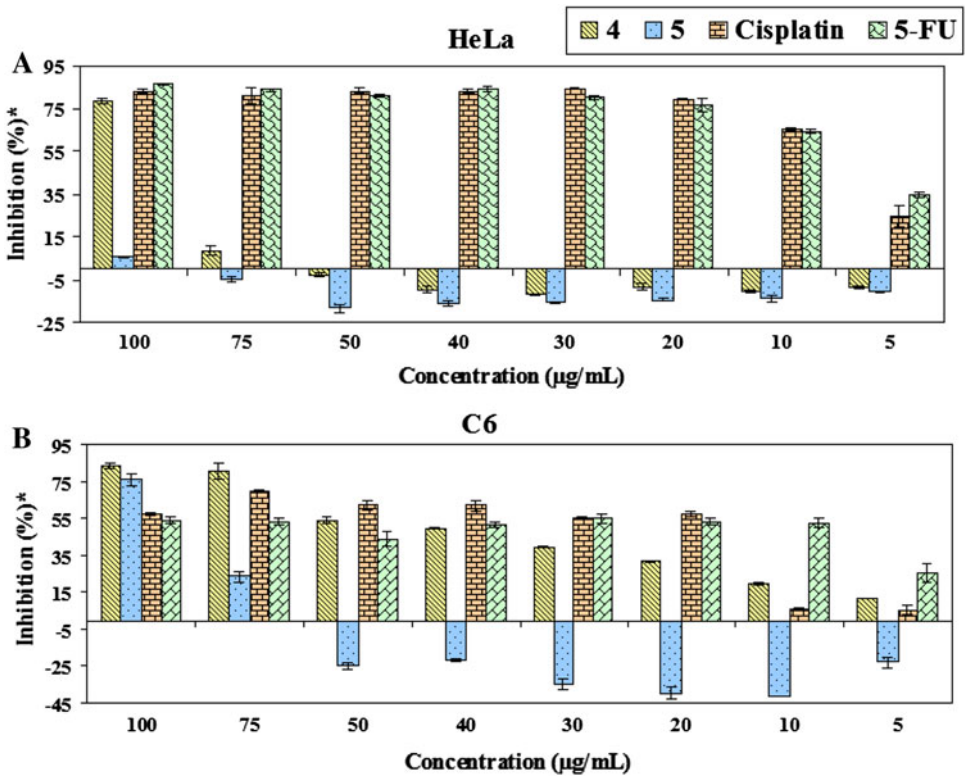


Fig. 3 Antiproliferative activity of (**4**–**5**) and standards against HeLa (**a**) and C6 (**b**) cell line. *Asterisks* each substance was tested twice in triplicates against cell lines. Data show average of two individual experiments ($p < 0.01$)

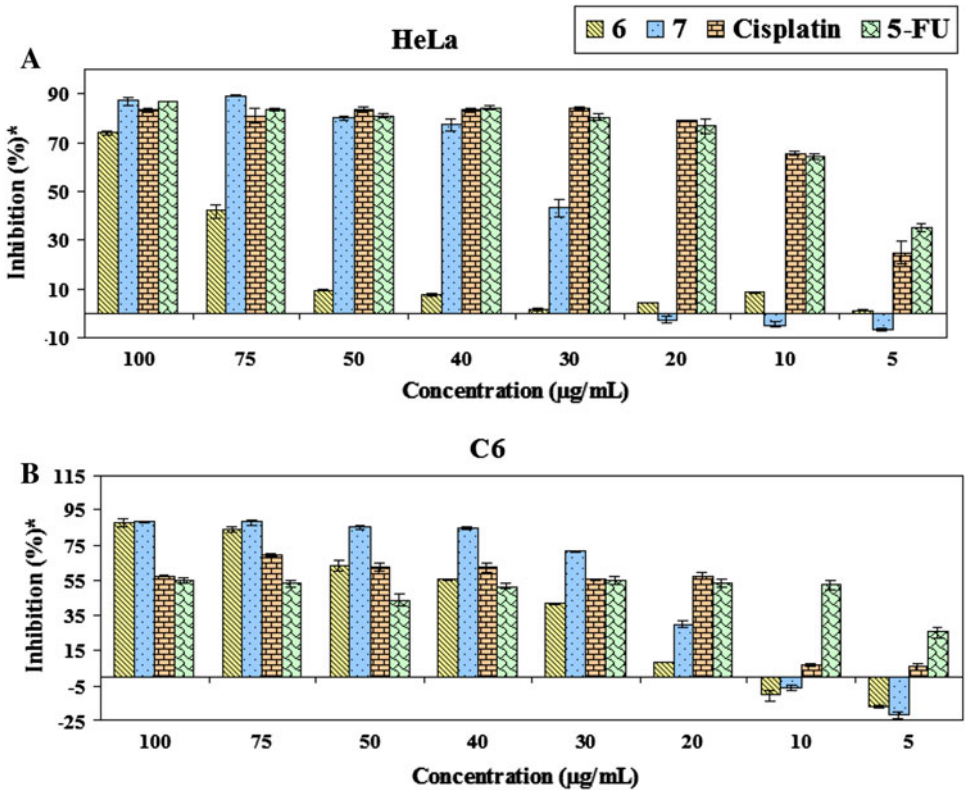


Fig. 4 Antiproliferative activity of (**6**–**7**) and standards against HeLa (**a**) and C6 (**b**) cell line. *Asterisks* each substance was tested twice in triplicates against cell lines. Data show average of two individual experiments ($p < 0.01$)

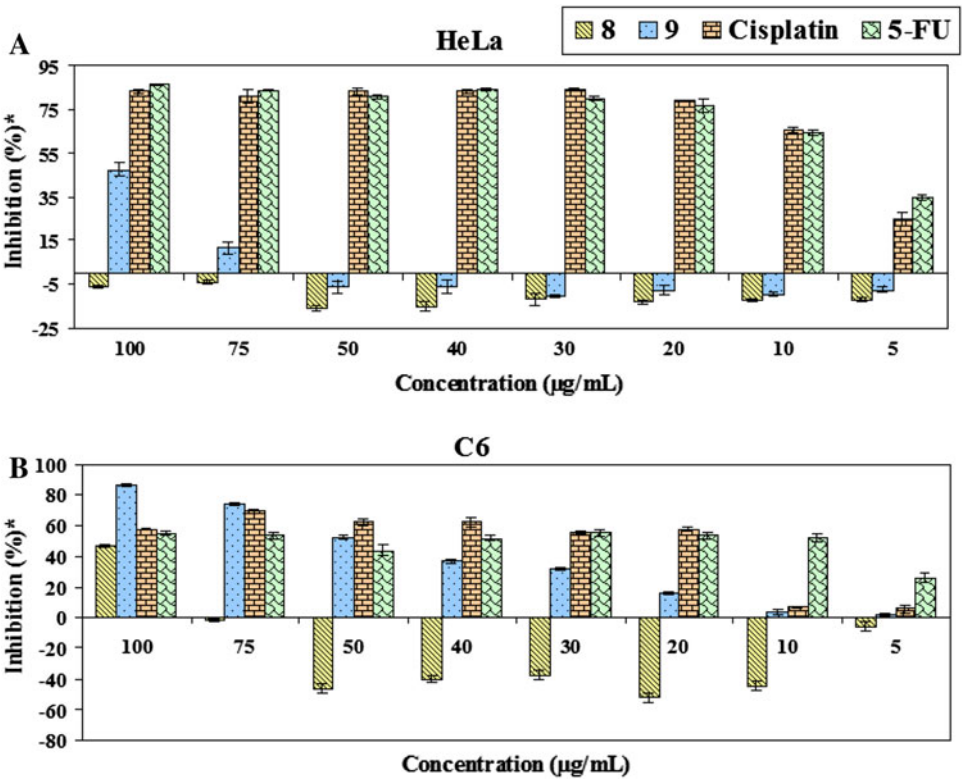


Fig. 5 Antiproliferative activity of (**8–9**) and standards against HeLa (**a**) and C6 (**b**) cell line. *Asterisks* each substance was tested twice in triplicates against cell lines. Data show average of two individual experiments ($p < 0.01$)

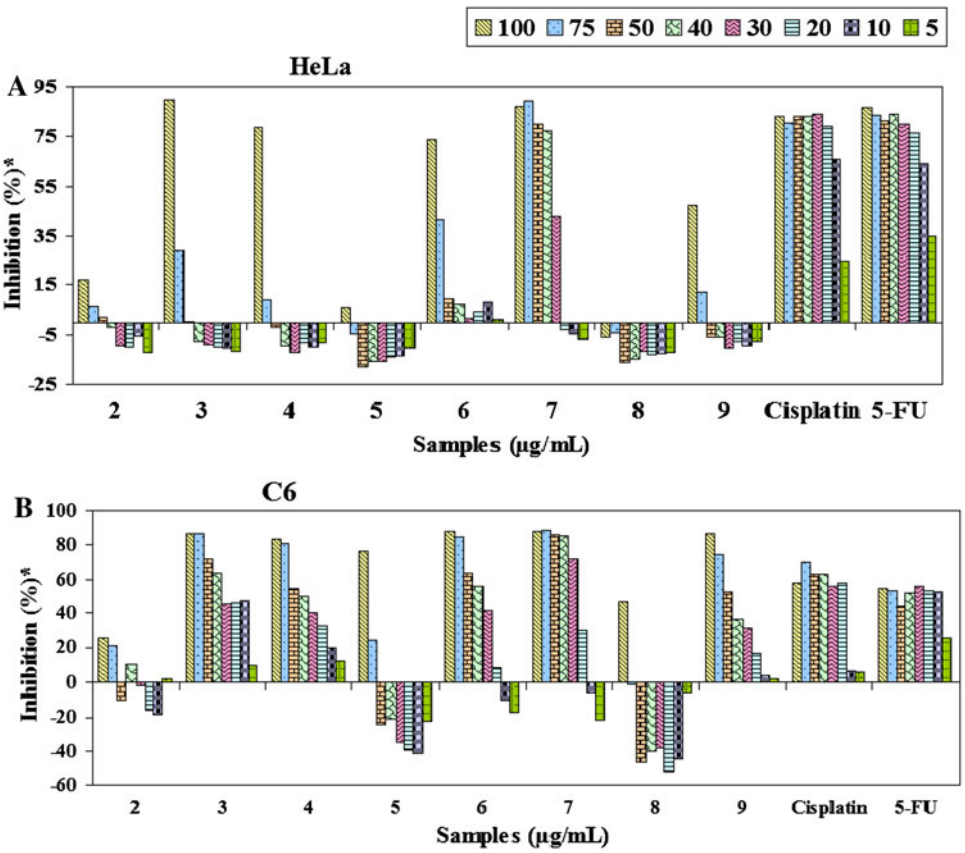


Fig. 6 Antiproliferative activity of (**2–9**) and standards against HeLa (**a**) and C6 (**b**) cell line. *Asterisks* each substance was tested twice in triplicates against cell lines. Data show average of two individual experiments ($p < 0.01$)

Table 2 Selectivity index of pyrazole-sulfonamide derivatives (**2–9**), cisplatin and 5-FU

	Compound names										Selectivity
	2	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**Cisplatin**	**5-FU**	
100	25	86	84	77	88	88	47	86	58	55	0.90
75	21	86	81	24	84	88	−2	74	69	53	1.00
50	−10	72	54	−25	63	86	−47	52	63	44	0.90
40	10	63	50	−21	56	85	−40	37	62	52	1.00
30	−2	45	40	−35	42	72	−37	32	55	55	0.90
20	−16	46	32	−39	9	30	−52	16	58	53	1.00
10	−19	47	20	−41	−11	−6	−44	4	7	52	1.00
5	2	10	12	−22	−17	−22	−6	2	6	26	0.90

*The comparison of antiproliferative activities of compounds (**2**), (**9**) and related structures against HeLa and C6 cell lines*

According to Fig. 6a, b, all compounds have cell selective effect against C6. However, compound (**7**) has only considerable antiproliferative activities against HeLa to compare with cisplatin and 5-FU. The potency of inhibition for HeLa is in the order of 5-FU > cisplatin > **7** > **3** > **4** > **6** > **9** > **2** > **5** > **8** and for C6, **7** > cisplatin > 5-FU > **3** > **4** > **6** ~ **9** > **5** > **8** > **2**.

The structure–activity relationships (SAR) can be drawn with a comparison between functional groups of the synthesized compounds. Compound (**3**) contains an iodine atom in the para position of the phenyl ring as different from the other compounds. According to the in vitro studies it seems that electron-donating resonance effect of this halogen atom contributes to the antiproliferative activity (Fig. 6a, b). This is consistent with the other results from the literature (Hou *et al.*, 2011; Parthiban *et al.*, 2011). The results of the activity study by Parthiban *et al.* (2011) indicate that halogen groups have more effect than hydrogen atoms (Br > Cl > F > H; IC_{50} 25.02, 48.97, 49.18, 112.72 against HeLa. Both (**4**) and (**5**) consist phenolic hydroxyl groups. These phenolic hydroxyl groups may be responsible for enhancement of inhibitory activity. But compound (**5**) also includes a carboxylate group next to the hydroxyl group. We suppose that carboxylate group decreases the activity. Furthermore, ethoxy groups existing at compounds (**7**) and (**9**) contribute substantially to the antiproliferative activity higher than methyl groups (at compound **8**). Parthiban *et al.*, (2011) observed increase in the antiproliferative activity hydrogen to isopropyl groups (isopropyl groups > ethyl groups > methyl groups > hydrogen atoms; 49.54, 57.27, 112.72, 120.68 are the IC_{50} values of alkyl groups against HeLa, respectively). These results are all consistent with our results related to compounds (**7**) and (**9**). Also Benchabane *et al.* (2009) determined that carbamate derivatives are more effective than acetamide derivatives against tumour CHO cells. In this study we found the carbamate derivative (**9**) has more antiproliferative activity than acetamide derivative (**8**) against HeLa and C6 cell lines (carbamate derivative > acetamide derivative, 83.13, 155.24 for HeLa cell line and 46.81, 104.78 for C6 cell line, respectively) (Table 1). The selectivity index values are given below (Table 2).

Experimental protocols

Materials and methods

Chemical compounds used in this research were at analytical purity, and the solvents were purified by using appropriate purifying agents and distillation. Tetrahydrofuran (THF) was distilled from sodium/benzophenone prior to use. All reactions were monitored by analytical thin-layer chromatography (TLC) on 0.25 mm precoated Kieselgel 60F 254 plates (E. Merck Co., Darmstadt, Germany) and compounds were visualized by TLC devices (Camag, Upland, CA, USA) UV (254 and 366 nm). Melting points (°C, uncorrected) were determined in open capillaries on a Barnstead Electrothermal 9200 melting point apparatus (Electrothermal Co, Essex, UK). Infrared (IR) spectra were recorded on Bruker Optics, Vertex 70 Fourier Transform Infrared Spectrometer (FT-IR) equipped with an Attenuated Total Reflection (ATR) device and the data were reported in reciprocal centimeters (cm^{-1}) (Bruker Optik GmbH, Ettlingen, Germany). ^{1}H NMR and ^{13}C NMR spectra were recorded on BRUKER DPX-400 (400 MHz for ^{1}H NMR and 100 MHz for ^{13}C NMR) spectrometers (Bruker BioSpin GmbH, Rheinstetten, Germany). Elemental analyses (C, H, and N) were performed on a Leco CHNS-932 elemental analyser (LECO Corporation, Saint Joseph, Michigan, USA).

Synthesis of target molecules

Compound (**1**) was previously prepared and it was used as a starting compound in this study (Kasimogullari *et al.*, 2011). Also the compounds 4-benzoyl-1-(2-methyl-1-(2-oxopropyl)-1*H*-indol-5-yl)-5-phenyl-*N*-(5-sulphamoyl-1,3,4-thiadiazol-2-yl)-1*H*-pyrazole-3-carboxamide (**2**), 4-benzoyl-1-(4-iodophenyl)-5-phenyl-*N*-(5-sulphamoyl-1,3,4-thiadiazol-2-yl)-1*H*-pyrazole-3-carboxamide (**3**), 4-benzoyl -1-(4-((4-hydroxyphenyl)diazenyl)phenyl)-5-phenyl-*N*-(5-sulphamoyl-1,3,4-thiadiazol -2-yl)-1*H*-pyrazole-3-carboxamide (**4**), and 4-benzoyl-1-(4-(2-(1,3-dioxo-1,3-diphenylpropan-2-ylidene)hydrazinyl)phenyl)-5-phenyl-*N*-(5-sulphamoyl-1,3,4-thiadiazol-2-yl)-1*H*-pyrazole-3-carboxamide (**6**) were synthesized and characterized in our previous work (Kasimogullari *et al.*, 2011).

*General procedure for the syntheses of compounds (**3**)–(**7**)*

1-(4-Aminophenyl)-4-benzoyl-5-phenyl-*N*-(5-sulfamoyl-1,3,4-thiadiazol-2-yl)-1*H*-pyrazole-3-carboxamide (**1**) (0.545 g, 1 mmol) was dissolved in a mixture of ethanol (50 ml) and concentrated hydrochloric acid (8 ml) and the solution was then cooled to 0–5 °C. Sodium nitrite (0.083 g, 1.2 mmol) in water (10 ml) was then added to this solution dropwise with vigorous stirring while cooling at 0–5 °C. After dissolving an aromatic or β-dicarbonyl compound (1 mmol) in a sufficient amount of ethanol (1 mmol KI was dissolved in water for compound **3**), the solution was cooled and added dropwise into the already prepared diazonium salt solution. The pH of the mixture, in each case, was maintained at 7–8 through the coupling process by adding aqueous sodium acetate. Stirring was continued for 1 h at 0–5 °C and 2 h at room temperature. The precipitated products were filtered off, washed with water several times, dried, and recrystallized from an appropriate solvent (see Scheme 1).

*5-((4-(4-Benzoyl-5-phenyl-3-(5-sulfamoyl-1,3,4-thiadiazol-2-ylcarbamoyl)-1H-pyrazol-1-yl) phenyl)diazenyl)-2-hydroxybenzoic acid (**5**)*

Salicylic acid (0.138 g, 1 mmol) was dissolved in ethanol and cooled to 0–5 °C and added dropwise to the diazonium salt solution of (**1**) which was prepared according to the general procedure. The precipitated yellow product was filtered and purified from ethanol. 431 mg, 62 %; mp 212–214 °C; IR (ν, cm^{-1}): 3600–2800 (COOH), 3358 (OH), 3249 (NH), 3061 (Ar CH), 1689 (C=O), 1598–1449 (Ar C=C and C=N), 1363 and 1172 (S=O, assym. and sym.); ^{1}H NMR (400 MHz, DMSO) δ (ppm): 13.72 (br, s, 1H, CONH), 12.79 (s, 1H, COOH), 8.29 (s, 2H, SO_2NH_2), 7.50 (s, 1H, OH), 7.80–7.24 (m, 17H, ArH); ^{13}C NMR (100 MHz, DMSO) δ (ppm): 190.59 (C=O, benzoyl), 177.55 (C=O, acid), 165.24 (C=O, amide), 161.64 and 160.58 (thiadiazol C-2 and C-5), 144.46, 143.29, 138.63, 137.79, 134.80, 133.98, 132.14, 130.55, 130.31, 129.97, 129.78, 129.60, 129.33, 129.04, 128.99, 128.75, 128.15, 127.97, 127.35, 126.11, 122.88; anal. calcd. for $C_{32}H_{22}N_8O_7S_2$: C, 55.33; H, 3.19; N, 16.13; S, 9.23; found: C, 55.25; H, 3.10; N, 16.18; S, 9.21.

*Ethyl 2-(2-(4-(4-benzoyl-5-phenyl-3-(5-sulfamoyl-1,3,4-thiadiazol-2-ylcarbamoyl)-1H-pyrazol-1-yl)phenyl)hydrazono)-3-oxohexanoate (**7**)*

Ethyl butyryl acetate (0.16 ml, 1 mmol) was dissolved in ethanol and cooled to 0–5 °C and added dropwise to the diazonium salt solution of (**1**) which was prepared according to the general procedure. The precipitated bright yellow product was filtered and purified from ethanol. 550 mg, 77 %; mp 162–164 °C; IR (ν, cm^{-1}): 3184 (NH), 3003 (Ar CH), 2968 (aliphatic CH), 1677 and 1656 (C=O), 1597–1449 (Ar C=C and C=N), 1368 and 1170 (S=O, assym. and sym.); ^{1}H NMR (400 MHz, DMSO) δ (ppm): 13.69 (br, s, 1H, CONH), 11.51 (s, 1H, Ar–NH–N=), 8.36 (s, 2H, SO_2NH_2), 7.79–7.22 (m, 14H, ArH), 4.32 (q, $J = 7.1$ Hz, 2H, OCH_2), 2.84 (t, $J = 7.2$ Hz, 2H, $CH_2CH_2CH_3$), 1.59 (hextet, $J = 7.4$ Hz, 2H, $CH_2CH_2CH_3$), 1.29 (t, $J = 7.0$ Hz, 3H, OCH_2CH_3), 0.91 (t, $J = 7.4$ Hz, 3H, $CH_2CH_2CH_3$); ^{13}C NMR (100 MHz, DMSO) δ (ppm): 196.46 and 190.58 (C=O, ketone), 165.27 (C=O, amide), 162.88 (C=O, ester), 161.53 and 160.51 (thiadiazol C-2 and C-5), 61.73 (OCH_2), 39.04 ($CH_2CH_2CH_3$), 17.86 ($CH_2CH_2CH_3$), 14.34 and 14.14 (CH_3) 144.48, 143.30, 143.14, 137.76, 133.97, 132.77, 130.28, 129.93, 129.58, 129.03, 128.97, 127.93, 127.57, 122.77, 116.68, 115.57; anal. calcd. for $C_{33}H_{30}N_8O_7S_2$: C, 55.45; H, 4.23; N, 15.68; S, 8.97; found: C, 55.34; H, 4.18; N, 15.71; S, 8.93.

*1-(4-Acetamidophenyl)-4-benzoyl-5-phenyl-N-(5-sulfamoyl-1,3,4-thiadiazol-2-yl)-1H-pyrazole-3-carboxamide (**8**)*

To a stirring solution of (**1**) (0.545 g, 1 mmol) and triethylamine (0.138 ml, 1 mmol) in dry THF (30 ml), acetylchloride (0.071 ml, 1 mmol) was added dropwise and the reaction mixture was refluxed for 5 h. After removal of the solvent, the crude product was obtained which was further purified by recrystallization from ethanol–water (1:1) mixture. 541 mg, 92 %; mp 188–190 °C; IR (ν, cm^{-1}): 3264 and 3198 (NH), 3026 (Ar CH), 2970 (aliphatic CH), 1691 and 1668 (C=O), 1605–1433 (Ar C=C and C=N), 1366 and 1167 (S=O, assym. and sym.); ^{1}H NMR (400 MHz, DMSO) δ (ppm): 13.77 (br, s, 1H, CONH), 10.17 (br, s, 1H, CONHAc), 8.34 (s, 2H, SO_2NH_2),

7.79–7.18 (m, 14H, ArH), 2.06 (s, 3H, CH_3); ^{13}C NMR (100 MHz, DMSO) δ (ppm): 190.61 (C=O, benzoyl), 169.19 and 165.29 (C=O, amide), 161.40 and 160.42 (thiadiazol C-2 and C-5), 24.53 (CH_3), 144.46, 142.92, 140.24, 137.66, 134.04, 133.45, 130.25, 129.91, 129.61, 129.08, 128.96, 127.88, 126.88, 122.69, 119.31; anal. calcd. for $C_{27}H_{21}N_7O_5S_2$: C, 55.19; H, 3.60; N, 16.69; S, 10.91; found: C, 55.05; H, 3.55; N, 16.70; S, 10.86.

*Ethyl 4-(4-benzoyl-5-phenyl-3-(5-sulfamoyl-1,3,4-thiadiazol-2-ylcarbamoyl)-1H-pyrazol-1-yl)phenylcarbamate (**9**)*

Compound (**9**) was synthesized from (**1**) (0.545 g, 1 mmol), triethylamine (0.138 ml, 1 mmol), and ethylchloroformate (0.095 ml, 1 mmol) as described for compound (**8**). The crude product was recrystallized from ethanol. 550 mg, 89 %; mp 128–130 °C; IR (ν, cm^{-1}): 3235 (NH), 3003 (Ar CH), 2972 (aliphatic CH), 1736 (C=O), 1602–1417 (Ar C=C and C=N), 1367 and 1169 (S=O, assym. and sym.); ^1H NMR (400 MHz, $CDCl_3$) δ (ppm): 11.57 (br, s, 2H, 2xCONH), 7.81 (s, 2H, SO_2NH_2), 7.58–6.60 (m, 14H, ArH), 4.23 (q, $J = 6.5$ Hz, 2H, OCH_2), 1.28 (t, $J = 7.1$ Hz, 3H, CH_3); ^{13}C NMR (100 MHz, $CDCl_3$) δ (ppm): 191.77 (C=O, benzoyl), 164.68 (C=O, amide), 161.40 and 158.55 (thiadiazol C-2 and C-5), 153.77 (C=O, carbamate), 61.55 (OCH_2), 14.50 (CH_3), 144.51, 141.56, 138.87, 137.02, 133.77, 133.18, 133.04, 129.55, 129.77, 128.63, 128.46, 127.27, 125.84, 122.45, 118.86; anal. calcd. for $C_{28}H_{23}N_7O_6S_2$: C, 54.45; H, 3.75; N, 15.87; S, 10.38; found: C, 54.31; H, 3.69; N, 15.91; S, 10.32.

Chemicals for biological activities

Cell proliferation ELISA, BrdU (colorimetric) kits were obtained from Roche Diagnostics GmbH (Mannheim, Germany). The antitumour drug 5-fluorouracil was provided from Sigma. Other antiproliferative chemicals used were in analytical grade and obtained from Sigma-Aldrich, Merck or Roche.

Preparation of sample solutions

Stock solutions of the samples, 5-fluorouracil, cisplatin were dissolved in DMSO and diluted Dulbecco's modified Eagle's medium (DMEM; 1:20). The final concentration of DMSO is below 1 % in all tests.

Cell culture and cell proliferation assay

HeLa (human cervix carcinoma) and C6 (rat brain tumor) cells were grown in Dulbecco's modified Eagle's medium (DMEM, Sigma), supplemented with 10 % (v/v) fetal bovine serum (Sigma, Germany) and PenStrep solution (Sigma, Germany) at 37 °C in a 5 % CO_2 humidified atmosphere. For proliferation assay, cells were plated in 96-well culture plates (COSTAR, Corning, USA) at a density of 30,000 cells per well. Vehicle (DMSO), 5-fluorouracil, cisplatin, and the samples in various concentrations (0–100 μg/mL) were added to each well. Cells were than incubated for overnight before applying the BrdU cell proliferation ELISA assay reagent (Roche, Germany) according to manufacturer's procedure. In brief, cells were pulsed with BrdU labeling reagent for 4 h followed by fixation in FixDenat solution for 30 min at room temperature. Thereafter, cells were incubated with 1:100 dilution of anti-BrdU–POD for 1.30 h at room temperature. The amount of cell proliferation was assessed by determining the 450 nm of the culture media after addition of the substrate solution by using a microplate reader (Ryto, China). Results were reported as percentage of the inhibition of cell proliferation, where the optical density measured from vehicle-treated cells was considered to be 100 % of proliferation. All assays were repeated at least twice using against HeLa and C6 cells. Percentage of inhibition of cell proliferation was calculated as follows: $[1 - (A_{\text{treatments}}/A_{\text{vehicle control}}) \times 100$.

Statistical analysis

The results of investigation in vitro are the mean ± SEM of six measurements for each cell type. Differences between treatment groups were tested way ANOVA and p values of <0.01 and 0.05 were considered significant.

Determination of IC_{50} and IC_{75} values

The half maximal inhibitory concentration (IC_{50}) is a measure of the effectiveness of a compound in inhibiting biological function. In this paper, IC_{50} and IC_{75} values were determined using ED50 plus v1.0.

Conclusions

The compounds tested in this work have cell selective effects against C6 cell line ($p < 0.01$). According to the results compound (**7**) especially have considerable antiproliferative activities against both Hela and C6 cell lines to compare with cisplatin and 5-FU. Compounds (**3**) and (**4**) have good antiproliferative activity against C6 at high concentrations. Also (**3**) and (**4**) tested for the inhibition over human cytosolic isozymes (CA I and CA II) in our previous work as in vitro (Kasimogullari *et al.*, 2011). Interestingly, these two compounds showed the greatest

effect over CA I and CA II between the tested compounds. According to the results these compounds (**3**), (**4**), and (**7**) may seen as candidate for the inhibition of the tumor-associated isozyme CA IX which is a recently discovered member of the CA gene family.

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