Research Article

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Evaluation of new sulfathiazole derivatives as antiproliferative agents

Yeni sülfatiyazol türevlerinin antiproliferatif ajan olarak değerlendirilmesi

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Abstract

Objectives: Sulfonamide group is an important scaffold used for generating new building blocks with diverse biological activities. Considering priority of the sulfonamide structure, seven new sulfathiazole derivatives were synthesized and evaluated for their antiproliferative activity, in this study.

Materials and methods: Compounds **2a–g** were synthesized using a two-step synthetic procedure starting from commercially available sulfathiazole. The antiproliferative activity of the compounds was investigated against A549 and NIH/3T3 cell lines by MTT assay, matrix metalloproteinase-9 (MMP-9) and cathepsin inhibition tests.

Results: Compound **2b** bearing triazole ring exhibited highest inhibitory activity (IC_{50}: 12.33 µg/mL) with selective profile which was better than cisplatin and it also inhibited MMP-9 with 53.67% percentage. Compounds **2c** and **2e** inhibited cathepsin L with percentages of 62.75 and 57.25%, whereas cathepsin D was poorly inhibited by the compounds.

Conclusions: Target compounds exhibited high to moderate antiproliferative activity and they displayed higher MMP-9 inhibition than cathepsin inhibition activity. **2b** and **2e** were identified as the most active compounds when evaluated, biologically.

Keywords: cathepsin inhibition; cytotoxicity; matrix metalloproteinase-9 inhibition; sulfathiazole; sulfonamide.

Öz

Amaç: Sülfonamid grubu, çeşitli biyolojik aktivitelere sahip yeni yapı taşları üretmek için kullanılan önemli bir yapı iskelesidir. Sülfonamid yapısının öncüliği göz önüne alınarak, bu çalışmada yedi yeni sülfatiyazol türevi sentezlenmiş ve antiproliferatif aktiviteleri değerlendirilmiştir.

Gereç ve Yöntem: **2a–g** bileşikleri, ticari olarak sağlanan sülfatiyazolden başlanarak iki aşamalı sentetik bir prosedürle sentezlenmiştir. Bileşiklerin antiproliferatif aktiviteleri A549 ve NIH/3T3 hücre dizilerine karşı MTT deneyi, matriks metaloproteinaz-9 (MMP-9) ve katepsin inhibisyon testleri ile belirlenmiştir.

Bulgular: Triazol halkası taşıyan bileşik **2b**, sisplatinden daha fazla olmak üzere seçici profile sahip en yüksek inhibitör aktiviteyi (IC_{50}: 12.33 µg/mL) sergilemiştir ve MMP-9'u % 53.67 ile inhibe etmiştir. Bileşik **2c** ve **2e** % 62.75 ve % 57.25'lik oranlarla katepsin L'yi inhibe ederken katepsin D bileşikler tarafından zayıf bir şekilde inhibe edilmiştir.

Sonuç: Hedef bileşikler, yüksek ila orta derecede antiproliferative aktivite sergilemiş ve katepsin inhibisyonuna göre daha yüksek MMP-9 inhibisyonu göstermiştir. Biyolojik açıdan değerlendirildiğinde **2b** ve **2e** en aktif bileşikler olarak tespit edilmiştir.

Anahtar kelimeler: Sülfatiyazol; sülfonamit; sitotoksisite; matriks metalloproteinaz-9 inhibisyonu; katepsin inhibisyonu.

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Introduction

Sulfonamides are essential scaffolds for medicinal chemistry since first oral antibiotic, prontosil which was discovered by Gerhard Domagk. Prontosil was determined to be metabolized in *p*-aminobenzenesulfonamide responsible for antibacterial activity under *in vivo* conditions. This discovery enabled its explorer to receive the Nobel prize in 1939 which was then pioneered to many pharmaceutical applications [1, 2]. A lot of sulfonamide containing heterocyclic compounds were acquired and used clinically of which about 30 sulfonyl including drugs are available on the market today [3–5]. Its derivatives have been documented in a wide array of pharmacological activities such as antimicrobial, antitubercular, antidiabetic, diuretics, anticonvulsant, antiinflammatory, antioxidant, anti-Alzheimer, anticancer, antiviral, antiobesity, antiglaucoma, antiparasitic, etc. [6–8]. Due to this expansive range of bioactivity and also multi-purpose structure of sulfonamides; similar and small molecules are of interest for the purpose of designing or reconstructing old drugs for use in various targets in areas where urgent need in medical chemistry [9]. In especial, antitumor activity of sulfonamides were extensively studied in recent years [10–16]. The reported mechanism of action for the antiproliferative activity of these compounds were matrix metalloproteinase (MMP) inhibition, carbonic anhydrase inhibition, cell cycle arrest in the G1 phase, disruption of microtubule assembly, functional suppression of the transcriptional activator NF-Y, inhibition receptor tyrosine kinase (TK) enzymes of vascular endothelial growth factor receptor and angiogenesis [17, 18].

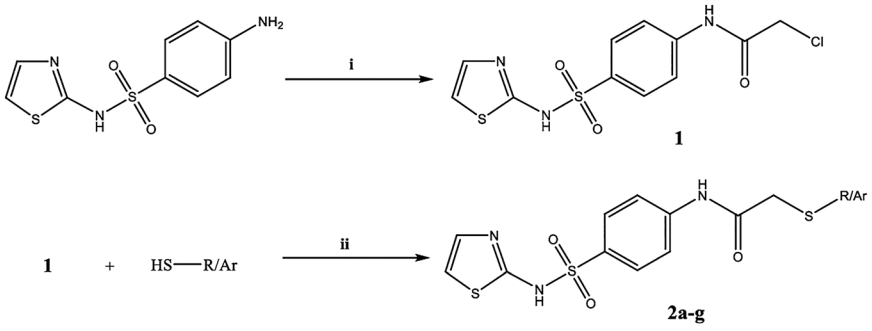
Indisulam (E7070), titled *N*-(3-chloro-7-indolyl)-1,4-benzenedisulfonamide, is a small promising sulfonamide antitumor agent which is in Phase I/II clinical trials. Phase I studies was successfully completed for the treatment of tumors through carbonic anhydrase inhibition. Besides, indisulam was established to block cyclin-dependent kinases responsible for cell progression in human tumor cells, which promotes promising results of it in phase II studies [19–21]. SLC-0111 (4-(4-fluorophenylureido)-benzenesulfonamide) is another small sulfonamide molecule which exhibited high carbonic anhydrase IX inhibition in tumor cells and it was represented with encouraging antiproliferative/antimetastatic activity in breast cancer [22], Pazopanib is an approved antineoplastic sulfonamide drug which acts as TK inhibitor wherefore used in the treatment of renal cancers since 2010 [23]. CGS-27023A and CGS-25966 are sulfonamide hydroxamate derivative compounds which inhibit tumor progress and angiogenesis as established in preclinical studies but failed in clinical trials. These compounds are first prototypes of the sulphonamide type of MMP inhibitors. MMPs are substantial enzymes which possess important roles in various biochemical processes in the organism such as wound healing, tissue formation, organ generation, angiogenesis. Overexpression of MMP-9 is an important indication for recognition and prognosis of many type of cancers including gastric, breast, lung, colon, prostate, and pancreatic cancers [24, 25]. MMP-9 inhibitor antitumor compounds have great interests in recent years that sulfonamide including new molecules have widely reported as MMP-9 inhibitors [26–29].

On the basis of above literature, we have acquired a series of sulfonamide derivatives and evaluated their antiproliferative activity, MMP-9 and cathepsin inhibitory activities.

Material and methods

Chemistry

The chemical and biological materials were provided from commercial sources Merck Chemicals (Merck KGaA, Darmstadt, Germany), Riedel-de Haen (Altmann Analytik GmbH & Co. KG, Munich, Germany) and Sigma-Aldrich Chemical Co (Sigma-Aldrich Corp., St. Louis, MO, USA) The course of reaction was controlled by thin layer chromatography (TLC) using TLC Silica gel 60 F_{254} Aluminum TLC plates (Merck KGaA, Darmstadt, Germany). In TLC, ethyl acetate–petroleum ether (1:3, 1:1) and more polar solutions were used as mobile phase. The physical



Scheme 1: The synthesis of the compounds **2a–g**. Reactants, reagents and conditions: **i:** $ClCH_2COCl$, TEA, THF, 0 °C, 3 h, then 1 h, r.t.; **ii:** K_2CO_3, Acetone, 5 h, r.t.

properties of the final compounds were determined, melting points (m.p.) were identified by MP90 digital melting point device (Mettler Toledo, Ohio, USA). Spectroscopic data were recorded to prove chemical structures of the compounds using the following instruments: IR, Shimadzu Affinity 1S spectrophotometer (Shimadzu, Tokyo, Japan); ^{1}H NMR (nuclear magnetic resonance) Bruker DPX 300 FT-NMR spectrometer, ^{13}C NMR, Bruker DPX 75 MHz spectrometer (Bruker Bioscience, Billerica, MA, USA), in DMSO-d_6 and chloroform-d_6; M+1 peaks were determined by Shimadzu 8040 LC/MS/MS system (Shimadzu, Tokyo, Japan). Elemental analyses were done on a Leco CHNS-932 Elementary Chemical Analyzer (Leco, Michigan, USA).

Synthesis of the compounds

Synthesis of the 2-chloro-*N*-[4-(*N*-(thiazol-2-yl)sulfamoyl)phenyl] acetamide (1): Yield: 65%. 0.05 mol (12.75 g) of sulfathiazole (analytical standard, ≥98.0%) was dissolved in 250 mL of tetrahydrofuran (for analysis EMSURE® ACS, Reag. Ph Eur) and 0.06 mol (8.4 mL) of triethylamine (for synthesis) was added to the solution. 0.06 mol (4.8 mL) of chloroacetyl chloride (for synthesis) which was diluted in 30 mL tetrahydrofuran was added dropwise to the reaction vessel on ice bath. After the dropping was complete, the reaction mixture was stirred at room temperature for 1 h. The end of the reaction was checked by TLC. After evaporation of the solvent, the raw product was washed with excess water and crystallized from ethanol (EtOH).

Synthesis of the 2-(arylthio)-*N*-[4-(*N*-(thiazol-2-yl)sulfamoyl)phenyl] acetamide derivatives (2a–g): 2-Chloro-*N*-[4-(*N*-(thiazol-2-yl) sulfamoyl) phenyl] acetamide (**1**) (1 mmol, 0.33 g) and the appropriate mercaptoaryl derivative (1 mmol) were stirred in 50 mL acetone (ACS reagent, ≥99.5%) in the presence of potassium carbonate (1 mmol, 0.14 g) at room temperature. The end of the reaction was checked by TLC control and it was allowed to evaporate the solvent to release from the mixture. The raw product was washed with water, dried and recrystallized from EtOH.

2-(Thiazolin-2-yl)-*N*-[4-(*N*-(thiazol-2-yl)sulfamoyl)phenyl]acetamide (2a): Yield: 70%. M.p. 94–95 °C. ^{1}H NMR (300 MHz, DMSO-d_6, ppm) δ: 3.46 (2H, t, J=8.1 Hz, N–CH_2), 4.08–4.14 (4H, m, CO_2CH_2 ve SCH_2), 6.78 (1H, d, J=4.53 Hz, Ar–H), 7.22 (1H, d, J=4.53 Hz, Ar–H), 7.67–7.75 (4H, m, Ar–H), 10.58 (1H, s, N–H), 12.71 (1H, brs, NH). ^{13}C NMR (75 MHz, DMSO-d_6, ppm) δ: 36.08 (CH_2), 37.38 (CH_2), 64.34 (CH_2), 108.51 (C), 119.02 (2C), 125.97 (C), 127.50 (2C), 137.34 (C), 142.24 (C), 163.21 (C), 166.51 (C), 169.23 (C=O). $C_{14}H_{14}N_4O_3S_4$ calculated: 40.56% C, 3.40% H, 13.52% N; found: 40.51% C, 3.42% H, 13.54% N. HRMS (m/z): $[M–H]^-$ calcld $C_{14}H_{14}N_4O_3S_4$: 412.9876; found: 412.9862.

2-(4-Methyltriazol-2-yl)-*N*-[4-(*N*-(thiazol-2-yl)sulfamoyl)phenyl] acetamide (2b): Yield: 72%. M.p. 129–131 °C. ^{1}H NMR (300 MHz, DMSO-d_6, ppm) δ: 3.54 (3H, s, CH_3), 4.04 (2H, s, CO_2CH_2), 6.44 (1H, d, J=3.87 Hz, Ar–H), 6.93 (2H, d, J=3.87 Hz, Ar–H), 7.53 (2H, d, J=8.73 Ar–H), 7.66 (2H, d, J=8.70 Hz, Ar–H), 10.52 (1H, s, N–H), 12.70 (1H, brs, NH). ^{13}C NMR (75 MHz, DMSO-d_6, ppm) δ: 31.29 (CH_3), 38.19 (CH_2), 107.66 (C), 118.60 (2C), 127.46 (2C), 137.15 (C), 140.53 (C), 140.89 (C), 146.75 (C), 149.10 (C), 166.55 (C), 170.00 (C=O). $C_{14}H_{14}N_6O_3S_3$ calculated: 40.96% C, 3.44% H, 20.47% N; found: 40.92% C, 3.46% H, 20.49% N. HRMS (m/z): $[M–H]^-$ calcld $C_{14}H_{14}N_6O_3S_3$: 409.0217; found: 409.0205.

2-(1-Methyltetrazol-5-yl)-*N*-[4-(*N*-(thiazol-2-yl)sulfamoyl)phenyl] acetamide (2c): Yield: 68%. M.p. 230–233 °C. ^{1}H NMR (300 MHz, DMSO-d_6, ppm) δ: 3.97 (3H, s, CH_3), 4.29 (2H, s, CO_2CH_2), 6.40 (1H, d, J=3.81 Hz, Ar–H), 6.90 (1H, d, J=3.81 Hz, Ar–H), 7.53 (2H, d, J=8.70 Ar–H), 7.66 (2H, d, J=8.70 Hz, Ar–H), 10.56 (1H, s, N–H), 12.70 (1H, brs, NH). ^{13}C NMR (75 MHz, DMSO-d_6, ppm) δ: 34.12 (CH_3), 38.15 (CH_2), 107.51 (C), 118.56 (2C), 127.51 (2C), 137.50 (C), 140.25 (C), 141.29 (C), 153.81 (C), 165.66 (C), 169.86 (C=O). $C_{13}H_{13}N_7O_3S_3$ calculated: 37.95% C, 3.18% H, 23.83% N; found: 37.90% C, 3.16% H, 23.80% N. HRMS (m/z): $[M–H]^-$ calcld $C_{13}H_{13}N_7O_3S_3$: 410.0169; found: 410.0155.

2-(5-Methylthiadiazol-2-yl)-*N*-[4-(*N*-(thiazol-2-yl)sulfamoyl)phenyl] acetamide (2d): Yield: 74%. M.p. 235–237 °C. ^{1}H NMR (300 MHz, DMSO-d_6, ppm) δ: 3.97 (3H, s, CH_3), 4.29 (2H, s, CO_2CH_2), 6.81 (1H, d, J=4.62 Hz, Ar–H), 7.25 (1H, d, J=4.62 Hz, Ar–H), 7.65–7.81 (4H, m, Ar–H), 10.52 (1H, s, N–H), 12.72 (1H, brs, NH). ^{13}C NMR (75 MHz, DMSO-d_6, ppm) δ: 15.66 (CH_3), 38.47 (CH_2), 108.61 (C), 119.15 (2C), 124.89 (C), 127.54 (2C), 137.14 (C), 142.31 (C), 164.70 (C), 166.21 (C), 166.31 (C), 169.21 (C=O). $C_{14}H_{13}N_5O_3S_4$ calculated: 39.33% C, 3.06% H, 16.38% N; found: 39.30% C, 3.07% H, 16.34% N. HRMS (m/z): $[M–H]^-$ calcld $C_{14}H_{13}N_5O_3S_4$: 425.9828; found: 425.9821.

2-(5-Methoxybenzimidazol-2-yl)-*N*-[4-(*N*-(thiazol-2-yl)sulfamoyl) phenyl]acetamide (2e): Yield: 72%. M.p. 161–163 °C. ^{1}H NMR (300 MHz, DMSO-d_6, ppm) δ: 3.75 (3H, s, OCH_3), 4.25 (2H, s, CO_2CH_2), 6.73–6.96 (2H, m, Ar–H), 7.24–7.35 (2H, m, Ar–H), 7.58–7.76 (5H, m, Ar–H), 10.85 (1H, s, N–H), 12.72 (1H, brs, NH), 13.39 (1H, brs, NH). ^{13}C NMR (75 MHz, DMSO-d_6, ppm) δ: 36.76 (CH_2), 55.92 (OCH_3), 94.79 (C), 108.61 (2C), 110.22 (C), 110.45 (C), 119.06 (3C), 124.91 (C), 127.54 (3C), 137.01 (C), 142.45 (C), 155.85 (C), 167.21 (C), 169.17 (C=O). $C_{19}H_{17}N_5O_4S_3$ calculated: 47.99% C, 3.60% H, 14.73% N; found: 47.95% C, 3.62% H, 14.70% N. HRMS (m/z): $[M–H]^-$ calcld $C_{19}H_{17}N_5O_4S_3$: 474.0370; found: 474.0355.

2-(5-Nitrobenzimidazol-2-yl)-*N*-[4-(*N*-(thiazol-2-yl)sulfamoyl)phenyl] acetamide (2f): Yield: 69%. M.p. 157–160 °C. ^{1}H NMR (300 MHz, DMSO-d_6, ppm) δ 4.39 (2H, s, CO_2CH_2), 6.81 (1H, d, J=4.59 Hz, Ar–H), 7.25 (1H, d, J=4.62 Hz, Ar–H), 7.58–7.81 (5H, m, Ar–H), 8.05 (1H, d, J=6.84 Hz, Ar–H), 8.30 (1H, s, Ar–H), 10.80 (1H, s, N–H), 12.72 (1H, brs, NH), 13.39 (1H, brs, NH). ^{13}C NMR (75 MHz, DMSO-d_6, ppm) δ: 36.66 (CH_2), 108.61 (C), 118.01 (C), 118.03 (2C), 119.13 (3C), 124.89 (C), 127.55 (3C), 137.08 (C), 142.39 (2C), 166.66 (2C), 169.19 (C=O). $C_{18}H_{14}N_6O_5S_3$ calculated: 44.07% C, 2.88% H, 17.13% N; found: 44.03% C, 2.90% H, 17.10% N. HRMS (m/z): $[M–H]^-$ calcld $C_{18}H_{14}N_6O_5S_3$: 489.0115; found: 489.0098.

2-(5-Chlorobenzothiazol-2-yl)-*N*-[4-(*N*-(thiazol-2-yl)sulfamoyl) phenyl]acetamide (2g): Yield: 75%. M.p. 155–158 °C. ^{1}H NMR (300 MHz, DMSO-d_6, ppm) δ: 4.41 (2H, s, CO_2CH_2), 6.45 (1H, d, J=4.53 Hz, Ar–H), 6.93 (1H, d, J=4.53 Hz, Ar–H), 7.40 (1H, d, J=8.58 Hz, Ar–H), 7.58 (2H, d, J=8.73 Hz, Ar–H), 7.68 (2H, d, J=8.67 Hz, Ar–H), 7.87 (1H, s, Ar–H), 8.04 (1H, d, J=8.58 Hz, Ar–H), 10.58 (1H, s, N–H), 12.71 (1H, brs, NH). ^{13}C NMR (75 MHz, DMSO-d_6, ppm) δ: 38.25 (CH_2), 107.63 (C), 118.64 (2C), 120.97 (C), 123.81 (C), 124.97 (2C), 127.53 (C), 131.67 (C), 134.02 (C), 136.72 (C), 140.53 (C), 140.94 (C), 153.88 (C), 165.72 (C), 169.54 (C), 169.87 (C=O).

$C_{18}H_{13}ClN_4O_3S_4$ calculated: 43.50% C, 2.64% H, 11.27% N; found: 43.54% C, 2.62% H, 11.23% N. HRMS (*m/z*): $[M–H]^-$ calcld $C_{18}H_{13}ClN_4O_3S_4$: 494.9486; found: 494.9471.

Biochemistry assays

MTT assay

3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide tetrazolium salt (MTT) assay is based on colorimetric measurement of the level of cellular reduction. The method was realized according to previously described in the literature [30]. A549 lung cancer and NIH/3T3 mouse embryo fibroblast cell lines were used for cytotoxicity determination. Compounds and positive control, cisplatin were tested in the range of 3.9–500 μg/mL for 24 h incubation. After transformation process of MTT to insoluble formazan crystals by mitochondrial succinate dehydrogenase, absorbance was assigned at 540 nm with a microtitre plate spectrophotometer (Bio-Tek plate reader). All test were triplicated and inhibition concentration (IC_{50}) values were defined as half concentration of the compounds that inhibit cell proliferation.

Matrix metalloproteinase-9 (MMP-9) inhibition

MMP-9, colorimetric kits were supplied from Enzo Life Sciences Inc. (Farmingdale, New York, NY, USA). All tests were realized according to the MMP Colorimetric Drug Discovery Kits manufacturer's manual [31].

Cathepsin inhibition assay

Screening for cathepsin inhibitors was performed with a fluorescence-based assay using the Cathepsin D Activity Fluorometric Assay Kit (Biovision, K143-100) and Cathepsin L Activity Fluorometric Assay Kit (Biovision, K142-100) according to the manufacturer's guidelines [32].

Results

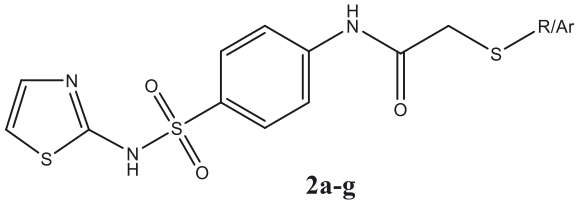
New seven 2-(arylthio)-*N*-[4-(*N*-(thiazol-2-yl)sulfamoyl)phenyl] acetamide derivatives (**2a–g**) were synthesized starting from commercially obtained sulfathiazole via acetylation and subsequent nucleophilic substitution reactions (Scheme 1). The molecular structures of the compounds (Table 1) were verified by spectroscopic techniques; ^1H NMR, ^{13}C NMR, HRMS and elemental analysis data. In the NMR spectra of the compounds, signals at 4.06–4.41 ppm and 36.66–38.47 ppm were assigned to methylene group whereas signal at about 169.17–170.00 ppm was appointed to carbonyl carbon. Amide proton gave signal at 10.52–10.80 ppm while sulfonamide proton gave at 12.70–12.72 ppm. Aromatic protons were observed at estimated regions with appropriate splitting patterns. In high resolution mass spectra of the compounds, negative ion peaks in accordance with molecular weights were detected. Elemental analysis results were also satisfying.

Seven final compounds were tested to determine antiproliferative activity via MTT, MMP-9 and cathepsin inhibition assays. Cytotoxicity was investigated against A549 lung cancer and NIH/3T3 mouse embryo fibroblast cell lines and the results were displayed in Table 1. Compound **2b** exhibited highest inhibitory activity (IC_{50}: 12.33 μg/mL) with selective profile which was better than cisplatin. Compounds **2c**, **2e** and **2g** showed cytotoxicity at IC_{50} concentrations between 53.57 and 71.67 μg/mL whereas remaining compounds did not display activity. Among these, compound **2e** showed selectivity which inhibited tumor cell line and did not inhibit healthy cell line. MMP-9 inhibition is an approach to determine antiproliferative activity. The MMP-9 inhibition percentages caused by the titled compounds were detected at 100 μg/mL concentration (Table 2). The percentages were calculated between ranges 8.10–63.67% whereas standard material, NNGH caused 93.36% inhibition at 1.3 μg/mL concentration. The most cytotoxic compounds **2b** bearing 4-methyltriazole and **2e** bearing 5-methoxybenzimidazole inhibited MMP-9 enzyme with percentages 53.67 and 40.67%, respectively. Cathepsin D and L inhibitory activities of the compounds were also investigated (Table 2). Compounds **2a** and **2b** did not inhibit both of the enzymes whereas **2f** and **2g** did not show cathepsin L inhibitory activity. The remaining compounds displayed weak cathepsin D inhibitory activity which was between the range of 14.87–31.47%. Compound **2c** and **2e** inhibited cathepsin L at most with percentages of 62.75 and 57.25%, respectively. Standard substances for cathepsin D was pepstatin and for cathepsin L was FF-FMK (Z-Phe-Phe-fluoromethyl ketone), they were tested at very low concentrations and inhibited enzymes more than 99%.

Discussion

Benzensulfonamides are versatile motifs which could be evaluated in various biological activities. Since it was discovered in early 1935, the scaffold has been used successfully in different molecules for bacterial infections. Over the years, a wide variety of pharmacological activities

Table 1: The chemical structures and IC_{50} values (μg/mL) of the compounds **2a–g**.



2a-g

Comp.	R/Ar	A549	NIH/3T3
2a	2-Thiazoline	>500	24.33 ± 8.14
2b	4-Methyl-1,2,4-triazole	12.33 ± 2.52	98.33 ± 7.64
2c	1-Methyltetrazole	71.67 ± 10.41	26.33 ± 1.53
2d	5-Methylthiadiazole	>500	>3.90
2e	5-Methoxybenzimidazole	66.67 ± 2.89	375.0 ± 106.07
2f	5-Nitrobenzimidazole	>500	112.50 ± 3.54
2g	5-Chlorobenzotiyazole	53.57 ± 15.50	27.67 ± 2.52
Cisplatin		13.50 ± 2.12	–

–: Not tested.

Table 2: MMP-9, Cathepsin D and L inhibition percentages of the compounds **2a–g** at 100 μg/mL.

Compounds	% Inhibition	Cathepsin D	Cathepsin L
2a	–––	–––	–––
2b	53.67 ± 0.47	–––	–––
2c	17.67 ± 0.47	16.23 ± 3.10	62.75 ± 0.35
2d	8.10 ± 0.95	15.40 ± 2.14	27.75 ± 1.77
2e	40.67 ± 1.54	17.29 ± 3.49	57.25 ± 1.77
2f	63.67 ± 2.35	31.47 ± 1.97	–––
2g	46.50 ± 1.18	14.87 ± 1.88	–––
NNGH (1.3 μM/mL)	93.36 ± 0.82	–	–
Pepstatin A (0.25 μM)	–	99.83 ± 0.25	–
FF-FMK (10 μM)		–	100

–: Not tested. –––: No inhibition.

have been studied on many different derivatives [33]. Numerous sulfonyl or sulfonamide containing analogues have been synthesized and studied for their different anticancer and cytotoxic activities. These derivatives possessed remarkable anticancer activity due to their structure diversity, low toxicities [34]. Hopefully, the studies provide to rise new alternative and more effective anticancer drugs avoiding the side effects of the available pharmacological agents [35]. Based on this purpose new derivatives of sulfathiazoles (**2a–g**) were synthesized and evaluated for antiproliferative activity, in this study. When we considered structures of the compounds, among seven derivatives except compound **2a**, all compound includes various aromatic rings. Compound **2a** contains 2-thiazoline heterocycle. This difference was seen in activity results that compound **2a** did not exhibit any biological activity. This condition expresses the importance of possessing aromatic ring for similar compounds. Among others, compound **2b** and compound **2e** displayed the highest cytotoxicity against A549 with selective profile. Compounds **2b** (4-methyltriazole), **2c** (1-methyltetrazole) and **2d** (1,3,4-thiadiazole) contain five-membered heteroaryl rings. When we evaluate these compounds among themselves, compound **2b** including triazole structure stands out with existing in many drug molecules. Also, triazole ring is reported to affect tumor cells with various biotargets and compounds integrating triazole scaffold with other heterocycles provides to development of drug congener molecules, which allows new biological targets to be achieved, either by increasing their action or lowering of toxicity [36]. Compounds **2e** (5-methoxybenzimidazole), **2f** (5-nitrobenzimidazole) and **2g** (5-chlorobenzothiazole) contain benzocondensed heterocycles. Among these compounds, **2f** did not exhibited cytotoxicity but another benzimidazole derivative compound **2e** showed the highest cytotoxicity. Benzimidazole derivatives are extensively studied molecules and especially the antitumor activity of them has been also working on by us. The methoxy group which is an electron donating group increased antiproliferative activity compared to nitro group (electron withdrawing group) [37].

MMPs are 26-membered zinc dependent endopeptidase enzymes which play essential roles in cell biochemistry. As they increase numerically, they cause impairment in extra cellular matrix (ECM) proteins such as collagen, laminin, elastin, fibronectin, etc. Correspondingly, various ordinary biological processes like cell proliferation, migration, apoptosis, differentiation, angiogenesis, and immune response are

damaged [38]. Among types of MMP enzyme; MMP-9, which is called gelatinase B, causes breakdown of gelatine, some collagens and tissue generation resulting in tumor invasion and metastasis. Essentially, it is considered as biosensors of cancer progression and metastasis, therapeutically. Herewith, MMP inhibition has been thought and evaluated as a new approach to prevent tumor formation, development and treat cancers. In this regard, various compounds were acquired to inhibit MMP-9. Sulfonyl and/or sulfonamide derivatives were also widely studied as MMP-9 inhibitors [39]. In this study, compounds **2f**, **2b** and **2g** exhibited the highest MMP-9 inhibition percentages although not as much as standard drug. Cathepsins are another family of protease enzymes which possess several members found in whole human body. Beside participating to terminal protein degradation, studies are revealed that they have major roles in cell modulation, i.e. of cell growth, adhesion, apoptosis, lipid balance and immune defense [40]. Cathepsin inhibition is another biochemical determination and prevention method for cancer disease. In this study, compounds **2c** and **2e** inhibited cathepsin L at most. In conclusion, we could evaluate that compound **2b** namely 2-(4-methyltriazol-2-yl)-*N*-[4-(*N*-(thiazol-2-yl)sulfamoyl)phenyl]acetamide and **2e** namely 2-(5-methoxybenzimidazol-2-yl)-*N*-[4-(*N*-(thiazol-2-yl)sulfamoyl)phenyl]acetamide are the most active compounds these compounds exhibit MMP-9 and/or cathepsin D/L inhibitions in noticeable levels based on the findings.

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