Synthesis and Anticancer Activity of Some Novel Tolmetin Thiosemicarbazides

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ABSTRACT
A novel series of new ten tolmetin hydrazide derivatives, N-Alkyl/Aryl substituted-2-[[1-methyl-5-(4-methylbenzoyl)-1H-pyrrol-2-yl]acetyl]hydrazinecarbothioamides [4a-j] have been synthesized in this study. The structures of the new compounds were determined by spectral (FT-IR, ¹H-NMR, ¹³C-NMR and 2D-NMR) methods and their purity were proven by elemental analysis and TLC. Tolmetin [1] and N-(4-fluorophenyl)-2-[[1-methyl-5-(4-methylbenzoyl)-1H-pyrrol-2-yl]acetyl]hydrazinecarbothioamide [4d] were evaluated for in vitro using the MTT colorimetric method for anticancer activity. Androgen-independent human prostate cancer cell line PC-3 (ATCC, CRL-1435), human colon cancer cell lines HCT-116 (ATCC, CCL-247) and HT-29 (ATCC, HTB-38) were used in this study. Compound 4d exhibited anticancer activity against PC-3 whereas Tolmetin showed minor activity comparing to compound 4d.

Keywords: Apoptosis, tolmetin, thiosemicarbazide, PC-3, colon cancer.

INTRODUCTION
Tolmetin, 2-[[1-methyl-5-(4-methylbenzoyl)-1H-pyrrol-2-yl] acetic acid is a non-steroidal anti-inflammatory drug. In addition, it has been reported to prevent proliferation of colon cancer cells (1) and inhibit the β-catenin functions (2). Duffy and co-workers evaluated that tolmetin and other (NSAID)s have effects on increasing the cytotoxic activity of the anti-cancer drugs (3). Thiosemicarbazides, which are intermediate products of the synthesis for bioactive heterocyclic compounds, have taken attention of the researchers because of their clinical use and diverse biological activities (4-14) (Fig 1). In this work, we synthesized novel ten tolmetin thiosemicarbazide derivatives (4a-j) and investigated their anticancer activity against cancer cell lines. The characterization of these compounds were identified with the help of elemental analysis, FT-IR, ¹H-NMR, ¹³C-NMR and 2D-NMR spectral data while their purities were analyzed by thin layer chromatography (TLC).
**Experimental**

**Chemistry**

Tolmetin sodium was generously provided by Santa Farma Pharmaceuticals (Istanbul, Turkey). All chemicals were purchased from Merck, Sigma-Aldrich, or Fluka. Reactions were monitored by TLC on silica gel plates purchased from Merck. Melting points of the synthesized compounds were determined in a Thermoscientific IA 9300 melting point apparatus and are uncorrected. The purity of the compounds was checked on TLC plates precoated with silica gel G using the solvent systems M1 (petroleum ether/acetone 50:50 v/v), M2 (petroleum ether/acetone 30:70 v/v). The spots were located under UV light (254 nm) (t = 21 °C). Elemental analyses were performed on a CHNS-932 (LECO). FT-IR spectra were recorded on a Shimadzu FT-IR-8400S spectrophotometer. **1H-NMR and 13C-NMR spectra were recorded on BRUKER 300 MHz (4a, 4b, 4c, 4d, 4i), 600 MHz (4e, 4f, 4g, 4h, 4j) and 75 MHz Ultrashield TM. NMR spectrometers using DMSO-d6 as solvent. Chemical shifts (d) are reported in parts per million (ppm). Data are reported as follows: chemical shift, multiplicity (s: singlet, d: doublet, m: multiplet and t: triplet, bs: broad singlet, b: broad, ss: singlet-singlet) coupling constants (Hz), integration.**

**Preparation of 2-[1-Methyl-5-(4-methylbenzoyl)-1H-pyrrol-2-yl]acetic acid [1], Methyl 2-[1-methyl-5-(4-methylbenzoyl)-1H-pyrrol-2-yl]acetate [2] and 2-[1-methyl-5-(4-methylbenzoyl)-1H-pyrrol-2-yl]acetohydrazide [3]**

Compounds [1-3] were prepared as described previously (15,16).

**General procedure for the synthesis of N-Alkyl/Arylsubstituted-2-[1-methyl-5-(4-methylbenzoyl)-1H-pyrrol-2-yl]acetylhydrazinecarbothioamides [4a-j]**

Compound 3 (0.001 mol) was dissolved in ethanol (10 ml) and equimolar amounts of appropriate aliphatic/aromatic isothiocyanates in absolute ethanol was heated under reflux for 2 to 6 hrs. The obtained precipitate was filtered off, dried, and recrystallized twice from ethanol.

**N-methyl-2-[[1-methyl-5-[(4-methylphenyl)carbonyl]-1H-pyrrol-2-yl]acetyl]hydrazinecarbothioamide (4a) [SGK 521]**

Light yellow solid. MW: 344.1306 g/mol. m.p. 160 °C. Yield 96.77%. Rf x 100 value: 60 (M1). FT-IR (v max, cm⁻¹): 3535 (Amide N-H), 1680 (C=O), 1257 (C=S). **1H NMR (300 MHz, DMSO-d6):** δ (ppm): 1.05 (t, CH₃, peak of ethanol held by the molecule); 2.38 (s, 3H, Ar-CH₃), 2.88 (d, 3H, CS-NH-CH₃), 3.66 (s, 2H, -CH₂-C=O), 3.85 (s, 3H, -N-CH₃) 3.55 (CH₂ peak of ethanol held by the molecule); 4.26 (–OH, peak of ethanol held by the molecule); 6.12 (d, 1H, pyrrol H₃, J=4.2 Hz); 6.56 (d, 1H, pyrrol H₄, J=4.2 Hz) 7.30 (d, 2H, o-protons, Ar-CH₃, J=8.1 Hz), 7.61 (d, 2H, m-protons, Ar-CH₃, J=8.1 Hz), 8.01 (s, 1H, CH-NH-CS and NH-C-SH), 10.04 (bs, 1H, NH-C=O). Anal calcd. for C₁₇H₂₀N₄O₂S. 1/2 C₂H₅OH: C, 58.85; H, 6.27; N, 15.26; S, 8.72%. Found: C, 58.66; H, 6.219; N, 15.41; S, 8.96%.

**N-ethyl-2-[[1-methyl-5-[(4-methylphenyl)carbonyl]-1H-pyrrol-2-yl]acetyl]hydrazinecarbothioamide (4b) [SGK 522]**

Lighy cream solid. MW: 358.1463 g/mol. m.p. 169-170 °C. Yield 87 %. Rf x 100 value: 59 (M1). FT-IR (v max, cm⁻¹): 3215 (Amide N-H), 1678 (C=O), 1263 (C=S). **1H NMR (300 MHz,
N-phenyl-2-[(1-methyl-5-[4-methylphenyl]carbonyl]-1H-pyrrolo-2-yl]acetyldrazincarbothioamide (4c) [SGK 523]

Grey solid. MW: 406.1463 g/mol. m.p. 170 °C. Yield 98%. Rf x 100 value: 49 (M.). FT-IR (ν_max, cm⁻¹): 3329–3280 (Amide N-H), 1654 (C=O), 1267 (C=S). ¹H NMR (300 MHz, DMSO-d₆): δ (ppm): 2.38 (3H, Ar-CH₃); 3.72 (2H, -CH₂-C=O); 3.88 (3H, -N-CH₃); 6.15 (1H, pyrrol H_j, J=3.9 Hz); 6.58 (1H, pyrrol H_j, J=3.9 Hz); 7.31 (2H, o-protons, Ar-CH₃, J=8.7 Hz); 7.62 (2H, m-protons, Ar-CH₃, J=8.1 Hz); 7.15-7.20; 7.35-7.45 (5H, Ar-H); 9.52 (1H, NH-aryl); 9.66, 9.74 (bs, 1H, -NH-aryl); 10.24 (s, 1H, -NH-C=O). Anal. calcd. for C₂₂H₂₂N₄O₃S: C, 65.00; H, 5.46; N, 7.15; S, 7.52%. Found: C, 64.64; H, 5.73; N, 13.67; S, 7.52%.

N-(4-fluorophenyl)-2-[(1-methyl-5-[4-methylphenyl]carbonyl]-1H-pyrrolo-2-yl]acetyldrazincarbothioamide (4d) [SGK 524]

Light purple solid. MW: 424.1369 g/mol. m.p. 206-208 °C. Yield 87%. Rf x 100 value: 52 (M.). FT-IR (ν_max, cm⁻¹): 3553 (Amide N-H), 1651 (C=O), 1269 (C=S). ¹H NMR (300 MHz, DMSO-d₆): δ (ppm): 2.39 (3H, Ar-CH₃), 3.73 (2H, -CH₂-C=O), 3.89 (3H, N-CH₃), 6.16 (1H, pyrrol H_j, J=3 Hz), 6.58 (1H, pyrrol H_j, J=3 Hz), 7.15 (2H, o-protons, 4-fluorophenyl, J=9 Hz), 7.37-7.59 (5H, Ar-H), 9.71 (2H, NHCSNH), 10.24 (s, 1H, NH-C=O). ¹³C-NMR (75 MHz) (DMSO-d₆/TMS) δ ppm: 21.52 (C-2), 32.35 (C-6), 33.53 (C-7), 109.60 (C-3), 110.01 (C-4), 115.29 (C-25, C-27), 122.16 (C-24, C-28), 128.59 (C-5), 129.20 (C-18, C-20), 129.42 (C-17, C21), 130.74 (C-2), 135.85 (C-23), 137.25 (C-16), 137.48 (C-26), 141.98 (C-19), 161 (thiosemicarbazide C=S), 168 (thiosemicarbazide C=O) 184.87 (C=O). D₂O (600 MHz) (D₂O/TMS) δ ppm: 2.34 (3H, Ar-CH₃), 3.71 (2H, -CH₂-C=O), 3.82 (3H, N-CH₃), 6.14 (1H, pyrrol H_j, J=3.6 Hz), 6.56 (1H, pyrrol H_j, J=3.6 Hz), 7.15 (2H, o-protons, 4-fluorophenyl, J=9 Hz), 7.37-7.59 (5H, Ar-H). Thiosemicarbazide protons were exchanged with docterium. Anal. calcd. for C₂₂H₂FN₄O₃S: C, 62.25; H, 4.99; N, 13.20; S, 7.55%. Found: C, 62.06; H, 5.05; N, 12.85; S, 6.97%.

N-(4-methoxyphenyl)-2-[(1-methyl-5-[4-methylphenyl]carbonyl]-1H-pyrrolo-2-yl]acetyldrazincarbothioamide (4e) [SGK 525]

Light yellow solid. MW: 436.1569 g/mol. m.p. 173-174 °C. Yield 50%. Rf x 100 value: 46 (M.). FT-IR (ν_max, cm⁻¹): 3396–3344 (Amide N-H), 1656 (C=O), 1246 (C=S). ¹H NMR (600 MHz, DMSO-d₆): δ (ppm): 2.39 (3H, Ar-CH₃), 3.72 (2H, -CH₂-C=O), 3.76 (3H, -N-CH₃), 3.88 (3H, -O-CH₃), 6.15 (1H, pyrrol H_j, J=4.2 Hz); 6.58 (1H, pyrrol H_j, J=4.2 Hz); 6.91 (2H, o-protons, Ar-CH₃, J=9 Hz); 7.25 (2H, m-protons, Ar-CH₃, J=9 Hz); 7.32 (2H, o-protons, Ar-CH₃, J=7.8 Hz); 7.62 (2H, m-protons, Ar-CH₃, J=7.8 Hz); 9.56 (bs, 1H, -NH-Ar), 9.60 (bs, 1H, -NH-CS-NH), 10.20 (s, 1H, -NH-C=O). Anal. calcd. for C₂₂H₂₂N₄O₃S: C, 63.28; H, 5.54; N, 12.83; S, 7.35%. Found: C, 63.27; H, 5.16; N, 12.43; S, 7.50%.

N-(4-chlorophenyl)-2-[(1-methyl-5-[4-methylphenyl]carbonyl]-1H-pyrrolo-2-yl]acetyldrazincarbothioamide (4f) [SGK 526]

White solid. MW: 463.9799 g/mol. m.p. 203-205 °C. Yield 82.4%. Rf x 100 value: 56 (M.). FT-IR (ν_max, cm⁻¹): 3396–3205 (Amide N-H), 1656 (C=O), 1267 (C=S). ¹H NMR (600 MHz, DMSO-d₆): δ (ppm): 1.33 (t, CH₃ peak of ethanol held by the molecule); 2.39 (3H, Ar-CH₃), 3.55 (CH₃ peak of ethanol held by the molecule); 3.73 (2H, -CH₂-C=O), 3.88 (3H, -N-CH₃), 4.5 (OH peak of ethanol held by the molecule); 6.15 (1H, pyrrol H_j, J=4.2 Hz); 6.58 (1H, pyrrol H_j, J=4.2 Hz); 7.31 (2H, o-protons, Ar-CH₃, J=7.8 Hz); 7.40–7.48 (4H, Ar-H); 6.73 (2H, m-protons, Ar-CH₃, J=7.8 Hz); 9.53, 9.77 (bs, 2H, NH-CS-NH), 10.25 (s, 1H, NH-C=O). Anal. calcd. for C₂₂H₁₇ClN₄O₃S: C, 59.54; H, 5.21; N, 12.08; S, 6.91%. Found: C, 59.80; H, 5.04; N, 12.09; S, 6.71%.

N-(4-nitrophenyl)-2-[(1-methyl-5-[4-methylphenyl]carbonyl]-1H-pyrrolo-2-yl]acetyldrazincarbothioamide (4g) [SGK 527]

Yellow solid. MW: 460.5058 g/mol. m.p. 207-208 °C. Yield 88%. Rf x 100 value: 64 (M.). FT-IR (ν_max, cm⁻¹): 3395–3333 (Amide N-H), 1680 (C=O), 1267 (C=S). ¹H NMR (600 MHz, DMSO-d₆): δ (ppm): 2.39 (3H, Ar-CH₃), 3.75 (2H, -CH₂-C=O), 3.89 (3H, -N-CH₃), 6.17 (1H, pyrrol H_j, J=4.2 Hz); 6.59 (1H, pyrrol H_j, J=4.2 Hz); 7.31 (2H, m-protons, Ar-CH₃, J=7.8 Hz); 7.63 (2H, m-protons, Ar-CH₃, J=7.8 Hz); 7.90 (2H, m-protons, Ar-nitro, J=9 Hz); 8.23 (2H, o-protons, Ar-nitro, J=7.8 Hz); 10.09 (bs, 1H, -NH-Ar), 10.32 (bs, 1H, -NH-CS-NH), 10.50 (bs, 1H, -NH-C=O). Anal. calcd. for C₂₂H₂₁N₄O₃S: C, 57.38; H, 4.82; N, 15.21; S, 6.96%. Found: C, 57.08; H, 4.46; N, 14.89; S, 6.68%.
N-(2,4-dichlorophenyl)-2-\{1-methyl-5-[(4-methylphenyl)carbonyl]-1H-pyrrol-2-yl\} acetyl hydrazinecarbothioamide (4h) [SGK 528]

White solid. MW. 420.527 g/mol. m.p. 203-205° C. Yield 96.42 %. Rf x 100 value: 64 (M 2). FT-IR (v max , cm⁻¹): 3363 (Amide N-H), 3286-3288 (Amine N-H), 1647 (C=O), 1638 (C=S). 1H NMR (600 MHz, DMSO-d 6): δ (ppm): 1.2 (–CH 3 , peak of ethanol held by the molecule); 3.54 (–CH 3 , peak of ethanol held by the molecule); 2.39 (s, 3H, Ar-CH 3 ); 3.73 (s, 2H, -CH 2 -C=O); 3.88 (s, 3H, -N-CH 3 ); 6.15 (d, 1H, pyrrol H 3 , J=3.6 Hz); 6.58 (d, 1H, pyrrol H 4 , J=4.2 Hz); 7.31 (d, 2H, o-protons, Ar-CH 3 , J=8.4 Hz); 7.42-7.45 (m, 2H, Ar-CH 3 ); 7.69-7.72 (m, 1H, Ar-H); 9.27 (s, 1/2 H, NH-CS-NH-Ar); 9.39, 9.89 (ss, 1H, NH-C=S and NH-C-SH); 10.32 (s, 1H, NH-C=O). Anal. calcd. for C 22 H 21 BrN 4 O 2 S: C, 56.15; H, 4.16; N, 11.44; S, 6.31%. Found: C, 56.42; H, 4.65; N, 11.24; S, 6.43%.

N-(benzyl)-2-\{1-methyl-5-[(4-methylphenyl)carbonyl]-1H-pyrrol-2-yl\} acetyl hydrazinecarbothioamide (4i) [SGK 529]

White solid. MW. 420.527 g/mol. m.p. 203-205° C. Yield 96.42 %. Rf x 100 value: 64 (M 2). FT-IR (v max , cm⁻¹): 3286-3138 (Amide N-H), 1680 (C=O), 1626 (C=S). 1H NMR (300 MHz, DMSO-d 6): δ (ppm): 2.38 (s, 3H, Ar-CH 3 ); 3.67 (s, 2H, -CO-CH 2 ), 3.83 (s, 3H, -N-CH 3 ); 4.75 (d, 2H, -NH-CH 2 -Ar), 6.12 (d, 1H, pyrrol H 3 , J=3.9 Hz), 6.56 (d, 1H, pyrrol H 4 , J=4.2 Hz). 7.21-7.63 (m, 9H, Ar-H), 8.57 (s, 1H, CS-NH-CH 2 -), 9.42 (s, 1H, NH-CS-NH-Ar), 10.08 (bs, 1H, CO-NH-). Anal. calcd. for C 23 H 24 N 4 O 2 S: C, 65.69; H, 5.75; N, 13.32; S, 7.62%. Found: C, 65.24; H, 5.98; N, 13.17; S, 7.42%.

N-(4-bromo phenyl)-2-\{1-methyl-5-[(4-methylphenyl)carbonyl]-1H-pyrrol-2-yl\} acetyl hydrazinecarbothioamide (4j) [SGK 530]

Dark White solid. MW. 484.0568 g/mol. m.p. 204-205 ° C. Yield 92.04 %. Rf x 100 value: 48 (M 2). FT-IR (v max , cm⁻¹): 3288 (Amide N-H), 1672 (C=O), 1257 (C=S). 1H NMR (600 MHz, DMSO-d 6): δ (ppm): 2.39 (s, 3H, Ar-CH 3 ); 3.73 (s, 2H, -CH 2 -C=O); 3.87 (s, 3H, -N-CH 3 ); 6.15 (d, 1H, pyrrol H 3 , J=4.2 Hz); 6.59 (d, 1H, pyrrol H 4 , J=4.2 Hz); 7.31 (d, 2H, o-protons, Ar-CH 3 , J=8.4 Hz); 7.44-7.54 (m, 4H, Ar-H) ); 7.63 (d, 2H, m-protons, Ar-CH 3 , J=8.4 Hz); 9.53; 9.78 (s, 2H, NH-C=S and NH-C-SH); 11.52; H, 4.16; N, 11.52; S, 6.17%.

Biological Activity

Cell Treatment:

Androjen-independent human prostate cancer cell line PC-3 (ATCC, CRL-1435), human colon carcinoma cell lines HCT-116 (ATCC, CCL-247), HT-29 (ATCC, HTB-38) and mouse embryonic fibroblast cell line NIH3T3 (ATCC, CRL-1658) were maintained in Dulbecco modified Eagle's medium (DMEM) supplemented with 10% FBS and 1% penicillin/streptomycin in the presence of 5% CO 2 in air at 37 °C. Tolmetin and compound 4d were dissolved in DMSO and the cells were treated with increasing doses of compounds for 24 h in 37 °C.

Cell Viability Assay:

Cell viability was determined by the MTT assay method. Briefly, PC-3 cells (1x10⁴ cells/well) were seeded into 96-well plates and incubated for 24 h at 37°C in CO 2 incubator. Then, the cells were grown in the absence or presence of increasing doses of tolmetin and compound 4d at 37°C. After 24 h treatment, the media was removed and then 10 μL of MTT (5 mg/mL in phosphate-buffered saline) was added to each well for an additional 4 hours. The precipitated formazan was dissolved in 100 μL of 10 % SDS and the absorbance was taken at 570 nm. The percentage of viability was calculated as the following formula: (viable cells)%=(OD of treated sample/OD of untreated sample)×100.

RESULTS AND DISCUSSION

Synthesis of Tolmetin Thiosemicarbazides

Tolmetin, 2-[1-methyl-5-(4-methylbenzoyl)-1H-pyrrol-2-yl]acetic acid was chosen as the starting compound to design several novel thiosemicarbazides. As shown in the synthesis scheme (Fig 2), tolmetin was first synthesized from tolmetin sodium dihydrate by hydrolysing the dihydrate in conditions. Methyl 2-[1-methyl-5-(4-methylbenzoyl)-1H-pyrrol-2-yl]acetate [2] has been synthesized from tolmetin [1] with methanol and few drops of concentrated sulfuric acid. 2-[1-Methyl-5-(4-methylbenzoyl)-1H-pyrrol-2-yl]acetohydrazide [3] was synthesized from compound [2], which is an ester, with hydrazine hydrate and methanol. N-Alkyl/Arylsulfonated-2-\{1-methyl-5-(4-methylbenzoyl)-1H-pyrrol-2-yl\}acetohydrazide [4a-j] were synthesized with the reaction of compound [3] and substituted alkyl/aryl isothiocyanates. The synthesized compounds were identified by FT-IR, 1H-NMR, 13C-NMR and 2D-NMR (only 4d) and their purity were proven by elemental analysis and TLC.
Fig 2. Synthesis of tolmetin thiosemicarbazides [4a-j]

2-[(1-methyl-5-(4-methylbenzoyl)-1H-pyrrol-2-yl)acetohydrazide was synthesized by Koç and co-workers (15), previously and –NH₂ protons of hydrazide were reported in 4.27 ppm. ¹H-NMR spectrum of the compounds [4a-j] proves the disappearance of –NH₂ protons which belong to –CONHNH₂ functional group. N₁, N₂ and N₄ protons which prove the presence of thiosemicarbazides were detected in ¹H-NMR spectrum (Fig 3) and the information given is supported by the literature. (17-22).

Fig 3. N₁, N₂ ve N₄ protons of tolmetin thiosemicarbazides

Thiosemicarbazide protons were detected as follows; N₁: 9.98-10.32 ppm, N₂: 9.21-10.50 ppm, respectively. N₄ protons were detected in different ppm values as this data can change depending on the substituent which can have aryl or alkyl functionality. The ppm values of N₄ protons is known to be in the aromatic field or outside of the aromatic field. Compounds [4a-b] bearing methyl and ethyl substituted, their N₄ protons were detected at 8.01-8.02 ppm. Other compounds bearing phenyl, 4-fluorophenyl, 4-methoxyphenyl, 4-chlorophenyl, 4-nitrophenyl, 2,4-dichlorophenyl, benzyl and 4-bromophenyl substituted, their N₄ protons were detected at 8.57-9.78 ppm. N₄ proton of compound [4h] has partly exchanged with deuterium. Ethyl derivative compound [4b] was studied ¹H-NMR at 300 MHz in DMSO-d₆ and the ethyl N-CH₂ peak was overlaped in the solution peak.

According to the ¹H-NMR of results some compounds (4a, 4c, 4e, 4f, 4h), it was determined that these compounds undergo thione-thiol tautomerism (Fig 4).

Fig 4. Thione-thiol tautomerism of tolmetin thiosemicarbazides

In addition, -NH proton of compound [4d] was observed to exchange with D₂O in the spectrum (Fig 5).

Fig 5. D₂O spectrum of compound [4d]

Compound [4d] was chosen as the prototype for the ¹³C NMR spectrum. The presence of C=O and C=S carbons in ¹³C-NMR spectrum proves the synthesis of thiosemicarbazide. C=O and C=S peak values can be seen in Fig 6. According to both HMBC and ¹³C-NMR data, C=O and C=S peaks were observed at 168 ppm and 161 ppm, respectively. (Fig 6-7).
Küçükgüzel and co-workers (16) studied the anticancer activity of Tolmetin [1] and N'-[(2,6-dichlorophenyl)methyldiene]-2-[(1-methyl-5-(4-methylbenzoyl)-1H-pyrrol-2-yl)aceto hydrazide against HCT-116 (ATCC, CCL-247) and HT-29 (ATCC, HTB-38) human colon cancer cell lines, using MTT assay. The results revealed that tolmetin hydrazone showed anticancer activity against HT-29 colon cancer cell line (dose depended) with IC$_{50}$ value of 76 µM. Tolmetin hydrazone was then used in further tests to investigate the apoptotic effect on HT-29 cancer cell line. Apoptosis is known to be an important mechanism of cancer growth and therefore, caspase 3, caspase 8 and caspase 9 enzyme levels were observed in apoptotic pathway. This study proves that tolmetin hydrazone triggers apoptosis using caspase 8 pathway in HT-29 cancer cell line.

Prostate cancer is known to be the second cancer disease after lung cancer with the incidence of 24.33/100.000 in our country. It is also reported as a common cancer type in USA and Europe (23). Demertzii et al. (24) reported the antineoplastic activity of thiosemicarbazones (TSC). TSC can bind the heavy metals with its two nitrogen and one sulphur atom, occuring a triple chelation. TSCs were reported to be strong inhibitors of ribonucleotide reductase (RR) which reduces ribonucleotides to deoxyribonucleotides (25). Ribonucleotide reductase is an important enzyme in cell division and tumor growth. TSCs, RR inhibitors, inhibits the DNA synthesis and DNA repair; therefore they show antineoplastic activity. (26, 27). Ferrari et al. (28) reported the biological activities of TSCs depends on the Van der Walls and hydrogen bonds between the molecule and DNA. Beytur et al. (29) studied the effects of TSCs and their metal complexes on LNCaP cell viability and found out all the metal complexes (except Ni complex) have dose depended effects on LNCaP cells. However, they did not observe any activity against PC-3 cell lines (except the 25 and 50 µM concentrations). The antitumor activity of TSCs against LNCaP cells depends on the DNA damage in the presence of androgen receptors.

N-(4-fluorophenyl)-2-[(1-methyl-5-[4-methylphenyl] carbonyl]-1H-pyrrol-2-yl]acetylhydrazinocarbothioamide [4d] bearing thiosemicarbazide and fluoro substitution and Tolmetin were chosen for the anticancer activity test in M.U. Faculty of Pharmacy, Department of Biochemistry. HCT-116 (ATCC, CCL-247) and HT-29 (ATCC, HTB-38) human colon cancer cell line and PC-3 (ATCC, CRL-1435) cell lines were used in this study. MTT assay results shows that, tolmetin thiosemicarbazide [4d] showed anticancer activity (dose depended) only to androgen independent prostate cancer cell line PC-3 with IC$_{50}$ value of 184.5 µM (Fig 8). Besides, tolmetin and compound 4d cytotoxicity tests were carried out with NIH-3T3 fibroblast cells (Fig 9).
Uncontrolled cell growth is not enough for cancer formation. The cell should gain the ability of invasion and metastasis. Extracellular matrix molecules (ECM) is the primer barrier molecule for the inhibition of tumor growth and tumor invasion (30). ECM has the structure of proteins and proteoglycans. Besides the ability of structural support of the cells, ECM has biological activity on cell proliferation, cell differentiation, migration, and adhesion, tissue morphogenesis. Cancer cells use metalloproteinases to get over that barrier. In order to complete invasion or metastasis, cancer cells should destroy the ECM molecules. Matrix metalloproteinases (MMP) are extracellular proteases that consist of 28 different enzymes and responsible for physiological and pathological tissue destruction. These enzymes were first identified by Jerome Gross and Charles Lapiere in 1962 (31).

The destruction of MMP, ECM, can be seen in different physiological and pathological situations (32). MMPs belong to neutral endopeptidases enzyme family and consist zinc ion, they also have the ability of destroying whole ECM members. Among these members, MMP-2 and 9 levels significantly increase in metastatic prostate cancer (33). These two enzyme levels decrease after the treatment and therefore, they can be evaluated for the efficacy of the treatment.

MMP inhibitors pharmacologically are divided into three different groups as peptides and non-peptides collagen-like inhibitors, Tetracycline and Biphosphonates. In early 1980’s, the first synthetic MMP inhibitor was developed as pseudopeptide derivative that imitate the collagen structure in MMP separation area. These inhibitors reversibly bind the MMP’s active side. They also create chelation with MMP’s active zinc ion with their hydroxamic acid. New molecules are developed from the derivatives of Tetracyclines and they have the ability of inhibiting MMPs rather than showing antibacterial activity. Tetracyclines could be preventing the avidity to zinc atom or they could inhibit the regulation of MMP during the transcription. Metastat (Col-3), minocyclin and doxycycline are the examples of these inhibitors (34).

Küçükgüzel and co-workers (16) synthesized N′-{[(2,6-dichlorophenyl)methyliden]-2-[1-methyl-5-(4-methylbenzoyl)-1H-pyrrol-2-yl]acetohydrazide and found activity against HT-29 colon cancer cell line with IC_{50} value of 76 µM. This information showed, against colon cancer cell line anticancer activity of tolmetin maintains whereas the thiosemicarbazide molecule from the same compound have no activity against colon cancer, instead, it shows activity against prostate cancer cells. We can conclude that, the electrophilic compounds in the cell bind the sulphur atom in the molecule. Furthermore, thiosemicarbazide may be creating a chelation with the zinc ion in the metalloproteinase enzyme as M. Öncel reported before (34). (Fig 10).

**Fig 10. Proposed chelation with zinc ion and thiosemicarbazide**

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**DECLARATION OF INTEREST**

The authors have declared no conflicts of interest. The authors alone are responsible for the content and writing of this article.
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