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# Synthesis, structure elucidation and cytotoxic activities of 2,5-disubstituted-1,3,4-thiadiazole and l,2,4-triazole-3-thione derivatives

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**ABSTRACT**: In this study, a series of 1,3,4-thiadiazole **(1b-9b)** and l,2,4-triazole-3-thione **(1c-9c)** derivatives were synthesized. The reaction proses was carried out with the cyclocondensation of suitable 1,4-disubstituted thiosemicarbazide derivatives (**1a-9a**). The structures of the synthesized compounds were confirmed by the data obtained from elemental analysis, HPLC, UV, IR, $^1$H-NMR and MS spectra. All of the compounds were tested for their cytotoxic activities against L929 fibroblast cells by MTT method. It was determined that the tested compounds 1a-9a, 1b-9b and 1c-9c were not cytotoxic at the studied concentrations (5.0 µg/mL and 10.0 µg/mL) in L929 cell lines. Compounds 1a-c, 2a-c, 3a-c, 4a-c, 5a-c and 8a-c showed increased growth inhibition whereas compounds 6a-c, 7a-c and 9a-c showed decreased growth inhibition on L929 cell lines

**KEYWORDS**: Acid hydrazide; cytotoxicity; 1,3,4-thiadiazole; 1,4-disubstituted thiosemicarbazide; 1,2,4-triazole-3-thione.

## 1. INTRODUCTION

Heterocyclic compounds are important structures due to their unique chemical properties and extensive biological activities. The 1,3,4-thiadiazole and 1,2,4-triazole derivatives, which constitute the skeleton of our study, have a broad spectrum of biological activities, including antibacterial [1], antifungal [2], antiviral [3], antiepileptic [4], antidiabetic [5], analgesic [6], and anti-inflammatory activities [7].

1,3,4-Thiadiazoles are aromatic compounds having a heterocyclic ring containing nitrogen and sulfur atoms as the isomer of the thiadiazole series. Due to the presence of the =N-C-S moiety, 1,3,4-thiadiazole derivatives are thought to have a variety of biological properties [8]. Researchers found out that the biological activity mostly depends on 1,3,4-thiadiazole aromaticity, which gives the ring a great *in vivo* stability and low toxicity [9], [10]. Acetazolamide, methazolamide, megazol and cefazolin are examples of drugs that contain a thiazole ring and are available in the market (Figure 1).

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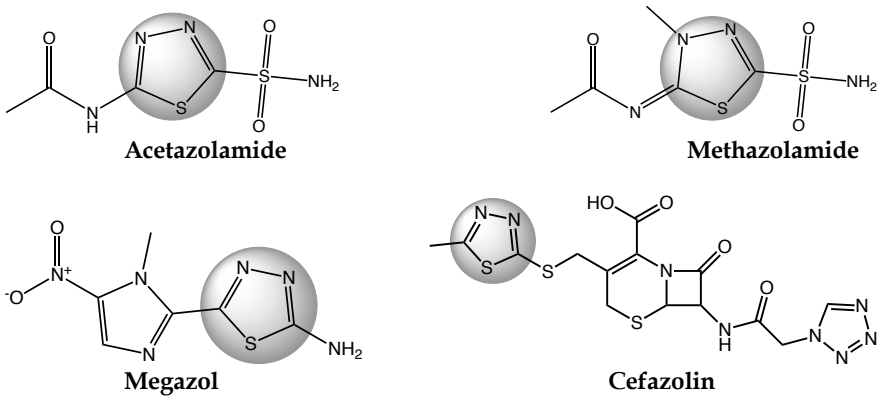


**Figure 1.** Some marketed drugs carrying a thiazole ring

1,2,4-triazole derivatives occupy a great place in medicinal chemistry field [11]. The triazole ring system presents a significant pharmacophore group with the ability of high receptor affinity the besides the weak cyctotoxic effect in human normal cells [12], [13]. These features mostly depend on their dipole moment, rigidity and solubility [14]. This ring system exist in a number of compounds used in clinical medicine, including anastrozole, fluconazole, itraconazole, trazodone, ribavirin and loreclezole as stable and difficult to cleave (Figure 2).

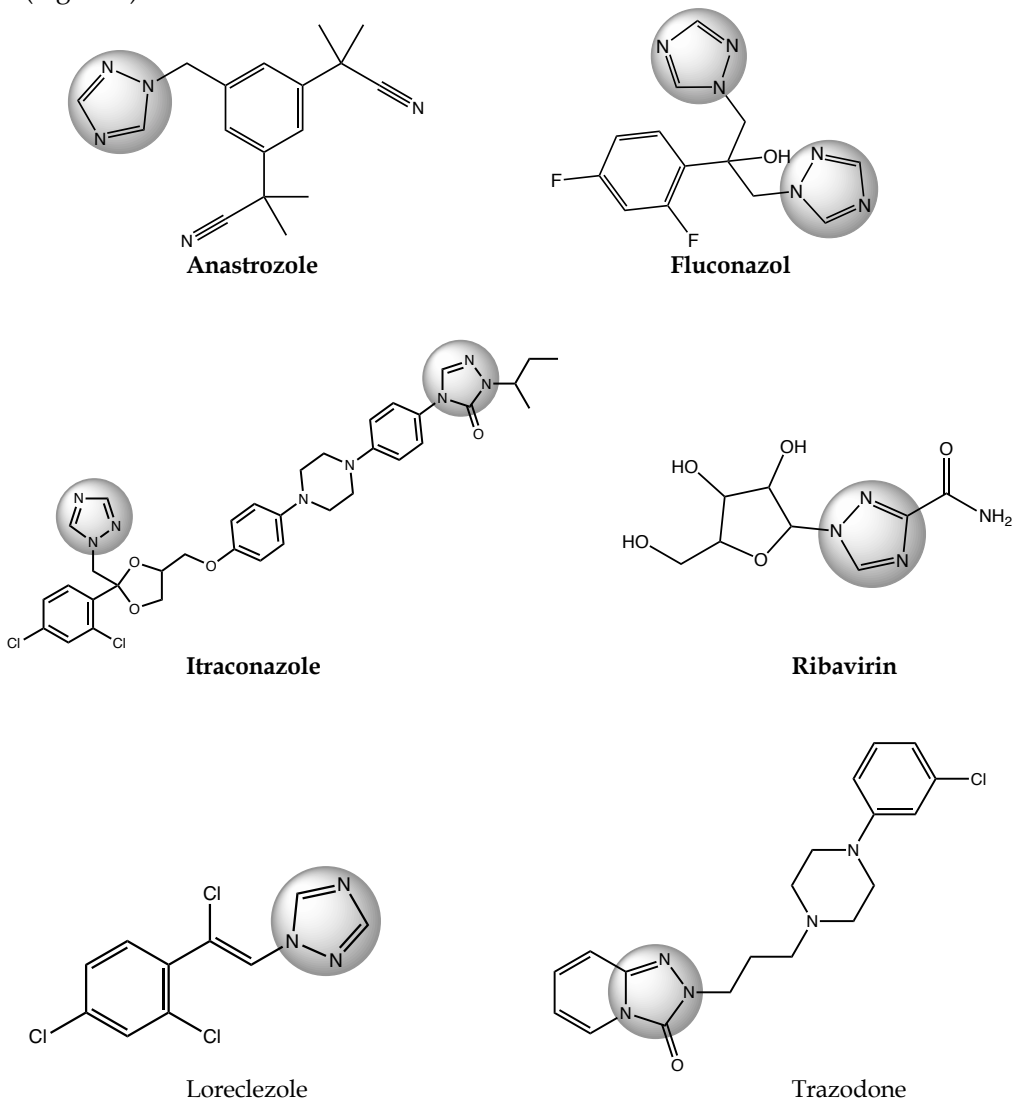


**Figure 2.** Some marketed drugs carrying a triazole ring

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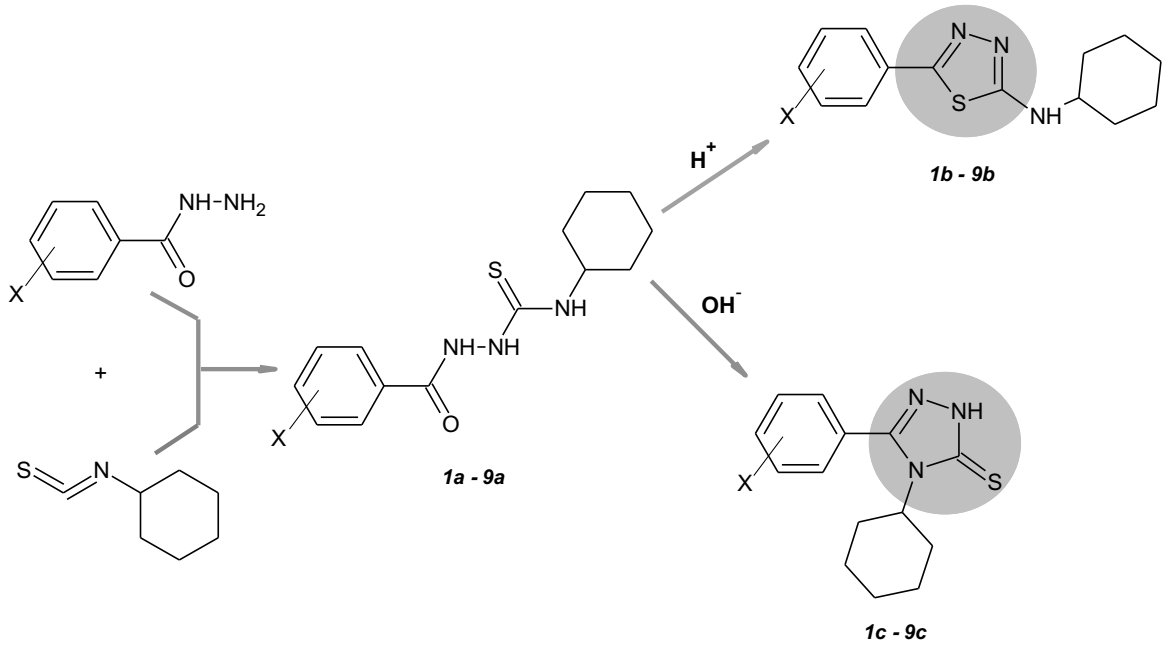
The increased technology and new medicinal development in health sciences, the ideal is believed to have cytotoxicity only at very high concentrations. On the other hand, the biological activity like antiviral, antibacterial and anticancer effects must be observed at very low concentrations. If a drug molecule does not have a selectivity on certain macromolecules, then it may show an undesirable adverse effect. Taking these into account, medicinal chemists seek rational design to develop new molecules based on medicinal agents with better activity and lower toxicity.

For this purpose, we designed, synthesized, and studied *in vitro* cytotoxic activities of a series of 1,3,4-thiadiazole **(1b-9b)** and 1,2,4-triazole-3-thione derivatives (**1c-9c**) against fibroblast cell line (L929). The structure of the target compounds was verified by the data obtained from elemental analysis, HPLC, UV, IR, $^1$H-NMR, and MS spectra. Further, the prediction of physicochemical and ADME properties of synthesized compounds was utilized by using the SwissADME tool.

## 2. RESULTS AND DISCUSSION

### 2.1. Chemistry

Our synthetic strategy for the target compounds is illustrated in Scheme 1. 1,4-Disubstituted thiosemicarbazide **(1a-9a)** which were obtained by the reaction of 2-fluoro/3-fluoro/4-fluoro/2-chloro/3-chloro/3-bromo/4-bromo/4-hydroxy/4-nitrophenyl hydrazide and cyclohexyl isothiocyanate in ethanol. Thereafter, 1,3,4-thiadiazole derivatives (**1b-9b**) were prepared by cyclization of **1a-9a** with conc. $H_2SO_4$. 2,4-dihydro-3*H*-1,2,4-triazole-3-thione derivatives (**1c-9c**) were synthesized as a result of base-catalyzed cyclization of **1a-9a** in ethanol (Scheme 1).




| 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 |
|---|---|---|---|---|---|---|---|---|
| 2-F | 3-F | 4-F | 2-CI | 3-CI | 3-Br | 4-Br | 4-OH | 4-$NO_2$ |

**Scheme 1.** The synthesis method of the target compounds

The structures of the synthesized compounds were confirmed by FT-IR, $^1$H-NMR, Mass spectroscopy and elemental analysis. The spectral data was consistent with the target structures.

According to IR spectra; characteristic C=O stretching vibrations bands of compounds **1a-9a** were detected in the range 1678-1636 cm$^{-1}$, this band has disappeared in the IR spectra of compounds **1b-9b** and **1c-9c**. NH and C=S stretching vibrations of compounds **1c-9c** were assigned in the range 3666-3177 cm$^{-1}$ and 1242-1103 cm$^{-1}$, respectively. According to the $^1$H-NMR spectra; N-H protons of 1,4-disubstituted thiosemicarbazide derivatives **(1a-9a)**, secondary amine NH protons of 2,5-disubstituted-1,3,4-thiadiazole derivatives **(1b-9b)** and NH protons of 2,4-dihydro-1,2,4-triazole-3-thiones (**1c-9c)** were determined in the range of 10.82-10.22, 9.70-9.31 and 8.30-7.85 ppm, 9.73.-7.20 ppm and 14.61-13.91 ppm in accordance with the literature [15], respectively.

The triazole NH peaks for the compounds **(1c-9c)** were observed in the range of 14.61-13.91 ppm; confirming the thione formation [16]. These results showed that compounds with the thione form were the predominant tautomer in both solid and solution form of the molecules.

The mass spectral fragmentation of the molecules has been studied by atmospheric pressure ionization-electrospray (API-ES) technique, the M-H$^+$ or M+H$^+$ peaks were characterized in agreement with structure of the compounds. Fragments in the mass spectra of **1b-9b** and **1c-9c** compounds were in accordance with the literature [17], [18], [19], [20], [21], [22], [23].

## 2.2. Cytotoxicity Studies

In this study, MTT cell proliferation, viability and cytotoxicity of the synthesized compounds were measured. L929 mouse fibroblast cell line was utilized for the evaluation of the cytotoxic activity. Dose-dependent cytotoxic activity was investigated using two different doses (5.0 µg/mL and 10.0 µg/mL) and the experiment was terminated in 48 hours. According to the findings obtained as depicted in Figures 3-5, cell viability between 90.69% and 68.08% for 5.0 µg/mL and cell viability between 83.01% and 53.97% for 10.0 µg/mL occurred. 1-(3-Fluorobenzoyl)-4-cyclohexylthiosemicarbazide **(2a)** showed lowest cytotoxicity at a concentration of 5.0 µg/mL with 90.7% cell viability value; the highest cytotoxicity activity was detected in compound 5-(4-nitrophenyl)-4-cyclohexyl-2,4-dihydro-3*H*-1,2,4-triazole-3-thione **(9c)** with 54% cell viability at a concentration of 10 µg/mL. When the compounds are evaluated in terms of cell viability, it decreases from **a** to **c** (a> b> c) in compounds numbered 1, 2, 3, 4, 5 and 8 and cell viability increases (a <b <c) as it passes from **a** to **c** in compounds 6, 7, 9. According to the results of the study performed in accordance with the ISO 10993-5 protocol, it was determined that based on all cell viability values above 50%, 27 synthesized compounds tested were not cytotoxic on normal cell at the studied concentrations [24].

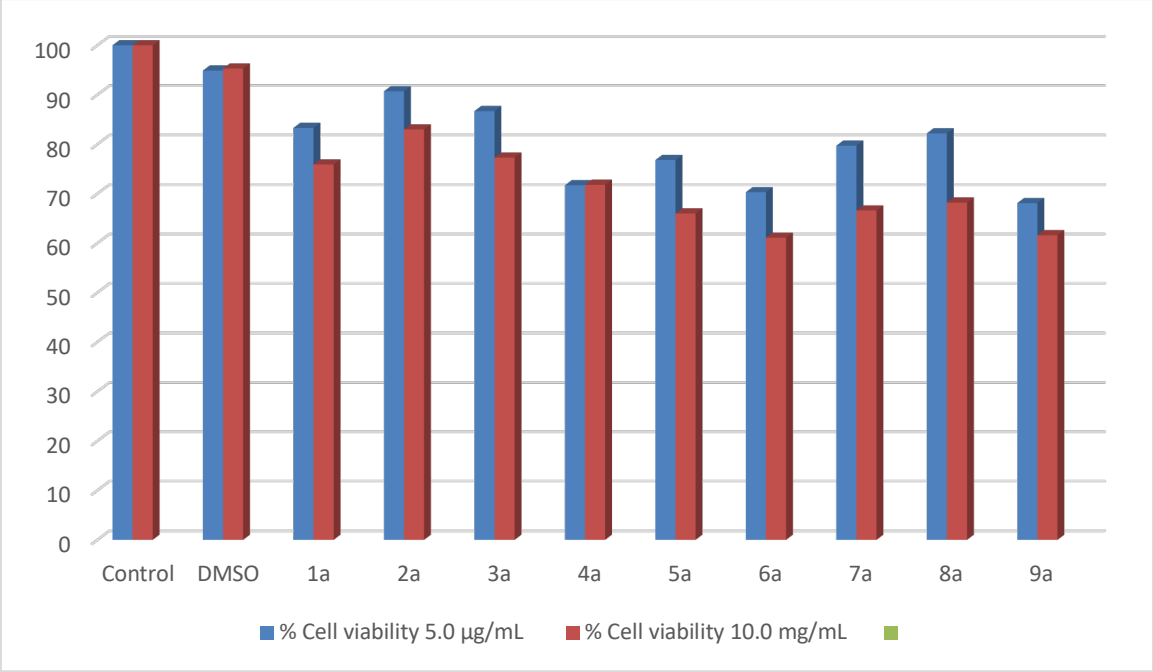



**Figure 3.** Effect of 1,4-disubstituted thiosemicarbazide derivatives on cell viability

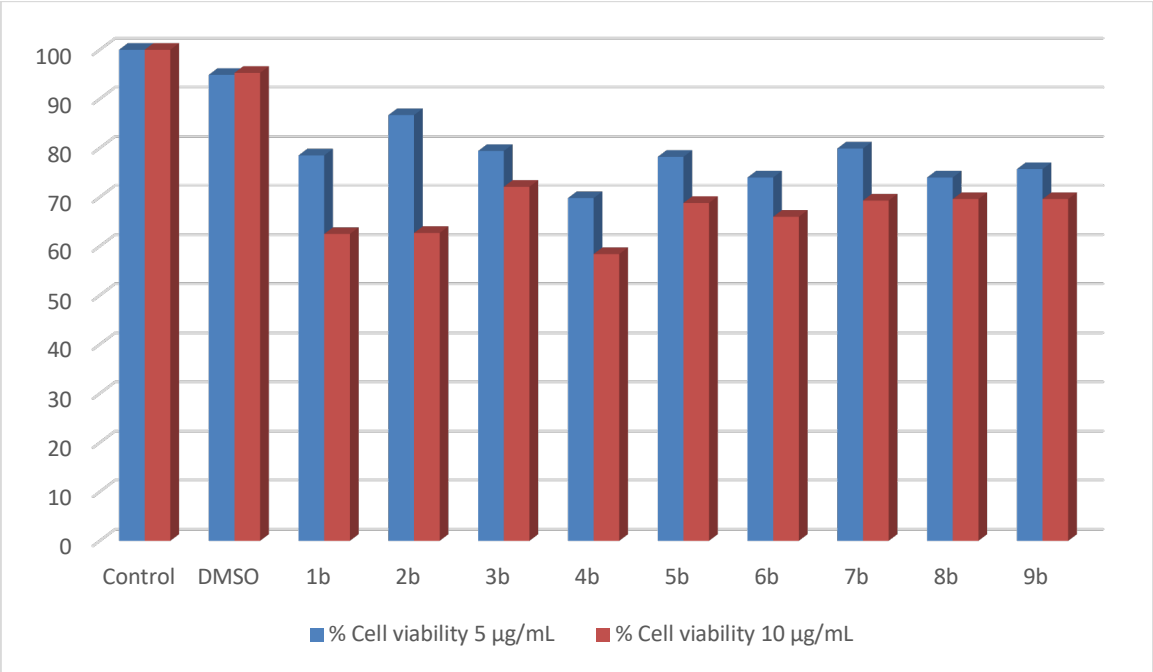



**Figure 4.** Effect of 2,5-disubstituted-1,3,4-thiadiazole derivatives on cell viability

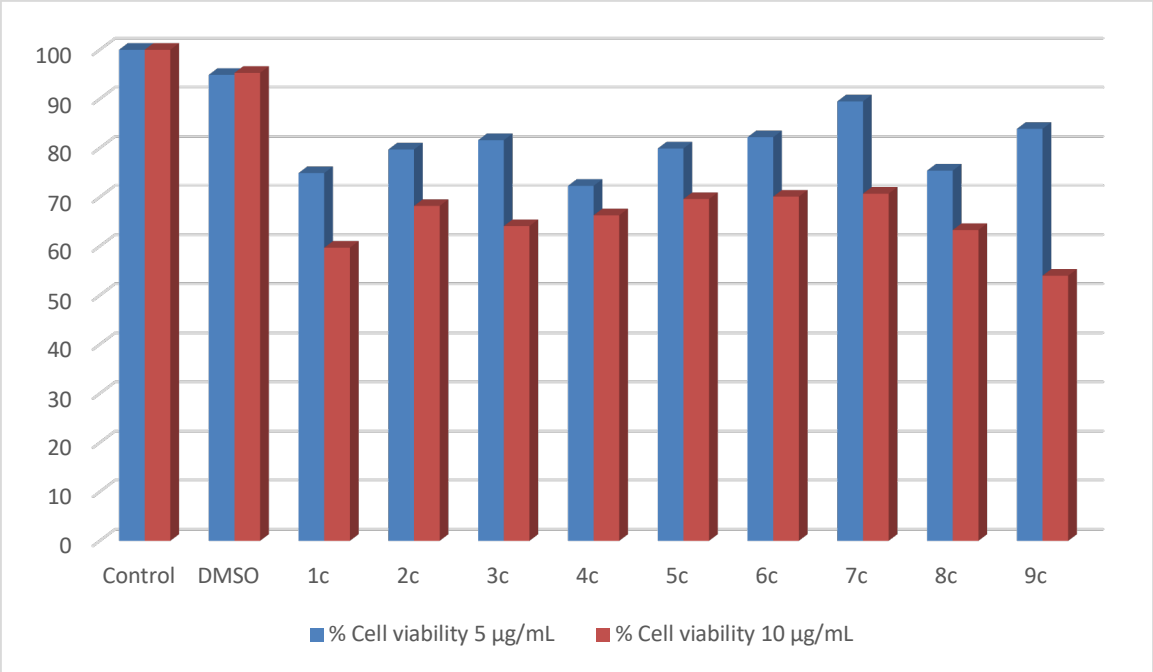



**Figure 5.** Effect of 1,2,4-triazol-3-thione derivatives on cell viability

### 2.3. ADME properties

Determining the pharmacokinetic and physicochemical properties of the compounds is very important for the development of new drug candidates. Lipinski Rules of five and Veber Rules can be used to evaluate the bioavailability of drugs by determining druglikeness properties. Considering the Lipinski Rules, molecular weight should not be higher than 500 da. There should not be hydrogen bond donors and acceptors more than 5 and 10, respectively. The values of the theoretical partition coefficient (cLog P) of compounds should be less than 5. Besides, according to Veber Rules; the number of rotatable bonds should not be larger than a maximum value of 10, polar surface area should not be larger than 140 Å2 [25]. Calculated values of the physicochemical properties of the synthesized compounds were given in Table 1.

Journal of Research in Pharmacy
Research Article

**Table 1.** Calculated physicochemical properties of target compounds

| Compound Code | MW | cLog P | H-bond acceptors | H-bond donors | #Rotatable bonds | TPSA |
|---|---|---|---|---|---|---|
| **1a** | 295.38 | 2.66 | 2 | 3 | 6 | 85.25 |
| **2a** | 295.38 | 2.66 | 2 | 3 | 6 | 85.25 |
| **3a** | 295.38 | 2.66 | 2 | 3 | 6 | 85.25 |
| **4a** | 311.83 | 2.79 | 1 | 3 | 6 | 85.25 |
| **5a** | 311.83 | 2.79 | 1 | 3 | 6 | 85.25 |
| **6a** | 356.28 | 2.91 | 1 | 3 | 6 | 85.25 |
| **7a** | 356.28 | 2.91 | 1 | 3 | 6 | 85.25 |
| **8a** | 293.38 | 1.70 | 2 | 4 | 6 | 105.48 |
| **9a** | 322.38 | 2.11 | 3 | 3 | 7 | 131.07 |
| **1b** | 277.36 | 3.33 | 3 | 1 | 3 | 66.05 |
| **2b** | 277.36 | 3.33 | 3 | 1 | 3 | 66.05 |
| **3b** | 277.36 | 3.33 | 3 | 1 | 3 | 66.05 |
| **4b** | 293.81 | 3.46 | 2 | 1 | 3 | 66.05 |
| **5b** | 293.81 | 3.46 | 2 | 1 | 3 | 66.05 |
| **6b** | 338.27 | 3.59 | 2 | 1 | 3 | 66.05 |
| **7b** | 338.27 | 3.59 | 2 | 1 | 3 | 66.05 |
| **8b** | 275.37 | 2.35 | 3 | 2 | 3 | 86.28 |
| **9b** | 304.37 | 2.73 | 4 | 1 | 4 | 111.87 |
| **1c** | 277.36 | 3.39 | 2 | 1 | 2 | 65.70 |
| **2c** | 277.36 | 3.39 | 2 | 1 | 2 | 65.70 |
| **3c** | 277.36 | 3.39 | 2 | 1 | 2 | 65.70 |
| **4c** | 293.81 | 3.52 | 1 | 1 | 2 | 65.70 |
| **5c** | 293.81 | 3.52 | 1 | 1 | 2 | 65.70 |
| **6c** | 338.27 | 3.64 | 1 | 1 | 2 | 65.70 |
| **7c** | 338.27 | 3.64 | 1 | 1 | 2 | 65.70 |
| **8c** | 275.37 | 2.41 | 2 | 2 | 2 | 85.93 |
| **9c** | 304.37 | 2.79 | 3 | 1 | 3 | 111.52 |

MW: Molecular weight; TPSA: Topological polar surface area; cLog P: calculated Log P.

## 3. CONCLUSION

1,3,4-Thiadiazole and l,2,4-triazole-3-thione derivatives were successfully synthesized by cyclization of thiosemicarbazides and spectroscopic methods confirmed the successful synthesis of these derivatives. ISO 10993-5 protocol determined that the tested compounds **1a-9a**, **1b-9b** and **1c-9c** were not cytotoxic at the concentrations studied for the fibroblast cell line (L929). These compounds could be considered for further investigations to obtain active and selective chemotherapeutic agents.

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## 4. MATERIALS AND METHODS

### 4.1. Chemistry

All the chemicals used in the study were purchased either from Fluka, Sigma, or Merck (Darmstadt, Germany). Chemical purities of all compounds were controlled by thin-layer chromatography (TLC), performed on commercially available silica gel (Kieselgel 60, F254) coated aluminum sheets (Merck) by using benzene: acetone (70:30, v/v) as a solvent system. The visualization on TLC was performed by ultra-violet (UV) light (λ= 254 nm). Melting points of the compounds were defined by the Mettler Toledo apparatus. The IR spectrum data of synthesized compounds were obtained on a Schimadzu. $^1$H-NMR spectra were performed in DMSO-$d_6$ by a Bruker Bruker VX400 BB 300 MHz with tetramethylsilane (TMS) as the internal reference standard. The mass spectral measurements were carried out by Waters. Elemental analysis (C, H, N and S) was performed on a Leco CHNS-932. Chromatographic analysis were performed by HPLC apparatus equiped with a Waters Model 600 pump, a Waters Model 481 UV detector and a Rheodyne Model 7725 injector and an integrator (Unicam 4880 Chromatography Data Handling System). AnACE $C_{18}$ column (25 cm x 4.6 mm) was used for the analysis. The mobile phase consisted of acetonitrile: water (80:20, v/v) was used. The solvent flow-rate was set as 0.5 mL/min.

#### *4.1.1. Synthesis of 1,4-thiosemicarbazide derivatives (1a-9a)*

Substituted acid hydrazides (0.05 mol) were heated with cyclohexyl isothiocyanate (0.05 mol) and refluxed for 2-3 h in ethanol (30 mL). The product obtained was filtered and crystallized from ethanol [26].

#### *4.1.2. Synthesis of 1,3,4-thiadiazole derivatives (1b-9b)*

Compounds 1a-9a (0.001 mol) were stirred with conc. $H_2SO_4$ (1mL) for 30 min room temperature and poured into ice-cold water. The precipitate product was washed with sodium bicarbonate solution and water. The product obtained was dried and crystallized from ethanol [27].

#### *4.1.3. Synthesis of 1,2,4-triazole-3-thione derivatives (1c-9c)*

Compounds 1a-9a (0.01 mol) was refluxed for 2 h in the presence of 2N NaOH (20 mL). The reaction content was neutralized with hydrochloric acid. The crude product was filtered, washed with water and crystallized from ethanol [28].

*1-(2-Fluorobenzoyl)-4-cyclohexylthiosemicarbazide (1a)*

M.p. 197.4 °C; yield 87.3%; white crystals; HPLC $t_R$ (min): 4.07; UV (EtOH, $\lambda_{max}$, nm): 249; IR ($\upsilon_{max}$, cm$^{-1}$): 3377 (N-H), 3121, 2928, 2855 (C-H), 1662 (C=O), 1614 (C=C), 1243 (C=S), 1198 (Ar-F); $^1$H-NMR (300 MHz, DMSO-$d_6$): 10.63 (1H, s, -CO-NH-), 9.51 (1H, s, -NH-), 8.07-7.67 (4H, m, Ar-H), 7.97 (1H, s, -NH-), 4.39-1.29 (11H, m, Cyclohexyl); *Anal*. Calcd. for $C_{14}H_{18}FN_3OS$: C, 56.92; H, 6.15; N, 14.25; S, 10.83%. Found: C, 56.28; H, 6.11; N, 14.12; S, 10.54%; API-ES (-) (m/z): 295.21 M-, 296.09 [M+H]$^-$, 294.21 [M-H]$^-$ (100%).

*1-(3-Fluorobenzoyl)-4-cyclohexylthiosemicarbazide (2a)*

M.p. 144.7 °C; yield 81.2%; white crystals; HPLC $t_R$ (min): 4.09; UV (EtOH, $\lambda_{max}$, nm ): 247; IR ($\upsilon_{max}$, cm$^{-1}$): 3304 (N-H), 3078, 2920, 2851 (C-H), 1636 (C=O), 1614 (C=C), 1215 (C=S), 1184 (Ar-F); $^1$H-NMR (300 MHz, DMSO-$d_6$): 10.34 (1H, s, -CO-NH-), 9.58 (1H, s, -NH-), 7.98 (1H, s, -NH-), 7.84-7.50 (4H, m, Ar-H), 4.30-1.33 (11H, m, cyclohexyl); *Anal.* Calcd. for $C_{14}H_{18}FN_3OS$: C, 56.93; H, 6.15; N, 14.25; S, 10.83%. Found: C, 6.46; H, 5.86; N, 14.13; S, 10.54%; API-ES (-) (m/z): 295.12 M$^+$, 296.09 [M+H]$^-$, 294.21 [M-H]$^-$ (100%), 158.82, 140.82, 124.75, 112.75, 110.69.

*1-(4-Fluorobenzoyl)-4-cyclohexylthiosemicarbazide (3a-Cas no: 205806-27-5)*

M.p. 202.9 °C; yield 79.9%; yellow crystals; HPLC $t_R$ (min): 4.03; UV (EtOH, $\lambda_{max}$, nm ): 250; IR ($\upsilon_{max}$, cm$^{-1}$): 3381 (N-H), 3065, 2922, 2857 (C-H), 1636 (C=O), 1599 (C=C), 1219 (C=S), 1167 (Ar-F); $^1$H-NMR (300 MHz, DMSO-$d_6$): 10.52 (1H, s, -CO-NH-), 9.43 (1H, s, -NH-), 8.23-7.53 (4H, m, Ar-H), 7.98 (1H, s, -NH-),

4.35-1.25 (11H, m, cyclohexyl); *Anal*. Calcd. for $C_{14}H_{18}FN_3OS$: C, 56.93; H, 6.15; N, 14.25; S, 10.83%. Found: C, 56.63; H, 6.04; N, 14.20; S, 10.59%; API-ES (-) (m/z): 295.21 $M^-$, 296.21 $[M+H]^-$, 294.27 $[M-H]^-$ (100%), 152.94, 110.63.

*1-(2-Chlorobenzoyl)-4-cyclohexylthiosemicarbazide (4a-Cas no: 26257-96-5)*

M.p. 156.3 °C; yield 90.2%; white crystals; HPLC $t_R$ (min): 4.19; UV (EtOH, $\lambda_{max}$, nm ): 251; IR ($\upsilon_{max}$, $cm^{-1}$): 3333 (N-H), 3063, 2953, 2847 (C-H), 1651 (C=O), 1591 (C=C), 1204 (C=S), 777 (Ar-Cl); $^1$H-NMR (300 MHz, DMSO-$d_6$): 10.50 (1H, s, -CO-NH-), 9.64 (1H, s, -NH-), 7.87 (1H, s, -NH-), 7.79-7.64 (4H, m, Ar-H), 4.30-1.36 (11H, m, cyclohexyl). *Anal*. Calcd. for $C_{14}H_{18}ClN_3OS$: C, 53.92; H, 5.82; N, 13.48; S, 10.26%. Found: C, 53.80; H, 5.54; N, 13.42; S, 9.62%; API-ES (-) (m/z): 312.15 $[M+H]^-$, 310.15 $[M-H]^-$ (100%), 211.01, 168.88, 140.82, 124.88, 112.69, 110.63.

*1-(3-Chlorobenzoyl)-4-cyclohexylthiosemicarbazide (5a-Cas no: 444145-39-5)*

M.p. 204.6 °C; yield 88.3%; white crystals; HPLC $t_R$ (min): 4.33; UV (EtOH, $\lambda_{max}$, nm): 252; IR ($\upsilon_{max}$, $cm^{-1}$): 3358 (N-H), 3071, 2980, 2855 (C-H), 1678 (C=O), 1568 (C=C), 1229 (C=S), 804 (Ar-Cl); $^1$H-NMR (300 MHz, DMSO-$d_6$): 10.57 (1H, s, -CO-NH-), 9.41 (1H, s, -NH-), 8.14 (1H, s, -NH-), 8.06-7.69 (4H, m, Ar-H), 4.31-1.21 (11H, m, cyclohexyl); *Anal*. Calcd. for $C_{14}H_{18}ClN_3OS$: C, 53.92; H, 5.82; N, 13.48; S, 10.26%. Found: C, 53.55; H, 5.56; N, 13.45; S, 9.39%; API-ES (-) (m/z): 312.21 $[M+H]^-$, 310.15 $[M-H]^-$ (100%), 169.01, 110.75.

*1-(3-Bromobenzoyl)-4-cyclohexylthiosemicarbazide (6a-Cas no: 632300-34-6)*

M.p. 201.1 °C; yield 72.8%; white crystals; HPLC $t_R$ (min): 4.42; UV (EtOH, $\lambda_{max}$, nm): 253; IR ($\upsilon_{max}$, $cm^{-1}$): 3348 (N-H), 3069, 2924, 2853 (C-H), 1676 (C=O), 1566 (C=C), 1229 (C=S), 671 (Ar-Br); $^1$H-NMR (300 MHz, DMSO-$d_6$): 10.58 (1H, s, -CO-NH-), 9.43 (1H, s, -NH-), 8.30 (1H, s, -NH-), 8.11-7.64 (4H, m, Ar-H), 4.33-1.23 (11H, m, cyclohexyl); *Anal*. Calcd. for $C_{14}H_{18}BrN_3OS$ x 0.5 $H_2O$: C, 46.03; H, 5.24; N, 11.50; S, 8.78%. Found: C, 45.87; H, 5.10; N, 11.45; S, 8.43%; API-ES (-) (m/z): 357.19 $M^-$ and 355.17 $M^-$, 356.18 $[M+H]^-$ (100%), 214.92, 213.03, 140.83, 110.65.

1-*(4-Bromobenzoyl)-4-cyclohexylthiosemicarbazide (7a-Cas no: 448232-94-8)*

M.p. 219.8 °C; yield 92.2%; white crystals; HPLC $t_R$ (min): 4.44; UV (EtOH, $\lambda_{max}$, nm): 250; IR ($\upsilon_{max}$, $cm^{-1}$): 3327 (N-H), 2988, 2934, 2849 (C-H), 1670 (C=O), 1587 (C=C), 1256 (C=S), 661 (Ar-Br); $^1$H-NMR (300 MHz, DMSO-$d_6$): 10.57 (1H, s, -CO-NH-), 9.43 (1H, s, -NH-), 8.07 (1H, s, -NH-), 8.00-7.91 (4H, m, Ar-H), 4.34-1.26 (11H, m, cyclohexyl); *Anal*. Calcd. for $C_{14}H_{18}BrN_3OS$: C, 47.20; H, 5.09; N, 11.79; S, 9.00%. Found: C, 46.95; H, 4.95; N, 11.71; S, 8.28%; API-ES (-) (m/z): 356.14 $[M-H]^-$ and 354.13 $[M-H]^-$, 158.92, 140.83, 124.83, 110.65.

*1-(4-Hydroxybenzoyl)-4-cyclohexylthiosemicarbazide (8a-Cas no: 26036-24-8)*

M.p. 206.7 °C; yield 70.4%; white crystals; HPLC $t_R$ (min): 4.24; UV (EtOH, $\lambda_{max}$, nm): 255; IR ($\upsilon_{max}$, $cm^{-1}$): 3302 (N-H), 3184 (Ar-OH), 2988, 2934, 2847 (C-H), 1653 (C=O), 1231 (C=S); $^1$H-NMR (300 MHz, DMSO-$d_6$): 10.29 (1H, s, -OH), 10.22 (1H, s, CONH), 9.32 (1H, s, -NH-), 8.00-7.00 (4H, m, Ar-H), 7.85 (1H, s, -NH-), 4.32 (1H, s), 1.79-1.23 (11H, m, cycloexyl); *Anal*. Calcd. for $C_{14}H_{19}N_3O_2S$: C, 57.31; H, 6.53; N, 14.32; S, 10.93%. Found: C, 57.15; H, 6.53; N, 14.17; S, 9.70%; API-ES 293.26 $M^-$, 294.20 $[M+H]^-$, 292.19 $[M-H]^-$ (100%), 292.19, 150.91, 110.71.

1-*(4-Nitrobenzoyl)-4-cyclohexylthiosemicarbazide (9a-Cas no: 17050-20-3)*

M.p. 197.6 °C; yield 95.8%; yellow crystals; HPLC $t_R$ (min): 5.43; UV (EtOH, $\lambda_{max}$, nm): 253; IR ($\upsilon_{max}$, $cm^{-1}$): 3335 (N-H), 3065, 2970, 2860 (C-H), 1670 (C=O), 1298 (C=S); $^1$H-NMR (300 MHz, DMSO-$d_6$): 10.78 (1H, s, -CO-NH-), 9.48 (1H, s, -NH-), 8.54-8.30 (4H, m, Ar-H), 8.04 (1H, s, -NH-), 4.31-1.20 (10H, m, cyclohexyl); *Anal*. Calcd. for $C_{14}H_{18}N_4O_3S$: C, 52.16; H, 5.63; N, 17.38; S, 9.95%. Found: C, 52.04; H, 5.43; N, 17.22; S, 8.68%; API-ES 322.15 $M^-$, 323.22 $[M+H]^-$, 321.22 $[M-H]^-$ (100%), 226.14, 180.01.

*2-Fluorophenyl-5-cyclohexylamino-1,3,4-thiadiazole (1b)*

M.p. 135.4 °C; yield 73.0%; white crystals; HPLC $t_R$ (min): 5.62; UV (EtOH, $\lambda_{max}$, nm): 328; IR ($\upsilon_{max}$, $cm^{-1}$): 3228 (N-H), 2926, 2855 (C-H), 1180 (Ar-F), 729 (thiadiazole C-S-C); $^1$H-NMR (300 MHz, DMSO-$d_6$): 9.30 (1H, s, -NH-), 7.86-7.52 (4H, m, Ar-H), 3.79-1.37 (11H, m, cyclohexyl); *Anal*. Calcd. for $C_{14}H_{16}FN_3S$: C, 60.63; H, 5.81; N, 15.15; S, 11.56%. Found: C, 60.06; H, 5.73; N, 14.93; S, 11.42%; API-ES (+) (m/z): 278.21 $[M+H]^+$ 159.01, 156.82 (100%).

*3-Fluorophenyl-5-cyclohexylamino-1,3,4-thiadiazole (2b)*

M.p. 163.0 °C; yield 94.3%; white crystals; HPLC $t_R$ (min): 5.62; UV (EtOH, $\lambda_{max}$, nm): 325; IR ($\upsilon_{max}$, $cm^{-1}$): 3201 (N-H), 3057, 2938, 2851 (C-H), 1184 (Ar-F), 743 (thiadiazole C-S-C).; $^1$H-NMR (300 MHz, DMSO-$d_6$): 8.34-7.71 (4H, m, Ar-H), 7.65 (1H, s, -NH-), 3.82-1.27 (11H, m, cyclohexyl); *Anal*. Calcd. for $C_{14}H_{16}FN_3S$: C, 60.63; H, 5.81; N, 15.15; S, 11.56%. Found: C, 60.04; H, 5.78; N, 14.89; S, 11.25%; API-ES (+) (m/z): 278.27 $[M+H]^+$, 159.07, 156.82 (100%).

*4-Fluorophenyl-5-cyclohexylamino-1,3,4-thiadiazole (3b)*

M.p. 114.9 °C; yield 56.0%; yellow crystals; HPLC $t_R$ (min): 5.49; UV (EtOH, $\lambda_{max}$, nm): 319; IR ($\upsilon_{max}$, $cm^{-1}$): 3228 (N-H), 3067, 2928, 2855 (C-H), 1157 (Ar-F), 741 (thiadiazole C-S-C).; $^1$H-NMR (300 MHz, DMSO-$d_6$): 8.35 (1H, s, -NH-), 8.01-7.47 (4H, m, Ar-H), 3.74-1.39 (11H, m, cyclohexyl); *Anal*. Calcd. for $C_{14}H_{16}FN_3S$: C, 60.63; H, 5.81; N, 15.15; S; 11.56%. Found: C, 60.95; H, 5.25; N, 15.78; S; 12.25%; API-ES (+) (m/z): 278.21 $[M+H]^+$, 159.01, 156.82 (100%).

2-*Chlorophenyl-5-cyclohexylamino-1,3,4-thiadiazole (4b-Cas no: 802862-37-9)*

M.p. 239.4 °C; yield 72.0%; white crystals; HPLC $t_R$ (min): 5.81; UV (EtOH, $\lambda_{max}$, nm): 322; IR ($\upsilon_{max}$, $cm^{-1}$): 3217 (N-H), 2936, 2851 (C-H), 750 (Ar-Cl), 731 (thiadiazole C-S-C); $^1$H-NMR (300 MHz, DMSO-$d_6$): 8.21-7.67 (4H, m, Ar-H), 7.82 (1H, s, -NH-), 3.79-1.43 (11H, m, cyclohexyl); *Anal*. Calcd. for $C_{14}H_{16}ClN_3S$: C, 57.23; H, 5.49; N, 14.30; S, 10.91%. Found: C, 56.73; H, 5.17; N, 14.04; S, 10.12%; API-ES (+) (m/z): 294.21 $[M+H]^+$, 176.01, 157.01 (100%).

*3-Chlorophenyl-5-cyclohexylamino-1,3,4-thiadiazole (5b-Cas no: 35313-92-9)*

M.p. 282.7 °C; yield 63.8%; white crystals; HPLC $t_R$ (min): 5.85; UV (EtOH, $\lambda_{max}$, nm): 327; IR ($\upsilon_{max}$, $cm^{-1}$): 3181 (N-H), 3061, 2926, 2853 (C-H), 779 (Ar-Cl), 733 (thiadiazole C-S-C); $^1$H-NMR (300 MHz, DMSO-$d_6$): 8.11 (1H, s, -NH-), 7.55-7.21 (4H, m, Ar-H), 3.31-0.94 (11H, m, cyclohexyl); *Anal*. Calcd. for $C_{14}H_{16}ClN_3S$: C, 57.23; H, 5.49; N, 14.30; S, 10.91%. Found: C, 56.63; H, 5.01; N, 14.18; S, 10.94%; API-ES (+) (m/z): 294.21 $[M+H]^+$, (100%), 257.08, 224.02, 157.01, 144.94, 136.94, 100.75.

*3-Bromophenyl-5-cyclohexylamino-1,3,4-thiadiazole (6b)*

M.p. 282.1 °C; yield 83.3%; white crystals; HPLC $t_R$ (min): 5.46; UV (EtOH, $\lambda_{max}$, nm): 328; IR ($\upsilon_{max}$, $cm^{-1}$): 3281 (N-H), 2937, 2854 (C-H), 802 (thiadiazole C-S-C), 696 (Ar-Br); $^1$H-NMR (300 MHz, DMSO-$d_6$): 8.94 (1H, s, -NH-), 8.15-7.66 (4H, m, Ar-H), 3.78 -1.51 (11H, m, cyclohexyl); *Anal*. Calcd. for $C_{14}H_{16}BrN_3S$: C, 49.71; H, 4.77; N, 12.42; S, 9.48%. Found: C, 49.62; H, 4.31; N, 12.45; S, 9.42%; API-ES (+) (m/z): 340.16 $[M+H]^+$ and 338.16 $[M+H]^+$, 157.02 (100%), 100.6.

*4-Bromophenyl-5-cyclohexylamino-1,3,4-thiadiazole (7b)*

M.p. 207.8 °C; yield 89.2%; white crystals; HPLC $t_R$ (min): 5.52; UV (EtOH, $\lambda_{max}$, nm): 328; IR ($\upsilon_{max}$, $cm^{-1}$): 3192 (N-H), 2988, 2924, 2851 (C-H), 758 (thiadiazole C-S-C), 665 (Ar-Br); $^1$H-NMR (300 MHz, DMSO-$d_6$): 7.60-7.46 (4H, m, Ar-H), 7.20 (1H, s, -NH-), 3.29-1.18 (11H, m, cyclohexyl); *Anal*. Calcd. for $C_{14}H_{16}BrN_3S$: C, 49.71; H,4.77; N, 12.34; S, 9.48%. Found: C, 49.27; H,4.31; N, 12.34; S, 9.30%; API-ES (+) (m/z): 340.13 $[M+H]^+$ and 338.13 $[M+H]^+$, 212.98, 171.96, 158.85, 140.96, 124.76, 110.71 (100%).

*4-Hydroxyphenyl-5-cyclohexylamino-1,3,4-thiadiazole (8b-Cas no: 773057-01-5)*

M.p. 119.9 °C; yield 67.7%; white crystals; HPLC $t_R$ (min): 4.62; UV (EtOH, $\lambda_{max}$, nm): 319; IR ($\upsilon_{max}$, $cm^{-1}$): 3169 (N-H), 3661 (Ar-OH), 2932, 2901, 2860 (C-H), 736 (thiadiazole C-S-C); $^1$H-NMR (300 MHz, DMSO-$d_6$): 10.33 (1H, s, -OH), 9.73 (1H, s, -NH-), 8.06-7.02 (4H, m, Ar-H), 3.80-1.44 (11H, m, cyclohexyl); *Anal*. Calcd. for $C_{14}H_{17}N_3OS$: C, 61.06; H, 6.22; N, 15.26; S, 11.64%. Found: C, 61.45; H, 6.38; N, 14.86; S, 12.25%; API-ES (+) (m/z): 276.18 $[M+H]^+$, 158.98 (100%), 156.90.

*4-Nitrophenyl-5-cyclohexylamino-1,3,4-thiadiazole (9b)*

M.p. 234.3 °C; yield 95.4%; white crystals; HPLC $t_R$ (min): 6.30; UV (EtOH, $\lambda_{max}$, nm): 382; IR ($\upsilon_{max}$, $cm^{-1}$): 3184 (N-H), 3057, 2918, 2853 (C-H), 752 (thiadiazole C-S-C); $^1$H-NMR (300 MHz, DMSO-$d_6$): 8.53-8.20 (5H, m, Ar-H and -NH-), 3.82-1.43 (11H, m, cyclohexyl); *Anal*. Calcd. for $C_{14}H_{16}N_4O_2S$: C, 55.25; H, 5.30; N, 18.41; S, 10.53%. Found: C, 55.07; H, 4.76; N, 18.32; S, 10.33%; API-ES (+) (m/z): 305.23 $[M+H]^+$, 157.03, 145.94, 136.86.

*4-Cyclohexyl-5-(2-fluorophenyl)-2,4-dihydro-3H-1,2,4-triazole-3-thione (1c-Cas no: 522606-86-6)*

M.p. 177.3 °C; yield 77.4%; white crystals; HPLC $t_R$ (min): 6.81; UV (EtOH, $\lambda_{max}$, nm): 261; IR ($\upsilon_{max}$, $cm^{-1}$): 3377 (triazole N-H), 3051, 2976, 2855 (C-H), 1242 (triazole C=S), 1198 (Ar-F); $^1$H-NMR (300 MHz, DMSO-$d_6$): 14.30 (1H, s, -NH-), 7.92-7.58 (4H, m, Ar-H), 4.45-1.05 (11H, m, cyclohexyl); *Anal*. Calcd. for $C_{14}H_{16}FN_3Sx0.5H_2O$: C, 58.72; H, 5.98; N, 14.67; S, 11.20%. Found: C, 57.90; H, 5.60; N, 14.47; S, 10.61%; API-ES (+) (m/z): 278.13 $[M+H]^+$ (100%), 262.26, 157.03, 155.07, 144.86.

*4-Cyclohexyl-5-(3-fluorophenyl)-2,4-dihydro-3H-1,2,4-triazole-3-thione (2c-Cas no: 925197-82-6)*

M.p. 177.3 °C; yield 87.4%; white crystals; HPLC $t_R$ (min): 6.85; UV (EtOH, $\lambda_{max}$, nm): 260; IR ($\upsilon_{max}$, $cm^{-1}$): 3304 (triazole N-H), 3051, 2941, 2857 (C-H), 1223 (triazole C=S), 1184 (Ar-F); $^1$H-NMR (300 MHz, DMSO-$d_6$): 14.31 (1H, s, -NH-), 7.92-7.58 (4H, m, Ar-H), 4.45-1.05 (11H, m, cyclohexyl); *Anal*. Calcd. for $C_{14}H_{16}FN_3S$: C, 60.63; H, 5.81; N, 15.15; S, 11.56%. Found: C, 60.86; H, 5.05; N, 15.23; S, 11.86%; API-ES (+) (m/z): 278.14 $[M+H]^+$ (100%), 153.94, 152.94, 132.94.

*4-Cyclohexyl-5-(4-fluorophenyl)-2,4-dihydro-3H-1,2,4-triazole-3-thione (3c-Cas no: 205806-32-2)*

M.p. 242.8 °C; yield 81.7%; white crystals; HPLC $t_R$ (min): 6.89; UV (EtOH, $\lambda_{max}$, nm): 262; IR ($\upsilon_{max}$, $cm^{-1}$): 3305 (N-H), 3048, 2936, 2857 (C-H), 1225 (C=S), 1186 (Ar-F); $^1$H-NMR (300 MHz, DMSO-$d_6$): 14.00 (1H, s, -NH-), 7.80-7.53 (4H, m, Ar-H), 4.39-1.05 (11H, m, cyclohexyl); *Anal*. Calcd. for $C_{14}H_{16}FN_3S \times 1H_2O$: C, 56.93; H, 6.14; N, 14.23; S, 10.86%. Found: C, 57.21; H, 5.44; N, 14.42; S, 10.38%; API-ES (+) (m/z): 278.26 $[M+H]^+$, 246,26, 196.05.

*5-(2-Chlorophenyl)-4-cyclohexyl-2,4-dihydro-3H-1,2,4-triazole-3-thione (4c-Cas no: 26029-19-6)*

M.p. 220.8 °C; yield 63.6%; white crystals; HPLC $t_R$ (min): 6.48; UV (EtOH, $\lambda_{max}$, nm): 259; IR ($\upsilon_{max}$, $cm^{-1}$): 3333 (N-H), 3034, 2938, 2855 (C-H), 1221 (C=S), 729 (Ar-Cl); $^1$H-NMR (300 MHz, DMSO-$d_6$): 14.24 (1H, s, -NH-), 7.92-7.62 (4H, m, Ar-H), 4.41-0.95 (11H, m, cyclohexyl); *Anal*. Calcd. for $C_{14}H_{16}ClN_3S$: C, 57.23; H, 5.49; N, 14.30; S, 10.91%. Found: C, 56.54; H, 5.37; N, 14.14; S, 10.56%; API-ES (+) (m/z): 294.21 $[M+H]^+$, 179.01, 157.01, 145.94, 136.82.

*5-(3-Chlorophenyl)-4-cyclohexyl-2,4-dihydro-3H-1,2,4-triazole-3-thione (5c-Cas no: 90657-90-2)*

M.p. 175.3 °C; yield 68.7%; white crystals; HPLC $t_R$ (min): 6.45; UV (EtOH, $\lambda_{max}$, nm): 262; IR ($\upsilon_{max}$, $cm^{-1}$): 3275 (N-H), 3036, 2938, 2855 (C-H), 1207 (C=S), 754 (Ar-Cl); $^1$H-NMR (300 MHz, DMSO-$d_6$): 14.03 (1H, s, -NH-), 7.90-7.71 (4H, m, Ar-H), 4.43-1.04 (11H, m, cyclohexyl); *Anal*. Calcd. for $C_{14}H_{16}ClN_3S$: C, 57.23; H, 5.49; N, 14.30; S, 10.91%. Found: C, 56.45; H, 5.29; N, 14.10%; S, 10.50; API-ES (+) (m/z): 294.20 $[M+H]^+$, 262.19.

*5-(3-Bromophenyl)-4-cyclohexyl-2,4-dihydro-3H-1,2,4-triazole-3-thione (6c-Cas no: 345971-84-8)*

M.p. 197.6 °C; yield 85.5%; white crystals; HPLC $t_R$ (min): 6.58; UV (EtOH, $\lambda_{max}$, nm): 261; IR ($\upsilon_{max}$, $cm^{-1}$): 3177 (N-H), 3057, 2928, 2851 (C-H), 1153 (C=S), 669 (Ar-Br); $^1$H-NMR (300 MHz, DMSO-$d_6$): 14.61(1H, s, -NH-), 8.49-7.87 (4H, m, Ar-H), 4.84-1.14 (11H, m, cyclohexyl); *Anal*. Calcd. for $C_{14}H_{16}BrN_3S$: C, 49.71; H, 4.77; N, 12.42; S, 9.48%. Found: C, 49.62; H, 4.57; N, 12.42; S, 9.04%; API-ES (+) (m/z): 340.15 $[M+H]^+$ and 338.15 $[M+H]^+$, 306.21, 308.21, 115.75, 106.81.

*5-(4-Bromophenyl)-4-cyclohexyl-2,4-dihydro-3H-1,2,4-triazole-3-thione (7c-Cas no: 333767-07-0)*

M.p. 206.2 °C; yield 61.9%; white crystals; HPLC $t_R$ (min): 6.58; UV (EtOH, $\lambda_{max}$, nm): 261; IR ($\upsilon_{max}$, $cm^{-1}$): 3260 (N-H), 3064, 2934, 2851 (C-H), 1186 (C=S), 669 (Ar-Br); $^1$H-NMR (300 MHz, DMSO-$d_6$): 14.10 (1H, s, -NH-), 8.07-7.71 (4H, m, Ar-H), 4.44-1.09 (11H, m, cyclohexyl); *Anal*. Calcd. for $C_{14}H_{16}BrN_3S$: C, 49.71; H,4.77; N, 12.42; S, 9.48%. Found: C, 49.62; H, 4.60; N, 12.19; S, 8.92%; API-ES (+) (m/z): 340.19 $[M+H]^+$ and 338.19 $[M+H]^+$, 256.06, 226.09, 224.01, 212.96, 197.04, 178.95, 161.94, 150.91, 134.85 (100%), 114.62, 108.00, 106.87.

*5-(4-Hydroxyphenyl)-4-cyclohexyl-2,4-dihydro-3H-1,2,4-triazole-3-thione (8c-Cas no: 26028-87-5)*

M.p. 270.8 °C; yield, 76.2%; white crystals; HPLC $t_R$ (min): 4.87; UV (EtOH, $\lambda_{max}$, nm): 264; IR ($\upsilon_{max}$, $cm^{-1}$): 3674 (O-H), 3240 (N-H), 3094, 2988, 2855 (C-H), 1180 (C=S); $^1$H-NMR (300 MHz, DMSO-$d_6$): 10.35 (1H, s, -OH), 13.95 (1H, s, -NH-), 7.54-7.10 (4H, m, Ar-H), 4.43-1.12 (11H, m, cyclohexyl); *Anal*. Calcd. for $C_{14}H_{17}N_3OS$: C, 61.06; H, 6.22; N, 15.26; S, 11.64%. Found: C, 61.23; H, 6.00; N, 15.11; S, 12.07%; API-ES (+) (m/z): 276.18 $[M+H]^+$, 179.02, 158.98, 156.77 (100%).

*5-(4-Nitrophenyl)-4-cyclohexyl-2,4-dihydro-3H-1,2,4-triazole-3-thione (9c-Cas no: 17050-54-3)*

M.p. 204.4; °C; yield, 66.1%; yellow crystals; HPLC $t_R$ (min): 6.31; UV (EtOH, $\lambda_{max}$, nm): 265; IR ($\upsilon_{max}$, $cm^{-1}$): 3666 (N-H), 3080, 2967, 2901 (C-H), 1103 (C=S); $^1$H-NMR (300 MHz, DMSO-$d_6$): 13.91 (1H, s, -NH-), 8.42-7.84 (4H, m, Ar-H), 4.27-1.00 (11H, m, cyclohexyl); *Anal*. Calcd. for $C_{14}H_{16}N_4O_2S$: C, 55.25; H, 5.30; N, 18.41; S, 10.53%. Found: C, 55.36; H, 5.25; N, 18.00; S, 11.17%; API-ES (+) (m/z): 305.29 $[M+H]^+$, 157.03, 136.86.

### 4.2. Cytotoxic Activity

MTT assay was performed using the mouse fibroblast cell line L-929 CCL-1 (ATCC) according to ISO 10993-5 protocol (ISO 10993-5, 2009) for the cytotoxic activities of the synthesized compounds. Cell Proliferation Kit I MTT (Roche) was used and the experiment was performed according to the manufacturer's instructions. For this assay, cells were maintained in 25 $cm^2$ flasks with antibiotic-free Eagle's MEM and RPMI 1640 medium containing 10% fetal bovine serum and 100 mM/L Glutamine Cells were plated in 96 well plates with 5x103 cells/well. When the cells, which were incubated at 37°C in an atmosphere of $CO_2$ overnight, reached 70% density, the synthesized compounds were added to the cells at 5.0 and 10.0 µg/mL. In addition, 0.05-0.10% dimethylsulfoxide (DMSO) was added to the cells to investigate the solvent's cytotoxicity. After 48 hours of incubation of cells, 10 µl of MTT solution was added to the cells, 100 µL of SDS solution was added to dissolve the formed crystals after 4 hours. The absorbance in the cells was measured at 550 and 690 nm after the crystals were completely dissolved. The obtained data were evaluated as cell viability in percentage compared to the control.

### 4.3. Calculation of pharmacokinetic and physicochemical properties

Pharmacokinetic and physicochemical properties of synthesized compounds were predicted by using the SWISSadme free website (http://www.swissadme.ch/).

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