ORIGINAL RESEARCH

Synthesis and cytotoxic activity of some 1,2,4-triazoline-3-thione and 2,5-disubstituted-1,3,4-thiadiazole derivatives

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ABSTRACT: In this search, a series of 5-[4-(4-fluorobenzoylamino)phenyl]-2-substitutedamino-1,3,4-thiadiazole (2a-g) and 5-[4-(4-fluorobenzoylamino)phenyl]-4-substituted-2,4-dihydro-3H-1,2,4-triazo-le-3-thione (3a-f) derivatives were synthesized and characteristed by elemental analysis, UV-visible, IR, ^{1}H-NMR, MS spectral data and X-ray crystallograpy (3b). Cytotoxic activity of six prototype compounds (2a, 2d, 2e, 3a, 3d and 3e) were evaluated by using HeLa (ATCC CCL-2) and normal cell lines according to procedures of MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide] Assay [Cell Proliferation Kit I (MTT) Roche-Germany].

KEY WORDS: Cytotoxic activity, 1,2,4-triazoline-3-thione, 2,5-disubstituted-1,3,4-thiadiazole, mtt assay, x-ray crystallograpy

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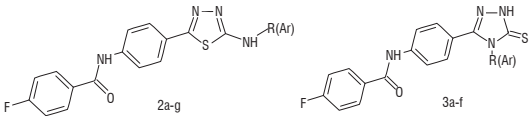
INTRODUCTION



SCHEME 1.

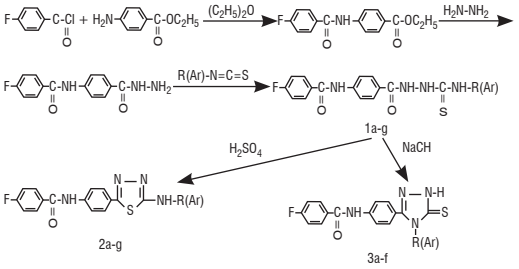
Substituted 1,3,4-thiadiazoles and 1,2,4-triazoles are important for pharmacological activity. These compounds show antibacterial (1-3), antifungal (4), antimicrobial (5-6), antimycobacterial (7-9), anti-inflammatory (10-11), antihypertensive (12), hypolipidemic (13) and anticancer (14-16) activity.

It has been reported that the thione form of 1,2,4-triazole-3-thiones were important for their antifungal activity (17). Pospisil et al. (18) explained that tautomers are often disregarded in computer – aided molecular modeling applications. Tautomeric states of molecules are rarely registered in chemical databases.

As a continuation of our earlier study (19) on the 1-[4-(4-fluorobenzoyl-amino)benzoyl]-4-substituted thiosemicarbazides (1a-f), their cyclization products 1,3,4-thiadiazoles (2a-g) and 1,2,4-triazole-thiones (3a-f) were synthesized and characterized. In addition to the thiole↔thione tautomerism of 3b was explained by using X-ray crystallography. From the synthesized compounds (2a, 2d, 2e, 3a, 3d and 3e) were tested for their cytotoxic activities. Cell viability and cytotoxic activity profile of the compounds were analyzed using the MTT assay.

RESULTS AND DISCUSSION

The starting compounds, (**1a-f**) were synthesized according to the previously reported procedure (19). 1,3,4-Thiadiazole **(2a-g)** were prepared from (**1a-f)** by reacting with concentrated sulphuric acid. Also 1,2,4-triazole derivatives **(3a-f)** were prepared from **1a-f** by reacting with 2N sodium hydroxide. The synthetic route for compounds **2a-g** and **3a-f** is presented in Scheme 2.



SCHEME 2. Synthetic route to 2a-g and 3a-f

All the synthesized compounds **2a-g** and **3a-f** were characterized by their melting points, elemental analysis and spectral data (UV, IR, ^{1}H-NMR and MS)**.** The UV spectra of **2a-g** exhibited three absorption maxima at 204-208, 221-228 (shoulder) and 326-341 nm.

According to IR spectra of compounds **2a-g** the bands between 1642-1670 cm^{-1} were assigned to the C=O group. The content of ^{1}H-NMR spectrum of compound **2c** includes a singlet at 10.40 ppm which was assigned to the –CONH- signal. The ^{1}H-NMR spectrum of **2g** exhibited a singlet at 10.40 ppm that was attributable to –NH- and –CONH- groups. The molecular ion peaks at m/z 328 (m.w.: 328.3), m/z 342 (m.w.: 342.4), m/z 356 (m.w.: 356.43), m/z 354 (m.w.: 354.41), m/z 396 (m.w.: 396.49) and m/z 390 (m.w.: 390.44) were obtained from MS spectra of compounds **2a**, **2b**, **2c** , **2d**, **2e** and **2g** respectively. Within the context of MS spectra of compounds **2b**, **2c** and **2g** the main fragmentation pattern existed as the removal of 4-fluorobenzoyl moiety in conformity with m/z 123 peak. The second fragmentation way is the removal of the substituent which was adjacent to amine moiety. The main MS fragmentation patterns of **2b** are shown in Scheme 3.

The UV spectra of **3a-f** showed three absorption maxima at 204-208, 243-260 and 263-293 nm. In the ^{1}H-NMR spectra of 1,2,4-tiazole-3-thione derivatives, signals for triazole NH proton were observed between the ranges 13.65-13.82 ppm**.** MS spectra of compounds **3a**, **3b** and **3f** gave molecular ion peaks at m/z 328 (m.w.: 328.39), m/z 342 (m.w.: 342.40) and m/z 390 (m.w.: 390.54) respectively. The main MS fragmentation patterns of **3a** are shown in Scheme 4.

3,4,5-trisubstituted-1,2,4-triazoles derived from thiosemicarbazide possess two possible tautomeric forms (20). UV and ^{1}H-NMR spectra were used to investigate the tautomerism of **3a-f**.

Solvent effects on the UV spectra of compounds **3a-g** were studied. The UV spectra of compound **3f** exhibited maximum absorbtions at 280, 281, 286 and 281 nm in ethanol, cyclohexane, sodium hydroxide and phosphate buffer (pH:7.4) respectively. The spectrum of compound **3f** that was measured in nonpolar solvent cyclohexane is similar to the other spectra evaluated by using the other solvents. This observation suggest that the compound exists in thione form (Table 1). Our suggestion constitutes a precedent of the literature by Kubota and Uda (21) that paraphrases; the band at 280 nm determined by measuring ethanolic solution of 1-methyl-3-phenyl-1,2,4-triazoline-5-thione belongs to thiocarbonile group of the compound.

The UV spectra of compounds **3a-e** were recorded in phosphate buffer (pH:7.4) and absorption maximas at 261, 257, 264, 254 and 281 nm were determined respectively. Consequently, compounds **3a-e** may exist in thione↔thiole tautomeric forms in biological fluids.

The presence of the peaks due to the NH function of the triazoline ring of compounds **3a-f** at 13.82, 13.69, 13.65 and 13.74 ppm supported the thione form (22, 23).

These results indicated that the thione form of compounds **3a-f** was the predominant tautomer in both solid and solution states. The substituent effect on tautomeric forms of these compounds was investigated. The thione form of compound **3f**, which has a phenyl substituent, was attended as the most stable tautomer. These results were in good agreement with the fact that certain of thiol ↔ thione tautomerism exists predominantly in the thione forms (20, 21).

Following the crystallization from ethanol the melting point of compound **3b** was detected as 245-246 ^{o}C hereafter the compound recrystallized from DMF in order to be analysed by X-ray and its melting point rised to 281 ^{o}C.

X-ray analysis showed that 5-[4-(4-fluorobenzoylamino) phenyl]-4-ethyl-2,4-dihydro-3H-1,2,4-triazole-3-thione **(3b)** (Fig 1) contains three planar rings namely two benzene rings [C1-C6 (A) and C8-C13 (B)] and a triazole ring (C). The structure that was formed by all these rings involves a substitued DMF molecule. DMF was not used in the process of compound **3b** synthesis, but, DMF might have been involved with the main structure during crystallization period.

The crystal structure is stabilized by intramolecular hydrogen bonds and intermolecular hydrogen bond, C-H…π and π…π type interactions. The hydrogen bonding details can be seen in Table 6. The C-H…π interaction involves triazole ring of a symmetry related molecule at (2-x, -y, 1-z)[C17…Cg1=3.580(5)Å,

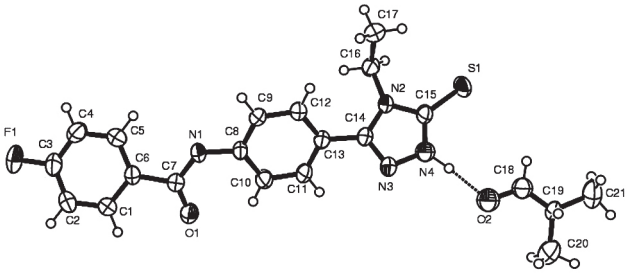


FIGURE 1. X-ray analysis of 5-[4-(4-fluorobenzoylamino)phenyl]-4-ethyl-2,4-dihydro-3H-1,2,4-triazole-3-thione (3b)

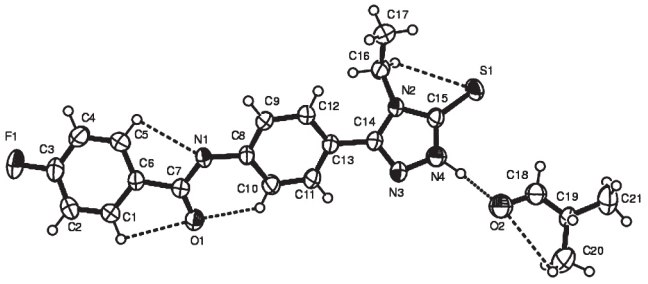


FIGURE 2. Intramolecular hydrogen bonds of 5-[4-(4-fluorobenzoylamino) phenyl]-4-ethyl-2,4-dihydro-3H-1,2,4-triazole-3-thione (3b)

TABLE 1. UV data of 3a-f

Compound	UVmax ethanol (1mg/100 mL)	UVmax cyclohexane (1mg/100 mL)	UVmax sodium hydroxide (1mg/100 mL)	UVmax phosphate buffer (1mg/100 mL)
3a	204, 260, 293	227, 275	217, 241, 272	206, 261
3b	208, 265	227, 277	216, 245, 265	205, 257
3d	204, 245, 287	227, 267	216, 244, 273	203, 264
3e	206, 263	227, 270	217, 245	204, 254
3f	204, 280	227, 281	217, 286	205, 281

H17C…Cg1=2.84Å and C17-H17C…Cg1=134°; Cg1 is the centroid of ring C at (2-x, -y, 1-z)]. Finaly, the π…π stacking interaction occurs between ring C at (x, y, z) and ring A at (x, y-1, z) [ring A…Ring B=3.717(2)Å] (Fig. 2, DMF molecules are excluded for clarity, Ct01 and Ct02 represent ring C and ring A, respectively).

Experimental

Melting points were determined by using a Büchi–530 melting point apparatus. Elemental analysis were performed on a Carlo Erba 1106. UV spectra were determined on a Shimadzu UV 2100 S spectrophotometer. IR spectra were run by a Perkin Elmer 5100 spectrophotometer as KBr pellets. ^{1}H–NMR spectra were obtained on a Bruker AC 200L spectrometer at 200 MHz using TMS as the internal reference. MS spectra were determined at 70 eV on a Kratos MS –9/50 spectrometer.

*General procedure for the preparation of 5-[4-(4-fluorobenzoylamino)phenyl]-2-substitutedamino-1,3,4-thiadiazoles (**2a-f**)*

To 0.001 mol of compounds **1a-f**, concentrated sulphuric acid (1ml) was added dropwise. The mixture was stirred for 30 min. The reaction content was poured into ice-water mixture. The precipitate was washed with sodium carbonate solution and water. The crude product was dried and recrystallized from ethanol (19).

*General procedure for the preparation of 5-[4-(4-fluorobenzoylamino)phenyl]-4-substituted-2,4-dihydro-3H-1,2,4-triazole-3-thiones (**3a-f**).*

To 0.01 mol of compounds **1a-f**, 2N NaOH (30 mL) was added and the mixture was heated at reflux for 1h. The reaction mixture was neutralized with hydrochloric acid. The precipitate was filtered, washed with water and recrystallized from ethanol (17).

***5-[4-(4-Fluorobenzoylamino)phenyl]-2-methylamino-1,3,4-thiadiazole* (2a)**

This compound was obtained as pale yellow powder (ethyl alcohol), yield 0.26 g (78%); mp 267-268 °C; UV: λ_{max} 208 nm (ε 26532), 228 nm (ε 21344), 326 nm (ε 50733); IR (KBr): 3320, 3170, 1642, 1600, 1530, 1225, 820 cm^{-1} ; MS (70 eV, electron impact) m/z: 328 (M^+), 327, 311, 293, 125, 100. *Anal*. Calcd. for $C_{16}H_{13}FN_4OS$: C, 58.52; H, 3.98; N, 17.06. Found: C, 59.17; H, 4.01; N, 16.63.

***5-[4-(4-Fluorobenzoylamino)phenyl]-2-ethylamino-1,3,4-thiadiazole* (2b)**

This compound was obtained as cream coloured needles (ethyl alcohol), yield 0.15g (64 %); mp 254-258 °C; UV: λ_{max} 208 nm (ε 33452), 226 nm (ε 27392), 266 nm (ε 16880), 328 nm (ε 59338); IR (KBr): 3320, 3178, 1650, 1570, 1230, 820 cm^{-1}; ^{1}H-NMR (DMSO-d_6): δ 0.94 (t, 3H, -$CH_2CH_2\underline{CH_3}$), 1.6 (m, 2H, -$CH_2\underline{CH_2}CH_3$), 3.27 (-$\underline{CH_2}CH_2CH_3$ was over shadow by DMSO peak), 7.35-8.05 (m, 9H, aromatic protons and –NH-$CH_2CH_2CH_3$), 10.40 (s, 1H, -CO<u>NH</u>-); MS (70 eV, electron impact) m/z: 342 (M^+), 326, 313, 241, 219, 117, 102, 95, 77, 69, 44, 28. *Anal*. Calcd. for $C_{17}H_{15}FN_4OS$: C, 59.63; H, 4.42; N, 16.36. Found : C, 59.32; H, 4.43 ; N, 16.31.

***5-[4-(4-Fluorobenzoylamino)phenyl]-2-propylamino-1,3,4-thiadiazole* (2c)**

This compound was obtained as yellow powder (ethyl alcohol), yield 0.32g (89 %); mp 233-237 °C; UV: λ_{max} 207 nm (ε 21564), 226 nm (ε 15148), 329 nm (ε 38102); IR (KBr): 3280, 3180, 2958, 2868, 1650, 1600, 1550, 1500, 1230, 830 cm^{-1} ;^{1}H-NMR (DMSO-d_6): δ 0.94 (t, 3H, -$CH_2CH_2\underline{CH_3}$), 1.6 (m, 2H, -$CH_2\underline{CH_2}CH_3$), 3.27 (-$\underline{CH_2}CH_2CH_3$ was over shadow by DMSO peak), 7.35-8.05 (m, 9H, aromatic protons and –<u>NH</u>-$CH_2CH_2CH_3$), 10.40 (s, 1H, -CO<u>NH</u>-); MS (70 eV, electron impact) m/z: 356 (M^+), 341, 327, 314, 241, 123, 119, 117, 95, 65, 63. *Anal*. Calcd. for $C_{18}H_{17}FN_4OS$: C, 60.65; H, 4.81; N, 15.72. Found : C, 60.56; H, 4.86; N, 15.30.

***5-[4-(4-Fluorobenzoylamino)phenyl]-2-allylamino-1,3,4-thiadiazole* (2d)**

This compound was obtained as yellow powder (ethyl alcohol), yield 0.26 g (72 %); mp 219-220 °C; UV λ_{max} 205nm (ε 14885), 226nm (ε 9923), 326nm (ε 27998); IR (KBr): 3270, 3030, 2920, 2840, 1650, 1600, 1550, 1500, 1230, 834 cm^{-1}; MS (70 eV, electron impact): m/z 354 (M^+), 353, 339, 325, 311, 127, 124. *Anal*. Calcd. for $C_{18}H_{15}FN_4OS \cdot 3/2.H_2O$: C, 56.68; H, 4.36. Found : C, 57.23; H, 4.35.

***5-[4-(4-Fluorobenzoylamino)phenyl]-2-cyclohexylamino-1,3,4-thiadiazole* (2e)**

This compound was obtained as pale yellow powder (ethyl alcohol), yield 0.36 g (69 %); mp 251 °C; UV λ_{max} 208nm (ε 25415), 226nm (ε 18833), 330 nm (ε 41354); IR (KBr): 3500-3000, 2922, 2843, 1670, 1600, 1525, 1500, 1230, 832 cm^{-1}; MS (70 eV, electron impact) m/z: 396 (M^+), 395, 346, 345, 339, 337, 325, 319, 318, 311, 269, 255, 217, 165, 135, 128. *Anal*. Calcd. for $C_{21}H_{21}FN_4OS$: C, 63.61; H, 5.33; N, 14.13. Found: C, 63.67; H, 5.46; N, 13.72.

***5-[4-(4-Fluorobenzoylamino)phenyl]-2-phenylamino-1,3,4-thiadiazole* (2f)**

This compound was obtained as yellow powder (ethyl alcohol), yield 0.25g (68 %); mp 293 °C; UV λ_{max} 207nm (ε 29947), 221nm (ε 21981), 341nm (ε 43651); IR (KBr): 3330, 3180, 3040, 1642, 1600, 1570, 1500, 1230, 840, 750 cm^{-1}; ^{1}H-NMR (DMSO-d_6): δ 7.02 (t, 1H, the proton of para position according to secondary amine), 7.36-8.10 (m, 12H, aromatic protons), 10.40 (s, 2H, -<u>NH</u>- and –CO<u>NH</u>-); MS (70 eV, electron impact) m/z 390 (M^+), 389, 267, 256, 150, 124, 123, 119, 95, 77, 32. *Anal*. Calcd. for $C_{21}H_{15}FN_4OS$: C, 64.61; H, 3.93; N, 14.11. Found: C, 64.60; H, 3.87; N, 14.35.

***5-[4-(4-Fluorobenzoylamino)phenyl]-2-phenylethylamino-1,3,4-thiadiazole* (2g)**

This compound was obtained as yellow powder (ethyl alcohol), yield 0.09 g (75 %); mp 185 °C; UV λ_{max} 204 nm (ε 32391), 227 nm (ε 15275), 328 nm (ε 28960); IR (KBr): 3280, 3180, 3040, 2922, 2860, 1650, 1600, 1560, 1500, 1235, 840, 750 cm^{-1}. *Anal*. Calcd. for $C_{23}H_{19}FN_4OS.3/2.H_2O$: C, 62.00; H, 4.63; N, 12.57. Found: C, 61.47; H, 4.50; N, 12.05.

***5-[4-(4-Fluorobenzoylamino)phenyl]-4-methyl-2,4-dihydro-3H-1,2,4-triazole-3-thione* (3a)**

This compound was obtained as cream powder (ethyl alcohol), yield 0.45 g (87 %); mp 250-253 °C; UV λ_{max} 204 nm (ε 33109), 260 nm (ε 24132), 293 nm (ε 23208); IR (KBr): 3286, 3197, 3098, 2937, 1641, 1603, 1505, 1480, 1447, 1439, 1241, 1181 cm^{-1}. ^{1}H-NMR (DMSO-d_6): δ 3.48 (s, 3H, -NH-$\underline{CH_3}$), 7.38-8.10 (m, 8H, aromatic protons), 10.49 (s, 1H, -CO<u>NH</u>-), 13.82 (s, 1H, triazole <u>NH</u>); MS (70 eV, electron impact) m/z 328 (M^+), 205, 133, 123, 117, 95, 89, 73. *Anal*. Calcd. for $C_{16}H_{13}FN_4OS \cdot H_2O$: C, 55.48; H, 4.36; N, 16.17. Found: C, 55.16; H, 4.25; N, 16.13.

5-[4-(4-Fluorobenzoylamino)phenyl]-4-ethyl-2,4-dihydro-3H-1,2,4-triazole-3-thione **(3b)**

This compound was obtained as dark yellow powder (ethyl alcohol), yield 0.28 g (80 %); mp 245-246 °C; UV λ_{max} 208 nm (ε 33106), 265 nm (ε 68049); IR (KBr): 3205, 3093, 2931, 1615, 1518, 1490, 1435, 1190. MS (70 eV, electron impact) m/z 342 (M^+), 341, 339, 337, 326, 325, 323, 311, 309, 297, 293, 265, 255, 253, 185, 145, 139, 125, 116 cm^{-1}. *Anal*. Calcd. for $C_{17}H_{15}FN_4OS$. H_2O: C, 56.65; H, 4.75; N, 15.54. Found: C, 56.41; H, 4.67; N, 15.38**.**

5-[4-(4-Fluorobenzoylamino)phenyl]-4-propyl-2,4-dihydro-3H-1,2,4-triazole-3-thione **(3c)**

This compound was obtained as dark yellow powder (ethyl alcohol), yield 0.53 g (67 %); mp 201-202 °C; UV λ_{max} 204 nm (ε 43435), 243 nm (ε 20669), 288 nm (ε 31790); IR (KBr): 3245, 3095, 2923, 2846, 1609, 1498, 1476, 1456, 1409, 1258, 1185 cm^{-1}. ^{1}H-NMR (DMSO-d_6): δ 0.78 (t, 3H, -$CH_2CH_2\underline{CH_3}$), 1.53 (m, 2H, -$CH_2\underline{CH_2}CH_3$), 4.00 (q, 2H, -$\underline{CH_2}CH_2CH_3$), 6.65-8.12 (m, 8H, aromatic protons), 10.54 (s, 1H, -CO<u>NH</u>-), 13.69 (widespread, 1H, triazole <u>NH</u>). *Anal*. Calcd. for $C_{18}H_{17}FN_4OS$. H_2O: C, 57.74; H, 5.11; N, 14.96. Found: C, 57.49; H, 4.92; N, 14.65.

5-[4-(4-Fluorobenzoylamino)phenyl]-4-allyl-2,4-dihydro-3H-1,2,4-triazole-3-thione **(3d)**

This compound was obtained as dark yellow powder (ethyl alcohol), yield 0.51 g (91 %); mp 207-208 °C; UV λ_{max} 204 nm (ε 38136), 245 nm (ε 17839), 287 nm (ε 26405); IR (KBr): 3228, 3115, 3095, 2952, 2974, 1634, 1608, 1516, 1487, 1471, 1443, 1418, 1196 cm^{-1}; ^{1}H-NMR (DMSO-d_6) δ: 4.68 (d, 2H, -CH_2), 4.89 (d, 1H, allyl=CH, *trans*, J:17.1Hz), 5.15 (d, 1H, allyl=CH, *cis*, J:10.3 Hz), 5.76-5.95 (m, 1H, CH=), 6.61-8.08 (m, 8H, aromatic protons), 10.45 (s, 1H, -CO<u>NH</u>-), 13.65 (s, 1H, triazole <u>NH</u>). *Anal*. Calcd. for $C_{18}H_{15}FN_4OS$. H_2O : C, 57.75; H, 5.12; N, 14.97. Found: C, 57.73; H, 4.44; N, 14.95.

5-[4-(4-Fluorobenzoylamino)phenyl]-4-cyclohexyl-2,4-dihydro-3H-1,2,4-triazole-3-thione **(3e)**

This compound was obtained as dark yellow powder (ethyl alcohol), yield 0.35 g (70 %); mp 232-235 °C; UV λ_{max} 206 nm (ε 30121), 263 nm (ε 50017); IR (KBr): 3233, 3109, 2927, 2850, 1608, 1506, 1489, 1456, 1404, 1254, 1190 cm^{-1}; ^{1}H-NMR (DMSO-d_6) δ: 0.94-2.38 (m, 10H, cyclohexyl protons), 4.25 (t, 1H, cyclohexyl proton), 6.63-7.97 (m, 8H, aromatic protons), 10.52 (s, 1H, -CO<u>NH</u>-), 13.74 (singlet, 1H, triazole <u>NH</u>). *Anal*. Calcd. for $C_{21}H_{21}FN_4OS$. H_2O : C, 60.85; H, 5.59; N, 13.52. Found : C, 60.61; H, 5.55; N, 13.63.

5-[4-(4-Fluorobenzoylamino)phenyl]-4-phenyl-2,4-dihydro-3H-1,2,4-triazole-3-thione **(3f)**

This compound was obtained as pale yellow powder (ethyl alcohol), yield 0.27 g (86 %); mp 258-260 °C; UV λ_{max} 204 nm (ε 36949), 280 nm (ε 22801); IR (KBr): 3105, 3090, 2929, 2887, 1652, 1627, 1606, 1581, 1436, 1410, 1244, 1183 cm^{-1}; ^{1}H-NMR (DMSO-d_6) δ: 7.04-8.10 (m, 13H, aromatic protons), 10.49 (s, 1H, -CO<u>NH</u>-); MS (70 eV, electron impact) m/z 390 (M^+), 389, 373, 273, 272, 253, 245, 243, 237, 235, 233, 229, 227, 223, 219, 213, 191, 189, 183, 181, 176, 173, 169, 151, 135, 128, 127, 126, 117, 115, 114, 109, 105, 100. *Anal*. Calcd. for $C_{21}H_{15}FN_4OS$. H_2O : C, 61.76; H, 4.19; N, 13.72. Found : C, 62.00; H, 4.11; N, 13.69.

X-ray crystallography analysis

The structure of title compound, 5-[4-(4-fluorobenzoylamino) phenyl]-4-ethyl-2,4-dihydro-3H-1,2,4-triazole-3-thione **(3b)** is shown in Fig 1 and crystal data are given in Table 2, together with refinement details. After, single crystals were selected and mounted on the tip of the glass fiber, preliminary examination and data collection were performed with Mo Kα radiation (λ=0.71073Å) on an Enraff-Nonius CAD4 kappa axis difractometer operating in ω/2θ scanning mode. The structure was determined by direct methods (SHELXS-97 (24)) and refined by full covariance matrix methods (SHELXL-97 (25)).

The fractional coordinates and mean temperature factors with estimated Standard deviations for non-hydrogen atoms are listed in Table 3 and selected bond lengths are given in Table 4, selected bond angles are given in Table 5. The hydrogen bonding details are shown in Table 6. The geometric calculations and preparing material for pablication were performed using the programs SHELX-97, PARST (26) and WinGX (27).

TABLE 2. Crystal data and summary of data collection and structure refinement

Empirical formula	$C_{20}H_{22}N_5O_2FS$
Formula weight	413.49
Crystal system	Triclinic
Space group	P-1
a(Å)	8.774(2)
b(Å)	10.720(2)
c(Å)	12.312(2)
α(°)	109.850(3)
β(°)	91.012(4)
γ(°)	108.742(4)
Cell volume, Å^3	1020.9(3)
Z	3
Density (calculated), mg/m^{-3}	2.018
F(000)	651
Absorption coefficient, mm^{-1}	0.289
Crystal size, mm	0.49X0.35X0.25
Radiation	Mokα, (λ= 0.71073Å)
Reflections measured	5790
Independent observed reflections	4037 (R_{int} = 0.022)
Limiting indices	-11≤H≤6, -10≤k≤14, -15≤l≤16
Θ range, deg	1,78-27,99
R [$i > 2\sigma(i)$]	R1=0.0638, wR2=0.1769
R (all data)	R1=0.0892, wR2=0.1936
Goodnes of fit on F^2	1.162
Largest diffraction peak and hole, e/Å^3	0.655 And –0.588

Symmetry transformations used to generate equivalent atoms: (none) x, y,z; (i) x, y-1, z; (ii) –x+1, -y, -z+1.

Sıx of the synthesized compounds (2a, 2d, 2e, 3a, 3d and 3e) were tested for their cytotoxic activities. Cell viability and cytotoxic activity profile of the compounds were analyzed using the MTT assay. MTT is cleaved to formazan by the "succinate-tetrazolium reductase" system (EC 1.3.99.1) which belongs to the mitochondrial respiratory chain and is active only in viable cells (28) .

Two cell lines (normal and cancerous) were used for the determination of cytotoxic activity. HeLa cell line was used for the determination of cytotoxic activity. HeLa cells are an <u>immortal cell</u>

TABLE 3. The fractional coordinates and mean temperature factors with estimated standart deviations for non-hydrogen atoms

	x	y	z	Ueq
C1	0.6365(4)	-0.4337(3)	0.9069(3)	0.0486(8)
C2	0.6951(4)	-0.5421(4)	0.8952(3)	0.0532(8)
C3	0.7022(4)	-0.6256(3)	0.7854(3)	0.0499(8)
C4	0.6554(4)	-0.6073(4)	0.6884(3)	0.0548(9)
C5	0.5953(4)	-0.4986(4)	0.7008(3)	0.0500(8)
C6	0.5865(4)	-0.4102(3)	0.8111(3)	0.0389(7)
C7	0.5365(4)	-0.2840(3)	0.8308(3)	0.0451(8)
C8	0.3940(3)	-0.1771(3)	0.7310(3)	0.0361(6)
C9	0.2935(4)	-0.2060(3)	0.6311(3)	0.0404(7)
C10	0.4470(4)	-0.0404(3)	0.8156(3)	0.0440(7)
C11	0.4017(4)	0.0632(3)	0.7956(3)	0.0430(7)
C12	0.2494(4)	-0.1020(3)	0.6121(3)	0.0425(7)
C13	0.3052(4)	0.0362(3)	0.6943(3)	0.0367(6)
C14	0.2556(4)	0.1504(3)	0.6827(3)	0.0367(7)
C15	0.2043(4)	0.2996(3)	0.6118(3)	0.0407(7)
C16	0.3075(4)	0.1247(3)	0.4733(3)	0.0446(7)
C17	0.1715(5)	0.0263(4)	0.3747(3)	0.0592(9)
C18	0.0680(4)	0.6307(4)	0.8255(3)	0.0487(8)
C19	0.0872(7)	0.8019(5)	1.0153(4)	0.0981(2)
C20	-0.0078(5)	0.8323(4)	0.8389(4)	0.0696(9)
N1	0.4406(3)	-0.2880(3)	0.7411(2)	0.0417(6)
N2	0.2511(3)	0.1838(2)	0.5837(2)	0.0367(6)
N3	0.2140(3)	0.2378(3)	0.7696(2)	0.0441(6)
N4	0.1806(3)	0.3279(3)	0.7238(2)	0.0444(6)
N5	0.0495(4)	0.7491(3)	0.8902(3)	0.0520(7)
O1	0.5840(4)	-0.1831(3)	0.9230(2)	0.0752(9)
O2	0.1173(3)	0.5558(3)	0.8631(2)	0.0613(7)
F1	0.7625(3)	-0.7316(2)	0.7718(2)	0.0783(7)
S1	0.1833(1)	0.3893(8)	0.5269(7)	0.0561(3)

line used in scientific research and were purchased from the American Type Culture Collection (ATCC CCL-2, Rockville, MD). The MTT metabolic assay was carried out in 96-well flat-bottom cell culture plates seeded with $1x10^4$ cells/well HeLa cells in 200µL MEM (Minumum Essential Medium) with 10% FBS (Fetal Bovine Serum). The following day, media was aspirated and the compounds were solved in DMSO and diluted with broth before they were added to the cell culteres at the concentrations of 5µg/mL and 10µg/mL. Cells were incubated for 48 hrs at 37 °C, 5% CO_2. After the incubation period, 10µL of the MTT labelling reagent (final concentration 0.5 mg/mL) was added to each well. After the incubation of the microplate for 4-12 hrs in a humidified atmosphere (e.g. 37 °C, 5.0 % CO_2) 100µL of the solubilization solution was added into each well. Following the plate was allowed to stand overnight in the incubator in a humidified atmosphere (e.g. 37 °C, 5.0 % CO_2) the formazan precipitates become soluable. Absorbance of formazan product was measured spectrophotometrically at 550 and 690 nm.

Based on the gained data evaluated from normal cell line procedure, four compounds which belong to our set of six compounds demonstrated inhibition between 1-10 % whereas the other two compounds demonstrated inhibition between 10-20 %.

Based on the gained data evaluated from cancerous cell line procedure, three compounds which belong to our set of six compounds demonstrated inhibition between 1-10 % whereas the other three compounds demonstrated inhibition between 10-30 %.

The highest inhibition was confirmed as 18.63 % and 16.97 % for the compounds *5-[4-(4-fluorobenzoylamino)phenyl]-2-cy-*

TABLE 4. Selected bond lenghts (Å)

N4	C15	1.342(4)
N4	N3	1.373(3)
O2	C18	1.231(4)
C18	N5	1.312(4)
S1	C15	1.686(3)
N2	C15	1.367(4)
N2	C14	1.385(3)
N2	C16	1.461(4)
N1	C7	1.356(4)
N1	C8	1.417(3)
F1	C3	1.363(4)
N3	C14	1.308(4)
O1	C7	1.221(4)
N5	C19	1.440(5)
N5	C20	1.452(4)

TABLE 5. Selected bond angles(o)

C15	N4	N3	113.0(3)
O2	C18	N5	125.0(3)
C15	N2	C14	107.4(2)
C15	N2	C16	123.6(2)
C14	N2	C16	128.4(2)
C7	N1	C8	127.8(3)
C14	N3	N4	104.2(2)
C11	C13	C12	117.8(3)
C11	C13	C14	118.7(2)
C12	C13	C14	123.3(3)
C9	C8	C10	118.9(3)
C9	C8	N1	117.8(2)
C10	C8	N1	123.3(3)
C9	C12	C13	120.5(3)
N4	C15	N2	104.4(3)
N4	C15	S1	127.2(2)
N2	C15	S1	128.3(2)
C12	C9	C8	121.3(3)
C11	C10	C8	119.3(3)
N3	C14	N2	111.0(2)
N3	C14	C13	122.5(3)
N2	C14	C13	126.4(2)
C6	C5	C4	120.1(3)
C1	C6	C5	118.7(3)
C1	C6	C7	118.6(3)
C5	C6	C7	122.6(3)
O1	C7	N1	123.2(3)
O1	C7	C6	120.3(3)
N1	C7	C6	116.6(3)
C18	N5	C19	120.8(3)
C18	N5	C20	121.7(3)
C19	N5	C20	117.5(3)
C10	C11	C13	122.2(3)
C6	C1	C2	121.6(3)
C3	C2	C1	117.7(3)
C4	C3	C2	123.3(3)
C4	C3	F1	117.9(3)
C2	C3	F1	118.8(3)
C3	C4	C5	118.7(3)
N2	C16	C17	113.7(3)

TABLE 6. Lengths (Å) and angles (o) of the hydrogen bonds

D-H…A	D…A	H…A	D-H…A
N4-H44…O2	2.698(4)	1.69(4)	172(4)
C10-H10…O1	2.834(5)	2.23(1)	121(1)
N1-H1'…S1^{I}	3.549(2)	2.73(3)	164(3)
C17-H17A…F1^{II}	3.304(5)	2.68(1)	123(1)

clohexylamino-1,3,4-thiadiazole **(2e)** and *5-[4-(4-fluorobenzoylamino)phenyl]-2-allylamino-1,3,4-thiadiazole* **(2d)** respectively at the end of the experiments on normal cell line.

The highest inhibition was confirmed as 29.70 % and 21.97 % for the compunds *5-[4-(4-fluorobenzoylamino)phenyl]-2-cyclohexylamino-1,3,4-thiadiazole* **(2e)** and *5-[4-(4-fluorobenzoylamino) phenyl]-2-allylamino-1,3,4-thiadiazole* **(2d)** respectively as a result of the experiments by using cancerous cell line.

The cytotoxic activity of the selected compounds towards cancerous cell line is higher than normal cell line. In normal cell line, the cytotoxic activity of 5-[4-(4-fluorobenzoylamino) phenyl]-2-allylamino-1,3,4-thiadiazole (**2d**) were found to be invariable according to the the means of dose variation. In cancerous cell line cytotoxic activity increases as a positive function of the increase in doses.

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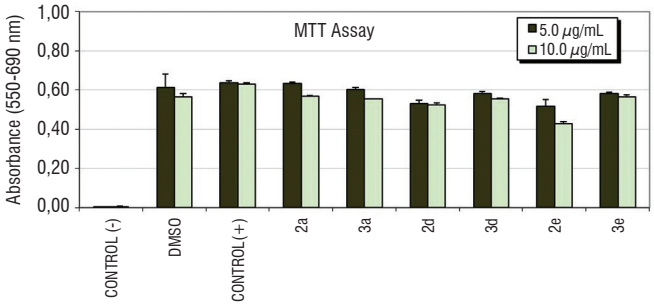


FIGURE 3. Absorbance values of after MTT assay formazan crystalls (a)

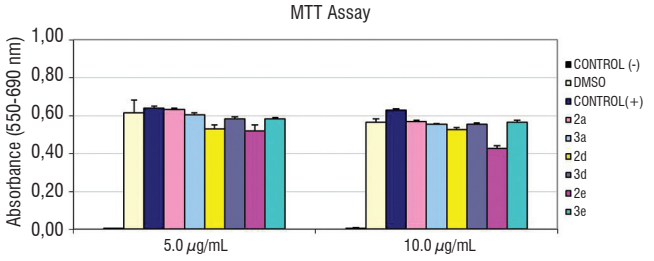


FIGURE 4. Absorbance values of after MTT assay formazan crystalls (b)

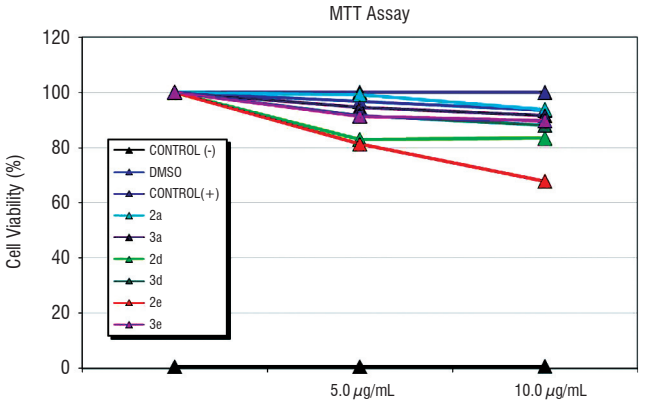
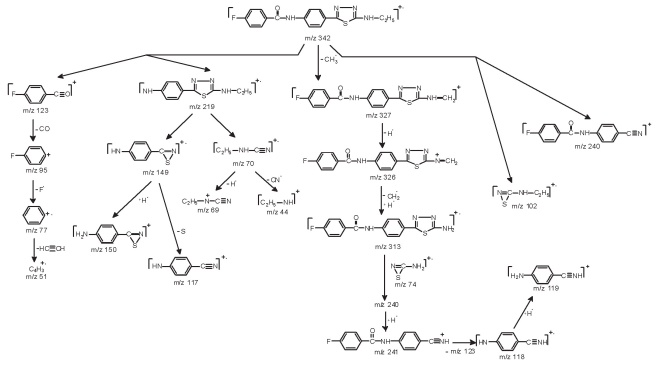
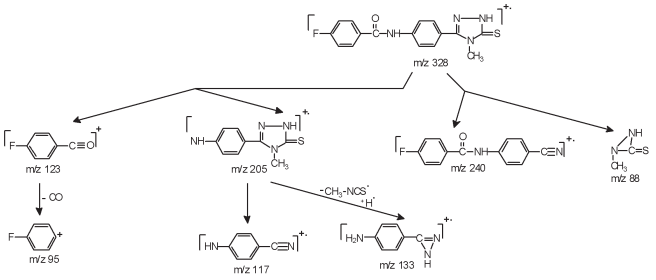


FIGURE 5. The effect of substances on cell viability



SCHEME 3. MS fragmentation patterns of compound 2b



SCHEME 4. MS fragmentation patterns of compound 3a

1,2,4-Triazolin-3-tiyon ve 2,5-disübstitüe-1,3,4-tiyadiazol türevlerinin sentezi ve sitotoksik aktiviteleri

ÖZET: Çalışmada bir seri 5-[4-(4-florobenzoilamino)fenil]-2-sübstitüeamino-1,3,4-tiyadiazol (2a-g) ve 5-[4-(4-florobenzoilamino)fenil]-4-sübstitüe-2,4-dihidro-3H-1,2,4-triazol-3-tiyon (3a-f) türevi bileşik sentez edilmiş ve yapıları elementel analiz, UV-visibl, IR, 1H-NMR, MS spektroskopik yöntemlerle kanıtlanmıştır. Ayrıca madde 3b'nin X-ray kristalografisi ile yapısı aydınlatılmıştır. Sentez edilen bileşikler arasından prototip seçilen altı bileşiğin (2a, 2d, 2e, 3a, 3d ve 3e) sitotoksik aktiviteleri HeLa (ATCC CCL-2) ve normal hücre hattı kullanılarak MTT [3-(4,5-dimetiltiyazol-2-il)-2,5-difenil tetrazolyum bromür] yöntemine göre [Hücre Proliferasyon Kit I (MTT) Roche-Germany] incelenmiştir.

ANAHTAR KELİMELER: Sitotoksik aktivite, 1,2,4-triazol-3-tiyon, 2,5-disübstitüe-1,3,4-tiyadiazol, MTT yöntemi, x-ray kristolografi

REFERENCES

1. Foroumadi A, Mansouri S, Kiani Z, Rahmani A. Synthesis and in vitro antibacterial evaluation of N-[5-(5-nitro-2-thienyl)-1,3,4-thiadiazole-2-yl]piperazinyl quinolones. Eur J Med Chem, 38: 851-854, 2003.
2. Foroumadi A, Soltani F, Moshafi MH, Ashraf-Askari R. Synthesis and in vitro antibacterial activity of some N-(5-aryl-1,3,4-thiadiazole-2-yl)piperazinylquinolone derivatives. Farmaco, 58: 1023-1028, 2003.
3. Thomasco LM, Gadwood RC, Weaver EA, Ochoada JM, Ford CW, Zurenko GE, Hamel JC, Stapert D, Moerman JK, Schaadt RD, Yagi BH. The synthesis and antibacterial activity of 1,3,4-thiadiazole phenyl oxazolidinone analogues. Bioorg Med Chem Lett, 13: 4193-4196, 2003.
4. Collin X, Sauleau A, Coulon J. 1,2,4-Triazolo mercapto and aminonitriles as potent antifungal agents. Bioorg Med Chem Lett, 13: 2601-2605, 2003.
5. Doğan HN, Duran A, Rollas S, Şener G, Uysal MK, Gülen D. Synthesis of new 2,5-disubstituted-1,3,4-thiadiazoles and preliminary evaluation of anticonvulsant and antimicrobial activities. Bioorg Med Chem, 10: 2893-2898, 2002.
6. Rollas S, Yılmaz N, Erdeniz H, Kiraz M. Antimicrobial activities of some 5-(4-nitro/aminophenyl)-1, 2, 4- triazoline-3-thione derivatives. Med Sci Res, 26: 83-84,1998.
7. Oruç E, Rollas S, Kandemirli F, Shvets N, Dimoglo AS. 1,3,4-Thiadiazole Derivatives Synthesis, Structure Elucidation and Structure-Antitüberculosis Activity Relationships Investigations. J Med Chem, 47: 6760-6767, 2004.
8. Shiradkar MR, Murahari KK, Gangadasu HR, Suresh T, Kalyan CA, Panchal D, Kaur R, Burange P, Ghogare J, Mokale V, Raut M. Bioorg Med Chem, 15: 3997-4008, 2007.
9. Küçükgüzel I, Tatar E, Küçükgüzel ŞG, Rollas S, Clercq ED. Synthesis of some novel thiourea derivatives obtained from 5-[(4-aminophenoxy)methyl]-4-alkyl/aryl-2,4-dihydro-3H-1,2,4-triazole-3-thiones and evaluation as antiviral/anti-HIV and anti-tuberculosis agents. Eur J Med Chem, 43: 381- 392, 2008.
10. Sharma S, Srivastava VK, Kumar A. Newer N-substituted anthranilic acid derivatives as potent anti-inflammatory agents. Eur J Med Chem, 37: 689-697, 2002.
11. Palaska E, Şahin G, Kelicen P, Durlu NT, Altınok G. Synthesis and anti-inflammatory activity of 1-acylthiosemicarbazides, 1,3,4-oxadiazoles, 1,3,4-thiadiazoles and 1,2,4-triazole-3-thiones. Farmaco, 57: 101-107, 2002.
12. Vio L, Mamolo MG, Laneve A. Synthesis and antihypertensive activity of some 1,3,4-thiadiazole derivatives. Farmaco, 44: 165-172, 1988.
13. Idrees GA, AlyGamal OM, El-Din A.A. Abuo-Rahma G, Radwan MF. Design, synthesis and hypolipidemic activity of novel 2-(naphtalen-2-yloxy)propionic acid derivatives as desmethyl fibrate analogs. Eur J Med Chem, 44: 3973-3980, 2009.
14. Mavrova AT, Wesselinova D, Tsenov YA, Denkova P. Synthesis, cytotoxicity and effects of some 1,2,4-triazole and 1,3,4-thiadiazole derivatives on immunocompetent cells. Eur J Med Chem, 44: 63-69, 2009.
15. Doğan HN, Duran A, Rollas S. Synthesis and preliminary anticancer activity of new 1H-4,5-dihydro-3-(3-hydroxy-2-naphthyl)-4-substituted-1,2,4-triazoline-5-thiones. Part II. Indian J Chem, 44B: 2301-2307, 2005.
16. Holla BS, Veerendra B, Shivananda MK, Poojary B. Synthesis characterization and anticancer activity studies on some Mannich bases derived from 1,2,4-triazoles. Eur J Med Chem, 38: 759-767, 2003.
17. Rollas S, Kalyoncuoğlu N, Sür-Altıner D, Yeğenoğlu Y. 5-(4-Aminophenyl)-4- substituted-2,4-dihydro-3H-1,2,4-triazole-3-thiones : Synthesis and antibacterial and antifungal activities. Pharmazie, 48: 308-309, 1993.
18. Pospisil P, Ballmer P, Scapozza L, Folkers G. Tautomerism in computer-aided drug design. J Recept Signal Transduct Res, 23: 361-371, 2003.
19. Durgun BB, Rollas S, Apaydın S, Öztürk R. Synthesis and antimicrobial activity of some new 1-[4-(4-fluorobenzoylamino)benzoyl]-4-substitutedthiosemicarbazides. Drug Metab and Drug Interact, 12: 145-150, 1995.
20. Sharba AHK, Al-Bayati RH, Rezki N, Aouad MR. Synthesis of thiadiazoles and 1,2,4-triazoles derived from cyclopropane dicarboxylic acid. Molecules, 10: 1153-1160, 2005.
21. Kubota S, Uda M. 1,2,4-Triazoles II. The tautomerism of 3-a-pyridyl-1,2,4-triazoline-5-thione and its methyl derivatives. Chem Pharm Bull, 20: 2096-2101, 1972.
22. Dobosz M, Pitucha M, Wujec M. The reactions of cyclization of thiosemicarbazide derivatives to 1,2,4-triazole or 1,3,4-thiadiazole system. Acta Pol. Pharm, 53: 31-38, 1996.
23. Rollas S. Synthesis and spectroscopic data of some 1,3,4-thiadiazoles. J Fac Pharm Istanbul, 17: 155-163, 1981.
24. Sheldrick GM. SHELXS-97, Program for solution of crystal structures, University of Göttingen, 1997.
25. Sheldrick GM. SHELXL-97, Program for solution of crystal structures, University of Göttingen, 1997.
26. Nardelli M. A system of Fortran routines for calculating molecular structure parameters from the results of crystal structure analyses. J Appl Cryst, 28: 659, 1995.
27. Farrugia LJ. WinGX suite for small-molecule single-crystal crystallography. J Appl Cryst, 32: 837, 1999.
28. Sgouras D, Duncan R. Methods for the Evaluation of Biocompatibility of Soluble Synthetic Polymer Which have potential for Biomedical Use: 1-Use of the Tetrazolium-Based Colorimetric assay (MTT) As a Preliminary Screen for Evaluation of In Vitro Cytotoxicity. J Mater Sci Mater Med, 1: 61-68, 1990.