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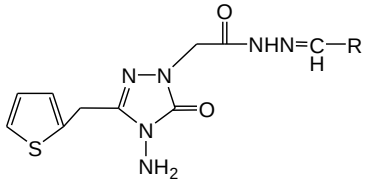

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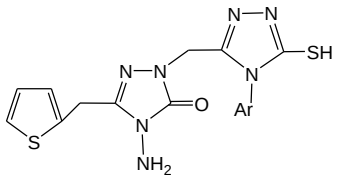

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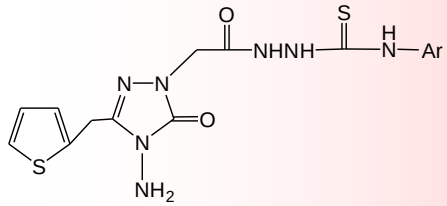

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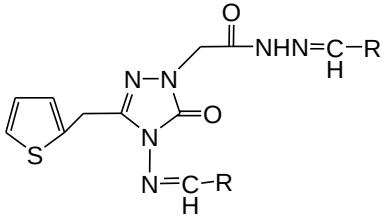

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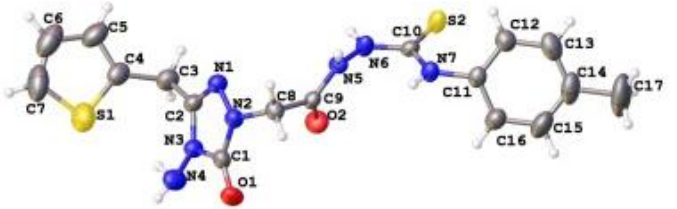

crystal structure of **4d**

New thiophene-1,2,4-triazole-5(3)-ones: highly bioactive thiosemicarbazides, structures of schiff bases and triazole-thiols

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ABSTRACT

Key compound 2-(4-amino-5-oxo-3-(thiophene-2-ylmethyl)-4,5-dihydro-1,2,4-tiazole-1-yl) acetohydrazide (**3**) was synthesized by reacting hydrazine hydrate with ethyl-2-(4-amino-5-oxo-3-(thiophene-2-ylmethyl)-4,5-dihydro-1,2,4-tiazole-1yl)acetate (**2**), obtained in basic media from 4-amino-5-(thiophene-2-ylmethyl)-2H-1,2,4-triazole-3(4H)-one (**1**). Compound **3** was converted to thiosemicarbazide derivatives (**4a-d)** and Schiff base derivatives **6a-e** and **7a-e**. The treatment of compound **4** with NaOH gave 4-amino-2-((4-(4-aryl)-5-mercapto-4H-1,2,4-triazole-3-yl)methyl)-5-(thiophene-2-ylmethyl)-2H-1,2,4-triazole-3(4H)-ones (**5a-d**). All newly compounds, well characterized by elemental analyses, IR, ^{1}H NMR, ^{13}C NMR and mass spectral studies were tested for their antioxidant and antimicrobial activities. Thiosemicarbazide derivatives (**4a-d**) were highly active in two antioxidant tests with 69.0-88.2% DPPH• scavenging and 503-1257 μM TEAC values, while the others showed lower or no activity. The results of the two antioxidant tests correlated well. Moreover, Thiosemicarbazide derivatives (**4a-d**) also showed antibacterial activity against *S. aureus*, *B. cereus*, and *M. smegmatis*. Thiosemicarbazide group deserves attention in the synthesis of bioactive compounds.

Keywords: Schiff base, Thiosemicarbazide, 1,2,4-triazole/thiol, Antioxidant, Antimicrobial

NOTE FOR GRAPHICAL ABSTRACT

Twenty novel triazole derivatives were synthesized. Compounds **4a-d** possessing triazole and thiosemicarbazide exhibited both antioxidant and antimicrobial activities. Crystal structures of **2** and **4d** were determined.

1. Introduction

Heterocyclic compounds that possess 1,2,4-triazole ring have relevance to the pharmacological properties. These properties are anti-inflammatory, antihypertensive fungicidal, antioxidant, antimicrobial and antiviral activities [1-5]. In addition to this kind of relevance, it was reported that Schiff bases that were originated from triazole compounds possessed some biological activities [6].

It has been stated that 1,2,4,-triazole-3-thiones possess biological activities which cover antimicrobial [7-10], antithyroid [11], hypoglycemic [12] and antidepressant [13,14].

Heterocyclic derivatives containing sulfur possess essential biological properties including anti-inflammatory [15], analgesic [16], antidepressant [17], antimicrobial [18] and anticonvulsant activities [19-22]. Antiepileptic drugs (AEDs) including tiagabine [19], etizolam [21], and brotizolam [22] contain thiophene moiety in their structures as active pharmacophore. Moreover, it has been stated that the presence of thiophene ring in its structure gives the higher activity of sodium phethenylate [20]. Furthermore, anticonvulsant activity of the compounds which contain hydrazones and thiosemicarbazones with different types of substitution has been well recorded. [23-27].

Antioxidants have capacity to protect organisms and cells from damage caused by oxidative stress during metabolism. For this reason, the synthetic compounds are extensively studied for their antioxidant activities using different methodologies. The search for active components that prevent or reduce the impact of oxidative stress on cells is a quite contemporary field. Exogenous chemicals involved in food systems and endogenous compounds involved in metabolic processes in human body produce highly reactive free radicals, particularly oxygen derived ones. They have the potential to oxidize biomolecules and cause cell death, consequently causing tissue damage. It is known that free radical oxidative processes also play a significant pathological role in causing many human diseases together with aging [28-29].

Thiosemicarbazide derivatives were reported to have stronger inhibitory effects on LP levels and more substantial scavenger effects on DPPH radical than those of their cyclic counterparts namely, 1,3,4-thiadiazoles and 2H-1,2,4-triazole-3(4H)-thiones [30].

Therefore, in this study, we proposed the synthesis of a new series of hybrid molecules which possess triazole-schiff base, triazole-triazol/tiol, triazole-thiosemicarbazide including thiophene for more efficacious biologically activities.

2. Chemistry

The synthesis of compounds **2**,**3**,**4**,**5**,**6** and **7** was carried out according to the steps shown in Scheme 1. Compound **1** with ethyl bromoacetate by refluxing in absolute ethanol in presence of sodium ethoxide afforded the ethyl-2-(4-amino-5-oxo-3-(thiophene-2-ylmethyl)-4,5-dihydro-1,2,4-triazole-1-yl) acetate (**2**) in good yield. Then, the compound **2** was converted to the corresponding compound, 2-(4-amino-5-oxo-3-(thiophene-2-ylmethyl)-4,5-dihydro-1,2,4-tiazol-1-yl)acetohydrazide (**3**) via the reaction with hydrazine hydrate. The treatment of acetohydrazide derivative (**3**) with several aromatic aldehydes (1 mol and 2 mol) in ethanol gave the Schiff bases **6a-e** and **7a-e** respectively. The reaction of compound **3** with 4-arylisothiocyanates produced 1-(2-(4-amino-5-oxo-3-(thiophene-2-ylmethyl)-4,5-dihydro-1,2,4-triazole-1-yl)acetyl)-4-(4-aryl)thiosemicarbazides (**4a-d**). The treatment of compound **4** with 2N NaOH caused the conversion of carbothioamide structure into 1,3,4-thiadiazole ring; thus, 4-amino-2-((4-(4-aryl)-5-mercapto-4H-1,2,4-triazole-3-yl)methyl)-5-(thiophene-2-ylmethyl)-2H-1,2,4-triazole-3(4H)-ones (**5a-d)** were obtained . The structures were confirmed by IR, ^{1}H NMR, ^{13}C NMR, elemental analyses. In addition, X-ray for compounds **2** and **4d** is presented in this paper.

3. Results and discussion

3.1. Synthesis

The main goal of the present study was to synthesize and investigate the antioxidant and antimicrobial activities of new thiophene-1,2,4- triazole-containing biologically active groups. Synthesis of the intermediate and target compounds was performed according to the reactions outlined in Scheme 1.

Following a previously reported literature procedure was performed, the starting compound 4-amino-5-thiophene-2-ylmethyl 2,4-dihydro[1,2,4]triazole-3-one (**1**) was prepared [6]. 4-amino-5-thiophene-2-ylmethyl 2,4-dihydro[1,2,4]triazole-3-one (**1)** with ethyl bromoacetate by refluxing in absolute ethanol in the presence of sodium ethoxide afforded the ethyl-2-(4-amino-5-oxo-3-(thiophene-2-ylmethyl)-4,5-dihydro-1,2,4-triazole-1-yl)acetate (**2**) in good yield. The ^{1}H and ^{13}C-NMR spectra of compound **2** exhibited additional signals originated from the $–CH_2CO_2Et$ group at the related chemical shift values.

The treatment of compound **2** resulted in the formation of hydrazide derivative **3** in good yield, and this was employed as key intermediate for synthesis of the target compounds. The

^{1}H-NMR spectrum of compound **3** did not display any signal belonging to the –OCH_2CH_3 group; instead, new signals originating from the hydrazide structure appeared at 4.06 ppm (-$NHNH_2$) and 9.21 ppm (-$NHNH_2$) integrating for 2 protons and 1 proton, respectively (controlled by changing with D_2O).

With the help of the reaction of isonicotinic acid hydrazides with 2-(4-amino-5-oxo-3-(thiophene-2-ylmethyl)-4,5-dihydro-1,2,4-tiazole-1-yl) acetohydrazide (**3**) phenyl/substitute phenyl thiosemicarbazides (**4a-d**) were obtained. In the ^{1}H-NMR spectra of compounds **4a-d,** additional NH- signal originating from thiosemicarbazide structures were observed at 9.18-9.68, 9.65-9.83 and 9.30-10.35 ppm, while the signal due to –NH_2 group of hydrazide structures did not appear. Additional signals belonging to the phenyl rings were observed in the aromatic region in the ^{1}H and ^{13}C-NMR spectra of compounds **4a-d**. Moreover, -C=S groups resonated at 181.32-181.64 ppm in the ^{13} C-NMR spectra of compounds **4a-d**.

When compounds **4a-d** were converted to 1,2,4-triazole-3-thiols (**5**) in basic media, the signal observed at 13.60-13.97 ppm in the ^{1}H-NMR spectra of compounds **5a-d** was attributed to –SH group, but -NH peak disappeared.

The synthesis of different Schiff base derivatives of compounds **6a-e** and **7a-e** were carried out by the reaction of compound **3** with several aromatic aldehydes (1 and 2 mol) in oil bath. Owing to the aromatic ring originated from aldehyde moiety at aromatic region, the ^{1}H and ^{13}C-NMR spectra of compounds **6a-e** and **7a-e** displayed additional signals, while the signal of –NH_2 group of hydrazide (for compound **6a-e**) and triazole –NH_2 (for compound **6a-e** and **7a-e**) structure did not appear.

3.2. Biological Activities

3.2.1. Antioxidant Activities

Compounds **2-7** were tested for their antioxidant activity based on the two methods most widely used in literature [30,32]. The first method applied was DPPH• scavenging, which may have disadvantage of dependence on the shape of the compound tested because the compound may have sterical hindrance to reach the radical site of DPPH•. DPPH method utilizes both single electron transfer (SET) and hydrogen atom transfer (HAT) reactions with antioxidants. The method is also known to have relatively slow kinetics. There are examples in which the test samples are inactive in DPPH• scavenging test, while they show high antioxidant activity in other test methods. Therefore, ferric reducing/antioxidant power

(FRAP) assay to measure antioxidant activity was also utilized. FRAP method utilizes only SET reaction mechanism and is widely used for determining antioxidant capacities of various samples including food, biological and synthetics.

DPPH radical scavenging activities were determined as %scavenging through 1 h incubation period, during which absorbance at 517 nm decreases as the active compound scavenges DPPH• present in the medium. The data is provided in Figure 1 for kinetic behavior of the reaction between only the active 12 compounds and DPPH•, and in Figure 2 for their comparison with the values only at 60 min incubation time. Because compounds **4a-d** were much more active in the pretests, their concentration was 10 μg/mL, while the concentration of the other compounds tested were 200 μg/mL.

The starting compounds **2** and **3**, all the thiosemicarbazides (**4a-d**), and triazole-thiols showed DPPH• radical scavenging activity at the test concentrations. Among the Schiff bases only **6d** and **7d** were slightly active and the others showed no activity at 200 μg/mL. With the DPPH scavenging compounds, especially highly active **4a-d**, the reaction appeared to be slow and continue even after 60 min incubation though at much reduced rates.

The comparatively high DPPH• scavenging activities of compounds **4a-d** can better be observed if the percentage scavenging values of the other active compounds (**2**, **3**, **5a-d**, **6d**, **7d**) were divided by 20, as the concentrations of **4a-d** were diluted 20 fold to see the kinetic behavior during 60 minute incubation. Therefore, the activities of **4a-d** were at least 20 fold higher than all other compounds. The activity order in the thiosemicarbazides based on the substituents was $–CH_3$ > –Br > –H > –F. However, the activity order in the triazole-thiols based on the substituents was different as –H > –F > $–CH_3$ > –Br, reflecting the importance of main structure in determination of the effect of substituent on DPPH• scavenging activity. The presence of N-H group on thiosemicarbazone moiety may provide hydrogen atom to DPPH• radical as stated by Nguyen et al. [33], which makes the compound a radical itself and reacts with other DPPH• radicals increasing the total antiradical capacity, and thus scavenging more radicals. The radical electron may delocalize towards the aromatic ring so as to stabilize the radical preventing it from self-destruction. Electron donating -CH_3 group attached to the aromatic ring (compound **4d**) resulted in highest antiradical activity in the thiosemicarbazide series probably by stabilizing the thiosemicarbazide radical, as opposed to the electron withdrawing group -F (compound **4c**) that reduced the antiradical activity. The presence of furan structure in Schiff bases appear to play a crucial role in antiradical activity as only the

furan derivatives (**6d** and **7d**) were active. Compounds **2** and **3** were only slightly active in DPPH• test.

With FRAP assay the sample compounds were tested for their abilities to reduce iron(III) to iron(II) ions, and the absorbance of TPTZ-Fe^{2+} complex was measured at 595 nm increasing with higher activity antioxidants. The absorbances measured for the samples were converted to Trolox equivalent antioxidant capacity (TEAC) values obtained from the absorbance – [Trolox] calibration graph, and the μM TEAC values are given in Figure 3. FRAP values show a similar trend with DPPH• scavenging results indicating higher activity with thiosemicarbazides (**4a-d**) and much lower activity with triazole-thiols (**5a-d**) and Schiff bases (**6**-**7**). The starting compound **2** also showed low activity as with DPPH• scavenging test. However, compound **3** was highly active in FRAP test as opposed to low activity in DPPH• scavenging test, which may be an indication of structural dependence of the antioxidation reaction in the latter case. The activity order for the thiosemicarbazides based on the substituents in compounds **4a-d** was same with DPPH• scavenging test as $–CH_3$ > –Br > –H > –F, reflecting the role of electron withdrawing and donating substituents as decreasing and increasing antioxidant capacity.

The results of the correlation of the two antioxidant tests are plotted (Figure 4), and a relatively good correlation was observed (R^2=0.83). The differences seen between the results of the two antioxidant methods are probably due to the differences between the reaction mechanisms and in dependence on the reaction conditions and sterical issues in the case of DPPH• test. The results of the antioxidant tests clearly show that thiosemicarbazide group cause a radical increase in antioxidant capacity, and its conversion to triazole thiol reduced the activity, which may be attributed to the disappearance of hydrogen atom on nitrogen of thiosemicarbazone structure. In many of the earlier antioxidant studies in the literature in which thiosemicarbazide structures appear as intermediate compounds towards the synthesis of triazoles or other products, thiosemicarbazides have not been tested for their biological activities including antioxidant activity [34,35]. However, as evident from our findings that the testing of their antioxidant activities must be reconsidered and the new structures to be included in the future literature must certainly be evaluated for their antioxidant activities.

3.2.2. Antimicrobial Activities

The compounds were tested against three Gram (-) bacteria (*E. coli*, *Y. pseudotuberculosis*, and *P. auroginosa*), four Gram (+) bacteria (*S. aureus*, *E. fecalis*, *L. monocytogenes*, and *B. cereus*), the mycobacterial model organism *M. smegmatis*, the yeast-like fungus *C. albicans*, and the yeast *S. cerevisiae*. Besides the activity of compounds **2** and **3** against *M. smegmatis*, activity was observed with only thiosemicarbazide derivatives at test concentrations and against only three microorganisms, two Gram (+) bacteria and the mycobacterium (Table 1). Thiosemicarbazide derivatives, being highly active in antioxidant tests, showed moderate activity, though all other derivatives were inactive. The reason for the Gram (-) bacteria to be resistant to all the compounds tested may be the relatively more impermeant structure of the cell wall including the more closely spaced peptidoglycan and the extra lipopolysaccharide layers.

4. Conclusion

In this study, the synthesis of some new 1,2,4-triazole-Schiff base, 1,2,4-triazole-thiosemicarbazide and 1,2,4-triazole-triazole/thiol compounds possessing thiophene ring was reported. The antioxidant studies revealed that all the compounds were active in FRAP assay, while thiosemicarbazide derivatives (**4a-d**) were very active and triazole-thiol derivatives (**5a-d**) and Schiff bases **6d** and **7d** showed low activity in DPPH• scavenging assay. Thiosemicarbazide derivatives also showed antibacterial activity against three microorganisms. As being potential pharmacophore, thiosemicarbazide groups in these types of compounds should be considered for the synthesis of lead compounds in search of active compounds against concomitant oxidative stress and bacterial infections.

5. Experimental

5.1. Chemistry

The ^{1}H-, and ^{13}C-Nuclear Magnetic Resonance spectra were recorded on a Varian-Mercury 200 MHz spectrometer, where TMS as an internal standard and DMSO-d6 as solvent are used. IR spectrum was recorded on a Perkin-Elmer Spectrum one FT-IR spectrometer (resolution 4) in KBr pellets. For elemental analysis, combustion/gas chromatography method was used. Elemental analyses was performed on a Hewlett-Packard 185 CHN analyzer; their values agreed with the

calculated ones. The MS spectrum was measured with an Micromass Quattro LC-MS/MS spectrometer with methanol as solvent. M.p. was measured on an electrothermal apparatus and are uncorrected. Compound **1** was prepared by the way reported earlier [6].

*5.1.1. Synthesis of the compound **2***

0.1 mol metallic sodium was added absolute alcohol and mixed until dissolved in room temperature. Then 0.1 mol 4-amino-5-(thiophene-2-ylmethyl)-2H-1,2,4-triazole-3(4H)-one was added this mixture and heated under reflux till observed the formation of salt. After that this mixture was cooled and 0.1 mol ethyl 2-bromoacetate was added in this mixture. Then mixture was heated under reflux for 6h. The reaction mixture was cooled refrigerator. White crude product was collapsed and recrystallized with water.

5.1.1.1. Ethyl-2-(4-amino-5-oxo-3-(thiophene-2-ylmethyl)-4,5-dihydro-1,2,4-triazole-1-yl) acetate ***(2)***. Yield: 78.02%, m.p.119-120 ^{o}C. IR (KBr, cm^{-1}): 3278-3216 (NH_2), 1752 (ester C=O), 1716 (C=O), 1667 (C=N), 1206 (C-O) ; ^{1}H NMR (200 MHz, DMSO-d_6) δ: 1.17 (t, 3H,CH_3), 4.05-4.16 (m, 4H, O-CH_2+N-CH_2), 4.52 (s, 2H, tyf-CH_2), 5.40 (s, 2H, NH_2), thiophen H [6.96 (s, 2H), 7.37 (bs, 1H)]; ^{13}C NMR (50MHz, DMSO-d_6) δ : 15.60 (CH_3), 25.98 (tyf-CH_2), 47.90 (N-CH_2), 61.95 (O-CH_2), Tyf-C [124.77 (CH), 126.13 (CH), 126.57 (CH), 137.95(C)], 146.10 (C=N), 153.10 (C=O), 169.51 (ester C=O); LC-MS (m/z): 284.09 (M^+,70%); Anal. Calcd for $C_{11}H_{14}N_4O_3S$: C, 46.80; H, 5.00; N, 19.85; Found: C,46.88; H,5.01; N,19.90.

*5.1.2.Synthesis of the compound **3***

0.1 mol ethyl 2-(4-amino-5-oxo-3-(thiophene-2-ylmethyl)-4,5-dihydro-1,2,4-triazole-1-yl)acetate and 0.1 mol hydrazine hydrate was mixed in butan-1-ol, then was heated under reflux for 6 h. At the end of the reaction, white solid product was collapsed and recrystallized with water.

5.1.2.1. 2-(4-amino-5-oxo-3-(thiophene-2-ylmethyl)-4,5-dihydro-1,2,4-triazole-1-yl)aceto hydrazide ***(3).*** Yield: 85.50%, m.p.196-197^{o}C. IR (KBr, cm^{-1}): 3417-3338 (NH_2), 3300-(NH), 1716 (trz C=O), 1650 (C=O), 1571 (C=N), 1524 (C=C); ^{1}H NMR (200 MHz, DMSO-d_6) δ:

4.20 (s, 2H, N-CH_2), 4.26 (s, 2H, tyf-CH_2), 5.32 (s, 2H, N-NH_2), 4.06 (s, 2H, NH-N$\underline{H_2}$), thiophen H [6.96 (d, 2H), 7.37 (d, 1H)], 9.21 (s, 1H, NH); ^{13}C NMR (50MHz, DMSO-d_6) δ : 25.66 (tyf-CH_2), 47.10 (N-CH_2), Tyf-C [125.77 (CH), 127.21 (CH), 127.57 (CH), 137.99(C)], 146.99 (C=N), 154.10 (trz C=O), 166.61 (C=O); LC-MS (m/z): 268.99 (M+2, 100%);Anal. Calcd for $C_9H_{12}N_6O_2S$: C, 40.29; H, 4.51; N, 31.32; Found: C,40.91; H, 4.76; N,31.01.

5.1.3. .General method for the synthesis of compounds ***4***

0.1 mol 2-(4-amino-5-oxo-3-(thiophene-2-ylmethyl)-4,5-dihydro-1,2,4-triazole-1-yl) acetohydrazide **(3)** was mixed with various isothiocyanates in ethanol. Then this mixture was heated under reflux for 6h. At the and of the reaction,crude product collapsed. The precipitate formed was filtered off and purified by recrystallization from ethanol and DMF.

5.1.3.1. 1-(2-(4-amino-5-oxo-3-(thiophene-2-ylmethyl)-4,5-dihydro-1,2,4-triazole-1-yl)acetyl)-4-phenylthiosemicarbazide ***(4a).*** Yield:80.25%, m.p.177-178 ^{o}C. IR (KBr, cm^{-1}) : 3413-3320 (NH_2), 3170 (NH), 3120 (NH), 1719 (C=0), 1705 (C=O), 1637 (C=N), 1597 (C=C), 1214 (C=S); ^{1}H NMR (200 MHz, DMSO-d_6) δ: 4.10 (s, 2H, N-CH_2), 4.50 (s, 2H, tyf-CH_2), 5.41 (s, 2H, NH_2), thiophen+ Arom. H [6.97 (bs, 2H), 7.20 (s, 1H), 7.40 (bs, 5H)], 9.68 (s, 1H, NH), 9.77 (s, 1H, NH), 10.32 (s, 1H, NH); ^{13}C NMR (50MHz, DMSO-d_6) δ (ppm): 25.69 (tyf-CH_2), 47.41 (N-CH_2), thiophen C [125.85 (CH), 127.30 (CH), 127.50 (CH), 137.82 (C)], Arom. C [127.96 (2CH), 128.83 (2CH), 137.41 (C),139.67 (C)], 147.24 (C=N), 154.14 (C=O), 167.23 (C=O), 181.42 (C=S); LC-MS (m/z): 403.98 (M^+,95%); Anal Calcd for $C_{16}H_{17}N_7O_2S_2$: C, 47.63; H, 4.25; N, 24.30, Found: C, 48.55; H, 5.23; N, 25.26.

5.1.3.2. 1-(2-(4-amino-5-oxo-3-(thiophene-2-ylmethyl)-4,5-dihydro-1,2,4-triazole-1-yl)acetyl)-4-(4-bromophenyl)thiosemicarbazide ***(4b).*** Yield:89.56%, m.p. 188-189 ^{o}C. IR (KBr, cm^{-1}): 3415-3329 (NH_2), 3251 (NH), 3221 (NH), 1713 (C=O), 1697 (C=O), 1675 (C=N), 1587 (C=C), 1225 (C=S) ; ^{1}H NMR (200 MHz, DMSO-d_6) δ: 4.11 (s, 2H, N-CH_2), 4.50 (s, 2H, tyf-CH_2), 5.41 (s, 2H,NH_2), thiophene + Arom. H [6.97 (s, 2H), 7.41- 7.52 (m, 5H)], 9.70 (s, 1H, NH), 9.82 (s, 1H, NH), 10.35 (s, 1H, NH); ^{13}C NMR (50MHz, DMSO-d_6)

δ (ppm): 25.68 (tyf-CH_2), 47.41 (N-CH_2), thiophene C [125.82 (CH), 127.29 (CH), 127.61 (CH), 137.79 (C)], Arom. C [131.67 (4CH), 139.08 (2C)], 147.24 (C=N), 154.11 (trz-C=O), 167.21 (C=O), 181.32 (C=S); LC-MS (m/z): 483.85 (M+1,65%); Anal Calcd for $C_{16}H_{16}BrN_7O_2S_2$: C, 39.84; H, 3.34; N, 20.33, Found: C, 41.00; H, 4.20; N, 21.39.

5.1.3.3. 1-(2-(4-amino-5-oxo-3-(thiophene-2-ylmethyl)-4,5-dihydro-1,2,4-triazole-1-yl)acetyl)-4-(4-fluorophenyl)thiosemicarbazide ***(4c).*** Yield:78.85%, m.p.180-181 ^oC. IR (KBr, cm^{-1}) : 3416-3345 (NH_2), 3231 (NH), 3211 (NH), 1708 (C=O), 1686 (C=O), 1609 (C=N), 1570 (C=N), 1215 (C=S); ^1H NMR (200 MHz, DMSO-d_6) δ: 3.62 (s, 2H, N-CH_2), 4.00 (s, 2H, tyf-CH_2), 4.91 (s, 2H,NH_2), thiophene + Arom. H [6.49 (s, 2H), 6.66- 6.74 (m, 2H),6.92 (bs,3H)], 9.83 (s, 1H, NH), 9.30 (s, 1H, NH), 9.18 (s, 1H, NH); ^{13}C NMR (50MHz, DMSO-d_6) δ (ppm): 25.66 (tyf-CH_2), 47.35 (N-CH_2), thiophene C [125.82 (CH), 127.27 (CH), 127.60 (CH), 137.82 (C)], Arom. C [115.27 (2CH), 115.72 (2CH), 135.96 (C), 136.01 (C)], 147.19 (C=N), 154.11 (C=O), 167.18 (C=O), 181.64 (C=S); LC-MS (m/z): 421.98 (M^+,100%); Anal Calcd for $C_{16}H_{16}FN_7O_2S_2$: C, 45.60; H, 3.83; N, 23.26, Found: C, 46.45; H, 5.01; N, 24.30.

5.1.3.4. 1-(2-(4-amino-5-oxo-3-(thiophene-2-ylmethyl)-4,5-dihydro-1,2,4-triazole-1-yl)acetyl)-4-p-tolylthiosemicarbazide ***(4d).*** Yield:89.34%, m.p.180-181 ^oC. IR (KBr, cm^{-1}) νmax: 3410-3340 (NH_2), 3226 (NH), 3210 (NH), 1705 (C=O), 1695 (C=O), 1673 (C=N), 1581 (C=C), 1225 (C=S) ; ^1H NMR (200 MHz, DMSO-d_6) δ: 2.27 (s, 3H, CH_3), 4.08 (s, 2H, N-CH_2), 4.47 (s, 2H, tyf-CH_2), 5.37 (s, 2H,NH_2), thiophene + Arom.H [6.95 (s, 2H), 7.11-7.37 (m, 5H)], 9.58 (s, 1H, NH), 9.65 (s, 1H, NH), 10.26 (s, 1H, NH); ^{13}C NMR (50MHz, DMSO-d_6) δ (ppm): 21.27 (CH_3), 25.65 (tyf-CH_2), 47.31 (N-CH_2), thiophene C [125.83 (CH), 127.28 (CH), 127.60 (CH), 137.82 (C)], Arom. C [126.62 (2CH), 129.28 (2CH), 135.18 (C), 137.06 (C)], 147.19 (C=N), 154.11 (C=O), 167.22 (C=O), 181.42 (C=S); LC-MS (m/z): 417.99 (M^+,100%); Anal Calcd for $C_{17}H_{19}N_7O_2S_2$: C, 48.90; H, 4.59; N, 23.48, Found: C, 50.21; H, 5.78; N, 24.60.

5.1.4. General method for the synthesis of compounds ***5***

Sodium hydroxide (2N, 50 mL) solution was added 0.1 mol isothiocyanato and heated under reflux for 3 h. Then this mixture was poured in ice-water mixture. After that, concentrated hydrogen chloride (%37) was added drop by drop this mixture and white crude products was filtered and dried.

5.1.4.1. 4-amino-2-((5-mercapto-4-phenyl-4H-1,2,4-triazole-3-yl)methyl)-5-(thiophene-2-yl methyl)-2H-1,2,4-triazole-3(4H)-one ***(5a).*** Yield:90.78%, m.p.288-289 ^{o}C. IR (KBr, cm^{-1}) : 3281-3176 (NH_2), 1696 (C=O), 1581 (C=N), 1535 (C=C),1214 (C=S); ^{1}H NMR (200 MHz, DMSO-d_6) δ: 3.99 (s, 2H, N-CH_2), 4.81 (s, 2H, tyf-CH_2), 5.14 (s, 2H, NH_2), thiophene+Arom.H [6.95 (s, 1H), 7.23-7.42 (m, 7H)], 13.95 (s, 1H, SH); ^{13}C NMR (50MHz, DMSO-d_6) δ (ppm): 25.50 (tyf-CH_2), 47.53 (N-CH_2), thiophene C [125.91 (CH), 127.34 (CH), 127.62 (CH), 137.66 (C)], Arom. C [128.28 (2CH), 129.85 (2CH),130.21 (CH), 133.43 (C)], 147.58 (C=N), 148.31 (C=N), 152.88 (C=N), 169.07 (C=O); LC-MS (m/z): 385.96 (M^+,100%); Anal Calcd for $C_{16}H_{15}N_7OS_2$: C, 49.85; H, 3.92; N, 25.44, Found: C, 51.34; H, 4.89; N, 26.33.

5.1.4.2. 4-amino-2-((4-(4-bromophenyl)-5-mercapto-4H-1,2,4-triazole-3-yl)methyl)-5-(thiophene-2-ylmethyl)-2H-1,2,4-triazole-3(4H)-one ***(5b).*** Yield:80.68%, m.p.199-200 ^{o}C. IR (KBr, cm^{-1}) : 3413-3312 (NH_2), 1697 (C=O), 1577 (C=N), 1510 (C=C), 1225 (C=S) ; ^{1}H NMR (200 MHz, DMSO-d_6) δ: 3.99 (s, 2H, N-CH_2), 4.82 (s, 2H, tyf-CH_2), 5.18 (s, 2H,NH_2), thiophene+ Arom. H [6.94 (s, 2H), 7.20 (d, 2H), 7.39 (s, 1H), 7.58 (s, 2H)], 13.98 (s, 1H, SH); ^{13}C NMR (50MHz, DMSO-d_6) δ (ppm): 25.53 (tyf-CH_2), 49.30 (N-CH_2), thiophene C [125.93 (CH), 127.35 (CH), 127.65 (CH), 137.63 (C)], Arom. C [130.70 (2CH), 132.72 (2CH), 123.04 (C),133.61 (C)], 147.72 (C=N), 149.55 (C=N), 152.95 (C=N), 168.90 (C=O; LC-MS (m/z): 463.26 (M^+,100%);Anal Calcd for $C_{16}H_{14}BrN_7OS_2$: C, 41.38; H, 3.04; N, 21.11, Found: C, 42.50; H, 4.11; N, 22.40.

5.1.4.3. 4-amino-2-((4-(4-fluorophenyl)-5-mercapto-4H-1,2,4-triazole-3-yl)methyl)-5-(thiophene-2-ylmethyl)-2H-1,2,4-triazole-3(4H)-one ***(5c).*** Yield:90.56%, m.p.133-134 ^{o}C. IR (KBr, cm^{-1}) : 3314-3194 (NH_2), 1697 (C=O), 1579 (C=N), 1509 (C=C), 1233 (C=S); ^{1}H NMR (200 MHz, DMSO-d_6) δ: 3.99 (s, 2H, N-CH_2), 4.80 (s, 2H, tyf-CH_2), 5.19 (s, 2H,NH_2), thiophene + Arom.H [6.94 (s, 2H), 7.22-7.25 (m, 4H), 7.40 (s, 1H)], 13.97 (s, 1H, SH); ^{13}C NMR (50MHz, DMSO-d_6) δ (ppm): 25.51 (tyf-CH_2), 47.39 (N-CH_2), thiophene C [125.93 (CH), 127.32 (CH), 127.63 (CH), 137.71 (C)], Arom. C [116.65 (2CH), 117.11 (2CH), 129.78 (C), 129.83 (C)], 147.66 (C=N), 148.29 (C=N), 152.91 (C=N), 169.31 (C=O); LC-MS (m/z): 403.95 (M^{+},60%); Anal Calcd for $C_{16}H_{14}FN_7OS_2$: C, 47.63; H, 3.50; F,4.71; N, 24.30; O,3.97; S,15.90. Found: C, 48.50; H, 2.39; N, 25.67.

5.1.4.4. 4-amino-2-((5-mercapto-4-p-tolyl-4H-1,2,4-triazole-3-yl)methyl)-5-(thiophene-2-yl methyl)-2H-1,2,4-triazole-3(4H)-one ***(5d).*** Yield:92.45%, m.p.208-209 ^{o}C. IR (KBr, cm^{-1}) : 3269-3129 (NH_2), 1734 (C=O), 1577 (C=N), 1517 (C=C), 1225 (C=S); ^{1}H NMR (200 MHz, DMSO-d_6) δ: 2.30 (s, 3H, CH_3), 3.99 (s, 2H, N-CH_2), 4.63 (s, 2H, tyf-CH_2), 5.12 (s, 2H,NH_2), thiophene + Arom. H [6.96 (d, 4H), 7.09 (d, 2H), 7.38 (s, 1H)], 13.60 (s, 1H, SH); ^{13}C NMR (50MHz, DMSO-d_6) δ (ppm): 21.32 (CH_3), 25.36 (tyf-CH_2), 47.44 (N-CH_2), thiophene C [125.91 (CH), 127.47 (CH), 127.77 (CH), 137.26 (C)], Arom. C [127.90 (2CH), 130.67 (2CH), 139.23 (C), 132.02 (C)], 147.57 (C=N), 147.76 (C=N), 153.05 (C=N), 168.00 (C=O); LC-MS (m/z): 399.98 (M^{+},100%); Anal Calcd for $C_{17}H_{17}N_7OS_2$: C, 51.11; H, 4.29; N, 24.54, Found: C, 52.20; H, 3.70; N, 24.90.

5.1.5. General procedure for the synthesis of the compounds ***6***

0.1 mol 2-(4-amino-5-oxo-3-(thiophene-2-ylmethyl)-4,5-dihydro-1,2,4-triazol-1-yl) acetohydrazide and 0.1 mol various aldehydes in ethonol was heated under reflux for 3 h. After three h., crude product collapsed in the reaction medium and recrystllized with DMF and alcohol.

5.1.5.1. 2-(4-amino-5-oxo-3-(thiophene-2-ylmethyl)-4,5-dihydro-1,2,4-tiazole-1-yl-N'(thiophene-2-ylmethylene)acetohydrazide ***(6a).*** Yield: 67.73%, m.p.244-245 ^{o}C. IR (KBr, cm^{-1}): 3461- 3414 (NH_2), 3320 (NH), 1719 (trz C=O), 1696 (C=O), 1617 (C=N), 1565 (C=C); ^{1}H NMR (200 MHz, DMSO-d_6) δ: 4.09 (s, 2H, tyf-CH_2), 4.40-4.73 (m, 2H, N-CH_2), 5.39 (s, 2H, N-NH_2), thiophene +Arom. H [6.96 (bs, 2H), 7.10- 7.12 (m,2H), 7.38-7.43 (m, 2H), 7.64 (s, 1H)], 8.16-8.39 (d, 1H, N=CH), 11.65 (s, 1H, NH); ^{13}C NMR (50MHz, DMSO-d_6) δ : 25.69 (tyf-CH_2), 46.92 (N-CH_2), thiophene+Arom. C [125.81 (CH), 127.22 (CH), 127.60 (CH), 128.63(CH), 129.38 (CH), 131.40 (CH), 137.93 (C), 139.26(C)], 143.18 (N=CH), 147.21 (C=N), 154.08 (trz C=O), 168.28 (C=O); LC-MS (m/z): 357.01 (M^+,100%); Anal. Calcd for $C_{14}H_{14}N_6O_2S_2$: C, 46.40; H, 3.89; N, 23.19; Found: C, 47.23; H, 4.56; N, 23.21.

5.1.5.2. 2-(4-amino-5-oxo-3-(thiophene-2-ylmethyl)-4,5-dihydro-1,2,4-triazole-1-yl-N'(furan-2-yl methylene)acetohydrazide ***(6b).*** Yield: 78.02%**,** m.p.140-141 ^{o}C. IR (KBr, cm^{-1}): 3448-3325 (NH_2), 3093 (NH), 1720 (trz C=O), 1698 (C=O), 1627 (C=N), 1567 (C=C); ^{1}H NMR (200 MHz, DMSO-d_6) δ: 3.79 (s, 3H, O-CH_3), 4. 08 (s, 2H, tyf-CH_2), 4.74 (bs, 2H, N-CH_2), 5.35 (s, 2H, trz-NH_2), thiophen+Arom. H [6.60 (bs, 2H), 6.90-6.95 (m, 3H), 7.35-7.38 (m, 1H), 7.80 (s, 1H)], 8.05 (bs, 1H, N=CH), 11.58 (bs, 1H, NH); ^{13}C NMR (50MHz, DMSO-d_6) δ : 27.86 (tyf-CH_2), 49.89 (N-CH_2), thiophene+Arom. C [127.96 (CH), 129.37 (CH), 129.76 (CH), 140.10 (C)], Arom-C [115.03 (CH), 116.56 (CH), 116.90 (CH), 149.34 (C)], 139.91 (N=CH), 151.93 (C=N), 156.44 (trz C=O), 170.65 (C=O); LC-MS (m/z): 387.01 (M^+,100%); Anal. Calcd for $C_{14}H_{14}N_6O_3S$: C, 48.55; H, 4.07; N, 24.26, Found: C, 49.92; H, 5.01; N, 22.90.

5.1.5.3. 2-(4-amino-5-oxo-3-(thiophene-2-ylmethyl)-4,5-dihydro-1,2,4-triazole-1-yl)-N'-benzylidene acetohydrazide ***(6c).*** Yield:89.12%, m.p.246-247 ^{o}C, IR (KBr, cm^{-1}): 3429-3210 (NH_2), 3131 (NH), 1719 (trz C=O), 1694 (C=O), 1670 (C=N), 1622 (C=C); ^{1}H NMR (200 MHz, DMSO-d_6) δ: 4.10 (s, 2H, tyf-CH_2), 4.84 (bs, 2H, N-CH_2), 5.39 (s, 2H, trz-NH_2), thiophene+Arom. H [6.96 (s, 2H), 7.40 (bs, 4H), 7.69 (bs, 2H)], 7.99-8.19 (m, 1H, N=CH), 11.68 (bs, 1H, NH); ^{13}C NMR (50MHz, DMSO-d_6) δ (ppm): 25.69 (tyf-CH_2), 47.69 (N-CH_2), thiophene C [125.80 (CH), 127.20 (CH), 127.60 (CH), 138.02 (C)], Arom. C [127.81 (CH), 129.51 (2CH), 130.69 (2CH), 134.61 (C)], 147.95 (N=CH), 147.15 (C=N), 154.31 (trz

C=O), 168.68 (C=O); LC-MS (m/z): 362.97 (M^+,90%); Anal. Calcd for $C_{16}H_{16}N_6O_2S$: C, 53.92; H, 4.52; N, 23.58, Found: C, 53.10; H, 3.62; N, 22.40.

5.1.5.4. N'-(4-methoxybenzylidene)-2-(4-amino-5-oxo-3-(thiophene-2-ylmethyl)-4,5-dihydro-1,2,4-triazole-1-yl)acetohydrazide ***(6d).*** Yield: 78.00%, m.p.232-233 ^oC. IR (KBr, cm^{-1}) : 3414-3320 (NH_2), 3217 (NH), 1709 (trz C=O), 1687 (C=O), 1607 (C=N), 1572 (C=C); ^1H NMR (200 MHz, DMSO-d_6) δ: 4.10 (s, 2H, tyf-CH_2), 4.82 (bs, 2H, N-CH_2), 5.38 (s, 2H, NH_2),), thiophene + Arom.H [6.97 (bs, 4H), 7.39 (bs, 1H), 7.63 (bs, 2H)], 8.14 (bs, 1H, N=CH), 11.52 (bs, 1H, NH); ^{13}C NMR (50MHz, DMSO-d_6) δ : 25.68 (tyf-CH_2), 47.68 (N-CH_2), 55.97 (O-CH_3), thiophene C [127.18(CH), 127.24 (CH), 129.24 (CH), 138.03 (C)], Arom. C [114.96 (2CH), 125.79 (2CH), 147.15 (C), 161.39 (C)], 144.60-147.86 (N=CH), 146.89 (C=N), 154.29 (trz C=O), 168.44 (C=O); LC-MS (m/z): 346.99 (M^+,100%);Anal. Calcd for $C_{17}H_{18}N_6O_3S$: C, 52.84; H, 4.70; N, 21.75, Found: C, 53.02; H, 4.85; N, 22.90.

5.1.5.5. 2-(4-amino-5-oxo-3-(thiophene-2-ylmethyl)-4,5-dihydro-1,2,4-triazole-1-yl)-N'-(pyridin-3-ylmethylene)acetohydrazid ***(6e).***Yield:67.88%, m.p.201-202 ^oC, IR (KBr, cm^{-1}) : 3337-3187 (NH_2), 3091 (NH), 1721 (trz C=O), 1674 (C=O), 1607 (C=N), 1573 (C=C); ^1H NMR (200 MHz, DMSO-d_6) δ: 4.09 (s, 2H, tyf-CH_2), 4.86 (bs, 2H, N-CH_2), 5.38 (s, 2H, NH_2), thiophene + Arom.H [6.96 (s, 2H), 7.39- 7.44 (m, 2H), 8.13 (d, 1H), 8.59 (s, 1H), 8.85 (s, 1H)], 8.24 (bs, 1H, N=CH), 11.81 (s, 1H, NH); ^{13}CNMR (50MHz, DMSO-d_6) δ (ppm): 25.68 (tyf-CH_2), 47.69 (N-CH_2), thiophene C [125.80 (CH), 127.19 (CH), 127.58 (CH), 137.93 (C)], Arom. C [124.65 (CH), 134.24 (CH), 149.28 (CH),151.25 (CH), 138.04 (C)], 145.31 (N=CH), 147.20 (C=N), 154.28 (trz C=O), 168.93 (C=O); LC-MS (m/z): 358.00 (M^+,100%); Anal. Calcd for $C_{15}H_{15}N_7O_2S$: C, 50.41; H, 4.23; N, 27.43, Found: C, 51.65; H, 4.90; N, 28.56.

5.1.6. *General procedure for the synthesis of the compounds* ***7***

0.1 mol 2-(4-amino-5-oxo-3-(thiophene-2-ylmethyl)-4,5-dihydro-1,2,4-triazole-1-yl) acetohydrazides **(4a-d)** and 0.2 mol different aldehydes in oil bath dry to dry was heated for 2 h. at 150 ^0C. At the and of the reaction, substance trituared with ethylacetete and was filtered and recrystallized with DMF and alcohol.

5.1.6.1. 2-(5-oxo-3-(thiophene-2-ylmethyl)-4-(thiophene-2-ylmethyleneamino)-4,5-dihydro-1,2,4-triazole-1-yl)-N'-(thiophen-2-ylmethylene)acetohydrazide ***(7a).*** Yield: 56.20%, m.p.228-229 ^{o}C. IR (KBr, cm^{-1}) : 3467 (NH), 1706 (trz C=O), 1686 (C=O), 1597 (C=N), 1525 (C=C); ^{1}H NMR (200 MHz, DMSO-d_6) δ: 4.24 (s, 2H, tyf-CH_2), 4.82 (bs, 2H, N-CH_2),), thiophene+Arom. H [6.83-7.45 (m, 4H), 7.68- 7.97 (m, 5H)], 8.43 (bs, 1H, N=CH), 9.82 (bs, 1H, N=CH), 11.74 (bs, 1H, NH); ^{13}C NMR (50MHz, DMSO-d_6) δ : 26.20 (tyf-CH_2), 47.61 (N-CH_2), thiophene+Arom. C [126.26 (CH), 127.45 (CH), 128.61 (CH), 129.06 (CH), 129.46 (CH), 129.89 (CH), 131.51 (CH), 132.11 (CH), 135.00 (CH), 137.53 (C), 138.51 (C), 139.18(C)], 143.45 (N=CH), 144.88 (C=N), 149.64 (N=CH), 150.78 (trz C=O), 167.88 (C=O); LC-MS (m/z): 445.01 (M^+,100%); Anal. Calcd for $C_{19}H_{16}N_6O_2S_3$: C, 49.98; H, 3.53; N, 18.41, Found: C, 50.24; H, 2.90; N, 18.96.

5.1.6.2. N'-(furan-2-ylmethylene)-2-(4-(furan-2-ylmethyleneamino)-5-oxo-3-(thiophene-2-ylmethyl)-4,5-dihydro-1,2,4-triazole-1-yl)acetohydrazide ***(7b).*** Yield:60.20%, m.p.160-161 ^{o}C. IR (KBr, cm^{-1}) : 3417 (NH), 1708 (trz C=O), 1690 (C=O), 1637 (C=N), 1613 (C=C); ^{1}H NMR (200 MHz, DMSO-d_6) δ: 2.71 (s, 3H, OCH_3), 2.86 (s, 3H,OCH_3), 4.21 (s, 2H, tyf-CH_2), 4.80 (bs, 2H, N-CH_2), Thiophene + Arom-H [6.58 (bs, 1H), 6.68 (s, 1H), 6.91-6.95 (m, 3H), 7.16 (s, 1H), 7.31-7.33 (m, 1H), 7.73 (bs, 1H), 7.86 (s, 1H)], 8.03 (bs,1H, N=CH), 9.46 (s, 1H, N=CH), 11.69 (bs, 1H, NH); ^{13}C NMR (50MHz, DMSO-d_6) δ (ppm): 26.06 (tyf-CH_2), 47.57 (N-CH_2), thiophene C [126.19 (CH), 127.60 (CH), 128.20 (CH), 137.30 (C)], Arom. C [112.59 (CH), 113.39 (CH), 114.54 (CH), 114.86 (CH), 118.82 (CH), 119.52 (CH), 148.87 (C), 149.57 (C)], 138.02 (N=CH), 149.71 (C=N), 143.70 (N=CH), 150.78 (trzC=O), 168.09 (C=O); LC-MS (m/z): 505.06 (M^+,100%); Anal. Calcd for $C_{19}H_{16}N_6O_4S$: C, 53.77; H, 3.80; N, 19.80, Found: C, 54.67; H, 4.27; N, 21.10.

5.1.6.3. N'-benzylidene-2-(4-(benzylideneamino)-5-oxo-3-(thiophene-2-ylmethyl)-4,5-dihydro-1,2,4-triazole-1-yl)acetohydrazide ***(7c).*** Yield:59.87%, m.p.250-251^{o}C. IR (KBr, cm^{-1}): 3414 (NH), 1708 (trz C=O), 1691 (C=O), 1637 (C=N), 1613 (C=C); ^{1}H NMR (200 MHz, DMSO-d_6) δ: 4.33 (s, 2H, tyf-CH_2), 4.94 (bs, 2H, N-CH_2), thiophene + Arom.H [6.90-7.02 (m, 2H), 7.41- 7.54 (m, 7H), 7.70 (bs, 2H), 7.87 (bs, 2H)], 8.21 (bs,1H, N=CH), 9.70 (s, 1H, N=CH), 11.74 (bs, 1H, NH); ^{13}C NMR (50MHz, DMSO-d_6) δ (ppm): 26.20 (tyf-CH_2), 48.89 (N-CH_2), thiophene C [125.80 (CH), 127.19 (CH), 127.65 (CH), 138.05 (C)], Arom. C

[127.84 (2CH), 127.89 (2CH), 129.53 (4CH),130.72 (CH), 130.81 (CH), 134.61 (C), 134.65 (C)], 148.01 (N=CH), 143.90 (C=N), 149.85 (N=CH), 150.79 (trz C=O), 168.35 (C=O); LC-MS (m/z): 456.96 (M^+,100%); Anal. Calcd for $C_{23}H_{20}N_6O_2S$: C, 62.15; H, 4.54; N, 18.91, Found: C, 63.21; H, 3.96; N, 20.01.

5.1.6.4. N'-(4-methoxybenzylidene)-2-(4-(4-methoxybenzylideneamino)-5-oxo-3-(thiophene-2-ylmethyl)-4,5-dihydro-1,2,4-triazole-1-yl)acetohydrazide ***(7d).*** Yield: 50.23%, m.p.260-261 ^oC. IR (KBr, cm^{-1}) : 3198 (NH), 1705 (trz C=O), 1687 (C=O), 1606 (C=N), 1587 (C=C) ; ^1H NMR (200 MHz, DMSO-d_6) δ: 4.29 (s, 2H, tyf-CH_2), 4.89 (d, 2H, N-CH_2), thiophene + Arom.H [6.94-7.08 (m, 5H), 7.35- 7.38 (m, 1H), 7.64 (d, 2H), 7.82 (d, 2H), 7.93 (s, 1H)], 8.14 (bs, 1H, N=CH), 9.59 (s, 1H, N=CH), 11.58 (bs, 1H, NH); ^{13}C NMR (50MHz, DMSO-d_6) δ : 26.25 (tyf-CH_2), 47.60 (N-CH_2), 56.13 (O-CH_2), thiophene C [126.11 (CH), 127.42 (CH), 127.59 (CH), 137.82 (C)], Arom. C[114.96 (2CH), 115.22 (2CH),129.29 (2CH),130.43 (2CH), 126.43 (C),127.17 (C), 161.41 (C), 161.61 (C)], 148.01 (N=CH), 145.18 (C=N), 150.92 (trz C=O), 154.57 (N=CH), 168.08 (C=O). LC-MS (m/z): 424.98 (M^+,100%); Anal. Calcd for $C_{25}H_{24}N_6O_4S$: C, 59.51; H, 4.79; N, 16.66, Found: C, 58.61; H, 5.90; N, 17.23.

5.1.6.5. 2-(5-oxo-4-(pyridin-3-ylmethyleneamino)-3-(thiophene-2-ylmethyl)-4,5-dihydro-1,2,4-triazole-1-yl)-N'-(pyridin-3-ylmethylene)acetohydrazide **(7e).** Yield:45.56%, m.p.215-216 ^oC. IR (KBr, cm^{-1}) : 3096 (NH), 1716 (trz C=O), 1703 (C=O), 1614 (C=N), 1587 (C=C); ^1H NMR (200 MHz, DMSO-d_6) δ: 4.39 (s, 2H, tyf-CH_2), 5.01 (bs, 2H, N-CH_2), thiophene + Arom.H [6.94-7.15 (m, 2H), 7.45- 7.62 (m, 3H), 8.25-8.38 (m, 2H), 8.45-9.11 (m, 4H)], 8.23 (1H, N=CH), 9.79 (s, 1H, N=CH), 11.82 (s, 1H, NH); ^{13}C NMR (50MHz, DMSO-d_6) δ (ppm): 26.14 (tyf-CH_2), 47.59 (N-CH_2), thiophene C [126.21 (CH), 127.54 (CH), 127.65 (CH), 137.53 (C)], Arom. C [124.59 (CH), 124.84 (CH), 129.98 (C), 130.50 (C), 134.27 (CH),135.09 (CH), 150.17 (CH), 151.28 (CH), 151.76 (CH), 152.80 (CH)], 145.53 (N=CH), 145.33 (C=N), 149.30 (N=CH), 150.70 (trz C=O), 168.52 (C=O); LC-MS (m/z): 447.04 (M^+, 100%); Anal. Calcd for $C_{21}H_{18}N_8O_2S$: C, 56.49; H, 4.06; N, 25.10, Found: C, 57.54; H, 5.11; N, 26.16.

5.2. Antioxidant Activity Tests

Different antioxidant test methods with varying strategies or chemistries are simultaneously used in antioxidant activity determinations since the results of different assays appear in many cases to be different due to differences in reaction mechanisms and kinetics, the effects of solvents, sterical sensitivities, and varying effects of temperature, pH, and matrix composition. Two of the most widely used antioxidant test methods were used in the current study. DPPH• radical scavenging assay has been developed and used widely for various samples including synthetic compounds [33]. The second method used was ferric reducing/antioxidant power (FRAP) assay [34,35], which has found application in many investigations with synthetic organics. The results of the two tests were comparatively evaluated.

5.2.1. DPPH• Scavenging Test

One of the most widely used spectrophotometric test methods, DPPH• radical scavenging test relies on the absorbance change of the radical when deactivated by antioxidants, which easily observable with naked eye as color changes from purple to yellow. DPPH• radical (2,2-diphenyl-1-picrylhydrazyl), a stable solid, was used in 50 μM final test concentration in methanol. The method developed by Cuendet et al was used with some modification [36]. The synthesized compounds were dissolved in ethanol-DMSO (3:2) at 200 μg/mL concentration. The working test concentrations were determined with a pretest observation, and all the compounds were tested at 200 μg/mL fixed concentration except thiosemicarbazides (compounds **4a-d**) which showed much higher activity in pretests and therefore diluted 20 fold to 10 μg/mL as test concentration. Equal volumes (750 μL) of DPPH• and sample solutions were mixed, vortexed, and incubated for a 60 min period at room temperature. The tests were made in duplicates. The absorbances were measured at 517 nm at 0.5, 20, 40, and 60 min incubation times and used for the calculation of percent DPPH• scavenging using the following equation:

$$\%\text{DPPH• Scavenging} = [\ (\ Abs_{initial} - Abs_{final}\ ) / Abs_{initial}\ ] \times 100$$

The absorbance of the reagent blanks at the end of incubation was used as $Abs_{initial}$. The absorbance of each sample blank, having solvent instead of DPPH•, was subtracted from the absorbance measured for the sample to obtain Abs_{final}. The percent DPPH scavenging values as a function of time were plotted in Figure 1, and the percent DPPH scavenging values at 60

min incubation time are given in Figure 2 for the activity comparison of the compounds synthesized.

5.2.2. Ferric reducing/antioxidant power (FRAP) Test

First developed as antioxidant test method by using $FeSO_4$ solutions [37], FRAP test method was later improved by using TPTZ (2,4,6-tris(2-pyridyl)-*s*-triazine) as ferrous ions complexing agent [38]. FRAP activities indicate total reducing potential of samples. The method utilizes the measurement of absorbance at 595 nm caused by Fe^{2+} - TPTZ complex.

To overcome expected solubility problems of the compounds when the sample solutions are mixed with FRAP reagent, the original method [38] was modified to contain methanol in 3:2 ratio in water as reagent solvent.

Fresh FRAP reagent was prepared by combining 300 mM acetate buffer (pH 3.6), 10 mM TPTZ in 40 mM HCl, and 20 mM $FeCl_3.6H_2O$ in 10:1:1 ratio, respectively. Calibration curve was prepared by using methanolic Trolox solutions at five different concentrations (1000 – 500 – 250 – 125 – 62.5 μM). The compound solutions of 200 μg/mL concentration and Trolox solutions (100 μL) were mixed with FRAP reagent (3 mL), vortexed, and incubated for 20 min. at room temperature. The absorbance was read at 595 nm against water. Reagent and sample blanks were also tested, and the sum of the absorbance of these two measurements was subtracted from the absorbance mean obtained by triplicate measurements with each sample. FRAP activities were expressed as Trolox Equivalent Antioxidant Capacity (TEAC, μM), calculated as the Trolox concentration from the graph corresponding to the absorbance observed with sample. Higher TEAC values means higher FRAP and thus higher antioxidant capacity.

5.3. Antimicrobial activity

5.3.1. Antimicrobial activity assessment

All test microorganisms were obtained from the Hifzissihha Institute of Refik Saydam (Ankara, Turkey) and were as follows: Ec: *Escherichia coli* ATCC 25922, Yp: *Yersinia pseudotuberculosis* ATCC 911, Pa: *Pseudomonas auroginosa* ATCC 10145, Sa: *Staphylococcus aureus* ATCC 25923, Ef: *Enterococcus fecalis* ATCC 29212, Li: *Listeria monocytogenes* ATCC 43251, Bc: *Bacillus cereus* 709 Roma, Ms: *Mycobacterium smegmatis* ATCC607, Ca: *Candida albicans* ATCC 60193, Sc: *Saccharomyces cerevisiae* RSKK 251. All the newly synthesized compounds were dissolved in DMSO to prepare stock solutions.

5.3.2. Minimum inhibition concentration

The agar dilution method, described by Vanden Berghe and Vietinck [39] was used for the antibacterial screening with slight modifications. Instead of 96 well microtitre plates 24 well tissue culture (Corning) plates were used. The crude extracts were dissolved in 70% ethanol and physiological Tris buffer (Amresco 0826-500G) 1:4, and mixed with an equal amount of 3% agar solution at 45 °C to a final concentration of 50, 25, 12.5, and 6.25 µg of sample compound/mL. From the solutions, 400 µL was transferred into each well of the tissue culture (Corning) plate. After solidification each well was inoculated with 10 µL of freshly prepared bacterial suspension of 10^8 bacteria/mL and incubated at 37 °C for 24 h. Ampicillin (10 µg) and Fluconasole (5 µg) were standard antibacterial and antifungal drugs, respectively. Dimethylsulphoxide (DMSO) was used as solvent control. The smallest concentration with which the growth of test microorganism was totally inhibited is reported as minimum inhibitory concentration (MIC, µg/mL) value (Table 1).

6. Crystal data for the compounds 2 and 4d

The crystallographic data were collected on a CCD system (Oxford Xcalibur Eos Mova) with a graphite monochromatized Mo K_α radiation (λ = 0.71073 Å). The crystals were mounted with grease on the top of a glass fiber. Data collection: CrysAlis PRO CCD [40]; cell refinement: CrysAlis PRO CCD; data reduction: CrysAlis PRO RED [41]. Cell constants and orientation matrix for data collection were obtained by least-squares refinement of the diffraction data in the range of 6.48 - 55.44° for 1 and 7 - 57.9° for 2. Using Olex2 [42], the structures were solved with the ShelXS [43] structure solution program using Direct Methods

and refined with the ShelXL [44] refinement package using F^2 by Least Squares minimization. Molecular figures were prepared by using ORTEP III [45] and OLEX 2. All non-H atoms were refined anisotropically. The H atoms were placed in geometrically idealized positions. The crystal data and structure refinement details are given in Table 2. Selected bond lengths and bond angles are listed in Table 3.

*6.1. Crystallographic study for **2** and **4d***

Both of the compounds crystallizes in the $P2_1/c$ space group and in a monoclinic crystal system, with one isolated molecule in the asymmetric unit cell. ORTEP III diagram of **2** and **4d** with the atom numbering scheme is shown in Figure 5 and Figure 6 respectively. While the molecular structure of **2** contains two five membered 1,2,4-triazole and thiophene rings linked through a methylene group, compound **4d** additionally has a aromatic ring. Dihedral angles between the rings are 69.08(3)º for **2** and 65.17(1)º for **4d**. The 1,2,4-triazole ring in the compound **4d** reveals a tendency of somewhat differences of some ring bonds in comparison with 1,2,4-triazole ring in compound **4d** (Table 3). All the bond lengths along the 1,2,4-triazole ring changes between 1.358 and 1.390 Å for **2**. Also, the N3-N4 bond lengths of **2** (1.396(3) Å) is shorter than that in **4d** (1.402(3)Å). The obtained results indicate a conjugation between the π-system of the 1,2,4-triazole ring and the 4-amino group for compound **2**. The C1–O1 bond length is practically the same as the both of the molecules [1.226(4) Å for **2** and 1.221(3) Å for **4d**] and within the values observed for a C=O double bond.

For compound **2**, the 4-amino group of 1,2,4-triazole ring is hydrogen bonded to the carbonyl O atom of symmetry related molecule by intermolecular hydrogen bond (Figure 7, 8 and Table 4). N4-H4a…O1^{i} hydrogen bonds with an $R_2^2(10)$ graph set notation (Fig. 7) [43]. The 4-amino group also hydrogen bonded to O3 atom as shown in Figure 7. The molecules are further interconnected into a 2D network by C8-H8b…N4^{ii} hydrogen bonds on the (100) plane (Figure 8, symmetry codes as in Table 4).

Inspection of molecular packing reveals that there are non-classical hydrogen bonds (N-H…X; X=N, O, S) in the crystal structure of **4d** (Table 4) which link the molecules to form three-dimensional network. N7—H7···O1^{iii} and N6—H6···S2^{iii} hydrogen bonds link the molecules with $R_2^2(8)$ and $R_2^2(20)$ graph set notation respectively [45], along the b axis (Figure 9). On the other hand, The non-classical hydrogen bond formed by N5 and

carboxylate O2 atoms are resulted in a chain along the c axis with *C*(4) notation (Figure 10). All these separate chains come together to form a 2D framework on the (100) plane as shown in Figure 11.

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7. Supplementary data

Full crystallographic data in CIF format for 2 and 4d have been deposited with the Cambridge Crystallographic Data Centre (CCDC No. 914599 for 2 and CCDC No. 915002 for 4d). Copies of the data can be obtained, free of charge, on application to CCDC, 12 Union Road, Cambridge, CB2 1EZ, U.K; Fax: +44(0)-1223-336033; or email: deposit@ccdc.cam.ac.uk.

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CAPTIONS

Scheme 1. Synthetic pathway for the preparation of target compounds **2,3,4,5,6 and 7**

Fig. 1. %DPPH scavenging of the compounds as a function of time (highly active compounds **4a-d** were tested at 10 μg/mL, while the others were at 200 μg/mL concentration, only the active compounds are shown).

Fig. 2. %DPPH scavenging of the compounds at 60 min incubation time (highly active compounds **4a-d** were tested at 10 μg/mL, while the others were at 200 μg/mL concentration, only the active compounds are shown).

Fig. 3. FRAP test results of all the synthesized compounds at 200 μg/mL concentration as μM TEAC (Trolox equivalent antioxidant capacity) values obtained from [Trolox]-absorbance calibration graph.

Fig. 4. The correlation graph of the results of FRAP test (TEAC values) and DPPH• scavenging test (percentage scavenging of DPPH• at 60 min incubation time).

Fig. 5. The molecular structure of the compound **2**, with the atom-numbering scheme. Displacement ellipsoids are drawn at the 50% probability level.

Fig. 6. The molecular structure of the compound **4d**, with the atom-numbering scheme. Displacement ellipsoids are drawn at the 50% probability level.

Fig. 7. A wiev of N4-H4a…O1$^{\#}$ hydrogen bond with an $R_2^2(10)$ graph set notation for compound **2**. Symmetry code #:1-x, -y, 2-z.

Fig. 8. Hydrogen bonds interaction and packing diagram along the c axis for compound **2**. Hydrogen atoms omitted for clarity except atoms H4a,H4b and H8b.

Fig. 9. A view of N7—H7···O1^{ii} and N6—H6···S2^{iii} hydrogen bonds for compound **4d**, with $R_2^2(8)$ and $R_2^2(20)$ graph set notation respectively. Symmetry codes #:-x, -y, 1-z; $: -x, -1-y, 1-z.

Fig. 10. A view of the N5-H5...O2$^{\#}$ interaction resulted in a chain along the c axis with *C*(4) notation for the structure **4d**. Symmetry codes #:#:-x, -y, 1-z; $: -x, -1-y, 1-z.

Fig. 11. Hydrogen bonds interaction and packing diagram along the c axis for compound **4d**. Hydrogen atoms omitted for clarity except atoms H5, H6, H7 and H4b.

Table 1. Screening for antimicrobial activity of the compounds

Compound No	Stock Concentration µg/mL	Microorganisms[a] and MIC values (µg/mL)									
		Ec	Yp	Pa	Sa	Ef	Li	Bc	Ms	Ca	Sc
2	31800	-	-	-	-	-	-	-	994	-	-
3	4500	-	-	-	-	-	-	-	994	-	-
4a	20200	-	-	-	2525	-	-	5050	1262	-	-
4b	7600	-	-	-	950	-	-	950	950	-	-
4c	19300	-	-	-	2412	-	-	-	-	-	-
4d	12900	-	-	-	3225	-	-	-	1612	-	-
5a	16000	-	-	-	-	-	-	-	-	-	-
5b	14000	-	-	-	1750	-	-	1750	1750	-	-
5c	12500	-	-	-	-	-	-	-	-	-	-
5d	10800	-	-	-	-	-	-	-	-	-	-
6a	23100	-	-	-	-	-	-	-	-	-	-
6b	7800	-	-	-	-	-	-	-	-	-	-
6c	8900	-	-	-	-	-	-	-	-	-	-
6d	3600	-	-	-	-	-	-	-	-	-	-
6e	4800	-	-	-	-	-	-	-	-	-	-
7a	18300	-	-	-	-	-	-	-	-	-	-
7b	7900	-	-	-	-	-	-	-	-	-	-
7c	7600	-	-	-	-	-	-	-	-	-	-
7d	8600	-	-	-	-	-	-	-	-	-	-
7e	12500	-	-	-	-	-	-	-	-	-	-
DMSO		g	g	g	g	g	g	g	g	g	g
Amp.[b]	at 100	ng	ng	ng	ng	ng	ng	ng	ng	nt	nt
Flu.[c]	at 100	nt	nt	nt	nt	nt	nt	nt	nt	ng	ng

[a] Ec: *Escherichia coli* ATCC 25922, Yp: *Yersinia pseudotuberculosis* ATCC 911, Pa: *Pseudomonas auroginosa* ATCC 10145, Sa: *Staphylococcus aureus* ATCC 25923, Ef: *Enterococcus fecalis* ATCC 29212, Li: *Listeria monocytogenes* ATCC 43251, Bc: *Bacillus cereus* 709 Roma, Ms: *Mycobacterium smegmatis* ATCC607, Ca: *Candida albicans* ATCC 60193, Sc: *Saccharomyces cerevisiae* RSKK 251.

[b] Amp.: Ampicillin

[c] Flu.: Fluconazole

symbols: (-): no activity at test concentrations, g: growth was observed, ng: no growth (total inhibition) was observed, nt: not tested.

Table 2. Crystal data and structure refinement for **2** and **4d.**

	Compound 2	**Compound 4d**
Empirical formula	C11H14N4O3S	C17H19N7O2S2
Formula weight	282.32	417.51
Temperature/K	293(2)	293(2)
Crystal system	monoclinic	monoclinic
Space group	P21/c	P21/c
a/Å	11.7829(4)	19.6992(7)
b/Å	14.525(4)	11.6204(4)
c/Å	7.9468(2)	8.8498(4)
α/°	90.00	90.00
β/°	97.7(3)	92.438(4)
γ/°	90.00	90.00
Volume/Å^3	1347.8(4)	2024.00(14)
Z	4	4
ρ_{calc}mg/mm^3	1.391	1.370
m/mm^{-1}	0.250	0.291
F(000)	592.0	872.0
Crystal size/mm^3	0.28 × 0.25 × 0.18	0.3 × 0.25 × 0.2
2Θ range for data collection	6.48 to 55.44°	7 to 57.9°
Index ranges	-8 ≤ h ≤ 15, -14 ≤ k ≤ 17, -10 ≤ l ≤ 10	-25 ≤ h ≤ 11, -8 ≤ k ≤ 14, -11 ≤ l ≤ 12
Reflections collected	5280	8153
Independent reflections	2711[R(int) = 0.0123]	4609[R(int) = 0.0301]
Data/restraints/parameters	2711/0/181	4609/0/255
Goodness-of-fit on F^2	1.074	1.018
Final R indexes [I>=2σ (I)]	R1 = 0.0662, wR2 = 0.2027	R1 = 0.0664, wR2 = 0.1449
Final R indexes [all data]	R1 = 0.0737, wR2 = 0.2123	R1 = 0.1138, wR2 = 0.1684
Largest diff. peak/hole / e Å^{-3}	0.62/-0.71	0.42/-0.34

Table 3. Geometric parameters for compounds **2** and **4d** (Å, °)

Compound 2			
S1—C7	1.673 (4)	C7—S1—C4	92.9 (3)
S1—C4	1.692 (3)	C9—O3—C10	116.8 (3)
O1—C1	1.226 (4)	C1—N1—N2	113.1 (2)
O2—C9	1.199 (4)	C1—N1—C8	125.5 (2)
O3—C9	1.324 (3)	N2—N1—C8	121.3 (2)
O3—C10	1.456 (4)	C2—N2—N1	104.1 (2)
N1—C1	1.358 (4)	C2—N3—C1	109.2 (2)
N1—N2	1.390 (4)	C2—N3—N4	124.8 (2)
N1—C8	1.439 (3)	C1—N3—N4	125.5 (2)
N2—C2	1.304 (3)	O1—C1—N1	129.5 (3)
N3—C2	1.365 (5)		
N3—C1	1.385 (3)		
Compound 4d			
S2—C10	1.683 (3)	C7—S1—C4	92.6 (2)
S1—C7	1.696 (5)	C9—N5—N6	118.0 (2)
S1—C4	1.704 (4)	C10—N7—C11	123.6 (2)
N5—C9	1.346 (4)	C2—N3—C1	108.6 (3)
N5—N6	1.382 (3)	C2—N3—N4	126.4 (3)

N7—C10	1.334 (4)	C1—N3—N4	124.4 (2)
N7—C11	1.417 (4)	C10—N6—N5	124.6 (2)
O1—C1	1.221 (3)	N2—C2—N3	111.6 (3)
O2—C9	1.212 (3)	N2—C2—C3	125.0 (3)
N3—C2	1.372 (4)	N3—C2—C3	123.3 (3)
N3—C1	1.376 (4)	C2—N2—N1	104.5 (2)
N3—N4	1.402 (3)	C1—N1—N2	111.9 (2)
N6—C10	1.345 (4)	C1—N1—C8	124.4 (3)
C2—N2	1.289 (4)	N2—N1—C8	117.5 (2)

Table 4. Hydrogen-bond geometry (Å, °) for **2** and **4d.**

Compound 2

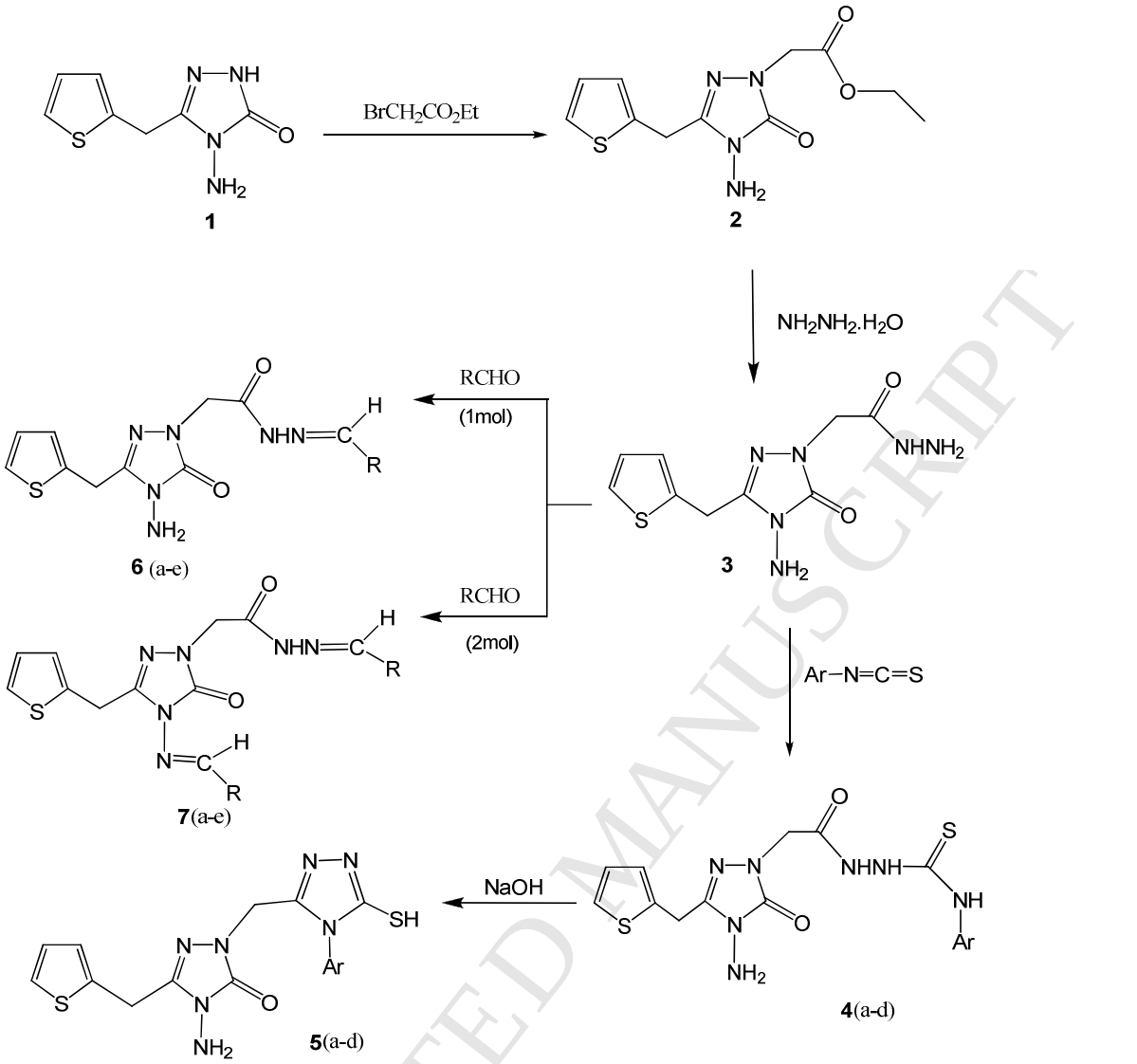
D—H···A	D—H	H···A	D···A	D—H···A
N4—H4A···O1^{i}	0.89 (4)	2.16 (4)	2.967 (3)	150 (3)
N4—H4B···O3^{ii}	0.78 (4)	2.52 (4)	3.153 (8)	140 (3)
C8—H8B···N4^{iii}	0.97	2.60	3.382 (5)	137

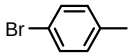
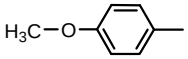
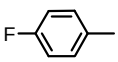
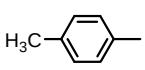
Symmetry codes: (i) −x+1, −y, −z+2; (ii) −x+1, y−1/2, −z+3/2; (iii) −x+1, y+1/2, −z+3/2.

Compound 4d

D—H···A	D—H	H···A	D···A	D—H···A
N5—H5···O2^{i}	0.86	2.18	2.748 (3)	123
N7—H7···O1^{ii}	0.86	2.12	2.834 (3)	140
N7—H7···N5	0.86	2.36	2.732 (3)	106
N6—H6···S2^{iii}	0.86	2.43	3.228 (3)	155
N4—H4B···S1	0.86	2.80	3.313 (3)	119

Symmetry codes: (i) x, −y−1/2, z+1/2; (ii) −x, −y−1, −z+1; (iii) −x, −y, −z+1.



4,5	Ar	6,7	R
a		a	
b		b	
c		c	
d		d	
		e	

Scheme 1. … Unver Y. et al.

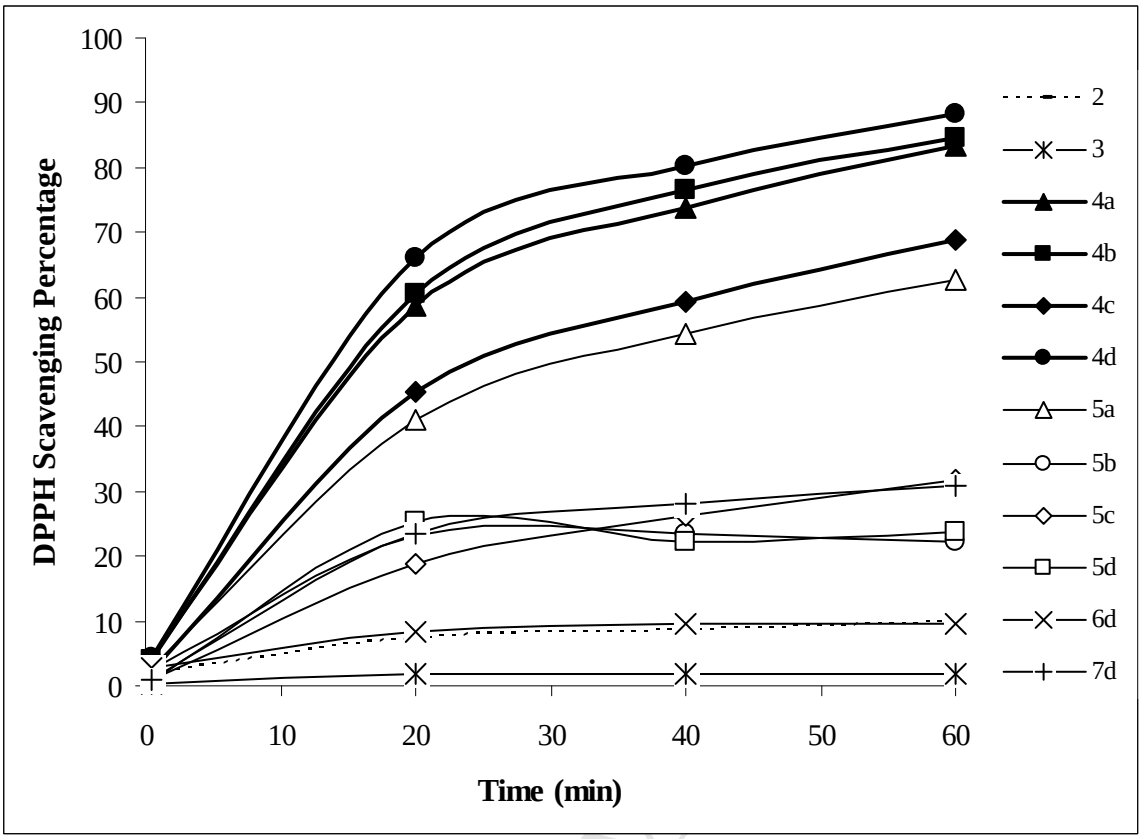
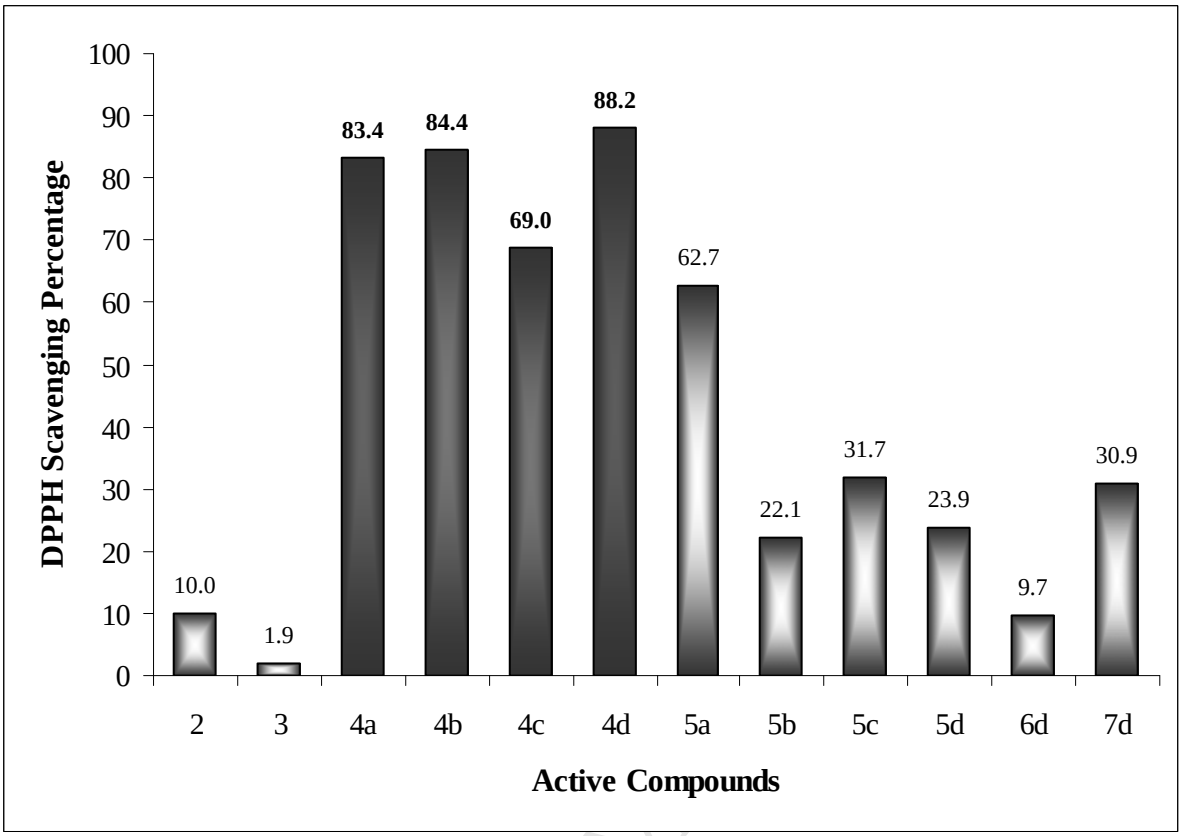


Fig. 1. … Unver Y. et al.



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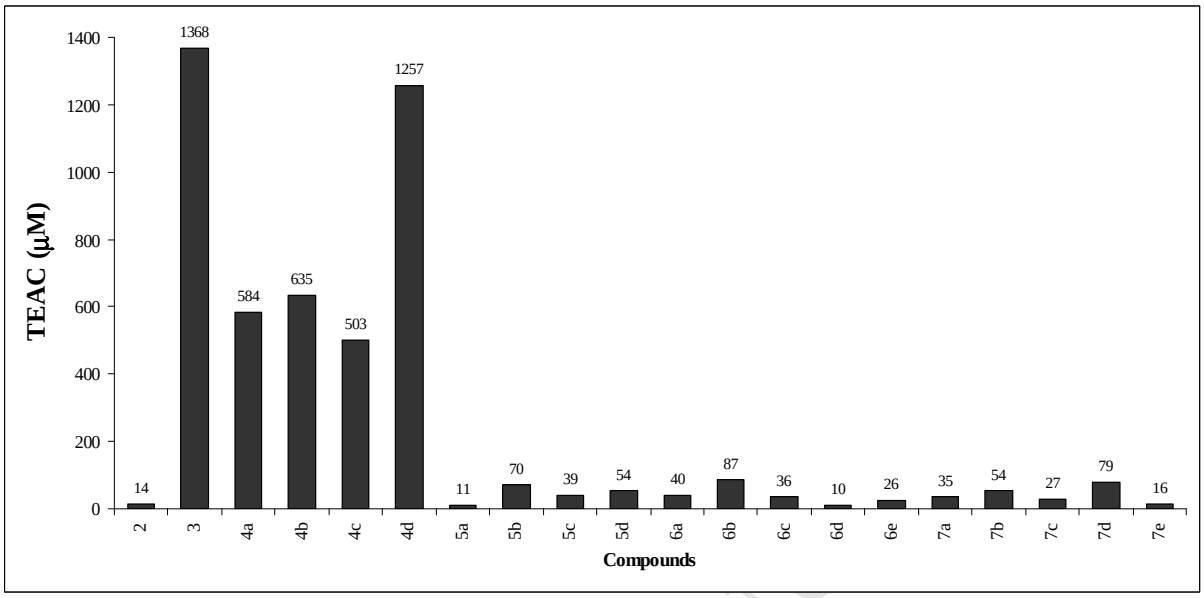

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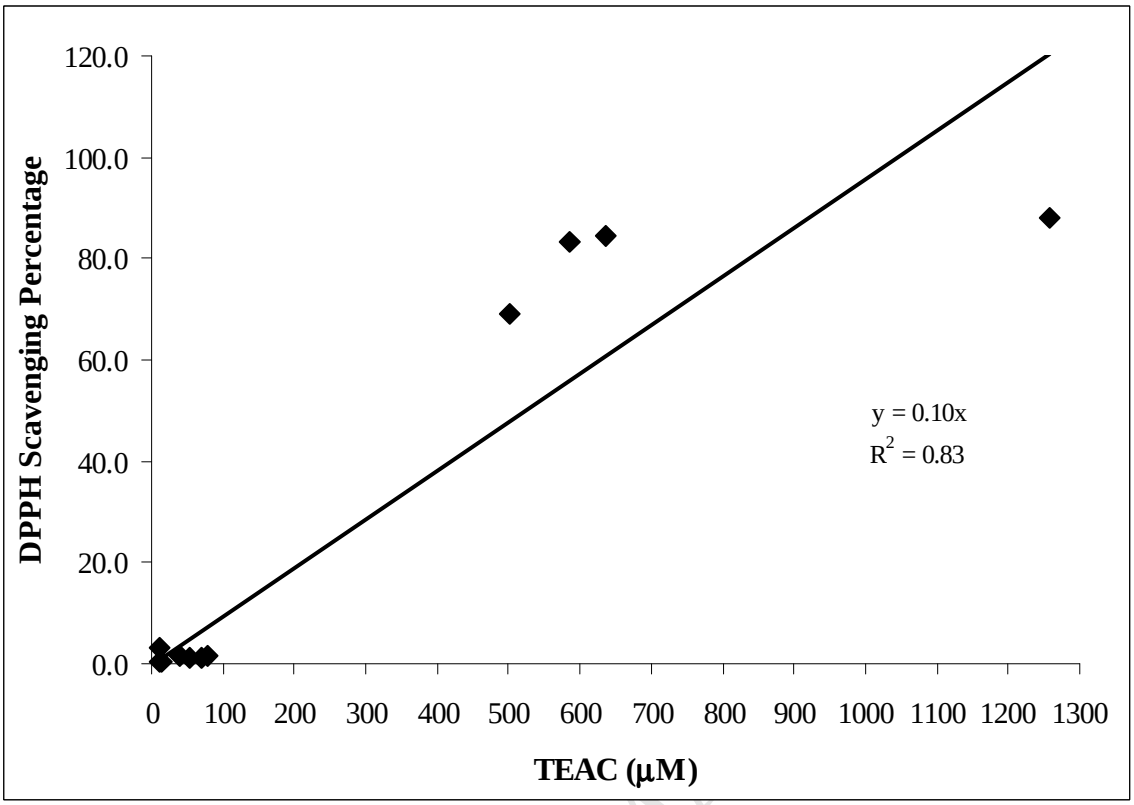

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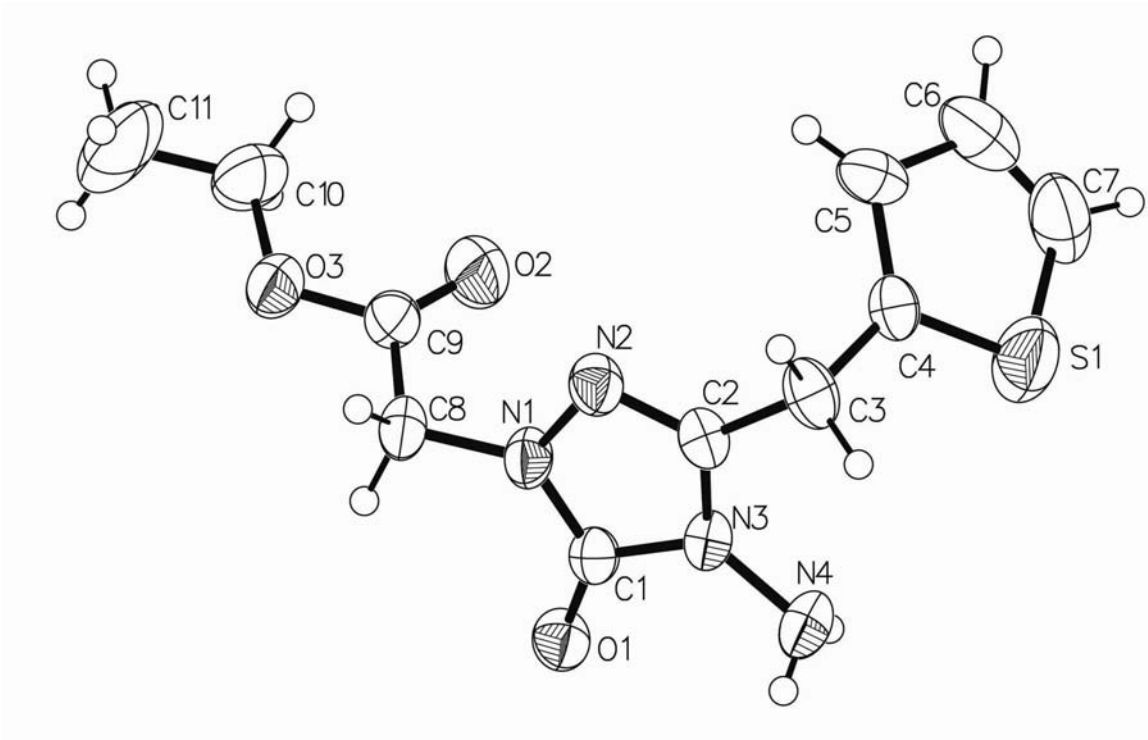

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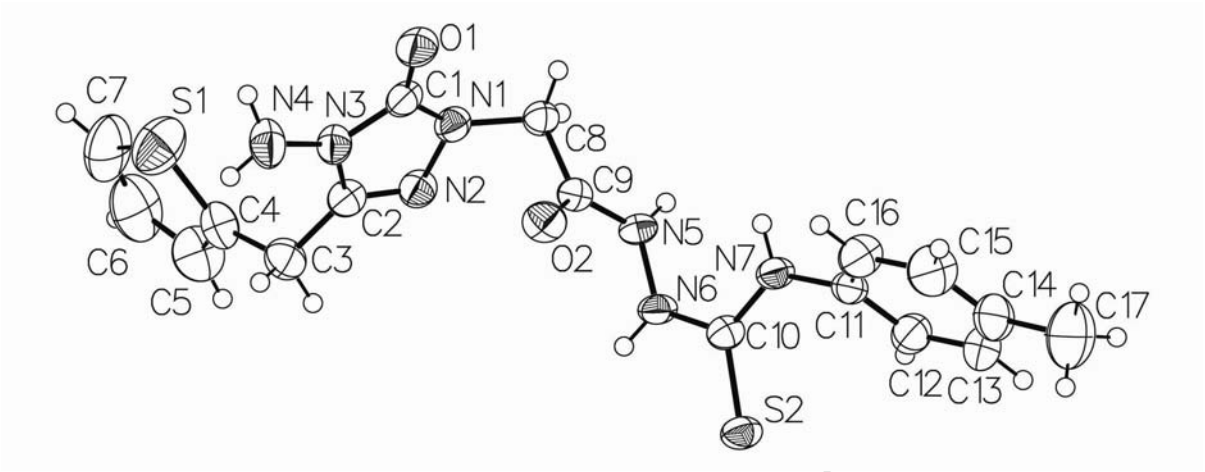

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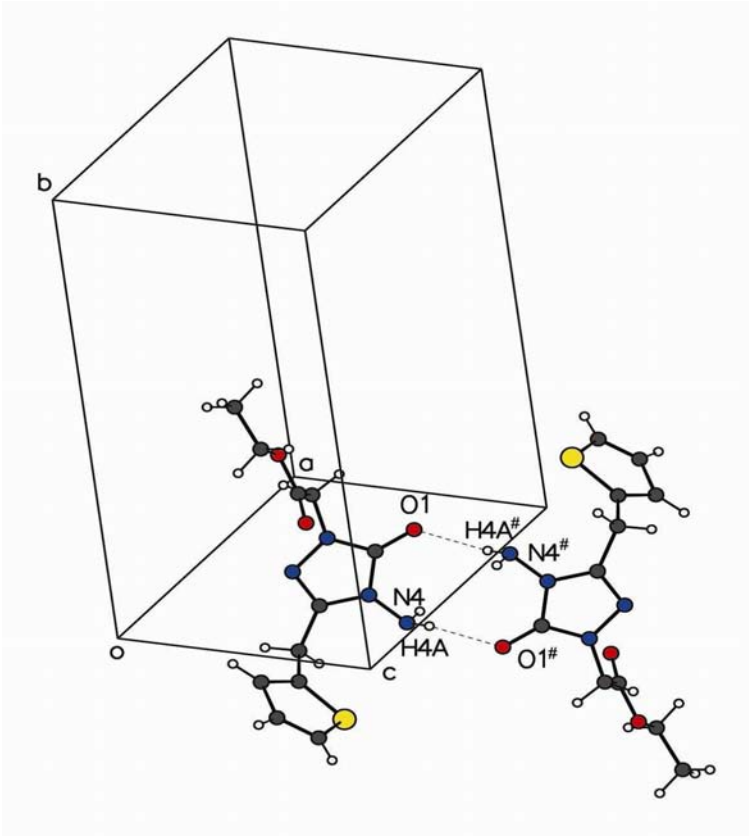

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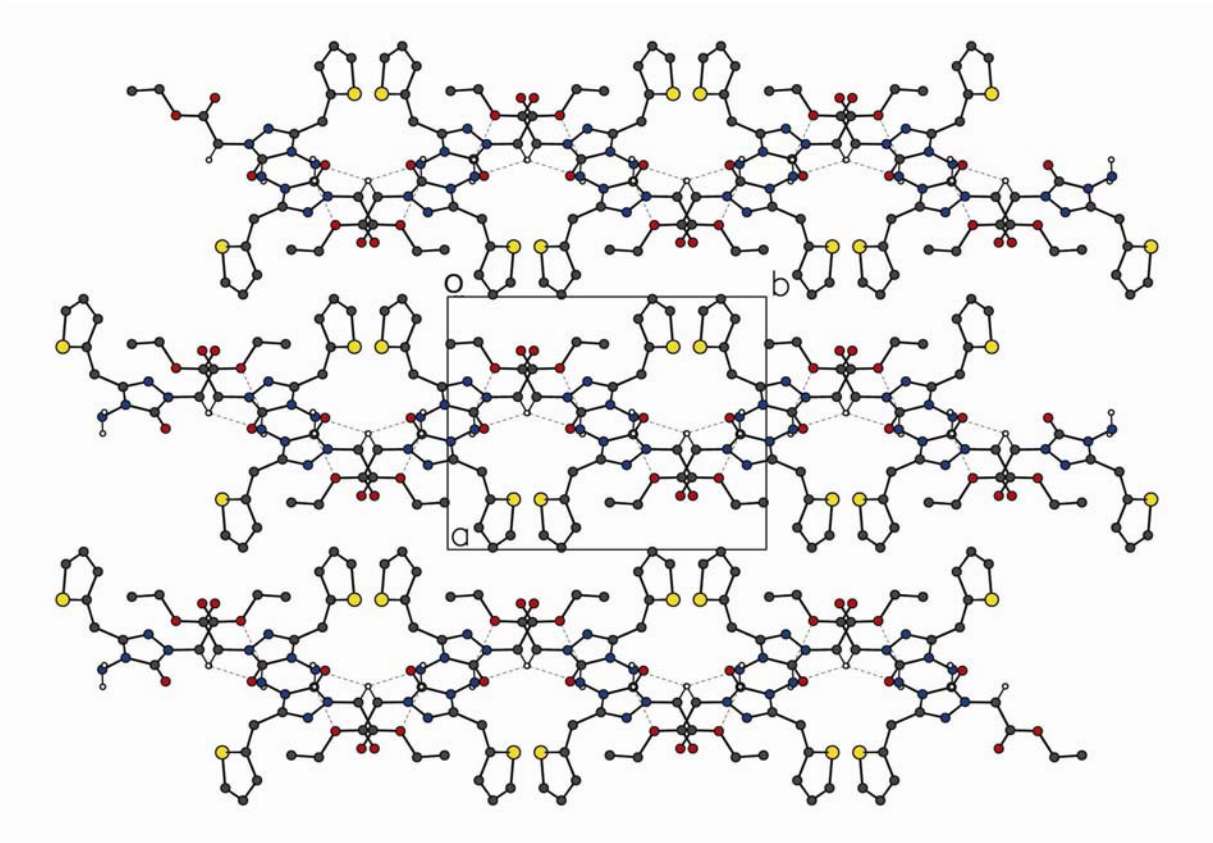

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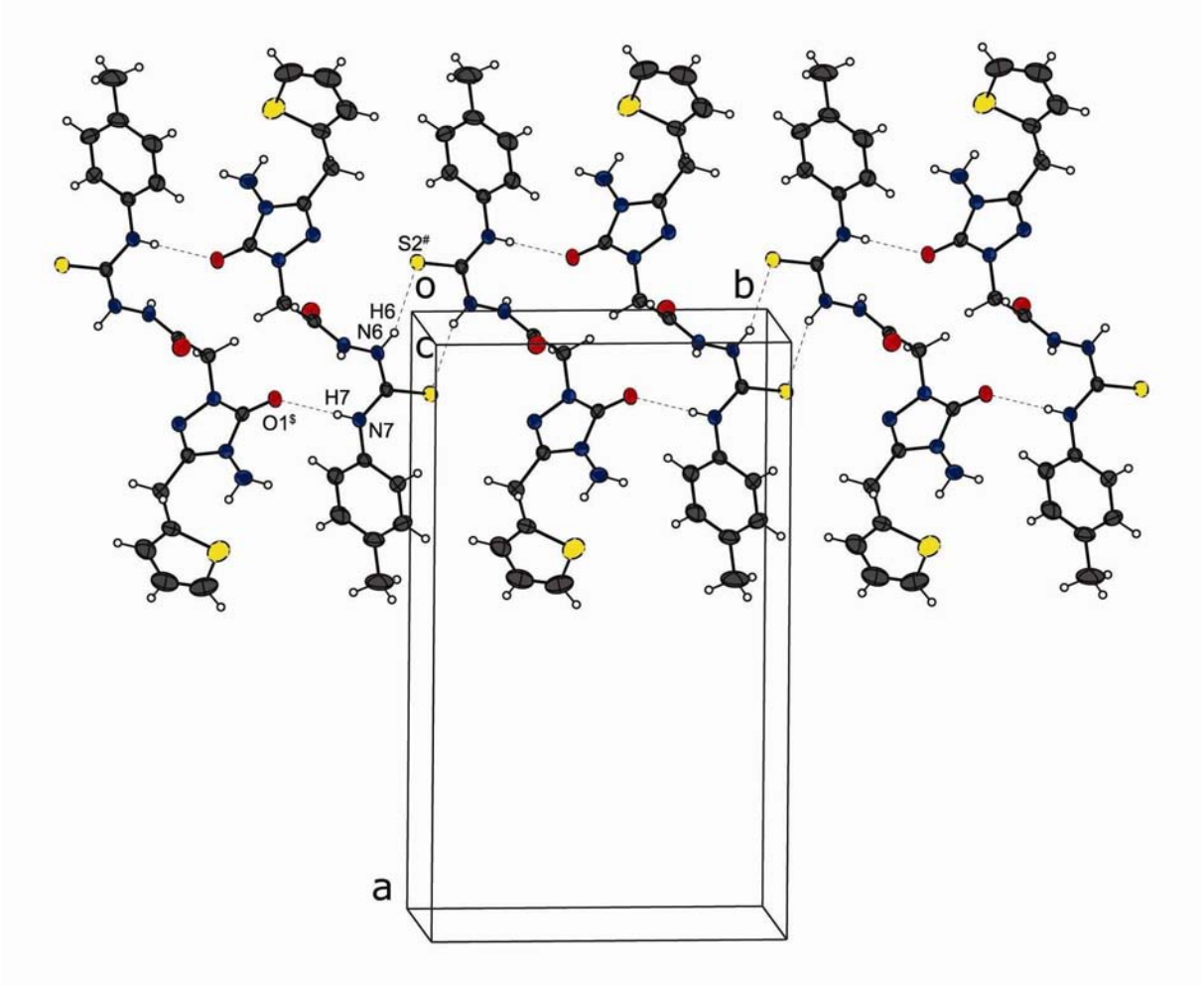

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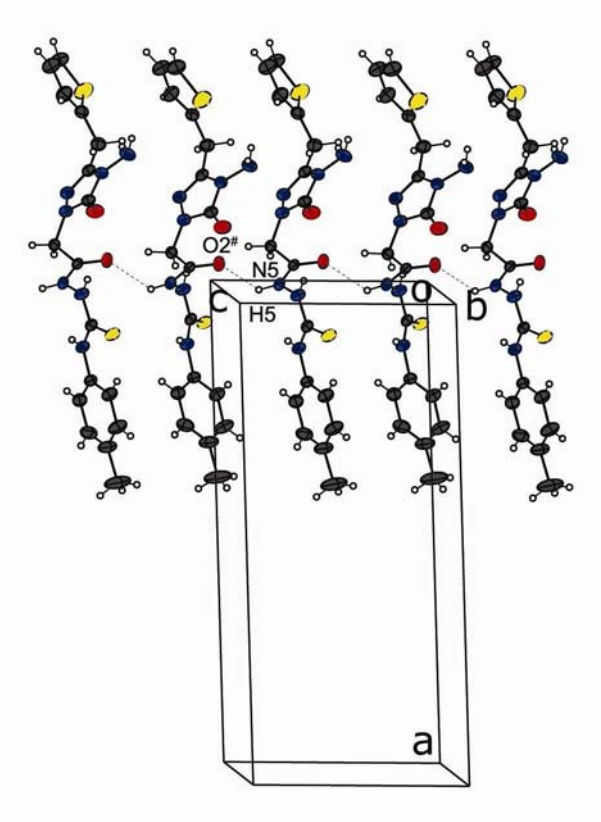

Fig. 10. … Unver Y. et al.

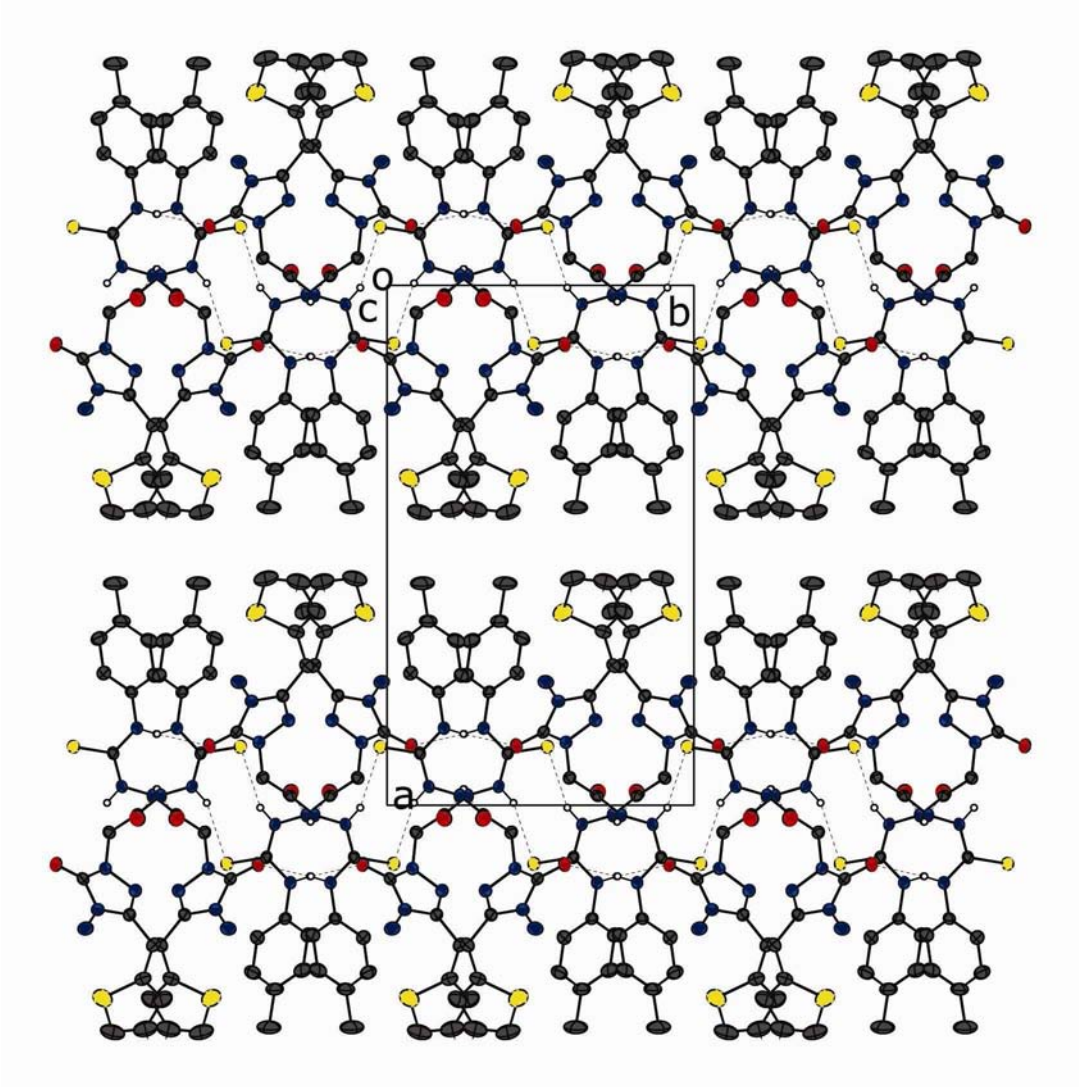

Fig. 11. … Unver Y. et al.

Highlights

- Bioactive 1,2,4-triazole-5(3)-one derivatives were synthesized.
- The compound **2** and **4d** were characterized by elemental analyses, IR, ^{1}H NMR, ^{13}C NMR and X-ray crystallography.
- All the compounds were tested for antimicrobial and antioxidant activities.
- Thiosemicarbazide group deserves attention in the synthesis of bioactive compounds.