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SYNTHESIS OF NEW FLUORINE-CONTAINING 1,2,4-TRIAZOLE-5-ON DERIVATIVES WITH THEIR ANTI-UREASE, ANTI-XANTHINE OXIDASE AND ANTIOXIDANT ACTIVITIES

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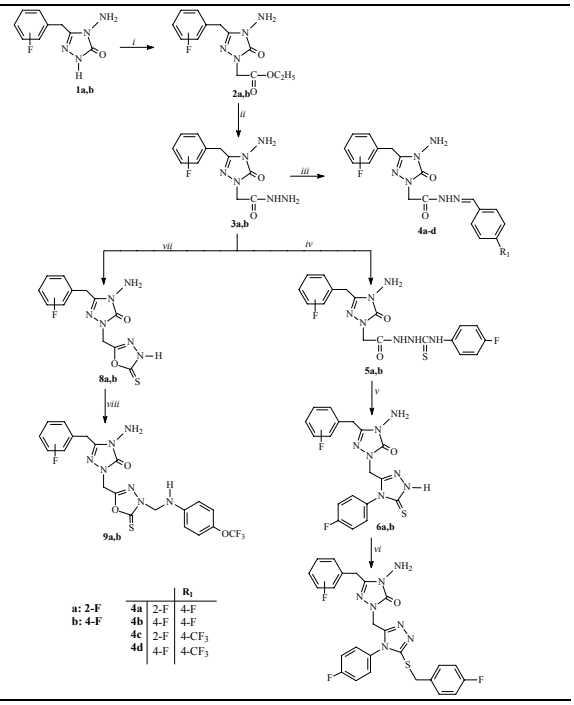
Acetohydrazide derivatives (**3a**, **b)** were obtained starting from 3-(2 or 4-fluorobenzyl)-4-amino-4,5-dihydro-1H-1,2,4-triazole-5-one (**1a**, **b**) by two steps. Compounds **3a**, **b** were converted to the corresponding methylideneacetohydrazide derivatives (**4a-d)**. The treatment of compounds **3a**, **b** with 4-fluorophenylisothiocyanate produced carbothioamide derivatives (**5a**, **b)**. The cyclization of compounds **5a**,**b** in the presence of NaOH resulted in the formation of compounds **6a**, **b**. The reactions of compounds **6a**, **b** with 4-fluorobenzyl bromide in the presence of sodium ethoxide afforded the corresponding S-(4-fluorobenzyl) derivatives (**7a**, **b**). On the other hand, the treatment of compounds **3a**, **b** with CS_2 in the presence of aqueous KOH afforded the 1,3,4-oxadiazole-5-thiones (**8a, b**) which were converted to N-Mannich bases **9a**, **b**. The synthesized compounds **1-9** were screened for their urease and xanthine oxidase inhibition activities and antioxidant activity were evaluated. Especially, according to the CUPRAC and ABTS$^{\bullet+}$ radical scavenging activity methods, **3a, 3b, 5a, 5b, 6a, 6b, 8a, 8b, 9a** and **9b** compounds showed strong antioxidant activity. Compound **4a** exhibited good xanthine oxidase (XO) inhibition. Also, compounds **9b** and **9a** showed efficient urease inhibition at various concentrations.



INTRODUCTION

Reactive oxygen species (ROS) are chemically reactive molecules containing oxygen. Types of ROS include the hydrogen peroxide (H_2O_2), hydroxyl radical (HO•), the superoxide anion radical ($\bullet O_2$), singlet oxygen ($^1O^2$), hypochlorite radical (ClO•), nitric oxide radical (NO), alkoxyl radical (RO•), alkylperoxy radical (RO_2•) and various lipid peroxides.[1] These molecules can damage DNA, proteins and lipids, resulting in diseases such as atherosclerosis, vasospasms, cancers, stroke, asthma, arthritis, heart attack, age pigments, dermatitis, cataractogenesis, retinal damage, hepatitis, liver injury, and periodontis.[2] Antioxidants delay or inhibit cellular dam-

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age mainly through their ROS scavenging property.[3]

Urease is a metalloenzyme containing nickel that catalyzes the hydrolysis of urea into ammonia and carbamate. Carbamate rapidly and spontaneously decomposes to yield a second molecule of NH_3 and CO2.[4] The reaction results in a pH increase, responsible for negative effects of urease activity in human health and agriculture.[5,6] Urease is responsible for urinary tract and gastrointestinal infections,[7] possibly causing severe diseases such as peptic ulcers and stomach cancer as in the case of Helicobacter pylori.[8] Urease is also involved in the development of urolithiasis, pyelonephritis, hepatic encephalopathy, hepatic coma, and urinary catheter encrustation.[9] Acetohydroxamic acid has been clinically used for the treatment of urinary tract infections by urease inhibition. However, it exhibits severe side effects such as difficulty breathing; swelling of your face, lips, tongue, or throat.[10]

Xanthine oxidase (XO) is a molybdenum-containing enzyme catalyzing the oxidation of hypoxanthine and xanthine to uric acid, which plays a crucial role in gout.[11] Allopurinol is a clinically used XO inhibitor in the treatment of gout. However, it may also cause side effects such as hypersensitivity problem,[12] Steven-Johnson syndrome,[13] renal toxicity[14] and fatal liver necrosis.[15]

A literature survey revealed that 1,2,4-triazole and its derivatives are important pharmacophores across a number of different therapeutic areas such asantifungal, antibacterial, anticancer, antitubercular, anti inflammatory, analgesic, anticonvulsant, antiviral, anti oxidant, enzyme inhibitor, herbicidal, insecticidal and plant growth activities.[4-15] The drugs like anastrozolo, letrozole and vorozole (antitumor),[16] difenoconazole and itraconazole (antifungal),[17] ribavirin (antiviral),[18] rizatriptan (antimigraine),[19] alprazolam (anxiolytic),[20] are the best examples of potent molecules possessing a 1,2,4-triazole nucleus. Moreover, Fluorinated substituted 1,2,4-triazoles have got a significant place in medicinal chemistry.[21] Some of the most well known fluorinated 1,2,4-triazoles drugs are albaconazole, fluconazole, posaconazole, ravuconazole, voriconazole, flusilazole, fluotrimazole, epoxiconazole and flutriafol.[22,23] Similarly, substituted 1,3,4-oxadiazoles are well known in medicinal chemistry due to their diverse biological properties like antibacterial, antitubercular, antifungal, anticonvulsant, anti-inflammatory, antioxidant, anti-allergic, enzyme inhibitor, analgesic, anticancer, insecticidal activities.[24-26] In addition to these, there are a number of marketing drugs containing a 1,3,4-oxadiazole ring in their structures structures such as zibotentan (anticancer),[27] raltegravir (antiretroviral),[28] tiodazosin[29] and nesapidil (antihypertensive),[30] and furamizol (antibiotic).[31] On the other hand, hydrazide and thiosemicarbazide derivatives attracted a lot of attention because they are considered as intermediates to synthesized five- and six membered heterocylic rings containing one, two or three of the same or different heteroatoms (O, N or S) which all were reported possess biological activities.[32,33] Apart from these, certain Mannich bases derived from 1,3,4-oxadiazoles exhibit good antioxidant and antimicrobial activity.[34-36] In view of these observations, we now report the synthesis of some fluorinated 1,2,4-triazole-5-on derivatives along with their antioxidant, urease and xanthine oxidase Inhibitiory activities.

EXPERIMENTAL

Melting points were determined in open capillaries on a Büchi oil-heated melting point apparatus (Büchi Labortechnik, Flawil, Switzerland) and are uncorrected. IR spectra were recorded as potassium bromide pellets using a Perkine-Elmer 1600 series FTIR spectrophotometer (Perkin-Elmer, Beaconfields, England). ^{1}H NMR and ^{13}C NMR spectra were recorded on a Varian-Mercury 200 MHz spectrometer (Varian Inc., Palo Alto, CA, USA**)** using tetramethylsilane as the internal reference. The elemental analysis was performed on a Costech Elemental Combustion System CHNS-O elemental analyzer (Valencia, CA 91355, USA). The reactions were monitored by thin layer chromatograph (TLC) using 0.2 mm precoated plates of silica gel G60 F254.

General procedure forthe synthesis of ethyl acetate derivatives (**2a**,**b**)

Compounds **1** (10 mmol) and Na (10 mmol) were refluxed in absolute ethanol for 2h. Then, ethyl bromoacetate (10 mmol) was added and stirred under reflux for 6 h, product formation was detected by TLC. After evaporating the solvent under reduced pressure, a solid appered. The solid product was recrystallized from ethanol-water (1:3) to afford the desired compounds.

Ethyl[4-amino-3-(2-fluorobenzyl)-4,5-dihydro-1H-1,2,4-triazol-5-one-1-yl]acetate (**2a**):

Yield 72 %, mp: 116-117 °C, IR (ν_{max}, cm^{-1}): 3317, 3211 (NH_2), 1748 (ester C=O), 1721 (triazole C=O), 1654 (C=N) and 1218 (C-O), ^{1}H NMR (DMSO-d_6, 200 MHZ) δ (ppm): 1.17 (t, 3H, J =7.0 Hz, CH_3), 3.96 (s, 2H, benzyl CH_2), 4.12 (q, 2H, J= 7.0 Hz, OCH_2), 4.50 (s, 2H, N-CH_2), 5.32 (s, 2H, NH_2) and Ar-H: [7.14-7.22 (m, 2H), 7.27-7.30 (m, 2H)]. ^{13}C NMR (DMSO-d_6, 50 MHZ): δ(ppm): 14.30 (CH_3), 24.53 (d, J_{CF}= 3.5 Hz, benzyl CH_2), 47.26 (N-CH_2), 61.74 (OCH_2), Ar-C: [115.92 (d, J_{CF}= 21.5 Hz, CH), 122.98 (d, J_{CF}= 21.5 Hz, C), 125.07 (d, J_{CF}= 2.5 Hz, CH), 129.70 (d, J_{CF}= 8.0 Hz, CH), 131.89 (d, J_{CF}= 3.5 Hz, CH), 161.06 (d, J_{CF}= 244.0 Hz, C)],

147.01 (triazole C-3), 153.97 (triazole C=O) and 168.61 (ester C=O). Anal. Calcd. for $C_{13}H_{15}FN_4O_3$: C, 53.06; H, 5.14 and N, 19.04; found: C, 53.28; H, 5.18 and N,18.93.

Ethyl[4-amino-3-(4-fluorobenzyl)-4,5-dihydro-1H-1,2,4-triazol-5-one-1-yl]acetate (**2b**):

Yield 80 %, mp: 135-136 °C, IR (ν_{max}, cm^{-1}): 3307, 3213 (NH_2), 1745 (ester C=O), 1714 (triazole C=O), 1602 (C=N) and 1228 (C-O), ^{1}H NMR (DMSO-d_6, 200 MHZ) δ (ppm): 1.17 (t, 3H, *J* =7.0 Hz, CH_3), 3.88 (s, 2H, benzyl CH_2), 4.11 (q, 2H, *J*= 7.0 Hz, OCH_2), 4.51 (s, 2H, N-CH_2), 5.36 (s, 2H, NH_2) and Ar-H: [7.12 (m, 2H), 7.29 (m, 2H)].^{13}C NMR (DMSO-d_6, 50 MHZ): δ (ppm): 14.27 (CH_3), 29.82 (benzyl CH_2), 46.90 (N-CH_2), 61.48 (OCH_2), Ar-C: [115.52 (d, J_{CF}= 21.0 Hz, 2CH), 131.12 (2 CH), 132.28, 159.36 (d, J_{CF}= 238.0 Hz, C)], 147.73 (triazole C-3), 153.75 (triazole C-5) and 168.39 (ester C=O). Anal. Calcd. for $C_{13}H_{15}FN_4O_3$: C, 53.06; H, 5.14 and N, 19.04; found C, 53.32; H, 5.21 and N,18.87.

General procedure for the synthesis of acetohydrazide derivatives (3a,b)

A mixture of **2** (0.01 mol) and hydrazine hydrate (0.025 mol) in 1-butanol (50 mL) was riflaxed for 3 h. The progress of the reaction was monitored by TLC. After cooling room temperature, a white solid appeared. The solid product was filtered and recrystallization from ethanol.

2-[4-Amino-3-(2-fluorobenzyl)-4,5-dihydro-1H-1,2,4-triazol-5-one-1-yl]acetohydrazide **(3a)**:

Yield 84 %, mp: 179-180 °C, IR (ν_{max}, cm^{-1}): 3313, 3205 (NH+$2NH_2$), 1726 (triazol C=O), 1671 (hydrazide C=O) and 1622 (C=N), ^{1}H NMR (DMSO-d_6, 200 MHZ) δ (ppm): 3.90 (s, 2H, benzyl CH_2), 4.20 (s, 2H, N-CH_2), 4.24 (s, 2H, hydrazide NH_2), 5.32 (s, 2H, NH_2), Ar-H: [7.14-7.18 (m, 2H), 7.23-7.31 (m, 2H)] and 9.16 (s, 1H, NH). ^{13}C NMR (DMSO-d_6, 50 MHZ): δ(ppm): 24.45 (d, J_{CF}=6.5 Hz, benzyl CH_2), 47.06 (N-CH_2), Ar-C: [115.90 (d, J_{CF}= 21.0 Hz, CH), 123.07 (d, J_{CF}= 15.5 Hz, C), 129.08 (d, J_{CF}= 3.5 Hz, CH), 129.64 (d, J_{CF}= 8.0 Hz, CH), 131.96 (d, J_{CF}= 4.5 Hz, CH), 161.00 (d, J_{CF}= 244.0 Hz, C)], 146.56 (triazole C-3), 154.04 (triazole C-5) and 166.66 (hydrazide C=O). Anal. Calcd. for $C_{11}H_{13}FN_6O_2$: C, 47.14; H, 4.68 and N, 29.99; found C, 47.28; H, 4.72 and N, 29.82.

2-[4-Amino-3-(4-fluorobenzyl)-4,5-dihydro-1H-1,2,4-triazol-5-one-1-yl]acetohydrazide (**3b**):

Yield 86 %, mp: 175-176 °C, IR (ν_{max}, cm^{-1}): 3310, 3190 (NH+NH_2), 1716 (triazol C=O), 1678 (hydrazide C=O) and 1651 cm^{-1} (C=N), ^{1}H NMR (DMSO-d_6, 200 MHZ) δ (ppm): 3.86 (s, 2H, benzyl CH_2), 4.21 (s, 2H, N-CH_2), 4.25 (s, 2H, hydrazide NH_2), 5.25 (s, 2H, NH_2), Ar-H: [7.07-7.15 (m, 2H), 7.26-7.33 (m, 2H)] and 9.17 (s, 1H, NH). ^{13}C NMR (DMSO-d_6, 50 MHZ): δ(ppm): 30.23 (benzyl CH_2), 47.09 (N-CH_2), Ar-C: [115.81 (d, J_{CF}= 21.5 Hz, 2CH), 131.39 (d, J_{CF}= 8.0 Hz, 2CH), 132.65 (d, J_{CF}= 3.0 Hz, C), 161.00 (d, J_{CF}= 241.0 Hz, C)], 147.57 (triazole C-3), 154.07 (triazole C-5) and 170.83 (hydrazide C=O). Anal. Calcd. for $C_{11}H_{13}FN_6O_2$: C, 47.14; H, 4.68 and N, 29.99; found C, 47.16; H, 4.69 and N, 29.71.

General procedure for the synthesis of methylideneacetohydrazide derivatives (4a-d)

To a solution of compound **3** (0.01 mol) in ethanol (50 mL) was added appropriate aldehyde (0.01 mol). Two drops of concentrated H_2SO_4 were added to a stirred solution, and the mixture was refluxed for 2 h (TLC-controlled). At the and of the reaction, crude product collapsed. The precipitate formed was filtered off and purified by recrystallization from dimethyl sulfoxide:water (1:2).

2-[4-Amino-3-(2-fluorobenzyl)-4,5-dihydro-1H-1,2,4-triazol-5-one-1-yl]-N'-[(4-fluorophenyl)methylidene]acetohydrazide (**4a**):

Yield 94 %, mp: 288-289 °C, IR (ν_{max}, cm^{-1}): 3331(NH), 3267, 3207 (NH_2),1718 (triazole C=O), 1695 (hydrazide C=O) and 1677, 1602 (C=N), ^{1}H NMR (DMSO-d_6, 200 MHZ) δ (ppm): 3.94 (s, 2H, benzyl CH_2), 4.83 and 4.42 (s, 2H, N-CH_2, *trans* and *cis* conformers), 5.36 (s, 2H, NH_2), Ar-H: [7.10-7.37 (m, 6H), 7.74-7.81 (m, 2H)], 7.99 and 8.19 (s, 1H, N=CH, *trans* and *cis* conformers) and 11. 61 (s, 1H, NH). ^{13}C NMR (DMSO-d_6, 50 MHZ): δ (ppm): 24.41 and 24.54 (d, J_{CF}=6.5 Hz, benzyl CH_2), 47.23 and 47.66 (N-CH_2, *trans* and *cis* conformers), Ar-C: [115.92 (d, J_{CF}= 21.5 Hz, CH), 116.54 (d, J_{CF}= 22.0 Hz, 2CH,) 123.14 (d, J_{CF}= 15.5 Hz, C), 125.09 (d, J_{CF}= 3.5 Hz, CH), 129.66 (d, J_{CF}= 9.5 Hz, CH), 130.04 (d, J_{CF}= 9.0 Hz, 2CH), 131.26 (d, J_{CF}= 3.5 Hz, C), 131.89 (d, J_{CF}= 3.5 Hz, CH), 161.03 (d, J_{CF}= 243.0 Hz, C), 163.72 (d, J_{CF}= 246.5 Hz, C)], 143.57 and 146.86 (-N=CH, *trans* and *cis* conformers), 146.50 and 146.74 (triazole C-3, *trans* and *cis* conformers), 154.38 and 163.85 (triazole C-5, *trans* and *cis* conformers) and 166.66 (hydrazide C=O). Anal. Calcd. for $C_{18}H_{16}F_2N_6O_2$: C, 55.96; H, 4.17 and N, 21.75; found C, 56.17; H, 4.33 and N, 21.56.

2-[4-Amino-3-(4-fluorobenzyl)-4,5-dihydro-1H-1,2,4-triazol-5-one-1-yl]-N'-[(4-fluorophenyl)methylidene]acetohydrazide (**4b**):

Yield: 95 %, mp: 276-277 °C, IR ((ν_{max}, cm^{-1}): 3325 (NH), 3265, 3206 (NH_2), 1720 (triazole C=O), 1698 (hydrazide C=O) and 1675, 1601 (C=N), ^{1}H NMR (DMSO-d_6, 200 MHZ) δ (ppm): 3.90 (s, 2H, benzyl CH_2), 4.85 and 4.43 (s, 2H, N-CH_2, *trans* and *cis* conformers), 5.37 (s, 2H, NH_2), Ar-H: [7.09-7.18 (m, 2H), 7.23-7.36 (m, 4H), 7.75-7.82 (m, 2H)], 7.99 and 8.20 (s, 1H, N=CH, *trans* and *cis* conformers) and 11. 67 (s, 1H, NH). ^{13}C NMR (DMSO-d_6, 50 MHZ): δ (ppm): 30.25 (benzyl CH_2), 47.22 and 47.66 (N-CH_2, *trans* and *cis* conformers), Ar-C: [115.81 (d, J_{CF}= 21.5 Hz, 2CH), 116.53 (d, J_{CF}= 21.5 Hz, 2CH), 129.85 (d, J_{CF}= 9.0 Hz, 2CH), 130.13, 131.36 (d, J_{CF}=8.5 Hz, 2CH), 131.26 (d, J_{CF}= 3.5 Hz, C),132.73, 161.04 (d, J_{CF}= 243.0 Hz, C), 163.72 (d, J_{CF}= 246.5 Hz, C)], 143.57 and 146.86 (-N=CH, *trans* and *cis* conformers), 147.48 and 147.73 (triazole C-3, *trans* and *cis* conformers), 154.41 and 164.20 (triazole C-5, *trans* and *cis* conformers) and 168.77 (hydrazide C=O). Anal. Calcd. for $C_{18}H_{16}F_2N_6O_2$: C, 55.96; H, 4.17 and N, 21.75; found C, 56.09; H, 4.26 and N,21.63.

2-[4-Amino-3-(2-fluorobenzyl)-4,5-dihydro-1H-1,2,4-triazol-5-one-1-yl]-N'-{[4-(trifluoromethyl)phenyl]methylidene}acetohydrazide (**4c**):

Yield 83%, mp: 264-265°C, IR (ν_{max}, cm^{-1}): 3333(NH), 3269, 3203 (NH_2),1717 (triazole C=O), 1695 (hydrazide C=O) and 1685, 1604 cm^{-1}(C=N), ^{1}H NMR (DMSO-d_6, 200 MHZ) δ (ppm): 3.95 (s, 2H, benzyl CH_2), 4.87 and 4.46 (s, 2H, N-CH_2, *trans* and *cis* conformers), 5.41 (s, 2H, NH_2), Ar-H: [7.15-7.37 (m, 4H), 7.76-7.80 (m, 2H), 7.92-7.96 (m, 2H)], 8.06 and 8.27 (s, 1H, N=CH, *trans* and *cis* conformers) and 11. 81 (s, 1H, NH). ^{13}C NMR (DMSO-d_6, 50 MHZ): δ (ppm): 24.50 and 24.57 (d, J_{CF}= 3.5 Hz, benzyl CH_2), 47.29 and 47.75 (N-CH_2, *trans* and *cis* conformers), 124.71 (q, J_{CF}= 275.5 Hz, CF_3), Ar-C: [115.90 (d, J_{CF}= 21.0 Hz, CH), 123.12 (d, J_{CF}= 15.0

Hz, C), 125.06 (d, J_{CF}= 3.0 Hz, CH), 126.35 (2CH), 128.33 (2CH), 129.64 (d, J_{CF}= 8.0 Hz, CH), 130.59, 131.89 (d, J_{CF}= 3.5 Hz, CH), 138.59, 161.05 (d, J_{CF}= 243.0 Hz, C)], 143.06 and 146.29 (-N=CH, *trans* and *cis* conformers), 146.57 and 146.81 (triazole C-3, *trans* and *cis* conformers), 154.39 and 164.17 (triazole C-5, *trans* and *cis* conformers) and 169.02 (hydrazide C=O). Anal. Calcd. for $C_{19}H_{16}F_4N_6O_2$: C, 52.30; H, 3.70and N, 19.26; found C, 52.31; H, 3.68 and N, 19.22.

2-[4-Amino-3-(4-fluorobenzyl)-4,5-dihydro-1H-1,2,4-triazol-5-one-1-yl]-N'-{[4-(trifluoromethyl)phenyl]methylidene}acetohydrazide (**4d**):

Yield 87 %, mp: 257-258°C, IR (ν_{max}, cm^{-1}): 3326(NH), 3274, 3214 (NH_2),1721 (triazole C=O), 1697 (hydrazide C=O) and 1678, 1605(C=N), ^{1}H NMR (DMSO-d_6, 200 MHZ) δ (ppm): 3.89 (s, 2H, benzyl CH_2), 4.87 and 4.46 (s, 2H, N-CH_2, *trans* and *cis* conformers), 5.34 (s, 2H, NH_2), Ar-H: [7.09-7.17 (m, 2H), 7.29-7.36 (m, 2H), 7.76-7.88 (m, 2H), 7.92-7.96 (m, 2H)], 8.07 and 8.27 (s, 1H, N=CH, *trans* and *cis* conformers), 11. 86 (s, 1H, NH). ^{13}C NMR (DMSO-d_6, 50 MHZ): δ (ppm): 30.25 (benzyl CH_2), 47.25 and 47.72 (N-CH_2, *trans* and *cis* conformers), 124.60 (q, J_{CF}= 275.0 Hz, CF_3,), Ar-C: [115.81 (d, J_{CF}= 20.5 Hz, 2CH,), 126.92 (2CH), 128.41 (2CH), 130.60, 131.36 (d, J_{CF}= 8.5 Hz, 2CH), 132.69 (d, J_{CF}= 3.0 Hz, C), 138.59, 156.77 (d, J_{CF}= 260.5 Hz, C)], 143.07 and 146.28 (-N=CH, *trans* and *cis* conformers), 146.55 and 146.77 (triazole C-3, *trans* and *cis* conformers), 154.41 and 164.20 (triazole C-5, *trans* and *cis* conformers) and 169.05 (hydrazide C=O). Anal. Calcd. for $C_{19}H_{16}F_4N_6O_2$: C,52.30; H,3.70 and N, 19.22; found C, 52.34; H, 3.71 and N, 19.16.

General procedure for the synthesis of carbothioamide derivatives (5a,b)

Compound **3** (0.01 mol) and 4-fluorophenyl isothiocyanate (0.01 mol) was refluxed in ethanol (100 mL) for 2 h. The progress of the reaction was monitored by TLC. The solution was cooled and a white solid appeared. The precipitated product was filtered and recrystallized from ethanol to afford the desired compounds.

2-{[4-Amino-3-(2-fluorobenzyl)-4,5-dihydro-1H-1,2,4-triazol-5-one-1-yl]acetyl}-N-(4-fluorophenyl)hydrazinecarbothioamid (**5a**):

ield 90 %, mp: 121-122 °C, IR (ν_{max}, cm^{-1}): 3313-3222 (3NH+NH_2), 1720 (triazole C=O), 1687 (thiosemicarbazide C=O), 1629 (C=N) and 1236 (C=S), ^{1}H NMR (DMSO-d_6, 200 MHZ) δ (ppm): 3.92 (s, 2H, benzyl CH_2), 4.46 (s, 2H, N-CH_2), 5.35 (s, 2H, NH_2), Ar-H: [7.13-7.28 (m, 4H), 7.32-7.39 (m, 4H)], 9. 64 (s, 1H, NH), 9. 72 (s, 1H, NH) and 10. 26 (s, 1H, NH). ^{13}C NMR (DMSO-d_6, 50 MHZ): δ (ppm): 24.56 (d, J_{CF}=4.0 Hz, benzyl CH_2), 47.35 (N-CH_2), Ar-C: [115.51 (d, J_{CF}= 22.5 Hz, 2CH), 115.93 (d, J_{CF}= 20.0 Hz, CH), 122.90 (d, J_{CF}= 16.0 Hz, C), 125.10 (d, J_{CF}= 2.5 Hz, CH), 128.75 (CH), 129.72 (d, J_{CF}= 8.5 Hz, 2CH,), 132.02 (d, J_{CF}= 3.5 Hz, CH), 135.93, 160.30 (d, J_{CF}= 241.0 Hz, C), 161.05 (d, J_{CF}= 243.5 Hz, C)],146.85 (triazole C-3), 154.19 (triazole C-5), 167.26 (C=O) and 181.68 (C=S). Anal. Calcd. for $C_{18}H_{17}F_2N_7O_2S$: C, 49.88; H, 3.95 and N, 22.62; found C, 49.92; H, 4.70 and N, 22.64.

2-{[4-Amino-3-(4-fluorobenzyl)-4,5-dihydro-1H-1,2,4-triazol-5-one-1-yl]acetyl}-N-(4-fluorophenyl)hydrazinecarbothioamid (**5b**):

Yield 92 %, mp: 194-195 °C, IR (ν_{max}, cm^{-1}): 3339-3203 (3NH+NH_2), 1717 (triazole C=O), 1682 (thiosemicarbazide C=O), 1621 (C=N) and 1238 (C=S), ^{1}H NMR (DMSO-d_6, 200 MHZ) δ (ppm): 3.90 (s, 2H, benzyl CH_2), 4.48 (s, 2H, N-CH_2), 5.37 (s, 2H, NH_2); Ar-H: [7.11-7.24 (m, 4H), 7.30-7.41 (m, 4H)], 9. 68 (s, 1H, NH), 9.80 (s, 1H, NH) and 10. 31 (s, 1H, NH). ^{13}C NMR (DMSO-d_6, 50 MHZ): δ (ppm):30.25 (benzyl CH_2), 47.34 (N-CH_2), Ar-C: [115.51 (d, J_{CF}= 22.0 Hz, 2CH), 115.83 (d, J_{CF}= 21.0 Hz, 2CH), 128.88 (2CH), 131.43 (2CH), 132.50, 135.96, 161.05 (d, J_{CF}= 243.0 Hz, C), 161.79 (d, J_{CF}= 241.0 Hz, C)],147.74 (triazole C-3), 154.20 (triazole C-5), 167.28 (C=O) and 181.64 (C=S). Anal. Calcd. for $C_{18}H_{17}F_2N_7O_2S$: C,49.88; H,3.95 and N, 22.62; found C, 50.06; H, 4.07 and N, 22.58.

General procedure for the synthesis of 1,2,4-triazol-5-thione derivatives (6a, b)

Compound **5** (0.01 mol) was dissolved in 2 N NaOH solution and riflaxed for 3 h. The solution was cooled to room temperature and acidified to pH 3-4 with 37 % HCl. The precipitated solid was filtered, washed thoroughly with water, dried, and recrystallized from ethanol-water (2:1).

4-Amino-5-(2-fluorobenzyl)-2-{[4-(4-fluorophenyl)-4,5-dihydro-1H-1,2,4-triazol-5-thione-3-yl]methyl}-2,4-dihydro-3H-1,2,4-triazol-3-one (**6a**):

Yield 82 %, mp: 207-208 °C, IR(ν_{max}, cm^{-1}): 3360 (NH), 3263, 3178 (NH_2), 1693 (triazole C=O), 1605, 1588 (C=N) and 1230 (C=S), ^{1}H NMR (DMSO-d_6, 200 MHZ) δ (ppm): 3.82 (s, 2H, benzyl CH_2), 4.77 (s, 2H, N-CH_2), 5.10 (s, 2H, NH_2), 7.18-7.25 (m, 8H, Ar-H) and 14.21 (s, 1H, NH). ^{13}C NMR (DMSO-d_6, 50 MHZ): δ (ppm): 24.49 (benzyl CH_2), 40.84 (N-CH_2), Ar-C: [115.92 (d, J_{CF}= 21.5 Hz, 2CH), 116.83 (d, J_{CF}= 20.0 Hz, CH), 122.75 (d, J_{CF}= 15.5 Hz, C), 125. 08 (CH), 129.80 (d, J_{CF}= 8.0 Hz, CH), 130.82 (d, J_{CF}= 8.5 Hz, 2CH), 131.11, 131.96 (d, J_{CF}= 3.5 Hz, CH), 161.57 (d, J_{CF}= 241.5 Hz, C), 162.40 (d, J_{CF}= 240.5 Hz, C)], 147.27 (triazole C-3, second ring), 148.12 (triazole C-3), 153.63 (triazole C=O) and 168.88 (triazole C=S). Anal. Calcd. for $C_{18}H_{15}F_2N_7OS$: C, 52.04; H, 3.64 and N, 23.60; found C, 52.14; H, 3.61 and N, 23.52.

4-Amino-5-(4-fluorobenzyl)-2-{[4-(4-fluorophenyl)-4,5-dihydro-1H-1,2,4-triazol-5-thione-3-yl]methyl}-2,4-dihydro-3H-1,2,4-triazol-3-one (**6b**):

Yield 85 %, mp: 220-221°C, IR (ν_{max}, cm^{-1}): 3423 (NH), 3280, 3135 (NH_2), 1692 (triazole C=O), 1604, 1569 (C=N) and 1226 (C=S), ^{1}H NMR (DMSO-d_6, 200 MHZ) δ (ppm): 3.77 (s, 2H, benzyl CH_2), 4.73 (s, 2H, N-CH_2), 5.14 (s, 2H, NH_2), 7.09-7.23 (m, 8H, Ar-H) and 14.23 (s, 1H, NH). ^{13}C NMR (DMSO-d_6, 50 MHZ): δ (ppm): 30.09 (benzyl CH_2), 40.95 (N-CH_2), Ar-C: [115.84 (d, J_{CF}= 21.5 Hz, 2CH,), 116.60 (d, J_{CF}= 22.5 Hz, 2CH), 128.55 (2CH), 130. 87 (2CH), 131.38, 132.52, 161.81 (d, J_{CF}= 241.0 Hz, C), 162.59 (d, J_{CF}= 240.0 Hz, C)], 147.81 (triazole C-3, second ring), 147.99 (triazole C-3), 153.04 (triazole C=O) and 169.03 (triazole C=S). Anal. Calcd. for $C_{18}H_{15}F_2N_7OS$: C, 52.04; H, 3.64 and N, 23.60; found C, 52.10; H, 3.64 and N, 23.57.

General procedure for the synthesis of benzythio derivatives (7a, b)

Compound **6** (10 mmol) was refluxed with an equivalent amount of sodium in absolute ethanol for 2h. Then, 4-fluorobenzyl bromide (10 mmol) was added and stirred under reflux for 5 h, product formation was detected by TLC. After evaporating the solvent under reduced pressure, a solid

appered. The solid product was recrystallized from ethanol-water (1:1).

4-Amino-5-(2-fluorobenzyl)-2-{[4-(4-fluorophenyl)-5-(4-fluorobenzythio)-4H-1,2,4-triazol-3-yl]methyl}-2,4-dihydro-3H-1,2,4-triazol-3-one (**7a**):

Yield 71 %, mp: 132-133 °C, IR (ν_{max}, cm^{-1}): 3308, 3201 (NH_2), 1712 (triazole C=O), 1602 and 1589(C=N), ^{1}H NMR (DMSO-d_6, 200 MHZ) δ (ppm): 3.82 (s, 2H, benzyl CH_2), 4.30 (s, 2H, N-CH_2), 4.86 (s, 2H, S-CH_2), 5.20 (s, 2H, NH_2) and 7.05-7.37 (m, 12H, Ar-H). ^{13}C NMR (DMSO-d_6, 50 MHZ): δ (ppm):24.44 (d, J_{CF}=3.5 Hz, benzyl CH_2), 35.89 (S-CH_2), 41.41 (N-CH_2), Ar-C: [115.90 (d, J_{CF}= 25.5 Hz, 3CH), 117.20 (d, J_{CF}= 23.5 Hz, 2CH), 122.88 (d, J_{CF}= 16.0 Hz, C), 125. 06 (d, J_{CF}= 3.0 Hz, CH), 129.12 (d, J_{CF}= 3.0 Hz, CH), 129.72 (d, J_{CF}= 8.5 Hz, CH,), 130.11 (d, J_{CF}= 9.5 Hz, 2CH,), 131.68 (d, J_{CF}= 9.0 Hz, 2CH), 131.97 (d, J_{CF}= 4.5 Hz, C), 134.05 (d, J_{CF}= 3.0 Hz, C), 160.98 (d, J_{CF}= 243.0 Hz, C), 162.14 (d, J_{CF}= 242.5 Hz, C), 163.12 (d, J_{CF}= 246.0 Hz, C)], 146.97 (triazole C-3),151.58 (triazole C-3, second ring), 152.00 (triazole C-5, second ring) and 153.01 (triazole C=O). Anal. Calcd. for $C_{25}H_{20}F_3N_7OS$: C,57.36; H,3.85 and N, 18.73; found C, 57.57; H, 3.89 and N, 18.63.

4-Amino-5-(4-fluorobenzyl)-2-{[4-(4-fluorophenyl)-5-(4-fluorobenzythio)-4H-1,2,4-triazol-3-yl]methyl}-2,4-dihydro-3H-1,2,4-triazol-3-one (**7b**):

Yield 75 %, mp: 145-146 °C, IR (ν_{max}, cm^{-1}): 3333, 3278 (NH_2), 1707 (triazole C=O), 1602 and 1583(C=N), ^{1}H NMR (DMSO-d_6, 200 MHZ) δ (ppm): 3.77 (s, 2H, benzyl CH_2), 4.31 (s, 2H, N-CH_2), 4.88 (s, 2H, S-CH_2), 5.15 (s, 2H, NH_2) and 7.09-7.37 (m, 12H, Ar-H). ^{13}C NMR (DMSO-d_6, 50 MHZ): δ (ppm): 30.10 (d, J_{CF}= 3.5 Hz, benzyl CH_2), 35.88 (S-CH_2), 41.35 (N-CH_2), Ar-C: [115.81 (d, J_{CF}= 21.5 Hz, 2CH), 115.92 (d, J_{CF}= 21.5 Hz, 2CH), 117.22 (d, J_{CF}= 22.5 Hz,2CH), 129.10 (d, J_{CF}= 3.0 Hz, C), 130.04 (d, J_{CF}= 9.0 Hz, 2CH), 130.41 (d, J_{CF}= 8.0 Hz,2CH), 131.69 (d, J_{CF}= 8.5 Hz, 2CH), 132.46 (d, J_{CF}= 3.0 Hz, CH), 134.04 (d, J_{CF}= 3.0 Hz,C), 161.81 (d, J_{CF}= 241.0 Hz,C), 162.14 (d, J_{CF}= 242.5 Hz,C), 163.11 (d, J_{CF}= 247.0 Hz,C)], 147.98 (triazole C-3),151.63 (triazole C-3, second ring), 152.02 (triazole C-5, second ring) and 153.01 (triazole C=O). Anal. Calcd. for $C_{25}H_{20}F_3N_7OS$: C, 57.36; H, 3.85 and N; 18.73. found C, 57.42; H, 3.80 and N, 18.62.

General procedure for the synthesis of 1,3,4-oxadiazole derivatives (8a, b)

A mixture of compound **3** (10mmol), potassium hydroxide (10mmol), CS_2 (10mmol) and ethanol (50 mL) was heated under reflux for 3 h. The solution was then concentrate, cooled and acidified with dilute HCl. The solid formed was filtered, washed with water and recrystallized from ethanol to afford the desired compound.

4-Amino-5-(2-fluorobenzyl)-2-[(4,5-dihydro-1,3,4-oxadiazol-5-thione-2-yl)methyl]-2,4-dihydro-3H-1,2,4-triazol-3-one (**8a**):

Yield 73 %, mp: 217-218 °C, IR (ν_{max}, cm^{-1}): 3318, 3207 (NH_2), 2592 (SH), 1677 (triazole C=O) and 1628, 1581,1520 (C=N), ^{1}H NMR (DMSO-d_6, 200 MHZ) δ (ppm): 3.92 (s, 2H, benzyl CH_2), 5.04 (s, 2H, N-CH_2), 5.45 (s, 2H, NH_2), 7.14-7.29 (m, 4H, Ar-H) and 14.57 (bs, 1H, SH). ^{13}C NMR (DMSO-d_6, 50 MHZ): δ (ppm):24.57 (d, J_{CF}=3.5 Hz, benzyl CH_2), 40.72 (N-CH_2), Ar-C: [115.95 (d, J_{CF}= 21.5 Hz, CH), 122.78 (d, J_{CF}= 15.5 Hz, C), 125.13 (d, J_{CF}= 3.5 Hz,CH), 129.75 (d, J_{CF}= 8.0 Hz,CH), 131.93 (d, J_{CF}= 4.5 Hz,CH), 161.02 (d, J_{CF}= 243.5 Hz,C)], 147.96 (triazole C-3), 153.57 (triazole C=O), 159.82 (oxadiazole C-2) and 178.68 (oxadiazole C-5). Anal. Calcd. for $C_{12}H_{11}FN_6O_2S$: C, 44.72; H, 3.44 and N, 26.07; found C, 44.78; H, 3.40 and N, 25.93.

4-Amino-5-(4-fluorobenzyl)-2-[(4,5-dihydro-1,3,4-oxadiazol-5-thione-2-yl)methyl]-2,4-dihydro-3H-1,2,4-triazol-3-one (**8b**):

Yield 75 %, mp: 223-224 °C, IR (ν_{max}, cm^{-1}): 3313, 3206 (NH_2), 2590 (SH), 1681 (triazole C=O) and 1604, 1584, 1510 (C=N), ^{1}H NMR (DMSO-d_6, 200 MHZ) δ (ppm): 3.89 (s, 2H, benzyl CH_2), 5.05 (s, 2H, N-CH_2), 5.38 (s, 2H, NH_2), 7.07-7.16 (m, 2H, Ar-H), 7.26-7.33 (m, 2H, Ar-H) and 14.63 (bs, 1H, SH). ^{13}C NMR (DMSO-d_6, 50 MHZ): δ (ppm):30.19 (benzyl CH_2), 40.70 (N-CH_2), Ar-C: [115.87 (d, J_{CF}= 21.5 Hz,2CH), 131.39 (d, J_{CF}= 8.0 Hz,2CH), 132.37 (d, J_{CF}= 3.0 Hz, C), 161.81 (d, J_{CF}= 241.5 Hz, C)], 148.93 (triazole C-3), 153.60 (triazole C=O), 159.84 (oxadiazole C-2) and 178.65 (oxadiazole C-5). Anal. Calcd. for $C_{12}H_{11}FN_6O_2S$: C, 44.72; H, 3.44 and N, 26.07; found C, 44.80; H, 3.42 and N, 25.97.

General procedure for the synthesis of Mannich bases (9a, b)

To 20 mL dimethyl formamide was added appropriate triazole (**8a** and **8b**) (10 mmol), formaldehyde (40%, 1.5 mL) and 4-(trifluoromethoxy)aniline (10 mmol), the reaction mixture was stirred 6 h, then distilled water was added and allowed to stand overnight in refrigerator. The precipitate so obtained was filtered, washed with cold waterand recrystallized from ethanol-water (1:2).

4-Amino-5-(2-fluorobenzyl)-2-({4-[(4-trifluoromethoxyphenylamino)methyl]-5-thioxo-1,3,4-oxadiazol-2-yl}methyl)-2,4-dihydro-3H-1,2,4-triazol-3-one (**9a**):

Yield 38 %, mp: 114-115 °C, IR (ν_{max}, cm^{-1}): 3364, 3280 (NH_2), 3065 (NH), 1714 (triazole C=O), 1609, 1585, 1511 (C=N), 1259 (OCF_3) and 1233 (C=S), ^{1}H NMR (DMSO-d_6, 200 MHZ) δ (ppm): 3.96 (s, 2H, benzyl CH_2), 4.70 (s, 2H, N-CH_2), 5.07 (bs, 1H, NH), 5.27 (s, 2H, NH_2), 5.69 (bs, 2H, N-CH_2-NH-), 6.97-7.14 (m, 3H, Ar-H), 7.17-7.29 (m, 2H, Ar-H) and 7.47-7.80 (m, 3H, Ar-H). ^{13}C NMR (DMSO-d_6, 50 MHZ): δ (ppm): 24.90 (d, J_{CF}=3.5 Hz, benzyl CH_2,), 47.132 (N-CH_2), 56.74 (N-CH_2-NH-), 122.52 (q, J_{CF}= 270.5 Hz, OCF_3), Ar-C: [115.95 (d, J_{CF}= 21.5 Hz, CH), 118.18 (2CH), 122.54 (2CH), 123.27, 125.09, 129.80, 131.88 (d, J_{CF}= 3.5 Hz, C), 132.12, 136.75, 161.88 (d, J_{CF}= 241.0 Hz, C)], 147.29 (triazole C-3), 153.46 (triazole C=O), 157.73 (oxadiazole C-2) and 178.74 (oxadiazole C-5). Anal. Calcd. for $C_{20}H_{17}F_4N_7O_3S$: C, 46.97; H, 3.35 and N, 19.17; found C, 47.14; H, 3.28 and N, 18.93.

4-Amino-5-(4-fluorobenzyl)-2-({4-[(4-trifluoromethoxyphenylamino)methyl]-5-thioxo-1,3,4-oxadiazol-2-yl}methyl)-2,4-dihydro-3H-1,2,4-triazol-3-one (**9b**):

Yield 32 %, mp: 129-130 °C, IR (ν_{max}, cm^{-1}): 3353, 3274 (NH_2), 3117 (NH), 1718 (triazol C=O), 1614, 1581, 1512 (C=N), 1259 (OCF_3) and 1233 (C=S), ^{1}H NMR (DMSO-d_6, 200 MHZ) δ (ppm): 3.46 (s, 2H, benzyl CH_2), 4.65 (s, 2H, N-CH_2), 5.10 (bs, 1H, NH), 5.40 (s, 2H, NH_2), 5.70 (bs, 2H, N-CH_2-NH-), 6.73-7.46 (m, 6H, Ar-H) and 7.60-7.76 (m, 2H, Ar-H). ^{13}C NMR (DMSO-d_6, 50 MHZ): δ (ppm): 30.55 (ben-

zyl CH_2), 47.36 (N-CH_2), 62.24 (N-CH_2-NH-), 123.87 (q, J_{CF}= 271.0 Hz, ,OCF_3), Ar-C: [115.77 (d, J_{CF}= 21.0 Hz, 2CH), 122.24 (2CH), 126.99 (2CH), 136.76, 128.35 (d, J_{CF}= 8.0 Hz, 2CH), 131.32 (d, J_{CF}= 3.0 Hz, C), 131.72, 161.85 (d, J_{CF}= 240.0 Hz, C)], 147.10 (triazole C-3), 152.89 (triazole C=O), 157.83 (oxadiazole C-2) and 177.13 (oxadiazole C-5). Anal. Calcd. for $C_{20}H_{17}F_4N_7O_3S$: C, 46.97; H, 3.35 and N, 19.17; found C, 46.73; H, 3.52 and N, 18.83.

Biological assays

Antioxidant Activity

In this study, the antioxidant activities of the synthesized compounds were determined using Cupric reducing antioxidant capacity assay, radical scavenging activities of the synthesized compounds ABTS (2,2-azinobis(3- ethylbenzothiazoline-6-sulfonic acid) and DPPH (1,1- diphenyl-2-picrylhydrazyl) systems.

Cupric reducing antioxidant capacity (CUPRAC) assay

The cupric reducing antioxidant capacity of the synthesized compounds was determined according to the method of Apak *et al.*[37] To a test tube 1 mL each of 10 mM Cu(II) chloride (Sigma Chemical Co, USA), 7.5 mM neocuprine (Sigma Chemical Co, USA), and NH_4Ac (Fluka Chemical Co., Switzerland buffer (1 M, pH 7.0) solutions were added. About 5 µL of compound solution in DMSO and 1.095 mL of water were added to the initial mixture so as to make the final volume 4.1 mL. The tubes were stoppered, and after 30 min, the absorbance at 450 nm was recorded against a reagent blank containing no compound. Trolox® (Sigma Chemical Co, USA) was also tested under the same conditions as a standard antioxidant compound. The standard curve was linear between 8 mg/mL and 0.125 mg/mL trolox (r^2=0.998). CUPRAC values were expressed as mg Trolox equivalent of 1 mg synthesized compound.

DPPH-Free radical scavenging assay

The 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical has been used widely in the model system to investigate the scavenging activities of several synthesized and natural compounds. The DPPH radical scavenging activity of the synthesized compounds was measured using the method of Brand-Williams.[38] Briefly, 0.1 mM DPPH (2,2-diphenyl-1-picrylhydrazyl, Aldrich-Germany) was prepared in methanol. 1200 microliters of this solution was added 300 microliters of the sythesized compound solution in DMSO. After, in the dark for 50 min incubation period at room temperature, the decrease in absorbance at 517 nm was measured, using a UV-Visible spectrophorometer (1601UV-Shimadzu, Australia). All determinations were carried out three times. Radical scavenging activity was measured by using ascorbic acid (AA), butylated hydroxy toluene (BHT) and catechin (Sigma Chemical Co, USA) as standards and all values are expressed as SC_{50} (µg compound per mL), the concentration of the samples that causes 50% scavenging of DPPH radical. The DPPH radical stock solution was prepared fresh daily.

$ABTS^{\bullet+}$ Radical Cation Decolorization Assay

The ability of the synthesized compound to scavenge $ABTS^{\bullet+}$ radical was determined according to the literature.[39] ABTS [2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid)] was dissolved in water to a 7 mM concentration. ABTS (Sigma Chemical Co, USA) radical cation ($ABTS^{\bullet+}$)was produced by reacting ABTS stock solution with 2.45 mM potassium persulfate (Sigma Chemical Co, USA), (final concentration) and allowing the mixture to stand in the dark for 16–18 h at room temperature. Before usage, the ABTS solution was diluted to get an absorbance of 0.700 ± 0.020 at 734 nm with PBS at pH 7.4. The compound solution of 200 µL was added to 1800 microliters of the resulting blue-green $ABTS^{\bullet+}$ radical solution. After, incubation 5 min in the dark at room temperature, the decrease of absorbance at 734 nm was measured by using a UV-Visible spectrophorometer (1601UV-Shimadzu, Australia). All determinations were carried out three times. ABTS radical scavenging activity was measured by using ascorbic acid, catechin and bht (Sigma Chemical Co, USA) as standards and the percentage scavenging was calculated from the formula

$$\% \text{ Scavenging} = [(OD_{control}\text{-}OD_{test})/(OD_{control})x100].$$

In vitro anti-xanthine oxidase assay

The inhibition of xanthine oxidase (XO) was measured by UV spectroscopy technique at 295 nm which attributes to released uric acid from xanthine. The inhibitory activity of each compound was determined using a slight modification of the reference methods.[40-43] Briefly, the reaction mixture consisted of 500 µL of the test compound solution in DMSO, 770 µL of phosphate buffer (pH 7.8) and 0.07 mL of bovine milk XO (Sigma–Aldrich, St. Louis, USA), which was prepared immediately before use. After preincubation at 25º C for 15 minutes the reaction was initiated by the addition 660 µL mL of substrate solution into the mixture. The assay mixture was incubated at 25º C for 15 min. The reaction was stopped by adding 200 µL of 0.5 N HCl and the absorbance was measured at 295 nm using UV/vis spectrophotometer (1601UV-Shimadzu, Australia).

A well known XO inhibitor (XOI), allopurinol (Sigma–Aldrich, St. Louis, USA) was used as a positive control for the inhibition test. The assay was done in triplicate. XO activity was expressed as percent remaining activity of XO, calculate as [(B/A) x 100], where A is the change in absorbance of the assay without the test samples. (Δ abs with enzyme -Δ abs without enzyme), and B is the change in absobance of the assay with the test sample (Δ abs with enzyme - Δ abs without enzyme). The assay was done in triplicate. The IC_{50} value was determined as the concentration of compound that give 50% inhibition of maximal activity.

Urease inhibition assay

Urease is an enzyme that caproductiontalyzes the hydrolysis of urea into carbon dioxide and ammonia. The production of ammonia was measured by indophenol method and used to determine the urease inhibitory activity.[44] Reaction mixtures including 100 µL of Jack Bean Urease, 400 µL of buffer (100 mM urea, 0.01 M K_2HPO_4, 1 mM EDTA and 0.01 M LiCl, pH 8.2) and 500 µL of the test compound solution in ethanol were incubated with at room temperature for 15 min. The phenol reagent (500 µL, 1% w/v phenol and 0.005% w/v sodium nitroprusside) and alkali reagent (500 µL, 0.5% w/v sodium hydroxide and 0.1% v/v NaOCl) were added to each tube and the increasing absorbance at 625 nm was measured after 50 min, using a UV/vis spectrophotometer (1601UV-Shimadzu, Australia). The percentage inhibition was calculated from the formula % Inhibition = $[(OD_{control}\text{-}OD_{test})/(OD_{control})x100]$. Thiourea was used as the standard inhibitor. In order to calcu-

late IC_{50} values, different concentrations of synthesized compounds and standard were assayed at the same reaction conditions.

RESULTS AND DISCUSSION

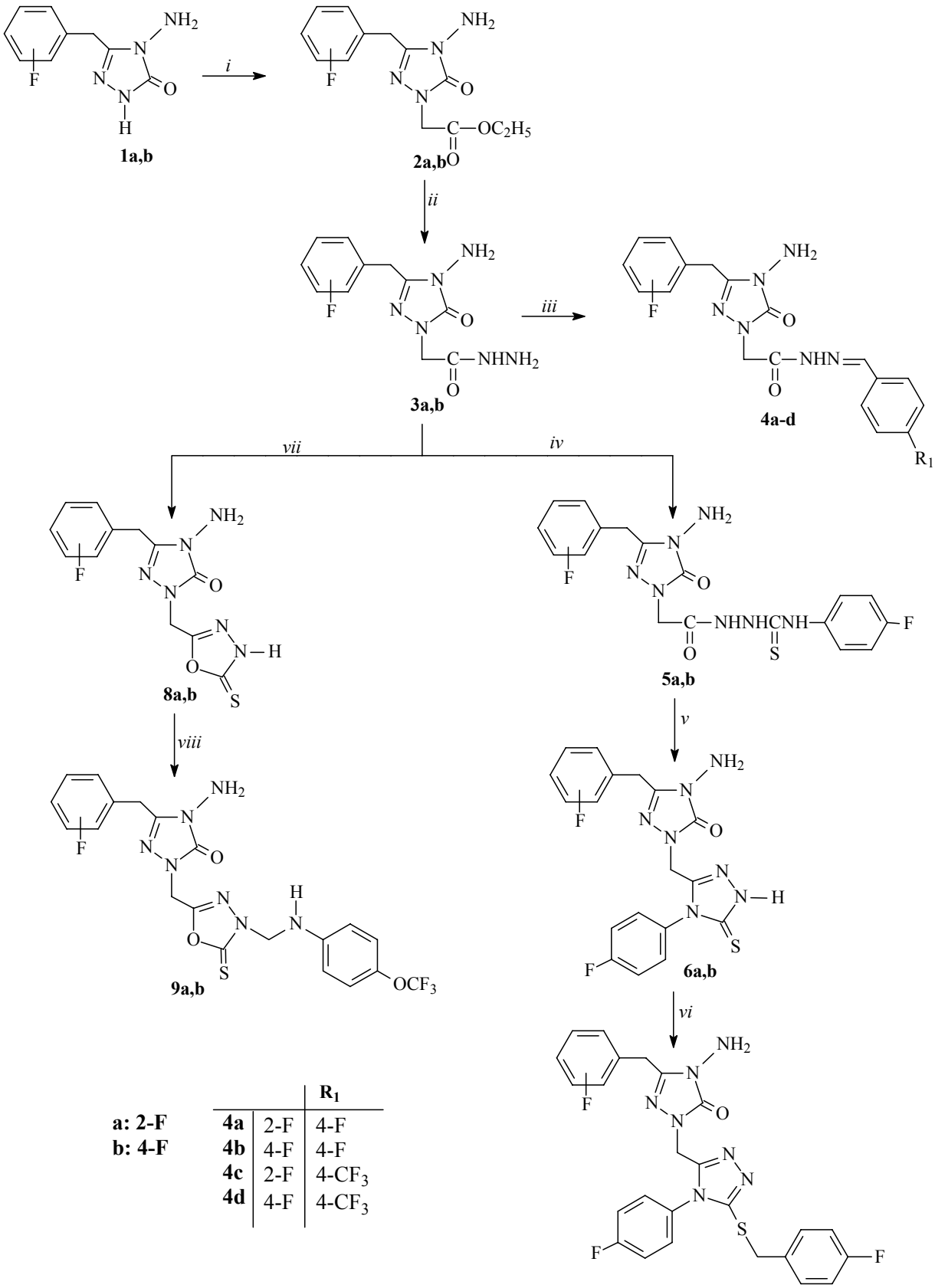
The synthetic routes to the target compounds **2-7** are illustrated in Scheme 1. Initially, the key intermediates, 3-(2-fluorobenzyl)-4-amino-4,5-dihydro-1H-1,2,4-triazole-5-one (**1a**) and 3-(4-fluorobenzyl)-4-amino-4,5-dihydro-1H-1,2,4-triazole-5-one (**1b**), were prepared following a previously reported literature procedure.[45] Reaction of compounds **1a, b**, with ethyl bromoacetate in the presence of sodium ethoxide in anhydrous ethanol afforded ethyl acetate derivatives (**2a, b**). These compounds treatment with hydrazine hydrate in 1-butanol yielded corresponding acid hydrazides (**3a, b)**. The compounds **3a, b** reacted with some aldehydes in ethanol afforded the corresponding methylidene acetohydrazide derivatives (**4a-d)**. In the IR spectra of compounds **4a-d**, we observed the bands at around 3330 cm^{-1}(NH), 3267 and 3205 cm^{-1} (triazole NH_2), 1720 cm^{-1} (triazole C=O) and 1695 cm^{-1} (hydrazide C=O). Furthermore, In the ^{1}H NMR spectra, the hydrazide $–NH_2$ protons signals (at around 4.24 ppm) was absent and triazole NH_2 protons observed at about 5.34 ppm. This observation showed that only hydrazide $–NH_2$ of compounds **3a, b** were reacted to aldehydes in the reaction conditions, although this compounds include 2 NH_2 groups in the structure. On the other hand, In the ^{1}H NMR spectra of compounds **4a-d**, NCH_2 and N=CH proton signals and NCH_2, triazole C-3, triazole C-5 and N=CH carbon signals in the ^{13}C NMR spectrum were recorded as double peaks. Because, methylidene acetohydrazide derivativesmay exist as *E*/*Z* geometrical isomers about C=N double bond and *cis*/*trans* amide confermers at the CO-NH single bond (Scheme 2)[46-50].

According to the literature, compounds containing an N=CH double bond restricts rotation and gives rise to the formation of *E* and *Z* isomers with the *E* isomer dominating, and that the equilibration rate is rather low. As is known, when methylidene acetohydrazide derivativesare dissolved in polar solvents (such as dimethyl-d_6 sulfoxide), the geometrical *E* isomers of these compounds undergo a rapid *cis*/*trans* amide equilibrium, in which the *trans* conformer predominates. The *E* isomers and the *cis*/*trans* confermer ratios can easily be determined by ^{1}H NMR integration. In the current study, the chemical shift values of *cis*/*trans* conformers belonging to protons of NCH_2 and N=CH in the ^{1}H NMR and to carbons of NCH_2, triazole C-3, triazole C-5 and N=CH in the ^{13}C NMR spectra of compounds **4a-d** and the percentage ratios of *cis*/*trans* conformers are given in Table 1. These data prove the *E* isomers and *trans* confermer structures (**I**) to be dominant forms among the four possible structures[46-50].

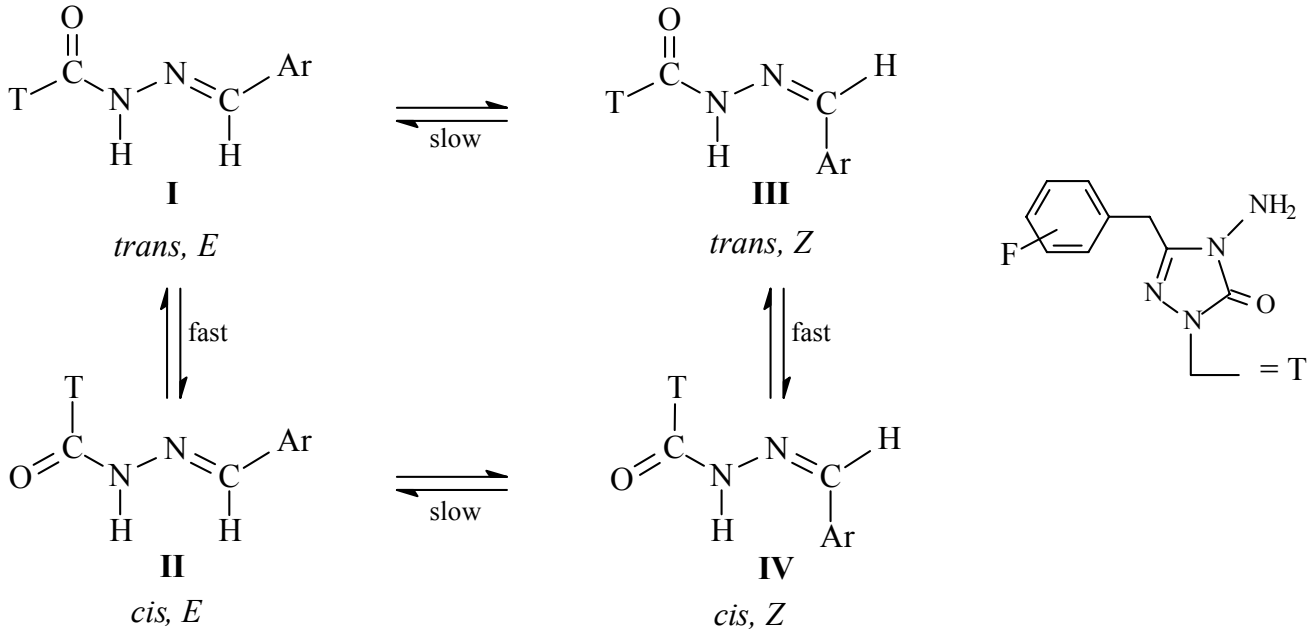
While *trans* conformer structures of the proton signals of N=CH are found in upfield, their *cis* conformer structures in downfield in the ^{1}H NMR spectra of compounds **4a-d**. In contrast, *trans* conformer structures of the protons of N-CH_2 are found in downfield, while their *cis* conformer structures in upfield in the ^{1}H NMR spectra, because of steric hindrance.[46-50]

Carbothioamide derivatives (**5a, b**) were obtained by the reaction of compounds **3a, b** with 4-fluorophenylisothiocyanate. The cyclization of compounds **5a, b** in the presence of 2N NaOH resulted in the formation of the 1,2,4-triazole-5-thiones (**6a,b**). Furthermore, reaction of hydrazides (**3a, b**) with CS_2 in the presence of aqueous KOH afforded the 1,3,4-oxadiazole-5-thiones (**8a,b**). The compounds **6a-b** and **8a-b** may exist in thiole and thione tautomeric forms[51]. According to the IR spectroscopic data of the compounds **6a**, **b** which have thione structure, the presence of C=S and N-H stretching bands at 1230 cm^{-1} and 3423 cm^{-1}, respectively and the absence of an absorption about in 2600-2550 cm^{-1} region for S-H group have proved that these compounds predominantly exist, in solid state, in the tautomeric thione form.[52-53] However, **8a**, **b** have thiole structure, the presence of S-H bands at 2590 cm^{-1}, and the absence of an absorption about 3100-3400 cm^{-1} region for N-H group have proved that these compounds predominantly exist, in solid state, in the thiole form.[43]

Compounds **6a, b** were converted to their corresponding S-(4-fluorobenzyl) derivatives (**7a, b**) by the reaction with 4-fluorobenzyl bromide in presence of sodium ethoxide. On the other hand, reaction of hydrazides (**3a, b**) with CS_2 in the presence of aqueous KOH afforded the 1,3,4-oxadiazole-5-thiones (**8a, b**). These compounds (**8a, b**) reacted with formaldehyde and 4-(trifluoromethoxy) aniline in DMF medium to give N-Mannich bases (**9a, b**).



Scheme 1 – Reagent and conditions: *i*: OEt/EtOH, $BrCH_2CO_2Et$, reflux; *ii*: H_2NNH_2/1-butanol, reflux; *iii*: Ar-CHO/EtOH, reflux; *iv*: 4-FC_6H_5-NCS isothiocyanate/EtOH, reflux; *v*: NaOH (2N), reflux, *vi*: 4-FC_6H_5-Br, OEt/EtOH, reflux; *vii*. CS_2, KOH, EtOH, reflux; *viii:* 4-$OCF_3C_6H_4$-NH_2 aniline, HCOH, DMF.



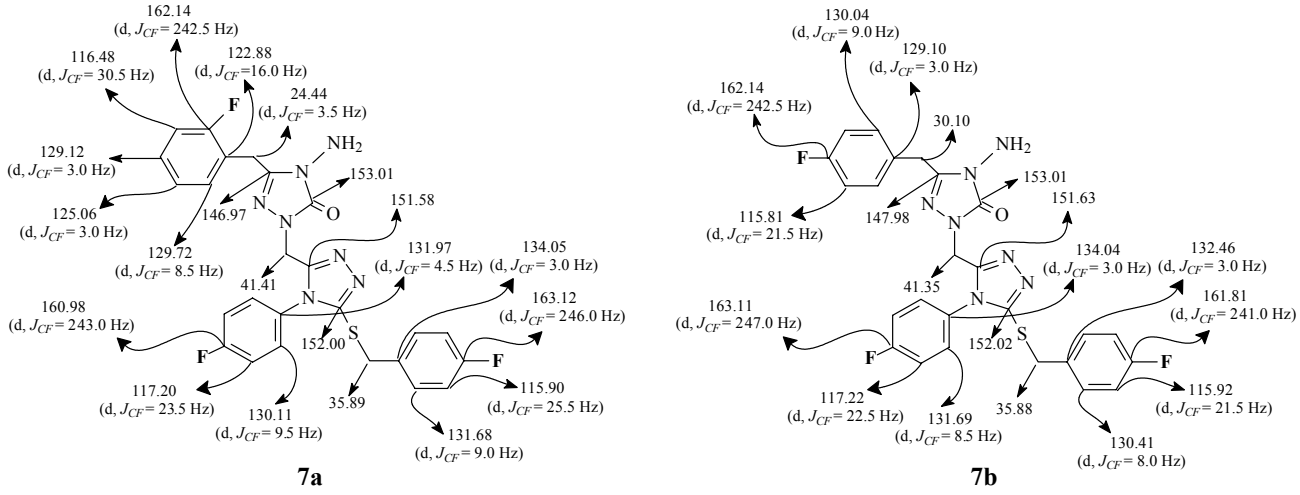
Scheme 2 – *E/Z* geometrical isomers and *cis*/*trans* amide conformers for compound **4**.

Table 1

Determination of chemical shifts and percentage of *cis*/*trans* conformers in ^{1}H NMR and ^{13}C NMR spectra at 200 MHz (in DMSO-d_6)

Comp.	Confor.	NMR	NCH_2	N=CH	Triazole C_3	Triazole C_5	Ratio (*trans*/*cis*)
4a	***trans***	H	4.83	7.99	-	-	% 70.94
		C	47.233	143.57	146.50	154.38	
	cis	H	4.42	8.19	-	-	% 29.06
		C	47.66	146.84	146.74	163.85	
4b	***trans***	H	4.85	7.99	-	-	% 71.78
		C	47.22	143.58	147.48	154.41	
	cis	H	4.43	8.17	-	-	% 28.22
		C	47.66	146.88	147.73	164.20	
4c	***trans***	H	4.87	8.06	-	-	% 72.20
		C	47.28	143.06	146.57	154.39	
	cis	H	4.46	8.26	-	-	% 27.80
		C	47.71	146.29	146.81	164.17	
4d	***trans***	H	4.87	8.07	-	-	% 73.15
		C	47.25	143.07	147.55	153.41	
	cis	H	4.46	8.27	-	-	% 26.85
		C	47.72	146.28	147.77	164.20	

Moreover, in the current study, in the ^{13}C NMR the aromatic carbons containing fluoro atom appear as doublets in the region 115-165 ppm due to C-F coupling. Because, ^{19}F has a nuclear spin of 1/2. This means that carbon signals are split into n+1 parts. Chemical shifts and J_{CF} coupling constants for **7a** and **7b** compunds bearing three fluorinated aromatic ring were given as examples in Scheme 3.



Scheme 3 – ^{13}C-NMR chemical shiftsand J_{CF} coupling constants for compounds **7a** and **7b**.

CUPRAC Antioxidant Activity Assay

The CUPRAC method, is based on the absorbance measurement of the CUPRAC chromophore, Cu(I)-neocuproine (Nc) chelate, formed as a result of the redox reaction of antioxidants with the CUPRAC reagent, bis(neocuproine)copper(II) cation [Cu(II)-Nc], where absorbance is recorded at the maximal light absorption wavelength of 450 nm. The orange–yellow colour is due to the Cu(I)-Nc charge-transfer complex formed.[37] The maximum antioxidant capacity in the CUPRAC method was observed for compound **9a.** According to the CUPRAC results, compounds **3a, 3b, 6b, 8a, 8b** and **9b** were evaluated as more dominant activity than **5a, 5b** and **6a** (Fig. 1). The compounds **2a, 2b, 4a, 4b, 4c, 4d, 7a** and **7b** exhibited very little antioxidant capacity in the CUPRAC method.

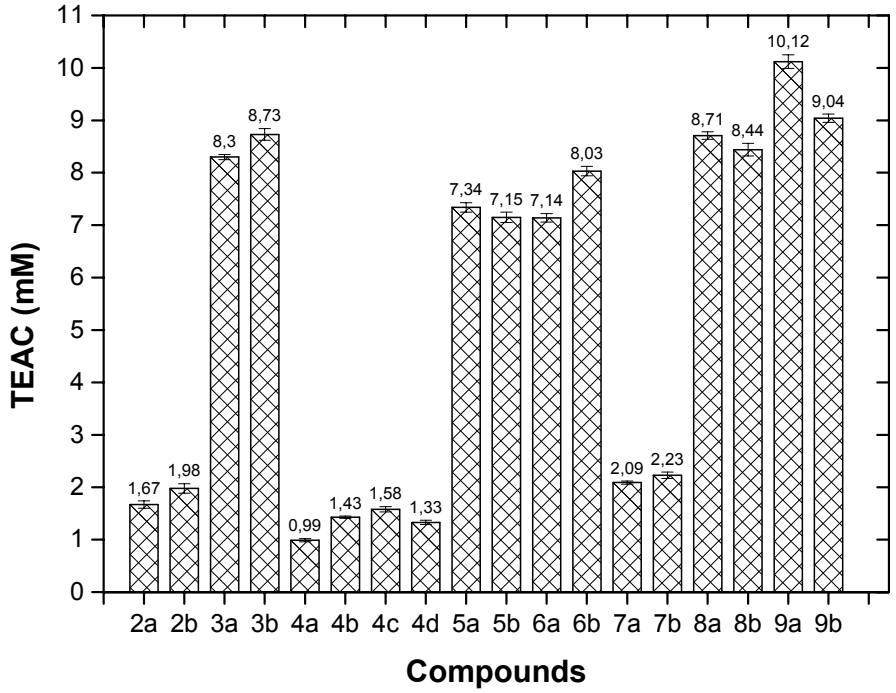


Fig. 1 – CUPRAC test results of all the synthesized compounds as mM TEAC (Trolox equivalent antioxidant capacity) values obtained from [Trolox]- absorbance calibration graph (r^2=0.998). CUPRAC values of compounds are expressed as the mean ±SD in triplicate.

DPPH Scavenging Assay

The DPPH method is based on the fact that the free radical is purple in color, and that the purple color of DPPH decays in the presence of an antioxidant. The color changed from purple to yellow after reduction, which can be quantified by its decrease of absorbance at wavelength 517 nm. The results were expressed as SC_{50} (μg/mL), which was calculated from the curves by plotting absorbance values, the SC_{50} values representing the concentration of the compound (μg/mL) required to inhibit 50% of the radicals. When the CUPRAC and DPPH scavenging activity methods were correlated by each others, compounds **9a-9b** could be seen as an efficient samples. On the other hand, the compounds **3a, 6b, 8a** and **8b** showed moderate activity, while the compounds **5b** and **6a** had weak activity (Figure 2). Because of the compounds **2a, 2b, 4a, 4b, 4c, 4d, 7a** and **7b** exhibited very little radical scavenging activity in the DPPH and ABTS methods, not depicted in Fig. 2.

$ABTS^{\bullet+}$ Radical Scavenging Activity

The pre-formed radical monocation of 2,2′-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid) ($ABTS^{\bullet+}$) is generated by oxidation of ABTS with potassium persulfate and is reduced in the presence of such hydrogen-donating antioxidants. The compounds **3a, 3b, 5a, 5b, 6b, 8a, 8b, 9a** and **9b** showed more efficient scavenging activity than bht and ascorbic acid, at concentration of 5.0 μg/mL. Also, compound **6a** exhibited very good scavenging activity at the same concentration (Fig. 3).

In vitro anti-xanthine oxidase activity results

All the synthesized compounds were evaluated with regard for bovine milk xanthine oxidase activity. The results had shown that the compound **4a** had good activity to inhibit XO up to 92.13 % at concentration of 31.25 μg/mL (Table 2). Among the synthesized compounds, **4a** displayed the best inhibitory effect against XO with an IC_{50} value of 36.37±0.11 μM. Also, compound **4c** exhibited good inhibition activity. The compounds **4b** and **4d** showed moderate XO enzyme inhibition activity. IC_{50} values of Allopurinol and the compound **4a** were calculated as 4.11±0.07 and 36.37±0.11 μM, respectively. The synthesized the compounds **4a** and **4b** can be evaluated as a good alternative to Allopurinol.

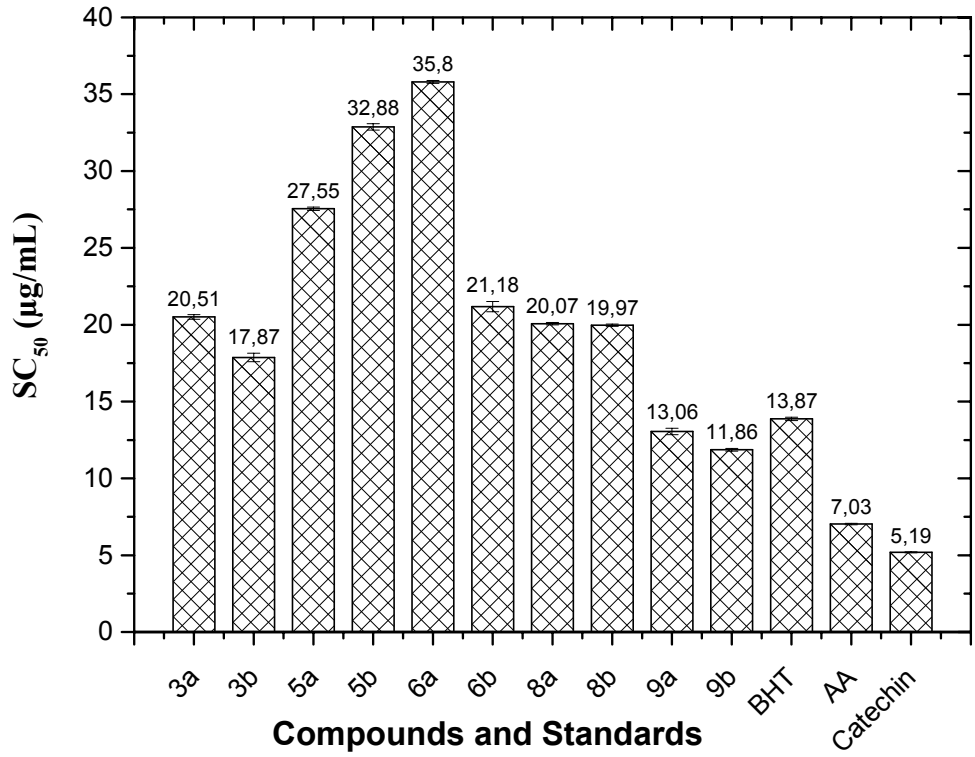


Fig. 2 – SC_{50} values to DPPH method of synthesized compounds and standards. SC_{50} values of compounds and standards are expressed as the mean ±SD in triplicate.

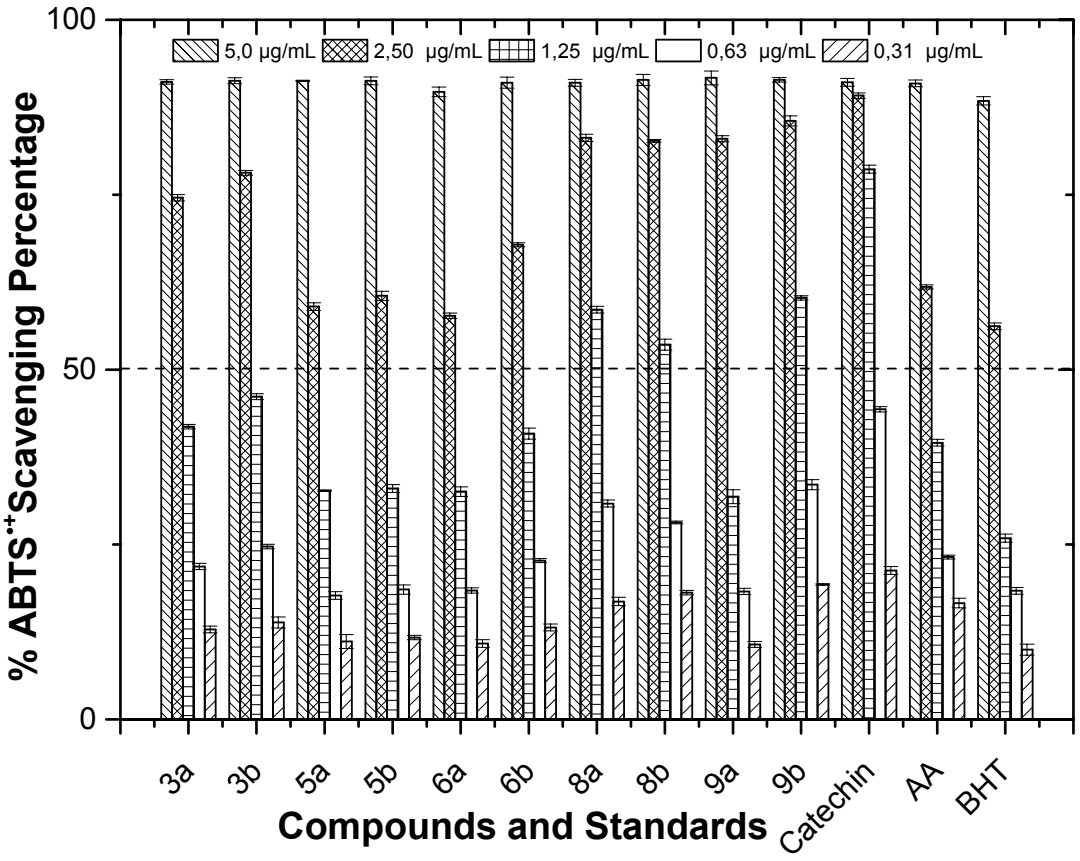


Fig. 3 – $ABTS^{\bullet+}$ Radical scavenging activity values of the synthesized compounds and standards.
% $ABTS^{\bullet+}$ scavenging percentage of compounds and standards are expressed as the mean ±SD in triplicate.

Table 2

Results of % remaining XO activity and IC_{50} values of the synthesized chemical compounds

Compounds	(31.25 µg/mL)	IC_{50} (µM)
Control	100.00	-
4a	7.87±0.21	36.37±0.11
4b	14.62±0.37	45.09±0.09
4c	10.94±0.23	37.03±0.35
4d	27.87±0.18	39.37±0.23
Allopurinol	0.00 ±0.00	4.11±0.07

Control, bovine milk xanthine oxidase without inhibitor; Allopurinol, positive control. Residual activities of compounds are expressed as the mean ±SD in triplicate.

Table 3

Inhibitory activities and IC_{50} values of the synthesized compounds against Jack Bean urease. Thiourea, positive control. % Inhibition values of compounds are expressed as the mean ±SD in triplicate

% Inhibition urease activity		
Compound No	(43.75 µg/mL)	IC_{50} (µM)
5a	87.21±0.61	35.23±0.19
5b	92.64±0.59	48.58±0.30
6a	74.07±0.23	70.69±0.55
6b	73.86±0.11	50.57±0.25
8a	94.29±0.31	49.67±0.17
8b	97.86±1.09	48.49±0.21
9a	97.14±0.27	29.34±0.36
9b	98.43±0.28	28.89±0.11
Thiourea	97.57±0.41	63.72±0.23

Anti-urease activity results

The synthesized compounds were assayed for their in vitro inhibitory activity against Jack Bean urease. Inhibitory effect of compounds and thiourea were measured at the range of 43.75 to 2.73 µg/mL concentrations. Thiourea with IC_{50} value 63.72±0.23 µM was used as standard inhibitor. Among the synthesized compounds, **9b** exhibited the best inhibitory effect against urease with IC_{50} value 28.89±0.11 µM (Table 3). Compound **9b** inhibited urease activity by 36.07±0.41%, 68.29±0.09% and 98.43±0.28 at concentrations of 10.94, 21.87 and 43.75 µg/mL, respectively. Furthermore, compound **9a** showed very good inhibition activity. These compounds, such as **9a** and **9b** can be considered as potential antibiotics to treat helicobacter pylori infections. On the other hand, compounds **5a**, **5b**, **6a**, **6b**, **8a** and **8b** demonstrated moderate urease inhibitory properties.

CONCLUSION

As the current, clinically used urease and xanthine oxidase inhibitor drugs have side-effects.[7,13-15] So, there is a need to investigate the new urease and XO inhibitors with good bioavailability and low toxicity. For this purpose, novel fluorinated 1,2,4-triazole-5(3)-on derivatives have been synthesized. The newly synthesized compounds were tested in vitro for antioxidant activity and, urease and XO inhibitory activities. The results of this study revealed that fluorinated 1,2,4-triazole-5-on derivatives can be used explored as anti-XO, anti-urease and antioxidant molecules.

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REFERENCES

1. J. Nordberg, E. S. J. Arnerand and K. J. A. Davies, *Free Rad. Biol. Med.,*2001, *319,*1287-1312.
2. K. J. Davies, *Bio Chem. Soc. Symp.,*1995, *61*, 1-31.
3. S.Vertuani, A. Angustiand and S. Manfredini, *Curr. Pharm. Des.,* 2004, *10*, 1677-1694.
4. C. P. Witte, S. A. Tiller, M. A. Taylorand and H. V. Davies, *Plant. Physiol.,* 2002, *128*, 1129-1136.
5. C. Montecuccoand and R. Rappuoli, *Nat. Rev. Mol. Cell. Biol.,* 2001, *2*, 457-466.
6. L. E. Zonia, N. E. Stebbins and J. C. Polacco, *Plant. Physiol.,* 1995, *107*, 1097-1103.
7. C. M. Collinsand and S. E. F. D-Orazio, *Mol. Microbiol.,* 1993, *9*, 907- 913.
8. C. Montecuccoand and R. Rappuoli, *Nat. Rev. Mol. Cell. Biol.,* 2001, *2*, 457-466.
9. R. P. Hausinger, P. A. Karplus, A. Messerschmidt, R. Hubber, T. Poulos and K.Wieghardt (Eds.), in "Handbook of Metalloproteins", Wiley: Chichester, 2001.
10. P.Kosikowska and L. Berlicki, *Expert. Opin. Ther. Pat.,* 2011, *26*, 945-957.
11. T.Hatano, T.Yasuhara, R.Yoshiharara, J.Agata, T. Noro and T.Okuda, *Chem. Pharm. Bull.,* 1990, *38*, 1224-1229.
12. V. L. Starand M. C. Hochberg,*Drugs,* 1993, 45, 212-22.
13. P. O. Fritsch and A. Sidoroff, *Am. J. Clin. Dermatol.,* 2000, *1*, 349-360.
14. H. Horiuchi, M. Ota, S. Nishimura, H. Kaneko, Y. Kasahara and T. Ohta, *Life Sci.,* 2000, *66*, 2051-2070.
15. S. Pereira, J. Almeida, A. O. Silva, M. Quintas, O. Candeias and F. Freitas, *Acta Med. Port.,* 1998, *11*, 1141-1144.
16. M. Clemons, R. E. Coleman and S. Verma, *Cancer Treat. Rev.,* **2004**, *30*, 325-332.
17. R. Fringuelli, F. Schiaffella and F. Bistoni, et al., *Bioorg. Med. Chem.,***1998**, *6*, 103-108.
18. T. C. Jenkins, I. J. Stratford and M. A. Stephens, *Anticancer Drug Des.,***1989**, *4*, 145-160.
19 C. G. H. Dahlof, A. M. Rapoport, F. D. Sheftell and C. R. Lines, *Clin.Ther.,***1999**, *21*, 1823-1836.
20. G. Griebel, D. C. Blanchard, A. Jung and J. C. Lee, *Neuropharmacology,***1995**, *34*, 1625-1633.
21. P. Shah and A. D.Westwell, *J. Enzym. Inhib. Med. Chem.,* **2007**, *22*, 527-540.
22. A. Sarro De, E. Camera La and M. T. Fera, *J. Chemotherapy.,* **2008**, *20*, 51-61.
23. C. C. Tang, Y. C. Li, B. Chen, H. Z. Yangand G. Y. Jin, "Pesticide Chemistry", 1st edition, Nankai University Publishing House, Tianjing, China, 1998.
24. V. K. R. Sahu, A. K. Singh and D. Yadav, *Int. J. Chem. Tech. Res.,* **2011**, *3*, 1362-1372.
25. J. Boström, A. Hogner, A. Llinàs, E. Wellner and A. T. Plowright, *J. Med. Chem.,* **2012**, *55*, 1817-1830.
26. K. C. Nagaraj, M. S. Niranjan and S. Kiran, *Int. J. Pharm. Pharm. Sci.,* **2011**, *3*, 9-16.
27. N. D. James and J. W. Growcott, *Drugs Future,***2009**, *34*, 624-633.
28. A. A.Savarino, *Expert Opin. Investig Drugs,* **2006**, *15*, 1507-1522.
29. S. Vardan, H. Smulyan, S. Mookherjee and R. Eich, *Clin. Pharmaco.lTher.,***1983**, *34*, 290-296.
30. R.Schlecker and P. C. Thieme, *Tetrahedron,***1988**, *44*, 3289-3294.
31. M. Ogata, H. Atobe, H. Kushi-Da and K. Yamamoto, *J. Antibiot.,***1971**, *24*, 443-451.
32. P. Majumdar, A. Pati, M. Patra, R. K. Behera and A. K. Behera*, Chem.Rev.,***2014**, *114*, 2942-2977.
33. G. A. Gazieva and A. N. Kravchenko, *Russ. Chem. Rev.,* **2012**, *81*, 494-523.
34. L.Ma, Y. Xiao, C. Li, Z. Xie, D. D. Li, Y. T. Wang, H. T. Ma, H. L. Zhu, M. H. Wang and Y. H. Ye, *Bioor. Med. Chem.,* **2013**, *21*, 6763-6770.
35. C. S. Naveena, P. Boja and N. S. Kumari, *Eur. J. Med. Chem.,* **2010**, *45*, 4708-4719.

36. K. Manjunatha, B. Poojary, P. L. Lobo, J. Fernandes and N. S. Kumari, *Eur. J. Med. Chem.,* **2010**, *45*, 5225-5233.
37. R. Apak, K. Güçlü, M. Özyürek and S. E. Karademir, *J. Agr. Food Chem.*, **2004**, *52*, 7970–7981.
38. W. Brand-Williams, M. E. Cuvelier and C. Berset, *LWT – Food Sci. Technol.*, **1995**, *28*, 25–30.
39. R. Re, N. Pellegrini, A. Proteggente, A. Pannala, M. Yang and C. Rice-Evans, *Free Radic. Biol. Med.*, **1999**, *26*, 1231-1237.
40. G. K. Kantar, N. Baltaş, E. Menteşe and S. Şaşmaz, *J. Organomet. Chem.*, **2015**, *787*, 8-13.
41. W. S. Chan, P. C. Wen and H. C. Chiang, *Anticancer Res.,* **1995**, *15*, 703-707.
42. D. E. C. Van Hoorn, R. J. Nijveldt, P. A. M. Van Leeuwen, Z. Hofman, L. M'Rabet and D. B. A. De Bont, *Eur. J. Pharmacol.*, **2002**, *451*, 111-118.
43. H. C. Chiang, Y. J. Lo and F. J. Lu, *J. Enzym. Inhib.*, **1994**, *8*, 61-71.
44. M. W. Weatherburn, *Anal. Chem.*, **1967**, *39*, 971-974.
45. O. Bekircan and H. Bektas, *Molecules,***2008**, *13*, 2126-2135.
46. G. Palla, G. Predieri and P. Domiano, *Tetrahedron,***1986**, 42, 3649-3654.
47. N.Demirbas, S. A.Karaoglu, A.Demirbas and K. Sancak, *Eur. J. Med. Chem.,***2004**, *39*, 793-804.
48. H. Bektas, A. Demirbas, N. Demirbas and S. A. Karaoglu, *Turk. J. Chem.,***2010**, *34*, 165-180.
49. E. Wyrzykiewicz and D. Prukah, *J. Het. Chem.,***1998**, *35*, 381-387.
50. A. Demirbas, D. Sahin, N. Demirbasand S. Alpay-Karaoglu, *Eur. J. Med.Chem.,***2009**, *44*, 2896-2903.
51. S. F. Barbuceanu, G. Saramet and L. A. Gabriela, et al., *Eur. J.Med. Chem.,* **2012**, *49*, 417-423.
52. S. Shelke, G. Mhaske, S. Gadakh and C. Gill, *Bioorg. Med. Chem. Lett.,* **2010**, *20*, 7200-7204.
53. A. Demirbas, D.Sahin and N. Demirbas, *Turk. J. Chem.,* **2010**, *34*, 347-358.