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Research Article

SYNTHESIS OF SUBSTITUTED 5-PHENYL-1-(5-PHENYL)-ISOXAZOL-3-YL)-1H-TETRAZOLE AS ANTIOXIDANT AGENTS

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ABSTRACT

A new series of substituted 5-phenyl-1-(5-phenyl)-isoxazol-3-yl)-1H-tetrazole have been synthesized from the reaction of sodium azide, benzonitrile and ammonium chloride followed by acetylation. The resulted intermediate was reacted with substituted aromatic aldehyde to yield substituted 1-(5-phenyl-1H-tetrazol-1-yl) prop-2-en-1-one which upon cyclization with hydroxylamine hydrochloride form titled compounds. The structures of synthesized compounds were characterized by IR, ^{1}H NMR and mass spectral data. All the synthesized compounds were investigated for their *in-vitro* antioxidant activity by scavenging of DPPH free radical. The result of antioxidant activity revealed that the compounds **21** and **23** have shown potent activity.

Keywords: Tetrazole, Sodium azide, Benzonitrile, Antioxidant activity.

1. INTRODUCTION

Antioxidants are extensively studies for their capacity to protect organisms and cells from damage induced by oxidative stress. Scientists in various disciplines have become more interested in new compounds, either synthesized or obtained from natural sources that could provide active component to prevent or reduce the impact of oxidative stress on cell [1]. Exogenous chemicals and endogenous metabolic process in human body or in food system might produce highly reactive free radicals, especially oxygen derived radicals, which are capable of oxidizing biomolecules, resulting in cell death and tissue damage. Oxidative damage plays a significant pathological role in human diseases for example; cancer, emphysema, cirrhosis, atherosclerosis and arthritis have all been correlated with oxidative damage. Also, excessive generation of ROS (Reactive Oxygen Species) induced by various stimuli and which exceeds the antioxidant capacity of the organism leads to a variety of pathophysioligical processes such as inflammation, diabetes, genotoxicity and cancer [2].

To protect the cells and organ systems of the body against reactive oxygen species, humans have evolved a highly sophisticated and complex antioxidant protection system. It involves a variety of components, both endogenous and exogenous in origin, that function interactively and synergistically to neutralize free radicals [3].

Development of tetrazole chemistry has been largely associated with wide scale of applications of these classes of compounds in medicine, biochemistry, agriculture [4, 5] and also a large number of medicinally important tetrazole heterocyclic incorporated drugs approved by FDA [6, 7]. The tetrazole functionality plays an important role in medicinal chemistry, primarily due to its ability to serve as bioisostere of the carboxylic acid group [8]. The chemistry of tetrazole as well as their medicinal applications has been covered in the literature [9]. The most direct method to synthesize tetrazole is *via* the formal [3+2] cycloaddition of azides and nitriles [10]. Tetrazole and its derivatives have attracted interest because of their unique structure and their applications as antibacterial, antifungal [11], anticancer [12], analgesic [13], anti-inflammatory [14], antidiabetic, antihyperlipidimic [15] and antitubercular agents [16].

The isoxazole moiety is a lead molecule in pharmaceutical development and has a wide range of biological activities like antimicrobial [17], anti-inflammatory, analgesic [18], antitubercular [19], antitumoral and antimycobacterial activity [20]. Isoxazoles are unique in their chemical behavior, not only among heterocyclic compounds but also among related azoles [21].

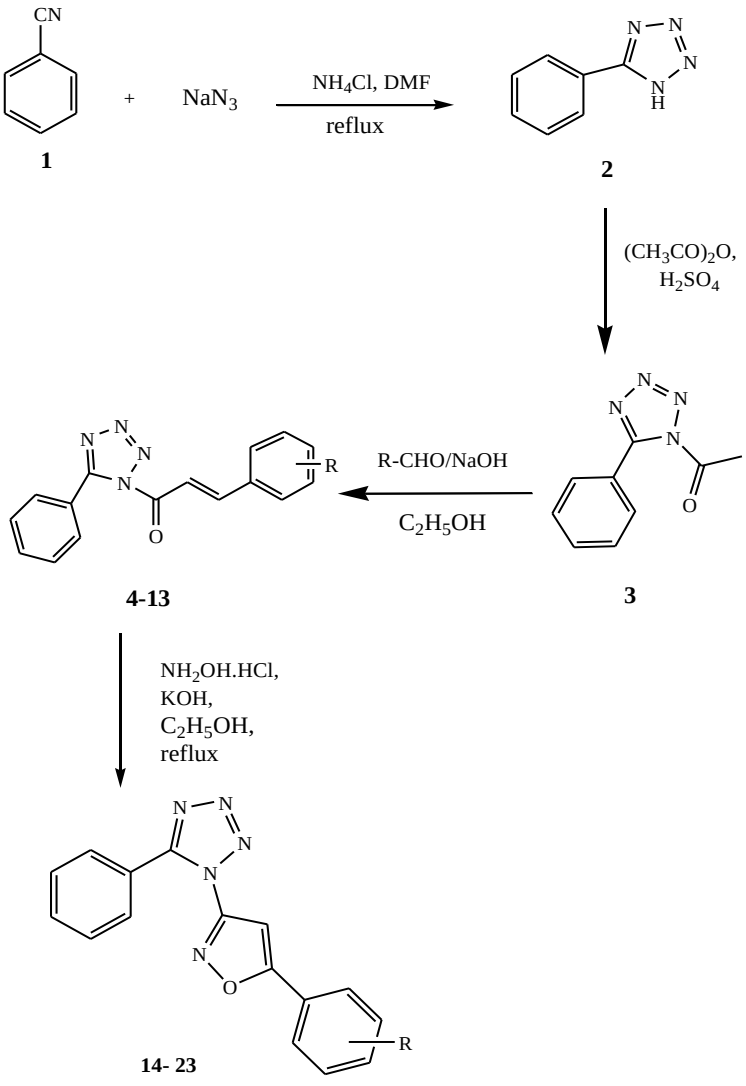
In the present attempt, various derivatives of tetrazole containing isoxazole moiety were synthesized and evaluated for their *in-vitro* antioxidant activity.

2. MATERIALS AND METHODS

All of the reagents were purchased from commercial sources and were used after being purified by standard procedures. Melting point was determined by open capillary method and is uncorrected. All the reactions were monitored

by thin layer chromatography by using toluene: ethyl acetate: formic acid in the ratio of (5:4:1) as mobile phase. IR spectra were recorded by using KBr disk on Shimadzu FTIR-8400S. ^{1}H NMR spectra was recorded on a JEOL AL300 FTNMR 300 MHz spectrophotometer by using tetramethylsilane as internal standard. The values of chemical shift (δ) are given in ppm. Mass spectra were carried out using Waters Micromass Q-Tof Micro. mass spectrometer equipped with electrospray ionization (ESI).

2.1. Reaction Scheme



14: 3-Br-4-NO_2 **19:** 2-Cl-4-F
15: 3,4,5-OCH_3 **20:** 2,4-OCH_3
16: 2,4- F **21:**2,4,6-OCH_3
17: 4-F **22:** 2-NO_2
18: 2-OH-5-NO_2 **23:** 3-Br-4-OCH_3

Figure 1. Synthetic scheme for synthesizing title compounds

2.2. General procedure for the synthesis of 5-phenyl tetrazole (*2*)

The equimolar quantities of sodium azide, ammonium chloride and benzonitrile were refluxed with dimethyl formamide at 125°C for 7-8 hours to obtain the 5-phenyl tetrazole **2**. The residue was dissolved in 100 ml of water and acidified with concentrated hydrochloric acid. The solution was cooled to 5°C, filtered, dried and recrystallized from ethanol.

IR (KBr, $\nu_{max,}$ cm^{-1}): 3348 (NH), 3062 (Ar-CH), 1628 (C=N), 1292 (N-N=N-); ^{1}H NMR ($CDCl_3$-d, δ, ppm): 8.72 (s, 1H, NH), 7.56- 7.18 (m, 5H, Ar-H).

2.3. General procedure for the synthesis of 5-phenyl-1-acetyl tetrazole (3)

5-phenyl tetrazole (**2**, 10 mmol) was mixed with acetic anhydride (10 mmol) and 2-3 drops of concentrated sulphuric acid. The reaction mixture was warmed at 60-70 ºC for 15-20 minutes on water bath. The content was cooled to room temperature and poured into ice cold water to obtain a white colored precipitate. The precipitate was filtered, washed, dried and recrystallized from ethanol.

IR (KBr, $\nu_{max,}$ cm^{-1}): 3058 (Ar-CH), 1730 (C=O), 1638 (C=N), 1285 (N-N=N-); ^{1}H NMR ($CDCl_3$-d, δ, ppm): 7.51-7.28 (m, 5H, Ar-H), 2.28 (s, 3H, CH_3).

2.4. General procedure for the synthesis of substituted 1-(5-phenyl-1H-tetrazol-1-yl) prop-2-en-1-one (4-13)

A solution of 5-phenyl-1-acetyl tetrazole (**3**, 10 mmol) and different substituted aromatic aldehyde (10 mmol) in ethanol (20 ml) was cooled to 5 to 10 ºC in an ice bath. The cooled solution was treated with drop wise addition of 40% sodium hydroxide solution. The reaction mixture was magnetically stirred for 30 minutes and then left over night in the refrigerator. The resulting dark solution was diluted with ice water and acidified using hydrochloric acid. The solution was filtered, washed with water and recrystallized with ethanol.

2.4.1. Synthesis of 3-(3-bromo-4-nitrophenyl)-1-(5-phenyl-1H-tetrazol-1-yl) prop-2-en-1-one (4)

Brown solid; Yield: 86%; m.p.: 260-262ºC; IR (KBr, $\nu_{max,}$ cm^{-1}): 3034 (Ar-CH), 1746 (C=O), 1618 (C=C), 1593 (C=N), 1570 (NO_2), 1284 (N-N=N-), 646 (C-Br); ^{1}H NMR ($CDCl_3$-d, δ, ppm): 7.94 (d, 1H, Ar-H), 7.53 (s, 1H, Ar-H), 7.47 (d, 1H, Ar-H), 7.40-7.28 (m, 5H, Ar-H), 7.23 (d, 1H, CH), 6.80 (d, 1H, CH); Elemental analysis cal. (%) for $C_{16}H_{10}BrN_5O_3$: C, 48.02; H, 2.52; Br, 19.97; N, 17.50; O, 11.99.

2.4.2. Synthesis of 3-(3, 4, 5-trimethoxyphenyl)-1-(5-phenyl-1H-tetrazol-1-yl) prop-2-en-1-one (5)

Yellowish Brown solid; Yield: 82%; m.p.: 230-232ºC; IR (KBr, $\nu_{max,}$ cm^{-1}): 3054 (Ar-CH), 1740 (C=O), 1630 (C=C), 1600 (C=N), 1248 (N-N=N-), 1221 (OCH_3); ^{1}H NMR ($CDCl_3$-d, δ, ppm): 7.47 (d, 1H, CH), 7.45-7.28 (m, 5H, Ar-H), 6.56 (s, 2H, Ar-H), 6.40 (d, 1H, CH), 3.66 (s, 9H, OCH_3); Elemental analysis cal. (%) for $C_{19}H_{18}N_4O_4$: C, 62.29; H, 4.95; N, 15.29; O, 17.47.

2.4.3. Synthesis of 3-(2, 4-difluorophenyl)-1-(5-phenyl-1H-tetrazol-1-yl) prop-2-en-1-one (6)

Creamy solid; Yield: 79%; m.p.: 234-236ºC; IR (KBr, $\nu_{max,}$ cm^{-1}): 3059 (Ar-CH), 1712 (C=O), 1614 (C=C), 1608 (C=N), 1268 (N-N=N-), 1163 (C-F); ^{1}H NMR ($CDCl_3$-d, δ, ppm): 7.68 (d, 1H, CH), 7.51-7.34 (m, 5H, Ar-H), 7.18 (d, 1H, Ar-H), 6.77 (s, 1H, Ar-H), 6.58 (s, 1H, Ar-H), 6.43 (d, 1H, CH); Elemental analysis cal. (%) for $C_{16}H_{10}F_2N_4O$: C, 61.54; H, 3.23; F, 12.17; N, 17.94; O, 5.12.

2.4.4. Synthesis of 3-(4-fluorophenyl)-1-(5-phenyl-1H-tetrazol-1-yl) prop-2-en-1-one (7)

Brown solid; Yield: 68%; m.p.: 224-226ºC; IR (KBr, $\nu_{max,}$ cm^{-1}): 3060 (Ar-CH), 1764 (C=O), 1620 (C=C), 1606 (C=N), 1310 (N-N=N-), 1166 (C-F); ^{1}H NMR ($CDCl_3$-d, δ, ppm): 7.48 (d, 1H, CH), 7.42-7.29 (m, 5H, Ar-H), 7.21 (d, 2H, Ar-H), 7.04 (d, 2H, Ar-H), 6.56 (d, 1H, CH); Elemental analysis cal. (%) for $C_{16}H_{11}FN_4O$: C, 65.30; H, 3.77; F, 6.46; N, 19.04; O, 5.44 .

2.4.5. Synthesis of 3-(2-hydroxy-5-nitrophenyl)-1-(5-phenyl-1H-tetrazol-1-yl) prop-2-en-1-one (8)

Brown solid; Yield: 63%; m.p.: 252-254ºC; IR (KBr, $\nu_{max,}$ cm^{-1}): 3583 (C-OH), 3055 (Ar-CH), 1680 (C=O), 1630 (C=C), 1610 (C=N), 1564 (NO_2), 1344 (N-N=N-); ^{1}H NMR ($CDCl_3$-d, δ, ppm): 7.76 (s, 1H, Ar-H), 7.57 (d, 1H, CH), 7.54 (d, 1H, Ar-H), 7.48-7.25 (m, 5H, Ar-H), 6.81 (d, 1H, Ar-H), 6.76 (d, 1H, CH), 4.92 (s, 1H, OH); Elemental analysis cal. (%) for $C_{16}H_{11}N_5O_4$: C, 56.98; H, 3.29; N, 20.76; O, 18.97.

2.4.6. Synthesis of 3-(2-chloro-4-fluorophenyl)-1-(5-phenyl-1H-tetrazol-1-yl) prop-2-en-1-one (9)

Reddish brown solid; Yield: 74%; m.p.: 220-222ºC; IR (KBr, $\nu_{max,}$ cm^{-1}): 3054 (Ar-CH), 1735 (C=O), 1630 (C=C), 1608 (C=N), 1285 (N-N=N-), 1178 (C-F), 786 (C-Cl); ^{1}H NMR ($CDCl_3$-d, δ, ppm): 7.72 (d, 1H, CH), 7.44-7.28 (m, 5H, Ar-H), 7.17 (d, 1H, Ar-H), 7.06 (s, 1H, Ar-H), 6.74 (d, 1H, Ar-H), 6.37 (d, 1H, CH); Elemental analysis cal. (%) for $C_{16}H_{10}ClFN_4O$: C, 58.46; H, 3.07; Cl, 10.78; F, 5.78; N, 17.04; O, 4.87.

2.4.7. Synthesis of 3-(2, 4-dimethoxyphenyl)-1-(5-phenyl-1H-tetrazol-1-yl) prop-2-en-1-one (10)

Brown solid; Yield: 78%; m.p.: 246-248ºC; IR (KBr, $\nu_{max,}$ cm^{-1}): 3054 (Ar-CH), 1666 (C=O), 1640 (C=C), 1606 (C=N), 1275 (N-N=N-), 1248 (OCH_3); ^{1}H NMR ($CDCl_3$-d, δ, ppm): 7.62 (d, 1H, CH), 7.41-7.30 (m, 5H, Ar-H), 7.16 (d, 1H, Ar-H), 6.71 (d, 1H, CH), 6.53 (d, 1H, Ar-H), 6.47 (d, 1H, Ar-H), 3.68 (s, 6H, OCH_3); Elemental analysis cal. (%) for $C_{18}H_{16}N_4O_3$: C, 64.28; H, 4.79; N, 16.66; O, 14.27.

2.4.8. Synthesis of 3-(2, 4, 6-trimethoxyphenyl)-1-(5-phenyl-1H-tetrazol-1-yl) prop-2-en-1-one (11)

Yellowish brown solid; Yield: 82%; m.p.: 212-214 ºC; IR (KBr, $\nu_{max,}$ cm^{-1}): 3064 (Ar-CH), 1666 (C=O), 1652 (C=C), 1606 (C=N), 1290 (N-N=N-), 1186 (OCH_3); ^{1}H NMR ($CDCl_3$-d, δ, ppm): 7.63 (d, 1H, CH), 7.45-7.30 (m, 5H, Ar-H), 6.67 (s, 2H, Ar-H), 6.63 (d, 1H, CH), 3.66 (s, 9H, OCH_3); Elemental analysis cal. (%) for $C_{19}H_{18}N_4O_4$; C, 62.29; H, 4.95; N, 15.29; O, 17.47.

2.4.9. Synthesis of 3-(2-nitrophenyl)-1-(5-phenyl-1H-tetrazol-1-yl) prop-2-en-1-one (12)

Yellow solid; Yield: 87%; m.p.: 228-230 °C; IR (KBr, $\nu_{max,}$ cm^{-1}): 3074 (Ar-CH), 1726 (C=O), 1620 (C=C), 1608 (C=N), 1578 (NO_2), 1248 (N-N=N-); ^{1}H NMR ($CDCl_3$-d, δ, ppm): 7.93 (d, 1H, CH), 7.78 (d, 1H, Ar-H), 7.58 (t, 1H, Ar-H), 7.61 (d, 1H, Ar-H), 7.53-7.32 (m, 5H, Ar-H), 7.28 (t, 1H, Ar-H), 6.65 (d, 1H, CH); Elemental analysis cal. (%) for $C_{16}H_{11}N_5O_3$: C, 59.81; H, 3.45; N, 21.80; O, 14.94.

2.4.10. Synthesis of 3-(3-bromo-4-methoxyphenyl)-1-(5-phenyl-1H-tetrazol-1-yl) prop-2-en-1-one (13)

Yellowish brown solid; Yield: 78%; m.p.: 164-166°C; IR (KBr, $\nu_{max,}$ cm^{-1}): 3034 (Ar-CH), 1660 (C=O), 1615 (C=C), 1608 (C=N), 1255 (N-N=N-), 1170 (OCH_3), 640 (C-Br); ^{1}H NMR ($CDCl_3$-d, δ, ppm): 7.47 (d, 1H, CH), 7.41-7.29 (m, 5H, Ar-H), 7.27 (s, 1H, Ar-H), 7.16 (d, 1H, Ar-H), 6.58 (d, 1H, CH), 6.64 (d, 1H, Ar-H); 3.77 (s, 3H, OCH_3); Elemental analysis cal. (%) for $C_{17}H_{13}BrN_4O_2$: C, 53.00; H, 3.40; Br, 20.74; N, 14.54; O, 8.31.

2.5. General procedure for the synthesis of substituted 5-phenyl-1-(5-phenyl)-isoxazol-3-yl)-1H-tetrazole (14-23)

A mixture of substituted 1-(5-phenyl-1H-tetrazol-1-yl) prop-2-en-1-one (**4-13,** 10 mmol), hydroxylamine hydrochloride (10 mmol) in ethanol and 40% potassium hydroxide solution were refluxed for 4-5 hours then reaction mixture was cooled and poured into crushed ice. The precipitate was separated by filtration, dried and recrystallized from ethanol.

2.5.1. Synthesis of 1-(5-(3-bromo-4-nitrophenyl) isoxazol-3-yl)-5-phenyl-1H-tetrazole (14)

Yellow solid; Yield: 76%; m.p.: 158-160°C; IR (KBr, $\nu_{max,}$ cm^{-1}): 3072 (Ar-CH), 2896 (CH), 1625 (C=N), 1560 (NO_2), 1502 (C=C), 1438 (N-O), 1261 (N-N=N-), 617 (C-Br); ^{1}H NMR (DMSO-d_6, δ, ppm): 7.92 (d, 1H, Ar-H), 7.83 (s, 1H, Ar-H), 7.71 (d, 1H, Ar-H), 7.56-7.28 (d, 5H, Ar-H), 7.04 (d, 1H, isoxazole); MS, m/z 411; Elemental analysis cal. (%) for $C_{16}H_9BrN_6O_3$: C,46.51; H, 2.20; Br, 19.34; N, 20.54; O, 11.62.

2.5.2. Synthesis of 1- (5-(3, 4, 5-trimethoxypheny)-isoxazol -3-yl)-5-phenyl-1H-tetrazole (15)

Yellow brown solid; Yield: 78%; m.p.: 136-138°C; IR (KBr, $\nu_{max,}$ cm^{-1}) 3051 (Ar-CH), 2918 (CH), 1691 (C=N), 1564 (C=C), 1485 (N-O), 1288 (N-N=N-), 1163 (OCH_3); ^{1}H NMR (DMSO-d_6, δ, ppm): 7.59-7.25 (m, 5H, Ar-H), 7.04 (s, 1H, isoxazole), 6.82 (d, 2H, Ar-H) 3.68 (s, 9H, OCH_3); MS, m/z 379; Elemental analysis cal. (%) for $C_{19}H_{17}N_5O_4$: C, 60.15; H, 4.52; N, 18.46; O, 16.87.

2.5.3. Synthesis of 1- (5-(2, 4-difluorophenyl)-isoxazol -3-yl)-5-phenyl-1H-tetrazole (16)

Brown solid; Yield: 68%; m.p.: 144-146°C; IR (KBr, $\nu_{max,}$ cm^{-1}): 3093 (Ar-CH), 2921 (CH), 1610 (C=N), 1561 (C=C), 1474 (N-O), 1282 (N-N=N-), 1118 (C-F); ^{1}H NMR (DMSO-d_6, δ, ppm): 7.48 (d, 1H, Ar-H), 7.41-7.29 (m, 5H, Ar-H), 6.97 (s, 1H, isoxazole), 6.91 (d, 1H, Ar-H), 6.80 (s, 1H, Ar-H); MS, m/z 325; Elemental analysis cal. (%) for $C_{16}H_9F_2N_5O$: C, 59.08; H, 2.79; F, 11.68; N, 21.53; O, 4.92.

2.5.4. Synthesis of 1-(5-(4-fluorophenyl)-isoxazol -3-yl)-5-phenyl-1H-tetrazole (17)

Yellowish white solid; Yield: 74%; m.p.: 153-155°C; IR (KBr, $\nu_{max,}$ cm^{-1}): 3063 (Ar-CH), 2896 (CH), 1682 (C=N), 1544 (C=C), 1485 (N-O), 1288 (N-N=N-), 1163 (C-F); ^{1}H NMR (DMSO-d_6, δ, ppm): 7.52 (d, 2H, Ar-H), 7.42-7.26 (m, 5H, Ar-H), 7.11 (d, 2H, Ar-H), 6.98 (s, 1H, isoxazole); MS, m/z 308; Elemental analysis cal. (%) for $C_{16}H_{10}FN_5O$: C, 62.54; H, 3.28; F, 06.18; N, 22.79; O, 4.92.

2.5.5. Synthesis of 4-nitro-2-(3-(5-phenyl -1H-tetrazol-1-yl)-isoxazol-5-yl) phenol (18)

Reddish brown solid; Yield: 73%; m.p.: 139-141°C; IR (KBr, $\nu_{max,}$ cm^{-1}): 3577 (OH), 3053 (Ar-CH), 2902 (CH), 1689 (C=N), 1564 (NO_2), 1514 (C=C), 1485 (N-O), 1284 (N-N=N); ^{1}H NMR (DMSO-d_6, δ, ppm): 7.92 (s, 1H, Ar-H), 7.74 (d, 1H, Ar-H), 7.56-7.27 (m, 5H, Ar-H), 7.13 (d, 1H, Ar-H), 7.05 (s, 1H, isoxazole), 4.92 (s, 1H, OH); MS, m/z 350; Elemental analysis cal. (%) for $C_{16}H_{10}N_6O_4$: C, 54.86; H, 2.88; N, 23.99; O, 18.27.

2.5.6. Synthesis of 1- (5-(2-chloro-4-fluorophenyl)-isoxazol-3-yl)-5-phenyl-1H-tetrazole (19)

White solid; Yield: 68%; m.p.: 145-147°C; IR (KBr, $\nu_{max,}$ cm^{-1}): 3095 (Ar-CH), 2848 (CH), 1610 (C=N), 1508 (C=C), 1465 (N-O), 1288 (N-N=N-), 1120 (C-F), 727 (C-Cl); ^{1}H NMR (DMSO-d_6, δ, ppm): 7.51 (d, 1H, Ar-H), 7.43-7.25 (m, 5H, Ar-H), 7.18 (s, 1H, Ar-H), 7.02 (s, 1H, isoxazole), 6.95 (s, 1H, Ar-H); MS, m/z 341; Elemental analysis cal. (%) for $C_{16}H_9ClFN_5O$: C, 56.24; H, 2.65; Cl, 10.37; F, 5.56; N, 20.49.

2.5.7. Synthesis of 1- (5-(2, 4-dimethoxyphenyl)-isoxazol-3-yl)-5-phenyl-1H-tetrazole (20)

Brown solid; Yield: 72%; m.p.: 172-174°C; IR (KBr, $\nu_{max,}$ cm^{-1}): 3056 (Ar-CH), 2880 (CH), 1608 (C=N), 1560 (C=C), 1485 (N-O), 1288 (N-N=N-), 1163 (OCH_3); ^{1}H NMR (DMSO-d_6, δ, ppm): 7.57-7.28 (m, 5H, Ar-H), 7.22 (d, 1H, Ar-H), 6.84 (s, 1H, isoxazole), 6.77 (d, 1H, Ar-H), 6.56 (s, 1H, Ar-H), 3.78 (s, 6H, OCH_3); MS, m/z 349; Elemental analysis cal. (%) for $C_{18}H_{15}N_5O_3$: C, 61.89; H, 4.33; N, 20.05; O, 13.74.

2.5.8. Synthesis of 1- (5-(2, 4, 6-trimethoxyphenyl)-isoxazol -3-yl)-5-phenyl-1H-tetrazole (21)

Brown solid; Yield: 76%; m.p.: 131-133ºC; IR (KBr, $\nu_{max,}$ cm^{-1}): 3055 (Ar-CH), 2877 (CH), 1606 (C=N), 1564 (C=C), 1485 (N-O), 1288 (N-N=N-), 1163 (OCH_3); ^1H NMR (DMSO-d_6, δ, ppm): 7.42-7.29 (m, 5H, Ar-H), 6.89 (s, 1H, isoxazole), 6.82 (s, 2H, Ar-H), 3.65 (s, 9H, OCH_3); MS, m/z 379; Elemental analysis cal. (%) for $C_{19}H_{17}N_5O_4$: C, 60.15; H, 4.52; N, 18.46; O, 16.87.

2.5.9. Synthesis of 1- (5-(2-nitrophenyl)-isoxazol -3-yl)-5-phenyl-1H-tetrazole (22)

Yield: 68%; m.p.: 174-176ºC; IR (KBr, $\nu_{max,}$ cm^{-1}): 3086 (Ar-CH), 2893 (CH), 1623 (C=N), 1548 (NO_2), 1517 (C=C), 1485 (N-O), 1276 (N-N=N-); ^1H NMR (DMSO-d_6, δ, ppm): 7.73-7.58 (m, 4H, Ar-H), 7.50-7.27 (m, 5H, Ar-H), 7.08 (s, 1H, isoxazole); MS, m/z 334; Elemental analysis cal. (%) for $C_{16}H_{10}N_6O_3$: C, 57.49; H, 3.02; N, 25.14; O, 14.36.

2.5.10. Synthesis of 1- (5- (3-bromo-4-methoxyphenyl)-isoxazol-3-yl)-5-phenyl-1H-tetrazole (23)

Creamy white solid; Yield: 73%; m.p.: 136-138ºC; IR (KBr, $\nu_{max,}$ cm^{-1}): 3066 (Ar-CH), 2877 (CH) 1602 (C=N), 1564 (C=C), 1465 (N-O), 1261 (N-N=N-), 1114 (OCH_3), 676 (C-Br); ^1H NMR (DMSO-d_6, δ, ppm): 7.48 (s, 1H, Ar-H), 7.42-7.25 (m, 5H, Ar-H), 7.21 (d, 1H, Ar-H), 7.12 (s, 1H, isoxazole), 6.91 (d, 1H, Ar-H), 3.68 (s, 3H, OCH_3); MS, m/z 397; Elemental analysis cal. (%) for :$C_{17}H_{12}BrN_5O_2$: C, 51.27; H, 3.04; Br, 20.07; N, 17.59; O, 08.04.

2.6. DPPH free radical scavenging activity

All the synthesized compounds were screened for their *in-vitro* antioxidant activity by scavenging of DPPH (2, 2-diphenyl-1-picrylhydrazyl) free radical. A stock solution of 100 μg/ml was prepared of all the test compounds as well as of standard. Different concentrations were made of 10, 20, 30, 40 and 50 μg/ml from stock solutions using methanol. 0.1mM solution of DPPH in methanol was prepared in a volumetric flask which was completely kept away from light. 1.0 ml of all concentration of test and standards were mixed with 1.0 ml of DPPH solution. This solution was kept for 30 minutes in dark place. Methanol with DPPH was used as control. Absorbance of all the samples was taken on UV-spectrophotometer at a λ_{max} of 517nm [22]. The free radical scavenging was expressed as the percentage inhibition and was calculated using the formula:

Percent inhibition % = [(Ao -A)/Ao) x100]

Where: Ao = Absorbance of control.

A = Absorbance of test or standard.

The percent inhibition was plotted against the sample or the standard concentration to obtain the amount of antioxidant necessary to decrease the initial concentration of DPPH to 50 % (IC_{50}). IC_{50} values were calculated from calibration curve. IC_{50} value is defined as the concentration of test compound required to achieve half maximal inhibition and lower IC_{50} value indicates greater antioxidant activity.

3. RESULTS AND DISCUSSION

In the present work the titled compounds were synthesized by the cyclization of sodium azide, benzonitrile in presence of ammonium chloride and DMF to form 5-phenyl tetrazole **2**, which on acetylation produce 5-phenyl-1-acetyl tetrazole **3**. A solution of 5-phenyl-1-acetyl tetrazole **3** and substituted aromatic aldehyde in ethanol was cooled to 5 to 10 ºC and this solution was treated with aqueous sodium hydroxide solution. The reaction mixture was magnetically stirred for 30 minutes and then left over night in the refrigerator. The resulting solution was diluted with ice water and acidified to form substituted 1-(5-phenyl-1H-tetrazol-1-yl) prop-2-en-1-one **4–13**. The compounds **4–13** were refluxed with hydrxoylamine hydrochloride in ethanol and potassium hydroxide for 4-5 hours to form substituted 5-phenyl-1-(5-phenyl)-isoxazol-3-yl)-1H-tetrazole **14–23**.

The structures of these synthesized compounds were confirmed by their spectral data analysis. The ^1H NMR spectrum of **18** in DMSO showed singlet at 7.92, 7.05 and 4.92 ppm for aromatic proton, proton of isoxazole ring and alcoholic proton respectively. Doublet at 7.74 and 7.13, a multiplet between 7.56-7.27 showed the presence of aromatic protons. IR spectra exhibited characteristic absorption bands at 3557 cm^{-1} due to OH group, 3053 cm^{-1} due to aromatic CH group, 2902 cm^{-1} due to CH group of isoxazole ring, 1689 cm^{-1} due to C=N group, 1564 cm^{-1} due to NO_2 group, 1514 cm^{-1} due to C=C group, 1485 cm^{-1} due to N-O group, and 1284 cm^{-1} due to N-N=N group of tetrazole ring. Mass spectrum of the compound showed the molecular ion peak at *m/z* 350.

All the synthesized compounds were screened for *in-vitro* antioxidant activity by scavenging of DPPH free radical, data of *in-vitro* antioxidant activity is summarized in table1. The investigation of antioxidant screening revealed that some of the tested compounds showed moderate to good antioxidant activity. Particularly, compounds **21** and **23** have shown more promising antioxidant activity and compounds **15, 20** shown moderate antioxidant activity as compared to standard, ascorbic acid. When the phenyl ring from aldehydic moiety is substituted with methoxy and bromo groups, the compounds exhibited good antioxidant activity.

Table 1. Data of antioxidants activity of synthesized compounds by DPPH scavenging assay

Compounds	Concentration µg/ml										IC_{50} value µg/ml
	10		20		30		40		50		
	*Abs	% inhibition	*Abs	% inhibition	*Abs	% inhibition	*Abs	% inhibition	*Abs	% inhibition	
Std.	0.168	46.15	0.126	59.61	0.092	70.51	0.07	77.56	0.057	81.73	10.78
14	0.177	43.26	0.163	47.75	0.151	51.6	0.139	55.44	0.122	60.89	25.83
15	0.185	40.76	0.15	51.92	0.127	59.29	0.116	62.82	0.09	71.15	19.97
16	0.227	27.24	0.206	33.97	0.164	47.43	0.151	51.6	0.135	56.73	38.62
17	0.228	26.92	0.203	34.93	0.151	51.6	0.117	62.5	0.096	69.23	30.85
18	0.258	17.3	0.235	24.67	0.199	36.21	0.172	44.87	0.157	49.67	48.19
19	0.27	13.46	0.255	18.26	0.211	32.37	0.197	36.85	0.174	44.23	56.16
20	0.189	39.42	0.145	53.52	0.133	57.37	0.12	61.53	0.101	67.62	20.85
21	0.166	46.79	0.153	50.96	0.136	56.41	0.117	62.5	0.107	65.7	16.88
22	0.223	28.52	0.206	33.97	0.163	47.75	0.138	55.76	0.099	68.26	33.1
23	0.163	47.75	0.144	53.84	0.119	61.85	0.103	66.98	0.067	78.52	14.21

*****Abs is Absorbance*

4. CONCLUSION

In summary, substituted 5-phenyl-1-(5-phenyl)-isoxazol-3-yl)-1H-tetrazole have been synthesized and characterized. Compounds were screened for *in-vitro* antioxidant activity by scavenging of DPPH free radical. Among the synthesized compounds, the compounds **21** and **23** have shown more promising antioxidant activity, the compounds **15** and **20** have shown moderate antioxidant activity as compared to standard. In future *in-vivo* antioxidant evaluation of these compounds can also be carried out for further study.

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