

# Synthesis, Antioxidant and Antidiabetic Activity of 1-[(5-Substituted phenyl)-4,5-dihydro-1H-pyrazol-3-yl]-5-phenyl-1H-tetrazole

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**Kaushik, et al.: Synthesis and Biological activity of 1-[(5-substituted phenyl)-4,5-dihydro-1H-pyrazol-3-yl]-5-phenyl-1H-tetrazole**

A series of 1-[(5-substituted phenyl)-4, 5-dihydro-1H-pyrazol-3-yl]-5-phenyl-1H-tetrazole derivatives (4a-j) have been synthesized by the reaction of 1-(5-substituted phenyl)-1H-tetrazol-1-yl prop-2-en-1-one (3a-j) with hydrazine hydrate in acetic acid under reflux for 5 h. Structure of the synthesized compounds was established by their spectral data. As a new strategy for alleviating the oxidative damage in diabetes mellitus, a growing interest has been observed in the usage of antioxidants. The synthesized compounds were screened for their *in vitro* antioxidant activity by DPPH (2,2-diphenyl-1-picrylhydrazyl) free radical scavenging method and *in vivo* antidiabetic activity by streptozotocin-induced model. Compounds 4b, 4g, 4h and 4j were exhibit prominent antioxidant as well as antidiabetic activity. Compound 4i was exhibit moderate antidiabetic activity.

**Key words:** Tetrazole, pyrazole, antioxidant activity, antidiabetic activity

Diabetes Mellitus (DM) is a multifactorial disease which is characterized by hypoglycemia<sup>[1]</sup>, lipoprotein abnormalities<sup>[2]</sup>, raised basal metabolic rate<sup>[3]</sup>, defect in reactive oxygen species scavenging enzymes<sup>[4]</sup> and high oxidative stress-induced damage to pancreatic beta cells<sup>[5]</sup>.

Although the mechanism of diabetic complications remains unclear, much attention has been focused to the role of oxidative stress. Oxidative stress may contribute to the pathogenesis of different diabetic complications<sup>[6]</sup>. Furthermore, with diabetes, several features appear including an increase in lipid peroxidation<sup>[7]</sup>, a decrease in the antioxidant enzyme activities. These changes indicate an oxidative stress caused by hyperglycemia<sup>[8]</sup>.

Oxidative stress is characterized by an increased concentration of intracellular oxidizing species, such as reactive oxygen species (ROS) and is often accompanied by the loss of antioxidant defense capacity<sup>[9]</sup>. It is well known that excess ROS attack many organs, and induce oxidative damage directly to critical biological molecules, such as lipoproteins,

proteins and nucleotides, causing lipid peroxidation and protein oxidation. Metabolic oxidative stress has been implicated, directly or indirectly, in the development of diseases and degenerative processes, including inflammation, cancer, dementia and physiological aging<sup>[10]</sup>. Moreover, oxidative stress also plays a central role in liver pathologies<sup>[11]</sup>. Antioxidant pharmacotherapy in various forms has emerged as a mean to minimize the bimolecular damage caused by attack of ROS on vital constituents of living organisms<sup>[12]</sup>.

In DM, oxidative stress seems mainly to be due to an increased production of free radicals and a sharp reduction of antioxidant defense<sup>[13]</sup>. Therefore, there is a great interest in looking for new tetrazole containing pyrazole moiety, which could represent a good

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pharmacological alternative to counteract oxidative stress.

Development of tetrazole chemistry offers wide scale of applications in the medicine, biochemistry and agriculture fields<sup>[14]</sup>. The chemistry of tetrazoles as well as their medicinal applications has been covered in literature. They have found use in pharmaceuticals as lipophilic spacer and carboxylic acid surrogates<sup>[15]</sup>. Tetrazole and its derivatives have attracted much interest in medicinal chemistry owing to unique structure. The tetrazole functionality plays an important role in medicinal chemistry primarily due to its ability to serve as bioisostere of the carboxylic acid group<sup>[16]</sup>. One of the well-known methods for synthesis of 5-substituted-1*H*-tetrazoles is by a [3+2] cycloaddition between hydrazoic acid and cyanides<sup>[17]</sup>. Their derivatives have been shown antibacterial, antifungal<sup>[18]</sup>, anticancer<sup>[19]</sup>, analgesic<sup>[20]</sup>, antiinflammatory<sup>[21]</sup>, antidiabetic, antihyperlipidemic<sup>[22-23]</sup> and antitubercular activities<sup>[24]</sup>. A large number of medicinally important drugs containing tetrazole moiety has been approved by USFDA<sup>[25]</sup>.

In addition the pyrazole moiety is a versatile lead molecule in pharmaceutical development and has a wide range of biological activities. Literature survey reveals that pyrazole derivatives are well known to have antibacterial<sup>[26]</sup>, antifungal<sup>[27]</sup>, anti-inflammatory<sup>[28]</sup>, antitubercular<sup>[29]</sup>, antidepressant, anticonvulsant<sup>[30]</sup>, antioxidant<sup>[31]</sup>, anticancer<sup>[32]</sup> and antiviral<sup>[33]</sup> activities. The pyrazolotetrazole nucleus has been studied by other researchers with different functional groups<sup>[34]</sup>. No detailed study has been carried out on the pyrazolotetrazole, in moderating oxidative stress associated with diabetes mellitus in experimental animals. Hence, the aim of present study is to synthesize various derivatives of tetrazole containing pyrazole moiety and evaluate their antioxidant and antidiabetic potential.

## MATERIALS AND METHODS

All of the reagents were purchased from commercial sources. Melting point was determined by open capillary method and was uncorrected. All the reactions were measured by thin layer chromatography (TLC). The compounds were analyzed for elemental analysis. Infrared (IR) spectra were recorded by using KBr disk on Shimadzu FTIR-8400S. <sup>1</sup>H NMR spectra was recorded on a JEOL AL300 FTNMR 300 MHz spectrophotometer by using tetramethylsilane as internal standard. The values of chemical shift ( $\delta$ ) are

given in ppm. Mass spectra were carried out utilizing Waters Micromass Q-ToF Micro Mass spectrometer equipped with electrospray ionization (ESI).

### General procedure for the synthesis of 5-phenyl tetrazole (1):

The equimolar quantities of sodium azide, ammonium chloride and benzonitrile were refluxed with dimethyl formamide at 125° for 7-8 h to obtain the 5-phenyl tetrazole (1). The residue was dissolved in 100 ml of water and acidified with concentrated hydrochloric acid. The solution was cooled, filtered, dried and recrystallized using ethanol.

Brown solid; % yield: 80; MP: 208-210°, IR (KBr)  $\nu$  (cm<sup>-1</sup>): 3348 (NH), 3062 (Ar-CH), 1628 (C=N), 1292 (N-N=N-); <sup>1</sup>H NMR (CDCl<sub>3</sub>)  $\delta$  (ppm): 7.18-7.56 (m, 5H, Ar-H), 8.72 (s, 1H, NH). Anal. Calcd. (%) for C<sub>7</sub>H<sub>6</sub>N<sub>4</sub>: C, 57.53; H, 4.14; N, 38.34. Found: C, 57.74; H, 4.18; N, 38.47.

### General procedure for the synthesis of 5-phenyl-1-acetyl tetrazole (2):

5-phenyl tetrazole (1, 10 mmol) was mixed with acetic anhydride (10 mmol), to this solution 2-3 drops of concentrated sulphuric acid was added. The reaction mixture was warmed at 60-70° for 15-20 min on water bath. The content was cooled to room temperature and then poured into ice cold water to obtain a white colored precipitate. The precipitate was filtered, washed, dried and recrystallized using ethanol.

White solid; % yield: 78; MP: 218-220°, IR (KBr)  $\nu$  (cm<sup>-1</sup>): 3058 (Ar-CH), 1730 (C=O), 1638 (C=N), 1285 (N-N=N-); <sup>1</sup>H NMR (CDCl<sub>3</sub>)  $\delta$  (ppm): 2.28 (s, 3H, CH<sub>3</sub>), 7.28-7.51 (m, 5H, Ar-H). Anal. Calcd. (%) for C<sub>9</sub>H<sub>8</sub>N<sub>4</sub>O: C, 57.44; H, 4.28; N, 29.77; O, 8.50. Found: C, 57.62; H, 4.31; N, 29.92.

### General procedure for the synthesis of 1-(5-substitutedphenyl-1*H*-tetrazol-1-yl) prop-2-en-1-one (3a-j):

A solution of 5-phenyl-1-acetyl tetrazole (2, 10 mmol) and different substituted aromatic aldehyde (10 mmol) in ethanol (20 ml) was cooled to 5 to 10° 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 min 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.

**3-(3-bromo-4-nitrophenyl)-1-(5-phenyl-1H-tetrazol-1-yl) prop-2-en-1-one (3a):**

Brown solid; % yield: 86; MP: 260-262°, IR (KBr)  $\nu$  (cm<sup>-1</sup>): 3034 (Ar-CH), 1736 (C=O), 1618 (C=C), 1593 (C=N), 1570 (NO<sub>2</sub>), 1284 (N-N=N-), 646 (C-Br). <sup>1</sup>H NMR (CDCl<sub>3</sub>)  $\delta$  (ppm): 6.80 (d, 1H, ethylenic), 7.23 (d, 1H, ethylenic), 7.28-7.40 (m, 5H, Ar-H), 7.47 (d, 1H, Ar-H), 7.53 (s, 1H, Ar-H), 7.94 (d, 1H, Ar-H). Anal. Calcd. (%) for C<sub>16</sub>H<sub>10</sub>BrN<sub>5</sub>O<sub>3</sub>: C, 48.02; H, 2.52; Br, 19.97; N, 17.50; O, 11.99. Found: C, 48.18; H, 2.54; N, 17.58.

**3-(3, 4, 5-trimethoxyphenyl)-1-(5-phenyl-1H-tetrazol-1-yl) prop-2-en-1-one (3b):**

Yellowish brown solid; % yield: 82, MP: 230-232°, IR (KBr)  $\nu$  (cm<sup>-1</sup>): 3054 (Ar-CH), 1740 (C=O), 1630 (C=C), 1600 (C=N), 1248 (N-N=N), 1221 (OCH<sub>3</sub>); <sup>1</sup>H NMR (CDCl<sub>3</sub>)  $\delta$  (ppm): 3.66 (s, 9H, -OCH<sub>3</sub>), 6.40 (d, 1H, ethylenic), 6.56 (s, 2H, Ar-H), 7.28-7.45 (m, 5H, Ar-H), 7.47 (d, 1H, ethylenic). Anal. Calcd. (%) for: C<sub>19</sub>H<sub>18</sub>N<sub>4</sub>O<sub>4</sub>: C, 62.29; H, 4.95; N, 15.29; O, 17.47. Found: C, 62.34; H, 4.98; N, 15.37.

**3-(2, 4-difluorophenyl)-1-(5-phenyl-1H-tetrazol-1-yl) prop-2-en-1-one (3c):**

Creamy solid; % yield: 79; MP: 234-236°, IR (KBr)  $\nu$  (cm<sup>-1</sup>): 3059 (Ar-CH), 1712 (C=O), 1614 (C=C), 1608 (C=N), 1268 (N-N=N-), 1163 (C-F); <sup>1</sup>H NMR (CDCl<sub>3</sub>)  $\delta$  (ppm): 6.43 (d, 1H, ethylenic), 6.58 (s, 1H, Ar-H), 6.77 (s, 1H, Ar-H), 7.18 (d, 1H, Ar-H), 7.34-7.51 (m, 5H, Ar-H), 7.68 (d, 1H, ethylenic). Anal. Calcd. (%) for C<sub>16</sub>H<sub>10</sub>F<sub>2</sub>N<sub>4</sub>O: C, 61.54; H, 3.23; F, 12.17; N, 17.94; O, 5.12. Found: C, 61.72; H, 3.26; N, 18.12.

**3-(4-fluorophenyl)-1-(5-phenyl-1H-tetrazol-1-yl) prop-2-en-1-one (3d):**

Brown solid; % yield: 68; MP: 224-226°, IR (KBr)  $\nu$  (cm<sup>-1</sup>): 3060 (Ar-CH), 1734 (C=O), 1620 (C=C), 1606 (C=N), 1310 (N-N=N-), 1166 (C-F); <sup>1</sup>H NMR (CDCl<sub>3</sub>)  $\delta$  (ppm): 6.56 (d, 1H, ethylenic), 7.04 (d, 2H, Ar-H), 7.21 (d, 2H, Ar-H), 7.29-7.42 (m, 5H, Ar-H), 7.48 (d, 1H, ethylenic). Anal. Calcd. (%) for C<sub>16</sub>H<sub>11</sub>FN<sub>4</sub>O: C, 65.30; H, 3.77; F, 6.46; N, 19.04; O, 5.44. Found: C, 65.57; H, 3.80; N, 19.12.

**3-(2-hydroxy-5-nitrophenyl)-1-(5-phenyl-1H-tetrazol-1-yl) prop-2-en-1-one (3e):**

Brown solid; % yield: 63; MP: 252-254°, IR (KBr)  $\nu$  (cm<sup>-1</sup>): 3583 (OH), 3055 (Ar-CH), 1680 (C=O), 1630 (C=C), 1610 (C=N), 1564 (NO<sub>2</sub>), 1344 (N-N=N-); <sup>1</sup>H

NMR (CDCl<sub>3</sub>)  $\delta$  (ppm): 4.92 (s, 1H, -OH), 6.76 (d, 1H, ethylenic), 6.81 (d, 1H, Ar-H), 7.25-7.48 (m, 5H, Ar-H), 7.54 (d, 1H, Ar-H), 7.57 (d, 1H, ethylenic), 7.76 (s, 1H, Ar-H). Anal. Calcd. (%) for C<sub>16</sub>H<sub>11</sub>N<sub>5</sub>O<sub>4</sub>: C, 56.98; H, 3.29; N, 20.76; O, 18.97. Found: C, 57.16; H, 3.31; N, 20.84.

**3-(2-chloro-4-fluorophenyl)-1-(5-phenyl-1H-tetrazol-1-yl) prop-2-en-1-one (3f):**

Reddish brown solid; % yield: 74; MP: 220-222°, IR (KBr)  $\nu$  (cm<sup>-1</sup>): 3054 (Ar-CH), 1735 (C=O), 1630 (C=C), 1608 (C=N), 1285 (N-N=N-), 1178 (C-F), 786 (C-Cl); <sup>1</sup>H NMR (CDCl<sub>3</sub>)  $\delta$  (ppm): 6.37 (d, 1H, ethylenic), 6.74 (d, 1H, Ar-H), 7.06 (s, 1H, Ar-H), 7.17 (d, 1H, Ar-H), 7.28-7.44 (m, 5H, Ar-H), 7.72 (d, 1H, ethylenic). Anal. Calcd. (%) for C<sub>16</sub>H<sub>10</sub>ClFN<sub>4</sub>O: C, 58.46; H, 3.07; Cl, 10.78; F, 5.78; N, 17.04; O, 4.87. Found: C, 58.62; H, 3.11; N, 17.18.

**3-(2, 4-dimethoxyphenyl)-1-(5-phenyl-1H-tetrazol-1-yl) prop-2-en-1-one (3g):**

Brown solid; % yield: 78; MP: 246-248°, IR (KBr)  $\nu$  (cm<sup>-1</sup>): 3054 (Ar-CH), 1666 (C=O), 1640 (C=C), 1606 (C=N), 1275 (N-N=N-), 1248 (OCH<sub>3</sub>); <sup>1</sup>H NMR (CDCl<sub>3</sub>)  $\delta$  (ppm): 3.68 (s, 6H, -OCH<sub>3</sub>), 6.47 (d, 1H, Ar-H), 6.53 (d, 1H, Ar-H), 6.71 (d, 1H, ethylenic), 7.16 (d, 1H, Ar-H), 7.30-7.41 (m, 5H, Ar-H), 7.62 (d, 1H, ethylenic). Anal. Calcd. for C<sub>18</sub>H<sub>16</sub>N<sub>4</sub>O<sub>3</sub>: C, 64.28; H, 4.79; N, 16.66; O, 14.27. Found: C, 64.36; H, 4.81; N, 16.78.

**3-(2, 4, 6-trimethoxyphenyl)-1-(5-phenyl-1H-tetrazol-1-yl) prop-2-en-1-one (3h):**

Yellowish brown solid; % yield: 82; MP: 212-214°, IR (KBr)  $\nu$  (cm<sup>-1</sup>): 3064 (Ar-CH), 1666 (C=O), 1652 (C=C), 1606 (C=N), 1290 (N-N=N-), 1186 (OCH<sub>3</sub>); <sup>1</sup>H NMR (CDCl<sub>3</sub>)  $\delta$  (ppm): 3.66 (s, 9H, OCH<sub>3</sub>), 6.63 (d, 1H, ethylenic), 6.67 (s, 2H, Ar-H), 7.30-7.45 (m, 5H, Ar-H), 7.63 (d, 1H, ethylenic). Anal. Calcd. (%) for C<sub>19</sub>H<sub>18</sub>N<sub>4</sub>O<sub>4</sub>: C, 62.29; H, 4.95; N, 15.29; O, 17.47. Found: C, 62.44; H, 4.97; N, 15.36.

**3-(2-nitrophenyl)-1-(5-phenyl-1H-tetrazol-1-yl) prop-2-en-1-one (3i):**

Yellow solid; % yield: 87; MP: 228-230°, IR (KBr)  $\nu$  (cm<sup>-1</sup>): 3074 (Ar-CH), 1726 (C=O), 1620 (C=C), 1608 (C=N), 1578 (NO<sub>2</sub>), 1248 (N-N=N-); <sup>1</sup>H NMR (CDCl<sub>3</sub>)  $\delta$  (ppm): 6.65 (d, 1H, ethylenic), 7.28 (t, 1H, Ar-H), 7.32-7.53 (m, 5H, Ar-H), 7.58 (t, 1H, Ar-H), 7.61 (d, 1H, Ar-H), 7.78 (d, 1H, Ar-H), 7.93 (d, 1H, ethylenic).

Anal. Calcd. (%) for  $C_{16}H_{11}N_5O_3$ : C, 59.81; H, 3.45; N, 21.80; O, 14.94. Found: C, 59.93; H, 3.47; N, 21.88.

**3-(3-bromo-4-methoxyphenyl)-1-(5-phenyl-1H-tetrazol-1-yl) prop-2-en-1-one (3j):**

Yellowish brown solid; % yield: 78; MP: 164-166°, IR (KBr)  $\nu$  ( $cm^{-1}$ ): 3034 (Ar-CH), 1660 (C=O), 1615 (C=C), 1608 (C=N), 1255 (N-N=N-), 1170 (OCH<sub>3</sub>), 640 (C-Br); <sup>1</sup>H NMR (CDCl<sub>3</sub>)  $\delta$  (ppm): 3.77 (s, 3H, OCH<sub>3</sub>), 6.58 (d, 1H, ethylenic), 6.64 (d, 1H, Ar-H), 7.16 (d, 1H, Ar-H), 7.27 (s, 1H, Ar-H), 7.29-7.41 (m, 5H, Ar-H), 7.47 (d, 1H, ethylenic). Anal. Calcd. (%) for  $C_{17}H_{13}BrN_4O_2$ : C, 53.00; H, 3.40; Br, 20.74; N, 14.54; O, 8.31. Found: C, 53.21; H, 3.44; N, 14.61.

**General procedure for the synthesis of 1-[(5-substituted phenyl)-4, 5-dihydro-1H-pyrazol-3-yl]-5-phenyl-1H-tetrazole (4a-j):**

A mixture of 1-(5-substitutedphenyl)-1H-tetrazol-1-yl) prop-2-en-1-one (3a-j, 10 mmol), hydrazine hydrate (10 mmol) and acetic acid (40 ml) was refluxed for 5 h and then poured into ice cold water. The precipitate was separated by filtration, dried and recrystallized from ethanol.

**1-(5-(3-bromo-4-nitrophenyl))-4, 5-dihydro-1H-pyrazol-3-yl)-5-phenyl-1H-tetrazole (4a):**

Brown solid, % yield: 76; MP: 148-150°, IR (KBr)  $\nu$  ( $cm^{-1}$ ): 3352 (NH), 3073 (Ar-CH), 2990 (CH), 1622 (C=N), 1580 (NO<sub>2</sub>), 1270 (N-N=N-), 615 (C-Br); <sup>1</sup>H NMR (CDCl<sub>3</sub>)  $\delta$  (ppm): 2.10 (d, 2H, CH<sub>2</sub>), 3.74 (t, 1H, CH), 6.85 (s, 1H, NH), 7.23 (d, 1H, Ar-H), 7.32 (s, 1H, Ar-H), 7.37-7.53 (m, 5H, Ar-H), 7.87 (d, 1H, Ar-H). MS  $m/z$ : 413 [M<sup>+</sup>], Anal. Calcd. (%) for  $C_{16}H_{12}BrN_7O_2$ : C, 46.39; H, 2.92; N, 23.67; O, 7.73; Br, 19.29. Found: C, 46.52; H, 2.94; N, 23.74.

**1-(4, 5-dihydro-5-(3, 4, 5-trimethoxyphenyl)-1H-pyrazol-3-yl)-5-phenyl-1H-tetrazole (4b):**

Brown solid, % yield: 72; MP: 145-147°, IR (KBr)  $\nu$  ( $cm^{-1}$ ): 3340 (NH), 3052 (Ar-CH), 2930 (CH), 1640 (C=N), 1292 (N-N=N-), 1218 (OCH<sub>3</sub>); <sup>1</sup>H NMR (CDCl<sub>3</sub>)  $\delta$  (ppm): 1.83 (d, 2H, CH<sub>2</sub>), 3.62 (s, 9H, OCH<sub>3</sub>), 3.78 (t, 1H, CH), 6.71 (s, 2H, Ar-H), 7.18 (s, 1H, NH), 7.28-7.47 (m, 5H, Ar-H). MS  $m/z$ : 381 [M<sup>+</sup>], MS  $m/z$ : 380 [M<sup>+</sup>], Anal. Calcd. (%) for  $C_{19}H_{20}N_6O_3$ : C, 59.99; H, 5.30; N, 22.09; O, 12.62. Found: C, 61.23; H, 5.34; N, 22.18.

**1-(5-(2, 4-difluorophenyl)-4, 5-dihydro-1H-pyrazol-3-yl)-5-phenyl-1H-tetrazole (4c):**

Yellowish brown solid, % yield: 78; MP: 150-152°, IR

(KBr)  $\nu$  ( $cm^{-1}$ ): 3356 (NH), 3064 (Ar-CH), 2942 (CH), 1652 (C=N), 1289 (N-N=N-), 1158 (C-F); <sup>1</sup>H NMR (CDCl<sub>3</sub>)  $\delta$  (ppm): 1.93 (d, 2H, CH<sub>2</sub>), 3.79 (t, 1H, CH), 6.69 (s, 1H, Ar-H), 6.73 (d, 1H, Ar-H), 6.78 (d, 1H, NH), 7.14 (d, 1H, Ar-H), 7.30-7.51 (m, 5H, Ar-H). MS  $m/z$ : 326 [M<sup>+</sup>], Anal. Calcd. (%) for  $C_{16}H_{12}F_2N_6$ : C, 58.89; H, 3.37; F, 11.64; N, 25.76. Found: C, 58.72; H, 3.39; N, 25.85.

**1-(5-(4-fluorophenyl)-4, 5-dihydro-1H-pyrazol-3-yl)-5-phenyl-1H-tetrazole (4d):**

Brown solid, % yield: 72; MP: 159-161°, IR (KBr)  $\nu$  ( $cm^{-1}$ ): 3312 (NH), 3094 (Ar-CH), 2963 (CH), 1682 (C=N), 1308 (N-N=N-), 1163 (C-F); <sup>1</sup>H NMR (CDCl<sub>3</sub>)  $\delta$  (ppm): 1.77 (d, 2H, CH<sub>2</sub>), 3.86 (t, 1H, CH), 6.81 (d, 2H, Ar-H), 6.88 (d, 1H, NH), 7.22 (d, 2H, Ar-H), 7.33-7.47 (m, 5H, Ar-H). MS  $m/z$ : 309 [M<sup>+</sup>], Anal. Calcd. (%) for  $C_{16}H_{13}FN_6$ : C, 62.33; H, 4.25; F, 06.16; N, 27.26. Found: C, 62.51; H, 4.27; N, 27.34.

**2-(4, 5-dihydro-3-(5-phenyl-1H-tetrazol-1-yl)-1H-pyrazol-5-yl)-4-nitrophenol (4e):**

Reddish brown solid, % yield: 84; MP: 130-132°, IR (KBr)  $\nu$  ( $cm^{-1}$ ): 3540 (OH), 3345 (NH), 3062 (Ar-CH), 2902 (CH), 1638 (C=N), 1588 (NO<sub>2</sub>), 1292 (N-N=N-); <sup>1</sup>H NMR (CDCl<sub>3</sub>)  $\delta$  (ppm): 1.74 (d, 2H, CH<sub>2</sub>), 3.75 (t, 1H, CH), 4.88 (s, 1H, OH), 6.85 (d, 1H, Ar-H), 6.93 (d, 1H, NH), 7.28-7.54 (m, 5H, Ar-H), 7.88 (d, 1H, Ar-H), 7.94 (s, 1H, Ar-H). MS  $m/z$ : 352 [M<sup>+</sup>], Anal. Calcd. (%) for  $C_{16}H_{13}N_7O_3$ : C, 54.70; H, 3.73; N, 27.91; O, 13.66. Found: C, 54.87; H, 3.76; N, 27.98.

**1-(5-(2-chloro-4-fluorophenyl)-4, 5-dihydro-1H-pyrazol-3-yl)-5-phenyl-1H-tetrazole (4f):**

Yellowish brown solid, % yield: 71; MP: 162-164°, IR (KBr)  $\nu$  ( $cm^{-1}$ ): 3360 (NH), 3052 (Ar-CH), 2942 (CH), 1680 (C=N), 1306 (N-N=N-), 1165 (C-F), 782 (C-Cl); <sup>1</sup>H NMR (CDCl<sub>3</sub>)  $\delta$  (ppm): 2.07 (d, 2H, CH<sub>2</sub>), 3.78 (t, 1H, CH), 6.75 (d, 1H, Ar-H), 6.84 (d, 1H, NH), 7.08 (s, 1H, Ar-H), 7.17 (d, 1H, Ar-H), 7.25-7.41 (m, 5H, Ar-H). MS  $m/z$ : 343 [M<sup>+</sup>], Anal. Calcd. (%) for  $C_{16}H_{12}ClFN_6$ : C, 56.07; H, 3.53; Cl, 10.34; F, 5.54; N, 24.52. Found: C, 56.26; H, 3.55; N, 24.61.

**1-(4, 5-dihydro-5-(2, 4-dimethoxyphenyl)-1H-pyrazol-3-yl)-5-phenyl-1H-tetrazole (4g):**

Brown solid, % yield: 65; MP: 168-170°, IR (KBr)  $\nu$  ( $cm^{-1}$ ): 3325 (NH), 3086 (Ar-CH), 2980 (CH), 1644 (C=N), 1275 (N-N=N-), 1266 (OCH<sub>3</sub>); <sup>1</sup>H NMR (CDCl<sub>3</sub>)  $\delta$  (ppm): 2.10 (d, 2H, CH<sub>2</sub>), 3.82 (s, 6H, OCH<sub>3</sub>), 3.87 (t, 1H, CH), 6.68 (d, 1H, Ar-H), 6.74 (d,



1H, NH), 6.76 (d, 1H, Ar-H), 7.08 (d, 1H, Ar-H), 7.30-7.54 (m, 5H, Ar-H). MS *m/z*: 350 [M<sup>+</sup>], Anal. Calcd. (%) for C<sub>18</sub>H<sub>18</sub>N<sub>6</sub>O<sub>2</sub>: C, 61.70; H, 5.18; N, 23.99; O, 9.13. Found: C, 61.88; H, 5.23; N, 24.12;

**1-(4, 5-dihydro-5-(2, 4, 6-trimethoxyphenyl)-1H-pyrazol-3-yl)-5-phenyl-1H-tetrazole (4h):**

Yellow solid, % yield: 68; MP: 141-143°, IR (KBr)  $\nu$  (cm<sup>-1</sup>): 3358 (NH), 3092 (Ar-CH), 2960 (CH), 1653 (C=N), 1263 (N-N=N-), 1236 (OCH<sub>3</sub>); <sup>1</sup>H NMR (CDCl<sub>3</sub>)  $\delta$  (ppm): 2.05 (d, 2H, CH<sub>2</sub>), 3.63 (s, 9H, OCH<sub>3</sub>), 3.72 (t, 1H, CH), 6.77 (d, 1H, NH), 6.82 (s, 2H, Ar-H), 7.32-7.46 (m, 5H, Ar-H). MS *m/z*: 380 [M<sup>+</sup>], Anal. Calcd. (%) for C<sub>19</sub>H<sub>20</sub>N<sub>6</sub>O<sub>3</sub>: C, 59.99; H, 5.30; N, 22.09; O, 12.62. Found: C, 60.15; H, 5.35; N, 22.01.

**1-(4, 5-dihydro-5-(2-nitrophenyl)-1H-pyrazol-3-yl)-5-phenyl-1H-tetrazole (4i):**

Reddish brown solid; % yield: 69; MP: 165-167°, IR (KBr)  $\nu$  (cm<sup>-1</sup>): 3308 (NH), 3098 (Ar-CH), 2936 (CH), 1682 (C=N), 1592 (NO<sub>2</sub>), 1276 (N-N=N-); <sup>1</sup>H NMR (CDCl<sub>3</sub>)  $\delta$  (ppm): 1.93 (d, 2H, CH<sub>2</sub>), 3.76 (t, 1H, CH), 6.88 (d, 1H, NH), 7.26-7.49 (m, 8H, Ar-H), 7.86 (d, 1H, Ar-H). MS *m/z*: 335 [M<sup>+</sup>], Anal. Calcd. (%) for C<sub>16</sub>H<sub>13</sub>N<sub>7</sub>O<sub>2</sub>: C, 57.31; H, 3.91; N, 29.24; O, 9.54. Found: C, 57.53; H, 3.96; N, 29.37.

**1-(5-(3-bromo-4-methoxyphenyl)-4, 5-dihydro-1H-pyrazol-3-yl)-5-phenyl-1H-tetrazole (4j):**

Brown solid, % yield: 71; MP: 138-140°, IR (KBr)  $\nu$  (cm<sup>-1</sup>): 3348 (NH), 3060 (Ar-CH), 2964 (CH), 1632 (C=N), 1282 (N-N=N-), 1250 (OCH<sub>3</sub>), 618 (C-Br); <sup>1</sup>H NMR (CDCl<sub>3</sub>)  $\delta$  (ppm): 1.92 (d, 2H, CH<sub>2</sub>), 3.72 (t, 1H, CH), 3.78 (s, 3H, OCH<sub>3</sub>); 6.79 (d, 1H, Ar-H), 6.91 (d, 1H, NH), 7.13 (d, 1H, Ar-H), 7.24 (s, 1H, Ar-H), 7.29-7.48 (m, 5H, Ar-H). MS *m/z*: 399 [M<sup>+</sup>], Anal. Calcd. (%) for C<sub>17</sub>H<sub>15</sub>BrN<sub>6</sub>O: C, 51.14; H, 3.79; Br, 20.01; N, 27.44; O, 04.01. Found: C, 51.32; H, 3.83; N, 27.56.

**Antioxidant 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  $\mu$ g/ml was prepared for all the test compounds as well as of standard, ascorbic acid. Different concentrations were made of 10, 20, 30, 40 and 50  $\mu$ g/ml from stock solutions using methanol. DPPH in methanol (0.1 mM) was prepared in a volumetric flask and was completely kept away from light. One milliliter of all concentration of test and standards were mixed with 1.0 ml of DPPH solution. This solution was

kept for 30 min in dark place. Methanol with DPPH was used as control. Absorbance of all the samples was taken on UV-spectrophotometer at a  $\lambda_{\max}$  of 517 nm. The free radical scavenging was expressed as the percentage inhibition and was calculated using the formula: percentage inhibition = [(Ao-A)/Ao]×100, 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<sub>50</sub>). IC<sub>50</sub> values were calculated from calibration curve, and defined as the concentration of test compound required to achieve half maximal inhibition<sup>[35-37]</sup>.

**Antidiabetic activity:**

All the synthesized compounds were screened for *in vivo* antidiabetic activity by streptozotocin induced model in albino rat<sup>[38-41]</sup>.

**Acute toxicity studies:**

Group of 6 rats, weighing 180-200 g were fasted overnight and treated per orally with the test compounds at the dose of 200, 500, 1000 and 2000 mg/kg of body weight. The animals were observed for 72 h for any signs of acute toxicity such as increased or decreased motor activity, tremors, convulsion, sedation etc. It was observed that more than 50% of animals were died at the dose of 2000 mg/kg of body weight. Thus for the screening of antidiabetic activity, the dose selected was 200 mg/kg (i.e. 1/10 of the 2000 mg/kg of body weight) as per OECD guidelines. All the animal experiments were performed by the approval of Institutional Animal Ethics Committee (IAEC), Meerut Institute of Engineering and Technology, Meerut, India. During the study period, guidelines of the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), Institutional Animals Ethics Committee were followed for the maintenance of animals. The research work was approved by IAEC No: 711/02/a/CPCSEA/3/2013.

**Streptozotocin-induced experimental diabetes:**

The rats were injected intraperitoneally with streptozotocin dissolved in freshly prepared citrate buffer at a dose of 60 mg/kg body weight. A blood glucose range of 200-300 mg/dl was used for the experiment. Hyperglycemia was confirmed in animals after 72 h of streptozotocin injection.

### Experimental design:

Animals were divided into 13 groups of 6 animals (n=6). Group 1 treated as normal control (vehicle) received 1.0 ml of 0.5% CMC. Group 2 treated as diabetic control. Group 3 treated as diabetic animals received rosiglitazone 100 mg/kg. Groups 4-13 diabetic animals received compounds 4a-j in a single dose of 200 mg/kg body weight per oral respectively for 7 days continuously. Blood was withdrawn from the tail vein each time. Blood glucose was measured at 0, 7th, 14th, 21st, and 28th day by Blood Glucose Monitoring System.

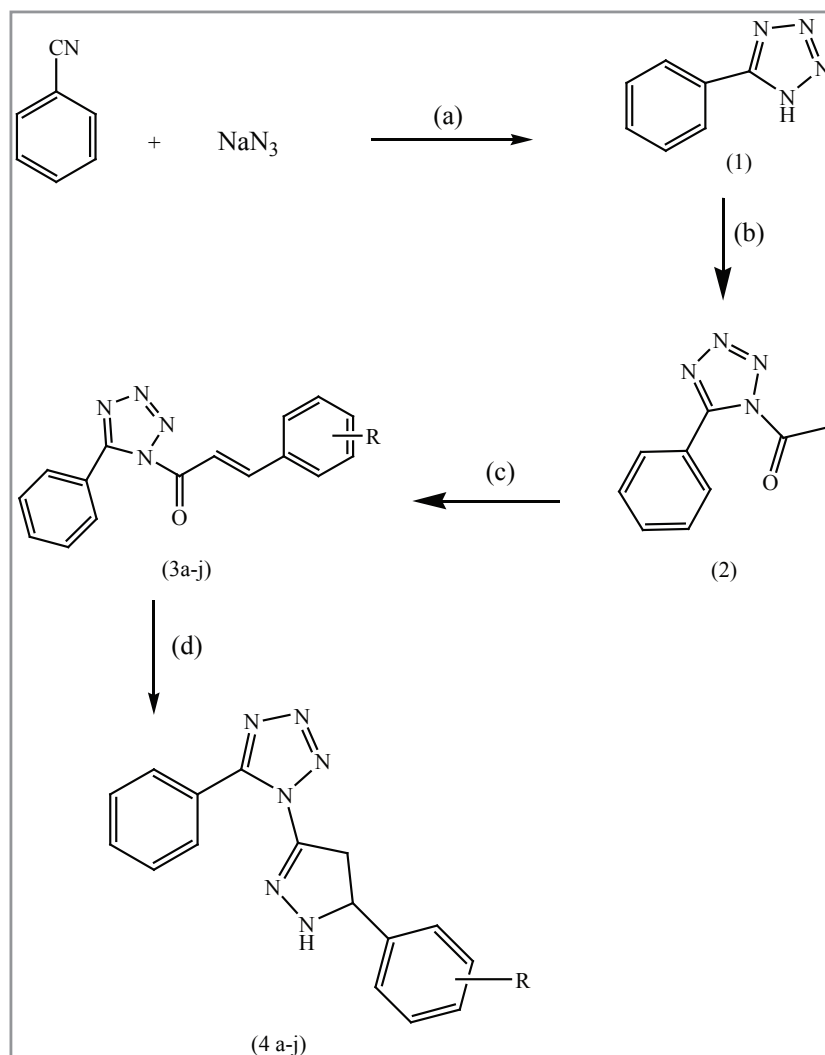
### Statistical analysis:

Values are represented as mean±SEM. Data were analyzed using analysis of variance and group means was compared with Bonferroni Post ANOVA test.

### RESULTS AND DISCUSSION

The reaction sequences used for the synthesis of titled compounds are shown in fig. 1. Several pyrazolotetrazole derivatives have been synthesized by Bhaskar *et al.* as target structures with different groups and evaluated for their biological activities like anticancer, anti-inflammatory and antimicrobial activity. They have found that pyrazolotetrazole nucleus could be a lead compound for drug development<sup>[42-43]</sup>. In the present study, an attempt was made to explore antioxidant and antidiabetic activity of synthesized compounds.

The compound, 5-phenyltetrazole (1) was synthesized by the reaction of sodium azide, benzonitrile, ammonium chloride and dimethylformamide, which after acetylation produce 5-phenyl-1-acetyl tetrazole (2). The compound 2 and substituted aromatic aldehyde in ethanol was cooled to 5 to 10° and further



**Fig. 1: Synthetic route of titled compounds.**

**Reagents and conditions:** (a) NH<sub>4</sub>Cl, DMF, Reflux; (b) (CH<sub>3</sub>CO)<sub>2</sub>O, H<sub>2</sub>SO<sub>4</sub>, heat at 60-70°; (c) R-CHO, ethanol, NaOH; (d) NH<sub>2</sub>NH<sub>2</sub>, H<sub>2</sub>O, acetic acid, reflux.

treated with aqueous sodium hydroxide solution. The resulting solution was diluted with ice cooled water and acidified to form 1-(5-substitutedphenyl)-*IH*-tetrazol-1-yl) prop-2-en-1-one 3a-j. The compounds 3a-j were refluxed with hydrazine hydrate and acetic acid to form 1-[(5-substituted phenyl)-4, 5-dihydro-*IH*-pyrazol-3-yl]-5-phenyl-*IH*-tetrazole (4a-j). Structures of synthesized compounds were confirmed by their spectral data interpretation.

The IR spectra of compound 3a-j showed characteristic C=O stretching at 1740-1666 cm<sup>-1</sup> and C=C stretching frequencies at 1652-1614 cm<sup>-1</sup>, characteristic absorption band at 1610-1593 cm<sup>-1</sup> and 1344-1248 cm<sup>-1</sup> due to the presence of C=N and N-N=N- group confirm the formation of tetrazole ring. In <sup>1</sup>H NMR spectra, the protons of  $\alpha$ ,  $\beta$ -unsaturated carbon atoms have displayed two doublets in the range of  $\delta$  6.40-6.80 and  $\delta$  7.23-7.93. Compounds 4a-j displayed characteristic absorption band in IR spectra at 3308-3360 cm<sup>-1</sup> due to NH functional group. <sup>1</sup>H NMR spectra of compounds 4a-j showed doublet in the range of  $\delta$  1.74-2.10 due to two methylene proton at C-5 of pyrazoline ring. The proton at C-4 position of pyrazoline ring also showed a triplet in the range of  $\delta$  3.74-3.87. All the aromatic protons were observed in the expected regions. Mass spectra of the compounds showed M+1 in agreement with their molecular formula.

All the synthesized compounds were screened for *in vitro* antioxidant activity by DPPH free radical scavenging method. Result obtained from *in vitro*

antioxidant activity is summarized in Table 1. The investigation of antioxidant screening revealed that some of the tested compounds showed moderate to good antioxidant activity. Particularly, compounds 4b, 4g, 4h and 4j have shown moderate antioxidant activity with IC<sub>50</sub> value at 13.19, 15.68, 22.41 and 16.14  $\mu$ g/ml as compared to standard, ascorbic acid.

All the synthesized compounds were screened *in vivo* for their antidiabetic activity by streptozotocin induced diabetic model in albino rat. Blood glucose levels in treatment of diabetic rats with synthesized derivatives were presented in Table 2. Among the synthesized compounds, only the compounds 4b, 4g, 4h, 4i and 4j

**TABLE 1: ANTIOXIDANT ACTIVITY OF STANDARD AND SYNTHESIZED COMPOUNDS**

Compound code	R	Antioxidant activity (IC <sub>50</sub> value in $\mu$ g/ml)
4a	3-bromo-4-nitro	74.81
4b	3,4,5-trimethoxy	13.19
4c	2,4-difluoro	54.11
4d	4-fluoro	40.93
4e	2-hydroxy-4-nitro	41.91
4f	2-chloro-4-fluoro	66.94
4g	2,4-dimethoxy	15.68
4h	2,4,6-trimethoxy	22.41
4i	2-nitro	34.59
4j	3-bromo-4-methoxy	16.14
Standard	Ascorbic acid	7.19

IC<sub>50</sub> - Concentration required to achieving half maximal inhibition

**TABLE 2: ANTIDIABETIC EFFECT OF SYNTHESIZED TEST COMPOUNDS**

Group	Blood glucose level (mg/dl)					% antidiabetic activity
	0 day	7 day	14 day	21 day	28 day	
Normal control	91.38±2.41	90.69±3.08	91.72±2.17	90.76±2.02	92.87±1.27	-
Diabetic control	267.40±4.23	270.65±4.33	276.44±3.88	284.20±3.35	287.45±4.80	-
4a	277.66±3.98	275.17±3.59	269.77±3.67	270.25±3.08	273.77±4.88	1.4
4b	268.99±2.55	254.02±4.86*	239.85±2.98*	165.12±3.70*	129.54±1.67*	51.84
4c	275.28±6.24	274.85±4.63	273.78±4.01	269.65±3.96	272.68±5.48	0.94
4d	277.33±4.69	272.03±6.27	265.15±5.30	269.51±4.59	273.28±3.91	1.28
4e	280.16±3.96	276.01±6.97	274.43±3.91	270.60±6.25	274.09±4.68	2.17
4f	274.81±4.41	267.05±4.17	265.23±3.72	270.45±5.35	273.44±4.92	0.49
4g	272.91±3.13	251.35±4.12*	235.44±5.13*	210.95±3.21*	174.49±4.70*	36.07
4h	270.42±2.94	249.43±4.01*	238.31±3.97*	215.54±4.38*	166.18±2.52*	38.54
4i	271.56±2.49	249.36±2.60*	217.06±4.92*	168.86±3.33*	161.67±3.27*	40.46
4j	281.24±2.77	250.11±2.38*	230.30±4.01*	180.91±2.78*	160.17±3.84*	43.04
Rosiglitazone	268.81±4.07	183.88±3.69*	153.65±3.50*	119.53±3.35*	110.81±3.53*	58.78

Values are expressed as mean±SEM (n=6). \*P<0.01, compared to diabetic control.

showed 51.84, 36.07, 38.54, 40.46 and 43.04% blood glucose lowering activity respectively.

In conclusion, a few number of new pyrazole containing tetrazole derivatives were synthesized by rapid and high yield synthetic route and evaluated for their *in vitro* antioxidant and *in vivo* antidiabetic activities. Presence of electron donating groups either at *o*-, *m*- or *p*-position, and electron withdrawing groups at *o*-, *m*-position of the phenyl ring attached to pyrazole nucleus as substituent elicits a moderate antioxidant and antidiabetic activity.

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### Conflicts of interest:

There are no conflicts of interest.

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