Synthesis of 4-Amino-1,5-dimethyl-2-phenylpyrazolone Derivatives and their Antioxidant Activity

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Summary: Schiff bases of 4-amino-1,5-dimethyl-2-phenylpyrazolone (**2-18**) were synthesized by treating with different benzaldehyde derivatives and tested for *in vitro* antioxidant activity. Synthesized Schiff base derivatives (**2-18**) were characterized by various spectroscopic techniques. Compounds specially having hydroxyl substituents showed good DPPH radical scavenging activity having IC_{50} values between 15.16 and 48.26 μM. Compounds **3-7** showed stronger potency than the standard (*n*-propyl gallate, IC_{50} = 30.27 ± 0.25 μM). However, in the superoxide anion radical assay, derivatives **2**, **4**, **5**, **6** and **7** showed better potency than the standard (*n*-propyl gallate, IC_{50} = 106.34 ± 1.60 μM).

Keywords: Pyrazolone; Antioxidant activity; DPPH; Superoxide radical scavenging assay.

Introduction

Heterocyclic compounds have great pharmacological and medicinal influences, the pyrazolone class is one of them. The compounds of this class exhibit antiinflamatory, antipyretic, analgesic [1,2], anticancer activities [3], and antimicrobial activities [4]. A number of pyrazolone derived compounds like antipyrine, dipyrone and aminopyrine are the known drugs used to have analgesic and antipyretic activities and the pharmacological molecular mechanism of these drugs have been widely invaestigated [5,6]. One of its renowned derivatives antipyrine has remarkable antipyretic and analgesic activities. Antipyrine is one of the popular drug in the world for treating infections like flu and fever [7]. Compounds of this sub-class recently showed tremendous anticorrosive activity which opens up new era between disciplines of material sciences and synthetic chemistry [8,9]. The importance of Schiff bases is extremely considerable in all classes of biological active molecule. Many of bases of other classes, showed good activities as β-glucuronidase inhibitors [10], DPPH radical and superoxide anion scavengers [11], phosphodiesterase-1 inhibitors [12], antiglycating [13] and antileishmanial [14]. The Schiff bases and their metal complexes showed intense and potent activities such as Gram-positive, Gram negative antibacterial, antifungal [15]. They are also found to have strong arantibacterial activity against *Staphylococcus aureus* [16].

Free radicals, known to be highly reactive molecules resulted *via* different biochemical reactions in the body. These free radicals lead the other metabolites to be oxidized and cause different diseases due to oxidative stress. Thus, it is essential to reduce the formation of free radicals with the help of antioxidants which can be either natural or synthetic compounds [17,18]. The main role of antioxidants is to prevent blood vessels membranes injury *via* inhibition or neutralization of the free radicals and ultimately avoid cardiovascular diseases. Thus providing first line defense against DNA damage and eventually prevent from the main cause of cancer [19,20]. Antioxidants acts *via* the radical scavenging activity by giving single electron to the radical and convert it into the risk-free molecule and thus defend cells from the oxidative damage and ultimately prevent the aging and diseases due to oxidative damage [21-23]. Biomolecules such as lipids, nucleic acids, proteins and various other classes like extra cellular matrix have strong attraction for free radicals. Literaure reveal that the pathology of arteriosclerosis, rheumatoid arthritis,

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malaria, neurodegenerative disease and aging have the major participation of free radicals [24-26].

Hence, the development of antioxidants, in order to supplement antioxidant system of the body, is an important area of interest in the current research.

Experimental

General Experimental

All solvents and reagents were of reagent grade and used directly without purification. The progress of all reactions was monitored by thin layer chromatography (TLC) which was performed on pre-coated silica gel aluminum plates (Kieselgel 60, 254, E. Merck, Germany). Chromatograms were visualized by UV at 254 and 365 nm. Electron impact mass spectra (EI MS) were recorded on a Finnigan MAT-311A, Germany (70eV) spectrophotometers and data are tabulated as *m/z*. ^{1}H-NMR spectra were recorded on Avance Bruker AM 300 and 400 MHz, respectively. Splitting patterns were as follows s = singlet; d = doublet; dd = double doublet, t = triplet; m = multiplet. Chemical shifts are reported in δ (ppm) and coupling constants are given in Hz. CHN analysis was performed on a Carlo Erba Strumentazione-Mod-1106, Italy.

Bioassay

The free radical scavenging activity of pyrazolone dervaitives series were measured by using 1,1-diphenyl-2-picrylhydrazil (DPPH) by following literature method reported in our previous paper [20]. A typical reaction mixture contains test sample (5 μL, 1 mM in DMSO) and 95 μL of DPPH (purchased from Sigma-Aldrich) in ethanol. A 96-well microtiter plate was used mixture incubation at 37º C for 30 min. Microtiter plate reader (Molecular Devices, USA) was used to meaured absorbance at 515 nm. A DMSO containign control only was compared to determine the radical scavenging activity. All the experiments were preformed in triplicate in dark. Inhibitory concentration at 50% inhibitition (IC_{50}) of DPPH were calculated and compared with *n*-Propyl gallate was used as a positive control. All the chemicals purchased from Sigma-Alrich.

General Procedure for the Synthesis of 4-Amino-1,5-dimethyl-2-phenylpyrazolone Derivatives **(2-18)**

Different derivatives of phenyl carbohydrazide (1 mmol) and phenyl pyrazolone **1** (1 mmol) were taken in ethanol (10 ml) with few drops of glacial acetic acid into a 100 ml round-bottommed flask. Than the reaction was refluxed for 2 to 4 h. TLC was monitored in order to check the completion of reaction (6:4 = EtOAc:Hexane). The solvent was evaporated on rotary evaporator in *vacuo* and then crude product were crystallized from a mixture of ethyl acetate and hexane. Final products were characterized by spectroscopic techniques (EIMS and NMR) and CHN analysis was also performed.

(E)-4-(2-Hydroxybenzylideneamino)-1,5-dimethyl-2-phenyl-1H-pyrazol-3(2H)-one **(1)**

^{1}H NMR (300 MHz, DMSO-d_6): δ 12.93 (s, 1H, N=CH), 9.69 (s, 1H, OH), 7.56 (s, 1H, ArCH=N), 7.53 (d, 1H, *J* = 7.8 Hz, H-3′), 7.47 (d, 1H, *J* = 7.5 Hz, H-6′), 7.41-7.36 (m, 3H, H-3, H-4, H-5), 7.33 (t, 1H, *J* = 7.8 Hz, H-5′), 7.26 (t, 1H, *J* = 7.5 Hz, H-4′) 6.93 (t, 1H, *J* = 7.5 Hz, H-2, H-6), 3.31 (s, 3H,RNCH_3), 2.49 (s, 3H,CH_3); ^{13}C NMR (75 MHz, DMSO-d6): δ 163.6 (CH, C=N), 161.2 (C, C-2′), 160.5 (C, C=O), 150.3 (C, C-9), 133.8 (C, C-1), 132.5 (CH, C-4′), 132.2 (CH, C-6′), 129.3 (CH, C-3), 129.3 (CH, C-5), 123.8 (CH, C-2), 123.8 (CH, C-6), 122.7 (CH, C-4), 121.3 (CH, C-5′), 118.4 (C, C-1′), 117.7 (CH, C-3′), 110.0 (C, C-10); Anal. Calcd for $C_{18}H_{17}N_3O_2$, C = 70.34, H = 5.58, N = 13.67, Found C = 70.33, H = 5.56, N = 13.69; EI MS *m/z* (% rel. abund.): 307 (100.0%).

(E)-4-(3-hydroxybenzylideneamino)-1,5-dimethyl-2-phenyl-1H-pyrazol-3(2H)-one **(2)**

^{1}H NMR (300 MHz, DMSO-d_6): δ 9.55 (s, 1H, OH), 9.44 (s, 1H, N=CH), 7.55 (t, 2H, *J* = 7.8Hz, H-2,H-6), 7.37 (t, 3H, *J* = 3.9 Hz, H-4′, H-5′, H-6′), 7.26 (m, 3H, H-3, H-4, H-5), 6.82 (d, 1H, *J* = 7.8 Hz, H-2′), 3.31 (s, 3H, RNCH_3), 2.49 (s, 3H, CH_3); ^{13}C NMR (75 MHz, DMSO-d6): δ 163.6 (CH, C=N), 160.8 (C, C=O), 158.7 (C, C-3′), 150.1 (C, C-9), 135.0 (C, C-1′), 133.8 (C, C-1), 130.1 (CH, C-5′), 129.3 (CH, C-3), 129.3 (CH, C-5), 123.8 (CH, C-2), 123.8 (CH, C-6), 122.7 (CH, C-4), 121.7 (CH, C-6′),118.1 (CH, C-4′), 114.8 (CH, C-2′), 110.0 (C, C-10); Anal. Calcd for $C_{18}H_{17}N_3O_2$, C = 70.34, H = 5.58, N = 13.67, Found C = 70.32, H = 5.57, N = 13.69; EI MS *m/z* (% rel. abund.): 307 (100.0%);

(E)-4-(2,4-dihydroxybenzylideneamino)-1,5-dimethyl-2-phenyl-1H-pyrazol-3(2H)-one **(3)**

^{1}H NMR (300 MHz, DMSO-d_6): δ 12.47 (s, 1H, 2′, OH), 9.90 (s, 1H, N=CH), 8.91 (s, 1H, 5′, OH), 8.62 (d, 1H, *J* = 8.4 Hz, H-3′), 7.71 (d, 1H, *J* = 1.8 Hz, H-5′), 7.55 (t, 1H, *J* = 7.8 Hz, H-6′), 7.52 (t,

3H, *J* = 7.8 Hz, H-2, H-6), 7.43 (t, 3H, *J* = 9.3 Hz, H-3, H-4, H-5), 3.32 (s, 3H, $RNCH_3$), 2.48 (s, 3H, CH_3); ^{13}C NMR (75 MHz, DMSO-d6): ∂ 163.8 (CH, C=N), 162.4 (C, C-2′), 162.3 (C, C-4′), 160.6 (C, C=O), 150.1 (C, C-9), 133.8 (C, C-1), 133.7 (CH, C-6′), 129.1 (CH, C-3), 129.1 (CH, C-5), 124.0 (CH, C-2), 124.0 (CH, C-6), 122.7 (CH, C-4), 111.0 (C, C-1′), 110.2 (C, C-10), 108.5 (CH, C-5′), 103.8 (CH, C-3′); Anal. Calcd for $C_{18}H_{17}N_3O_2$, C = 66.86, H = 5.30, N = 13.00, Found C = 66.82, H = 5.33, N = 13.03; EI MS *m/z* (% rel. abund.): 323 (100.0%);

(E)-4-((1-(2,4-dihydroxyphenyl)ethylidene)amino)-1,5-dimethyl-2-phenyl-1H-pyrazol-3(2H)-one **(4)**

^{1}H NMR (300 MHz, DMSO-d_6): ∂ 12.47 (s, 1H, 2′, OH), 8.90 (s, 1H, 5′, OH), 8.61 (d, 1H, *J* = 8.4 Hz, H-3′), 7.70 (d, 1H, *J* = 1.8 Hz, H-5′), 7.54 (t, 1H, *J* = 7.8 Hz, H-6′), 7.51 (t, 3H, *J* = 7.8 Hz, H-2, H-6), 7.42 (t, 3H, *J* = 9.3 Hz, H-3, H-4, H-5), 3.31 (s, 3H, $RNCH_3$), 2.46 (s, 3H, CH_3), 2.32 (s, 3H, CH_3); ^{13}C NMR (75 MHz, DMSO-d6): ∂ 164.5 (C, C=N), 162.4 (C, C-2′), 162.3 (C, C-4′), 160.8 (C, C=O), 150.3 (C, C-9), 133.8 (C, C-1), 133.7 (CH, C-6′), 129.3 (CH, C-3), 129.3 (CH, C-5), 123.8 (CH, C-2), 123.8 (CH, C-6), 122.7 (CH, C-4), 111.0 (C, C-1′), 110.0 (C, C-10), 108.5 (CH, C-5′), 103.6 (CH, C-3′), 16.7 (CH_3, C); Anal. Calcd for $C_{18}H_{17}N_3O_2$, C = 67.64, H = 5.68, N = 12.46, Found C = 67.63, H = 5.66, N = 12.48; EI MS *m/z* (% rel. abund.): 337 (100.0%);

(E)-4-(2,5-dihydroxybenzylideneamino)-1,5-dimethyl-2-phenyl-1H-pyrazol-3(2H)-one **(5)**

^{1}H NMR (300 MHz, DMSO-d_6): ∂ 12.50 (s, 1H, 2′, OH), 9.60 (s, 1H, N=CH), 8.94 (s, 1H, 5′, OH), 7.55 (t, 2H, *J* = 7.5 Hz, H-2, H-6), 7.40 (t, 3H, *J* = 7.8 Hz, H-3, H-4, H-5), 6.80 (s, 1H, H-6′), 6.73 (s, 2H, H-3′,H-4′), 3.32(s, 3H,$RNCH_3$), 2.49 (s, 3H,CH_3); ^{13}C NMR (75 MHz, DMSO-d6): ∂ 163.6 (CH, C=N), 160.8 (C, C=O), 153.6 (C, C-2′), 151.1 (C, C-5′), 150.1 (C, C-9), 133.8 (C, C-1), 129.1 (CH, C-3), 129.1 (CH, C-5), 123.7 (CH, C-2), 123.7 (CH, C-6), 122.7 (CH, C-4), 120.3 (CH, C-3′), 119.8 (C, C-1′), 119.5 (CH, C-4′), 116.4 (CH, C-6′), 110.0 (C, C-10); Anal. Calcd for $C_{18}H_{17}N_3O_2$, C = 66.86, H = 5.30, N = 13.00, Found C = 66.88, H = 5.31, N = 13.03; EI MS *m/z* (% rel. abund.): 323 (100.0%);

(E)-4-(1-(2,5-dihydroxyphenyl)ethylideneamino)-1,5-dimethyl-2-phenyl-1H-pyrazol-3(2H)-one **(6)**

^{1}H NMR (300 MHz, DMSO-d_6): ∂ 12.49 (s, 1H, 2′, OH), 8.93 (s, 1H, 5′, OH), 7.54 (t, 2H, *J* = 7.5 Hz, H-2, H-6), 7.40 (t, 3H, *J* = 7.8 Hz, H-3, H-4, H-5), 6.79(s, 1H, H-6′), 6.72 (s, 2H, H-3′,H-4′), 3.31(s, 3H,$RNCH_3$), 2.48 (s, 3H,CH_3), 2.33 (s, 3H, CH_3); ^{13}C NMR (75 MHz, DMSO-d6): ∂ 164.5 (C, C=N), 160.8 (C, C=O), 155.2 (C, C-2′), 151.1 (C, C-5′), 150.3 (C, C-9), 133.8 (C, C-1), 129.3 (CH, C-3), 129.3 (CH, C-5), 123.8 (CH, C-2), 123.8 (CH, C-6), 122.7 (CH, C-4), 120.3 (CH, C-3′), 120.1 (C, C-1′), 119.5 (CH, C-4′), 116.2 (CH, C-6′), 110.1 (C, C-10), 16.7 (CH_3, C); Anal. Calcd for $C_{18}H_{17}N_3O_2$, C = 67.64, H = 5.68, N = 12.46, Found C = 67.62, H = 5.69, N = 12.48; EI MS *m/z* (% rel. abund.): 337 (100.0%);

(E)-4-(4-methoxybenzylideneamino)-1,5-dimethyl-2-phenyl-1H-pyrazol-3(2H)-one **(7)**

^{1}H NMR (300 MHz, DMSO-d_6): ∂ 9.50 (s, 1H, N=CH), 7.76 (d, 2H, *J* = 8.7 Hz, H-2, H-6), 7.54 (d, 2H, *J* = 7.2 Hz, H-2′, H-6′), 7.37-7.32 (m, 3H, H-3, H-4, H-5), 7.01 (d, 2H, *J* = 8.7 Hz, H-3′, H-5′), 3.80 (s, 3H, OCH_3), 3.13 (s, 3H, $RNCH_3$), 2.49 (s, 3H, CH_3); ^{13}C NMR (75 MHz, DMSO-d6): ∂ 163.6 (CH, C=N), 162.7 (C, C-4′), 160.6 (C, C=O), 150.1 (C, C-9), 133.8 (C, C-1), 130.1 (CH, C-2′), 130.1 (CH, C-6′), 129.1 (CH, C-3), 129.1 (CH, C-5), 126.2 (C, C-1′), 123.8 (CH, C-2), 123.8 (CH, C-6), 122.7 (CH, C-4), 114.3 (CH, C-3′), 114.0 (CH, C-5′), 110.0 (C, C-10), 55.7 (OCH_3, C); Anal. Calcd for $C_{18}H_{17}N_3O_2$, C = 71.01, H = 5.96, N = 13.08, Found C = 71.03, H = 5.98, N = 13.07; EI MS *m/z* (% rel. abund.): 321 (100.0%);

(E)-1,5-dimethyl-4-((4-(methylthio)benzylidene)amino)-2-phenyl-1H-pyrazol-3(2H)-one **(8)**

^{1}H NMR (300 MHz, DMSO-d_6): ∂ 9.48 (s, 1H, N=CH), 7.75 (d, 2H, *J* = 8.7 Hz, H-2, H-6), 7.54 (d, 2H, *J* = 7.2 Hz, H-2′, H-6′), 7.37-7.33 (m, 3H, H-3, H-4, H-5), 7.02 (d, 2H, *J* = 8.7 Hz, H-3′, H-5′), 3.79 (s, 3H, SCH_3), 3.12 (s, 3H, $RNCH_3$), 2.48 (s, 3H, CH_3); ^{13}C NMR (75 MHz, DMSO-d6): ∂ 163.8 (CH, C=N), 160.7 (C, C=O), 150.3 (C, C-9), 141.8 (C, C-4′), 133.8 (C, C-1), 130.2 (C, C-1′), 129.5 (CH, C-2′), 129.5 (CH, C-6′), 129.3 (CH, C-3), 129.3 (CH, C-5), 126.8 (CH, C-3′), 126.8 (CH, C-5′), 123.7 (CH, C-2), 123.7 (CH, C-6), 122.9 (CH, C-4), 110.2 (C, C-10), 14.7 (SCH_3, C); Anal. Calcd for $C_{18}H_{17}N_3O_2$, C = 67.63, H = 5.68, N = 12.45, Found C = 67.65, H = 5.67, N = 12.46; EI MS *m/z* (% rel. abund.): 337 (100.0%);

(E)-4-(2-hydroxy-3-methoxybenzylideneamino)-1,5-dimethyl-2-phenyl-1H-pyrazol-3(2H)-one **(9)**

^{1}H NMR (300 MHz, DMSO-d_6): ∂ 13.01 (s, 1H, 2′, OH), 9.67 (s, 1H, N=CH), 7.56 (t, 2H, *J* = 7.5 Hz, H-2, H-6), 7.41 (t, 3H, *J* = 8.1Hz, H-3, H-4, H-5), 7.06 (t, 2H, *J* = 6.9 Hz H-4′, H-6′), 6.87 (t, 1H, *J* = 7.8Hz, H-5′), 3.80 (s, 3H, OCH_3), 3.31(s, 3H,$RNCH_3$), 2.49 (s, 3H,CH_3);

^{13}C NMR (75 MHz, DMSO-d6): ∂ 163.6 (CH, C=N), 160.8 (C, C=O), 150.3 (C, C-9), 150.0 (C, C-2′), 149.1 (C, C-3′), 133.8 (C, C-1), 129.3 (CH, C-3), 129.3 (CH, C-5), 124.5 (CH, C-6′), 123.8 (CH, C-2), 123.8 (CH, C-6), 122.7 (CH, C-4), 119.4 (C, C-1′), 116.7 (CH, C-5′), 115.1 (CH, C-4′), 110.0 (C, C-10), 56.2 (OCH_3, C); Anal. Calcd for $C_{18}H_{17}N_3O_2$, C = 67.64, H = 5.68, N = 12.46, Found C = 67.66, H = 5.67, N = 12.48; EI MS *m/z* (% rel. abund.): 337 (100.0%);

(E)-4-(3-ethoxy-2-hydroxybenzylideneamino)-1,5-dimethyl-2-phenyl-1H-pyrazol-3(2H)-one **(10)**

^{1}H NMR (300 MHz, DMSO-d_6): ∂ 13.08 (s, 1H, 2′, OH), 9.67 (s, 1H, N=CH), 7.56 (t, 2H, *J* = 7.8 Hz, H-2, H-6), 7.41 (t, 3H, *J* = 8.1Hz, H-3, H-4, H-5), 7.04 (t, 2H, *J* = 7.5 Hz H-4′, H-6′), 6.87 (t, 1H, *J* = 7.8Hz, H-5′), 4.05 (q, 2H, *J* = 6.9 Hz, OCH_2), 2.40 (s, 3H, $RNCH_3$), 2.49 (s, 3H,CH_3), 2.72 (s, 3H,OCH_2CH_3); ^{13}C NMR (75 MHz, DMSO-d6): ∂ 163.6 (CH, C=N), 160.8 (C, C=O), 150.1 (C, C-9), 150.3 (C, C-2′), 148.2 (C, C-3′), 133.8 (C, C-1), 129.1 (CH, C-3), 129.1 (CH, C-5), 123.8 (CH, C-2), 123.8 (CH, C-6), 123.6 (CH, C-6′), 122.7 (CH, C-4), 119.2 (C, C-1′), 118.0 (CH, C-4′), 116.1 (CH, C-5′), 110.0 (C, C-10), 64.8 (OCH_2, C), 14.7 (CH_3, C); Anal. Calcd for $C_{18}H_{17}N_3O_2$, C = 68.36, H = 6.02, N = 11.96, Found C = 68.35, H = 6.04, N = 11.95; EI MS *m/z* (% rel. abund.): 351 (100.0%);

(E)-1,5-dimethyl-2-phenyl-4-(pyridin-4-ylmethyleneamino)-1H-pyrazol-3(2H)-one **(11)**

^{1}H NMR (300 MHz, DMSO-d_6): ∂ 9.53 (s, 1H, N=CH), 8.64 (d, 2H, *J* = 5.4 Hz, H-3′, H-5′), 7.73 (d, 2H, *J* = 5.7 Hz, H-2, H-6), 7.56 (t, 2H, *J* = 7.5 Hz, H-2′, H-6′), 7.42 (q, 3H, *J* = 7.2 Hz, H-3, H-4, H-5), 3.31 (s, 3H, $RNCH_3$), 2.49 (s, 3H, CH_3); ^{13}C NMR (75 MHz, DMSO-d6): ∂ 163.7 (CH, C=N), 160.6 (C, C=O), 150.1 (C, C-9), 149.3 (CH, C-2′), 149.3 (CH, C-6′), 144.2 (C, C-6′), 133.8 (C, C-1), 129.1 (CH, C-3), 129.1 (CH, C-5), 123.8 (CH, C-2), 123.8 (CH, C-6), 122.7 (CH, C-4), 120.2 (CH, C-3′), 120.2 (CH, C-5′), 110.3 (C, C-10); Anal. Calcd for $C_{18}H_{17}N_3O_2$, C = 69.85, H = 5.52, N = 19.17, Found C = 69.86, H = 5.55, N = 19.18; EI MS *m/z* (% rel. abund.): 292 (100.0%);

(E)-1,5-dimethyl-2-phenyl-4-(pyridin-3-ylmethyleneamino)-1H-pyrazol-3(2H)-one **(12)**

^{1}H NMR (300 MHz, DMSO-d_6): ∂ 9.60 (s, 1H, N=CH), 8.92 (s, 1H, H-2′), 8.59 (d, 1H, *J* = 3.9 Hz, H-4′), 8.21 (d, 1H, *J* = 7.8 Hz, H-5′), 7.55-7.35 (m, 6H, H-6′, H-2, H-6, H-3, H-4, H-5), 3.32 (s, 3H, $RNCH_3$), 2.49 (s, 3H, CH_3); ^{13}C NMR (75 MHz, DMSO-d6): ∂ 163.9 (CH, C=N), 160.8 (C, C=O), 151.7 (CH, C-6′), 150.3 (C, C-9), 149.1 (CH, C-2′), 133.7 (C, C-1), 133.5 (CH, C-4′), 130.3 (C, C-3′), 129.0 (CH, C-3), 129.0 (CH, C-5), 123.8 (CH, C-2), 123.8 (CH, C-6), 123.7 (CH, C-5′), 122.6 (CH, C-4), 110.2 (C, C-10); Anal. Calcd for $C_{18}H_{17}N_3O_2$, C = 69.85, H = 5.52, N = 19.17, Found C = 69.84, H = 5.53, N = 19.15; EI MS *m/z* (% rel. abund.): 292 (100.0%);

(E)-4-(4-fluorobenzylideneamino)-1,5-dimethyl-2-phenyl-1H-pyrazol-3(2H)-one **(13)**

^{1}H NMR (300 MHz, DMSO-d_6): ∂ 9.56 (s, 1H, N=CH), 7.88 (dd, 2H, *J* = 6.0Hz, H-2, H-6), 7.55 (t, 2H, *J* = 7.8 Hz, H-3′, H-5′), 7.37 (t, 3H, H-3, H-4, H-5), 7.30 (t, 2H, *J* = 8.7 Hz, H-2′, H-6′), 3.30 (s, 3H, $RNCH_3$), 2.49 (s, 3H, CH_3); ^{13}C NMR (75 MHz, DMSO-d6): ∂ 165.3 (C, C-4′), 163.5 (CH, C=N), 160.6 (C, C=O), 150.1 (C, C-9), 133.8 (C, C-1), 130.7 (CH, C-2′), 130.7 (CH, C-6′), 129.4 (C, C-1′), 129.0 (CH, C-3), 129.0 (CH, C-5), 123.7 (CH, C-2), 123.7 (CH, C-6), 122.6 (CH, C-4), 115.7 (CH, C-3′), 115.5 (CH, C-5′), 110.0 (C, C-10); Anal. Calcd for $C_{18}H_{17}N_3O_2$, C = 69.89, H = 5.21, N = 13.58, Found C = 69.88, H = 5.22, N = 13.59; EI MS *m/z* (% rel. abund.): 309 (100.0%)

(E)-4-(2,6-dichlorobenzylideneamino)-1,5-dimethyl-2-phenyl-1H-pyrazol-3(2H)-one **(14)**

^{1}H NMR (300 MHz, DMSO-d_6): ∂ 9.58 (s, 1H, N=CH), 7.77 (d, 2H, *J* = 8.2 Hz, H-2, H-6), 7.39-7.35 (m, 3H, H-3, H-4, H-5), 7.20 (t, 1H, *J* = 7.8 Hz, H-4′), 7.02 (d, 2H, *J* = 8.6 Hz, H-3′, H-5′), 3.81 (s, 3H, OCH_3), 3.14 (s, 3H, $RNCH_3$), 2.50 (s, 3H, CH_3); ^{13}C NMR (75 MHz, DMSO-d6): ∂ 163.6 (CH, C=N), 160.9 (C, C=O), 150.1 (C, C-9), 133.7 (C, C-1), 133.4 (C, C-2′), 133.4 (C, C-6′), 131.7 (C, C-1′), 129.1 (CH, C-3), 129.1 (CH, C-5), 129.0 (CH, C-4′), 128.1 (CH, C-3′), 128.1 (CH, C-5′), 123.8 (CH, C-2), 123.8 (CH, C-6), 122.7 (CH, C-4), 110.3 (C, C-10); Anal. Calcd for $C_{18}H_{17}N_3O_2$, C = 60.01, H = 4.20, N = 11.66, Found C = 60.00, H = 4.22, N = 11.68; EI MS *m/z* (% rel. abund.): 359 (100.0%), 361 (64.4%);

(E)-4-(2,4-dichlorobenzylideneamino)-1,5-dimethyl-2-phenyl-1H-pyrazol-3(2H)-one **(15)**

^{1}H NMR (300 MHz, DMSO-d_6): ⌯ 9.91 (s, 1H, N=CH), 8.64 (d, 1H, *J* = 8.4 Hz, H-3′), 7.70 (d, 1H, *J* = 1.8 Hz, H-5′), 7.55 (t, 1H, *J* = 7.8 Hz, H-6′), 7.53 (t, 3H, *J* = 7.8 Hz, H-2, H-6), 7.41 (t, 3H, *J* = 9.3 Hz, H-3, H-4, H-5), 3.31 (s, 3H, $RNCH_3$), 2.49 (s, 3H, CH_3); ^{13}C NMR (75 MHz, DMSO-d6): ⌯ 163.6 (CH, C=N), 160.9 (C, C=O), 150.1 (C, C-9), 133.7 (C, C-1), 131.4 (C, C-1′), 131.0 (C, C-2′), 129.3 (CH, C-3), 129.3 (CH, C-5), 129.1 (CH, C-3′), 129.0 (CH, C-6′), 128.2 (C, C-4′), 127.3 (CH, C-5′), 123.8 (CH, C-2), 123.8 (CH, C-6), 122.7 (CH, C-4), 110.0 (C, C-10); Anal. Calcd for $C_{18}H_{17}N_3O_2$, C = 60.01, H = 4.20, N = 11.66, Found C = 60.02, H = 4.23, N = 11.67; EI MS *m/z* (% rel. abund.): 359 (100.0%), 361 (66.5%);

(E)-1,5-dimethyl-4-(4-nitrobenzylideneamino)-2-phenyl-1H-pyrazol-3(2H)-one **(16)**

^{1}H NMR (300 MHz, DMSO-d_6): ⌯ 9.60 (s, 1H, N=CH), 7.79 (d, 2H, *J* = 8.2 Hz, H-2, H-6), 7.56 (d, 2H, *J* = 7.2 Hz, H-2′, H-6′), 7.39-7.35 (m, 3H, H-3, H-4, H-5), 7.03 (d, 2H, *J* = 8.6 Hz, H-3′, H-5′), 3.82 (s, 3H, OCH_3), 3.15 (s, 3H, $RNCH_3$), 2.51 (s, 3H, CH_3); ^{13}C NMR (75 MHz, DMSO-d6): ⌯ 163.6 (CH, C=N), 160.8 (C, C=O), 150.1 (C, C-9), 150.0 (C, C-4′), 139.7 (C, C-1′), 133.8 (C, C-1), 129.3 (CH, C-3), 129.3 (CH, C-5), 127.7 (CH, C-2′), 127.7 (CH, C-6′), 124.1 (CH, C-3′), 124.1 (CH, C-5′), 123.8 (CH, C-2), 123.8 (CH, C-6), 122.7 (CH, C-4), 110.2 (C, C-10); Anal. Calcd for $C_{18}H_{17}N_3O_2$, C = 64.28, H = 4.79, N = 16.66, Found C = 64.26, H = 4.77, N = 16.67; EI MS *m/z* (% rel. abund.): 336 (100.0%);

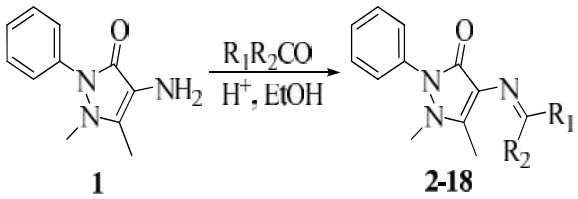
(E)-1,5-dimethyl-4-(3-nitrobenzylideneamino)-2-phenyl-1H-pyrazol-3(2H)-one **(17)**

^{1}H NMR (300 MHz, DMSO-d_6): ⌯ 9.60 (s, 1H, N=CH), 9.80 (s, 1H, H-2′), 8.40 (d, 1H, *J* = 8.1 Hz, H-4′), 8.23 (t, 2H, *J* = 7.5 Hz, H-2, H-6), 7.77 (t, 1H, *J* = 7.8 Hz, H-5′), 7.60 (d, 1H, *J* = 7.8 Hz, H-6′), 7.32-7.12 (m, 3H, H-3, H-4, H-5), 3.32 (s, 3H, $RNCH_3$), 2.50 (s, 3H, CH_3); ^{13}C NMR (75 MHz, DMSO-d6): ⌯ 163.5 (CH, C=N), 160.6 (C, C=O), 150.0 (C, C-9), 148.1 (C, C-3′), 135.2 (CH, C-6′), 134.5 (C, C-1′), 133.7 (C, C-1), 129.8 (CH, C-5′), 129.1 (CH, C-3), 129.1 (CH, C-5), 126.0 (CH, C-4′), 123.8 (CH, C-2), 123.8 (CH, C-6), 122.7 (CH, C-4), 121.5 (CH, C-2′), 110.0 (C, C-10); Anal. Calcd for $C_{18}H_{17}N_3O_2$, C = 64.28, H = 4.79, N = 16.66, Found C = 64.27, H = 4.80, N = 16.67; EI MS *m/z* (% rel. abund.): 336 (100.0%);

Results and Discussion

Chemistry

By keeping in mind, the multi-dimensional activities of Schiff bases, we synthesized different phenyl hydrazones derivatives of phenyl pyrazolone by reacting it with the different derivatives of phenyl carbohydrazide in ethanol with glacial acetic acid as catalyst under reflux for 2 to 4 h (Scheme 1). The products obtained in good yield. Pure products were obtained after crystallization of crude product from ethanol. The structures of phenyl hydrazones of phenyl pyrazolone **2-18** were deduced by utilizing different spectroscopic techniques including EIMS, IR, and NMR. All compounds gave satisfactory elemental analyses in good concurrence with calculated values.



Scheme 1: Synthesis of Schiff base derivatives of N-Phenyl Pyrazolone.

Antioxident Activity

DPPH Radical Scavenging Activity

The synthesized derivatives **2-18** were tested for *in vitro* free radical scavenging activity by following the procedure reported in our previous publication [20]. The compounds demonstrated the IC_{50} values within the range of 15.16-48.26 μM (Table 1). From bioactivity data, the compounds having hydroxyl groups are found active. Moreover, the DPPH activity of dihydroxy pyrazolones is found higher than monohydroxy derivaitives. The dihydroxyl compound **7** having hydroxyl substituent at 2' and 5' positions (IC_{50} = 15.16 ± 1.09 μM) is the most active compound in the series showed two-folds more activity than the standard (IC_{50} = 30.30 ± 0.25 μM). The 2' and 5' positions of hydroxyl groups plays an important role in activity, possibly through stabilizing the free radicals generated from **7**.

Table-1: *In Vitro* DPPH Radical Scavenging and Superoxide Anion Radical Scavenging Activities of Schiff bases of Phenyl Pyrazolone **2-18**.

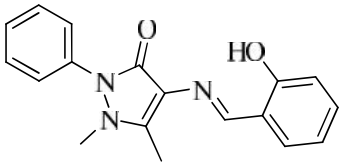
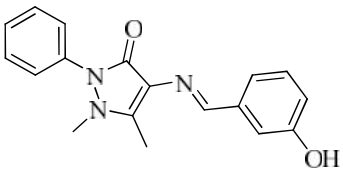
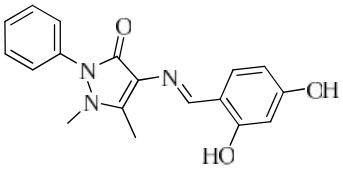
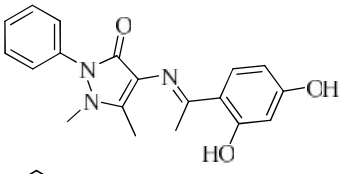
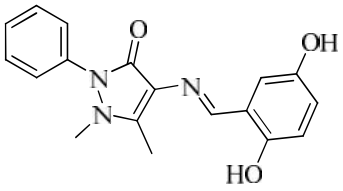
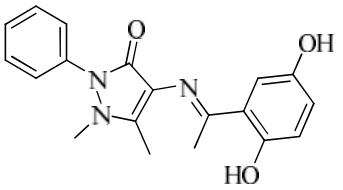
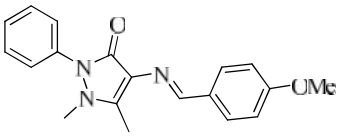
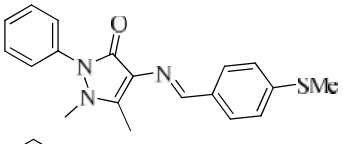
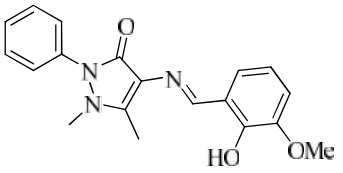
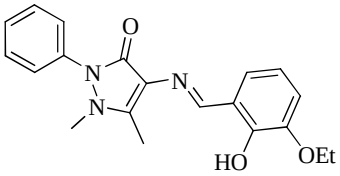
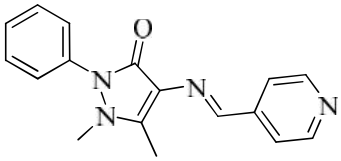
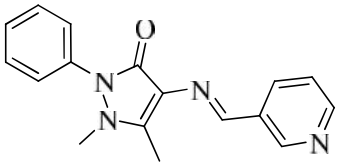
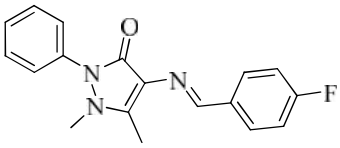
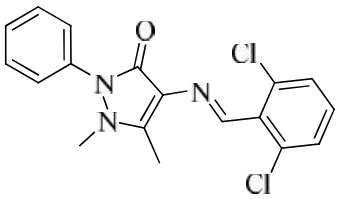
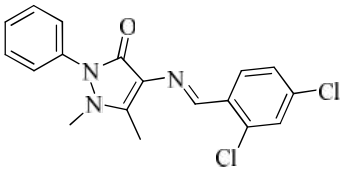
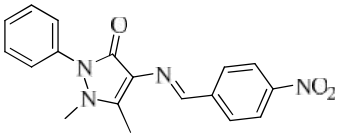
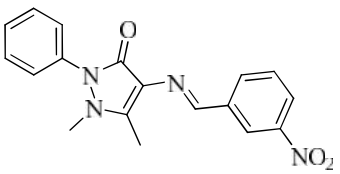
S. No.	Structures of Compounds	IC50 (μM ± SEM[a]) DPPH	IC50 (μM ± SEM[a]) Superoxide Anion
2		**42.12 ± 0.08**	**76.14 ± 1.50**
3		**27.14 ± 1.01**	**226.12 ± 2.10**
4		**20.14 ± 0.90**	**90.45 ± 0.83**
5		**19.12 ± 2.20**	**96.15 ± 0.92**
6		**17.14 ± 1.05**	**45.16 ± 1.40**
7		**15.16 ± 1.09**	**98.14 ± 1.02**
8		**N.A.[b]**	**N.A.[b]**
9		**N.A.[b]**	**N.A.[b]**
10		**N.A.[b]**	**N.A.[b]**

Table-1: continued…

11		**48.26 ± 2.08**	**120.12 ± 1.56**
12		**N.A.[b]**	**N.A.[b]**
13		**N.A.[b]**	**N.A.[b]**
14		**N.A.[b]**	**N.A.[b]**
15		**N.A.[b]**	**N.A.[b]**
16		**N.A.[b]**	**N.A.[b]**
17		**N.A.[b]**	**N.A.[b]**
18		**N.A.[b]**	**N.A.[b]**
19	***n*-propyl gallate[c]**	**30.27 ± 0.25**	**106.34 ± 1.60**

SEM[a] is the standard error mean, NA. [b] = Not active, *n*-propyl gallate[c] = standard drug for the DPPH assays and superoxide anion radical scavenging assays.

Compound **6** (IC_{50} = 17.14 ± 1.05 μM) which is structurally similar with the compound **7** but absence of methyl graoup on imine carbon slightly reduced the activity to IC_{50} = 17.14 ± 1.05 μM. The compound **5** having hydroxyl substituents at 2' and 4' positions (IC_{50} = 19.12 ± 2.20 μM) showed slight decline in the activity compared to compound **7** (Table-1). The compound 4 having hydrogen atom in place of methyl group on imine carbon possess IC_{50} value of 20.14 ± 0.90 μM which almost equal to **5**. These observations showed that the methyl group on imine carbon is also playing an important role in the activity. For the mono-hydroxyl compounds, compounds **2** and **3** both showed activity (IC_{50} = 42.12 ± 0.08 μM) and (IC_{50} = 27.14 ± 1.01 μM), respectively. Compound **3** have hydroxyl at 3' position illustrated good activity than its analogue **2** have hydroxyl at 2' position. Compound **11** (IC_{50} =

48.26 ± 2.08 μM) showed moderate activity having 3'-ethoxy group while 3'-methoxy analogue **10** found to be completely inactive. The bulky 3'-ethoxy group may have role in activity. The compounds having halogens subtituents (**14**, **15** and **16**), nitro substiuents (**17** and **18**), heterocyclic ring like pyridine (**12** and **13**) and methoxy and thiomethoxy derivatives showed no activity at all due less capability of stabilization of free radicals.

Superoxide Anion Radical Scavenging Activity

Compounds **2**, **4**, **5**, **6** and **7** demonstrated good activity than the standard *n*-propylgallate (Table 1). Compounds **2** and **11** showed moderate activities. The better activity of compounds **2**, **4**, **5**, **6** and **7** may be because of more stabilizing ability of these compounds for free radicals formed during bioassay. DPPH radical scavenging activity and superoxide anion radical scavenging activity are mainly depend on the hydroxyl substituent position as well as number of hydroxy groups present in the molecule [27,28].

Conclusion

Schiff bases of 4-amino-1,5-dimethyl-2-phenylpyrazolone (**2-18**) were evaluated for *in vitro* antioxidant activity. The compounds specially having hydroxyl substituents showed moderate to good DPPH scavenging activity and superoxide scavenging activity ranging from 15.16–48.26 μM and 45.16-226.12 μM, respectively.

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