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# Synthesis and antioxidant activities of some 4-benzylidenamino-4,5-dihydro-1*H*-1,2,4-triazol-5-one derivatives

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Eight 3-alkyl(aryl)-4-(3,4-dihydroxybenzylidenamino)-4,5-dihydro-1*H*-1,2,4-triazol-5-ones **2** having 4,5-dihydro-1*H*-1,2,4-triazol-5-one ring are synthesized by the reactions of 3-alkyl(aryl)-4-amino-4,5-dihydro-1*H*-1,2,4-triazol-5-ones **1** with 3,4-dihydroxy-benzaldehyde and their antioxidant activities are investigated. In addition, compounds **2b**, **2f** and **2h** are titrated potentiometrically with tetrabutylammonium hydroxide in non-aqueous solvents and the half-neutralization potential values and the corresponding $pK_a$ values are determined. The structures of new compounds are established from spectral data.

**Keywords:** Antioxidant activities, 1,2,4-triazol-5-ones, $pK_a$ values

**IPC**: **Int.Cl.**[7] **C 07 D**

Compounds of the 1,2,4-triazole and 4,5-dihydro-1*H*-1,2,4-triazol-5-one type show a broad spectrum of biological activity[1-6], and many reports of the synthesis of N-arylidenamino-1,2,4-triazole and N-arylidenamino-4,5-dihydro-1*H*-1,2,4-triazol-5-one derivatives are currently known[2,7-11].

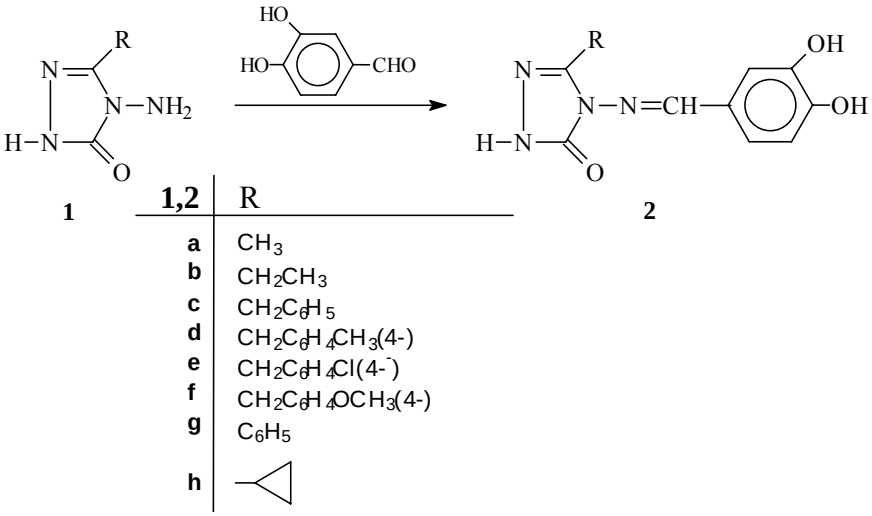
Antioxidants inhibit or delay oxidation which appears to have a role in the prevention of many diseases[12]. DPPH (2,2-diphenyl-1-picrylhydrazyl) free radical scavenging measurements were used to determine antioxidant capacity with butylated hydroxytoluene (BHT) as reference[13].

In addition, some 1,2,4-triazole and 4,5-dihydro-1*H*-1,2,4-triazol-5-one derivatives were titrated potentiometrically against tetrabutylammonium hydroxide in non-aqueous solvents, and the $pK_a$ values of the compounds were determined[7-11,14,15].

A series of 3-alkyl(aryl)-4-(3,4-dihydroxybenzyl-idenamino)-4,5-dihydro-1*H*-1,2,4-triazol-5-ones **2** was synthesized, out of which three compounds are reported for the first time, from the reactions of 3-alkyl(aryl)-4-amino-4,5-dihydro-1*H*-1,2,4-triazol-5-ones **1** with 3,4-dihydroxybenzaldehyde (**Scheme I**). Furthermore, antioxidant activity by the DPPH method for **2a** and **2c-h** were determined. The $pK_a$ values of the compounds **2b**, **2f** and **2h** were found out by potentiometric titration against tetrabutyl-ammonium hydroxide (TBAH) in four non-aqueous solvents, *viz.* isopropyl alcohol, *tert*-butyl alcohol, acetonitrile and N,N-dimethylformamide (DMF). The data obtained from the potentiometric titrations was interpreted, and substituent effects at the C-3 position in 4,5-dihydro-1*H*-1,2,4-triazol-5-one ring as well as solvent effects were studied[7-11,14-16].

## Results and Discussion

DPPH is a stable free radical, and any molecule that can donate an electron or hydrogen to DPPH can react with it and thereby bleach the DPPH absorption. The results of the DPPH in the samples is given in **Figure 1**. There is a reverse correlation between $IC_{50}$ values and free radical scavenging activity. All the tested compounds except for compound **2d**, which is not shown in **Figure 1** because of its inactivity, showed considerable DPPH radical scavenging activity. $IC_{50}$ values, and thus, antioxidant activity of compounds **2a**, **2c** and **2e** were the same and the highest.




**Scheme I**

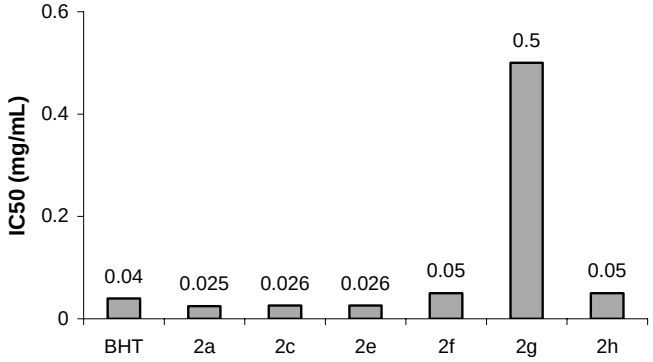



**Figure 1 –** DPPH radical scavenging activity of the triazole compounds. The results are compared with standard antioxidant BHT.

Compounds **2b**, **2f** and **2h** were titrated potentiometrically against tetrabutylammonium hydroxide (TBAH) in the non-aqueous solvents, *viz.* isopropyl alcohol, *tert*-butyl alcohol, acetonitrile and DMF. Among the non-aqueous solvents, acetonitrile and DMF are aprotic solvents whereas isopropyl alcohol and *tert*-butyl alcohol are amphiprotic neutral solvents. The mV values of each titration were drawn against TBAH volumes (mL) to obtain the potentiometric titration curves. From the titration curves, the half-neutralization potential (HNP) values and hence, the corresponding $pK_a$ values were obtained.

The HNP values and the corresponding $pK_a$ values for compounds **2b**, **2f** and **2h** were obtained from the potentiometric titrations against 0.05 M TBAH in isopropyl alcohol, *tert*-butyl alcohol, acetonitrile and DMF. The results are given in **Table I**.

The *p*H of the weak acids are given by the following equation:

$$pH = pK_a + \log[A^-] / [HA]$$

$pH = pK_a$ occurs when $[A^-]$ is equal to [HA] at the half-neutralization point. Therefore, the *p*H values can be regarded as $pK_a$ at the half-neutralization points.

When the dielectric permittivity of solvents is taken into consideration, the acidic arrangement can be expected as follows: DMF (ε=37) > acetonitrile (ε=36) > isopropyl alcohol (ε=19.4) > *tert*-butyl alcohol (ε=12). As seen in **Table I**, the arrangement for compounds **2b** and **2f** is isopropyl alcohol > *tert*-butyl alcohol > DMF > acetonitrile, while the arrangement for compound **2h** is acetonitrile > isopropyl alcohol > *tert*-butyl alcohol > DMF. For compound **2f**, the $pK_a$ value has not been obtained in acetonitrile.

As it is well known, the acidity of a compound depends on several factors. The two most important factors are the solvent effect and molecular structure[7-11,14-16]. **Table I** shows that the HNP values and the corresponding $pK_a$ values obtained from potentiometric titrations depend on the type of non-aqueous

**Table I –** Half-neutralization potential (HNP) values and the corresponding $pK_a$ values of compounds **2b**, **2f** and **2h** in isopropyl alcohol, *tert*-butyl alcohol, acetonitrile and DMF

| Compd | Isopropyl alcohol | | *Tert*-butyl alcohol | | DMF | | Acetonitrile | |
|---|---|---|---|---|---|---|---|---|
| | HNP (mV) | $pK_a$ | HNP (mV) | $pK_a$ | HNP (mV) | $pK_a$ | HNP (mV) | $pK_a$ |
| **2b** | -252 | 11.09 | -260 | 11.43 | -346 | 12.52 | -356 | 12.80 |
| **2f** | -238 | 10.49 | -257 | 10.94 | -335 | 12.07 | -506 | - |
| **2h** | -237 | 10.74 | -273 | 11.59 | -360 | 12.78 | -174 | 9.45 |

solvents used and molecular structure of the compound tested.

## Experimental Section

### Synthesis

Melting points were determined on an electrothermal digital melting point apparatus and are uncorrected. IR spectra were recorded using KBr disks on a Perkin-Elmer 1600 FTIR spectrometer. $^1$H NMR and $^{13}$C NMR spectra were recorded in DMSO-$d_6$ with TMS as internal standard on a Varian Mercury spectrometer at 200 MHz and 50 MHz, respectively. UV-Vis absorption spectra were measured for ethanol solutions in 10 mm quartz cells between 200 and 400 nm using a Shimadzu UV-1201 spectrophotometer.

The starting compounds **2b**, **2f** and **2h** were prepared by the reaction of the corresponding ester ethoxycarbonylhydrazones with hydrazine hydrate[17,18]. In addition, the compounds **2a**, **2c-e** and **2g** were synthesized according to literature[8].

**General method for the preparation of 3-alkyl(aryl)-4-(3,4-dihydroxybenzylidenamino)-4,5-dihydro-1*H*-1,2,4-triazol-5-ones**. 2,3-Alkyl(aryl)-4-amino-4,5-dihydro-1*H*-1,2,4-triazol-5-one **1** (0.01 mole) was dissolved in acetic acid (15 mL) and treated with 3,4-dihydroxybenzaldehyde (1.38 g, 0.01 mole). The mixture was refluxed for 1 hr and then concentrated at 50-55°C *in vacuo*. Repeated recrystallizations of the residue from an appropriate solvent gave the pure compound **2**.

**3-Ethyl-4-(3,4-dihydroxybenzylidenamino)-4,5-dihydro-1*H*-1,2,4-triazol-5-one 2b**: Yield 89% (2.21 g), m.p. 261-62°C; IR (KBr): 3440, 3150 (OH, NH), 1710 (C=O), 1610 and 1580 cm$^{-1}$ (C=N); $^1$H NMR (DMSO-$d_6$): δ 1.20 (t, 3H, $CH_3$), 2.64 (q, 2H, $CH_2$), 6.83 (d, 1H, Ar-H, *J*=8.1 Hz), 7.06 (d, 1H, Ar-H, *J*=7.9 Hz), 7.28 (s, 1H, Ar-H), 9.14 (s, 1H, OH), 9.42 (s, 1H, CH), 9.70 (s, 1H, OH), 11.76 (s, 1H, NH); $^{13}$C NMR (DMSO-$d_6$): δ 10.57 ($CH_3$), 19.08 ($CH_2$), 113.32, 116.10, 122.27, 125.26, 148.44, 149.74 (aromatic carbons), 146.26 (triazole $C_3$), 151.97 (N=CH), 155.40 (triazole $C_5$); UV-Vis: nm (ε, M$^{-1}$cm$^{-1}$) 321 (20560), 236 (11800), 214 (18650).

**3-*p*-Methoxylbenzyl-4-(3,4-dihydroxybenzylidenamino)-4,5-dihydro-1*H*-1,2,4-triazol-5-one 2f**: Yield 87%, m.p. 289-90°C; IR (KBr): 3412, 3298 (OH, NH), 1694 (C=O), 1610, 1590 (C=N) and 807 cm$^{-1}$ (1,4-disubstituted benzenoid ring); $^1$H NMR (DMSO-$d_6$): δ 2.40 (s, 3H, $OCH_3$), 3.60 (s, 2H, $CH_2$), 6.74-7.16 (m, 7H, Ar-H), 9.30 (s, 1H, OH), 9.32 (s, 1H, N=CH), 9.64 (s, 1H, OH), 11.78 (s, 1H, NH); $^{13}$C NMR (DMSO-$d_6$): δ 30.31 ($CH_2$), 55.05 ($OCH_3$), 112.97, 113.90 (2C), 115.67, 121.90, 124.80 (2C), 127.67, 129.96 (2C), 145.86, 149.36 (aromatic carbons), 146.54 (triazole $C_3$), 151.41 (N=CH), 154.59 (triazole $C_5$); UV-Vis: nm (ε, M$^{-1}$cm$^{-1}$) 318 (18933), 284 (14333).

**3-Cyclopropyl-4-(3,4-dihydroxybenzylidenamino)-4,5-dihydro-1*H*-1,2,4-triazol-5-one 2h**: Yield 85%, m.p. 277-78°C; IR (KBr): 3437, 3284 (OH, NH), 1698 (C=O), 1610 and 1591 cm$^{-1}$ (C=N); $^1$H NMR (DMSO-$d_6$): δ 0.82-0.91 (m, 4H, $2CH_2$), 2.41-1.44 (m, 1H, CH), 6.76 (d, 1H, Ar-H), 7.01 (d, 1H, Ar-H), 7.22 (s, 1H, Ar-H), 9.31 (s, 1H, OH), 9.34 (s, 1H, N=CH), 9.65 (s, 1H, OH), 11.65 (s, 1H, NH); $^{13}$C NMR (DMSO-$d_6$): δ 5.62 ($2CH_2$), 6.37 (CH), 112.85, 115.53, 121.75, 124.63, 145.70, 149.20 (aromatic carbons), 147.96 (triazole $C_3$), 151.35 (N=CH), 155.35 (triazole $C_5$); UV-Vis: nm (ε, M$^{-1}$ cm$^{-1}$) 317 (27535).

### Antioxidant activity

#### Chemicals

All the reagents used were of analytical grade. 2,2-diphenyl-1-picrylhydrazyl (DPPH) was purchased from Fluka. Butylated hydroxytoluene (BHT) and dimethylsulfoxide (DMSO) were purchased from Sigma Chem. Co.

#### Free radical scavenging activity

Briefly, 750 μL sample solution of various concentrations (0.0625-1.0000 mg/mL) was added to

750 μL 0.004% methanolic DPPH solution. After a 30 min incubation period at rt, the absorbance was read against a blank at 517 nm[13]. A lower absorbance of the reaction mixture indicates higher DPPH radical scavenging activity. 50% inhibitory compound concentration ($IC_{50}$, mg/mL), was calculated from the curve drawn by plotting absorbance values for corresponding sample concentration. This corresponds to the sample concentration that scavenge the half of the radicals present and was used to evaluate radical scavenging activities of the compounds. All sample solutions were prepared in DMSO.

### Half neutralization potential and *p*$K_a$ value determination

For the potentiometric titrations, a Jenway 3040 ion analyser *p*H meter (calibrated according to the instructions of the manufactures) equipped with an Ingold *p*H electrode was used. During the titrations, the titrant was added in increments of 0.05 mL after each stable reading, and the corresponding mV values were recorded. After purification, isopropyl alcohol was used to prepare 0.05 M tetrabutylammonium hydroxide (TBAH). For all potentiometric titrations, 0.05 M TBAH in isopropyl alcohol was used.

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