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In vitro biological activity of some new 1,2,4-triazole derivatives with their potentiometric titrations

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In this study, 3-alkyl(aryl)-4-amino-4,5-dihydro-1*H*-1,2,4-triazol-5-ones 3 have been reacted with 4-methoxy-3-(*p*-toluenesulfonyloxy)-benzaldehyde **1** to afford the corresponding nine new 3-alkyl(aryl)-4-[4-methoxy-3-(*p*-toluenesulfonyloxy)-benzylidenamino)-4,5-dihydro-1*H*-1,2,4-triazol-5-ones **4**. Then, the acetylation reactions of compounds **4** have been investigated and **5** type compounds have been obtained. The structures of fifteen new compounds have been characterized by IR, ^{1}H and ^{13}C NMR, MS and UV-Vis spectral data. The synthesized compounds have been analyzed for their *in vitro* potential antioxidant activities by three different methods. Those antioxidant activities have been compared to standard antioxidants such as BHA, BHT and α-tocopherol. Compounds **4b**, **4d** and **5d** show best activity for iron binding. In addition, the compounds **4** have been titrated potentiometrically with tetrabutylammonium hydroxide (TBAH) in four non-aqueous solvents. Furthermore, these fifteen new compounds have been screened for their antimicrobial activities.

Keywords: 4,5-Dihydro-1*H*-1,2,4-triazol-5-one, Schiff base, acetylation, antimicrobial activity, antioxidant, potentiometric titrations

1,2,4-Triazole and 4,5-dihydro-1*H*-1,2,4-triazol-5-one derivatives are reported to possess a broad spectrum of biological activities such as anti-inflammatory[1], antibacterial[2,3], antioxidant[4,5], antifungal[6], anticancer[7], analgesic[8], anticonvulsant[9], antiparasitic[10], antiviral[11], anti-HIV[12], antihypertensive and diuretic[13] properties. In addition, several articles reporting the synthesis of some *N*-arylidenamino-4,5-dihydro-1*H*-1,2,4-triazol-5-one derivatives have been published so far[4,5,14-16].

On the other hand, it is known that 1,2,4-triazole and 4,5-dihydro-1*H*-1,2,4-triazol-5-one rings have weak acidic properties, so that some 1,2,4-triazole and 4,5-dihydro-1*H*-1,2,4-triazol-5-one derivatives were titrated potentiometrically with TBAH in non-aqueous solvents, and the pK_a values of the compounds were determined[4,5,14-18].

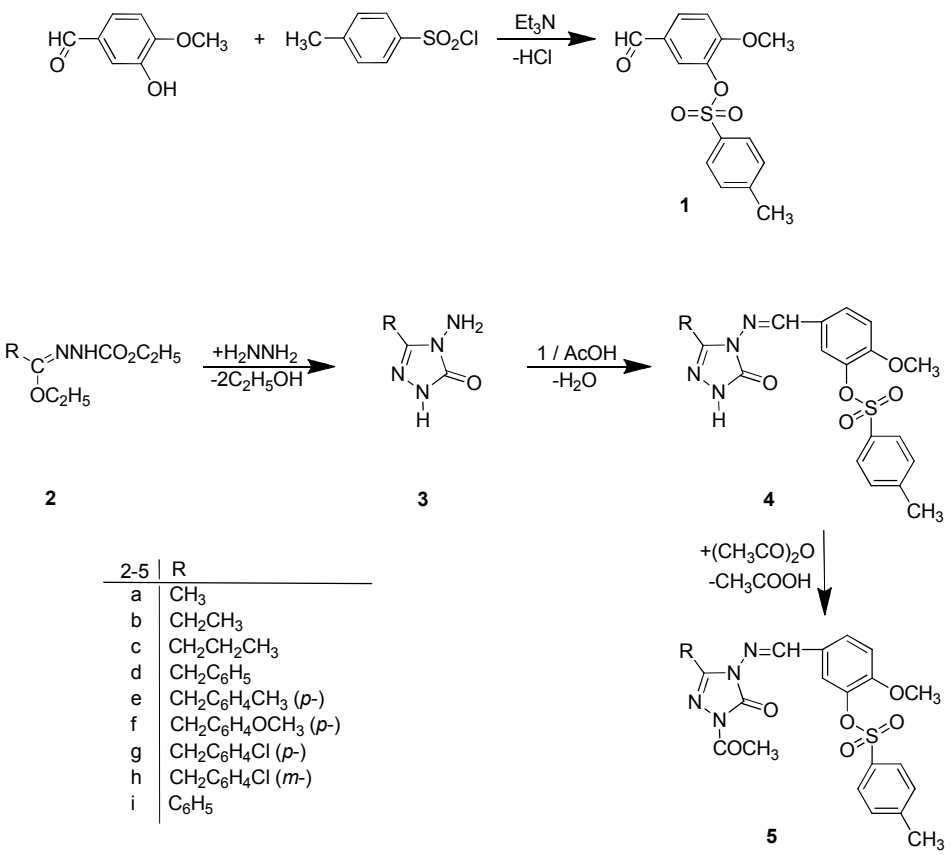
In order to investigate the antimicrobial and antioxidant activity of some 4,5-dihydro-1*H*-1,2,4-triazol-5-ones; nine new 3-alkyl(aryl)-4-[4-methoxy-3-(*p*-toluenesulfonyloxy)-benzylidenamino)-4,5-dihydro-1*H*-1,2,4-triazol-5-ones and six new 1-acetyl-3-alkyl(aryl)-4-[4-methoxy-3-(*p*-toluenesulfonyloxy)-benzylidenamino)-4,5-dihydro-1*H*-1,2,4-triazol-5-ones synthesized. For that purpose, 4-methoxy-3-(*p*-toluenesulfonyloxy)-benzaldehyde **1** were synthesized by the reactions of 3-hydroxy-4-methoxybenzaldehyde with *p*-toluenesulfonyl chloride by using trimethylamine. The 3-alkyl(aryl)-4-[4-methoxy-3-(*p*-toluenesulfonyloxy)-benzylidenamino)-4,5-dihydro-1*H*-1,2,4-triazol-5-ones **4a-i** were obtained from the reactions of compounds 3-alkyl(aryl)-4-amino-4,5-dihydro-1*H*-1,2,4-triazol-5-ones **3a-i** with 4-methoxy-3-(*p*-toluene-sulfonyloxy)-benzaldehyde **1**. Then, the reactions of compounds **4a-e** and **4g** with acetic anhydride were investigated, and compounds **5a-e** and **5g** were prepared (Scheme I). On the other hand, the newly synthesized **4a-i** compounds were titrated potentiometrically with tetrabutylammonium hydroxide (TBAH) in non-aqueous solvents and the pK_a values of the compounds were determined.

Results and Discussion

In this study, the structures of nine new 3-alkyl(aryl)-4-[4-methoxy-3-(*p*-toluenesulfonyloxy)-benzylidenamino)-4,5-dihydro-1*H*-1,2,4-triazol-5-ones **4a-i** and six new 1-acetyl-3-alkyl(aryl)-4-[4-methoxy-3-(*p*-toluenesulfonyloxy)-benzyliden-amino)-4,5-dihydro-1*H*-1,2,4-triazol-5-ones **5a-e**, **5g** were identified by using IR, ^{1}H and ^{13}C NMR, UV-Vis and MS data.

Antioxidant Activity

The antioxidant activities of fifteen new compounds **4a-i**, **5a-e** and **5g** were determined. Several methods have been used to determine



2-5	R
a	CH_3
b	CH_2CH_3
c	$CH_2CH_2CH_3$
d	$CH_2C_6H_5$
e	$CH_2C_6H_4CH_3$ (*p*-)
f	$CH_2C_6H_4OCH_3$ (*p*-)
g	$CH_2C_6H_4Cl$ (*p*-)
h	$CH_2C_6H_4Cl$ (*m*-)
i	C_6H_5

Scheme I — Synthetic pathway of the target compounds **4** and **5**

antioxidant activities and the methods used in the study are given below:

Total reductive capability using the potassium ferricyanide reduction method

The reductive capabilities of compounds were assessed by the extent of conversion of the Fe^{3+} / ferricyanide complex to the Fe^{2+} / ferrous form. The reducing powers of the compounds were observed at different concentrations, and results were compared with BHA, BHT and α-tocopherol. It has been observed that the reducing capacity of a compound may serve as a significant indicator of its potential antioxidant activity[19]. The antioxidant activity of putative antioxidant has been attributed to various mechanisms, among which are prevention chain initiation, binding of transition metal ion catalyst, decomposition of peroxides, prevention of continued hydrogen abstraction, reductive capacity and radical scavenging[20]. In the study, examined compounds did not show the reductive activities. In other words, all the amount of the compounds showed lower absorbance than standard antioxidants such as BHA, BHT and α-tocopherol. Hence, no activities were observed to reduce metal ions complexes to their lower oxidation state or to take part in any electron transfer reaction.

DPPH$^{\cdot}$ radical scavenging activity

The model of scavenging the stable DPPH radical model is a widely used method to evaluate antioxidant activities in a relatively short time compared with other methods. The effect of antioxidants on DPPH radical scavenging was thought to be due to their hydrogen donating ability[21]. DPPH is a stable free radical and accepts an electron or hydrogen radical to become a stable diamagnetic molecule[22]. The reduction capability of DPPH radicals was determined by decrease in its absorbance at 517 nm induced by antioxidants. The absorption maximum of a stable DPPH radical in ethanol was at 517 nm. The decrease in absorbance of DPPH radical was caused by antioxidants because of reaction between antioxidant molecules and radical, progresses, which resulted in the scavenging of the radical by hydrogen donation. It is visually noticeable as a discoloration from purple to yellow. Hence, DPPH$^{\cdot}$ is usually used as a substrate to evaluate antioxidative activity of antioxidants[23].

Antiradical activities of compounds and standard antioxidants such as BHA and α-tocopherol were determined by using DPPH· method. Scavenging effect values of compounds **4** and **5** with BHA and α-tocopherol at different concentrations are respectively given in Figure 1 and Figure 2. The newly synthesized compounds showed no activity as radical scavengers.

Ferrous ion chelating activity

The chelating effect towards ferrous ions by the compounds and standards was determined. Ferrozine can quantitatively form complexes with Fe^{2+}. In the presence of chelating agents, the complex formation is disrupted with the result that the red colour of the complex is decreased. Measurement of colour reduction therefore allows estimation of the chelating activity of the coexisting chelator[24]. Transition metals have pivotal role in the generation oxygen free radicals in living organism. The ferric iron (Fe^{3+}) is the relatively biologically inactive form of iron. However, it can be reduced to the active Fe^{2+}, depending on condition, particularly *p*H[25] and oxidized back through Fenton type reactions with the production of hydroxyl radical or Haber-Weiss reactions with superoxide anions. The production of

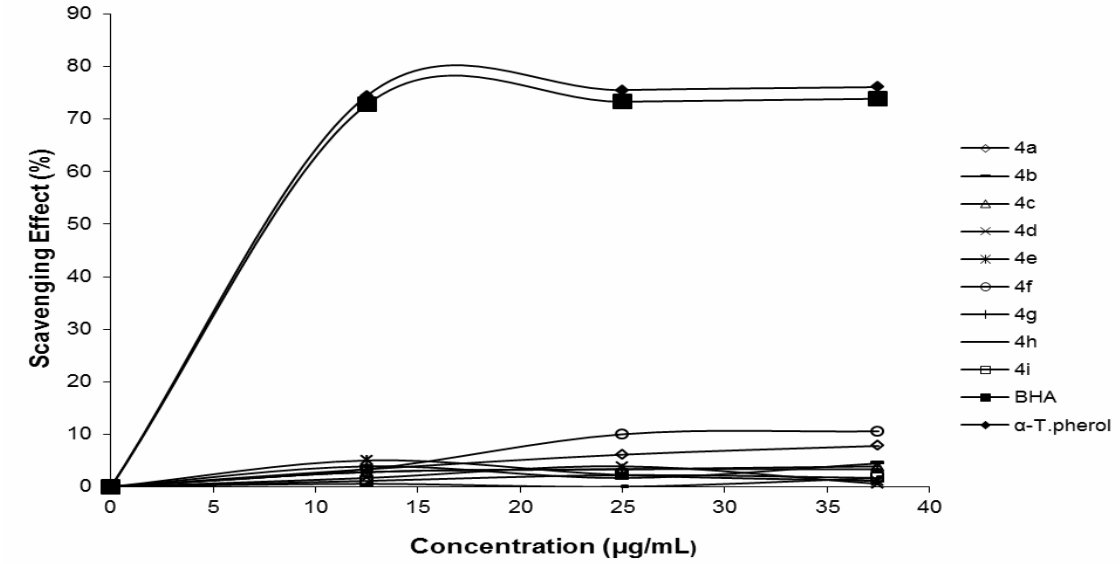


Figure 1 — Scavenging effect of compounds **4a-i**, BHA and α-tocopherol at different concentrations (12.5-25-37.5 µg/mL)

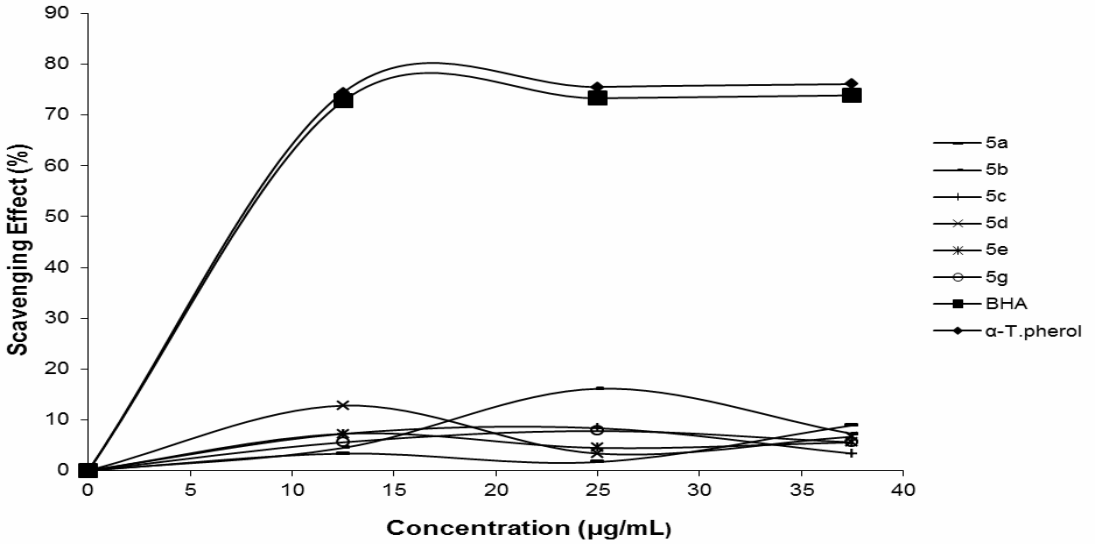


Figure 2 — Scavenging effect of compounds **5a-e**, **5g**, BHA and α-tocopherol at different concentrations (12.5-25-37.5 µg/mL)

these radicals may lead to lipid peroxidation, protein modification and DNA damage. Chelating agents may not activate metal ions and potentially inhibit the metal-dependent processes[26]. Also, the production of highly active ROS such as $O_2^{\cdot -}$, H_2O_2 and $OH^{\cdot}$ is also catalyzed by free iron though Haber-Weiss reactions:

$$O^{\cdot}_2 + H_2O_2 \rightarrow O_2 + OH^- + OH^{\cdot}$$

Among the transition metals, iron is known as the most important lipid oxidation pro-oxidant due to its high reactivity. The ferrous state of iron accelerates lipid oxidation by breaking down the hydrogen and lipid peroxides to reactive free radicals *via* the Fenton reactions:

$$Fe^{2+} + H_2O_2 \rightarrow Fe^{3+} + OH^- + OH^{\cdot}$$

Fe^{3+} ion also produces radicals from peroxides, even though the rate is tenfold less than that of Fe^{2+} ion, which is the most powerful pro-oxidant among the various types of metal ions[27]. Ferrous ion chelating activities of the compounds **4**, **5**, BHT and BHA are respectively shown in Figure 3 and Figure 4.

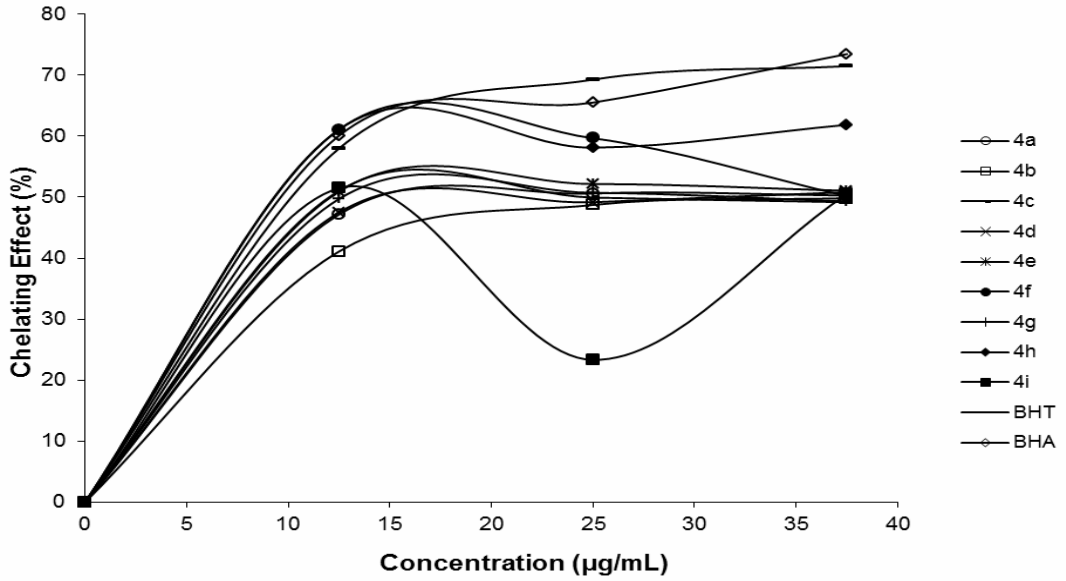


Figure 3 — Metal chelating effect of different amount of the compounds **4a-i**, BHT and BHA on ferrous ions

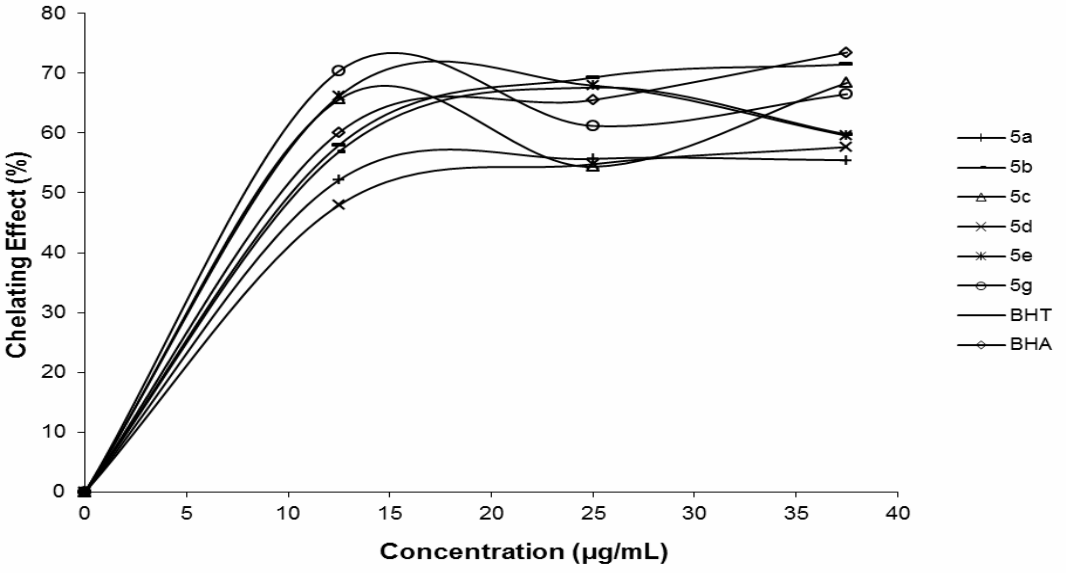


Figure 4 — Metal chelating effect of different amount of the compounds **5a-e**, **5g**, BHT and BHA on ferrous ions

In this study, metal chelating capacity was significant since it reduced the concentrations of the catalyzing transition metal. It was reported that chelating agents that form σ-bonds with a metal are effective as secondary antioxidants because they reduce the redox potential thereby stabilizing the oxidized form of metal ion[28]. The data obtained from Figure 3 and Figure 4 reveal that the compounds, especially **4b**, **4d** and **5d** demonstrate a marked capacity for iron binding, except **4i**, suggesting that their action as peroxidation protectors may be related to their iron binding capacity. On the other hand, free iron is known to have low solubility and a chelated iron complex has greater solubility in solution, which can be contributed solely by the ligand. Furthermore, the compound-iron complex may also be active, since it can participate in iron-catalyzed reactions.

Potentiometric titrations

In order to determine the pK_a values of the compounds **4a-i**, they were titrated potentiometrically with TBAH in four non-aqueous solvents: isopropyl alcohol, *tert*-butyl alcohol, acetone and DMF. The mV values read in each titration were plotted against 0.05 M TBAH volumes (mL) added, and potentiometric titration curves were obtained for all the cases. From the titration curves, the HNP values were measured, and the corresponding pK_a values were calculated. The data obtained from the potentiometric titrations was interpreted, and the effect of the C-3 substituent in 4,5-dihydro-1*H*-1,2,4-triazol-5-one ring as well as solvent effects were studied.

As an example for the potentiometric titration curves for 0.001 M solutions of compounds **4b** titrated with 0.05 M TBAH in isopropyl alcohol, *tert*-butyl alcohol, DMF and acetone are shown in Figure 5.

When the dielectric permittivity of solvents is taken into consideration, the acidity order can be given as follows: DMF (ε=36.7) > acetone (ε=36) > isopropyl alcohol (ε=19.4) > *tert*-butyl alcohol (ε=12).

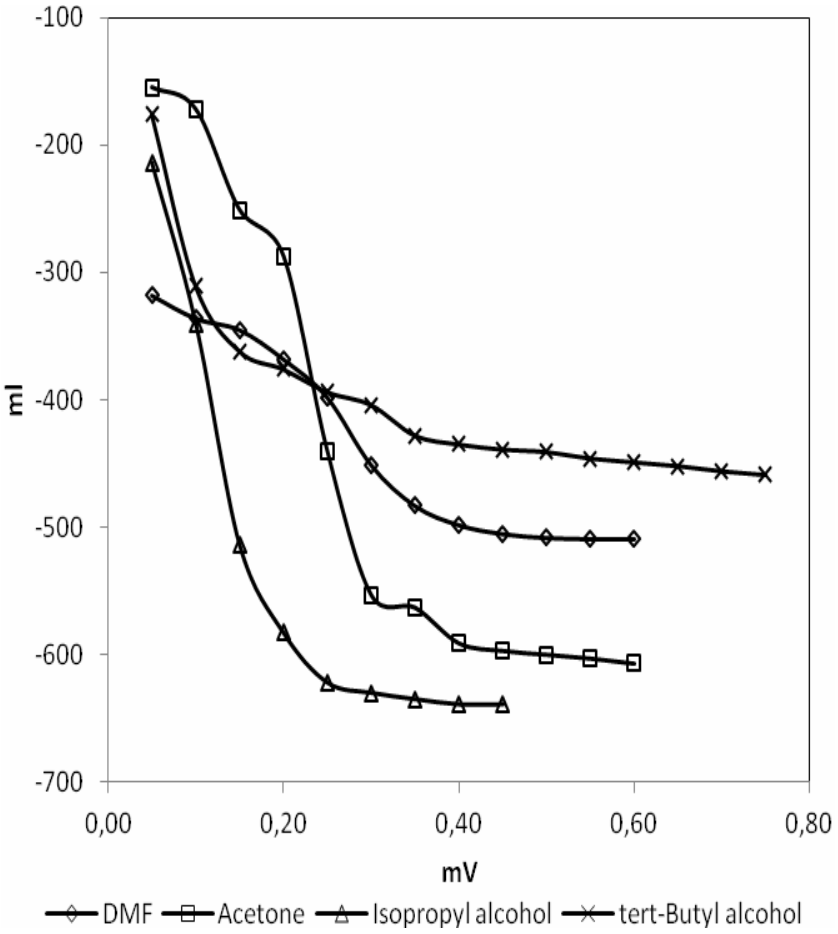


Figure 5 — Potentiometric titration curves of 0.001 M solutions of compound **4b** titrated with 0.05 M TBAH in isopropyl alcohol, *tert*-butyl alcohol, DMF and acetone at 25°C

As seen in Table I, the acidity order for compound **4a** is: acetone > DMF > *tert*-butyl alcohol, for compound **4b** it is: acetone > *tert*-butyl alcohol > DMF , for compounds **4c** and **4d** it is: acetone > DMF, for compound **4e** it is: acetone > isopropyl alcohol > DMF > *tert*-butyl alcohol, for compound **4f** it is: acetone > isopropyl alcohol > DMF, while the order for compound **4g** is: acetone > DMF > *tert*-butyl alcohol > isopropyl alcohol and for compound **4h** it is: DMF > acetone and for compound **4i** it is: *tert*-butyl alcohol > acetone > DMF > isopropyl alcohol. Moreover, as seen in Table I, for compounds **4a**, **4b** and **4c** in isopropyl alcohol, for compounds **4c**, **4d**, **4f** and **4h** in *tert*-butyl alcohol the HNP values and the corresponding pK_a values were not obtained.

As it is well known, the acidity of a compound depends on some factors. The two most important factors are the solvent effect and molecular structure[4,5,14-18,29]. Table I and Figure 5 show that the HNP values and corresponding pK_a values obtained from the potentiometric titrations depend on the non-aqueous solvents used and the substituents at C-3, in 4,5-dihydro-1*H*-1,2,4-triazol-5-one ring.

Antimicrobial Activity

The microbiological results are summarized in Table II. Microbiology results are not promising; only compounds **4a**, **5b** and **5d** showed low antimicrobial activity (10µg/100 µL) against *Mycobacterium smegmatis* and *Bacillus cereus*.

Experimental Section

Chemical reagents used in this study were purchased from Merck AG, Aldrich and Fluka. The

Table I — The HNP and the corresponding pK_a values of compounds **4a-i** in isopropyl alcohol, *tert*-butyl alcohol, DMF and acetone

Compd	DMF		Acetone		*tert*-Butyl alcohol		Isopropyl alcohol	
	HNP (mV)	pK_a	HNP (mV)	pK_a	HNP (mV)	pK_a	HNP (mV)	pK_a
4a	–364	15.32	–256	13.08	–515	18.88	–	–
4b	–340	15.04	–172	10.58	–214	10.73	–	–
4c	–381	16.13	–184	11.08	–	–	–	–
4d	–334	14.9	–243	10.06	–	–	–137	9.31
4e	–343	15.45	–105	8.82	–339	15.16	–312	14.09
4f	–351	14.92	–201	10.02	–	–	–259	11.85
4g	–301	13.67	–266	13.21	–408	15.61	–413	16.32
4h	–316	14.50	–451	17.70	–	–	–276	12.36
4i	–327	14.75	–221	11.84	–42	7.60	–331	14.81

Table II — Antimicrobial and antifungal activity of the compounds **4** and **5**

Compd	Stock solution (µg/mL)	Microorganisms and zone of inhibition (mm)								
		Ec	Yp	Pa	Sa	Ef	Bc	Ms	Ca	Sc
4a	10.000	–	–	–	–	–	–	10	–	–
4b	10.000	–	–	–	–	–	–	–	–	–
4c	10.000	–	–	–	–	–	–	–	–	–
4d	10.000	–	–	–	–	–	–	–	–	–
4e	10.000	–	–	–	–	–	–	–	–	–
4f	10.000	–	–	–	–	–	–	–	–	–
4g	10.000	–	–	–	–	–	–	–	–	–
4h	10.000	–	–	–	–	–	–	–	–	–
4i	10.000	–	–	–	–	–	–	–	–	–
5a	10.000	–	–	–	–	–	–	–	–	–
5b	10.000	–	–	–	–	–	6	–	–	–
5c	10.000	–	–	–	–	–	–	–	–	–
5d	10.000	–	–	–	–	–	6	–	–	–
5e	10.000	–	–	–	–	–	–	–	–	–
5g	10.000	–	–	–	–	–	–	–	–	–
Amp.		10	18	18	35	10	15			
Strep.Flu.								35		
Flu									25	>25

starting materials **3a-i** were prepared from the reactions of the corresponding ester ethoxycarbonyl-hydrazones **2a-i** with an aqueous solution of hydrazine hydrate as described in the literature[30,31]. Melting points were determined in open glass capillaries by using a Stuart SMP-30 melting point apparatus and are uncorrected. The IR spectra were obtained by an ALPHA-P BRUKER FT-IR spectrometer. ^{1}H and ^{13}C NMR spectra were recorded in DMSO-d_6 with TMS as internal standard using a Bruker 400 NMR spectrometer at 400 MHz for ^{1}H and 100 MHz for ^{13}C NMR with TMS as internal standard. UV-Vis absorption spectra were measured in 10 mm quartz cells between 200 and 400 nm using a PG Instruments Ltd T80 UV-Vis spectrometer. Extinction coefficients (ε) are expressed in L.mol^{-1}.cm^{-1}. Electrospray ionisation mass spectrometry (ESI-MS) was performed on a TSQ Quantum Access Max Triple Stage Quadrupole Mass Spectrometer.

General procedure for the synthesis of compounds 4

3-Hydroxy-4-methoxybenzaldehyde (0.01 mol) dissolved in ethyl acetate (20 mL) was treated with *p*-toluenesulfonyl chloride (0.01 mol) and to this solution was slowly added triethylamine (0.01 mol) with stirring at 0-5°C. The process of stirring continued for 2 h, and then the mixture was refluxed for 3 h and filtered. The filtrate was evaporated *in vacuo*, and the crude product was washed with water and recrystallized from acetic acid-water to afford compound **1** (Ref 32), m.p. 144-46°C. IR: 2843 and 2740 (CHO), 1720 (C=O), 1360 and 1179 (SO_2), 813 (1,4-disubstituted benzenoid ring) cm^{-1}; ^{1}H NMR (DMSO-d_6): δ 2.09 (s, 3H, CH_3), 2.43 (s, 3H, $PhCH_3$), 3.62 (s, 3H, OCH_3), 7.28 (d, 1H, ArH), 7.47 (d, 2H, ArH), 7.62 (m, 1H, ArH), 7.72 (d, 2H, ArH), 7.88-7.90 (s, 1H, ArH), 9.86 (s, 1H, CH); UV-Vis λ_{max} (ε): 268 (14458), 232 (15190), 220 (14125). The corresponding compound **3** (0.01 mol) was dissolved in acetic acid (20 mL) and treated with 4-methoxy-3-(*p*-toluenesulfonyloxy)-benzaldehyde **1** (0.01 mol). The mixture was refluxed for 2 h and then the solvent evaporated at 50-55°C *in vacuo*. Several recrystallizations of the residue from an appropriate solvent gave pure compounds 3-alkyl(aryl)-4-[4-methoxy-3-(*p*-toluenesulfonyloxy)-benzylidenamino)-4,5-dihydro-1*H*-1,2,4-triazol-5-ones **4** as colorless crystals.

3-Methyl-4-[4-methoxy-3-(*p*-toluenesulfonyloxy)-benzylidenamino)-4,5-dihydro-1*H*-1,2,4-triazol-5-one, 4a: Yield 3.90 g (97%). m.p.196-98°C. IR: 3148 (NH), 1689 (C=O), 1599 (C=N), 1361, 1179 (SO_2), 813 cm^{-1} (1,4-disubstituted benzenoid ring); ^{1}H NMR (DMSO-d_6): δ 2.21 (s, 3H, $PhCH_3$), 3.62 (s, 3H, OCH_3), 7,19 (d, 1H, ArH), 7.27 (d, 2H, ArH), 7.47 (m, 1H, ArH), 7.72 (d, 2H, ArH), 7.89 (d, 1H, ArH), 9.86 (s, 1H, CHO); ^{13}C NMR (DMSO-d_6): δ 11.44 (CH_3), 21.61 ($PhCH_3$), 56.45 (OCH_3), 114.02, 121.35, 126.73, 128.75 (2C), 129.87, 130.38 (2C), 132.51, 138.50, 144.59, 154.33 (arom-C), 146.18 (triazole C_3), 151.65 (N=CH), 152.63 (triazole C_5); UV-Vis λ_{max} (ε): 270 (20427), 228 (24688), 212 (20323) nm; MS (70 eV): *m/z* (%) 426 (M+1+23), 425 (M+23, 100), 404 (M+2), 403 (M+1, 60), 398, 361, 330, 329, 307, 282, 277, 261, 243, 234, 186, 155, 139, 104.

3-Ethyl-4-[4-methoxy-3-(*p*-toluenesulfonyloxy)-benzylidenamino)-4,5-dihydro-1*H*-1,2,4-triazol-5-one, 4b: Yield 4.10 g (99%). m.p.200-201°C. IR: 3183 (NH), 1694 (C=O), 1591 (C=N), 1365, 1180 (SO_2), 820 cm^{-1} (1,4-disubstituted benzenoid ring); ^{1}H NMR (DMSO-d_6): δ 1.18 (t, 3H, CH_2CH_3), 2.43 (s, 3H, $PhCH_3$), 2.60 (q, 2H, CH_2CH_3), 3.62 (s, 3H, OCH_3), 7,19 (d, 1H, ArH), 7.47, 7.50 (m, 3H, ArH), 7.70, 7.75 (m, 3H, ArH), 9.60 (s, 1H, N=CH), 11.83 (s, 1H, NH); ^{13}C NMR (DMSO-d_6): δ 10.43 (CH_2CH_3), 19.00 (CH_2CH_3), 21.60 ($PhCH_3$), 56.45 (OCH_3), 114.04, 121.24, 126.76, 128.72 (2C), 129.87, 130.37 (2C), 132.54, 138.54, 146.16, 154.36 (arom-C), 148.35 (triazole C_3), 151.81 (N=CH), 152.64 (triazole C_5); UV-Vis λ_{max} (ε): 308 (19656), 226 (22854), 222 (22646) nm; MS (70 eV): *m/z* (%) 440 (M+1+23), 439 (M+23, 100), 434, 418 (M+2), 417 (M+1, 45), 361, 329, 307, 289, 284, 276, 243.

3-*n*-Propyl-4-[4-methoxy-3-(*p*-toluenesulfonyloxy)-benzylidenamino)-4,5-dihydro-1*H*-1,2,4-triazol-5-one, 4c: Yield 4.20 g (98%). m.p.207-208°C. IR: 3177 (NH), 1692 (C=O), 1590 (C=N), 1363, 1179 (SO_2), 813 cm^{-1} (1,4-disubstituted benzenoid ring); ^{1}H NMR (DMSO-d_6): δ 0.93, 0.96 (m, 3H, $CH_2CH_2CH_3$), 1.63, 1.68 (m, 2H, $CH_2CH_2CH_3$), 2.43 (s, 3H, $PhCH_3$), 2.57, 2.60 (m, 2H, $CH_2CH_2CH_3$), 3.60 (s, 3H, OCH_3), 7,18, 7.20 (m, 1H, ArH), 7.47, 7.53 (m, 3H, ArH), 7.72, 7.75 (m, 3H, ArH), 9.60 (s, 1H, N=CH), 11.84 (s, 1H, NH); ^{13}C NMR (DMSO-d_6): δ 13.91 ($CH_2CH_2CH_3$), 19.37 ($CH_2CH_2CH_3$), 21.61 ($PhCH_3$), 27.09 ($CH_2CH_2CH_3$), 56.46 (OCH_3), 114.09, 121.41, 126.78, 128.73 (2C), 129.79, 130.37 (2C), 132.50, 138.52, 146.17, 154.33 (arom-C), 147.25 (triazole C_3), 151.74 (N=CH), 152.76 (triazole C_5); UV-Vis λ_{max} (ε): 308 (18000), 226 (21065), 220 (20183) nm; MS (70 eV): *m/z* (%) 454 (M+1+23), 453 (M+23, 100), 432 (M+2), 431 (M+1, 65), 428, 397, 361, 330, 329, 324, 296, 291, 275, 245, 211, 183, 155, 128.

3-Benzyl-4-[4-methoxy-3-(*p*-toluenesulfonyloxy)-benzylidenamino)-4,5-dihydro-1*H*-1,2,4-triazol-5-one, 4d: Yield 4.60 g (96%). m.p.204-205°C. IR: 3173 (NH), 1691 (C=O), 1586 (C=N), 1365, 1179 (SO_2), 838 (1,4-disubstituted benzenoid ring), 765, 708 cm^{-1} (monosubstituted benzenoid ring); ^{1}H NMR (DMSO-d_6): δ 2.38 (s, 3H, $PhCH_3$), 3.58 (s, 3H, OCH_3), 3.99 (s, 2H, CH_2Ph), 7,16 (d, 1H, ArH), 7.22, 7.25 (m, 1H, ArH), 7.28, 7.33 (m, 4H, ArH), 7.46 (d, 2H, ArH), 7.59 (s, 1H, ArH), 7.67 (d, 1H, ArH), 7.74 (d, 2H, ArH), 9.57 (s, 1H, N=CH), 11.96 (s, 1H, NH); ^{13}C NMR (DMSO-d_6): δ 21.57 ($PhCH_3$), 31.63 (CH_2Ph), 56.41 (OCH_3), 113.98, 121.51, 126.75, 127.25, 128.72 (2C), 128.93 (2C), 129.32 (2C), 129.91, 130.34 (2C), 132.52, 136.16, 138.49, 146.18, 154.27 (arom-C), 146.63 (triazole C_3), 151.66 (N=CH), 152.42 (triazole C_5); UV-Vis λ_{max} (ε): 308 (13190), 220 (19700) nm; MS (70 eV): *m/z* (%) 502 (M+1+23), 501 (M+23, 100), 480 (M+2), 479 (M+1, 72), 328, 320, 315, 299, 244, 243, 187.

3-*p*-Methylbenzyl-4-[4-methoxy-3-(*p*-toluenesulfonyloxy)-benzylidenamino)-4,5-dihydro-1*H*-1,2,4-triazol-5-one, 4e: Yield 4.75 g (97%). m.p.209-10°C. IR: 3122 (NH), 1695 (C=O), 1588 (C=N), 1357, 1180 (SO_2), 835 cm^{-1} (1,4-disubstituted benzenoid ring); ^{1}H NMR (DMSO-d_6): δ 2.25 (s, 3H, $PhCH_3$), 2.39 (s, 3H, $PhCH_3$), 3.59 (s, 3H, OCH_3), 3.93 (s, 2H, CH_2Ph), 7.10, 7.12 (m, 2H, ArH), 7.16, 7.18 (m, 3H, ArH), 7.46 (d, 2H, ArH), 7.59 (s, 1H, ArH), 7.66, 7.69 (m, 1H, ArH), 7.75 (d, 2H, ArH), 9.56 (s, 1H, N=CH), 11.94 (s, 1H, NH); ^{13}C NMR (DMSO-d_6): δ 21.09 (CH_2PhCH_3), 21.59 ($PhCH_3$), 31.22 (CH_2Ph), 56.43 (OCH_3), 114.02, 121.48, 126.76, 128.73 (2C), 129.18 (2C), 129.50 (2C), 129.94, 130.36 (2C), 132.51, 133.05, 136.30, 138.50, 146.21, 154.26 (arom-C), 146.78 (triazole C_3), 151.61 (N=CH), 152.42 (triazole C_5); UV-Vis λ_{max} (ε): 308 (24750), 226 (28400), 214 (24638) nm; MS (70 eV): *m/z* (%) 516 (M+1+23), 515 (M+23, 100), 494 (M+2), 493 (M+1, 70), 491, 437, 408, 393, 355, 329, 327, 322, 306, 244, 243, 187.

3-*p*-Methoxybenzyl-4-[4-methoxy-3-(*p*-toluenesulfonyloxy)-benzylidenamino)-4,5-dihydro-1*H*-1,2,4-triazol-5-one, 4f: Yield 4.48 g (89%). m.p.218-19°C. IR: 3125 (NH), 1694 (C=O), 1600 (C=N), 1358, 1180 (SO_2), 830 cm^{-1} (1,4-disubstituted benzenoid ring); ^{1}H NMR (DMSO-d_6): δ 2.39 (s, 3H, $PhCH_3$), 3.59 (s, 3H, OCH_3), 3.71 (s, 3H, OCH_3), 3.91 (s, 2H, CH_2Ph), 6.87 (d, 2H, ArH), 7.16, 7.21 (m, 3H, ArH), 7.46 (d, 2H, ArH), 7.60 (s, 1H, ArH), 7.67, 7.76 (m, 3H, ArH), 9.57 (s, 1H, N=CH), 11.93 (s, 1H, NH); ^{13}C NMR (DMSO-d_6): δ 21.58 ($PhCH_3$), 30.78 (CH_2Ph), 55.44 (CH_2PhOCH_3), 56.43 (OCH_3), 114.02, 114.32 (2C), 121.47, 126.78, 127.92, 128.74 (2C), 129.98, 130.36 (2C), 130.39 (2C), 132.49, 138.50, 146.21, 154.26, 158.56 (arom-C), 146.93 (triazole C_3), 151.67 (N=CH), 152.42 (triazole C_5); UV-Vis λ_{max} (ε): 308 (18696), 274 (19826), 232 (26467), 214 (22717) nm; MS (70 eV): *m/z* (%) 532 (M+1+23), 531 (M+23, 80), 510 (M+2), 509 (M+1, 48), 507, 488, 456, 415, 393, 362, 361, 330, 329 (100), 307, 272, 258, 250, 234, 187, 155.

3-*p*-Chlorobenzyl-4-[4-methoxy-3-(*p*-toluenesulfonyloxy)-benzylidenamino)-4,5-dihydro-1*H*-1,2,4-triazol-5-one, 4g: Yield 4.95 g (97%). m.p.207-209°C. IR: 3161 (NH), 1698 (C=O), 1602 (C=N),; 1355, 1183 (SO_2), 833 cm^{-1} (1,4-disubstituted benzenoid ring); ^{1}H NMR (DMSO-d_6): δ 2.39 (s, 3H, $PhCH_3$), 3.58 (s, 3H, OCH_3), 4.00 (s, 2H, CH_2Ph), 7.17 (d, 1H, ArH), 7.31, 7.38 (m, 4H, ArH), 7.46 (d, 2H, ArH), 7.59 (s, 1H, ArH), 7.67, 7.69 (m, 1H, ArH), 7.74 (d, 2H, ArH), 9.58 (s, 1H, N=CH), 11.98 (s, 1H, NH); ^{13}C NMR (DMSO-d_6): δ 21.59 ($PhCH_3$), 30.94 (CH_3Ph), 56.44 (OCH_3), 114.05, 121.57, 126.69, 128.75 (2C), 128.86 (2C), 129.95, 130.35 (2C), 131.25 (2C), 131.93, 132.47, 135.16, 138.46, 146.21, 154.29 (arom-C), 146.30 (triazole C_3), 151.63 (N=CH), 152.60 (triazole C_5); UV-Vis λ_{max} (ε): 308 (17381), 224 (27737) nm; MS (70 eV): *m/z* (%) 537 (M+2+23, 20), 535 (M+23, 45), 514 (M+2), 513 (M+1), 493, 453, 424, 402, 362, 361, 337, 332, 329 (100), 324, 307, 267, 258, 250, 229, 215, 104.

3-*m*-Chlorobenzyl-4-[4-methoxy-3-(*p*-toluenesulfonyloxy)-benzylidenamino)-4,5-dihydro-1*H*-1,2,4-triazol-5-one, 4h: Yield 4.90 g (96%). m.p.184-86°C. IR: 3168 (NH), 1702 (C=O), 1594 (C=N), 1364, 1185 (SO_2), 826 (1,4-disubstituted benzenoid ring), 785, 687 cm^{-1} (monosubstituted benzenoid ring); ^{1}H NMR (DMSO-d_6): δ 2.38 (s, 3H, $PhCH_3$), 3.59 (s, 2H, OCH_3), 4.03 (s, 2H, CH_2Ph), 7.17 (d, 1H, ArH), 7.26, 7.37 (m, 4H, ArH), 7.45, 7.47 (m, 2H, ArH), 7.58 (m, 1H, ArH), 7.68, 7.70 (m, 1H, ArH), 7.74 (d, 2H, ArH), 9.57 (s, 1H, N=CH), 11.99 (s, 1H, NH); ^{13}C NMR (DMSO-d_6): δ 21.58 ($PhCH_3$), 31.14 (CH_2Ph), 56.44 (OCH_3), 114.03, 121.66, 126.66, 127.32, 128.20, 128.74 (2C), 129.26, 129.86, 130.35 (2C), 130.74, 132.51, 133.45, 138.49, 138.53, 146.12, 154.34 (arom-C), 146.18 (triazole C_3), 151.61 (N=CH), 152.72 (triazole C_5); UV-Vis λ_{max} (ε): 308 (16280), 222 (21370) nm; MS (70 eV): *m/z* (%) 537 (M+2+23, 18), 535 (M+23, 55), 514 (M+2), 513 (M+1), 477, 410, 353, 346, 345,

329, 324, 316, 307, 258, 242, 236, 220, 204, 195 (100), 179, 163, 142, 134, 129.

3-Phenyl-4-[4-methoxy-3-(*p*-toluenesulfonyloxy)-benzylidenamino)-4,5-dihydro-1*H*-1,2,4-triazol-5-one, 4i: Yield 4.00 g (86%). m.p.185-86°C. IR: 3161 (NH), 1702 (C=O), 1600 (C=N), 1372, 1180 (SO_2), 842 (1,4-disubstituted benzenoid ring), 765, 716 cm^{-1} (monosubstituted benzenoid ring); ^1H NMR (DMSO-d_6): δ 2.36 (s, 3H, $PhCH_3$), 3.59 (s, 3H, OCH_3), 7.20 (d, 1H, ArH), 7.41, 7.43 (m, 2H, ArH), 7.51, 7.59 (m, 4H, ArH), 7.70, 7.74 (m, 3H, ArH), 7.86, 7.88 (m, 1H, ArH), 9.56 (s, 1H, N=CH), 12.37 (s, 1H, NH); ^{13}C NMR (DMSO-d_6): δ 21.58 ($PhCH_3$), 56.45 (OCH_3), 114.15, 122.03, 126.58, 127.07, 128.33 (2C), 128.69 (2C), 128.96 (2C), 129.82, 130.32 (2C), 130.61, 132.32, 138.43, 144.90, 155.50 (arom-C), 146.19 (triazole C_3), 151.80 (N=CH), 154.44 (triazole C_5); UV-Vis λ_{max} (ε): 308 (19869), 274 (22464), 232 (29095), 224 (27809) nm; MS (70 eV): *m/z* (%) 488 (M+1+23), 487 (M+23, 100), 466 (M+2), 465 (M+1, 65), 333, 329, 313, 300, 244, 243 (50), 187.

General procedure for the synthesis of compounds 5

The corresponding compound **4** (0.01 mol) was refluxed with acetic anhydride (15 mL) for 30 min. After addition of absolute ethanol (50 mL), the mixture was refluxed for 1 h more. Evaporation of solvent from the resulting solution at 40-45°C *in vacuo* and several recrystallizations of the residue from EtOH gave pure compounds **5a-e** and **5g** as colourless crystals.

1-Acetyl-3-methyl-4-[4-methoxy-3-(*p*-toluenesulfonyloxy)-benzylidenamino)-4,5-dihydro-1*H*-1,2,4-triazol-5-one, 5a: Yield 3.89 g (88%). m.p.201-203°C. IR: 1769, 1696 (C=O), 1606 (C=N), 1370, 1180 (SO_2), 823 cm^{-1} (1,4-disubstituted benzenoid ring); ^1H NMR (DMSO-d_6): δ 2.29 (s, 3H, CH_3), 2.44 (s, 3H, $PhCH_3$), 2.49 (s, 3H, $COCH_3$), 3.62 (s, 3H, OCH_3), 7.21 (d, 1H, ArH), 7.49 (d, 2H, ArH), 7.56 (s, 1H, ArH), 7.74, 7.78 (m, 3H, ArH), 9.48 (s, 1H, N=CH); ^{13}C NMR (DMSO-d_6): δ 11.60 (CH_3), 21.63 ($PhCH_3$), 23.91 ($CO\mathit{C}H_3$), 56.42 (OCH_3), 114.14, 121.76, 126.17, 128.76 (2C), 130.34 (2C), 130.41, 132.46, 138.51, 146.25, 154.95 (arom-C), 147.07 (triazole C_3), 148.31 (N=CH), 154.75 (triazole C_5), 166.51 ($COCH_3$); UV-Vis λ_{max} (ε): 306 (27125), 292 (25443), 232 (28170) nm; MS (70 eV): *m/z* (%) 468 (M+2), 467 (M+1, 98), 446 (M+2), 445 (M+1), 426, 425 (100), 403, 402, 361, 329, 307, 303, 282, 269, 243, 223, 206, 182, 164.

1-Acetyl-3-ethyl-4-[4-methoxy-3-(*p*-toluenesulfonyloxy)-benzylidenamino)-4,5-dihydro-1*H*-1,2,4-triazol-5-one, 5b: Yield 3.90 g (85%). m.p.144-46°C. IR: 1771, 1695 (C=O), 1604 (C=N), 1371, 1184 (SO_2), 805 cm^{-1} (1,4-disubstituted benzenoid ring); ^1H NMR (DMSO-d_6): δ 1.23 (t, 3H, $CH_2C\mathit{H}_3$), 2.44 (s, 3H, $PhCH_3$), 2.50 (s, 3H, $COCH_3$), 2.68 (q, 2H, $C\mathit{H}_2CH3$), 3.62 (s, 3H, OCH_3), 7.22 (d, 1H, ArH), 7.49 (d, 2H, ArH), 7.56 (s, 1H, ArH), 7.70, 7.78 (m, 3H, ArH), 9.48 (s, 1H, N=CH); ^{13}C NMR (DMSO-d_6): δ 9.87 ($CH_2\mathit{C}H_3$), 19.01 ($\mathit{C}H_2CH_3$), 21.62 ($PhCH_3$), 23.91 ($CO\mathit{C}H_3$), 56.53 (OCH_3), 114.14, 121.61, 126.19, 128.73 (2C), 130.34 (2C), 130.39, 132.49, 138.54, 146.22, 154.87 (arom-C), 148.54 (triazole C_3), 150.53 (N=CH), 154.77 (triazole C_5), 166.45 ($\mathit{C}OCH_3$); UV-Vis λ_{max} (ε): 308 (20848), 292 (18293), 226 (25087) nm; MS (70 eV): *m/z* (%) 482 (M+1+23), 481 (M+23, 100), 460 (M+2), 459 (M+1), 439, 417, 404, 384, 362, 329, 305, 289, 284, 276, 243, 227.

1-Acetyl-3-*n*-propyl-4-[4-methoxy-3-(*p*-toluenesulfonyloxy)-benzylidenamino)-4,5-dihydro-1*H*-1,2,4-triazol-5-one, 5c: Yield 3.87g (82%). m.p.161-65°C. IR: 1691 (C=O), 1593 (C=N), 1368, 1183 (SO_2), 810 cm^{-1} (1,4-disubstituted benzenoid ring); ^1H NMR (DMSO-d_6): δ 0.96 (t, 3H, $CH_2CH_2C\mathit{H}_3$), 1.70 (sext, 2H, $CH_2\mathit{C}H_2CH_3$), 2.43 (s, 3H, $PhCH_3$), 2.51 (s, 3H, $COCH_3$), 2.59 (t, 2h, $C\mathit{H}_2CH_2CH_3$), 3.61 (s, 3H, OCH_3), 7.22 (d, 1H, ArH), 7.48 (d, 2H, ArH), 7.59 (s, 1H, ArH), 7.73, 7.79 (m, 3H, ArH), 9.49 (s, 1H, N=CH); ^{13}C NMR (DMSO-d_6): δ 13.91 ($CH_2CH_2\mathit{C}H_3$), 19.94 ($CH_2\mathit{C}H_2CH_3$), 21.63 ($PhCH_3$), 23.95 ($CO\mathit{C}H_3$), 27.09 ($\mathit{C}H_2CH_2CH_3$), 56.53 (OCH_3), 114.19, 121.80, 126.22, 128.73 (2C), 130.24, 130.39 (2C), 132.48, 138.52, 146.22, 155.01 (arom-C), 148.50 (triazole C_3), 149.42 (N=CH), 154.74 (triazole C_5), 166.48 ($\mathit{C}OCH_3$); UV-Vis λ_{max} (ε): 308 (12954), 268 (19648), 224 (17880) nm; MS (70 eV): *m/z* (%) 496 (M+1+23), 495 (M+23, 95), 474 (M+2), 473 (M+1), 453 (100), 431, 426, 404, 349, 329, 307, 291, 283, 250, 234.

1-Acetyl-3-benzyl-4-[4-methoxy-3-(*p*-toluenesulfonyloxy)-benzylidenamino)-4,5-dihydro-1*H*-1,2,4-triazol-5-one, 5d: Yield 4.78 g (92%). m.p.164-66°C. IR: 1779, 1720 (C=O), 1602 (C=N), 1374, 1182 (SO_2), 798 (1,4-disubstituted benzenoid ring), 756, 704 cm^{-1} (monosubstituted benzenoid ring); ^1H NMR (DMSO-d_6): δ 2.39 (s, 3H, $PhCH_3$), 2.50 (s, 3H, $COCH_3$), 3.59 (s, 3H, OCH_3), 4.08 (s, 2H, CH_2Ph), 7.18 (d, 1H, ArH), 7.26, 7.34 (m, 5H, ArH), 7.46 (d, 2H, ArH), 7.59 (s, 1H, ArH), 7.71, 7.75 (m, 3H,

ArH), 9.45 (s, 1H, N=CH); ^{13}C NMR (DMSO-d_6): δ 21.59 ($PhCH_3$), 23.98 ($CO\mathit{C}H_3$), 31.58 (CH_2Ph), 56.48 (OCH_3), 114.07, 121.87, 126.20, 127.51, 128.72 (2C), 128.93, 129.00 (2C), 129.53 (2C), 130.36 (2C), 132.48, 135.03, 138.49, 146.23, 154.67 (arom-C), 148.70 (triazole C_3), 151.65 (N=CH), 152.45 (triazole C_5), 166.50 ($\mathit{C}OCH_3$); UV-Vis λ_{max} (ε): 308 (25684), 226 (30618), 222 (29803) nm; MS (70 eV): *m/z* (%) 544 (M+1+23), 543 (M+23, 100), 522 (M+2), 521 (M+1, 75), 501, 479, 349, 328, 307, 289, 243, 236, 204, 195 (90), 163.

1-Acetyl-3-*p*-methylbenzyl-4-[4-methoxy-3-(*p*-toluen sulfonyloxy)-benzylidenamino)-4,5-dihydro-1*H*-1,2,4-triazol-5-one, 5e: Yield 4.84 g (89%). m.p.190-92°C. IR: 1725 (C=O), 1602 (C=N), 1371, 1182 (SO_2), 840 cm^{-1} (1,4-disubstituted benzenoid ring); ^{1}H NMR (DMSO-d_6): δ 2.26 (s, 3H, $CH_2PhC\mathit{H}_3$), 2.39 (s, 3H, $PhCH_3$), 2.51 (s, 3H, $COC\mathit{H}_3$), 3.59 (s, 3H, OCH_3), 4.02 (s, 2H, CH_2Ph), 7.13 (d, 1H, ArH), 7.18, 7.22 (m, 4H, ArH), 7.46 (d, 2H, ArH), 7.63 (s, 1H, ArH), 7.73, 7.75 (m, 3H, ArH), 9.45 (s, 1H, N=CH); ^{13}C NMR (DMSO-d_6): δ 21.12 ($CH_2Ph\mathit{C}H_3$), 21.60 ($PhCH_3$), 24.00 ($CO\mathit{C}H_3$), 31.19 (CH_2Ph), 56.49 (OCH_3), 114.11, 121.85, 126.21, 128.73 (2C), 129.18, 129.39 (2C), 129.50, 129.58, 130.37 (2C), 131.89, 132.46, 136.62, 138.49, 146.25, 154.66 (arom-C), 148.47 (triazole C_3), 148.51 (N=CH), 154.60 (triazole C_5), 166.48 ($\mathit{C}OCH_3$); UV-Vis λ_{max} (ε): 308 (14091), 222 (22761) nm; MS (70 eV): *m/z* (%) 558 (M+1+23), 557 (M+23, 60), 536 (M+2), 535 (M+1, 40), 516, 515 (100), 493, 456, 344, 343, 335, 327, 322, 306, 244, 243, 241, 187.

1-Acetyl-3-*p*-chlorobenzyl-4-[4-methoxy-3-(*p*-toluenesulfonyloxy)-benzylidenamino)-4,5-dihydro-1*H*-1,2,4-triazol-5-one, 5g: Yield 5.06 g (91%). m.p. 174-77°C. IR: 1727 (C=O), 1603 (C=N), 1375, 1181 (SO_2), 835 cm^{-1} (1,4-disubstituted benzenoid ring); ^{1}H NMR (DMSO-d_6): δ 2.39 (s, 3H, $PhCH_3$), 2.51 (s, 3H, $COCH_3$), 3.58 (s, 3H, OCH_3), 4.10 (s, 2H, CH_2Ph), 7.19 (d, 1H, ArH), 7.38, 7.40 (m, 4H, ArH), 7.47 (d, 2H, ArH), 7.63 (m, 1H, ArH), 7.71, 7.75 (m, 3H, ArH), 9.46 (s, 1H, N=CH); ^{13}C NMR (DMSO-d_6): δ 21.60 ($PhCH_3$), 23.98 ($CO\mathit{C}H_3$), 30.89 (CH_2Ph), 56.49 (OCH_3), 114.12, 121.95, 126.16, 128.74 (2C), 128.92 (2C), 130.36 (2C), 131.25, 131.47 (2C), 132.21, 132.44, 134.06, 138.46, 146.25, 154.68 (arom-C), 146.25 (triazole C_3), 148.41 (N=CH), 154.68 (triazole C_5), 166.50 ($\mathit{C}OCH_3$); UV-Vis λ_{max} (ε): 308 (22304), 292 (19391), 230 (26761), 220 (25109) nm; MS (70 eV): *m/z* (%) 579 (M+2+23, 40), 577 (M+23, 100), 556 (M+2), 555 (M+1, 45), 537, 535 (75), 513 (60), 467, 423, 405, 375, 361, 338, 332, 329 (55), 307, 241, 240. Anal. Calcd for $C_{26}H_{23}ClN_4O_6S$ (555.01): C, 56.27; H, 4.18; N, 10.09. Found: C, 57.05; H, 4.60; N, 9.70%.

Antioxidant Activity: Chemicals

Butylated hydroxytoluene (BHT) was obtained from E. Merck. Ferrous chloride, α-tocopherol, 1,1-diphenyl-2-picryl-hydrazyl ($DPPH^{\cdot}$), 3-(2-pyridyl)-5,6-bis(phenylsulfonic acid)-1,2,4-triazine (ferrozine), butylated hydroxyanisole (BHA) and trichloroacetic acid (TCA) were obtained from Sigma-Aldrich.

Reducing power

The reducing power of the synthesized compounds was determined according to the method of Oyaizu[33] as explained in the literature[4,5].

Free radical scavenging activity

Free radical scavenging activity of compounds was measured by $DPPH^{\cdot}$, using the method of Blois[34] as explained in the literature[4,5].

Metal chelating activity

The chelation of ferrous ions by the synthesized compounds and standards were estimated by the method of Dinis *et al*.[35] as explained in the literature[4,5].

Potentiometric Titrations

A Jenco model ion analyzer and an Ingold *p*H electrode were used for potentiometric titrations. For each compound that was titrated, the 0.001 M solution was separately prepared in each non-aqueous solvent. The 0.05 M solution of TBAH in isopropyl alcohol, which is widely used in the titration of acids, was used as titrant. The mV values that were obtained in *p*H-meter were recorded. Finally, the HNP values were determined by drawing the mL (TBAH)-mV graphic.

Antimicrobial activity

All bacterial and yeast strains were obtained from the Hifzissihha Institute of Refik Saydam (Ankara, Turkey) and were as follows: (*Escherichia coli* ATCC 25922, *Yersinia pseudotuberculosis* ATCC 911, *Pseudomonas aeruginosa* ATCC 43288, *Staphylococcus aureus* ATCC 25923, *Enterococcus faecalis* ATCC 29212, *Bacillus cereus* 702 Roma, *Mycobacterium smegmatis* ATCC607, *Candida albicans* ATCC 60193, *Saccharomyces cerevisiae).* Simple susceptibility screening test using agar well diffusion method was used[36,37]. Each microorganism was suspended in Mueller

Hinton (MH) (Difco, Detroit, MI) broth and diluted approximately 10^6 colony forming unit (cfu)/mL. They were 'flood-inoculated' onto the surface of MH agar and Sabouraud Dextrose Agar (SDA) (Difco, Detriot, MI, USA) and then dried. For *C. albicans*, SDA was used. Five-millimeter diameter wells were cut from the agar using a sterile cork-borer, and 50 µL of the sample solutions was delivered into the wells. The plates were incubated for 18 h at 35°C. Antimicrobial activity was evaluated by measuring the zone of inhibition against the test organism. Ampicillin (10 µg) and fluconazole (5 µg) were standard antibacterial and antifungal agents, respectively. DMSO was used as solvent control.

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