Russian Journal of Bioorganic Chemistry, Vol. 31, No. 4, 2005, pp. 387–397. Translated from Bioorganicheskaya Khimiya, Vol. 31, No. 4, 2005, pp. 430–440.
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Synthesis and Biological Activities of New 1,2,4-Triazol-3-one Derivatives[1]

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Received December 1, 2004; in final form, March 14, 2005

Abstract—New 2-phenacyl-1,2,4-triazol-3-ones were obtained by the reaction of 5-alkyl-1,2,4-triazol-3-ones with α-bromoacetophenone in an alkaline medium. Selective reduction of the side chain carbonyl group to hydroxy group was achieved with $NaBH_4$. The reaction of some compounds containing a phenolic hydroxyl with 4-toluenesulfonyl chloride or benzyl bromide in the presence of NaOH led to tosylated or benzylated derivatives. The tosylation or benzylation at the alcoholic hydroxyl was carried out in the presence of sodium metal. Some of the newly synthesized compounds revealed an antimicrobial activity; 6 of 14 new compounds that were studied by the National Cancer Institute were found to possess antitumor activity.

Key words: antimicrobial activity, antitumor activity, benzylation, reduction, tosylation, 1,2,4-triazol-3-one

INTRODUCTION

In the last decades, the drug resistance to the commonly used antibiotics has become a wide-spread and serious problem. Therefore, the synthesis of new compounds that could be used for the effective treatment of infectious diseases without side effects is an urgent biomedical problem.

1,2,4-Triazoles are a new class of synthetic therapeutic agents. For instance, fluconazole and itraconazole are used as antimicrobial drugs in medicine [1, 2]. Moreover, vorozole, letrozole and anastrozole are nonsteroidal drugs used for the treatment of cancer [3]. In addition, a number of triazole derivatives have been reported to possess pharmacological, insecticidal, fungicidal and herbicidal activities [4–11]. Some *N*-alkylated derivatives of 1,2,4-triazoles were obtained in our laboratory as antimicrobial agents by the reaction of 5-alkyl-1,2,4-triazol-3-one with the corresponding alkyl halide in the presence of sodium ethoxide [5, 6]. The *S*-alkylation of 1,2,4-triazol-3-thiones was achieved in aqueous sodium hydroxide in the case of the precursors that have a good solubility in water [12]. Furthermore, some Schiff base derivatives of 1,2,4-triazol-3-ones and their reduced derivatives were obtained as antitumor agents in our laboratory [7, 13, 14]. The highest activity was observed for the compounds containing a phenylethylidenamino- or phenylethylamino group in the position 4 of the 1,2,4-triazol-3-one ring. On the other hand, the compounds containing an arylidene-hydrazide group in position 2 of the 1,2,4-triazol-3-one ring exhibit a selective antitumor activity only towards breast cancer [8].

Sulfonates, which have been used as important intermediates in organic synthesis and possess antiviral, anti-HIV or anticancer activity [15, 16], are usually obtained by treatment of the corresponding alcohols with sulfonating agents in the presence of pyridine or triethylamine. Moreover, tetramethylalkylidenediamine and silver(I) oxide were reported to be efficient reagents for the sulfonylation of alcohols in the presence of sulfonyl chlorides; the latter reagent is being used in the presence of a catalytic amount of potassium iodide [17]. In addition, several sulfonylation reactions were achieved in aqueous sodium hydroxide when sufficiently hydrophilic alcohols were used [18, 19].

These facts allow us to hope for the developing bioactive compounds and, in this study, to synthesize a series of new 1,2,4-triazol-3-one derivatives.

RESULTS AND DISCUSSION

We first synthesized ten new 1,2,4-triazol-3-one derivatives (**IIa**)–(**IIj**) by the reaction of (**Ia**)–(**Ij**) with α-bromoacetophenone in ethanolic sodium ethoxide (Scheme 1). The structures of new compounds were confirmed by IR, ^1H and ^{13}C NMR and mass (for some selected compounds) spectra. The spectral data were consistent with the proposed structures. In the NMR spectra of (**IIa**)–(**IIj**), additional signals from acetophenone moiety were observed, while the NH signal was absent. The IR spectra of these compounds display an additional absorption peak belonging to exocyclic carbonyl function and no signals belonging to NH group. In addition, (**IIa**), (**IIc**), and (**IIg**) gave good molecular ion peaks.

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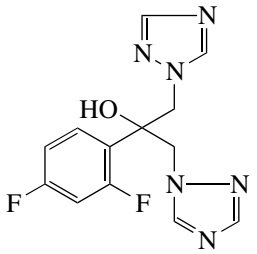
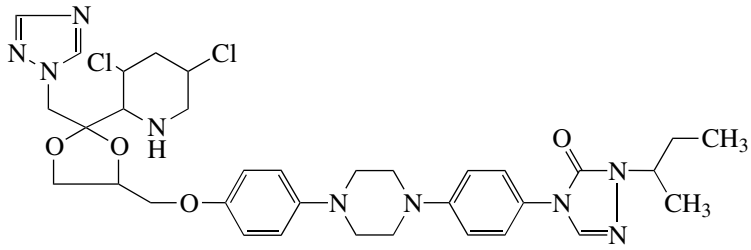
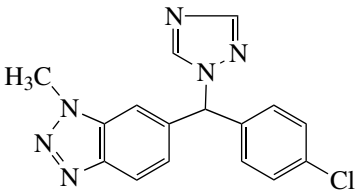


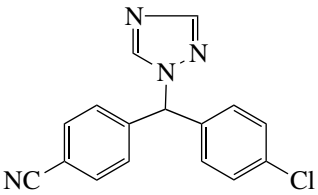
Fluconazol



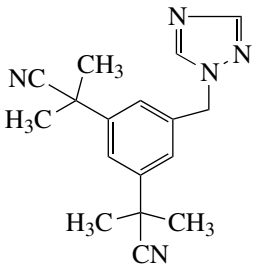
Itraconazol



Vorozol



Letrozol



Anastrozol

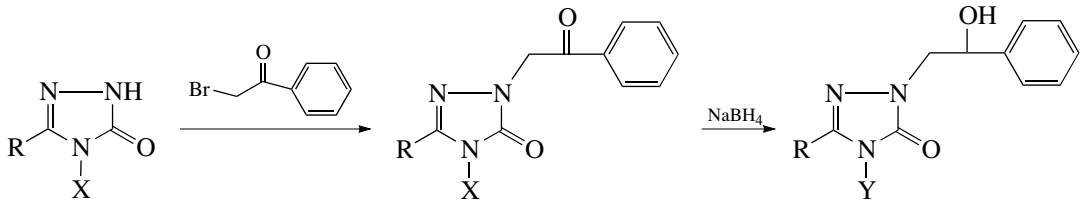
Compounds (**IIIa**)–(**IIIj**) were obtained by the selective reduction by $NaBH_4$ of exocyclic carbonyl group in (**IIa**)–(**IIj**) and imine bond in (**IIc**)–(**IIj**) (Scheme 1). No reduction of heterocyclic ring took place in the course of these reactions since $NaBH_4$ was used as the reducing agent [20]. Note that the reduction of (**IIa**)–(**IIj**) was achieved in milder conditions then similar reactions carried out in our laboratory [7, 13, 14]. The spectra of (**IIIa**)–(**IIIj**) are very informative. In the IR spectra of (**IIIa**)–(**IIIj**), only one carbonyl absorption band is observed as a result of selective reduction of exocyclic carbonyl in (**IIa**)–(**IIj**). In the ^{1}H NMR spectra of reduced compounds (**IIIa**)–(**IIIj**), the CHOH group resonates at 4.9–5.4 ppm (1 H) and 5.5–5.7 ppm (1 H), $NC\underline{H}_2$ group exhibits doublets centered at 3.6–4.3 and 3.8–4.5 ppm (2 H), $NH\underline{C}H_2$ proton resonates at 3.7–4.2 ppm for (**IIIc**)–(**IIIg**) and 6.2–6.3 ppm for (**IIIh**)–(**IIIj**).

In the ^{13}C NMR spectra of these compounds, the $\underline{C}H_2$–NH carbons of Y substituent resonate at 47–49 ppm for (**IIIc**)–(**IIIj**), carbons of CH–OH group, at 69–71 ppm, while the N=CH and exocyclic C=O carbon signals were absent.

Tosylated compounds (**IV**), (**V**) and (**VI**) were synthesised by the reaction of the corresponding (**IIg**), (**IIj**), and (**IIIj**) with 4-toluenesulfonyl chloride in ethanol in the presence of NaOH; no acylation reaction took place under these conditions at alcoholic OH of (**IIIj**). On the other hand, the tosylation of (**IIIb**) could be achieved in dry toluene in the presence of sodium metal (Scheme 2). The IR spectra of (**IV**) and (**V**) display no absorption bands derived from aromatic OH group. The proton signal belonging to aromatic OH is absent from the ^{1}H NMR spectra of these compounds, while additional new signals due to a tosyl group with the corresponding chemical shifts appear. In the ^{1}H NMR spectrum of (**VI**), the resonance assigned to alcoholic OH is observed at 5.98 ppm. On the other hand, the resonance of alcoholic OH of starting (**IIIb**) disappears from the ^{1}H NMR spectrum of (**VII**). In addition, (**IV**), (**V**) and (**VI**) gave molecular ion peaks consistent with their structures.

The derivatives (**IIg**) and (**IIIg**) were benzylated by the treatment with benzyl bromide in ethanol in the presence of sodium hydroxide. The NMR spectra of the resulting (**VIII**) and (**IX**) exhibit no signals from phenolic hydroxyl; new signals assigned to benzyl group appear instead. On the other hand, the benzylation of (**IIIa**) and (**IIId**) in the presence of sodium metal in dry toluene led to (**X**) and (**XI**), respectively (Scheme 3). As a result, the signals of alcoholic hydroxyls are absent from the IR and ^{1}H NMR spectra of these compounds; instead, additional signals are observed due to benzyl group at the corresponding chemical shifts. On the other hand, the signal assigned to alcoholic OH is present in the ^{1}H NMR spectrum of (**IX**), while the resonance from phenolic OH is absent.

Among the compounds tested, (**IIc**), (**IIh**), and (**IIIj**) exhibit good activities against *Staphylococcus aureus* and *Bacillus subtilis* with the MIC values of 1 μg/ml. Moreover, (**IIc**) exhibits a good activity against *Enterococcus faecalis*, whereas (**IIj**) is active against *B. subtilis* and moderately active against *E. faecalis* and *S. aureus* with the MIC values of 1, 9, and 4 μg/ml, respectively. An increased activity toward *S. aureus* was observed with the MIC value of 1 μg/ml, when (**IIj**) was converted to (**IIIj**). The tosylation of phenolic hydroxyl in (**IIj**) highly increased the antimi-



(**Ia**)–(**Ij**) (**IIa**)–(**IIj**) (**IIIa**)–(**IIIj**)

(**I**)–(**III**)	**R**	**X**	**Y**
a	CH_3	NH_2	NH_2
b	$CH_2C_6H_5$	NH_2	NH_2
c	CH_3	$N{=}CHC_6H_5$	$NHCH_2C_6H_5$
d	C_6H_5	$N{=}CHC_6H_5$	$NHCH_2C_6H_5$
e	$CH_2C_6H_5$	$N{=}CHC_6H_3Cl(2,4)$	$NHCH_2C_6H_3Cl(2,4)$
f	$CH_2C_6H_4Cl(\text{-p})$	$N{=}CHC_6H_3Cl(2,4)$	$NHCH_2C_6H_3Cl(2,4)$
g	CH_3	$N{=}CHC_6H_4OH(\text{o-})$	$NHCH_2C_6H_4OH(\text{o-})$
h	C_2H_5	$N{=}CHC_6H_4OH(\text{o-})$	$NHCH_2C_6H_4OH(\text{o-})$
i	C_3H_7	$N{=}CHC_6H_4OH(\text{o-})$	$NHCH_2C_6H_4OH(\text{o-})$
j	$CH_2C_6H_5$	$N{=}CHC_6H_4OH(\text{o-})$	$NHCH_2C_6H_4OH(\text{o-})$

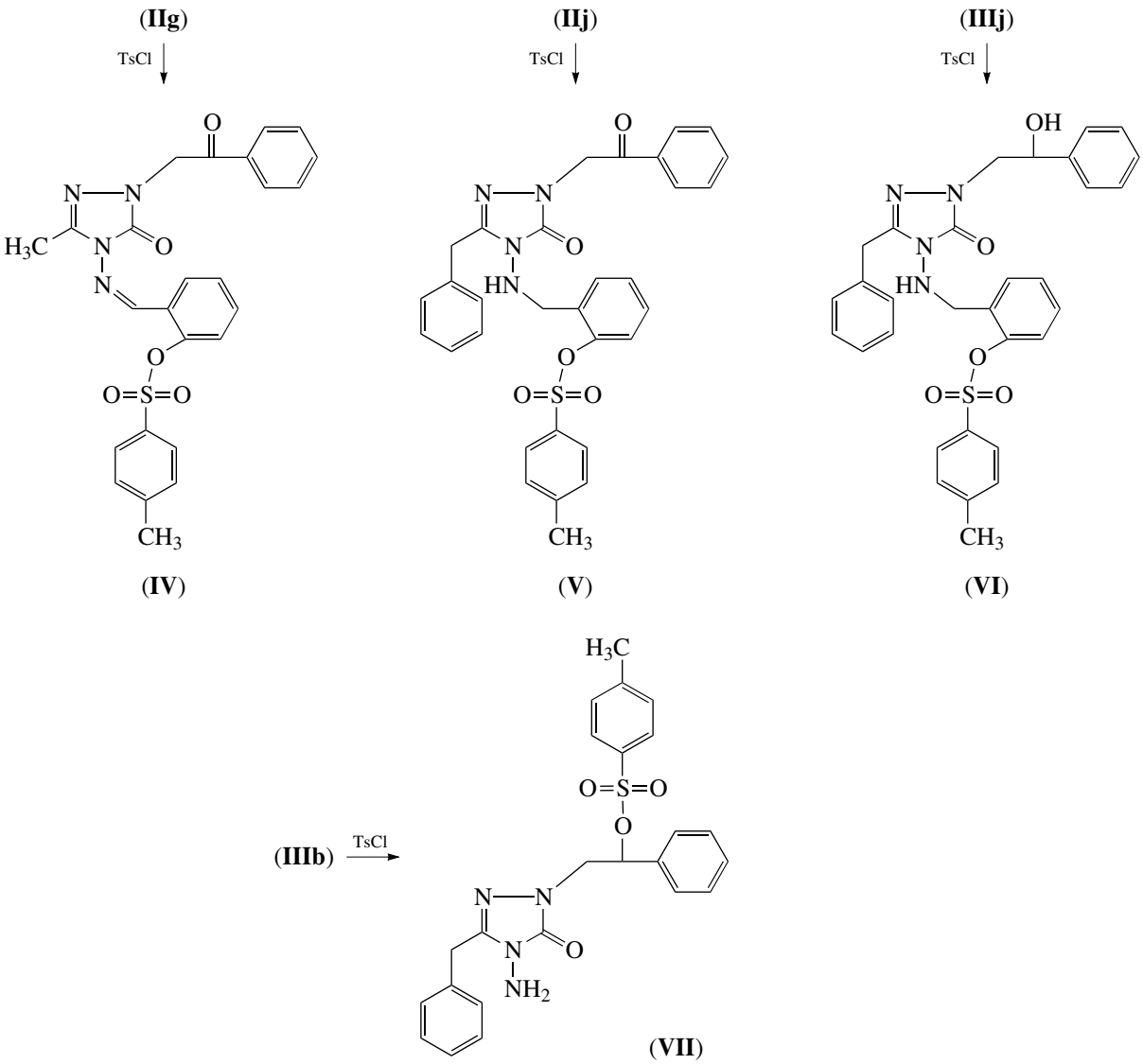
Scheme 1.

crobial effect of the resulting (**V**) toward *E. faecalis*, while decreasing the activity against *B. subtilis.*

Similarly, tosylation of (**IIIj**) increased the activity against *E. faecalis* and decreased the observed effects for *S. aureus* and *B. subtilis.* Compound (**IIb**) manifested a good activity against *Escherichia coli* (MIC value of 2 μg/ml) and moderate activities for *E. faecalis*, *S. aureus*, and *B. subtilis* (MIC values = 8, 4 and 4 μg/ml). The reduction of (**IIb**) to (**IIIb**) decreased the activity against *E. coli* and *S. aureus* up to MIC values of 8 μg/ml. Decreased activities toward *E. coli*, *E. faecalis*, *S. aureus*, and *B. subtilis* (up to MIC values of 16, 16, 32 and 16 μg/ml, respectively) were observed for (**VII**), the derivative of (**IIIb**) tosylated at alcoholic OH. On the other hand, the effect of tosyl group at phenolic hydroxyl is more complex, since it increases the activity against *E. faecalis*, while decreasing the antimicrobial effect toward *S. aureus* and *B. subtilis.* In addition, (**IV**), the tosylated derivative of (**IIg**), demonstrates decreasing activities against *E. faecalis*, *S. aureus*, and *B. subtilis* in comparison with the parent compound. A similarly decreased activity in the same tests was observed for (**IIIc**), a reduced derivative of (**IIc**). However, the reduction of (**IIh**) to (**IIIh**) increases the activity against *E. faecalis*, while decreasing the activity against *S. aureus* and *B. subtilis.* Moderate activities were observed for (**IIa**) and (**IIf**) against *E. faecalis*, *S. aureus*, and *B. subtilis* with the MIC values of 4 μg/ml.

The benzylation of (**IIg**), (**IIIc**) and (**IIIg**) led to a decrease in antimicrobial activity. On the contrary, the benzylation of (**IIId**) at alcoholic hydroxyl led an increase in the activity against *E. faecalis*, *S. aureus*, and *B. subtilis* (MIC values of 0.3 μg/ml). All the tested compounds exhibited no activity against *Candida albicans*, *C. tropicalis*, and *C. glabrata.* DMSO, acetone, and ethanol showed no inhibitory effect on Gram-positive and Gram-negative bacteria.

Among the compounds with a tosyl group in their molecules (**IV**)–(**VII**), the reduced derivatives (**VI**) and (**VII**) were found to possess antitumor activity, whereas (**IV**) and (**V**) that simultaneously contain a tosyl group and an Schiff base exhibited no antitumor activity. Among the benzylated compounds (**VIII**)–(**XI**), only (**IX**), reduced and benzylated at phenolic hydroxyl, displayed an antitumor activity. These results do not permit any evaluation of structure–antitumor activity relationship within the series of (**IIa**)–(**IIj**) and (**IIIa**)–(**IIIj**), since (**IIi**) displays an antitumor activity, while its reduced derivative (**IIIi**) not. On the contrary, (**IIf**) was found to be inactive, while its reduced derivative (**IIIf**) do possess an antitumor activity.



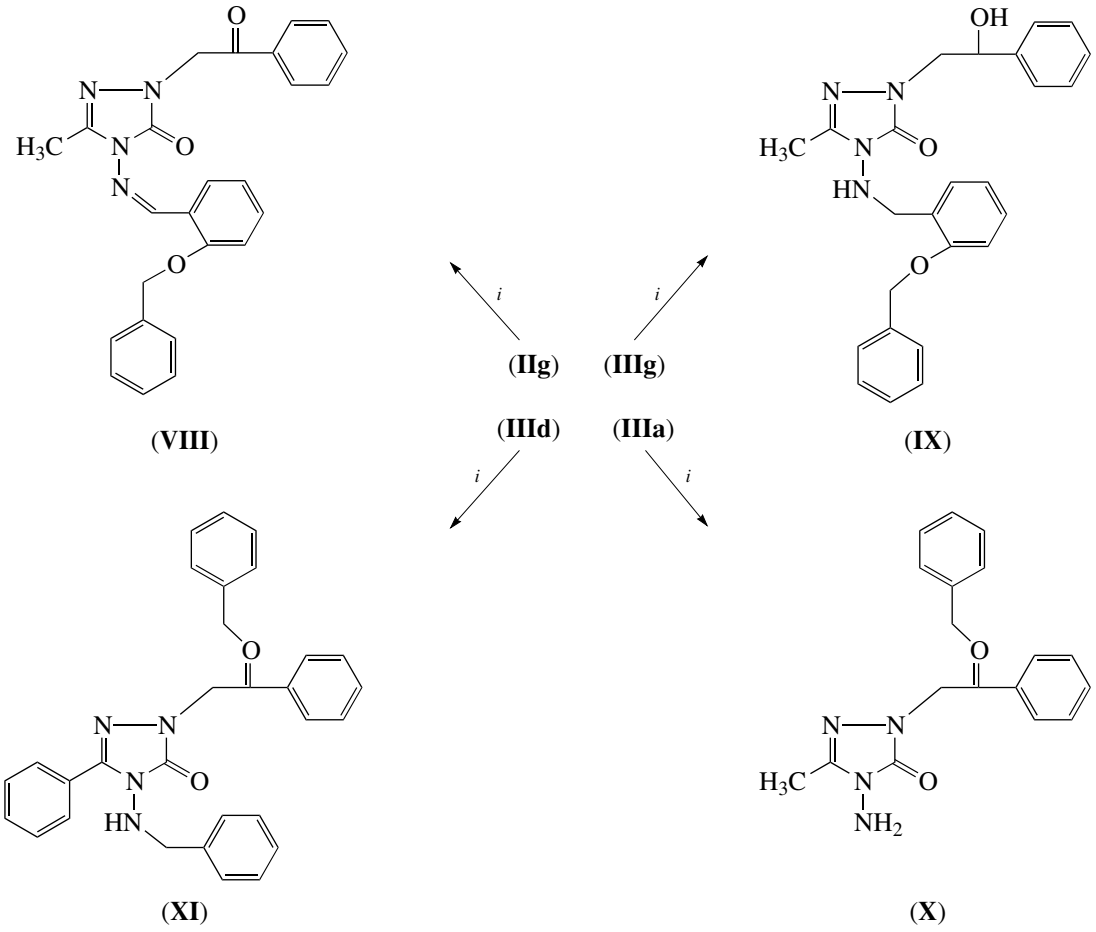
Scheme 2.

EXPERIMENTAL

Melting points were determined on a Gallenkamp melting point apparatus and are uncorrected. ^{1}H NMR and ^{13}C NMR spectra were recorded on a Varian-Mercury 200 MHz spectrometer in DMSO-d_6, chemical shift values are given in ppm (δ scale), and tetramethylsilane was used as an internal standard. IR spectra were recorded in potassium bromide pellets on a Perkin-Elmer 1600 FTIR spectrometer (ν, cm^{-1}). Mass spectra were recorded on a Quattro LC-MS (70 eV) Instrument. All the chemicals were obtained from Fluka Chemie AG Buchs (Switzerland). Compounds (**Ia**)–(**Ij**) were synthesized by the earlier published method [23].

A general procedure for the synthesis of 5-alkyl-4-X-2-phenacyl-2,4-dihydro-*3H*-1,2,4-triazol-3-ones (IIa–IIj). The corresponding 5-alkyl(or aryl)-4-amino(or arylidenamino)-2,4-dihydro-3*H*-1,2,4-triazol-3-one (**I**) (0.01 mol) was refluxed with equivalent amount of sodium metal in absolute ethanol for 2 h. Then, α-bromoacetophenone (0.01 mol) was added, and the mixture was refluxed for additional 5 h. After evaporation at 35–40°C in a vacuum, the resulting solid was recrystallized from an appropriate solvent.

4-Amino-5-methyl-2-phenacyl-2,4-dihydro-3*H*-1,2,4-triazol-3-one (IIa); yield 82.20%, mp 182–183°C (from ethanol); IR: 1723 and 1699 (2 C=O), 1611 (C=N) cm^{-1}; ^{1}H NMR: 1.85 (3 H, s, CH_3), 5.43 (2 H, s, NCH_2), 5.52 (2 H, s, NH_2), 7.20–7.31 (3 H, m, Ar), 7.43–7.58 (2 H, m, Ar); ^{13}C NMR: 22.34 (CH_3), 56.66 (NCH_2), Ar: 128.01 (CH), 128.36 (2 CH), 129.61 (CH), 130.73 (CH), 131.24 (C), 143.04 (C3 of triazole), 154.18 (C5 of triazole), 194.21 ($CH_2\underline{C}$=O); MS, *m/z*



Scheme 3.

(*I*, %): 238.08 $[M + 1]^+$ (16), 233.08 $[M]^+$ (100), 127.08 (33), 119.11 (23), 115.15 (25).

4-Amino-5-benzyl-2-phenacyl-2,4-dihydro-3*H*-1,2,4-triazol-3-one (IIb); yield 80.12%, mp 188–189°C (from 1 : 1 ethyl acetate–petroleum ether); IR: 3308 and 3206 (NH_2), 1706 and 1682 (2 C=O), 1652 (C=N) cm^{-1}; ^1H NMR: 3.85 (2 I, s, CH_2), 5.38 (2 H, s, NCH_2), 5.42 (2 H, s, NH_2), 7.28–7.36 (3 H, m, Ar), 7.58–7.72 (2 H, m, Ar), 7.69–7.98 (5 H, m, Ar); ^{13}C NMR: 37.87 (CH_2), 51.66 (NCH_2), Ar: 128.01 (2 CH), 128.12 (2 CH), 128.69 (2 CH), 129.32 (CH), 129.67 (CH), 130.12 (CH), 130.45 (CH), 131.19 (2 C)], 142.52 (C3 of triazol), 152.34 (C5 of triazol), 193.71 ($CH_2\underline{C}$=O).

5-Methyl-4-(phenylmethyliden)amino-2-phenacyl-2,4-dihydro-3*H*-1,2,4-triazol-3-one (IIc); yield 74.13%); mp 136–137°C (from ethanol); IR: 1703 and 1691 (2 C=O), 1611 and 1595 (2 C=N) cm^{-1}; ^1H NMR: 2.35 (3 H, s, CH_3), 5.41 (2 H, s, NCH_2), 7.54–7.59 (4 H, m, Ar), 7.62–7.68 (2 H, m, Ar), 7.85–8.00 (2 H, m, Ar), 8.05–8.09 (2 H, m, Ar), 9.72 (H, s, N=CH); ^{13}C NMR: 10.93 (CH_3), 51.62 (NCH_2), Ar: 127.58 (CH), 127.74 (2 CH), 128.08 (2 CH), 128.87 (2 CH), 128.93 (2 CH), and 131.55 (CH), 133.18 (2C), 143.63 (C3 of triazol), 150.12 (C5 of triazol), 154.16 (N=CH), 192.84 ($CH_2\underline{C}$=O); MS, *m*/*z* (*I*, %): 321.04 $[M]^+$ (100), 248.99 (50), 238.77 (14), 123.78 (14), 121.91 (27), 115.89 (12), 65.00 (36), 63.12 (60), 60.24 (18).

5-Phenyl-4-(phenylmethyliden)amino-2-phenacyl-2,4-dihydro-3*H*-1,2,4-triazol-3-one (IId); yield 81.62%; mp 159–160°C (from ethanol); IR: 1701 and 1692 (2 C=O), 1597 and 1544 (2 C=N) cm^{-1}; ^1H NMR: 5.61 (2 H, s, NCH_2), 7.56–7.74 (6 H, m, Ar), 7.75–7.84 (3 H, m, Ar), 7.87–7.93 (3 H, m, Ar), 8.05–8.12 (3 H, m, Ar), 9.67 (H, s, N=CH); ^{13}C NMR: 54.13 (NCH_2), Ar: 125.89 (C), 127.88 (3 CH), 127.92 (3 CH), 128.11 (2 CH), 128.56 (2 CH), 128.86 (CH), 129.02 (CH), 130.31 (CH), 131.77 (CH), 132.96 (CH), 134.00 (C), and 134.11 (C), 143.68 (C3 of triazol), 150.22 (C5 of triazol), 157.09 (N=CH), 192.64 ($CH_2\underline{C}$=O).

Table 1. Antimicrobial activity of the compounds tested (μg/ml)

Compound	*Ec*	*Kp*	*Yp*	*Pa*	*Ef*	*Sa*	*Bs*	*Ca*	*Ct*	*Cg*
(**IIa**)	>1250	39	156	156	4	4	4	–	–	–
(**IIb**)	2	48	3125	3125	8	4	4		–	–
(**IIc**)	625	78	156	>1250	2	1	1	–	–	–
(**IIf**)	312	156	156	156	4	4	4	–	–	–
(**IIg**)	312	156	156	156	9	4	4	–	–	–
(**IIh**)	312	156	>1250	312	9	2	2	–	–	–
(**IIj**)	312	156	156	625	9	4	1	–	–	–
(**IIIb**)	8	781	3125	3125	8	8	4	–	–	–
(**IIIc**)	312	156	312	312	19	9	4	–	–	–
(**IIId**)	625	156	625	>1250	19	19	19	–	–	–
(**IIIh**)	312	156	156	>1250	4	4	4	–	–	–
(**IIIj**)	312	156	156	312	39	1	1	–	–	–
(**IV**)	16	48	3125	3125	32	32	16	–	–	–
(**V**)	16	3125	6250	3125	0.5	4	4	–	–	–
(**VI**)	8	48	3125	3125	8	4	4	–	–	–
(**VII**)	16	97	3125	3125	16	32	16	–	–	–
(**VIII**)	16	48	3125	3125	32	32	16	–	–	–
(**IX**)	16	48	3125	3125	32	32	32	–	–	–
(**X**)	32	48	3125	3125	32	32	32	–	–	–
(**XI**)	>1250	12	94	>1250	0.3	0.3	0.3	–	–	–
Amp.	8	32	32	>128	2	2	<1			
Flu.	32							<1	8	64

Note: Abbreviations: *Ec*, *E. coli* ATCC 25922; *Kp*, *K. pneumoniae* ATCC 13883; *Yp*, *Y. pseudotuberculosis* ATCC 911; *Pa*, *P. aeruginosa* ATCC 10145; *Ef*, *E. faecalis* ATCC 29212; *Sa*, *S. aureus* ATCC 25923; *Bs*, *B. subtilis* ATCC 6633; *Ca*, *C. albicans* ATCC 60193; *Ct*, *C. tropicalis* ATCC 13803; *Cg*, *C. glabrata* ATCC 66032. Amp., Ampicillin; Flu., Fluconazole; and (–), not determined (5 mg/ml).

5-Benzyl-4-[(2,4-dichlorophenyl)methyliden]amino-2-phenacyl-2,4-dihydro-3*H*-1,2,4-triazol-3-one (IIe); yield 84.21%; mp 167–168°C (from ethanol); IR: 1709 and 1697 (2 C=O), 1595 and 1583 (2 C=N) cm^{-1}; ^1H NMR: 4.10 (2 I, s, CH_2), 5.47 (2 H, s, NCH_2), 7.27–7.29 (5 H, m, Ar), 7.58–7.61 (3 H, m, Ar), 7.69–7.72 (2 H, m, Ar), 7.98–8.06 (3 H, m, Ar), 10.09 (H, s, N=CH); ^{13}C NMR: 36.64 (CH_2), 58.76 (NCH_2), Ar: 126.74 (CH), 128.05 (3 CH), 128.43 (3 CH), 128.68 (3 CH), 128.83 (3 CH), 129.54 (C), 133.96 (C), 134.06 (C), 135.04 (C), 136.69 (C), 144.42 (C3 of triazol), 147.95 (N=CH), 150.02 (C5 of triazol), 192.69 ($CH_2\underline{C}$=O).

4-[(2,4-Dichlorophenyl)methyliden]amino-5-(4-chlorophenyl)methyl-2-phenacyl-2,4-dihydro-3*H*-1,2,4-triazol-3-one (IIf); yield 77.35%; mp 186–187°C (from ethanol); IR: 1705 and 1698 (2 C=O), 1587 and 1563 (2 C=N) cm^{-1}; ^1H NMR: 4.18 (2 H, s, CH_2), 5.46 (2 H, s, NCH_2), 7.39 (4 H, br.s., Ar), 7.58–7.61 (5 H, m, Ar), 7.80–8.03 (3 H, m, Ar), 9.89 (H, s, N=CH); ^{13}C NMR: 29.98 (CH_2), 51.78 (NCH_2), Ar: 128.05 (3 CH), 128.37 (2 CH), 128.84 (2 CH), 129.55 (CH), 129.59 (C), 130.66 (2 CH), 131.46 (C), 133.94 (C), 134.07 (2 CH), 134.19 (C), 135.07 (C), 136.73 (C), 145.15 (C3 of triazol), 148.10 (N=CH), 150.00 (C5 of triazol), 192.56 ($CH_2\underline{C}$=O).

4-[(2-Hydroxyphenyl)methyliden]amino-5-methyl-2-phenacyl-2,4-dihydro-3*H*-1,2,4-triazol-3-one (IIg); yield 81.38%; mp 175–176°C (from 1 : 1 ethyl acetate–petroleum ether); IR: 3327 (OH), 1724 and 1692 (2 C=O), 1614 and 1590 (2 C=N) cm^{-1}; ^1H NMR: 2.37 (3 H, s, CH_3), 5.48 (2 H, s, NCH_2), 7.14–7.31 (2 H, m, Ar), 7.33–7.38 (2 H, m, Ar), 7.45–7.62 (3 H, m, Ar), 7.70–7.95 (2 H, m, Ar), 9.83 (H, s, N=CH), 10.29 (H, s, OH); ^{13}C NMR: 19.74 (CH_3), 53.06 (NCH_2), Ar: 118.82 (CH), 119.29 (2 CH), 119.57 (CH), 124.86 (CH),126.17 (CH), 128.21 (CH),129.48 (2 CH), 132.11 (CH), 134.05 (C), and 142.11 (C), 146.92 (C3 of triazol), 155.37 (C5 of triazol), 162.63 (N=CH), 191.49 ($CH_2\underline{C}$=O); MS, *m*/*z* (*I*, %): 337.09 $[M]^+$ (67), 304.17 (46), 282.17(44), 254.96 (21), 232.82 (14), 218.84 (13), 196.90 (11), 176.84 (16), 160.66 (11), 158.78 (59), 155.77 (100), 152.95 (51), 139.78 (16), 121.79 (13), 119.78 (34).

4-[(2-Hydroxyphenyl)methyliden]amino-5-ethyl-2-phenacyl-2,4-dihydro-3*H*-1,2,4-triazol-3-one (IIh); yield 68.66%, mp 155–157°C (from ethanol); IR: 3355 (OH), 1712 and 1693 (2 C=O), 1602 and 1591 (2 C=N) cm^{-1}; ^1H NMR: 1.22 (3 H, t, *J* 7.0 Hz, CH_3), 2.50 (2 H, q, *J* 7.0 Hz, CH_2), 5.41 (2 H, s, NCH_2), 6.93–6.98 (2H, m, Ar), 7.36–7.54 (H, m, Ar), 7.68–7.72 (4 H, m, Ar), 8.02–8.07 (2 H, m, Ar), 9.94 (H, s, N=CH), 10.40 (H, s, OH); ^{13}C NMR: 19.62 (CH_2), 28.55 (CH_2), 52.03 (NCH_2), Ar: 116.11 (CH), 118.82 (CH), 119.43 (C), 124.78 (CH), 127.14 (CH), 128.82 (CH), 132.12 (CH), 133.28 (2 CH), 134.06 (2 C), 145.92 (C), 147.11 (C3 of triazol), 156.74 (C5 of triazol), 162.69 (N=CH), 192.67 ($CH_2\underline{C}$=O).

4-[(2-Hydroxyphenyl)methyliden]amino-5-propyl-2-phenacyl-2,4-dihydro-3H-1,2,4-triazol-3-one (IIi); yield 66.52%, mp 156–157°C (from ethanol); IR: 3355 (OH), 1708 and 1685 (2 C=O), 1601 and 1584 (2 C=N) cm^{-1}; ^1H NMR: 0.96 (3 H, t, *J* 7.0 Hz, CH_3), 1.34 (2 H, m, CH_2), 2.57–2.64 (2 H, d, *J* 6.0 Hz, CH_2), 5.42 (2 H, s, NCH_2), 6.93–6.97 (2 H, m, Ar), 7.33–7.43 (H, m, Ar), 7.55–7.85 (4 H, m, Ar), 8.03–8.07 (2 H, m, Ar), 9.95 (H, s, N=CH), 10.35 (H, s, OH); ^{13}C NMR: 13.28 (CH_3), 19.62 (CH_2), 28.52 (CH_2), 51.68 (NCH_2), Ar: 116.32 (CH), 119.33 (CH), 119.43 (C), 126.00 (CH), 128.03 (CH), 128.83 (CH), 130.71 (2 CH), 133.13 (CH), 134.05 (2 C), 146.07 (C), 147.26 (C3 of triazol), 157.51 (C5 of triazol), 162.69 (N=CH), 192.84 ($CH_2\underline{C}$=O).

5-Benzyl-4-[(2-hydroxyphenyl)methyliden]amino-2-phenacyl-2,4-dihydro-3*H*-1,2,4-triazol-3-one (IIj); yield 63.17%; mp 151–152°C (from ethanol); IR: 3294 (OH), 1702 (2 C=O), 1604 and 1594 (2 C=N) cm^{-1}; ^1H NMR: 3.86 (2 H, s, CH_2), 5.42 (2 H, s, NCH_2), 6.90–6.97 (2 H, m, Ar), 7.28–7.39 (3 H, m, Ar), 7.48–7.54 (5 H, m, Ar), 8.02–8.11 (4 H, m, Ar), 9.92 (H, s, N=CH), 10.33 (H, s, OH); ^{13}C NMR: 36.22 (CH_2), 54.04 (NCH_2), Ar: 119.45 (CH), 119.92 (C), 122.01 (CH), 124.83 (2 CH), 126.24 (2 CH), 128.07 (CH), 128.66 (2 CH), 129.12 (2 CH), 131.77 (2 CH), 133.72 (CH), 134.02 (2 C), 136.18 (C), 142.52 (C3 of triazol), 152.34 (C5 of triazol), 154.23 (N=CH), 192.12 ($CH_2\underline{C}$=O).

General procedure for the synthesis of 5-alkyl[aryl]-4-Y-2-(2-hydroxy-2-phenethyl)-2,4-dihydro-3*H*-1,2,4-triazol-3-ones (IIIa–IIIj). A solution of the corresponding compound (**IIa**)–(**IIj**) (0.01 mol) in absolute ethanol (40 ml) was treated with a solution of $NaBH_4$ (0.03 mol) in absolute ethanol (30 ml). The mixture was refluxed for 8 h and then poured in 500 ml of water. A cooling in a deep freezer led to precipitation of a solid of (**IIIa**)–(**IIIj**), which was recrystallized from 1 : 1 ethanol–water mixture.

4-Amino-2-(2-hydroxy-2-phenethyl)-5-methyl-2,4-dihydro-3*H*-1,2,4-triazol-3-one (IIIa); yield 48.77%; mp 98–99°C (from ethanol); IR: 3365–3238 (OH + NH_2), 1688 (C=O), 1614 and 1519 (2 C=N) cm^{-1}; ^1H NMR: 1.93 (3 H, s, CH_3), 3.90 (H, d, NCH_2), 4.07 (H, d, NCH_2), 5.43 (H, br. s, CH), 5.56 (H, d, *J* 4.0 Hz, OH), 6.52 (H, s, NH), 7.03–7.22 (3 H, m, Ar), 7.28–7.42 (2 H, m, Ar); ^{13}C NMR: 23.65 (CH_3), 57.61 (NCH_2), 69.76 (CH), Ar: 126.11 (CH), 128.36 (CH), 129.19 (CH), 128.91 (CH), 133.96 (CH), and 144.48 (C), 144.27 (C3 of triazol), 153.73 (C5 of triazol).

Table 2. Antitumor activity of some compounds

Compound	Number assigned by NCI	Growth percentage of tumor cell, %			Activity
		MCF7	NCI-H460	SF-268	
(**IIa**)	729340	122	102	99	–
(**IIb**)	729341	40	96	90	–
(**IIg**)	731549	46	93	121	–
(**IIf**)	729346	62	96	96	–
(**IIi**)	729348	29	94	80	+
(**IIIb**)	729343	1	32	23	+
(**IIIf**)	729347	1	4	3	+
(**IIIi**)	729901	138	156	96	–
(**IV**)	734565	97	104	106	–
(**V**)	734567	95	86	89	–
(**VI**)	734566	7	48	85	+
(**VII**)	734568	0	6	53	+
(**VIII**)	734568	110	112	118	–
(**IX**)	734564	1	30	45	+

4-Amino-5-benzyl-2-(2-hydroxy-2-phenethyl)-2,4-dihydro-3*H*-1,2,4-triazol-3-one (IIIb); yield 50.23%; mp 164–165°C; IR: 3362–3332 (OH + NH_2), 1693 (C=O), 1630 (2 C=N) cm^{-1}; ^1H NMR: 3.92 (2 H, s, CH_2), 4.31 (H, d, NCH_2), 4.38 (H, d, NCH_2), 5.02 (H, m, CH), 5.38 (2 H, s, NH_2), 6.68 (H, br. s, OH), 7.31–7.40 (3 H, m, Ar), 7.51–7.62 (2 H, m, Ar), 7.73–8.07 (5 H, m, Ar); ^{13}C NMR: 34.65 (CH_2), 55.45 (NCH_2), 69.21 (CH), Ar: 124.18 (2 CH), 125.22 (2 CH), 126.51 (2 CH), 127.29 (2 CH), 127.93 (CH), 128.65 (CH), 130.08 (2 C), 142.15 (C3 of triazol), 152.18 (C5 of triazol); MS, *m*/*z* (*I*, %): 311.12 $[M]^+$ (13), 293.08 (26), 282.18 (13), 155.71 (14), 152.83 (100).

4-(Benzylamino)-5-methyl-2-(2-hydroxy-2-phenethyl)-2,4-dihydro-3*H*-1,2,4-triazol-3-one (IIIc); yield 48.77%; mp 115–116°C; IR: 3231 (OH + NH), 1678 (C=O), 1584 and 1510 (2 C=N) cm^{-1}; ^1H NMR: 1.93 (3 H, s, CH_3), 3.58 (H, d, NCH_2), 3.83 (H, d, NCH_2), 4.03 (2 H, br. s, $NHC\underline{H}_2$), 5.29 (H, br. s, CH), 5.56 (H, d, *J* 4.0 Hz, OH), 6.52 (H, br. s, NH), 7.20–7.52 (10 H, m, Ar); ^{13}C NMR: 24.15 (CH_3), 48.85 ($NHCH_2$), 58.78 (NCH_2), 70.46 (CH), Ar: 126.07 (2 CH), 127.25 (CH), 127.42 (CH), 129.59(CH), 128.23 (CH), 128.64 (CH), 129.81 (CH), 129.96 (2 CH),

137.14 (C), 142.65 (C), 144.13 (C3 of triazol), 152.31 (C5 of triazol); MS, *m*/*z* (*I*, %): 324.37 $[M]^+$ (4), 311.12 (6), 110.98 (100).

4-(Benzylamino)-2-(2-hydroxy-2-phenethyl)-5-phenyl-2,4-dihydro-3*H*-1,2,4-triazol-3-one (IIId); yield 42.19%; mp 110–111°C; IR: 3251 (OH + NH), 1689 (C=O), 1583 and 1550 (2 C=N) cm^{-1}; ^1H NMR: 3.70–3.93 (2 H, dd, NCH_2), 4.15 (2 H, br. s, $NHCH_2$), 4.95 (H, m, CH), 5.62 (H, d, *J* 4.1 Hz, OH), 6.60 (H, br. s, NH), 7.05–7.22 (6 H, m, Ar), 7.23–7.45 (5 H, m, Ar), 7.65–7.78 (4 H, m, Ar); ^{13}C NMR: 49.18 ($NHCH_2$), 52.31 (NCH_2), 70.13 (CH), Ar: 126.04 (3 CH), 126.34 (C), 127.19 (3 CH), 127.27 (3 CH), 128.02 (3 CH), 128.81 (2 CH), 129.58 (CH), 136.25 (C), and 142.50 (C), 144.13 (C3 of triazol), 152.31 (C5 of triazol); MS, *m*/*z* (*I*, %): 387.12 $[M]^+$ (13), 369.11 (36), 265.07 (16), 249.10 (100), 177.03 (10).

5-Benzyl-4-(2,4-dichlorobenzyl)amino-2-(2-hydroxy-2-phenethyl)-2,4-dihydro-3H-1,2,4-triazol-3-one (IIIe); yield 51.5%; mp 114–115°C; IR: 3446 (OH), 3232 (NH), 1688 (C=O), 1586 (2 C=N) cm^{-1}; ^1H NMR: 3.68 (2 H, s, CH_2), 3.71 (2 H, br. s, $NHC\underline{H}_2$), 4.05 and 4.29 (2 H, dd, NCH_2), 4.96 (H, t, *J* 12.0, CH), 5.54 (H, br. s, OH), 6.47 (H, br. s, NH), 7.03–7.16 (3 H, m, Ar), 7.21–7.33 (5 H, m, Ar), 7.33–7.39 (5 H, m, Ar); ^{13}C NMR: 35.82 (CH_2), 48.91 ($NHCH_2$), 51.83 (NCH_2), 70.43 (CH), Ar: 126.09 (CH), 128.63 (2 CH), 127.18 (3 CH), 127.98 (3 CH), 128.18 (2 CH), 128.63 (2 CH), 132.70 (C), 132.99 (C), 133.50 (C), 134.34 (C), 135.49 (C), 145.78 (C3 of triazol), 152.68 (C5 of triazol); MS, *m*/*z* (*I*, %): 504.98 $[M + 1]^+$ (25), 486.97 (44), 475.24 (57), 454.22 (14), 453.22 (52), 249.02 (39), 227.08 (43), 209.02 (12), 164.90 (13), 116.96 (13), 113.95 (20), 100.11 (29), 79.18 (100), 60.32 (47).

5-(4-Chlorobenzyl)-4-(2,4-dichlorobenzyl)amino-2-(2-hydroxy-2-phenethyl)-2,4-dihydro-3*H*-1,2,4-triazol-3-one (IIIf); yield 50.32%; mp 126–127°C; IR: 3263–3084 (OH + NH), 1692 (C=O), 1612 and 1602 (2 C=N) cm^{-1}; ^1H NMR: 3.48 (2 H, s, CH_2), 3.63–3.89 (2 H, m, NCH_2), 4.07 (H, s, $NHC\underline{H}_2$), 4.89 (H, br. s, CH), 5.63 (H, d, *J* 4.0 Hz, OH), 6.64 (H, br. s, NH), 7.00–7.05 (3 H, m, Ar), 7.16–7.33 (7 H, m, Ar), 7.53 (2 H, br. s, Ar); ^{13}C NMR: 34.12 (CH_2), 48.88 ($NH\underline{C}H_2$), 54.85 ($N\underline{C}H_2$), 70.11 (CH), Ar: 126.25 (2 CH), 127.18 (CH), 127.24 (2 CH), 127.99 (CH), 128.07 (2 CH), 128.64 (CH), 129.13 (2 CH), 130.11 (CH), 131.17 (C), 132.69 (C), 133.04 (C), 133.44 (2CH), 134.34 (C)], 145.37 (C3 of triazol), 152.21 (C5 of triazol); MS, *m*/*z* (*I*, %): 504.98 $[M + 1]^+$ (25), 486.97 (44), 475.24 (57), 454.22 (14), 453.22 (52), 249.02 (39), 227.08 (43), 209.02 (12), 164.90 (13), 116.96 (13), 113.95 (20), 100.11 (29), 79.18 (100), 60.32 (47).

4-(2-Hydroxybenzyl)amino-2-(2-hydroxy-2-phenethyl)-5-methyl-2,4-dihydro-3*H*-1,2,4-triazol-3-one (IIIg); yield 45.16%; mp 154–155°C; IR: 3341 (2 OH), 3256 (NH), 1684 (C=O), 1595 (2 C=N) cm^{-1}; ^1H NMR: 2.34 (3 H, s, CH_3), 3.74 (H, br. s, $NHC\underline{H}_2$), 4.24 (H, d, NCH_2), 4.47 (H, d, NCH_2), 4.97 (H, br. s, CH), 5.47 (H, d, *J* 4.2 Hz, OH), 6.19 (H, br. s, NH), 6.86–6.94 (3 H, m, Ar), 7.02–7.13 (H, m, Ar), 7.21–7.26 (5 H, m, Ar), 9.49 (H, s, $PhO\underline{H}$); ^{13}C NMR: 19.65 (CH_3), 47.34 ($NH\underline{C}H_2$), 56.31 (NCH_2), 70.27 (CH), Ar: 116.38 (CH), 119.31 (CH), 123.62 (C), 125.24 (2CH), 128.03 (2CH), 129.28 (CH), 129.03 (CH), 130.21 (CH), 137.09 (C), and 139.11 (C), 145.56 (C3 of triazol), 153.68 (C5 of triazol).

4-(2-Hydroxybenzyl)amino-2-(2-hydroxy-2-phenethyl)-5-ethyl-2,4-dihydro-3*H*-1,2,4-triazol-3-one (IIIh); yield 57.30%; mp 117–119°C; IR: 3246 (2 OH + NH), 1688 (C=O), 1593 (2 C=N); ^1H NMR: 0.98 (3 H, t, *J* 7.4 Hz, CH_3), 2.25 (2 H, q, *J* 7.4 Hz, CH_2), 3.60 (H, d, NCH_2), 3.85 (H, d, NCH_2) 4.06 (2 H, br.s, $N\underline{H}CH_2$), 4.94 (H, br. s, CH), 5.59 (H, d, *J* 4.1 Hz, OH), 6.21 (H, br. s, NH), 6.72–6.80 (3 H, m, Ar), 6.84–6.94 (H, m, Ar), 6.98–7.11 (H, m, Ar), 7.30–7.37 (4 H, m, Ar), 9.53 (H, s, PhOH); ^{13}C NMR: 18.63 (CH_3), 25.69 (CH_2), 47.33 ($NHCH_2$), 55.81 (NCH_2), 70.15 (CH), Ar: 115.14 (CH), 118.64 (2 CH), 123.09 (C), 126.17 (CH), 127.33 (2 CH), 127.59 (CH), 128.55 (CH), 133.52 (CH), 140.67 (C), 144.46 (C), 146.94 (C3 of triazol), 153.02 (C5 of triazol).

4-(2-Hydroxybenzyl)amino-2-(2-hydroxy-2-phenethyl)-5-propyl-2,4-dihydro-3*H*-1,2,4-triazol-3-one (IIIi); yield 56.07%; mp 144–145°C; IR: 3246 (2 OH + NH), 1684 (C=O), 1595 (2 C=N) cm^{-1}; ^1H NMR: 0.75 (3 H, t, *J* 7.4 Hz, CH_3), 1.32–1.43 (2 H, m, CH_2), 2.06 (2 H, t, *J* 7.6 Hz, CH_2), 3.76 (2 H, br. s, $NHCH_2$), 3.60 and 3.85 (2 H, dd, NCH_2), 4.88 (H, br. s, CH), 5.52 (H, d, *J* 4.0, OH), 6.19 (H, br. s, NH), 6.69–6.78 (3 H, m, Ar), 7.05–7.09 (H, m, Ar), 7.18–7.28 (5 H, m, Ar), 9.47 (H, s, PhOH); ^{13}C NMR: 13.38 (CH_3), 18.63 (CH_2), 25.66 (CH_2), 47.37 ($NHCH_2$), 55.92 (NCH_2), 70.15 (CH), Ar: 114.88 (CH), 118.58 (CH), 123.08 (C), 126.03 (2 CH), 127.17 (2 CH), 127.97 (CH), 128.53 (CH), 130.70 (CH), 142.66 (C), 145.68 (C), 146.96 (C3 of triazol), 152.44 (C5 of triazol); MS, *m*/*z* (*I*, %): 369.02 $[M]^+$ (27), 351.08 (17), 285.90 (15), 284.96 (100), 248.96 (44), 244.98 (32), 214.09 (20), 121.80 (13), 105.88 (11).

5-Benzyl-4-(2-hydroxybenzyl)amino-2-(2-hydroxy-2-phenethyl)-2,4-dihydro-3*H*-1,2,4-triazol-3-one (IIIj); yield 47.60%; mp 139–140°C; IR: 3296 (2 OH), 3239 (NH), 1686 (C=O), 1609 (2 C=N) cm^{-1}; ^1H NMR 3.78 (2 H, s, CH_2), 3.76 (H, br.s, $NHC\underline{H}_2$), 4.06 and 4.31 (2 H, dd, NCH_2), 5.11 (H, t, *J* 12.0 Hz, CH), 5.49 (H, br. s, OH), 6.29 (H, br. s, NH), 6.71–6.82 (3 H, m, Ar), 7.09–7.12 (2 H, m, Ar), 7.16–7.25 (4 H, m, Ar), 7.28–7.36 (5 H, m, Ar), 9.54 (H, s, PhOH); ^{13}C NMR: 34.11 (CH_2), 48.12 ($NHCH_2$), 49.09 (NCH_2), 71.06 (CH), Ar: 121.81 (CH), 122.26 (2 CH), 123.66 (2 CH), 124.07 (C), 126.05 (CH), 126.17 (2 CH), 127.94 (2 CH), 128.53 (2 CH), 130.72(2 CH), 136.14 (C),

141.97 (C), 143.12 (C), 145.45 (C3 of triazol), 155.71 (C5 of triazol); MS, *m*/*z* (*I*, %): 417.06 $[M]^+$ (20), 381.17 (18), 380.16 (49), 364.16 (11), 305.17 (22), 304.17 (100), 302.16 (18), 282.14 (72), 265.07 (13), 256.79 (35), 240.85 (22), 228.05 (16), 198.81 (13), 176.78 (19), 160.78 (13), 158.77 (33), 152.94 (79), 142.71 (30), 141.77 (16), 119.80 (29), 110.77 (33), 100.79 (64).

A general procedure for the synthesis of compounds (IV)–(VII). A solution of the corresponding compound (**IIg**), (**IIj**), or (**IIIj**) (0.01 mol) in ethanol [dry toluene for (**IIIb**)] was refluxed with equivalent amount of NaOH [sodium metal for (**IIIb**)] for 1 h. Then, *p*-toluenesulfonyl chloride (0.01 mol) was added, and the reaction mixture was refluxed for an additional 5 h. After the removal of solvents at a reduced pressure, a solid was obtained, which was recrystallized from ethanol.

5-Methyl-4-[(2-tosyloxyphenyl)methyliden]amino-2-phenacyl-2,4-dihydro-3*H*-1,2,4-triazol-3-one (IV); yield: 48.28%, mp 181–182°C; IR: 1719 and 1693 (C=O), 1615 and 1594 (C=N) cm^{-1}; ^1H NMR: 1.88 (3 H, s, CH_3), 2.47 (3 H, s, CH_3), 5.28 (2 H, s, NCH_2), 7.08–7.14 (3 H, m, Ar), 7.17–7.25 (2 H, m, Ar), 7.33–7.39 (6 H, m, Ar), 7.45–7.62 (2 H, m, Ar), 9.03 (H, s, N=CH); ^{13}C NMR: 12.34 (CH_3), 22.15 (CH_3), 48.31 (NCH_2), Ar: 119.36 (CH), 119.48 (CH), 121.84 (2 CH), 123.36 (CH), 124.73 (C), 125.95 (2 CH), 127.72 (CH), 128.37 (2 CH), 130.02 (3 CH), 131.71 (2 C), 139.12 (C), 145.93 (C), 143.17 (C3 of triazol), 145.65 (C5 of triazol), 155.12 (N=CH), 192.48 (C=O); MS, *m*/*z* (*I*, %): 491.21 $[M]^+$ (100), 320.23 (10), 305.19 (11), 304.18 (52), 283.23 (9), 282.22 (37), 156.86 (11), 155.86 (17), 152.96 (22).

5-Benzyl-4-[(2-tosyloxyphenyl)methyliden]amino-2-phenacyl-2,4-dihydro-3*H*-1,2,4-triazol-3-one (V); yield: 47.85%; mp 141–142°C; IR: 1715 and 1684 (2 C=O), 1623 and 1597 (C=N) cm^{-1}; ^1H NMR: 2.33 (3 H, s, CH_3), 3.94 (2 H, s, CH_2), 5.39 (2 H, s, NCH_2), 6.79–6.93 (3 H, m, Ar), 6.97–7.08 (2 H, m, Ar), 7.18–7.25 (9 H, m, Ar), 7.32–7.47 (4 H, m, Ar), 9.21 (H, s, N=CH); ^{13}C NMR: 21.66 (CH_3), 33.59 (CH_2), 51.67 (NCH_2), Ar: 120.52 (C), 122.18 (2 CH), 125.34 (2 CH), 126.82 (2 CH), 127.31 (2 CH), 128.29 (2 CH), 128.63 (2 CH), 129.42 (2 CH), 129.76 (2 CH), 133.71 (C), 137.12 (2 CH), 138.43 (C), 139.12 (CH), 141.61 (C), 142.56 (C), 143.14 (C3 of triazol), 145.52 (C5 of triazol), 152.65 (N=CH), 194.45 (C=O); MS, *m*/*z* (*I*, %): 567.12 $[M]^+$ (17), 304.19 (11), 282.10 (7), 153.91 (15), 152.90 (100).

5-Benzyl-2-(2-hydroxy-2-phenethyl)-4-[(2-tosyloxybenzyl)amino]-2,4-dihydro-3*H*-1,2,4-triazol-3-one (VI); yield 66.13%; mp 124–125°C; IR: 3268 (OH), 1709 and 1691 (2 C=O), 1594 and 1581 (2 C=N) cm^{-1}; ^1H NMR: 2.24 (3 H, s, CH_3), 3.62 (2 H, s, $PhCH_2$), 3.55 and 3.70 (2 H, dd, NCH_2), 4.26 (H, br. s, $NHCH_2$), 5.74 (H, br. s, CH), 5.98 (H, d, *J* 4.0, OH), 6.11 (H, br. s, NH), 6.84–6.92 (3 H, m, Ar), 6.98–7.09 (2 H, m, Ar), 7.11–7.25 (5 H, m, Ar), 7.28–7.46 (3 H, m, Ar), 7.51–7.56 (3 H, m, Ar), 8.01–8.07 (2 H, m, Ar); ^{13}C NMR: 21.98 (CH_3), 35.21 ($PhCH_2$), 44.35 ($NHCH_2$), 51.51 (NCH_2), 73.28 (CH), Ar: 121.70 (C), 125.93 (CH), 126.12 (CH), 126.84 (2 CH), 127.05 (2 CH), 127.76 (2 CH), 127.85 (2 CH), 127.92 (2 CH), 128.16 (2 CH), 129.17 (4 CH), 129.66 (2 C), 134.62 (C), 134.79 (C), 141.11 (C), 143.17 (C3 of triazol), 155.67 (C5 of triazol); MS, *m*/*z* (*I*, %): 569.82 $[M]^+$ (100), 567.87 (20), 537.85 (43), 304.18 (24), 282.20 (30), 276.98 (18), 274.97 (19), 218.88 (13), 200.85 (16), 155.93 (47), 116.87 (16).

4-Amino-5-benzyl-2-(2-tosyloxy-2-phenethyl)-2,4-dihydro-3*H*-1,2,4-triazol-3-one (VII); yield: 54.36%; mp 167–169°C; IR: 3352–3318 (NH_2), 1698 (C=O), 1612 (2 C=N) cm^{-1}; ^1H NMR: 2.38 (H, s, CH_3), 3.92 (2 H, s, $PhCH_2$), 4.01 and 4.25 (2 H, dd, NCH_2), 5.39 (2 H, s, NH_2), 5.53 (H, m, CH), 6.69 (H, br. s, OH), 7.24–7.38 (3 H, m, Ar), 7.48–7.60 (2 H, m, Ar), 7.71–7.97 (5 H, m, Ar); ^{13}C NMR: 22.32 (CH_3), 35.23 ($PhCH_2$), 49.56 (NCH_2), 72.21 (CH), Ar: 126.31 (2 CH), 127.32 (2 CH), 128.35 (2 CH), 129.29 (2 CH), 130.84 (CH), 133.58 (CH), 141.00 (2 C), 143.82 (C3 of triazol), 145.61 (C5 of triazol).

A general procedure for the synthesis of compounds (VIII)–(XI). A solution of the corresponding compound (**II**) or (**III**) (0.01 mol) in ethanol [(**IIg**) and (**IIIg**)] or dry toluene [(**IIIa**) and (**IIId**)] was refluxed with equivalent amount of NaOH [(**IIg**) and (**IIIg**)] or sodium metal [(**III**) and (**IIId**)] for 1 h. Then, benzyl bromide (0.01 mol) was added and reflux was prolonged for additional 5 h. After the removal of solvents at a reduced pressure, a solid was obtained, which was recrystallized from ethanol.

4-[(2-Benzyloxyphenyl)methyliden]amino-5-methyl-2-phenacyl-2,4-dihydro-3*H*-1,2,4-triazol-3-one (VIII); yield 47.63%; mp 179–180°C; IR: 1721 and 1684 (2 C=O), 1612 and 1597 (2 C=N) cm^{-1}; ^1H NMR 1.99 (3 H, s, CH_3), 4.44 (2 H, s, NCH_2), 4.64 (2 H, s, OCH_2), 6.89–6.93 (2 H, m, Ar), 7.13–7.21 (2 H, m, Ar), 7.28–7.35 (3 H, m, Ar), 7.46–7.62 (4 H, m, Ar), 7.67–7.84 (3 H, m, Ar), 9.72 (H, s, N=CH); ^{13}C NMR: 13.26 (CH_3), 49.45 (NCH_2), 68.13 (OCH_2), Ar: 114.61 (2 CH), 118.75 (2 CH), 120.14 (2 CH), 123.66 (CH), 125.17 (CH), 127.34 (CH), 128.21 (CH), 128.66 (C), 129.48 (2 CH), 132.11 (CH), 134.05 (C), 136.53 (CH), 139.16 (C), 142.11 (C), 144.16 (C3 of triazol), 154.53 (C5 of triazol), 163.25 (N=CH), 194.08 (C=O); MS, *m*/*z* (*I*, %): 427.11 $[M]^+$ (100), 375.00 (13), 337.04 (13), 304.16 (18), 282.17 (31), 156.84 (14), 152.96 (59), 142.81 (24).

4-[2-(Benzyloxy)benzyl]amino-2-(2-hydroxy-2-phenethyl)-5-methyl-2,4-dihydro-3*H*-1,2,4-triazol-3-one (IX); yield: 42.54%; mp 132–134°C; IR: 3324 (OH), 3256 (NH), 1693 (C=O), 1598 (2 C=N) cm^{-1}; ^1H

NMR: 2.01 (3 H, s, CH_3), 4.16 (H, m, $NHCH_2$), 3.88 and 4.13 (2 H, dd, NCH_2), 4.99 (H, br. s, CH), 5.14 (2 H, s, OCH_2), 5.57 (H, br. s, OH), 6.83 (H, br. s, NH), 6.89–6.93 (3 H, m, Ar), 7.11–7.17 (H, m, Ar), 7.19–7.38 (10 H, m, Ar); ^{13}C NMR: 21.01 (CH_3), 44.98 ($NHCH_2$), 54.24 (NCH_2), 70.99 (CH), 72.01 (OCH_2), Ar: 119.28 (2 CH), 121.96 (CH), 124.36 (C), 126.21 (4 CH), 129.45 (3 CH), 129.91 (2 CH), 130.42 (2 CH), 132.21 (C), 138.23 (C), 141.67 (C), 143.60 (C3 of triazol), 155.43 (C5 of triazol); MS, *m/z* (*I*, %) 431.12 $[M]^+$ (100), 413.10 (24), 410.10 (18), 364.05 (16), 363.05 (69), 342.07 (9), 341.07 (46), 324.05 (11), 323.04 (53), 307.03 (16), 304.14 (13), 282.09 (18), 256.97 (14), 216.91 (41), 196.87 (10), 158.75 (11), 152.91 (36), 114.86 (29).

4-Amino-2-[2-(benzyloxy)-2-phenethyl]-5-methyl-2,4-dihydro-3*H*-1,2,4-triazol-3-one (X); yield: 58.38%; mp 139–140°C; IR: 3334–3218 (NH_2), 1693 (C=O), 1617 and 1543 (2 C=N) cm^{-1}; ^{1}H NMR: 1.96 (3 H, s, CH_3), 3.52 and 3.79 (2 H, dd, NCH_2), 4.67 (2 H, s, OCH_2), 5.43 (H, br. s, CH), 5.56 (H, br. s, OH), 6.56 (H, s, NH), 7.21–7.34 (6 H, m, Ar), 7.42–7.49 (2 H, m, Ar), 7.51–7.56 (2 H, m, Ar); ^{13}C NMR: 23.05 (CH_3), 57.61 (NCH_2), 73.34 (CH), Ar: 126.35 (2 CH), 127.11 (CH), 128.93 (CH), 129.19 (CH), 129.75 (CH), 130.63 (CH), 130.91 (CH), 135.25 (2 CH), 138.91 (C), 145.26 (C), 143.62 (C3 of triazol), 154.11 (C5 of triazol).

4-Benzylamino-2-[2-(benzyloxy)-2-phenethyl]-5-phenyl-2,4-dihydro-3*H*-1,2,4-triazol-3-one (XI); yield 54.87%; mp 188–189°C; IR: 3251 (NH), 1702 (C=O), 1597 and 1508 (C=N) cm^{-1}; ^{1}H NMR: 3.36 (H, br. s, $NHCH_2$), 3.62–3.81 (2 H, dd, NCH_2), 4.27 (2 H, s, OCH_2), 4.92 (H, t, *J* 12.0 Hz, CH), 6.12 (H, t, *J* 4.0 Hz, NH), 6.73 (3 H, br. s, Ar), 6.91–7.06 (3 H, m, Ar), 7.17–7.47 (8 H, m, Ar), 7.51–7.66 (4 H, m, Ar), 8.01–8.05 (2 H, m, Ar); ^{13}C NMR: 64.05 (OCH_2), 49.27 ($NHCH_2$), 52.00 (NCH_2), 79.27 (CH), Ar: 125.72 (C), 126.31 (2 C), 127.06 (2 CH), 127.27 (2 CH), 127.71 (2 CH), 127.81 (2 CH), 128.09 (2 CH), 128.22 (CH), 128.69 (2 CH), 128.87 (CH), 129.00 (2 CH), 133.43 (2 CH), 134.66 (C), 135.75 (C), 137.09 (C), 145.21 (C3 of triazol), 152.13(C5 of triazol); MS, *m/z* (*I*, %): 477.07 $[M]^+$ (6), 476.13 (29), 475.06 (100), 454.17 (8), 453.16 (27), 100.06 (19), 91.01 (19), 79.01 (50), 64.06 (79).

Pharmacology

Antimicrobial activity assessment. All test microorganisms were obtained from the Hifzissihha Institute of Refik Saydam (Ankara, Turkey) and were as follows: *Escherichia coli* ATCC 25922, *Yersinia pseudotuberculosis* ATCC 911, *Klebsiella pneumoniae* ATCC 13883, *Pseudomonas aeruginosa* ATCC 10145, *Enterococcus faecalis* ATCC 29212, *Staphylococcus aureus* ATCC 25923, *Bacillus subtilis* ATCC 6633, *Candida albicans* ATCC 60193, *Candida tropicalis* ATCC 13803, and *Candida glabrata* ATCC 66032. All the newly synthesized compounds were dissolved in DMSO and acetone and 95% ethanol to prepare stock solutions with 100-mg/ml concentration.

The antimicrobial effects of the substances were tested quantitatively in respective broth media by using double dilution, and the minimal inhibition concentration (MIC) values (μg/ml) were determined [21]. The antibacterial and antifungal assays were performed in the Mueller–Hinton broth (MH) (Difco, Detroit, MI) at pH 7.3 and buffered Yeast Nitrogen Base (Difco, Detroit, MI) at pH 7.0, respectively. The MIC was defined as the lowest concentration that showed no growth. Ampicillin and fluconazole were used as standard antibacterial and antifungal drugs, respectively. DMSO, acetone and 95% ethanol were used as solvent controls. The results are given in Table 1.

Antitumor screening studies. The screening experiments were carried out by the Developmental Therapeutic Program of the National Cancer Institute (NCI), Bethesda, Maryland, United States. Fourteen compounds (**IIa**), (**IIb**), (**IIg**), (**IIf**), (**IIi**), (**IIIb**), (**IIIf**), (**IIIi**), (**IV**), (**V**), (**VI**), (**VII**), (**VIII**) and (**IX**) were selected by NCI for screening toward 3 human tumor cell lines: *vic.*, breast cancer (MCF7), non small cell lung cancer (NCI-H460) and CNS (SF-268). Each cell line was inoculated and preincubated on a microtiter plate. Test agents were then added at a single concentration and culture incubated for 48 h. End point determinations were made with Alamar Blue [22]. The screening results are summarized in Table 2. Results for each test agent are reported as the percent of growth of the treated cells compared with the untreated control cells. The compounds that reduce the growth any one of the cell lines to approximately 32% or less were regarded as having antitumor activity.

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