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Synthesis of 3-Alkyl(Aryl)-4-alkylidenamino-4,5-dihydro-1H-1,2,4-triazol-5-ones and 3-Alkyl-4-alkylamino-4,5-dihydro-1H-1,2,4-triazol-5-ones as Antitumor Agents

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Abstract—A series of 3-alkyl-4-phenylethylidenamino- (**8**) and 3-alkyl-4-(3-phenylallylidenamino)-4,5-dihydro-1H-1,2,4-triazol-5-ones (**9**) was synthesized from the reaction of the corresponding 3-alkyl(aryl)-4-amino-4,5-dihydro-1H-1,2,4-triazol-5-ones (**1**), with phenylacetaldehyde and cinnamaldehyde. 3-Alkyl-4-(2-phenylethylamino)- (**10**) and 3-alkyl-4-(3-phenylpropylamino)-4,5-dihydro-1H-1,2,4-triazol-5-ones (**11**) were obtained from the selective reduction of compounds (**8**) and (**9**) with $NaBH_4$. The in vitro antitumor activity of the novel compounds was screened and the highest inhibition of tree tumor cell lines was observed for the compounds containing phenylethylenamino and phenylethylamino groups at position 4 of 1,2,4-triazol ring.

Introduction

There exist a number of 1,2,4-triazoles and 4,5-dihydro-1H-1,2,4-triazol-5-ones having a wide range of biological activities such as analgesic,[1] antibacterial,[2–7] fungicidal,[8,9] anti-inflammatory,[10,11] antihypertensive,[12,13] herbicidal,[14,15] coccidiostatic,[16] antiviral,[17] antagonistic,[18] controgestational[19] and antitumoral.[20–22] Moreover, several compounds involving 1,2,4-triazole moiety and having divers pharmacological and antitumorial activities were reported.[23–29]

The chemistry of 3-alkyl(aryl)-4-amino-4,5-dihydro-1H-1,2,4-triazol-5-one compounds (**1**), studied in detail by Ikizler and coworkers[30] was reported to be good nucleophiles in most reactions. For example, 3-alkyl (aryl)-4-alkyliden(aryliden)amino-4,5-dihydro-1H-1,2,4-triazol-5-ones were obtained via nucleophilic attack of amino nitrogen at position 4 on the 1,2,4-triazol-5-one ring to carbonyl carbon of various aldehydes.[30–32] It has also been reported that the conversation of the amino group in 4 position at 1,2,4-triazol ring into arylidenamino group causes antitumor activity.[21] Several alkyliden (aryliden)amino compounds were synthesized by using type **1** compounds and reported to be antitumor active agents. Among these, the compounds having the highest activity contain phenyl and *p*-tolyl groups at position 3 of the heterocycle and *p*-nitrophenylmethylenamino, *o*-chlorophenylmethylenamino and phenylmethylenamino groups at position 4 of the arylidenamino structure (**2**–**4**) (Chart 1).[20] In another study, some 3-alkyl-4-(2-hydroxy-1-naphthylidenamino)- (**5**) and *N*,*N*′-bis(3-alkyl-4,5-dihydro-1H-1,2,4-triazol-5-on-4-yl)-1,4-butanediimines (**6**) having antitumor activities were obtained from the reaction of type **1** compounds with 1-hydroxynaphthaldehyde and 2,5-dimethoxytetrahydrofuran.[21] Moreover, *N*,*N*′-bis(3-alkyl-4,5-dihydro-1H-1,2,4-triazol-5-on-4-yl)-1,4-xylenediimines (**7b** and **7c**), having cytostatic activity against some of the 60 tumor cell lines were synthesized in our laboratories from the reaction of type **1** compounds with terephthalaldehyde (Chart 1).[22] Recently, 3-alkyl(aryl)-4-alkylamino-4,5-dihydro-1H-1,2,4-triazol-5-ones have been synthesized by the reduction of 4-alkylidenamino compounds by using $NaBH_4$ as a selective reducing agent.[33]

We report here the synthesis and testing results of potentially antitumor active 3-alkyl(aryl)-4-alkyliden (aryliden)amino-, 3-alkyl(aryl)-4-alkyl(aryl)amino- and 1-acetyl-3-alkyl(aryl)-4-alkyliden(aryliden)amino-4,5-dihydro-1H-1,2,4-triazol-5-ones. 3-alkyl-4-(phenylethylidenamino)-4,5-dihydro-1H-1,2,4-triazol-5-ones (**8**) and 3-alkyl-4-(3-phenylallylidenamino)-4,5-dihydro-1H-1,2,4-triazol-5-ones (**9**) were synthesized by the reaction of type (**1**) compounds with phenylacetaldehyde and cinnamaldehyde. 3-alkyl-4-(2-phenylethylamino)-4,5-dihydro-

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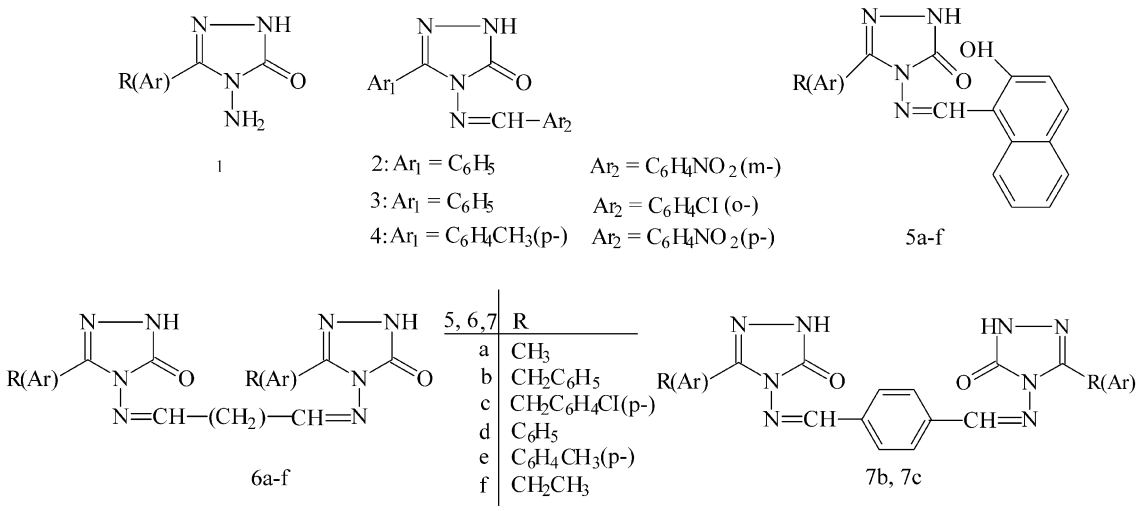
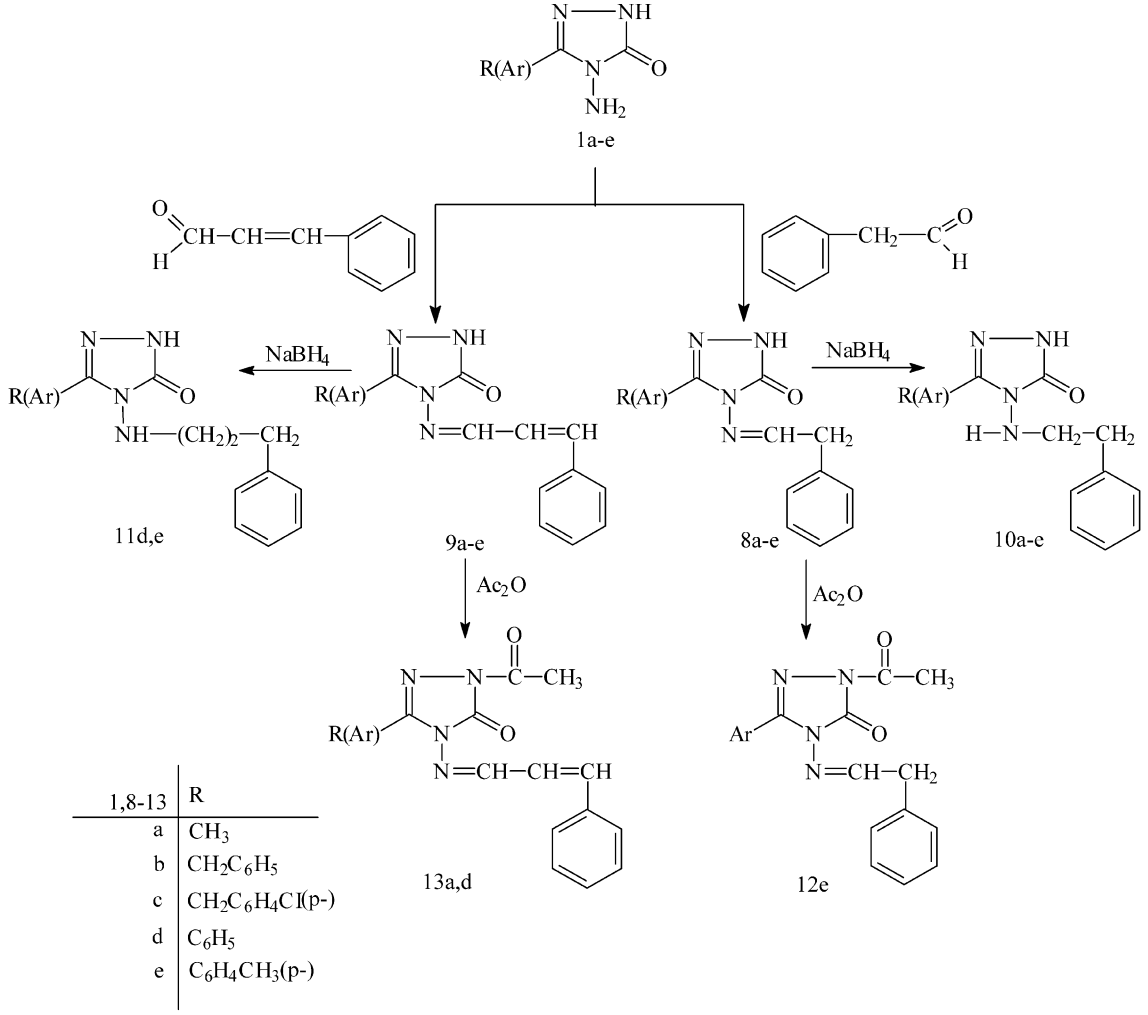


Chart 1.

1H-1,2,4-triazol-5-ones (**10**) and 3-alkyl-4-(3-phenylpropylamino)-4,5-dihydro-1H-1,2,4-triazol-5-ones (**11**) were obtained by the reduction of type **8** and **9** compounds with $NaBH_4$. Furthermore, the acetylation of compounds **8e**, **9a** and **9d** was performed and subsequently 1-acetyl-3-*p*-tolyl-4-(2-phenylethylidenamino)-4,5-dihydro-1H-1,2,4-triazol-5-ones (**12e**), 1-acetyl-3-aryl-4-(3-phenylethylidenamino)-4,5-dihydro-1H-1,2,4-triazol-5-ones (**13a** and **13d**) were synthesized. (Scheme 1). All compounds, except **8d**, **9e**, **11d** and **11e**, were selected by the National Cancer Instutite, USA and screened for their in vitro antitumor activities.

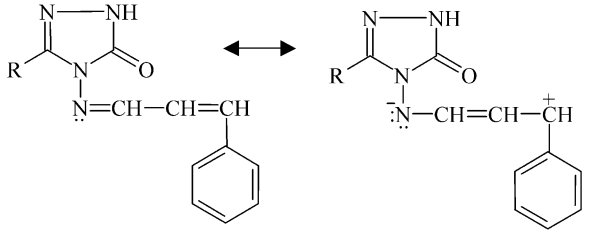


Scheme 1. Synthetic pathway for the preparation of compounds **8**–**13**.

Results and Discussion

Previously, the reduction of some 4-arylidenamino compounds obtained by the reaction of type **1** compounds with diverse halogenobenzaldehydes and *p*-tolualdehyde has been reported.[33] In the present study, in order to improve the method, arom-aliphatic aldehydes such as phenylacetaldehyde and cinnamaldehyde were used to synthesize type **8** and **9** compounds and subsequently these products were reduced with $NaBH_4$ to obtain 3-alkyl-4-(2-phenylethylamino)-4,5-dihydro-1H-1,2,4-triazol-5-ones (**10**) and 3-alkyl-4-(3-phenylpropyl-amino)-4,5-dihydro-1H-1,2,4-triazol-5-ones (**11**). It has been previously reported that resonance structures may exist in the compounds containing azomethyne groups and these compounds may form *E* and *Z* isomers together in diverse percentage.[34] In particular the type **9** compounds, a resonance associated with both azomethyne and allyl groups may exist (Scheme 2). Together with azomethyne group, the carbon–carbon double bond in type **9** compounds was probably due to this resonance, reduced with $NaBH_4$. As a result of these resonance structures, one carbon atom of carbon–carbon double bond in compounds **9** should be more electropositive than the other. Therefore, this situation may facilitate the attack of hydride ion of $NaBH_4$ to this carbon atom. Furthermore, only one signal was observed in ^{13}C NMR spectra indicating the formation of either *E* or *Z* isomer.

The exocyclic–NH signals were observed at 5–6 ppm in the compounds **10**, **11** while endocyclic –NH proton signals were seen at 10–12 ppm as expected for all. In presence of D_2O, the singlet signals disappeared in ^1H NMR spectra and the –N=CH proton signal of compounds **8** and **9** were recorded at 9–10 ppm. These signals disappeared when the compounds **10** and **11** formed. Instead, a new signal at 3–3.5 ppm belonging to methylenic protons of $–NH–CH_2$ group of compounds **10** and **11** was seen. In the ^1H NMR spectra of compounds **12** and **13**, a new additional signal belonging to methyl protons of acetyl group was recorded at 2.5 ppm. The signals belonging to carbonyl carbon and methyl carbon of acetyl group were seen at 166 ppm and 23.5 ppm in ^{13}C –NMR spectra of compounds **12** and **13**. The –N=CH carbon signal of compounds **8**, **9**, **12** and **13** was recorded at 156–160 ppm. The disappearance of this signal in the case of compounds **10** and **11** indicates that this signal belongs to the carbon atom of –N=CH group of compounds **8** and **9**, respectively, and at the same time, this is an evidence for the reduction of this carbon atom in compounds **8** and **9** to form **10** and **11**. The signal of the reduced ($–NH–CH_2$) carbon atom of the compounds **10** and **11** was observed at 50 ppm in ^{13}C NMR spectra.



Scheme 2. Resonance structures of 4-(3-phenylallylidenamino) derivatives **9**, **13**.

The growth inhibition properties of the selected compounds by the National Cancer Institute, USA were screened on three human tumor cell lines, breast Cancer (MCF7), non small cell lung cancer (NCI-H460) and CNS (SF-268) as listed in Table 1. From the point of a structure–activity relationship, the results obtained reveal that an antitumor property appears as a result of the conversion of 4-amino-4,5-dihydro-1H-1,2,4-triazol-5-ones **1** into 4-alkylidenamino- or 4-alkylamino-4,5-dihydro-1H-1,2,4-triazol-5-ones and 1-acetyl-derivative of compound **8e** whereas type **1** compounds are known as antitumor inactive.[21] The highest activity was observed for phenylethylidenamino and phenylethylamino groups at position 4 of the heteroring. The compounds **9b** and **9c** containing 3-phenylallylidenamino group at position 4 of 1,2,4-triazol ring have less cytostatic effect against two cell line than formers.

According to the results obtained, 3-alkyl-4-alkyliden (or alkyl)amino-4,5-dihydro-1H-1,2,4-triazol-5-ones can be described as a new class of antitumor agents. Which served us to search structural modifications in 4,5-dihydro-1H-1,2,4-triazol-5-one ring for designing potentially antitumor active 1,2,4-triazol-5-one systems.

Experimental

Chemistry

Melting points were determined on a Gallenkamp melting point apparatus and are uncorrected. ^1H NMR and ^{13}C NMR spectra were recorded on a Varian-Mercury 200 MHz spectrophotometer. The IR spectra were measured as potassium bromide pellets using a Perkin-Elmer 1600 series FTIR spectrophotometer. The ultraviolet

Table 1. Antitumor screening data for the selected compounds

Compd	Number assigned by NCI	Growth percentage of tumor cell		
		MCF7	NCI-H460	SF-268
8a	S723464	111	0	0
8b	S723465	120	0	116
8c	S723466	130	0	0
8e	S723467	0	0	0
9a	S723036	94	93	81
9b	S723038	37	17	49
9c	S723220	73	67	98
9d	S723039	32	22	52
10a	S723461	0	0	0
10b	S723462	135	0	0
10c	S723463	105	0	0
10d	S723459	0	0	0
10e	S723460	118	0	0
12e	S723468	0	0	0
13a	S723217	119	99	118
13d	S723218	121	95	119

absorption spectra were measured between 200–400 nm on a Schimadzu 1202 UV–vis spectrometer using 10 mm quartz cells. Combustion analysis was performed on a Carlo Erba 1106 elemental analyzer. The chemicals were obtained from Fluka Chemie AG Buchs (Switzerland). Compounds **1** and **2** were synthesized according to the published methods.[4,22]

General method for synthesis of 3-alkyl(aryl)-4-phenylethylidenamino-4,5-dihydro-1H-1,2,4-triazol-5-ones (8)

The corresponding 3-alkyl-4-amino-4,5-dihydro-1H-1,2,4-triazol-5-one (**1**) (0.01 mol) was heated in an oil bath with phenylacetaldehyde (2.11 mL, 0.01 mol) at 150–160 °C for 2 h. After cooling the mixture to room temperature, a solid appeared, and it was recrystallized from an appropriate solvent to afford the desired compound.

3-Methyl-4-phenylethylidenamino-4,5-dihydro-1H-1,2,4-triazol-5-one (8a). Following the general procedure reported above, a white solid was obtained. It was recrystallized from benzene–petroleum ether (1:3) to afford the desired compound (yield: 1.06 g, 49.23%). Mp 126–127 °C. Analysis (% calcd/found): for $C_{11}H_{12}ON_4$ C: 61.09/60.75, H: 5.59/5.96, N: 25.91/26.71; IR (KBr) cm^{-1}: 3182 (ν_{NH}), 1899 ($\nu_{C=O}$), 1593 ($\nu_{C=N}$); ^1H NMR (DMSO-d_6) δ 2,30 (s, CH_3), 3.70 (d, CH_2, $J=5.8$ Hz), 9.20 (t, N=CH, $J=5.8$ Hz), 7.75–7.36 (m, 5H), 10.25 (s, NH); ^{13}C NMR (DMSO-d_6) δ 158.90 (N=CH), 152.30 (triazole C_3), 145.40 (triazole C_5), Ar C : [135.40 (C), 129.02 (2CH), 128.85 (2CH), 127.07 (CH)], 40.19 CH_2), 11.52 (CH_3).

3-Benzyl-4-phenylethylidenamino-4,5-dihydro-1H-1,2,4-triazol-5-one (8b). Following the general procedure reported above, a white solid was obtained. This was recrystallized from benzene to afford the desired compound (yield: 1.35 g, 46.23%). M.p. 163–164 °C. Analysis (% calcd/found): for $C_{17}H_{16}ON_4$ C: 69.84/70.01; H: 5.52/5.78, N: 19.17/19.20; IR (KBr) cm^{-1}: 3182 (ν_{NH}), 1690 ($\nu_{C=O}$), 1550 ($\nu_{C=N}$); ^1H NMR (DMSO-d_6) δ 3.65 (d, CH_2, $J=5.8$ Hz), 4.00 (s, CH_2), 9.10 (t, N=CH, $J=5.4$ Hz), 7.10–7.35 (m, 10H), 10.60 (s, NH); ^{13}C NMR (DMSO-d_6) δ 158.90 (N=CH), 152.30 (triazole C_3), 147.20 (triazole C_5), Ar C: [135.30 (2C), 129.10 (2CH), 129.04 (2CH), 128.722 (CH), 128.53 (2CH), 126.97 (2CH)], 40.19 (CH_2), 11.52 (CH_3).

3-(*p*-Chlorobenzyl)-4-phenylethylidenamino-4,5-dihydro-1H-1,2,4-triazol-5-one (8c). Following the general procedure reported above, a white solid was obtained. It was recrystallized benzene to afford the desired compound (yield: 1.52 g, 46.62%). Mp 189–190 °C. Analysis (% calcd/found): for $C_{17}H_{15}ON_4Cl$ C: 62.48/63.051; H: 4.62/4.71, N: 17.14/16.93; IR (KBr) cm^{-1}: 3168 (ν_{NH}), 1690 ($\nu_{C=O}$), 1595 ($\nu_{C=N}$); ^1H NMR ($CDCl_3$) δ 3.70 (d, CH_2, $J=5.8$ Hz), 3.98 (s, CH_2), 9.05 (t, N=CH, $J=5.4$ Hz), 7.15–7.40 (m, 9H), 10.90 (s, NH); ^{13}C NMR ($CDCl_3$) δ 158.10 (N=CH), 151.05 (triazole C_3), 145.05 (triazole C_5), Ar C: [135.10(C), 134.02 (2CH), 130.95 (2CH), 130.02 (2CH), 128.02 (2CH), 127.95 (2CH), 127.90 (2CH), 126.50 (C)], 40.01 (CH_2), 29.95 (CH_2).

3-Phenyl-4-phenylethylidenamino-4,5-dihydro-1H-1,2,4-triazol-5-one (8d). Following the general procedure reported above, a white solid was obtained. It was recrystallized from benzene to afford the desired compound (yield: 1.40 g, 50.35%). Mp 176–177 °C. Analysis (% calcd/found): for $C_{16}H_{14}ON_4$ C: 69.05/68.27, H: 5.07/4.92, N: 20.13/20.97; IR (KBr) cm^{-1}: 3167 (ν_{NH}), 1691 ($\nu_{C=O}$), 1552 ($\nu_{C=N}$); ^1H NMR (DMSO-d_6) δ 3.75 (d, CH_2, $J=5.2$ Hz), 9.20 (t, N=CH, $J=5.2$ Hz), 7.86 (bs, 2H), 7.28–7.43 (m, 10H), 10.92 (s, NH); ^{13}C NMR (DMSO-d_6) δ 161.01 (N=CH), 152.40 (triazole C_3), 145.95 (triazole C_5), Ar C :[135.30 (C), 130.31 (CH), 129.18 (2CH), 128.88 (2CH), 128.34 (4CH), 127.12 (CH), 126.50 (C)], 40.16 (CH_2).

3-(*p*-Tolyl)-4-phenylethylidenamino-4,5-dihydro-1H-1,2,4-triazol-5-one (8e). Following the general procedure reported above, a white solid was obtained. It was recrystallized from ethylacetate to afford the desired compound (yield: 1.31 g, 44.86%). Mp 176–177 °C. Analysis (% calcd/found): for $C_{17}H_{16}ON_4$ C: 69.84/69.80, H: 5.52/5.42, N: 19.17/18.63; IR (KBr) cm^{-1}: 3164 (ν_{NH}), 1694 ($\nu_{C=O}$), 1590 ($\nu_{C=N}$); ^1H NMR (DMSO-d_6) δ 2.40 (s, CH_3), 3.75 (d, CH_2, $J=5.6$ Hz), 9.16 (t, N=CH, $J=5.6$ Hz), 7.74–7.10 (m, 7H), 7.78 (d, 2H, $J=8.0$ Hz), 10.34 (s, NH); ^{13}C NMR (DMSO-d_6) δ 160.79 (N=CH), 152.24 (triazole C_3), 145.88 (triazole C_5), Ar C: [140.53 (C), 135.02 (C), 129.16 (2CH), 129.11 (2CH), 128.23 (2CH), 127.09 (CH), 123.580 (C)], 40.16 (CH_2), 21.51 (CH_3).

General method for synthesis of 3-alkyl(aryl)-4-(3-phenylallylidenamino)-4,5-dihydro-1H-1,2,4-triazol-5-ones (9)

The corresponding 3-alkyl-4-amino-4,5-dihydro-1H-1,2,4-triazol-5-one (**1**) (0.01 mol) was heated in an oil bath with cinnamaldehyde (1.25 mL, 0.01 mol) at 120–130 °C for 2 h. After cooling the mixture to room temperature, a solid appeared. It was recrystallized from an appropriate solvent to afford the desired compound.

3-Methyl-4-(3-phenylallylidenamino)-4,5-dihydro-1H-1,2,4-triazol-5-one (9a). Following the general procedure reported above, a white solid was obtained. It was recrystallized from chloroform–ethylacetate (1:3) to afford the desired compound (yield: 1.90 g, 83.33%). Mp 221–224 °C. Analysis (% calcd/found): for $C_{12}H_{12}ON_4$ C: 63.14/62.58, H: 5.30/5.10, N: 24.55/24.92; IR (KBr) cm^{-1}: 3177 (ν_{NH}), 1703 ($\nu_{C=O}$), 1595 ($\nu_{C=N}$); ^1H NMR ($CDCl_3$) δ 2.21 (s, CH_3), 7.10 (dd, CH, $J^{ab}=9.4$, $J^{bc}=16$), 7.40 ($J^{bc}=16$), 9.55 (d, N=CH, $J=9.4$ Hz), 6.97–7.10 (m, Ar–H, 6H), 11.81 (s, NH); ^{13}C NMR (DMSO-d_6) δ 156.33 (N=CH), 151.34 (triazole C_3), 144.10 (triazole C_5), Ar C: [135.47 (C), 129.05 (2CH), 127.73 (2CH), 125.05 (CH)], 11.38 (CH_3).

3-Benzyl-4-(3-phenylallylidenamino)-4,5-dihydro-1H-1,2,4-triazol-5-one (9b). Following the general procedure reported above, a white solid was obtained. It was recrystallized from ethyl acetate to afford the desired compound

(yield: 2.50 g, 82.23%). Mp 199–200 °C. Analysis (% calcd/found): for $C_{13}H_{16}ON_4$ C: 71.03/71.62, H: 5.30/4.92, N: 18.4/17.91; IR (KBr) cm^{-1}: 3165 (ν_{NH}), 1689 ($\nu_{C=O}$), 1588 ($\nu_{C=N}$)); ^1H NMR (DMSO-d_6) δ 4.10 (s, CH_2), 7.69 (dd, CH, $J^{ab}=9.3$ Hz, $J^{bc}=16$ Hz, 7.10 $J^{bc}=16$ Hz), 9.55 (d, N=CH, $J=9.4$ Hz), 7.20–7.45 (m, Ar–H, 8H), 7.50 (d, Ar–H, 2H), 10.60 (s, NH); ^{13}C NMR ($CDCl_3$) δ 156.70 (N=CH), 152.08 (triazole C_3), 147.28 (triazole C_5), Ar C : [135.40 (C), 134.97 (C), 129.03 (2CH), 128.80 (2CH), 127.40 (2CH), 127.01 (CH), 125.04 (CH)], 31.53 (CH_2).

3-(*p*-Chlorobenzyl)-4-(3-phenylallylidenamino)-4,5-dihydro-1H-1,2,4-triazol-5-one (9c). Following the general procedure reported above, a white solid was obtained. It was recrystallized from chloroform–ethyl acetate (1:2) to afford the desired compound (yield: 2.82 g, 82.84%). Mp 229–230 °C. Analysis (% calcd/found): for $C_{13}H_{16}ON_4Cl$ C: 63.81/63.27, H: 4.46/4.82, N: 16.53/16.82; IR (KBr) cm^{-1}: 3177 (ν_{NH}), 1703 ($\nu_{C=O}$), 1595 ($\nu_{C=N}$); ^1H NMR ($CDCl_3$) δ 4.00 (s, CH_2), 7.05 (dd, CH $J^{ab}=9$ Hz, $J^{bc}=16$), 7.25 (d, $J=16$ Hz), 9.50 (d, N=CH, $J=9.2$ Hz), 7.30–7.50 (m, Ar–H, 7H), 7.65–7.70 (m, 2H), 12.00 (s, NH); ^{13}C NMR (DMSO-d_6) δ 156.05 (N=CH), 151.30 (triazole C_3), 145.50 (triazole C_5), Ar C: [135.47 (C), 134.50 (C), 131.40 (C), 130.70 (2CH), 128.22 (2CH), 127.48 (2CH), 124.70 (CH)], 30.03 (CH_2).

3-Phenyl-4-(3-phenylallylidenamino)-4,5-dihydro-1H-1,2,4-triazol-5-one (9d). Following the general procedure reported above, a white solid was obtained. It was recrystallized from benzene to afford the desired compound (yield: 1.95 g, 67.24%). Mp 196–197 °C. Analysis (% calcd/found): for $C_{17}H_{14}ON_4$ C: 41.42/41.88, H: 2.85/3.12, N: 11.32/11.82; IR (KBr) cm^{-1}: 3158 (ν_{NH}), 1694 ($\nu_{C=O}$), 1623 ($\nu_{C=N}$); ^1H NMR (DMSO-d_6) δ 6,95 (dd, CH $J^{ab}=9$ Hz, $J^{bc}=16$ Hz, 7.15 (d, $J=16$ Hz), 9.60 (d, N=CH, $J=9.2$ Hz), 7.20–7.60 (m, Ar–H, 5H), 7.90–8.02 (m, Ar–H, 5H), 10.82 (s, NH); ^{13}C NMR ($CDCl_3$) δ 158.50 (N=CH), 152.50 (triazole C_3), 146.01 (triazole C_5), 144.02 (CH), 129.30 (CH), Ar C: [135.30 (C), 130.03 (C), 128.80 (2C), 128.48 (2C), 127.40 (2C), 126.50 (C), 124.80 (C)].

3-(*p*-tolyl)-4-(3-phenylallylidenamino)-4,5-dihydro-1H-1,2,4-triazol-5-one (9e). Following the general procedure reported above, a white solid was obtained. It was recrystallized from benzene to afford the desired compound (yield: 2.10 g, 69.07%). Mp 215–216 °C. Analysis (% calcd/found): for $C_{18}H_{16}ON_4$ C: 70.03/70.86, H: 5.30/5.32, N: 18.41/18.30; IR (KBr) cm^{-1}: 3163 (ν_{NH}), 1694 ($\nu_{C=O}$), 1625 ($\nu_{C=N}$); ^1H NMR ($CDCl_3$) δ 2.40 (s, CH_3), 7.10 (dd, CH $J^{ab}=9.0$ Hz, $J^{bc}=16$ Hz), 7.30 (d, $J=16$ Hz), 9.45 (d, N=CH, $J=9.0$ Hz), 7.30–7.50 (m, Ar–H, 5H), 7.62–7.90 (m, Ar–H, 4H),12.30 (s, NH); ^{13}C NMR (DMSO-d_6) δ 158.03 (N=CH), 150.08 (triazole C_3), 143.80 (triazole C_5), 143.05 (CH), 129.03 (CH), Ar C: [134.02 (C), 130.01 (C), 129.03 (2C), 128.01 (2C), 127.02 (2C), 127.00 (2C), 124.03 (C), 126–3.05 (C)], 20.05 (CH_3).

General method for synthesis of 3-alkyl(aryl)-4-(2-phenylethylamino-4,5-dihydro-1H-1,2,4-triazol-5-ones (10)

A solution of corresponding compound (**8**) (0.01) in 40 mL diglime was treated with a solution of $NaBH_4$ (1.11 g, 0.03 mol) in 30 mL diglime. Then, the mixture was refluxed for 8 h and subsequently, was poured in 500 mL water. On cooling in deep freeze, a solid appeared. It was recrystallized from an appropriate solvent to afford the desired compound.

3-Methyl-4-(2-phenylethylamino)-4,5-dihydro-1H-1,2,4-triazol-5-one (10a). Following the general procedure reported above, a white solid was obtained. It was recrystallized from benzene–*n*-hexane (1:3) to afford the desired compound (yield: 1.09 g, 50.0%). Mp 115–116 °C. Analysis (% calcd/found): for $C_{18}H_{14}ON_4$ C: 60.53/60.17, H: 6.47/7.07, N: 25.67/25.93; IR (KBr) cm^{-1}: 3177, 3250 (ν_{NH}), 1706 ($\nu_{C=O}$), 1590 ($\nu_{C=N}$); ^1H NMR (DMSO-d_6) δ 2.05 (s, CH_3), 2.70–2.80 (m, CH_2), 3.20–3.30 (m, $NH\underline{CH_2}$), 6.05 (s,NNH), 7.20–7.38 (m, Ar-H, 5H), 11.40 (s, NH); ^{13}C NMR (DMSO-d_6) δ 154.03 (triazole C_3), 145.63 (triazole C_5), Ar C: [139.48 (C), 128.38 (2CH), 126.66 (2CH), 128.66 (2CH), 126.13 (CH)], 50.52 (CH_2), 33.79 (CH_2), 10.86 (CH_3).

3-Benzyl-4-(2-phenylethylamino)-4,5-dihydro-1H-1,2,4-triazol-5-one (10b). Following the general procedure reported above, a white solid was obtained. It was recrystallized from acetone–water (1:3) to afford the desired compound (yield: 1.66 g, 56.46%). Mp 115–116 °C. Analysis (% calcd/found): for $C_{17}H_{18}ON_4$ C: 69.37/68.65, H: 6.16/6.54, N: 19.04/18.31; IR (KBr) cm^{-1}: 3231, 3166 (ν_{NH}), 1702 ($\nu_{C=O}$), 1585 ($\nu_{C=N}$); ^1H NMR (DMSO-d_6) δ 2.52–2.65 (m, CH_2), 3.75 (s, CH_2) 3.20–3.30 (m, $NH\underline{CH_2}$), 6.05 (bs, NNH), 7.10–7.40 (m, Ar–H, 10H), 11.52 (s, NH), ^{13}C NMR ($CDCl_3$) δ 153.52 (triazole C_3), 147.08 (triazole C_5), Ar C: [138.86 (C), 135.61 (C), 128.29 (C), 128.07 (2CH), 128.04 (2CH), 127.92 (2CH), 127.78 (CH), 124.14 (CH)], 49.89 (CH_2), 33.02 (CH_2), 30.30 (CH_2).

3-(*p*-Chlorobenzyl)-4-(2-phenylethylamino)-4,5-dihydro-1H-1,2,4-triazol-5-one (10c). Following the general procedure reported above, a white solid was obtained. It was recrystallized from acetone–water (1:3) to afford the desired compound (yield: 1.70 g, 51.82%). M.p. 151–152 °C. Analysis (% calcd/found): for $C_{17}H_{18}ON_4Cl$ C: 62.10/61.71: H: 5.21/4.88, N: 17.03/17.70; IR (KBr) cm^{-1}: 3229, 3179 (ν_{NH}), 1727 ($\nu_{C=O}$), 1590 ($\nu_{C=N}$); ^1H NMR ($CDCl_3$) δ 2.60–2.75 (m, CH_2), 3.75 (s, CH_2), 2.90–3.10 (m, $NH\underline{CH_2}$), 4.54 (t, NNH, $J=6.4$ Hz), 7.05–7.30 (m, Ar–H, 9H), 10.30 (s, NH); ^{13}C NMR ($CDCl_3$) δ 154.03 (triazole C_3), 147.05 (triazole C_5), Ar C:[138.03 (C), 135.02 (C), 133.28 (C), 130.12 (2CH), 128.60 (2CH), 128.48 (2CH), 126.31 (CH)], 50.03 (CH_2), 33.90 (CH_2), 30.80 (CH_2).

3-Phenyl-4-(2-phenylethylamino)-4,5-dihydro-1H-1,2,4-triazol-5-one (10d). Following the general procedure reported above, a white solid was obtained. It was recrystallized from chloroform–*n*-hexane (1:3) to afford the desired compound (yield: 50%). Mp 166–167 °C. Analysis (% calcd/found): for $C_{16}H_{16}ON_4$ C: 68.55/68.75: H: 5.75/5.60, N: 19.99/19.52; IR (KBr) cm^{-1}: 3237,3154 (ν_{NH}), 1702 ($\nu_{C=O}$), 1519 ($\nu_{C=N}$); ^1H NMR (DMSO-d_6) δ 2.60–2.70 (m, CH_2), 2.95–3.10 (m,

NHCH$_2$), 6.20 (t, NNH, $J=6.5$ Hz), 7.18–7.50 (m, Ar–H, 8H), 7.85 (d, 2H, Ar–H, $J=8.0$ Hz), 11.90 (s, NH); ^{13}C NMR (DMSO-d_6) δ 154.05 (triazole C$_3$), 144.08 (triazole C$_5$), Ar C:[139.05 (C), 129.08 (2CH), 128.80 (2CH), 128.29 (2CH), 128.25 (2CH), 127.04 (2CH), 127.03 (C), 126.11 (CH)], 50.08 (CH$_2$), 33.50 (CH$_2$).

3-(*p*-tolyl)-4-(2-phenylethylamino)-4,5-dihydro-1H-1,2,4-triazol-5-one (10e). Following the general procedure reported above, a white solid was obtained. It was recrystallized from acetone–water (1:3) to afford the desired compound (yield: 1.94 g, 65.68%). Mp 163–164 °C. Analysis (% calcd/found): for $C_{17}H_{18}ON_4$ C: 69.37/69.05, H: 6.59/5.97, N: 19.04/19.28; IR (KBr) cm^{-1}: 3274,3156 (ν_{NH}), 1702 ($\nu_{C=O}$), 1518 ($\nu_{C=N}$); ^{1}H NMR (CDCl$_3$) δ 2.37 (s, CH$_3$), 2.77 (t, CH$_2$, $J=7.0$ Hz), 3.22–3.35 (m, NHCH$_2$), 5.00 (t, NNH, $J=6.4$ Hz), 7.05–7.30 (m, Ar–H, 7H), 7.85 (d, 2H, Ar–H, $J=8.2$ Hz), 11.05 (s, NH); ^{13}C NMR (CDCl$_3$) δ 155.03 (triazole C$_3$), 145.08 (triazole C$_5$), Ar C: [140.08 (C), 138.80 (C), 129.44 (2CH), 128.99 (2CH), 128.74 (2CH), 127.51 (2CH), 126.66 (CH), 123.90 (C)], 51.97 (CH$_2$), 34.33 (CH$_2$), 19.95 (CH$_3$).

General method for synthesis of 3-alkyl(aryl)-4-(3-phenylpropylamino)-4,5-dihydro-1H-1,2,4-triazol-5-ones (11)

A solution of corresponding compound (**9**) (0.01) in 40 mL diglime was treated with a solution of NaBH$_4$ (1.11 g, 0.03 mol) in 30 mL diglime. Then, the mixture was refluxed for 8 h and subsequently was poured in 500 mL water. On cooling in deep freeze, a solid appeared. It was recrystallized from an appropriate solvent to afford the desired compound.

3-Phenyl-4-(3-phenylpropylamino)-4,5-dihydro-1H-1,2,4-triazol-5-one (11d). Following the general procedure reported above, a white solid was obtained. It was recrystallized from ethanol–water (1:3) to afford the desired compound (yield: 1.50 g, 51.02%) Mp 147–148 °C. Analysis (% calcd/found): for $C_{17}H_{18}ON_4$ C: 69.37/68.87, H: 6.16/15.97, N: 19.04/18.6; IR (KBr) cm^{-1}: 3248,3152 (ν_{NH}), 1703 ($\nu_{C=O}$), 1515 ($\nu_{C=N}$); ^{1}H NMR (CDCl$_3$) δ 1.65–1.80 (m, CH$_2$), 2.50–2.65 (m, CH$_2$), 3.37 (q, NHCH$_2$, $J=7.0$ Hz), 5.08 (t, NNH, $J=5.4$ Hz), 6.95–8.10 (m, Ar–H, 10H), 11.30 (s, NH); ^{13}C NMR (CDCl$_3$) δ 154.32 (triazole C$_3$), 145.35 (triazole C$_5$), Ar C: [131.05 (C), 129.98 (2C), 128.53 (2C), 127.41(3C), 127.13 (2C), 125.79 (C)], 48.60 (CH$_2$), 32.59 (CH$_2$), 29.14 (CH$_2$).

3-(*p*-Tolyl)-4-(3-phenylpropylamino)-4,5-dihydro-1H-1,2,4- triazol-5-one (11e). Following the general procedure reported above, a white solid was obtained. It was recrystallized from ethanol–water (1:3) to afford the desired compound (yield: 1.53 g, 49.67%). Mp 147–149 °C. Analysis (% calcd/found): for $C_{18}H_{20}ON_4$ C: 70.10/69.64, H: 6.54 /6.89, N: 18.17/18.76; IR (KBr) cm^{-1}: 3250, 3163 (ν_{NH}), 1703 ($\nu_{C=O}$), 1509 ($\nu_{C=N}$); ^{1}H NMR (DMSO-d_6) δ 2.35 (s, CH$_3$),1.50–1.70 (m, CH$_2$), 2.45–2.50 (m, CH$_2$), 3.13.30 (m, NHCH$_2$), 6.20 (t, NNH, $J=5.4$ Hz), 6.98–7.93 (m, Ar–H, 9H), 11.94 (s, NH); ^{13}C NMR (DMSO-d_6) δ 154.43 (triazole C$_3$), 145.50 (triazole C$_5$), Ar C: [132.20 (C), 132.30 (C),129.41 (2CH), 128.32 (3CH), 127.33 (2CH), 126.24 (CH), 125.67 (CH), 124.38 (C)], 48.70 (CH$_2$), 32.65 (CH$_2$), 29.29 (CH$_2$), 21.08 (CH$_3$).

1-Acetyl-3-(*p*-tolyl)4-phenylethylydenamino-4,5-dihydro-1H-1,2,4-triazol-5-one (12e). Compound (**8e**) (2.92 g, 0.01 mol) was refluxed with 10 mL acetic anhydride for 2 h. Then, the mixture was cooled to room temperature and added 40 mL ethanol and than refluxed 30 min. On cooling the mixture in the deep-freeze a solid appeared. This crude product was crystallized from benzene–petroleum ether (1:2) to give compound (**12e**) (yield: 2.00 g, 59.88%). Mp 102–103 °C. Analysis (% calcd/found): for $C_{19}H_{18}O_2N_4$ C: 68.24/69.64, H: 5.42/5.02, N: 16.76/16.92; IR (KBr) cm^{-1}, 1731, 1725 ($\nu_{C=O}$), 1517 ($\nu_{C=N}$); ^{1}H NMR (DMSO-d_6) δ 2.64 (s, CH$_3$), 2.42 (s, CH$_3$), 3.78 (d, CH$_2$, $J=5.6$ Hz), 9.09 (d, N=CH, $J=5.6$ Hz), 7.40–7.82 (m, Ar–H, 9H); ^{13}C NMR (CDCl$_3$) δ 162.31 (triazole C$_3$), 148.90 (triazole C$_5$), Ar C: [141.82 (C), 134.90 (C), 129.14 (3CH), 129.12 (3CH), 127.90 (CH), 127.21 (2CH), 122.40 (C)], 40.08 (CH$_2$), 23.70 (CH$_3$), 21.58 (CH$_3$).

General method for synthesis of 1-acetyl-3-alkyl(aryl)-4-(3-phenylallylydenamino)-4,5-dihydro-1H-1,2,4-triazol-5-ones (13)

The corresponding compound (**9**) (0.01 mol) was refluxed with 10 mL acetic anhydride for 2 h. Then, the mixture was cooled to room temperature and added 40 mL ethanol and than refluxed 30 min. After evaporated at 35–40 °C under reduced pressure a solid appeared. This was recrystallized from an appropriate solvent to afford desired compound.

1-Acetyl-3-phenyl-4-(3-phenylallylydenamino)-4,5-dihydro-1H-1,2,4-triazol-5-one (13a). Following the general procedure reported above, a white solid was obtained. It was recrystallized from benzene–petroleum ether (1:2) to afford desired compound (yield: 1.82 g, 67.40%) Mp 168–169 °C. Analysis (% calcd/found): for $C_{14}H_{14}O_2N_4$ C: 62.21/69.64, H: 5.22/5.20, N: 20.73/19.98; IR (KBr) cm^{-1}, 1727 ($\nu_{2C=O}$), 1616 ($\nu_{C=N}$); ^{1}H NMR (CDCl$_3$) δ 2.36 (s, CH$_3$), 2.60 (s, CH$_3$), 9.48 (d, N=CH, $J=9.4$ Hz), 7.58–7.50 (m, Ar–H, 2H); 7.33–7.42 (m, Ar–H, 2H); ^{13}C NMR (CDCl$_3$) δ 157.52 (triazole C$_3$), 147.50 (triazole C$_5$), 148.20 (N=CH), 144.45 (CH), 125.60 (CH), Ar C: [135.12 (C), 129.89 (CH), 128.91 (CH), 127.53 (2CH), 124.60], 23.28 (CH$_3$), 11.658 (CH$_3$).

1-Acetyl-3-phenyl-4-(3-phenylallylidenamino)-4,5-dihydro-1H-1,2,4-triazol-5-one (13d). Following the general procedure reported above, a white solid was obtained. It was recrystallized from from benzene–petroleum ether (1:2) to afford desired compound (yield: 2.50 g, 75.30%) Mp 167–168 °C. Analysis (% calcd/found): for $C_{19}H_{16}O_2N_4$ C: 68.66/69.03, H: 4.85/4.80, N: 16.86/16.70; IR (KBr) cm^{-1}, 1780, 1778 ($\nu_{C=O}$), 1619 ($\nu_{C=N}$); ^{1}H NMR (DMSO-d_6) δ 2.57 (s, CH$_3$), 9.28 (d, N=CH, $J=9.2$ Hz), 7.95–7.85 (m, Ar–H, 2H), 7.80–7.65 (m, Ar–H, 2H), 7.65–7.45 (m, Ar–H, 3H), 7.45–7.40 (m, Ar–H, 3H); ^{13}C NMR (CDCl$_3$) δ 161.00 (N=CH), 148.02 (triazole C$_3$), 145.28 (triazole C$_5$), 145.23 (CH), 124.14

(CH), Ar C: [135.00 (2C), 131.50 (CH), 129.98 (CH), 128.52 (2CH), 127.76 (4CH), 125.98 (CH)], 23.47 (CH_3).

Pharmacology

All compounds, except **8d**, **9e**, **11d** and **11e**, were selected by the National Cancer Instutite, USA for screening towards in vitro three human tumor cell lines, 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.[35]

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