Synthesis and Antioxidant Properties of Some New 3-(4-Chlorophenyl)-5-(pyridin-4-yl)-4*H*-1,2,4-triazole Derivatives

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Z. Naturforsch. **2008**, *63b,* 548 – 554; received November 27, 2007

A series of new 3-(4-chlorophenyl)-5-(pyridin-4-yl)-4-(arylmethyleneamino)-4*H*-1,2,4-triazole derivatives **3** were prepared in good yields by treatment of 4-amino-3-(4-chlorophenyl)-5-(pyridine-4-yl)-4*H*-1,2,4-triazole (**2**) with selected aldehydes. Compounds **3** were reduced with $NaBH_4$ to afford the corresponding 3-(4-chlorophenyl)-5-(pyridin-4-yl)-4-(arylmethylamino)-4*H*-1,2,4-triazole derivatives **4**. Eighteen new compounds were synthesized and characterized by elemental analyses, IR, ^{1}H NMR and ^{13}C NMR spectral data. The compounds were screened for their antioxidant and antiradical activities.

Key words: 4*H*-1,2,4-Triazoles, 4-Amino-4*H*-1,2,4-triazoles, 4-Arylmethyleneamino-4*H*-1,2,4-triazoles, 4-Arylmethylamino-4H-1,2,4-triazoles, Antioxidant Activity

Introduction

A large number of heterocyclic compounds containing the 1,2,4-triazole ring are associated with diverse pharmacological properties, such as anticonvulsant, antifungal, antimicrobial, antihypertensive, analgesic, antiviral, anti-inflammatory, antioxidant, antitumor and anti-HIV activity [1 – 15]. Some compounds containing a 1,2,4-triazole ring are known as drugs. For example, fluconazole is used as an antimicrobial drug in medicine [16], vorozole, letrozole and anastrozole are non-steroidal drugs used for the treatment of cancer [17], and loreclezole is used as an anticonvulsant drug [18]. Furthermore, in recent years, some Schiff base derivatives of 1,2,4-triazoles and their reduced derivatives have also been found to possess pharmacological activities [19 – 26]. It is also observed that incorporation of an aryloxymethyl and a halogen substituent into the heterocyclic ring systems augments the biological activities considerably [26]. These biological data prompted us to synthesize new 1,2,4-triazole derivatives. The synthesized compounds were evaluated for antioxidant and free radical scavenging activity. Furthermore, we observed that some compounds possess more potent antioxidant and antiradical scavenging activities than butylated hydroxyanisole (BHA) and butylated hydroxytoluene (BHT).

Results and Discussion

In the current study, ethyl *p*-chlorobenzoate 4-pyridinoylhydrazone (**1**) was synthesized from the reaction of ethyl imido-*p*-chlorophenylbenzoate hydrochloride with isonicotinohydrazide. 4-Amino-3-(4-chlorophenyl)-5-(pyridin-4-yl)-4*H*-1,2,4-triazole (**2**) was obtained from the reaction of compound **1** with hydrazine hydrate. Compound **2** was treated with some aromatic aldehydes in acetic acid to give 3-(4-chlorophenyl)-5-(pyridin-4-yl)-4-(arylmethyleneamino)-4*H*-1,2,4-triazoles (**3**). Subsequently, compounds **3** were converted to 3-(4-chlorophenyl)-5-(pyridin-4-yl)-4-(arylmethylamino)-4*H*-1,2,4-triazoles (**4**) by treatment with $NaBH_4$ in methanol (Scheme 1).

It was reported that Schiff bases can be obtained as their *E* and *Z* geometrical isomers at the C=N double bond [27 – 30]. According to literature data, the signals of the azomethine protons of the *E* isomers of triazole derivatives appear at higher field with respect to the corresponding signals of the *Z* isomers [27 – 30]. On the basis of these findings, we assigned the *E* configuration to the isomers with the higher percentage in the mixtures. The percentage of each isomer was calculated using the integral values of each singlet pair in Table 1. Moreover, in the ^{13}C NMR spectra of compounds **3a** – **h** the N=CH

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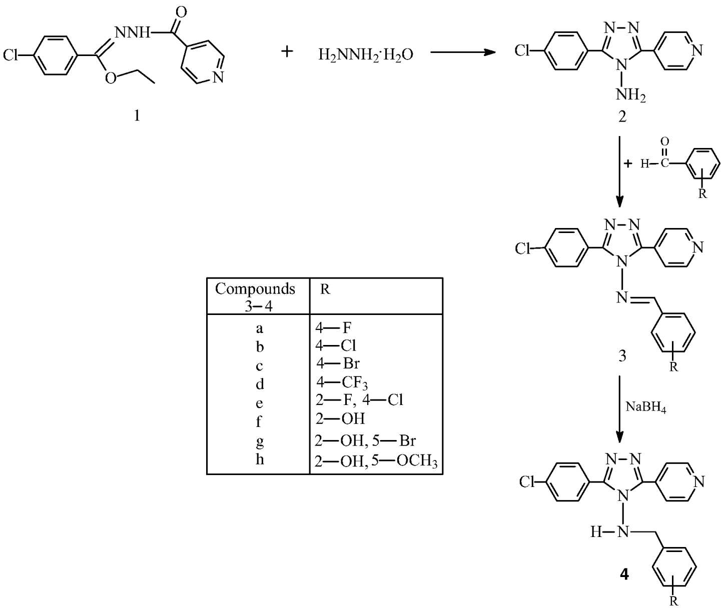

Compounds 3–4	R
a	4—F
b	4—Cl
c	4—Br
d	4—CF_3
e	2—F, 4—Cl
f	2—OH
g	2—OH, 5—Br
h	2—OH, 5—OCH_3

Scheme 1. Synthetic pathway for the preparation of target compounds **2**–**4**.

Table 1. Determination of the configuration of the Schiff bases **3a**–**h** by assignment of the azomethine hydrogen signals and their relative integrals in the ^1H NMR spectra ([D_6]-DMSO, 200 MHz).

Compound	— δ (ppm) — *E*-isomer	*Z*-isomer	Ratio *E*:*Z*
3a	8.69	8.64	7.5 : 1
3b	8.73	8.70	9 : 1
3c	8.69	8.63	5 : 1
3d	8.76	8.72	8.3 : 1
3e	8.72	8.67	15 : 1
3f	8.73	8.69	7.2 : 1
3g	8.67	8.63	11.2 : 1
3h	8.74	8.71	3.8 : 1

signals of the *E*, *Z* isomers appeared at 170.64/170.17, 170.64/170.58, 170.79/170.30, 170.17/169.62, 163.94/163.17, 167.93/167.47, 165.57/165.45 and 164.34/164.27 ppm, respectively.

The reduction of **3** with $NaBH_4$ occurred at the imino group exclusively, without affecting the hetero ring [23, 24, 31]. The characteristic ^1H NMR signals for the -NH–CH_2- group of reduced compounds **4a**–**e** were observed as triplets at δ = 7.40 ppm (t, 1H, NH) and doublets at δ = 3.75 ppm (d, 2H, CH_2). The -NH–CH_2- carbon signals of compounds **4a**–**e** were recorded between δ = 47.14 and 53.75 ppm.

Antioxidant activity

The antioxidant activity of some new 3-(4-chlorophenyl)-5-(pyridin-4-yl)-4*H*-1,2,4-triazole derivatives and standard compounds was determined by the thiobarbituric acid method (TBA) in linoleic acid. The TBA method was used to measure the amount of peroxide produced during the initial stages of oxidation. The effects of some of the new compounds (50 μg mL^{-1}) on lipid peroxidation was found to range from 44.1 to 75.4 % (Table 2), higher than that of BHA (63.7 %) and BHT (67.8 %). Compounds **3b**, **e**–**h** and **4b**, **c**, **e**–**h** showed the highest activities, with compounds **4h**, **4b** and **3f** bearing 2-hydroxy-5-methoxy, 4-chloro, and 2-hydroxy substituents, respectively, being the most active ones.

Antiradical activity

Radical scavenging activities are an important property because of the deleterious role of free radicals in foods and biological systems. Excessive formation of free radicals accelerates the oxidation of lipids in foods and decreases food quality and consumer acceptance [32]. The chosen assay is considered valid and easy for the evaluation of the free radical scavenging activity of antioxidants, since the radical compound is stable and does not have to be generated as for other radical scavenging assays [33]. With this method it was possible to determine the antiradical power of an antioxidant by measuring the decrease of absorbance of $DPPH^\bullet$ at 517 nm. Compounds **4g**, **2**, **4h**, **3g**, **3f**, **4f**, **3h**, **3b**, and **4e** showed highest interactions with the $DPPH^\bullet$ radical, even better than BHA and BHT

Table 2. The antioxidant and antiradical activities of some new 3-(4-chlorophenyl)-5-(pyridin-4-yl)-4H-1,2,4-triazole derivatives.

Compound	Antioxidant activity	Antiradical activity
1	56.84 ± 1.43	68.30 ± 0.85
2	73.22 ± 1.37	93.38 ± 1.04
3a	61.88 ± 0.98	80.60 ± 0.30
3b	70.39 ± 0.66	89.70 ± 0.71
3c	47.05 ± 3.19	79.77 ± 0.57
3d	44.06 ± 1.02	57.84 ± 1.89
3e	73.64 ± 1.13	85.28 ± 6.50
3f	74.04 ± 1.52	91.84 ± 0.40
3g	71.44 ± 0.54	92.54 ± 0.70
3h	70.87 ± 0.50	90.83 ± 0.30
4a	64.13 ± 0.47	79.47 ± 1.05
4b	74.71 ± 0.91	65.69 ± 5.20
4c	71.73 ± 1.30	80.94 ± 0.30
4d	54.57 ± 3.54	70.47 ± 2.30
4e	72.11 ± 0.92	89.30 ± 0.25
4f	73.62 ± 0.28	91.24 ± 0.65
4g	73.43 ± 0.96	94.71 ± 0.45
4h	75.42 ± 0.55	92.64 ± 0.71
BHA	63.71 ± 0.79	87.29 ± 0.85
BHT	67.77 ± 0.61	88.63 ± 0.72

(Table 2). The inhibition values for these compounds were 89.6 – 94.7 % at 50 μg mL^{-1}. Compounds **4g**, **2**, **4h** and **3g** had the strongest interactions with the stable free radical DPPH$^{\bullet}$.

Experimental Section

Melting points were determined on a Barnstead Electrothermal melting point apparatus and are uncorrected. ^{1}H NMR and ^{13}C NMR spectra (δ, ppm) were recorded on a Varian-Mercury 200 MHz spectrometer using tetramethylsilane as an internal reference. IR spectra (ν, cm^{-1}) were run on a Perkin-Elmer 1600 FTIR spectrometer by using KBr pellets. Elemental analyses were performed on an ECS 4010 elemental combustion system CHNS-O. The necessary chemicals were purchased from Merck and Fluka.

Synthesis of compound 1

A solution of isonicotinic acid hydrazide (0.01 mol) in 25 mL of absolute ethanol was added to a solution of ethyl imido-*p*-chlorobenzoate hydrochloride (0.01 mol) in 25 mL of absolute ethanol. The mixture was stirred for 6 h at 0 – 5 °C and subsequently for 2 h at r. t. The reaction mixture was poured into a beaker containing 40 mL of cold water and 10 g of ice. The precipitate formed was washed with 50 mL of ice-water and then dried. The product was recrystallized from benzene-petroleum ether (40 – 60 °C) (1 : 2) to give pure compound **1**. Yield: 85 %, m. p. 80 – 81 °C. – IR (KBr): ν = 3203 (NH), 1671 (C=O), 1617 (C=N), 838, 752, 706 (arom. ring) cm^{-1}. – ^{1}H NMR ([D$_6$]-DMSO): δ = 1.35 (t, 3H, CH$_3$), 4.33 (q, 2H, OCH$_2$), 7.47 – 7.27 (m, 5H, Ar-H), 8.63 – 8.74 (m, 3H, Ar-H), 10.97 (s, 1H, NH). – ^{13}C NMR ([D$_6$]-DMSO): δ = 14.00 (CH$_3$), 63.33 (OCH$_2$), 121.10 (2C), 128.78 (2C), 129.45, 129.66, 135.21, 140.76 (2C), 150.03 (2C) (all Ar-C), 160.75 (C=N), 164.34 (C=O).

Synthesis of compound 2

Compound **1** (0.005 mol) was added to a solution of hydrazine hydrate (0.01 mol) in 50 mL of 1-propanol and the mixture was refluxed for 24 h. On cooling, a precipitate was formed. This product was filtered and, after drying, was washed with 20 mL of benzene. The remaining solid was recrystallized from 1-propanol to afford pure compound **2**. Yield: 54 %, m. p. 261 – 262 °C. – IR (KBr): ν = 3311, 3184 (NH$_2$), 1602 (C=N), 835, 731, 699 (arom. ring) cm^{-1}. – ^{1}H NMR ([D$_6$]-DMSO): δ = 6.44 (s, 2H, NH$_2$), 7.61 – 7.65 (d, 2H, Ar-H), 8.03 – 8.09 (m, 4H, Ar-H), 8.74 – 8.77 (m, 2H, Ar-H). – ^{13}C NMR ([D$_6$]-DMSO): δ = 121.83 (2C), 125.46, 128.59 (2C), 129.96 (2C), 134.13, 134.67, 149.97 (2C) (all Ar-C), 152.25 (triazole C$_3$), 154.08 (triazole C$_5$). – Anal. for $C_{13}H_{10}N_5Cl$ (271.7): calcd. C 57.47, H 3.71, N 25.78; found C 57.77, H 3.72, N 25.48.

General method for the synthesis of compounds 3a – 3h

The corresponding aldehyde (0.005 mol) was added to a solution of compound **2** (0.005 mol) in 20 mL of glacial acetic acid, and the mixture was refluxed for 4 h. After cooling, the mixture was poured into a beaker containing 100 mL of ice-water. The precipitate formed was filtered. After drying *in vacuo*, the product was recrystallized from an appropriate solvent to give the desired compound.

3-(4-Chlorophenyl)-5-(pyridin-4-yl)-4-(4-fluorobenzylidenamino)-4H-1,2,4-triazole (3a)

Recrystallized from ethanol-water (1 : 1), yield 55 %, m. p. 230 – 231 °C. – IR (KBr): ν = 1560, 1602 (C=N), 829, 770, 686 (arom. ring) cm^{-1}. – ^{1}H NMR ([D$_6$]-DMSO): δ = Ar-H [7.34 – 7.44 (m, 3H), 7.57 – 7.61 (m, 3H), 7.80 – 7.97 (m, 6H)], 8.69 and 8.64 (s, 1H, -N=CH, *E* and *Z* isomers). – ^{13}C NMR ([D$_6$]-DMSO): δ = 116.82, 121.72 (2C), 124.62, 127.66 (2C), 129.02 (2C), 129.96 (2C), 132.02 (2C), 133.41, 134.85, 148.30 (2C), 167.70 (all Ar-C), 149.87 (triazole C-3), 150.31 (triazole C-5), 170.64 and 170.17 (N=CH, *E* and *Z* isomers). – Anal. for $C_{20}H_{13}N_5ClF$ (377.8): calcd. C 63.58, H 3.47, N 18.54; found C 63.59, H 3.48, N 18.10.

3-(4-Chlorophenyl)-5-(pyridin-4-yl)-4-(4-chlorobenzylidenamino)-4H-1,2,4-triazole (3b)

Recrystallized from ethanol-water (1 : 1), yield 71 %, m. p. 233 – 234 °C. – IR (KBr): ν = 1559, 1603 (C=N), 850, 823, 773, 687 (arom. ring) cm^{-1}. – ^{1}H NMR ([D$_6$]-DMSO):

δ = Ar-H [7.56 – 7.64 (m, 6H), 7.81 – 7.88 (m, 6H)], 8.73 and 8.70 (s, 1H, -N=CH, *E* and *Z* isomers). – ^{13}C NMR ([D$_6$]-DMSO): δ = 121.74 (2C), 124.56, 129.02 (2C), 129.48 (2C), 129.77, 129.97 (2C), 130.82 (2C), 133.36, 134.87, 138.45, 148.29 (2C) (all Ar-C), 149.84 (triazole C-3), 150.31 (triazole C-5), 170.64 and 170.58 (N=CH, *E* and *Z* isomers). – Anal. for $C_{20}H_{13}N_5Cl_2$ (394.3): calcd. C 60.93, H 3.32, N 17.76; found C 60.71, H 3.32, N 17.85.

*3-(4-Chlorophenyl)-5-(pyridin-4-yl)-4-(4-bromobenzylidenamino)-4H-1,2,4-triazole (**3c**)*

Recrystallized from ethanol-water (1 : 1), yield 68 %, m. p. 186 – 187 °C. – IR (KBr): ν = 1588, 1602 (C=N), 840, 822, 724, 689 (arom. ring) cm^{-1}. – ^{1}H NMR ([D$_6$]-DMSO): δ = Ar-H [7.56 – 7.85 (m, 12H)], 8.69 and 8.63 (s, 1H, -N=CH, *E* and *Z* isomers). – ^{13}C NMR ([D$_6$]-DMSO) δ = 121.43 (2C), 123.58, 129.00 (2C), 129.87 (2C), 129.98, 130.92, 131.79 (2C), 132.44 (2C), 133.15, 134.90, 147.63 (2C) (all Ar-C), 150.24 (triazole C-3), 150.33 (triazole C-5), 170.79 and 170.30 (N=CH, *E* and *Z* isomers). – Anal. for $C_{20}H_{13}N_5BrCl$ (438.7): calcd. C 54.76, H 2.99, N 15.96; found C 54.66, H 3.10, N 15.56.

*3-(4-Chlorophenyl)-5-(pyridin-4-yl)-4-(4-trifluoromethylbenzylidenamino)-4H-1,2,4-triazole (**3d**)*

Recrystallized from ethanol-water (1 : 1), yield 64 %, m. p. 177 – 178 °C. – IR (KBr): ν = 1572, 1601 (C=N), 829, 750, 721 (arom. ring) cm^{-1}. – ^{1}H NMR ([D$_6$]-DMSO): δ = Ar-H [7.57 – 7.62 (m, 4H), 7.82 – 7.93 (m, 8H)], 8.76 and 8.72 (s, 1H, -N=CH, *E* and *Z* isomers). – ^{13}C NMR ([D$_6$]-DMSO): δ = 121.84 (2C), 126.19, 129.00 (2C), 129.73 (2C), 129.86, 129.96 (2C), 130.06, 133.35, 134.76 (2C), 134.96, 149.34 (2C) (all Ar-C), 124.84 (CF$_3$), 149.85 (triazole C-3), 150.35 (triazole C-5), 170.17 and 169.62 (-N=CH-, *E* and *Z* isomers). – Anal. for $C_{21}H_{13}N_5ClF_3$ (427.8): calcd. C 58.96, H 3.06, N 16.37; found C 58.97, H 3.19, N 16.64.

*3-(4-Chlorophenyl)-5-(pyridin-4-yl)-4-(2-fluoro-4-chlorobenzylidenamino)-4H-1,2,4-triazole (**3e**)*

Recrystallized from ethanol-water (1 : 1), yield 73 %, m. p. 187 – 188 °C. – IR (KBr): ν = 1562, 1607 (C=N), 828, 755, 722, 689 (arom. ring) cm^{-1}. – ^{1}H NMR ([D$_6$]-DMSO): δ = Ar-H [7.48 – 7.67 (m, 5H), 7.80 – 7.84 (m, 4H), 8.01 – 8.09 (m, 2H)], 8.72 and 8.67 (s, 1H, -N=CH-, *E* and *Z* isomers). – ^{13}C NMR ([D$_6$]-DMSO): δ = 117.04, 118.00, 121.92 (2C), 124.64, 126.00, 128.98 (2C), 129.81, 130.13 (2C), 133.45, 134.93, 139.36, 149.27 (2C), 158.79 (all Ar-C), 149.77 (triazole C-3), 150.32 (triazole C-5), 163.94 and 163.17 (-N=CH-, *E* and *Z* isomers). – Anal. for $C_{20}H_{12}N_5Cl_2F$ (412.3): calcd. C 58.27, H 2.93, N 16.99; found C 58.27, H 2.93, N 16.56.

*3-(4-Chlorophenyl)-5-(pyridin-4-yl)-4-(2-hydroxybenzylidenamino)-4H-1,2,4-triazole (**3f**)*

Recrystallized from ethanol-water (1 : 1), yield 63 %, m. p. 215 – 216 °C. – IR (KBr): ν = 3131 (OH), 1570, 1607 (C=N), 831, 764, 725 (arom. ring) cm^{-1}. – ^{1}H NMR ([D$_6$]-DMSO): δ = Ar-H [6.86 – 6.95 (m, 2H), 7.40 – 7.61 (m, 4H), 7.82 – 7.95 (m, 6H)], 8.73 and 8.69 (s, 1H, -N=CH-, *E* and *Z* isomers), 10.43 (s, 1H, OH). – ^{13}C NMR ([D$_6$]-DMSO): δ = 117.44, 118.24, 120.53 (2C), 122.67, 126.00, 128.26 (2C), 129.73 (2C), 130.72, 134.50, 135.35, 136.08, 150.12 (2C), 159.60 (all Ar-C), 150.64 (triazole C-3), 151.09 (triazole C-5), 167.93 and 167.47 (-N=CH-, *E* and *Z* isomers). – Anal. for $C_{20}H_{14}N_5OCl$ (375.8): calcd. C 63.92, H 3.75, N 18.63; found C 63.97, H 4.16, N 18.48.

*3-(4-Chlorophenyl)-5-(pyridin-4-yl)-4-(2-hydroxy-5-bromobenzylidenamino)-4H-1,2,4-triazole (**3g**)*

Recrystallized from ethanol-water (1 : 1), yield 95 %, m. p. 254 – 255 °C. – IR (KBr): ν = 3043 (OH), 1597, 1602 (C=N), 828, 775, 728 (arom. ring) cm^{-1}. – ^{1}H NMR ([D$_6$]-DMSO): δ = Ar-H [6.84 – 6.88 (d, 2H), 7.58 – 7.62 (m, 4H), 7.80 – 7.96 (m, 5H)], 8.67 and 8.63 (s, 1H, -N=CH-, *E* and *Z* isomers), 10.78 (s, 1H, OH). – ^{13}C NMR ([D$_6$]-DMSO): δ = 110.85, 119.00 (2C), 119.31, 124.80, 125.09, 128.98, 129.00 (2C), 129.99 (2C), 130.09, 134.80, 137.50, 149.81 (2C), 157.29 (all Ar-C), 150.29 (triazole C-3 and triazole C-5), 165.57 and 165.45 (-N=CH-, *E* and *Z* isomers). – Anal. for $C_{20}H_{13}N_5OBrCl$ (454.7): calcd. C 52.83, H 2.88, N 15.40; found C 53.09, H 2.99, N 14.93.

*3-(4-Chlorophenyl)-5-(pyridin-4-yl)-4-(2-hydroxy-5-methoxybenzylidenamino)-4H-1,2,4-triazole (**3h**)*

Recrystallized from ethanol-water (1 : 1), yield 56 %, m. p. 215 – 216 °C. – IR (KBr): ν = 3235 (OH), 1573 – 1603 (C=N), 823, 750, 654 (arom. ring) cm^{-1}. – ^{1}H NMR ([D$_6$]-DMSO): δ = 3.85 (s, 3H, OCH$_3$), Ar-H [6.98 (d, 2H), 7.36 (d, 2H), 7.57 – 7.63 (m, 3H), 7.86 – 7.95 (m, 4H)], 8.74 and 8.71 (s, 1H, -N=CH-, *E* and *Z* isomers), 10.22 (s, 1H, OH). – ^{13}C NMR ([D$_6$]-DMSO): δ = 53.72 (OCH$_3$), 121.65 (2C), 124.65, 125.26, 127.83, 128.64, 128.72 (2C), 129.70 (2C), 133.86, 134.70, 139.85, 149.97 (2C), 153.08, 153.70 (all Ar-C), 152.01 (triazole C-3 and triazole C-5), 164.34 and 164.27 (-N=CH-, *E* and *Z* isomers). – Anal. for $C_{21}H_{16}N_5O_2Cl$ (405.8): calcd. C 62.15, H 3.97, N 17.26; found C 62.37, H 3.96, N 17.25.

*General method for the synthesis of compound **4a**–**4h***

The corresponding compound **3a** – **h** (0.005 mol) was dissolved in 50 mL of dried methanol, and NaBH$_4$ (0.005 mol) was added in small portions to this solution. The mixture was refluxed for 20 min and then allowed to cool. After evaporation at 25 – 30 °C under reduced pressure, the solid residue was washed with cold water. After drying *in vacuo*, the solid product was recrystallized from an appropriate solvent to afford the desired compound.

*3-(4-Chlorophenyl)-5-(pyridin-4-yl)-4-(4-fluorobenzylamino)-4H-1,2,4-triazole (**4a**)*

Recrystallized from ethanol-water (1 : 1), yield 85 %, m. p. 215 – 216 °C. – IR (KBr): ν = 3245 (NH), 1560 – 1603 (C=N), 830, 733 (arom. ring) cm^{-1}. – ^1H NMR ([D_6]-DMSO): δ = 3.75 (d, 2H, CH_2), Ar-H [6.68 – 6.82 (m, 4H), 7.58 – 7.68 (m, 2H), 7.78 – 8.07 (m, 4H), 8.72 – 8.78 (m, 2H)], 7.42 (t, 1H, NH). – ^{13}C NMR ([D_6]-DMSO): δ = 53.75 (CH_2), 114.91 (2C), 121.62 (2C), 125.37, 128.74 (2C), 129.55 (2C), 129.65 (2C), 130.75 (2C), 134.93, 149.96 (2C), 159.07 (all Ar-C), 152.02 (triazole C-3), 153.69 (triazole C-5). – Anal. for $C_{20}H_{15}N_5ClF$ (379.8): calcd. C 63.25, H 3.98, N 18.44; found C 63.29, H 3.99, N 18.72.

*3-(4-Chlorophenyl)-5-(pyridin-4-yl)-4-(4-chlorobenzylamino)-4H-1,2,4-triazole (**4b**)*

Recrystallized from ethanol-water (1 : 1), yield 84 %, m. p. 262 – 263 °C. – IR (KBr): ν = 3270 (NH), 1601, 1565 (C=N), 836, 810, 824, 780 (arom. ring) cm^{-1}. – ^1H NMR ([D_6]-DMSO): δ = 3.72 (d, 2H, CH_2), Ar-H [6.72 – 6.76 (d, 2H), 7.065 (d, 2H), 7.56 – 7.62 (m, 2H), 7.83 – 7.93 (m, 4H), 8.70 (d, 2H)], 7.38 (t, 1H, NH). – ^{13}C NMR ([D_6]-DMSO): δ = 53.40 (CH_2), 121.65 (2C), 125.34, 127.88 (2C), 128.74 (2C), 129.59 (2C), 129.68 (2C), 130.62, 132.31, 134.00, 134.96, 149.97 (2C) (all Ar-C), 152.01 (triazole C-3), 153.69 (triazole C-5). – Anal. for $C_{20}H_{15}N_5Cl_2$ (396.3): calcd. C 60.62, H 3.82, N 17.67; found C 60.44, H 3.85, N 17.75.

*3-(4-Chlorophenyl)-5-(pyridin-4-yl)-4-(4-bromobenzylamino)-4H-1,2,4-triazole (**4c**)*

Recrystallized from ethanol-water (1 : 1), yield 60 %, m. p. 241 – 242 °C. – IR (KBr): ν = 3248 (NH), 1602, 1547 (C=N), 834, 825, 732 (arom. ring) cm^{-1}. – ^1H NMR ([D_6]-DMSO): δ = 3.72 (d, 2H, CH_2), Ar-H [6.72 (d, 2H), 7.21 (d, 2H), 7.43(d, 2H), 7.62 – 7.90 (m, 4H), 8.73 (bs, 2H)], 7.40 (bs, 1H, NH). – ^{13}C NMR ([D_6]-DMSO): δ = 53.52 (CH_2), 120.96, 121.73 (2C), 125.37, 128.80 (2C), 129.74 (2C), 130.86 (2C), 131.01(2C), 134.44, 134.75, 135.03, 149.98 (2C) (all Ar-C), 150.12 (triazole C-3 and triazole C-5). – Anal. for $C_{20}H_{15}N_5BrCl$ (440.7): calcd. C 54.51, H 3.43, N 15.89; found C 54.68, H 3.53, N 16.19.

*3-(4-Chlorophenyl)-5-(pyridin-4-yl)-4-(4-trifluoromethylbenzylamino)-4H-1,2,4-triazole (**4d**)*

Recrystallized from ethanol-water (1 : 1), yield 85 %, m. p. 216 – 217 °C. – IR (KBr): ν = 3256 (NH), 1603, 1550 (C=N), 825, 770, 733 (arom. ring) cm^{-1}. – ^1H NMR ([D_6]-DMSO): δ = 3.82 (d, 2H, CH_2), Ar-H [6.94 (d, 2H), 7.33 (d, 2H), 7.53 – 7.60 (m, 2H), 7.81 – 7.91 (m, 4H), 8.69 (d, 2H)], 7.48 (t, 1H, NH). – ^{13}C NMR ([D_6]-DMSO): δ = 53.74 (CH_2), 121.67 (2C), 125.56(2C), 128.67 (2C), 128.76 (2C), 129.62 (2C), 129.71, 133.87, 134.72, 134.97, 139.88, 149.99 (2C) (all Ar-C), 124.75 (CF_3), 152.03 (triazole C-3), 153.10 (triazole C-5). – Anal. for $C_{21}H_{15}N_5ClF_3$ (429.8): calcd. C 58.68, H 3.52, N 16.29; found C 59.05, H 3.53, N 16.19.

*3-(4-Chlorophenyl)-5-(pyridin-4-yl)-4-(2-fluoro-4-chlorobenzylamino)-4H-1,2,4-triazole (**4e**)*

Recrystallized from ethanol-water (1 : 1), yield 66 %, m. p. 234 – 235 °C. – IR (KBr): ν = 3254 (NH), 1610, 1582 (C=N), 825, 820, 734 (arom. ring) cm^{-1}. – ^1H NMR ([D_6]-DMSO): δ = 3.78 (d, 2H, CH_2), Ar-H [6.74 – 6.98 (m, 3H), 7.55 – 7.61 (m, 3H), 7.84 – 7.94 (m, 3H), 8.70 (d, 2H)], 7.47 (t, 1H, NH). – ^{13}C NMR ([D_6]-DMSO): δ = 47.27 (CH_2), 115.14, 121.50 (2C), 125.09, 128.49, 128.60 (2C), 129.47, 129.58 (2C), 132.54, 133.67, 134.64, 134.91, 149.84 (2C), 162.87 (all Ar-C), 151.93 (triazole C-3), 153.66 (triazole C-5). – Anal. for $C_{20}H_{14}N_5Cl_2F$ (414.3): calcd. C 57.99, H 3.41, N, 16.91; found C 57.99, H 3.42, N 16.48.

*3-(4-Chlorophenyl)-5-(pyridin-4-yl)-4-(2-hydroxybenzylamino)-4H-1,2,4-triazole (**4f**)*

Recrystallized from ethanol-water (1 : 1), yield 80 %, m. p. 293 – 294 °C. – IR (KBr): ν = 3265 (NH), 3070 (OH), 1607, 1557 (C=N), 825, 756, 733 (arom. ring) cm^{-1}. – ^1H NMR([D_6]-DMSO): δ = 3.75 (d, 2H, CH_2), Ar-H [6.50 – 6.70 (m, 3H), 7.14 (bs, 1H), 7.59 – 7.62 (m, 3H), 8.00 – 8.06 (m, 4H), 8.70 (bs, 1H)], 7.00 (t, 1H, NH), 9.47 (s, 1H, OH). – ^{13}C NMR ([D_6]-DMSO) δ = 49.14 (CH_2), 114.67, 118.44, 121.09 (2C), 125.49, 128.48, 128.58 (2C), 128.96, 129.66 (2C), 130.56, 134.01, 134.74, 149.82 (2C), 155.71 (all Ar-C), 152.91 (triazole C-3), 153.62 (triazole C-5). – Anal. for $C_{20}H_{16}N_5OCl$ (377.8): calcd. C 63.58, H 4.27, N 18.54; found C 63.67, H 4.30, N 18.32.

*3-(4-Chlorophenyl)-5-(pyridin-4-yl)-4-(2-hydroxy-5-bromobenzylamino)-4H-1,2,4-triazole (**4g**)*

Recrystallized from ethanol-water (1 : 1), yield 83 %, m. p. 287 – 288 °C. – IR (KBr): ν = 3260 (NH), 3080 (OH), 1603, 1537 (C=N), 826, 820, 735 (arom. ring) cm^{-1}. – ^1H NMR ([D_6]-DMSO): δ = 3.73 (d, 2H, CH_2), Ar-H [6.48 (m, 1H), 6.80 (bs, 1H), 7.05 (m, 1H), 7.26 (bs, 1H), 7.57 (m, 3H), 7.93 (d, 4H)], 7.15 (t, 1H, NH), 9.69 (s, 1H, OH). – ^{13}C NMR ([D_6]-DMSO): δ = 48.17 (CH_2), 109.38, 116.61, 123.96 (2C), 125.37, 128.55 (2C), 129.57, 129.68 (2C), 131.17, 132.96, 134.56, 134.79, 149.81 (2C), 155.01 (all Ar-C), 151.96 (triazole C-3), 153.08 (triazole C-5). – Anal. for $C_{20}H_{15}N_5OBrCl$ (456.7): calcd. C 52.60, H 3.31, N 15.33; found C 52.49, H 3.47, N 15.02.

*3-(4-Chlorophenyl)-5-(pyridin-4-yl)-4-(2-hydroxy-5-methoxybenzylamino)-4H-1,2,4-triazole (**4h**)*

Recrystallized from ethanol-water (1 : 1), yield 78 %, m. p. 283 – 284 °C. – IR (KBr): ν = 3258 (NH), 3082 (OH), 1604, 1540 (C=N), 826, 803, 734 (arom. ring) cm^{-1}. – ^{1}H NMR ([D$_6$]-DMSO): δ = 3.45 (s, 3H, OCH$_3$), 3.73 (d, 2H, CH$_2$), Ar-H [6.22 (bs, 1H), 6.50 (d, 2H), 7.54 – 7.60 (m, 3H), 7.97 – 8.00 (m, 4H), 8.65 (bs, 1H)], 7.16 (t, 1H, NH), 8.92 (s, 1H, OH). – ^{13}C NMR ([D$_6$]-DMSO): δ = 48.87 (CH$_2$), 54.85 (OCH$_3$), 114.37, 115.23, 121.52 (2C), 125.50, 128.45, 128.53 (2C), 129.58, 129.68 (2C), 134.44, 134.69, 149.35, 149.81 (2C), 153.72 (all Ar-C), 151.89 (triazole C-3), 153.01 (triazole C-5). – Anal. for $C_{21}H_{18}N_5O_2Cl$ (407.9): calcd. C 61.84, H 4.45, N 17.17; found C 61.49, H 4.72, N 17.53.

Assay of antioxidant activity

The compound, 50 μg mL^{-1}, and a standard sample (BHA, BHT) in 2.5 mL of potassium phosphate buffer (0.04 M, pH = 7.0) were added to a linoleic acid emulsion in potassium phosphate buffer (2.5 mL, 0.04 mM, pH = 7.0) [34]. As a reference 5 mL of a control fluid consisting of linoleic acid emulsion (2.5 mL) and potassium phosphate buffer (2.5 mL, 4 mM, pH = 7.0) were used. The reaction mixture was incubated in a glass flask at 37 °C in the dark. The solution without compounds or standard was used as a blank sample. The lipid peroxide formation was then monitored [35]. To 1 mL solution, 20 % trichloroacetic acid (2 mL) and thiobarbituric acid (2 mL) were added. This mixture was then placed in a boiling water bath for 25 min. After cooling, it was centrifuged at 3000 rpm for 20 min. The absorbance of the supranatant was measured at 532 nm [36]. Analysis of all samples was done in triplicate and averaged. The inhibition of lipid peroxidation in percent was calculated by the following the equation:

Inhibition of lipid peroxidation (in %) = $100 \times (A_0 - A_1)/A_0$, where A_0 is the absorbance of control and A_1 is the absorbance of compounds or standards.

Assay of antiradical activity

Some new 3-(4-chlorophenyl)-5-(pyridin-4-yl)-4H-1,2,4-triazole derivatives and standard compounds were tested for their ability to bleach the stable radical DPPH$^\bullet$ (1-diphenyl-2-picryl-hydrazyl) as described by Blois at 50 μg mL^{-1} [37]. 1 mL of DPPH$^\bullet$ solution [0.1 mmol L^{-1}, in 95 % ethanol (v/v)] was incubated with different concentrations of extract. Thirty minutes later, the absorbance was measured at 517 nm. Lower absorbance of the reaction mixture indicated the higher free radical scavenging activity. The DPPH$^\bullet$ concentration (mM) in the reaction medium was calculated from a calibration curve, determined by linear regression (R^2 = 0.9998): Absorbance = $6.5781 \times [\text{DPPH}^\bullet] + 0.058$.

The activity to scavenge the DPPH$^\bullet$ radical was calculated using the equation:

DPPH$^\bullet$ scavenging effect (in %) = $100 \times (A_0 - A_1)/A_0$, where A_0 is the absorbance of control and A_1 is the absorbance of compounds or standards. The DPPH$^\bullet$ solution without sample solution was used as a control. Triplicate samples were run for each set and averaged.

Acknowledgement

The authors are thankful to Prof. Dr. N. Yaylı, Department of Chemistry, Faculty of Arts and Science, Karadeniz Technical University, Trabzon, Turkey, for elemental analyses.

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